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Clinical & Translational Immunology (2014) 3, e17; doi:10.1038/cti.2014.11

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REVIEW

Adoptive T-cell therapy: adverse events and safety switches

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The potential of adoptive T-cell therapy in effecting complete and durable responses has been demonstrated in a number of malignant and infectious diseases. Ongoing progress in T-cell engineering has given cause for optimism in the broader clinical applicability of this approach. However, the development of more potent T cells is checked by safety concerns, highlighted by the occurrence of on-target and off-target toxicities that, although uncommon, have been fatal on occasions. Timely pharmacological intervention is effective in the management of a majority of adverse events but adoptively transferred T cells can persist long term, along with any unwanted effects. A recently validated cellular safety switch, inducible caspase 9 (iCasp9), has the potential to mitigate the risks of T-cell therapy by enabling the elimination of transferred T cells if required. In haematopoietic stem cell transplantation, iCasp9-modified donor T cells can be rapidly eliminated in the event of graft-versus-host disease. This review presents an overview of the risks associated with modern T-cell therapy and the development, clinical results and potential future application of the iCasp9 safety switch.
Clinical & Translational Immunology (2014) **3,** e17; doi:10.1038/cti.2014.11; published online 20 June 2014

The first clinical validation of adoptive T-cell transfer came in the early 1990s when it was demonstrated that donor lymphocyte infusions could bring about disease remission in patients with relapsed chronic myeloid leukaemia following allogeneic bone marrow transplantation.[1–3] Around the same time, a number of investigators showed that the adoptive transfer of *in vitro*-expanded donor-derived virus-specific T cells was effective in the treatment of virus reactivation and Epstein–Barr virus-associated post-transplant lymphoproliferative disorder.[4–6] These findings were soon extended to the autologous setting with the demonstration that autologous virus-specific T cells could also be effective in the prevention and treatment of Epstein–Barr virus-associated post-transplant lymphoproliferative disorder following solid organ transplantation.[7,8]

The development of autologous T-cell therapy for malignancies that arise in immunocompetent patients is more challenging. In the early days, the generation of tumour-specific T cells largely relied on the *in vitro* expansion of antigen-specific precursors found in the peripheral blood[9] or tumour-infiltrating lymphocytes.[10] The arrival of clinical gene transfer technology in the past decade has seen intense interest in redirecting polyclonal T cells towards tumour targets. Intracellular antigens can be targeted by transducing polyclonal T cells with T-cell receptors (TCRs) that recognise specific peptide epitopes. For example, T cells transduced with TCR α and β chains specific for a human leukocyte antigen (HLA)-*0201-restricted MART-1 epitope can bring about melanoma regression.[11] TCR transfer, however, is limited by HLA restriction and much of the focus has now shifted to chimeric antigen receptors (CARs). CARs are composed of an extracellular domain that recognises cell surface antigens, which is linked to an intracellular signalling domain via a transmembrane sequence. The extracellular domain usually consists of the antigen-binding variable regions (Fv) from the heavy and light chains of a monoclonal antibody that are fused into a single protein known as a single-chain variable fragment (scFv).[12,13] The intracellular signalling domain is usually derived from the TCR complex and can include one or more costimulatory molecules to enhance its antitumour effect.

CAR T cells can be highly efficacious and their efficacy can be further increased with the addition of lymphodepleting chemotherapy before cell transfer. Striking responses have been observed in acute and chronic B-cell malignancies treated with CD19-targeted CAR T cells. At the same time, adverse events, such as cytokine release syndrome and prolonged B-cell depletion, have emerged.[14–18] Whereas the drug concentration and biological effects of conventional pharmaceuticals fall with time, adoptively transferred T cells can persist long term and even expand with time, with the potential for prolonged effects, both therapeutic and deleterious. The introduction of cellular safety switches, also known as suicide genes, may mitigate the risks by enabling the elimination of transferred T cells if required. This review will present an overview of the risks that are associated with modern T-cell therapy and the development, clinical results and potential future application of a recently validated safety switch, inducible caspase 9 (iCaps9).

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Received 7 April 2014; revised 8 May 2014; accepted 8 May 2014

RISKS OF T-CELL THERAPY

The infusion of T cells is generally well tolerated. Infusional adverse events are infrequent and mild, and are mostly due to the cryoprotectant, dimethyl sulphoxide, or concomitant medication.[19] The main concern of T-cell therapy is the potential for delayed side effects. This became evident from the early days of allogeneic bone marrow transplantation when T cells were recognised as the central mediators of graft-versus-host disease (GVHD).[20–22] Donor T-cell infusion in patients with post-transplant relapse can bring about disease remission through a graft-versus-leukaemia effect but this is generally associated with the development of GVHD as a result of alloimmunity against non-haematopoietic tissues.[1–3] Although the antigenic targets in adoptive T cell therapy are much better defined, the potential for adverse effects, both on-target and off-target, remains.

On-target but off-tumour adverse effects

T cells targeting differentiation antigens can be expected to also recognise nonmalignant cells that express the same antigens, resulting in adverse events (Table 1). For example, melanoma patients treated with T cells targeting melanocyte differentiation antigens, such as MART-1 and gp100, often developed vitiligo and uveitis. These on-target toxicities have been observed across all forms of therapeutic approaches, including tumour-infiltrating cells,[23] *in vitro*-expanded T-cell clones[24] and TCR-transgenic cells.[25] In general, on-target autoimmunity is associated with tumour regression[23,24] and is more prominent in treatment approaches that are more efficacious. Melanoma patients treated with T cells transduced with a high-avidity MART-$1_{(27-35)}$ TCR and murine-derived gp100 TCR had a significantly higher rate of tumour response than an earlier cohort of patients treated with T cells transduced with a lower-avidity MART-$1_{(27-35)}$ TCR.[25] Not unexpectedly, the rate of autoimmunity was also correspondingly higher: of the 20 patients treated with the high-avidity TCR transgenic T cells, 11 (55%) developed uveitis and 10 (50%) developed a transient steroid-responsive hearing loss; the latter was not observed in previous studies and was attributed to the presence of melanocytes in the striae vascularis of the inner ear.[25] A similar pattern was seen in CD19 CAR T-cell therapy, where better disease response was associated with long-term B-cell depletion and hypogammaglobulinaemia.[15,16]

On-target but off-tumour toxicities can be immediately life-threatening. A patient with colorectal cancer with lung and liver metastases developed respiratory distress within 15 min of HER2-specific CAR T-cell infusion and subsequently died from multiorgan failure 5 days later.[26] It was postulated that the T cells recognised HER2 expressed by normal lung tissues, leading to the release of inflammatory cytokines, pulmonary toxicity and a cascading cytokine storm that cumulated in multiorgan failure. This adverse event was not foreseeable as it has not been observed in HER2 vaccine trials or the many breast cancer patients treated with the HER2 monoclonal antibody, trastuzumab. It is thought that the fatal toxicity was a function of the high potency of the CAR construct that contained CD28 and 4-1BB co-stimulatory molecules, and the use of prior non-myeloablative chemotherapy that further enhanced treatment effect.

On-target toxicities that are not immediately life-threatening can still be treatment-limiting. Carbonic anhydrase-IX (CAIX)-specific CAR T cells, which were studied in patients with metastatic renal cell carcinoma, were associated with dose-limiting liver toxicity because of low-level CAIX expression in bile duct epithelium.[27,28] Similarly, a study using T cells transduced with a high-avidity murine TCR against human carcinoembryonic antigen in patients with metastatic colorectal carcinoma was halted after all three patients developed severe transient colitis caused by the recognition of normal levels of

Table 1 Adverse events attributed to on-target effects

Technology	*Antigen*	*Disease*	*Off-tumour target*	*Adverse event*	*Treatment*	*Reference*
Antigen-specific T cells	MART-1 gp100	Melanoma	Melanocytes in skin and eyes	Vitiligo Uveitis	Topical steroids (eye drops)	23,24
	MART-1 gp100	Melanoma	Melanocytes in skin, eyes and inner ear	Vitiligo Uveitis Hearing loss	Topical steroids (eye drops and intratympanic injection)	25
High-avidity TCR gene transfer (+ lymphodepleting chemotherapy and high-dose IL-2)	CEA	Colorectal cancer	Normal colonic epithelium	Colitis	Systemic steroids	29
	MAGE-A3	Melanoma Oesophageal cancer	MAGE-A12 expressed in brain cells	Seizures, coma in 3 of 9 patients; fatal in 2	Various. Systemic steroids and antiepileptics	30
CAR T cells	CAIX (+IL-2)	Renal cell carcinoma	CAIX in bile duct epithelium	Raised liver enzymes	Corticosteroids Pretreatment with CAIX monoclonal antibody	27,28
	HER2 (+ lymphodepleting chemotherapy)	Colorectal cancer (metastatic)	HER2 expressed by normal lung tissues	Acute pulmonary infiltrates (fatal)	Corticosteroids and supportive care	26
	CD19 (± lymphodepleting chemotherapy)	B-cell lymphoma and acute lymphoblastic leukaemia	Normal B cells	B-cell depletion, hypogammaglobulinaemia	Replacement intravenous gammaglobulin	15
			Malignant B cells (tumour target)	Cytokine release syndrome	Systemic steroids or interleukin-6 receptor antibody	14–18

Abbreviations: CAR, chimeric antigen receptor; CAIX, carbonic anhydrase-IX; IL-2, interleukin-2; TCR, T-cell receptor.

carcinoembryonic antigen in the colonic mucosa.[29] The hepatitis and colitis in both studies were either self-limiting or responsive to corticosteroids and there were no treatment-related deaths.[27–29] More recently, however, fatal on-target toxicities were reported in patients treated with anti-MAGE-A3 TCR-transduced T cells.[30] In this study, HLA-A*0201 transgenic mice were immunised with a MAGE-A3 peptide epitope to generate a high-avidity TCR that recognised not only MAGE-A3 but also MAGE-A9 and MAGE-A12. Nine patients with various malignancies were treated on this protocol and three developed altered mental status within a few days, two of whom became comatose and died. Autopsy showed necrotising leukoencephalopathy with extensive white matter defects associated with $CD8^+$ T-cell infiltration, caused by the previously unrecognised low-level expression of MAGE-A12 in human brain.[30] In each of these cases, the adverse effects occurred despite relatively low levels of antigen expression in the off-tumour sites, thus highlighting the potential for harm in using redirected T cells with high avidity and potency.

Cytokine release syndrome

As T-cell therapy becomes more effective, acute toxicities have also become more evident. Cytokine release syndrome, which is characterised by fevers, rigors, hypotension and hypoxia, has been observed in a number of CD19 CAR T-cell studies as a result of large-scale T-cell activation upon the recognition of $CD19^+$ malignant cells.[14–18] The symptoms usually begin a few days following T-cell infusion but can be as early as 24 h, depending on the co-stimulatory domains, and coincide with the *in vivo* expansion of CD19 CAR T cells and the elevation of a number of serum cytokine levels, including interferon-γ, soluble interleukin-2 receptor α, interleukin-2, interleukin-6 and tumour necrosis factor.[14,17,18,31] Some patients also develop features of macrophage activation syndrome, including very high ferritin levels, histological features of haemophagocytic lymphohistiocytosis, hepatosplenomegaly and disseminated intravascular coagulation.[32] A significant proportion of patients develop alarming but reversible neurological symptoms, including delirium and seizure-like activity, the reason for which is not fully understood but thought to be related to generalised T cell-mediated inflammation rather than direct toxicity of CAR T cells on the brain.[14,15,17,18] In general, patients with evidence of persistent disease at the time of T-cell infusion are more likely to develop cytokine release syndrome.[17,18]

Off-target adverse effects

In mid-2011, a patient with metastatic melanoma suffered a sudden cardiac death 4 days after an infusion of autologous T cells transduced with an affinity-enhanced HLA-A1-restricted MAGE-A3 TCR.[33] This high-affinity TCR was generated by introducing mutations into the α chain of a MAGE-A3 TCR that was isolated from another patient from a previous vaccination study. The α chain mutations increased the potency of the T cells against MAGE-A3-expressing targets *in vitro* and *in vivo* while maintaining a high level of specificity *in vitro*.[34] Investigations into the death did not identify any evidence of direct T cell-mediated toxicity and a second patient was enrolled the following year. This patient also suffered a cardiac death 5 days after T-cell infusion and was found to have extensive myocardial necrosis on autopsy.[33] Further investigation using a combination of amino acid substitution and *in silico* screening showed that the MAGE-A3 TCR also recognised a peptide from an unrelated muscle protein, Titin, which is important for the contraction of striated muscles.[34] As it turned out, this off-target toxicity would have been very difficult to predict: Titin expression was undetectable in cardiac-derived primary cell lines and could only be detected in a more elaborate beating myocyte culture system,[34] and the toxicity would not have been detected in HLA-A1 transgenic mouse models either because there was no reactivity against the equivalent mouse Titin peptide.[34] Although this remains the only example of off-target toxicity, the difficulties in predicting such toxicity and the fatal outcome are very concerning. Another potential source of off-target toxicity is the mispairing of transgenic α or β TCR chains with endogenous TCR, which can potentially give rise to TCRs with new specificities and autoreactivity.[35] This has been demonstrated in murine models[36] but has not been observed in human clinical studies encompassing >100 patients to date.[37]

Insertional mutagenesis

The integration of viral vectors proximate to growth-promoting genes can result in the transactivation of proto-oncogenes and malignant transformation. Acute leukaemia as a result of insertional mutagenesis has plagued a number of gene therapy studies for primary immunodeficiency disorders, including X-linked severe combined immunodeficiency,[38,39] chronic granulomatous disease[40] and Wiskott–Aldrich syndrome.[41] In contrast, there has not been any report of insertional mutagenesis arising from gene-modified T cells that have been administered to hundreds of patients. This vast difference in genotoxicity profile may be related to the nature of the transgenes involved and the pluripotency of haematopoietic stem cells, which may render them much more susceptible to malignant transformation than mature T cells.

MANAGEMENT OF ADVERSE EVENTS

Supportive treatment

Treatment of adverse events is not always required or desired. On-target toxicities that require treatment, such as uveitis and colitis, can often be managed with topical or systemic steroids. Hypogammaglobulinaemia can be managed with gammaglobulin replacement. Cytokine release syndrome, commonly seen in CD19 CAR T-cell therapy, generally responds well to either high-dose corticosteroids or the interleukin-6 receptor-blocking antibody, tocilizumab.[14,18] However, treatment with high-dose corticosteroids is associated with a loss of CD19 CAR T cells and consequent disease relapse, whereas tocilizumab did not appear to have the same deleterious effect.[18] A set of diagnostic criteria for cytokine release syndrome has been recently proposed that should lead to a more uniform definition of the syndrome and the development of management guidelines that can abrogate the symptoms without loss of antileukaemic activity.[18]

ENGINEERING SAFETY

One of the attractions of T-cell therapy is the potential for the transferred cells to persist and expand, thus mediating sustained therapeutic effects. However, any adverse effects will also be similarly sustained and can worsen as the cells proliferate. Although the infused T cells can be eliminated *in vivo* with antithymocyte globulins or other pharmaceutical means, the effect of these drugs is generally delayed, incomplete and nonspecific. Concerns about prolonged unwanted effects have led to the development of cellular suicide genes, also known as safety switches, that enable the conditional elimination of transferred T cells in the event of adverse effect.

The first suicide gene to be clinically tested is herpes simplex virus thymidine kinase (HSVtk) that mediates the conversion of ganciclovir to ganciclovir triphosphate, which is toxic to dividing cells.[42–44] In haematopoietic stem cell transplantation, the insertion of HSVtk into donor T cells enables them to be eliminated by the administration

Table 2 Comparison of HSVtk and iCasp9 safety switch

	HSVtk	*iCasp9*
Mechanism of action	Disrupts DNA synthesis	Dimerisation of caspase 9 domains resulting in the activation of downstream effector caspases, leading to apoptosis
Requirement for cell kill	Only kills dividing cells	Killing independent of cell division but high level of killing (>90–99%) only seen in cells with high level of iCasp9 expression. Relative sparing of quiescent T cells expressing low/intermediate level of iCasp9.
Time to cell kill	Takes several days	> 90% killing within 30 min for cells with a high level of transgene expression
Agent to trigger killing	Ganciclovir—also used in the treatment of cytomegalovirus reactivation	Biologically inert small molecule dimeriser, AP1903
Immunogenicity	Foreign antigen with known $CD4^+$ and $CD8^+$ T cell-mediated immune destruction	Largely endogenous, hence less likely to be immunogenic. No evidence of immune-mediated destruction to date (>2 years follow-up)

Abbreviations: HSVtk, herpes simplex virus thymidine kinase; iCasp9, inducible caspase 9.

of ganciclovir with subsequent abrogation of acute GVHD.[42–45] However, HSVtk as a safety switch has a number of drawbacks (Table 2): its mechanism of action is dependent on DNA synthesis and, hence, killing is restricted to dividing cells and can take days to weeks;[42,43,45] it is also a foreign protein and therefore a target for $CD4^+$ and $CD8^+$ T cell-mediated destruction.[46] In addition, the cells are unintentionally eliminated when ganciclovir is required for the treatment of cytomegalovirus reactivation. Ganciclovir resistance can also occur as a result of cryptic splice donor and acceptor sites that give rise to truncated, ganciclovir-resistant HSVtk.[47]

Development of iCasp9 safety switch

The iCasp9 is a new suicide gene that has recently undergone successful clinical testing.[48] Its development began 20 years ago when ligand-mediated dimerisation was first proposed as a means to control intracellular signalling.[49] This technology is based on a member of the immunophilin receptor family, FK506 Binding Protein (FKBP12). The physiological function of FKBP12 is to bind to and inactivate calcineurin. In order to create a synthetic ligand that will dimerise FKBP12 without the unwanted effects of calcineurin inhibition, a dimeric FK506 analogue that does not bind calcineurin was developed.[49] This prototype was subsequently improved upon with the introduction of an ethyl 'bump' into FK506, resulting in a compound that binds poorly to wild-type FKBP12 but has subnanomolar affinity to a redesigned FKBP12 binding pocket that has a valine residue instead of the bulkier phenylalanine reside (FKBP12-F36V).[50] This redesigned FKBP12/FK506 interface effectively eliminates ligand interaction with wild-type FKBP12, resulting in a synthetic ligand that interacts strongly with FKBP12-F36V but is otherwise biologically inert.

A number of proteins have been investigated as potential mediators of dimerisation-induced apoptosis. These include the Fas receptor, the death effector domain of Fas-associated protein, FADD, and the caspases 1, 3, 7, 8 and 9.[51–53] The upstream, membrane proximal proteins, such as Fas receptor and FADD, are less robust because of the presence of downstream inhibitors of apoptosis such as c-FLIP, bcl-2 and bcl-X_L. The downstream terminal effector caspases will provide more robust killing but it is difficult to express these at functional levels, presumably because of basal toxicity from ligand-independent dimerisation.[54] This led to the use of caspase 9, which is a distal component of the intrinsic apoptotic pathway, directly upstream of the terminal caspases. Activated caspase 9 activates the terminal effector caspase, caspase 3, leading rapidly to apoptosis.

The optimised iCasp9 molecule consists of an FKBP12-F36V domain linked, via a flexible Ser-Gly-Gly-Gly-Ser linker, to a caspase 9 molecule, without the caspase activation and recruitment domain (CARD) (Figure 1a).[54] CARD is the physiological dimerisation domain and is now superfluous. The iCasp9 has a good balance of low dimeriser-independent basal activity and high sensitivity to dimeriser-induced apoptosis. A single 10 nM dose of the dimeric FK506 analogue, AP1903 or AP20187, induces apoptosis within hours in >99% of cells that express high levels of iCasp9. T cells that express low or intermediate levels of iCasp9 are also susceptible to dimeriser-induced killing but to a lesser degree.[54]

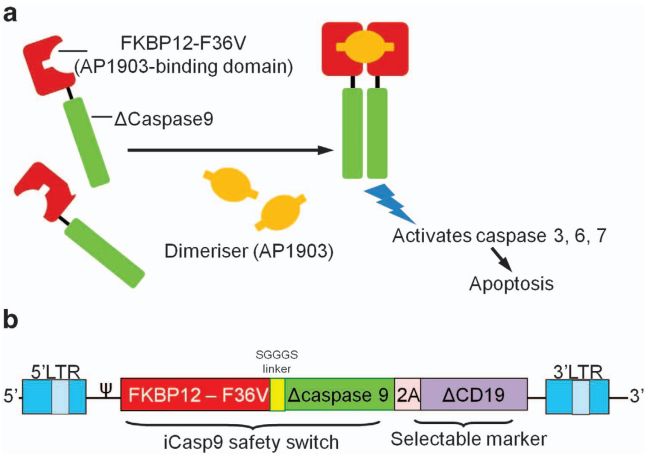

Figure 1 (**a**) The iCasp9 molecule consists of a drug-binding domain, FK506-F36V, joined, via a short linker, to ΔCaspase 9, which is caspase 9 without its physiological caspase recruitment domain (CARD). Binding of a small molecule dimeriser, AP1903, results in the dimerisation of ΔCaspase 9 that activates downstream effector caspases, leading to apoptosis. (**b**) The iCaps9.2A.ΔCD19 retroviral insert consists of iCasp9, joined via a 2A-like sequence, to ΔCD19, which serves as a surface selectable marker.

Clinical validation of iCasp9 safety switch in haploidentical stem cell transplantation

The iCasp9 safety switch was recently validated in a small cohort of patients who required donor T-cell add-back following haploidentical stem cell transplantation.[48] Haploidentical stem cell transplants are matched in only 5/10 to 8/10 HLA loci and are associated with very high rates of fatal acute GVHD unless specific measures are taken. Extensive T-cell depletion of the stem cell graft is very effective in preventing GVHD but also results in profoundly delayed T-cell immune reconstitution with consequently high risks of infection and disease relapse. The add-back of donor T cells can help accelerate immune reconstitution but is hampered by the risk of life-threatening

acute GVHD, thus limiting it to doses that are generally insufficient for clinical antiviral or antileukaemic effect.

The insertion of iCasp9 gene into donor T cells allows the safe add-back of larger doses of T cells by providing a means for their conditional elimination in patients who develop GVHD. Donor T cells are transduced with a gammaretroviral vector carrying the iCaps9.2A.ΔCD19 cassette that consists of iCasp9 joined, via a 2A-like linker, to ΔCD19 (Figure 1b).[55] ΔCD19 is used as a surface selectable marker that enables the gene-modified cells to be immunomagnetically enriched using a clinical grade cell selection device, thus ensuring that the majority of the infused T cells carry the iCasp9 gene. The intracytoplasmic domain of CD19 has been truncated from 242 to 19 amino acids, with the removal of all conserved tyrosine residues to eliminate the potential for intracellular signalling. The 2A-like linker is a 60-nucleotide sequence from an insect virus that results in the synthesis of two discrete proteins, iCasp9 and ΔCD19, without a joining peptide bond, though a 'ribosomal skip' mechanism.[56]

In this first-in-human study, patients who received a T cell-deplete haploidentical stem cell transplant were given an infusion of 1×10^6 to 1×10^7/kg iCasp9-transduced donor T cells starting from 30 days after stem cell infusion.[48] The iCasp9 T cells expanded *in vivo* and constituted a majority of the T cells in the first couple of weeks. They had antiviral specificity and were able to control clinical virus reactivation. Four patients developed acute GVHD and were treated with a single infusion of the dimeriser, AP1903. This resulted in the elimination of 90% of the iCasp9-transduced T cells within 30 min and another 0.5 to 1 log cell elimination in the next 24 h, with the complete resolution of GVHD within 24 to 48 h.[48] Interestingly, the residual iCasp9-transduced T cells re-expanded with time but did not cause further GVHD. The sparing of quiescent non-alloreactive T cells is a result of the downregulation of retroviral long terminal repeat-driven transgene expression in quiescent cells,[57] thus reducing the susceptibility of quiescent cells to dimeriser-mediated killing.[55] These residual T cells included virus-specific T cells[48] and remained susceptible to dimeriser-induced killing following TCR activation *in vitro*.[55] The iCasp9-transduced T cells have persisted for at least 2 years in surviving patients and there is no evidence of T-cell immune response against either iCasp9 or the 2A sequence to date.[58]

In this first-in-human study, the iCasp9-transduced T cells were first depleted of alloreactive T cells as an additional safety measure.[55] With the clinical validation of the iCaps9 safety switch, selective allodepletion can be omitted and clinical studies using non-allodepleted iCasp9-transduced donor T cells are currently in progress in a number of centres.

Safety switches in antigen-redirected T cells and other T-cell therapies

The iCasp9 safety switch has been incorporated into a number of CAR T-cell studies, including those targeting the disialoganglioside, GD2, in sarcoma and neuroblastoma (ClinicalTrials.gov Identifiers NCT01822652 and NCT01953900). The ability of safety switches to mitigate unexpected catastrophic toxicities such as the MAGE-A3 TCR-associated sudden cardiac deaths is likely to be limited. The main role of safety switches is likely to be in adverse effects that develop less precipitously, such as GVHD and the long-term depletion of B cells by CD19 CAR T cells. In the case of late adverse effects, more than one dose of AP1903 may be required for the complete elimination of transduced T cells because of reduced killing in quiescent cells but complete cell kill may be neither necessary nor desirable, as cancer progression remains the principal risk faced by these patients.

Safety switches can be especially useful where new technology or cell types are involved. Examples include the generation of CAR T cells from induced pluripotent stem cells[59] and the *in vitro* expansion or induction of regulatory T cells for use in patients with autoimmune diseases or GVHD.[60,61] In the case of regulatory T cells, there is a concern that *in vitro*-expanded regulatory T cells can revert of convert into effector T cells *in vivo*, which can potentially exacerbate rather than ameliorate the clinical problem.[62] Hence, a safety switch would be very useful in this setting, particularly in early-phase trials. The complexity of gene transfer, including regulatory demands, does unfortunately reduce the appeal of such an approach, especially where gene transfer is not central to the process. However, the improvement in safety and the potential for increased scientific return through gene marking are strong arguments for their use in selected circumstances.[63]

CONCLUSION

As T-cell therapies increase in efficacy, so have their risks. The potential for adverse events has led to calls for a more conservative, step-wise approach in clinical testing: dose escalation should be gradual, new co-stimulatory domains or other functional domains should be introduced in a step-wise manner, as should CARs with novel antigenic targets, and the routine use of lymphodepletion may need to be reconsidered, particularly when new CAR constructs are involved.[64] The iCasp9 safety switch is not a panacea for all the safety concerns but will facilitate the introduction of new technologies and help push the pace for therapeutic advances.

CONFLICT OF INTEREST

The author declares no conflict of interest.

ACKNOWLEDGEMENTS

This work is supported by an Australian National Health and Medical Research Council (NH&MRC) Early Career Clinical Fellowship (APP1054786) and project grant funding (APP1053135) to S-KT.

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