A multi-omics approach to overcoming resistance to the direct inhibition of mutant KRAS^{G12C}

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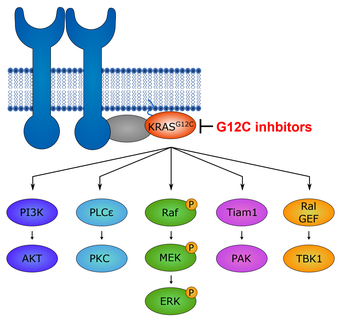
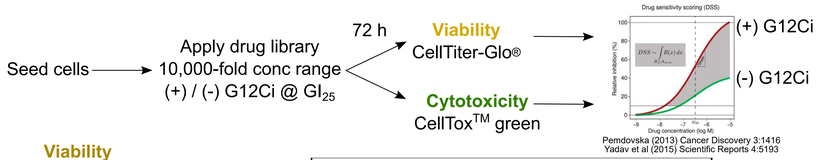
INTRODUCTION

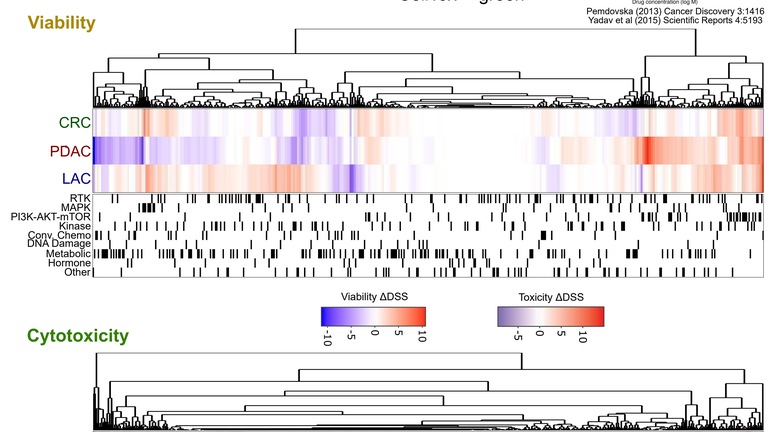
The recent development of small molecule inhibitors selective for one specific KRAS mutant, KRAS^{G12C}, with four currently under clinical evaluation, has begun to challenge the perception that KRAS is 'undruggable'. However, as targeted therapies are limited by acquired resistance, combination treatment strategies will be needed for G12C inhibitors (G12Ci) to maximize the extent and duration of anti-tumor efficacy. To determine strategies to overcome resistance to direct KRAS^{G12C} inhibition, we performed proteomic, transcriptomic, and high-throughput drug combination profiling to identify combination strategies.



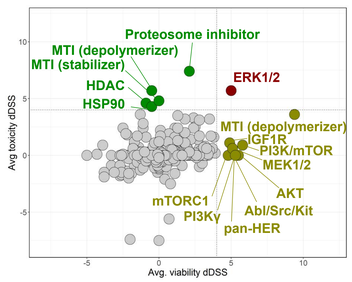
High-throughput drug sensitivity screen identifies therapeutic combinations with KRAS^{G12C} inhibition

A high-throughput drug screen of 528 clinically relevant compounds was performed on LAC, CRC, and PDAC cell lines in the presence and abscence of a low dose of KRAS^{G12C} inhibitor. Assay endpoints were both cell viability and cytotoxicity.



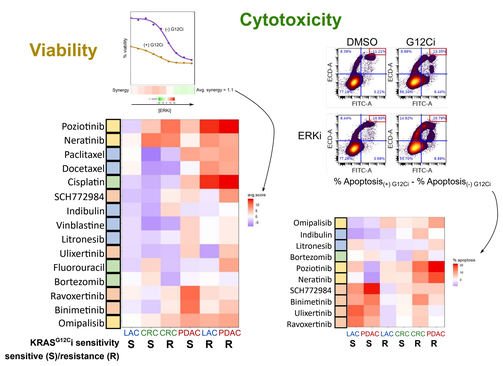


Effective combinations were identified using Drug Sensitivity Scores (DSS). A drugs' DSS was used to deteremine whether KRAS^{G12C} inhibition caused a sensitization towards the combination drug. Inhibitors that performed well in viability did not always score well for increasing toxicity (see graph to the right). We found agents that inhibited RTK and downstream KRAS effector pathways (e.g. PI3K-AKT-mTOR and ERK-MAPK) performed will in viability screens. However, we observed that microtubule inhibitors (MTIs), HDAC, and HSP90 inhibitors induced more cyctotoxicity. Only the ERK1/2 inhibitor, SCH772984, scored high in both screens.



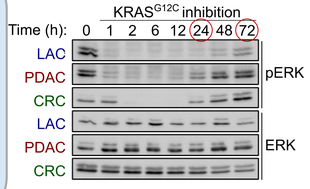
Validation screens are ongoing, and are designed to investigate viability and toxicity (apoptosis) effects

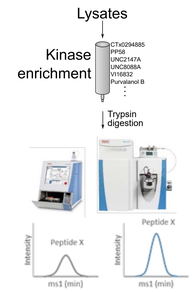
Drug	Target
Poziotinib	HER2
Neratinib	HER2
Omipalisib	p110/mTOR
Binimetinib	MEK1/2
Ravoxertinib	ERK1/2
Ulixertinib	ERK1/2
SCH772984	ERK1/2
Litronesib	KIF11A
Docetaxel	MTI (stabilizing)
Cisplatin	MTI (stabilizing)
Vinblastine	MTI (destabilizing)
Indibulin	MTI (destabilizing)
Cisplatin	DNA synthesis
Fluorouracil	TS
Bortezomib	26S Proteosome

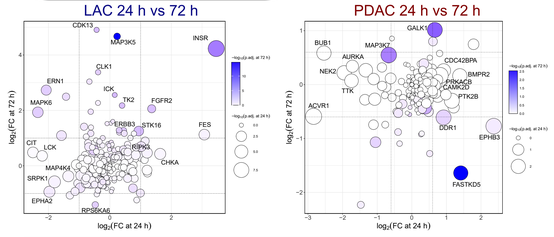


Kinome and phosphoproteomics profiling reveals adapative resonse to KRAS^{G12C} inhibition

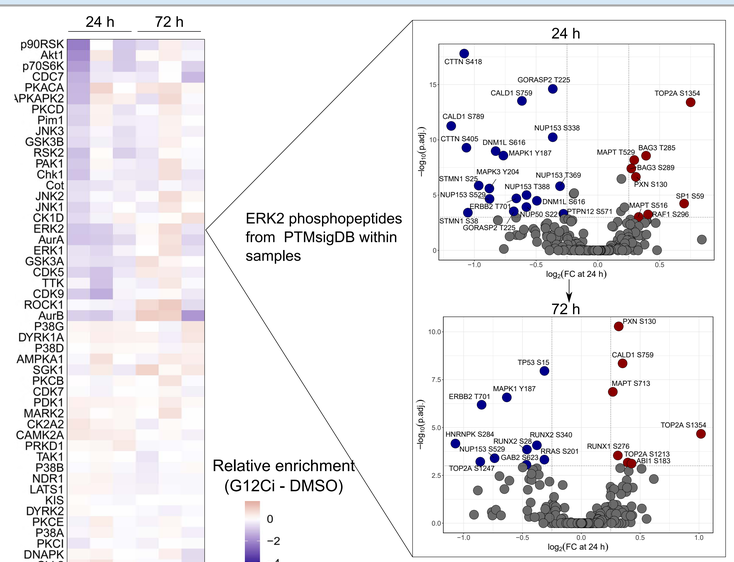
Adaptive resistance to KRAS^{G12C} inhibitors is associated with ERK-MAPK reactivation. To address this, we used multiplexed kinase inhibitor beads coupled with LC-MS/MS (MIB-MS) to profile changes in the kinome at times when cells are adapting to KRAS^{G12C} inhibition and reactivating ERK (see blot to the right). We found that there was considerable heterogeneity at these time points representing distinct trends in kinase activities.





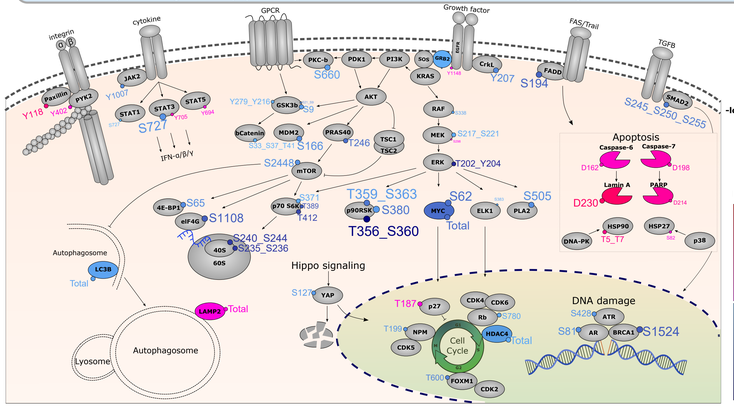


In addition to kinome profiling, where we detected kinases, we also analyzed samples for phosphopeptides using TiO_2 enrichment and samples were multi-plexed using tandem mass tag (TMT) labeling. Using this technique, we identified ~25,000 unique phosphopeptides. We used single sample gene set enrichment analysis (ssGSEA) and the PTMsigDB phosphosite sets to determine the relative enrichment of phosphopeptides attributed to the 108 kinases within PTMsigDB.

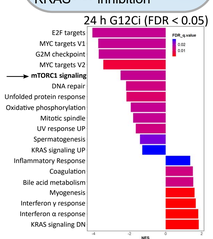


Reverse phase protein aray (RPPA) and RNAseq analyses identify the inhibition of mTORC1 and activation of autophagy as a targetable response to KRAS^{G12C} inhibition

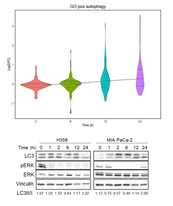
Pathway analysis of significantly (p<0.05) altered proteins/phosphoproteins following 24 h of KRAS^{G12C} inhibition



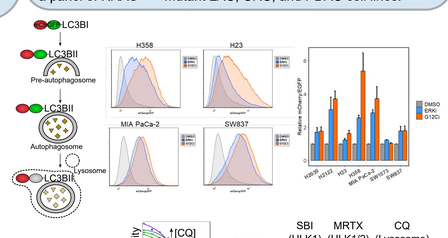
GSEA of hallmark gene sets reveals mTORC1 gene set depleted following KRAS^{G12C} inhibition



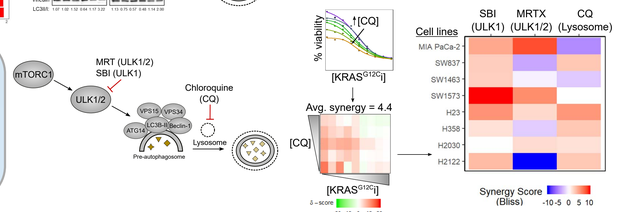
Transcripts of autophagy related genes increase with KRAS^{G12C} inhibition that follows LC3BI to LC3BII conversion



Flow cytometry of the autophagy reporter (mCherry-EGFP-LC3, cartoon below) is a read-out of autophagic flux. Autophagic flux is increased following 24 h of KRAS^{G12C} inhibition or ERK inhibition in a panel of KRAS^{G12C} mutant LAC, CRC, and PDAC cell lines.



Autophagy inhibition through the targeting of ULK1/2 (MRT/SBI) or lysosomal acidification (CQ) is an effective therapeutic strategy in combination with KRASG12C in a panel of LAC, CRC, and PDAC cell lines *in vitro*.



Conclusions

- Acquired resistance for KRAS^{G12C} inhibitors will be a significant limitation for their efficacy.
- Across KRAS^{G12C} mutant cell lines, ERK-MAPK reactivation occurs rapidly.
- Inhibition of metabolic changes (e.g. autophagy) that follow KRAS^{G12C} inhibition remains a promising strategy.
- Combination therapies that inhibit the receptor tyrosine kinases HER2/3 and serine-threonine kinases ERK1/2 are effective at inhibiting proliferation and promoting apoptosis in a panel of KRAS^{G12C} mutant cell lines.

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