Citation: Molecular Therapy — Oncolytics (2016) **3,** 16028; doi:10.1038/mto.2016.28
Official journal of the American Society of Gene & Cell Therapy

www.nature.com/mto

EDITORIAL

Next frontiers in CAR T-cell therapy

Molecular Therapy — Oncolytics (2016) **3**, 16028;
doi:10.1038/mto.2016.28; published online 30 November 2016

Chimeric antigen receptor (CAR) T-cell therapy is entering a new era, transitioning from an experimental approach being tested in a handful of centers to a more mainstream and broadly investigated therapeutic platform with significant efforts directed towards commercial translation. CARs are synthetic receptors engineered and transduced into T cells to redirect T-cell cytotoxicity by recognition of cell surface antigens expressed on cancer cells.[1,2] Ongoing innovations into the design and application of CAR T cells are aimed at improving antitumor potency and, at the same time, ensuring safety of this promising therapy.[3] In this issue of *Molecular Therapy – Oncolytics*, we have invited preeminent authors to focus on the specific issues that comprise the next frontiers in CAR T-cell therapy.

The concept and clinical promise of CAR T-cell therapy is best illustrated in the success of CD19-targeted therapies for refractory/relapsed B-cell malignancies (reviewed in refs. 4–6). The remarkable clinical efficacy demonstrated with CD19-CARs has been achieved at multiple institutions, each evaluating their own CAR T-cell platforms and trial designs. Founding principles that have arisen from this wealth of clinical experience has helped shape our thinking about the parameters key to achieving therapeutic success, as well as management of potential toxicity risks. The application of these concepts to other malignancies is a major focus of current investigations. The reviews presented in this Special Issue address challenges facing successful CAR T-cell therapy: CAR bioengineering,[7] T-cell manufacturing,[8] application of CAR T cells for the treatment of solid tumors,[9] toxicity and safety management (Curran *et al*.[10]), and immune monitoring to gain comprehensive understanding of therapeutic outcomes (Kalos *et al*.[11]).

BIOENGINEERING OF CARS

Abate-Daga and Davila[7] discuss the structure of the CAR as a hybrid antigen receptor, part antibody and part T-cell receptor, comprising an extracelluar antigen-binding domain and intracellular signaling domain(s). The antibody single chain variable fragment (scFv) directs T-cell binding to a tumor antigen and the intracellular domain, usually consisting of costimulatory and CD3ζ endodomains, initiates T-cell activation. The modifications of scFv, hinge/spacer length, and intracellular domains can influence T-cell recognition of differential antigen expressed on cancer cells versus normal cells, affinity, proliferation, persistence, and prevention of exhaustion. New generations of CARs, such as ligand CARs (IL-13 receptor), universal CAR systems, and bispecific CARs that can either be activated by two different antigens or inhibitory bispecific CARs that can prevent normal tissue destruction, are discussed. Additionally, third-generation CARs or TRUCKs (joint expression of CARs and accessory genes either in *cis*- or *trans*-, secretion of IL-12, IL-15, IL-7, or IL-21, either constitutively or induced) are developed with strong preclinical evidence of enhanced functionality and persistence, which are currently being translated into clinic. Davila and colleagues also highlight the careful thought process required in the incorporation of accessory molecules as safety switches (huEGFRt and iCasp9) in their alignment in the CAR structure.

CLINICAL MANUFACTURING OF CAR T CELLS

With the success of CD19-targeted CAR T cells in early-phase clinical trials and with industry-academia partnerships, clinical manufacturing of CAR T cells for late-phase clinical trials is rapidly developing. As a mostly autologous cell therapy, CAR T-cell manufacturing starts from apheresis and proceeds through systematic steps of T-cell selection, activation prior to gene transfer, and T-cell expansion. Expanded CAR-transduced T cells are formulated and cryopreserved to be administered to the patient. Although early in clinical development, several systems are available for T-cell activation such as cell-based, bead-based, antibody-coated magnetic beads, nanobeads, and expamer technologies. Genetic modification of T cells are routinely performed either by use of retroviral or lentiviral vectors. The advantages and limitations of both T-cell activation methods and vectors are discussed comprehensively by Wang *et al*.[8] Additionally, transposon/transposase and messenger RNA transfer system were discussed. Following gene transfer, expansion protocols use a variety of bioreactors. More importantly, after manufacturing several quality standards are developed prior to administering T cells to the patient. Riviere and colleagues describe the above steps in a comprehensive manner. With the introduction of newer costimulatory domains into CARs and selective transduction of specific T-cell subsets, the knowledge, advantages, and limitations of each system are becoming increasingly important.

CARS FOR SOLID MALIGNANCIES

CAR T-cell therapy for solid malignancies is an exciting front that has yet to be realized and must overcome several barriers specific to the tumor microenvironment. Newick, Moon, and Albelda discuss the primary hurdles to CAR T-cell therapy in the solid tumor microenvironment[9] and present an eloquent summary of the current approaches to overcome solid tumor barriers. The elements necessary for effective solid tumor CAR T-cell therapy—trafficking to the tumor, successful infiltration and engagement of tumor antigens, overcoming CAR T-cell intrinsic, and extrinsic factors that can influence the potency and persistence of CAR T cells—are discussed in detail.

Selection of an optimal solid tumor-associated antigen for targeting by CAR T cells requires that an antigen: (i) is overexpressed in the majority of solid tumors; (ii) displays limited expression in normal tissues; and (iii) imparts tumor aggression thereby reducing the likelihood for tumor escape.[12,13] Newick *et al*.[9] highlight the lack of a "dispensable antigen" in solid tumors and discusses tumor-selective versus tumor-specific antigens that are being targeted in clinical trials. While neoantigens may avoid the common problem of choosing an antigen target for CAR T-cell therapy, they are not practical to apply to a large cohort of patients. Even after selecting an optimal antigen target, the function of a CAR is dependent upon the scFv avidity and ability to prevent tumor escape of immunogenic epitopes. Albelda and colleagues next discuss the important issue of T-cell trafficking that is dependent upon appropriate expression

of chemokine receptors on T cells and "matching" tumor-secreted chemokines. "Engineered-matching" by overexpression of a tumor-specific chemokine receptor or genetic inhibition of protein kinase A activation to promote CAR T-cell infiltration are some of the highlighted strategies. To improve CAR T-cell trafficking and infiltration of solid tumors, our group has published the clinical and biological advantages of regional administration of CAR T cells; this approach is now being tested in clinical trials.[14]

Dr. Albelda's laboratory has contributed novel approaches to overcome T-cell extrinsic tumor microenvironmental factors such as stromal fibroblasts, Tregs, TGFβ, and PGE2 by FAP CAR T cells, systemic blockade of TGFβ, and genetic inhibition of protein kinase A, respectively.[9] In addition to discussing the rationale for these approaches, other novel methods, such as TGFβ dominant negative receptor and tumor-restricted secretion of IL-12, are also highlighted. Suppressive immune cells such as Tregs, myeloid-derived suppressor cells, tumor-associated macrophage, and tumor-associated neutrophil can be better addressed in an immunocompetent mouse model that requires mouse CAR T cells; the translational potential of such CARs to the human setting is indirect. Our laboratory has highlighted the differential functional potency and persistence of CD28 or 4-1BB costimulated mesothelin CARs in a solid tumor environment and developed translational approaches to overcome T-cell intrinsic inhibitory mechanisms such as PD-1/PD-L1/2.[15] Other approaches, including antibody-mediated PD-1 blockade and PD-1 "switch receptors," are also discussed.

The potential for well described severe "cytokine storm" in hematological malignancies, immunogenic reaction from transduced genes of murine origin resulting in "HAMA reaction" or feared "on-target, off-tumor" events in solid tumor therapy are still unknown. Approaches to address these issues including "self-limiting" and "activation-induced elimination" of CAR T cells are discussed by Newick *et al*.[9]

TOXICITY MANAGEMENT

The success of CD19 CAR T-cell therapy owes, in part, to the development of early recognition and better treatment strategies for CAR T-cell toxicities. Theoretical toxicities, such as clonal expansion secondary to insertional mutagenesis, graft versus host disease, and off-target antigen recognition, luckily have been rare. However, cytokine release syndrome, due to T-cell activation and subsequent cytokine secretion, is more prevalent and requires early recognition and expert management. Currently used therapeutic approaches, such as IL-6 blockade and corticosteroids, and their mechanisms of action were discussed in this issue by Curran *et al*.[10] Neurological toxicity is the least understood or studied, yet a common toxicity in CD19 studies. Neurological studies, such as electroencephalogram and brain scans, have not been helpful. Ongoing investigations are testing the cytokine concentration in cerebrospinal fluid, CAR T-cell accumulation in cerebrospinal fluid, and central nervous system leukemia. With expansion of CAR T-cell therapy solid tumors, identification of an ideal tumor-restricted antigen is rare. On-target, off-tumor toxicities are feared especially if the target tissue is expressed in crucial tissues such as lungs, heart, or liver. Anaphylaxis due to murine components of the vectors have been described. Bonifant *et al*.[10] discuss treatment strategies for toxicity management. The current approaches include pharmacological immunosuppression by use of tocilizumab or corticosteroids. A variety of suicide genes have been incorporated into CAR designs such as HSV-tk, iCaspase-9, and EGFR or CD20 mutations.

IMMUNE MONITORING

Robust monitoring of CAR T cells following adoptive transfer to elucidate their bioactivity and mechanism of action are critical for understanding the molecular underpinnings of both therapeutic success, as well as failure. Broad interrogation of patient responses is particularly significant for early-phase clinical trials with limited patient numbers, in order to facilitate rational refinement of next-generation CAR T-cell approaches and clinical trial designs. In the article by Novosiadly and Kalos[11], emerging platforms for molecular profiling of T-cell therapies are extensively reviewed. Discussed within are assays focused on evaluation of CAR T-cell frequency and phenotype, alterations in endogenous immune responses, changes in inflammatory cytokine levels, and differences in tumor antigen expression and microenvironment. Emphasis is placed on new technologies for high-throughput and multiplex analyses, including T-cell receptor sequencing to follow clonal T-cell populations, gene-expression platforms, mass cytometry methodologies (*e.g.*, CyTOF) for comprehensive multi-channel phenotyping, and multiplexed microbead immunoassays for simultaneous cytokine detection. Additional emerging approaches, including whole body/tumor PET imaging techniques for detecting immune cells (*e.g.*, Immuno-PET) and interrogation of the microbiome, while not yet applied to the evaluation of CAR T-cell responses, have the potential to add new dimensions to our level of understanding. These next generation methodologies are of great benefit in expanding identification of multiple surrogate markers of clinical responses, thus increasing the information gleaned from individual patients. As these assays become standard in the field and are harmonized to better compare patient responses between institutions, this wealth of immune monitoring information will help drive successful evolution of next-generation T cells.

CONCLUSIONS

Along with the authors in this special issue, we share the excitement and enthusiasm for rapidly developing and changing paradigms in CAR T-cell therapy. In addition to the potential treatment benefits for therapy-refractory patients ("bench-to-bedside"), one spin-off of CAR T-cell therapy has been the advancement of the understanding of cellular immunology ("bedside-to-bench"). Concurrent developments in the understanding of tumor immunology and advances in other immunotherapies, such as checkpoint blockade, will result in combination strategies that will hopefully keep even the most difficult to treat cancers at bay.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

Christine E Brown[1] and Prasad S Adusumilli[2,3]
[1]T cell Therapeutics Research Laboratory, Department of Hematology & Hematopoietic Transplantation, Beckman Research Institute at City of Hope National Medical Center, Duarte, California, USA; [2]Center for Cell Engineering, Memorial Sloan Kettering Cancer Center, New York, New York, USA; [3]Thoracic Service, Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, New York, USA. Correspondence: PS Adusumilli (adusumip@mskcc.org) or CE Brown (cbrown@coh.org)

REFERENCES

1. Sadelain, M (2016). Chimeric antigen receptors: driving immunology towards synthetic biology. *Curr Opin Immunol* **41**: 68–76.
2. June, C, Rosenberg, SA, Sadelain, M and Weber, JS (2012). T-cell therapy at the threshold. *Nat Biotechnol* **30**: 611–614.
3. Sadelain, M, Brentjens, R and Rivière, I (2013). The basic principles of chimeric antigen receptor design. *Cancer Discov* **3**: 388–398.
4. Ruella, M and June, CH (2016). Chimeric antigen receptor T cells for B cell neoplasms: choose the right CAR for you. *Curr Hematol Malig Rep* **11**: 368–384.
5. Davila, ML and Sadelain, M (2016). Biology and clinical application of CAR T cells for B cell malignancies. *Int J Hematol* **104**: 6–17.
6. Park, JH, Geyer, MB and Brentjens, RJ (2016). CD19-targeted CAR T-cell therapeutics for hematologic malignancies: interpreting clinical outcomes to date. *Blood* **127**: 3312–3320.
7. Abate-Daga, D and Davila, ML (2016). CAR models: next-generation CAR modifications for enhanced T-cell function. *Mol Ther Oncolytics* **3**: 16014.
8. Wang, X and Rivière, I (2016). Clinical manufacturing of CAR T cells: foundation of a promising therapy. *Mol Ther Oncolytics* **3**: 16015.
9. Newick, K, Moon, E and Albelda, SM (2016). Chimeric antigen receptor T-cell therapy for solid tumors. *Mol Ther Oncolytics* **3**: 16006.
10. Bonifant, CL, Jackson, HJ, Brentjens, RJ and Curran, KJ (2016). Toxicity and management in CAR T-cell therapy. *Mol Ther Oncolytics* **3**: 16011.
11. Novosiadly, R and Kalos, M (2016). High-content molecular profiling of T-cell therapy in oncology. *Mol Ther Oncolytics* **3**: 16009.
12. Morello, A, Sadelain, M and Adusumilli, PS (2016). Mesothelin-targeted CARs: driving T cells to solid tumors. *Cancer Discov* **6**: 133–146.
13. Priceman, SJ, Forman, SJ and Brown, CE (2015). Smart CARs engineered for cancer immunotherapy. *Curr Opin Oncol* **27**: 466–474.
14. Adusumilli, PS, Cherkassky, L, Villena-Vargas, J, Colovos, C, Servais, E, Plotkin, J *et al.* (2014). Regional delivery of mesothelin-targeted CAR T cell therapy generates potent and long-lasting CD4-dependent tumor immunity. *Sci Transl Med* **6**: 261ra151.
15. Cherkassky, L, Morello, A, Villena-Vargas, J, Feng, Y, Dimitrov, DS, Jones, DR *et al.* (2016). Human CAR T cells with cell-intrinsic PD-1 checkpoint blockade resist tumor-mediated inhibition. *J Clin Invest* **126**: 3130–3144.