# Multiomic Drug Response Profiling of PRISM-Multiplexed Cancer Cell Lines

Houlin Yu[1,2], Guoping Wang[1,2], Stephanie Yaung[3], Heejo Choi[1], Megan Rogers-Peckham[4], Michael Kartje[3], Matthew Rees[1], Paul Lund[5], Brian Haas[1,2], Charlotte Yang[4], Laura Doherty[1], Lacey McGee[4], Kendall Berg[4], Cynthia Cech[4], Salka Barrett[4], Anasha Arryman[4], Joanne Leadbetter[4], Taylor Lehmann[4], John Mannion[3], Chen Zhao[3], Marc Prindle[4], Melud Nabavi[4], Carolyn Morrison[5], Peter Smibert[5], Kit Nazor[6], Todd Golub[1,7], Victoria Popic[1,2], Katie Campbell[1], Jennifer Roth[1], Dean Proctor[1], Niall Lennon[1,2], Mark Kokoris[4] and Aziz M. Al'Khafaji[1,2]

[1]Broad Institute, Cambridge, MA; [2]Broad Clinical Labs, Burlington, MA; [3]Roche Sequencing Solutions, Inc, Santa Clara, CA; [4]Roche Sequencing Solutions, Inc, Seattle, WA; [5]10x Genomics, Pleasanton, CA; [6]Proteintech Genomics, San Diego, CA; [7]Department of Pediatric Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA

## Introduction

RAS is one of the most frequently mutated oncogenes, playing a key role in driving tumor growth across many cancer types. It was considered undruggable for years, but recent advances in small molecule inhibitors have finally made it possible to target specific mutant forms. Clinical outcomes are promising, but drug resistance remains a big problem, and we are trying to solve this problem by understanding and overcoming drug resistance in RAS-driven cancers.

## Materials & Methods

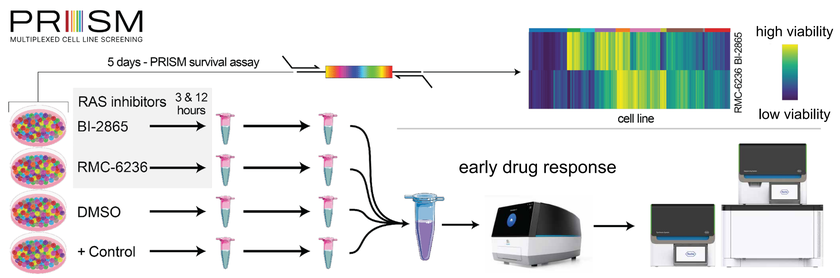


**Figure 1. Pooled multiomic drug response profiling workflow**

We conducted a RAS inhibitor response profiling, using a pool of 418 cell lines across 22 tissues types (**Figure 1**). Each cell line expresses a unique barcode that can be sequenced and identified. This barcoding system allows us to do single cell profiling as a pool instead of just one cell line at a time, which minimizes batch effects, reduces cost, and allows parallel profiling of diverse disease models. We treated the PRISM pools with our RAS inhibitor compounds and negative and positive controls, and performed single-cell assays on 3 and 12 hour time points and survival assays after 5 days. This experimental set up allows us to link cell line survival outcomes with early drug responses. We captured ~340 protein targets using a discovery panel and whole transcriptome using the 10X FLEX. We sequenced these samples on Roche Prototype SBX platform (Kokoris et al. 2025) – capturing ~320 billion reads to target 1 million single cells.

## Results

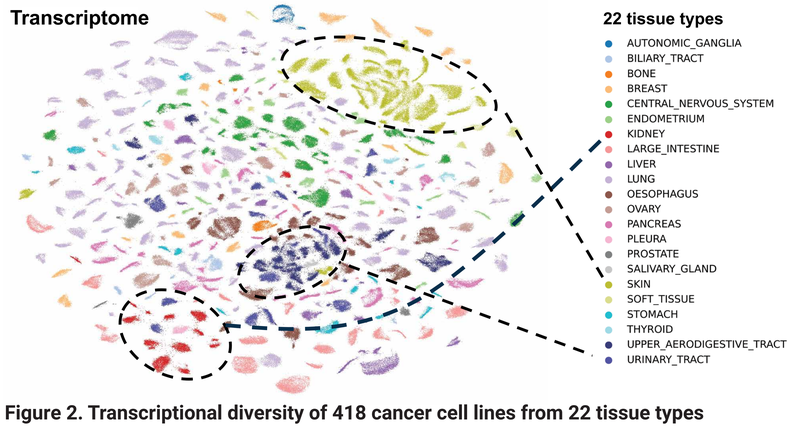


**Figure 2. Transcriptional diversity of 418 cancer cell lines from 22 tissue types**

The high coverage of cell counts, UMIs (Median UMIs per cell: 58,555) and features (Median Features Detected per cell: 8,080) reveals a detailed transcriptomic landscape across diverse cancer cell lines (**Figure 2**). Cell lines from same tissues are more transcriptionally similar.

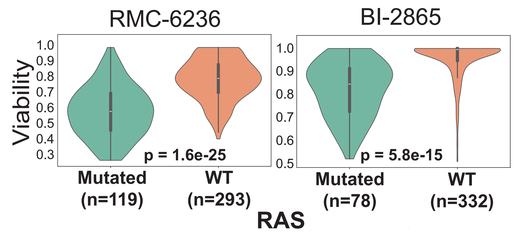


**Figure 3. RAS mutations shaping drug response**

BI-2865 inhibits multiple KRAS mutated cell lines, while RMC-6236 inhibits KRAS, NRAS, and HRAS mutated cell lines. RAS mutations are associated with lower cell viability under both RAS inhibitor treatments, confirming on-target activity. However, resistance was found in some cell lines with drug targeted RAS mutations.

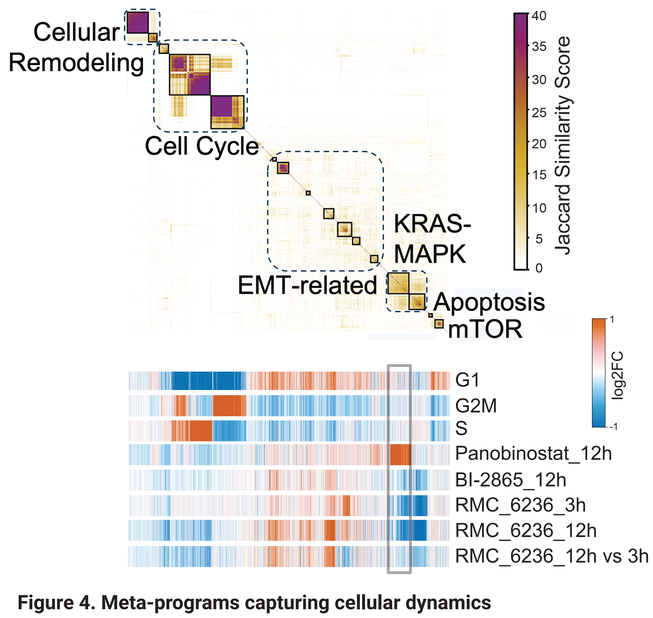


**Figure 4. Meta-programs capturing cellular dynamics**

To link transcriptional features to cell viability phenotypes, we first performed NMF on each cell line individually, then constructed meta-programs by clustering and grouping all programs from all cell lines (**Figure 4**). KRAS inhibitors perturbed various programs, including down-regulating a program related to the KRAS-MAPK pathway.

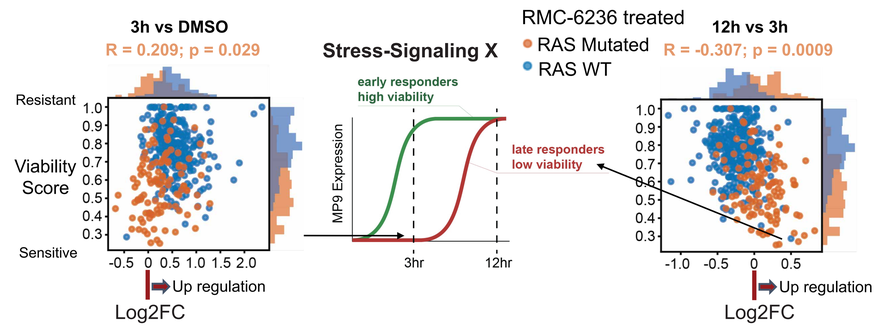


**Figure 5. Dynamics of Stress-Signaling X associated with cell viability**

Analysis of the Stress-Signaling X Metaprogram dynamics reveals that the timing of signaling may be critical for RMC-6236 response in RAS-mutant cell lines. An early (3h vs. DMSO) up-regulation is associated with higher cell viability. Furthermore, cell lines that were unresponsive at 3 hours later displayed a delayed response (12h vs. 3h), and this late signaling is strongly associated with cell sensitivity (**Figure 5**).

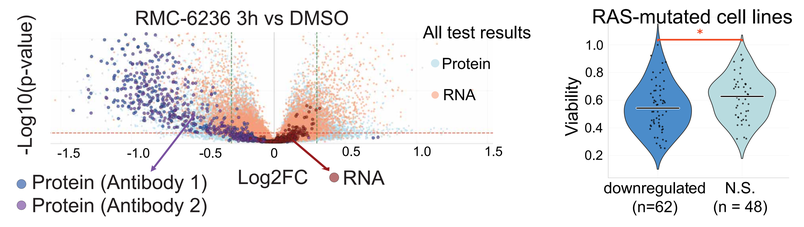


**Figure 6. Feature Y down-regulation at protein but not RNA level after 3h RMC-6236 treatment**

Differential Expression (DE) tests comparing RMC-6236 (3h) to DMSO showed a significant down-regulation of protein Y in over half the cell lines, contrasting sharply with mostly non-significant changes in the corresponding RNA transcripts. Crucially, in RAS-mutated cell lines, this protein down-regulation was directly associated with significantly lower cell viability.

## Conclusions

1) RAS mutations shape drug response - key for downstream stratification.
2) Various programs are responsive to RAS inhibitors, some of these correlate with drug sensitivity.
3) Early time points are critical for understanding transient mediators of drug response.
4) Joint RNA and protein profiling reveals signals not captured by RNA alone.
5) Numerous putative mediators were identified for downstream validation.

## Disclosure

We received support for this research from Roche* and 10x Genomics;
*Data shown in this presentation was generated on a prototype SBX platform (in development).

## Reference

Kokoris et al. 2025 bioRxiv https://doi.org/10.1101/2025.02.19.639056