frontiers in Oncology

REVIEW
published: 09 December 2021
doi: 10.3389/fonc.2021.799957

The Research Progress of PD-1/PD-L1 Inhibitors Enhancing Radiotherapy Efficacy

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Approximately 60%–70% of patients with malignant tumours require radiotherapy. The clinical application of immune checkpoint inhibitors (ICIs), such as anti-PD-1/PD-L1, has revolutionized cancer treatment and greatly improved the outcome of a variety of cancers by boosting host immunity.However, radiotherapy is a double-edged sword for PD-1/PD-L immunotherapy. Research on how to improve radiotherapy efficacy using PD-1/PD-L1 inhibitor is gaining momentum. Various studies have reported the survival benefits of the combined application of radiotherapy and PD-1/PD-L1 inhibitor. To fully exerts the immune activation effect of radiotherapy, while avoiding the immunosuppressive effect of radiotherapy as much as possible, the dose selection, segmentation mode, treatment timing and the number of treatment sites of radiotherapy play a role. Therefore, we aim to review the effect of radiotherapy combined with anti-PD-1/PD-L1 on the immune system and its optimization.

Keywords: radiotherapy, immunotherapy, PD-1/PD-L1, inhibitors, abscopal effect

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Specialty section:
This article was submitted to Cancer Immunity and Immunotherapy, a section of the journal Frontiers in Oncology

Received: *22 October 2021*
Accepted: *15 November 2021*
Published: *09 December 2021*

Citation:
Wen L, Tong F, Zhang R, Chen L, Huang Y and Dong X (2021) The Research Progress of PD-1/PD-L1 Inhibitors Enhancing Radiotherapy Efficacy. Front. Oncol. 11:799957. doi: 10.3389/fonc.2021.799957

INTRODUCTION

Radiotherapy is the main treatment option for tumours. Approximately 60%–70% of patients with malignant tumours require radiotherapy. The radiosensitivity of tumour cells is the key to its curative effect. Radiotherapy can directly act on tumour cell DNA, killing the cells, and it can also change the tumour microenvironment by producing *in situ* tumour vaccines that induce immune activation, triggering anti-tumour responses, and inducing the potential of tumour regression in non-irradiated areas, which is called abscopal effect (1–5). In some cases, the specific anti-tumour effect induced using radiotherapy is limited and radiotherapy efficacy is unsatisfactory. Additionally, while radiotherapy kills tumour cells, it can also damage the immune cells in the irradiated area. Therefore, radiotherapy is considered to be a double-edged sword. Radiotherapy can up-regulate PD-L1 expression and inhibit T cell activity (6). Radiotherapy can also activate the anti-tumour immune response (7–10). For example, radiotherapy can release a large number of tumour-related antigens by killing tumour cells, inducing an increase in tumour-infiltrating lymphocytes, and enhancing the anti-tumour immune response mediated by CD8+ T cells (9). Moreover, radiotherapy can promote the activation and maturation of dendritic cells. It also promotes antigen presentation by up-regulating MHC I expression tumour cell surface (7, 8). Therefore, it is feasible to combine radiotherapy and immunotherapy based on the immune-stimulating properties of radiotherapy.PD-1/PD-L1 inhibitor has been approved for the treatment of oesophageal, head and neck, melanoma, kidney, bladder, lung cancers and other tumours. Some tumours are treated with PD-1/PD-L1 inhibitor alone, which has good sensitivity and efficacy rate < 25% (11–33). Some tumours are almost ineffective, such as microsatellite stable colorectal cancer and EGFR (+) lung cancer (1, 34). The combined applications of PD-1/PD-L1 monoclonal

antibody and radiotherapy have been reported to have good efficacy (35). Such combination therapies can enhance the body's anti-tumour immune response and increase the abscopal effect on distant tumour inhibitions (36). However, when radiotherapy is combined with PD-1/PD-L1 treatment, it is necessary to consider the maximization of the immune activation effect of radiotherapy and avoidance of the immunosuppressive effect of radiotherapy. Therefore, the segmentation mode, dosage, combined action mechanism, and radiotherapy treatment part numbers need to be studied. For patients with multiple metastatic tumours, the practice of irradiating a single metastasis and expecting the abscopal effect should be abandoned. Instead, systemic therapy based on the PD-1/PD-L1 inhibitor and multi-site radiotherapy to enhance its efficacy should be considered (37). This article reviews the optimization of the combined PD-1/PD-L1 and radiotherapy treatment option (**Figure 1**).

MECHANISM OF PD-1/PD-L1 PROMOTING RADIOTHERAPY EFFICACY

Clinically, immunotherapy can be combined with radiotherapy. Radiotherapy can induce tumour antigen release, enhance tumour cell immunogenicity, activate immune cells, secrete immune factors and promote tumour-related antigen presentation, and thereby effectively activating the anti-tumour immune response. Moreover, studies have shown that radiotherapy can up-regulate the expression of PD-1 on T cells and PD-L1 on tumour cells, leading to the inactivation and depletion of CD8+ T cells, suppression of immune responses and development of radiotherapy tolerance (38). If PD-1/PD-L1 monoclonal antibody is administered at the early stage of radiotherapy, it can restore T cell activity and enhance the anti-tumour immune response. Various studies have reported that TGF-β secretion increases after radiotherapy, leading to Treg infiltration and immune response suppression (39, 40). Radiotherapy combined with immunotherapy can reduce Treg numbers, increase CD8+T/Treg ratio and enhance tumour cell killings (41). Additionally, radiotherapy can promote HMGB1 release, stimulate calreticulin transportation to the cell surface and induce immunogenic cell death(ICD). Radiotherapy can increase protein breakdown, induce increased MHC I expression on the tumour cell surface and promote TAAs recognition by CTL cells. Radiotherapy causes tumour cell death, inducing the release of DAMPs, TAAs and inflammatory cytokines in cell debris and activating antigen-presenting cells, such as dendritic cells, to present TAAs to immune cells in the lymph nodes. Therefore, combined immunotherapy can enhance the radiotherapeutic immune induction and cooperates with radiotherapy to inhibit tumour growth, achieving an effect of 1 + 1 > 2 (42, 43). Additionally,

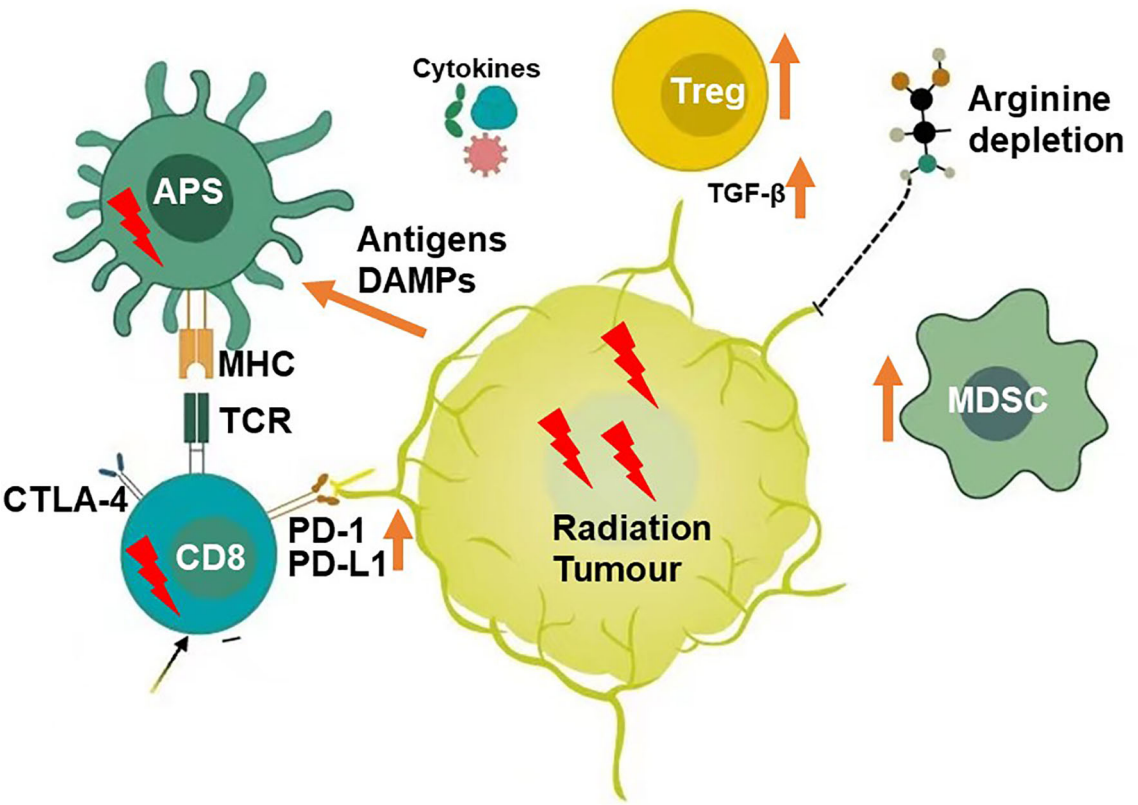

FIGURE 1 | Microenvironment Modifiers(Radiation).

radiotherapy also plays various roles in combination therapy for tumours of different stages and types. When the tumour burden is small and limited, radiotherapy can be used as a local radical treatment, aiming to cooperate with a systemic PD-1 inhibitor for curing cancer. In other cases, such as massive metastases or multiple metastatic tumours, radiotherapy can be used as an adjuvant for PD-1/PD-L1 inhibitors. Therefore, the combination of radiotherapy and PD-1/PD-L1inhibitor is diversified (**Figure 2**).

EFFICACY OF DIFFERENT COMBINATIONS OF RADIOTHERAPY AND PD-1/PD-L1

The dose and division mode of radiotherapy combined with PD-1/PD-L1 have attracted widespread attention. Precise irradiation using hypofractionated radiotherapy (HFRT) can minimize the damage to the surrounding normal tissues. HFRT can induce a stronger immune response and abscopal effect than conventional radiotherapy. It is more suitable for combining with immunotherapy, and this theory has been confirmed by various studies. In 2020, Professor Lu You reported that HFRT can induce stronger local and systemic anti-tumour immune effects than conventional fractionated radiation therapy (CFRT) by inhibiting the VEGF/VEGFR signalling pathway, reducing MDSCs recruitment to the tumour microenvironment, mediating lower PD-L1 expression, decreasing tumour cells for immune escape through the PD-L1/PD-1 axis, increasing CD8+ T cell levels around tumour tissues and in peripheral blood and maintaining their tumour cell killing activity (44). Studies have further reported that HFRT combined with anti-PD-L1 antibody therapy can significantly improve the tumour (local and radiotherapy field lesions) control rate and survival rate in tumour-bearing mice (44). Clinically, the increase in Tregs affects the local control at 15 Gy/F and 1 F. The 7.5~10.0 Gy/F, 2~3 F regimen can maintain a low level of Treg, and it can better stimulate the body's immune response safely (45, 46). The PEMBRO-RT study used 8 Gy/F, 3 F mode combined with PD-1 inhibitor to treat advanced non-small cell lung cancer (NSCLC). The ORR rate at 12-weeks was 36%, which increased more than once, compared with the control group's progression-free survival (PFS) and overall survival (OS) rates (47). But, McBride et al. shown no improvement in response and no evidence of an abscopal effect with the addition of SBRT to nivolumab in unselected patients with metastatic HNSCC. Although the efficiency of immunotherapy may be improved by combined with radiotherapy, but the ORR of PD-1 antibody monotherapy for HNSCC is high, so it is necessary to further expand the sample size to reflect the difference between the experimental and control groups.

A consensus is yet to be reached over the optimal timing for the use of the combination therapy of radiotherapy and PD-1/PD-L1 inhibitor. Dovedi et al. found that simultaneous administration of anti-PD-1/PD-L1 antibody with conventional split RT has a higher survival rate than that of sequential administration (38). A phase I clinical study for advanced metastatic urothelial carcinoma reported that the effect of the simultaneous treatment group using PD-1 inhibitor receiving 8 Gy/F, 3F stereotactic body radiation therapy (SBRT) before the third cycle was significantly better than that of the sequential treatment group (48). The COSINR Phase I trial evaluates the simultaneous or sequential application of CTLA-4 inhibitor, PD-1 inhibitor and SBRT in patients with stage IV NSCLC. The trial's latest data showed that the median PFS period of the sequential and contemporaneous groups was 5.9 and6.2 months, respectively (49). However, a study by Herter-Sprie et al. reported that the OS was similar to that of sequential administration

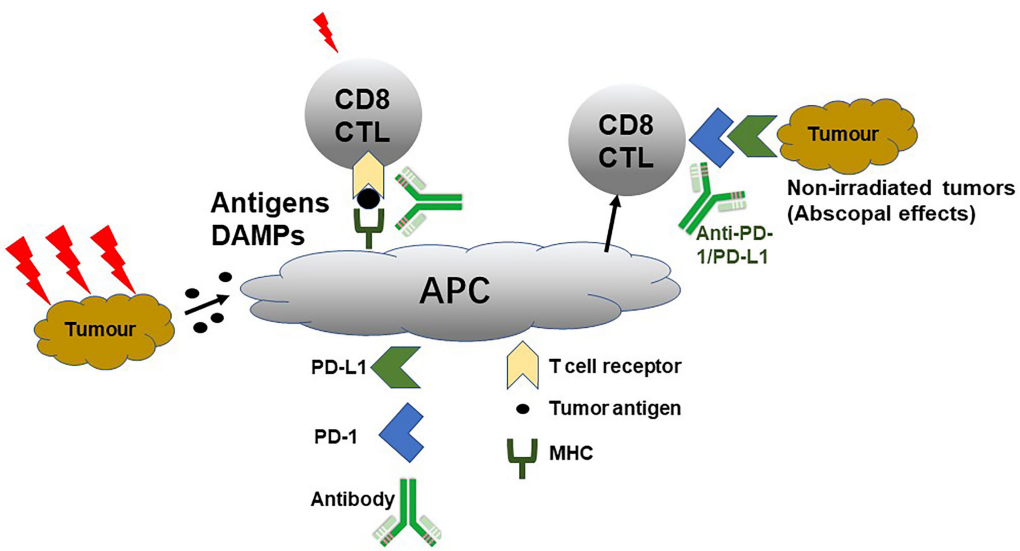

FIGURE 2 | PD-1/PD-L1 Promoting Radiotherapy.

(PD-1 antibody administration on the 7th day after radiotherapy) regardless of the simultaneous administration from either the 1st or 5th day of radiotherapy (50). Some preclinical studies report contradictory results on the simultaneous or sequential use of radiotherapy combined with PD-1/PD-L1 inhibitor; however, an incline towards simultaneous use is observed. The subgroup analysis of the PACIFIC study showed that the PFS benefit trend of receiving PD-L1 monoclonal antibody within 14 days after concurrent radiotherapy and chemotherapy for stage III unresectable NSCLC was more significant than that of receiving PD-L1 antibody treatment for14 days (51–53). Radiotherapy combined with immune checkpoint inhibitors (ICIs) in patients with melanoma brain metastases showed that the combined ICI therapy within 4 weeks after treatment with stereotactic radiosurgery (SRS) had significantly better results than those in patients with SRS > 4 weeks (54). Evidence indicates that sequential treatment and PD-1/PD-L1 inhibitor administration after radiotherapy can increase the clinical benefit.

The timing of radiotherapy combined with anti-PD-1/PD-L1 therapy is affected by adverse effects along with therapeutic effectiveness. In the MDACC study, two patients with simultaneous HFRT or SBRT combined with PD-1 inhibitor had Grade 4 adverse effects, which may be attributed to the simultaneous medication. A study by ESMO 2020 showed that the administration of anti-PD-1 drugs before or during radiotherapy for thoracic tumours increased the incidence of radiation pneumonitis (60% 28%, P = 0.01) compared with the administration of anti-PD-1 drugs after radiotherapy (55, 56). However, the occurrence of adverse effects is closely related to factors such as radiotherapy dose, volume and location. Therefore, whether synchronization will increase adverse reactions than sequential treatment needs to be further confirmed by clinical studies.

For multiple metastatic tumours, there have been no large randomized controlled data on the number of lesions irradiated for the generation of the greatest monosensitization effect. Current methods include the partial irradiation of large tumours (46, 57), SBRT combined with low-dose irradiation (58, 59) and multiple periodic irradiations of different metastatic lesions. You et al. proposed for the first time a combination group of primary tumours receiving HFRT and secondary tumours receiving low-dose radiation therapy (LDRT), combined with ICIs. Compared with HFRT alone, secondary tumour growth in mice receiving LDRT combined treatment showed a significant decline in growth. LDRT strongly promotes the local infiltration of T cells into tumours and induces the lower recruitment of MDSCs; however, LDRT also promotes the up-regulation of immune activation related gene expression (antigen presentation related genes and T cell activation related genes) and T cell-related chemokine expression. It has also been confirmed in mouse CT26 and MC38 colon cancer models that the triple treatment group achieved the best secondary tumour growth control. Therefore, it has been proposed that the secondary tumour receiving LDRT can promote the migration of effector T cells into the tumour, reshape the local tumour microenvironment, amplify the abscopal effect of HFRT and increase the efficacy of combined immunotherapy.

RADIOTHERAPY COMBINED WITH IMMUNOTHERAPY INCREASE ABSCOPAL EFFECT

The abscopal effect was proposed by Mole in 1953. The abscopal effect is achieved through the activation of the immune system, which may be closely related to the increase in T cell activating factors, increase in existing tumour-specific antibodies and formation of new anti-tumour antibodies (60) The production of the abscopal effect by radiotherapy alone is rare in clinical practice and has been reported only in a few individual cases. Recently, radiotherapy combined with immunotherapy has caused a significant increase in the abscopal effect, but the mechanism of action remains unclear. Studies have found that PD-1/PD-L1 monoclonal antibody combined with radiotherapy can inhibit distant tumours through the abscopal effect. A study of melanoma (B16-OVA) mice reported that PD-1 monoclonal antibody combined with Stereotactic ablative brachytherapy (SABT) can decrease the primary lesion close to CR, and the tumour volume at the non-irradiated site is also reduced by 66% (61–63). SBRT can easily induce the abscopal effect compared to the conventional segmentation mode and is more suited for combining with immunotherapy (64). Attesting to this, Deng et al. reported that breast cancer mice receiving radiotherapy combined with PD-L1 monoclonal antibody treatment showed a reduction in the volume of distant tumours outside the radiotherapy site, and thereby producing a lasting immune memory (65). You et al. reported, for the first time, that HFRT induces primary tumour cell apoptosis, produces an "*in situ* vaccination" effect and sensitizes tumour-specific T cells. Moreover, LDRT promotes the migration of tumour-specific T cells into the secondary tumour. The combination of these two therapies (HFRT and LDRT) produces CD8+T cell-dependent immune effects. Meanwhile, PD-1 inhibitor restores the tumour-killing activity of T cells by releasing the "inhibitory brake" on the surface of T cells, further enhancing the systemic anti-tumour immune effect (58). Additionally, the results from the 2015 "Lancet" clinical trial on local radiotherapy combined with immunotherapy confirmed the production of the abscopal effect in approximately a quarter of patients with advanced tumours (including NSCLC, breast cancer and thymic cancer). Patients producing the abscopal effect had more obvious survival rates (60, 66).

ALTERNATE METHODS TO ENHANCE RADIOTHERAPY EFFICACY

Enhancement of the radiotherapy-related anti-tumour immune response can be performed *via* various methods as elucidated by the reports on the treatment mode of PD-L1 and CTLA-4 monoclonal antibodies combined with radiotherapy (67). In melanoma mice receiving radiotherapy combined with CTLA-4 monoclonal antibody, the tumour cell PD-L1 expression was significantly up-regulated. Therefore, the combination of PD-L1

TABLE 1 | Immunotherapy agents under clinical investigation in combination with radiation.

Category	Reagent	Diseases	No. of current studies
Checkpoint inhibitors	Anti-CTLA-4	Cervix, melanoma, head and neck, NSCLC, pancreas, liver, lung, Breast, colon	100
	PD-1/PD-L-1	Esophageal, NSCLC, Malignant glioma, melanoma (brain metastases), invasive bladder, oligometastatic breast, head and neck, pancreas, gastric, colorectal, follicular lymphoma, Extensive Stage Small Cell Lung, Prostate, urothelial, Gastroensophageal, HCC, Pancreatic, renal, colon, glioblastoma	
Vaccines/ oncolytic Viruses	AdV-tk, Sipluleucel-T, G207, ADV/HSV-tk, Oncolytic Adenovirus Ad5-yCD/ mutTKSR39rep-hIL12, and Ad5-yCD/ MutTKSR39rep-AD	Prostate, pancreas, malignant supratentorial neoplasms, NSCLC, triple negative breast, prostate, glioma, ovarian, sarcoma, glioblastoma, oesophageal	28
Cytokines	IL-2, IFN, GM-CSF, and TGF-beta blockade	Metastatic breast, NSCLC, glioblastoma, follicular lymphoma, and pancreas, renal, advanced hepacellular carcinoma, oesophageal, colorectal, melanoma, glioma	155
Other targeted immune Rx	OX40 antibody, CDX-301, GITR, and TLR-4,7,9 agonists	Melanoma, renal cell carcinoma, NSCLC, breast, sarcoma, cutaneous T-cell and recurrent lymphoma, breast, colorectal, fibrosarcoma, fibrosarcoma, lung, melanoma, osteosarcoma, renal, B-cell lymphomas	27

monoclonal antibodies can restore the function of T cells, increase the ratio of CD8+T/Treg and increase the CR rate of mice to 80%. This study aims to reveal that triple therapy synergistically enhances the anti-tumour effects. Additionally, IL-2, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-α and tumour necrosis factor-α (TNF-α) participate at different steps in the synergistic effect of radiotherapy and immunotherapy on tumour cells. IL-2 can promote the proliferation and activation of T cells and also activate NK cells. Radiotherapy can up-regulate the expression of MHC-I molecules and promote the formation of memory T cells. In the models of mouse melanoma, colon cancer and breast cancer, HFRT combined with IL-2 complex can produce a significant synergistic effect, enhancing the anti-tumour effects of CD8+ T and NK cells (68). Phase I clinical studies have reported that combining SBRT with IL-2 for the treatment of metastatic renal cell carcinoma and melanoma has a remission rate of 66. 6%. The response rate of melanoma was 71.4% (69, 70). Moreover, the combination of IL-2 and radiotherapy can synergistically control the combined treatment of local and distant lesions (69–73). GM-CSF promotes monocytes/M1 macrophage and DC differentiation, enhances antigen presentation and amplifies the body's immune effect (74). A clinical trial has reported that local radiotherapy combined with GM-CSF subcutaneous injection induced the abscopal effect at a rate of 22.2% in NSCLC and OS showed significant prolongation (60). A clinical study of patients with advanced cholangiocarcinoma who received PD-1 inhibitor combined with GM-CSF showed that the 6-month PFS rate reached 35% (75). A prospective clinical study of single-arm HFRT combined with PD-1 inhibitor and GM-CSF in the treatment of advanced multiple metastatic solid tumours is being carried out. The median PFS stage is 4.0 months. The current study is still in progress (ChiCTR1900026175) (76).

TNF-α is produced by activated macrophages and can induce immune cell activation. A phase I clinical study found that the combined treatment of TNF-α and radiotherapy improved OS and PFS in oesophageal cancer, head and neck cancer and other solid tumours (77).

CONCLUSION

Immunotherapy has a dramatic impact on the field of oncology. Many pre-clinical data show that radiotherapy combined with immunotherapy enhances tumour killing through the vaccine effect, attraction effect and fragility effect. The synergistic effects of PD-1 and PD-L1 monoclonal antibodies combined with radiotherapy have been confirmed in various preclinical trials. Such combination therapies can enhance the body's anti-tumour immune response and increase the abscopal effect on distant tumour inhibitions. However, this treatment model is still in its infancy, it is necessary to consider the maximization of the immune activation effect of radiotherapy and avoidance of the immunosuppressive effect of radiotherapy (78). Radiotherapy dose, segmentation method, irradiation site, radiotherapy volume, intervention point of immunotherapy, selection of immunodrugs and disease/patient all need to be demonstrated by more sufficient clinical trial data. There are many clinical studies at home and abroad that are actively trying to add radiotherapy to various immunotherapy strategies to determine the best therapy combination (**Table 1**). Further studies on answering the current problems in combination therapy and making radiotherapy combined with immunotherapy clinically effective are required.

AUTHOR CONTRIBUTIONS

LW is the main writer of this review. FT and RZ participate in the analysis and sorting of literature data. LC and YH complete the collection and analysis of relevant literature data. XD conceptulize this review. All authors contributed to the article and approved the submitted version.

FUNDING

This research was supported by the grants from the National Key R&D Program of China (Grant No 2019YFC1316205).

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