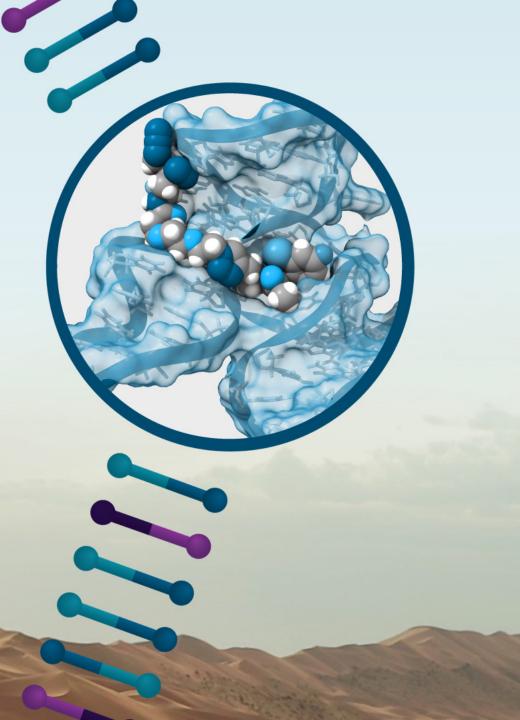


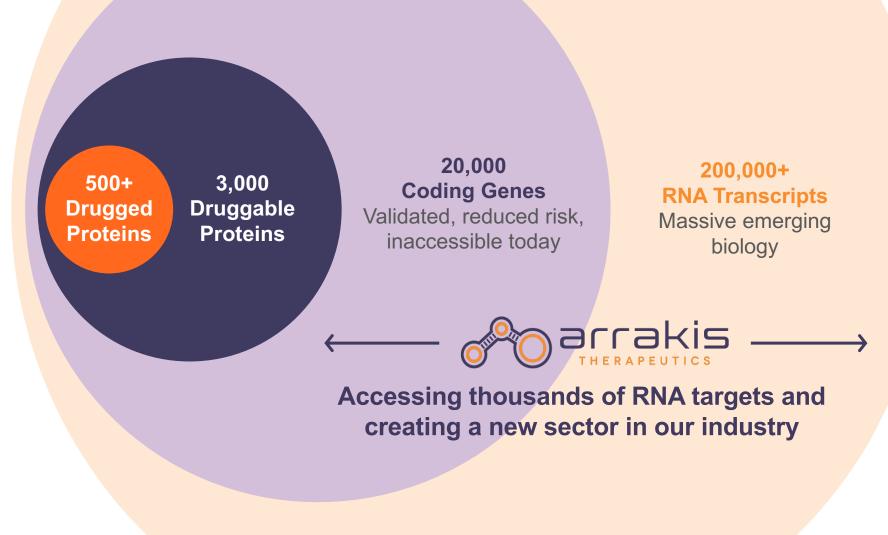
Application of IPA for interpreting RNA-targeted small molecule treatment datasets

Alex Amlie-Wolf, PhD IPA User's Group meeting 09/22/22



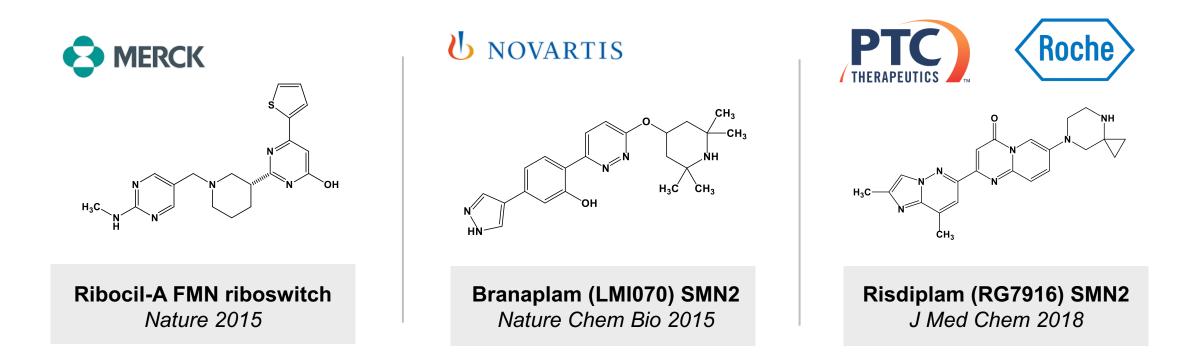
The ability to drug RNA will vastly increase our therapeutic targets

- Targeting RNA extends small-molecule medicines beyond the domain of well-studied protein targets, tapping into validated but previously inaccessible biology
- Goal: ligand RNA with drug-like (oral) small molecules
- This requires building a toolkit for identifying and optimizing RNAtargeted small molecules ("rSMs")





Bioactive, RNA-targeting, drug-like SMs already exist

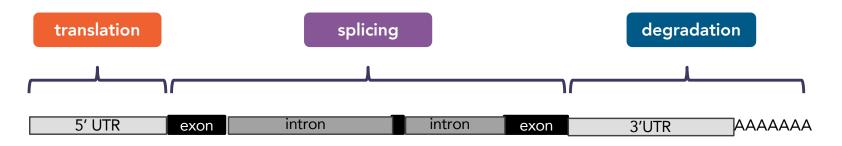


- These compounds were identified in phenotypic screens and **later** discovered to act on RNA targets
- Branaplam is in Phase 1/2 clinical testing and risdiplam was recently approved by the FDA for the treatment of spinal muscular atrophy (SMA) and is marketed under the name Evrysdi[™]
- Our approach is to start with an RNA structure and screen small-molecule libraries to identify selective binders followed by assessment of their activity in cells



Our approach to drugging mRNA

- Focus on mRNA to modulate the expression of undruggable proteins
- Compounds could affect expression by acting at any stage of the mRNA lifecycle splicing, translation, mRNA decay



Use bioinformatics data from public and proprietary sources to select targets:

- mRNA isoform complexity
- Conservation
- Human SNPs

- Transcriptional start sites
- Translation start sites
- Translational efficiency (e.g., ribosomal profiling)

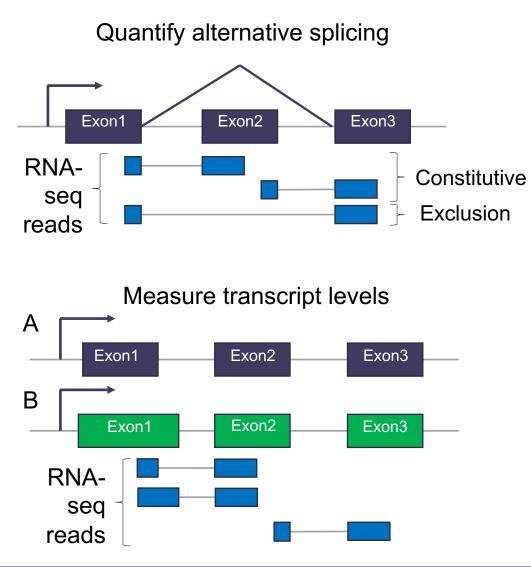
- Splicing efficiencies at each intron, assessing basal and induced mis-splicing
- RBPs (binding and functional impact)

- 2D RNA folding
- 3D structure analysis to identify potential ligandable pockets



Goal: characterize and understand off-target rSM effects

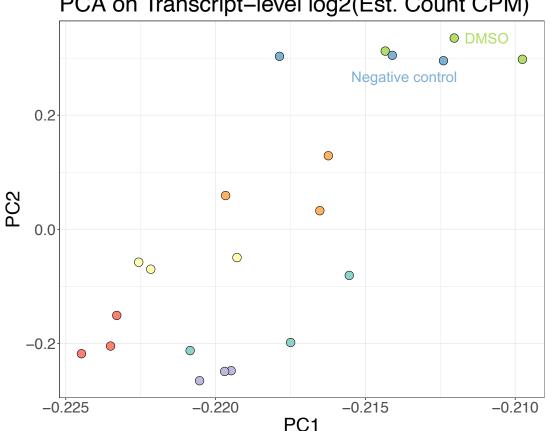
- Whatever the mechanism of action, rSMs interact with RNA molecules
- Once we screen and identify rSMs with quantifiable RNA binding, we need to understand their function
- Specific hypotheses about how our molecules act on RNA can be sensitively tested with bespoke assays
- However, ultimately we need to know what happens when rSMs get into cells!
- RNA-seq and proteomics are essential tools for understanding global effects on other RNAs and on proteins, respectively





Case study: early compound for internal program

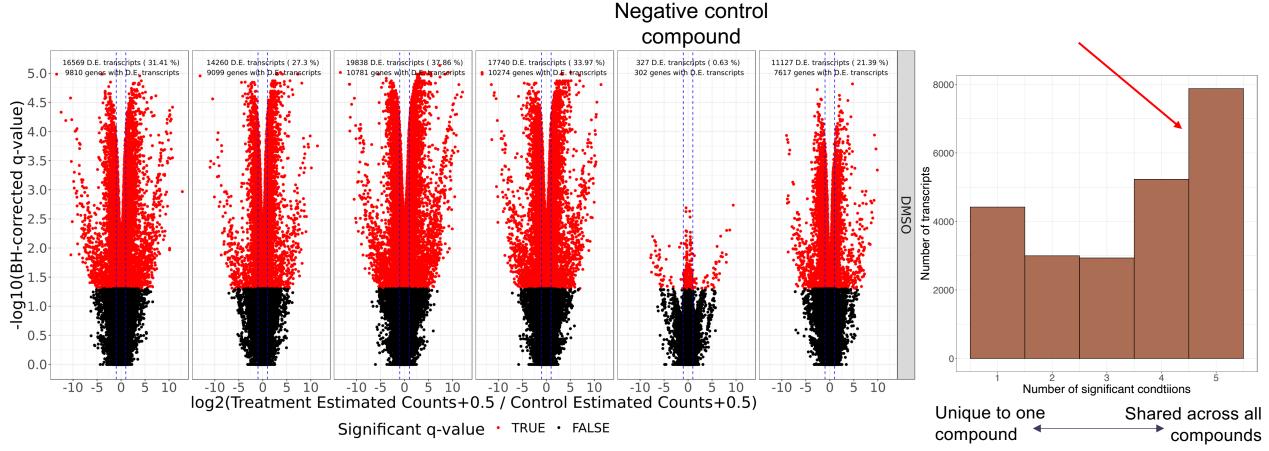
- Testing 5 analogs of cell active compound, along with • structurally-related negative control compound against DMSO
- Three replicates per condition, 85-140M reads per • sample
- Differential expression analysis used Kallisto • estimates followed by Sleuth, with p-value correction across multiple condition comparisons
 - Found similar results with htseq-counts + DESeq2







Differential expression analysis shows widespread and shared compound effects



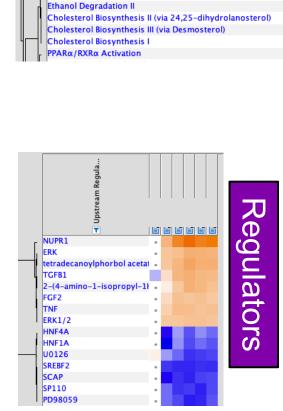
Widespread transcriptomic dysregulation is apparent, can we use IPA to pick out specific processes?

Uses Kallisto estimated counts with 100 bootstraps for transcript estimation to perform response error linear modeling and quantify differential expression with Sleuth tool, statistics based on likelihood ratio test between model with and without treatment variable



IPA characterizes common and compound-specific effects

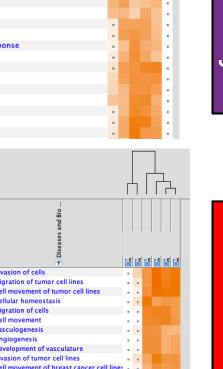
- For all 6 compounds, identify transcripts with significant differential expression and at least 2-fold change in either direction. then collapse to genes
- Maximum fold change of a given transcript is used for collapsing (under advanced analysis settings)



1 Cyclins and Cell Cycle Regulation

Serotonin Degradation





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Bio function S



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Pathway analysis of compound treatment effects

- Hierarchical clustering identifies sets of consistently downregulated (blue) and upregulated (orange) pathways
- Most strongly enriched pathways were found across all compound treatments
- Broadly seems to suggest cell stress / death pathways (upregulation in signaling and senescence pathways, downregulation of biosynthesis especially cholesterol and cell cycle)

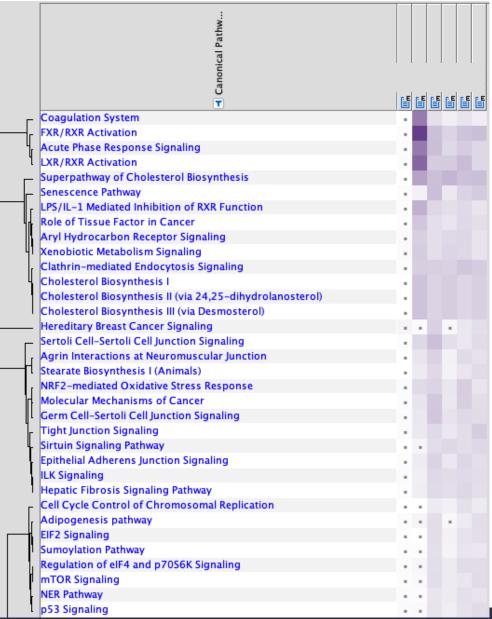
	Canonical Pathw					
	T	E	E	E	li I	5
1	Cyclins and Cell Cycle Regulation					
г	Serotonin Degradation					
h	Ethanol Degradation II					
	Cholesterol Biosynthesis II (via 24,25-dihydrolanosterol)					
	Cholesterol Biosynthesis III (via Desmosterol)					
	Cholesterol Biosynthesis I					
	PPARa/RXRa Activation					
	Pyrimidine Ribonucleotides Interconversion					
	Pyrimidine Ribonucleotides De Novo Biosynthesis					
	Pyrimidine Deoxyribonucleotides De Novo Biosynthesis I					
1	Cell Cycle Control of Chromosomal Replication					
4 .	Glycine Betaine Degradation					
	γ-linolenate Biosynthesis II (Animals)					
	Gluconeogenesis I					
	Glycolysis I					
	Bile Acid Biosynthesis, Neutral Pathway					
1	Glutathione Redox Reactions I					
Цr	RhoGDI Signaling					
	Thyroid Hormone Metabolism II (via Conjugation and/or Degradation)		•			
11	Intrinsic Prothrombin Activation Pathway					
ΠL	Stearate Biosynthesis I (Animals)					
	Noradrenaline and Adrenaline Degradation					
חך	PPAR Signaling					
1	Glutathione-mediated Detoxification					
1	Heparan Sulfate Biosynthesis (Late Stages)					
ΥΠ	Superpathway of Geranylgeranyldiphosphate Biosynthesis I (via Mevalonate)					
	Valine Degradation I					
11	Mevalonate Pathway I					
1 I	Colanic Acid Building Blocks Biosynthesis					
h	Isoleucine Degradation I					
	Ketogenesis					
	Glutaryl-CoA Degradation					
U'	Tryptophan Degradation III (Eukaryotic)					
ľ	Ethanol Degradation IV					
	Dopamine Degradation					
Ľ	Histamine Degradation					
ľ	Tryptophan Degradation X (Mammalian, via Tryptamine)					
	Fatty Acid α-oxidation					
ľ	Fatty Acid β-oxidation I Oleate Biosynthesis II (Animals)					
ľ	Oleate Biosynthesis II (Animais) Zymosterol Biosynthesis					
	Sucrose Degradation V (Mammalian)					
	Oxidative Ethanol Degradation III					
	oxidative Ethanor Degradation in					

Canonical Pathw	
r ERK5 Signaling	
Colorectal Cancer Metastasis Signaling	
Actin Nucleation by ARP-WASP Complex	
Integrin Signaling	
Systemic Lupus Erythematosus In B Cell Signaling Pathway	
Ephrin Receptor Signaling	
Cholecystokinin/Gastrin-mediated Signaling	
Phospholipase C Signaling	
, iNOS Signaling	
TNFR1 Signaling	
TNFR2 Signaling	
Opioid Signaling Pathway	
Senescence Pathway	
Role of NFAT in Regulation of the Immune Response	
Cardiac Hypertrophy Signaling (Enhanced)	
Agrin Interactions at Neuromuscular Junction	
Tec Kinase Signaling	
Reelin Signaling in Neurons	
HMGB1 Signaling	
ILK Signaling	
IL-8 Signaling	
Hepatic Fibrosis Signaling Pathway	
Actin Cytoskeleton Signaling	
Signaling by Rho Family GTPases	
Ephrin B Signaling	
VEGF Signaling	
PKC0 Signaling in T Lymphocytes	
Regulation of Cellular Mechanics by Calpain Protease	
Oncostatin M Signaling	
PI3K/AKT Signaling	
UVC-Induced MAPK Signaling	
Angiopoietin Signaling	
L-3 Signaling	
Thrombopoietin Signaling	
ERK/MAPK Signaling	
CXCR4 Signaling	
PAK Signaling	
NRF2-mediated Oxidative Stress Response	
Macropinocytosis Signaling	
¹ Gα12/13 Signaling	
Paxillin Signaling	
G Beta Gamma Signaling	
Neuregulin Signaling	
ErbB Signaling	
GNRH Signaling	1 C C C C C C C C C C C C C C C C C C C
IL-6 Signaling	
Telomerase Signaling	



Strongest pathways by p-value

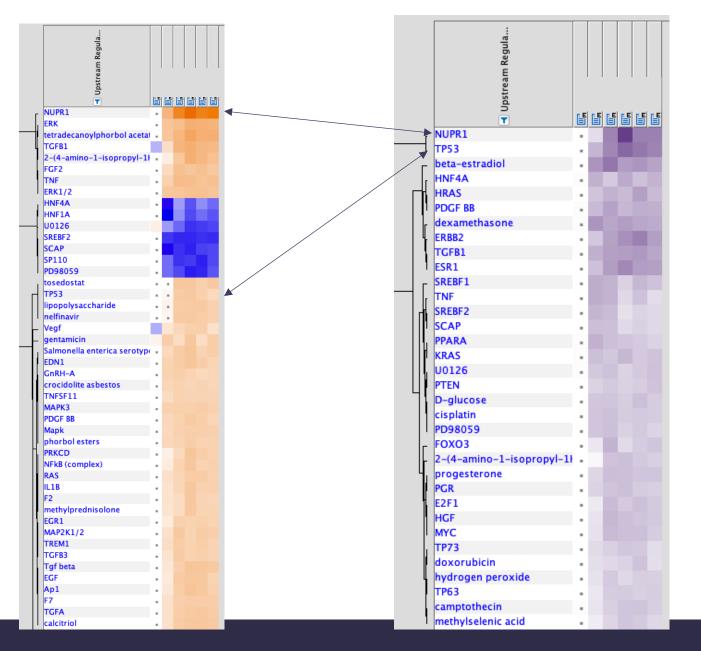
- Enrichment p-value as opposed to fold change-based Z-score encodes enrichment of affected genes in a pathway
- Picks out coagulation, RXR and phase response signaling as specific effect of one compound, senesecence and cholesterol biosynthesis more broadly





Upstream regulator analysis supports common effects mediated by specific factors

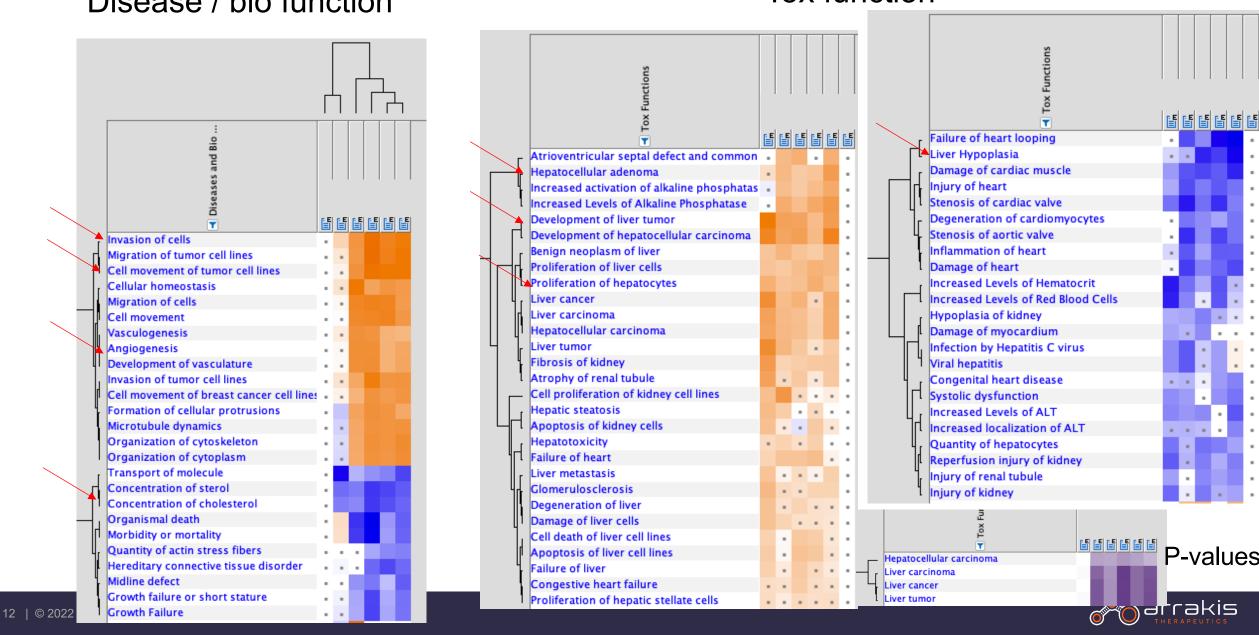
Visualizing Z score (blue is downregulated, orange is up) shows clusters of both up-regulated and down-regulated regulatory pathways



Visualizing corrected p-value with hierarchical clustering shows strong NUPR1 (high z-score) and TP53 (moderate z-score) signals across compounds



Toxicology and disease functions suggest broad carcinogenic effects Disease / bio function



Challenges in interpreting pathway analysis results

- With such widespread transcriptomic compound effects, it can be overwhelming to look at pathway enrichments
 - Hard to avoid the pitfall of looking for your favorite/expected enrichments, but on the other hand can help to give hints about MoA
 - Any other strategies and tips to get closer to interpretable results?
- Combination of p-value and z-score results can help to tease out 'real' enrichments
- Upstream regulator analysis is powerful approach to try to explain observed changes, even with widespread cellular effects





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