therapy approaches have now been tested, albeit with variation in the definition of standard or lower-risk acute GVHD. These and other agents should be considered in development of randomized trials, and such investigation should capture a full extent of treatment benefit and risks, including steroid exposure, infectious complications, and patient-reported outcome measures. Taken together, there is significant promise to advance the field toward personalized therapy of acute GVHD.

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Comment on Cappelli et al, page 503

Newer insights on how to TEC down T-ALL

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In this issue of *Blood*, Cappelli et al[1] investigated the interplay between T-cell acute lymphoblastic leukemia (T-ALL) and endothelial cells (ECs) with the hypothesis that the tumor microenvironment (TME), especially ECs, may affect drug responses.

T-ALL is an aggressive and often incurable disease which is treated with a similar chemotherapeutic backbone as used for B-cell ALL. Unlike B-cell ALL, there has not been an explosion of novel therapies for T-ALL. This is especially concerning for early T-cell precursor (ETP) ALL, which has significantly worse outcome. The options for salvage are limited, mainly conventional chemotherapy and hematopoietic stem cell transplantation (HSCT). Nelarabine (compete response rates: 30% to 40%) has enabled some patients with relapsed/refractory T-ALL to undergo HSCT and achieve long-term survival.[2]

The TME has a significant influence on cancer development and progression. It is well established that cancer pathogenesis involves genomically transformed cancer cells interacting with and benefiting from recruited accessory cells in the TME.[3,4] In particular, fibroblastic, endothelial, and other stromal cell components are driving forces in tumor development.[3,4] In the TME host-leukemia interaction, there is a vulnerability that can be exploited to develop personalized treatments. As major gatekeepers of cellular transmigration, ECs profoundly impact tumor angiogenesis and even peripheral immune cell trafficking into tumor compartments. The investigators used a nontransformed serum/xenobiotic free EC platform called E4ORF1[5] which allows studying leukemia-host interactions while maintaining their angiogenic potential. First, by performing a drug screen, they identified T-ALL liabilities using 22 patient-derived xenograft (PDX) models in vitro and in vivo. They then performed coculture experiments and generated bulk and single-cell RNAseq gene set enrichment analyses and hierarchical clustering and pathway analysis. Tumor-associated endothelial cells (TECs) have been shown to modulate T-ALL aggressiveness via multiple synergistic mechanisms, including SDF1α/CXCR4, DLL4JAG1-2/NOTCH, and IGFBP7/IGF1 prosurvival pathways. It has been known that therapies targeting the host endothelial cells impact the clinical responses and the potential side effects[6] (the Endothelial Activation and Stress Index score in cellular therapy). Additionally, their significance motivates therapeutic targeting of TECs via pharmacological or immunologic approaches. Identifying EC-associated molecular pathways and alterations may enable the development of novel therapeutic targets to evade TEC-mediated chemotherapeutic resistance and to enhance the efficacy of already established agents. Using high throughput screening of 433 compounds from clinically active and US Food and Drug Administration–approved agents, the investigators performed experiments focusing on signaling pathways, epigenetic changes, and antiapoptotic pathways. Redundancy testing using multiple drugs targeting the same pathways was used to confirm the results. A subset of these agents was examined in vitro, whereas others, including irinotecan, ruxolitinib, tofacitinib, bortezomib, panabinostat, daunorubicin, and OSI906 (linsitinib), were tested in vivo using T-ALL PDX. They found that the TECs were able to sustain T-ALL in stress conditions and counteracted the antiapoptotic effects of several drugs. Furthermore, TEC and T-ALL remained engaged at the genomic level undergoing reciprocal transcriptomic changes. At the single-cell resolution, this was characterized as "education signatures" associated with the bidirectional enhancement of canonical pathways (T-ALL: JAK-STAT, MAPK, TGFB, EGFR, NOTCH, and, MYC; TEC: JAK-STAT, NF-κB, TNFα, and VEGF); up-/downregulation of genes driving multiple pathogenetic processes

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(T-ALL: IGF1R, RPS6, RPL11, HMGB2, MALAT1, and RHOH; TEC: DLL1, JAG1, CD34, ETS1, ETS2, IGFBP-4, IGFBP-7, CD63, and CD9); and phenotypic changes (T-ALL: immature/stem-like; TEC: tumor-endothelial cell-like). Finally, an IGF1-decoy (IGFBP-7) abrogated the TEC-rescue for selected compounds suggesting a dependency on the IGF1-IGFR1 pathway. This is a seminal observation of the plastic nature of TEC to directly enhance tumor-promoting abilities through genetic reprogramming. More importantly, this can be therapeutically targeted to overcome this coconspiracy between T-ALL and TECs.

As discussed in this paper (and elsewhere[1-5]), ECs lining the vessels of TMEs deserve consideration for inclusion in the roster of functionally significant TME cells. Data from this study show the general principles underlying the dynamic and transcriptional control of leukemia cells in the TME and offer a platform to explore TEC as a tumor-state-specific modulator. The prospective use of a molecularly characterized endothelial-leukemia platform to identify drug responses and uncover the subverted role of tumor vascular niches is an effort in the right direction. Some future considerations include (1) focused studies on overcoming resistance in ETP-ALL which represents ~5% to 15% of T-ALL and has significantly worse survival; and (2) studying the common origins of leukemic clones and the endothelial cells from the common hemangioblastic progenitor in the context of endothelial to mesenchymal transition. The use of multi-omic profiling using innovative technology platforms is expected to provide this differentiation and to investigate how ECs facilitate tumor hallmark-enabling tumor progression not just for T-ALL but for other hematological malignancies.

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Comment on Hengeveld et al, page 519

Looking for a needle in the haystack of CLL

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In this issue of *Blood*, Hengeveld et al describe an openly available, highly sensitive next-generation sequencing approach developed to track minimal residual disease (MRD) in chronic lymphocytic leukemia (CLL).[1] This tool improved patients' outcome prediction in comparison with the traditionally available MRD tools.

Allele-specific oligonucleotide quantitative polymerase chain reaction (ASO-qPCR) targeting immunoglobulin (IG) gene rearrangements has been for years the criterion standard for monitoring minimal residual disease (MRD) in several lymphoproliferative diseases. Its wide use relied on strict standardization among centralized laboratories by defining common strategies for patient-specific assay designs and strict guidelines for data analysis.[2] This standardization process was undertaken by the international EuroMRD group, which since 2001 has been coordinating quality control rounds twice a year among the 69 MRD-PCR laboratories spread across 26 countries in Europe, Asia, Australia, and North and South America (www.euromrd.org).

However, while ASO-qPCR has considerable sensitivity (up to 10^{-5}, which means detection of 1 clonal cell out of 100 000 analyzed cells) it also has several shortcomings, specifically, technical complexity, labor-intensiveness, and the need for patient-tailored primer design. Next-generation sequencing (NGS) technology is currently used to overcome the limitations of MRD ASO-qPCR by using high-throughput consensus primers and deeper analysis, able to achieve superimposable sensitivity levels as the gold standard method.[3] Recently, a company-dependent IG-based NGS platform (ClonoSEQ) was developed for MRD monitoring and risk stratification in lymphoproliferative disorders, which claimed a sensitivity up to 10^{-6}, a claim that has been questioned. Several papers have extolled the potential advantage of the approach but ignored the large DNA input required to achieve such a cutoff.[4-6] The ClonoSEQ platform has recently received regulatory approval for the detection of MRD in acute lymphoblastic leukemia, multiple myeloma, and CLL. However, this approach is available only commercially, so the protocols and primer sequences are not at the disposal of the scientific community.[7] Alternatively, many research groups have implemented different NGS-based approaches for MRD: for example, international development and standardization efforts in this field are ongoing within the EuroClonality-NGS working group.[8]

In this issue of *Blood*, Hengeveld et al address several unresolved issues in this field. Starting from the EuroClonality-NGS experience, they showed the feasibility of an academically developed,

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