CRISPR-Cas9 in oncology

Will gene editing unmask synthetic lethal targets?

The RNA-guided nuclease Cas9 represents a revolution in mammalian molecular biology as, finally, facile targeted mutagenesis allows the power of genetics to be applied to human cell biology, disease modelling and target identification and validation. Functional genomics is emerging as a major application for CRISPR-Cas9: screens performed with lentiviral-delivered guide RNA libraries are linking new genes to phenotypes, revealing knock-out driven modes of drug resistance and identifying cohorts of essential genes.[1, 2] The Broad Institute,[3] Sanger Institute[4] and start-up company KSQ Therapeutics Inc have defined cohorts of essential genes in hundreds of cancer cell lines with the motivation of finding the next generation of oncology targets. Most new targets will fit into the synthetic lethality paradigm, an emerging concept for drug discovery that has arisen from genetics in model systems and already delivered one class of drugs, the PARP (poly ADP ribose polymerase) inhibitors. But how many of these targets will survive the validation process and can we expect to see a new wave of synthetic lethal therapies advancing towards approval?

The last 25 years of cancer drug discovery have been characterised by two major trends. The first, dominant from the early 1990s to around 2010, began with the realisation that cancer cells might be selectively killed by drugs that inhibited the activity of overactive oncogenes.[5] Of the many drugs that entered the clinic, the best performing group were those that directly targeted proteins encoded by oncogenes mutated in malignancy; mere overexpression of a target was rarely associated with the clinical success of its inhibitors. Successful therapies versus cancers harbouring mutations in druggable oncogenes include imatinib (Glivec) vs BCR-ABL translocations, erlotinib (Tarceva) vs mutated EGFR and, for a non-kinase example, enasidenib (Idhifa) vs IDH2 mutant acute myeloid leukaemia (AML).

The second trend in cancer therapeutics that has dominated since 2010, is to restore or enhance the ability of the active immune system to fight malignant cells.[6] Interest in this approach has been driven by the very durable responses observed in some patients treated with antibodies vs CTLA4 and PD1/PDL1. Additional immune checkpoint inhibitors are now being tested in the clinic, the field of cancer vaccines has been rejuvenated and major investments are being made to re-direct T cells and stimulate tumour infiltrating lymphocytes.

But can immunotherapy approaches work everywhere if optimised? It is instructive that, in colon cancer patients, anti-PD1 antibodies have only been effective against tumours that have mismatch repair deficiency and therefore very high mutational loads.[7] Immune checkpoint inhibitors require neoantigens to be effective and it appears logical that a tumour's mutation load will influence the probability of expressing a neoantigen. Typical mutation loads vary massively between different tumour sites[8] and immunotherapy has met the most success in the indications that have the highest mutation loads, namely melanoma and lung cancer. Most cancers have equivalent or lower mutation loads than the non-responsive mismatch-repair-competent majority of colon cancers. This argues that immunotherapy will have its limits.

So what new therapeutic approaches can meet the need of patients whose cancers have a low mutation burden? Unfortunately, gain-of-function mutations in druggable oncogenes are observed in only a minority of cancers. This seam of drug discovery opportunities may now be 'mined out' with the approval of larotrecinib (Vitrakvi) for the rare but diverse set of cancers with NTRK translocations. Instead, the overall landscape of cancer-driver mutations[8] is dominated by mutations in so-far undruggable oncogenes, such as KRAS, and inactivating mutations in tumour suppressors such as VHL, ARID1A or RB1. Only in exceptionally rare cases will it be possible to restore the function of an inactive tumour suppressor with a drug. However for patients whose ovarian cancers have mutations in the tumour suppressors BRCA1 and BRCA2, we do now have molecularly targeted therapies in the form of olaparib (Lynparza) and the other PARP inhibitors. BRCA mutant tumours have defective homologous recombination, which creates an intense dependence on the alternative DNA-repair pathway of non-homologous end joining (NHEJ) and therefore a *non-oncogene dependence* on enzymes critical for NHEJ like PARP.

The term 'synthetic lethality' dates back to the 1940s, when it was used to describe the situation where mutations in two genes were lethal in combination but had no impact on cell viability if they occurred independently. As the most prominent use of the term has moved from genetics in model organisms to cancer biology, it has come to also encompass the situation where a combination of a tumour-specific mutation and a drug are able to induce cell death.[9] The success of olaparib and other PARP inhibitors in cancers with homologous recombinant defects demonstrates this approach can be exploited in the development of anticancer therapeutics. What remains unclear is whether this class of drug will be unique, or whether it is the tip of the iceberg in terms of a new class of breakthrough therapeutics that operate through the mechanism of synthetic lethality. The first step in answering this question is to look for new non-mutated cancer-dependency targets that map to particular mutated genetic biomarkers.

The first cancer dependency target ID screens relied on the (then novel) adaptation of RNA interference (RNAi) to high-throughput functional genomics. We now know that RNAi, especially when applied to genome-scale shRNA screens, is plagued by two opposing issues. First, the partial knockdown achieved leads to frequent failures to observe a true link between a gene and function. Second, the knockdown of off-target mRNA leads to a false attribution of a phenotype to a gene. This results in a lack of reproducibility of findings between labs as evidenced by the identification of a slew of genes as putative synthetic

lethal targets, such as STK33, that have not gone on to validate.[10] Looking to overcome the limitations of RNAi, many researchers have switched to CRISPR-Cas9 as a more penetrant and more precise screening tool. Both the Sanger and Broad Institutes have completed genome-wide CRISPR screening in hundreds of cell lines,[3,4] giving rise to powerful data sets that can be stratified by biomarker mutation and searched for associated dependencies. Whether these activities will lead to an avalanche of synthetic lethal drug targets remains unclear, but several new companies are now operating in this space.

CRISPR has major advantages over RNAi for target ID and validation in terms of both improved penetrance and reduced off-target effects.[11,12] However, it is important to remember that the latest CRISPR screens remain based on the culture of clonal cell lines as monolayers on plastic support and in this respect carry the same caveats for identifying 'real' targets as the historic RNAi work.

At Horizon we sought to identify novel synthetic lethal targets in the major colon cancer genotypes, including PIK3CA and KRAS mutant cancers. We invested significant effort to ensure the cell culture conditions of these screens were such that cell growth was dependent on oncogenic signalling. Our primary pooled CRISPR screens revealed a long list of potential targets, which we progressed into a high-throughput validation pipeline discarding any targets where the initial synthetic lethal hypothesis was not validated or where we did not find evidence for a major quantitative impact on cell growth or survival. Around 15 potential targets survived this process and we sought a drug discovery partner to exploit their potential as targets for cancer therapeutics. In December 2018, we announced a partnership with C4X Discovery Ltd that supports further target validation and will allow C4X's innovative high-productivity drug discovery engine to rapidly progress chemistry programmes, with the view to delivering high value pre-clinical licensable assets for partnering.

What else is in the industry pipeline in the way of synthetic lethal therapies and targets? Loss of the VHL tumour suppressor in renal cancers leads to unregulated and oncogenic expression of the transcription factor HIF-2, which surprisingly has proved druggable. A small molecule inhibitor of HIF2, PT2325 from Peloton Therapeutics Inc, is now in Phase 2 clinical trials.[13] Some groups of researchers are keeping close to the original BRCA/PARP paradigm and looking for additional synthetic lethal relationships in cancers with DNA repair defects, where there are both precedents of success and theoretical reasons to anticipate better penetrance.[14] Several drug discovery projects are targeting the POLQ DNA polymerase following hypothesis-led work leading to its validation as a druggable synthetic lethal target in homologous recombination deficient cancers.[15] A combined RNAi and CRISPR-screening approach has revealed the nucleases FEN1and APEX2 as additional synthetic lethal targets for BRCA2 mutant cancers.[16] Furthermore, an early target emerging from the Sanger Institute CRISPR screens is the RecQ family helicase, WRN-1, which has been reported to be synthetic lethal in mismatch repair deficient cancers.[4]

The Sanger and Broad Institute CRISPR datasets, along with the higher quality RNAi datasets from the Broad Institute and Novartis contain near genome-wide dependency data for hundreds of cancer cell lines and mining of these data reveals many potential opportunities for drug discovery. Our experience, however, is that most reasonable looking synthetic lethal associations between biomarker and target evaporate upon validation. Furthermore, many of the better target dependency-biomarker links in the CRISPR dataset were already known from years of hypothesis-led work. Our impression is that novel and tractable synthetic lethal targets may actually be rather rare, which may add to the value of the drug discovery programmes that succeed in drugging them.

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