blood commentary

IMMUNOBIOLOGY AND IMMUNOTHERAPY

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CAR-T and ibrutinib vs CLL: sequential or simultaneous?

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In this issue of *Blood*, Gauthier and colleagues present the results of a pilot study evaluating the safety and feasibility of administrating ibrutinib concurrently with CD3zeta, 4-1BB-signaling anti-CD19 chimeric antigen receptor (CAR) T-cell therapy in relapsed/refractory chronic lymphocytic leukemia (CLL).[1] Minimal residual disease (MRD) assessment was performed on bone marrow (BM) at an early time point (4 weeks) following CAR T-cell infusion. The study enrolled 19 CLL patients, one of whom died 4 days after infusion from a presumed ibrutinib-related cardiac arrhythmia during grade 2 cytokine release syndrome (CRS). At the 4-week time point, 15 of the remaining 18 patients responded, with 4 patients achieving a complete response. Remarkably, 11 patients had undetectable BM MRD as measured by *IGH* sequencing. One-year progression-free survival (PFS) of the 18 evaluable patients was 38%. The authors compared these results to 19 CLL patients who were previously treated with a very similar regimen but without concurrent ibrutinib[2] and found that severity of CRS was lower with concomitant ibrutinib, but PFS was unchanged.

This study raises several questions.

First, with the novel highly successful target therapies available for CLL, do we actually need complex strategies such as CAR T-cell treatment? Novel drugs that inhibit either key kinases of the B-cell receptor–signaling pathway, such as the Bruton tyrosine kinase inhibitor ibrutinib, or specific anti-apoptotic proteins, such as the Bcl-2 inhibitor venetoclax, have recently been shown to be valuable additions to the therapeutic arsenal of CLL. However, these agents are costly and are to be taken continuously with potential long-term toxicities and development of resistance. Novel combinations of such agents aimed at early treatment cessation are promising but cannot, at this point, be expected to be curative.

Therefore, indeed an unmet need exists for the development of additional effective yet tolerable treatment options with alternative mechanisms of action.

Second, is there a need to improve CAR T-cell therapeutic regimens in CLL? A proof-of-concept that T-cell–based therapies have curative potential in CLL comes from observations that allogeneic hematopoietic stem cell therapy, which can induce long-term remissions through T cell–mediated graft-versus-leukemia effects. However, the applicability of allogeneic T cells is severely hampered by high levels of treatment-related morbidity and mortality due to treatment-related toxicity and graft-versus-host disease. An additional impediment is the limited availability of HLA-matched

donors. CAR-reprogrammed patient T cells that provide a cellular antitumor response might, therefore, be a highly attractive approach for CLL. However, CAR T-cell treatment regimens have shown disappointingly low response rates in CLL.[3] A likely factor in the limited responses in CLL is the acquired T-cell dysfunction that we, and others, have described. T-cell abnormalities include impaired synapse formation, impaired proliferative capacity, an exhausted phenotype, and diminished T-cell cytotoxicity.[4,5] Increasing evidence suggests that T-cell dysfunction in CLL occurs through direct and indirect interactions of CLL cells with both CD4$^+$ and CD8$^+$ T cells through mechanisms that are yet not fully understood. By studying the metabolic function of T cells in CLL, we recently linked the acquired abnormalities of CLL-derived T cells to the lack of CAR T-cell persistence and clinical responses.[6] Hence, acquired T-cell dysfunction in CLL currently hampers successful implementation of CAR T-cell therapy in CLL.[7]

What strategies could improve CAR T-cell efficacy in CLL? When CAR T-cells are used in the setting of active disease, both during the leukapheresis process and during product infusion, interactions between T cells and CLL cells are expected to occur. Ibrutinib does not only target Bruton tyrosine kinase, but also interleukin-2–inducible T-cell kinase, and both in vitro and ex vivo observations showed improved T-cell function upon ibrutinib treatment.[8] More recently, also in the context of CAR T-cell therapy in CLL, ibrutinib was shown to augment responses: first, ibrutinib improved the expansion of CAR T cells, which was associated with decreased expression of programmed cell death protein 1 (PD1) on T cells and of CD200 on CLL cells. Second, in a human xenograft model of CLL, ibrutinib exposure improved CAR T-cell engraftment and survival.[9] Gauthier and colleagues' observations are very instructive in that the rescue of ibrutinib-nonresponsive CLL patients with anti-CD19 CAR T cells,[2] and the concurrent

treatment of ibrutinib-refractory CLL patients with ibrutinib and CD19 CAR T cells result in comparable response rates. Disappointingly, the complete response rates were not higher than previously reported by others.[7]

Third, what are possible other strategies to improve CAR T-cell efficacy in CLL? As T-cell function in CLL is not intrinsically imprinted but can be restored following depletion of leukemia cells,[5,6] 1 approach might be to use CAR T-cell therapy not as salvage therapy for highly refractory patients but as consolidation treatment following successful tumor debulking. A better alternative therefore may be to use ibrutinib, possibly combined with therapeutic apheresis to remove mobilized tumor cells. Preclinical studies from Fraietta et al demonstrated that the enhanced antitumor efficacy of CLL CD19 CAR T cells was only observed after at least 5 prior cycles on ibrutinib.[9] Hence, the combined debulking with ibrutinib with concurrent T-cell function recovery might represent the next iteration in CLL-directed precision targeting. Along the same line, we recently observed normalization of T-cell function and a specific decrease of follicular T-helper cells, regulatory T cells, and PD1+ CD8+ cells following 1 year of treatment with a venetoclax containing regimen.[10]

Hence, Gauthier and colleagues have here and in their previous study provided support for a synergistic approach of CLL targeting with both CAR-reprogrammed T cells and ibrutinib; the question remains, however, whether the concurrent treatment of patients with both drugs really results in superior responses.

REFERENCES

1. Gauthier J, Hirayama AV, Purushe J, et al. Feasibility and efficacy of CD19-targeted CAR T cells with concurrent ibrutinib for CLL after ibrutinib failure. *Blood.* 2020;135(19):1650-1660.

2. Turtle CJ, Hay KA, Hanafi LA, et al. Durable molecular remissions in chronic lymphocytic leukemia treated with CD19-specific chimeric antigen receptor-modified T cells after failure of ibrutinib. *J Clin Oncol.* 2017;35(26):3010-3020.

3. Porter DL, Hwang WT, Frey NV, et al. Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. *Sci Transl Med.* 2015;7(303):303ra139.

4. Riches JC, Davies JK, McClanahan F, et al. T cells from CLL patients exhibit features of T-cell exhaustion but retain capacity for cytokine production. *Blood.* 2013;121(9):1612-1621.

5. de Weerdt I, Hofland T, Lameris R, et al. Improving CLL Vγ9Vδ2-T-cell fitness for cellular therapy by ex vivo activation and ibrutinib. *Blood.* 2018;132(21):2260-2272.

6. van Bruggen JAC, Martens AWJ, Fraietta JA, et al. Chronic lymphocytic leukemia cells impair mitochondrial fitness in CD8(+) T cells and impede CAR T-cell efficacy. *Blood.* 2019;134(1):44-58.

7. Fraietta JA, Lacey SF, Orlando EJ, et al. Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. *Nat Med.* 2018;24(5):563-571.

8. Long M, Beckwith K, Do P, et al. Ibrutinib treatment improves T cell number and function in CLL patients. *J Clin Invest.* 2017;127(8):3052-3064.

9. Fraietta JA, Beckwith KA, Patel PR, et al. Ibrutinib enhances chimeric antigen receptor T-cell engraftment and efficacy in leukemia. *Blood.* 2016;127(9):1117-1127.

10. de Weerdt I, Hofland T, de Boer R, et al. Distinct immune composition in lymph node and peripheral blood of CLL patients is reshaped during venetoclax treatment. *Blood Adv.* 2019;3(17):2642-2652.

HEMATOPOIESIS AND STEM CELLS

Comment on Jinnouchi et al, page 1661

SIRPAssing other xenograft murine models?

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In this issue of *Blood*, Jinnouchi et al report a new xenograft mouse model with enhanced human hematopoietic and tumor engraftment, created by knocking the human *SIRPA* gene into immunodeficient mice (see figure).[1]

The ability of human hematopoietic cells injected into mice to engraft and establish multilineage human hematopoiesis is a remarkable feat that has facilitated seminal discoveries in hematopoiesis, cancer, infectious disease, and immunology.[2,3] Thanks to decades of research to identify and overcome barriers to human-mouse engraftment and further optimize murine xenograft strains, numerous robust models are currently available to researchers. However, limitations in some xenograft models still exist, including incomplete myeloid reconstitution, low capacity to support long-term serially transplantable human engraftment, and/or reduced longevity of xenografted animals. Jinnouchi et al sought to address some of these limitations via humanization of the murine phagocytic cells.

Mice can reject human cells via innate and adaptive immune responses, and these murine defenses must be overcome to create robust humanized models. Depletion of T and B cells via either mutation of the *Prkdc* gene or deletion of *Rag1/2*null and ablation of NK cell function, most commonly via deletion of *IL2rg*null, can prevent rejection of the transplanted human cells. An additional necessity for human-mouse engraftment is so-called phagocytic tolerance, mediated by the signal-regulatory protein α (SIRPA)–CD47 axis.[4] SIRPA is a transmembrane protein expressed on macrophages. When SIRPA binds CD47, a ubiquitously expressed cell surface protein, it sends a "do not eat" signal to the macrophage; in its absence, phagocytosis is triggered, and the unrecognized cell is engulfed and destroyed. SIRPA-CD47