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Progress in Novel Cellular Therapy Options for CLL: The MD Anderson Perspective

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Abstract

Over the past several years there has been considerable progress in the number and breadth of therapeutic options for patients with chronic lymphocytic leukemia (CLL). In particular, cumulative experience with stem cell transplantation and immunotherapy has made these modalities more available to a broader range of patients. Advances in genetic engineering and *ex vivo* expansion techniques have sculpted cellular therapy products to optimize the combination of specificity and toxicity. In this brief review, we will discuss recent cutting-edge experience with chimeric antigen receptor T cells as well as developing cellular therapy products at our institution.

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is the most common adult hematologic malignancy. While some patients can demonstrate a long, indolent course without significant morbidity, a significant number of patients eventually require treatment. Though there has been a remarkable development in the treatment options for CLL over the past 15 years, many patients, particularly those with high-risk features, often succumb to their disease.

Allogeneic hematopoietic stem cell transplantation (HSCT) has emerged as a viable treatment strategy for CLL patients and is still considered the only curative option. Advances in preparative regimens have now made this procedure more tolerable for older patients. In addition, reduced intensity regimens underscore the importance of the graft versus leukemia (GVL) effect in CLL. Though this regime has been highly successful with some centers

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Conflict of Interest: Dr. Cooper founded and owns InCellerate, Inc.; InCellerate is not involved in the development of CAR T cells. He has patents with Sangamo BioSciences with artificial nucleases. He consults with Targazyme, Inc. (American Stem cells, Inc.), GE Healthcare, Ferring Pharmaceuticals, Inc., and Bristol-Myers Squibb.

reporting 39–70% long term survival,¹ the issue of relapse still remains, as does minimizing graft versus host disease (GVHD) while maximizing GVL.

Building on the beneficial effect of GVL seen in CLL, numerous investigators have been developing strategies to optimize cell therapy to yield a more durable and eventually less toxic approach to eradicating this disease. In this brief report we will discuss the results of early novel cell therapy trials in CLL as well as unveil new strategies being developed at our institution and elsewhere.

CHIMERIC ANTIGEN RECEPTOR T CELLS

One of the most highly publicized and exciting developments over the past few years has been engineering of T cells to express chimeric antigen receptors (CARs). The CAR technology combines the specificity of antibody therapy with the cytotoxicity of T cells to directly target tumor cells. Each CAR is expressed on a T cell as a result of a genetic integration of a specific construct with the native DNA.

The basic design of a CAR involves an extracellular domain which is antigen-specific and an intracellular signaling domain which prompts cytotoxicity. The antigen specific extracellular domain is most commonly derived from the variable region of the respective antibody produced by B-cells. Thus, recognition of an antigen by these CAR T cells does not depend on presentation by HLA molecules. In the case of CLL, CD19 has been the tumor associated antigen (TAA) of choice, as it is essentially limited to cells of B-lineage. The signaling portion of the CAR relies on machinery more native to the T cell receptor, though the optimal sequence of signaling domains is still evolving. The more recent modifications of this technology have employed the CD3 ζ activation domain combined with that of one or more co-stimulatory receptors such as CD28 and 4-1BB. The transduction of T cells by the CAR genetic construct is accomplished by various means, including retroviral vectors and transposases.

Thus far there have been several clinical trials of CAR T cells that have included patients with CLL. The majority of these are early phase studies to establish safety and feasibility of this technology. In 2011 Kalos et al reported a pilot trial of 3 patients with advanced CLL who were treated with autologous CD19-specific CAR T cells with the CD3 ζ /4-1BB construct.² Overall 2 patients achieved a complete response (CR) and 1 patient achieved a partial response (PR). An recent update of these results in abstract format demonstrated responses in 8/14 CLL patients.³ In addition, an ongoing dose-optimizing study from the same institution has demonstrated a 40% response rate in relapsed/refractory CLL patients.⁴ A similar trial conducted by Kochenderfer et al enrolled 8 patients with B- cell malignancies, 4 of whom had CLL.⁵ Patients received CD19-specific CAR T cells (CD3 ζ /CD28 endodomain) and supplemental IL-2. Of the 4 CLL patients included in this trial 1 achieved a CR, 2 achieved a PR and 1 patient had stable disease (SD). Brentjens et al treated 8 patients with CD19-specific CAR T cells (CD3 ζ /CD28 endodomain) and reported 3 patients with SD.⁶ Importantly, this study demonstrated trafficking of T cells to tumor sites and persistence up to 8 days after infusion. The toxicity in these studies has varied; several

patients have experienced hypotension and other signs of cytokine release storm (CRS). Preliminarily, this CRS may actually be a surrogate for clinical response.⁷

More recently, there have been clinical trials employing allogeneic CAR T cells. Kochenderfer et al treated 10 allogeneic HSCT recipients with donor-derived CD19-specific CAR T cells.⁸ Of the 4 CLL patients included in this trial, 1 achieved a CR and another maintained SD without additional GVHD. Similarly, the group at Baylor College of Medicine has demonstrated the ability to transduce virus-specific allogeneic T-cells with CD19-specific CAR.⁹ This study included 4 patients with CLL who had relapsed after allogeneic HSCT. Of these 4 patients, 1 achieved a PR and another achieved SD. Importantly, there were no infusion-related toxicities and no associated GVHD. These studies thus presented a new platform for treating CLL patients with allogeneic CD19-specific T cell therapy, opening the door for patients who relapse after allogeneic HSCT or as a part of early post-HSCT consolidation.

At our institution we are building on the growing knowledge in this field to offer patients both autologous and allogeneic CAR T cell products. Because of the increased flexibility in rapidly developing new constructs we are exploring the Sleeping Beauty transposon/transposase system.¹⁰ This system has the dual advantage of stable introduction of the CD19-specific CAR sequence and deletion of the α or β T cell receptor (TCR) chains, thus limiting unwanted, non-specific allo-reactivity. With this technology we have been able to generate CD19-specific T cells which are not responsive to TCR stimulation. Using an artificial antigen presenting cell (APC) culture system, we have been able to expand these CAR T cells >1000-fold. We are currently conducting 4 clinical trials of either autologous or allogeneic CD19-specific CAR T cells: 2 trials for B cell malignancies,¹¹ a cord blood-CAR-CD19 trial and a trial of autologous CAR T cells specifically for CLL patients in the non-transplant setting. To date there have been no significant toxicities in any of these trials while efficacy data is still being matured. In select patients, the CAR T cells have been detectable several months post-infusion.

While CD19 is an attractive target for CAR technology, additional TAAs are being studied. In collaboration with other institutions we are also developing a CAR against receptor tyrosine kinase-like orphan receptor-1 (ROR1). This protein is expressed highly on CLL cells as well as mantle cell lymphoma cells with some additional, lower level expression on adipose tissue.¹² Importantly, ROR1 is not expressed on mature B cells. This target thus has the potential advantage of malignant B-cell specificity without total depletion of all B cell stores. In addition ROR1 may also be expressed on solid tumor cells.¹³ Though there is the potential for low level ROR1 expression on normal tissue cells, there are ROR1 antibodies in development that do not bind to normal tissues. In preliminary studies, we have been able to transduce normal donor T cells with a ROR1-specific CAR (containing either a CD3 ζ /CD28 or CD3 ζ /4-1BB endodomain) with subsequent logarithmic expansion of T cells. These cells demonstrate *in vivo* activity in a murine model of B cell malignancy.

NATURAL KILLER CELLS

Natural Killer (NK) cells are increasingly becoming recognized as important players in innate anti-tumor immunity. These CD56⁺/CD3⁻ lymphocytes are thought to become activated after engagement with cells which lack MHC class I (the “missing-self hypothesis”).¹⁴ They can thus recognize tumor cells which tend to down regulate MHC class I. Furthermore, in an allogeneic HSCT setting, certain mismatches between donor-recipient HLA alleles are associated with theoretical NK cell alloreactivity and improved clinical outcome *without* concomitant increases in GVHD.^{15,16} In addition, NK cell recovery after allogeneic HSCT has been linked to decreased acute GVHD, decreased non-relapse mortality and improved relapse-free survival.¹⁷ To date the majority of clinical trials with NK cell therapy have focused on myeloid malignancies but there is a growing interest in the potential application of NK cells against CLL.

While autologous NK cell generation from CLL patients would seem attractive, this is challenging due to several factors. The absolute number of NK cells from CLL patients is limited, particularly with the relative increase in B cells, thus impairing the generation of a clinically meaningful NK cell dose. In addition, there is growing evidence to suggest that autologous NK cells from CLL patients are hypofunctional with decreased Nkp30 expression.¹⁸

Though the field of NK cell therapy has traditionally relied on peripheral blood-derived NK cells we have been interested in harvesting NK cells from umbilical cord blood (CB), a known source of hematopoietic stem cells. Recently our group has developed a system for large-scale, GMP-compliant *ex vivo* expansion of CB-derived NK cells from a fraction of a frozen CB unit.¹⁹ These cells demonstrate an activated phenotype and are cytotoxic against K562 cells as well as multiple myeloma cells.

Preliminary data from our laboratory suggests that CB-derived NK cells are also active against CLL. After *ex vivo* expansion, CB-NK cells appear to form strong synapses with target primary CLL cells and demonstrate *in vitro* cytotoxicity (Figure 1A and B). In a murine model of CLL that is under development in our lab, these CB-NK cells appear to impair *in vivo* expansion of CLL cells and subsequent lymphoid infiltration of the spleen (Figure 2A and B).

There is also emerging data from our laboratory and others^{20,21} suggesting that NK cell function may be augmented by lenalidomide. Our preliminary findings demonstrate increased *in vitro* CB-NK cytotoxicity against CLL after incubation with lenalidomide (Figure 3). Though the mechanisms underlying this interaction are not clear, multiple hypotheses have been proposed, including disinhibition of NK cell signaling,²² increase in NK cell activating receptors²³ and enhancement of NK-CLL synapse formation.²⁴ These results suggest that the eventual combination of immunomodulatory and cellular therapies may optimize immune therapy for CLL.

Finally, we have recently transduced CB-NK cells with a CD19-retroviral CAR construct that also expresses IL15. In preliminary experiments these NK-CAR-CD19 cells have shown

significantly higher levels of cytotoxicity against CD19+ target cells than non-transduced NK cells. Once confirmed, translation of NK-CAR therapy to the clinic is planned.

CONCLUSION

In conclusion, the therapeutic options for patients with CLL have increased dramatically in the last several years. While there has been an unprecedented development in pharmacologic agents, there has been an equally exciting progress in cellular therapy. The multiple clinical trials and modifications of CAR T cells are beginning to converge on the optimal strategy for this technology. In addition, the advances in *ex vivo* expansion and immunomodulation have brought within reach NK cell therapy. As we continue to improve specificity and limit toxicity, we can move closer to the ideal goal of long-term remission for our patients.

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Figure 1.

A. *Ex vivo* expanded CB-NK cells form immunological synapses with primary CLL cells.

B. CB-NK cells demonstrate dose-dependent cytotoxicity against primary CLL cells in a standard Chromium-51 assay.

Figure 2. CFSE-labeled CLL cells were injected into NOD/SCID IL-2R γ^{null} (NSG) mice with or without CB-NK cell adoptive transfer. A. Representative plot at day 12 demonstrating impaired proliferation of CFSE-labeled CLL cells in the mice who received CB-NK cells. B. Hematoxylin and eosin staining showing reduced lymphocytic infiltration of murine spleen after treatment with CB-NK cells.

Figure 3.
Chromium-51 assay demonstrating augmented CB-NK cytotoxicity against primary CLL cells in the presence of lenalidomide.