Beyond the genotype: integrating single-cell multiomics with clinical data to predict and explain venetoclax-azacitidine response in AML

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Abstract

Introduction: Single-cell profiling resolves cell diversity driving heterogeneity in cancers like acute myeloid leukemia (AML). Yet such data are rarely combined with clinical variables in prognostic models given high feature dimensionality and small cohorts. RefinedScience's CITE-seq dataset of 1,151,472 cells from 248 AML samples addresses this gap. We tested whether integrating single-cell features (cell type proportions, gene expression, pathway activity, and ADTs) with clinical data adds prognostic and target-discovery value for venetoclax plus azacitidine treatment.

Methods: Using 91 diagnosis samples, we developed a processing and screening pipeline that reduces single-cell dimensionality while preserving high-resolution cell-states. We built integrated risk stratification and treatment-response models, evaluating against clinical- and single-cell-only baselines. Lastly, we built a single-cell-based algorithm to segment patients for target discovery.

Results: Integrated risk models separated favorable, intermediate, and adverse groups modestly better than clinical-only models. Similarly, single-cell features improved cross-validated response prediction over clinical features. Known venetoclax-response mechanisms measurable in our single-cell data associated with both response and survival. A simple single-cell risk score met or exceeded clinical-only models, and the resulting patient-segmentation could enhance target discovery.

Conclusion: Integrating single-cell with clinical data yielded prognostic and translational insights largely unavailable from either modality alone, motivating research that leverages both.

Motivation

- Clinical data (e.g., genotype) predict outcome but don't tell you what to treat
  - Does single-cell data (CITE-seq) improve response prediction?
  - Can it translate non-targetable variables into targetable biology?

RefinedScience provides rich multimodal single-cell and clinical resources

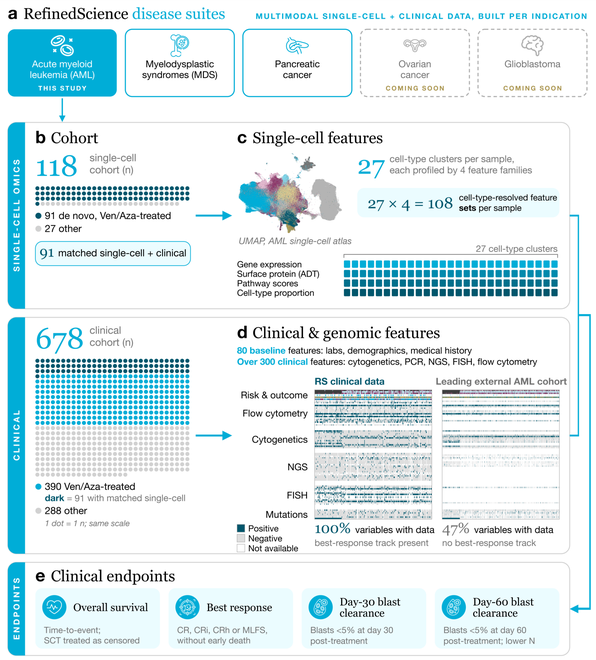
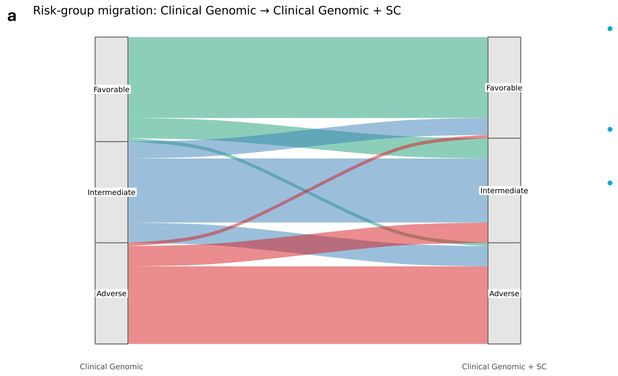
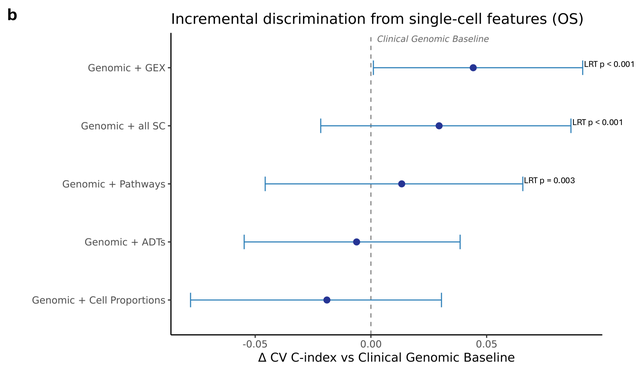


Figure 1. (a) RefinedScience disease suites and (b) cohorts of 118 single-cell and 678 clinical patients, 92 of them matched (1 dot = 1 patient). (c) Each sample is resolved into 27 cell-type clusters with gene expression, surface protein, pathway and proportion features, alongside (d) 80 baseline and 300+ clinical features, giving high data availability versus 47% in a leading public AML cohort. (e) These data support four clinical endpoints: overall survival, best response, and day-30 and day-60 blast clearance.

Single-cell features add prognostic value beyond clinical models





- Single cell features can improve overall survival, best response, and day 30 post-treatment blast clearance prediction.
- Single cell features influence model-based risk grouping.
- Specific single cell features of prognostic interest can be investigated for deeper biological insights.

Figure 2. a) Patient change in risk group based on clinical only vs clinical and single cell data. Risk groupings are based on risk scores from a ridge-penalized Cox model for OS, using tertiles to assign patients into favorable, intermediate, and adverse risk strata. b) Predictive performance is measured by change in c-index compared to a clinical feature only baseline, all models used a ridge-penalized Cox model. C-index represents performance across 20 repeat, 5-fold CV of 82 patients as the holdout 10 patient set yielded unreliable performance measures, with a patient-resampling confidence interval formed by resampling patients and recomputing the C-index. Lymphocyte-related cell type single cell features were dropped from these analyses as the nature of feature selection may have arbitrarily inflated lymphocyte-related features.

Single-cell data recovers known Ven/Aza biology, and a subsequent score predicts response without model training

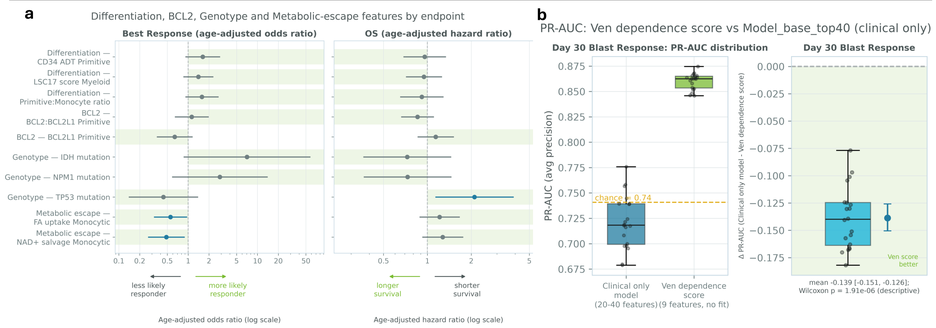


Figure 3. (a) Forest plots of odds and hazard ratios coming from age-adjusted logistic and unpenalized Cox regression, respectively. single-cell features and genotypes corresponding to known Ven/Aza biology in the literature[1-3], with green highlights representing the expected direction. (b) Boxplots of models Area Under the Precision-Recall Curve (PR-AUC) for predicting Day 30 post-treatment blast response. ROC-AUC showed similar results; PR-AUC was chosen due to class-imbalance. Ven-dependence-score is a weight-normalized mean of signed z-scores of 8 single-cell features and 1 mutation feature. Clinical only model is an elastic-net logistic regression on the clinical data, restricted within each training fold to the 40 features with the highest Spearman rho to the endpoint. Both are scored on identical 20 repeated 5-fold outer splits (each dot). Right shows the paired difference in PR-AUC.

- Features expected to be predictive of Ven/Aza response were translated into single-cell features in our data set and show expected associations with both best response and overall survival.
- A simple weight-normalized mean of single-cell features representing known Ven/Aza biology can outperform low-feature clinical-based models.
- Models promoting target discovery for Ven/Aza treatment should be compared to known biology baselines.

Single-cell data reveals a cell state that might modify NPM1-associated survival

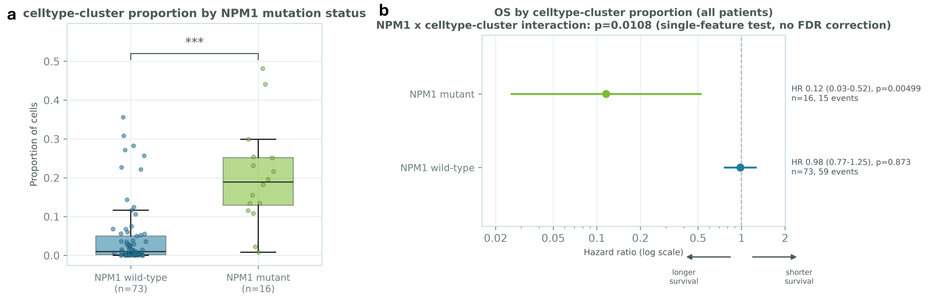


Figure 4. A) Boxplots of proportion of cells in each sample (dot) belonging to a specific cell-type cluster. *** corresponds to the scCODA[4] cross-reference credibility (fraction of reference cell types for which the compositional effect stayed credible being >95%). B) Forest plot for the cell-type cluster, with 95% confidence intervals. CIs left of the dotted line (Hazard Ratio, HR=1) indicate higher proportions of the cell-type cluster associate with longer survival.

- NPM1 mutation status associates with higher response and survival through an unknown mechanism[5]
- A cell-type cluster made up a statistically significantly higher proportion of NPM1-mutant samples than WT (both based on X and scCODA[4]) (A)
- Higher proportions of this same cell-type cluster associated with patients showing higher overall survival (B) in NPM1-mutant but not clearly in NPM1-WT patients
- Cell-type cluster microenvironment and characterization from single-cell data can reveal possible biological mechanisms and confounders for potential target discovery

Mechanism-based stratification enables testing of distinct therapeutic hypotheses

MOLECULAR SCORING	CLINICAL ENDPOINT	TARGET DISCOVERY FOCUS	N
Ven dpt, no escape	Deep Response	**Any** BCL2i predicted to work	6
	Short-lived Response	**Candidate for more potent BCL2i**	14
Ven dpt, escape	Mixed	**Combination with BCL2 inhibitor**	24
Not Ven dpt	Responder	Response plausibly **Aza-driven or unknown Ven mechanism**	30
	No response	**Different mechanism** needed	16

- Stratification based on clinical endpoint **and** multiomics data reveals more focused populations for target discovery
- E.g. Separating patients with **known** biology explaining relapse vs **those for novel target discovery**
- Such stratifications can be used for more refined clinical markers

Figure 5. Patients are split based on both molecular scoring (Ven dependency and escape scores) and clinical endpoints. A Deep Response is defined as a Day-30 post-treatment blast <5% and Best response achieved. A Short-lived Response is defined as only a Day-30 blast <5%. The "target discovery focus" notes what type of target that population best supports.

Conclusions and Limitations

Single-cell integrated multimodal data unveils insights largely unavailable from one modality alone.

1. Adds predictive signal beyond even rich clinical data
2. Recovers known Ven/Aza biology and configures a simple weight-normalized score out-performing clinical-only models of Ven/Aza Response
3. Captures heterogeneity well-enough to translate untreatable genotypes into targetable mechanisms

Limitations:

- Prognostically important single-cell features for risk stratification are not easily applicable to a clinical context given model size and risk score tertile usage, however, features are intended for retrospective target discovery.
- Despite this group being one of the largest **de-novo Ven-Aza** treated ones, it is still relatively small for optimal training across many single-cell features. Current work involves optimizing feature-filtering/engineering with both published and novel approaches.

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