POINTS TO CONSIDER WHEN DESIGNING NEW TRIALS USING CHIMERIC ANTIGEN RECEPTORS

INTRODUCTION

On June 15, 2010, the NIH Recombinant DNA Advisory Committee (RAC) convened a symposium titled *Gene-Modified T Cells: Challenges in Clinical Trial Design*. The conference convened leading investigators conducting studies of a novel approach for cancer immunotherapy that uses gene modified autologous or allogeneic T cells carrying a chimeric antigen receptor (CAR). A CAR combines an antibody-antigen receptor linked to one or more T cell receptor signaling domains. The CAR is directed against tumor antigens and some are based on monoclonal antibodies that are already used in clinical practice.

The impetus for this conference, in part, was a report of a death on a gene transfer trial that was attributed to these gene modified T cells (OBA Protocol #0804-920). This was the second death to have occurred shortly after dosing on trials using chimeric antigen receptors.[1] These events prompted the Office of Biotechnology Activities (OBA) to hold this conference to review the reported treatment-associated toxicities and to discuss trial design methods to improve safety and efficacy. The conference focused on key areas of trial design identified by members of the RAC and investigators. The attached publication summarizes the discussions of that conference and the webcast and slide presentations are also available on OBA's website. The points to consider outlined below represent the RAC's recommendations based on data available in June 2010.

SUMMARY

The conference began with a brief review of the current state of the field, the types of CARs being used in clinic, and the rationale for the evolution in CAR design. A chimeric antigen receptor is a modified T cell receptor that typically contains an immunoglobulin variable fragment (scFv) linked to a TCR signaling domain with or without additional intracellular co-signaling motifs. It offers recognition of surface tumor antigens by T cells in a non-human leukocyte antigen restricted manner. Earlier versions of CARs are referred to as first generation. These CARs have an immunoglobulin signaling chain linked to the epsilon, gamma or zeta signaling sequences of the T cell receptor. In trials with these early constructs, the modified cells did not persist and there were limited demonstrations of efficacy. However, despite the limited persistence at least one trial demonstrated that recognition of low levels of the target antigen in normal tissue could lead to unexpected toxicity.[2]

Lack of persistence of the modified T cells was identified as a potential barrier to efficacy in early studies with first generation CARs. This problem has been addressed by modifications to

the CAR itself and through trial design. Second generation CARs incorporate an additional cosignaling moiety (e.g. CD28, CD134 or 4-1BB) and third generation CARs incorporate two additional cosignaling moieties. Another strategy to promote persistence and engraftment of the modified T cells is to use virus-specific T cells, such as Epstein Barr virus-specific T cells that may be continually activated through the viral T cell receptor. Such viral specific CARs may also contain additional cosignaling moieties, such as CD28.

In addition to modification of the CARs, investigators have added lymphodepleting chemotherapy to "make space" for the transduced T cells and to alter the cytokine milieu. Some trials have also used additional cytokine support for the T cells after infusion, predominately with interleukin-2 (IL-2).

POINTS TO CONSIDER

The conference was not designed to be a consensus conference, but the RAC endorsed several points to consider regarding the design of these trials. These points are based on data available at the time of the conference.

Dosing

One of the vexing questions raised by the adverse events seen in these clinical trials is how to determine an appropriate starting dose. Initial starting doses have ranged from $5x10^5$ cells/kg to 10^7 cells/kg; however, it is difficult to compare dosing across trials, as not all studies report weight based dosing. At this time it is not possible to generalize about a safe starting dose. In addition to considering the preclinical data, it is important to consider the distribution of the antigen in normal tissue and the potential for T cell activation against normal tissues expressing the target antigen. This potential for "on-target, off-tissue" activity may warrant a more conservative dose escalation. The initial dose may vary depending upon the type of CAR utilized. For example, given the lack of persistence of most first generation CARs, a higher starting dose may be more appropriate with a first generation CAR compared to second or third generation CAR, but still the potential for acute toxicity must be considered with first generation CARs as well.[3] Dosing of second, third and virus specific modified T cells should take into the account the possibility for expansion of these cells, especially in the setting of preconditioning chemotherapy.

One strategy being explored to enhance safety is splitting of initial doses, for example giving 10-20% of the dose initially and then monitoring the subject for a period of time before administering the remaining dose. The goal is to identify a subject who is at risk of a severe acute reaction, for example, cytokine release, prior to giving the full dose. While such strategies continue to be explored there are not yet data to definitively recommend for or against splitting doses.

Precise recommendations regarding starting doses across protocols are not possible as the initial dose should take into account a number of protocol specific factors including the type of CAR, whether the target antigen is only expressed on tumor tissue or is more widely expressed, and the use of preconditioning. There is insufficient data to recommend for or against splitting of doses to reduce the risk of an acute toxicity.

Monitoring

Analysis of the death that occurred on NIH OBA Protocol #920, revealed a dramatic rise in certain cytokines within hours of dosing, in particular interferon γ (IFN- γ), tumor necrosis factor-α (TNF- α), granulocyte macrophage colony-stimulating factor (GM-CSF) and interleukin-6 (IL-6). While in that case, the cytokine elevations were significant and correlated with an adverse clinical outcome, a lingering question is how much is known about the effect of these therapies on cytokine levels and whether measuring of cytokines can be used as an early predictor of potential toxicity during dose escalation. As many trials only enroll a small number of subjects, elucidating the role of cytokines may require pooling of data across trials.

All protocols should have clear monitoring plans that include, at a minimum, routine laboratory tests of sera and urine, including tests that evaluate for target organ toxicity based on the antigen being targeted, collection of plasma and peripheral blood mononuclear cells for cryopreservation and collection of cytokine data, including, for example, IFN-γ, IL-6, TNF-α. The RAC encourages investigators to develop mechanisms to share this data and to consider input from experts outside of gene transfer research who study the effects of cytokines and inflammation.

Strategies to Improve Persistence and Engraftment of Modified T cells

The inclusion of co-signaling moieties as a strategy to enhance T cell persistence and possibly efficacy is often combined with lymphodepleting chemotherapy to enhance engraftment. The optimum combination of co-signaling moieties and chemotherapy is not yet known. It may or may not be necessary to use lymphodepleting chemotherapy with viral-specific T cells as at least one study has demonstrated persistence of the cells in the absence of lymphodepleting chemotherapy.[4] In order to support persistence of the cells many protocols also employ cytokine support, but different strategies and doses are employed.

Strategies to achieve optimum engraftment while minimizing the potential for acute and long-term toxicity should continue to be studied as there is no consensus on the optimum combination of co-signaling moieties, lymphodepleting chemotherapy and cytokine support. Protocols should include a discussion of the rationale for the combination being employed based on the available data.

Enhancing Safety

A potential strategy to mitigate both acute and long-term toxicity of the gene modified T cells is to include suicide genes. It is not clear, however, that a suicide gene will be effective in preventing an acute toxicity as was seen in OBA protocol #920. There are risks to including suicide genes, for example the potential immunogenicity of viral based suicide genes that will need to be weighed against the alternative approaches for addressing non-acute toxicities.

There are not data to recommend suicide genes as a mechanism to prevent acute toxicities. The potential for suicide genes to be used to manage long term toxicities remains an open research question.

Ethical Considerations

To date, trials using CARs have enrolled subjects with end-stage malignancies. These subjects are potentially vulnerable given their limited therapeutic options. The RAC recognizes the tension in developing new agents for this population of patients, which is not unique to gene transfer. On one hand investigators need to proceed slowly to establish the safety of the approach, and yet there is a desire to provide these patients, even in early trials, an opportunity for clinical benefit. Whether the acceptable level of risk should be adjusted in relation to the disease prognosis for a given patient cohort remains a subject worthy of future debate.

As many protocols using CARs enroll subjects with terminal cancer and few alternative options, special attention should be paid to ensuring that the informed consent process is structured to avoid therapeutic misconception. The term gene therapy should be avoided and the potential risks should be detailed, including the rationale for the chosen elements of study design, e.g. preconditioning, cytokine support and split dosing.

CONCLUSION

These points to consider reflect the findings at the time of the conference in June 2010. At that time, there were approximately 30 trials registered with OBA that had enrolled subjects and most were early phase trials involving a small number of subjects. Moreover, most trials have targeted hematologic malignancies and have used first or second generation CARs, and therefore available data are still quite preliminary regarding the use of CARs against solid tumor antigens and the use of third generation CARs. OBA will continue to provide data on new trials through GeMCRIS, including summaries of amendments to trial design. A summary of serious safety reports that are considered possibly related to the products will continue to be provided on a quarterly basis as part of the Data Management Report posted with on the OBA's Website at each OBA - RAC Meetings.

The RAC emphasized that, in evaluating the risks and benefits of new trials, one must not lose sight of the fact that even the standard therapies for the conditions being targeted carry significant risk. Elimination of risk may not be possible, but the goal should be to take reasonable steps to minimize risk, especially in these early trials where benefit is also less likely.

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[2] Lamers C. H. J., Sleijfer S., Vulto A. G., *et al.*, Treatment of Metastatic Renal Cell Carcinoma With Autologous T-Lymphocytes Genetically Retargeted Against Carbonic Anhydrase IX: First Clinical Experience. J Clin Oncol. 24:e20-22, 2006.

[3] Kershaw, M. H., Westwood, J. A., Parker, L. L., *et al.*, Phase I Study on Adoptive Immunotherapy Using Gene-Modified T Cells for Ovarian Cancer. Clinical Cancer Research, 12: 6106-6115, 2006.

[4] Pule, M., Savoldo, B., Meyers, G. D., *et al.*, Virus-Specific T cells engineered to co-express tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma, Nature Medicine, 14(11): 1264-1270, 2008.