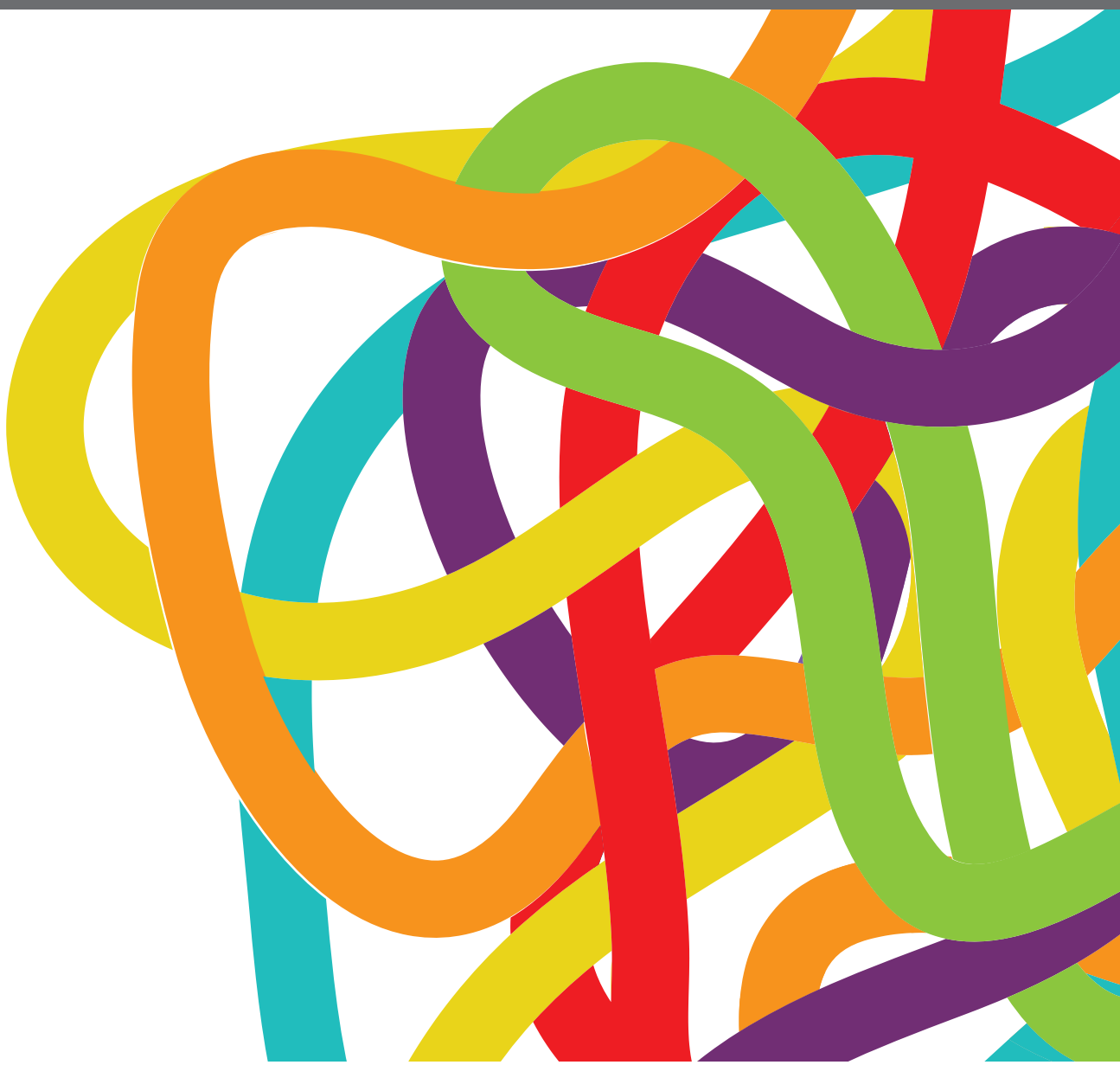
CUTANEOUS T-CELL LYMPHOMAS

EDITED BY: Basem M. William and Catherine Grace Chung
PUBLISHED IN: Frontiers in Oncology



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88966-589-1
DOI 10.3389/978-2-88966-589-1

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

CUTANEOUS T-CELL LYMPHOMAS

Topic Editors:
Basem M. William, The Ohio State University, United States
Catherine Grace Chung, The Ohio State University, United States

Citation: William, B. M., Chung, C. G., eds. (2021). Cutaneous T-cell Lymphomas. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88966-589-1

Table of Contents

frontiers in Oncology

EDITORIAL
published: 09 February 2021
doi: 10.3389/fonc.2021.649714

Editorial: Cutaneous T-Cell Lymphomas

Catherine G. Chung[1,2*] *and Basem M. William*[3]

[1] Departments of Pathology, The Ohio State University Wexner Medical Center and The James and Solove Cancer Hospital, Columbus, OH, United States, [2] Medicine, Division of Dermatology, The Ohio State University Wexner Medical Center and The James and Solove Cancer Hospital, Columbus, OH, United States, [3] Division of Hematology, The Ohio State University Wexner Medical Center and The James and Solove Cancer Hospital, Columbus, OH, United States

Keywords: cutaneous T cell lymphoma, brentuximab, PD-L1, mycosis fungoides, CAR T cell

Editorial on the Research Topic

Cutaneous T-Cell Lymphomas

Cutaneous T-cell lymphomas (CTCL), mycosis fungodies (MF) and its leukemic counterpart Sezary syndrome (SS), remain incurable malignancies with significant impact on patients' quality of life (1). These are however exciting times for clinicians caring for patients with CTCL with approval of two highly effective agents in the past few years. Brentuximab vedotin (BV), a chimeric anti-CD30 monoclonal antibody conjugated to monmethyl auristatin E (MMAE) toxin, was approved by the US Food and Drug Administration (FDA) in 2017 based on the results of the phase III study (ALCANZA) that showed a superior overall response, lasting 4 months of 56.3% as compared to 12.5% for physician's choice of methotrexate or bexarotene in patients with relapsed CTCL (2). Mogamulizumab, a humanized IgG1 anti-CCR4 monoclonal antibody, was also approved by the FDA in 2018, based on the phase III study (MAVORIC) that showed superior progression-free survival of mogamulizumab of 7.7 compared to 3.1 months with vorinostat in patients with relapsed CTCL (3).

In this Research Topic of *Frontiers in Oncology* focused on CTCL, Khan and Sawas review ongoing studies of antibody-directed therapies for MF/SS and the rationale for their use; among them are PD-1 and PD-L1 inhibitors nivolumab and pembrolizumab, both of which have had increasing applications in the treatment of various solid and hematologic malignancies. PD-L1 is expressed in a subset of patients with MF/SS, and pembrolizumab has shown a promising ORR of 38% in patients, in CITN-10 trial, with relapsed CTCL with a subset of patients experiencing very durable responses (4). Despite the emergence of multiple new targeted agents for the treatment of CTCL, skin-directed therapy remains the mainstay of treatment for early disease, and is reviewed in this issue by Tarabadkar and Shinohara, including current evidence and updated management recommendations of United States Cutaneous Lymphoma Consortium (USCLC).

The pathogenesis of CTCL remains incompletely understood and largely unknown. In this issue, several authors lend insights to our current understanding of lymphomagenesis in MF/SS. Ghazawi et al. reviews recent epidemiological data evaluating risk factors in the development of CTCL, including sex, age, race and various environmental, infectious, and iatrogenic exposures that shed light on possible mechanisms of malignancy in MF/SS. Gantchev et al. reports on the aberrant activation of meiosis genes in cells undergoing mitosis (a process termed "meiomitosis") in the setting of CTCL, and demonstrate that there is differential gene expression of meiosis-specific cancer testis (meiCT) genes in a cohort of SS patients compared to healthy controls; these findings suggest that malignant cells in SS undergo meiomitosis, which may allow for the development of

OPEN ACCESS

Edited and reviewed by:
Pierluigi Porcu, Sidney Kimmel Cancer Center, United States

***Correspondence:**
Catherine G. Chung
catherine.chung@osumc.edu

Specialty section:
This article was submitted to Hematologic Malignancies, a section of the journal Frontiers in Oncology

Received: *05 January 2021*
Accepted: *15 January 2021*
Published: *09 February 2021*

Citation:
Chung CG and William BM (2021) Editorial: Cutaneous T-Cell Lymphomas. Front. Oncol. 11:649714. doi: 10.3389/fonc.2021.649714

novel diagnostic tests that accurately distinguish CTCL from benign inflammatory conditions, as well as targeted therapies. Ferranti et al. discuss immunomodulation in CTCLs, and note that in a recent investigation of HIV-infected and non-HIV-infected patients with MF/SS, individuals with HIV demonstrated significantly higher survival and decreased risk of overall mortality compared to those without HIV. The authors also discuss exploiting retrovirus infection mechanisms for gene therapy in the treatment of CTCL, and review current studies to this end. In addition to identifying specific genetic aberrations in MF/SS involving TP53, the NFκB pathway, and the JAK3/STAT3 signal transduction pathways that may provide specific targets for future therapies, these technologies have led to the identification of multiple distinct subpopulations with different drug sensitivities within a single patient (5), suggesting that the ideal treatment for CTCL may involve combination therapies informed by a patient's unique malignant T-cell population. Phyo et al. summarizes new findings in the understanding of the biology of CTCL based on newer technologies that allow more precise molecular investigations of malignant T-cells including whole genome and whole exome sequencing and single cell RNA sequencing The evolving role of chimeric antigen receptor (CAR) T-cell therapy is summarized by Scarfo et al. highlighting the unique challenges in applying CAR-T cell therapy in the setting of T-cell malignancies including the consequences of T-cell aplasia and the killing of CAR-expressing cells by each other; a phenomenon described as "fratricide."

This Research Topic of *Frontiers in Oncology* provides an overview of key issues relating to the pathobiology, current, and future, management of CTCL and should help identify areas of common interest between dermatologist, hematologists, and cutaneous biologists thus encouraging collaboration in both basic science research and translation into practice through national and international clinical trials. We hope you find this issue interesting and informative.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

REFERENCES

1. William BM, Vose JM. *T-Cell Lymphomas. Lymphoma*. Humana Press, Totowa, NJ: Springer (2013) p. 211–29.
2. Prince HM, Kim YH, Horwitz SM, Dummer R, Scarisbrick J, Quaglino P, et al. Brentuximab vedotin or physician's choice in CD30-positive cutaneous T-cell lymphoma (ALCANZA): an international, open-label, randomised, phase 3, multicentre trial. *Lancet (Lond Engl)* (2017) 390(10094):555–66. doi: 10.1016/S0140-6736(17)31266-7
3. Kim YH, Bagot M, Pinter-Brown L, Rook AH, Porcu P, Horwitz SM, et al. Mogamulizumab versus vorinostat in previously treated cutaneous T-cell lymphoma (MAVORIC): an international, open-label, randomised, controlled phase 3 trial. *Lancet Oncol* (2018) 19(9):1192–204. doi: 10.1016/S1470-2045(18)30379-6
4. Khodadoust MS, Rook AH, Porcu P, Foss F, Moskowitz AJ, Shustov A, et al. Pembrolizumab in Relapsed and Refractory Mycosis Fungoides and Sézary Syndrome: A Multicenter Phase II Study. *J Clin Oncol* (2020) 38(1):20–8. doi: 10.1200/JCO.19.01056
5. Buus TB, Willerslev-Olsen A, Fredholm S, Blümel E, Nastasi C, Gluud M, et al. Single-cell heterogeneity in Sézary syndrome. *Blood Adv* (2018) 2(16):2115–26. doi: 10.1182/bloodadvances.2018022608

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

MINI REVIEW
published: 11 April 2019
doi: 10.3389/fonc.2019.00260

Skin Directed Therapy in Cutaneous T-Cell Lymphoma

Erica S. Tarabadkar[†] *and Michi M. Shinohara*[*†]

Division of Dermatology, University of Washington, Seattle, WA, United States

Skin directed therapies (SDTs) serve important roles in the treatment of early stage cutaneous T-cell lymphoma (CTCL)/mycosis fungoides (MF), as well as managing symptoms and improving quality of life of all stages. There are now numerous options for topical therapies that demonstrate high response rates, particularly in early/limited MF. Phototherapy retains an important role in treating MF, with increasing data supporting efficacy and long-term safety of both UVB and PUVA as well as some newer/targeted methodologies. Radiation therapy, including localized radiation and total skin electron beam therapy, continues to be a cornerstone of therapy for all stages of MF.

Keywords: cutaneous T-cell lymphoma, mycosis fungoides, skin-directed therapy, nitrogen mustard, phototherapy

OPEN ACCESS

Edited by:
Catherine Grace Chung, The Ohio State University, United States

Reviewed by:
Alberto Fabbri, Azienda Ospedaliera Universitaria Senese, Italy
Sabarish Ayyappan, The Ohio State University, United States

***Correspondence:**
Michi M. Shinohara
mshinoha@uw.edu

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

Specialty section:
This article was submitted to Hematologic Malignancies, a section of the journal Frontiers in Oncology

Received: *15 December 2018*
Accepted: *22 March 2019*
Published: *11 April 2019*

Citation:
Tarabadkar ES and Shinohara MM (2019) Skin Directed Therapy in Cutaneous T-Cell Lymphoma. Front. Oncol. 9:260. doi: 10.3389/fonc.2019.00260

INTRODUCTION

Skin directed therapies (SDTs) in cutaneous T-cell lymphoma (CTCL)/mycosis fungoides (MF) serve important roles in treating disease, but also in treating symptoms. Although SDTs can be used to cure CTCL in some patients with limited or early stage MF (stage 1A, 1B), they are most often used with palliative intent at all stages (1), with adjunct roles for both treatment and symptom management in more advanced MF, particularly managing pruritus and maintaining the skin barrier. The current National Comprehensive Cancer Network (NCCN) guidelines (1) recommend a general list of SDTs, but do not dictate the order in which they should be selected, allowing flexibility for selection based on both practitioner and patient factors.

One of the most important considerations when selecting a SDT is the extent of skin involvement (T stage). Although most SDT are appropriate for patients with any stage MF, topical preparations may be most practical (and therefore utilized with the highest compliance and best response) for those with limited skin body surface area involvement compared to patients with generalized skin involvement. In addition, a patient's ability to use a treatment as prescribed must be considered; a patient who lives alone may not be able to apply a topical therapy to their back, and someone who cannot stand without assistance may not be able to safely comply with phototherapy. Lastly, there are regional differences in preference for particular SDTs, and not all therapies are available in all regions worldwide.

Many of the studies evaluating SDTs were performed before standard definitions of clinical end points and response criteria were defined for CTCL/MF (2). As such, the difficulty comparing efficacy rates of different therapies should be considered when evaluating treatment options. A summary of the significant studies supporting the use of the various SDTs reviewed in this article is included in **Table 1**.

TOPICAL THERAPIES

Topical Corticosteroids

Topical corticosteroids have been reported in CTCL since the 1960s (16). A prospective study of 79 patients demonstrated overall response rates (ORR) of 94% for T1 (IA) disease and 82% for T2 (IB) disease, with complete response (CR) rates of 63% and 25%, respectively (3). In an updated report, 200 patients with early MF were treated with class I corticosteroids, with ORR of 80–90% (17). Less potent topical steroids may also be effective when used under occlusion (18).

Cutaneous side effects to topical corticosteroids in CTCL are common. In Zackheim et al.'s series, 10–20% of patients using class I steroids for $\geq$3 months developed irritant dermatitis or purpura, and some developed atrophy and striae (17). Reversible suppression of cortisol levels can also occur with topical steroids in CTCL (3).

Imidazoquinolines (Imiquimod and Resiquimod)

Imiquimod is a toll-like receptor-7 (TLR) agonist with antiviral and antitumor properties. Topical imiquimod leads to local production of interferon (IFN)-α, tumor necrosis factor-α, interleukin (IL)-1, and IL-6, (19–21) and induces direct tumor cell death and apoptosis (19, 22).

Published data on imiquimod in CTCL is limited. In a case series of 20 patients with IA-IIB mycosis fungoides (MF), treatment with imiquimod 5% yielded an ORR of 80%, with 45% CR and 35% partial response (PR). Twenty percent of patients did not respond (4). Side effects of imiquimod are typically skin-limited, including pain, redness, local irritation, ulceration, and pruritus (4). There are rare reports of patients experiencing flu like symptoms and fatigue (23, 24). Most adverse events resolve after the first few weeks of treatment, or with short treatment interruption (25, 26).

Resiquimod is a potent agonist of TLR-7 and TLR-8, leading to production of IFN-α, IL-12, and IL-15 (21). In a Phase I trial of 12 patients with IA-IIA CTCL, treatment with 0.03 and 0.06% topical resiquimod gel resulted in clinical improvement in 75% of treated lesions. Three patients had responses in untreated lesions, suggesting systemic activity. Adverse events were mostly skin limited, but 2 patients developed fever. Ninety percent of patients had a decrease in malignant T cell clones in treated lesions (5).

Mechlorethamine Hydrochloride (Nitrogen Mustard)

Mechlorethamine hydrochloride [nitrogen mustard (NM)] is one of the most studied treatments for CTCL, with studies reporting on the topical use of NM for CTCL from the late 1950s onward (27, 28). NM is a cytotoxic alkylating agent thought to act directly on malignant cells by causing apoptosis, though the exact mechanism of action of topical NM in CTCL is unknown (22, 29).

Topical NM is a first line treatment for localized and generalized MF (1). NM can be used as a compounded solution or ointment, or a commercial gel formulation. Data on efficacy of NM vary by study and preparation, but all topical preparations have high response rates in MF, including significant rates of CR. For NM solution, 50–60% CR rates are reported (6–8). Responses are similar with aqueous solution and ointment (8). Median time to CR with NM is on the order of 6–12 months (7, 8).

The phase 2 trial leading to FDA approval of topical mechlorethamine gel (Valchlor) included 260 stage IA to IIA MF patients treated with 0.02% gel daily for up to 12 months. The ORR (CR + PR) using Composite Assessment of Index Lesion Severity (CAILS) was 58.5%, with 13.8% CR. Responses were durable; 85.5% had ongoing responses at 12 months (9).

Overall, topical NM is well tolerated, with side effects minor and skin limited (8, 9, 30). Common side effects include contact dermatitis, which is most frequent with the aqueous solution (64.7% in one series) (31). The rate of allergic contact dermatitis (ACD) with ointment preparations is significantly lower (<10%), though irritant reactions still occur in about 25% (particularly on the face and intertriginous areas). Decreasing frequency or concentration of NM may improve tolerability (8, 29). The rate of ACD with commercial NM gel was 16.4%, though 25% experienced skin irritation (9). The use of topical steroids and NM together may improve tolerability and allow for less frequent NM application (30).

The risk of secondary malignancies in patients treated with topical NM is conflicting and difficult to assess, as many patients also receive concurrent treatments (29). There are reports of MF patients with no significant risk factors developing skin cancer after topical NM (6, 32), while at least one study has showed no significant increase in secondary skin cancers with long duration of NM therapy (33).

Carmustine

Carmustine (bis-chloroethyl-nitrosourea; BCNU) is an alkylating agent that cross-links DNA, causing apoptosis (34, 35). Zackheim reported 143 patients with CTCL treated with topical carmustine solution at variable dosing (from 2 mg/mL local up to 60 mg total body daily) (10). The ORR in patients with T1 (IA) disease was 98%, with 86% CR and 12% PR. In patients with T2 (IB) disease, ORR was 84%, with 47% CR and 37% PR. The median time to CR for all patients was 11.5 weeks (range 3–104 weeks) (10).

Cutaneous adverse events with BCNU are common, with frequent erythema, particularly in the intertriginous skin. Contact dermatitis can also occur. Telangiectasias can develop in areas of prior erythema, and may be permanent. Mild bone marrow suppression (leukopenia and anemia) can occur with widespread BCNU therapy; monthly monitoring of complete blood counts is recommended for these patients (10).

Topical Retinoids

Bexarotene

Bexarotene is a retinoid X receptor agonist. Topical bexarotene 1% gel is FDA approved for the treatment of stage IA and IB persistent or refractory CTCL. The mode of action of bexarotene in CTCL is unclear, but it has been reported to cause apoptosis in CTCL cell lines (36, 37).

Topical bexarotene demonstrates benefit for early MF, however, adverse events and intolerance are common. The Phase 1/2 trial of bexarotene gel enrolled 67 patients with stage IA-IIA CTCL/MF. ORR was 63%, with CR in 21% and PR in 42%. Local

TABLE 1 | Studies and treatment responses to topical therapies for CTCL.

Treatment	Study	Study design	Stage	Response rate	Adverse events
Topical steroids (class I-III)	Zackheim et al. (3)	Prospective	$n = 79$, IA and IB, patch and plaque	T1: ORR 94%, CR 63%, PR 31% T2: ORR 82%, CR 25%, PR 57%	Temporary depression of cortisol levels ($n = 10$), minor skin irritation, reversible skin atrophy
Imiquimod	Shipman et al. (4)	Case series and review	$n = 20$, IA–IIB	ORR 80%, CR 45%, PR 35%	Application site reaction, rare fatigue, flu-like symptoms
Resiquimod gel	Rook et al. (5)	Open label, phase 1 trial	$n = 12$, IA–IIA patch, plaque, folliculotropic	ORR 75%, CR 33%, PR 42%	Local skin irritation, low grade fever
Mechlorethamine solution	Vonderheid et al. (6)	Retrospective	$n = 324$, I–IV or Sezary syndrome[§]	T1 CR 80% T2 CR 62%	Allergic contact dermatitis, increased risk of cSCC
Mechlorethamine solution	Ramsay et al. (7)	Retrospective	$n = 117$, Stage I–III*	60.8% CR (all stages) stage I CR 75.8% stage II CR 44.6%	Delayed hypersensitivity reaction
Mechlorethamine ointment or solution	Kim et al. (8)	Retrospective	$n = 203$, T1–T4[§]	ORR 83%, CR 50%, PR 33%	Irritant or allergic contact dermatitis
Mechlorethamine	Lessin et al. (9)	Randomized, controlled, trial	$n = 260$, IA–IIA	ORR 58.5%, CR 13.8%, PR 44.6%	Skin irritation, pruritus, contact dermatitis
Carmustine	Zackheim et al. (10)	Retrospective	$n = 143$, IA–IVA[§]	IA: ORR 98%, CR 86%, PR 12% IB: ORR 84%, CR 47%, PR 37%	Mild bone marrow suppression (<10%), local skin erythema and tenderness, telangiectasia
Bexarotene gel	Breneman et al. (11)	Phase 1/2 dose escalation trial	$n = 67$, IA–IIA	ORR 63%, CR 21%, PR 42%	Local rash, pruritus, pain, rash
Bexarotene gel	Heald et al. (12)	Phase III trial	$n = 50$, IA–IIA	ORR 54%, CR 10%, PR 44%	Irritant dermatitis
Tazarotene cream	Morin et al. (13)	Open-label, prospective study	$n = 10$, IA–IIA	CR 60%	Pruritus, burning, erythema, desquamation
Tazarotene gel	Apisarnthanarax et al. (14)	Open-label pilot study	$n = 19$, Patch or plaque (<20% BSA)	ORR 58%, index lesions cleared in 35%	Skin irritation (erythema, burning, peeling)

ORR, objective response rate; CR, complete response; PR, partial response; cSCC, cutaneous squamous cell carcinoma.
[§]Staging according to 1978 Mycosis Fungoides.
Cooperative Group-National Cancer Institute Workshop staging classification (15).
**Other staging system used (7).*

adverse events occurred in 87% of patients, and included rash, pruritus, pain, and vesiculobullous rash (11). A phase III trial of topical bexarotene 1% gel showed similar findings, with an ORR of 54%, clinical CR in 10%, and frequent dose related irritant dermatitis (12).

Tazarotene

Tazarotenic acid binds retinoic acid receptors (RAR)-β and RAR-γ, exerting anti-proliferative and anti-inflammatory affects in the skin (38, 39). The first study of topical tazarotene as monotherapy in CTCL was published in 2016. Ten patients with early stage CTCL/MF were treated with tazarotene 0.1% cream to index lesions every other day for 2 weeks, then once daily for 6 months. Sixty percent of patients had a CR, with mean time to CR 3.8 months. Seventy percent of patients reported grade I or II side effects including pruritus, burning, erythema, and desquamation, and two patients withdrew from study because adverse events (13). Topical tazarotene 0.1% gel has also been evaluated in 19 patients as adjuvant therapy, with an ORR of 58% with once daily application for 24 weeks. Local skin irritation was reported in 84% (14).

PHOTOTHERAPY

Several groups have reviewed the existing studies and published guidelines for the use of phototherapy in CTCL/MF (40, 41). The United States Cutaneous Lymphoma Consortium (USCLC) recommends phototherapy as monotherapy for patients with early (stages IA–IIA) CTCL/MF, and in combination with systemic therapies for refractory early disease or advanced disease (41). The choice of nbUVB vs. PUVA as the initial therapy may be dictated by patient preference or by access issues. UVA has better skin penetration than UVB, and patients with thicker plaques or folliculotropic disease (41) or darker skin (42) may get more benefit from PUVA.

The USCLC offers expert consensus recommendations for phototherapy treatment protocols (41). In general, phototherapy for CTCL includes clearance, consolidation, and maintenance phases. The USCLC defines the goal of the clearance phase

as 100% clearance (41). The clearance phase for CTCL may take longer than for other skin diseases that are treated with phototherapy. Patients with CTCL may also benefit from a 1–3 month long "consolidation phase" between clearance and maintenance phases, in which the frequency and dose of treatments is held constant (41). The consolidation phase may maximize the potential of histologic and molecular clearance (including loss of the dominant T-cell clone), which can lag behind clinical clearance (43, 44). Inclusion of a prolonged maintenance phase after clearance of MF may prolong the time to relapse (45) and reduce relapse rates (46), but remains controversial given the potential for increased UV exposure and a lack of significant data supporting a decrease in relapse (40).

Contraindications and Side Effects of Phototherapy

General contraindications to phototherapy include photosensitive disorders, including xeroderma pigmentosa, lupus erythematosus, and porphyrias. PUVA should not be used in pregnant or lactating women, or those with a history of melanoma or multiple non-melanoma skin cancers. Relative contraindications to phototherapy in general include chronic actinic dermatitis and claustrophobia. Phototherapy should be used cautiously in those who are immunosuppressed, in children, or those who take photosensitizing medications (41).

Common side effects during phototherapy for CTCL include erythema and pruritus. Pruritus can be a particularly bothersome issue after starting phototherapy, particularly during PUVA ("PUVA itch") (47). Photodamage is also a common side effect of phototherapy, particularly with PUVA, in which at least 27% of CTCL/MF patients show signs of photodamage (48).

One of the most serious potential side effects of phototherapy is secondary skin cancer. There are meta-analyses and large studies of patients assessing the risk of skin cancer with psoriasis and other skin disorders treated with UVB. In general, the risk of skin cancer overall does not appear to be significantly increased with UVB phototherapy alone (49, 50), though there may be an increase in basal cell carcinomas (BCCs) and genital skin cancers in patients who have received both UVB and PUVA (50). Patients treated with PUVA do have an increased risk of skin cancer, particularly squamous cell carcinoma (SCC) and BCC (51). In a long term study of a cohort of CTCL patients that received PUVA, the incidence of skin cancer was 26% (48).

Ultraviolet radiation induces UV-signature DNA mutations, and there is a theoretical concern that phototherapy itself could induce progression of CTCL. Hoot et al analyzed their cohort of 345 MF patients, and found that patients treated with phototherapy had a longer time to tumor progression (3.5 years) compared to those who did not receive phototherapy (1.2 years), arguing that phototherapy does not appear to increase the risk of tumor progression (52).

Psoralen Plus UVA (PUVA)

PUVA was the first type of phototherapy used to treat CTCL (53), and is still the initial phototherapy choice preferred by many CTCL experts (54). PUVA is effective for early MF, with estimated response rates of 85% for stage IA, and 65% for stage IB (41). Patients with phototypes I or II may respond better to PUVA compared to patients with skin of color (45). Time to CR with PUVA therapy is reported in the 2–4 month range when patients are treated 2–3 times weekly (48). Those with thicker or infiltrated plaques may require longer times to clearance compared to thin plaques or patches, and PUVA is not as effective for tumor stage MF (55). Patients with hand and/or foot lesions can be treated with hand/foot bath PUVA alone or in addition to whole body treatment.

Evidence supports the use of maintenance therapy with PUVA after attaining complete clearance. Inclusion of a maintenance period is associated with longer time until relapse (45), though doesn't appear to impact overall relapse rates or survival (48).

In addition to the general contraindications and side effects of phototherapy listed above, 8-methoxypsoralen (8-MOP; methoxsalen, Oxsoralen Ultra), the most commonly used psoralen in the United States, can cause nausea and abdominal pain. 5-methoxypsoralen (5-MOP) shows similar efficacy to 8-MOP and with fewer side effects (56), but is not currently available in the United States. Psoralens can accumulate in the lens of the eye and theoretically increase the likelihood of cataracts. When eye protection is used at the current recommendation of 12–24 h after ingestion of psoralens, the risk of cataracts with PUVA therapy does not appear to be significantly increased (57).

UVB

Broadband UVB (bbUVB) was historically used to treat CTCL/MF, with high clearance rates (71% for patients with stage IA and 44% of patients with IB), but frequent (70%) relapses (46). Among lymphoma experts, bbUVB has largely been replaced by nbUVB (54).

Narrowband UVB (nbUVB) has complete response rates in the 54–90% range (41), with patches responding better than plaques (44). Among CTCL experts, nbUVB is the initial phototherapy treatment of choice for patients with stage IA disease and fair (phototypes I and II) skin (45). Patients with the hypopigmented variant of MF may not respond as well compared to other variants of MF (42).

Whether maintenance therapy with nbUVB delays relapse is unclear. The USCLC recommends maintenance therapy given that there does seem to be decrease in relapse rate when patients undergo maintenance after complete clearance with nbUVB compared to those who do not receive maintenance (41).

In general, nbUVB is better tolerated than PUVA, with fewer reported side effects (45).

Other Types of Phototherapy

Excimer (MEL)

There are several reports supporting the use of the monochromatic excimer light (308 nm) ("MEL") for patch MF. Two series reported high response rates to MEL, with one study reporting 4/4 patients with complete clinical and histologic response (58) and another with complete clinical response in 4/5 patients (59). Follow up times were short. MEL may have a particularly useful role for sanctuary sites or sites that are not as

easily accessible by phototherapy or topical preparations, such as acral surfaces (60) or intertriginous areas.

UVA1

Longwave or narrowband UVA (UVA1) penetrates the skin at the level of the dermis, and does not require adjunct psoralen, resulting in lower phototoxicity compared to PUVA (61). Several small series have reported on the use of UVA1 for early (stage IA–IIA) MF. The largest included 19 patients, with CR in 63% and PR in 37% with a treatment protocol of 30 J/cm^2 5 times a week for 5 weeks. Relapses occurred in 58% within 3 months of completing therapy (62). Prior reports have suggested higher response rates, but with low clarity on the definition of response and no follow up interval reported (63). Given low toxicity, UVA1 therapy may be a safe alternative or adjunct to other skin directed therapies.

Photodynamic Therapy

Photodynamic therapy (PDT) uses visible light to activate a topically applied photosensitizing agent, leading to the generation of reactive oxygen species and cell death in the affected cells. PDT demonstrates activity treating actinic keratosis and non-melanoma skin cancers (64). There are several reports supporting the use of PDT for MF. The most commonly reported photosensitizer used in MF is 5-aminolevulinic acid (ALA). In one series, plaque and tumor lesions from 10 patients were treated. Seven out of nine treated plaques had complete clinical response; tumors did not respond. A commonly reported side effect of PDT is pain; in this series, one patient dropped out because of pain, and most patients reported erythema and local edema (65).

RADIATION THERAPY

MF is highly radiosensitive, and radiation therapy is effective in most stages of MF. Radiotherapy can be used with curative intent in patients with single (unilesional) early stage MF, and with palliative intent in all stages, with the goal of improving symptoms and cosmesis (66).

Electron beam (e-beam) is the preferred type of radiation therapy for MF, except in certain instances such as exophytic tumors or complex surfaces, in which X-rays or photons may be superior (67). The International Lymphoma Radiation Oncology Group (ILROG) recommends guidelines for radiation therapy for MF (67). When patients with single lesions of MF are treated with the intent to cure, CR rates can be as high as 100%, with no recurrences at treated sites (68). Radiation therapy is utilized most frequently as a palliative treatment in MF. Palliative doses of 8–12 Gy are recommended, given CR rates of >90% at doses ≥8 Gy, and higher non-response rates at lower doses (1, 69). Higher doses may be required for tumor stage or large cell transformed MF (69). For patients traveling long distances or with difficulty accessing radiation therapy, treatment can occur in a single fraction, while retaining a high CR rate (94.4%) and at significant cost savings (70). Localized radiotherapy can be especially helpful for otherwise difficult to treat sites, such as the eyelids, and the hands and feet (71).

Side effects of local radiation therapy are dose dependent, and at very low doses (2 Gy) radiotherapy to the skin can have essentially no side effects (69). Even at higher doses, side effects can be minimal (72); commonly reported side effects include erythema, desquamation, atrophy, and skin dryness (68).

Total skin electron beam (TSEBT) is a technique of delivering electron beam radiation to the entire skin surface, usually by positioning patients and exposing to multiple intersecting beams (73). TSEBT has been described as the single most effective SDT for MF (74). Traditionally, doses as high as 36 Gy ("conventional dose") have been used, with complete response rates in the 75–95% range (73). Relapses are frequent after TSEBT, occurring in the majority of patients. Common side effects of conventional dosing TSEBT include erythema, desquamation, alopecia, nail changes, and lower extremity edema (73). Higher dose TSEBT is also associated with irreversible alopecia and low sperm counts in men (73).

Hoppe et al. published pooled data on the use of low dose (12 Gy) TSEBT for CTCL. Potential advantages to the use of low dose TSEBT over conventional dose TSEBT include lower toxicity and the potential for repeat treatment courses. ORR with low dose TSEBT was 88%, with 27% CR. Side effects were mild, reversible and less severe than reported with 36 Gy dosing. Of note, 12% of patients developed skin infections with *Staphylococcus aureus* during treatment (74); coverage with anti-staphylococcal antibiotics during TSEBT may be warranted.

The use of adjunct treatments to "consolidate" TSEBT may improve the durability. Data from the Stanford group suggest that following a TSEBT-induced CR with topical nitrogen mustard allows for longer freedom from relapse (but did not impact survival) (75). The addition of extracoroporeal photopheresis (ECP) concurrently or immediately after TSEBT has also been shown to be helpful, improving survival for patients with erythrodermic MF (76).

The literature evaluating the risk of secondary skin malignancy in CTCL is limited, but some reports suggest a possible increase in both melanoma non-melanoma skin cancer in CTCL patients with prior TSEBT (77).

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

1. *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines), Primary Cutaneous Lymphomas.* (2018). Available online at: https://www.nccn.org/professionals/physician_gls/pdf/primary_cutaneous.pdf (Accessed November 21, 2018).

2. Olsen EA, Whittaker S, Kim YH, Duvic M, Prince HM, Lessin SR, et al. Clinical end points and response criteria in mycosis fungoides and sezary syndrome: a consensus statement of the international society for cutaneous lymphomas, the united states cutaneous lymphoma consortium, and the cutaneous lymphoma task force of the european organisation for research and treatment of

cancer. *J Clin Oncol.* (2011) 29:2598–607. doi: 10.1200/JCO.2010.32.0630
3. Zackheim HS, Kashani-Sabet M, Amin S. Topical corticosteroids for mycosis fungoides. Experience in 79 patients. *Arch Dermatol.* (1998) 134:949–54. doi: 10.1001/archderm.134.8.949
4. Shipman AR, Scarisbrick J. New treatment options for mycosis fungoides. *Indian J Dermatol.* (2016) 61:119. doi: 10.4103/0019-5154.174085
5. Rook AH, Gelfand JM, Wysocka M, Troxel AB, Benoit B, Surber C, et al. Topical resiquimod can induce disease regression and enhance T-cell effector functions in cutaneous T-cell lymphoma. *Blood.* (2015) 126:1452–61. doi: 10.1182/blood-2015-02-630335
6. Vonderheid EC, Tan ET, Kantor AF, Shrager L, Micaily B, Van Scott EJ. Long-term efficacy, curative potential, and carcinogenicity of topical mechlorethamine chemotherapy in cutaneous T cell lymphoma. *J Am Acad Dermatol.* (1989) 20:416–28. doi: 10.1016/S0190-9622(89)70051-7
7. Ramsay DL, Halperin PS, Zeleniuch-Jacquotte A. Topical mechlorethamine therapy for early stage mycosis fungoides. *J Am Acad Dermatol.* (1988) 19:684–91. doi: 10.1016/S0190-9622(88)70223-6
8. Kim YH, Martinez G, Varghese A, Hoppe RT. Topical nitrogen mustard in the management of mycosis fungoides: update of the Stanford experience. *Arch Dermatol.* (2003) 139:165–73. doi: 10.1001/archderm.139.2.165
9. Lessin SR, Duvic M, Guitart J, Pandya AG, Strober BE, Olsen EA, et al. Topical chemotherapy in cutaneous T-cell lymphoma: positive results of a randomized, controlled, multicenter trial testing the efficacy and safety of a novel mechlorethamine, 0.02%, gel in mycosis fungoides. *JAMA Dermatol.* (2013) 149:25–32. doi: 10.1001/2013.jamadermatol.541
10. Zackheim HS, Epstein EH, Jr, Crain WR. Topical carmustine (BCNU) for cutaneous T cell lymphoma: a 15-year experience in 143 patients. *J Am Acad Dermatol.* (1990) 22(5 Pt 1):802–10. doi: 10.1016/0190-9622(90)70112-U
11. Breneman D, Duvic M, Kuzel T, Yocum R, Truglia J, Stevens VJ. Phase 1 and 2 trial of bexarotene gel for skin-directed treatment of patients with cutaneous T-cell lymphoma. *Arch Dermatol.* (2002) 138:325–32. doi: 10.1001/archderm.138.3.325
12. Heald P, Mehlmauer M, Martin AG, Crowley CA, Yocum RC, Reich SD. Topical bexarotene therapy for patients with refractory or persistent early-stage cutaneous T-cell lymphoma: results of the phase III clinical trial. *J Am Acad Dermatol.* (2003) 49:801–15. doi: 10.1016/S0190-9622(03)01475-0
13. Besner Morin C, Roberge D, Turchin I, Petrogiannis-Haliotis T, Popradi G, Pehr K. Tazarotene 0.1% cream as monotherapy for early-stage cutaneous T-cell lymphoma. *J Cutan Med Surgery.* (2016) 20:244–8. doi: 10.1177/1203475415626686
14. Apisarnthanarax N, Talpur R, Ward S, Ni X, Kim HW, Duvic M. Tazarotene 0.1% gel for refractory mycosis fungoides lesions: an open-label pilot study. *J Am Acad Dermatol.* (2004) 50:600–7. doi: 10.1016/j.jaad.2003.09.005
15. Bunn PA, Jr, Lamberg SI. Report of the committee on staging and classification of cutaneous T-cell lymphomas. *Cancer Treat Rep.* (1979) 63:725–8.
16. Farber EM, Cox AJ, Steinberg J, McClintock RP. Therapy of mycosis fungoides with topically applied fluocinolone acetonide under occlusive dressing. *Cancer.* (1966) 19:237–45.
17. Zackheim HS. Treatment of patch-stage mycosis fungoides with topical corticosteroids. *Dermatol Ther.* (2003) 16:283–7. doi: 10.1111/j.1396-0296.2003.01639.x
18. Farber EM, Zackheim HS, McClintock RP, Cox AJ, Jr. Treatment of mycosis fungoides with various strengths of fluocinolone acetonide cream. *Arch Dermatol.* (1968) 97:165–72. doi: 10.1001/archderm.1968.01610080069013
19. Schon MP, Schon M. Immune modulation and apoptosis induction: two sides of the antitumoral activity of imiquimod. *Apoptosis.* (2004) 9:291–8. doi: 10.1023/B:APPT.0000025805.55340.c3
20. Dummer R, Urosevic M, Kempf W, Hoek K, Hafner J, Burg G. Imiquimod in basal cell carcinoma: how does it work? *Br J Dermatol.* (2003) 149(Suppl 66):57–8. doi: 10.1046/j.0366-077X.2003.05630.x
21. Huen AO, Rook AH. Toll receptor agonist therapy of skin cancer and cutaneous T-cell lymphoma. *Curr Opin Oncol.* (2014) 26:237–44. doi: 10.1097/CCO.0000000000000048
22. Kim EJ, Hess S, Richardson SK, Newton S, Showe LC, Benoit BM, et al. Immunopathogenesis and therapy of cutaneous T cell lymphoma. *J Clin Invest.* (2005) 115:798–812. doi: 10.1172/JCI24826
23. Lewis DJ, Byekova YA, Emge DA, Duvic M. Complete resolution of mycosis fungoides tumors with imiquimod 5% cream: a case series. *J Dermatol Treat.* (2017) 28:567–9. doi: 10.1080/09546634.2017.1294728
24. Deeths MJ, Chapman JT, Dellavalle RP, Zeng C, Aeling JL. Treatment of patch and plaque stage mycosis fungoides with imiquimod 5% cream. *J Am Acad Dermatol.* (2005) 52:275–80. doi: 10.1016/j.jaad.2004.04.049
25. Coors EA, Schuler G, Von Den Driesch P. Topical imiquimod as treatment for different kinds of cutaneous lymphoma. *Eur J Dermatol.* (2006) 16:391–3.
26. Suchin KR, Junkins-Hopkins JM, Rook AH. Treatment of stage IA cutaneous T-Cell lymphoma with topical application of the immune response modifier imiquimod. *Arch Dermatol.* (2002) 138:1137–9. doi: 10.1001/archderm.138.9.1137
27. Remission of lesions in mycosis fungoides following topical application of nitrogen mustard. By John R. Haserick, Joseph H. Richardson, and Douglas J. Grant. *Cleve Clin Q.* (1983) 50:91–5.
28. Watson JI, Wilkinson RD, Craig GE. Topical nitrogen mustard in cutaneous lymphosarcoma (mycosis fungoides). *Can Med Assoc J.* (1962) 87:1284–5.
29. Kim YH. Management with topical nitrogen mustard in mycosis fungoides. *Dermatol Ther.* (2003) 16:288–98. doi: 10.1111/j.1396-0296.2003.01640.x
30. de Quatrebarbes J, Esteve E, Bagot M, Bernard P, Beylot-Barry M, Delaunay M, et al. Treatment of early-stage mycosis fungoides with twice-weekly applications of mechlorethamine and topical corticosteroids: a prospective study. *Arch Dermatol.* (2005) 141:1117–20. doi: 10.1001/archderm.141.9.1117
31. Lindahl LM, Fenger-Gron M, Iversen L. Topical nitrogen mustard therapy in patients with mycosis fungoides or parapsoriasis. *J Eur Acad Dermatol Venereol.* (2013) 27:163–8. doi: 10.1111/j.1468-3083.2011.04433.x
32. Lee LA, Fritz KA, Golitz L, Fritz TJ, Weston WL. Second cutaneous malignancies in patients with mycosis fungoides treated with topical nitrogen mustard. *J Am Acad Dermatol.* (1982) 7:590–8. doi: 10.1016/S0190-9622(82)70138-0
33. Lindahl LM, Fenger-Gron M, Iversen L. Secondary cancers, comorbidities and mortality associated with nitrogen mustard therapy in patients with mycosis fungoides: a 30-year population-based cohort study. *Br J Dermatol.* (2014) 170:699–704. doi: 10.1111/bjd.12620
34. Tong WP, Kirk MC, Ludlum DB. Formation of the cross-link 1-[N3-deoxycytidyl),2-[N1-deoxyguanosinyl]ethane in DNA treated with N,N'-bis(2-chloroethyl)-N-nitrosourea. *Cancer Res.* (1982) 42:3102–5.
35. Kohn KW. Interstrand cross-linking of DNA by 1,3-bis(2-chloroethyl)-1-nitrosourea and other 1-(2-haloethyl)-1-nitrosoureas. *Cancer Res.* (1977) 37:1450–4.
36. Zhang C, Hazarika P, Ni X, Weidner DA, Duvic M. Induction of apoptosis by bexarotene in cutaneous T-cell lymphoma cells: relevance to mechanism of therapeutic action. *Clin Cancer Res.* (2002) 8:1234–40.
37. Schadt CR. Topical and oral bexarotene. *Dermatol Ther.* (2013) 26:400–3. doi: 10.1111/dth.12087
38. Talpur R, Cox K, Duvic M. Efficacy and safety of topical tazarotene: a review. *Exp Opin Drug Metabol Toxicol.* (2009) 5:195–210. doi: 10.1517/17425250902721250
39. Chandraratna RA. Tazarotene–first of a new generation of receptor-selective retinoids. *Br J Dermatol.* (1996) 135(Suppl 49):18–25. doi: 10.1111/j.1365-2133.1996.tb15662.x
40. Grandi V, Fava P, Rupoli S, Alberti Violetti S, Canafoglia L, Quaglino P, et al. Standardization of regimens in Narrowband UVB and PUVA in early stage mycosis fungoides: position paper from the Italian task force for cutaneous lymphomas. *J Eur Acad Dermatol Venereol.* (2018) 32:683–91. doi: 10.1111/jdv.14668
41. Olsen EA, Hodak E, Anderson T, Carter JB, Henderson M, Cooper K, et al. Guidelines for phototherapy of mycosis fungoides and Sezary syndrome: a consensus statement of the United States cutaneous lymphoma consortium. *J Am Acad Dermatol.* (2016) 74:27–58. doi: 10.1016/j.jaad.2015.09.033
42. Gathers RC, Scherschun L, Malick F, Fivenson DP, Lim HW. Narrowband UVB phototherapy for early-stage mycosis fungoides. *J Am Acad Dermatol.* (2002) 47:191–7. doi: 10.1067/mjd.2002.120911
43. Dereure O, Picot E, Comte C, Bessis D, Guillot B. Treatment of early stages of mycosis fungoides with narrowband ultraviolet B. A clinical, histological and molecular evaluation of results. *Dermatology.* (2009) 218:1–6. doi: 10.1159/000161114

44. Gokdemir G, Barutcuoglu B, Sakiz D, Koslu A. Narrowband UVB phototherapy for early-stage mycosis fungoides: evaluation of clinical and histopathological changes. *J Eur Acad Dermatol Venereol.* (2006) 20:804–9. doi: 10.1111/j.1468-3083.2006.01635.x
45. Nikolaou V, Sachlas A, Papadavid E, Economidi A, Karambidou K, Marinos L, et al. Phototherapy as a first-line treatment for early-stage mycosis fungoides: the results of a large retrospective analysis. *Photodermatol Photoimmunol Photomed.* (2018) 34:307–13. doi: 10.1111/phpp.12383
46. Pavlotsky F, Barzilai A, Kasem R, Shpiro D, Trau H. UVB in the management of early stage mycosis fungoides. *J Eur Acad Dermatol Venereol.* (2006) 20:565–72. doi: 10.1111/j.1468-3083.2006.01557.x
47. Roelandts R, Stevens A. PUVA-induced itching and skin pain. *Photodermatol Photoimmunol Photomed.* (1990) 7:141–2.
48. Querfeld C, Rosen ST, Kuzel TM, Kirby KA, Roenigk HH Jr, Prinz BM, et al. Long-term follow-up of patients with early-stage cutaneous T-cell lymphoma who achieved complete remission with psoralen plus UV-A monotherapy. *Arch Dermatol.* (2005) 141:305–11. doi: 10.1001/archderm.141.3.305
49. Hearn RM, Kerr AC, Rahim KF, Ferguson J, Dawe RS. Incidence of skin cancers in 3867 patients treated with narrow-band ultraviolet B phototherapy. *Br J Dermatol.* (2008) 159:931–5. doi: 10.1111/j.1365-2133.2008.08776.x
50. Lee E, Koo J, Berger T. UVB phototherapy and skin cancer risk: a review of the literature. *Int J Dermatol.* (2005) 44:355–60. doi: 10.1111/j.1365-4632.2004.02186.x
51. Archier E, Devaux S, Castela E, Gallini A, Aubin F, Le Maître M, et al. Carcinogenic risks of psoralen UV-A therapy and narrowband UV-B therapy in chronic plaque psoriasis: a systematic literature review. *J Eur Acad Dermatol Venereol.* (2012) 26(Suppl 3):22–31. doi: 10.1111/j.1468-3083.2012.04520.x
52. Hoot JW, Wang L, Kho T, Akilov OE. The effect of phototherapy on progression to tumors in patients with patch and plaque stage of mycosis fungoides. *J Dermatol Treat.* (2018) 29:272–6. doi: 10.1080/09546634.2017.1365113
53. Gilchrest BA, Parrish JA, Tanenbaum L, Haynes HA, Fitzpatrick TB. Oral methoxsalen photochemotherapy of mycosis fungoides. *Cancer.* (1976) 38:683–9.
54. Carter J, Zug KA. Phototherapy for cutaneous T-cell lymphoma: online survey and literature review. *J Am Acad Dermatol.* (2009) 60:39–50. doi: 10.1016/j.jaad.2008.08.043
55. Briffa DV, Warin AP, Harrington CI, Bleehen SS. Photochemotherapy in mycosis fungoides. A study of 73 patients. *Lancet.* (1980) 2:49–53. doi: 10.1016/S0140-6736(80)92937-2
56. Wackernagel A, Hofer A, Legat F, Kerl H, Wolf P. Efficacy of 8-methoxypsoralen vs. 5-methoxypsoralen plus ultraviolet A therapy in patients with mycosis fungoides. *Br J Dermatol.* (2006) 154:519–23. doi: 10.1111/j.1365-2133.2005.07008.x
57. Malanos D, Stern RS. Psoralen plus ultraviolet a does not increase the risk of cataracts: a 25-year prospective study. *J Am Acad Dermatol.* (2007) 57:231–7. doi: 10.1016/j.jaad.2007.04.027
58. Mori M, Campolmi P, Mavilia L, Rossi R, Cappugi P, Pimpinelli N. Monochromatic excimer light (308 nm) in patch-stage IA mycosis fungoides. *J Am Acad Dermatol.* (2004) 50:943–5. doi: 10.1016/j.jaad.2004.01.047
59. Passeron T, Zakaria W, Ostovari N, Perrin C, Larrouy JC, Lacour JP, et al. Efficacy of the 308-nm excimer laser in the treatment of mycosis fungoides. *Arch Dermatol.* (2004) 140:1291–3. doi: 10.1001/archderm.140.10.1291
60. Jin SP, Jeon YK, Cho KH, Chung JH. Excimer laser therapy (308 nm) for mycosis fungoides palmaris et plantaris: a skin-directed and anatomically feasible treatment. *Br J Dermatol.* (2010) 163:651–3. doi: 10.1111/j.1365-2133.2010.09793.x
61. Krutmann J, Morita A. Mechanisms of ultraviolet (UV) B and UVA phototherapy. *J Invest Dermatol Symp Proc.* (1999) 4:70–2. doi: 10.1038/sj.jidsp.5640185
62. Adisen E, Tektas V, Erduran F, Erdem O, Gurer MA. Ultraviolet A1 phototherapy in the treatment of early mycosis fungoides. *Dermatology.* (2017) 233:192–8. doi: 10.1159/000458149
63. Olek-Hrab K, Silny W, Danczak-Pazdrowska A, Osmola-Mankowska A, Sadowska PA, Polanska A, et al. Ultraviolet A1 phototherapy for mycosis fungoides. *Clin Exp Dermatol.* (2013) 38:126–30. doi: 10.1111/ced.12001
64. Ross K, Cherpelis B, Lien M, Fenske N. Spotlighting the role of photodynamic therapy in cutaneous malignancy: an update and expansion. *Dermatol Surgery.* (2013) 39:1733–44. doi: 10.1111/dsu.12319
65. Edstrom DW, Porwit A, Ros AM. Photodynamic therapy with topical 5-aminolevulinic acid for mycosis fungoides: clinical and histological response. *Acta Dermato Venereol.* (2001) 81:184–8. doi: 10.1080/000155501750376276
66. Prince HM, Whittaker S, Hoppe RT. How I treat mycosis fungoides and Sezary syndrome. *Blood.* (2009) 114:4337–53. doi: 10.1182/blood-2009-07-202895
67. Specht L, Dabaja B, Illidge T, Wilson LD, Hoppe RT. Modern radiation therapy for primary cutaneous lymphomas: field and dose guidelines from the International Lymphoma radiation oncology group. *Int J Rad Oncol Biol Phys.* (2015) 92:32–9. doi: 10.1016/j.ijrobp.2015.01.008
68. Micaily B, Miyamoto C, Kantor G, Lessin S, Rook A, Brady L, et al. Radiotherapy for unilesional mycosis fungoides. *Int J Radiat Oncol Biol Phys.* (1998) 42:361–4. doi: 10.1016/S0360-3016(98)00218-1
69. Neelis KJ, Schimmel EC, Vermeer MH, Senff NJ, Willemze R, Noordijk EM. Low-dose palliative radiotherapy for cutaneous B- and T-cell lymphomas. *Int J Radiat Oncol Biol Phys.* (2009) 74:154–8. doi: 10.1016/j.ijrobp.2008.06.1918
70. Thomas TO, Agrawal P, Guitart J, Rosen ST, Rademaker AW, Querfeld C, et al. Outcome of patients treated with a single-fraction dose of palliative radiation for cutaneous T-cell lymphoma. *Int J Radiat Oncol Biol Phys.* (2013) 85:747–53. doi: 10.1016/j.ijrobp.2012.05.034
71. Goddard AL, Vleugels RA, LeBoeuf NR, O'Farrell DA, Cormack RA, Hansen JL, et al. Palliative therapy for recalcitrant cutaneous T-cell lymphoma of the hands and feet with low-dose, high dose-rate brachytherapy. *JAMA Dermatol.* (2015) 151:1354–7. doi: 10.1001/jamadermatol.2015.3028
72. Piccinno R, Caccialanza M, Cuka E, Recalcati S. Localized conventional radiotherapy in the treatment of mycosis fungoides: our experience in 100 patients. *J Eur Acad Dermatol Venereol.* (2014) 28:1040–4. doi: 10.1111/jdv.12254
73. Jones GW, Kacinski BM, Wilson LD, Willemze R, Spittle M, Hohenberg G, et al. Total skin electron radiation in the management of mycosis fungoides: consensus of the European Organization for Research and Treatment of Cancer (EORTC) cutaneous lymphoma project group. *J Am Acad Dermatol.* (2002) 47:364–70. doi: 10.1067/mjd.2002.123482
74. Hoppe RT, Harrison C, Tavallaee M, Bashey S, Sundram U, Li S, et al. Low-dose total skin electron beam therapy as an effective modality to reduce disease burden in patients with mycosis fungoides: results of a pooled analysis from 3 phase-II clinical trials. *J Am Acad Dermatol.* (2015) 72:286–92. doi: 10.1016/j.jaad.2014.10.014
75. Chinn DM, Chow S, Kim YH, Hoppe RT. Total skin electron beam therapy with or without adjuvant topical nitrogen mustard or nitrogen mustard alone as initial treatment of T2 and T3 mycosis fungoides. *Int J Radiation Oncol Biol Phys.* (1999) 43:951–8. doi: 10.1016/S0360-3016(98)00517-3
76. Wilson LD, Jones GW, Kim D, Rosenthal D, Christensen IR, Edelson RL, et al. Experience with total skin electron beam therapy in combination with extracorporeal photopheresis in the management of patients with erythrodermic (T4) mycosis fungoides. *J Am Acad Dermatol.* (2000) 43(1 Pt 1):54–60. doi: 10.1067/mjd.2000.105510
77. Licata AG, Wilson LD, Braverman IM, Feldman AM, Kacinski BM. Malignant melanoma and other second cutaneous malignancies in cutaneous T-cell lymphoma. The influence of additional therapy after total skin electron beam radiation. *Arch Dermatol.* (1995) 131:432–5. doi: 10.1001/archderm.1995.01690160060009

Conflict of Interest Statement: MS has served as principle investigator for Actelion and Soligenix.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

MINI REVIEW
published: 16 April 2019
doi: 10.3389/fonc.2019.00259

CAR-Based Approaches to Cutaneous T-Cell Lymphoma

Irene Scarfò[1,2], *Matthew J. Frigault*[1,2] *and Marcela V. Maus*[1,2,3]*

[1] Cellular Immunotherapy Program, Massachusetts General Hospital Cancer Center, Charlestown, MA, United States, [2] Harvard Medical School, Boston, MA, United States, [3] Broad Institute of Harvard and MIT, Cambridge, MA, United States

Cutaneous T cell lymphomas (CTCL) are a heterogeneous group of malignancies characterized by the expansion of a malignant T cell clone. Chimeric Antigen Receptor (CAR) T cell therapy has shown impressive results for the treatment of B-cell tumors, but several challenges have prevented this approach in the context of T cell lymphoma. These challenges include the possibilities of fratricide due to shared T-cell antigens, T cell immunodeficiency, and CAR transduction of malignant cells if CAR T are manufactured in the autologous setting. In this review, we discuss these and other challenges in detail and summarize the approaches currently in development to overcome these challenges and offer cellular targeting of T cell lymphomas.

Keywords: T cell lymphomas/leukemias, CAR T cells, adoptive cell therapy, immunotherapy, cutaneous

OPEN ACCESS

Edited by:
Catherine Grace Chung, The Ohio State University, United States

Reviewed by:
Yona Keisari, Tel Aviv University, Israel
Yago Nieto, University of Texas MD Anderson Cancer Center, United States

***Correspondence:**
Marcela V. Maus
mvmaus@mgh.harvard.edu

Specialty section:
This article was submitted to Hematologic Malignancies, a section of the journal Frontiers in Oncology

Received: *21 January 2019*
Accepted: *22 March 2019*
Published: *16 April 2019*

Citation:
Scarfò I, Frigault MJ and Maus MV (2019) CAR-Based Approaches to Cutaneous T-Cell Lymphoma. Front. Oncol. 9:259. doi: 10.3389/fonc.2019.00259

INTRODUCTION

Cutaneous T-cell lymphomas (CTCL) are a heterogeneous group of malignancies of T-cell origin that occur primarily in the skin. They are the second most common form of extranodal Non-Hodgkin lymphomas (NHL) and their incidence has been increasing over the time (1). Mycosis fungoides (MF) and Sézary syndrome (SS) are the most common subtypes, which represent about 70–75% of CTCL (2, 3); other frequent types include primary cutaneous CD30+ T-cell lymphoproliferative disorders, adult T-cell leukemia/lymphoma and a portion of peripheral T-cell lymphoma not otherwise specified (PTCL-NOS). Although both cutaneous and/or systemic therapies have been developed for these tumors, long-term outcomes are characterized by high relapse rates with advanced forms of CTCL considered incurable. Recent progress has been made using immunotherapy approaches. These include the development of monoclonal antibodies, such as brentuximab vedotin, which has shown clinical efficacy leading to FDA approval in certain disease sub-types (4–6). Here, we discuss recent advances in adoptive Chimeric Antigen Receptor (CAR) T cell therapy and summarize the challenges and opportunities for the treatment of CTCL.

HURDLES IN THE DEVELOPMENT OF ADOPTIVE CELL THERAPY FOR THE TREATMENT OF CTCL

Immunotherapy has emerged as groundbreaking approach for the treatment of cancer and includes monoclonal antibodies, immune checkpoint blockade, tumor vaccines, and most recently, CAR T cell therapy. CAR-T cell therapy redirects a poly-clonal T-cell population against a tumor specific antigen in an MHC independent fashion. Based on impressive phase I/II data, CAR-T cell therapies are now approved for CD19+ B-cell malignancies including pediatric acute lymphoblastic leukemia (B-ALL) and large cell lymphoma (7, 8). Although B-cell aplasia is a common and manageable side effect of CD19 directed CAR-T cell therapy, T-cell aplasia is less tolerable in the long term, and can

be life-threatening if it is not reversed or ameliorated. Furthermore, in the case of CTCL, malignant and normal T cells express many shared surface markers, limiting our ability to differentially target these populations. For this reason, special considerations have to be made when designing CARs for the treatment of T-cell lineage malignancies. First, permanent T cell aplasia is not acceptable, but approaches using either transient CAR T cell expression or persistence or suicide genes could be used to eliminate the CAR cells and allow for T cell immune reconstitution (**Figure 1A**). Second, killing of CAR-expressing cells by each other, known as fratricide, can undermine the *ex/in vivo* expansion of modified T cells and the generation of CAR T cell products (**Figure 1B**). Third, circulating tumor cells can contaminate leukapheresis products and be transduced with CARs during manufacturing, which could be associated with a growth advantage for the transduced tumor cells or resistance to CAR-T cell mediated cytotoxicity (**Figure 1C**). This phenomenon has been recently documented in a B-ALL patient relapsed after CTL019 treatment (9), whereby transduction of the tumor cells with the CAR led to "masking" the expression of the CD19 target antigen and therefore resistance to the CAR T cell-mediated killing. All these aspects need to be considered for the development of CAR T cell therapy against CTCL. However, the unmet need in T cell lymphomas is great, and effective treatments would represent a significant therapeutic advance.

CAR T CELLS AGAINST T CELL ANTIGENS

It has been difficult to identify targets uniquely expressed on malignant but not on normal T cells. One strategy has been to target molecules expressed by a subpopulation of T cells, or which are downregulated when T cells are activated. This approach has been adopted for the design of CAR against CD4, CD5, CD7, CD30, CD37, CCR4, and the 2 alleles of the T cell receptor beta chains (TRBC1/TRBC2) (**Table 1**).

CD4

CD4 was one of the first molecules selected as target for CAR T cell therapy design. It is highly and uniformly expressed by T cell lymphomas, including CTCL which are predominantly comprised of peripheral CD4 positive T cells. In 2016, Pinz and colleagues showed preclinical efficacy of anti-CD4 CAR T cells *in vitro* and in a xenograft mouse model of ALCL (10). Although this approach demonstrated the potential for CAR-T cells in ALCL, ongoing CD4 depletion could lead to a T cell immunodeficiency similar to that observed in the acquired immunodeficiency syndrome (AIDS) induced by the human immunodeficiency virus (HIV).

CD5

CD5 is another highly expressed antigen on malignant T cells (24, 25). In normal mature T cells, it has a costimulatory role in synergy with CD28 and TCR/CD3 (26–28); previous studies have shown that its expression is post-transnationally regulated (29). Anti-CD5 CAR T cells have been tested in two configurations. The first, designed by Mamonkin et al. included CD28 as costimulatory domain and showed a transient fratricide and a limited bystander killing of normal T cells due indeed to surface downregulation of CD5 protein (11). These CAR T cells demonstrated preclinical activity *in vitro* against different TCL and T-ALL cell lines, including the HUT78 Sézary syndrome cells, but only partial clearance of T-ALL xenograft tumor, suggesting a lack of CAR-T cell persistence. For this reason, Mamonkin and colleagues designed a second version of the CAR using 4-1BB as costimulatory domain. Interestingly, they reported a higher fratricide when expanding 4-1BB CAR T cells compared to CD28 CAR T cells. The authors demonstrated that 4-1BB upregulates ICAM-1 molecule increasing the stability of the immunological synapse and consequent killing (12). In order to regulate CD5 targeted killing, the authors put their 4-1BB CAR under an inducible promoter allowing for transient expression and therefore killing. This approach demonstrated complete elimination of T-ALL xenograft tumors, but raised concerns about the clinical safety and the immunogenicity of transactivator proteins. Moreover, CD5 is not expressed by many malignant T cell clones and can be easily down regulated, potentially leading to antigen escape.

CD7

CD7 is a transmembrane glycoprotein which is a primary marker for acute T-ALL and is highly expressed in a subset of T cell lymphomas (24, 30, 31). In normal tissues, CD7 expression is confined to T and natural killer (NK) cells. Recently, various groups have independently shown the potential of targeting CD7, however, all the studies reported a lack of CD7 downregulation on effector T-cells which resulted in extensive fratricide. Given the near universal expression of CD7 on normal T-cells, Gomes-Silva et al. used CRISPR/Cas9 system to disrupt the CD7 locus. Genetic knockout (KO) of CD7 led to normal expansion of CD7 specific CAR T cells without detectible fratricide of gene disrupted T cells. More importantly, they also demonstrated that anti-CD7 CAR T cells retained anti-viral activity *in vitro* which may provide protection in the context of T and NK ablation (13). These data led to the opening of a first in human phase I clinical trial (NCT03690011) of CD7 specific CAR-T cells in T cell leukemia and lymphoma.

A second group designed an elegant method to prevent membrane expression of CD7 protein called protein expression blocker (PEBL) by coupling an intracellular retention domain KDEL to an anti-CD7 single chain variable fragment. Transduction of anti-CD7 PEBL lead to abrogation of CD7 expression and inhibition of fratricide of PEBL CAR T cells. These modified T cells showed anti leukemic activity in cell-lines and patient derived xenograft (PDX) models of T-ALL (14). An additional advantage of this approach would be the possible direct translation to the clinic using existing current good manufacturing practice (cGMP) manufacturing processes.

Finally, Cooper and colleagues designed the first allogeneic CAR T cell product for the treatment of CD7 positive T cell tumors (15). They developed a CRISPR/Cas9 multiplex system targeting the T cell receptor alpha chain (TRAC) and CD7 genes. These UCART7 T cells were fratricide-resistant and killed PDX

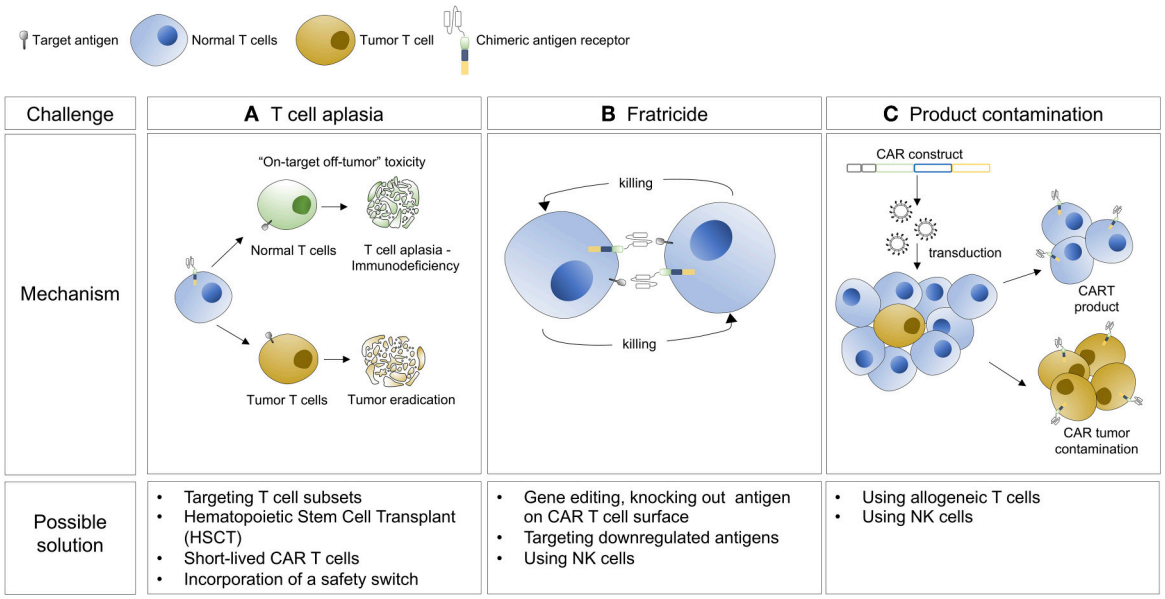


FIGURE 1 | Hurdles associated with the development of CAR T cell therapy for the treatment of CTCL and possible solutions.

model of T-ALL *in vivo* without inducing xenogeneic graft-vs.-host disease (GvHD).

CD30

CD30 is a more selectively expressed T lineage marker, present in all anaplastic large cell lymphomas (ALCLs) and some T-ALL (32, 33). Antibody drug conjugates such as brentuximab vedotin have been used to treat cutaneous lymphomas and advanced stage mycosis fungoides with clinical success (34, 35). Despite this success, significant drug conjugate mediated toxicities, including neuropathy (62%) and cytopenias (upwards of 78%), as well as limited durability of response, have led to the development of anti-CD30 CAR T cells. Although the majority of CD30. CAR-T cell trials have focused on Hodgkin lymphomas, results of 2 ALCL patients treated with CD30.CAR-Ts are promising and include one complete remission for 9 months without impaired virus-specific immunity (16).

CCR4

CCR4 is a chemokine receptor abundantly expressed on the surface of T cell tumors including T-ALL, PTCL, and CTCL and is responsible for the homing of malignant cells to the skin. Various anti-CCR4 monoclonal antibodies are in development including mogamulizumab which already has approval in Japan for the treatment of relapsed/refractory adult T-cell leukemia/lymphoma. CCR4 has been validated as a CAR-T target as Perera and colleagues have demonstrated the ability of an anti-CCR4-CAR to lyse multiple T-cell lines *in vitro* and clear T-ALL tumor in a xenograft mouse model (18). Furthermore, since CCR4 is also expressed by T regulatory (Tregs) cells, the authors speculate that anti-CCR4 CAR T cells would be able to eliminate Tregs cells which have typically an immunosuppressive role in the tumor microenvironment. Nevertheless, given the wide expression of CCR4, safety questions about the toxicity profile of these cells still need to be addressed.

CD37

CD37 is a four-passage transmembrane protein belonging to the tetraspanin superfamily. Our group and others have shown that it is expressed not only on B-cell tumors but on T cell malignancies as well (17). We generated CAR37 T cells and demonstrated specific *in vitro* killing of T cell lines, including HUT78 cells. We did not detect significant fratricide of CAR+ and/or untransduced T-cells *in vitro* lessening the concern for CAR-T mediated T cell aplasia. *In vivo* experiments and an upcoming phase I trial of CAR-37 will further evaluate CD37 as a therapeutic target.

TRBC1 and TRBC2

Another promising approach is the targeting of the T-cell receptor beta-chain constant domain, type 1(TRBC1) or 2 (TRBC2). Maciocia and colleagues showed that T-cell malignancies are clonally restricted to either TRBC1 or TRBC2, akin to kappa/lambda clonality in multiple myeloma. They subsequently developed anti-TRBC1 CAR T cells and demonstrated specific killing of TRBC1 expressing normal and malignant T cells while sparing TRBC2 restricted cells (19). Given the near 50:50 distribution of TRBC1:TRBC2 expression, this approach offers the potential to spare an adequate number of normal T cells to maintain polyclonal T-cell immunity. An ongoing phase I/II clinical trial is evaluating the safety and efficacy of TRBC1 specific CAR-T cells in TRBC1 positive malignancies (NCT03590574). This group is also developing a novel TRBC2 binder and anti-TRBC2 CAR for TRBC2 restricted clones (20). These strategies open new opportunities for the treatment of T cell malignancies.

TABLE 1 | CAR T/NK cells for the treatment of CTCL.

	Target antigen	CAR construct	Modifications	Models	Clinical trials	References
CAR T cells	CD4	αCD4 CD28 41BB CAR	–	*in vitro*: KARPAS-299; Sézary syndrome, PTCL *in vivo*: KARPAS-299	–	(10)
	CD5	αCD5 CD28 CAR	–	*in vitro*: MOLT-4, CCRF-CEM, Jurkat, HuT78, SUP-T1; T-ALL *in vivo*: Jurkat	–	(11)
		αCD5 41BB CAR	–	*in vitro*: CCRF-CEM, Jurkat *in vivo*: Jurkat	–	(12)
	CD7	αCD7 CD28 CAR	CD7 CRISPR/Cas9 KO	*in vitro*: MOLT-4, CCRF, Jurkat, HuT78, SUP-T1; T-ALL *in vivo*: CCRF-CEM	NCT03690011	(13)
		αCD7 41BB CAR	CD7 protein expression blocker (PEBL)	*in vitro*: MOLT-4, CCRF-CEM, Jurkat, Loucy, KG1a *in vivo:* CCRF-CEM; ETP-ALL PDX		(14)
		αCD7 C CD28 41BB AR	CD7, TRAC CRISPR/Cas9 KO	*in vitro*: MOLT-4, MOLT-3, HSB-2, T-ALL *in vivo*: CCRF-CEM; T-ALL PDX		(15)
	CD30	αCD30 CD28 CAR	–	*in vitro*: KARPAS-299, HDLM-2, L428, L540, KH-M2, L1236 *in vivo*: KARPAS-299	NCT03049449, NCT01316146, NCT02690545, NCT02917083	(16)
	CD37	αCD37 41BB CAR	–	*in vitro*: FEPD, HuT78, PTCL	–	(17)
	CCR4	αCCR4 41BB CAR	–	*in vitro*: HH, HuT78, ML, HuT102, JB-6, Karpas299, SUDHL-1, SR-786, SUP-M2, DEL, Mac-1, Mac-2A, Mac-2B *in vivo*: ATL41214	–	(18)
	TRBC1	αTRBC1 CD28-OX40 CAR	–	*in vitro*: T-PLL, PTCL-NOS, ATLL *in vivo*: Jurkat	NCT0359054	(19)
	TRBC2	αTRBC2 CD28-OX40 CAR	–	*in vitro*: Loucy, MOLT13, BE13 *in vivo*: Loucy	–	(20)
CAR NK cells	CD3	αCD3 41BB CD28 CAR	NK-92 cells	*in vitro*: KARPAS-299, CCRF-CEM, Jurkat; PTCL, Sézary syndrome *in vivo*: Jurkat	–	(21)
	CD4	αCD4 CD28 41BB CAR	NK-92 cells	*in vitro*: KARPAS-299, HL60, CCRF-CEM; Sézary syndrome, T-ALL *in vivo*: KARPS-299	–	(22)
	CD5	αCD5 41BB CD28 CAR	NK-92 cells	*in vitro*: MOLT-4, CCRF-CEM, Jurkat; T-ALL, Sézary syndrome *in vivo*: Jurkat	NCT03081910	(23)
	CD7	αCD7 CD28 41BB CAR	NK-92 cells		NCT02742727	

CAR NK CELLS AGAINST T CELL ANTIGENS

There is an expanding interest in using natural killer cells as source of autologous and allogeneic cellular therapy. NK cells are cytotoxic immune cells which represent our first line of defense against pathogens and malignant cells. NK cells express CD56, CD16, and CD7 but lack TCR, CD3, and CD5 expression (36). Notably, they are able to kill target cells in a non-antigen dependent fashion, do not cause GVHD and are short-lived relative to their T-cell counterparts. All these characteristics make them attractive candidates for genetic engineering with CAR molecules, especially in the context of T cell malignancies. As NK cells do not share as many common T-cell antigens, fratricide would not be expected during cellular manufacturing. Additionally, their short lifespan may prevent long-term T cell aplasia while their lack of a of TCR make them a potential source of allogeneic products.

For these reasons CAR NK cells targeting CD3, CD4, CD5, and CD7 have been explored (**Table 1**). Chen et al. utilized a NK-92 human cell line transduced with a 3rd generation anti-CD3 CAR and demonstrated *in vitro* activity against T-ALL cell lines and primary PTCL samples. Although these NK-92 CAR cells were unable to completely eradicate leukemic cells in a xenograft mouse model, improvements *in vivo* persistence may improve on the short lived nature of CD3CAR NK-92 cells (21). This same approach utilizing the NK-92 cell line for CAR therapy has been demonstrated for CD4 and CD5 positive lymphomas as well as with improvements in both *in vivo* persistence as well as activity in other disease such as T-ALL and PTCL (22, 23). Although NK-92 CARs carry promise, there is a significant safety concern regarding the use of a transformed human NK lymphoma cell line as

an NK cell source. Approaches utilizing irradiated CAR NK-92 cells are being evaluated in clinical trials (NCT03081910, NCT02742727), however any approach meant to limit the persistence and expansion of an immortalized cell line *in vivo* will have similarly detrimental effects on CAR persistence and efficacy.

CONCLUSIONS

The major challenges in the development of adoptive cell therapy for T cell tumors, as mentioned above, remain fratricide, T cell aplasia and the potential for leukemic transduction or poor T cell function if used in the autologous setting. Approaches to overcome fratricide include the genetic modification and deletion of the T cell antigen in the case of long-term CAR-T cell persistence or regulated CAR-T expression. To ensure restoration of T cell immunity, transient CAR expression can be achieved incorporation of a CAR suicide gene, transient CAR expression using mRNA electroporation, or short-lived NK cell lines. Finally, given that these toxicities may be tolerable initially, CAR-T cells followed by an ablative hematopoietic stem cell transplant may allow for hematologic rescue following CAR-T mediated disease clearance. As most of the models to date have utilized normal donor human T cells for CAR manufacturing, we must also consider the underlying fitness of the starting cell product. Peripheral T-cells from patients with underlying T cell malignancy routinely demonstrate impairments in T cell function as well as reduced quantities in peripheral blood, given the extensive prior treatment burden and immune-dysregulation. Approaches utilizing allogeneic donors and gene-editing techniques to remove the endogenous TCR, or CAR products generated from autologous or allogeneic NK cells may offer creative solutions. These include the potential for multiple allogeneic sources, such as peripheral blood, umbilical cord blood, or effector cells generated from induced pluripotent stem cells (iPCSs). Regardless of cell source, target antigen, and the challenges and obstacles each approach may carry, CAR-effector cells as a treatment option for T-cell lymphomas may provide an exciting opportunity for these diseases.

AUTHOR CONTRIBUTIONS

IS wrote and edited the manuscript. MF and MM contributed to manuscript planning and editing.

REFERENCES

1. Wilcox RA. Cutaneous T-cell lymphoma: 2016 update on diagnosis, risk-stratification, and management. *Am J Hematol*. (2016) 91:151–65. doi: 10.1002/ajh.24233
2. Bradford PT, Devesa SS, Anderson WF, Toro JR. Cutaneous lymphoma incidence patterns in the United States: a population-based study of 3884 cases. *Blood*. (2009) 113:5064–73. doi: 10.1182/blood-2008-10-184168
3. Willemze R, Jaffe ES, Burg G, Cerroni L, Berti E, Swerdlow SH, et al. WHO-EORTC classification for cutaneous lymphomas. *Blood*. (2005) 105:3768–85. doi: 10.1182/blood-2004-09-3502
4. Argnani L, Broccoli A, Zinzani PL. Cutaneous T-cell lymphomas: focusing on novel agents in relapsed and refractory disease. *Cancer Treat Rev*. (2017) 61:61–9. doi: 10.1016/j.ctrv.2017.10.007
5. Folkes AS, Feng M, Zain JM, Abdulla F, Rosen ST, Querfeld C. Targeting CD47 as a cancer therapeutic strategy: the cutaneous T-cell lymphoma experience. *Curr Opin Oncol*. (2018) 30:332–37. doi: 10.1097/CCO.0000000000000468
6. Prince HM, Kim YH, Horwitz SM, Dummer R, Scarisbrick J, Quaglino P, et al. Brentuximab vedotin or physician's choice in CD30-positive cutaneous T-cell lymphoma (ALCANZA): an international, open-label, randomised, phase 3, multicentre trial. *Lancet*. (2017) 390:555–66. doi: 10.1016/S0140-6736(17)31266-7
7. Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N Engl J Med*. (2017) 377:2531–44. doi: 10.1056/NEJMoa1707447
8. Schuster SJ, Svoboda J, Chong EA, Nasta SD, Mato AR, Anak Ö, et al. Chimeric antigen receptor T cells in refractory B-cell lymphomas. *N Engl J Med*. (2017) 377:2545–54. doi: 10.1056/NEJMoa1708566
9. Ruella M, Xu J, Barrett DM, Fraietta JA, Reich TJ, Ambrose DE, et al. Induction of resistance to chimeric antigen receptor T cell therapy by transduction of a single leukemic B cell. *Nat Med*. (2018) 24:1499–503. doi: 10.1038/s41591-018-0201-9
10. Pinz K, Liu H, Golightly M, Jares A, Lan F, Zieve GW, et al. Preclinical targeting of human T-cell malignancies using CD4-specific chimeric antigen receptor (CAR)-engineered T cells. *Leukemia*. (2016) 30:701–7. doi: 10.1038/leu.2015.311
11. Mamonkin M, Rouce RH, Tashiro H, Brenner MK. A T-cell-directed chimeric antigen receptor for the selective treatment of T-cell malignancies. *Blood*. (2015) 126:983–992. doi: 10.1182/blood-2015-02-629527
12. Mamonkin M, Mukherjee M, Srinivasan M, Sharma S, Gomes-Silva D, Mo F, et al. Reversible transgene expression reduces fratricide and permits 4-1BB costimulation of CAR T cells directed to T-cell malignancies. *Cancer Immunol Res*. (2018) 6:47–58. doi: 10.1158/2326-6066.CIR-17-0126
13. Gomes-Silva D, Srinivasan M, Sharma S, Lee CM, Wagner DL, Davis TH, et al. CD7-edited T cells expressing a CD7-specific CAR for the therapy of T-cell malignancies. *Blood*. (2017) 130:285–96. doi: 10.1182/blood-2017-01-761320
14. Png YT, Vinanica N, Kamiya T, Shimasaki N, Coustan-Smith E, Campana D. Blockade of CD7 expression in T cells for effective chimeric antigen receptor targeting of T-cell malignancies. *Blood Adv*. (2017) 1:2348–60. doi: 10.1182/bloodadvances.2017009928
15. Cooper ML, Choi J, Staser K, Ritchey JK, Devenport JM, Eckardt K, et al. An "off-the-shelf" fratricide-resistant CAR-T for the treatment of T cell hematologic malignancies. *Leukemia*. (2018) 32:1970–83. doi: 10.1038/s41375-018-0065-5
16. Ramos CA, Ballard B, Zhang H, Dakhova O, Gee AP, Mei Z, et al. Clinical and immunological responses after CD30-specific chimeric antigen receptor-redirected lymphocytes. *J Clin Invest*. (2017) 127:3462–71. doi: 10.1172/JCI94306
17. Scarfo I, Ormhøj M, Frigault MJ, Castano AP, Lorrey S, Bouffard AA, et al. Anti-CD37 chimeric antigen receptor T cells are active against B- and T-cell lymphomas. *Blood*. (2018) 132:1495–506. doi: 10.1182/blood-2018-04-842708
18. Perera LP, Zhang M, Nakagawa M, Petrus MN, Maeda M, Kadin ME, et al. Chimeric antigen receptor modified T cells that target chemokine receptor CCR4 as a therapeutic modality for T-cell malignancies. *Am J Hematol*. (2017) 92:892–901. doi: 10.1002/ajh.24794
19. Maciocia PM, Wawrzyniecka PA, Philip B, Ricciardelli I, Akarca AU, Onuoha SC, et al. Targeting the T cell receptor beta-chain constant region for immunotherapy of T cell malignancies. *Nat Med*. (2017) 23:1416–23. doi: 10.1038/nm.4444
20. Onuoha S, Ferrari M, Bulek A, Bughda R, Manzoor S, Srivastava S, et al. *Structure Guided Engineering of Highly Specific Chimeric Antigen Receptors for the Treatment of T Cell Lymphomas*. ASH Annual Meeting, San Diego, CA (2018).

21. Chen KH, Wada M, Firor AE, Pinz KG, Jares A, Liu H, et al. Novel anti-CD3 chimeric antigen receptor targeting of aggressive T cell malignancies. *Oncotarget*. (2016) 7:56219–32. doi: 10.18632/oncotarget.11019
22. Pinz KG, Yakaboski E, Jares A, Liu H, Firor AE, Chen KH, et al. Targeting T-cell malignancies using anti-CD4 CAR NK-92 cells. *Oncotarget*. (2017) 8:112783–96. doi: 10.18632/oncotarget.22626
23. Chen KH, Wada M, Pinz KG, Liu H, Lin KW, Jares A, et al. Preclinical targeting of aggressive T-cell malignancies using anti-CD5 chimeric antigen receptor. *Leukemia*. (2017) 31:2151–60. doi: 10.1038/leu.2017.8
24. Campana D, van Dongen JJ, Mehta A, Coustan-Smith E, Wolvers-Tettero IL, Ganeshaguru K, et al. Stages of T-cell receptor protein expression in T-cell acute lymphoblastic leukemia. *Blood*. (1991) 77:1546–54.
25. Pui CH, Behm FG, Crist WM. Clinical and biologic relevance of immunologic marker studies in childhood acute lymphoblastic leukemia. *Blood*. (1993) 82:343–62.
26. Kroesen BJ, Bakker A, van Lier RA, The HT, de Leij L. Bispecific antibody-mediated target cell-specific costimulation of resting T cells via CD5 and CD28. *Cancer Res*. (1995) 55:4409–15.
27. Vandenberghe P, Ceuppens JL. Immobilized anti-CD5 together with prolonged activation of protein kinase C induce interleukin 2-dependent T cell growth: evidence for signal transduction through CD5. *Eur J Immunol*. (1991) 21:251–9. doi: 10.1002/eji.1830210203
28. Vandenberghe P, Verwilghen J, Van Vaeck F, Ceuppens JL. Ligation of the CD5 or CD28 molecules on resting human T cells induces expression of the early activation antigen CD69 by a calcium- and tyrosine kinase-dependent mechanism. *Immunology*. (1993) 78:210–7.
29. Lu X, Axtell RC, Collawn JF, Gibson A, Justement LB, Raman C. AP2 adaptor complex-dependent internalization of CD5: differential regulation in T and B cells. *J Immunol*. (2002) 168:5612–20. doi: 10.4049/jimmunol.168.11.5612
30. Murphy K, Weaver C. *Janeway's immunobiology*. New York, NY: Garland Science/Taylor and Francis Group, LLC (2016). p. 904.
31. Gorczyca W. *Atlas of Differential Diagnosis in Neoplastic Hematopathology*. (2014). CRC Press; Taylor & Francis Group.
32. Falini B, Pileri S, Pizzolo G, Dürkop H, Flenghi L, Stirpe F, et al. CD30 (Ki-1) molecule: a new cytokine receptor of the tumor necrosis factor receptor superfamily as a tool for diagnosis and immunotherapy. *Blood*. (1995) 85:1–14.
33. Zheng W, Medeiros LJ, Young KH, Goswami M, Powers L, Kantarjian HH, et al. CD30 expression in acute lymphoblastic leukemia as assessed by flow cytometry analysis. *Leuk Lymphoma*. (2014) 55:624–7. doi: 10.3109/10428194.2013.820293
34. Kim YH, Tavallaee M, Sundram U, Salva KA, Wood GS, Li S, et al. Phase II investigator-initiated study of brentuximab vedotin in mycosis fungoides and sezary syndrome with variable CD30 expression level: a multi-institution collaborative project. *J Clin Oncol*. (2015) 33:3750–8. doi: 10.1200/JCO.2014.60.3969
35. Duvic M, Tetzlaff MT, Gangar P, Clos AL, Sui D, Talpur R. Results of a phase II trial of brentuximab vedotin for CD30+ cutaneous T-cell lymphoma and lymphomatoid papulosis. *J Clin Oncol*. (2015) 33:3759–65. doi: 10.1200/JCO.2014.60.3787
36. Daher M, Rezvani K. Next generation natural killer cells for cancer immunotherapy: the promise of genetic engineering. *Curr Opin Immunol*. (2018) 51:146–53. doi: 10.1016/j.coi.2018.03.013

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

PERSPECTIVE
published: 18 April 2019
doi: 10.3389/fonc.2019.00300

Environmental and Other Extrinsic Risk Factors Contributing to the Pathogenesis of Cutaneous T Cell Lymphoma (CTCL)

Feras M. Ghazawi[1], *Nebras Alghazawi*[2], *Michelle Le*[2], *Elena Netchiporouk*[2], *Steven J. Glassman*[1], *Denis Sasseville*[2] *and Ivan V. Litvinov*[2]*

[1] Division of Dermatology, University of Ottawa, Ottawa, ON, Canada, [2] Division of Dermatology, McGill University, Montréal, QC, Canada

OPEN ACCESS

Edited by:
Catherine Grace Chung,
The Ohio State University,
United States

Reviewed by:
Himanshi Bhatia,
Washington University in St. Louis,
United States
Vemika Chandra,
Children's Hospital of Philadelphia,
United States

***Correspondence:**
Ivan V. Litvinov
ivan.litvinov@mcgill.ca

Specialty section:
This article was submitted to Hematologic Malignancies, a section of the journal Frontiers in Oncology

Received: 23 January 2019
Accepted: 01 April 2019
Published: 18 April 2019

Citation:
Ghazawi FM, Alghazawi N, Le M, Netchiporouk E, Glassman SJ, Sasseville D and Litvinov IV (2019) Environmental and Other Extrinsic Risk Factors Contributing to the Pathogenesis of Cutaneous T Cell Lymphoma (CTCL). Front. Oncol. 9:300. doi: 10.3389/fonc.2019.00300

The applications of disease cluster investigations in medicine have developed rather rapidly in recent decades. Analyzing the epidemiology of non-random aggregation of patients with a particular disease fostered identification of environmental and external exposures as disease triggers and promoters. Observation of patient clusters and their association with nearby exposures, such as Dr. John Snow's astute mapping analysis in the mid-1800's, which revealed proximity of cholera patients in London to a contaminated water pump infected with *Vibrio cholerae*, have paved the way for the field of epidemiology. This approach enabled the identification of triggers for many human diseases including infections and cancers. Cutaneous T-cell lymphomas (CTCL) represent a group of non-Hodgkin lymphomas that primarily affect the skin. The detailed pathogenesis by which CTCL develops remains largely unknown. Notably, non-random clustering of CTCL patients was reported in several areas worldwide and this rare malignancy was also described to affect multiple members of the same family. These observations indicate that external factors are possibly implicated in promoting CTCL lymphomagenesis. Here, we review the epidemiology of CTCL worldwide and the clinical characteristics of CTCL patients, as revealed by global epidemiological data. Further, we review the known risk factors including sex, age, race as well as environmental, infectious, iatrogenic and other exposures, that are implicated in CTCL lymphomagenesis and discuss conceivable mechanisms by which these factors may trigger this malignancy.

Keywords: cutaneous T-cell lymphoma, CTCL, epidemiology, incidence, environmental risk factors

CUTANEOUS T-CELL LYMPHOMA

Cutaneous T-cell lymphomas (CTCL) are a class of non-Hodgkin lymphomas. CTCL represents a heterogeneous group of lymphoproliferative disorders that are characterized by the infiltration of activated bystander and malignant CLA^+ $CCR4^+$ T cells into the skin (1, 2). Patients with CTCL, depending on the disease subtype, can present with a spectrum of clinical morphologies including erythematous, hyper- or hypo-pigmented patches with or without atrophy, or present with thickened plaques, that may mimic benign, inflammatory, or autoimmune disorders such as

eczema, psoriasis, morphea, pityriasis lichenoides chronica, pityriasis rubra pilaris, drug eruptions, poikiloderma, panniculitis, vitiligo, and pigmented purpuric dermatoses. Thus, CTCL is considered a "great mimicker" in dermatology. In fact, CTCL is often challenging to diagnose, especially in early and erythrodermic stages, and on average it takes ∼6 years to establish a definitive diagnosis since its initial presentation (3). As the malignancy progresses in a subset of patients, the disease can spread to lymph nodes and other organs. While mycosis fungoides (MF) and Sézary syndrome (SS) are the most commonly recognized subtypes, other variants of CTCL were documented by the 2016 World Health Organization classification of primary cutaneous lymphomas (4) and include angioimmunoblastic T-cell lymphoma, subcutaneous panniculitis-like T-cell lymphoma, adult T-cell leukemia/lymphoma (ATLL), mature T-cell lymphoma not otherwise specified (NOS), cluster of differentiation 30 positive ($CD30^+$) T-cell lymphoproliferative disorders of the skin and extranodal natural killer (NK)/T-cell lymphoma, nasal type (ENKL). Despite the aforementioned variability in clinical presentation, the majority of MF lesions develop on the trunk and lower extremities often asymmetrically and follow a "1930's bathing suit" distribution on skin not commonly exposed to the sun.

DEMOGRAPHICS AND CLINICAL CHARACTERISTICS OF CTCL PATIENTS

While CTCL has been shown to affect individuals of almost all ethnicities and age groups in both sexes, extensive epidemiological studies have consistently reported that CTCL preferentially affects patients of "typical" profiles and in classic body sites. Indeed, the mean age at the time of diagnosis is in the mid- to late-fifties. The mean age of diagnosis was reported in Canada as 59.4 years (5) and the median age of diagnosis in the United States, United Kingdom and Switzerland is in the range of 54–57.5 years, with marked differences between different ethnicities (6–9). In addition, more males are diagnosed with CTCL than females. In fact, the reported incidence-rate-ratio (IRR) of males-to-female patients in the United States during 2001–2005 and 2005–2009 years were 1.7:1 and 1.6:1, respectively (10, 11). The IRR of males-to-females in Canada during 1992–2010 was 1.4:1 (5). There are also important differences in CTCL incidence and prognosis between different ethnic backgrounds. The average age of CTCL diagnosis in African-Americans is significantly lower compared to Caucasians (6–9, 12). In one study that examined the clinical characteristics of 4,496 patients diagnosed with cutaneous lymphoma between 2004 and 2008 in the United States, the mean age at the time of diagnosis for Caucasian, African-American Asian/Pacific Islander, and Native American/other/unknown patients were 59.2, 51.5, 51.3, and 53.8 years, respectively (9). In fact, CTCL was reported to occur at a significantly higher rate in individuals of African-American descent, when compared to individuals of European or Asian origin. In a population-based study in the United States, the age-adjusted incidence rates of CTCL in 8 states from 2001 to 2005 were the highest among African-Americans (10.0/1,000,000 person-years), followed by non-Hispanic whites (8.1), Hispanic whites (5.1), and Asian/Pacific Islander (5.1) (10). Further, in these individuals, the disease was often associated with worse prognosis (9, 12).

In terms of subtype prevalence, MF, SS, $CD30^+$ lymphoproliferative disorders and primary cutaneous peripheral T cell lymphomas not otherwise specified (PCTCL-NOS) are the most common and well-recognized variants of CTCL. Indeed, MF is the predominant subtype of CTCL and is corroborated by several studies showing that it accounts for 44–60% of all CTCL cases (1, 8).

OVERALL INCIDENCE PATTERNS FOR CTCL

The incidence of CTCL worldwide has demonstrated a 2 to 3-fold increase during the last 25–30 years (9, 13), and ranged between ∼5 and 11 cases per million individuals per year depending on the CTCL subtypes and the examined year range. In the United States, incidence rates of CTCL were on the rise from 1973 until 1998 and stabilized at 10.2 cases per million individuals per year (11). In Canada, we recently reported that the incidence rate of CTCL increased steadily during the 1990s and then stabilized at ∼11 cases per million individuals per year, demonstrating geographical continuity between the two North American countries (5). The incidence rates of CTCL in other parts of the world including Norway, Wales, France, Kuwait, and Japan are summarized in **Table 1**.

CLUSTERING OF CTCL PATIENTS

Despite the rarity of this malignancy, clustering of CTCL patients was reported in several regions around the world including the Västernorrland county of Sweden (21) as well as in Pittsburgh, Pennsylvania (22) and in Texas (23) in the United States. Based on the analysis of 1990 patients using two cancer registries (MD Anderson Cancer Center and Texas Cancer Registry), we previously reported geographic clustering of patients in several communities in the state of Texas including in cities of Katy, Beaumont, and Spring, where CTCL incidence rates were 3–20 times higher than the US national average (23, 24). Notably clustering of patients was observed in our recent study in Canada in the areas of heavy industrial presence (e.g., in Winnipeg, MB, Hamilton and Oakville, ON, etc.), while cities with limited industrial presence including Ottawa, Gatineau and Quebec City were relatively spared by CTCL (5). Similarly, Moreau et al. demonstrated CTCL case clustering in certain location of the Pittsburgh metropolitan area (22). Further, familial aggregation of this rare malignancy were reported among Jewish nationals in Israel (25). The non-random distribution of CTCL patients argues that external environmental, occupational, behavioral, and communicable triggers may indeed exist for this malignancy.

TABLE 1 | Reported CTCL incidence rates worldwide (rates are denoted as cases per 1 million individuals per year).

Country	Incidence of CTCL	Incidence of CTCL subtypes	Study year range	Data source	References
USA	2.8–10.5		1973–2009	SEER (6,230 patients from Los Angeles, California; San Jose, California; Alaska and rural Georgia)	(11)
USA		*MF* = 5.5 *SS* = 0.1	2005–2008	SEER program (17 data sets representing ∼10% of the US population: Connecticut, Hawaii, Iowa, New Mexico, Utah, Detroit, San Francisco/Oakland, Seattle/Puget Sound and Atlanta)	(14)
USA	6.4		1973–2002	SEER; 13 registries covering ∼14% of the US population (Los Angeles and San Jose, California; Alaska; and rural Georgia).	(13)
USA	7.7	*MF* = 4.1 *SS* = 0.1 CD30 + LPD = 1.1 *SPTC* = 0.1 *pcPTL* = 2.2	2001–2005	16 SEER registries representing ∼26% of the US population (Connecticut, Hawaii, Iowa, Kentucky, Louisiana, New Jersey, New Mexico, and Utah), greater California, rural Georgia, and 6 metropolitan areas (Atlanta, Detroit, Los Angeles, San Francisco–Oakland, San Jose–Monterrey, and Seattle–Puget Sound)	(10)
USA		MF 1973–1974 = 1.8 1975–1976 = 2.3 1977–1978 = 2.8 1979–1980 = 2.8 1981–1982 = 4.1 1983–1984 = 4.8 1985–1986 = 4.3 1987–1988 = 4.2 1989–1990 = 4.9 1991–1992 = 4.5	1973–92	SEER (specific states not mentioned)	(15)
Canada	11.3		1992–2010	Canadian Cancer Registry and Le Registre Québécois du Cancer	(5)
Norway	1.6 (1980–84) 2.9 (2000–03)	*MF/SS* = 1.5–1.8	1980–2003	Cancer Registry of Norway on non-Hodgkin lymphomas	(16)
France	2.0–5.7	*MF* = 1.3–2.5	1980–2003	Doubs cancer registry (France)	(17)
Kuwait		*MF* = 4.3	1991–2006	National Dermatology Department (193 patients)	(18)
Wales	4.8		2003–2011	All Wales Lymphoma Panel (120 Patients)	(19)
Japan		*MF/SS* = 1.0–1.5	2008–2015	National Cutaneous Lymphoma Registry (391 patients)	(20)

SEER, Surveillance, Epidemiology, and End Results; MF, mycosis fungoides; SS, Sézary syndrome; CD30$^+$ LPD, CD30$^+$ T-cell lymphoproliferative disorders of the skin; SPTCL, Subcutaneous panniculitis-like T-cell lymphoma; pcPTL, Primary cutaneous peripheral T-cell lymphoma; AITCL, Angioimmunoblastic T-cell lymphoma; MTCL-NOS, Mature T-cell lymphoma NOS; CD30$^+$ALCL, CD30-positive anaplastic large-cell lymphoma; ENKTL-NT, Extranodal NK/T-cell lymphoma, nasal type; ATCLL, Adult T-cell leukemia/lymphoma; EBVANK/TCL, Epstein-Barr virus-associated natural-killer/T-cell lymphoma.

ENVIRONMENTAL AND EXTERNAL RISK FACTORS OF CTCL

Analysis of the epidemiology and spatial distribution of diseases enabled the identification of occupational, communicable and environmental exposures as initiators/promoters of many malignancies, such as arsenic triggering cutaneous squamous cell carcinomas, benzene exposure contributing to the carcinogenesis of Acute Myelogenous Leukemia, and asbestos accounting for the majority of mesothelioma cases, reviewed in Ghazawi et al. (26, 27).

A number of external triggers/disease promoting factors such as hydrochlorothiazide diuretics, immunosuppression, as well as several putative bacterial and viral agents have been proposed for CTCL (28) and are summarized in **Supplementary Table 1**. Early evidence shows that air pollution and chemical exposure, including pesticides (e.g., Glyphosate in Roundup®, Monsanto Inc.) and detergents may increase one's risk of developing MF, SS, and other Non-Hodgkin Lymphomas (29, 30). Indeed, as mentioned above, our previous analysis on the distribution of CTCL patient clusters in Texas revealed that many of the patients were residing along the same streets/highways and/or rivers/streams. Similarly, analysis of CTCL distribution by postal codes in Canada identified several regions with elevated CTCL incidence rates that were located in the proximity to heavy industrial factories and major transportation hubs (5, 31). The same trend was observed by Moreau et al. in Pittsburgh, PA area (22). These combined results argue that common exposures may be promoting this cancer in certain communities. Furthermore, our study in Texas demonstrated that two densely populated

adjacent zip codes located in a sunny desert climate near El Paso, Texas were completely spared by CTCL (23, 24).

In other population-based studies in the United States, low household income, types of owner-occupied housing units and foreign birth (although, the countries of birth were not detailed in the cited study) were correlated with increased incidence of CTCL (11). Incidence of CTCL has also been correlated with high physician density (correlation coefficient (r) = 0.6; $p = 0.04$) and high family income ($r = 0.7$; $p = 0.01$) (13). In addition, body mass index, tobacco use, personal history of eczema, family history of multiple myeloma, crop, and vegetable farming activities, painting, woodworking and carpentering occupations have all been linked to an increased risk of MF and SS. Alcohol use and sun exposure were also reported as exacerbating and protective lifestyle risk factors for MF, respectively (32). Regarding sun exposure being a protective factor, one plausible hypothesis is centered on low vitamin D levels in CTCL patients. A study by Talpur et al. reported that low vitamin D levels were present in 76.9% of the MF/SS patients, comparable to the levels in other cancer patients (75.2%) (33).

As mentioned earlier, iatrogenic immunosuppression with conventional systemic or newer biologic (i.e., anti TNF-α) therapies increases ones likelihood of developing MF/SS and other lymphomas (28). The use of hydrochlorothiazide was also evaluated in MF and SS patients with hypertension and was found to be a possible trigger of disease in a subset of patients with early MF (34). Although not proving causality, hydrochlorothiazide use has been linked to increased severity in MF and SS cases. The discontinuation of hydrochlorothiazide in these patients has led to the clearing or amelioration of their MF; when re-challenged with this medication, a subset of these patients had a reoccurrence of their MF lesions (34). Other medications that were proposed as possible triggers for MF include antihistamines, antiepileptics, antihypertensives, and serotonin reuptake inhibitors (28).

Familial clustering studies showed an increased incidence of CTCL by analyzing the allele frequency of HLA DQB1*03 in first-degree relatives (25). Furthermore, a few cases have reported that organ transplant recipients (albeit these patients are on immunosuppressive drugs) (35) and patients with HIV-related immunodeficiency had an increased risk of developing CTCL (36).

Based on current literature, infections may play more than one role in natural CTCL disease course. Specifically, some infections were proposed to trigger/promote the disease. At the same time, as the malignancy progresses to more advanced stages the host becomes susceptible to an increasing number of infections that ultimately lead to a demise of a patient. Several studies reported a significant incidence of skin infections in CTCL patients with an association between the pathogenic burden and disease severity (37, 38). *Staphylococcus aureus, Mycobacterium leprae, Chlamydophila pneumoniae,* and dermatophytes are among some of the infectious agents implicated as triggers/promoters of CTCL. Moreover, certain viruses investigated for their role in triggering CTCL include *Human T-cell leukemia/lymphotropic virus type 1 (HTLV-1),* which was consistently associated with Adult T-Cell Lymphoma (39), but not MF/SS (40–46). Further, *HTLV-2* (41, 47), *HIV* (36, 48, 49), *Epstein-Barr virus* (50–61), *Cytomegalovirus* (62, 63), *Human Herpesvirus (HHV)-6*, *HHV-7* (57, 64–66), *HHV-8* (67) and even *Polyomaviruses* including *Merkel cell polyomavirus (MCV)* (68–70) were also proposed to play an important role in disease pathogenesis. However, some of these studies have yielded conflicting results, as highlighted by Mirvish et al. (71), and ultimately failed to report a clear explanation for CTCL pathogenesis (71).

HOW COULD EXTERNAL FACTORS PROMOTE OR TRIGGER CTCL?

While the precise triggers are not yet identified/confirmed, and the mechanism of lymphomagenesis remains enigmatic, several studies have investigated a number of different hypotheses (72). Chromosomal instability as well as dysregulated expression of many genes such as cancer testis and meiomitosis genes, Suppressor of cytokine signaling 3 (SOCS3), B-Raf proto-oncogene, serine/threonine kinase (BRAF), Interleukin-2 receptor common gamma chain, Thymocyte selection-associated high-mobility group box (TOX), among others [reviewed in (72, 73)] were reported in CTCL patients. Aberrant expression of SOCS3, a regulator of the Jak-3/STAT disrupts the normal expression of several cytokines including IL-5, IL-10, IL-17A, and IL-17F and tumor suppressor microRNAs such as miR-22 further highlighting the important role of the cytokine milieu in disease pathogenesis (74). As disease progresses, an important switch from a Th1 to Th2 profile immune response is observed in patients with subsequent eosinophilia and superinfections with *S. aureus* (75, 76). On the other hand, a recent study by Fanok et al. demonstrated that T-cell receptor engagement is critical for malignant transformation of T lymphocytes in the setting of presumed bacterial trigger (77). *S. aureus,* being a common pathogen residing on the skin, is thought to promote malignant inflammation. Lack of Th1 immune response is a driving factor for the growth of *S. aureus* on the CTCL skin. Following this event, a Th2 immune-mediated response is precipitated by the downregulation of STAT4 and upregulation of STAT5 and STAT3 by oncomiRs (miR-155) making CTCL patients more susceptible to *S. aureus* colonization and prolonged antigenic stimulation (75, 76, 78, 79).

Further, a recent study described the mechanism by which *S. aureus* activates oncogenic STAT3 signaling in malignant T cells (80). Staphylococcal enterotoxin A (SEA) was shown to impact malignant T cells in an indirect mechanism by promoting infiltrating bystander non-malignant T cells to produce IL-2 and other regulatory cytokines, thus upregulating JAK3/STAT3 signaling and leading to proliferation of malignant T cells in the skin (28, 80). Finally, Staphylococcal toxins have been implicated in the activation of malignant T cells in SS patients by acting as superantigens and binding to a TCR-v β chain, leading to T cell activation and proliferation (81–83). Collectively, it is likely that patients' genetic profiles and skewed cytokine milieu in response to select infectious agents may predispose individuals to develop MF/SS.

Also, central to CTCL pathogenesis remains chronic and persistent antigen stimulation of skin-homing $CD4^+$ memory T-cells by external factors. The resultant chronic inflammation drives the immune system toward the proliferation and expansion of specific T cell malignant clone(s) (84). This was reinforced by a collection of different observations. For instance, it was reported that one patient, who had implanted gravel in the hip from a traumatic injury developed a chronic plaque, which histologically appeared as parapsoriasis and progressed in 15 years to MF (85). The fact that, after about 10 years on average, some patients developed MF at the sites previously inoculated with foreign substances also supports the notion that CTCL may develop after chronic antigen stimulation (86). Consistent with these observations, it is well-established that implant associated-Anaplastic Lage Cell Lymphoma (ALCL), an indolent disease similar to primary cutaneous ALCL, can arise as a result of breast implants, tibial implants, dental implants, chest injection ports, gluteal implants, shoulder repairs, and a gastric band placement (87). It is now standard procedure to include a discussion of ALCL risk before any breast implant procedures and inform patients of a possible onset of ALCL within a median time frame of 8 to 10 years, albeit this disease affects 1 in 2,000 to 1 in 70,000 implant recipients (87). Notably, textured prostheses have higher risk of ALCL then smooth implants (87).

The reviewed implicated factors in **Supplementary Table 1** clearly indicate that more than one antigen may stimulate CTCL. Additional evidence of this comes from studies that established a widespread repertoire of the stimulated CTCL clones (or single-cell heterogeneity in Sézary Syndrome) between different patients (88). Therefore, particular antigen engagement, coupled with the combination of aberrant cytokine milieu and chronicity of antigenic-stimulation, may collectively play critical roles in the development of cutaneous lymphoma.

CONCLUSIONS

Epidemiological studies have repeatedly helped identify definitive triggers for several diseases. As highlighted in this perspective report, previous studies strongly argue for the interplay between intrinsic factors and putative preventable extrinsic triggers/promoters for CTCL. Given the evidence of geographical regional clustering of CTCL patients, CTCL occurrence in unrelated family members and recent evidence implicating *S. aureus* in the pathogenesis/progression of CTCL, more research is needed to decipher the precise mechanism by which specific environmental exposures may be driving the pathogenesis of this malignancy. Hopefully, such knowledge of potential triggers and perpetuating factors for this cancer would enable us at some point to significantly decrease CTCL incidence. Therefore, it is important to take into consideration the effects of hydrochlorothiazide diuretics and immunosuppressive medications in patients with definite or suspected diagnosis of CTCL (e.g., recalcitrant eczematous patches/plaques that appear in a bathing-suit distribution) (89). Patients living near heavy industrial factories may consider an air filtration system, if feasible, to decrease their risk of developing a malignancy. Minimizing daily exposure to pesticides/herbicides containing chemicals that are listed as probable or possible (Groups 1-2B) carcinogens on the International Agency for Research on Cancer (IARC) database, such as glyphosate, is also important. Any traumatically implanted in the skin or subcutaneous tissue foreign objects (especially textured objects) should be surgically removed. Furthermore, given our further understanding of *S. aureus* in the pathogenesis of CTCL progression, clinicians may consider decolonizing patients using various techniques already used for patients with atopic dermatitis such as bleach baths as well as topical and systemic antibiotics. Similarly, patients with detectable dermatophytes may similarly benefit from anti-fungal treatments to avoid developing CTCL.

AUTHOR CONTRIBUTIONS

FG, NA, ML, EN, SG, DS, and IL have contributed by participating in reviewing the literature on the topic, preparing the tables summarizing existing evidence, writing and critically reviewing the manuscript.

FUNDING

This work was supported by the Cole Foundation Grant to Dr. Litvinov, Canadian Dermatology Foundation research grants to Dr. Sasseville and Dr. Litvinov and the Fonds de la recherche en santé du Québec (FRSQ# 34753 and 36769) research grants to Dr. Litvinov. No funding bodies had any role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2019.00300/full#supplementary-material

REFERENCES

1. Willemze R, Jaffe ES, Burg G, Cerroni L, Berti E, Swerdlow SH, et al. WHO-EORTC classification for cutaneous lymphomas. *Blood.* (2005) 105:3768–85. doi: 10.1182/blood-2004-09-3502
2. Campbell JJ, Clark RA, Watanabe R, Kupper TS. Sezary syndrome and mycosis fungoides arise from distinct T-cell subsets: a biologic rationale for their distinct clinical behaviors. *Blood.* (2010) 116:767–71. doi: 10.1182/blood-2009-11-251926
3. Kirsch IR, Watanabe R, O'malley JT, Williamson DW, Scott LL, Elco CP, et al. TCR sequencing facilitates diagnosis and identifies mature T cells as the cell of origin in CTCL. *Sci Transl Med.* (2015) 7:308ra158. doi: 10.1126/scitranslmed.aaa9122
4. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood.* (2016) 127:2375–90. doi: 10.1182/blood-2016-01-643569
5. Ghazawi FM, Netchiporouk E, Rahme E, Tsang M, Moreau L, Glassman S, et al. Comprehensive analysis of cutaneous T-cell lymphoma (CTCL)

incidence and mortality in Canada reveals changing trends and geographic clustering for this malignancy. *Cancer.* (2017) 123:3550–67. doi: 10.1002/cncr.30758
6. Kim YH, Liu HL, Mraz-Gernhard S, Varghese A, Hoppe RT. Long-term outcome of 525 patients with mycosis fungoides and Sezary syndrome: clinical prognostic factors and risk for disease progression. *Arch Dermatol.* (2003) 139:857–66. doi: 10.1001/archderm.139.7.857
7. Agar NS, Wedgeworth E, Crichton S, Mitchell TJ, Cox M, Ferreira S, et al. Survival outcomes and prognostic factors in mycosis fungoides/Sezary syndrome: validation of the revised International Society for Cutaneous Lymphomas/European Organisation for Research and Treatment of Cancer staging proposal. *J Clin Oncol.* (2010) 28:4730–9. doi: 10.1200/JCO.2009.27.7665
8. Jenni D, Karpova MB, Seifert B, Golling P, Cozzio A, Kempf W, et al. Primary cutaneous lymphoma: two-decade comparison in a population of 263 cases from a Swiss tertiary referral centre. *Br J Dermatol.* (2011) 164:1071–7. doi: 10.1111/j.1365-2133.2010.10143.x
9. Wilson LD, Hinds GA, Yu JB. Age, race, sex, stage, and incidence of cutaneous lymphoma. *Clin Lymphoma Myeloma Leuk.* (2012) 12:291–6. doi: 10.1016/j.clml.2012.06.010
10. Bradford PT, Devesa SS, Anderson WF, Toro JR. Cutaneous lymphoma incidence patterns in the United States: a population-based study of 3884 cases. *Blood.* (2009) 113:5064–73. doi: 10.1182/blood-2008-10-184168
11. Korgavkar K, Xiong M, Weinstock M. Changing incidence trends of cutaneous T-cell lymphoma. *JAMA Dermatol.* (2013) 149:1295–9. doi: 10.1001/jamadermatol.2013.5526
12. Sun G, Berthelot C, Li Y, Glass DA 2nd, George D, Pandya A, Kurzrock R, et al. Poor prognosis in non-Caucasian patients with early-onset mycosis fungoides. *J Am Acad Dermatol.* (2009) 60:231–5. doi: 10.1016/j.jaad.2008.09.063
13. Criscione VD, Weinstock MA. Incidence of cutaneous T-cell lymphoma in the United States, 1973-2002. *Arch Dermatol.* (2007) 143:854–9. doi: 10.1001/archderm.143.7.854
14. Imam MH, Shenoy PJ, Flowers CR, Phillips A, Lechowicz MJ. Incidence and survival patterns of cutaneous T-cell lymphomas in the United States. *Leuk Lymphoma.* (2013) 54:752–9. doi: 10.3109/10428194.2012.729831
15. Weinstock MA, Gardstein B. Twenty-year trends in the reported incidence of mycosis fungoides and associated mortality. *Am J Public Health.* (1999) 89:1240–4. doi: 10.2105/AJPH.89.8.1240
16. Saunes M, Nilsen TI, Johannesen TB. Incidence of primary cutaneous T-cell lymphoma in Norway. *Br J Dermatol.* (2009) 160:376–9. doi: 10.1111/j.1365-2133.2008.08852.x
17. Riou-Gotta MO, Fournier E, Mermet I, Pelletier F, Humbert P, Danzon A, et al. Primary cutaneous lymphomas: a population-based descriptive study of 71 consecutive cases diagnosed between 1980 and 2003. *Leuk Lymphoma.* (2008) 49:1537–44. doi: 10.1080/10428190802136368
18. Alsaleh QA, Nanda A, Al-Ajmi H, Al-Sabah H, Elkashlan M, Al-Shemmari S, et al. Clinicoepidemiological features of mycosis fungoides in Kuwait, 1991-2006. *Int J Dermatol.* (2010) 49:1393–8. doi: 10.1111/j.1365-4632.2010.04567.x
19. Abbott RA, Aldridge C, Dojcinov S, Piguet V. Incidence of primary cutaneous T-cell lymphoma in wales. *Br J Dermatol.* (2013) 169:1366–7. doi: 10.1111/bjd.12516
20. Hamada T, Nomura H, Iwatsuki K. Regional incidences of adult T-cell leukemia/lymphoma with cutaneous involvement in Japan. *J Dermatol.* (2018) 45:58–63. doi: 10.1111/1346-8138.14100
21. Gip L, Nilsson E. Clustering of mycosis fungoides in the County of Vasternorrland. *Lakartidningen.* (1977) 74:1174–6.
22. Moreau JF, Buchanich JM, Geskin JZ, Akilov OE, Geskin LJ. Non-random geographic distribution of patients with cutaneous T-cell lymphoma in the greater pittsburgh area. *Dermatol. Online J.* (2014) 20. Retrieved from: https://escholarship.org/uc/item/4nw7592w
23. Litvinov IV, Tetzlaff MT, Rahme E, Habel Y, Risser DR, Gangar P, et al. Identification of geographic clustering and regions spared by cutaneous T-cell lymphoma in Texas using 2 distinct cancer registries. *Cancer.* (2015) 121:1993–2003. doi: 10.1002/cncr.29301
24. Litvinov IV, Tetzlaff MT, Rahme E, Jennings MA, Risser DR, Gangar P, et al. Demographic patterns of cutaneous T-cell lymphoma incidence in Texas based on two different cancer registries. *Cancer Med.* (2015) 4:1440–7. doi: 10.1002/cam4.472
25. Hodak E, Klein T, Gabay B, Ben-Amitai D, Bergman R, Gdalevich M, et al. Familial mycosis fungoides: report of 6 kindreds and a study of the HLA system. *J Am Acad Dermatol.* (2005) 52:393–402. doi: 10.1016/j.jaad.2003.12.052
26. Ghazawi FM, Glassman SJ, Sasseville D, Litvinov IV. Using patient registries to identify triggers of rare diseases. In Heston TF, editor. *eHealth - Making Health Care Smarter*. IntechOpen (2018). Available online at: https://www.intechopen.com/books/ehealth-making-health-care-smarter/using-patient-registries-to-identify-triggers-of-rare-diseases
27. Ghazawi FM, Le M, Cyr J, Netchiporouk E, Rahme E, Alakel A, et al. Analysis of acute myeloid leukemia incidence and geographic distribution in Canada from 1992 to 2010 reveals disease clusters in Sarnia and other industrial US border cities in Ontario. *Cancer.* (2019). doi: 10.1002/cncr.32034. [Epub ahead of print].
28. Litvinov IV, Shtreis A, Kobayashi K, Glassman S, Tsang M, Woetmann A, et al. Investigating potential exogenous tumor initiating and promoting factors for cutaneous T-cell lymphomas (CTCL), a rare skin malignancy. *Oncoimmunology.* (2016) 5:e1175799. doi: 10.1080/2162402X.2016.1175799
29. Fischmann AB, Bunn PA Jr., Guccion JG, Matthews MJ, Minna JD. Exposure to chemicals, physical agents, and biologic agents in mycosis fungoides and the Sezary syndrome. *Cancer Treat Rep.* (1979) 63:591–6.
30. Chang ET, Delzell E. Systematic review and meta-analysis of glyphosate exposure and risk of lymphohematopoietic cancers. *J Environ Sci Health B.* (2016) 51:402–34. doi: 10.1080/03601234.2016.1142748
31. Ghazawi FM, Netchiporouk E, Rahme E, Tsang M, Moreau L, Glassman S, et al. Distribution and clustering of cutaneous T-cell lymphoma (CTCL) cases in Canada during 1992 to 2010. *J Cutan Med Surg.* (2018) 22:154–65. doi: 10.1177/1203475417745825
32. Morales Suarez-Varela MM, Olsen J, Kaerlev L, Guenel P, Arveux P, Wingren G, et al. Are alcohol intake and smoking associated with mycosis fungoides? A European multicentre case-control study. *Eur J Cancer.* (2001) 37:392–7. doi: 10.1016/S0959-8049(00)00383-X
33. Talpur R, Cox KM, Hu M, Geddes ER, Parker MK, Yang BY, et al. Vitamin D deficiency in mycosis fungoides and Sezary syndrome patients is similar to other cancer patients. *Clin Lymphoma Myeloma Leuk.* (2014) 14:518–24. doi: 10.1016/j.clml.2014.06.023
34. Jahan-Tigh RR, Huen AO, Lee GL, Pozadzides JV, Liu P, Duvic M. Hydrochlorothiazide and cutaneous T cell lymphoma: prospective analysis and case series. *Cancer.* (2013) 119:825–31. doi: 10.1002/cncr.27740
35. Pomerantz RG, Campbell LS, Jukic DM, Geskin LJ. Posttransplant cutaneous T-cell lymphoma: case reports and review of the association of calcineurin inhibitor use with posttransplant lymphoproliferative disease risk. *Arch Dermatol.* (2010) 146:513–6. doi: 10.1001/archdermatol.2010.60
36. Biggar RJ, Engels EA, Frisch M, Goedert JJ. Risk of T-cell lymphomas in persons with AIDS. *J Acquir Immune Defic Syndr.* (2001) 26:371–6. doi: 10.1097/00126334-200104010-00015
37. Axelrod PI, Lorber B, Vonderheid EC. Infections complicating mycosis fungoides and Sezary syndrome. *JAMA.* (1992) 267:1354–8. doi: 10.1001/jama.1992.03480100060031
38. Bonin S, Tothova SM, Barbazza R, Brunetti D, Stanta G, Trevisan G. Evidence of multiple infectious agents in mycosis fungoides lesions. *Exp Mol Pathol.* (2010) 89:46–50. doi: 10.1016/j.yexmp.2010.05.001
39. Amar L, Le M, Ghazawi FM, Rahme E, Segal A, Netchiporouk E, et al. Human T-Cell Lymphotropic Virus-1 (HTLV-1) infection prevalence in Canada. *Curr Oncol.* (2019) 26:1–3. doi: 10.3747/co.26.4593
40. Hall WW, Liu CR, Schneewind O, Takahashi H, Kaplan MH, Roupe G, et al. Deleted HTLV-I provirus in blood and cutaneous lesions of patients with mycosis fungoides. *Science.* (1991) 253:317–20. doi: 10.1126/science.1857968
41. Zucker-Franklin D, Hooper WC, Evatt BL. Human lymphotropic retroviruses associated with mycosis fungoides: evidence that human T-cell lymphotropic virus type II (HTLV-II) as well as HTLV-I may play a role in the disease. *Blood.* (1992) 80:1537–45.
42. Pancake BA, Zucker-Franklin D, Coutavas EE. The cutaneous T cell lymphoma, mycosis fungoides, is a human T cell lymphotropic

virus-associated disease. A study of 50 patients. *J Clin Invest.* (1995) 95:547–54. doi: 10.1172/JCI117697
43. Khan ZM, Sebenik M, Zucker-Franklin D. Localization of human T-cell lymphotropic virus-1 tax proviral sequences in skin biopsies of patients with mycosis fungoides by *in situ* polymerase chain reaction. *J Invest Dermatol.* (1996) 106:667–72. doi: 10.1111/1523-1747.ep12345488
44. Pancake BA, Wassef EH, Zucker-Franklin D. Demonstration of antibodies to human T-cell lymphotropic virus-I tax in patients with the cutaneous T-cell lymphoma, mycosis fungoides, who are seronegative for antibodies to the structural proteins of the virus. *Blood.* (1996) 88:3004–9.
45. Shohat M, Hodak E, Hannig H, Bodemer W, David M, Shohat B. Evidence for the cofactor role of human T-cell lymphotropic virus type 1 in mycosis fungoides and Sezary syndrome. *Br J Dermatol.* (1999) 141:44–9. doi: 10.1046/j.1365-2133.1999.02919.x
46. Netchiporouk E, Gantchev J, Tsang M, Thibault P, Watters AK, Hughes JM, et al. Analysis of CTCL cell lines reveals important differences between mycosis fungoides/Sezary syndrome vs. HTLV-1(+) leukemic cell lines. *Oncotarget.* (2017) 8:95981–98. doi: 10.18632/oncotarget.21619
47. Poiesz B, Dube D, Dube S, Love J, Papsidero L, Uner A, et al. HTLV-II-associated cutaneous T-cell lymphoma in a patient with HIV-1 infection. *N Engl J Med.* (2000) 342:930–6. doi: 10.1056/NEJM200003303421304
48. Bachelez H, Hadida F, Gorochov G. Massive infiltration of the skin by HIV-specific cytotoxic CD8+ T cells. *N Engl J Med.* (1996) 335:61–2. doi: 10.1056/NEJM199607043350118
49. Wilkins K, Turner R, Dolev JC, Leboit PE, Berger TG, Maurer TA. Cutaneous malignancy and human immunodeficiency virus disease. *J Am Acad Dermatol.* (2006) 54:189–206; quiz 207-10. doi: 10.1016/j.jaad.2004.11.060
50. Lee PY, Charley M, Tharp M, Jegasothy BV, Deng JS. Possible role of Epstein-Barr virus infection in cutaneous T-cell lymphomas. *J Invest Dermatol.* (1990) 95:309–12. doi: 10.1111/1523-1747.ep12485017
51. Borisch B, Boni J, Burki K, Laissue JA. Recurrent cutaneous anaplastic large cell (CD30+) lymphoma associated with Epstein-Barr virus. A case report with 9-year follow-up. *Am J Surg Pathol.* (1992) 16:796–801. doi: 10.1097/00000478-199208000-00009
52. Dreno B, Celerier P, Fleischmann M, Bureau B, Litoux P. Presence of Epstein-Barr virus in cutaneous lesions of mycosis fungoides and Sezary syndrome. *Acta Derm Venereol.* (1994) 74:355–7.
53. Cho KH, Kim CW, Lee DY, Sohn SJ, Kim DW, Chung JH. An Epstein-Barr virus-associated lymphoproliferative lesion of the skin presenting as recurrent necrotic papulovesicles of the face. *Br J Dermatol.* (1996) 134:791–6. doi: 10.1111/j.1365-2133.1996.tb06994.x
54. Mouly F, Baccard M, Rybojad M, Lebbe C, Morinet F, Morel P. Aggressive cutaneous T-cell lymphoma associated with the presence of Epstein-Barr virus. 2 cases. *Ann Dermatol Venereol.* (1996) 123:574–6.
55. Jumbou O, Mollat C, N'guyen JM, Billaudel S, Litoux P, Dreno B. Increased anti-Epstein-Barr virus antibodies in epidermotropic cutaneous T-cell lymphoma: a study of 64 patients. *Br J Dermatol.* (1997) 136:212–6. doi: 10.1111/j.1365-2133.1997.tb14898.x
56. Jumbou O, Huet S, Bureau B, Litoux P, Dreno B. Epstein-Barr virus research by *in situ* hybridization in 65 cutaneous T cell epidermotropic lymphomas. *Ann Dermatol Venereol.* (1998) 125:90–3.
57. Nagore E, Ledesma E, Collado C, Oliver V, Perez-Perez A, Aliaga A. Detection of Epstein-Barr virus and human herpesvirus 7 and 8 genomes in primary cutaneous T- and B-cell lymphomas. *Br J Dermatol.* (2000) 143:320–3. doi: 10.1046/j.1365-2133.2000.03657.x
58. Tournadre A, D'incan M, Dubost JJ, Franck F, Dechelotte P, Souteyrand P, et al. Cutaneous lymphoma associated with Epstein-Barr virus infection in 2 patients treated with methotrexate. *Mayo Clin Proc.* (2001) 76:845–8. doi: 10.1016/S0025-6196(11)63231-X
59. Noorali S, Yaqoob N, Nasir MI, Moatter T, Pervez S. Prevalence of mycosis fungoides and its association with EBV and HTLV-1 in Pakistanian patients. *Pathol Oncol Res.* (2002) 8:194–9. doi: 10.1007/BF03032394
60. Novelli M, Merlino C, Ponti R, Bergallo M, Quaglino P, Cambieri I, et al. Epstein-Barr virus in cutaneous T-cell lymphomas: evaluation of the viral presence and significance in skin and peripheral blood. *J Invest Dermatol.* (2009) 129:1556–61. doi: 10.1038/jid.2008.396
61. Park S, Lee DY, Kim WS, Ko YH. Primary cutaneous Epstein-Barr virus-associated T-cell lymphoproliferative disorder-2 cases with unusual, prolonged clinical course. *Am J Dermatopathol.* (2010) 32:832–6. doi: 10.1097/DAD.0b013e3181d68381
62. Herne KL, Talpur R, Breuer-Mcham J, Champlin R, Duvic M. Cytomegalovirus seropositivity is significantly associated with mycosis fungoides and Sezary syndrome. *Blood.* (2003) 101:2132–6. doi: 10.1182/blood-2002-07-2247
63. Ballanger F, Bressollette C, Volteau C, Planche L, Dreno B. Cytomegalovirus: its potential role in the development of cutaneous T-cell lymphoma. *Exp Dermatol.* (2009) 18:574–6. doi: 10.1111/j.1600-0625.2008.00817.x
64. Brice SL, Jester JD, Friednash M, Golitz LE, Leahy MA, Stockert SS, et al. Examination of cutaneous T-cell lymphoma for human herpesviruses by using the polymerase chain reaction. *J Cutan Pathol.* (1993) 20:304–7. doi: 10.1111/j.1600-0560.1993.tb01266.x
65. Erkek E, Senturk N, Dincer I, Olut AI, Kocagoz T, Bukulmez G, et al. Identification of herpes simplex virus DNA and lack of human herpesvirus-8 DNA in mycosis fungoides. *Acta Derm Venereol.* (2002) 82:214–6. doi: 10.1080/00015550260132569
66. Ponti R, Bergallo M, Costa C, Quaglino P, Fierro MT, Comessatti A, et al. Human herpesvirus 7 detection by quantitative real time polymerase chain reaction in primary cutaneous T-cell lymphomas and healthy subjects: lack of a pathogenic role. *Br J Dermatol.* (2008) 159:1131–7. doi: 10.1111/j.1365-2133.2008.08811.x
67. Kreuter A, Bischoff S, Skrygan M, Wieland U, Brockmeyer NH, Stucker M, et al. High association of human herpesvirus 8 in large-plaque parapsoriasis and mycosis fungoides. *Arch Dermatol.* (2008) 144:1011–6. doi: 10.1001/archderm.144.8.1011
68. Shuda M, Arora R, Kwun HJ, Feng H, Sarid R, Fernandez-Figueras MT, et al. Human Merkel cell polyomavirus infection I. MCV T antigen expression in Merkel cell carcinoma, lymphoid tissues and lymphoid tumors. *Int J Cancer.* (2009) 125:1243–9. doi: 10.1002/ijc.24510
69. Andres C, Puchta U, Sander CA, Ruzicka T, Flaig MJ. Prevalence of Merkel cell polyomavirus DNA in cutaneous lymphomas, pseudolymphomas, and inflammatory skin diseases. *Am J Dermatopathol.* (2010) 32:593–8. doi: 10.1097/DAD.0b013e3181ce8beb
70. Kreuter A, Silling S, Dewan M, Stucker M, Wieland U. Evaluation of 4 recently discovered human polyomaviruses in primary cutaneous B-cell and T-cell lymphoma. *Arch Dermatol.* (2011) 147:1449–51. doi: 10.1001/archdermatol.2011.330
71. Mirvish JJ, Pomerantz RG, Falo LD Jr., Geskin LJ. Role of infectious agents in cutaneous T-cell lymphoma: facts and controversies. *Clin Dermatol.* (2013) 31:423–31. doi: 10.1016/j.clindermatol.2013.01.009
72. Bagherani N, Smoller BR. (2016). An overview of cutaneous T cell lymphomas. *F1000Res.* 5:F1000 Faculty Rev-1882. doi: 10.12688/f1000research.8829.1
73. Tsang M, Gantchev J, Netchiporouk E, Moreau L, Ghazawi FM, Glassman S, et al. A study of meiomitosis and novel pathways of genomic instability in cutaneous T-cell lymphomas (CTCL). *Oncotarget.* (2018) 9:37647–61. doi: 10.18632/oncotarget.26479
74. Sibbesen NA, Kopp KL, Litvinov IV, Jonson L, Willerslev-Olsen A, Fredholm S, et al. Jak3, STAT3, and STAT5 inhibit expression of miR-22, a novel tumor suppressor microRNA, in cutaneous T-Cell lymphoma. *Oncotarget.* (2015) 6:20555–69. doi: 10.18632/oncotarget.4111
75. Litvinov IV, Cordeiro B, Fredholm S, Odum N, Zargham H, Huang Y, et al. Analysis of STAT4 expression in cutaneous T-cell lymphoma (CTCL) patients and patient-derived cell lines. *Cell Cycle.* (2014) 13:2975–82. doi: 10.4161/15384101.2014.947759
76. Netchiporouk E, Litvinov IV, Moreau L, Gilbert M, Sasseville D, Duvic M. Deregulation in STAT signaling is important for cutaneous T-cell lymphoma (CTCL) pathogenesis and cancer progression. *Cell Cycle.* (2014) 13:3331–5. doi: 10.4161/15384101.2014.965061
77. Fanok MH, Sun A, Fogli LK, Narendran V, Eckstein M, Kannan K, et al. Role of dysregulated cytokine signaling and bacterial triggers in the pathogenesis of cutaneous T-cell lymphoma. *J Invest Dermatol.* (2018) 138:1116–25. doi: 10.1016/j.jid.2017.10.028
78. Kopp KL, Ralfkiaer U, Gjerdrum LM, Helvad R, Pedersen IH, Litman T, et al. STAT5-mediated expression of oncogenic miR-155 in cutaneous T-cell lymphoma. *Cell Cycle.* (2013) 12:1939–47. doi: 10.4161/cc.24987

79. Litvinov IV, Pehr K, Sasseville D. Connecting the dots in cutaneous T cell lymphoma (CTCL): STAT5 regulates malignant T cell proliferation via miR-155. *Cell Cycle.* (2013) 12:2172–3. doi: 10.4161/cc.25550
80. Willerslev-Olsen A, Krejsgaard T, Lindahl LM, Litvinov IV, Fredholm S, Petersen DL, et al. Staphylococcal enterotoxin A (SEA) stimulates STAT3 activation and IL-17 expression in cutaneous T-cell lymphoma. *Blood.* (2016) 127:1287–96. doi: 10.1182/blood-2015-08-662353
81. Detmar M, Pauli G, Anagnostopoulos I, Wunderlich U, Herbst H, Garbe C, et al. A case of classical mycosis fungoides associated with human T-cell lymphotropic virus type I. *Br J Dermatol.* (1991) 124:198–202. doi: 10.1111/j.1365-2133.1991.tb00434.x
82. Tokura Y, Yagi H, Ohshima A, Kurokawa S, Wakita H, Yokote R, et al. Cutaneous colonization with staphylococci influences the disease activity of Sezary syndrome: a potential role for bacterial superantigens. *Br J Dermatol.* (1995) 133:6–12. doi: 10.1111/j.1365-2133.1995.tb02485.x
83. Jackow CM, Cather JC, Hearne V, Asano AT, Musser JM, Duvic M. Association of erythrodermic cutaneous T-cell lymphoma, superantigen-positive *Staphylococcus aureus*, and oligoclonal T-cell receptor V beta gene expansion. *Blood.* (1997) 89:32–40.
84. Tan RS, Butterworth CM, Mclaughlin H, Malka S, Samman PD. Mycosis fungoides–a disease of antigen persistence. *Br J Dermatol.* (1974) 91:607–16. doi: 10.1111/j.1365-2133.1974.tb12449.x
85. Duvic M. Current treatment of cutaneous T-cell lymphoma. *Dermatol. Online J.* (2001) 7. Retrieved from: https://escholarship.org/uc/item/3bs957xs
86. Paul LJ, Duvic M. Mycosis fungoides following skin trauma. *J Am Acad Dermatol.* (2012) 67:e148. doi: 10.1016/j.jaad.2011.11.953
87. Dixon JM, Clemens M. Breast implants and anaplastic large cell lymphoma. *BMJ.* (2018) 363:k5054. doi: 10.1136/bmj.k5054
88. Buus TB, Willerslev-Olsen A, Fredholm S, Blumel E, Nastasi C, Gluud M, et al. Single-cell heterogeneity in Sezary syndrome. *Blood Adv.* (2018) 2:2115–26. doi: 10.1182/bloodadvances.2018022608
89. Peck JR, Frank MP, Peck LR. Was treatment the trigger? Mycosis fungoides. *Am J Med.* (2013) 126:1048–9. doi: 10.1016/j.amjmed.2013.08.007

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

ORIGINAL RESEARCH
published: 31 May 2019
doi: 10.3389/fonc.2019.00429

The Ectopic Expression of Meiosis Regulatory Genes in Cutaneous T-Cell Lymphomas (CTCL)

*Jennifer Gantchev†, Amelia Martínez Villarreal†, Pingxing Xie†, Philippe Lefrançois, Scott Gunn, Elena Netchiporouk, Denis Sasseville and Ivan V. Litvinov**

Division of Dermatology, McGill University, Montréal, QC, Canada

OPEN ACCESS

Edited by:
Catherine Grace Chung, The Ohio State University, United States

Reviewed by:
Himanshi Bhatia, Washington University in St. Louis, United States
Lubos Cipak, Slovak Academy of Sciences, Slovakia

***Correspondence:**
Ivan V. Litvinov
ivan.litvinov@mcgill.ca

†These authors have contributed equally to this work

Specialty section:
This article was submitted to Hematologic Malignancies, a section of the journal Frontiers in Oncology

Received: *23 January 2019*
Accepted: *07 May 2019*
Published: *31 May 2019*

Citation:
Gantchev J, Martínez Villarreal A, Xie P, Lefrançois P, Gunn S, Netchiporouk E, Sasseville D and Litvinov IV (2019) The Ectopic Expression of Meiosis Regulatory Genes in Cutaneous T-Cell Lymphomas (CTCL). Front. Oncol. 9:429. doi: 10.3389/fonc.2019.00429

Cancer testis (CT) antigens, under normal circumstances are uniquely expressed in testicular germ cells. Recent research has shown that meiosis-specific CT (meiCT) antigens are ectopically expressed in cutaneous T-cell lymphoma (CTCL) and may contribute to increased genomic instability. The aberrant activation of meiosis genes in a mitotic cell is now recognized as a distinctive process, "meiomitosis." We have previously demonstrated the ectopic expression of several meiCT antigens in nine patient-derived CTCL cell lines and in expanded peripheral T lymphocytes isolated from Sézary Syndrome patients. In this study we analyzed the transcriptional expression of meiCT genes in Sézary Syndrome patients and healthy controls using publicly-available RNA sequencing (RNA-Seq) data. We corroborated our *in silico* analysis by examining the expression of 5 meiCT proteins in formalin-fixed, paraffin-embedded (FFPE) lesional samples from CTCL patients. Our results show significant differential gene expression of *STAG3*, *SGO2*, *SYCP3,* and *DMC1* in a cohort of Sézary Syndrome patients when compared to healthy controls. Additionally, our study demonstrates a heterogenous expression of meiCT genes involved in initiation (*STRA8*), sister chromatin cohesion (*STAG3, SGO2*), homologous chromosome synapsis (*SYCP3*) and homologous recombination (*DMC1*) in atypical lymphocytes in FFPE samples. Our results further confirm the ectopic expression of meiCT genes in CTCL which indicates that CTCL malignant cells likely undergo the process of cancer meiomitosis, as opposed to a typical mitotic division. The ectopic expression of meiCT genes together with investigations into the functional mechanisms of cancer meiomitosis will help provide a foundation to develop novel diagnostic tests to distinguish CTCL from benign inflammatory dermatoses and may enable us to develop additional targeted therapies for patients with this malignancy.

Keywords: mycosis fungoides (MF), Sézary Syndrome (SS), cutaneous T-cell lymphomas (CTCL), cancer/testis antigens, germ cell proteins, meiCT, meiomitosis

INTRODUCTION

Cutaneous T-cell lymphoma (CTCL) is an extranodal non-Hodgkin's lymphoproliferative disease characterized by the infiltration of malignant T-cells into the skin (1). The two most commonly recognized forms of CTCL are mycosis fungoides (MF) and a leukemic variant, Sézary Syndrome (SS). MF, the predominant subtype of cutaneous lymphomas, presents with localized erythematous scaly patches and plaques on the trunk. Overall, this CTCL subtype has an indolent course and a good prognosis. MF patients often live >10–15 years with no further progression until the later stages of the disease. Histologically, the epidermis may show hyperkeratosis, acanthosis, the presence of Pautrier's microabscesses and a notable lack of significant spongiosis. Additionally, a dermal lichenoid infiltrate of atypical T lymphocytes (with atypical/cerebriform nuclei) and minimal papillary dermal fibrosis are often observed (2–4). SS is an aggressive subtype of CTCL with a poor prognosis and a mean disease-specific survival of only 2–4 years (5). SS patients present with erythroderma, pruritus, generalized lymphadenopathy, and circulating malignant T cells (6). The disease develops either *de novo* or evolves from an idiopathic erythroderma (7).

Genomic CTCL studies have been steadily increasing in number and revealed extensive chromosomal instability in malignant T cells from patients and patient-derived cell lines (8–10). In fact, an essential criterion for SS diagnosis is the presence of a chromosomally abnormal malignant T cell clone (11). Consistent with prior studies, we have characterized distinct genomic and transcriptional heterogeneity in MF and SS, that were demonstrated by widespread genomic alterations including translocations, insertions, deletions, and extensive copy number variations (9, 10, 12–16).

Current research has demonstrated the ectopic expression of a variety of CT antigens in CTCL, including the *MAGE* (17, 18), *CAGE* (19), and *GAGE* proteins (20) that confer several hallmarks of cancer including sustained growth, angiogenesis, evasion of apoptosis, tissue invasion, and metastasis (21). With regards to genomic instability, the family of CT antigens involved in meiosis is of particular interest. Genes that regulate meiosis are exclusively expressed during oocyte development/spermatogenesis and become transcriptionally silent in normal somatic tissues. Our lab has shown that several meiCT genes including *SPO11, REC8, SYCP3*, and *SYCP1* are ectopically expressed in CTCL (10, 22). We propose that the malignant lymphocytes in this cancer are undergoing meiomitosis, a process defined by the clashing of the mitotic and meiotic molecular machineries in malignancy. It has been proposed that the partial re-expression of meiotic genes could cause the increased genomic instability seen in dividing cancer cells (10, 23), but studies demonstrating this statement in human cancers are lacking.

The mechanisms driving meiotic gene expression in CTCL are largely unknown. The ectopic expression of meiotic proteins involved in crossing over, meiotic DSB formation and repair, may promote genomic rearrangements (21, 24), Moreover, mitotic expression of meiotic cohesins could promote aneuploidy by eliciting an aberrantly assembled mitotic spindle that can result in the shearing and mis-segregation of DNA (23, 25, 26). We have recently demonstrated the ectopic expression and function of meiotic genes in patient-derived CTCL cell lines representing both MF and SS, and in lymphocytes isolated from stage IV SS patients (10). We demonstrated that the ectopic expression of *STRA8, SYCP1, 3,* and other proteins in CTCL is tightly regulated throughout the cell cycle and that their expression is the result of a loss of epigenetic transcriptional repression. Moreover, we proposed that the extensive chromosomal instability seen in CTCL may result from the aforementioned ectopic expression of these normally restricted meiotic CT antigens (10). The current study aims to broaden the understanding of ectopic expression patterns of meiCT genes in CTCL by analyzing RNA-Seq expression data from Sézary Syndrome patients and corroborating these results in 32 histologically-confirmed CTCL skin samples.

METHODS

Patients and Tissues

All patients were enrolled in this study with written informed consent and in accordance with the Declaration of Helsinki from The Ottawa Hospital (REB study #20150896-01H), McGill University Health Centre and affiliated hospitals (REB study #A09-M81-10A) and Laval University (REB study # 2011HES-22808). For immunohistochemistry, we analyzed a total of 32 samples from 29 patients with CTCL.

Diagnosis was histologically verified for all patients by a dermatopathologist and patients were staged according to the most recent consensus of the WHO-EORTC/ISCL staging system (5). Tissue samples were obtained by punch biopsy of the skin and were immediately frozen in liquid nitrogen, as previously described (22, 27, 28).

Histology

Human skin biopsies were processed into formalin-fixed, paraffin-embedded (FFPE) tissue blocks using standard fixation and embedding techniques. All tissue samples were sectioned and prepared on positively charged slides. Immunohistochemistry staining was performed on FFPE tissue sections using the Leica Bond™ system and the standard protocol F. Sectioned slides were stained with the following anti-human antibodies: rabbit anti-*STRA8* (Novus Biologicals), rabbit anti-*STAG3* (Proteintech), rabbit anti-*DMC1* (Proteintech), rabbit anti-*SGO2* (Novus Biologicals), and rabbit anti-*SYCP3* (Abcam) using either heat mediated antigen retrieval with sodium citrate buffer (pH 6, epitope retrieval solution 1 ER1) for 20 min or with an EDTA buffer (pH 9, epitope retrieval solution 2 ER2) for 20 min. The sections were then incubated for 30 min at room temperature using the dilutions listed in **Table S1** and detected using an HRP conjugated compact polymer system. Slides were then stained using DAB as the chromogen, counterstained with Hematoxylin, mounted, and cover slipped.

Slides were analyzed by a team of four investigators on a multi-head microscope (Nikon Eclipse Ni-U) with a 20X objective lens using a consensus scoring method. The investigators were blind to diagnosis and clinical stage of the sample. Positive

cells were verified (using a 40x lens) for enlarged/atypical nuclei or cerebriform nuclear contours, characteristic of malignant T lymphocytes. Nuclear and/or cytoplasmic staining of irregular cells with characteristic malignant T cell morphology was considered positive staining. The percentage of positive cells were assigned as Zero−0% (negative); I = <5%; II = 5–10% and III = >10%. The intensity of staining was scored as 1 (weak), 2 (moderate), and 3 (strong). The scores for percentage and intensity were multiplied for each sample to give a final expression score of 1–4 (weak-moderate staining with 10% or less positive cells) or 6–9 (strong staining with >10% positive cells).

Sézary Syndrome Patient Gene Expression and Analysis

Gene expression data for Sézary Syndrome patients was obtained from publicly-available RNA-Seq data (12, 16). The data from Choi et al. (12) and Ungewickell et al. (16) were pooled together to increase the sample size for analysis. Datasets were converted from TPM (transcripts per million) to a normalized rank. A non-parametric resampling method (Bootstrapping) was used to compute *p*-values comparing the means of non-parametric rank fraction values between Sézary patients and normal controls, as previously described (29). *P*-values were corrected for multiple hypothesis testing using a Bonferroni correction.

Statistical Analysis

Fisher's Exact test (FET) was used to analyze categorical outcomes in the histological analysis for gene expression scoring results between MF Stage I, StageII-III and aggressive forms of CTCL (i.e., SS, primary cutaneous $CD8^+$ positive aggressive epidermotropic T- cell lymphoma, peripheral T-cell lymphoma, or primary cutaneous gamma-delta T-cell lymphoma). All values were calculated using RStudio version 1.1.456. All statistical significance tests were two-sided. *P*-values <0.05 were considered statistically significant.

RESULTS

Expression of meiCT Genes in Sézary Syndrome Patients From External Cohorts

Using RT-PCR, western blot analysis and immunofluorescence, we have previously shown that meiCT genes and proteins are ectopically expressed in nine patient-derived cell lines representing both MF and SS, and in lymphocytes isolated from three SS patients (10, 22). We wanted to expand those observations with a different method to attain patient data. We used publicly-available RNA-Seq datasets from two studies focusing on Sézary Syndrome patients (12, 16), as previously described (29). Mean transcripts per million (TPM) values were calculated for each gene for the SS and control groups for Choi et al. (12) and Ungewickell et al. (16) cohorts and then combined the values as one dataset for analysis. Mean TPM values were plotted on bar graphs to compare gene expression levels for Sézary Syndrome vs. normal control patients. Overall, we observed increased gene expression in Sézary patients for all tested genes (**Figure 1**). Using a rank-based non-parametric approach, these genes showed higher relative expression levels in SS patients compared to controls (**Table 1**; $p < 10^{-5}$ for all genes except for *SGO2*; $p = 0.035$ for *SGO2*).

Expression of meiCT Genes in CTCL Lesional Biopsies

To further support our RNA-Seq based statistical analysis of meiCT gene expression in SS patients, we used immunohistochemical staining of FFPE skin tissues biopsied from patients with different stages of CTCL, including SS. We analyzed IHC staining for 5 meiCT genes that are expressed at various stages of meiosis, ranging from initiation to homologous recombination, in 32 CTCL samples isolated form 29 patients. Patient age ranged from 21 to 90 years with a median of 65 ± 18 years with a male to female ratio of 1:1.9. Control samples included normal human skin, and human testis.

The percentage and intensity of positively stained cells of the atypical lymphoid infiltrate was recorded for each sample and for all five genes (**Table 2**). Positive cells displayed characteristic features of malignant T-lymphocytes including atypical/cerebriform nuclear contours and enlarged and/or hyperchromatic nuclei within the papillary dermis (mixed lichenoid infiltrate).

STRA8 is an important of initiator of meiosis. Positive control expression of this gene in human testis shows intense nuclear expression pattern (**Figure 2A**). As expected, its expression is not detectable in normal skin except for diffuse non-specific background staining (**Figure 2B**). MF early stage samples and $CD8^+$ MF (**Figures 2C,D,F**) show appreciable nuclear staining in a number of dermal atypical lymphocytes abutting the dermal-epidermal junction, while a number of atypical T cells in Sézary Syndrome patients exhibit specific nucleolar staining (**Figure 2E**).

Similarly, meiotic cohesion proteins *STAG3* (**Figures 3A,B**) and *SGO2* (**Figures 4A,B**) demonstrate mostly nuclear staining in the testis and background non-specific staining in the epidermal keratinocytes, respectively. More precisely, *STAG3* shows intense non-specific staining in the granular layer, while *SGO2* shows diffuse staining throughout the epidermis and intense staining in the corneal layer of normal skin. Overall, staining for *STAG3* was mostly weak and ranged from pan-cellular expression pattern (**Figure 3C**) to mostly cytoplasmic (**Figure 3D**). In some cases, only faint nucleolar staining was observed (**Figures 3E,F**). In contrast, *SGO2* staining was more robust and demonstrated specific nuclear expression in a number of atypical cells (**Figures 4C–F**).

Synaptonemal complex protein *SYCP3* is expressed predominantly in the nuclei>cytoplasm of spermatogonia (**Figure 5A**) and only non-specific background staining is observed in normal skin (**Figure 5B**). Notably, atypical lymphocytes in CTCL express *SYCP3* in a nuclear and in some cases mis-localized pan-cellular pattern (**Figures 5C–F**).

Finally, for *DMC1*, meiotic recombination protein, nucelar>cytoplasmic expression is seen in spermatogonia (**Figure 6A**), while malignant lymphocytes in CTCL demonstrate strong nuclear >>> cytoplasmic expression for this protein (**Figures 6C–F**).

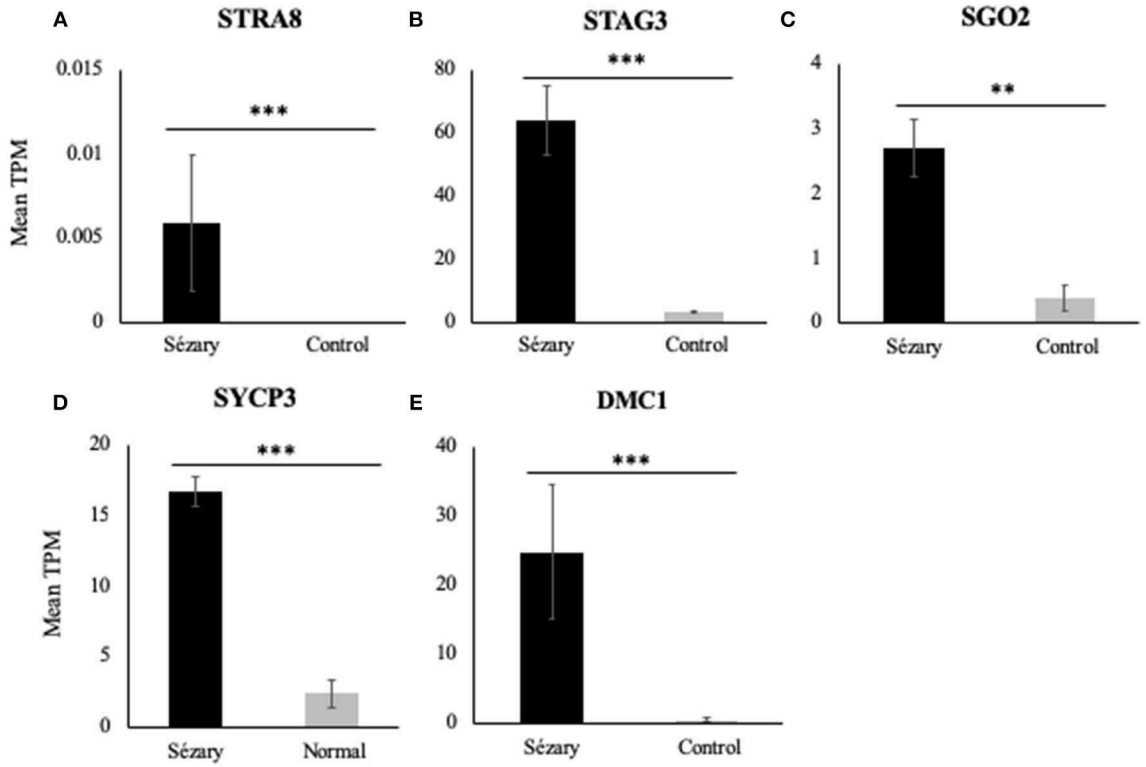


FIGURE 1 | RNA-Seq gene expression of meiCT genes **(A)** *STRA8,* **(B)** *STAG3*, **(C)** *SGO2*, **(D)** *SYCP3,* and **(E)** *DMC1* in Sézary Syndrome patients compared to normal/control subjects based on pooled data from Choi et al. (12) and Ungewickell et al. (16) datasets. Data was normalized to a mean TPM value for each cohort. Asterisks indicate statistical significance.

TABLE 1 | RNA-sequencing results for differentially expressed meiCT genes in two pooled independent cohorts of Sézary Syndrome patients (12, 16).

Genes	Ratio mean TPM SS/mean TPM Normal	Fraction Genes with TPM > GOI-Normal	Fraction Genes with TPM > GOI-Sézary	*p*-value
STRA8	∞	0	0.95	$<10^{-5}$
STAG3	20.52	0.40	0.17	$<10^{-5}$
SGO2	6.96	0.55	0.51	0.035
SYCP3	7.04	0.62	0.41	$<10^{-5}$
DMC1	43.33	0.67	0.41	$<10^{-5}$

P-values were determined using non-parametric testing and are corrected for multiple hypothesis testing using Bonferroni's correlation. TPM, Transcripts Per Million; SS, Sézary Syndrome; GOI, Gene Of Interest.

Detailed scoring for all genes across all lesional CTCL skin samples is presented in **Table 2**. While most proteins appear to be upregulated only weakly across different stages, *DMC1* shows robust staining across samples from all clinical stages of CTCL. Unfortunately, due to limited sample size, we did not observe meaningful differences between protein expression and clinical staging of patients (**Table 2**).

DISCUSSION

There is a unique set of meiosis specific genes that are temporally activated in germ cell development and are subsequently silenced in somatic cells. In this study we expanded our previous research by performing statistical analysis on a combined RNA-Seq dataset from Choi et al. (12) and Ungewickell et al. (16) that confirmed the differential expression of *STRA8, STAG3, SGO2, SYCP3,* and *DMC1* genes in Sézary patients when compared to healthy controls. We further corroborated our analysis with protein expression patterns of the meiCT genes in stained FFPE lesional skin samples isolated from patients diagnosed with various stages and subtypes of CTCL. We have shown that most of these proteins are expressed in atypical lymphocytes, albeit at weak to moderate levels. However, this pattern is expected for tightly-regulated meiCT genes whose expression occurs at specific time points in actively dividing cells. Furthermore, *DMC1* showed robust strong expression in biopsy samples

TABLE 2 | Results of MeiCT gene expression in lesional skin biopsy samples of patients with CTCL.

Gene	Disease	Expression score[a] 1–4 <10% cells positive; weak-moderate staining	Expression score 6–9 ≥10% cells positive; moderate -strong staining	*p*-value
STRA8	MF Stage I	17/18	0/18	0.05
	MF Stage II/III	3/6	1/6	
	Aggressive[b]	5/7	1/7	
STAG3	MF Stage I	11/19	1/19	0.6
	MF Stage II/III	3/6	0/6	
	Aggressive[b]	2/7	0/7	
SGO2	MF Stage I	12/19	1/19	0.9
	MF Stage II/III	3/6	0/6	
	Aggressive[b]	5/7	0/7	
SYCP3	MF Stage I	16/19	0/19	1
	MF Stage II/III	5/6	0/6	
	Aggressive[b]	6/7	0/7	
DMC1	MF Stage I	9/19	10/19	0.2
	MF Stage II/III	4/6	2/6	
	Aggressive[b]	6/7	1/7	

Expression score 1–4 corresponds to weak-moderate staining with <10% of positive cells. Expression score 6–9 corresponds to strong staining with ≥10% positive cells.

[a]*Intensity * Percentage = Expression score.*

Intensity: 1 mild, 2 weak, 3 strong.

Percentage positive: 0, 0%; 1, ± and <5%; 2, 5–10%; 3 >10%.

[b]*Aggressive disease includes SS (n = 2), primary cutaneous gamma/delta T-cell lymphoma (n = 2), Primary cutaneous $CD8^+$ positive aggressive epidermotropic T- cell lymphoma (n = 2) and Peripheral T-cell Lymphoma (n = 1).*

representing different stages of CTCL. Our findings corroborate and extend our recent work regarding the exploration of the ectopic expression and functional analysis of meiCT antigens in CTCL (10).

Under normal circumstances, *STRA8* is a vital protein to initiate meiosis in spermatocytes/oocytes in response to RA (retinoic acid) signaling (30–36). STRA8 expression is upregulated when germ cells switch from a mitotic state to meiosis (35) and spermatocytes that are depleted of STRA8 fail to enter meiosis. However, the role of STRA8 in cancer is yet to be defined. In germ cells, *STRA8* protein is primarily localized to the cytoplasm. In this study, we demonstrated that STRA8 is heterogeneously expressed in the nucleus of malignant T lymphocytes from CTCL patients. Other studies have shown expression of this protein in the nucleus with potential double stranded DNA binding (37, 38). Hence, STRA8 may have transcription regulatory activity (38) given reports that STRA8 expression is found in the nucleus of primordial germ cells and teratocarcinoma cell lines (37, 38). Additionally, two studies have shown that STRA8 binds double-stranded DNA (37, 38) and may have a role in transcription (38).

Throughout meiosis, a number of proteins form complexes along sister chromatids to orchestrate cohesion and release in a tightly regulated process to ensure the proper distribution of chromatids into gametes. The meiosis-specific cohesin subunit protein, *STAG3* (*Stromal antigen 3*), binds sister chromatids arms in meiosis I (39) and provides stability of meiosis-specific cohesion complexes (40). The cohesion complex is prudently protected by *SGO2* (*Shugoshin 2*) to avoid untimely degradation by separases. Although *SGO2* can also be expressed in mitotic cells, *SGO2* deficient mice form aneuploid gametes that result in infertility (41). However, lack of *SGO2* does not affect cohesion formation or development in cultured adult somatic cells and fibroblasts (41). We observed positive IHC staining of the two cohesion molecules in <5% of the atypical lymphoid infiltrate present in the papillary dermis. These results are not surprising since few T-cells are actively undergoing cell division at any given time in CTCL.

Similar results were found for the synaptonemal complex protein *SYCP3*. *SYCP3* forms the synaptonemal complex (SC) which is considered one of the cardinal features of meiosis. The SC a highly ordered assembly of proteins that comprise the interface between paired homologous chromosomes in meiosis. This meticulous complex stabilizes the interactions between homologs through a process called synapsis and promotes recombination (42). Staining of *SYCP3* was found in <5% of atypical lymphocytes in the papillary dermis.

As meiosis progresses, *DMC1* (Disrupted Meiotic cDNA 1) catalyzes strand exchange by localizing to double-stranded DNA (DSB) break sites and facilitates efficient homologous recombination DNA repair (43). *DMC1* localizes to meiotic DNA break sites to initiates strand exchange (43) and was expressed in 10–15% of the lymphoid infiltrate. We hypothesize that the expression of meiotic genes is tightly regulated and temporally expressed by the malignant T-cell in CTCL patients. Based on our previous work, we have demonstrated the temporal expression of meiCT genes at the onset of the cell cycle in patient-derived cell lines (10). Therefore, our IHC positive cells have the potential to undergo meiomitotic cell division.

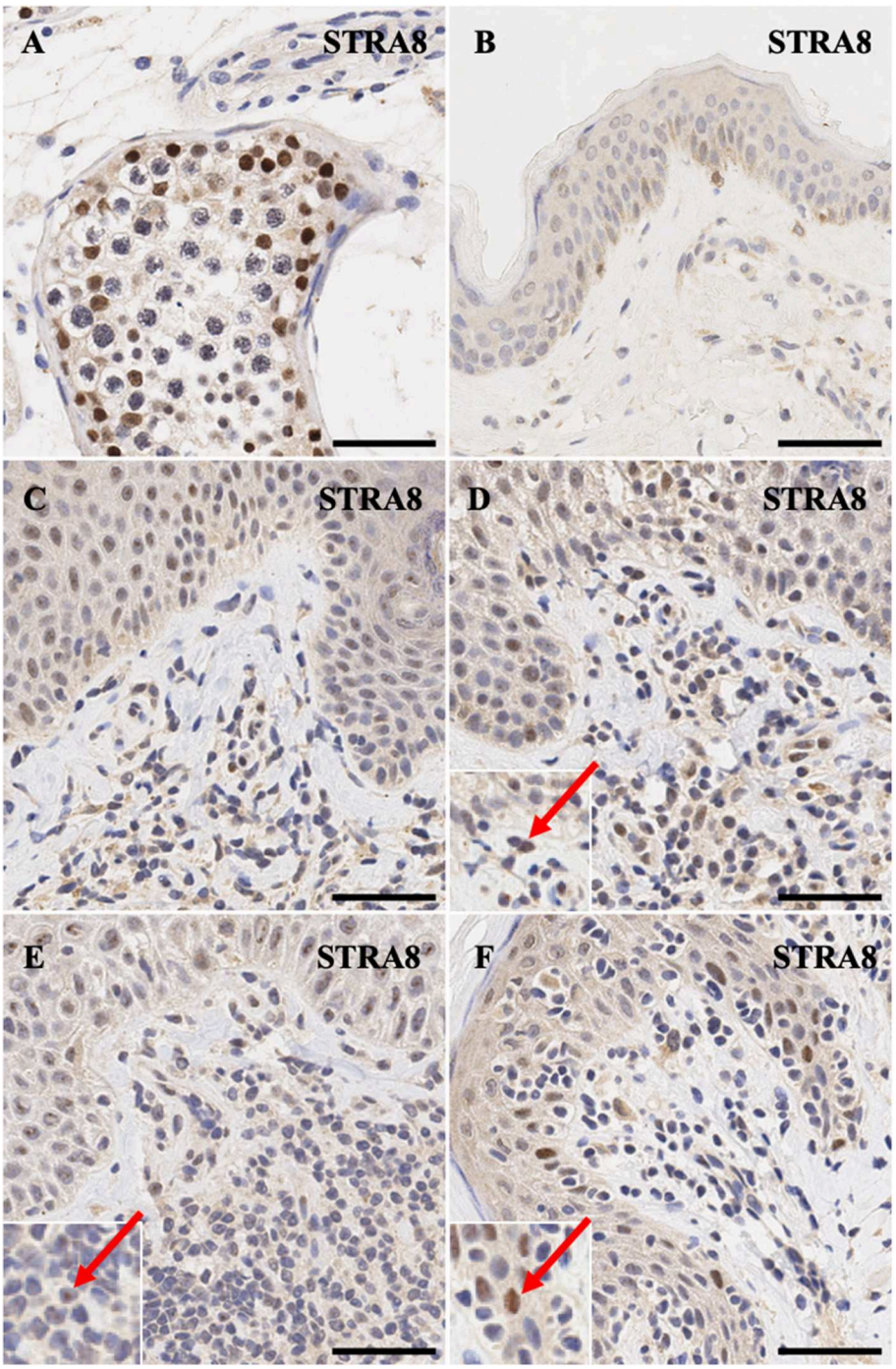


FIGURE 2 | Immunohistochemistry staining of *STRA8* in **(A)** normal human testis (positive control), **(B)** normal skin **(C)** stage IB MF lesional skin, **(D)** stage IA MF lesional skin. **(E)** Sézary Syndrome **(F)** $CD8^{+}$ MF lesional skin. Scale bars are 50 μm. Nuclear staining in malignant lymphocytes is highlighted (red arrow).

The transcriptional activation of meiCT genes is not fully understood, however studies have demonstrated two mechanisms of reactivation: the hypomethylation of meiCT gene promoter regions in lung adenocarcinoma (44), and increased expression of select meiCT genes following a genotoxic stress in cervical cancer, melanoma and lymphoma cell lines (25, 45, 46). Studies have demonstrated the ectopic expression of meiCT genes in polyploid lymphoma and cervical cells during depolyploidization (25, 45). CTCL cells may express meiCT genes to promote survival by maintaining stable ploidy and the proliferative states of the malignant T cells. Additionally, the concurrent activation of both the meiotic and mitotic

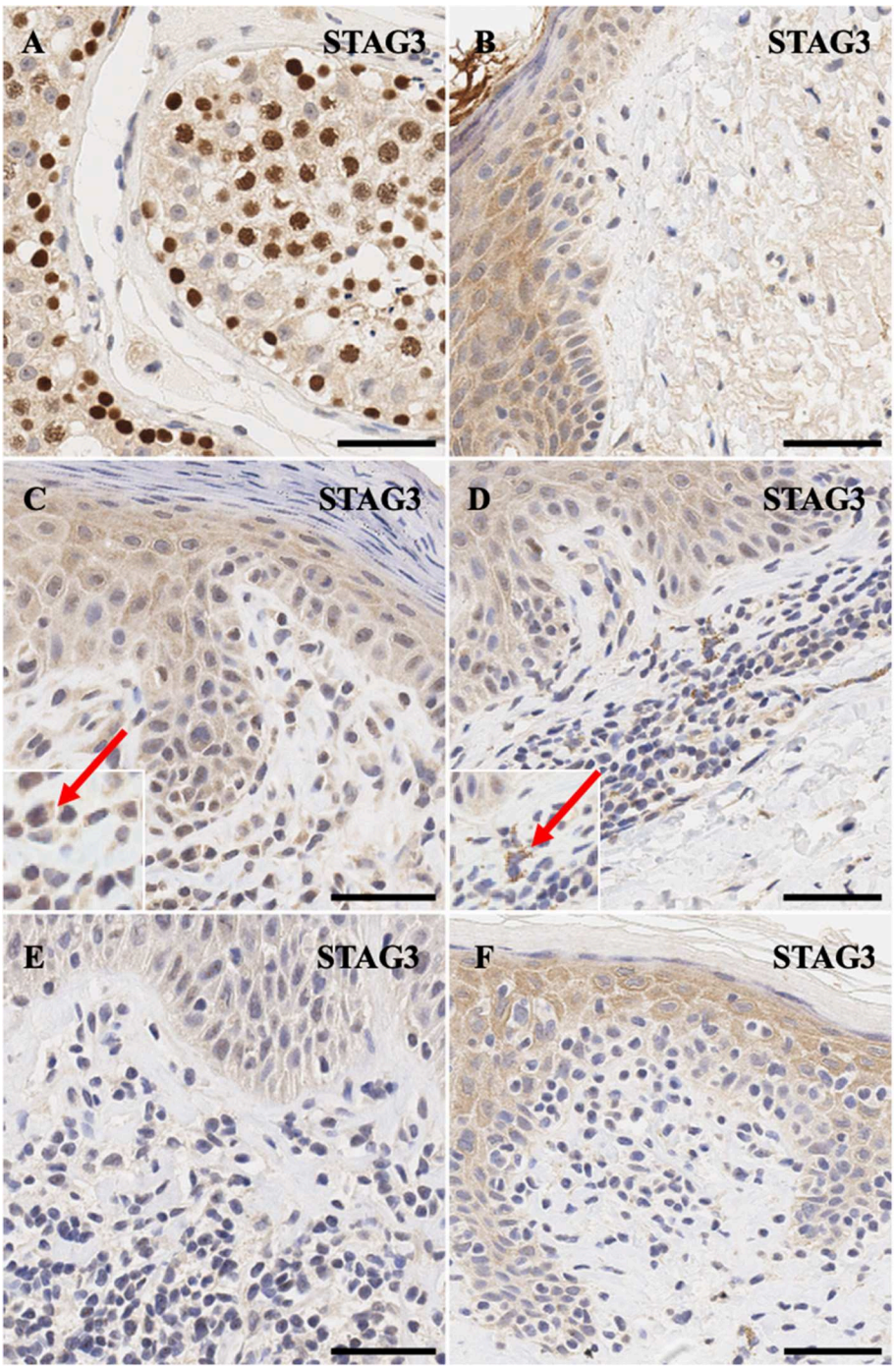


FIGURE 3 | Immunohistochemistry staining of *STAG3* in **(A)** normal human testis (positive control), **(B)** normal skin **(C)** stage IIA MF lesional skin, **(D)** stage IB MF lesional skin. **(E)** Sézary Syndrome **(F)** $CD8^{+}$ MF lesional skin. Scale bars are 50 μm. Red arrow highlights cytoplasmic staying of this protein in a number of cells.

pathways may represent an adapted regulatory pathway for a reductional division.

The expression of the homologous recombination *DMC1*gene and cohesin *SGO2* factor have the propensity to contribute to genomic rearrangements and increase aneuploidy, another potential mechanism for survival in a dynamic lesional/tumor environment. Previous studies have shown that CT antigens have the potential to modulate chromosomal aberrations and contribute to increased genomic instability (10, 47). Deregulation or even the presence of the meiotic proteins like *DMC1* may initiate a partial meiotic program in mitotic cells and have the potential to influence

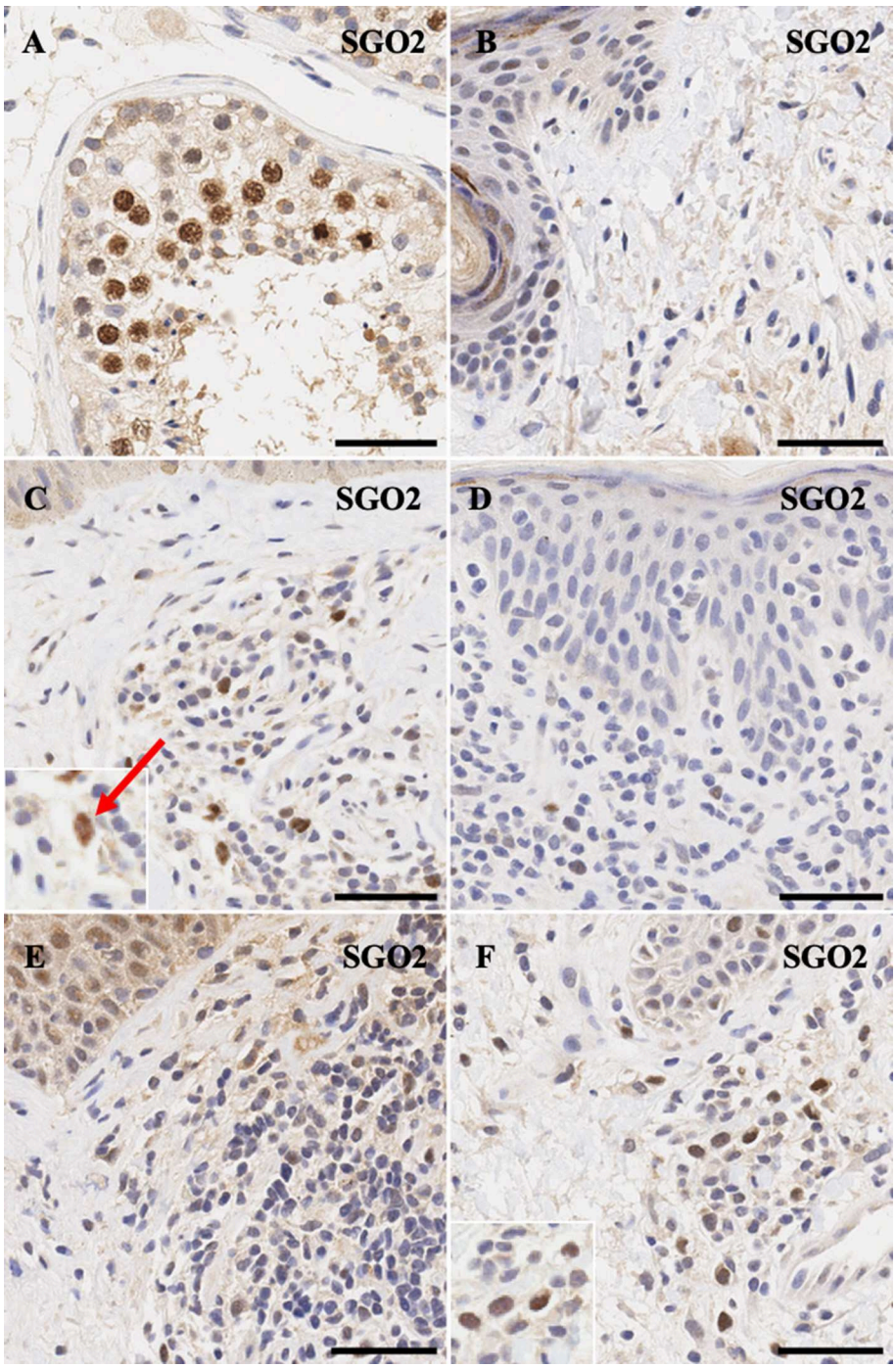


FIGURE 4 | Immunohistochemistry staining of *SGO2* in **(A)** normal human testis (positive control), **(B)** normal skin **(C)** stage IIA MF lesional skin, **(D)** $CD8^+$ MF lesional skin, **(E)** Sézary Syndrome (**F**) peripheral T-Cell Lymphoma. Scale bars are 50 μm. Nuclear staining in malignant lymphocytes is highlighted (red arrow).

genomic translocations, insertions and deletions during cell division.

We would like to address an important limitation, that due to a relatively small sample size of biopsy samples, we did not detect a correlation between CTCL clinical disease stage and protein expression from IHC staining. Future studies with a larger sample size would be able to estimate expression levels more precisely across different CTCL stages for each protein. In addition, another limitation is the semi-quantitative nature of IHC staining. Due to the variable staining patterns we adopted a semi-quantitative consensus scoring method. Unfortunately, we were not able to provide a quantitative analysis of the analyzed IHC stained slides.

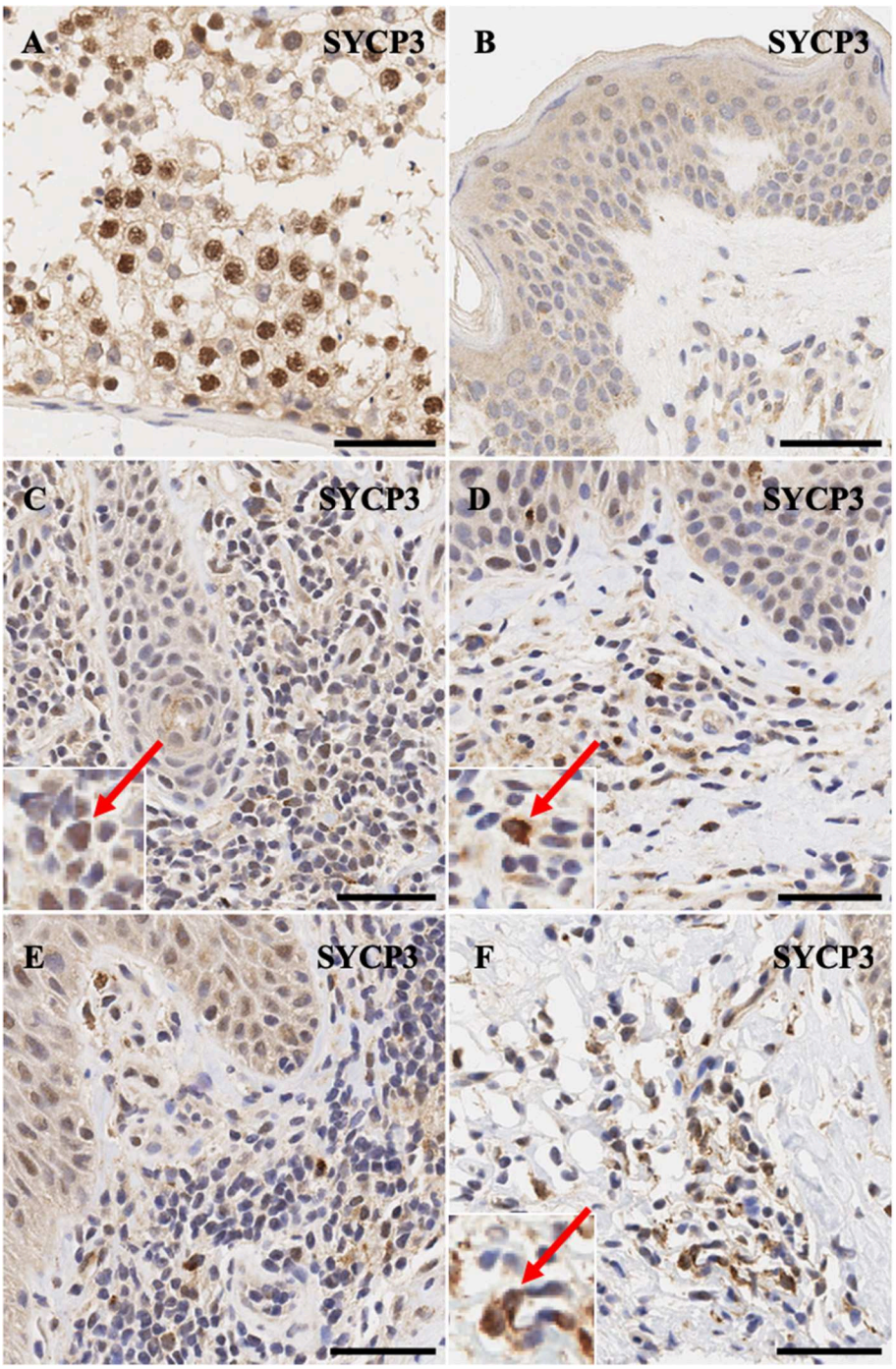


FIGURE 5 | Immunohistochemistry staining of *SYCP3* in **(A)** normal human testis (positive control), **(B)** normal skin **(C)** stage IB MF lesional skin, **(D)** stage IB MF lesional skin, **(E)** Sézary Syndrome **(F)** stage IA MF lesional skin. Scale bars are 50 μm. Nuclear>cytoplasmic staining in malignant lymphocytes is highlighted (red arrow).

In conclusion, the ectopic expression of meiCT genes in CTCL raises the possibility that malignant infiltrating T lymphocytes are undergoing a process termed meiomitosis. Further studies of the functional mechanisms of meiCT genes might shed light on the role of meiomitosis in carcinogenesis for various malignancies including CTCL. Further investigation of meiCT protein roles in carcinogenesis may lead to the development of small molecule inhibitors targeting this aberrant meiomitosis cell division. This can ultimately lead to the development of biomarkers for CTCL and novel targeted therapies.

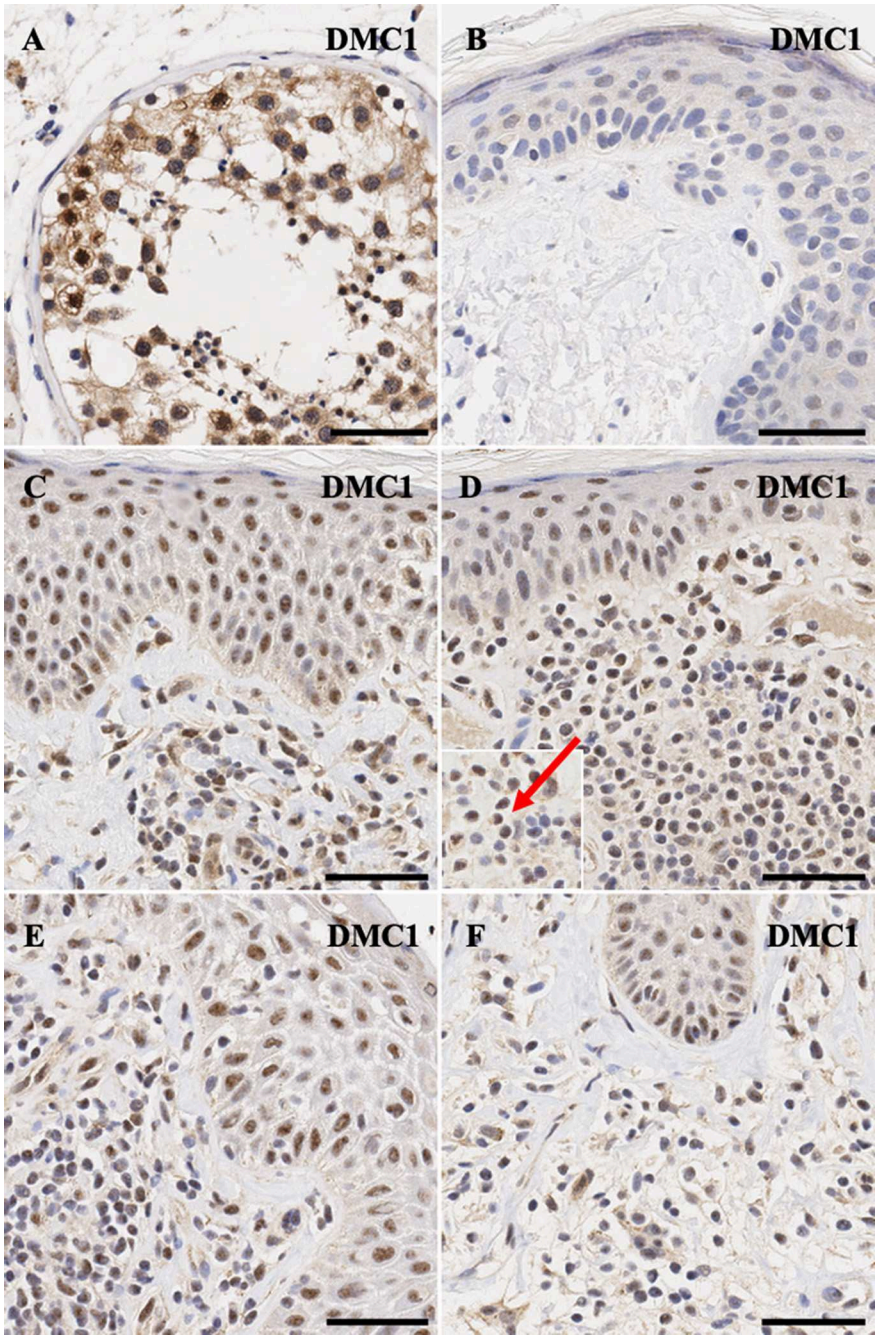


FIGURE 6 | Immunohistochemistry staining of *DMC1* in **(A)** normal human testis (positive control), **(B)** normal skin **(C)** stage IA MF lesional skin, **(D)** stage IA MF lesional skin, **(E)** Sézary Syndrome **(F)** $CD8^+$ MF lesional skin. Scale bars are 50 μm. Nuclear staining in malignant lymphocytes is highlighted (red arrow).

ETHICS STATEMENT

All patients were enrolled in this study with written informed consent and in accordance with the Declaration of Helsinki from The Ottawa Hospital (REB study #20150896-01H), McGill University Health Centre and affiliated hospitals (REB study #A09-M81-10A) and Laval University (REB study # 2011HES-22808).

AUTHOR CONTRIBUTIONS

JG, AM, SG, and PL performed histological scoring. PL and PX performed the data acquisition and analysis of Sézary patient cohort. PL, PX, and JG performed statistical analyses. AM and JG prepared the figures. JG wrote the first draft of the manuscript. All authors contributed to the revision and approval of the submitted manuscript. IV,

EN, and DS contributed to the conception and design of the study.

FUNDING

This work was supported by the Canadian Dermatology Foundation research grants to DS and IL, Joan Sealy Trust Cancer Research grant to IL and the Fonds de la recherche en santé du Québec (FRSQ) research grant to DS (FRSQ# 22648) and FRSQ research grants to IL (# 34753 and 36769). Consejo Nacional de Ciencia y Tecnología (CONACYT) provided Ph.D. scholarship to AM.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2019.00429/full#supplementary-material

REFERENCES

1. Siegel RS, Gartenhaus RB, Kuzel TM. Human T-cell lymphotropic-I-associated leukemia/lymphoma. *Curr Treat Options Oncol.* (2001) 2:291–300. doi: 10.1007/s11864-001-0022-8
2. Kazakov DV, Burg G, Kempf W. Clinicopathological spectrum of mycosis fungoides. *J Eur Acad Dermatol Venereol.* (2004) 18:397–415. doi: 10.1111/j.1468-3083.2004.00937.x
3. Pimpinelli N, Olsen EA, Santucci M, Vonderheid E, Haeffner AC, Stevens S, et al. Defining early mycosis fungoides. *J Am Acad Dermatol.* (2005) 53:1053–63. doi: 10.1016/j.jaad.2005.08.057
4. Furmanczyk PS, Wolgamot GM, Kussick SJ, Sabath DE, Olerud JE, Argenyi ZB. Diagnosis of mycosis fungoides with different algorithmic approaches. *J Cutan Pathol.* (2010) 37:8–14. doi: 10.1111/j.1600-0560.2009.01289.x
5. Olsen E, Vonderheid E, Pimpinelli N, Willemze R, Kim Y, Knobler R, et al. Revisions to the staging and classification of mycosis fungoides and Sezary syndrome: a proposal of the International Society for Cutaneous Lymphomas (ISCL) and the cutaneous lymphoma task force of the European Organization of Research and Treatment of Cancer (EORTC). *Blood.* (2007) 110:1713–22. doi: 10.1182/blood-2007-03-055749
6. Kim EJ, Lin J, Junkins-Hopkins JM, Vittorio CC, Rook AH. Mycosis fungoides and sezary syndrome: an update. *Curr Oncol Rep.* (2006) 8:376–86. doi: 10.1007/s11912-006-0061-1
7. Hwang ST, Janik JE, Jaffe ES, Wilson WH. Mycosis fungoides and Sezary syndrome. *Lancet.* (2008) 371:945–57. doi: 10.1016/S0140-6736(08)60420-1
8. Karenko L, Hahtola S, Ranki A. Molecular cytogenetics in the study of cutaneous T-cell lymphomas (CTCL). *Cytogenet Genome Res.* (2007) 118:353–61. doi: 10.1159/000108320
9. Netchiporouk E, Gantchev J, Tsang M, Thibault P, Watters AK, Hughes JM, et al. Analysis of CTCL cell lines reveals important differences between mycosis fungoides/Sezary syndrome vs. HTLV-1(+) leukemic cell lines. *Oncotarget.* (2017) 8:95981–98. doi: 10.18632/oncotarget.21619
10. Tsang M, Gantchev K, Netchiporouk E, Moreau L, Ghazawi FM, et al. A study of meiomitosis and novel pathways of genomic instability in cutaneous T-cell lymphomas (CTCL). *Oncotarget.* (2018) 9:37647–61. doi: 10.18632/oncotarget.26479
11. Vonderheid EC, Bernengo MG. The Sezary syndrome: hematologic criteria. *Hematol Oncol Clin North Am.* (2003) 17:1367–89. doi: 10.1016/S0889-8588(03)00120-5
12. Choi J, Goh G, Walradt T, Hong BS, Bunick CG, Chen K, et al. Genomic landscape of cutaneous T cell lymphoma. *Nat Genet.* (2015) 47:1011–9. doi: 10.1038/ng.3356
13. da Silva Almeida AC, Abate F, Khiabanian H, Martinez-Escala E, Guitart J, Tensen CP, et al. The mutational landscape of cutaneous T cell lymphoma and Sezary syndrome. *Nat Genet.* (2015) 47:1465–70. doi: 10.1038/ng.3442
14. Kiel MJ, Sahasrabuddhe AA, Rolland DC, Velusamy T, Chung F, Schaller M, et al. Genomic analyses reveal recurrent mutations in epigenetic modifiers and the JAK-STAT pathway in Sezary syndrome. *Nat Commun.* (2015) 6:8470. doi: 10.1038/ncomms9470
15. McGirt LY, Jia P, Baerenwald DA, Duszynski RJ, Dahlman KB, Zic JA, et al. Whole-genome sequencing reveals oncogenic mutations in mycosis fungoides. *Blood.* (2015) 126:508–19. doi: 10.1182/blood-2014-11-611194
16. Ungewickell A, Bhaduri A, Rios E, Reuter J, Lee CS, Mah A, et al. Genomic analysis of mycosis fungoides and Sezary syndrome identifies recurrent alterations in TNFR2. *Nat Genet.* (2015) 47:1056–60. doi: 10.1038/ng.3370
17. De Plaen E, Arden K, Traversari C, Gaforio JJ, Szikora JP, De Smet C, et al. Structure, chromosomal localization, and expression of 12 genes of the MAGE family. *Immunogenetics.* (1994) 40:360–9. doi: 10.1007/BF01246677
18. Zendman AJ, Ruiter DJ, Van Muijen GN. Cancer/testis-associated genes: identification, expression profile, and putative function. *J Cell Physiol.* (2003) 194:272–88. doi: 10.1002/jcp.10215
19. Por E, Byun HJ, Lee EJ, Lim JH, Jung SY, Park I, et al. The cancer/testis antigen CAGE with oncogenic potential stimulates cell proliferation by up-regulating cyclins D1 and E in an AP-1- and E2F-dependent manner. *J Biol Chem.* (2010) 285:14475–85. doi: 10.1074/jbc.M109.084400
20. De Backer O, Arden KC, Boretti M, Vantomme V, De Smet C, Czekay S, et al. Characterization of the GAGE genes that are expressed in various human cancers and in normal testis. *Cancer Res.* (1999) 59:3157–65.
21. Gjerstorff MF, Andersen MH, Ditzel HJ. Oncogenic cancer/testis antigens: prime candidates for immunotherapy. *Oncotarget.* (2015) 6:15772–87. doi: 10.18632/oncotarget.4694
22. Litvinov IV, Cordeiro B, Huang Y, Zargham H, Pehr K, Dore MA, et al. Ectopic expression of cancer-testis antigens in cutaneous T-cell lymphoma patients. *Clin Cancer Res.* (2014) 20:3799–808. doi: 10.1158/1078-0432.CCR-14-0307
23. Grichnik JM. Melanoma, nevogenesis, and stem cell biology. *J Invest Dermatol.* (2008) 128:2365–80. doi: 10.1038/jid.2008.166
24. Chen YT, Venditti CA, Theiler G, Stevenson BJ, Iseli C, Gure AO, et al. Identification of CT46/HORMAD1, an immunogenic cancer/testis antigen encoding a putative meiosis-related protein. *Cancer Immun.* (2005) 5:9.
25. Ianzini F, Kosmacek EA, Nelson ES, Napoli E, Erenpreisa J, Kalejs M, et al. Activation of meiosis-specific genes is associated with depolyploidization of human tumor cells following radiation-induced mitotic catastrophe. *Cancer Res.* (2009) 69:2296–304. doi: 10.1158/0008-5472.CAN-08-3364
26. Cappell KM, Sinnott R, Taus P, Maxfield K, Scarbrough M, Whitehurst AW. Multiple cancer testis antigens function to support tumor cell mitotic fidelity. *Mol Cell Biol.* (2012) 32:4131–40. doi: 10.1128/MCB.00686-12
27. Litvinov IV, Jones DA, Sasseville D, Kupper TS. Transcriptional profiles predict disease outcome in patients with cutaneous T-cell lymphoma. *Clin Cancer Res.* (2010) 16:2106–14. doi: 10.1158/1078-0432.CCR-09-2879
28. Litvinov IV, Netchiporouk E, Cordeiro B, Dore MA, Moreau L, Pehr K, et al. The use of transcriptional profiling to improve personalized diagnosis and management of Cutaneous T-cell Lymphoma (CTCL). *Clin Cancer Res.* (2015) 21:2820–9. doi: 10.1158/1078-0432.CCR-14-3322
29. Lefrancois P, Xie P, Wang L, Tetzlaff MT, Moreau L, Watters AK, et al. Gene expression profiling and immune cell-type deconvolution highlight robust disease progression and survival markers in multiple cohorts of CTCL patients. *Oncoimmunology.* (2018) 7:e1467856. doi: 10.1080/2162402X.2018.1467856
30. Oulad-Abdelghani M, Bouillet P, Decimo D, Gansmuller A, Heyberger S, Dolle P, et al. Characterization of a premeiotic germ cell-specific cytoplasmic protein encoded by Stra8, a novel retinoic acid-responsive gene. *J Cell Biol.* (1996) 135:469–77. doi: 10.1083/jcb.135.2.469
31. Miyamoto T, Sengoku K, Takuma N, Hasuike S, Hayashi H, Yamauchi T, et al. Isolation and expression analysis of the testis-specific gene, STRA8,

stimulated by retinoic acid gene 8. *J Assist Reprod Genet.* (2002) 19:531–5. doi: 10.1023/A:1020963919596
32. Baltus AE, Menke DB, Hu YC, Goodheart ML, Carpenter AE, de Rooij DG, et al. In germ cells of mouse embryonic ovaries, the decision to enter meiosis precedes premeiotic DNA replication. *Nat Genet.* (2006) 38:1430–4. doi: 10.1038/ng1919
33. Vernet N, Dennefeld C, Guillou F, Chambon P, Ghyselinck NB, Mark M. Prepubertal testis development relies on retinoic acid but not rexinoid receptors in Sertoli cells. *EMBO J.* (2006) 25:5816–25. doi: 10.1038/sj.emboj.7601447
34. Bowles J, Koopman P. Retinoic acid, meiosis and germ cell fate in mammals. *Development.* (2007) 134:3401–11. doi: 10.1242/dev.001107
35. Anderson EL, Baltus AE, Roepers-Gajadien HL, Hassold TJ, de Rooij DG, van Pelt AM, et al. Stra8 and its inducer, retinoic acid, regulate meiotic initiation in both spermatogenesis and oogenesis in mice. *Proc Natl Acad Sci USA.* (2008) 105:14976–80. doi: 10.1073/pnas.0807297105
36. Zhou Q, Nie R, Li Y, Friel P, Mitchell D, Hess RA, et al. Expression of stimulated by retinoic acid gene 8 (Stra8) in spermatogenic cells induced by retinoic acid: an *in vivo* study in vitamin A-sufficient postnatal murine testes. *Biol Reprod.* (2008) 79:35–42. doi: 10.1095/biolreprod.107.066795
37. Tedesco M, La Sala G, Barbagallo F, De Felici M, Farini D. STRA8 shuttles between nucleus and cytoplasm and displays transcriptional activity. *J Biol Chem.* (2009) 284:35781–93. doi: 10.1074/jbc.M109.056481
38. Choi Y-J, Yoon J-W, Pyo C-W, Kim J-A, Bae S-H, Park S-S. A possible role of STRA8 as a transcription factor. *Genes Genomics.* (2010) 32:521–6. doi: 10.1007/s13258-010-0059-9
39. Prieto I, Suja JA, Pezzi N, Kremer L, Martínez AC, Rufas JS, et al. Mammalian STAG3 is a cohesin specific to sister chromatid arms in meiosis I. *Nat Cell Biol.* (2001) 3:761–6. doi: 10.1038/35087082
40. Hopkins J, Hwang G, Jacob J, Sapp N, Bedigian R, Oka K, et al. Meiosis-specific cohesin component, Stag3 is essential for maintaining centromere chromatid cohesion, and required for DNA repair and synapsis between homologous chromosomes. *PLoS Genet.* (2014) 10:e1004413. doi: 10.1371/journal.pgen.1004413
41. Llano E, Gomez R, Gutierrez-Caballero C, Herran Y, Sanchez-Martin M, Vazquez-Quinones L, et al. Shugoshin-2 is essential for the completionof meiosis but not for mitotic cell division in mice. *Genes Dev.* (2008) 22:2400–13. doi: 10.1101/gad.475308
42. Zickler D, Kleckner N. Recombination, pairing, and synapsis of homologs during meiosis. *Cold Spring Harb Perspect Biol.* (2015) 7:1–26. doi: 10.1101/cshperspect.a016626
43. Howard-Till RA, Lukaszewicz A, Loidl J. The recombinases Rad51 and Dmc1 play distinct roles in DNA break repair and recombination partner choice in the meiosis of Tetrahymena. *PLoS Genet.* (2011) 7:e1001359. doi: 10.1371/journal.pgen.1001359
44. Nichols BA, Oswald NW, McMillan EA, McGlynn K, Yan J, Kim MS, et al. HORMAD1 is a negative prognostic indicator in lung adenocarcinoma and specifies resistance to oxidative and genotoxic stress. *Cancer Res.* (2018) 78:6196–208. doi: 10.1158/0008-5472.CAN-18-1377
45. Kalejs M, Ivanov A, Plakhins G, Cragg MS, Emzinsh D, Illidge TM, et al. Upregulation of meiosis-specific genes in lymphoma cell lines following genotoxic insult and induction of mitotic catastrophe. *BMC Cancer.* (2006) 6:6. doi: 10.1186/1471-2407-6-6
46. Erenpreisa J, Cragg MS, Salmina K, Hausmann M, Scherthan H. The role of meiotic cohesin REC8 in chromosome segregation in gamma irradiation-induced endopolyploid tumour cells. *Exp Cell Res.* (2009) 315:2593–603. doi: 10.1016/j.yexcr.2009.05.011
47. Nielsen AY, Gjerstorff MF. Ectopic expression of testis germ cell proteins in cancer and its potential role in genomic instability. *Int J Mol Sci.* (2016) 17:1–5. doi: 10.3390/ijms17060890

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

MINI REVIEW
published: 30 July 2019
doi: 10.3389/fonc.2019.00645

Antibody-Directed Therapies: Toward a Durable and Tolerable Treatment Platform for CTCL

*Shaheer Khan and Ahmed Sawas**

Department of Medicine, Center for Lymphoid Malignancies, Columbia University Medical Center, The New York Presbyterian Hospital, College of Physician and Surgeons, New York, NY, United States

Cutaneous T-cell lymphomas (CTCL) are a rare group of heterogeneous disorders characterized by cutaneous involvement of monoclonal T-lymphocytes. Although indolent at early stages, CTCL can confer significant morbidity, and mortality when advanced. There is an unmet need for tolerable and durable treatments with antibodies recently gaining promise. Here we review approved systemic therapies and discuss select antibodies in development.

Keywords: CTCL, antibody-directed therapy, brentuximab vedotin, mogamulizumab, PD-1, E777, TTI-621, AFM13

OPEN ACCESS

Edited by:
Basem M. William,
The Ohio State University,
United States

Reviewed by:
John C. Reneau,
Michigan Medicine, University of Michigan, United States
Belinda Pinto Simoes,
University of São Paulo, Brazil

***Correspondence:**
Ahmed Sawas
as4386@columbia.edu

Specialty section:
This article was submitted to Hematologic Malignancies, a section of the journal Frontiers in Oncology

Received: *23 April 2019*
Accepted: *01 July 2019*
Published: *30 July 2019*

Citation:
Khan S and Sawas A (2019) Antibody-Directed Therapies: Toward a Durable and Tolerable Treatment Platform for CTCL. Front. Oncol. 9:645. doi: 10.3389/fonc.2019.00645

INTRODUCTION

Cutaneous T-Cell lymphomas (CTCL) are a heterogeneous group of disorders that are characterized by cutaneous involvement of monoclonal T-lymphocytes. Mycosis fungoides (MF) is the most common form, accounting for ∼50% of cases. Primary cutaneous CD30+ anaplastic large cell lymphoma, lymphomatoid papulosis, and Sezary syndrome (SS) constitute the other most common forms (1). There are ∼2,500 new cases of MF and SS per year in the United States (2).

MF usually presents as erythematous patches that occur predominantly in sun-shielded areas including the torso and lower extremities. In about two thirds of patients, the disease is diagnosed at an early stage (stage IA-IIB) with a relatively indolent course. In patients with early stage MF, median survival can exceed 25 years (3). Progression to advanced stage (IIB-IV) occurs in 30% of MF cases. Sezary syndrome exhibits a more aggressive course in which systemic involvement of neoplastic T-lymphocytes results in leukemic involvement, exfoliative dermatitis, and lymph node enlargement. In advanced MF and SS, median survival has been described as short as 1.5 years (4).

Currently, there is no cure for CTCL and it is characterized by a chronic, relapsing course that requires repeat treatment regimens. Patients with early stage disease are treated with skin-directed therapies which include topical corticosteroids, phototherapy, topical chemotherapy, topical bexarotene, and radiotherapy. Patients with advanced stage disease are offered palliative systemic therapies to control symptoms and achieve disease control. Systemic therapies include bexarotene, Interferon-alpha, extracorporeal photopheresis, histone deacetylase inhibitors, chemotherapy, monoclonal antibodies (Mab), and allogeneic transplantation (5).

Most studies examining systemic treatments in CTCL have been small, single-arm studies with various response criteria. Most commonly, overall response rate (ORR) has been the primary endpoint, resulting in studies with little to no information on extended durability and tolerability. There remains a substantial need for treatments that are both tolerable and offer durable disease control.

Among therapies that have been studied in this setting, antibodies have recently gained prominence with the recent approval of two agents, increasing the limited number of approved systemic therapies in CTCL (see **Table 1**). In this review, we will discuss the approved antibodies as well as select agents in development.

APPROVED ANTIBODY THERAPIES

Brentuximab Vedotin

Brentuximab vedotin (Bv) is a chimeric monoclonal antibody-drug conjugate consisting of an anti-CD30 antibody linked to an anti-microtubule agent, monomethyl auristatin E (MMAE). CD30 is relatively specific for activated leukocytes and certain hematological malignancies including Hodgkin lymphoma and ALCL. CD30 is variably expressed in MF and SS, with higher expression noted in 24% of patients with advanced MF (15, 16). Binding of the antibody to CD30 results in endocytosis of MMAE and cell death ensues via cell cycle arrest and apoptosis. Based on the positive results seen in the SG035-003 study, brentuximab vedotin was approved in 2011 in patients with relapsed/refractory Hodgkin lymphoma and systemic ALCL (17).

Based on the benefit seen in patients with Hodgkin lymphoma and ALCL, two phase II studies of Bv in CTCL were initiated. In the first study, 30 patients with MF or SS with variable CD30 expression were included with objective global response as the primary endpoint. Patients received Bv 1.8 mg/kg IV every 3 weeks for a maximum of 16 doses. Objective global response was seen in 21 patients (70% in all patients, 50% in SS patients), with one patient achieving a complete response (CR). There was no statistically significant difference in response rates between early or advanced stages. However, patients with a CD30 expression of <5% had a much lower likelihood of response compared to higher CD30 levels. The 12-month progression free survival (PFS) rate was 54% including 5 patients who remained on therapy at time of analysis. Median duration of response was not reported. The most common adverse events were peripheral neuropathy (66%), fatigue (47%), nausea (28%), alopecia (22%), and neutropenia (19%). Ten patients had a dose delay and/or reduction, and six patients (19%) were terminated from the study prematurely due to toxicities, with peripheral neuropathy as the most likely cause (18).

In the second phase II study, 48 patients with CTCL and lymphomatoid papulosis were treated with Bv 1.8 mg/kg IV every 3 weeks with ORR as the primary endpoint (19). An ORR of 73% with a CR rate of 35% was observed. In the MF and SS cohorts, ORR was >50% regardless of the degree of CD30 expression. Median duration of response (DoR) in all patients was 7.4 months (<1–23 months). Peripheral neuropathy was the most common side effect with grade 1 to 2 peripheral neuropathy observed in 65% of patients. Grade 3–4 events included neutropenia, nausea, chest pain, deep vein thrombosis, transaminitis, and dehydration. Dose reductions to 1.2 mg/kg were instituted due to neuropathy, transaminitis, arthralgias, and fatigue.

In the first frontline phase III study comparing a novel systemic therapy with standard therapies in CTCL, the randomized, open-label brentuximAb vedotin phase III trial for Cutaneous T-cell lymphomA aNalyZing pAtient outcomes (ALCANZA) study compared Bv to physician's choice of methotrexate or bexarotene in previously untreated CD30 positive CTCL (13). The primary endpoint was objective response lasting at least 4 months (ORR4). A total of 131 patients were randomized, with 128 patients in the intent-to-treat population and 64 patients assigned to each arm. Results demonstrated a clear benefit in ORR4 of 56.3 vs. 12.5% and in CR of 16 vs. 2%. Median PFS was also significantly longer (16.7 vs. 3.5 months). In patients with MF, the ORR4 rate was 50 vs. 10%. This benefit extended across all disease compartments including patients with skin-only disease (67.7 vs. 16.7%) and skin and other involvement (45.5 vs. 8.8%). CD30 expression ranged between 12 and 67.5% with responses seen at all levels. Median PFS in the MF arm was 15.9 vs. 3.5 months.

Overall, toxicity of Bv was comparable to standard therapies. Serious drug related adverse events (grade ≥3) were seen in 29% in both groups. Toxicities specific to the Bv arm included peripheral neuropathy (67 vs. 4%) resulting in discontinuation of treatment in 9 patients. Discontinuation due to adverse events occurred in 24% patients in the BV group vs. 8% in the PC group.

In November 2017, the FDA approved brentuximab vedotin for use in relapsed/refractory CD30+ mycoises fungoides or primary cutaneous ALCL (20).

Brentuximab vedotin is a durable and effective targeted treatment for patients with CD30+ CTCL with a response rate <50%. Importantly, this benefit was seen across all levels of CD30 expression above 10%. Peripheral neuropathy is the most significant toxicity.

Mogamulizumab

Mogamulizumab is a humanized IgG1 monoclonal antibody which selectively binds to CCR4 (21). CCR4 is normally expressed on type 2 helper and regulatory (Treg) T-cells and has been found to be overexpressed on the surface of tumor cells in various T-cell malignancies, including in up to 52% of patients with different stages of MF and SS (22, 23). In addition to the stimulation of antibody-dependent cellular cytotoxicity, the suppression of Treg cells is thought to play an important role in the efficacy of mogamulizumab (24).

In a phase II study performed in Japan, 37 patients with relapsed/refractory CCR4+ peripheral T-cell lymphoma ($n = 29$) and MF ($n = 7$) received mogamulizumab at a dose of 1 mg/ kg IV once per week for 8 weeks with ORR as the primary endpoint. The ORR was 34% and median OS was 14.2 months. Amongst the MF subgroup, 2/7 patients had a response (25). Median PFS for the group was not reached, however, for the PTCL group it was 14.2 months. Based on these results, mogamulizumab was approved in Japan in 2014 for relapsed/refractory CCR4-positive peripheral T-cell lymphoma and cutaneous T-cell lymphoma (26).

Subsequently, a multi-center phase I/II study was performed in the US which enrolled 41 patients with CTCL. Patients received mogamulizumab at a dose of 1 mg/kg IV once per week

TABLE 1 | Approved systemic therapies.

Class	Drug	Response rate %	Median DoR	Major toxicities
Immunomodulatory	Extracorporeal photopheresis (6)	5	Not well-defined	Fluid shifts, hypotension, infection, anemia
Antimetabolite	Methotrexate (7)	33	15 months	mucositis, myelosuppression, hepatic, renal toxicity
Retinoid	Bexarotene (8)	45-55	10–13 months	hyperlipemia, hypercholesterolemia hypothyroidism
HDAC inhibitor	Vorinostat (9)	30	6 months	fatigue, diarrhea, nausea, thrombocytopenia, anorexia, taste abnormalities, weight loss, and muscle spasms
HDAC inhibitor	Romidepsin (10, 11)	34	13.7–15 months	nausea vomiting, fatigue, myelosuppression, QT interval changes
IL-2 Fusion Protein	Denileukin diftitox (12)	44	7.8 months	nausea, pyrexia, fatigue, rash, LFT abnormalities, vision changes, and capillary leak syndrome
Antibody-Drug conjugate	Brentuximab vedotin (13)	56	15.1 months	peripheral neuropathy, GI upset (nausea, diarrhea, vomiting), alopecia, pruritus, pyrexia, decreased appetite, and fatigue
Monoclonal antibody	Mogamulizumab (14)	28	15.5–25.5 months	infusion-related reactions, drug rash, diarrhea, and fatigue

for 4 weeks and then every 2 weeks until disease progression. Among 38 evaluable patients (21 MF, 17 SS), no dose-limiting toxicities were observed, and the maximum tolerated dose was not reached. In the phase II component the observed ORR was 37%. Patients with SS had a higher response rate (48%) compared with MF (28.6%). Responses were noted in all compartments in both MF and SS. Median PFS and duration of response (DOR) for the entire group were 11.4 and 10.4 months, respectively. The majority of toxicities were grade 1 or 2 with nausea, chills, infusion reaction, pyrexia, and fatigue being the most common. 7 patients (17%) developed a cutaneous skin eruption necessitating study withdrawal (27).

This led to the randomized, open-label phase 3 study comparing Mogamulizumab vs. VORinostat In patients with previously treated CTCL (MAVORIC). In this study, 372 patients with relapsed/refractory MF or SS who had failed at least one line of systemic therapy were randomized to receive 1 mg/kg of mogamulizumab IV weekly for the first 4 weeks and then every 2 weeks, or vorinostat 400 mg po daily. PFS was the primary endpoint. Results demonstrated superior PFS in the mogamulizumab arm with a median PFS of 7.7 months compared to 3.1 months in the vorinostat group. The hazard ratio for patients with MF was 0.72 (0.51–1.01) compared to 0.32 in patients with SS (0.21–0.49). Also noted was an increased ORR of 28% with mogamulizamab compared to 4.8% with vorinostat. The response rate was higher amongst patients with SS (37%) than in patients with MF (21%). Duration of response also differed, with median of 13.1 months in MF compared with 17.3 months in SS. Patients achieved responses in skin (42%), blood (68%), and lymph nodes (17%). Adverse events were higher with mogamulizumab, with the most common being infusion reaction (34%), and skin eruption (24%). Discontinuation of treatment due to drug rash occurred in 7% of patients. There were also two reported cases of Stevens-Johnson syndrome, including one that resulted in death. These reactions are thought to be the result of dramatic decreases in the number of Treg cells resulting in suppression of their immunosuppressive properties (14).

Based on the results of these studies, mogamulizumab received FDA approval in August, 2018 for previously treated patients with mycosis fungoides or Sézary syndrome (28).

Mogamulizumab is a generally tolerable and effective therapy in CTCL with particularly high response in leukemic disease and well-demonstrated durability that is applicable to the majority of patients with CTCL. Skin toxicity remains a prominent concern and careful monitoring and assessment of skin rashes on therapy is important to distinguish toxicity against progression of disease.

Denileukin Diftitox

Denileukin diftitox or DAB389IL-2, is a genetically engineered IL-2 fusion toxin fusion protein designed to direct the cell-killing action of diphtheria toxin to cells which express the IL-2 receptor, of which CD25 is a component. CD25 expression occurs in approximately 50% of patients with CTCL. DAB389IL-2 binds selectively to CD25 after which it is internalized and eventually released in the cytosol where it inhibits protein synthesis and leads to cell death (29).

A phase I trial of an initial version of denileukin diftitox (DAB486IL-2) was conducted in patients with CD25 expressing hematological malignancies with demonstration of safety and tolerability and a ORR of 17% in CTCL patients, including 1 CR (30). A subsequent phase II study involving 14 patients with advanced CTCL resulted in 1 partial response (PR) and 2 patients with SS who demonstrated major cutaneous improvement without change in circulating Sezary cells (31).

A form of the fusion protein DAB389IL-2 with higher affinity to CD25 was then developed. In a phase I/II clinical trial of patients with NHL, HL, or CTCL with IL-2R expression (>20% by IHC), 35 patients received DAB389IL-2 in a dose escalation manner on days 1–5 of each 21-day cycle. Doses of <31 mcg/kg/day were well tolerated. DAB389IL-2 demonstrated significant activity in patients with CTCL with an ORR of 37%, including 5 CR. Infusion-related reactions developed in 74% of patients. Fever, chills, nausea, asthenia, and mild hypotension were noted in 50% of patients (32). Following these results, a randomized phase III multicenter trial compared two different doses of DAB389IL-2 in 73 patients with advanced stage recurrent CTCL who had received a median of 5 lines of previous therapy. Patients were randomly assigned to receive 9 or 18

mcg/kg/day IV for five consecutive days and treatment was repeated every 21 days for up to 8 cycles. Results demonstrated an ORR of 30% with 10% of patients achieving a CR and a mDOR of 4.4 months. There was no association between response and dose. Infusion reactions were the most common side effect (74%) with a significant number of patients developing vascular leak syndrome (23%), skin rash (42%), and grade 3/4 transaminitis (38%) (33).

A subsequent placebo-controlled phase III clinical trial was conducted in 144 patients with stage IA to III CD25+ CTCL, 123 of which had MF and 9 with SS (12). Patients received either 9 or 18 μg/kg/day for 5 days every 21 days. ORR was significantly higher in the 18 mcg/kg/day arm at 44 (10% CR) vs. 15.9%. Median PFS was also significantly longer compared with placebo (26.1 vs. 4 months). Median DOR was 7.8 months compared to 2.7 months. Significant reported adverse events included nausea (10%), pyrexia (11%), fatigue (12%), and capillary leak syndrome (4%). Discontinuation due to treatment was seen in 17% of patients.

DAB389IL-2 received full FDA approval in 2008 for patients with resistant and recurrent CTCL (34). Unfortunately, production of DAB389IL-2 was discontinued in 2014 due to production issues related to the bacterial expression system and it is currently not available for clinical use.

Denileukin diftitox (Ontak) is an active and durable treatment option for patients with CD-25 positive CTCL, although currently unavailable. Given the substantial potential benefit, however, novel agents targeting the IL2-R mechanism are worthy of further development.

EXPERIMENTAL ANTIBODY THERAPIES

PD-1/PD-L1

Following the demonstration of Programmed Death-ligand 1 (PD-L1) expression in a subset of patients with MF and SS (35), a phase 2 study of pembrolizumab in 24 patients with MF ($n = 9$) and SS ($n = 15$) who were previously treated with at least 1 systemic therapy was initiated. Patients received 2 mg/kg IV every 3 weeks for up to 2 years. In preliminary results published in abstract form after a median follow up of 51 weeks, the ORR was 38% with 1 CR and 8 PR (56% RR in MF, 27% in SS). The median duration of response was 14 months and 2-year PFS rate was 69%. The toxicity profile was consistent with immune-mediated adverse events seen in other studies with one case of grade 2 pneumonitis and one case of grade 3 diarrhea (36). More recently, a phase II study assessed pembrolizumab in 18 patients with relapsed or refractory systemic T-cell lymphomas. In 13 evaluable patients (7 with PTCL-NOS, 3 with MF), the ORR was 33% with 4 patients achieving complete response. The median PFS was 3.2 months and median OS was 10.6 months. The trial was halted early after a preplanned futility analysis for PFS. Of note, 1 out of the 3 patients with MF had an ongoing complete response (37). Nivolumab has been studied in a phase Ib study in patients with relapsed or refractory hematologic malignancies, including 13 patients with MF. An ORR of 15% (2/13) was noted with both being partial responses. Median PFS was 10 weeks (38). There is an ongoing phase II study combining pembrolizumab with IFN-gamma in patients with previously treated MF or SS (NCT03063632).

Early data suggests that immunotherapy with anti-PD1/PD-L1 agents offers a promising approach in CTCL with ongoing research combining these agents with other immunomodulatory agents in an effort to achieve a synergistic response.

IL-2 Fusion Toxin

After the discontinuation of denileukin diftitox, several novel IL-2 fusion proteins have been developed and are currently in pre-clinical testing. E777 is a recombinant cytotoxic fusion protein composed of diphtheria toxin fragments A and B and human IL-2. In a phase 1 open-label study in 17 patients with CTCL (13 with MF, 4 with SS) patients received doses ranging from 6 to 15 mcg/kg IV for the first 5 days of every 21-day cycle. Results of the lead-in portion demonstrated an ORR in 5 patients including 3 patients with stage IV disease (39). A phase 1 study of E7777 was conducted in Japan in 13 patients with relapsed/refractory PTCL ($n = 10$) and CTCL ($n = 3$, all with MF). Patients received 6, 12, and 9 mcg/kg IV with the same schedule. 9 mcg/kg/day was the maximum tolerated dose. Partial response was observed in 5/13 patients including 1/3 patients with MF. It was generally well tolerated at the recommended dose and with steroid pre-treatment with decreased rates of capillary leak syndrome, and infusion reaction compared to denileukin diftitox (40). There is an ongoing phase 2 study assessing the safety and efficacy of E7777 in persistent/recurrent CTCL (NCT01871727).

E777 represents a new class of IL-2 fusion toxin agents which may offer the benefit of denileukin diftitox with an improved toxicity profile.

Alemtuzumab

Alemtuzumab is a humanized IgG1 antibody directed against CD52, which is expressed on most malignant lymphocytes but is not expressed on hematopoietic stem cells. Binding of the antibody results in cell death via various proposed mechanism including antibody-dependent cellular cytotoxicity, complement-mediated cell lysis, and apoptosis (41) (28). In a phase 2 study of 22 patients with advanced MF ($n = 15$) and SS ($n = 7$), patients were treated with a rapidly escalating dose regimen, followed by 30 mg 3 times a week for up to 12 weeks. This resulted in an ORR of 52% in all patients, of which 32% achieved CR. In addition, 6/7 patients with blood involvement had a response. Median time to treatment failure was 12 months (5–32+ months). CMV reactivation occurred in 18% of patients and another 27% of patients had other infections (42). A retrospective study in 39 patients with MF ($n = 16$) and SS ($n = 23$) who received alemtuzumab 30 mg two to three times per week for a median duration of 12 weeks (range 1–35)

demonstrated an ORR of 51% in all patients, with a 70% response rate in patients with SS compared to 25% in patients with MF (43). A small number of patients exhibited a durable response. However, severe infectious complications were common. 62% of patients had a grade 3 or higher infectious adverse event resulting in treatment discontinuation in 44% and 2 deaths.

In 2012, alemtuzumab was withdrawn from market in the US and Europe and has since been reintroduced as a treatment for multiple sclerosis with availability on a compassionate use basis in hematologic and oncologic indications.

Alemtuzumab is an effective therapeutic option, especially in patients with leukemic disease however it's use is limited due to high rates of serious infectious complications.

IPH4102

KIR3DL2 negatively modulates immune effector cell functions through binding to HLA Class-1 ligands (44). Normally, KIR3DL2 expression is restricted to minor subpopulations of NK cells and T cells. However, KIR3DL2 has been found to be expressed in all subtypes of CTCL, with the highest prevalence of expression in SS and transformed MF (45). IPH4102 is an anti-KIR3DL2 antibody developed for the treatment of cutaneous T-cell lymphoma (46).

Preliminary results in the SS subset of a phase I dose-escalation and expansion study of IPH4102 in advanced CTCL have been published in abstract form (47). Patients received 10 dose levels up to 10 mg/kg in an accelerated 3 + 3 design with recommended cohort expansion dose of 750 mg. Results demonstrate excellent tolerability and an ORR of 42.9% with median duration of response of 13.8 months and median progression-free survival of 11.7 months (47, 48). Clinical activity was associated with a substantial improvement in quality of life and displayed a favorable safety profile.

Based on these results, the FDA has granted fast track designation to IPH4102 for the treatment of adult patients with relapsed or refractory Sézary syndrome who have received at least two prior systemic therapies. A multi-cohort phase II study in T-cell lymphoma subtypes will be initiated in the first half of 2019 (NCT03902184).

IPH4102 is an immune NK cell engager with a CTCL specific target that has demonstrated significant promise toward offering a durable and tolerable treatment response.

TTI-621

CD47 is expressed on all normal cells and interacts with SIRPα on the surface of myeloid cells, resulting in inhibition of macrophage phagocytosis. CD47 has been found to be overexpressed on cancer cells, suggesting CD47 antagonists may offer a new potential therapy targeted toward malignancies (49). Recent studies of anti-CD47 antibodies have been proven to have therapeutic effects against the tumor microenvironment in CTCL and there are several CD47 antagonists in active phase I clinical trials (50).

TTI-621 is a recombinant fusion protein composed of human SIRPa fused to the Fc receptor of IgG1 (51). In preliminary results of a phase 1 study (52), nine adult patients with mycosis fungoides and Sezary syndrome were treated with a single intratumoral injection of TTI-621 at a dose of 1, 3, or 10mg. TTI-621 was well tolerated with fatigue, chills, and decreased appetite being the main adverse effects and no dose-limiting toxicities noted. All patients experienced declines in tumor size and had decreased circulating Sezary cells with one patient achieving a CR that was ongoing at time of publication (4.2 months). These results suggest that direct antitumoral injection of TTI-621 can elicit local and systemic anti-tumoral activity. There is also an ongoing phase I study of systemic TTI-621 in hematologic malignancies and select solid tumors, including a cohort with CTCL, which is currently recruiting patients (NCT02663518).

Checkpoint inhibition with anti-CD47 antibodies offer an exciting new potential therapy in many malignancies including CTCL, with evidence of durable and tolerable responses seen with intratumoral administration.

AFM13

AFM13 is a bispecific antibody designed to bind to CD30 and to CD16A, a receptor on NK cells that results in NK-mediated killing of tumor cells (53).

A phase 1 study assessed the safety and tolerability of AFM13 in 28 patients with Hodgkin Lymphoma in the relapsed/refractory setting (54). Patients received doses of 0.01–7 mg/kg. Most adverse events were mild to moderate and the maximum tolerated dose was not reached. Of the 26 evaluable patients, 11.5% had a partial remission and 50% had stable disease. There are multiple additional studies ongoing in patients with relapsed/refractory HL.

An investigator-sponsored phase 1/2 study in patients with relapsed/refractory CD30+ CTCL is also ongoing (NCT03192202). Initial results of the first three dose cohorts (9 patients dosed at 1.5–7.0 mg/kg) demonstrated that AFM13 was well tolerated and showed therapeutic activity as a single agent, with an ORR of 44% (4/9) including one CR, three PR, and two patients with SD (55).

AFM13 is an exciting new immunotherapy that is well-tolerated and in preliminary data has demonstrated activity in patients who have failed standard therapies and have limited to no remaining treatment options.

ARGX-110

Overexpression of CD70 has been documented in a variety of cancers, including PTCL and CTCL (56). Signaling mediated by CD70-CD27 is thought to induce proliferation and survival of tumor cells via activation of the NF-κB pathway. In addition, T-reg cells expressing CD27 can be activated by CD70 cells and contribute to an immunosuppressive tumor microenvironment

TABLE 2 | Experimental antibody therapies.

Class	Drug	ORR	# of patients	Study phase
IL-2 fusion protein	E777	29%	17	Phase I (39)
		38%	13	Phase I (40)
Anti-CD52 Mab	Alemtuzumab	52%	22	Phase II (42)
		51%	39	Retrospective (43)
Anti-KIR3DL2 Mab	IPH4102	43%	13	Phase I (47)
Anti-PD-1/PD-L1 Mab	Pembrolizumab	38%	24	Phase II (36)
	Nivolumab	15%	13	Phase I (38)
Anti-CD47 Mab	TTI-621	Not reported	9	Phase I (52)
Bispecific CD30-CD16 ab	AFM13	44%	9	Phase I (55)
Anti-CD70 Mab	ARGX-110	56%	16	Phase I (60)

(57, 58). ARGX-110 is a human, defucosylated IgG_1 monoclonal antibody that binds to CD70 and blocks signaling mediated by its interaction with CD27. In addition to interrupting CD70-CD27 signaling, ARGX-110 also induces antibody-dependent cellular cytotoxicity in CD70+ tumor cells (58). A phase 1b trial focused on the use of ARGX-110 in T-cell malignancies is currently ongoing (NCT01813539). Initial results have demonstrated overexpression of CD70 in 28/26 patients. ARGX-110 has been administered to 16 patients with CTCL with disease control and response seen in 9 patients, including 3 PR. Treatment has been well tolerated with no grade 3 toxicities noted to date (59, 60).

ARG-110X offers an attractive potential therapy with early evidence of anti-tumor activity that is well tolerated.

CONCLUSION

There is a significant need for new systemic treatments that offer durable and tolerable responses in patients with CTCL. Antibody-directed therapies offer significant potential in these incurable illnesses with the potential for lasting responses and manageable toxicity. The recent approval of the CD30 directed antibody-drug conjugate brentuximab vedotin and anti-CCR4 antibody mogamulizumab represent ground-breaking developments in this process. There are additional promising modalities currently under study including anti-PD-1/PD-L1 therapy, KIR3DL2 inhibition, and novel IL-2 fusion toxins, amongst others (see **Table 2**). These advances have been based on extensive work understanding the molecular characteristics, intracellular signaling pathways, and interaction with the tumor microenvironment in CTCL, which continue to offer new targets of potential treatment. Toxicities remain a prominent concern with these therapies and will need to be closely examined in the evaluation of novel agents and combination regimens.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

1. Teras LR, DeSantis CE, Cerhan JR, Morton LM, Jemal A, Flowers CR. 2016 US lymphoid malignancy statistics by World Health Organization subtypes. *CA Cancer J Clin.* (2016) 66:443–59. doi: 10.3322/caac.21357
2. Wilcox RA. Cutaneous T-cell lymphoma: 2016 update on diagnosis, risk-stratification, and management. *Am J Hematol.* (2016) 91:151–65. doi: 10.1002/ajh.24233
3. Kim YH, Liu HL, Mraz-Gernhard S, Varghese A, Hoppe RT. Long-term outcome of 525 patients with mycosis fungoides and Sezary syndrome: clinical prognostic factors and risk for disease progression. *Arch Dermatol.* (2003) 139:857–66. doi: 10.1001/archderm.139.7.857
4. Kim YH, Bishop K, Varghese A, Hoppe RT. Prognostic factors in erythrodermic mycosis fungoides and the Sezary syndrome. *Arch Dermatol.* (1995) 131:1003–8. doi: 10.1001/archderm.131.9.1003
5. Trautinger F, Knobler R, Willemze R, Peris K, Stadler R, Laroche L, et al. EORTC consensus recommendations for the treatment of mycosis fungoides/Sezary syndrome. *Eur J Cancer.* (2006) 42:1014–30. doi: 10.1016/j.ejca.2006.01.025
6. Lim HW, Edelson RL. Photopheresis for the treatment of cutaneous T-cell lymphoma. *Hematol Oncol Clin.* (1995) 9:1117–26. doi: 10.1016/S0889-8588(18)30062-5
7. Zackheim HS, Kashani-Sabet M, McMillan A. Low-dose methotrexate to treat mycosis fungoides: a retrospective study in 69 patients. *J Am Acad Dermatol.* (2003) 49:873–8. doi: 10.1016/S0190-9622(03)01591-3
8. Duvic M, Hymes K, Heald P, Breneman D, Martin AG, Myskowski P, et al. Bexarotene is effective and safe for treatment of refractory advanced-stage cutaneous T-cell lymphoma: multinational phase II-III trial results. *J Clin Oncol.* (2001) 19:2456–71. doi: 10.1200/JCO.2001.19.9.2456
9. Olsen EA, Kim YH, Kuzel TM, Pacheco TR, Foss FM, Parker S, et al. Phase IIb multicenter trial of vorinostat in patients with persistent, progressive, or treatment refractory cutaneous T-cell lymphoma. *J Clin Oncol.* (2007) 25:3109–15. doi: 10.1200/JCO.2006.10.2434
10. Whittaker SJ, Demierre MF, Kim EJ, Rook AH, Lerner A, Duvic M, et al. Final results from a multicenter, international, pivotal study of romidepsin in refractory cutaneous T-cell lymphoma. *J Clin Oncol.* (2010) 28:4485–91. doi: 10.1200/JCO.2010.28.9066
11. Piekarz RL, Frye R, Turner M, Wright JJ, Allen SL, Kirschbaum MH, et al. Phase II multi-institutional trial of the histone deacetylase inhibitor

romidepsin as monotherapy for patients with cutaneous T-cell lymphoma. *J Clin Oncol.* (2009) 27:5410–7. doi: 10.1200/JCO.2008.21.6150
12. Prince HM, Duvic M, Martin A, Sterry W, Assaf C, Sun Y, et al. Phase III placebo-controlled trial of denileukin diftitox for patients with cutaneous T-cell lymphoma. *J Clin Oncol.* (2010) 28:1870–7. doi: 10.1200/JCO.2009.26.2386
13. Prince HM, Kim YH, Horwitz SM, Dummer R, Scarisbrick J, Quaglino P, et al. Brentuximab vedotin or physician's choice in CD30-positive cutaneous T-cell lymphoma (ALCANZA): an international, open-label, randomised, phase 3, multicentre trial. *Lancet.* (2017) 390:555–66. doi: 10.1016/S0140-6736(17)31266-7
14. Kim YH, Bagot M, Pinter-Brown L, Rook AH, Porcu P, Horwitz SM, et al. Mogamulizumab versus vorinostat in previously treated cutaneous T-cell lymphoma (MAVORIC): an international, open-label, randomised, controlled phase 3 trial. *Lancet Oncol.* (2018) 19:1192–204. doi: 10.1016/S1470-2045(18)30379-6
15. Duvic M. CD30+ neoplasms of the skin. *Curr Hematol Malig Rep.* (2011) 6:245–50. doi: 10.1007/s11899-011-0096-8
16. Diamandidou E, Colome-Grimmer M, Fayad L, Duvic M, Kurzrock R. Transformation of mycosis fungoides/Sezary syndrome: clinical characteristics and prognosis. *Blood.* (1998) 92:1150–9.
17. Younes A, Gopal AK, Smith SE, Ansell SM, Rosenblatt JD, Savage KJ, et al. Results of a pivotal phase II study of brentuximab vedotin for patients with relapsed or refractory Hodgkin's lymphoma. *J Clin Oncol.* (2012) 30:2183–9. doi: 10.1200/JCO.2011.38.0410
18. Kim YH, Tavallaee M, Sundram U, Salva KA, Wood GS, Li S, et al. Phase II investigator-initiated study of brentuximab vedotin in mycosis fungoides and sezary syndrome with variable CD30 expression level: a multi-institution collaborative project. *J Clin Oncol.* (2015) 33:3750–8. doi: 10.1200/JCO.2014.60.3969
19. Duvic M, Tetzlaff MT, Gangar P, Clos AL, Sui D, Talpur R. Results of a phase II trial of brentuximab vedotin for CD30+ cutaneous T-cell lymphoma and lymphomatoid papulosis. *J Clin Oncol.* (2015) 33:3759–65. doi: 10.1200/JCO.2014.60.3787
20. FDA, FDA approves first-line treatment for peripheral T-cell lymphoma under new review pilot. (2018).
21. Ishii T, Ishida T, Utsunomiya A, Inagaki A, Yano H, Komatsu H, et al. Defucosylated humanized anti-CCR4 monoclonal antibody KW-0761 as a novel immunotherapeutic agent for adult T-cell leukemia/lymphoma. *Clin Cancer Res.* (2010) 16:1520–31. doi: 10.1158/1078-0432.CCR-09-2697
22. Jones D, Patel K, Hering M, Aguilar RJ, Ueda R, Inagaki H, et al. Upregulation of the chemokine receptor CCR4 in cutaneous T-cell lymphoma and nodal T-cell lymphoma: implications for targeted therapy. *Blood.* (2004) 104:2646.
23. Sugaya M, Morimura S, Suga H, Kawaguchi M, Miyagaki T, Ohmatsu H, et al. CCR4 is expressed on infiltrating cells in lesional skin of early mycosis fungoides and atopic dermatitis. *J Dermatol.* (2015) 42:613–5. doi: 10.1111/1346-8138.12852
24. Ni X, Langridge T, Duvic M. Depletion of regulatory T cells by targeting CC chemokine receptor type 4 with mogamulizumab. *Oncoimmunology.* (2015) 4:e1011524. doi: 10.1080/2162402X.2015.1011524
25. Ogura M, Ishida T, Hatake K, Taniwaki M, Ando K, Tobinai K, et al. Multicenter phase II study of mogamulizumab (KW-0761), a defucosylated anti-cc chemokine receptor 4 antibody, in patients with relapsed peripheral T-cell lymphoma and cutaneous T-cell lymphoma. *J Clin Oncol.* (2014) 32:1157–63. doi: 10.1200/JCO.2013.52.0924
26. Kyowa Hakko Kirin Co. Ltd. *Approval for Additional Indication for Chemotherapy-Native CCR4-Positive ATL of Mogamulizumab* (2014).
27. Duvic M, Pinter-Brown LC, Foss FM, Sokol L, Jorgensen JL, Challagundla P, et al. Phase 1/2 study of mogamulizumab, a defucosylated anti-CCR4 antibody, in previously treated patients with cutaneous T-cell lymphoma. *Blood.* (2015) 125:1883–9. doi: 10.1182/blood-2014-09-600924
28. FDA, POTELIGEO® (mogamulizumab-kpkc) injection, for intravenous use Initial U.S. Approval: 2018. 2018.
29. Nichols J, Foss F, Kuzel TM, LeMaistre CF, Platanias L, Ratain MJ, et al. Interleukin-2 fusion protein: an investigational therapy for interleukin-2 receptor expressing malignancies. *Eur J Cancer.* (1997) 33 (Suppl. 1):S34–6. doi: 10.1016/S0959-8049(96)00327-9
30. LeMaistre CF, Meneghetti C, Rosenblum M, Reuben J, Parker K, Shaw J, et al. Phase I trial of an interleukin-2 (IL-2) fusion toxin (DAB486IL-2) in hematologic malignancies expressing the IL-2 receptor. *Blood.* (1992) 79:2547–54.
31. Foss FM, Borkowski TA, Gilliom M, Stetler-Stevenson M, Jaffe ES, Figg WD, et al. Chimeric fusion protein toxin DAB486IL-2 in advanced mycosis fungoides and the Sezary syndrome: correlation of activity and interleukin-2 receptor expression in a phase II study. *Blood.* (1994) 84:1765–74.
32. Saleh MN, LeMaistre CF, Kuzel TM, Foss F, Platanias LC, Schwartz G, et al. Antitumor activity of DAB389IL-2 fusion toxin in mycosis fungoides. *J Am Acad Dermatol.* (1998) 39:63–73. doi: 10.1016/S0190-9622(98)70403-7
33. Olsen E, Duvic M, Frankel A, Kim Y, Martin A, Vonderheid E, et al. Pivotal phase III trial of two dose levels of denileukin diftitox for the treatment of cutaneous T-cell lymphoma. *J Clin Oncol.* (2001) 19:376–88. doi: 10.1200/JCO.2001.19.2.376
34. FDA, Ontak Label (2008).
35. Wilcox RA, Feldman AL, Wada DA, Yang ZZ, Comfere NI, Dong H, et al. B7-H1 (PD-L1, CD274) suppresses host immunity in T-cell lymphoproliferative disorders. *Blood.* (2009) 114:2149–58. doi: 10.1182/blood-2009-04-216671
36. Khodadoust MS, Rook AH, Porcu P, Foss F, Moskowitz A, Shustov AR, et al. Pembrolizumab in mycosis fungoides and Sezary syndrome: updated results of the CITN multicenter phase 2 study. *Eur J Cancer.* (2018) 101:S37.
37. Barta SK, Zain J, MacFarlane AW, Smith SM, Ruan J, Fung HC, et al. Phase II study of the PD-1 inhibitor pembrolizumab for the treatment of relapsed or refractory mature T-cell lymphoma. *Clin Lymphoma Myeloma Leuk.* (2019) 19:356–364.e3. doi: 10.1016/j.clml.2019.03.022
38. Lesokhin AM, Ansell SM, Armand P, Scott EC, Halwani A, Gutierrez M, et al. Nivolumab in patients with relapsed or refractory hematologic malignancy: preliminary results of a phase Ib study. *J Clin Oncol.* (2016) 34:2698–704. doi: 10.1200/JCO.2015.65.9789
39. Duvic M, Kuzel TM, Dang NH, Prince M, Feldman T, Foss FM, et al., A dose finding lead-in study of E7777 (Diphtheria toxin fragment-Interleukin-2 Fusion Protein) in persistent or recurrent cutaneous T-cell lymphoma (CTCL). *Blood.* (2014) 124:3097.
40. Ohmachi K, Ando K, Ogura M, Uchida T, Tobinai K, Maruyama D, et al. E7777 in Japanese patients with relapsed/refractory peripheral and cutaneous T-cell lymphoma: a phase I study. *Cancer Sci.* (2018) 109:794–802. doi: 10.1111/cas.13513
41. Gilleece MH, Dexter TM. Effect of campath-1H antibody on human hematopoietic progenitors *in vitro*. *Blood.* (1993) 82:807–12.
42. Lundin J, Hagberg H, Repp R, Cavallin-Ståhl E, Fredén S, Juliusson G, et al. Phase 2 study of alemtuzumab (anti-CD52 monoclonal antibody) in patients with advanced mycosis fungoides/Sezary syndrome. *Blood.* (2003) 101:4267–72. doi: 10.1182/blood-2002-09-2802
43. de Masson A, Guitera P, Brice P, Moulonguet I, Mouly F, Bouaziz JD, et al. Long-term efficacy and safety of alemtuzumab in advanced primary cutaneous T-cell lymphomas. *Br J Dermatol.* (2014) 170:720–4. doi: 10.1111/bjd.12690
44. Thielens A, Vivier E, RomagneF. NK cell MHC class I specific receptors (KIR): from biology to clinical intervention. *Curr Opin Immunol.* (2012) 24:239–45. doi: 10.1016/j.coi.2012.01.001
45. Bagot M, Moretta A, Sivori S, Biassoni R, Cantoni C, Bottino C, et al. CD4(+) cutaneous T-cell lymphoma cells express the p140-killer cell immunoglobulin-like receptor. *Blood.* (2001) 97:1388–91. doi: 10.1182/blood.V97.5.1388
46. Marie-Cardine A, Viaud N, Thonnart N, Joly R, Chanteux S, Gauthier L, et al. IPH4102, a humanized KIR3DL2 antibody with potent activity against cutaneous T-cell lymphoma. *Cancer Res.* (2014) 74:6060–70. doi: 10.1158/0008-5472.CAN-14-1456
47. Bagot M, Porcu P, Ram-Wolff C, Khodadoust M, Battistella M, Marie-Cardine A, et al. First-in-human, multicenter phase I study of IPH4102, First-in-class humanized anti-KIR3DL2 monoclonal antibody, in relapsed/refractory cutaneous T-cell lymphomas: preliminary safety, exploratory and clinical activity results. *Blood.* (2016) 128:1826–6.
48. Van Der Weyden C, Bagot M, Neeson P, Darcy PK, Prince HM. IPH4102, a monoclonal antibody directed against the immune receptor molecule KIR3DL2, for the treatment of cutaneous T-cell lymphoma. *Expert Opin Investig Drugs.* (2018) 27:691–7. doi: 10.1080/13543784.2018.1498081

49. Matlung HL, Szilagyi K, Barclay NA, van den Berg TK. The CD47-SIRPalpha signaling axis as an innate immune checkpoint in cancer. *Immunol Rev*. (2017) 276:145–64. doi: 10.1111/imr.12527
50. Huang Y, Ma Y, Gao P, Yao Z. Targeting CD47: the achievements and concerns of current studies on cancer immunotherapy. *J Thorac Dis*. (2017) 9:E168–74. doi: 10.21037/jtd.2017.02.30
51. Petrova PS, Viller NN, Wong M, Pang X, Lin GH, Dodge K, et al. TTI-621 (SIRPalphaFc): a CD47-blocking innate immune checkpoint inhibitor with broad antitumor activity and minimal erythrocyte binding. *Clin Cancer Res*. (2017) 23:1068–79. doi: 10.1158/1078-0432.CCR-16-1700
52. Querfeld C, Thompson J, Taylor M, Pillai R, Johnson LD, Catalano T, et al. A single direct intratumoral injection of TTI-621 (SIRPαFc) induces antitumor activity in patients with relapsed/refractory mycosis fungoides and sézary syndrome: preliminary findings employing an immune checkpoint inhibitor blocking the CD47 "do not eat" signal. *Blood*. (2017) 130(Suppl. 1):4076.
53. Reusch U, Burkhardt C, Fucek I, Le Gall F, Le Gall M, Hoffmann K, et al. A novel tetravalent bispecific TandAb (CD30/CD16A) efficiently recruits NK cells for the lysis of CD30+ tumor cells. *MAbs*. (2014) 6:728–39. doi: 10.4161/mabs.28591
54. Rothe A, Sasse S, Topp MS, Eichenauer DA, Hummel H, Reiners KS, et al., A phase 1 study of the bispecific anti-CD30/CD16A antibody construct AFM13 in patients with relapsed or refractory Hodgkin lymphoma. *Blood*. (2015) 125:4024–31. doi: 10.1182/blood-2014-12-614636
55. NVA. *Affimed Presents Data at ASH 2018 Substantiating Opportunity for AFM13 as Mono- and Combination Therapy in CD30-Positive Tumors*. San Diego, CA (2018).
56. Boursalian TE, McEarchern JA, Law CL, Grewal IS.Boursalian TE, et al., Targeting CD70 for human therapeutic use. *Adv Exp Med Biol*. (2009) 647:108–19. doi: 10.1007/978-0-387-89520-8_7
57. Silence K, Dreier T, Moshir M, Ulrichts P, Gabriels SM, Saunders M, et al. ARGX-110, a highly potent antibody targeting CD70,eliminates tumors via both enhanced ADCC and immune checkpoint blockade. *MAbs*. (2014) 6:523–32. doi: 10.4161/mabs.27398
58. Awada A, Rolfo CD, Rottey S, de Lendonck LY, Schroyens WA, Offner F,et al., A phase I, first-in-human study of ARGX-110, a monoclonal antibody targeting CD70, a receptor involved in immune escape and tumor growth in patients with solid and hematologic malignancies. *J Clin Oncol*. (2014) 32 (15_suppl):3023. doi: 10.1200/jco.2014.32.15_suppl.3023
59. Michot J-M, Maerevoet M, Aftimos PG, Rottey S, Rolfo D, Offner F, et al. Clinical response observed in a phase I study in T cell lymphoma patients treated with anti-CD70 SIMPLE Antibody ARGX-110. *J Clin Oncol*. (2016) 34(15_suppl):7556. doi: 10.1200/JCO.2016.34.15_suppl.7556
60. Hultberg A, Gandini D, Bagot M, Maerevoet M, Zwanenpoel K, De Winne K, et al. CD70 EXPRESSION IN CUTANEOUS T CELL LYMPHOMA (CTCL) PATIENTS AND MECHANISMS OF ACTION OF ARGX-110 IN SKIN: HISTOPATHOLOGICAL AND CLINICAL DATA. *Hematol Oncol*. (2017) 35:275. doi: 10.1002/hon.2438_148

Conflict of Interest Statement: AS: Consultation: Seattle Genetics and Kyowa Kirin. Research support: Seattle Genetics, Affimed.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

OPINION
published: 09 October 2019
doi: 10.3389/fonc.2019.01025

Immunomodulation in Cutaneous T Cell Lymphoma

*Martina Ferranti, Giulia Tadiotto Cicogna, Irene Russo and Mauro Alaibac**

Unit of Dermatology, University of Padua, Padua, Italy

Keywords: cutaneous T-cell lymphoma, immunosuppression, HIV, CD30 cutaneous T-cell lymphoma, mycosis fungoides

Primary cutaneous lymphomas (PCL), defined as lymphomas limited to the skin with no evidence of extracutaneous disease at the time of diagnosis, are a heterogeneous group of lymphoproliferative disorders. They encompass cutaneous T-cell lymphomas (CTCL) (65% of all cases), cutaneous B-cell lymphomas (CBCL) (25% of all cases) and other rarer variants (1, 2).

The most common CTCL subtype is mycosis fungoides (MF) which together with Sézary syndrome (SS) accounts for about 65% of all CTCLs (1). The neoplastic cells of CTCL usually express a CD3+CD4+ mature helper T-cell phenotype. CTCLs include also the group of primary cutaneous CD30+ lymphoproliferative disorders which represent at least 25% of all CTCLs (1–3). These conditions are generally characterized by expression of the CD30 molecule by more than 75% of neoplastic lymphocytes. In particular, the group of CD30+ lymphoproliferative disorders of the skin includes two subtypes: lymphomatoid papulosis (LyP) and primary anaplastic large cell lymphoma (PALCL) (4). However, these two entities differ significantly in several aspects. In contrast to LyP, PALCL may spread to extracutaneous sites in 10–20% of the patients, whereas the classical type of LyP is characterized clinically by recurrent self-healing lesions and there is no risk of spread to extracutaneous sites. The presence of clonality by TCR gene rearrangements is detected only in about 40% LyP cases in contrast to ALCL where it is observed in about 90% of cases (4). Moreover, in a study investigating the prevalence of PCLs in solid organ transplant recipients it has been demonstrated a significant increase of PALCL in this group of patients whereas only one case of LyP was reported (5). This is consistent with view that LyP is probably a reactive conditions whose onset is hampered by the immunosuppressive therapy, whereas ALCL is a true lymphoma and consequently its development is favored by the immunosuppressive regimen. On the other hand, there are data demonstrating an increased risk of MF in patients with LyP (6). Gene expression analysis in these disorders may help to shed light on this issue.

The role of viral agents in CTCLs is still debated, but it has not possible to date to convincingly establish a causal correlation between a viral infection and CTCL development (7). A recent study investigated CTCLs in HIV-infected and non-HIV-infected patients (8). Data were obtained from the Surveillance, Epidemiology, and End Results program, 1973–2013, of the U.S. National Cancer Institute and showed that HIV-infected patients with CTCL experienced significantly higher survival and a decreased risk of overall mortality than non-HIV-infected patients. Moreover, these results indicated that HIV infection was an independent protective factor. These epidemiological data suggest that a HIV-based therapeutic approach could be appropriate for CTCL. To this regard, previous studies generated a retroviral vector which specifically transfers genes into CD4+ cells by pseudotyping of murine leukemia virus (MLV) capsid particles with a variant of the HIV-1 envelope protein (9, 10). These vectors are suitable for gene therapy of CTCL, because they are able to deliver therapeutic genes exclusively to target cells. A subsequent study developed a xenograft mouse model to study CTCL and generated MLV/HIV-pseudotyped vectors encoding the herpes simplex virus thymidine kinase suicide gene (HSV-TK) (11). Vector particles were administered intratumorally into human CTCL xenografts in nude mice which then underwent systemic

OPEN ACCESS

Edited by:
Catherine Grace Chung, The Ohio State University, United States

Reviewed by:
Brian Poligone, Rochester General Hospital Research Institute (RGHRI), United States

****Correspondence:***
Mauro Alaibac
mauro.alaibac@unipd.it

Specialty section:
This article was submitted to Hematologic Malignancies, a section of the journal Frontiers in Oncology

Received: *15 December 2018*
Accepted: *23 September 2019*
Published: *09 October 2019*

Citation:
Ferranti M, Tadiotto Cicogna G, Russo I and Alaibac M (2019) Immunomodulation in Cutaneous T Cell Lymphoma. Front. Oncol. 9:1025. doi: 10.3389/fonc.2019.01025

treatment with ganciclovir (GCV). Tumor development was significantly delayed in HSV-TK-transduced and GCV-treated tumors. These data demonstrate that the use of MLV/HIV-pseudotyped vectors could be an appropriate approach for the treatment of CTCLs.

Interferons (IFNs) are naturally occurring proteins released by host cells which act as a part of the innate immune response (12). Although IFNs were first identified for their capacity to induce resistance to virus, they also exert a wide range of immunomodulatory and antitumor effects (11). In particular IFN-alpha is an effective treatment for the two main subtypes of CTCL, MF and SS (13). To this regard, IFN alpha promotes the anti-tumor activity of NK cells and CD8+ T lymphocytes (12). However, the efficacy of IFN-alpha is limited by loss of response in a significant proportion of patients. An alternative or adjunctive strategy could be the utilization of the immune checkpoint inhibitors, such as anti-CTLA4 and anti-PD1/PDL1, which have become a new promising way of immunotherapy (14). CTLA4 and PD1 are two immunomodulatory receptors expressed on T cell and involved in the inhibition of immune system. The interaction between the receptors PD1 and CTLA4 and their ligands (PDL1 and CD80/CD86, respectively) induces a downregulation of T cell effector functions leading to the inhibition of the antitumor immune response (14). Thus, PD1 inhibitors (nivolumab and pembrolizumab) and CTLA4 inhibitors (ipilimumab) enhance anti-tumor immune response delaying tumor growth and facilitating tumor rejection. The relationship between immune checkpoint inhibitors and CTCL has not yet been clarified. A significant increase of PD1 expression in peripheral blood malignant T cells has been observed in SS, whereas data regarding overexpression of PD1 and CTLA4 in MF patients are conflicting (15). To this regard, the inhibition of PD1 and CTLA4 may have an important role in controlling the progression of some CTCL and could be investigated as a potential immunotherapy for SS and MF (16). Currently clinical trials over the use of pembrolizumab and nivolumab for the treatment of MF/SS are ongoing (17, 18). Promising results in the use of ipilimumab for the treatment of CTCL, specifically SS, have also been observed (19). Interestingly a case of complete regression of MF after ipilimumab therapy for advanced melanoma has been reported (20). On the other hand, a case of cutaneous CD56 + T cell lymphoma that developed during pembrolizumab treatment for metastatic melanoma has been described by Zheng et al. (21). Recently, the approval of mogamulizumab, a humanized defucosylated anti-CC chemokine receptor 4 (CCR4) monoclonal antibody, has expanded the landscape of drugs for the treatment of advanced CTCL (22). CCR4 is expressed in the vast majority of CTCLs, especially when peripheral blood involvement is present. This receptor plays an essential role in T-lymphocyte migration into the skin. In a recent study mogamulizumab significantly improved progression-free survival in advanced CTCL, especially in those with SS which is a subtype of CTCL characterized by peripheral blood involvement (23). Furthermore, a number of clinical trials are currently focusing on evaluating mogamulizumab with checkpoint inhibitors (NCT03309878, NCT02476123) as a means of improving antitumor immunity. For less advanced MF, topical resiquimod gel, a Toll-like receptor 7/8 agonist, has been used (24). This topical approach triggers innate immune responses that in turn support the induction of tumor-specific immunity. Lesions of MF treated with resiquimod gel significantly improved in 75% of patients and 30% of patients showed complete resolution of all treated lesions (25). Furthermore, in some patients resiquimod promoted distant response of untreated lesions indicating a systemic antitumor effect of the gel (25).

AUTHOR CONTRIBUTIONS

MA conceived the idea. MF, GT, IR, and MA contributed to the design of the article and to the writing of the manuscript.

REFERENCES

1. Willemze R, Jaffe ES, Burg G, Cerroni L, Berti E, Swerdlow SH, et al. WHO-EORTC classification for cutaneous lymphomas. *Blood.* (2005) 105:3768–85. doi: 10.1182/blood-2004-09-3502
2. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood.* (2016) 127:2375–90. doi: 10.1182/blood-2016-01-643569
3. Maurelli M, Tessari G, Colato C, Schena D, Girolomoni G. Incidence and ten-year follow-up of primary cutaneous lymphomas: a single-centre cohort study. *Eur J Dermatol.* (2018) 28:44–9.
4. Chen C, Gu YD, Geskin LJ. A review of primary cutaneous CD30+ lymphoproliferative disorders. *Hematol Oncol Clin North Am.* (2019) 33:121–34. doi: 10.1016/j.hoc.2018.08.003
5. Seçkin D, Barete S, Euvrard S, Francès C, Kanitakis J, Geusau A, et al. Primary cutaneous posttransplant lymphoproliferative disorders in solid organ transplant recipients: a multicenter European case series. *Am J Transplant.* (2013) 13:2146–53. doi: 10.1111/ajt.12281
6. Cordel N, Tressières B, D'Incan M, Machet L, Grange F, Estève É, et al. (2016) Frequency and risk factors for associated lymphomas in patients with lymphomatoid papulosis. *Oncologist* 21:76–83. doi: 10.1634/theoncologist.2015-0242
7. Mirvish JJ, Pomerantz RG, Falo LD Jr, Geskin LJ. Role of infectious agents in cutaneous T-cell lymphoma: facts and controversies. *Clin Dermatol.* (2013) 31:423–31. doi: 10.1016/j.clindermatol.2013.01.009
8. Wang J, Liang R1, Hao C, Liu X, Zhang N, Duan X, et al. Survival outcomes of primary cutaneous T-cell lymphoma in HIV-infected patients: a national population-based study. *J Investig Med.* (2018) 66:762–7. doi: 10.1136/jim-2017-000636
9. Schnierle BS, Stitz J, Bosch V, Nocken F, Merget-Millitzer H, Engelstädter M, et al. Pseudotyping of murine leukemia virus with the envelope glycoproteins of HIV generates a retroviral vector with specificity of infection for CD4-expressing cells. *Proc Natl Acad Sci USA.* (1997) 94:8640–5. doi: 10.1073/pnas.94.16.8640
10. Thaler S, Schnierle BS. A packaging cell line generating CD4-specific retroviral vectors for efficient gene transfer into primary human T-helper lymphocytes. *Mol Ther.* (2001) 4:273–9. doi: 10.1006/mthe.2001.0445
11. Thaler S, Burger AM, Schulz T, Schnierle BS. MLV/HIV-pseudotyped vectors: a new treatment option for cutaneous T cell lymphomas. *Mol Ther.* (2003) 8:756–61. doi: 10.1016/S1525-0016(03)00241-7

12. Pestka S. The interferons: 50 years after their discovery, there is much more to learn. *J Biol Chem.* (2007) 282:20047–51. doi: 10.1074/jbc.R700004200
13. Spaccarelli N, Rook AH. The use of interferons in the treatment of cutaneous T-cell lymphoma. *Dermatol Clin.* (2015) 33:731–45. doi: 10.1016/j.det.2015.05.008
14. Sadreddini S, Baradaran B, Aghebati-Maleki A, Sadreddini S, Shanehbandi D, Fotouhi A, et al. Immune checkpoint blockade opens a new way to cancer immunotherapy. *J Cell Physiol.* (2018) 234:8541–9. doi: 10.1002/jcp.27816
15. Kantekure K, Yang Y, Raghunath P, Schaffer A, Woetmann A, Zhang Q, et al. Expression patterns of the immunosuppressive proteins PD-1/CD279 and PD-L1/CD274 at different stages of cutaneous T-cell lymphoma/mycosis fungoides. *Am J Dermatopathol.* (2012) 34:126–8. doi: 10.1097/DAD.0b013e31821c35cb
16. Alaibac M. Monoclonal antibodies against cutaneous T-cell lymphomas. *Expert Opin Biol Ther.* (2017) 17:1503–10. doi: 10.1080/14712598.2017.1369951
17. Naidoo J, Page DB, Li BT, Connell LC, Schindler K, Lacouture ME, et al. Toxicities of the anti-PD-1 and anti-PD-L1 immune checkpoint antibodies. *Ann Oncol.* (2015) 26:2375–91. doi: 10.1093/annonc/mdv383
18. Lesokhin AM, Ansell SM, Armand P, Scott EC, Halwani A, Gutierrez M, et al. Nivolumab in patients with relapsed or refractory hematologic malignancy: preliminary results of a phase Ib study. *J Clin Oncol.* (2016) 34:2698–704. doi: 10.1200/JCO.2015.65.9789
19. Sekulic A, Liang WS, Tembe W, Izatt T, Kruglyak S, Kiefer JA, et al. Personalized treatment of Sézary syndrome by targeting a novel CTLA4:CD28 fusion. *Mol Genet Genomic Med.* (2015) 3:130–6. doi: 10.1002/mgg3.121
20. Bar-Sela G, Bergman R. Complete regression of mycosis fungoides after ipilimumab therapy for advanced melanoma. *JAAD Case Rep.* (2015) 1:99–100. doi: 10.1016/j.jdcr.2015.02.009
21. Zheng YJ, Lee A, Pincus L, Ho W, Vujic M, Ortiz-Urda S. Cutaneous CD56+ T-cell lymphoma developing during pembrolizumab treatment for metastatic melanoma. *JAAD Case Rep.* (2018) 4:540–2. doi: 10.1016/j.jdcr.2018.01.016
22. Ollila TA, Sahin I, Olszewski AJ. Mogamulizumab: a new tool for management of cutaneous T-cell lymphoma. *Onco Targets Ther.* (2019) 12:1085–94. doi: 10.2147/OTT.S165615
23. Kim YH, Bagot M, Pinter-Brown L, Rook AH, Porcu P, Horwitz SM, et al. Mogamulizumab versus vorinostat in previously treated cutaneous T-cell lymphoma (MAVORIC): an international, open-label, randomised, controlled phase 3 trial. *Lancet Oncol.* (2018) 19:1192–204. doi: 10.1016/S1470-2045(18)30379-6
24. Killock D. Haematological cancer: resiquimod—a topical CTCL therapy. *Nat Rev Clin Oncol.* (2015) 12:563. doi: 10.1038/nrclinonc.2015.142
25. Rook AH, Gelfand JM, Wysocka M, Troxel AB, Benoit B, Surber C, et al. Topical resiquimod can induce disease regression and enhance T-cell effector functions in cutaneous T-cell lymphoma. *Blood.* (2015) 126:1452–61. doi: 10.1182/blood-2015-02-630335

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

MINI REVIEW
published: 12 May 2020
doi: 10.3389/fonc.2020.00765

Update on Biology of Cutaneous T-Cell Lymphoma

Zaw H. Phyo[1], *Satish Shanbhag*[2] *and Sima Rozati*[3]*

[1] Johns Hopkins University School of Medicine, Baltimore, MD, United States, [2] Departments of Oncology and Medicine, Johns Hopkins University, Baltimore, MD, United States, [3] Department of Dermatology, Johns Hopkins University, Baltimore, MD, United States

Cutaneous T cell lymphomas (CTCL) comprise of a heterogeneous group of non-Hodgkin lymphomas derived from skin-homing T cells. Variation in clinical presentation and lack of definitive molecular markers make diagnosis especially challenging. The biology of CTCL remains elusive and clear links between genetic aberrations and epigenetic modifications that would result in clonal T cell expansion have not yet been identified. Nevertheless, in recent years, next generation sequencing (NGS) has enabled a much deeper understanding of the genomic landscape of CTCL by uncovering aberrant genetic pathways and epigenetic dysregulations. Additionally, single cell profiling is rapidly advancing our understanding of patients-specific tumor landscape and its interaction with the surrounding microenvironment. These studies have paved the road for future investigations that will explore the functional relevance of genetic alterations in the progression of disease. The ultimate goal of elucidating the pathogenesis of CTCL is to establish effective therapeutic targets with more durable clinical response and treat relapsing and refractory CTCL.

Keywords: cutaneous T cell lymphoma (CTCL), mycosis fungoides, sezary syndrome, single cell profiling, precision medicine, next generation sequencing, tumor microenvironment

OPEN ACCESS

Edited by:
Alessandra Romano,
University of Catania, Italy

Reviewed by:
Robert Gniadecki,
University of Alberta, Canada
Cosimo Di Raimondo,
Policlinico Tor Vergata, Italy
Tanya Hundal,
Mayo Clinic, United States

***Correspondence:**
Sima Rozati
srozati1@jhmi.edu

Specialty section:
This article was submitted to Hematologic Malignancies, a section of the journal Frontiers in Oncology

Received: *25 October 2019*
Accepted: *21 April 2020*
Published: *12 May 2020*

Citation:
Phyo ZH, Shanbhag S and Rozati S (2020) Update on Biology of Cutaneous T-Cell Lymphoma. Front. Oncol. 10:765. doi: 10.3389/fonc.2020.00765

INTRODUCTION

Cutaneous T cell lymphomas (CTCL) are a heterogeneous group of lymphoid malignancies derived from skin-homing T cells. The incidence rate of CTCL is 6.4 per million persons and has experienced increases in recent years in part due to better diagnosis (1). Highest incidence rates are seen among African Americans and older individuals, with a four-fold increase in incidence of patients over 70 (2, 3). Mycosis fungoides (MF) is the most common form of CTCL characterized by skin-homing CD4+ T cells. Sezary syndrome (SS) is an aggressive CTCL variant with varying levels of clonal lymphocytes in the blood. MF and SS together account for more than 50% of all CTCL cases (4). Diagnosis of CTCL is challenging due to the polymorphic nature of disease presentation, and a lack of a single definitive diagnostic procedure. Management of limited-stage MF involves more conservative approaches such as skin directed therapies while advanced-stage MF/SS patients are often treated with targeted and systemic chemotherapies, which are often short-lived and associated with adverse reactions (1). Large cell transformation (LCT) is a complication found in advanced-stage MF/SS that is histologically defined by the presence of large, atypical lymphocytes exceeding 25% of lymphoid infiltrate and is historically associated with significantly lower median survival compared to non-transformed MF (5). In addition, expression of CD30 antigen does not manifest in significant prognostic significant differences (6). However, brentuximab vedotin, a CD30 targeting antibody-drug conjugate, has demonstrated significant clinical response in variable $CD30^+$ CTCL patients (7, 8).

Beyond differences in clinical presentation, the biology of malignant T cells in MF and SS are thought to be at least in part distinct due to expression of cell surface markers consistent with skin resident effector memory T cells and central memory T cells, respectively (9). However, recent single-cell profiling studies reveal phenotypic plasticity and tumor heterogeneity, suggesting that MF and SS may belong to the same disease spectrum. The molecular and cellular biology of this spectrum of malignancies has yet to be fully decoded. In this review, we will discuss advances in understanding the genomic landscape of CTCL with emphasis on recent NGS studies in further elucidating the pathogenesis of MF and SS. We acknowledge that the fast pace of evolving technologies, such as single-cell profiling, will provide further insights to patient-specific tumor biology.

ORIGIN OF MALIGNANT T CELLS

External Risk Factors

Despite isolated case series of familial mycosis fungoides (10) and links to potential HLA alleles (11, 12), there is no concrete evidence for genetic predisposition. Additionally, infectious agents such as viruses, viral particles (13–15), environmental, geographical (16) or occupational exposure (17) have been contemplated to be triggers for the rise of CTCL but a strong link has not been identified.

Bacterial infections, particularly with staphylococcus aureus, are frequently noted in patients with CTCL and antibiotic therapy usually results in clinical improvement (18–20) Housing mice with CTCL-like features in germ-free conditions led to attenuated tumor burden which was reversible when co-housed with conventional mice (21). A recent prospective study examining 8 patients with advanced-stage CTCL found that transient antibiotic treatment is associated with a decrease in fraction of neoplastic T cells, decreased cell proliferation and a decreased in STAT3 signaling (22). While the mechanistic role is unclear, these data indicate that the commensal microbiome may play a role in malignant T cell transformation and consequently may serve as a therapeutic target.

Tumor Microenvironment and Dysregulated Cytokine Signaling

T cell receptors (TCRs) recognize and bind to a specific antigen presented by the major histocompatibility complex molecule (MHC); this interaction induces a cascade of phosphorylation and gene expression that results in T cell survival and proliferation. The hyperactivity of the TCR pathway, either by genetic alteration (23–25) and/or continuous contact with antigen presenting cells (26, 27) can result in robust proliferation and continuous activation of T cells leading to disease progression. Malignant T cell clones in MF can be traced to peripheral blood by TCR sequencing and the heterogeneity of malignant T cells between skin lesions might be partially attributed to variation in seeding patterns by peripheral blood (28). The growth and viability of the CTCL cell is partly dependent on direct contact with immature dendritic cells (DC), through interaction between CD40 located on DC and CD40 ligand located on CTCL cells (26). Macrophages and mast cells are also investigated for the roles they play in the tumor microenvironment. *In vivo* mouse studies demonstrated slower rates of progression of human CTCL tumor cells in mice depleted of mast cells (29) and macrophages (30). The malignant T cells also facilitate shaping the tumor microenvironment that is supportive of disease progression. Multiple ligand/receptor interactions, including VEGF/VEGFR (31) and CXCR4/CXCL12 (32), have been characterized for their role in development of a vascular niche conducive to growth of neoplastic T cells. Further research is needed for potential utilization of these vascular niche factors in improving diagnosis and targeted anti-angiogenic therapy. Malignant T cells also secrete galectin-1 and−3, which have been linked to decreased skin barrier function and uncontrolled epidermal proliferation (33), which explains the increased incidence of bacterial skin infections observed in CTCL patients. The functional state of T cell is crucial in the dynamic state of tumor microenvironment. In cancer, T cells operate in a chronic inflammatory state and ultimately enter a hypo-responsive state called T cell exhaustion which is in part characterized by expression of inhibitory receptors (34). Indeed, malignant T cells derived from patients across all CTCL stages display increased expression of inhibitory receptors including PD-1 (35–37), CTLA-4 (38), and LAG-3 (37). The role of inhibitory receptors in T cell exhaustion implies that they can be targeted to effectively reinvigorate effector T cells. Nivolumab (anti-PD-1) was found to be well-tolerated patients with relapsed or refractory hematologic malignancy, which included patients with MF (39). More recently, a multicenter phase II trial of pembrolizumab (anti-PD-L1) led to favorable outcomes in patients with advanced MF or SS (40). In 2018, the FDA approved Mogamulizumab (anti-CTLA-4) for treatment of relapsed and refractory MF and SS, after a randomized, multicenter phase III clinical trial revealed superior investigator-assessed progression-free survival compared to vorinostat (41).

Investigation of the role of cytokine profile in CTCL stemmed from the observation that atopic dermatitis, a classically Th2-skewed disease, is more prevalent in family history of MF patients (42). PBMCs from SS patients of various stages revealed decreased IL-4, IL-2, and IFN-γ, suggesting that malignant T cells in CTCL resemble the cytokine profile found in Th2 cells (43). Th1 pattern, found to be prevalent in early stage of the disease, may allow antitumor response to local disease. In later stage, there is Th2 and Th17 bias with global depression in cytokine expression, which may signify loss of immune function and T cell exhaustion (44). Gata-3 and JunB, Th2 cells-specific transcription factors, are expressed starting in early disease (45). Induction of Th2-dominant biology is partially linked to expression of extracellular matrix proteins periostin and thymic stromal lymphopoietin (TSLP) by dermal fibroblasts, which subsequently activates release of Th2-specific cytokines in CTCL cells (46).

The immune responses that dictate CTCL progression or inhibition are largely unknown and our understanding is complicated by conflicting results in the literature. For example, pro-inflammatory responses, such as Th17 are thought to promote tumor progression and limit anti-cancer Th1 response (47). Recently, a few case series have shown that TNF-inhibitors

and IL-17 inhibitors promoted the development or progression of MF in patients with inflammatory bowel disease, rheumatoid arthritis or misdiagnosed psoriasis (48). Contrary to previous reports, these results suggest that inhibition of Th17 mediated immune responses lead to CTCL disease progression.

On the other hand, regulatory T cells (Tregs) have been associated with Sezary syndrome and are thought to be an indicator of poor outcome (49). However, a recent single-cell profiling study of CTCL identified Treg transcription factor Foxp3 as the strongest predictor of early rather than late-stage Sezary syndrome (50). These data indicate that tumor FoxP3 expression may suppress CTCL disease rather than promote progression as previously thought. Therefore, it is crucial to investigate the factors that drive Th17 and Treg immunity in CTCL to better understand the mechanisms that affect disease outcome.

Our current knowledge on CTCL immunophenotype, cytokine profile and its interactions within the host immune system denote an intricate tumor microenvironment and present numerous potential targets for therapy.

GENOMIC LANDSCAPE OF CTCL

Genetic Aberrations

In the past few years, multiple groups have applied deep sequencing techniques including whole genome and whole exome sequencing to explore the genomic landscape alterations in cutaneous lymphoma (23–25, 51–53). These results have broadened our horizon in the understanding of the pathogenesis of this heterogeneous group of malignancies by identification of new somatic mutations, and common mutagenic pathways.

TP53 is a tumor suppressor, which responds to DNA damage and other stress signals and is often dysregulated in cancer (54). While a unifying oncogenic driver is absent, TP53 is a notable tumor suppressor in CTCL with a somatic mutation (23) and gene deletion (24, 25) on chromosome 17p detected in 19 and 37% of studied CTCL patients, respectively (55). However, TP53 mutation status does not endow any changes in prognosis in primary SS patients (56). The constitutive activation of nuclear factor kappa B (NF-κB) pathway, located downstream of TCR signaling, has been implicated to play a key role in tumor resistance to apoptosis in CTCL (57). Recent genomic sequencing by multiple groups have reported on alterations in PLCG1 (58), CARD11 (25), TNFRSF1B (23) and KIT (55) that are involved in NF-κB pathway. These alterations are involved in regulating T cell survival and proliferation and/or control transcriptional programs downstream of key T cell signaling. The involvement of the NF-κB pathway served as a rationale for the potential use of bortezomib, an inhibitor of NF-κB signaling, which exhibited 67% overall response rate with acceptable drug tolerability in a phase II clinical trial (59).

To address the low incidence rate of CTCL and small patient cohorts with variations in geographic or subtype origin found in most NGS studies, Chang et al. created an integrated CTCL genomic dataset by collecting and re-analyzing raw genomic data of 139 patients with CTCL from seven different sequencing studies of MF/SS (55). Consolidation of previous NGS cohorts improved statistical power and revealed insights to specific patterns of genetic aberrations. TP53 mutations were found to be mutually exclusive from NF-κB pathway gene mutations, indicating that tumor variants might arise from distinct genetic backgrounds. Moreover, mutual exclusivity was observed within the NF-κB pathway genes, suggesting that CTCL tumorigenesis may be triggered by one pathway alone. Cases that did not carry p53 or NF-κB mutational changes did not have any other significant abnormalities, indicating that other important changes in the transcriptome or epigenome may have a major role in tumorigenesis.

In addition to the NF-κB pathway, the JAK3/STAT3 signal transduction pathway is also well-characterized for its role in survival and proliferation of malignant T cells (60–62). Genomic studies have shown that gain of function point mutations and copy number gains in this pathway are frequent and correlate with gene expression of STAT3 and increased expression of pro-inflammatory cytokines IL17 and IL22, downstream targets of STAT3 activation that likely play a role in tumor progression (25, 63). In a mouse model of CTCL, transgenic STAT3 hyper-activation in T cells was linked to IL-17 and IL-22 expression and phenotypic features of CTCL (21, 64). Staphylococcal enterotoxin A (SEA) has been shown to drive IL-17 expression through a JAK3/STAT3-dependent pathway in malignant T cells when co-cultured with non-malignant T cells, suggesting that SEA-driven cross talk between malignant and non-malignant T cells are needed for oncogenic activation of STAT3 (64).

Beyond somatic mutations characterized previously, genes may also be amplified or deleted due to somatic copy number variants (SCNVs). Compared to other cancers, CTCL is unique in that it harbors a disproportionately high number of SCNV compared to somatic mutations (24). In addition to 17p deletion involving TP53, other tumor suppressors such as RB1, PTEN and CDKN1B have been reported, along with amplification of STAT3 (17q) and MYC (8q) (23–25, 65–67).

Epigenetics

Epigenetic abnormalities have been recognized for their role in altering gene expression of oncogenes and tumor suppressors and ultimately contributing to malignant cell transformation in cancer (68). Both hypo-methylation and hyper-methylation signatures have been observed in CTCL. DNMT3A, a gene encoding a methyltransferase, is often mutated or deleted in CTCL (55), signifying that genetic aberrations may underlie epigenetic dysregulation. The association between DNMT3A and mutated genes highlights the importance of integrating findings from sequencing studies and epigenetic findings. Histone deactylases (HDACs) remodel chromatin architecture by removing acetyl groups from histones and have been characterized as a therapeutic target in cancer (69). Vorinostat (70) and Romidepsin (71) inhibit HDACs which leads to gene expression of cell cycle regulators and promotes tumor cell apoptosis.

MiRNAs are non-coding RNA involved in epigenetic mechanisms that are implicated in essential cellular processes (72). The miRNA expression profile has been investigated through different CTCL populations. Increased expression of

miR-213, miR-486, and miR-21 were proposed to promote apoptotic resistance in CTCL cell lines (73). Notably, multiple groups have identified miR-155 for its role in pathogenesis of MF (74–76). Later, a causal link was established between JAK/STAT signaling and expression of miR-155 and its host gene BIC (B cell integration cluster), implying that STAT5/BIC/miR-155 can be targeted for therapy (77). MiR-155 inhibitors have been assessed in phase I-II clinical trials for their safety, tolerability and clinical activity with encouraging results (78, 79). MiRNAs may also play a role in predicting prognosis, with multiple groups demonstrating that a panel of miRNAs could be used to effectively stratify patients based on prognosis (80, 81). These types of prognostic markers must be validated in large multi-centered, ideally prospective cohort, studies. While many miRNAs have been implicated in CTCL, further research is needed to delineate the mechanisms in which miRNAs are deregulated and how it impacts disease progression.

Emerging technologies, such as transcript—index ATAC-seq allows researchers to interrogate epigenetic signatures indexed by TCR sequence-based T cell clonality, further refining single-cell resolution in dissecting tumor heterogeneity of CTCL (82).

Emerging Frontiers in CTCL

Emerging technologies in NGS allows researchers to interrogate the DNA sequence and transcriptomes of tumors at the resolution of single cells and has provided an unprecedented view of cellular processes. These advances in technology will rapidly evolve our understanding of tumor transformation and progression.

Single cell RNA-sequencing provides an in-depth view of gene expression profile of each tumor cell as well as an insightful perspective of major cellular components in relation to the tumor microenvironment. The heterogeneity of tumors between patients with CTCL has been well-documented at a clinical and molecular level in the literature. This knowledge has been further cemented by striking distinct gene expression profiles seen in advanced CTCL patients (83). Nevertheless, Gaydosik et al. also identified a 17-gene expression signature that was common between highly proliferative tumor cells in all samples. Interestingly, these signatures overlap with expression of TOX, a previously reported marker for identifying malignant lymphocytes in CTCL (83). Others have utilized single-cell sequencing in conjunction with artificial intelligence (AI)-based learning to create a framework that broadens the clinical applicability of their results in CTCL (50).

Using a single-cell flow cytometry-based assay, Buss et al. isolated malignant cells from 8 treatment-naive patients with SS and assessed the expression of 240 surface antigens and single-cell RNA sequencing for 110 T-cell-relevant genes. Based on surface antigen expression, malignant T cells were divided into distinct subpopulations and exhibited different sensitivities to HDACi treatment (84). The presence of multiple subpopulations with variable sensitivity to a single agent lends further support for the need for combination treatments that are informed by the patient's unique malignant clonal characteristics. The synergistic epigenetic-modulatory effect of histone acetylation of DNA demethylation results in global CpG methylation alterations as well as reexpression of tumor suppressor genes that was not achieved by single agent treatments (85). Preclinical and clinical investigations have demonstrated the combinatorial use of different epigenetic modulators together or in combination with other treatments (85–90).

Mutual exclusivity in CTCL reported by Chang et al. (55) as well as single-cell RNA sequencing analysis may contribute to the basis for molecular subtyping of the disease similar to other hematologic malignancies such as systemic diffuse large B cell lymphoma (DLBCL) (91) or acute leukemia (92). Combination approaches in therapy targeting multiple signaling pathways and clonal subpopulations can lead to unprecedented improvements in survival and quality of life.

Emerging technologies are further refining single-cell resolution in dissecting tumor heterogeneity of CTCL. Applications of these technologies could enable novel therapies or treatment strategies that have previously been deemed unlikely in CTCL. One such example is chimeric antigen receptor (CAR) T cell therapy, which primes the patient's own T cells to activate upon recognition of a tumor-specific antigen (93). CAR T therapies targeting CD19 in B cell malignancies led to durable remissions for refractory B-ALL (94) and DLBCL (95) patients. In contrast, immunophenotypic methods yield high overlap in surface markers between malignant and normal T cells, which presents a major challenge in targeting cancerous cells. Future studies could reveal more subtle phenotypic differences in T cells as NGS technologies enables higher resolution of clonotypic T cells.

CONCLUSION

We have yet to identify a major oncogenic driver or a constellation of genetic and epigenetic alterations that lead to malignant clonal T cell expansion seen in CTCL patients. However, recent NGS data including single cell sequencing have identified genetic aberrations in major signaling pathways and epigenetic components that play an important role in pathogenesis of CTCL. They have provided biomarker signatures that could be utilized in the future to identify early disease, predict disease progression, and tailor treatments to individual patients. It is important to ask the right type of research questions when performing such studies to extract relevant data and improve clinical outcomes. Further understanding of tumor biology in CTCL is imperative in developing patient-specific treatment with minimal side effects, a cornerstone of precision medicine. We are entering a new area of discovery that will optimize our management for this heterogeneous group of malignancies.

AUTHOR CONTRIBUTIONS

ZP and SR performed literature search and wrote most parts of the manuscript. SS performed literature search and wrote some parts of the manuscript. SR conceived the framework of this review article, provided insights, and edited the manuscript.

REFERENCES

1. Wilcox RA. Cutaneous T-cell lymphoma: 2016 update on diagnosis, risk-stratification, and management. *Am J Hematol.* (2016) 91:151–65. doi: 10.1002/ajh.24233
2. Criscione VD, Weinstock MA. Incidence of cutaneous T-cell lymphoma in the United States, 1973-2002. *Arch Dermatol.* (2007) 143:854–9. doi: 10.1001/archderm.143.7.854
3. Agar NS, Wedgeworth E, Crichton S, Mitchell TJ, Cox M, Ferreira S, et al. Survival outcomes and prognostic factors in mycosis fungoides/Sézary syndrome: validation of the revised International society for cutaneous lymphomas/European organisation for research and treatment of cancer staging proposal. *J Clin Oncol.* (2010) 28:4730–9. doi: 10.1200/JCO.2009.27.7665
4. Bradford PT, Devesa SS, Anderson WF, Toro JR. Cutaneous lymphoma incidence patterns in the United States: a population-based study of 3884 cases. *Blood.* (2009) 113:5064–73. doi: 10.1182/blood-2008-10-184168
5. Salhany KE, Cousar JB, Greer JP, Casey TT, Fields JP, Collins RD. Transformation of cutaneous T cell lymphoma to large cell lymphoma. A clinicopathologic and immunologic study. *Am J Pathol.* (1988) 132:265–77.
6. Arulogun SO, Prince HM, Ng J, Lade S, Ryan GF, Blewitt O, et al. Long-term outcomes of patients with advanced-stage cutaneous T-cell lymphoma and large cell transformation. *Blood.* (2008) 112:3082–7. doi: 10.1182/blood-2008-05-154609
7. Duvic M, Tetzlaff MT, Gangar P, Clos AL, Sui D, Talpur R. Results of a phase II trial of brentuximab vedotin for CD30+ cutaneous T-cell lymphoma and lymphomatoid papulosis. *J Clin Oncol.* (2015) 33:3759–65. doi: 10.1200/JCO.2014.60.3787
8. Kim YH, Tavallaee M, Sundram U, Salva KA, Wood GS, Li S, et al. Phase II investigator-initiated study of brentuximab vedotin in mycosis fungoides and sézary syndrome with variable CD30 expression level: a multi-institution collaborative project. *J Clin Oncol.* (2015) 33:3750–8. doi: 10.1200/JCO.2014.60.3969
9. Campbell JJ, Clark RA, Watanabe R, Kupper TS. Sezary syndrome and mycosis fungoides arise from distinct T-cell subsets: a biologic rationale for their distinct clinical behaviors. *Blood.* (2010) 116:767–71. doi: 10.1182/blood-2009-11-251926
10. Hodak E, Klein T, Gabay B, Ben-Amitai D, Bergman R, Gdalevich M, et al. Familial mycosis fungoides: report of 6 kindreds and a study of the HLA system. *J Am Acad Dermatol.* (2005) 52(3 Pt 1):393–402. doi: 10.1016/j.jaad.2003.12.052
11. Safai B, Myskowski PL, Dupont B, Pollack MS. Association of HLA-DR5 with mycosis fungoides. *J Invest Dermatol.* (1983) 80:395–7. doi: 10.1111/1523-1747.ep12553615
12. Jackow CM, McHam JB, Friss A, Alvear J, Reveille JR, Duvic M. HLA-DR5 and DQB1*03 class II alleles are associated with cutaneous T-cell lymphoma. *J Invest Dermatol.* (1996) 107:373–6. doi: 10.1111/1523-1747.ep12363352
13. Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc Natl Acad Sci USA.* (1980) 77:7415–9. doi: 10.1073/pnas.77.12.7415
14. van der Loo EM, van Muijen GN, van Vloten WA, Beens W, Scheffer E, Meijer CJ. C-type virus-like particles specifically localized in langerhans cells and related cells of skin and lymph nodes of patients with mycosis fungoides and Sezary's syndrome. A morphological and biochemical study. *Virchows Arch B Cell Pathol Incl Mol Pathol.* (1979) 31:193–203. doi: 10.1007/BF02889936
15. Morales MM, Olsen J, Johansen P, Kaerlev L, Guenel P, Arveux P, et al. Viral infection, atopy and mycosis fungoides: a European multicentre case-control study. *Eur J Cancer.* (2003) 39:511–6. doi: 10.1016/S0959-8049(02)00773-6
16. Litvinov IV, Tetzlaff MT, Rahme E, Habel Y, Risser DR, Gangar P, et al. Identification of geographic clustering and regions spared by cutaneous T-cell lymphoma in Texas using 2 distinct cancer registries. *Cancer.* (2015) 121:1993–2003. doi: 10.1002/cncr.29301
17. Fischmann AB, Bunn PA, Guccion JG, Matthews MJ, Minna JD. Exposure to chemicals, physical agents, and biologic agents in mycosis fungoides and the Sézary syndrome. *Cancer Treat Rep.* (1979) 63:591–6.
18. Jackow CM, Cather JC, Hearne V, Asano AT, Musser JM, Duvic M. Association of erythrodermic cutaneous T-cell lymphoma, superantigen-positive *Staphylococcus aureus*, and oligoclonal T-cell receptor V beta gene expansion. *Blood.* (1997) 89:32–40. doi: 10.1182/blood.V89.1.32.32_32_40
19. Talpur R, Bassett R, Duvic M. Prevalence and treatment of *Staphylococcus aureus* colonization in patients with mycosis fungoides and Sezary syndrome. *Br J Dermatol.* (2008) 159:105–12. doi: 10.1111/j.1365-2133.2008.08612.x
20. Tokura Y, Yagi H, Ohshima A, Kurokawa S, Wakita H, Yokote R, et al. Cutaneous colonization with staphylococci influences the disease activity of Sezary syndrome: a potential role for bacterial superantigens. *Br J Dermatol.* (1995) 133:6–12. doi: 10.1111/j.1365-2133.1995.tb02485.x
21. Fanok MH, Sun A, Fogli LK, Narendran V, Eckstein M, Kannan K, et al. Role of dysregulated cytokine signaling and bacterial triggers in the pathogenesis of cutaneous T-cell lymphoma. *J Invest Dermatol.* (2018) 138:1116–25. doi: 10.1016/j.jid.2017.10.028
22. Lindahl LM, Willerslev-Olsen A, Gjerdrum LMR, Nielsen PR, Blümel E, Rittig AH, et al. Antibiotics inhibit tumor and disease activity in cutaneous T-cell lymphoma. *Blood.* (2019) 134:1072–83. doi: 10.1182/blood.2018888107
23. Ungewickell A, Bhaduri A, Rios E, Reuter J, Lee CS, Mah A, et al. Genomic analysis of mycosis fungoides and Sézary syndrome identifies recurrent alterations in TNFR2. *Nat Genet.* (2015) 47:1056–60. doi: 10.1038/ng.3370
24. Choi J, Goh G, Walradt T, Hong BS, Bunick CG, Chen K, et al. Genomic landscape of cutaneous T cell lymphoma. *Nat Genet.* (2015) 47:1011–9. doi: 10.1038/ng.3356
25. da Silva Almeida AC, Abate F, Khiabanian H, Martinez-Escala E, Guitart J, Tensen CP, et al. The mutational landscape of cutaneous T cell lymphoma and Sézary syndrome. *Nat Genet.* (2015) 47:1465–70. doi: 10.1038/ng.3442
26. Berger CL, Hanlon D, Kanada D, Dhodapkar M, Lombillo V, Wang N, et al. The growth of cutaneous T-cell lymphoma is stimulated by immature dendritic cells. *Blood.* (2002) 99:2929–39. doi: 10.1182/blood.V99.8.2929.h8002929_2929_2939
27. Krejsgaard T, Lindahl LM, Mongan NP, Wasik MA, Litvinov IV, Iversen L, et al. Malignant inflammation in cutaneous T-cell lymphoma-a hostile takeover. *Semin Immunopathol.* (2017) 39:269–82. doi: 10.1007/s00281-016-0594-9
28. Iyer A, Hennessey D, O'Keefe S, Patterson J, Wang W, Wong GK, et al. Skin colonization by circulating neoplastic clones in cutaneous T-cell lymphoma. *Blood.* (2019) 134:1517–27. doi: 10.1182/blood.2019002516
29. Wu X, Schulte BC, Zhou Y, Haribhai D, Mackinnon AC, Plaza JA, et al. Depletion of M2-like tumor-associated macrophages delays cutaneous T-cell lymphoma development *in vivo*. *J Invest Dermatol.* (2014) 134:2814–22. doi: 10.1038/jid.2014.206
30. Rabenhorst A, Schlaak M, Heukamp LC, Förster A, Theurich S, von Bergwelt-Baildon M, et al. Mast cells play a protumorigenic role in primary cutaneous lymphoma. *Blood.* (2012) 120:2042–54. doi: 10.1182/blood-2012-03-415638
31. Miyagaki T, Sugaya M, Oka T, Takahashi N, Kawaguchi M, Suga H, et al. Placental growth factor and vascular endothelial growth factor together regulate tumour progression via increased vasculature in cutaneous T-cell lymphoma. *Acta Derm Venereol.* (2017) 97:586–92. doi: 10.2340/00015555-2623
32. Kremer KN, Dinkel BA, Sterner RM, Osborne DG, Jevremovic D, Hedin KE. TCR-CXCR4 signaling stabilizes cytokine mRNA transcripts via a PREX1-Rac1 pathway: implications for CTC. *Blood.* (2017) 130:982–94. doi: 10.1182/blood-2017-03-770982
33. Thode C, Woetmann A, Wandall HH, Carlsson MC, Qvortrup K, Kauczok CS, et al. Malignant T cells secrete galectins and induce epidermal hyperproliferation and disorganized stratification in a skin model of cutaneous T-cell lymphoma. *J Invest Dermatol.* (2015) 135:238–46. doi: 10.1038/jid.2014.284
34. Wherry EJ. T cell exhaustion. *Nat Immunol.* (2011) 12:492–9. doi: 10.1038/ni.2035
35. Wada DA, Wilcox RA, Harrington SM, Kwon ED, Ansell SM, Comfere NI. Programmed death 1 is expressed in cutaneous infiltrates of mycosis fungoides and Sézary syndrome. *Am J Hematol.* (2011) 86:325–7. doi: 10.1002/ajh.21960
36. Cetinözman F, Jansen PM, Vermeer MH, Willemze R. Differential expression of programmed death-1 (PD-1) in Sézary syndrome and mycosis fungoides. *Arch Dermatol.* (2012) 148:1379–85. doi: 10.1001/archdermatol.2012.2089

37. Querfeld C, Leung S, Myskowski PL, Curran SA, Goldman DA, Heller G, et al. Primary T cells from cutaneous T-cell lymphoma skin explants display an exhausted immune checkpoint profile. *Cancer Immunol Res.* (2018) 6:900–9. doi: 10.1158/2326-6066.CIR-17-0270
38. Wong HK, Wilson AJ, Gibson HM, Hafner MS, Hedgcock CJ, Berger CL, et al. Increased expression of CTLA-4 in malignant T-cells from patients with mycosis fungoides – cutaneous T cell lymphoma. *J Invest Dermatol.* (2006) 126:212–9. doi: 10.1038/sj.jid.5700029
39. Lesokhin AM, Ansell SM, Armand P, Scott EC, Halwani A, Gutierrez M, et al. Nivolumab in patients with relapsed or refractory hematologic malignancy: preliminary results of a phase Ib study. *J Clin Oncol.* (2016) 34:2698–704. doi: 10.1200/JCO.2015.65.9789
40. Khodadoust MS, Rook AH, Porcu P, Foss F, Moskowitz AJ, Shustov A, et al. Pembrolizumab in relapsed and refractory mycosis fungoides and Sézary syndrome: a multicenter phase II study. *J Clin Oncol.* (2020) 38:20–8. doi: 10.1200/JCO.19.01056
41. Kim YH, Bagot M, Pinter-Brown L, Rook AH, Porcu P, Horwitz SM, et al. Mogamulizumab versus vorinostat in previously treated cutaneous T-cell lymphoma (MAVORIC): an international, open-label, randomised, controlled phase 3 trial. *Lancet Oncol.* (2018) 19:1192–204. doi: 10.1016/S1470-2045(18)30379-6
42. Tuyp E, Burgoyne A, Aitchison T, MacKie R. A case-control study of possible causative factors in mycosis fungoides. *Arch Dermatol.* (1987) 123:196–200. doi: 10.1001/archderm.123.2.196
43. Vowels BR, Cassin M, Vonderheid EC, Rook AH. Aberrant cytokine production by Sezary syndrome patients: cytokine secretion pattern resembles murine Th2 cells. *J Invest Dermatol.* (1992) 99:90–4. doi: 10.1111/1523-1747.ep12611877
44. Chong BF, Wilson AJ, Gibson HM, Hafner MS, Luo Y, Hedgcock CJ, et al. Immune function abnormalities in peripheral blood mononuclear cell cytokine expression differentiates stages of cutaneous T-cell lymphoma/mycosis fungoides. *Clin Cancer Res.* (2008) 14:646–53. doi: 10.1158/1078-0432.CCR-07-0610
45. Kari L, Loboda A, Nebozhyn M, Rook AH, Vonderheid EC, Nichols C, et al. Classification and prediction of survival in patients with the leukemic phase of cutaneous T cell lymphoma. *J Exp Med.* (2003) 197:1477–88. doi: 10.1084/jem.20021726
46. Takahashi N, Sugaya M, Suga H, Oka T, Kawaguchi M, Miyagaki T, et al. Thymic stromal chemokine TSLP acts through Th2 cytokine production to induce cutaneous T-cell lymphoma. *Cancer Res.* (2016) 76:6241–52. doi: 10.1158/0008-5472.CAN-16-0992
47. Wolk K, Mitsui H, Witte K, Gellrich S, Gulati N, Humme D, et al. Deficient cutaneous antibacterial competence in cutaneous T-cell lymphomas: role of Th2-mediated biased Th17 function. *Clin Cancer Res.* (2014) 20:5507–16. doi: 10.1158/1078-0432.CCR-14-0707
48. Yoo J, Shah F, Velangi S, Stewart G, Scarisbrick JS. Secukinumab for treatment of psoriasis: does secukinumab precipitate or promote the presentation of cutaneous T-cell lymphoma? *Clin Exp Dermatol.* (2019) 44:414–7. doi: 10.1111/ced.13777
49. Berger CL, Tigelaar R, Cohen J, Mariwalla K, Trinh J, Wang N, et al. Cutaneous T-cell lymphoma: malignant proliferation of T-regulatory cells. *Blood.* (2005) 105:1640–7. doi: 10.1182/blood-2004-06-2181
50. Borcherding N, Voigt AP, Liu V, Link BK, Zhang W, Jabbari A. Single-cell profiling of cutaneous T-cell lymphoma reveals underlying heterogeneity associated with disease progression. *Clin Cancer Res.* (2019) 25:2996–3005. doi: 10.1158/1078-0432.CCR-18-3309
51. Wang L, Ni X, Covington KR, Yang BY, Shiu J, Zhang X, et al. Genomic profiling of Sézary syndrome identifies alterations of key T cell signaling and differentiation genes. *Nat Genet.* (2015) 47:1426–34. doi: 10.1038/ng.3444
52. Woollard WJ, Pullabhatla V, Lorenc A, Patel VM, Butler RM, Bayega A, et al. Candidate driver genes involved in genome maintenance and DNA repair in Sezary syndrome. *Blood.* (2016) 127:3387–97. doi: 10.1182/blood-2016-02-699843
53. Izykowska K, Przybylski GK, Gand C, Braun FC, Grabarczyk P, Kuss AW, et al. Genetic rearrangements result in altered gene expression and novel fusion transcripts in Sezary syndrome. *Oncotarget.* (2017) 8:39627–639. doi: 10.18632/oncotarget.17383
54. Muller PA, Vousden KH. p53 mutations in cancer. *Nat Cell Biol.* (2013) 15:2–8. doi: 10.1038/ncb2641
55. Chang LW, Patrone CC, Yang W, Rabionet R, Gallardo F, Espinet B, et al. An integrated data resource for genomic analysis of cutaneous T-cell lymphoma. *J Invest Dermatol.* (2018) 138:2681–3. doi: 10.1016/j.jid.2018.06.176
56. Gros A, Laharanne E, Vergier M, Prochazkova-Carlotti M, Pham-Ledard A, Bandres T, et al. TP53 alterations in primary and secondary Sézary syndrome: a diagnostic tool for the assessment of malignancy in patients with erythroderma. *PLoS ONE.* (2017) 12:e0173171. doi: 10.1371/journal.pone.0173171
57. Sors A, Jean-Louis F, Pellet C, Laroche L, Dubertret L, Courtois G, et al. Down-regulating constitutive activation of the NF-kappaB canonical pathway overcomes the resistance of cutaneous T-cell lymphoma to apoptosis. *Blood.* (2006) 107:2354–63. doi: 10.1182/blood-2005-06-2536
58. Vaqué JP, Gómez-López G, Monsálvez V, Varela I, Martínez N, Pérez C, et al. PLCG1 mutations in cutaneous T-cell lymphomas. *Blood.* (2014) 123:2034–43. doi: 10.1182/blood-2013-05-504308
59. Zinzani PL, Musuraca G, Tani M, Stefoni V, Marchi E, Fina M, et al. Phase II trial of proteasome inhibitor bortezomib in patients with relapsed or refractory cutaneous T-cell lymphoma. *J Clin Oncol.* (2007) 25:4293–7. doi: 10.1200/JCO.2007.11.4207
60. Sommer VH, Clemmensen OJ, Nielsen O, Wasik M, Lovato P, Brender C, et al. *In vivo* activation of STAT3 in cutaneous T-cell lymphoma. Evidence for an antiapoptotic function of STAT3. *Leukemia.* (2004) 18:1288–95. doi: 10.1038/sj.leu.2403385
61. Verma NK, Davies AM, Long A, Kelleher D, Volkov Y. STAT3 knockdown by siRNA induces apoptosis in human cutaneous T-cell lymphoma line Hut78 via downregulation of Bcl-xL. *Cell Mol Biol Lett.* (2010) 15:342–55. doi: 10.2478/s11658-010-0008-2
62. Bagherani N, Smoller BR. An overview of cutaneous T cell lymphomas. *F1000Res.* (2016) 5:F1000. doi: 10.12688/f1000research.8829.1
63. Park J, Yang J, Wenzel AT, Ramachandran A, Lee WJ, Daniels JC, et al. Genomic analysis of 220 CTCLs identifies a novel recurrent gain-of-function alteration in RLTPR (p.Q575E). *Blood.* (2017) 130:1430–40. doi: 10.1182/blood-2017-02-768234
64. Willerslev-Olsen A, Krejsgaard T, Lindahl LM, Litvinov IV, Fredholm S, Petersen DL, et al. Staphylococcal enterotoxin A (SEA) stimulates STAT3 activation and IL-17 expression in cutaneous T-cell lymphoma. *Blood.* (2016) 127:1287–96. doi: 10.1182/blood-2015-08-662353
65. Vermeer MH, van Doorn R, Dijkman R, Mao X, Whittaker S, van Voorst Vader PC. Novel and highly recurrent chromosomal alterations in Sézary syndrome. *Cancer Res.* (2008) 68:2689–98. doi: 10.1158/0008-5472.CAN-07-6398
66. Laharanne E, Oumouhou N, Bonnet F, Carlotti M, Gentil C, Chevret E, et al. Genome-wide analysis of cutaneous T-cell lymphomas identifies three clinically relevant classes. *J Invest Dermatol.* (2010) 130:1707–18. doi: 10.1038/jid.2010.8
67. Lin WM, Lewis JM, Filler RB, Modi BG, Carlson KR, Reddy S, et al. Characterization of the DNA copy-number genome in the blood of cutaneous T-cell lymphoma patients. *J Invest Dermatol.* (2012) 132:188–97. doi: 10.1038/jid.2011.254
68. Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. *Carcinogenesis.* (2010) 31:27–36. doi: 10.1093/carcin/bgp220
69. Meier K, Brehm A. Chromatin regulation: how complex does it get? *Epigenetics.* (2014) 9:1485–95. doi: 10.4161/15592294.2014.971580
70. Mann BS, Johnson JR, Cohen MH, Justice R, Pazdur R. FDA approval summary: vorinostat for treatment of advanced primary cutaneous T-cell lymphoma. *Oncologist.* (2007) 12:1247–52. doi: 10.1634/theoncologist.12-10-1247
71. Grant C, Rahman F, Piekarz R, Peer C, Frye R, Robey RW, et al. Romidepsin: a new therapy for cutaneous T-cell lymphoma and a potential therapy for solid tumors. *Expert Rev Anticancer Ther.* (2010) 10:997–1008. doi: 10.1586/era.10.88
72. Garzon R, Marcucci G, Croce CM. Targeting microRNAs in cancer: rationale, strategies and challenges. *Nat Rev Drug Discov.* (2010) 9:775–89. doi: 10.1038/nrd3179
73. Narducci MG, Arcelli D, Picchio MC, Lazzeri C, Pagani E, Sampogna F, et al. MicroRNA profiling reveals that miR-21, miR486 and miR-214 are

upregulated and involved in cell survival in Sézary syndrome. *Cell Death Dis.* (2011) 2:e151. doi: 10.1038/cddis.2011.32
74. Ralfkiaer U, Hagedorn PH, Bangsgaard N, Løvendorf MB, Ahler CB, Svensson L, et al. Diagnostic microRNA profiling in cutaneous T-cell lymphoma (CTCL). *Blood.* (2011) 118:5891–900. doi: 10.1182/blood-2011-06-358382
75. van Kester MS, Ballabio E, Benner MF, Chen XH, Saunders NJ van der Fits L, et al. miRNA expression profiling of mycosis fungoides. *Mol Oncol.* (2011) 5:273–80. doi: 10.1016/j.molonc.2011.02.003
76. Maj J, Jankowska-Konsur A, Sadakierska-Chudy A, Noga L, Reich A. Altered microRNA expression in mycosis fungoides. *Br J Dermatol.* (2012) 166:331–6. doi: 10.1111/j.1365-2133.2011.10669.x
77. Kopp KL, Ralfkiaer U, Gjerdrum LM, Helvad R, Pedersen IH, Litman T, et al. STAT5-mediated expression of oncogenic miR-155 in cutaneous T-cell lymphoma. *Cell Cycle.* (2013) 12:1939–47. doi: 10.4161/cc.24987
78. Seto AG, Beatty X, Lynch JM, Hermreck M, Tetzlaff M, Duvic M, et al. Cobomarsen, an oligonucleotide inhibitor of miR-155, co-ordinately regulates multiple survival pathways to reduce cellular proliferation and survival in cutaneous T-cell lymphoma. *Br J Haematol.* (2018) 183:428–44. doi: 10.1111/bjh.15547
79. Foss FM, Querfeld C, Kim YH, Pinter-Brown LC, William BM, Porcu P, et al. Ph 1 study of MRG-106, an inhibitor of miR-155. *J Clin Oncol.* (2018) 36(Suppl. 15):2511. doi: 10.1200/JCO.2018.36.15_suppl.2511
80. Lindahl LM, Besenbacher S, Rittig AH, Celis P, Willerslev-Olsen A, Gjerdrum LMR, et al. Prognostic miRNA classifier in early-stage mycosis fungoides: development and validation in a Danish nationwide study. *Blood.* (2018) 131:759–70. doi: 10.1182/blood-2017-06-788950
81. Shen X, Wang B, Li K, Wang L, Zhao X, Xue F, et al. MicroRNA signatures in diagnosis and prognosis of cutaneous T-cell lymphoma. *J Invest Dermatol.* (2018) 138:2024–32. doi: 10.1016/j.jid.2018.03.1500
82. Satpathy AT, Saligrama N, Buenrostro JD, Wei Y, Wu B, Rubin AJ, et al. Transcript-indexed ATAC-seq for precision immune profiling. *Nat Med.* (2018) 24:580–90. doi: 10.1038/s41591-018-0008-8
83. Gaydosik AM, Tabib T, Geskin LJ, Bayan CA, Conway JF, Lafyatis R, et al. Single-cell lymphocyte heterogeneity in advanced cutaneous T-cell lymphoma skin tumors. *Clin Cancer Res.* (2019) 25:4443–54. doi: 10.1158/1078-0432.CCR-19-0148
84. Buus TB, Willerslev-Olsen A, Fredholm S, Blumel E, Nastasi C, Gluud M, et al. Single-cell heterogeneity in Sezary syndrome. *Blood Adv.* (2018) 2:2115–26. doi: 10.1182/bloodadvances.2018022608
85. Rozati S, Cheng PF, Widmer DS, Fujii K, Levesque MP, Dummer R. Romidepsin and azacitidine synergize in their epigenetic modulatory effects to induce apoptosis in CTCL. *Clin Cancer Res.* (2016) 22:2020–31. doi: 10.1158/1078-0432.CCR-15-1435
86. Kim SR, Lewis JM, Cyrenne BM, Monico PF, Mirza FN, Carlson KR, et al. BET inhibition in advanced cutaneous T cell lymphoma is synergistically potentiated by BCL2 inhibition or HDAC inhibition. *Oncotarget.* (2018) 9:29193–207. doi: 10.18632/oncotarget.25670
87. Samimi S, Morrissey K, Anshelevich S, Evans K, Gardner J, Musiek A, et al. Romidepsin and interferon gamma: a novel combination for refractory cutaneous T-cell lymphoma. *J Am Acad Dermatol.* (2013) 68:e5–6. doi: 10.1016/j.jaad.2011.06.043
88. Vu K, Wu CH, Yang CY, Zhan A, Cavallone E, Berry W, et al. Romidepsin plus liposomal doxorubicin is safe and effective in patients with relapsed or refractory T-cell lymphoma: results of a phase I dose-escalation study. *Clin Cancer Res.* (2020) 26:1000–8. doi: 10.1158/1078-0432.CCR-19-2152
89. Froehlich TC, Müller-Decker K, Braun JD, Albrecht T, Schroeder A, Gülow K, et al. Combined inhibition of Bcl-2 and NFκB synergistically induces cell death in cutaneous T-cell lymphoma. *Blood.* (2019) 134:445–55. doi: 10.1182/blood.2019001545
90. Zhao L, Okhovat JP, Hong EK, Kim YH, Wood GS. Preclinical studies support combined inhibition of BET family proteins and histone deacetylases as epigenetic therapy for cutaneous T-cell lymphoma. *Neoplasia.* (2019) 21:82–92. doi: 10.1016/j.neo.2018.11.006
91. Lenz G, Wright GW, Emre NC, Kohlhammer H, Dave SS, Davis RE, et al. Molecular subtypes of diffuse large B-cell lymphoma arise by distinct genetic pathways. *Proc Natl Acad Sci USA.* (2008) 105:13520–5. doi: 10.1073/pnas.0804295105
92. Ng SW, Mitchell A, Kennedy JA, Chen WC, McLeod J, Ibrahimova N, et al. A 17-gene stemness score for rapid determination of risk in acute leukaemia. *Nature.* (2016) 540:433–37. doi: 10.1038/nature20598
93. Almåsbak H, Aarvak T, Vemuri MC. CAR T cell therapy: a game changer in cancer treatment. *J Immunol Res.* (2016) 2016:5474602. doi: 10.1155/2016/5474602
94. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N Engl J Med.* (2018) 378:439–48. doi: 10.1056/NEJMoa1709866
95. Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N Engl J Med.* (2017) 377:2531–44. doi: 10.1056/NEJMoa1707447

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership