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Review

Farnesyl transferase inhibitors as anticancer agents

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Abstract

Protein farnesylation catalysed by the enzyme farnesyl protein transferase involves the addition of a 15-carbon farnesyl group to conserved amino acid residues at the carboxyl terminus of certain proteins. Protein substrates of farnesyl transferase include several G-proteins, which are critical intermediates of cell signalling and cytoskeletal organisation such as Ras, Rho, PxF and lamins A and B. Activated Ras proteins trigger a cascade of phosphorylation events through sequential activation of the PI3 kinase/AKT pathway, which is critical for cell survival, and the Raf/Mek/Erk kinase pathway that has been implicated in cell proliferation. Ras mutations which encode for constitutively activated proteins are found in 30% of human cancers. Because farnesylation of Ras is required for its transforming and proliferative activity, the farnesyl protein transferase inhibitors were designed as anticancer agents to abrogate Ras function. However, current evidence suggests that the anticancer activity of the farnesyl transferase inhibitors may not be simply due to Ras inhibition. This review will discuss available clinical data on three of these agents that are currently undergoing clinical trials.

Keywords: Farnesyl transferase inhibitors; Ras; Rho B; R115777; SCH66336; BMS-214662; Signal transduction

1. Introduction

Advances in molecular biology over the past decade have identified a number of novel targets for cancer therapy. One such target is the enzyme farnesyl protein transferase (FT), which catalyses a key step in the addition of an aliphatic isoprenoid side chain to a number of proteins. A novel class of antineoplastic agents, the farnesyl transferase inhibitors (FTIs), have recently been developed to specifically inhibit FT. These inhibitors were designed to target Ras, a G-protein with 4 isoforms (H-, N- and K-RasA/K-RasB) mutated in a large number of cancers, which requires prenylation for function [1]. However, it has become clear in many instances that Ras may not be the critical target of FTIs. For instance, in human tumour cell lines, the antitumour activity of FTIs does not correlate with a mutated Ras status [2,3]. The findings that K- and N-Ras can be alternatively prenylated by geranylgeranyl protein transferase (GGT) also argues against Ras as the target, as preclinical models bearing these mutations are sensitive to FTI treatment [4–7]. To date, more than a hundred polypeptides possessing a 'CAAX' (tetrapeptide motif, where C is cysteine, A is any alphatic amino acid and X is serine, leucine, glutamine or methironine) sequence that can potentially be farnesylated have been identified [8]. Theoretically, the inhibition of farnesylation of any of these polypeptides could result in the antiproliferative effects of the FTIs in human tumours.

Despite uncertainty about the true target of FTIs, these agents demonstrate anticancer activity as single agents and in combination with standard cytotoxic chemotherapy [8–18]. In addition, FTIs synergise with gamma irradiation, and may have a role in chemoprevention [19–21]. This review will focus on the FTIs that are currently undergoing clinical investigations. Published results of these clinical trials will be discussed. Recent investigations into the targets of FTIs will also be discussed.

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2. Farnesyl protein transferase

FT is a heterodimeric metalloenzyme whose activity is dependent on magnesium and zinc, the latter of which is required for coordination of the farnesyl-accepting cysteine of the target protein and the active site of the enzyme [22]. FT catalyses the addition of a 15-carbon isoprenyl farnesyl moiety, to the carboxy terminus of proteins containing the peptide target motif 'CAAX'. Similarly, the related protein geranylgeranyl transferase type I (GGT-1) catalyses the addition of a 20-carbon geranylgeranyl group to CAAX-containing proteins, where 'C' is cysteine and 'A' is any aliphatic residue. The specificity between the two enzymes is dependent on the 'X' residue. Proteins containing serine, glutamine or methionine are farnesylated and proteins containing leucine or isoleucine are geranylated [23–26]. Likewise, amino acid specificity at the A2 position is strict such that a CAAX tetrapeptide, which contains an aromatic side chain in the A2 position generates a competitive inhibitor of FT [27]. Although there is selective substrate specificity of FT and GGT-1 for prenyl donors and acceptors under usual intracellular conditions, this is not absolute. As an example, geranylgeranylation of proteins can arise as a 'salvage mechanism' for normally farnesylated proteins in the absence of FT activity [6]. FT consists of a 49 kDa (kilo Dalton) α-subunit and a 46 kDa β-subunit [28]. The genes for the alpha and beta subunits reside on chromosome bands 8p22–q11 and 14q23–q24, respectively [29]. The α-subunit of FT is identical to GGT-1 and promotes the catalysis of the prenylation reaction. The β-subunit of FT, which shares only 30% sequence homology with GGT-1, provides specificity in recognising peptide substrates and contains both the isoprenoid and peptide binding sites [30–33].

3. Protein farnesylation

The addition of a farnesyl group to conserved amino acid residues at the carboxy terminus is necessary for the proper functioning of many farnesylated proteins. The farnesylation reaction is part of a series of modifications necessary for plasma membrane association of various proteins (Fig. 1). While it is generally believed that prenylation confers a hydrophobic core necessary to anchor proteins to cell membranes, more investigation is needed to understand the entire spectrum of function that ensues after the addition of prenyl groups. Farnesyl pyrophosphate (FPP), an intermediate in the cholesterol biosynthetic pathway, becomes a substrate for adding a farnesyl group to the C-terminal cysteine of the CAAX motif via FT. The farnesylated protein is then further modified, as the AAX motif is removed and the farnesylated cysteine is methylated by a specific CAAX protease and methyl transferase (MT), respectively. In the case of Ras, the protein is then translocated to the plasma membrane where it undergoes palmitoylation at multiple upstream cysteine residues (except for K-Ras B) [31,34–36]. Subsequently, activated Ras interacts with downstream effectors that mediate several signalling pathways involved in cell proliferation or survival (Fig. 2). In addition to Ras, other well-characterised farnesylated proteins, some of which have roles in signal transduction, include the retinal proteins transducin, rhodopsin kinase and cGMP phosphodiesterase, nuclear lamin A and B, skeletal muscle phosphorylase kinase, the peroxisomal protein Pxf, the chaperone protein HDJ-2 and others (see Table 1) [37,38].

4. Mechanism of FTI cytotoxicity

More than 70% of cancer cell lines have shown sensitivity to FTI treatment. FTIs inhibit the anchorage-independent growth of this large variety of transformed cell lines [2,39–41]. In addition, while FTIs clearly abrogate FT activity both in cultured cell lines and in surrogate tissue [9,15] or tumour cells [9,15] from patients, it has become increasingly clear that FTIs do not target only Ras, but may have other protein targets as well. As mentioned earlier in the introduction, there is no correlation between the sensitivity to FTIs and the presence of an activating Ras mutation. FTIs appear to inhibit mutated, as well as wild-type Ras, with cells over-expressing native Ras largely being more sensitive [2]. In addition, geranylgeranylated forms of K-Ras and N-Ras, which are themselves capable of transforming cells, are observed in cells treated with FT inhibitors [6,43]. Despite this alternative prenylation pathway, FT inhibitors inhibit the proliferation of K-Ras transformed cells *in vitro* and *in vivo* [4,5,42].

Furthermore, cells harbouring Ras isoforms engineered to be N-myristylated are still sensitive to FTIs [43]. Collectively, these findings argue that inhibition of the farnesylation of other proteins may also contribute to the observed antitumour properties of these agents.

Currently, existing data implicate at least three proteins as putative targets of FTIs. One potential target whose role is supported by a large body of evidence is RhoB. This is a 21-kDa G-protein which regulates receptor trafficking, and may be a downstream effector of Ras (Fig. 2). RhoB activation leads to $p21^{Waf1/Cip1}$ inactivation, cell cycle progression to S phase and ras-mediated malignant transformation [44]. Interestingly, RhoB can be farnesylated or geranylgeranylated [45]. After FTI treatment, the geranylgeranyl form of RhoB accumulates. This form, in contrast to the growth-promoting farnesylated form, appears to stimulate growth inhibition and induction of apoptosis. For example, a

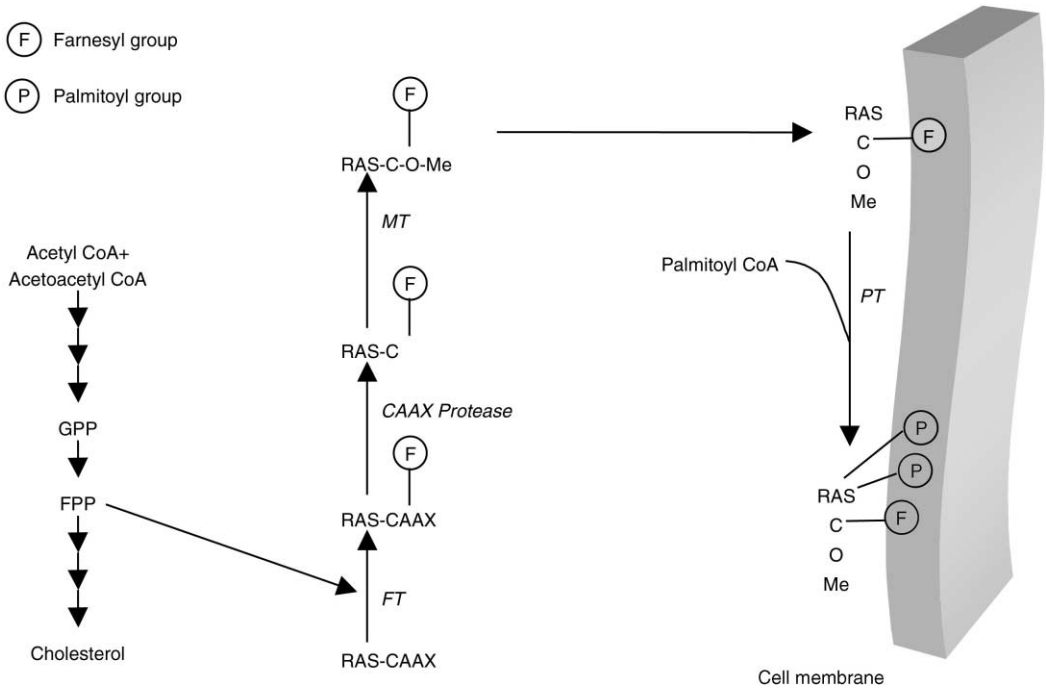

Fig. 1. Mechanism of Ras prenylation—A farnesyl group is covalently linked to the C-terminal cysteine residue by FT using farnesylpyrophosphate, an intermediate in the cholesterol biochemical pathway. The C-terminal AAX residues are then cleaved by a specific CAAX protease. The resulting C-terminal carboxylate is then methylated by a specific methyl transferase. The farnesylated Ras then translocates into the cell membrane. In all Ras proteins, except K-Ras B, one or more palmitoyl groups are added to upstream cysteine residues [1,35,36].

RhoB/Rho chimeric protein that is exclusively geranylgeranylated is growth inhibitory [46]. Furthermore, a myristylated form of RhoB not prenylated by FT was shown to prevent FTIs from reversing Ras transformation [47]. However, other evidence argues against RhoB as the target of FTI inhibition. Treatment of cells with GGT-1 inhibitors, or GGT-1 inhibitors in combination with FTIs suppresses tumour cell growth [4,48]. In addition, RhoB is not mutated in human cancers, nor is it required for Ras transformation [49,40].

A second possible target of the FTI-mediated antitumour effects is an as yet unknown farnesylated protein associated with the phosphoinositide 3′-kinase (PI-3K)/Akt growth factor- and adhesion-dependent survival pathways (Fig. 2). The findings described below occur primarily in H-Ras transformed cells, in which the primary survival pathway appears to be through PI-3K/Akt. In addition, alternative prenylation of this Ras isotype does not occur [50,51]. In these H-Ras transformed human cancer cell lines treated with FTI-277, Akt-2 levels correlate with ability of FTI to induce apoptosis. Cell lines that over-express Akt-2 undergo brisk apoptosis after treatment with the FTI.

This FTI-induced apoptosis is also rescued by the addition of activated Akt-2, suggesting that an upstream farnesylated protein in this pathway is targeted. Consistent with these findings, FTIs block the activation of ribosomal s6 kinase (p70s6k), a downstream effector of Akt-2 [52]. Furthermore, FTI-277 treatment inhibits phosphorylation of the proapoptotic protein BAD, another downstream effector of Akt-2 (Fig. 2) [53]. The dephosphorylated form of BAD, which binds bcl-2, abrogates the anti-apoptotic effects of bcl-2 homodimerisation [54,55].

Third, the centromeric proteins CENP-E and CENP-F have been implicated as potential targets of the FTIs. Except for cells harbouring H-*ras* mutations, human tumour cell lines sensitive to FTIs accumulate in G_2 to M, suggesting inhibition of a mitotic checkpoint protein. Prenylation of CENP-E and CENP-F has been shown to be inhibited in A549 lung carcinoma cell lines treated with the FTI SCH66336. This inhibition leads to an altered association between CENP-E and microtubules, affecting microtubule–centromere interaction [56]. Other studies, however, have argued against CENP-E or CENP-F as FTI targets [57,58]. In H-*ras* transformed cells, for instance, treatment with SCH66336 leads to accumulation in G_1 phase [59].

5. Preclinical/clinical studies

Three FTIs (Fig. 3) are undergoing clinical testing currently as monotherapy, and in combination with

standard cytotoxic agents (Table 2). An additional agent, L-778,123, was introduced into the clinic, but its further development has been halted due to toxicity. At least three other compounds are in late preclinical testing and may be introduced into clinical trials shortly.

5.1. R115777 (Zarnestra™)

R115777, initially developed as an antifungal agent, is a non-peptidomimetic quinolone analogue. *In vitro* inhibition of FT with R115777 occurs with lamin B and K-RasB as peptide substrates, with IC_{50}s of 0.86

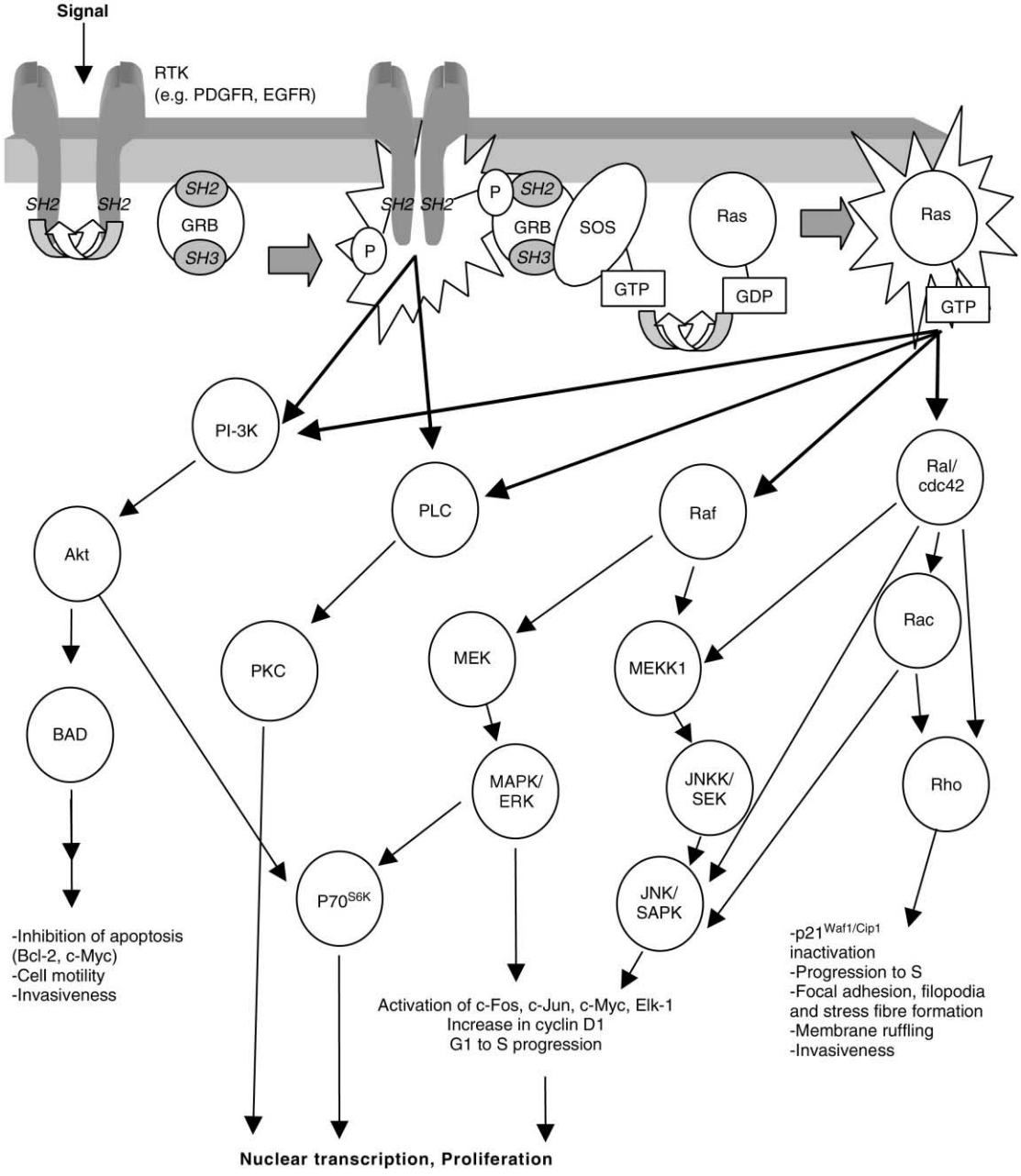

Fig. 2. Ras signal transduction pathway—in this simplified schematic, RTK is stimulated by an extracellular signal (i.e. growth factor) which leads to dimerisation and autophosphorylation of Src-homology-2 (SH2) domains on the intracellular surface of the protein. This leads to binding of GRB, an adapter protein, via its SH2 domains. Through its association with GRB, son of sevenless (SOS) is brought to the cell membrane surface where it is able to interact with Ras. Through their interaction, guanosine diphosphate (GTP)–guanoside diphosphate (GDP) exchange occurs. The activated GTP-Ras is then able to stimulate proliferative cellular processes through the activation of multiple pathways including, Raf, mitogen-activated ERK kinase (Mek)/Jun N-terminal kinase (JNK), Rac/Rho, phospholipase C (PLC) and phosphoinositide 3′ kinase (PI-3K). Multiple channels of cross-talk exist between the pathways. Some pathways, such as PLC and PI-3K can be activated directly by receptor tyrosine kinase (RTK).

and 7.9 nM, respectively. R115777 has shown activity in 75% of a large number of cell lines investigated. Cell lines bearing N- and H-*Ras* or no *Ras* mutations are most sensitive, whereas K-*Ras* mutants are relatively resistant. R115777 also decreases proliferation of H-*Ras* transfected NIH 3T3 cells, K-*Ras* transformed CAPAN-2 pancreatic cancer cells and several colon cancer lines [60,61]. Growth of tumours containing H-*Ras*, K-*Ras* and wild type *Ras* in nude mice were inhibited at doses ranging from 6.25 to 100 mg/kg [62]. Preclinically, human tumours showed a variety of responses based on the tumour type. Colon tumours displayed an anti-angiogenic response and an antiproliferative response was observed in pancreatic tumours histologically [62].

Clinically, R115777 is given orally on a twice daily (BID) dosing schedule [63]. Phase I single agent studies in solid tumours have established the maximum tolerated dose (MTD) in the range of 300–500 mg BID depending on the dosing schedule [12,63–65]. The dose-limiting toxicities have been myelosuppression and neurological complications including neurosensory/motor deficits and neuropathy [12,63]. Skin sensitivity and rash have also been documented as significant toxicity [12,66]. In general, this agent was well tolerated at doses that generated pharmacologically significant plasma concentrations [9]. Evidence of activity was seen in a minority of patients including those with non-small cell lung cancer (NSCLC), colorectal cancer (CRC) and pancreatic cancer (Table 2) [12,63].

Phase I single agent trials in patients with refractory leukaemias resulted in striking objective responses. 10 of 34 patients (29%) responded including 2 complete remissions. 8 of the 10 responders, including the 2 complete remissions, were patients with acute myelogenous leukaemia (AML) [9]. The recommended phase II dose in leukaemia is 600 mg orally BID for up to 3 weeks out of every 4 weeks. At the high doses (900 and 1200 mg) utilised in leukaemia, relatively rare toxicities such as ataxia and confusion were seen. Preliminary results from phase I combination studies have also been reported with gemcitabine, topotecan, docetaxel, capecitabine, irinotecan, trastuzamab and 5-fluorouracil (5-FU)/leucovorin [11,14,67–71]. The MTDs of R115777 have been in the range of 200–400 mg BID, with enhancement of myelosuppression of the cytotoxic agents.

Some of these studies have demonstrated impressive antitumour activity.

For example, a 21% response rate was seen when R115777 (BID d1–14, q21days) was combined with docetaxel (d1 q21 days), including one complete response (CR) in breast. Partial responses (PR) (11%) were seen in combination with capecitabine in colon, gall bladder, breast and head/neck cancers. In this study, the DLTs were diarrhoea and hand–foot syndrome, known toxicities of capecitabine [14].

The combination of R115777 on days 1–14 with gemcitabine 1000 mg/m^2 on days 1 and 8 plus cisplatin on day 1 has led to responses in 10 (9 PR and 1 CR) of 27 evaluable patients [72]. The toxicities of this combination, which were predominantly myelosuppression, nausea, vomiting and electrolyte abnormalities, were similar to those one would expect from cisplatin and gemcitabine alone.

Data from a phase II single agent study in advanced breast cancer have been reported. R115777 was initially administered at 400 mg BID, later reduced to 300 mg

Table 1
Farnesylated mammalian proteins

Protein	Function	Ref.
H-, K- and N-Ras	Growth, differentiation and inhibition of apoptosis	[24,105–111]
Rho-B and -E	Cell cycle regulation	[112–114]
PTP-CAAX1 and 2	Growth and differentiation, cell regulatory protein tyrosine phosphorylase	[115,116]
Rap2A	GTPase, unknown function (? suppress Ras-mediated signal transduction)	[117–119]
CENP-E and -F	Centromere binding proteins; G2→M transition	[56]
HDJ2/dj2/HSDJ/rdj1/hsj2	Chaperone protein	[61,120,121]
DJ3/CPR3/dnj3/HIRIP4/rdj2	Chaperone protein	[122]
Lamin A and B	Nuclear membrane structure	[109,123]
PxF	Peroxisomal protein	[124]
PEX19p	Peroxisomal biogenesis	[125]
cGMP	Visual signal transduction	[126]
Transducin	Visual protein	[127]
Rhodopsin kinase	Visual protein	[128]
Phosphorylase kinase α and β	Glycogenolysis, membrane phospholipid metabolism, platelet function	[61,129]

orally (p.o.) BID for 21 days every 4 weeks after dose-limiting myelosuppression was seen in all patients treated at the 400 mg BID dose. 27 women were enrolled. Objective partial responses occurred in 3 patients for a response rate of 11%. Disease stabilisation for six cycles or greater was observed in 9 patients (35%). The major toxicity was grade 3–4 self-limited myelosuppression in roughly 30%, with lesser grades of paresthesias, diarrhoea, skin rash and fatigue in 10–30% of patients [9].

Phase III studies of R115777 in pancreatic and colorectal cancers have completed accrual [37]. Two studies compare R115777 plus gemcitabine versus gemcitabine plus placebo in advanced pancreatic cancer, one with survival as the endpoint, the other with time to deterioration as the endpoint. The colorectal cancer study is a 2:1 randomisation to R115777 plus best supportive care versus best supportive care in patients who have received two or more previous chemotherapy regimens.

5.2. SCH66336 (SARASAR*)

SCH66336 is a tricyclic, non-peptidomimetic, specific reversible inhibitor of FT [73]. *In vitro*, this agent competitively inhibits FT ability to farnesylate H-Ras and K-Ras B with IC_{50}s of 1.9 and 5.2 nM, respectively. SCH66336 inhibits the transformed growth properties of human fibroblasts and cell lines expressing mutant K-*Ras*. It also suppresses the anchorage-independent growth of non-Ras-transformed cancer cell lines [74]. In glioblastoma cell lines, SCH66336 inhibits viability and anchorage-independent growth in a time- and dose-dependent manner. Gliobastoma cells engineered to overexpress the epidermal growth factor receptor (EGFR) were more sensitive to SCH66336 than the parental cell line [75]. SCH66336 also showed a significant prolongation of survival in a BCR/ABL-positive murine acute lymphoblastic leukaemia (ALL)

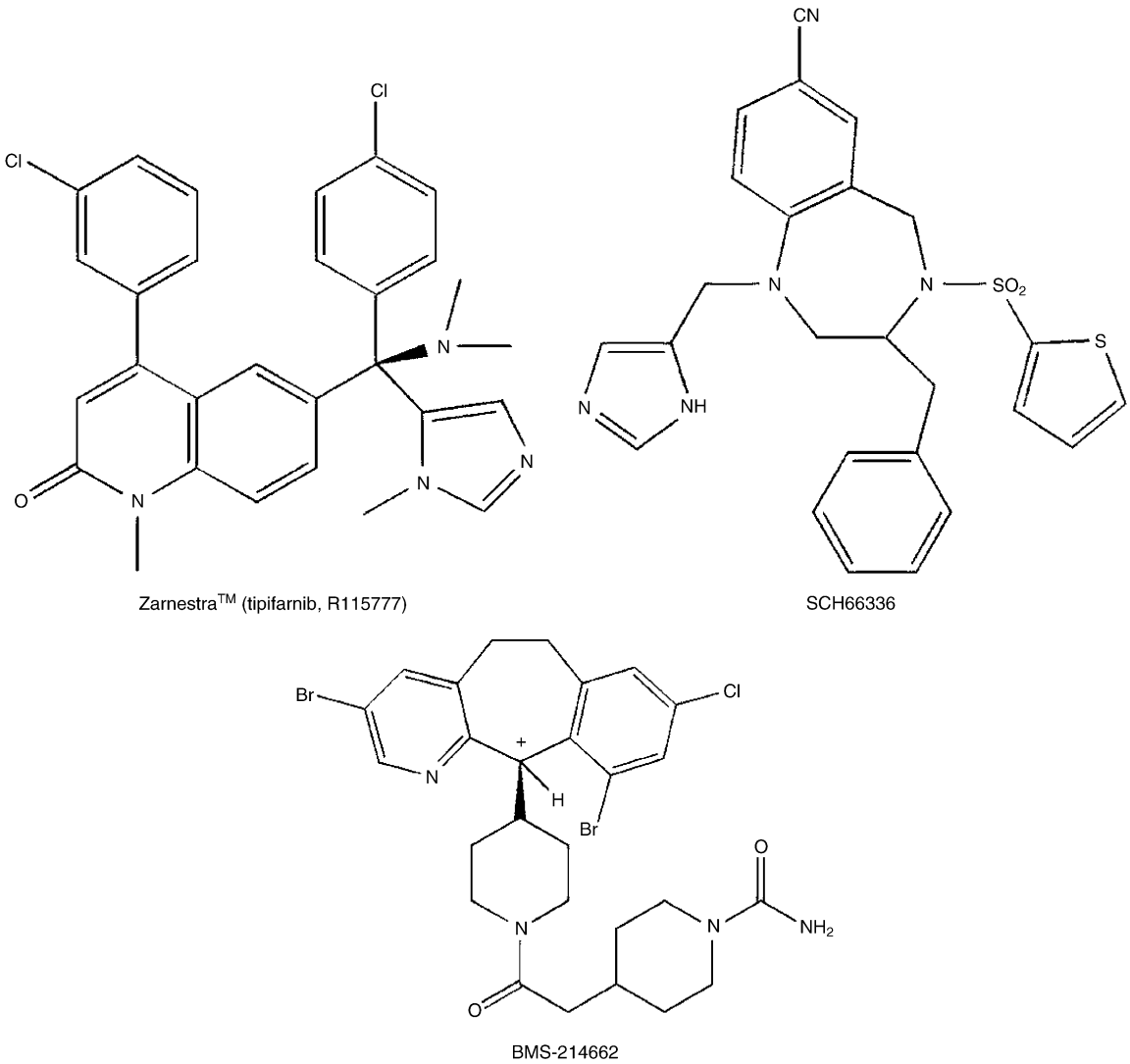

Fig. 3. Chemical structure of farnesyl transferase inhibitors currently undergoing clinical trials.

Table 2
Results of FTI in clinical trials

Study type (phase)	Tumour type	Schedule/MTD	Toxicities (DLTs are in italics)	Responses	Ref.
R115777					
Mono (I)	Solid	Oral 50–500 BID, continuous; MTD—300 BID	*Neutropenia, thrombocytopenia, neurosensory/motor,* skin hypersensitivity, leucopenia	1/18:PR in NSCLC 3/18: evidence of activity in CRC, pancreatic.	[12]
Mono (I)	Solid	Oral solution 25–850 mg BID or cap 500–1300 mg BID 5d q14d; MTD—500 mg BID	*Neuropathy, fatigue*, nausea, vomiting, headache, anaemia, and hypotension	1/27: SD in CRC	[63]
Mono (I)	Solid	Oral 100–400 mg BID×21d q28d; MTD—300 mg BID	*Neutropenia/leucopenia,* fatigue, fever, anorexia, nausea, vomiting, and increasing alkaline phosphatase	–	[64]
Mono (I)	Solid	Oral 200–300 mg BID×28d q35–42 d; MT—D-300 mg BID	Myelosuppression	-	[65]
Mono (I) Paediatric	Solid and plexiform neurofibromas	Oral 150–375 mg/m^2 BID×21d q28d; MTD—200 mg/m^2 BID	*Neutropenia, thrombocytopenia, rash, hypofibrinogenemia, vomiting, diarrhoea,* abdominal pain, headache, fatigue, anaemia, nausea	-	[66]
Mono (I)	Leukaemia	Oral 100–1200 mg BID; MTD-1200 mg BID×21 days	*Neurotoxicity —ataxia, confusion, and dysarthria,* nausea, renal insufficiency, paresthesia, polydipsia, myelosuppression	2/34: CR 8/34: PR	[9,13]
Combo with gemcitabine (I)	Solid	FTI—oral 100–300 mg BID and gemcitabine—i.v. 1000 mg/m^2 days 1, 8 and 15 q28d; MTD—200 mg BID	*Neutropenia,* thrombocytopenia, nausea, vomiting, fatigue	None	[67]
Combo with topotecan (I)	Solid	FTI—oral 200–300 BID×21d q28d and topotecan 1.0–1.25 mg/m^2 qd×5d q28d; MTD—200/1.0	*Neutropenia, thrombocytopenia,* fatigue, nausea, diarrhoea	–	[68]
Combo with docetaxel (I)	Solid	FTI—oral 200–300 mg BID continuous, ×7 and ×14d and docetaxel 75 mg/ m^2×3d q21d; MTD—not reached	Asthenia, diarrhoea, myalgia, mucositis, neurotoxicity	1/24: CR in breast; 4/24; PR in breast, NSCLC, unknown primary, 6/24: SD	[11]
Combo with capecitabine (I)	Solid	FTI—oral 100–300 mg BID×14d q21d and capecitabine oral 2000–2500 mg/m^2 qd×14d q21d; MTD—300/2250	*Diarrhoea, hand–foot syndrome*, nausea, mucositis, neutropenia, thrombocytopenia	3/27: PR in colon, GB, breast, head/neck; 9/27: SD	[14]
Combo with irinotecan (I)	Mostly Colorectal	FTI—Oral 200–300 mg BID continuous, ×14d q21d and Irinotecan 200–350 mg/ m^2 q21d; MTD—350 mg/m^2 for irinotecan, for FTI—not reached	*Neutropenia, thrombocytopenia,* nausea, vomiting, rash, diarrhoea	–	[69]
Combo with trastuzumab (Herceptin) (I)	Solid (4/7 breast)	FTI—oral 200–400 mg BID×14d q21d and trastuzumab i.v. 4 mg/kg day 1, then 2 mg/kg q7d; MTD—400 mg BID	*Neutropenia, thrombocytopenia*, nausea, vomiting, headache, fatigue	–	[70]
Combo with 5-FU/LV (I)	CRC, pancreas	FTI—oral 200–500 BID×21d q28d or continuous, 5FU-IV 400 mg/m^2 bolus + 600 mg/m^2 22 h infusion q14d and LV-i.v. 200 mg/m^2 q14d; MTD—200 BID intermittent	*Neutropenia, fatigue, paresthesia,* nausea, vomiting, anorexia, diarrhoea	–	[71]

Table 2 (*continued*)

Study type (phase)	Tumour type	Schedule/MTD	Toxicities (DLTs are in italics)	Responses	Ref.
Mono (II)	Breast	Oral 300–400 mg BID	*Myelosuppression,* paresthesia, diarrhoea, rash, fatigue	3/26: PR; 9/26: SD	[8]
SCH66336					
Mono (I)	Solid	Oral 25–400 mg BID; MTD—350 mg BID×7d q21d	*Nausea, vomiting, diarrhoea, fatigue,* renal insufficiency, anorexia	1/20: PR in NSCLC 8/20: SD	[15]
Mono (I)	Solid	Oral 25–400 mg BID, MTD—300 mg BID continuous	*Myelosuppression, neurocortical toxicity, nausea, vomiting, diarrhoea, renal insufficiency, anorexia*	2/24: SD in pseudomyxoma peritonei and thyroid CA	[79]
Mono (IB)	SCC of head/neck	Randomised to oral 100–300 mg BID×8–14 d or no therapy prior to surgery; MTD—not reached	Nausea, diarrhoea, dehydration, anorexia	3/17 treated: PR	[10]
Combo with gemcitabine (I)	Solid	FTI—oral 100–200 mg BID continuous and gemcitabine—600–1000 mg/m^2 d1, 8, 15 q28d; MTD—100–150 mg FTI and 1000 mg/m^2 gemcitabine	*Nausea, vomiting, diarrhoea, myelosuppression*	2/25: PR in pancreas; 2/25: MR in pancreas, mesothelioma; 11/25: SD	[16]
Combo with paclitaxel (Taxol) (I)	Solid	FTI—oral 100–150 BID, paclitaxel 135–175 mg/m^2; MTD—100 BID/175 mg/m^2	Dehydration, hyperbilirubinaemia, neutropenia with fever, myelosuppression, diarrhoea	8/21: PR in NSCLC, salivary gland; 7/21: SD	[17,18]
Mono (II)	Transitional cell	Oral 200 mg BID continuous (dose escalation q28d)	*Anaemia, neutropenia, thrombocytopenia,* fatigue, anorexia, nausea, vomiting, confusion, dyspnoea, dehydration	1/14: SD, 6/14: PROG	[81]
Mono (II)	Pancreas	Randomised to FTI—oral 200 mg BID or gemcitabine 1000 mg/m^2 q7d×7, then a week off	Nausea, vomiting, diarrhoea, neutropenia, thrombocytopenia (Gem only)	FTI- 2/33 PR, 6/33 SD; Gem—1/30 PR, 11/30 SD	[80]
BMS-214662					
Mono (I)	Solid	i.v. 36–225 mg/m^2 over 1 h q21d and single oral 36–168 mg/m^2 at course 2; MTD—not reached	Nausea, vomiting, diarrhoea, transaminase elevation, somnolence, fatigue, anorexia	1/38: MR in NSCLC, disease reduction in breast, CRC, 'several' pts with SD	[85]
Mono (I)	Solid	i.v. 28–102 mg/m^2 qweek ×4 q6weeks; MTD—not reached	No grade 3 or 4 toxicities	2/12: SD, 6/12: PROG	[86]
Mono (I)	Solid	i.v. 50 mg 1 week prior to oral 50–150/d on qd or BID dosing×14d q21d; MTD—50 mg BID	*Nausea, diarrhoea,* vomiting, abdominal cramping, anorexia, fatigue and fever	1/23: SD; others PROG	[84]
Mono (I)	Solid	i.v. 56–278 mg/m^2 weekly, MTD—278 mg/m^2	*Vomiting, diarrhoea, renal insufficiency,* hypotension, transaminase elevation, neutropenia	1/30: MR in breast	[87,88]
Combo with paclitaxel (I)	Solid	FTI – i.v. 80–120 mg/m^2 over 1 h qweek and paclitaxel 80 mg/m^2 over 1 h qweek; MTD—not reached	Neutropenia, nausea	2/6: ';response' in laryngeal and prostate	[83]
Combo with cisplatin (I)	Solid	FTI—i.v. 126–225 mg/m^2 over 1 h q3 weeks and cisplatin i.v. 75–100 mg/m^2 over 1 or 4 h q3 weeks; MTD—not reached	*Transaminase elevation,* nausea, vomiting, stomatitis, lethargy, neutropenia	2/10: SD	[89]

Table 2 (*continued*)

Study type (phase)	Tumour type	Schedule/MTD	Toxicities (DLTs are in italics)	Responses	Ref.
L-778,123					
Mono (I)	Solid	i.v. continuous for 2 weeks at 140–840 or 4 weeks at 560 mg/m^2/day followed by 1 week off; MTD—840 mg/m^2/day on 2-week schedule	*Prolonged QTc, neutropenia*	None	[90]
Combo with XRT (I)	Solid	i.v. 280–560 mg/m^2/d week 1,2,4 and 5 of 7-week radiotherapy regimen; MTD—not reached	None reported	2/13: CR in head/neck; 2/13: PR in NSCLC	[92]
Combo with paclitaxel (I)	Solid	FTI—i.v. continuous 280 mg/m^2/d×7d and paclitaxel i.v. 175 mg/m^2 bolus	Hypersomnolence, tachycardia, hypotension, sensory neuropathy, dyspnoea, neutropenia with sepsis, mild abnormalities in the electroretinogram, increases in QTc	None	[91]

CA, cancer; combo, combined with; 5-FU, 5-fluorouracil; LV, leucovorin; SCC, squamous cell carcinoma; pts, patients; PROG, progressed; XRT, radiotherapy; CR complete response; PR, partial response; MR, minimal response, i.v., intravenous; MTD, maximum tolerated dose; DLT, dose-limiting toxicity; BID, twice daily; NSCLC, non-small cell lung carcinoma?; CRC, colorectal carcinoma; SD stable disease; d, day; qd, daily; GB, gall bladder; QTc, corrected QT interval on electrocardiogram; FTI, farnesyl transferase inhibitor.

model without any detectable side-effects [76]. This agent is orally absorbed in multiple animal models, with favourable pharmacokinetics. In addition to the preclinical systems discussed above, SCH66336 has activity in a wide variety of human tumour xenografts including colon, lung, pancreas, prostate and urinary bladder. The efficacy of SCH66336 *in vivo* is enhanced by cytotoxic chemotherapy agents, including cyclophosphamide, 5-FU, vincristine and the taxanes [74]. Sequence-dependent synergy between SCH66336 and cisplatin in NSCLC and glioblastoma cell lines has also been demonstrated [77]. SCH66336 was also able to sensitise previously resistant wap-Ras/F mammary tumours in transgenic mice to paclitaxel [78].

SCH66336 is orally active on a BID dosing schedule. The MTD in 3 phase I studies varied based on schedule, although gastrointestinal side-effects were shared as the DLT, including vomiting and diarrhoea [10,15,79]. The first phase I monotherapy study published administered the agent BID for 7 days every 3 weeks. The DLTs were observed at 400 mg BID and comprised of nausea, vomiting, diarrhoea and fatigue. The recommended phase II dose based on this schedule was 350 mg BID. Of the 20 people treated in this study, 1 patient with NSCLC experienced a PR and remained on the study for 14 months. 8 others experienced disease stabilisation. Using prelamin A as a surrogate marker for FT inhibition, SCH66336 was shown to inhibit farnesylation at clinically relevant doses [15].

In another phase I study, SCH66336 was administered p.o. BID on a continuous schedule. In addition to gastrointestinal effects, myelosuppression, renal and neurological toxicities were also observed at 300 mg BID dose level (Table 2). Pharmacokinetic analysis suggested accumulation of the drug with prolonged dosing, perhaps contributing to the additional toxicity. The suggested phase II dosing based on this schedule was 200 mg BID. Out of 24 patients treated, 2 patients experienced stable disease for 12 weeks or more [79].

A phase IB monotherapy study was also undertaken in patients with squamous cell carcinoma of the head and neck, who were randomised to receive 100–300 mg SCH66336 BID or best supportive care for up to 14 days prior to surgery. Gastrointestinal side-effects were observed, but no dose-limiting toxicities (DLTs) were reported. PRs were observed in 18% (3/17) of patients treated. Analysis of the surrogate marker DNA-J (HDJ-2), a farnesylated chaperone protein, in surgical samples revealed an increase in unfarnesylated protein in patients treated with SCH6336 [10].

Preliminary data have been reported from phase I combination studies with gemcitabine and paclitaxel (Table 2) [16–18]. SCH66336 was administered continuously in combination with gemcitabine given weekly for 3 weeks in a 4- week cycle. The DLTs were diarrhoea, nausea, vomiting and myelosuppression. The recommended phase II doses were 150 mg a.m. and 100 mg p.m. of SCH66336 with 1000 mg/m^2 of gemcitabine. Responses were encouraging with 4/25 (16%) patients experiencing a partial or minor responses, including 3/9 patients with pancreatic cancer. Disease stabilisation for 6 or more months was seen in an additional 11 patients [16].

A preliminary report of a combination study of SCH66336 and paclitaxel has been reported. The recommended phase II dose on this study is 100 mg BID of oral SCH66336 daily, with 175 mg/m^2 of paclitaxel every 3 weeks. The most common toxicities on this study were myelosuppression and diarrhoea. Other toxicities were dehydration and hyperbilirubinaemia. Promising preliminary evidence of efficacy was documented. 8 out of 21 evaluable patients achieved a PR. Notably, 3 of these patients had either progressed on, or after, taxane therapy [17,18]. These data are consistent with *in vitro* and xenograft studies in lung tumours. These studies demonstrated synergistic cytotoxicity between SCH66336 and paclitaxel [78].

Preliminary data are also available from two phase II single-agent studies using SCH66336 at the 200 mg continuous BID dose level [80,81]. In the phase II study in transitional cell carcinomas previously failing chemotherapy, myelosuppression was dose-limiting with patients experiencing additional toxicities of fatigue, anorexia, nausea, vomiting, confusion, dyspnoea and dehydration. Despite significant toxicities, no responses were observed [81].

In another phase II study, patients with metastatic pancreatic cancer were randomised to SCH66336 200 mg BID or gemcitabine 1000 mg/m^2 weekly for 7 weeks in an 8-week cycle. Gastrointestinal toxicities, including vomiting and diarrhoea were observed in both groups and were not dose limiting. Haematological toxicity was also observed with gemcitabine. Activity was observed in both groups, with a larger number of stable or responsive disease seen in the gemcitabine-treated group (40% versus 24%) [80]. Further studies are to be performed with gemcitabine in combination with SCH66336 in patients with pancreatic cancer.

5.3. BMS-214662

BMS-214662 is a benzodiazepine-type FTI. It is a highly selective inhibitor of FT with over a 1000-fold selectivity for FT over GGT. *In vitro*, FT is inhibited by BMS-214662 with a concentration inhibiting 50% of activity (IC_{50}) of 1.35 nM. Inhibition of H-*Ras*-transformed Rat-1 cell growth and of the anchorage-independent growth of H-*Ras*-transformed NIH 3T3 cells occurs with a concentration that elicits 50% of maximal response (EC_{50}) values of 25 nM. In a tumour xenograft model using K-*Ras* transformed HCT-116 human colon tumour cells, BMS-214662 cured 8/8 mice treated with

the oral agent [82]. Furthermore, sequence-dependent synergy has been shown in combination with paclitaxel [83].

BMS-214662 has been investigated in phase I single agent studies as an intermittent infusion or as a p.o. administered agent in patients with solid tumours [84–88]. In the only phase I study using oral BMS-214662 given once or twice daily for 2 weeks in a 3-week cycle, patients experienced DLTs of nausea and diarrhoea with additional toxicities of vomiting, abdominal cramping, anorexia, fatigue and fever. Of the 23 patients treated, all but 1 had progressive disease. Thus, while pharmacokinetics demonstrated favourable oral bioavailability, the oral form has been abandoned secondary to gastrointestinal intolerance [84].

Other dosing schedules have included infusion over 1 hour every 3 weeks, 1 h weekly and 24 hours weekly. In a phase I study administering BMS-214662 intravenously (i.v.) over 1 h every 3 weeks, evidence of activity was observed. A greater than 40% shrinkage in the tumour occurred in 1 patient with NSCLC. In addition, tumour regressions occurred in patients with colorectal and breast cancer. Several other patients had disease stabilisation for up to 10 cycles. Toxicities were mostly gastrointestinal, including anorexia, nausea, vomiting, nausea and diarrhoea, but somnolence and fatigue were also reported. The MTD and recommended phase II dose has not yet been reported [85].

In another phase I monotherapy trial, BMS-214662 was initially administered over 1 h weekly to a total of 30 patients. A minor response was reported in one patient with chemotherapy-refractory breast cancer. At the 278 mg/m^2 dose level, vomiting, diarrhoea and renal insufficiency were dose-limiting. Additional toxicities included hypotension, neutropenia and transaminase elevation. The tentatively recommended phase II dose was 245 mg/m^2 [88]. Pharmacodynamic studies in this trial indicated that FT inhibition in patient tumour samples occurs at 2 h, but wanes by 24 h. Thus, further recruitment is occurring with patients receiving 24-h infusions weekly [87].

Finally, preliminary results have been reported in another phase I study, administering BMS-214662 weekly for 4 weeks in a 6-week cycle. Although the MTD has not yet been reached, 2 of 8 evaluable patients have had stable disease (SD). No commom toxicity criteria (CTC) grade 3 or 4 toxicities have been reported. Accrual continues at the 154 mg/m^2 dose level [86].

Data from two phase I combination trials with weekly BMS-214662 infusion and cytotoxic chemotherapy have been reported. In an ongoing study combining weekly paclitaxel over 1 h with weekly BMS-214662, partial responses have been reported in 2 patients with laryngeal and prostate cancer, out of the first 6 patients treated. Minimal toxicity has been documented so far [83]. Patients in the other combination study are given BMS-214662 and cisplatin every 3 weeks. The MTD in this study has not been reported, although transaminase elevation has been dose-limiting in 1 patient treated at the BMS-214662 225 mg/m^2 and cisplatin 75/mg/m^2 dose level. Other toxicities reported are nausea, vomiting, stomatitis, lethargy and neutropenia. SD has been described in 2 out of 10 evaluated patients [89]. There are currently no published phase II trials with this agent.

5.4. L-778,123

L-778,123 is a peptidomimetic inhibitor of FT that is administered as a continuous infusion. It is a potent inhibitor of FT with an IC_{50} of 2 nM and has some cross-activity against GGT *in vitro* with an IC_{50} of 98 nM.

L-778,123 has been tested clinically as a 5-day continuous infusion as a single agent and in combination with radiation and paclitaxel [90–92]. Despite showing responses in head/neck and NSCLC, clinical investigations have been discontinued due to the evidence of cardiac conduction abnormalities, manifested as a prolongation of the QTc interval. This toxicity does not appear to be a common finding with the other FTIs that have been investigated to date in clinical trials [37,90–92].

6. FTIs in combination with ionising radiation

Ras oncogenes have been reported to confer resistance to ionising radiation [93,94]. Since the FTIs were presumed to be inhibiting ras proteins, their role as radiosensitising agents, have been evaluated. In spite of the uncertainty surrounding their ras inhibitory effects, the FTIs have been shown to synergise with gamma radiation in preclinical models.

Because activated H-ras expression had been shown to markedly increase radiation resistance in some transformed cells and FTI-277 was shown to inhibit the farnesylation of H-ras, this FTI was tested in combination with gamma irradiation in H-*ras* transformed rat embryo cells. Treatment with FTI-277 resulted in higher levels of apoptosis after irradiation and increased radiosensitivity in these cells [95].

Based on the known radioresistance of hypoxic cells, the oxygenation of tumour xenografts in nude mice after treatment with another FTI, L744,832, has been evaluated. Results indicated that FTI treatment markedly improved the oxygenation of xenografts from tumour cell lines with H-*ras* mutations. In contrast, xenografts from tumours without *ras* mutations had equivalent hypoxia regardless of the treatment. These results suggested that FTI treatment might be useful in the radiosensitisation of tumours with H-*ras* activation [96]. Other studies indicate that radiosensitisation of

tumours with K-*ras* mutations requires the use of FTIs and GGT-1 inhibitors [97], whereas tumours with wild-type ras can also be sensitised to gamma irradiation by FTIs [98].

It must be noted, however, that other studies have failed to demonstrate synergistic cell killing when FTIs are combined with radiation in tumours with wild-type ras [97]. Taken together, these data suggest that the FTIs can synergise with gamma irradiation in certain human tumours. The predominant ras isoform in the tumour is a critical determinant of the radiosensitising ability of FTIs. Tumours with an activated H-*ras* isoform appear to be the most sensitive. Tumours with wild-type *ras* may also be sensitive, while tumours with K-ras mutations may be relatively resistant to the radiosensitising properties of FTIs. These data should be evaluated carefully as studies with FTI-radiation combinations are planned. One phase I study combining gamma radiation with the FTI L778,123 has been reported in abstract form. This study demonstrated that such a combination was feasible with minimal toxicity. More importantly, 2 CR in head and neck cancer (70 Gy over 7 weeks with L778,123 280 mg/m^2/day on weeks 1, 2, 4, 5) and 2 PR in NSCLC (65 Gy over 7 weeks with L778123 280 mg/m^2/day on weeks 1, 2, 4, 5) were observed [99]. Unfortunately, the clinical development of L778,123 has been halted. Studies with radiation therapy and the clinically relevant FTIs are ongoing, and the results are awaited with interest.

7. Conclusions

FTIs are a promising class of novel antineoplastic agents. Although initially designed as anti-Ras agents, mounting evidence indicates that other farnesylated targets are involved in the cytotoxic effects of these agents. Identification of the critical protein target(s) of FTIs is currently an active area of research [2,38,60–62,74,76,100,101]. The FTIs are generally well tolerated at clinically effective doses [102]. Some of their toxicities, such as nausea, vomiting and diarrhoea, may be class effects, since they occur with all the agents discussed, albeit with varying frequency and severity. Other toxicities which are specific to individual agents, such as the cardiac toxicity of L778, 123, may be structurally related.

As single agents, the FTIs have significant activity in myeloid leukaemias. Activity in other haematological malignancies is being investigated. In solid tumours, single-agent activity appears to be modest, and these agents will probably need to be studied in combination with cytotoxic agents. For example, the FTIs have demonstrated synergistic cytotoxicity with paclitaxel both preclinically and clinically [83,103,104]. A number of the initial phase II and phase III studies with these agents targeted tumours with K-*ras* mutations such as colorectal, non-small cell lung and pancreatic cancers. With current knowledge that tumours harbouring K-*ras* mutations are relatively more resistant to FTIs, it is probable that a number of these studies will be negative. It is imperative that these interesting compounds be studied in more appropriate tumours such as those with wild-type *ras* and not be abandoned based on such results.

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