Under the surface: scratching the acute lymphoblastic leukemia niche

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In this issue of *Haematologica*, Barz *et al.* use three-dimensional (3D) imaging to pinpoint the localization of xenografted primary B-cell progenitor acute lymphoblastic leukemia (BCP-ALL) and T-cell ALL (T-ALL) cells in the bone marrow (BM) before and after chemotherapy.[1]

Despite significant improvements in chemotherapy regimens for ALL treatment, many children and up to 50% of adults will relapse and succumb to the disease.[2] Large-scale genomic studies of diagnostic, remission and relapsed disease have led to two major theories of relapse: (i) that rare chemotherapeutic resistant subclones exist at diagnosis and are selected by therapy[3] or (ii) that leukemic cells develop resistance to therapy during treatment.[4] Alternatively, some patients are refractory to treatment at diagnosis and fail to reach remission. Additionally, it is becoming increasingly appreciated that niches can offer protection from treatment in a wide range of cancers.

Indeed, prior *in vivo*[5-7] mouse and patient-derived xenograft (PDX) models of ALL have identified specific niche factors,[5] cell populations,[6] and proximity to the endosteum[7] as important for T-ALL and BCP-ALL survival before or after therapy. However, using time-lapse imaging of the niche before and after chemotherapy, Hawkins *et al.*[8] challenged the notion of a specific tissue localization of chemotherapeutic-resistant cells by demonstrating that T-ALL cells remain motile in the niche before and after treatment. To investigate the distribution of primary cells in the bone, the authors employed 3D microscopy to image the cells' localization before, during, and after chemotherapy.

First, the team established PDX models in NSG mice from nine genetically heterogeneous BCP-ALL and five T-ALL samples engineered to express luciferase for live tracking of disease burden. Engraftment of immunodeficient miceby human ALL cells, does not require conditioning thus ensuring that the niche is unharmed before engraftment. Bioluminescence imaging confirmed ALL localization to the proximal and distal metaphyses of the BM at 1 day after transplantation. Impressively, the authors established a 28-day model of induction chemotherapy including dexamethasone, doxorubicin, and vincristine, three of the mainstays of the human induction therapy regimen. The induction regimen successfully reduced disease burden in all xenograft models, while leaving detectable minimal residual disease (MRD) to allow imaging of post-chemotherapy ALL cell localization.

Next, the team used 3D confocal imaging of clarified femur to reveal the BM localization of BCP-ALL, T-ALL and $CD34^+$ healthy cord blood hematopoietic stem and progenitor cells in the absence of treatment to compare the localization of healthy and leukemic cells. While both subtypes of ALL cluster alongside sinusoidal cells, BCP-ALL cells were observed closer to BM sinusoids and T-ALL cells more scattered throughout the BM with some in closer vicinity to bone endosteal regions. Importantly, hematopoietic stem and progenitor cells displayed overlapping but distinct localizations compared to ALL cells, suggesting that distinct niche factors are required for these populations, which could be exploited for therapeutic benefit.

After chemotherapy treatment, residual BCP-ALL localized closely with sinusoids, whereas T-ALL cells were scattered throughout the niche, but with more cells found in the bone endosteal region. Interestingly, at later stages of leukemic cell engraftment and/or after the 28-day chemotherapy regimen, BM sinusoids were remodelled to a denser, swollen phenotype when compared to those of untreated and un-engrafted animals. Excitingly, the vascular changes were reversed within as little as 4 days after chemotherapy, suggesting that the vascular niche can bounce back from prolonged stress to support normal hematopoiesis. Follow-up experiments should address the ability of this compartment to support normal hematopoietic output upon remission.

Next, the group demonstrated that residual cells were capable of recapitulating primary disease after serial transplantation into secondary hosts. To test if chemotherapy selected for subclones with greater resistance to treatment, the authors transplanted MRD cells into secondary

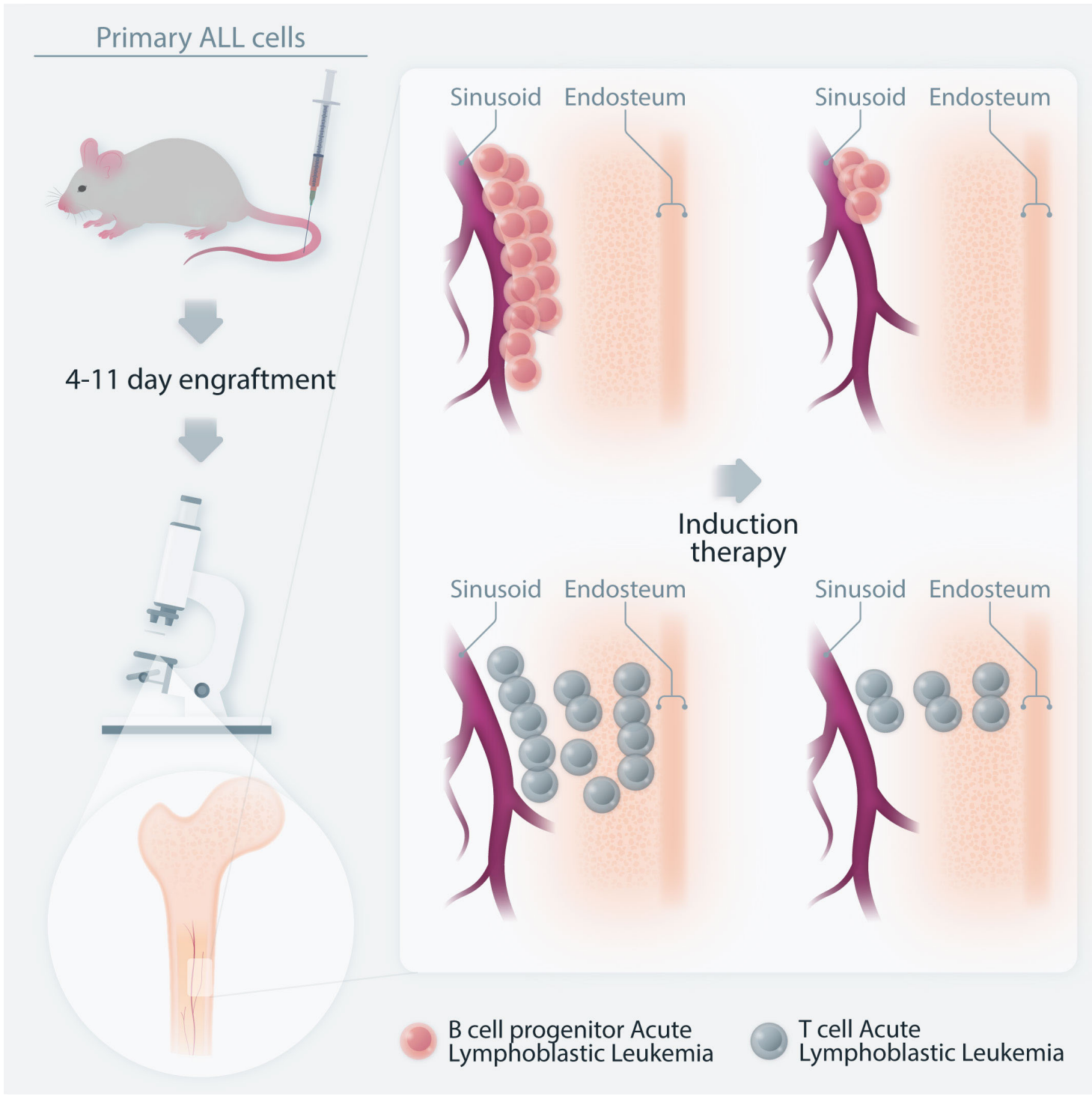

Figure 1. Characterizing the B-cell precursor and T-cell acute lymphoblastic leukemia niches before and after induction therapy. Patient-derived xenografts were established in NSG mice and allowed to engraft for 4-11 days prior to three-dimensional imaging of clarified bone marrow from the femur. B-cell precursor acute lymphoblastic leukemia (BCP-ALL) and T-cell acute lymphoblastic leukemia (T-ALL) cells were both found in close proximity to sinusoids, but T-ALL cells were also found close to the endosteum. After a model induction therapy regimen including vincristine, doxorubicin, and dexamethasone, residual BCP-ALL and T-ALL cells did not localize to or concentrate in new areas of the niches.

immunodeficient hosts and observed no delay in engraftment or response to the induction regimen *in vivo*. Two interpretations can be drawn, either niche interactions, rather than cell-intrinsic changes, drive chemotherapy resistance in this model, or the therapeutic regimen implemented is insufficient to kill all cells regardless of resistance mechanisms.

Finally, the authors aimed to identify whether cells survived chemotherapy by remaining dormant. To address this question, transplanted xenograft cells were pre-labeled with CFSE, a fluorescent dye whose signal dilutes out over multiple cell divisions, and mice were treated short-term (3 days) or with the full induction regimen prior to imaging and flow cytometry analysis to identify CFSE label-retaining cells (LRC). Three days after treatment initiation cells in chemotherapy-treated mice showed slightly higher CFSE retention than those in untreated mice. In contrast to the findings of Ebinger *et al.*, who identified LRC residing proximal to the endosteum after treatment,[7] the authors found that no LRC could be harvested from the BM after induction therapy, indicating that cells continued to proliferate during therapy. Imaging following short-term treatment demonstrated that $CFSE^{high}$ cells were not found closer to the endosteum than $CFSE^{low}$ cells. Overall, similar to results reported by Hawkins *et al.*,[8] the authors were unable to identify a population of dormant MRD cells or a tissue localization supporting MRD cells after chemotherapy.

The authors utilized a comprehensive 3D imaging approach

to study the BM microenvironment of BCP-ALL and T-ALL, demonstrating unique tissue localization of cells from each disease. Furthermore, this works brings into question prior work that demonstrated that residual ALL cells survive chemotherapy by remaining dormant.[7] However, since the current and prior studies model induction therapy differently, the difference in findings could be dependent on the chemotherapeutic agents used. It would be interesting to determine whether LRC reside in peripheral organs such as the spleen and central nervous system after treatment, since the authors noted that residual cells were found in these tissues. Work by Cahu *et al.* identified the adipose-rich tail BM niche as a reservoir for chemotherapeutic-resistant ALL cells.[6] Overall, the thought-provoking work by Barz *et al.* provides a beautifully detailed 3D view of primary human ALL cells in the BM niche and challenges the notion that specific BM niches promote dormancy to drive chemotherapy resistance. Future studies should seek to build on this research by determining the functional interactions between ALL cells and the niche that are required for leukemic progression and therapy resistance.

Disclosures

No conflicts of interest to disclose.

Contributions

MG and ANT co-wrote the manuscript.

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