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LYMPHOID NEOPLASIA

Comment on Duell et al, page 685

Lymphoma immunotherapy: the garden of forking paths

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In this issue of Blood, Duell et al[1] describe their effort to trace complex evolutionary trajectories of both aggressive and indolent B-cell lymphomas as these tumors escape elimination by CD19-directed chimeric antigen receptor-armed T cells (CART-19) and bispecific CD20xCD3 T-cell engagers. The authors observed considerable spatial and temporal heterogeneity in target antigen expression during relapses. Notably, in 1 patient CD20+ tumor cells disappeared from the main lesion, only to reemerge later in some, but not all, distant lymph nodes. This striking example of tumor evolution evokes the steady unraveling of seemingly conflicting scenario in the short story "The Garden of Forking Paths" by Jorge Luis Borges. Its protagonist believes that "this web of time—the strands of which approach one another, bifurcate, intersect or ignore each other through the centuries—embraces every possibility ... in some you exist and not I, while in others I do, and you do not." This quote aptly describes the natural history of many relapsing or primarily refractory blood cancers treated with potent immunotherapeutics.

In the mid-2010s, harnessing the power of autologous T lymphocytes to kill cancer cells proved to be a game changer in the field of hematologic malignancies. At the time, CART-19 cells and bispecific CD19xCD3 T-cell engagers (such as blinatumomab) were among the few effective treatments available to patients with relapsed or refractory B-cell leukemias and lymphomas. Although these therapies have generally improved overall survival, treatment failure is frequently observed in patients with leukemia as well as in those with lymphoma, often due to poor CAR T-cell persistence and autochthonous T-cell exhaustion. However, a significant percentage of relapsing neoplasms exhibit loss of cognate epitopes. This can occur by mechanisms ranging from deleterious gene mutations to messenger RNA (mRNA) splicing aberrations to epigenetic reprogramming causing transdifferentiation of infant leukemia with KMT2A gene rearrangements into myeloid lineages[2] and mantle cell lymphomas into sarcomas.[3]

Fortunately, besides CD19, there are other highly specific B-cell lineage markers. CD19− relapses can now be routinely treated with immunotherapies directed against other cluster of differentiation (CD) antigens, such as CD22, CD79B, and CD20. In fact, the oldest form of lymphoma immunotherapy is the anti-CD20 monoclonal antibody rituximab. Beyond rituximab and several next-generation CD20-directed monoclonal antibodies, the antilymphoma armamentarium now includes CD20-directed CAR T cells and bispecific CD20xCD3 T-cell engagers, such as glofitamab and mosunetuzumab. The latter bispecific was granted accelerated approval by the Food and Drug Administration in December 2022 for relapsed or refractory follicular lymphoma based on the results of a large multicenter clinical trial in which the overall response rate approached 80% and complete response rate approached 60%.[4] However, recent data still suggest that resistance due to epitope loss remains an ongoing problem, with up to 25% of patients relapsing with CD20− disease.[5] What drives postmosunetuzumab relapses remained an open question, motivating the study by Duell et al.

The samples available for this study included sequential biopsies from 7 patients with relapsed B-cell neoplasms (4 diffuse large B-cell, 2 follicular, and 1 transformed follicular lymphomas). These relapses came in 2 varieties: CD20− (with epitope loss) and CD20+ (with epitope retention). The 4 that were CD20− (as judged by flow cytometry and immunohistochemistry) arose due to various deleterious events affecting the MS4A1 gene, which in humans encodes the CD20 protein. These events included deep deletions, frameshift mutations, splice sites alterations, and even an apparent chromosomal translocation. Their functional impact was easy to interpret, although 1 of the longitudinal samples exhibited the loss of MS4A1 mRNA without any corresponding alterations in the gene structure. All this was to be expected because similar events were previously found to drive CD19− and CD22-dim relapses in pediatric patients with B-cell acute lymphoblastic leukemia.[6]

In contrast, the 3 CD20+ relapses appeared to rely on nongenetic, non–tumor cell autonomous mechanisms, such as T-cell exhaustion. Specifically, the authors observed that the exhaustion scores were significantly higher in CD8 effector T cells from 2 CD20+ relapses than from 1 CD20−

relapse. Despite the very small sample size, this observation raises interesting questions about evolutionary trajectories of lymphoma cells facing the selective pressures of T cell–based immunotherapies. Is it more effective for a cancer cell to lose its cognate epitope or to persist unaffected despite the onslaught of CD8^{+} killer cells, to the point at which these T lymphocytes simply exhaust themselves? The answer likely depends both on the cell of origin and on the nature of targeted antigen: although CD19^{−} and CD20^{−} relapses are fairly common, inactivating mutations in the CD22 gene are not routinely observed, at least not in childhood leukemia.

This epitope-positive vs epitope-negative dichotomy, although useful, needs to be approached with care. In 1 patient treated with CART-19 before enrollment in the Duell et al study, lymphoma cells were uniformly CD19^{−}/CD20^{+}. After treatment with mosunetuzumab and eventual relapse, all cells appeared to be double-negative by flow cytometry. However, after 8 weeks of salvage chemotherapy, 7% of cancer cells were CD20^{+}, according to single-cell RNA sequencing (RNA-seq) and immunohistochemistry (see figure). In another case, the clonal evolution was even more complex. That patient initially relapsed on CD20-directed bispecific antibodies with CD20^{+} disease, then received salvage therapy with a CART-19 product, and then relapsed again with CD19^{+} disease but now with a small subset of CD20^{−} clones. These convoluted trajectories highlight the complex evolutionary pressures applied on lymphoma cells by T cell–based immunotherapeutics.

Several practical questions arise from this and similar studies. For example, how should apparently epitope-negative relapses be treated? Are clinical flow cytometry and immunohistochemistry assays sensitive enough to detect meaningful immunotherapy-responsive levels of expression of cognate antigens? As Duell et al point out, in both ZUMA-1 and JULIET trials of 2 distinct CART products, objective responses were observed in patients deemed CD19^{−} at baseline.[7,8] With respect to CD20 as a target, we reported in a recent issue of *Blood* that lymphoma cells expressing alternatively spliced, translation-deficient *MS4A1* mRNA isoforms were resistant to killing by rituximab and mosunetuzumab. Strikingly, they were still sensitive to killing by CD20-directed CAR T cells.[9] As sequential losses of B-cell lineage markers become commonplace occurrences,[10] there is a need to incorporate into clinical practice successive biopsies aided by high-resolution immunohistochemical, flow cytometrical, and single-cell RNA-seq analyses. These analyses could
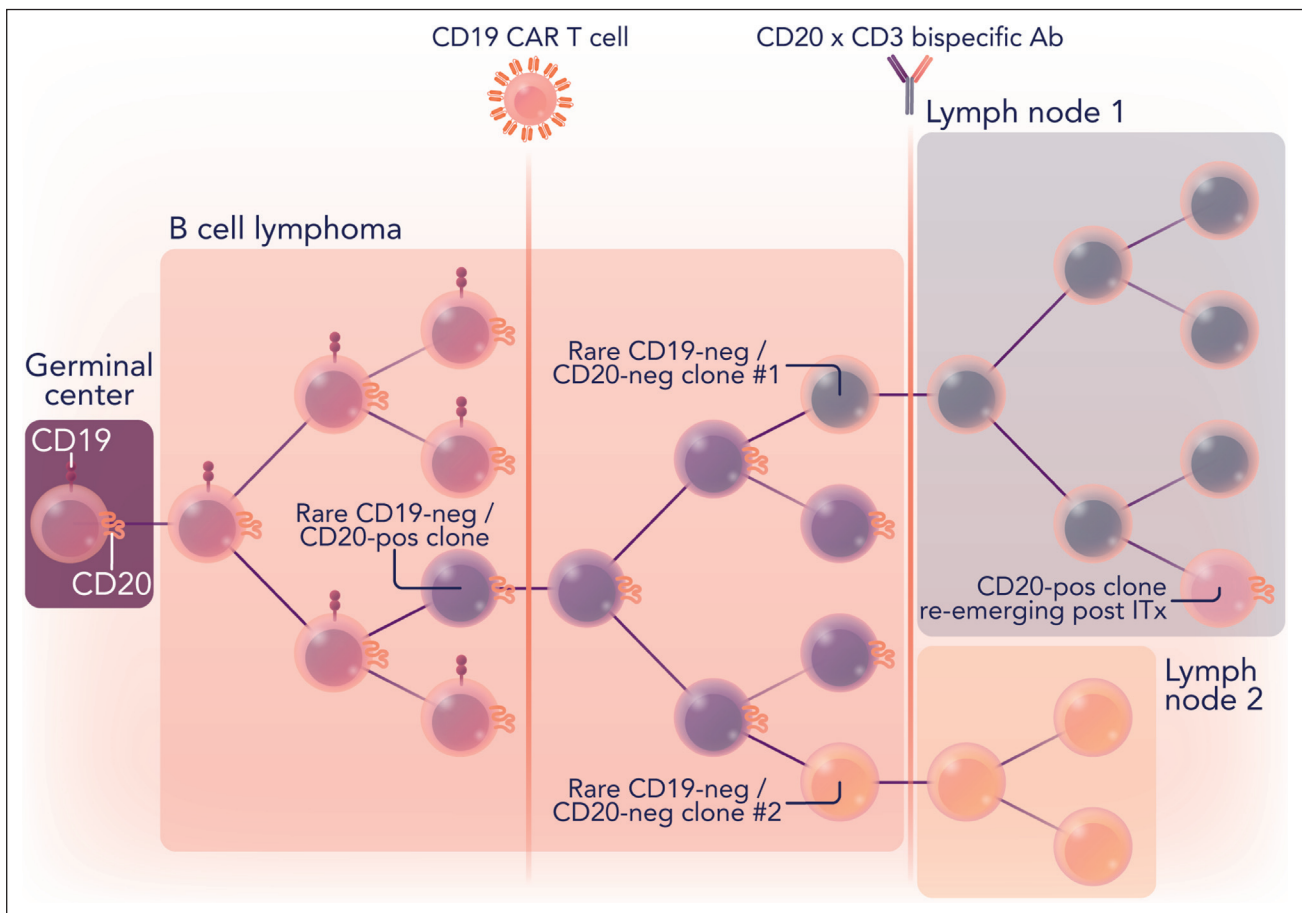
Branching evolution of B-cell lymphomas surviving successive rounds of CD19- and CD20-directed immunotherapies. B-cell lymphomas originate in germinal centers of the secondary lymphoid organs and are originally CD19/CD20 double-positive. Upon immunotherapy (ITx) with CD19-directed CAR T cells and/or bispecific CD20xCD3 T-cell engagers (represented by vertical lines), they routinely lose 1 or both antigens. However, CD20^{+} clones can reemerge in some of the distant lymph nodes upon discontinuation of ITx. The dendrogram illustrates one of the cases described by Duell et al. Ab, antibody; neg, negative; pos, positive. Professional illustration by Somersault18:24.

inform the selection of appropriate immunotherapeutics and thereby improve outcomes.

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MYELOID NEOPLASIA

A new SAGA for AML: targeting SGF29 in AML

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In this issue of *Blood*, Barbosa et al[1] discover that the chromatin reader protein SGF29 is a novel dependency for leukemias with upregulation of the MEIS1/HOX pathway. Chromosomal translocations involving MLL (the mixed lineage leukemia gene) as well as CALM (the clathrin assembly lymphoid myeloid gene) are enriched in infant/pediatric and therapy-related[2] leukemias and are often correlated with worse outcomes. MLL- and CALM-rearranged leukemias are characterized by upregulation of the posterior homeobox A (HOX A) gene cluster through recruitment of epigenetic modifiers,[3] the best described being DOT1L, a histone H3 lysine 79 (H3K79) methyltransferase.[4] Mechanistic dissection of how MLL fusion oncoproteins drive gene dysregulation and leukemia has resulted in several novel therapeutic for leukemias. For instance, discovery of the requirement of DOT1L and menin for HOXA gene upregulation in MLL-rearranged leukemias has led to clinical development of DOT1L and menin inhibitors (the latter of which appear very promising for the treatment of acute myeloid leukemia [AML][5]).

Although expression of posterior *HOXA* genes causes expansion of hematopoietic stem cells,[6] it is also known that HOXA genes require coexpression of the related homeobox protein MEIS1 to drive overt leukemia development.[7] Armed with this knowledge, Barbosa et al utilized an innovative approach of performing phenotypic drug and CRISPR screens to identify factors that silence expression of MEIS1 in an AML cell line expressing the CALM-AF10 fusion. Specifically, the authors tagged the endogenous *MEIS1* gene with a sequence encoding an in-frame green fluorescent protein (GFP), which enabled them to perform high-throughput screens of genes and proteins that led to silencing of GFP.

The author's initial drug screen utilized previously known drugs targeting an array of epigenetic modifying enzymes. However, the previously known drugs did not affect MEIS1-GFP expression, which led the authors to use an epigenetic enzyme-targeted CRISPR library to broaden their search. Results from their CRISPR knock-down screen identified genes involved in 6 highly enriched chromatin complexes that appeared to regulate MEIS1 expression, including DOT1L, ENL, and CK2 as well as multiple members of the SAGA, KMT2A, and HBO complexes. These top hits were then evaluated with an orthogonal CRISPR screen in single cells, allowing for simultaneous evaluation of genetic suppression and readout of gene expression. This additional screen identified the gene SGF29 (SAGA-associated factor of 29 kDa), which both downregulated MEIS1 and other oncogene expression while simultaneously increasing myeloid differentiation gene expression signatures. Importantly, when the top hits, including SGF29, were validated across AML cell lines, they limited proliferation of MEIS1/HOXA upregulated forms of AML including CALM- and MLL-rearranged AML cell lines.

SGF29 is a member of multiple chromatin regulatory complexes, including the SAGA (Spt-Ada-Gcn5-acetyltransferase) and ATAC (ADA2A-containing) complexes. SGF29 contains 2 tandem C-terminal Tudor domains, which bind histone H3 lysine 4 trimethyl (H3K4me3), a histone modification enriched at promoters, and recruits the SAGA complex for