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Immunotherapy Using CAR T Cells: Current Challenges and Solutions to Overcome On-Target, Off-Tumor Toxicity

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ABSTRACT

Blood cancers like leukemia and lymphoma currently affect approximately 1.3 million Americans and account for about 10% of all cancer related deaths in the US. However, immunotherapies such as chimeric antigen receptor (CAR) T cells have been developed to provide a cure for many of these patients where refractory/relapsed disease was previously a death sentence. Current approaches for CAR T treatment involve the modification of autologous T cells *ex vivo* in order to engineer these cells with a CAR which binds to a B cell cancer antigen and results in T cell mediated clearance of the target tumor cell. With exception to CD19 and other B cell antigens, targeting other tumor cell types has been largely unsuccessful for many reasons, including antigen escape and on-target off-tumor toxicity (OTOT), which occurs when the target cancer antigen is co-expressed on healthy tissue cells and becomes targeted by the CAR T cell therapy. The purpose of this review is to understand the challenges in antigen escape and OTOT. We will discuss current strategies to overcome these obstacles which allow CAR T therapy to be more applicable to other tumor cell types. We will highlight novel approaches such as logic gated CARs and epitope editing as potential solutions to overcome antigen escape and OTOT.

Introduction - Blood Cancers and Current Treatment

Most hematological cancers start in the bone marrow as this is where blood cells are produced. These blood cancers arise when abnormal blood cells begin to multiply out of control, which among other consequences interferes with the normal blood cells' ability to fight off infections and generate new blood cells (Allart-Vorelli et al., 2015). Different types of blood cancers may arise depending on the type, location, and number of cells affected. Hematological cancers can consist of lymphoma, leukemia, or multiple myeloma. Leukemia and multiple myeloma occur in the bone marrow cells, while lymphoma originates in the lymphatic system and spreads to target the lymph nodes and the lymphatic tissue (Allart-Vorelli et al., 2015). Formerly healthy blood cells become cancerous due to deleterious mutations in the DNA of the host cell. These mutations consist of insertions or deletions of nucleotide bases, which can greatly alter the DNA sequence of the cell. While most mutations end up having a neutral effect, if the altered DNA sequence is significant to the amino acid structure, it can lead to either gain of function or loss of function of important proteins which normally keep the cell in a regulated cell cycle. These alterations can cause aberrant regulation of the cell cycle, resulting in generation of cancer cells and potential expansion into a tumor mass (Yates & Campbell, 2012). A different type of mutation can result in inactivation of the tumor suppressor genes which typically regulate a healthy cell cycle. The loss of these genes allows the cell to uncontrollably grow and multiply and thus become cancerous (Yates & Campbell, 2012).

Through understanding the biology of blood cancer formation, clinicians have been treating blood cancers for decades. More traditional approaches consist of broadly targeted chemotherapy, radiation, and other

nonspecific therapeutics. However, more advanced targeted immunotherapies have been developed over the last decade and will be discussed throughout this review. Primary treatment typically consists of chemotherapy which puts cancer into remission by killing all fast-growing cells. The reason that chemotherapy is generally the first option is that it frequently and effectively terminates the vast majority of all cancer cells. Unfortunately, not infrequently the cancer cells mutate and survive chemotherapy, and the patient now has a weakened immune system, as chemotherapy destroys many healthy cells, which can lead to a rampantly growing cancer (Sweeney & Vyas, 2019). This is where the second treatment option arises: an allogeneic bone marrow transplant. After first clearing the host bone marrow niche using chemotherapy, new donor bone marrow cells are infused in hope that they will generate a new immune system that will be able to terminate the cancer (Guillaume et al., 1998). This process is known as the Graft vs. Leukemia effect, where the donor T cells along with other immune cells are able to eliminate any residing leukemia cells after chemotherapy treatment. With this treatment there is also a risk of Graft vs Host Disease (GVHD), as the donor immune cells could begin to target and eliminate healthy body cells (Sweeney & Vyas, 2019). Should this second treatment end up ineffective and the patient relapses, the third line of treatment is considered. This treatment is a type of immunotherapy where T cells can be modified to specifically bind with and terminate cancer cells and it is the purpose of this review.

Interactions Between the Immune System and Cancer

A healthy immune system can identify a threat, eliminate a pathogen, and later recognize it resulting in a swift response and immunity upon second exposure. This process starts with the innate immune system which is the immediate response. The innate system is not able to distinguish specific antigens from each other but is still able to launch attacks on foreign antigens via Group 1 Innate Lymphoid cells (ILCs), including natural killer (NK) cells and ILC1s. ILC1s rapidly produce interferon gamma (IFNγ) very early on during viral infection, which non-specifically impacts viral multiplication (Hildreth & O'Sullivan, 2019). The second system is the adaptive immune system which is able to target specific antigens. This system can recognize different antigens via specialized B and T cells, but because these cells need to be primed, this response takes longer to activate than the innate response. Once the T cells have been activated by antigen presenting cells (APC), typically dendritic cells, they begin a targeted response to eliminate the foreign antigen, such as cancer.

During an immune response, T cells expand, proliferate, but also can contract in response to inhibitory signals such as a programmed cell death receptor 1 (Jiang et al., 2015). This latter action is important in order to not have autoimmunity after mounting an immune response. However, due to high expression of inhibitory markers (such as PDL1, LAG3, TIM3 and TIGIT), many T cells can be impaired where their antitumor functions are severely reduced. These T cells are known to be "exhausted" and incapable of eliminating cancer cells (Jiang et al., 2015). In order to overcome exhaustion, immunotherapy treatments have been developed to reinvigorate the immune response against cancer.

CAR T Immunotherapy as A Tool to Cure Cancer

CAR's are synthetic receptors derived from antibodies and T cell signaling components which are expressed *ex vivo* and then reintroduced into the patient in order to better target and kill cancer cells. The first attempt at cell therapy occurred in the 1950's with bone marrow transplants as it was the first-time living cells were implanted into patients for cancer control. In 1989 the first CAR T receptor was described and theorized to be implanted into T cells by Yoshihisa Kuwana (Maman & Witz, 2018). In 2011, a CAR T treatment resulted in complete remission in many patients with chronic lymphocytic leukemia (Melenhorst et al., 2022). A major milestone occurred in 2017 when the U.S. Federal Drug Administration (FDA) approved CAR T cell therapy treatments

for children with acute lymphoblastic leukemia (ALL). As time goes on, more and more CAR T treatments for a wider variety of cancers have been developed and approved.

Current Methods to Produce CAR T Cell Therapy

The current protocol for manufacturing CAR T cells begins by isolating T cells from the donor via leukapheresis, the CAR is integrated into the host genome via lentiviral transduction (an innocuous virus mediated DNA alteration), the CAR expressing T cells are then expanded *in vitro*, and then re-infused into the patient **(Fig. 1)** (Rafiq et al., 2020). Donors are either autologous, meaning the T cells are from the patient, or allogeneic where the T cells are derived from a healthy donor. Autologous treatments were the first to be approved and provide a very effective anti-tumor response. Because these cells are patient derived, they are much less likely to be rejected by the host immune system and are capable of maintaining long term persistence. The downsides of this treatment include the very high cost and a wait time of approximately three weeks (Depil et al., 2020). In some extreme cases of acute leukemia, three weeks of manufacturing time is too long, and the patient is at risk of life-threatening complications. As such, the option of having cryopreserved allogeneic CAR T treatments from a healthy donor ready to go "off-the-shelf" is rather enticing. Allogeneic CAR T cells can be sourced from either Peripheral Blood Monocellular Cells (PBMC) or Umbilical Cord Blood (UCB). PBMC cells from a healthy donor can be multiplied and modified to have various human leukocyte antigen (HLA) complexes in order to maximize the chances of a ready match with a patient (Depil et al., 2020). T cells donated from UCB are a more "naive" cell form as these cells haven't had much exposure to foreign pathogens. The benefit of these cells is that they have less incidence and severity of GVHD, one of the major issues with allogeneic CAR T therapies (Depil et al., 2020). In GVHD the graft begins to attack the host. The opposite can also happen where the host immune system recognizes the graft as a threat and eliminates the CAR before it ever targets cancer. An upcoming model of allogeneic T cells to potentially fix these issues is a syngeneic CAR T cell. This is when a patient receives donated T cells from an identical twin (Zoine et al., 2022). The idea behind this is that the identical genes of the cells will enable the graft to quickly and effectively work with the host immune system to terminate cancer. Hopefully, it will also remove the risk of the host cells terminating the graft as it will recognize the graft as its own cells. The trials for this are currently ongoing with syngeneic mouse models but have shown promise and could be a viable option in the future (Zoine et al., 2022).

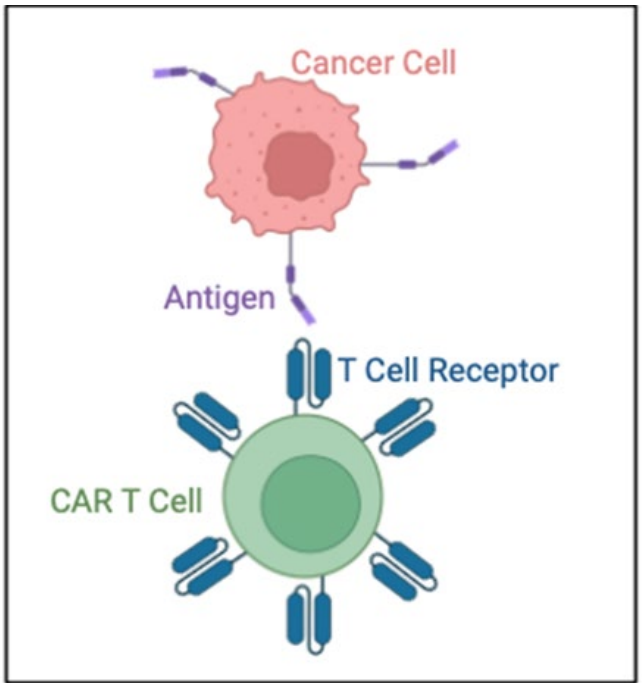

Figure 1. CAR T Cell structure and basis of action. T cells are removed from the donor/patient and then implanted with specific T Cell receptors *ex vivo.* These T cells are then placed into the patient's immune system where they will interact with the local cells. Should the cells have the target antigen present the CAR T cell will be triggered to release signals that terminate these cells, leading to a targeted cancer treatment.

A Major Obstacle in CAR T Cell Therapy: On-Target Off-Tumor Toxicity

An unfortunate issue that occurs with CAR T cell therapies is OTOT. This began with specific CD19 or B Cell Maturation Antigen (BCMA) CAR T therapies that have been approved to treat B cell malignancies. Once these T cell therapies have been modified to treat B cell tumors OTOT begins to occur; as the targeted receptors on antigens are typically co-expressed on non-malignant tissues such as healthy B cells (Flugel et al., 2023). CAR T cells are not able to differentiate the cancer cells from somatic cells and can end up terminating all of the patient's healthy B cells. While a patient can still survive without their B cells, their bodies/immune systems will be significantly less effective at fighting off bacteria and viruses. Their body will also lose the "memory" function of their antibodies that makes a secondary response so quick and effective. Fortunately, patients are able to live relatively normal lives without B cells with the addition of Intravenous Immunoglobulin (IVIG) to supply antibodies to their immune system. Because the expression of other antigens on non-dispensable somatic cells cannot simply be replaced, OTOT remains a huge hurdle when designing CAR T therapies to these antigens. A current strategy that is being implemented to address this issue is to deliver the CAR T cells directly to the target antigen, via specific delivery routes that minimize off-target toxicity, with less exposure to somatic cells, and maximization of tumor suppression. Unfortunately, many solid tumor sites are not easily accessible to local delivery methods (Flugel et al., 2023).

Another method used to reduce off-target toxicity is improving the specificity of the CAR by having it target multiple antigens. This is beneficial as the CAR would only be activated when all of these antigens are present, a method that can be used to target specific cancer cells that express multiple antigens, while leaving the non-malignant cells unaffected (Rafiq et al., 2020). The CAR could also be modified to only be activated in the presence of factors that are more common in tumor cells, such as phospho antigens that can be recognized

via T cell receptors (TCRs), or in the tumor microenvironment (TME) like the immunosuppressive cytokine Interleukin 4 (IL-4) (Rafiq et al., 2020). A third method to overcome OTOT toxicity focuses on modifying affinity to selectively target the receptor CD38 which is highly expressed on multiple myeloma cells, and also expressed on healthy immune cells, although to a much lower degree (Drent et al., 2017). This study designed a single chain fragment variable (scFv) with severely reduced affinity for CD38 (which still allowed for effective tumor cell killing of abundantly CD38 expressing MM cells), while healthy but less CD38+ rich cells were spared in their study. In vivo analysis of these CAR T cells infused with scFvs has clearly shown a construct that is able to effectively target tumors while having minimal cytotoxicity towards healthy blood cells (Drent et al., 2017).

Antigen Escape and How Tumors May Hide from The Immune System

An additional scientific phenomenon that can occur with CAR T cell therapies is known as antigen escape. This occurs when the tumor develops a resistance to CAR T cells that have only been modified to target a single antigen. While these single antigen CAR T cells are initially very effective, malignant cells frequently adapt. This can lead to either a partial or complete loss of target antigen expression (Sterner & Sterner, 2021). One example of this occurs when CD19 is downregulated and CD19 leukemia cells are able to evade the CAR T therapy as they no longer express CD19. A solution to this problem involves CD19/CD20 autologous bispecific CAR T cell therapy derived from naive and memory T cells, which targets CD20 (also a marker of malignant leukemia cells) in addition to CD19. The trials with this approach have demonstrated strong efficacy, with a 90% overall response rate and a 70% complete response rate (Larson et al., 2023).

A more comprehensive current solution to this issue is to implement a dual or tandem CAR construct (two chimeric T cell changes instead of the typical one). This leads to a single CAR construct that contains two scFvs which allows it to bind specifically and individually with two target antigens. The recent trials of CD19/CD22 CAR constructs have shown effective results for adults with acute lymphoblastic leukemia (ALL) (Sterner & Sterner, 2021). This demonstrates the possibility of optimizing CARs to have a more effective anti-tumor response while also decreasing the chance of antigen escape.

Another method to overcome this issue is to further modify the CAR T cells to secrete bi-specific T cell engagers (biTes). These modified CAR T cells typically consist of two scFvs, one specific to CD3 and the other to a tumor associated antigen. This enables the CAR T to create a physical link to the target antigen. These have been noted to be effective at preventing antigen escape in models of leukemia and solid tumors (Rafiq et al., 2020).

The Latest Advances in Overcoming Tumor Resistance to Immunotherapy

Epitope Editing

Epitope editing is a cutting-edge solution to issues with immunotherapy treatments of cancers like ALL, a cancer of the blood and bone marrow. The immunotherapy treatments for this cancer target antigens expressed by hematopoietic stem progenitor cells (HSPCs) or differentiated myeloid cells. As such an intolerable amount of OTOT builds up with standard CAR T therapy (Casirati et al., 2023). This is where epitope editing comes into play as scientists can edit donor HSPCs (used for bone marrow transplants) ex vivo to have selective resistance to CAR T or monoclonal antibodies, without affecting their protein structure or function (Fig. 2). This editing enables the CAR T cells to target genes that are essential for leukemia survival/development that are also co-expressed on HSPCs, but concomitantly reducing the severity and risk of OTOT (Casirati et al., 2023). The true significance of this approach is that as opposed to treatments that aim to remove or truncate the target

molecule, which can only be done to dispensable genes (like CD33), epitope editing enables the CAR to target genes that are essential to leukemia survival but are also essential in healthy hematopoiesis. An example of this would be cytokine receptors, which are necessary signaling receptors in acute myeloid leukemia (AML) (Casirati et al., 2023). A truly exciting development occurred more recently with epitope editing of CD45, as CD45 is expressed in all leukocytes, including cancer cells, making it a great target for CAR T. Unfortunately, the associated toxicities have made this CAR construct too dangerous to be usable in clinical practice until now (Wellhausen et al., 2023). CRISPR adenine base editing can be used to create a mutated CD45 that is able to evade the CAR T cells and carry out their somatic roles. These epitope edited CD45 CAR T cells were found to be fratricide resistant (sparing the somatic HSPCs), while effective against AML, B cell lymphoma, and acute T cell leukemia (Wellhausen et al., 2023). In recent animal studies, mice with AML were treated with this modified CAR T 45 as leukemia cells universally express CD45. Using an AML cell line model effective killing and T cell proliferation were observed and within three weeks of this therapy leukemia was eradicated (Wellhausen et al., 2023). As CD45 is expressed on most hematological malignancies, the development of epitope editing of CD45 yields the fascinating possibility of a universal and effective blood cancer immuno-therapy treatment.

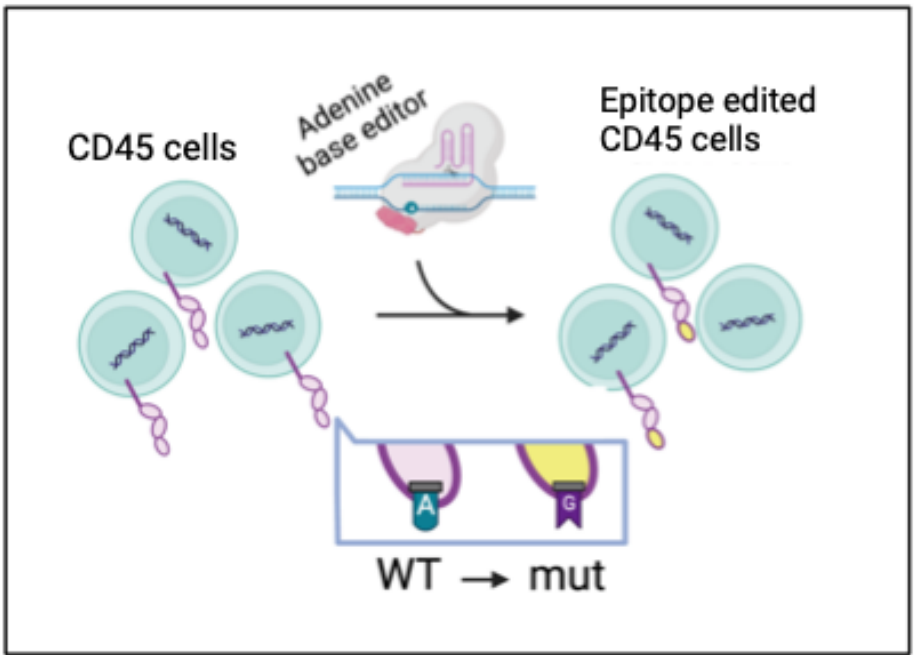

Figure 2. Base editing strategy to undergo epitope editing. By using an adenine base editor, the adenine of the wild type CD45 molecule (Purple outline) can be edited into guanine creating a mutated CD45 cell. This edit does not affect the structure or the function of the CD45 receptor on the HSPCs. As proteins are very shape specific this small edit is still significant enough to stop the CAR T cells from binding with somatic cells while still enabling the CD45 HSPCs to carry out their daily somatic functions.

Logic Gating CAR T Therapy

Another technique used to overcome OTOT relies on CAR constructs to use multiple markers to terminate instead of a single definitive one. This process is known as logic-gating and works by integrating a NOT gate which uses specific antigens to protect normal tissues via inhibitory receptors **(Fig. 3)**. These NOT gates can be integrated into T cells with a NOT-gate dual-signal integrator (DiAndreth et al., 2022). This creates a CAR

construct with a high killing specificity in a mixed group of malignant and non-malignant cells. As the T cells have two components, a CAR or a TCR acts as the activating receptor. Then an inhibitory receptor based on the LIR-1 protein, expressed primarily on macrophages, inhibits the T cells from terminating these healthy body cells (DiAndreth et al., 2022). Thus, when the CAR T cell binds with the target antigen it becomes activated and begins terminating the cell. The CAR T will carry out this process with malignant cells, but with healthy cells that possess unique proteins not found in the cancer cells, the NOT-gate will be activated. This inhibits the T cell's response and halts the termination of the normal somatic cell. One example of this would be to replace traditional CD3ζ domains with intracellular proximal T cell signaling molecules. Certain CAR constructs, such as ZAP-70 CAR, can work with T cells to terminate tumor cells while bypassing signaling proteins like CD3ζ. With this the main function of ZAP-70, to phosphorylate LAT, can be exploited to create a logic-gated CAR that will continue to target cancer cells while preventing OTOT (Tousley et al., 2023).

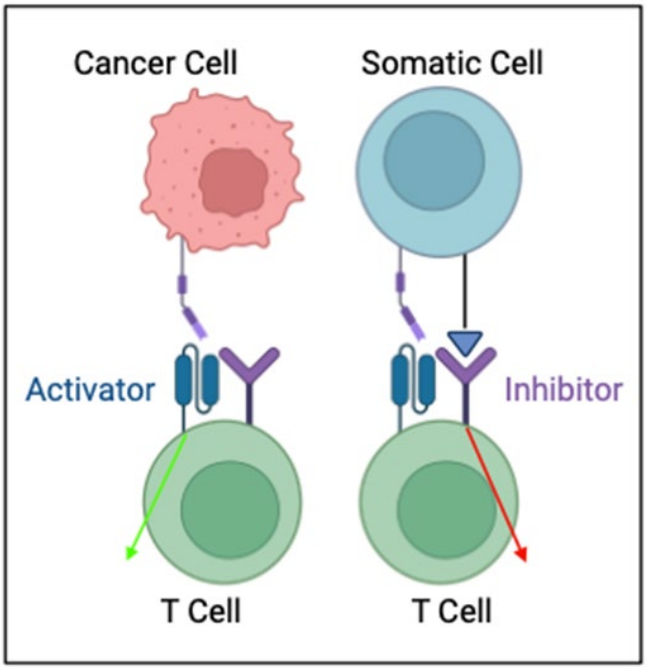

Figure 3. Utilizing an inhibitory NOT logic gate. In this NOT gate the inhibitor that is integrated into the T cell is not activated by the cancer cell. The cancer cell only possesses the antigen specific to the CAR construct, as such the CAR T cell is activated and begins to terminate which is seen with the green arrow. While the somatic cell possesses this antigen it also has unique proteins that activate the T cell's inhibitor, suppressing the termination of this cell which is seen with the red "stop" arrow.

Conclusion

Hematological cancers, which occur when blood cells begin to multiply and grow out of control, remain a major public health issue and a therapeutic challenge. Traditional first line (chemotherapy) and relapse (allogeneic bone marrow transplant) are not always effective. If the cancer relapses, then the third step includes immunotherapy treatments like CAR T cell therapies, which can be sourced from either autologous or allogeneic donors. While very promising, there are a few issues associated with these novel treatments like antigen escape and OTOT, for which exciting and highly effective, complex immunological solutions are being investigated. Antigen escape occurs when the tumor develops a resistance to the CAR T cells; current remedies include

bispecific CAR T cells and a tandem CAR construct. OTOT occurs when the target antigen is expressed on non-malignant cells which results in targeting of these healthy somatic cells. Toxicity on these somatic tissues carrying low levels of the target antigen can be highly detrimental for these patients. Current solutions for this issue include epitope editing where the target antigen is removed from the somatic cells without affecting its function. Another solution is logic gating where the CAR is implanted with a NOT gate that is activated by antigen only present on non-malignant cells; once this NOT gate is activated the CAR becomes inhibited and the somatic cells are not terminated. While these solutions are proving to be viable the world of CAR T is constantly developing and will continue to until cancer can be effectively cured.

References

Allart-Vorelli, P., Porro, B., Baguet, F., Michel, A., & Cousson-Gélie, F. (2015). Hematological cancer and quality of life: A systematic literature review. *Blood Cancer Journal*, *5*(4), e305. https://doi.org/10.1038/bcj.2015.29

Casirati, G., Cosentino, A., Mucci, A., Salah Mahmoud, M., Ugarte Zabala, I., Zeng, J., Ficarro, S. B., Klatt, D., Brendel, C., Rambaldi, A., Ritz, J., Marto, J. A., Pellin, D., Bauer, D. E., Armstrong, S. A., & Genovese, P. (2023). Epitope editing enables targeted immunotherapy of acute myeloid leukemia. *Nature*, *621*(7978), 404–414. https://doi.org/10.1038/s41586-023-06496-5

Depil, S., Duchateau, P., Grupp, S. A., Mufti, G., & Poirot, L. (2020). 'Off-the-shelf' allogeneic CAR T cells: Development and challenges. *Nature Reviews Drug Discovery*, *19*(3), 185–199. https://doi.org/10.1038/s41573-019-0051-2

DiAndreth, B., Hamburger, A. E., Xu, H., & Kamb, A. (2022). The Tmod cellular logic gate as a solution for tumor-selective immunotherapy. *Clinical Immunology*, *241*, 109030. https://doi.org/10.1016/j.clim.2022.109030

Drent, E., Themeli, M., Poels, R., de Jong-Korlaar, R., Yuan, H., de Bruijn, J., Martens, A. C. M., Zweegman, S., van de Donk, N. W. C. J., Groen, R. W. J., Lokhorst, H. M., & Mutis, T. (2017). A Rational Strategy for Reducing On-Target Off-Tumor Effects of CD38-Chimeric Antigen Receptors by Affinity Optimization. *Molecular Therapy*, *25*(8), 1946–1958. https://doi.org/10.1016/j.ymthe.2017.04.024

Flugel, C. L., Majzner, R. G., Krenciute, G., Dotti, G., Riddell, S. R., Wagner, D. L., & Abou-el-Enein, M. (2023). Overcoming on-target, off-tumour toxicity of CAR T cell therapy for solid tumours. *Nature Reviews Clinical Oncology*, *20*(1), 49–62. https://doi.org/10.1038/s41571-022-00704-3

Guillaume, T., Rubinstein, D. B., & Symann, M. (1998). Immune Reconstitution and Immunotherapy After Autologous Hematopoietic Stem Cell Transplantation. *Blood*, *92*(5), 1471–1490. https://doi.org/10.1182/blood.V92.5.1471

Hildreth, A. D., & O'Sullivan, T. E. (2019). Tissue-Resident Innate and Innate-Like Lymphocyte Responses to Viral Infection. *Viruses*, *11*(3), 272. https://doi.org/10.3390/v11030272

Jiang, Y., Li, Y., & Zhu, B. (2015). T-cell exhaustion in the tumor microenvironment. *Cell Death & Disease*, *6*(6), Article 6. https://doi.org/10.1038/cddis.2015.162

Larson, S. M., Walthers, C. M., Ji, B., Ghafouri, S. N., Naparstek, J., Trent, J., Chen, J. M., Roshandell, M., Harris, C., Khericha, M., Schweppe, T., Berent-Maoz, B., Gosliner, S. B., Almaktari, A., Ceja, M. A., Allen-Auerbach, M. S., Said, J., Nawaly, K., Mead, M., … Chen, Y. Y. (2023). CD19/CD20 Bispecific Chimeric Antigen Receptor (CAR) in Naive/Memory T Cells for the Treatment of Relapsed or Refractory Non-Hodgkin Lymphoma. *Cancer Discovery*, *13*(3), 580–597. https://doi.org/10.1158/2159-8290.CD-22-0964

Maman, S., & Witz, I. P. (2018). A history of exploring cancer in context. *Nature Reviews Cancer*, *18*(6), 359–376. https://doi.org/10.1038/s41568-018-0006-7

Melenhorst, J. J., Chen, G. M., Wang, M., Porter, D. L., Chen, C., Collins, M. A., Gao, P., Bandyopadhyay, S., Sun, H., Zhao, Z., Lundh, S., Pruteanu-Malinici, I., Nobles, C. L., Maji, S., Frey, N. V., Gill, S. I., Loren,

A. W., Tian, L., Kulikovskaya, I., … June, C. H. (2022). Decade-long leukemia remissions with persistence of CD4+ CAR T cells. *Nature*, *602*(7897), Article 7897. https://doi.org/10.1038/s41586-021-04390-6

Mosti, L., Langner, L. M., Chmielewski, K. O., Arbuthnot, P., Alzubi, J., & Cathomen, T. (2021). Targeted multi-epitope switching enables straightforward positive/negative selection of CAR T cells. *Gene Therapy*, *28*(9), Article 9. https://doi.org/10.1038/s41434-021-00220-6

Rafiq, S., Hackett, C. S., & Brentjens, R. J. (2020). Engineering strategies to overcome the current roadblocks in CAR T cell therapy. *Nature Reviews Clinical Oncology*, *17*(3), 147–167. https://doi.org/10.1038/s41571-019-0297-y

Sterner, R. C., & Sterner, R. M. (2021). CAR-T cell therapy: Current limitations and potential strategies. *Blood Cancer Journal*, *11*(4), 69. https://doi.org/10.1038/s41408-021-00459-7

Sweeney, C., & Vyas, P. (2019). The Graft-Versus-Leukemia Effect in AML. *Frontiers in Oncology*, *9*, 1217. https://doi.org/10.3389/fonc.2019.01217

Tousley, A. M., Rotiroti, M. C., Labanieh, L., Rysavy, L. W., Kim, W.-J., Lareau, C., Sotillo, E., Weber, E. W., Rietberg, S. P., Dalton, G. N., Yin, Y., Klysz, D., Xu, P., de la Serna, E. L., Dunn, A. R., Satpathy, A. T., Mackall, C. L., & Majzner, R. G. (2023). Co-opting signaling molecules enables logic-gated control of CAR T cells. *Nature*, *615*(7952), Article 7952. https://doi.org/10.1038/s41586-023-05778-2

Wellhausen, N., O'Connell, R. P., Lesch, S., Engel, N. W., Rennels, A. K., Gonzales, D., Herbst, F., Young, R. M., Garcia, K. C., Weiner, D., June, C. H., & Gill, S. I. (2023). Epitope base editing CD45 in hematopoietic cells enables universal blood cancer immune therapy. *Science Translational Medicine*, *15*(714), eadi1145. https://doi.org/10.1126/scitranslmed.adi1145

Yates, L. R., & Campbell, P. J. (2012). Evolution of the cancer genome. *Nature Reviews Genetics*, *13*(11), Article 11. https://doi.org/10.1038/nrg3317

Zoine, J. T., Moore, S. E., & Velasquez, M. P. (2022). Leukemia's Next Top Model? Syngeneic Models to Advance Adoptive Cellular Therapy. *Frontiers in Immunology*, *13*, 867103. https://doi.org/10.3389/fimmu.2022.867103