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Does lineage plasticity enable escape from CAR-T cell therapy? Lessons from *MLL*-r leukemia

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Abstract

The clinical success of engineered, CD19-directed chimeric antigen receptor (CAR) T cells in relapsed, refractory B-cell acute lymphoblastic leukemia (B-ALL) has generated great enthusiasm for the use of CAR T cells in patients with cytogenetics that portend a poor prognosis with conventional cytotoxic therapies. One such group includes infants and children with mixed lineage leukemia (*MLL1, KMT2A*) rearrangements (*MLL*-r), who fare much worse than patients with low- or standard-risk B-ALL. Although early clinical trials using CD19 CAR T cells for *MLL*-r B-ALL produced complete remission in most patients, relapse with CD19-negative disease was a common mechanism of treatment failure. Whereas $CD19^{neg}$ relapse has been observed across a broad spectrum of B-ALL patients treated with CD19-directed therapy, patients with *MLL*-r have manifested the emergence of AML, often clonally related to the B-ALL, suggesting that the inherent heterogeneity or lineage plasticity of *MLL*-r B-ALL may predispose patients to a myeloid relapse. Understanding the factors that enable and drive myeloid relapse may be important to devise strategies to improve durability of remissions. In this review, we summarize clinical observations to date with *MLL*-r B-ALL and generally discuss lineage plasticity as a mechanism of escape from immunotherapy.

Cellular and immunotherapy approaches show promise for *MLL*-r B-ALL

Relapsed and refractory B-cell acute lymphoblastic leukemia (B-ALL) remains a leading cause of cancer mortality in children despite the successful iterative development of

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PE owns Amgen stock. TF is employed by Sana Biotechnology and is an inventor on immunotherapy patents owned by the National Institutes of Health.

risk-adapted, multi-agent chemotherapeutic regimens [1]. Years of sophisticated molecular characterization of childhood ALL [2,3] make it possible to identify subsets of patients with a high likelihood of relapse at diagnosis. Among the poor prognostic groups are those with rearrangements of the mixed lineage leukemia (*MLL1, KMT2A*) gene at 11q23 [4,5]. *MLL* rearrangements occur as the initial or only genetic lesion in >75% of infants with B-ALL [1]. Because of the elevated risk of relapse and resistance, the development of targeted therapies has been a priority to improve outcomes for this population. Although multiple small molecule inhibitors thought to be selective for MLL fusion oncoproteins have reached clinical trials, immunotherapy has also begun to have an impact in this patient group.

Two relatively new immunotherapies use the patient's immune system to target the CD19 cell surface protein, which is coupled to B-cell identity and therefore highly expressed on B-ALL. First, blinatumomab is a CD3/CD19 bispecific T-cell engager (BiTE), or bispecific antibody that redirects a patient's T cells to kill CD19+ cells. Blinatumomab was approved by the U.S. Food and Drug Administration (FDA) for relapsed, refractory, or Philadelphia chromosome−positive B-ALL in 2014, and approval was expanded in 2018 for broader use in B-ALL as a second-line treatment [6,7]. Second, chimeric antigen receptors (CARs) recognizing CD19 can direct the patient's T cells to kill CD19+ B-ALL. The CAR construct is introduced into patient T cells during ex vivo manufacturing, which endows the T cell with directed specificity using an antibody-derived target binding domain and T-cell receptor signaling domains (Figure 1). Tisagenlecleucel (formerly CTL019) was approved by the FDA in 2017 for the treatment of relapsed or refractory B-ALL in pediatric/young adult patients based on the remarkable success of the phase II trial (NCT02435849) [8]. Multiple clinical trials using a variety of CD19-directed CAR T cell products have indicated complete remission rates of 70%−90% in pediatric patients with multiply relapsed and/or highly refractory B-ALL [8−12]. Longer follow-up in these trials revealed that patients receiving CD28-containing CAR T cells lost functional CAR T activity within 2 months of infusion. These patients had a high risk of post-CAR relapse without further treatment with a consolidative hematopoietic stem cell transplant [11]. For patients receiving CARs containing the 4–1BB co-stimulatory signaling domain (Figure 1), persistence of CAR T cells could be observed for months to years [8−10]. Follow-up studies of patients who received 4–1BB CAR T cells in clinical trials, as well as in postapproval "real world" studies, have reported that, despite the high initial rate of complete remission, only ~50% of patients remain leukemia-free 1 year after treatment because of post-CAR T cell relapses [9,13−15]. For patients receiving either CD28- or 4–1BB-containing CARs, two major patterns of relapse have been observed: antigen-positive (CD19+) relapse occurring in the absence of ongoing CAR T cell activity, and $CD19^{neg}$ relapse in which the loss of the target antigen allows the leukemic cells to survive and expand in the presence of a persistent and functional CAR T cell population [16]. Multiple studies have now found that patients treated with CD19-directed BiTEs or CD19-directed CAR T cells can relapse with $CD19^{neg}$ disease, which can arise via multiple mechanisms [9,16−18]. A poorly understood mechanism of $CD19^{neg}$ relapse is "lineage switching," in which leukemia undergoes global changes resulting in the loss of multiple lymphoid markers and the acquisition of a myeloid phenotype (Table 1) 18−29,30−33 Lineage switching relapses have been reported after both CD19-directed BiTE and CAR T-cell therapy and tend to be enriched

in *MLL* rearrangements, although cases harboring other translocations have been reported [18,31,33,34]. Because of the relatively recent implementation of immunotherapy and the rarity of *MLL*-r subsets within all B-ALL, it is too early to know whether $CD19^{neg}$ relapse occurs more or less frequently than in other subtypes. However, *MLL* rearrangements are associated with increased risk of relapse in pediatric ALL patients and occur in most infant ALL patients [35]; thus, it is increasingly likely that more children and infants harboring *MLL* rearrangements will receive immune-based therapies, possibly resulting in an increased number of patients experiencing lineage switch relapses. Interestingly, infants with *MLL*-r B-ALL and detectable residual disease at the end of induction chemotherapy may have better outcomes if myeloid consolidation regimens are used, suggesting that preventing myeloid relapse would result in an overall benefit [36]. Understanding the factors contributing to relapse from this otherwise effective therapy will be critical to improving on the initial success of this approach. Below, we address the clinical observations and potential underlying mechanisms of relapse. Specifically, we focus on the concept of lineage plasticity in generating $CD19^{neg}$ relapse in a poor-prognosis patient group in which this phenomenon has been documented.

Lineage identity and plasticity in *MLL*-r B-ALL

Rearrangements of the *MLL1* gene, including internal tandem duplications, occur in adults and children, producing leukemia with mixed myeloid, B-lymphoid, or T-lymphoid characteristics, hence the original designation of “mixed lineage” or “bi-phenotypic” leukemia. Whereas about half of older adults present with acute myelogenous leukemia (AML), the ratio of ALL to AML in infants with *MLL*-r leukemia is nearly 6 to 1 [37]. In infants, approximately 90% of *MLL*-r ALL is arrested at a CD19+ pro-B/pre-B stage [37]. Coexpression of myeloid genes and stage of B-cell differentiation (pro-B/pre-B cells) varies as a function of age of the patient [38]. As early as 1986, undifferentiated, mixed lineage features of *MLL*-r leukemia were appreciated and interpreted to reflect transformation of a multipotent progenitor [39]. The first transcriptional signatures illustrated the distinct identity of pediatric *MLL*-r B-ALL overall, which occupied a position in principal component space (cell identity) between lymphoid and myeloid leukemia [40]. Both immunophenotypic and genomic features of childhood *MLL*-r ALL have been interpreted to indicate that the cell of origin is a primitive fetal progenitor rather than a committed B-cell progenitor, given the association of *MLL* rearrangements with young age [41,42].

It is thus not surprising that *MLL*-r leukemia is common among cases of relapse-related lineage switching [43]. Reviewing pediatric cases from the 1980s to the 2010s, two groups carefully documented lineage switching after chemotherapy and found frequencies of 1%−6% of any lineage switch posttreatment, which was predominantly from pro-/pre-B-ALL to AML [44,45]. *MLL*-r leukemia accounted for 78% of the cases that switched lineages posttherapy in the latter study [45]. These clinical observations suggest an underlying heterogeneity or lineage plasticity inherent in cells transformed by MLL-fusion oncoproteins. The current designation of mixed phenotype acute leukemia (MPAL) includes a specific category for *MLL*-r, as well as BCR-ABL+ leukemia, because of their prevalence in this mixed lineage group [46,47]. Consistent with this concept of high lineage plasticity in MLL-r leukemia, evasion of CD19-targeted immunotherapy through relapse with myeloid

markers has been reported under treatment with blinatumimab [20−27,32] or treatment with CD19 CAR T cells (Table 1) [10,18,30,31,33]. Loss of not only the CD19 cell surface protein but complete loss of all B-lineage markers and acquisition of a myeloid phenotype characterizes these cases (Table 1). In addition to these published data, two recent abstracts focusing on infant B-ALL revealed that of the 14 CD19 CAR-treated patients, the majority (79%) were MLL-r, and 4 exhibited a conversion to AML either during the primary CAR response or during relapse, and two of these were *MLL*-r [48]; and of 14 *MLL*-r infants treated with CD19 or CD19 × CD22 CARs, three relapsed with B-ALL and one relapsed with AML, overall suggesting a frequency that might be higher than that observed for chemotherapy [30]. However, it is important to emphasize that most patients in these studies remained in remission, and it is unclear how the lineage of the relapse relates to overall outcome.

In some reports, clonal relationships between the B-ALL and the AML are discussed through detailed characterization of the *MLL* rearrangement as well as immunoglobulin heavy chain rearrangements (Table 1) [18,23,26,32,33]. Recent single-cell genomics data suggest that pre-existing myeloid-primed B-ALL cells could be the source of such relapses (see Figure 2) [49]. In addition to *MLL*-r B-ALL, myeloid lineage-switched relapse has been reported for B-ALL harboring a Ph+ or Ph-like phenotype [35], as well as several other B-ALL categories with unique cytogenetics [31,50].

Model systems for studying *MLL*-r B-ALL evolution under CAR T cell pressure

Given the incomplete understanding of mechanisms that control lineage plasticity in *MLL*-r or other acute leukemias, a model system to study mechanisms of escape from CD19-directed immunotherapy would be immensely informative. Although *MLL*-r B-ALL can be efficiently generated in vitro from human cord blood or human fetal liver progenitors, [51,52] B-ALL, as modeled in xenograft systems, lacks the appropriate niche and endogenous immune components to accurately model in vivo evolution. Furthermore, xenograft models fail to recapitulate many conditions that impact CAR T-cell responses in vivo, such as the lack of CAR stimulation by non-malignant B cells expressing human CD19 antigen, limited cognate interactions between human T cells and the murine innate immune system, requirement for supraphysiological doses of CAR T cells, and the presence of xeno-reactivity between the human TCR and murine MHC complexes, which confounds the study of immune interactions and limits long-term studies because of lethal xenograft-versus-host disease. Ideally, a murine syngeneic system would allow testing of a variety of leukemia-intrinsic, niche, or CAR T cell pathways in the progression and relapse of B-ALL.

Some progress in developing such a system was presented in 2018, with the development of a TCF3-PBX1 cell line based on a model initially developed by Bijl and Sauvageau [53]. This in vitro−adapted CD19+ B-ALL consistently engrafts with as few as 100 cells in a syngeneic murine system. CD19 CAR T cells at doses comparable to those used clinically ($2-2.5 \times 10^6$/kg) can completely cure animals engrafted with this TCF3-PBX1 line [54,55]. However, $CD19^{neg}$ “late relapses” were observed exhibiting features of a myeloid gene

expression program [56]. These CD19^{neg} relapse samples remain CD19 ^{neg} on secondary transfer and at the genomic level, reflect an epigenomic reprogramming away from B-cell identity toward myeloid identity [56]. This novel model system provided initial insights into the conditions that promote CD19^{neg} relapse; however, it is unclear whether it reflects the actual processes underlying myeloid relapse in patients. First, TCF3−PBX1 fusions are not common and do not represent a poor prognostic group within pediatric B-ALLs, and patients harboring this fusion oncoprotein are not particularly prone to relapse with AML [57−59] Second, the CD19+ B-ALL line is adapted to culture and is unlikely to exhibit the same genomic plasticity as observed for primary B-ALL. Nonetheless, this model system provided a platform to study the epigenomic changes that occur on CD19^{neg} relapse and the impact of CAR T-cell dose on this process.

In contrast to *TCF3-PBX1* translocations, *MLL* translocations are enriched in myeloid relapses in B-ALL [18,45]. The propensity for lineage switching, particularly toward a myeloid identity, is likely an underlying property of *MLL*-r B-ALL, based on historical clinical observations [18,20−27,31−33]. It would clearly be beneficial to study processes leading to CD19^{neg} relapse under immunotherapeutic pressure using a model system in which B-ALL is driven by *MLL*-r. B-ALL initiated by MLL fusion oncoproteins has been surprisingly difficult to produce in mouse models despite nearly 30 years of investigator efforts [60]. The first animal models of *MLL*-r leukemia in the 1990s used γ retroviruses to introduce MLL-fusion oncoproteins into murine bone marrow progenitors [61]. Retroviral introduction of MLL fusions such as MLL-AF9 and MLL-ENL was sufficient to induce leukemia in 100% of mice, but AML was produced, independent of cell type transduced [60]. In addition, transgenic expression of fusion oncoproteins from the endogenous *Mll1* locus generally produced myeloid leukemia, even in conditions expressing fusions predominantly found in human B-ALL, and even when the fusion oncoprotein is directed selectively to lymphoid progenitors [62]. In some cases, B-ALL (defined by immunophenotype) can be produced using γ retroviral models of *MLL*-r leukemia, best exemplified by So et al. [63] in which an MLL-GAS7 fusion produced mixed lineage/B-ALL in conjunction with added FMS-like tyrosine kinase 3 ligand (FLT3L) and interleukin (IL)-7. Generally, the murine system (together with the gene expression program imposed by MLL fusion oncoproteins) strongly drives AML rather than B-ALL.

Nonetheless, recent publications have reported that using fetal cell types and carefully regulated levels of MLL fusion oncoproteins may improve the lineage fidelity of murine *MLL*-r leukemia model systems. In one case, inducible *Mll*-AF4 knock-in models that must be kept on a mixed genetic background exhibit ~30% B-ALL with many still succumbing to AML [64,65] Using knock-in models from the Rabbitts' laboratory, several groups have found that progenitors exhibit an embryonic period of sensitivity to *Mll*-AF4 or *Mll*-ENL transformation [66,67]. For example, Malouf and Ottersbach [66] and Barrett et al. [68] reported that fetal liver lymphoid-primed progenitors exhibit a preleukemic phenotype dependent on *Mll*-AF4 expression but fall short of producing full-blown B-ALL. Okeyo-Owuor et al. [67] similarly found, using a distinct MLL-ENL knock-in, that a perinatal progenitor exhibited the peak sensitivity to *MLL-ENL*-mediated transformation; however, AML was the outcome in these mice. In an effort to study the role of the leukemia-extrinsic environment in lineage specification, Rowe et al. [69] found that serially transplanting

MLL-AF9- or MLL-ENL−transduced progenitors through newborn mouse recipients (rather than adult recipients) enhanced the frequency of B-ALL−like phenotypes. This effect was attributed to an excess of myeloid-promoting chemokines and cytokines such as Ccl5 in the adult bone marrow niche relative to the newborn niche.

In contrast to these murine systems, both viral transduction of human umbilical cord blood progenitors [51,52,70,71] and CRISPR/Cas9 editing of human fetal cells [72] easily produce a B-ALL depending on the cytokines supplied during in vitro culture and on ontogeny [71] These observations collectively argue that the combination of fusion oncoprotein and the murine micro-environment produce a myeloid bias that does not accurately reflect the conditions found during early human development (Figure 2). Altering the collective conditions such that pediatric-relevant B-ALLs can be reliably and reproducibly generated in a mouse model would contribute significantly to discovering better cellular or immunotherapeutics for the most common *MLL*-r pediatric leukemia and, importantly, enable discovery of more effective methods for inducing long-term remission following targeted immunotherapy.

Can we anticipate and prevent lineage switching-related relapse?

To address this critical clinical question, it is important to understand key features of early B-cell fate commitment and resolution of B-cell versus myeloid identity. Hematopoietic differentiation occurs through a continuum of fate restriction events, and some of the earliest-defined B-cell progenitor−enriched populations including common lymphocyte progenitors (CLPs) and lymphoid primed multipotent progenitors (LPMPs) retain myeloid potential [73−76]. Interestingly, murine fetal CLPs and LPMPs exhibit more robust myeloid priming or myeloid potential than their adult counterparts [77,78], a phenomenon shared with human fetal progenitors [79]. The molecular basis for B-cell commitment is understood best at the level of transcriptional antagonistic and feed-forward hierarchies featuring well-studied transcription factors such as E2A, EBF, and PAX5 [80]. Latent myeloid potential of committed or even transformed pre-B cells was revealed in classic experiments manipulating PAX5 and/or the transcription factors of the C/EBPα family [81−85] through a mechanism that involves coordinated regulation of enhancers co-bound by EBF and C/EBPα. From these studies, it is clear that stochastic fluctuations in key fate-determining transcription factors could have a dramatic impact on the propensity of B-ALL to escape CD19-directed therapy through myeloid differentiation. In addition, the MLL fusion−dependent transcriptional network may perturb the B-cell fate network to tip the balance toward myelopoiesis, or the transformation of a particular progenitor stage may preserve an active enhancer network that retains the ability to respond to myeloid transcription factor networks (Figure 2).

Findings from the murine TCF3−PBX1 model system described earlier [54,86] suggested that late CD19^{neg} relapses may have arisen from cellular reprogramming in the leukemia niche in which the CAR T cells are just one component. Extracellular signals elaborated in the CAR T cell/leukemia/niche such as inflammatory cytokines could promote activation of the *Cebpa* enhancer in B-ALL, influencing the cell's propensity to adopt a myeloid fate. How could one apply these fundamental observations to the treatment of B-ALL? One

approach could be to identify and target the signaling pathways leading to *Cebpa* enhancer activation, specifically signaling to activate myeloid-specific *Cebpa* enhancer elements [87]. Targeting such signals may block feed-forward transcriptional networks that promote myelopoiesis.

Another strategy for patients considered to be at risk for relapse of AML would be to anticipate this problem by applying immunotherapies that simultaneously target CD19 and myeloid-lineage antigens such as FLT3, CD33, or CD123 [88−90]. Given the ongoing development of myeloid-directed CAR T cells for AML, this approach may be closest to clinical use, but combinatorial toxicity would have to be carefully considered. FLT3 CARs have been effective in preclinical studies [91] and are particularly relevant because of the high expression levels on pediatric *MLL*-r B-ALL [91−96] as well as myeloid leukemias [98−100]. The optimal strategy for simultaneously targeting FLT3 and CD19 has not yet been determined, because approaches using a mixture of antigen-specific CAR T cells, CAR molecules with multiple single-chain variable fragments (ScFvs: antigen-binding domains), and T cells expressing multicistronic CAR molecules have shown efficacy in preclinical models as well as in patients [86,100−106]. Although these approaches would be expected to broaden the CAR response, allowing for the targeting of leukemia cells with either a B-ALL or myeloid phenotype, this would also broaden the “on-target, off-tumor” toxicity beyond B-cell aplasia to likely include myelosuppression. To test any of these strategies, there is a need for suitable preclinical model systems in which to test combinatorial B- and myeloid-directed CAR T cell strategies in a syngeneic, immunocompetent model to carefully test efficacy and toxic effects, because such studies are not possible in existing human−murine xenografts.

Outlook

Immunotherapies including CAR T cell therapies have provided new hope for treating many refractory malignancies. Despite remarkable successes in B-ALL, relapses remain a significant challenge. The mechanisms of resistance to immunotherapy are beginning to be elucidated [16,107,108], and it appears that lineage plasticity plays a role not only in hematologic cancers as described here but also in multiple tumor types; therefore, understanding the factors that influence lineage plasticity should lead to better anticipatory treatment strategies to combine with immunotherapies [109−112].

In the case of *MLL*-r B-ALL, there remain several unanswered questions relating to the impact of lineage switching and relapse with AML. First, the actual frequency of this event (as opposed to $CD19^{neg}$ relapse with B-cell phenotype) is unknown because of the lack of consistent documentation of this phenomenon in clinical trials and the relatively short time that CAR T cells have been in use. Because of the sporadic nature of case reports, it is unlikely that the true frequency of relapse as AML on CD19-directed therapies is well represented in the literature, particularly because *MLL* rearrangements are relatively rare. We would advocate for a more uniform, international system for documenting AML relapses using CD19-directed therapies such that frequencies and outcomes can be quantitatively determined. Cases of incomplete lineage conversion during relapse may go undetected because of the limited phenotypic analysis typically performed by standard

flow cytometry, which may result in an underappreciation of the true frequency of lineage switch post-CAR treatment, a feature that genomic approaches may better capture [49]. Furthermore, whether lineage-switching relapse is worse than other forms of relapse remains an open question. Relevant to this question, a retrospective study of more than 200 infants diagnosed with *MLL*-r B-ALL treated on the Interfant-06 protocol provides clues that myeloid fate postinduction requires more aggressive therapy. Eighty percent of patients with high minimal residual disease (MRD) at the end of induction chemotherapy had B-ALL expressing at least one myeloid marker and shorter survival times as compared with patients with low MRD, who had lower frequencies of myeloid markers and survived longer. Indeed, use of myeloid-type chemotherapy was subsequently reported to improve outcomes in these patients [113]. These observations suggest that an underlying capacity for myeloid fate conversion in *MLL*-r B-ALL corresponds to poor survival and that this would pose a challenge for CD19-targeted immunotherapy as well. Understanding these issues for immunotherapy for B-ALL may have a broad impact as targeted immunotherapies are extended to other forms of cancer with underlying lineage plasticity.

An additional unanswered question is whether lineage switching relapse frequency differs between adult and infant B-ALL. One might expect that the developmental origin or history of the transformed cell could influence lineage fidelity, even under the influence of MLL fusion oncoproteins, based on the human and murine studies discussed earlier [67−69,71,73]. Again, the small numbers of such patients treated with immunotherapy/CAR T cell therapy lineage-switch relapses preclude such analysis at this time. An interesting approach to deducing the developmental history of the cell of origin in B-ALL was to derive a transcriptional signature distinguishing murine B1 (more prevalent during fetal development) versus B2 B-cell subtypes and ask whether pediatric B-ALL subtypes were enriched in this signature. Surprisingly, such an analysis revealed that *MLL*-r B-ALL exhibits a more B2-like transcriptional signature, despite its fetal origins [114]. Whether such a signature reflective of distinct fetal origins can be used to infer lineage fidelity or not must be experimentally determined.

One approach to preventing relapse from an inherently lineage-plastic B-ALL is to employ bispecific CARs to achieve higher selectivity and deeper killing of variants. In fact, CARs including CD133 have been suggested for *MLL*-r leukemia [115] because CD133 is a direct target of MLL-fusion oncoproteins [116] therefore, loss of both CD19 and CD133 would be very unlikely even if leukemia evolves. Similarly, FLT3 is highly expressed on most *MLL*-r B-ALLs, and FLT3 CARs are already well developed for use in AML [90,91]. Alternative cellular strategies such as CAR-NK and CAR-iNKT cells [117] may differentially affect the immune/niche/leukemia microenvironment and therefore have a distinct impact on AML relapse, but these strategies should be methodically tested in an appropriate model system.

There are two main hurdles to using the *MLL*-r leukemia paradigm to understand how to predict risk for and prevent relapse through lineage switch evasion of CD19 CAR T killing. First, an animal model system is desperately needed in which syngeneic or spontaneously arising B-ALL can be produced through induction of MLL fusion oncoproteins. Current model systems favor myelopoiesis and therefore do not accurately model the cellular or genomic features of human pediatric B-ALL driven by MLL fusion oncoproteins. Exciting

new data are emerging that describe the B-ALL niche and how it changes postrelapse [49,118], but these systems are not as experimentally tractable as a syngeneic murine system, which would enable dynamic and robust assessments of the complete leukemia niche, as well as genetic manipulation of individual niche components.

Second, although there is a wealth of data surrounding transcriptional and epigenetic networks that control B-cell differentiation and latent myeloid potential, how to realistically manipulate those pathways in a clinical setting is unclear. Further characterization of the signals that exist in the leukemia niche and how they impact myeloid versus B-cell enhancer activity, for example, will be important to identify strategies to activate or suppress particular transcriptional networks. In this sense, reprogramming cancer cells could take a page from the pluripotency field, where cellular reprogramming studies have moved toward identifying small molecules that can replace some of the initial transcription factor manipulations [119−123]. In addition to preventing or treating antigen-negative relapse, such fate-regulating small molecules may also be helpful in combination with other targeted therapeutics to control or reverse tumor plasticity.

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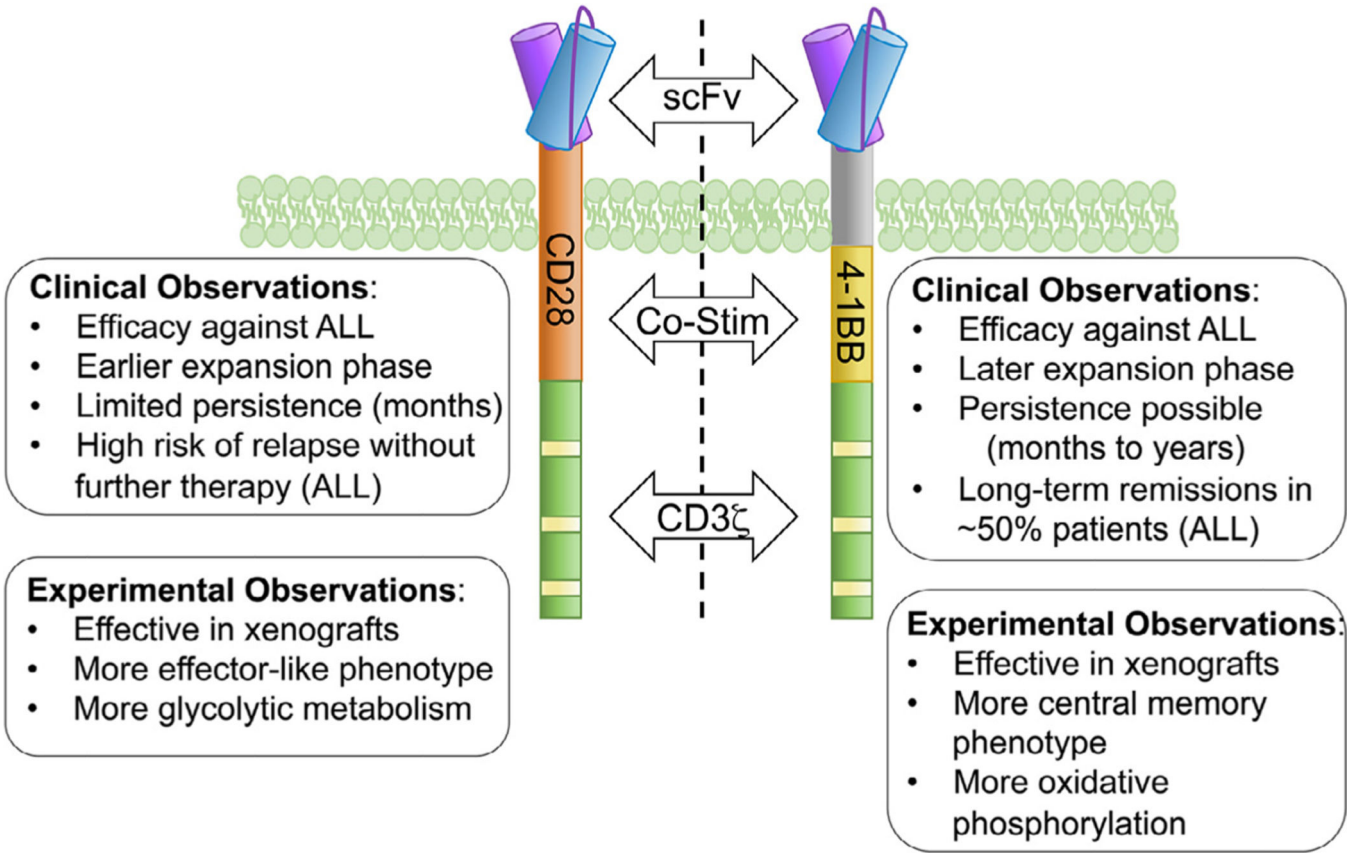

Figure 1.

Clinical and biological differences in CD28- and 4–1BB−containing CAR T cells. Second-generation CARs, consisting of an antigen-binding domain (scFv) connected via an extracellular and transmembrane domain to a co-stimulatory domain (derived from either CD28 or 4–1BB) and the intracellular portion of the CD3z chain. Both CAR formats successfully activate T cells leading to leukemic clearance in preclinical models and in patients; however, each co-stimulatory molecule elicits differences in persistence, T-cell phenotype, and metabolism.

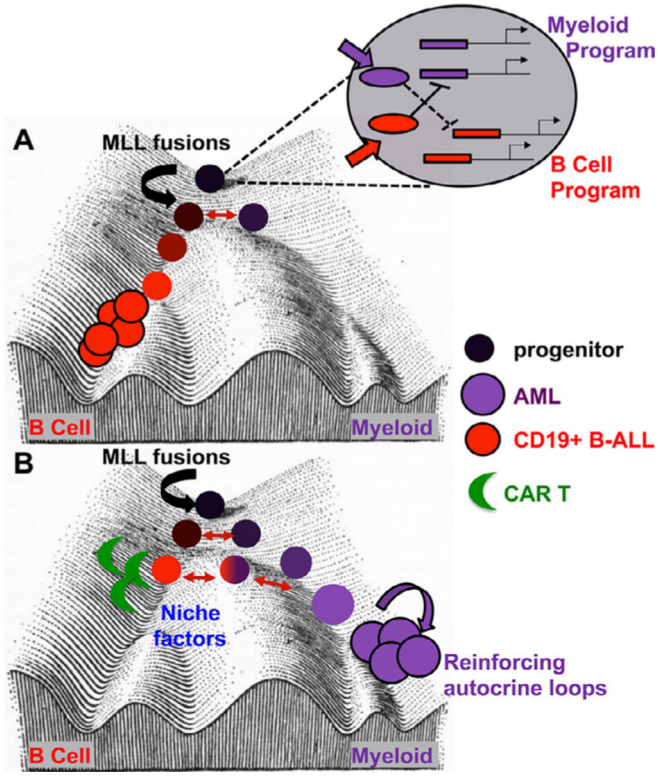

Figure 2.

Waddington landscape depicting the impact of MLL fusion oncoproteins and extrinsic factors on leukemia lineage. **(A)** The expanded progenitor cell diagram reveals myeloid- or lymphoid-promoting signals (*filled arrows*) promoting transcription factors (*ovals*) acting on lineage-directing enhancers (*filled rectangles*) to maintain exclusive lineage identity. *Red double-headed arrows* indicate the latent myeloid potential of transformed B-ALL which can overcome the activation energy to lose B-cell characteristics and gain myeloid identity. **(B)** Lineage switching on CD19-directed therapy (*green crescent*) as influenced by direct killing of the CD19+ B-ALL and/or impact of the immunotherapy and the niche on lineage decisions within the remaining B-ALL cells.

Table 1.

Published cases of CD19^{neg} relapse with myeloid phenotype after CD19-directed immunotherapies, both CAR T and BiTEs

Immunotherapy	Total relapse cases	CD19-negative relapse cases	No. of myeloid switch cases	Phenotype after CD19-directed immunotherapy		IgH clonal relationship	Time between immunotherapy and lineage switch	Cytogenetics	Age/Sex	Ref.
CD19 CAR-T	2	2	2	Case 1	CD19−, CD13 (dim)+, CD64+, HLA-DR (dim)+, CD15+, CD33+, CD71, MPO+	Yes	22 d	t(4;11)(q21;q23) MLL/AF4	52 y/F	18
				Case 2	CD19−, CD4+, CD56+, CD64+, CD13+, CD33+, CD38+, HLA-DR+, CD34+, CD45+,CD71+	No	21 d	ins(11;10)(q23;p12p1?1.2) MLL/MLLT10	18 mo/F	
	4	2	1	CD19 negative myeloid phenotype switch		Yes	No data reported	t(4;11)(q21;q23) MLL/AF4	52 y/not reported	33
	1	1	1	CD13+, CD34+. CD117+, CD123+, CD11b+, CD38(mod), CD7+, CD19−, CD10−, CD22−, TdT−, CD24−, CD20−, MPO−		No data reported	8 mo	TCF3(Ex11)-ZNF384(Ex2) fusion	13 mo/M	31
BiTEs	1	1	1	CD19−, CD34−, CD10−, CD3−, CD16−, CD117 −, HLADR−, nTdT−, CD2−, CD7−, CD38−, cCD22−, cCD79a−, cCD3−, CD45+, CD13+, CD15+, CD33+, CD56+, CD36+, CD64 (partial)+, cMPO+		No data reported	9 d	t(4;11)(q21;q23) MLL/AF4	40 y/F	20
	1	1	1	sCD19low, CD33+;CD34, CD14++, CD15++, CD11b++, CD64+		No data reported	9 d	t(4;11)(q21;q23) MLL/AF4	5 mo/F	21
	1	1	1	CD19−, PAX5−, CD33+, CD43+, lysozyme+		No data reported	8 mo	t(4;11)(q21;q23) MLL/AF4	77 y/M	22
	1	1	1	CD19−, CD34−, CD10−, CD38+, cMPO+, CD33+, CD13(low)+, CD64 +, CD65+, cCD79+		Yes	53 d	t(4;11)(q21;q23) MLL/AF4	46 y/F	23
	1	1	1	CD19−, CD34−, CD79a−, TdT−,CD33+ CD11b+, CD14 (subset/dim)+, CD64+, MPO+;CD13(dim)+, CD22(dim),+ CD33(dim),+ CD38+, HLA-DR+		No data reported	15 d	t(4;11)(q21;q23) MLL/AF4	3 mo/not reported	24
	1	1	1	CD19−, CD20−, CD22−, CD24+;cyIGM−, cyCD79a−, CD2−, CD3−, CD7−, CD8−, cyCD3−, CD13+, CD33+, CD15+, cyMPO+, CD117−, CD66c+, CD10+, CD34−, CD45+, TdT−, Cd38+, CD52−		No data reported	3 wk	No	8 y/F	25
	1	1	1	CD19−, cCD79a−, CD22−, CD34−, CD33(low)+, CD65+, CD15+		Yes	28 d	t(4;11)(q21;q23) MLL/AF4	15 y/M	26
	1	1	1	CD19−, PAX5−, CD34−, lysozyme+, CD33+, CD64(dim)+, CD13+, myeloperoxidase+, cytoCD79a-		No data reported	1 mo	t(4;11)(q21;q23) MLL/AF4	40 y/F	27

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Immunotherapy	Total relapse cases	CD19-negative relapse cases	No. of myeloid switch cases	Phenotype after CD19-directed immunotherapy	IgH clonal relationship	Time between immunotherapy and lineage switch	Cytogenetics	Age/Sex	Ref.
	1	1	1	CD19−,CD33+, CD11b+, CD14+, CD64+, HLADR+, CD38+, CD56+, CD4+, minor clone CD19+, CD22+, CD24+, CD38+	Yes	15 d	t(1;11)(p32;q23) MLL/EPS15	6 m/F	32

F=Female; *M*=Male; y=year; mo=month; d=day