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## Update: KRAS Inhibition in Cancer Management

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## Abstract

With >20% prevalence across all human tumors, mutant KRAS has been a primary target of oncological drug development for nearly 40 years. Until recently, attempts at pharmacologic inhibition of this protein did not lead to any clinically-approved inhibitors; as a result, KRAS had been deemed “undruggable”. However, during the past decade there has been a surge of direct and indirect KRAS inhibitors in clinical trials. This update article will summarize the recent data on these drugs, including (1) small molecule inhibitors targeting the *KRAS*<sup>G12C</sup> mutant (2) RNA therapeutics targeting *KRAS*<sup>G12D</sup>, (3) mRNA vaccines targeting a variety of KRAS mutants, (4) pan-KRAS inhibition targeting SOS1, an upstream activator of KRAS, and (5) adoptive T-cell therapy targeting the G12D and G12V mutants. These nascent treatments may bring new anti-cancer options against a key oncologic regulator which formerly had been considered undruggable.

## Introduction

The KRAS protein was discovered in extracts of virally-transformed cells in 1979.<sup>1</sup> Within a few years, the *KRAS* genetic sequence was published,<sup>2</sup> human genes homologous to the viral genes were identified,<sup>3,4</sup> the oncogenicity of Ras mutants in human tumors was demonstrated,<sup>5-7</sup> and a high frequency of KRAS mutation was detected in human cancers.<sup>8-11</sup> Thereafter, mutant KRAS became a target for cancer therapy.<sup>12</sup> Members of the Ras family (which includes *KRAS*, *HRAS*, and *NRAS*) are among the frequently mutated genes in cancer, with a prevalence of ~25% in all human tumors<sup>13</sup>. Specifically, *KRAS* (for Kirsten Rat Sarcoma virus) mutants are involved with oncogenesis in 90% of pancreatic cancers, 30-40% of colorectal cancers (CRC), and 5-20% of lung cancers<sup>14,15</sup>. The GTPase function of the KRAS protein acts as powerful “master” switch in major cellular pathways, including RAF/MAPK and PI3K/Akt, which regulate essential cellular functions such as proliferation, differentiation, survival, immunological responses, and stromal integrity (Fig. 1). Activated KRAS mutants<sup>16,17</sup> (and, to a lesser extent, amplification of wild-type KRAS<sup>18-20</sup>) dysregulate these systems, promoting tumor proliferation and protecting from anti-tumor mechanisms. Despite years of attempts to produce a pharmacologic inhibitor of KRAS or its mutants, no approved therapy has made it to the clinic; KRAS was deemed to be “undruggable”.<sup>12,21,22</sup>

The past decade has seen a resurgence in the development of pharmacologic KRAS inhibitors. The intent of this update is to outline the various inhibitor mechanisms and the current experimental therapeutics that are in clinical trials, including: (1) small-molecule direct inhibitors of the KRAS G12C mutant; (2) small interfering RNA (siRNA) targeting the KRAS G12D mutant; (3) An mRNA vaccine targeting common oncogenic KRAS mutations; (4) A pan-KRAS

inhibitor working through GEF blockade; and (5) adoptive T-cell therapy against tumors with KRAS G12D or G12V mutations.

## **KRAS Biology and Signaling**

There are three isoforms of the 21 kDa Ras protein: HRAS (Harvey Rat Sarcoma), NRAS (Neuroblastoma Ras), and KRAS.<sup>13</sup> There are two copies of the *KRAS* gene: *KRAS1* and *KRAS2*; the latter (simply referred to as *KRAS*) is the proto-oncogene.<sup>13</sup> KRAS has six exons, which can be alternatively spliced into two transcripts, *KRAS4A* and *KRAS4B*; the latter is translated into the KRAS protein of interest.<sup>23</sup> KRAS has three primary domains, including the G-domain (residues 1-166), which contains a highly conserved GDP-GTP binding region with two biologically-relevant “Switch” loops (I and II).<sup>24</sup> The other two domains (C-terminal domain and the CAAX motif), are more variable and participate in post-translational modifications and membrane trafficking.

KRAS activation occurs when this protein binds to GTP, producing a conformational change in KRAS, which then results in a series of protein-protein interactions at the plasma membrane, followed by propagation of signaling to downstream effectors, including the MAPK and PI3K/Akt pathways, which results in upregulation of cellular survival and proliferation (Fig. 1), along with other effects. Displacement of GDP by GTP to produce this active state is facilitated by Guanine Nucleotide Exchange Factors (GEFs). Hydrolysis of the bound GTP to GDP switches KRAS back to the inactive state; this inactivation is catalyzed by GTPase-Activating Proteins (GAPs). The reversible binding and exchange of the guanine nucleotides

within the G-domain produces the molecular switch function of KRAS, in which KRAS-GTP is “on” (signal propagation) and KRAS-GDP is “off” (no signal propagation).

Most KRAS-activating mutations in cancer are single nucleotide substitutions at codons 12, 13, and 61.<sup>14</sup> Codons 12 and 13 account for nearly 90% of KRAS-driven cancers. The single amino acid substitution of glycine at position 12 (G12) produces steric interference with GAP binding to the Switch-II pocket of KRAS, thus preventing catalytic hydrolysis of GTP, which results in constitutive activation of KRAS.<sup>14</sup> Inhibition of GTP binding to KRAS mutants with a small molecule appeared to be a natural anti-cancer approach; however, this strategy proved difficult due to the protein’s relatively smooth surface and shallow binding pockets, along with its picomolar affinity for GTP.<sup>21,25</sup> Research focus in this area gradually shifted to other participants in pathways influenced by mutant KRAS.<sup>26</sup>

The RAF-MAPK pathway became a primary main target due to its regulation of cell cycle progression and mitotic activation.<sup>27</sup> Inhibition of BRAF, MEK, or ERK was found to have clinical efficacy in a small subset of cancer patients, but was not efficacious in KRAS-mutant cancers.<sup>14,28</sup> This inhibitor resistance was due to the fact that *(i)* KRAS drives oncogenesis through multiple different pathways, while downstream inhibitors are only specific to the RAF/MAPK pathway, and *(ii)* tumor cells develop bypass mechanisms that allow for the RAF/MAPK pathway to remain constitutively activated while specific participants remain inhibited.<sup>29</sup> While this former circumstance can be overcome by the direct inhibition of mutant KRAS, the latter circumstance theoretically could compensate for direct KRAS inhibition, thereby decreasing its anticancer efficacy.<sup>30</sup> Some clinical trials currently underway are attempting to impair these bypass mechanisms through simultaneous targeting of multiple participants in a given pathway (vertical polytherapy).<sup>31</sup>

## Direct Inhibitors

The KRAS G12C mutation is the most common mutation in non-small cell lung cancer, with a prevalence of approximately 10-11%; this mutation also is present in 3-4% of colorectal adenocarcinomas and 1-3% of other solid tumors.<sup>32</sup> Drug discovery efforts found that quinazolinone-based entities could dock into a “cryptic pocket” (associated with residues H95/Y96/Q99) and covalently bind to the cysteine residue at position 12,<sup>25</sup> thus locking KRAS in the GDP-bound state and blocking constitutive activation. The proof-of-concept drug to exploit this cryptic pocket in experimental tumor models was ARS-1620, which then led to AMG 510.<sup>25,33</sup>

### *Sotorasib (AMG 510)*

Amgen’s experimental therapeutic sotorasib (AMG 510) has a structure which exploits the cryptic groove associated with the KRAS His95 residue, producing a 10-fold increase in binding potency over ARS-1620 in mutant cell lines.<sup>34</sup> Treatment of cell lines expressing KRAS G12C with sotorasib decreased phosphorylation of ERK, Akt, and S6 and increased Caspase-3 cleavage, with inhibition of cellular proliferation and survival.<sup>35</sup> Sotorasib had minimal to no effect on cell lines that did not express KRAS G12C. In murine *KRAS*<sup>G12C</sup> solid tumor models, sotorasib treatment along with PD-1 inhibition produced durable (112 day) tumor regression. Of note, mice lacking competent T cells had regression but not a durable “cure”,<sup>35</sup> implying an important role of immune response in these models.

In 2018, sotorasib became the first direct KRAS inhibitor to enter clinical testing.<sup>36</sup> In a phase 1 dose escalation and expansion trial involving 129 patients with advanced, heavily-pretreated solid tumor patients with demonstrated *KRAS*<sup>G12C</sup> mutation,<sup>37</sup> daily oral administration of sotorasib resulted in treatment-related adverse events in 57% of patients (most commonly diarrhea, fatigue, and nausea; 11.6% grade 3-4), but no treatment-related deaths. Objective response and disease control rates were 32% and 88% in patients with NSCLC and 32% and 74% in patients with colorectal cancer, respectively. Median duration of response was 10.9 months and 5.4 months in NSCLC and colorectal cancer, respectively. Other clinical trials are underway (Table 1), including: (1) a phase 1 study of combined treatment with sotorasib and other chemotherapeutics (including other components of the KRAS pathway or immune checkpoint inhibition) in advanced solid tumors with KRAS G12C (NCT04185883); (2) a phase 1/2 study of sotorasib mono- and combined therapy in solid tumors with the G12 C mutation (NCT03600883); and (3) a phase 3 study of sotorasib vs. docetaxel in the treatment of NSCLC with the G12C mutation (NCT04303780). The clinical outcome of combined therapy with AMG sotorasib and immune checkpoint inhibition will be particularly interesting, considering the preclinical efficacy of this combination in the above murine models,<sup>35</sup> which underlines the topical importance of anti-cancer T-cell activity.<sup>38</sup>

### *MRTX849*

Mirati Therapeutics' MRTX849 is another direct KRAS inhibitor that with potential efficacy in the treatment of KRAS G12C mutant cancers. Similar to sotorasib, MRTX849 (adagrasib) forms a covalent bond with the cysteine substituted at position 12, locking KRAS in

its inactive state.<sup>39</sup> MRTX849 has been shown to specifically inhibit KRAS G12C in mutant cell lines, while preserving function of wild-type KRAS in non-cancerous cells; in addition, this inhibitor had durable efficacy against KRAS G12C tumor xenografts in nude mice (55 days of complete regression).<sup>40</sup>

MRTX849 is being evaluated as monotherapy or in various combinations in a phase I/II clinical trial of advanced solid tumor patients (NCT03785249), with some partial responses reported.<sup>41</sup> As of August 2020, 79 patients with NSCLC were treated in the study;<sup>42</sup> the most common treatment-related adverse events include nausea, diarrhea, vomiting and fatigue. The only grade 3-4 serious adverse event was hyponatremia, which occurred in 3% of patients. Among 14 evaluable patients, the objective response rate was 43% with 4/6 patients having an ongoing response of greater than 11 months. The recommended phase 2 dose was identified as 600 mg twice daily.

Another phase I/II clinical trial is studying MRTX849 in combination with TNO155 (NCT04330664; Table 1); the latter is an inhibitor of SHP2 (Src homology region 2 domain-containing phosphatase-2, also known as PTPN11 or PTP-1D). SHP2 is a tyrosine-protein phosphatase that promotes RTK-KRAS signaling in KRAS-driven cancers<sup>20,43,44</sup> through a variety of mechanisms, including GEF complexation (Fig.1).<sup>45</sup> Combined inhibition of mutant KRAS and SHP2 may have synergistic effects in KRAS-driven cancer (another example of vertical polytherapy).

#### *Other Direct KRAS Inhibitors*

Two novel KRAS G12C inhibitors, LY349446 (Eli Lilly and Company,) and JNJ-74699157 (Johnson & Johnson/ Janssen Research & Development,) also entered phase I clinical trials in patients with advanced solid tumors at the end of 2019 (NCT04165031 and NCT04006301, respectively).<sup>46</sup>

### *pan-KRAS inhibition*

Another approach to disrupt KRAS signaling is through inhibition of SOS1 (Son Of Sevenless 1), a GEF that promotes KRAS activation. SOS1 facilitation of the GDP-GTP switch on KRAS is increased through growth factor signaling and tyrosine kinase activity, working through the mediator protein Grb2 (Fig. 1). BI 1701963 is a small molecule that binds SOS1 and prevents interaction with KRAS-GDP, thus preventing the switching-on of KRAS.<sup>47-49</sup> As a pan-KRAS inhibitor and the first in clinical trials (NCT04111458), BI 170963 potentially could be used in nearly 15% of all cancers (i.e., those in which a KRAS mutation plays a major role). There is interest in combining BI 1701963 with other KRAS pathway inhibitors,<sup>49</sup> such as trametinib (a MEK inhibitor with clinical efficacy against melanoma with *BRAF*<sup>V600E/K</sup>).<sup>50</sup> The downfall of inhibiting of pan-KRAS inhibition, however, is the effect on wild-type KRAS in normal cells;<sup>48</sup> data on this potential issue should be available upon completion of the ongoing Phase 1 trial (NCT04111458; Table 1).

### **RNA Therapeutics**

Therapy with RNA interference (RNAi) involves the delivery of small interfering RNA (siRNA; <30 base pair length) to cells expressing a complementary mRNA template, which then complex to form double stranded RNA. As dsRNA typically is found only in virally infected cells, a mechanism has evolved to digest and silence intracellular dsRNA (i.e., dsRNA is perceived as foreign genetic material). RNAi therapy takes advantage of this defense mechanism by targeting siRNA to mRNA that code for disease-relevant proteins, thus decreasing or preventing translation of the targeted protein (“knockdown”). In 2018, the first RNAi-based drug (patisiran) was approved for the treatment of transthyretin amyloidosis.<sup>51</sup> Unfortunately, therapy with RNAi remains limited by pharmacokinetic issues, including a short serum half-life of siRNA, inefficient delivery of the siRNA to the tumor, and intracellular digestion of these sequences once delivered.<sup>52</sup> While most of the small molecule inhibitors of the KRAS protein target the G12C mutation that primarily is found in NSCLC and colorectal cancer, RNAi therapy has focused primarily on the G12D mutation, a predominant driver of pancreatic ductal adenocarcinoma (PDAC). The substitution of aspartate at the glycine-12 position has a prevalence of nearly 30% of all PDAC patients, and is associated with a worse overall survival compared to other *KRAS* mutations seen in PDAC.<sup>23</sup>

### *AZD4785*

AZD4785 was the first RNA therapeutic targeting mutant KRAS to reach clinical trials in cancer patients (NCT03101839). This entity is an antisense oligonucleotide (i.e., not an siRNA) which utilizes a mechanism similar to RNAi, though the route of degradation is different.<sup>53</sup> AZD4785 demonstrated anti-tumor efficacy in KRAS-mutant cell- and patient-derived

xenografts within nude mice, and was capable of targeting multiple isoforms of mutant KRAS.<sup>54</sup> In the phase 1 trial, however, 15/28 subjects did not complete the 6-month observation period secondary to death or early decision to withdraw. It is not clear whether further clinical study with AZD4785 will be undertaken.<sup>55</sup>

### *iExosomes*

Exosomes are extracellular signaling vesicles (30-150 nm diameter) utilized by multiple cell types, and can carry a wide range of molecules; exosomes can participate in the maintenance, progression, and metastasis of cancer cells.<sup>56</sup> Exosomal delivery of therapeutics has been studied in a range of diseases, including cancer.<sup>57</sup> iExosomes are engineered exosomes<sup>58</sup> which contain KRAS G12D siRNA and possess a transmembrane signaling ligand CD47. The latter binds to SIRP-alpha on phagocytes and thereby inhibits phagocytosis,<sup>58</sup> thus prolonging exosome half-life. iExosomes demonstrated enhanced pancreatic tumor uptake compared to liposomes and other nanoparticles utilized for RNAi delivery, knocked down KRAS G12D in murine tumors models to nearly undetectable levels after 30 days of treatment, and also prolonged survival in these models.<sup>58,59</sup> iExosome delivery of KRAS G12D siRNA is in a phase I clinical trial for patients with metastatic PDAC (NCT03608631).

### *LODER™*

The LODER™ (Local Drug EluteR) devices consists of a biodegradable polymeric matrix containing KRAS G12D siRNA.<sup>60</sup> This matrix can be manually embedded/injected into

the tumor, and then provides a sustained local release of siRNA for approximately four months.<sup>60</sup> A Phase I/II clinical trial of 15 patients with locally advanced PDAC studied LODER™ administered in combination with standard chemotherapeutics (NCT01188785) demonstrated a 60% partial response and 40% no progression. There were some apparent drug-related adverse effects including pancytopenia and colonic obstruction, as well as procedure-related adverse effects such as cholangitis.<sup>61</sup> The LODER matrix is currently in a phase II clinical trial in combination with chemotherapy for locally advanced PDAC (NCT01676259); patient accrual is expected to be completed in December 2020. The original study design would have compared standard chemotherapy with and without LODER™, but after an interim analysis this design was revised so that all patients would receive LODER™,<sup>62</sup> suggesting some efficacy has been observed. Of note, LODER™ was designed for treatment of local disease, and would not be suitable for metastatic cancer,<sup>63</sup> in contrast to systemic treatments such as the above iExosome therapy.

## **Immunotherapy**

Current efforts to enhance immune attack of KRAS-driven tumors focus on cytotoxic (T-cell mediated) mechanisms, whether through augmentation of native T-cell response with a KRAS tumor vaccine or by infusion of engineered, expanded T-cells that attack KRAS tumor cells.

### *mRNA Vaccine*

Moderna and Merck are collaborating on a vaccine (mRNA-5671/V941) that would target common KRAS mutations.<sup>64</sup> For decades it has been known that that presentation of mutant KRAS peptides in patients can induce a specific T-cell immune response which kills KRAS-mutant tumor cells but not normal cells,<sup>65-69</sup> implying that KRAS appears on the cell surface during processing. However, past trials of KRAS vaccines so far have not demonstrated adequate anti-tumor immune responses or efficacy;<sup>70</sup> active investigation into a KRAS vaccine therefore continues. The above mRNA vaccine consists of mutant KRAS mRNA housed in lipid nanoparticles, which is taken up by antigen presenting cells. The APCs would translate the KRAS mRNA and present the resultant protein with the major histocompatibility complex, thus eliciting a T-cell mediated response against tumor cells that express the KRAS mutant protein. With sequences specific for KRAS mutations G12C, G12D, G12V, and G13D, mRNA-5671 potentially can target ~83% of all KRAS-driven tumors.<sup>71</sup> Similar to RNAi therapeutics, the vaccine is dependent on a delivery system that must protect the nucleotides from degradation while engaging the APCs.<sup>72</sup> The mRNA-5671 vaccine currently is in a phase I clinical (with or without the PD-1 inhibitor pembrolizumab) in patients with KRAS Mutant NSCLC, colorectal cancer, or PDAC (NCT03948763). Combination of this mRNA vaccine with a PD-1 inhibitor (another immunotherapeutic, but with a different mechanism) may prove beneficial in the upregulation of T-cell response against tumors expressing mutant KRAS, hopefully providing clinically impactful anti-tumor activity.

*ACT with Engineered mTCR*

Human T lymphocytes can be modified with murine T cell receptors (mTCRs; derived from transgenic HLA mice) that are engineered to recognize human tumor epitopes with HLA restriction; infusion of these modified T cells results in specific killing of cells with the targeted epitope.<sup>73</sup> Anti-tumor treatment with such engineered T cells is an example of Adoptive Cellular Therapy (ACT), alternatively known as adoptive T-cell therapy, or adoptive immunotherapy. In early clinical trials of ACT with engineered mTCRs, toxicity secondary to destruction of normal tissue was noted.<sup>74,75</sup> This toxicity is a reflection of the fact that many tumor antigens are also expressed to a variable degree in normal tissue.<sup>76</sup> In a case report of a patient with metastatic colorectal cancer, treatment with autologous *ex vivo*-expanded Tumor-Infiltrating Lymphocytes (TILs) that specifically targeted the KRAS G12D mutant produced regression of metastatic lung lesions,<sup>77</sup> which suggested that adoptive transfer of T cells targeting the KRAS mutant could have anti-tumor efficacy with less risk of toxicity. Currently, ACT with mTCRs specific for the KRAS G12D and G12V mutants is being evaluated in phase I/II clinical trials involving patients with metastatic or unresectable cancer (NCT03745326; NCT03190941). Therapy with T lymphocytes expressing engineered Chimeric Antigen Receptors (CAR-T)<sup>78</sup> specific for KRAS mutants has not yet reached the clinical trial stage.

## **Conclusion**

The KRAS oncogene contributes to approximately 125,000 cancer patient deaths per year in the US.<sup>14</sup> Previous attempts to inhibit KRAS yielded no approved direct inhibitor therapies, and the protein was deemed “undruggable”. However, persistent efforts to develop such an inhibitor has produced some promising candidates which now are in clinical trials. These

candidate drugs utilize an array of mechanistic approaches to decrease KRAS activity, including blockade of critical binding sites on the mutant protein itself, inhibition of other proteins critical for KRAS signal transduction, degradation of mutant KRAS mRNA, vaccines against mutant KRAS, and adoptive T-cell therapy. Preliminary data suggest that KRAS inhibitors (such as sotorasib and adagrasib) have acceptable toxicity profiles and promising clinical activity in patients with advanced solid tumors harboring KRASG12C mutations. Additional novel KRAS inhibitors in preclinical testing (not discussed here) will likely enter clinical trials in the near future. There now is renewed hope that a specific inhibitor for mutant KRAS will become available. As an archetype of precision medicine, such an inhibitor would be intended for the patient whose tumor possessed the specific mutation targeted by the inhibitor. Such treatment should be both efficacious and well-tolerated.

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## References

1. Shih TY, Weeks MO, Young HA, Scholnick EM. Identification of a sarcoma virus-coded phosphoprotein in nonproducer cells transformed by Kirsten or Harvey murine sarcoma virus. *Virology*. 1979;96(1):64-79. PMID: 223311. DOI: 10.1016/0042-6822(79)90173-9
2. Tsuchida N, Ryder T, Ohtsubo E. Nucleotide sequence of the oncogene encoding the p21 transforming protein of Kirsten murine sarcoma virus. *Science*. 1982;217(4563):937-939. PMID: 6287573. DOI: 10.1126/science.6287573
3. Chang EH, Gonda MA, Ellis RW, Scolnick EM, Lowy DR. Human genome contains four genes homologous to transforming genes of Harvey and Kirsten murine sarcoma viruses. *Proc Natl Acad Sci U S A*. 1982;79(16):4848-4852. PMID: 6289320. DOI: 10.1073/pnas.79.16.4848
4. Ellis RW, Defeo D, Shih TY, Gonda MA, Young HA, Tsuchida N, Lowy DR, Scolnick EM. The p21 src genes of Harvey and Kirsten sarcoma viruses originate from divergent members of a family of normal vertebrate genes. *Nature*. 1981;292(5823):506-511. PMID: 6265801. DOI: 10.1038/292506a0
5. Santos E, Tronick SR, Aaronson SA, Pulciani S, Barbacid M. T24 human bladder carcinoma oncogene is an activated form of the normal human homologue of BALB- and Harvey-MSV transforming genes. *Nature*. 1982;298(5872):343-347. PMID: 6283384. DOI: 10.1038/298343a0
6. Taparowsky E, Suard Y, Fasano O, Shimizu K, Goldfarb M, Wigler M. Activation of the T24 bladder carcinoma transforming gene is linked to a single amino acid change. *Nature*. 1982;300(5894):762-765. PMID: 7177195. DOI: 10.1038/300762a0
7. Der CJ, Cooper GM. Altered gene products are associated with activation of cellular *ras*<sup>k</sup> genes in human lung and colon carcinomas. *Cell*. 1983;32(1):201-208. PMID: 6825168. DOI: 10.1016/0092-8674(83)90510-x

8. Bos JL, Fearon ER, Hamilton SR, Verlaan-de Vries M, van Boom JH, van der Eb AJ, Vogelstein B. Prevalence of ras gene mutations in human colorectal cancers. *Nature*. 1987;327(6120):293-297. PMID: 3587348. DOI: 10.1038/327293a0
9. Forrester K, Almoguera C, Han K, Grizzle WE, Perucho M. Detection of high incidence of K-ras oncogenes during human colon tumorigenesis. *Nature*. 1987;327(6120):298-303. PMID: 2438556. DOI: 10.1038/327298a0
10. Rodenhuis S, Slebos RJ, Boot AJ, Evers SG, Mooi WJ, Wagenaar SS, van Bodegom PC, Bos JL. Incidence and possible clinical significance of K-ras oncogene activation in adenocarcinoma of the human lung. *Cancer Res*. 1988;48(20):5738-5741. PMID: 3048648. DOI:
11. Smit VT, Boot AJ, Smits AM, Fleuren GJ, Cornelisse CJ, Bos JL. KRAS codon 12 mutations occur very frequently in pancreatic adenocarcinomas. *Nucleic Acids Res*. 1988;16(16):7773-7782. PMID: 3047672. DOI: 10.1093/nar/16.16.7773
12. Uprety D, Adjei AA. KRAS: From undruggable to a druggable Cancer Target. *Cancer Treat Rev*. 2020;89:102070. PMID: 32711246. DOI: 10.1016/j.ctrv.2020.102070
13. Hobbs GA, Der CJ, Rossman KL. RAS isoforms and mutations in cancer at a glance. *J Cell Sci*. 2016;129(7):1287-1292. PMID: 26985062. DOI: 10.1242/jcs.182873
14. Liu P, Wang Y, Li X. Targeting the untargetable KRAS in cancer therapy. *Acta Pharm Sin B*. 2019;9(5):871-879. PMID: 31649840. DOI: 10.1016/j.apsb.2019.03.002
15. Cancer Facts & Figures. *American Cancer Society Journal*. 2019. PMID: DOI:
16. Jančík S, Drábek J, Radzioch D, Hajdúch M. Clinical Relevance of KRAS in Human Cancers. *Journal of Biomedicine and Biotechnology*. 2010;2010:150960. PMID: DOI: 10.1155/2010/150960
17. Zander DS, Farver CF. Chapter 18 - Molecular Basis of Pulmonary Disease. In: Coleman WB, Tsongalis GJ, editors. *Molecular Pathology (Second Edition)*: Academic Press, 2018, pp. 347-386. ISBN: 978-0-12-802761-5.

18. Mita H, Toyota M, Aoki F, Akashi H, Maruyama R, Sasaki Y, Suzuki H, Idogawa M, Kashima L, Yanagihara K, Fujita M, Hosokawa M, Kusano M, Sabau SV, Tatsumi H, Imai K, Shinomura Y, Tokino T. A novel method, digital genome scanning detects KRAS gene amplification in gastric cancers: involvement of overexpressed wild-type KRAS in downstream signaling and cancer cell growth. *BMC Cancer*. 2009;9:198. PMID: 19545448. DOI: 10.1186/1471-2407-9-198
19. Valtorta E, Misale S, Sartore-Bianchi A, Nagtegaal ID, Paraf F, Lauricella C, Dimartino V, Hobor S, Jacobs B, Ercolani C, Lamba S, Scala E, Veronese S, Laurent-Puig P, Siena S, Tejpar S, Mottolese M, Punt CJ, Gambacorta M, Bardelli A, Di Nicolantonio F. KRAS gene amplification in colorectal cancer and impact on response to EGFR-targeted therapy. *Int J Cancer*. 2013;133(5):1259-1265. PMID: 23404247. DOI: 10.1002/ijc.28106
20. Wong GS, Zhou J, Liu JB, Wu Z, Xu X, Li T, Xu D, Schumacher SE, Puschhof J, McFarland J, Zou C, Dulak A, Henderson L, Xu P, O'Day E, Rendak R, Liao WL, Cecchi F, Hembrough T, Schwartz S, Szeto C, Rustgi AK, Wong KK, Diehl JA, Jensen K, Graziano F, Ruzzo A, Fereshetian S, Mertins P, Carr SA, Beroukhim R, Nakamura K, Oki E, Watanabe M, Baba H, Imamura Y, Catenacci D, Bass AJ. Targeting wild-type KRAS-amplified gastroesophageal cancer through combined MEK and SHP2 inhibition. *Nat Med*. 2018;24(7):968-977. PMID: 29808010. DOI: 10.1038/s41591-018-0022-x
21. Kessler D, Gmachl M, Mantoulidis A, Martin LJ, Zoephel A, Mayer M, Gollner A, Covini D, Fischer S, Gerstberger T, Gmaschitz T, Goodwin C, Greb P, Häring D, Hela W, Hoffmann J, Karolyi-Oezguer J, Knesl P, Kornigg S, Koegl M, Kousek R, Lamarre L, Moser F, Munico-Martinez S, Peinsipp C, Phan J, Rinnenthal J, Sai J, Salamon C, Scherbantin Y, Schipany K, Schnitzer R, Schrenk A, Sharps B, Siszler G, Sun Q, Waterson A, Wolkerstorfer B, Zeeb M, Pearson M, Fesik SW, McConnell DB. Drugging an undruggable pocket on KRAS. *Proceedings of the National Academy of Sciences*. 2019;116(32):15823-15829. PMID: DOI: 10.1073/pnas.1904529116

22. McCormick F. KRAS as a Therapeutic Target. *Clin Cancer Res.* 2015;21(8):1797-1801. PMID: 25878360. DOI: 10.1158/1078-0432.CCR-14-2662
23. Bryant KL, Mancias JD, Kimmelman AC, Der CJ. KRAS: feeding pancreatic cancer proliferation. *Trends Biochem Sci.* 2014;39(2):91-100. PMID: 24388967. DOI: 10.1016/j.tibs.2013.12.004
24. Pantzar T. The current understanding of KRAS protein structure and dynamics. *Comput Struct Biotechnol J.* 2020;18:189-198. PMID: 31988705. DOI: 10.1016/j.csbj.2019.12.004
25. Lanman BA, Allen JR, Allen JG, Amegadzie AK, Ashton KS, Booker SK, Chen JJ, Chen N, Frohn MJ, Goodman G, Kopecky DJ, Liu L, Lopez P, Low JD, Ma V, Minatti AE, Nguyen TT, Nishimura N, Pickrell AJ, Reed AB, Shin Y, Siegmund AC, Tamayo NA, Tegley CM, Walton MC, Wang H-L, Wurz RP, Xue M, Yang KC, Achanta P, Bartberger MD, Canon J, Hollis LS, McCarter JD, Mohr C, Rex K, Saiki AY, San Miguel T, Volak LP, Wang KH, Whittington DA, Zech SG, Lipford JR, Cee VJ. Discovery of a Covalent Inhibitor of KRASG12C (AMG 510) for the Treatment of Solid Tumors. *Journal of Medicinal Chemistry.* 2020;63(1):52-65. PMID: DOI: 10.1021/acs.jmedchem.9b01180
26. Wang J, Yao X, Huang J. New tricks for human farnesyltransferase inhibitor: cancer and beyond. *MedChemComm.* 2017;8(5):841-854. PMID: 30108801. DOI: 10.1039/c7md00030h
27. Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. *Oncogene.* 2007;26(22):3291-3310. PMID: DOI: 10.1038/sj.onc.1210422
28. Havel JJ. MEK Inhibitors in Lung Cancer—You Can Teach an Old Drug New Tricks. *Cancer Research.* 2019;79(22):5699-5701. PMID: DOI: 10.1158/0008-5472.Can-19-2590
29. Yaeger R, Solit DB. Overcoming adaptive resistance to KRAS inhibitors through vertical pathway targeting. *Clinical Cancer Research.* 2020:clincanres.4060.2020. PMID: DOI: 10.1158/1078-0432.Ccr-19-4060
30. Hata AN, Shaw AT. Resistance looms for KRASG12C inhibitors. *Nature Medicine.* 2020;26(2):169-170. PMID: DOI: 10.1038/s41591-020-0765-z

31. Chatterjee N, Bivona TG. Polytherapy and Targeted Cancer Drug Resistance. *Trends Cancer*. 2019;5(3):170-182. PMID: 30898264. DOI: 10.1016/j.trecan.2019.02.003
32. The AACR Project GENIE Consortium. AACR Project GENIE: Powering Precision Medicine through an International Consortium. *Cancer Discov*. 2017;7(8):818-831. PMID: 28572459. DOI: 10.1158/2159-8290.CD-17-0151
33. Janes MR, Zhang J, Li L-S, Hansen R, Peters U, Guo X, Chen Y, Babbar A, Firdaus SJ, Darjania L, Feng J, Chen JH, Li S, Li S, Long YO, Thach C, Liu Y, Zariéh A, Ely T, Kucharski JM, Kessler LV, Wu T, Yu K, Wang Y, Yao Y, Deng X, Zarrinkar PP, Brehmer D, Dhanak D, Lorenzi MV, Hu-Lowe D, Patricelli MP, Ren P, Liu Y. Targeting KRAS Mutant Cancers with a Covalent G12C-Specific Inhibitor. *Cell*. 2018;172(3):578-589.e517. PMID: DOI: <https://doi.org/10.1016/j.cell.2018.01.006>
34. Gentile DR, Rathinaswamy MK, Jenkins ML, Moss SM, Siempelkamp BD, Renslo AR, Burke JE, Shokat KM. Ras Binder Induces a Modified Switch-II Pocket in GTP and GDP States. *Cell Chemical Biology*. 2017;24(12):1455-1466.e1414. PMID: DOI: 10.1016/j.chembiol.2017.08.025
35. Canon J, Rex K, Saiki AY, Mohr C, Cooke K, Bagal D, Gaida K, Holt T, Knutson CG, Koppada N, Lanman BA, Werner J, Rapaport AS, San Miguel T, Ortiz R, Osgood T, Sun J-R, Zhu X, McCarter JD, Volak LP, Houk BE, Fakih MG, O'Neil BH, Price TJ, Falchook GS, Desai J, Kuo J, Govindan R, Hong DS, Ouyang W, Henary H, Arvedson T, Cee VJ, Lipford JR. The clinical KRAS(G12C) inhibitor AMG 510 drives anti-tumour immunity. *Nature*. 2019;575(7781):217-223. PMID: DOI: 10.1038/s41586-019-1694-1
36. Govindan R, et al, editor PHASE 1 STUDY OF AMG 510, A NOVEL MOLECULE TARGETING KRAS G12C MUTANT SOLID TUMORS. European Society for Medical Oncology 2019 Congress; 2019 September 28, 2019; Barcelona, Spain. Oncology Pro2019.
37. Hong DS, Fakih MG, Strickler JH, Desai J, Durm GA, Shapiro GI, Falchook GS, Price TJ, Sacher A, Denlinger CS, Bang YJ, Dy GK, Krauss JC, Kuboki Y, Kuo JC, Coveler AL, Park K, Kim TW, Barlesi F, Munster PN, Ramalingam SS, Burns TF, Meric-Bernstam F, Henary H,

- Ngang J, Ngarmchamnanrith G, Kim J, Houk BE, Canon J, Lipford JR, Friberg G, Lito P, Govindan R, Li BT. KRAS(G12C) Inhibition with Sotorasib in Advanced Solid Tumors. *N Engl J Med*. 2020;383(13):1207-1217. PMID: 32955176. DOI: 10.1056/NEJMoa1917239
38. Sun L, Zhang L, Yu J, Zhang Y, Pang X, Ma C, Shen M, Ruan S, Wasan HS, Qiu S. Clinical efficacy and safety of anti-PD-1/PD-L1 inhibitors for the treatment of advanced or metastatic cancer: a systematic review and meta-analysis. *Scientific Reports*. 2020;10(1):2083. PMID: DOI: 10.1038/s41598-020-58674-4
39. Goebel L, Müller MP, Goody RS, Rauh D. KRasG12C inhibitors in clinical trials: a short historical perspective. *RSC Med Chem*. 2020;11:760-770. PMID: DOI: 10.1039/D0MD00096E
40. Hallin J, Engstrom LD, Hargis L, Calinisan A, Aranda R, Briere DM, Sudhakar N, Bowcut V, Baer BR, Ballard JA, Burkard MR, Fell JB, Fischer JP, Vigers GP, Xue Y, Gatto S, Fernandez-Banet J, Pavlicek A, Velastagui K, Chao RC, Barton J, Pierobon M, Baldelli E, Patricoin EF, Cassidy DP, Marx MA, Rybkin II, Johnson ML, Ou S-HI, Lito P, Papadopoulos KP, Jänne PA, Olson P, Christensen JG. The KRAS<sup>G12C</sup> Inhibitor MRTX849 Provides Insight toward Therapeutic Susceptibility of KRAS-Mutant Cancers in Mouse Models and Patients. *Cancer Discovery*. 2020;10(1):54-71. PMID: DOI: 10.1158/2159-8290.Cd-19-1167
41. Kettle JG, Cassar DJ. Covalent inhibitors of the GTPase KRASG12C: a review of the patent literature. *Expert Opinion on Therapeutic Patents*. 2020;30(2):103-120. PMID: DOI: 10.1080/13543776.2020.1709443
42. Jänne PA, Rybkin II, Spira AI, Riely GJ, Papadopoulos KP, Sabari JK, Johnson ML, Heist RS, Bazhenova L, Barve M, Pacheco JM, Leal TA, Velastagui K, Cornelius C, Olson P, Christensen JG, Kheoh T, Chao RC, Ou SHI. KRYSTAL-1: Activity and Safety of Adagrasib (MRTX849) in Advanced/Metastatic Non–Small-Cell Lung Cancer (NSCLC) Harboring KRAS G12C Mutation. *Eur J Cancer*. 2020;138S2:S1. PMID: DOI:
43. Ruess DA, Heynen GJ, Ciecieski KJ, Ai J, Berninger A, Kabacaoglu D, Gorgulu K, Dantes Z, Wormann SM, Diakopoulos KN, Karpathaki AF, Kowalska M, Kaya-Aksoy E, Song L, van der

- Laan EAZ, Lopez-Alberca MP, Nazare M, Reichert M, Saur D, Erkan MM, Hopt UT, Sainz B, Jr., Birchmeier W, Schmid RM, Lesina M, Algul H. Mutant KRAS-driven cancers depend on PTPN11/SHP2 phosphatase. *Nat Med.* 2018;24(7):954-960. PMID: 29808009. DOI: 10.1038/s41591-018-0024-8
44. Hao HX, Wang H, Liu C, Kovats S, Velazquez R, Lu H, Pant B, Shirley M, Meyer MJ, Pu M, Lim J, Fleming M, Alexander L, Farsidjani A, LaMarche MJ, Moody S, Silver SJ, Caponigro G, Stuart DD, Abrams TJ, Hammerman PS, Williams J, Engelman JA, Goldoni S, Mohseni M. Tumor Intrinsic Efficacy by SHP2 and RTK Inhibitors in KRAS-Mutant Cancers. *Mol Cancer Ther.* 2019;18(12):2368-2380. PMID: 31439712. DOI: 10.1158/1535-7163.MCT-19-0170
45. Yang H, Liang S-Q, Schmid RA, Peng R-W. New Horizons in KRAS-Mutant Lung Cancer: Dawn After Darkness. *Frontiers in oncology.* 2019;9:953-953. PMID: 31612108. DOI: 10.3389/fonc.2019.00953
46. Nagasaka M, Li Y, Sukari A, Ou SI, Al-Hallak MN, Azmi AS. KRAS G12C Game of Thrones, which direct KRAS inhibitor will claim the iron throne? *Cancer Treat Rev.* 2020;84:101974. PMID: 32014824. DOI: 10.1016/j.ctrv.2020.101974
47. Evelyn CR, Duan X, Biesiada J, Seibel WL, Meller J, Zheng Y. Rational design of small molecule inhibitors targeting the Ras GEF, SOS1. *Chemistry & biology.* 2014;21(12):1618-1628. PMID: 25455859. DOI: 10.1016/j.chembiol.2014.09.018
48. Hillig RC, Sautier B, Schroeder J, Moosmayer D, Hilpmann A, Stegmann CM, Werbeck ND, Briem H, Boemer U, Weiske J, Badock V, Mastouri J, Petersen K, Siemeister G, Kahmann JD, Wegener D, Böhnke N, Eis K, Graham K, Wortmann L, von Nussbaum F, Bader B. Discovery of potent SOS1 inhibitors that block RAS activation via disruption of the RAS-SOS1 interaction. *Proceedings of the National Academy of Sciences of the United States of America.* 2019;116(7):2551-2560. PMID: 30683722. DOI: 10.1073/pnas.1812963116
49. Gerlach D, Gmachl M, Ramharther J, Teh J, Fu S-C, Trapani F, Kessler D, Rumpel K, Botesteanu D-A, Ettmayer P, Arnhof H, Gerstberger T, Kofink C, Wunberg T, Vellano CP, Heffernan TP,

Marszalek JR, Pearson M, McConnell DB, Kraut N, Hofmann MH. BI-3406 and BI 1701963: Potent and selective SOS1::KRAS inhibitors induce

regressions in combination with MEK inhibitors or irinotecan [abstract]. *Canc Res.* 2020;80(16 Suppl):Abstract nr 1091. PMID: DOI: 10.1158/1538-7445.AM2020-1091

50. Long GV, Hauschild A, Santinami M, Atkinson V, Mandala M, Chiarion-Sileni V, Larkin J, Nyakas M, Dutriaux C, Haydon A, Robert C, Mortier L, Schachter J, Schadendorf D, Lesimple T, Plummer R, Ji R, Zhang P, Mookerjee B, Legos J, Kefford R, Dummer R, Kirkwood JM. Adjuvant Dabrafenib plus Trametinib in Stage III BRAF-Mutated Melanoma. *N Engl J Med.* 2017;377(19):1813-1823. PMID: 28891408. DOI: 10.1056/NEJMoa1708539
51. Setten RL, Rossi JJ, Han S-p. The current state and future directions of RNAi-based therapeutics. *Nature Reviews Drug Discovery.* 2019;18(6):421-446. PMID: DOI: 10.1038/s41573-019-0017-4
52. Strand MS, Krasnick BA, Pan H, Zhang X, Bi Y, Brooks C, Wetzel C, Sankpal N, Fleming T, Goedegebuure SP, DeNardo DG, Gillanders WE, Hawkins WG, Wickline SA, Fields RC. Precision delivery of RAS-inhibiting siRNA to KRAS driven cancer via peptide-based nanoparticles. *Oncotarget.* 2019;10(46):4761-4775. PMID: 31413817. DOI: 10.18632/oncotarget.27109
53. Yamakawa K, Nakano-Narusawa Y, Hashimoto N, Yokohira M, Matsuda Y. Development and Clinical Trials of Nucleic Acid Medicines for Pancreatic Cancer Treatment. *Int J Mol Sci.* 2019;20(17):4224. PMID: 31470511. DOI: 10.3390/ijms20174224
54. Ross SJ, Revenko AS, Hanson LL, Ellston R, Staniszewska A, Whalley N, Pandey SK, Reville M, Rooney C, Buckett LK, Klein SK, Hudson K, Monia BP, Zinda M, Blakey DC, Lyne PD, Macleod AR. Targeting KRAS-dependent tumors with AZD4785, a high-affinity therapeutic antisense oligonucleotide inhibitor of KRAS. *Sci Transl Med.* 2017;9(394). PMID: 28615361. DOI: 10.1126/scitranslmed.aal5253

55. Phase I Dose-Escalation Study of AZD4785 in Patients with Advanced Solid Tumours. Available from: <https://astrazenecagrouptrials.pharmacm.com/ST/Submission/View?id=24623>.
56. Osaki M, Okada F. Exosomes and Their Role in Cancer Progression. *Yonago acta medica*. 2019;62(2):182-190. PMID: 31320822. DOI: 10.33160/yam.2019.06.002
57. Luan X, Sansanaphongpricha K, Myers I, Chen H, Yuan H, Sun D. Engineering exosomes as refined biological nanoplatforms for drug delivery. *Acta Pharmacol Sin*. 2017;38(6):754-763. PMID: 28392567. DOI: 10.1038/aps.2017.12
58. Kamekar S, LeBleu VS, Sugimoto H, Yang S, Ruivo CF, Melo SA, Lee JJ, Kalluri R. Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. *Nature*. 2017;546(7659):498-503. PMID: 28607485. DOI: 10.1038/nature22341
59. Crunkhorn S. RNA-based approaches target KRAS. *Nature Reviews Drug Discovery*. 2017;16(8):529-529. PMID: DOI: 10.1038/nrd.2017.137
60. Zorde Khvalevsky E, Gabai R, Rachmut IH, Horwitz E, Brunschwig Z, Orbach A, Shemi A, Golan T, Domb AJ, Yavin E, Giladi H, Rivkin L, Simerzin A, Eliakim R, Khalaileh A, Hubert A, Lahav M, Kopelman Y, Goldin E, Dancour A, Hants Y, Arbel-Alon S, Abramovitch R, Shemi A, Galun E. Mutant KRAS is a druggable target for pancreatic cancer. *Proceedings of the National Academy of Sciences*. 2013;110(51):20723-20728. PMID: DOI: 10.1073/pnas.1314307110
61. Golan T, Khvalevsky EZ, Hubert A, Gabai RM, Hen N, Segal A, Domb A, Harari G, David EB, Raskin S, Goldes Y, Goldin E, Eliakim R, Lahav M, Kopleman Y, Dancour A, Shemi A, Galun E. RNAi therapy targeting KRAS in combination with chemotherapy for locally advanced pancreatic cancer patients. *Oncotarget*. 2015;6(27):24560-24570. PMID: 26009994. DOI: 10.18632/oncotarget.4183
62. Varghese AM, Ang C, Dimaio CJ, Javle MM, Gutierrez M, Yarom N, Stemmer SM, Golan T, Geva R, Semenisty V, Khamaysi I, Ligresti R, Rotkopf S, Gabai-Malka R, Galun E, Shemi A, Schattner M, O'Reilly EM. A phase II study of siG12D-LODER in combination with chemotherapy in patients with locally advanced pancreatic cancer (PROTACT). *Journal of*

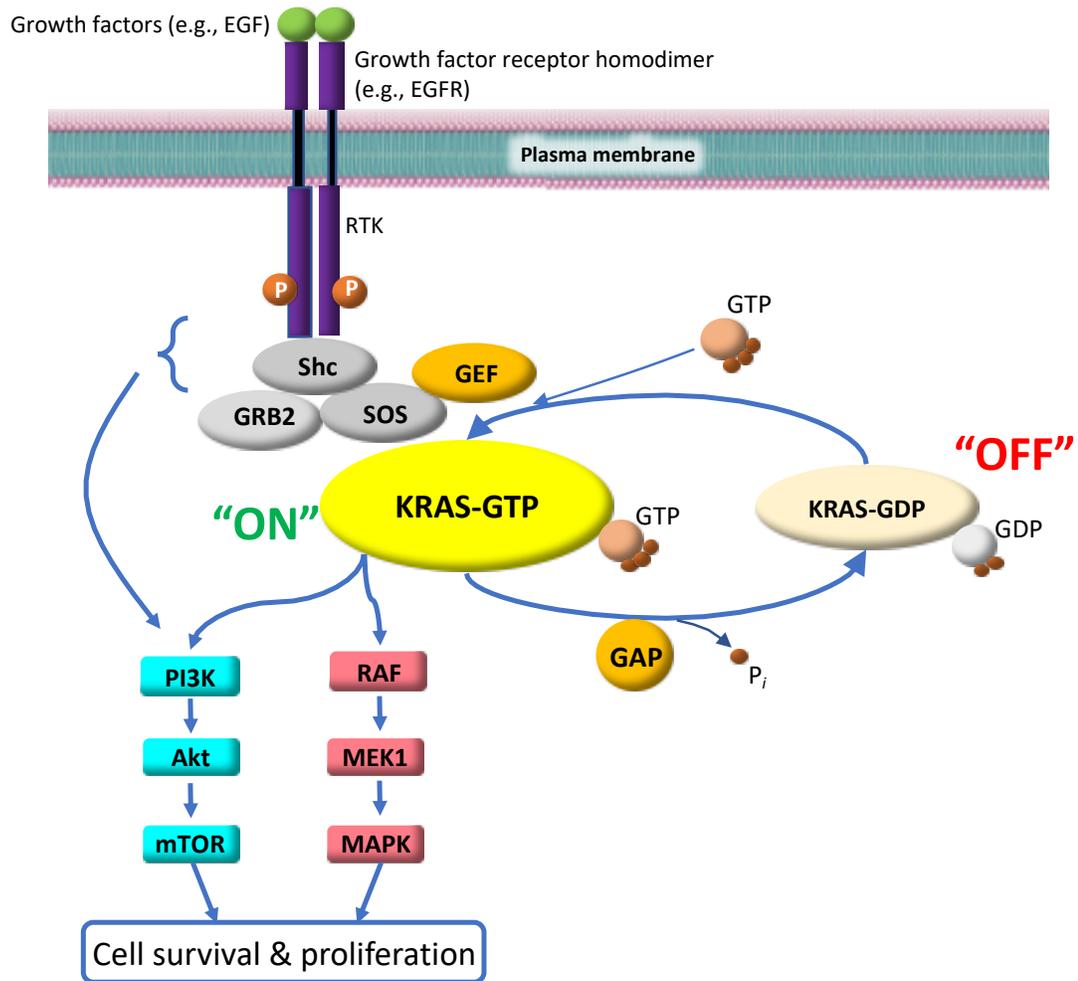
- Clinical Oncology*. 2020;38(15\_suppl):TPS4672-TPS4672. PMID: DOI:  
10.1200/JCO.2020.38.15\_suppl.TPS4672
63. Titze-de-Almeida R, David C, Titze-de-Almeida SS. The Race of 10 Synthetic RNAi-Based Drugs to the Pharmaceutical Market. *Pharmaceutical Research*. 2017;34(7):1339-1363. PMID: DOI: 10.1007/s11095-017-2134-2
64. Faghfuri E, Pourfarzi F, Faghfour AH, Abdoli Shadbad M, Hajiasgharzadeh K, Baradaran B. Recent developments of RNA-based vaccines in cancer immunotherapy. *Expert Opin Biol Ther*. 2020:1-18. PMID: 32842798. DOI: 10.1080/14712598.2020.1815704
65. Khleif SN, Abrams SI, Hamilton JM, Bergmann-Leitner E, Chen A, Bastian A, Bernstein S, Chung Y, Allegra CJ, Schlom J. A phase I vaccine trial with peptides reflecting ras oncogene mutations of solid tumors. *J Immunother*. 1999;22(2):155-165. PMID: 10093040. DOI: 10.1097/00002371-199903000-00007
66. Gjertsen MK, Buanes T, Rosseland AR, Bakka A, Gladhaug I, Soreide O, Eriksen JA, Moller M, Baksaas I, Lothe RA, Saeterdal I, Gaudernack G. Intradermal ras peptide vaccination with granulocyte-macrophage colony-stimulating factor as adjuvant: Clinical and immunological responses in patients with pancreatic adenocarcinoma. *Int J Cancer*. 2001;92(3):441-450. PMID: 11291084. DOI: 10.1002/ijc.1205
67. Carbone DP, Ciernik IF, Kelley MJ, Smith MC, Nadaf S, Kavanaugh D, Maher VE, Stipanov M, Contois D, Johnson BE, Pendleton CD, Seifert B, Carter C, Read EJ, Greenblatt J, Top LE, Kelsey MI, Minna JD, Berzofsky JA. Immunization with mutant p53- and K-ras-derived peptides in cancer patients: immune response and clinical outcome. *J Clin Oncol*. 2005;23(22):5099-5107. PMID: 15983396. DOI: 10.1200/JCO.2005.03.158
68. Toubaji A, Achta M, Provenzano M, Herrin VE, Behrens R, Hamilton M, Bernstein S, Venzon D, Gause B, Marincola F, Khleif SN. Pilot study of mutant ras peptide-based vaccine as an adjuvant treatment in pancreatic and colorectal cancers. *Cancer Immunol Immunother*. 2008;57(9):1413-1420. PMID: 18297281. DOI: 10.1007/s00262-008-0477-6

69. Kubuschok B, Neumann F, Breit R, Sester M, Schormann C, Wagner C, Sester U, Hartmann F, Wagner M, Remberger K, Schilling M, Pfreundschuh M. Naturally occurring T-cell response against mutated p21 ras oncoprotein in pancreatic cancer. *Clin Cancer Res.* 2006;12(4):1365-1372. PMID: 16489095. DOI: 10.1158/1078-0432.CCR-05-1672
70. Zhang Y, Ma JA, Zhang HX, Jiang YN, Luo WH. Cancer vaccines: Targeting KRAS-driven cancers. *Expert Rev Vaccines.* 2020;19(2):163-173. PMID: 32174221. DOI: 10.1080/14760584.2020.1733420
71. Stolze B, Reinhart S, Bullinger L, Fröhling S, Scholl C. Comparative analysis of KRAS codon 12, 13, 18, 61 and 117 mutations using human MCF10A isogenic cell lines. *Scientific Reports.* 2015;5(1):8535. PMID: DOI: 10.1038/srep08535
72. Wadhwa A, Aljabbari A, Lokras A, Foged C, Thakur A. Opportunities and Challenges in the Delivery of mRNA-based Vaccines. *Pharmaceutics.* 2020;12(2):102. PMID: 32013049. DOI: 10.3390/pharmaceutics12020102
73. Cohen CJ, Zhao Y, Zheng Z, Rosenberg SA, Morgan RA. Enhanced antitumor activity of murine-human hybrid T-cell receptor (TCR) in human lymphocytes is associated with improved pairing and TCR/CD3 stability. *Cancer Res.* 2006;66(17):8878-8886. PMID: 16951205. DOI: 10.1158/0008-5472.CAN-06-1450
74. Johnson LA, Morgan RA, Dudley ME, Cassard L, Yang JC, Hughes MS, Kammula US, Royal RE, Sherry RM, Wunderlich JR, Lee CC, Restifo NP, Schwarz SL, Cogdill AP, Bishop RJ, Kim H, Brewer CC, Rudy SF, VanWaes C, Davis JL, Mathur A, Ripley RT, Nathan DA, Laurencot CM, Rosenberg SA. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. *Blood.* 2009;114(3):535-546. PMID: 19451549. DOI: 10.1182/blood-2009-03-211714
75. Parkhurst MR, Yang JC, Langan RC, Dudley ME, Nathan DA, Feldman SA, Davis JL, Morgan RA, Merino MJ, Sherry RM, Hughes MS, Kammula US, Phan GQ, Lim RM, Wank SA, Restifo NP, Robbins PF, Laurencot CM, Rosenberg SA. T cells targeting carcinoembryonic antigen can

- mediate regression of metastatic colorectal cancer but induce severe transient colitis. *Mol Ther.* 2011;19(3):620-626. PMID: 21157437. DOI: 10.1038/mt.2010.272
76. Finn OJ. Cancer immunology. *N Engl J Med.* 2008;358(25):2704-2715. PMID: 18565863. DOI: 10.1056/NEJMra072739
77. Tran E, Robbins PF, Lu Y-C, Prickett TD, Gartner JJ, Jia L, Pasetto A, Zheng Z, Ray S, Groh EM, Kriley IR, Rosenberg SA. T-Cell Transfer Therapy Targeting Mutant KRAS in Cancer. *New England Journal of Medicine.* 2016;375(23):2255-2262. PMID: 27959684. DOI: 10.1056/NEJMoa1609279
78. June CH, Sadelain M. Chimeric Antigen Receptor Therapy. *N Engl J Med.* 2018;379(1):64-73. PMID: 29972754. DOI: 10.1056/NEJMra1706169
79. Nussinov R, Wang G, Tsai CJ, Jang H, Lu S, Banerjee A, Zhang J, Gaponenko V. Calmodulin and PI3K Signaling in KRAS Cancers. *Trends Cancer.* 2017;3(3):214-224. PMID: 28462395. DOI: 10.1016/j.trecan.2017.01.007
80. Riely GJ, Marks J, Pao W. KRAS mutations in non-small cell lung cancer. *Proc Am Thorac Soc.* 2009;6(2):201-205. PMID: 19349489. DOI: 10.1513/pats.200809-107LC
81. Chuang HC, Huang PH, Kulp SK, Chen CS. Pharmacological strategies to target oncogenic KRAS signaling in pancreatic cancer. *Pharmacol Res.* 2017;117:370-376. PMID: 28077300. DOI: 10.1016/j.phrs.2017.01.006
82. Zhu Z, Golay HG, Barbie DA. Targeting pathways downstream of KRAS in lung adenocarcinoma. *Pharmacogenomics.* 2014;15(11):1507-1518. PMID: 25303301. DOI: 10.2217/pgs.14.108
83. Wang Y, Kaiser CE, Frett B, Li HY. Targeting mutant KRAS for anticancer therapeutics: a review of novel small molecule modulators. *J Med Chem.* 2013;56(13):5219-5230. PMID: 23566315. DOI: 10.1021/jm3017706
84. Collins MA, Pasca di Magliano M. Kras as a key oncogene and therapeutic target in pancreatic cancer. *Front Physiol.* 2013;4:407. PMID: 24478710. DOI: 10.3389/fphys.2013.00407

85. Drosten M, Barbacid M. Targeting the MAPK Pathway in KRAS-Driven Tumors. *Cancer Cell*. 2020;37(4):543-550. PMID: 32289276. DOI: 10.1016/j.ccell.2020.03.013
86. Martini M, De Santis MC, Braccini L, Gulluni F, Hirsch E. PI3K/AKT signaling pathway and cancer: an updated review. *Ann Med*. 2014;46(6):372-383. PMID: 24897931. DOI: 10.3109/07853890.2014.912836
87. Wood K, Hensing T, Malik R, Salgia R. Prognostic and Predictive Value in KRAS in Non-Small-Cell Lung Cancer: A Review. *JAMA Oncol*. 2016;2(6):805-812. PMID: 27100819. DOI: 10.1001/jamaoncol.2016.0405
88. Fayard E, Tintignac LA, Baudry A, Hemmings BA. Protein kinase B/Akt at a glance. *J Cell Sci*. 2005;118(Pt 24):5675-5678. PMID: 16339964. DOI: 10.1242/jcs.02724
89. Ahmed SBM, Prigent SA. Insights into the Shc Family of Adaptor Proteins. *J Mol Signal*. 2017;12:2. PMID: 30210578. DOI: 10.5334/1750-2187-12-2

Figure 1.



**Fig. 1. KRAS activation and effector network.** Akt: Ak strain Transforming (aka PKB, Protein Kinase B); EGF: Epidermal Growth Factor; EGFR: Epidermal Growth Factor Receptor; GAP: GTPase-activating protein; Grb2: Growth factor receptor-bound protein 2; GEF: Guanine Nucleotide Exchange Factor; GDP: guanosine-5'-diphosphate; GTP: guanosine-5'-triphosphate; KRAS: Kirsten Rat Sarcoma; MAPK: Mitogen-Activated Protein Kinase (aka ERK or Extracellularly Regulated Kinase); MEK1: MAPK/ERK Kinase (aka MAPKK or MAP2K); mTOR: Mammalian Target of Rapamycin;  $P_i$ : inorganic Phosphate; PI3K: Phosphatidylinositol-3 Kinase; RAF: Rapidly Accelerated Fibrosarcoma; RTK: Receptor Tyrosine Kinase; Shc: Src

Homology and Collagen; SoS: Son of Sevenless. Figure was composited from multiple sources.<sup>23,79-89</sup>

**Table 1. Drugs currently in clinical trials for KRAS cancers.**

| Drug                | Company                        | Mechanism                          | CLINICAL TRIAL(S)  |
|---------------------|--------------------------------|------------------------------------|--|
| AMG 510 (Sotorasib) | Amgen                          | G12C Inhibitor                     | <b>NCT04303780:</b> A Phase 3 Multicenter, Randomized, Open Label, Active-controlled, Study of AMG 510 Versus Docetaxel for the Treatment of Previously Treated Locally Advanced and Unresectable or Metastatic NSCLC Subjects With Mutated KRAS p.G12C<br><b>NCT04185883:</b> A Phase 1b, Protocol Evaluating the Safety, Tolerability, Pharmacokinetics, and Efficacy of AMG 510 (pINN) Sotorasib Monotherapy and in Combination With Other Anti-cancer Therapies in Subjects With Advanced Solid Tumors With KRAS p.G12C Mutation (CodeBreak 101)<br><b>NCT03600883 :</b> A Phase 1, Open-label Study Evaluating the Safety, Tolerability, Pharmacokinetics, and Efficacy of AMG 510 (pINN Sotorasib) in Subjects of Chinese Descent With Advanced/Metastatic Solid Tumors With KRAS p.G12CMutation (CodeBreak 105) |
| MRTX849             | Mirati Therapeutics            | G12C Inhibitor                     | <b>NCT04330664:</b> A Phase 1/2 Trial of MRTX849 in Combination With TNO155 in Patients With Advanced Solid Tumors With KRAS G12C Mutation<br><b>NCT03785249:</b> A Phase 1/2 Multiple Expansion Cohort Trial of MRTX849 in Patients With Advanced Solid Tumors With KRAS G12C Mutation KRYSTAL-1  |
| JNJ-74699157        | J&J and Wellspring Biosciences | G12C Inhibitor                     | <b>NCT0400630:</b> A First-in-Human Study of the Safety, Pharmacokinetics, Pharmacodynamics, and Preliminary Antitumor Activity of JNJ-74699157 in Participants With Advanced Solid Tumors Harboring the KRAS G12C Mutation  |
| LY3944996           | Eli Lilly and Company          | G12C Inhibitor                     | <b>NCT04165031:</b> A Phase 1/2 Study of LY3499446 Administered to Patients With Advanced Solid Tumors With KRAS G12C Mutation   |
| iExosomes           | Codiak Biosciences             | siRNA-G12D                         | <b>NCT03608631:</b> Phase I Study of Mesenchymal Stromal Cells-Derived Exosomes With KrasG12D siRNA for Metastatic Pancreas Cancer Patients Harboring KrasG12D Mutation  |
| LODER               | Silenseed ltd                  | siRNA-G12D                         | <b>NCT01188785:</b> Phase 2 Study Evaluating the Efficacy, Safety, Tolerability and PK of siG12-LODER in the Treatment of Patients With Unresectable LAPC When Used in Conjunction With Standard Chemotherapy (Gemcitabine+Nab-Paclitaxel) Versus Chemotherapy Alone   |
| mRNA 5671           | Moderna                        | RNA Vaccine G12D,G12C, G12V, G13D  | <b>NCT03948763:</b> A Phase 1, Open-Label, Multicenter Study to Assess the Safety and Tolerability of mRNA-5671/V941 as a Monotherapy and in Combination With Pembrolizumab in Participants With KRAS Mutant Advanced or Metastatic Non-Small Cell Lung Cancer, Colorectal Cancer or Pancreatic Adenocarcinoma   |
| BI 1701963          | Boehringer Ingelheim           | SOS1 Inhibitor                     | <b>NCT04111458:</b> A Phase I Open-label Dose Escalation Trial of BI 1701963 as Monotherapy and in Combination With Trametinib in Patients With KRAS99 Mutated Advanced or Metastatic Solid Tumours  |
| mTCR                | National Cancer Institute      | Adoptive T-Cell Therapy G12D, G12V | <b>NCT03745326:</b> A Phase I/II Study Administering Peripheral Blood Lymphocytes Transduced With a Murine T-Cell Receptor Recognizing the G12D Variant of Mutated RAS in HLA-A*11:01 Patients   |

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|  |  |  | <b>NCT03190941: A Phase I/II Study Administering Peripheral Blood Lymphocytes Transduced With a Murine T-Cell Receptor Recognizing the G12V Variant of Mutated RAS in HLA-A*11:01 Patients</b> |
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