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Review Article

Relapse and Resistance to CAR-T Cells and Blinatumomab in Hematologic Malignancies

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ABSTRACT

Application of immunotherapeutic modalities changed paradigms in the treatment of hematologic malignancies, with regards to drug manufacturing, treatment protocols, short- and long-term toxicities. FDA-approved therapies, including blinatumomab, tisagenlecleucel, and axicabtagene ciloleucel, target T cells to attack healthy and malignant cells expressing CD19, leading to high response rates in previously heavily treated patients, and to durable remissions in the absence of further therapies. Nevertheless, despite paucity of long-term data, some patients are resistant to these agents, and many relapse. This review will discuss the mechanisms of failure of these immune-based therapies, and offer guidelines to the practicing physician.

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1. INTRODUCTION

The immunotherapy revolution in hematologic malignancies is primarily due to the success in synthetic immunotherapy—a term applied when an immune response is generated against new targets [1]. Immune-targeting of B-cell specific cell surface markers by bispecific antibodies (BiTEs) or chimeric-antigen receptor expressing T (CAR-T) cells has resulted in high remission rates in previously heavily treated patients, leading to survival advantage, and to FDA approval of blinatumomab, tisagenlecleucel, and axicabtagene ciloleucel for B-cell acute lymphoblastic leukemia (ALL) and lymphoma [2–6]. Early clinical trials of other CAR-T targets against hematologic malignancies are promising, including CD22 CAR-T cells for ALL [7] and BCMA CAR-T cells for multiple myeloma [8]. The common mechanism of action is based on induction of T-cell based cytotoxicity against a predefined target via granzyme and perforin, leading to proliferation of effector and helper T cells, cytokine production, and target cell death [9]. Remission rates reported in ALL vary between 40% and 94% with either blinatumomab or CAR-T cells targeting CD19 or CD22 [2–4,7,10–15]. In non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia (CLL) the rates are slightly lower when patients with measurable disease are treated, and vary between 30% and 60% [5,6,16–20]. The long-term 5+ year follow-up is still missing, especially real-life data. Nevertheless, even in ALL, the disease with the highest response rates to CD19 CAR-T cells, less than 50% of infused patients remain in remission more than one year after CAR-T therapy, in the absence of additional treatment.

Overall, resistance to targeted synthetic immunotherapy can be divided into two major mechanism: T-cell failure, or target-antigen modulation (Table 1).

Table 1 | Resistance mechanisms to CD19 and CD22 CAR-T cells.

	Proposed Mechanism	Ref.
T-Cell Failure		
Production failure	Failure of T-cell expansion and transduction in culture	
Primary T-cell failure	Low memory/stem-cell memory T cells in the leukapheresis	[21]
	Lack of multi-cytokine producing cells	[22]
	Increased exhaustion of cells	[21,23–25]
	Increased regulatory T cells	[26]
Secondary T-cell failure	Nature of the costimulatory domain	[27,28]
	Anti-CAR immune response	[10,13]
	Suppressive microenvironment in extramedullary leukemia	[15,29]
Target Antigen Modulation		
Loss of CD19 expression	Mutations in CD19	[30,31]
	Splice variants of CD19	[30]
	Lineage switch to myeloid leukemia	[32–35]
	Defective trafficking to the cell membrane	[36]
	Leukemia transduction by CAR masking CD19 expression	[37]
CD22 down modulation	Unknown posttranscriptional effects	[7]

Abbreviation: CAR-T cells: chimeric-antigen receptor expressing T cells.

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2. T-CELL FAILURE WITH CAR-T CELLS OR BITES

The generation of CAR-T cells from patient material, namely leukapheresed lymphocytes, adds new layers of complexity to medicinal production, and may result in failure. This occurs in upto 10% of cases, and may be related to low T-cell numbers in the patient (after prior T-cell depleting chemotherapy), poor functionality of autologous T cells in heavily pre-treated patients, or through monocyte-driven suppression during production [38]. Guidelines recommending timing of leukapheresis in regards to recent therapy have been developed, aiming to reduce production failure rates [39]. Several methods for T-cell selection of the initial product, starting with plastic-adherence for monocyte depletion [38], but also CD4:CD8 positive isolation of the starting material may improve transduction and clinical outcome [12,13,40]. Also, the patient may deteriorate during the production time required for CAR-T cells. For example, 17 out of 92 patients enrolled on the ELIANA study did not receive the product, due to production failure (n = 7), clinical deterioration leading to death (n = 7) or adverse events (n = 3) [4].

More commonly, the failure is a result of either primary failure to generate an effective immune response *in vivo* or failure of the T cells to persist. The mechanisms of primary T-cell failures have not been well studied, but are not entirely dose-dependent, as increasing the CAR-T cell dose resulted in increased toxicity without increased response in some studies [10,12]. The PD-1/PD-L1 axis is known to limit response to blinatumomab [23], and may limit CAR-T cells, more often in CD28-containing CAR-T cells [27,41]. However, PD-1 inhibitory antibodies failed to improve expansion or persistence of a third-generation GD-2 CAR-T cells [42]. Early data in ALL show responses to PD-1 inhibition in patients with either loss of B-cell aplasia or extramedullary (EM) relapse, but not in patients with initial CAR-T cell failure [24]. Patients with DLBCL who received axicabtagene ciloleucel with the PD-L1 inhibitor atezolizumab on the ZUMA-6 trial had a similar response rate to those treated with the CAR-T cells alone, but with improved expansion and persistence of the CAR-T cells [43]. Genetic engineering eliminating PD-1 via Crispr/Cas9 targeted *pdcd1* disruption in CAR-transduced T cells may further improve their function *in vivo* and prevent their loss [41]. Addition of checkpoint inhibitors to blinatumomab may improve response rates in nonresponding patients [23,25]. In CLL, where response rates are generally lower, determinants of CAR-T cell phenotype may affect the chances of remission or primary failure. Complete responders had higher proportion of memory $CD8^+CD27^+CD45RO^-$ T cells in the leukapheresis product, and lower percentages of exhausted $CAR^+CD8^+CD27^+PD1^+$ cells in the infusion product [21]. Phenotypic data correlated with gene expression data, where upregulation of genes related to exhaustion, glycolysis and apoptosis was seen in CAR-T products of non-responders [21]. Thus, improved response may be seen if the starting material is selected for stem-cell memory T cells [44]. Recently, using single-cell cytokine analysis, a profile of polyfunctional T cells (producing several cytokines) was identified, and correlated with response to CAR-T cells [22]. This may enable prediction of response to a CAR-T cell-based product.

Secondary failure of CAR-T cells is due to poor T-cell persistence. Three factors were identified so far to affect persistence. A higher disease burden (>15% leukemic blasts) was reported to be associated with improved persistence by the Seattle group [12]. A conditioning regimen comprised of fludarabine and cyclophosphamide (flu/cy) was also shown to improve efficacy and durability of CAR-T cells by several groups [12,13,45]. Though not well studied, improved response after flu/cy conditioning may be a result of depletion of regulatory T cells, known to limit BiTEs [26], which are given without conditioning, or through increase in homeostatic cytokines [46]. The most studied factor to contribute to persistence is the CAR-T costimulatory chain. CAR-T cells encoding a 4-1BB costimulatory moiety are associated with longer persistence than CAR-T cells containing a CD28 costimulatory domain [27], as shown in elegant preclinical models [27,47,48], mostly through effects on cell metabolism and memory subsets. Nevertheless, in ALL, 41BB-CAR-T cells have been reported to have prolonged persistence [4,11,12,49], more than seen with CD28-CAR-T cells [10,15,50]. Interestingly, in DLBCL, long-term persistence was also seen in CAR-T cells with CD28 [5,16,51]. A single small clinical trial has compared head to head these moieties and showed similar response with different kinetics [52]. Third-generation CAR-T cells, combining both moieties, showed better persistence when co-infused with second-generation CD28-contaning CAR-T cells for NHL [28]. It is unclear whether this benefit arises from combining the two moieties, or solely from the 41BB domain.

Another mechanism to limit CAR-T cell persistence is immune rejection of the CAR, as commercially-available CAR-T cells include single-chain variable fractions from murine antibodies. The immune reaction is mostly of T-cell origin (since B cells are depleted), and may be responsible for failures in T-cell re-infusion in patients who lost CAR persistence [10,13]. Early data from humanized versions of CD19 CAR-T cells show success in reinfusion, and may lead to less immune reaction and superior persistence [53,54].

Importantly, long-term persistence is not a guarantee for cure following immunotherapy in ALL. Both CD28-based CAR-T cells and blinatumomab have shown, in patients treated with low-burden disease, long-term survival in the absence of further therapy [50,55,56], and the unique resistance mechanisms described below may lead to relapse despite the presence CAR-T cells. It may be the case that in NHL durable remissions do not require long-term persistence of CAR-T cells [57].

Last, unique extramedullary relapse forms have been seen after blinatumomab [29]. These have been susceptible to CAR-T cells [15], suggesting that their occurrence may be related to leukemia protection by the suppressive microenvironment, which is at least partially targeted via flu/cy conditioning and inherent co-stimulation in CAR-T cells.

3. TARGET-ANTIGEN MODULATION

Long-term persistence, though attractive, may not be sufficient when targeting a single leukemic antigen. CD19 was thought to be essential for B-cell precursor leukemia and lymphoma. Nevertheless, antigen modulation of CD19 and CD22 has been reported by various mechanisms following targeted immunotherapy.

Initially, CD19 mutations as well as exon 2, 4, 5 or 6 alternative splicing were observed to confer resistance, as these alter the immunogenic part of CD19 which is targeted by the CAR and is identified by the flow-cytometry antibodies [30,31]. Surprisingly,

CD19 splicing variants lacking exon 2 appear in leukemia prior to treatment, making it difficult to predict response or resistance prior to CD19-directed therapy [58]. A recent report from Novartis suggested that CD19 mutations, rarely seen before, accounted for the majority of CD19 non-expressing cases [31]. Several groups have reported lineage switch of leukemia to occur following CD19-directed therapy, especially in cases where the leukemia cell of origin is not a precursor B-cell *per se* (such as MLL-rearranged leukemia) [32–35]. Unique mechanisms, such as defects in the CD81-CD19 co-trafficking from the endoplasmic reticulum to the cell surface [36], or co-transduction of leukemia with CAR-T cells thereby CAR-CD19 interactions occurring in cis and masking CD19 presentation from T cells [37], have also been reported. Of note, CD19 loss has also been seen in NHL following CD19 CAR therapy [59,60].

CD22 is the second molecule in ALL targeted by CAR-T cells, showing promising remission rates in patients who have mostly relapsed after CD19-directed therapy [7]. CD22 is a molecule known to internalize upon stimulation, thus serving as a platform for immunotoxins, some of which are FDA-approved [61,62]. CD22 is not as abundant on ALL cells as CD19 [63], and down-modulation was seen in relapse after CD22 CAR, though in the majority of cases this was not complete cell surface negativity, and despite ongoing CAR-T cell persistence [7]. As shown in several models, CAR-T cells require high antigen density on the target cell surface, and a mere reduction in the amount of antigens per cell may result in failure of the CAR-T cells [64].

Despite this variety of antigen loss mechanisms, these occur mostly after prolonged immune pressure, and are thus mostly seen in patients with long-persisting 41BB-containing CAR-T cells or sequential antibody and CAR therapies. Still, the majority of relapse events with blinatumomab or short-term CAR-T cells express CD19 [65].

4. GUIDELINES TO THE CLINICIAN AND FUTURE THOUGHTS

CAR-T cell and BiTE therapies hold many promises in contemporary hematology, and are expected to become a larger part of our practice. Through the study of mechanisms of resistance, we can offer tools for evaluation and, hopefully, prevention of relapse, and to improve future development.

Some measures can be taken to try to prevent primary T-cell failure (table 2). Early referral and leukapheresis before any T-cell lytic therapy is administered will increase the likelihood of successful CAR-T cell production. Lymphodepletion using fludarabine and cyclophosphamide has proven itself as the best regimen to-date prior to CAR-T cell infusion, reducing the rate of primary and secondary T-cell failure. Failure after blinatumomab may be reversed by adding checkpoint inhibitors. Such combinations, as well as combinations of CAR-T cells with checkpoint inhibitors, should still be evaluated in large clinical trials. Selection of T-cell stem-cell memory subsets for production, suggested to be an additional measure to prevent primary T-cell failure, is still complex and has yet to be adopted in real-life clinical settings.

Early detection of CAR failure may be important. Flow-cytometry based minimal-residual disease (MRD) detection is extremely valuable to guide the investigation of resistance mechanisms, as well as for planning future potential therapies based on antigen expression. In ALL, since CD19 expression may be lost, alternative gating strategies not solely based on CD19 expression should be designed, with the goal to identify resistant clones. As these clones may be small, we routinely add VDJ-rearrangement-based MRD techniques (polymerase chain reaction, PCR, or next-generation sequencing, NGS), which may enable earlier detection of resistance, and guide further therapy. In addition to MRD detection, duration of B-cell aplasia as a surrogate marker for CAR persistence can inform on the timing for introduction of additional therapy, such as checkpoint inhibitors or an allogeneic hematopoietic stem cell transplant (HSCT). A consolidative allogeneic-HSCT has shown benefit for short-term CAR-T cells in some studies [15,45], especially in transplant-naïve patients [66]. When using a short-term CAR for ALL, we routinely recommend it [15]. Since most patients referred to CAR-T cell treatment have exhausted standard chemotherapy options, establishing the antigen expression pattern on leukemic blasts is crucial. If CD19 is present, another CD19-targeting CAR may be used if it includes a different ScFv, or if the patient has had an allogeneic HSCT following the first CART cells—to avoid potential anti-CAR-T cells. BiTEs may also be tried. For patients who lack CD19 expression, CD22-targeted therapy is an option, but has so far showed limited durability [7,61].

Table 2 | Recommendations to reduce relapse following CAR-T cell therapy.

Prior to CAR-T cell production:
• Early referral and leukapheresis
• Fludarabine/Cyclophosphamide-based lymphodepletion
Following CAR-T cell administration:
• In ALL, use of PCR or NGS-based MRD detection along with flow cytometry
• Routine CAR persistence monitoring by flow cytometry for B-cell recovery using both CD19 and CD20
• In short-term CAR-T cells, especially in transplant-naïve ALL patients, consider consolidative HSCT

Abbreviations: CAR-T: Chimeric-antigen receptor expressing T cells, ALL: Acute lymphoblastic leukemia, NGS: next-generation sequencing, MRD: minimal-residual disease, HSCT: hematopoietic stem cell transplant.

Finally, similar to how our practice to prevent antibiotic or chemotherapy resistance led to combination therapies, multiple antigen targeting along with immunomodulatory strategies will further improve current results, and several bi-specific CAR-T cells are being tested in clinical trials. Results of these trials, as well as trials combining synthetic immunotherapy with checkpoint inhibitors, may shed more light on the management of relapsed and resistant B-cell malignancies.

CONFLICT OF INTEREST

Advisory board for Novartis Israel.

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