JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

A REVIEW OF KRAS G12D INHIBITORS: FROM DISCOVERY TO CLINICAL TRIALS

[1]Komal Korde, [2]Rohini Kamble
[1]M Pharm student, [2]M Pharm student
[1&2]Department of Pharmaceutical Chemistry
[1&2]Y.B. Chavan College of Pharmacy, Aurangabad, India

ABSTRACT: Kristen Rat Sarcoma Virus [KRAS] belongs to family of guanine nucleotide binding proteins. KRAS consist of mutation as G12D, G12V, G13D and Q61H which originates in biliary tract, small intestine, colorectal, lung and pancreatic cancer. KRAS G12D converts inactive GDP to active GTP by GEF which is responsible and tumor formation for this reason it becomes the most targeted site. Targeting the KRAS G12D mutation previously remained a challenge due to lack of druggable pockets on the surface of RAS. Recently, the first selective non covalent inhibitor MRTX1133 of KRAS G12D was depicted to show activity in preclinical trial and currently it in under phase 1or 2 studied under Mirati Therapeutics Co. Ltd. Various KRAS G12D inhibitors were discovered as HRAS-4642, BI-1701963, BT-1823911, BI-2852, TH-2801, TH-2816 which are under clinical trials. In this, review we summarize the KRAS G12D mutant importance in solid tumors, prior attempts at inhibiting mutant KRAS, and the current promising targets agents being investigated in clinical trials.

I. INTORDUCTION

KRAS is one of the greatest repeatedly mutated oncogenes in all social malignancies and is seen in 1 in 7 of all human tumours [1]. The oncogenic effects of the KRAS gene were first described in 1980s, producing KRAS one of the first recognized oncogenes [2]. The human RAS gene family encompasses KRAS, HRAS and NRAS, all of about change 21 k Da small GTPase proteins that are stimulated on the inner cell membrane and transduce extracellular progress stimuli signals to intracellular effector signalling cascades [3]. It is superficial that mutations at additional places in KRAS, including codon 12, 13, 59, 61, 117, and 146 mutations [4]. We believe noncovalent allosteric inhibition will be required to mark some of the salient mutations in KRAS including G12D, G12V, G13D, and Q61H originate in biliary tract, small intestine, colorectal, lung, and pancreatic cancers [5]. G12D mutations predominate in pancreatic channel adenocarcinoma and carcinomas of the colon and rectum [6].

1.1 MOLECULAR CELL BIOLOGY OF RAS

RAS proteins (G-Protein) cycle between an active GTP bound and an inactive GDP bound state [7]. KRAS has been known as a KRAS-1 pseudogene on short arm of chromosome 6 and KRAS-2 gene, situated on the short arm of chromosome 12 (12p11.1-12p12.1) [8]. KRAS-2 coding region extents across six exons and measures over 45 kB. Two protein isoforms of KRAS 2, KRAS-4A and KRAS-4B are produced due to alternate merging on its fourth exon, principal to 188 and 189 monomeric amino acid sequences, respectively. Ras proteins belong to the small GTPase family and are involved in transmitting growth, survival, and proliferation signals within cells. Two regions of Ras proteins, Switch I (residues 30-40) and Switch II (residues 60-76), undergo significant conformational changes and form effector-protein interaction surfaces upon GTP binding. KRAS shifts between an inactive, guanosine diphosphate (GDP)-bound state, and an active, guanosine triphosphate (GTP)-bound state. While bound to GDP, KRAS is inactive. Receptor activation leads the stimulation of a family of guanine nucleotide exchange factors (GEFs), which cause GDP's exchange with GTP. GTP-bound KRAS transduces signals to downstream effectors and stimulates multiple signalling pathways, including the RAF family of kinases and phosphatidylinositol 3-kinase (PI3K) that in turn activate a cascade [9]. Under normal physiological conditions, the KRAS gene encodes the GTPase transductor protein, which plays a key role in the signal transduction cascades that control cell growth, proliferation, migration, differentiation, survival, and apoptosis [10].
In Normal cell, after activation of receptor RAS protein convert inactive RAS-GDP into active RAS-GTP by guanine nucleotide exchange factors (GEF) further activation of downstream signalling RAF/MEK/ERK and Ral A/B pathway that regulate the cell division, differentiation and growth of cell. Completion of cell growth the active form of RAS-GTP is hydrolysed by GTPase activating protein (GAP) to RAS-GDP inactive form, but in cancer cell RAS-GTP remains in "ON" state as active form and continuously formation of cell growth, proliferation which it's converts into tumour cancer cell and invasive spreading in body.

Shown in **Figure 1.**

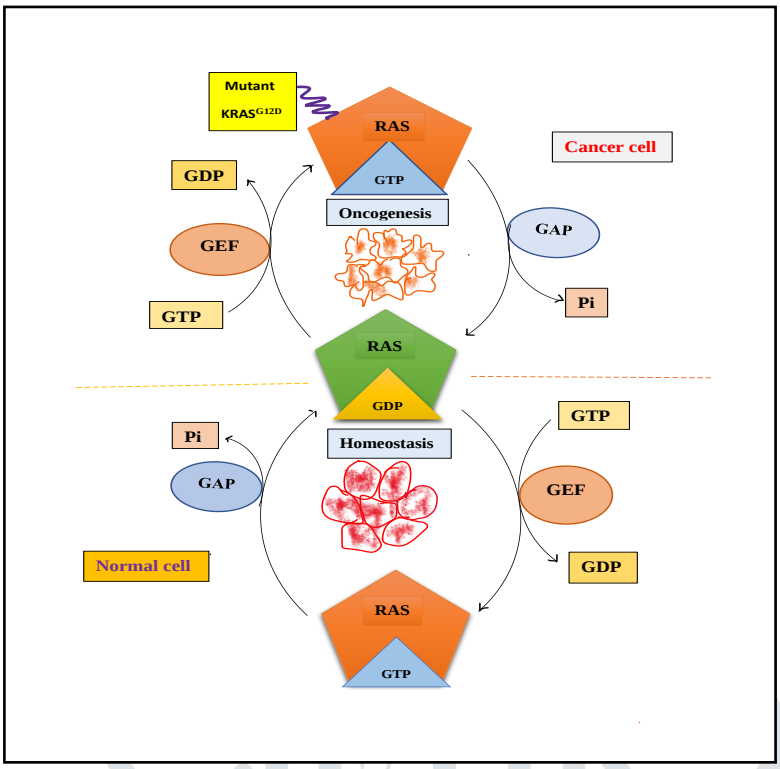


Fig.1 RAS: Oncogene protein, KRAS: Kirsten Rat Sarcoma Viral Proto-Oncogene, GAP: GTPase-activating protein, GEF: Guanine nucleotide-exchange factor, GDP: Guanosine diphosphate, GTP: Guanosine-5'-triphosphate.

1.2 KRAS MUTATION LEADS TO CANCERS

Pancreatic cancer is the lethal gastrointestinal malignancy, as the fourth foremost source of cancer-related death and with one of the lowermost 5-year survival rates, at only 11% [8]. The four most generally mutated genes in pancreatic cancer are KRAS, CDKN2A, TP53, and SMAD41]. Lung cancer, poses a serious hazard to human health, as directed by the top-ranking morbidity and mortality rates worldwide and in China [11]. NSCLC was further classified into different subtypes giving to the incidence of gene mutation, such as EGFR, ALK, MET, ROS-1, KRAS (Chen et al., 2014) 10]. only 58% of NSCLC patients with KRAS-mutant NSCLC were shown to have a high expression of PD-L1 and penetration of CD8+ tumour-infiltrating lymphocytes (TILs), and not all patients helped from anti-PD-1 immunotherapy. Colorectal Cancer had the second main KRAS mutation rate [11].

1.3 DETAILS ACCOUNTS OF ABOUT RAS ONCOGENE

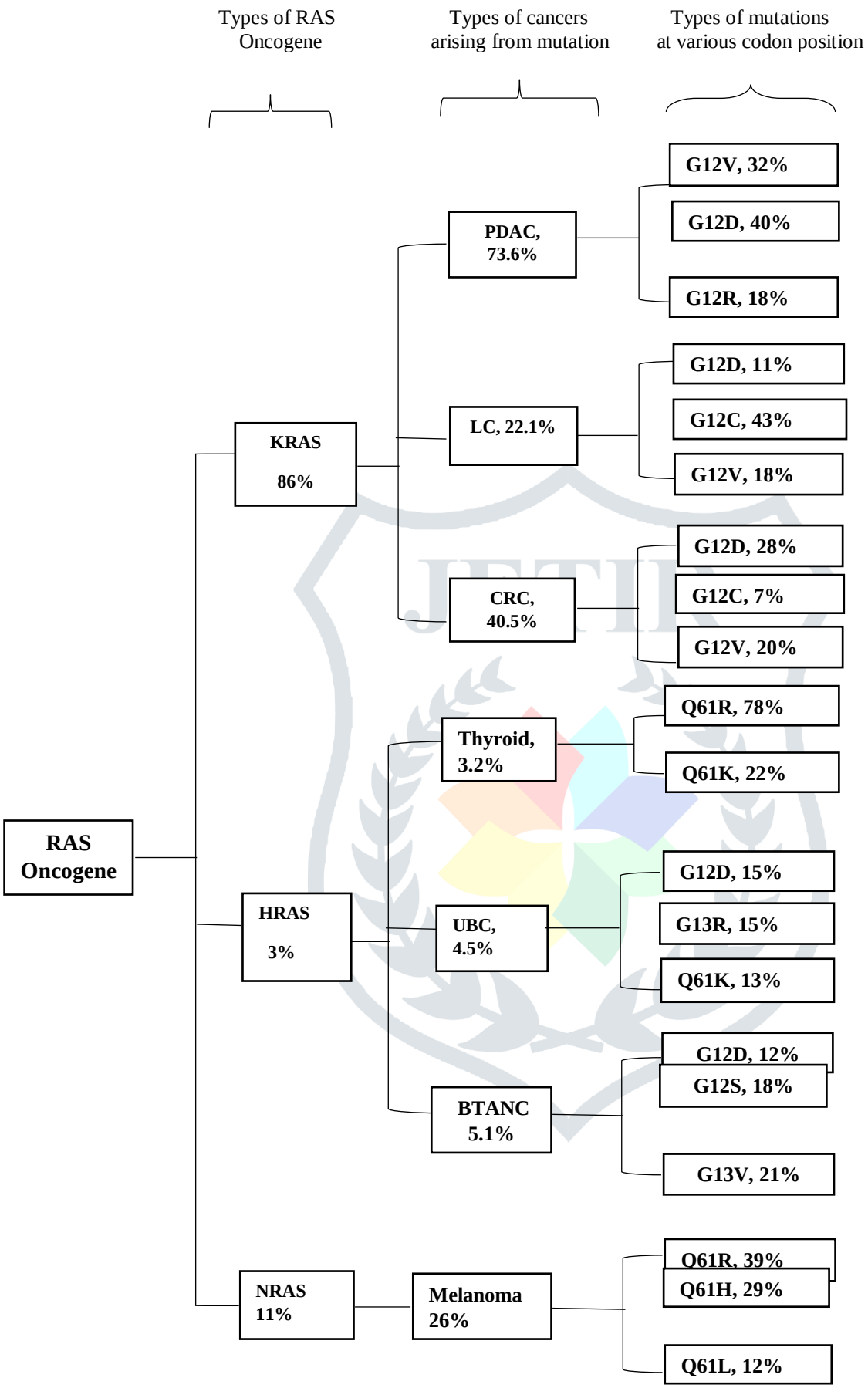


Fig.2 RAS: Protein Oncogene; KRAS: Kirsten Rat Sarcoma Viral Proto-Oncogene; HRAS: Harvey Rat Sarcoma Viral Oncogene Homolog; NRAS: Neuroblastoma RAS Viral Oncogene Homolog; PDAC: Pancreatic duct adenocarcinoma; LC: Lung cancer; CRC: Colorectal cancer; UBC: Urinary bladder cancer; BTNAC: Brain tumour and neck cancer.

II. PATIENTS SURVIVAL AND MORTALITY ANALYSIS

Marco H. Hofmann et al. reported that KRAS mutations and/or KRAS wild-type amplifications are often found in colorectal cancer (United States ~45%, China ~49% of cases), pancreatic cancer (United States ~90%, China ~87% of cases), and NSCLC (subtype adenocarcinoma: United States ~35%, China ~13% of cases. About 166,000 newly analysed tumours harbour a KRAS driver mutation, around 9,000 transfer a KRAS wild-type magnification, and 4,000 patients show several KRAS variations. There were 138 patients in total who were not KRAS G12D, including KRAS wild-type patients (59.5%) [4]. KRAS mutations are most mutual in PDAC, with 81.72% of patients offering KRAS mutations [12]. Pancreatic cancer, of which pancreatic ductal adenocarcinoma (PDAC) characterizes the huge mainstream, is a strangely aggressive and toxic disease with an overall 5-year survival rate of only near 8%. The incidence of this disease in the US is assessed to rise to 53,670 new cases in 2017 and it is presently the fourth important cause of cancer mortality in both males and females [13]. Qian Yu He et al. reported that amongst all types of enmities, the frequency of PDAC is at an impartially high level (2.6% ranked 14th in 2020), while the mortality rate is much higher (4.7% ranked 7th in 2020). In many countries, the trend in the occurrence and mortality of PDAC has moreover continued basically the same or has amplified to some extent, which may be due to obesity, diabetes, alcohol, and other factors. patients with resectable or borderline resectable PDAC, postoperative adjuvant chemotherapy for six months with fluorouracil and leucovorin progresses medium survival (19.7 months in 238 patients vs. 14.0 months in 235 patients. Ogura et al. analysed 242 patients with unresectable pancreatic cancer and found that those in the KRAS mutation group had a significantly shorter survival related to the wild-type group. On the contrary, a recent study on 219 patients with advanced PDAC found that there was no significant difference in survival between the mutant KRAS and KRAS wild-type groups. However, overall, as confirmed in a recent meta-analysis, patients with KRAS mutation had lesser overall survival [14]. KRAS is a significant driver gene in the development of NSCLC, and the mutation rates of KRAS in Western 20%-30% and Asian 10%- 15% patients with NSCLC [11].

III. HISTORY OF KRAS INHIBITORS UPTO RECENT

KRAS mutation arises in almost 30% of human malignancies, yet the maximum prevalent and oncogenic KRAS G12D variant still lacks inhibitors [1]. The common strategies adopted for anti-RAS drug development can be broadly classified into direct and indirect approaches [15]. Direct methods comprise compounds that bind directly to RAS and interrupt the interactions between RAS and GEFs/effectors. Indirect strategies comprise: (1) hindering proteins such as farnesyltransferase or PDEδ that encourage the association of RAS by plasma membrane; (2) pointing proteins complex in RAS downstream signalling; (3) preventing synthetic mortal interaction partners for mutant RAS and (4) targeting progressions that are RAS controlled, for example, micropinocytosis and autophagy [6]. Unacceptable clinical trial results from the indirect strategies have failed to offer any RAS drugs, hence, targeting RAS genes directly has been considered as a practical approach [16].

3.1 DRUG RESISTANCE

HTS has been used to recognize frequent compounds capable of troublemaking the KRAS signalling pathway. However, no therapy capable of specifically and effectively targeting mutant KRAS is now available. The problem is not due to a lack of therapeutic targets, but rather due to a lack of a complete understanding of the biology ultimate the disease. Cell signalling is often depicted as a linear cascade, and this model is used to identify potential therapeutic targets. However, the linear depiction of cell signalling is a simplification. In reality, cell signalling is complex and dynamic, in which one protein interacts with many others in a non-linear, circuit-like fashion. Cancer is not caused by just one genetic mutation or abnormal protein that drives the disease. As an alternative, several mutations remaining together form and drive the progression of cancer. Thus, effective treatment will necessitate the regulation of many targets to achieve a continuous response. Therefore, a therapy capable of inhibiting multiple pathways/targets is likely the best option for treating mutant KRAS-driven cancers. KRAS can activate both PI3K and RAF, which lie in different signalling cascades. If only one pathway is withdrawn, the cell can circumvent this event by overexpressing the other pathway, and thereby create a resistant phenotype. For example, treatment with just the multi-kinase inhibitor PP-121,120 which is active against PI3K without blocking the RAS/RAF pathway, will likely be ineffective in patients. A better treatment option would be blocking both the PI3K and the RAS/RAF pathways, which could be achieved by the combination of a MEK inhibitor, such as 14, with an AKT inhibitor, such as MK-2206 .120 Inhibition of both pathways simultaneously may be able to produce better efficacy in mutant KRAS-driven cancers, as opposed to the inhibition of only one pathway. The design of a successful cancer therapeutic relies on an understanding of the biology underlying the disease. The idea that cells signal in a linear fashion is quickly becoming antiquated and is being replaced with circuit-type models. Likewise, cancer treatments need to be designed under the notion of circuit signalling. We expect that employing this rationale will lead to better treatments, more effective drug combinations, and better outcomes for patients.

IV. KRAS G12D INHIBITORS

4.1 Mechanism of Action of KRAS G12D Inhibitors:

The G12D is a more common mutation, especially in pancreatic cancer, colon and lung cancers. Due to the deficiency of the mutated cysteine, it is of great task to target KRAS G12D [14]. It is estimated that pancreatic cancer will turn out to be the second leading

cause of cancer-related deaths by 2030, which highlights the need to develop new treatment drugs to improve the survival rate of patients with pancreatic cancer.

1) KRAS G12D is the most common KRAS mutation, inducing an aggressive phenotype via the activation of MAPK, PI3K, and Ras-like GEF (RalGEF) pathways.

2) However, when compared to KRAS G12C, selective inhibition of KRAS G12D presents a substantial challenge due to the requirement of inhibitors to bind KRAS G12D with high adequate affinity to avoid the necessity for covalent interactions with the mutant KRAS protein. The common strategies approved for anti-RAS drug development can be generally classified into direct and indirect approaches. Direct approaches comprise compounds that directly bind to RAS and disturb the interactions between RAS and GEFs/effectors. Indirect approaches include: (a) Inhibiting proteins such as farnesyltranseferase or PDE that encourages the association of RAS with plasma membrane. (b) Targeting proteins involved in RAS downstream signalling.

(c) Inhibiting synthetic lethal interaction partners for mutant RAS. (d) Targeting processes that are RAS regulated, for example, macropinocytosis and autophagy.

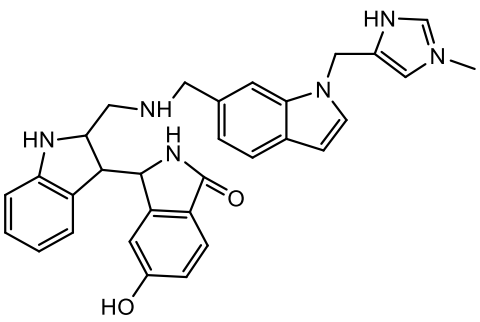
3) Although KRAS G12D is an excellent drug discovery target for many cancers, no drug directly targeting KRAS G12D has been clinically approved. Targeting the KRAS G12D mutation with a small molecule still remains a challenge due to deficiency of druggable pockets on the surface of RAS. However, with the study of RAS binding to low molecular weight organic molecules and the recent FDA approval of AMG 510 (Lumakras), a KRAS G12C inhibitor binding to GDP, there has been a resurgence of research surrounding RAS. 4) Recent studies have revealed that mutated KRAS expression is associated with reduced anticancer activity of gemcitabine and paclitaxel, the drugs currently used in the clinical treatment of pancreatic cancer, and new therapeutic strategies of targeting KRAS should inhibit tumour cell growth and combat drug resistance.

5) KRAS mutations lead to a reduction in intrinsic GTPase activity, which further reductions the rate of GTP hydrolysis and ultimately remains to activate downstream pathways and produce carcinogenesis. The intrinsic hydrolysis rate of the KRAS G12C mutation is equivalent to approximately 70% of that of the wild-type KRAS, while the intrinsic hydrolysis rate of the KRAS G12D mutation is only less than 30%. This disadvantage poses a challenge for the design of KRAS G12D inhibitors. It is similarly challenging to determine whether the inhibitor has sufficient affinity for 12-aspartate involved in the KRAS G12D mutant to evade binding to wild-type KRAS [9].

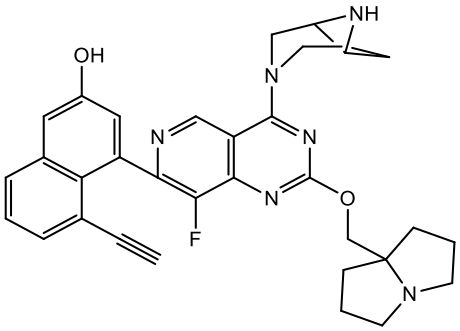
4.2 Recent Progresses of KRAS G12D Inhibitors:

1. Kessler et al. discovered BI-2852 as a direct inhibitor of KRAS that targets the region between switch I and II. BI-2852 was shown to form polar interactions with Glu37, Ser39, and Asp54, and by doing so, it blocks the interaction of KRAS with SOS and also a bridged pERK and pAKT levels. BI-2852 reduced pERK and pAKT levels in a dose-dependent manner, leading to an antiproliferative effect in NCI-H358 cells. The effects of this were established to be KRAS-driven and not undefined effects, through the constant data generated for the 10-fold weaker distomer. This compound exhibited a significantly reduced ClogP of 2.6 and had a solubility of 18 μg/mL at pH 6.8 while maintaining nanomolar binding affinity to GTP-KRAS G12D (KD = 750 nM) as measured by ITC and with an IC50 of 450 nM in the Alpha Screen [9] Using biochemical assays, they investigated whether this inhibited 3 of the 4 PPIs important for KRAS cycling. Namely, a) GDP KRAS interaction with the catalytic site of SOS b) GTP KRAS interaction with the allosteric site of SOS and c) GTP-KRAS interaction with downstream effectors (CRAF and PI3Kα). They were unable to establish a biochemical assay for the fourth intervention point, namely, d) GTP-KRAS interaction with its GAPs. As a reference compound for these assays, they used the KRAS G12C-specific covalent inhibitor ARS-1620. BI-2852 inhibited all 3 RAS cycle intervention points (1 to 3) in a dose-dependent manner in the range of 100 to 770 nM. This highlights the potential for significant improvement beyond the current potency of BI-2852(e.g., IC50 of 180 nM for the PPI between active KRAS G12D and CRAF) and indicates that the SI/II-pocket is indeed druggable. The SI/II-pocket is involved in interactions with GEFs, GAPs, and downstream effectors, and they provide evidence that BI-2852 inhibits all of these PPIs. The effects of BI-2852 were established to be KRAS-driven and not off-target through the constant data generated. They expect BI-2852 to serve as a beneficial chemical probe for the learning of RAS biology in an in vitro setting, and it is available to the scientific community. BI-2852 is likewise an ideal starting point for the design of more-potent and selective RAS inhibitors. It was observed that BI-2852 bound to GTP-KRAS G12D with a nanomolar binding affinity (IC50 of 450 nM). (Kessler et al., 2019; Chen et al., 2020) [9].
2. Vasta et al. (2022) used the NanoBiT protein-protein interaction assay to assess the inhibitory effect of MRTX-EX185 on KRAS G12D in cells and demonstrated that MRTX-EX185 is a potent KRAS G12D inhibitor, albeit with low affinity [14].
3. Welsch et al. designed a small molecule compound, named 3144, had affinity in the micromolar range, but toxicity and off-target activity of compound 3144 were detected in cells and mice. In addition, its low water solubility made it problematic to use in some contexts (Welsch et al., 2017; Khan et al., 2020).
4. TH Z835 was designed based on salt-bridge and induced fit pocket formation for KRAS G12D targeting and inhibited the proliferation of cancer cells and significantly reduced the tumour volume. However, THZ835 had an off-target effect because the inhibition was not fully dependent on KRAS mutation status (Mao et al., 2022).
5. Mirati Therapeutics announced a selective non-covalent inhibitor, MRTX1133 **of** KRAS G12D. The structure of MRTX1133 is based on MRTX849, a KRASG12C inhibitor established by Mirati Therapeutics. Based on the structure of MRTX849, the electrophilic receptor, acrylamide, was substituted with piperazine to form intermolecular ion pair force. MRTX1133 binds to the switch-II pocket and prevents the protein−protein interactions necessary for the activation of the downstream pathway of KRAS. Jianxin Cheng et al., found that MRTX1133 could bind to both the GDP-bound state (inactive) and the GTP-bound (active) state of KRAS G12D and inhibit the active function of KRAS G12D. It is straight forward that the binding of MRTX1133 to the GDP (inactive) bound state of KRAS G12D may stabilize the KRAS G12D in an inactive form. Further remarkably, how MRTX1133 bound to GTP-bound state of KRAS G12D and not employ the active function of KRAS G12D has not been fully understood. Compared with KRAS G12C inhibitors, the development of KRAS G12D inhibitors faces greater challenges due to the deficiency of covalent bound anchor. The KRAS G12D small molecule inhibitor MRTX1133 has revealed remarkable antitumor effects. In summary, inhibitor MRTX1133 stabilize the switch II region and leave the switch I region dynamically in an inactive conformation [17].

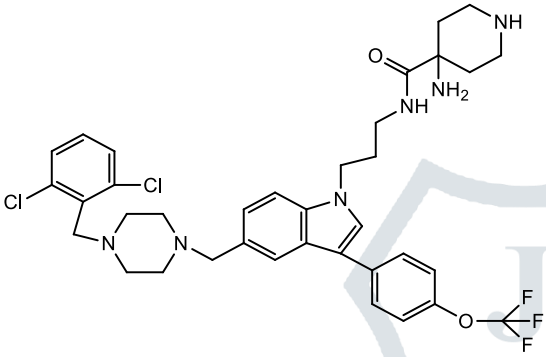
6. The investigators introduced a salt bridge between the inhibitor and 12-aspartate to enhance the reversible affinity for KRAS G12D. This strengthened the selectivity of the inhibitor for KRAS G12D through a series of modifications to avoid binding to wild-type KRAS. Compared to several KRAS G12C inhibitors whose alterable affinity for the target is in the micromolar range {Canon, J.et al. (2019); & Fell, J.B.et al (2020)}, MRTX1133 has a picomolar range of reversible affinity for KRASG12D. At present, this is in clinical phase and additional studies is going on [15].
7. Michael J et al combined structure-based design with a battery of cell and biophysical assays to determine a novel pyrazolopyrimidine-based allosteric KRAS inhibitor that binds to activated KRAS with sub-micromolar affinity and interrupts effector binding, thereby inhibiting KRAS signalling and cancer cell. Pyrazolopyrimidine-based compounds may represent a first-in-class allosteric noncovalent inhibitor of KRAS. The inhibitory potential of compounds was tested in monoclonal baby hamster kidney (BHK) cell lines stably expressing monomeric green fluorescence protein (mGFP)-tagged KRAS G12D. Compound (2- [4-(8-methyl-3,9- diphenyl-2,6,7-triazabicyclo[4.3.0]nona-2,4,7,9-tetraen-5-yl)- piperazin-1-yl]ethanol) is druglike (drug likeness = 4.1) and somewhat polar with six hydrogen bond donors and two acceptors (clogP = 0.87). It has a pyrazolopyrimidine core rather than an indole or imidazole ring typical in published ligands. Compound binds to GTP KRAS with submicromolar affinity, inhibits MAPK signaling, and decreases the growth of cancer cells expressing mutant KRAS more competently than those expressing HRAS. They propose that the piperazine ethanol group interacts with switch 1 of KRAS and plays a serious role in revoking effector binding, whereas the potentially switch 2-interacting nonpolar moieties attached to the pyrazolopyrimidine core modulate GEF activity and contribute to high-affinity binding. These insights provide ideal starting points for further optimization of our highly promising lead compounds. Compound may characterize the first small molecule to selectively bind GTP-bound KRAS with high affinity. Compound dose-dependently decreases both p-ERK and p-cRaf levels in BHK cells expressing KRAS G12D, suggesting inhibition of RAS signalling via the MAPK pathway [18].
8. Yutingwang et.al selected 69 compounds of pharmacophore-base screening were docked into the KRAS G12D binding site. TH-Z835 was used as the positive control and has a docking score of - 10.04 kcal/mol. Based on docking scores, four top compounds (namely, hit compounds 1–4) below -10.04 kcal/mol and other three hit compounds (namely, hit compounds 5–7) ranking below them were further selected for predicting the three-dimensional interaction modes similar to THZ835. Hit compounds 1–4 exhibited hydrogen-bond interactions with the key amino acids in the active site including His95, Glu62, Gly60, and Asp12. Compared with TH-Z835, halogen atoms in the skeleton of hit compounds 1–4 not only could show stronger hydrophobic interactions with Val9, but also could greatly increase molecular liposolubility and metabolic stability. TH-Z835, hit 3 with the IC50 value of 43.80 nM exhibited a stronger anti-proliferative activity on Panc 04.03 cells. Therefore, hit compound 3 was carefully chosen as a lead compound and further used for anti-tumor experimental evaluation in vivo. In this experiment, the MTT method was used to detect the properties of hit compounds 1–4 on the proliferation of human pancreatic cancer cells (Panc 04.03). The results showed that hits 1–4 had a dose dependent effect on the proliferation of Panc 04.03 cells. In addition, their IC50 values were further calculated. Compared with other hits and the positive control. The MST experimental result showed that hits 1–4 had sub nanomolar affinities for KRAS G12D. Particularly, hit compound 3 had a remarkable anti-proliferation effect on human pancreatic carcinoma and significantly inhibited tumour growth in tumour bearing mice. Since hit compound 3 is the most promising lead compound targeting KRAS G12D, structural optimization of hit compound 3 is currently under way in their laboratory to further discover structurally novel and more active KRAS G12D inhibitors [14].
9. Various strategies have been testified for targeting KRAS G12D, including those using indole-based small molecules to target a switch-I/II pocket compound (KAL-21404358) to target the P110 site, a pan-RAS inhibitor (compound 3144) to target the A59 site, and a cyclic peptide (KD2) to target KRAS G12D none of these molecule's targets KRAS G12D with appropriately low (micromolar) concentration. Rey-Ting Guo et al., speculated that a compound targeting the aspartic acid residue of KRAS G12D may in some way bind to KRAS G12D (similar to that formed between inhibitors and the cysteine residue of KRAS G12C), as a result may induce an allosteric pocket. They successfully developed a series of small molecule inhibitors of KRAS G12D, which function by inducing an allosteric S-IIP and forming a salt bridge bonding with Asp12 residue, as established by crystallographic studies. These inhibitors bind to both GDP-bound and GTP-bound KRAS G12D, efficiently disrupt KRAS–CRAF interaction. They synthesized TH-Z801, which exert inhibition (IC50 = 115 μM), assessed based on the GDP exchange rate of KRAS G12D as catalysed by SOS. Further chemical investigation focusing on piperazine substitution yielded TH-Z816 (wherein the piperazine was (R)-methyl-substituted), which had relatively strong inhibition activity (IC50 = 14 μM). A salt bridge-based strategy for targeting KRAS G12D with a methyl-substituted piperazine inhibitor. Assuming that the α-carboxylic acid moiety of Asp12 is deprotonated under physiological conditions, we chased a strategy based on the formation of a strong interaction (salt-bridge) between Asp12 and an alkyl amine moiety on an inhibitor. They further conducted isothermal titration calorimetry (ITC) assays to test whether TH-Z816 can bind directly to KRAS G12D. Indeed, the detected binding affinity (KD) of TH-Z816 with KRAS G12D (GDP-bound) was 25.8 μM [2].
10. HRS-4642 is also an inhibitor which treats advanced solid tumours with KRAS G12D mutation. Recently this inhibitor is under phase 1 of clinical trial. Jiangsu HengRui Medicine Co, Ltd. Company carries out further study and evaluate safety and tolerability of HRS-4642 to estimate maximum tolerated dose (MTD) and biologically active dose within investigated subject population groups.
11. BI-1701963 is a small molecule protein-protein interaction inhibitor that prevents the interaction between KRAS and SOS
12. Binding of BI-1701963 to the catalytic site of SOS1 inhibits binding of SOS1 to RAS-GDP, thereby hindering activation of KRAS proteins.
13. Preclinical studies demonstrated cytostatic effects for BI-1701963 in cancer cells with an activated KRAS pathway, and combination with a MEK inhibitor resulted in a more pronounced effect.
14. Boehringer Ingelheim Company studies this inhibitor and currently it is under phase 1 of clinical trial.
15. BI-1823911 used for Advanced or Metastatic solid tumour with KRAS G12D Mutation and currently it is under phase 1 of clinical trial. Karyopharm Therapeutics Inc. Company studied KPT-8602 inhibitor for metastatic colorectal cancer which is under phase 1 and 2 of clinical trial. Sequentially we have mentioned in below.



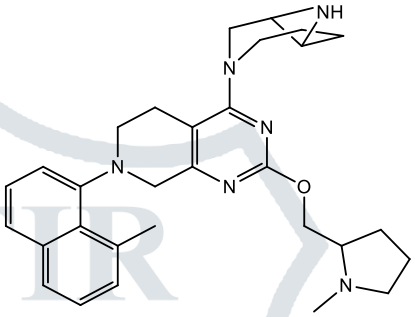
A



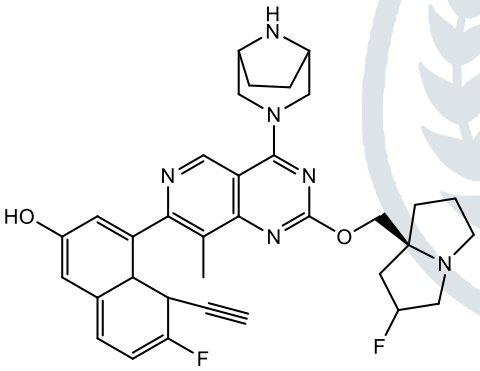
B



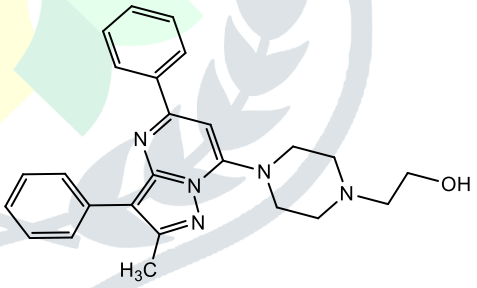
C



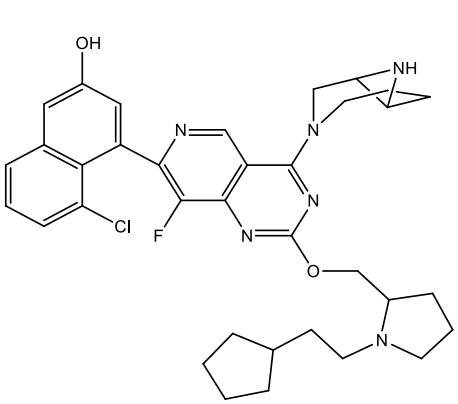
D



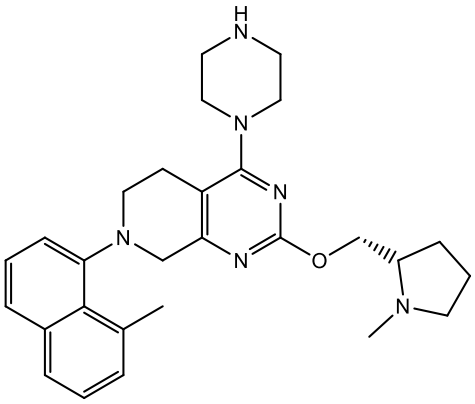
E



F



G



H

A= BI-2852

B= MRTX-EX185

C= 3144

D= TH-Z835

E= MRTX1133

F= (2-[4-(8-methyl-3,9-diphenyl-2,6,7-triazabicyclo[4.3.0]nona-2,4,7,9-tetraen-5-yl)- piperazin-1-yl]ethanol)

G= 4-(4-((7S)-3, 8-diazabicyclo[5.1.0]octan-3-yl)-2-((1-(2-cyclopentylethyl)pyrrolidin-2-yl)methoxy)-8-fluoropyrido[4,3-d]pyrimidin-7-yl)-5-chloronaphthalen-2-ol

H= TH-Z801

DRUG	TUMOUR TYPE	IC 50	PHASE	DESCRIPTION	Clinical trial.gov. identifier
MRTX1133	Advanced Solid Tumors with KRAS G12 D Mutation	2nM	½	MRTX1133 binds to the switch-II pocket and inhibits the protein−protein interactions necessary for the activation of the downstream pathway of KRAS.	NCT05737706
HRS-4642	Advanced solid tumors' harboring KRAS G12D Mutation	---	1	To evaluate safety and tolerability of HRS-4642. To estimate maximum tolerated dose (MTD) and biologically active dose within investigated subject population groups.	NCT05533463
BI-1701963	Metastatic Colorectal Cancer	50 mg	1	Monotherapy safety run-in part, combination dose escalation part, a randomization will be included for the expansion therapy part.	NCT04627142
BI-1823911	Advanced or Metastatic solid tumor with KRAS G12D Mutation	50 mg	1	The purpose of study is to find the highest dose of BI 1823911 that people can tolerate when taken alone and together with BI-1701963.	NCT04973163
KPT-8602	Metastatic colorectal cancer (Mcrc)	--	Phase 1 Phase 2	First in human dose escalation (phase 1) & dose expansion (phase2), to assess preliminary safety, tolerability, and efficacy.	NCT02649790
BI-2852	Advanced Solid Tumors with KRAS G12D Mutation	450	Preclinical Phase	Direct inhibitor of KRAS that targets the region between switch I and II.	---
Compound 11	Advanced Solid Tumors with KRAS G12D Mutation	1.3μM	Preclinical Phase	Compound 11 binds to GTPKRAS with sub micro molar affinity , and reduces the growth of cancer cells expressing mutant KRAS more efficiently	---
pyrazolopyrimidine-based inhibitor	Advanced Solid Tumours with KRAS G12 D Mutation	43.80	Preclinical Phase	Had a remarkable anti-proliferation effect on human pancreatic cancer cells and significantly inhibited tumor growth in tumor bearing KRAS G12D mutation	---
TH-Z801	Advanced Solid Tumors with KRAS G12 D Mutation	115 Mm	Preclinical Phase	This Inhibitors bind to both GDP-bound and GTP-bound KRAS G12D, efficiently disrupt KRAS–CRAF interaction.	----
TH-Z816	Advanced Solid Tumors with KRAS G12 D Mutation	14 μM	Preclinical Phase	This Inhibitors bind to both GDP-bound and GTP-bound KRAS G12D, efficiently disrupt KRAS–CRAF interaction.	----

Table 4.2: List of Discovered KRAS G12D Inhibitors

V. FUTURE DIRECTONS AND CONCLUSION

Specific KRAS G12D inhibitors will change the therapeutic landscape of KRAS-driven tumours, benefiting many patients with KRAS mutations. Undesirably innate and acquired resistance to KRAS inhibitors has delayed their development, rendering these new drugs less effective or even ineffective. In preclinical studies, possible resistance mechanism for KRAS mutation therapy include secondary

mutations in the KRAS binding site, reactivation of multiple upstream and downstream effectors, cell-cycle dysregulation, and immune deficiency. Importantly, this mechanism of drug resistance appears to be tissue specific.

It was thought that KRAS protein plays an on/off role in the GDP/GTP cycle. However, this concept oversimplifies the complex interactions between the states of individual molecules and the resulting dynamic protein conformations, which is unique to each KRAS mutant determines the active state of entire KRAS protein, in the cells. Our current understanding of the impact of different mutations on the biochemical activity of KRAS proteins is based on the results of extensive structure- function studies conducted over the past 40 years, while biological validation of functional differences has only begun in recent years.

We have mentioned various KRAS G12D inhibitors in this review. Kessler et al., reported BI-2852 but due to less cellular activity it is in preclinical trial; Vasta et al. reported MRTX-EX185 which is potent KRAS G12D inhibitor with low affinity and then Welsch et al. and khan Khan et al. reported compound 3144 but due to its low solubility it made some difficulties to use: Mao et al. reported TH-Z835 which had an off-target effect on KRAS mutation. One of the inhibitors MRTX-1133 for KRAS G12 D showed activity in preclinical trial and currently it is in under phase 1/2 of clinical trial phase. MRTX-1133 binds to the switch 2 pocket and inhibits the protein-protein interactions necessary for the activation of the downstream pathway of KRAS. This inhibitor is studied under Mirati therapeutics co. ltd. Recently clinical studies of various inhibitors such as HRS-4642, BI-1701963, BI-1823911, KPT-8602 is under process of activity are still remaining in preclinical trials. It is believed that in near future KRAS G12D inhibitors will provide a new prospective on curing cancers.

REFERENCES

[1] Shen, H.; Lundy, J.; Strickland, A.H.; Harris, M.; Swan, M.; Desmond, C.; Jenkins, B.J.; Croagh, D. *KRAS* G12D Mutation Subtype in Pancreatic Ductal Adenocarcinoma: Does It Influence Prognosis or Stage of Disease at Presentation? *Cells* **2022**, *11*, 3175. https://doi.org/10.3390/cells11193175

[2] Mao, Z., Xiao, H., Shen, P. *et al.* KRAS (G12D) can be targeted by potent inhibitors via formation of salt bridge. *Cell Discov* **8**, 5 (2022). https://doi.org/10.1038/s41421-021-00368-w

[3] Kodaz H, Kostek O, Hacioglu M, Erdogan B, Elpen Kodaz C, Hacibekiroglu I, et al. Frequency of Ras Mutations (Kras, Nras, Hras) in Human Solid Cancer. EJMO. 2017; 1(1): 1-7

[4] He Q, Liu Z, Wang J. Targeting KRAS in PDAC: A New Way to Cure It? Cancers (Basel). 2022 Oct 11; 14(20):4982. doi: 10.3390/cancers14204982. PMID: 36291766; PMCID: PMC9599866.

[5] Feng H, Zhang Y, Bos PH, Chambers JM, Dupont MM, Stockwell BR. K-Ras^{G12D} Has a Potential Allosteric Small Molecule Binding Site. Biochemistry. 2019 May 28; 58(21):2542-2554. doi: 10.1021/acs.biochem.8b01300. Epub 2019 May 14. PMID: 31042025; PMCID: PMC8158984.

[6] Xie C, Li Y, Li LL, Fan XX, Wang YW, Wei CL, Liu L, Leung EL, Yao XJ. Identification of a New Potent Inhibitor Targeting KRAS in Non-small Cell Lung Cancer Cells. Front Pharmacology. 2017 Nov 14; 8:823. doi: 10.3389/fphar.2017.00823. PMID: 29184501; PMCID: PMC5694459.

[7] Kulkarni, A.M.; Kumar, V.; Parate, S.; Lee, G.; Yoon, S.; Lee, K.W. Identification of New KRAS G12D Inhibitors through Computer-Aided Drug Discovery Methods. *Int. J. Mol. Sci.* **2022**, *23*, 1309. https://doi.org/10.3390/ijms23031309

[8] Wang Y, Zhang H, Li J, et al. Discovery of potent and noncovalent KRAS^{G12D} inhibitors: Structure-based virtual screening and biological evaluation. Frontiers in Pharmacology. 2022; 13:1094887. DOI: 10.3389/fphar.2022.1094887. PMID: 36618907; PMCID: PMC9815544.

[9] Pirlog R, Calin GA. KRAS mutations as essential promoters of lymph angiogenesis via extracellular vesicles in pancreatic cancer. J Clin Invest. 2022 Jul 15; 132(14):e161454. doi: 10.1172/JCI161454. PMID: 35838046; PMCID: PMC9282924.

[10] Chang HH, Moro A, Takakura K, Su HY, Mo A, Nakanishi M, Waldron RT, French SW, Dawson DW, Hines OJ, Li G, Go VLW, Sinnett-Smith J, Pandol SJ, Lugea A, Gukovskaya AS, Duff MO, Rosenberg DW, Rozengurt E, Eibl G. Incidence of pancreatic cancer is dramatically increased by a high fat, high calorie diet in KrasG12D mice. PLoS One. 2017 Sep 8; 12(9):e0184455. doi: 10.1371/journal.pone.0184455. PMID: 28886117; PMCID: PMC5590955.

[11] Pirlog R, Calin GA. KRAS mutations as essential promoters of lymph angiogenesis via extracellular vesicles in pancreatic cancer. J Clin Invest. 2022 Jul 15; 132(14):e161454. doi: 10.1172/JCI161454. PMID: 35838046; PMCID: PMC9282924.

[12] Liu C, Zheng S, Wang Z, Wang S, Wang X, Yang L, Xu H, Cao Z, Feng X, Xue Q, Wang Y, Sun N, He J. KRAS-G12D mutation drives immune suppression and the primary resistance of anti-PD-1/PD-L1 immunotherapy in non-small cell lung cancer. Cancer Common (Lond). 2022 Sep; 42(9):828-847. doi: 10.1002/cac2.12327. Epub 2022 Jul 11. PMID: 35811500; PMCID: PMC9456691.

[13] Wang, Xiaolun, et al. "Identification of MRTX1133, a noncovalent, potent, and selective KRASG12D inhibitor." *Journal of medicinal chemistry* 65.4 (2021): 3123-3133.

[14] Negri F, Bottarelli L, de'Angelis GL, Gnetti L. KRAS: A Druggable Target in Colon Cancer Patients. Int J Mol Sci. 2022 Apr 8; 23(8):4120. doi: 10.3390/ijms23084120. PMID: 35456940; PMCID: PMC9027058.

[15] Zhu, C., Guan, X., Zhang, X. *et al.* Targeting KRAS mutant cancers: from druggable therapy to drug resistance. *Mol Cancer* **21**, 159 (2022). https://doi.org/10.1186/s12943-022-01629-2

[16] Yang, Y.; Zhang, H.; Huang, S.; Chu, Q. KRAS Mutations in Solid Tumors: Characteristics, Current Therapeutic Strategy, and Potential Treatment Exploration. *J. Clin. Med.* **2023**, *12*, 709. https://doi.org/10.3390/jcm12020709.

[17] Wang X, Allen S, Blake JF, Bowcut V, Briere DM, Calinisan A, Dahlke JR, Fell JB, Fischer JP, Gunn RJ, Hallin J, Laguer J, Lawson JD, Medwid J, Newhouse B, Nguyen P, O'Leary JM, Olson P, Pajk S, Rahbaek L, Rodriguez M, Smith CR, Tang TP, Thomas NC, Vanderpool D, Vigers GP, Christensen JG, Marx MA. Identification of MRTX1133, a Noncovalent, Potent, and Selective KRAS^{G12D} Inhibitor. J Med Chem. 2022 Feb 24;65(4):3123-3133. doi: 10.1021/acs.jmedchem.1c01688. Epub 2021 Dec 10. PMID: 34889605.

[18] McCarthy MJ, Pagba CV, Prakash P, Naji AK, van der Hoeven D, Liang H, Gupta AK, Zhou Y, Cho KJ, Hancock JF, Gorfe AA. Discovery of High-Affinity Noncovalent Allosteric KRAS Inhibitors That Disrupt Effector Binding. ACS Omega. 2019 Feb 28;4(2):2921-2930. doi: 10.1021/acsomega.8b03308. Epub 2019 Feb 8. PMID: 30842983; PMCID: PMC6396121.

[19] Saliakoura M, Konstantinidou G. Lipid Metabolic Alterations in KRAS Mutant Tumors: Unmasking New Vulnerabilities for Cancer Therapy. Int J Mol Sci. 2023 Jan 16;24(2):1793. doi: 10.3390/ijms24021793. PMID: 36675307; PMCID: PMC9864058.
[20] Shen, H.; Lundy, J.; Strickland, A.H.; Harris, M.; Swan, M.; Desmond, C.; Jenkins, B.J.; Croagh, D. *KRAS* G12D Mutation Subtype in Pancreatic Ductal Adenocarcinoma: Does It Influence Prognosis or Stage of Disease at Presentation? *Cells* **2022**, *11*, 3175. https://doi.org/10.3390/cells11193175
[21] Hofmann MH, Gerlach D, Misale S, Petronczki M, Kraut N. Expanding the Reach of Precision Oncology by Drugging All KRAS Mutants. Cancer Discov. 2022 Apr 1;12(4):924-937. doi: 10.1158/2159-8290.CD-21-1331. PMID: 35046095; PMCID: PMC9394389.
[22] Wang Y, Zhang H, Li J, Niu MM, Zhou Y, Qu Y. Discovery of potent and noncovalent KRAS^{G12D} inhibitors: Structure-based virtual screening and biological evaluation. Front Pharmacol. 2022 Dec 22;13:1094887. doi: 10.3389/fphar.2022.1094887. PMID: 36618907; PMCID: PMC9815544.
[23] Shai A, Galouk E, Miari R, Tareef H, Sammar M, Zeidan M, Rayan A and Falah M: Inhibiting mutant KRAS G12D gene expression using novel peptide nucleic acid-based antisense: A potential new drug candidate for pancreatic cancer. Oncol Lett 23: 130, 2022
[24] Zhu GQ, Tang Z, Huang R, Qu WF, Fang Y, Yang R, Tao CY, Gao J, Wu XL, Sun HX, Zhou YF, Song SS, Ding ZB, Dai Z, Zhou J, Ye D, Wu DJ, Liu WR, Fan J, Shi YH. CD36^{+} cancer-associated fibroblasts provide immunosuppressive microenvironment for hepatocellular carcinoma via secretion of macrophage migration inhibitory factor. Cell Discov. 2023 Mar 6;9(1):25. doi: 10.1038/s41421-023-00529-z. PMID: 36878933; PMCID: PMC9988869.
[25] Parikh, K., Banna, G., Liu, S.V. *et al*. Drugging KRAS: current perspectives and state-of-art review. *J Hematol Oncol* **15**, 152 (2022). https://doi.org/10.1186/s13045-022-01375-4
[26] Michael J. McCarthy, Cynthia V. Pagba, Priyanka Prakash, Ali K. Naji, Dharini van der Hoeven, Hong Liang, Amit K. Gupta, Yong Zhou, Kwang-Jin Cho, John F. Hancock, and Alemayehu A. Gorfe *ACS Omega* **2019** *4* (2), 2921-2930 DOI: 10.1021/acsomega.8b03308