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Stem cells make leukemia grow again

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Leukemic stem cells were hypothesized to play a critical role in acute myeloid leukemia relapse, but data to support this were lacking. In a recent study elegantly combining sequencing with functional xenograft assays, Shlush *et al* (2017) identified two distinct origins of leukemic relapse. They provided direct experimental evidence linking relapse to cancer stem cell clones already present before therapeutic intervention.

See also: **LI Shlush *et al*** (July 2017)

The term "cancer stem cell" (CSC) was coined in 2001 (Reya *et al*, 2001). This concept was mostly based on data obtained in acute myeloid leukemia (AML), and it was hypothesized that most tumors are organized in a hierarchical manner with CSCs sitting on top of that hierarchy (Bonnet & Dick, 1997; Reya *et al*, 2001). Moreover, it predicts that CSCs are functionally distinct from other cells within the same tumor clone (i.e., sharing the same mutational profile) caused by their differential self-renewal capacity and inherent resistance to anti-proliferative therapies (Kreso & Dick, 2014). A prime example for this concept is AML, in which LSCs can be functionally identified using xenotransplantation assays (Bonnet & Dick, 1997; Ng *et al*, 2016). Molecular characterization of these cells revealed that they harbor a transcriptional profile related to hematopoietic stem cells (HSCs), which can be used to estimate the LSC burden in AML patients and can serve as an excellent predictor of clinical parameters including overall survival (Ng *et al*, 2016). Although these data established a clinical relevance of LSCs for patient survival, their role during therapeutic interventions and disease relapse remained unexplored.

A major obstacle in the treatment of AML patients is the development of relapse. The CSC hypothesis would predict that this is caused by the presence of chemotherapy resilient AML subpopulations. Previous studies with informative paired diagnostic and relapse samples have already suggested that relapse arises from re-emergence or clonal evolution of a pre-existing clone generated before treatment and whose clonal selection is shaped by chemotherapy (Ding *et al*, 2012; Parkin *et al*, 2013; Perry *et al*, 2013). These studies and others hint to the fact that both the dominating clone at diagnosis as well as pre-existing AML subclones have to be eradicated to control the disease. LSCs have been linked to this phenomenon, as their self-renewal and dormancy features make them prone to survive anti-proliferative therapies (Trumpp *et al*, 2010; Pollyea & Jordan, 2017). Shlush *et al* (2017) now shed some new light on the role of relapse-relevant LSC populations in the leukemic hierarchy. Using a sophisticated approach combining sequencing with xenograft assays, they characterized paired diagnostic/relapse AML samples and identified two distinct origins of relapse clones, which differ in their immunophenotypic, transcriptomic, and functional characteristics.

The origin of leukemic cells in relapse samples can be traced back by analyzing their mutational profile and by using specific mutations as lineage tracing marks. Therefore, cells within the relapse and diagnostic samples sharing the same mutational profile will most likely originate from the same founder LSC. For their study, Shlush *et al* (2017) determined the mutational landscape of bulk AML samples obtained at diagnosis and relapse as well as non-leukemic lymphocytes to distinguish between pre-leukemic and leukemic mutations and calculate their individual variant allele frequencies (VAFs). Additionally, they performed xenotransplantation of the diagnostic samples, allowing them to characterize the LSC populations and their respective mutational spectrum present at diagnosis. VAFs were then used to follow the evolution of LSC clones from diagnosis to relapse. Interestingly, this analysis showed that LSC clones giving rise to the relapse clone were already present to varying degrees at diagnosis. These data provide evidence for the hypothesis that some of the LSCs were resistant to the therapy and importantly were not induced by the therapeutic intervention.

Interestingly, Shlush *et al* (2017) identified two major types of LSC populations giving rise to relapses differing in their stage of differentiation as well as in their transcriptomic profile (Fig 1). In the first group, called relapse origin-primitive (RO_P), the relapse-relevant LSC is characterised by a hematopoietic stem and progenitor (HSPC) like phenotype. In these patients, mutations found in the relapse blasts were not detectable in diagnostic blasts but could only be identified in HSPCs (e.g., HSC/MPP, MLP, GMP), as well as in the leukemias grown in the xenotransplantations derived from the diagnostic sample. Therefore, the LSCs responsible for the relapse are rare, and score in the xenograft assay, but importantly do not significantly contribute to the leukemic blasts at diagnosis. However, these excruciatingly rare LSCs (e.g., 1/5,000) are apparently resilient to the standard chemotherapy and re-initiate the leukemia during relapse.

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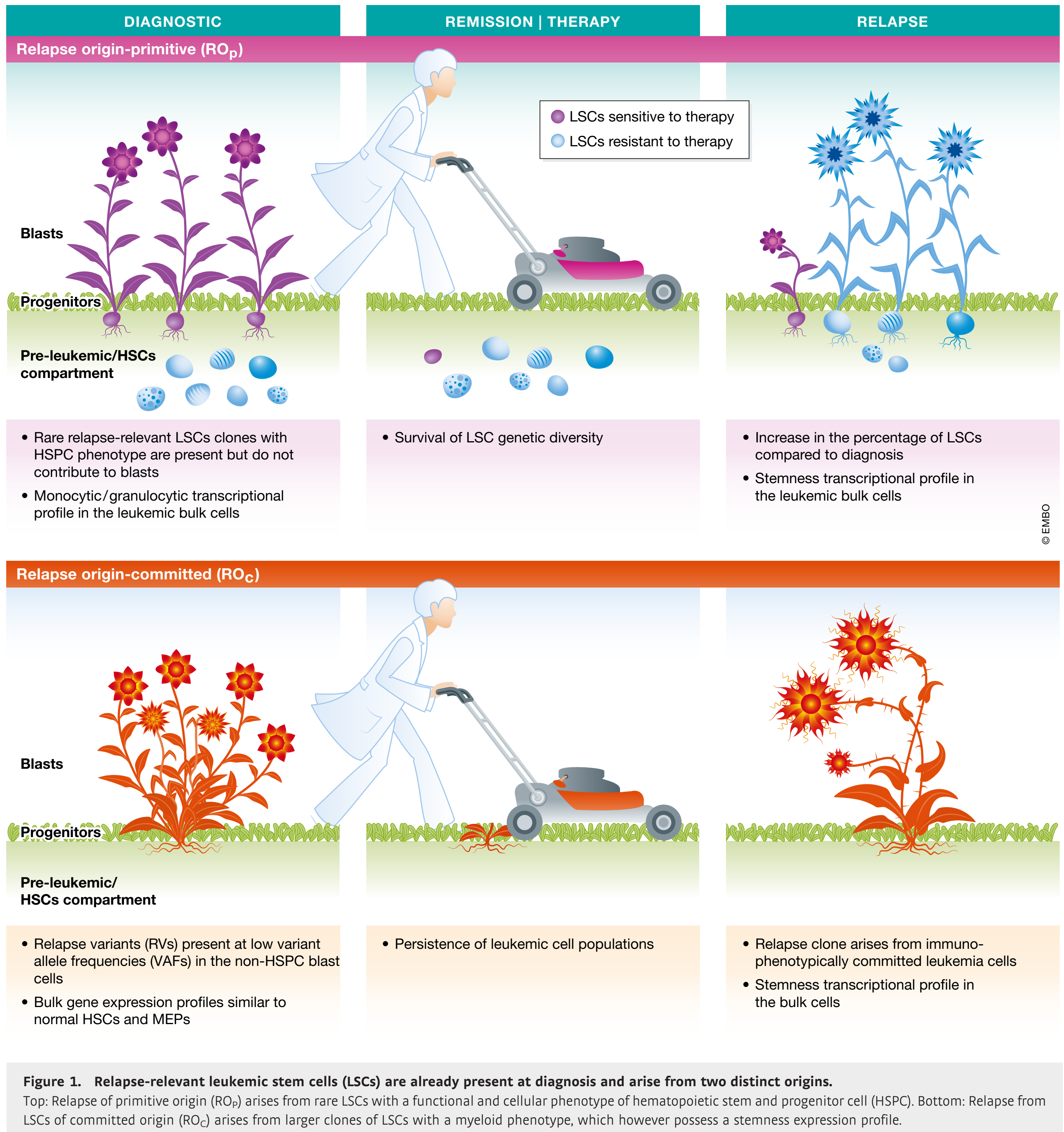

Figure 1. Relapse-relevant leukemic stem cells (LSCs) are already present at diagnosis and arise from two distinct origins.
Top: Relapse of primitive origin (RO$_P$) arises from rare LSCs with a functional and cellular phenotype of hematopoietic stem and progenitor cell (HSPC). Bottom: Relapse from LSCs of committed origin (RO$_C$) arises from larger clones of LSCs with a myeloid phenotype, which however possess a stemness expression profile.

In the second group, called relapse origin-committed (RO$_C$), the relapse originated from cells with an immunophenotype of a more committed progenitor, however possessing a strong stemness signature. These cells are more closely related to the major clone present at diagnosis and reflect a more flat hierarchy from which the relapse clone develops. Two additional types of relapse patterns were identified: one in which the diagnostic and relapse clone showed very little evolution, possibly due to an inefficient therapy response and outgrowth right after the end of therapy, and another in which the relapse clones showed no resemblance to the leukemia present at diagnosis.

The identification of the former two sources of LSCs responsible for relapse has clinical implications. In order to treat patients of the RO$_P$ group, the apparently dormant relapse-relevant LSC clone has to be targeted at diagnosis to ensure long-term remission or even cure. In contrast, in patients of the RO$_C$ group, the major clone

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present at diagnosis and its closely related relapse-relevant LSC clone still present during remission have to be targeted (Pollyea & Jordan, 2017; Shlush *et al*, 2017). In conclusion, this study suggests that tracing LSC populations during conventional or targeted therapy approaches might be helpful to understand the development of potential resistant mechanisms.

In addition, this work now queries for the molecular and cellular mechanism of how the relapse-relevant LSC populations develop already before therapy, as their mutational spectrum is enriched for nucleotide transversions, which are typically the result of mutagen exposure. Given previous studies, it is surprising that the majority of protein-damaging variants are already present in the pre-leukemic and leukemic cells at diagnosis, whereas they are not substantially increased over time despite DNA-damaging chemotherapy (Ding *et al*, 2012). Another important question is whether the predicted LSC dormancy is induced by the major leukemic clone due to competition or whether it is an intrinsic feature of these LSCs similar to normal HSCs. Potentially, this can be distinguished in xenotransplantation assays, as the number of competitor LSC populations can be reduced experimentally. While a state of dormancy in LSCs is likely but still experimentally unproven, dormancy in normal mouse HSCs and other stem cells is better characterized and can be mediated by differential activity of retinoic acid, TGF-beta or c-MYC (Scognamiglio *et al*, 2016; Cabezas-Wallscheid *et al*, 2017). It will be interesting to see whether similar pathways are mediating LSC dormancy, as this may open the direction towards possible targeting schemes.

Regardless of the many new interesting questions this work is provoking, a clear message arises: Irrespectively of the cellular origin of the relapse (RO_P or RO_C), the common feature is the stemness property of relapse-relevant clones, a characteristic that should be considered a priority when designing new prospective trials and approaches to prevent and target relapse. Collectively, this work suggests that AML follows a complex clonal evolution itinerary shaping the pre-leukemic HSC and LSC compartments and this complexity can, at least in part, be deconvoluted by a combination of xenotransplant assays and deep sequencing.

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