Abstract #1888

Identification and optimization of novel small molecule modulators of immune checkpoint resistance with a unified representation space for genomic and chemical perturbations

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0 ABSTRACT

Immune checkpoint inhibitors have revolutionized cancer treatment, producing a durable response consistent with immunologic memory in a subset of patients. However, the majority of patients demonstrate innate or acquired resistance that must be characterized and overcome to induce successful treatment. Advancements in human reverse translation and scaled in vivo CRISPR screening have uncovered novel molecular and genomic correlates of resistance, and promising druggable mechanisms - driven by highly complex interactions between tumor cells and the immune system. It is this core biology that must be disentangled to build the treatment paradigms of the future. Here we demonstrate a technique for massively parallel prioritization of new immuno-oncology hypotheses using industrial-scale experimentation and machine learning. Leveraging high-content imaging data from whole-genome CRISPR knockout and a library of >250,000 compounds, a deep learning model was trained to construct a batch-invariant low dimensional representation of each perturbation. Millions of perturbations in multiple cell types were embedded in a unified representation space that was leveraged to increase the rate of discovery, accelerate reverse translation, yield novel biological insights, and guide the advancement of lead molecular series through structure-activity relationship (SAR). Here we highlight multiple discovery programs driven by inferred relationships between small molecules and gene knockout with translation from inference to in vivo efficacy. Specifically, we prioritize molecules with activity in STK11-deficient tumors and additional immune checkpoint sensitizers.

1 PHENOMIC PLATFORM FOR SCALED DISCOVERY AND EXPLORATION OF IMMUNE CHECKPOINT SENSITIZERS

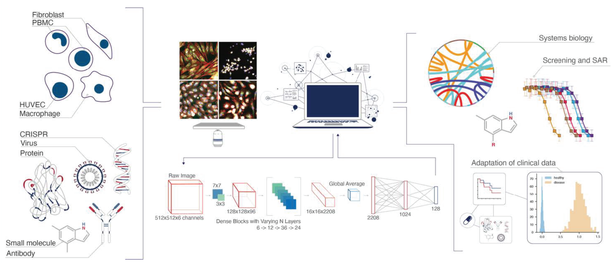
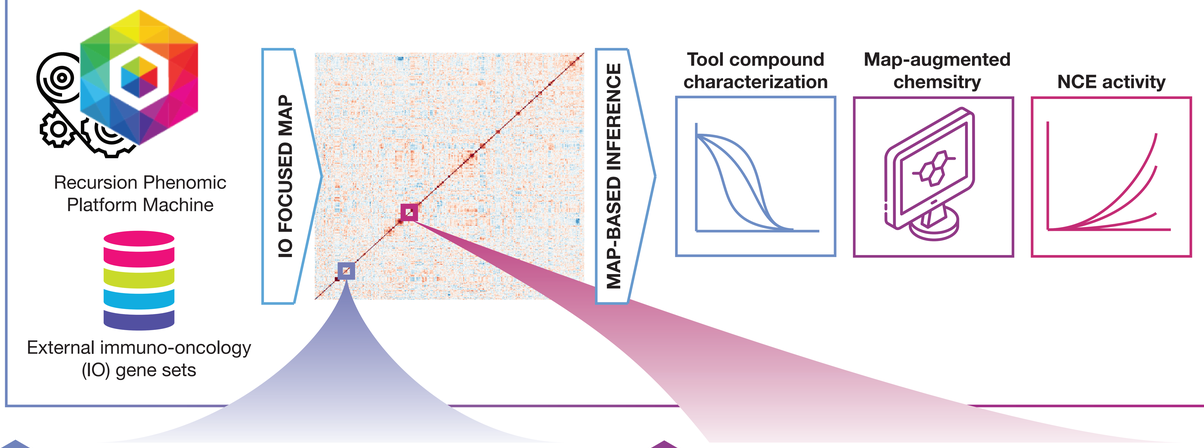


Figure 1. Phenomic platform for scaled discovery and exploration. Various cell types (top left) are treated with a range of biological perturbants and treatments (bottom left), including CRISPR-based genetic modifications and small molecules. High-throughput fluorescence microscopy (middle-top) and deep-learning-enabled image featurization (middle-bottom) generate high-dimensional phenoprints that are used for interrogating a range of experimental questions. Vector representations of millions of multi-channel fluorescence microscopy images generated using a proprietary analytics workflow based on an extension of a DenseNet-161 are analyzed (right) to map out gene-gene and gene-compound relationships, including protein complex membership, pathway regulation, target identification, and structure-activity relationship (SAR).

2 USING BIOLOGICAL MAPS TO DRIVE ITERATIVE CHEMICAL OPTIMIZATION FROM TOOL COMPOUNDS TO NEW CHEMICAL ENTITIES

Figure 2. Using biological maps to drive iterative chemical optimization from tool compounds to new chemical entities (NCE). Biological relationships captured in our maps are prioritized based on functional roles in cancer. By relating maps of gene knockouts and extensive compound libraries, we can identify tool compounds predicted to modulate genes implicated in resistance to immuno-oncology (IO) therapies. Iterative structure-activity relationship (SAR) cycling and map updates allow us to drive chemical optimization from tool compounds to New Chemical Entities (NCEs) with meaningful effect sizes in vivo. Inferential-search of the IO focused map has identified a program for the treatment of immune checkpoint resistance in KRAS/STK11 mutant non-small cell lung cancers (purple insert, Figure 3), and a series to enhance anti-tumor immune response leveraging a novel target insight (magenta insert, Figure 4).



3 ORALLY BIOAVAILABLE, SMALL MOLECULE TO ENHANCE ANTI-PD-1 RESPONSE OF STK11 MUTANT CANCERS

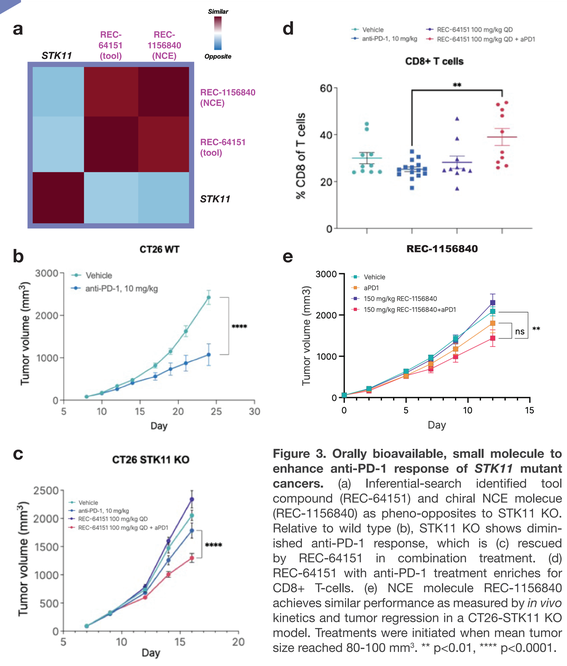


Figure 3. Orally bioavailable, small molecule to enhance anti-PD-1 response of *STK11* mutant cancers. (a) Inferential-search identified tool compound (REC-64151) and chiral NCE molecue (REC-1156840) as pheno-opposites to STK11 KO. Relative to wild type (b), STK11 KO shows diminished anti-PD-1 response, which is (c) rescued by REC-64151 in combination treatment. (d) REC-64151 with anti-PD-1 treatment enriches for CD8+ T-cells. (e) NCE molecule REC-1156840 achieves similar performance as measured by *in vivo* kinetics and tumor regression in a CT26-STK11 KO model. Treatments were initiated when mean tumor size reached 80-100 mm^3. ** p<0.01, **** p<0.0001.

4 SMALL MOLECULE TO ENHANCE ANTI-PD-1 RESPONSE IN THE PRESENCE OF CHECKPOINT RESISTANCE MUTATIONS

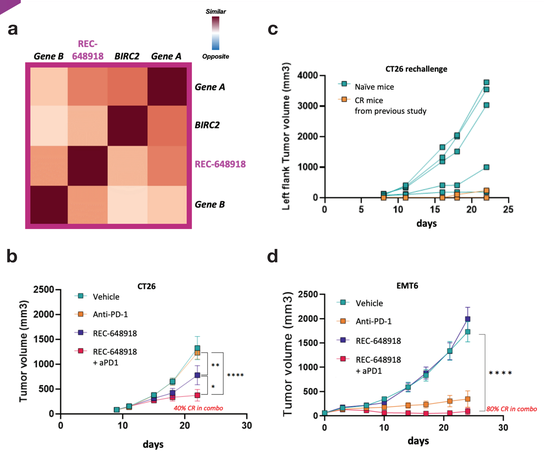


Figure 4. Small molecule to enhance anti-PD-1 response in the presence of checkpoint resistance mutations. (a) A novel compound (REC-648918) was identified with similarity to knockout of potential immunotherapy resistance gene targets (Gene A, Gene B). (b) Efficacy demonstrated in CT26 checkpoint resistance mouse model. (c) The majority of mice (75%) with complete response (CR) reject tumor growth when rechallenged. (d) Efficacy demonstrated in EMT6 mouse model. CT26: mouse colon carcinoma. REC-648918 was dosed PO, QD for 5 weeks at 100mg/kg. Anti-PD-1 was dosed IP, BIW for 5 weeks at 10mg/kg. 10 mice per group. Re-challenge was initiated 2 weeks after final treatment. Combination treatment in EMT6 resulted in 8 CR and 8 rejections on re-challenge. * p<0.05, ** p<0.01, **** p<0.0001.

5 CONCLUSIONS

- Maps of biological relationships derived from high content imaging enable us to identify gene-compound relationships that translate to druggable, efficacious early discovery drug programs.
- With no therapies currently approved for enhancing checkpoint sensitivity in tumors harboring *STK11* mutations, map-based inference identified a tool compound (REC-64151) and a Recursion-generated NCE (REC-1156840) that rescues *STK11* knock out.
- REC-64151 presents an opportunity for treatment in combination with anti-PD-1 therapy in both checkpoint refractory and treatment naive metastaic cancers.
- Querying an IO focused phenomap identified a novel compound (REC-648918) with similarity to knockout of potential immunotherapy resistance gene targets.
- Treatment with REC-648918 reduced tumor growth relative to anti-PD-1 alone, showing 40% and 80% complete response rates in CT26 checkpoint resistance and EMT6 models, respectively.

6 ACKNOWLEDGEMENTS

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7 REFERENCES

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