HSC fates without altering self-renewal or that they alter lineage fate in more downstream progenitors. In addition, it remains unclear if Reg1/3 regulate lymphoid-myeloid fate decisions in other contexts associated with altered lympho-myeloid output including infection and aging. Finally, although the ASO studies provide support for a potential strategy to modulate hematopoietic output, they were performed under nonphysiologic conditions in which oligonucleotide was transfected directly into HSPCs followed by an in vitro readout. Whether or not oligonucleotide strategies can be utilized to effectively target *Nfkbiz* in vivo is presently unclear, but given the challenges of therapeutic targeting of the bone marrow using oligonucleotide strategies,[7] this may prove to be quite challenging.

Taken together, these impressive studies provide important new insights into the importance of posttranscriptional mechanisms in lineage fate decisions in the most primitive hematopoietic cells. Given the importance of this pathway in regulating lineage fates during steady-state and posttransplantation hematopoiesis, further investigation is warranted on the role of Reg1/3 in HSPCs in the context of aging as well as acute and chronic inflammatory stimuli including infection and metabolic stressors such as hyperglycemia and hyperlipidemia.[8,9] Indeed, it would be important to ascertain whether the Reg1/3 DKO phenotypes themselves depend on the cell-intrinsic induction of local or systemic inflammation, given the critical role of *Nfkbiz* in regulating NF-kB, a well-described mediator of inflammation.[10] Finally, given the large body of literature demonstrating that driver mutations in myeloid neoplasms frequently induce myeloid-biased maturation that is important to promote the selection of mutant clones, it also would be of interest to determine whether the Reg1/3-*Nfkbiz* axis plays a role in this process.

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LYMPHOID NEOPLASIA

Comment on Sakemura et al, page 258

CD19 occupancy may drive CARs further

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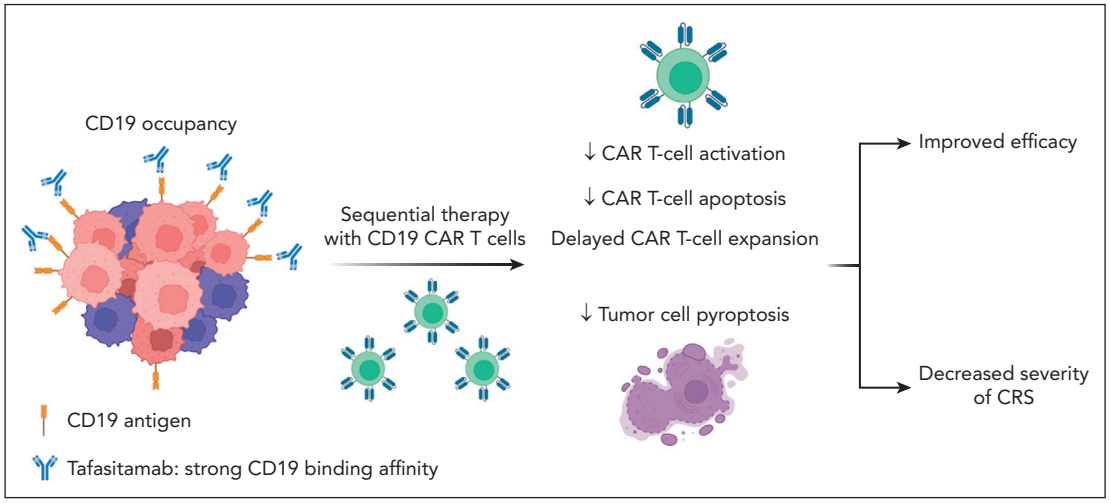
In this issue of *Blood*, Sakemura et al[1] report that treatment with the CD19-targeting monoclonal antibody tafasitamab followed by CD19-targeted chimeric antigen receptor T (CAR T) cells leads to reduced early CAR T-cell activation and apoptosis, with robust, but delayed, CAR T-cell expansion, resulting in improved efficacy, diminished tumor cell pyroptosis, and decreased severity of cytokine release syndrome (CRS) in preclinical models (see figure).

CD19 CAR T-cell therapy demonstrated high response rates in patients with relapsed and/or refractory large B-cell lymphoma (LBCL) and is now approved for the treatment of adults as early as second-line therapy. CD19 CAR T-cell activity is dependent on antigen density,[2] and lower pretreatment CD19 cell surface density was associated with progressive disease after CAR T-cell therapy in LBCL.[3] Treatment with the CD3-CD19 bispecific T-cell engager blinatumomab before CD19 CAR T-cell therapy was associated with diminished or partial CD19 expression and worse outcomes.[4] With the approvals of tafasitamab and loncastuximab terisine for patients with LBCL, whether the administration of these CD19-targeted antibodies before CD19 CAR T-cell therapy impacts its efficacy is a clinically relevant question.

Sakemura et al examined the impact of CD19 targeting with a monoclonal antibody on subsequent CD19 CAR T-cell therapy in preclinical models. They demonstrate that concomitant treatment with tafasitamab and CD19 CAR T cells inhibited T-cell effector function due to competition for CD19 binding. Conversely, prior treatment with tafasitamab improved the efficacy of CD19 CAR T cells due to delayed and robust CAR T-cell expansion. Sequential treatment with tafasitamab resulted in lower CD19 antigen availability and reduced early CAR T-cell activation and apoptosis. CAR T cells display tonic signaling, leading to impaired T-cell function and exhaustion,[5] and are prone to activation-induced cell death.[6] To enhance functionality, several approaches have been used to modulate CAR T-cell activation, including use of



Schematic representation of the effects of CD19 occupancy after sequential treatment with tafasitamab and CD19 CAR T cells in preclinical models.

lower-affinity CAR,[7] and transient inhibition of CAR signaling.[8] The current study adds transient CD19 occupancy as another strategy. Of note, the effects of antigen modulation on CAR T-cell activation may be dependent on CD19-binding affinity, as reduced CAR T-cell activation and apoptosis were not observed with the CD19 monoclonal antibody clone used in blinatumomab, which has weaker binding affinity compared with tafasitamab.

Sequential therapy with tafasitamab and CD19 CAR T cells also decreased the severity of CRS in a CAR T-cell toxicity mouse model. The development of CRS is closely associated with CAR T-cell activation and proliferation, and the release of inflammatory cytokines from both the infused T cells and bystander immune cells, with preclinical data suggesting that monocytes/macrophages play a key role in the pathogenesis of CRS.[9] Prior treatment with tafasitamab resulted in lower tumor burden at the time of CAR T-cell infusion, delayed CAR T-cell expansion with a modest increase in proinflammatory cytokines, and diminished tumor cell pyroptosis. Pyroptosis activates macrophages, resulting in the production of CRS-related cytokines,[10] and, again, lower levels of pyroptosis-released factors were only observed after treatment with tafasitamab.

Treatment with tafasitamab did not result in copy number variations or focal deletions in the CD19 locus, and CD19 expression recovered within 3 days after stopping the drug. However, given CD19 expression heterogeneity in LBCL,[2] and potential clonal selection due to immune pressure and trogocytosis, studies to accurately evaluate CD19 expression, such as quantitative flow cytometry, on tumor cells from patients treated with CD19-targeting monoclonal antibodies are warranted. The results of such studies may influence the sequencing of these novel therapies. Furthermore, a better understanding of the antigen density threshold for CAR T-cell activity and efficacy will help inform the optimal time interval between CD19-targeting monoclonal antibody and CD19 CAR T-cell therapy. This study provides a proof of concept that sequential treatment with tafasitamab and CD19 CAR T cells may represent a clinically viable strategy to modulate CD19 antigen density and, therefore, CAR T-cell activation and tumor cell pyroptosis.

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PLATELETS AND THROMBOPOIESIS

Comment on Asquith et al, page 272

Megakaryocytes in the lung: guests or ghosts?

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In this issue of *Blood*, Asquith et al reveal that only a marginal fraction of extramedullary megakaryocytes (MKs) are found within the lung and the spleen in mice, suggesting that the bone marrow (BM) is the primary site for platelet production.[1]

MKs are large, polyploid cells, primarily responsible for platelet production,[2] a process that occurs first in the yolk sac during embryogenesis, progressing to the fetal liver and ultimately to the adult BM. Despite MKs being observed in multiple tissues, the role of the contribution of the extramedullary MKs to circulating platelet mass has only recently been raised, with data suggesting a significant contribution by lung MKs.[3]

A study published in 2017 challenged the belief that platelets are produced primarily in the BM sinusoids. Using intravital microscopy and reporter mice to explore platelets and MKs, the authors found via direct visualization that MKs extended fine proplatelet structures within the lung. Based on imaging calculations, they estimated that lung MKs could contribute up to 50% of the platelet production in mice.[3] Subsequent publications reported the presence of intravascular MKs dedicated to platelet production in addition to the resident MK populations with immunoregulatory functions in lung parenchyma.[4]

In this work, Asquith and colleagues reassert the BM as the primary site for thrombopoiesis, presenting compelling evidence of the critical function of the BM for platelet production. They demonstrate in mice that (1) BM exhibits significantly higher counts of MKs and progenitor cells compared with spleens or perfused lungs; (2) fetal liver and BM have the highest concentration of MKs, with fewer in the spleen and only a nominal presence in the lung identified by in situ fluorescent labeling of cryosections using antibodies targeting canonical MK markers, such as CD41, glycoprotein IX (GPIX), and platelet factor 4 (PF4); (3) confirmation by 2-photon intravital and light-sheet microscopy that there are greater number of MKs in the fetal liver and BM than in the spleen or lung; (4) MKs cultured ex vivo from the BM display a superior proplatelet-formation capacity in static or microfluidic assays, compared with MKs from other sources; and (5) only a limited number of cells from murine and human lung single-cell RNA-sequencing (scRNA-seq) data sets express MK markers.

The strength of this study lies in its comprehensive approach, including whole-mount light sheet and quantitative histological imaging, flow cytometry, intravital imaging, and examination of scRNA-seq databases. These methods were used to distinguish the relative abundance of MKs in various organs and to estimate their potential role in maintaining the platelet population under steady-state conditions. Based on cell frequencies, immunophenotypes, and functional characteristics of MKs from the different tissues, the study concluded that lung and spleen MKs may make only a minimal contribution to physiological platelet generation compared with their better established counterparts derived from the BM and fetal liver during development.

The observed low frequency of MKs in the spleen is in line with the existing understanding of splenic MKs serving as a significant source of platelets in inflammatory conditions, during immune responses following infections or in cases of failed BM hematopoiesis.[5] What adds intrigue to this investigation is the sporadic detection and characterization of lung MKs using both conventional and cutting-edge imaging techniques, which have generated contradictory results in the literature. Previous studies have identified lung MKs using antibodies such as anti-CD42c antibody (GPIBB)[4] or anti CD41/CD42d (GPV),[6] whereas the current research used CD41, GPIX, and PF4 staining, which detected only a small number of MKs. Furthermore, direct evidence of physiological platelet release in the lung was reported based on in vivo MK tracking in PF4-Cre x mT/mG reporter mice,[3] but physiologic platelet release was not consistently observed in vWF-eGFP reporter mice used in this study. Hence, lung MKs appear to be a distinct group from those in the adult BM and fetal liver. Recent studies have characterized lung MKs as inflammatory cells.[3] Platelets are also considered inflammatory cells, and there is a well-known association between inflammation in the lung and platelets.[7] Emerging evidence, from scRNA-seq studies, is revealing distinct MK subpopulations,[8] which may share markers despite being heterogeneous in terms of morphology, function, and ontogeny.

In this intricate landscape, it is valuable to draw insights from clinical observations. Through the analysis of countless human peripheral blood smears, we know that under normal physiological conditions, circulating MKs are a rarity, except in pathological conditions, such as myeloproliferative neoplasms. Can MKs potentially originate directly from the lung in myeloproliferative neoplasms?[9] Clinical experience emphasizes that hematological diseases typically originate in the BM, with alterations there leading to changes