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Novel agents for multiple myeloma

Edited by
Claudio Cerchione, Hans C. Lee, Giovanni Martinelli, María-Victoria Mateos and Kenneth Anderson

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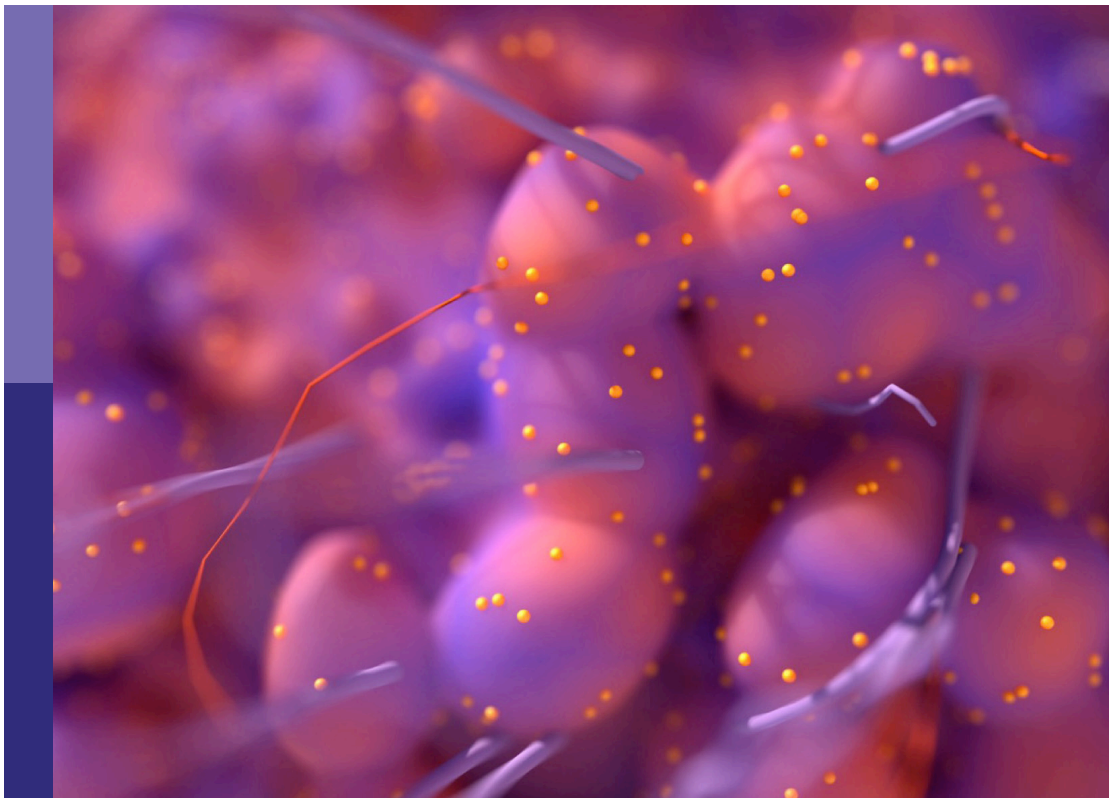

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Novel agents for multiple myeloma

Topic editors

Claudio Cerchione — Scientific Institute of Romagna for the Study and Treatment of Tumors (IRCCS), Italy

Hans C. Lee — University of Texas MD Anderson Cancer Center, United States

Giovanni Martinelli — University of Bologna, Italy

María-Victoria Mateos — University Hospital of Salamanca, Spain

Kenneth Anderson — Dana–Farber Cancer Institute, United States

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REVIEW
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Multiple Myeloma Outpatient Transplant Program in the Era of Novel Agents: State-of-the-Art

Massimo Martino[1*†], *Annalisa Paviglianiti*[1], *Mara Memoli*[2,3], *Giovanni Martinelli*[4] *and Claudio Cerchione*[4*†]

[1] Stem Cell Transplant Program, Clinical Section, Department of Hemato-Oncology and Radiotherapy, Grande Ospedale Metropolitano "Bianchi-Melacrino-Morelli", Reggio Calabria, Italy, [2] Department of Hematology and Cellular Therapy, Saint Antoine Hospital, AP-HP, Paris, France, [3] Department of Medicine and Surgery, Hematology and Hematopoietic Stem Cell Transplant Center, University of Naples Federico II, Naples, Italy, [4] Hematology Unit, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy

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***Correspondence:**
Massimo Martino
massimo.martino@ospedalerc.it
Claudio Cerchione
claudio.cerchione@irst.emr.it

†ORCID:
Massimo Martino
0000-0002-3987-419X
Claudio Cerchione
0000-0002-9104-5436

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Multiple myeloma (MM) is the most common indication for autologous stem cell transplantation (ASCT), and outpatient models have been widely developed in this setting. Although numerous studies have demonstrated the safety and feasibility of outpatient ASCT, it is not a routine procedure. Stringent guidelines for patient selection and clinical management, including functional status, caregiver support, and psychological aspects, are essential to identify eligible patients. However, there is still no general agreement on these criteria. Quality of life data are limited and contradictory. There is considerable variability in outpatient transplant models, and there are no randomised studies supporting the use of one over the other. Studies evaluating results in terms of long-term survival, transplant toxicity in comparison with a standard approach are lacking. The procedure is cost-effective within the context of a hospital budget, but an in-depth analysis of the real cost of these programmes has yet to be performed.

Keywords: multiple myeloma, autologous stem cell transplantation, outpatient, inpatient, novel agents, cost-effectiveness

INTRODUCTION

Multiple myeloma (MM) is an incurable blood cancer. Considered as a chronic condition, it can be treated to slow its spread.

In last decades, the introduction of bortezomib as first-line therapy have provided considerable improvements in treatment and prognosis of patients with MM.

Although novel agents, including monoclonal antibodies, were recently introduced into clinical practise, high-dose chemotherapy followed by autologous stem cell transplantation (ASCT) remains the standard of care for eligible patients (1–4). High-dose melphalan (HDM) (200 mg/m^2) is the standard conditioning regimen (5) and the procedure is characterised by a very low transplant-related mortality (TRM) (6).

Healthcare systems are always faced with problems due to the counterbalance between demand and supply. Since request arrives randomly, it can generate waiting lists unless treatment capacity

exceeds demand levels. In recent years, a significant increase in hospital waiting lists and times in recent years, causing concerns about the appropriate use of healthcare resource was observed (7).

Several randomised trial have demonstrated the feasibility of outpatient ASCT as an optimal approach to managing hospital length of stay (8–21). This procedure is not feasible in several settings, such as low or middle in-come countries.

The ease of administration of HDM, the relatively low extra-haematological toxicity, and the short duration of neutropenia post-chemotherapy make patients with MM a perfect candidate (5).

ASCT OUTPATIENT MODELS

Patients scheduled for ASCT are commonly admitted to Transplant Units on an inpatient basis. In this setting, the central venous catheter (CVC) insertion, HDC administration, ASCT, and supportive care during neutropenia performed in positive-pressure reverse isolation rooms with a hospital stay of 3 to 4 weeks. Several trials have investigated different outpatient models to evaluate the safety, efficacy, and potential cost- saving of reducing hospital stay for patients undergoing ASCT (**Tables 1** and **2**).

Early-Discharge Model

In this model, CVC insertion, HDC administration, and ASCT are carried out in positive pressure reverse isolation rooms, whereas supportive care of the aplastic phase is performed on an outpatient basis.

Ferrara et al. reported outcomes of 28 patients with MM who underwent ASCT using an early-discharge model (EDM). There were no cases of early TRM, and the readmission rate was 36% (9). The same authors described a series of 161 MM patients submitted to ASCT on an outpatient basis and managed post-procedure with either post-transplant single-dose PEG-filgrastim (n = 48) or conventional daily granulocyte colony-stimulating factors (G-CSF) (n = 113) (13). The conditioning regimen was HDM (140 - 200 mg/m2). Overall, a second hospitalisation was required in 32% of cases (36/161 procedures). There was no difference in the rate of readmissions between the PEG-filgrastim and filgrastim groups (12% vs. 26%, respectively, p = 0.06), however the low number of patients prevents to draw firm conclusions.

Faucher et al. carried out the first and only randomised trial to date comparing an EDM with standard inpatient ASCT in 131 patients with MM, lymphomas, or solid tumours. In both arms, high-dose chemotherapy (HDC) was administered, and ASCT performed during hospitalisation. Patients in the EDM arm were discharged on day 0, looked after at home by a caregiver, and followed up on an outpatient basis. The study reported a readmission rate of 86%, mainly during the first week (87% of re-admitted patients) and before haematological recovery (93%). Although safe and feasible, the procedure was highly dependent on economic-social factors. Of the 131 patients, 39% with an indication for HDC was not be discharged early for social or psychological reasons such as lack of a caregiver, living far away from the hospital, or patient's own request. The study demonstrated that the EDM model was highly dependent on caregivers and that only some patients could benefit from it (8).

Martino et al. analysed the outcome of 382 patients with MM who underwent ASCT in EDM in Italy between 1998 and 2012. Overall, TRM was 1%. A second hospital admission during the aplastic phase occurred in 98 (18.8%) patients. Neutropenic fever (NF) was observed in 161 cases (30.8%) and required readmission in 76. The incidence of grade 3-4 mucositis was 9.6%. In multivariate analysis, independent predictor factors of readmission were fever, grade 3–4 mucositis, and delayed transplantation. No centre effect was observed (p = 0.36) (16). In 2015, Paul et al. analysed 301 ASCT procedures carried out for MM, including patients with a ≤ 4-day hospitalisation (n = 82) and with a ≥ 5-day stay in hospital (n = 219) (17). Amongst the shorter stay patients, 67% required readmission before day + 100. They also had a lower cumulative number of days in hospital than the longer stay group (9 vs.18 day, respectively, $p < 0.0001$), a lower infection rate (22% vs. 46%, $p < 0.001$) and fewer admissions to the Intensive Care Units (0% vs. 5.9%, respectively, p = 0.02). The 100- day mortality rate was 1.8% (p = 0.6) in the longer stay group, whereas no patients died in the short stay group. Subsequently, an Asian study analysed the efficacy, cost-effectiveness, and safety of EDM ASCT (n = 10) compared to inpatient ASCT (n = 11) in a MM cohort treated in a single centre with relatively good healthcare resources and easy

TABLE 1 | Outpatient Autologous Stem Cell Transplantation Models.

Model	Central venous catheter insertion	High-dose chemotherapy administration	Stem cell infusion	Management of aplastic phase	Comments
Early Discharge	Inpatient Clinic	Inpatient Clinic	Inpatient Clinic	Outpatient clinic	The most widely used model worldwide
Delayed Admission	Outpatient clinic	Outpatient clinic	Outpatient clinic	Inpatient Clinic	The model does not significantly reduce the duration of hospitalisation and its costs when compared to other models.
Total Outpatient	Outpatient clinic	Outpatient clinic	Outpatient clinic	Outpatient clinic	This approach is associated with the shorter stay in hospital
Mixed inpatient-Outpatient	Outpatient clinic	Outpatient clinic	Admitted to the Inpatient Unit for stem cell infusion on day 0 for 2 days	Outpatient clinic	This programme was primarily designed and used in Italy. Inpatient stem cell infusion is mandatory to obtain the optimal reimbursement according to the Italian diagnosis-related group (DRG) system
At-Home	Inpatient Clinic	Inpatient Clinic	Inpatient Clinic	Outpatient Clinic	The most attractive model for the future

TABLE 2 | Clinical studies evaluating the management and outcome of Outpatient Autologous Stem Cell Transplantation in Multiple Myeloma.

Author(Year)	Study Design	Specific eligibility criteria	Regimen	Model	No. of Transplants	No. of read-mission%	Reasons for hospitalisation	TRM %	Comments
Ferrara (13)	Prospective	Presence of a caregiver, patient adherence, home within a 45-min travelling distance from the hospital	MEL	EDM	48 (PEG) 113 (G- CSF)	(PEG) 88 (G-CSF) 74	FN and severe mucositis. PEG group 12% G-CSF group 26%	PEG= 0 G-CSF=0.8	The administration of single-dose PEG resulted in similar outcome in terms of safety and efficacy with respect to 8 days of G-CSF.
Faucher (8)	Randomized	Available family caregiver 24 h a day, and living within 45 min driving distance to and from the hospital. Patients not meeting these criteria were not discharged	MEL	EDM, IN	66 (EDOM) 65 (IN)	15 (EDOM) 18(IN)	unknown	0	First randomised study comparing EDOM with standard inpatient ASCT. About 40% of patients in the EDOM arm were not discharged for social or psychological reasons
Martino (15)	Retrospective	Caregiver on a 24-h basis; home within easy reach of the transplantation centre; adequate activities of daily living	MEL	EDM	522	18.8	Fever: 14.6% Mucositis: 1.7% Diarrhoea: 1.7% Arrhythmia:0.4 %; TIA: 0.2% Cutaneous haemorrhage: 0.2%	1	No centre effect was observed
Paul (16)	Retrospective	Availability of Caregiver at home; short distance to transplant centre; patient and physician preference; ECOG≦1	MEL	EDM	301 (n=82, ≤4days; n=219,≥5 days)	67	Fever: 87% Inability to maintain hydration: 7% Other: 65%	0	Carefully selected patients were managed with a brief initial hospitalisation and outpatient follow-up, with low morbidity and mortality
Abid (18)	Case-Control study	<65 years of age; newly diagnosed, transplant-eligible MM patients	MEL	EDM, IN	10 (EDM), 11 (IN)	Unknown	FN	0	Findings demonstrated that outpatient ASCT can be considered in Asia in carefully selected patients
DAM									
Anastasia (12)	retrospective		MEL	DAM	123	93		0	The model was not associated with a significant reduction in length of hospital stay
TOM									
Kassar (10)	Retrospective	Patients with a primary care provider	MEL	TOM	90	58	Fever: 33% No Primary Care Provider:13% Mucositis:6% Other: 6%	0	80% of patients remained neutropenic for 5 days or less, and no patient had neutropenia for more than 7 days. The study confirmed the relatively low extra-haematological toxicity and the short period of post-high-dose melphalan neutropenia
Gerzt (11)	Prospective non-randomised study	Availability of a chaperone or Caregiver with them	MEL	TOM	716	39	Declining performance status, mucositis infection with hemodynamic instability	1.1	Younger patients and those with serum creatinine levels less than 1.5 mg/dl were more likely to complete the programme as outpatients
Holbro (14)	Retrospective	Availability of a caregiver and residence close to the hospital	MEL	TOM	91	84	Fever: 85% Mucositis: 6% Other: 9%	0	The cost savings was $19,522 per patient

(Continued)

TABLE 2 | Continued

Author(Year)	Study Design	Specific eligibility criteria	Regimen	Model	No. of Transplants	No. of read-mission%	Reasons for hospitalisation	TRM %	Comments
Shah (19)	Retrospective. Outpatient versus inpatient ASCT	Patients <70 years old with normal organ function, committed Caregiver and residence within 30 min of the cancer centre were considered for outpatient management	MEL or MEL+BU	TOM	Inpatient *n*=669 Outpatient *n*=377	55	The leading cause (42%) of these admissions was neutropenic fever.	Inpatient= 1.5 Outpatient= 0.3	Patients transplanted as outpatients were significantly younger and more likely to have a HCTCI score <2and creatinine <2. The inpatient group experienced significantly more adverse events. Two-year PFS was significantly longer in the out- patient group. Two-year OS was also longer in the outpatient group.
Kodad (20)	Retrospective	Patients with MM, POEMS syndrome, amyloidosis	MEL	TOM	752	245 (32.6%)		0.4	Low transplant-related mortality. Overall resource utilization significantly lower than that of inpatient ASCT. Model requiring a multidisciplinary approach with close follow-up.
Yip (21)			MEL	TOM	54	100	FN and gastrointestinal toxicity	0	The number of post-transplant admissions and the high complications reported are not in line with other studies in this sector
MIOM									
Morabito (2002)	Retrospective	Psychosocial evaluation to establish skills and compliance of patients and caregivers.	MEL	MIOM	60	56.7	FN	0	Patients were admitted for HPC infusion for 2 days for reimbursement purposes.
Home-Care									
Martino (17)	Three-arm prospective, non-randomised study	Availability of a caregiver who was willing to stay at home and help, and approval of the home by the medical staff of the SCT Unit	MEL	HC/ ED OM/IN	HC=15 EDOM=25IN=40	HC=13 EDOM=8	FN	0	Vey Innovative approach

FN, Febrile neutropenia; EDM, early discharge model; TIA, transient ischemic attack; ECOG, Eastern Cooperative Oncology Group; DAM, delayed admission model; MIOM, mixed Inpatient-Outpatient model; TOM, total Outpatient Clinic; HCTCI, Hematopoietic Stem Cell Comorbidity Index; PFS, progression-free survival: OS, overall survival; HPC, hematopoietic progenitor cell; MEL, melphalan, BU, busulfan; SCT, Stem Cell Transplant; HM, home-care, IN, in-patient.

and prompt access to care due to thanks to its small size and excellent emergency medical services. Mortality was 0%. The authors demonstrated that outpatient ASCT could be considered a viable/valid option in Asia in selected patients. The cost of treatment was significantly lower in the outpatient's arm than in the inpatient's arm because of higher hospitalisation-related costs for the latter (19).

Delayed Admission Model

In this model, first proposed in a study by Anastasia et al., HDC and ASCT were performed on an outpatient basis, whereas supportive care for the aplastic phase is provided positive-pressure reverse isolation rooms. The discharge was planned on day one and scheduled re-hospitalisation on day 5. One hundred and forty-four patients with various haematological and non-haematological malignancies entered the programme. The early discharge was feasible in 86% of cases, and only 5% of discharged patients were re-hospitalised before day 5, mainly due to severe mucositis or fever. The delayed admission model (DAM) did not result in a significant reduction and cost of hospitalisation when compared with other models (12).

Total Outpatient Model

The total outpatient model (TOM) is associated with the shortest duration of hospitalisation. In this case, conditioning chemotherapy and ASCT are performed on an outpatient basis. After ASCT, patients are followed daily in the Outpatient Clinic during the aplastic phase. The feasibility of TOM was first reported by Gerzt et al. (11) in 716 MM patients submitted to ASCT at the Mayo Clinic in Rochester, Minnesota (US). With a median hospitalisation time of 4 days, the study showed that 39% of patients did not require admission. However, the majority of patients who lived too far away from the Centre to commute every day were forced to find accommodation in local hotels to attend the Outpatient Clinic. This approach may substantially increase the out-of-pocket cost burden for patients.

Using a TOM model, Kassar et al. (10) showed the time and duration of neutropenia after HDM (140–200 mg/m^2). Nearly two-thirds of patients became neutropenic on day 5 and neutropenia lasted 5 days in 80% of the patients, and 7 days in the remaining 20%.

A retrospective analysis of 91 patients with MM who underwent outpatient ASCT showed that TOM ASCT could be performed safely (14). The majority required hospital admission during the first 100 days. Patient age and creatinine > 2 mg/dl were predictive factors for hospitalisation.

Shah et al. compared outcomes of 1,046 MM patients receiving ASCT as an inpatient procedure (n = 669) with those treated as outpatients (n = 377) (19). Although over half of the outpatients eventually had to be hospitalised (and thus only 20% of patients completed the procedure as outpatients), the overall incidence of adverse events was far lower than that of the inpatient's arm, with no difference in TRM. Two-year progression-free survival (PFS) and overall survival (OS) were significantly better in the outpatient group (60% vs. 50%, p = 0.005 and 83% vs. 77%, respectively, p = 0.01). The differences observed were associated with baseline characteristics of patients. Inpatients were older with more comorbidities and more advanced disease.

Outpatient ASCT for MM is the standard of care at the Vancouver General Hospital, given the increasing volume of patients and longer waiting times. Kodad et al. (20) evaluated the number of patients requiring hospital admission, duration of hospitalisation, patient characteristics, TRM, and OS in 724 patients who underwent ASCT. The majority of patients received HDM, the day 100all-cause mortality rate was 0.9%, and the TRM 0.4%. Yip et al. analysed the outpatient programme in 70 patients with MM who underwent HDC and ASCT in London's hospital (21). The authors concluded that the outpatient transplant programme was a safe procedure for eligible patients. An innovative care pathway has been established in the Mayo Clinic Stem Cell Transplantation Program (22), reducing day 100 mortality (all-cause) to 0.3%. Patients underwent transplantation with a median hospital duration of 0 days and with only 25% of patients requiring hospitalisation ≥ 5 days.

Mixed Inpatient-Outpatient Model

The mixed inpatient-outpatient model (MIOM) was used in Italy for the first time (9). Ferrari et al. reported that CVC insertion, fluid infusion, HDM, and supportive care during the aplastic phase were carried out in the Outpatient Clinic. Patients with MM were admitted to the hospital for two days during which ASCT was performed. An inpatient setting was mandatory for the procedure in order to obtain the highest reimbursement according to the Italian diagnosis-related group (DRG) system (23). Clinical outcomes were compared with a retrospective cohort of MM patients traditionally transplanted using an inpatient procedure. Patients in the MIOM programme showed a significant reduction in the length of hospitalisation and no increased toxicity. Overall, 6.7% of patients were not discharged after the ASCT, and, amongst those discharged as planned, 43% were re-hospitalised for a median of 9 days, significantly shorter than 20 days observed for patients undergoing conventional inpatient ASCT.

At-Home Model

The hospital-at-home is it is a model by which healthcare professionals provide the same level of care at the patient's home as a traditional hospital model. Martino et al. (17) published the preliminary results of a three-arm, prospective, non- randomised study to evaluate the feasibility and safety of a home-ASCT in 80 patients with MM. In the Home-Care arm (n=15), the patients were discharged the day after the transplant and managed daily at home. The mandatory condition for this type of model, in addition to the consent and availability of a caregiver 24/24 h, was the home no more than 20-min drive from the hospital. Patients who did not have a home near the hospital were discharged to a residential facility the day after the transplant and were treated as Outpatients. There were no cases of TRM and no differences in mucositis rates between the three arms of the study. FN incidence was lower in the outpatient (28%) and home- care cohorts (40%) than in inpatients (75%). Re-hospitalisations were necessary for 8%

and 15% of outpatients and home-care patients, respectively, all caused by fever

GENERAL RECOMMENDATIONS

The recommend inclusion criteria for MM Outpatient Autologous Stem Cell Transplantation are summarised in **Table 3**. Supportive care (such as management of nausea and vomiting, hydration, analgesic therapy) should not differ from recommended conventional ASCT guidelines (24).

Antimicrobial prophylaxis for outpatient ASCT should not differ from that required for conventional inpatient ASCT (25–27). Levofloxacin prophylaxis is associated with decreased risk of infection and fever (28), and primary antifungal prophylaxis is not recommended (29). Antiviral prophylaxis is recommended at least up to 3 months after transplantation, or until there is a satisfactory immunological recovery (CD4+ lymphocytes 4200/mmc), as well as *Pneumocystis jiroveci* prophylaxis. The first clinical evaluation should be on day +5 after discharge, and then twice weekly until sustained haematological and clinical recovery. Patients, caregivers, and family members should be adequately trained on the careful monitoring of fever and other infectious signs/symptoms.

International guidelines (30, 31) indicate that G-CSFs should be used as primary prophylaxis after chemotherapy when the risk of FN is > 20%, as happens after HDC and ASCT. Short-acting G-CSFs are the standard molecules for enhancing neutrophil recovery after ASCT but the long-acting G-CSF, pegfilgrastim, can also be used (32), Pegfilgrastim is more useful in an outpatient programme (33) as it is given in single doses, thus facilitating the work of staff and caregivers by reducing the total number of drug administrations needed. Recently, a study provided evidence of the superior efficacy of lipegfilgrastim over short-acting G-CSFs for the prevention of severe neutropenia in an MM ASCT setting (34). Lipegfilgrastim is a new, long-acting, once-per-cycle G-CSF for reducing the duration of neutropenia and the incidence of FN in adult cancer patients treated with chemotherapy. It recently received European Union marketing approval.

TABLE 3 | Suggested Inclusion Criteria for Multiple Myeloma Outpatient Autologous Stem Cell Transplantation.

Age ≤ 65 years
ECOG ≤ 2
Normal cardiac, lung, liver, and renal function
Absence of advanced disease
Absence of gram-negative MDR pathogens colonisation or infection during the 3 months prior to the scheduled transplant
Severe infection not completely resolved
Signed written informed consent
Availability of a caregiver 24 h/24 h
Detailed SOP for the Caregiver and patient
Distance from house to the hospital ≤ 1 h
Outpatient clinic available 24 h/day or bed reserved in the Transplant Unit
A specific phone line 24 h/24 h

ECOG, Eastern Cooperative Oncology Group; MDR, multidrug resistance; SOP, standard operating procedure.

READMISSION CRITERIA AFTER OUTPATIENT ASCT

Criteria for a readmission include severe mucositis unresponsive to outpatient management and/or fever > 38.3°C. In case of illness during neutropenia, patients must be evaluated within 1–2 h and blood pressure, O2 saturation, and vital signs carefully monitored. Patients without symptoms can be followed as outpatients after 6 h of clinical monitoring. The guidelines strongly advise the availability of a 24/7 active phone line to the haematologist on call in the Bone Marrow Transplant Unit (24). The clinical examination can be performed either in the Outpatient Clinic by the general practitioner or in Emergency Department in case of clinical worsening. In either case, immediate feedback can be given to the haematologist on call, and, if needed, oral antibiotic treatment can be started. A detailed standard operating procedure (SOP) in the event of FN should be made available to the patient, Caregiver, and general practitioner. Patient should undergo physical exam, blood cultures, and imaging studies when clinically indicated.

The Multinational Association for Supportive Care in Cancer (MASCC) score (35) could be used to evaluate the risk of chemotherapy-associated complications/febrile neutropenia, but this index has yet to be validated in an ASCT setting. The authors concluded that, although a MASCC score of 21 or less (high-risk patients) could be considered a criterion for rapid readmission, a score of 22 or more was not a sufficient criterion *per se* for defining patients at low risk whose readmission could be delayed (35).

Suggested criteria for readmission are reported in **Table 3**: hemodynamic instability (e.g., tachycardia and low blood pressure), impaired respiratory function (increased respiratory frequency and low oximetry on room air), oliguria, altered mental status and other signs of clinical instability**;** Grade > 2 oral mucositis and diarrhoea**;** colonisation by extended-spectrum beta-lactamases producing Enterobacteriaceae (colonisation by other multidrug resistance (MDR) pathogens); fever persisting after two days of broad-spectrum antibacterial therapy; and low patient compliance".

The use of empiric antibacterial therapy must follow internationally accepted guidelines for patients with FN and haematologic malignancies (36–38). Empiric broad-spectrum antibacterial therapy should be initiated within 1 h of the clinical evaluation, and as soon as the fever workup was completed. Outpatient oral antibiotic therapy (i.e., amoxicillin-clavulanate) can be considered (39), although intravenous antibiotics are preferred and should be chosen in the light of clinical and laboratory findings.

In 2018, a meta-analysis of 1940 patients with MM or lymphoma who underwent ASCT compared the risk of FN in outpatients and inpatients (40). The study showed a lower risk of FN, grade 2-3 mucositis, and septicemia in outpatient ASCT. In 2017, a retrospective study evaluating performance status and hematopoietic cell transplantation comorbidity index (HCT-CI) in 448 patients MM patients undergoing outpatient ASCT reported a lower Karnofsky performance status and higher

HCT-CI score in inpatient groups than in outpatients ($p < 0.004$) (41).

QUALITY OF LIFE

The Patient's Side

Transplant is usually associated with physical and psychological sequelae that can contribute to a dramatic decline in patients' quality of life (QoL) during the 3–4 weeks of isolation after stem cell infusion. Few trials have focused on patient QoL during an outpatient approach, often with conflicting results. Despite de lack of robust clinical data, the impression is that an outpatient transplant approach is correlated with better patients' QoL.

Summers et al. reported significantly better emotional well-being and QoL in outpatients than in inpatients, supporting outpatient ASCT approach as an ideal form of care for those with appropriate physical and psychological motivation (42). Conversely, overall QoL was not significantly different between outpatients and inpatients in a cohort of MM patients during the first 30 days after ASCT (43). Schulmeister et al. showed that the QoL decreased immediately post-treatment but then increased to above pre-treatment levels by six months (44). An excellent clinical outcome following ASCT was associated with better QoL and greater satisfaction with care. These studies, however, have a significant limitation in that they were observational, non-randomised studies, and patients could choose the type of transplantation procedure (outpatient or inpatient). Thus, subjective QoL outcomes were influenced by the initial choice.

The Caregiver's Side

Caregivers include parents, siblings, children, partners, and friends who play a critical role in the recovery from ASCT and are intimately involved in the patient's care (45). Foster et al. reported that, when a caregiver was involved during the hospitalisation phase of ASCT, the patient outcome in terms of OS was significantly better than in the group without a caregiver (46). Moreover, the cost-cutting and feasibility associated with the outpatient approach appear to be mediated mainly by the efforts of caregivers whose involvement is needed to decrease the need for hospital readmission (47). The majority of centres offering outpatient ASCTs require the availability of a caregiver 24/7 during the post-ASCT period to take on the many responsibilities traditionally shouldered by professionals (24). Such an agreement involves the total dedication of the Caregiver to the patient, which obviously impacts multiple areas of the carer's life (45).

Whilst several systematic reviews have evaluated the burden of transplant on caregivers (48, 49), only a few have included caregivers of patients receiving stem cell transplantations (SCTs) in the outpatient setting. Overall, the studies have corroborated existing literature on the experience of a significant burden amongst SCT caregivers across the SCT trajectory, highlighting the emotional costs of outpatient ASCT on caregivers and the need to identify caregivers at high risk of strain and distress (45, 50). With these premises, it is essential to design and conduct studies that can critically analyse the emotional burden of the caregivers, and the impact it may have on the clinical outcome of the outpatient transplant (51–53).

COST DATA

Several trials have shown that outpatient ASCT is cost-effective, mainly because of the shorter duration of hospitalisation (8, 54, 55). Holbro et al. reported a cost savings of $19,522 (Canadian dollars) per outpatient ASCT compared to inpatient procedure, with an annual savings of approximately $740,000 (14). Ghatnekaret al. showed that the major contributor to the total cost of MM treatment was the cost of inpatient care (56).

Clemmons et al. reported a reduction of about US $ 2000 per transplant when a mixed inpatient-outpatient ASCT model was applied (57) with a total annual cost saving of US$ 90,000. Shah et al. showed that the average cost of the procedure was $292,572 and$416,154 for the outpatient and the inpatient transplant group, respectively (19).

In the late 1980s, a tariff-calculating method was created using a diagnosis-related group (DRG) system based on the international classification of diseases, patients' characteristics as gender and age, presence of comorbidities, diagnosis procedures, and discharge status (23). Italian Regions pay the cost of hospitalisation based on the length of hospital stay and the identified DRG, based on a fixed price (58). The impact of new, costly therapies has made the DRG not the best method to evaluate the actual cost of a health service. Activity-based costing (ABC) is a tool developed to improve efficiency and control costs (59, 60). ABC endeavours to assign values to each activity and source, making it easier to understand and administrate total costs.

The use of the ABC system allows scrutiny of the complete map of activities and the relationships that connect them. Its implementation in healthcare centres has been hypothesised since the early 1990s, and now over 20% of hospitals in the U.S. and Canada use this method (61).

Martino et al. calculated the cost of ASCT in MM patients using the ABC method, and showed a charge of €28,615.15 and €16,499.43, in inpatient and outpatient ASCT, respectively. If we considered that in Calabria Region (south of Italy), the DRG reimbursement for a transplant is €60,000, the estimated cost saving per patient is €31,190.85 for the inpatient approach and €43,306.57 for the outpatient model.

Dunavin et al. using a merged dataset of the Center for International Blood and Marrow Transplant Research (CIBMTR) observational database and Centers for Medicare & Medicaid Services Medicare administrative claims data to analyse reimbursement, service utilisation and patient financial responsibility amongst Medicare beneficiaries in 1640 patients with MM who underwent ASCT in inpatient and outpatient settings (62). Total reimbursement and patient responsibility were analysed for patient and disease characteristics. Of the 1640 patients, 1445 (88%) underwent inpatient ASCT and 195 (12%), outpatient ASCT. The adjusted total mean reimbursement was

higher for inpatients than outpatients ($82,368 vs. $46,824, respectively; $p < 0.0001$). Adjusted total mean patient responsibility was $4,736 for inpatients and $6,944 for outpatients ($p< 0.0001$). Within 100 days of ASCT, 107 (55%) of the 195 outpatient recipients had required at least one readmission compared with 348 (24%) of the 1445 inpatients. Reimbursement, service utilisation, and financial responsibility varied on the basis of the ASCT setting.

DISCUSSION

Outpatient ASCT is feasible and safe with a TRM of 1% for MM, making it an appealing alternative to the standard inpatient ASCT. The popularity of outpatient ASCT is limited by concerns that the lack of protective isolation used during inpatient ASCT could predispose outpatients to a higher risk of toxicities, in particular infections. Although several studies have reported a lower incidence of FN in outpatient ASCT, it has yet to be established as a routine procedure, and many haematologists are still reluctant to adopt this approach. The extensive use of outpatient ASCT models in MM could contribute to making ASCT more competitive, especially when compared with the high cost of some new drugs. Opinion leaders should commit to writing specific reference recommendations/guidelines, and rigorous criteria for patient selection, such as stringent selection criteria with emphasis on functional status, caregiving support, and psychosocial aspects.

The main critical points of the outpatient transplant approach are the following: there are no randomised studies that clearly indicate which model is better than another; there are no studies that have analysed survival outcomes after extended follow-up; the real costs of these programmes still need to be calculated. One could speculate that the outpatient procedure is cost effective in terms of hospital budget, but prospective randomised trials are needed to draw firm conclusions.

Some authors report direct savings of between 10% and 50% that are highly influenced by the release of hospital beds and low readmission rates (14, 54, 55). Data available on QoL are limited and contradictory (42, 43). The majority of centres offering outpatient ASCT call for the availability of a caregiver 24/7, at least for the duration of the aplastic phase. In this way, caregivers spend much of their time with patients, which affects multiple areas of their life. Caregivers must prepare their homes or residential facility to avoid potential infectious complications and are responsible for the administration of medications, monitoring of vital signs, and intake and output of fluids, tasks traditionally carried out by professionals. Caregivers of outpatient ASCT patients may also be required to facilitate daily visits to the Outpatient Clinic. Some studies have shown that OS is significantly better in ASCT patients with a caregiver. Therefore, feasibility and safety of an outpatient approach appears to be mediated in large part by the efforts of caregivers. Nevertheless, the lack of a caregiver is the most common reason for a patient's refusal to take part in an outpatient programme, and this should be considered a bias. The last crucial point is that the majority of trials were not randomised, controlled trials. The characteristics of the patients were different across the groups, and sometimes the decision for an outpatient or inpatient approach was according a subjective physician opinion. This means that the observed difference in the risks of infection may have been a consequence of the different baseline characteristics rather than the effect of the treatment strategy. The eligibility criteria of some trials indicated that patients in the outpatient group were required to have good performance status, no organ failure, and an age ≤65 years. This may have introduced a bias in the form of a selection of only healthier subjects for the outpatient arm. Furthermore, patients could choose the type of transplantation procedure (outpatient or inpatient), and outcomes may thus have been influenced by this choice.

In conclusion, outpatient ASCT for MM is a safe and feasible approach and should be considered by healthcare providers. Given that it is difficult to carry out randomised trials in this setting, rigorous selection criteria are mandatory for the routine use of the outpatient approach. Caregivers play a crucial role in the success of the outpatient procedure. Useful tools to assess the QoL of patients and caregivers are needed to evaluate this aspect of care.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

Conceptualization, methodology, design, and writing: MMa, AP, MMe, GM, and CC. Supervision: MMe, CC, and GM. All authors contributed to the article and approved the submitted version.

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Cytotoxic T Cell Responses Induced by CS1/CRT Fusion DNA Vaccine in a Human Plasmacytoma Model

Xueshi Ye[1*†], *Wanli Li*[2†], *Jinwen Huang*[1], *Lifei Zhang*[1] *and Ye Zhang*[1]

[1] Department of Hematology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou, China,
[2] Department of Orthopedics, The Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China

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María-Victoria Mateos, University Hospital of Salamanca, Spain

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***Correspondence:**
Xueshi Ye
yexueshi2008@zju.edu.cn

[†]*These authors have contributed equally to this work*

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To date, multiple myeloma remains an incurable disease. Immunotherapy is an encouraging option in the development of multiple myeloma (MM) therapy. CS1 is a specific myeloma antigen, which is highly expressed in myeloma cells. Calreticulin (CRT) is a key determinant of cell death, which can influence antigen presentation and promote cellular phagocytic uptake. In the current study, we constructed a DNA vaccine encoding both CS1 and CRT. Our results show that the PcDNA3.1-CS1/CRT vaccine was able to induce cytotoxic T cell responses against myeloma cells *in vivo*, and the tumor growth was significantly suppressed in mice immunized with this vaccine. Therefore, our findings indicate that the CS1/CRT fusion DNA vaccine may represent a promising novel myeloma therapy, and the potential for combining the CS1/CRT vaccine with other myeloma treatments.

Keywords: myeloma, immunotherapy, DNA vaccine, CS1, calreticulin

INTRODUCTION

Multiple myeloma (MM) is a plasma cell malignancy that accounts for 10%–15% of hematopoietic neoplasms, and 20% of deaths due to hematological malignancies. During the past several years, novel drugs (e.g., proteasome inhibitors and immunomodulatory drugs [IMiDs]) together with hematopoietic stem cell transplantation have been extensively applied in a clinical setting (1). As a result, the myeloma treatment response rates continue to improve and the survival time has been prolonged for myeloma patients. However, since myeloma remains an incurable disease, the patient will eventually relapse and die because the vast majority of the remaining tumor cells are multidrug-resistant plasma cell clones. Thus, novel treatment strategies for myeloma are urgently required. For example, aggressive multidrug combination chemotherapy, which aims at generating a complete response, strives for much longer survival and even a potential cure. However, due to the presence of treatment-related toxicity and side-effects, the improved response rates are not necessarily associated with a survival benefit. Therefore, additional attention is required to obtain a balance between treatment efficacy and patient quality of life. Therefore, novel less aggressive and more effective therapeutic approaches may represent a promising treatment direction for myeloma.

Immunotherapy is an encouraging option in the development of treatment strategies for MM, because it has a mechanism of action that is distinct from cytotoxic chemotherapy (2–6). It is important for immunotherapeutic approaches to induce a specific anti-myeloma immune response, specifically eliminate myeloma cells, and provide long-lasting protection. DNA vaccines have been demonstrated to generate long-term gene expression and induce both humoral and cellular immune responses against the encoded cancer antigens. In a DNA vaccine, tumor antigens can be presented by DNA in a suitable molecular form to elicit effective T cell-mediated anti-tumour responses. Therefore, DNA vaccines have emerged as an attractive immunotherapeutic approach for the treatment of myeloma (7–9). In addition, DNA vaccines are more economical compared to other vaccines and can be designed to encode other antigens (e.g., various immunomodulatory molecules) to enhance the resulting immune response. The safety of DNA vaccines has also been substantiated in both animal models and human clinical trials (10–12).

Although DNA vaccines are associated with several advantages, the most important factor limiting their effectiveness against cancer is poor immunogenicity. Therefore, a key property of a DNA vaccine is to select specific tumor antigens and an effective immune adjuvant which can amplify the specific anti-tumor immune response. In previous studies, the cell-surface glycoprotein, CS1 (CD2 subset 1, CRACC, SLAMF7, CD319, or 19A24), is universally and highly expressed in normal plasma cells and myeloma cells (13, 14). Plasma cell malignancy in the bone marrow, tissue, and blood all appeared to express high levels of CS1. Moreover, CS1 is not expressed in normal tissue parenchyma or in a variety of solid tumors (13). Together, these findings make CS1 an optimal target antigen for vaccination strategies against myeloma.

In this study, we also selected calreticulin (CRT, a multifunctional protein predominantly located in endoplasmic reticulum) as an immune adjuvant to amplify the specific anti-myeloma immune response elicited by the CS1-DNA vaccine. Previous studies have demonstrated that CRT plays an important role for the destruction of cancer cells *via* immune activation, and CRT exposure increases cancer immunogenicity (15–17). CRT expression on the cell surface is considered as an activating signal for multiple human cancers, whereas CRT suppression by siRNA could inhibit anthracycline-induced phagocytosis by dendritic cells and destroy the immunogenicity of tumor cells in mice.

In the present study, we constructed a DNA fusion gene vaccine (CS1/CRT) designed to target the specific myeloma antigen, CS1. We aimed to explore whether a CS1/CRT fusion DNA vaccine could induce a specific anti-myeloma immune response and control myeloma cell growth in a human plasmacytoma model.

MATERIALS AND METHODS

DNA Vaccine Construction

The cDNA of the human CS1 gene (1,019 bp) and CRT gene (1,265 bp) were synthesized by Takara. The CS1 gene was amplified using PCR, and Hind III/EcoR I enzyme cutting sites were added to both ends of the CS1 gene. The amplified DNA fragment was cloned into the Hind III/EcoR I sites of pCDNA3.1 to generate PcDNA3.1-CS1. The CRT gene was amplified using PCR, and EcoR I enzyme cutting sites were added to both ends of the DNA fragment. Finally, the amplified DNA fragment containing the CRT gene was cloned into PcDNA3.1-CS1 to construct the DNA vaccine, PcDNA3.1-CS1/CRT, which encoded CRT linked to the specific myeloma antigen, CS1. Primer sequences for specific gene amplification are listed in **Table 1**. The accuracy of all constructs was confirmed by DNA sequencing.

Western Blot Analysis, Fluorescence Microscopy, and Flow Cytometry

PcDNA3.1, PcDNA3.1-CS1 and PcDNA3.1-CS1/CRT were transfected into 293T cells using Lipofectamine 2000 (Invitrogen). Following transfection for 48 h, the cells were lysed and the expression of CS1/CRT was detected by Western blot. The transfected 293T cells were respectively stained with an anti-calreticulin mAb (Abcam, ab2907) and anti-CS1 mAb (Santa Cruz biotechnology, sc-47748), observed under a fluorescence microscope (OLYMPUS IX71), and analyzed by flow cytometry (BD Accuri C6, FlowJo was used for the data analysis).

Establishment of a Human Plasmacytoma Model

The human MM cell line, OPM2 [ATCC, with high expression of CS1 (18)] was cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS, Gibco) at 37°C, in an atmosphere containing 5% CO_2. OPM2 cells were collected during the logarithmic growth period. A total of 1×10 (7) OPM2 cells/mouse were subcutaneously injected into the right leg of BALB/c mice (Male, 5-week-old, weight 16 g–18 g, purchased from Shanghai Sippe-Bk Lab Animal Co., Ltd.). The mass growth of BALB/c mice was observed after an injection of OPM2 cells, and the tumor size was measured every other day.

Mouse Vaccination and Tumor Challenge

A small mass was palpable under the skin of the right leg 10 days after OPM2 cells were subcutaneously injected into BALB/c mice. BALB/c mice were intramuscularly vaccinated around the mass with 100 μg DNA in 100 μL saline on day 11. The tumor sizes were measured with vernier calipers every other day, and the tumor volume (mm^3) was calculated using the following formula: $0.5 \times$ length (mm) $\times$ width (mm) (2). BALB/c mice were divided into three groups (n=6: 1) Group 1 was vaccinated with the pcDNA3.1-CS1 plasmid; 2) Group 2 was vaccinated with the pcDNA3.1-CS1/CRT plasmid; 3) Group 3 was the control group, which received injections of pcDNA3.1. A booster injection with

TABLE 1 | List of primer sequences.

Gene name	Sequence (5' to 3')
CS1	F: AGGGAGACCCAAGCTTATGGCTGGTTCCCCAACATG
	R: GATATCTGCAGAATTCCAAGATAACATTCTCATAGGC
CRT	F: TGTTATCTTGGAATTCTGGATGCTGCTATCCGTGCCGC
	R: TGATGGATATCTGCACACCAGCTCGTCCTTGGCCTGG

the same dose was administered seven days after the first injection. Mice were sacrificed when there was the first mouse with maximum diameter of tumor up to 15 mm occurred in control group.

Analysis of the T Lymphocyte Subsets

The splenocyte suspension was prepared after the mice were sacrificed. The percentage of $CD4^+$ and $CD8^+$ T cells in the splenocytes from the three groups of mice (described above) was detected by flow cytometry. The $CD4^+$ and $CD8^+$ T cells were also sorted by flow cytometry for the subsequent experiments which detected the CTL response against OPM2 cells.

IFN-γ Assay

The isolated splenocytes (described above) were cultured in the upper chamber of the Transwell culture system, whereas OPM2 cells were seeded into the lower chamber. The ratio of splenocytes to OPM2 cells was 5:1. The cells were incubated at 37°C for 72 h. The supernatants were collected, and the level of IFN-γ was measured using a commercially available ELISA kit (Elabscience Biotechnology Co., Ltd, China).

Cytotoxicity Assay *via* the Lactate Dehydrogenase (LDH)-Releasing Method

The cytotoxic lymphocyte (CTL) response against the target OPM2 cells was detected using a standard LDH method according to the manufacturer's protocol (Cloud-clone Corp, USA). $CD4^-CD8^+$ T cells were sorted by flow cytometry (described above) and plated into 96-well U-bottom plates as the effector cells. Both the effector and target cells (OPM2 cells) were added to a final volume of 100 µl. In the experimental wells, the effector cells were co-cultured with the target cells at a ratio of 40:1 and incubated for 4 h at 37°C. Target spontaneous and maximal releasing wells were distinguished by the presence of either 100 µl medium or 2.5% Triton, respectively. The supernatant was harvested and transferred to a fresh plate to test the LDH-releasing rate. Finally, the absorbance was measured at 450 nm. The level of CTL cytotoxicity (% killing) was calculated using the following formula: A450 nm (experimental) − A450 nm (target spontaneous)/A450 nm (target maximal releases) - A450 nm (target spontaneous) × 100%.

Statistical Analysis

An unpaired Student's *t*-test was used to compare the data from various experimental groups. P-values < 0.05 were considered to be statistically significant.

RESULTS

Identification of the Recombinant Plasmids

The DNA sequencing results showed that the human CS1 and CRT gene fragments were successfully inserted into the pCDNA3.1 plasmid to construct PcDNA3.1-CS1 and PcDNA3.1-CS1/CRT. The DNA sequencing directions and primers of inserted into the CS1 and CRT gene fragment are shown in **Supplementary Figure 1**.

Detection of CS1/CRT Expression by Western Blot, Fluorescence Microscopy, and Flow Cytometry

A Western blot was performed to detect the level of CS1/CRT protein expression in plasmid-transfected 293T cells (**Figure 1**). The Western blot results showed a high level CS1 expression in 293T cells transfected with PcDNA3.1-CS1. High levels of both CS1 and CRT protein expression were detected in 293T cells transfected with PcDNA3.1-CS1/CRT.

After the plasmids were transfected into the 293T cells for 48 h, 293T cells were collected and detected by fluorescence microscopy (**Figure 2**) and flow cytometry (**Supplementary Figure 2**). The results showed that CS1 protein was significantly expressed in the cells transfected with PcDNA3.1-CS1, whereas both CS1 and CRT protein were significantly expressed in the cells transfected with PcDNA3.1-CS1/CRT.

Vaccination With the CS1/CRT Fusion DNA Vaccine Significantly Suppressed the Growth of Myeloma Cells

To detect the therapeutic efficacy of the CS1/CRT fusion DNA vaccine, we generated a xenograft mouse model of human plasmacytoma. Ten days after the OPM2 cells were subcutaneously injected into BALB/c mice, a small mass was palpable under the skin of the right leg in some mice. These mice were intramuscularly vaccinated around the mass with 100 µg DNA in 100 µl saline on day 11. The experimental mice were divided into three groups, which were respectively vaccinated with either the pcDNA3.1-CS1 plasmid, pcDNA3.1-CS1/CRT plasmid, or the pcDNA3.1 plasmid as a control. A booster injection with the same dose was administrated seven days after the first injection. The tumor size was measured and recorded every other day. The first mouse with maximum

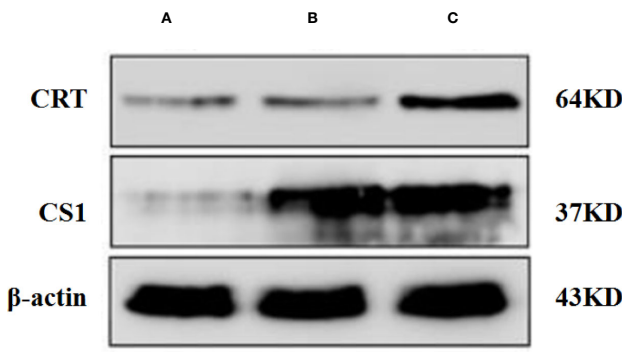

FIGURE 1 | Western blot analysis. **(A)** Control, 293T cells transfected with PcDNA3.1; **(B)** 293T cells transfected with PcDNA3.1-CS1, high level CS1 expression was showed; **(C)** 293T cells transfected with PcDNA3.1-CS1/CRT, High levels of both CS1 and CRT protein expression were detected.

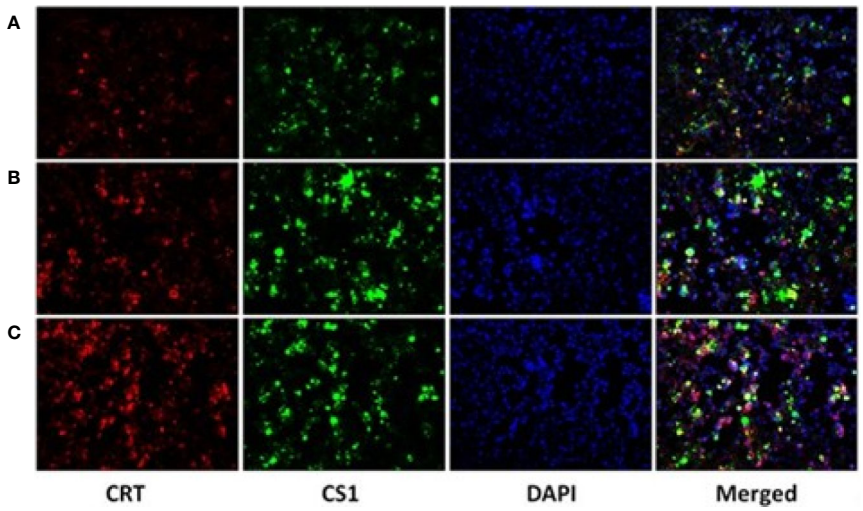

FIGURE 2 | CS1 and CRT protein expression observed by fluorescence microscopy (× 200). **(A)** Control, 293T cells transfected with PcDNA3.1; **(B)** the density of green fluorescence increased significantly in 293T cells transfected with PcDNA3.1-CS1, revealing high levels of CS1 protein expression in PcDNA3.1-CS1 transfected cells; **(C)** the density of both green and red fluorescence increased significantly in 293T cells transfected with PcDNA3.1-CS1/CRT, revealing high expression of both the CS1 and CRT proteins in PcDNA3.1-CS1/CRT-transfected cells.

diameter of tumor up to 15 mm occurred in the control group eight days after the booster injection. At this time, all mice were sacrificed for further experimentation.

The results showed that the mean volumes of the tumor mass in the control group were much higher than that of the pcDNA3.1-CS1 and pcDNA3.1-CS1/CRT groups (**Table 2**, **Figure 3**). These data demonstrate that the DNA vaccines significantly suppressed the growth of myeloma cells. The inhibition mediated by the CS1/CRT fusion DNA vaccine on the tumor cells was greater than that of the pcDNA3.1-CS1 plasmid, suggesting that the use of CRT as an immune adjuvant may enhance the inhibitory effect of the CS1-DNA vaccine on myeloma cells.

TABLE 2 | The mean volume (mm^3) [1]of tumor mass following immunization.

	Control group[2]	pcDNA3.1-CS1	pcDNA3.1-CS1/CRT
D11[3]	10.34 ± 4.87	10.77 ± 3.93	11.31 ± 5.62
D13	43.43 ± 20.09	41.95 ± 16.48	43.07 ± 11.08
D15	130.17 ± 44.57	108.61 ± 30.16	81.36 ± 35.36
D17	251.30 ± 62.53	114.76 ± 26.39	100.85 ± 49.32
D18[4]	415.17 ± 104.57	138.59 ± 29.70	116.05 ± 47.66
D20	751.30 ± 152.53	271.35 ± 65.11	129.17 ± 47.09
D22	1251.30 ± 252.53	312.19 ± 62.03	242.75 ± 47.84
D24	1496.70 ± 194.04	437.01 ± 92.89	260.95 ± 54.88
D26	1707.86 ± 269.95	491.09 ± 78.02	324.96 ± 64.55

[1]*Data are presented as the mean ± SD; n=6/group.*

[2]*BALB/c mice were vaccinated with the control plasmid, pcDNA3.1.*

[3]*There was no significantly different between the two experiment groups and the control group (mean ± SD. P > 0.05, respectively) in the size of tumor at the time of the first immunization.*

[4]*The group mean tumor volumes were significantly different between the two experiment groups and the control group (mean ± SD. P < 0.01, respectively), and there was also significant difference between the CS1 and CS1/CRT group (mean ± SD. P < 0.05), when the booster injection was administrated on D18 (seven days after the first injection).*

Analysis of T Lymphocyte Subsets

The splenocyte suspension was prepared after all the mice had been sacrificed. The percentage of $CD4^+$ and $CD8^+$ T cells among the splenocytes was detected by flow cytometry. The results (**Figure 4**) showed that the percentage of $CD4^-CD8^+$ cells significantly increased in the CS1 DNA vaccine group and CS1/CRT fusion DNA vaccine group, compared with the control group ($P < 0.05$). But, there was no significant statistical difference when we compared CS1 group and CS1/CRT group ($P > 0.05$). The percentage of $CD4^+CD8^-$ cells also increased in both DNA vaccine groups, but only the CS1 DNA vaccine group was significantly different when compared with the control group ($P < 0.05$).

IFN-γ Assay

The results of the ELISA (**Figure 5**) revealed that the levels of IFN-γ in both the CS1 DNA vaccine group and CS1/CRT fusion DNA vaccine group were significantly increased; however, only the CS1/CRT fusion DNA vaccine group was significantly different compared with the control group ($P < 0.01$).

Effect of the CS1/CRT-DNA Vaccine on the CTL Response

To examine the CTL response induced by the CS1/CRT-DNA vaccine, we used the the standard LDH method with OPM2 cells used as target cells to detect the cytotoxicity of $CD4^-CD8^+$ cells sorted from the splenocytes by flow cytometry. The results (**Figure 6**) showed a significant difference in the killing rate of both group, compared with the control group ($P < 0.001$). These data confirm that both the CS1 DNA vaccine and CS1/CRT fusion DNA vaccine can induce a specific CTL response targeting myeloma cells. Moreover, the CS1/CRT fusion DNA vaccine

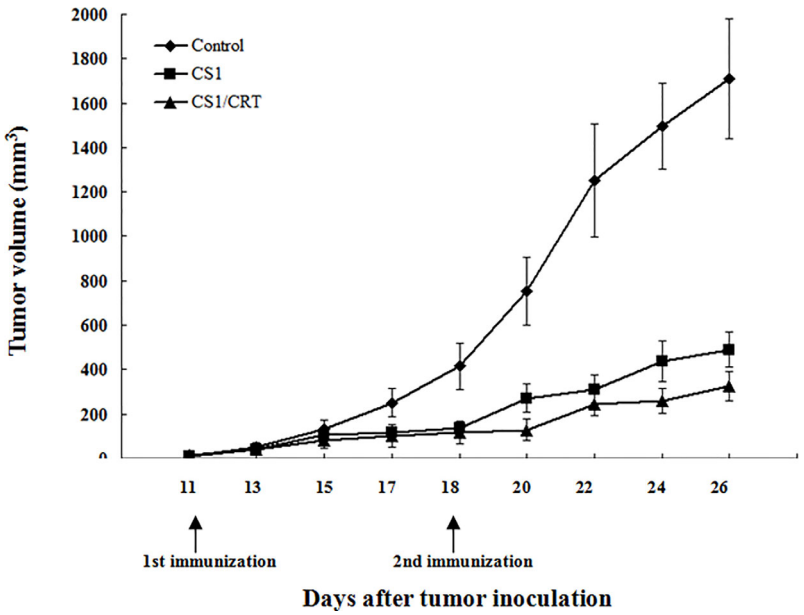

FIGURE 3 | Effect of the DNA vaccine on tumor growth: The mean volume of the tumor mass in the control group (immunized with pcDNA3.1, n=6) was much larger than that of the pcDNA3.1-CS1 (n=6) and pcDNA3.1-CS1/CRT group (n=6). The group mean tumor volumes were significantly different between the two experiment groups and the control group (mean ± SD. $P < 0.01$, respectively), and there was also significant difference between the CS1 and CS1/CRT group (mean ± SD. $P < 0.05$), when the booster injection was administrated on D18 (seven days after the first injection). DNA vaccines significantly suppressed the growth of myeloma cells and the inhibition of the CS1/CRT fusion DNA vaccine on the tumor cells was more obvious than that of the CS1 vaccine.

induced a significantly stronger CTL response compared to the CS1 DNA vaccine ($P < 0.001$). These findings suggest that the use of CRT as an immune adjuvant can amplify the anti-myeloma immune response induced by the CS1-DNA vaccine.

DISCUSSION

Since MM typically affects the elderly, many patients may be too frail to undergo intensive chemotherapy (19, 20). To enhance the therapeutic effect and avoid serious complications, a combination of low intensity therapies with different mechanisms may be considered. For example, low intensity chemotherapy combined with immunotherapy may be beneficial. In general, the immune function of patients with myeloma is considered to be severely compromised, which results in an increased infection rate and reduced immune surveillance for tumor cells (21–24). The mechanisms of immune evasion of myeloma cells include the weak expression of tumor antigens, enhanced expression of inhibitory ligands (e.g., PD-L1), as well as increased numbers of regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSC), which can inhibit CTL function. Therefore, immunotherapy research for myeloma should focus on how to enhance the immune system, to recover and enhance the level of immune surveillance for myeloma cells.

Myeloma vaccines are classified as active immunotherapies, which target tumor-associated antigens and induce antitumor immune responses. Immunotherapy approaches with patient-specific protocols mean more expense, more difficult to operate, and need to take longer time. Thus, an off-the-shelf-based immunotherapy that is more cost-effective for patients is highly desired. It makes a DNA vaccine an attractive choice. The key to improve the efficacy of a DNA vaccine is associated with enhancing its immunogenicity, and promoting the T cell-mediated antitumor immune response. In our study, CS1 was selected as the specific target antigen. CS1 is highly expressed in over 95% of cases of MM (13, 18) and CS1 expression has also been found to remain high following treatment with bortezomib, or in patients who relapse after transplantation. Thus, high levels of CS1 expression are generally a universal and persistent feature in MM (14). This feature makes CS1 an attractive target for the treatment of MM. To enhance the immune response, we constructed a recombinant vector encoding CS1 and the immune adjuvant, CRT, to investigate its antitumor effects in a MM mouse model. CRT is a key determinant of the immunogenic forms of cell death, can influence antigen presentation to CTLs and promote cellular phagocytic uptake. Tumor protection requires cell surface CRT, as well as CD8+ and CD4+ T cells (15–17). Therefore, we attempted to increase CRT expression on the surface of tumor cells using a CS1/CRT DNA vaccine, thereby enhancing the T cell-mediated anti-myeloma immune response.

In our study, we constructed a recombinant plasmid PcDNA3.1-CS1/CRT. The results from the Western blot analysis demonstrated that the recombinant plasmid PcDNA3.1-CS1/CRT was highly expressed in 293T cells (**Figure 1**). Observations using fluorescence microscopy also revealed high levels of CS1 and CRT protein expression in 293T cells transfected with PcDNA3.1-CS1/CRT (**Figure 2**). Furthermore, flow cytometry revealed that the level

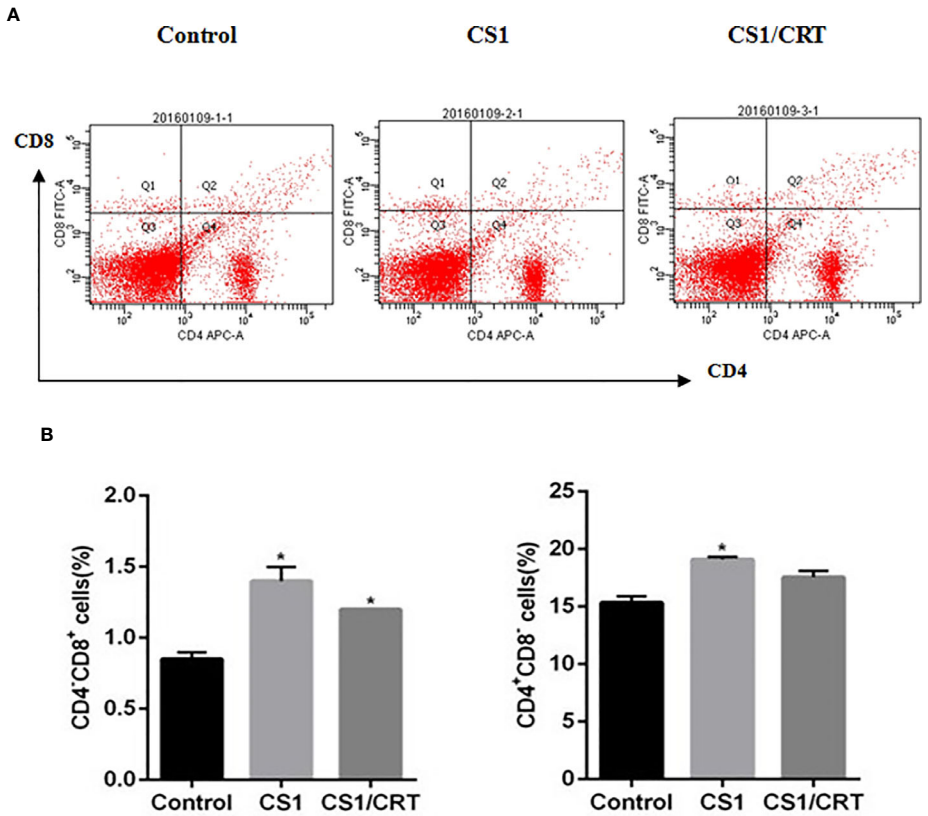

FIGURE 4 | Analysis of T lymphocyte subsets from vaccinated mouse spleens. **(A)** FACS analysis was used to measure the percentage of $CD4^+$ and $CD8^+$ T cells. **(B)** The data are expressed as the mean ± SEM; $^*P < 0.05$, compared with the control group.

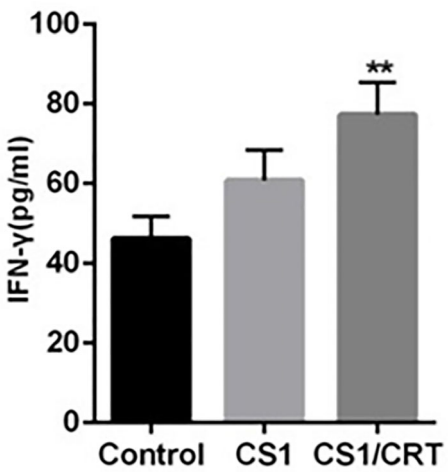

FIGURE 5 | IFN-γ assay. The level of IFN-γ was using an ELISA. The data are presented as the mean ± SEM; $^{**}P < 0.01$ vs control.

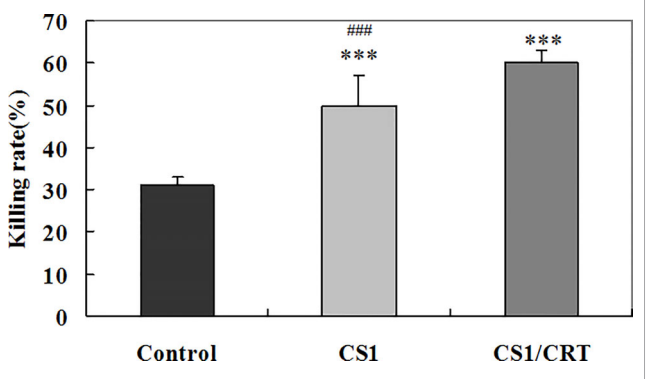

FIGURE 6 | Specific anti-myeloma CTL cytotoxicity induced by the CS1 and CS1/CRT fusion DNA vaccines. The data is presented as the mean ± SD; $^{***}P < 0.001$ vs control; $^{\#\#\#}P < 0.001$ vs CS1/CRT.

of CS1 and CRT protein expression was significantly increased on the surface of transfected 293T cells (**Supplementary Figure 2**). Collectively, the above experimental results all demonstrate that the recombinant plasmid PcDNA3.1-CS1/CRT could transfect cells to express high levels of both CS1 and CRT protein.

In our study, to investigate the immune attack efficacy of the CS1/CRT vaccine on myeloma cells in a short term, 5-week-old male BALB/c mice were challenged with OPM2 cells (the human MM cell line, which express high levels of CS1) to establish a human

plasmacytoma xenograft mouse model. The animal experiment results showed that the tumor growth was significantly suppressed in the immunized mice ($P < 0.01$, compared with the control group, **Figure 3**), and such suppression was more obvious in the CS1/CRT vaccine group compared to the CS1 vaccine group ($P < 0.05$, **Figure 3**). These findings suggest that the CS1 vaccine can effectively suppress myeloma cells, and its antitumor effects can be further enhanced by combining it with CRT as an immune adjuvant. Immunological studies revealed that an increased number of CD8+ cells among the splenocytes isolated from immunized mice (**Figure 4**). But there was no significant statistical difference when we compared CS1 group and CS1/CRT group ($P > 0.05$). The experimental data also showed markedly increased levels of IFN-γ after the splenocytes from immunized mice were inoculated with myeloma cells for 72 h (**Figure 5**). In addition, the cytotoxicity assay confirmed that our DNA vaccine can induce a specific CTL response targeting myeloma cells, and the use of CRT as an immune adjuvant can further amplify the anti-myeloma immune response induced by the CS1-DNA vaccine (**Figure 6**). So, we can see that the CS1/CRT fusion DNA vaccine induced increased production of IFN-γ and stronger CTL response, but no increase of the amount of CD8+ cells, compared to the CS1 DNA vaccine. We think that the difference may be owing to the following reasons: CRT mainly influences antigen presentation and promote cellular phagocytic uptake, while it cannot significantly increase the amount of T cells; the statistical difference may appear after the number of samples increases more.

It has historically been considered that the anti-tumor effect of immunotherapy for myeloma may be limited by the compromised immune function of myeloma patients. Some recent studies have shown that the immune system of myeloma patients with long-term disease control can recover, even to similar levels of age-matched controls (25–28). These results suggest that the immune status of myeloma patients can recover toward normal following successful treatment. This evidence provides a theoretical basis for the application of a myeloma vaccine as maintenance therapy in patients following intensive therapy to generate an effective anti-myeloma immune response and maintain long-term tumor control. Based on our results, we also consider that a myeloma vaccine may be applied as a form of pre-emptive treatment for smoldering myeloma to delay or prevent its progression into symptomatic myeloma, or for high-risk MGUS (Monoclonal gammopathy of undetermined significance) patients to prevent its conversion to MM. In addition, a myeloma vaccine can be used repeatedly to sustain an effective immune response.

Recently, some studies have shown that IMiDs combined with a cancer vaccine can enhance the anti-myeloma immune response. This effect may be due to the ability of IMiDs to enhance the immunologic milieu in patients with myeloma by promoting T cell proliferation and suppressing inhibitory factors (29–31). These results suggest that the combination of myeloma vaccines with other therapies (e.g., IMiDs) may represent a novel strategy for the treatment of refractory myeloma. Since the expression of death signals on the surface of myeloma cells induced by chemotherapeutic drugs can promote immune recognition of tumor cells, our CS1/CRT fusion vaccine combined with low dose chemotherapeutic drugs may achieve a superior anti-myeloma immune response and ultimately better tumor control (32).

In conclusion, our study demonstrates for the first time, that CS1 can be used as a target antigen in a DNA vaccine to successfully induce specific cytotoxic T cell responses against myeloma cells and suppress tumor growth *in vivo*. Furthermore, the CS1/CRT fusion DNA vaccine could enhance the anti-myeloma immune response and substantially suppress tumor growth. These findings highlight the need to explore the combination of this myeloma DNA vaccine with IMiDs or chemotherapeutic drugs for the treatment of myeloma in future studies. Thus, this study presents convincing evidence to support the application of a CS1/CRT fusion DNA vaccine in myeloma, and the potential for its use in combination with other treatments for myeloma.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors to any qualified researcher.

ETHICS STATEMENT

The animal study was reviewed and approved by Experimental animal welfare ethics committee of Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, China.

AUTHOR CONTRIBUTIONS

XY and WL conceived and designed the study. XY and WL performed the experiments. XY did the final statistical analysis, interpreted the data and wrote the manuscript. XY and WL obtained funding. JH contributed to the implementation of the study, scheduling of the study participants. LZ and YZ provided technical assistance. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.587237/full#supplementary-material

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REVIEW
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PET/CT in Multiple Myeloma: Beyond FDG

Federica Matteucci[1]*, *Giovanni Paganelli*[1], *Giovanni Martinelli*[2] and *Claudio Cerchione*[2]*

[1] Nuclear Medicine Unit, Istituto Scientifico Romagnolo per lo studio e la cura dei tumori (IRST) IRCCS, Meldola, Italy,
[2] Hematology Unit, Istituto Scientifico Romagnolo per lo studio e la cura dei tumori (IRST) IRCCS, Meldola, Italy

Recent advances in the diagnosis and treatment of multiple myeloma (MM) have highlighted the importance of imaging methods, not only in the localization and extent of the disease but also in prognostic stratification and assessment of response to therapy. In this context, PET/CT, combining both morphological and functional information, is particularly useful in this pathology. The tracer mostly used is 18F-FDG, a glucose analog, which provides extremely accurate information with a sensitivity ranging from 80 to 100%. However, this tracer has some limitations, mostly related to the physiological uptake of FDG in the bone marrow and brain, which reduce its effectiveness. For this reason, some studies in the literature have evaluated the effectiveness of other PET tracers, which provide information on protein metabolism or the synthesis of metabolic plasma membranes, such as choline and methionine, as well as innovative radiopharmaceuticals, directed against receptors expressed by cells of myeloma, including tracers directed to the chemokine receptor. This review analyzes the characteristics and accuracy of non-FDG tracers in the management of patients with multiple myeloma.

Keywords: myeloma, choline positron emission tomography/computed tomography, 18F-fluorodeoxyglucose-positron emission tomography/computed tomography, new tracers, methionine positron emission tomography/computed tomography, multiple myeloma, FDG-PET/CT

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***Correspondence:**
Federica Matteucci
Federica.matteucci@irst.emr.it
Claudio Cerchione
claudio.cerchione@irst.emr.it

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INTRODUCTION

Multiple myeloma (MM) is a neoplastic disease characterized by the uncontrolled clonal proliferation of plasma cells in the bone marrow. Bone involvement occurs in approximately two thirds of patients at diagnosis and in nearly all patients during their disease in the form of focal osteolytic lesions (1).

For this reason, imaging provides useful information in the detection of both intramedullary and extramedullary disease, both in the differentiation between solitary plasmacytoma (SP) and MM and finally in the predictive evaluation of the progression from smoldering myeloma (SMM) to active disease. The limitations of planar radiography, which has long been the examination of choice in these patients, have been largely due to the use in clinical practice of new imaging modalities, represented by computed tomography (CT), magnetic resonance imaging (MRI), and from positron emission tomography/computed tomography (PET/CT). At present, the role of PET/CT with 18F-fluorodeoxyglucose (18F-FDG PET/CT) in MM has reached an extremely significant level of evidence, so much so that it is considered a method of choice both in the diagnostic phase and for prognosis, as well as in the assessment of response to treatment. In particular, the role of 18F-FDG

PET/CT has been extensively studied both in the diagnostic phase and in the prognostic evaluation of the disease and in the response to treatment, reaching very significant levels of evidence.

According to the update of the International Myeloma Working Group (IMWG), the presence of one or more osteolytic lesions evident on CT or PET/CT is indicative of bone disease, thus requiring specific treatment (2).

18F-FDG PET/CT represents a modality of choice in the various phases of MM: studies in the literature report a high sensitivity and specificity both in the evaluation of bone marrow and extramedullary disease, ranging from 80% to 100% (3–5).

In other studies, a comparison was made between the diagnostic performance of FDG-PET and MRI, highlighting how the sensitivity of FDG-PET is substantially equal to or slightly lower than that of pelvic-spinal MRI (PR-MRI) both in the evidence of diffuse infiltration of the spinal cord than in the visualization of focal lesions (6–10). In particular, Zamagni et al. (9) in a study on 46 patients showed how the FDG PET was not able to detect the infiltration of the bone marrow highlighted on MRI in 30% of patients, while the PET, performed in whole body, showed lesions located outside the MRI field of view in 35% of cases. The combination of the two methods allowed a correct diagnosis in 92% of the patients.

In patients with solitary plasmacytoma (SP), FDG-PET is instead able to detect additional lesions compared to MR, with greater sensitivity and specificity (8, 11).

In addition to its diagnostic value, FDG-PET has proved to be a fundamental tool for prognostic purposes, with undoubted usefulness in an era oriented towards precision medicine. In particular, in a study by Bartel et al. (12) found that the only imaging test significantly associated with an adverse prognosis for both overall survival (OS) and event-free survival (EFS) was FDG-PET when the number of focal lesions was greater than three at baseline. Furthermore, Fouquet et al. showed that the presence of at least two hypermetabolic lesions by FDG-PET was predictive of rapid progression to MM (13). Also in smoldering multiple myeloma (SMM), a positive FDG-PET in the absence of evident osteolytic lesions on transmission CT may be predictive of progression to symptomatic MM. In a series of 122 patients with SMM, Siontis et al. (14) showed that the probability of progression to MM within 2 years for patients with FDG-PET positive (uptake with or without lytic lesions on transmission CT) was 75%, *vs.* 30% for patients with negative PET.

Finally, PET/CT 18F-FDG is a reliable tool for evaluating therapy in MM. Studies published in the literature have shown that obtaining complete metabolic remission (CMR) on FDG PET/CT in an interim evaluation before or after autologous stem cell transplantation (ASCT) is associated with a better survival rate, especially in patients with a complete biological response (15, 16). For these reasons, the IMWG strongly advised to consider 18F-FDG PET/CT as the preferred imaging technique to assess response to therapy in MM (16, 17). Despite the promising results reported by several groups, however, there are currently no unambiguous standard interpretation criteria. In fact, in many studies the interpretation of the images is mainly based on semi-quantitative analysis and in others on visual evaluation or on both methods.

Recently Zamagni et al. (18) published a study that aimed to establish unique criteria to define the complete metabolic response (CMR) to PET after therapy in a subgroup of newly diagnosed MM patients eligible for transplantation. The results confirmed that Deauville score can also be used in this subgroup of patients and that liver background can be a useful reference to identify CMR on PET after therapy.

However, the use of 18F-FDG PET/CT is not exempt from certain limitations: in particular in relation to its metabolic characteristics, the 18F-FDG appears less sensitive in highlighting a diffuse infiltration of the bone marrow and in the visualization of the lesions of the cranial theca, given the physiological uptake of the tracer in the brain.

In addition, the uptake of 18F-FDG, as an analogue of glucose, can present both false positive lesions (due to inflammation, post-surgical areas, recent use of chemotherapy, fractures, etc.) that falsely negative lesions (in the presence of high levels of blood glucose, or following administration of high-dose steroids, etc.). To overcome the limitations of 18F-FDG, many other PET tracers have been proposed in patients with MM: the aim of our review is to provide an overview of the new non-FDG PET tracers currently used in the management of patients with MM.

OLD TRACERS FOR NEW INDICATIONS: CHOLINE AND METHIONINE

Choline, a component of phosphatidylcholine, is an indicator of the synthesis of plasma membranes; its use in oncology is linked to the evidence that uptake is greater in proliferating cells in relation to the growth of plasma membranes. Choline PET imaging is used in clinical practice in prostate cancer diagnostics.

Methionine, labeled with 11C, is an amino acid PET tracer used mainly in oncological diseases of the central nervous system. The rationale for its use in MM is related to the evidence that radiolabeled amino acids show rapid metabolic absorption and incorporation into newly synthesized immunoglobulins.

Studies in the literature have highlighted a possible role of PET with both choline and methionine in the management of patients with MM.

In particular, the first experience, that have evaluated the use of choline in myeloma, is due to the Bologna group, which, following the occasional finding of a PET choline positive myelomatous lesion in a patient studied for prostate cancer, compared the diagnostic performance of PET with 11C-choline with those of FDG-PET (19).

The study, conducted in a small cohort of 10 patients at different times of the disease, showed a difference, although not statistically significant, in the average number of lesions detected in the two methods, with a consequent change in the management of these patients.

About 10 years later, Cassou-Mounat (20) studied 21 MM patients with both FCH-PET/CT and FDG PET/CT, showing a significant difference in the number of choline PET *versus* FDG positive lesions [8.1 *vs* 4.6 for FDG ($p < 0.001$)] with a higher target/background ratio.

The difference in uptake between 18FDG and choline does not yet find an exhaustive explanation even if several hypotheses have been formulated: in particular, the finding of high choline uptake and low FDG accumulation could be linked to a lower expression of hexokinase-2, enzyme that catalyzes the phosphorylation of FDG, preventing its back diffusion through the cell membrane (21).

In this study carried out on 221 patients, the authors showed a low expression of the gene coding for hexokinase-2, in PET false-negative cases (5.3-fold change, $P < 0.001$), so provides a possible explanation for this feature.

Furthermore, a heterogeneity of the accumulation of the different tracers in the same patient has been highlighted in several studies, suggesting the simultaneous presence of multiple spatially separated clones that coexist in the same patient and the need to use more than one tracer in some situations that are difficult to interpret.

At present, choline-PET is not used in clinical practice for the management of patients with MM and its possible inclusion in flow charts will only be possible after validation of diagnostic accuracy in larger prospective studies.

Similar considerations can also be made in relation to the use of PET with methionine in patients with MM: PET/CT with 11C-methionine seems to have better performance than 18F-FDG in detecting myeloma lesions even if the literature is very limited and therefore insufficient for the use of this tracer in clinical practice.

The first study comparing PET with methionine and 18F-FDG PET/CT was published in 2013 by Nakamoto et al. in 20 patients (15 with MM and 5 with plasmacytoma), reporting a greater sensitivity of PET with methionine than FDG (89 *vs* 78%) (22).

These results were amply confirmed by the work published by the Würzburg group in 2017 (23), which analyzed 78 patients (4 solitary plasmacytoma, 5 SMM, 69 MM symptomatic), reporting a significantly greater ability of MET-PET to highlight both medullary and extramedullary lesions than FDG PET/CT (respectively 75.6 *vs* 60.3%; $p < 0.01$).

The authors also highlighted that both MET-PET correlates with the number of intramedullary lesions highlighted in iliac crest biopsies to a greater extent than 18F-FDG PET/CT (Spearman's r respectively equal to 0.832 and 0.635).

PET MET also appears to be superior to PET choline: the same authors recently published a head-to-head comparison study of 11C-methionine and 11C-choline for metabolic imaging of MM in 19 patients with a history of MM ($n = 18$) or solitary bone plasmacytoma ($n = 1$). The results obtained showed that MET-PET is more sensitive, detecting a greater number of intramedullary lesions in about 40% of patients (24).

NEW TRACERS

Molecular imaging has made significant progress in recent years with the development of innovative tracers that assess metabolic pathways other than those considered in the past or that evaluate the expression of specific plasmacellular receptors.

Currently, some specific biomarkers for plasma cell disorders have been studied for both diagnostic and therapeutic purposes: in particular the chemokine receptor 4 (CXCR4) and the differentiation cluster 38 (CD38).

In particular, it looks very interesting CXCR4, a G protein-coupled member of the chemokine receptor family (25), expressed on hematopoietic stem and progenitor cells in the bone marrow niche. CXCL12 (stromal cell-derived factor 1) binds to CXCR4 and forms various downstream signaling pathways, resulting in multiple responses necessary for tumor growth and development, including chemotaxis and gene transcription. The CXCL12/CXCR4 pathway is also involved in cell migration, the return of hematopoietic stem cells to the bone marrow, angiogenesis and cell proliferation. It has been shown that CXCR4 is overexpressed in MM (26), correlating both with progression and outcome of the disease (27).

Pentixafor is a peptide with high affinity for CXCR4 and represents an extremely promising ligand, in relation to its theragnostic characteristics: in fact it can be marked with both 68Ga, becoming an ideal PET tracer and with beta-emitting isotopes, such as 90Y or 177 Lu, becoming a therapeutic tracer.

Table 1 summarizes the main studies available about the role of 68Ga-Pentixafor PET/CT in MM patients.

In particular, Lapa et al. in 2017 (28) published a study on 35 patients with MM, who underwent 68Ga-Pentixafor-PET/TC for the evaluation of any radiometabolic therapy, comparing with [18F]FDG-PET/CT and laboratory data.

The results showed positivity to 68Ga-Pentixafor-PET/CT in 66% of the patients studied, in 8/23 (34.8%) with intramedullary disease, in 13/23 (56.5%) with both intra- and extramedullary lesions and in 2/23 (8.7%) with extramedullary lesions only.

The result of PET/CT was not correlated with the different myeloma subtypes or with other serological parameters. Positivity to 68Ga-Pentixafor PET/CT was instead a negative prognostic factor (OS 181 ± 41 d in PET positive patients; median OS in negative patients not reached).

In the 19 patients in whom a comparison with 18F-FDG PET/CT was possible, 68Ga-Pentixafor PET/CT was able to highlight a greater number of lesions in 21% of cases, while 18F-FDG PET/CT was superior in 7/19 (37%). In the remaining 8/19 patients (42%), both tracers detected an equal number of lesions ($p = 0.018$).

Based on the results obtained, albeit with the limitations linked to the retrospective nature of the study and the small size of the sample also subjected to the 18F-FDG PET/CT, the authors concluded that 68Ga-Pentixafor PET/CT could represent a useful tool for selection of patients to be referred to radiometabolic therapy and prognostic stratification, while no real benefit for diagnostic purposes is currently evident. Recently, some Authors have compared 68Ga-Pentixafor PET/CT and 18F-FDG PET/CT in patients with newly diagnosed multiple myeloma (29). In this retrospective study, conducted in 30 homogeneous patients with a recent diagnosis of multiple myeloma (7 pts in stage I, 4 in stage II, and 19 in stage III), a comparison was made between PET/CT with 68Ga-Pentixafor and 18F-FDG PET/CT, using both qualitative and semi-quantitative criteria.

The visual analysis of the images showed the positivity of 68Ga-Pentixafor PET/CT compared to 18F-FDG PET/CT in a greater number of patients (28/30 *vs* 16/30, respectively).

TABLE 1 | 68Ga-Pentixafor PET/CT: Summary of analyzed papers.

Author	Year	N° pts	Pathology	Control	Principal results
Lapa et al. (28)	2017	35	MM	FDG-PET (in 19 pts)	Overall Pentixafor-PET positivity 23/35 (66%) Pentixafor-PET>FDG-PET in 4/19 pts (21%), FDG-PET>Pentixafor-PET in 7/19 (37%). In 8/19 (42%) patients, both tracers detected an equal number of lesions.
Pan et al. (29)	2020	30	MM	FDG PET	Pentixafor-PET positive in 28/30 (93.3%), FDG-PET positive 16/30 (53,3%) Diffuse bone marrow lesions (n = 17): Pentixafor-PET positive in 88.2%), FDG-PET positive in 29.4% Focal bone marrow lesions (n = 13): Pentixafor-PET positive in 92.3%, FDG-PET positive in 69.2%
Zhou et al. (30)	2020	10	SMM	FDG-PET MET-PET	MET-PET positive in 2/10 pts, Pentixafor-PET positive in 5/10 pts, FDG-PET negative in all pts. Correlation between BMPC infiltration rate and SUVmean in MET-PET and Pentixafor-PET; no correlation with FDG-PET.
Philippe-Abbrederis et al. (26)	2015	14	MM	FDG-PET	FDG-PET positive in 9/14 pts (64.3%), Pentixafor-PET positive in 10/14 pts (71.4%). Lesions comparable in 3 pts, Pentixafor-PET>FDG-PET in 7 pts, FDG-PET> Pentixafor-PET in 2 pts, FDG-PET and Pentixafor-PET complementary in 2 pts.

MM, multiple myeloma; SMM, smoldering multiple myeloma; BMPC, bone marrow plasma cells; FDG-PET, 18F-FDG PET/CT; MET-PET, 11C-Methyonine PET/CT; Pentixafor-PET, 68Ga-Pentixafor PET/CT.

The semi quantitative parameters measured with 68Ga-Pentixafor PET/CT showed a significant correlation with the organ damage score (CRAB criteria), while the same correlation did not exist considering the semi-quantitative parameters of 18F-FDG PET/CT. Based on the results obtained, the authors concluded that the quantification of 68Ga-Pentixafor PET/CT could be a promising biomarker and superior to 18F-FDG PET/CT in the evaluation of the tumor burden of newly diagnosed MM.

However, the study certainly presented various limitations, mainly related to the lack of a correlation between the tumor burden highlighted on 68Ga- Pentixafor PET/CT and magnetic resonance imaging, which still represents the gold standard for the evaluation of widespread involvement of the bone marrow of the spine.

Recently, Zhou et al. (30) evaluated for the first time the role of 11C-Met PET/CT and 68Ga-Pentixafor PET/CT in 10 smoldering multiple myeloma patients, compared to 18F-FDG PET/CT.

The correlation between the percentage of plasma cell infiltration and the PET uptake, expressed by the mean SUV value, measured in the lumbar spine, was analyzed: the results showed a significant correlation of 11C-MET PET/CT and 68Ga-Pentixafor PET/CT, but not 18F-FDG PET/CT.

The authors therefore highlighted a greater sensitivity of 11C-Met PET/CT and 68Ga-Pentixafor PET/CT in the evaluation of bone marrow involvement in patients with SMM, suggesting studies in larger cohorts and prospectively the role of these methods in early identification of patients with high-risk SMM.

The theragnostic approach for individualized therapy today represents one of the main objectives in the oncology field: from this perspective, the development of a ligand of the CXCR4 peptide that can be labeled with α or ß- isotopes is extremely interesting.

The first studies have reported significant results, highlighting a good tolerance of therapy with high initial response rates (31, 32). Further future developments should include the study of therapy in patients with multiple myeloma in the early stages of the disease, alone or in combination with other conventional therapies.

A new frontier in the field of molecular imaging lies in the possibility of labeling antibodies with positron-emitting isotopes, in what is commonly defined as immuno-PET.

It is known that multiple myeloma cells express CD38, a transmembrane glycoprotein, which is the target of immunotherapy with Daratumumab.

The possibility of labeling daratumumab with positron-emitting radioisotopes such as Copper-64 (64Cu) and Zirconium-89 (89Zr) could therefore allow the creation of PET tracers ideal for MM imaging.

The studies currently present in the literature were carried out on animal models: in the only first-in-human phase I study in six patients, 89Zr-DFO-daratumumab PET/CT demonstrated an excellent ability to highlight known myeloma lesions as well as locations unknown to previous investigations carried out (33).

Obviously, prospective studies will be necessary to validate these first experiences, which however appear extremely promising for the use of this PET tracer, especially with the aim of identifying those patients with MM who could benefit from this immunotherapy.

CONCLUSION

At present, PET/CT with 18F-FDG is recognized as a useful tool in the management of patients with MM both in the diagnostic phase and in the assessment of response to therapy and in the prognostic stratification of patients.

However, the method is not free from some limitations and for this reason several alternative PET tracers have been studied for the detection of MM. Some of these radiotracers have provided promising results, such as 18F-choline and 11C-choline, 11C-methionine, 68 Ga-pentixafor, and 89Zr-Daratumumab, but most studies are currently based on small patient cohorts and therefore the evidence will need to be validated in further prospective clinical trials.

AUTHOR CONTRIBUTIONS

FM and CC provided for bibliographic research and critical reading of the existing literature. FM and CC provided the draft. FM, CC, GP, and GM checked the paper. All authors contributed to the article and approved the submitted version.

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REVIEW
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Beyond Andromeda: Improving Therapy for Light Chain Amyloidosis

Gregory P. Kaufman[1*] *and Claudio Cerchione*[2*]

[1] Department of Lymphoma & Myeloma, The University of Texas MD Anderson Cancer Center, Houston, TX, United States,
[2] Hematology Unit, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy

Therapy for light chain amyloidosis (AL) continues to evolve, and a new standard of care for the disease is rapidly forming. The risk of early death however, mainly from cardiac complications, remains an important benchmark yet to be definitively improved upon. This brief review explores recent advances in plasma cell directed therapy for AL, highlighting unique factors specific to these patients and AL biology driving differences in treatment strategies and clinical development compared with multiple myeloma. Improving upon proteasome inhibitor based upfront therapy combinations with the addition of anti-CD38 antibodies has shown promise with improved response rates in the ANDROMEDA (NCT03201965) study. Though depth and kinetics of achieving deep hematologic response as well as rates of biomarker defined organ response were improved with the addition of daratumumab to the combination of bortezomib, cyclophosphamide, and dexamethasone, death rates in each arm remained similar. Evaluation of other targeted and novel therapies in AL is ongoing, and we highlight efforts evaluating B-cell maturation antigen (BCMA) directed therapy, BCL-2 family inhibitors, and other novel agents in the field. We also look ahead to efforts to reimagine the clinical development of anti-fibrillar therapies after late phase study failures. Upcoming anti-amyloid fibril antibody studies explore opportunities to improve outcomes for the sickest AL patients with advanced cardiac disease, focusing on improving overall patient survival and reducing the risk of early death in this uniquely frail population.

Keywords: amyloidosis, daratumumab, NEOD001, CAEL-101, BCMA, CD-38

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***Correspondence:**
Gregory P. Kaufman
gpkaufman@mdanderson.org
Claudio Cerchione
claudio.cerchione@irst.emr.it

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INTRODUCTION AND BRIEF REVIEW

Progressive organ dysfunction driven by amyloid deposition and risk of early death is a hallmark of AL amyloidosis, particularly for those with cardiac involvement, which includes the majority of patients (~70%) (1). Plasma cell directed therapy has improved outcomes for these patients given the intrinsic linkage between hematologic response rates, organ response rates, and survival (2). Oft quoted is the poor prognosis of advanced cardiac amyloid nearing 4 months in advanced involvement, though studies have shown improved survival with successfully achieving hematologic response endpoints (3, 4). Still, selection bias and clinical status have often excluded many advanced cardiac patients from prospective clinical trials and some retrospective series. Manwani et al. reported in a retrospective series of 915 patients with AL treated with upfront bortezomib, 51% percent of whom had stage III cardiac disease, a complete hematologic response

rate of 15%, and a cardiac organ response rate of 32% (4). These results were similar to an upfront study of CyBorD in stage III cardiac AL, which did of note include patients with baseline NT-proBNP greater than 8,500 ng/L, that demonstrated a 40% VGPR or CR response rate and 1 year overall survival of 57% for this high risk cohort, though notably found marked inferior survival outcomes among the 40% of patients with baseline NT-proBNP >9,500 ng/L (5). Based on these and similar findings, bortezomib based upfront therapy, primarily the CyBorD combination, has become a highly used standard of care for the initial treatment of systemic AL (6). Of note, given the risk of worsening neuropathy in this population, the oral proteasome inhibitor ixazomib has been evaluated in the TOURMALINE-AL1 study of relapsed AL with encouraging secondary endpoints favoring ixazomib–dexamethasone over physician's choice combinations in terms of patient survival and preservation of organ function, although hematologic response rates were similar (7). Ixazomib is currently being evaluated in a phase II study in combination with cyclophosphamide and dexamethasone for treatment of naïve AL, with early results demonstrating 57% hematologic overall response rates including 26% VGPR and 14% amyloid CR (8).

High dose melphalan and autologous stem cell transplantation in appropriately selected AL patients can achieve excellent long term disease control, though often advanced cardiac AL will preclude eligibility evaluation for transplant unless upfront therapy enables clinical improvement (9). Sequential CyBorD induction and proceeding to stem cell transplant for patients with an unsatisfactory response is a proven treatment strategy, though often driven by the benefit for patients without stage III cardiac disease (10–12). A multicenter retrospective cohort of 22 patients (86% of whom were stage III with regard to cardiac status at diagnosis) has shown the feasibility of deferred stem cell transplantation, either for consolidation or relapse, in patients initially deemed transplant ineligible due to cardiac status (13). While this cohort demonstrates the feasibility of improving patients' clinical status with successful upfront bortezomib based therapy, overall hematologic CR rates for patients with revised Mayo stage IIIA or IIIB cardiac amyloid treated with CyBorD plateau at around 14–23% (6). Combined with the knowledge of data showing that around 80% of patients obtaining a hematologic CR will reach the threshold of a cardiac organ response, the majority of advanced cardiac AL patients have significant room for improvement in outcomes (2).

DARATUMUMAB EMERGES AS A TREATMENT

Daratumumab has demonstrated responses of high clinical interest in retrospective and prospective reported studies in heavily pretreated AL patients with reported rates of VGPR/CR of 47–86% (14–16). These encouraging hematologic response rates, many of which were achieved in patients who had never previously achieved a deep level of free light chain control to prior line therapies, resulted in cardiac organ response rates of 50–55% (16, 17). The randomized prospective ANDROMEDA study (NCT03201965) aims to show the potential improvement in hematologic response rates with the addition of subcutaneous daratumumab to CyBord and how this will translate to meaningful improvement in long term patient outcomes in the newly diagnosed AL setting. Of note patients with NT-proBNP greater than 8,500 ng/L were not eligible for ANDROMEDA, though 71% of patients had cardiac involvement with 37% of all patients having stage III disease (18, 19). Primary results from ANDROMEDA have as of now been reported at the 2020 EHA meeting. With the use of subcutaneous daratumumab, systemic administration reactions were low (4% of patients with grade 1 cough and hypotension), and the need for intravenous fluids with intravenous daratumumab was avoided in this potentially volume sensitive cardiac population. Patients randomized to the daratumumab combination arm had significantly longer median duration on treatment for their initial therapy (9.6 *vs* 5.3 months), with only 9.7% of Dara-CyBorD randomized patients receiving subsequent therapy compared to 41% of patients in the CyBorD alone arm (with 60% of these patients effectively crossing over to receive daratumumab). Overall hematologic response rates as well as VGPR/CR rates both significantly favored the Dara-CyBorD arm (ORR 92 *vs* 77%; VGPR/CR 79 *vs* 49%). These results support the use of Dara-CyBorD as upfront therapy in systemic AL and demonstrate the ability to achieve a VGPR/CR for the majority of patients for the first time. The composite time to event endpoint of progression free survival and major organ deterioration also favored the Dara-CyBorD combination (HR 0.58; CI 0.36–0.93, P = 0.022). Cardiac organ response rates at six months favored the addition of the anti-CD38 antibody as well, with the near doubling of patients achieving a cardiac response (42 *vs* 22%). Notable side effects included a low rate (7%) of grade 1–2 administration-related reactions with the use of subcutaneous daratumumab as well as slightly higher rates of pneumonia (8 *vs* 4%), lymphocytopenia (13 *vs* 10%), and diarrhea (6 *vs* 4%) with the addition of daratumumab. Still, despite the significant improvement in response parameters and overall tolerability with the addition of daratumumab to the CyBorD backbone, patient deaths were relatively equal between the two arms (27 patients in the Dara-CyBorD arm and 29 patients in the CyBorD arm) (18). Additional questions remain about the specific outcomes of the subgroup of patients with stage IIIA cardiac AL, as well as to the generalizability to patients with Stage IIIB disease. Several additional studies are ongoing in the upfront setting for patients with advanced cardiac AL. These include a study of daratumumab monotherapy in patients with Stage IIIB AL (NCT04131309) being conducted primarily in Europe, the combination of daratumumab, bortezomib, and dexamethasone in revised Mayo stage IV patients (NCT04474938), as well as a study of daratumumab, ixazomib, and dexamethasone for both newly diagnosed and previously treated systemic AL patients (NCT03283917). While the addition of the anti-CD38 antibody daratumumab to CyBorD clearly improves surrogate response endpoints, such as depth of hematologic response, in newly diagnosed systemic AL, its exact role in upfront therapy and patient selection for alternative therapies including high dose

melphalan and autologous stem cell transplant remain the central questions to improve outcomes for many AL patients.

ADDITIONAL THERAPY OPPORTUNITIES IN AL

Alkylating agents have long held a central role in the therapy of systemic AL. Both cyclophosphamide based combination therapy and melphalan have been used extensively in the early treatment of patients (20). The question of whether cyclophosphamide adds value and improves survival when added to bortezomib and dexamethasone for the upfront treatment of AL has recently been explored with multiple evaluations showing no clear benefit with respect to survival or depth of hematologic and organ response rates (21, 22). Other alkylating therapies are being explored in the previously treated AL setting. Bendamustine is an alkylating agent that has been evaluated in previously treated AL with modest results, with an overall hematologic response rate of 32–57%, and only 12–29% of patients achieving an organ response (23, 24). Melphalan Flufenamide (Melflufen) is a novel peptide-drug conjugate that is metabolized inside malignant plasma cells to melphalan, and in the setting of previously treated multiple myeloma has shown efficacy even in alkylator resistant cases (25, 26). Evaluation of Melhalan Flufenamide in combination with dexamethasone in patients with previously treated AL is currently being evaluated (NCT04115956). Given the historical success of delivering high dose melphalan to the relatively indolent plasma cell neoplasm underlying AL, as compared with multiple myeloma, with long term survival in AL patients being a well described experience, there is high excitement about the potential for melflufen in this setting (27).

The large structural chromosomal abnormality t(11;14) is overrepresented in the plasma cell neoplasm underlying AL compared with multiple myeloma, with about 50% of AL pati ents showing t(11;14) positivity compared to about 15% in newly diagnosed multiple myeloma (28–31). Patients with AL amyloidosis and t(11;14) have been found to have inferior hematologic and organ response rates as well as inferior five-year overall survival compared to non-t(11;14) AL patients in the setting of upfront bortezomib based therapies (32). The presence of t(11;14) is associated with a high BCL-2/MCL-1 ratio and confers sensitivity to venetoclax in clinical studies of relapsed or refractory multiple myeloma (33, 34). Given this, there has been interest in venetoclax and other BCL-2 family inhibitory compounds for plasma cell directed therapy in AL. Given the availability of venetoclax for off-label use, several retrospective case reports and series have been reported showing high hematologic response rates and general tolerability (35). Reported hematologic response rates in t(11;14) AL have been high, with Sidiqi et al. reporting seven of eight evaluable patients achieving either a CR or VGPR among a heavily pretreated population; additionally venetoclax at a dose of either 400 mg or 800 mg daily was generally well tolerated with the majority of patients experiencing low grade GI toxicity. As this was a small retrospective reported series in which venetoclax was used in varying therapy combinations, it is difficult to generalize when speaking of response rates, though ongoing planned studies of venetoclax and other BCL-2 targeted agents are planned in both previously treated and newly diagnosed AL harboring t(11;14).

B-cell maturation antigen (BCMA) is overexpressed on the surface of neoplastic plasma cells, and has been validated as a target in the treatment of multiple myeloma (36, 37). Likewise, early studies have shown preservation of high membrane bound BCMA expression on the clonal plasma cells underlying AL (38, 39). One study of bone marrow specimens in 28 patients with AL amyloidosis demonstrated universal plasma cell BCMA expression of >50% with a median of 65% (50–80) (39). Other studies have shown that like multiple myeloma, gamma secretase inhibitors can increase membrane bound BCMA expression on clonal plasma cells in AL. In one study the gamma secretase inhibitor LY-411575 increased both mBCMA expression on ALMC-1 cells *in vitro* (mBCMA expression increased from 84 to 99%), as well as on CD138 selected cells from bone marrow aspirates of AL patients from 36 to 68% (40). Soluble BCMA has been shown to correlate with disease activity and may play a role in mediating resistance to BCMA targeted therapies; as such the relatively lower burden of clonal plasma cells in AL may represent a therapeutic opportunity for BCMA targeted therapies. Therapies targeting BCMA are undergoing extensive development in the myeloma field, and as of late 2020 belantamab mafodotin, an antibody drug conjugate, has been FDA approved for the treatment of relapsed multiple myeloma. Development of antibody drug conjugates, bispecific antibodies, and T cell engager therapies as well as CAR-T cell therapies are under consideration and are in the early stages at this time.

While plasma cell directed therapies have shown the ability to induce hematologic and organ responses that correlate with improvements in patient survival, the ability to directly target soluble and deposited amyloidogenic light chains and fibrils would present an attractive opportunity to uniquely target AL pathogenesis. NEOD001, also known as birtamimab, was developed based on the binding to an epitope of serum amyloid A protein, though it also demonstrated reactivity to AL amyloid extracts and fibrils consisting of light chain immunoglobulins (41). In a phase 1/2 study of NEOD001 conducted in patients with persistent organ dysfunction following plasma cell directed therapy, 57% of eligible patients achieved an NT-proBNP defined cardiac organ response, and 60% met the threshold for renal response (42, 43). While these organ response rates were seen as encouraging in relation to expectations with historically treated patients of a similar population, questions remained about the clinical significance of biomarker defined organ responses and a randomized phase III study with a primary endpoint containing survival outcomes was planned to evaluate efficacy. The VITAL study randomized patients with treatment naïve AL with cardiac dysfunction to standard of care CyBorD as plasma cell directed therapy with or without NEOD001, with a primary composite endpoint of all-cause mortality or cardiac hospitalization (44). The study was terminated early after an interim futility analysis showed lack of

efficacy and risk of increased harm in the cohort receiving NEOD001. However, a *post hoc* analysis focusing on advanced cardiac AL patients, looking at overall survival without regard to cardiac hospitalizations, showed potential benefit with a hazard ratio of 0.498 (95% CI 0.24–1.03) P = 0.055, among 77 Mayo stage IV patients (45). Based on these results, further studies in advanced cardiac AL focusing on overall survival are planned. CAEL-101 is another promising anti-light chain antibody in development with phase I and II studies showing encouraging results. In dose escalation and expansion cohorts, CAEL-101 showed no high grade toxicity and demonstrated rapid organ responses in 60–80% of patients (46). Follow-up data confirms 78% of patients are alive at 37 months of median follow-up, with high rates of sustained organ response (47). Based on these and confirmatory phase II dosing studies, twin randomized phase III studies in treatment naïve Mayo stage IIIA or IIIB cardiac AL patients are being conducted (NCT04512235 and NCT04504825).

DISCUSSION

Therapy for light chain amyloidosis is rapidly evolving, and due to distinct disease pathophysiology and biology, the paradigm of developing combination therapies and sequences is further diverging from multiple myeloma. Upcoming trials evaluating anti-CD38 monoclonal antibody combinations, BCMA and BCL-2 family targeting agents, anti-amyloid fibril targeting antibodies, and other novel therapies are poised to generate a new standard of care, so urgently needed in this devastating disease.

AUTHOR CONTRIBUTIONS

GK and CC conceptualized and wrote the article. All authors contributed to the article and approved the submitted version.

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REVIEW
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Daratumumab for the Management of Newly Diagnosed and Relapsed/ Refractory Multiple Myeloma: Current and Emerging Treatments

*Massimo Offidani[1], Laura Corvatta[1], Sonia Morè[1], Davide Nappi[2], Giovanni Martinelli[3], Attilio Olivieri[1] and Claudio Cerchione[3]**

[1] Clinica di Ematologia Azienda Ospedaliero-Universitaria, Ospedali Riuniti di Ancona, Ancona, Italy, [2] Department of Hematology and Cell Bone Marrow Transplantation (CBMT), Ospedale di Bolzano, Bolzano, Italy, [3] Hematology Unit, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Meldola, Italy

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***Correspondence:**
Claudio Cerchione
claudio.cerchione@irst.emr.it
orcid.org/0000-0002-9104-5436

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Immunotherapy is changing the paradigm of multiple myeloma (MM) management and daratumumab is the first-in-class human monoclonal antibody targeting CD38 approved for the treatment of this malignancy. Daratumumab exerts anti-myeloma activity by different mechanisms of action as antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), complement-dependent cytotoxicity (CDC), direct apoptosis, and immunomodulation. After GEN501 and SIRIUS trials showed efficacy of daratumumab monotherapy in heavily pretreated relapsed-refractory multiple myeloma (RRMM), in patients with at least two previous line of therapy, two phase III trials demonstrated superior overall response rate (ORR) and progression free survival (PFS) using triplets daratumumab–bortezomib–dexamethasone (DVd) *vs* Vd (CASTOR) or daratumumab–lenalidomide–dexamethasone (DRd) *vs* Rd (POLLUX) in relapsed-refractory MM patients; so these combinations have been approved and introduced in clinical practice. The ongoing phase III CANDOR is evaluating the triplet daratumumab–carfilzomib–dexamethasone (DKd) *vs* Kd whereas phase III APOLLO trial is exploring daratumumab–pomalidomide–dexamethasone (DPd) *vs* PD. Many other trials exploring daratumumab combinations in relapsed-refractory MM are ongoing, and they will provide other interesting results. In newly diagnosed transplant-eligible patients, phase III CASSIOPEIA trial found the combination daratumumab–bortezomib–thalidomide–dexamethasone (Dara-VTd) significantly improves stringent Complete Response (sCR) rate and PFS compared with VTD, whereas in the phase II GRIFFIN study, comparing daratumumab–bortezomib–lenalidomide–dexamethasone (Dara-VRD) *vs* VRD, sCR rate was significantly higher using quadruplet combination. Many studies are evaluating daratumumab in consolidation and maintenance therapy after autologous stem cell transplantation (ASCT). As regard patients ineligible for ASCT, a great efficacy of daratumumab-containing combinations was reported by the phase III trials ALCYONE and MAIA, exploring daratumumab–bortezomib–melphalan–prednisone (DVMP) *vs* VMP and daratumumab–lenalidomide–dexamethasone (DRd) *vs* Rd, respectively. These studies

provided results never seen before in this setting. The aim of this paper is to critically review the results obtained with regimens containing daratumumab both in relapsed-refractory and in newly diagnosed MM.

Keywords: daratumumab, multiple myeloma, relapsed refractory multiple myeloma, newly diagnosed multiple myeloma, anti CD38

INTRODUCTION

Immunotherapy is changing the paradigm of MM management and daratumumab is the first-in-class human monoclonal antibody targeting CD38 approved for the treatment of this malignancy. Daratumumab exerts anti-myeloma activity by different mechanisms of action as antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), complement-dependent cytotoxicity (CDC), direct apoptosis and immunomodulation. After the GEN501 and SIRIUS trials showed efficacy of daratumumab monotherapy in heavily pretreated RRMM, in patients with at least two previous lines of therapy, two phase III trials demonstrated superior ORR and PFS using triplets daratumumab–bortezomib–dexamethasone (DVd) *vs* Vd (CASTOR) or daratumumab–lenalidomide–dexamethasone (DRd) *vs* Rd (POLLUX) in relapsed-refractory MM patients; so these combinations have been approved and introduced in clinical practice. The ongoing phase III CANDOR is evaluating the triplet daratumumab–carfilzomib–dexamethasone (DKd) *vs* Kd whereas phase III APOLLO trial is exploring daratumumab–pomalidomide–dexamethasone (DPd) *vs* PD. Many other trials exploring daratumumab combinations in relapsed-refractory MM are ongoing, and they will provide other interesting results.

In newly diagnosed transplant-eligible patients, phase III CASSIOPEIA trial found the combination daratumumab–bortezomib–thalidomide–dexamethasone (Dara-VTd) significantly improves sCR rate and PFS compared with VTD, whereas in the phase II GRIFFIN study, comparing daratumumab–bortezomib–lenalidomide–dexamethasone (Dara-VRD) *vs* VRD, sCR rate was significantly higher using quadruplet combination. Many studies are evaluating daratumumab in consolidation and maintenance therapy after ASCT. As regard patients ineligible for ASCT, a great efficacy of daratumumab-containing combinations was reported by the phase III trials ALCYONE and MAIA exploring daratumumab–bortezomib–melphalan–prednisone (DVMP) *vs* VMP and daratumumab–lenalidomide–dexamethasone (DRd) *vs* Rd, respectively. These studies provided results never seen before in this setting.

TRANSPLANT-ELIGIBLE NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

In young MM patients, ten-year survival increased from 18% in 2002–2006 to 35% in 2012–2016 (1), and this improvement has been related to the growing number of available therapeutic options since the 2000s. Previously, the development of autologous stem cell transplantation (ASCT) in the 1990s (2) had already contributed to a significantly increased survival, and now triplet novel agent regimens followed by ASCT represent the standard treatment for eligible patients. This therapeutic approach led to a ten-year survival of 60% (3). In Europe bortezomib, thalidomide dexamethasone (VTD) and bortezomib, cyclophosphamide, dexamethasone (VCD) combinations represent the most used regimens as induction therapy before ASCT, whereas in the USA bortezomib, lenalidomide, dexamethasone (VRD) is the preferred regimen according to the latest National Comprehensive Cancer Network (NCCN) guidelines.

The impact of daratumumab in combination with VTd (D-VTd) *vs* VTd as induction and consolidation therapy post ASCT was assessed in the phase III CASSIOPEIA trial (4) including 1,085 patients enrolled in 111 European sites. The primary endpoint of the study was the rate of sCR after consolidation, whereas secondary goals were minimal residual disease (MRD) negativity and ≥CR rates, PFS, and OS. Patients were randomized to four induction cycles and two consolidation cycles with VTd including bortezomib (1.3 mg/m^2 on days 1, 4, 8, 11), thalidomide (100 mg daily), and dexamethasone or D-VTd with intravenously daratumumab at a dose of 16 mg/kg once weekly in induction cycles 1 and 2 and once every 2 weeks during all the other cycles. Patients achieving at least a PR at day 100 post-ASCT were further randomized to observation or maintenance therapy with daratumumab every 8 weeks for 2 years. Median age of patients receiving VTd and D-VTd were 58 and 59 years and high-risk cytogenetics were documented in 16 and 15% of patients, respectively. Stringent CR rate after consolidation was significantly better in the D-VTd group compared with VTd (29 *vs* 20%; p = 0.0010), and this superiority was consistent across all subgroups of patients except for those with high-risk cytogenetics and ISS stage III (4). As regard MRD status after consolidation, a higher proportion of patients treated with D-VTd achieved MRD negativity assessed by multiparametric flow cytometry (MFC, 10^{-5}) (64 *vs* 44%; p <0.0001), and this benefit was documented also in high-risk cytogenetics (60 *vs* 44%, OR = 1.88) and ISS stage III (64 *vs* 46%, OR = 2.14) subgroups (5). The assessment of MRD status with next-generation sequencing (NGS, 10^{-6}) showed a negativity in 39% of patients receiving D-VTd *vs* 23% VTd, (p < 0.0001) (6). In the CASSIOPET companion study (7) including 268 patients enrolled in CASSIOPEIA trial, more patients with a response ≥CR receiving D-VTd *vs* VTd achieved PET/CT and MRD double negativity after consolidation (41.7 *vs* 25%; p = 0.0206). Regarding survival

measures, PFS at 18 months was 93% in the D-VTd group *vs* 85% in the VTd group (HR = 0.47; p < 0.0001), whereas the short median follow-up (18.8 months) makes survival data immature. However, it should be outlined that no maintenance was planned in the VTd arm, and lenalidomide maintenance, actually a standard therapy post-ASCT, could have prolonged PFS in patients enrolled in the standard arm. Overall, toxicity was not increased when adding daratumumab to VTd, and the most common grade 3–4 side effect was neutropenia occurring in 28 and 15% of patients treated with D-VTd and VTd, respectively. Among non-hematologic toxicities, grade 3–4 infections occurred in 22% of D-VTd patients *vs* 20% in VTd whereas grade 3–4 peripheral neuropathy developed in 9% of both groups. The rate of treatment discontinuation due to side effects was 7% in the D-VTd group and 8% in VTd (4). Of note, a comparison between patients with baseline conventional "CRAB" diagnostic criteria and those with "slim" only criteria showed no significant differences in terms of response rates, MRD-negativity rates, and PFS (8). Based on these results, both FDA and EMA regulatory agencies approved D-VTd in the early 2020.

No randomized trials have directly compared D-VTd to VRD but a matching-adjusted indirect comparison (MAIC) of 543 patients receiving four courses of D-VTd plus ASCT *vs* 350 patients enrolled in the IFM2009 trial and treated with three cycles VRD plus ASCT, has been presented at the last International Myeloma Workshop (9). This MAIC showed that D-VTd plus ASCT significantly improves PFS and MRD negativity compared to VRD plus ASCT.

Lenalidomide, instead of thalidomide, in combination with bortezomib, dexamethasone and daratumumab (D-VRd) has been evaluated in the randomized phase II GRIFFIN trial (10) whose primary endpoint was the sCR rate by the end of post-ASCT consolidation. All patients were assigned to receive four induction cycles, ASCT and two consolidation cycles with VRd (bortezomib 1.3 mg/m^2 on days 1, 4, 8, 11; lenalidomide 25 mg daily on days 1–14; dexamethasone 20 mg on days 12, 2, 8, 9, 15, 16) or D-VRd (VRD plus daratumumab 16 mg/kg on days 1, 8, 15 in the induction cycles and on day 1 in the consolidation cycles). After consolidation, maintenance therapy until progression or up to 2 years consisted in lenalidomide for the VRd group and lenalidomide plus daratumumab in the D-VRd group. Among 103 patients receiving VRd and 104 treated with D-VRd, 14 and 16%, respectively, were at high-risk cytogenetics. A sCR post consolidation was achieved in 42.4 and 32% of D-VRd and VRd patients, respectively (p = 0.068, statistically significant at the preset one-sided α of 0.10). However, achievement of sCR after consolidation could be debatable as primary endpoint considering that it was foregone that a quadruplet combination including daratumumab, mostly well tolerated, would have resulted in higher response rates than triplet combinations. MRD status would have represented a more significant primary endpoint being a surrogate biomarker for PFS. As regard MRD negativity (10^{-5} threshold), it resulted in 51% in the D-VRd group *vs* 20.4% in the VRd at the last follow-up. Responses deepened over time in both groups of patients, the rate of D-VRd patients with a sCR being 62.6 *vs* 45.4% of VRd patients after a median follow-up of 22.1 months. However, it has to be outlined that a lower percentage of patients receiving D-VRd underwent ASCT (90.4 *vs* 75.7%). Median PFS was not reached in either study arm, but it is presumable that follow-up is too short for detecting a significant difference. As regard toxicity, the most common grade 3–4 side effect was neutropenia (D-VRd 41.4%; VRd 21.6%) whereas the incidence of grade 3–4 infections was 23.2 *vs* 21.6%. The ongoing phase III PERSEUS trial, a collaborative study with European Myeloma Network (EMN) (NCT03710603) with the same study design of GRIFFIN, is evaluating D-VRd (with daratumumab administered subcutaneously) *vs* VRd in 690 patients. The results are awaited since, if efficacy of D-VRd is confirmed, another therapy for patients eligible for ASCT will be available in the future. Another ongoing phase III study (CEPHEUS, NCT03652064) is assessing D-VRd *vs* VRd in patients of all age for whom transplant is not intended as initial therapy, and it will probably provide some answers about the role of ASCT as frontline therapy.

Very important results have been recently reported with the triplet KRd in which a second generation proteasome inhibitor as carfilzomib replaces bortezomib. In a phase II study (11) including 76 patients receiving four cycles of KRd as induction, ASCT, four cycles KRd as consolidation, and 10 cycles of KRd as maintenance, after consolidation 90% of patients achieved at least a VGPR, 60% sCR and 61% MRD negativity assessed by next generation sequencing (NGS) with $<10^{-5}$ sensitivity. After a median follow-up of 56 months, 5-year PFS and OS were 72 and 84%, respectively. These results are similar to those reported by the phase II randomized FORTE trial (12, 13) in which 474 newly diagnosed MM patients were randomized to receive four KRd induction cycles, ASCT, four consolidation KRd cycles; 12 KRd cycles or four KCd induction cycles, ASCT, four KCd consolidation cycles. The rates of post consolidation ≥VGPR, ≥CR, and MRD negativity (at a cut-off of at least 10^{-5}) were 89, 60, and 58%, respectively. The addition of daratumumab to KRd was found to be tolerated in a phase 1b study (NCT01998971) (14) including newly diagnosed MM patients regardless of transplant eligibility. Patients received a median of 11 cycles of quadruplet D-KRd that yielded an ORR of 100% with 91% of patients achieving at least VGPR and 43% a CR. Based on these results, a phase II study in which 24 cycles of D-KRd is administered as initial therapy for patients of all ages is ongoing (NCT 03500445).

A phase II study of KRd-D with carfilzomib administered weekly (20 mg/m^2 on day 1 of cycle 1, 56 mg/m^2 on days 8 and 15 of cycle 1 and days 1, 8, and 15 of cycles 2–8) for eight cycles has been presented at the last American Society of Hematology (ASH) (15). Peripheral stem cell collection was recommended after four to six cycles of therapy for eligible patients but wKRd-D was continued for a total of eight cycles. Thirty patients with a median age of 57 years (range 36–70 years) were enrolled. MRD negativity rate (at level of 10^{-5}), the primary endpoint of study, was 75% in the 24 patients who completed eight cycles (ORR = 100%; ≥VGPR = 92%). These data are very interesting considering that 49% of the patients were at high-risk

cytogenetics. The phase III ADVANCE trial (NCT04268498) is evaluating wKRd-D *vs* wKRd and VsRD.

In the ongoing phase II MASTER trial (16) 101 patients received four cycles of D-KRd (daratumumab 16 mg/kg on days 1, 8, 15, and 22 of cycles 1 and 2 and less frequently in the subsequent cycles; carfilzomib 56 mg/m^2 on days 1, 8, and 15; lenalidomide 25 mg days 1–21; dexamethasone 40 mg on days 1, 8, 15, and 22) as induction, ASCT and 0, 4, or 8 cycles of D-KRd consolidation according to MRD status evaluated by NGS assay ($<10^{-5}$) at each phase of therapy. Patients who received therapy until two consecutive assessments were negative for MRD status, whereas patients who were MRD positive at the end of consolidation received lenalidomide maintenance. MRD negativity rate was 42% post induction, 73% post ASCT, and 82% during consolidation MRD-adapted. Of note, MRD negativity rates were similar between the standard and the high-risk cytogenetic groups. Most common grade 3–4 side effects were neutropenia (25%) and infections (12%).

An all oral regimen with ixazomib, the first approved oral proteasome inhibitor, lenalidomide and dexamethasone, has been assessed in combination with daratumumab in a phase II study (17) including MM patients irrespective of their transplant eligibility. Treatment consisted of 12 cycles with D-IxaRd as induction (ixazomib 4 mg days 1, 8, 15; lenalidomide 25 mg days 1–21; dexamethasone 40 mg weekly and daratumumab 16 mg/kg weekly for two cycles, every other week during cycles 3–6 and every 4 weeks afterwards) followed by 24 courses of daratumumab 16 mg/kg every 4 weeks plus ixazomib on days 1, 8, and 15 as maintenance. In patients who were ASCT eligible, stem cells were collected after four D-IxaRd cycles. The median age of 40 enrolled patients was 64.5 years (range 33–81 years). After a median follow-up of 10.1 months, response rates ≥VGPR and ≥CR were documented in 69 and 19% of patients, respectively, with 28% achieving MRD negativity. Treatment was well tolerated and the main toxicities were grade 3–4 neutropenia occurring in 16% of patients and infections in 3%. Rash developed in 48% of patients, but it was mainly of grades 1–2 (45%).

The triplet VCD, as mentioned above, represents another regimen frequently used as induction in patients eligible for ASCT, although a phase III trial (18) and a retrospective analysis by GIMEMA and European Myeloma Network (EMN) (19) reported a higher quality of response with VTD than VCD. However, as well as for the other triplets, also VCD has been evaluated in combination with daratumumab. In the phase II LYRA study (20) 86 patients, irrespective of eligibility for ASCT, (median age 63, range 41–82 years; 37% with high-risk cytogenetics) received 4–8 cycles of induction therapy with bortezomib 1.5 mg/m^2 on days 1, 8, and 15; oral cyclophosphamide 300 mg/m^2 on days 1, 8, 15, and 22; dexamethasone 40 mg weekly and daratumumab 16 mg/kg weekly for two cycles, every two weeks for four cycles and every 4 weeks for the last two cycles. After induction patients could receive ASCT at the discretion of the investigator and afterwards a maintenance with monthly daratumumab for 12 cycles. The rate of CR + VGPR after four cycles (the primary endpoint) was 44% with an ORR of 79%. The same combination was assessed in a phase 1b study (21) in which 18 patients received four induction cycles with CyBorD-Dara, ASCT, two consolidation cycles with CyBorD-Dara, and a maintenance with daratumumab every 4 weeks until progression. Overall, treatment was safe, and 94 and 44% of patients, respectively, achieved at least VGPR and CR after ASCT. Remarkably, 44% of patients obtained MRD negativity at a level of 10^{-5} after consolidation. The ongoing phase II randomized trial EMN 18 (NCT03896737) is testing a therapeutic strategy including four cycles of Dara-VCD as induction, ASCT, four cycles of Dara-VCD as consolidation *vs* four VTd, ASCT, four VTd. At the end of the consolidation the patients are randomized to receive a maintenance with daratumumab alone or plus ixazomib for up 24 months.

After a meta-analysis (22) confirmed the advantage in terms of PFS and OS of lenalidomide maintenance post-ASCT, several trials are evaluating daratumumab alone or in combination with other agents in this setting. In **Figure 1** we reported results of the main daratumumab-containing combinations in MM patients eligible for ASCT, whereas in **Table 1** we summarized other not mentioned ongoing clinical trials.

TRANSPLANT-INELIGIBLE NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

Daratumumab recently has obtained good results also in the setting of newly diagnosed MM patients not eligible for ASCT since it has been approved in combination with bortezomib–melphalan–dexamethasone (D-VMP) and with lenalidomide–dexamethasone (D-Rd).

In the phase III trial ALCYONE (23, 24) 706 patients (median age 71 years) were randomized to receive nine cycles with VMP (bortezomib 1.3 mg/m^2 twice weekly in cycle 1 and once weekly in cycles 2–9; melphalan 9 mg/m^2 on days 1–4 and dexamethasone 60 mg/m^2 on days 1–4) or D-VMP (the same schedule of VMP plus daratumumab 16 mg/kg weekly in cycle 1, every 3 weeks in cycles 2–9 and every 4 weeks subsequently). After a median follow-up of 40.1 months median PFS, the primary endpoint of the study was 36.4 in the D-VMP group *vs* 19.3 in the VMP group (HR = 0.42; $p < 0.0001$). Remarkably, PFS curve of VMP arm starts to show a much higher slope if compared with D-VMP curve just after nine cycles, emphasizing the benefit of daratumumab maintenance *vs* fixed duration therapy. The lack of maintenance in the VMP arm also explains such a high difference (58%) in the risk of progression/death between the two regimens.

The superiority of D-VMP was consistent across all subgroups of patients including those older than 75 years or with ISS stage III, whereas hazard ratio was lower in patients with standard-risk *vs* high-risk cytogenetics (0.39 *vs* 0.78). Overall, after a longer follow-up, a benefit for OS was also observed since patients receiving D-VMP showed a 40% reduction in the risk of death with an estimated 42-month OS rate of 75% with D-VMP *vs* 62% with VMP (HR = 0.60; $p = 0.0003$). The D-VMP group had higher overall response rates (91 *vs* 74%; $p < 0.0001$), ≥CR rate (46 *vs* 25%; $p < 0.0001$), and MRD negativity rate (28 *vs* 7%;

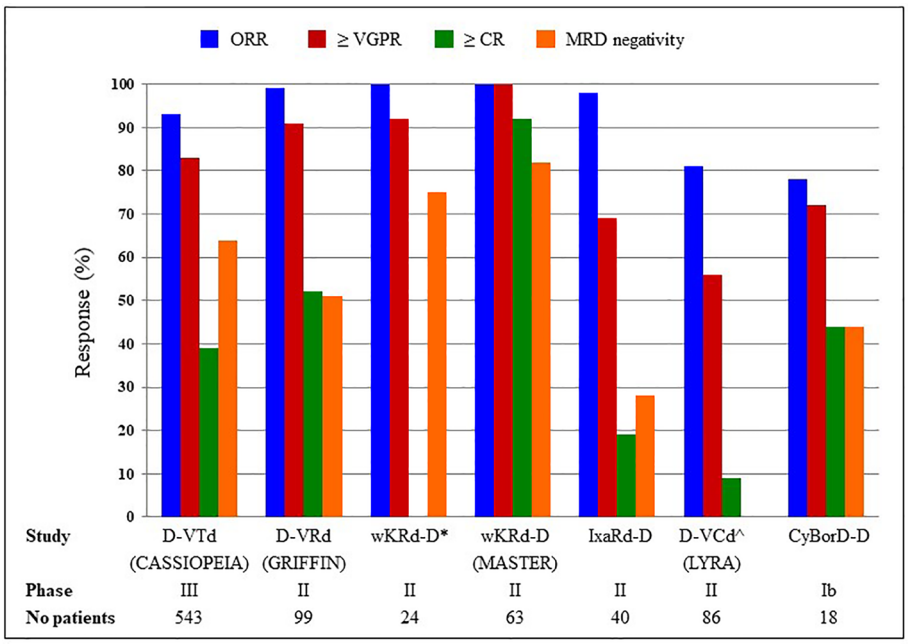

FIGURE 1 | D-VTd, daratumumab, bortezomib, thalidomide, dexamethasone; D-VRd, daratumumab, bortezomib, lenelidomide, dexamethasone; wKRd_D, weekly carfilzomib, lenalidomide, dexamethasone, daratumumab; IxaRd-D, ixazomib, lenalidomide, dexamethasone, daratumumab; D-VCd and CyBorD-D, daratumumab, cyclophosphamide, bortezomib, dexamethasone. *≥ CR not available; ^MRD status not available.

$p < 0.0001$) compared with the VMP group. Remarkably, in a subgroup analysis a sustained MRD negativity for at least 12 months *vs* <12 months was associated with better outcomes in terms of PFS and OS (24). As for safety, infections were most commonly reported in grade 3–4 adverse event (23% in the D-VMP group and 14.7% in the VMP group), particularly pneumonia (11.3 *vs* 4%). In addition, fewer daratumumab-treated patients discontinued treatment due to adverse events, compared with the VMP-treated patients (5 *vs* 9%). During daratumumab monotherapy in the D-VMP group, the most frequent any grade adverse events were upper respiratory infections (19%) and bronchitis (15%). Simultaneously, the phase III trial MAIA (25, 26) compared the standard of care lenalidomide–dexamethasone (Rd) with Rd plus daratumumab (D-Rd) in 737 newly diagnosed MM patients with a median age of 73 years. Patients enrolled in the Rd arm received lenalidomide 25 mg on days 1–21 plus dexamethasone 40 mg on days 1, 8, 15 and 22; patients allocated in the D-Rd arm were treated with Rd plus daratumumab at a dose of 16 mg/kg once weekly during cycles 1,2, every two weeks during cycles 3–6 and every 4 weeks thereafter. Treatment was continued until progression or unacceptable toxicity. In the last update of the study, after a median follow-up of 36.4 months, D-Rd demonstrated a significant PFS benefit since median PFS was not reached *vs* 33.8 months in D-Rd and Rd groups, respectively (HR = 0.56; $p < 0.0001$). Of note, 36-months PFS was 68% in the D-Rd group *vs* 46% in the Rd group. Although comparison between different trials should be made with caution, median PFS of the population treated with Rd in the MAIA trial is quite similar to that treated with Dara-VMP in the ALCYONE one (33.8 *vs* 36 months). This further outlines the better performance of Dara-Rd compared with Dara-VMP despite the higher HR (0.56 *vs* 0.42). The ORR was 93 *vs* 82%, with CR rates or better 50 *vs* 27% ($p < 0.0001$), respectively. MRD negativity was also significantly more frequent in patients treated with D-Rd *vs* Rd, being 29 *vs* 9% ($p < 0.0001$).

In the forest plot for PFS, D-Rd turned out to be favorable in all subgroups, but its benefit seemed less strong in the high-risk cytogenetic compared to the standard-risk patients (HR 0.52 *vs* 0.49). The most frequent grade 3–4 hematological adverse event reported in the study was neutropenia (50% in the D-Rd group *vs* 35.3% in the Rd group) whereas among non-hematologic toxicities grade 3–4 infections developed in 32 and 23.3% of D-Rd and Rd patients, respectively, with pneumonia occurring in 13.7 *vs* 8%. The addition of daratumumab to Rd did not increase the incidence of second primary malignancies (8.8 *vs* 7.1%).

D-VMP and D-Rd are actually recommended by the NCCN Guidelines as the preferred Category 1 therapeutic options for newly diagnosed MM patients not eligible for ASCT. However, considering that infections, mainly pneumonia, represent a frequent adverse event in patients receiving Dara-VMP and D-Rd, a recent pooled retrospective analysis of ALCYONE and MAIA trials assessed predictive markers of grade ≥3 and serious infection occurring during the first 6 months of treatment. Using four parameters (age, LDH, albumin, and baseline ALT) patients were classified as low- and high-risk with infection rates of 15.7 and 29.3%, respectively (HR = 2.11; $p = 0.0001$) (27).

Recently, the PEGASUS study (28) made an anchored indirect treatment comparison (ITC) in terms of PFS among

TABLE 1 | Ongoing clinical trial with daratumumab in transplant eligible newly diagnosed MM patients.

Trial	Phase	Characteristics of patients	Design	ClinicalTrials N.
MUK Nine b: OPTIMUM Treatment Protocol (MUKnineb)	II	Transplant eligible with high-risk NDMM and plasma cell leukemia	CVRDd × 4-6 (induction)→ SCT→DVRd x 6 (consolidation part 1) → DVR × 12 (consolidation part 2)→ DR (maintenance)	03188172
Study association of lenalidomide, ixazomib, dexamethasone and daratumumab in newly diagnosed standard risk multiple myeloma (IFM2018-01)	II	Transplant eligible with standard-risk NDMM	IxaRd-D (induction) → SCT → IxaRdD (consolidation) → R (maintenance)	03669445
Daratumumab, carfilzomib, lenalidomide, and low dose dexamethasone (DKRd) in newly diagnosed, multiple myeloma	II	Transplant and non-transplant eligible NDMM	DKRd x 24 cycles	03500445
Ixazomib citrate, lenalidomide, dexamethasone, and daratumumabintreating patients with newly diagnosed multiple myeloma	II	Transplant and non-transplant eligible NDMM	IxaRdD × 12 (induction) → IxaD for up to 36 months (maintenance)	03012880
Daratumumab, ixazomib, and dexamethasone or daratumumab, bortezomib, and dexamethasone in patients with newly diagnosed multiple myeloma (DeRIVE)	II	Transplant and non-transplant eligible NDMM	Arm 1: IxaDd x 8 (induction) → ± SCT → IxaDd for up to 24 months (maintenance) Arm 2: DVd x 3 followed by IxaDd x 5 (induction) → ± SCT → IxaDd for up to 24 months (maintenance)	03944224
An intensive program with with quadruplet induction and consolidation plus tandem autologous stem cell transplantation in newly diagnosed high-risk multiple myeloma patients (IFM 2018-04)	II	Transplant eligible with high-risk NDMM	DKRd × 6 (induction) → tandem SCT → DKRd × 4 (consolidation) → DR (maintenance)	03606577
Study of daratumumab combined with carfilzomib, lenalidomide, and dexamethasone for newly diagnosed multiple myeloma	II	Transplant and non-transplant eligible NDMM	DKRd x 8 (induction) → MRD based therapy (post-induction)	04113018
2015-12: A study exploring the use of early and late consolidation/ maintenance therapy	II	Transplant eligible with high-risk NDMM	DKTd-PACE ×→ SCT → DKd ± SCT (consolidation 1) → D (consolidation 2) → DKd alternating with DRd in 3-month blocks	03004287
Adaptive strategy in treatment for newly diagnosed multiple myeloma with upfront daratumumab-based therapy	II	Transplant and non-transplant eligible NDMM	DRd (induction) → DVRd (consolidation MRD based) → DR→R (maintenance)	04140162
Daratumumab in treating transplant-eligible partecipants with multiple myeloma	II	Transplant eligible with NDMM who have received any prior induction therapy	D × 2 (consolidation 1) → SCT (consolidation 2) → DR × 12→D (maintenance)	03477539
Short course daratumumab in patients with multiple myeloma	II	Transplant with NDMM who have achieved VGPR or better after induction ± consolidation/SCT	DR × 6 months	03490344
A study of daratumumab plus lenalidomide *versus* lenalidomide alone as maintenance treatment in participants with newly diagnosed multiple myeloma who are minimal residual disease positive after frontline ASCT (AURIGA)	III	Transplant eligible with NDMM who have received induction ± consolidation and SCT	DR *vs* R until progression	03901963
S1803, daratumumab/rHuPh20+/– lenalidomide as post-ASCT maintenance for MM w/MRD to direct therapy duration (DRAMATIC)	III	Transplant eligible with NDMM who have received induction and SCT	DR *vs* R, duration guided by MRD status	04071457

C, cyclophosphamide; V, bortezomib; R, lenalidomide; D, daratumumab; d, dexamethasone; Ixa, ixazomib; K, carfilzomib; T, thalidomide; SCT, stem cell transplant.

patients treated with D-Rd in the MAIA trial and those receiving VRd or Vd in real life. This analysis demonstrated that D-Rd reduced the risk of progression or death compared to either VRd (HR 0.68; p = 0.04) or Vd (HR 0.48; p < 0.001) in transplant-ineligible patients.

Daratumumab was also studied in association with ixazomib and low dose dexamethasone in phase II HOVON-143 trial (29) for unfit and frail patients according to the International Myeloma Working Group Frailty Index (IMWG-FI). Treatment consisted of nine cycles with ixazomib (4 mg on days 1, 8, 15), daratumumab (16 mg/kg weekly cycles 1 and 2; every two weeks cycles 3–6; day 1 cycles 7–9), dexamethasone (in combinations with daratumumab 10 mg). Maintenance therapy until progression or for maximum of 2 years included daratumumab plus ixazomib. Results of the 65 frail patients enrolled in the study (median age 81 years, range 70–92 years) have been recently presented at the last European Hematology Association (EHA) Congress (30). Overall response rate, primary endpoint, was 78% with 36% of the patients achieving at least a VGPR. However, 12 patients (15%) died due to toxicity, and among them six died early (≤60 days). After a median follow-up of 16.3 months, median PFS was 13.8 months, and 1-year OS was 78%.

Several ongoing phase II and III studies are assessing daratumumab-based combinations in elderly patients. In a phase II US study (NCT 04052880), patients older than 70 years receive subcutaneous daratumumab, dose-attenuated bortezomib, lenalidomide, and dexamethasone until progression with the aim to evaluate response VGPR or better after 8 cycles. In another

phase II study (NCT04151667), patients 65 years and older are treated with a response adapted approach, receiving subcutaneous daratumumab plus dexamethasone for 2 months and a subsequent therapy according to response. A phase II randomized clinical trial (NCT04009109) will evaluate 12 cycles with ixazomib plus D-Rd followed by either lenalidomide maintenance or maintenance with lenalidomide, ixazomib, daratumumab for at least 2 years. The IFM 2017-03 phase III trial (NCT03993912) compares subcutaneous daratumumab associated with lenalidomide to Rd until progression in frail patients. Another phase III trial by PETHEMA group (NCT03742297) enrolling elderly fit patients aged between 65 and 80 years randomizes patients to nine cycles VMP followed by nine Rd *vs* 18 cycles KRd *vs* 18 cycles D-KRd.

As regard the key question whether daratumumab is able to improve outcome in patients with high-risk cytogenetics, a recent meta-analysis analyzed six randomized phase III trials, three for newly diagnosed MM (ALCYONE, MAIA, CASSIOPEIA) and three for relapsed/refractory MM (CASTOR, POLLUX, CANDOR). The addition of daratumumab to backbone regimens led to improved PFS among patients with high-risk newly diagnosed MM (pooled HR = 0.67; p = 0.02). However, hazard ratio was better (0.45, p < 0.01) in patients with standard-risk cytogenetics (31).

DARATUMUMAB IN SMOLDERING MULTIPLE MYELOMA

Smoldering multiple myeloma (SMM) represents a very heterogeneous entity, and the question whether patients with SMM should be treated or not remains unresolved. Since the risk of progression for this disease is not uniform over time (32), several studies have been conducted with the aim of recognizing predictive factors and thus of evaluating the risk of progression (33–35). The last risk model by Mayo Clinic (36) categorizes patients in low risk (0 factor), intermediate risk (1 factor) or high risk (2–3 factors) using as risk factors bone marrow plasma cells >20%, serum monoclonal protein >2 g/dl and an involved to uninvolved serum-free light chain ratio >20 (20/2/20 model). The median TTP for low-, intermediate-, and high-risk groups were 110, 68, and 29 months, respectively (p < 0.0001). Current therapeutic approach in patients with smoldering myeloma (SMM) is active monitoring until progression to MM, but different treatments favoring disease control or disease eradication have been evaluated in several studies (37–39), and they are under investigation in other ongoing clinical trials. Based on activity and safety of daratumumab monotherapy in relapsed refractory MM (40), Landgren et al. recently reported results of a randomized, multicenter, phase II study (CENTAURUS) (41) including 123 patients with high or intermediate risk SMM who were randomized to receive three different daratumumab dosing schedules (intense, intermediate, and short). The co-primary endpoint of CR rate >15% was not met since CR rate was lower in all arms of the study, whereas the other co-primary end point of a median PFS ≥24 months in all arms was met. Of note, the 24-month PFS rates were 90, 82, and 75% in the intense, intermediate, and short arm, respectively. The ongoing phase III AQUILA trial (NCT03301220), testing subcutaneous daratumumab *vs* active monitoring will provide further data regarding the efficacy of daratumumab alone in SMM. Another phase II study (NCT03236428) is evaluating daratumumab monotherapy in patients with high-risk MGUS and low-risk SMM with the aim to determine if this agent is able to prevent MM development. In MM setting, daratumumab has also been evaluated in combination with lenalidomde and proteasome inhibitors also in SMM. In the phase II ASCENT study (NCT03289299) high-risk MM patients receive six cycles of induction with D-KRd followed by six consolidation cycles with the same regimen and a maintenance therapy with daratumumab plus lenalidomide for 12 months. Finally, the phase III DETER-MM (NCT 03937635) is assessing, in high-risk SMM, DRd *vs* Rd for 24 cycles.

However, it was emphasized that, at now, no reliable predictive markers of evolution of SMM in overt MM are available. Therefore, we cannot exclude that a not negligible portion of SMM patients treated with the above mentioned trials would never progress to MM.

Relapsed/Refractory Multiple Myeloma

Daratumumab-based three-drug regimens or as single-agent are treatment options highly efficacious in relapsed/refractory multiple myeloma (RRMM). As described in detail below, several combination treatment strategies with daratumumab, able to prolong PFS when administered until progression are now approved. Combinations with lenalidomide–dexamethasone (DRd) or bortezomid–dexamethasone (DVd) were both firstly approved by both the FDA and EMA. More recently, also combinations with carfilzomib–dexamethasone (DKd) and pomalidomide-dexamethasone (DPd) were approved by the FDA. Single-agent use is labeled for patient refractory to previous lines proteasome inhibitors and immunomodulating-containing agents.

In **Table 2** we summarized the main clinical trials in relapsed/refractory setting.

Daratumumab–Lenalidomide–Dexamethasone

DRd regimen was explored in POLLUX trial, a phase 3, randomized, open-label, multicenter study evaluating the safety and efficacy of Rd and DRd in patients with RRMM, with a median of 1 prior treatment line (42). 569 patients with relapsed/refractory MM were randomly assigned to receive Rd with or without daratumumab, each administered until disease progression or unacceptable toxicity. Lenalidomide was given 25 mg PO on days 1 through 21 of each cycle and dexamethasone 40 mg weekly in the Rd arm. In the DRd arm, daratumumab was given at 16 mg/kg IV weekly for 8 weeks in cycles 1 and 2, every 2 weeks for 16 weeks in cycles 3–6, and then every 4 weeks, along with Rd. Safety and efficacy were evaluated after a median follow-up of 54.8 months, with a treatment median duration of 34.3 and 16.0 months in the DRd and Rd groups, respectively (43). PFS in

TABLE 2 | Major ongoing clinical trial with daratumumab in refractory/relapsed MM patients.

Trial	Phase	Characteristcs of pts.	Design	ClinicalTrials N
PLEIADES (MMY2040): non-randomized trial exploring daratumumab in combination with various treatment regimens, including Rd	II	RRMM patients ≥1 prior treatment line	RRMM patients received DRd in 28 day-cycle until PD or intolerable toxicity	NCT03412565
MMY1001 trial exploring daratumumab when administered in combination with various treatment regimens, including Kd	Ib	RRMM patients 1–3 prior lines of therapy) carfilzomib-naïve;	RRMM patients received DKd in 28 day-cycle until PD or intolerable toxicity	NCT01998971
CANDOR randomized trial evaluating DKd *vs* Kd in RRMM patients	III	RRMM; 1–3 prior therapies with ≥PR to ≥1 prior therapy	DKd *vs* Kd in 28 day-cycle until PD or intolerable toxicity	NCT03158688
LYNX (MMY2065): randomized trial evaluating DKd *versus* Kd, also for daratumumab-exposed patients	II	RRMM who have received 1–2 prior lines of therapy, daratumumab included	DKd *vs* Kd in 28 day-cycle until PD or intolerable toxicity	NCT03871829
MMY1001trial exploring daratumumab when administered in combination with various treatment regimen, including pomalidomide	IIb	RRMM patients ≥2 prior lines, including V and R	RRMM patients received DPd in 28 day-cycle until PD or intolerable toxicity	NCT01998971
APOLLO: randomized trial evaluating daratumumab + Pd *vs* Pd	III	RRMM ≥1 prior treatment with both lenalidomide and a PI	DPd *vs* PD in 28 day-cycle until PD or intolerable toxicity	NCT03180736
MM-014 non-randomized trial evaluating DPd and Pd in RRMM	II	RRMM patients 1 or 2 prior lines of therapy, including lenalidomide	RRMM patients received DPd in 28 day-cycle until PD or intolerable toxicity	NCT01946477
Randomized trial evaluating daratumumab, cyclophosphamide, dexamethasone plus or not pomalidomide in RRMM	II	RRMM patients ≥1 prior treatment line	Arm A (DCdP) *vs* arm B (DCd plus P, if progressive disease)	NCT03215524
LYRA single arm trial evaluating daratumumab + CyBorD in MM patients, including RRMM	II	RRMM patients 1 treatment line	RRMM patients received DCyborD until progression	NCT02951819
Non-randomized 2-parts trial evaluating venetoclax and daratumumab–dexamethasone plus or not bortezomib i	I/II	RRMM patients with (part-1) or regardless t(11;14) (part-2)	Part-1: VenDd in patients RRMM ≥1 prior line; Part-2: VenDVd in patients RRMM 1–3 prior lines of therapy (no PI)	NCT03314181
CA209-755: randomized trial evaluating nivolumab and daratumumab with or without low-dose cyclophosphamide in patients with RRMM	II	RRMM patients ≥2 prior therapies	Part A: run-in phase + randomization; Part B: randomization; NDC *vs* ND	NCT03184194

C, cyclophosphamide; V, bortezomib; R, lenalidomide; D, daratumumab; d, dexamethasone; Ixa, ixazomib; K, carfilzomib; P, pomalidomide; N, nivolumab, Ven, venetoclax; CyBorD, cyclophosphamide–bortezomib–dexamethasone.

the ITT population for the DRd *vs* Rd groups was 45.0 *vs* 17.5 months (P < 0.0001) respectively, with a 48-month PFS rate of 48% DRd *vs* 21% Rd and an ORR of 93% for DRd (n = 281) *vs* 76% for Rd (n = 276) (P < 0.0001). In patients exposed to one prior treatment line, PFS was 53.3 months in the DRd arm *vs* 19.6 months in the Rd arm (HR 0.42, P < 0.0001). MRD negativity rates (10^{-5}) for DRd *vs* Rd were 33 *vs* 7% (P < 0.0001) in the ITT population. Regarding safety profile, grade 3/4 neutropenia was the most relevant hematologic AE, with 57 *vs* 42% in DRd and Rd respectively, followed by anemia and thrombocytopenia (19 *vs* 22% and 15 *vs* 16%, respectively). Non-hematologic AE was dominated by diarrhea 59 *vs* 38% and pneumonia 25 *vs* 17%, respectively in the DRd and Rd arms. Infusion reactions were reported in 48% of patients and were mostly mild; the majority (92%) occurred at the first administration. An updated efficacy and safety data of DRd based on cytogenetic risk status from POLLUX after a median follow-up of 44.3 months showed DRd significantly improved ORR, PFS, and MRD-negativity rates *vs* Rd in patients with both standard and high cytogenetic risk (44). A sub-analysis for elderly patients of POLLUX trials, divided in two groups of 65–74 years and ≥75 years, showed an improvement in PFS, ORR, and MRD-negativity rates for DRd *vs* Rd (45). Regarding safety, hematological AEs were superimposable to other age groups; daratumumab infusion reaction rate was similar in ITT population, but only with 14 and 5% of grade 3/4, respectively, with treatment discontinuation. Overall, in the POLLUX trial, the evidence of the greatest clinical benefit of DRd observed in patients that had received one prior line of therapy supports the use of DRd in patients with RRMM at first relapse. Despite a higher incidence of neutropenia and pneumonia in the DRd arm, treatment discontinuation rate was similar (17 *vs* 15%). PLEIADES is an ongoing, phase 2, non-randomized, multicenter study evaluating the clinical benefit of DRd in RRMM with ≥1 prior line of therapy (46). Daratumumab subcutaneous is administered weekly at 1,800 mg in cycles 1 and 2, then on days 1 and 15 of cycles 3–6, and on day 1 of cycles 7+; lenalidomide at 25 mg PO on days 1–21 of each cycle; dexamethasone: 40 mg PO weekly. An ORR of 93.8%, the primary end-point, was met for the DRd cohort with response rates similar to the POLLUX study. Most common AEs were neutropenia (49%), thrombocytopenia (14%), and pneumonia (12%).

Daratumumab–Bortezomib–Dexamethasone

The DVd combination was first explored by CASTOR, a phase 3, open-label, randomized, multicenter study evaluating the safety

and efficacy of bortezomib–dexamethasone (Vd) alone and plus IV daratumumab (DVd) in 498 patients with RRMM (47). Regarding the administration schedule, in Vd: bortezomib 1.3 mg/m^2 subcutaneously on days 1, 4, 8, and 11 over each 21-day cycle for eight cycles; dexamethasone 20 mg PO or IV on days 1, 2, 4, 5, 8, 9, 11, and 12 of the eight cycles. In the DVd arm: Vd plus daratumumab 16 mg/kg IV weekly for the first three cycles, once every 3 weeks of cycles 4–8, and every 4 weeks thereafter. Updated results after a median follow-up of 50.2 months showed a median PFS of 16.7 months *vs* 7.1 months (HR: 0.31, P < 0.0001) with DVd and Vd respectively, and regarding patients who received one previous therapy line, the benefit was 27.0 *vs* 7.9 months (HR: 0.21, P < 0.0001) (48). In patients with evaluable response, ORR was 85 *vs* 63% (P < 0.0001), and for those receiving one previous therapy ORR was 92 *vs* 74% (P = 0.0007), respectively. Overall, as in POLLUX, the safety profile of CASTOR trial was marked by a slightly higher incidence in thrombocytopenia and neutropenia, but not translated in a significative rate of discontinuation in the DVd arm *vs* Vd (10 and 9%, respectively). Regarding cytogenetic risk: in high-risk patients, median PFS was 12.6 months with DVd *vs* 6.2 months with Vd (HR: 0.41; P = 0.0106), while in standard cytogenetic risk median PFS was 16.6 *vs* 6.6 months (HR: 0.25; P < 0.0001). Regarding safety profile, most common grade 3/4 hematologic AEs were, for DVd and Vd arm, thrombocytopenia (46 *vs* 33%), anemia (both 16%) and neutropenia (14 *vs* 15%). Non-hematologic AEs comprised mainly of peripheral neuropathy (all grade 50 *vs* 38% for DVd and Vd), upper respiratory tract infections, pneumonia, and hypertension (36 *vs* 18%, 16 *vs* 13%, and 11 *vs* 13%, respectively). Secondary solid or hematological malignancies were reported in 15 (6%) patients who received DVd *vs* four (2%) patients who received Vd. As in POLLUX, also CASTOR trial was analyzed for the elderly population, divided in two groups by age (65 to 74 years and ≥75 years) showing the advantage of DVd over Vd in terms of PFS and ORR of both groups, with a safety profile similar to that of the younger patients (45).

Daratumumab–Carfilzomib–Dexamethasone

Given the effectiveness of daratumumab with bortezomib-containing regimens, it was also evaluated with the second-generation proteasome-inhibitor carfilzomib (DKd) in the six-arm phase 1b study, proving its efficacy and safety in 85 RRMM patients receiving DKd (49). In each 28-day cycle, daratumumab was administered at 16 mg/kg IV every week on cycles 1–2, every 2 weeks on cycles 3–6, and every 4 weeks thereafter; carfilzomib was administered weekly on days 1, 8, and 15 of each cycle at 20 mg/m^2 on day 1-cycle 1 and escalated to 70 mg/m^2 on day 8-cycle 1; dexamethasone: 40 mg/week. With a median follow-up of 16.6 months, an ORR of 84% was obtained in the whole cohort. Major grade >3 AEs were: thrombocytopenia (31%), anemia (21%), neutropenia (21%), hypertension (18%), and asthenia (12%). Infusion reaction rate was higher when the first infusion of daratumumab was administered as a single dose compared to a split dose (60 *vs* 43%). Updated results after 23.7 months of median follow-up were: ORR was 84%, median PFS was 25.7 months, and median OS was not reached (50). Relevant grade 3/4 AEs were: thrombocytopenia (32%), anemia (21%), neutropenia (21%), hypertension (20%), and upper respiratory tract infections (4%). Multicentric phase 3 CANDOR trial evaluated DKd *vs* Kd allocating in a randomized 2:1 mode to receive DKd or Kd in 28-day cycles until disease progression (51). Carfilzomib was given on days 1, 2, 8, 9, 15, and 16 of each cycle to all patients at 20 mg/m^2 on days 1 and 2 during cycle 1 and 56 mg/m^2 thereafter, as IV infusion. Daratumumab (8 mg/kg) was administered as IV infusion on days 1 and 2 of cycle 1 and at 16 mg/kg weekly for the remaining doses of the first two cycles, then every 2 weeks for four cycles (cycles 3 to 6), and every 4 weeks thereafter. Dexamethasone was administered PO or IV at 40 mg weekly (20 mg for patients ≥75 years). A total of 466 patients received either DKd (n = 312) or Kd (n = 154). Median PFS follow-up was 16.9 *vs* 16.3 months, and median PFS was not evaluable and 15.8 months for DKd and Kd, respectively. Treatment with DKd resulted in a 37% reduction in the risk of progression or death (HR, 0.63; P = 0.0027). The ORR in the DKd group was 84 *vs* 75% in the Kd group (P = 0.0080). Severe (grade >3) hematologic and non-hematologic AEs of interest in DKd group *vs* Kd, group were: thrombocytopenia (24 *vs* 18%), respiratory tract infections (27 *vs* 15%), acute renal failure (5 *vs* 7%), cardiac failure (4 *vs* 9%). Updated results of this trial approximately 36 months after the enrollment beginning show a median PFS follow-up was 28.6 *vs* 15.2 months, resulting in a 13.4-month improvement in median PFS which was observed in the DKd arm, with safety data consistent with the previous analysis (52). LYNX is an ongoing, randomized, open-label, multicenter, phase 2 study evaluating the safety and efficacy of DKd (subcutaneous daratumumab) *versus* Kd alone in RRMM patients who were previously exposed to a IV daratumumab-containing therapy, with the scope to evaluate daratumumab retreatment (53, 54). Enrolled patients (expected 230) received one to two prior lines of therapy with at least one prior treatment exposure to daratumumab IV (but not exposed to carfilzomib) and are randomized 1:1 in order to receive DKd or Kd. All patients will receive 28-day cycles of Kd until PD or intolerable toxicity as follows: carfilzomib 20 mg/m^2 IV on day 1-cycle 1, escalated to 70 mg/m^2 on days 8 and 15-cycle 1, and 70 mg/m^2 on days 1, 8, and 15 for each subsequent cycle; dexamethasone 40 mg IV or oral on days 1, 8, 15, and 22 up to cycle 9, then on days 1, 8, and 15 for subsequent cycles. DKd will receive also daratumumab–hyaluronidase 1,800 mg subcutaneous once weekly in cycles 1 and 2, then once every 2 weeks in cycles 3–6, and once monthly for each subsequent cycle. Primary endpoint is rate of ≥VGPR. Exposing again patients to daratumumab, even if by another route of administration, is an attractive opportunity to evaluate how the immune system acts in these conditions and if any kind of immunogenicity could be raised against this monoclonal antibody that potentially could affect a retreatment strategy.

Daratumumab–Pomalidomide–Dexamethasone

The same trial also evaluated another treatment combination: daratumumab plus pomalidomide–dexamethasone (DPd) (49, 55). Patients in the DPd arm (n = 103) received 28-day cycles of

intravenously daratumumab 16 mg/kg (weekly for cycles 1–2, every 2 weeks for cycles 3–6) in combination with pomalidomide 4 mg (on days 1–21) and dexamethasone 40 mg weekly. Among responder patients, ORR was 66%, and the median duration of response was 21.5 months, median PFS was 9.9 months, median OS was 25.1 months with a median follow-up of 28.1 months. Safety profile showed relevant grade >3 AEs as follows: neutropenia (78.6%), anemia (28.2%), thrombocytopenia (19.4%), upper respiratory tract infections (2.9%). MM-0146 is an ongoing, phase 2, non-randomized, multicenter, open-label clinical study evaluating the safety and efficacy of DPd and Pd RRMM patients (N = 112) previously exposed to one or two prior lines of therapy including lenalidomide (56). Patients in the DPd arm will receive 28-day cycles of intravenously daratumumab 16 mg/kg in combination with pomalidomide 4 mg PO daily (days 1–21) and dexamethasone 40 or 20 mg/day, depending on age, on days 1, 8, 15, and 22. Daratumumab was administered on cycles 1–2 weekly, twice weekly for cycles 3–6, and every 4 weeks thereafter. After a median follow-up of 17.2 months, in the ITT population (N = 112), ORR was 77.7%, PFS was not reached. Safety analysis reported that the most common grade 3/4 AEs were neutropenia (62.5%), anemia (17.9%), and pneumonia (13.4%). RRMM patients undergoing a daratumumab-containing regimen are often previously exposed to IMIDs. Therefore, effectiveness of pomalidomide in overcoming IMID resistance could potentially enhanced by daratumumab co-administration, giving a new chance to use also IMID activity on myeloma cells.

Daratumumab–Cyclophosphamide–Dexamethasone

The alkylating agent cyclophosphamide was challenged with daratumumab in different modalities. A phase II clinical trial enrolling 120 patients with RRMM who had received at least one line of prior therapy randomize patients in two arms. In the A arm patients receive daratumumab, weekly low dose of cyclophosphamide, dexamethasone, and pomalidomide (DCdP); in the B arm patients receive DCd and pomalidomide only at progression of disease (57). In the DCdP arm patients were randomized to receive daratumumab 16 mg/kg weekly cycles 1–2, every 2 weeks cycles 3 to 6, monthly on cycle 7 and beyond: dexamethasone 40 mg PO weekly, cyclophosphamide 400 mg PO weekly and pomalidomide 4 mg PO days 1–21 of 28-day cycles. In the DCd arm patients received daratumumab, cyclophosphamide, and dexamethasone at the same dose; pomalidomide was added after proof of disease progression. After a median of 8.2 months, ORR in the DCdP arm was 88.5% compared with 50.8% for DCd arm, and PFS was not reached for the DCdP arm. Incidence of grade 3/4 hematologic toxicities included a high incidence of neutropenia 74 *vs* 30%, and thrombocytopenia was 4.9 and 13.6% in DCdp *vs* DCdP, respectively. Infectious AEs were: febrile neutropenia was 8.2 *vs* 6.8% and pneumonia 18 *vs* 16.9%, respectively. Daratumumab was also evaluated with cyclophosphamide–bortezomib–dexamethasone (DCyBorD) in a small number of patients. LYRA, an ongoing, phase 2, single-arm, open-label, multicenter study evaluates the safety and efficacy of this regimen either for the treatment of MM in patients who have not received previous treatment and for one RRMM of one treatment line (n = 14) (58, 59). Daratumumab was administered at 8 mg/kg intravenously on days 1–2 of cycle 1, then 16 mg/kg weekly in cycle 1 (day 8) and cycle 2, then twice weekly in cycles 3–6, then every 4 weeks in cycles 7–8. Cyclophosphamide was given as 300 mg/m^2 PO weekly on days 1, 8, 15, and 22 of each cycle; bortezomib at 1.5 mg/m^2 subcutaneously weekly on days 1, 8, and 15; dexamethasone: 40 mg weekly. With a median follow-up of 26.6 months, 79% of RMM patients obtained ORR, and median PFS was not reached (60). In RRMM patients, hematological and non-hematological grade >3 relevant reported AEs were: neutropenia (21%) and diarrhea (7%).

Daratumumab–Venetoclax–Dexamethasone

The BCL-2 inhibitor venetoclax, largely adopted in other lymphoproliferative disorders, is on evaluation in a phase 1/2 trial also in patient with RRMM with and without t(11;14) (61). Venetoclax is given in combination with daratumumab and dexamethasone with or without bortezomib (VenDd or VenDVd) and patients (n = 48) are divided in two cohorts of patients, depending on t(11;14) status. With a median follow-up of 10 and 9 months for VenDd and VenDVd respectively, ORR was 96 and 92%, and median PFS was not reached. Grade ≥3 AEs were neutropenia (17%), hypertension (12%), fatigue and hyperglycemia (both 8%) for patients on VenDd, and insomnia (21%), diarrhea and thrombocytopenia (both 8%) for patients on VenDVd. A phase 1/2 study enrolling RRMM patients is designed to administer DVd with or without venetoclax and evaluate MRD rates and the role of t(11;14) as marker of disease (62).

Daratumumab–Nivolumab/Daratumumab–Nivolumab–Cyclophosphamide

Anti-PD1 nivolumab is another molecule with a promising anti-myeloma activity, as shown by two ongoing trials. CA209-755, an ongoing phase 2, randomized, multicenter study, is expected to enroll 60 patients with RRMM receiving daratumumab–nivolumab with or without cyclophosphamide (DN *vs* DNc) (63). In a 28-day cycle: daratumumab IV weekly is given 16 mg/kg for cycles 1–2, then every 2 weeks for cycles 3–6, then every 4 weeks thereafter; nivolumab 240 mg IV every 2 weeks in cycles 1–6 and 480 mg weekly subsequently. When added, cyclophosphamide was given 50 mg orally once daily on days 1–28. A total of 40 patients were randomized in two consecutive phases and after a median follow-up of 8.6 months: ORR (>SD) was 85 and 80% for DN and DNc, respectively. Most relevant toxicity was infections. CA209-039 is another phase 1/2 ongoing trial investigating the role of nivolumab in several hematological neoplasm, RRMM included, as monotherapy or in combination regimens across various associations (64). Patients with RRMM are being assigned to one of the following arms: daratumumab + nivolumab or daratumumab + nivolumab + pomalidomide and dexamethasone. The aim of the trial is to evaluate the safety of these combinations. A limitation of this trial is that nivolumab is not challenged with another agent that is commonly adopted in

combination in clinical practice (bortezomib, lenalidomide), but only with cyclophosphamide.

Daratumumab–Durvalumab

The human monoclonal antibody anti-PD-L1 durvalumab, already adopted in lung neoplasm, is currently being tested in MEDI4736-MM-003, a safety and efficacy trial of daratumumab IV when administered in combination with daratumumab (DD) for the treatment of RRMM (65). The study will also conduct a preliminary analysis of the addition of pomalidomide and low-dose dexamethasone to DD either in patients with progressive disease with DD or as upfront therapy. Daratumumab is also under evaluation with another humanized monoclonal antibody anti-PD-L1, atezolizumab, in GO29695 trial (66, 67). This phase 1b, open-label, non-randomized, multicenter study is expected to enroll approximately 300 patients exposed to different drug combinations. Three arms are planned: daratumumab–atezolizumab (DA) alone (explored in a run-in and expansion phases), DA–lenalidomide, DA–pomalidomide. In a 28-day cycle, daratumumab and atezolizumab are administered intravenously at 16 mg/kg and 840 mg, respectively, lenalidomide and pomalidomide at different dosages. A total of 24 patients were enrolled in the study and treated; ORR was 67% in the DA (run-in phase) cohort, 57% in the DA + lenalidomide (dose escalation) cohort, and 67% (n = 4) in the DA + pomalidomide (dose escalation phase) cohort. Regarding AEs, grades 3–4 occurred in 33% of patients in the DA (run-in phase) cohort, 75% in the DA (expansion phase), 86% in the DA + lenalidomide (dose escalation phase) cohort and 100% of DA + pomalidomide (dose escalation phase).

Daratumumab–Ixazomib

Finally, the new generation oral PI ixazomib was evaluated with daratumumab and dexamethasone as interim efficacy analysis of the phase 2 trial, without published results at the moment (68).

TOXICITY PROFILE

Daratumumab generally shows a favorable toxicity profile with easily manageable AEs. Being part of the anti-myeloma monoclonal antibody class, daratumumab mostly shows AE and a toxicity profile commonly found in this category of compounds (es. elotuzumab). In clinical practice, a relevant topic when using daratumumab, and generally monoclonal Abs, is the infusion-related reactions (IRRs). In the SIRIUS trial, single agent daratumumab had a 45% IRR rate, represented by respiratory symptoms, such as nasal congestion, rhinitis cough, throat irritation, and dyspnea, mostly grades 1–2 (40). IRRs are characterized by a typical onset timing: they usually occur with maximum incidence at first infusion (96%) or, at least, at the second one, but with lower incidence (7%). The same IRR rate and timing of onset is found also when daratumumab is combined with other anti-myeloma agents. In the CASTOR trial, DVd treatment is associated with an IRR rate of 45%, with almost all events occurring during the first administration (47). Moreover, in the POLLUX trial, a 48% of IRRs were reported for daratumumab when infused as combined regimen DRd, a 50% in MMY1001 when daratumumab is administered as DPd (42, 69). Overall, IRRs are easily both preventable and manageable with adequate pre- and post-medications as antihistamines, corticosteroids, montelukast acetaminophen as well as interrupting and slowing down the infusion rate of daratumumab (70). Minimizing the possibility of IRR by a slow rate of intravenous infusion of daratumumab is routinely adopted but is also time-consuming: 7 h at first–second administration to 3 h subsequently. A way to possibly reduce the IRR rate together with a faster administration modality is subcutaneous injection, as it was explored by COLUMBA trial (71). This multicenter, open-label, non-inferior, randomized, phase 3 trial showed that in RRMM patients, a 1,800 mg subcutaneous flat dose of daratumumab delivered in 5 min is not inferior in terms of efficacy compared to the intravenous route. With the limitation of a non-blinded trial (for both patients and clinicians), grade 3 IRR occurred in 2% of patients, and no grade 4 or 5 IRRs in the subcutaneous group were reported. As reported about the safety profiles of the trials cited in this review, other common ADRs are mostly hematological or related to hematological toxicity such as anemia, neutropenia, thrombocytopenia, fatigue, pyrexia, pneumonia, and upper respiratory tract infections. It is predictable and intuitable that these types of AEs are notably influenced by the daratumumab-associated anti-myeloma agent in a certain regimen adopted. A combined analysis of five phase III randomized controlled trials showed that relative risk of all grades of neutropenia and leukopenia in patients undergoing daratumumab-based regimens was higher than that in the control arms despite lower RR of anemia (72). Finally, not properly definable as toxicity or AD, daratumumab can affect the indirect Coombs test when performed as blood group compatibility test, due to the expression of its target CD38 on red blood cells (73).

CONCLUSIONS

The effectiveness and the favorable toxicity profile of daratumumab for the treatment of both NDMM and RRMM have led to a wide spreading of the use of this new immunotherapeutic agent alone and in combination with standard of care anti-MM treatment. Emerging data from clinical trials are crucial to define newer possible treatment combination since combination treatments involving molecules with different therapeutic target on myeloma cells. The improvement rates of CR with the adoption of novel drugs are nowadays to be considered as a chronic disease relapse eventually appears along the clinical history of almost each patient. Simultaneously, the disease refractoriness to a specific class of drug is a concerning issue for clinicians. The advent of daratumumab, anti-CD38 antibody, gave to physicians one more effective molecule to treat this through the phase of the clinical course of MM. The toxicity profile of daratumumab is also favorable and easily manageable by clinicians. Ongoing trials are giving the

opportunity of exploring its effectiveness also combined with other mechanism of actions, such as cyclophosphamide, venetoclax, and molecules acting on the PD-1 pathway. Given the effectiveness of daratumumab combination and its safety profile still adopted in clinical practice, efforts are mandatory to conduct these (and future) trials to explore other combinations.

AUTHOR CONTRIBUTIONS

Conceptualization, methodology, design, and writing: MO, LC, SM, DN, GM, AO, and CC. Supervision: MO, GM, AO, and CC. All authors contributed to the article and approved the submitted version.

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Daratumumab as Single Agent in Relapsed/Refractory Myeloma Patients: A Retrospective Real-Life Survey

Uros Markovic[1,2], Alessandra Romano[1,3*], Vittorio Del Fabro[2], Claudia Bellofiore[1,2], Anna Bulla[1,2], Marina Silvia Parisi[2], Salvatore Leotta[2], Massimo Gentile[4], Clotilde Cangialosi[5], Iolanda Vincelli[6], Giuseppe Mineo[7], Marco Rossi[8], Massimo Poidomani[9], Giuseppina Uccello[10], Cinzia Maugeri[2,11], Donato Mannina[12], Vanessa Innao[13], Francesco Di Raimondo[1,2,3] and Concetta Conticello[2*]*

[1] Postgraduate School of Hematology, University of Catania, Catania, Italy, [2] Division of Hematology, University Hospital Policlinico Vittorio Emanuele, Catania, Italy, [3] Department of General Surgery and Medical-Surgical Specialties, University of Catania, Catania, Italy, [4] Unit of Clinical Hematology, Cosenza Hospital, Cosenza, Italy, [5] Unitá Operativa Complessa Ematologia, Azienda Ospedaliera Ospedali Riuniti Villa Sofia Cervello, Palermo, Italy, [6] Unità Operativa Complessa di Ematologia, Grande Ospedale Metropolitano Bianchi Melacrino Morelli, Reggio Calabria, Italy, [7] Unitá Operativa Semplice Dipartimentale Ematologia, Ospedale San Vincenzo, Taormina, Italy, [8] Department of Clinical and Experimental Medicine, Magna Græcia University of Catanzaro, Catanzaro, Italy, [9] Servizio di Immunoematologia e Medicina Trasfusionale, Ematologia ASP Ragusa, Ragusa, Italy, [10] Unità Operativa Complessa Ematologia, Garibaldi Nesima Hospital, Catania, Italy, [11] Division of Hematology, Sant'Elia Hospital, Caltanissetta, Italy, [12] Division of Hematology, Papardo Hospital, Messina, Italy, [13] Division of Hematology, Department of Human Pathology in Adulthood and Childhood, Policlinico "G. Martino", University of Messina, Messina, Italy

Background: The anti-CD38 monoclonal antibody daratumumab is approved as a single agent for the treatment of patients with relapsed/refractory multiple myeloma (RRMM) who received at least three prior lines of therapy, including proteasome inhibitor and immunomodulatory agent. A retrospective multicentric study was designed to evaluate feasibility, tolerability, and efficacy of daratumumab in monotherapy in RRMM.

Methods: This study included 44 consecutive RRMM patients that underwent daratumumab monotherapy after a median number of four prior therapies (range 2–9). Patients were treated in seven Sicilian centers, as part of Sicilian Myeloma Network and three Calabrian centers outside of controlled clinical trials from August 2016 through July 2020.

Results: The regimen was well tolerated with few grade 3–4 haematological and rare non-haematological adverse events, such as pneumonia. Definitive discontinuation was due to disease progression in 25 (57%) patients. Since three patients did not complete at least one full cycle, a total of 41 patients was evaluated for response. Overall response rate was 37%, and the disease control rate (stable disease or better) was high (73%). The best achieved responses within 6 months were very good partial remission or better (27%), partial remission (10%), minimal response (14%) and stable disease (22%). After a median follow up of 7.8 months, median progression free survival (PFS) was 7.2 months and overall survival (OS) 7.8 months. Univariate analysis showed that patients with PR or better

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***Correspondence:**
Uros Markovic
urosmarkovic09041989@gmail.com
Alessandra Romano
sandrina.romano@gmail.com
Concetta Conticello
ettaconticello@gmail.com

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after 6 months of therapy had longer median PFS and OS (respectively 29.5 vs 3.6 months, p=0.0001 and 30.6 vs 3.9 months p=0.0001), confirmed by multivariate analysis. Furthermore, standard cytogenetic risk and biochemical relapse type had prolonged median PFS, but not OS (respectively unreached vs 2.6, p=0.03 and 23.9 vs 6.2, p=0.05) in both univariate and multivariate analysis. Additionally, univariate analysis showed that patients treated with carfilzomib-lenalidomide-dexamethasone prior to daratumumab had significantly shorter PFS compared to pomalidomide-dexamethasone (3.4 months vs 9.3 months, p=0.03), that multivariate analysis failed to confirm.

Conclusions: Our findings indicate that daratumumab as single agent is safe and well-tolerated regimen in real-life, associated to prolonged PFS and OS in responding patients. No new safety signals were identified.

Keywords: multiple myeloma, relapsed/refractory, salvage treatment, immunotherapy, daratumumab

INTRODUCTION

Multiple myeloma (MM) is a chronic plasma cells disease characterized by several relapses that require new treatments. Even in the era of novel agents belonging to different classes of mechanism of actions like pomalidomide, carfilzomib, and ixazomib as single agents or in combination regimens, the treatment response remains highly variable. It has been supposed that the progressively shorter duration and lack of response is probably caused by an increasing use of different drugs and their combinations, with growing drug cross-resistance after each relapse (1). Thus, the disease remains incurable in most cases with a constantly growing number of relapsed and refractory multiple myeloma (RRMM) patients in later lines of therapy. The biggest challenge remains the choice of the most suitable salvage therapy in this setting.

Increase in overall survival (OS) in MM patients along with constant therapy improvement, have brought in evidence a new population of frail patients, that could benefit little from the use of novel agents especially in combination, due to their fitness, medical history, previous drug toxicity, adverse events, relapse type, etc (2).

In this setting, recent randomized trials have shown feasibility, sufficient effectiveness and safety of novel drugs as single agents or in combination with dexamethasone in heavily pre-treated RRMM patients, including pomalidomide (MM-002, MM-003) (3, 4), carfilzomib (CHAMPION-1, ENDEAVOR) (5–7), and daratumumab (GEN-501, SIRIUS) (8, 9). On the other hand, elotuzumab (10) and panobinostat (11) did not demonstrate sufficient efficacy as single agents.

Even with very encouraging results with new drugs, the main difference between randomized studies and real-life experience remains the selection of patients. Subjects followed outside of clinical trials often have several comorbidities like impaired kidney, hepatic or heart function, high performance status (PS) score according to Eastern Cooperative Oncology Group (ECOG) and persistent drug toxicity like peripheral neuropathy, recurrent deep vein thrombosis, reduced bone marrow reservoir, etc. This makes hard to personalize the appropriate therapy in advanced stages, estimating not only the disease aggressiveness, but also the patients' conditions, without data from every-day experience (12).

Daratumumab is a human IgG1 monoclonal antibody that binds CD38-expressing malignant cells with high affinity. It induces tumor cell death through diverse mechanisms of action, including complement-dependent cytotoxicity, antibody-dependent cell-mediated cytotoxicity, antibody-dependent cellular phagocytosis, apoptosis, and to a lesser extent, inhibition of the enzymatic activity of CD38 (13–15). The drug may also target other CD38-expressing immune cells, thereby exerting an immunomodulatory effect relevant not only at diagnosis, but also during subsequent lines of therapy (16, 17). Depletion of regulatory B cells, certain regulatory T cells and myeloid-derived suppressor cells (MDSCs), along with increase of both CD4+ and CD8+, can lead to improved adaptive immune response (18–20).

Monoclonal antibody treatment with daratumumab was available in Calabria for relapsed/refractory multiple myeloma (RRMM) patients since August 2016, and in Sicily in November 2017. The efficacy and safety was evaluated with our real-life experience in heavily pre- treated patients, most of them being unfit and with important comorbidities.

METHODS

Patient Selection

In this real-life retrospective survey, 44 RRMM patients were treated with salvage regimen based on daratumumab single-agent between August 2016 and July 2020 in seven Sicilian centers (part of the Sicilian Myeloma Network) and three Calabrian centers. Database lock was 31st July 2020. The study was approved by an independent ethics committee of the coordinating center (*Policlinico Catania 1*, n.34/2019/PO) and was conducted in accordance with International Conference on Harmonization Guidelines on Good Clinical Practice and the principles of the Declaration of Helsinki. All patients have provided written informed consent to data recording and collection before being treated with daratumumab. Primary

endpoint was the overall response rate (ORR). Secondary endpoints were rate of best responses, time to progression or relapse, progression-free survival, overall survival and safety.

Procedures and Drug Administration

All patients received daratumumab monotherapy according to the schedule of SIRIUS trial: daratumumab (DARA) 16 mg/kg i.v. per week for 8 weeks (cycles 1 and 2), then every 2 weeks for 16 weeks (up to cycle 6), and every 4 weeks thereafter. First infusion was prepared and divided in two 500 ml diluitions of DARA preceded by standard premedication. The first infusion was started at 50 ml/h, followed by dose escalation up to 200 ml/h, in the absence of infusion-related reactions (IRRs) as manufacturer suggestions. Subsequent infusions were diluited in 500 ml and started from 50 ml/h in second infusion or 100 ml/h in subsequent infusions with an increase up to 200 mL/h. According to the SIRIUS trial, treatment was continued until progression.

To prevent IRRs, patients received premedication 1 h prior to administration of daratumumab as follows: methylprednisolone (100 mg i.v. for the first and second infusion, and 60 mg thereafter in the absence of infusion related reactions (IRRs) during the first two infusions), paracetamol (650–1,000 mg) and diphenhydramine (25–50 mg) or equivalent antihistamine drug, according to SIRIUS trial and local guidelines. Oral methylprednisolone (20 mg) or equivalent was administered for two days after all daratumumab infusions. In order to prevent IRRs, in 21 patients the first infusion of DARA on cycle 1, day 1 was given as a split dose in two days.

Treatment was discontinued in cases of disease progression, unacceptable adverse events or consent withdrawal.

Concomitant Medications

Seven patients (16%) received treatment with bisphosphonates every 4 weeks during daratumumab treatment. Antibiotic and antiviral prophylaxis was carried out with trimethoprim and sulfamethoxazole (800 mg + 160 mg twice a day, twice a week) and acyclovir 200, 400, or 800 mg daily, according to the policy of each center. Supportive therapy with erythropoietin (EPO) and granulocyte colony-stimulating factor (G-CSF) was administered according to ASH/ASCO guidelines and policy of each single center (21, 22).

Safety and Efficacy Assessment

Each patient's medical history was recorded on day 1 of each cycle. Physical examinations were conducted, and blood samples were collected for hematology, renal and liver function tests on day 1 of each cycle and whenever it was considered necessary. Adverse events (23) were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) (24).

Efficacy assessment was recorded on day 1 starting from cycle 2 and every cycle thereafter. Response evaluation and progression assessment were reported according to International Myeloma Working Group consensus criteria (25), including complete remission (CR, 100% reduction in M protein according to electrophoresis, with negative immunofixation), very good partial response (VGPR, ≥90% reduction in serum M protein, and less than 100 mg urine M protein per day), partial response (PR, ≥50% reduction in serum M protein, and less than 100 mg urine M protein per day), stable disease (SD), progression disease (PD); not valuable (NV). Minimal response (MR) was defined according to European Society for Blood and Marrow Transplantation criteria (26).

According to IMWG criteria, biochemical relapse was defined as an increase of M protein at least 25% from nadir in serum (absolute increase at least ≥0.5 g/l) and/or urine paraprotein (absolute increase at least ≥200 mg/24 h) in 2 consecutive measurements. A 25% increase in the difference between involved and uninvolved free light chain (FLC) with an abnormal ratio and absolute increase of at least 10 mg/dl was also considered as biochemical relapse. On the other hand, clinical relapse was defined as the presence of at least one of the CRAB criteria, namely hypercalcemia, renal insufficiency, anemia and bone lesions (27).

Statistical Analysis

Descriptive statistics were generated for data analysis and two-sides p-values of 0.05 or less were considered significant. Qualitative results were summarized in counts and percentages. Overall response rate (ORR) was defined as PR or better (CR + VGPR + PR), while disease control (DCR) rate was defined as a response equal or better than stable disease (≥SD).

Descriptive analysis was performed by frequency distribution for continuous variables. Survival analysis were estimated with the Kaplan–Meier method and compared by the log-rank test. The impact of the following factors was evaluated with univariate analysis: age (≤65 years or >65 years), gender, ECOG performance status (<3 or ≥3), number of previous treatment lines (<5 or ≥5), immunoglobulin type (IgG or other), cytogenetic risk (high versus standard risk), previous autologous stem cell transplantation, creatinine clearance level (<60 ml/min versus ≥60 ml/min), baseline hemoglobin level (<10 g/dL versus ≥10 g/dL), baseline lactic acid dehydrogenase level (normal or increased), last treatment line in terms of doublets versus triplets and pomalidomide-dexamethasone versus carfilzomib-lenalidomide-dexamethasone, relapse type (biochemical versus clinical), best response achieved at 6 months of therapy and grade 3/4 hematological adverse events. Cox proportional hazard model was used to assess association between patients, disease characteristics, namely best response achieved at six months, relapse type, last treatment (KRD vs Poma-Dex), and grade 3/4 hematological adverse events, along with progression free survival (PFS); confidence intervals were at 95%. PFS was calculated from the time of daratumumab start until the date of progression, relapse, relapse- related death or date the patient was last known to be in remission. OS was calculated from the start of daratumumab therapy until the date of death for any cause or the date the patient was last known to be alive. PFS and OS were calculated for patients that completed at least one complete 28-day cycle. All calculation were performed using Stat View (CA, USA) and MedCalc version 12.30.0.0 (Producer: MedCalc Software bvba, Ostend (Belgium), www.medcalc.org).

RESULTS

Patients' Characteristics and Treatment

This survey included 44 patients treated with daratumumab as single agent outside of clinical trial, from August 2016 until July 2020, and evaluated according to an intention-treat-analysis; 41 patients received at least 1 complete 28-day cycle and were evaluated for efficacy analysis as well (**Figure 1**).

The baseline demographics are summarized in **Table 1**. The median age was 65 years (range 49–82). All patients had measurable disease due to secreted paraprotein; IgG-heavy chain was present in more than half of cases, while in 6 patients the paraprotein was light-chain only.

At the time of the last relapse, a poor performance status (ECOG score of 3 or more) was present in 14 patients (32%), while three (7%) had impaired renal function (creatinine clearance <30 ml/min), requiring hemodialysis as supportive care. Data on cytogenetic abnormalities, detected by fluorescence in-situ-hybridization (FISH) on highly purified bone marrow plasma cells, were available in 15 patients (34%) at time of relapse, with 6 cases showing a high cytogenetic risk [including del 17p, t(4;14) and t(14;16)].

The median number of prior therapies was four (range 2–9), including 15 (34%) patients who had received five or more. All patients have previously received proteasome inhibitors (PI) and immunomodulatory agents (IMiDs). In most cases, the last regimen received was based on a combination of PI and IMiD (e.g., KRd, 39%) and less frequently on a single novel agent, including pomalidomide (18%), lenalidomide and bortezomib (7% each), or chemotherapy (16%) alone or in associaton with a novel agent. The median time from MM diagnosis to daratumumab monotherapy was 5 years (range 1–22 years). Most patients started immunotherapy suffering from CRAB symptoms at relapse (75%), while only eleven patients were treated for asymptomatic biochemical relapse. Most patients included in the study (75%) were double-refractory to both PIs and IMIDs.

A median number of 6 cycles (range 1–32) per patient was completed; three patients received one incomplete cycle and progressed two died from progression), thus they were excluded

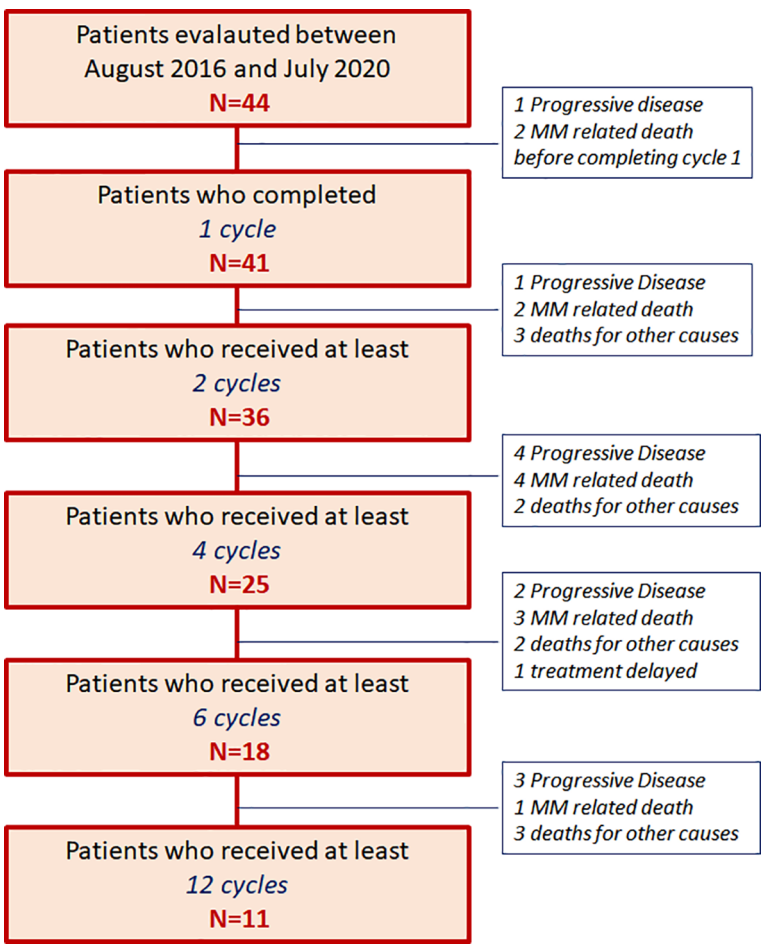

FIGURE 1 | Patients' allocation [44 patients with relapsed/refractory multiple myeloma (RRMM) patients from August 2016 until July 2020].

TABLE 1 | Patients' clinical characteristics in 44 patients with relapsed/refractory multiple myeloma (RRMM) patients treated with daratumumab as single agent [Cytogenetic high risk was defined as the presence of t(4;14), t(14;16) or del17p documented by FISH].

Age	
Median in years (range)	65 (49–82)
< 64 years, N (%)	22 (50)
65–75 years, N (%)	17 (39)
> 75 years, N (%)	5 (11)
Gender	
Male, N (%)	24 (55)
Female, N (%)	20 (45)
Paraprotein (isotype)	
secreting, N (%)	44 (100)
micromolecolar, N (%)	6 (14)
IgG-heavy chain, N (%)	29 (66)
IgA-heavy chain, N (%)	9 (20)
Number of prior therapies	
Median n. of prior therapies, N (range)<5 therapies, N (%)≥5 therapies, N (%)	4 (2–9)29 (66)15 (34)
ECOG (Performance Status at baseline)	
0-2, N (%)	30 (68)
3 or more, N (%)	14 (32)
Risk class at relapse according to IMWG (15 patients)	
High, N (%)	6 (40)
Standard, N (%)	9 (60)
Creatinine clearance	
≥60 ml/min, N (%)	25 (57)
<60 ml/min, N (%)	19 (43)
Dialysis	3 (7)
Double refractory MM patients (PIs and IMIDs)	
Yes, N (%)	33 (75%)11 (25%)
No, N (%)	
Extramedullary lesions	
Yes, N (%)	2 (5)
No, N (%)	42 (95)
Relapse type	
Biochemical	11 (25)
Clinical (CRAB)	33 (75)

In bold: ECOG Performance Status, Eastern Cooperative Oncology Group Performance Status; IMWG, International Myeloma Working Group; MM, Multiple Myeloma; PIs, Proteasome Inibitors; IMIDs, Immunomodulatory drugs.

from further analysis of efficacy. In one patient daratumumab administration was delayed and reduced due to toxicity and in eight (18%) patients treatment was delayed due to adverse events (in one case not yet recovered), but no definitive discontinuation was recorded.

After a median follow-up of 7.9 months (range 1.1–34.3 months), 12 (27%) patients are still in treatment (11 of them received at least 12 cycle), 10 (23%) patients progressed and shifted to further salvage regimen, 22 (50%) patients died, 12 (27%) for MM progression, and 10 (23%) for other causes: two patients died from myocardial infarction, one from stroke, one sudden intestinal bleeding, one case of pneumonia, two cardiac arrest, three patients from unknown causes, as shown in the patients' allocation diagram in **Figure 1**.

Safety

Daratumumab was relatively well tolerated (**Table 1**, **Supplementary Materials**). IRRs were observed in 12 patients (27%), with two of them having more than one episode. In half of these episodes (six patients, 13.5%) grading was 1–2, including short breathness and pruritus, and was safely managed with appropriate supportive care. Severe infusion-related reactions (grade 3 or higher) occurred in remaining patients (six patients, 13.5%) and required temporary suspension (three patients, 7%) or delayed administration (one patient, 2%). All 21 patients who received the first dose split in 2 days, completed the drug infusion as planned without IRRs. Grade 3 or 4 hematological AEs occurred in 14 patients (32%). The most common grade 3–4 hematological AE was anemia, present in 10 patients, and associated with thrombocytopenia in four of them. Nine patients required red blood cell transfusion (20%), and in three (7%) platelet transfusion support was performed. None of the patients developed severe neutropenia, whereas grade 1–2 neutropenia occurred in seven patients (16%). Supportive care with growth factors such as EPO or G-CSF (filgrastim 30 MU) was required in 19 (43%) and seven (16%) patients, respectively, all of them with reduced bone marrow reserve. As for non-hematological AEs, infectious complications were recorded as follows: pneumonia in four patients (9%), severe in two of them (4.5%) requiring hospitalization, fever in four patients, while diarrhea and reactivation of varicella virus (the patient did not assume antiviral prophylaxis) were present in one patient each, respectively. Grade 3 adverse events, both hematological and pneumonia, occurred in the first three months of the treatment, and were recorded only in the patients that did not achieve at least PR ("non responders"). One patient had an atrial fibrillation episode during treatment. No patient undergoing concomitant antibiotic and antiviral prophylaxis had Herpes zoster reactivation or suffered from Pneumocysitis jirovecii related pneumonia.

Efficacy

Forty-one patients that completed at least one 28-day cycle were evaluated for response (**Table 2**, **Supplementary Materials**). The ORR was 37%, while the disease control rate was high (73%). The best- achieved responses were VGPR+CR in 27%, while 10% attained partial response. In 11 (25%) patients treatment is still ongoing, seven of which achieved at least PR. Median duration of response (DOR) in patients who obtained at least PR (N=15) was 16 (range 4.1–32.3) months, significantly longer than in those not achieving this level of response (N=26), which was 11.5 (range 1–32) months (p=0.04).

In the whole cohort, median PFS was 7.2 months (CI 95% 3.6–29.5) and median OS 7.8 months (CI 95% 3.9–34.3). Univariate analysis showed that patients with PR or better after 6 months of therapy ("responders") had a prolonged median PFS (range 29.5 vs 3.6 months, p=0.0001) and OS (30.6 vs 3.9 months, p<0.0001) compared to the "non responders", regardless of the depth of response (**Figure 2**, **Table 3**, **Supplementary Materials**). Both PFS and OS were not affected by age, gender, monoclonal protein type, previous autologous stem cell transplantation, number of prior lines of treatment, baseline LDH, ECOG, creatinine clearance, and last therapy (doublet versus triplet) prior to daratumumab. Standard cytogenetic risk, biochemical relapse type and a previous treatment with pomalidomide-dexamethasone (Poma-Dex),

compared to carfilzomib-lenalidomide- dexamethasone (KRd, **Figure 3**), were associated with prolonged median PFS, but not OS (respectively unreached vs 2.6, p=0.03, 23.9 vs 6.2, p=0.05 and 9.3 vs 3.4 months, p=0.03). In addition, despite no differences in PFS, patients who had baseline hemoglobin levels lower than 10 g/dL before daratumumab, had shorter median OS (respectively 5.6 vs 9.5 months, p=0.05) (**Table 3**, **Supplementary Materials**). Most patients with low baseline hemoglobin level did not respond to daratumumab (22 out of 29 patients, 75%). On the other hand, four "responders" with low hemoglobin level eventually recovered along with treatment response in the first 3 months of daratumumab. Patients who experienced hematological adverse events grade 3 or more had inferior PFS, than those who did not (respectively 3.7 vs 9.3 months, p=0.03), without significant difference in OS.

In frail patients, with performance status (PS) ECOG equal or more than 3, both median PFS and OS were shorter compared to patients with PS-ECOG 0-2 (respectively, 4.1 -95% CI 2.7–12.1- versus 7.6 -95% CI 3.7–29.5-, p=0.21 and 4.1 -95% CI 4.7–8.1- versus 9.5 months -95% CI 3.9–30.6-, p=0.09, data not shown).

In multivariate analysis for PFS the following covariates were included: cytogenetic risk, best response achieved at 6 months, relapse type, last treatment (KRD vs Poma-Dex) and grade 3/4 hematological adverse events (**Table 4**, **Supplementary Materials**). High risk cytogenetics and previous treatment with KRd regimen were indipendently associated to shorter PFS (HR respectively 19.2, 95% CI 1.6–233.4, p=0.02 and 15.9, 95% CI 1.6–155.5, p=0.018).

In multivariate analysis for OS, we found that achievement of at least partial remission after 6 months of therapy was associated to longer OS at 24 months.

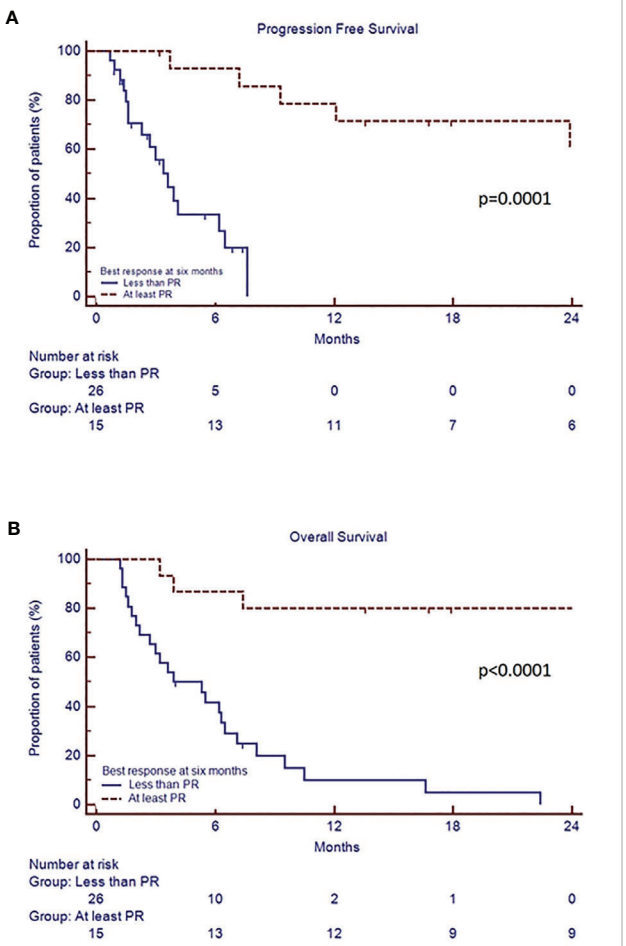

FIGURE 2 | Progression free survival **(A)** and overall survival **(B)** according to best response achieved by 6th cycle.

DISCUSSION

About five years ago, the introduction of daratumumab as single agent was associated with highly positive results in terms of ORR and median time to response in heavily pre-treated MM patients, with limited toxicities. The accelerated approval of anti-CD38 immunotherapy after phase II clinical trial (9) by both the FDA in November 2015 and EMA in May 2016, opened a new chapter in RRMM management. Soon, the triplet combination with either bortezomib (28) or lenalidomide (29) and dexamethasone demonstrated improved efficacy and response duration, therefore quickly extending the use of daratumumabin combination by FDA in November 2016 and EMA in April 2017. Since then, the broad use of combination therapy has greatly improved both the PFS and OS of RRMM patients, but the use of daratumumab in monotherapy was limited. Therefore, the experience of daratumumab monotherapy in real-life with the exact mechanism of efficacy in population of "responders" (PR or better) is still unknown. In this perspective, retrospective studies outside of clinical trials could help define the population of patients who can benefit from monotherapy with daratumumab, especially in multi-refractory patients with important comorbidities, who are not eligible for combination therapies.

In the drug-approval studies GEN501 and SIRIUS, a total of 148 heavily pretreated patients received daratumumab 16 mg/kg, with a median follow-up of 20.7 months (range, 0.5–27.1 months), as shown in **Table 5** in **Supplementary Materials**. Patients had received a median of 5 prior therapies (range 2–14) and 86.5% of patients were double refractory to both a PI and an IMID. The ORR was 31%, with 14% achieving VGPR or better. PFS was 3.4 months (range, 0.03–26.0 months), rising up to 15 months in responding patients with at least PR (30). Although controlled clinical trials aided greatly in improving the experience of drug mechanism and efficacy, patient selection was limited on the basis of age and comorbidities such as severe renal impairment or performance status, in comparison to real-life population.

On the other hand, real-life studies on daratumumab evaluated the efficacy and tolerability in overall population, thus elaborating the drug's every-day use. Even though

different population studies had significant variation in patient size, double-refractory status and follow-up (31–35), ORR and PFS described in the majority of real-life studies were at least equal, if not superior compared to clinical trials (**Table 5**, **Supplementary Materials**) (30).

In this real-life study we retrospectively evaluated the efficacy and tolerability of daratumumab as single agent in 44 RRMM patients from seven Sicilian centers (belonging to the Sicilian Myeloma Network, SMN) and three Calabrian centers from August 2016 until July 2020, outside of controlled clinical trials. The present study population represents the largest real-life cohort of patients on daratumumab monotherapy. Compared to other studies, the ORR was comparable, if not superior (37%, of which VGPR or better in 27% of patients), together with DCR (73%) and PFS (7.2 months) (**Table 5**, **Supplementary Materials**). Interestingly, patients who achieved at least PR by six months ("responders"), regardless of the depth of response, had a significantly prolonged both PFS and OS.

It is known that CD38 is highly expressed on myeloma cells (36), but it is also present on MDSC (myeloid-derived suppressor cells), Treg (regulatory T cell) and regulatory B cells (18). The presence of myeloma cells in the bone marrow causes important modulation of the environment, leading to immune escape through MDSC and Treg immune suppression (37), with NK and T cell immune dysfunction (38). Daratumumab exhibits lytic activity versus myeloma cells through different immunologic mechanisms: antibody-dependent cellular cytotoxicity (ADCC), complement- dependent cytoxicity (CDC), induction of apoptosis through Fc-mediated cross linking and antibody-dependent cellular phagocytosis (ADCP) (13, 14). However, additional immunomodulatory mechanism has been demonstrated through decrease of CD38 positive immunosuppressive regulatory cells, following an increase in both the effector T cell population and T cell receptor clonality.18 Immunomodulatory functions of daratumumab are complex and probably have a continuous influence on bone marrow microenviroment where myeloma cells find their niche, supporting the role of continuative daratumumab treatment. Also in our experience, there is a significant fraction of RRMM patients who are still benefiting of long-term exposure to DARA.

Given the small number of patients we have not found any disease or patient's characteristic that brings together all these patients. Our data suggest that DARA could be of benefit in patients refractory to pomalidomide, thus aiding in the optimal sequential strategy for RRMM. It can be hypothesized that the immunomodulatory mechanism of IMIDs could help improve the efficacy of daratumumab priming MM cytotoxicity through loss of Ikaros and Aiolos (39). Also, the contrasting influence of PIs and IMIDs on MDSCs in MM microenviroment could further explain inferior response to DARA monotherapy in KRd refractory patients (17). This subgroup analysis represents a novelty among real-life observations in DARA exposed patients. In general, evaluation of the impact of the previous treatment explored in the real-life setting is emerging as a powerful tool to optimize the sequential treatment in MM (40).

The International Myeloma Working Group (IMWG) has discussed previously about the importance of cytogenetic abonormalities by FISH and high risk cytogenetic abnormalities, namely t(4;14), t(14;16) and del(17p), already included in revised International Staging System (R- ISS) (41, 42). Therefore, FISH analysis prior to change in treatment strategy represents a clinically relevant prognostic factor. In the present real-life study cohort even though only a third of subjects had cytogenetic risk status prior to daratumumab, the advantage in terms of PFS was demonstrated by both univariate and multivariate analysis, confirminig the negative prognostic impact of high-risk cytogenetic abnormalities and the opportunity to perform FISH analysis even in relapsed/refractory MM patients.

The importance of early biochemical relapse detection, compared to clinical relapse with end- organ damage in improving subsequent survival and quality of life, has already been described (43), also by our group (Markovic, EHA 2020, abstract n. EP1001). As for our retrospective study, univariate analysis demonstrated statistically significant advantage in terms of PFS in patients treated with daratumumab early at biochemical relapse compared to the clinical, although the advantage was not confirmed in terms of OS and multivariate analysis.

As for the drug's safety profile, therapy was well tolerated with less than one third of cohort with grade 3-4 hematological (anemia, platelet reduction) and around one fifth having non-hematological AEs, that were in line with the results of GEN501 and SIRIUS (8, 9). Regarding the infectious AEs, compared to other real-life studies, the incidence of grade 3–4 events was lower (31–35). We can hypothesize that the use of antiviral and antibiotic prophylaxis, together with on demand G-CSF supportive therapy could have been of aid in reducing the incidence of infectious complications, similar to our previous real-life experience with Poma-Dex (44) and KRd regimen (40). The presence of grade 3/4 hematological adverse events was also significant in terms of PFS in univariate analysis. However, due to their presence only in "non-responding" patients (less than PR), the benefit was not confirmed in multivariate analysis. It can be presumed that the lack of response led to increased bone marrow failure, thus contributing to hematological toxicity. On the other hand, all "responding" patients (PR or better) resolved their baseline low hemoglobin level (less than 10 g/dL) in the first three months of daratumumab, as mentioned before, thus confirming the importance of tumor burden. Therapy was delayed due to hematological AEs in only one patient, whereas definitive discontinuation in our series was due to disease progression and MM related death. Despite low numbers that could not allow us to understand how performance status at baseline could affect clinical outcome, treatment was also tolerated well in compromised patients with renal insufficiency and PS-ECOG grade 3 or higher than 3, making daratumumab single agent a suitable treatment also in this subset of patients.

The limitations of the study include retrospective observational study design, together with a limited follow-up time. Furthermore, cytogenetic analysis was available in relatively small proportion of patients.

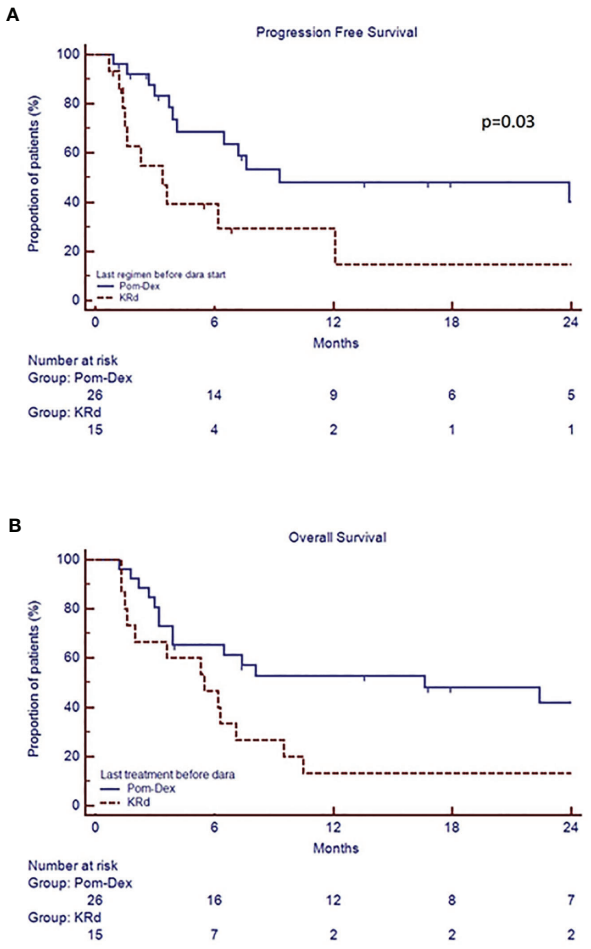

FIGURE 3 | Progression free survival **(A)** and overall survival **(B)** according the type of last therapy (KRd versus Poma-Dex).

CONCLUSIONS

Our findings indicate that daratumumab as single agent is a safe and well-tolerated regimen in real-life, associated to prolonged PFS and OS in responding patients. No new safety signal was identified. Our real-life results confirmed the efficacy of single-agent daratumumab in advanced patients with RRMM in comparison with data from clinical trials. Achievement of PR within the first six cycles is associated to longer PFS and OS.

Taken together, our data suggest that RRMM patients with standard risk cytogenetics and previous exposure to pomalidomide could have large benefit from long-term exposure to daratumumab.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Policlinico Catania 1, n.34/2019/PO. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors have made substantial contributions to all of the following: Project administration: CC. Methodology: AR. UM: interpreted the data and drafted the final article. CC, AR, UM, VDF, CB, AB, MP, SL, MG, CCa, IV, GM, MR, MP, GU, CM, DM, and VI: selected patients, acquired, analyzed, and interpreted the data. CC, AR, and FR: revised the article for important intellectual content and approved the final version for submission. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.624405/full#supplementary-material

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Pegylated Liposomal Doxorubicin in Vindesine-Based and Bortezomib-Based Regimens for Patients With Newly Diagnosed Multiple Myeloma: A Retrospective Study of Efficacy and Safety

Yujia Zhai[1,2], *Dai Yuan*[1,2,3,4,5], *Xueling Ge*[1,2,3,4,5], *Shunfeng Hu*[1,2], *Peipei Li*[1,2,3,4,5], *Xiaosheng Fang*[1,2,3,4,5], *Ying Li*[1,2,3,4,5,6], *Xiangxiang Zhou*[1,2,3,4,5,6*] and *Xin Wang*[1,2,3,4,5,6*]

[1] Department of Hematology, Shandong Provincial Hospital, Cheeloo College of Medicine, Shandong University, Jinan, China, [2] School of Medicine, Shandong University, Jinan, China, [3] Department of Hematology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, China, [4] Shandong Provincial Engineering Research Center of Lymphoma, Jinan, China, [5] Branch of National Clinical Research Center for Hematologic Diseases, Jinan, China, [6] National Clinical Research Center for Hematologic Diseases, The First Affiliated Hospital of Soochow University, Suzhou, China

Purpose: Although pegylated liposomal doxorubicin (PLD) has been approved in combination with bortezomib for relapsed/refractory multiple myeloma (MM), the antitumor efficacy and tolerability of PLD in different regimens for patients with newly diagnosed MM (NDMM) have not been fully defined.

Methods: A total of 249 NDMM patients diagnosed between January 2008 and October 2019 were included in this retrospective study. Among them, 112 patients received vindesine-based chemotherapy (35 vDD and 77 vAD) and 137 received bortezomib-based chemotherapy (58 VDD and 79 VD).

Results: In bortezomib-containing regimens, the complete response rate (48.3 *vs.* 30.4%, p = 0.033) and very good partial response or better rate (74.1 *vs*. 57.0%, p = 0.038) of VDD were significantly higher than those of VD subgroup. While no superior survival was found between VDD and VD subgroup. In vindesine-containing regimens, no statistical significance was identified between vDD and vAD in terms of response rate and survival. The occurrence rates of all cardiac AEs were similar between VDD and VD.

Conclusions: The vDD regimen was similar with vAD in the aspect of response rate, survival, and toxicity in NDMM patients. The addition of PLD to VD brought deeper response without increased toxicity, while no superior survival was found.

Keywords: pegylated liposomal doxorubicin, multiple myeloma, efficacy, survival, toxicity

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Dinesh Pendharkar, Asian Cancer Institute, India

***Correspondence:**
Xin Wang
xinw007@126.com
Xiangxiang Zhou
xiangxiangzhou@sdu.edu.cn

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INTRODUCTION

Multiple myeloma (MM) is a malignant tumor that ranks second among all hematological tumors worldwide (1). It is characteristic of abnormal proliferation of bone marrow plasma cells, production of clonal immunoglobulin, and destruction of the bones (2). Chemotherapy is the main therapeutic strategy for MM. The conventional first-line chemotherapy mostly uses anthracycline containing doxorubicin, which has a certain effect and less damage to stem cells, but the side effects of conventional anthracycline are obvious (3). With the advances in cytogenetic investigations, various chemotherapy regimens based on novel drugs are emerging, which are expected to improve the prognosis of MM patients.

Pegylated liposomal doxorubicin (PLD) is a liposomal form of doxorubicin, with doxorubicin packaged in liposomes with surface-bound methoxypolyethyleneglycol in the course of pegylation (4). It has a pharmacokinetic feature characterized as longer circulation time and diminished volume of distribution to promote tumor uptake (5). On one hand, interactions between diverse circulating plasma components and the liposome surface are decreased by the hydrophilic coating of PLD formulation, thus blocking the uptake of circulating liposomes mediated by reticuloendothelial system. This allows circulating liposomes to better reach tumors which have increased vascular permeability (4). On the other hand, PLD has a particle size window of 20–200 nm, which seems to be the best opportunity to take advantage of the difference in permeability between normal and tumor vessels (6).

Clinical studies have been carried out using PLD in relapsed/refractory multiple myeloma (RRMM). PLD has been approved in combination with bortezomib for RRMM in many countries (7). In patients with RRMM, although PLD and bortezomib combination did not improve the overall survival (OS) in long-term follow-up compared to bortezomib alone (8), the results from the interim analysis showed that PLD and bortezomib significantly reduced the risk of disease progression by 45% and prolonged the median time of progression by 3 months (9). However, in Asian countries like Japan, the tolerability of dose levels which were approved in many other countries of PLD and bortezomib combination was not confirmed in RRMM patients (10). This combination treatment was prematurely discontinued in all three Japanese patients with RRMM in a phase I study due to adverse events (AEs) including Grade 3 bronchiolitis, Grade 3 peripheral sensory neuropathy, and Grade 2 stomatitis with all achieved partial response (PR). A retrospective study of 28 patients with RRMM showed PLD, bortezomib, and intravenous dexamethasone (DVD) appeared to represent a well-tolerated regimen, with only six patients (21%) showed aggravation of their baseline peripheral neuropathy (PN) and a high overall response rate (ORR) of 61%, which included one (4%) complete response (CR), three (11%) very good partial responses (VGPR), eight (29%) PR, and five (18%) minimal responses (11). In addition, DVD combination was safe and effective in elderly patients of a median age of 75 years with RRMM, with the ORR of 80% (20/25) and progression-free survival (PFS) of 8 months (12).

However, in terms of patients with newly diagnosed MM (NDMM), the antitumor efficacy and tolerability of PLD in different chemotherapy regimens have not been fully defined yet. In traditional vincristine combination regimens, compared with VAd (vincristine + doxorubicin + dexamethasone), DVd (PLD + vincristine + dexamethasone) was related to significantly less toxicity like Grade 3/4 neutropenia, a lower occurrence rate of sepsis, and less supportive care like antibiotic use, while similar efficacy, as objective response rates, PFS, and OS were similar (3). In contrary to RRMM, PLD + bortezomib therapy in a phase II study for NDMM patients did not meet the near CR/CR rate specified in the protocol, which was 7% out of 61 patients, and was associated with increased AEs in older patients (13). However, the three drug regimen VDD (bortezomib + PLD + dexamethasone) in patients with NDMM revealed well tolerance and high efficacy for induction treatment followed by HSCT in appropriate MM patients (14).

In this study, we investigated the efficacy and safety of PLD in different combination therapies based on vindesine or bortezomib in NDMM patients.

PATIENTS AND METHODS

Patients

Patients with NDMM who received at least one cycle of chemotherapy, including PLD (PLD + vindesine + dexamethasone and PLD + bortezomib + dexamethasone) or excluding PLD (epirubicin + vindesine + dexamethasone and bortezomib + dexamethasone), between January 2008 and October 2019 in Shandong Provincial Hospital affiliated to Shandong University (SPHASU) were eligible in this retrospective analysis. The inclusion criteria were as follows: 1) newly diagnosed with symptomatic MM based on International Myeloma Working Group (IWMG) criteria (15); 2) previously untreated patients; 3) complete clinical data available for basic information as well as assessment of response and survival; 4) without clinically cardiac insufficiency (New York Heart Association Class II or greater); 5) without previous or concomitant tumor. These patients were identified through the hospital discharge registry system and electronic medical records. This study was approved by the Medical Ethical Committee of Shandong Provincial Hospital affiliated to Shandong University. All data of the recruited patients were obtained with written informed consent in accordance with the Declaration of Helsinki.

Study Design and Treatment

This single-center, retrospective study investigated the efficacy and safety of PLD in vindesine-based regimens and bortezomib-based regimens as initial treatment for NDMM. The primary objective was CR which was assessed after every cycle of chemotherapy and before HSCT. CR was defined as: immunofixation electrophoresis (IFE) in serum and urine was negative; there was no soft tissue plasmacytoma; and the proportion of bone marrow plasmacytoma was less than 5% (16).

The chemotherapy regimens of included NDMM patients mainly consisted of vindesine-based regimens and bortezomib-based regimens, each of which was with or without PLD. The vindesine-based regimens contained vDD (PLD + vindesine + dexamethasone) and vAD (epirubicin + vindesine + dexamethasone). Patients chose vindesine-based regimens or bortezomib-based regimens mainly for economic reasons. After bortezomib entering China's medical insurance system in 2017, most patients could use bortezomib regimens without economic pressure. The vDD regimen consisted of PLD 40 mg/m^2 intravenously (IV) over 1 h on Day 1, as well as vindesine 1 mg/day and dexamethasone 20 mg/day orally on Days 1–4 of each 28-day cycle. The vAD regimen contained vindesine 1 mg/day and epirubicin 10 mg/day IV on Days 1–4 with dexamethasone 20 mg/day orally on Days 1–4 of each 28-day cycle. The bortezomib-based regimens contained VDD (PLD + bortezomib + dexamethasone) and VD (bortezomib + dexamethasone). The VD regimen was composed of bortezomib 1.3mg/m^2 IV on Days 1, 4, 8, 11 and dexamethasone 20 mg/day orally on Days 1, 2, 4, 5, 8, 9, 11, 12 of every 21-day cycle. The VDD regimen consisted of the same VD regimen with PLD 40 mg/m^2 IV on Day 1 of each 21-day cycle.

All patients' subsequent therapies were not limited. After those who completed four to six cycles of the enrolled initial treatment, eligible patients ≤65 years without severe organ dysfunction were offered the opportunity to HSCT. HSCT was not widely used until 2016 limited by transplantation technologies and conditions. Similarly, eligible patients chose HSCT or not as for their own wishes and economic reasons. Those who refused or were not eligible for HSCT progressed to thalidomide, cyclophosphamide, lenalidomide, melphalan, ixazomib, and even cross-group therapies. Genetic abnormalities including gain (1q21), t (4;14), del (17p13) and del (13q14) were detected by fluorescence *in situ* hybridization (FISH).

Study Assessments

Efficacy

We compared the treatment response and the survival time in vindesine regimens (vDD *vs*. vAD) and bortezomib regimens (VDD *vs*. VD), respectively. Response to the treatment was assessed according to the IMWG consensus criteria for response (16). The treatment response was assessed after every cycle during induction chemotherapy and before HSCT and the follow-up was conducted every 3 months during consolidation and maintenance treatment. PFS referred to the time from the beginning of treatment to disease progression or death for any cause. The definition of OS was the time from the beginning of treatment to death for any cause.

Safety

Safety assessment included AE monitoring, vital signs, physical examination, and clinical laboratory tests. All AEs were evaluated at each visit and graded based on the National Cancer Institute Common Terminology Criteria of Adverse Events (NCI-CTCAE), version 5.0.

Statistical Analyses

Chi-square test or Fisher exact test was employed for categorical variables. The Kaplan–Meier method was employed to estimate the survival analysis. The log-rank test was used to calculate the PFS and OS. Statistical analyses were performed by SPSS software for Windows Version 25.0 (SPSS Inc, Chicago, IL, USA). P-value of <0.05 was considered statistically significant.

RESULTS

Clinical Characteristics of Patients

A total of 410 NDMM patients was presented in SPHASU between January 2008 and October 2019. Among them, 309 NDMM patients received at least one cycle of chemotherapy, including vindesine-based regimens (vDD and vAD) and bortezomib-based regimens (VDD and VD). Based on the inclusion criteria, 249 patients were finally included in this study. We excluded patients whose clinical information data were incomplete for assessment, those with clinically cardiac insufficiency (New York Heart Association Class II or greater) and those with previous or concomitant tumor (**Figure 1**). The median age at diagnosis was 58 years for vAD, 59 years for vDD and VD, and 56 years for VDD subgroup, respectively. 46 (59.8%) patients in vAD subgroup, 27 (77.1%) in vDD subgroup, 70 (88.6%) in VD subgroup and 35 (60.3%) in VDD subgroup were male. Among all, 112 patients received vindesine-based regimens, including 35 in vDD (with PLD) and 77 in the vAD subgroup (without PLD). The median number of treatment cycles patients received was three (range, 1–11). Four patients of vDD (11.4%) and six patients of vAD subgroup (7.8%) received HSCT, respectively. 137 of the included patients were treated with bortezomib-based regimens, 58 with PLD (VDD regimen) and 79 without PLD (VD regimen). The median number of treatment cycles received was four (range, 1–8) in VDD and three (range, 1–11) in the VD subgroup. Among these two subgroups, 23 (39.7%) of the VDD subgroup and 12 (15.2%) of the VD subgroup proceeded to HSCT. The baseline clinical characteristics of patients are presented in **Table 1**.

Response Rates

The response rates for each subgroup were summarized in **Table 2**. In the vindesine-based group, the ORR was 65.7% (23/35) in the vDD subgroup and 63.6% (49/77) in the vAD subgroup, including 17.1% (6/35) patients achieved CR, and 25.7% (9/35) patients achieved ≥VGPR in the vDD subgroup compared to 11.7% (9/77) of CR and 24.7% (19/77) of ≥VGPR in the vAD subgroup, which were all considered not statistically significant.

As for bortezomib-based group, although ORR between the VDD subgroup (91.4%) and VD subgroup (84.8%; p=0.249) had no significant difference, the rates of achieving CR and ≥VGPR were both significantly different between these two subgroups. 48.3% (28/58) of the patients achieved CR and 74.1% (43/58) achieved ≥VGPR in the VDD subgroup, compared to 30.4% (24/79) achieving CR (p = 0.033) and 57.0% (45/79) ≥VGPR in the VD subgroup (p = 0.038).

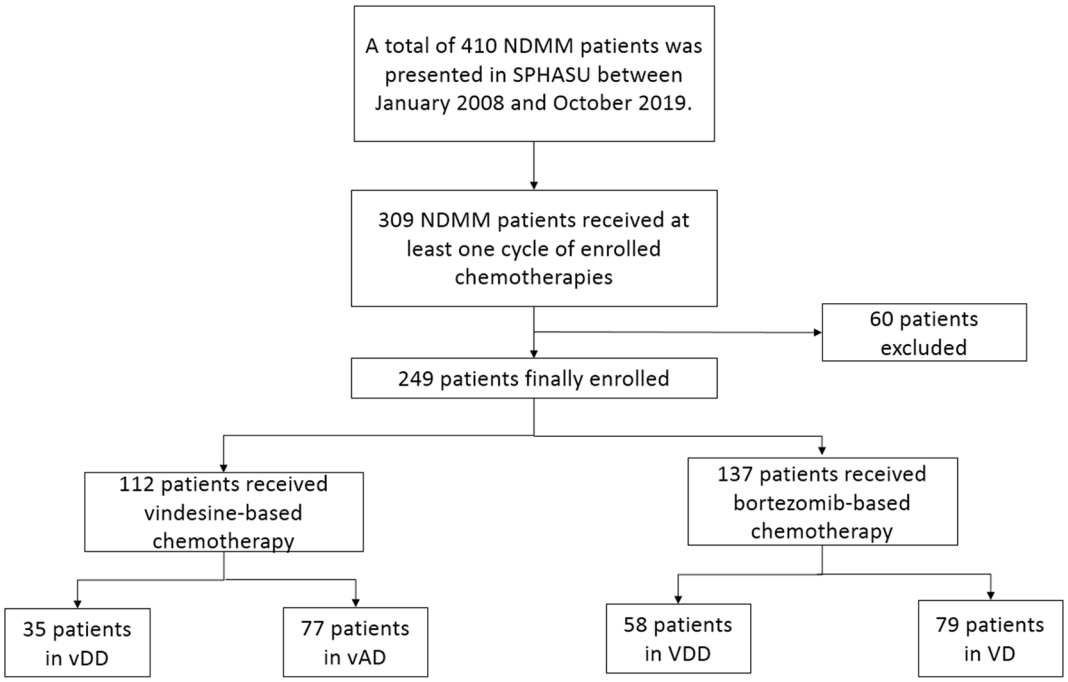

FIGURE 1 | This is a flow diagram of all patients with newly diagnosed multiple myeloma enrolled and their regimen groups in this study.

Survival

The median follow-up time for patients in the vindesine-based group was 25 months (range, 1–125 months), and the median follow-up time for patients in bortezomib-based group was 16 months (range, 1–134 months). Between the vDD and vAD subgroups, the median PFS was 28 months (95% CI, 13–42 months) in the vDD with 25 months (95% CI, 19–30 months) in the vAD. The median OS was 46 months (95% CI, 28–63 months) in the vAD while the median OS not reached in the vDD subgroup partly because of the later use of PLD. However, neither PFS nor OS differed significantly between vDD and vAD subgroup (p = 0.135, p = 0.240, respectively; **Figures 2A, B**). As for bortezomib regimens, the median PFS was 45 months (95% CI, 23–66 months) and the median OS was 52 months (95% CI, 32–71months) in the VD subgroup; comparing the median PFS and the median OS both did not reach in the VDD subgroup. Similarly, no significant difference was found between the VDD and VD subgroups in either PFS (p = 0.875) or OS (p = 0.448) (**Figures 2C, D**).

Safety

In the bortezomib regimen group, with the addition of PLD, the occurrence rates of Grade 3/4 hematological toxicities, including thrombocytopenia (19.0%), neutropenia (15.5%), and anemia (5.2%), as well as infection, including pneumonia (56.9%) and urinary tract infection (5.2%), in the VDD subgroup were significantly higher than those in the VD subgroup (p = 0.004 and p = 0.005, respectively). Gastrointestinal toxicities including vomiting, diarrhea, abdominal distension, and intestinal obstruction were significantly more frequent in the VDD subgroup (46.6 *vs.* 22.8%, p = 0.003). While no treatment-related deaths occurred, and these side events can be controlled in the supportive care. The occurrence rates of all cardiac AEs were similar between the VDD and VD subgroups (p = 0.509). Among patients receiving VDD regimen, 3.4% experienced heart failure and 1.7% experienced left ventricular systolic dysfunction. The addition of PLD also did not raise the occurrence rates of herpes zoster and PN. Among all enrolled patients, only one in the VD subgroup developed pulmonary embolism.

For the vindesine-based group, the occurrence rate of infection in the vDD was significantly higher than in the vAD subgroup (51.4 *vs.* 24.7%, p = 0.005), partly because of more Grade 3/4 hematological toxicities caused by PLD. Other AEs including gastrointestinal toxicities, cardiac toxicities, herpes zoster, and PN were all without statistical significances. The results of all AEs in each group are shown in **Tables 3** and **4**.

DISCUSSION

In this present study, we retrospectively analyzed the efficiency and safety of PLD in different combination therapies for patients with NDMM. In therapies based on vindesine, PLD did not show superior antitumor efficacy compared to epirubicin, with similar ORR, CR and ≥VGPR rate. Replacement of epirubicin with PLD combined with vindesine and dexamethasone did not bring about significantly longer PFS or OS.

TABLE 1 | Baseline characteristics of patients.

Characteristics	vAD (n = 77)	vDD (n = 35)	P-value	VD (n = 79)	VDD (n = 58)	P-value
Median age, years(range)	58 (37–75)	59 (37–77)	0.272	59 (32–76)	56 (41–74)	0.218
Gender (male)	46 (59.8%)	27 (77.1%)	0.073	70 (88.6%)	35 (60.3%)	<0.001
ECOG PS						
0–1	65(84.4%)	31(88.6%)	0.560	72(91.1%)	52(89.7%)	0.770
≥2	12(15.6%)	4(11.4%)	–	7(8.9%)	6(10.3%)	–
MM subtype						
IgG kappa	23 (29.9%)	10 (28.6%)	0.889	22 (27.8%)	15 (25.9%)	0.796
IgG lambda	17 (22.1%)	7 (20.0%)	0.804	17 (21.5%)	12 (20.7%)	0.907
IgA kappa	8 (10.4%)	6 (17.1%)	0.317	7 (8.9%)	12 (20.7%)	0.048
IgA lambda	8 (10.4%)	4 (11.4%)	0.869	3 (3.8%)	4 (6.9%)	0.416
IgD kappa	0 (0.0%)	0 (0.0%)	–	1 (1.3%)	0 (0.0%)	0.390
IgD lambda	2 (2.6%)	0 (0.0%)	0.336	3 (3.8%)	3 (5.2%)	0.698
Lambda light chain only	9 (11.7%)	6 (17.1%)	0.432	13 (16.5%)	8 (13.8%)	0.669
Kappa light chain only	2 (2.6%)	0 (0.0%)	0.336	11 (13.9%)	3 (5.2%)	0.095
Non-secretory	8 (10.4%)	2 (5.7%)	0.421	2 (2.5%)	1 (1.7%)	0.750
Genetic abnormalities						
Yes	10 (13.0%)	18 (51.4%)	<0.001	28 (35.4%)	19 (32.8%)	0.744
No	4 (5.2%)	6 (17.1%)	0.040	22 (27.8%)	24 (41.4%)	0.098
Not accessible	63 (81.8%)	11 (31.4%)	<0.001	29 (36.7%)	15 (25.9%)	0.179
ISS stage II/III	45 (58.4%)	28 (80.0%)	0.026	70 (88.6%)	42 (72.4%)	0.015
Durie-Salmon stage II/III	64 (83.1%)	34 (97.1%)	0.037	70 (88.6%)	54 (93.1%)	0.375
Median number of therapy cycles (range)	3 (1–11)	3 (1–10)	0.369	3 (1–11)	4 (1–8)	0.422
Subsequent transplant after treatment	6 (7.8%)	4 (11.4%)	0.532	12 (15.2%)	23 (39.7%)	0.001

MM, multiple myeloma; ECOG PS, Eastern Cooperative Oncology Group performance status; Ig, immunoglobulin; ISS, International Staging System; Genetic abnormalities including gain (1q21), t (4;14), del (17p13), del (13q14) were detected by Fluorescence in situ hybridization (FISH).

On the other hand, the addition of PLD to bortezomib and dexamethasone demonstrated a significantly better CR rate of 48.3%, ≥VGPR rate of 74.1%, and a slightly improvement in ORR, which is in accordance with the research by Wang et al. (17), which suggested that addition of PLD resulted in a deeper remission. Preclinical studies demonstrated that the synergistic effect between bortezomib and anthracycline through the caspase-8 pathway and dexamethasone through the caspase-9 pathway provided the rationale for combining PLD to bortezomib and dexamethasone (18).

PLD is an anthracycline compound, which is used as topoisomerase II inhibitor and DNA damaging agent. The upregulation of nuclear factor-κB (NF-κB), which results in transcription of genes involved in oncogenes is one major mechanism that inhibits the effect of PLD. Several researches have verified that bortezomib enhances the anti-MM effects of doxorubicin by suppressing the degradation of the NF-κB inhibitor to inhibit the activation of NF-κB (18). Proteasome inhibitors can also stimulate the MKP-1 to anti-apoptosis. Preclinical studies suggested that anthracyclines attenuated the induction of MKP-1, thus inhibiting its antiapoptotic effect (19). Various tumor model preclinical studies showed a synergy of increased apoptotic activity in combination of doxorubicin and bortezomib, and prevented anti-apoptotic activity which both the transcription factor NF-κB and the MKP-1 involved are observed (4). Thus, the combination of proteasome inhibitors bortezomib and anthracyclines PLD can increase their both efficacy (11).

Clinical data based on therapeutic approaches of MM have already indicated that the quality of response could predict long-term outcomes (20), with deeper responses, such as achievement of ≥VGPR, are associated with longer PFS and OS (14). On one hand, deeper responses of VDD did not show longer PFS or OS than the VD subgroup in our study of the total survival data. It is possible the unlimited subsequent therapy and inadequate follow-up time covered this benefit to some extent. After receiving the observed cycles of specific regimens, different patients progressed to varied therapies including HSCT, thalidomide, cyclophosphamide, lenalidomide, melphalan, ixazomib, and even cross-group therapies.

TABLE 2 | Overall response rates of vindesine and bortezomib regimens.

	vDD (n = 35)	vAD (n = 77)	P-value	VDD (n = 58)	VD (n = 79)	P-value
CR	6 (17.1%)	9 (11.7%)	0.432	28 (48.3%)	24 (30.4%)	0.033
≥VGPR	9 (25.7%)	19 (24.7%)	0.906	43 (74.1%)	45 (57.0%)	0.038
PR	14 (40.0%)	30 (39.0%)	0.917	10 (17.2%)	22 (27.8%)	0.147
ORR	23 (65.7%)	49 (63.6%)	0.832	53 (91.4%)	67 (84.8%)	0.249
SD	11 (31.4%)	19 (24.7%)	0.454	3 (5.2%)	8 (10.1%)	0.292
PD	1 (2.9%)	9 (11.7%)	0.129	2 (3.4%)	4 (5.1%)	0.648

CR, complete response; ≥VGPR, very good partial response or better; PR, partial response; ORR, overall response rate; SD, stable disease; PD, progressive disease.

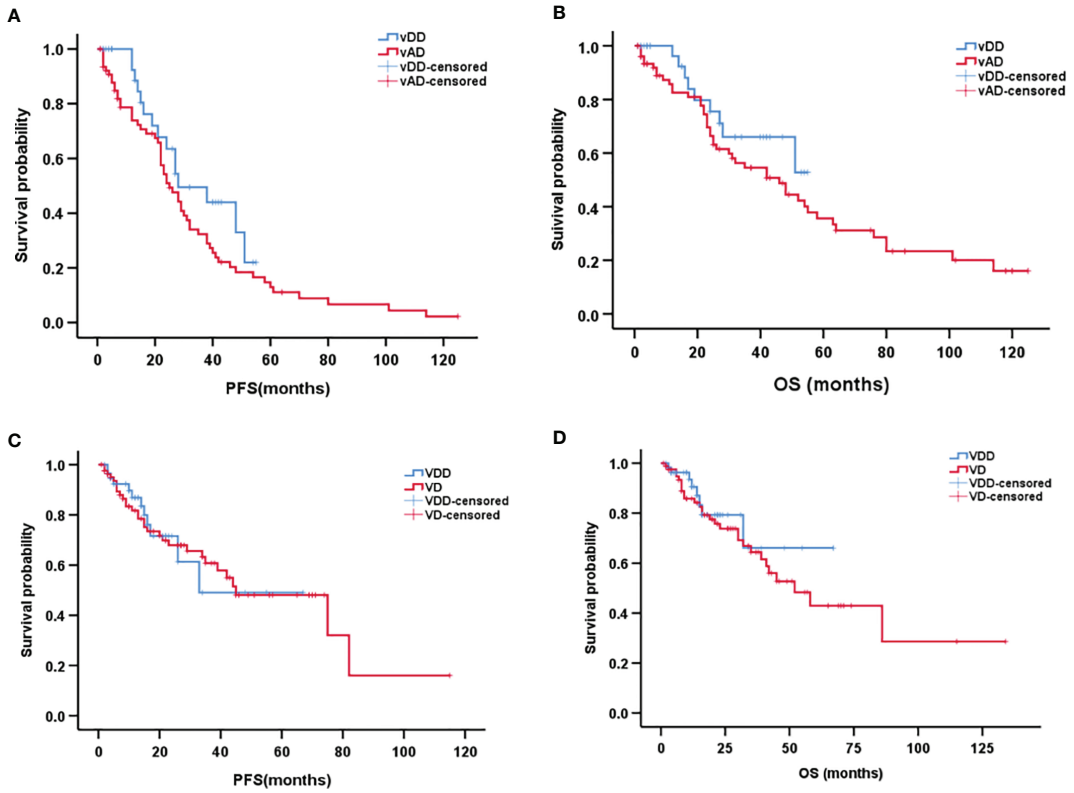

FIGURE 2 | Kaplan–Meier curve of progression-free survival **(A)** and overall survival **(B)** between vDD and vAD in patients with newly diagnosed multiple myeloma. Kaplan–Meier curve of progression-free survival **(C)** and overall survival **(D)** between VDD and VD in patients with newly diagnosed multiple myeloma.

TABLE 3 | Treatment-related adverse events of bortezomib-based regimens.

Adverse events	VD (n = 79)	VDD (n = 58)	P-value
Hematologic events (Grade 3/4)	14 (17.7%)	23 (39.7%)	0.004
Neutropenia	2 (2.5%)	9 (15.5%)	0.006
Thrombocytopenia	8 (10.1%)	11 (19.0%)	0.139
Anemia	4 (5.1%)	3 (5.2%)	0.977
Cardiotoxicity	8 (10.1%)	4 (6.9%)	0.509
Arrhythmia	4 (5.1%)	0 (0.0%)	0.082
Heart failure	3 (3.8%)	2 (3.4%)	0.914
Left ventricular systolic dysfunction	0 (0.0%)	1 (1.7%)	0.241
ECG QT corrected interval prolonged	1 (1.3%)	1 (1.7%)	0.825
Infection	30 (38.0%)	36 (62.1%)	0.005
Pneumonia	30 (38.0%)	33 (56.9%)	0.028
Urinary tract	0 (0.0%)	3 (5.2%)	0.041
Thromboembolism	2 (2.5%)	0 (0.0%)	0.222
Deep vein thrombosis	1 (1.3%)	0 (0.0%)	0.390
Pulmonary embolus	1 (1.3%)	0 (0.0%)	0.390
Gastrointestinal	18 (22.8%)	27(46.6%)	0.003
Vomiting	1 (1.3%)	4 (6.9%)	0.082
Diarrhea	8 (10.1%)	15 (25.9%)	0.015
Abdominal distension	6 (7.6%)	4 (6.9%)	0.877
Intestinal obstruction	3 (3.8%)	4 (6.9%)	0.416
Hepatic disorders	4 (5.1%)	4 (6.9%)	0.651
Skin	21 (26.6%)	13 (22.4%)	0.577
Herpes zoster	13 (16.5%)	8 (13.8%)	0.669
Rash	8 (10.1%)	5 (8.6%)	0.766
Peripheral neuropathy	21 (26.6%)	16 (27.6%)	0.896

ECG, electrocardiogram.

TABLE 4 | Treatment-related adverse events of vindesine-based regimens.

Adverse events	vAD (n = 77)	vDD (n = 35)	P-value
Hematologic events (Grade 3/4)	16 (20.8%)	12 (34.3%)	0.126
Neutropenia	11(14.3%)	11 (31.4%)	0.034
Thrombocytopenia	2 (2.6%)	1 (2.9%)	0.937
Anemia	3 (3.9%)	0 (0.0%)	0.237
Cardiotoxicity	8 (10.4%)	5 (14.3%)	0.551
Arrhythmia	5 (6.5%)	2 (5.7%)	0.875
Heart failure	2 (2.6%)	3 (8.6%)	0.156
Left ventricular systolic dysfunction	1 (1.3%)	0 (0.0%)	0.498
Infection	19 (24.7%)	18 (51.4%)	0.005
Pneumonia	16 (20.8%)	17 (48.6%)	0.003
Urinary tract	3 (3.9%)	1 (2.9%)	0.784
Thromboembolism	0 (0.0%)	0 (0.0%)	–
Deep vein thrombosis	0 (0.0%)	0 (0.0%)	–
Pulmonary embolus	0 (0.0%)	0 (0.0%)	–
Gastrointestinal	21 (27.3%)	5 (14.3%)	0.131
Vomiting	1 (1.3%)	1 (2.9%)	0.564
Diarrhea	5 (6.5%)	1 (2.9%)	0.428
Constipation	12 (15.6%)	3 (8.6%)	0.312
Intestinal obstruction	3 (3.9%)	0 (0.0%)	0.237
Hepatic disorders	4 (5.2%)	0 (0.0%)	0.170
Skin	5 (6.5%)	2 (5.7%)	0.875
Herpes zoster	1 (1.3%)	2 (5.7%)	0.180
Rash	4 (5.2%)	0 (0.0%)	0.170
Peripheral neuropathy	10 (13.0%)	2 (5.7%)	0.249

In terms of safety, PLD showed favorable hematological and non-hematological toxicity profile in clinical studies. A significantly decreased incidence of bone marrow suppression or neutropenic fever, less alopecia and decrease in cardiac function was observed in patients who received vDD regimen compared to those who received vAD (3). Treatment of vDD was significantly related to higher incidence of mostly Grades 1 and 2 hand–foot syndrome (3). Unfortunately, vDD regimen did not present any superior to vAD regimen in terms of safety in our study. Although the addition of PLD to bortezomib increased toxicity compared to bortezomib alone, mostly caused by increased myelosuppression and GI events, these toxicities were predictable and manageable through dose adjustment and supportive treatment (9). The increased non-hematological toxicities were more significant in NDMM patients aged ≥65 years (CALGB (Alliance) 10301) (13). The VDD regimen was associated with frequent grade 3/4 AEs including neutropenia, thrombocytopenia, pneumonitis and a high incidence of PN in several clinical trials (11). Yet PN was proved to be reversible (21) and dose-limiting (22). In our study, with the addition of PLD to VD, in accordance with the above studies, the incidence of Grade 3/4 hematological toxicities, infection, and gastrointestinal toxicities in VDD were significantly higher than those in the VD subgroup, which can be controlled in the supportive care like GSF and antibiotic use. Moreover, the occurrence rates of all cardiac AEs were comparable between VDD and VD subgroups, which means the addition of PLD did not raise the cardiac toxicity.

Multivariate analysis indicated that PLD could be an effective component in other regimens like bortezomib, cyclophosphamide, PLD, and dexamethasone combination (VCDD) (23, 24). Novel therapies such as the second-generation proteasome inhibitor carfilzomib, PLD, and dexamethasone (KDD), the deacetylase inhibitor vorinostat, PLD and bortezomib, the immunomodulatory drugs lenalidomide (25, 26) or pomalidomide (27) with PLD and bortezomib and so on all have high efficiency and well tolerance for RRMM patients. Especially, KDD demonstrated the ORR of 83% (20/24) and ≥VGPR rate of 54% (13/24), with the median PFS of 13.7 months (7). The vorinostat + PLD + bortezomib regimen produced an ORR of 65%, with the median PFS 13.9 months and the 3-year OS rate of 77% (28).

In this study, the regimens were the initial inductive therapies, and the sequential treatment was not limited, while VDD followed by TD (thalidomide + dexamethasone) or VTD was decided to be active in a clinical study, making more patients including those clinically defined high-risk cohort achieving maximal response before transplant (29). These better-quality responses were maintained following transplant and were related to a trend toward longer TTP and OS.

In conclusion, vDD is similar with vAD in response rate, survival, and toxicity for NDMM. The addition of PLD to VD brings deeper response without increased toxicity. However, superior survival in a long term was not proved in our study. There were still some limitations to our study, which was a retrospective one in a single center, and the amounts of recruited patients were confined. Thus, we included a mixed population of both receiving HSCT and not, which may have led to potential bias that could weaken the results. The heterogeneity in sequential regimens may have contributed to the relative variation in our findings. Moreover, because of the later use of PLD, the follow-up time of the regimens including PLD was inadequate and unequal with that of the regimens excluding

PLD. Prospective randomized studies in larger population cohorts and extended follow-up time are necessary for further verifying the prognostic effect of PLD in different regimens.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

This study was approved by the Medical Ethical Committee of Shandong Provincial Hospital affiliated to Shandong University. All data of the recruited patients were obtained with written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

XW designed the study. YZ, DY, XG, and SH collected the clinical data. PL, XF, and YL analyzed the data. YZ wrote the paper. XZ and XW revised the paper. All authors contributed to the article and approved the submitted version.

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Employment of Artificial Intelligence Based on Routine Laboratory Results for the Early Diagnosis of Multiple Myeloma

*Wei Yan[1], Hua Shi[1], Tao He[2], Jian Chen[2], Chen Wang[2], Aijun Liao[1], Wei Yang[1] and Huihan Wang[1]**

[1] Haematology Department of Shengjing Hospital, China Medical University, Shenyang, China, [2] Neusoft Research Institute, Northeastern University, Shenyang, China

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Hans C. Lee,
University of Texas MD Anderson Cancer Center, United States

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Kristofer Jennings,
University of Texas MD Anderson Cancer Center, United States

***Correspondence:**
Huihan Wang
240333079@qq.com

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Objective: In order to enhance the detection rate of multiple myeloma and execute an early and more precise disease management, an artificial intelligence assistant diagnosis system is developed.

Methods: 4,187 routine blood and biochemical examination records were collected from Shengjing Hospital affiliated to China Medical University from January 2010 to January 2020, which include 1,741 records of multiple myeloma (MM) and 2,446 records of non-myeloma (infectious diseases, rheumatic immune system diseases, hepatic diseases and renal diseases). The data set was split into training and test subsets with the ratio of 4:1 while connecting hemoglobin, serum creatinine, serum calcium, immunoglobulin (A, G and M), albumin, total protein, and the ratio of albumin to globulin data. An early assistant diagnostic model of MM was established by Gradient Boosting Decision Tree (GBDT), Support Vector Machine (SVM), Deep Neural Networks (DNN), and Random Forest (RF). Out team calculated the precision and recall of the system. The performance of the diagnostic model was evaluated by using the receiver operating characteristic (ROC) curve.

Results: By designing the features properly, the typical machine learning algorithms SVM, DNN, RF and GBDT all performed well. GBDT had the highest precision (92.9%), recall (90.0%) and F1 score (0.915) for the myeloma group. The maximized area under the ROC (AUROC) was calculated, and the results of GBDT (AUC: 0.975; 95% confidence interval (CI): 0.963–0.986) outperformed that of SVM, DNN and RF.

Conclusion: The model established by artificial intelligence derived from routine laboratory results can accurately diagnose MM, which can boost the rate of early diagnosis.

Keywords: multiple myeloma, artificial intelligence, early diagnosis, gradient boosting decision tree, machine learning

INTRODUCTION

As a hematological malignancy, multiple myeloma (MM) accounts for 1% of all cancer and 13% of hematological tumors with the characteristics of proliferation of malignant plasma cells in the bone marrow (BM), presence of anemia, renal dysfunction, hypercalcemia, and lytic lesions (1). The involvement of other disciplines such as orthopedics, nephrology and hematology often cause misdiagnoses (2). In addition, due to the sub-par distribution of state-of-the-art medical and diagnostic equipment, rural health centers and primary care providers register a high rate of misdiagnoses and missed diagnoses. Howell et al. reported that the time from the appearance of first symptoms to the first instance of seeking medical help ranged from 1 to 7 months, and the time from help-seeking to diagnosis ranged from 2 weeks to 17 months in MM patients. Patients reported between one and ten primary care consultations with what they considered (in hindsight) to be myeloma symptoms, before the referral leading to diagnosis (3). The delay in diagnosis will deprive the patient of the optimal opportunity for treatment and can lead to the development of complications which can only at times be reversed. Increased tumor burden, symptoms, and organ damage all affect the treatment results and the capacity for myeloma patients to receive treatment (4). Improving the time to diagnosis of MM is a sine qua non condition to fulfill to give patients a fair chance of recovery especially in community hospitals and primary care clinics.

Being human-made, artificial intelligence (AI) can simulate intellectual work such as humans' thoughts and judgments and has thus revolutionized the medical field (5). Hence, there is an increasing attention on the application of AI for the diagnosis and treatment of cancer (6, 7). In terms of settling the problems of classification and regression, the gradient boosting decision tree (GBDT) is regarded as a powerful ensemble learning technique (8). This model outperforms other models as the direction of the negative gradient is followed in order to train the residuals of each iteration, which can avoid the over-fitting problem. Furthermore, the GBDT model has performed well for knowledge discovery in various fields (9, 10). The current study is the first time that the artificial intelligence technology, including GBDT, has been used constructing a multiple myeloma early screening model based on a large amount of clinical conventional examination data. With the help of AI technology, the knowledge and experience of authoritative experts will better benefit the public, and effectively improve the current diagnosis rate of myeloma in the areas short of experience, which is of very important clinical significance.

PATIENTS AND METHODS

The Medical Ethics Committee at the Shengjing Hospital of China Medical University approved the present study (2020PS055J) according to the principles of the Declaration of Helsinki. The requirement for personal informed consent has been waived by the Ethics Committee as electronic medical records are researched retrospectively.

Patient and Data Selection

In this retrospective research, our institutional databases were screened to investigate the patients admitted to our hospital for the first routine blood checks, hepatic function panel, renal function tests and immunoglobulin tests from January 2010 to January 2020. These included 1,741 records of multiple myeloma (MM) and 2,446 records of non-myeloma (infectious disease, rheumatic immune system disease, hepatic disease and renal disease). We also collected the data for these laboratory items from January 2020 to November 2020, including 68 records of newly diagnosed multiple myeloma (MM) and 70 records of non-myeloma aimed at testing the theory of generalizability. The diagnosis was made based on the 2014 International MM Working Group criteria (IMWG) (11). Nine variables (hemoglobin, serum creatinine, serum calcium, immunoglobulin (A, G and M), albumin, total protein, and ratio of albumin to globulin) were collected based on the use of current diagnostic criteria and medical judgment. Because immunoglobulin assays are not part of routine laboratory tests, we have therefore used six variables to try the alternative, cheaper model.

Data Processing

Based on the diagnostic criteria and doctor-assisted judgments, the related factors for Multiple Myeloma risk prediction have been determined, and the original data related to the prediction have been extracted from the HIS and LIS databases. After extracting the correlating factors, the original sample set could not be directly applied in training machine learning models as the sample set still required further preprocessing of data.

Handling the Missing Value in the Sample Set

The presence of empty values in the extracted raw data is first confirmed. A patient is eliminated from the pool when the number of missing values is larger than the designated threshold. We initially tested the number of missing values from 0–8. The results are depicted in **Supplementary Figure 1**. It was concluded that with the increase of the threshold value, F1 score increases followed by a decrease. This is due to the fact that fewer samples remain after data cleaning through the reduction of the number of the threshold, which results in the weakening of the generalization ability. However, when setting the threshold value as a sufficiently large value, more lost features are filled with normal values, resulting in the decrease of the F1 score. Considering these two factors, we finally set the threshold as 3 in our algorithm. If more than three factors are empty, the sample will be deleted, and if the missing value is below or equal to 3, this sample is deemed as fit to be retained. Through application of the same method, when six variables are considered, the threshold value is set to 2. Due to the fact that reserved data still contain missing values, its missing item will be filled with a normal value. With this missing data processing, we thus can reduce the possible deviation caused by using an abnormal value, and ensure there are no missing values in this data either for training or testing. We have implemented this

method as a specific data processing module in our designed learning model.

Expanding the Number of Positive Classes

Based on the real data extracted from the system, the number of positives is much lower than the negatives. The Synthetic Minority Oversampling Technique (SMOTE) algorithm can be used to fix this unbalanced classification problem, by producing synthetic examples to increase the number of positive classes (12). The SMOTE algorithm analyzes and simulates the minority samples, uses the k-nearest neighbor (KNN) algorithm to synthesize new minority samples and adds the synthesized new samples into the training data, which can expand the sample size (13). The following steps are carried out by the SMOTE algorithm to synthesize new samples: the nearest neighbor algorithm is used and the number of the nearest neighbors for each minority is calculated; random number of samples are selected to randomly implement linear interpolation, and construct new minority samples; finally, new samples are synthesized with original data to produce new training sets (**Figure 1**). Here we obtained 580 synthetic samples based on 1,741 myeloma samples with SMOTE algorithm. These 580 synthetic samples were further integrated with original positive samples for model construction and testing.

Expanding the Number of Correlation Features

Since the newly generated features can reflect the deviation degree of the detection items to its normal range, we thus utilize this feature as the part of the features to build the model. It should be noted that each index feature has a normal range in our system. Subsequently, the data standardization method was expanded by subtracting the upper and lower limits with the normal value. Moreover, we applied the outlier detection method after standardization method to reduce some outlier values in the sample data set. The relationship between the testing instance and its normal reference range is used to carry out feature correlation and expansion. For example, we assume the detection value of this feature index i as d_i. Each index feature has a normal range $[L_d^i, U_d^i]$. By comparing the differences between d_i and L_d^i and between U_d^i, two novel features l_d^i *and* u_d^i are generated by

$$l_d^i = \begin{cases} 0, & \mathrm{d}_i > L_d^i \\ d_i - l_d^i, & \text{otherwise} \end{cases} \quad (1)$$

$$u_d^i = \begin{cases} 0, & \mathrm{d}_i > L_d^i \; U_d^i \\ d_i - d_i, & \text{otherwise} \end{cases} \quad (2)$$

where l_d^i and u_d^i reflect the deviation of the detection value from the normal range. The greater the detection value from the normal range, the larger l_d^i or u_d^i will be obtained. By utilizing the l_d^i, u_d^i, l_d^i as the input features, more detailed and expanded features are thus obtained. Experiments show that this method can reduce the prediction variance of the model.

Building the Prediction Model

Ensemble learning is a popular paradigm employed to leverage the strength of individual classifiers and mitigate their weaknesses. Ensemble techniques consist of combining more than one single classifier under a specific combination rule to solve the same task (14). As a common algorithm for ensemble learning, GBDT is composed of Decision Tree and Gradient Boosting (15) Because this tree model is characterized by high bias, low variance and small depth, highly pruned version of CART tress is thus utilized as the base classifiers for GBDT in each iteration (16).

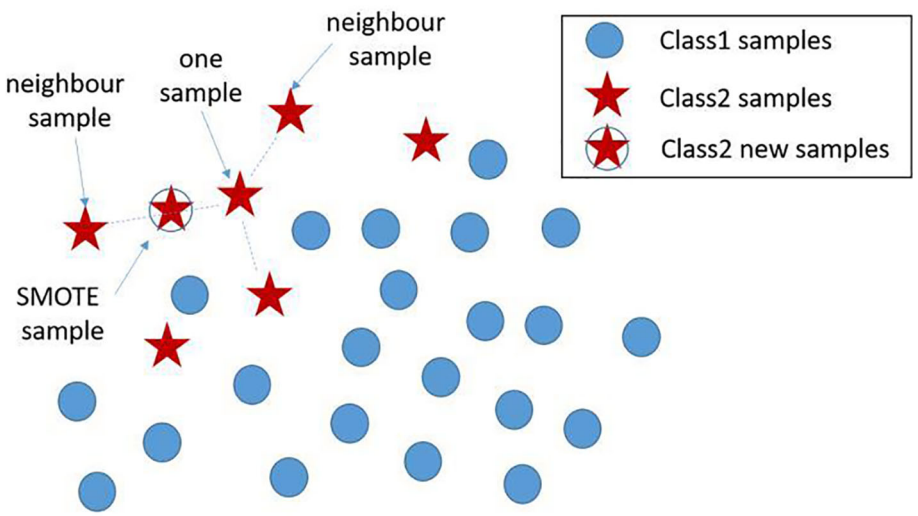

FIGURE 1 | New sample generation with SMOTE algorithm. A new sample was generated using two existing samples, where the newly generated samples are denoted by 'stars'. SMOTE, Synthetic Minority Oversampling Technique.

The aggregated classifier using the additive modeling structure is as follows (17):

$$\hat{y}_i = F(x_i) = \Sigma_{k=1}^{K} \gamma_k f_k(x_i) \quad (3)$$

where $\hat{y}_i$ and $F(x_i)$ represent the predicted value of ith sample, γ_k represents the weight of the kth tree, $f_x(x_i)$ represents the prediction result of the kth regression tree for samples, x_i represents the independent values used in fitting each regression tree, K is the number of CART model trees.

For Binary Classification problems, logarithmic loss function, which is also called as the log-likelihood loss function is utilized as the loss function:

$$L(y, F(x)) = log(1 + exp(-2yF(x))) \quad (4)$$

where $F(x)$ is given by Equation (3), and x is a generalization of x_i, y represents the true value of the sample.

With this loss function, the common applied gradient descent method is applied to find the optimal model. By calculating the negative gradient, we can obtain the moving direction brings has the steepest decline in the value of the loss function. The optimal model, through an iterative manner, can be found in this moving direction. With each iteration, the gradient descent method first calculates the negative gradient of the current model on all samples, and then trains a new base classifier with the value as the target for quasi merging, thus to calculate the weight of the base classifier. By utilizing this method iteratively, we finally realize the updating of the model.

In order to ensure the generalization ability of the model, the negative data and positive data are first mixed and shuffled, thus changing the original order. Then, using random extraction we obtain the training set and test set, which can ensure the independence of these two data sets. In our algorithm, the data volume ratio of these two data sets is 4:1. For the GBDT algorithm, the important super parameters include the maximum depth of the decision tree and the number of decision trees. The grid search method is then applied on the validation set, and the calculated optimal number of decision trees is 81, while the maximum depth for the decision trees is 6. All these performance results are obtained in the test data set. The complete training pipeline is demonstrated in **Figure 2**.

Moreover, Support Vector Machine (SVM) (13), Deep Neural Networks (DNN) (18), and Random forest (RF) (19) were also applied for performance comparisons. These three algorithms used the same training set and test set. For SVM algorithm, the Gaussian kernel function was utilized and the "gamma" parameter was set to 1. Gamma is a regularized super parameter. A larger gamma value denotes a more irregular decision boundary. Concurrently, a smaller gamma denotes a smoother boundary. Consequentially, we found that the gamma value needs to be adjusted when the model is over-fitted or otherwise (13). For DNN, we found that the model with more than four hidden layers seemed to be over fitted, and two hidden layers would not fit well. Therefore, a network with three hidden layers was constructed, where each layer contained 256 neurons and the ReLU activation function was applied. For random forest algorithm, we had tried the number of trees within set {50,100,300,500,600,800,1,000} and the depth of trees within set {5,10,15,20,30,50}. By testing all the combination on the validation set, we set the number of trees as 500 with its depth as 15.

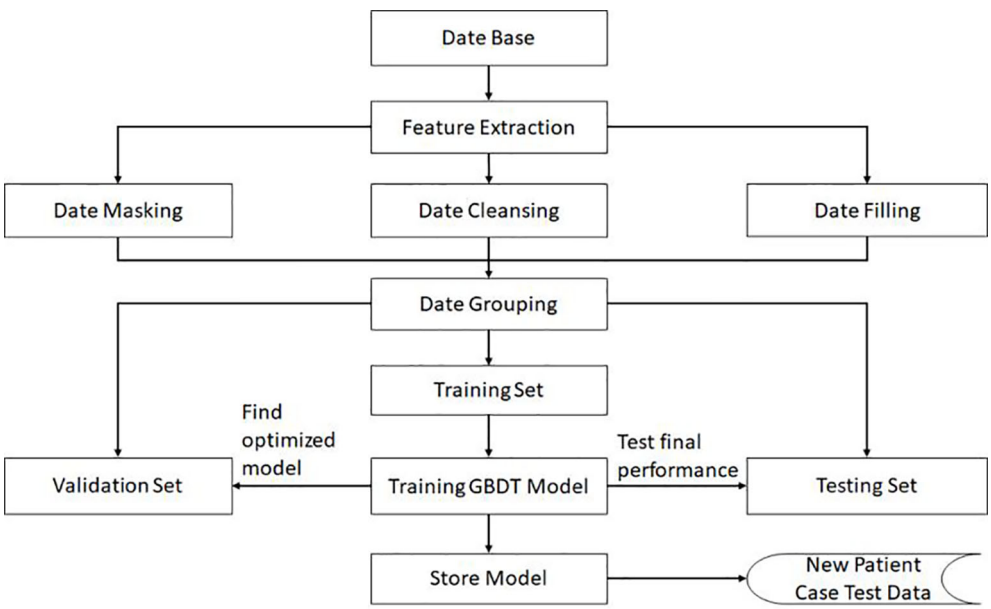

FIGURE 2 | The flowchart and the complete training pipeline of the GBDT model.

Precision P, recall R, and F1 score are three common metrics used to evaluate the performance of a model in Machine Learning. Their formulas are as follows:

$$P = \frac{TP}{TP + FP} \tag{5}$$

$$R = \frac{TP}{TP + FN} \tag{6}$$

$$F_1 = 2 \times \frac{P \times R}{P + R} \tag{7}$$

From the formula, P stands for the proportion between the number of correctively predicted positives myeloma and the total number of positives in both myeloma and non-myeloma classes, P therefore represents the prediction of myeloma in our model; R stands for the proportion between the correctively predicted myeloma and the total number of actual myeloma patients; and F1-score is the harmonic mean of precision and recall (20). High F1-score only occurs when both recall and precision are high.

Among them, the clinical interpretation for evaluation criteria is as followed: For the category of myeloma, true positives (TP) indicate that the myeloma patient is correctly predicted to be in this myeloma class, a false positive (FP) indicates that it is not a myeloma patient and it has been incorrectly predicted as a myeloma class, and false negatives (FN) is the total number of incorrect predictions for a certain true myeloma class. For the non-myeloma category, TP represents the non-myeloma is correctly predicted in the non-myeloma class, FP represents the number of samples predicted as non-myeloma but actually myeloma, FN represents the number of non-myeloma incorrect predicted to be myeloma. The threshold for calculating TP and FN is the default value of 0.5.

The balance between the positive and negative samples can potentially cause the model to be impartial to positive and negative cases. Conversely, if the number of negative samples is larger than the number of positive samples, it will result in the deviation to the negative direction due to the over-exposure to negative samples. In our algorithm, the enhanced data is treated as normal data for model construction and testing without special treatment.

The curve of the receiver operator characteristics (ROC) is another important evaluation metric with regard with binary classification problems, and is a probability curve that plots the true positive rate (TPR) against false positive rate (FPR) at various threshold values.

All the experimental programs in this paper have been written in the Python language, and Python version 3.6 was applied as the interpreter. The machine learning development kit using in this paper is scikit learn version 0.20. The random forest applied in this paper is based on sklearn. ensemble class. The support vector machine is based on the SVM class of sklearn, and DBDT is based on Gradient Boosting Classifier class of sklearn. ensemble. The deep learning is operated on the tensorflow 1.12, with numpy 1.15.4 used to process and transform arrays, and Matplotlib 3.0.2 used to draw ROC curves.

RESULTS

Some 1,741 records of multiple myeloma and 2,446 records of non-myeloma (infectious diseases, rheumatic immune system diseases, hepatic disease and renal disease) were analyzed. The basic assay indicators are shown in **Table 1**.

Moreover, we compared performance with or without data standardization and outlier detection. The results are shown in **Supplementary Table 1**. The contents of the table indicate performance comparison of utilizing three features u_d^i, d_i and l_d^i or utilizing only feature d_i with multiple tests, where both the mean value and standard deviation are compared. As can be seen from the statistics, using u_d^i, d_i and l_d^i can reduce the standard deviation. It can be concluded that our method increases the reliability of prediction performance. By taking the F1 scores of positive samples as example, a smaller standard deviation is yielded, indicating the estimation is more stable.

For comparison, SVM, DNN, RF and GBDT models were trained and tested on the same dataset using nine variables as hemoglobin, serum creatinine, serum calcium, immunoglobulin (A, G and M), albumin, total protein, and ratio of albumin to globulin. Among the four machine learning algorithms, GBDT yielded the highest precision 0.929 and 0.899 for the myeloma and non-myeloma respectively. GBDT also has the highest recall (0.900) and F1 score (0.915) for myeloma. The value of P, R, and F1 of the four machine learning algorithms are shown in **Table 2**, and can be calculated according to Reference (21). The influence weight of each variable on classification calculated by GBDT is shown in **Supplementary Table 2**. The weight of each feature in the table is automatically calculated by machine learning algorithm, which shows the importance of each feature for disease prediction. A larger value indicates a bigger influence on classification by this variable.

An immunoglobulin assay is not part of routine laboratory queries, we have therefore used six variables namely: hemoglobin, serum creatinine, serum calcium, albumin, total protein, and ratio of albumin to globulin) to train the model. If the immunoglobulin were considered unwarranted, the model would eventually have 0.797 precision, 0.726 recall and 0.760 F1 score, which are lower than the nine variable model fit with immunoglobulin based on the GBDT model. The value of P, R, and F1 related to six variable data were shown in **Supplementary Table 3** and **Supplementary Figure 2**. For the clinics and hospitals where immunoglobulin (A, G and

TABLE 1 | Subject characteristics.

Variable	Multiple myeloma dataset Mean (SD)	Control dataset Mean (SD)
Creatinine (umol/L)	137.97 (4.62)	119.85 (2.83)
Serum β_2 microglobulin (mg/L)	7.51 (0.30)	6.12 (0.66)
Urine β_2 microglobulin (mg/L)	22.35 (1.24)	16.75 (5.16)
IgA (g/L)	4.43 (0.37)	2.89 (0.04)
IgG (g/L)	14.45 (0.48)	12.11 (0.14)
IgM (g/L)	0.67 (0.80)	1.23 (0.02)
Albumin (g/L)	35.91 (0.26)	29.70 (0.30)
Total protein (g/L)	68.60 (0.63)	58.93 (0.46)
Serum calcium (mmol/L)	2.20 (0.08)	2.04 (0.00)
Hemoglobin (g/L)	107.38 (0.70)	114.59 (0.47)

TABLE 2 | Results of Testing Group based on 9 variables.

Method	Class	P	R	F_1
GBDT	Non-myeloma	0.899	0.928	0.913
	Myeloma	0.929	0.900	0.915
RF	Non-myeloma	0.884	0.903	0.908
	Myeloma	0.901	0.90	0.906
SVM	Non-myeloma	0.830	0.827	0.829
	Myeloma	0.836	0.839	0.837
DNN	Non-myeloma	0.850	0.783	0.815
	Myeloma	0.772	0.842	0.805

M) can be routinely measured, the 9-variable model should be used to achieve higher accuracy. For health centers where immunoglobulin examination is not commonly ordered, the 6-variable model should be applied as a precautionary measure should indications of further immunoglobulin investigations appear.

With the ROC curve, the area under the curve (AUC) can be calculated to measure a classifier's ability to distinguish classes. The higher the AUC, the better the model will be at classifying. **Figure 3** illustrates the performance of comparing the AUC with these four algorithms. We can observe that the classifier with GBDT obtains an AUC of 0.975 [95% confidence interval (CI): 0.963–0.986], and has the best performance when compared to the other three algorithms.

The model we trained was also tested on the new data set, and the performance index obtained is similar with the result on the original data set, but for January to November in 2020. GBDT once more showed the highest recall (0.909), precision (0.952) and F1 score (0.930) for the myeloma set. Also, GBDT showed recall (0.954), precision (0.912) and F1 score (0.932) for the non-myeloma set with the threshold 0.5. The values of P, R, F1 of the 68 newly diagnosed MM and 70 non-myelomas by machine learning algorithms are shown in **Supplementary Table 4**. We can observe that the classifier with GBDT obtained an AUC of 0.974 in the new data shown in **Supplementary Figure 3**. From this analysis, it can be inferred that the model trained by the original data set has a strong generalization ability.

DISCUSSION

Multiple myeloma has an incidence rate of about 28,000 new cases per year in China (22) and due to various clinical presentations, diagnosis is a challenge. Data shows that more than 90% of patients suffer from bone pain and fractures in the early stages of the disease or during the course of disease progression, while about 50% of patients have renal impairment (23–25). The public do not understand MM and with a slow onset of the disease and a lack of typical symptoms in China, MM can hardly be distinguished from other diseases in other departments. This thus leads to delayed treatment and the poor prognosis of patients (26). In order to improve early diagnosis of MM, IMWG recommends the application of a percentage of clonal plasma cell into the bone marrow, serum-free light chain ratios and MRI focal lesions as additional biomarkers for the disease (27). However, in China, these programs are not considered routine inspection programs, and some primary medical care centers do not even carry out the relevant tests. We hope that based on the integration of data from routine laboratory tests in clinics, an early warning system for MM can be designed to make it easier to proceed.

As artificial intelligence develops at an extraordinarily pace, countless applications have been created in the past decade (28–30). Recently, AI has been increasingly adopted to diagnose and predict some diseases, while the medical image analysis community has paid particular attention to the success of machine learning in computer vision (31, 32). Some researchers have been initiated into applying AI techniques to the quantification of early rheumatoid arthritis using Magnetic Resonance Imaging (MRI) data (33). Ni et al. achieved a general accuracy of 84.48% when using radionics analysis based on the LASSO + GBDT method for the noninvasive diagnosis of microvascular invasion in hepatocellular carcinoma [34]. Zhang et al. used LASSO + GBDT to examine the ability of radionics characteristics from MRI in differentiating anaplastic oligodendroglioma (AO) from atypical low-grade oligodendroglioma (35). The majority of researchers have performed quantitative analysis of multi-modality image data

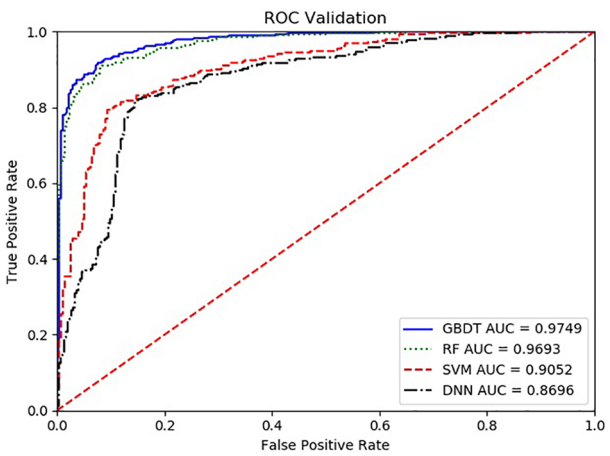

FIGURE 3 | The ROC comparison of four algorithms based on nine variables. The classifier with GBDT obtains an AUC of 0.975 [95% confidence interval (CI): 0.986–0.963], and has the best performance when comparing with the other three algorithms. ROC, Receiver Operating Characteristic; GBDT, Gradient Boosting Decision Tree; RF, Random Forest; DNN, Deep Neural Networks. Nine items are hemoglobin, serum creatinine, serum calcium, immunoglobulin (A, G and M), albumin, total protein, and ratio of albumin to globulin.

for diagnosis and prognosis by using artificial intelligence methods, but few of them have focused on the routine laboratory tests that easily obtained from clinic.

AI techniques have been applied to the treatment of multiple myeloma. Ji et al. constructed a hybrid multi-scale agent-based model (HABM) model to provide new insight into the development of myeloma in a bone marrow micro-environment that is the basis of the immune system, and also build an efficient computational platform for prediction of drug response for discovering the optimal dose combination (36). Zhang et al. built a more efficient approach by combining the standard ordinal logistic regression and the hierarchical modeling. This method can simultaneously analyze numerous variables for detecting important predictors and for predicting multi-level drug response (37). Tang et al. established and validated a novel mathematical model of multiple myeloma cell dynamics. The clinical data compounded with mathematical modeling, suggested that bortezomib-based therapy exerted a selection pressure on myeloma cells (38) Bouchnita et al. developed a hybrid discrete-continuous model to predict the response of MM tumors to treatment with gefitinib and 6-aminonicotinamide (6-AN) (39). There is no published early diagnosing, laboratory results-based AI models as of yet.

According to our preliminary test based on the data of 1,000 myeloma patients and 2,000 non-myeloma patients in our hospital, the predictive value of artificial intelligence can reach more than 90% with the prospect of having a wide application. Our research also indicates that the SVM algorithm is suitable for classifying small-size data, while the DNN algorithm is suitable for classifying large-size data. By efficiently extracting the sample features, the GBDT algorithm can simultaneously train some decision trees on the ability to sort out the features based on their importance, so as to obtain the best performance when comparing with the other three algorithms (40).

Taking the integration of test data as the breakthrough point, this project adopts the methods of big data analysis and artificial intelligence, so as to propose the automatic integration of routine test reports, establish multiple myeloma screening models, give early warnings for multiple myeloma, and improve the diagnosis rate. The research contents are innovative in the medical, information and business fields.

CONCLUSION

In this study, routine exam results obtained from general hospitals are utilized to train machines to realize automatic screening, identify patients at a high risk of diagnosed multiple myeloma and provide early warnings through the big data platform, artificial intelligence and other technologies. This technology can be widely used in general hospitals and primary medical care to improve the early diagnosis rate of myeloma and prevent the occurrence of missed diagnosis and misdiagnosis. At the end, an early warning and screening system for myeloma based on artificial intelligence will be formed.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

Study conception and design: WYan and HW. Literature review and data extraction: HS. Quality control: WYan and AL. Model construction and statistical analysis: JC and TH. Manuscript preparation: WYan and HW. Manuscript review: WYan, AL, WYang, and HW. Editing and revisions: WYan, HW, JC, TH, and CW. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.608191/full#supplementary-material

Supplementary Figure 1 | Performance comparison with different threshold in the four algorithms.

Supplementary Figure 2 | The ROC comparison of four algorithms based on 6 variables. Six variables are hemoglobin, serum creatinine, serum calcium, albumin, total protein, and ratio of albumin to globulin.

Supplementary Figure 3 | The ROC comparison of four algorithms based on 9 variables from new cases set in 2020.

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OPINION
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Treatment of Lenalidomide Exposed or Refractory Multiple Myeloma: Network Meta-Analysis of Lenalidomide-Sparing Regimens

Cirino Botta[1], Enrica Antonia Martino[1], Concetta Conticello[2], Francesco Mendicino[1], Ernesto Vigna[1], Alessandra Romano[3], Giuseppe Antonio Palumbo[3], Claudio Cerchione[4], Giovanni Martinelli[4], Fortunato Morabito[5], Francesco Di Raimondo[3] and Massimo Gentile[1]**

[1] Hematology Unit, "Annunziata" Hospital of Cosenza, Cosenza, Italy, [2] Division of Hematology, AOU Policlinico, Catania, Italy, [3] Department of Medical and Surgical Sciences and Advanced Technologies "G.F. Ingrassia", University of Catania, Catania, Italy, [4] Department of Medical Oncology, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy, [5] Hematology and Bone Marrow Transplant Unit, Hemato-Oncology Department, Augusta Victoria Hospital, East Jerusalem, Israel

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Cyrus Khandanpour, University Hospital Münster, Germany

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***Correspondence:**
Cirino Botta
cirino.botta@gmail.com
Massimo Gentile
m.gentile@aocs.it

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INTRODUCTION

Over the past 10 years, the treatment of multiple myeloma (MM) dramatically changed due to the introduction of a number of new agents and combination regimens both in the frontline and in the relapsed/refractory setting. Currently, at least 11 classes of therapeutic agents, including steroids, alkylators (melphalan and cyclophosphamide), proteasome inhibitors (PI: bortezomib, carfilzomib, ixazomib), immunomodulatory agents (thalidomide, lenalidomide, pomalidomide), monoclonal antibodies (mAbs: elotuzumab, daratumumab), HDAC-inhibitors (panobinostat), BCL2 inhibitors (venetoclax), selective inhibitors of nuclear export (selinexor), drug-conjugated mAbs (belantamab mafodotin), bispecific agents and CAR-T, are approved (or are going to be approved) alone or in different combinations for the treatment of this disease, while few or no data are available to guide the therapeutic strategy to adopt at diagnosis or relapse (1). The choice of the treatment at relapse (2), in particular, poses particular challenges, and is currently dependent on patients (age, comorbidities, fitness, renal impairment, frailty) and disease characteristics (aggressive vs biochemical relapse, cytogenetics, presence of extra-medullary disease), previous treatments (classes of agents, duration of response, progression while on therapy), regional drug access (approval of combinations, reimbursement, costs) and, finally, patient's choice. Unfortunately, there is a lack of trials specifically designed to help in this choice, and often, pre-planned subgroup analyses, do not include a sufficient number of patients to reach statistical evidence. Recently, since lenalidomide is progressively becoming the preferred one-line option to treat MM patients (and often, it is administered until progression), the choice of the treatment to be offered at relapse should be carefully evaluated. Interestingly, it has been reported that the longest prior lenalidomide treatment duration (>12 months) and IMiD-free interval (>18 months) could positively impact patients' outcome (3), making the choice of a lenalidomide-sparing regimen of particular interest in this setting. On the bases of these premises, we performed a systematic review and a frequentist network meta-analysis in R [by using the *netmeta* package (4)] comparing direct and indirect

evidence on the efficacy of seven different lenalidomide-sparing regimens (bortezomib-dexamethasone, VD; daratumumab-VD, DVD; carfilzomib-D, KD; daratumumab-KD, KdD; pomalidomide-VD, PVD; isatuximab-KD, IKD; selinexor-VD, SVD) in lenalidomide-exposed and lenalidomide-refractory patients, to provide statistical evidence to support clinical decision making (**Supplementary Figure 1**).

EVIDENCE FROM CLINICAL TRIALS

Overall, we included 1,616 relapsed refractory MM patients (RR/MM) previously exposed to lenalidomide (lena-exposed) and 984 RR/MM patients reported to be lenalidomide refractory (lena-refractory) included in six randomized phase 3 trials (5–10). **Figure 1A** (and **Supplementary Figure 1**) reports the distribution of patients according to treatment and the presence of direct comparisons. All the groups were well balanced for presence of lena-refractory patients (about 70%, with the exception of the Castor trial which, within the lena-exposed population, only included about 50% of lena-refractory patients), exposure to bortezomib (about 65%, with the exception of the aCD38_KD group were about 90% of patients have been previously exposed to bortezomib) (**Table 1**) and patients treated in second line (about 45% in all trials, data not shown). Hazard ratios for PFS were included in our study. As reported in **Figures 1B** and **C**, all the treatments appear to be significantly superior to VD in both the lenalidomide exposed and refractory setting (with the exception of KD in the refractory group). Interestingly, DVD resulted to be significantly better than VD, KD, and PVD, slightly better than SVD (without reaching the statistical significance) and equal to both IKD and KdD in the lena-exposed population (**Supplementary Figure 2**). The same results are observed within the lena-refractory population, where DVD shows a trend of superiority over PVD and a significant advantage over both KD and VD. Looking at the P scores (the equivalent of the SUCRA score in frequentist NMA (4)), the triplets including an anti-CD38 mAb and a PI, always outperforms PVD and the doublets VD and KD (**Figure 1D**). These results are in line with

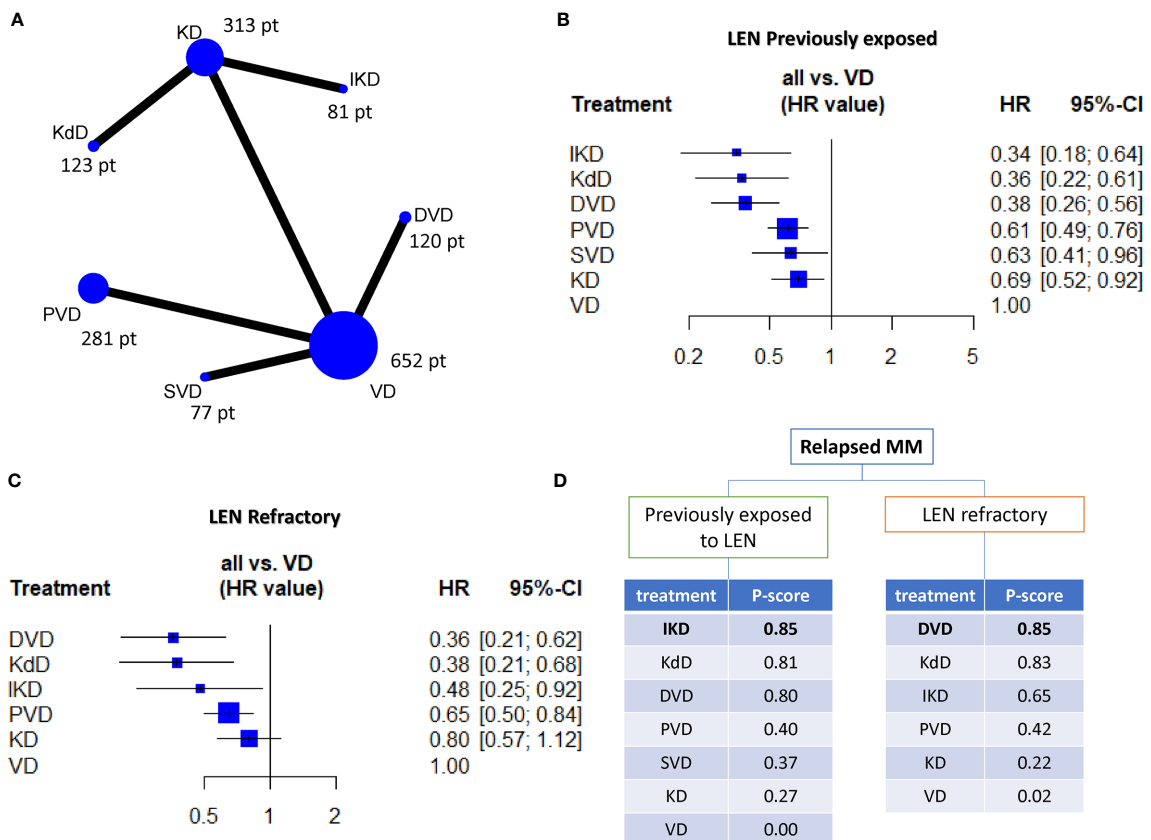

FIGURE 1 | **(A)** Network plot showing all the direct comparisons and the number of patients included in each node (i.e. the total number of patients receiving the treatment indicated in the node). **(B, C)** forest-plots indicating the efficacy of each regimen (in terms of hazards ratio (HR) and 95% confidence intervals) by using VD as comparator arms. **(D)** Ranking charts of all the evaluated regimens based on the P-score and grouped according to previous exposition or resistance to lenalidomide. VD, bortezomib-dexamethasone; DVD, daratumumab-VD; SVD, selinexor-VD; PVD, pomalidomide-VD; KD, carfilzomib-dexamethasone; KdD, daratumumab-KD; IKD, isatuximab-KD.

TABLE 1 | Main characteristics and previous treatments of the clinical trials included in the network meta-analysis.

Trials/Authors	treatment	total pts	LEN previously exposed	LEN refractory	%	BORT previously exposed	%
Castor/Palumbo	DVD	251	89	45	50,6	162	64,5
	VD	247	120	60	50,0	164	66,4
Endeavor/Dimopoulos	KD	464	177	113	63,8	250	53,9
	VD	465	177	122	68,9	252	54,2
Optimismm/Richardson	PVD	281	281	200	71,2	201	71,5
	VD	278	278	191	68,7	203	73,0
Candor/Dimopoulos	KdD	312	123	99	80,5	287	92,0
	KD	154	74	55	74,3	134	87,0
Ikema/Moreau	IKD	179	81	57	70,4	166	92,7
	KD	123	62	42	67,7	105	85,4
Boston/Dimopoulos	SVD	195	77			134	68,7
	VD	207	77			145	70,0

VD, bortezomib-dexamethasone; DVD, daratumumab-VD; SVD, selinexor-VD; PVD, pomalidomide-VD; KD, carfilzomib-dexamethasone; KdD, daratumumab-KD; IKD, isatuximab-KD.

our previous work where we demonstrated in pairwise meta-analysis the advantage of triplets over doublets in the RRMM setting (1).

DISCUSSION

Currently, no guidelines exist, which help decision making in the lenalidomide exposed or refractory setting. The last European guideline (11), indicates, due to the plethora of new agents currently available for MM treatment, to perform a class-switch whenever possible at the time of relapse, without indicating the best regimen to choose. The absence of precise indications mainly depends on the lack of direct comparisons between the available regimens together with the lack of preplanned subgroup analysis "numerically" designed to answer these questions. To overcome this limitation, we used the NMA approach, demonstrating that, whenever possible, the combination of an anti-CD38 agent with a PI should represent the first choice in order to achieve the best result in term of PFS in both the lena-exposed and lena-refractory RRMM population. However, while these results are of strong clinical interest, some limitations due to the methodology should be taken into account: for instance, the NMA is based on indirect evidence (other than on direct evidence), which could intrinsically introduce biases and on data retrieved from published studies rather than from individual patients. Additionally, while patients' characteristics are very similar between the studies included in the NMA, small or unknown differences, such as the distribution of patients according to treatment line, could impact the final results: e.g., the CASTOR trial (10) includes, within the lena-exposed group, about 50% of patients refractory to lenalidomide, which is a lower than what reported in the other trials; however the advantage of DVD was confirmed even in the lena-refractory subgroup, rendering this difference acceptable. Furthermore, few or no data are currently available on the activity of lenalidomide-based triplets or quadruplets (thus excluded from this analysis) in lena-refractory patients as well as on the efficacy of lenalidomide ramp-up in patients progressing during 10 mg maintenance. Along the same line, the efficacy of the new pomalidomide/mAbs combo regimens, which look very promising, could not be evaluated with this approach, mainly due to the fact that all the investigational clinical trials have been performed in more advanced settings (from the third line of therapy) by using (always) PD as control arm (12–14). Translational investigations, which shed light on the biologic interplay which take place within the bone marrow microenvironment (15–19) are eagerly awaited and could help to develop new therapeutic approaches in this setting. Finally, this work should be considered a snapshot of current evidence, taking into account that some of these drugs will probably move to the frontline setting.

To the best of our knowledge, this is the first NMA designed to compare the efficacy of lena-sparing regimens in RRMM previously exposed or refractory to lenalidomide. Our findings suggest that among the currently approved regimens, DVD (or KdD/IKD when available) has the highest probability of being the best treatment in both lenalidomide previously exposed or refractory setting, further underscoring how mAbs represents a very important addition to the therapeutic armamentarium available for the treatment of MM patients. However, taking into account that, even with these regimens, the reported median PFS is about 9 months, prospective randomized trials investigating new agents and combinations are needed to identify better therapeutic options for this high-risk MM population.

AUTHOR CONTRIBUTIONS

CB designed the research and analyzed data. MG and FMe supervised the analysis. EM, CCo, CCe, and AR provided analysis and discussion inputs. CB and MG wrote the paper. FD, EV, FMe, GM, GP, and FMo revised the paper and improved the discussion of the results. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.643490/full#supplementary-material

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Ocular Toxicity of Belantamab Mafodotin, an Oncological Perspective of Management in Relapsed and Refractory Multiple Myeloma

Ahsan Wahab[1†*], *Abdul Rafae*[2‡], *Kamran Mushtaq*[3‡], *Adeel Masood*[4‡], *Hamid Ehsan*[5‡], *Maria Khakwani*[6‡] *and Aqsa Khan*[7‡]

[1] Internal Medicine/Hospital Medicine Department, University of Alabama at Birmingham, Montgomery, AL, United States, [2] Internal Medicine Residency Program, McLaren Regional Medical Center, Flint, MI, United States, [3] Internal Medicine/Hospital Medicine Department, Northeast Internal Medicine Associates, LaGrange, IN, United States, [4] Hospital Medicine, TidalHealth Peninsula Regional, Salisbury, MD, United States, [5] Biomedical Sciences/Biohazardous Threat Agents & Emerging Infectious Diseases Department, Georgetown University, Washington, DC, United States, [6] Department of Medicine, Lahore Medical and Dental College, Lahore, Pakistan, [7] Department of Medicine, Fatima Jinnah Medical University, Lahore, Pakistan

Belantamab mafodotin (belamaf), an antibody-drug conjugate approved for the treatment of relapsed and refractory multiple myeloma (RRMM), is an anti B-cell maturation antigen (BCMA) agent. DREAMM-1, a first in-human trial of belamaf, reported several ocular toxicities requiring dose adjustments, dose delays and treatment discontinuations. In DREAMM-1, 53% of patients in part-1 and 63% of patients in part-2 had ocular toxicity. Similarly, 73% of patients in DREAMM-2 had keratopathy (71% in 2.5 mg/kg *versus* 75% in 3.4 mg/kg) with the most common symptoms being blurred vision and dry eyes. Ocular toxicity of belamaf is attributed to microtubule-disrupting monomethylauristatin-F (MMAF), a cytotoxic payload of the drug that causes an off-target damage to the corneal epithelial cells. Ocular adverse events (AEs) of belamaf are more frequent at higher doses compared with lower doses. Higher belamaf dose, history of dry eyes and soluble BCMA are associated with increased risk of corneal toxicity. Absence of ocular symptoms does not exclude the possibility of belamaf-induced ocular toxicity, so patients need slit lamp and Snellen visual acuity testing to detect microcytic-like epithelial changes and visual decline. Corticosteroid eyes drops for 4-7 days prior to belamaf dose do not prevent ocular AEs and may cause steroid-related AEs instead. Keratopathy and Visual Acuity scale (*KVA*) is recommended to document the severity of belamaf-induced ocular toxicity and make treatment adjustments. Management of toxicity includes dosage modifications, treatment interruption or discontinuations and preservative-free artificial tears along with close ophthalmology and hematology-oncology follow-ups.

Keywords: belantamab mafodotin, B-cell maturation antigen, ocular toxicity, multiple myeloma, management

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****Correspondence:***
Ahsan Wahab
drahsan.wahab@gmail.com

[†]This author has first authorship

[‡]These authors have contributed equally to this work

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INTRODUCTION

Belantamab mafodotin (belamaf, GSK2857916) is an antibody-drug conjugate (ADC) approved by the Food and Drug Administration (FDA) in August 2020 for the treatment of relapsed and refractory multiple myeloma (RRMM) (1). It counteracts B-cell maturation-antigen (BCMA) activity in multiple myeloma (MM). BCMA glycoprotein is naturally expressed as a cell surface transmembrane receptor of B-cells along with its ligands including proliferation-inducing ligand (APRIL) or B-cell activating factor (BAFF). BCMA regulates the differentiation of B-cells into both benign and malignant plasma cells (PCs) and is required for their longevity and survival (2, 3). Malignant PCs have a higher BCMA expression compared with non-malignant PCs. Its expression is also restricted on other organs making it an attractive target for MM therapeutics. The evidence from *in-vitro* and *in-vivo* studies indicates that overly expressed BCMA augments the proliferation and survival of malignant PCs and generates a bone marrow microenvironment that is conducive to myeloma cells proliferation (4).

BMA117159/DREAMM-1 (DRiving Excellence in Approaches to Multiple Myeloma-1) was the first in-human belamaf trial, the results of which were published in November 2018 (5). Investigators split the trial into two parts, i.e., dose-escalation and dose-expansion. There were no dose-limiting toxicities reported in the dose-escalation part, however corneal events occurred in 53% of patients. In the dose-expansion part, corneal events occurred even more frequently at 63% (5). These adverse events (AEs) were diverse including blurred vision, dry eyes, photophobia/eye pain, abnormal visual activity, and keratitis. The majority of the events were mild but caused frequent dose adjustments in the dose-expansion part. Therefore, although the results of DREAMM-1 were promising (≥ PR: 60.0%; 95% CI, 42.1–76.1, VGPR: 40%, CR: 9% and sCR: 6%, median progression-free survival: 12 months; 95% CI, 3.1-not-reached), ocular toxicity was recognized as an emerging concern, especially with larger belamaf doses (6). That is why the DREAMM-2 evaluated both the 3.4 mg/kg dose (recommended phase-2 dose (RP2D) in DREAMM-1) and 2.5 mg/kg dose (lower than RP2D) of belamaf for comparison (7). The patients in DREAMM-2 underwent systematic ocular history collection, National Eye Institute Visual Function questionnaire-25 (NEI-VFQ-25) and ophthalmic examinations. As a result of feedback from the FDA, ocular events were thoroughly evaluated in DREAMM-2 and the goal was successful development of ocular toxicity scale to guide toxicity mitigation and management strategies. Future DREAMM sequels will evaluate the role of belamaf in RRMM. This brief review focuses on belamaf-induced ocular toxicity, its mechanism of action, presentation, patient's perspectives, preventive, and management strategies.

Mechanism of Ocular Toxicity

Microtubule-disrupting monomethylauristatin-F (MMAF) is the cytotoxic component of belamaf that is linked to a monoclonal antibody *via* protease-resistant maleimidocaproyl (mc) linker. MMAF is proposed as an attributable cause of ocular toxicity along with other ADCs that used the MMAF (8, 9). ADCs may cause ocular toxicity *via* on-target or off-target processes (10). Belamaf has been previously detected in animal tears, circumstantially pointing toward its off-target damage site as the cornea lacks BCMA (11). Factors implicated to promote off-target ocular toxicity by ADCs may include linker instability or premature cleavage in extracellular environments, linker-cytotoxin intracellular metabolism, and Fc-receptor mediated cellular uptakes (10, 12). These mechanistic aspects of ocular toxicity caused by MMAF-containing ADCs may contribute to the ocular toxicity effects of belamaf. Though belamaf has a non-cleavable linker of maleimidocarproyl (mc) that provides resistance against degradation in extracellular environment, the combination of mc-MMAF in various ADCs such as SGN-75 and SGN-CD19A has known evidence of ocular toxicity (9, 13). One example of linker-cytotoxin metabolism in mc-MMAF is the intracellular liberation of ionized cytotoxic metabolite that is not capable of diffusing across the cell membrane and hence may promote localized cytotoxicity due to intracellular entrapment (10). Belamaf induces apoptosis of myeloma cells but may cause a concomitant off-target apoptosis of corneal epithelial cells due to microtubulin inhibition caused by MMAF. These corneal changes mirror as microcyst-like corneal epithelial changes (MECs) or keratopathy on slit lamp (11). MECs may occur following the first dose of belamaf but usually occur more frequently after subsequent exposures. MECs require medium to high magnification power when examined *via* slit lamp. On *in-vivo* confocal microscopy these changes may appear as hyper-reflective opacities (11). When belamaf encroaches the cornea *via* limbus vasculature or tears, it undergoes the process of solute internalization or cellular uptake *via* macropinocytosis (i.e., the process of pericellular belamaf eating without receptor-ligand interaction on cell surface) into corneal basal epithelial layer. Once belamaf is internalized into corneal cells it inhibits their proliferation eventually leading to their apoptosis. These corneal cells with swallowed-up belamaf travel away from the basal layer of cornea and approach its anterior or central parts and reflect their apoptosis with subsequent extrusion of dead cells (11). The migration of belamaf-carrying cells in the line of visual axis interferes with visual activity and causes ocular symptoms depending on their corneal location. Belamaf-containing cells and MECs are initially found at the periphery of cornea but eventually migrate in a centripetal fashion and then vanish due to extrusion (14). Theoretically, the inhibition of belamaf macropinocytosis might reduce occurrence of ocular toxicity but the practical role of such inhibition is limited. In animal and laboratory studies, the inhibition of macropinocytosis occurs *via* inhibition of membrane ruffle formation due to certain pharmacological agents such as imipramine (15). However, the clinical significance of such drugs remains unexplored in *in-vivo* and clinical trials. The mechanism of ocular toxicity requires further elucidation.

Presentation of Ocular Toxicity

In part-1 of DREAMM-1 trial where belamaf dose ranged between 0.03 mg/kg to 4.60 mg/kg, the ocular toxicity occurred

more frequently at larger doses than at smaller doses (5). Ocular toxicity occurred in 63% of patients in part-2 who received belamaf dose of 3.40 mg/kg. Blurred vision was reported in 29% (n=11) of patients in part-1 *versus* 46% (n=16) in part-2. Dry eyes were reported in 24% (n=9) of patients in part-1 *versus* 34% (n=12) in part-2 of DREAMM-1. In DREAMM-2, keratopathy was the most common ocular toxicity (73%, n=141) irrespective of its grades (71% in 2.5 mg/kg *versus* 75% in 3.4 mg/kg) and the most common complaints were also blurred vision (22% for 2.5 mg/kg *versus* 30% for 3.4 mg/kg) and dry eyes (14% for 2.5 mg/kg *versus* 23% for 3.4 mg/kg) (7). The patients with a prior history of dry eyes were more prone to develop corneal changes as shown in DREAMM-2.

When corneal changes occur in the form of keratopathy, the majority of patients are symptomatic. The absence of corneal symptoms, however, does not rule out the existence of keratopathy as indicated by the slit lamp and visual acuity testing data of DREAMM-2 participants. In the dosing cohort of 2.5 mg/kg of DREAMM-2, 72% of patients had MECs and 54% had vision changes. Contrarily, only 25% of those patients reported blurred vision and 15% reported dry eyes. Similarly, in the 3.4 mg/kg cohort of belamaf, 77% of patients had MEC, but 33% of those reported blurred vision and 25% reported dry eyes (7). This means that ocular toxicity requires active surveillance irrespective of symptoms. Even patients with grade (G)-3 or 4 keratopathies, i.e., severe superficial keratopathy and corneal ulcers may be asymptomatic. Such patients may continue to receive belamaf with ongoing toxicity unless screened *via* slit lamp (11). Keratopathy may occur somewhere between 9 days to 9 months after receiving belamaf with a median of 36 days. These events resolved in 36% of patients at median of 71 days (range, 57-99) in the dosing cohort of 2.5 mg/kg and in 28% of patients at median of 96 days (range, 70-127) in the dosing cohort of 3.4 mg/kg (7).

The exposure-safety analyses of DREAMM-2 evaluated the likelihood of G-2/G-3 corneal AEs and their relationship with belamaf concentration (16). Higher belamaf Ctau (the predicted concentration on day 21 at the end of first cycle) was associated with a lower threshold of developing G-2/G-3 corneal AEs in addition to an earlier onset of these events. A history of dry eyes and lower baseline serum concentration of soluble BCMA (sBCMA) were associated with an increased risk of G-2/G-3 corneal AEs. A history of dry eyes and baseline keratopathy prior to belamaf use were associated with a higher risk of any grade of blurred vision. Baseline keratopathy was also associated with an earlier onset of blurred vision along with an increased probability of ≥2 G-2 blurred vision (16).

The Common Terminology Criteria for Adverse Events (CTCAE v5), usually employed to grade AEs based on patients' symptoms and interference with daily functioning, may under-estimate the severity of ocular toxicity (17). Due to the limitations of CTCAE, *Keratopathy and Visual Acuity (KVA)* scale devised in DREAMM-2 (11) outlines the objective findings of slit-lamp examination and best corrected visual acuity (BCVA) regardless of symptoms. Eighteen percent (n=17) of DREAMM-2 patients (n=97) in dosing cohort of 2.5 mg/kg experienced a BCVA decline to 20/50 or worse. The transient BCVA decline was relatively common with definite worsening in a considerably smaller proportion, i.e., 82% (n=14) *versus* 18% (n=3) (11). Two patients had BCVA decline to 20/200, which is considered to be legally blind in the United States (11, 18). It is important to consider the residual ocular sequelae of previous lines of therapies among patients who receive belamaf. About 67% of patients (n=49) in DREAMM-1 and 83% of patients (n=163) in DREAMM-2 had received at least 5 lines of prior therapies (5, 7). One hundred percent patients in DREAMM-1 and 98% of patients in DREAMM-2 had received bortezomib (5, 7). Bortezomib has previously been reported to cause eye disorders such as chalazion, blepharitis, and conjunctivitis (19).

The ocular health data of DREAMM-2 shows a huge burden of ocular problems in RRMM patients prior to belamaf use perhaps due to previously used steroids and bortezomib (20). These ocular abnormalities included 60% prevalence of cataract and 43% prevalence of corneal epithelial abnormalities followed by 20% blepharitis and 6% prevalence of glaucoma. Such a poor baseline ocular status of RRMM population might be partly responsible for their predisposition to higher rates of ocular toxicity seen in DREAMM-2 in addition to belamaf. Notably, the increased number of blepharitis and dry eyes in these patients may be associated with prior bortezomib use (20). In an ongoing DREAMM-6 study (NCT03544281), belamaf is being used in combination with lenalidomide-dexamethasone (Arm-A) or bortezomib-dexamethasone (Arm-B). As of March 30, 2020, 18 patients had received treatment in parts-1 & 2 of Arm-B, i.e., belamaf-bortezomib-dexamethasone. The AEs related to cornea/eye were responsible for dose interruptions or delays in 83% of patients and dose reductions in 39% of patients (21). No patients have discontinued treatment thus far due to ocular toxicity.

Patients' Perspective and Experience of Belantamab Mafodotin

In patient-reported experience analyses of 104 patients during and following treatment in DREAMM-2, 57% of patients reported some degree of visual problems whereas 40% reported symptoms of eye irritation (dry or itchy eyes and foreign body sensation) (22). About 12% of patients reported eye pain/soreness and burning eyes. Among 26 patients who were interviewed at the end of treatment, six patients considered stopping the treatment and two of those reported an actual discontinuation based on ocular symptoms. Despite ocular complaints, patients reported high satisfaction while on treatment and expressed desire to remain on treatment (22). In patient-reported outcome (PRO) measures of DREAMM-2 (n=92), two vision-related PRO questionnaires including NEI-VFQ-25 and Ocular Surface Disease Index (OSDI) were used to evaluate the patients' symptoms and visual function both at baseline and every three weeks thereafter while on belamaf. 49.5% of patients reported 12.5-point or greater worsening on OSDI from baseline with median time to worsening of 44 days. Meaningful recovery of self-reported changes from the worst post-baseline severity was reported in 72% of patients with median time to improvement at 24 days (23).

Mitigation Strategy for Ocular Toxicity

In previous clinical trials, the use of topical corticosteroids to mitigate MMAF-related ocular toxicity showed some benefit (10, 24). Borrowing the same concept, corticosteroid eye drops were used in DREAMM-1 for 4 days starting one day before the first dose of belamaf and then before subsequent doses. As there was no clear benefit from the use of steroids in DREAMM-1, DREAMM-2 ocular sub-study (n=30) further investigated the role of steroid eye drops. The duration of topical steroids was increased from 4 days to 7 days in DREAMM-2, but their use remained ineffective in preventing corneal changes (7). Apart from being an ineffective preventive option, topical steroids may potentially cause secondary ocular AEs. The long-term follow-up of five patients in DREAMM-1 showed the development of secondary cataracts and glaucoma due to frequent steroid use requiring cataract extractions and ocular pressure lowering drugs (25). We do not recommend use of steroid eye drops as a mitigation strategy to reduce ocular toxicity of belamaf. We also recommend baseline ophthalmic examination prior to the first dose and then before subsequent doses even in the absence of symptoms per DREAMM-2 protocol (11). Though baseline eye examination and then symptom-triggered evaluations were previously thought to be sufficient to monitor ocular toxicity, the increasing evidence of asymptomatic corneal toxicity warrants ophthalmic examination before each dose to detect early corneal changes (22). To complement ophthalmic examinations, NEI-VFQ-25 and OSDI questionnaires may be used to document quality of life changes but should not be used as the only guide to treatment modifications (26). Among 17 interviewees from DREAMM-1 who received 3.4 mg/kg of belamaf, about 76% had blurred vision but 93% of them did not consider it bad enough to discontinue the treatment (27). Therefore, prescribers should incorporate the objective evidence of ocular toxicity such as the *KVA* scale into treatment-related decisions. Using this scale, mild, moderate, severe superficial keratopathy and corneal epithelial defects on slit lamp correspond to G-1 to G-4 toxicity, respectively. Decline in BCVA from baseline up to 1 line, 2-3 lines, >3 lines and 20/200 as determined by Snellen chart correspond to G1-4 ocular toxicity (11). The role of cooling eye masks and vasoconstrictors prior to the belamaf infusion is unclear and should be used at the discretion of prescriber. Cooling eye masks were used in DREAMM-2 prior to each drug infusion to reduce corneal concentration. The philosophy behind such measures is to reduce the diffusion of belamaf into the cornea and therefore minimize the ocular concentration (25, 28). We strongly recommend the use of preservative-free artificial tears among all patients at least four times a day from the day of belamaf infusion and throughout the treatment course. Patients with pre-existing corneal diseases are at higher risk of ocular toxicity. Therefore, the use of belamaf in such population is cautioned and the use of contact lens should be avoided. Patients with baseline ocular abnormalities and prior corticosteroid or bortezomib use (98-100% of belamaf population) should be monitored closely with more extensive screening and regular ocular examination.

DISCUSSION

Belamaf-induced ocular toxicity is managed with dosage modifications (dose delays, reductions) or discontinuations in addition to preservative-free artificial tears (29). **Table 1** shows the incidence of keratopathy, and treatment changes in DREAMM-1, 2 and 6 trials.

Previously many studies have documented the resolution of corneal changes associated with MMAF use after treatment changes (9, 10).We recommend that belamaf-induced ocular toxicity be managed with close ophthalmology and hematology-oncology follow-ups and use of the *KVA* scale for treatment guidance. So far there are a few reports of permanent discontinuation of belamaf based on ocular toxicity, but no case of permanent blindness has been reported. In DREAMM-1, two patients discontinued belamaf in part-1 and one discontinued belamaf in part-2 due to ocular toxicity (5, 6). Among 66% of dose reductions in part-2, blurred vision was the cause in 34% of cases whereas it caused dose interruption or delays in 40% of cases (6). Keratitis and photophobia caused belamaf interruption or delays in 9% of cases for each category. Overall, corneal events in part-2 were responsible for dose reductions in 46% and dose interruptions/delays in 49% of participants (6). In DREAMM-2, keratopathy led to belamaf discontinuation in 1% and 3% of cases in 2.5 mg/kg and 3.4 mg/kg cohorts, respectively. Keratopathy caused dose reduction in 23% and 27% of cases in 2.5 mg/kg and 3.4 mg/kg cohorts whereas it caused dose delays

TABLE 1 | Summary of belantamab mafodotin related treatment changes in DREAMM (1, 2, 6) trials due to ocular toxicity.

Trial	Belamaf cohort/arm	Incidence of keratopathy	Time to onset of keratopathy Median days (range)	Time to resolution of keratopathy Median days (range)	Treatment holidays	Dose reduction	Discontinuation of therapy
DREAMM-1 (4, 5)	Dose-expansion cohort (3.4 mg/kg)	69% (n=24/35)	23 (1–84)	35 (5-442)	49% (n=17/35)	46% (n=16/35)	2.9% (n=1/35)
DREAMM-2 (6, 15)	Cohort1 (2.5 mg/kg)	70% (n=67/95)	36 (19–143)	71 (57–99)	47% (n=45/95)	23% (n=22/95)	1% (n=1/95)
	Cohort2 (3.4 mg/kg)	74% (n=74/99)	23 (9–150)	96 (70–127)	48% (n=48/99)	27% (n=27/99)	3% (n=3/99)
DREAMM-6 (18)	Arm B (belamaf 2.5 mg/kg + Bortezomib-dexamethasone)	100% (n=18/18)	NA	NA	83% (n=15/18)	39% (n=7/18)	0% (n=0/18)

NA, not available.

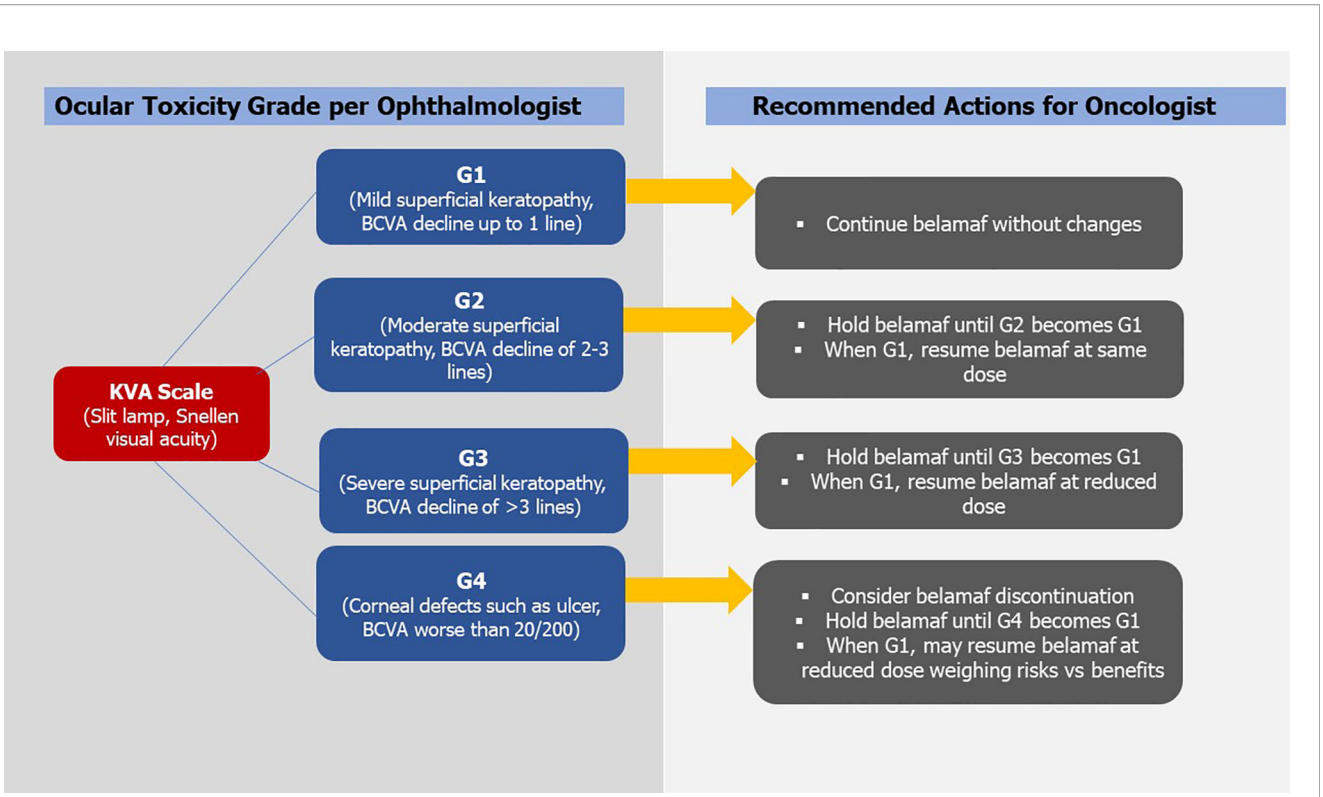

FIGURE 1 | Showing Keratopathy and Visual Acuity Scale (*KVA*) and recommended treatment modifications of Belantamab Mafodotin (BCVA, best corrected visual acuity; belamaf, belantamab mafodotin; G, grade. *Figure is derived from DREAMM-2 guidelines).

or interruptions in 47% and 48% cases, respectively (7). Corneal findings identified on either KVA or BCVA assessments can be used to make treatment modifications (11). The worst *KVA* grade in the worst eye should guide such changes. For G-1 *KVA* toxicity, belamaf may be continued without any modification. For G-2/G-3 toxicity, belamaf should be stopped until corneal/BCVA findings return to ≤ G-1. For G-2, belamaf can be resumed at the same dose but for G-3, belamaf should be resumed at a reduced dose ensuring that G-2 or G-3 ocular toxicity has improved to ≤ G-1. The G-4 *KVA* findings may require belamaf discontinuations. If the decision based on risk *vs* benefit assessment is to resume belamaf, it should be resumed at a reduced dose after the G-4 toxicity has improved to ≤ G-1 (11). When reduction in belamaf dose is required, few authors have recommended a 25% reduction in the dose, i.e., from a standard approved dose of 2.5 mg/kg to ~1.9 mg/kg (7, 25). For judicious and transparent use of belamaf considering its ocular toxicity, GlaxoSmithKline devised a Risk Evaluation and Mitigation Strategy *(REMS)* program called *BLENREP REMS* that is endorsed by the FDA (1). *REMS* ensures that prescribers are certified in the program and wholesale distributors are distributing belamaf to certified entities only. Schematic guidance of treatment modification based on the *KVA* scale derived from DREAMM-2 and *BLENREP REMS* is shown in **Figure 1**.

CONCLUSION

Belantamab mafodotin is associated with significant ocular toxicity especially in the presence of baseline ocular abnormalities. Serial ophthalmic examinations employing the *KVA* scale and artificial tears are the best strategies to mitigate toxicity. Ocular side-effects of belamaf should be managed with dose modifications, interruptions, or discontinuations. Patients should be monitored closely with ophthalmology and hematology-oncology follow-ups to ensure the safe use of belamaf.

AUTHOR CONTRIBUTIONS

AW developed the idea of study and formulated a study plan and manuscript writing. He was also involved in manuscript editing from the beginning till the end and his main section of the article were Mitigation Strategy for Ocular Toxicity and Discussion. AR helped in research strategy and literature review. KM and AM reviewed the literature and wrote the introduction section. HE, MK, and AK were involved in writing the subsections Mechanism of Ocular Toxicity, Presentation of Ocular Toxicity, and Patients' Perspective and Experience of Belantamab Mafodotin, respectively. All authors contributed to the article and approved the submitted version.

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HLA-E Binding Peptide as a Potential Therapeutic Candidate for High-Risk Multiple Myeloma

*Ying Yang, Zhuogang Liu, Hongtao Wang and Guojun Zhang**

Department of Hematology, Shengjing Hospital of China Medical University, Shenyang, China

Human leukocyte antigen-E (HLA-E) has been putatively associated with the pathogenesis of multiple myeloma (MM). Our study first showed that HLA-E was differentially expressed on MM and normal plasma cells (39.27 ± 27.01 and 11.28 ± 0.79, respectively). Based on the median value of HLA-E expression, we further stratified MM patients into high and low-expression groups, and then found high expression of HLA-E was correlated with advanced ISS stage (p = 0.025) and high-risk cytogenetics risk stratification (p = 0.000) by the Pearson Chi-square test, suggesting that HLA-E could be considered as a biomarker for high-risk MM. Furthermore, peptide 3 (P3) from our previous study was confirmed to possess a high affinity to HLA-E positive MM cells. Taken together, HLA-E could be considered as a new marker and candidate treatment target for MM, while peptide P3 may act as a potential treatment choice for targeting MM cells.

Keywords: HLA-E, high risk, multiple myeloma, clinical outcomes, target-binding peptide

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Tijana Martinov, Fred Hutchinson Cancer Research Center, United States

***Correspondence:**
Guojun Zhang
zhanggj@sj-hospital.org

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INTRODUCTION

Multiple myeloma (MM) is a common malignant hematological disease originating from plasma cells (1), and its prognosis has remarkably improved as treatment regimens have evolved into currently more popularized immunotherapies (2–6). As one type of immunotherapeutic regimens, monoclonal antibodies, such as Daratumumab (CD38 antibody), has exhibited significant treatment efficacy in both patients with MM and with relapsed/refractory MM (RRMM) (7). However, a certain percentage of MM patients have been profiled as high-risk for RRMM with much shorter progression-free survival (PFS) and overall survival (OS). Therefore, early identification of myeloma patients with a high-risk of refractory or relapse and development of targeted treatment regimen remain the priorities in the study of MM.

HLA-E is a non-classical major histocompatibility complex (MHC) class I molecule characterized by lower polymorphism, which plays a critical role in the immune response by both inhibiting and activating the function of natural killer (NK) cells (8). Studies have shown that HLA-E expression correlates with worse progression-free survival in newly diagnosed, treatment-naïve MM patients. Based on a bioinformatics analysis in our previous work, we suggest HLA-E as a potential therapeutic target for the treatment of MM (9) and designed peptides to bind HLA-E by analyzing its interaction with CD94/NKG2A. Thereafter, a peptide library was built upon the strategy of randomly replacing non-key amino acids to enhance the affinity of peptides (10),

in which the top three peptides were subjected to molecular docking analysis. Subsequently, a peptide designated as P3 (NALDEYCEDKNR) was found to have the highest affinity for HLA-E, indicating that P3 could be considered as a potential inhibitor to specifically target MM cells (9). Thus, the present study aims to continue our investigation on the clinical meaning of HLA-E expression in MM patients and further explore whether peptide P3 could target HLA-E positive myeloma cells.

MATERIALS AND METHODS

General Information

This study, which included 30 newly diagnosed multiple myeloma (NDMM) patients from January 1, 2018 to November 31, 2019, was approved by the ethics committee of Shengjing hospital of China Medical University (2020PS215K). Following the diagnoses of MM according to the International Myeloma Working Group (IMWG) guidelines for symptomatic MM (11) and acquiring patients' consents, all bone marrow samples were collected. All patients were classified according to the staging criteria (12). Patients were excluded from this study if they had histories of any immune deficiency disease, transplantation or other malignant tumor, or previous immunosuppressive therapy. For the purpose of analysis, the baseline data of gender, age, clinical stage, typing, and immunoglobulin heavy chain (IgH) quantity were recorded, while bone marrow from non-malignant patients was collected for use as the control.

Flow Cytometric (FCM) Analysis

The expressions of HLA-E, CD138, and CD45 were determined by a flow cytometer (FACS Calibur; Becton Dickinson, San Diego, CA, USA) with mouse antihuman fluorescent monoclonal antibodies [fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin-chlorophyll-protein (Percp) and allophycocyanin (APC)]. The antibodies were purchased from BD Pharmingen (San Diego, CA, USA). After incubation with antibodies in the dark for 15 min, flow cytometry with CD138++ /CD45 was performed on at least 50,000 cells for gating the viable cells. The HLA-E antigen expression was further analyzed with CellQuest software (Becton Dickinson). Target-binding peptides labeled by FITC (peptide M and peptide P3) were synthesized by Chinese Peptide (Hangzhou, China). Then, the affinity of peptides against HLA-E on the collected bone marrow from the MM patients was detected by FCM, and the binding affinity was further analyzed on the positive portion of the target-binding peptide.

Statistical Analysis

The Mann–Whitney U test was used to compare the difference between non-normal distribution data, while the Pearson chi-square test was employed to compare the correlation between HLA-E expression and clinic-pathologic parameters. MM patients with HLA-E expression were divided into high-expression and low-expression groups based on the mean value. SPSS24.0 (Chicago, IL, USA) and GraphPad PRISM 6.0 (La Jolla, CA, USA) were used for statistical analysis, and $p < 0.05$ was considered statistically significant.

RESULTS

General Characteristics

A total of 30 patients diagnosed with MM, according to IMWG guidelines, were evaluated, including 18 males and 12 females with a median age of 65 years (47-83 years). All patients received Bortezomib-based regime as the standard chemotherapy. The general characteristics of these MM patients are summarized in **Table 1**. Additionally, bone marrow from seven patients with a non-malignant hematological disease were selected as a control for the present study. High-risk cytogenetic features of MM patients were detected by fluorescence *in situ* hybridization (FISH).

Identification of High Expression of HLA-E Protein on Multiple Myeloma

HLA-E was detected in 30 MM patients and in 7 non-malignancy control patients by FCM. In MM patients, CD138 and CD38 were strongly positive in abnormal plasma cells, thus indicating that the CD138 antigen can be used to identify MM cells. According to the quantitative analysis of FCM results, the mean fluorescence intensity of HLA-E was 39.27 ± 27.01 (15.4-152.61) in MM cells and 11.28 ± 0.79 (8.82-14.33) in control cells with positive CD138. These results show that HLA-E was highly expressed in MM patients ($p < 0.05$) (**Figure 1**).

High HLA-E Expressed in Advanced Stage Multiple Myeloma

The relationship between HLA-E expression and age, gender, stage, and cytogenetic risk stratification in the 30 patients with MM was further analyzed. Considering the median expression value of HLA-E protein of 31.77, median age of 65 years old, and R-ISS staging system, the MM patients were stratified into high-expression and low-expression groups, older and younger age groups, and early stage and advanced stage groups, respectively. Furthermore, the early stage was divided into stages I and II, which included 12 patients, and the advanced stage was categorized as stage III with 18 patients. Patients were also divided into two groups based on cytogenetic risk. The Pearson Chi-square test showed that high HLA-E expression was correlated with advanced ISS stage ($p = 0.025$) and high cytogenetic risk ($p = 0.000$) (**Table 2**). Therefore, the high expression of HLA-E in advanced stage, high-risk MM patients may predict poor prognosis, indicating that HLA-E could be considered as a treatment target, especially for high-risk MM patients.

Binding Frequency of the Target-Binding Peptides to HLA-E in Multiple Myeloma

As mentioned, the results show that HLA-E was highly expressed on myeloma cells. In our previous work, the Molecular Operating Environment (MOE) software was employed to screen four target-

TABLE 1 | General characteristics of the patients with newly diagnosed MM.

Characteristics	NDMM
Gender (male/female)	18/12 (60/40%)
Age (years)	65 (47–83)
Immunoglobin types (n/%)	
IgG	12/40%
IgA	5/16.7%
IgD	1/3.3%
Light chain	11/36.7%
Non-secretory	1/3.3%
DS staging system (n/%)	
Stage I	3/10%
Stage II	9/30%
Stage III A	12/40%
Stage III B	6/20%
ISS staging system (n/%)	
Stage I	3/10%
Stage II	8/26.7%
Stage III	19/63.3%
R-ISS staging system (n/%)	
Stage I	2/6.7%
Stage II	10/33.3%
Stage III	18/60%
Cytogenetic risk factors * (n/%)	
Standard risk	21/70%
High risk	9/30%

**According to Mayo Clinic mSMART 3.0: Classification of active MM. The genetic abnormalities for high risk of MM include t(4;14); t(14;16); t(14;20); Del 17p; Gain 1q.*

binding peptides, namely M and P1-P3, for their affinity to HLA-E. Furthermore, these four peptides were synthesized and labeled with FITC fluorescein, for which the amino acid sequences are listed in **Figure 2**. The results from our previous work also indicate that P3 specifically binds to HLA-E highly expressed in cell lines with the highest affinity compared to the three other peptides. The purity and molecular weights of these peptides are provided in **Supplementary Material**. As shown in **Figure 3A**, CD138+HLA-E+ and CD138+HLA-E- myeloma cells were cultured with peptide M and P3. It was found that both peptide M and P3 could interact with CD138+HLA-E+ cells but not with CD138+HLA-E- myeloma cells. Specifically, the proportions of FITC-labeled peptide M and P3 on CD138+HLA-E+ cells were 21.97% and 53.1%, respectively (**Figures 3B, D**), but were only 3.17% and 1.65% on CD138+ HLA-E- cells (**Figures 3C, E**).

DISCUSSION

Immune function plays an important role in MM (13), whereby the absolute lymphocyte count (ALC) is related to the prognosis of MM patients (14). Therefore, a high level of ALC in NDMM patients leads to a better prognosis even in the new immunotherapy era. Although HLA-E has been screened as the key membrane antigen in the development of MM by the bioinformatics method (9), the clinical meaning of the different expressions of HLA-E in MM patients remains unknown. In the present study, we found that the HLA-E protein is highly expressed on MM cells and is linked to high-risk MM. Thus, HLA-E could be considered as both a marker of high-risk MM and a targeted candidate in a new treatment regimen for MM patients.

As a non-classical major histocompatibility complex (MHC) molecule, HLA-E can interact with NK cells and T cells (15, 16).

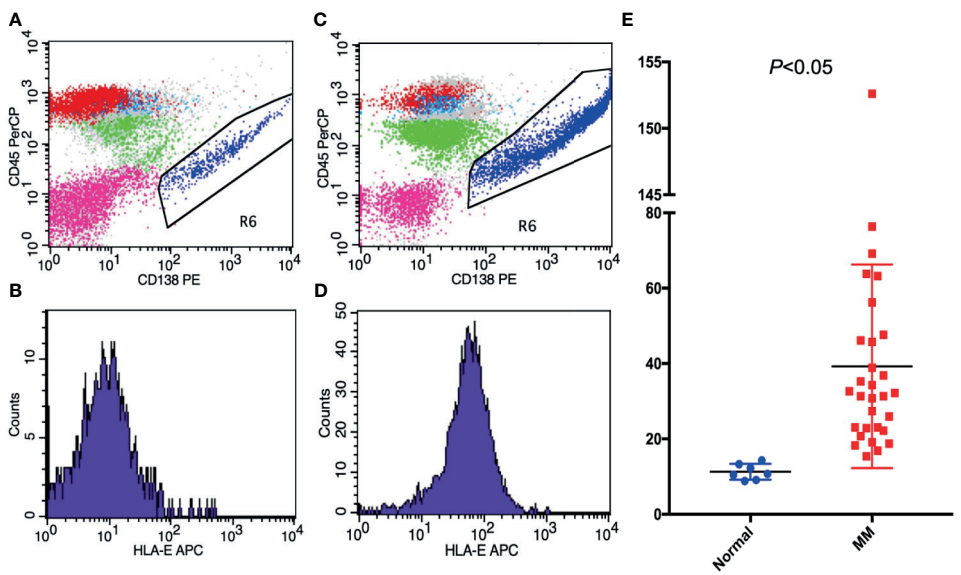

FIGURE 1 | The expressions of **(A, B)** HLA-E on non-malignant hematological patients; **(C, D)** HLA-E in MM patients; and **(E)** HLA-E between MM patients and non-malignant patients presented by mean fluorescence intensity.

TABLE 2 | The relationship between HLA-E and clinical parameters.

Characteristics	Expression of HLA-E		
	Low expression n (%)	High expression n (%)	*p*
Age (years)			
≤65	7 (43.8%)	9 (56.2%)	0.464
>65	8 (57.1%)	6 (42.9%)	
Gender			
Male	8 (44.4%)	10 (55.6%)	0.456
female	7 (58.3%)	5 (41.7%)	
R-ISS staging system			
Early stage (stage I–II)	9 (75%)	3 (25%)	**0.025**
Advanced stage (stage III)	6 (33.3%)	12 (66.7%)	
Cytogenetics risk			
Standard risk	15 (71.4%)	6 (28.6%)	**0.000**
High risk	0 (0%)	9 (100%)	

**The cutoff value of HLA-E was 31.77 based on the median expression value.*
Bold value means having statistically significant.

It has been suggested that the overexpression of HLA-E on cells could inhibit the immune clearance function (17). On some tumors, including MM, inflammatory cells and senescent cells with highly expressed HLA-E could escape the NK and T-cell immune surveillance and stay alive in the host body (18–21). Herein, we found that HLA-E was expressed much higher on MM cells than normal plasma cells, especially in NDMM patients. While cytogenetic risk factors are typically used to predict the prognosis of MM patients (22), introducing new medication, such as protease inhibitor, immunomodulatory agents, and monoclonal antibodies, could significantly improve prognoses (7, 23). However, some patients still suffer from disease progression despite these new therapies. Therefore, new prognostic markers and novel therapeutic targets should be investigated for identifying the patients with high-risk MM and improving treatment efficacy.

Furthermore, we divided MM patients into two groups based on the median HLE-A expression value of 31.77. On one hand, no statistical difference existed between the different age and sex groups. On the other hand, the group with a high risk of MM and the standard-risk group showed a significant difference in the expression of HLA-E. Comparatively, HLA-E was expressed much higher in the high-risk group than the low-risk one. This indicates that HLA-E could be considered as a marker for predicting whether the patients have high-risk myeloma or not. Besides, HLA-E might be taken as a potential treatment target for MM, especially for high-risk patients. Since HLA-E overexpression could inhibit the immune clearance function of NK cells, we propose that MM cells could be eliminated by either targeting HLA-E or inhibiting the interaction between HLA-E and NKG2A using peptide P3 (15, 24, 25). In our future work, we aim to examine the effect of peptide P3 on recovering the killing function of NK cells by inhibiting the interaction between HLA-E and NKG2A. Yet, if this recovery cannot be done by peptide P3, HLA-E could be considered as a target to find MM cells, and then a peptide drug conjugate could be produced to target MM cells.

Target-binding peptides M and P1-3, which were designed and synthesized in our previous work, could interact with the HLA-E protein in MM cell lines (9). Comparatively, peptide M exhibited the lowest binding affinity with HLA-E, while P3 showed the highest affinity. The present study further verified this finding in the bone marrow of MM patients. The results confirm that peptide P3 could bind to HLA-E positive cells but cannot interact with HLA-E negative cells in bone marrow.

CONCLUSIONS

The results of this study reveal the overexpression of HLA-E on MM cells, especially of high-risk patients, and the high binding frequency of peptide P3 to HLA-E on MM patients *in vitro*. From a therapeutic perspective, HLA-E can be considered as an effective targeting therapy against MM cells, while P3 specifically binds with HLA-E. Consequently, this interrupts the interaction between HLA-E and the inhibitory receptor

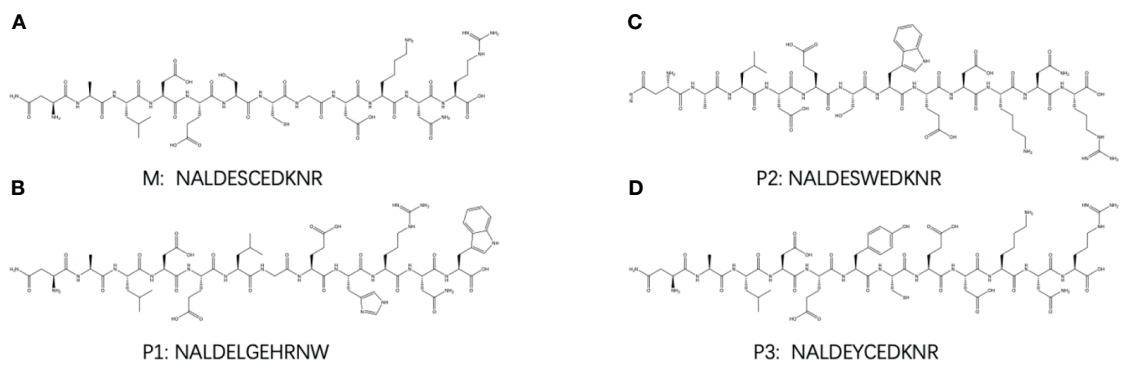

FIGURE 2 | The structure of HLA-E targeted binding peptides: **(A)** M, **(B)** P1, **(C)** P2, and **(D)** P3.

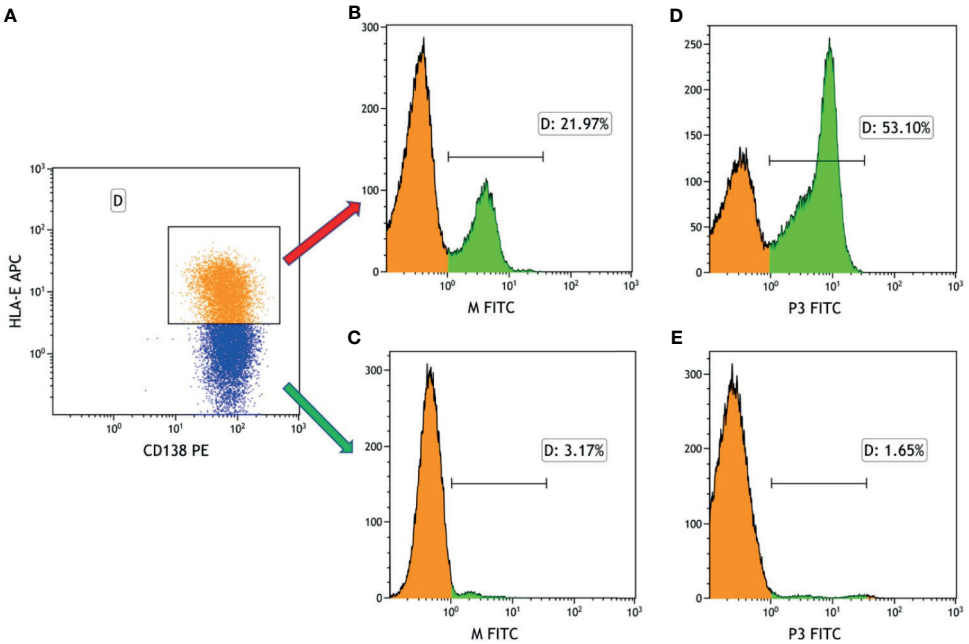

FIGURE 3 | The binding frequency of HLA-E-targeted binding peptides to the bone marrow cells from the patients with NDMM. **(A)** Myeloma cells were divided into HLA-E+ cells and HLA-E- cells. **(B)** The binding affinity of peptide M to HLA-E positive myeloma cells was 21.97%. **(C)** The affinity of peptide M to HLA-E negative myeloma cells was 3.17%. **(D)** The binding affinity of peptide P3 to HLA-E positive myeloma cells was 53.10%. **(E)** The binding affinity of peptide M to HLA-E negative myeloma cells was 1.65%.

NKG2A, providing a promising strategy to improve the immune clearance of MM cells.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by ethics committee in Shengjing hospital, 2020PS215K. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

HW and YY conceived and designed the study. YY and GZ performed the experiment and collected the data. GZ and ZL performed the data analysis and statistical analysis. YY, HW, and GZ revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.670673/full#supplementary-material

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REVIEW
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Mechanisms of Action of the New Antibodies in Use in Multiple Myeloma

Alessandra Romano[1†], Paola Storti[2†], Valentina Marchica[2], Grazia Scandura[1], Laura Notarfranchi[2], Luisa Craviotto[2,3], Francesco Di Raimondo[1,4] and Nicola Giuliani[3*]*

[1] Department of Surgery and Medical Specialties, University of Catania, Catania, Italy, [2] Department of Medicine and Surgery, University of Parma, Parma, Italy, [3] Azienda Ospedaliero-Universitaria di Parma, Parma, Italy, [4] U.O.C. Ematologia, A.O.U. Policlinico–San Marco, Catania, Italy

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***Correspondence:**
Nicola Giuliani
nicola.giuliani@unipr.it
Francesco Di Raimondo
diraimon@unict.it

†These authors have contributed equally to this work and share first authorship

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Monoclonal antibodies (mAbs) directed against antigen-specific of multiple myeloma (MM) cells have Fc-dependent immune effector mechanisms, such as complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent cellular phagocytosis (ADCP), but the choice of the antigen is crucial for the development of effective immuno-therapy in MM. Recently new immunotherapeutic options in MM patients have been developed against different myeloma-related antigens as drug conjugate-antibody, bispecific T-cell engagers (BiTEs) and chimeric antigen receptor (CAR)-T cells. In this review, we will highlight the mechanism of action of immuno-therapy currently available in clinical practice to target CD38, SLAMF7, and BCMA, focusing on the biological role of the targets and on mechanisms of actions of the different immunotherapeutic approaches underlying their advantages and disadvantages with critical review of the literature data.

Keywords: monoclonal antibodies, multiple myeloma, CD38, SLAMF7, BCMA, antibody-drug conjugate, bispecific antibodies

INTRODUCTION

Multiple Myeloma (MM) is the second most frequent hematological neoplasm, due to uncontrolled proliferation of neoplastic plasma cells (PCs) in and out the bone marrow (BM), surrounded by a permissive and protective tumor microenvironment (TME) (1, 2). The cross talk between MM cells and their surrounding TME has been a major obstacle for the development of immunotherapy. However, thanks to increasing body of evidence about the molecular arms of MM/TME interaction and the introduction of multiple novel agents (3), median patient survival prolonged from 3 to 8–10 years (4). MM PCs are strictly dependent on BM microenvironment cells and they express different molecules on the surface as receptors and adhesion molecules that exploit the function of crosstalk and adhesion with the BM microenvironment (5). Some of these molecules, such as Cluster of Differentiation 38 (CD38), signaling lymphocyte activation molecule family member 7 (SLAMF7), and B cell maturation antigen (BCMA), are highly expressed by MM PCs characterizing them as good target for novel therapeutic strategies as monoclonal antibodies (6–8).

Monoclonal antibodies (mAbs) are a group of agents with immune-based mechanism of actions that in recent years have changed the management of newly diagnosed and relapsed/refractory MM

(RRMM) (6, 9). Moreover, the development of a new generation of mAbs, including antibody-drug conjugates (ADCs) and bispecific antibodies (bsAbs) has the potential to additional improve the clinical outcome of MM patients (6, 10). Isotype dictates mAbs activity (11), and most anti-MM mAbs are IgG antibodies. The IgG subclass, allotype, and glycosylation pattern are the main factors involved in the interaction strength of the IgG-Fc domain with Fc engaging molecules, including the classical IgG-Fc receptors (FcγR), the neonatal Fc-receptor (FcRn), the Tripartite motif-containing protein 21 (TRIM21), the first component of the classical complement cascade (C1), the Fc-receptor-like receptors (FcRL4/5). The effector potential strength of the interaction between IgG mAbs and Fc engaging molecules will not be described, being out of scope of this manuscript. Several extensive and updated reviews are available about this topic (12, 13).

This review will describe main therapeutic targets in MM cells and the BM microenvironment and the mAbs in use in the anti-MM therapy focusing on their mechanism of actions and strategies to improve their efficacy.

CD38

Target Definition

Human Cyclic ADP ribose hydrolase, also known as CD38, is a 43.7 kDa type II transmembrane glycoprotein, encoded by CD38 gene located on chromosome 4 (14). E.L. Reinherz, S. Schlossman and colleagues, first identified this surface molecule in 1980 during their analysis of the human lymphocyte surface using mAbs in search of the T-cell receptor (15). Therefore, at the beginning, it was considered a marker of T cells; afterwards, it was exploited as a phenotypic marker to recognize and classify T and B leukemia (16).

Physiological Expression and Function of CD38

This molecule is widely expressed in lymphoid and myeloid lineages (14, 17–19). Resting natural killer (NKs) cells and monocytes express it at low levels, as well as other cell types belonging to the hematopoietic lineage (**Table 1**): erythrocytes, platelets, and dendritic cells (DCs) (16, 20–22). Moreover, CD38 is expressed by different T cells subtypes T cell precursors as $CD4^+CD8^+$ double-positive thymocytes (19). Within the circulating pool, CD38 is expressed by $CD4^+/CD45RA^+$ naive T cells as well as by subset of $CD4^+CD25^+FoxP3^+$ regulatory T cells (Tregs) and by a subset of memory T cells (19, 23). CD38 is also a marker of activated T cells (19). Among CD8 T cells, CD38 is strongly expressed during chronic infection. CD38 is also expressed at high levels by peripheral blood mononuclear cells upon *in vitro* and *in vivo* activation (24). Subsequentially, CD38 expression is also modified during different stages of B cell differentiation. It is present at high levels on BM B cell precursors (immature or transitional) and is downregulated in mature B cells and is expressed at high level in terminally differentiated PC (19). Moreover, CD38 is expressed at high levels in a subset of B regulatory (Bregs) $CD19^+CD24^{hi}$ cells and on IL-10-producing plasmablast with regulatory functions, on the other hand memory B cell population show a low expression of CD38 ($CD24^{hi}CD38^{lo}CD27^+$) (25). CD38 is also expressed on

TABLE 1 | Expression of CD38, SLAMF7, and BCMA in cells circulating in peripheral blood.

Cell Type		CD38	SLAMF7	BCMA
T-cells	Precursor/double positive	+	+/–	–
	$CD4^+/CD45RA^+$ naive	+	+	–
	$CD4^+CD25^+FoxP3^+$ regulatory	+ *(subset)*	+	–
	Memory	+ *(subset)*	+	–
	Activated $CD8^+$	+	++	–
B-cells	Immature/transitional	+		–
	Mature	+/–	+	+
	Memory $CD24^{hi}CD27^+$	–/+	+	+/–
	Plasma cells	++	+	++
	$CD19^+CD24^{hi}$ regulatory	++ *(subset)*	+	–
	$IL\text{-}10^+$ Plasmablast	+/– *(subset)*	+	–
NK-cells	Progenitor	+	+	–
	Resting	+	+	–
	Activated	+	+	–
Monocyte		+	+	–
Macrophage		+	+	–
Dendritic cells	Immature	+/–	+/–	–
	Mature	+	+	–
Erythrocytes		+	–/+	–
Platelets		+	+/–	–

(+: positive; -: negative; +/-: weak positivity; -/+: mostly negative).

pathological cells such as chronic lymphocytic leukemia cells, where a presence of a major clone $CD38^+$ positive is correlated with an unfavorable prognosis, and on MM cells (26). Analysis of CD38 distribution within MM bone niche revealed that only PCs express CD38 at high levels (27). Nevertheless, some studies demonstrated that CD38 expression is highly heterogeneous on MM cells, without a difference between newly diagnosed and relapsed/refractory MM patients (28). Moreover, in the MM bone microenvironment, CD38 decreases during osteoblasts (OBs) differentiation (29) and recently has been demonstrated that CD38 is expressed on the surface of early osteoclasts (OCs) progenitors but it is lost during *in-vitro* differentiation toward OCs (27).

CD38 has a dual function of receptor and enzyme. As receptor, it regulates cellular adhesion, signal transduction, and calcium signaling. CD38 interacts with hyaluronic acid and the non-substrate ligand CD31, which is constitutively expressed by endothelial cells, leading to the activation of NF-kB, ZAP-70, and ERK1/2 pathways (26, 30). It has been generally known as a receptor despite a very short cytoplasmic tail that led to an inability to transduce the signal (6). Indeed, to act as a receptor, CD38 needs to be redirected to lineage-depended receptors of the cell membrane: BCR/CD19/CD21 in B cells, CD3/TCR in T cells, and CD16/CD61 in NK cells (26, 31).

The extracellular domain of CD38 acts as an ectoenzyme that, depending on the pH, is involved in the catabolism of nicotinamide adenine dinucleotide (NAD+) and nicotinamide adenine dinucleotide phosphate (NADP+) generating calcium signaling molecules, such as adenosine (ADO), that have immunosuppressive functions (32, 33).

All these data indicate multiple roles of CD38 in MM, becoming one of the most attractive antibody targets of the immunotherapeutic approaches to inhibit MM cell growth and survival and revert immunosuppression in MM patients.

Monoclonal Antibodies Anti-CD38: *In Vitro* Molecular Rationale for Use

Several anti-CD38 antibodies have been developed in the last decade with different mechanisms of action. CD38-targeting antibodies such as daratumumab (DARA), MOR202, and isatuximab (ISA), have high single agent activity in heavily pretreated MM patients by pleiotropic mechanisms of actions (**Table 2**).

DARA is the first anti-CD38 mAb approved in MM therapy. It is a fully human immunoglobulin G1 kappa (IgG1ƙ) mAb (38) that binds two sequences of a unique CD38 epitope outside the catalytic domain (33, 39). It entered in clinical trial in 2015, for use as a monotherapy in the treatment of MM patients who had received at least three previous lines of therapy (40), now the use has been expanded to newly diagnosed MM.

ISA is a humanized IgG1k mAb, which binds to a specific 23-amino acid discontinuous epitope that include a part of the CD38 catalytic site and it has been selected due to its multiple mechanisms of actions (33, 41). ISA is now approved in combination with pomalidomide and dexamethasone, for the treatment of MM patients with who have received at least two prior therapies (42).

Anti-CD38 antibodies exert their anti-MM activity by different mechanisms of action including classical FC-dependent immune effector mechanisms, namely the antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis

TABLE 2 | Monoclonal antibodies against CD38 and SLAMF7 (Major clinical trials with published data).

Drug	Target	Manufacturer	Therapeutic format	Mechanism of action	Dose	Dose schedule	Clinical outcome in Monotherapy	Reference
Daratumumab	CD38	Janssen	naked mAb	ADCP, ADCC, CDC, cross-linking, immunomodulatory effect	16 mg/kg i.v.	Cycle 1–2 days 1, 8, 15, 22, cycles 3–6 days, cycle 7+ day 1	RRMM: ORR: 31.1%, Median PFS: 4.0 months (95% CI, 2.8–5.6 months). Median OS: 20.1 months (95% CI, 16.6 months to NE)[1]	(34)
Isatuximab	CD38	Sanofi-Aventis	naked mAb	ADCP, ADCC, CDC, direct apoptosis, adenosine inhibition	10 mg/kg i.v.	Cycle 1–4 days 1, 8, 15, 22, 29, cycle 4+ days 1, 15, cycle 18+ day 1	RRMM: ORR: 20% Median PFS: 4.6 months Median OS: 18.7 months[2]	(35)
Felzartamab (MOR202)	CD38	MorphoSys	naked mAb	ADCP, ADCC, CDC,	16 mg/kg i.v.	Days 1, 8, 15, and 22 of 28 days cycle	RRMM: ORR: 28% (+DEX)	(36)
Elotuzumab	SLAMF7	Bristol Myers Squibb/ Celgene	naked mAb	ADCC, NK cells activation	0.5–20 mg/kg	Days 1, 15	RRMM: ORR: z10%	(37)

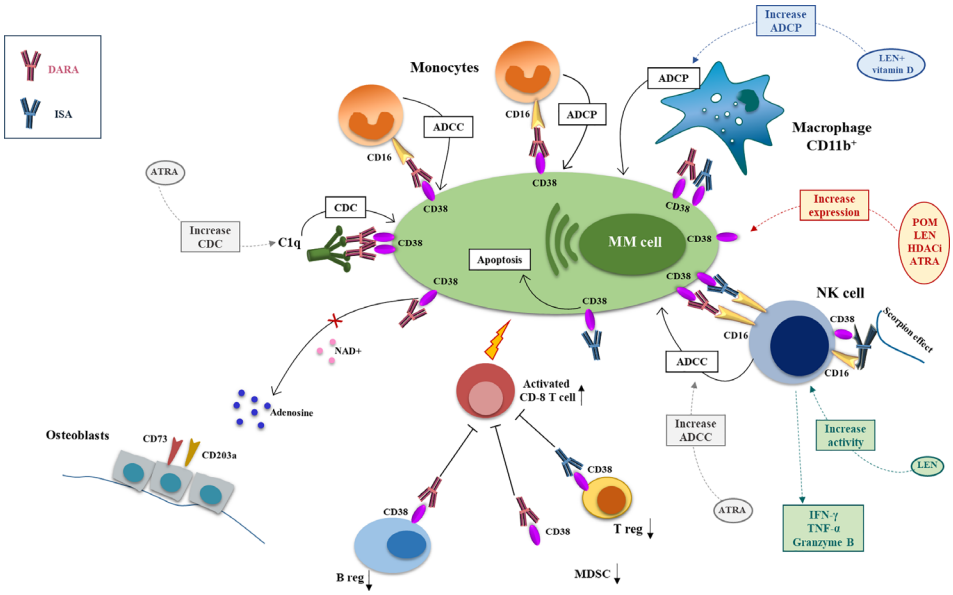

FIGURE 1 | Mechanism of action and major drug combination of anti-CD38 mAbs, daratutmumab, and isatuximab. The anti-CD38 mAbs exert their antimyeloma activity through different mechanisms of actions that can be potentiate by different anti-MM drugs. CDC is activated by engagement of the C1q by DARA and initiates the classical complement cascade and the recognition of MM cells by phagocytic cells and the production of the anaphylatoxins. This mechanism can be increased by ATRA. ADCC involves NK cell and monocytes that through CD16 recognize the anti-CD38 mAbs on MM cell surface and activate the cytotoxic process. ISA can activate directly the NK cells through the scorpion effect. NK cell activity can be boosted by ATRA and LEN. ADCP is carried by CD16+ monocytes and CD11b+ macrophage; LEN+ vitamin D can enhance anti-CD38 mAbs-mediated macrophages phagocytic activity. ISA can also have a direct anti-MM effect inducing MM cell apoptosis. DARA has also an immunomodulatory function downregulating the immunosuppressor ADO, diminishing Breg and MDSCs and activating CD8+ T cells. ISA exerts its immunomodulating potential downregulating Treg (DARA, daratumumab; ISA, isatuximab; CDC, complement depend cytotoxicity; ADCC, antibody depend cytotoxicity; ADCP, antibody depend phagocytosis; ATRA, all-trans retinoic acid; LEN, lenalidomide).

(ADCP) and the complement-dependent cytotoxicity (CDC), and direct and immunomodulatory effects (43) (**Figure 1**).

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

Anti-CD38 antibodies can bind the Fc gamma receptors (FcγRs) (44) on the immune effector cells inducing the ADCC (40).

The binding with the Fc fragment of the anti-CD38 mAbs produces the intracellular phosphorylation of the tyrosine-based activating motifs of the FcγRs that leads in the lysis of MM cells (45). In particular the cell types FcγRs-expressing that are mainly involved in the ADCC-anti-CD38 mAbs mediated are NK cells which express CD32 and CD16, monocytes expressing CD16 and macrophages $CD64^+$ (45).

NK cells are probably the main mediator of ADCC by mAbs. *In vitro* and *ex-vivo* data demonstrated that, DARA, by its binding with CD16, induces NK cells activation through the induction of STAT1 phosphorylation and activation of NF-kB p65 (46). Activated NK produced pro-inflammatory cytokines, as interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) that lead to the recruitment of immune cells and MM cells killing (45, 46). Recent *ex vivo* data report the important role of the BM adaptive NK cell, characterized by a lower expression of CD38 and high expression of NKG2C, an activating NK receptor, in the response to DARA treatment of newly diagnosed MM patients (47). In particular, this NK subset sorted from BM of MM patients have higher ADCC capacity to kill MM cell coated with DARA compared to the conventional NK cell and adaptive NK cell frequencies is correlated with DARA response *ex vivo* (47).

Moreover, DARA could also enhance $CD38^+$ NK cell apoptosis through a fratricide NK-to-NK ADCC without the involvement of tumor cells (46). This mechanism could be the basis of the rapid depletion of NK cell in MM patients after DARA treatment. Finally, also $CD14^+CD16^+$ monocytes can induce ADCC against MM cell coated by DARA (43, 48, 49).

On the other hand, *in vitro* data support that ISA induces ADCC by NK cells more efficiently against MM cells with higher density of CD38 on the surface, leading to the production of INF-γ and TNF-α (50). Moreover, it is hypothesized that ISA can directly activate NK cells through the cross-link of CD38 and CD16 on their surface (the scorpion effect) and activated NK cells can kill $CD38^{low}$ and $CD38^-$ target cells (50–52). Finally, Moreno et al. suggest that the depletion of NK cells after ISA treatment could be imputed to an exhaustion of these cells due to the higher ISA-mediated activation, rather than a fratricide mechanism (51).

Antibody-Dependent Cellular Phagocytosis (ADCP)

Phagocytosis contributes to the anti-MM activity of the anti-CD38 mAbs, as well (43). *In vitro* studies have demonstrated that

DARA-coated MM cells are rapidly engulfed by tumor-associated macrophages (53). Recently, it has been demonstrated in an *ex-vivo* assay that the $CD16^+$ subset of monocytes is essential in DARA MM cells-killing activity and the inhibition of the anti-phagocytic signal CD47-SIRPα significantly improves the DARA effect mediated by $CD16^+$ monocytes (49).

On the other hand, Moreno et al. demonstrated that *in vitro* ISA triggers ADCP by $CD11b^+$ macrophages only on MM cells that present a high level of CD38 molecules on the surface and the ability of ISA to induces ADPC also in NOD/scid/$\gamma c^{-/-}$ (NSG) mice (51).

Complement-Dependent Cytotoxicity (CDC)

The Fc tail of the anti-CD38 mAbs engage the C1q molecule and initiates the classical complement cascade, leading the deposition of C3b on MM cell surface inducing the CDC, the recognition by phagocytic cells and the production of the anaphylatoxins C3a and C5a (40, 45). This effect could be imputed to a mechanism recently described by different groups on anti-CD20 mAbs: the establishments of non-covalent interactions between the mAbs Fc tails resulting in the formation of antigen dimerization that adjuvate the constitution of antibody hexamers after antigen binding on cells that recruit and activated C1 (54–56). DARA is the most effective inducer of CDC, while ISA can induce CDC only in a few MM samples with high expression of CD38 on PCs (51). The *in vitro* CDC induction by both DARA and ISA is reduced in presence of high level of inhibitory complement regulatory proteins CD59 and CD55 on MM cells (28, 50).

Immunomodulatory Effects

As CD38 is expressed on several immune cells, anti-CD38 mAbs have also immunomodulatory effects. DARA treatment reduced $CD19^+C24^+CD38^+$ Bregs in MM patients and *in vitro* generated MDSCs ($CD11b^+CD14^-HLA\text{-}DR^-CD33^+CD15^+CD38^+$) causing a modification of the antitumor response (57). Moreover, DARA induces $CD4^+$ and $CD8^+$ T cells expansion in MM patients and in particular the effector memory $CD8^+$ T cells concomitant with a decrease of naïve T cells subset (57). Indeed, the reduction of immunosuppressive cells could lead to an increase in T-cell numbers, T-cell clonality, as well as T-cell activity contain higher levels of granzyme B after exposure to DARA (57, 58).

Like DARA, ISA reduces T regulatory cells (Tregs) and blocks the production of immune inhibitory cytokines like interleukin (IL)-10 (59). Recently it has been demonstrated that ISA also depletes $CD38^{hi}$ B lymphocyte precursors and NK cells (51).

Finally, DARA treatment possibly modulates the enzymatic activity of CD38 by reducing the ADO levels. The axis CD38/CD203a/CD73 converts NAD^+ to ADO: NAD+ reduction leads to the development of exhausted T cells and adenosine has an immunosuppressive effect on NK and $CD8^+$ cells (60, 61). Van de Donk et al. showed that DARA reduces CD38 cyclase activity, increasing NAD^+ levels and decreasing ADO levels (58). Indeed, targeting CD38 with anti-CD38 mAbs could restore the immune functions.

Direct Effects

ISA was selected initially based on its *in vitro* ability to directly induce MM cell death independently of effector cells and independently of Fc fragment binding to FcRs by binding the CD38 activating the classical caspase, lysosome death pathways, lysosomal membrane permeabilization, and cathepsin hydrolase release (62). Moreover, it is reported that ISA could induce reactive oxygen species production and promote to MM cell death (62). In contrast with these data, a recent paper reported no direct killing activity on ISA on MM cell *in vitro* (51).

In contrast, DARA did not show a direct killing effect on MM cells (43).

Mechanisms to Potentiate the Effects of Anti-CD38 mAbs in MM

Several pre-clinical studies indicate that the effects of anti-CD38 may be potentiated with other drugs and compounds. A synergistic effect between DARA and lenalidomide (LEN) in the induction of ADCC cytotoxicity has been previously demonstrated (63). Indeed, it is known that LEN stimulates NK cell increasing their production of IFN-γ, TNF-α, and granzyme B (64). Interestingly, an up-regulation of DARA-dependent ADCC was described in peripheral blood mononucleated cells (PBMCs) isolated from MM patients during or just after LEN treatment, thus further supporting the potential benefits from this combination (63).

Other studies showed that LEN enhances DARA-induced MM cell lysis by an increased frequency of CD3−CD56+ NK cells, with no alterations of T cell and monocyte compartments, even in patients refractory to LEN (65). Consistently, data obtained in humanized mice engrafted with MM cells from LEN refractory patients confirmed the capacity of LEN to potentiate the DARA effect (65). Accordingly, Van der Veer et al. (66) showed that the synergism between DARA and LEN/bortezomib treatment was more prominent in $CD138^+$ $CD38^+$ cells of MM patients refractory to LEN (66).

More recently, it has been also suggested that also vitamin D can potentiate the synergism between LEN and anti-CD38 mAbs combination mediated by the increase of the ADCP (67) due to LEN ability to induce CYP27B1 expression in macrophages (68). DARA-LEN synergism could be also due to the decrease of the frequency of inhibitory T cell populations induced by LEN (69). Indeed, it has been demonstrated that LEN up-regulates CD38 expression on Tregs and increases the fraction of CD38-high Tregs sensitizing this population to the anti-CD38, ISA (59).

Our group previously demonstrated that LEN and pomalidomide (POM) up-regulate CD38 expression by MM cells (70). This finding was recently confirmed by others (71): showing that the activity of DARA in combination with LEN was correlated by the increased CD38 surface expression by MM cells but not by NK cells by LEN (71). Lastly, Jiang et al. (62) showed that POM, enhances anti-CD38 mAbs effect both by the direct killing of MM cells, and by the indirect cytotoxicity effect (62). Interestingly it has been also reported that POM synergized with ISA in CD38-high MM cells with mutated p53 (62) supporting

the use of POM and ISA combination in this type of high-risk MM patients.

Panobinostat is a pan-HDACi able to increase the expression of CD38 by PCs but not T cells (72) and consequently to potentiate the effect of DARA (72). Ricolinostat also increases CD38 expression on the surface of MM cells and it augments the ADCC by DARA against MM cell lines but not CDC effect (73).

Other drugs could be also used to increase the efficacy of anti-CD38 mAbs through the modulation of CD38 expression by MM cells and/or the effector cells. Different studies showed that agents such all-trans-Retinoic acid (ATRA) can be used to improve the effect of DARA and to overcome its resistance by increasing the expression of CD38 in MM cell (28). Nijhof et al. showed that treatment with ATRA significantly increased the expression of CD38 enhancing DARA-induced ADCC and CDC (28). The mechanism involving the modulation of CD38 by ATRA can be explained by the presence of a retinoic acid responsive element located in the first intron of the CD38 gene (74). Interestingly, treatment with ATRA also reduced the expression of CD55 and CD59 in MM cells (75). These studies clearly provide the rationale to design clinical trial with ATRA and DARA in refractory MM patients.

Bispecific Antibodies Against CD38

An antibody containing two different antigen-binding sites within one molecule is known as a bispecific antibody (BsAb). In particular, Bispecific T-cell engaging (BiTE) antibodies are a new class of drugs that can bind both a specific antigen on the surface of the tumor cells and the CD3e chain on T cells (76). BsAbs that target CD38 are in developing the last years and some of them are under evaluation in Phase I studies.

AMG424 is a novel CD38/CD3 BiTE, and recently it has been reported that AMG 424 can kill cancer cells expressing high and low levels of CD38 *in vitro* and increases T-cell proliferation, but with attenuated cytokine release (77). However, since CD38 is expressed in normal immune cells and non-hematopoietic tissues, it is associated with off-target toxicity (77). A phase 1 first-in-human trial (NCT03445663) of the drug in patients with R/R MM started in July 2018.

This year, it has been published a new BiTe against CD38: Bi38-3. Bi38-3 is made of two single-chain variable fragments anti-human CD38 and CD3e; it activates T-cell-mediated lysis of $CD38^+$ MM cells *in vitro*, *ex vivo*, and *in vivo*. Moreover, it has been reported that it has no toxicity on B, T, and NK cells *in vitro* (78). Furthermore, Bi38-3 triggers the killing of MM cells from resistant patients and, since it recognizes a specific epitope on the Fc region of CD38, could be efficient also in patients after daratumumab therapy (78).

SLAMF7/CS1/CD319

Target Definition

The signaling lymphocyte SLAMF7, also known as CRACC or CD319 is encoded by SLAMF7 gene present on chromosome 1 at locus 1q23-24 (79, 80), is a 66kDa glycoprotein member of the SLAM superfamily. The SLAM family include several related CD2 subset of the immunoglobulin superfamily of receptors expressed on the surface of a wide variety of hematopoietic cells, including CD150, CD48, CD244, CD229, CD84 NK-T-B-antigen (NTB-A), also known as SF2000 in human or Ly108 in mouse, CD352, CD319, B lymphocyte activator macrophage expressed (BLAME, Slamf8), and SF2001 (CD84H, Slamf9) (79).

Like most SLAM receptors, SLAMF7/CS1 is a self-ligand, which exert activating or inhibitory influences on cells of the immune system depending on cellular context and the availability of effector proteins (81, 82).

SLAMF7 contains a membrane proximal C-type Ig fold and a membrane distal V-type Ig, a cytoplasmic region including two immunoreceptor tyrosine-based switch motifs (ITSM). The phosphorylation of tyrosine-based motifs in SLAMF7 induces downstream molecules activation including PLCγ1, PLCγ2, and PI3K kinases, regulating a variety of cell functions. SLAM receptors triggered by homo- or heterotypic cell-cell interactions control the activation and differentiation of a wide variety of immune cells and the interplay between innate and adaptive immune response. Downstream signaling is mediated by recruitment of small cytoplasmic adapter proteins, namely SH2D1A/SAP and/or SH2D1B/EAT-2. In humans, SLAMF7/CS1 has two splice variants, constitutively expressed on NK cells, namely a long-form CS1-L and a short form CS1-S, which lacks an ITSM motif required for NK cells activation described above (83). CS1L mediates NK cell activation through a SH2D1B/EAT-2 dependent, SH2D1A/SAP-independent extracellular signal-regulated ERK-mediated pathway. Thus, SLAMF7 can act as an activator if it can bind EAT-2, otherwise it is an inhibitor of downstream signaling (82).

Physiological Expression and Function of SLAMF7

SLAMF7, first described in NK cells (84, 85) and macrophages, is involved in numerous functions, including PCs survival, cell adhesion, NK cell– and CD8 T cell–mediated cytotoxicity (79). Several other hematopoietic cells express SLAMF7, including myeloid cells, activated T cells, most B cells, including antibody-producing PCs (81).

In NK cells, SLAMF7 is usually a positive regulator of NK cell activation, as consequence of the binding with the SAP family adaptor Ewing's sarcoma-associated transcript 2 (EAT-2) *via* phosphorylated tyrosine 281 (Y281) in its cytoplasmic segment, thereby triggering activating signals involving phospholipase C-γ (PLC-γ) (86, 87) to induce polarization of cytotoxic granules (86). In the absence of EAT-2, or in excess of another adapter protein SAP (87), SLAMF7 recruits SHIP-1 and mediates inhibitory effects, as found in NK cells derived from EAT-2-deficient mice, and in normal activated T cells (81) and MM PCs (82), which lack EAT-2.

Expression and Function of SLAMF7/CS1 in Multiple Myeloma

Primary myeloma cells and human myeloma cell lines express higher levels of SLAMF7 than the normal or reactive

counterpart, as consequence of genetic derangements (**Table 1**). In particular, cancer cells carrying the translocation t(4; 14) seem to have a greater expression of SLAMF7 and the *in vitro* inhibition of the expression of SLAMF7 in these cells is able to reduce the formation of colonies and to induce apoptosis and arrest of cells in G1, thus indicating an important role of this receptor in the proliferation of MM cells (88). The promoter region of SLAMF7/CS1 can bind the identity marker of PCs Blimp-1 (B-lymphocyte-induced maturation protein-1), which is dysregulated in MM, to enhance SLAMF7/CS1 transcription (89). This finding could explain why increased expression of SLAMF7 has been reported also in other B-cell disorders, like chronic lymphocytic leukemia and diffuse large B cell lymphoma (90). High levels of soluble SLAMF7 (sCS1) (91, 92) and increased mRNA of SLAMF7 in purified PCs have been documented in patients affected by monoclonal gammopathies in the entire spectrum, from monoclonal gammopathy of unknown significance (MGUS) through smoldering-, active-, and relapsed-refractory MM relapsed patients (93) and in autoimmune diseases, like systemic lupus erythematosus (94), or systemic infections in response to IFN-α stimulation (80).

In 199 newly diagnosed MM patients, the amount of sCS1 was positively associated to active MM and not appreciated in healthy or stage I MM patients (95). Increased sCS1 was associated to most aggressive presentation, in both newly diagnosed and relapsed MM-patients, associated to lower probability to achieve deep response and reduced progression free survival, even if it could not be shown as an emerging independent prognostic factor (91).

Monoclonal Antibodies Anti-SLAMF7: *In Vitro* Molecular Rationale for Use of Elotuzumab

Elotuzumab is a humanized IgG1 mAb directed selectively against SLAMF7, unable to induce direct or complement-mediated lysis of MM cells, as shown *in vitro* and by absence of clinical activity as single agent (82, 96) (**Table 2**). In MM cells, high SLAMF7 expression is not able to induce neither proliferation nor apoptosis, due to lack of both EAT-2 (required to activate downstream signaling) and SHIP-2 (required to inhibit of downstream signaling) (82). Targeting SLAMF7/CS1 *in vitro* inhibited cell viability of human MM cell lines co-cultured with bone-marrow stromal cells (BMSCs) in a dose-dependent fashion, overcoming the stimulatory and protective effects of microenviroment on MM growth and survival (95), implying that its efficacy occurred through an indirect mechanism.

Elotuzumab can induce SLAMF7 expression in NK cells and acting as self-ligand can amplify its molecular effect (97). Favoring the homotypic SLAMF7-SLAMF7 interaction between NK and MM cells, elotuzumab further promotes natural cytotoxicity in a CD16-independent manner (85). Indeed, an Fc mutant form of Elotuzumab, unable to bind CD16, could promote cytotoxicity of SLAMF7+ target cells by NK cells derived from healthy donors, in particular when these cells were previously exposed to IL2. Therefore, it is likely that additional effects of Elotuzumab on immune system are based on involvement of other SLAMF7+ cells such as CD8+ lymphocytes (98), dendritic cells, activated monocytes, and dendritic plasmacytoid cells (99), as shown by reduced efficacy in CD8+ T- cells-depleted mice (98). Elotuzumab may inhibit the plasmocytoid dendritic cells that play an important role within the microenvironment that support MM cells growth and survival (99).

In Vivo Effects of Elotuzumab in MM and Strategies to Improve Clinical Efficacy of Immunotherapy Addressed Against SLAMF7

In vivo, most activity of elotuzumab can be attributed to NK-cells engagement *via* two main mechanisms. First, ADCC triggering, *via* engagement of FCγRIIIa/CD16a (93, 95). Second, a direct binding of elotuzumab's Fab domain with the SLAMF7 receptor enhances EAT-2 recruitment to promote NK cells activation (81, 82, 84, 85, 87, 97, 100, 101). For this reason, differently from daratumumab, another mAb used in the same setting of relapsed/refractory (RRMM) patients, elotuzumab does not affect viability of NK cells (102), explaining the increasing interest for this molecule in the emerging adoptive CAR NK-cell therapies (103) (**Figure 2**).

To increase the cytotoxic effect of elotuzumab several strategies have been explored. In general, the combination with drugs able to induce SLAMF7 expression, recruit NK-cells and promoting ADCC is been extensively evalauted and it is highly recommended. In the pivotal studies, Van Rhee et al. treated SCID-human xenograft mice and documented that antitumor activity was enhanced if the MM cells were pretreated with bortezomib, even if pretreatment with bortezomib did not affect SLAMF7 expression (96).

In mice, the combination of lenalidomide and elotuzumab was very effective in reducing tumor volume and increasing the infiltration of NK cells into the tumor microenvironment, an effect enhanced by IL-2 secreted by T cells and TNF-alpha produced by monocytes (101) and macrophages (104). According to observation that Elotuzumab is able to reduce tumor burden and prolong survival in a MM model with SCID-beige mouse lacking B-, T-cells and with a reduced NK function, a further mechanism of action has been recently proposed. Elotuzumab could recruit monocytes, promote the infiltration of M1-polarized tumor-associated macrophages with enhanced ADCP of MM cells through engagement of the Fcγ receptor (104).

Immunomodulatory drugs (IMiDs) look like the ideal backbone to combine with Elotuzumab for their direct and indirect effects on both T- and NK-cells function (64, 105, 106), as shown in the relapsed/refractory setting by trials ELOQUENT-2, which tested efficacy and safety of elotuzumab combined to lenalidomide and dexamethasone (107, 108) and ELOQUENT-3 study, which tested efficacy and safety of elotuzumab combined to pomalidomide and dexamethasone (109). Unfortunately, the ELOQUENT-1 trial, which evaluated elotuzumab combined to lenalidomide and dexamethasone in

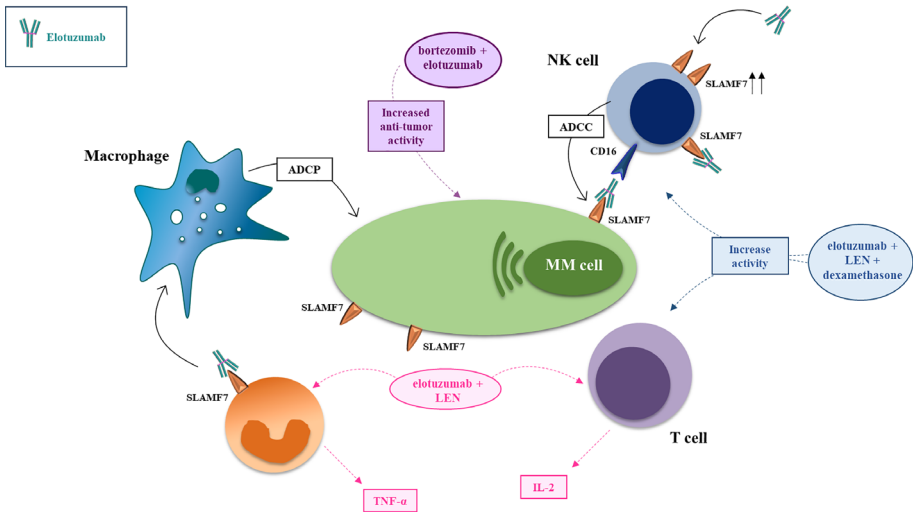

FIGURE 2 | Mechanism of action and major drug combination of the anti-SLAMF7 mAb elotuzumab. The anti-SLAMF7 mAb elotuzumab exerts anti-MM effects *via* several indirect mechanisms: (i) promoting macrophage-mediated antibody-dependent cellular phagocytosis (ADCP) engaging co-stimulatory signaling to enhance ADCP in macrophages expressing both SLAMF7 and EAT-2; (ii) facilitating NK cell-mediated antibody-dependent cellular cytotoxicity (ADCC) of myeloma cells through Fc-dependent interactions with CD16 (FcγRIIIA); (iii) enhancing co-stimulatory signaling in NK cells, thereby potentiating natural cytotoxicity of myeloma cells, *via* simultaneous engagement of ITAM-linked activating receptors on NK cells (e.g. NKp46 or CD16) with ligands on myeloma cells; (iv) tagging myeloma cells for cell recognition; (v) elimination of immunosuppressive CD8+CD28–CD57+ Tregs which overexpress SLAMF7. In combination with proteasome inhibitors (e.g. bortezomib, carfilzomib) or immunomodulators (e.g. lenalidomide, pomalidomide), elotuzumab enhances anti-tumor effects *via* activation of T-cells and NK-cells.

the setting of newly diagnosed, transplantation-ineligible MM patients, failed to demonstrate additive clinical activity of elotuzumab (110).

Elotuzumab can be combined with other mAbs to increase the activity of effector cells, like the checkpoint inhibitor pembrolizumab (111) to promote tumor-infiltrating NK and CD8+ T-cell activation, intratumoral cytokine and chemokine release (98).

Elotuzumab has ben used to arm an anti-CD3 (OKT3) antibody to develop a bispecific antibody-armed activated T cell to induce adaptive cellular and humoral immune responses in MM patients, to mediate MM cytotoxicity independently from major histocompatibility complex. *Ex vivo* arming unarmed activated T cells avoids the need to administer large quantities (mg/kg) of bispecific antibody to reduce adverse events, like cytokine release syndrome. This strategy utilizes humoral antibody targeting by ATC. Secretion of Th1 cytokines upon binding of the effector cells to the myeloma cells not only augments tumoricidal activity directed at the malignant B cells, but may increase local cytokine and chemokine secretion that leads to shifting the tumor microenvironment to recruit endogenous immune effectors and induce an endogenous immune response (112). The targeting domain derived from elotuzumab has been used to develop T cells expressing an SLAMF7 CAR, with promising activity in preclinical models *in vitro* and *in vivo*, leading to ongoing phase 1/2a clinical trials CARAMBA and MELANI-01 (113).

Finally, a SLAMF7-targeted mAb ahs been conjugated with a payload drug (e.g. DM1, DM4, SN38, MMAE, MMAF) through a linker (e.g. SMCC, SPDB, MC-Vc-PAB). Azintuxizumab vedotin (ABBV-838) was the first-in-class antibody-drug conjugate (ADC) in which a SLAMF7-targeted mAb was linked to monomethyl auristatin E (MMAE) *via* a cathepsin B-cleavable peptide linker. Two phase I clinical trials have been started but in June 2017 AbbVie decided to terminate the phase-I/Ib trial NCT02462525 for insufficient clinical activity (114), with only 10% of overall response rate (115).

THE BAFF-APRIL-BCMA SYSTEM

Target Definition

The persistence of normal and neoplastic plasma cells (PCs) depends on survival factors provided in the bone marrow as consequence of direct contact to mesenchymal stromal cells (116) or the B-Cell Maturation Antigen (*BCMA*) triggering, induced by its two ligands, namely, B-cell-Activating Factor (*BAFF*; *BLys* and *CD257*) (117) and A PRoliferation-Inducing Ligand (*APRIL; CD256*), that are respectively produced mainly by macrophages (118) and osteoclasts (119, 120).

BCMA (also referred as TNFRSF17, CD269) is a transmembrane glycoprotein belonging to the tumor necrosis factor superfamily, selectively induced during B-cell differentiation into plasmablasts and bone marrow PCs (121), neoplastic PCs (120, 122), while it is nearly absent on naive and memory B cells (121, 122) CD34 stem cells, and other normal tissue cells (123) (**Table 1**). In normal and neoplastic mouse plasma cells, and in the human MM cell line MM1.s, the BCMA

expression is under control of the master plasma cell gene IRF4 (124, 125), even if the post-translational regulation of BCMA can be largely compensated for reduced transcription, by mechanisms still under investigation (124).

Following stimulation with APRIL or BAFF, BCMA becomes a trimer, eliciting a signaling cascade involved in the activation of MAP kinases and the induction of anti-apoptotic proteins, such as Bcl-2, Bcl-XL, and the antiapoptotic protein myeloid cell leukemia 1 (MCL-1) (126).

The BAFF-APRIL-BCMA System Regulates Plasma Cells Homeostasis

BAFF is required for homeostasis and maintaining normal B-cell development, and survival of malignant B- and PCs, by increasing the levels of the pro-survival molecules B cell lymphoma 2 (Bcl-2) and Bcl-x and by decreasing the levels of the proapoptotic molecule Bcl-2-homologous antagonist/killer (Bak) (127, 128). $BCMA^{ko}$ mice have shorter survival of long-lived bone marrow PCs compared to wild-type controls while maintaining a normal phenotype (129). BAFF binds mainly the BAFF Receptor (BAFF-R), which triggers naïve B cell survival and maturation. During B-cell development BAFF-R is first expressed on immature B cells with the highest expression levels on transitional and mature B cells and decreased levels on germinal center B cells, while BCMA and TACI (Transmembrane activator and CAML interactor) are expressed in a more restricted manner and support the survival of PCs. BAFF/BCMA binding activates NF-kB and the MAPK8/JNK signaling pathways, to sustain long-term humoral immunity, survival, and proliferation to regulate B cell antibody responses, isotype switching, and homeostasis (130). While BAFF is required for B cell homeostasis, the excessive production of BAFF is detrimental to the host. Transgenic mice overexpressing BAFF suffer from increased production of autoantibodies and symptoms of autoimmune diseases (130–132).

APRIL binds to BCMA with higher affinity interaction than BAFF to prevent activation of the endoplasmic reticulum (ER)-associated Casp12 contributing to maintenance of long-lived PCs in the niche (116). APRIL can also bind heparin sulfate proteoglycans to potentiate TACI and BCMA activation through its multimerization (133, 134), but the underlying molecular mechanisms are still largely unknown.

BAFF and APRIL are equally potent in inducing bone marrow plasma cell survival. *TACI* mediates the BAFF- and APRIL-induced generation of PCs and T cell-independent immunoglobulin isotype switching and secretion, whereas the function of *BCMA* is restricted to the maintenance of PCs and antigen presentation by B cells, through the activation of AKT, MAPK, and *via* NF-kB (120).

BCMA is shed from the surface of PCs *via* γ-secretase–mediated cleavage, with consequent releases of soluble BCMA (sBCMA). sBCMA acts as a decoy neutralizing APRIL (135) and sequesters B-cell activating factor BAFF (136), thereby preventing it from performing its signaling to stimulate normal B-cell and plasma cell development, resulting in reduced polyclonal antibody levels (136).

The BAFF-APRIL-BCMA System in Multiple Myeloma

BCMA is detectable in malignant PCs throughout the duration of the disease, with progressive increased expression from monoclonal gammopathy of uncertain significance (MGUS) to smoldering myeloma to active MM, with the highest levels correlated to the worst prognosis (137). In MM patients, BCMA mRNA is upregulated in PCs, and in CD138– progenitor cells responsible for recurrences (138). Conversely, a downregulation of BCMA reduces the viability and formation of myeloma colonies (120). *In vivo*, BCMA-overexpressing tumors increased neo-angiogenesis and transcription of genes crucial for osteoclast activation, adhesion, and angiogenesis/metastasis, as well as genes mediating immune inhibition including programmed death ligand 1 (PDL1), transforming growth factor β (TGF- β), and interleukin 10 (IL-10) to orchestrate the complex interplay between myeloma and microenvironment cells (120).

Soluble BCMA (sBCMA) is higher in supernatants of mononuclear cell cultures of MM-BM than the marrow of healthy subjects (135), progressively increased from healthy, MGUS, and active MM, and higher in patients with progressive disease, associated to reduced overall survival (139). In mice, sBCMA levels correlated with the change in tumor volume in response to melphalan or cyclophosphamide with bortezomib (139). *In vivo*, sBCMA correlated with the percentage of bone marrow PCs, even in patients with non-secretory myeloma and with the depth of response to treatment. Patients with levels above the median had shorter progression free survival and overall survival (140). Normalization of sBCMA during any treatment was predictor of increased overall response rate, overall survival (141), and achievement of complete response (141). Currently, sBCMA (139, 140) and sTACI are investigated as novel biomarker of disease activity in B-cell disorders with prognostic value (142), with two main advantages: independence from renal kidney and shorter half-life (24–36 h) than monoclonal components IgG (21 days) and IgA (7 days), or free light chains (140, 141).

Based on the above-mentioned circuitry, BCMA has been recently evaluated as highly selective antigen for neoplastic PCs, representing that tumor associated antigen ideal for the development of target therapy (143). Moreover, due to lack of expression on B-cell precursors, a rapid recovery of cell B immunity could be expected upon discontinuation of anti BCMA treatment.

How to Target the BAFF-APRIL-BCMA System in Multiple Myeloma: Tabalumab

Tabalumab (LY 2127399) is an-anti BAFF human mAb developed by Eli Lilly and Company, designed for the treatment of autoimmune diseases and B cell malignancies (144). In MM, two phase II studies, conducted in US and Japanese cohorts, failed to show any clinical improvement in progression free survival (145–147), probably for high BAFF concentrations in RRMM patients or the induction of compensatory pathways *via* APRIL/BCMA engagement (148–

151), suggesting the need to combine tabalumab with anti-APRIL antibody or TACI-Fc fusion protein, a potent inhibitor of both BAFF and APRIL, to augment clinical efficacy in RRMM.

How to Target BCMA in Multiple Myeloma: The Antibodies Drug Conjugates

Currently, the development of immunotherapy against BCMA is directed towards three approaches: antibodies drug conjugates (ADCs), the bispecific antibodies, and the CAR-T cells, that have been recently described in a comprehensive review (**Tables 3** and **4**) (163).

ADC technologies combine *mAbs* (generally IgG1 due to the availability of multiple lysines required for optimal conjugation), selective for the antigen on the target cell, with *toxic payloads* (generally targeting microtubules or DNA duplication) (164, 165) and a *linker* between the antibody and the cytotoxic agent, as extensively described in excellent reviews (166). Based on ADC design, linkers can be stable in serum or in the circulation, thus after the initial internalization antigen/ADC complex is followed by its complete degradation in the lysosome (166). Otherwise, linkers could be cleaved only under certain specific conditions to ensure drug delivery. For example, the hydrazine linkers are susceptible to acidic conditions, the disulfide linkers to reducing equivalents (glutathione), and the peptide linkers to proteases (164, 165, 167, 168).

The first anti-BCMA mAb *cSG1* was initially evaluated by Seattle Genetics in 2007, developed both as a naked mAb (not further tested in large clinical trials) as well as with a drug

TABLE 3 | BsAbs against BCMA in clinical development (Major clinical trials with published data).

Drug	Target	Manufacturer	Therapeutic format and Mechanism of action	Dose	Dose schedule	Clinical outcome in Monotherapy	Reference
AMG420 (former BI 836909)	BCMA/ CD3ϵ	Boehringer Ingelheim/ Amgen	Bispecific single-chain variable fragment with hexahistidine tag antibody	0.2–800 µg/day I.V.	4 weeks of continuous I.V. infusion over a 6-weeks treatment cycle	RRMM, ORR 31%, 70% at 400 µg/d (N = 7)	(152)
Pavurutamab (AMG701)	BCMA/ CD3ϵ	Amgen	Bispecific single-chain variable fragment with hexahistidine tag antibody	Phase I dose-escalation study	4 weeks of continuous I.V. infusion over a 6-weeks treatment cycle	RRMM, ORR 26%, 83% at 18 mg dose (N = 6)	(153)
CC-93269 (former BCMA-TCB2/ EM-901)	BCMA/ CD3 (Dual BCMA binding site)	Celgene	Asymmetric two-arm IgG1-based human bispecific T-cell engaging antibody. In EM 901the heterodimeric Fc region has intact FCRn binding site	Phase I dose-escalation study	I.V. @ on days 1, 8, 15, and 22 of cycles 1 to 3, on days 1 and 15 of cycles 4 to 6, and on day 1 of cycle 7	RRMM, ORR 43%, 89% at 10 mg dose (N = 9)	(154)
TNB-383B	BCMA/ CD3 (Dual BCMA binding site)	TeneoBio and Abbvie	T-cell engaging bispecific antibody, with unique selective activating anti-CD3 moiety, two heavy-chain-only anti-BCMA moieties for a 2:1 tumor associated antigen to CD3 stoichiometry, with an IgG4 silenced backbone to reduce nonspecific T-cell activation	Phase I dose-escalation study	1–2 h I.V. infusions every 3 weeks	RRMM, ORR 47%, 80% at 40–60 mg doses (N = 15)	(155)
Elranatamab (PF-06863135)	BCMA/ CD3	Pfizer Alexo Therapeutics Kodiak Sciences	Fully human IgG CD3 bispecific molecule, with IgG2A backbone	Phase I dose-escalation study, 80–360 µg/kg (SC) 0.1–50 µg/kg (I.V.)	Weekly subcutaneous	RRMM, ORR 53%, 80% at 215–1,000 µg/kg mg doses (N = 20)	(156)
Teclistamab (JNJ-64007957)	BCMA/ CD3	Janssen Pharmaceutical Companies	DuoBody. Bispecific IgG1 molecule generated by controlled Fab-arm exchange of two separated mAbs	80–3,000 µg/kg (SC) 0.3–720 µg/kg (I.V.)	Weekly I.V./SC	RRMM ORR 64%,	(157)
REGN5458	BCMA/ CD3	Regeneron and Sanofi	BCMA x CD3 bispecific antibody	Phase I dose-escalation study 3–96 mg	Weekly I.V. ×16, then every 2 weeks	RRMM ORR 39%, 63% at 96 mg dose (N = 8)	(158)

TABLE 4 | ADCs against BCMA in clinical development.

Drug	Target	Manufacturer	Therapeutic format and Mechanism of action	Dose	Dose schedule	Clinical outcome in Monotherapy	Reference
Belantamab (former GSK2857916)	BCMA	GSK	mAb: afucosylated IgG1 humanized αBCMA linker: non-cleavable, protease resistant payload: MMAF	3.4 mg/kg 2.5 mg/kg	30–60 min I.V. infusions every 3 weeks	RRMM ORR 60% RRMM ORR 31%	(159) (160)
AMG224	BCMA	Amgen	mAb: IgG1 linker: not cleavable payload: mertansine	Phase I dose-escalation study, 30–300 mg	60 min I.V. infusions every 3 weeks	RRMM ORR 23%	(161)
MEDI2228	BCMA	AstraZeneca	mAb: IgG1 linker: valine-alanine protease cleavable payload: tesirine	Phase I dose-escalation study 0.0125–0.20 mg/kg	I.V. infusions every 3 weeks	RRMM ORR 66% at 0.14 mg/kg dose (N = 41)	(162)

conjugate (ADC) (143). Alone, or in combination with bortezomib or lenalidomide, cSG1was able to induce cytotoxicity of myeloma cells *in vitro*, even in the presence of BMSCs, and to reduce the migratory capacity of MM cells through the inhibition of NFkB (120).

The first-in-class anti-BCMA ADC investigated in clinical trials is *Belantamab Mafodotin* (GSK2857916). The *Belantamab Mafodotin* platform has three peculiarities: i) afucosylated IgG1to ensure the highest affinity for the FCγRIIIa/CD16a receptor of the effector cells to mediate ADCC; ii) a protease non-cleavable linker, to avoid serum degradation and release of the payload outside the cell of interest. The linker is cleaved inside the cell; iii) a powerful cytotoxic agent as payload, monomethyl auristatin (MMAF), designed to be much more active when actively delivered inside cells with a mAb, compared to treatment in the untargeted form. Thanks to its peculiar structure, *Belantamab Mafodotin* has different mechanisms of action:

1) induces the arrest of MM cells in G2/M phase resulting in apoptosis
2) induces a powerful ADCC *via* binding of the defucosylated Fc fragment of NK and PBMC cells
3) induces ADCP *via* binding of the defucosylated Fc fragment of macrophages
4) competes with BAFF and APRIL, reducing their signal of activation of NFkB
5) reduces activity of $BCMA^+$ dendritic plasmacytoid cells which support proliferation and drug resistance of MM cells (169).

Belantamab Mafodotin was first tested in both disseminated and subcutaneous human MM xenograft mouse models where it was shown to induce a complete eradication of the neoplasm without inducing weight loss of the mice, thus confirming the absence of toxicity (170). Subsequently, it was investigated in phase I (171) and phase II trials (160), with encouraging about 30% of overall response in penta-refractory patients and now is being tested in combination with lenalidomide and pomalidomide for patients with relapsed/refractory MM (143), as recently described in several recent comprehensive reviews (172–180). The most common grade 3–4 adverse events include: keratopathy (181, 182), thrombocytopenia, and anemia (160). Blurred vision, keratitis, dry eye, and microcystic epithelial damage are typically associated to ADCs due to o non-specific ADC uptake into actively dividing basal epithelial limbal stem cells residing in the basal epithelial layer of the cornea (183).

Future developments to improve drug-induced toxicities include the combination of Belantamab Mafodotin with both immunomodulatory drugs and proteasome inhibitors, extending dosing intervals (i.e. every 4–6 week dosing versus every 3 week dosing) (179), together with the clinical studies involving other anti-BCMA ADCs with several promising different payloads (184).

Other BCMA-targeting ADCs include AMG-224, CC-99712, SG1-auristatin, MEDI228, and HDP-101, as summarized in very recent comprehensive reviews (172, 185–187).

AMG 224 is an antihuman BCMA IgG1 antibody conjugated with mertansine, an antitubulin maytansinoid, through a non-cleavable linker. In the dose escalation NCT02561962 phase 1 trial, 40 patients received intravenous AMG 224 every 3 weeks at prespecified doses of 30–300 mg in a 3 + 3 design, with no mandated pre-medications. The objective response rate (ORR) for the study was 23%, including six responses in dose escalation and three responses in the dose expansion. In the dose escalation cohort, the most common AEs include thrombocytopenia, fatigue, nausea, AST increase, and anemia (161).

In *MEDI2228* a fully human BCMA-binding IgG1 antibody is conjugated to DNA-damaging agent pyrrolobenzodiazepine (PBD) *via* a protease-cleavable linker, showing higher clinical activity than to a monomethyl auristatin F (MMAF) analog, also in the presence of high levels of sBCMA, due to induced DNA damage responses (DDR) and synergized with multiple DDR-inhibitors. A phase 1 dose-escalation/-expansion study of MEDI2228 as monotherapy in relapsed/refractory patients is currently ongoing (NCT03489525).

In *HDP-101* a BCMA-specific antibody is conjugated to the RNA polymerase inhibitor amanitin, a synthetic derivative belonging to the amatoxin family, identified more than 40 years ago in the mushroom *Amanita phalloides*. These substances, responsible for severe hepatotoxicity secondary to the ingestion of these fungi, bind with high affinity to RNA Polymerase II, thus reducing transcription and protein synthesis and are effective against both rapidly dividing and resting cells.

Based on preclinical studies, HDP-101 has large clinical activity in models with a knockout of tumor suppressor TP53 and knockdown of RNA polymerase POLR2A, which mimics the deletion of 17p in a subtype of high-risk MM patients. Preclinical data have shown that HDP-101 has significant anti-tumor activity both *in vitro* and on xenograft models and results of the clinical study are expected in 2021 (188).

How to Target BCMA in Multiple Myeloma: The Bispecific Antibodies and Beyond

A further challenge of immunotherapy is retargeting the effector cells (NK-cells, macrophages, T-cells) to provide rapid activation, robust and durable cytotoxic responses, and potentially generate immunologic memory (189). The engagement of CD3 (part of the T-cell receptor) induces both proliferation of CD4 and CD8 T-cells and cytotoxic activity by CD8 and in part CD4 cells against the target. The engagement of CD3 is the major proliferation signal, even though there may be additional indirect mechanism of proliferation induction by cytokines. Thus, the interaction between the patient's own T lymphocytes and the tumor cells expressing a specific antigen could be facilitated, to eliminate cancer without genetic alteration of the T cells or need for *ex vivo* expansion/manipulation, providing off-the-shelf immuno-oncotherapy (189, 190). In this scenario, the class of bispecific antibodies (bsAbs), also known as dual-targeting molecules, includes antibodies or derived proteins engineered to have multiple binding sites, each with a unique antigen specificity, to different epitopes, to physically bridge two or more cells.

There are two major factors which could affect pharmacokinetics (191) of bsAbs immunotherapy: i) the binding to FcRn (neonatal Fc receptor), which in turn mediates the long half-life of IgG molecules *in vivo* and it is involved in transcytosis from the vascular space out into tissue compartments; ii) the potential higher immunogenicity of anti-drug antibodies due to the presence of non-natural structural motifs (192). To this end, Fc mutations have been heavily employed in new generation mAbs to modify interaction with FC-γ-receptors, to increase or decrease CDC, ADCC, and ADCP. Mutations to modify FcRn binding are also attempted by different groups to modify pharmacokinetics, but these have not reached the clinic (191). ADC hapten-like structure across eight molecules tested in 11 phase I–II clinical trials do not appear to increase patient immune responses beyond those generally observed for mAb biotherapeutics (193), but data lack in MM setting. It is still under investigation if larger molecules could hard penetrate the tumor, especially when extramedullary bulky masses are present.

Two formats of bsAbs have been extensively studied in MM: BiTE (Bispecific T-cell engager, developed by Amgen, Thousand Oaks, CA, USA) (10, 184, 190, 194) and DuoBody (developed by Genmab A/S, Copenhagen, Denmark). In the BiTE molecules, binding domains are two single-chain variable fragment (scFv) regions, arised from mAbs, joined by a flexible peptide linker: one, to recognize tumor-expressed antigens, and another to engage effector T-cells. The second scFv binding domain is always specific for CD3, the invariable part of the T-cell receptor complex. When a BiTE molecule engages both a cytotoxic T cell and a tumor cell, the T cells start to proliferate, increasing overall numbers of effector cells and strengthening the potency of BiTE therapy (195). Once the cytolytic synapse has occurred, the T cells release perforin and granzyme B, thus inducing the apoptosis of the tumor cells. Furthermore, the activation of lymphocytes induces the release of cytokines that amplify the immunological response by involving other immune cells and induce a proliferation of T cells (189, 190, 196).

AMG 420 (formerly BI 836909) was the first anti-BCMA BiTE used in relapsed/refractory MM patients. AMG 420 is a bispecific single-chain variable fragment consisting of two linked single-chain variable fragments (scFvs) (197). The BCMA scFv is positioned N-terminally, and the CD3εscFvC-terminally followed by a hexahistidine (His6tag) (197). *In vitro* experiments have documented that both T lymphocyte subpopulations ($CD4^+$ and $CD8^+$) contribute to the antibody-induced lysis of MM cells, associated to autologous T-cell activation, documented by increased secretion of IFNγ, IL-2, IL-6, IL-10, and TNFα in a dose dependent manner, in T-cells obtained from both newly diagnosed and RRMM patients (**Figure 3**). The maximum cytolytic activity was reached between 16 and 24 h, greater in presence of peripheral blood mononuclear cells (PBMCs), suggesting the engagement of other blood cells. The cytolytic activity of AMG420 was not affected by the co-culture of MM cells with stromal cells, that usually confer drug resistance, or in presence of soluble APRIL and BCMA, which could interfere with or bind the antibody. The same encouraging results have been obtained *in vivo*, in both mouse xenograft models with the insertion of human T cells and in cynomolgus monkeys, where a dose-dependent decrease of bone marrow PCs could be documented as well (197). Clinical application of AMG420 was very promising in RRMM setting, with a response rate of 70% at the maximum tolerated dose, including for half patients the achievement of MRD-negative complete response (152). However, further clinical development has been stopped, due to the short half-life of AMG420, requiring a continuous infusion of 4 weeks, and the high rate of infections, mainly due to the requirement of a catheter for i.v. injection.

In the attempt to improve drug manageability and prolong its clearance, new bsAbs have been engineered to have a Fc moiety which makes them more like a complete antibody (198). Among them, *AMG701* has a mean elimination half-life of 112 h (4.7 days) and it is able to induce a potent T-cell-dependent cellular toxicity against BCMA positive MM cell lines, together with a dose-dependent T-cell activation a cytokine secretion. *In vivo*, it was able to inhibit growth of tumor xenografts, prolong survival of an orthotopic mouse xenograft model and deplete MM cells in cynomolgus monkeys (199). In RRMM patients, AMG 701 potently induced autologous cell lysis, T-cell proliferation, and differentiation leading to higher CD8/CD4 ratios, acting synergistically with lenalidomide and pomalidomide to prevent myeloma relapse *in vivo* (200). The interim analysis of the Phase 1 dose escalation trial (NCT03287908) presented at ASH 2020 (153) provided encouraging signs of activity as a single agent in

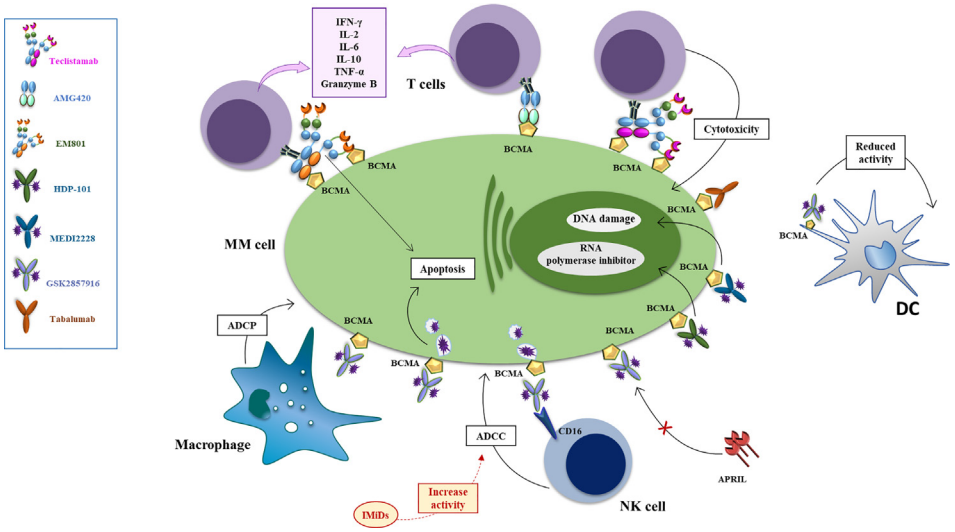

FIGURE 3 | Mechanisms of action of anti-BCMA mAb, antibody drug conjugates and bispecific antibodies. The antibody drug conjugate Belantamab mafodotin exerts anti-MM effect *via* several mechanisms: i. inducing ADCP *via* binding of the defucosylated Fc fragment of macrophages the arrest of MM cells in G2/M phase resulting in apoptosis; ii. inducing a powerful ADCC *via* binding of the defucosylated Fc fragment of NK and PBMC cells (an effect enhanced by combination with lenalidomide) iii. competing with BAFF and APRIL, reducing their signal of activation of NFkB in MM cells (an effect enhanced by combination with bortezomib) iv. reducing activity of BCMA+dendritic plasmacytoid cells which support proliferation and drug resistance of MM cells. Upon binding with MM cells *via* BCMA, MEDI2228 releases pyrrolobenzodiazepine to promote DNA damage and cell death, while HDP-101 releases the RNA polymerase inhibitor amanitin, to reduce transcription and protein synthesis, resulting in apoptosis of both rapidly dividing and resting cells. AMG 224 is an antihuman BCMA IgG1 antibody conjugated with mertansine, to inhibit the assembly of microtubules with consequent cell death. Tabalumab (LY 2127399) is an-anti BAFF human naked monoclonal antibody that neutralizes the membrane-bound and soluble forms of this factor, reducing their signal of activation of NFkB in MM cells. Bispecific monoclonal antibodies can simultaneously bind to two different types of antigen to engage effector cells against neoplastic cells. EM-801 and AMG-420 are two examples of BCMA/CD3 bispecific T-cell engager. Teclistamab is a BCMA/CD3 DuoBody.

heavily pre-treated MM patients. AMG 701 was given to 85 R/R MM patients who had received at least three prior lines of therapy, and a median of six lines. The response rate was 36% at doses of 3–18 mg with responses lasting up to 26 months in one patient. Six of seven patients, who were tested for minimal residual disease (MRD), were MRD-negative. In the most recent evaluable cohort, there was an 83% ORR, with 4/5 responders being triple refractory (153).

EM801 is an asymmetric IgG1 bsAb first developed by EngMab AG with Celgene. EM801 incorporates bivalent binding to BCMA, a head-to-tail fusion of BCMA- and CD3ε-binding Fab domains and an engineered Fc region, with completely abolished binding to FcγRs and C1q, carrying a heterodimeric Fc region with intact FcRn binding. The molecular structure enables prolonged half-life to approximately 4 days, thus allowing the possibility of intravenous or subcutaneous administration once a week (201). *In vitro*, EM801 induces a strong, dose dependent bond between T lymphocytes and MM cells with consequent activation of T cells, documented by the hyper-expression of CD25 and CD69 and release of granzyme B and inflammatory cytokines such as IFNγ, TNFα, and IL-2 (**Figure 3**). At an E:T ratio of 2:1, 5:1, and 10:1 EM801 induces a killing of MM cells of 60, 70, and 80% respectively and the depletion of a subpopulation of T lymphocytes ($CD4^+$ or $CD8^+$) not significantly reduces the cytocidal effect of the antibody. Experiments conducted on primary MM cells have documented that EM801 was able to induce mortality in 77 and 83% primary samples obtained respectively from newly diagnosed and RRMM patients, without toxicity on microenvironmental cells. *In vivo*, anticancer activity has been documented in both mouse models and in cynomolgus monkeys, where a single administration induced a significant reduction of BCMA+ cells after only 24 h. A clinical study documented almost a 90% overall response rate, including 44.4% sCR/CR and more than 90% of MRD negativity achievement, with a good toxicity profile (184).

The further development of *CC-93269 (former EM901)*, a human IgG1-based T-cell engager that binds to BCMA and CD3 epsilon in a 2 + 1 format, is promising as well, as shown by Dr. Costa at ASH 2019 (154). In a dose-escalation trial of 30 patients with relapsed or refractory disease, the 10-mg dose of CC-93269 induced responses in 89% of patients, including complete or stringent complete remission in 44%. Minimal residual disease negativity was achieved by 92% of responders. Although cytokine-release syndrome occurred in about three-quarters of patients, cases were mostly grade 1 or 2, and it tapered off after the first dose.

TNB-383B and *TNB-384B* have been developed by Tenebio based on *in silico* analysis of heavy chain only/fixed light chain antibody sequences (202). *TNB-383B* is a BCMA x CD3 bispecific T-cell redirecting antibody incorporating an activating a unique anti-CD3 moiety (selective in the -383B platform, pan-T-cell activator in the -384B platform), two heavy-

chain-only anti-BCMA moieties for a 2:1 tumor associated antigen to CD3 stoichiometry, and a silenced human IgG4 Fc tail (203). The bivalent BCMA binding reduces APRIL competition, conferring high specificity and avidity to the anti-BCMA moieties. Differently from other pan-T cell activating T-bsAbs that can overstimulate T cells, *TNB-383B* preferentially activates effector over regulatory T-cells and uncouple cytokine release from anti-tumor activity, induce PC lysis, regardless of very high or low E:T ratio (203), reducing the CRS risk, as shown in further clinical development (155, 202, 203). Data presented at last 2020 ASH meeting showed a favorable safety profile in patients with R/R MM and achieved an overall response rate of 80% at doses ≥40 mg every 3 weeks. The most common adverse events were cytokine release syndrome, fatigue, headache, anemia, infection, and nausea. TNB-383B was well tolerated at doses up to 40 mg, without the need for step/split dosing. A preliminary ORR of 52% (12/23) was observed at doses ≥5.4 mg, including deep (6 PR/3 VGPR/3 CR) and durable (up to 24 weeks) responses despite dosing only every 3 weeks (155).

PF-06863135 (Elranatamab) is an anti-BCMA x anti-CD3 BsAb that consists of targeting arms within an IgG2a Fc backbone, given with a weekly subcutaneous (SC) infusion to allow higher doses than intravenous administration without increasing adverse events (156, 204). Recent update about its safety and efficacy in RRMM patients has been reported at ASH 2020 by Dr. Lesokhin. Responses were achieved with SC dosing of Elranatamab in 6 of 8 (75%) patients at the two highest dose levels evaluated. However, the enrollment of the phase-2 trial MagnetisMM-3 has been paused in May 2021 in US due to peripheral neuropathy.

Novel mechanisms of drug resistance are emerging, like the loss or reduction of BCMA antigen, requiring alternative antigens to target. A promising antigen is the G-protein-coupled receptor class 5 member D (GPRC5D), expressed selectively at high levels in MM cells and associated to inferior outcome in MM patients, independently from BCMA expression, even if its function and ligand is still largely known (205).

Talquetamab (JNJ-64407564) is a GPRC5D x CD3 DuoBody able, *in vitro*, to induce cytotoxicity independently from the number of BCMA receptors or the amount of sBCMA, and, *in vivo*, to recruit T cells at the tumor site, without affecting humoral immunity due to lack of expression on B memory cells. Robust preclinical data provided the rationale for the ongoing phase NCT03399799I clinical trial (122).

Cevostamab (BFCR4350A) is a new bispecific antibody developed by Roche that simultaneously binds to the CD3 protein on immune T-cells and a portion of the Fc receptor-like protein 5 (FcRH5), a protein receptor found in nearly all myeloma cells, more highly expressed in cell carrying 1q21 abnormalities (206). Preliminary data discussed at ASH 2020 showed ORR of about 53%, irrespective of target expression level in patients. Deep and durable responses were observed in patients with high-risk cytogenetics, triple-class refractory disease, and/or prior exposure to anti-CD38 monoclonal antibodies, CAR T cells, or antibody-drug conjugates (207). In peripheral blood, Cevostamab induced robust CD8+ T-cell activation and proliferation and IFN-γ induction at active doses (3.6 mg), up to 20-fold higher than at baseline. CD8+ tumor-infiltrating T-cell levels were higher on treatment in responders than in non-responders, and T-cell expansion by end of the first cycle was more pronounced in responders than in non-responders, irrespective of baseline CD8+ T-cell levels (208).

The recent discovery that, under physiological conditions, IgG4 can engage Fab-arms exchange (209), has prompted the technology of DuoBody platform (195, 210, 211). In Duobodies, introducing matched mutations at the CH3 interfaces creates a IgG1 bispecific antibody favoring and then stabilizing Fab arm exchange, for the generation of stable bispecific IgG1 antibodies in which heavy and light chain homodimers from two different antibodies form a single heterodimeric bispecific antibody (211–214).

Teclistamab (JNJ-64007957) is a DuoBody bsAb that induces T cell-mediated cytotoxicity against BCMA-expressing myeloma cells, independently from the amount of sBCMA, APRIL, or BAFF (Pillarisetti et al., 2020). *Teclistamab* has shown to be highly active *in vitro* on immortalized and primary myeloma cells, obtained also from daratumumab-refractory patients (215). Teclistamab is currently being evaluated in a Phase 2 clinical study for the treatment of relapsed or refractory multiple myeloma (NCT04557098) and is also being explored in combination studies (NCT04586426, NCT04108195). At ASCO 2020 congress, Dr. Usmani presented excellent preliminary results from the ongoing study of weekly teclistamab in RRMM (NCT03145181), with manageable safety across all doses explored and 78% overall response rate (102). However, the efficacy of Teclistamab was inversely related to the PDL-1 expression on RRMM cells, thus ongoing trials are investigating the possibility of overcoming this resistance using a combination with PDL1 inhibitors (216). Additional partners for combination therapy include γ-secretase inhibitors which potentiate Teclistamab killing capacity by elevating BCMA surface expression (215).

HPN217 is a tri-specific T cell activating construct (TriTAC) consisting of three binding domains: an N-terminal single domain antibody (sdAb) that binds to human BCMA, a middle sdAb that binds to human serum albumin (HSA), and a C-terminal single chain Fv (scFv) that binds to CD3ϵ of the T cell receptor (TCR) complex (217). The *in vitro* pharmacological activity of HPN217 was evaluated by T cell-dependent cellular cytotoxicity (TDCC) assays. In co-cultures of T cells from normal human or cynomolgus monkey donors, target tumor cells, and HSA, HPN217 mediated dose-dependent and BCMA-dependent cytotoxicity with EC50 values ranging from 0.05 to 0.7 nM. Killing was dependent on expression of BCMA on target tumor cells.

Non-clinical *in vivo* properties of HPN217 were evaluated in xenograft models and a single dose pharmacokinetic (PK) study in cynomolgus monkeys. HPN217 mediated dose-dependent growth suppression against the RPMI-8226 MM model and Jeko-1 mantle cell lymphoma model expressing relatively low

levels of 5,600 and 2,200 copies of BCMA per cell, respectively. Serum half-life, volume of distribution, and clearance appeared to be independent of dose. HPN217 was demonstrated to be stable and remained intact up to 3 weeks *in vivo* as demonstrated by a functional ligand binding assay using recombinant CD3ϵ and BCMA, respectively, to capture and detect HPN217. Importantly, serum samples collected 1 week after dosing were as potent as stock HPN217 in MM tumor cell killing in TDCC assays.

RO7297089 is a potent therapeutic agent *in vitro* and selectively kills BCMA expressing MM-PCs by activating innate immunity, *via* ADCC and ADCP, with low incidence of acute cytokine release. In a 1-month repeat-dose study in cynomolgus monkeys, RO7297089 was well tolerated, and there were no test article-related adverse effects at up to 50 mg/kg, with no significant cytokine release. RO7297089 represents a novel and promising MOA with a favorable safety profile, distinct from the T cell-based BCMA-targeting modalities in the clinic (218, 219).

CTX-4419, is a first-in-class NKp30xBCMA bispecific, able to induce cytokine production, NK-cell proliferation, and potent tumor cell killing of target cells, independently from high, intermediate, or low BCMA expression. Differently BCMA-IgG1 mAbs can activate NK cells in the absence of CD16A engagement (220).

2A9-MICA consists of human MICA extracellular region and a single-chain antibody fragment (scFv) that targets BCMA generated by phage display technology. *In vitro*, 2A9-MICA activated NK cell-mediated cytotoxicity and induced NK cells to kill BCMA-positive human myeloma cells. Moreover, in BCMA-positive, MM-bearing nude mice, 2A9-MICA specifically targeted tumor tissue, where it effectively recruited immune cells and inhibited tumor tissue growth showed superior antitumor activity (221).

BLOCKING PD-1/PDL-1 AXIS BY MABS IN MM

Several experimental pre-clinical data indicate that PD-L1/PD-1 blockade by mAbs provided promising anti-MM effects. *In vitro* PD-L1/PD-1 blockade overcame BM MSC-mediated MM growth and directly enhanced NK and T cell mediated anti-MM responses (222, 223). MM cells by PD-L1 expression inhibit the activity of CTLs, acquiring a proliferative advantage which results in immune evasion and resistance to anti-myeloma drugs (224). *In vivo* PD-L1 blockade prolonged mice survival after stem-cell transplantation (225–228) as well as PD-1 blockade also prolonged the survival in disseminated myeloma-bearing mice (228, 229), by mainly acting on $CD4^+$ or $CD8^+$ T cells (229). In these models, PD-1 expression on both $CD8^+$ and $CD4^+$ T cells was higher in mice with advanced MM as compared to non-tumor bearing ones.

mAbs targeting the PD-1/PD-L1 axis can be divided into two different groups: (i) those against the PD-1 receptor and (ii) those against the ligands (PD-L1/PD-L2). Nivolumab, pembrolizumab, and pidilizumab are the main anti-PD-1 mAbs used whereas anti-PD-L1 mAbs are durvalumab and atezolizumab. However, despite promising pre-clinical data, the use of mAbs antiPD-1/PD-L1 mAbs as single agents did not show a significant clinical effect in relapsed refractory MM. On the other hand, the phase III trial evaluating lenalidomide and dexamethasone in combination with pembrolizumab in patients with MM presented unexpected safety findings and was discontinued. Accordingly, the other clinical trials anti PD-1/PD-L1 mAbs in combination with IMiDs have been interrupted. Actually the identification of the best MM patients candidate to the treatment with PD-1/PD-L1 blockade is still be unknown.

CONCLUSIONS: CHALLENGES AND PERSPECTIVES OF IMMUNOTHERAPY IN MULTIPLE MYELOMA

Immunotherapy is revolutionizing the therapeutic scenario of both newly diagnosed and refractory-relapsed MM patients. Novel challenges are emerging on how to choose the target and the therapeutic format as summarized in **Table 5**, the best sequential approach, timing, and patients' characteristics.

CAR-T cell therapy against BCMA is one of the most powerful single-agent for RRMM patients (with ORR range 50–90% across the studies), but it is affected by logistical constrains, with up to 20% drop-out rate in the manufacturing time (4–7 weeks) for complications associated with disease progression (230). To overcome these limitations, reduce the high costs and face with exhaustion of manufacturing capacities of centralized and highly specialized production facilities, some technological improvements are ongoing, including virus-free gene transfer, automated point-of-care production and allogeneic cell products to provide off-the-shelf CAR-T cells products (113, 231, 232).

The limited persistence and lack of survival plateau is an additional limitation of CAR-T cells in MM. Some authors suggest to identify upfront (e.g. high risk patients with extramedullary presentation or adverse cytogenetics or biallelic TP53 inactivation) or early during the treatment (e.g. after induction or at first minimal residual disease detection after autologous stem cell transplantation) those patients with the highest chance of benefiting from T-cells redirecting therapies with the final goal of cure (231) or achievement of persistence of minimal residual disease negativity (233). However, T-cell function looks like compromised since the asymptomatic phase of disease (234–236), arising the question about how to improve the efficacy of current immunotherapy approaches and their toxicity profile. For example, T cell proliferation decreased in presence of mature neutrophils (234, 236–238). The cytotoxic potential of T cells engaged by EM801 increased notably with the depletion of mature neutrophils (236), arising the question if immunotherapy should be adapted to an extensive immune profiling not limited to T-cells only.

In immunotherapy of both solid and hematological cancers, there is an increasing evidence about the prominent role of

TABLE 5 | Vantages and Disadvantage of monoclonal antibodies, Bispecific antibodies, Antibody drug conjugated and CAR-T cells.

Target	Therapeutic format	Advantage	Disadvantage
CD38	Naked monoclonal antibody	High clinical activity in triplets and quadruplets (dara-based regimens are novel standard of care for elderly patients). The target is generally unaffected by disease stage	Reduction of CD38+ activated T-cells. Perturbation of T-cell compartment.
	Bispecific antibody	• No lymphodepletion regimen required • No delay in treatment because they are "off the shell" products	• Neurotoxicity, cytokine release syndrome (CRS) • Short half-life and they need continuous infusion
SLAMF7	Naked monoclonal antibody (elotuzumab)	The target is slightly reduced during disease progression. However, SLAMF7 expression is retained in MM patients with relapsed/refractory disease, and after intensive prior therapy.	Lack of relevant clinical efficacy of elotuzumab as single agent or in triplets given frontline; it requires to be part of combination regimens
	Bispecific antibody	T-cell mediated cytolysis independent of major histocompatibility complex.	• Short half-life and they need continuous infusion • Multiple dosing is expected to elicit a durable response, with intermittent infusions (usually every 3 weeks)
	CAR-T cells	• A virus-free CAR gene transfer using advanced Sleeping Beauty (SB) transposon technology. SB transposition in CAR-T engineering is attractive due to the high rate of stable CAR gene transfer enabled by optimized hyperactive SB100X transposase and transposon combinations, encoded by mRNA and minicircle DNA, respectively, as preferred vector embodiments (CARAMBA PROJECT). • The allogenic anti-SLAMF7-CAR T cell (UCARTCS1) is the first 'off-the-shelf' CAR T-cell product in MM	• Restrictive eligibility criteria (adequate heart, liver, and kidney function) • SB technology requires lower biosafety level translating to lower infrastructure costs for manufacturing and quality control and high modularity
BCMA	Antibody drug conjugated	Off-the-shelf products, immediately available for patients with aggressive disease Action independent from autologous T-cell fitness and host immune function (ideal for elderly patients).	• Toxicity due to linker-payloads constructs (keratopathy for ADCs using anti mitotic agents). • Potential lower response rate as single agents. • Multiple dosing is expected to elicit a durable response, with intermittent infusions (usually every 3 weeks)
	Bispecific antibody	• Off-the-shelf products, immediately available for patients with aggressive disease • Limited CRS (AMG420), extended half-life from dosing once a week (AMG701, CC-93269) to every 3 weeks (TNB-383B). • Subcutaneous administration is intended to allow higher doses than intravenous administration without increasing adverse events and limited CRS (PF-06863135).	• Cytokine release syndrome (CRS) • Immune effector cells associated neurotoxicity syndrome (ICANS) • Higher doses required for antigen target modulation • AMG420: continuous I.V. infusion limits the patients' compliance and quality of life, increased risk of catheter-related infections, neurological toxicity. • PF-06863135: polyneuropathy • Short half-life and they need continuous infusion Mechanisms of resistance: antigen loss or downregulation; immune response against BsAbs constructs; interference with sBCMA
	CAR-T cells	• Usually only one infusion is needed • The most potent single agent available in the RRMM setting • CRS and neuropathy are usually grade 1–2 and manageable	• Logistical challenges: lag time because of manufacturing • Lymphodepleting conditioning chemotherapy required • Cytopenias (sometimes severe and persistent) • Limited persistence given the dependence on autologous T-cell fitness and host immune function • Short-term remission duration • Requirement of defined T-cell subset compositions and humanized targeting domains to reduce immunogenicity and promote engraftment and in *vivo* expansion • High costs • Exhaustion of manufacturing capacities of centralized and highly specialized GMP production facilities

antibody's constant region, much of which is mediated through interaction of the Fc with FcγRs, that could be engineered to modify their pharmacokinetics and pharmacodynamics. Neutrophils could positively affect the activity of several mAbs, *in vitro* and *in vivo*, *via* the recognition of IgA-opsonized tumor targets by FcαRI/CD64, as recently reviewed (239). However, the mechanisms of neutrophil-induced tumor killing are still under debate and the role of neutrophils, either positive or negative, is far from clear.

Reducing progressively the costs, potential toxicity, and therapy complexity is the major strength of off-the-shelf immunotherapeutic strategies like bispecific antibodies and ADCs, which function is largely independent from autologous T-cell fitness and host immune function. Cytokine release syndrome and immune effector cells associated neurotoxicity syndrome can be managed at lower dosages or changing the format to reduce non-specific T-cell activation (as in TNB-383B).

While clinical trials are not yet mature for a direct comparison of several classes of agents and we still need larger series and further confirmation, the favorable safety profile of the first-in-class belantamab mafodotin, makes it a potential great choice for the elderly patients (232), especially when the therapeutic goal is not disease eradication but long-term control of disease, with repeated infusions. The most relevant challenge for the ADCs development is to reduce toxicity related to linker-payloads constructs, like keratopathy associated to ADCs with anti-mitotic agents and neuropathy (240).

The mechanisms involved in the acquired resistance to anti-CD38 mAbs are not fully understood but could involve the downregulation of the CD38 on cell surface, NK and T cells number and exhaustion, overexpression of complement inhibitory proteins, and expression of inhibitory pathways as CD47-SIRPα (45, 49). The therapy in combination with immunomodulatory drugs seems to potentiate the effect of the anti-CD38 mAbs compared with single-agent treatment, increasing the activity of NK cells and on macrophage (9). These mechanisms lead to reach significant response even in relapsed/refractory MM patients. Other drugs (HDACs and ATRA) could overcome the resistance to anti-CD38 mAbs increasing the expression of the CD38 molecule (28, 72, 73).

In contrast to BCMA, CD38 and SLAMF7 antigens show stable expression levels throughout the successive lines of MM treatments, making their contemporary dual targeting an emerging therapeutic option. Hemibodies are a pair of complementary antibody fragments that redirect T cells against cancer-defining antigen combinations. Hemibodies addressing CD38 and SLAMF7 recruit T cells for the exquisite elimination of dual antigen positive multiple myeloma cells while leaving single antigen positive bystanders unharmed. Differently from T-cell redirecting therapies targeting only CD38 and SLAMF7 targeting hemibodies do not induce massive cytokine release and T cell fratricide reactions, translating into very low off-tumor toxicity in clinical settings (241).

The unique mechanisms of action of monoclonal antibodies make them a perfect component to be used alone or in combination with present therapeutic treatments, which could improve the efficacy of the treatment and probably overcome resistance (114). In the upcoming years, a robust selection of patients, based on both genomic and immune profiling to test respectively the clonal architecture and the host immune fitness, combined to multi-target immunotherapy could induce a further major paradigm shift to offer long-term control of disease and hopefully cure to most of MM patients.

AUTHOR CONTRIBUTIONS

Conceptualization: FR and NG. PS and AR, writing the paper; FR and NG—writing and editing. VM prepared the figures. LN and GS prepared the tables. LC revised the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Novel Approaches Outside the Setting of Immunotherapy for the Treatment of Multiple Myeloma: The Case of Melflufen, Venetoclax, and Selinexor

Nicola Sgherza[1]*, *Paola Curci*[1], *Rita Rizzi*[1,2] and *Pellegrino Musto*[1,2]

[1] Unit of Hematology and Stem Cell Transplantation, Azienda Ospedaliero Universitaria Consorziale (AOUC) Policlinico, Bari, Italy, [2] Department of Emergency and Organ Transplantation, "Aldo Moro" University School of Medicine, Bari, Italy

Although the survival rate of patients with multiple myeloma has significantly improved in the last years thanks to the introduction of various classes of new drugs, such as proteasome inhibitors, immunomodulatory agents, and monoclonal antibodies, the vast majority of these subjects relapse with a more aggressive disease due to the acquisition of further genetic alterations that may cause resistance to current salvage therapies. The treatment of these often "triple" (or even more) refractory patients remains challenging, and alternative approaches are required to overcome the onset of that resistance. Immunotherapies with novel monoclonal, drug-conjugated, or bi-specific antibodies, as well as the use of chimeric antigen receptor T cells, have been recently developed and are currently investigated. However, other non-immunologic therapeutic regimens based on melfluflen, venetoclax, or selinexor, three molecules with new mechanisms of action, have also shown promising results in the setting of relapsed/refractory myeloma. Here we report the most recent literature data regarding these three drugs, focusing on their efficacy and safety in multiple myeloma.

Keywords: multiple myeloma, relapsed/refractory disease, melflufen, venetoclax, selinexor

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***Correspondence:**
Nicola Sgherza
nicolasgherza@libero.it

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INTRODUCTION

Multiple myeloma (MM) is the second most common hematological cancer (1). Despite the survival of patients affected by this plasma cell neoplasm has improved over the past years thanks to the advent of very effective drugs, such as proteasome inhibitors (PIs), immunomodulatory agents (IMiDs), and monoclonal antibodies (MoAbs), most of these subjects usually experience an alternation of remission and relapse (2, 3) as they cycle through therapeutic options. Typically,

Abbreviations: MM, multiple myeloma; RRMM, relapsed/refractory multiple myeloma; NDMM, newly diagnosed multiple myeloma; PIs, proteasome inhibitors; IMiDs, immunomodulatory agents; mAb, monoclonal antibodies; ORR, overall response rate; sCR, stringent complete response; CR, complete response; VGPR, very good partial response; PR, partial response; SD, stable disease; PD, progressive disease; CBR, clinical benefit rate; mOS, median overall survival; mPFS, median progression free-survival; mDOR, median duration of response; MTD, maximum tolerated dose; RP2D, recommended phase 2 dose; TRAEs, treatment-related adverse events; AE, adverse event; NR, not reached; ASCT, autologous stem cell transplant.

each remission is usually shorter than the last as the tumor becomes more aggressive, with progression and treatment resistance driven by clonal evolution and genomic instability within myeloma clones (4, 5). Moreover, since MM patients are usually elderly, they often present with comorbidities, such as disabilities, diabetes, and pulmonary and cardiovascular diseases, which not only further impact the quality of life of the patient but also limit the therapy options (6, 7). Treatments for relapse largely depend on prior therapy, according to previous response and tolerability, with class switching often prioritized (8). Many new approaches that aim to overcome or bypass resistance mechanisms are currently under investigation for patients with relapsed and/or refractory MM (RRMM). Among these, the development of novel monoclonal, drug-conjugated, or bi-specific antibodies (9), as well as the use of chimeric antigen receptor (CAR) T cells (10), have recently opened a new immune-therapeutic scenario for MM patients, ideally integrating or even substituting other "conventional" chemotherapy or PIs/IMiDs-based treatments characterized by a well-known toxicity profile mainly resulting in cytopenia, neurologic symptoms, and thrombophilia. On the other hand, novel, non-immunologic therapeutic regimens based on melfluflen, venetoclax, or selinexor, three molecules with different mechanisms of action, have also shown promising results in the setting of RRMM. These drugs may have the possible advantage of avoiding some specific side effects related to immunological approaches (*i.e.*, cytokine release syndrome, infusion-related reactions, central nervous system complications, or unusual infections), thus warranting evaluation as possible alternative options or, even better, as partners for new combinations. In this review, we provide an overview of the efficacy and safety, from main clinical trials and real-world experiences, of melfluflen, venetoclax, and selinexor in the setting of RRMM.

MELFLUFEN

Melflufen (melphalan flufenamide) is a first-in-class peptide–drug conjugate that, through the hydrolytic activity of intracellular aminopeptidases, releases alkylating agents into tumor cells (11, 12). Melflufen is rapidly taken up by myeloma cells due to its high lipophilicity; once inside the cell, aminopeptidases cleave melflufen into melphalan and p-fluorophenylalanine; melphalan accumulates in myeloma cells and, within the nucleus, induces irreversible DNA damage and apoptosis (**Figure 1**) (12–14). Melflufen increases p53 levels, but its cytotoxic activity is not dependent on the activation of p53 function, unlike melphalan; this justifies the activity of melflufen in melphalan-resistant cells. Moreover, since p53 mutations/deletions can be present at the presentation (10–15%) or at the progression of a disease, a therapeutic approach including melflufen could be considered even in MM patients carrying these genetic alterations (11). Melflufen has also demonstrated an anti-angiogenic activity in *in vitro* and *in vivo* models, inhibitory action on myeloma cell migration, and capacity to overcome the cytoprotective effects of the bone marrow microenvironment. Finally, the combination of melflufen with bortezomib or dexamethasone or lenalidomide triggered a

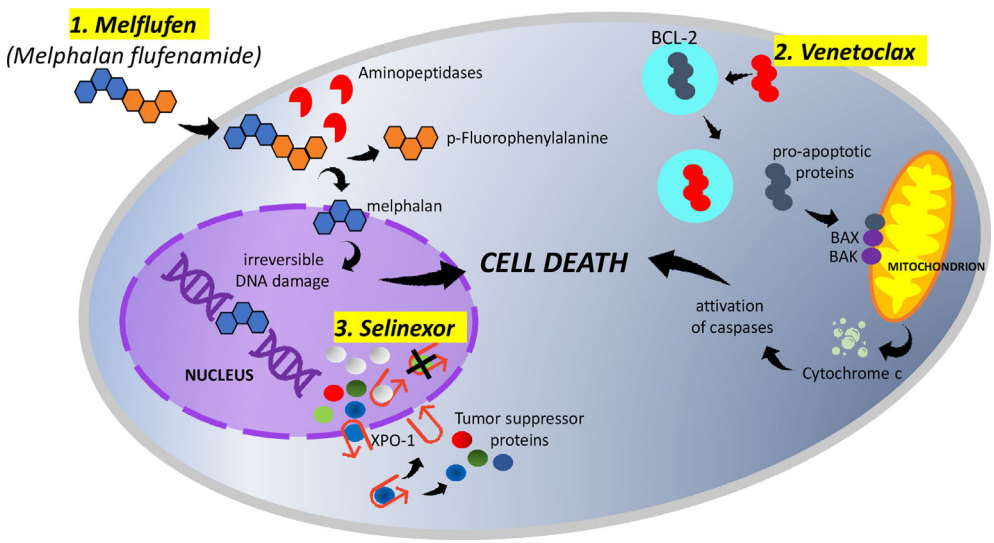

FIGURE 1 | 1. Melphalan flufenamide (melflufen) is highly lipophilic and rapidly diffuses across the membranes of myeloma cells. Once inside the cell, aminopeptidases cleave melflufen into melphalan and p-fluorophenylalanine. melphalan accumulates in myeloma cells and, within the nucleus, induces irreversible DNA damage and apoptosis. 2. Venetoclax binds selectively to BCL-2, freeing pro-apoptotic proteins. The released pro-apoptotic proteins associate with the apoptotic effectors BAX and BAK and induce the permeabilization of the mitochondrial outer membrane. The cytochrome c released activates caspases and triggers cell death. 3. Myeloma cells overexpress XPO-1, causing the increased export of tumor-suppressor proteins from the nucleus. Selinexor (represented by white spheres), binding to XPO-1, inhibits the nuclear export of tumor-suppressor proteins (represented by green, blue, and red spheres). The accumulation of tumor suppressors in the nucleus ultimately leads to cell cycle arrest and apoptosis of multiple myeloma cells.

synergistic anti-MM activity *in vitro* (11, 15–17). Preclinical studies provided the framework for different clinical trials. A detailed summary of main clinical trials on melflufen monotherapy or in combination in the setting of RRMM, including schedules and doses, can be found in **Table 1**.

O-12-M1 (NCT01897714) is the first study evaluating melflufen in RRMM patients. It is a phase 1/2, multicenter, dose escalation, and dose expansion clinical trial of melflufen +/- dexamethasone in patients who had received two or more prior lines of therapy, including lenalidomide and bortezomib, and were refractory to the last line of therapy (18). In phase 1, among the four doses evaluated (15, 25, 40, and 55 mg), the established melflufen maximum tolerated dose (MTD) was 40 mg; in phase 2, 13 patients received single-agent melflufen and 45 received melflufen plus dexamethasone. With a median follow-up of 28 months, among the 45 patients receiving melflufen plus dexamethasone, the overall response rate (ORR) was 31% (very good partial response, VGPR: five patients; partial response, PR: nine patients), the median progression free-survival (mPFS) was 5.7 months, and the median overall survival (mOS) was 20.7 months. Among the 13 patients who received single-agent melflufen, the ORR was 8%, the mPFS was 4.4 months, and the mOS was 15.5 months. At the last update, with a median follow-up of 46 months, in the arm melflufen plus dexamethasone, mOS and mPFS were unchanged at 20.7 and 5.7 months, respectively (24).

HORIZON (OP-106; NCT02963493) is a pivotal, single-arm, multicenter, phase 2 study evaluating the efficacy and safety of melflufen and dexamethasone in heavily pretreated and poor-risk patients with RRMM refractory to pomalidomide or an anti-CD38 MoAb or both (19). Among 157 efficacy-evaluable patients, ORR was 29%, the median duration of response (mDOR) was 5.5 months, the mPFS was 4.2 months, and the mOS was 11.6 months at a median follow-up of 14 months.

ANCHOR (OP-104; NCT03481556) is a phase 1/2 study evaluating the safety and efficacy of melflufen and dexamethasone in combination with daratumumab or bortezomib in patients with RRMM. In the daratumumab arm, the patients could not have received prior anti-CD38 MoAb therapy; in the bortezomib arm, the patients could not have been PI-refractory. The patients are treated until progressive disease (PD) or unacceptable toxicity. In the daratumumab arm (20), with a median treatment duration of 8.4 months (1.0–23.7), ORR was 70%, including one stringent complete response (sCR), one CR, 10 VGPRs, and 11 PRs. At a median follow-up of 11.9 months, mPFS was 11.5 months and mDOR was 12.5 months. In the bortezomib arm (21), with a median treatment duration of 6.5 months (range: 1.4–29) and 8.7 months (range: 2.1–19.6), ORR was 50%, and it was 71% for melflufen 30 and 40 mg, respectively.

The ongoing, randomized, open-label, phase 3 multicenter study OCEAN (OP-103; NCT03151811) (22) will enroll patients with RRMM following two to four lines of prior therapy and who are refractory to lenalidomide in the last line of therapy. The patients will be randomized to either one of two arms: melflufen plus dexamethasone *versus* pomalidomide plus dexamethasone. The patients will be treated until confirmed PD, unacceptable toxicity, or when the patient or investigator decides to discontinue the therapy.

BRIDGE (OP-107; NCT03639610) is a phase 2 study evaluating the pharmacokinetics of melphalan during treatment with melflufen and dexamethasone in patients with RRMM, following two to four prior lines of therapy and a renal function (creatinine clearance by Cockcroft–Gault formula) between ≥30 to <45 ml/min in cohort 1 and ≥15 to <30 ml/min in cohort 2. The preliminary results on 31 patients have been reported at the 2021 EHA congress with encouraging results; ORR was 48%, and the clinical benefit rate was 58%, with stable renal function (23).

To date, there is no data (or active clinical trials) evaluating the role of melflufen in newly diagnosed MM (NDMM) as well as on any potential impact on stem cells and stem cell collection.

VENETOCLAX

The discovery that an increased expression of the oncogene *BCL-2*, located on chromosome 11, prevents cell death and that it is an important factor in tumor survival through the regulation of apoptosis subsequently led to the hypothesis of this pathway as a target for anti-cancer activity (25). Venetoclax (ABT-199), a potent selective inhibitor of the BCL-2 protein, has previously shown an antitumor activity in acute myeloid leukemia (26), non-Hodgkin lymphoma (27), and chronic lymphatic leukemia (28, 29), receiving following approval from FDA and EMA for sub-categories of patients affected by these hematological malignancies. Focusing the attention on the mechanism of action, venetoclax binds selectively to BCL-2, freeing the pro-apoptotic proteins. These molecules associate with the apoptotic effectors BAX and BAK and induce the permeabilization of the mitochondrial outer membrane. Finally, the released cytochrome c activates caspases and triggers cell death (**Figure 1**). Since about 20% of MM patients demonstrate a t(11;14) (that activates BCL-2) and an overexpression of BCL-2, a possible anti-myeloma activity of venetoclax in MM has been investigated. Preclinical studies demonstrated the sensitivity to venetoclax mainly, but not exclusively, in *in vitro* MM cells harboring t(11;14) (30, 31). Moreover, the sensitivity of MM cells to venetoclax would be improved by the addition of dexamethasone (32); venetoclax would enhance bortezomib activity as well. A detailed summary of the main clinical trials on venetoclax monotherapy or in combination in the setting of RRMM, including schedules and doses, can be found in **Table 2**.

Venetoclax Single Agent

The phase 1 trial NCT01794520 evaluated the safety of venetoclax monotherapy in 66 patients with RRMM (33). Thirty patients were enrolled in the dose escalation part of the trial, while 36 patients were enrolled in the safety expansion phase. The patients received a median of 5 prior therapies (range: 1–15); approximately 60% of patients were bortezomib and lenalidomide double refractory. Thirty (46%) patients were

TABLE 1 | Summary of findings of main clinical trials with melflufen in relapsed/refractory multiple myeloma.

	Phase/ number of patients	Dosing	Median number of prior lines (range)	Efficacy	Adverse events (grades 3 and 4)	Reference
Melflufen +/- Dexamethasone (O-12-M1; **NCT01897714**)	I/23 II/58	**Phase I: M** (15 or 25 or 40 or 55 mg IV) on day 1 of each 21-day cycle; **Dexamethasone** (40 mg) on days 1, 8, and 15 of each 21-day cycle				Richardson PG et al. (18)
		Phase II: **a) M** (40 mg IV) on day 1 of each 21- or 28-day cycle; **Dexamethasone** (40 mg) on days 1, 8, and 15 of each 21-day cycle (for any pts on the 28-day treatment schedule, an additional dose of 40 mg dexamethasone was administered on day 22 of each cycle) (**45 pts**)	IIa: 4 (3–5) IIb: 5 (4–6)	**IIa** ORR: 31% CBR: 49% VGPR: 11% PR: 20% mPFS: 5.7 months mOS: 20.7 months	**IIa:** thrombocytopenia (62%), neutropenia (58%)	
		b) M (40 mg IV) on day 1 of each 28-day cycle (**13 pts**)		**IIb:** ORR: 8% CBR: 23% PR: 8% mPFS: 4.4 months mOS: 15.5 months	**IIb:** neutropenia (69%), thrombocytopenia (62%)	
Melflufen plus Dexamethasone (HORIZON, OP-106; **NCT02963493**)	II/157	**M** (40 mg IV): day 1 of each 28-day cycle; **Dexamethasone** (40 mg or reduced dose for patients 75 years or older) on days 1, 8, 15, and 22 of each 28-day cycle	5 (2–12)	ORR: 29% mDOR: 5.5 months mPFS: 4.2 months mOS: 11.6 months	Neutropenia (79%), thrombocytopenia (76%), anemia (43%), pneumonia (10%)	Richardson PG et al. (19)
Melflufen plus Dexamethasone and Daratumumab or Bortezomib (ANCHOR, OP-104; **NCT03481556**)	I-II/ 46	**Daratumumab arm (33 pts):** **M** (30, 40, or 20 mg IV) on day 1 of each 28-day cycle; **Daratumumab** (16 mg/kg) weekly for 8 doses, every other week for 8 doses, and then once every 4 weeks until PD; **Dexamethasone** (20 mg pre-daratumumab and 20 mg/day after-daratumumab; 20 mg total for pts 75 years or older)	2 (1–4)	ORR: 70%, mDOR: 12.5 months mPFS: 11.5 months	Neutropenia (58%), thrombocytopenia (55%), anemia (24%)	Ocio EM et al. (20)
		Bortezomib arm (13 pts): **M** (30, 40, or 20 mg IV) on day 1 of each 28-day cycle; **Bortezomib** (1.3 mg/m^2) on days 1, 4, 8, and 11; **Dexamethasone** (20 or 12 mg for pts 75 years or older) on days 1, 4, 8, and 11; 40 or 20 mg for pts 75 years or older on days 15 and 22 of each 28-day cycle	M (30 mg): 3.5 (2–4) M (40 mg): 2 (1–4)	M (30 mg): ORR: 50% M (40 mg): ORR: 71%	M (30 mg): thrombocytopenia (50%), neutropenia (33%) M (40 mg): thrombocytopenia (100%), neutropenia (71%)	Hajek R. et al. (21)
Melflufen plus Dexamethasone *versus* Pomalidomide plus Dexamethasone (OCEAN, OP-103; **NCT03151811**)	III/ongoing	**Arm A: M** (40 mg IV) on day 1; **Dexamethasone** (40 or 20 mg for pts 75 years or older) on days 1, 8, 15, and 22 of each 28-day cycle. **Arm B: Pomalidomide** (4 mg orally, daily) on days 1 to 21; **Dexamethasone** (40 or 20 mg for pts 75 years or older) on days 1, 8, 15, and 22 of each 28-day cycle	NA	NA	NA	Schjesvold F. et al. (22)
Melflufen plus Dexamethasone (BRIDGE, OP-107; **NCT03639610**)	II/31	**Arm 1A: M** (40 mg IV) on day 1 of each 28-day cycle; **Dexamethasone** (40 or 20 mg for pts 75 years or older) on days 1, 8, 15, and 22 of each 28-day cycle	NA	ORR: 48% CBR: 58%	Thrombocytopenia (58%), neutropenia (42%), anemia (35%)	Pour L et al. (23)
		Arm 1B: M (30 mg IV) on day 1 of each 28-day cycle; **Dexamethasone** (40 or 20 mg for pts 75 years or older) on days 1, 8, 15, and 22 of each 28-day cycle				
		Arm 2A: M (20 mg IV) on day 1 of each 28-day cycle; **Dexamethasone** (40 or 20 mg for pts 75 years or older) on days 1, 8, 15, and 22 of each 28-day cycle	NA	NA	NA	

(Continued)

TABLE 1 | Continued

	Phase/ number of patients	Dosing	Median number of prior lines (range)	Efficacy	Adverse events (grades 3 and 4)	Reference
		Arm 2b: M (30 mg IV) on day 1 of each 28-day cycle; **Dexamethasone** (40 or 20 mg for pts 75 years or older) on days 1, 8, 15, and 22 of each 28-day cycle				

pts, patients; M, melflufen; ORR, overall response rate; VGPR, very good partial response; PR, partial response; PD, progressive disease; CBR, clinical benefit rate; mPFS, median progression free-survival; mOS, median overall survival; mTTP, median time to progression; mDOR, median duration of response; NR, not reached; NA, not available; IV, intravenous.

TABLE 2 | Summary of findings of main clinical trials with venetoclax in relapsed/refractory multiple myeloma (RRMM).

Regimen (trial ID)	Phase/ number of patients	Dosing	Median number of prior lines (range)	Efficacy	Adverse events (grades 3 to 4)	Reference
Venetoclax Monotherapy (**NCT01794520**)	I/66	**Venetoclax:** dose escalation cohort (30 pts): 300 to 1,200 mg daily until progression **Venetoclax:** safety expansion cohort (36 pts): 1,200 mg daily until progression	5 (1–15)	**Pts (30): with t(11;14)** ORR: 40%, > VGPR: 27% mTTP: 6.6 months (3.9–10.2) mDOR: 9.7 months **Pts (33) without t(11;14):** ORR: 6%, > VGPR: 6% mTTP: 1.9 months (1.2–2.3)	Thrombocytopenia (26%), neutropenia (21%), anemia (14%), and leukopenia (14%)	Kumar S et al. (33)
Venetoclax plus Dexamethasone (**NCT01794520**)	I/20 II/31	**Venetoclax:** 800 mg daily; **Dexamethasone** 40 mg oral (20 mg for pts ≥75 years of age) on days 1, 8, and 15 of each 21-day cycle	3 (1–7)/ 5 (2–12)	ORR: 60%/48% mTTP: 12.4 months/ estimated mTTP: 10.8 months/ mDOR: 12.4 months/ estimated DOR at 12 months: 61%	Lymphopenia (20%), thrombocytopenia (10%), neutropenia (10%), anemia (12%), and hypophosphatemia (10%)	Kaufman JL et al. (34)
Venetoclax plus Bortezomib and Dexamethasone (**NCT01794507**)	I/66	**Venetoclax:** dose escalation cohort (54 pts): 100–1,200 mg daily until progression; safety expansion cohort (12 pts): 800 mg daily until progression; **Bortezomib** (1.3 mg/m^2) on days 1, 4, 8, and 11 during cycles 1 to 8 and days 1, 8, 15, and 22 during cycles 9 to 11); **Dexamethasone** (20 mg on days 1, 2, 4, 5, 8, 9, 11, and 12 during cycles 1 to 8 and on days 1, 8, 15, and 22 during cycles 9 to 11	3 (1–13)	ORR: 67% > VGPR: 42% mTTP: 9.5 months mDOR: 9.7months	Thrombocytopenia (29%) anemia (15%)	Moreau et al. (35)
Venetoclax or Placebo plus Bortezomib and Dexamethasone (BELLINI, **NCT02755597**)	III/291	**Venetoclax** (800 mg daily) (194 pts) or **Placebo** (97 pts); **Bortezomib** (1.3 mg/m^2) on days 1, 4, 8, and 11 during cycles 1 to 8 and days 1, 8, 15, and 22 during cycles 9 and beyond; **Dexamethasone** (20 mg) on days 1, 2, 4, 5, 8, 9, 11, and 12 during cycles 1 to 8 and on days 1,2, 8, 9, 15, 16, 22, and 23 during cycles 9 and beyond. Treatment was given in 21-day cycles for the first eight cycles and 35-day cycles from the ninth cycle until PD, unacceptable toxicity, or patient withdrawal	2 (1–3)	mPFS (venetoclax): 23.2 months; mPFS (placebo): 11.4 months mOS (venetoclax): 33.5 months; mPFS (placebo): NR	Neutropenia (21%/8%), thrombocytopenia (15%/30%), anemia (16%/15%), diarrhea (15%/12%), and pneumonia (18%/13%)	Kumar SK et al. (36, 37)
Venetoclax plus Carfilzomib and	II/43	**Cohort 1: Venetoclax** (400 mg daily), **Carfilzomib** (27 mg/m^2) on days 1, 2, 8, 9, 15, and 16; **Dexamethasone** (40 mg) on days 1, 8, 15, and 22	2 (1–3)	ORR: 79%, ≥CR rate: 40% ≥VGPR rate: 64%	Lymphopenia (23%), pneumonia (16%),	Costa L et al. (38, 39)

(Continued)

TABLE 2 | Continued

Regimen (trial ID)	Phase/ number of patients	Dosing	Median number of prior lines (range)	Efficacy	Adverse events (grades 3 to 4)	Reference
Dexamethasone (**NCT02899052**)		**Cohort 2: Venetoclax** (800 mg daily), **Carfilzomib** (27 mg/m^2) on days 1, 2, 8, 9, 15, and 16; **Dexamethasone** (40 mg) on days 1, 8, 15, and 22 **Cohort 3: Venetoclax** (800 mg daily), **Carfilzomib** (70 mg/m^2) on days 1, 8, and 15; **Dexamethasone** (40 mg) on days 1, 8, 15, and 22 **Cohort 4: Venetoclax** (800 mg daily), **Carfilzomib** (56 mg/m^2) on days 1, 2, 8, 9, 15, and 16; **Dexamethasone** (40 mg) on days 1, 2, 8, 9, 15, 16, 22, and 23			hypertension (16%), and hypophosphatemia (12%)	
Venetoclax–Dexamethasone *vs*. Pomalidomide–Dexamethasone in t (11;14)–positive RRMM (CANOVA, **NCT03539744**)	III/ongoing	**Venetoclax** (800 mg daily) or **Pomalidomide** (4 mg daily on days 1–21 of 28-day cycles); **Dexamethasone** (40 mg; 20 mg for patients ≥75 years once weekly)	NA	NA	NA	Mateos M et al. (40)
Venetoclax plus Pomalidomide and Dexamethasone (**NCT03567616**)	II/ongoing	**Part 1:** dose escalation; **Part 2:** dose expansion. For part 2, the participants will be divided into two cohorts based on the presence of t(11;14).	NA	NA	NA	https://clinicaltrials.gov/ (41)
Venetoclax plus Ixazomib and Dexamethasone (**NCT03399539**)	I/II ongoing	**Phase 1:** determine the MTD of **Venetoclax** in combination with **Ixazomib** and **Dexamethasone**; **Phase 2:** evaluate the therapeutic activity of this triplet in patients with relapsed MM	NA	NA	NA	https://clinicaltrials.gov/ (41)
Venetoclax plus Daratumumab and Dexamethasone (VenDd), +/- Bortezomib (V) (**NCT03314181**)	I/II ongoing	**Part 1a: Venetoclax** (various doses administered once daily), **Daratumumab** (1,800 mg SC (preferred) or 16 mg/kg IV; **Dexamethasone** (40 or 20 mg weekly, if necessary, as described in the protocol) **Part 1b: Venetoclax** (at a dose determined by the dose escalation phase), **Daratumumab** [1,800 mg SC (preferred) or 16 mg/kg IV], **Dexamethasone** (40 or 20 mg weekly, if necessary, as described in the protocol) **Part 2a: Venetoclax** (at various doses administered once daily), **Daratumumab** [1,800 mg SC (preferred) or 16 mg/kg IV], **Bortezomib** (1.3 mg/m^2) cycles 1–8, days 1, 4, 8, and 11; **Dexamethasone** (20 mg) cycles 1–3: days 1, 2, 4, 5, 8, 9, 11,12, and 15; cycles 4–8: days 1, 2, 4, 5, 8, 9, 11, and 12; cycle 9+ weekly (40 or 20 mg weekly, if necessary as described in the protocol) **Part 2b: Venetoclax** (at a dose determined by the dose escalation phase), **Daratumumab** [1,800 mg SC (preferred) or 16 mg/kg IV], **Bortezomib** (1.3 mg/m^2) cycles 1–8, days 1, 4, 8, and 11; **Dexamethasone** (20 mg) cycles 1–3, days 1, 2, 4, 5, 8, 9, 11, 12, and 15; cycles 4–8: days 1, 2, 4, 5, 8, 9, 11, and 12; cycle 9+ weekly (40 or 20 mg, if necessary as described in the protocol) **Part 3: Venetoclax** (400 or 800 mg once daily), **Daratumumab** [1,800 mg SC (preferred) or 16 mg/kg IV], **Dexamethasone** (40 or 20	NA	ORR (VenDd/VenDVd): 96%/92% ≥VGPR rate (VenDd/VenDVd): 96%/79% mPFS and mDOR: NR	**VenDd:** neutropenia (17%), hypertension (12%), fatigue (8%), hyperglycemia (8%) **VenDVd:** insomnia (21%), diarrhea (8%), thrombocytopenia (8%)	Kaufman JL et al. (42)

(Continued)

TABLE 2 | Continued

Regimen (trial ID)	Phase/ number of patients	Dosing	Median number of prior lines (range)	Efficacy	Adverse events (grades 3 to 4)	Reference
		mg weekly, if necessary, as described in the protocol) *versus* **Daratumumab** [1,800 mg SC (preferred) or 16 mg/kg IV], **Bortezomib** (1.3 mg/m^2) cycles 1–8: days 1, 4, 8, and 11; **Dexamethasone** cycles 1–3 (20 mg), days 1, 2, 4, 5, 8, 9, 11,12, and 15; cycles 4–8 (40 or 20 mg weekly, if necessary as described in the protocol): days 1, 2, 4, 5, 8, 9, 11, and 12; cycle 9+ (20 mg) monthly day 1				
Cobimetinib +/- Venetoclax +/- Atezolizumab (**NCT03312530**)	Ib/II 49 (ongoing)	**Arm A: Cobimetinib** (60 mg, daily) on days 1–21 of each 28-day cycle until disease progression. **Arm B: Cobimetinib** (40 mg, daily) on days 1–21 of each 28-day cycle; **Venetoclax** (800 mg, daily) on days 1–28 of each 28-day cycle Arm C: **Cobimetinib** (40 mg, daily) on days 1–21 of each 28-day cycle; **Venetoclax** (800 mg daily) on days 1–28 of each 28-day cycle; **Atezolizumab** (at a fixed dose of 840 mg IV) on days 1 and 15 of each 28-day cycle	4 (3–5)	OS (A/B/C): 12.9/12.4/23.3 months ORR (A/B/C):0%/27%/29%	Arms A/B/C: neutropenia (0/14%/38%), anemia (0/23%/24%), thrombocytopenia (0/ 18%/24%), pneumonia (0/ 14%/14%)	Schjesvold F. et al. (43)

pts, patients; ORR, overall response rate; VGPR, very good partial response; PR, partial response; PD, progressive disease; CBR, clinical benefit rate; mPFS, median progression free-survival; mOS, median overall survival; MTD, maximum tolerated dose; mTTP, median time to progression; mDOR, median duration of response; NR, not reached; NA, not available; IV, intravenous; SC, subcutaneous.

positive for t(11;14). In terms of response, the ORR was 21% (14/66), and 15% achieved ≥VGPR. Most responses (12/14, 86%) were reported in patients with t(11;14). In this group, ORR was 40%, with 27% of patients achieving ≥VGPR. The MTD was not reached (NR), and the dose of 1200 mg/day was selected for the expansion cohort.

A real-world experience of 18 RRMM patients with t(11;14) at diagnosis treated with venetoclax as a single agent (starting with a dose of 100 mg daily and increasing to a maximum dose of 400 mg daily) was recently reported (44). Six patients (33%) achieved a response ≥PR; the dominant nonhematological adverse event (AE) was nausea, while the hematological AEs were neutropenia and thrombocytopenia.

Venetoclax Plus Dexamethasone

The safety and efficacy of venetoclax was also evaluated in combination with dexamethasone in 51 RRMM patients with t(11;14) in an open-label phase 1/2 study (NCT01794520) (34). The phase 1/2 patients had respectively received a median of 3/5 lines of prior therapy, and 20/87% were refractory to daratumumab. At a median follow-up of 12.3/9.2 months, ORR was 60/48%. The DOR, estimated at 12 months, was 50/ 61%, and the median time to progression (mTTP) was 12.4/ 10.8 months.

Venetoclax in Combination With Other Drugs

A single-center, retrospective study reported data on 47 patients with RRMM treated with off-label venetoclax (45) after a median of 7 (range: 3–13) lines of therapy; prior treatments also included autologous stem cell transplant (ASCT) in 39 patients (83%). Most patients (87%) received venetoclax plus a PI, though there was heterogeneity in the venetoclax-containing regimens. Eighteen patients (38%) were positive for t(11;14). The ORR was 39%, with 17% achieving ≥VGPR. In the t(11;14) group, ORR was 71%, with 24% achieving ≥VGPR. OS was 15.6 months, and mPFS was 2.1 months.

Venetoclax has been evaluated in combination with bortezomib and dexamethasone in 66 RRMM patients enrolled in a phase 1b study (NCT01794507) (35). In the dose escalation part of the study, 54 patients received venetoclax orally from 100 to 1,200 mg/day until progression after a 1-week lead-in period. In the safety expansion phase, 12 patients received venetoclax 800 mg daily until progression. The median number of prior lines of treatment was three. Nine patients (14%) were positive for t (11;14). Thirty-nine percent of the participants were refractory to bortezomib, and 53% were refractory to lenalidomide. Approximately 60% previously underwent ASCT. In terms of efficacy, ORR was 67%, including 20% CR/sCR and 23% VGPR. In the subgroup of patients that were not refractory to bortezomib and who had one to three prior therapies, ORR of 97% and ≥VGPR of 73% were observed.

In the randomized, double-blind, multicenter phase 3 trial BELLINI (NCT02755597), 291 patients with RRMM who had received one to three previous therapies were enrolled to receive venetoclax (194 patients) or placebo (97 patients) with

bortezomib and dexamethasone (36). Treatment was given in 21-day cycles for the first eight cycles and 35-day cycles from the ninth cycle until PD, unacceptable toxicity, or patient withdrawal. Randomization was stratified by previous exposure to a PI and the number of previous therapies. ORR was 82% (venetoclax arm) *versus* 68% (placebo arm), and ≥VGPR was seen in 59 *versus* 36% of patients, respectively. In patients with t (11;14), ORR was 90% (venetoclax group) *versus* 47% (placebo group). mDOR was NR with venetoclax compared with 12.8 months with placebo. At the last update (37), with a median follow-up of 28.6 months, mPFS was 23.2 months with venetoclax *versus* 11.4 months with placebo; mOS was 33.5 months in the venetoclax group, while it was NR with the placebo group. There was an increased mortality in the venetoclax group (14 treatment-emergent deaths *versus* one in the placebo arm) mainly due to a higher rate of infection; as a consequence, in March 2019, FDA suspended the enrollment of new patients in this trial.

Venetoclax (800 mg/day), in combination with a standard dose of bortezomib and dexamethasone, was administered until PD or unacceptable toxicity in a real-life experience recently reported (46). Eleven patients with RRMM and highly pretreated with a median of 7 (range: 4–10) previous lines of therapy were included; all patients were negative for t(11;14). ORR was 27% (3/11), with one (9%) patient reaching VGPR and two (18%) patients reaching PR; two (18%) patients had a stable disease (SD), and six (54%) patients had PD. The mPFS of the whole cohort was 2 months. Nevertheless, the mPFS of those who responded with PR or better was 9 months *versus* 1.5 months for non-responders. The mOS of the whole cohort was 12 months (NR for PR or better *versus* 5 months for non-responders). The main AEs included gastrointestinal toxicities, especially nausea, thrombocytopenia, and infections.

In a phase 2 ongoing trial (NCT02899052), 43 patients with RRMM and no prior carfilzomib exposure were enrolled to receive venetoclax in combination with carfilzomib and dexamethasone (38, 39). The treatment continued until PD or unacceptable toxicity. Eight patients (19%) were positive for t(11;14). The median number of prior lines of therapy was 2 (range: 1–3). ORR was 79%, ≥CR rate was 40%, and ≥VGPR rate was 64% for all patients.

A real-world experience of 14 RRMM patients treated with venetoclax, carfilzomib, and dexamethasone was recently reported (47). The median previous number of therapies was 5 (range: 2–9). Five patients were positive for t(11;14). Regarding efficacy, ORR among all patients was 35,7%, with all responding patients in VGPR or better. Strikingly, these five responders specifically corresponded to the five t(11;14)-positive patients, resulting in 100% ORR for this particular cytogenetic subgroup and contrasting with the absence of response ≥PR in t(11;14)-negative patients. A rapid but short-lived response was reported in two further cases of patients with RRMM carrying t(11,14) and treated with venetoclax, carfilzomib, and dexamethasone (48).

At the 2021 EHA congress, real-world data of 50 MM patients with t(11;14) have been reported; most patients received venetoclax in combination with a PI and dexamethasone (49). The ORR was remarkably high (48/50 patients responded to the treatment with CR of 28%, VGPR of 38%, and PR of 30%), given that 33 patients (66%) of this group were heavily pretreated. The calculated PFS and OS were 15.5 and 24 months, respectively. The most common AEs were cytopenia, gastrointestinal toxicities, and infections.

Notably, a phase 1/2 study (NCT03399539) aiming to determine the MTD of venetoclax in combination with ixazomib and dexamethasone (phase 1) and to evaluate the therapeutic activity of this triplet in patients with RRMM (phase 2) has been temporarily closed (by FDA and IRB) to enrollment due to safety-related findings (41).

Regarding the combination of venetoclax plus pomalidomide, in the ongoing multicenter, randomized, open-label phase 3 study CANOVA (NCT03539744), RRMM patients with t(11;14) will be randomized 1:1 to venetoclax or pomalidomide plus dexamethasone (40). The treatment will continue until PD, unacceptable toxicity, or withdrawal from the study. The patients will be stratified at screening and before randomization according to age, prior lines of therapy, and International Staging System stage. Furthermore, in another phase 2 trial (NCT03567616), venetoclax will be combined with pomalidomide and dexamethasone in RRMM patients with at least one prior line of therapy (41). The study will include a dose escalation phase and a dose expansion phase, where the participants will be divided into two cohorts based on the presence of t(11;14).

Some studies are exploring the role of venetoclax in combination with MoAbs. An ongoing phase 1/2, non-randomized, multicenter study (NCT03314181) is evaluating the safety, efficacy, and pharmacokinetics of venetoclax, daratumumab, and dexamethasone (VenDd) +/- bortezomib (V) in RRMM (42). The study consists of three distinct parts: part 1 and 2 include patients with t(11;14) or irrespective of t(11;14), respectively, who receive VenDd; part 3 enrolls patients with t(11;14) who receive VenDd +/- bortezomib. The median follow-up time (VenDd/VenDVd) was 10 and 9 months. The ORR in VenDd/VenDVd was 96/92%, and 96/79% had ≥VGPR rate. The mPFS and mDOR were not reached.

An open-label, randomized, multicenter, three-arm phase 1b/2 study (NCT03312530) of cobimetinib (a MEK inhibitor) administered as a single agent and in combination with venetoclax +/- atezolizumab (an engineered MoAb of IgG1 isotype against protein programmed cell death-ligand 1) is currently under investigation in 49 RRMM patients who had received three to five prior therapies, including a PI and an IMiD (43). The patients are randomized 1:2:2 to cobimetinib (arm A), cobimetinib+venetoclax (arm B), or cobimetinib+venetoclax+atezolizumab (arm C). The median prior line of therapy was 4 (range: 3–5), with prior ASCT in 43% and prior daratumumab in 41% of patients, respectively. Twenty-four percent of the patients had high-risk cytogenetics. The ORR was 0% (arm A), 27% (arm b), and 29% (arm C), while the mOS in the three arms were 12.9, 12.4, and 23.3 months, respectively.

Finally, various case reports have been published about the use of venetoclax monotherapy or in combination with other

drugs in patients with advanced RRMM, particularly in patients with primary or secondary plasma cell leukemia (50–60).

To date, there is no data (or active clinical trials) evaluating the role of venetoclax in NDMM; there is no data as well on any potential impact on stem cells and stem cell collection. A trial (NCT03785184) aimed to evaluate the safety and preliminary efficacy of venetoclax when combined with lenalidomide and dexamethasone in patients with NDMM and positive for t(11;14), first available on *ClinicalTrials.gov* in December 2018, was withdrawn (41).

SELINEXOR

Selinexor is a first-in-class, oral, slowly reversible, highly specific inhibitor of exportin-1 (XPO-1) which is an important nuclear exporter for more than 200 proteins, including many tumor-suppressor proteins (TSPs). The overexpression of XPO-1 in myeloma cells, as in most cancer cells, makes selinexor a promising targeted therapy (61) for MM patients. It prevents the transport of TSPs from the nucleus to the cytoplasm, leading to the accumulation of TSPs in the nucleus with consequent cell cycle arrest and apoptosis of MM cells (**Figure 1**) (62, 63), without affecting the normal cells (64). The anticancer activity of XPO-1 inhibitors (including selinexor) is p53 mutation independent (65) and is synergistically increased when combined with other chemotherapies and targeted therapies (66–69); the combination with glucocorticoids would intensify the anti-myeloma activity, too (70). Moreover, selinexor, inhibiting NF-kB, seems to reduce in the microenvironment of cytokines which are vital for the survival of MM cells, like IL-6, IL-10, and VEGF (65). Selinexor has recently been approved by the US FDA in combination with dexamethasone for RRMM patients who have received at least four prior therapies and whose disease is refractory to at least two PIs, at least two IMiDs, and an anti-CD38 mAb (71). A detailed summary of the main clinical trials on selinexor monotherapy or in combination in the setting of RRMM, including schedules and doses, can be found in **Table 3**.

The multicenter phase I clinical trial (NCT01607892) was conducted in advanced hematological malignancies to assess the safety, efficacy, and recommended phase 2 dose of selinexor. In the dose escalation phase, 22 patients with heavily pretreated MM and three with Waldenstrom macroglobulinemia were administered with selinexor as a single agent. In the dose expansion phase, 59 patients with MM received selinexor in combination with dexamethasone. Considering all patients, the ORR was 10%; considering patients treated with selinexor at 45 mg/m^2 twice weekly plus dexamethasone, the ORR was 50% (63).

The single-arm, open-label, multicenter phase 2b study STORM (NCT02336815) evaluated selinexor plus dexamethasone in patients with MM previously treated with lenalidomide, pomalidomide, bortezomib, carfilzomib, and daratumumab and refractory to prior treatment with glucocorticoids, an IMiD, a PI, and daratumumab (70). This study consisted of two parts: part 1 included 79 patients with both quad-refractory MM and penta-refractory MM, and part 2 included 122 patients with penta-refractory MM only. Regarding part 1, the ORR was 21%, mDOR was 5 months, and mPFS and mOS were 2.3 and 9.3 months, respectively. Regarding part 2, the ORR was 26%, mDOR was 4.4 months, and mPFS and mOS were 3.7 and 8.6 months, respectively (67).

The MAMMOTH study evaluated the efficacy of selinexor and dexamethasone in a cohort of patients similar to those enrolled in the STORM study *versus* other multi-agent combinations in RRMM patients treated in academic centers after they became refractory to anti-CD38 mAbs (including a subset of patients who were triple-class refractory) (83). In this retrospective analysis, selinexor plus dexamethasone improved OS (10.4 *versus* 6.9 months) and ORR (32.8 *versus* 25%) with respect to contemporary care (without selinexor).

The single-arm phase 2 MARCH study (NCT03944057) evaluated selinexor and dexamethasone in RRMM patients in China. At the last update (72), 60 patients have been enrolled; the ORR was 26.7%, mDOR was 4.6 months, mPFS was 3.7 months, mOS was NR, and the OS rate at 9 months was 68.5%.

STOMP (NCT02343042) is a phase Ib/II multicenter, open-label, clinical trial with the goals of determining the MTD, the recommended phase 2 dose (RP2D), and the efficacy and safety of selinexor and dexamethasone in combination with various widely used anti-myeloma drugs (bortezomib, pomalidomide, lenalidomide, carfilzomib, daratumumab, *etc.*) in patients with RRMM or NDMM.

Sixty-five RRMM patients were enrolled in the STOMP trial (NCT02343042) to receive selinexor, dexamethasone, and pomalidomide after a median of 3 (range: 1–10) prior therapies (73). The RP2D was selinexor 60 mg, pomalidomide 4 mg, and dexamethasone 40 mg. Among pomalidomide-naïve patients (n = 44), the ORR was 57% (1 sCR, 1 CR, 8 VGPRs, and 15 PRs), and mPFS was 12.2 months. In patients treated with RP2D (n = 20), the ORR was 65% (1 sCR, 5 VGPRs, and 7 PRs); mPFS was NR, with a median follow-up time of 3.9 months. In pomalidomide-refractory patients (n = 16) and those with prior exposure to daratumumab (N = 15), the ORR was 44 and 60%, respectively.

Twenty-four RRMM patients were enrolled in the STOMP trial (NCT02343042) to receive selinexor, dexamethasone, and lenalidomide (74). The median number of prior treatments was 1.5 (range: 1–8). RP2D was set at 60 mg of selinexor, dexamethasone 40 mg, and lenalidomide 25 mg. Regarding outcome, among the lenalidomide-naïve patients (n = 12), the ORR was 92%, including one sCR, four VGPR, and six PR. PFS has not been reached, with a median follow-up period of 7.8 months. For patients with prior lenalidomide treatment (n = 8), the ORR was 13%, suggesting that selinexor–lenalidomide–dexamethasone is effective for patients with RRMM who have not been previously exposed to lenalidomide.

Selinexor, in combination with daratumumab and dexamethasone, has been evaluated, within the STOMP trial (NCT02343042), in 34 RRMM patients who had received three or more prior lines of therapy, including a PI and an IMiD, or

TABLE 3 | Summary of findings of main clinical trials with **Selinexor** in relapsed/refractory multiple myeloma.

Regimen (trial ID)	Phase/ number of patients	Dosing	Median number of prior lines (range)	Efficacy	Adverse events (grade 3 and 4)	Reference
Selinexor +/- Dexamethasone (**NCT01607892**)	I/84	**Dose escalation phase** (25 patients: MM (22) or/and Waldenstrom macroglobulinemia (3): **Selinexor** (3–60 mg/m^2) in eight or 10 doses per 28-day cycle. **Dose expansion phase** (59 MM patients): **Selinexor** (45 or 60 mg/m^2) plus **Dexamethasone** (20 mg), twice weekly in 28-day cycles, or **Selinexor** (40 or 60 mg flat dose) without corticosteroids in 21-day cycles	6 (1–16)	ORR: 10% mDOR: 5 months (2–11)	Thrombocytopenia (45%), anemia (23%), neutropenia (23%)	Chen C et al. (63)
Selinexor plus Dexamethasone (STORM, **NCT02336815**)	IIb/201	**Part 1** (79 patients): (A) **Selinexor** (80 mg), **Dexamethasone** (20 mg) twice weekly on days 1 and 3 for 3 weeks of each 4-week cycle (B) **Selinexor** (80 mg), **Dexamethasone** (20 mg) twice weekly continuously in 4-week cycles	7 (3–17)	ORR: 21% mDOR was 5 months mPFS: 2.3 months mOS: 9.3 months	Thrombocytopenia (59%), anemia (28%), neutropenia (23%), hyponatremia (22%), leukopenia (15%), and fatigue (15%)	Vogl DT et al. (70)
		Part 2 (122 patients): **Selinexor** (80 mg), **Dexamethasone** (20 mg) twice weekly on days 1 and 3, until disease progression	7 (3–18)	ORR: 26% mDOR: 4.4 months mPFS: 3.7 months mOS: 8.6	Thrombocytopenia (59%), anemia (44%), hyponatremia (22%), neutropenia (21%), nausea (10%)	Chari A et al. (67)
Selinexor pus Dexamethasone (MARCH, **NCT03944057**)	II/60	**Selinexor** (80 mg twice weekly of each 28-day cycle), **Dexamethasone** (20 mg twice weekly of each 28-day cycle)	5 (1–16)	ORR: 26.7% mDOR: 4.6 months mPFS: 3.7 months mOS: NR	anemia (60%), thrombocytopenia (55%), leukopenia (42%), lymphopenia (42%), neutropenia (38%), hyponatremia (28%), and pneumonia (23%)	Fu W et al. (72)
Selinexor plus Pomalidomide and Dexamethasone (STOMP, **NCT02343042**)	Ib/II 65	**Selinexor** (once weekly: 60, 80, or 100; twice weekly: 60 or 80 mg), **Pomalidomide** (2, 3, or 4 mg) on days 1–21 of each 28-day cycle; **Dexamethasone** (20 mg twice weekly or 40 mg once weekly) **RP2D: Selinexor** (60 mg once weekly), **Pomalidomide** (4 mg) on days 1–21 of each 28-day cycle, **Dexamethasone** (40 mg once weekly)	3 (1–10)	**Pomalidomide-naïve** (44 patients) ORR: 57% mPFS: 12.2 months **Pomalidomide-exposed** (16 patients) ORR:44%	Neutropenia (55%), anemia (32%), thrombocytopenia (31%), fatigue (11%), decreased appetite (2%), nausea (2%)	White DJ et al. (73)
Selinexor plus Lenalidomide and Dexamethasone (STOMP, **NCT02343042**)	Ib/II 24	**Selinexor** (once weekly: starting dose 80 mg; twice weekly: starting dose 60 mg), **Lenalidomide** (25 mg) on days 1–21 of each 28-day cycle, **Dexamethasone** (40 mg once weekly or 20 mg twice weekly) **RP2D: Selinexor** (60 mg once weekly), **Lenalidomide** (25 mg) on days 1–21 of each 28-day cycle, **Dexamethasone** (40 mg once weekly)	1.5 (1–8)	**Lenalidomide-naïve** (12 patients) ORR: 92% PFS: NR **Lenalidomide-exposed** (eight patients) ORR: 13%	Thrombocytopenia (63%), neutropenia (63%), nausea (4%), fatigue (17%), decreased appetite (8%), weight loss (8%)	White DJ et al. (74)
Selinexor plus Daratumumab and Dexamethasone (STOMP, **NCT02343042**)	Ib/II 34	**Selinexor** (once weekly: 100 mg; twice weekly: 60 mg) in 28-day cycles; **Daratumumab** (16 mg/kg weekly for weeks 1–8, every 2 weeks for weeks 9–24, then every 4 weeks for weeks ≥25); **Dexamethasone** (40 mg once weekly) **RP2D: Selinexor** (100 mg once weekly), **Daratumumab** (16 mg/kg weekly for weeks 1–8, every 2 weeks for weeks 9–24, then every 4 weeks for weeks ≥25), **Dexamethasone** (40 mg once weekly)	3 (2–10)	**Daratumumab-naïve** (32 patients) ORR: 73% mPFS: 12.5 months	Thrombocytopenia (47.1%), anemia (32.4%), leukopenia (32.4%), neutropenia (26.5%), fatigue (17.6%), nausea (8.8%), hyponatremia (11.8%)	Gasparetto C et al. (75)

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TABLE 3 | Continued

Regimen (trial ID)	Phase/ number of patients	Dosing	Median number of prior lines (range)	Efficacy	Adverse events (grade 3 and 4)	Reference
Selinexor plus Carfilzomib and Dexamethasone (STOMP, **NCT02343042**)	Ib/II 27	**Selinexor** (80 or 100 mg once weekly), **Carfilzomib** (56 or 70 mg/m^2) on days 1, 8, and 15 of 28-day cycle; **Dexamethasone** (40 mg) once weekly **RP2D: Selinexor** (80 mg once weekly), **Carfilzomib** (56 mg/m^2) on days 1, 8, and 15 of 28-day cycle; **Dexamethasone** (40 mg) once weekly	4 (1–8)	ORR: 78% mPFS: 23.7 months	Thrombocytopenia (56%), anemia (19%), neutropenia (7%), fatigue (7%), anorexia (4%)	Gasparetto C et al. (76)
Selinexor plus Bortezomib and Dexamethasone (STOMP, **NCT02343042**)	Ib/II 42	**Cohort 1. Selinexor** (80 or 100 mg once weekly in a 35-day cycle), **Dexamethasone** (40 mg) once weekly, **Bortezomib** (1.3/m^2) on days 1, 8, 15, and 22 **Cohort 1. Selinexor** (80 mg once weekly in a 21-day cycle), **Dexamethasone** (40 mg) once weekly, **Bortezomib** (1.3/m^2) on days 1, 4, 8, and 11 **Cohort 2. Selinexor** (60 or 80 mg twice weekly in a 35-day cycle); **Dexamethasone** (20 mg) on days 1, 3, 8, 10, 15, 17, 22, 24, 29, and 31; **Bortezomib** (1.3/m^2) on days 1, 8, 15, and 22 **RP2D: Selinexor** (100 mg once weekly), **Bortezomib** (1.3 mg/m^2) once weekly for 4 weeks, **Dexamethasone** (40 mg) once weekly per 35-day cycle	3 (1–11)	**Global ORR: 63%** ORR PI non-refractory: 84% ORR PI refractory: 43% **Global mPFS: 9.0 months** mpfs PI non-refractory: 17.8 months, mPFS PI refractory: 6.1 months	Thrombocytopenia (45%), neutropenia (24%), fatigue (14%), anemia (12%)	Bahlis NJ et al. (77)
Selinexor plus Ixazomib and Dexamethasone (**NCT02831686**)	I/18	**Selinexor** Cohort A: 40 and 60 mg on days 1, 3, 8, 10, 15, and 17 of a 28-day cycle Cohort B: 80 and 100 mg on days 1, 8, 15, and 22 of each 28- day cycle **Ixazomib** (4 mg) on days 1, 8, and 15 of each 28-day cycle **Dexamethasone**: the same days as selinexor	5 (1–11)	ORR: 22%, maximum DOR: 14 months	Thrombocytopenia (61%), neutropenia (28%), anemia (17%), nausea (11%), vomiting (11%), fatigue (11%)	Salcedo M et al. (78)
Selinexor plus Carfilzomib and Dexamethasone (SINE, **NCT02199665**)	I/21	**Selinexor** (20, 30, 40, and 60 mg) on days 1, 3, 8, 10, 15, and 17 of a 28-day cycle; **Carfilzomib** (20, 20/27, 20/36, 20/45, and 20/56 mg/m^2): cycle 1–8 on days 1 and 2, 8 and 9, 15 and 16; cycle 9+: on days 1 and 2, 15 and 16; **Dexamethasone**: 40 mg weekly (cycle 1–4), 20 mg weekly (cycle 5+) **RP2D: Selinexor** (60 mg) on days 1, 3, 8, 10, 15, and 17, **Carfilzomib** (20/27 mg/m^2) on days 1, 2, 8, 9, 15, and 16; **Dexamethasone** (20 mg; 10 mg from cycle 5 afterwards) on days 1, 2, 8, 9, 15, 16, 22, and 23 on a 28-day cycle	4 (2–10)	ORR: 48% CBR: 71% mPFS: 3.7 months mOS: 22.4 months	Thrombocytopenia (71%), anemia (33%), neutropenia (33%), lymphopenia (33%), infections (24%)	Jakubowiak AJ et al. (79)
Selinexor plus Doxorubicin and Dexamethasone (**NCT02186834**)	I/27	Loading phase (1 to 2 weeks): A: **Selinexor**, **Dexamethasone** twice weekly for 2 weeks or B: one dose of **Selinexor** and **Dexamethasone** Induction phase: **Doxorubicin** (20 mg/m^2 IV) on day 1, **Selinexor**, and **Dexamethasone** (once weekly) Maintenance phase: **Selinexor** and **Dexamethasone** (once weekly) **RP2D: Selinexor** (80 mg on days 1, 8, and 15), **Doxorubicin** (20 mg/m^2 on day 1), and **Dexamethasone** (40 mg on days 1, 8, and 15)	6 (2–10)	ORR: 15% CBR: 26%	Thrombocytopenia 33%, neutropenia 33%, hyponatremia 30%, anemia 26%, nausea/vomiting 11%, hyperglycemia 11%, diarrhea 7%, fatigue 7%	Baz R et al. (80)

(Continued)

TABLE 3 | Continued

Regimen (trial ID)	Phase/ number of patients	Dosing	Median number of prior lines (range)	Efficacy	Adverse events (grade 3 and 4)	Reference
Selinexor, Bortezomib, and Dexamethasone (SVd) *vs*. Bortezomib and Dexamethasone (Vd) (BOSTON, **NCT03110562**)	III/402	**SVd (195 patients): Selinexor** (100 mg once weekly), **Bortezomib** (1–3 mg/m^2 once weekly), **Dexamethasone** (20 mg twice weekly) **Vd (207 patients): Bortezomib** (1–3 mg/m^2 twice weekly for the first 24 weeks and once weekly thereafter), **Dexamethasone** (20 mg four times per week for the first 24 weeks and twice weekly thereafter)	2 (1–3)	**SVd:** mPFS: 13.93 months ORR: 76.4% **Vd:** mPFS: 9.46 months ORR: 62.3%	**SVd:** thrombocytopenia: 39%, fatigue 13%, anemia 16%, pneumonia 11% **Vd:** thrombocytopenia: 17%, fatigue 1%, anemia 10%, pneumonia 11%	Grosicki S et al. (81)
Selinexor plus Bortezomib, Dexamethasone, Daratumumab (SELIBORDARA, **NCT03589222**)	II/ongoing	**Selinexor** (100 mg weekly out of each 4-week cycle), **Dexamethasone** (40 or 20) with each dose of selinexor, **Daratumumab** (16 mg/kg IV) on days 1, 8, 15, and 22 during the first two cycles; on days 1 and 15 during cycles 3 to 6 and on day 1 thereafter; **Bortezomib** (1.3 mg/m^2) on days 1, 8, 15, and 22 starting from the first cycle and on days 1 and 15 since cycle 9. Each cycle is 4 weeks in duration	NA	NA	NA	https://clinicaltrials.gov/ (41)
Selinexor, Cyclophosphamide, Prednisolone *vs*. Cyclophosphamide and Prednisolone (MUKTWELVE, **ISRCTN15028850**)	II/ongoing	**SCP: Selinexor** (100 mg once a week) on days 1, 8, 15, and 22; **Cyclophosphamide** (oral 50 mg once daily, starting on day 1), **Prednisolone** (oral 30 mg every other day, starting on day 1) **CP: Cyclophosphamide** (oral 50 mg once daily, starting on day 1), **Prednisolone** (oral 30 mg every other day, starting on day 1), followed by SCP combination	NA	NA	NA	Brown SR et al. (82)

pts, patients; ORR, overall response rate; VGPR, very good partial response; PR, partial response; PD, progressive disease; CBR, clinical benefit rate; mPFS, median progression free-survival; mOS, median overall survival; mTTP, median time to progression; mDOR, median duration of response; NR, not reached; NA, not available; IV, intravenous.

whose MM was refractory to a PI and an IMiD (75). The median number of prior therapies was 3 (range: 2–10). The RP2D was selinexor 100 mg weekly, daratumumab 16 mg/kg (weekly for weeks 1–8, every 2 weeks for weeks 9–24, and then every 4 weeks for weeks ≥25), and dexamethasone 40 mg weekly. The ORR was 73%, and mPFS was 12.5 months in daratumumab-naïve patients (n = 32).

Twenty-seven RRMM patients were enrolled in the STOMP trial (NCT02343042) to receive selinexor, carfilzomib, and dexamethasone (76). The median number of prior regimens was 4 (range: 1–8). The RP2D was selinexor 80 mg, carfilzomib 56 mg/m^2, and dexamethasone 40 mg. The ORR was 78% (5 CRs, 8 VGPRs, and 8 PRs), and mPFS was 23.7 months.

Another study evaluating the efficacy of selinexor in combination with carfilzomib and dexamethasone is the phase 1 SINE trial (NCT02199665). Twenty-one RRMM patients had been enrolled after a median of four prior lines of therapy, whereas 95% had received carfilzomib and 81% were dual-class refractory (PI and IMiD) and previously exposed to bortezomib, carfilzomib, lenalidomide, and pomalidomide (79). The RP2D was set at 60 mg of selinexor, carfilzomib at 20/27 mg/m^2, and dexamethasone at 20 mg. The ORR was 48%, CBR was 71%, and mPFS and mOS for all enrolled patients were 3.7 and 22.4 months, respectively.

Returning to the STOMP trial (NCT02343042), 42 patients with RRMM were enrolled to receive selinexor, dexamethasone, and bortezomib (77). The median number of prior lines of therapy was 3 (range: 1–11). Fifty percent of the patients were refractory to a prior PI (bortezomib, carfilzomib, or ixazomib), and 45% were refractory to both a PI and an IMiD (lenalidomide, pomalidomide, or thalidomide). The RP2D was set as selinexor at 100 mg, bortezomib at 1.3 mg/m^2, and dexamethasone at 40 mg. The ORR for the entire population was 63%: 84% ORR for PI non-refractory and 43% for PI-refractory patients. The mPFS for all patients was 9.0 months; 17.8 months for PI non-refractory and 6.1 months for PI-refractory patients.

In the open-label phase 3 trial BOSTON (NCT03110562), 402 RRMM patients were randomly allocated to receive bortezomib, dexamethasone (Vd) +/- selinexor (S) (SVd: 195 patients; Vd: 207 patients) (81). Randomization was done using interactive response technology and stratified by previous PI exposure, lines of treatment, and MM stage. Crossover to SVd upon progression on Vd was allowed. The median number of prior lines of therapy was 2 (range: 1–3). After a median follow-up period of 13.2 months for SVd and 16.5 months for Vd, mPFS was significantly longer in the SVd group (13.93 months) than in the Vd group (9.46 months). The ORR in the SVd group was 76.4% (*versus* 62.3% of the Vd group) and included 19 sCR, 14 CR, 54 VGPR, and 62 PR. mDOR was longer with SVd (20.3 months) than with Vd (12.9 months). Furthermore, the median time to next anti-MM treatment was longer in the SVd group (16.1 months) than in the Vd group (10.8 months). Efficacy was consistent across various patient subgroups, including patients with high-risk cytogenetic abnormalities. At the 2021 ASCO congress, a *post-hoc* analysis of this study comparing the survival benefits in patients ≥65 *versus* <65 years of age was reported; for patients ≥65 years, mOS was NR with SVd, while it was 28.6 months with Vd; for patients <65 years, there was no difference in terms of OS (84). Another *post-hoc* analysis (85) reported an improved ORR, PFS, and time-to-next-treatment in the SVd group *versus* Vd regardless of the documented refractory status to lenalidomide or any IMiDs.

In a real-life experience report, eight RRMM, heavily treated patients and with a median of 11 prior lines of therapy (range: 6–18), received a treatment based on the dosing schedule of SVd of the BOSTON trial (86). The responses included one CR, one VGPR, two PR, three SD, and one PD. The mPFS was 91 days (range: 58–350), while OS was 300 days (range: 68–376). The treatment-related adverse effects (TRAEs) included fatigue, thrombocytopenia, and neutropenia, which were managed with selinexor dose adjustment and supportive care.

Another real-world experience included 13 RRMM patients, heavily treated and with a median of 7 (range: 4–10) prior lines of therapy; the patients received selinexor (40–80 mg), dexamethasone (20–40 mg), and bortezomib (1.3 mg/m^2) once a week (87). The ORR was 23% (the responses included three VGPR, one MR, five SD, and four PD). The adverse events were in line with the known safety profile of each of the components.

Selinexor was administered in combination with ixazomib and dexamethasone to 18 heavily pretreated MM patients in a phase I, open-label trial (NCT02831686) (78). Cohort A had a bi-weekly dosing of selinexor with two dose levels (40 and 60 mg). Cohort B had a weekly dosing of selinexor with two dose levels (80 and 100mg). The patients had a median of five prior lines of therapy, and 83% were PI refractory. The ORR was 22%, and the maximum DOR was 14 months. The once-weekly schedule was preferred due to better tolerability, and the selinexor MTD was determined at 80 mg.

In a multicenter, open-label phase I/II clinical trial (NCT02186834), selinexor was administered in combination with doxorubicin and dexamethasone in 27 RRMM patients (80). The median number of prior regimens was 6 (range: 2–10). The RP2D was selinexor (80 mg), doxorubicin (20 mg/m^2), and dexamethasone (40 mg). The ORR was 15%, and CBR was 26%.

The ongoing open-label, multicenter phase II trial, SELIBORDARA (NCT03589222), aims to evaluate the efficacy and safety of the combination of selinexor, bortezomib, dexamethasone, and daratumumab in RRMM patients (41).

The ongoing randomized, controlled, open, parallel group, multi-center phase II trial, MUKTWELVE (ISRCTN15028850), aims instead to evaluate the clinical efficacy of selinexor in combination with cyclophosphamide and prednisolone in patients with RRMM (82). A maximum of 60 participants will be recruited.

Among other selinexor trials with available results, seven patients received a selinexor-based regimen (one selinexor–dexamethasone, one selinexor–bortezomib–dexamethasone, and five selinexor-carfilzomib-dexamethasone) after

progression on CAR T cell therapy (88). All of them were heavily pretreated, with a median of 10 prior lines of treatment; four were penta-refractory and had a rapidly progressive disease. The responses to selinexor-based regimens were one sCR, three VGPR, two PR, and one minimal response. Although preliminary, these data suggest the effectiveness of the selinexor-based regimen also after CAR T cell therapy.

Regarding the role of selinexor in the treatment of NDMM, limited data are available as well as data on any potential impact on stem cell collection. In the STOMP trial (NCT02343042), eight NDMM patients were enrolled to receive the RP2D of selinexor (60 mg once weekly), lenalidomide (25 mg, on days 1–21 of each 28-day cycle), and dexamethasone (40 mg once weekly) (74). All seven patients evaluable for efficacy achieved a response, with an ORR of 100%, including 1 CR, 4 VGPR, and 2 PR. With a median follow-up of 10.2 months, the median PFS has not been reached. The common TRAEs grade ≥3 were thrombocytopenia (38%), neutropenia (75%), fatigue (50%), and decreased appetite (13%). Out of these seven patients, three withdrew their consent to transit to successful autologous stem cell collection and transplantation.

Twelve patients were enrolled in phase I/II of NCT02780609 to receive selinexor (dose level 1: 40 mg, dose level 2: 60 mg, and dose level 3:80 mg) on days -3 and -2 before melphalan, in combination with high-dose melphalan (100 mg/m^2 IV on days -3 and -2), as a conditioning regimen for hematopoietic cell transplant (89). The primary objective was to establish the MTD and identify the RP2D. The combination with selinexor 80 mg (RP2D) with high-dose melphalan at 100 mg/m^2 on days -3 and -2 was well tolerated, and the engraftment kinetics were not altered (neutrophil engraftment occurred with a median of 11 days, and platelet engraftment occurred with a median of 15 days). The trial is proceeding to phase II to assess the efficacy of this combination.

SeaLAND (ALLG MM23) is an ongoing randomized phase 3 trial regarding maintenance after ASCT in NDMM. It aims to compare standard lenalidomide maintenance after ASCT with a low dose of selinexor and lenalidomide to find any benefits in terms of CR, minimal residual disease negativity rate, and PFS (90).

Considering the promising results of selinexor, a second-generation oral selective inhibitor of nuclear export, eltanexor (KPT-8602), is being evaluated in RRMM patients for safety and tolerability; 36 patients were enrolled in a phase I/II open-label study NCT02649790 (91). Based on preliminary data, eltanexor has been shown to have a potentially improved adverse effect profile with similar efficacy compared with selinexor, although more clinical data are needed at this time.

CONCLUSION

Recent therapeutic regimens based on melflufen, venetoclax, or selinexor provide a promising novel approach to patients with RRMM, even outside of the strict immunotherapy treatments. In particular, melflufen, in combination with dexamethasone alone or with a third agent, has shown effectiveness in triple-class refractory patients and in extramedullary disease that represent a major issue in the context of aggressive MM progression (92). Venetoclax appears to be particularly effective in patients with t (11,14), which is present in approximately 20% of MM (93). Selinexor also shows promising outcomes in terms of ORR; the responses observed in selinexor-based three-drug regimens are higher as compared to two-drug regimens, providing a benchmark for further studies (94). Regarding the side effects, TRAEs are generally reversible by applying dose modification and appropriate supportive care (95) to reduce their incidence and maximize the effectiveness of therapy. However, there have been treatment-emergent AEs associated with agents such as venetoclax and Selinexor, and therefore, in some circumstances, the risk–benefit profile may not be favorable compared to currently approved regimens. Obviously, patient selection is necessary for determining the optimal combination of melflufen, venetoclax, and selinexor with other approved agents according to MM biology and status, previous drugs, disease biomarkers, and patient clinical features. Well-designed, pivotal clinical trials are needed to further investigate these agents, preferably in combination and possibly in earlier lines of treatment where these agents could provide a higher benefit. If so, the exact position of these drugs in the therapeutic path of patients with MM will become evident. Currently, potent next-generation cereblon E3 ligase modulators (CELMods), such as iberdomide and CC-92480, not strictly considered as immunotherapy approaches, are in clinical development (96). Though outside of the scope of our review, these new agents have the potential to replace backbone IMiDs and PIs and should also be considered within the expanding number of active agents as a further opportunity and challenge to combine and sequence therapies to maximize long-term patient survival and quality of life.

AUTHOR CONTRIBUTIONS

NS and PM analyzed the data and conceived and wrote the paper. PC and RR reviewed pertinent literature and provided criticisms and suggestions. All authors contributed to the article and approved the submitted version.

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Dual Targeting of Multiple Myeloma Stem Cells and Myeloid-Derived Suppressor Cells for Treatment of Chemotherapy-Resistant Multiple Myeloma

Fatih M. Uckun[1,2,3]*

[1] Department of Developmental Therapeutics, Immunology, and Integrative Medicine, Drug Discovery Institute, Ares Pharmaceuticals, St. Paul, MN, United States, [2] Clinical Research Program, Aptevo Therapeutics, Seattle, WA, United States, [3] Translational Oncology Program, Reven Pharmaceuticals, Westminster, CO, United States

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***Correspondence:**
Fatih M. Uckun
fatih.uckun@aresmit.com

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Here we review the insights and lessons learned from early clinical trials of T-cell engaging bispecific antibodies (BsABs) as a new class of biotherapeutic drug candidates with clinical impact potential for the treatment of multiple myeloma (MM). BsABs are capable of redirecting host T-cell cytotoxicity in an MHC-independent manner to malignant MM clones as well as immunosuppressive myeloid-derived suppressor cells (MDSC). T-cell engaging BsAB targeting the BCMA antigen may help delay disease progression in MM by destroying the MM cells. T-cell engaging BsAB targeting the CD38 antigen may help delay disease progression in MM by depleting both the malignant MM clones and the MDSC in the bone marrow microenvironment (BMME). BsABs may facilitate the development of a new therapeutic paradigm for achieving improved survival in MM by altering the immunosuppressive BMME. T-cell engaging BsiABs targeting the CD123 antigen may help delay disease progression in MM by depleting the MDSC in the BMME and destroying the MM stem cells that also carry the CD123 antigen on their surface.

Keywords: tumor microenvironment (TME), multiple myeloma (MM), bispecific T-cell engagers (BiTEs), bispecific antibodies (BsABs), bone marrow microenvironment (BMME), myeloid-derived suppressor cells (MDSC)

MULTIPLE MYELOMA AND DRUG RESISTANCE

MM is a heterogenous hematologic malignancy and relapses due to resistant disease are common (1–4). Resistance of the malignant clones to multiple drugs hampers a more successful treatment outcome after contemporary standard of care regimens in MM (1–4). Personalized therapy platforms have been designed to overcome the drug resistance, including precision medicines, kinase inhibitors, CAR-T cells, and antibody therapeutics (5–8). Effective treatment of patients with drug-resistant relapsed disease continues to be an unmet medical need (1–4).

IMMUNOSUPPRESSIVE BONE MARROW MICROENVIRONMENT IN MULTIPLE MYELOMA

The immunosuppressive bone marrow microenvironment (BMME) in MM contains cellular elements that facilitate the immune evasion of malignant MM clones (9–13). These immunosuppressive cells include MDSCs, an immature myeloid cell population capable of inhibiting effector cytotoxic T-cell (CTL) populations as well as natural killer (NK) cells and contribute to the T-cell exhaustion which is a hallmark of the BMME in MM patients (4, 14–20). In addition, regulatory T cells (Tregs), regulatory B-cells (Bregs), and tumor-associated macrophages (TAM) also contribute to BMME-associated immunosuppression (20). The immunosuppressive BMME in MM has been implicated in clonal evolution and immune evasion of MM cells accelerating disease progression (4, 20).

Expanded populations of MDSC, representing $CD33^{+}CD123^{+}$ immature myeloid cells within the bone marrow mononuclear cell fraction contribute to the immunosuppressive BMME by inhibiting both memory and cytotoxic effector T-cell populations as well as natural killer (NK) cells, thereby promoting the immune evasion of MM clones (4, 9–15, 20) (**Figure 1**). MDSCs along with MM cell derived interleukin 10 (IL-10), TGF-β and IL-6 inhibit dendritic cell (DC) maturation and their antigen-presenting function, which further aggravates the immunosuppression (6). The abundance of MDSCs is associated with a higher risk of rapidly progressive disease and poor survival outcomes in MM (9–11). MDSCs are activated by exosomes and support the development of Tregs, promote angiogenesis and growth of MM cells besides inhibiting the immune effector cells (21–26).

Several strategies are being explored to overcome the immunosuppressive cellular elements of the BMME in MM patients, including the use autologous hematopoietic stem cell transplantation (AHSCT) (4, 20, 27–29) to remodel the BMME by establishing a more favorable ratio between effective MM-specific CTLs *versus* Tregs and other immunosuppressive cells. Treatment strategies aimed at further enhancing the anti-MM immunity can be employed as post-AHSCT interventions, including MM- or MDSC-directed monoclonal antibodies (MoAb) (20, 27, 28). New generation multi-parameter minimal residual leukemia (MRD) detection techniques provide a unique opportunity to evaluate the effect of new treatment modalities that are applied as part of AHSCT or post-AHSCT on the quality and length of complete remission in both newly diagnosed high-risk MM as well as RR MM (29, 30).

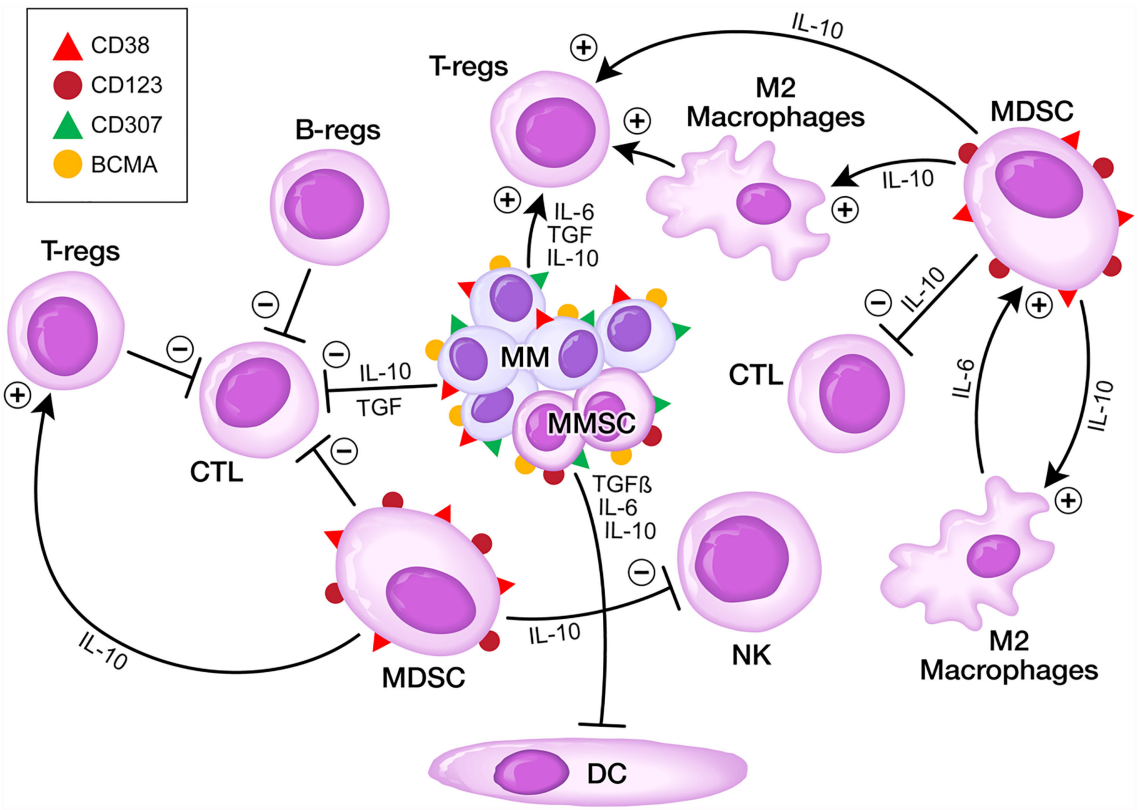

FIGURE 1 | Targeting the Immunosuppressive TME in MM. MM stem cells (MMSC) express BCMA, CD38, CD307 and CD123 antigens on their surface. MDSCs express CD38 and CD123 antigens on their surface.

DUAL TARGETING OF MM CELLS AND MDSCS

Dual targeting of MM cells and MDSCs using biotherapeutic agents has emerged as a very promising new therapeutic platform with a particularly high clinical impact potential. For example CD38 antigen is present on MM cells as well as MDSCs (4, 20, 31–34). Daratumumab, a complement-activating anti-CD38 MoAb capable of causing antibody-dependent cellular cytotoxicity (ADCC) and apoptosis in MM cells, showed significant single agent activity in relapsed MM patients and improved the survival outcome when used in combination with other active anti-MM agents such as bortezomib and dexamethasone or lenalidomide and dexamethasone (4, 20, 31–34). Similar results were obtained using alternative anti-CD38 MoAbs, such as Isatuximab (4, 20). Daratumumab has been shown to expand the immunoreactive CTL populations *via* depletion of $CD38^+$ immunosuppressive cellular elements of the BMME (33). The immunomodulatory effects of Daratumumab improved the clinical responses of previously resistant MM patients to standard combination therapy (4, 20). Unfortunately, increased expression levels of complement inhibitors CD55 and CD59 as well as decreased cell surface expression levels of CD38 on MM cells may decrease the clinical activity of anti-CD38 antibodies (4, 35).

CLINICAL IMPACT POTENTIAL OF BISPECIFIC T-CELL ENGAGERS

BsABs capable of redirecting host T-cell cytotoxicity in an MHC-independent manner to malignant clones as well as immunosuppressive MDSCs (14–20, 35–39) are being explored as a new class of drug candidates in various hematologic malignancies (40). Bispecific CD3xBCMA antibodies targeting the B-cell maturation antigen (BCMA; CD269/TNFRS17) on MM cells, such as EM801, REGN5458 (NCT03761108) and AMG 420 (NCT03836053) showed single agent activity in relapsed/refractory MM patients (41–46) (**Figure 2**). CD3xBCMA BsABs, Elranatamab (PF-06863135) and Teclistamab are being evaluated in R/R MM patients (NCT04649359 and NCT03145181/NCT04557098). In the MajesTEC-1 Phase 1 study of the BCMAxCD3 BsAB Teclistamab in R/R MM (NCT03145181), both intravenous and subcutaneous (s.c) administration schedules were evaluated, and the recommended phase 2 dose was identified as 1.5 mg/kg administered s.c. once a week. At this dose level, the overall response rate was 65%. Grade 3-4 neutropenia was observed in 40% and Grade 1-2 CRS was observed in 70% of the patients (47). The second-generation CD3xBCMA BsAB AMG701 that has an Fc domain to extend its half-life (48) is being evaluated in an Phase 1/2 clinical study (NCT03287908). TNB-383B has been designed to reduce the risk of the class-specific AE cytokine release syndrome (CRS) (49). TeneoBio has shown that TNB-383B causes significantly lower cytokine release from activated T-cells. A clinical proof of concept study (Clinicaltrials.gov identifier: NCT0302577) was designed to study the effects of reducing the levels of γ-secretase cleaved soluble BCMA in MM patients by using a γ-secretase inhibitor because soluble BCMA interferes with the mechanism of action of BCMA-targeting bispecific antibodies.

Bispecific CD3xCD38 antibodies have also been developed (50, 51) and entered clinical trials in patients with relapsed or refractory MM, such as AMG424 (NCT0344566) (51) and GBR1342 (NCT0330911). A CD38-reactive tri-specific antibody targeting CD3 and CD28 co-receptors on T-cells has also been developed to achieve augmented and sustained T-cell activation *via* CD28 engagement (52). A bispecific T-cell engaging CD3xCD307 antibody, named BFCR4350A, was developed targeting the FcRH5/CD307 antigen (53) on MM cells and it is currently being evaluated in a Phase 1 clinical trial (NCT03275103) (54).

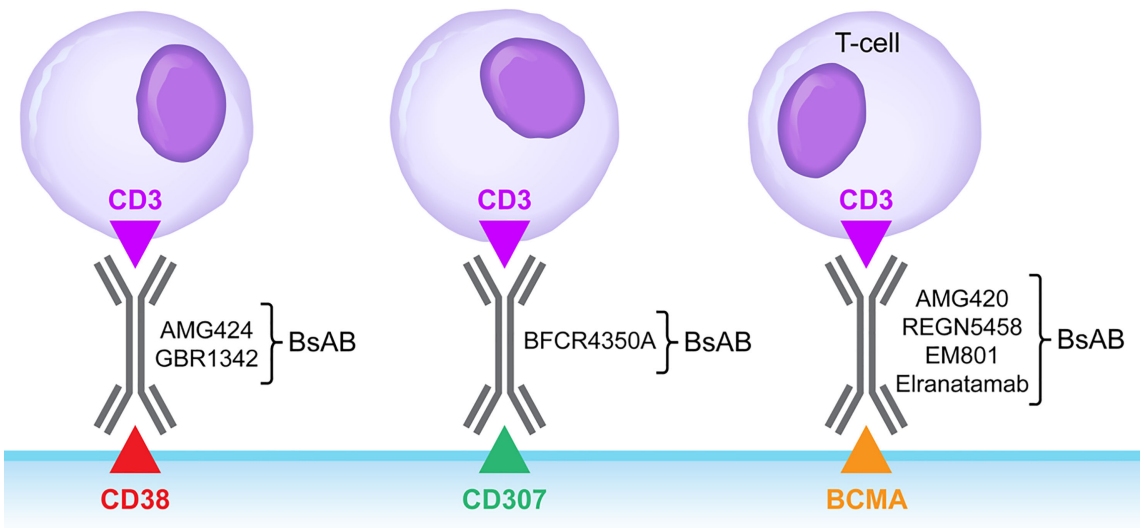

FIGURE 2 | Bispecific Antibodies targeting MM cells. BsAB, bispecific antibody.

Talquetamab is a GPRC5DxCD3 BsAB targeting the orphan G protein-coupled receptor GPRC5D that is abundantly expressed on MM cells. In a Phase 1 study in R/R MM patients testing both IV and SC administration schedules (NCT03399799), the RP2D was identified as 405 mcg/kg administered SC on a weekly basis (55). The RP2D level was well tolerated and exhibited promising activity with an overall response rate of 63%. CRS (79%), neutropenia (64%), anemia (57%) and dysgeusia (57%) were the most common treatment-emergent AEs. Furthermore, 7% of patients developed neurotoxicity and 32% developed infections. The overall response rate at the RP2D was 63% (55).

TARGETING CD123 ON MDSC

The α-chain of the IL-3 receptor, also known as the CD123 antigen, is abundantly expressed on MDSC (20). Furthermore, CD123 is also expressed on plasmacytoid dendritic cells (PDCs) that contribute to the growth of MM cells as well as cancer stem-like cells and osteoclast progenitors (56). Several biotherapeutic agents targeting CD123 have been developed, including the CD123-directed recombinant human IL3 fusion toxin Tagraxofusb (SL-401), MoAbs, BsABs targeting CD123 antigen, such as bispecific T-cell engagers (BiTEs), dual-affinity retargeting antibodies (DARTs), bispecific killer cell engagers, and tri-specific killer cell engagers (40, 57–59).

Targeting the BMME in MM with SL-401 has been shown to reduce the viability of PDCs and inhibit PDC-induced MM cell growth, impair the viability of $CD123^+$ MM stem cells, and prevent osteoclastogenesis in preclinical model systems (60). SL-401 is being assessed in combination with standard of care in a clinical study (NCT02661022) in relapsed/refractory MM patients with promising early evidence of clinical activity (61, 62). Seroproteomics analysis of MM patient serum samples reportedly showed a reduction of PDC-derived soluble proteins in SL-401 treated patients (63).

CD123-targeting, CD3-engaging BsAB, such as Flotetuzumab (59) and APVO436 (64) bring cytotoxic T-cells (CTLs) within close vicinity of target $CD123^+$ cells to create "cytolytic synapses" as a short bridge between target cells and CTLs, triggering CTL activation and destruction of targeted cells (**Figures 2**, **3**). These dual-function anti-MM drug candidates are currently in clinical trials for treatment of CD123-expressing hematologic malignancies with early clinical proof of concept for their ability to destroy CD123 + malignant clones, including CRs in relapsed or refractory AML patients (NCT02152956, NCT03647800). However, the clinical potential of a CD123xCD3 bispecific antibody in MM therapy may be limited as the bulk population of MM cells lack CD123 and depletion of $CD123^+$ MM stem cells alone is unlikely to be an effective strategy for monotherapy. Therefore, clinical feasibility and efficacy studies of combinations of CD123 targeting BsAB with active anti-MM drugs such as pomalidomide that appeared to have augmented activity in the presence of the anti-CD123 fusion toxin tagraxofusp (3, 4, 8, 60), biotherapeutic agents, such as CD3xBCMA BsABs, daratumumab, elotuzumab (4–7, 20), and CAR-T cells (4, 65) are needed to gain insights into the clinical impact potential of CD123xCD3 BsABs.

Notably, MDSCs have been shown to significantly suppress the CTL engaging activity of a BCMAxCD3 BsAB, but the MDSC-suppressed CTL activity could be restored by addition of a hypomethylating agent (HMA) capable of epigenetically altering the MDSC transcriptome *via* reversal of the aberrant DNA methylation (66). Therefore, MDSC-targeting BsAB could be potentiated by HMAs. It is noteworthy that a combination of the CD123xCD3 BsAB APVO436 with azacitidine is being evaluated in one of the cohorts in the ongoing expansion phase of a Phase 1B AML study (NCT03647800).

CYTOKINE RELEASE SYNDROME (CRS)

CD3-engaging BsABs act as agonists and activate T-cells in the presence of tumor cells expressing the target tumor-associated antigen, which can lead to excessive T-cell activation with release of inflammatory cytokines and development of the potentially life-threatening systemic inflammation, known as cytokine release syndrome (CRS) (67–71). For example, BsAB AMG330 binds CD33 antigen on AML cells as well as MDSCs cells and CD3ε on T-cells. In an open-label Phase 1 study (Clinicaltrial.gov identifier: NCT#02520427), AMG330 was given at doses ranging from 0.5–720 μg/d in the manner of continuous IV infusion among 55 patients with R/R AML (NCT02520427). AMG 330-related AEs included CRS (67%; Grade ≥3 in 13%) as the most frequent AEs (72). Similarly, CRS was observed in 63% of AML patients treated with AMG673, a new version of AMG330 (Grade ≥3 in 18%; Clinicaltrial.gov identifier: NCT03224819) (72). Flotetuzumab (MGD006) is a bispecific, dual-affinity re-targeting (DART) antibody reactive with both CD3 antigen on T-cells and CD123 antigen on AML cells and MDSCs. This CD3 engaging bispecific antibody exhibited promising single agent activity in therapy-refractory AML patients with primary induction failure as well as patients with an early first relapse. CRS was observed in all AML patients treated with Flotetuzumab (73) and 58% of AML patients treated with Vibecotamab (XmAb14045), another CD3xCD123 BsAB (74). By comparison, only 10 of 46 patients (21.7%) treated with the CD3xCD123 BsAB APVO436 developed CRS (64).

IL-6 is one of the driving pro-inflammatory cytokines that contribute CRS and its pulmonary, cardiovascular, renal, and neurologic complications (60, 64–71). Cytokine profiling in patients who developed CRS after APVO436 infusion indicates that the predominant cytokine in this inflammatory cytokine response is IL-6, which agrees with our current knowledge regarding CRS that occurs in the context of BsAB therapy (20, 60, 64–71, 75). Within 1-2 days following the first dose of APVO436, the mean serum IL-6 concentration in these patients who developed CRS was elevated 145-fold over baseline (755 *vs* 5.2) and at the end of one week it was still elevated 83-fold over baseline. In most cases, CRS events were transient and medically manageable with standard of care including the use of dexamethasone and anti-IL-6:IL-6R antibody Tocilizumab or anti-IL-6 antibody Siltuximab (antibody against IL-6). However, CRS can be life-threatening even with the use of Tocilizumab or Siltuximab (60, 64–71). Therefore, development of consistently effective prevention and treatment regimens against CRS

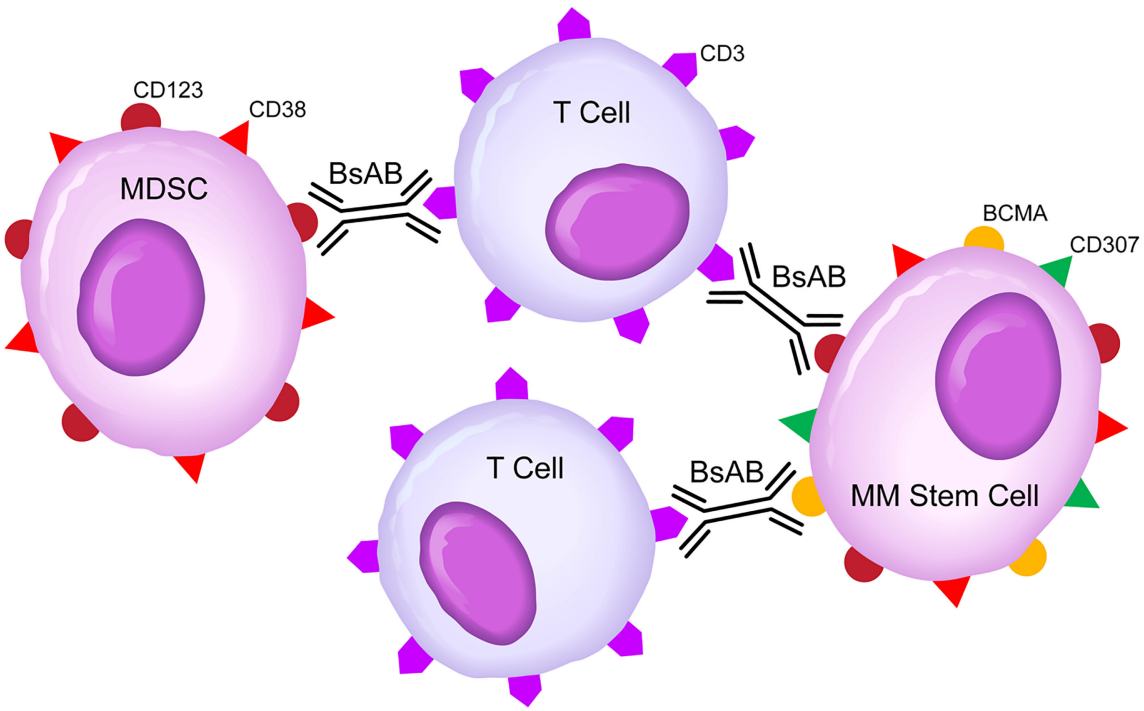

FIGURE 3 | Bispecific CD3xCD123 Antibodies For Dual Targeting of MM Stem Cells Clones and MDSC Cells in High-Risk MDS Patients. BsAB, bispecific antibody; MM, Multiple myeloma; MDSC, Myeloid-derived suppressor cell. See text for a detailed discussion of the rationale of targeting the CD123 and CD38 antigens that are expressed on both MM stem cell clones and MDSCs.

remains an urgent and unmet medical need. Identification of such regimens would further advance the field of immunotherapy. We recently reported the robust anti-inflammatory activity of RJX in animal models of CRS (76). RJX has been shown to block the production of IL-6, TNF-α, as well as TGF-β and reverse inflammation-induced tissue injury and multi-organ damage in mouse models of sepsis and CRS (76). RJX is currently being evaluated for its ability to prevent COVID-19 associated CRS in a double-blind randomized clinical study (NCT04708340). Because of its safety and easy use, RJX may emerge as an attractive adjunct to BsAB platforms to mitigate the risk of severe CRS.

NEUROTOXICITY

Neurotoxicity is a treatment-emergent adverse event (AE) for BsABs, and it is often associated with CRS (77). The signs and symptoms vary from patient to patient and include headache, tremor, confusion, expressive and nominal dysphasia, impaired attention, apraxia, and lethargy occurring as early and common manifestations (77–79). Consensus grading criteria were developed by the ASTCT based on the use of the Immune Effector Cell-Associated Encephalopathy (ICE) screening tool (78). The CD19xCD3 BsAB blinatumumab has been reported to cause neurotoxicity in 70% of patients with B-lineage non-Hodgkin's lymphomas (NHL). By comparison, it is less common with CD20xCD3 or CD123xCD3 BsABs (80). In a recent Phase 1 dose escalation study of the CD123xCD3 BsAB APVO436, APVO436-related transient neurotoxicity occurred only in 5 of 46 patients (10.9%). It occurred during the first cycle in 4 of the 5 patients and in Cycle 8 in the remaining patient. It was mild with Grade 1 AEs including headache, tremor, dizziness, lethargy, insomnia, memory loss, and confusion (64). A single case of Grade 3 confusion was encountered on the first day of treatment and resolved within a day. Neurotoxicity did not show any dose-dependence. Gender, race, age, absolute lymphocyte count or percentage of lymphocytes in peripheral blood did not predict neurotoxicity. Neurotoxicity occurred in 3 patients who also experienced CRS and in 2 patients who did not develop CRS (75). Conversely, of 10 patients who developed CRS, 7 did not experience any neurotoxicity (75).

CONCLUSION

Recombinant T-cell engaging humanized BsABs redirect host T-cell cytotoxicity in an target antigen-expressing cells in patients with hematologic malignancies. They can be used both for targeting drug-resistant MM clones as well as the immune-suppressive cell populations in the BMME (**Figure 3**). Dual targeting of drug-resistant MM clones and immunosuppressive MDSC has the potential to change the therapeutic landscape for MM and improve the survival outcomes of high-risk as well as relapsed/refractory MM patients. The definition of optimal strategies for overcoming the

immunosuppressive BMME in MM may require randomized clinical studies with parallel cohorts and adaptive trial designs. CD3xCD123 BsAB have clinical impact potential in MM as they may help treatment outcomes by blocking immune evasion *via* depletion of $CD123^+$ MDSC and by reducing the drug-resistant tumor load *via* CTL-mediated MHC-independent destruction of MM stem cells.

AUTHOR CONTRIBUTIONS

FMU conceived the review, analyzed the contents of relevant publications, wrote the original draft of the manuscript revised the manuscript, provided final approval for submission of the final version. No medical writer or editor was involved.

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Conflict of Interest: FU was employed by Ares Pharmaceuticals, and he was a consultant for Aptevo Therapeutics and for Reven Pharmaceuticals.

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Successful BCMA CAR-T Therapy for Multiple Myeloma With Central Nervous System Involvement Manifesting as Cauda Equina Syndrome—A Wandering Road to Remission

Yiyun Wang[1,2,3,4], *Linqin Wang*[1,2,3,4], *Yifan Zeng*[5], *Ruimin Hong*[1,2,3,4], *Cheng Zu*[1,2,3,4], *Elaine Tan Su Yin*[1,2,3,4], *Houli Zhao*[1,2,3,4], *Guoqing Wei*[1,2,3,4], *Li Yang*[1,2,3,4], *Aiyun Jin*[1,2,3,4], *Yongxian Hu*[1,2,3,4]* *and He Huang*[1,2,3,4]*

[1] Bone Marrow Transplantation Center, the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China, [2] Institute of Hematology, Zhejiang University, Hangzhou, China, [3] Zhejiang Province Engineering Laboratory for Stem Cell and Immunity Therapy, Hangzhou, China, [4] Zhejiang Laboratory for Systems & Precision Medicine, Zhejiang University Medical Center, Hangzhou, China, [5] Department of Neurology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China

Multiple myeloma (MM) with central nervous system (CNS) involvement is rare with only 1% incidence. So far, there is no standard or effective treatment for CNS MM, and the expected survival time is fewer than 6 months. Here, we report a case of MM with CNS involvement presented with cauda equina syndrome (CES) who achieved complete remission after anti-B-cell maturation antigen (BCMA) chimeric antigen receptor T (CAR-T) cell therapy (Chictr.org.cn, ChiCTR1800017404). The expansion of BCMA CAR-T cells was observed in both peripheral blood (PB) and cerebrospinal fluid (CSF). The CAR-T cells peaked at 2.4×10^6/l in CSF at day 8 and 4.1×10^9/l in PB at day 13. The peak concentration of interleukin (IL)-6 in CSF was detected 3 days earlier, and almost five times higher than that in PB. Next, morphological analysis confirmed the elimination of nucleated cells in CSF 1 month after CAR-T cell treatment from 300 cells/μl, and the patient achieved functional recovery with regressed lesion shown in PET-CT. The case demonstrated that BCMA CAR-T cells are effective and safe in this patient population.

Keywords: multiple myeloma, central nervous system, chimeric antigen receptor (CAR T), immunotherapy, cauda equina syndrome

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Chandraditya Chakraborty, Dana-Farber Cancer Institute and Harvard Medical School, United States

***Correspondence:**
He Huang
huanghe@zju.edu.cn
Yongxian Hu
1313016@zju.edu.cn

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INTRODUCTION

Multiple myeloma (MM) is a clonal plasma cell malignancy which accounted for 10% of hematologic malignancies. In MM, extramedullary diseases in the central nervous system (CNS) are rare and are diagnosed in less than 1% of MM patients (1). The mechanism of CNS infiltration remains unclear—two hypotheses were suggested: the hematogenous spread of malignant plasma cells and the direct invasion from proximal lesions (2). So far, there is no standard or effective

regimen for CNS MM. Conventional treatment methods like systemic chemotherapy, local radiotherapy, and intrathecal injection were used to treat MM patients (1, 3, 4). Nonetheless, the MM patients' prognosis with CNS infiltration is abysmal, and the expected survival time is fewer than 6 months (1–6).

On the other hand, chimeric antigen receptor T (CAR-T) cell therapy has become a promising method to treat hematological malignancies (7–9). It is noteworthy that CNS involvement was considered one of the exclusion criteria for CAR-T clinical trials concerning severe local severe cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) in early time (10–12). However, an increasing number of reports suggested that CAR-T is effective and safe to ALL and lymphoma patients (13, 14). According to previous studies, anti-B-cell maturation antigen (BCMA) CAR-T in MM has achieved CR rates higher than 80%, but the efficacy of BCMA CAR-T in MM CNS patients has not been reported yet (15–19).

In this study, we report a case of refractory/relapsed MM with CNS involvement, manifesting as cauda equina syndrome (CES), and demonstrate the safety and effectiveness of BCMA CAR-T therapy in this patient population.

METHODS

Patient

The patient was a 60-year-old man diagnosed with IgA/λ MM, positive for monoclonal IgH gene rearrangement, 1q21 amplification, and P53 mutation. He was given 3 cycles of chemotherapy with bortezomib, cyclophosphamide, and dexamethasone (VCD) and 3 cycles of bortezomib, lenalidomide, and dexamethasone (VRD). PET-CT scan showed a new osteolytic lesion in the left transverse process of the 7th thoracic vertebra which extended into the spinal canal. Accordingly, he received 7 cycles of local radiation combined with 15 cycles of chemotherapy (3 cycles of bortezomib + dexamethasone, etoposide, doxorubicin, cisplatin (DEAP); 10 cycles of melphalan, cyclophosphamide, and prednisone (MCP); and 2 cycles of dexamethasone, etoposide, cyclophosphamide, cisplatin (DECP). At that time, the patient achieved complete remission (CR) in bone marrow confirmed by morphological examination and flow cytometry, as well as negative serum and urine immunofixation, while the extramedullary lesion showed only partial regression shown by PET-CT. Four months later, he complained of pain and weakness in bilateral lower limbs accompanied by urinary incontinence and was diagnosed with secondary CES, which is a rarely reported complication of MM (20). He was therefore enrolled in BCMA CAR-T therapy trial (Chictr.org.cn, ChiCTR1800017404, details regarding the design of this trial are accessible at https://www.chictr.org.cn/showproj.aspx?proj=28864) after the approval by the ethics committee of the First Affiliated Hospital of Zhejiang University.

BCMA CAR-T Cell Generation, Therapy, and Detection

The single-chain fragment variable (scFv) sequence of BCMA CAR was obtained from a murine hybridoma cell line raised against BCMA. In addition to the scFv, a 4-1BB co-stimulatory domain and a CDζ3-signaling domain were inserted into a lentiviral vector as well. The construct of this second-generation anti-BCMA CAR is shown in **Figure 1**.

The peripheral blood mononuclear cells were obtained from the patient by leukapheresis. The blood cells were transduced with BCMA CAR using lentivirus. He received a fludarabine- (30 mg/m^2, day -4 to -2) and cyclophosphamide- (500 mg/m^2, day -3 to -2) based lymphodepletion regimen before CAR-T cell infusion. After preconditioning chemotherapy, he received BCMA CAR-T cells at a dose of 7.1 × 10^6/kg. Furthermore, an Ommaya reservoir was installed to obtain CSF samples once a day for consecutive 4 weeks. The grading of CRS was based on the Penn grading scale (8), and the grading of neurotoxicity was based on the Common Terminology Criteria for Adverse Events 5.0 (CTCAE 5.0) (21). The patient's response was assessed 1 month after CAR-T therapy. Subsequently, the patient's condition and MRD detection were being followed up in outpatient departments at 2, 3, 6, 12, 18, 24, 36, and 48 months. The expansions of *in vivo* CAR-T cells in the peripheral blood and CSF were continuously detected by flow cytometry. The methods to assess the treatment response included morphological analysis, flow cytometry, and MRI.

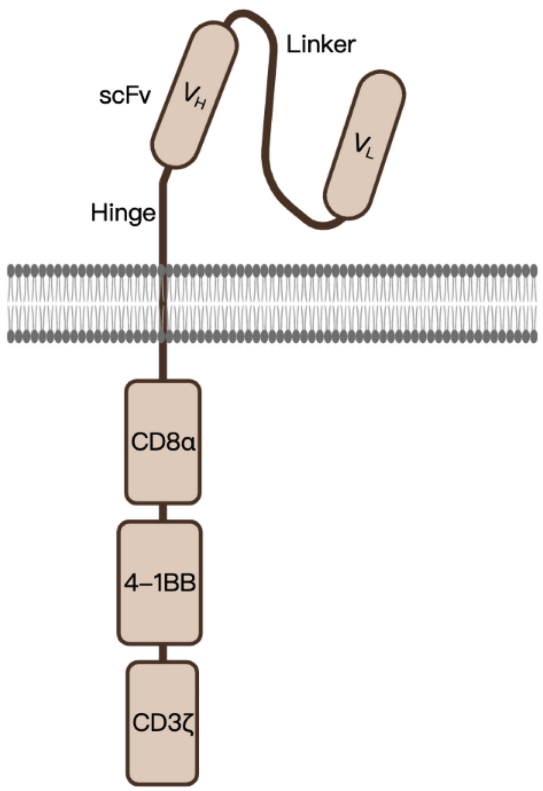

FIGURE 1 | A schematic diagram of anti-BCMA CAR design. scFv, single-chain variable fragment, serving as the BCMA-recognition domain; CD8α, in which the hinge (transmembrane domain) was derived; 4-1BB, co-stimulatory domain which enhances the function of CAR; CD3ζ, serves as the signal-transduction domain.

RESULTS AND DISCUSSION

The flowchart of the patient's treatment history and CAR-T therapy is shown in **Figure 2A**. The infiltration of MM into CNS was confirmed by enhanced lumbar MRI (**Figure 2B**) and CSF examination. Morphological analysis further revealed the presence of 300 nucleated cells/μl (35% plasmablasts and 54% proplasmacytes) in CSF (**Figure 2C**) and 96.873% of plasma cells expressing BCMA (**Figure 2D**). Therefore, he was enrolled in the clinical trial of BCMA CAR-T cell therapy. To assess the response continually and safely, the patient was installed with an Ommaya reservoir. After a preconditioning chemotherapy, he received CAR-T cells at a dose of 7.1×10^6/kg.

As a result, the patient had a high fever (38°C) 8 h after CAR-T cell infusion, indicating CRS onset. In the following weeks, the proliferation of CAR-T cells was observed in both peripheral blood (PB) and CSF. The CAR-T cells peaked at 2.4×10^6/l in CSF at day 8 and 4.1×10^9/l in PB at day 13 (**Figures 2E**, **3A, B**). Moreover, $CD8^+$ cells were predominant in the PB after infusion, while it returned to normal proportion in CSF in 7 days (**Figure 4**).

The predominance of $CD8^+$ CAR-T after infusion is in accord with the findings of previous publications (16, 22). These results indicate that $CD8^+$ CAR-T cells may play a more central role in the elimination of tumor cells. The predominance was not as significant in CSF as in PB, which suggests that the penetration of $CD4^+$ and $CD8^+$ CAR-T cells across the BBB may be regulated by discrete mechanisms. Several trials found that an appropriately defined $CD4^+/CD8^+$ ratio can improve the efficacy of CAR-T cells (23, 24). Thus, the penetration ability should be taken into consideration when a preferred ratio was defined for the treatment of CNS-infiltrated malignancies.

Along with cell proliferation, cytokines increased rapidly. Remarkably, IL-6 in CSF increased fast to 3,953.44 pg/ml at day 10 and reached a peak of 823.11 pg/ml in PB at day 13. The peak of IL-6 in CSF was observed 3 days earlier and almost five times higher than that in PB. Meanwhile, the IL-10, TNF-α, and IFN-γ levels were much higher in PB, suggesting a major difference in the mechanisms of cytokine secretion between these two environments (**Figures 3C–H**).

To our surprise, the patient merely suffered a mild grade 2 systemic CRS without any manifestation of neurotoxicity. Zhang et al. reported a similar patient who suffered relatively severe neurotoxicity, including headache, lethargy, chemosis, stiff neck, aphasia, pupil asymmetry with loss of light reflex, and obtundation which further developed into stupor (25). Such a huge gap in the severity of neurotoxicity resulted from, we suppose, the different locations of CNS lesions. Our patient's primary lesion was sited at the cauda equina, while the patient in the report by Zhang et al. had lesions located in the occipital lobe, thoracolumbar spine, and leptomeninges of the brainstem, which may account for the symptoms of impaired consciousness and cranial nerves.

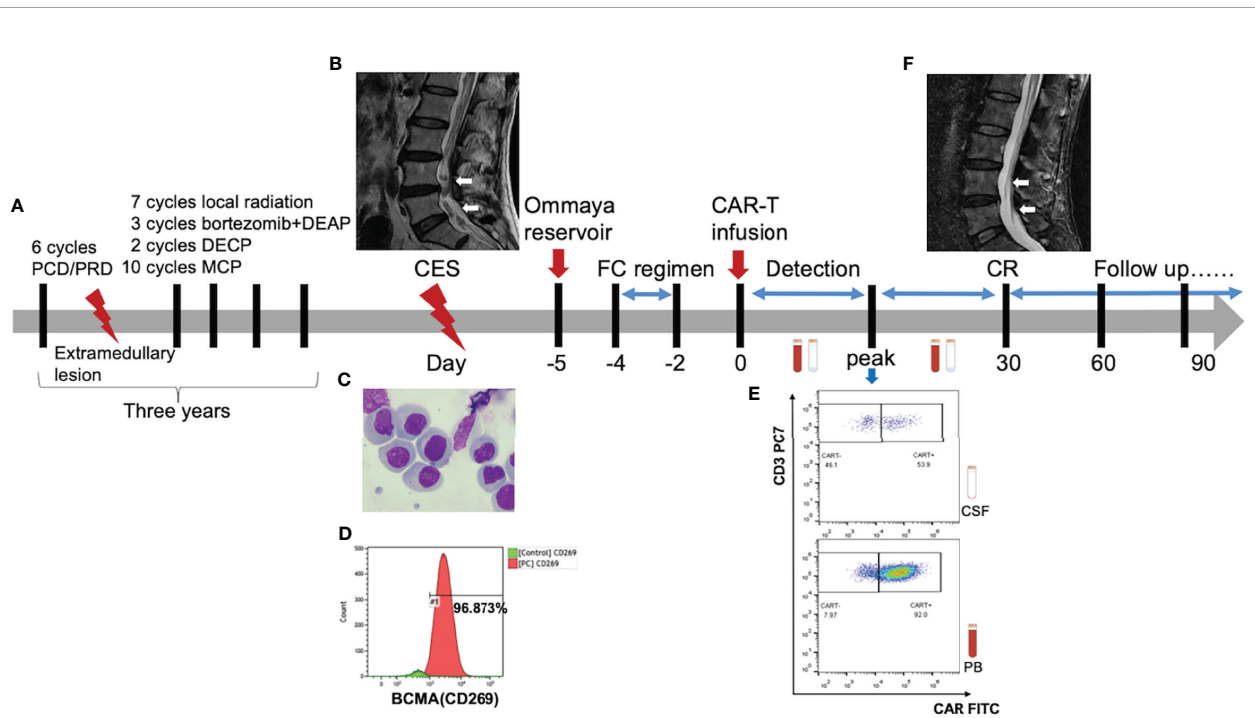

FIGURE 2 | **(A)** The flowchart of patient's treatment history and CAR-T therapy. **(B)** Pretreatment lumbar MRI showed multiple diffuse abnormal signal nods in the cauda equina at lumbar and caudal level. **(F)** The lesion disappeared in the post-treatment imaging, indicating a complete remission (CR) [high-resolution T2-weighted image]. **(C)** Abnormal plasma cells were observed under the microscope. There were 300 nucleated cells/μL in the cerebrospinal fluid of which 35% were plasmablast and 54% were proplasmacyte. **(D)** The flow cytometry indicated that 96.873% of the plasma cells were expressed with BCMA antigens. **(E)** The CAR-T cells peaked 53.9% in CSF at day 8 and 92.0% in PB at day 13.

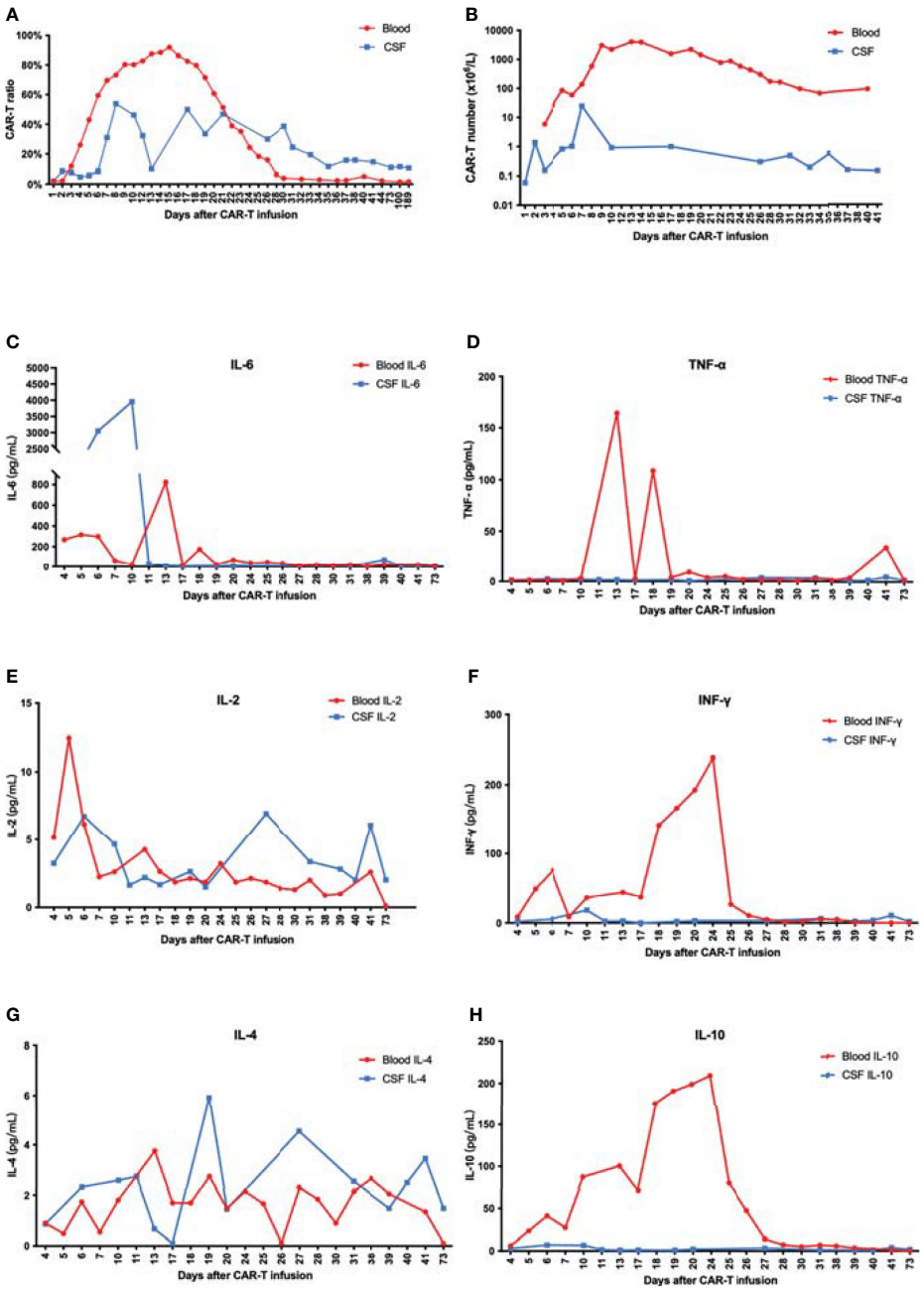

FIGURE 3 | **(A)** The proportion of CAR-T cells in CD3-positive cells increased and reached a peak at 7 or 15 days after CAR-T infusion. **(B)** The number of CAR-T cells in peripheral blood far exceeded that in CSF. **(C–H)** IL-6 increased rapidly in CSF and was significantly higher than that in blood. There was no significant difference of IL-2 and IL-4 between peripheral blood and CSF. Moreover, IL-10, TNF-α, and IFN-γ in peripheral blood significantly exceeded that in CSF.

Next, morphological analysis showed that the nucleated cells were eliminated in CSF 1 month after CAR-T cell treatment. Imaging evaluation demonstrated that the extramedullary lesion had entirely regressed (**Figures 2F**, **5**) and that he achieved CR with relief of previous manifestations of CES (pain and weakness in both lower limbs, and urinary incontinence).

Unfortunately, plasmacytoma reappeared in his cerebral parenchyma 317 days after infusion, of which he died, at 392 days after infusion, despite of further localized cerebrospinal radiotherapy and anti-CD38 monoclonal antibody treatment.

Most importantly, our report is an unprecedented case of CES by CNS MM, which was successfully treated by BCMA

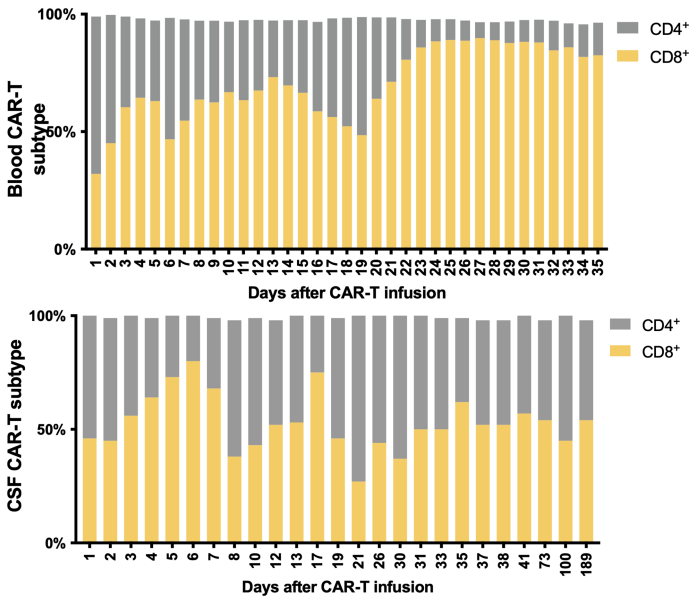

FIGURE 4 | Subtypes of CAR-T cells in peripheral blood and CSF. $CD8^+$ cells were predominant in the PB after CAR-T therapy, while it returned to normal CSF proportions in 7 days.

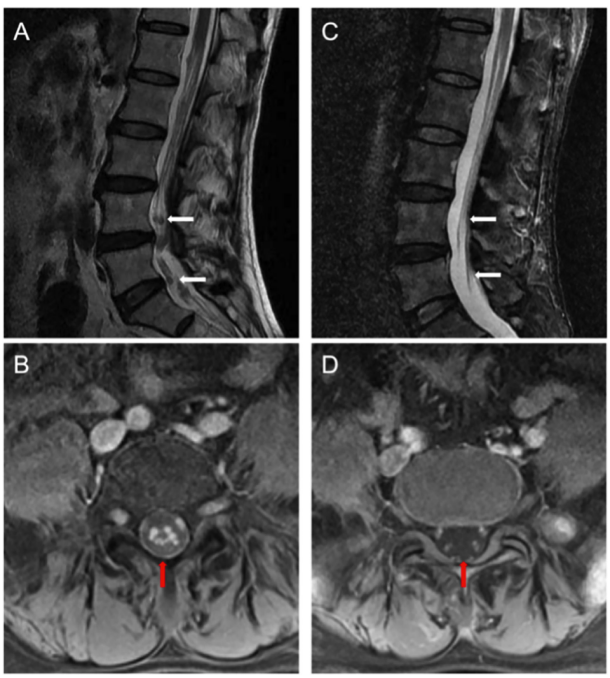

FIGURE 5 | **(A)** Pretreatment lumbar MRI showed multiple diffuse abnormal signal nods in the cauda equina at lumbar and caudal level. **(B)** Enhanced transversal scanning. **(C)** Lesion disappeared in the post-treatment imaging, indicating a complete remission (CR). **(D)** Enhanced transversal scanning also verified complete remission of these lesions after BCMA CAR-T cell therapy [high-resolution T2-weighted image].

CAR-T therapy. With the installation of the Ommaya reservoir, we could record and track the most detailed CAR-T treatment dynamics data in a CNS-involved patient. The clinical data strongly suggested that BCMA CAR-T cells are capable of entering the blood–brain barrier, amplifying and exerting cytotoxicity in CSF, which is an irreplaceable advantage of BCMA CAR-T cells compared to chemotherapy and surgical treatment. The case demonstrated that BCMA CAR-T cells are effective and safe in multiple myeloma with central nervous system involvement. Furthermore, the latest preclinical trials supported that the intracerebroventricular injection of CAR-T cells may achieve more durable tumor cell eradication for mice with CNS-involved malignancies (26). Hence, we deduced that the intra-cerebroventricular injection of CAR-T cells for patients with CNS involvement *via* an Ommaya reservoir could offer a even more valuable new strategy in the future.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of the First Affiliated Hospital

of Zhejiang University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

YW, YH, and HH designed the study. YW, RH, LW, and HZ analyzed and interpreted the data. YW, YZ, EY, CZ, YH, and HH drafted the article. GW, LY, AJ, YH, and HH provided CAR-T cell treatment and care to patient. All authors contributed to the article and approved the submitted version.

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REVIEW
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Gene Expression Profiling in Multiple Myeloma: Redefining the Paradigm of Risk-Adapted Treatment

Claudio Cerchione[1], Saad Z. Usmani[2], A. Keith Stewart[3], Martin Kaiser[4,5], Leo Rasche[6], Martin Kortüm[6], María-Victoria Mateos[7], Andrew Spencer[8], Pieter Sonneveld[9] and Kenneth C. Anderson[10]**

[1] Hematology Unit, IRCCS Istituto Scientifico Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola, Italy, [2] Division of Hematologic Malignancies, Memorial Sloan Kettering Cancer Center, New York, NY, United States, [3] Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada, [4] Division of Molecular Pathology, The Institute of Cancer Research, London, United Kingdom, [5] Department of Haematology, The Royal Marsden Hospital, London, United Kingdom, [6] Department of Internal Medicine II, University Hospital of Würzburg, Würzburg, Germany, [7] Department of Hematology, University Hospital of Salamanca, Salamanca, Spain, [8] Malignant Haematology and Stem Cell Transplantation Service, Alfred Hospital-Monash University, Melbourne, Australia, [9] Department of Hematology, Erasmus MC Cancer Institute Rotterdam, Rotterdam, Netherlands, [10] Jerome Lipper Multiple Myeloma Center, Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, United States

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***Correspondence:**
Claudio Cerchione
claudio.cerchione@irst.emr.it
Kenneth C. Anderson
Kenneth_Anderson@dfci.harvard.edu

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Multiple myeloma is a blood cancer characterized by clonal proliferation of plasma cells in the bone marrow. In recent years, several new drugs have been added to the therapeutic landscape of multiple myeloma, which have contributed to increased survival rates. However, while the use of therapeutics has evolved, there is still a group of high-risk patients who do not benefit from current treatment strategies. Risk stratification and risk-adapted treatment are crucial to identify the group of patients with urgent need for novel therapies. Gene expression profiling has been introduced as a tool for risk stratification in multiple myeloma based on the genetic make-up of myeloma cells. In this review we discuss the challenge of defining the high-risk multiple myeloma patient. We focus on the standardized analysis of myeloma cancer cells by gene expression profiling and describe how gene expression profiling provides additional insights for optimal risk-adapted treatment of patients suffering from multiple myeloma.

Keywords: multiple myeloma, gene expression profiling (GEP), risk-adapted treatment, novel agents, SKY92, risk stratification

INTRODUCTION: RISK STRATIFICATION IN MULTIPLE MYELOMA

Multiple myeloma (MM) is a blood cancer characterized by clonal proliferation of plasma cells in the bone marrow (1). MM accounts for 1.2% of all cancers and 17.1% of blood cancers in Europe, North America, Australia and New Zealand. In these countries, 80,498 patients were newly diagnosed in 2018. In addition, the global prevalence of MM in 2018 was estimated at 159,985.[1] MM is more common in the elderly, with a median age at diagnosis of 70 years. As a consequence,

[1] GLOBOCAN database from the International Agency for Research on Cancer (IARC). Available at: https://gco.iarc.fr. Accessed July 30, 2020.

the number of myeloma patients is expected to rise as it follows the ageing population worldwide (2).

In the last 10 years, new therapies and novel mechanisms of action have been introduced in the clinical landscape of MM. The inclusion of immunomodulatory drugs (e.g. lenalidomide, thalidomide and pomalidomide), proteasome inhibitors, (e.g. bortezomib, carfilzomib) and therapeutic monoclonal antibodies (e.g. daratumumab, isatuximab) led to a significant improvement in survival (3). The median overall survival for newly diagnosed patients treated with high-dose therapy is between 4 and 10 years (4). Although some patients may have long term remission or "functional cure", MM is a chronic relapsing disease for the majority of patients. By combining up to 4 drugs (*quadruplet regimens*) with different mechanisms of action, the list of possible treatment regimens is endless, creating a complex decision tree in the clinical path for a newly diagnosed patient (5).

Despite the enormous progress that has been made in prolonging survival in MM, there is a fraction of patients who do not respond to any of the available treatments or relapse rapidly after an initial response and have reduced survival. In literature, these patients are referred to as *high-risk* patients. The definition of high risk has evolved over time and there are still many variations on how to describe high-risk disease characteristics. Risk stratification is crucial for better understanding of the disease prognosis and rational use of therapies to achieve risk-adapted treatment. Additionally, risk stratification is essential for understanding the risk-based diversity of patients in clinical trials – why do certain patients respond better than others.

Studying the genetics of MM offers more insight into the cancer cells and molecular risk stratification. About 20% of newly diagnosed multiple myeloma (NDMM) patients have molecular abnormalities that account for high risk. Fluorescence *in situ* hybridization (FISH) can detect chromosomal aberrations, such as deletion, translocation and gain. Presence of one these aberrations (*single hit*) have been associated with worse outcomes in MM patients. Furthermore, presence of more (*double or triple hit*) of such aberrations indicate serious genomic instability and a very aggressive disease (6, 7). Gene expression profiling (GEP) has also been introduced as a tool for risk stratification in MM based on the genetic make-up of myeloma cells and offers additional prognostic subgroups (4).

The recent introduction of novel agents with multiple modes of action has primarily benefited patients with standard-risk disease defined by current criteria. Although the treatment outcome of patients with high-risk disease has improved, the unfavorable impact of high-risk FISH abnormalities has not been abrogated. Therefore, this review will focus on the standardized analysis of myeloma cancer cells by GEP and describe how GEP can provide additional insights for optimal risk-adapted treatment of MM patients.

THE CHALLENGE OF DEFINING THE HIGH-RISK PATIENT

One of the controversies that limit risk-adapted treatment in MM is the challenge of defining the high-risk patient. Stratifying patients into different risk groups depends on several aspects. Molecular abnormalities are one way to determine high risk, but the clinical behavior of the patient is another one. In order to identify the group of patients that are not receiving the right treatment, both patient clinical behavior and molecular abnormalities need to be integrated into the definition of high risk.

Clinical Risk

Patient frailty, renal failure, extramedullary disease, tumor burden, early relapse and minimal residual disease can all predict high-risk disease (8–11). The Durie-Salmon staging system, introduced in 1975, reflects the tumor burden by using immunoglobulin levels, hemoglobin and calcium concentration and the number of bone lesions as the classification criteria (9). Although widely accepted from its time of publication, the Durie-Salmon staging system lacks reproducibility and has problematic performance in patients under treatment (9, 12).

In the following years, the relevance of two important and highly prognostic factors appeared in the clinical field. The first is proliferation rate and disease severity indicated by albumin and its inverse relation with interleukin-6 – a known growth and survival factor of myeloma cells (13, 14). The second is tumor burden and renal function reflected by β-2 microglobulin (15–22). Serum albumin and β-2 microglobulin have shown to be better indicators of prognosis and have outperformed the Durie-Salmon system (23). In 2005, a large international consortium of myeloma key opinion leaders defined a staging system on the basis of 10,750 patients from three continents that was about to be the new standard: the International Staging System (ISS), that based its three-group stratification on a combination of the two most powerful and reproducible markers – albumin and β-2 microglobulin (24). The role of tumor burden is however affected by age. Data from 3894 patients uniformly treated in the Myeloma XI trial shows that ISS plays the major role in older patients when defining the survival risk and is of less importance in younger patients (25).

Early relapse, that is a relapse occurring within 12 months from autologous stem cell transplantation (ASCT), is a marker of high-risk disease. Early relapse is associated with reduced survival even after an intensive first line of treatment (8, 26). The first line of treatment in MM is considered crucial in order to prolong the duration of response and survival (8). In similar fashion, patients who do not achieve long lasting minimal residual disease (MRD) negativity are also considered high-risk (11). MRD negativity has become the new end point in treatment, especially for NDMM. Additionally, highly sensitive MRD monitoring may allow for better prediction of early relapse.

Molecular Risk

In parallel with defining staging systems on the basis of clinical variables, the role of cytogenetics in multiple myeloma was being investigated. Cytogenetics has shown to play a role in other hematologic malignancies but was difficult to study in MM mainly due to low proliferation of myeloma cells, which hampered karyotyping (27). The emergence of FISH enabled the analysis of genetic aberrations independent from cell cycle phases and thereby allowed research of the prognostic value of

single chromosomal abnormalities (28). Primary genetic events involved in MM include immunoglobulin heavy chain gene translocations and hyperdiploidy (29, 30). In general, patients with translocations t(4;14) or t(14;16) are considered high-risk (31–35), whereas patients with t(11;14) are considered standard-risk (31, 32, 36, 37) and have a better prognosis. As MM progresses, secondary genetic aberrations develop including mutations and copy number abnormalities, del(13q) (31, 38–40), del(17p) (31–34), del(1p) and gain of 1q (34, 41–43).

With chromosomal abnormalities obtained through FISH adding information to the prognosis of MM patients, the International Myeloma Working Group proposed the revised ISS (R-ISS) in order to add the presence or absence of cytogenetic markers t(4;14), t(14;16), del(17p) and serum lactate dehydrogenase (LDH) (44). Higher levels of LDH are a proxy for high proliferation rates or the presence of tumor mass leading to extramedullary and extraosseous disease and have shown prognostic value in various treatment settings (45–49).

To further add to the prognostic arsenal of clinical and biological markers, various molecular gene classifiers were developed to stratify patients on the basis of up- and downregulated genes (**Table 1**). In 2007, Shaughnessy et al. reported a 70-gene signature with a 17-gene subset that predicts prognosis and stratifies patients in two risk groups (50). This 70-gene classifier is known as GEP70 or UAMS70 and has the brand name MyPRS. In 2008, Decaux et al. published a 15-gene signature that stratifies patients in low or high-risk group, developed by the Intergroupe Francophone du Myélome and called IFM15 (52). In 2010, Dickens et al. defined a 97-gene signature containing cell death genes and reflecting prognosis. A subset of 6 genes were identified to predict poor prognosis and formed the MRCIX6 gene classifier (53). In 2011, Shaughnessy et al. published GEP80 model, that could identify an additional 9% of fast progressing high-risk patients in the patient group defined low risk by GEP70 (51). Also in 2011, Hose et al. reported a gene expression based proliferation index stratifying patients in a low-, intermediate- and high-risk group for disease progression (54). In 2012, Kuiper et al. defined a 92-gene signature that stratifies patients in a standard and high-risk group (4). This 92-gene classifier, called SKY92, is commercially available under the brand name SKY92 or MMprofiler.

The Challenge of Defining High Risk in MM

Globally, there is no consensus on the definition of high risk, nevertheless clinical experts agree that it is never a single marker. Furthermore, clinical experts seem to also agree on an escalated treatment paradigm for high-risk patients with prolonged planned maintenance (55–58). The National Comprehensive Cancer Network (NCCN) states "patients with cytogenetically and molecularly defined high-risk disease do not receive the same benefit from certain approaches as the low-risk patients and need alternative therapies".[2] The International Myeloma Working Group (IMWG) concludes that "risk stratification in MM is important to predict survival and to define a treatment strategy" (59). In none of the guidelines preferred risk-stratified treatment pathways have been described.

The challenge of defining high risk is also reflected in clinical trials studying the efficacy of treatment (combinations) (**Figure 1**). We have performed a search on August 11, 2021 on ClincialTrials.gov on condition "multiple myeloma" in combination with the terms "newly diagnosed" and "high risk". The search resulted in 78 studies that were "not yet recruiting", "recruiting", "enrolling by invitation", "active, not recruiting" or "completed". We further analyzed 17 trials mentioning high-risk as eligibility criteria for enrollment and found 29 different high-risk markers. **Figure 1** lists all 29 markers and shows the diversity in selecting markers to define high risk across clinical trials in MM.

GENE EXPRESSION PROFILING IDENTIFIES A UNIQUE GROUP OF HIGH-RISK PATIENTS

The developed GEP signatures provide additional insights into risk stratification in a robust manner. The clinical application of the GEP signatures in myeloma has been stagnant because of the lack of standardization and user-friendly platforms (60). Clinical development of SKY92 has overcome these issues, by providing a standardized, analytically validated and user-friendly tool (61). At this moment, the SKY92 signature is the only fully accredited GEP signature and has consistent performance in detecting high risk in MM (61). Therefore, from this section onwards we will focus on SKY92.

The GEP-Based Marker SKY92

The prognostic GEP-based marker SKY92 was developed as EMC-92 based on the HOVON65/GMMG-HD4 trial using a cohort of 290 NDMM patients. The prognostic power in the combination of 92 genes (including several known cancer genes) was generated by supervised principal component analysis in combination with simulated annealing (4). SKY92 provides a binary outcome and classifies a patient at either high risk with poor survival or at standard risk (61).

SKY92 is clinically validated for accurately predicting the prognosis of NDMM and relapsed/refractory multiple myeloma (RRMM) patients for both overall survival (OS) as well as progression free survival (PFS). After discovery in 2012, SKY92 has been independently validated in 16 patient cohorts totaling 3,339 patient cases (**Table 2**) (73). The validation cohorts cover a wide variety of geographies and treatments, clinical trial and non-trial real world settings, and both transplant eligible as well as non-transplant eligible patients. The proportion of high-risk patients identified by SKY92 remains stable around 20% in the NDMM validation sets and is slightly higher for the poorer performing RRMM patients.

Longitudinal analysis of two publicly available MM patient data sets aimed to investigate the evolution of SKY92 risk classification with disease progression (74). SKY92 risk classification was compared between same-patient samples at

[2] Kumar SK. et al. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) Multiple Myeloma Version 4.2020 – May 8, 2020. Available at: https://nccn.org. Accessed August 3, 2020.

TABLE 1 | GEP risk signatures and their characteristics.

Scientific name (Brand name)	Platform	Classification	Utility	Performance*	Availability
UAMS70/ GEP70 (50) (MyPRS/ MyPRS Plus)	ThermoFisher U133Plus2.0 microarray	Continuous score with a cutoff such that patients are either: - High risk for disease progression - Low risk for disease progression	Predicts event-free survival and OS at the moment of diagnosis or at relapse.	High-risk score present in 13% of patients. HR = 5.16 (p<0.001) in training set and HR = 4.75 (p<0.001) in the test cohort.	In research setting only.
UAMS80/ GEP80 (51)	ThermoFisher U133Plus2.0 microarray	Dichotomous score such that patients are either: - High risk with significantly inferior PFS and OS - Low risk with significantly better PFS and OS	Predicts PFS and OS at the moment of diagnosis.	GEP80 identifies 9% of high-risk patients in the GEP70 low-risk group and 41% of low-risk patients in the GEP70 high-risk group.	In research setting only.
IFM15 (52)	Custom designed platform	Dichotomous score such that patients are either: - High risk with significant shorter OS - Very low risk with significant better OS	Predicts OS at the moment of diagnosis.	Survival at 3 years was 90.5% (95% CI, 85.6%-95.3%) for the very-low risk group and 47.4% (95% CI, 33.5%-60.1%) for the high-risk group; as estimates of rates from training, test and external validation cohorts.	In research setting only.
EMC92/ SKY92 (4) (MMprofiler)	ThermoFisher U133Plus2.0 microarray	Dichotomous score such that patients are either: - High risk of early relapse - Standard risk of early relapse	Predicts PFS and OS at the moment of diagnosis or at relapse.	High-risk patients showed reduced OS with HR=3.40 (95% CI, 2.19-5.29) for TT2; 5.23 (95% CI, 2.06-4.39) for TT3; 2.38 (95% CI, 1.65-3.43) for MRC-IX and 3.01 (95% CI, 2.06-4.39) for APEX patient cohort. All with p<0.0001.	Both in research setting and commercially (CLIA validated Laboratory Developed Test in the US and CE-IVD certified in Europe).

CI, confidence interval; HR, hazard ratio; OS, overall survival; PFS, progression-free survival.
**Performance as described by the authors in respective discovery papers.*

diagnosis and relapse in the Multiple Myeloma Research Foundation (MMRF) CoMMpass data set and the University of Arkansas for Medical Sciences (UAMS) Total Therapy cohort (TTx). In the analysis of the CoMMpass data set, 31% of patients were classified high-risk at diagnosis. The number of high-risk patients increased to 46% at the second timepoint and 58% for patients with the latest timepoint more than 12 months after baseline. Furthermore, almost all patients in the CoMMpass data set died within 12 months after being classified as high-risk. In the TTx cohort the percentage of high-risk patients increased significantly from 12% at diagnosis to 28% at relapse. Analysis of these data sets show that repeated testing of risk signature provides additional prognostic information for standard-risk patients.

The PROMMIS trial (NCT02911571) investigated the impact of SKY92 on risk stratification and treatment plan (73). This

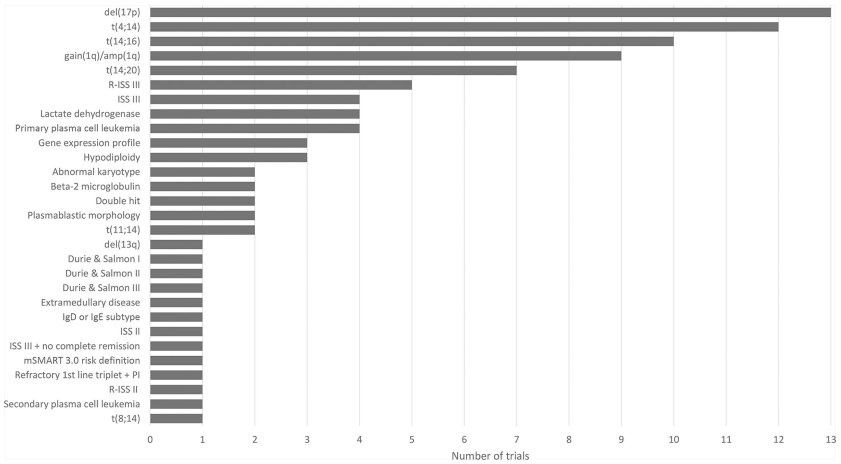

FIGURE 1 | Overview of 29 high-risk markers for multiple myeloma resulting from the analysis of 17 clinical trials (NCT00570180, NCT00691704, NCT00793572, NCT01341262, NCT01668719, NCT02217163, NCT02685826, NCT03004287, NCT03104842 (GMMG-CONCEPT), NCT03188172 (OPTIMUM), NCT03441958, NCT03606577 (IFM 2018-04), NCT03641456, NCT04133636 (CARTITUDE-2), NCT04196491 (KarMMa-4), NCT04579523, NCT04935580). Figure shows the diversity in high-risk marker selection. For each marker, the number of trials selecting the marker to define high-risk disease is shown.

TABLE 2 | SKY92 clinical validation studies.

Cohort	MM type*	N	SKY92 high risk (%)	Hazard ratio OS (p-value)	Hazard ratio PFS (p-value)
HOVON-65/GMMG-HD4 (4)	ND	329	–		
TT2 (4)	ND	351	68 (19%)	3.4 (<0.0001)	
APEX (4)	RR	264	43 (16%)	3.0 (<0.0001)	1.7 (0.0058)
TT3 (62)	ND	254	47 (19%)	4.5 (<0.0001)	
MMGI (63)	ND	91	19 (21%)	8.2 (<0.0001)	
GIMEMA-MMY-3006 VTD (64)	ND	114	23 (20%)	4.0 (0.0037)	
CoMMpass (65)	ND	632	116 (18%)	3.1 (<0.0001)	
HOVON-87/NMSG-18 (66)	ND	190	26 (14%)	2.6 (<0.0001)	2.4 (<0.0001)
KRd trial (67)	ND	16	5 (31%)		8.2 (0.017)
CarThaDex trial (68)	ND	20	5 (25%)		2.8 (0.12)
EMN-02/HOVON-95 (69)	ND	179	36 (20%)		
E-MTAB-1038 (70)	ND/RR	66	13 (20%)	2.6 (0.044)	
TT6 (70)	RR	55	11 (20)	10.3 (0.00015)	
MMpredict non-trial set (71)	ND/RR	155	34 (22%)	4.5 (<0.0001)	2.7 (<0.0001)
MUKseven trial (72)	RR	48	9 (25%)		2.9 (0.037)
MRC-IX (34)	ND	246	51 (21%)	2.2 (<0.0001)	
MRC-XI (34)	ND	329	81 (25%)	3.9 (<0.0001)	2.6 (<0.0001)
Total		3,339	587		

ND, newly diagnosed; RR, relapsed/refractory.

prospective observational study showed that SKY92 added more information on risk stratification, compared to currently used standards. In the PROMMIS trial, physicians first classified the MM patients according to their own local standard and determined the patient's treatment path accordingly. Then, after unblinding SKY92 results, physicians had the opportunity to reclassify the patients and adapt treatment. Overall, Unblinding SKY92 results led to a change of risk status for 42% of patients (62/147). More specifically, 16 patients received a SKY92 high-risk result while previously being assigned standard risk – all of these patients (100%) were reclassified high risk after unblinding SKY92; 46 patients received a SKY92 standard-risk result while previously being assigned high risk – 30 out of 46 (65%) were reclassified standard risk after unblinding SKY92. Treatment plans were changed for 37% of patients (54/147). After knowing SKY92 results, physicians were more confident in their final treatment plan, even when SKY92 confirmed their initial risk classification. For 89% of patients (131/147), the final risk classification assigned by the physician matched the SKY92 result, showing the added value of SKY92 in clinical decision making.

Combining GEP-Based Biomarkers With (R-)ISS

Besides the use of univariate prognostic markers such as (R-)ISS, single cytogenetic abnormalities or single gene expression classifiers, the combination of prognostic markers is being

increasingly investigated (34, 75). This allows for more specific risk classifications, shifting from the ability to determine if a patient is high risk (and otherwise standard risk) towards a multi-categorical classification.

In a multivariate analysis, 20 clinical and biological risk markers were used independently to find the strongest predictor for prognosis as either a stand-alone marker or in combination (62). A total number of 4,750 patients were included from the APEX, HOVON65/GMMG-HD4, IFM-G, MRC-IX, TT2 and TT3 cohorts. The research showed that ISS is a valuable partner to both GEP classifiers and FISH markers. Ranking all existing and combined risk markers showed that GEP+ISS is the strongest predictor for OS, resulting in a 4-group risk classification. In this setting GEP, by means of SKY92, stratified patients into the high-risk and the standard-risk group. ISS sub-stratified the standard-risk patients into two intermediate-risk groups (ISS II + ISS III) and a low-risk group (ISS I group). The median survival was 24 months for the high-risk group, 47 and 61 months for the intermediate-risk groups, and the median survival was not reached after 96 months for the low-risk group.

The GEP classifier SKY92 has also been combined with R-ISS in the HOVON-87/NMSG-18 trial in an analysis of 168 older myeloma patients (76). Combining the R-ISS with SKY92 resulted in 3 risk groups: SKY-RISS I (SKY92 standard risk + R-ISS I, 15%), SKY-RISS III (SKY92 high risk + R-ISS II/III, 11%) and SKY-RISS II (all other patients, 74%). The 3-year OS rates for SKY-RISS I to III were 88%, 66% and 26% ($p=6x10^{-7}$) and were validated in the elderly patient subset from the CoMMpass dataset. Combining SKY92 with R-ISS resulted in a superior prognostic marker compared to either marker separately.

Unique Group of High-Risk Patients Is Identified With GEP

In order to make sure that a high-risk patient is correctly identified, newer, more robust, standardized and reliable technologies should be incorporated for risk stratification. There is substantial evidence that GEP is indispensable for correct assessment of high-risk MM patient population after which the well-established clinical and cytogenetic markers can further distinguish the low-risk from the intermediate-risk patients.

In 2019, Kuiper et al. analyzed PFS and OS related to high-risk outcomes based on ISS and SKY92 in non-transplant eligible patients from the HOVON87/NMSG18 study (66). In this cohort, 26% of patients were classified as high risk by ISS (ISS III). SKY92 classified 14% of patients as high-risk, with an overlap between the two groups of 5%. Thus, 9% of high-risk patients are misclassified as lower risk (ISS I or ISS II). As the R-ISS is becoming the preferred staging system, in 2020 Kuiper et al. compared prognostication between R-ISS and SKY92 in older myeloma patients (76). In the HOVON87/NMSG18 cohort, R-ISS III identified 7% of patients as high risk, where SKY92 identified 13%. Furthermore, in the CoMMpass cohort these percentages were 13% and 26%, respectively.

Several multivariate analyses have established that SKY92 is an independent prognostic marker and that combining SKY92 with ISS or R-ISS results in a marker with improved performance compared to either marker separately (34, 66, 76). But how does the prognostic power of SKY92 relate to several high-risk cytogenetic markers such as del(17p) and t(4;14)? Combining six datasets, for a total of 805 patients, SKY92 combined with ISS identified three risk groups: low, intermediate and high (77). The OS of the high-risk group was significantly shorter than the low-risk group (hazard ratio 6.0, $p<0.001$). For all three risk groups, the comparison of FISH-positive and FISH-negative patients resulted in a non-significant OS difference. In the high-risk group (n=169), 53% of patients (90/169) were FISH-negative. The high-risk status of these 90 patients was overlooked by using only FISH for risk stratification.

In 2020, Shah et al. examined the combined predictive value of high-risk chromosomal abnormalities, including t(4;14), t(14;16), t(14;20), gain(1q) and del(17p), and SKY92 in 329 NDMM patients from the NCRI Myeloma XI trial who received intensive therapy and validated the findings in Medical Research Council (MRC) Myeloma IX trial (34). SKY92 identified 24.6% high-risk patients (81/329) with a significantly shorter PFS (median 16 versus 33.8 months; hazard ratio 2.6, CI 95% 2.0-3.5; $p=4.1x10^{-11}$) and OS (median 36.7 months versus not reached; hazard ratio 3.9, 95% CI: 2.7-5.7; $p=2.5x10^{-13}$), regardless of induction regimen and posttransplant randomization. There was a partial overlap between patients with SKY92 and chromosomal high-risk markers, with 6.1% (20/329) of patients identified as SKY92 high risk in the absence of chromosomal high-risk markers. Furthermore, 161 patients carried no chromosomal high-risk marker, of which 12% (20/161) were SKY92 high risk. These 20 patients had significantly shorter PFS (median 15.8 versus 41.7 months; hazard ratio 3.18, 95% CI: 1.86-5.46; $p=2.6x10^{-5}$) and OS (estimated 4-year OS 55% versus 86%; hazard ratio 2.42, 95% CI: 1.04-5.67; p=0.04). The study demonstrated the prospective prognostic validity of SKY92 in the wider context as a means of identifying patients at diagnosis who have high-risk MM. Furthermore, the study highlighted that SKY92 combined with chromosomal profiling at diagnosis can predict clinical outcome with significant precision. The authors of the study also acknowledge that the identification of high-risk patients opens up the possibility of risk-adapted treatment.

UK OPTIMUM trial (MUKnine, NCT03188172) is a prospective study from 39 UK hospitals designed to identify ultra high-risk patients and provide risk-adapted treatment. Patients were centrally profiled for GEP high-risk signature and/or double hit disease. These patients were considered ultra high-risk and were treated with daratumumab, cyclophosphamide, bortezomib, lenalidomide, dexamethasone (Dara-CVRd) induction, augmented high-dose melphalan and ASCT (78). MRD status was 64% MRD-negative, 14% MRD-positive and 22% not evaluable at day 100 post ASCT (assessed by flow cytometry, sensitivity 10^{-5}). Despite overall high MRD-negativity, some early progressions indicate a group of patients with unmet clinical need. Furthermore, OPTIMUM trial is a digital comparator arm trial. PFS at 18 months was compared between patients in the OPTIMUM trial and matched ultra

high-risk myeloma patients from the Myeloma XI trial treated with carfilzomib, lenalidomide, cyclophosphamide and dexamethasone, ASCT and lenalidomide maintenance or observation. Patients from the OPTIMUM trial, treated with the five-drug combination, were found to have significantly improved PFS; with an 18 months estimate of 81.7% (95% CI: 74.2-89.1) and 65.9% (95% CI: 57.3-74.4) for the OPTIMUM and Myeloma XI trial, respectively (79). The analyses of the OPTIMUM trial have shown that identifying the ultra high-risk patients and adapting the treatment can lead to high MRD negativity rates and improved survival in comparison to the standard of care. Lastly, OPTIMUM trial demonstrated the feasibility of incorporating GEP into clinical patient pathway across institutions and showed the value of combining cytogenetic information with GEP.

DISCUSSION

Several new drugs have been added to the therapeutic landscape of MM in recent years, which have contributed to increased PFS and OS. However, while the use of therapeutics in the clinical scenario has evolved, there is still a group of high-risk patients who do not benefit from current treatment strategies. The international guidelines on MM all recognize the relevance of having detailed information on the patient's disease and associated risk for progression in order to better evaluate the individual clinical care pathway (59). It has become clear that there is not a single marker capable of independently and accurately defining the high-risk MM patient. For this reason, multiple combinations are postulated by different study groups, but no real consensus has been formulated and used in clinical trials.

In this review, we aimed to describe the impact of the genetic make-up of cancer cells as a molecular risk factor in MM. In this section, we will discuss the integration of both clinical variables and molecular markers into the definition of high risk and the outlook of risk-adapted treatment in MM.

GEP-based marker SKY92 is a standardized tool for risk stratification that provides additional information to the anamneses of patients with MM. SKY92 allows for risk stratification in relatively homogenous subgroups of patients and provides added value in combination with clinical markers and FISH, which by itself does not capture the genomic complexity of MM (27, 34). SKY92 stratifies MM patients into high-risk or standard-risk group irrespective of treatment regime and relapse setting. Risk stratification using SKY92 is important at diagnosis in order to choose the optimal first line of treatment for maximum effect and prevention of relapse.

Longitudinal monitoring of risk assessment using SKY92 allows for dynamic risk stratification (74). GEP in combination with FISH is a great way to track changes in molecular risk over time and should be performed at diagnosis and every relapse to correctly identify high-risk patients and guide treatment (80, 81).

Risk can also change during the course of disease depending on response to treatment. Early relapse has strong association with reduced survival in both high-risk and standard-risk cytogenetic groups (8). MRD also plays a big role in patient prognosis. MRD-negative patients with high-risk cytogenetics have similar outcome as standard-risk patients (82). However, further research is needed to investigate the proper actions that should be taken regarding de-escalation of therapy of patients with sustained MRD-negativity.

There are still some limitations of using GEP in practice. Bone marrow sample availability is one of the challenges, as well as the lack of guidelines for optimal risk classification and corresponding treatment strategy. Additionally, SKY92 was developed and validated for active and symptomatic MM, therefore the prognostic value of SKY92 has yet to be assessed in (asymptomatic) smoldering myeloma. Lastly, a cost-benefit analysis of SKY92 still needs to be performed because current evidence is lacking. Such analysis should be done to investigate the cost impact of SKY92 on the use of high-cost anti-myeloma drugs.

Despite the current limitations, there is substantial evidence that GEP is an important tool in providing the most accurate risk assessment identifying the true high-risk population and can be used in combination with the well-established clinical and cytogenetic markers. After GEP risk assessment, ISS and FISH can further distinguish the intermediate-risk from the low-risk patients. Without GEP, many patients are misclassified using existing tools. Therefore, a new era beckons in which patients are routinely and accurately assigned risk and its relevance, considering the available treatment opportunities. In this new era:

- GEP is used to identify high-risk MM patients;
- GEP is part of routine diagnostic work-up to allow for risk-adapted strategies;
- Risk-adapted treatment is investigated in both clinical trials and the real-world setting.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

With the availability of new techniques and increasing knowledge of MM biology, the definition of high risk is evolving and should include personalized assessment of both clinical and molecular markers. Treatment should focus on biology of high risk in younger patients and on clinical behavior in older patients. Treatment intensification in molecular high-risk patients and dose reduction and deliverability in clinical high-risk and frail patients need to be researched.

Treatment combining several modes of action and incorporation of novel immunotherapies, for example CAR T-cell therapy or bispecific antibodies (83), could be the next area to explore for the high-risk patients that represent the group with unmet clinical need. Risk-adapted therapy is crucial to achieve deep and sustained response in high-risk MM patients. In order to develop therapeutic strategies for specific risk groups, it is of utmost importance to use GEP as an eligibility marker. In many trials the risk groups are stratified along different randomization

arms or inclusion criteria. For both misclassified high-risk patients in a low-risk trial and low-risk patients in high-risk trials, the final conclusions on the effectiveness of the investigated therapeutic regimen will be influenced. Clinical trials focusing on high-risk MM patients are crucial for identification of optimal therapy for improved survival.

AUTHOR CONTRIBUTIONS

CC wrote the manuscript. AKS, LR, and PS reviewed and edited the manuscript. SU, MKa, MKo, MVM, AS, and KA reviewed the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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BRIEF RESEARCH REPORT
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Efficacy of Daratumumab-Containing Regimens Among Patients With Multiple Myeloma Progressing on Lenalidomide Maintenance: Retrospective Analysis

Hira Mian[1], Christine Eisfeld[2], Christopher P. Venner[3], Esther Masih-Khan[4,5], Moustafa Kardjadj[5], Victor H. Jimenez-Zepeda[6], Cyrus Khandanpour[2], Georg Lenz[2], Arleigh McCurdy[7], Michael Sebag[8], Kevin Song[9], Richard LeBlanc[10], Darrell White[11], Julie Stakiw[12], Anthony Reiman[13], Martha Louzada[14], Muhammad Aslam[15], Rami Kotb[16], Engin Gul[5] and Donna Reece[4,5]*

[1] Department of Oncology, Juravinski Cancer Center, Hamilton, ON, Canada, [2] Department of Medicine, University Hospital Münster, Münster, Germany, [3] Cross Cancer Institute, University of Alberta, Edmonton, AB, Canada, [4] Department of Medical Oncology and Hematology, Princess Margaret Cancer Centre, Toronto, ON, Canada, [5] Canadian Myeloma Research Group, Toronto, ON, Canada, [6] Arnie Charbonneau Cancer Institute, University of Calgary, Calgary, AB, Canada, [7] Department of Medicine, The Ottawa Hospital, Ottawa, ON, Canada, [8] Department of Medicine, McGill University, Montreal, QC, Canada, [9] BC Cancer Agency, Vancouver General Hospital, Vancouver, BC, Canada, [10] Maisonneuve-Rosemont Hospital Research Centre, University of Montreal, Montreal, QC, Canada, [11] Queen Elizabeth II Health Sciences Centre, Dalhousie University, Halifax, NS, Canada, [12] Saskatoon Cancer Centre, University of Saskatchewan, Saskatoon, SK, Canada, [13] Department of Medicine, Saint John Regional Hospital, Saint John, NB, Canada, [14] Department of Haematology, London Regional Cancer Center, London, ON, Canada, [15] Department of Medical Oncology, Allan Blair Cancer Centre, Regina, SK, Canada, [16] Department of Medical Oncology & Hematology, Cancer Care Manitoba, Winnipeg, MB, Canada

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Giovanni Martinelli,
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***Correspondence:**
Esther Masih-Khan
Esther.masih-khan@uhnresearch.ca

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Background: Daratumumab, a monoclonal antibody directed against CD38 is a recent class of drugs introduced into the multiple myeloma therapeutic landscape. While clinical trial data have shown a remarkable impact on outcomes, the efficacy of daratumumab combination therapies in specific clinically relevant subgroups including among patients refractory to lenalidomide maintenance remains unknown.

Methods: In this study, retrospective data were reviewed from the Canadian Myeloma Research Group and the German Munster Myeloma databases to identify patients that received daratumumab in combination with pomalidomide (DPd), lenalidomide (DRd), and bortezomib (DVd) in a population that had relapsed on lenalidomide maintenance postautologous stem cell transplant. The primary aim of the study was to look at outcomes of these patients in different daratumumab combinations.

Results: A total of 73 patients were identified. The median age of the patients at the time of daratumumab initiation was 60 (38-72) and 64.4% (n = 47) were men. In the selected cohort, 43.8% (n = 32) were treated with DRd, 31.5% (n = 23) with DVd, and 24.7% (n = 18) with DPd regimen. The median progression-free survival (PFS) of the entire cohort was 15.8 months (95% CI, 12.9–37.1 months). The median PFS of the individual regimens was as follows: DPd 18.9 months (95% CI, 13.7-not reached), DRd 21.7 months (95% CI, 11.6-not reached), and DVd 12.9 months (95% CI, 3.1-not reached).

Conclusions: Daratumumab-containing therapies are effective regimens in patients progressing on lenalidomide maintenance. Additional studies are required to decide the optimal regimen post-lenalidomide maintenance.

Keywords: multiple myeloma, lenalidomide, maintenance, daratumumab (DARA), relapsed/refractory

INTRODUCTION

Multiple myeloma is an incurable plasma cell neoplasm characterized by the clonal proliferation of malignant plasma cells within the bone marrow (1). The clinical manifestations of multiple myeloma, which reflect end organ damage, include renal impairment, hypercalcemia, lytic bony lesions, and anemia. Treatment modalities for multiple myeloma have led to pivotal improvements in patient outcomes in the past decades with many new therapeutic agents entering the landscape (2).

Lenalidomide maintenance following autologous stem cell transplant (ASCT) remains a standard of care among transplant-eligible patients with newly diagnosed multiple myeloma (NDMM) (3). The monoclonal antibody, daratumumab, represents a novel class of drugs that has shown remarkable efficacy among both newly diagnosed and relapsed patients in landmark clinical trials (4, 5). Daratumumab-containing triplet regimens have been introduced for patients in the relapsed setting, including in combination with dexamethasone and pomalidomide (DPd), lenalidomide (DRd), or bortezomib (DVd). The efficacy of these daratumumab-containing regimens in patients progressing on low-dose maintenance lenalidomide following the first line of therapy is largely undescribed. Landmark randomized clinical trials such as APOLLO, POLLUX, or CASTOR have either excluded or included only a very small proportion of patients progressing specifically on lenalidomide maintenance (4, 6, 7). To our knowledge, there is no prospective data to allow comparison of the efficacy of these three regimens in patients specifically progressing on lenalidomide maintenance. This is an increasingly common clinical scenario, and understanding outcomes and gaps of various combination regimens can further improve clinical decision-making.

In order to fulfill this knowledge gap, we report on the outcomes of DPd, DRd, and DVd regimens given as a second-line therapy for patients progressing specifically on lenalidomide maintenance following frontline ASCTs. Using two large disease-specific databases, we aimed to understand the response rates, progression-free survival, and overall survival in patients treated with DPd, DRd, and DVd following progression on lenalidomide maintenance.

MATERIALS AND METHODS

Data Source

The Canadian Myeloma Research Group Database is a prospectively maintained disease-specific database with over 7,000 patients enrolled from 14 academic sites across Canada with legacy data collected from 2007. The Munster Myeloma database collects myeloma-specific information in a German academic center and currently contains data from 800 patients from 2005. All patients treated with daratumumab-based regimens in second line, including those treated on clinical trial protocols, following relapse on lenalidomide maintenance were included in the analysis from the two databases analyzed up to June 30, 2020. Local research ethics boards at every contributing site approve entry of source data into the CMRG-DB. The approval for review of this specific dataset was obtained from the University Health Network Research Ethics Board (UHN REB) as per the approved governance structure of the CMRG-DB, and the analysis was conducted in accordance with the Declaration of Helsinki.

Included Patient Cohort

Multiple myeloma patients progressing on or within 60 days of last receiving lenalidomide-based maintenance therapy after high-dose chemotherapy and ASCT who were then treated with DPd, DRd, or DVd between Jan 2015 and Jun 2020 were identified using the Canadian Myeloma Research Group Database and the Munster Myeloma database. As this was a retrospective study, patients treated on clinical trials were also included in our cohort.

Study Outcomes

The primary endpoint of this study was progression-free survival (PFS). PFS was defined from the date of daratumumab-based regimen initiation until the date of progression or death, whichever came first. Secondary endpoints included response (response assessed as per standard International Myeloma Working Group Response Criteria) (8) and overall survival (OS). OS was calculated from the date of daratumumab-based regimen initiation to date of death or censored at date of last follow-up.

Statistical Plan

Patient-baselined demographics, disease characteristics, and treatment details (induction therapy, maintenance therapy, and daratumumab-based regimens) were analyzed using descriptive statistics. Categorical variables and continuous variables were analyzed using Fisher's exact test and the Wilcoxon ran-sum test, respectively. The Kaplan-Meier method and log rank test was used to estimate the time-to-event endpoints and between group comparisons for PFS and OS. Statistical analyses were performed using R (4.1.0) and RStudio (1.4.1717) for Windows. All p-values were two sided; $p < 0.05$ was considered to indicate a statistically significant result.

RESULTS

A total of 1,380 NDMM patients who received lenalidomide maintenance were identified in the two databases. Of those, 73 patients were treated with a daratumumab-containing regimen on progression in second line. In the included cohort, 32 patients (43.8%) were treated with DRd, 23 (31.5%) with DVd, and 18 (24.7%) with DPd regimen. The baseline characteristics for the entire cohort as well as for each group (DPd, DRd, DVd) are summarized in **Table 1**. The median age for the entire cohort was 60 years and 47 (64.4%) were male. The most common myeloma subtype was IgG in 40 (54.8%) of the patients. The majority of patients were ISS stage II at diagnosis 47.1% (n = 32). High-risk status based on the presence or absence of t(4:14) or t(14:16) or deletion17p was available in 86% (n = 63) of patients of which 17.5% were high risk. Most patients on DPd had their therapy initiated in 2017 (88.9%), whereas those on DRd (75.0%) and DVd (60.9%) initiated it most commonly in 2019.

Regarding the individual subgroups, the median age for the group receiving DPd and with the most clinical trial patients (94%, n = 17) was 53 years (range 38–68), younger than the other two groups. IgG MM subtype was the most common in all the three subgroups. The ISS stage was well balanced between the three groups. There was a slightly higher proportion of patients with high-risk cytogenetics in the DVd arm; however, the overall number of patients were small in each cytogenetic risk group.

Regarding outcomes of first-line therapy preceding a daratumumab-based regimen, the details are outlined in **Table 2**. Most patients (>80%) received CyBorD as induction and had a greater than 90% overall response on it in the entire cohort. Three patients received tandem ASCTs, 1 in the DPd group and 2 in the DRd groups. Post-ASCT, all patients received lenalidomide maintenance as a single agent. Patients received single-agent lenalidomide doses between 5 and 15 mg, 48% (n = 35) on a 21- of 28-day-cycle schedule and others (52% n = 38) on a continuous 28 of 28-day-cycle schedule. All patients progressed while on maintenance; however, the most frequent maintenance dose was 10 mg (90.1%) at the time of progression. Maintenance median duration was 21.1 months (range, 1.0–77.6 months) for the entire cohort. Among the specific subgroups, the median duration on lenalidomide maintenance was 23.9 months (range, 3.1–77.2) for the DPd group, 19.0 months (range, 1.4–67.7) for the DRd group, and 16.4 months (range, 1.0–77.6) for the DVd group. For the entire cohort, the median PFS on first-line treatment was 33.8 months (95% CI, 30.5–37.5).

Baseline lab values at initiation of daratumumab are summarized in **Table 2**. The median follow-up for the entire cohort from the time of daratumumab initiation was 21.0 months (range, 0.9–30.2). The median follow-up for DPd, DRd, and DVd regimen was 41.8 months (range, 13.6–53.6 months), 21.6 months (range, 7.5–32.0 months) and 13.8 months (range, 0.9–30.2 months), respectively. The response of each daratumumab-containing regimen is outlined in **Table 3**. A higher proportion of patients in the DPd arm (76.5%) obtained a CR/VGPR compared with DRd (58.1%) or DVd (28.6%). The median PFS of the entire cohort was 15.8 months (95% CI, 12.9–37.1 months). The median PFS of the individual regimens was as follows: DPd 18.9 months (95% CI, 13.7-not reached), DRd 21.7 months (95% CI, 11.6-not reached), and DVd 12.9 months (95% CI, 3.1-not reached) as demonstrated in **Figure 1A** (p-value = 0.18).

TABLE 1 | Patient characteristics at diagnosis for the daratumumab combination treatment groups.

Characteristics	All (N = 73)	DPd (N = 18)	DRd (N = 32)	DVd (N = 23)
Age at initiation of daratumumab [median (range)]	60 (38–72)	53 (38–68)	59 (39–71)	64 (47–72)
Male [*n* (%)]	47 (64.4)	15 (83.3)	19 (59.4)	13 (56.5)
MM subtype [*n* (%)]				
IgG	40 (54.8)	14 (77.7)	14 (43.8)	12 (52.2)
IgA	17 (23.3)	3 (16.7)	7 (21.9)	7 (30.4)
FLC	14 (19.2)	1 (5.6)	10 (31.2)	3 (13.1)
Other	2 (2.7)	0	1 (3.1)	1 (4.3)
ISS stage at diagnosis [*n* (%)]				
I	23 (33.8)	4 (23.5)	11 (36.7)	8 (38.1)
II	32 (47.1)	10 (58.8)	12 (40.0)	10 (47.6)
III	13 (19.1)	3 (17.7)	7 (23.3)	3 (14.3)
Unknown	5	1	2	2
High-risk FISH[a] [*n* (%)][b]				
Present	11 (17.5)	2 (12.5)	4 (14.8)	5 (25.0)
Not present	52 (82.5)	14 (87.5)	23 (85.2)	15 (75.0)
Unknown	10	2	5	3
Initiation year [*n* (%)]				
2015–2016	2 (2.7)	1 (5.6)	1 (3.1)	0
2017	17 (23.3)	16 (88.9)	1 (3.1)	0
2018	9 (12.3)	1 (5.6)	5 (15.6)	3 (13.0)
2019	38 (52.1)	0	24 (75.0)	14 (60.9)
2020	7 (9.6)	0	1 (3.1)	6 (26.1)

[a]*High-risk cytogenetics defined as del 17p, t(4;14), and/or t(14;16).*

[b]*Denominator for percentage calculations do not include unknown values.*

ISS, International Staging System; CR, complete response; VGPR, very good partial response; PR, partial response; MR, minimal response; SD, stable disease; PD, progressive disease; Len, lenalidomide.

TABLE 2 | Summary of frontline therapy for the daratumumab combination treatment groups.

Frontline therapies	All (*N* = 73)	DPd (*N* = 18)	DRd (*N* = 32)	DVd (*N* = 23)
Induction therapy				
Regimen [*n* (%)]				
CyBoRD	61 (83.6)	16 (89)	26 (81.3)	19 (82.6)
VD	4 (5.5)	1 (6)	1 (3.1)	2 (8.7)
RVD	2 (2.7)	1 (6)	1 (3.1)	0
Other	6 (8.2)	0	4 (12.5)	2 (8.7)
Best response on induction [*n* (%)]				
ORR (>PR)	67 (94.4)	16 (94.1)	30 (93.8)	21 (95.4)
CR/VGPR	41 (57.8)	5 (29.4)	24 (75.0)	12 (54.5)
PR	26 (36.6)	11 (64.7)	6 (18.8)	9 (40.9)
MR/SD	4 (5.6)	1 (5.9)	2 (6.2)	1 (4.5)
Unknown	2	1	0	1
Maintenance therapy				
Len maintenance dose at progression [*n* (%)]				
5 mg	4 (5.6)	0	4 (12.9)	0
10 mg	64 (90.1)	17 (94.4)	25 (80.6)	22 (100.0)
15 mg	3 (4.3)	1 (5.6)	2 (6.5)	0
Unknown	2	0	1	1
Tandem ASCT	3	1	2	0
Len maintenance duration (months; median, range)	21.1 (1.0–77.6)	23.9 (3.1–77.2)	19.0 (1.4–67.7)	16.4 (1.0–77.6)
Median PFS for first-line treatment (months, 95% CI)	33.8 (30.5–37.5)	35.6 (31.9–56.5)	32.5 (26.9–55.4)	31.1 (23.0–38.2)

Denominator for percentage calculations do not include unknown values.
Cy, cyclophosphamide; Bor, bortezomob; V, velcade; D, dexamethasone; Len, lenalidomide; CR, complete response; VGPR, very good partial response; PR, partial response; MR, minimal response; SD, stable disease; PD, progressive disease; PFS, progression-free survival; CI, confidence interval.

TABLE 3 | Baseline lab values at initiation and responses on daratumumab based therapy.

Daratumumab combination therapy	All (*N* = 73)	DPd (*N* = 18)	DRd (*N* = 32)	DVd (*N* = 23)
Lab values at initiation of daratumumab therapy				
Hemoglobin [g/L, median (range)]	120 (72–164)	128 (87–154)	120 (82–162)	120 (72–164)
White blood cell count $\times 10^9$/L [median (range)]	4.0 (1.9–15.5)	4.4 (2.5–15.5)	4.1 (1.4–10.9)	3.3 (1.9–6.9)
Absolute neutrophil count $\times 10^9$/L [median (range)]	2.0 (0.5–10.2)	2.0 (1.2–10.2)	2.2 (0.5–6.9)	1.8 (0.9–5.4)
Platelet count ×109/L [median (range)]	143 (16–371)	159 (32–275)	149 (42–371)	127 (16–218)
Calcium [mg/dl, median (range)]	2.0 (1.9–3.4)	2.3 (2.1–2.4)	2.4 (2.0–2.7)	2.3 (2.0–3.4)
Creatinine [μmol/L, median (range)]	87 (54–620)	96 (58–136)	85 (58–225)	86 (54–620)
Albumin [g/L, median (range)]	38 (19–46)	38 (30–42)	40 (22–46)	36 (19–44)
LDH [U/L, median (range)]	167 (93–861)	179 (102–861)	181 (115–262)	156 (93–449)
Patients treated on a clinical trial	21 (28.8)	17 (94.0)	3 (9.4)	1 (4.5)
Best response to second-line treatment [*n* (%)]				
CR/VGPR	37 (53.6)	13 (76.5)	18 (58.1)	6 (28.6)
PR	16 (23.2)	2 (11.8)	6 (19.4)	8 (38.1)
MR/SD	8 (11.6)	1 (5.8)	2 (6.5)	5(23.8)
PD	8 (11.6)	1 (5.8)	5 (16.1)	2 (9.5)
Unknown	4	1	1	2

LDH, lactate dehydrogenase; CR, complete response; VGPR, very good partial response; PR, partial response; MR, minimal response; SD, stable disease; PD, progressive disease; PFS, progression-free survival; CI, confidence interval.

The median OS for the entire cohort was 49.1 months (95% CI, 43.7-not reached). The median OS for DPd was 49.1 months (2-year OS, 72.2%) and was not reached for DRd (2-year OS, 84.0%) or DVd (2-year OS, 69.2%) as shown in **Figure 1B**.

DISCUSSION

Our study compares the outcomes of DPd, DRd, and DVd regimens in patients progressing on lenalidomide maintenance. The results from this study provide a benchmark for outcomes expected with these regimens in this specific clinical setting and highlights opportunities for further improvement.

Daratumumab-containing regimens are shown to be effective post-lenalidomide maintenance (9); however, the efficacy of specific combinations remains unknown. The results presented here are in line with recent subanalyses of studies examining patients progressing on lenalidomide following one prior line of treatment. In the phase II nonrandomized MM-014 trial, DPd was evaluated in patients following one to two prior lines of treatment. DPd was associated with a median PFS of 21.8 months among those with lenalidomide refractoriness (10) in keeping with our results (median PFS of 18.9 months). DPd was also evaluated in

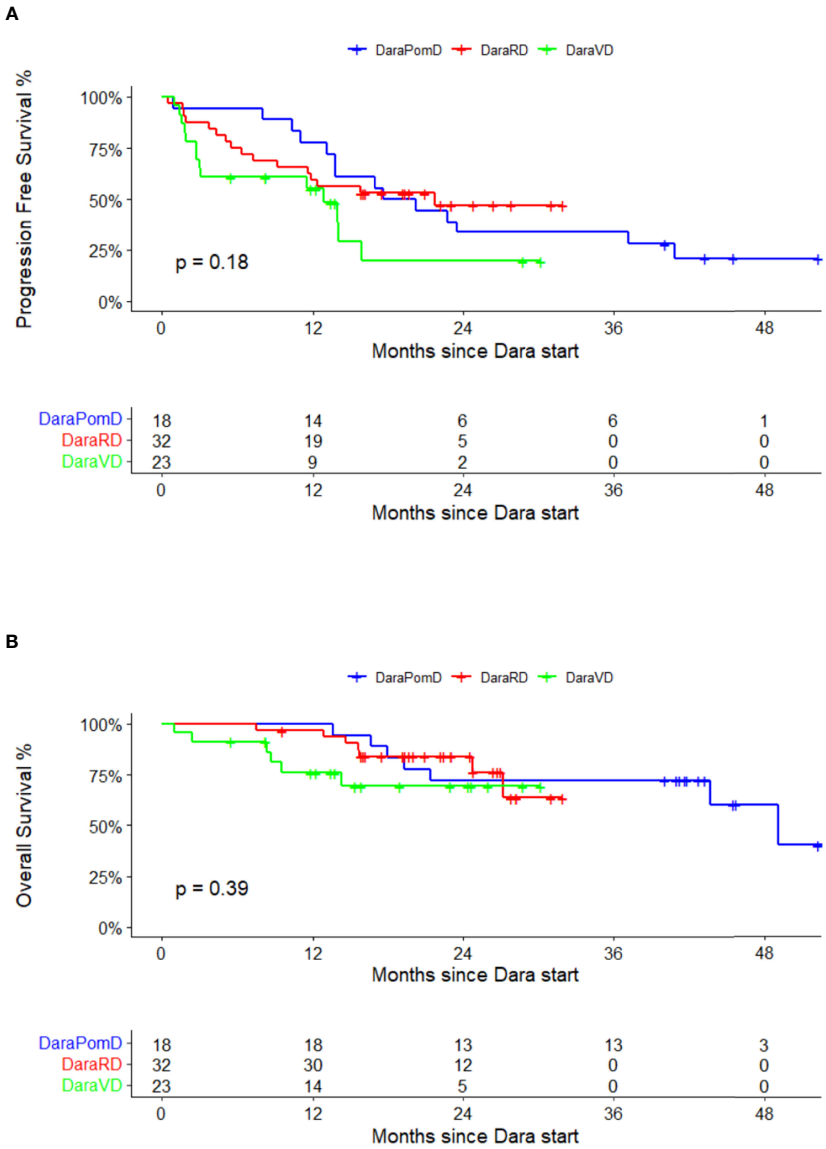

FIGURE 1 | Daratumumab-containing regimens post-lenalidomide maintenance. **(A)** Progression-free survival. **(B)** Overall survival.

the phase III APOLLO trial in patients who had received one or more prior lines of treatment including lenalidomide and a proteasome inhibitor and demonstrated a median PFS of 12.6 months (6). Although 62% of patients included in the APOLLO study were refractory to lenalidomide as the last previous line of therapy, only 11% of them had received one prior line of therapy, limiting our ability to understand the efficacy of this regimen specifically among patients refractory to lenalidomide maintenance given at first line.

The pivotal trial POLLUX evaluated DRd among patients with one to three prior lines of treatment but excluded patients with lenalidomide refractory disease (4). Kunacheewa et al. evaluated the outcomes of lenalidomide retreatment with triplet regimens among 64 patients progressing on lenalidomide maintenance (11). In this study, ORR was 58% and median PFS was 20.2 months among patients treated with novel triplets following one line of treatment; however, only eight patients were treated with DRd following lenalidomide maintenance (11). Lastly, in the phase III CASTOR study, DVd had a median PFS of 7.8 months among the 60 patients refractory to lenalidomide (12). However, the included patients in the CASTOR study were heterogeneous with more than one prior line of treatment as compared with our study that included patients progressing on lenalidomide maintenance in first line and showed a median PFS of 12.9 months.

Our results demonstrated that daratumumab-based regimens remain effective in patients progressing on lenalidomide maintenance. DPd was effective providing a median PFS of 18.9 months; however, additional data on patients treated off

clinical trial are needed to understand the efficacy of this regimen in the "real world". Moreover, our study also examines the specific use of DRd after progression on lenalidomide maintenance therapy with the results demonstrating that the increased dose of lenalidomide along with the addition of daratumumab can still lead to clinical meaningful disease control in a subset of patients. Additionally, this response appeared to be at least as favorable as DVd, which is commonly used and reimbursed regimen for the treatment of lenalidomide refractory patients based upon the CASTOR registrational trial.

The strength of our study is robust information on myeloma-specific variables on patients' progressing on lenalidomide maintenance, a subgroup with a paucity of data in the literature. The limitation of our study is that while our study consists of real-world patients, patients on clinical trials were also included. Clinical trial patients may have different outcomes compared to patients not eligible for clinical trials (13); however, further subgroup analysis based upon additional factors including trial participation, cytogenetic risk, and response to first-line treatment could not be conducted in our study due to the sample size. Furthermore, the exact reason why one regimen was picked over another available regimen cannot be elucidated from our study. Given the retrospective collection of this data, toxicities including infection rates were not collected precluding our ability to comment on the safety profile of each regimen. Lastly, our study does not contain information on other emerging daratumumab-containing regimens, such as in combination with carfilzomib (DKd) (14).

In conclusion, our study shows the effectiveness of daratumumab-containing regimens among patients refractory to lenalidomide maintenance following first line ASCT. Additional studies with longer follow up are required to assess the optimal daratumumab based regimen use in this growing population of patients relapsing after lenalidomide maintenance.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because Research Ethics Boards at data contributing sites do not allow patient level data to be shared. Any aggregate data supporting the findings of this study can be made available from the corresponding author upon reasonable request. Requests to access the datasets should be directed to esther.masih-khan@uhnresearch.ca.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University Health Network Research Ethics Board. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

HM, CE, CV, EM-K, and DR designed the research, performed the research, collected, analyzed, and interpreted data, and wrote the manuscript. MK performed statistical analysis and interpreted data. All authors contributed to data collection, interpreted the data, and reviewed the manuscript.

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REVIEW
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Harnessing the T Cell to Treat Multiple Myeloma: Dawn of a New Therapeutic Paradigm

Alana L. Keller[1], *Daniel W. Sherbenou*[1,2], *Peter A. Forsberg*[1,2] *and Tomer M. Mark*[1,2*]

[1] Division of Hematology, Department of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, United States, [2] Comprehensive Cancer Center, University of Colorado, Aurora, CO, United States

Multiple myeloma is an incurable hematologic malignancy. The typical disease course for myeloma patients is characterized by initial response to treatment followed by eventual development of resistance. Subsequent cycles of remission and relapse proceed as long as patients have new lines of therapy available to them. This reality has prompted development of many novel immunotherapeutics. Many of these drugs exploit the cytotoxic capabilities of the patients' own T cells, effectively redirecting them to myeloma cells that are otherwise evading immune attack. Approaches including CAR T cell therapy and bispecific antibodies have displayed impressive efficacy in clinical trials for myeloma patients. This review examines the different approaches that utilize T cells in multiple myeloma therapy and investigates the benefits and risks of these exciting new strategies.

Keywords: bispecific antibodies, CAR T cell therapy, T cells, B cell maturation antigen, immunotherapy, myeloma

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***Correspondence:**
Tomer M. Mark
tomer.mark@cuanschutz.edu

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INTRODUCTION

Multiple myeloma (MM), a cancer of plasma cells, is the second most common hematologic malignancy in the United States after non-Hodgkin lymphoma, accounting for approximately 35,000 new diagnoses each year (1). The uncontrolled proliferation of clonal plasma cells in MM leads to anemia, lytic bone lesions that easily fracture, renal failure, impaired humoral immunity, hypercalcemia, and early death (2, 3). MM is a disease of older persons, with a median age at diagnosis of 70. Unfortunately, the disease is incurable in the vast majority of those afflicted leading to over 12,000 deaths yearly in the United States (1). The incidence of MM has been gradually rising in recent decades, with an increase of 126% in cases globally from 1990 to 2016 (4).

The first treatments used for MM, dating from the 1940s, included nitrogen mustard-based alkylating chemotherapies, anthracycline compounds, and glucocorticoids (5, 6). With these agents, life expectancy averaged only 20 months, and extended courses of treatment could not be given due to adverse effects such as chemotherapy-induced myelodysplastic syndrome and opportunistic infections (6). Autologous stem cell transplant (ASCT), using high dose melphalan for myeloablation followed by hematopoietic stem cell rescue, became a widespread treatment modality in the 1990s as it was the first treatment shown to have a survival benefit in MM in randomized clinical trials and confirmed by meta-analysis (7–10). However, the survival benefit of ASCT is modest, averaging 11 months, and adverse events such as prolonged fatigue,

immunosuppression, and delayed blood count recovery limited its use to those patients who were generally fitter and younger.

The late 1990s and early 2000s saw the advent of the "novel agents" to treat MM: proteasome inhibitors (PIs) and immunomodulatory agents (IMiDs) (11). Bortezomib was the first PI to be used in the clinic leading to responses and improved overall survival in the treatment of both upfront and relapsed MM. Other PIs, carfilzomib and ixazomib, with different chemical structures and binding properties to the proteasome, have since been FDA approved and incorporated into clinical use (12). Thalidomide was the first IMiD to be used clinically for MM, initially tried at the urging of a patient's wife after she had read that thalidomide had anti-angiogenic properties (13). A derivative of thalidomide, lenalidomide, was later shown to be both directly cytotoxic to the MM cells and also a powerful activator of T cells, potentially leading to more MM cell death (14, 15). The observation that use of lenalidomide maintenance therapy after allogeneic stem cell transplant leads to increased incidence and severity of graft vs host disease points to the T cell activating properties of IMiDs (16). In addition to T cell activation, lenalidomide also alters the cytokine milieu to decrease the inflammation which fuels MM growth through inhibition of IL-6 and TNF-alpha secretion (17). A later derivative of thalidomide, named pomalidomide, was demonstrated to have efficacy even in the setting of lenalidomide resistance (18). Unfortunately, while overall survival for MM has certainly improved with use of these novel agents, it has historically been dismal once patients become refractory to both IMiD and PI therapy with a median overall survival of only 13 months (19).

Monoclonal antibodies were the first immunotherapeutics in MM, the application of which we have reviewed previously (20). These have arrived relatively recently, with the first monoclonal antibody (MoAb) against CD38, daratumumab, gaining FDA approval in the use of multiply-relapsed or refractory multiple myeloma (RRMM) in 2015 based on a single-agent response rate of 30% (21, 22). CD38 is a cell surface protein present on plasma cells and red blood cells that acts both as an adhesion molecule and an ectoenzyme involved in calcium metabolism (23, 24). In RRMM, daratumumab added in combination with either IMiDs led to improved response rates and progression free survival over the backbone regimens alone in multiple studies (25–27). In subsequent studies, when daratumumab is combined with either IMiDs or PIs (or both) as part of initial treatment for newly diagnosed MM, the overall response to treatment rises dramatically with nearly 90% of patients achieving tumor reduction and 20-30% reaching minimal residual disease negativity by next-generation sequencing or flow cytometry-based detection (28–33). After daratumumab, isatuximab became the second anti-CD48 MoAb FDA-approved for use in MM (34). Isatuximab is an anti-CD38 monoclonal antibody approved for relapsed or refractory MM in combination with either pomalidomide or carfilzomib (35, 36). Moving beyond targeting CD38, elotuzumab is another monoclonal antibody approved for MM instead directed against Signaling Lymphocytic Activation Molecule Family member 7 (SLAMF7) (37, 38). The clinical observation that elotuzumab does not work well as a single agent, but can reduce chance of relapse by 30-50% in combination with lenalidomide or pomalidomide, supports the important role of NK and T cell activation in anti-tumor efficacy (39–41). All in all, success of daratumumab, isatuximab, and elotuzumab served as proof-of-concept for the many adaptive immune-mediated therapies being developed for relapsed/refractory myeloma.

Following the discovery of the power of the immune checkpoint inhibitors in other malignancies, and the success of T-cell activating treatments in other hematologic malignancies, there was an intensive effort to exploit T cells in combating myeloma. What follows is a discussion of the evolution of immunotherapy in MM with a focus on T cell-directed strategies that have been tested in MM, including immune checkpoint inhibitors, bispecific antibodies, and chimeric antigen receptor (CAR) T cells.

IMMUNE CHECKPOINT INHIBITORS

Cytotoxic $CD8^+$ T cells require two signals to become activated to kill a foreign, infected, or cancerous cell. The first signal is engagement of the T cell receptor (TCR) with major histocompatibility complex (MHC) Class 1 expressed on the target cell (42). A second signal results from CD28 engagement on the T cell with CD80/86 on professional antigen-presenting cells (APCs), which is required to promote ongoing T cell stimulation and survival (42). This requirement for T cell co-stimulation has been exploited in anti-cancer therapy in two different ways. The first is *via* the inhibition of cytotoxic T-lymphocyte-associated protein 4 (CTLA4), which is found on CD4 and CD8 T cells (43). The expression of CTLA4 increases with the level of TCR activation and general T cell stimulation *via* cytokines such as IL-2 (44). CTLA4 has a higher affinity for co-stimulatory ligands CD80/86 on APCs than CD28, thus outcompeting and acting as a brake on T cell activation (45). It follows that blockade of CTLA4 on T cells would lead to increased activity of cytotoxic T cells and more tumor killing. This has turned out to be the case for certain tumor types and the anti-CTLA4 antibody, ipilimumab, has been FDA approved for the treatment of metastatic melanoma (46).

Another means by which cancer cells evade T cell immune surveillance is *via* binding to the programmed cell death 1 (PD-1) protein. PD-1 is a cell surface protein expressed on T-lymphocytes, acting as another brake on their activation *via* interaction with its ligand PD-L1. This interaction can lead to T cell exhaustion and differentiation to regulatory T cells. Physiologically, PD-L1 is expressed on most normal cells, and abnormal expression may be linked to autoimmune disease *via* unchecked T cell activity (47). Pathologically, PD-L1 can be aberrantly expressed by tumor cells, including MM cells, to avoid this normal checkpoint that identifies and eliminates abnormal, cancerous cells. Monoclonal antibodies against PD-L1 and PD-1 have been developed, such as pembrolizumab, nivolumab, atezolizumab, and durvalumab, and are in clinical use either

alone or in combination with anti-CTLA4 treatment or chemotherapy for several malignancies, including bladder cancer, renal cell carcinoma, and lung cancer. A predictable adverse effect of checkpoint blockade would be development of auto-reactive T cells. Indeed, this has been observed in clinical practice, with treatment-related side effects including thyroiditis, pneumonitis, and in rare cases cerebritis. Interestingly, it appears that patients with immune-related adverse events during PD-1 axis blockade treatment may have better tumor responses (48).

CTLA4 and PD-L1 have both been found to be highly expressed in bone marrow samples from MM patients, however the focus thus far on checkpoint inhibition treatment for MM has been on the PD-1 pathway (49, 50). A phase 1b study of nivolumab (anti-PD-1 MoAb) was performed in advanced hematologic malignancies, with a subset of 27 participants with relapsed MM (51). Of these patients, 60% had stabilization of the MM for 11 weeks, but none had significant reduction in tumor burden. Postulating that more T cell stimulation would be useful to enhance tumor response, PD-1 inhibition was combined with IMiD treatment. In the initial phase 1 study [KEYNOTE-023] of pembrolizumab (another anti-PD-1 MoAb) and lenalidomide for relapsed MM, there was an overall response rate of 44% with an additional 50% of patients achieving stable disease; the 1-year survival rate was 82.6% (52). Notably, 93% of the patients involved in this study had been exposed to and progressed after prior lenalidomide treatment. Another phase 1/2 study of pembrolizumab, pomalidomide, and dexamethasone in more heavily pre-treated MM [HD-00061522] produced an impressive response rate of 60% and median PFS of 17.4 months, albeit most patients in this study had not been previously treated with pomalidomide (53). Although very promising initially, randomized studies of pembrolizumab with or without IMiDs were halted by the FDA in 2019 due to excess deaths in those who received the checkpoint inhibitor. In the phase 3 study of lenalidomide/dexamethasone with or without pembrolizumab as initial treatment in newly diagnosed MM, [KEYNOTE-185], overall responses between the control and pembrolizumab arms were similar at 62% vs 64%, and 82% vs 87% of subjects were without disease progression at 6 months respectively (54). Nine out of 51 (19%) patients in the pembrolizumab arm had died during the study, as compared to only 6% in the control arm. In the relapsed setting, the phase 3 study of pomalidomide/dexamethasone with or without pembrolizumab [KEYNOTE-183] showed an inferior overall response rate in the investigational arm (34% vs 40% for pomalidomide/dexamethasone alone), and again there were more deaths in the pembrolizumab arm at 23% vs 17% (55). Notably, approximately a third of the deaths in the pembrolizumab arms of both KEYNOTE phase 3 studies were due to immune-related adverse events such as myocarditis or Stevens-Johnson syndrome. Increased infection rates were also noted. Thus, in MM compared to other tumors, it appears that the risks of checkpoint inhibition outweigh the benefits, at least when combined with IMiDs. Further development of immune checkpoint strategies in MM treatment was slowed significantly and a role for checkpoint inhibitors in MM at this point appears unlikely.

BISPECIFIC ANTIBODIES

Bispecific antibodies (bsAbs) are engineered, bivalent monoclonal antibodies that comprise a diverse 'zoo' of options displaying great variation in structure (56). The earliest formats included Bispecific T cell engagers (BiTEs), which are comprised of two single-chain variable fragments (scFv) connected by a short, flexible linker. Blinatumumab, a CD19-targeting BiTE used in acute lymphoblastic leukemia, was the first bsAb approved in oncology (57, 58). However, these constructs have a short half-life and require burdensome continuous intravenous infusions (59). More recently, the prominent agents in trials take forms more similar to MoAbs, in which Fc regions are included to prolong molecule half-life and allow for periodic dosing (60, 61). Generally, bsAbs can be separated into groups based on their binding partners: (1) those that bind two immune targets, (2) those binding two tumor-associated antigens (TAA), and (3) those that bind one TAA and one immune target (62, 63). The majority of bsAbs in development for MM belongs to the third category, with the anti-MM cell targeting arm binding the TAAs including BCMA, FcRH5, or GPRC5D (64). As shown in **Table 1**, we will highlight the bsAbs furthest in clinical development for each target.

There are currently several BCMA-directed bsAbs showing promise in ongoing clinical trials. The first bispecific antibody tested clinically in MM was AMG-420, a BiTE directed against CD3/BCMA that showed an excellent overall response rate of 70% in a phase I trial for relapsed or refractory MM (70). However, 2 weeks of continuous intravenously infused AMG-420 was onerous and further development was abandoned. Instead, AMG-420 was reformulated to AMG-701, a bsAb with an Fc region and once-weekly dosing that produced an impressive overall response of 83% at target dosing (68). However, since AMG-420 results became public, the competition has intensified in anti-MM bsAbs. In a phase 1/2 clinical trial of teclistamab (anti-CD3/BCMA), patients who had progressive disease were treated after a median of 5 prior lines of therapy, and an overall response rate of 65% was seen at the recommended phase 2 dosing (67). While overall follow-up has been short, it is encouraging that among the responders, over 90% maintained the response for 6.5 months. These results appear to be corroborated in the phase 2 extension. Another anti-CD3/BCMA bispecific, elranatamab, displayed high response rates in a phase 1 clinical trial in patients with a median of 6 prior lines of therapy (65). The observed overall response rate of patients receiving the recommended phase 2 dose (RP2D) was 83%. Remarkably, 75% of patients previously treated with BCMA-targeted therapies still achieved response. Other anti-BCMA bsAbs such as REGN5458, TNB-383B, and CC-93269 have demonstrated initial promise as well (**Table 1**). Given the diversity of anti-BCMA bsAbs in clinical development, it is unclear how many will proceed through late-phase

TABLE 1 | Preliminary Clinical Data of Bispecific Antibodies in Multiple Myeloma.

Drug	Targets	Company	Grade 3-4 TEAE	CRS	Response Rate	Survival (e.g. PFS, DOR, OS)
Elranatamab (PF-06863135) (65)	anti-CD3/ BCMA	Pfizer	neutropenia 60%, anemia 38%, lymphopenia 64%, thrombocytopenia 31%	83%	At RP2D- ORR: 83%; sCR: 83%	median DOR not reached
REGN5458 (66)	anti-CD3/ BCMA	Regeneron	anemia 9%, lymphopenia 7%, infections 20%	38%	At all doses-ORR: 36%; CR: 31%	43.8% of responders DOR > 4 m; 18.8% responders DOR > 8 m
Teclistamab (67)	anti-CD3/ BCMA	Janssen	neutropenia 45%, anemia 27%, thrombocytopenia 18%, fatigue 2%	At RP2D-67%	At RP2D- ORR: 65%; ≥VGPR: 60%; ≥CR: 40%	median DOR not reached; 6 m DOR: 90% (95% CI 63-97)
AMG 701 (68)	anti-CD3/ BCMA	Amgen	CRS 7%, atrial fibrillation 1%, acidosis 1%, thrombocytopenia 1%	61%	At 3-12mg- ORR: 36%; sCR: 4%	median DOR: 3.8 m (ongoing in 14/17 pts); maximum DOR: 23 m
TNB-383B (69)	anti-CD3/ BCMA	TeneBio/ Abbvie	CRS 3%	52%	At ≥40 mg- ORR: 64%; ≥VGPR: 43%; CR: 16%	At ≥40 mg- ORR: 64%; ≥VGPR: 43%; CR: 16%
AMG420 (70)	anti-CD3/ BCMA	Amgen	CRS 2%, polyneuropathy 5%, edema 2%	38%	At all doses- ORR: 31%; ≥CR: 21%	median DOR > 8.4 m
CC-93269 (71)	anti-CD3/ BCMA	Celgene	neutropenia 43%, anemia 37%, infections 30%, thrombocytopenia 17%	77%	At all doses- ORR: 43%; ≥CR: 17%	DOR: 5.3-40.6 m
Talquetamab (72)	anti-CD3/ GPRC5D	Janssen	405 μg/kg weekly: CRS 3%, neutropenia 60%, infections 3% 800ug/kg biweekly: neutropenia 35%, infections 4%	405 μg/kg weekly: 73% 800ug/kg biweekly: 78%	At 405 μg/kg weekly- ORR: 70%; ≥VGPR: 57% At 800 μg/kg biweekly dose- ORR: 71%; ≥VGPR rate: 53%	6 m DOR for pts given 405ug/kg dose: 67% [95% CI: 41-84]; median DOR not reached
Cevostamab (BFCR4350A) (73)	anti-CD3/ FcRH5	Genentech	CRS 1%, anemia 22%, neutropenia 16%, infections 19%	80%	At 160mg dose- ORR: 55% At 90mg dose- ORR: 37%	median DOR: estimated 15.6 m (95% CI: 6.4-21.6 m)

TEAE, treatment-related adverse events; CRS, cytokine release syndrome; RP2D, recommended phase 2 dose; ORR, overall response rate; sCR, stringent complete response; CR, complete response; VGPR, very good partial response; PFS, progression-free survival; DOR, duration of response; OS, overall survival; m, month; yr, year; pts, patients.

investigation and how differences in described efficacy, dosing schedule, and toxicity profiles may ultimately drive clinical utilization.

In addition to BCMA, other MM antigens have emerged as promising targets for bsAbs. Fc Receptor Homolog 5 (FcRH5) is another attractive target, as it is expressed exclusively in B-lineage cells, mature plasma cells, and MM cells (74). FcRH5 is a protein that plays a role in isotype selection and proliferation in activated B cells (75). In a phase 1 study of cevostamab (anti-CD3/FcRH5) in patients with a median of 6 prior lines of therapy, 55% achieved response at the higher dose level of 160mg, and estimated median duration of response was 15.6 months (73). Another target, the orphan G protein coupled-receptor class C group 5 member D (GPRC5D), is a cell surface protein that is highly expressed on malignant plasma cells as well as in hard keratinized tissues such as hair and nails. The function of GPRC5D is currently unknown, although its high expression correlates with poor prognosis in MM patients (76). Updated results of a phase 1 study of talquetamab (anti-CD3/GPRC5D) in subjects with relapsed/refractory multiple myeloma (RRMM) were excellent, with a response rate of 70% seen in the 405 μg/kg weekly RP2D cohort and 71% at the 800 μg/kg every-other-week RP2D (72). It is also worth noting that due to off-target GPRC5D expression on keratinized tissues, oral and dermatologic adverse events were frequently observed, but they have been described as manageable (77). Initial testing has begun to explore the safety and efficacy of various MM bsAbs in combination with additional agents including MoAbs and other bsAbs with different TAAs. In summary, there are several bispecific antibodies showing very promising results in early clinical trials, and data from larger randomized studies is eagerly anticipated.

CAR T CELLS

Historically, cell-based therapies in MM have consisted of stem cell transplants. High-dose myeloablative chemotherapy with autologous stem cell transplantation (ASCT), has been used since the early 1990s as a means to achieve tumor reduction. ASCT has been a long-standing standard of care as a result of randomized clinical trials showing a survival benefit compared to conventional chemotherapy (7, 8, 78). Despite often deep and durable responses after ASCT, relapse is largely inevitable, with a median response of 54 months when administered with high dose chemotherapy and 50 months with supporting lenalidomide, bortezomib, and dexamethasone (8, 78). The use of post-transplant lenalidomide maintenance extended response to a median of 40 months (79, 80). Allogeneic stem cell transplant has also been tested in MM to induce a graft vs tumor effect mediated by donor T cells. However, there is a high treatment-related mortality in up to 40% of patients in early studies. With more current approaches, estimates of treatment-

related mortality are between 5-10% due to complications of acute graft vs host disease (GvHD) and prolonged immunosuppression (81, 82). Furthermore, although graft vs tumor effect has been demonstrated in MM by response to donor lymphocyte infusion or lifting of immunosuppression after allogeneic stem cell transplant, graft vs tumor effect is relatively weaker in MM when compared to other hematologic malignancies (83, 84). Until recently, the development of novel cell-based therapies was limited.

Chimeric antigen receptor (CAR) T cells are a new cellular therapy in MM that utilize autologous engineered T cells for anti-tumor effect. CAR T cells are T lymphocytes with an artificial receptor engineered to target a specific TAA. A CAR construct allows a patient's T cells to attack their own malignant cells in an MHC-independent fashion, bypassing the tumor's immune evasion mechanisms and avoiding acute and chronic GvHD. CAR T constructs have a single chain variable scFv linked to the TCR transmembrane region and intracellular signal activating domains. The intracellular domain can activate downstream signaling in T cells to promote activation and pro-inflammatory cytokine release (e.g. IL-2, TNF-alpha, IL-6). The intracellular domain of early CAR T constructs was solely CD3ζ, which led to some activity, but limited duration due to lack of a proliferation signal (85). Newer CAR T constructs include additional co-stimulatory domains (most often 4-1BB and CD28) in addition to CD3ζ, greatly enhancing persistence and activation (86, 87). In the process of CAR T cell development, a patient stops any chemotherapies and corticosteroids for a short period, then undergoes lymphocyte apheresis (88, 89). After lymphocyte collection, the cells are sent for CAR T manufacturing *via* lentiviral transduction of a DNA cassette encoding for the chimeric TCR. Once created, CAR T cells are selected and expanded in culture to provide the significant cell number needed for infusion. This process takes approximately 4-6 weeks, during which the patient may receive a therapy "bridge" while waiting for the CAR T product. Once ready, the patient is then given a 3-day course of lymphodepletion chemotherapy, usually fludarabine and cyclophosphamide, to ensure that the infused CAR T cells are not immediately destroyed by the recipient's immune system (90). The patient is then monitored closely for signs and symptoms of cytokine release syndrome (CRS) and neurotoxicity. These T cell therapy-specific complications are described in a separate section to follow.

CAR T cells have been shown to have remarkable activity in several hematologic malignancies (91). There are four anti-CD19 CAR T products currently approved for use in B cell acute lymphoblastic leukemia (brexucabtagene autoleucel and tisagenelcleucel) and non-Hodgkin B cell lymphomas (axicabtagene ciloleucel and lisocabtagene maraleucel) (92). The first CAR T product for relapsed MM, idecabtagene vicleucel (ide-cel), was approved in 2021 for patients who have received 4 or more prior lines including an IMiD, a PI, and a CD38-directed MoAb. As most MM cells lack CD19, ide-cel is a BCMA-directed CAR T that possesses a 4-1BB costimulatory domain. A phase 1 study of ide-cel showed dose-dependent efficacy in RRMM, with depth and duration of response improving as infused cell numbers increase (93). In 128 patients with MM that progressed after prior PI, IMiD, and anti-CD38 MoAb treatment, ide-cel led to an overall response rate of 73%, with 33% achieving complete remission at doses ranging from 150-450 x 10^6 CAR T cells/kg. At target dosing of 450x10^6 CAR T cells/kg, ide-cel had an 83% overall response rate and progression-free survival of 12.1 months. Phase 3 studies comparing ide-cel vs. other standard-of-care treatments for RRMM are currently underway. In early 2022, another BCMA-directed CAR T product with a 4-1BB costimulatory domain, ciltacabtagene autoleucel (cilta-cel), became the second CAR T product approved for use in RRMM with a similar indication as ide-cel. Cilta-cel may have even better activity than ide-cel, potentially due to its bivalent binding region. In a phase 1/2 study of cilta-cel, the overall response to the target dosing of 0.75x10^6 CAR T cells/kg was 98% in MM patients with a median of six prior lines of therapy (94). The 12-month progression-free survival was excellent at 77% with a 1-year survival rate of 89%. Cilta-cel is also currently being explored in more clinical settings in several ongoing trials.

Beyond ide-cel and cilta-cel, there are several BCMA CAR T cell therapies in clinical trials that use novel manufacturing approaches designed to reduce toxicity and improve response (**Table 2**). P-BCMA-101 is manufactured using a transposon-based technology called piggyBac that favors production of T cells with the stem cell memory phenotype and reduces toxicity (100). The overall response rate of patients in the corresponding phase 1/2 clinical trial was 57% (95). An updated version of the ide-cel product, bb21217, has also been evaluated in early phase trials. Unlike ide-cel, bb21217 is cocultured with a PI3K inhibitor to increase the number of memory-like T cells and remove senescent cells from the CAR T product (96). So far, an ORR of 69% has been observed in a phase 1 trial. Recently the development of both P-BCMA-101 and bb21217 have been discontinued demonstrating some of the challenges of iterative CAR T development. CT053 and CT103A both use a human anti-BCMA scFv and clinical trial results reported ORRs of 87.5% and 100% respectively (97, 98). However, the small patient cohort included in the CT103A phase 1 trial makes it difficult to interpret the high ORR. ALLO-715 is an allogenic CAR T product that is engineered with a modified T cell receptor and CD52 to reduce GvHD in the 'off-the-shelf' product (99). Current phase 1 data reflects an ORR of 62%. CAR T cells directed to other MM targets, such as CD38, SLAMF7, GPCR5D, CD56, and CD138 have also been developed, but clinical data has yet to emerge (101). Thus far, although there are clear potential improvements to make upon the lead CAR T products in MM (especially allogenic CAR Ts), these have yet to bear out in trials.

While CAR T cell therapy has now been approved in MM, development is ongoing and other modalities are still being explored. For example, TCR-engineered cells are patient-derived T cells modified to target a specific tumor-associated antigen or neoantigen (102). Unlike CARs, whose artificial receptors allow efficacy independent of MHC presentation, which is often downregulated by immunosuppressive tumor cells, TCR-engineered T cells rely on native TCR biology (103). Production of TCR-engineered T cells is a similar process to CAR T production, expensive, and can take approximately 4-6 weeks to produce (102, 104).

TABLE 2 | Clinical Response of BCMA CAR T Cell Therapies in Multiple Myeloma.

Drug	Company	Grade 3-4 TEAE	CRS	Response Rate	Survival (e.g. PFS, DOR, OS)
Idecabtagene vicleucel (ide-cel) (93)	Bristol Myers Squibb/bluebird bio	CRS 5%, Neurotoxicity 3%,	84%	ORR: 73%; CR: 33%	median DOR: 10.7 m; median PFS: 8.8 m (95% CI, 5.6-11.6); OS 78% at 12 m (estimates)
Ciltacabtagene autoleucel (cilta-cel) (94)	Janssen	neutropenia (94.8%), anemia (68.0%), leukopenia (60.8%), thrombocytopenia (59.8%), and lymphopenia (49.5%)	95%	ORR: 98%; ≥VGPR: 95%; sCR: 80.4%	median DOR: 21.8 m – NE; 2-yr PFS: 60.5% (95% CI, 22.8 m – NE)
P-BCMA-101 (95)	Poseida Therapeutics	neutropenia 79%, thrombocytopenia 30%, anemia 30%	17%	ORR: 57%	Responses ongoing
bb21217 (96)	Bristol Myers Squibb/bluebird bio	CRS 1%, neurotoxicity 4%	75%	ORR: 69%; ≥VGPR: 58%; sCR/CR: 28%	estimated median DOR: 27.2 m
CT053 (97)	CARsgen Therapeutics	neutropenia 100%, leukopenia 100%, thrombocytopenia 36%	86%	ORR: 87.5%; CR: 79%	median DOR: 21.8 m
CT103A (98)	Nanjing IASO Biotherapeutics	leukopenia 100%, neutropenia 100%, lymphopenia 100%, anemia 89%, thrombocytopenia 94%	94%	ORR: 100%; CR/ sCR: 72%	1-yr PFS: 58.3%
ALLO-715 (99)	Allogene Therapeutics	CRS 2%, infections 13%	52%	ORR: 62%; VGPR: 39%	median DOR: 8.3 m (95% CI: 1.5 – not reached)

TEAE, treatment-related adverse events; CRS, cytokine release syndrome; ORR, overall response rate; CR, complete response; sCR, stringent complete response; VGPR, very good partial response; PFS, progression-free survival; DOR, duration of response; OS, overall survival; m, month; yr, year; NE, not estimable.

TCR-engineered T cells have shown early promise in leukemia and are being evaluated in a few MM clinical trials in a small number of patients (102, 105–107). One study, in which TCRs are engineered to target a shared sequence between antigens New York esophageal squamous cell carcinoma-1 (NY-ESO-1) and L-antigen family member 1 (LAGE-1), has reported an objective response rate of 80% at day 42 and median progression free survival of 13.5 months in 25 relapsed/refractory myeloma patients with at least one adverse cytogenetic abnormality (107). This area is rapidly evolving, and more clinical trial data is expected to emerge.

TOXICITIES OF T CELL DIRECTED THERAPY

As discussed in the immune checkpoint inhibition section above, immune-mediated side effects can be significant in patients with MM, likely responsible for the early termination of clinical studies of PD-1 inhibitors. Representing the consequences of T cell hyperactivation, cytokine release syndrome (CRS) and a spectrum of neurologic symptoms dubbed immune effector cell-associated neurotoxicity syndrome (ICANS) are the unique adverse effects of CAR T treatment. Hematologic toxicities (cytopenia, hypogammaglobulinemia) although not unique to T cell modalities, represent other CAR T-associated toxicities resultant from lymphodepletion regimens preceding CAR T infusion (108). CRS has been seen with CAR T, bispecific antibodies, and haploidentical (5/10 HLA matched) allogeneic stem cell transplant. It can also occur as an adverse effect from checkpoint inhibitors and anti-thymocyte globulin (ATG), albeit much less frequently. The symptoms of CRS unfold when unchecked T cell activity leads to an outpouring of pro-inflammatory cytokines into the circulation (IFN-gamma initially, then IL-6, TNF-alpha, and IL-10), resulting in a sepsis-like syndrome with fevers and potential for distributive shock characterized by hypotension, delirium, disseminated intravascular coagulation, hypoxia, and even death without treatment (48). The role of IL-6 in CRS is paramount, as demonstrated by the success of the neutralizing treatment tocilizumab, a MoAb which blocks the IL-6 receptor.

The earlier tocilizumab is given the in course of CRS, the less severe and durable the CRS. Fortunately, it does not appear that clinical efficacy of the CAR T infusion is hindered by early tocilizumab (109). The vast majority of CRS is mild and self-limited, but the timing of onset and duration can vary depending on the therapy used. In the pivotal KarMMa-2 trial ide-cel was associated with an 84% rate of any grade CRS and only 5% rate of grade 3 or higher CRS (93). The median time to onset of CRS was 1 day with a median duration of 3 days. In the CARTITUDE-1 trial cilta-cel was associated with a 95% rate of any grade CRS and only 4% rate of grade 3 or higher CRS (110). Median time to onset of CRS was 7 days and median duration was 4 days. For bsAbs, AMG701 had an overall rate of CRS of 61%, only 7% of moderate severity or greater; talquetamab was associated with a 73% rate of CRS at the 405 μg/kg weekly dose and 78% at the 800μg/kg biweekly dose with very few cases that were more than mild in intensity (68, 72). The anti-FcRH5 bispecific cevostamab had a slightly higher reported rate of CRS at 80%, but also with few severe cases (73). For the bsAbs, the onset of CRS is quick with intravenous vs subcutaneous administration (24 vs 48 hours) and median duration of symptoms likewise was 24-48 hours (65, 68, 111, 112). Due to similar findings, many clinical trials for bsAbs in MM have attempted different steps including planned dose escalation steps and use of subcutaneous administration in attempts to reduce CRS rate and severity. In most cases, CRS tended not to recur with dosing beyond the first infusion at target dose. Unfortunately, the unpredictable and wide range of timing for CRS complicates these T-cell activating treatments. Currently, many patients undergoing CAR T are hospitalized for a planned observation period, although there is effort being made now to dose and manage CAR T in the outpatient setting.

ICANS, another commonly observed toxicity, may present as headache, confusion, difficulty with word finding and speech, and in severe cases seizures, encephalopathy and obtundation. ICANS may be difficult to parse from CRS and both issues may occur simultaneously. The pathophysiology of ICANS is still unclear, but evidence from a mouse model suggested it may occur through inflammatory changes to the endothelium at the blood-brain barrier, leading to capillary leak of inflammatory cytokines and clotting factors into the central nervous system (113). For bsAbs teclistamab and talquetamab the ICANS rate was low, at 3% and 6%, respectively (67, 112). Cevostamab, on the other hand, was associated with a higher rate of neurotoxicity at 13% (73). In the case of CAR T treatment, it appears that there may be an association between the use of a CD28 costimulatory domain and risk of ICANS, as these CAR T products in use for treatment of lymphoma generally have a higher rate and severity of ICANS than the 4-1BB CAR T cells. The CD28 CAR T products axicabtagene ciloleucel and brexucabtagene autoleucel (used for non-Hodgkin lymphomas) had neurotoxicity rates of approximately 65%, compared to approximately 20% for the 4-1BB CAR T products idecabtagene and ciltacabtagene (93, 110, 114, 115). In KarMMa-2 ide-cel had an associated rate of 18% with only 3% of patients experiencing grade 3 events, median time to onset of events was 2 days with a median duration of 3 days (93). Cilta-cel has been associated with a neurotoxicity rate of 21% with 9% grade 3/4 events (110). In CARTITUDE-1 the neurotoxicity observed with Cilta-cel included both ICANS in 17% of patients with median time to onset of 8 days and median duration of 4 days as well as other neurotoxicity in 12% of patients, all of whom had previous CRS and 2/3s of whom had prior ICANS. This other neurotoxicity occurred later with a median onset of 27 days with variable associate symptoms including a cluster of movement and neurocognitive treatment-emergent events. Further exploration to understand and minimize the frequency to this other neurotoxicity is ongoing and a recent description of BCMA expression on neurons and astrocytes in the basal ganglia may represent a possible mechanistic explanation (116). Most cases of ICANS can be managed successfully with a short course of corticosteroids. There has been some concern that the steroids may affect CAR T cell function and quality, but thus far steroid use to control ICANS does not appear to affect clinical outcomes (117). Due to CRS and ICANS associated with CAR T and bsAbs, their administration in MM has largely been restricted to transplant centers experienced in managing these syndromes.

DISCUSSION

The advent of the first MoAb with clinical efficacy in myeloma, daratumumab, led to an explosion of new research on methods to harness the host's immune system to enhance treatment response and overall survival (118). BsAbs and CAR T have given new options and hope for patients running out of treatment choices after failing PI, IMiD, and anti-CD38 antibody therapies. However, each therapeutic modality has unique pros and cons which could be more or less suitable for different patient populations. Bispecific antibodies offer the advantage of being "off the shelf" products that can be used promptly to treat MM, whereas CAR T administration involves a 4-6 week process of lymphocyte collection, manufacturing, and infusion (119). During these 4-6 weeks "bridging" chemotherapy or immunotherapy may need to be used to prevent disease progression while patients are waiting (120). While CAR T production takes time, it is typically a single infusion with a substantial depth and duration of response, even in the most refractory of patients (93, 110). On the other hand, bsAbs require regular dosing to maintain efficacy, meaning they are eventually less convenient to patients through ongoing treatment in clinic (121).

There remain many outstanding clinical questions about the role of T cell-mediated treatment in MM. The optimal timing sequence for CAR T and bsAbs is unknown. Potential roles for T cell therapies could include consolidation after induction chemotherapy or ASCT in an effort to achieve the deepest possible remissions or, alternatively, as salvage treatment in those patients failing IMiDs and PIs. Trials of CAR T and bsAbs in early phases of myeloma treatment of MM are also currently ongoing. It is also unknown how much the use of a prior anti-BCMA targeted therapy such as anti-BCMA CAR T affects the efficacy of a subsequent anti-BMCA treatment with a different modality such as bsAbs, and vice-versa. CAR T therapy at this point does not appear to be curative, and relapses may occur due to selection of BCMA-negative MM cells as well as antigen escape *via* secretion of BCMA into the bloodstream through the action of gamma-secretase (88). Gamma-secretase inhibitors are currently being tested with anti-BCMA CAR T to combat this potential mechanism of resistance (122). Already, we have seen an increase in activity when the antibody-drug conjugate belantamab is combined with the PI bortezomib. More than likely, combination therapies of IMiDs, PIs, bsAbs, and CAR T will be used in the future. Treatments will be tailored to be patient and tumor-type specific. Newer technologies involving trivalent CAR T, CAR-NK cells, and more advanced co-stimulatory domains are under exploration and may enhance the efficacy and reduce the toxicity of immunotherapy. Truly, it is an exciting era in MM therapy with a brighter future for patients.

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J. Luis Espinoza,
Kanazawa University, Japan

REVIEWED BY
Jill Adamski,
Mayo Clinic Arizona, United States

*CORRESPONDENCE
Yandy Marx Castillo-Aleman
yandy.castillo@adscc.ae

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Beyond 8-methoxypsoralen as the photosensitizer for extracorporeal photopheresis

Yandy Marx Castillo-Aleman*

Department of Immunology, Abu Dhabi Stem Cells Center (ADSCC), Abu Dhabi, United Arab Emirates

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Introduction

Since the United States Food and Drug Administration (FDA) approved the use of extracorporeal photopheresis (ECP) in the palliative treatment of cutaneous T-cell lymphoma (CTCL) in 1988 (1), many other indications have been successfully explored. However, most of these approaches still demand further randomized clinical trials, including graft-vs.-host disease (GvHD), rejection of solid organ transplantation, and a wide range of autoimmune diseases.

ECP is a leukapheresis-based therapy where the patient's whole blood is collected and separated into its different components; the collected leukocytes are treated extracorporeally with a photosensitizing agent and then exposed to ultraviolet-A (UV-A) irradiation before reinfusion.

Interestingly, despite the vast marketing experience of the ECP therapy gathered over more than 30 years and the manufacturing improvement of photopheresis devices, no significant changes have occurred around the photosensitizing drugs, which remain restricted to 8-methoxypsoralen (8-MOP, methoxsalen).

Few preclinical and early phase clinical trials have explored different photosensitizers during the last decade. Other ECP photosensitizing agents with equivalent (or better) safety, efficacy, and cost profiles could be available in the marketing landscape of low-income regions that cannot afford the current financial and economic costs of these promising therapies.

This Opinion paper aims to raise some regulatory considerations regarding the conventional photosensitizing agent (8-MOP), emphasizing the potential usefulness of other drugs and non-pharmacological systems for photosensitization during ECP.

ECP regulatory landscape

The FDA considers a photopheresis system a "combination product" comprising two regulated components: a drug (photosensitizer) and a device (leukapheresis machine) (2). Furthermore, the European Medicines Agency (EMA) refines the regulation of medicinal products used with a medical device by defining "integral," "co-packaged," and "referenced" combinations. Those referenced products "combine two (or more) product information of the medicinal product refers to a specific medical device to be used, and the specified medical device is obtained separately by the user of the medicinal product" (3).

For instance, 8-MOP branded as UVADEX® Sterile Solution (Mallinckrodt Pharmaceuticals, Ireland- United States) has been granted FDA authorization in combination with one specific photopheresis device only (UVAR®, and its more recent superseding device, THERAKOS® CELLEX®, both Therakos Inc., Mallinckrodt Pharmaceuticals) (4) that correspond to closed, "online" ECP methods. In Europe, ECP products manufactured using open "offline" methods are subject to Advanced Therapeutic Medical Product (ATMP) regulations (5).

Nevertheless, it is widely accepted that a combination product's primary mode of action (PMOA) is "the single mode of action expected to make the most significant contribution to the overall intended therapeutic effects of the combination" (2). In ECP, the PMOA relies on photosensitizers, comprised of drugs and irradiation systems, rather than the leukapheresis device. A few online and integrated systems for cellular collection, photoactivation, and infusion are available (e.g., THERAKOS® CELLEX® device and AMICUS® Blue ECP system, Fresenius Kabi, Germany); however, evidence also shows that ECP has effectiveness using different marketed photosensitizers and photoactivation devices (e.g., MacoGenic G2 irradiation device, Macopharma, France). Those offline platforms treat leukocytes harvested by other apheresis machines not necessarily intended for ECP protocols (e.g., SPECTRA OPTIA® Apheresis System, Terumo BCT, Japan) and are not approved as combination products, as mentioned earlier.

Discussion

Irradiation devices for ECP

Photoactivation devices play pivotal roles in ECP PMOA. The UV-A irradiation dose (intensity and duration) and the plate film thickness can polarize to either the immunizing or tolerogenic effects exerted by ECP (6, 7). These dual capabilities are crucial for clinical conditions that can be treated with photopheresis: while enhanced immune responses are desirable in cancer scenarios (e.g., CTCL as the primary approved indication), ECP tolerizing effects are required in cases such as solid organ transplantation, GvHD, and autoimmune diseases.

Photosensitizer drugs for ECP: Capabilities beyond 8-MOP

Unfortunately, there is no consensus as to what in-process and pharmaceutical controls should be performed for ECP products; therefore, there is a critical need for harmonized quality-control assays, as stated and summarized as (5):

- Cellular composition of ECP products;
- Induction of apoptosis in lymphocytes;
- Monocyte polarization;
- Psoralen photoadducts;
- T cell suppression; and,
- Capacity for antigen cross-presentation.

Although many questions about PMOA of photosensitizers and ECP as a whole procedure remain inconclusive, the main advances in pharmacological development, manufacturing, quality controls, and preclinical and clinical uses, correspond to 8-MOP.

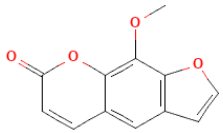
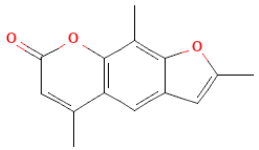
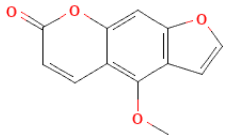
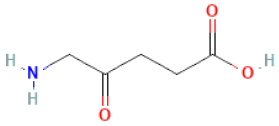
Like other psoralens, 8-MOP, upon UV-A photoactivation (wavelength: 315-400 nm), conjugates and forms covalent bonds with DNA that lead to monofunctional (addition to a single strand) and bifunctional (crosslinking of psoralen to both DNA strands) photoadducts. Lastly, it results in inhibition of DNA synthesis and cell division (4, 8). The apoptotic fate of extracorporeally exposed cells consists of the ECP PMOA generating antigen-specific immune and clinical responses, which are not described here (8).

Traditionally, the combined administration of psoralens (orally or topically) with UV-A (PUVA) has been widely used to manage psoriasis and CTCL patients. Eventually, non-8-MOP psoralens and other photosensitizer drugs, such as those used in photodynamic therapy (PDT), can be applied in ECP (9). Table 1 summarizes distinctive characteristics, preclinical, and clinical evidence of current and potential photosensitizers for ECP.

In 1996, Wolf et al. described the successful treatment of a patient with photoaccentuated erythroderma and idiopathic CD4 T lymphocytopenia using 5-methoxypsoralen (5-MOP)-PUVA, and ECP with 8-MOP concomitantly (10). However, there are no publications of ECP procedures delivered with 5-MOP or trioxsalen (trimethylpsoralen) to date.

In contrast, PDT photosensitizers, such as 5-aminolevulinic acid (5-ALA), have emerged in preclinical (11–14) and early phase clinical trials (9) as an alternative to the authorized 8-MOP for ECP.

TABLE 1 Current and potential photosensitizer drugs for ECP.

Photosensitizer	ATC code	Chemical structure	Current indications	Required light (wavelength, nm)	ECP regulatory status	Preclinical [P] and clinical [C] evidence on ECP
Psoralens for systemic use						
8-methoxypsoralen (8-MOP, methoxsalen)	D05BA02		- Psoriasis - Vitiligo - CTCL (ECP)	UV-A (315-400)	- Marketing authorization (CTCL)	- Vast post-authorization experience [C] - Ongoing clinical trials for new indications (e.g., NCT05168384, NCT05413005) [C]
Trioxsalen (trimethylpsoralen, trisoralen)	D05BA01		- Vitiligo (discontinued)	UV-A (315-400)	- Unknown or not reported	- Unknown or not reported
5-methoxypsoralen (5-MOP, bergapten)	D05BA03		- Psoriasis - Vitiligo - CTCL	UV-A (315-400)	- Unknown or not reported	- (10) [C] *
Sensitizer used in PDT [$]						
5-aminolevulinic acid (5-ALA)	L01XD04		- Actinic keratosis - Glioma (intraoperative optical imaging agent)	Visible light (400-635)	- Preclinical studies - Early phase clinical trials	- (11) [P] ** - (12) [P] - (13) [P] - (14) [P] - (9) [C] - NCT04164849 [C]
Electromagnetic spectrum of light applied for ECP						

*Case report on concomitant use of 5-MOP (PUVA) and 8-MOP (ECP); **_In vitro_ administration of hexaminolevulinate, an ester of 5-ALA

[$]Other drugs in this group include: porfimer sodium, methyl aminolevulinate, temoporfin, efaproxiral, padeliporfin (unknown or not reported use for ECP)

ATC, Anatomical Therapeutic Chemical; CTCL, Cutaneous T-Cell Lymphoma; ECP, Extracorporeal Photopheresi; PDT, Photodynamic therapy; PUVA, Psoralen and UV-A; UV-A, Ultraviolet-A Light (Source: PubMed, PubChem, and ClinicalTrials.gov databases).

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PDT typically involves systemic or topical administration of a lesion-localizing photosensitizer (e.g., 5-ALA) and its subsequent activation by visible light (400-780 nm, represented by blue and red arrowheads in the electromagnetic spectrum of Table 1), primarily resulting in a singlet oxygen-induced photodamage in the exposed cells (12, 14). Nevertheless, further preclinical research is needed to clarify ECP mechanisms of action, resulting in appropriate harmonized quality controls for these photosensitizers.

The alternative use of 5-ALA is supported by *ex vivo* investigations that show that this photosensitizer affects T-cells from chronic GvHD patients more selectively and efficiently than those treated with 8-MOP-ECP, through the formation of protoporphyrin IX. Consequently, reducing the number of ECP treatments can be achieved using 5-ALA (9). This finding occurs even with a UV-A light source resembling emission spectral wavelengths to those of the built-in certified UV-A commercial photopheresis systems (340-410 nm) (12, 14).

The clinical applications of novel photosensitizing drugs are limited based on the number of published clinical trials, procedures, and patients enrolled. For instance, the cited work of Christensen et al. reported 82 ECP treatments with 5-ALA in five chronic GvHD patients who responded poorly to 8-MOP-photopheresis after a minimum of three months of treatment. Although safety and tolerability were found to be adequate (9), no additional clinical evidence is yet available. Only another phase I/II clinical trial using 5-ALA-ECP has been registered on ClinicalTrials.gov (NCT04164849) for patients with active Crohn's disease, as shown in Table 1. Still, more clinical studies are required to demonstrate differences in safety, efficacy, and cost profiles compared to the approved 8-MOP.

Apoptosis induced by light: Are ECP-photosensitizers still needed?

Insulting agents that trigger "immediate" pre-programmed cell death (pre-PCD) apoptosis include PDT, UV-A1 (340-400 nm), and agents that generate singlet-oxygen damage to mitochondrial membranes (e.g., with 5-ALA). "Intermediate" apoptosis occurs to a significant extent within 4 h, but requires more than 30 min, and high doses of either UV-B (290-320 nm) or UV-C (200-290 nm) radiation, and any agent that activates a membrane receptor containing a death domain, such as Fas/CD95/APO-1. "Delayed" apoptosis occurs well after 4 h (or days), and examples of agents that induce primarily delayed PCD apoptosis are UV-B, UV-C, X-rays, and any agent that causes significant DNA damage (15).

However, different wavelength photons have been used to treat various diseases without the need for photosensitizing drugs, resulting in necrosis but also inducing cell apoptosis (desired PMOA of ECP), with consequent changes in the production of soluble mediators (cytokine profiles), modulation of the expression of cell-surface associated molecules, and damage in pathogenetically relevant cells (cytotoxicities) (15, 16). It has been demonstrated that *in vitro* UV-A1 and UV-B irradiation alone induced T cell apoptosis, which reduces inflammatory infiltrates in T cell-mediated skin diseases (16). Still, the chances to use other types of radiations to induce apoptosis extracorporeally require further photoimmunologic studies.

Conclusions

Additional developments in manufacturing and evaluating innovative ECP-photosensitizing drugs are rising needs that should accompany the development of photopheresis medical devices. Moreover, alternative non-pharmacological systems for *in vitro* photosensitization and apoptosis induction are strategies that should be further explored. Compliance with regulatory requirements derived from the emerging knowledge might extend the new indications for ECP, benefiting more patients and healthcare systems.

Author contributions

YMCA is the sole author and agrees to be accountable for the content of the work.

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Conflict of interest

The author is an Investigator and Principal Investigator of two clinical trials examples of new indications of ECP using 8-MOP (NCT05168384 and NCT05413005, respectively; Table 1).

Publisher's note

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