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Mechanisms of resistance to CAR T cell therapies

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Abstract

Chimeric antigen receptor (CAR)-engineered T cells have demonstrated remarkable success in the treatment of B cell malignancies. FDA approval of these therapies represents a watershed moment in the development of therapies for cancer. Despite the successes of the last decade, many patients will unfortunately not experience durable responses to CAR therapy. Emerging research has shed light on the biology responsible for these failures, and further highlighted the hurdles to broader success. Here, we review the recent research identifying how interactions between cancer cells and engineered immune cells result in resistance to CAR therapies.

Keywords

Chimeric antigen receptor; resistance

Introduction

The first landmark clinical studies of chimeric antigen receptor (CAR)-engineered T cells in the treatment of hematologic malignancies were published less than a decade ago.^{1–3} Two of these studies report outcomes in three adult patients with relapsed or refractory chronic lymphocytic leukemia (CLL), a recalcitrant disease that is highly chemotherapy resistant,

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Conflicts of interest

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treated as part of a phase I study of CD19-targeted CAR T cells. All three patients experienced disease regression, and two achieved complete disease remission. These remarkable results paved the way for trials in pediatric patients with relapsed and refractory acute lymphoblastic leukemia (ALL), another highly challenging disease setting. Independent studies performed at multiple centers have demonstrated remarkably similar outcomes of CD19 CAR T cells in pediatric ALL, with complete remission rates consistently between 70–90%.^{4–7} Investigation of CD19 CAR T cells has been expanded to non-Hodgkin lymphoma, where response rates have been more modest than those seen in ALL, however remain impressive.^{1,8,9} These results led to the FDA approval of two CD19 CAR T cell products in 2017: axicabtagene ciloleucel, approved for adults with relapsed or refractory non-Hodgkin lymphoma, and tisagenlecleucel, approved for the same indication in adults as well as in children with relapsed ALL. Integration of these new treatments into the clinical armamentarium has fundamentally changed the approach to patients with difficult-to-treat disease, and the flood of clinical trials evaluating new CAR-based products provides a glimpse into the future of this therapy and its role in modern management of hematologic malignancies.

We are now nine years removed from the initial reports of success using CAR T cells, and larger clinical trials have had time to mature. Close examination of the clinical trial data in lymphoma and ALL reveal important clues into the nature of CAR T cell success and failure. Outcomes in Non-Hodgkin's Lymphoma (NHL) and chronic lymphocytic leukemia (CLL) reveal that clinical failures are primarily characterized by an up-front lack of disease response. Patients that experience anti-tumor responses, however, appear to maintain those responses (Figure 1), with long-term remission occurring in ~26–40% of patients treated. Intriguingly, outcomes in patients with ALL appear quite different. Long-term data confirm that ~80% of patients will achieve complete remission, but that ~40% of these patients will eventually relapse. Furthermore, targeted treatment of multiple myeloma with anti-B cell maturation antigen (BCMA)-specific CARs contributes to our experience in the field and provides additional insight into mechanisms of response and resistance to autologous, CAR-engineered T cell therapies. This difference in durability of response has many potential mechanisms, related to tumor-intrinsic and -extrinsic pathways to resistance.

In this review, we summarize the most recent clinical and pre-clinical work that has been done to identify the tumor-intrinsic and -extrinsic mechanistic bases of resistance to CAR T cell therapy. We focus on how interactions between tumor cells and T cells govern failure of these therapies, using models of both hematologic and solid malignancies. Finally, we highlight exciting areas of active research that may challenge traditional dogma about the fundamental mechanisms driving resistance.

Tumor-intrinsic resistance

Genetic mechanisms—A large proportion of patient with ALL treated with CD19 CAR T cells will relapse after achieving an initial complete remission. A seminal observation was that many of these relapsed leukemias demonstrated lack of surface CD19 expression (Figure 2).^{5,10} CD19 status is usually assessed by flow cytometry of CD19 surface expression,¹¹ categorizing patients as either CD19+ or CD19-. In CD19+ relapse surface

CD19 is maintained and relapse is instead associated with lack of persistence of CAR T cells, often due to poor T cell function as discussed later in this review. In CD19- relapse, the absence of CD19 on the cell surface enables the tumor to evade CAR T-mediated recognition and clearance, despite CAR T cell persistence. Retreatment with an anti-CD19 therapy has not been successful for CD19- relapse, and patient outcomes are poor.

To date, there have been multiple reported mechanisms for CD19- relapse, including antigen escape via loss of function mutations accompanied by loss of heterozygosity (LOH) at the *CD19* locus,¹² alternative splicing of *CD19*,¹³ lineage switching,^{14,15} and CD19 masking.¹⁶ There have also been reports of antigen loss by dysregulated membrane trafficking of CD19¹⁷ and reduction of target cell antigen concentration by trogocytosis.¹⁸

The combination of loss of function mutations coupled with LOH in *CD19* has been identified as a primary resistance mechanism for CD19- relapse in two phase II anti-CD19 CAR T cell clinical trials in children and young adults with B-ALL.¹² A cohort of 12 patients with relapsed leukemia that entirely or almost entirely lacked surface CD19 expression was evaluated. DNA and/or RNA sequencing was performed on bone marrow and/or peripheral blood specimens collected at the time of screening and within 14 days of relapse from each of the 12 patients. DNA and RNA sequencing were used to identify short nucleotide variants (SNVs), as well as insertions or deletions across CD19 and other B cell related genes. DNA sequencing was further utilized for LOH and focal copy number alteration calling. At the time of relapse, leukemias from all patients were identified to harbor mutations in the CD19 extracellular or transmembrane (TM) coding regions that were not present at the time of screening. In addition to genetic mutations, LOH at *CD19* was observed in a majority of patients at relapse, suggesting a mechanism for biallelic gene silencing. The authors concluded that mutations were present in the vast majority of resistant ALL cells, and predictive modeling suggested that these specific mutations lead to either a truncated or non-functional TM domain, with consequent loss of cell surface CD19.

In a preceding report, Sotillo et al. performed DNA and RNA sequencing on relapse specimens from four CD19- relapsed leukemias. Loss of function mutations in *CD19* after disease relapse were reported in three of the patients and LOH was reported in two of these three patients. The one wild-type relapse was found in a later report to be resistant due to CD19 masking.¹⁶ The authors hypothesized that the frequency of mutations found in the three relapse patients alone could not fully explain the complete loss of surface CD19. RNA sequencing analysis suggested the existence of *CD19* isoforms with alternative splicing of exon 2, encoding the binding site of the anti-CD19 CAR, or exons 5 and 6, encoding the transmembrane domain. *In vitro* modeling demonstrated that increased alternative splicing of exon 2, 5 and 6 in *CD19* led to loss of surface CD19 expression. The authors hypothesized that exon 2 splicing eliminates the epitope binding site, and that employment of a CAR T targeting an alternative CD19 ectodomain (outside of exon 2) would be a possible approach for treating these patients.¹³

CD19- relapse has also been observed following other CD19-directed immunotherapies, suggesting that CD19 loss is not CAR-specific but driven by target-dependent selective pressure.^{19,20} It is unknown whether the mechanism of CD19 loss are the same in CAR T

cell therapy and other CD19-targeting modalities. In one study, 5/17 patients treated in three phase 2 and one phase 3 clinical trials with blinatumomab, a CD19-directed monoclonal antibody, were found to have *CD19* mutations. 4/5 patients had tumor samples compatible with LOH and in 4/4 patients LOH was present in *CD19*.²¹ The implication from this study is that some patients treated with blinatumomab experience CD19- relapse from *CD19* mutations with LOH, as seen with CAR T cell treated patients. However, other patients in this study did not appear to have mutations in *CD19*. The authors suggested that this may be due to stronger selection pressure from CAR T cell therapy compared to blinatumomab, which also has a lower incidence of CD19- relapse. The 12/17 patients without mutations may lose CD19 protein expression by alternative splicing of *CD19*, or other post-transcriptional mechanisms. In another study of a CD19- relapse following blinatumomab, disrupted CD19 membrane export was identified. Interestingly in this study, neither *CD19* mutations nor alternative splicing of *CD19* at the time of relapse were identified.¹⁷ As CD19-targeting strategies expand, it will be important to integrate knowledge about resistance mechanisms to gain a more complete understanding of what drives CD19 loss in each treatment setting.

It remains unclear if the genetic mechanisms driving CD19- relapse exist in cancer cells *a priori* or are acquired following treatment. In Orlando et al. and Sotillo et al., *CD19* mutational and transcriptional variants were not detected in the pre-treatment samples.^{12,13} However, B-ALL cells with *CD19* variants may exist prior to treatment at frequencies that are below the limit of detection of next generation sequencing. Treatment with CD19-directed immunotherapy may allow selective survival of pre-existing CD19-negative clones. An alternative hypothesis is that the *CD19* variants are acquired post-treatment upon selective pressure of the therapy. MRD detection via deep IgH-V(D)J next generation sequencing may now shed light on these questions.²²

Finally, overcoming resistance driven by target antigen loss is not a unique challenge to CD19 targeted therapies but has been seen across a wide range of targeted therapies. For example, loss of *CD20* expression was seen after treatment with rituximab, a monoclonal anti-CD20 antibody.²³

Much work is now being focused on methods to avert CD19- relapse. Since mutations have been reported throughout *CD19*, retreatment or targeting an alternative CD19 ectodomain is unlikely to be successful. Significant effort is being dedicated to dual-antigen targeting as a means of evading antigen-negative relapse. In Orlando et al., all CD19- relapsed disease was CD22+ at screening and continued to have CD22+ cells present at relapse, suggesting that a combination CAR approach, such as anti-CD22 CAR T cells in combination with CAR19 therapy, may be effective.

Antigen density—Unlike peptide antigen-specific CD4+ or CD8+ T cell responses where mutations in the antigen processing and presentation machinery have frequently been observed,²⁴ those pathways do not play any role in the surface expression of CAR-targeted antigens. Instead, the mechanism of escape from CAR T cell detection likely reflects the functional avidity of the CAR. For instance, work from our laboratory (Zhao Y, Lundh, Maus, Melenhorst, June et al., unpublished observations) has revealed that minute quantities

of CD19 protein are required to elicit lytic activity by CAR T-cells. Hence, only complete loss of antigen allows a CD19-expressing tumor to escape CAR T cell detection.^{4,5,10} Over the years both epigenetic^{14,15} and genetic^{12,13} mechanisms have been elucidated in this setting as outlined elsewhere in this paper.

Antigen escape in CAR T-cell therapies targeting the B cell antigen CD22, however, appear to be mechanistically distinct. Fry and colleagues recently reported their findings of anti-CD22 CAR T cell therapy in B-ALL wherein 12/21 patients achieved complete remission; 9/12 experienced an MRD-negative CR. Eight of 12 patients, however, relapsed 1.5–12 months post-infusion.²⁵ Intriguingly, all relapses were associated with reduced CD22 protein expression. Genomic analysis demonstrated that CD22 copy number profiles remained stable in the two patients studied, and none of the samples revealed the acquisition of mutations within the CD22 locus. RNA-sequencing analysis of samples prior to and following CAR T cell infusion did not identify a reduced CD22 expression level after treatment. Furthermore, whereas Sotillo and colleagues previously identified alternative splicing as a contributing factor to anti-CD19 CAR T cell escape¹³, Fry and colleagues observed no evidence of alternative CD22 isoforms underlying the reduced CD22 protein expression levels on tumor cells. These data therefore suggested that CD22 surface protein expression levels on tumor cells were regulated at the post-transcriptional level. A pre-clinical model consisting of a human ALL cell line reconstituted with low, intermediate, or high CD22 expression levels showed that mice infused with CD22^{low} leukemia cells demonstrated negligible responses to CD22 CAR T cell therapy. Things findings suggest that the role of antigen density may vary depending on the target antigen, the CAR itself, or both.

CD20-specific CAR T cells also appear to require higher antigen expression levels for full activation.²⁶ Beyond surface recognition of target antigens, binding of soluble antigen to the CAR may impede the ability of CAR T cells to eliminate target cells. A recent report brings this hypothesis into question for CAR T cells targeting the plasma cell protein B cell maturation antigen (BCMA). This protein is over-expressed by myeloma cells, and in some cases at several logs higher than on normal plasma cells in healthy individuals. BCMA is, however, also shed by plasma cells and can be used as a biomarker for myeloma tumor burden. Theoretically, high levels of soluble BCMA could interfere with CAR T cell function by coating CAR T cells and thereby function as an “antigen masking” mechanism. However, no evidence exists that this negatively impacts BCMA-targeted CAR T cells,²⁷ and the growing number of clinical trials targeting BCMA will certainly shed more light on this evolving topic.

Antigen masking—The current method of manufacturing autologous T cell therapy products begins with collection of peripheral blood leukocytes via leukapheresis, followed by T cell enrichment. T cells are then activated using CD3/CD28 stimulatory beads, and the CAR transgene is delivered using a viral gene therapy vector (most often retrovirus or lentivirus). Engineered cells are then expanded *in vitro* and formulated/cryopreserved at the conclusion of manufacturing. The variation of starting material of patients comes from difference in composition of T lymphocytes (differences in memory subtypes, expression of exhaustion markers^{28,29} and number of regulatory T cells³⁰) and non-T cell leukocytes. The

presence of non-T cell leukocytes such as myeloid cells, monocytes and granulocytes from patients may dampen the T cell stimulation and expansion during the manufacturing^{31–35}. In addition, circulating malignant cells may also serve as another inhibitor of T cell proliferation²⁸. In the absence of fluorescence- or magnetic cell sorting, common density- and size-based methods are incapable of separating T cell subsets from non-T leukocytes prior to genetic engineering. Thus, it is still plausible that non-T cell leukocytes present in the apheresis product could undergo genetic engineering during CAR T cell manufacturing. Despite these variations in the starting material, multiple groups have demonstrated that final manufactured products are regularly >80% T cells, despite variations in manufacturing protocols themselves^{36–38}.

Our group recently reported the unintentional transduction of a single leukemia cell with the anti-CD19 CAR transgene, and re-infusion of CAR19 T cells along with CAR19-modified tumor cells in a patient with B-cell ALL¹⁶. This case was brought to light after the patient was identified to have relapsed with a seemingly CD19-negative leukemia. Molecular analysis revealed that these leukemic cells did not harbor mutations in *CD19* or the co-receptor *CD81*, necessary for CD19 membrane delivery, or demonstrate evidence of alternative *CD19* splicing. Intriguingly, while CD19 CAR T cells underwent contraction in the peripheral blood, qPCR analysis revealed persistence of the CAR transgene. Further investigation demonstrated that the relapsed ALL had been engineered with two copies of the CD19 CAR transgene, which resulted in the persist expression of the CAR in *cis* with CD19 on the leukemic cell surface. Binding of the CAR to CD19 on the same cell resulted in masking of the CAR epitope on CD19, preventing detection by CD19-targeted CAR T cells, and allowing escape of a seemingly antigen-negative leukemic clone. This finding, although the only such identified case thus far, highlights the urgent need for increasing the efficiency of T cell enrichment prior to genetic transduction during manufacture, specifically as clinical CAR T cell manufacturing is broadened to more centers. This also underscores the need for the development of consistent manufacturing processes that will prevent such cases in the future.

Tumor microenvironment—The tumor microenvironment (TME) encompasses both non-cancerous cells and non-cellular tissue. In brain tumors, these components include myeloid cells, with a macrophage predominance, endothelial cells, pericytes, and fibroblasts.³⁹ Little is known regarding the response of the TME to immunotherapies, other than that it plays a significant role in tumor response.⁴⁰ There have been very few neoadjuvant clinical trials that allow for surgical sampling of tumor tissue and in-depth analysis of the TME after immunotherapy, including immune checkpoint blockade, peptide vaccines or cell therapies. Additionally, relatively few CAR T cell clinical trials have evaluated TME composition after treatment. In glioblastoma (GBM), baseline immunosuppression within the TME has been demonstrated in several studies.^{41,42} Systemic delivery of Epidermal Growth Factor Receptor variant III (EGFRvIII)-targeted CAR T cells in patients with refractory GBM has demonstrated significant limitations in T cell entry, *in situ* proliferation and activation, as well as EGFRvIII antigen loss.⁴⁰ Evaluation of primary patient tissue collected 7–14 days after CAR T cell infusion revealed that functional CAR T activation was accompanied by a robust immunosuppressive response that was felt to limit efficacy of CAR T cell infusion.

The GBM TME showed induction of PD-1, PDL-1 and IDO-1 along with a large FoxP3⁺ T cell population, suggesting significant upregulation of immunosuppressive surface proteins as well as regulatory T cell infiltration. An ongoing Phase I trial targeting ROR1-expressing non-small cell lung cancers and triple negative breast cancers found upregulation of immunosuppressive receptors on the CAR T cells at the time of peak expansion.⁴³ Conversely, CEA-targeted CAR T cells isolated from liver metastases of CEA⁺ tumors demonstrated a decrease in PD-L1 and IDO.⁴⁴ The CAR T cells were administered directly to the hepatic blood supply and the resultant CAR T activity promoted a regional pro-inflammatory milieu in the vicinity of the tumors. Collectively, these observations suggest that the microenvironment may play distinct roles depending on tumor histology.

A significant limitation in modeling of CAR T cell efficacy is preclinical evaluation in immunodeficient NOD-scid-IL-2R γ ^{null} (NSG) mice.⁴⁵ Interactions between the TME, tumor, and CAR T cells are limited by the lack of endogenous immune system. Despite these limitations, important observations regarding immune cell interactions can be made using the NSG system. Several *in vivo* studies in NSG mice have shown immune checkpoint upregulation upon tumor challenge with CAR T cells. CAR T cells targeting EGFRvIII or IL13R α 2 demonstrated increased expression of immunosuppressive surface markers PD1, TIM3 and CTLA4 after co-culture with patient-derived xenograft D270 glioma models.⁴⁶ This *in vitro* assessment was corroborated by increased survival in D270-bearing animals when CAR T cells were combined with distinct immune checkpoint blockade. In a complementary study, hepatocellular carcinoma cells (PLC/PRF/5) exhibited increased PD-L1 expression when treated with GPC3-targeting CAR T cells.⁴⁷

Several important points emerge from these early observations. First, acquisition of tissue post-CAR T treatment will continue to provide critical insight into comprehensive TME responses to adoptive cell therapy. This may require prioritizing different clinical trial designs, with an emphasis on surgical resection and TME evaluation after CAR delivery. This approach has already provided valuable insight into tumor infiltrating lymphocyte composition after peptide vaccine therapy⁴⁸ and checkpoint blockade therapy.⁴⁹ Second, if additional studies confirm that the response of the TME in solid cancers is immunosuppressive and antagonistic, additional hurdles to successful treatment will need to be defined. Lastly, technologies now exist to explore the TME in great depth and at the single-cell level to more articulately define the cellular architecture of the TME pre- and post-adoptive cell therapy. Rational design of future immuno-oncology combinations that may be more likely to result in clinical efficacy should follow.

T cell failure

CAR immunogenicity—Persistence of infused CAR T cells is an important correlate of anti-tumor efficacy. Until recently, most CARs evaluated clinically have derived their single chain variable fragment (scFv) from non-human species, and mounting evidence suggests that this may play a direct role in limiting persistence of adoptively transferred cells. Kershaw et al.⁵⁰ targeted the alpha-folate receptor, overexpressed on ovarian cancer cells, using a first-generation CD3 ζ CAR. The scFv was based on the murine monoclonal antibody (mAb) MOv18. In this phase I trial, three of the tested six cases developed an

inhibitory serum factor which blocked the anti-tumor response (**Figure 3**). Interestingly, protein G pre-incubation in the post-treatment serum completely removed this inhibitory effect, suggesting that anti-CAR antibodies were responsible for this inhibition. Similarly, Lamers et al.^{51,52} targeting renal cell carcinoma with an anti-carbonic anhydrase-IX (CAIX) first-generation murine scFv (mAb G250)-based CAR observed persistence for a few weeks. They demonstrated that most patients developed anti-CAR B cell and T cell responses. Similar observations were made targeting CD19 and CD20 CARs.⁵³ Though these studies revealed an adaptive immune response after CAR T cell infusion, these data were correlative in nature. Furthermore, the limited persistence observed of the infused cells could also be explained by the design of the CAR, which lacked a co-stimulatory domain. More recently 17 patients with multiple myeloma were treated with a 4-1BB/CD3 ζ -signaling CAR containing two B cell maturation antigen (BCMA)-specific, camelid-derived antibody fragments.⁵⁴ The data indicated that among seven patients harboring a high-level of anti-CAR antibody response, six cases relapsed or progressed 6 months after infusion. This incidence was significantly higher than that in patients without detected humoral immunogenicity. In patients with a high proportion of anti-CAR antibody, the CAR-T levels were very low. A similar clinical correlation between reduced clinical efficacy and CAR immunogenicity were made by Turtle et al., who demonstrated that the re-infusion of lymphoma and ALL patients with the original second-generation CAR did not result in their expansion and tumor reduction.^{9,55} These authors were able to map the anti-CAR T cell response to a specific domain in the murine FMC63-based CAR, offering the opportunity of de-immunizing this CAR for enhanced clinical efficacy.⁵⁶

Several groups have used cytoreducing agents to enhance engraftment and prolong the tumor-targeting effect of CAR T cells. Indeed, the addition of fludarabine to the lymphodepletion regimen strongly enhanced the CAR-T cells proliferation and improved response duration.^{9,55,57} One trial in multiple myeloma found that among seven patients treated with cyclophosphamide plus fludarabine, only one patient had an anti-CAR immune response, while in 8 evaluated patients receiving single agent cyclophosphamide, 6 had detectable anti-CAR responses. This evidence further indicated that fludarabine may reduce, inhibit, or delay anti-CAR antibody production.⁵⁴ However, the underlying mechanism of fludarabine in reduced immunogenicity deserves further investigation.

Since the possibility of reduced anti-tumor activity of a non-human antibody-based CAR looms large over the field, researchers have begun to develop fully human CAR to overcome the issue of transgene immunogenicity.^{58,59} A fully human scFv-based anti-CD19 CAR (JCAR021) has been initially investigated in 7 patients with ALL or NHL enrolled in a phase I trial ([NCT03103971](#)). The clinical toxicity was favorable and 4 out of 7 patients achieved a partial or complete response. Importantly, JCAR021 persisted in peripheral blood for >4 months post infusion in all patients.⁶⁰ Other groups have also transitioned to using humanized or fully human CAR T cells targeting BCMA^{61,62} or mesothelin ([NCT03054298](#) and [NCT03323944](#)). Although it remains pre-mature to draw conclusions based on these early trials, trends suggest that use of human scFvs may enhance CAR T cell function.

T cell product dysfunction—Unlike conventional therapies, CAR T cells and other cell-based therapeutics are influenced by conditions that affect cellular fitness, such as metabolic

stress, the presence of immune-suppressive cytokines and cell-to-cell interactions. In addition, intrinsic cell fitness must be considered, as CAR T cells made from “low quality” cells have been shown not to perform as well as CAR T cells made from “high quality” cells.^{63,64} The effect of T cell quality on CAR T cell therapy is best underscored by comparing the differences in response between CART19-treated ALL patients and CLL patients. While many factors differentiate ALL from CLL, the same CART19 products that yield >80% complete remissions in pediatric ALL result in a response rate of only 26% in adults with CLL, highlighting a stark distinction in efficacy between these two cell therapy products.⁶⁵ Beyond fundamental differences in cancer cell biology, it has been shown that cancer type has a direct influence on CAR T cell fitness.^{64,66}

Several factors likely contribute to the development of T cell dysfunction in CLL. First, CLL patients have a median age of diagnosis of 70 years, making their T cells chronologically older and less fit as a result of a waning immune system and a higher proportion of terminally differentiated effector memory cells.⁶⁷ These cells inherently have limited immune responsiveness and secrete cytokines such as IFN γ and IL-4 that support the growth of the tumor cells and have been shown to induce the expression of anti-apoptotic proteins such as BCL-2 in CLL cells themselves.^{68,69} These cells also demonstrate signs of functional impairments beyond simple aging; previous work has shown that chronic lymphoid malignancies directly deplete the circulating naïve T cell pool.⁶⁴ Further highlighting the importance of T cell quality as defined by memory state, it is possible to predict which CLL patients will respond based on the frequency of naïve or early memory cells present prior to CAR T cell manufacturing; patients with a higher proportion of naïve or early memory CD8⁺ T cells have a greater response rate than patients with few or no such T cells.⁶³

In addition to skewing of memory populations, T cells from CLL patients demonstrate impaired cytotoxic function, impaired immune synapse formation, low-level, dysregulated cytokine production, and prolonged expression of inhibitory receptors.^{28,70} This dysfunctional state shares similarities to the classical T cell exhaustion seen in chronic infection models.⁷⁰ Actin polymerization and polarization are critical for the formation of the immune synapse, and exposure to CLL has been shown to be sufficient to impair synapse formation in even healthy donor T cells.^{71,72} This co-culture alters Rho GTPase signaling in T cells by upregulating Cdc42 and downregulating Rac1 and RhoA. Cdc42 is a negative regulator and Rac1/RhoA are positive regulators of the integrin LFA-1, a critical component of the immune synapse. In concert, this results in significantly decreased adhesive function of LFA-1, preventing effective synapse formation.⁷³

Altered cytokine production of T cells from CLL patients contributes to tumor escape in several ways. First, while CLL T cells do produce a variety of effector cytokines, the levels of production are significantly lower than those produced by healthy T cells.^{28,63} This may be due to decreased cytokine production or direct uptake of IL-2 by CLL cells themselves. Second, CLL cells are known to exert immunosuppressive cytokine activity, secreting TGF β and serving as an IL-2 sink.^{74,75} This IL-2 sinking further impairs immune responses, as IL-2 is required for full T cell activation. Furthermore, CLL cells express and release high level of the inhibitory molecule CD200.⁷⁶ Tumor cells from poor prognosis CLL patients

expressed significantly higher levels of this inhibitory molecule.⁷⁷ Additionally, CD200 blocking stabilized T cell-CLL cell interactions, suggesting that the CD200-CD200R axis contributes to reduced T cell reactivity against CLL and possibly other tumors as well. Finally, T cells from CLL patients have high, sustained expression of inhibitory receptors including PD-1, TIM3, and LAG3. Inhibitory receptor expression can both destabilize the immune synapse and suppress functional immune responses.^{67,78,79}

Abortive CAR T cell activation—During a normal immune response, co-stimulatory molecule expression on antigen presenting cells is a requirement for inducing a productive T cell response. When the TCR is engaged in the absence of co-stimulation, T cells become anergic and fail to differentiate into mature cytotoxic T lymphocytes.⁸⁰ CAR T cells are unique in that the chimeric receptor contains co-stimulatory intracellular signaling domains. Currently, there is no evidence that CAR T cells require additional co-stimulation from tumor cells. However, co-stimulatory signaling molecule expression varies by disease type. ALL blasts express high levels of CD86, CD40, CD27, and CD70, whereas CLL cells express low levels of CD80 and CD86, and high levels of CD40.^{81,82} Interestingly, CLL cells can promote the down-regulation of CD40 ligand on CD4 T cells. Activated T cells upregulate CD40, which serves as another important costimulatory factor, and its loss may further blunt T cell responses in this disease. In addition, constitutive CD40 ligand expression on CAR T cells has been shown to enhance their tumor-targeting capability.⁸³ Further work is necessary to determine if the differences in co-stimulatory molecule expression between different tumor types contribute to the poor responses seen in CLL, and potentially other diseases. We may predict that if CAR T cells are deprived of necessary co-stimulatory signals not present on their targets, these cells may become anergic similar to their native counterparts, impairing anti-tumor function.

Conclusions and Future Directions

CAR-based T cell immunotherapy represents a paradigm-shift in the therapeutic approach to patients with cancer. It was recognized as the “Advance of the Year” by the American Society of Clinical Oncology in 2018, highlighting its significance in the field of oncology. Now that the “honeymoon” has ended, however, it is our responsibility to reflect on the current status of this therapy and recognize its limitations.

CAR T cell product manufacturing remains a highly-specialized, labor-intensive and individualized practice. While many centers have become certified to deliver this therapy, it is far from broadly available across the US, not to mention less-developed countries. Beyond the barriers to accessibility, successful manufacturing of a clinically-viable product remains a significant hurdle for many patients.⁶⁴ While these therapies represent an exciting new addition to the oncologist’s armamentarium, how they integrate with current therapies, specifically hematopoietic cell transplantation, remains unresolved. Finally, the high cost also places a new strain on the healthcare system. A recent study has attempted to evaluate cost-effectiveness of this therapy, with mixed conclusions.⁸⁴

Nestled within these other obstacles is the clinical and scientific hurdle of therapeutic failure. The traditional approach to failure has categorized it into two forms: lack of response

or emergence of CD19+ relapse attributed to T cell failure, and CAR immunogenicity, or adaptive resistance and CD19- relapse attributed to tumor evasion (Figure 1). As outlined in this review, several mechanisms have been identified as leading to each of these outcomes. While it has been instructive to consider these as distinct biological entities leading to therapeutic failure, the reality is likely not so simple. Our group has recently identified that tumor-intrinsic qualities can lead to resistance to CAR T cell cytotoxicity, and that this resistance in turn leads to the development of CAR T cell dysfunction and failure as a result of persistent antigen stimulation.⁸⁵ This mechanism highlights the dynamic interplay between T cells and tumor cells that can lead to therapeutic failure, and offers a new model for resistance to CAR T cell therapy.

A great deal of effort has now been dedicated to surmounting resistance. Several groups have developed strategies to target two antigens on the tumor cell surface in an effort to avert antigen-negative escape.^{25,86–88} Targeting of the TME has already shown benefit in the efficacy of CAR therapies in solid tumors,⁸⁹ highlighting a pathway to overcome some of the hurdles imposed by immunosuppressive non-cancerous tissue. Finally, combination therapies are emerging as an exciting approach to overcome limitations in CAR T cell efficacy and toxicity. Thus far these have included ibrutinib,²⁹ dasatinib⁹⁰ and checkpoint inhibitor antagonists;^{91,92} surely this list will continue to grow as novel combinations are evaluated pre-clinical and in patients.

Resistance to CAR T cells is a primary barrier to its broader efficacy. Rapidly evolving research into the mechanisms responsible, as well as strategies to overcome resistance, provide great promise to patients in need of these innovative therapies.

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Figure 1. Identified mechanisms of tumor evasion to CAR T cells.

Summary of recent data of the cancer cell biology responsible for resisting CAR T cells (center, green): (1) loss or reduction of surface antigen expression, (2) masking of target antigen by co-expression of CAR and antigen on the leukemic cell surface, and (3) expression of immunosuppressive surface receptors and secreted factors.

Figure 2. Identified mechanisms of T cell failure.

Summary of recent data on the T cell biology responsible for inherent resistance to CAR T cells. (1) anti-CAR immune responses mediated by the endogenous immune system, (2) low quality of the collected T cells used to manufacture the CAR product, and (3) ineffective co-stimulation of transferred T cells.