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Viewpoint

Drugging the Next Undruggable KRAS Allele-Gly12Asp

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ABSTRACT: Since its discovery as the first human oncogene in 1983, the small GTPase KRAS has been a major target of cancer drug discovery. The paper reported in this issue describes a long-awaited small molecule drug candidate of the oncogenic KRAS (G12D) mutant for the treatment of currently incurable pancreatic cancer.

Why has it taken almost 40 years to identify a ligand which binds and inhibits KRAS (G12D) as reported in acs.jmedchem.1c01688 in this issue?[1] KRAS drug discovery has been challenged by the seeming lack of a druggable pocket on the protein for 30 years. In late 2012 and early 2013, four seminal papers described candidate small molecules with the ability to bind to three regions of KRAS. The Genentech group[2] and the Vanderbilt group headed by Stephen Fesik[3] used structure activity relationship by NMR (SAR by NMR) screening to identify indole containing lead fragments that bound to a pocket near two mobile elements (switch I and switch II) of KRAS. The next papers, one from Nathanael Gray at the Dana Farber Cancer Institute[4] and one from our lab at UCSF,[5] exploited a particular oncogenic cysteine mutant of KRAS (G12C), which is particularly frequent in lung cancer. The Gray lab developed GTP analogs bearing reactive warheads in place of the γ-phosphate that uniquely reacted with the oncogenic KRAS (G12C) and inhibited its function. Subsequently, the challenge of competing with the pM affinity of KRAS for its native nucleotide and cell permeability challenges of GDP and GTP analogs prevented further development of this approach. The KRAS (G12C) inhibitors from our lab reported in 2013 revealed a pocket under switch II, which is the site where the reversible ligands reported in this issue bind (Figure 1). Targeting the switch II pocket of KRAS (G12C) was clinically validated by Amgen (AMG510-sotorasib) and Mirati (MRTX849-adagrasib). Sotorasib was approved in May 2021, and many other KRAS (G12C) inhibitors are currently under clinical study; however, it was not immediately possible to develop inhibitors of the other KRAS oncogenic alleles, such as G12D, which predominates in pancreatic cancer.

Why did it take eight years to convert an irreversible KRAS (G12C) inhibitor into a reversible inhibitor of the most common KRAS mutant (G12D)? It is useful to consider the extremes of irreversible inhibitors—those driven by high reversible affinity and slow covalent bond formation compared to those with weak reversible affinity and rapid covalent bond formation. The early examples of KRAS (G12C) inhibitors showed weak reversible affinity for the protein and first order rate constants for cysteine covalent bond formation. The subsequent medicinal chemistry efforts to improve potency (captured by the overall second order rate constant) by almost 5 orders of magnitude. However, the surprising result of this medicinal chemistry campaign was that the improvement was almost exclusively in the k_{inact} (1/s)—the reversible K_i values for the first reported G12C inhibitor and ARS-1620 were >64 μM.[6]

The two key medicinal chemistry questions regarding reversible targeting of KRAS (G12D) were (1) whether improvements in affinity were achievable or whether a paucity of interactions in the switch II pocket of KRAS would preclude high affinity recognition and (2) whether interactions with the 12-aspartate could be made sufficiently selectively to limit wildtype (WT) KRAS binding.

The medicinal chemistry campaign began with a key idea to introduce a salt bridge between the SIIP inhibitor and Asp-12 to compensate for the lack of the acrylamide−Cys12 covalent bond. The Mirati G12C clinical molecule adagrasib contains an unusual piperazine C-2 cyanomethyl group not shared with the sotorasib or ARS-1620. In terms of k_{inact}, this group increased the electrophilicity of the acrylamide cysteine warhead.[7] This led to the inclusion of the 2-fluoro acrylamide to reduce the electrophilicity through warhead modification, leading to adagrasib. In terms of reversible binding affinity, the X-ray cocrystal structure of adagrasib showed the cyano N: making an interaction with the carbonyl of Gly-10 and displacing a water in the bottom of the switch II pocket. Furthermore, adagrasib contains an *N*-methylpyrrolidine attached at C-2 of the core, and this basic moiety forms a new ionic interaction with the side chain of Glu-62. These unique features of adagrasib compared to sotorasib and ARS-1620 appear to have provided one of the important steps toward achieving reversible binding affinity.

As is often the case, the continued optimization of a lead involves one step forward and several steps backward in order

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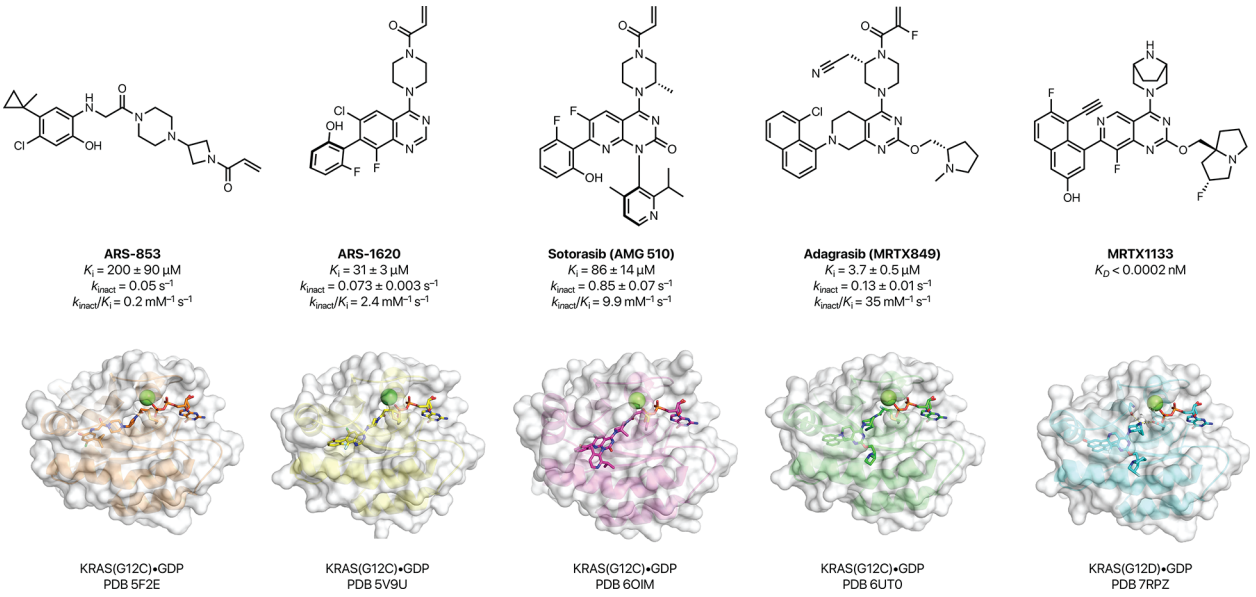

Figure 1. Evolution of switch II pocket inhibitors of KRAS oncogenes.

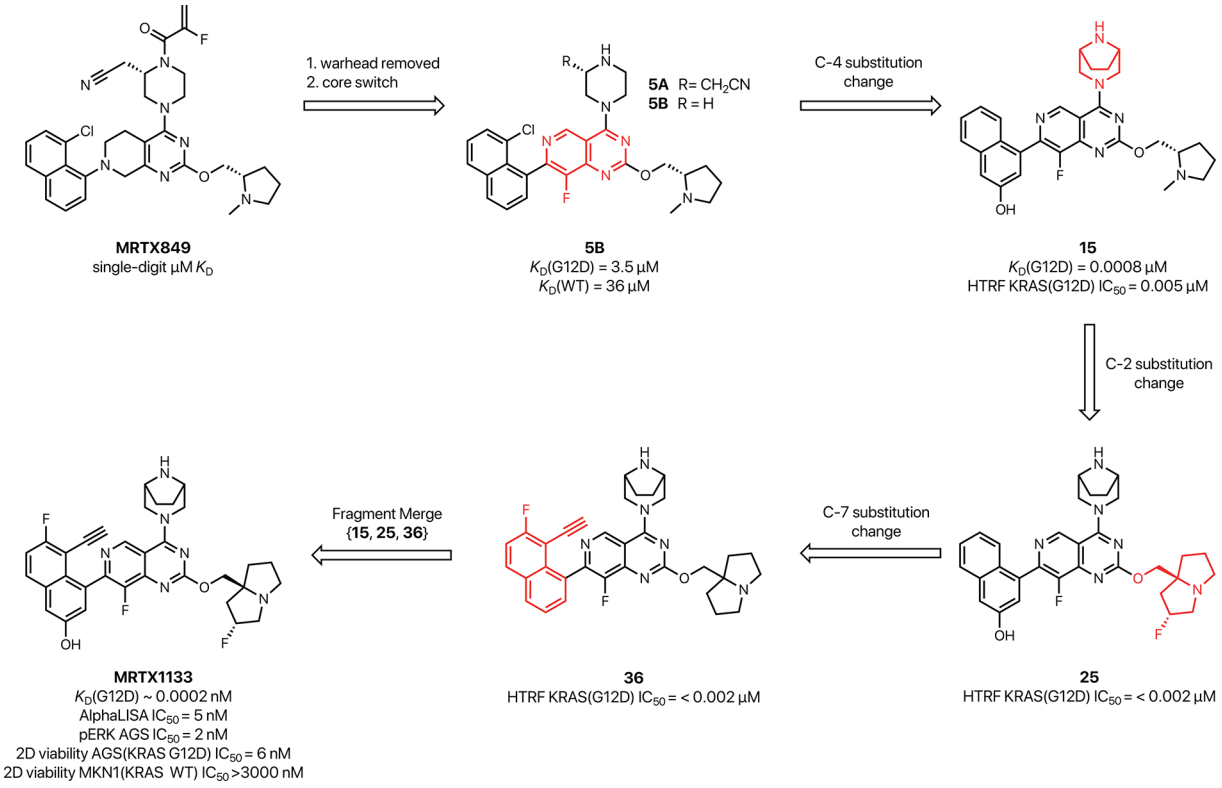

Figure 2. Medicinal chemistry campaign toward MRTX1133.

to achieve the desired goal. As evidence of this, the cyanomethyl piperazine was less active against the KRAS (G12D) target, when the core tetrahydropyridopyrimidine from adagrasib was substituted with a pyrido[4,3-*d*]pyrimidine core[8] (Figure 2, **5A** vs **5B**). These modifications led to a reversible measured binding affinity of 3.5 μM against G12D compared to 36 μM against WT KRAS. The cocrystal structure of **5B** with KRAS (G12D) showed a direct salt bridge contact between the piperidinyl N and the carboxylate of Asp-12 along with interactions with the Gly-60 carbonyl. A 10× preference for the oncogenic G12D compared to WT and the low micromolar reversible affinity demonstrated strong early evidence that the switch II pocket could be mined for improved reversible affinity and oncogene selectivity.

The cocrystal structure of **5B** with KRAS (G12D) also suggested a potential improvement could be gained by interacting with the carbonyl of Gly-10, as the cyanomethyl substituent of adagrasib did, but this would require exploration of more analogs of the piperazine. This effort led the group to discovery of **15**, which contains a [3.2.1]bicyclic diamino substituent replacement of piperazine. **15** broke the micromolar affinity barrier and exhibited improved selectivity for

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G12D over WT. The cocrystal structure of **15** with KRAS (G12D) revealed that the rigid, bicyclic amine is positioned to preserve the Gly-60 and Asp-12 salt bridge interactions, while the two carbon bridge displaces the bound water, and one of the endo C-H's forms a nonclassical hydrogen bond to the Gly-10 carbonyl.

Further iterative exploration of the C-2 and C-7 substituents identified and explored extensively in other KRAS (G12C) programs led to MRTX1133. A late surprise in the final push toward MRTX1133 was the emergence of an 8-ethynl substituent on the C-7 naphthyl. The cocrystal structure revealed an intricate hydrogen bond network with the Gly-10 bound water molecule that was displaced in the early optimization efforts but now reappeared in the cocrystal structure with the more optimized lead. The systematic interrogation of elements of the switch II pocket is an excellent example of modern structure guided medicinal chemistry on a challenging and clinically important target.

One aspect which we have not addressed in this Viewpoint is the nucleotide state specific nature of the switch II pocket. In the case of KRAS (G12C), the clinical agents sotorasib and adagrasib only interact with the GDP (OFF) state of the protein. What is the situation with MRTX1133 and related highly reversible affinity ligands? Our lab recently collaborated with scientists at Promega to address this question and found that in fact the new G12D ligands are able to bind to the GTP (ON) state, albeit with weaker affinity than for the GDP state.[9] This will be another aspect of drugging RAS to continue to explore especially in the context of clinical efficacy and combination therapies.

It is exciting to enter 2022 with the high likelihood of seeing derivatives based on MRTX1133 enter clinical trials in pancreatic cancer patients with KRAS (G12D) driven tumors. If the experience with KRAS (G12C) inhibitors over the last four years is repeated, we can expect multiple companies to be mining the interactions in the switch II pocket and hopefully see multiple trials opening in the KRAS (G12D) space.

What else is on the horizon for direct KRAS targeted agents? Boehringer Ingelheim scientists have mined the early work from Genentech and Fesik laboratories to develop ligands to the switch I/II pocket and attach E3 recruiting elements. Completely new approaches based on using cyclophilin recruitment to KRAS (G12C) and other alleles are approaching the clinic from Revolution Medicines. It is exciting to imagine how many new modalities of drug discovery are now being applied to this long-known target, and—if the exciting responses of KRAS (G12C) patients to sotorasib and adagrasib are an indication—we should see clinical benefits in challenging KRAS diseases.

Taking a step back from KRAS for a moment, what does the MRTX1133 story tell us about the state of medicinal chemistry in 2022? Traditional structure-based design, iterative synthesis, and testing of analogs has been the mainstay of drug discovery (since the work from Merck scientists who discovered the first HIV protease inhibitor in the 1980s), and this same basic strategy continues to tackle the hardest problems in medicine. In this era of unparalleled computational power and achievements, such as AlphaFold predicting the structure of every protein, when will chemists see a fundamental change in the way drugs are discovered? KRAS would be an excellent testing ground for modern computational chemistry methods, but we doubt they will beat the traditional approach to the clinic.

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Notes

The authors declare the following competing financial interest(s): K.M.S. has several conflicts related to KRAS drug discovery due to patent filings by the University of California, San Francisco related to the switch II pocket of KRAS and stock and cash compensation from the following companies: Apertor, BioTheryX, BridGene Biosciences, Denali Therapeutics, eFFECTOR Therapeutics, Erasca, G Protein Therapeutics, Genentech/Roche, Ikena, Initial Therapeutics Janssen Pharmaceuticals, Kumquat Biosciences, Kura Oncology, Merck, Mitokinin, Nested, Nextech, Radd Pharma, Turning Point, Type6 Therapeutics, and Wellspring Biosciences (Araxes Pharma).

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