Gene-Modified Cells for Stem Cell Transplantation and Cancer Therapy

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Introduction

AS RESEARCHERS, WE LIKE TO BELIEVE that our training and the investigative paths we elect to follow are dictated by logical and conscious decisions. In reality, our scientific destiny is a consequence of both our will and our fate. A successful research career depends on accepting and balancing this interplay of internal and external controls. Unfortunately, scientific training has become increasingly prolonged and the flexibility to move easily between disciplines ever more circumscribed, making it even harder to strike this balance (Alberts *et al.*, 2014). In hopes of encouraging perseverance with this career strategy, I present my personal “retrospective analysis,” which describes my own—intermittently successful—efforts to combine firmness of purpose with flexibility in response to opportunities.

The Early Years

After completing my medical qualification and postgraduate training in 1978, I returned to Cambridge in 1978 to study for a PhD in immunology. The 1970s were a pivotal time for immunology—the first monoclonal antibodies had been created in Cambridge by Kohler and Milstein (1975), while Doherty and Zinkernagel had discovered the phenomenon of major histocompatibility complex (MHC) restriction, by which T cells recognized their antigens on target cells (Zinkernagel and Doherty, 1974). Investigators were rapidly generating monoclonal antibodies to targets on human cells (Kung *et al.*, 1979), and it was anticipated that the molecular analysis of the T-cell receptor would yield similar advances in our ability to manipulate this arm of the immune system (Minden and Mak, 1986). My own research reflected these advances, as it focused on the mechanisms by which B cells interacted with T cells to produce antibody, while in an adjacent laboratory another graduate student, Cliona Rooney, was exploring how T cells interacted with and killed virus-infected targets. To paraphrase Oscar Wilde, “Life imitates science,” and our study of cellular interactions was soon mirrored by our own social interactivity.

After receiving my PhD in 1981, I was faced with the dilemma of all physician scientists: how does one succeed in

two separate fields, science and medicine, both of which require full-time commitment to attain true excellence. I first tried to resolve this dilemma by choosing an area in which the laboratory research was on patient material and the clinical practice was limited in extent and strongly influenced by the progress in the laboratory. Acquired immunodeficiency met those requirements, since at that time most patients with this disorder had common variable hypogammaglobulinemia. The UK Medical Research Council’s Clinical Research Center had opened a division of clinicians and investigators (lead by the late Geoffrey Asherson and by David Webster) that was focusing on this relatively uncommon disease, and I became a member of that group. Within 2 years, of course, the world of acquired immunodeficiency had been overturned forever by the identification of AIDS.

When AIDS was first described, its cause was unknown. But the epidemiology and rate of spread made two things clear: AIDS would soon come to dominate all other forms of acquired immunodeficiency, and its origin was likely to be a transmissible agent, ultimately amenable to intervention with antimicrobial or antiviral agents. Since my interest had primarily been in the development of cellular therapies for immune disorders, I decided to change fields. Bone marrow transplantation (now called hemopoietic stem cell transplantation or HSCT), seemed especially attractive, as its major associated problems are immunological—graft rejection, graft versus host disease (GVHD), and severe infections caused by persistent posttransplant immunodeficiency. In the United Kingdom, as in the United States, HSCT was almost exclusively the province of trained hematologists/oncologists. Since my own background was in immunology and general medicine, I was not considered to be suitably trained or adequately experienced, resulting in my rejection by all of the transplant centers I initially contacted. Fortunately, a senior-level training position had opened jointly at the Royal Free Hospital and The Hospital for Sick Children, London. Although many on the selection committee opposed my appointment given my lack of background in the field, the will of A. Victor Hoffbrand, the department chair, won out, and after a crash course in the basics of hematology, I was dispatched as a senior registrar (junior attending physician)

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to the Hospital for Sick Children, London, a leading pediatric hospital in the United Kingdom.

A Niche Opens and Is Occupied

It took only a few months as a pediatric hematologist for me to realize my inadequacies in that field and to consider a return to the safe harbor of immunodeficiency. Unexpectedly, however, a New Zealand-trained physician at the Royal Free Hospital who was serving as their HSCT medical co-coordinator was offered a position back in New Zealand, and I applied for and was given her position, to my own and my pediatric colleagues' relief. This new position proved to be the ideal blend of clinical and research activity. The clinical side was pure stem cell transplantation, without the encumbrance of hematology and oncology, enabling me to work with H. Grant Prentice and George Janossy, on T-lymphocyte depletion to reduce GVHD (Prentice *et al.*, 1984; Wimperis *et al.*, 1986). In the lab we were joined by a "replacement" New Zealander, Helen Heslop, and together we developed and then clinically tested an approach to enhance immune recovery after T-cell-depleted stem cell transplantation by immunizing both donor and recipient pretransplant (Heslop *et al.*, 1988; Wimperis *et al.*, 1990). This work was the forerunner of our later efforts to improve antiviral and antitumor immunity by adoptive transfer of T cells.

In the meantime (1984–1989), Clio Rooney had moved to Bristol, then Birmingham, and then Yale Universities for postdoctoral fellowships on the immunobiology of Epstein Barr virus (EBV), discovering the target antigens for T cell killing and investigating their effects on transformed B cells. By the time she returned to a position at the Ludwig Institute in London, I had become fascinated by the potential linkage between HSCT and gene therapy. In principle, if a therapeutic transgene could be integrated into even a single hemopoietic stem cell (HSC), then transplantation of this cell could repopulate all blood lineages for the lifetime of the patient, offering the chance to permanently cure a substantial range of monogenic disorders. There were, however, significant obstacles to putting this concept into practice. In the late 1980s, the United Kingdom lacked a regulatory environment to allow gene therapy of human subjects. Although there was no legislation that expressly forbid it, the absence of a procedural framework for approvability for work with such significant legal, ethical, and medical implications meant that only a much bolder and better established investigator than myself would have been able to implement the approach.

I have never enjoyed being subjected to excessive regulation, and the increasing sclerosis of many aspects of our professional lives is certainly a disservice to our patients, but clearly any new scientific and medical strategy as potentially controversial as gene therapy needed to be discussed and agreed upon by all stakeholders before it could begin. Fortunately, the scientific leadership in the United States had thought ahead on this issue, to the extent that the NIH and FDA had set up a complementary system for public and private evaluation of human gene therapy, respectively, the Human Gene Therapy Subcommittee of the Recombinant DNA Advisory Committee and the FDA Center for Biologics Evaluation and Research (CBER).

To take advantage of the regulatory environment, Clio and I began to seek positions in the United States. My initial choice of U.S. institution did not work out, but Clio was offered a position in the Virology Department of St. Jude Children's Research Hospital. I then met with the institutional director, Joe Simone, who agreed with some trepidation to employ me as the "ornamental" partner and to let me restart the institution's dormant stem cell transplant program. Helen also moved to St. Jude, initially working as a postdoctoral fellow with James Ihle. Although this move provided me with a regulatory pathway to approval of a clinical gene transfer protocol, it did not affect the second major obstacle to successful stem cell gene therapy—the lack of efficient vectors.

Gene Transfer: From Half Empty to Half Full

James Wilson and Marina Cavazzana have already described the limitations of retroviral vectors in the early 1990s (Cavazzana, 2014; Wilson, 2014), in particular their poor transduction of HSCs, most of which are non-proliferating. This limitation severely constrained efforts to correct severe monogenic disorders of hemopoietic cells and their progeny. Thus, I looked instead for an application in which even a limited degree of gene transfer could be informative.

When I arrived at St. Jude, there was a considerable interest in improving the outcome of pediatric acute myeloblastic leukemia (AML). Although this is a much less frequent form of childhood leukemia than the acute lymphoblastic variant (ALL), the toxicity of treatment was great and the cure rate lower than for ALL. One option was to treat the patients with chemotherapy until they were in remission, remove a portion of their stem cells, and then give a supralethal dose of chemotherapy and radiation to eradicate any residual cancer cells, before rescuing the patients from the effects of this treatment by autologous (Auto) HSCT using the harvested stem cells. The obvious concern was that the infused stem cells would be contaminated with residual malignant cells and would simply reintroduce the leukemia. Investigators tried to prevent this by purging the marrow with various chemicals and toxins, but these efforts inevitably reduced the viability of the normal stem cells, delaying engraftment. Since nobody knew whether malignant cells capable of reintroducing leukemia were actually present in the harvested stem cells, there was considerable debate in the field about whether autologous stem cell transplant could be of value for AML, and if benefit or harm would be produced by purging the HSCs before the procedure.

If we could genetically mark the harvested autologous stem cells before infusion, and look at the characteristics of the relapse in patients who were unfortunate enough to have a disease recurrence, then the presence of the marker gene in the malignant cells would mean that the autograft had indeed reintroduced cancer and had indeed been infused with the transplant and that we needed to develop effective purging techniques if autografting for AML was to have optimal benefit. Importantly, even marking a tiny percentage of any infused malignant cells would have the chance of providing a positive result at relapse, since the malignant clones undergo such massive expansion when disease recurs.

After a year or more of flying back and forth to Washington to meet with federal regulators, and with considerable help from W. French Anderson and the scientists and executives of Genetic Therapy, Inc., we received approval for the study in 1992. Using a gamma retroviral vector that encodes the neomycin phosphotransferase (*neo*) gene, we transduced only a portion of the infused stem cells in case the transduction protocol itself damaged the ability of the normal stem cells to engraft. Two of the treated patients relapsed, one of whom had malignant cells containing both a unique malignancy marker (*AML/ETO* fusion) and the *neo* marker malignant transgene. The relapse cells of the remaining patient were also *neo* positive, but we could not definitively say that these *neo*-positive cells were truly the malignant blasts as there was no coexisting genetic marker for the malignant clone (Brenner *et al.*, 1993). The results thus confirmed that the harvested marrow could indeed contribute to relapse, and that effective purging might be beneficial to outcome. More importantly, however, this was the first gene transfer study to be conducted outside of NIH and the first anywhere to use HSCs as the target of transduction, and it provided useful safety and feasibility data to the field.

Changing Outcomes of Transplantation

We were also interested in using gene marking to improve the treatment of allogeneic HSCT, in which a partially or fully MHC-matched donor is the source of cells. While these allogeneic stem cells lack contamination with malignancy, they are alloreactive and may cause severe, sometimes lethal, GVHD. As I described above, removal of T lymphocytes from the donor graft could prevent GVHD but left the recipient vulnerable to a range of infections and can increase the risk of relapse. As we began to extend the degree of acceptable HLA mismatching for an allograft (in order to increase the probability of finding a suitable donor), we ran into more and more problems with viral disease. Of these, EBV-induced lymphoproliferative disease was the most intractable. EBV causes a mild illness in most people with normal immune systems but becomes latent for the lifespan of the individual. Occasionally, the virus can cause malignant transformation in the lymphocytes (resulting in Hodgkin or non-Hodgkin lymphomas) or epithelial cells (most commonly producing nasopharyngeal carcinoma [NPC]) of the host. But in patients who are severely immunocompromised after stem cell transplantation, uncontrolled viral replication in B lymphocytes causes aggressive proliferation progressing to fatal immunoblastic lymphoma (Gottschalk *et al.*, 2005). In 1992 there was no effective treatment for this disorder, which was fatal in 7–20% of recipients of T-cell-depleted stem cell transplants, and was a serious problem in the St. Jude program (Hongeng *et al.*, 1997).

Helen and I had previously worked on adoptive transfer following T-cell-depleted stem cell transplantation in the United Kingdom, and given Clio's extensive expertise in the analysis of cellular immunity to EBV, we developed an approach to generate EBV-specific T cells from the donor by culturing them with the donors' own EBV-transformed B cells. Almost all immunoblastic lymphomas after HSCT are of donor origin and express the same range of antigens as EBV-transformed normal B cells (termed type III latency).

Our approach gave us donor T cells that were specific for the posttransplant EBV-induced lymphomas. Moreover, because these donors were now directed to viral antigens, we hoped that they would lack alloreactivity and so would be unable to cause GVHD.

Destined to Study Fate

Cells have no will, but they certainly have a fate. To discover what happened to the EBV-specific T cells when they were infused, we used our retroviral marking vector to transduce them before administration. The results were striking (Rooney *et al.*, 1995; Heslop *et al.*, 1996). On the basis of the semiquantitative PCRs then available, we found that there was an estimated 10,000-fold expansion of the cells *in vivo* (Rooney *et al.*, 1998) within 7–10 days of administration, and biopsies confirmed trafficking to tumor sites. In 11 of 13 patients, there was permanent eradication of advanced tumors, while among 100+ patients who received the cells at an earlier stage of EBV reactivation or as disease prophylaxis, none developed EBV lymphoma (Heslop *et al.*, 2010). In contrast to reports of toxicities from chimeric antigen receptor (CAR)-expressing T cells, we saw no evidence of any cytokine storms, even in patients with extensive disease at the time of treatment, although we did observe local inflammation at some disease sites (Rooney *et al.*, 1998; Heslop *et al.*, 2010). Prolonged follow-up showed that these gene-marked EBV-specific cytotoxic T lymphocytes persisted in peripheral blood for up to 10 years or more, and that when the patients reactivated EBV at any time, the gene-marked T-cell population expanded and controlled EBV reactivation and prevented EBV disease (Heslop *et al.*, 2010).

These results were extremely encouraging and helped to establish the “ground rules” for effective T-cell treatment of malignant diseases. Antigen-specific T cells undergo their greatest expansion and persistence and are likely to have their greatest benefit when the following conditions are met. The T cells should be infused into an environment that favors lymphocyte proliferation, for example, after stem cell transplantation when lymphocytic homeostasis is active; they should recognize and encounter strong and unique antigens presented on potent antigen-presenting cells so that they receive the necessary receptor stimulation and cellular costimulation; and the T cells should contain or be able to form a memory population that can persist long-term and re-expand should infection or malignancy resurge.

Houston Beckons

In 1998, Clio, Helen, and I were all recruited to Houston by Ralph Feigin, president of Baylor College of Medicine (BCM), and we moved to start the Center for Cell and Gene Therapy at BCM, Texas Children's Hospital, and The Methodist Hospital (now Houston Methodist Hospital). This move increased the clinical translational resources at our disposal and gave us the opportunity to extend our work in genetically modified cell therapy.

An early priority was to extend our studies on EBV-associated malignancies to immunocompetent individuals, who may develop different types of EBV-associated lymphomas and epithelial tumors, including Hodgkin or non-Hodgkin lymphomas or nasopharyngeal carcinomas. These

cancers are associated with the expression of a more restricted array of EBV-associated antigens (type 2 latency), including LMP1 and LMP2, and are less immunostimulatory than type 3 latency tumors. We reasoned that these malignancies might nonetheless respond to reinfusion of T cells if the EBV-specific T cells were expanded *ex vivo* away from the local immunosuppressive effects of the tumors and were enriched for effector cells directed to type 2 latency-associated antigens. In a series of studies, we showed that even in the absence of posttransplant lymphodepletion, EBV-specific T cells could expand (although only modestly) and produce complete and generally sustained tumor responses in approximately 50% of patients with relapsed Hodgkin or non-Hodgkin lymphoma (Bollard *et al.*, 2004, 2007, 2014). The success rate in NPC was significantly lower (Straathof *et al.*, 2005a; Louis *et al.*, 2009, 2010), but evidence from independent phase I and phase II studies at several centers suggested that these T cells may nonetheless be beneficial (Heslop, 2014; Chia *et al.*, in press).

Because of the promising results we had obtained with EBV-specific and other virus-directed cytotoxic T cells (Leen *et al.*, 2006), we wondered whether this strategy could be used as a platform for CARs, and thus potentially could be directed to a broad array of malignancies. Although described over 20 years ago (Eshhar *et al.*, 1993; Hwu *et al.*, 1993), initial clinical studies of CAR-modified T cells had been disappointing, with limited *in vitro* persistence and expansion and little clinical evidence for antitumor activity (Kershaw *et al.*, 2006). It had become clear that providing a single stimulatory signal through a CAR was insufficient to induce full T-cell activation and proliferation. T cells instead rely on a large number of “checkpoint” signals that provide costimulation and up- or downregulation of the initial signals received during receptor engagement. Fortunately, it was soon found that incorporation of such costimulatory signals in the CAR greatly augmented the T-cell activation and proliferation *in vivo* (Maher *et al.*, 2002), leading to dramatic tumor responses in patients with B-cell malignancies who have received T cells with a CD19-directed CAR that also incorporates a costimulatory signaling domain (Kalos *et al.*, 2011; Brentjens *et al.*, 2013).

Under physiological conditions, T-cell activation, regulation, costimulation, and inhibition occur in a choreographed temporospatial sequence. It is difficult to safely replicate this sequence by using a transgene in which one or more costimulatory intracellular domains are constitutively activated whenever the receptor is engaged. When B cells are the targets of attack (e.g., by a CD19-specific CAR-modified T cell), this limitation of transgenic signaling *in cis* may be of little importance, since normal and some malignant B cells express a broad array of costimulatory molecules. They may thereby provide the necessary signals, in the correct sequence, *trans*-complementing the *cis* costimulation from the CAR. Most solid tumors are less obliging, so that a CAR directed to an antigen on these cells will not bring additional costimulation once the target cell is engaged.

From our experience with EBV-specific T cells, we raised the question whether we could provide an alternative means by which CAR-modified T cells could obtain the required physiological costimulation. The idea was to use the signaling that occurs when the native receptor of a

virus-specific T cell (VST) engages a professional antigen-presenting cell that is expressing viral antigens together with the required costimulatory molecules. This encounter should result in a fully activated/licensed CAR-expressing VST that could then recognize and kill tumor cells through its chimeric receptor (Fig. 1) (Rossig *et al.*, 2002). Such doubly specific T cells could be further stimulated by administration of a viral vaccine containing the antigens to which the T cell was specific (Melcher *et al.*, 2011) or even by an oncolytic virus.

Although the above approach holds clinical promise (Pule *et al.*, 2008; Louis *et al.*, 2011; Cruz *et al.*, 2013), it has—like most cancer immunotherapy strategies for solid tumors—proved to be less straightforward than hoped. It is clear that solid tumors deploy a broad array of active and passive immune defenses that can defeat even a well-targeted antitumor response from a highly activated effector cell. Our research is now focused on developing countermeasures to the tumor inhibitory environment, for example, by making the T cells resistant to the inhibitory cytokine TGF- β (Bollard *et al.*, 2002) or by generating chimeric cytokine receptors that can convert a cytokine signal that would normally favor development of noncytotoxic T cells into one that enhances the growth of oncolytic effector cells (Leen *et al.*, 2014). We are also developing approaches by which a single T cell can target multiple tumor antigens simultaneously to reduce the risk of tumor immune escape (Gerdemann *et al.*, 2011; Hegde *et al.*, 2013). Once combined, these approaches should substantially increase the effectiveness of genetically modified T-cell therapy for cancer, and the combination of such engineered T cells with available checkpoint inhibitor antibodies should allow the adoptively transferred T cells to reach their maximum expansion and activity without the constraints of physiological or tumor-derived checkpoint signals to limit their growth and persistence.

Looking further ahead, it is likely that the introduction of Boolean logic into T cells will enable them to recognize complex patterns of antigenic and environmental cues and thus discriminate between the identical antigen expressed on both a normal cell and a tumor.

FIG. 1. T cells expressing a native receptor for a viral antigen and a chimeric receptor for a tumor-associated antigen will receive stimulation through their native receptor and costimulation from viral antigen-expressing antigen-presenting cells. These expanded and activated T cells may then be able to destroy tumor cells recognized through their chimeric antigen receptor.

Controlling Cell Fate

All of us are aware that gene and cell therapies have been held to very high standards of safety (Wilson, 2014). Unlike small molecules for which adverse effects generally diminish if the drug is withdrawn, complications from gene and cell therapy can persist long-term and even worsen if the (gene-modified) cells expand. A classic example of this phenomenon is GVHD after allogeneic stem cell transplantation, in which the damage to skin, gut, or liver both persists and often intensifies over time. More recently, investigators have reported substantial toxicities from infusions of cells modified to express transgenic native or chimeric receptors. Some of these toxicities are because of intense activation of the T cells and innate immune system (systemic inflammatory response syndrome or cytokine storm), while others are caused by (cross) reactivity of the infused cells with normal host tissues (Linette *et al.*, 2013; Morgan *et al.*, 2013; Davila *et al.*, 2014; Papadopoulos *et al.*, 2014). As we engineer T cells for higher potencies, concerns over immediate or delayed toxicities will undoubtedly be an increasing drag on development and implementation, and it will be crucial for the field to develop means by which we can obtain effective and fast control over the transferred cells.

About 15 years ago, David Spencer and colleagues developed a dimerizable component of the intrinsic apoptosis pathway called inducible caspase 9 (iC9) (Fan *et al.*, 1999). Clio became interested in the possibilities of modifying this molecule so that it could be used to kill the T cells we were giving to patients should the need arise, and after considerable effort, an approach was developed that seemed acceptable for use in the clinic (Straathof *et al.*, 2005b). Indeed our first study of this approach after stem cell transplantation proved highly successful (Di Stasi *et al.*, 2011; Zhou *et al.*, 2014). In that trial, the goal was to correct the profound immunodeficiency that occurs after HLA haploidentical, T-cell-depleted HSCT by administering increasing numbers of donor T cells to the recipients after their procedure. To prevent these T cells from causing GVHD, we ensured that they also contained the *iC9* gene, the product of which could be activated by dimerization with an otherwise bioinert small molecule. The engineered T cells engrafted well and provided antiviral activity, and in the three patients in whom they also caused GVHD, the complication could be rapidly and permanently reversed by a single dose of the dimerizing drug (Di Stasi *et al.*, 2011). Importantly, 90% of the iC9-expressing T cells were killed within 30 min of dimerizer infusion, and 99% by 24 hr (Di Stasi *et al.*, 2011), suggesting that prompt administration of the dimerizing drug would be able to speedily reverse even the acute toxicities of other T-cell therapies. It is likely that this and several other safety or suicide strategies now in clinical development (Ciceri *et al.*, 2009) will make a significant contribution to the implementation of effective cell-based therapies in the next decade.

Conclusions

Regulatory, cost, and safety concerns mean that all medical progress is slower than one would hope, and the idiosyncrasies of drug development associated with individualized and complex biological therapies such as gene-modified T cells mean that the way forward is even more

tortuous than usual. Nonetheless, I hope that my account has made it clear that this path to successful cell therapies is now at least visible, and that by combining equal measures of flexibility and fortitude, we can ensure that these therapies ultimately produce the benefits for which we all hope.

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Author Disclosure Statement

MKB has stock or stock options in Bluebird Bio, Adcyte, and FF Canvac which are cell and gene therapy companies. MKB is an advisor to Cellectis and his spouse is an advisor to Cell Medica. These are also cell and gene therapy companies.

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