The CAR T Cell Story

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This article was received on August 29, 2019.
This article was published on September 10, 2019.
DOI: 10.36000/hbT.OH.2019.01.001
ISSN: 2673-2092 (Print) and 2673-2106 (Online)

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During the entire 20th century, chemotherapy and irradiation were the mainstay of non-surgical cancer treatment. In the past 20 years, however, cancer immunotherapy has been revolutionizing the field of oncology with ever-increasing pace. During this time, various molecular pathways have successfully been exploited to re-direct the immune system to fight cancer. This shift of treatment paradigms was acknowledged by the Nobel committee, which awarded the 2018 Nobel Prize in Physiology or Medicine to James Allison and Tasuku Honjo for their discoveries leading to the development of checkpoint inhibitors.[1] A different approach, however, has gained widespread attention inside as well as outside the medical community: the regulatory approval of chimeric antigen receptor (CAR) T cells for the treatment of leukemia and lymphoma in 2017. While the adoptive transfer of genetically engineered human cells in clinical routine has been limited to individual indications, the unprecedented efficacy of genetically modified T cells in refractory patients was inconceivable a few years ago. The groundwork for the clinical implementation of CAR T cells was laid in the past three decades in terms of progress that was made in the fields of immunology as well as in cellular and gene therapy. Here, we provide a brief overview of fundamental discoveries, from basic to translational research, that ultimately led to the clinical approval of CAR T cells for the treatment of cancer patients.

THE EARLY DAYS OF IMMUNOTHERAPY

The first documented case of immunotherapy was performed by Dr William Coley, a bone surgeon and oncologist, in the 1890s in New York. He documented a correlation between accidental infections and spontaneous tumor regressions in cancer patients.[2] Based on these retrospective observations, he successfully treated a 35-year old man with a terminal case of neck cancer with repeated intra-tumoral injections of live bacteria. Although, at that time, the function of the immune system was barely recognized, experts today agree that the effect induced by Dr Coley should be considered as an early form of immunotherapy. Despite these promising results, immunotherapeutic approaches were overtaken by developments in radio- and chemotherapy in the first half of the 20th century. The ground-breaking work of Dr Old, who sought to treat cancer by improving the body's natural defenses against it, was therefore widely considered odd. Dr Old successfully performed intravesical injections of Bacillus Calmette-Guérin (BCG), a live vaccine against tuberculosis, in patients with bladder cancer. This is considered to be the first direct demonstration that the immune system can be used against cancer, and today, BCG is approved for the treatment of bladder cancer.[3,4]

T CELLS RECOGNIZE AND ATTACK CANCER

Starting from the 1950s, many of the fundamental immunological mechanisms were discovered, which have become textbook knowledge today. For instance, the thymus was recognized as an immunological organ in which T cells develop.[5] The identification of protective immunity conveyed by lymphocytes against sarcoma cells in an experimental setting in 1960 added an unexpected aspect to the repertoire of T cell functions.[6] In human patients, T cells were observed to accumulate in cancer tissue, further indicating a surveillance function of lymphocytes against malignantly transformed host cells. In the 1980s, Dr Steven Rosenberg from the National Institute of Health (NIH) pioneered the field of adoptive T cell transfer as he extracted tumor-infiltrating lymphocytes (TILs) from resected melanomas, expanded them *in vitro* and achieved durable remissions upon re-administration into patients (**Figure 1**).[7,8] Despite the curative potential of adoptively transferred TILs,[8] the complex manufacturing process as well as the limited efficacy prevented a widespread clinical implementation of this technic. Functionally, TIL-mediated recognition of antigens relies on an endogenous T cell receptor, which exhibits moderate affinity to cancer antigens and requires the presence of major histocompatibility complex (MHC) molecules.[9] Much later, tumor immune evasion mechanisms, such as the down-regulation of MHC molecules or the up-regulation of negative immune regulators on tumor cells were demonstrated to critically limit the anti-tumor functions of unmodified T-cells.[10]

With the knowledge gained from studying the human immunodeficiency virus (HIV), replication-defective retroviruses were developed as a novel method for gene transfer by the late 1980s. With this technology, foreign genes could be stably introduced into eukaryotic cells for the first time.[11,12] Additionally, with the development of polymerase-chain reaction (PCR), DNA sequencing and molecular cloning techniques, the generation of artificial/synthetic genes became possible. With these new possibilities, the idea of combining the specificity of antibodies with the effector strength of T cells, using an intentionally designed and synthetically produced chimeric gene, was born. Kuwana et al., envisioned that the expression of an antibody-like molecule on the surface of T cells could be used to re-direct them against target cells in a non-MHC restricted manner.[13] The design was subsequently refined by Eshhar et al.[14] Albeit major differences existed in the design of the artificial gene in these pioneering works in comparison to modern CAR T cells, Eshhar's modified cells were able to kill antigen-expressing target cells *in vitro* and thus provided evidence for the feasibility of targeted redirection of T cells for the first time. Notably, these cells did not contain an intrinsic activation domain and depended on integration of the CAR into the endogenous TCR complex for signaling. In 1991, three separate reports introduced the intracellular CD3ζ chain as the activating domain in different chimeric constructs, which facilitated the CAR design and drastically increased the activation potential.[15–17]

HOW DOES CAR T CELL THERAPY WORK?

Patient-derived T cells are collected by leukapheresis — a process used to separate white blood cells from whole blood. Subsequently, T cells are activated *in vitro* by stimulation with antibody-coated beads that act as "artificial dendritic cells" (**Figure 2**). Activated T cells are then infected with retroviral vectors, which stably integrate the CAR gene into the T cell genome. CAR expressing T cells are further expanded *in vitro* by stimulation with recombinant growth factors (e.g. Interleukin-2) and transfused back into the patient. To prevent rejection of CAR T cells by the recipient's immune system, mild chemotherapy ("lymphodepletion") is administered prior to CAR T cell infusion.

FROM BENCH TO BEDSIDE

The current design of CARs is derived from these reports and consists of three different modules: an extracellular antigen-binding domain (1) that is linked via a stalk and transmembrane part (2) to an intracellular signaling domain (3). In all clinically relevant CAR constructs, the extracellular antigen-binding domain is formed by an antibody-like molecule, the so-called single-chain fragment variable (scFv). The design of the transmembrane domain differs and the length of the stalk must be optimized empirically for the respective antigen of choice. The diverging biological properties of different CAR T cells mainly result from the composition of the intracellular signaling domain. A common feature of all CAR T cell constructs is the incorporation of a CD3ζ stimulation domain, which mediates signal transduction from the T cell receptor (TCR) to the intracellular compartment in normal T cells.[18] CAR T cells which only incorporated a CD3ζ chain in their signaling domain (first-generation CARs), demonstrated anti-cancer activity *in vitro* but failed to persist and to induce long-term remissions in clinical trials despite exogenous application of Interleukin-2.[19]

To overcome these limitations, CARs have been gradually refined. Importantly, the addition of co-stimulatory moieties in the signaling domain resulted in more robust and persistent CAR T cell activation. In fact, different co-stimulatory domains were introduced that imitate the activation mechanism of normal T cells, which is generally considered a three-step process. The first signal is the ligation of the TCR to an antigen-MHC complex on the surface of antigen-presenting cells. The second signal is mediated by ligation of a co-stimulatory molecule such as CD28 or CD137 (4-1BB) to their cognate ligands. These signals induce the synthesis of cytokines such as interleukin (IL)-2, which are essential for T cell differentiation and survival (signal three).

In seminal studies, Dr Michel Sadelain, Dr Hinrich Abken, Dr Weir, and Dr Roberts independently designed second-generation CAR T cells which incorporated a co-stimulatory CD28 chain in the signaling domain.[20,21] These cells conferred proliferative advantage over first generation CAR T cells as well as an increased release of immunostimulatory cytokines, while maintaining their cytolytic potential. Increased biological potency of anti-CD19 second-generation CD28-CD3ζ CAR T cells compared to their first-generation counterparts became evident in pre-clinical models of acute lymphoblastic leukemia (ALL).[22] With the potential of expansion in vivo, the foundation for the use of CAR T cells as "living drugs" was laid. Since then, several other co-stimulatory signals have been integrated into CARs including CD134/OX40, CD137/4-1BB and CD27.[18] As mentioned before, the biological properties (i.e. cytokine release, T cell expansion and persistence) relate from the respective co-stimulatory domain. Finally, multiple co-stimulatory domains

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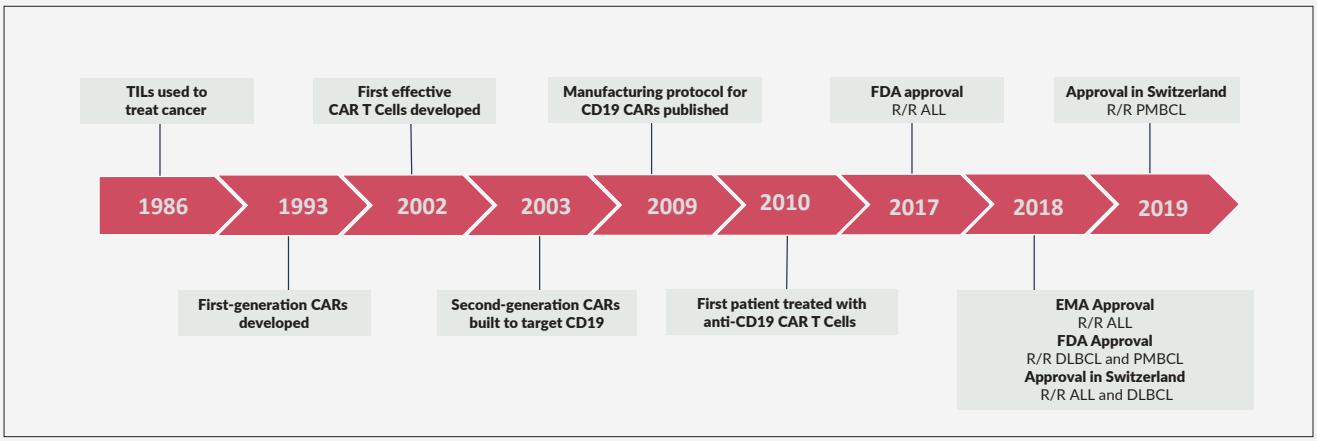

Figure 1. Timeline depicting key events leading to the establishment of CAR T cells as a novel class of therapeutics. ALL, acute lymphoblastic leukemia; CAR, chimeric antigen receptor; DLBCL, diffuse large B-cell lymphoma; EMA, European Medicines Agency; FDA, Food and Drug Administration; PMBCL, primary mediastinal large B-cell lymphoma; R/R, relapsed/refractory; TILs, tumor-infiltrating lymphocytes.

have been combined into a single construct (third-generation CARs). However, a clinical advantage of these constructs is yet to be shown.

Building on the promising pre-clinical data, first-in-human CAR T cell trials were initiated. The two initial studies, however, failed due to different reasons. CAR T cells targeting the ovarian cancer-associated antigen folate receptor alpha (FRα) were evaluated for the treatment of patients with metastatic ovarian cancer.[23] Despite the manageable safety profile, responses were limited and not durable. In contrast, the infusion of CAR T cells targeting the carboxy-anhydrase-IX (CAIX) in patients with metastatic renal cell carcinoma (RCC) resulted in severe liver toxicities leading to treatment discontinuation in 2 out of 3 patients.[24] Therefore, it became evident that the choice of target antigen was of paramount importance for clinical effectivity of CAR T cell therapy.

In 2003, Dr Sadelain's group introduced CAR T cells targeting CD19, an antigen expressed by healthy and malignant B cells and thus set the stage for a revolutionary era in the treatment of hematological malignancies.[25] On the basis of convincing pre-clinical data, large academic cancer institutes in the USA subsequently initiated trials with anti-CD19 CAR T cells in different entities of B cell malignancies such as B cell non-Hodgkin lymphoma (B-NHL) or adult and childhood B cell ALL (B-ALL). In all trials, unprecedented remission rates were achieved and many patients with highly refractory diseases were cured. In 2012, Emily Whitehead, a then 7-year old girl with relapsed, refractory B-ALL was successfully treated by Dr Carl June at the University of Pennsylvania. Her story raised widespread media attention and raised awareness for the potential of CAR T cell therapy. Emily Whitehead is now 14 years old and cancer free for 7 years. With her charity foundation, she supports the development of novel therapies for childhood cancer.

During these initial trials, however, it became evident that the anti-cancer efficacy of CAR T cells comes at the price of drastic systemic toxicities. The most frequently encountered adverse event is the cytokine release syndrome (CRS), a SIRS-like systemic inflammatory syndrome that can be fatal and regularly requires intensive care treatment. Glucocorticosteroids and the IL-6 antagonist tocilizumab can mitigate the toxicity and are now routinely administered in the clinical setting. Additionally, some patients develop a neurological deterioration, termed as CAR-related encephalopathy syndrome (CRES). These symptoms are preliminary in most cases but can be fatal in some. The underlying changes are poorly understood, and effective pharmacological treatment options are not yet established.

While the entire pre-clinical and early clinical development of CAR T cell therapy was driven by academic institutions, larger phase III trials required the financial potency and the logistics that only big pharmaceutical companies could provide. Therefore, academic CAR constructs were licensed to Novartis (University of Pennsylvania, tisagenlecleucel, Kymriah®) and Kite Pharma (National Cancer Institute, Axicabtagen-Ciloleucel, Yescarta). Results of the ELIANA study[26], the first global

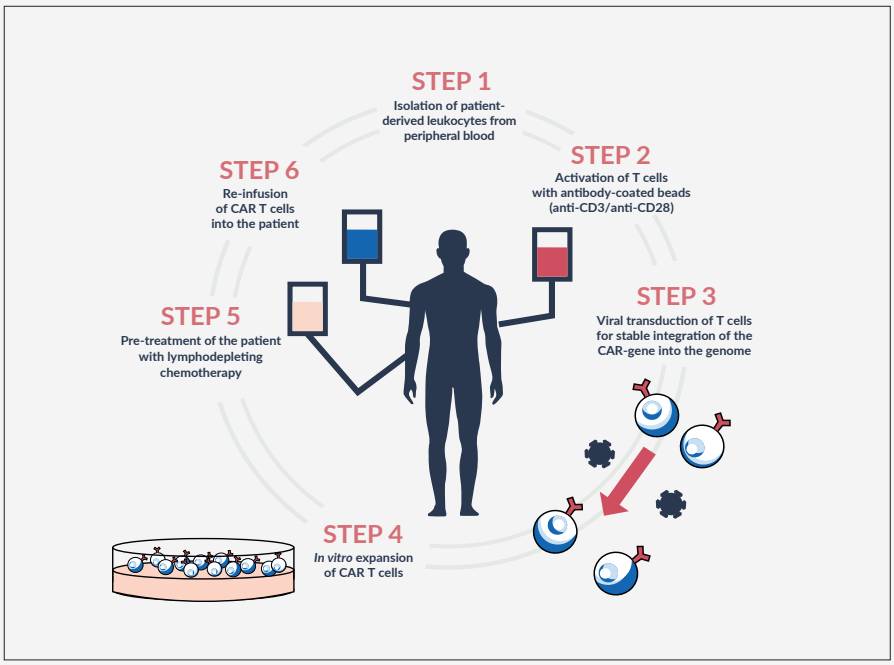

Figure 2. CAR T cell therapy: From T cell isolation to infusion of engineered cells back into the patient. CAR, chimeric antigen receptor.

CAR T cell registration trial in pediatric patients, showed that 41 out 50 patients infused with anti-CD19 CAR T cells achieved complete and durable remissions. Based on these data, tisagenlecleucel received FDA approval for the treatment of pediatric and young adult patients with refractory or relapsed B-ALL (**Figure 1**).[27] Hence, tisagenlecleucel was the first genetically engineered cellular therapy to enter the market. In addition, the JULIET trial[28] led to the approval of tisagenlecleucel for the treatment of adult patients with relapsed or refractory diffuse large B-cell lymphoma (DLBCL) in the USA and Europe.[29] Similarly, based on the results of the ZUMA-1 trial[30], axicabtagene ciloleucel gained approval for the treatment of patients with refractory DLBCL, primary mediastinal B-cell lymphoma or transformed follicular lymphoma (**Figure 1**).[31]

CHALLENGES AND FUTURE DIRECTIONS

While clinical experience has validated the unprecedented therapeutic potential of CAR T cell therapy to combat cancer, many challenges persist. For instance, concerns about long-term safety of genetically engineered cells and off-target toxicity remain a topic of debate. The frequency and the severity of adverse events such as infections, CRS and CRES limit CAR T cell therapy to patients that are otherwise refractory to conventional treatment. The main problem with regards to toxicity is the lack of control that treating physicians have over CAR T cells, once they are re-infused into the patients. Different methods to terminate the activity of CAR T cells in case of toxicity or after successful treatment are therefore being developed. A simple approach would be the targeted depletion of CAR T cells with monoclonal or polyclonal antibodies. More elegant approaches use "switchable" CAR T cells that require an adapter molecule to recognize their targets. By modifying the extrinsic administration of the adapter-molecule, physicians would be able to fine-tune the CAR T cell response and adapt to the individual toxicity profile.[32] Another main obstacle for the widespread clinical implementation of CAR T cells, not only in developing countries, is the high financial burden that this therapy inflicts on the health care system. With a price range of $371,000 −$475,000 for a single infusion, novel pricing and reimbursement strategies need to be implemented. Additionally, different approaches exist to cut down the price tag of CAR T cell manufacturing. For example, point-of-care production within academic centers or the development of allogeneic off-the-shelve CAR T cells that do not require the sophisticated logistics of individualized products are promising concepts.

To date, clinical efficacy of CAR T cell therapy is largely limited to hematological malignancies of the B and plasma cell lineage. Other hematological cancers such as myelodysplastic syndrome or acute myeloid leukemia (AML) are more difficult to target due to toxicity against healthy hematopoietic stem and progenitor cells (HSPC). Even more difficult is the translation of CAR T cell therapy for the treatment of solid cancers. In addition to the identification of targetable tumor antigens, impaired access of CAR T cells into the tumor as well as the immunosuppressive tumor microenvironment are major challenges that still need to be overcome.[33]

In summary, despite certain drawbacks, the CAR T cell story is a paradigm for the successful translation of advancements in basic science for the benefit of critically ill patients. In turn, the unprecedented efficacy of this therapy in the clinical setting has had a fundamental impact on the cancer research community. With lessons learned under "real-world" conditions, scientists around the globe are now working on improving CAR T cell constructs by lowering toxicity profiles, improving control options or reducing manufacturing costs. With the ever-increasing pace of scientific discoveries, the future of immunotherapy in general and adoptive CAR T cell therapy in particular remains to be exciting.

CONCLUSIONS

CAR T cells have emerged as a powerful new class of therapeutics and have marked the beginning of a new era in cancer immunotherapy. Over decades, advances in both basic and translational research have contributed to the successful development of one of the first examples of truly "personalized medicine". To date, progress in the CAR T cell field has offered new hope for terminally ill patients, who were refractory to conventional therapies. Future developments will certainly improve the safety of CAR T cells and expand the spectrum of targetable cancer types, especially in the field of solid oncology. Finally, a significant reduction of manufacturing costs will be a premise to enable widespread implementation of this technology outside as well as inside highly industrialized countries. In this context, the development of "universal" CAR T cells that are produced from healthy donors and used as "off-the-shelf-product" might mitigate costs and shorten the time span until patients can receive the potentially life-saving cells.[26]

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