The promise of synthetic lethality in precision oncology

Kathryn L Chapman, Head of Discovery Biology & **Jürgen Moll** CSO at Tessellate Bio explore alternative approaches to synthetic lethality to target cancer cells that use alternative lengthening of telomeres.

Synthetic lethality and targeted drugs are a clinically established approach

Synthetic lethality (SL) is an established, clinical and commercial approach to targeting and treating cancer. Originally, SL was based upon the premise that cancer cells have a loss of function (LoF) of a tumour suppressor gene, e.g. by mutation or deletion, and concurrent inhibition by pharmacological inhibitors of a second gene results in loss of viability within that cell. Healthy cells, however, display full expression of the first gene and therefore the pharmacological inhibitor does not affect their viability. This approach offers safety benefit to the patient over established chemotherapies through targeting mutated cancer cells only.

The most established SL mechanism in the clinic is poly ADP-ribose polymerase (PARP) inhibitors which are used for the treatment of BRCA1/2 LoF and 'BRCAness' cancers, which is defined as a deficiency in homologous recombination repair within a cell that mimics BRCA1 or BRCA2 LoF phenotype and defects in replication fork protection. In healthy cells, BRCA1/2 play a pivotal role in homologous recombination repair of DNA double strand breaks. In their absence, cells are unable to effectively repair double-strand DNA breaks (DSBs), which causes an increase in genomic instability and homologous recombination deficiency (HRD), driving the formation and progression of cancer cells. Whilst cancer cells require and can tolerate a certain level of genomic instability, PARP inhibitors disable single strand break repair, increasing DSB's further, exacerbating genomic instability and driving the cells into cell death. Since the first PARP inhibitor was approved in 2014 (Lynparza), many cancer patients have benefited and second-generation molecules, e.g. with increased selectivity for PARP1, are expected to have a better safety profile and preliminary data in the clinic is encouraging.

The success of a PARP inhibitor approval in the BRCA1/2 LoF setting has paved the way for the development of further SL drugs, which have been mainly focused on the HRD patient segment, such as drugs targeting e.g. poly(ADP-ribose) glycohydrolase (PARG) or ubiquitin specific peptidase (USP1). However, HRD patients account for only 15% of total cancer patients, highlighting a huge patient population with an unmet medical need and often without molecular targeted drugs. For these cancer patients beyond HRD, there

are a wealth of SL-based drug development opportunities in DNA damage response (DRR).

Redefining synthetic lethality to treat patients beyond HRD

With increasing use of CRISPR screening and open access of genomic data (e.g. DepMap and Open Targets) to inhibit or inactivate single genes, novel SL targets have been identified which show selectivity within subsets of cell lines. However, elucidating a single gene that predicts SL in a significant patient population remains rather the exception. This has driven the scientific community to redefine SL beyond a single gene interaction.

FANCM as a synthetic lethal target in alternative lengthening of telomeres

One highly promising approach to expanding SL beyond HRD is to focus on cancers that rely on Alternative Lengthening of Telomeres (ALT). Telomeres are long dsDNA consisting of 5'-TTAGGG-3' sequential repeats at the ends of each chromosome, and that protect the genomic DNA from degradation or fusions. Following each cell division, the telomeres on the chromosomes are shortened as the replication machinery cannot duplicate the DNA completely to the end of the strand. Cumulative telomere shortening results in replicative senescence and cell death, and thereby acts as a barrier to unlimited proliferation and tumorigenesis. To overcome this barrier, a substantial proportion of cancers utilise ALT, which is a heterogenous and complex mechanism whereby the cells' telomere length varies including some which are extremely long. Importantly, ALT-dependent cancer cells must deal with and counteract elevated levels of DNA damage, indicative of heightened telomeric replication stress in ALT cells. The deterioration of stalled replication forks to double strand breaks in telomeres is thought to provide a substrate for homology-directed repair, resulting in break-induced telomere synthesis and replication fork collapse at ALT telomeres. This leads to the formation of extrachromosomal circular telomeric DNA derived from C-rich strands (so called C-circles), which are a hallmark of ALT cancers[1] and can be used to identify patients who will benefit from targeting ALT[3]. The fine balance of replicative stress in ALT telomeres can be shifted from a level that is sufficient to trigger telomere elongation to a level that induces cell death by inhibiting fanconi anaemia, complementation group M (FANCM). FANCM is an ATPase and translocase that forms an anchoring complex at ALT telomeres at the site of stalled replication and restricts ALT phenotype. Inhibition of FANCM results in 'taking the brakes off' an ALT checkpoint, exacerbating the ALT phenotype and resulting in cellular death[2,4,5]. This first-in-class target is the best scoring synthetic-lethal target when comparing ALT+ with Telomerase positive (TEL+) cell lines from DepMap CRISPR data and ALT+ cells are selectively killed upon knockdown (Figure 1). This concept has been validated and published by Professor Hilda Pickett (Children's Medical Research Institute, Sydney) and Professor Claus M. Azzalin (Instituto de Medicina Molecular Joao Lobo Antunes, Lisbon). In addition, they have identified two specific regions within FANCM that play a key role in this process, the BLM-TOP3A-RMI (BTR) complex interaction domain and the FANCM ATPase domain.

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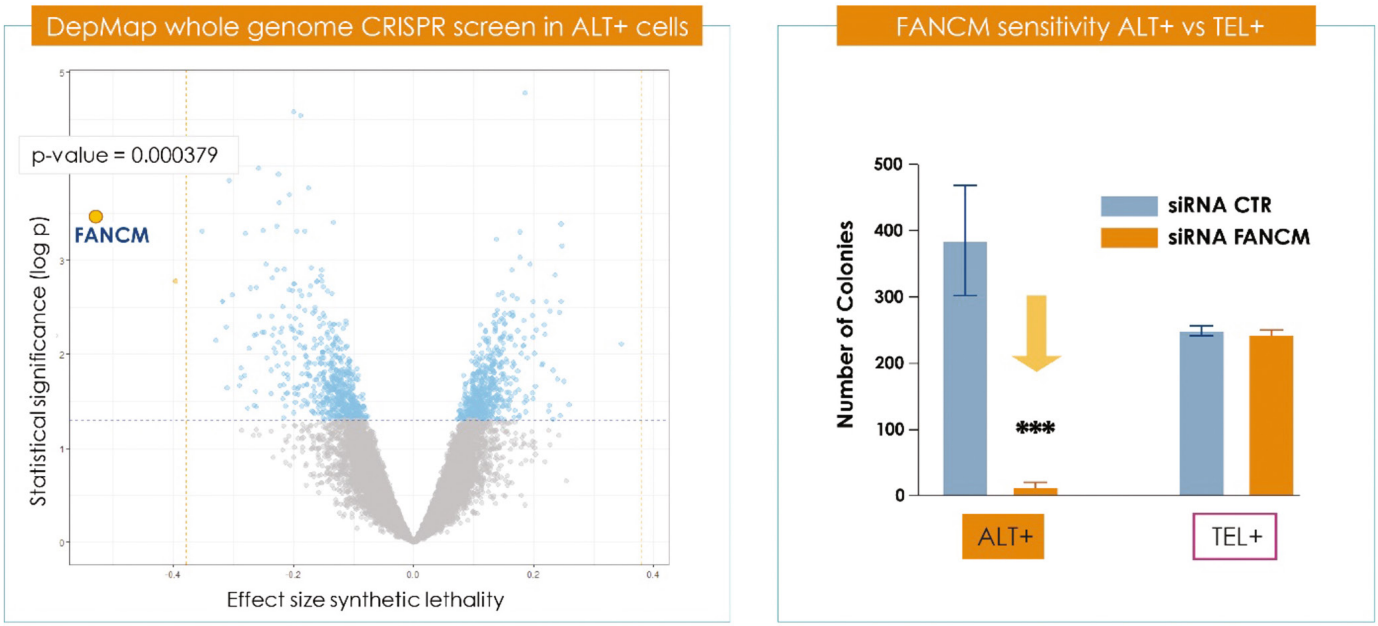
1) Inhibition of FANCM interaction with BTR complex

FANCM contains multiple protein-protein interaction domains including a binding site for the RMI1-RMI2 subcomplex of BTR (Bloom's complex) which consists of BLM (helicase), TOP3A (type 1A topoisomerase), and RMI1/2 (oligonucleotide-binding fold proteins). BTR is thought to play a pivotal role in the stabilisation of stalled forks and alleviates replication stress at ALT telomeres. This has been genetically validated where mutation or removal of the MM2 domain of FANCM which interacts with BTR complex results in SL with ALT+ cell lines.

Figure 1: *DepMap analysis comparing ALT+ with TEL+ cell lines (left). Specific inhibition of colony growth in an ALT+ cell line (right).*



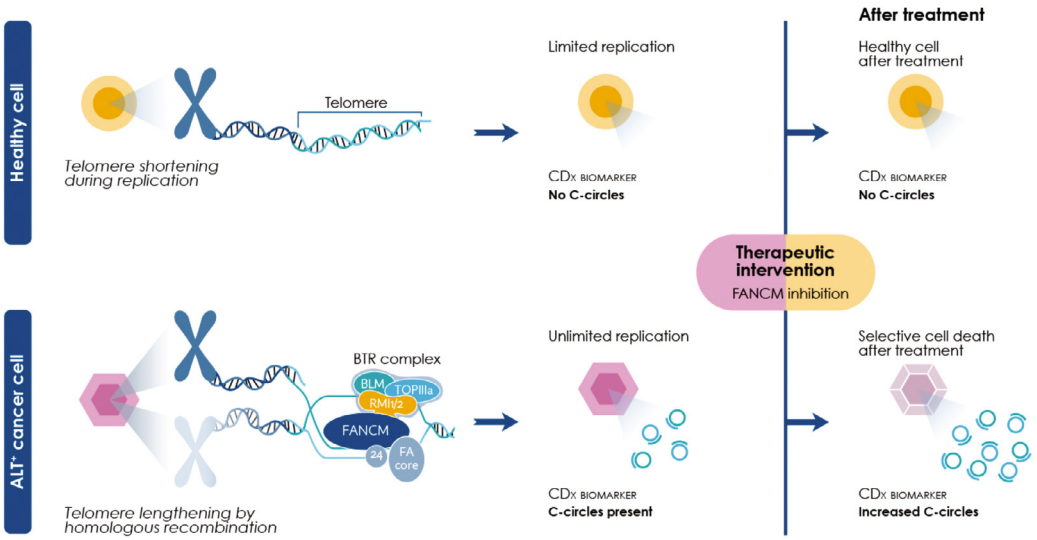


Figure 2: *Synthetic Lethality of FANCM in ALT+ cancers in comparison with healthy cells.*

2) FANCM Translocase and ATPase activity[5]

FANCM uses ATP hydrolysis to power branchpoint translocation, promoting fork reversal at telomeres in ALT+ cells. Mutations within the ATPase domain result in reduced activity. Moreover, when wild-type FANCM was replaced by a mutated form, a SL interaction was observed within ALT+ cell lines.

These unique academic insights are now being built on by Tessellate BIO to identify small molecules targeting FANCM in ALT+ cancers as defined by cells that generate C-Circles (ALTness) with the primary goal of entering the clinic and providing safe therapeutics for patients within high unmet needs such as sarcomas.

Our data suggest that short-term inhibition of FANCM may have a rapid onset cytotoxic effect selectively in ALT-dependent cancer cells. Importantly, data from humans with a biallelic non-functional FANCM mutation as well as FANCM knockout mice point towards a benign safety profile.

Identification of ALT+ patients: a need for a clinically feasible CDx

For a drug to be efficacious and have a positive impact for patients, it is a necessity to establish in parallel a companion diagnostic (CDx) to help determine which patient populations respond to the drug. This is a key challenge which needs to be addressed early in a drug discovery programme to ensure precision oncology success. Since single interactions in cells often do not translate into a patient setting, extensive efforts are required to identify genetic signatures or phenotypes. This poses many research challenges and, despite bioinformatics and AI advancements, can sometime be insurmountable due to the complexity and heterogeneity of pathways and cancers. For ALT, the best predictive biomarker is the generation of C-circles, and through academic and commercial collaboration, a clinical trial assay has been successfully developed. Clinical validation is performed in collaboration with the OMICO initiative in Australia and targets detection of ALT activity in clinical samples from multiple indications, including osteosarcoma patients. This CDx is currently the only clinically appropriate assay for the identification of ALT cancers (Figure 2).

Final comments

Synthetic lethal small molecule inhibitors in BRCA1/2 LoF have shown efficacy and good safety profiles in the clinic and on the market. It is important now to apply these learnings to patients beyond HRD. There are very exciting advancements in SL for the development of new drugs focusing beyond LoF of a single gene, but rather at phenotypes. At Tessellate BIO we are exploring this new target space and further benefits of these drugs for patients will be explored as a therapeutic in both ALT+ and 'ALTness' type of cancers. Concomitant development of a CDx will be critical to a precision oncology approach.

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About the author:

Jürgen Moll is a drug hunter with a proven track record in cancer drug discovery. He has held leadership positions at major pharmaceutical companies including Sanofi, Boehringer Ingelheim, and Pfizer. In his current role as Chief Scientific Officer at Tessellate BIO, Moll is responsible for strategically building and strengthening the company's pipeline of innovative cancer therapies, ensuring that Tessellate BIO's research efforts are focused on the most promising areas of cancer drug development.

About the author:

Katie Chapman leads the Discovery Research activities at Tessellate BIO. She is a skilled *in vitro* pharmacologist with experience and a track record of delivery on diverse drug targets. She has a passion for enabling technologies which help in unlocking challenging proteins and has been successful in grant funding. Chapman has worked in large pharmaceutical companies, CROs, and in drug discovery for universities. She completed her PhD at the University of Leeds on the structure and function of GPCRs.