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by Mark Gower and Anastasia Tikhonova

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Under the surface: scratching the ALL niche

Mark Gower[1] and Anastasia N. Tikhonova[1]

[1]Princess Margret Cancer Center, University Health Network

Corresponding author: Anastasia N. Tikhonova – e-mail anastasia.tikhonova@uhnresearch.ca

In this issue of Haematologica, Capron, Behrmann Barz, and Zuchtriegel *et al.* use 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 treatment[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 relapse disease have put forward two major theories of relapse: 1) That rare chemotherapeutic resistant subclones exist at diagnosis and are selected by therapy[3] or 2) That leukemic cells evolve 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 endosteum proximity[7] as important for T-ALL and BCP-ALL survival pre-therapy or post-therapy. However, using time-lapse imaging of the niche pre- and post-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 pre- and post-treatment. To investigate the distribution of primary cells in the bone, the authors employed 3D microscopy to image their localization before, during, and after chemotherapy.

First, the team established PDX models in NSG mice from 9 genetically heterogenous BCP-ALL and 5 T-ALL samples engineered to express luciferase for live tracking of disease burden. Engraftment of immunodeficient mice by human leukemic cells does not require conditioning, ensuring the niche is unharmed before engraftment. Bioluminescence imaging confirmed ALL localization to the proximal and distal metaphysis of the BM at one day post-transplant. 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 (HSPCs) in the absence of treatment to compare healthy and leukemic cell localization. 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, HSPCs display 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 untreated and un-engrafted animals. Excitingly, the vascular changes were reversed within as little as 4 days post-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 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 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 if 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 LRCresiding 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 post-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 demonstrates residual ALL cells survive chemotherapy by remaining dormant[7]. However, since these studies model induction therapy differently, these differential findings could be chemotherapeutic agent dependent. Additionally, it would be interesting to determine if LRC reside in peripheral organs such as spleen or CNS after treatment, since the authors noted that residual cells were found in these tissues. Indeed, work by Cahu *et al.* identified the adipose rich tail BM niche as a reservoir for

chemotherapeutic resistant ALL cells[6]. Overall, this thought-provoking work 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.

References

1 Barz MJ, Behrmann L, Capron D, et al. B and T cell acute lymphoblastic leukemia evade chemotherapy at distinct sites in the bone marrow. Haematologica. xxx.

2 Palomero T, Ferrando A. Therapeutic targeting of NOTCH1 signaling in T-cell acute lymphoblastic leukemia. Clin Lymphoma Myeloma. 2009;9 Suppl 3(Suppl 3):S205-S210.

3 Mullighan CG. Molecular genetics of B-precursor acute lymphoblastic leukemia. J Clin Invest. 2012;122(10):3407-3415.

4 Li B, Brady SW, Ma X, et al. Therapy-induced mutations drive the genomic landscape of relapsed acute lymphoblastic leukemia. Blood. 2020;135(1)41-55.

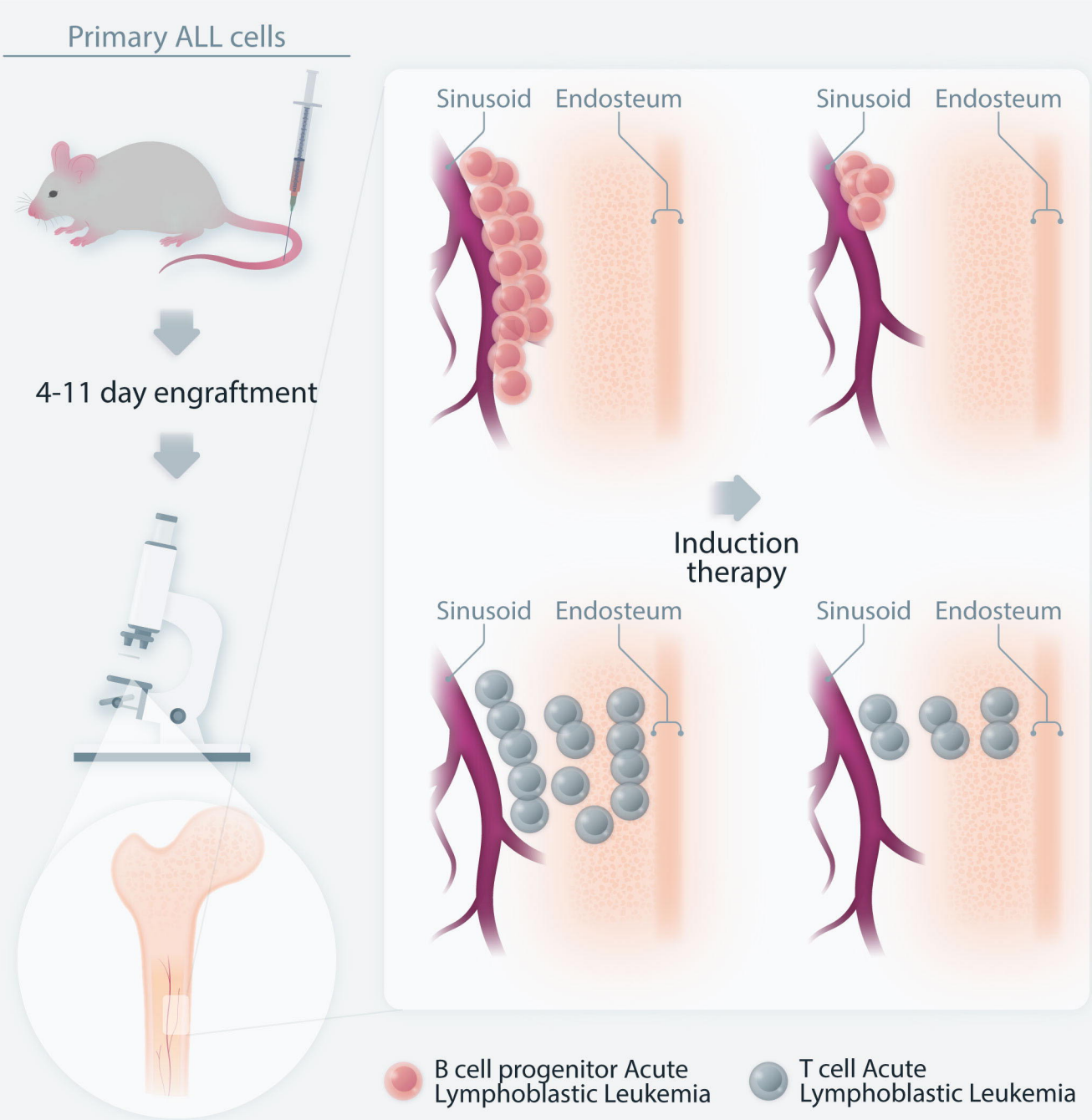
5 Pitt LA, Tikhonova AN, Hu H, et al. CXCL12-Producing Vascular Endothelial Niches Control Acute T Cell Leukemia Maintenance. Cancer Cell. 2015;27(6):755-768.

6 Cahu X, Calvo J, Poglio S, et al. Bone marrow sites differently imprint dormancy and chemoresistance to T-cell acute lymphoblastic leukemia. Blood Adv. 2017;1(20)1760-1772.

7 Ebinger S, Özdemir EZ, Ziegenhain C, et al. Characterization of Rare, Dormant, and Therapy-Resistant Cells in Acute Lymphoblastic Leukemia. Cancer Cell. 2016;30(6):849-862.

8 Hawkins ED, Duarte D, Akinduro O, et al. T-cell acute leukaemia exhibits dynamic interactions with bone marrow microenvironments. Nature. 2016;538(7626):518-522.

Figure 1: Characterizing the BCP-ALL and T-ALL niche pre- and post-induction therapy. Patient derived xenografts were established in NSG mice and allowed to engraft for 4-11 days prior to 3D imaging of clarified bone marrow from the femur. BCP-ALL and 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 niche.


Primary ALL cells
4-11 day engraftment
Sinusoid
Endosteum
Sinusoid
Endosteum
Induction therapy
Sinusoid
Endosteum
Sinusoid
Endosteum
B cell progenitor Acute Lymphoblastic Leukemia
T cell Acute Lymphoblastic Leukemia