Advances in radioimmunotherapy 4

Enhancing CAR T-cell therapy through cellular imaging and radiotherapy

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Chimeric antigen receptor (CAR) T-cell therapy is one of the most remarkable advances in cancer therapy in the last several decades. More than 300 adoptive T-cell therapy trials are ongoing, which is a testament to the early success and hope engendered by this line of investigation. Despite the enthusiasm, application of CAR T-cell therapy to solid tumours has had little success, although positive outcomes are increasingly being reported for these diseases. In this Series paper, we discuss the short-term strategies to improve CAR T-cell therapy responses, particularly for solid tumours, by combining CAR T-cell therapy with radiotherapy through the use of careful monitoring and non-invasive imaging. Through the use of imaging, we can gain greater mechanistic insights into the cascade of events that must unfold to enable tumour eradication by CAR T-cell therapy.

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This is the fourth in a **Series** of five papers on advances in radioimmunotherapy

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Introduction

The addition of immunotherapy to standard treatment approaches (surgery, radiotherapy, and chemotherapy) for the management of cancer is a recent and highly complementary advance. Immunotherapy harnesses the machinery of living cells and modifies it in ways that can enable the eradication of malignant cells in the body. Cancer immunotherapy has several pillars, including vaccines, immune checkpoint inhibitors, antibody-based reagents (including those that are antibody–drug conjugates or are fitted with therapeutic radionuclides), exogenous cytokines, and adoptive T-cell therapies.[1] Results with some of those immunotherapies, particularly with immune checkpoint inhibitors and chimeric antigen receptor (CAR) T cells, have been some of the most promising forms of cancer treatment from clinical trials in decades.[2–7] Because durable responses to checkpoint inhibitor therapy are now possible, the appearance of metastatic melanoma or non-small-cell lung cancer is no longer promptly followed by the risk of death. However, such responses occur in only 20–30% of patients.[2] In the case of CAR T cells, many patients will relapse because persistence of the re-purposed T cells varies, tumours escape by antigen loss or modulation, and immunological memory is not guaranteed.[8,9] Therefore, improvements in cancer immunotherapy are needed. Improving responses to CAR T-cell therapy requires careful attention not only to the choice of tumour-associated antigen to target but also to the precise mechanism by which the CAR T cell engages and kills its cellular prey at the immunological synapse.[10] To see this form of immunotherapy successful in solid tumours, there is a need to further understand the tumour microenvironment in which CAR T cells must operate.[10–12] For example, tumour immunoediting can be both site and organ specific,[8] with profound implications for CAR T-cell activity in different tissues such as bone marrow and lymph nodes.

CAR T cells are fitted with a chimeric synthetic receptor consisting of a single-chain variable fragment presented on its surface that binds to a tumour-associated antigen. This element is connected to a transmembrane domain, which is attached to distinct signalling domains that mediate T-cell activation and co-stimulation. Engagement of CAR with the target antigen leads to proliferation of the engineered T cells resulting in logarithmic expansion, and enables cells to promote apoptosis of the engaged target cell through cytolysis and the production of toxic cytokines such as TNF (tumor necrosis factor) α and interferon (IFN)γ (figure 1).[12,13] Four generations of CAR T cells have been described, each representing an enhancement related to activity and cell selectivity.[10,11] This is an autologous, highly personalised, and unique approach in which the patient's own T cells are harvested and reengineered to be MHC-independent and ready to attack only cells expressing the tumour-associated antigen in the hope of avoiding on-target and off-tumour toxicity.[11] There are many steps in enabling this highly complex cellular therapy, which includes not only genetic reprogramming and expansion of engineered cells, but also lymphocyte depletion of patients before the cells are reintroduced to them.[14,15] There is room for improvement in each step, from target selection to promotion of longevity of administered T cells at the tumour site, which in turn can produce immunological memory.[16] Questions arise at each step of the CAR T-cell therapeutic cascade regarding whether and when T cells engage their target, how many such cells are present, whether they remain viable and active at the target, and for how long they must reside within the tumour to eradicate it. Such questions are beginning to be answered through imaging, including at the microscopic level. In preclinical models, nuanced events, including the directed secretion of lytic granules into the targeted cell, can be studied in detail.[10] By answering these questions, we can begin to determine who could benefit from CAR T-cell therapy, refine and optimise dosing levels, enable prognosis, and possibly affect clinical outcomes. We might also be able to mitigate toxicity, including cytokine release syndrome,

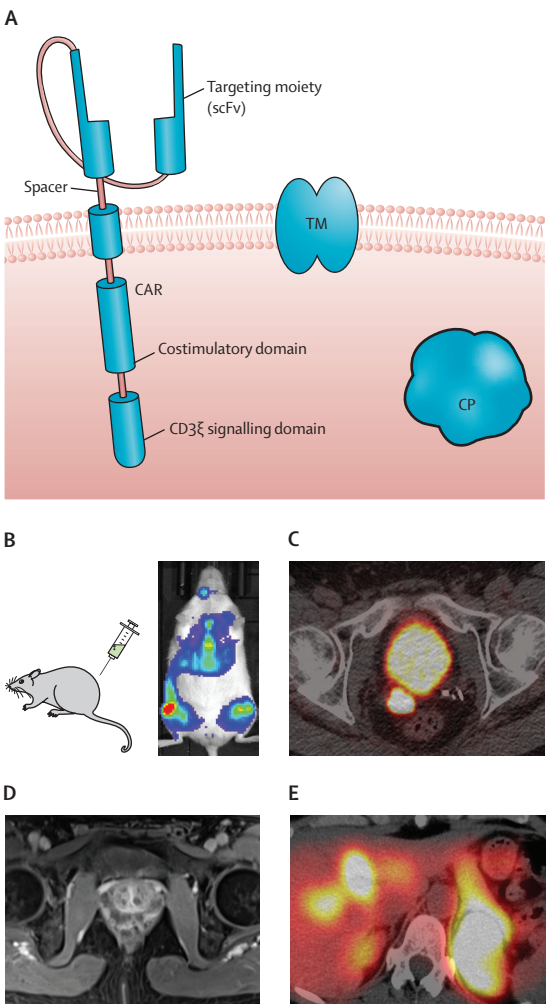

***Figure 1*:CAR-based T-cell imaging**
(A) CAR with co-expressed TM or CP imaging reporter targets. (B) Bioluminescence imaging—preclinical only. (C) PET. (D) MRI. (E) Single photon emission CT. scFv=single-chain variable fragment. CAR=Chimeric antigen receptor. TM=transmembrane. CP=cytoplasmic.

tumour lysis syndrome, and neurotoxicity, which has led to several deaths with the use of CAR T-cell therapy.[17–19]

The limited ability of CAR T cells to treat solid tumours when used as a single-agent treatment has been profoundly disappointing given their robust activity against haematological malignancies.[12,14] Although durable remissions and high overall survival rates can be achieved in diseases such as relapsed and refractory B-cell acute lymphoblastic leukaemia (50% event-free survival and 76% overall survival 12 months after a single infusion of anti-CD19 CAR-T cells),[20] multiple attempts at translating CAR T-cell therapy to solid tumours expressing a variety of targets have generally shown low efficacy.[21,22] Nevertheless, there remains substantial interest in replicating the largely positive outcomes of CD19-targeted CAR T cells in haematological malignancies with CAR T cells engineered to target antigens expressed on solid tumours. The reasons for CAR T-cell therapy being ineffective for solid tumour treatment are many and include overall low or absent expression of tumour-associated antigen; hetero-geneous expression of tumour-associated antigen on cells composing solid tumours, facilitating antigen escape; loss of tumour-associated antigen from tumour cells, precipitating relapse; presence of immunosuppressive cells and molecules within the tumour microenvironment,[23] including avid consumption of metabolites by tumour cells, limiting T-cell migration and function; and, tumour neovascular endothelium that is non-adhesive and, in combination with the extracellular matrix, can restrict T-cell migration into solid tumours.[11,14,24–26]

Immunotherapy, including CAR T cells, is a very expensive form of treatment and, for resource-limited environments, including many health systems in the USA, whatever tools are available to simplify treatment processes must be used. One way to enhance CAR T-cell therapy is through careful monitoring of the disposition and function of cells in vivo after administration. Using a variety of imaging modalities, some of which are clinically prevalent, can localise CAR T-cell therapies in the service of optimising dose delivered, determining the pharmacokinetics of the cells, and measuring their expansion and time of viable sequestration where needed, both within the tumour and at metastatic deposits. In addition to imaging and an in-depth understanding of the mechanisms, combination therapeutic regimens that include CAR T cells along with standard and readily available therapy, such as radiotherapy, could lead to further improvements.

Combination therapy: focus on CAR T cells with radiotherapy

The combination of radiotherapy with CAR T-cell therapy could be more effective than CAR T-cell therapy alone. Radiotherapy has been proposed as an adjunct to multiple types of immunotherapies, including check-point inhibitors and tumour vaccines.[27] The efficacy of radiotherapy in the context of a combination approach could be, at least in part, mediated by an increased presence of MHC class I molecules on the tumour cell surface and peptide synthesis and concomitant antigen presentation, and heightened recognition by cytotoxic T lymphocytes.[28] But CAR T cells are already programmed against a specific antigen, which might or might not be enhanced by radiation. For CAR T-cell therapy, radiation might tame the tumour microenvironment. Radiation activates NFκB and type 1 IFN, leading to the release of pro-inflammatory cytokines.[29] Combined with the activation of dendritic cells, improvement of neoantigen presentation, and increased expression of MHC class I, T cells resident within the tumour can be induced to upgrade tumouricidal responses.[25] So long as expression of the tumour-associated antigen can be maintained and the immune-related changes of radiation are applied, it

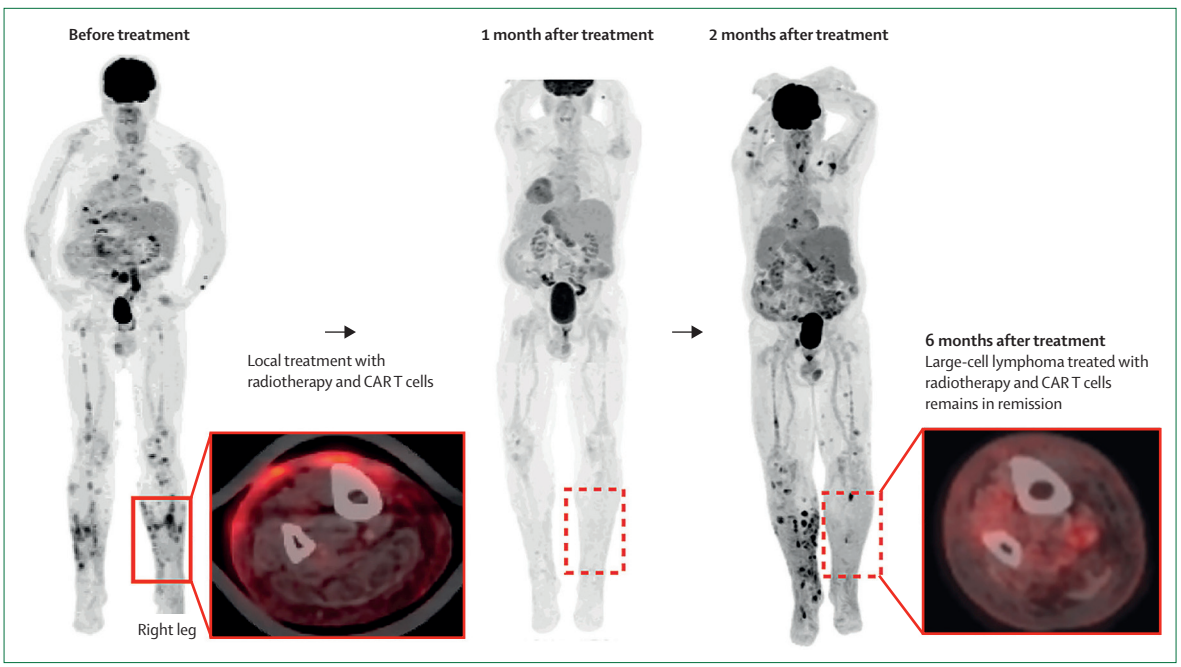

***Figure 2*: Patient with large-cell lymphoma treated with palliative radiotherapy and CD19-expressing CAR T cells**
Fluorodeoxyglucose-PET before leg treatment and 1, 2, and 6 months after treatment with radiotherapy and systemic CAR T cells. Reproduced from DeSelm et al[25] by permission of Elsevier. CAR=chimeric antigen receptor.

can be reasonably hypothesised that radiotherapy with CAR T-cell therapy can have additive or synergistic effects. The widespread clinical use of radiotherapy, including external beam, brachytherapy, and ligand-mediated endoradiotherapy, in a wide variety of cancer types would aid the adoption of combination radiotherapy and CAR T-cell therapy approaches.

An example of a combination approach is a phase 2 trial (NCT03196830) done by Qu and colleagues[30] in which they compared priming with intensive chemotherapy or radiotherapy before infusion of CAR T cells for patients with relapsed or refractory diffuse large B-cell lymphoma and who had a high tumour burden. The authors found that priming with radiotherapy (40 Gy in 20 fractions) enhanced efficacy and decreased neurotoxicity more than priming with lymphodepletion chemotherapy did, suggesting that radiotherapy is more effective when used in combination with CAR T cells than is chemotherapy.[30] Early results from preclinical data support the hypothesis that radiotherapy can facilitate the activity of CAR T cells in solid tumours. Weiss and colleagues[31] found that CAR T cells expressing the NKG2D (NKG2-D type II integral membrane protein) acted synergistically with radiotherapy to treat an orthotopic mouse model of glioblastoma. NKG2D-based CAR T cells might be of particular value because NKG2D can interact with multiple ligands on both glioblastoma and tumour-associated cells, decreasing the likelihood of antigen escape. These ligands are also induced by radiotherapy, likely explaining the synergy observed between the two methods.[31] However, radiation can also induce NKG2D ligands on non-malignant cells, potentially exacerbating off-tumour killing.

Activation of T cells by pro-inflammatory cytokines and improved neoantigen presentation induced by radiation can still lead to low antitumour activity if the malignant cells can avoid detection through antigen escape. De Selm and colleagues[25] investigated an orthotopic pancreatic cancer model that was composed of 25% cells that were absent of sialyl Lewis A, the tumour-associated antigen for the CAR T cells constructed for their study. Those heterogeneous tumours could not be eradicated by CAR T-cell monotherapy, although when mice bearing the orthotopic tumours were first treated with low-dose sensitising radiation, higher rates of complete and partial responses to CAR T-cell therapy were observed.[25] The authors showed that higher response rates were in part mediated by the proapoptotic TRAIL ligand, which is produced by activated CAR T cells and is effective at inducing death in tumour cells that have been exposed to radiation. This finding suggests that there is a mechanism by which the combination of radiotherapy and CAR T cells can overcome antigen escape and effectively eliminate tumours with heterogeneous tumour-associated antigen expression. The authors tested these findings in a single patient with refractory B-cell lymphoma whose response pattern might have been in keeping with the preclinical experimental results (figure 2). Specifically, PET imaging showed that the patient had an excellent response 1 month after CAR T-cell therapy: cancer rebounded elsewhere, minimally if at all at the irradiated site. Although the case does not

provide evidence of synergy, because radiotherapy alone could have produced a similar pattern of response and eventual progression, further exploration of this approach is warranted in dedicated prospective studies. Dovetailing of the known underlying biological mechanism of radiation-mediated immune response and preliminary findings of the potential synergy of radiotherapy with CAR T-cell therapy suggests that there is a need for continued research on the topic.

New approaches to enhance CAR T-cell therapy for solid tumours

The tumour microenvironment of solid cancers develops a cadre of pro-tumourigenic activities that negatively affect CAR T-cell function. Alongside CAR T-cell therapy with radiotherapy, further engineering of CAR T-cells to express additional effectors, and other approaches that combine CAR T-cell therapy with other immune modulators, have been tested to overcome those barriers.

Modification of CAR T cells

Manufacturing CAR T cells is an ex-vivo process that requires at least one step for the introduction of exogenous genes to harvested cells in which additional genes to further improve cells could be added at such a step. Co-expression of cytokines, such as interleukin (IL)-12[32] and IL-18[23,33] can enhance the antitumour effect of CAR T cells in animal models. The opposite approach can also be taken in which inhibition of negative regulators produced in the tumour microenvironment can be pursued. Ligtenberg and colleagues[34] introduced CAR T cells co-expressing catalase to remove reactive oxygen species in the tumour microenvironment. CAR T cells co-expressing catalase exhibited antitumour efficacy superior to standard CAR T cells that were in the presence of high local concentrations of hydrogen peroxide.[34] Immunosuppressive TGFβ signalling was blocked by expressing the dominant negative form of TGFβ receptor 2 in CAR T cells, resulting in enhanced performance of prostate-specific membrane antigen (PSMA)-targeted CAR T cells in an experimental model of human prostate cancer.[35] Prostaglandin E_2 and adenosine can inhibit T-cell receptor activation through localisation of PKA (protein kinase A) to the immune synapse. That immunosuppressive process requires interaction between PKA and ezrin, which can be blocked by RAID. Newick and colleagues[36] introduced RAID to CAR T cells and improved their effector function and degree of tumour infiltration in mice who have tumours. A strategy to convert a pro-tumourigenic modulator into an antitumour effector has been tested.[37,38] A switch receptor composed of a truncated PD-1 extracellular domain, a transmembrane domain, and a CD28 signalling domain can increase CAR T-cell activity in an animal model of human prostate cancer.[37] Last, co-expressing chemokine CCR4 with a CD30-targeting CAR improved homing of CAR T cells to the tumour, as well as efficacy in an experimental model of Hodgkin disease.[38]

Combination of CAR T-cell therapy with other immune modulators

CAR T-cell therapy is being studied in combination with a wide variety of therapies other than radiotherapy, mainly in the preclinical setting. A mechanism of T-cell exhaustion is acquired expression of receptors for immune checkpoints on the T-cell surface. Moon and colleagues[39] showed expression of PD-1, LAG-3, TIM-3, and natural killer cell receptor 2B4 on ineffective CAR-T cells that nevertheless infiltrated a tumour.[39] Combination of CAR T cells with an anti-PD-1 antibody enhanced the eradication of HER2-expressing tumours in two experimental models.[40,41] Li and colleagues[42] applied the same concept by engineering CAR T cells to co-express secreted anti-PD-1 single-chain variable fragment, enabling prolonged survival of treated animals. Myeloid-derived suppressor cells (MDSC) express PD-L1 and can inactivate PD-1-expressing CAR T-cells. Combination therapy with all-trans retinoic acid and DG2-targeted CAR T cells exhibited improved anti-sarcoma efficacy by eliminating MDSC.[43]

Non-invasive methods to assess CAR T-cell therapy in vivo

Imaging can be a valuable adjunct to CAR T-cell therapy by providing three-dimensional spatial information enhanced by adding time domain through video-microscopy,[44] dynamic whole-body imaging, or acquisition of serial images over the time course of treatment. In-vivo cellular imaging has been covered in several instructive articles and reviews.[45–50]

Optical imaging

Optical techniques dominate preclinical cellular imaging, including for in-vivo studies. Diffraction-limited and super-resolution microscopy can be used to study degranulation or visualise the leading edge of a CAR T cell as it engages a target in vitro.[10] Bioluminescent gene reporter or near-infrared imaging can follow cellular pharmacokinetics in small populations of between one and 10 000 cells in live animals.[51–53] Bioluminescence imaging, a strictly preclinical method, has been valuable in studying the disposition of phagocytic and malignant cells. Although occasionally used to follow T cells in vivo,[54] bioluminescence imaging has more frequently been used for gene tagging of the target tumour cells rather than for lymphocytes.[55,56] Although difficult to transfect, T cells have been successfully transfected with a variety of optical reporters, including functional reporters such as those based on granzyme B,[55] and improved light-emitting enzymes, such as a cell surface luciferase[57] that can enhance signal over ten-fold making T-cell tracking in vivo possible. Optical reporters are often coupled with reporters that use other imaging

modalities such as PET and MRI,[44] but are restricted to the preclinical realm.

Magnetic resonance cytometry and imaging

MRI is often criticised for its low sensitivity despite its exquisite spatial resolution. However, depending on the reagent and technique used, which will determine the degree of background signal, 5000 cells per voxel can be identified in vivo.[44] Furthermore, magnetic resonance techniques are readily translational because magnetic resonance scanners are clinically ubiquitous. There are several magnetic resonance-based approaches, which include those that use agents that provide positive contrast upon promoting T1-weighted (spin-lattice) relaxation of water; those that provide a negative signal, such as microcrystalline iron oxide and superparamagnetic iron oxide nanoparticles; and those that have very little background signal, such as perfluorinated nanoemulsions, which are used in conjunction with ^{19}F nuclear magnetic resonance or MRI.[58] MRI is the basis of magnetic resonance cytometry, a preclinical method that has enabled the study of the pharmacokinetics of anti-EGFR variant III-targeted CAR T cells.[59] Sensitivity is based on the experimental details (ranging from 1000 to 100 000 cells per voxel) because ^{19}F detection is inherently low, an issue partially addressed with the use of perfluorinated agents that boost local concentration of ^{19}F.[58] A clinical trial using such perfluorinated agents is currently underway (NCT02035085), although for an indication other than CAR T-cell tracking. With the advent of preclinical and clinical PET and magnetic resonance scanners, which enable both methods concurrently, the exquisite spatial resolution of magnetic resonance can be combined with the high sensitivity of PET detection to possibly provide the most informative assessment of CAR T-cell disposition in vivo.

Radionuclide imaging

Because a small number of CAR T cells are infused clinically, there is a need for a sensitive in-vivo imaging technique to follow their early pharmacokinetics and ultimate expansion. Standard protocols recommend only 1 000 000–10 000 000 CAR T cells administered per kg because of potential toxicities.[60] Considerations in radionuclide imaging of CAR T cells include using a labelling method: direct or indirect (genetic reporter-based) and a choice of isotope.

Weist and colleagues[61] used a direct labelling method with the ^{89}Zr-oxine complex to monitor cell-based therapies in a variety of experimental models with PET. ^{89}Zr-oxine has also been used for PET imaging of CAR T cells in preclinical models of glioblastoma (IL13Ra2-CAR-T) and prostate cancer (PSCA-CAR-T).[61] The radio-labelling method was robust yet biocompatible, and because of the long physical half-life of ^{89}Zr (3·27 days) cells could be followed for nearly a week in vivo. Parente-Pereira and colleagues[62] used ^{111}In-tropolonate to label MUC1-specific CAR T cells ex vivo and showed that they could be imaged by single-photon emission CT (SPECT) for up to 96 h after injection. Other examples using the direct approach involve dual-modality agents such as those used by Zanzonico and colleagues.[63] The authors used a superparamagnetic iron oxide nanoparticle for magnetic resonance and ^{64}Cu for PET, which allowed imaging of the targeting of CD19-expressing lymphoma. Careful optimisation of transfection was required, and radiosensitivity of the T cells was revealed, necessitating very low doses of the ^{64}Cu isotope. T cells are radiosensitive;[63] therefore, care must be taken to limit continuous exposure to ionising radiation when a radionuclide-based method is used. Another challenge with using such a dual modality agent, and most nanotechnology-based agents, including the ^{19}F-containing nanoemulsion above, is that signal is halved with each cell division. Furthermore, upon death, passively labelled cells or particles can be consumed by phagocytes, confounding the cell of origin for the signal detected. Many of these issues can be addressed through a reporter gene strategy for radionuclide-based CAR T-cell imaging.

Reporter gene cell imaging with radionuclides has been used elsewhere,[53] but is now being applied to the study of CAR T cells. Advantages to this method over the direct radio-labelling approach include a transient exposure to ionising radiation because the radio-labelling occurs in vivo only periodically and at tracer levels; no dilution of label because the imaging reporter is genetically encoded through stable viral transduction. Other desirable properties for a CAR T-cell radionuclide imaging reporter system include no background or non-specific radioactivity, especially radioactivity that does not emanate specifically from the living CAR T cell; no interference with CAR T-cell function, including expansion and cell killing ability; and the ability for the reporter to provide information on not only relevant to location or number of cells but also to their functional status. Disadvantages of a reporter-based imaging system include the complex nature of manufacturing and obtaining regulatory approval for engineered plasmids for human use and, in some cases, immunogenicity of the construct. However, as an adoptive cell therapy, there is a unique opportunity to introduce reporter constructs ex vivo to CAR T cells before re-introduction to affected patients.

A variety of PET reporters for imaging CAR T-cell activity exists. The most advanced of those is herpes simplex virus type 1 and thymidine kinase (HSV-1-TK), which has been used for molecular genetic imaging of cells for many years.[63–66] Najjar and colleagues[67] used the HSV-1-TK/[^{18}F]FEAU imaging reporter probe pair for CD19-targeted CAR T cells using the sleeping beauty (non-viral) transposon system and firefly luciferase (fLuc) as a co-reporter for optical imaging. Expressing those reporters did not compromise the cytotoxic effect of the construct.[67] Injected CAR T cells were successfully detected using both reporters. The minimal number of cells detected from a sample taken from a mouse flank

was $7 \cdot 5 \times 10^6$ cells in 100 µL. Investigators also showed that CAR T cells expressing HSV-1-TK could be selectively treated with ganciclovir in vitro for targeted elimination. Unique to the HSV-1-TK system is that it has been studied in patients, specifically in those who were treated with IL13Ra2-CAR T cells for recurrent glioma.[68] The study[67] showed not only proof of principle for imaging CAR T cells in patients during therapy, but also unveiled certain challenges. One challenge was the background, non-specific uptake of the imaging agent [^{18}F]FHBG, which provided signal in the brain even before CAR T-cell infusion. Background signal is not an uncommon problem with reporter/probe pairs and, along with potential immunogenicity of HSV-1-TK, has prompted a search for alternative reporter/probe pairs.

SSTR2 is a marker for neuroendocrine tumours with a targeted theranostic pair, ^{68}Ga/^{177}Lu-DOTATATE, which has received regulatory approval in the USA, and is available for PET imaging and targeted radiotherapy.[69] Because of its relatively limited expression in normal tissues,[70] SSTR2 has been explored as an imaging reporter for CAR T-cells targeting ICAM-1 using ^{68}Ga-DOTATOC, a compound similar to ^{68}Ga-DOTATATE, as the companion imaging probe.[71] The authors used an experimental model of metastatic human thyroid cancer (8505C encoding fLuc and green fluorescent protein) to track the responsive CAR T cells.[71] They found that the timing of CAR T-cell expansion was crucial for successful clinical outcome on the basis of the longitudinal imaging analyses of both the tumour burden, measured by bioluminescence imaging, and of CAR T-cell expansion, determined by PET. The sensitivity of detection was estimated at 50 000 cells per 100 µL. Endogenous expression of SSTR2 in kidney, cerebrum, and gastrointestinal tract[72] can represent a limitation of this reporter. SSTR2 is expressed on T cells and acts as a negative regulator for T-cell activation, which might interfere with its use as a CAR T-cell reporter.[73] Other immune cells (eg, B cells and macrophages) also express SSTR2, suggesting that other potential problems regarding cell type specificity exist.[73] However, these concerns are mostly theoretical, and SSTR2 is still considered a viable clinical imaging reporter currently.

HNIS (human sodium/iodide symporter) was first used as an endogenous reporter for thyroid cancer.[74] Because hNIS can transport iodide, pertechnetate, perrhenate, and astatine, both imaging (^{123}I, ^{124}I, ^{125}I, and ^{99}mTc) and therapeutic (^{131}I, ^{125}I, ^{211}At, ^{186}Re, and ^{188}Re) radionuclides can be used for targeting cells expressing hNIS.[75] Emami-Shahri and colleagues[16] did a preclinical validation study using the hNIS/^{99}mTcO^4 pair for real-time tracking of CAR T-cells. The authors engineered prostate-specific membrane antigen (PSMA)-targeting CAR T (4P28ζ) cells to express hNIS (4P28ζN). 4P28ζN cells were able to eliminate PSMA-expressing tumours in immunocompromised mice, indicating that expression of the reporter did not affect efficacy. SPECT/CT imaging enabled non-invasive monitoring of infused CAR T cells for a duration of 14 days. In-vivo phantom studies[16] showed that as few as 15 000 cells could be visualised, although the volume in which they were assayed was not reported. SPECT/CT images confirmed that there was robust uptake by thyroid, stomach, and salivary glands due to endogenous expression of hNIS, suggesting difficult T cells tracking in these areas.

We used PSMA as an imaging reporter for cell tracking because of the availability of highly specific clinical radiotracers that target PSMA for detection of prostate cancer,[76,77] and because it is a human protein. In a recent report, we leveraged PSMA as an imaging reporter for cell tracking and compared its sensitivity with that of hNIS and HSV-1-TK.[78] The PSMA/[^{18}F]DCFPyL (PyL) reporter/probe pair worked as well or better than hNIS and HSV-1-TK and their respective imaging agents with respect to sensitivity of detection[78] In addition, there are also readily available small-molecule PSMA-targeted radiotherapeutics that can be used as a rapid off-switch if the transduced cells, particularly CAR T cells, and induce severe toxicity.[79] We tested PSMA as a reporter for CD19-expressing CAR T cells.[80] PSMA is internalised and recirculates to the cell surface of cells naturally expressing PSMA,[81] a factor that we needed to consider because such activity can affect CAR T-cell function. To minimise these effects, we generated a truncated version that did not recirculate and showed that CD19-tPSMA(N9del) CAR T cells maintained their full cytotoxic capacity in vitro. We also did phantom studies.[80] that indicated a detection limit of 2000 cells per 50 µL using a preclinical PET scanner. We generated an experimental model of human B cell leukaemia (Nalm6-eGFP-fLuc) to evaluate the efficacy of CD19-tPSMA(N9del) CAR T cell in vivo, and used PyL PET serially to track the location and expansion of these cells. Nalm6-eGFP-fLuc cells develop spontaneous metastases in bone marrow, spleen, and liver when injected into immunocompromised mice subcutaneously. Despite the genetic identity of the mice, which received identical numbers of Nalm6-eGFP-fLuc cells, the tPSMA(N9del) CAR T cells showed different kinetics in most cases with respect to which organ they would migrate and expand initially (predominatly in tumour and bone marrow) and how long it would take for that expansion to occur. Such heterogeneity mirrored what Cazaux and colleagues[8] showed in their CD19-expressing tumour Eμ-myc transgenic mice, particularly the differential tissue-dependent tumour cell killing ability of CAR T-cells, which is likely due to anatomical and regional differences in immunoediting.[8] We also noted no correlation between the number of tPSMA(N9del) CAR T cells recovered from tumours relative to those recovered from circulation, attesting to the fact that blood assays might not provide a complete picture of CAR T-cell disposition and that other, more relevant biomarkers (ie, in situ biomarkers), might be more informative.

Assessment of functional status of CAR T cells

Despite the salutary effects of CAR T-cell therapy in CD19-expressing lymphoblastic leukaemia, non-Hodgkin lymphoma, chronic lymphocytic leukaemia, and B-cell maturation antigen-positive multiple myeloma, many patients do not respond well to treatment.[82–85] In addition to localising and monitoring expansion of CAR T cells in vivo, real-time information regarding their functional status might prove helpful in determining the cause for treatment failure. Reporter gene-based imaging can provide such information by placing the reporter under the control of a promoter responsive to T-cell activation signals. Ponomarev and colleagues[86] tested the concept using a synthetic promoter consisting of four repeats of nuclear factor of activated T-cells response elements and a minimal cytomegalovirus promoter. The study showed feasibility of imaging T-cell function using a fusion reporter (TKGFP) with fluorescence and PET imaging in vivo. Uchibori and colleagues[87] developed an enhanced version of a nuclear factor of activated T cells responsive synthetic promoter with reduced background expression to increase promoter activity providing an inducible reporter (iReporter). The authors generated CAR T/iReporter cells and showed that the construct was activated only at the site of the tumour-associated antigen-expressing lesion.

Perspective

Durable responses in an expanding array of clinical cases with immunotherapy, particularly immune checkpoint inhibitors and CAR T cells, is arguably the biggest advance in cancer therapy in the past generation. Unlike the blunt instruments of surgery, radiotherapy, and chemotherapy, which themselves continue to be refined, these engineered cells carry substantially more personalised information, and are accordingly far more nimble in their ability to move toward eradicating cancer from the body. Cellular therapies, such as CAR T cells, present many possibilities for further engineering, such as outfitting them with cytokines to complement their inherently cytotoxic qualities, in the form of CAR T cells with inducible release of a transgenic payload.[88] CAR T cells are potent and therefore could cause severe and uncontrollable toxicity, which must be addressed by mitigating or eliminating their activity swiftly. One way to deal with these potential side-effects is to further engineer CAR T cells, such as UniCAR T cells, which are inactive unless exposed to the antigen.[89,90] Small molecules with rapid pharmacokinetics outfitted with therapeutic radionuclides can be mobilised quickly to remove dangerous cells with few side-effects.[91] Imaging can be helpful with respect to not only understanding the choreography of CAR T-cell activity in vivo, but also to measure and to predict the extent of side-effects that may occur. An example is the use of a PET radiotracer that targets the CSF1R receptor, which is present on microglia[92] and is activated during neuroinflammation—a particularly lethal but fortunately rare side-effect of CAR T-cell therapy. Other such imaging agents targeting neuroinflammation are being researched.[93,94]

Search strategy and selection criteria

We searched PubMed for artciles published before March 31, 2019. We searched terms "CAR-T", "radiation", "imaging", "cell imaging", "immuno-oncology", and "theranostic". Articles were also identified through searches of the authors' own files. Only papers published in English were reviewed. The final reference list was generated on the basis of originality and relevance to the broad scope of this Series paper.

Conclusion

CAR T-cell therapy is still in its early stages of development and is imperfect in terms of both efficacy and toxicity. However, if their current trajectory is maintained, including further engineering and synergy with other treatment methods such as radiotherapy, it will not be long before we possibly see improved responses, fewer side-effects, and hopefully lower costs as a result of more streamlined production processes and because only patients who are likely to respond will be treated, in part, because of imaging.

Contributors

IM and SPR performed the literature search and wrote the manuscript; MGP performed the literature search, wrote the manuscript, and generated figures.

Declaration of interests

MGP is a co-inventor on a US Patent covering [18F]DCFPyL and is entitled to a portion of any licensing fees and royalties generated by this technology. This arrangement has been reviewed and approved by the Johns Hopkins University in accordance with its conflict-of-interest policies. All other authors declare no competing of interest.

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