

Cell therapy for treatment of cancer

The universal excitement over cancer immunotherapy is well founded. Impressive clinical results over the last few decades and recently approved immunotherapy treatments have shown unprecedented responses and survival rates in many difficult-to-treat cancers.

While these breakthroughs continue to improve patient outcomes, immunotherapies must still overcome several major challenges, such as improving toxicity management, increasing and sustaining efficacy and improving response prediction. This intriguing and rapidly evolving field is further expanding to also explore the safety and efficacy of these therapies in additional hematological malignancies and in solid tumors.

This white paper provides the current position on the key cell types and their role in cancer immunotherapies, with a more in-depth focus on chimeric antigen receptor (CAR) T-cell therapies. This will include a review of toxicity management guidelines for CAR T-cell therapies, the future outlook on improving safety and efficacy profiles, current CAR T cell limitations and potential use for solid tumors.

Understanding the cellular interplay in cancer immunotherapy

Cellular immunotherapies can rely on several different cell types, each with their own characteristics and peculiarities in the context of cell-mediated tumor immunology. These include the following:

B Cells

B cells are produced in the bone marrow. They represent approximately 5-25% of all circulating lymphocytes. Circulating B cells live only a few days in blood, but may interact and be activated by T cells in association with antigen-presenting dendritic cells. Activated B cells live longer and they enter the follicles where they can proliferate forming germinal centers. Then they differentiate into plasma cells specialized for antibody production and long-lived memory B cells.

T Cells

T cells are produced in the thymus and can live longer than B cells – up to several decades. Also, CAR T cells have been detected in patients for up to four years post-infusion, indicative of sustained presence with which to elicit a therapeutic response. T cells have the ability to recognize and kill foreign cells, based primarily on T cell receptor (TCR) binding to “non-self” antigen, which then activates the adaptive immune response.

There are several subtypes of T cells, the most common being the CD4, CD8 and T regulatory cells. CD4 are also called T helper cells. Among other functions, they activate B cells and can also help activate the CD8 cytotoxic T cells. T cells are activated in the context of human leukocyte associated (HLA) antigens and costimulatory molecules. The CD4 and CD8 co-receptors determine whether T cells recognize antigens presented by major histocompatibility complex (MHC) proteins, of class I or II types. T regulatory cells, commonly referred to as “T regs,” downregulate the immune response. They are involved in immune-tolerance mechanisms, are akin to an antidote to the effect of cytotoxic T cells and serve as a key player of the many checks and balances in the human immune system.

Memory T cells are also relevant, specifically stem memory T cells (TSCM), which exhibit therapeutic potential due to their long lifespan, robust proliferative potential and self-renewal capacity. These cells have generated much research and clinical interest particularly for therapeutic use.

Antigen Presenting Cells (APCs)

Antigen presenting cells include dendritic cells, macrophages, Langerhans cells and B cells, and have also been utilized in the development of cellular therapy for cancers. APCs activate T helper cells and express HLA class II molecules, together with antigen portions.

Natural Killer (NK) Cells

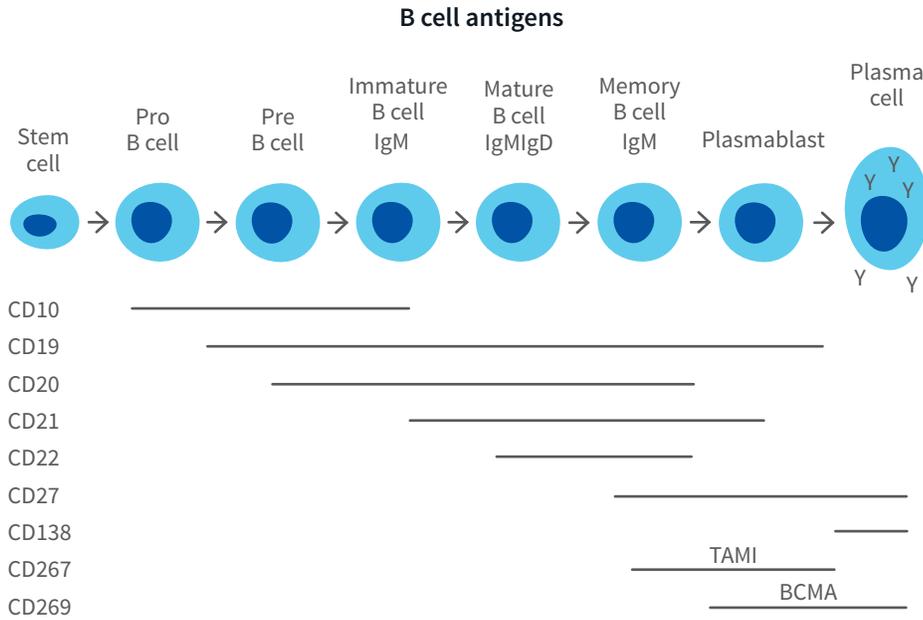
Natural killer cells are also cytotoxic immune cells with a lifespan of less than two weeks. They are the effectors of the innate immune system, a more primitive immunity that plays an important role in killing tumor cells. The advantage of NK cell mediated cytotoxicity is that unlike cytotoxic T lymphocytes; they do not require the presence of MHC class I molecules, but just co-stimulatory signals to be present, which allow for a more immediate killing effect. NK cells also have attributes of adaptive immunity in line with expression of activating and inhibitory receptors, adaptation of their reaction to the different environment, and the ability to develop a form of antigen-specific immunologic memory.

Dendritic Cells (DC)

Dendritic cells are cells of the innate immune system, bridging between innate and adaptive immune systems, and represent a suitable choice for cancer immunotherapy. DCs include two major subsets: myeloid DCs and plasmacytoid DCs, expressing different toll-like receptors and secreting mainly IL-12 (myeloid DCs) or IFN α (plasmacytoid DCs). Mature DCs present foreign antigens in the context of MHC I or MHC II to naive CD8 or CD4 T cells respectively, causing T cell activation. Following antigen presentation, DCs undergo apoptosis. The overall life span of these cells is approximately 14 days.

An overview of B cell antigens and malignancies

As B cells mature, many different antigens are sequentially expressed with each playing a specific role in cell signaling pathways. These antigens can be used as biomarkers for diagnostic purposes, and can also serve as a target for immunotherapies when dealing with cancer of B cell lineage. For example, B-lymphocyte antigen CD19 is expressed over a long range of the B normal cell lineage, but is also detectable in B cell lymphoma and B cell leukemia, both acute and chronic.



Since CD19 is expressed over a number of B cell derived leukemias and lymphomas, it was chosen as the target for the three currently approved CAR T-cell therapies. With the wide range of expression, CD19-targeted treatments can kill cancer cells, but at the same time also impact healthy B cells harboring CD19. This does not make the CD19 a target exclusively specific to cancer cells, and this “on target-off cancer” effect leads to the common adverse event of prolonged lymphopenia after CAR T cell treatment infusion.

Additional B cell antigens have been explored in the context of cellular immunotherapy for cancer. These include CD20, CD22, B cell maturation antigen (BCMA). BCMA only appears toward the end of the B cell lineage, when a B cell matures and moves toward the plasma cell stage, and is used in the currently approved myeloma CAR T-cell therapies. BCMA is a precise target since myeloma has abnormal plasma cells; on the other hand, BCMA is not expressed on the earlier B lineages and therefore wouldn't affect the earlier stages of B cell maturation.



CAR T cell background and aspects in common with the first cellular immunotherapy

CAR T cells are genetically modified T cells, redirected against a specific tumor antigen, aimed to equip patients' T lymphocytes with a genetically modified receptor (CAR). These cells recognize the specific antigen present on one's own cancer cells (e.g., CD19 on B cell neoplasia) with the selectivity of an antibody, while rapidly activating a cytotoxic reaction through the transduction function of the modified T cell receptor. The CAR harbors an extracellular domain, a transmembrane domain and an intracellular signaling domain. The CAR external portion consists of a single-chain variable fragment directed against the tumor antigen (CD19 in approved CAR T cells). This redirects the specificity of the T cell receptor to be activated upon recognition of specific antigens, independent of MHC protein expression.

The first cellular immunotherapy (sipuleucel-T, Provenge®) was approved in 2010 for advanced prostate cancer, and was designed on patients' own APCs and the PAP-GM-CSF protein. The original cell product was collected via leukapheresis, similarly to CAR T-cell therapies' initial step for production. Provenge requires the presence of MHC class I class II proteins and subsequent indirect T cell activation; its tumor shrinkage effects are relatively slow, taking two to three months to manifest. It wasn't until 2017 that cell-based therapies took the next leap forward, with the approval of CD19 CAR T-cell therapies KYMRIAH® and YESCARTA®. These therapies showed unprecedented response rates in B cell malignancies, including R/R DLBCL and B-ALL (ranging from 60 to 80%, and including B-ALL and DLBCL), together with a specific and expensive manufacturing process and rather unique toxicities. In addition, KYMRIAH, in the ELIANA study on 75 patients with R/R B-ALL aged <22 years showed an overall remission rate of 81% in the first three months with EFS and OS of 50% and 76% respectively at 12 months of follow-up. In contrast to Provenge, these CAR T-cell therapies are re-engineered with a CAR in the absence of MHC dependent antigen stimulation and costimulatory molecules for improved signaling. As a result, CAR T cells work in a faster and more direct way than Provenge against cancer cells, as shown by patients with relapsed or refractory acute lymphoblastic leukemia (ALL) who became cancer-free within one week following a single dose of a CAR T-cell therapy. More recently, in July 2020, another CD19 CAR T-cell therapy, TECARTUS™ was approved by the FDA, based on the safety and efficacy findings from a multi-center clinical trial of 60 adults with refractory or relapsed MCL. Patients responded to a single infusion of TECARTUS, with an ORR of 87% and with 62% of patients achieving a CR. Follow-up of at least six months after the first OR suggests durable response, with a median duration of response not yet reached.

TIL (tumor-infiltrating lymphocyte) therapy is another area of interest as it basically exploits the intrinsic power of the T cell but focuses on expansion of available cells, rather than manipulation followed by expansion, and consists of all lymphocytes invading the tumor tissue.

Challenges with approved CAR T-cell therapies

YESCARTA, KYMRIAH, and TECARTUS are three approved CAR T-cell products approved in the US which target CD-19. However the CD19 target is not exclusive to B cell cancers but is shared with normal B cell lineage. Compared to standard chemotherapies, these three novel cell based immunotherapies showed superiority in response rate that led to their approval. However this comes with frequent and severe side effects, such as B cell aplasia, cytokine release syndrome (CRS), and immune effector cell-associated neurotoxicity syndrome (ICANS).

CAR T cell anti-CD19 therapy causes frequent, long-lasting B-lymphocyte aplasia that persists at six months in over 80% of patients. This is due to the target being shared with normal B cells expressing CD19 and getting killed as “collateral damage” by the anti-CD19 CAR T cells, which can survive for years and sustain a prolonged lymphopenia. The majority of patients will require replacement therapy with intravenous immunoglobulin and prophylactic antibiotics to reduce infectious complications due to lymphopenia. CAR T-cell therapies are also associated with the unique toxicities of CRS and neurologic toxicity, which are also the most life-threatening toxicities associated with CAR T cells treatment.

CRS is observed in approximately 50% of patients and represent a severe systemic inflammatory response due to massive T cell stimulation. CRS tends to occur two to three days (and a range of one to ten days) after CAR T cell administration, with a median duration around eight days. Patients with CRS can experience mild flu-like symptoms, ranging up to life-threatening reactions, with high fever, chills, hypotension, hypoxia, tachycardia, nausea and vomiting that can last several days, and in some cases with fatal outcome. Multiple cytokines levels are increased during CRS, including TNF α , IL-6 and IFN γ , reflecting the severe inflammation and underlying “cytokine storm.” The initial tumor burden, type of conditioning for lymphodepletion, CAR T cell dose, CD28 vs. 4-1BB costimulatory domains, have all been associated to different extent with risk of developing CRS.

Neurotoxicity or ICANS (Immune-effector Cell Associated Neurotoxicity Syndrome) is observed in approximately 20% of patients, six to eight days after CAR T cell infusion; it can occur in overlap or in absence of CRS, and has a median duration of 17 days. It manifests with different severity and with various combinations of neurological symptoms and signs, including toxic encephalopathy, delirium, aphasia, seizures, confusion, cerebral edema or coma. The underlying mechanism of ICANS is largely unknown, with a clinical course generally reversible, although rare lethal cases have occurred.

Tocilizumab is approved for the treatment of CAR T cell-induced severe or life-threatening CRS, and acts by specifically inhibiting IL-6 at the receptor level. In absence of improvement within 24 hours after starting tocilizumab, administration of corticosteroids is recommended for the treatment of CRS. Treatment for neurotoxicity other than a local standard practice symptomatic approach, can include the use of tocilizumab (if concurrent CRS) and/or corticosteroids for moderate, severe or life-threatening neurologic involvement.



Due to the unpredictability of the onset of potentially life threatening CRS and neurotoxicity, limited treatment options are available, and the need to immediately address CRS to save a patient's life, patients need to be hospitalized and closely monitored for at least a week, and in some cases far longer, following CAR T cell infusion.

Developing a uniform toxicity grading system for CRS and neurotoxicity

The assessment and grading of CRS and neurotoxicity varies across different clinical studies and institutions, due to the different grading systems used over time. Evaluation of cytokines and inflammatory markers to assess CRS are relevant and are being included in clinical trial testing. These markers include ferritin, CRP levels and cytokines including IL-6, IL-15, IFN γ , GM-CSF, IL-8, MIP-1, among others. However, the mechanisms related to neurotoxicity are still unclear.

In practice, the variability of assessment and grading has limited the comparison of CAR T cell safety profiles across different products and studies. Since CRS and neurotoxicity treatment algorithms are based on the severity grade of the event, different grading systems could result in more or less aggressive treatment of the same event, depending on the criteria adopted at each institution. Early clinical trials modified the Common Terminology Criteria for Adverse Events (CTCAE) grading of CRS, to better refine CRS criteria and reflect hypoxia complication, and led to what are known as the Lee criteria in 2014. Further improvement of the criteria to reflect progression of CRS with additional organ involvement and treatment needs, including vasopressor use, multi organ failure, disseminated intravascular coagulation, requirement of mechanical ventilation are reflected in subsequently developed criteria, including Penn and MSKCC criteria. More recently, an additional effort to uniform further the grading systems for CRS and neurotoxicity, lead a group of experts to agree to the ASTCT consensus criteria, named after the American Society for Transplantation and Cellular Therapy that supported and coordinated the effort. The use of ASTCT criteria across all CAR T cell treatments will allow for comparison of toxicities across different CAR T cell treatments and studies.



CAR T cell in solid tumors

Currently, CAR T-cell therapies have only been approved in hematologic malignancies, due to failures or mixed responses in solid tumors and a number of challenges typical of solid tumors. A key obstacle is the immunoevasion by solid tumors, which includes impaired molecular trafficking into the tumor (with pro-tumoral endothelium, altered chemokine profile and stroma), microenvironment alterations (hyperacidity and increased potassium level, presence of myeloid derived suppressor cells (MDSCs) and tumor-associated macrophages (TAM), and their cytokines), and modulation of tumor-specific molecular pathways (JAK, PI3K, IFN γ , TNF α , microRNAs and other) that each contribute to the tumor microenvironment being difficult to penetrate and elicit an immune response.

There are additional challenges that drug developers face in the solid tumor setting. The number of specific targets is limited, meaning fewer antigenic epitopes are available that are completely unique and specific to the cancer cells, and not shared in other healthy tissues. Although the CD19 for approved CAR T cells is expressed in healthy circulating B-lymphocytes, this “on-target off-cancer” effect is manageable through the use of replacement immunoglobulins and prophylactic antibiotics, in contrast to the potentially sustained, chronic organ damage that would occur with a CAR T cell targeting a solid tumor antigen, shared with the healthy tissue of the organ.

Given that CAR T cells can survive for years, it is imperative to have a solid tumor target that is specific and not expressed in other healthy cell types. In addition, for a treatment to reach the solid tumor, the T cells must penetrate it. Compared to hematologic malignancies, whose cells are already in the vasculature, reaching a solid tumor is much more difficult to achieve. This is due to the aforementioned mechanism of immunoevasion, and the hostile and immunosuppressive tumor microenvironment that is prone to destroy any cells that might invade it.

Some potential solutions are being explored to address these major issues. Selection of the specific tumor antigen is a key approach, together with the need to overcome the immunosuppressive tumor microenvironment, and the choice of a particular T cell subset for the generation of CAR T cells. T cells with dual/multiple CARs could also help address the heterogeneity of tumor antigens and the shared presence across healthy and tumor tissues.

Currently, the success of CAR T cell approaches in the solid tumor space remains limited. There have been objective responses reported in trials, but the response rates have generally been comparable to traditional chemotherapies, rather than achieving the impressive results seen in hematologic malignancies.

To be effective in solid tumors, it is likely that the CAR T cells will also have to be given in combination with other products that affect directly or indirectly the tumor microenvironment. One example is combining CAR T cells with anti-PD-1 drugs that target checkpoint inhibitors, which can increase the efficacy of the CAR T cells. If promising combinations can be identified, CAR T-cell therapies have the potential to be as successful in solid tumors as they are in hematologic tumors. Other platforms of interest could include natural killer (NK) cells and dendritic cells, which use similar construct approach to T cells but with higher viability in solid tumors. A combination of T cells and NK cells or a highly engineered NKs could also be promising.



The Future of CAR T Cells: Limiting Relapse, Exploring New Targets and Indications

Selecting different targets in CAR T cells can have a major impact on both efficacy and safety aspects, also depending on the specificity and degree of expression of the new target in healthy tissue.

Recent CAR T cell products in development target several additional antigens beyond CD19, including B cell maturation antigen (BCMA), CD20, CD22 and others. Anti-BCMA CAR T cells (bb2121) showed high efficacy in multiple myeloma patients with an ORR of 89% and only one progression among 21 heavily pre-treated patients. When using BCMA as the target rather than CD19, two-thirds of the patients still develop CRS and ICANS, but at milder grades, giving BCMA-targeted treatments a slightly improved safety profile. Anti-CD22 CAR T-cell therapy resulted in high remission rates in ALL patients, although short lived in the majority, likely due to 57.5% of the patients having received prior anti-CD19 CAR T cell treatment.

Drug development sponsors are trying to further improve the safety profile of CAR T cells with the hope of decreasing life threatening complications and therefore reducing hospitalization requirements. Current studies can also rely on the more recent uniform consensus definitions and grading for CRS and ICANS.

Relapses can still occur following CAR T-cell therapy. There are several mechanisms by which these may occur, including the loss of CAR T cells. It is established that shorter CAR T cells persistence is associated with higher risk of relapse, with early B cell recovery post-infusion (one to three months) being a potential predictor of immune-mediated rejection. An additional mechanism for relapse is the development of CD19-negative clones. In this case, the leukemic blasts escape the anti-CD19 CAR T cell by no longer expressing the target antigen. Alternative therapies are required in this case, including potential use of CAR T cells targeted against other B cell markers, like anti-CD22 CAR T cell. Furthermore, bivalent CARs targeting CD22 and CD19 are currently in clinical development.

While first generation CAR T cells basically consist of the CD3 ζ domain alone in the intracellular portion, the second generation adds additional costimulatory signaling domains (CD28, 4-1BB, and others) and include the three CAR T cell treatment currently approved, the third generation CAR T cell combines two costimulatory domains. However, there are already fourth-generation CAR T cell, referred to as TRUCKs (T cells redirected for universal cytokine-mediated killing), that are genetically “armored” with antitumor activity maximized through additional genetic modification, including knock-out, or knock-in of different genes that can control CAR expression and activity, including transgenes for cytokine secretion (e.g., IL-12, 8, 9, 11, 12, 15, and IL-18). This approach is aimed to ultimately improve CAR T cell expansion and survival, while making them resistant to the immunosuppressive tumor environment.

Beside modification of CAR constructs and viral vector-mediated random insertion of additional genes, recent strategies incorporate more targeted gene editing technologies, such as TALEN and CRISPR/Cas-9 to modify the T cell genome, by knocking out negative T cell regulators, through specific gene disruption as well as by adding transgenes. Finally, the long-term effects of CAR T cells and the possibility of integration-mediated oncogenesis, still remain unknown. While CAR T cells are weaponized directly against cancer cells, without requiring prior MHC I/II dependent antigen presentation and signaling through co-stimulatory molecules, another form of T cell immunotherapy based on T cell receptors (TCR) has also shown evidence of potential benefit in the context of solid tumors; with TCR therapies, a patient’s T lymphocytes can be modified ex vivo and then reinfused into the patient like CAR T cell. However, the main difference is in their mechanisms for recognizing antigens, which may overcome the limit of CAR T cell to penetrate and deliver the anti-tumor effect in solid tumors, by targeting the TCR-peptide/MHC interaction. Intracellular tumor-related antigens can be presented as peptides in the MHC on the cell surface, which interact with the TCR on antigen-specific T cells to stimulate an anti-tumor response. TCR therapy, by using the natural mechanisms that T cells use to recognize the antigen may be better suited to act within the solid tumor and overcome the immune suppressive microenvironment.

Another important development in this space is the use of allogeneic cells (cells from a donor and not the patient) as opposed to autologous cells (cells from the patient). The currently approved CAR T-cell therapies use genetically modified autologous T cells, which require about two to three weeks to manufacture and return to the patient. If off-the-shelf allogeneic cells could be used instead, then the product would become more accessible and readily available “off the shelf.” Patients could be treated faster and the product would potentially cost less. However, there are still concerns on whether allogeneic products can be engineered without a risk of graft versus host disease or a risk of a reduced efficacy.

With expanded indications, explorations in the solid tumor space and lessons learned from previous therapies and clinical trials, this area of novel immunotherapies will continue to rapidly evolve. CAR T cell immunotherapies are not yet the standard treatment option for their indications, highlighting the need for ongoing research to improve their safety profile and better understand how to optimize their efficacy.

References

1. Berdeja JG et al. Durable Clinical Responses in Heavily Pretreated Patients with Relapsed/Refractory Multiple Myeloma: Updated Results from a Multicenter Study of bb2121 Anti-Bcma CAR T-cell Therapy. *Blood* 2017; 130(Suppl 1):740 LP-740.
2. Lee DW et al. Current concepts in the diagnosis and management of cytokine release syndrome. *Blood*. 2014;124:188-195.
3. Lee DW et al. ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells. *Biol Blood Marrow Transplant*. 2019;25(4):625-638.
4. Nayar S. et al. Extending the lifespan and efficacies of immune cells used in adoptive transfer for cancer immunotherapies. *Oncol Immunology* 4:4, e1002720; April 2015
5. Neelapu SS. Managing the toxicities of CAR T-cell therapy. *Hematological Oncology*. 2019; 37 (S1):48-52.
6. Park JH et al. Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. *N Engl J Med*. 2018;378:449-459.
7. Porter D et al. Grading of cytokine release syndrome associated with the CAR T-cell therapy tisagenlecleucel. *J Hematol Oncol*. 2018;11:35.
8. Schmidts A, Maus MV. Making CAR T Cells a Solid Option for Solid Tumors. *Front. Immunol*, 08 November 2018;9:2593
9. Shimabukuro-Vornhagen et al. Cytokine release syndrome. *Journal for ImmunoTherapy of Cancer*. 2018;6:56
10. Tontonoz M, Stallard J. Driving Armored CARs at Cancer. Accessed 14 Jul 2020. <https://www.mskcc.org/blog/driving-armored-cars-cancer>
11. Titov A. et al. Advancing CAR T-cell Therapy for Solid Tumors: Lessons Learned from Lymphoma Treatment. *Cancers* 2020;12:125
12. Wrona E, Potemski P. A novel immunotherapy — the history of CAR T-cell therapy. *Oncology in Clinical Practice*, 2019;15(4):202-207.

Learn more at biopharma.labcorp.com.