Editorial Commentary

CRISPR screens and the best cancer drug targets: picking the needle out of the haystack

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In the past two decades, the clinical management of cancer patients, mostly based on the use of surgery, chemotherapy and radiotherapy, has been revolutionized by the development of precision medicine and new immunotherapy approaches, two new pillars in cancer therapeutics. The ways to treat cancer have enormously increased providing more tailored approaches to cancer patients, who can be offered a wider range of therapeutic options. Despite such progress, however, the huge heterogeneity of tumors and their ability to develop resistance to therapies make cancer a moving target. Cancer-specific dependencies and the best drug targets need to be identified to increase even more the arsenal of weapons that can be used at different stages of treatment in a sequential manner to defeat the disease or make it become chronic.

The recent discovery of the prokaryotic CRISPR (clustered regularly interspaced short palindromic repeats)-Cas 9 adaptive immune system as a revolutionary genome editing tool (1,2), provided an invaluable means for fast pace discoveries through both forward and reverse genetics approaches (3).

Only few years ago, through the rapid generation of human somatic cell knockout of thousands of genes, the first studies showed the potential of CRISPR-Cas9-based genome scale lentiviral libraries for the identification, and subsequent functional characterization, of the molecular underpinnings of biological and pathological processes (4-7), anticipating a myriad of following studies. However, identifying the most promising drug targets among hundreds of potential candidates deriving from such high-throughput screens remains challenging. Helping to figure out how to pick some needles out of the haystack, Behan, Iorio and Picco, in a recently published article, established a framework to prioritize and rank actionable cancer genes (8). The authors started by performing CRISPR-Cas9 screens, targeting nearly 20,000 genes, in 339 cancer cell lines deriving from tumors of 19 different tissues, spanning from lung, breast, colon, stomach, ovary and others, to difficult-to-treat tumors such those of the nervous system and pancreas. Selected cells, stably expressing Cas9 at a level of activity above 75%, were transduced with lentiviral-packaged whole genome sgRNA library to achieve optimal library coverage. Following stringent quality control analyses, the authors were able to evaluate a final set of 324 cell lines, deriving from 30 tumor types, which were screened for genes that determine cancer cell fitness, being essential to sustain cancer cell growth and viability. The analysis retrieved a median of 1,459 fitness genes in each cell line.

The authors reasoned that fitness genes common to a cancer type and required for most of the tested cell lines (defined as core fitness genes) are likely associated to fundamental processes in cells and their inactivation could probably result in high toxicity. Conversely, better actionable drug targets could consist of genes required only in specific contexts because their inactivation would likely spare healthy tissues from toxic effects. So, to classify such core fitness genes and identify the context-specific ones, Behan and colleagues designed an algorithm, based on a previously described model (9,10), which was named Adaptive Daisy Model (ADaM). Through ADaM, which was released for public use (8), the authors were able to

adaptively determine statistically a core fitness gene by calculating the minimal number of cell lines, of a certain cancer type, which were dependent on it. Similarly, ADaM allowed to determine the minimal number of cancer types for which a gene could be classified as pan-cancer core fitness gene. The large dataset of this study allowed the authors to refine the classification of essential genes compared with previous works (10-12), resulting, overall, in a median of 866 cancer-type specific genes and 533 pan-cancer core fitness genes, the latter being essential in at least 12–13 cancer types. Ideal drug targets are supposedly those context-specific fitness genes that are highly expressed in single tumor types but not in matched healthy tissues, therefore less likely to induce toxicity if targeted.

To narrow down to a manageable list of potential cancer-specific drug candidates, the authors developed a prioritization framework taking into account multiple factors, each contributing with a different weight to assign every gene a priority score, ranging from 0 to 100. In particular, 70% of the priority score was assigned based on a first set of criteria, including the fitness effect size derived from the CRISPR-Cas9 screen in dependent cell lines, the significance of fitness loss, target gene expression/mutational status, and record of other fitness genes within the same pathway. The remaining 30% of the priority score was assigned through a second set of criteria based on the identification of genomic features correlated with fitness genes: in particular, target somatic mutations in primary tumors and the presence of genetic biomarkers associated with the target dependency were taken into account. For such biomarker analysis the associations between fitness genes and the presence of cancer driver events or microsatellite instability (MSI), were evaluated through a systematic ANOVA, both at the pan-cancer and individual cancer-type level, categorizing genes into different classes—A, B, C or weaker—according to the significance and effect size thresholds of their associations. So, the integrated analysis of public data with their own allowed the authors to short-list 628 priority targets, 617 of which were cancer-type specific. Core fitness genes, which are likely poor drug targets because of potential high toxicity, were scored 0 and ruled out along with non-expressed or homozygously deleted genes that could test as false positive. The data were made available through the project Score website, further contributing, with such wide CRISPR-Cas9 screen, to draw the Cancer Dependency Map, a sort of genome-scale rule book of cancer-specific weaknesses that can be used as Achilles' heel for synthetic lethal precision medicine strategies (12-14).

Going further, to hunt down the most promising targets, the authors integrated the list of 628 priority targets with data relative to their potential tractability—which relates to the ability of a target to be drugged with a therapeutically useful level of affinity, efficacy and safety by a small molecule or an antibody (15). The authors started from a previously developed genome-wide target tractability assessment pipeline, which collects publicly available data and finally ranks and assigns human genes to different buckets of tractability depending on their likelihood of being targeted through small molecules, antibodies or other approaches (15). So, the 628 priority targets were cross-referenced with their tractability data and categorized into three tractability groups. Forty priority targets were classified in group 1; these included targets of already approved cancer drugs or of compounds in pre- or clinical development. Interestingly, while some of these targets already have a drug developed in the same cancer in which they induced dependency, others have drugs developed for other conditions, suggesting new repurposing strategies. Approximately 33% of these candidates were also associated with a class A biomarker further supporting the fact that they are indeed highly recommended targets. Another set of 277 priority targets were classified in tractability group 2, which gathered targets without drugs in the current clinical development pipeline but with high potential for druggability. Approximately 18% of these were also associated with a class A biomarker. Finally, 311 priority targets ended up in group 3 for which no supportive information were available concerning tractability.

From a functional point of view, priority targets in group 1 were significantly enriched in protein kinases, which are classic oncogenic drivers and probably the most attractive targets of precision medicine approach so far, which justifies their allocation in the group with already available drugs. Whereas priority targets in group 3 were significantly enriched in transcription factors, which are probably more difficult to target with conventional strategies. The authors therefore reasoned that priority targets within tractability group 2 had the better chance for novel drug development. So, among priority targets within this class, they zoomed in on Werner (WRN), a member of the RecQ subfamily of DNA helicases, which is involved in the maintenance of genome integrity and whose germline loss-of-function mutations cause a syndrome characterized by premature aging and cancer predisposition (OMIM #277700) (16).

From the study analysis, the authors identified at the pan-

cancer level a dependency on WRN, which showed a class A association with the MSI biomarker. MSI arises owing to defects of the DNA mismatch repair system, which is common to various tumor types and, along with tumor mutational burden and expression of immune checkpoint factors, is being considered as an important predictive factor for immunotherapy approaches (17). Indeed, the proof that MSI tumors, with a faulty mismatch repair and subsequent high mutational load, were susceptible to immune checkpoint blockade (18,19), led to the first tissue-agnostic drug approval by the Food and Drug Administration in 2017 (20).

Through their study, Behan and colleagues found that microsatellite unstable colon and ovarian cancer cells, in particular, showed a dependency on WRN. The association of MSI and WRN requirement was not significant in gastric cancer cells and could not be assessed in endometrial cancer cells because of the small sample size. However, further validation analysis showed that CRISPR-Cas9 based knock out of WRN through four different sgRNAs potently affected cell fitness (with an effect size similar to the one exerted by core fitness genes) in all MSI colon, ovarian, gastric and endometrial cells tested but not in satellite stable cells from the same cancer types. The efficacy of such synthetic lethal therapeutic strategy was also confirmed *in vivo*, in a xenograft model of colorectal cancer. Finally, through expression of sgRNA-resistant mutants in the WRN knock out MSI cells, the authors showed that WRN helicase activity was required to rescue the loss-of-fitness effect, suggesting that drug development should aim towards the protein domain endowed with such activity (8).

Three other studies almost concomitantly found that WRN is a synthetic lethal target in tumors bearing MSI (21-23), further supporting not only the potential of this new precision medicine approach but also the validity of such a streamlined method to identify and prioritize promising cancer drug candidates.

There is no doubt that CRISPR-Cas9 based screens will discover in the near future new cancer genes at an unprecedented pace. Generally, however, the costs for pharmaceutical companies to produce new drugs are very high and only a small percentage of these gain final approval by regulatory agencies. Among all pharmaceuticals, oncology drugs show the lowest success rate, as confirmed by recent studies estimating the rates and reasons for such high attrition (24,25). Although efforts are ongoing to improve the design of clinical trials favoring a more rapid drug assessment and eventually accelerating approval (26,27), the initial selection of the best drug candidates seems of utmost importance in the drug development process. The work from Behan, Iorio and Picco provides a valuable tool to derive a genome-scale data-driven framework (integrating open data from various sources including genomic features and tractability datasets) for the prioritization of drug candidates holding potential for a rapid development and clinical translation.

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Footnote

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