Abstract # 113

Leveraging the Stabl framework for anti-PD1 response biomarker discovery in head and neck squamous cell carcinoma using the TruTumor ex vivo platform

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Background

The traditional method of biomarker identification often fails to capture the complexity of the Tumor Immune Microenvironment (TIME) response, particularly in small datasets. SurgeCare's Stabl framework addresses this by using noise injection for reproducible, quantitative, and high-throughput biomarker selection. In this study, we applied the Stabl framework to anti-PD1 response data generated using the Farcast TruTumor ex vivo platform to identify activity biomarkers and subsequent analyses to further filter out efficacy biomarkers that constitute a response biosignature.

Study Design

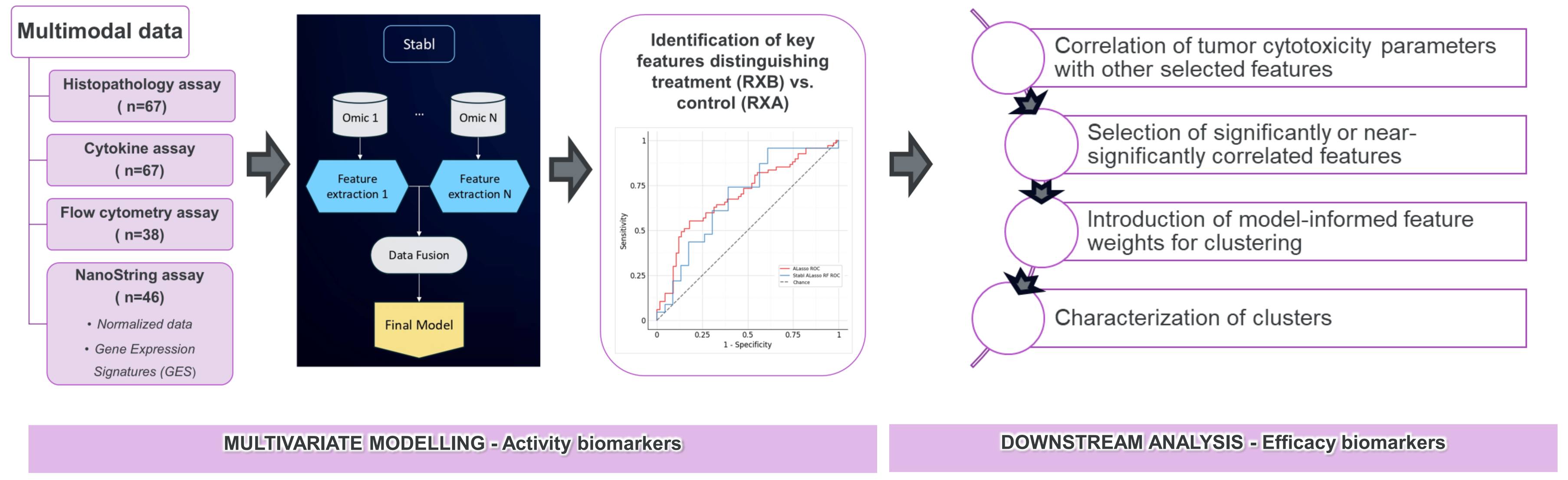


Figure 1: Schematic representation of work-flow using SurgeCare's Stabl framework on Farcast[TM] TruTumor Histoculture platform sample data

Methods

Patient tissue samples: Fresh, surgically resected HNSCC tissue samples (n=67) were collected from consented patients along with matched blood samples.

Histo-Culture workflow: The tumor sample was processed to generate thin explants, without enzymatic digestion retaining the native tumor microenvironment. Tumor explants were cultured with media and autologous plasma for 72 hours, with anti-PD1 (Nivolumab:132 μg/ml) treatment every 24 hours[1].

Cytokine Analysis: The culture supernatants were collected every 24 hours (T0, T24, T48, T72 time points) and analysed for the presence of cytokines, namely interferon gamma (IFNg), Granzyme B, and Perforin using Luminex MAGPIX instrument. Data was analysed using MILLIPLEX Analyst software.

Flow Cytometry Analysis: The tumor explants were dissociated post culture into single cells and stained with Live/Dead dye, and cocktail of immune cell lineage and activation marker antibodies (anti-CD45, anti-CD8, anti-CD68, anti-FoxP3, anti-Ki67 (BD Bioscience), anti-CD3, anti-CD4, anti-PD1, anti-GranzymeB, anti-CTLA4, anti-CD56, anti-CD206, anti-CD15, anti-CD14 (Biolegend), anti-panCK (Novus Biologicals)). Data was acquired using BD LSR Fortessa Flow cytometer with appropriate compensation controls and data analyzed using FlowJo software.

NanoString Analysis: Post treatment RNA was extracted, arm-wise, from the Tissue Micro Array (TMA) FFPE block and quantified using Tape Station. 50ng of RNA based on DV200 concentration was used for running on the nCounter PanCancer IO 360 panel. Data was normalized (NS normalised) and analyzed using the nSolver Data Analysis and Gene Expression Signature (NS GES) was generated using Log2(mRNA count) of each gene for the select gene signatures[1,2]

Feature Selection: Selected features were derived from multimodal assay data using Stabl[3] .The Stabl methodology involved preprocessing omics datasets using variance thresholds, missing value filters, median imputation, and z-score standardization, implemented with sci-kit-learn v1.2. Five repetitions of 5-fold Monte Carlo cross-validation were done to ensure stratified sampling. Feature selection was conducted using Knockoffs or Random Permutation with Logistic Regression or Random Forest, along with Adaptive Lasso for penalized regression. These methods identified features distinguishing control (RXA) from anti-PD1 treated (RXB) arms. A responder signature was derived from 23 samples (with complete multimodal data) by correlating selected features with tumor cytotoxicity markers, such as decrease in tumor content and/or increased cleaved caspase 3 expression.

Statistical analysis: All data analysis and graphical representations were done using GraphPad Prism (Version 9). Mann-Whitney t-test was performed to generate p-values. p-value significance is represented as # ($0.05 \le p \le 0.08$) and *($p \le 0.05$) **($p \le 0.01$) ***($p \le 0.001$) ****($p \le 0.0001$).

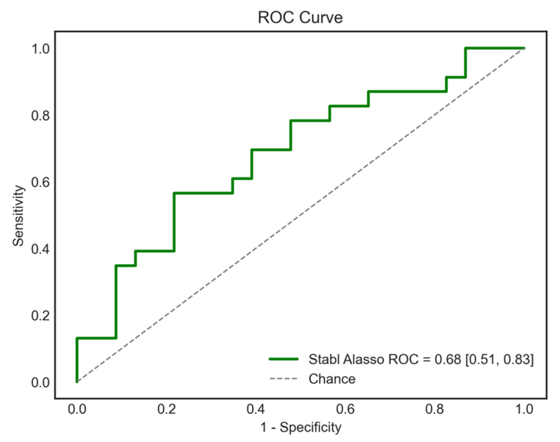
Results

Identification of key features distinguishing treatment (RXB) from control (RXA) using Stabl

A

Data	Source type	AUROC	P-value	Total no. of features	No. of selected features
Cytokine assay	Single-assay	0.65 [0.56, 0.74]	2.89e-03	18	5
Nanostring Normalized		0.67 [0.55, 0.77]	5.8e-03	770	5
Nanostring GES		0.65 [0.54, 0.77]	1.2e-02	36	6
Histopathology data		0.55 [0.46, 0.65]	2.72e-01	7	4
Flow cytometry		0.57 [0.44, 0.69]	2.8e-01	18	5
Multi-modal assay data	Multi-assay	0.68 [0.51, 0.83]	3.69E-02	849	25

B



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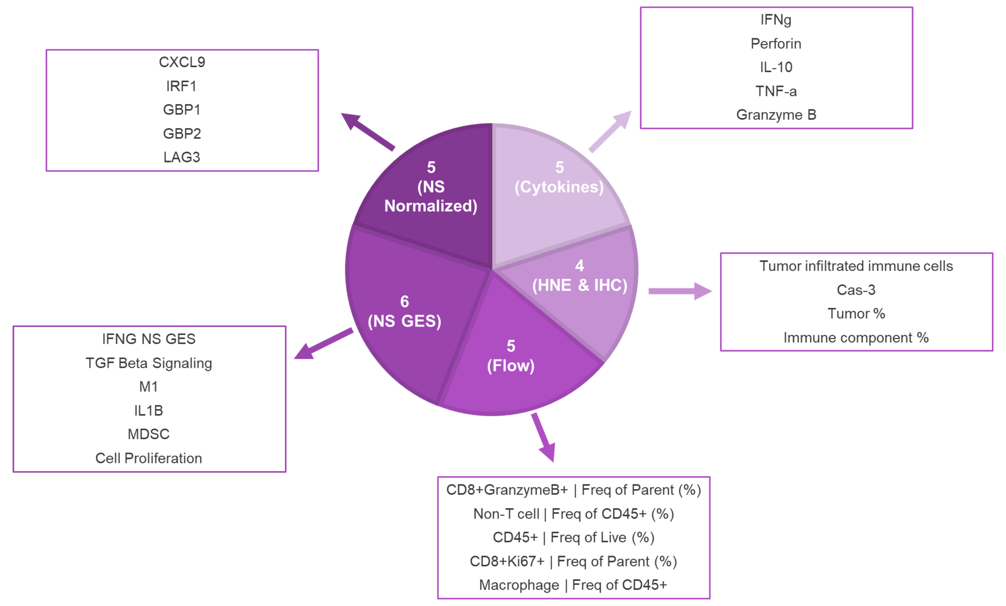

Figure 2: (A) Stabl framework performance metrics: AUROC, pvalue, and feature selected from multi modal and individual assay data. (B) Stable Alasso model built using multi-modal assay data achieved an AUROC of 0.68 [0.51–0.83], selecting 25 informative features out of 849 total. (C) Pie chart representing assay wise distribution of selected features.

Model-Weighted Clustering Identifies distinct Response-enriched cohorts

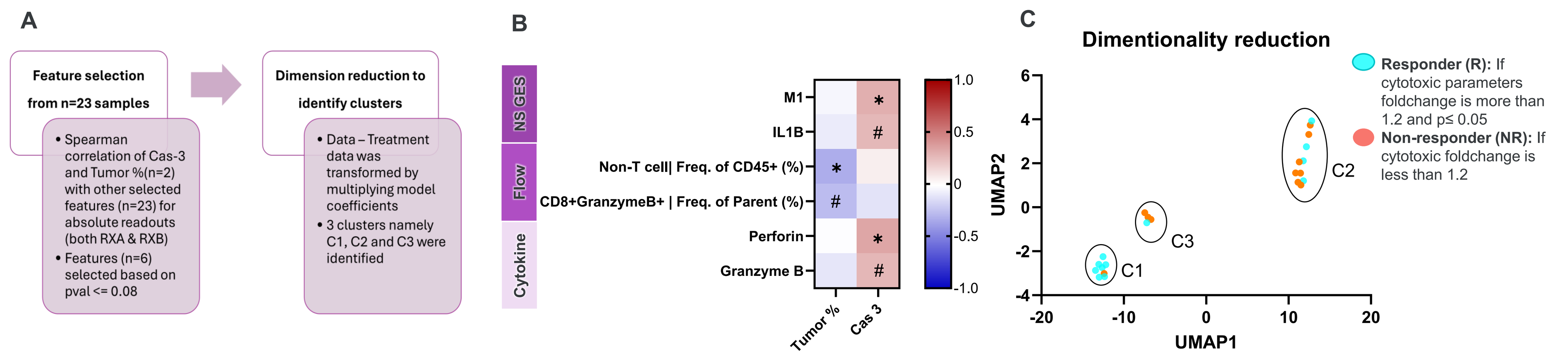

Figure 3: (A) Workflow for identification of response-linked cohorts and features. (B) Features were selected based on Spearman correlation with tumor cytotoxicity, using a p-value cutoff of ≤ 0.08. (C) UMAP of the transformed RXB dataset using the six selected features revealed three distinct clusters, highlighting heterogeneous response patterns.

Non T-cell and activated CTL (CD8+GranymeB+) proportions significantly differed between C1 and C2 clusters

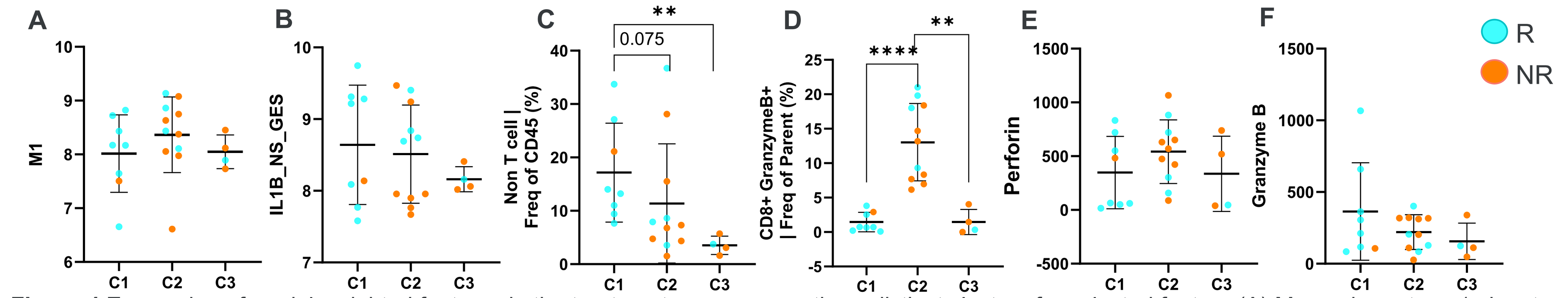

Figure 4:Expression of model-weighted features in the treatment group across three distinct clusters for selected feature (A) Macrophage type 1 signature(M1) (B) Interleukin 1 beta signature (IL1B) (C) Non T-cell population (D) Activated CD8+ T-cell population (CD8+ GranzymeB+ CTL) (E) Perforin expression (F) Granzyme B expression. Mann Whitney unpaired U-test was performed to assess the significance.

Refinement of Responder Classification and Differential mechanism driving response phenotype across clusters

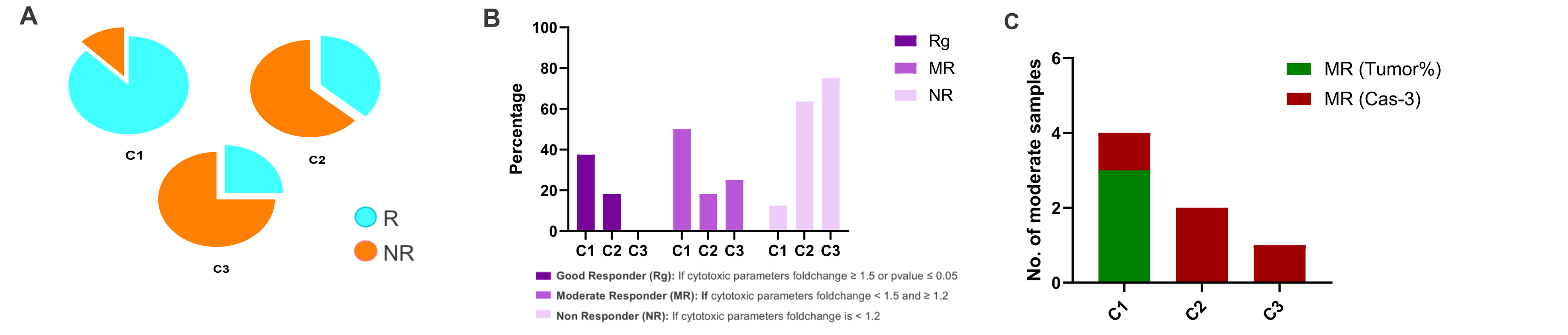

Figure 5: (A) Characteristics of response phenotype across different clusters, top left panel is C1 with highest responder rate (n=7/8) followed by C2 (n=4/11) in top right panel and at bottom is C3 with least responder rate (n=1/4) (B) Refined responder classification (C) Bar graph representing MR based on tumor and Cas-3 expression.

Enhanced response in C1 cluster is driven by immune cell rich TME

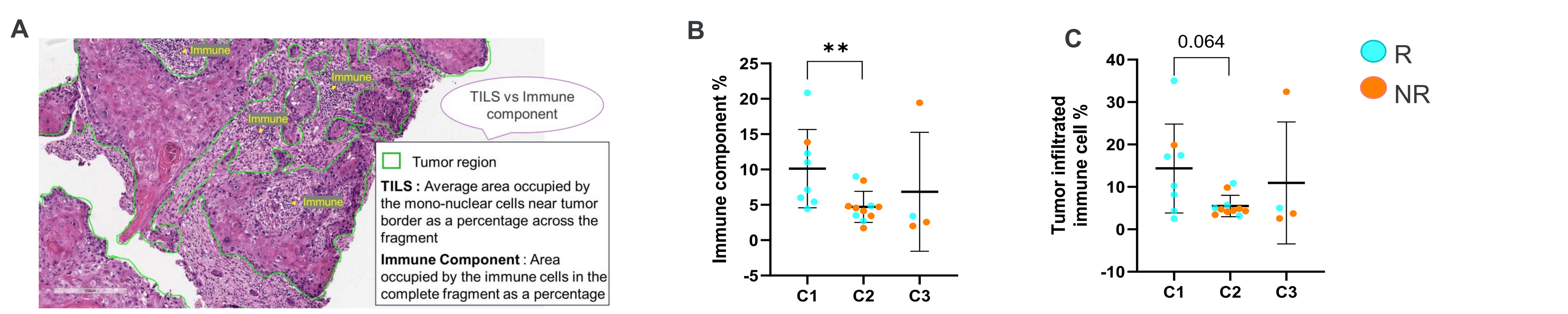

Figure 6: (A) Representative images describing Tumor infiltration of immune cells (TILS) and immune component Expression of (B) Immune component, (C) TILS across different clusters.

C2 exhibits stronger early activity markers compared to C1

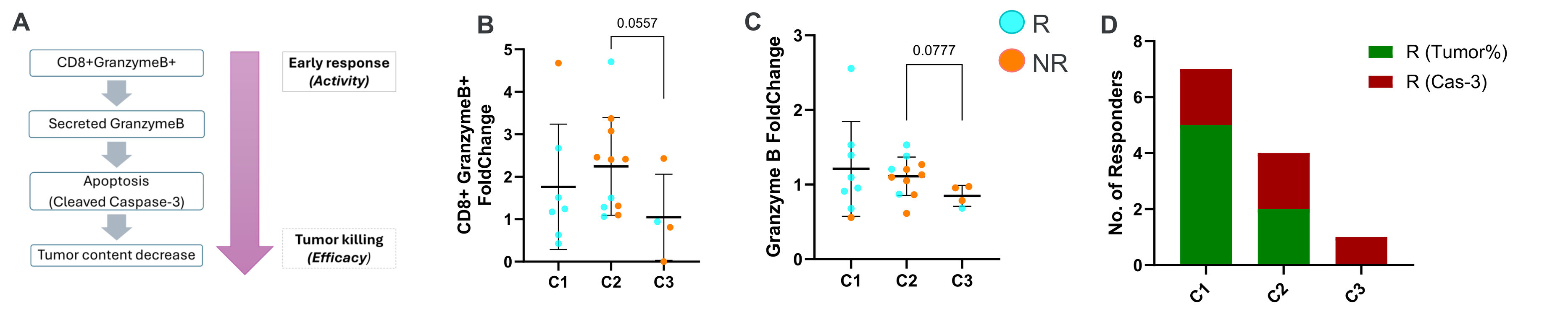

Figure 7: (A) Mechanism of tumor killing by activated CD8+ T cells. Foldchange of RXB with respect to RXA for (B) activated CD8+ T cells and (C) Granzyme B. Mann Whitney U-test was performed to calculate significance. (D) Bar graph representing samples response based on Tumor% and Cas-3

Conclusion

The complex response phenotypes observed from the TruTumor ex vivo platform that can generate multi-dimensional datasets combined with the ability of Stabl framework to work with small (n) data set facilitates considerable derisking of novel therapies at late pre-clinical stage.

Reference

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3. Hédou et al (2024) Discovery of sparse, reliable omic biomarkers with Stabl. *Nat Biotechnol* 42, 1581

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