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Targeting the Ras pathway in pediatric hematologic malignancies

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Abstract

Purpose: Ras pathway mutations are one of the most common type of alterations in pediatric hematologic malignancies and are frequently associated with adverse outcomes. Despite ongoing efforts to use targeted treatments, there remain no food and drug administration (FDA) approved medications specifically for children with Ras pathway mutated leukemia. This review will summarize the role of Ras pathway mutations in pediatric leukemia, discuss the current state of RAS pathway inhibitors and highlight the most promising agents currently being evaluated in clinical trials.

Recent findings: Efficacy of RAF and MEK inhibitors has been demonstrated across multiple solid and brain tumors and these are now considered the standard of care for treatment of certain tumor types in adults and children. Clinical trials are now testing these medications for the first time in pediatric hematologic disorders such as acute lymphoblastic leukemia, juvenile myelomonocytic leukemia and histiocytic disorders. Novel inhibitors of the Ras pathway, including direct RAS inhibitors, are now being tested in clinical trials across a spectrum of pediatric and adult malignancies.

Summary: Activation of the Ras pathway is a common finding in pediatric hematologic neoplasms. Implementation of precision medicine with a goal of improving outcomes for these patients will require testing of Ras pathway inhibitors in combination with other drugs in the context of current and future clinical trials.

Keywords

Ras; targeted therapy; pediatric leukemia

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Introduction

Activation of the Ras signaling pathway is one of the most common findings in cancer. RAS proteins act as molecular switches that cycle between the active, GTP-bound, state, and the inactive, GDP-bound, state. RAS is activated by guanine nucleotide exchange factors (GEFs), and the GTP bound RAS interacts with a number of effectors. GTPase-activating proteins (GAPs) downregulate RAS by accelerating its intrinsic GTPase activity. Alterations including point mutations, insertion/deletions, rearrangements, amplifications and deletions have been reported in nearly every gene in this pathway. *NRAS, KRAS, HRAS, NF1, PTPN11, BRAF, and CBL* are among the most commonly altered genes and lead to hyperactive signaling in effector pathways including PI3K/mTOR/AKT and RAF/MEK/ERK which are mitogen-activated protein kinases (MAPK). The final result is upregulation of pro-survival transcription factors resulting in increased cellular proliferation and enhanced survival (Figure 1).

In pediatrics, Ras pathway mutations are among the most common genomic alterations in both solid and hematologic malignancies. This review will focus on pediatric hematological malignancies with an emphasis on recent advances in pharmacological targeting of the Ras signaling pathway.

Incidence of Ras mutations in pediatric leukemia

Ras pathway activation is common in pediatric hematological malignancies (Table 1). Ras pathway mutations are found in over 30% of infant B-cell acute lymphoblastic leukemia (B-ALL)(1–3), non-infant B-ALL(4, 5) and high hyperdiploid B-ALL(6–8). Ras pathway and receptor tyrosine kinase (RTK) mutations are present in 70% of near haploid B-ALL(9). Ras (*NRAS, KRAS, BRAF, NF1,* and *PTPN11*) mutations are present in approximately 14% of T-ALL(10) and up to 30% of early T-cell precursor ALL(11). Ras mutations are also frequent in myeloid malignancies with nearly half of pediatric myelodysplastic syndromes (MDS)(12) and one-third of acute myeloid leukemia (AML)(13, 14) patients harboring Ras mutations. In ALL and AML, the mutations are often subclonal and can be gained or lost at diagnosis and relapse(1, 15). Despite the frequent sub-clonal nature at diagnosis, Ras mutations appear to retain prognostic relevance. Ras pathway mutations have been associated with early relapse, chemotherapy resistance and poor survival in both patients with infant KMT2A-rearranged ALL(3), non-infant ALL(16) and AML(17). While not all Ras mutations from diagnosis persist at relapse, de novo mutation in Ras pathway genes can also appear at the time of disease progression(18). In distinction to ALL and AML, the Ras pathway is universally activated in juvenile myelomonocytic leukemia (JMML), with 95% of patients harboring founding Ras pathway mutations that always persist in the event of relapse(19).

While mutations in the Ras pathway are the most common mechanism of upregulated RAS signaling, other mechanisms have been reported as well. Methylation, leading to silencing of *RASSF1A* and *RASD1* gene expression have also been implicated in RAS activated hematologic diseases, most notably multiple myeloma(20, 21). Point mutations and fusions involving RTKs, such as *CSF1R, ALK, PDGFRB* and *FLT3,* have been reported in a variety

of myeloid and lymphoid neoplasms leading to constitutive activation of the Ras pathway with elevated levels of phosphorylated ERK(22–26).

Associations with specific subtypes of leukemia

Ras pathway mutations are often found in association with other genetic features in acute leukemia. For *KMT2A* rearranged acute leukemia, Ras mutations are found in over 30% of ALL (1, 27) and AML(14, 28). These mutations appear to cooperate in leukemogenesis as the presence of *NRAS* p.G12D and *FLT3* mutations have been shown to accelerate leukemia onset in a *KMT2A-MLLT3* driven AML and lead to a more aggressive disease(29).

Patients with hyperdiploid ALL have excellent outcomes with overall survival greater than 90% at 5 years(30). Despite the favorable prognosis, hyperdiploid ALL is responsible for a disproportionate number of relapses because it is one of the most common subtypes of ALL in children. Studies have implicated the presence of Ras mutations, in particular *KRAS* and *NRAS,* as poor predictors of outcome when they co-exist with *CREBBP* even if they are all subclonal at diagnosis. This “malicious liaison” of *CREBBP* with Ras mutations has been associated with early relapse due to a possible combinatorial effect that leads to resistance to chemotherapy(8).

Pediatric patients with Ph-like, or *BCR-ABL1*-like, disease have inferior outcomes compared to those without these gene expression signatures(26, 31). While most patients with this subtype of ALL have *CRLF2* over-expressing or *ABL* class kinase fusion lesions, approximately 5% of patients have been found to harbor Ras mutations leading to a Ph-like designation (26). One recent study among pediatric Korean patients demonstrated that 68% of patients with a *BCR-ABL1*-like signature harbored mutations in the Ras pathway, higher than any other subtype in that study(32).

FDA approved compounds targeting the MAPK signaling pathway

Given the frequent prevalence of Ras mutations in cancer, efforts to pharmacologically target this pathway have been a longstanding goal in cancer therapy. Despite early setbacks in targeting RAS itself, novel insights into molecular biology and advances in structural chemistry have led to the development of clinically relevant medications for the treatment of Ras pathway mutant cancers (Table 2). First, we will review two classes of FDA approved inhibitors of the Ras pathway, RAF and MEK inhibitors. Despite FDA approval for their use in subsets of solid tumors, RAF and MEK inhibitors do not have FDA approval for any hematological malignancy.

RAF inhibitors

BRAF is the most frequently mutated gene in the MAPK pathway with nearly 10% of all human cancers harboring an alteration. Mutations in *ARAF* and *CRAF* are infrequent compared to BRAF, which can be categorized into 3 distinct classes (I, II and III). Under physiologic conditions, RAS proteins interact with BRAF, undergo dimerization and subsequent activation. Mutations can alter this balanced process and the effects are dependent on the type of mutation. Class I mutations affect codon V600 and represent ~90% of all *BRAF* alterations. These mutations allow BRAF to signal as a monomer, resulting in

constitutive activation and upregulation of MAPK signaling leading to elevated levels of phosphorylated ERK, independent of RAS. Class II mutations include activating point mutations, fusions and in-frame deletions which all lead to activated BRAF dimers that also function independent of RAS activation. Class III alterations are unique in that they are dependent on RAS activation, frequently co-occur with *N/K/HRAS* mutations and lead to high receptor tyrosine kinase activity.

There are reports of *BRAF* alterations in chronic lymphocytic leukemia, chronic myeloid leukemia and hairy cell leukemia, all diseases that predominantly affect adults. *BRAF* mutations in pediatric AML, ALL, MDS are also rare but are frequent in histiocytic disorders(33). Histiocytic disorders can be broadly grouped into Langerhans cell histiocytosis (LCH) and non-Langerhans cell histiocytoses (non-LCH) of which several varieties have been named including Rosai Dorfman disease, Erdheim-Chester disease (ECD), juvenile xanthogranulomatous disease and histiocytic sarcoma. In contrast to acute leukemias, histiocytic disorders harbor *ARAF* alterations alone or in combination with other MAPK mutations (most commonly *NRAS*) in approximately 20% of cases (34, 35). *BRAF* p.V600E mutations occur in approximately 65% of LCH patients(36). *NRAS* and *KRAS* mutations are also seen in both LCH and non-LCH patients. More recent reports have identified mutations in the gene *MAP2K1* (which encodes for MEK1) in ~ 20% of LCH and ECD patients without *BRAF* or *NRAS/KRAS* mutations. Elevated MAPK signaling is the hallmark of LCH and non-LCH and therefore attempts to treat these disorders with both RAF inhibitors and MEK inhibitors have been tested. LCH patients with *BRAF* p.V600E mutations have been treated with single agent RAF inhibitor, vemurafenib, with one basket study demonstrating an overall response rate (ORR) of 43% in LCH and ECD(37). Another ongoing study in pediatrics combines vemurafenib with cytarabine and cladribine in the treatment of newly-diagnosed *BRAF* p.V600E mutated LCH (NCT03585686). Vemurafenib and dabrafenib are FDA approved for the treatment of *BRAF* mutant melanoma.

MEK inhibitors

MEK is downstream of RAS in the MAPK pathway and has been shown to be upregulated in a variety of leukemias. While directly inhibiting RAS has been vexing, pre-clinical data provided the justification to test inhibiting the downstream effector, MEK, as a strategy in pediatric leukemia. Trametinib, cobimetinib, selumetinib and binimetinib inhibited the growth of primary Ras pathway mutant leukemia cells *in vitro* and *in vivo* in different subtypes of ALL(5, 16, 38). Interestingly, *KMT2A*-rearranged samples without Ras mutations have also been noted to respond to MEK inhibition, suggesting activation of the pathway via other mechanisms besides genetic mutations(38, 39).

An adult phase 1 study tested the MEK inhibitor trametinib in adult patients with a variety of relapsed or refractory hematologic malignancies, including MDS, chronic myelomonocytic leukemia and AML. Responses were seen in nearly 30% of Ras mutated leukemias but only 3% of non-mutated cases(40). Cobimetinib has been tested in adults with advanced histiocytic disorders with an ORR of nearly 90%, irrespective of genotype(41). Due to the unique dependency on the Ras pathway in JMML, a phase II trial of trametinib in patients with relapsed or refractory disease is under way (NCT03190915). While Ras pathway

mutant ALL has been associated with steroid resistance (42), glucocorticoids and the MEK inhibitor selumetinib, have been shown to be synergistic in pre-clinical models(43) and led to the ongoing Seludex clinical trial (NCT03705507). There are four FDA approved MEK inhibitors including trametinib, selumetinib, binimetinib and cobimetinib in a variety of solid tumors. Selumetinib is FDA approved for patients greater than 2 years old with NF1 and plexiform neurofibromas.

Combination therapy with MEK inhibitors

Signaling pathway inhibition along two nodes has the potential for more durable inhibition and prevention of upregulation of pathway enzymes as a mechanism of resistance to a single drug. BRAF inhibition causes upregulation of ERK signaling pathway, supporting targeting of both proteins. This approach may be efficacious in targeting the signaling pathway even in the setting of wildtype BRAF(44). The combination of a BRAF inhibitor with a MEK inhibitor has proven effective in melanoma(45, 46) and the combination of dabrafenib and trametinib was recently tested in adult patients with ECD (NCT02281760).

Given the prevalence of Ras pathway mutations in hematological malignancies, combining inhibition of this pathway with other novel compounds may offer additional therapeutic benefit. For example, the oral BCL2 inhibitor, venetoclax, has transformed treatment of both newly diagnosed and relapsed adult patients with AML. Venetoclax has now been tested in combination with the hypomethylating agents, decitabine and azacitidine (47) as well as with conventional chemotherapy including cytarabine(48). The first published prospective study in relapsed pediatric AML using venetoclax was in combination with cytarabine with or without idarubicin and demonstrated an overall response rate of 69% in the 35 patients treated on the phase I/II study(49). However, resistance to venetoclax is common and combinatorial approaches beyond hypomethylating agents and cytarabine are therefore being explored. Preclinical testing demonstrated synergy between venetoclax and the MEK1/2 inhibitor, cobimetinib, in 7 of 11 AML cell lines tested including in lines resistant to the individual medications(50). One mechanism for this synergy was the downregulation of MCL-1 protein levels and disruption of BCL2:BIM complexes, leading to the release of BIM and eventual cell death. A clinical trial is now underway in adults testing the combination of venetoclax, azacitidine and trametinib for patients with relapsed or refractory AML or MDS (NCT04487106). Similar work in ALL had previously demonstrated synergy of BCL-2/BCL-XL inhibitors, venetoclax or ABT-263, and the MEK1/2 inhibitor, trametinib, across multiple B-ALL cell lines with or without MAPK mutations(51). That preclinical study also identified BIM as the potential mediator of synergy. BIM is dephosphorylated as a result of MEK inhibition which then neutralizes MCL-1, and eventually leads to BCL2 mediated apoptosis. A phase 1B trial combined cobimetinib with venetoclax in elderly patients with relapsed or refractory AML but data from the trial are not yet published (NCT02670044). Combinations of MAPK targeted agents with venetoclax and/or other novel drugs will need to be explored further in clinical trials.

Novel Ras pathway inhibitor development

While Ras isoforms have historically been considered directly “undruggable”, recent efforts have focused on alternate strategies. Here we will discuss drugs targeting the Ras pathway that are still in clinical development and therefore not FDA approved for use outside of clinical trials.

Farnesyl transferase inhibitors

Another approach to target RAS focuses on inhibiting its necessary interaction with the inner plasma membrane. This interaction is mediated by the addition of a farnesyl lipid to its carboxy-terminal CAAX motif. Efforts to directly interfere with RAS GTPase have centered on farnesyltransferase, a member of the prenyltransferase family, involved in catalyzing the chemical reaction between farnesyl diphosphate and protein-cysteine during the protein post-translational modification, with RAS being one of its targets. This is one of the first modification steps leading to the active RAS protein. Farnesyltransferarse inhibitors (FTIs) were trialed in several phase 3 studies across different cancers but few if any responses were seen in KRAS or NRAS mutated cancers(52). Specifically in pediatric hematologic malignancies, tipifarnib, an FTI, was tested in newly diagnosed JMML patients in a window setting followed by stem cell transplant. However, outcomes were not significantly different than in prior studies and the addition of an FTI was not determined to be beneficial(53). Tipifarnib was also tested in older adults with AML with acceptable toxicities but limited efficacy(54). A mechanism of resistance to FTIs was identified which involved an alternative post-translational modification by an enzyme geranylgeranyltransferase I (GGTase I) via a process called prenylation. Recently however, there has been interest in resurrecting FTIs for *HRAS* mutated cancers because they lack this alternative mechanism in response to FTI. Tipifarnib and a more potent FTI, lonafarnib, are now being tested in more rationally designed clinical trials focusing on *HRAS* mutant cancers including within the MATCH study being conducted through the Children’s Oncology Group (NCT04284774) (55, 56).

KRAS G12C inhibitors

RAS proteins act as a binary switch in either the active or inactive state. In the active state, Switch I (residues 32–38) and Switch II (residues 60–75) regions undergo conformational changes leading to activation of downstream signaling of the MAPK pathway(57). Oncogenic mutations in *KRAS* at codon G12, G13 and Q61 result in decreased stimulation of GAPs and therefore higher levels of GTP-bound KRAS. This active form of the protein leads to downstream activation of signaling pathways via interactions at the GTPase-binding-domain (GBD) of effector proteins including RAF, MEK and ERK among others. GTPases can be recharged by GEFs, which weaken the binding of GDP and catalyze its replacement with GTP.

Previous attempts at inhibiting RAS included strategies to identify GTP-competitive inhibitors of RAS(58). However, GTP binds to RAS proteins with picomolar activity, effectively precluding that as a feasible approach. Several groups have recently demonstrated that allele specific inhibition of KRAS may be a tractable approach(59). Small molecules were discovered that covalently bind to G12C mutant form of KRAS during a screen

utilizing GDP-bound KRAS-G12C tethering approach. The compounds bind to a region of the Switch 2 region and block nucleotide exchange and therefore decrease the binding of RAS to both BRAF and CRAF. These compounds were found to have selectivity for *KRAS* p.G12C mutated cancers. Drugs in this class have been rapidly shepherded into the clinic and are now the focus of several ongoing studies(60). One phase I/II clinical trial testing sotorasib, enrolled 129 patients who harbored *KRAS* G12C mutations across a variety of solid tumors. No dose limiting toxicity was observed. Activity of sotorasib was most pronounced in patients with non-small cell lung cancer where 88% of patients had disease control which included objective responses and stable disease. Responses were also observed in a smaller proportion of patients with colorectal, pancreatic, endometrial, appendiceal cancers and melanoma. However, median progression-free survival was 4 months(61). *KRAS* p.G12C is a rare variant in leukemia in general and even more so in pediatric patients. As such, there have yet to be any clinical trials specifically designed using an allele specific approach for patients with hematologic malignancies but a proof of principle regarding direct inhibition of RAS has now been demonstrated.

SHP-2 inhibitors

The protein tyrosine phosphatase SHP-2, encoded by *PTPN11,* is a critical regulator of the Ras signaling pathway. Activating, somatic mutations in *PTPN11* are the most common cause of JMML and germline mutations are the most common cause of Noonan syndrome. SHP099 is a selective small-molecule SHP-2 inhibitor, which stabilizes SHP-2 in an auto-inhibited conformation(62). SHP099 suppresses RAS-ERK signaling and has been shown to have activity *in vitro* and *in vivo*(63). Although *PTPN11* mutations are common in AML and JMML, SHP099, an allosteric inhibitor does not have activity for the most common alterations including D61Y, A72V and E76K due to conformational selection to the closed state that reduces drug affinity(64).

Previous studies have demonstrated that compensatory upregulation of upstream pathways including FGFR1 are possible mechanisms of resistance to long-term MEK inhibition. In a preclinical study using KRAS mutant colorectal cancer cell lines, SHP099 blocked activation of RAS signaling through several RTKs. In addition, synergy was noted between combined MEK and SHP2 inhibition, revealing a potentially strategy to prevent MEK inhibitor mediated resistance(65). A clinical trial involving the allosteric SHP2 inhibitor furthest along in clinical development, RMC-4630, is now being tested in combination with cobimetinib in adults relapsed and refractory solid tumors (NCT03989115). Early studies of TNO155, an allosteric inhibitor of SHP-2, are currently in clinical trials for adults with solid tumors (NCT03114319, NCT04000529, NCT04330664, NCT04294160).

SOS1 inhibitors

SOS1 is a KRAS activator and a major control point for Ras pathway regulation. SOS1 can be activated in response to MEK inhibition, a mechanism of resistance to RAS pathway inhibition. SOS1 inhibitors, BI-3406 and BAY293, interfere with SOS1 function. BI-3406 binds to the catalytic domain of SOS1, preventing its interaction with KRAS(66). *KRAS* mutant cells lines with a variety of G12 and G13 mutations, are sensitive to BI-3406. Cell lines with *KRAS/NRAS* Q61 mutations are less sensitive to BI-3406, possibly due to their

lowest intrinsic GTPase activity. Interestingly, 7 out of 14 tested cell lines carrying *NF1* aberrations were also sensitive to BI-3406(66). Sensitive cell lines show sustained inhibition of ERK1/2 phosphorylation. BI-3406 attenuates feedback activation of MEK signaling in response to MEK inhibitors, enhancing sensitivity to MEK inhibitors and promising for potential combination use(66). BAY293 is another inhibitor of the KRAS::SOS1 interaction, interfering with the KRASG12C-SOS1 complex and showing synergy when combined with a covalent KRAS G12C inhibitor, ARS-853(67). BAY293 did not show selectivity for *KRAS* mutant cells when tested head to head with BI-3406(66).

PI3K/AKT inhibitors

The PI3K/mTOR/AKT signaling pathway is upregulated in many Ras pathway mutant leukemias. In addition, there are several types of genomic alterations in the pathway itself, including loss of function alterations in the tumor suppressor *PTEN*, activating mutations in *PI3K*, and amplifications that can also result in increased signaling. One effector protein in this pathway is AKT and there have been several attempts to target this protein. MK-2206, an oral, allosteric inhibitor of AKT, was evaluated in a phase II clinical trial for adult patients with relapsed or refractory AML. Among the 18 patients evaluated, only 1 had an objective response leading to study termination(68). In addition, correlative biology assays performed on the study using a reverse phase protein array indicated that even at the maximum tolerated dose there was only modest decrease in phosphorylated levels of AKT. Upregulation of upstream signaling pathways including PI3K and mTOR were also noted as a possible mechanism of resistance. There have more recently been attempts to trial traditional ATP-competitive AKT inhibitors such as ipatasertib which is now being tested in adults with a variety of solid tumors. Afuresertib, another oral, reversible, ATP-competitive, pan-AKT kinase inhibitor was first tested in a phase 1 trial for adults with a variety of hematologic malignancies with the most promising results seen in patients with multiple myeloma (69). A phase IIa study using the same compound was then tested in adult LCH patients with an ORR of 33% and 28% in treatment naive and relapsed/refractory patients, respectively(70). PTX-200 is a synthetic tricyclic nucleoside inhibitor that inhibits the phosphorylation of AKT1/2/3 but does not have activity on the kinase itself. There is an ongoing trial of PTX-200 (triciribine) in combination with cytarabine for adult patients with relapsed or refractory AML (NCT02930109). In general, development of clinically active AKT inhibitors has lagged behind inhibitors of RAF, MEK and more recently ERK owing to structural limitations in targeting AKT.

ERK inhibitors

ERK1 and ERK2 are encoded by the same gene and are splice variants. When GTP-bound RAS recruits and activates RAF, it phosphorylates and activates MEK which then phosphorylates and activates ERK which eventually translocates to the nucleus. There are multiple substrates of ERK including transcription factors and kinases that regulate key cellular functions including differentiation, proliferation and death. There are multiple feedback mechanisms in this pathway; ERK1/2 can phosphorylate BRAF/CRAF which then inhibit phosphorylation of MEK. Additionally, Sprouty proteins and dual-specificity phosphatases provide negative feedback by dephosphorylating ERK1/2. ERK is the last effector in the MAPK pathway and is therefore an attractive treatment strategy in Ras

mutated cancers. Targeting ERK may also overcome resistance mechanisms that arise in the setting of inhibiting MEK which include amplification of RAF or downregulation/mutations in MEK. Ulixertinib is an oral ERK1/2 inhibitor that is furthest along in clinical trial development. *In vitro*, ulixertinib resulted in reduced proliferation and inhibited phosphorylation of target substrates despite increased phosphorylation of ERK1/2. *In vivo* studies demonstrated efficacy even in models with acquired resistance to MEK or combined BRAF and MEK therapy(71). A phase 1 study was completed in patients with advanced solid tumors that resulted in an overall response rate of 15%(72). A phase 1/2 study of ulixertinib in adults with AML and MDS has been completed but results are not yet published (NCT02296242). An ongoing study in pediatrics using ulixertinib is being conducted in patients with relapsed or refractory solid tumors and histiocytic disorders with MAPK alterations (NCT03698994).

Conclusion

Despite initial obstacles in targeting the MAPK pathway for the treatment of cancer, there has been a recent surge in the development of compounds with potential to inhibit the Ras signaling pathway including KRAS itself. There is newfound optimism that the success of Ras pathway targeting agents in solid tumors will be translated to the treatment of pediatric patients with hematological malignancies, where Ras pathway mutations are among the most common genomic alterations. Translation to pediatric leukemia patients has the greatest likelihood of being effective by using a combination of the agents described above, thereby preventing upregulation of parallel or upstream pathways.

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Key points:

- Ras mutations are among the most common alterations in pediatric hematologic malignancies and are frequently associated with adverse outcomes.
- RAF and MEK inhibitors are the Ras pathway inhibitors furthest along in clinical development and are now being tested in pediatric leukemia patients.
- Novel Ras pathway inhibitors including directly inhibiting *KRAS* p.G12C are now being tested in adults with solid tumors.
- A combination of agents is the most likely approach to have clinical activity in Ras mutated pediatric leukemia.

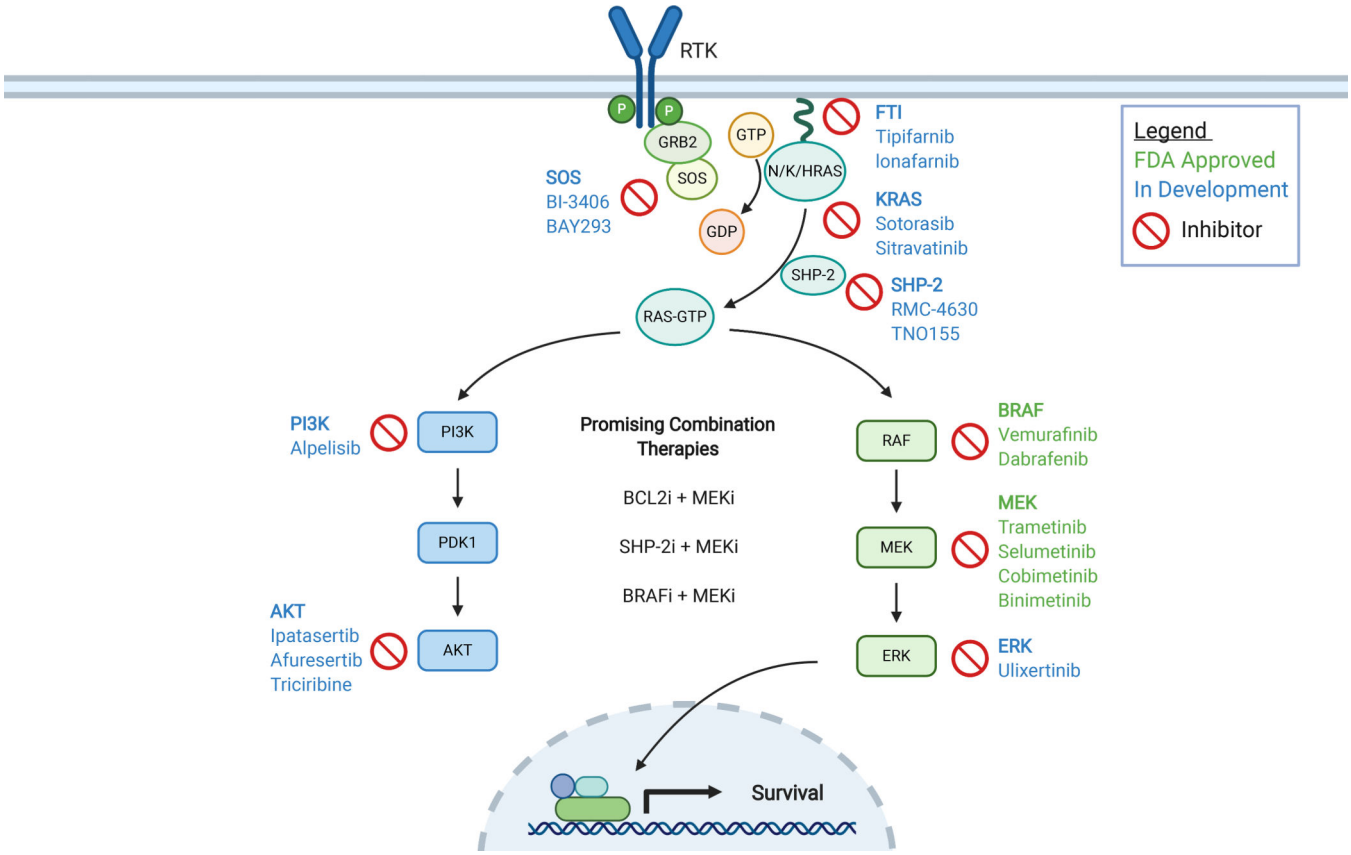

Figure 1. Clinically relevant inhibitors of the RAS pathway.

Approved inhibitors are listed in green and inhibitors currently in development are listed in blue. Figure was created in Biorender.com.

Abbreviations: FTI, farnesyltransferase inhibitor; i, inhibitor; RTK, receptor tyrosine kinase; GTP, guanosine triphosphate; GDP, guanosine diphosphate.

Table 1:

Frequency of Ras pathway mutations in pediatric hematological malignancies.

Disease	Genes	Ras pathway mutation frequencies	References
B-ALL	*FLT3, KRAS, NRAS, PTPN11*	35% diagnosis, 25% relapse	(4)
	KRAS and *NRAS*, codons 12 and 13	20%	(6)
	KRAS, NRAS, PTPN11	28% (35/125)	(15)
	KRAS, NRAS	30% (18/60)	(15)
	KRAS, NRAS, NF1, PIK3CA, PIK3R1, PTPN11	42%	(1)
Infant	*KRAS, NRAS*	22% in KMT2Ar ALL	(2)
	BRAF, KRAS, NRAS	14% overall; 24% in KMT2A-AF4	(3)
iAMP21	*BRAF, CBL, KRAS, MAPK1, NRAS, NF1, PTPN11*	57% (24/42)	(5)
	KRAS and NRAS, codons 12 and 13	30%	(6)
High hyperdiploid	*FLT3, KRAS, NRAS, PTPN11*	53%	(7)
	KRAS, NRAS, PTPN11	52% (26/50)	(8)
Near haploid	*FLT3, KRAS, NRAS, MAPK1, NF1, PTPN11*	71%	(9)
T-ALL	*BRAF, KRAS, NF1, NRAS, PTPN11*	14%	(10)
ETP	*BRAF, KRAS, NF1, NRAS, PTPN11*	28% (18/64)	(11)
AML	*KRAS, NRAS, PTPN11*	>30%	(14)
	KRAS, NRAS, PTPN11	43% (diagnosis) 49% (relapse)	(13)
MDS	Ras/MAPK pathway	55%	(12)
JMML	*CBL, KRAS, NRAS, NF1, PTPN11, RRAS, RRAS2*	95%	(19)

Table 2.

Clinical trials in pediatric hematologic malignancies testing RAS pathway inhibitors.

Trial identifier	Study name	Disease	Targeted agent	Class
Hematologic malignancy				
NCT03190915	Trametinib in Treating Patients With Relapsed or Refractory Juvenile Myelomonocytic Leukemia	JMML	Trametinib	MEK inhibitor
NCT03705507	International Trial of Selumetinib in Combination With Dexamethasone for the Treatment of Acute Lymphoblastic Leukaemia (SeluDex)	ALL	Selumetinib	MEK inhibitor
NCT03585686	A Combination of Vemurafenib, Cytarabine and 2-chlorodeoxyadenosine in Children With LCH and BRAF V600E Mutation	LCH	Vemurafenib	BRAF inhibitor
Pediatric MATCH				
NCT04284774	Tipifarnib for the Treatment of Advanced Solid Tumors, Lymphoma, or Histiocytic Disorders With HRAS Gene Alterations, a Pediatric MATCH Treatment Trial	Solid Tumors, non-Hodgkin lymphoma, or histiocytic Disorders with HRAS mutations	Tipifarnib	Farnesyltransferase inhibitor
NCT03220035	Vemurafenib in Treating Patients With Relapsed or Refractory Advanced Solid Tumors, Non-Hodgkin Lymphoma, or Histiocytic Disorders With BRAF V600 Mutations (A Pediatric MATCH Treatment Trial)	Solid Tumors, non-Hodgkin lymphoma, or histiocytic Disorders with BRAF V600E mutations	Vemurafenib	BRAF inhibitor
NCT03213691	Selumetinib Sulfate in Treating Patients With Relapsed or Refractory Advanced Solid Tumors, Non-Hodgkin Lymphoma, or Histiocytic Disorders With Activating MAPK Pathway Mutations (A Pediatric MATCH Treatment Trial)	Solid Tumors, non-Hodgkin lymphoma, or histiocytic Disorders with MAPK pathway mutations	Selumetinib	MEK inhibitor
NCT03698994	Ulixertinib in Treating Patients With Advanced Solid Tumors, Non-Hodgkin Lymphoma, or Histiocytic Disorders With MAPK Pathway Mutations (A Pediatric MATCH Treatment Trial)	Solid Tumors, non-Hodgkin lymphoma, or histiocytic Disorders with MAPK pathway mutations	Ulixertinib	ERK inhibitor