**Question**:

**Critically discuss both past failed and current successful strategies of targeting Ras as new cancer drugs. Include the name of one specific approved drug.**

**Ans:**

*RAS*, or Rat-associated sarcoma, is a gene frequently mutated in cancers (25% -30 %) and is, in fact, one of the hallmarks of cancer (Cox & Der, 2010). Ras proteins are members of the monomeric GTPases family that act as a molecular switch to transduce the growth proliferating signal downstream of the surface receptors to activate a series of pathways for cell division and growth (Milburn *et al*., 1990); Vetter & Wittinghofer, 2001). In the classical EGFR-RAS-RAF-MEK-ERK pathway (MAPK- pathway), for example, a signal from growth factors such as EGF (Epidermal growth factor) or IGF (Insulin-like growth factor) causes activation of the receptor tyrosine kinase (EGFR) by dimerization and autophosphorylation (Cully & Downward, 2008). The activated EGFR activates SOS (sister of seven less) by phosphorylation, and SOS recruits Ras to the scene. SOS is a guanine nucleotide exchange factor (GEF). Normally, the Ras protein exists in an inactive GDP-bound state in the cell. However, upon activation, the SOS promotes the dissociation rate of GDP such that an exchange of GDP/GTP occurs. Because the natural concentration of GTP is higher in the cytoplasm, it replaces bound GDP from the Ras protein, and the inactive Ras-GDP becomes an active Ras-GTP. Ras in its active form activates MAPKKK (Map kinase kinase kinase) or Raf, which activates MEK or MAPKK (Map kinase), which then activates Erk (MAPK) or Map kinase. Erk, which normally resides in the cytoplasm, upon activation, goes inside the nucleus and activates a group of transcription factors such as c-fos or jun or AP-1, which ultimately increase the transcription of certain genes, such as Cyclin D. Genes such as Cyclin D are required for cell cycle progression. Ras has an intrinsic GTPase activity, potentiated by a GAP (GTPase activating protein) protein. Hence, the signal by the growth factor has a short half-life under normal circumstances.

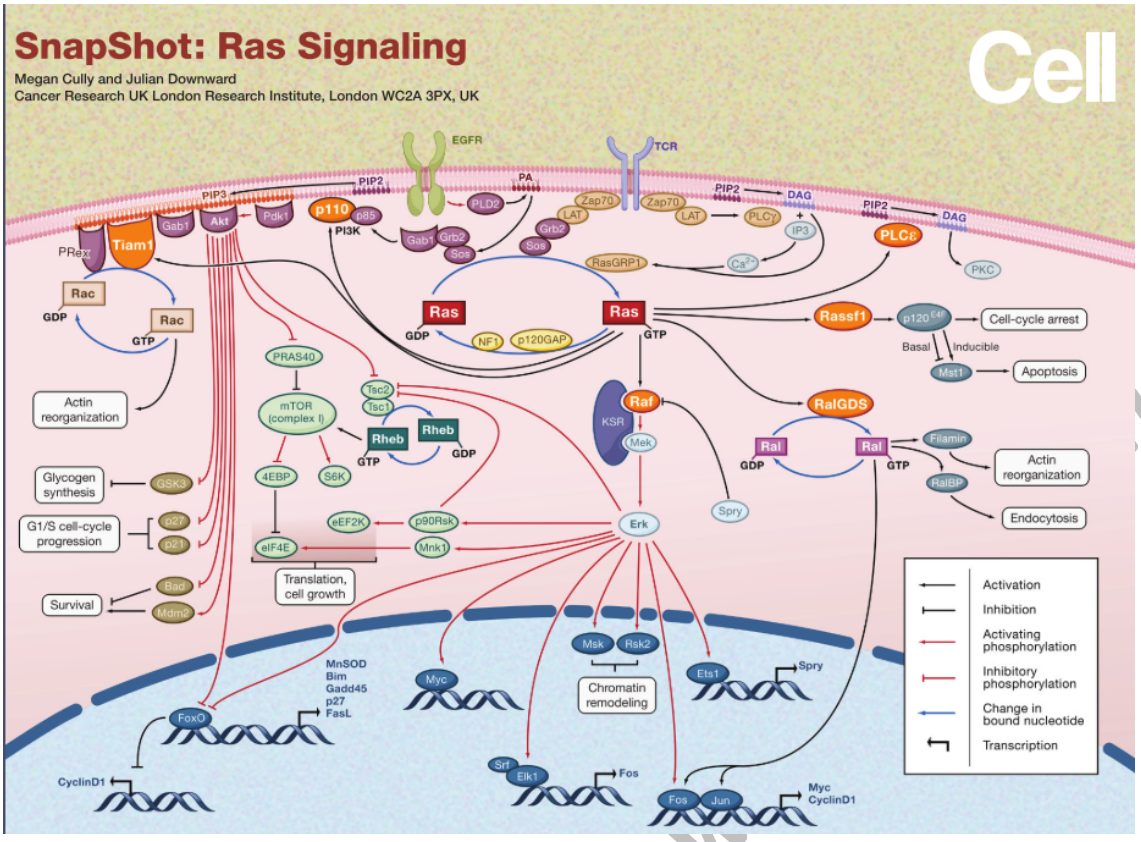



*Figure 1A snapshot of Ras signalling (Cully & Downward, 2008).*

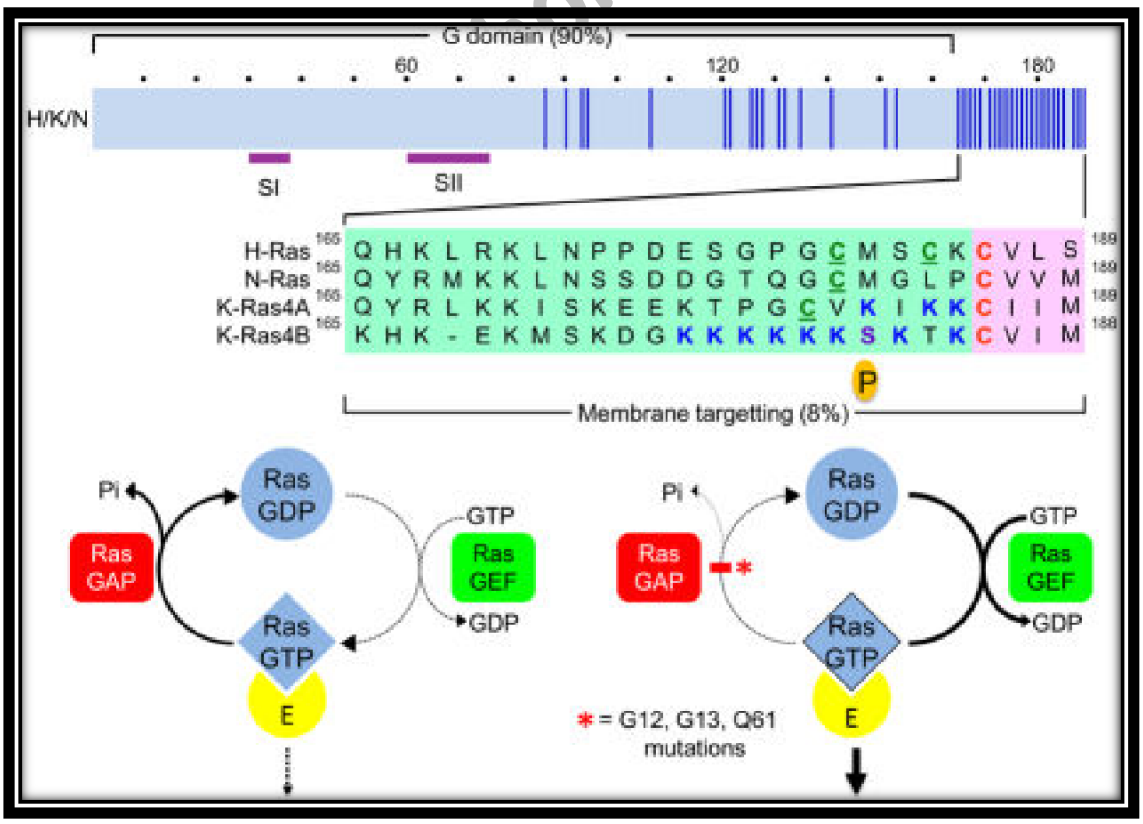
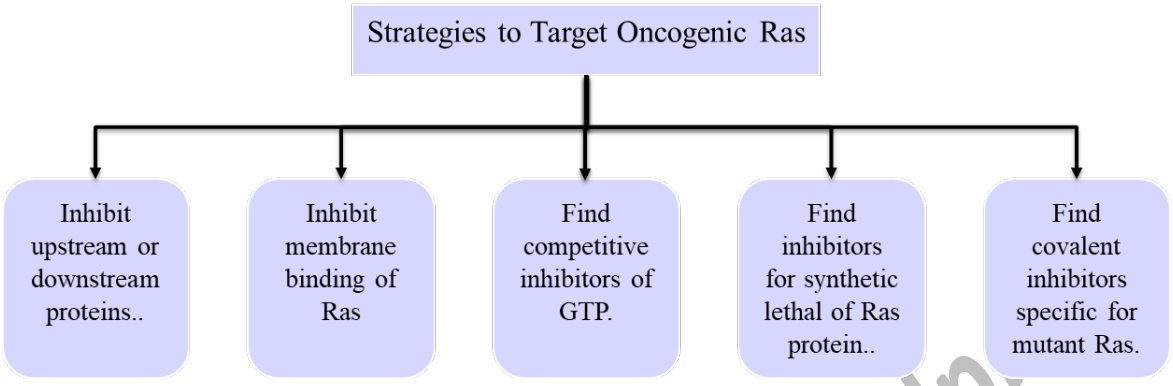



*Figure 2Ras GTP cycle in the wild-type Ras and the mutant Ras (*Cox et *al*., 2014*).*

In tumors, an activating mutation in the RAS gene (mostly at the 12th amino acid glycine or, 13th Glycine or 61st Glycine) causes the mutant Ras protein to be refractory to GAP. This results in a substantially longer half-life of Ras-GTP; thus, the downstream signal is persistent, thereby giving the tumour cell a proliferation advantage.




Many inhibitors of this classical cellular proliferation pathway have been developed (Moore *et al*., 2020). However, most have been against either the upstream (like EGFR) targets or the downstream (Raf, Mek, or Erk) targets. However, the effect of upstream inhibitors might not be full if the Ras is mutated and activated without needing the upstream receptor. Similarly, the downstream effect may not be sufficient for inpatient patients who acquire resistance due to mutation in these proteins, so the inhibitor cannot bind.

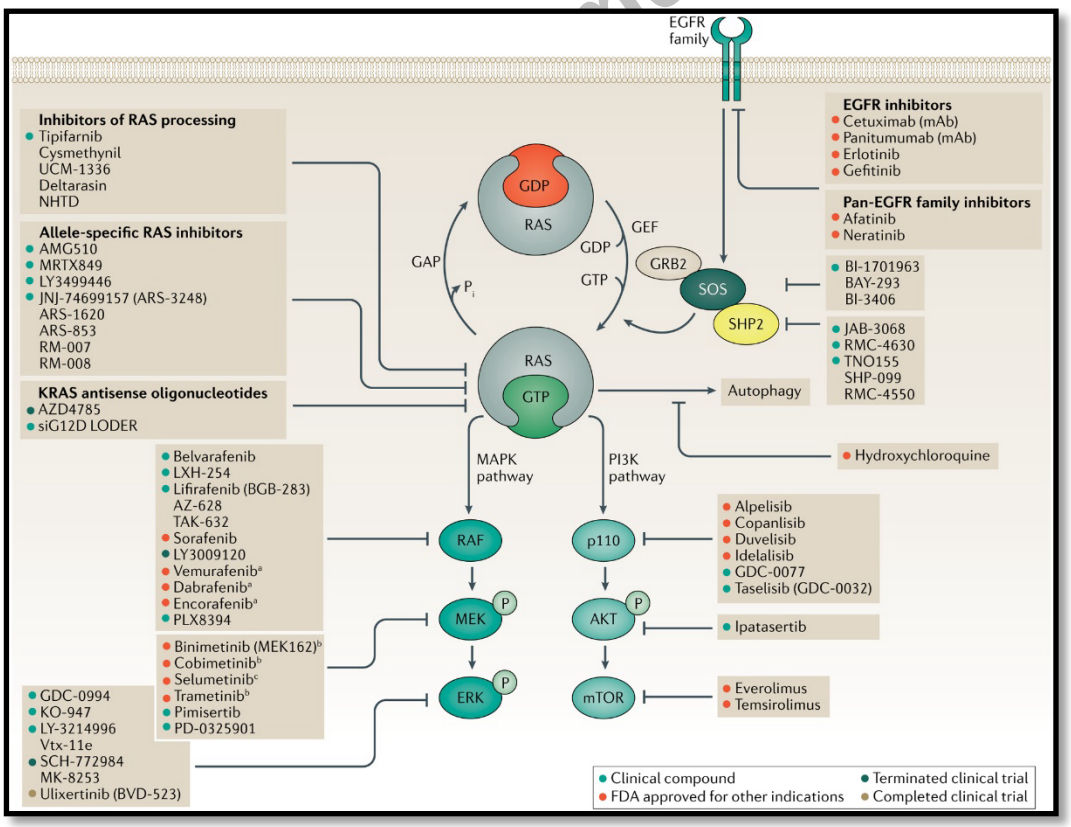



*Figure 3Inhibitors of the Ras Pathway (*Moore *et al*., 2020*).*

The other strategies to target Ras oncoprotein can be to block its membrane association, tackle the oncogenic Ras-associated metabolic changes, and find the "synthetic lethal" proteins. Ras, however, at least till 2013, had been considered "undruggable" (Matikas *et al*., 2017; Moore *et al*., 2020). This is because, for effective therapeutic results, a mutant selective inhibitor is required to minimize the side effects. This is important because the Ras pathway is required for cell survival, and cells die in the absence of this pathway. Thus, if an inhibitor against mutant Ras also acts against the wild-type Ras, it will be toxic to the patient.

Further, since the Ras proteins are highly conserved and mutation is just a point mutation, it was hard to develop a mutant selective inhibitor. The structure obtained via X-ray crystallography is of the Raf binding domain (RBD), not the full Ras protein. Moreover, a clear-cut allosteric site differentiative of wild-type and mutant Ras was not reported, and the Ras has a very high affinity for Guanine nucleotides (with picomolar affinity). Hence, designing a specific inhibitor of mutant Ras had been a challenge.

The initial attempt to develop Ras-specific inhibitors was focused on finding a competitive inhibitor of GTP, targeting the nucleotide-binding site of the Ras protein (Christensen *et al*., 2020). However, since GTP has a very high affinity (in the picomolar range), finding an inhibitor with a higher affinity to Ras was more difficult than finding an inhibitor with a higher affinity to the guanine nucleotide.

However, later, a compound, N-Hydroxysulfanilamides SCH-54292, was identified that binds to an allosteric site and mitigates the nucleotide "exchange" reaction in the Ras (Taveras *et al*., 1997). Nevertheless, the attempts to co-crystalize the Ras protein bound to the inhibitor failed, and an appreciative success concerning clinical development could not be achieved.

Subsequently, a strategy to find a compound that binds specifically to the mutant amino acid (for example, cysteine in G12G mutant) was adopted so that the mutant Ras could be specifically targeted (Ostrem *et al.*, 2013; Ostrem & Shokat, 2016). By adopting a “tethering” approach in which the nucleophilic character of the -SH group of cysteine was utilized to bind different compounds, Osterm *et a*l. reported inhibitors that bind to the allosteric site that doesn't overlap with the bound GDP. These compounds are bound to the mutant cysteine residue, and even in the presence of 1mM GDP in the presence of EDTA, they remain in the bound state. Moreover, these compounds do not bind to the wild-type Ras. Thus, mutant-specific inhibitors were obtained. The crystal structures of such compounds revealed an extra pock in the switch II region of Ras (called switch II pocket) where these compounds reside, and they also affect the Gly60 residue, which is required for nucleotide exchange reaction. These compounds also disturb the metal coordination, altering the three-dimensional position of switch I and switch II regions of Ras such that GTP can not bind. In other words, these compounds inhibited the activation of the mutant Ras.

Based on the above finding, a covalent inhibitor of mutant Ras, ARS-853, was subsequently identified that bound to the Ras-GDP state, thereby preventing the GTP exchange and activation of the mutant Ras (Patricelli *et al*., 2016). The authors also show that the Ras-GTP is not static. Rather, Ras exist in a "hyperexcitable" state, in which the exchange of GDP/GTP occurs. Thus, the inhibitor binds to the GDP state and prevents GTP from exchanging; hence, it works more efficiently than the previous inhibitors. Three years later, the same group reported another inhibitor, ARS-1620, an atropisomer-selective KRASG12C inhibitor with desirable pharmacokinetics (Janes *et al*., 2018). They also reported that their inhibitor was

active in *vivo*. The crystal structure revealed that the inhibitor binds to the shallow switch II groove, although the catalysis by the K-Ras protein leads to the covalent bond formation (Hansen *et al*., 2018). Despite this, the inhibitor was not found suitable for clinical testing.

The crystal structure of Ras with ARS-1620 revealed that ARS-1620 forms hydrogen bonds with His95 of the Ras protein. His95, in another orientation, forms a pocket in Ras protein in which an aromatic ring can be fit (Canon *et al*., 2019). Thus, including an aromatic ring can enhance the potency of the inhibitor. This approach led to the discovery of an acrylamide-based covalent inhibitor called AMG-510, which binds to the novel His95 groove and the P2 pocket of Ras.

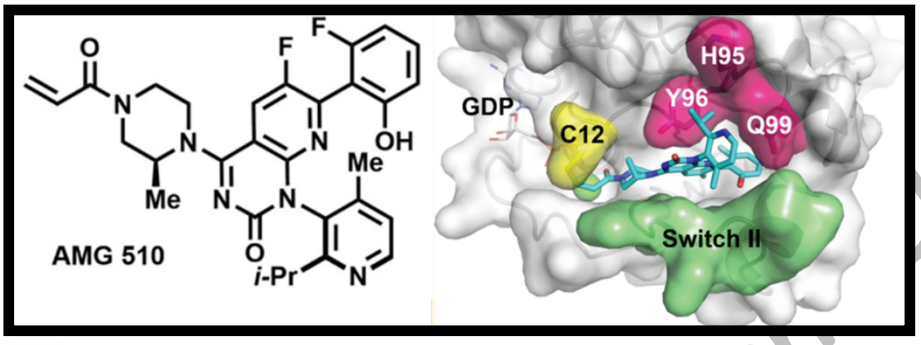



*Figure 4 The AMG-10 inhibitor with an aromatic ring and its crystal structure revealed the binding to the His95 groove near the switch II pocket on Ras (*Lanman *et al*., 2019*)*

The AMG-510 was shown to find relief in solid tumours with oncogenic Kras (Lanman *et al*., 2019). In addition, another inhibitor, MRTX849, was also shown to inhibit tumours in patients by reconditioning the tumour microenvironment and making the tumours more sensitive to checkpoint inhibition therapy (Hallin *et al*., 2020; Briere *et al*., 2021). The inhibitor MRTX849 (adagrasib) sought permission for clinical use. In contrast, the inhibitor AMG 510 (sotorasib) has been approved for clinical use ("FDA grants accelerated approval to sotorasib for KRAS G12C mutated NSC", 2022; Mirati Therapeutics, 2022).

However, the story is yet incomplete. Evolution is the rule of nature, and resistance in tumours against the Ras inhibitors has been observed (Tanaka *et al*., 2021). These resistances develop due to novel KRAS switch-II pocket mutations and polyclonal alterations that lead to the re-activation of the Ras-Mapk pathway in patients.

Thus, the researchers continue to find that therapy and the race against evolution are ongoing. Further inhibitors keep on being discovered. An inhibitor, ASP2453, has recently shown promise in the preclinical model (Nakayama *et al*., 2021). Whether it would be successful is yet to be observed.

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