APPLICATION NOTE

Pooled phenotypic CRISPR screening enables the identification of synergistic drug combinations for cancer therapy.

Authors

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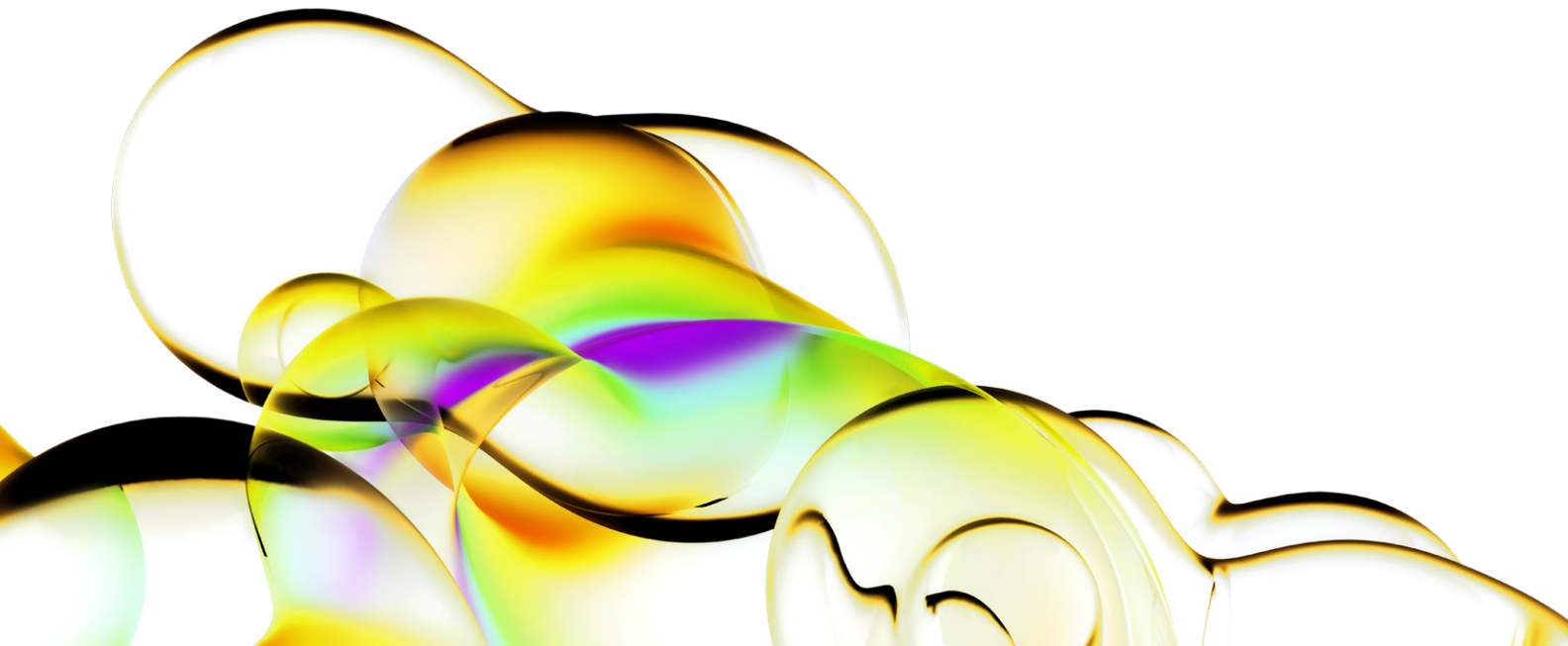
Single agent therapies have often limited clinical benefit, therefore identifying targets that synergize with a given drug is crucial for identifying combination therapy approaches that can maximize treatment efficiency. Cancer drugs, such as Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) inhibitors, often lead to proliferative arrest rather than cell death (Elkabets et al. 2013), leaving a reservoir of live tumor cells, some of which might be resistant to the drug or lead to the development of drug resistance (Klempner et al. 2013). Identifying targets that can induce cell death under these circumstances could be used to eliminate cancer cells and to prevent or delay the development of drug resistance.

Pooled CRISPR-Cas9 knock-out screens are valuable tools for new drug target identification. However, finding potential new therapeutic interactions through looking for gene deletions that cause cell death in the presence of a given drug is challenging. Negative selection screens rely on the dropout of sgRNA sequences from the treated population compared to a control, untreated population. As guide RNA loss is limited by the coverage of the sgRNA library, subtle but significant changes in guide abundancy can be difficult to detect. More importantly, negative selection dropout screens do not allow one to distinguish between gene loss that causes cell cycle arrest and gene loss that causes cell death, as both lead to a loss of cells from the expanding cell population.



A powerful adaptation of CRISPR screening is provided by the coupling of pooled NGS-linked screening strategies to phenotypic measurements. By using this combination of techniques, the screening strategy can be targeted to detect genes which drive complex biological responses by a biomarker signal read out and then deep sequencing sorted populations separately. Revvity have previously developed FACS-linked approaches for this, and here we show that a positive selection CRISPR death screen using magnetic beads can also be deployed.

Looking for synergies with PI3K inhibitors in TNBC cell lines

The treatment of triple negative breast cancer cell lines with PI3K inhibitors does not induce robust cell death in the majority of the treated cells. Thus, we treated MDA-MD-231 cells with the pan PI3K inhibitor GDC-0941 for 24 hours under low serum conditions and then separated into Annexin positive and Annexin V negative populations. These two populations were analyzed for sgRNA abundance using NGS (Figure 1).

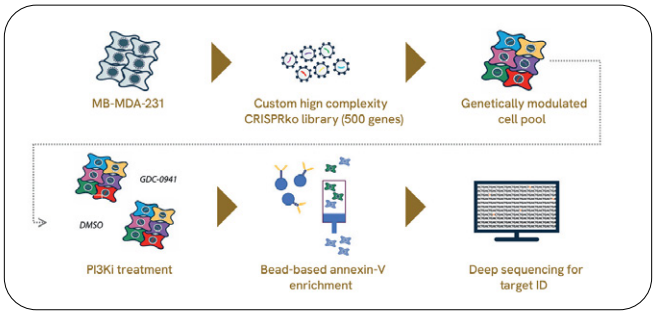


Figure 1: CRISPR death screen setup. sgRNA abundancies are determined in the death cell population, allowing the identification of sgRNAs that induce cell death in the presence of the pan PI3K inhibitor GDC-0941 and enabling the identification of targets suitable for combination therapy.

Disease annotation analysis of the top enriched genes in the Annexin V positive population, based on Log2 fold change, revealed mammary neoplasm as the most abundantly associated disease with six of the top 22 enriched genes associated with breast cancer. EGFR is one of the top enriched genes (Figure 2) and notably, EGFR inhibition is known to synergize with PI3K-AKT pathway inhibitors (Jacobsen et al., 2017; She et al., 2016; Yi et al. , 2013) and this combination is currently in clinical trials. We also found a kinase among the top enriched targets (Target 9, see Figure 2) that is known to promote cell survival by inhibition of the pro-apoptotic protein BAD and thus Target 9 is a potentially interesting novel therapeutic target in TNBC.

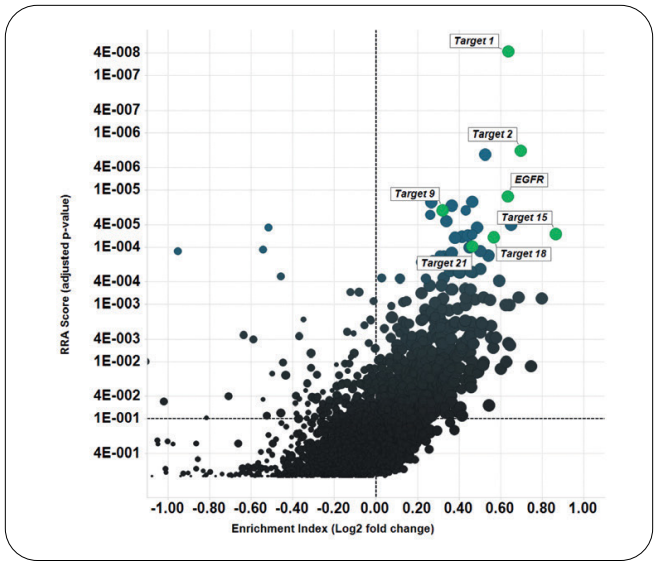


Figure 2: A CRISPR death screen. Carried out in the triple negative breast cancer (TNBC) cell line MDA-MB-231 revealed known and novel targets that synergize with the pan PI3K inhibitor GDC-0941.

Positive Log2 fold changes were observed which indicate that target loss promotes death when plotted with the robust ranking aggregation score for each gene (Li et al., 2014). Compelling hits which pass the threshold of FDR<0.1 are highlighted in green and blue.

Analysis of guide behaviour for some of the potential hits indicated that the guides behave consistently in the cell death screen, and provide confidence that selection of Annexin V positive cells is a robust method for identifying potential drug targets that synergize with PI3K inhibitors (Figure 3).

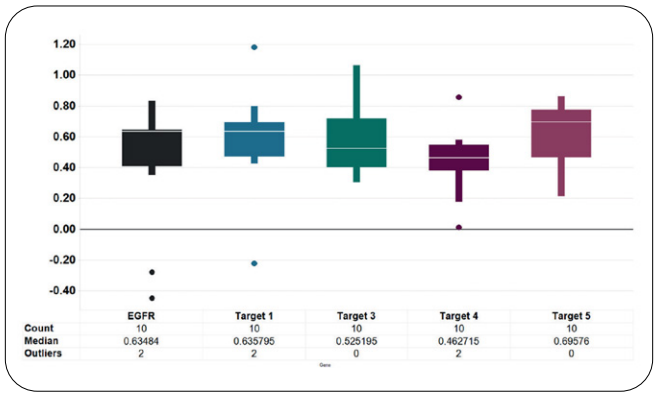


Figure 3: Death screen guide analysis. Guide analysis for the top 5 enriched genes in the Annexin V positive cell population in the presence of PI3K inhibition revealed consistent sgRNA behaviour for at least 8 out of 10 guides.

The cell death screen also allowed analysis of cells that were surviving in low serum conditions in the presence of GDC-0941. Analysis of the guide RNAs enriched in this cell population identified six tumor suppressors among the top 14 depleted targets (Target 1C, Target 5C, Target 8C, Target 10C, Target 13C, Target 14C), indicating that these genes might be required to induce cell death in response to PI3K inhibition. These findings are consistent with many tumour suppressors having an effect on the induction of cell death in cancer cell lines.

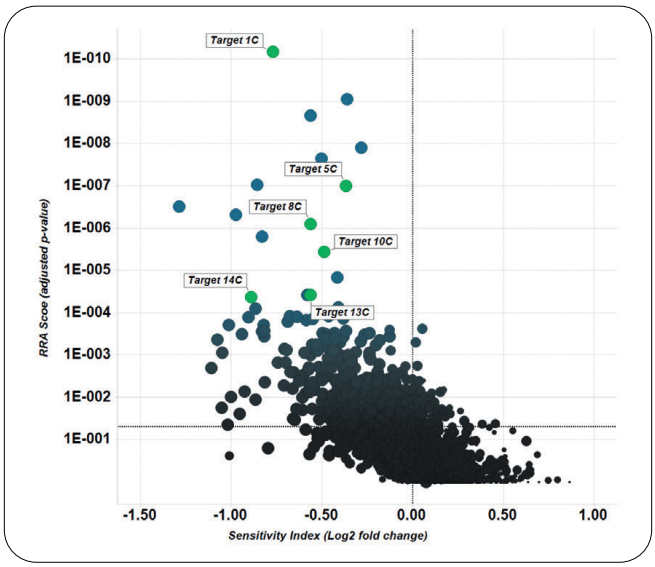


Figure 4: A CRISPR death screen revealed known and novel targets that are required for cell death induction in the presence of GDC-0941. Negative Log^2 fold changes indicate that target loss protects from cell death.

Summary and conclusion

A positive selection screen where dead cells are enriched based on their Annexin V status has enabled the identification of known as well as previously unknown genes whose loss synergizes with PI3K inhibition in TNBC cell lines.

This same screen also identified genes that are required for cell death induction under low serum conditions in the presence of GDC-0941. This approach identified known interactions that synergize with PI3K inhibitors and novel interactions that have not previously been linked to induction of cell death. These findings will aid the identification of new drug combinations and to better understand the development of drug resistance to single agent PI3K inhibitors.

Methods

MDA-MD-231 cells were infected with Revvity's optimized CRISPR lentiviral library (Cross et al. 2016) targeting the "druggable" genome at low multiplicity of infection (MOI). Infected cells were selected for 3 days with 0.5 µg/mL puromycin, after which cells were expanded for an additional 10 days, before cells were transferred to media containing low serum concentration (2% FBS). After 24 hrs, cells were treated for an additional 24 hrs with 1 µM GDC-0941 and DMSO, respectively. Annexin V+ve dead cells and Annexin V-ve cells were purified using magnetic Annexin V microbeads and positive selection LS columns (Miltenyi Biotec). Genomic DNA was isolated from all samples, the sgRNA sequences were amplified by PCR and sgRNA abundances in each sample were analyzed by NGS. MAGeCK was used for hit calling (Li et al. 2014).

References

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