Editorial

# Allogeneic anti-CD19 CAR T cells: new perspectives in the treatment of B-cell malignancies that progress after allogeneic stem cell transplantation?

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Allogeneic stem cell transplantation (ASCT) remains the ultimate curative option for numerous lymphoid malignancies, especially acute lymphoblastic leukemia (ALL). When relapse occurred after ASCT, prognosis is dismal especially in ALL and therapeutic options are limited (1). Donor lymphocyte infusions (DLIs) of unmanipulated allogeneic lymphocytes from the transplant donor are then commonly used. While DLIs demonstrated efficacy in non-aggressive diseases such as chronic lymphoid leukemia (CLL) and follicular lymphoma (FL), with response rate ranging from 70% to 80% (2), complete remission (CR) rates observed after DLIs in patients with ALL were of less than 20%, probably due to a lack of graft-versus-leukemia or immune escape of the relapsing clone by loss of human leukocyte antigen (HLA) expression (3). Furthermore, one major issue with DLIs is the onset of significant acute graft-versus-host disease (aGVHD) in one third of cases, with a treatment-related mortality approaching 10% (4). There is therefore an urgent need for innovative therapies in B-cell malignancies that relapse after ASCT.

Over the past years, chimeric antigen receptor (CAR) T cells have emerged as a promising new therapeutic approach. Initially described by Eshar in 1989 at the Weizmann Institute, the concept is based on recombinant receptor molecules genetically transferred, redirecting T cells against a specific tumor-associated antigen (5). CARs are composed of three different parts, with specific functions: an extracellular domain formed by a single chain variable fragment (scFv), derived from the fused variable heavy and light chains of an antibody; a transmembrane domain composed of a hinge or 'spacer' to provide flexibility and stable expression of the scFv; and an intracellular signaling domain usually derived from the CD3ζ-chain of the T-cell receptor (TCR)-CD3 complex, which plays a key role in the activation of CAR T cells. CAR T cells overcome some primordial limitations of TCR by targeting antigens in a non-major histocompatibility complex (MHC) manner, and can then recognize tumor cells independently of HLA molecules (6). In B-cell lineage malignancies, CD19 represents an ideal target antigen. It is expressed in almost all B-cell malignancies and long-term B-cell depletion is generally manageable. Based on pre-clinical data with first- and second-generation CARs, trials using anti-CD19 CARs were initiated in patients with recurrent indolent non-Hodgkin lymphoma (NHL) or CLL, and reported promising first results with prolonged CR durations (7-9). The initial enthusiasm was hampered in further trials showing no CR achievement and very limited CAR T cells persistence especially in NHL (8,10). Discrepancies among studies could be attributed to suppressive effects of the tumor microenvironment, differences in treatment history, pretreatment tumor burden, potential tumor resistance to the lymphocyte depleting agent, or impaired T-cell immunological function in lymphomas (11). More recently, CD19-CAR T cells appeared as a valuable choice for the treatment of B-cell malignancies. In a recent study

testing anti-CD19 CAR T cells in patients with relapsed or refractory CD19$^+$ lymphomas, the overall response rate was 47% in diffuse large B-cell lymphomas (DLCBL) and 73% in indolent lymphomas. None of DLCBL patients who achieved CR relapsed at a median follow-up of 14.5 months (12). Very promising results were also observed in B-cell lineage ALL, with overall CR rates ranging from 80% to 90% (11,13). The major adverse event encountered with CAR T cells, was cytokine release syndrome (CRS), which appears associated with tumor burden, but is usually manageable (14).

In a recent issue of the *Journal of Clinical Oncology*, Brudno *et al.* reported interesting clinical results regarding a new approach involving anti-CD19 CARs (15). Twenty patients with various B-cell malignancies that progress after ASCT were treated with allogeneic donor-derived anti-CD19 CAR T cells. The overall response rate was 40% (8/20) with 30% of CR. Four of the five patients with ALL achieved molecular remission. Patients with ALL who obtained CR showed recovery of normal polyclonal B cells after clearance of CAR T cells. Responses were less observed in aggressive or indolent lymphomas with only one CR in patients with DLCBL and one CR lasting 30 months in one patient with CLL. High peak blood CAR19 T-cell levels were predictive of an anti-malignancy response. Interestingly, this peak was not determined by initial T-cell dose infused, suggesting a potential role of endogenous CD19$^+$ B cells in CAR T-cell expansion. The originality of the paper was the immunomonitoring of the CD19$^+$ CAR T cells after infusion. The authors showed that these cells acquired progressively a more differentiated phenotype (close to that of effector memory and effector memory RA T cells) and, as expected, displayed markers such as the programmed cell death protein-1 (PD-1), an inhibitory receptor expressed on T cells. Moreover, the CD8:CD4 ratio was up to 15 fold higher in responders, reflecting the major role of cytotoxic T cells in CAR T-cell efficacy. Altogether, these data reflected that engineered CAR T cells behave as normal T cells *in vivo*. However, while normal effector memory cells remain after antigenic stimulation, effector memory-like CAR T cells ultimately disappear after few weeks. Allogeneic CAR19 T cells appeared to enhance the graft-versus-malignancy potency without worsening new-onset aGVHD after infusion.

Despite these encouraging results, some issues should be mentioned. First, CD19 gene-modified T-cell therapy seems insufficient as a stand-alone therapy. From literature, very heterogeneous CAR T-cell persistence has been reported (from 1 to 18 months), probably depending on CAR constructs (13,16,17). However, it seems clear that persistence and *in vivo* expansion of adoptively transferred T cells are strongly correlated with outcome (18). The incidence of relapse has also been related to CAR T-cell disappearance, suggesting that this immunotherapy is not able to eradicate the initiating clone, even in ALL cases with negative minimal residual disease (MRD). In Brudno's paper, authors realized a dynamic CAR T-cell monitoring. They showed that after CAR T-cell infusion, the fraction of CAR19 T cells with naïve or central memory phenotypes decreased, while the fraction of T cells with more-differentiated effector memory and effector memory RA phenotypes increased. This rapid expansion was associated with PD-1 up-regulation in blood CAR19-expressing T cells, a chronic activation and T-cell anergy marker. Beside antigen presenting function, the interaction between MHC and TCR is mandatory for T-cell activation (19). Even if CAR T cells are chronically activated through the endogeneous production of CD19$^+$ B cells, CAR T-cell TCRs are probably not similarly engaged as normal T cells. It can therefore be hypothesized that CD19$^+$ B-cell aplasia, TCR-MHC interaction and CAR T-cell anergy are major factors involved in CAR19-expressing T-cell persistence. B-cell aplasia persistence is a sign of persistent anti-tumor response. In Brudno's study, virtually all patients ultimately relapsed without another ASCT after CR achievement. This is likely related to the abrogation of CAR T-cell persistence.

The second issue raised by Brudno's study is inherent to the CD19$^+$ CAR T-cell production and administration. It is well known that TCR engagement (signal 1) without co-stimulatory signal (signal 2) lead to T-cell anergy and rapid activated induced cell death. Signal 2 is provided by engagement of the co-stimulatory domain, which is critical for CAR T-cell expansion. As numerous different CAR T cells exist, it seems unlikely that each construct can be tested in all hematological settings. The choice might probably depend on pre-clinical data and CAR subunit properties. Regarding second generation CAR T cells, CD28 co-stimulatory endodomain seems to be more suitable for rapid expansion while 4-1BB endodomain seems recommended for persistence. Brentjens *et al.* highlighted that only CD28-transfected CAR T cells rather than 4-1BB, OX40 or DAP10 have enhanced proliferative activity and cytokine production compared to CD19z1 (first generation CAR T cells) (20). In the other hand, Milone *et al.* showed that 4-1BB signal transduction endodomain exhibited the

 

greatest anti-leukemic and prolonged survival *in vivo* (21). In acute leukemia, most of the current clinical trials have used CD28 or 4-1BB endodomain, but comparisons among intracellular signaling domains should take into account multiple representatives of epitope location, density, affinity and CAR scaffold to reach suitable protocols for routine clinical application. Most clinical trials use lymphocyte depletion before CAR T-cell infusion. Despite a direct anti-malignancy activity, this model is based on the concept of homeostatic proliferation, which mimics chronic antigenic stimulation. After lymphocyte depletion, homeostatic proliferation is the mechanism by which a lymphocyte population can fill the gap in order to generate an equivalent lymphocytic pool. This mechanism depends on TCR/MHC self-peptide interaction and cytokines such as interleukin (IL)-7 and IL-15. Moreover, lymphocyte depletion by chemotherapy or total body irradiation suppresses T regulators and other repressive cell populations leading to rapid anergy of genetically modified T-cells, but also of cells competing for stimulatory cytokines (IL2, IL7, IL15, IL21) (22). In prior studies, lymphocyte depletion by chemotherapy was associated with higher response rates especially in CLL and NHLs, likely due to a direct anti-tumor effect of chemotherapy (17). In Brudno's study, CAR T cells were administered to recipients without lymphocyte depletion in order to avoid severe aGVHD. Lymphocyte depletion before CAR infusion appeared then not requested for their expansion and for higher CD19 CAR T-cell peak levels observed in presence of blood B cells. As the initial peak levels of blood CAR19 T cells were significantly higher in patients who obtained remissions than in those who did not, it could be suggested that lymphocyte depletion before CAR infusion may be necessary only in case of high tumor burden at the time of relapse.

Overall, Brudno *et al.*'s results argue that CAR19 T-cell infusion is superior to standard DLIs at eradicating malignancy. Despite these very promising results, this treatment seems still not enough as sole therapy, but can serve as a potentially life-saving bridge to further therapy when relapse occurred after ASCT. In this setting, increasing peak blood levels of CD19 CAR T cells appears as a major goal, suggesting the administration of CAR T cells as planned repeated infusions after stem-cell infusion. Combinations with agents targeting more than one antigenic determinant, gene mutation, or signal transduction pathway might be shown as effective therapeutic strategies. The coming years should undoubtedly see the development of such therapeutic approaches and the development of CAR T cells with optimization of CAR constructs and harmonization in their manufacturing procedures.

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