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What's next for the synthetic lethality drug discovery engine?

As a surge of cancer drugs with new synthetic lethality targets enter the clinic, the opportunities and challenges of the underlying discovery strategies are coming into focus.

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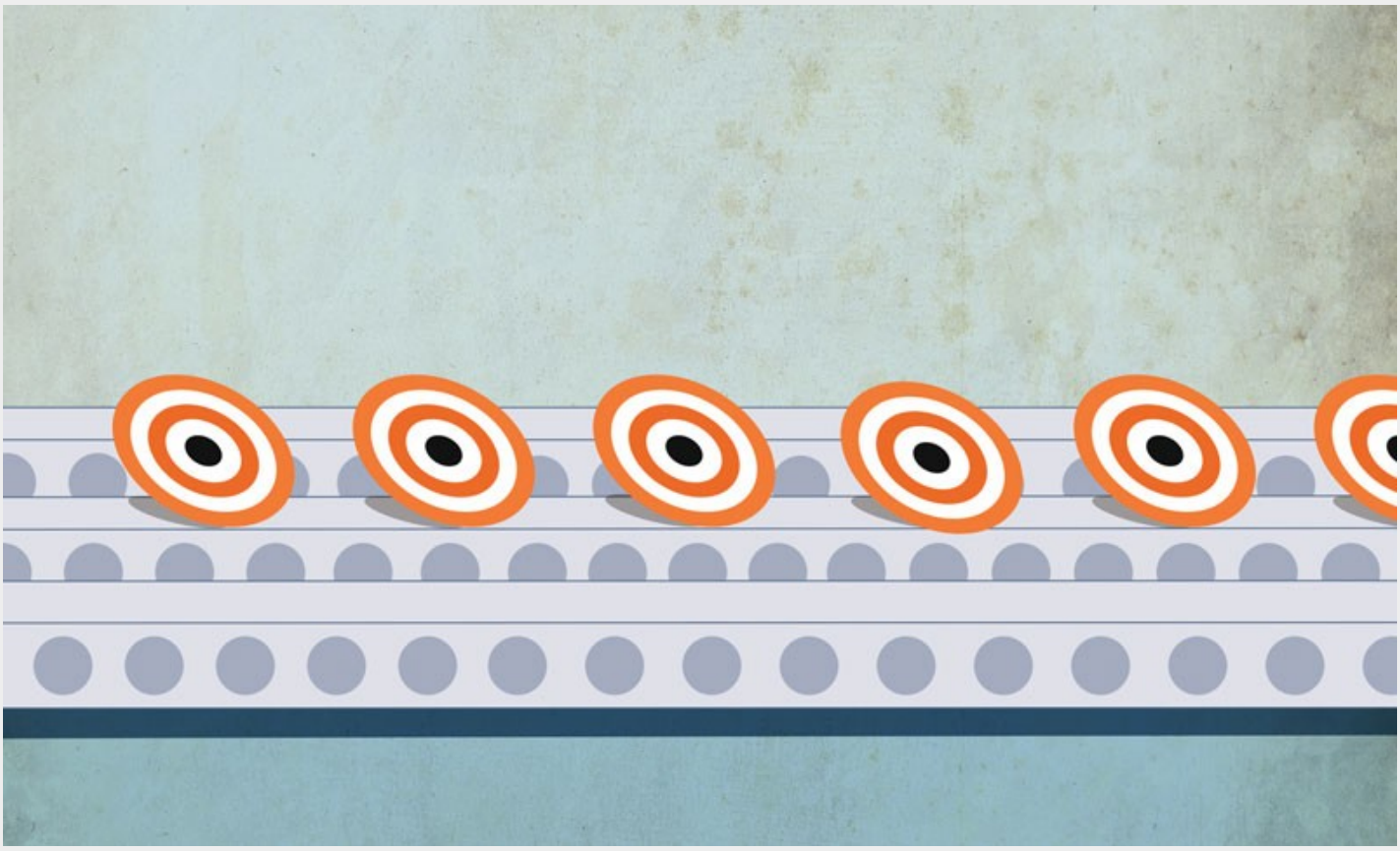

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Drug developers are always on the lookout for the next generation of cancer targets. The low-hanging fruits have been picked. Known higher-hanging targets — including a long list of tumour suppressor genes that are frequently neutralized by loss-of-function mutations — remain out of reach. After all, how do you drug a mutated protein that is no longer working, or in some cases not being produced at all?

When Bill Sellers and Frank Stegmeier were considering this question at Novartis about a decade ago, the answer was clear: "The only way to go after loss-of-function mutations is with a synthetic lethal approach," says Stegmeier, now CSO at KSQ Therapeutics.

The BRCA1/2–PARP interaction is the poster child of this approach. When cancer cells lose these DNA-repairing BRCA proteins, they become dependent on the PARP1 enzyme to keep DNA damage in check. PARP inhibitors kick out the second leg of the DNA repair pathway, toppling cancer cells with loss-of-function BRCA mutations.

The rise of genome-wide knockout screens has now created a discovery engine to identify otherwise hard-to-find synthetic lethal pairings. By systematically depleting every gene, across hundreds of cancer cell lines, researchers can map out cancer-specific vulnerabilities. Novartis's RNAi-induced knockdown Project DRIVE, launched by Stegmeier and Sellers, helped pioneer this approach, and CRISPR gene editing tools have supercharged these efforts. A surge of synthetic lethal candidates are in and approaching the clinic — taking on polymerase theta (POLQ), USP1, PKMYT1, PRMT5, MAT2A and WRN (Table 1).

Table 1 | Selected synthetic lethal interactions

Drug	Sponsor	Status
POLQ* × *BRCA		

Drug	Sponsor	Status
ART4215	Artios	Phase I/II
NA	Ideaya/GSK	Preclinical
NA	Repare	Preclinical
Novobiocin	Varsity	Preclinical
USP1 × BRCA		
KSQ-4279	KSQ	Phase I
NA	Tango	Preclinical
PKMYT1 × CCNE1		
RP-6306	Repare	Phase I
PRMT5 × MTAP		
AMG 193	Amgen	Phase I
MRTX1719	Mirati	Phase I
PRT811 and PRT543	Prelude	Phase I
TNG908	Tango	Phase I
MAT2A × MTAP		

Drug	Sponsor	Status
IDE397	Ideaya/GSK	Phase I
WRN* × *MSI		
NA	Ideaya/GSK	Preclinical
NA	Vividion/Roche	Preclinical

NA, not available; MSI, microsatellite instability.

"It's very satisfying to see these candidates move forward," says Bill Sellers, now at the Broad Institute of MIT and Harvard. "Those are all exciting opportunities."

But the biggest unmet needs — synthetic lethality targets for commonly mutated tumour suppressor genes like *TP53*, *PTEN* and *RB1* — remain elusive, he cautions. "We've found things that are quite cool, but they are not the things we initially thought we would be finding," says Sellers.

The polymerase path

Synthetic lethality started regaining traction in 2015. The FDA had approved AstraZeneca's PARP1/2 inhibitor olaparib for patients with BRCA-mutant ovarian cancer the year before, showcasing the path to market for these drugs. CRISPR screens were becoming more commonplace. Investments surged, and companies emerged. KSQ and Ideaya were both founded in 2015, joined shortly after by Artios Pharma, Repare Therapeutics, Tango Therapeutics and others.

POLQ was in the pipeline from the start — with no need for a boost from genome-wide screens. The polymerase's path to becoming a synthetic lethal target instead mirrored that of PARP. Both proteins initially attracted drug developers because of their potential

as chemosensitizers. Chemotherapies kill cancers by damaging the DNA of rapidly dividing cells. By blocking the proteins that repair this damage, drug developers hoped to overload the cells.

But because of the complex nature of DNA damage repair, researchers hypothesized that synthetic lethal interactions might link some of the proteins in this pathway. In 2005, two teams showed that combined perturbation of BRCA and PARP killed cancer cells – kick-starting the field (Fig. 1). A decade on, two teams hypothesized and then showed that dual hits on POLQ and BRCA kill cancer cells too.

Fig. 1 | **Synthetic lethality.** In healthy cells, loss of gene A or gene B alone does not affect cell survival. But in tumour cells, a mutation in one gene leaves the cell vulnerable to disruption of the other gene. These synthetic lethal relationships create drug discovery opportunities, pointing the way to candidates that can kill cancer cells while sparing normal tissue. Figure adapted from F. L. Rehman et al. *Nat. Rev. Clin. Oncol.* **7**, 718–724; 2010 Springer Nature Ltd.

Multiple PARP inhibitors are now on the market, and patients with DNA repair-deficient cancers already have access to safe and effective drugs. Next-generation PARP inhibitors are on the way too. But many of these cancers still develop resistance to PARP blockade – creating a need and potential paths for new strategies. Last year, for example, Artios's CSO Graeme Smith and colleagues reported that PARP resistance, via the loss of the 53BP1/SHLD complex, sensitizes cells to POLQ inhibitors.

"In this PARP inhibitor resistance setting, cells are very, very dependent on POLQ for survival, and therefore very sensitive to POLQ inhibition," says Smith.

Another group at the Dana-Farber Cancer Institute has also shown that BRCA-deficient PARP-resistant cancers are sensitive to POLQ inhibition.

Artios has advanced a first POLQ inhibitor, ART4215, into the clinic as a monotherapy and in combination with PARP inhibitors. Varsity and Repare are in pursuit with inhibitors too.

Ideaya is taking a different tack. POLQ has both a helicase function that unwinds DNA and a polymerase function that repairs breaks. But genetic knockouts alone do not show which of these functions needs to be targeted. So Ideaya is advancing a small-molecule degrader that ablates POLQ entirely, more closely mimicking the biological consequences of a genetic knockout.

Sellers welcomes the addition of targeted protein degraders to the drug discovery toolbox, but cautions that they are no panacea for synthetic lethality targets. If chemists hit a potency wall with an inhibitor, turning that candidate into a degrader is unlikely to provide the needed leg-up. The pharmacology of degraders is also more complex than that of inhibitors, with many pitfalls in the path to clinical effect.

"People have to go into degradation purposefully," says Sellers, who is on the scientific advisory board of Ideaya.

Screen time

Other programmes showcase the types of targets that genetic knockout screens find. Repare used a genome-wide knockout approach, for example, to pinpoint PKMYT1 as another opportunity in the DNA damage repair space.

This programme started with a focus on *CCNE1*-amplified cancers — a common cancer signature that correlates with poor prognosis. *CCNE1* encodes the cell cycle regulator protein cyclin E, and oncogenic over-expression of this protein speeds up cell growth and destabilizes the genome. But cyclin E drives its effect through a protein–protein interaction with CDK2, and this interaction has been hard to hit with direct inhibitors. CDK2 inhibitors have proven challenging to develop too — held back by lack of selectivity and off-target effects — although Pfizer and Blueprint Medicines are each making progress with these.

Synthetic lethality could provide an alternative means of attack, hypothesized Daniel Durocher, a molecular geneticist at the University of Toronto and co-founder of Repare

Therapeutics. After first making cyclin E-overexpressing cancer cell lines, his team used a genome-wide CRISPR knockout approach to identify the genes that these cell lines depended on the most. *PKMYT1*, another controller of the cell cycle, lit up.

"We were elated when we saw it was a kinase," adds Durocher. "It's that diamond in the rough that we can run with."

A PKMYT1 inhibitor has potent activity in *CCNE1*-amplified cells, Durocher and his colleagues have reported, showing that knockout screens can create opportunities even beyond loss-of-function alterations. Cyclin E amplification puts cancer cells on edge, and PKMYT1 inhibition induces catastrophic levels of genomic instability.

Phase I trials of Repare's PKMYT1 inhibitor RP-6306 in solid tumours are now ongoing, testing the drug as monotherapy and in combination with other agents including chemotherapy.

KSQ used a different search strategy to arrive at USP1.

Rather than focus on a set cancer signature, their starting filter was a predetermined "gene effect". Some genes, when knocked out across hundreds of cell lines, will kill nearly everything. Others will only kill a subset of cells with a specific mutational profile. For Stegmeier, this second group of genes — called selective essential genes — are the sweet spot.

Inhibit these and you can kill cancer cells with exploitable mutations, with minimal risk of off-tissue effects.

"We didn't go out looking for USP1, but we found it in the [selective essential] box," says Stegmeier.

A literature review showed that USP1 acts in the DNA damage response pathway, and the team was on their way. USP1 is a de-ubiquitinase that regulates levels of two DNA clamps that help identify DNA damage, and USP1 blockade disrupts the function of these sensors. BRCA1-mutant and homologous recombination-deficient cancers are particularly sensitive to USP1 inhibition, the KSQ team found.

KSQ's experience highlights the risk that synthetic lethality screens will point to targets beyond those that are typically tractable with small-molecule drugs. "Of course the temptation is to be drawn towards druggable families like kinases, because of their perceived feasibility," says Andrew Wylie, head of oncology at KSQ. "But when we are looking through our screens, what we see are many other classes of proteins and enzymes that people are often a little hesitant to go after."

This trend might become more pronounced, adds Stegmeier. "The extremely low-hanging fruits are gone. It's not like there are dozens of easily druggable kinases that are amazing synthetic lethal targets."

In the case of the de-ubiquitinase USP1, the availability of tool compounds helped clear the path. But it still "took the blood, sweat and tears of an army of medicinal chemists to identify novel chemical matter and work it through," says Wylie.

KSQ-4279 is the first selective de-ubiquitinase inhibitor into the clinic, he adds. The drug is in phase I, as monotherapy and in combination with various agents including PARP inhibitors.

Tango is also advancing a USP1 inhibitor towards the clinic.

Genetics versus pharmacology

As interest in synthetic lethality opportunities picks up, Stegmeier worries that the field is getting ahead of itself. "If we take programmes into the clinic that don't really test the

fundamental hypothesis, and these fail, then investors will say 'well, clearly synthetic lethality doesn't pan out'," he explains. "I think we were almost at that point."

ATR inhibitors showcase the ambiguity. These kinase inhibitors also modulate the DNA repair pathway, and are in clinical trials for patients with loss of function of *ATM*, another node in the DNA damage response. But CRISPR knockout data show that *ATR* is an essential gene. Inhibiting ATR, therefore, might be broadly cytotoxic.

"It's not that you can never develop a successful drug from these types of targets," says Stegmeier. "But these drugs are not going to have the broad therapeutic index that synthetic lethality promises."

Sure enough, ATR inhibitors are facing toxicity challenges in the clinic — although as yet these have not been show-stopping.

But genetic screens don't show the whole picture, counters Chris Lord, a cancer researcher at the Institute of Cancer Research, who helped to unravel the BRCA–PARP interaction in 2005. While CRISPR knockout screens can provide a compelling black-and-white perspective, he explains, small molecules provide a means of distinguishing between the shades of grey.

"Clearly catalytic inhibition of ATR is not generally lethal in the way genetic ablation of ATR is," says Lord. ATR development continues apace — both in monotherapy and combination treatment settings.

PKMYT1 also scores as broadly essential when knocked out with CRISPR across hundreds of cell lines. But Durocher's team was able to readily make viable PKMYT1-knockout cell lines, he adds, offsetting concerns about broad cytotoxicity for PKMYT1 inhibitors. The target is also not pan-essential when depleted by RNAi, he adds, and the preclinical toxicity of RP-6306 in mice looks promising.

"I don't think there is anything wrong with having 'essential genes' as targets," says Durocher. "Pharmacology is not identical to genetics. Pharmacology gives you options."

"I would argue that we need to retool ourselves, and think a bit more about how we can do synthetic lethal target discovery from a high-throughput, medicinal chemistry perspective," adds Lord.

Beyond DNA damage repair

Work on MTAP synthetic lethality partners — a rare example of interactions that act outside of the DNA damage repair pathway — demonstrates the importance of small-molecule nuance.

MTAP is a methylthioadenosine phosphorylase that metabolizes and salvages methionine and adenine. Although loss of *MTAP* leads to the accumulation of the metabolite MTA in cancer cells, this process is not thought to be oncogenic. Instead, *MTAP* deletions are passenger mutations, a result of the gene's proximity to the frequently deleted tumour suppressor gene *CDKN2A*.

An early synthetic lethality project by Sellers and Stegmeier at Novartis looked at RNAi knockdown data to search for synthetic lethal partners for *CDKN2A*. Instead, they discovered an interaction between co-deleted *MTAP* and *PRMT5*. Another team at the Broad Institute found the same thing.

PRMT5, for its part, is a methyltransferase that takes a methyl group from *S*-adenosyl-l-methionine (SAM) to coordinate multiple regulatory programmes, including cell growth regulation. The knockout screens provided a path to druggability: co-deletion of MTAP leads to accumulation of MTA; MTA partially cripples PRMT5, putting pressure on the cell viability; PRMT5 inhibitors can leverage this dysregulated metabolic state to push cancer cells over the edge.

But PRMT5 is also pan-essential by CRISPR knockout, and a first round of PRMT5 inhibitors has faced toxicity challenges — possibly because they act independently of MTA. The Novartis team proposed in their first PRMT5 paper that inhibitors that preferentially bind the target in the presence of MTA would be the best way forward. Although these have been harder to find, Mirati recently reported that its MRTX1719 acts by stabilizing the interaction between PRMT5 and MTA. A phase I trial of this drug is ongoing.

Amgen, Tango and Prelude also have PRMT5 inhibitors in the clinic.

Ideaya has advanced an alternative approach to MTAP-depleted cancers, also highlighted by early RNAi knockdown screens. PRMT5's methyl source, SAM, is produced by the enzyme MAT2A. MAT2A inhibitors like IDE397 prevent the production of SAM, starving PRMT5 of its substrate to exploit the MTA imbalance in cancer cells. SAM is used broadly by multiple methyltransferases, however, leading to concerns about the potential off-tissue effects of these drugs.

A trickle or a tsunami?

A few other synthetic lethal targets are up and coming, including Werner syndrome helicase in microsatellite unstable cancers. But the bigger tumour suppressor targets on the drug discovery wish lists remain, as yet, out of reach. "Given our first experience, I'm not convinced there's some tsunami coming," cautions Sellers.

Multi-way synthetic interactions could still open new avenues, he adds. So far, the synthetic lethality community has mostly focused on two-way interactions — knocking out one gene at a time, across cancer cell lines — because of the impracticalities of double- and triple-knockout screens. But tools to screen for more complex interactions are in development.

Sellers, for example, has pivoted to paralogue knockout — using CRISPR to deplete related proteins that provide redundant activity. Knock out just one member of a

paralogue family, and cancer cells can survive. Knock out a few at a time, and lethal interactions can emerge. Last year, he showed, for example, that dual knockout of the phosphatases *DUSP4* and *DUSP6* creates a synthetic lethal interaction in *NRAS*- and in *BRAF*-mutated cancers.

"Paralogues are a first stab at functional redundancy analysis," says Sellers.

As data accumulate, machine learning could identify more complex multi-way synthetic lethality patterns too, adds Aviv Regev, the head of research and early development at Genentech. "This has become a personal obsession of mine," says Regev. "There's still plenty out there that we're not yet leveraging."

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