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*The novel concept of modulation of k-ras by inhibitors of the mevalonate pathway may potentiate the EGFR therapy by altering the **KRAS** phenotype.*

Therapeutic modulation of k-ras signaling in colorectal cancer

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***KRAS* has an important role in colorectal carcinogenesis and mutant *KRAS* leads to a permanently activated k-ras protein. To exert its biological activity, k-ras requires post-translational modification by prenylation. K-ras modulation has become a promising concept for new therapies, mostly by interference with the mevalonate pathway and subsequently by the prenylation of k-ras. Clinical data of agents interfering with the mevalonate pathway and the prenylation of ras are summarized and suggest that these agents might be effective when administered in combination with anticancer drugs that target k-ras. Here, we discuss the novel concept that modulation of k-ras might potentiate EGFR therapy by altering the *KRAS* phenotype.**

Introduction

Colorectal cancer (CRC) is the second most common tumor type in the USA and accounts for 49,920 cancer deaths each year. It is, therefore, the second most common cause of cancer-related mortality in the USA, causing nearly 9% of all cancer-related deaths [1].

If diagnosed early, colorectal tumors can be cured by radical resection. Unfortunately, many patients are diagnosed with (distant) metastasis either during follow-up or at first presentation. A small subset of patients with metastasis confined to a single organ (mostly the liver) can be cured by resection. For the majority of patients with metastasized disease, however, the only treatment option is palliative systemic treatment. In the past decade, new chemotherapeutic agents for CRC have become available, such as irinotecan and oxaliplatin. For advanced or metastasized CRC patients failing 5-FU (or capecitabine or UFT (ftorafur plus uracil)), oxaliplatin and irinotecan, therapy with a monoclonal antibody against the epidermal growth factor receptor (EGFR) is advised, but only in patients with tumors not harboring an activating mutation in the *KRAS* gene. *RAS* has a key role in carcinogenesis, signal transduction and proliferation in colorectal carcinoma. Mutations in *RAS* are found in 30% of all cancers and are a potential target for therapy. This review focuses on the role of *KRAS* and the novel concept of modulating k-ras with statins,

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farnesyltransferase inhibitors, geranylgeranyltransferase inhibitors and bisphosponates in human colorectal carcinomas.

Search strategy

A systematic literature search in PubMed was conducted on 3 April 2009 using the following keywords and combinations: *KRAS*, (colorectal) carcinoma, farnesyl transferase inhibitors, geranylgeranyltransferase inhibitor, bisphosphonates, statins, EGFR inhibitors, cetuximab and panitumumab. Results were assessed by reviewing titles and abstracts, and relevant articles were retrieved. Cited references in these articles were used to find further relevant articles.

RAS proto-oncogenes

The *RAS* gene family consists of proto-oncogenes, which control cell growth in mammalian cells. Three different kinds of *RAS* oncogenes are known: *Kirsten RAS* (*KRAS*), *Harvey RAS* (*HRAS*) and *Neuroblastoma RAS* (*NRAS*); these members of the *RAS* gene family are closely related and function in a similar way [2]. The *KRAS* gene encodes for a 21 kDa membrane-bound guanosine triphosphate (GTP)/guanosine diphosphate (GDP)-binding G protein. The k-ras protein serves as a switch between the EGFR and the nucleus, controlling downstream processes. To be active, hydrophilic k-ras requires post-translational modification by prenylation. Ras terminates in a CAAX sequence: a cysteine (C), two aliphatic amino acids (A) and any amino acid (X). The CAAX sequence is subject to post-translational farnesylation or geranylgeranylation. A 15-carbon chain from farnesylpyrophosphate (FPP) is added to the cysteine residue close to the carboxyl terminus, and this process is catalyzed by the enzyme farnesyl protein transferase (FTase). When FTase is inhibited, k-ras will be geranylgeranylated, thereby a 20-carbon chain of geranylgeranylpyrophosphate (GGPP) is added to ras catalyzed by geranylgeranyltransferase (GGTase) [3,4]. After isoprenylation of ras, the endopeptidase RCE1 protease removes the AAX amino acids at the end of the carboxyl terminus. The new terminus is methylated by isoprenylcysteine carboxyl methyltranferase (ICMT) before ras is transported to the cellular membrane. In n-ras and k-ras, the SH-group of cysteine residue is palmitoylated before transport to the membrane. As a consequence of post-translational modifications, k-ras becomes more hydrophobic and translocates from the cytosol to attach to the cell membrane by its farnesylgroup or geranylgeranylgroup [5–7] (Fig. 1). Membrane association of k-ras is crucial for its function in signaling and transforming activities. Both FPP and GGPP are isoprenoids formed during the mevalonate pathway. FPP is a precursor for cholesterol, heme A, dolichols and ubiquinones, and GGPP can be formed out of FPP [8]. Inactivated k-ras is bound to GDP; activation occurs by the conversion of GDP to GTP by guanine exchange factors. In normal cells, the ratio of GDP and GTP is controlled by guanine exchange factors and GTPase-activating proteins (GAPs). Active k-ras is hydrolyzed by GAPs to return to an inactive state [9].

K-ras signaling

K-ras is situated in the inner cell membrane. Binding of a ligand to the EGFR activates a downstream process to the nucleus. This process activates major pathways in the cell: the ras–raf–mitogen-activated protein kinase (MAPK) and the PI3 kinase pathway (Fig. 2). *KRAS* has a key role in the ras–raf–MAPK pathway. Son of sevenless (SOS) is conformationally modificated by interaction with growth factor receptor bound protein 2. Activated SOS induces the k-ras pathway [10]. In ras–raf–MAPK signaling, k-ras activates serine–threonine kinase raf 1, which phosphorylates two MAPK kinases. These in turn phosphorylate other MAPKs. MAPKs translocate to the nucleus and activate transcription factors involved in proliferation [8,11].

Signaling via the PI3 kinase pathway activates AKT and thereby phosphoproteins, for example, p-GSK3 and p-AKT [12]. The tumor suppressor gene *PTEN* inhibits the PI3 kinase pathway.

KRAS mutations in cancer

KRAS mutations have an important role in tumorigenesis. In CRC, *KRAS* somatic mutations are thought to be involved in the transition of adenoma into carcinoma, contributing to tumor growth and atypia [13,14]. Mutant *RAS* is present in approximately 30% of all human cancers. *KRAS* mutational rate is high in some tumors; however, it is low in others (Table 1). Approximately 40% of CRCs have mutations in *KRAS*.

Mutations are found in primary tumors and matched metastases. Most mutations are found in the primary tumor, indicating a role in early tumorigenesis. Mutations are occasionally found only in metastases, however, thus indicating such mutations can also occur during a later stage of disease [15].

The most frequent mutations in *KRAS* are guanine to adenine transitions and guanine to thymine transversions [16] with 90% of the somatic point mutations occurring in hotspot codon 12 (70%) or 13 (30%) in exon 1. Other, less frequent, mutations are known in codon 61, 62 and 146. The most frequent mutations in codon 12 and 13 are listed in Table 2. 6.6% of the somatic mutations are found outside codon 12 or 13 in codons 8, 9, 10, 15, 16, 19, 20 or 25

TABLE 1

Tumor type	*RAS*	Frequency (%)
Colorectal carcinoma	*KRAS*	50
Lung adenocarcinoma (NSCLC)	*KRAS*	30
Pancreatic carcinoma	*KRAS*	90
Melanoma	*NRAS*	20
Thyroid carcinoma	*KRAS, NRAS, HRAS*	50
Myeloid disorders	*NRAS* (less frequently *KRAS, HRAS*)	30

Abbreviations: KRAS, Kirsten RAS gene; NSCLC, non-small cell lung carcinoma; NRAS, neuroblastoma RAS gene; HRAS, Harvey RAS.
Mutations of NRAS, KRAS and HRas in different tumor types [32,93].

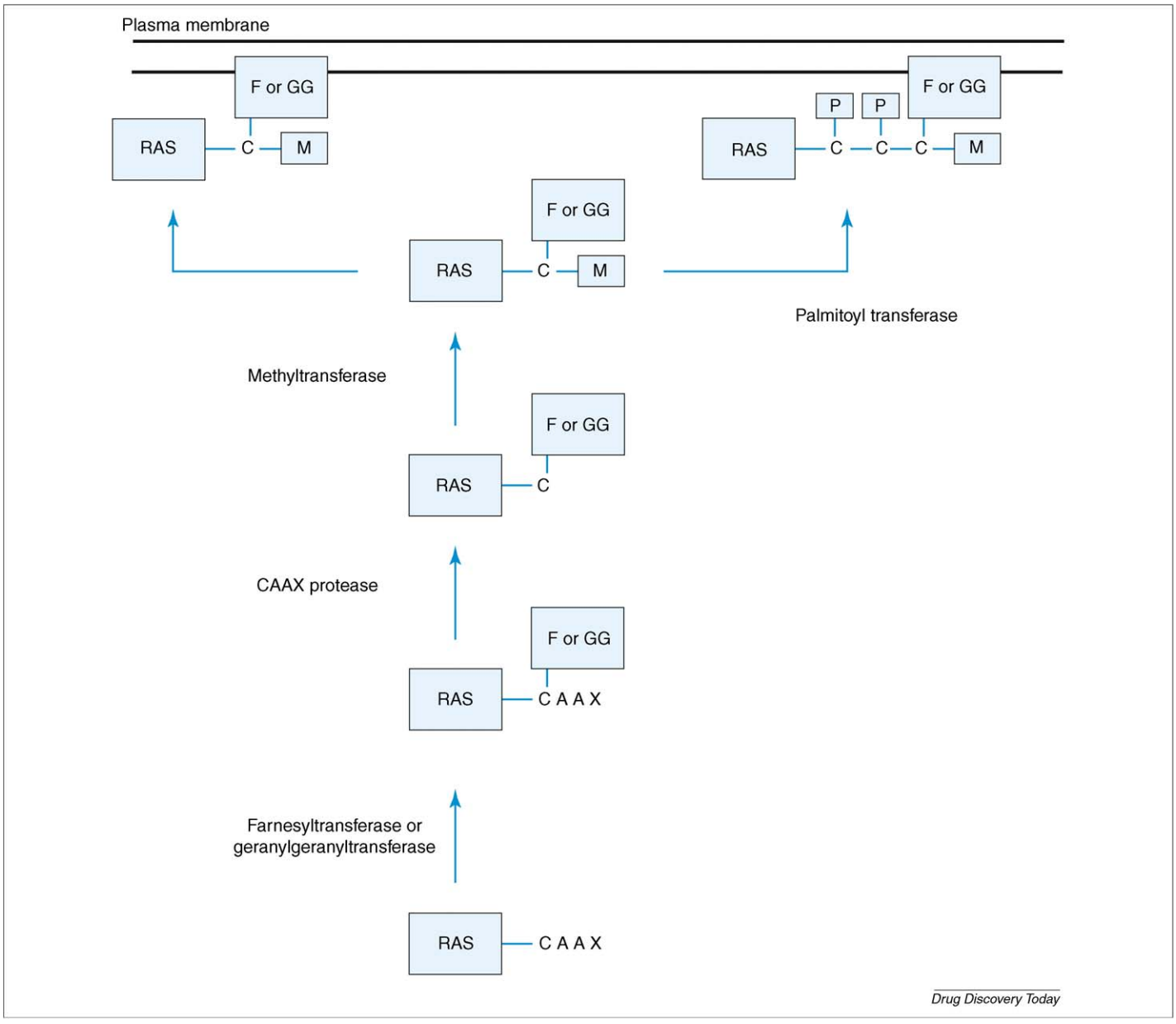

FIGURE 1

Post-translational modification of ras. Abbreviations: F, farnesyl pyrophosphate; GG, geranylgeranylpyrophosphate; M, methylgroup; P, palmitoylgroup.

[16]. A recent study showed mutations in 59, 61, 117 and 163 [17]. During tumor progression, more *KRAS* codon 12 mutations and fewer codon 13 mutations are found. In normal tissue, however, there is a balanced codons 12 and 13 mutation ratio [18].

Different mutations in codon 12 or 13 have various effects on disease progression [19]. Guanine to adenine point mutations are associated with methylguanine methyltransferase epigenic silencing [20]. Mutations leading to a 12-glycine residue (without a side chain) toward a residue with a side chain interfere with the geometry of k-ras and the ability of GTP to be hydrolyzed to return to an inactive state. These mutations cause impaired GTPase activity: k-ras binds GAP, but there is no activation of the GAP because of steric hindrance [21], and they permit a permanently active state causing growth and proliferation [22,23]. Consequently, mutant k-ras operates independently of activation of the EGFR and causes downstream processes [24].

No clear conclusions can be drawn from the studies regarding the influence of *KRAS* on the progression of colon cancer and, thus, the prognostic impact of *KRAS* mutation in colorectal carcinoma is unclear. Several studies link *KRAS* to worse prognosis, whereas others do not implicate a prognostic role for *KRAS* [25–31]. The RASCAL study was initiated to determine whether the presence of *KRAS* mutations in CRC patients is associated with poor prognosis. Initial results of this study suggested that *KRAS* mutational status is indeed associated with poorer disease-free survival and overall survival. The RASCAL II study, however, reported that only one specific mutation reduces disease-free and overall survival statistically significant and that *KRAS* mutational status in general is not a prognostic marker. Nevertheless,

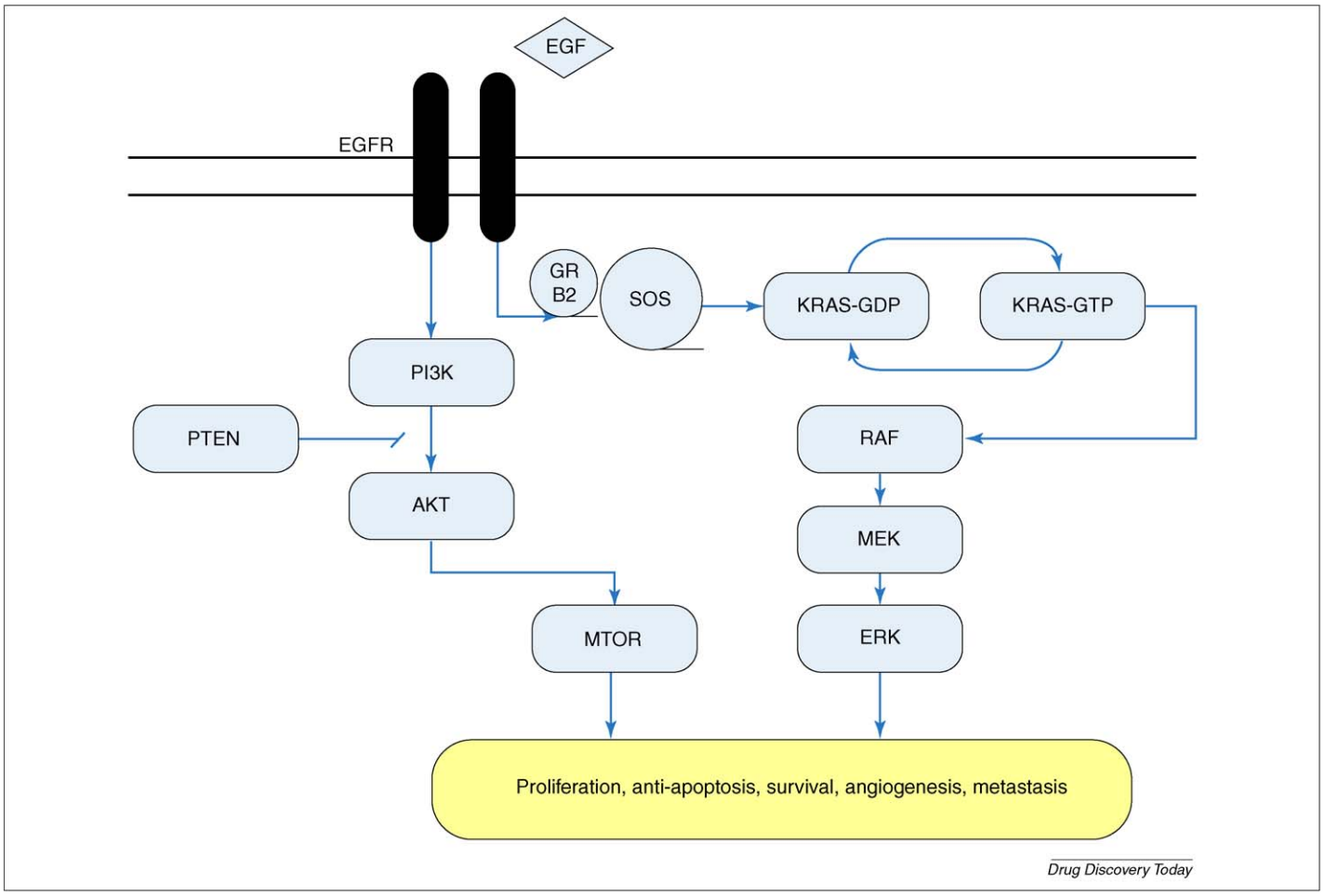

FIGURE 2

Overview of EGFR-dependent intracellular signaling. Abbreviations: AKT, protein kinase B; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular signal-related kinase; GRB2, growth factor bound protein 2; k-ras-GDP, k-ras bound to guanine diphosphate; k-ras-GTP, k-ras bound to guanine triphosphate; MEK, mitogen-activated protein kinase; MTOR, mammalian target of rapamycin; PI3K, phosphatidyl-inositide-3-kinase; PTEN, phosphatase and tensin homolog; RAF, V-raf murine sarcoma viral oncogene homolog; SOS, son of sevenless.

mutational status of *KRAS* is of great clinical relevance in CRC patients in predicting response to EGFR-inhibitor-based therapy. The RASCAL II study showed that only glycine to valine transversion on codon 12 had a statistically significant influence on interval between operation and relapse or death from any cause and on overall survival [19,32]. *Post hoc* analyses of two trials evaluating the EGFR inhibitors panitumumab and cetuximab in CRC showed lack of response to these agents in *KRAS* mutant patients [33,34]. Nowadays, EGFR inhibitor therapy in CRC is indicated only in patients free of mutations in codons 12 and 13 of the *KRAS* gene.

TABLE 2

Codon 12 mutations	
GGT (glycine) → AGT (serine)	G–A transition
GGT (glycine) → GAT (aspartate)	G–A transition
GGT (glycine) → TGT (cysteine)	G–T transversion
GGT (glycine) → GTT (valine)	G–T transversion
GGT (glycine) → CGT (arginine)	G–C transversion
GGT (glycine) → GCT (alanine)	G–C transversion
Codon 13 mutations	
GGC (glycine) → GAC (aspartate)	G–A transition
GGC (glycine) → TGC (cysteine)	G–T transversion
GGC (glycine) → GTC (valine)	G–T transversion
GGC (glycine) → CGC (arginine)	G–C transversion
GGC (glycine) → GCC (alanine)	G–C transversion
GGC (glycine) → AGC (serine)	G–A transition

Abbreviations: A, adenine; C, cytosine; G, guanine; T, thymine.
Common transitions and transversions in KRAS codon 12 and 13.

Testing for *KRAS* gene mutations

Currently, testing for *KRAS* mutations is not standardized. For the identification of *KRAS* mutations, different methods are being used; however, data about the accuracy of different tests are limited [12]. *KRAS* testing currently focuses on codon 12 or 13 mutations. Seven mutations in these codons contribute to more than 95% of all *KRAS* mutations. In real-time polymerase chain reactions, probes for the most common mutations in codons 12, 13 and sometimes 61 are applied. In direct sequencing analysis, all possible mutations of *KRAS* can be identified [35].

Many methods of *KRAS* testing are laboratory-based methods. The following methods are used for *KRAS* testing: gel electrophoresis assays, sequencing, allele-specific PCR assays and allele-discrimination-based allele-specific ligation detection reaction.

Allele discrimination is based on discrimination amplification efficiencies at low melting temperatures. Some assays are commercially available [36,37]. Juan *et al.* [38] compared testing methods (Histogenex, Genzyme, Invitek and Gentrix) from four independent commercial laboratories with their internal direct sequencing, and all but one (Invitek) were comparable with the internal direct sequencing method.

Tol *et al.* [36] compared two commonly used *KRAS* mutation tests – real time PCR and sequencing – in DNA extracted from CRC samples. Both sequencing and real-time PCR are reliable *KRAS* testing assays with a sensitivity of 95.5% (95% confidence interval 91.7–97.9%) and 96.5% (95% confidence interval 93.0–98.6%), respectively.

A difficulty in *KRAS* testing occurs when a low volume of tumor material is available, for example because of pre-treatment with radiotherapy. In samples with less than 30% tumor cells, a *KRAS* mutation can be missed by sequencing. Obviously, high-quality *KRAS* testing is necessary because the *KRAS* status of a patient is used to determine clinical opportunities. The European Society of Pathology has started a Quality Assessment program for *KRAS* testing because of the lack of procedures and standardization (http://esp-pathology.org).

KRAS and pathogenetic pathways in CRC

In the progression toward CRC, pathological genetic changes occur. This review focuses on *KRAS*; however, other genetic changes have an important role and interplay in colorectal carcinogenesis. Early genetic abnormalities arise in adenomatous polyposis coli, KRAS and BRAF (v-raf murine sarcoma viral oncogene homolog B1). Mismatch repair gene mutation and MLH1 mutation contribute to microsatellite instability. These pathological genetic changes lead to dysplastic crypt and (early) adenoma formation.

Further positive selection occurs for the mutation of TGFβ receptor 2, insulin-like growth factor 2 receptor, BAX, loss of SMAD4, TP53 and PIK3CA, which lead to further progression to carcinoma.

KRAS, *BRAF*, PTEN and PIK3CA are mediators of the downstream signaling of the EGFR. Genetic alterations in these genes contribute to a different EGFR signaling. Oncogenic mutations in *RAS* and *BRAF* activate the MAPK signaling pathway. BRAF mutations occur in 13% of CRCs. PIK3CA encodes for PI3 kinase. PI3 kinase is controlled by PTEN, which could be lost in colorectal carcinoma. Figure 3 overviews the pathogenic changes and interplay in colorectal carcinoma [39,40].

Targeting k-ras as an anticancer therapy

Modulating k-ras signaling has become a promising concept for new cancer therapies. A variety of approaches – mostly interfering with the mevalonate pathway, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA) reductase and prenylation of k-ras – have been studied [41]. The mevalonate metabolites, FPP and GGPP, play an important part in the post-translational modification of k-ras and have become a target for different anticancer approaches. The effects of statins, bisphosphonates, FTIs, GGTIs, Rce1 inhibitors and ICMT inhibitors on the mevalonate pathway and indirectly on prenylation of k-ras (Fig. 4) and the results of phase I, II and III clinical studies are discussed.

Statins

Statins are HMG-CoA inhibitors, which suppress the cholesterol biosynthesis in humans by their inhibitory effect on the mevalonate pathway, thereby inhibiting the formation of low-density lipoprotein (LDL). Owing to upregulation of LDL receptors, the blood clearance of LDL also enhances, increasing the lipid-lowering effect of statins.

Besides the cholesterol-lowering effects, statins are believed to inhibit tumor cell growth and angiogenesis, induce apoptosis and impair tumor metastasis. Through inhibition of HMG-CoA, statins inhibit the formation of mevalonate, thereby affecting the synthesis of the isoprenoids FPP and GGPP. These substrates are used for farnesylation and geranylgeranylations of ras and rho. In addition,

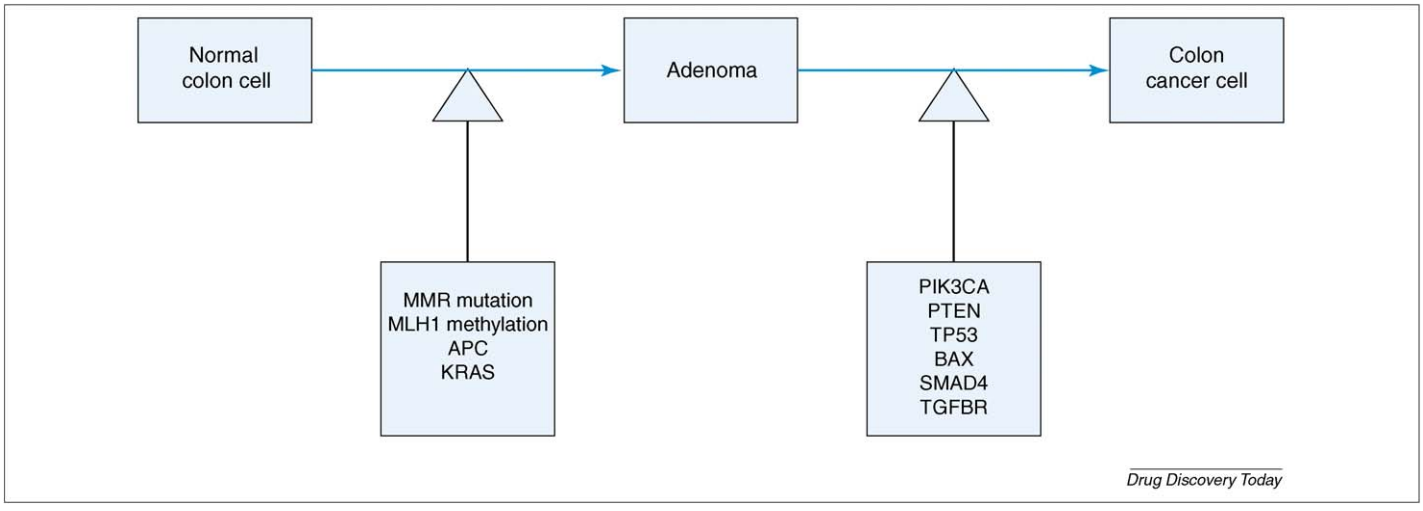

FIGURE 3

Genetic alterations in colorectal carcinoma. Abbreviations: APC, adenomatous polyposis coli; BAX, BCL2-associated X protein; BRAF, V-raf murine sarcoma viral oncogene homolog; KRAS, Kirsten RAS gene; MMR, mismatch repair; MLH1, human mutL homolog 1; PIK3CA, phosphoinositide-3-kinase; catalytic, alpha polypeptide; PTEN, phosphatase and tensin homolog; SMAD4, SMAD family member 4; TGFBR2, transforming growth factor, beta receptor II; TP53, tumor protein p53.

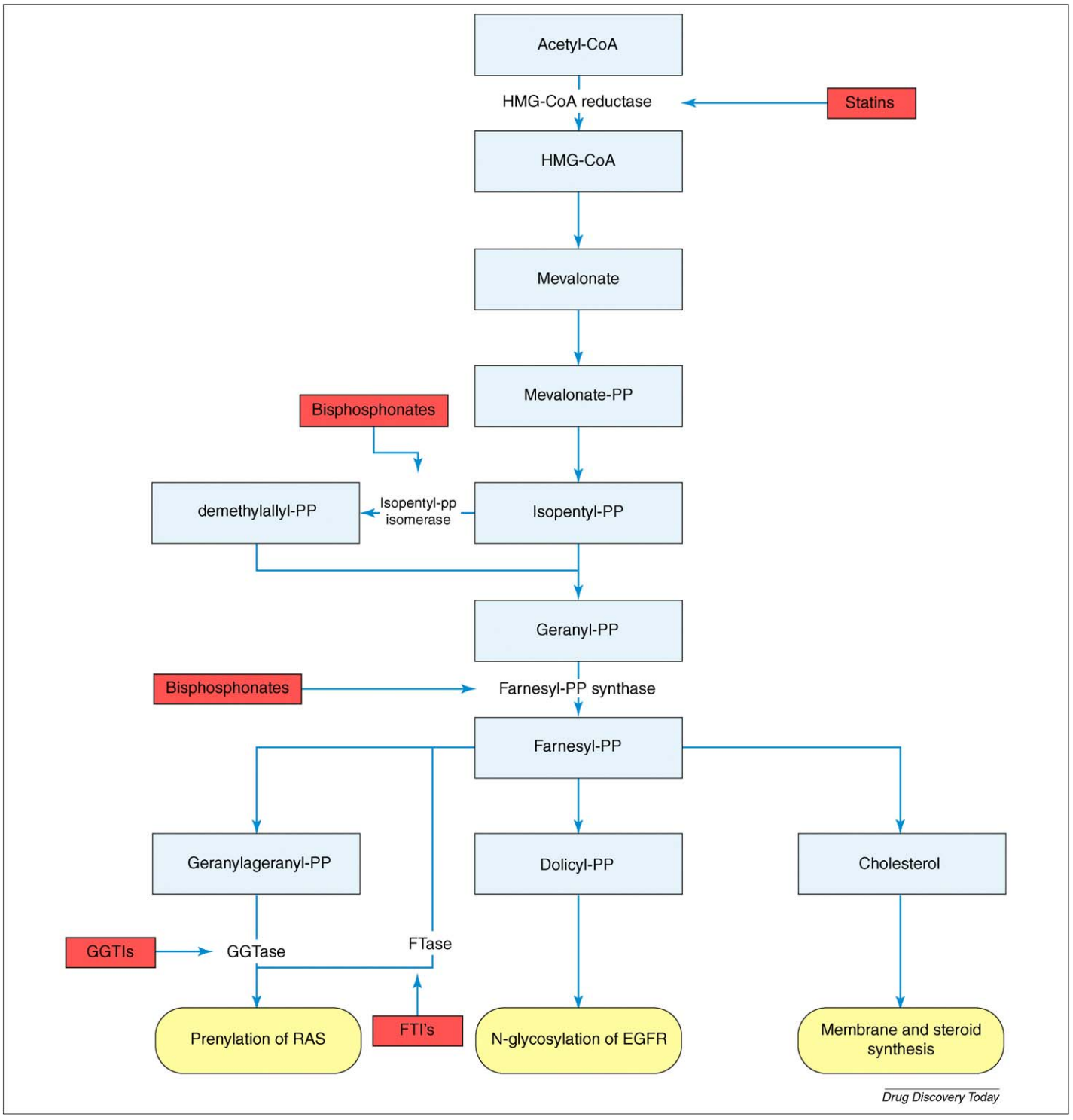

FIGURE 4

Overview of the mevalonate pathway and inhibitors. The mevalonate pathway causes prenylation of ras, N-glycosylation of EGFR and membrane and steroid synthesis. Statins, bisphosphonates, farnesyltransferase inhibitors and geranylgeranyltransferase inhibitors have inhibitory effects on the mevalonate pathway and thus on prenylation of k-ras. Abbreviations: Acetyl-CoA (acetyl coenzyme A); EGFR, epidermal growth factor receptor; FTase, farnesyltransferase; FTIs, farnesyltransferase inhibitors; GGTase, geranylgeranyltransferase; GGTIs, geranylgeranyltransferase inhibitors; HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) (reductase),; -PP, -pyrophosphate.

statins affect both angiogenesis and inflammation processes [5,42] and exert a role in chemoprevention by the inhibition of HMG-CoA reductase, which is upregulated in colon cancer cells [43]. *In vitro* studies have shown that statins suppress growth and induce apoptosis [44,45].

The clinical characteristics of colon cancer among statin users differ from non-users. The former have a lower tumor state, have a lower frequency of metastases, more frequently have a right-sited location of the tumor and have a significantly improved five-year survival rate (37% versus 33%, P-value < 0.01) [46].

TABLE 3

Phase I, II, and III trials evaluating statins in cancer treatment

Study	Refs	Study design	Tumor type	Agent	Additional agent	*n*	Main results
Lee	[51]	Phase II	CRC	Simvastatin	FOLFIRI	49	TTP possibly prolonged; no effect on RR or OS
Graf	[50]	Phase III	HCC	Pravastatin	TACE	183	mOS 20.9 months versus 12.0 months
Lopes-Aguilar	[94]	Phase II	Brain stem tumors (pediatric)	Fluvastatin	Chemotherapy + thalidomide	9	RR 78%
Sondergaard	[47]	Phase II	Multiple myeloma	Simvastatin	None	6	RR 0%
van der Speck	[48]	Phase II	Multiple myeloma	Simvastatin	VAD	12	RR 8%
Schidmaier	[49]	Phase II	Multiple myeloma	Simvastatin	Bortezomib or bendamustine	6	RR 0%
Knox	[95]	Phase I	SCCHN/cervical carcinoma	Lovastatin	None	26	RR 0%; CBR 23%
Lersch	[96]		HCC	Pravastatin versus octreotide versus gemcitabine		58	mOS 7.2 versus 5 versus 3.5 months
Kim	[97]	Phase II	Gastric adenocarcinoma	Lovastatin	None	16	RR 0%
Kawata	[98]	Phase III	HCC	Pravastatin	TAE + oral 5FU	91	mOS 18 months versus 9 months
Larner	[99]	Phase I/II	Astrocytoma/GBM	Lovastatin	±Radiation	18	RR 11%; CBR 17%
Thibault	[100]	Phase I	Solid tumors	Lovastatin	None	88	Lovastatin well tolerated up to 25 mg/kg/day

Abbreviations: (m)OS, (median) overall survival; CBR, clinical benefit rate (i.e. complete and partial remission and stable disease); CRC, colorectal carcinoma; FOLFIRI, irinotecan, leucovorin and 5-FU; GBM, glioblastoma multiforme; HCC, hepatocellular carcinoma; RR, response rate (i.e. complete and partial remission); SCCHN, squamous cell carcinoma of head and neck; TA(C)E, transcatheter arterial (chemo)embolization; TTP, time to progression; VAD, vincristine, adriamycin, dexamethasone.

The anticancer effects of statins have been studied in phases I, II, and III clinical trials in various malignancies (Table 3), with statin doses from 20 mg/day up to 45 mg/kg/day. Results vary, showing no (additional) effect of statins in multiple myeloma [47–49] and promising results in hepatocellular carcinoma [50]. Graf *et al.* [50] studied the addition of statins to transcatheter arterial chemoembolization (TACE) in hepatocellular carcinoma and found a significant gain in overall survival compared to TACE alone (median overall survival 20.9 months versus 12.0 months, $P = 0.003$).

Lee *et al.* [51] recently reported results of a trial adding simvastatin to irinotecan, leucovorin and 5-FU (FOLFIRI) as first-line therapy in CRC patients. They based the hypothesis on a synergistic effect of these therapies in preclinical research. Response rates and overall survival were similar to historical results of FOLFIRI alone, but time to progression was prolonged (9.9 months versus 6.7–8.5 months), and there was no additional toxicity.

These trials show promising activity of statins in solid tumors, yet further studies on statins in cancer therapy are needed.

Farnesyltransferase inhibitors

Prenylation is a necessary post-translational step for functional k-ras; for that reason, farnesyltransferase inhibitors (FTIs) and geranylgeranyltranferase inhibitors (GGTIs) have been developed as anticancer therapy. Besides k-ras, other GTPases that promote tumor progression are prenylated. FTase can recognize and prenylate tetrapeptides with a CAAX sequence. FTIs act through two mechanisms. FPP analogs selectively compete with FPP for binding to FTase and the CAAX sequence of k-ras. The peptidemimetics competes with ras-CAAX for FTase; some FTIs compete via both mechanisms. By these mechanisms, FTIs inhibit farnesylation of not only ras proteins but also various other polypeptides, such as nuclear lamins A and B, skeletal muscle phophorylase kinase, transducin, cGMP phophodiesterase and the cell regulatory protein tyrosine phosphatases [52].

Four FTIs were tested in clinical trials worldwide: lonafarnib and tipifarnib (both oral compounds) have been tested in phase II and phase III studies (listed in Table 4), and BMS-214662 and L-778,123, administered intravenously, were tested in phase I studies. Some of the trials listed in Table 4 tested tipifarnib and lonafarnib in solid tumors, such as breast, pancreatic, colorectal, urothelial and brain tumors, but the results of these trials were disappointing. Sparano *et al.* recently published the results of a phase II trial testing the addition of tipifarnib to neo-adjuvant doxorubicin–cyclophosphamide in patients with clinical stage IIB–IIIC breast cancer. The trial included 44 patients, and a pathological complete remission was seen in 25%, compared to 10–15% for chemotherapy alone according to historical results. Still, the role of tipifarnib in the treatment of solid tumors remains unclear and further study is needed. In hematologic malignancies, however, tipifarnib did show some single-agent activity, especially in elderly patients with poor risk and previously untreated acute myeloid leukemia. Lancet *et al.* [54] tested tipifarnib monotherapy in this population and observed a response rate of 23%. Tipifarnib was submitted to the FDA for the treatment of acute myeloid leukemia in elderly patients not applicable for standard chemotherapy in January 2005. In June 2005, however, the FDA filed a Not Approvable Letter, awaiting the results of subsequent phase III trials of tipifarnib for this indication [55–57]. Recently, the

TABLE 4

Phase II and III trials evaluating FTIs in cancer treatment

Author	Refs	Study design	Tumor	Agent	Additional agent	*n*	Endpoints and results
Harrousseau	[58]	Phase III	AML	Tipifarnib	None	457	No effect on survival
Sparano	[53]	Phase II	Breast cancer	Tipifarnib	Doxorubicin and cyclophosphamide	44	RR 77%
Li	[101]	Phase II	Breast cancer	Tipifarnib	Fulvestrant	33	CBR 52%; target CBR (70%) not achieved
Lustig	[102]	Phase II	GBM	Tipifarnib	Radiotherapy	28	RR 0%; CBR 29%
Eckhardt	[103]	Phase II	Pancreatic cancer	Tipifarnib versus placebo	Gemcitabine	244	No effect of the addition of tipifarnib on survival
Ravoet	[104]	Phase II	MDS/AML	Lonafarnib	None	16	RR 6%
Feldman	[105]	Phase II	MDS/CML	Lonafarnib	None	67	RR 4%; HI 19%
Karp	[106]	Phase II	AML	Tipifarnib	None (maintenance)	48	mDFS 13.5 months
Fouladi	[107]	Phase II	Glioma	Tipifarnib	None	97	RR 2%
Johnston	[108]	Phase II	Breast cancer	Tipifarnib	None	120	RR 12%
Harousseau	[109]	Phase II	AML	Tipifarnib	None	252	RR 4%
Lancet	[54]	Phase II	AML	Tipifarnib	None	158	RR 23%
Cloughesy	[110]	Phase II	Glioma	Tipifarnib	None versus + EIAEDs	89	10% had PFS > 6 months; RR > 7%
Whitehead	[111]	Phase II	CRC	Tipifarnib	None	55	RR 7%
Borthakur	[112]	Phase II	CML	Lonafarnib	None	13	RR 18%
Macdonald	[113]	Phase II	Pancreatic cancer	Tipifarnib	None	53	mOS 2.6 months
Kim	[114]	Phase II	NSCLC	Lonafarnib	Paclitaxel	33	RR 10%; CBR 48%
Theodore	[115]	Phase II	Urothelial cancer	Lonafarnib	Gemcitabine	31	RR 32%
Winquist	[116]	Phase II	Urothelial cancer	Lonafarnib	None	19	RR 0%
Rosenberg	[117]	Phase II	Urothelial cancer	Tipifarnib	None	34	RR 6%; CBR 44%
Rao	[118]	Phase III	CRC	Tipifarnib versus placebo	None	268	CBR 24% versus 13%; no effects on PFS and OS
Heymach	[119]	Phase II	SCLC	Tipifarnib	None	22	RR 0%; mPFS 1.4 months
Van Cutsem	[120]	Phase III	Pancreatic cancer	Tipifarnib versus placebo	Gemcitabine	688	mOS 193 days versus 182 days
Kurzrock	[121]	Phase II	MDS	Tipifarnib	None	28	RR 11%; severe toxicity
Alsina	[122]	Phase II	Multiple myeloma	Tipifarnib	None	43	RR 0%; CBR 64%
Johnston	[108]	Phase II	Breast cancer	Tipifarnib	None	76	RR up to 14%
Adjei	[123]	Phase II	NSCLC	Tipifarnib	None	44	RR 0%; CBR 16%
Cohen	[124]	Phase II	Pancreatic cancer	Tipifarnib	None	20	RR 0%; mOS 19.7 weeks
Cortes	[125]	Phase II	Multiple myeloma/CML	Tipifarnib	None	40	RR 18%
Sharma	[126]	Phase II	CRC	Lonafarnib	None	21	RR 0%; CBR 14%

Abbreviations: (m)DFS, (median) disease-free survival; (m)OS, (median) overall survival; (m)PFS, (median) progression-free survival; (N)SCLC, (non) small cell lung carcinoma; AML, acute myeloid leukemia; CBR, clinical beneficial rate (i.e. complete remission, partial remission and stable disease); CML, chronic myeloid leukemia; CRC, colorectal cancer; EIAEDs, enzyme-inducing antiepileptic drugs; GBM, glioblastoma multiforme; MDS, myelodysplastic syndrome; HI, hematologic improvement; RR, response rate (i.e. complete and partial remission).

results of a phase III trial comparing tipifarnib with best supportive care in newly diagnosed acute myeloid leukemia in patients of 70 years or older were published. The results showed no effect of tipifarnib on survival (median survival, 107 days versus 109 days; *P*-value, 0.843) [58].

Activation of k-ras by mutation is associated with radiotherapy resistance. Preclinical studies *in vitro* and *in vivo* with FTIs showed that the radiosensitivity of cells might be improved. The potential synergistic effect for radiosensitization might be the inhibition of activated k-ras by the FTIs [59–61].

A phase I trial of L-778,123 (an FTI and GGTI) and radiotherapy in 12 patients with pancreatic cancer showed acceptable toxicity. In a patient-derived pancreatic cell line, radiosensitization was observed. In total, eight patients completed treatment, one patient showed partial response for six months, five patients showed stable disease (>2 months) and two patients were progressive [62].

Another phase I trial with L-788,123 with radiotherapy in nine patients with locally advanced head and neck or lung cancer showed a complete response in one patient and five patients with a partial response [63].

GGTase inhibitors

Only inhibition of the farnesylation of k-ras by FTIs does not considerably affect its function, because k-ras can be geranylgeranylated as well. GGTase I geranylgeranylates k-ras when FTases are inhibited by FTIs. This fact triggered the development of GGTIs. GGPP analogs and CAAL peptidomimetics both act as GGTIs. Inhibition of k-ras prenylation might require co-treatment of FTIs with GGTIs and might explain the limited efficacy of the FTIs as single drug [56]. Moreover, in contrast to FTIs, GGTIs are able to block phosphorylation of both PDGF- and EGF-dependent tyrosine kinase receptors. GGTase inhibitors have been tested in preclinical studies and showed decreased tumor growth (cell-cycle arrest in G1 and apoptosis) *in vivo* and *in vitro* [65–67]. Possibly because of the preclinical toxicity of GGTase I inhibitors, up till now they have not proceeded to clinical stages.

Bisphosphonates

Bisphosphonates (BPs) inhibit isopentenyl diphosphatase isomerase and FPP synthase and probably also GGPP synthase, two metabolites in the mevalonate pathway. The newer nitrogen-containing BPs (e.g. pamidronate and zoledronic acid), inhibited farnesylation and geranylgeranylation of k-ras, resulting in a decrease of downstream signaling, inducing apoptosis [5,64]. Other observed effects of BPs on tumor cells are inhibition of migration through and adhesion and invasion to the extracellular matrix, so-called 'MMP activity'. At low concentrations, BPs inhibit the mevalonate pathway, whereas at higher concentrations, MMP activity is inhibited [68]. Furthermore, effects on the mevalonate pathway BPs reduce complications such as osteoporosis and skeletal morbidity caused by metastatic bone disease in metastatic and non-metastatic disease. In non-metastatic disease, BPs might prevent bone metastasis [69]; in metastatic disease, BPs might delay or prevent the complications caused by bone metastasis [70,71]. Clinical studies on BPs in cancer treatment have been performed, mainly focusing on endpoints regarding skeletal-related events such as fractures and bone pain. Some of these trials also focus on response-related endpoints, to investigate the role of BPs in survival in cancer.

Table 5 shows the phase II/III clinical trials on BPs in cancer treatment, not (only) focusing on skeletal-related events. The largest and most recent trial was published by Gnant *et al.* [72], who tested the effects of the addition of zoledronic acid to either goserelin and tamoxifen or goserelin and anastrozole in pre-menopausal women with endocrine-responsive early breast cancer. After a median follow-up of 47.8 months, a disease-free survival rate of

TABLE 5

Phase II and III clinical trials evaluating the effect of bisphosphonates on response related endpoints in malignancies

Author	Refs	Study design	Tumor	Agent	Additional agent	*n*	Endpoints and results
Gnant	[72]	Phase III	Breast	Zoledronic acid	Tamoxifen and goserelin versus anastrozole and goserelin	1803	Significantly longer disease-free survival with zoledronic acid
James	[127]	Phase III	Prostate	Zoledronic acid	Androgen suppression ± docetaxel/±celecobrix	Ongoing trial	
Diel	[128]	Phase III	Breast	Clodronate	Adjuvant therapy	290	At 55 months follow up significantly improved PFS and OS with clodronate
Kristensen	[129]	Phase III	Breast	Pamidronate	Adjuvant chemotherapy and/or radiotherapy	953	No effect on occurrence of bone metastases
Kattan	[130]	Phase II	Prostate	Zoledronic acid	Docetaxel estramustine	27	PSA response in 52%RR 21%
Mason	[131]	Phase III	Prostate	Clodronate versus placebo	None	508	No effects on OS and bone metastases-free survival
Pavlu	[132]	Phase I/II	CML	Zoledronic acid	Imatinib	10	RR 0%
Di Lorenzo	[133]	Phase II	Prostate	Zoledronic acid	Docetaxel vinorelbine	40	PSA response in 32%RR in 40%
Di Lorenzo	[134]	Phase II	Prostate	Zoledronic acid	Gemcitabine prednisone	22	PSA response in 23%RR in 14%
Mitsiades	[135]	Phase III	Prostate	Zoledronic acid	None versus somatostatin analog and dexamethasone	38	RR 0% versus 65%.PFS and OS significantly improved
Lewis	[136]	Phase II	Melanoma	Apomine	None	42	RR 0%, mPFS 6.1 months
Bertelli	[137]	Phase II	Prostate	Zoledronic acid	Docetaxel	25	PSA response in 48%, mild toxicity
Figg	[138]	Phase II	Prostate	Alendronate	Ketoconazole and hydrocortisone	72	No significant differences in PFS, OS and RR
Tiffany	[139]	Phase II	Prostate	Zoledronic acid	Imatinib	15	No effects on pain and PSA
Dearnaley	[140]	Phase III	Prostate	Clodronate	None	311	Non-significant betterBPFS and OS
Mardiak	[141]	Phase III	Breast	Clodronate versus placebo	Standard chemotherapy	73	Time to development of (bone) metastases 13 months versus 28 months

Abbreviations: (m)PFS, (median) progression-free survival; BPFS, bone progression-free survival; CML, chronic myeloid leukemia; OS, overall survival; PSA, prostate-specific antigen; PSA response, >50% PSA decline; RR, response rate (i.e. complete and partial remission).

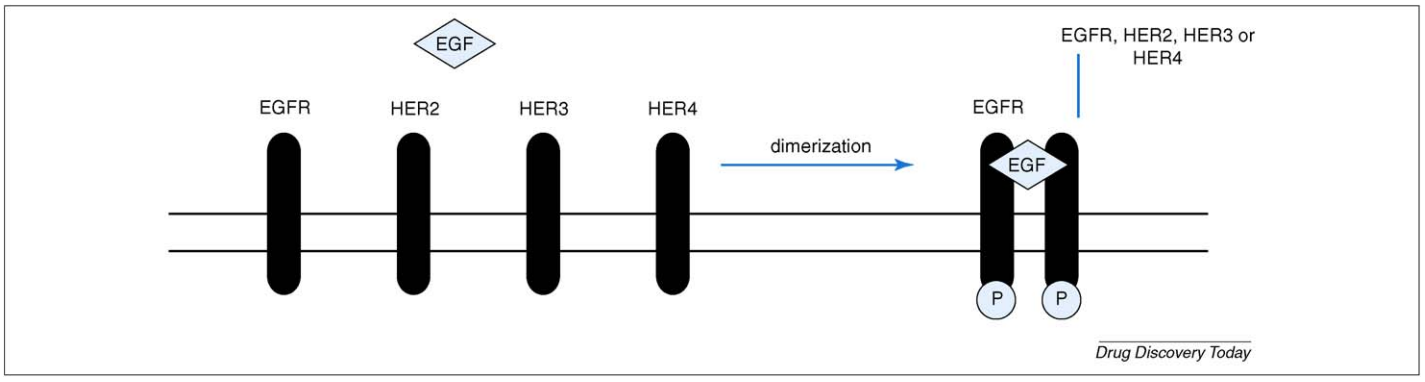

FIGURE 5

Dimerization of the EGFR. The binding of a specific ligand (e.g. EGF) causes a conformational change and results in homodimer or heterodimer formation. Abbreviations: EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; HER, human epidermal growth factor receptor.

94.0% was seen in the group receiving endocrine therapy with zoledronic acid, compared to 90.8% in the group receiving only endocrine therapy ($P = 0.01$) [72].

Nowadays, BPs are known to reduce bone loss owing to hormone therapy (such as for breast and prostate cancer) and prevent skeletal-related events [70]. Despite the results published by Gnant *et al.* [72], however, there is no consensus about the effect of BPs on survival.

Other post-prenylation inhibitors

After prenylation, k-ras undergoes endoproteolytic processing by the RCE1 protease and carboxyl methylation by ICMT. These enzymes, which act on both farnesylated and geranylgeranylated enzymes, could be targets for anticancer therapy.

Few small-molecule inhibitors of RCE1 and ICMT have been described so far. RPI, a prenylated CAAX peptide, competitively inhibits RCE1 as substrate analogs. Two types of ICMT inhibitors have been developed; both types act as mimics of substrates. The S-adenosylhomocysteines bind to methyltransferases and competitively inhibit the enzyme. In preclinical studies with cell lines, a partial block of proliferation was shown. Membrane-associated k-ras was reduced by 66% in one study, resulting in a decrease of downstream MEK/ERK signaling [73,74]. The second group of ICMT inhibitors contains derivatives of prenylcysteine: for example, *N*-acetyl-*S*-farnesyl-L-cysteine and *N*-acetyl-*S*-geranylgeranyl-L-cysteine. These substrates act also as substrates for ICMT; however, they target other processes in the cell as well [75].

EGFR antibodies and *KRAS*

The EGFR is a target for anticancer therapy. EGFR is expressed in normal tissues and different tumors. The EGFR is a 170-kDa transmembrane receptor with an extracellular ligand binding domain, a transmembrane domain and an intracellular tyrosine kinase membrane. There are four EGFR-related receptors; EGFR (HER1), HER2, HER3 and HER4. The binding of the ligand to the ligand-binding domain results in a conformational change, enabling the receptor to form an EGFR–EGFR homodimer or an EGFR–HER2, EGFR–HER3 or EGFR–HER4 heterodimer (Fig. 5). The active dimer causes ATP-dependent phosphorylation of EGFR through tyrosine kinases, which cause proliferation, inhibition of apoptosis, invasion and metastasis [76].

Monoclonal anti-EGFR antibodies bind the extracellular domain of EGFR, thereby blocking the ligand-binding region, and as a result, the EGFR tyrosine kinase activation is halted and ras signaling is inhibited [76,77]. Cetuximab can induce antibody-dependent cell-mediated cytotoxicity (ADCC) and downregulation and degradation of EGFR and in this way exerts its anti-tumor activity. For panitumumab, no ADCC has been described [78].

Two EGFR antibodies, cetuximab and panitumumab, have been registered. Cetuximab is registered for the treatment of metastasized colorectal carcinoma with EGFR overexpression in *KRAS* wild-type patients (monotherapy or in combination with chemotherapy), head and neck squamous cell carcinomas in combination with radiotherapy, and metastasized head and neck squamous cell carcinomas in combination with cisplatin-based chemotherapy. Panitumumab is registered for colorectal carcinoma with EGFR overexpression in *KRAS* wild-type patients. Retrospective analysis of clinical trials showed a lack of clinical activity of cetuximab and panitumumab in patients with mutant *KRAS* because mutant k-ras operates independently of activation of the EGFR [24,33,34,79–88]. Table 6 represents clinical studies on the efficacy of cetuximab or panitumumab in patients with CRC

TABLE 6

Studies *KRAS* and cetuximab and panitumumab and *KRAS* status in colorectal carcinoma and outcome in panitumumab or cetuximab treated patients

Study	Refs	Treatment	*KRAS* status	RR	Median PFS	Median OS
Douillard	[91]	FOLFOX4 ± panitumumab	*KRAS* mutant	N/A	7.3 months	N/A
			KRAS wild type	(55)	9.6 months	N/A
Peeters	[142]	FOLFIRI ± panitumumab	*KRAS* mutant	N/A	N/A	N/A
			KRAS wild type	(35)	5.9 months	14.5 months

TABLE 6 (*Continued*)

Study	Refs	Treatment	*KRAS* status	RR	Median PFS	Median OS
Van Cutsem	[143,144]	FOLFIRI ± cetuximab	*KRAS* mutant	102 (59.3)	7.6 months	17.5 months
			KRAS wild type	38 (36.2)	9.9 months	24.9 months
Bokemeyer	[81]	FOLFOX-4 ± cetuximab	*KRAS* mutant	17 (33)	5.5 months	N/A
			KRAS wild type	37 (60)	7.7 months	N/A
Tol	[88]	Capecitabine + oxaliplatin + bevacizumab ± cetuximab	*KRAS* mutant	(45.9)	8.1 months	17.2 months
			KRAS wild type	(61.4)	10.5 months	21.8 months
Amado	[33]	Panitumumab versus BSC	*KRAS* mutant	0 (0)	7.4 months	4.5 months
			KRAS wild type	21 (17)	12.3 weeks	6.8 months
Karapetis	[34]	Cetuximab versus BSC	*KRAS* mutant	(1.2)	1.9 months	4.8 months
			KRAS wild type	(1.28)	3.7 months	9.5 months
Lievre 2008		Cetuximab ± chemotherapy	*KRAS* mutant	0 (0)	9 weeks	10.1 months
			KRAS wild type	34 (43.6)	31.4 weeks	14.3 months
Lievre 2006	[85]	Cetuximab ± chemotherapy	*KRAS* mutant	(0)	N/E	6.9 months
			KRAS wild type	(65)	N/E	16.3 months
De Roock	[82]	Cetuximab ± irinotecan	*KRAS* mutant	0 (0)	12 weeks	27.3 weeks
			KRAS wild type	27 (21)	24 weeks	43 weeks
Khambata-Ford	[24]	Cetuximab	*KRAS* mutant	3 (10)	59 days	N/E
			KRAS wild type	24 (48)	61 days	N/E
Di Fiore	[145]	Cetuximab plus chemotherapy	*KRAS* mutant	0 (0)	3 months	N/E
			KRAS wild type	12 (27.9)	5.5 months	N/E
Benvenuti	[79]	Cetuximab/panitumumab	*KRAS* mutant	1 (6.2)	N/A	N/E
			KRAS wild type	10 (31.2)	N/A	N/E
Frattini	[146]	Cetuximab	*KRAS* mutant	1 (10)	N/A	N/E
			KRAS wild type	9 (53)	N/A	N/E
Hecht	[147]	Bevacizumab + irinotecan based chemotherapy ± panitumumab	*KRAS* mutant	30	8.3 months	17.8 months
			KRAS wild type	54	10 months	N/A
		Bevacizumab + oxaliplatin based chemotherapy ± panitumumab	*KRAS* mutant	47	10.4 months	19.3 months
			KRAS wild type	50	9.8 months	20.7 months
Garm Spindler	[84]	Irinotecan + cetuximab	*KRAS* mutant	0 (0)	2.3 months	8.7 months
			KRAS wild type	(40)	8.0 months	11.1 months
Bibeau	[80]	Panitumumab versus BSC	*KRAS* mutant	1 (4)	3.0 months	8.7 months
			KRAS wild type	10 (27)	5.5 months	10.8 months
Prenen	[87]	Irinotecan ± cetuximab	*KRAS* mutant	1 (1.3)	12 weeks	26 weeks
			KRAS wild type	37 (30.3)	24 weeks	45 weeks
Laurent-Puig	[148]	Cetuximab, remaining therapy unspecified	*KRAS* mutant	0 (0)	8.6 weeks	
			KRAS wild type	24 (68.4)	32 weeks	
Moroni	[149]	Chemotherapy ± cetuximab/panitumumab	*KRAS* mutant	2 (20)	N/E	N/E
			KRAS wild type	8 (38)	N/E	N/E
Loupakis	[150]	Irinotecan + cetuximab	*KRAS* mutant	N/A	3.1 months	6.1 months
			KRAS wild type	N/A	4.2 months	13.5 months
Cappuzzo	[151]	Chemotherapy ± cetuximab	*KRAS* mutant	4 (9.5)	4.4 months	9.5 months
			KRAS wild type	10 (26.3)	5.4 months	10.8 months
Finocchiaro	[152]	Cetuximab	*KRAS* mutant	(6.3)	3.7 months	8.3 months
			KRAS wild type	(26.5)	6.3 months	10.8 months
Freeman	[153]	Panitumumab	*KRAS* mutant	0 (0)	N/A	N/A
			KRAS wild type	(10.5)	N/A	N/A
Di Nicolantonio	[83]	Chemotherapy ± cetuximab/panitumumab	*KRAS* mutant	2 (6)	N/A	N/A
			KRAS wild type	22 (28)	N/A	N/A
Tabernero	[154]	Cetuximab	*KRAS* mutant	0 (0)		
			KRAS wild type	(27.6)		
		Chemotherapy + cetuximab	*KRAS* mutant	(31.6)	5.6 weeks	
			KRAS mutant	(55.2)	9.4 weeks	

Abbreviations: BSC, best supportive care; N/A, not available (yet); N/E, not evaluated; OS, overall survival; PFS, progression-free survival; RR, response rate.
The values in parentheses are the percentages of patients with RR.

with either mutant or wild-type *KRAS* tumors. These results indicate that the efficacy of panitumumab and cetuximab (mono-) therapy is limited to patients with wild-type *KRAS* tumors [33,34,89,90].

Alternative strategies

An alternative strategy to attack *KRAS*-mutated cells would be to inhibit targets downstream of ras, such as MTOR (using RAD001), PI3 kinase (using BEZ235) or raf (using BAY 43-9006). One could consider combining inhibitors of targets within the ras–raf–MAPK and PI3 kinase pathway, thereby possibly creating inhibition comparable to targeting of the EGFR. Inhibitors of various targets within these pathways have been tested *in vivo* and are currently being studied in phase I/II clinical trials (http://www.clinicaltrials.gov). Because the efficacy of these agents has not been proved yet, however, none of them are standard in cancer therapy. Such alternative strategies might be relevant in the future in the treatment of patients harboring *KRAS* mutations.

Future perspectives

KRAS mutation status has an impact on the therapeutic opportunities for patients with colorectal carcinoma. Both cetuximab and panitumumab are effective only in *KRAS* wild-type patients, and in *KRAS* mutant patients, a worse response has been reported [81,91]. Modulation of k-ras prenylation in *KRAS* mutant tumors might potentiate EGFR therapy [92] because the metabolites formed during the mevalonate pathway have a key role in prenylation and thereby post-translational activation of k-ras. Indeed, inhibition of the mevalonate pathway could influence the potential of k-ras to translocate from the cytosol toward the membrane and, thus, alter the *KRAS* phenotype toward the wild type. Combinations of EGFR antibodies to target the EGFR with k-ras modulators such as statins, BPs, FTIs or GGTIs inhibitors targeting ras–raf–MAPK signaling might augment the effect in patients with *KRAS* mutations. In (pre)clinical studies, further investigation should be done to elucidate the role of statins, FTIs, GGTIs, BPs, RCE1 inhibitors and ICMT inhibitors in CRC and the possibilities of therapeutic modulation of *KRAS* mutations.

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