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Drive to survive: gene dependency in T-cell acute lymphoblastic leukemia

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In this issue of *Haematologica*, Meyers *et al*.[1] present a genetic screen at the single-cell resolution to better explore the clonal fitness and the transcriptional remodeling caused by recurrent loss-of-function alterations in T-ALL. A tremendous effort has been made over the past three decades to uncover the main drivers of T-ALL, an aggressive hematological class of tumors with complex oncogenetic traits that contribute to stratifying patient outcomes[2–4]. While the impact of oncogenic activation of NOTCH1, JAK/STAT, or PI3K signaling pathways on leukemogenesis and clinical outcome of T-ALL have been extensively studied[5,6], the functional consequences of dozens of additional recurrent alterations remain an unresolved conundrum.

While primary tumors and patient-derived xenografts are the archetypes for understanding cancer cell evolution and resistance to treatments, these samples are inadequate to deconvolve the cooperative oncogenic networks that participate in transforming thymocytes and the onset of T-ALL. In the era of single-cell genomics, the field still lacks robust models to study gene dependencies and oncogenic cooperations that drive leukemogenesis.

To address this, the authors developed a model using primary pro-T cells, obtained from the *ex vivo* differentiation of lineage-negative hematopoietic stem cells, engineered to undergo single-cell genetic screening over time combined with transcriptomics in a wild-type background. First, this experimental setup allows to identify and validate essential genes for pro-T cells. Second, it permits the evaluation of the impact on the transcriptome and the cellular fitness – using cell proliferation as a readout – induced by the loss of function of each candidate gene with high granularity.

The authors first validated that their model consistently identifies essential and tumor-suppressor genes by applying this strategy to an initial panel of 17 key T-cell and T-ALL genes. As expected, the genetic inactivation of *Myc*, *Spi1*, *Kit*, *Notch1, Il7r* or *Stat5b* strongly impedes pro-T cell expansion. Conversely, *Pten*-deleted cells rapidly expand and represent the dominant clone within 7 days of screening. Additionally, this system suggested mild tumor-suppressing roles for another set of genes including *Runx1*, *Fos*, *Jun*, *E2f1* or *Ptprc*. Because it combines single-cell transcriptomics with clonal fitness, this

system allows the exploration of perturbation-associated regulons. By applying network inference algorithms to their data, the authors revealed that cooperative transcriptomic signatures driven by *Myc* and *E2f* were downregulated upon *Spi1* silencing whereas *Ets1*-related programs were found induced, raising the question of potential compensation by other Ets transcription factors.

In a second screening, the authors adapted their single-cell screening method to evaluate the impact of the loss of function of 42 genes recurrently found altered in T-ALL on the clonal fitness and transcriptome adaptation. A relevant observation from this approach is that most of the single-gene perturbations lead to the upregulation of the JAK/STAT signature, in line with the central role of this pathway in T-ALL leukemogenesis and maintenance[7]. Another interesting observation is that the silencing of several transcription factors and epigenetic regulators results in the uncoupling of NOTCH1 activation and proliferative programs driven by MYC and E2F. Given the major roles of NOTCH1-Myc axis T-ALL, and the failure of anti-NOTCH1 strategies in the clinics, identifying targets amenable to uncoupling Notch1 and its target effector c-Myc is relevant.

BCL11B is an essential driver of T-cell lineage commitment and its tumor suppressor functions have notably been reported in T-ALL, where frequent loss-of-function alterations are reported[8,9]. As for other tumor suppressor genes, the haploinsufficiency of BCL11B elicits T-ALL development, while the biallelic inactivation is lethal. These tumor-specific oncogenetic traits may represent an extensive source of potential novel vulnerability to target cancer cells[10]. In their model, Meyers *et al.*[1] report that *Bcl11b* loss of function is associated with elevated NF-kB- and STAT-driven inflammatory signatures and the induction of several anti-apoptotic regulators of the BCL2 family. These findings were also observed in T-ALL patients, suggesting that *BCL11B* haploinsufficiency may cooperate with oncogenic JAK/STAT activation to drive T-ALL onset and maintenance. Besides, the authors present that pro-T cells with *BCL11B* inactivation respond less to the JAK inhibitor ruxolitinib, which may be informative in the treatment of T-ALL harboring these lesions.

It is well-established that leukemogenesis does not result from single-gene alterations, but rather requires several cooperating oncogenic lesions to initiate and maintain the disease. Moreover, these alterations are probably acquired in a specific sequence, as some mutations only exhibit their oncogenic potential in a favorable genetic and epigenetic environment. Nevertheless, the elegant functional screening of gene dependency proposed by the authors sheds light on the contribution of several recurrent alterations in T-ALL and provides a novel methodology to resolve the oncogenetic networks that drive the disease.

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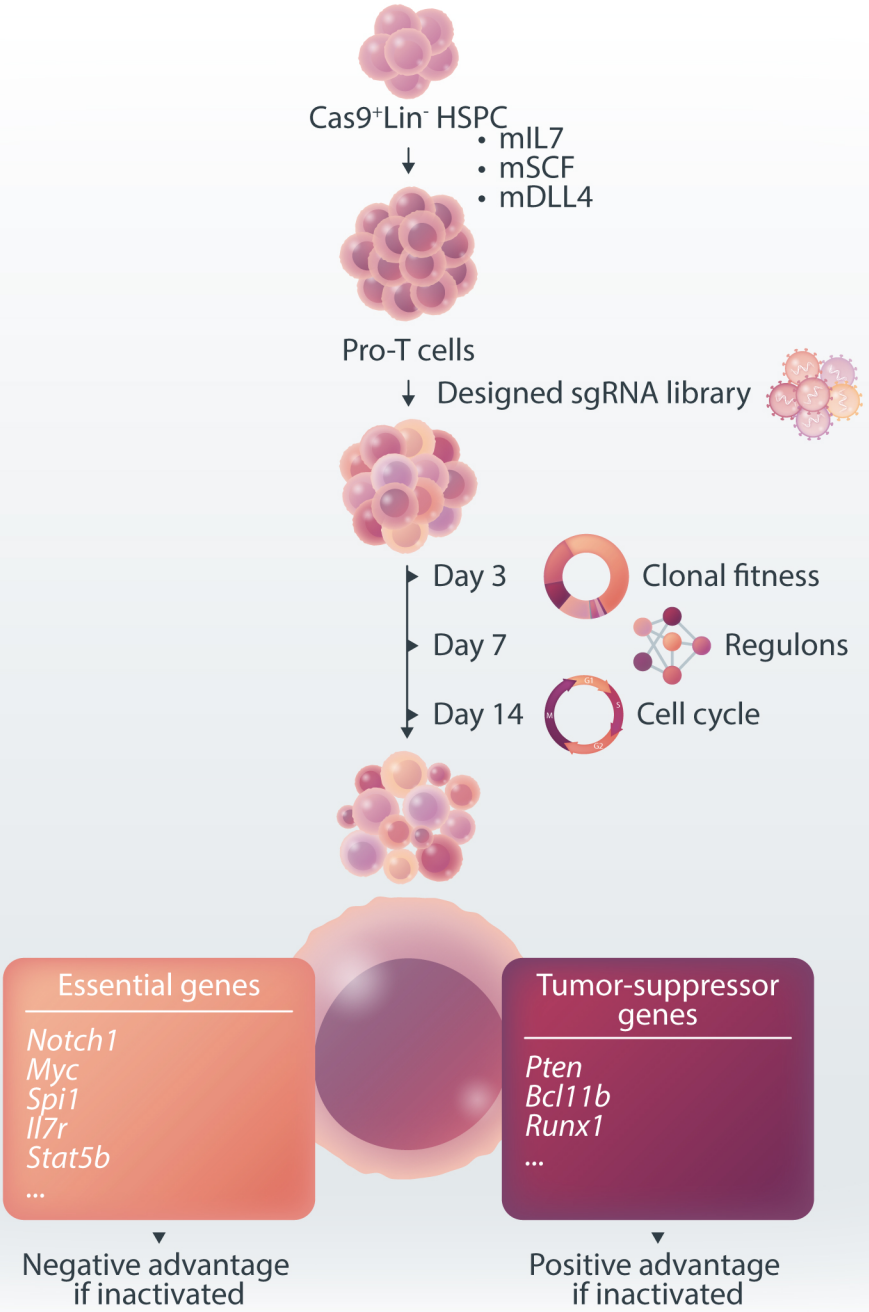
Figure 1. A single-cell functional screening of T-ALL gene dependency in pro-T cells.


Cas9+Lin- HSPC
• mIL7
• mSCF
• mDLL4
Pro-T cells
Designed sgRNA library
Day 3
Day 7
Day 14
Clonal fitness
Regulons
Cell cycle
Essential genes
Notch1
Myc
Spi1
Il7r
Stat5b
...
Tumor-suppressor genes
Pten
Bcl11b
Runx1
...
Negative advantage if inactivated
Positive advantage if inactivated