that ibrutinib is active at these lower doses after employing this step-down strategy.

However, enthusiasm for ibrutinib dose reduction must be tempered by the fact that there were no clinical end points in this study, and duration of follow-up was short at <3 months. It is important to realize what these data do not demonstrate. The study was not designed to examine for potential differences in response, adverse events, or development of resistance at the lower doses of ibrutinib compared with the standard 420-mg dose. Data from this small study with only laboratory end points are insufficient to recommend ibrutinib dose reduction in routine clinical practice except when required to manage intolerable side effects. Given the financial burden of ibrutinib, which is a frequent concern of patients, it will be tempting to consider a lower-dose approach, and this must be resisted until a study with clinical end points is completed.

This pilot data support that a larger clinical trial with ibrutinib step-down dosing is warranted, and it is imperative that this study be done. Ideally this would be a randomized trial comparing reduction to 140 mg to the standard 420 mg so that differences in response profile, progression-free survival, and toxicity can be determined. If a prospective study were to demonstrate equivalent clinical outcomes with lower doses of ibrutinib, this would be a major benefit to patients by sparing side effects and cost, as well as lessen the burden on the health care system, where the economic impact would be substantial. This study by Chen et al is an important step toward this possibility.

Conflict-of-interest disclosure: K.A.R. receives research funds from Genentech and consulted for Acerta. ■

REFERENCES

1. Chen LS, Bose P, Cruz ND, et al. A pilot study of lower doses of ibrutinib in patients with chronic lymphocytic leukemia. *Blood*. 2018;132(21):2249-2259.
2. Cervantes-Gomez F, Kumar Patel V, Bose P, Keating MJ, Gandhi V. Decrease in total protein level of Bruton's tyrosine kinase during ibrutinib therapy in chronic lymphocytic leukemia lymphocytes. *Leukemia*. 2016;30(8):1803-1804.
3. Byrd JC, Furman RR, Coutre SE, et al. Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. *N Engl J Med*. 2013;369(1):32-42.
4. Mato AR, Samp JC, Gauthier G, Terasawa E, Brander DM. Drivers of treatment patterns in patients with chronic lymphocytic leukemia stopping ibrutinib or idelalisib therapies. *Cancer Biol Ther*. 2018;19(7):636-643.
5. Mato AR, Timlin C, Ujjani C, et al. Comparable outcomes in chronic lymphocytic leukaemia (CLL) patients treated with reduced-dose ibrutinib: results from a multi-centre study. *Br J Haematol*. 2018;181(2):259-261.

DOI 10.1182/blood-2018-10-878579

LYMPHOID NEOPLASIA

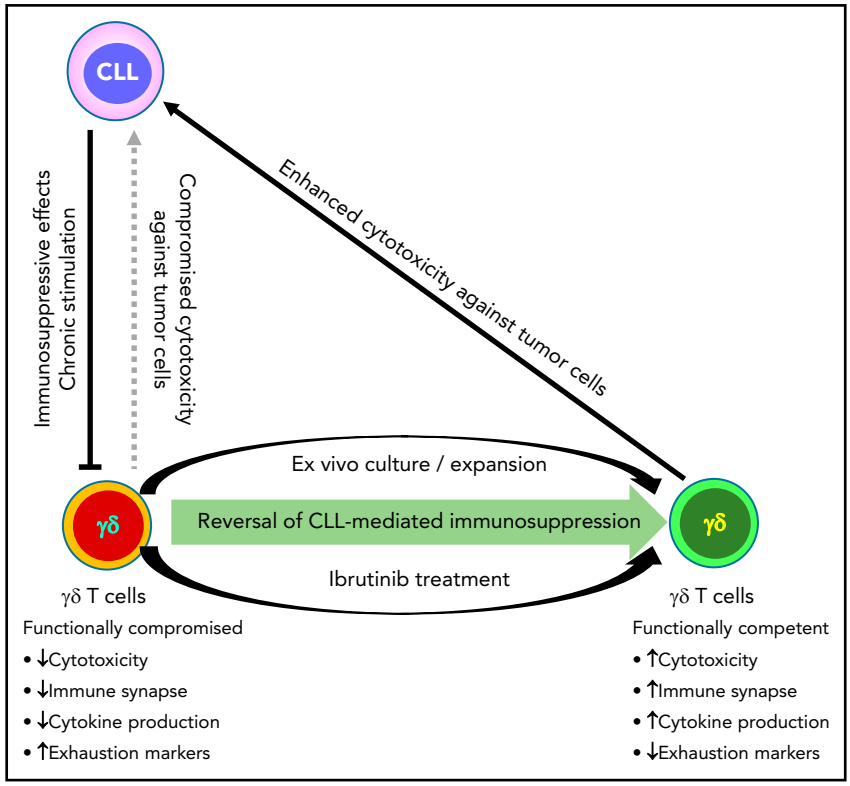
Comment on de Weerdt et al, page 2260

γδ T cells for immunotherapy

Meixiao Long | Ohio State University Medical Center

In this issue of *Blood*, de Weerdt et al report that in chronic lymphocytic leukemia (CLL) patients, the γδ T cells are functionally compromised. These γδ T cells can recover their function and demonstrate cytotoxicity against tumor cells after in vitro activation/expansion and/or ibrutinib treatment, which can be further explored as immunotherapy for CLL patients.[1]

Most T cells are αβ T cells, which have T-cell receptors (TCRs) composed of α and β TCR chains. γδ T lymphocytes, on the other hand, have TCRs made up of γ and δ chains. Up to 10% of T cells in peripheral blood are γδ T cells. Unlike the conventional αβ T cells, γδ T cells have the unique capability to recognize and kill cells under stressed conditions, demonstrating both innate and adaptive immune properties.[2] For example, under stressed conditions, γδ T cells recognize phosphoantigens that are produced as intermediate metabolites during cellular



γδ T cells from CLL patients are functionally impaired and cannot efficiently lyse tumor cells. Their functional competency and cytotoxicity against CLL cells can be recovered after ex vivo stimulation or ibrutinib treatment.

stress and malignant transformation. One major limitation for immunotherapy with conventional αβ T cells is that each clone of tumor-specific T cells can only recognize a single antigen epitope, in a HLA-restricted manor. For example, αβ T cells have been engineered to express TCRs specific for NY-ESO-1 or MART-1 (both are tumor-associated antigens). However, these αβ T cells can only recognize tumor cells that are of certain HLA types (eg, HLA-A2) and express high levels of the cognate antigens.[3] γδ T cells, on the other hand, hunt and kill tumor cells in a manner similar to innate immune cells: they are not major histocompatibility complex restricted and are capable of recognizing a broader spectrum of tumor cells rather than a single specific antigen epitope.[2] These features make γδ T cells a more "flexible" approach for cancer immunotherapy, especially for diseases like CLL, which is known to have limited options for tumor-associated antigens or mutation-specific "neo"-antigens and does not respond well to checkpoint blockade.[4] In this paper, the authors reported the potential of ex vivo activated γδ T cells in immunotherapy of CLL patients.

CLL cells profoundly suppress T-cell immunity via multiple mechanisms, such as expression of inhibitory surface molecules (eg, CD200, HLA-G, and PD-L1), production of immunosuppressive cytokines, and induction of regulatory T cells and myeloid-derived suppressor cells.[5] Dysfunction of "conventional" αβ T cells in CLL patients has been well studied. In CLL patients, these T cells demonstrate features of "exhaustion," with significant upregulation of checkpoint molecules and exhaustion markers such as PD-1 and CD160. T-cell "exhaustion" usually results from repeated chronic stimulation.[6] It has been postulated that CLL cells may cause chronic aberrant stimulation of T cells by inducing a CLL-specific immune response, or by modifying T-cell response to chronic infections such as cytomegalovirus.[7] In line with this, CLL patients' T-cell subsets are skewed toward a terminally differentiated phenotype, with significant reduction in naive T cells and expansion of more terminal differentiated effector memory/effector T cells. CLL also causes profound functional defects in T cells, including reduced cytotoxicity of CD8 T cells and defective immunologic synapse formation. T-cell response and functional status are skewed toward the Th2 polarization, which is considered to be detrimental in the scenario of antitumor immunity. However, little is known about how CLL affects γδ T cells.

In this paper, the authors reported that, in CLL patients, phenotypic and functional aberrations similar to those that occur in αβ T cells also occur in γδ T cells. They also demonstrated an exhausted phenotype, with subset distribution skewed toward a more terminal differentiated effector memory phenotype. Functionality wise, the γδ T cells have compromised cytokine production capability and limited cytotoxicity against CLL cells. Strikingly, the abovementioned dysfunctions can be induced in normal γδ T cells after being cocultured with CLL cells, which strongly suggests leukemia induced immune suppression. Moreover, γδ T-cell dysfunctions in CLL patients can be reversed after in vitro stimulation and both of the in vitro stimulation methods tested by the authors can also be used for clinical scale expansion for immunotherapy (see figure).

Ibrutinib, designed as a Bruton tyrosine kinase inhibitor for treatment of B-cell malignancies, has been found to have favorable immunomodulatory effects in multiple tumor models.[8] For conventional αβ T cells, ibrutinib enhances Th1/Tc1 response[9] in part through inhibition of interleukin-2 related tyrosine kinase (ITK). It also promotes the expansion and persistence of activated T cells by protecting them from activation-induced cell death.[10] In this paper, the authors demonstrated similar findings for γδ T cells: ibrutinib binds to ITK in γδ T cells, and in vitro treatment with ibrutinib enhances markers of Th1/Tc1-like function and cytotoxicity in γδ T cells.

These new data suggest that γδ T cells could be a promising approach for immunotherapy in CLL patients; however, there are still questions to be addressed. Unlike αβ T cells, much less is known about γδ T cells. For example, the mechanism by which γδ T cells recognize targets is different. How costimulatory/coinhibitor signals regulate the activation vs tolerance of γδ T cells is also less understood. We cannot simply extrapolate the findings from αβ T cells to γδ T cells. For safe immunotherapies, it is crucial to ensure that the tumor cells are killed efficiently while the normal tissue/cells are spared (ie, a safe therapeutic window). More mechanistic studies are needed to understand how to test and improve γδ T cells' specificity and efficacy against autologous tumor cells while sparing normal organs/cell types. In addition, for an immunotherapy to achieve clinical efficacy, the effector cells have to localize to the tumor site and battle with the immunosuppressive tumor microenvironment. Again, because most of our knowledge on these issues is from conventional αβ T cells, much more research about γδ T cells is needed.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. 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.
2. Simões AE, Di Lorenzo B, Silva-Santos B. Molecular determinants of target cell recognition by human γδ T cells. *Front Immunol*. 2018;9:929.
3. Ping Y, Liu C, Zhang Y. T-cell receptor-engineered T cells for cancer treatment: current status and future directions. *Protein Cell*. 2018;9(3):254-266.
4. Ding W, LaPlant BR, Call TG, et al. Pembrolizumab in patients with CLL and Richter transformation or with relapsed CLL. *Blood*. 2017;129(26):3419-3427.
5. Cutucache CE. Tumor-induced host immunosuppression: special focus on CLL. *Int Immunopharmacol*. 2013;17(1):35-41.
6. Zenz T. Exhausting T cells in CLL. *Blood*. 2013; 121(9):1485-1486.
7. Forconi F, Moss P. Perturbation of the normal immune system in patients with CLL. *Blood*. 2015;126(5):573-581.
8. Davids MS, Brown JR. Ibrutinib: a first in class covalent inhibitor of Bruton's tyrosine kinase. *Future Oncol*. 2014;10(6):957-967.
9. Dubovsky JA, Beckwith KA, Natarajan G, et al. Ibrutinib is an irreversible molecular inhibitor of ITK driving a Th1-selective pressure in T lymphocytes. *Blood*. 2013;122(15): 2539-2549.
10. 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.

DOI 10.1182/blood-2018-10-876078