Multimodal atlas of paired diagnosis and relapse AML reveals surface antigens for multi-specific immunotherapy

Amanda Halfond, Matthew Ung, Julia Etchin, Julia DiFazio, Yonina Keschner, Alyssa Pyclik, Mariana Silva, Antonino Montalbano, Brikena Gjeci, Ermin Zhao, Guy Mundelboim, Julian Scherer, Huanying Gary Ge, John Lydeard, Tirtha Chakraborty

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Vor Bio, 100 Cambridgepark Dr, Cambridge, MA, USA 02140

INTRODUCTION

- Targeted immunotherapy of Acute Myeloid Leukemia (AML) has been limited due to lack of tumor-specific antigens resulting in "on-target, off-tumor" effects that can lead to severe cytopenia
- To unlock the full potential of targeted treatments, we engineer hematopoietic stem cells (HSCs) by genetically ablating target antigens from healthy, donor-derived HSCs for hematopoietic stem cell transplant. This allows compatible immuno-therapy to specifically kill leukemic cells bearing the AML target-antigen while sparing the antigen-null allogenic graft[1-5]
- Combinatorial targeting of AML antigens may be required to avoid antigen escape and address tissue heterogeneity, while multiplex genome editing of these antigens is necessary to protect the transplanted healthy graft (Figure 1)
- To identify combinations of cell surface antigens that can be targeted to eliminate the heterogeneous AML subclones, we designed a novel approach to quantify intra- and inter- patient antigen expression at diagnosis and relapse from the same patient, profiling 81 AML antigens, four being evaluated in clinical trials and 77 identified in literature[6-9]

OBJECTIVE

- Profile 81 prospective AML antigens in a longitudinal assessment of 400,000 cells in AML patient bone marrow mononuclear cell (BMMC) samples from 26 patients at diagnosis and relapse (Figure 2) and 10 healthy donors to understand tumor heterogeneity and therapeutic targetability
- Develop machine learning model to expand number of quantifiable antigens by correlating flow cytometry and antibody derived oligo-tags (ADT) from cellular indexing of transcriptomes and epitopes sequencing (CITE-seq)[10]
- Identify possible antigens and combinations to be targeted by a paired therapeutic with low on-target, off-tumor cytotoxicity
- Determine targetability of single antigens and multi-specific targeting by *in vitro* 2˚ antibody drug conjugate (ADC) cytotoxicity assay and chimeric antigen receptor (CAR) T cells

Figure 1. Vor Multiplex Platform

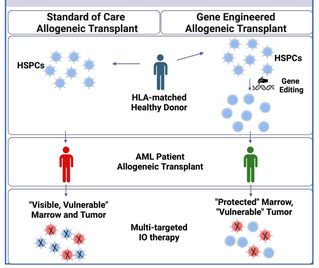
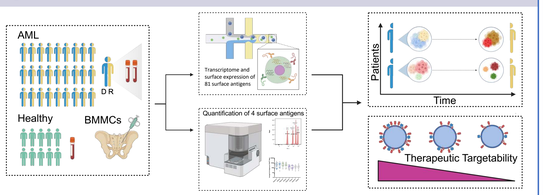


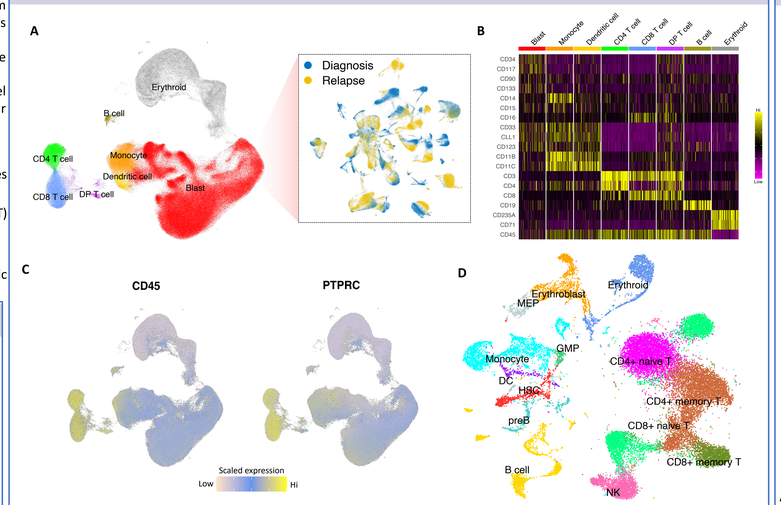
Figure 2. Overview of AML Atlas



- Schema bridging flow cytometry of four surface antigens and CITE-seq of 81 surface antigens from AML BMMCs to predict antigen number in 77 remaining antigens at diagnosis and relapse
- Identification of blast population heterogeneity within and across patients over time
- Leveraging the power of surface quantification to understand therapeutic targetability

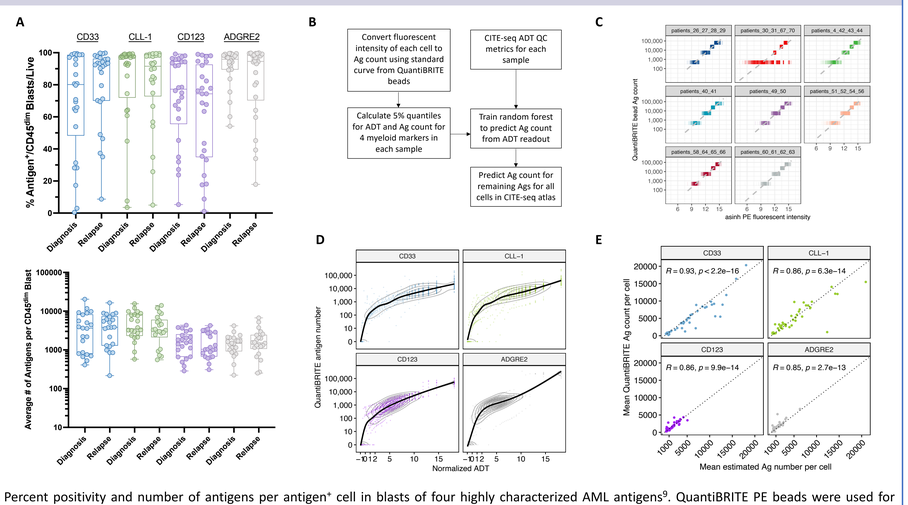
RESULTS: IDENTIFICATION AND ANALYSIS OF AML BLASTS

Figure 3. Identification of blast population



A. Clustering and identification of subcellular populations of patient AML BMMCs including blasts from CITE-seq by RNA expression. Further analysis of the blast population showed discrete clustering of diagnosis and relapse cells. **B**. Phenotypic confirmation of identified subpopulations by surface protein through antibody-derived oligo tags (ADT). **C.** Surface protein and transcript analyses show reduced expression of CD45 in the predicted blast population by CITE-seq. **D.** Healthy donor BMMC analysis reveals distinct compartments of the bone marrow that can be utilized for AML BMMC cell state phenotypes.

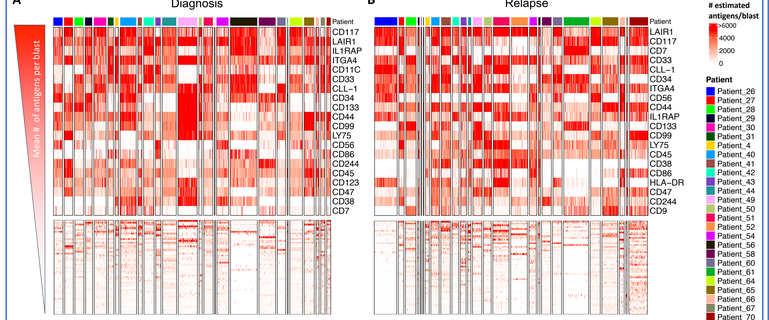
Figure 4. Machine learning and modality integration estimates number of antigen molecules on blasts



A. Percent positivity and number of antigens per antigen+ cell in blasts of four highly characterized AML antigens[9]. QuantiBRITE PE beads were used for antigen quantitation. Data shown as median ± standard error of mean (SEM). **B**. Overview of machine learning model used to surface antigen count per blast by integrating flow cytometry and CITE-seq ADT readouts of four antigens. **C.** Mapping individual cell fluorescent intensities to QuantiBRITE standard curve produces antigen count measurements for each cell. **D.** Quantile mapping (5% quantiles) of normalized ADT distribution to QuantiBRITE antigen count distribution. **E.** Random forest model trained on data from 3 of the 4 antigens accurately estimates antigen count for the remaining antigen[11]. Plots show correlation between mean estimated count (x-axis) and the true mean count (y-axis) across blasts for each patient sample.

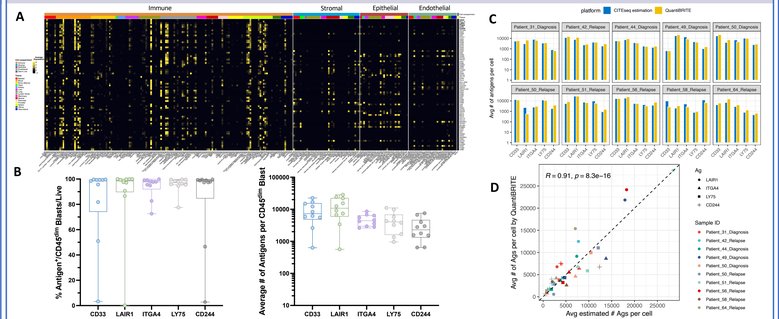
RESULTS: MODEL VALIDATION AND *IN VITRO* CYTOTOXICITY

Figure 5. Ranking of antigens at diagnosis and relapse from predicted average antigen number per cell



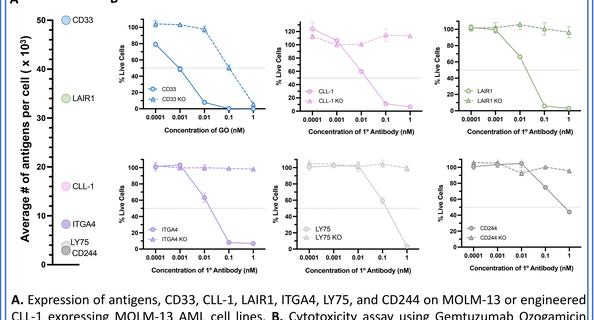
A-B. Systematic ranking of 81 antigens in 26 patient BMMC samples at diagnosis (left) and relapse (right) from CITE-seq. Top 20 most highly expressed antigens are labeled and all display >2000 antigens per blast on average based on machine learning prediction.

Figure 6. Antigen prioritization and machine learning model validation



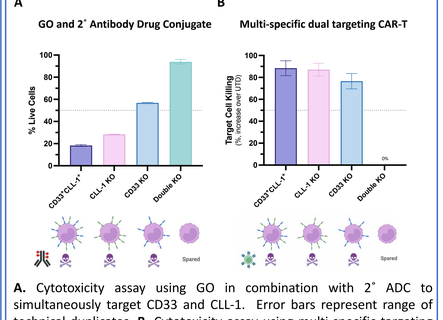
A. Healthy tissue analysis from single-cell data base Tabula Sapiens showing expression of antigens on heme and non-heme tissue prioritizing antigens that have high antigen intensity, large coverage of blasts, and low expression on non-heme tissue[12] **B.** Flow cytometry validation of four antigens, LAIR1, ITGA4, LY75, CD244 in patient samples that were previously run with CITE-seq and machine learning. **C-D.** Average antigen count per cell by flow cytometry compared to predicted antigen count by CITE-seq. Both modalities show high correlation with an R = 0.91 and p = 8.3E-16

Figure 7. Cytotoxicity of prioritized antigens



A. Expression of antigens, CD33, CLL-1, LAIR1, ITGA4, LY75, and CD244 on MOLM-13 or engineered CLL-1 expressing MOLM-13 AML cell lines. **B.** Cytotoxicity assay using Gemtuzumab Ozogamicin (GO) or 2˚ ADC bound to 1˚ specific antibody showing targetability of varying average antigen counts per cell, CD33, CLL-1, LAIR1, ITGA4, LY75, and CD244 with an IC50 ≤1 nM for all six antigens. Error bars represent SEM of technical triplicates.

Figure 8. Representative multi-specific antigen targeting



A. Cytotoxicity assay using GO in combination with 2˚ ADC to simultaneously target CD33 and CLL-1. Error bars represent range of technical duplicates. **B.** Cytotoxicity assay using multi-specific targeting CAR-T cells to simultaneously target CD33 and CLL-1 in HL-60 AML cell lines. Error bars represent range of experimental duplicates. This CAR has demonstrated efficacy *in vivo*[14]

CONCLUSIONS

- Multimodal AML atlas of paired patient BMMC samples collected at diagnosis and relapse from a large patient cohort, reveal inter- and intra-patient blast antigen heterogeneity
- Candidate therapeutic target antigens were systematically ranked by AML expression (% blasts and number of target molecules per blast) and filtered for hematopoietic-specific expression to limit the risk of multi-organ targeting on non-heme tissue
- Well-characterized AML antigens CD33 and CLL-1; as well as four less-characterized targets LAIR1, ITGA4, LY75, and CD244; are among the highest expressing antigens in AML blasts at diagnosis and relapse timepoints
- *In vitro* studies demonstrate the potential of the identified antigens as promising immunotherapy targets, either alone or in combination with ADC or CAR-T therapies, to overcome antigen escape and address AML heterogeneity
- By combining multi-specific targeting therapies (such as a multi-specific CAR-T) with Multiplex-edited HSPCs, we aim to develop a transformative therapeutic approach to address the challenges of AML treatment, including antigen escape and relapse

References

1. Zhu X, et al. Nat Struct Mol Biol. 2019;26(8):679-685. 2. Borot F, et al. Proc Natl Acad Sci U S A. 2019;116(24):11978-11987. 3. Humbert O, et al. Sci Transl Med. 2019;11(503):eaaw3768. 4. Kim MY, et al. Cell. 2018;173(6):1439-1453.e19., 5. Lydeard J, et al. Methods & Clinical Dev. 2023; 31: 101135 6. Jayavelu et al. Cancer cell 2022; 40(3) 301-317 7. de Boer et al. Cancer Cell 2018; 34(4): 674-689 8. Hu et al. Nat Cancer 2021; 2(12): 1406-1422 9. Perna F, et al. Cancer Cell 2017; 32(4): 506-519.e5 10. Stoickieus et al. Nat Methods 2017; 14(9): 865-868 11. Hao et al. Nat Biotech 2023; 42 (293-304) 12. Tabula Sapiens Consortium 13. GTEx Portal 14. Silva M, et al. ESGCT, 2024

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Disclosures

All authors are current employees of Vor Bio

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