

Overall, the work from Thoms et al suggests that transcriptional networks dynamically control cell fate decisions and shape unique subcircuits during phenotype transition in healthy blood and leukemia. The study also raises several interesting questions for future study. The elegant work of the authors shows that the chromatin accessibility state of only a handful of enhancers can classify essentially all major stages of early hematopoiesis. They also succeeded in the identification of a unique transcriptional subcircuit during erythroid differentiation. Based on this, it will be interesting to identify and study other types of subcircuits which may govern the transition to precursors of other lineages. Another intriguing question is what precise mechanisms are harnessed to dynamically regulate the transcriptional networks in HSPCs, and which ultimately drive the transition from HSCs to precursors (eg, recent work has monitored low-level co-fluctuations of PU.1, Gata1, and Gata2 in murine HSPCs) and found that transcriptional stochasticity of these TFs played a vital role in the maintenance of transcriptional plasticity.⁷ Future single-cell studies of changes of epigenetic states and transcriptional heterogeneity in the heptad circuit may lead to novel insights in that regard.

Another interesting question and future challenge are how the advances presented by Thoms et al could potentially be leveraged for therapeutic purposes. Although TFs are challenging drug targets in general, new approaches with small molecules or modified biologics are emerging.⁸⁻¹⁰ If future studies were able to identify AML-specific subcircuits which are not critical in normal hematopoiesis, the therapeutic targeting of such key TFs may be a highly promising strategy to tackle the generation of leukemic cell fates at its root.

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LYMPHOID NEOPLASIA

Comment on Herrera et al, page 1456

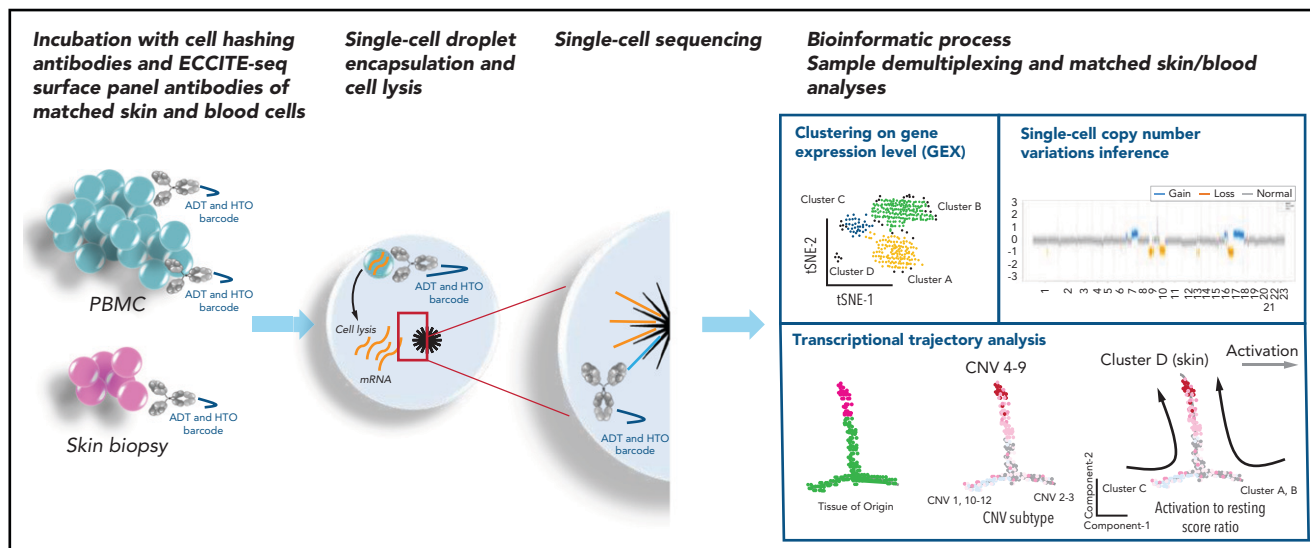
Single-cell trajectories in Sézary syndrome

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In this issue of *Blood*, Herrera et al¹ identified a restricted phylogeny together with a proliferation and T-cell activation signature in the skin compartment of leukemic cutaneous T-cell lymphoma (CTCL) by utilizing a multimodal single-cell analysis of paired blood and skin samples.

In recent years, both phenotypic analysis and next-generation sequencing have revealed significant interpatient diversity of Sézary syndrome (SS).²⁻⁴ Assessing blood tumor burden has always been a challenge in CTCL, requiring international efforts to define malignant cells because of their aberrant phenotype and/or T-cell receptor gene clonotype. Herrera et al employed a CRISPR-compatible cellular indexing of transcriptomes and epitopes by sequencing (ECCITE-seq), combining cell hashing plus phenotypic analysis by antibody-derived tags (ADT) with single-guide RNA capture of individual cells identified by molecule barcoding (see figure). This multimodal approach permits the detection of transcriptome, T-cell receptor α/β (TCR α/β) and TCR γ/δ clonotype, and surface proteins expression at the single-cell level.⁵

Thanks to integrated bioinformatics tools, Herrera et al established the transcriptional profile of individual skin and blood clonal T cells in 4 patients with SS and 1 patient with leukemic mycosis fungoides (MF), thus defining clusters in both compartments. Interestingly, malignant skin T cells mainly clustered together, whereas blood contained several clusters revealing a greater transcriptional heterogeneity. This approach also demonstrated inter- and intraindividual heterogeneity according to the transcriptional and phenotypic profile of the malignant T cells. Although the expression of transcription factors resulted in a relatively homogeneous T helper 2 cell (Th2) or Th17 signature, the study confirmed the heterogeneity of SS cells according to their naive, central, or effector memory phenotypes as previously shown using comparative phenotypic and molecular profiling of skin and blood



Skin biopsy (pink) and peripheral blood mononuclear cells (PBMCs) (light green) were collected. PBMC and skin-dissociated cells were incubated with ubiquitous antibodies conjugated with hashtag oligonucleotides (HTO) for cell hashing and with surface panel antibodies conjugated with ADT for ECCITE-seq. Stained and washed cells were pooled and sequenced on Illumina platforms. After sequencing, bioinformatics analysis demultiplexed samples by their HTO. Malignant T cells were defined on their clonal TCR β CDR3 sequence and on their distinct transcriptome and ADT. Then, transcriptional analysis defined clusters at the single-cell level from the same patient, and CNVs were inferred to build phylogenetic trees of subclones. Transcriptional trajectory analysis between blood and skin-derived malignant cells in the same patient revealed a marked proliferation and T-cell activation signature in the skin compartment.

CD4⁺ cells.² This suggested that skin SS cells exhibit a more advanced maturation pattern than do their circulating counterparts, but the present data support that SS cells may be not fixed at a specific cell of origin or maturation stage, as recently observed in SS preclinical models.⁶

In this study, Herrera et al assessed single-cell copy number variation (CNV) by evaluating gene expression data. The cytogenetic profile inferred from single-cell transcriptome with gains in 8q and 17q and losses in 10q and 17q is highly concordant with data obtained by bulk comparative genomic hybridization of SS samples.⁷ Among CTCL, the characteristic cytogenetic profile of SS contrasts with the diversity seen in its mutational landscape except for some notable recurrent alterations, such as those of the *TP53* gene.^{3,4,8} The study did not investigate correlations with these specific gene mutations. Interestingly, CNV at the *TP53* locus was absent in their leukemic MF case and in 1 SS sample. As discussed, a limitation of the CNV inference method may be the detection of small-scale alterations, especially focal deletions that are highly recurrent in SS.⁸ However, phylogenetic trees based on CNV analysis have shown parallel and shared branches of clonal evolution generating subclones supporting a continuous or stepwise

migration between skin and blood. This approach did not show a clear monodirectional relationship to support a tissue of origin of the disease.

However, pseudo-time trajectory analysis of matched blood and malignant skin T cells revealed a tissue-dependent transcriptional signature masking subclonal differences among malignant skin T cells characterized by a strong and consistent upregulation of T-cell activation and proliferation signatures. This implies that the skin microenvironment determines the transcriptional program of malignant T cells and may be critical for disease initiation, as suggested by the presence of a UV signature in the mutational profile of SS cells.^{3,4,8} Both skin pathobionts and the cutaneous niche may support malignant T-cell activation and expansion. The data are in accordance with differences in the proliferation index of SS between blood and skin compartments.⁹ The quiescent status of blood SS cells and the proliferative status of cutaneous SS cells were associated with the expression of CD-62L or PD-1, respectively. These findings also support the need for a multi-agent therapeutic approach rather than the sequential single-agent therapies commonly used for refractory advanced-stage CTCL. For example, the response rate to mogamulizumab, an anti-CCR4

mono-clonal antibody, is lower in the skin than in the blood.¹⁰ Indeed, the ECCITE-seq analysis should be applied to pre- and posttherapeutic samples to further monitor the therapeutic response in the blood and skin compartments, especially in early or limited CTCL stages. A sequential trajectory analysis would identify the transcriptional and genetic features associated with either therapeutic response or primary or secondary resistance to multi-modal or single-agent therapies.

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PLATELETS AND THROMBOPOIESIS

Comment on Bye et al, page 1481

Sugar and spike: not so nice

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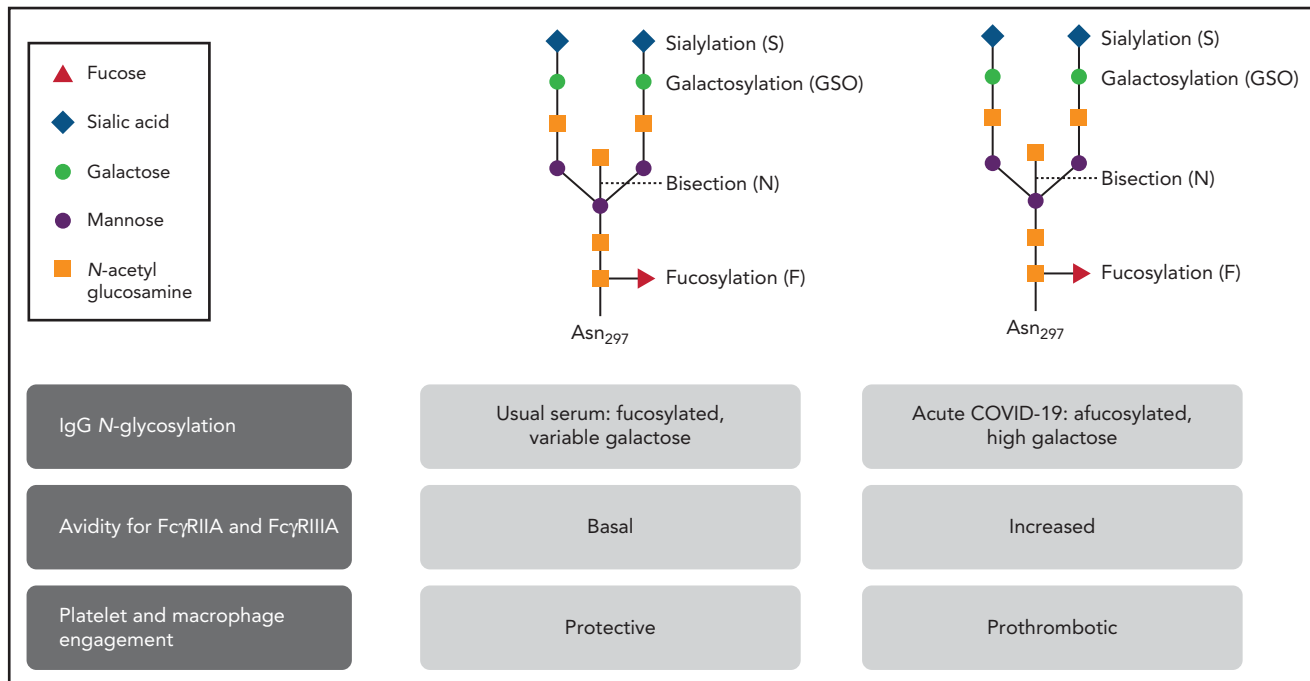
Bye and colleagues employed a new way to look at the thrombosis that accompanies COVID-19 infection.¹ In this issue of *Blood*, they have identified that aberrant glycosylation of the anti-spike IgG leads to greater prothrombotic platelet activation via FcγRIIA.

Over the past 2 decades, the interplay among immunity, inflammation, hemostasis, and thrombosis has been more clearly appreciated. Advances in fundamental immunology have translated into

improved understanding of the relationship of human thrombotic disorders and immune stimuli. This work by Bye and colleagues fits nicely into that tradition. Thrombosis related to COVID-19 is

important and incompletely understood. It is clearly multifactorial, with alterations of endothelial cells and activation of leukocytes, platelets, and coagulation. Since the beginning of the pandemic, clinicians have recognized that critical pulmonary (acute respiratory distress syndrome with pulmonary vascular thrombosis) and systemic (deep vein thrombosis) manifestations of COVID-19 infection are often pronounced when adaptive immunity has begun. IgG is an important component of the adaptive response. Immunoglobulin (IgG) undergoes N-glycosylation of the heavy chain in the Fc region during biosynthesis (see figure). The nature of these sugars in circulating IgG has been well studied, consistent with fucose and galactose residues being regulated in a narrow range. New fundamental studies have identified reduced fucosylation and increased galactosylation status of the IgG in response to certain viral infections, including HIV and dengue, and indicate that it may be a generalizable feature of the early IgG response to enveloped viruses that bud from cells.²⁻⁴

Enter the human IgG response to the COVID-19 spike protein. Bye et al build on the data of reduced fucosylation and increased galactosylation of anti-SARS-CoV-2 IgG directed against the spike protein to pursue the prothrombotic effects on



The N-glycosylation of IgG in severe acute COVID-19 infection has a variant pattern that has greater avidity for FcγRIIA and FcγRIIIA. As a result, platelet and macrophage engagement is prothrombotic. Professional illustration by Patrick Lane, ScEYEnce Studios.