Combination of CAR-T cell therapy and radiotherapy: Opportunities and challenges in solid tumors (Review)

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Received March 3, 2023; Accepted April 28, 2023

DOI: 10.3892/ol.2023.13867

Abstract. Chimeric antigen receptor (CAR) T cell therapy has emerged as a new and breakthrough cancer immunotherapy. Although CAR-T cell therapy has made significant progress clinically in patients with refractory or drug-resistant hematological malignancies, there are numerous challenges in its application to solid tumor therapy, including antigen escape, severe toxic reactions, abnormal vascularization, tumor hypoxia, insufficient infiltration of CAR-T cells and immunosuppression. As a conventional mode of anti-tumor therapy, radiotherapy has shown promising effects in combination with CAR-T cell therapy by enhancing the specific immunity of endogenous target antigens, which promoted the infiltration and expansion of CAR-T cells and improved the hypoxic tumor microenvironment. This review focuses on the obstacles to the application of CAR-T technology in solid tumor therapy, the potential opportunities and challenges of combined radiotherapy and CAR-T cell therapy, and the review of recent literature to evaluate the best combination for the treatment of solid tumors.

Contents

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Key words: CAR-T cell therapy, radiotherapy, immunotherapy, solid tumor clinical trials

1. Introduction

Chimeric antigen receptor (CAR) T-cell therapy is a successful adoptive cell therapy strategy that has significantly improved the treatment and prognosis of hematological malignancies. So far, the Food and Drug Administration (FDA) has approved seven CAR-T cell products to treat hematological malignancies (1-3). CAR-T cells can have a single chain variable fragment (scFv) on their surface that recognizes tumor-related surface antigens after genetic engineering modification and pass through CD3ξ, 4-1BB (a costimulatory protein, a component of CAR intracellular modules), CD28 and other intracellular signal domains to initiate an anti-tumor immune response and release INF-γ, TNF-α, perforin and granzyme can kill tumor cells (4-7). Compared with traditional T-cell receptors (TCRs), CARs can independently recognize tumor antigens and do not rely on antigen-derived peptides presented by class I molecules of the major histocompatibility complex (MHC). Moreover, when CARs are added to T-cells, they can be designed to specifically recognize and bind to these proteins or gangliosides on the surface of cancer cells (8). This recognition triggers the activation of the T-cells and leads to the destruction of the cancer cells; to date, four generations of CARs have been created (9). The first-generation CARs only possess an intracellular signaling domain of CD3ξ to activate T cells (10). In the second-generation CARs, the intracellular signaling domain added a co-stimulatory domain to CD3ξ, usually 4-1BB (CD137) or CD28, to enhance T cell activation and proliferation (11). CD3ζ is a T-cell-activated switch in CARs, and the co-stimulatory domain increases the effectiveness and durability of the CAR (12). The third-generation CARs have three intracellular signaling domains, with two co-stimulatory signaling domains [4-1BB (CD137) and CD28] used in conjunction with the CD3ξ domain. The fourth-generation CARs are referred to as T cells redirected for universal cytokine-mediated killing. Here, the addition of cytokine expression cassettes to the second-generation CARs enables the secretion of proteins, including interleukin (IL)-2, IL-12,

IL-10, IL-17 and IL-15, and promotes enhanced T cell activation, while recruiting a patient's immune cells (4,13).

CAR-T cell therapy is an up-and-coming adoptive immunotherapy technique with remarkable efficacy in the treatment of hematological tumors. However, despite its potential, several obstacles still limit its effectiveness in hematological and solid tumors (14,15). There is a need for continued research to address these challenges and optimize the use of CAR-T cell therapy in the treatment of hematological and solid tumors. This includes developing strategies to overcome antigen escape, reducing the toxicity associated with CAR-T cell therapy, improving the penetration of CAR-T cells into solid tumors, and enhancing the long-term efficacy of CAR-T cell therapy. By addressing these challenges, CAR-T cell therapy has the potential to become an even more effective and widely used therapy for cancer treatment.

2. Limitations of CAR-T cell therapy

CAR-T cell therapy is a promising cancer treatment that genetically modifies a patient's T cells to recognize and attack cancer cells. While CAR-T cell therapy has been reported to show great success in treating certain types of cancer, such as lymphoma, this approach has certain limitations and challenges (16). Reportedly, the main limitations of CAR-T cell therapy for hematological malignancies are antigen escape, CAR-T cell associated toxicities (17). There are also challenges such as insufficient CAR-T cell infiltration and immunosuppression when it is used to treat solid tumors (18).

Antigen escape and toxicity are significant challenges associated with CAR-T cell therapy when used alone or in combination with radiotherapy (19). One approach to reducing antigen escape is to develop dual-targeting CAR-T cells that target multiple antigens on tumor cells, which increase the breadth of the CAR-T cell response and reduce the likelihood of antigen escape. CAR-T cell therapy can also target antigens which are essential for cancer cells, which reduces the risk of antigen escape, and controlled expression of the target antigen, such as through inducible promoters or RNA interference, helps to reduce off-target effects and toxicity (20). Optimizing toe dose of CAR-T cells also helps to reduce toxicity and the risk of antigen escape. Previous studies have reported that lower doses of CAR-T cells were more effective than higher doses, as higher doses caused more severe toxicity and increased the risk of off-target effects (21). Reducing the risk of antigen escape and toxicity associated with CAR-T cell therapy when used alone or in combination with radiotherapy is a critical area of research. These strategies can help improve CAR-T cell therapy's safety and efficacy and reduce the risk of treatment-related side effects (22).

Antigen escape. The key obstacle affecting the effectiveness of CAR-T cell therapy is antigen escape. During follow-up of a clinical trial on the treatment of recurrent and/or refractory acute lymphocytic leukemia with CD19 targeted CAR-T cells, CD19 antigen was reported to be downregulated in tumor cells, which led to therapy resistance (23,24). Although CAR-T cells targeting a single antigen have shown good anti-tumor efficacy in the early stage of hematological malignancies, the recurrence rate is high due to the tumor cells partially or completely losing the expression of target antigen in the late stages (15,25). However, the mechanism of antigen escape is still unclear. It may be that the CAR induces antigen loss upon the activation of T cells through phagocytosis of target antigens and promoter DNA hypermethylation (26-28). During antigen escape, the density of target antigen on the surface of tumor cells decreases below the threshold required for CAR-T lymphocyte activity, and tumor cells develop resistance to CAR-T cell therapy (29). Solid tumors are more prone to antigen escape and therapy resistance because of their low expression level of tumor antigens and obvious tumor heterogeneity (30). It has been previously suggested that CAR-T cells combined with radiotherapy may cause epitope diffusion to overcome immune escape (31).

Toxicity: On target/on tumor toxicity. The main toxic reactions to CAR-T cell therapy are on target/on tumor toxicity and on target/off-tumor toxicity. Cytokine release syndrome (CRS) is one of the side effects of CAR-T cell therapy with high incidence and lethality (32). CRS is characterized by the release of a great quantity of inflammatory cytokines, which are closely related to CAR-T cells and bystander cells (such as monocytes/macrophages) that have recognized the antigens on target tumor cells. The occurrence and severity of CRS depend on the tumor load, CAR structure, CAR-T cell number and other factors (33,34). Controlled CRS can improve the remission rate and progression free survival (PFS) of cancer patients and improve the effectiveness of CAR-T cell treatment. In addition, the severity of CRS is reported to be positively correlated with the tumor load of patients (35). Bridging therapy can reduce the tumor burden before CAR-T cells are used to reduce the occurrence of lethal CRS (36). CRS is mainly mediated by the cytokine IL-6 (37,38). The use of tocilizumab, an IL-6 receptor antagonist, blocks IL-6 and relieves the clinical symptoms of CRS. In addition, the use of IL-1 receptor antagonists, such as anakinra and granulocyte-macrophage colony-stimulating factor, have been reported to alleviate the symptoms of CRS (39). Differently to hematological tumors, target antigens in patients with solid tumors are often delivered to different levels by normal tissues (40). Targeting of antigens expressed on normal cells by CAR-T cells causes harm to normal tissues and organs. Therefore, the specificity of CAR-T cells for the target antigen is the key to reduce its toxicity (41). The on-target/off-tumor toxicity can also be reduced by adjusting the affinity of the scFv (42).

Insufficient tumor infiltration of CAR-T cells. The number of CAR-T cells transported to solid tumor tissue determines whether the treatment of CAR-T cells is successful. Firstly, local tissue hypoxia develops in solid tumors on account of the hyperproliferation of tumor cells, abnormal vascularization and insufficient blood supply in the expanded tumor tissue (43). The conveyance and activation of CAR-T cells depend on tumor blood vessels; therefore, CAR-T cells have difficulty in reaching tumor tissues far away from blood vessels, which results in therapy resistance (44). At the same time, in the hypoxic tumor microenvironment (TME), tumor cells produce cytokines, which are recruited to immunosuppressive cells to deplete CAR-T cells (45). Hypoxia promotes the expression of HIF-1α, which activates the secretion of

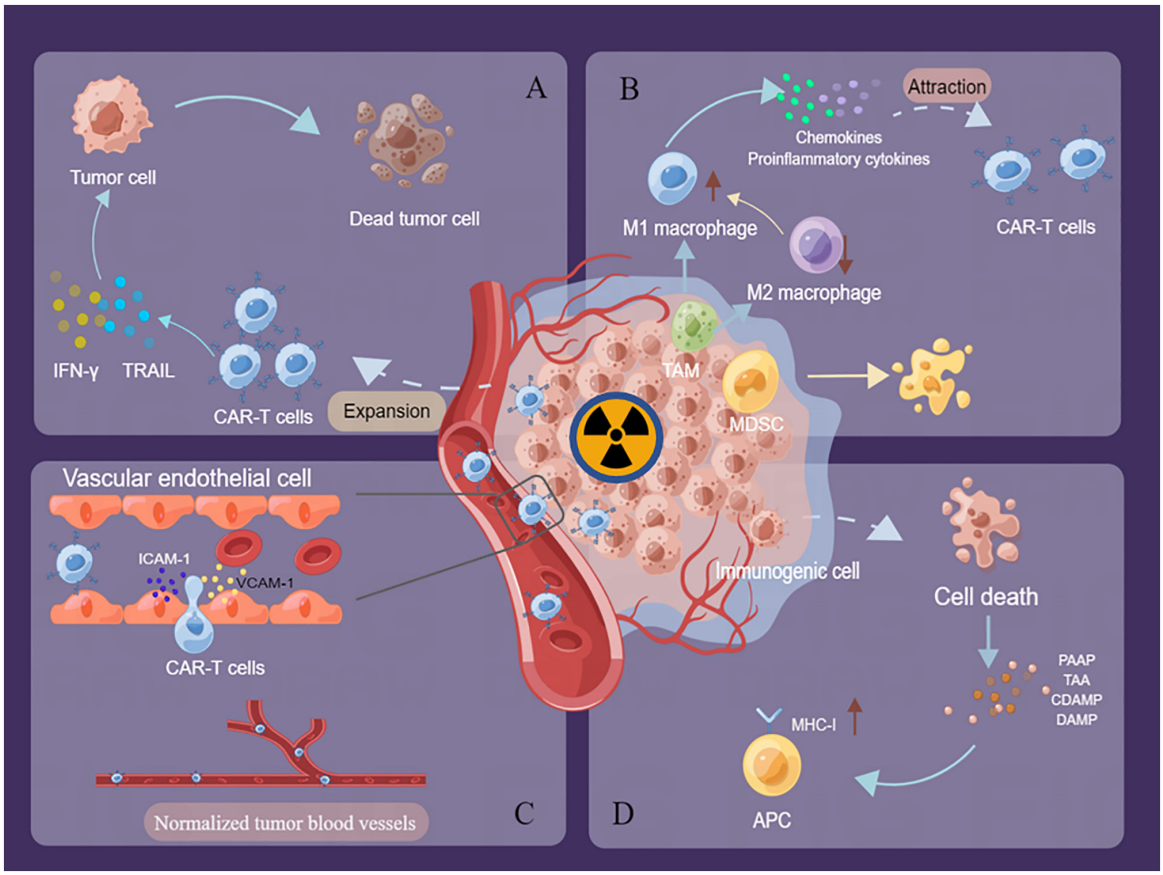

Figure 1. Advantages of the combination of radiotherapy and CAR-T cell therapy. (A) Radiotherapy promotes the expansion of CAR-T cells and enhances their killing effect on tumor cells. (B) Radiotherapy modulates the inflammatory TME and increases the secretion of chemokines and proinflammatory cytokines, leading to the homing of CAR-T cells. (C) Radiotherapy induces an increase in the expression of the integrins ICAM-1 and VCAM-1 in vascular endothelial cells, which facilitates the migration of CAR-T cells across the vascular endothelium into the tumor tissue and normalizes tumor blood vessels. (D) Radiotherapy potentially improves the efficacy of CAR-T cell therapy by activating and enhancing endogenous target antigen-specific immune responses. CAR, chimeric antigen receptor; TME, tumor microenvironment; ICAM, inter-endothelial cell adhesion molecule; VCAM, vascular cell adhesion protein; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; TAM, tumor-associated macrophages; MDSC, myeloid-derived suppressor cells; APC, antigen-presenting cell; MHC, major histocompatibility complex; PAAP, prostatic acid phosphatase; TAA, tumor-associated antigen; CDAMP, cancer-associated damage-associated molecular pattern; DAMP, damage-associated molecular pattern.

pro-angiogenic factors such as VEGF-A. VEGF-A inhibits the release of inter-endothelial cell adhesion molecule (ICAM)-1 and vascular cell adhesion protein (VCAM)-1, and inhibits the migration of CAR-T cells across the vascular endothelial cell barrier into the tumor tissue (46,47).

Immunosuppression. After CAR-T cells surmount obstacles and successfully arrive at the tumor tissue, suppressive immune cell subsets in the TME can deplete and render the CAR-T cells dysfunctional. Suppressive immune cell subsets are mainly recruited by cytokines secreted by tumor cells (48). Regulatory T cells (Tregs), an important subgroup of suppressive immune cells, are recruited to tumor tissue by the chemokines CXCL12 or SDF1α, which inhibit the activity of effector T cells and CAR-T cells (49). Myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs) have been reported to be immunosuppressive subsets that affect CAR-T cell function (50). MDSCs produce and secrete cytokines and metabolic factors, such as IL-10, IL-1 receptor antagonist, TGF-β and nitric oxide, which hinder the anti-tumor immune response. MDSCs also express immune checkpoint molecules that promote T cell and CAR-T cell exhaustion (51,52).

TAMs can be taken up by tumor cells and polarized into an anti-inflammatory M2-subtype by secreting TGF-β, prostaglandin E2, IL-10, indoleamine 2,3-dioxygenase IDO, and chemokines such as CCL17, CCL18 and CCL22, which lead to the inhibition of T cell responses and infiltration of Tregs (53-55). To overcome the factors which hinder the application of CAR-T cell therapy in solid tumors, a combination therapy strategy has been proposed, in which CAR-T cells are administered alongside radiotherapy.

3. Advantages of radiotherapy

Radiotherapy (RT) is a traditional first-line anti-tumor therapy. In addition to directly killing the tumor cells, radiotherapy also activates immune responses in the TME (56) (Fig. 1D). Dying immunogenic cells secrete radiation-associated antigen proteins, tumor-associated antigens, and cell death-associated molecular patterns that increase target antigen expression, which leads to the activation of immune surveillance (6,57). Radiotherapy also increases the number of MHC molecules on antigen-presenting cells (APCs), which leads to the translocation of calreticulin (CRT), which releases high mobility group

Table I. Summary of chimeric antigen receptor T cell therapy and radiotherapy in clinical and preclinical studies.

A, Preclinical studies

First author, year	Tumor model	Target	Scheme	(Refs.)
Weiss *et al*, 2018	Glioblastoma	NKG2D	4 Gy x1	(31)
DeSelm *et al*, 2018	Pancreatic cancer	sLeA	2 Gy x1	(80)
Murty *et al*, 2020	Glioblastoma	GD2	5 Gy x1	(99)

B, Clinical studies

First author, year	Tumor model	Target	Scheme	(Refs.)
Sim *et al*, 2019	Diffuse large B-cell lymphoma	CD19	2-4 Gy/fraction (range, 6.0-36.5 Gy)	(61)
Smith *et al*, 2019	Multiple myeloma	BCMA	4 Gy x5	(103)
Qu *et al*, 2020	Diffuse large B-cell lymphoma	CD19/CD20/ CD22	2 Gy x20	(89)
Saifi *et al*, 2022	Relapsed and/or refractory NHL	CD19	Median 20 Gy in 5 fractions	(105)

NHL, non-Hodgkin lymphoma; NKG2D, natural killer group 2D; sLeA, sialyl Lewis A; GD2, disialoganglioside; BCMA, B-cell maturation antigen.

box 1 protein as well as ATP and heat shock protein, which activated dendritic cells, APCs, etc., and enhanced endogenous target antigen-specific immunity (13,58).

In addition, radiation converts immunosuppressive cells in the TME into immune-promoting cells, which strengthens the anti-tumor behavior of CAR-T cells (59) (Fig. 1B). Radiotherapy increases the number of M1-like macrophages and decreases M2-like macrophages and MDSCs in the TME (6). Although radiotherapy offers strong advantages in tumor treatment, it also promotes radio resistance in tumor cells, which results in the failure of local cancer treatment (60). However, radioresistant tumor cells are sensitive to T-cell retargeting, therefore, combining radiation with CAR-T cell immunotherapy can achieve the greatest efficacy. According to previous reports, radiotherapy boosts the efficacy of CAR-T cells through multiple mechanisms (61,62).

In the TME, radiotherapy triggers M1-type macrophages to secrete more chemokines, such as CXCR3, CXCL9-11 or CXCL16, in response to IFN-γ, which promotes CAR-T cell recruitment (63). Irradiation also induces an increase in the production of the integrins ICAM-1 and VCAM-1 in vascular endothelial cells, which facilitates the movement of CAR-T cells across the vascular endothelium into the tumor tissue (64,65) (Fig. 1C). In addition, radiotherapy normalizes tumor blood vessels and improves tumor reoxygenation, which improves hypoxia-induced therapy resistance (60). Radiotherapy also promotes the local expansion of CAR-T cells in tumors (Fig. 1A). In conclusion, radiotherapy may improve the effectiveness of CAR-T cell therapy by activating and enhancing endogenous target antigen-specific immune responses, which promotes CAR-T cell recruitment and expansion, and modulates the inflammatory TME (66,67).

4. Challenges associated with radiotherapy

Although there is ample evidence that the combination of radiotherapy and CAR-T cell therapy exerts synergistic antitumor effects, radiation therapy can cause immunosuppression and CAR-T cell death, which reduces the efficacy of the combination therapy. Radiotherapy induces the transformation of immune cells to immunosuppressive cells, such as Tregs, and enhances the effect of immunosuppressive molecules, such as IL-10, TGF-β and adenosine in the TME (68-70). Tregs are key cells that promote immunosuppression in the tumor immune microenvironment. Radiotherapy causes increased Tregs infiltration in irradiated tumor areas, and Tregs show increased Akt expression, which makes them more radioresistant than other T cell subsets (71). A preclinical study reported that targeted depletion of Tregs after radiotherapy promoted long-lasting antitumor effects (72). Notably, it has been reported that the infiltration efficiency of Tregs depends on the dose at each administration rather than the total dose and that this is positively correlated with each radiation dose (73). Therefore, when combining radiotherapy with CAR-T cell therapy, the optimal fractionation and radiation dose should be evaluated to maximize the efficacy and minimize the toxic side effects (74).

In addition, radiotherapy also directly damages CAR-T cells. Although there are few reports on the direct damaging effects of radiotherapy on CAR-T cells, high-dose radiotherapy administration immediately after the infusion of CAR T cells needs to be carefully evaluated for its effects on the health of the CAR-T cells (75). Notably, in a clinical study, it was shown that administration of 2 Gy of radiotherapy per dose induced *in vivo* expansion of CAR-T cells (76).

5. Combination of radiotherapy and CAR-T cell therapy

Radiotherapy is a double-edged sword, with differences in dose and fractionation times resulting in different immunomodulatory responses. On the one hand, it synergistically promotes anti-tumor effects when used in combination with CAR-T cell treatment (77). On the other hand, radiotherapy promotes an immunosuppressive microenvironment and reduces the anti-tumor effects of CAR-T cells (78). Therefore, the timing, total radiation dose and the number of fractions of radiation therapy during CAR-T cell treatment are critical for the success of the combination therapy (67). When CAR-T-cell therapy is used in union with radiotherapy, the standard total radiation dose and fractionations need to be optimized (56,79). Few previous studies have explored this, but the conclusions of studies on other immunotherapies such as immune checkpoint blockage and their combination with radiotherapy can be used for reference. Existing research indicates that the dose-fraction regimen of 8 Gy x3 is the best standard fractionation regimen for immunotherapy combinations (80,81). However, certain studies have suggested that low-dose radiotherapy (<2-4 Gy) may be a better complement to CAR-T cell treatment based on the radioimmunological effects (82).

Substantial evidence suggests that hypo-fractionated RT (>2 Gy/fraction) or standard fractionated RT (1.8-2 Gy/fraction) enhances the ability of radiation to promote anti-tumor immune response by the mechanisms described above (18,83). Hypo-fractionated irradiation has been reported to be the best radiotherapy method for extending the benefits of immunotherapy (84). CAR-T cell treatment combined with photon radiation is the type of combination therapy that has reported to demonstrate the best anti-tumor effects and minimal side effects (85). However, other types of radiation therapy such as iodine 125 seed brachytherapy combined with Robo1 (a member of the axon guidance receptor family that has also been reported to play a role in modulating chemotaxis of T cells and tumor angiogenesis) dimeric antigen receptor-natural killer cells has also been reported to suppress human pancreatic tumor growth in mice (39,86). Minimizing immunosuppression and CAR-T cell death due to radiotherapy and optimizing the dose and fractionation of radiotherapy when combined with CAR-T cell therapy are essential considerations for enhancing treatment efficacy and reducing toxicity (87). These strategies are still being studied, and more research is needed to fully understand the optimal parameters for combining radiotherapy and CAR-T cell therapy (88).

6. Implementation and feasibility of combined radiotherapy and CAR-T cell therapy

The implementation and feasibility of combined radiotherapy and CAR-T cell therapy is an area of active research and clinical investigation. The combination of these two therapies has the potential to enhance treatment efficacy; however, certain challenges and considerations need to be addressed (89). One potential benefit of combining radiotherapy and CAR-T cell therapy is that radiotherapy can help to create a more favorable TME for CAR-T cells to function. Radiation can cause DNA damage in cancer cells, which leads to the release of tumor-associated antigens and the activation of immune cells (90). One concern is that radiation can cause damage to CAR-T cells, which may compromise their function and reduce treatment efficacy. Several clinical trials to investigate the feasibility and efficacy of combining radiotherapy and CAR-T cell therapy to treat various types of cancer are understood to be currently underway (60,91,92). These studies are reported to be evaluating dosing schedules, radiation techniques and CAR-T cell products. The combination of radiotherapy and CAR-T cell therapy has the potential to be a powerful treatment option for certain types of cancer such as prostate cancer, pancreatic cancer and glioblastoma (93). The below section enumerates and summarizes the existing preclinical and clinical studies which have reported on the combination of CAR-T cell therapy and radiotherapy, to understand its safety and efficacy and to assess the optimal timing, total radiation dose and the number of fractionations of radiotherapy, and CAR-T cell infusion.

A preclinical study of natural killer group 2-member D (NKG2D) CAR-T cells and 4 Gy x1 RT in glioblastoma. In a preclinical study of glioma, GL-261 cells were intravenously injected with NKG2D CAR T cells 5, 7 days and 10 days after implantation of the GL-261 cells into the brains of mice, and local skull radiotherapy was performed on the 7th day after tumor cell implantation, with a single dose of 4 Gy (31). The NKG2D system serves an indispensable role in cancer immune surveillance, and NKG2D-ligand is delivered on the surface of glioblastoma cells and radiotherapy is known to induce its expression (31,94). After systemic administration, chNKG2D CAR-T cells moved to the brain tumor site through the blood brain barrier, and no adverse events were reported (95). The results indicated that local tumor irradiation promoted the movement of CAR-T lymphocytes to the tumor site and increased the expression of IFN-γ in a NKG2D-based chimeric antigen receptor construct (chNKG2D) in CAR-T cells. Moreover, the survival of tumor bearing mice was prolonged and certain of the treated mice demonstrated complete tumor regression (31).

A preclinical study of GD2 CAR-T cells and whole-body irradiation (WBI) or localized 5 Gy x1 RT in glioblastoma. A preclinical study in glioblastoma compared the anti-tumor effects of GD2 CAR-T cells combined with a sub-lethal 5 Gy WBI or localized 5 Gy radiotherapy (96). *In vitro*, the combination treatment induced substantial cytotoxic effects on GD2-overexpressing mouse GBM cell lines. In the *in vivo* experiments, a single intravenous infusion of GD2 CAR-T cells within 1-3 h of 5 Gy WBI, significantly improved the anti-tumor response and survival of tumor bearing mice compared with the other groups (97). Ten weeks after the first treatment, the same GD2+ tumor cells were inoculated in the contralateral tumor hemisphere of mice, and only the group which received the combination of WBI and GD2 CAR-T cell therapy showed tumor regression, which indicated antigen-specific T cell memory. However, treatment with GD2 CAR-T cells 4 days after WBI indicated no anti-tumor therapeutic effects. Therefore, the timing of radiotherapy and CAR-T cell injection may be critical. The combination of localized 5 Gy RT and GD2 CAR-T cells indicated significant complete anti-tumor responses only in mice that received the

combined treatment compared with the single-arm control group (98).

Imaging with intravital microscopy indicated that WBI enabled rapid extravasation of GD2 CAR-T-cells from the vasculature, which promoted their local expansion and durable immune responses in the TME. In the two aforementioned preclinical studies on GD2 CAR-T cells joined with large fraction radiotherapy for glioma bearing mice, local treatment joined with CAR-T cells was confirmed to promote tumor regression, and no serious toxic effects were observed. However, when combining systemic radiotherapy and CAR-T cells, the anti-tumor effects and treatment timing were crucial owing to the impact of the timeliness of radiotherapy in promoting local infiltration and migration of CAR-T cells (99).

A preclinical study of sialic acid Lewis-a (sLeA) CAR-T cells and 2 Gy x1 RT in pancreatic cancer. In a study with sLeA-expressing pancreatic cancer tumor-bearing mice, a strategy which combined sLeA-targeted CAR-T cells with 2 Gy single low-dose radiotherapy was used. The study reported that local radiation sensitized tumor cells to activated CAR-T cells, and that combination therapy upregulated the sensitivity of sLeA- and sLeA+ tumor cells to TRAIL-mediated cell death (80). Pancreatic cancer is considered as a cold tumor, having the characteristics of low mutation load and low response to T cell infiltration, which indicate its low response to immunotherapy. The combination of low-dose radiotherapy with CAR-T cells increased the sensitivity of pancreatic cancer cells to the immune response. The potential mechanism may involve the activation of the innate and adaptive immunity (mainly CD4+effector T cells), which enable NKG2D dependent tumor growth control and reprogramming of the immunosuppressive tumor immune microenvironment (100). However, evidence for the immunogenicity of 1.8-2 Gy conventional radiotherapy in the TME is insufficient. The complex TME of solid tumors is a serious obstacle to CAR-T cell treatment, which necessitates its combination with other anti-tumor therapies to achieve improved therapeutic efficacy (91). Although the combination therapy with radiotherapy and CAR-T cells has been evaluated in animal tumor models, none of the CAR-T cell products have been approved for treating for solid tumors in the clinic.

A clinical study of CD19 CAR-T cell therapy and different doses of bridging radiotherapy on diffuse large B-cell lymphoma (DLBCL). To achieve improved anti-tumor effects between the time when autologous T cells were collected and final CD19 CAR-T-cell therapy was administered to treat patients with DLBCL, the use of bridging radiotherapy was proposed (101). Relapsed/refractory (R/R) DLBCL patients accepted bridging radiation treatment prior to the administration of Axicabtagene ciloleucel, and all patients received 2-4 Gy per session, with a median dose of 20 Gy (range, 6.0-36.5 Gy). A subset of patients received concurrent chemotherapy (fludarabine/cyclophosphamide). The results of the study indicated that patients did not experience disease progression prior to CAR-T cells treatment. Therefore, these results indicated that bridging radiation treatment provided adequate local disease control prior to CAR-T cell injection. In addition, no obvious toxicity was observed during the bridging treatment and 27% of the patients developed CRS after the infusion of Axicabtagene ciloleucel (61).

A case of B-cell maturation antigen (BCMA) CAR-T cell therapy and 4 Gy x5 radiotherapy for multiple relapsed and refractory myeloma. In a study which evaluated the targeting of CAR-T cells by autologous BCMA, it was reported that radiotherapy combined with CAR-T cells, unexpectedly, exerted synergistic anti-tumor effects through an abscopal-like response (102). In this clinical trial, a patient with multiple relapsed refractory myeloma received BCMA CAR-T cell therapy. Shortly after the treatment, emergency high-dose steroids and palliative radiotherapy (total dose, 20 Gy, 5 times, per site) were given to the affected thoracic vertebrae and whole brain due to spinal cord compression. The authors reported that after the use of a high dose of steroids, there was no decline in the therapeutic efficacy of CAR-T cells. Moreover, compared with other patients with multiple relapsed refractory myeloma who only received BCMA CAR-T cells, this patient presented with a specific delayed CRS. The time of onset for CRS-like clinical symptoms and inflammatory markers was consistent with the expansion of new TCR clones after radiotherapy (103). Therefore, this combination treatment showed synergistic effects, which lead to an abscopal-like response.

A phase II clinical trial of CD19/CD20/CD22 BCMA CAR-T cell therapy and 2 Gy x20 bridging radiotherapy in R/R DLBCL. In a phase II clinical trial which evaluated the safety and efficacy of CAR-T cells, the investigators enrolled ten patients with R/R DLBCL, who had high tumor burden and received bridging radiotherapy (2 Gy x20) and bridging chemotherapy (104). CAR-T cells have three types of targets, namely, CD19, CD20 and CD22. Dual-targeted CAR-T cells are selected according to the two antigens with higher expression at relapse. Patients who underwent radiotherapy showed a higher overall response rate and the toxicity was lower than in patients who underwent chemotherapy (89). This suggested that radiation treatment was an effective and safe treatment for tumor reduction prior to CAR-T cell therapy. These findings indicated that bridging radiotherapy was an effective and safe option prior to CAR-T cell therapy. In addition, in patients with R/R DLBCL and a high tumor burden, radiotherapy prior to CAR-T cell treatment may reduce the severity of CRS and neurotoxicity associated with the treatment.

A clinical study of CD19 CAR-T cell therapy and different doses of bridging radiotherapy in non-Hodgkin lymphoma. In a retrospective analysis of CD19 CAR-T cells, the leading cause of treatment failure after CAR-T cell infusion in patients with non-Hodgkin lymphoma was reported to be the progression of the primary tumor site. Patients who accepted CAR-T cell infusion after bridging radiotherapy had higher rates of local tumor control and durable responses. Thus, higher doses of bridging radiotherapy may be beneficial in patients with a high risk of recurrence (105). In a clinical study on radiotherapy and CAR-T cells, radiotherapy, as a bridge treatment, was reported to reduce the tumor load, improve the curative effect of CAR-T cell therapy, and reduce the occurrence of CAR-T cell related serious toxic reactions (106) (Table I). Fortunately, abscopal

effects were also observed, which together with CAR-T cells could have promoted the anti-tumor immune effects. The combination of bridging radiation treatment and CAR-T cell treatment was a clinically relevant treatment strategy, but there were few relevant clinical studies exploring this at present (96). Further clinical studies which evaluated the efficacy of the above combination treatment strategy are required to validate its safety and therapeutic efficacy in the clinic (107).

7. Conclusions

CAR-T cell therapy is a revolutionary and promising immunotherapy technology. Mature CAR-T cell products have been approved for treating hematological tumors and have demonstrated promising results in the clinic. However, its application in hematological and solid tumors faces many unexpected difficulties and reduces the therapeutic effect. As a conventional anti-tumor therapy, radiotherapy can overcome certain obstacles by enhancing the specific immunity against endogenous tumor antigens, which promotes the amplification and expansion of CAR-T cells and improves the hypoxic TME (108). However, radiotherapy has advantages and disadvantages, and differences in dose and the number of divisions generates different immune regulatory responses. Therefore, the timing of radiotherapy, the total radiation dose and the number of fractionations, and the type of radiation are critical parameters that influence the success of the combination therapy (107).

In preclinical and clinical studies on the combination of CAR-T cells and radiotherapy, radiotherapy has been reported to promote the rapid exosmosis and migration of CAR-T cells from the vascular system into the tumor site, activate anti-tumor immunity, reduce tumor load, improve the therapeutic effect and reduce the occurrence of CAR-T cell related severe toxic reactions. Although the antitumor effects of this combined treatment strategy are definite, there is still a long way to go before a standard treatment plan is put forward, and more research is needed to fully understand the optimal parameters for combining therapies to address the associated challenges for clinical translation.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

Not applicable.

Authors' contributions

LZ was responsible for conceptualization and the writing of the original draft. YL wrote and revised the manuscript. TAM was responsible for visualization, generation of the figures, and was involved in drafting and revising the intellectual content of the manuscript. YW was responsible for revising the manuscript, and for project administration and supervision. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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