VIEWPOINT

Drugging the 'undruggable' cancer targets

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Abstract | The term 'undruggable' was coined to describe proteins that could not be targeted pharmacologically. However, progress is being made to 'drug' many of these targets, and therefore more appropriate terms might be 'difficult to drug' or 'yet to be drugged'. Many desirable targets in cancer fall into this category, including the RAS and MYC oncogenes, and pharmacologically targeting these intractable proteins is now a key challenge in cancer research that requires innovation and the development of new technologies. In this Viewpoint article, we asked four scientists working in this field for their opinions on the most crucial advances, as well as the challenges and what the future holds for this important area of research.

Q *What would you say are the key so-called undruggable targets in cancer (and why)?*

Chi V. Dang. Tremendous progress in sequencing thousands of cancer genomes through The Cancer Genome Atlas (TCGA) and advances in cancer biology have uncovered many drivers of tumorigenesis[1]. Many of the drivers, particularly kinases, have provided druggable targets that have yielded significant clinical benefits over the past several decades. However, many known drivers such as RAS, MYC and fusion transcription factors commonly seen with paediatric cancers have been deemed undruggable owing to large protein–protein interaction (PPI) interfaces or their lack of deep protein pockets[2,3]. As a result, drugging these intractable targets is now one of the key challenges to cancer research along with the barriers to fully understand tumour heterogeneity and drug sensitivity and resistance mechanisms. In fact, targeting fusion proteins in paediatric cancers and a richer understanding of tumour heterogeneity and the microenvironment are two key areas of investigation recommended for acceleration by the Cancer Moonshot Blue Ribbon Panel charged by former US Vice-President Joseph Biden (see Further information). Notwithstanding the challenges of targeting difficult targets, new concepts in drug development and a richer understanding of synthetic lethality interactions hold promise for new drug classes that could be highly effective when used in combinations that would be lethal for the cancer cells.

Cancer drugs have evolved since 1939, when Charles Huggins used synthetic hormones to treat prostate cancer. The first chemotherapy drug, mustine, was used in 1942, followed by Sidney Farber's famed used of antifolate to treat leukaemia[4]. In subsequent years, chemotherapeutic drugs targeting DNA (alkylating agents), DNA synthesis (nucleoside analogues) and microtubules (vincristine, vinblastine and taxol) as well as anthracyclines (directed at various cellular targets) were the mainstay war chest for cancer treatment. Although responses with these drugs were remarkable, they were not without major side effects. The 1990s were marked by the targeted therapy revolution. Imatinib, which inhibits the tyrosine kinase activity of BCR–ABL, a fusion protein derived from the Philadelphia chromosome translocation, was invented and discovered to be effective against chronic myeloid leukaemia (CML) and it gained FDA approval in 2001. The advent of monoclonal antibody therapies, such as rituximab for treatment of haematological malignancies, and approval of the aromatase inhibitor anastrozole for breast cancer further mark the advances of the 1990s. The 2000s witnessed further FDA approvals of monoclonal antibodies, most recently those targeting immune checkpoints (cytotoxic T lymphocyte associated antigen 4 (CTLA4), programmed cell death protein 1 (PD1) and PD1 ligand 1 (PDL1))[5], underscoring remarkable advances in immunotherapy that include the use of dendritic cell vaccines and chimeric antigen receptor (CAR) T cells for the treatment of leukaemia and lymphoma. Notwithstanding these amazing advances, key oncogenic drivers such as KRAS and MYC still harbour threats for many cancer patients. These targets have been deemed undruggable, but this label has not deterred the US National Cancer Institute (NCI) from launching its assault on KRAS through the RAS Initiative anchored at the Frederick National Laboratory for Cancer Research (see Further information).

E. Premkumar Reddy. The term undruggable is somewhat of an exaggeration and a more appropriate term might be difficult to drug. Our experience teaches us that many so-called undruggable targets were eventually successfully targeted, with several of these products having reached the market. A good example is the BCL-2 family of proteins, which were at one time considered undruggable. Today, there is at least one drug that has reached the market with several more likely to follow. These undruggable targets became druggable because of major strides made in the basic understanding of the biochemical and biological properties of these proteins and the availability of structural insights provided by X-ray crystallography and nuclear magnetic resonance (NMR).

Today, there are many cancer targets that are considered undruggable. A large percentage of these targets fall under the category of transcription factors, for example, MYC, MYB and nuclear factor-κB (NF-κB), which have long been recognized to play crucial roles in cell proliferation and development[6,7]. The vast majority of cancers are driven by these transcription factors, and inhibition of these proteins has proved difficult because of their intracellular (often nuclear) localization

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Laura Soucek graduated in 1996 in biological sciences from University La Sapienza in Rome, Italy. She obtained her Ph.D. in genetics and molecular biology at the National Research Center, in Rome. In 2001 she joined Gerard Evan's laboratory at the University of California San Francisco, USA, initially as a postdoctoral fellow and later, in 2006, as an assistant researcher. There she published in some of the most prestigious international journals. Since early 2011, she has headed the Mouse Models of Cancer Therapies group at the Vall d'Hebron Institute of Oncology (VHIO), in Barcelona, Spain. She received prestigious awards and grants from the American Association for Cancer Research, the Miguel Servet Program, the FERO Foundation, the Association for International Cancer Research, the European Research Council, FIS and BBVA. In October 2014 she was appointed Institució Catalana de Recerca I Estudis Avançats (ICREA) Research Professor. In December 2014 she founded a spin-off company, Peptomyc S.L. In April 2015, she became Associate Professor at the Universitat Autònoma de Barcelona.

and their mechanism of action, which involves association with a large number of co-factors to form a transcriptional complex. A second major category of undruggable targets is the RAS family of proteins[8], with the three RAS oncogene products (KRAS, NRAS and HRAS) being the most intensively studied proteins because of their mutation in approximately 30% of human cancers[8]. These proteins act as binary molecular switches that interact with a large number of catalytically distinct downstream effectors such as RAF, PI3K and Ral guanine nucleotide dissociation stimulator (RALGDS). These effectors, which are activated by their interaction with RAS, in turn regulate cytoplasmic signalling, leading to gene expression and cell cycle progression. Importantly, it is not just the RAS proteins that are relevant in this context. The RAS superfamily actually consists of about 150 related proteins, such as RAC and RHO, which also regulate multiple signal transduction pathways[9]. In addition, the α-subunits of several heterotrimeric G proteins, which are related to RAS, mediate their effects by binding to multiple effectors, and some of these genes, such as those encoding Gα12 and Gα13, are mutated in several cancers and constitute important cancer targets[9].

Kevan M. Shokat. Undruggable is such a great word because it really focuses our attention on overcoming significant challenges in drug discovery. There are two aspects to identification of a target as undruggable. First, the target must be currently chemically intractable. Next, there must be strong data suggesting that making a small molecule against the target would be clinically meaningful. To my mind, this requires evidence from human genetics. In cancer, the target must be a known oncogene or a known tumour suppressor.

With these criteria in mind, my current shortlist of undruggable oncogenes is: $KRAS^{G12D/V}$, *MYC* and the androgen receptor (*AR*) variant 7 (*AR-V7*).

KRAS represents the most frequently mutated oncogene across all cancer types. The $KRAS^{G12D}$ and $KRAS^{G12V}$ mutated alleles are found in 90% of patients with pancreatic cancer, which is a major unmet need[10]. Aresurgence of interest in targeting KRAS has emerged from the NCI, leading to the RAS Initiative led by Frank McCormick[10]. Our efforts have led to progress on drugging one particular allele, $KRAS^{G12C}$, by relying on the presence of a nucleophilic cysteine residue for drug binding[11–13]. $KRAS^{G12C}$ accounts for an estimated 29,700 new cancer diagnoses (lung and colon most frequently) compared with 53,700 and 39,100 for $KRAS^{G12D}$ and $KRAS^{G12V}$, respectively[10]. The current need is to develop KRAS inhibitors that do not rely on covalent attachment to Cys12, that is, the Asp12 and Val12 mutants.

MYC is a transcription factor that orchestrates a potent pro-cancer programme across multiple cellular pathways. Other authors in this Viewpoint article have provided more details on the importance of MYC and the myriad attempts to overcome its lack of a druggable binding pocket. As MYC itself is so challenging, much effort has been focused on indirect targeting strategies. We were motivated by the work of Martin Eilers showing that MYCN stability in cells is controlled by the Aurora kinase A (AURKA)[14]. In collaboration with my colleague William Weiss's lab we carried out a targeted screen of AURKA inhibitors in search of amphosteric inhibitors of MYC[15]. An amphosteric inhibitor denotes one that is simultaneously both orthosteric (inhibits kinase activity) and allosteric (disrupts PPIs). No crystal structure of the complex between AURKA and MYCN was known when we reported the first amphosteric MYC inhibitor, making further optimization somewhat challenging. Fortunately, Richard Bayliss's lab recently revealed the co-complex of AURKA bound to MYCN, which will greatly facilitate further chemical optimization of this strategy for drugging MYC[16]. As MYC is often overexpressed in late-stage cancer, targeting it for degradation is an attractive strategy in many settings.

The AR drives prostate cancer cell growth and survival. Advanced prostate cancer therapies include drugs that suppress the production of androgens and/or suppress androgen binding to the AR ligand binding domain (LBD). Patient responses to these drugs are outstanding. However, resistance emerges, leading to hormone-refractory prostate cancer for which we currently do not have therapies[17]. Among the resistance mechanisms is a splice variant of *AR* termed *AR-V7* (REF. 18). Mediated by splicing of cryptic exons, *AR-V7* produces a form of AR that contains the canonical DNA binding domain but lacks the LBD. The splicing out of the drug binding site of AR effectively removes the druggable domain, making AR-V7 undruggable. I chose this example of an undruggable protein because it highlights the challenge we face when we see approval of one effective therapy leading to emergence of resistance in the form of a new undruggable form of the driver oncogene.

Laura Soucek. The term undruggable is really becoming a question of semantic debate and I would probably erase it, if only to avoid the risk of discouraging innovation and development of new and valuable technology. However, if I must adopt the term at least for the sake of discussion, I would prefer to distinguish between two possible categories. The first includes those targets that should not be tampered with because they are equally shared by normal and cancer cells, and interfering with them could therefore cause severe side effects in normal tissues; the second is a much more extensive category of targets that have not yet been targeted, because they have simply proved extremely challenging to effectively attack and control so far.

The first category is curiously the one we have historically targeted the most. Indeed, standard chemotherapy is usually based on non-specific poisons, which inhibit critical processes associated with cell division. In this case, the partial or total success of the treatment comes at a high price for normal proliferating tissues. Nevertheless, this still represents the most common approach available in cancer treatment. Personalized targeted medicine, which acts on specific molecular targets altered in cancer cells and limits the damage to other tissues, is a reality for only a few cancer types.

The second category, though, is the one we should probably focus on to really grasp the magnitude of therapeutic opportunity that remains to be exploited. To date, our therapeutic strategies have focused on the use of two broad classes of drug: small molecules (usually <100 atoms) and biologics such as peptides, antibodies, nucleic acids and vaccines[19]. These have mainly been developed to modulate targets with hydrophobic pockets, or those that reside on the cellular surface or are secreted, leaving around 80% of potential existing protein targets untouched[20]. Among the major obstacles is the fact that these proteins function through PPIs and often fall into the category of partially or completely intrinsically disordered proteins, whose 3D structure and architecture are very labile and dependent on their interaction with functional partners. Preventing such interaction usually means targeting proteins that do not have a defined and sufficient interaction surface that would enable drugs to be specific and efficient[21].

In both categories, we find notable and infamous players in cancer initiation and progression that are not treatable by conventional therapies, such as transcription factors (for example, p53, MYC, E2F or Kruppel like factor 4 (KLF4)), phosphatases (for example, PP2δ, PP2A or PTP1B) or the well-known RAS family, which, despite being identified as the first human mutated cancer gene, remains undruggable even after more than 30 years of research.

Q *What are the potential benefits of targeting these molecules or pathways?*

C.V.D. The *KRAS* oncogene is mutated in 90% of human pancreatic ductal adenocarcinomas (PDACs) and many lung cancers, while the *MYC* oncogene is amplified in 40% of ovarian cancers and broadly overexpressed in many cancer types[3,22]. Preclinical validation of RAS or MYC as a therapeutic target is provided by loss of function analysis through gene expression manipulation or use of a molecular disruptor such as Omo-MYC, which behaves in a dominant-negative fashion against MYC[2,3]. Although mutated KRAS is necessary for driving tumorigenesis in transgenic mouse PDACs and is prevalent in human PDACs, whether human pancreatic cancer requires KRAS for tumour maintenance is less certain. However, the use of Omo-MYC as a preclinical molecular tool suggests that MYC is necessary for tumour maintenance even if the oncogenic driver was the *KRAS* oncogene[23]. Notwithstanding the uncertainty of the roles of RAS or MYC in human tumour maintenance, their potent oncogenic activity provides the rationale for targeting these undruggable targets.

E.P.R. Targeting these pathways is considered essential as mutations or amplification of these genes and/or pathways is observed in a large percentage of human cancers where they serve as the driver mutations. It is now well documented that tumour cells often circumvent the action of targeted therapies (especially kinase inhibitors) through the activation of alternative signalling pathways. Interestingly, a good percentage of these resistant tumours appear to activate the RAS pathway to overcome the effects of these kinase inhibitors[24]. Similarly, MYC overexpression or NF-κB activation in tumour cells is often causal in resistance to targeted therapies. This observation, combined with the fact that many cancers exhibit constitutive MYC or NF-κB activity before treatment, suggests that effective tumour growth inhibition can be addressed only through the development of inhibitors of these pathways[6,7].

K.M.S. The immediate benefit of having drugs against the undruggable targets I mention is the potential to treat patients with diseases that currently have no targeted therapies. By targeting the driver oncogene (*KRAS*, *MYC* or *AR-V7*) we would expect profound clinical benefit based on the addiction of the tumours to these oncogenes[25]. Another benefit of directly targeting these players is patient selection. Unfortunately, for example, although we can identify patients with $KRAS^{G12D}$ mutation, this is not currently actionable. Overcoming the undruggable nature of these targets would make them immediately actionable.

L.S. As mentioned above, most current targets in cancer therapy reside in the most degenerate and redundant compartments of cells (that is, surface-associated receptors or subsequent signalling pathways). Their modulation, therefore, often results in the emergence of resistance and even exacerbation of the cancer phenotype as a consequence of selection pressure exerted by the therapy itself. Such redundancy seems to be notably less present downstream, with signals funnelling through some essential and unique nodes that are crucial for cancer maintenance and progression (that is, MYC[26]). Channelling our efforts towards the inhibition of those nodes would likely be rewarded by less resistance to therapy. Moreover, as these nodes often serve as conduits for multiple oncogenic signals, they would give us the opportunity to 'kill two [or more] birds with one stone', reducing the number of agents required for multiple types of cancer. It would clearly be a more practical solution compared with the increasing stratification of patients on the basis of a multitude of different driver oncogenic lesions. Last but not least, being able to attack this new category of targets would enable us to access a much wider range of therapeutic opportunities impinging on other aspects of tumorigenesis besides cell division, such as metabolism, transcription and translation, survival, resistance to therapy, immune tolerance and immune reprogramming, among others. With our available suite of novel technologies, it is unacceptable that we are still targeting only 10–20% of potential protein candidates within a cell[20].

Q *Where are we now in terms of making these targets druggable?*

C.V.D. The deregulated cancer transcriptome, proteome and metabolome downstream of oncogenic KRAS or MYC provide

potential opportunities through synthetic lethal or essential interactions with specific genes, including those involved in metabolic pathways[22]. Oncogenic KRAS drives pathways that activate MYC or stimulate macropinocytosis[27]. Oncogenic MYC, on the other hand, drives many metabolic pathways that in specific instances could cause MYC-dependent cells to be vulnerable to metabolic inhibition[22]. Although these opportunities are not specific to targeting either RAS or MYC, understanding these connections is crucially important as a foundation for targeting the drivers themselves. This is particularly important because diminishing RAS or MYC may make cancer cells less vulnerable to other therapies that may be synthetically lethal when RAS or MYC is oncogenic. Because targeting RAS is under way with significant support from the NCI and is covered by others in this Viewpoint, I will focus on MYC as an undruggable target.

The regulation of *MYC* expression is complex, involving a promoter densely packed with transcription factor sites and variable flanking enhancer regions. Although the bromodomain and extraterminal (BET) inhibitors have been touted to target MYC, their pleiotropic effect complicates matters and in some instances their activities are independent of an effect on MYC[28]. Notwithstanding this limitation, the use of BET inhibitors to target MYC should be accompanied by clear biomarkers that are associated with interrupting MYC function. The use of antisense oligonucleotide technology has been disappointing in targeting MYC, due primarily to the lack of efficient means to deliver the drug effectively and safely. Similarly, the specificity of targeting MYC protein stability is challenging[29]. However, advances in this arena are palpable, and once prime therapeutic candidates are identified, they will have to be tested for specificity of their effects in well-defined biological systems.

MYC is a pervasive human oncogene that is amplified, translocated or overexpressed in many types of cancer[22]. The gene produces the MYC protein, which functions as a transcription factor by dimerizing with MAX to bind to target DNA sequences (E-box: 5′-CACGTG-3′) and activate (or suppress) transcription, primarily through RNA polymerase II pause release and transcriptional elongation. MAX is involved in an extended transcription factor network by binding to other partners, such as the MAD and MNT proteins that can antagonize specific MYC transcription factor functions. In addition to serving as a transcription factor, MYC may have non-transcriptional function such as cap-dependent translation or microtubule regulation in the cytoplasm. As dominant-negative MYC could curb tumorigenesis and Omo-MYC can inhibit tumour formation *in vivo*, targeting the interaction between MYC and MAX seems attractive[23]. In this regard, small molecules have been generated with a spectrum of activity *in vitro* and in some cases, *in vivo*[2,30]. The greatest challenge to date is the low potency of these molecules, which challenge the advancement of the field towards the clinic. Because Omo-MYC is a small peptide, it has been developed as a potential therapeutic agent by coupling it with cell import peptides[2]. This approach could be refined, for example, by using Omo-MYC as a scaffold that could be tweaked in a semi-random manner by unbiased amino acid substitutions and screened in a high-throughput fashion to identify more active derivatives.

Another potential approach is to use small molecules that bind to MYC and link them via proteolysis-targeting chimaera (PROTAC) technology to target MYC for proteasomal degradation[31]. PROTAC relies on linking a drug that binds to a target to a small-molecular moiety that is recognized by the cereblon (CRBN) or von Hippel–Lindau (VHL) pathway for proteasomal degradation. However, this approach will need proof-of-concept that the PROTAC can drag MYC along for degradation. Because the MYC protein is largely unstructured, adifferent strategy to target disordered proteins could be fruitful. Unstructured proteins such as MYC adopt a conformation upon binding to a protein partner (MAX) and/or DNA that results in a lower entropic state that is compensated by an enthalpic gain for a favourable thermodynamic outcome[32]. Small molecules can interact with a disordered protein and create multiple conformations of the protein in order to increase entropy (ΔS) to drive the reaction thermodynamically. Favoured small-molecule interactions are those with decreased Gibbs free energy ($\Delta G<0$) defined by $\Delta G = \Delta H - T\Delta S$, where H is enthalpy and T is the temperature in Kelvin. In this regard, an entropy-driven drug-binding scenario does not require high-affinity binding, but rather increased entropy. This inherently creates a problem with specificity of the drug; however, in the case of MYC, mutagenesis studies have indicated that binding of a small molecule to disordered MYC is contained within a specific region of the MYC helix–loop–helix leucine zipper region[33]. Further, EWS–FLI, a disordered fusion transcription factor that is pivotal for the pathogenesis of Ewing sarcoma, is inhibited by interaction with the small molecule YK-4-279. Intriguingly, only one enantiomer of YK-4-279 is active, indicating structural specificity for inhibition of EWS–FLI through an interaction with the disordered protein[34]. The main challenge with this strategy, however, is to develop high-throughput read-outs and methodologies for library screening and for structure–activity relationships for lead compound derivatives.

E.P.R. The past few years have witnessed substantial progress in developing new approaches to develop RAS inhibitors. An important breakthrough in developing compounds that bind to RAS and inhibit its activity was achieved by Kevan Shokat's group, who showed that the $KRAS^{G12C}$ mutation creates a pocket that could be exploited to synthesize a covalent inhibitor specific to this mutant protein[11]. Although the initial compound required high micromolar concentrations for biological activity in cell culture assays, second- and third-generation compounds were much more active in their ability to inhibit KRAS signalling[12,13]. More recently, the group headed by Brent Stockwell was successful in designing compounds that bind simultaneously to two or more adjacent sites on RAS proteins and exhibit pan-RAS inhibitory activity[35]. Biophysical and biochemical assays suggested that their lead compound (3144), binds with an affinity in the low micromolar range, induces apoptosis of RAS-mutant cell lines and inhibits tumour growth in mouse xenograft models. There is a strong expectation that there will be several high-affinity RAS-binding compounds in the near future with sufficient biological activity to enter clinical trials.

A second and equally attractive approach is blockade of downstream effector signalling. In the absence of a molecule that directly inhibits RAS, initial efforts focused on targeting the MAPK and PI3K pathways[8]. Unfortunately, clinical trials with these inhibitors (as single agents) have shown little or no antitumour activity in RAS-mutant cancers. Although combination therapies inhibiting both these pathways have shown promise in animal studies, clinical trials have been disappointing, with toxicity to normal tissue being a limiting concern[36]. However, given that the switch regions of RAS proteins associate with a large number (50–100) of effector proteins via their RAS-binding domains (RBDs), it has been

possible for us to exploit this biological process and develop small molecules that effectively block these interactions[37]. One such compound, rigosertib, acts as a small-molecule RAS mimetic that binds to RBDs of multiple RAS effectors to block their interaction with RAS. NMR spectroscopy revealed that this compound binds to the BRAF RBD at essentially the same location as the RAS switch I region. Rigosertib was found to inhibit both wild-type and mutant RAS signalling and is currently in phase III clinical trials for the treatment of myelodysplastic syndrome and acute myeloid leukaemia (AML).

Whereas targeting RAS appears to be within reach, there has been little or no success in developing compounds that bind directly to MYC or NF-κB with high affinity and inhibit their activity at levels that are adequate to enter clinical trials. However, there has been substantial progress in developing inhibitors that block *MYC* transcription. A small-molecule inhibitor (JQ1) of the BET family member BRD4 was found to downregulate MYC by displacing the bromodomain chromatin regulators from the large superenhancers of genes like *MYC*[38]. JQ1 effectively inhibits *Myc* transcription, resulting in significant antitumour activity in mouse models of cancer. Although it is suspected that some of these BET inhibitors may inhibit other oncogenic pathways, several BET inhibitors are currently in early-phase clinical trials for the treatment of haematological malignancies. Undoubtedly, these trials will provide substantial information on the utility of these inhibitors in treating MYC-driven cancers.

An important advance that is likely to have a major impact on targeting undruggable targets is the advent of PROTACs, which are bifunctional small molecules that simultaneously bind to a target protein and an E3 ubiquitin ligase, thereby causing ubiquitylation and degradation of the target protein[39]. The ability of PROTACs to degrade proteins regardless of their function makes this approach highly attractive, especially for those targets for which compounds can be developed that bind to a given target without inhibiting its activity. Degradation of the target protein by PROTACs is also suitable for targets that overcome the effect of an inhibitor by overexpression, which is often seen in cancer. A recent study shows that although BRD4 inhibitors rapidly lose efficacy owing to increased BRD4 expression, BRD4-PROTACs are unaffected by such a mechanism[39].

K.M.S. I firmly believe these undruggable targets are 'yet to be drugged'. Drug discovery requires a long-term outlook, and these targets will not succumb easily. When will we know that a target has been cracked? There are many false starts in drugging difficult targets. The research community is coming to define what constitutes a validated chemical tool, which is akin to the first step in a drug discovery project. For a list of criteria necessary to call a molecule a good lead, also called a chemical probe, see Stephen Frye's commentary[40] and also follow Derek Lowe's 'In the Pipeline' blog (see Further information).

I rely on three go-to criteria in evaluating first reports of a new probe or drug. The first is a consistent dose-dependent relationship between biochemical target engagement and cellular activity. The second is the availability of a co-crystal structure showing the drug binding to the protein. Many first reports do not include a co-crystal structure, and substitute this with other forms of data, but in my experience this is far less convincing than a crystal structure. The third criteria is proof that the first drug can be modified and its biochemical and cellular activity improved in a manner consistent with the structural model. Note that I do not include a requirement that the molecule work in an animal model. In my opinion, too many first reports describe animal efficacy data, long before the first three criteria are established, leading to false-positive proof of target inhibition. Something to look for if the report was published more than a year ago, is whether a follow-up study has appeared showing an improved version of the molecule and further proof of target engagement. If nothing appears after several years in the peer-reviewed literature, *bioRxiv* or published patent applications, you can bet the molecule was an artefact and the target remains undrugged.

L.S. We should remember that even protein tyrosine kinases were considered difficult targets only a couple of decades ago whereas today they represent the main weaponry in our arsenal of personalized anti-cancer medicines. Our frontiers are constantly expanding and we continue to push boundaries.

In general, with current technologies, we understand more aspects of protein structure production and stability, and we have more tools to finely tune some of these elements. Most drug discovery approaches used so far have relied on high-throughput screening (HTS), which has provided us with most of today's enzyme-targeting molecules. These are now rapidly being updated and adapted to incorporate non-enzyme targets. To do so, these approaches make use of new techniques, such as affinity-based techniques, including NMR-based screens, in which target-interacting molecules cause perturbations of the chemical shifts associated with N–H or C–H bonds within the target; this can provide insights into how to disrupt contact surfaces in PPIs[21]. Other techniques are also employed to assess changes in protein stability, and could be used to identify compounds that can increase the activity of tumour suppressors or promote degradation of oncoproteins. One example is differential scanning fluorimetry (DSF), which measures changes in the thermostability of a protein as a consequence of drug exposure[21]. Further improvements are achieved when HTS is preceded by *in silico* methods, especially 3D molecular modelling, to first enrich HTS with potential candidates based on structural predictions[21]. This approach appears to be more effective and selective.

One more issue to be solved to tackle more undruggable targets is the delivery of compounds to the appropriate tissue (for example, brain) or even cellular compartment (for example, nucleus). Cells in general are more permeable to small molecules than to biologics, and important advances have also been made towards more effective cell-penetrating peptide design, encapsulation in nanoparticles or viral delivery[2].

Overcoming the fear of the undruggable is by no means trivial, but there are already some success stories that should help us to keep an open mind and remain hopeful. Targeting the BCL-2 family of pro-survival factors perfectly exemplifies a positive outcome despite its premises: from a chemical standpoint, the BCL-2 family does not present hydrophobic pockets, has an intracellular location and possesses a rather flat contact surface; in principle a very unappealing target. BH3 mimetics have since been developed and proved capable of inhibiting BCL-2 clinically. For example, venetoclax (also known as ABT-199) was effective in clinical trials for refractory chronic lymphocytic leukaemia and is now advancing towards multiple trials for other malignancies[41]. In addition, preclinical studies indicate that targeting other pro-survival BCL-2 family members, particularly myeloid cell leukaemia 1 (MCL1)[42], could be beneficial for the treatment of multiple cancer types and could quickly follow the same path. This precedent should be emulated.

In general, new approaches other than small molecules are constantly emerging. To name a few: some researchers are revisiting older techniques but with more effective delivery systems (that is, siRNA[43] or antisense oligonucleotides in nanoparticles[44]), others are tweaking the ubiquitin system to hijack the degradation machinery and target specific cancer drivers[45], and some are developing small and large peptides to interfere with PPIs and overcome the limitations of small interacting surfaces[46].

What are the future challenges for this field of research?

C.V.D. This field of research is inherently technically challenging, but the bigger challenge could be the commitment of resources from stakeholders to invest in a high-risk area of research. Technically, target specificity of these new drug classes could be challenging, and the on-target effect on normal proliferating tissues would be another potential barrier to clinical development. For that reason, public research funding support will be essential to advance this field.

E.P.R. Despite dramatic responses to targeted therapies, the vast majority of patients develop resistance to these drugs and exhibit disease progression within 1–2 years. Resistance is a major challenge that needs to be fully addressed for the targeted therapies to succeed and we can presume that tumour cells will develop resistance to drugs targeting proteins such as RAS and MYC. We can infer this from studies with inducible KRAS-mutant PDAC model systems. Studies by Ronald DePinho's group[47] showed that some tumours induced by $Kras^{G12D}$ undergo spontaneous relapse following $Kras^{G12D}$ ablation owing to amplification and overexpression of the Yes-associated protein 1 (*Yap1*) transcriptional coactivator, which drives $Kras^{G12D}$-independent tumour maintenance. Using a similar $Kras^{G12D}$ model in a $Trp53^{+/Lox}$ background, Viale *et al.*[48] showed that a subpopulation of dormant tumour cells survives following *Kras* ablation, leading to tumour relapse. This relapse appears to be due to the expression of genes governing mitochondrial function, autophagy and lysosome activity, leading to decreased dependence on glycolysis for cellular energetics. We can expect similar resistance mechanisms for RAS antagonists. Similarly, in mouse models of BET inhibitor-resistant AML, activation of the WNT pathway was found to be the principal mediator of JQ1 resistance[49]. Again, similar mechanisms are likely to mediate resistance to MYC antagonists.

Another major challenge in treating patients with solid tumours is the heterogeneity of resistance mechanisms, and it is becoming clear that a single patient may have tumour cells with different mechanisms driving resistance at different sites or even within the same tumour. A recent study of epidermal growth factor receptor (*EGFR*)-mutated lung carcinomas revealed the existence of multiple resistance mutations in some patients, suggesting the presence of multiple but separate resistant clones or a single cell harbouring multiple resistance mechanisms[50].

The role that the immune system plays in conferring resistance to therapies adds another layer of complexity. Immune checkpoint inhibitors that are expected to revitalize the host's immune system seem to be effective in only a fraction of patients and even fewer achieve sustained responses[51]. Emerging evidence suggests that oncogenes such as *KRAS* modulate the immune system and thus subvert the host's immune response. A more thorough understanding of the relationship between oncogenic mutations and mechanisms by which they subvert the immune response will hopefully guide the development of sound and rational combination therapy regimens that achieve durable responses in cancer patients.

K.M.S. I think new chemical approaches might provide opportunities for overcoming the undruggable nature of these targets. I put these approaches into two buckets: new strategies inspired by serendipity and expanding our view of drug space.

The serendipitous discovery that 'imide' drugs such as thalidomide and lenalidomide bind to the E3 ubiquitin ligase CRBN, which causes CRBN to target neo-substrates for degradation, is transformational. Importantly, the targets for degradation can be proteins for which no known binding ligand exists[52]. Thus, using an imide endows CRBN with the ability to degrade a tough target. The field of degraders, especially the designer degraders created by Craig Crews termed PROTACs[53], could be ideal for MYC or AR-V7.

A very hot area is the expansion of chemical space, enabling targeting of proteins in new ways. One way for drugs to bind to proteins without traditional binding pockets is to rely on interactions beyond H-bonds, salt bridges and Van der Waals interactions. There has been a great deal of recent innovation in the area of covalent targeting. It is often assumed that covalent drugs are non-specific because they react with many targets based on their electrophilic nature. My favourite trick to overcoming this challenge comes from my colleague Jack Taunton who developed reversible covalent drugs to target non-conserved cysteines[54]. A covalent bond formed with proteins complementary to the rest of the drug molecule is more stable than bonds formed with proteins that are not able to bind to the remainder of the drug, thus simultaneously enhancing on-target and limiting off-target engagement. Expansion beyond the hypernucleophile cysteine is also gaining traction (for example, N-terminal amine, lysine or tyrosine). One intriguing example is a drug (GBT440) being developed for treatment of sickle cell disease that covalently binds to the N-terminal α-chain of haemoglobin S by way of an optimized aromatic aldehyde, and is based on earlier reports of similar chemotypes that increase the affinity of haemoglobin for oxygen[55]. That this simple functional group can so rapidly and selectively bind to the N-terminal α-chain in the presence of so many other competing amines certainly suggests that there are many opportunities to expand our repertoire of such modules. I look forward to many more advances in this area in the coming years.

L.S. I believe that the biggest challenge is scepticism. Changing paradigms and breaking dogmas are crucial to innovating and progressing in our field. To publish data that openly contradict previous concepts and the literature is, however, challenging and rarely possible. In this respect, as a young scientist (allow me to define myself so at least for a few more years) and as somebody working in the field of MYC inhibition, Ihave encountered more than one obstacle. MYC has for a long time been considered untouchable and one of those targets that allegedly falls into both aforementioned categories: a protein that should not be tampered with and a target too challenging to be inhibited effectively. Both beliefs have since been radically changed by the research that we have led over the past decade[2,56]. We have demonstrated MYC inhibition as an efficient therapeutic strategy in cancer, causing only very mild, well-tolerated and completely reversible side effects in normal tissues. Nevertheless, the challenges standing in the way of making a MYC inhibitor a reality in the clinic are not yet resolved — and not only for technical reasons. I am of the opinion, for example,

that high-risk projects presented by a junior principal investigator are seldom funded. Such research is expected and typically only funded when it comes from established scientists with a long and successful track record. How can we expect new ideas to come with such limitations imposed? We are not only ignoring many therapeutic opportunities in cancer by calling some targets undruggable, but also potential breakthrough science by not allowing younger researchers to pursue their ideas. If the concept is good and supported by valid science and preliminary data, the project should be fundable.

Many major historic milestones came from a little dose of recklessness combined with genius. After all, we would have never been able to fly if we had limited our tools to what Mother Nature had given to us terrestrial bipeds. Let us be brave, and identify novel tools to take us to new heights in targeting these crucial proteins. Mark my words: the undruggable is about to be drugged.

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