GENETICALLY MODIFIED T-CELLS EXPRESSING CHIMERIC ANTIGEN RECEPTORS IN THE TREATMENT OF CANCER

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ABSTRACT

Dr. Carl June and his colleagues at the University of Pennsylvania have succeeded in treating patients with Chronic Lymphocytic Leukemia using gene therapy. Two of the three patients treated sustained a complete remission and one a partial remission. The procedure involved transducing the patients' T cells to express chimeric antigen receptors which target a particular protein found on both healthy and cancerous B cells. Following infusion of the newly transduced T cells, each patient developed clinical symptoms associated with an intense immune response. Shortly thereafter, tumors were completely eliminated in two of the patients and partially eliminated in the third. All three patients were pre-treated with conventional therapies and responded poorly. This study coalesces volumes of research in genetics, immunology, and molecular biology in what might just be the future of cancer treatment.

INTRODUCTION

Harnessing the body's own immune system to battle cancer has always been understood by medical scientists as the ideal avenue for treatment. An astounding Phase-1 Clinical Trial recently conducted at the University of Pennsylvania (UP) may prove to be a breakthrough in this area. The study represents a culmination of twenty years of intense research in immunology and gene therapy. Dr. Carl June and his colleagues at UP treated three patients having advanced chemotherapy-resistant CLL (chronic lymphocytic leukemia), a cancer of B-Cells. The trial involved transducing the patients' own T-cells to express chimeric antigen receptors, a fairly recent biomolecular invention, generally referred to as CARs (June et al. 2011). CARs, genetically engineered receptors, are generally comprised of a constant region that is similar to that of any T-cell receptor and a variable region that targets a specific antigen (Eshhar et al. 1989). With the correct genetic engineering and biomolecular construction, T-cells can be induced to express CARs that target a gamut of antigens. In the trial led by Dr. June, the patients' T-cells were transduced via an HIV-1 based lentiviral vector to express a CAR with specificity for CD19 (cluster of differentiation 19), a protein found on both healthy and cancerous B-cells. The modified cells are referred to as CART19 (chimeric antigen receptor T 19) cells.

Following exogenous transduction, the patients' T-cells were infused intravenously. Within one to three weeks, all three patients developed symptoms that were indicative of a serious immune response. The CAR T-cells expanded *in vivo* at least a thousand fold and successfully eliminated both healthy and cancerous B-cells. The effector-to-target (E/T) ratios observed in the patients were 1: 93,000; 1:2,200; and 1:1,000. Hence, in the patient with an E/T ratio of 1:93,000, an infusion of 1.4 x 10^{11} CART19 cells resulted in the remarkable elimination of 1.3 x 10^{12} CLL cells. Two of the three patients sustained complete remission, and one patient experienced a partial remission. Furthermore, in the two patients achieving complete remission, a population of memory CART19 cells was observed months after treatment, indicating the possibility of prolonged immuno-surveillance (June et al. 2011).

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CHIMERIC ANTIGEN RECEPTORS

The original construct of T-cells expressing chimeric antigen receptors was designed two decades ago by Dr. Zelig Eshhar and his colleagues at the Weizmann Institute of Science (Bridgeman et al. 2010). Their CAR T-cell was transduced with rearranged gene segments coding for the variable domain (V_H and V_L chains) of an anti-trinitrophenyl antibody attached to constant region (either alpha or beta) of a T-cell receptor. The transmembrane and cytoplasmic domains of a typical T-cell receptor were maintained. The resulting T-cells then produced an efficient immune response when exposed to trinitrophenyl. In response to the hapten, the CAR T-cells proliferated, produced interleukin 2, and targeted cell lysis (Eshhar et al. 1989).

The fact that T-cells can be endowed with antibody-type specificity is highly significant. Typically, T-cells only respond to an antigen that is bound to MHC (major histocompatibility) protein, either I or II. When antigen-presenting cells, such as macrophages; B cells; and dendritic cells, encounter an antigen, they process it. The procedure involves ingesting the foreign molecule, synthesizing an MHC molecule, fusing the two, and attaching this antigen-MHC complex to the plasma membrane of the cell. T-cells only respond to an antigen presented in this way. More specifically, CD4 cells react to antigens bound to MHC-II molecules, and CD8 cells respond to antigens that are bound to MHC-I molecules. The necessity of having an antigen bound to an MHC molecule in order to elicit an immune response in T-cells is known as MHC restriction (Tortora and Derrickson 2009).

MHC restriction can be circumvented with CARs. Since the CAR has the variable region of an antibody, it acts as one. Antibodies react with antigens in their native state to elicit an immune response. Thus, CARs can be used to target antigens that would normally not be presented with an MHC molecule (Eshhar et al. 1989), such as in the UP trial where the CARs targeted the CD19 protein found on B cells. In addition, in situations where MHC-I may be somewhat down-regulated by tumors as part of a strategy to inhibit immuno-surveillance, the use of CARs in the treatment of cancer may be more favorable (Bridgeman et al. 2010).

T-Cell activation usually requires two signals. One is delivered by the TCR-CD3 complex which interacts with the MHC-antigen complex. The other is delivered by co-stimulatory domain CD28 when it interacts with the co-stimulatory molecules (such as CD80 or CD86) found on the antigen presenting cells. Other co-stimulatory domains, such as CD137 or CD134 may also be necessary.

The optimal construction of CARs has been explored during the past two decades (Eshhar et al. 2001). Naturally, the precise design would depend on the target antigen and efficacy of the CAR. As illustrated in Figure 1, first generation CARs consist of the single-chain variable fragment (scFv) of an immunoglobulin specific for antigen (usually a tumor antigen), bound to a hinge region that crosses the cell membrane. The hinge region is attached only to the CD3- ζ chain of the TCR-CD3 complex which plunges into the cytoplasm and acts as the signaling domain.

To enhance effectiveness, second generation CARs have a co-stimulatory signaling domain, such as CD28, CD137 (4-1 BB), or OX40, inserted between the hinge region and the CD3- ζ chain (Urba and Longo 2011). For example, inclusion of a CD137 co-stimulatory signaling domain significantly enhanced *in vivo* persistence of CARs and antitumor activity in preclinical trials (Kalos et al. 2011, as described in

June et al. 2009). Third generation CARs incorporate various combinations of co-stimulatory domains (Urba and Longo 2011).

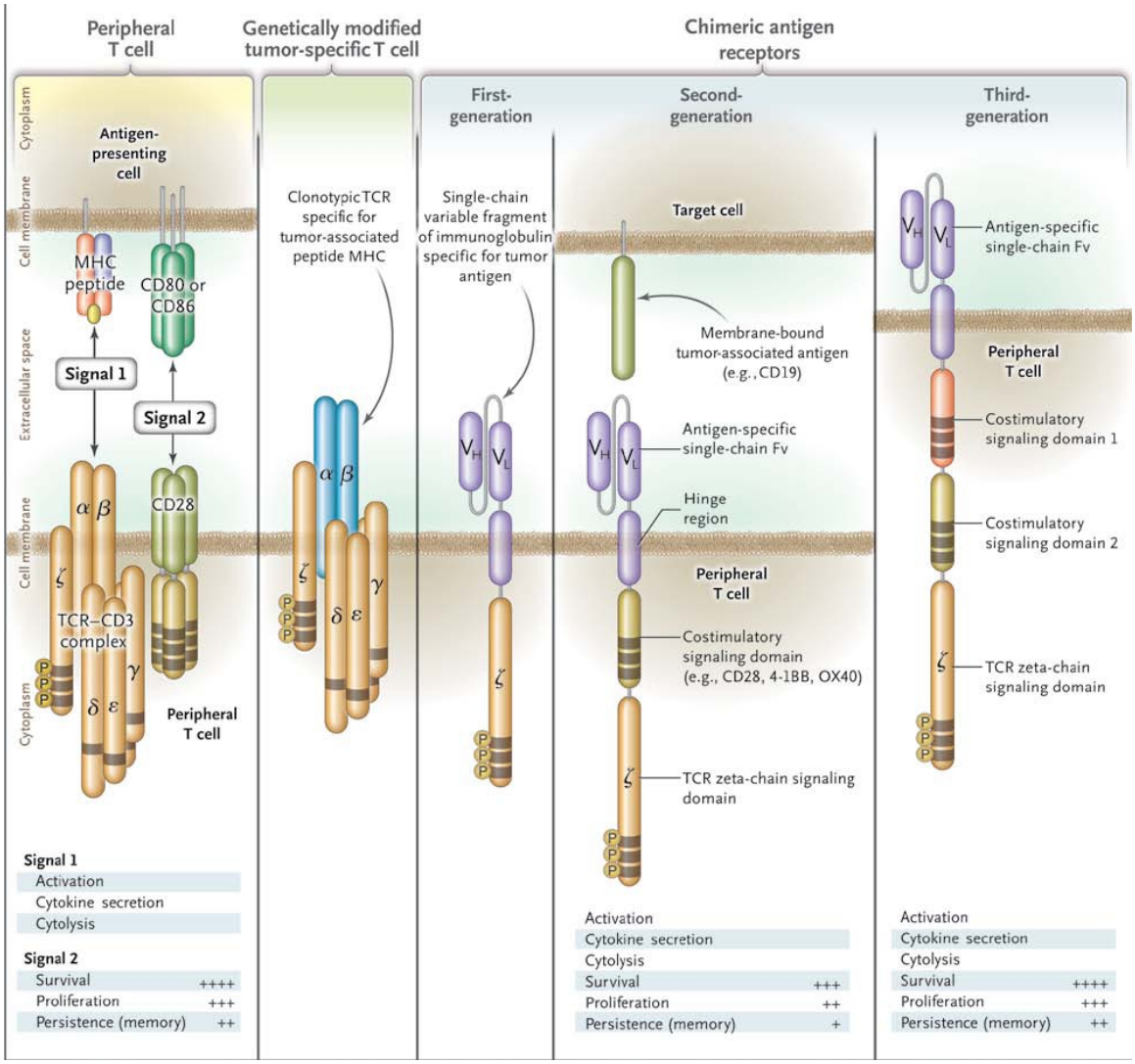

Figure 1: T-Cell Activation.
"Optimal T-cell activation requires a minimum of two signals. Signal 1 is delivered by the TCR–CD3 complex through interaction of the T-cell receptor (TCR) alpha and beta chains as they recognize peptide presented by a class I (CD8 T cells) or class II (CD4 T cells) major histocompatibility complex (MHC) molecule. Signal 2 is most commonly provided by the engagement of CD28 on the T cell with the costimulatory molecule CD80 or CD86 on the antigen-presenting cell. CD137 (4-1BB) and CD134 (OX40) also provide costimulation to T cells. The optimal combination of effector function, proliferation, and survival requires both signals. Delivery of signal 1 without costimulation, which often occurs for tumor-infiltrating lymphocytes and transgenic T cells encountering antigen on a solid tumor devoid of costimulatory molecules, leads to anergy and apoptosis, thereby limiting the antitumor response. The first-generation chimeric antigen receptors usually comprise of a single-chain variable fragment of an antibody specific for tumor antigen, linked to the transmembrane and intracellular signaling domain of CD3-zeta. Second-generation chimeric antigen receptors were developed to incorporate the signaling domain of a costimulatory molecule to improve T-cell activation and expansion. Third-generation chimeric antigen receptors include combinations of costimulatory domains." (Data are from Keith Bahjat, Ph.D.) Source: Urba and Longo 2011

Currently, expression of CARs on non T-cells is limited due to difficulties in transfection, although developments are underway. Furthermore, CARs only target molecules expressed on cell surfaces. Thus, intracellular tumor specific antigens would require a different approach (Bridgeman et al. 2010).

HIV-1 Based Lentiviral Vectors

A key factor in effectively transducing T-cells, as in any other aspect of gene therapy, is choice of the vector. Safety; long-term stability; versatility; and, sometimes, the ability to transduce non-dividing cells are all taken into account (Lu et al. 2004). In the UP trial, an HIV-1 based lentiviral vector was used. Lentiviruses (lenti is the Latin word for "slow") are thus named because of the long incubation period between infection and the onset of disease. What makes lentiviruses unique among retroviruses is their ability to infect non-dividing cells (Durand and Cimarelli 2011). Typically, a retrovirus must wait until the S phase of the cell cycle when it is afforded the opportunity of penetrating the nuclear membrane. Thus, it cannot infect the cell unless the cell is dividing. However, lentiviruses have the ability to integrate into the host's genome by penetrating the nuclear membrane on their own (Lu et al. 2004). This makes them highly useful in gene therapy whose main targets include the brain, lungs, liver, muscles, and hematopoietic system (Zufferey et al. 1998). In addition, transcriptional silencing has been observed in the use of onco-retroviral vectors and not in the use of lentiviral vectors. In fact, lentiviral vectors have successfully integrated into a variety of tissues (Vigna and Naldini 2000). Furthermore, these vectors are capable of carrying large transgenes of up to 18,000 bases (Coleman et al. 2003). One drawback of lentiviral vectors is that they integrate into the host's genome at random locations. Sometimes, this may activate nearby oncogenes. A number of patients in a clinical trial undergoing gene therapy for SCID – γC (severe combined immunodeficiency) syndrome developed leukemia as a result of the use of lentiviruses (Durand and Cimarelli 2011). Only one virus is known to incorporate itself at a specific site in the human genome—the adeno-associated virus that partially integrates into the human chromosome 19q13.42. Scientists are attempting to find a way to transpose the site-specificity of the adeno-associated virus to lentiviruses (Durand and Cimarelli 2011).

HIV Structure

The HIV virus (Figure 2) contains two copies of a single-stranded RNA measuring 9,749 nucleotides long enclosed in a capsid. Bound to the RNA are nucleocapsid proteins p6 and p7 (which prevent the RNA from digestion by nucleases) and enzymes such as reverse transcriptase and integrase. These enzymes allow the virus to transcribe DNA off its RNA and then integrate into

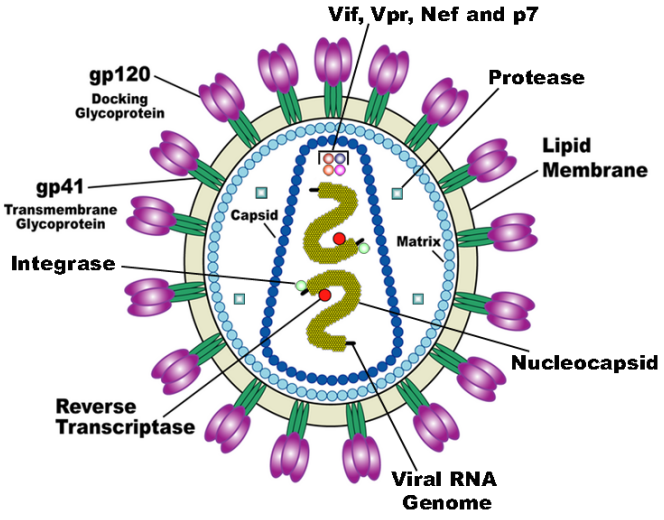

Figure 2: HIV Structure. Source: Henderson C.

the host's genetic material, respectively (Kuiken et al. 2008). Along the viral envelope are protein units that aid in attachment to the host cell. Each unit consists of three transmembrane subunits of glycoprotein 41 attached to three external subunits of glycoprotein 120 (Chan et al. 1997). Surrounding the capsid and anchoring these glycoproteins into the envelope is a matrix composed of the protein p17 (Kuiken et al. 2008).

HIV Genome

The typical HIV genome contains nine genes flanked by LTRs (long terminal repeats). The *gag*, *pol*, and *env* genes code for viral structural proteins. *Gag* codes for a p17 presursor, capsid protein p24, necleocapsid proteins p6 and p7, and spacer peptides. *Pol* codes for HIV protease, integrase, and reverse transcriptase. The *env* (envelope) gene codes for glycoprotein 160, a precursor to gp41 and gp 120 which are necessary for viral attachment to the host cell (Watts et al. 2009).

Tat and *rev* are two regulatory genes. *Tat* activates expression of the viral RNA, and *rev* promotes cytoplasmic export of *gag*, *pol*, and *env* transcripts. *Vif*, *nef*, *vpu*, and *vpr* are accessory genes critical for pathogenesis but not for replication (Vigna and Naldini 2000).

Construction of Safe HIV-1 Based Vectors

Constructing safe vectors presents many challenges. The virus must retain those genes necessary for efficient transduction of target cells. At the same time, genes that would enable the virus to reproduce and infect other cells following transduction must be eliminated.

Effective transduction using the two-plasmid production approach involves the introduction of two genomic constructs into the target cell: a transfer vector construct (Figure 3) and a packaging construct, also known as the helper plasmid (Figure 4). The transfer vector construct contains *cis*- acting sequences that are vital for the transfer and integration of the

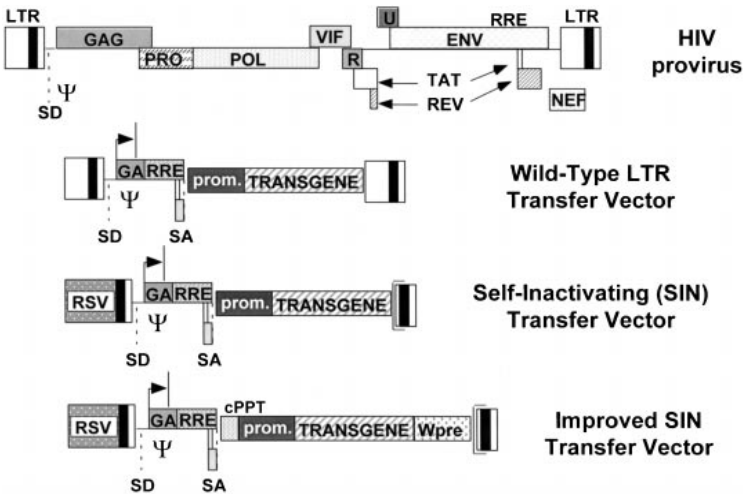

Figure 3: Various HIV-1 derived transfer vector constructs. Source: Vigna and Naldini 2000

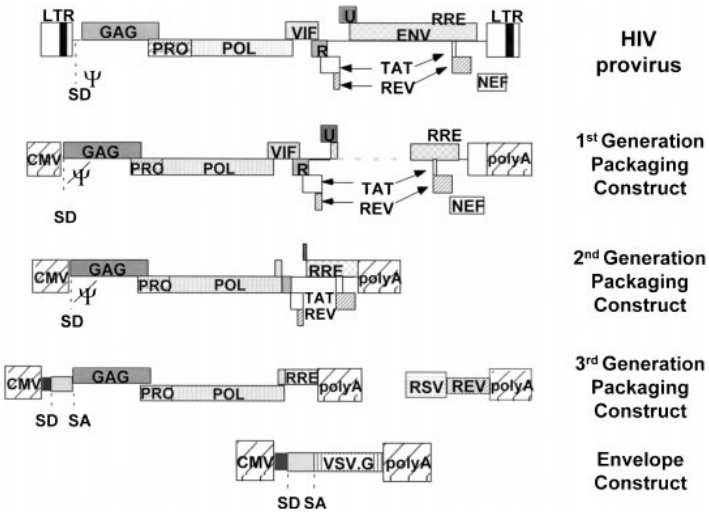

Figure 4: Various HIV-1 derived packaging constructs/helper plasmids. Source: Vigna and Naldini 2000

viral genome into a host's genome. The transgene is hooked onto the transfer vector construct. The packaging construct contains *trans*-acting genes that code for the essential viral proteins. When both constructs are introduced into the same cell, they express proteins necessary for encapsulation and integration of the transfer vector. As these vectors are carefully engineered to adhere to the highest standards of biosafety, this infectious process happens only once. This is due to efficient removal of genetic material that would enable the virus to proliferate and infect other cells.

In first generation HIV-1 derived packaging constructs, all genes necessary for the production of structural and accessory proteins are maintained; only *env* is eliminated. In second-generation constructs, the accessory genes, *vif*, *nef*, *vpu*, and *vpr* are also absent. In the third generation, *tat* and *rev* are eliminated as well, although *rev* is expressed by a separate construct that is flanked by a rous sarcoma virus promoter and a polyadenylation signal. Thus, only three out of the original nine genes are expressed.

Transfer vectors house the transgene as well as GA and RRE, which are placed adjacently between an LTR and the internal promoter. GA is a derivative of the *gag* gene, while RRE (rev-responsive element) is a portion of the *env* gene (Vigna and Naldini 2000).

Elimination of most viral genes is necessary in order to avoid RCR (replication competent recombinants), which can happen if the *trans*-acting viral genes in the packaging constructs merge with the *cis*-acting sequences in the transfer vector. In fact, the biosafety of a vector depends on the extent of successful separation between the functions of the *cis*-acting and *trans*-acting viral genomes that comprise the transfer vector construct and packaging construct, respectively.

To avoid the emergence of RCR, self-inactivating (SIN) transfer vectors have been designed. In a wild-type LTR transfer vector (non-SIN), the LTRs are maintained. In the SIN transfer vector, there is a large deletion in the U3 region of the 3' LTR. Upon transduction, this deletion is duplicated upstream, resulting in inactivation of both LTRs (Vigna and Naldini 2000). Thus, during reverse transcription, the deletion is transferred to the proviral DNA, resulting in ineffective transcription of the LTRs. This avoids the hazardous production of complete vector RNA, since the LTRs contain promoter and enhancer sequences. In addition, sabotage of the LTRs prevents aberrant expression of sequences in the host's genome that are adjacent to the vector integration site. Transcription of the transgene is, instead, driven by an internal promoter that is nestled safely away from the cell's native genome (Zufferey et al. 1998).

The improved SIN transfer vector has some additional constituents. The central polypurine tract (cPPT) sequence (which provides increased transduction efficiency and transgene expression) of the *pol* gene is inserted just before the internal promoter in order to improve gene transfer performance. The post-transcriptional regulatory element of the woodchuck hepatitis virus (Wpre), which improves the performance of vectors, is inserted just after the transgene to enhance its expression (Vigna and Naldini 2000).

Lentiviral vectors, as observed by stable expression of marker genes *in vivo*, can successfully transduce a spectrum of cells. Neurons of adult rat brains were the first cells in which stable expression of lentiviral tranduction was observed (Vigna and

Naldini 2000, as described in Verma et al. 1996). Other cells that have been successfully transduced with lentiviral vectors include cells of the retina, liver cells of rodents (Miyoshi et al. 1997), human dendritic cells and macrophages (Schroers et al. 2000), and human CD34+ and CD38- hematopoietic cells (Case et al. 1999).

First Lentiviral Vector Evaluated in Human Cells,

VRX496 and its sister VRX496 were the first lentiviral vectors to be evaluated in human cells. The trial was conducted by Xiaobin Lu and his colleagues at the Sydney Kimmel Comprehensive Cancer Center of the John Hopkins School of Medicine. The HIV *env* gene is not eliminated from VRX496 and 496; instead, these vectors express a 937-base antisense to silence the gene. VRX496 contains a 186-base sequence that acts as a molecular marker and is derived from the Green Fluorescence Protein (GFP) gene. VRX496 has an enhanced GFP gene.

The cells transduced in this experiment were human CD4+ T lymphocytes. Human blood was obtained, and CD4+ cells were isolated with the magnetic activated cell-sorting system. Flow cytometry indicated a purity >95%. The cells were then cultured in X-vivo 15 media containing 10% human serum and the antibiotic gentamycin. In preparation for transduction, the cells were plated in a 24-well plate at 1 x 10^6 cells per well. VRX496 was added to the cells at 20 tranducing units per cell. Concurrently, to ensure activation and expansion of the cells, immobilized anti-CD3/CD28 (iCD3/28) antibodies were added at a ratio of three beads per cell as well as 100 U/ml of interleukin 2. The cells were then cultured for three days during which they were washed three times to remove the vector. The iCD3/28 beads were removed four days later. Finally, the lymphocytes were replated at half a million cells per ml. At this point, the culture was able to be maintained for a significant amount of time.

After seven days in culture, the tranduced cells were assessed for GFP expression which would indicate successful integration. Flow cytometry indicated that 99.4% of tranduced cells were positive for vector gene expression, while 99.9% of the control cells were negative.

To measure stability of transduction, another batch of CD4+ T lymphocytes were transduced at 20 TU/cell as well. The cells were allowed to expand 1.5-million-fold over 36 days during which GFP expression was monitored. GFP expression remained stable throughout the entire culture period, demonstrating consistent vector payload expression. To date, no RCR generation has been reported in the use of VRX496 and VRX496 (Lu et al. 2004).

Chimeric Antigen Receptors and Lentiviral Vectors in the Treatment of Cancer

The recent pilot study conducted by Dr. Carl June and his colleagues at UP involved three patients with chemotherapy-resistant CLL. Two of them had p53-deficient CLL, a 17p deletion that usually indicates poor response to conventional treatment. Before enrollment in the study, all the patients underwent standard therapies, such as rituximab, fludarabine and bendamustine. Nevertheless, all had significant tumor burdens right before the trial, including bone marrow infiltration and lymphoadenopathy. One patient also had peripheral lymphocytosis.

The lentiviral vector used, GeMCRIS 0607-793 (Figure 5), was produced by Lentigen Corp. using a three-plasmid approach. It contained the transgene CD-19-BB- ζ which coded for a second-generation chimeric antigen receptor (Kalos et al.

2011). The receptor was comprised of the single-chain variable fragment (scFv) from the human CD19-specific murine antibody (FMC63), a human CD8α hinge region, a human 4-1BB (CD137) co-stimulatory signaling domain, and a human CD3-ζ signaling domain (June et al. 2011). As previously mentioned, the inclusion of the 4-1BB signaling domain considerably enhanced anti-tumor activity and in vivo persistence of CARs in preclinical trials. This effectiveness was also observed in the clinical trial.

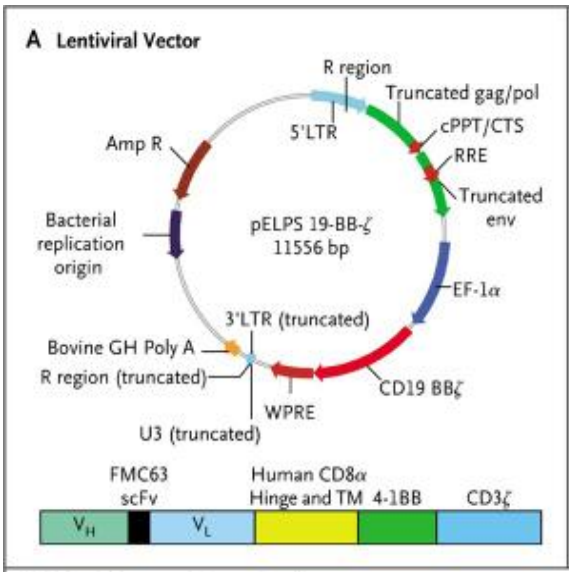

Figure 5: Lentiviral transfer vector construct used in the UP trial. The major functional elements of the transgene (represented by the sectional arrow labeled CD19BB ζ) are outlined in the bar at the bottom of Figure 5. Source: June et al. 2011

The patients' cells were obtained via leukapheresis. Anti-CD3/CD28 mAB-coated paramagnetic beads were used to positively select and activate T cells while remaining leukemic cells were depleted. GeMCRIS 0607-793, the lentiviral vector housing the transgene, was added to the culture and washed out three days later. The transduced cells were then allowed to expand for eight to ten days. Finally, the magnetic beads were removed by passing them through a magnetic field. The CART19 cells were collected, washed, concentrated, and cryopreserved in infusible medium (Kalos et al. 2011).

PATIENTS' RESPONSES

One to four days preceding the infusion of tranduced T-cells, all the patients underwent a round of lymphodepleting chemotherapy. Subsequently, each patient was infused intravenously with the transduced T cells over a three-day period as follows: 10%, 30%, and 60% of the dosage was infused on days one, two, and three, respectively. Patient UPN 03, the focus of this paper, began to have low-grade fevers associated with grade-2 fatigue two weeks after the infusion. Over the next few days, his temperature increased. Other symptoms, such as diarrhea, nausea, anorexia, and diaphoresis, were also observed. On day 22, he was diagnosed with tumor lysis syndrome, a metabolic complication that results when the kidneys are overburdened with a large load of destroyed tumor cells. The patient's uric acid and lactate dehydrogenase levels were above normal at 10.6 mg/dL and 1130 U/L, respectively. A creatinine level of 2.6 mg/dL indicated acute kidney injury. The patient was hospitalized and treated. His uric acid level returned to normal within 24 hours and the creatinine level within three days. He was discharged on the fourth day, and lactate dehydrogenase levels gradually decreased and returned to normal within a month.

On day 23 after infusion of the CART19 cells, CLL was absent from the bone marrow (BM) of UPN 03. By day 28, adenopathy was not palpable, and on day 31, CT scanning showed its resolution. In 198 out of 200 cells examined, FISH testing was negative for the p53 deletion. Flow cytometry indicated B-cell aplasia and no residual CLL. Three months later, CT scanning showed sustained remission. In addition, at

three and six months after infusion, studies of BM indicated no evidence of CLL as well as normal B cells (Figure 6). As of the publication of the study, remission has been sustained for ten months (June et al. 2011).

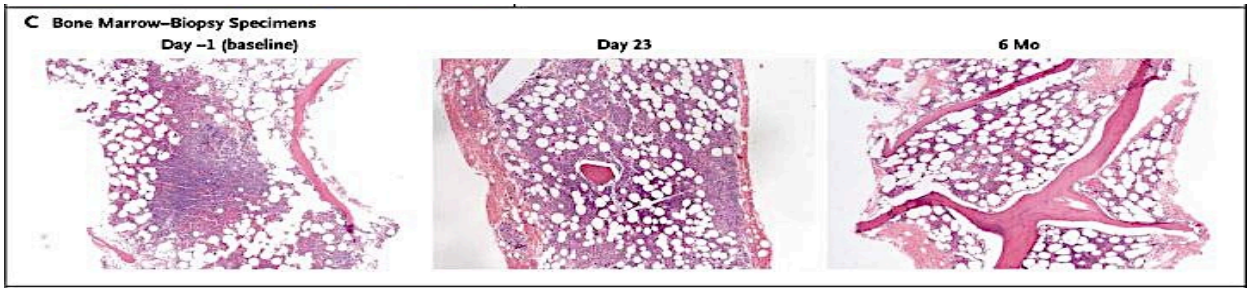

Figure 6: Bone marrow specimens of patient UPN 03. "The baseline specimen shows hypercellular bone marrow (60%) with trilineage hematopoiesis, infiltrated by predominantly interstitial aggregates of small, mature lymphocytes that account for 40% of total cellularity. The specimen obtained on day 23 shows residual lymphoid aggregates (10%) that were negative for chronic lymphoid leukemia (CLL), with a mixture of T cells and CD5-negative B cells. The specimen obtained 6 months after infusion shows trilineage hematopoiesis, without lymphoid aggregates and continued absence of CLL." Source: June et al. 2001

Patient UPN 02 also developed fevers and was hospitalized after CART19 infusion. Adenopathy was reduced somewhat, and p53 deficient CLL cells were eradicated from peripheral blood (PB). However, one month later, his BM was still infiltrated extensively with CLL cells. Thus, he had only sustained partial remission.

Patient UPN 01 also developed symptoms upon infusion. No CLL cells were detected in his blood one and sixth months later. BM studies were performed one, three, and six months after infusion and indicated total absence of CLL cells. Adenopathy was resolved as observed by CT scans one and three months after infusion. He remained in remission for over ten months as of the publication of the study (Kalos et al. 2011).

CART19 Effector-to-CLL Target Cell Ratio

In preclinical trials involving humanized mice, 2.2×10^7 CAR T cells were able to destroy tumors containing 1×10^9 cells (Kalos et al. 2011, as discussed in June et al. 2009). However, these calculations did not take into account *in vivo* expansion of the T cells. In the aforementioned UP trial, the three patients had their tumor loads estimated before infusion of the CAR T cells. This was done by calculating CLL cells in blood, bone marrow, and secondary lymphoid tissue. Patient UPN 03 had an estimated 8.8×10^{11} CLL cells in his bone marrow and 4.4×10^{11} CLL cells in secondary lymphoid tissue, totaling approximately 1.3×10^{12} tumor cells. His infusion contained only 1.4×10^7 CART19 cells. An astounding effector-to-target (E/T) ratio of 1:93,000 resulted in complete elimination of CLL cells. The overwhelming effectiveness of the CART19 cells is most likely due, in part, to their in vivo expansion. The E/T ratios observed in patients UPN 01 and UPN 02 were 1:2200 and 1:1000, respectively (Kalos et al. 2011).

Safety of CART19 Cells

No long-term toxicity, other than B cell aplasia, was observed as a result of CART19 infusion. The patients did, however, develop transient febrile reactions and other short-term symptoms indicative of a serious immune response that coincided with tumor destruction (June et al. 2011).

CYTOKINES

The patients' immune responses were accompanied by sharp increases in cytokines. Peripheral blood and bone marrow samples were analyzed and significant increases in interleukin-6, (IL-6), IL-8, IL-10, and interferon-γ (IFN- γ) were observed in patients UPN 01 and 02. Levels of chemokines, such as CXCL9 and CXCL10, also rose. Cytokine and CART19 cell levels both peaked at the same time, coinciding with the patients' clinical symptoms and eradication of tumor cells.

The chimeric antigen receptor used in this trial contained a 4-1BB signaling domain as opposed to CD28. CARs containing a CD28 signaling domain are associated with increased levels of IL-2 and tumor necrosis factor-α both of which are undesirable. Previous studies have shown that high levels of IL-2 suppress CAR T cells, and TNF-α is associated with cytokine-storm effects. Levels of IL-2 and TNF-α did not rise in any of the patients (Kalos et al. 2011).

IN VIVO EXPANSION, PERSISTANCE, AND BONE MARROW TRAFFICKING

On the first day after infusion, real-time PCR detected expression of the anti-CD19 CARs in patient UPN 03. The doubling time of CART19 cells was approximately 1.2 days. By day 21, a 3-log expansion of the cells was observed. CART19 cells comprised over 20% of circulating lymphocytes at peak levels, coinciding with the elevated levels of serum cytokines and the tumor lysis syndrome. Six months after infusion, CART19 levels were still significantly high, although they decreased by a factor of ten. The elimination half-life of the cells was 31 days. CART19 cells were also detected in the bone marrow beginning on day 23 and remained there for at least six months. Their half-life in the BM was 34 days, slightly longer than those in circulation. Three months after treatment, no CD19 or normal B cells were detected (June et al. 2011). Interestingly, none of the patients had an immune response targeting the CART19 cells even though they contained murine-derived segments. This may be due to the patients' severely compromised immune systems resulting from heavy pretreatment of CLL (Kalos et al. 2011).

LONG-TERM EXPRESSION AND ESTABLISHMENT OF MEMORY

In previous studies that have been conducted, CART cells have not been effective for prolonged periods of time. The long-term success of CART19 cells in the UP trial may be due to improved construction of the CAR. Several months after infusion, the values obtained by PCR for the prevalence of the CAR transgene closely matched those obtained by flow cytometry for the frequency of circulating CART19 cells. Blood and BM samples of UPN 03 that were analyzed by flow cytometry 169 days after infusion indicated the presence of CART19 cells and complete absence of B cells. In all three patients, PCR indicated that CART19 cells persisted for at least four months. At 71 days after infusion, 5.7% of the T cells in the blood of patient UPN 01 expressed CARs, and on day 286, 1.7% expressed CARs. Although small, these percentages indicate long-term expression of a CART19 population, possibly indicating long-term immunity too.

Polychromatic flow cytometry was also performed to study the function and phenotype of CART19 cells in patient UPN 03. On day 56, CART19 CD8+ cells expressed an effector memory phenotype which is normally stimulated by prolonged

exposure to an antigen. By day 169, some of the CART19 CD4+ cells expressed a central memory phenotype, as indicated by CCR7 and CD127 expression, both of which are associated with memory T cells. B cell progenitors in the BM could ensure that CART19 cells maintain a memory population, thereby providing long-term immunity to CLL with the use of CART19 cells (June et al. 2011).

CONCLUSION

Decades of research in genetics, immunology, and molecular biology have culminated in the ability of medical scientists to treat patients with CLL with autologous T cells. In the UP trial of Dr. Carl June and his colleagues, two out of three patients enrolled in the study have reached total remission, and one achieved partial remission. The engineering of chimeric antigen receptors, the ability to use lentiviral vectors to transduce T cells to express them, and the *in vivo* expansion and persistence of these CAR cells all represent monumental breakthroughs that will, hopefully, be transposed to other areas of medicine as well.

REFERENCES

Bridgeman JS, Hawkins RE, Hombach AA, Abken E, Gilham DE. 2010. Building better chimeric antigen receptors for adoptive T cell therapy. Current Gene Therapy 10:77-90.

Case SS, Price MA, Jordan CT, Yu XJ, Wang L, Bauer G, Haas DL, Xu D, Stripecke R, Naldini L, Kohn DB, Crooks GM. 1999. Stable transduction of quiescent CD34(+)CD38(-) human hematopoietic cells by HIV-1-based lentiviral vectors. Proceedings of the National Academy of Sciences 96:2988-2993.

Chan DC, Fass D, Berger JM, Kim PS. 1997. Core structure of gp41 from the HIV envelope glycoprotein. Cell 89:263-273.

Coleman JE, Huentelman MJ, Kasparov SY, Metcalfe BL, Paton JFR, Katovich MJ, Semple-Rowlan SL, Raizada MK. 2003. Efficient large-scale production and concentration of HIV-1-based lentiviral vectors for use in vivo. Physiological Genomics 12:221-228.

Durand S, Cimarelli A. 2011. The inside out of lentiviral vectors. Viruses 3(2):132-159.

Eshhar Z, Waks T, Dendavid A, Schindler DG. 2001. Functional expression of chimeric receptor genes in human T cells. Journal of Immunological Methods 248:67-76.

Eshhar Z, Waks T, Gross G. 1989. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. Proceeding of the National Academy of Sciences, USA 86:10024-10028.

Henderson C. Retrieved December 27, 2011 from: http://en.wikipedia.org/wiki/File:HIV_Virion-en.png.

June CH, Porter DL, Levine BL, Kalos M, Bagg A. 2011. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. New England Journal of Medicine 365(8):725-733.

Kalos M, Levine BL, Porter DL, Katz S, Grupp SA, Bagg A, June CH. 2011. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. Science Translational Medicine 3(95):1-11.

Kuiken C, Foley B, Marx P, Wolinsky S, Leitner T, Hahn B, McCutchen F, Korber B. 2008. HIV Sequence Compendium 2008. Retrieved December 27, 2011 from: http://www.hiv.lanl.gov/content/sequence/HIV/COMPENDIUM/2008/frontmatter.pdf.

Levine BL, Humeau LM, Boyer J, MacGregor RR, Rebello T, Lu X, Binder GK, Slepushkin V, Lemiale F, Mascola JR, Bushman FD, Dropulic B, June CH. 2006. Gene transfer in humans using a conditionally replicating lentiviral vector. Proceedings of the National Academy of Sciences 103:173723-17377.

Lu X, Humeua L, Slepushkin V, Binder G, Yu Q, Slepushkina T, Chen Z, Merling R, Davis B, Chang YN, Dropulic B. 2004. Safe Safe two-plasmid production for the first clinical lentivirus vector that achieves >99% transduction in primary cells using a one-step protocol. Journal of Gene Medicine 6:963-973.

Miyoshi H, Takahashi M, Gage FH, Verma IM. 1997. Stable and efficient gene transfer into the retina using an HIV-based lentiviral vector. Proceedings of the National Academy of Sciences 94:10319-10323.

Schroers R, Sinha I, Segall H, Schmidt-Wolf IGH, Rooney CM, Brenner MK, Sutton RE, Chen SY. 2000. Transduction of human PBMC-derived dendritic cells and macrophages by an HIV-1-based lentiviral vector system. Molecular Therapy 1(2):171-179.

Tortora GJ, Derrickson B. 2009. Principles of Anatomy and Physiology. 850-854.

Urba WJ, Longo DL. 2011. Redirecting T cells. New England Journal of Medicine 365(8):754-757.

Vigna E, Naldini L. 2000. Lentiviral vectors: excellent tools for experimental gene transfer and promising candidates for gene therapy. Journal of Gene Medicine 2(5):308-316.

Watts JM, Kristen KD, Gorelick RJ, Leonard CW, Bess JWJ, Swanstrom R, Burch C, Weeks KM. 2009. Architecture and secondary structure of an entire HIV-1 RNA genome. Nature 460:711-716.

Zufferey R, Dull T, Mandel RJ, Bukovsky A, Quiroz D, Naldini L, Trono D. 1998. Self-inactivating lentivirus vector for safe and efficient in vivo gene delivery. Journal of Virology 72(12):9873-9880.