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Importance of genetic screens in precision oncology

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ABSTRACT

Precision oncology aims to distinguish which patients are eligible for a specific treatment in order to achieve the best possible outcome. In the last few years, genetic screens have shown their potential to find the new targets and drug combinations as well as predictive biomarkers for response and/or resistance to cancer treatment. In this review, we outline how precision oncology is changing over time and describe the different applications of genetic screens. Finally, we present some practical examples that describe the utility and the limitations of genetic screens in precision oncology.

ADVANTAGES AND LIMITATIONS OF PRECISION ONCOLOGY

Precision medicine is defined as administering the right medicine at the right dose at the right time to the right patient. Despite being used in different medical fields, it is most commonly applied to oncology. Prasad and Gale analysed the use of precision oncology in the biomedical literature by classifying 50 articles over three time intervals.[1] Between 2005 and 2010, the term precision oncology was mainly used to describe the use of targeted therapies such as epidermal growth factor receptor (EGFR) inhibitors or BCR/ABL1 inhibitors like gefitinib/erlotinib and imatinib. In 2013, precision oncology was used to describe the use of therapies based on specific biomarkers, like the administration of crizotinib for patients with lung cancer whose tumour had an EML-ALK rearrangement. By 2016, the definition of precision oncology referred to the use of next generation sequencing to guide the treatment choice. Regardless of this evolution in terminology, precision oncology has always been referred to the use of a certain drug based on molecular aberrations carried by the tumour. Historically, treatment decisions were made based on the histology of the tumour while nowadays are also based on mutation analysis.[2] Genomic projects, like The Cancer Genome Atlas,[3] have identified the main drivers of most solid and haematological malignancies, thus improving the diagnostic and the classification process, as well as the therapeutic approaches (table 1).

The observation that many genomic aberrations are recurrent across multiple cancer types has led to the design of both basket and umbrella trials. The inhibition of HER2 in breast, gastric and colon cancer is, in this context, a successful example[4–6] Molecular profiling of tumours has clearly shown to be beneficial for treatment decision-making. Indeed, several trials have shown that an individualised approach based on the molecular profiling of the tumour, can result in a better progression-free survival (PFS) when compared with the PFS of the previous regimen received by the patients.[7–9] This benefit has been observed in adult as well as in paediatric cohorts.[10 11] Nevertheless, in spite of these encouraging results, none of these trials were randomised. The molecularly targeted therapy based on tumour molecular profiling versus conventional therapy for advanced cancer (SHIVA) trial, which is the only completed randomised phase II basket trial for precision oncology, showed that the use of molecularly targeted agents outside their indications did not improve the clinical outcome of heavily pretreated patients as compared with the treatment according to clinician's choice.[12] However, the effect of targeted anticancer drugs outside their approved indications is still under evaluation in big international precision oncology initiatives like the Target Agent and Profiling Utilization Registry, the Molecular Analysis of Therapy Choice and the Secured Access to Innovative Therapies Programme.[13–15]

There are several other limitations that interfere with a broader success of precision oncology. For example, from the DNA-sequencing data, we have learnt that fewer than 10% of patients with advanced cancer have a simple actionable mutation.[16 17] Moreover, although some targets might appear to be interesting, the activity of drugs that inhibit them can be limited. Davis *et al*[18] clearly showed that most drugs that entered the market in the period between 2009 and 2013 did not show a benefit in overall survival (OS) or in quality of life after 3 years follow-up.

Table 1 List of current approved molecular-driven treatments

Disease	Gene	Drug
CML	ABL	Imatinib
Resistant CML	mutant ABL	Dasatinib
HES	PDGFRa	Imatinib
CMML	PDGFRb	Imatinib
Myelofibrosis	JAK2	Ruxolitinib
AML	FLT3	Quizartinib
Gastrointestinal stromal tumour	KIT	Imatinib
Lung cancer	EGFR	Erlotinib, Gefitinib
Kidney cancer	VEGFR	Sunitinib, Sorafenib
Breast cancer	HER2	Trastuzumab/Pertuzumab
Lung cancer	ALK	Crizotinib
Melanoma	BRAF	Vemurafenib/Trametinib
Ovarian cancer	BRCA	Olaparib
Gastric cancer	HER2	Trastuzumab

AML, acute myeloid leukaemia; CML, chronic myeloid leukaemia; CMML, chronic myelomnocytic leukaemia; HES, hypereosinophilic syndrome.

Furthermore, we know from basket trials that the histological context can be an important determinant of response to targeted agents.[19–21] This means that we cannot completely ignore the histology of the tumour nor the molecular context in which the mutation has been detected. Second, even if the tumour depends on that aberration, meaning that we can block the tumour growth specifically, mechanisms of acquired resistance might still emerge,[22 23] which again may be tissue specific. Finally, the use of gene expression profiling is not yet a clinical standard, drugs targeting a specific aberration might not be effective, combinatorial approaches might be toxic to the patient and tumours are heterogeneous in space and time.

One way to overcome these limitations is to further dissect the biology of cancer aberrations by using synthetic lethal interaction approaches. In this review, we will describe the different approaches using functional genetic screens and their applications in precision medicine. We will summarise the current evidence showing that synthetic lethality can help to understand some of the limitations and lead to improve the success rate of precision oncology. Most importantly, we will also highlight the limitations of such approaches and the difficulties to translate preclinical findings into clinical practice.

SYNTHETIC LETHALITY AND GENETIC SCREENS

The understanding of cancer biology as well as advances in precision oncology heavily relies on preclinical research. Approaches that exploit synthetic lethality can help understand cancer vulnerabilities, mechanisms of primary and secondary resistance to treatment, the role of specific aberrations (mutations, amplifications, gene silencing) and their dependence on the tissue context.

Synthetic lethality is described as a phenomenon where a deficiency of two genes leads to cell death, but the deficiency of either one does not impair cell viability[24 25] (figure 1). The deficiency can be due to a loss-of-function mutation, epigenetic silencing or pharmacological inhibition of the protein.

The first clinically relevant example of a synthetic lethal interaction in cancer was the one between mutations in the genes encoding BRCA 1 and 2 and inhibition of the enzymes of the Poly (ADP-ribose) polymerase (PARP) family of enzymes. Its discovery came from the observation that PARP null mice are viable, but rely heavily on BRCA-mediated homologous recombination to repair the DNA damage. This led to the hypothesis that the inverse was true as well, meaning that BRCA deficient cells would depend more on PARP. This hypothesis-driven approach of predicting a synthetic lethal interaction turned out to be true. Actually, tumours that harbour BRCA1 or 2 loss-of-function mutations are especially sensitive to PARP inhibitors.[26 27] These results were followed by the investigation and approval of PARP inhibitors for the treatment of patients with a germline BRCA1/2 mutated ovarian and breast cancer.

This interaction is an example of genotype-specific synthetic lethality, where a mutation in a tumour cell causes dependency on another pathway in order to maintain viability. When the compensatory pathway is inhibited, either genetically or pharmacologically, viability is

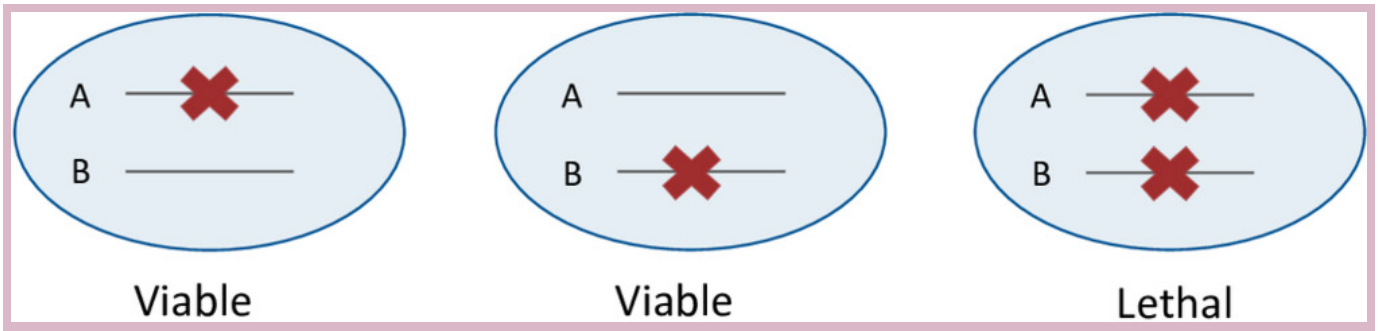

Figure 1 Synthetic lethality is defined as a phenomenon where a loss of either gene A or gene B is tolerated by the cell, but the loss of A and B is lethal.

impaired. Those types of interactions are of great importance for cancer treatment, since they offer selective targeting of mutated cancer cells over normal cells. In addition, drug-specific synthetic lethality can be exploited to identify rational combinational treatment. In this case, a combination of two drugs can be more effective than each of the drugs alone.[28]

A valuable tool to discover novel synthetic lethal interactions are functional genetic screens. Screens can offer an unbiased insight into complex biological processes, identify cancer vulnerabilities and biomarkers of resistance and sensitivity to the specific treatment. A genetic screen can be performed only with the help of techniques that allow large-scale gene perturbations, for example, RNA interference (RNAi), clustered regularly interspaced palindromic repeats (CRISPR) or transposons.

The first breakthrough technology that allowed for a systematic screening of multiple thousands of genes was RNAi using small interfering RNA to degrade selected transcripts.[29 30] Although revolutionary at the time, the biggest drawback of this technology was the transient and unstable silencing. A significant improvement was achieved by the introduction of the short hairpin RNA (shRNA) technologies, that was characterised by a more stable and durable knockdown, and by the possibility to pool the shRNAs which simplified the screening procedure.[31–33] Despite the success of shRNA-based genetic screens, also this technology had its drawbacks, mainly off-target effects.[34]

Lastly, CRISPR technology got adapted for precise genome editing in mammalian cells. The CRISPR-Cas9 system, which was originally discovered in bacteria as a form of primitive immune system to protect against viral infections,[35] consists of two parts: an endonuclease Cas9 and a single-guide RNA (sgRNA) molecule. When they are both present in the cell, they form a complex, which is guided to the target genomic DNA location by the sgRNA. Next, Cas9 cuts the DNA resulting in a double strand break. As the cells try to repair the double strand break, small insertions and deletions (indels) can arise at the break site. These indels can lead to loss-of-function mutation in the targeted gene. Therefore, the knockout of the gene is a direct consequence of error-prone DNA-repair mechanisms and not due to the double strand break. By using a variety of sgRNAs that target any gene in the genome, we can create knockout mutations in every gene. In addition, the Cas9 protein has been modified, allowing to also perform transcriptional silencing known as CRISPR interference (CRISPRi) or activation (CRISPRa) screens.[36 37] The main limitations of CRISPR screens include big difference in efficiency between sgRNAs leading to variable editing and mismatch tolerance, also producing some off-target effects.[38] In comparison to shRNA, CRISPR shows better on-target activity and is nowadays widely used for screening.[39]

In addition to RNAi and CRISPR, transposons can also be used to disrupt genes. Transposons have been modified to allow the performance of insertional mutagenesis screens. In this type of screens, the enzyme transposase randomly cuts and pastes the transposon sequences across the genome, thus disrupting genes. The most widely used transposon systems are PiggyBac and Sleeping Beauty systems.[40] Even if this system is less effective for studying recessive phenotypes, the use of a haploid cell line HAP1, that contains only one copy of each gene,[41] can circumvent this limitation. Overview of their molecular mechanisms is depicted in figure 2.

Independent of the technology used, genetic screens can answer a variety of biological questions by changing the setup and read-out of the screen. To identify novel synthetic lethal interactions with a certain gene alteration we can perform synthetic lethal or ‘drop-out’ screens.

We can make use of isogenic cell line pairs or large panels of cell lines where one group carries the mutation while the other one does not.[42] In addition to loss-of-function mutations, those aberrations also include gain-of-function mutations, gene amplifications, overexpression, gene signatures and epigenetic changes.[43–46] Moreover, we can use drop-out screens to find genes whose loss can confer sensitivity to a certain drug treatment, thus uncovering mechanisms of primary resistance. The potential clinical utility of those screens lies in discovering new combinational treatment strategies that overcome primary resistance[47] or identification of predictive biomarkers of response that can be used to select the group of patients that is most likely to benefit from that treatment.[48] In contrast to drop-out screens, positive selection screens or ‘enrichment screens’ can be used to identify mechanisms of secondary resistance to a certain drug and identify which genes upon loss confer resistance to the specific treatment (figure 3).[49] Besides genetic screens, other approaches can help in uncovering synthetic lethal interactions and finding new combinations of treatment, for example, drug screens and computational approaches.[50 51]

One of the major advantages of functional genetic screens is that they can be applied to any biological process. However, they do require an extensive in vitro and in vivo validation, as well as clinical trials before a novel finding can be translated into clinical practice.

APPLYING GENETIC SCREENS TO PRECISION ONCOLOGY

Targeted therapies like Braf and MEK inhibitors revolutionised the treatment of BRAF (V600E) metastatic melanoma and have been shown to be active in other malignancies as well.[52] Paradoxically, although the same point mutation occurs in about 8%–10% of colorectal cancer (CRCs), these tumours do not respond to the BRAF (V600E) inhibitor (vemurafenib) when used as single agent.[20] The mechanism underlying this unresponsiveness has been elegantly uncovered using a synthetic lethal screen. In particular, Prahallad *et al*[47] performed a drop-out screen in BRAF (V600E) CRC cell lines looking for kinases that could sensitise cells to vemurafenib. With this approach, they discovered a feedback reactivation of

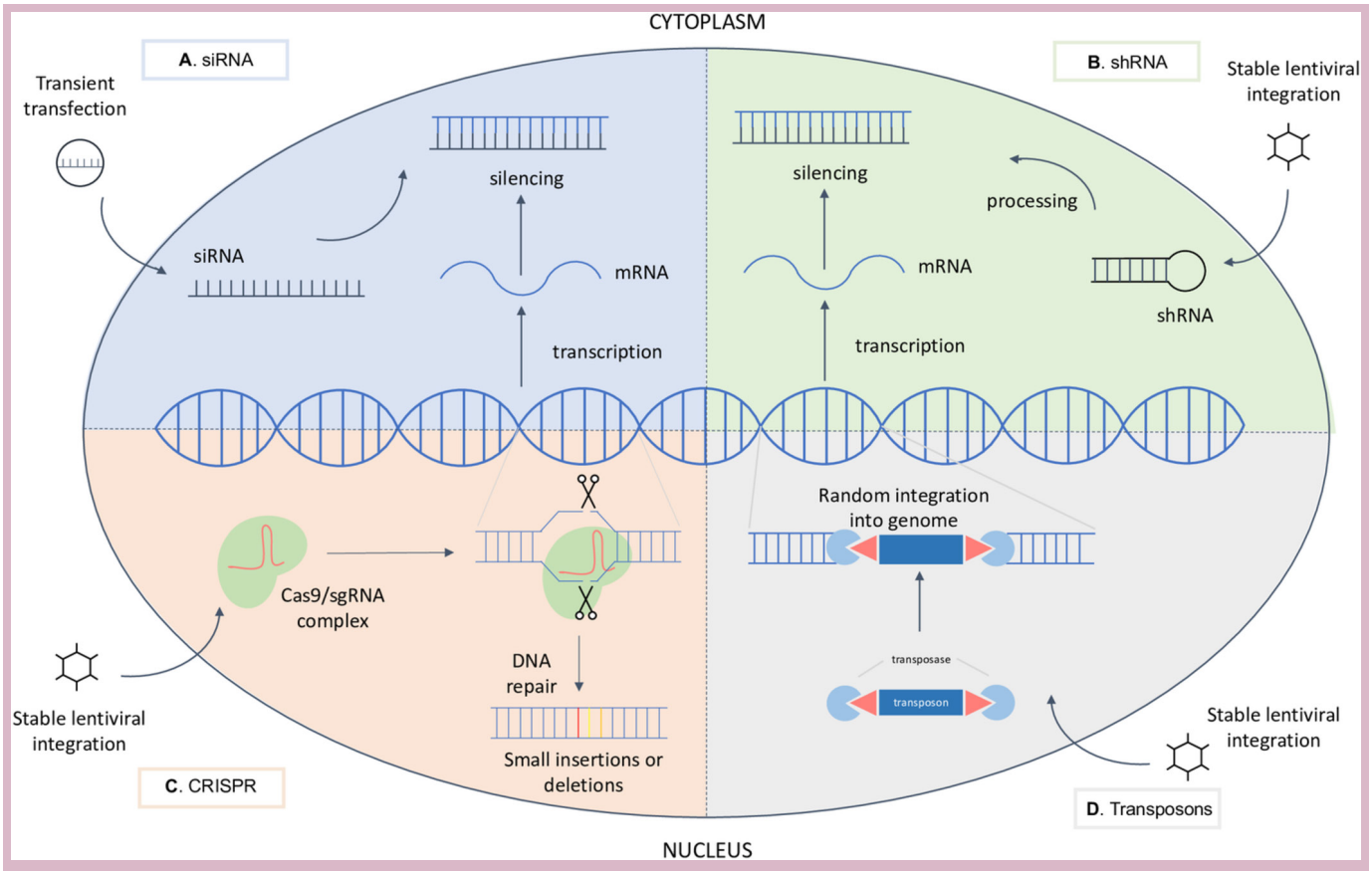

Figure 2 Molecular mechanisms of genetic perturbations (A) siRNA molecule is transiently transfected into the cell, where it binds and thus silences the target mRNA molecule. (B) shRNA is introduced in the cell trough viral infection. Upon stable integration into the genomic DNA, it is processed into an siRNA that silences the target mRNA. (C) CRISPR system is generally introduced in the cell trough viral infection. Upon stable integration into the genomic DNA, both Cas9 and the sgRNA are expressed. The endonuclease Cas9 and a sgRNA form, therefore, a complex causing a double-strand DNA break at a target location. Mistakes during DNA repair can cause mutations at the break site. (D) Upon viral infection, transposon and transposase enzyme integrate into the genomic DNA and lead to random insertions in the genome, thus disrupting genes. CRISPR, clustered regularly interspaced palindromic repeats;mRNA, messenger RNA; sgRNA, single guide RNA; siRNA, small interfering RNA ; shRNA, short hairpin RNA.

EGFR on BRAF inhibition in BRAF (V600E) CRC cells as the driver of unresponsiveness to such treatment. These results led to the hypothesis that BRAF inhibitors need to be administered in combination with EGFR inhibitors to effectively kill these tumours. This hypothesis has been extensively validated both in vitro and in vivo. Most importantly, the results of this preclinical work have led to the design of several clinical trials, where BRAF (V600E) metastatic CRC (mCRC) patients have been treated either with a dual combination of Braf and EGFR inhibitors or a triple combination of a BRAF, EGFR and MEK or PI3K inhibitors.[53–55] The results of these studies have clearly shown that the dual and triple blockade improved response rates and outcome as compared with BRAF inhibition alone. Simultaneously, a phase 1b study and a phase II study evaluated the combination of targeted therapies with chemotherapy in a three-drug regimen of vemurafenib, cetuximab and irinotecan.[56 57] The addition of a BRAF inhibitor showed an increase of response rate and PFS when compared with the standard combination of anti-EGFR treatment and chemotherapy. Finally, the BEACON CRC[58] is the first phase III trial that compares the triple combination (BRAF, MEK and EGFR inhibitors) versus dual combination (BRAF and EGFR inhibitor) versus a control arm (EGFR inhibitor and chemotherapy) as second or third-line treatment for BRAF (V600E) mCRC patients. Recently, an update of the safety lead of the study confirmed the triple combination to be safe. Clinical activity was characterised by 48% of overall response rate and efficacy by an improved PFS and OS as compared with standard of care.[59] Based on these data, the US Food and Drug Administration (FDA) has granted breakthrough therapy designation of the triple combination as second or third-line treatment to patients affected by BRAF (V600E) mCRC.[60] Nevertheless, the responses and the outcome benefit were not observed in the entire cohort of patients enrolled and secondary resistance occurred. Recently, CRCs have been classified into four distinct consensus molecular subtypes (CMS): CMS1 characterised by microsatellite instability and immune infiltration (14%), CMS2, known as canonical with Wingless/int1 (WNT) and myelocytomatosis oncogene (MYC)

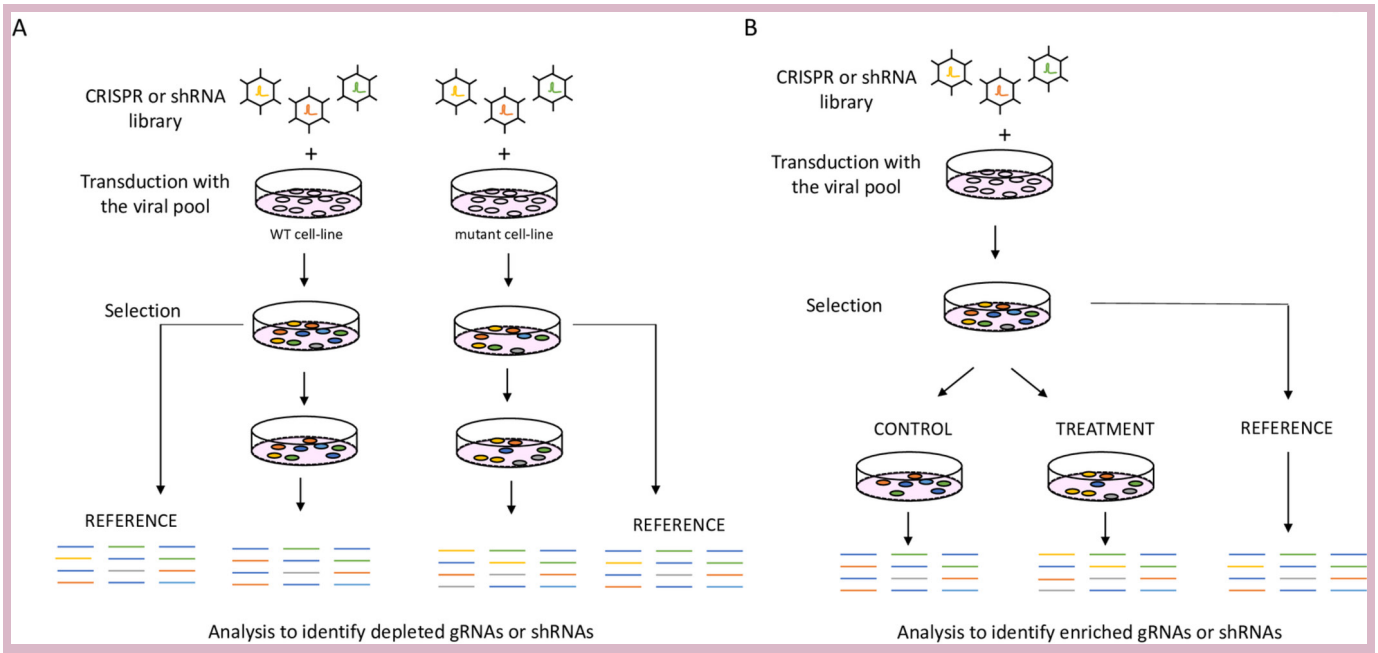

Figure 3 Functional genetic screens (A) Drop-out screen, an isogenic cell line pair infected with the CRISPR library, selected and cultured alongside. Next, gRNA or shRNA barcodes are recovered, and the abundance of barcodes between the cell lines and the reference samples is compared. (B) Resistance screen where a cell line that is sensitive to the test treatment is used. After infection and selection, the cell population is split to treated and control arm. Next, barcodes are recovered from remaining cells and compared between reference sample, treated and untreated samples. CRISPR, clustered regularly interspaced palindromic repeats; gRNA, guide RNA; shRNA, short hairpin RNA; WT, wild type.

signalling activation (37%), CMS3 harbouring metabolic dysregulation (13%) and CMS4 with mesenchymal characteristics (23%).[61] Notably, BRAF (V600E) mutations are present across all four different CRC molecular subtypes.

In addition, Oddo *et al*[62] described that mutations leading to reactivation of the MAPK pathway represent the major mechanisms of secondary resistance. Therefore, tumour heterogeneity and clonal evolution could partially explain the heterogeneous response to both, the dual and triple blockade, observed in those clinical trials.

Prior to the CMS classification[61] two independent groups found BRAF (V600E) colon cancers (CCs) to be characterised by a distinct gene expression profile when compared with KRAS-mutant and KRAS-BRAF double wild type (WT2) CCs. These tumours were defined as BRAF-mutant like by a transcriptional signature.[63 64] To note, this gene signature identified BRAF (V600E) CCs and subsets of KRAS-mutant (30%) and WT2 (13%) CCs. The relevance of this transcriptional signature relies on the fact that the BRAF-mutant like tumours harbour similar poor prognosis regardless of the presence of BRAF (V600E) mutation.[63 64] The signature has been further validated in a larger cohort of BRAF (V600E) CC patients[65] and its biological implication has been investigated by using a synthetic lethal screen. By performing a drop-out screen, Vecchione *et al*[46] identified RANBP2 to be synthetic lethal with the BRAF-like signature in CC cell lines. Further investigation of the function of this protein in CC cell lines led to the hypothesis that BRAF-like CC cells lines could be more vulnerable to antimitotic agents. This concept was extensively validated in vitro and in vivo models and is currently under investigation in the Motricolor consortium.[66] Immediately after this finding, a prospective multicentre phase II clinical study started, where chemorefractory BRAF (V600E) mCRC patients were treated with vinorelbine[67] A total of 20 patients were enrolled. Unfortunately, no responses were observed, with only one stable disease reported. In contrast, Masuishi *et al*[68] reported tumour shrinkage in four BRAF (V600E) mCRC patients treated with eribulin as third and fifth line of treatment. Based on the results of these four cases, the BRAVERY study is now investigating the activity of eribulin as second line treatment in BRAF (V600E) mCRC.[69]The hypothesis generated from Vecchione *et al*[46] is still far from being applicable, highlighting how complex is to translate preclinical findings into clinical practice.

An example of enrichment screen performed by Berns *et al*[70] identified loss of phosphatase and tensin homolog (PTEN) as well as activating mutations in PIK3CA to induce resistance to trastuzumab in HER2 amplified breast cancer cell lines. These findings were further validated in a small cohort of HER2 amplified patients with breast cancer where both PIK3CA mutations and low PTEN expression correlated with poor prognosis after trastuzumab treatment. Moreover, similar preclinical results were obtained by other independent groups and with different HER2 inhibitors supporting the relevance of these discovery.[71 72] Based on these results, the role of PIK3CA mutations and loss of PTEN in HER2 amplified

patients with breast cancer treated with anti-HER2 antibody has been investigated, both in neoadjuvant and metastatic setting. The combined analysis of the GeparQuattro, GeparQuinto and GeparSixto trials showed PIK3CA mutant HER2 amplified breast tumours to have reduced pathological complete response (pCR) when compared with PIK3CA WT tumours[73] Similarly, Majewski *et al*[74] found lower pCR rate on trastuzumab and lapatinib monotherapy or in combination in PIK3CA mutant HER2 amplified patients with early breast cancer versus PIK3CA WT tumours. Additionally, PIK3CA mutated HER2 positive patients with metastatic breast cancer treated with capecitabine and lapatinib showed lower PFS compared with PIK3CA WT HER2 positive patients.[75] To increase statistical power, Loibl *et al*[76] performed a pooled analysis including approximately 1000 HER2 amplified patients with breast cancer whose PIK3CA status was known and were treated with anti-HER2 antibody. They confirmed PIK3CA mutant tumours to have lower chances to achieve a pCR when treated with HER2 blockade. Interestingly, this is especially significant in hormone receptor (HR) positive group as compared with the HR negative group. Importantly, none of the studies observed differences in outcome between PIK3CA mutant and PIK3CA WT tumours. Finally, the biomarker analysis of the NeoSphere study[77] found only PIK3CA mutations in exon 9 to be associated with resistance to HER2 blockade. Overall, even if the results of the preclinical genetic screen are clear and robust, PIK3CA mutations are not used as predictive biomarker yet. Furthermore, other clinical variables might be considered to better understand its role in predicting resistance to HER2 blockade in breast cancer.

More recently, a group of researchers discovered loss of E-Cadherin, a frequently mutated gene in breast (>13%) and gastric cancer (>14%), to be synthetic lethal with ROS1 inhibitors, such as crizotinib. Authors used lobular breast cancer models for a perturbation screen with a focused library and a compound screen with drugs that are either approved in the clinic or that are being tested. As a result, E-cadherin loss became a potential biomarker for treatment with ROS1 inhibitors in a significant subset of patients with poor prognosis.[78] Currently, a phase II clinical trial is testing crizotinib as a monotherapy in diffuse gastric cancer as well as crizotinib in combination with fulvestrant in lobular breast cancer.[79]

Another example of frequent genetic alteration that cannot be selectively targeted yet is KRAS mutations. The development of MEK inhibitors became a promising option for the treatment of these aggressive tumours. Lamentably, KRAS mutant tumours harbour different mechanisms of primary resistance to those inhibitors. In an attempt to identify genes whose loss could synergise with MEK inhibitors in KRAS mutant cancer cells, Corcoran *et al* performed a loss-of-function genetic screen. They identified Bcl-XL, a member of BH-3 antiapoptotic family, to be synthetic lethal with MEK inhibitors in KRAS mutant cell lines. These data were further validated in preclinical models by using a Bcl-XL inhibitor (navitoclax).[80] At present, a clinical trial is recruiting patients with advanced or metastatic solid tumours to test MEK inhibitor (trametinib) in combination with navitoclax.[81]

In the last few years, checkpoint inhibitors have shown encouraging results that have changed the therapeutic approach of certain tumours, like non-small cell lung cancer, melanoma and microsatellite instable mCRC.[82–84] In spite of this success, the efficacy and responsiveness to anti PD1, PD-L1 and CTLA-4 varies among different tumour types and across individual patients. Therefore, establishment of predictive biomarkers for checkpoint blockades as well as identification of novel targets for cancer immunotherapy are key to maximise therapeutic benefits. In this context, the use of genetic screens could be of great support. For example, by using a pooled loss-of-function in vivo genetic CRISPR-Cas9 screen to unravel genes responsible for sensitivity and resistance, Manguso *et al* demonstrated that loss of PTPN2 in cancer cells enhances interferon-γ-mediated effects on antigen presentation and growth suppression, thus increasing the efficacy of immunotherapy in a mouse transplantable tumour model.[85] Similarly, another group performed an enrichment genome-scale CRISPR/Cas9 screen in coculture with activated cytotoxic CD8 +T-lymphocytes seeking for genes whose loss evoke resistance to adaptive immune response. The authors identified the expression of five negative regulators of the MAPK pathway as responsible for resistance to immunotherapy.[86] On the opposite, loss of genes belonging to the SWI/SNF complex, the nuclear factor κB (NF-κB) pathway and metabolic pathway were shown to confer sensitivity to immunotherapy in a mouse melanoma model. Patel *et al*[87] confirmed that loss of genes with a role in antigen presentation pathway as well as in interferon-γ signalling are responsible for immunotherapy resistance. Among the validated genes, they identified that loss of APLNR reduces the efficacy of adoptive cell transfer and checkpoint blockade by interacting with JAK1, thus, modulating interferon-γ responses. Finally, Mezzadra *et al*[88] used an haploid genetic screen to seek for regulators of PD-L1 protein. They identified CMTM4 and CMTM6 as new potential target to block the PD-1 pathway. Altogether, these data highlight the importance of genetic screens to unveil mechanisms of responsiveness to immunotherapy as well as new potential targets to exploit therapeutically. Nevertheless, none of those results have been validated in the clinic yet. A schematic overview of the preclinical findings and the clinical studies reported above is depicted in table 2.

FUTURE DIRECTIONS

Precision oncology is based on molecular profile of cancer cells. Defining genetic alterations helps to establish a precise molecular diagnosis of the tumour and to predict the course of the disease. Moreover, it allows the administration of a tailored therapy in accordance to the genomic aberrations carried by that individual tumour.

Table 2 From bench to the bedside

Preclinical findings	Clinical trials	Clinical practice changing
EGFR loss is synthetic lethal with BRAF (V600E) in CRC in vivo and in vitro models[47]	53–59	FDA breakthrough therapy designation[60]
BRAF-like CCs are vulnerable to antimitotic agents[46]	66–68	Controversial data. Waiting for further studies
PTEN loss and PIK3CA mutations confer resistance to trastuzumab in HER2 amplified breast cancer cell lines[70]	73–77	Not yet
E-cadherin loss is synthetic lethal with ROS1 inhibitors in lobular breast cancer preclinical models[78]	79	Trial not yet recruiting
Loss of BCL-XL is synthetic lethal with MEK inhibition in KRAS mutant preclinical models[80]	81	Trial ongoing
Loss of PTPN2 synergises with immunotherapy in mouse transplantable tumour models[85]	No trials ongoing nor retrospective analysis of already closed trials	Not yet
Identification of biomarkers of response and resistance to immunotherapy in a mouse melanoma model[86 87]	No trials ongoing nor retrospective analysis of already closed trials	Not yet
Identification of novel targets for immunotherapy[88]	No trials ongoing nor retrospective analysis of already closed trials	Not yet

Depicts preclinical findings followed by clinical trials and clinical practice implementation.
CCs, colon cancers; CRC, colorectal cancer; EGFR, epidermal growth factor receptor; FDA, Food and Drug Administration.

The development of targeted therapies requires several years of intense multidisciplinary effort, from understanding the cancer biology to testing a new drug in a phase III study. Nevertheless, large phase III clinical trials are often not feasible for rare tumour subtypes. In this context, a possible solution are basket trials, which can accelerate the translation into clinical practice. Moreover, several limitations need to be considered during this complex process, like unpredicted toxicity of combinatorial treatments, tumour evolution, cancer heterogeneity, context dependency and the tumour microenvironment. In addition, due to the ever increasing number of FDA-approved cancer drugs, the number of possible drug combinations increases exponentially. This poses a conundrum that can only be solved by upfront selection of the most potent combinations. We have argued here that genetic screens can be a useful tool to identify such powerful drug combinations. A second potential clinical use regards the notion that not all patients treated with a specific drug will benefit from it. As we have discussed as well, genetic screens can help to identify biomarkers of response or resistance.

After almost two decades from the introduction of the RNAi technology in human cancer cells, we are starting to witness the benefits of the use of genetic screens. As a result, we see new therapies being implemented for some malignancies that were untreatable before. Indeed, some clinical trials are finding strong correlation in what has been described in vitro.[47] In addition, organoids and in vivo screens are now being exploited as techniques to study the complex interplay between the tumour and its stroma. Although in vivo screens are technically a huge challenge, they have become a valuable tool, especially when looking for targets that are related with the immune system.

The technology that allows genome-wide screens has become easily available, cheaper and relatively simple to implement. As a consequence, there has been an exponential increase in the number of screens performed. Even though there are indications that functional genetic screens can play a role in clinically relevant discoveries, there are a lot of hurdles to deal with, when translating the preclinical observation from a genetic screen into clinical practice.

First, genetic screens are often long and complicated. It should also be remembered that complete removal of a protein from a cell is not necessarily the same as pharmacological inhibition of the protein, as proteins can also have scaffolding functions. Thus, nor CRISPR or shRNA technologies can simulate drug inhibition. On the one hand, shRNAs are prone to off-target effects. On the other hand, CRISPR screens have less off-target effects, however, drugs seldom inhibit a protein for the full 100%, which is the result of a CRISPR knockout. Moreover, even if a genetic screen unveils a new target, the development of small molecule inhibitors needed to clinically validate can often take years. Therefore, a great number of genetic screens with potential clinical utility still remain to be proven relevant for the patients.

Second, to overcome limitations like context dependency, heterogeneity and tumour evolution, the use of a comprehensive and integrated analysis can be of great help. Combining genetic approaches with cell line analysis and patient data, when available, could help to overcome these problems in order to focus on clinical relevant targets.[89]

As a result, we need to design smarter and better screens, to maximise the outcomes while minimising the costs. Since screens can be adapted to answer a wide variety of questions, we can use them to investigate complex biological processes. We can use different reporter systems for phenotype selection,[90] or knock-in of a selection marker to a target locus.[91] Now, we can design screens that are not focused only on cell death or proliferation. For example, a flow cytometry-based read-out allows separation of the population of cells based on any protein for which an antibody is available. Currently, CRISPR technology offers a diverse toolkit to modify gene expression. In addition, CRISPRa and CRISPRi, introduction of diverse point mutations or epigenetic reprogramming is possible. Subsequently, it is expected that screens adopting these technologies will offer novel insights into the complex biology of the cancer cell in the near future.

Another important aspect that applies to precision oncology is that the phenotype and behaviour of a certain tumour might be the consequence of the activity of multiple genes. For example, it may not be the aberration in gene X that plays a role in that specific tumour context, but rather the combination with other gene aberrations. To better model this, we need to develop systems that allow perturbations of more than one gene at the time. In that respect, there have been significant improvements in the last years to develop screens that allow screening for interactions, both with shRNAs and CRISPR.[92–94] Additionally, a dual system that combines activation of transcription with knockout has recently been developed, which can further expand our understanding of genetic interactions.[95] Furthermore, we can couple pooled genetic screens with single cell RNA sequencing, for example, Perturb-seq, which allows immediate transcriptional profiling of genetically diverse populations.[96–98]

In conclusion, genetic screens have already shown to be a relevant tool to find new therapeutic options and to predict treatment response. Nevertheless, it is an early technology that we are still improving. Therefore, optimising and integrating this technology with other analysis would potentially bring us to the new era of precision oncology.

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