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Farnesyltransferase Inhibitors
Potential Role in the Treatment of Cancer

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Abstract

New targets for drug discovery have been identified rapidly as a result of the many recent and rapid advances in the understanding of signal transduction pathways that contribute to oncogenesis. In particular, oncogenic Ras proteins have been seen as an important target for novel anti-cancer drugs. Since the decade-old identification and cloning of farnesyltransferase (FTase), a critical enzyme that post-translationally modifies Ras and other farnesylated proteins, FTase inhibitors (FTIs) have been under intense investigation designed to bring them to clinical practice for cancer therapy.

FTIs can inhibit the growth of tumour cells in culture and in animal models, and are now in clinical trials. Interestingly, their mechanism of action is not as simple as originally envisioned, and Ras is probably not the most important farnesylated protein whose modification is inhibited as a result of FTI treatment. Although K-Ras can escape inhibition of processing by FTIs, tumours with oncogenically mutated K-Ras proteins can still be inhibited by FTI treatment. Indeed, Ras mutation status does not correlate with FTI sensitivity or resistance. Instead, it now appears likely that inhibition of the processing of other farnesylated proteins such as RhoB and the centromere-binding proteins CENP-E and CENP-F can explain the ability of FTIs to cause cell cycle arrest and apoptosis in preclinical studies, and even to cause regression in animal tumour models.

Preclinical studies suggest the likelihood that FTIs will be useful in combination therapies with conventional treatment modalities including cytotoxics (especially paclitaxel) and radiation. Phase I combination trials are underway, and early phase II/III trials using FTIs as monotherapy are open for patients with a wide variety of cancers. Early preclinical results also suggest the possibility of using FTIs as chemopreventive agents.

Studies to be completed over the next 2 or 3 years should define the appropriate patient populations, administration and scheduling necessary to optimise the use of these novel anticancer agents.

1. Ras and Cancer

Ras proteins are guanine triphosphate (GTP)-regulated switches that regulate signal transduction pathways controlling biological processes as diverse as cellular proliferation, differentiation and apoptosis.[1] Oncogenic mutations result in Ras proteins that are constitutively GTP-bound and therefore chronically active; typically, these mutations occur at hot spots such as codons 12, 13 or 61. Although *Ras* is the most frequently mutated oncogene in human tumours, this varies widely de-

pending on the tumour type.[2] Loss of the mutated *Ras* allele in cell lines derived from human colon carcinoma[3] or fibrosarcoma[4] results in impairment of the transformed phenotype despite the continued presence of all the other genetic aberrations in these tumour cell lines; these results show that Ras can be a good target for pharmacological intervention for cancer treatment. Earlier studies also showed that either pharmacological blockade or mutagenesis which prevented Ras protein association with the plasma membrane impaired Ras transformation.[5] Thus, drugs that could target Ras should be good candidates for anti-tumour agents.

2. Farnesyltransferase (FTase) as a Target for Anti-Cancer Drug Design

All Ras proteins absolutely require covalent modification by a farnesyl isoprenoid lipid for their proper membrane localisation and biological activity.[5,6] Farnesyltransferase (FTase) modifies newly synthesised Ras proteins by the transfer of a farnesyl pyrophosphate (FPP) donor to the cysteine of the carboxyterminal CAAX motif (where C = cysteine, A = aliphatic and X = any amino acid) of the substrate protein (fig. 1). Attachment of FPP, which is derived from farnesol, an obligate intermediate in the cholesterol biosynthetic pathway,[7] is the first and obligate step in post-translational processing that ultimately results in the localisation of mature Ras proteins to the inner leaflet of the plasma membrane.[5,6] Ras proteins that are not so localised are impaired in transformation. Thus, compounds that could block FTase were immediately envisioned as potential anti-cancer drugs.

2.1 Development of FTase Inhibitors

The cloning of the FTase enzyme, and the finding that a tetrapeptide sequence (i.e., the CAAX sequence) was both necessary and sufficient to serve as its substrate, drew the attention of pharmaceutical companies and academic researchers to designing small molecule inhibitors of FTase.[8-11] The earliest generation of rationally designed FTase inhibitors (FTIs) were peptidomimetics of CVIM, the CAAX sequence of K-*ras*4B. Such peptidomimetic inhibitors could selectively block FTase compared with the related enzyme geranylgeranyltransferase I (GGTase I), which attaches a geranylgeranyl isoprenoid to proteins, and were good inhibitors of enzymatic activity *in vitro*. Current generation FTIs are largely nonpeptide, non–thiol-containing compounds with nanomolar potency. They represent a variety of different structures, including improved molecules based on the rationally designed CAAX peptidomimetics, FPP analogues and nonpeptidic molecules identified by random screening of libraries generated from natural compounds and by combinatorial chemistry.

Preclinical studies in animal tumour models, using both H-Ras-transformed fibroblasts grown as xenografts in nude mice, as well as in transgenic mice that stochastically developed mammary and salivary tumours secondary to having the H-*ras* transgene, showed that FTIs could inhibit the growth of

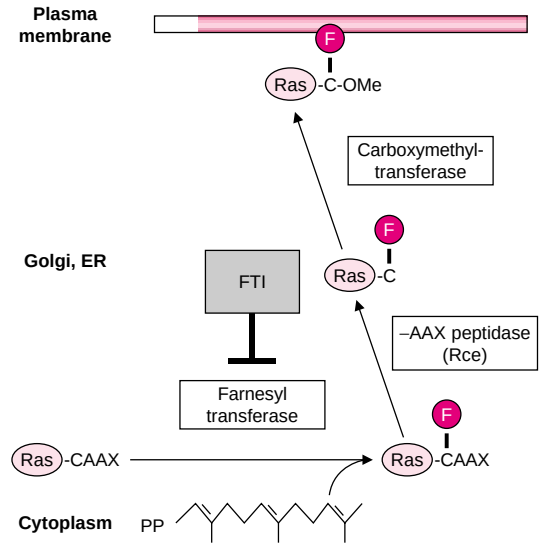

Fig. 1. Farnesyltransferase (FTase) modifies newly synthesised Ras proteins by the transfer of a farnesyl pyrophosphate (FPP) donor to the cysteine of the carboxyterminal CAAX motif (where C = cysteine, A = aliphatic and X = any amino acid) of the substrate protein. FTI blocks FTase activity and therefore blocks all modification steps. **ER** = endoplasmic reticulum; **FTI** = farnesyltransferase inhibitor; **PP** = pyrophosphate; **Rce** = Ras converting enzyme.

such tumours. In the transgenic H-*ras* models, FTI treatment even caused tumour regression,[12,13] a surprising feat for compounds that were expected initially to be cytostatic. A wide variety of structurally distinct FTIs all showed similar results in preclinical studies, suggesting that the tumour inhibition was mechanism-based and a consequence of inhibiting FTase. These results were highly encouraging to those interested in using FTIs as anticancer drugs for Ras-containing tumours. However, because all the early tests were carried out using H-Ras, rather than N-Ras or K-Ras, certain surprises did not emerge until later and suggested a less simple mechanism of action than originally envisioned.

2.2 K-Ras is Resistant to FTase Inhibitor (FTI) Inhibition of Processing and Becomes Alternatively Prenylated

Because of alternate splicing, there are 4 forms of Ras proteins: H-Ras, N-Ras, K-Ras4A and K-Ras4B. Although it was very easy and straightforward to inhibit both processing of and transformation as a result of oncogenic H-Ras, this was not the case with K-Ras. The basis for the resistance of K-Ras to FTIs was not only that its affinity for the FTase enzyme was high but also that, in the presence of FTIs, it could become a substrate for alternative prenylation by the related enzyme, GGTase I *in vivo*.[14,15] This provides a mechanism whereby K-Ras processing could escape FTI inhibition; nevertheless, tumours containing mutated K-Ras proteins could be inhibited by FTIs.[16] These results suggested that either those tumours that were sensitive to FTIs were not dependent on K-Ras function, or FTIs inhibited the farnesylation of one or more non-Ras proteins to exert their anti-tumour effects.

2.3 Ras Mutation Status Does Not Predict FTI Sensitivity or Resistance

Some preclinical studies on the response of cultured human tumour cells to FTI treatment found that the type of mutant Ras protein present was predictive of relative tumour cell responsiveness.[17] However, others showed the then totally unexpected result that *ras* mutation status did not predict FTI sensitivity or resistance to blockade of both anchorage-dependent and anchorage-independent growth.[16] All possible combinations could be found, such that tumour cell lines with mutated *ras* genes were both sensitive and resistant, and the same was true of tumour cell lines containing only wild type *ras*.

As indicated in section 2.1, these results forced consideration of the possibility that Ras might not be the most important farnesylated protein whose function was altered by inhibiting the FTase enzyme. Other results also suggested this possibility. For example, the dramatic tumour regression seen in H-*ras* transgenic animals[12,13] was a surprise, given that FTIs were expected to be cytostatic, rather than cytotoxic, and that FTI treatment of H-*ras* xenografts led to tumour stasis but not regression. The expectation of cytostasis was based on the anticipation that FTI treatment would lead to the loss of function of newly synthesised Ras proteins, but not to the clearance of processed Ras proteins which were already present, and even more importantly on the concept that loss of Ras function would produce a G1 cell cycle arrest but not cell death.[5]

Although widely accepted today, the possibility that Ras might not be the most important downstream target of FTase inhibitors was a tremendous paradigm shift, considering that the push for FTIs had originally been seen as a logical way in which to block oncogenic Ras proteins associated with a wide spectrum of cancers. Indeed, given that K-Ras processing has turned out to be so resistant to FTI inhibition, the existence of a target potentially more important than Ras is now seen as a positive outcome that bodes well for the utility of FTIs in a spectrum of cancers.

2.4 FTI-Mediated Arrest versus Apoptosis

In transformed cells, FTIs have now been shown to mediate a G2/M growth arrest under some circumstances[18] and an apoptotic response under others.[19,20] Neither of these consequences

appears to be dependent upon inhibiting Ras processing. Apoptosis of transformed cells can be induced by FTIs in preclinical models when an additional stressor such as growth factor or anchorage deprivation is involved.[19,20] Apoptosis may be increased as a consequence of up-regulating proapoptotic Bcl family proteins and activating caspase-3 by undefined mechanisms,[20] and/or by blocking the farnesylation of a protein(s) regulating the PI3-K/Akt lipid kinase-mediated survival pathway.[21,22] Interestingly, the FTI BMS-214662 promotes regression and increased levels of apoptosis in preclinical xenograft models where others do not.[23,24] It is unclear at present whether this is a unique response due to additional activities in this particular compound not possessed by others.

2.5 Searching for Downstream Targets of FTIs

Despite their unexpected consequences in terms of blocking Ras processing and biology, it is currently agreed that the FTIs under development are true inhibitors of the FTase enzyme, and that the most important consequence of their activity may be altering the processing of other unidentified farnesylated protein(s).[5,8-11,25] The identification of such proteins is thought to be important in order to improve the targeting of potential patient populations that may benefit from FTI treatment, and also to identify one or more targets for a new round of drug discovery and development.

Currently, most attention is focused on specific proteins already known to be farnesylated, including members of the Ras-related family of Rho proteins, especially RhoB,[25] and proteins important for cell cycle progression, especially the centromere-binding proteins CENP-E and CENP-F.[26] The mitotic arrest seen in transformed cells correlates with inhibition of the farnesylation of the CENP-E and CENP-F proteins, which are responsible for mitotic spindle segregation.[26] Why the division of transformed cells should be more susceptible to inhibition of these proteins than that in normal cells is not clear. Of the Rho family, there is evidence that RhoB, an immediate-early protein, functions differently depending on whether it is modified by a farnesyl or geranylgeranyl isoprenoid, and that geranylgeranylated RhoB, the only type of prenylated RhoB present under conditions of FTI treatment, is growth inhibitory to Ras-transformed but not normal fibroblasts.[21] However, others have shown that both farnesylated and geranylgeranylated forms of RhoB are growth inhibitory in human tumour cell lines,[27] and the role of RhoB in the mechanism of FTI sensitivity is controversial. Additional work to clarify the roles of these proteins in the FTI response is ongoing.

It is likely that there will be multiple relevant proteins that determine FTI responsiveness, depending on the tumour type treated or the phenotype inhibited (e.g. proliferation *vs* metastasis *vs* radiation sensitisation). For example, very recent work has demonstrated genetically that RhoB is required for an apoptotic response to FTIs, whereas it is not required for FTI-mediated growth inhibition.[28] Tumours that are more sensitive to FTIs may display differential regulation or dependence upon RhoB and/or the CENPs than those that are more resistant. Studies designed to determine whether this is indeed the case are underway. Nevertheless, identification of the exact downstream targets is not required to move forward with the development of FTIs for clinical use.

3. FTIs Inhibit Growth of Tumour Cells in Preclinical Studies When Used as Single Agents

Regardless of the mechanism, FTIs are indeed capable of inhibiting, and in some cases even causing regression of, a wide variety of tumour cells both in culture and in various animal models. Tumour cells include those derived from model cell lines such as transfected rodent fibroblast or epithelial cells, as well as diverse human tumour cell lines grown in monolayer culture, in soft agar, or as xenografts in nude mice.

Mice transgenic for H-*ras* alone[12] or in combination with *p53* or *myc*[29] have shown tumour shrinkage or growth inhibition, respectively, with FTI treatment. In addition, transgenic mice overexpressing N-*ras* have shown both reduction in tu-

mour growth rates and decreased incidence of lymphoma with FTI treatment.[30] Recent work with newly developed transgenic K-*ras* mouse models shows that the growth of their tumours (mammary tumours resulting from expression of Ras from the MMTV promoter) also becomes static with FTI treatment.[31] Therefore, there is reason to hope that many tumours may be targeted successfully by FTIs.

Overall, nearly 60% of human tumour cell lines demonstrate some sensitivity to FTI growth inhibition, suggesting that a variety of different tumours may also prove to be clinically amenable to FTI treatment.

3.1 FTIs Can be Radiosensitisers

Given that FTIs have not met the initial expectations of excellent antitumour activity in patients in early clinical trials of FTIs as single agents for anticancer treatment (see section 4), it seems likely that they will find their greatest utility in combination with other treatment modalities. Because Ras proteins induce radioresistance,[32] inhibiting Ras function by means of FTIs might produce important radiosensitising effects.

The first study to test the potential of FTIs as radiosensitisers showed that FTIs could modestly radiosensitise H-Ras transformed rodent fibroblasts,[33] and that this effect correlated with the ability to block H-Ras processing. A follow-up study showed that FTIs could also radiosensitise human tumour cell lines, although it suggested that for tumours with oncogenic K-Ras mutations, the addition of an inhibitor of GGTase I was also required.[34] For growth inhibition of tumours with oncogenic K-Ras mutations, the addition of GGTase I inhibitor did not provide additional efficacy,[35] which may reflect a difference in the roles of Ras in transformation and radioresistance. However, an FTI was also shown to cause radiosensitisation even in transformed cells containing only wild type Ras,[36] suggesting that FTIs may influence multiple pathways regulating radioresistance. Interestingly, long pretreatment with FTI prior to irradiation does not appear to be required, which is puzzling from a mechanistic point of view but bodes well for the logistics of combination therapy using FTIs.

3.2 FTI Synergy with Standard Anti-Cancer Agents

Several preclinical studies also examined whether FTIs could be used in conjunction with standard cytotoxic agents. Comparisons were made between FTIs alone and in combination with different classes of standard chemotherapies, including antimetabolites (fluorouracil), alkylating agents such as cytoxan and cisplatin, DNA intercalators (doxorubicin), and various microtubule binding agents (vinblastine and vincristine, taxanes and epothilones).[13,37-40]

In general, FTIs did not interfere with the action of cytotoxic drugs. Instead, FTIs exhibit additive effects when used in combination with most cytotoxics, and can even exhibit synergistic effects when used in combination with taxanes.

Several different cell types treated with both FTI and paclitaxel, for example, display increased G1 or G2/M arrest, and are apparently sensitised to mitotic block by the combination treatment. The mechanism behind this synergy is unclear, but points to the importance of one or more farnesylated proteins in controlling mitotic checkpoints. As described in section 2, the centromere-binding proteins CENP-E and -F are under investigation as potential mediators of these effects. Whether different sequencing or administration schedules could improve the activity of the various combinations must also be tested more rigorously.

4. Clinical Trials

The earliest phase I clinical trials using FTIs as single agents successfully identified dosages appropriate for the phase II and phase III trials that are now underway. Delivery by both oral and intravenous routes has been accomplished, and pharmacokinetic and pharmacodynamic studies have shown that it is possible to achieve plasma drug concentrations that are in the target range for inhibiting FTase activity using either of these meth-

ods. Preclinical testing suggested that FTIs were likely to be extremely nontoxic; surprisingly, the therapeutic index has been narrower than expected from preclinical models. Reversible myelosuppression, largely grade 3 neutropenia and thrombocytopenia, is the most commonly reported toxicity and is presumably mechanism-based.[41-43] Other toxicities reported have been unique to each structurally distinct FTI, for example diarrhoea with SCH-66336,[44] and therefore are likely not to be mechanism-based.

Although not the goal of Phase I studies, it is always hoped that some efficacy will be seen even at this stage. To date, few partial responses have been reported for trials using FTI as monotherapy, lending additional support to the possibility that FTIs will best be used in combination therapy with conventional chemotherapy, radiation and biological neoadjuvants. Some investigators have reported additive or synergistic effects with combination FTI and paclitaxel in preclinical models,[37,40] and partial responses have been reported in phase I trials using SCH-66336 in combination with both paclitaxel and gemcitabine in patients with pancreas cancer and non-small cell lung cancer.[45,46] A recent report of a phase II trial showing partial responses in patients with breast cancer treated with R-115777 as a single agent,[47] as well as a phase I trial showing both complete and partial short term (3 months) responses to R-115777 in relapsed or refractory acute leukaemias,[48] suggests that tumours other than the initially targeted carcinomas such as pancreas and colon may be more amenable to therapy with FTIs. R-115777 and SCH-66336 are also currently in a variety of nonblind phase II/III studies using FTIs as single agents.

Other FTIs undergoing clinical evaluation include those from Merck (L-778123),[49] Bristol-Myers Squibb (BMS-214662)[50] and Pfizer (CP-609754). A variety of phase I combination trials using FTIs with radiation, paclitaxel and other standard cytotoxics are also open, and additional trials are planned to determine the most effective use of FTIs.

Clinical issues that must still be addressed in analysing results of trials already underway and in planning new trials include different administration, scheduling and sequencing parameters that are only now beginning to be compared. Particularly important are the comparisons between continuous and intermittent administration; original suspicions that continuous administration would be necessary have now given way to the expectation that intermittent administration is preferable. It is possible that such differences may account for whether a given schedule provides additive effects or synergy, and these must be taken into account when planning new trials. In addition, whether FTIs are cytotoxic or cytostatic may have profound implications for study design, as the appropriate end-points would differ substantially.

Finally, issues of patient selection may be more important than originally envisioned for demonstration of FTI responsiveness. Many trials were originally planned based on the idea that FTIs would be anti-Ras drugs, which is now known not to be the case. Thus, there may have been an unintended bias against potentially sensitive tumour types. Until the most critical downstream target(s) are identified, this may continue to be a problem for FTI development.

4.1 Surrogate Markers of Efficacy

Another conundrum is the identification of optimal surrogate markers for monitoring efficacy of FTase inhibition.[51] Directly monitoring FTase levels in peripheral blood mononuclear cells (PBMC) or biopsy material has been attempted by some.[48,50] Such studies are necessary to determine what levels of inhibition of FTase are required for FTIs to be effective. However, in the absence of simple and reliable assays that can be widely used for monitoring FTase activity directly in patient samples, currently the emphasis is to analyse inhibition of processing of farnesylated proteins such as H-Ras or N-Ras, hDNAJ2 or HSDJ, or prelaminA or lamin B in PBMC or in buccal mucosa cells.

5. Potential Role in Cancer Prevention

Dietary isoprenoids, as well as the isoprenoid-related monoterpenes *d*-limonene and its active metabolite perillyl alcohol, have shown efficacy in preclinical chemopreventive studies[52,53] and are in clinical trials for prevention of mammary malignancies. The terpenes have also shown activity as chemotherapeutic agents in the settings of both breast and pancreatic carcinoma.[54] They cause tumour regression, increase apoptosis, and preferentially induce pro-apoptotic proteins such as Bak in pancreatic adenocarcinoma cells rather than in non-malignant pancreatic ductal cells.[55] However, the antitumour effect of these monoterpenes is probably a result of interfering with GGTase rather than FTase, so they also block prenylation of cell-growth regulating proteins that are likely to include not only Ras but most of the Rho family as well. How well such studies can be used to predict FTI activity is unknown.

The possibility of using FTIs to prevent cancer has also been recently tested.[56] Early results showed that both the number and size of tumours were reduced in mouse models of skin, lung and colon cancer when BMS-214662 was administered during the tumour promotion phase.[56] Whether FTIs can find utility as chemopreventive agents remains to be seen. The possibility of developing resistance is real[57] and may affect this presumably long term use of FTIs.

6. Potential Role in Cancer Treatment

The potential role of FTIs as single agents in cancer treatment should become clearer in the relatively near term as results of phase II/III trials become available. However, more extensive use of FTIs in different contexts will be required to learn whether long term administration will be required, whether the development of resistance will be a problem, and what schedules for administration should be used.

Historically, when FTIs were viewed as likely anti-Ras agents, it was thought that pancreatic cancers would be the most likely tumour types to respond to FTIs because of their extremely high percentage (≈90%) of K-*ras* mutations. More recently, with the additional information that H-Ras processing is easily blocked whereas K-Ras becomes alternatively prenylated in the presence of FTIs, the pendulum of thought has swung again such that conventional wisdom now says that head and neck tumours (where any mutated Ras protein is likely to be H-Ras) may be the most susceptible to growth inhibition with FTIs. However, until the most important farnesylated target of FTase inhibition has been identified in a rational and mechanistic manner, it remains critical to study different types of tumours to learn empirically which tumours are susceptible and which are not.

Clearly preclinical studies with some pancreatic tumours and others where mutated K-*ras* is present have shown responses, so it is important that clinical trials currently underway continue to include all types of diseases. In addition, the transgenic mouse data have shown that FTIs cause different effects on cell cycle and apoptosis depending upon the context of the mutated *ras* gene. Thus, it will be important to investigate the context of genetic mutations of the treated tumours as thoroughly as practical, so that combinations may be assembled more rationally.

Investigators are awaiting the results of future clinical trials with cautious optimism. Because of the variety of trials planned or underway, it seems reasonable to expect that appropriate target populations and schedules may be identified within the next 2 or 3 years. To aid in the identification of appropriate target populations, it will be important to collect data not only on clinical response but also on degree of inhibition of FTase, as defined by surrogate markers, and on genetic mutations including not only Ras status but also *p53, p16* and other tumour suppressor genes. With the advent of gene array chips, it would also be desirable to bank tumour biopsies for future investigations, although this certainly will not be practical for most trials now supported. Meanwhile, it will be important to obtain as much information as possible regarding the efficacy of FTIs in combination with different

conventional and novel treatment modalities, to learn how best to use this promising new class of antitumour agent.

7. Other Uses

In addition to cancer prevention and treatment, there are other tantalising uses that may be envisioned for FTIs in the future. First, trypanosomes and other parasites such as the agents responsible for leishmaniasis and malaria rely on FTases that can be targeted by FTIs.[58,59] Thus, FTIs may find utility as single agents in anti-parasitic therapy. Secondly, FTIs can in some specific cases act as anti-inflammatory agents by inhibiting macrophage motility. In particular, topical application of FTI in a mouse preclinical model has shown efficacy for healing corneal wounds.[60] Thirdly, a wide variety of plants have been shown to possess specific FTases,[61-63] and their FTase activity is important for characteristics as diverse as cell cycle progression and drought resistance.[64] It is possible that FTIs will find agricultural utility and, because of the extensive degree of conservation of FTase enzymes in evolution, their study will provide new insights into the mechanism of FTI action in mammalian cells.

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