Are CRISPR Screens Providing the Next Generation of Therapeutic Targets?

Francisca Vazquez[1] and William R. Sellers[1,2,3]

ABSTRACT

CRISPR screens combined with molecular and genetic profiling of large panels of cell lines are helping to systematically identify cancer vulnerabilities. These large-scale screens, together with focused *in vivo* and isogenic cell line screens, have identified a growing number of promising targets and led directly to numerous target-specific drug discovery programs, several of which have reached clinical testing. However, systematic loss-of-function studies are still in their early stages. Genetic redundancy, the limitation of cell line models for many cancer types, and the difficulty of conducting complex *in vitro* and *in vivo* screens remain opportunities for discovery. We expect that over the next few years, efforts like the Cancer Dependency Map along with more focused screens will play a significant role in the creation of a roadmap of oncology therapeutic targets.

Introduction

The success of precision cancer medicine hinges on our ability to find and therapeutically target specific vulnerabilities in patient tumors. Ideally, we will identify all target vulnerabilities in human cancers, the combination of such targets that sustain cancer viability, and develop drugs to inhibit each of them. Finally, we will need non–cross-resistant therapeutic combinations to overcome the underlying subclonal heterogeneity found in human tumors. We are still far from this goal because we do not fully understand cancer vulnerabilities and we lack drugs to target the majority of identified vulnerabilities. Two advances now make possible more robust progress in identifying all of the cancer vulnerabilities.

First, the availability and characterization of large panels of cancer cell models that, while still incomplete, can begin to model the diversity of human cancer. Here, the Cancer Cell Line Encyclopedia has markedly changed the ability to profile therapeutic activity across large sets of highly characterized cell lines (1–3). This collection is now annotated with datasets encompassing more than 1,700 cell lines (available at depmap.org). Recent advances in model generation should make it possible to greatly expand this diversity. The second revolution is the ability to induce gene-specific loss of function in the absence of small-molecule inhibitors. Moreover, with the emergence of genome-scale CRISPR screens, it is now possible to alter the function of each gene efficiently in a pooled format. CRISPR screens are now being routinely performed both *in vitro* in large panels of cell lines or isogenic cell line pairs and *in vivo* in syngeneic mouse models (4–6). These approaches have identified therapeutic targets that are being prosecuted in drug discovery programs or where new inhibitors are in clinical development (**Fig. 1**). Perhaps equally important is the ability to now determine the broader validity or invalidity of specific therapeutic hypotheses allowing therapeutic development programs to focus on targets having a higher probability of success.

Drug Discovery Impact

Targets for which therapeutics are now in clinical trials

Therapeutics for three targets that were entirely discovered using RNAi or CRISPR screens are in clinical development. *PRMT5* and *MAT2A* were identified using *in vitro* RNAi screens in large panels of cell lines or in isogenic pairs as synthetic lethal targets in *CDKN2A/MTAP*-deleted tumors. MTAP, enzyme methylthioadenosine phosphorylase, generates the PRMT5 substrate S-adenosylmethionine (SAM) and is frequently codeleted with the tumor suppressor gene CDKN2A due to its proximity, resulting in a dependency in both *PRMT5* and *MAT2A* (7). Two SAM-cooperative PRMT5 inhibitors (GSK-3326595 and JNJ-64619178; NCT02783300 and NCT03573310) are in phase I/II clinical trials for multiple indications. Results from these trials showed that adverse effects were common while multiple tumor types responded to therapy with partial responses or stable disease. Notably, it has not been clear that SAM-cooperative inhibitors are the appropriate approach to exploiting the MTAP deficient state. The MAT2A inhibitor AG-270 (Agios Pharmaceuticals) has now completed phase I dose escalation with the appropriate decreases in biomarkers and one confirmed partial response in a patient with a neuroendocrine tumor (NCT03435250). Combination trials with taxanes have now been initiated. A second MAT2A inhibitor, IDE397 (Ideaya Biosciences) has also entered phase I testing (NCT04794699). It is worth noting that although both *PRMT5* and *MAT2A* depletion are selective essential in MTAP-deficient tumors when queried using RNAi, they are pan-essential with CRISPR raising the question of whether a sufficient therapeutic index will be achieved with PRMT5 and or MTAP inhibitors.

PTPN2, a tyrosine phosphatase, was identified from *in vivo* CRISPR–Cas9 screens in murine syngeneic tumors treated with or without anti-PD1 antibodies (6). Deletion of *PTPN2* in tumor cells increased the efficacy of checkpoint blockade by enhancing IFNγ-mediated effects on antigen presentation. PTPN2 inhibitors (ABBV-CLS-579 and ABBV-CLS-484; Abbvie and Calico Life Sciences) are currently in Phase I development (NCT04417465 and NCT04777994). Unlike *PRMT5* and *MAT2A*, *PTPN2* is a selective dependence in CRISPR screens, suggesting that suppression of *PTPN2* might be well tolerated.

[1]Broad Institute of MIT and Harvard, Cambridge, Massachusetts. [2]Dana-Farber Cancer Institute, Boston, Massachusetts. [3]Harvard Medical School, Boston, Massachusetts.

Corresponding Author: Francisca Vazquez, Cancer Program, Broad Institute of Harvard and MIT, 415 Main Street, Cambridge, MA, 02142. E-mail: vazquez@broadinstitute.org

Cancer Res 2021;81:5806–9

doi: 10.1158/0008-5472.CAN-21-1784

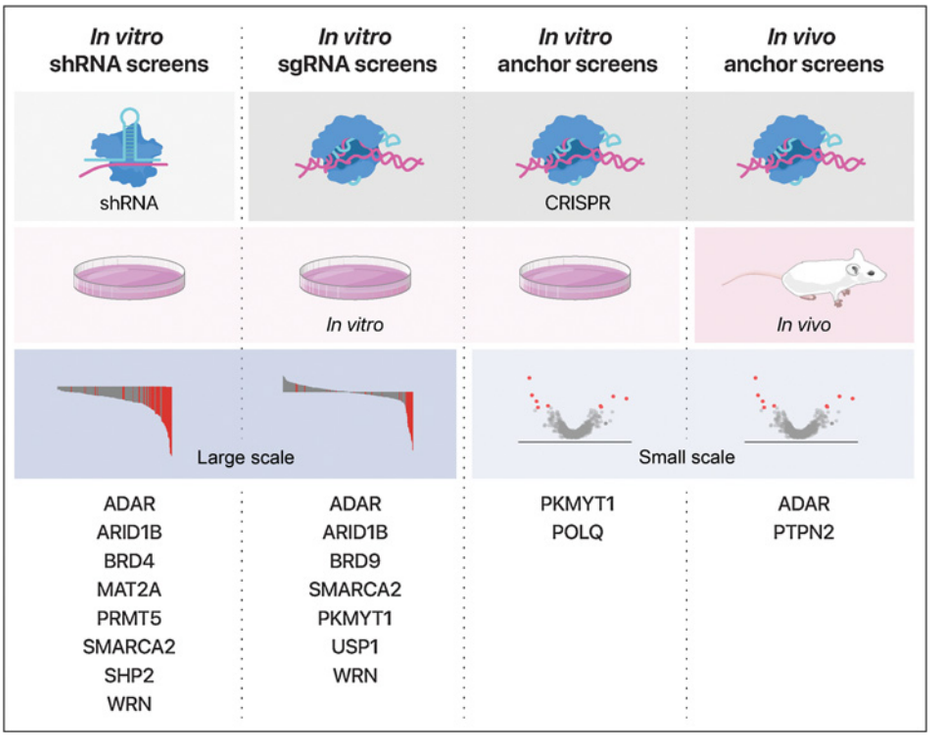

Figure 1.
Illustration depicting the different types of functional genomics screens and the targets emerging from each that are described in this article. Targets emerging from large-scale screens are typically identified as dependencies in subsets of cell lines. In small-scale screens, pairs of cell lines with or without the genomic alteration or the perturbation are compared.

The nonreceptor protein tyrosine phosphatase SHP2, encoded by *PTPN11*, was also found to be a dependency in cell lines with activated receptor tyrosine kinases (8). Multiple SHP2 inhibitors (BBP-398, JAB-3068, TNO155) are currently in clinical trials. Subsequent investigations have suggested a role for SHP2 inhibition in the RAS^{G12C} setting as well.

PKMYT1 kinase, a negative regulator of CDK1, was recently described as a synthetic lethal target in cyclin E amplified tumors using a combination of screens in isogenic pairs of cell lines and data from DepMap (9). A phase I clinical trial was recently initiated with RP-6306, a PKMYT1 inhibitor. On the other hand, *PKMYT1* is a pan-essential gene in the CRISPR DepMap dataset, thus the therapeutic index of such inhibitors might be compressed.

Targets with disclosed drug discovery programs

A growing number of drug discovery programs are underway for novel targets that were discovered using CRISPR screens. In particular, several members of the SWI/SNF complexes were identified as vulnerabilities in selected contexts. BRG (*SMARCA2*) and *ARID1B* were identified as synthetic lethal targets in tumors with mutations in their paralogs BRM (*SMARCA2*) and *ARID1A*, respectively. The noncanonical SWI/SNF complex member, BRD9, was validated as a marked dependency in synovial and rhabdoid tumors. Suppression of epigenetic regulators might be slower in causing a viability effect, and thus pooled screens might underestimate the viability effect as they are competing with faster dependencies. Hence, it will be of interest to see if differences in dependency profiles translate into differences in efficacy for these epigenetic regulators.

A promising class of synthetic lethal targets is emerging among genes involved in DNA repair with several targets in drug discovery programs. *POLQ* was identified as a synthetic lethal target in homologous recombination–deficient cells and later discovered using short hairpin RNA (shRNA) and CRISPR screens in *BRCA2*-mutant isogenic cell lines (10). *WRN* was recently described as a synthetic lethal target in the context of microsatellite instability (MSI; refs. 5, 11). WRN is required to resolve secondary structures that form due to large expansion of TA-dinucleotide in MSI tumors. Multiple programs are ongoing to find inhibitors of WRN. *WRN* dependence is highly specific for MSI tumors having no effect in microsatellite stable (MSS) cell lines supporting the notion that a high therapeutic index might be achieved with this target. *USP1* was discovered using CRISPR screens as a target in *BRCA*-deficient ovarian and breast cancer, and an inhibitor against USP1, a ubiquitin-specific peptidase that regulates DNA damage response, was recently reported by KSQ therapeutics (12). The success of PARP inhibitors in the context of BRCA1 exemplifies the potential of these DDR targets.

ADAR was identified as a potential target with both tumor cell–intrinsic and -extrinsic mechanisms of action. First, in large-scale RNAi screens, a cell state of chronic IFN signaling activation was shown to render tumors susceptible to *ADAR* loss as a response to the accumulation of double-strand RNA (dsRNA). Second, in *in vivo* CRISPR screen in immunocompetent mice ADAR was found to increase sensitivity and overcome resistance to immunotherapy (13). ADAR acts as an innate immune checkpoint in tumor cells by preventing dsRNA-induced type 1 IFN signaling. Several drug discovery programs are ongoing for this target. While *ADAR* suppression appears to cause a fitness effect in a large proportion of cell lines in DepMap, a subset of cells is more dependent, suggesting a patient selection strategy and positive therapeutic index might be possible.

Therapeutic indication expansion

As more loss-of-function data is generated in an expansive number of contexts, we can use the information to expand therapeutic indications for a given therapeutic target beyond the original context. For example, CRISPR screens in pediatric cell lines showed that pediatric tumors, especially rhabdoid and Ewing sarcoma, that are often *TP53*

wild-type (WT), are dependent on *MDM2* or *MDM4* suppression (14, 15). This led to the initiation of a phase I clinical trial with the dual MDM2/MDMX dual inhibitor ALRN-6924 in children with relapsed/refractory cancer.

Target invalidation

An underappreciated aspect of larger-scale CRISPR screening is the reevaluation of target hypotheses originally proposed based on limited cell line profiling (e.g., 1–2 cell line models) either with therapeutic compounds or with knockout (KO) or knockdown reagents. Indeed, anecdotally pharmaceutical companies frequently turn to such datasets when presented with new target hypotheses. In keeping with the power of larger-scale profiling, targets such as HDACs, *AURKA*, *AURKB*, *PLK1*, and *KIF11* are now seen to be broadly cytotoxic when depleted rather than having selective activity as originally proposed.

On the other hand, RNAi reagents also cause unintended off-target effects. Several targets initially identified using this technology were subsequently invalidated (16). In particular, maternal embryonic leucine zipper kinase (MELK), a kinase involved in cell-cycle regulation, was shown to decrease cell viability in several cancer cell lines using RNAi reagents. This work supported the development of tool inhibitors MELKi and NVS-MELK8a, as well as OTS167, which reached clinical trials. Using CRISPR this kinase was later shown to be dispensable for cell growth, indicating that these inhibitors were acting through an off-target mechanism. Other candidate oncology therapeutic targets, including TAK1, TBK1, STK33, HDAC6, MAPK14, PAK4, PBK, are likewise suspected of having resulted from off-target effects of RNAi reagents. In depmap.org the CRISPR dependency profiles for *TBK1, STK33, HDAC6, PAK4*, and *PBK* shows that complete suppression of these targets causes no or only a weak loss-of-fitness effect across more than 1,000 cell lines. Similarly, PHGDH, a target originally found to be essential in a subset of breast cancer cell lines using RNAi, does not score when targeted by CRISPR guides.

Finally, in large-scale RNAi and CRISPR experiments dependence on metabolic genes including cholesterol regulators (e.g., *SCAP*), amino acid transporters (e.g., *SLC7A1*), and iron regulatory genes (e.g., *TFRC*) are strongly linked to variations in media compositions that can vary from cell to cell and thus, the selective dependencies are likely artifacts of *in vitro* growth conditions until proven otherwise (2).

What Are We Missing?

Although the scale of CRISPR screening efforts might seem large, there are reasons to believe that we have only identified a small fraction of cancer vulnerabilities in human cancer.

First, cancer is heterogeneous, and many tumor types are underrepresented or entirely missing. Thus, we remain severely limited in the ability to understand dependencies by the availability of cell line models. In addition, to underrepresented cancers including neuroendocrine tumors, oligodendroglioma, adenoid cystic carcinomas, and many pediatric malignancies; the representation of important subsets of major cancers also remains inadequate. For example, estrogen receptor–positive breast, prostate, esophageal, and *EGFR* and *ALK* mutant lung cancer are all represented by only a handful of cell lines in the current datasets. Recent advances in the generation of patient-derived models including 3D organoids coupled with methods enabling genome-scale screens in this context will allow a significant expansion in the number of tumor types profiled.

Second, the majority of screens have been performed *in vitro* using simplified one-cell type culture systems with one selected culture media. Likely, many vulnerabilities that are due to ligand-receptor interactions in distinct cell types remain to be discovered. Similarly, dependencies that are sensitive to microenvironmental changes such as nutrient conditions, oxygen tension, or cytokines would have been missed. On the other hand, vulnerabilities that are linked to artificial media conditions could be eliminated using *in vivo* screens or screening in more physiologic relevant media conditions. *In vivo* screens using syngeneic murine tumor models have uncovered *PTPN2* as a novel tumor-expressed modulator of the immune response (17). Single-cell technologies, such as perturb sequencing could provide opportunities to screen heterogeneous cell populations and screen using phenotypes beyond cell viability (18). Thus, despite the difficulty of such complex *in vivo*, 3D, or coculture screens, the discovery value is likely high.

Third, we have not discovered new tractable synthetic lethal nodes relevant to many of the commonly mutated oncogenic pathways including the RAS, PI3K, and WNT pathways. This may highlight a limitation in the use of single-gene CRISPR KO. Specifically, the majority of these pathways are comprised of multiple paralogous genes providing redundant signaling functions. Indeed, the profiles of MEK1 or MEK2 KOs do not overlap with the activity profile of MEK inhibitors. Thus, the expression of a paralogous gene can mask dependency if both paralogs are not simultaneously suppressed. This limitation could be overcome by using combinatorial CRISPR screens to target paralog genes simultaneously in large panels of cell lines (19). Beyond paralogs, combinatorial CRISPR screens could help uncover new vulnerabilities that only manifest when two genes or more are suppressed, although the potential combinations are very large and only a subset of the genome can be interrogated in any particular effort. Similarly, drug-CRISPR combinations could uncover drug-induced vulnerabilities. Indeed, a drug combination of BRAF and EGFR inhibitors was recently approved in colorectal cancer based on preclinical data using genetic screens.

One way to help accelerate these efforts is to make use of the current datasets and other approaches to come to a complete set of fully validated CRISPR guides for every gene in the genome. Combined with approaches that can combine two or more single-guide RNAs (sgRNA) in the same construct, this would enable greatly reduced library sizes, which will be important for combinatorial, complex screens. Such validated guide sets would also enable a reduction in false-positive hits, and a reduction in low, but important false-negative rates.

Fourth, targets having a pan-essential profile as defined by CRISPR might be presumed to have a narrow therapeutic index where therapeutics targeting the same gene would suffer from increased toxicity. However, few therapeutics ever achieve complete target inhibition and thus complete gene KOs might miss differential sensitivities induced by partial or temporally restricted loss of function. For example, *DHFR* and TOP2A are pan-essential genes in CRISPR screens, yet are targets for highly effective chemotherapeutic agents. Thus, at the moment we are unable to discover whether subsets of pan-essential gene targets when partially or temporally inhibited would exhibit robust and selective dependence. Additional methods to induce such phenotypes are likely needed. Here, CRISPR interference (CRISPRi) could be used as an alternative approach to RNAi by using mismatched sgRNA to partial inhibit gene expression (20). Additionally, chemically induced degron tags are likely to prove a powerful tool to manipulate gene necessity in these two dimensions.

Lastly, perturbation of gene function by gene KOs is not equivalent to occupying a binding site by a small molecule. Thus, more sophisticated tools including base editing might provide the genetic tools to

allow us to more directly mimic the functional consequences of small molecules.

In conclusion, much work remains to be done but CRISPR screens and functional genomics more generally will play a major role in the mapping of the next generation targets for precision cancer medicine.

Authors' Disclosures

F. Vazquez reports grants from Novo Ventures and Dependency Map Consortium outside the submitted work; in addition, F. Vazquez has a patent for WRN pending. W.R. Sellers reports grants from Pfizer Pharmaceuticals, Ridgeline Discovery; grants and personal fees from Ideaya Biosciences; grants from Merck Pharmaceuticals, Boehringer-Ingelheim, Novartis during the conduct of the study; personal fees from Civetta Therapeutics, Red Ridge Bio, Bluebird Bio, Epidarex Capital, Peleton Therapeutics, Syndax Pharmaceuticals, Servier Pharmaceuticals, Scorpion Therapeutics; and personal fees from Genentech outside the submitted work.

Acknowledgments

The authors thank Andrew Tang for the figure.

Received June 3, 2021; revised July 20, 2021; accepted September 27, 2021; published first December 1, 2021.

References

1. Ghandi M, Huang FW, Jané-Valbuena J, Kryukov GV, Lo CC, McDonald ER III, et al. Next-generation characterization of the Cancer Cell Line Encyclopedia. Nature 2019;569:503–8.
2. Li H, Ning S, Ghandi M, Kryukov GV, Gopal S, Deik A, et al. The landscape of cancer cell line metabolism. Nat Med 2019;25:850–60.
3. Barretina J, Caponigro G, Stransky N, Venkatesan K, Margolin AA, Kim S, et al. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature 2012;483:603–7.
4. Tsherniak A, Vazquez F, Montgomery PG, Weir BA, Kryukov G, Cowley GS, et al. Defining a cancer dependency map. Cell 2017;170:564–76.
5. Behan FM, Iorio F, Picco G, Gonçalves E, Beaver CM, Migliardi G, et al. Prioritization of cancer therapeutic targets using CRISPR-Cas9 screens. Nature 2019;568:511–6.
6. Lane-Reticker SK, Manguso RT, Haining WN. Pooled in vivo screens for cancer immunotherapy target discovery. Immunotherapy 2018;10:167–70.
7. Huang A, Garraway LA, Ashworth A, Weber B. Synthetic lethality as an engine for cancer drug target discovery. Nat Rev Drug Discov 2019;19:23–38.
8. Chen Y-NP, LaMarche MJ, Chan HM, Fekkes P, Garcia-Fortanet J, Acker MG, et al. Allosteric inhibition of SHP2 phosphatase inhibits cancers driven by receptor tyrosine kinases. Nature 2016;535:148–52.
9. Gallo D, Young JTF, Fourtounis J, Martino G, Álvarez-Quilón A, Bernier C, et al. CCNE1 amplification is synthetic-lethal with PKMYT1 kinase inhibition. bioRxiv 2021[cited 2021 May 4]. Available from: https://www.biorxiv.org/content/10.1101/2021.04.08.438361v1.
10. Ceccaldi R, Liu JC, Amunugama R, Hajdu I, Primack B, Petalcorin MIR, et al. Homologous-recombination-deficient tumours are dependent on Polθ-mediated repair. Nature 2015;518:258–62.
11. Chan EM, Shibue T, McFarland JM, Gaeta B, Ghandi M, Dumont N, et al. WRN helicase is a synthetic lethal target in microsatellite unstable cancers. Nature 2019;568:551–6.
12. Cadzow L, Brenneman J, Sullivan P, Liu H, Shenker S, McGuire M, et al. Development of KSQ-4279 as a first-in-class USP1 inhibitor for the treatment of BRCA-deficient cancers. Eur J Cancer 2020;138:S52.
13. Bhate A, Sun T, Li JB. ADAR1: a new target for immuno-oncology therapy. Mol Cell 2019;73:866–8.
14. Howard TP, Arnoff TE, Song MR, Giacomelli AO, Wang X, Hong AL, et al. MDM2 and MDM4 are therapeutic vulnerabilities in malignant rhabdoid tumors. Cancer Res 2019;79:2404–14.
15. Stolte B, Iniguez AB, Dharia NV, Robichaud AL, Conway AS, Morgan AM, et al. Genome-scale CRISPR-Cas9 screen identifies druggable dependencies in TP53 wild-type Ewing sarcoma. J Exp Med 2018;215:2137–55.
16. Lin A, Giuliano CJ, Palladino A, John KM, Abramowicz C, Lou Yuan M, et al. Off-target toxicity is a common mechanism of action of cancer drugs undergoing clinical trials. Sci Transl Med. 2019;11:eaaw8412.
17. Manguso RT, Pope HW, Zimmer MD, Brown FD, Yates KB, Miller BC, et al. In vivo CRISPR screening identifies Ptpn2 as a cancer immunotherapy target. Nature 2017;547:413–8.
18. Schraivogel D, Gschwind AR, Milbank JH, Leonce DR, Jakob P, Mathur L, et al. Targeted Perturb-seq enables genome-scale genetic screens in single cells. Nat Methods 2020;17:629–35.
19. Ito T, Young M, Li R, Jain S, Wernitznig A, Krill-Burger JM, et al. Paralog CRISPR screens reveal synthetic lethality in MAPK-mutant cells. Nat Genet 2021. In press.
20. Jost M, Santos DA, Saunders RA, Horlbeck MA, Hawkins JS, Scaria SM, et al. Titrating gene expression using libraries of systematically attenuated CRISPR guide RNAs. Nat Biotechnol 2020;38:355–64.