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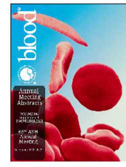

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The 65th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

802.CHEMICAL BIOLOGY AND EXPERIMENTAL THERAPEUTICS

Transcription Factor Redistributors Pharmacologically Actuate Non-Canonical Gene Networks to Drive AML Differentiation

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Aberrant transcriptional networks are hallmarks of aging and cancer, yet our ability to target these aberrations is poor. PU.1 is one such transcription factor (TF) who's transcriptional networks are corrupted in disease, including in >50% of Acute Myeloid Leukemia (AML) cases. In this study we investigate an unappreciated pharmacological approach to target the aberrant PU.1 network, by employing novel small molecules which competitively inhibit PU.1:DNA interactions.
We performed an extensive multiomics-driven, molecular characterization of AML cells following exposure to PU.1-DNA binding inhibitors (e.g. DB2115). Unexpectedly, we found that such compounds not only led to a reduction of many canonical PU.1 transcripts, but also (seemingly paradoxically) caused increases in alternative PU.1-driven transcripts. We discovered that this two-sided response was a result of robust cistromic repositioning of PU.1 chromatin binding rather than global inhibition. From kinetic CUT&Tag and ATAC sequencing studies, we identified that PU.1 redistribution involved rapid PU.1 loss (~1hr) followed by delayed PU.1 gain (~4-12hrs), causing subsequent opening of the chromatin, thus demonstrating endogenous PU.1's powerful pioneering ability. Through development of CLICK-on-CUT&Tag, we identified exclusive drug binding at displaced PU.1 binding sites, and identified unique, sequence-specific ETS motifs dictating PU.1 gain or loss. By employing these motifs in eGFP reporter assays and CRISPRd systems, we identified direct PU.1-mediated transcriptional control of known and novel functionally relevant genes, including *MYC*, *POMP* and *STRAP*. Finally, we establish that primary AML samples are sensitive to pharmacological PU.1 redistribution upon exposure to DB2115, and the rewiring of the PU.1 network in these cells drives differentiation down the myeloid lineage (Figure 1).
Overall, we uncover a novel biological phenomenon we describe as: Pharmacological transcription factor (TF) redistribution. "TF Redistributors", such as DB2115 and next generation derivatives, are an unprecedented investigative and pharmacological tool which allow the study of complex and fast transcription factor dynamics without disrupting the structure or levels of the TF itself. Furthermore, we discover that pharmacological binding-site restriction and consecutive repositioning of the PU.1 network, cascades into an extensive rewiring of transcriptional circuits which ultimately drives myeloid differentiation of AML cells, potentially providing a novel therapeutic intervention for PU.1-corrupted AML.

Disclosures Steidl: *Pfizer:* Consultancy; *Stelexis Therapeutics:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *Roche:* Consultancy; *Novartis:* Consultancy; *Aileron Therapeutics:* Consultancy, Research Funding; *Bayer Healthcare:* Research Funding; *GlaxoSmithKline:* Research Funding.

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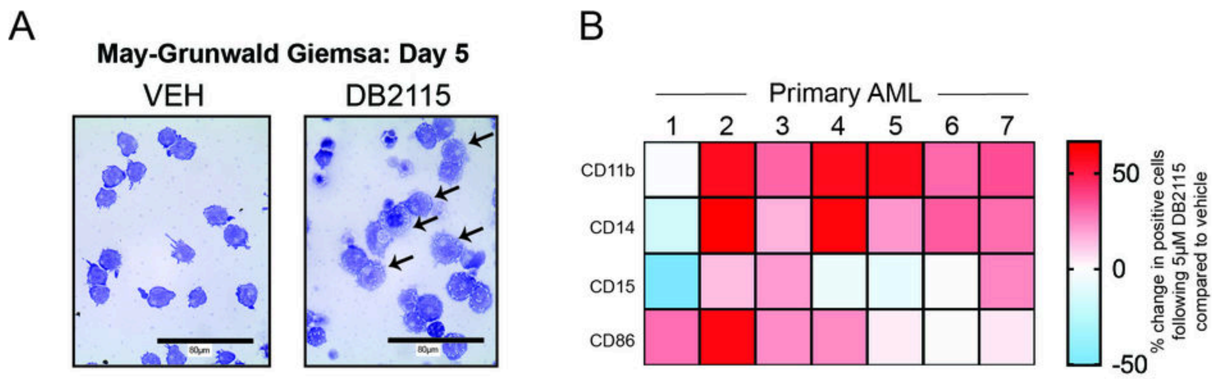

Figure 1: Induction of myeloid differentiation by pharmacological PU.1 redistribution in primary AML samples (A) May-Grunwald Giemsa cytospin image of MOLM13 cells treated with vehicle or 1µM DB2115 for 5 days displaying evidence of myeloid differentiation. (B) Heatmap of the percentage change in myeloid CD marker expression (CD11b, CD14, CD15 and CD86) in alive primary AML cells following ~1 week of 5µM DB2115 exposure compared to vehicle.

Figure 1

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