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Prenyltransferase Inhibitors: Treating Human Ailments from Cancer to Parasitic Infections

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Abstract

The posttranslational modification of protein prenylation is a covalent lipid modification on the C-terminus of substrate proteins that serves to enhance membrane affinity. Oncogenic proteins such as Ras have this modification and significant effort has been placed into developing inhibitors of the prenyltransferase enzymes for clinical therapy. In addition to cancer therapy, prenyltransferase inhibitors have begun to find important therapeutic uses in other diseases, including progeria, hepatitis C and D, parasitic infections, and other maladies. This review will trace the evolution of prenyltransferase inhibitors from their initial use as cancer therapeutics to their expanded applications for other diseases.

1. Introduction

The biochemical mechanism of protein prenylation has been extensively studied since the initial discovery of this post-translational modification on the mammalian protein lamin B in 1989.¹ Following the initial reports of protein farnesylation (15 carbon isoprenoid modification), proteins modified with a geranylgeranyl group (20 carbon isoprenoid modification) were discovered in mammalian cells shortly thereafter (Figure 1).² Together, the post-translational modifications of farnesylation and geranylgeranylation are referred to as prenylation. The specific process of protein prenylation encompasses three distinct enzymes: farnesyltransferase (FTase), geranylgeranyltransferase type I (GGTase I), and Rab geranylgeranyltransferase (Rab GGTase or GGTase II). Prenylation using FTase and GGTase I involves the addition of either a C₁₅ (farnesyl) or C₂₀ (geranylgeranyl) isoprenoid moiety, respectively, onto a C-terminal cysteine residue of a protein that bears a CA₁A₂X (herein referred to as CAAX) consensus motif at its C-terminus (Figure 1), where ‘C’ represents cysteine, ‘A₁’ and ‘A₂’ represent aliphatic amino acids, and ‘X’ directs whether the protein will be farnesylated or geranylgeranylated. ‘X’ residues of cysteine, methionine, alanine, serine, or glutamine target farnesylation while leucine, isoleucine, and phenylalanine target the protein to be geranylgeranylated, although there are many exceptions to this rule.^{3–5} For instance, the RhoB protein, with a CKVL CAAX box, is found in both farnesylated (30% of total RhoB) and geranylgeranylated (70% of total RhoB) forms in mammalian cells.⁶ Additionally, it has been shown that while the ‘A₁’ CAAX position can be virtually any amino acid, the ‘A₂’ residue plays a significant role in determining the type of prenylation.^{7–9}

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Another type of prenylation exists that is specifically present on Rab proteins, which are responsible for membrane transport and fusion in the cell.¹⁰ While substrate proteins for FTase and GGTase I have well defined consensus sequences, prenylation by the enzyme Rab geranylgeranyltransferase (RabGGTase or GGTase II) has a less distinct consensus sequence. RabGGTase specifically di-geranylgeranylates Rab proteins that bear two cysteine residues at their C-terminus, with the following possible motifs: CC, CXC, CCX, CCXX, or CCXXX; additionally, some Rab proteins can be mono-geranylgeranylated by this same enzyme (with a C-terminus of CXXX).¹¹ Further differentiating this process from prenylation by FTase and GGTase I, Rab geranylgeranylation requires the Rab Escort Protein (REP) for prenylation. The REP binds to Rab proteins and facilitates their formation of a ternary complex with RabGGTase so prenylation can occur (see section 2.1 and Figure 3).¹²

The three prenyltransferase enzymes are all heterodimers, and while FTase and GGTase I share an identical α -subunit, they are only 25% sequence identical in the β -subunit.¹³ In contrast, the RabGGTase α -subunit is only 27% identical to FTase, while the β -subunit is 29% identical, despite all three enzymes sharing nearly identical topology (Figure 2).¹⁴

Following the prenylation step, further protein processing is required for newly prenylated proteins. First, the three C-terminal 'AAX' residues are cleaved by the proteases Ras-converting enzyme 1 (Rce1) or Ste24p, two functionally related enzymes that differ in primary sequence but that perform the same function.¹⁵ Second, the newly exposed C-terminal carboxylic acid is methylated by isoprenylcysteine carboxymethyl transferase (ICMT, Figure 1). Using an artificial membrane *in vitro* assay, Ghomashchi and coworkers showed that the K-Ras4B peptide has a 70-fold higher affinity for the membrane upon farnesylation, and further proteolysis and C-terminal methylation leads to an additional 150-fold increase in membrane affinity.¹⁶ Thus, it appears that the main purpose for this modification is to ensure membrane association of many proteins, but prenylation has also been shown to mediate important protein-protein interactions.¹⁷

Approximately 2% of mammalian proteins, an estimated 150 different proteins, receive the prenylation modification.^{18,19} Extensive interest in protein prenylation was spurred by the finding that the potentially oncogenic Ras family of proteins were prenylated²⁰ and that in order to maintain malignant, transforming activity, Ras requires prenylation.²¹ Initial therapeutic intervention focused on inhibiting the prenylation of Ras to stop malignant cell activity by utilizing farnesyltransferase inhibitors (FTIs). The success of these compounds in clinical trials has been rather disappointing; however, some patients respond to FTIs while others do not.⁶ In addition, prenyltransferase inhibitors may be used as potential therapeutics for several other human afflictions. This review serves to trace the evolution of FTI development from the initial studies as cancer therapeutics through the more recent use of prenyltransferase inhibitors in various human disorders, such as progeria, parasitic infections, hepatitis C and D, and other maladies.

2. Prenyltransferase Inhibitor Development

The first crystal structure of mammalian PFTase was solved in 1997, and this opened the door to structure-activity relationship (SAR) studies to develop more potent enzyme inhibitors.²² Considerable effort has been placed in understanding the biochemistry and enzymology of the prenyltransferases so that efficient inhibition of them may lead to new therapeutics. Studying the mechanism of the prenyltransferase reactions, as well as exact placement of substituents in the active site, has guided the development of very potent FTIs. This section will follow the evolution of prenyltransferase inhibitors and summarize the current status of the field.

2.1 Biochemistry of Protein Prenylation

FTase and GGTase I use the same mechanism to prenylate their respective substrates (Figure 3). The prenylation reaction begins when the prenyltransferase enzyme binds the isoprenoid substrate, either the C₁₅ farnesyl or the C₂₀ geranylgeranyl, and forms a binary enzyme-isoprenoid diphosphate complex. Following binary complex formation, the CAAX protein substrate binds adjacent to the isoprenoid molecule in the enzyme active site. As the CAAX protein binds, the cysteine residue coordinates to a Zn²⁺ ion (as a thiolate) in the active site; the Zn²⁺ is necessary for catalysis.^{23,24} This ternary complex is short lived, as the cysteine thiolate reacts at the C-1 position of the isoprenoid, forming a stable, covalent, thioether linkage to the isoprenoid. Upon binding of an additional prenyl diphosphate molecule, the isoprenoid portion of the newly formed prenylated protein translocates into an exit groove in the enzyme, and subsequently the prenylated protein product is released from the enzyme; this last step (product release) is the rate-limiting step for the protein prenylation reaction. In addition, FTase requires millimolar levels of Mg²⁺ for efficient catalysis to occur, as this ion stabilizes the diphosphate leaving group of FPP during the reaction. In GGTase I, however, a positively charged lysine residue near the diphosphate stabilizes it and removes the requirement of exogenous Mg²⁺.

In terms of the chemical mechanism for the enzymatic reaction, both FTase and GGTase I catalyze C-S bond formation with inversion of configuration at C-1 of the isoprenoid.^{25,26} Fluorine substituted FPP analogues manifest an attenuated rate suggesting a transition state with some cationic character.^{27,28} Kinetic isotope effect measurements are also consistent with partial C-O bond cleavage as well as partial C-S bond formation in the transition state.^{29,30} QM/MM calculations indicate that no stable carbocationic intermediate occurs in the reaction pathway.³¹ Although not observed crystallographically, single turnover experiments and calculations suggest that a conformational change in the enzyme and/or substrate(s) occurs prior to C-S bond formation.^{32,33}

The RabGGTase reaction proceeds with some similarities and some key differences (Figure 3). The major difference is that RabGGTase only recognizes a Rab substrate protein when it is bound to a Rab escort protein (REP). Similar to FTase and GGTase I, Rab prenylation begins when RabGGTase is loaded with a molecule of GGPP. The next step involves a portion of the REP, the Rab-binding platform (RBP), recognizing a Rab protein substrate and binding to it. This binding is enhanced when the C-terminal binding region (CBR) of the REP interacts with the CBR interacting motif (CIM) on the Rab protein substrate, enhancing

the affinity of the REP for the Rab protein substrate (further information on the CBR/CIM interaction can be found in section 2.2). The binary complex then binds with the RabGGTase and GGPP binary complex to form a quaternary complex of REP/Rab/RabGGTase/GGPP, in which the C-terminal cysteine of the Rab substrate binds to the active site Zn^{2+} of RabGGTase as a thiolate. Catalysis then occurs, with the thiolate of the Rab substrate reacting with C-1 of the GGPP isoprenoid to form a covalent thioether linkage. Next, an additional molecule of GGPP binds to the active site of RabGGTase by displacing the previous isoprenoid and the second round of prenylation occurs. Substrate release occurs analogously to FTase and GGTase I, in which the binding of an additional GGPP molecule forces displacement of the REP/di-prenylated Rab complex from RabGGTase (this is also the rate limiting step in Rab geranylgeranylation).¹² The REP/di-prenylated Rab complex is then trafficked to its cognate target membrane by a yet unknown mechanism. The REP protein is not only responsible for Rab protein binding and escort, but also for solubilization of the di-prenylated Rab product until its delivery to a membrane.³⁴ Since Rab proteins lack the C-terminal AAX motif, they are not subject to proteolysis and methylation the way FTase and GGTase I substrates are.

All three prenyltransferase enzymes manifest nanomolar affinities for their respective isoprenoid substrates. Additionally, the rate-limiting step in the prenylation reaction is the release of product, which relies on the binding of an additional isoprenoid molecule. These two factors combine to give the prenyltransferases relatively slow turnover rates of approximately 3 min^{-1} , depending on the substrate.¹⁹ The rates of C-S bond formation catalyzed by the three enzymes (obtained from pre-steady state measurements) span an order of magnitude, with FTase reacting the fastest, followed by GGTase I and lastly RabGGTase (k_{chem} values are $12\text{--}17 \text{ s}^{-1}$, 0.5 s^{-1} , and 0.16 s^{-1} for the three enzymes, respectively).³⁵ Furthermore, RabGGTase catalyzes its first geranylgeranylation four times faster than the second ($k_1 = 0.16 \text{ s}^{-1}$ and $k_2 = 0.04 \text{ s}^{-1}$).³⁵ This difference in rates is likely due to steric hindrance between the first prenylated cysteine residue and the next, as the two C-terminal cysteine residues are generally adjacent to one another.

2.2 Substrate Specificity

The specificity of the FTase and GGTase I catalyzed reactions arises mainly from the CAAX motif on the substrate protein, although analogs of FPP can alter the substrate specificity of the FTase enzyme.³⁶ Recently, methods for preparing C-terminal peptide libraries have been developed that have considerable potential for future experiments aimed at defining the global determinants of protein sequence specificity.³⁷ Additionally, structural differences in FTase and GGTase I account for the ability of the enzymes to discriminate between FPP or GGPP. In the isoprenoid binding pocket of FTase in the β -subunit, there are two residues that limit the length of isoprenoid that can bind: a Trp102 at the end of the binding cavity and a Tyr365 that prevents anything longer than a farnesyl group from rotating to fit (Figure 4A). In GGTase I, these same positions are occupied by less bulky residues, Thr49 and Phe324, respectively. These smaller residues, especially the Thr49, allow the longer GGPP substrate to fit into the active site. This simple ‘molecular ruler’ allows GGTase I to bind its longer, cognate isoprenoid. In contrast, FTase uses a different mechanism to control isoprenoid selectivity. While FTase binds GGPP with high affinity,

the longer isoprenoid bulges out into the protein binding site due to the limited depth of the binding pocket (Figure 4B). The resulting binary complex cannot bind a protein substrate and is hence catalytically inactive.³⁸

Much less is known about the precise determinants of substrate specificity in the Rab GGTase reaction. In contrast to FTase and GGTase, RabGGTase does not act on simple peptide substrates. The first crystal structure of RabGGTase in complex with the REB wasn't solved until 2003.³⁹ This has hampered detailed mechanistic studies of the RabGGTase reaction and how this enzyme recognizes its substrates. Recently, Guo and coworkers solved RabGGTase structures complexed with substrates and products which enabled a more complete picture to be revealed.³⁵ RabGGTase has the broadest lipid substrate specificity among all prenyltransferases (i.e. it is the most promiscuous of the prenyltransferases).⁴⁰ Even though the CBR of REP is a participating protein not involved directly in catalysis, it has been proposed that the interactions between the CIM of the Rab protein and the CBR of the REP are responsible for the substrate specificity of RabGGTase, analogous to the 'AAX' residues in FTase and GGTase I.³⁵ The REP controls the specificity of RabGGTase by selecting and binding to Rab proteins and facilitating their prenylation. The RabGGTase enzyme itself is highly promiscuous and operates stochastically, geranylgeranylating the protein substrate whenever a cysteine happens to be in its active site.³⁵

2.3 Types of Prenyltransferase Inhibitors

Synthetic inhibitors of FTase and GGTase I fall into one of three categories: isoprenoid analogs, peptidomimetics (also known as CAAX competitive inhibitors), and bi-substrate inhibitors combining both the isoprenoid analog and peptidomimetic portions (Figure 5). Specific inhibitors of RabGGTase include those that are phosphonocarboxylates as well as peptidomimetics (Figure 5). Highly potent natural product-derived inhibitors of these enzymes have also been identified.⁴¹

Isoprenoid analog inhibitors of all three prenyltransferases have been the least developed and studied, because of three important reasons:⁴² 1) The affinity of the isoprenoids for the prenyltransferases makes it difficult to design inhibitors that can be competitive with the native substrate (for example, the K_D of FPP for FTase is 2 nM⁴²). 2) The diphosphate moiety is negatively charged and this may affect the uptake properties of the molecule versus a prodrug or uncharged molecule.⁴³ While biosynthetic intermediates in the isoprenoid pathway (present as diphosphates) and anionic inhibitors clearly enter the cell, the precise mechanism by which this occurs remains unclear.⁴⁴ 3) Several additional enzymes (e.g. squalene synthase, undecaprenyl pyrophosphate synthase, heme O synthase, etc.) utilize isoprenoids as substrates and an isoprenoid analog inhibitor may have significant off-target effects. Bisubstrate inhibitors that contain both a CAAX competitive portion and an isoprenoid analog portion have also not been well developed, as complications in synthesis and water solubility have hampered their progress. The next section will focus on those inhibitors that are peptidomimetic as they have been the most studied and refined of all the classes of prenyltransferase inhibitors.

2.4 Evolution of FTIs

Peptidomimetic FTIs have been developed by three strategies: “reengineering” of substrate peptides,⁴⁸ use of rigid nonpeptidic scaffolds that mimic peptide secondary structural elements,^{49,50} and large scale screening of compound libraries.⁵¹ The initial development of peptidomimetic FTIs began with the native K-Ras CAAX box CVIM peptide by mutating the A₂ position to a phenylalanine (CVFM), giving an inhibitor with a 25 nM IC₅₀ (Figure 6).⁵² Placing bulky substituents in the A₂ position displaces the peptide in the binding pocket resulting in a catalytically inactive conformation and enzyme inhibition. Other inhibitors of this type have been able to achieve low nanomolar inhibition of FTase (see L-739,750, Figure 6),⁵³ but the peptide based scaffold made them less than ideal for *in vivo* use due to the possibility of proteolytic degradation. The next generation of FTIs generally contained fewer amide bonds in the backbone to circumvent this problem. For example, FTI-276 (Figure 6) was prepared from a related scaffold and binds in a similar manner to the FTase active site as L-739,750, achieving a 0.5 nM IC₅₀ (with respect to FTase and 50 nM with respect to GGTase I) without potential degradation issues.⁵⁴ Interestingly, changing the C-terminal methionine of FTI-276 to a leucine changes the specificity of inhibition (25 nM for FTase and 5 nM for GGTase I, Figure 6).⁵⁵

It was presumed in the early development of FTIs that a thiol moiety was necessary in the inhibitor structure to preserve the zinc coordination. Thiols can be oxidized to sulfoxides and sulfones, and the search for thiol replacements for zinc coordination led to the development of FTIs containing phenols, carboxylic acids, and nitrogen heterocycle derivatives. The most potent of these are the pyridine⁵⁶ and imidazole⁵⁷ based inhibitors (see Figure 7) with low nanomolar IC₅₀ values. Placing the zinc-coordinating imidazole in a cyclic structure led to one of the most potent FTIs developed with an IC₅₀ of 20 pM (Figure 7).⁵⁸

The last evolutionary step for FTIs occurred by removing the zinc-coordination on the peptidomimetic inhibitor. A class of inhibitors from Schering-Plough, originally based on an antihistamine scaffold, exemplifies the effects of removing the zinc-coordination (Figure 8). SCH 66366 (lonafarnib) lacks any zinc-coordinating moiety but still has an impressive IC₅₀ of 1.9 nM.⁵⁹ When a similar scaffold was modified slightly to contain an imidazole zinc-coordinating moiety, the IC₅₀ improved nearly 50-fold to 0.04 nM (SCH 211618).⁶⁰

Tuning the FTI to contain various structural elements has resulted in the creation of hundreds of FTIs with low nanomolar IC₅₀ values. Several of the best FTIs that combine potency, oral-bioavailability, and efficacy in animal models have entered clinical trials for cancer treatment (see Section 3). Additionally, an important goal in the development of FTIs has been to provide specificity for FTase over GGTase I, as they share considerable similarity, especially in the active site. Section 2.5 will discuss inhibitors that have been developed specifically to target GGTase I while having minimal impact on FTase.

2.5 Inhibitors for GGTase I

Providing selective GGTase I inhibitors was initially as simple as modifying the peptide backbone residues to contain larger, more hydrophobic elements to better fit into the

GGTase I active site and exclude them from FTase (see Figure 6). This strategy only provided approximately 5-fold selectivity for GGTase I over FTase. Structure-activity design efforts to introduce non-oxidizable zinc-coordinating groups, such as an imidazole, into the scaffold from GGTI-287 has led to the development of an inhibitor with greater than 250-fold selectivity for GGTase I over FTase (GGTI-2154, Figure 9).⁶¹ Additional tuning and optimization of this C-terminal leucine scaffold led to a dramatic selectivity increase of over 5,000-fold for GGTase I over FTase (GGTI-2418, Figure 9).⁶² Clearly, the use of structure-activity relationships has been successful for the development of selective inhibitors of GGTase I; furthermore, the use of library screening has proven immensely beneficial. The inhibitor P5-H6 was developed after screening a 171 compound library for inhibitors of GGTase I (Figure 9).⁶³ This compound exhibits an approximate 100-fold selectivity for GGTase I over FTase. Many potent and selective inhibitors for GGTase I have been developed, however, only one inhibitor, GGTI-2418 (Figure 9), has advanced to clinical trials (see Section 3).

2.6 RabGGTase inhibitor development

The design of inhibitors of RabGGTase has progressed significantly more slowly, as the main effort has been in pursuing FTIs for cancer therapy based on the importance of Ras proteins in malignancy. However, recently, RabGGTase has been shown to be a valid cancer target as many Rab proteins and RabGGTase itself, are overexpressed in several cancer types, such as ovarian,⁶⁴ breast,⁶⁴ hepatocellular carcinoma,⁶⁵ and thyroid adenomas.⁶⁶ The difficulty in the development of RabGGTase inhibitors has been in creating molecules that specifically inhibit only this enzyme, as there is considerable similarity with the other two prenyltransferases (Figure 2). Further, RabGGTase has the largest amount of lipid promiscuity of all three prenyltransferases and doesn't recognize a single, direct substrate, but rather a dimer of Rab and REB, complicating inhibitor development. Nonetheless, progress has been made to identify specific RabGGTase inhibitors with high potency, with the current generation achieving low nanomolar inhibition.

The first generation of RabGGTase inhibitors was based on the scaffold of risedronate, a bisphosphonate drug that is used to strengthen bone and treat osteoporosis through osteoclast inhibition, leading to increased bone mass.⁶⁷ Interestingly, the molecular target of risedronate is farnesyl diphosphate synthase (FPPS), which is responsible for the synthesis of farnesyl diphosphate in the cell.⁶⁸ Inhibition of FPPS directly decreases the available pool of FPP, in addition to GGPP, which prevents geranylgeranylation of several proteins and leads to osteoclast inhibition.⁶⁹ Inhibition of geranylgeranylated proteins in osteoclasts is crucial for the treatment of osteoporosis, and this drug specifically inhibits the isoprenoid pathway to prevent protein geranylgeranylation, while also inhibiting the RabGGTase enzyme, thus functioning via a dual mode of action.^{70,71}

Structure-activity relationship studies on risedronate led to the synthesis and evaluation of phosphonocarboxylate derivatives of the bisphosphonates with low micromolar inhibition of RabGGTase (Figure 10).⁷² The first such compound was 3-PEHPC (also known as NE10790), which was originally reported to have an *in vitro* IC₅₀ value of approximately 600 μ M;⁴⁶ however, in later assays with a modified buffer system and optimized

concentrations of RabGGTase, isoprenoid and protein substrates, and Rab escort protein, the newly reported *in vitro* IC₅₀ value of 3-PEHPC for RabGGTase was 24.1 μ M.⁷² It was believed that the α -hydroxyl in 3-PEHPC was important for bone mineral binding, so a series of halogen substitutions on the α -carbon was synthesized with the goal of improving binding and enhancing RabGGTase inhibition; this, however, only led to minor improvements (Figure 10).⁷² Substitution of the pyridine ring structure in 3-PEHPC with a imidazo[1,2-a]pyridine cyclic moiety led to the most potent inhibitor of RabGGTase in this compound class, with an IC₅₀ value of 1.3 μ M, nearly 25-fold better than the initial inhibitor structure.⁷³ It was recently discovered that this class of inhibitors inhibits only the second geranylgeranylation step of RabGGTase, not the first, and it was suggested that the phosphonocarboxylate inhibitors bind in a site adjacent to the active site, preventing the mono-geranylgeranylated protein from translocating to provide room for the second prenylation step to occur.⁷³ Some Rab proteins are only mono-geranylgeranylated and as a consequence are not inhibited by the phosphonocarboxylates; the development of specific inhibitors of all Rab protein geranylgeranylation has become a high priority.

In addition to the phosphonocarboxylate derivatives, several attempts to make specific peptidomimetic inhibitors for RabGGTase have been attempted, with recent generations performing exceptionally well. Bon and coworkers developed the most selective RabGGTase inhibitors to date by modifying the scaffold of the inhibitor BMS3 (Figure 11), which, at the time of their study, was the most potent RabGGTase inhibitor, but that was not selective as it also inhibited FTase.⁷⁴ Through structure-activity relationship guided design, their best molecule inhibited RabGGTase with an *in vitro* IC₅₀ of 42 nM (Figure 11) and displayed high selectivity, as it showed no inhibition of FTase or GGTase I at concentrations up to 9,700 nM and 99,500 nM, respectively; the compound also inhibited the proliferation of several cancer cell lines, demonstrating potency in cell-based assays.⁷⁴ To achieve this remarkable selectivity, the authors capitalized on a structural element present only in RabGGTase, called the TAG tunnel. This tunnel provides extra space in the active site for molecular diversity; placing a benzylcarbamate substituent in position A fills this TAG tunnel in RabGGTase, while excluding the ability of the molecule to bind FTase or GGTase I (Figure 11). Additional modification of the cyano group (B) with a furonitrile derivative provides hydrophobic interactions with the lipid substrate pocket further enhancing RabGGTase selectivity. Continued development of RabGGTase inhibitors in this manner should allow potent inhibitors to be developed with the potential to be used in a clinical setting.

3. Prenyltransferase Inhibitors in Cancer

Several inhibitors of FTase and one inhibitor of GGTase have entered clinical trials to test their efficacy in treating various types of cancer in patients. The following sections discuss why these compounds are being tested for cancer treatment and the current status of these inhibitors in clinical trials.

3.1 FTIs as cancer therapeutics

Initial interest in developing farnesyltransferase inhibitors (FTIs) was spurred by the finding that the oncogenic Ras protein is farnesylated²⁰ and that the farnesyl group is necessary for

membrane association and proper function of Ras.⁷⁵ Activating mutations in Ras, and specifically K-Ras, are involved in more than 30% of all human cancers and have been implicated in several tissue specific cancers with alarmingly high frequency; for example, greater than 90% of pancreatic cancers and 50% of colorectal cancers harbor K-Ras mutations.⁷⁶

The first report of an inhibitor of FTase was published in 1990⁷⁷ and since this time hundreds of different FTIs have been developed, with four progressing to clinical trials: tipifarnib, lonafarnib, BMS-214662 and L-778,123 (Figure 12).⁶ In the 75 different clinical trials using these four compounds alone or in combination with other anti-cancer drugs, a mere 2.3% of all patients treated had an objective response (either a partial or complete response) with monotherapy, while only 11.4% had a similar response with combination therapy.⁶ The results of these studies have clearly been a disappointment. The most probable explanation for these unanticipated results is likely due to the fact that the majority of clinical trials enrolled patients harboring mutations in the K-Ras oncogene. In the presence of FTIs, both the K- and N-Ras proteins can be alternatively prenylated by GGTase I to yield geranylgeranylated forms that retain their biological function.^{78–81} Patients that had been given an FTI to treat their tumors bearing K-Ras mutations likely suffered from this unfortunate biochemical event. Additionally, a majority of the patients enrolled in these clinical trials had advanced or metastatic disease, that may have been too advanced for effective treatment with an FTI. As an example, mice bearing H-Ras mammary tumors showed tumor regression upon daily FTI administration, but when the FTI was withdrawn the tumor reappeared; this may indicate that treating H-Ras bearing tumors early, prior to metastasis, may prove effective.⁸² It is likely that clinical trials utilizing more selective patient enrollments, especially with those patients harboring H-Ras mutations may have been more effective.

3.2 FTIs currently in clinical trials

Only two clinical trials remain active, both of which are using tipifarnib. The first is a Phase 2 trial to treat patients with relapsed or refractory lymphoma, while the second is a Phase 3 observational trial to determine whether tipifarnib or observation alone is better at preventing cancer recurrence in patients with acute myeloid leukemia (www.clinicaltrials.gov).⁸⁷ With the remaining two trials nearing completion, it is likely that clinical trials with FTIs will no longer be a priority (see section 3.4).

3.3 GGTase I inhibitor development for cancer

In early 2009, GGTI-2418 (Figure 9) entered Phase I clinical trials investigating tolerability, dosing, and toxicity; patients that had metastatic tumors for which the current therapies were unsuccessful or unavailable were selected for participation. Early results demonstrated that this compound was well-tolerated and had minimal toxicity.⁸⁸ However, the Phase I trial of GGTI-2418 has been stopped due to its lack of efficacy in patients (<http://kiraxcorp.com/ggti2418.html>, personal communication with Kirax Corp. representative). GGTI-2418 was the first GGTase I inhibitor to enter clinical development. Given the ability of FTase to circumvent GGTase inhibition and vice versa, monotherapy with these inhibitors remains a distinct challenge.

3.4 Future of prenyltransferase inhibitors in cancer

As discussed previously, inhibitors of both FTase and GGTase I have been ineffective in clinical trials for cancer treatment and have had disappointing results. To date, no inhibitors of RabGGTase have entered clinical trials, as this enzyme was only recently shown to be a potential cancer target (see section 2.6). One of the biggest problems in these clinical trials has been how patients have been selected (see section 3.1–3.2); however, an even more pressing issue is finding the exact molecular targets of prenyltransferase inhibitors. It has been shown that in most systems tested, from cells in culture to human patients, the ability of FTIs to inhibit tumor growth does not correlate with mutations in Ras.^{83,89,90} This information indicates that even though the Ras protein is oncogenically mutated, it is the inhibition of other prenylated proteins that is responsible for the anti-tumor activity of prenyltransferase inhibitors. Methods to detect the specific proteins that are affected by prenyltransferase inhibition will be required to develop more potent and specific inhibitors as well as to design clinical trials with patients that are likely to have the most success with prenyltransferase inhibitor treatment. Some strategies for addressing this goal have been developed, including various proteomic approaches and expression analysis (see references 40,91–97 for examples). More work needs to be conducted to identify specific proteins affected by prenyltransferase inhibition for more effective cancer therapeutics to be developed that will impact cancer therapy significantly.

4. Farnesyltransferase Inhibitors in Hutchinson-Gilford Progeria Syndrome

4.1 Hutchinson-Gilford Progeria

Hutchinson-Gilford progeria syndrome (HGPS, progeria) is a rare, autosomal dominant, fatal disorder characterized by early onset aging.⁹⁸ Approximately 1 in 4,000,000 children is stricken with this disease and display other phenotypes as a result, such as osteoporosis and alopecia. Those suffering from progeria have a mean survival time of 12.6 years.⁹⁹ This disease is the result of a single base pair mutation (C to T) at nucleotide 1824 in the gene (LMNA) coding for the nuclear lamin A protein.^{100,101} The lamin A protein is initially synthesized as a precursor protein, called prelamin A, that undergoes a series of modifications (Figure 13). First, prelamin A is farnesylated, followed by proteolysis of the 'AAX' residues and methylation of the C-terminus. Lastly, an internal cleavage by the enzyme ZMPSTE24 gives rise to non-farnesylated, mature lamin A.¹⁰² In HGPS, the single base pair mutation results in a cryptic mRNA splice site that results in an internal 50 amino acid deletion in prelamin A.¹⁰³ Because this form of prelamin A still contains the CAAX box, it is still farnesylated, proteolyzed (by –AAX removal), and methylated; however, the internal 50 amino acid deletion causes the loss of the ZMPSTE24 internal cleavage site resulting in a permanently farnesylated form of lamin A called progerin (Figure 13).¹⁰⁴ Progerin is targeted to the nuclear rim where it interferes with the nuclear lamina, causing mis-shaped nuclei and chromosomal disorganization.¹⁰⁵

4.2 Possible therapies for progeria using FTIs

The finding that the permanently farnesylated progerin was responsible for the phenotype of progeria led to considerable interest in the use of FTIs for treating this disease;¹⁰⁶ if farnesylation of prelamin A could be prevented, progerin would not form. Initial studies

testing this hypothesis in cell culture were successful; the nuclear blebbing phenotype of HGPS was lost when fibroblasts from patients were treated with an FTI.¹⁰⁷ Following these *in vitro* studies, a mouse model of HGPS was developed, and it was shown that treatment of these mice with an FTI improved progeria-like disease phenotypes, such as rib fractures and reduced bone density).^{106,108,109} However, the amelioration of these symptoms was not complete, and eventually all of the mice developed severe disease and died. Importantly, the amelioration of the progeria-like symptoms in these mouse models was directly due to blocking the farnesylation of progerin with an FTI.¹¹⁰

The prospect of using FTIs as a treatment for progeria in the clinic has been questioned recently due to the finding that progerin can be alternatively geranylgeranylated in the presence of an FTI.¹¹¹ Varela and coworkers used a combination of a statin, to deplete endogenous isoprenoids, and a bisphosphonate, to inhibit the farnesyl diphosphate synthase enzyme. This combination served to deplete farnesylated proteins, including lamin A.¹¹¹ These investigators showed that this combination improved progeria-like disease phenotypes in mice. Based on the current evidence, it appears that any therapy targeting protein farnesylation may improve the symptoms of progeria, but it is unclear whether it will completely ameliorate the disease in humans.

4.3 Clinical trials for the treatment of progeria with FTIs

Characterization of all HGPS phenotypes, especially those resulting in vascular problems, has been performed^{112,113} and clinical trials are underway to test the treatment of progeria with FTIs. The first was a phase 2 trial, started in 2007, that enrolled 26 children to receive the FTI lonafarnib.¹¹⁴ The trial ended in early 2010 and the results have recently been published.¹¹⁵ In this study, children with progeria were administered lonafarnib every 12 hours for up to two years. The therapy was well tolerated and no children were removed from the study because of toxicity.¹¹⁵ Exciting successful outcomes of the study included decreases in arterial pulse wave velocity and carotid artery echodensity and increases in skeletal rigidity and sensorineural hearing, one or more of which occurred in all children in the study.¹¹⁵ For instance, the carotid-femoral pulse wave velocity, PWV_{cf} , measures the arterial stiffness in an individual, which normally increases with age. Those suffering from HGPS typically have PWV_{cf} values equivalent to individuals aged 60–69 (3.5 times greater than normal pediatric values); in this study the PWV_{cf} decreased in 18 patients by a median of 35%.¹¹⁵ Furthermore, areal bone mineral density (aBMD) demonstrated a clinically significant 3% increase from pretherapy to end of therapy at one or more sites in 76% of children.¹¹⁵ Although therapy with lonafarnib is not a cure, these results suggest that it may be a suitable therapy to improve vascular stiffness and bone structure in patients with progeria. Finally, it should also be noted that while there is good evidence to support the notion that inhibition of lamin A farnesylation is physiologically relevant for the treatment of this disease, the authors of the above study noted that there was “a lack of correlation between the pharmacodynamics marker used (HDJ-2) and clinical response”. This observation highlights the need for improved methods for examining the effects of FTIs on the prenylation status of multiple proteins simultaneously as noted in section 3.4.

A second phase 2 clinical trial began in 2009; 45 patients with HGPS received a triple combination of zoledronic acid (a bisphosphonate), pravastatin (a statin), and lonafarnib (an FTI).¹¹⁶ The trial lasted two years and was scheduled for completion in February 2012, with the results of the trial now pending. Because lonafarnib showed earlier success as a single agent in clinical therapy,¹¹⁵ using combination therapy to ensure complete farnesylation inhibition is an optimistic approach that may prove beneficial. The results from this study as well as the previous study should provide valuable information to better develop treatments for Hutchinson-Gilford progeria syndrome.

5. Farnesyltransferase Inhibitors in Parasitic Diseases

Parasitic infections and their impact on human health are particularly important in the developing world. In some cases, no therapy is available while in others resistance to current modalities has developed. Chief among this latter group is malaria, which has seen widespread drug resistance, leading to increased mortality rates, especially in Africa.¹¹⁷ Several parasitic pathogens contain protein prenylating enzymes, including *Plasmodium falciparum* (malaria), *Trypanosoma brucei* (African sleeping sickness), *Trypanosoma cruzi* (Chagas disease),¹¹⁸ *Leishmania major* (leishmaniasis), *Toxoplasma gondii* (toxoplasmosis), *Giardia lamblia* (giardiasis), and *Entamoeba histolytica* (amebiasis).^{119–123} The growth of these parasites is severely impaired by the inhibition of protein farnesylation with FTIs,^{124–127} which suggests that developing inhibitors specific for the parasitic FTase over mammalian FTase may be a viable treatment option to remove the parasitic infection. The structures of the FTase enzyme between parasitic species and metazoans is quite similar,^{128–130} with subtle differences in the substrate specificity. Further, many of the FTIs developed thus far are more potent against cultured parasites than mammalian cells.¹³¹ The reasons for this species specificity are unknown, but it may be because the parasites lack GGTase I and thus the protein substrates cannot be alternatively prenylated as in mammalian cells.¹³¹

Because mammals, like parasites, contain FTase, significant side effects may occur when treating humans with FTIs designed to kill a parasitic infection. For this reason, it is important to develop selective, potent inhibitors of the parasitic FTase while not affecting the FTase in the host organism (humans). Significant progress has been made in this area, especially in studies of *Plasmodium falciparum* FTase (*Pf*FTase). Some initial studies have developed FTIs with approximately 10-fold to 25-fold selectivity for *Pf*FTase over its mammalian counterpart.^{132,133} Using structure-activity relationship information based on ethylenediamine analogue scaffolds, Fletcher *et al.* have developed FTIs that inhibit *Pf*FTase with an IC₅₀ of 1 nM that display 136-fold selectivity for *Pf*FTase over mammalian FTase.¹²⁸ Substantial effort has been focused on the development of specific inhibitors for *Pf*FTase due to the high incidence of drug-resistant malaria, although significant progress on other parasitic diseases has also been made.^{118,134,135} With continued development it may be a short matter of time before FTIs to fight parasitic infections appear in clinical trials.

6. Prenyltransferase Inhibitors in Hepatitis

Hepatitis C virus (HCV) is a persistent infection, often leading to chronic liver inflammation, that affects approximately 200 million people worldwide.¹³⁶ The HCV genome encodes a single polypeptide that is posttranslationally processed into at least 10 distinct viral proteins.¹³⁷ Addition of a GGTase I inhibitor to hepatoma cultured cells induces dissolution of the HCV replication complex.¹³⁸ Because the HCV genome does not encode a geranylgeranylated protein, it may be that a geranylgeranylated host protein is required for viral replication and suggests a GGTase I inhibitor may be a viable therapeutic strategy.¹³⁸

In addition, hepatitis D (caused by the hepatitis D virus, HDV) requires concomitant infection with the hepatitis B virus and is the most difficult to treat of the viral hepatitis.¹³⁹ The large antigen of HDV is farnesylated *in vitro* and in animal cells¹⁴⁰ and two different FTIs have proven effective at preventing the infection of HDV virions of two different genotypes, including the one that is associated with the most severe form of HDV (HDV genotype III virions).^{141,142} Both studies concluded that FTIs were a novel class of antivirals with therapeutic potential against these diseases.

7. Other Potential Therapies using Prenyltransferase Inhibitors

There are many other potential applications for the therapeutic use of prenyltransferase inhibitors and early evidence indicates that several diseases, such as multiple sclerosis¹⁴³ and osteoporosis,¹⁴⁴ could be treated with them. Additionally, fungal pathogens such as *Cryptococcus neoformans* utilize FTase to farnesylate key signal transduction proteins and inhibition of FTase has shown to be a promising treatment for invasive fungal infections.^{129,145} Prenyltransferase inhibitors may also be relevant in non-disease settings, for example in preventing restenosis after balloon angioplasty.¹⁴⁶

8. Future Outlook

The field of protein prenylation has been extensively studied for more than 20 years and inhibitors of the prenyltransferases have been in development for the same duration. Despite more than 75 clinical trials using farnesyltransferase inhibitors for cancer treatment, little success has been achieved. Indeed, the use of prenyltransferase inhibitors has been investigated in a number of diseases other than cancer and the results are either mixed or unknown at this time. The reasons for these unfortunate setbacks are largely unknown, and the key question stalling the field of prenyltransferase inhibitor development is this: what are the relevant downstream molecular targets of prenyltransferase inhibitors? While there is no doubt that these compounds inhibit prenyltransferases, it is unclear which prenylated proteins are germane to specific diseases. The beneficial effects from prenyltransferase inhibitor treatment *in vitro* or in animal disease models aren't necessarily from the prenylated protein that was originally targeted (such as Ras proteins). Further, a subset of patients do in fact respond to FTIs, which suggests that some human tumors depend on farnesylated proteins for survival.⁶ Additionally, it has yet to be shown conclusively that the beneficial effects of prenyltransferase inhibitor treatment are due to inhibiting prenylation on a specific protein. It is clear, however, in many instances, that these inhibitors ameliorate or

completely remove disease in several animal models. The key to better utilizing prenyltransferase inhibitors in a clinical setting is to identify the specific proteins responsible for the beneficial affects and link together the molecular mechanisms of the inhibitors with the clinical phenotypes observed. Once this information is known, more targeted approaches can be used to inhibit a specific protein(s) for more efficient treatment with minimal side effects.

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Figure 1.
Schematic representation of protein prenylation carried out by farnesyltransferase (C_{15} isoprenoid) or geranylgeranyltransferase type I (C_{20} isoprenoid).

Figure 2.

Alignment of the crystal structures of all three prenyltransferase enzymes. FTase: yellow, PDB 2BED. GGTase I: green, PDB 1N4P. RabGGTase: magenta, PDB 3C72. Structures were overlaid and aligned using the PyMOL program.

Prenylation by FTase (and GGTase I): **Prenylation by RabGGTase:** **Figure 3.**

Cartoon scheme of the mechanism of prenylation for all three prenyltransferase enzymes. FTase, farnesyltransferase; GGTase I, type 1 geranylgeranyltransferase; RabGGTase, Rab geranylgeranyltransferase (type II geranylgeranyltransferase); REP, Rab escort protein; CBR, c-terminal binding region; CIM, CBR interacting motif;

Figure 4.

A) Overlay of the crystal structures of FTase and GGTase I showing the active site residues that control isoprenoid binding in the ‘molecular ruler’ hypothesis. In FTase, the tryptophan at 102 prevents the longer GGPP substrate from binding in a catalytically competent manner, while in GGTase this position is occupied by a threonine, allowing GGPP to fit in the active site with higher affinity. The FTase (green residues, PDB entry 1KZO) and GGTase (magenta residues, PDB entry 1N4P) structures were aligned in PyMOL. B) Overlay of space-fill structure of FTase with bound GGPP (magenta) and bound FPP (green), demonstrating the bulging of the isoprenoid into the peptide binding pocket preventing catalysis. Structure made in PyMOL based on entry 1O1R (GGPP) and 2ZIS (FPP).

Structures of FTase and GGTase I Inhibitors:

Isoprenoid Analog:

α -hydroxyfarnesyl phosphonic acid

Peptidomimetic:

L-744,832

Bisubstrate Analog:

Peptidomimetic

Structures of RabGGTase Inhibitors:

Phosphonocarboxylate:

3-PEHPC

Peptidomimetic:

compound 4

Figure 5.

Select structures of the various classes of protein prenylation inhibitors. α -hydroxyfarnesyl phosphonic acid and L-744,832 are commercially available, Schl-872 was described by Schlitzer,⁴⁵ 3-PEHPC was synthesized by Coxon et al.,⁴⁶ and compound 4 was described by Guo et al.⁴⁷

Figure 6.

Structures of FTIs and a type I GGTI showing the specificity of inhibition by subtle changes in the compound structure. CVIM represents the natural K-Ras substrate peptide, and changing the isoleucine for phenylalanine gives an FTase inhibitor with a 25 nM IC_{50} .⁵² L-739,750 represents an analog with reduced amide bonds in the backbone to improve stability.⁵³ FTI-276 has a 10-fold selectivity for FTase inhibition over GGTase I⁵⁴ but subtly changing the C-terminal methionine to leucine changes the specificity to 5-fold for GGTase I over FTase.⁵⁵

Figure 7.

Structures of non-thiol FTIs that still allow for coordination to the active site zinc residue. Pyridine based inhibitors⁵⁶ and imidazole based inhibitors⁵⁷ have similar, low IC_{50} values as their thiol containing counterparts. A cyclic version of an imidazole based inhibitor has a remarkably low IC_{50} of 20pM.⁵⁸ Removing the thiol leads to stabilization from oxidation.

Figure 8.

The effects of zinc-coordinating moieties on FTIs. SCH 66336 (lonafarnib) does not contain a zinc-coordinating moiety but still has an impressive IC₅₀ value,⁵⁹ while a very close structural analog, SCH 211618, has a zinc-coordinating moiety and an approximately 50-fold lower IC₅₀.⁶⁰

Figure 9.

Inhibitors of GGTase I that are selective for GGTase over FTase. GGTI-2154 exhibits 250-fold selectivity,⁶¹ while GGTI-2418 is 5,000-fold more selective and is the only GGTI to have entered clinical trials.⁶² P5-H6 is an example of a selective GGTI that arose from high throughput screening.⁶³

Figure 10.

Structures and inhibition values of phosphonocarboxylate derivatives of risedronate as inhibitors of RabGGTase. Risedronate, 3-PEHPC, and Cl-3-PEHPC have been described in Marma et al.⁷² while 3-IPEHPC was described in Baron et al.⁷³ Importantly, these compounds were found to only inhibit the second geranylgeranylation step by RabGGTase, and thus are ineffective for mono-geranylgeranylated proteins by RabGGTase.⁷³ Additionally, the (+)-enantiomer of 3-IPEHPC has been found to be more potent than the (–)-enantiomer; however, the more active absolute configuration for the remaining bisphosphonates has yet to be determined.⁷³

Figure 11.

Structures of RabGGTase inhibitors used by Bon et al.⁷⁴ Starting with the crystal structure of BMS3 bound to RabGGTase, the circled positions in BMS3 were mutated to larger moieties as RabGGTase has room to accommodate the larger substituents but FTase and GGTase I do not. Their best compound displayed remarkable selectivity for RabGGTase with a potent *in vitro* inhibitory value.

Tipifarnib

IC₅₀: FTase - 0.9 nM
GGTase I - >50 μM

Lonafarnib

IC₅₀: FTase - 1.9 nM
GGTase I - >50 μM

BMS-214662

IC₅₀: FTase - 1.35 nM
GGTase I - >1 μM

L-778,123

IC₅₀: FTase - 2 nM
GGTase I - 98 nM

Figure 12.

Structures of the four FTIs that have been studied in clinical trials for cancer: tipifarnib,⁸³ lonafarnib,⁸⁴ BMS-214662⁸⁵ and L-778,123.⁸⁶

Figure 13.

Post-translational processing of prelam A to form mature lamin A. In the left side of the figure the normal processing route from prelam A to lamin A is shown. In HGPS, a 50 amino acid deletion in prelam A results from a cryptic splice site in the prelam pre-mRNA (right side). The consequence of this deletion is the loss of the internal cleavage site for the ZMPSTE24 enzyme (depicted in red), resulting in permanently farnesylated lamin A, called progerin. Because mutant prelam A still contains the CAAX box, it is still farnesylated.