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Dynamic Duo: Synergy between Cancer Radiation Therapy and Immunotherapy

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Abstract

Immunotherapy is increasingly being applied in conjunction with any of the three commonly accepted treatment modalities of surgery, chemotherapy, and radiation therapy in the treatment of solid and hematological malignancies. Radiation therapy, which can be applied as a monotherapy, has immunostimulatory properties that are specifically augmented when used together with immunotherapy. High-dose irradiation has significant effects on both the targeted tumor and the immune system that are mutually enhancing when applied appropriately. Immunogenic cell death and changes in gene expression in irradiated tumor cells fuel the immune system, allowing for immune-mediated killing that leads to improved overall survival. Herein, we discussed the recent advances in radiation therapy and immunotherapy, covering myeloid-derived suppressor cells (MDSCs), radioimmunotherapy, immunocheckpoint inhibition, cancer treatment vaccine, and chimeric antigen receptor (CAR) engineered T cells and NK cells. We put forth our current understanding of the mechanisms behind how immunotherapy and radiation therapy complement each other, and suggest some topics that are worthy of further exploration.

Keywords: Immunotherapy; Radiation therapy; Myeloid-derived suppressor cells; Radioimmunotherapy; Cytokines; Antibodies; Vaccines; Adoptive T-cell therapy; CTL; CAR T or NK cells; Synergy

Introduction

Cancer remains a major health concern; effective cancer therapy in most types of cancer is still a challenge for researchers at both the bench and bedside. Cancer immunotherapy activates the endogenous anti-tumor innate and adaptive immune response by using cytokines, antibodies, vaccines, or even allogeneic immune cells including cytotoxic T cells (CTL) or natural killer (NK) cells. Cancer radiation therapy, on the other hand, not only directly kills tumor cells (cytotoxicity) but also leads to systemic response at distant sites, a phenomenon known as the abscopal effect [1,2], which has been implicated in the induction and enhancement of the anticancer immune response. Radiation therapy and immunotherapy and the combination treatment with these two have advanced rapidly in recent years. The preclinical and clinical outcomes of some of the studies, especially the combination treatment are very promising. In this article, we will review the advances in these two fields, and discuss how these two treatment methods interact with and compensate for each other to achieve superior therapeutic effect with minimal toxicity.

Radiation Therapy: from EBRT to Brachytherapy, to SBRT, to Radioimmuno Therapy (RIT)

There are two classic methods of delivery of radiation therapy, external beam radiation therapy (EBRT) in which high energy rays are directly aimed at the tumor or internal radiation (brachytherapy) for which radioactive sources are implanted at the tumor site [3]. The standard EBRT dosage is 1.8-2 Gy per day for 5-9 weeks with variations depending on numbers of fractions [4]. In recent years, interest has risen in stereotactic body radiation therapy (SBRT), which allows precise image-guided delivery of radiation to small tumors [5]. The limited radiation field of SBRT allows for much higher doses with minimal damage to surrounding tissues [6], challenging original perceptions of radiation therapy as a form of therapy with negative systemic repercussions.

RIT delivers monoclonal antibody-conjugated radionuclides to tumor tissues that specifically express the antigens recognized by the antibody, permitting the delivery of high-dose therapeutic radiation to cancer cells while minimizing the exposure of normal cells [7]. Clinically, RIT is mostly applied to the most radiosensitive tumors, including leukemias and lymphomas. Solid tumors are more resistant and require higher (5 to 10 times) deposited radiation dose than that required in hematopoietic malignancies [7].

A perfect molecular target expressed on the cell surface is a key for successful RIT. This molecular target must be expressed only on surfaces of cancer cells, not normal cells. In practice, those targets that have higher expression levels in cancer cells than in normal cells are considered as good targets, and can be identified through protein expression profiling and database mining of many readily available bioinformatics databases. Equally important, the antigen (molecular target)-antibody binding affinity must be higher in cancer cells than that in normal cells, as some proteins similarly expressed in both cancer and normal cells differ in their binding affinities to specific antibodies. Unfortunately this kind of data is very scarce. Lastly, the metabolism of the antibody- antigen complex ultimately determines the therapeutic activity of the radionuclides.

Metabolism of the antibody-antigen complex by the cell may either enhance the anticancer effects by retaining the radionuclide within lysosomes or storage proteins, such as using cluster of differentiation (CD) antigen 20 (CD20), or reduce the radiation effects by expelling the radionuclide from the cell, such as using CD5 or prostate-specific membrane antigen (PSMA). Therefore selecting the appropriate antibody-antigen complex is very important for the optimal radionuclide delivery in RIT [7].

Several excellent targets have been identified for lymphoma, including CD20 and CD22 for B-cell non-Hodgkin lymphoma (B-NHL), as well as CD33 and CD45 for acute myeloid leukemia. Clinical trials using CD20 antibodies conjugated to radionuclides, ^{131}I or ^{90}Y produce higher overall response rates (ORRs, 60-80%) and complete response rates (CRs, 15-40%) in relapsed NHL than rituximab, the humanized CD20 antibody, alone [7-10]. The median remission duration with non-myeloablative RIT has been 1 to 2 years in most studies with 15-20% of patients achieving sustained remission and in some cases, remission duration of 10 years or more [7,11].

Radiation Therapy: How does it affect the Immune System?

Radiation causes DNA damage, which leads to cell death via apoptosis, mitotic catastrophe via p53-Caspases-cytochrome c cascade, necrosis (less common than apoptosis) via TNFα-PARP-JNK-Caspases pathway, senescence, and autophagy via PI3K-Akt-mTOR cascade [3]. As these cells die, they are taken up by scavenger cells called antigen-presenting cells, such as dendritic cells (DCs). These antigen-presenting cells (APCs) then travel to regional lymph nodes where they present antigen to T cells, initiating or potentiating anti-tumor immune response. Activated tumor-specific T cells can then traffic to the tumor to participate in immune-mediated tumor killing [12]. At the same time, irradiation can cause (a) upregulation of chemokines and adhesion molecules, providing signals for T cells to migrate towards the tumor, (b) upregulation of MHC molecules and tumor-associated antigens, making it easier for T cells to recognize tumor, and (c) upregulation of death signal receptor, Fas and downregulation of regulatory T cells (Tregs), making it easier for tumor specific cytotoxic T cells (CTL) to kill tumor [12].

Immunogenic cell death (ICD) requires interaction between calreticulin, a multifunctional calcium-binding protein commonly associated with the endoplasmic reticulum, and dendritic cells [4,13]. ICD may be induced by some dose of radiation therapy [4]. Most importantly, the cross presentation of tumor antigens by dendritic cells to T cells as a result of ICD during radiotherapy is currently being exploited as an "*in situ* vaccine", which has been shown to be effective even in patients for whom the passive immunotherapy of anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) has been ineffective [14]. In addition to generation of "*in situ* vaccines", radiation has been used to generate whole-cell vaccines that are then injected into patients. For example, algenpantucel-L, a vaccine consisting of irradiated HAPa-1 and HAPa-2 (allogeneic pancreatic cancer cell lines) transfected with murine α-1,3-galactosyltransferase for easy recognition by endogenous antibodies, has been proven to improve survival and is currently undergoing phase III trial [15]. Whole-cell lysates are often preferred over peptides due to possible immune evasion through selection for tumor cells not expressing the targeted peptide [16].

The Immunosuppressive Effect of Radiation Therapy: How to overcome it?

Unfortunately, in addition to the anti-tumor immune response, radiation therapy causes extensive immunosupression that compromises therapeutic efficacy. Radiotherapy does indeed affect the natural killer cell (NK) activity in cancer patients predominantly when the irradiation site includes the mediastinum [17]. It is widely accepted that external radiotherapy suppresses NK activity while brachytherapy has little influence on NK activity alteration [18].

In recent years, characterization or manipulation of myeloid-derived suppressor cells (MDSC) that exist in the tumor microenvironment has emerged as a very interesting research topic [19-21]. The moderate therapeutic efficacies of most of the current therapies including radiation therapy can be explained by activation of the MDSCs.

MDSC is a collective term used to describe a heterogeneous population of immature myeloid cells that have immunosuppressive properties [19-21]. There are two general populations of MDSCs that reflects the two lineages of myeloid cells: monocytic and granulocytic. In mice, monocytic MDSCs (m-MDSC) are $CD11b^{+}Ly6C^{high}Ly6G^{-}$, granulocytic MDSCs (g-MDSC) are $CD11b^{+}Ly6C^{low}Ly6G^{+}$, while human MDSCs are defined as $CD11b^{+}CD14^{-}CD133^{+}$ [21]. MDSCs in the serum and tumors correlate with poor clinical outcomes [21]. MDSCs directly suppress anti-tumor immune response through expression of iNOS and arginase. iNOS generates NO, which inhibits signaling through the IL-2 receptor, interfering with T cell activation and proliferation. Arginase depletes L-arginine that is crucial for T-cell function. Additionally, iNOS and arginase mediate the generation of other reactive nitrogen species such as peroxybitrite, which can modify the T cell receptor (TCR) of tumor-killing T cells, making them unable to bind to their cognate MHC-peptide antigen. The reactive nitrogen species can also modify chemokines, inactivating them and preventing the recruitment of tumor-killing immune cells [21]. Besides, MDSCs indirectly suppress anti-tumor immunity by generating inhibitory cytokines, such as IL-10 and transforming growth factor (TGF)-β, which can suppress the anti-tumor function of tumor-infiltrate lymphocytes (TILs), generate Tregs in the tumor, and convert DCs into regulatory phenotype [20,21].

Ionizing radiation profoundly affects MDSCs. Depending on radiation dose and tumor models, the effect of radiation therapy on MDSCs can be characterized as: removal, recruitment, reorganization, repolarization, and representation [21]. Clearly, except for removal, all other four effects should be supressed to improve therapeutic efficacy.

In a recent clinical study, patients with oligometastases of various cancer types were found to have elevated g-MDSCs and certain subsets of m-MDSCs. Treatment with sunitinib (Sutent), a multitargeted kinase inhibitor drug, resulted in a significant reduction in m-MDSCs ($CD33^{+}CD14^{+}CD16^{+}$), their arginase levels, and Tregs. Patients responding to the treatment also had an increase in T-cell proliferative activity while nonresponding patients did not. SBRT synergized the therapeutic effects of sunitinib, which were not observed in patients receiving SBRT alone [22]. Obviously SBRT benefited from sunitinib-inhibited MDSCs in this study.

Cancer Immunotherapy: Activate or Direct the Immune System to Kill Cancer Cells

Cytokine cancer immunotherapy

Many spontaneous and experimental cancers naturally express ligands for the lectin-like type-2 transmembrane stimulatory NKG2D immunoreceptor. In addition to stimulating proliferation of CTL or NK cells, interleukin-2 (IL-2) or IL-12 suppresses tumor metastases largely via NKG2D ligand recognition and perforin-mediated

cytotoxicity [23]. The infusion of IL-2 at low or high doses for multiple cycles in patients with metastatic melanoma and renal carcinoma was the first successful immunotherapy for cancer, proving that the immune system could completely eradicate tumor cells under certain conditions, which encouraged the use of other IL-2 family cytokines, such as IL- 7, IL-15, and IL-21 in clinical trials with some obtaining measurable early success. These cytokines regulate the development, proliferation, and function of specific subsets of lymphocytes at different stages of differentiation [24]. IL-18, on the other hand, is another potent immunoregulatory cytokine that was initially described as an IFN-γ–inducing factor. IL-18 enhances T and NK cell cytokine production, proliferation, and cytolytic activity and the expression of Fas ligand (FasL) and FasL- or perforin-mediated antitumor activity. Systemic administration of IL-18 has demonstrated considerable therapeutic activity in several murine tumor models [23,25]. Therefore a cytokine combination treatment with IL-2 family members and IL-18 may reach anticancer synergy by stimulating both perforin and FasL effector mechanisms [23].

Because focal high-dose radiation makes tumors more immunogenic, treatment followed by IL-2 or other cytokines including IL-18 may achieve significantly improved therapeutic effect. In a phase I study, patients with metastatic melanoma or renal cell carcinoma (RCC) who had received no previous medical therapy received one, two, or three doses of SBRT (20 Gy per fraction) with the last dose administered 3 days before starting IL-2. IL-2 (600,000 IU per kilogram by means of intravenous bolus infusion) was given every 8 hours for a maximum of 14 doses with a second cycle after a 2-week rest. Patients with regressing disease received up to six IL-2 cycles. Eight of 12 patients (66.6%) achieved a complete (CR) or partial response (PR) (1 CR and 7 PR). Six of the patients with PR on computed tomography had a CR. Five of seven (71.4%) patients with melanoma had a PR or CR, and three of five (60%) with RCC had a PR [26]. A phase II clinical trial to treat patients with metastatic melanoma with high dose IL-2 compared to high dose IL-2 plus SBRT (NCT01416831) in currently undergoing, and expected to conclude in 2016.

Antibody therapy

Tumors have an escape mechanism that involves activating the immunosuppressive signals to dampen lymphocytic activity. The inhibitory receptors on CTL or NK cells have the capacity to abrogate anti-tumor immune response, and therefore are termed as immune checkpoints [27,28]. Programmed death 1 (PD-1) and CTLA-4 are the best characterized inhibitory receptors for CTL [27]. As T cells become activated, CTLA-4 expression increases which inhibits their proliferative capacity and effector function. CTLA-4 is also essential for suppression of effector T cell responses by $Foxp3^+$ $CD4^+$regulatory T cells. PD-1-mediated inhibition of cytotoxic T cell responses occurs in tumors as: (1) T cells experience chronic antigen stimulation, resulting in persistent PD-1 upregulation; (2) T cells secrete interferon-γ (IFN-γ) upon antigen recognition, which leads to programmed death-ligand 1 (PD-L1) upregulation on cancer and tumor stromal cells; and (3) T cells lose effector function through negative PD-1/PD-L1 interactions [27].

What will happen if these inhibitory receptors are blocked? Humanized antibodies against PD-1, PD-L1 and CTA-4 have been developed and have shown very promising therapeutic efficacies in various clinical trials. In a phase I trial, the overall response rate to anti-PDL1 antibody, MPDL3280A treatment in patients with metastatic urothelial bladder cancer was shown to be dependent on the amount of PDL1 expressed by the tumors. Patients with high-PDL1-expressing tumors had a response rate of 43% versus 11% for patients with low-PDL1-expressing tumors [29,30]. Recently the FDA have approved the use of anti-PD1 antibodies, nivolumab (OPDIVO®) and pembrolizumab (Keytruda®) for the treatment of non-small cell lung cancer (NSCLC) and advanced melanoma [30]. Notably, it was observed that in mouse tumors PDL1 expression was increased after irradiation, and the addition of anti-PDL1 IgG led to rapid decrease in tumor volume compared with radiation or PDL1 blockade alone, by promoting the secretion of TNF-α by $CD8^+$ cells, which in turn suppressed the number of tumor infiltrating MDSCs [30-32]. A different study also found that dual anti-PD1 antibody therapy with stereotactic radosurgery (SRS, 10Gy) in mice with implanted glioma resulted in significantly improved overall survival; biopsy of the tumors from the combination treatment showed higher numbers of tumor-infiltrating lymphocytes and lower numbers of Tregs [30,33].

Humanized antibodies against CTLA4 (ipilimumab, tremelimumab) are considered as the first checkpoint inhibitors approved by the FDA [30, 34]. In a phase III trial, it was found that patients with advanced melanoma receiving ipilimumab had longer overall survival than those treated with gp-100 vaccine (10.1 months vs. 6.4 months, p=0.003) [30,35]. Preclinical studies in mouse tumor models with dual anti-CTLA4 antibody and radiation treatment obtained similar results compared to anti-PD1/PDL1 antibodies, which led to a number of clinical trials [30,34,36,37]. One of these was done using ipilimumab with radiation in patients with metastatic, castration-resistant prostate cancer. In this study, up to three bone metastases were treated with single 8 Gy radiation fractions with ipilimumab (at 3 or 10 mg/kg) given every 3 weeks for a total of four doses. Of 50 patients who received high- dose ipilimumab, one patient achieved complete response, and an estimated 15% experienced declines in prostate-specific antigen levels [30,38].

Notably, cancer cells also overexpress a wide variety of oncogenes, such as the epidermal growth factor receptor (EGFR) family members EGFR and Her2 [39,40]. High-levels of oncogene expression means poor prognosis in cancers including head and neck cancer (HNSCC) [41,42], colon cancer [43,44], and breast cancer [45]. Humanized antibodies against EGFR, such as cetuximab, and against Her2, such as Herceptin, have been approved by the FDA to treat these cancers. These humanized antibodies block cancer cell proliferation/survival/metastasis signaling. More importantly, these antibodies trigger antibody-dependent cell-mediated cytotoxicity (ADCC) of cancer cells. In HNSCC, at least, it has been discovered that radiation increases the expression of EGFR [46,47], and blockade of EGFR with cetuximab sensitizes cancer cells to the effects of radiation. Many clinical trials have proven that cetuximab in combination with radiation therapy significantly improves HNSCC patient survival, compared with either treatment alone [48,49], that the combination treatment has now been accepted as the standard care of HNSCC patients with high-levels of EGFR expression.

Cancer treatment vaccine

Producing effective cancer treatment vaccines has proven much more difficult and challenging than developing cancer preventive vaccines. To be effective, cancer treatment vaccines must achieve two goals. First, like traditional vaccines and cancer preventive vaccines, cancer treatment vaccines must stimulate specific immune responses against the correct target. Second, the immune responses must be

powerful enough to overcome the barriers that cancer cells use to protect themselves from attack by B cells and killer T cells (CTL) [50,51]. Sipuleucel-T (Provenge®), manufactured by Dendreon, inc., is so far the only FDA approved cancer treatment vaccine for certain types of metastatic prostate cancer.

Sipulecucel-T is actually an autologous, APC-based immunotherapy, in which the APCs of each individual patients are isolated from blood through leukapheresis and sent to Dendreon, where they are cultured with a defined prostate antigen, prostatic acid phosphatase (PAP) linked to granulocyte-macrophage colony-stimulating factor (GM- CSF) that stimulates the immune system and enhances antigen presentation. APCs cultured with PAP-GM-CSF constitute the active component of sipulecucel-T. Each patient's cells are then returned for infusion. Patients receive three treatments, usually 2 weeks apart [50,52-54]. A phase III clinical trial using sipulecucel-T in metastatic castration-resistant prostate cancer (mCRPC) showed a 4.1 month improvement in median survival over placebo (25.8 vs. 21.7 months). T cell and humoral immune responses to the prostate antigen were observed in patients receiving sipulecucel-T and correlated with survival [52,54,55].

Radiation treatment enhances antigen presentation, therefore it is expected that sipulecucel-T in combination with radiation therapy has significantly improved therapeutic efficacy. In fact, a randomized phase II clinical trial (NCT01807065) is underway to access this in mCRPC patients, and expected to finish in June, 2017 [52].

ACT, CAR T and CAR NK Cells

The condition of immunocompromised patients and the immunosuppressive activity of radiation therapy may be remediated by the infusion of allogeneic or autologous killer T cells or NK cells that are expanded and activated *ex vivo*.

Adoptive T-cell therapy (ACT) was first developed by Steven Rosenberg at NCI/NIH. His initial trials in patients with melanoma resulted in 50% clinical response rates among those for whom T-cell isolation and expansion worked after lymphodepletion. He also reported synergistic effects for patients with metastatic melanoma given total body irradiation and ACT. In that study of 93 patients, 40% of those given a high dose of 12 Gy as total body irradiation had a durable complete response as opposed to only 12% for patients not given total body irradiation. Among the 20 patients with complete responses, the 5-year survival rate was 93% [30,56]. The efficacy of ACT may be further enhanced by α-radioimmunotherapy (α-RIT), a type of internal radiotherapy using α–particles that are highly efficient to destroy small cluster of cancer cells with minimal impact on surrounding healthy tissues. In a murine model of multiple myeloma which express the tumor antigen CD 138 and ovalbumine (OVA), significant tumor growth inhibition and improved survival were observed in mice treated with anti- CD138 coupled to bismuth - 213 (to generate α–particles), followed by an adoptive transfer of OVA-specific $CD8^+$ T cells [57].

Normally, T cell receptors (TCRs) must bind to cognate antigens presented in the context of major histocompatibility complex (MHC) for specific T cells to be activated. T cells engineered to express chimeric antigen receptors (CARs) have the ability to directly target a particular antigen without requiring this MHC-TCR interaction, thereby granting CAR T cells the ability to kill tumor cells more efficiently after antigen encounter [30,58-61]. The prototypical CAR has four components, each of which is required to trigger the activation and killing functions of the engineered T cells: 1). The antigen specific domain, typically derived from the single chain variable fragment (scFv) of a monoclonal antibody that recognizes specific tumor cell surface antigen; 2) The spacer that links the heavy chain (VH) and light chain (VL) of the scFV; 3) The transmembrane domain; 4) The signaling domain that sustains/improves the T cell effector function. The first generation CARs deployed in clinical trials contained a CD3ζ chain, as derived from the TCR signaling complex, whereas more advanced second- or third-generation CARs contain additional costimulatory domains such as CD24, OX40 (CD134), or 4-1BB (CD137) [30,58-61]. A clinical trial using CD19-targeting CAR T cells in patients with relapsed or advanced lymphoblastic leukemia (ALL) showed profound clinical response, in which fourteen out of 16 patients entered remission phase. CD19 is highly overexpressed on ALL cell surfaces [30,58,60,61]. Multiple clinical trials using CAR T cells targeting various tumor antigens are currently under way [30,58,61].

However, CAR T cells have the following disadvantages:
1. The risk of inducing graft versus host diseases (GvHD) when using T cells from allogeneic donors [59]; 2. On- target/off tumor side effect [62]. Some cancer cell specific antigens may still be expressed in normal cells, even though the expression levels may be lower than in cancer cells, such as CD19 in normal B cells and interleukin 3 receptor α (IL3R) in bone marrow cells; using CD19 CAR T cells can cause a profound and long-lasting B-cell deficiency as they eliminate normal B cells while killing ALL cells, and using IL3R CART T cells kill not only leukemic cells but also bone marrow cells, leading to prolonged and profound marrow suppression. As a matter of fact, the anticancer activity of CAR T cells is related to and dependent on their persistence in patient circulation and malignant tissues, making this side effect worse. Furthermore, cancer cells lose their specific antigens after immunotherapeutic interventions, but the specific antigen CAR T cells may still render the on-target/off tumor effect; 3. Cytokine storm (high-level cytokine release) [62]. Cancer treatment using CAR T cells leads to T-cell expansion *in vivo*, which can in turn lead to the release of toxic levels of cytokines including TNF-α, IL-1, and IL-6, referred to variously as cytokine storms or cytokine release syndrome (CRS), this may damage local normal tissues and sometimes is fatal [63]; 4. CAR T cells have relatively long lifespans *in vivo*, so suicide genes are required for engineering CAR T cells that they can cleared as soon as their existence causes normal tissue damage [59,62,64].

CAR NK cells, on the other hand, do not have these disadvantages. Normally allogeneic NK cells are expected to induce an immune response and be rejected after a few days, and even autologous NK cells should disappear relatively rapidly from the circulation, owing to their limited lifespans [62]. Importantly, while T lymphocytes only kill their targets by a CAR-specific mechanism, NK cells are endowed with spontaneous cytotoxic activity and can trigger the demise of target cells in a tumor antigen-unrestricted manner via specific natural cytotoxicity receptors (NCRs), including NCR3 (NKp30), NCR2 (NKp44), NCR1 (NKp46), and killer cell lectin-like receptor subfamily K, member 1 (KLRK1, or NKG2D). NK cells also express the Fc fragment of IgG, low affinity III, receptor (FcγRIII) that binds the Fc fragment of antibodies to elicit ADCC. This specific feature of NK cells would enable the combination of two targeted therapies recognizing different (or the same) tumor antigens, namely CAR-expressing NK cells and a tumor antigen-specific monoclonal antibody [62]. Most importantly, NK cells produce a host of cytokines that are different from those produced by T cells, including interferon- γ (IFN- γ) and granulocyte macrophage colony stimulating factor (GM-CSF) [62],

which do not cause normal tissue damage. Intriguingly, it is also known that NK cells are "serial killers." Time-lapse video microscopy studies have shown that NK cells diligently move from one target to the next one, killing as many as 7–10 target cells. Evidence for such serial killing by T cells is lacking [62]. Moreover, NK-92 [45,65], NKL [30,65] , and YTS [22], continuously-growing, highly-active, NK cell-derived cell lines, are readily available for CAR engineering.

Multiple preclinical studies have shown the promising anticancer activity of CAR NK cells. The cell-surface glycoprotein, CS1 is highly and nearly ubiquitously expressed on multiple myeloma (MM) cell surface. CS1-CAR NK cells displayed enhanced MM cytolysis and IFN-γ production *in vitro*, and showed specific CS1-dependent recognition of MM cells. CS1-CAR NK cells also showed similarly enhanced activities when responding to primary MM tumor cells *ex vivo*. More importantly, in an aggressive orthotopic MM xenograft mouse model, adoptive transfer of NK-92 cells expressing CS1-CAR efficiently suppressed the growth of human IM9 MM cells and also significantly prolonged mouse survival [65]. Her2/ErbB2 is overexpressed in various cancer types. NK-92 cells engineered with CAR that contains the scFV of erbB2 specific antibody, FRP5, efficiently lysed ErbB2-expressing tumor cells *in vitro* and exhibited serial target killing. Specific recognition of tumor cells and antitumor activity were retained *in vivo*, resulting in selective enrichment of ErbB2-CAR NK cells in orthotopic breast cancer xenografts, and reduction of pulmonary metastasis in a renal cell carcinoma model, respectively [45]. There are two reports testing the anticancer efficacy of EGFR variant III (EGFRvIII)-CAR in glioblastoma multiforme (GBM). The first one engineered YTS cells with CAR containing the scFV from an EGFRvIII specific antibody (MR1.1) and DAP12 (DNAX-activation protein 12), a signaling adaptor protein involved in signal transduction of activating NK cell receptors [66] (MR1.1-DAP12). Infusion of YTS cells expressing MR1.1-DAP12 caused a moderate but significantly delayed tumor growth and increased median survival time in U87-EGFRvIII- overexpressing subcutaneous tumor xenograft model. Further engineering of these cells with the chemokine receptor CXCR4 conferred a specific chemotaxis to CXCL12/SDF-1α secreting U87 cells. Most importantly, the administration of such NK cells through tail vein injection resulted in complete tumor remission in a number of mice and a significantly increased mouse survival [22]. The second study transduced NK-92, NKL, and primary NK cells with a second generation CAR targeting both EGFR and EGFRvIII. EGFR-CAR NK cells displayed enhanced cytolytic activity and IFN-γ production when co-cultured with GBM cells or patient-derived GBM stem cells in an EGFR-dependent manner. In two orthotopic GBM xenograft mouse models, intracranial administration of NK-92-EGFR-CAR cells resulted in efficient suppression of tumor growth and significantly prolonged the tumor-bearing mouse survival [30]. However, it remains unknown if the CAR NK cells in these two studies are able to pass the blood brain barrier. No data from any clinical trials using CAR NK cells is available at this moment.

Radiation treatment increases the expression of lots of tumor specific antigens, such as EGFR in HNSCC [46,47], extracellular mesothelin in epidermoid carcinoma [30,67], Her2/ErbB2 in breast cancer [30,45,68], c-MET in NSCLC [30,69], etc., which justifies that the combination treatment of cancer with radiation therapy and CAR T, especially CAR NK cells may achieve synergistic anticancer activity. Nevertheless, no such work has been reported yet.

Conclusion

While much remains to be understood about the synergy between immunotherapy and radiation therapy, many advances have been made in recent years to uncover the mechanisms behind this powerful interplay. This combination therapy holds particular promise for tumors for which standard surgical resections and/or chemotherapy may be difficult and/or insufficient, such as hypogammaglobulinemia (HGG) [70]. It appears that radiation therapy directly kills cancer cells and in the meantime makes the body more susceptible to immunotherapy while its side effects such as increased expression of oncogenes including EGFR and Her2 may enhance immunotherapy. Immunotherapy, on the other hand, suppresses/blocks other side effects of radiation therapy including increased number of MDSCs and increased expression of PDL1. The safety profile of radiation therapy has improved especially with the use of SBRT or RIT while significant toxicities (such as severe inflammatory reaction) are relatively rarely observed with immunotherapy [16], which has advanced to using checkpoint inhibitors and CAR T or CAR NK cells in various levels of preclinical and clinical trials, although selecting a more specific tumor antigen still remains a challenge. Used together and with attention to dosage and timing, radiation therapy and immunotherapy have proven to be effective for both solid and hematological malignancies and continue to deserve our interest and anticipation.

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