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ORAL ABSTRACTS

603.LYMPHOID ONCOGENESIS: BASIC

Transient Differentiation-State Plasticity during Initiation of Pediatric Acute Lymphoblastic Leukemia

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Introduction

Leukemia is characterized by oncogenic lesions that result in a block of differentiation while at the same time intratumor phenotypic plasticity is retained. It is unclear how these two phenomena arise during leukemogenesis in humans. Here we characterized T-cell acute lymphoblastic leukemia (T-ALL), as a model disease to investigate the coherence between leukemia initiating cells (LICs), developmental arrest, and phenotypic plasticity.

Methods

We characterized 31 primary T-ALL samples for differentiation state heterogeneity using multi parameter flow cytometry. Subsequently, for 5 patients, phenotypic subpopulations were single cell sorted. As leukemic cells do not expand *in vitro* primary template-directed amplification (PTA) was used to amplify the genome of single blasts to obtain enough DNA for WGS analysis with nucleotide resolution. Shared and unique mutations were used to construct retrospective lineage trees and assess the hierarchy of phenotypically diverse blasts.

Results

To assess phenotypic differentiation state heterogeneity in T-ALL, we designed a 17-parameter flow cytometry panel to discriminate T-lymphoid developmental cell populations. We characterized 31 samples (median blast count 98% (IQR = 94 - 99)), and defined immunophenotypic subpopulations as populations with a size of >10% of the total CD7+ population. Differentiation state heterogeneity (> 2 subpopulations) was observed in 26 out of the 31 T-ALL patients (83.9%). Interestingly, the most immature CD4-CD8-CD3- "DN-like" differentiation state was found in all samples, suggesting that developmental hierarchy is maintained. We then questioned whether genetic determinants were driving the phenotypic diversity we observed. Therefore 5 T-ALL patients harboring translocations of oncogenes commonly seen in T-ALL were selected, namely, *TLX1* (n=1) , *LMO2* (n=2) , *TAL1* (n=2) and *TLX3* (n=1) . For each patient cells of each differentiation state were single cell and bulk sorted for single cell WGS and bulk WGS, respectively. Structural variant analysis confirmed presence of the oncogenic translocation in all single blasts, validating their (pre-)leukemic origin. Moreover, driver analysis showed that type B mutations in e.g., *NOTCH1* and *PHF6* were shared among all blasts. Interestingly, there were no potential genetic drivers found that were unique to a subpopulation. Neutral passenger mutations were therefore used to construct phylogenetic trees. Herein, blasts with similar phenotypic differentiation states were genetically closer related than blasts of different phenotypes, revealing the heritability of phenotypes (Fig. 1A). The biased distribution of immunophenotypes across the different branches of the phylogenetic tree was then confirmed in bulk WGS samples of sorted phenotypic populations. Suggesting that switching between phenotypes is not a frequent process. Next, the plasticity of T-ALL was assessed by identifying the phenotypic cell state of the LIC. We assessed V(D)J recombination per single blast and identified monoclonal V(D)J recombination for most of the blasts. Moreover, in *TAL1* T-ALL we observed monoclonal *TRG, TRB* and *TRA* translocation. This suggested that the LIC had undergone complete V(D)J recombination and was a more mature T-cell progenitor. However, blasts with an immature CD4-CD8- (DN) phenotype had undergone the same V(D)J recombination suggesting phenotypic de-differentiation of the LIC. Additionally, in *TLX1* T-ALL, the blasts with the more mature CD4+CD8+ double positive (DP) phenotype had monoclonal *TRG* and *TRB* gene recombination, but polyclonal *TRA* recombination, whereas cells with a more immature DN phenotype had no *TRA*

recombination (Fig. 1B). This indicated an immature T-cell progenitor as the LIC and continued differentiation after leukemia initiation resulting in DP cells with unique *TRA* recombination.

Conclusion

In conclusion, we show immunophenotypic differentiation state heterogeneity in diagnostic primary T-ALL samples. By coupling phenotypic data with novel single cell whole genome sequencing techniques, we identified heritability of phenotypic cell states. Moreover, we reveal the ability of the LIC to differentiate and de-differentiate after leukemia initiation. Taken together our results demonstrate a transient period of plasticity during leukemia initiation where phenotypic switches appear unidirectional.

Disclosures Meijerink: *Acerta Pharma:* Current Employment, Other: full time senior director biotech.

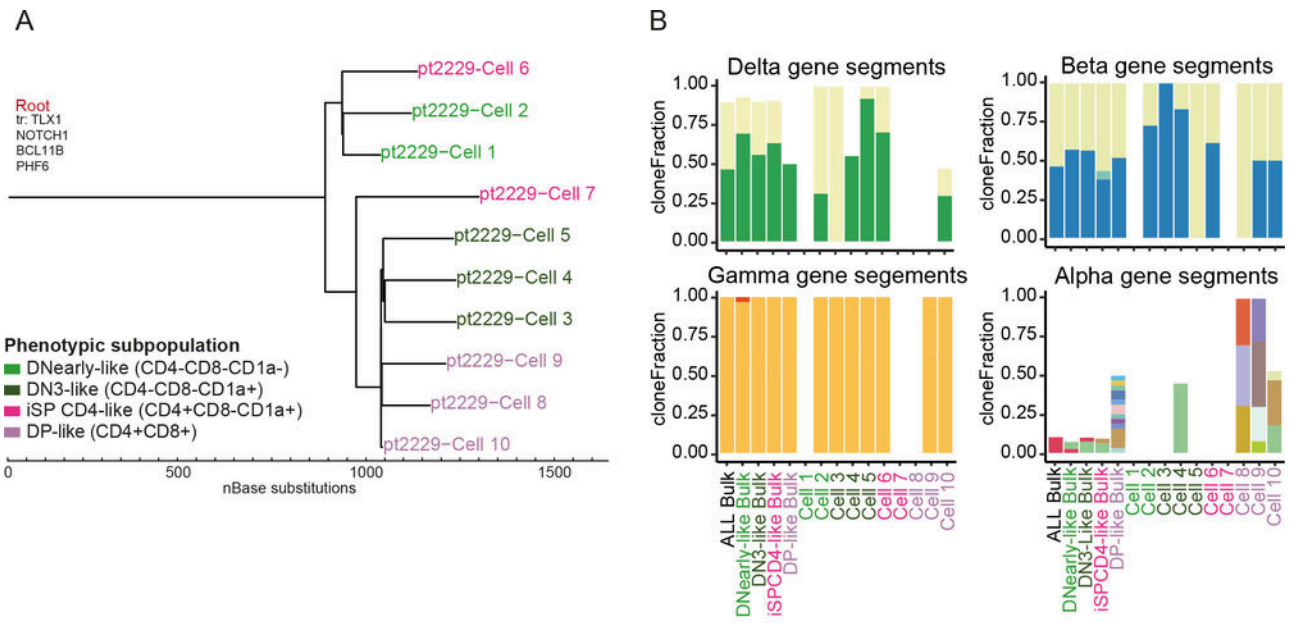

Figure 1: A) Phylogenetic tree of T-ALL single blasts of pt2229. Length of the branches indicates the number of somatic base substitutions. Text colors indicate the phenotypic subpopulation. B) The relative contribution of V(D)J clones per gene segment to sequenced bulk populations and single cells of pt2229. Colors indicate unique recombination per allele. Text colors indicate phenotypic subpopulation.

Figure 1

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