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From oncogene to drug: development of small molecule tyrosine kinase inhibitors as anti-tumor and anti-angiogenic agents

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The confluence of two distinct but related activities in the past 10 years has dramatically accelerated efforts towards the discovery and development of novel drugs to treat cancer. The first is a rapidly emerging understanding that a number of distinct tyrosine kinases play roles in diverse but fundamentally important aspects of tumor progression (growth, survival, metastasis and angiogenesis). The second is the discovery that small molecule compounds have the capacity to potently and selectively inhibit the biochemical function of tyrosine kinases by competing for ATP binding at the enzyme catalytic site. These observations have been conjoined in major efforts to bring forward into clinical development novel cancer drugs with the potential to provide both clinical efficacy and improved tolerability. The focus of this review is on the development of small molecule tyrosine kinase inhibitors, and does not extend to other approaches that could be applied to disrupt the same pathways in clinical tumors (receptor and/or ligand-competitive antibodies, intrabodies, antisense ribonucleotides, ribozymes, phosphatase inhibitors or SH2/SH3-directed agents). Selected tyrosine kinase inhibitors, known or believed to be in development in cancer treatment trials, are summarized as are some of the key issues that must be addressed if these compounds are to be developed into clinically useful cancer chemotherapeutic agents. *Oncogene* (2000) **19,** 6574–6583.

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Origin of species – brief overview of substrate-based inhibitors of protein tyrosine kinases

Among all non-traditional (non-DNA-directed) cancer targets for which pharmacological intervention is feasible, there are none that have generated as much widespread interest, and have invoked as much resource investment in both the public and private sectors in the past 7 years, as have the tyrosine kinases. Several excellent recent reviews have described the functions of various tyrosine kinases in the key pathways that drive tumor progression, from first genetic insult to disseminated disease (Hanahan and Weinberg, 2000; Hunter, 2000; Gibbs, 2000). Key among these are the receptor tyrosine kinases which initiate signal transduction in tumor cells or endothelial cells following the binding of the growth factors EGF, PDGF and VEGF. There are also several excellent reviews that provide detailed overviews of the work accomplished to date to understand the molecular pharmacology of small molecule inhibitors of receptor tyrosine kinases (Sedlacek, 2000; Fry, 2000; Bridges, 1999; Levitzki, 1999; Lawrence and Niu, 1998). Without summarizing each of these important reviews, they provide an appropriate context for understanding the obstacles and triumphs that have led, very recently, to the first reproducible, objective clinical responses in cancer patients treated with tyrosine kinase inhibitors.

The catalytic function of protein tyrosine kinases involves the simple transfer of the gamma phosphate of ATP to hydroxyl group of a tyrosine residue of proteins (or peptides) encompassing a diversity of primary sequences and tertiary structures (Songyang and Cantley, 1998). Each of the substrates in the phosphotransfer reaction, the tyrosine hydroxy group and ATP, represent reasonable pharmacological starting points for the design of substrate analogs and competitive inhibitors of tyrosine kinases. A diverse set of pharmacophores, including natural products (lavendustins and erbstatins) and synthetic tyrosine mimetics, have all been characterized on the basis of their ability to competitively inhibit tyrosine kinase function (Levitzki, 1999). These compounds tended to have poor potency (particularly in cells), to yield relatively flat structure-activity relationships, and to be somewhat non-specific in their kinase inhibition (Fry, 2000). Attacking this reaction from the other side, by identifying compounds that mimic ATP, was originally thought to be even less tractable. As reviewed by Lawrence and Niu (1998), the theoretical obstacles were immense. First, the primary sequence of the ATP-binding pocket of all kinases is highly conserved, and therefore selectivity, if not specificity, represents a significant technical challenge. Secondly, the intracellular concentration of ATP can exceed 5 mM, particularly in tumor cells, while the K_m for ATP in most kinase active sites is in the micromolar range, thus ensuring full-time saturation by ATP. ATP-competitive inhibitors would need to exhibit at least nanomolar inhibitory kinetic constants to effectively compete in this circumstance (Lawrence and Niu, 1998). Finally, there are multiple non-kinase ATP-dependent enzymes important to normal physiology, and so an indiscriminant ATP mimetic would likely have toxicities that were pharmacologically and medically unacceptable.

This theoretical logjam was broken in convincing fashion when the tyrosine kinase inhibitory activities of anilinoquinazolines were first described in 1994 by three separate groups (Fry *et al*., 1994; Ward *et al*., 1994; Osherov and Levitzki, 1994). For example, the work of Fry *et al*. (1994) at Warner Lambert revealed

of the EGFR tyrosine kinase with good cell activity and profound biochemical selectivity relative to other kinases within the tyrosine kinase family. Further elaboration of structure-activity relationships rich in new possibilities resulted in ATP-competitive inhibitors of the EGFR tyrosine kinase with K_i values in the single digit picomolar range. It is interesting to note that the Michaelis-Menten equation could not be used to derive the K_i values of these molecules. So avid was the binding of compound to the ATP site, the conventional approximation that total and free enzyme concentrations were equivalent did not apply under these conditions. These accomplishments, which may be among the most important in pharmacology for the last 10 years, were largely achieved by empirical screening and iterative medicinal chemistry. Even more new chemotypes may emerge as structure-based design becomes more commonly applied to the identification of both active site- and allosteric site-directed inhibitors for an ever-widening slate of tyrosine kinase targets. While these early lead molecules had biopharmaceutical properties which were by-and-large incompatible with oral bioavailability and good duration of exposure *in vivo*, the results spurred on a number of groups, which have since identified and developed tyrosine kinase inhibitors with significant potential to treat clinical cancer.

Selected development candidates – updates

PDGFR inhibitors: STI 571 and SU101

STI 571 (CGP57148B) Among all of the candidates currently in clinical development, perhaps none has provided as much 'proof of concept' for the clinical efficacy and tolerability of small molecule tyrosine kinase inhibitors as has STI 571. Originally disclosed by Novartis as a multitrophic tyrosine kinase inhibitor, STI 571 was described by Druker *et al.* (1996); and Druker and Lydon (2000) as having potent activity *vs* the translocation product *bcr-abl*, the transforming tyrosine kinase found in virtually all CML cells expressing the Philadelphia chromosome (Kurzrock *et al.*, 1988; Kelliher *et al.*, 1990). The inhibition of v-*abl*, *bcr-abl* and PDGFR autophosphorylation by the 2-phenylaminopyrimidine STI 571 (Figure 1) at nanomolar concentrations was found to translate to both *in vivo* anti-tumor activity, and to the inhibition of clonogenicity of blasts from CML patients (le Coutre *et al.*, 1999; Druker *et al.*, 1996). The results of a clinical trial in which STI 571 was administered to CML and ALL patients expressing *bcr-abl* in their leukemic blasts were most recently summarized in May 2000 (Talpaz *et al.*, 2000). STI 571 was used to treat 33 acute leukemia patients, which included 21 myeloid blast crisis CML patients and 12 *bcr-abl*-positive ALL or lymphoid blast crisis CML patients. Clinical responses, as defined by a decrease in the percentage of patients achieving reduction in bone marrow blasts to 15% of pre-treatment levels, were observed in 55% of myeloid blast crisis patients, with complete responses in 22% of these patients. The response rates in patients with *bcr-abl* positive ALL and lymphoid blast crisis of CML were higher (82% with 55% complete responses), but all of the patients with lymphoid leukemias relapsed on drug between 45 and 81 days. Of 19 responding patients, 10 experienced Grade 3–4 neutropenia. This response rate, and the incidence of Grade 3–4 toxicity, compares very favorably to the standard of care cytotoxic chemotherapies for CML. As such, more definitive trials assessing the efficacy and safety of STI 571 are ongoing in CML.

It is interesting to speculate as to the biochemical basis for both the efficacy and the toleration profile of STI 571. Two other tyrosine kinases potently inhibited by STI 571, c-*kit* and PDGFR, are both believed to play important roles in maintaining bone marrow stroma–progenitor cell interactions (Ashman, 1999; Sungaran *et al.*, 2000). Thus, inhibition of c-*kit* and PDGFR could also account for some of the compelling clinical activity of STI 571 in CML, as well as for its toxicity profile (neutropenia). Treatment of a c-*kit* expressing a human myeloid leukemia cell line, M-07e, with STI 571 before stimulation with *kit* ligand inhibited c-*kit* autophosphorylation, activation of mitogen-activated protein (MAP) kinase, and activation of Akt, with an IC_{50} of 100 nM (Heinrich *et al.*, 2000). STI 571 was even more potent in a human mast cell leukemia cell line (HMC-1) expressing an activated mutant form of c-*kit*. Similar results have also recently been reported in non-hematopoietic tumor cells (Wang *et al.*, 2000). The efficacy and safety hypotheses for inhibition of c-*abl* in CML may perhaps only be addressed with a more selective abl tyrosine kinase inhibitor. Given the apparent therapeutic benefit of STI 571, this may be largely an academic question, but one with important implications as one tries to rationalize the desired selectivity profiles of tyrosine kinase inhibitors most likely to generate both efficacy and safety in humans.

SU101 (leflunomide; HWA 486) Leflunomide was originally described and developed as an inhibitor of dihydroorotate dehydrogenase, a key enzyme in the de novo synthesis of pyrimidines, for use as an immunosuppressive or anti-arthritic agent (Bartlett and Schleyerbach, 1985; Kuo *et al.*, 1996). Leflunomide has shown significant activity as a treatment for rheumatoid arthritis (Smolen and Emery, 2000; Cohen *et al.*, 2000b), and was launched by Aventis as Arava™ in the US and elsewhere beginning in 1998. Extending the work of others (Mattar *et al.*, 1993; Xu *et al.*, 1995), Shawver and co-workers reported that micromolar concentrations of leflunomide inhibited the autophosphorylation of the tyrosine kinase receptors for PDGF and VEGF (Shawver *et al.*, 1997). The compound was also effective at blocking mitogenesis stimulated by both PDGF and EGF, but exogenous uridine could not reverse the effect of leflunomide on PDGF mitogenesis, suggesting that inhibition of the receptor tyrosine kinase, and not inhibition of pyrimidine pools, was a key pharmacological activity. The inhibition of EGF-induced mitogenesis by leflunomide was reversed in part by uridine (Shawver *et al.*, 1997), despite the fact that leflunomide and close-in analogs also have inhibitory activity *vs* the EGFR tyrosine kinase (Ghosh *et al.*, 1999).

Leflunomide/SU101 is clearly a tyrosine kinase inhibitor with multiple biochemical effects, and readily generates a predominant active metabolite (SU0020 or A771726; Figure 1) that has a complex inhibitory profile of its own (Hamilton *et al.*, 1999). SU101 was

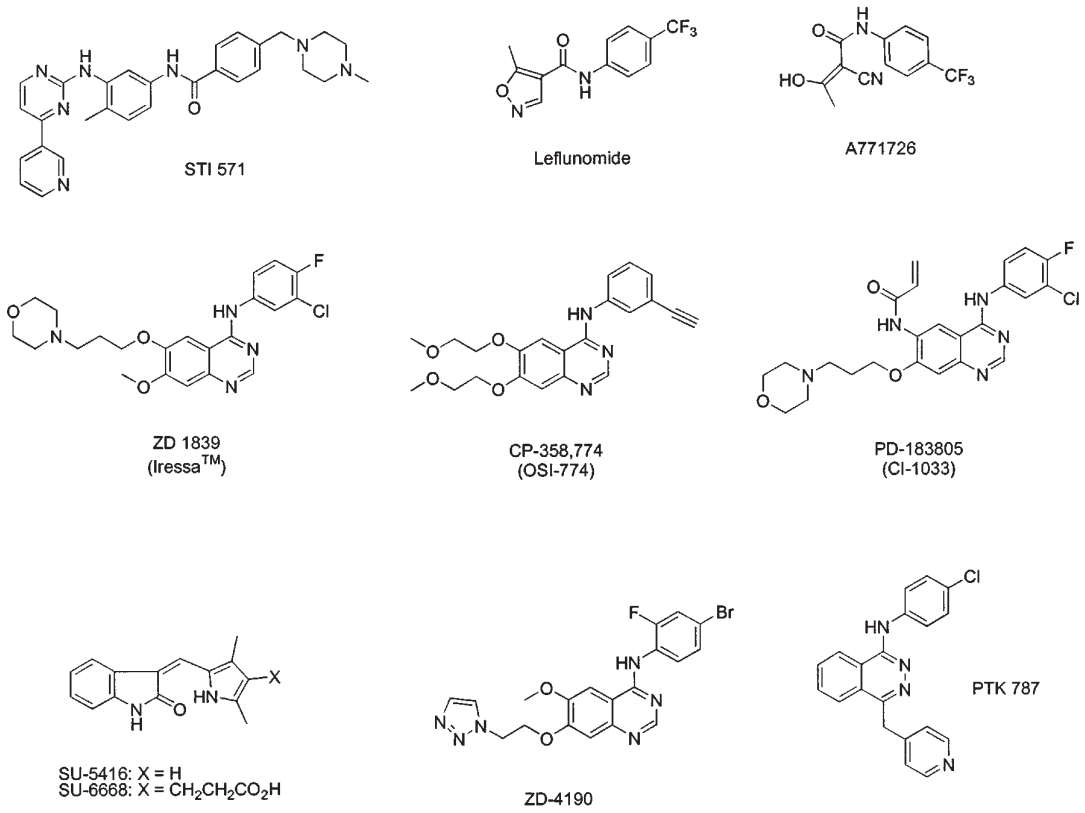

Figure 1 Structures of selected tyrosine kinase inhibitors in clinical development for cancer

nonetheless, progressed into clinical trials by SUGEN (now part of Pharmacia). A Phase I study in cancer patients revealed that SU 101 was well-tolerated as a 24 h continuous i.v. infusion at doses up to 443 mg/m^2/wk. At this dose, the plasma concentration of the active metabolite was maintained at levels sufficient to block both PDGFR and EGFR signaling, as well as pyrimidine biosynthesis (Eckhardt *et al*., 1999). Toxicities were relatively minor (Grade 1–2 nausea, vomiting and fever in approximately 20% of all courses given). Surprisingly, hematopoietic toxicities and hemolysis, which had been noted in the preclinical experience with SU 101, were not seen in this Phase I population. One partial response was seen in 26 patients receiving an average of two courses each; the responding patient received 13 courses (52 infusions) to treat an anaplastic astrocytoma, and had a notable (>50%) reduction in one measurable lesion (Eckhardt *et al*., 1999). SU 101 has been reported to be in advanced trials for multiple solid tumor types, but recent disclosures (Garber, 2000) indicate that Phase III trials in at least one tumor type (glioblastoma) have been abandoned. The status of other trials (ongoing Phase II trials for ovarian and NSCLC; planned Phase III trials for prostate, colon and NSCLC) is uncertain at the present time.

EGFR inhibitors: Iressa™ (ZD1839), OSI-774 (CP-358,774) and CI-1033 (PD183805)

Iressa™ (ZD1839) While STI 571 has provided notable clinical proof-of-concept for the clinical efficacy and safety of tyrosine kinase inhibitors, the early clinical findings with AstraZeneca's ZD1839 (Iressa™) have been equally compelling. The pharmacological characteristics of Iressa™ were first described in 1996 (Wakeling *et al*., 1996; Woodburn *et al*., 1997) as a potent and selective inhibitor of the EGFR tyrosine kinase. This quinazoline-based compound (Figure 1) is an ATP-competitive inhibitor of the EGFR tyrosine kinase (IC_{50} 25 nM) with 50-fold selectivity relative to closely homologous *erb*B family members (IC_{50} for *erb*B2 1–3 μM) and even greater selectivity for more divergent tyrosine kinases. It demonstrates good cellular potency (80 nM IC_{50} for inhibition of EGF-dependent mitogenesis) and robust, dose-dependent anti-tumor efficacy in a variety of human tumor xenografts (Woodburn *et al*., 1997). These results have been most recently extended to show that Iressa™ has *in vivo* efficacy in a diverse human tumor xenograft models both with (Ciardello *et al*., 2000) and without (Sirotnak *et al*., 2000) highly activated EGFR signaling pathways. Of equal interest are the observations that Iressa™ combines with standard cytotoxic agents (platinums, taxanes, topoisomerase I inhibitors, etc.) to produce additive or supra-additive anti-tumor efficacy *in vivo* without exacerbation of the toxicity of the co-administered cytotoxics. The findings that tumor EGFR density does not predict efficacy when the compound is used in conjunction with cytotoxic agents have significantly impacted the development strategy employed by AstraZeneca as Iressa™ moves towards pivotal clinical trials.

Multiple Phase I trials with Iressa™ have been summarized, and the results revealed reasonable pharmacokinetics, good toleration and the first signs

of clinical efficacy when used as a single agent in patients with advanced disease (Ferry *et al*., 2000; Baselga *et al*., 2000; Kelly *et al*., 2000). Following oral administration of a single dose (50 mg), maximum plasma drug concentrations (mean 45 ng/ml) occurred 1–5 h post-dose. The mean terminal $t_{1/2}$ was 34 h. Inter-subject variability in exposure was significant following single and multiple administration (up to sevenfold at each dose level), but exposure increased proportionally with dose, with no apparent change in terminal $t_{1/2}$ across the dose range tested (Kelly *et al*., 2000). In a larger dose-escalation trial, Ferry and collaborators administered Iressa™ at doses of 50–700 mg once daily, given orally for 14 days followed by 14 days of observation (Ferry *et al*., 2000). In total, 64 patients with advanced disease, who had each progressed while on prior chemotherapy, completed 145 cycles. C_{max} and $AUC_{0\text{-}24h}$ were proportional across the entire dose range (mean values 113–2255 ng/ml and 1.8–38.5 mg.h/ml, respectively). As in single dose studies, Iressa™ showed a long terminal elimination half-life (mean of 46 h). Iressa™ was very well-tolerated in this study; the most common adverse events were diarrhea and acne-like skin rash (Grade 1–2). Acne-like skin rashes have emerged as a common, mechanism-based adverse event for EGFR inhibitors, but the specific toxicological effect in the skin is not yet well understood. Grade 3–4 adverse events were shown to be rare with Iressa™ treatment, and were generally ascribed to disease progression. The dose-limiting toxicity, defined at the 700 mg dose level, was Grade 3 diarrhea (Ferry *et al*., 2000).

A compelling level of efficacy was also revealed in these early trials (Ferry *et al*., 2000). Anti-tumor responses were most evident among the 16 NSCLC patients treated with Iressa™–two had an objective partial response, two patients had significant regression of disease and two patients had stable disease. Similar pharmacokinetic and safety profiles were noted in a separate study (Baselga *et al*., 2000), one that also revealed the potential for efficacy from Iressa™ in patients with advanced prostatic and head-neck cancers. These early results added importantly to the proof-of-concept that selective tyrosine kinase inhibitors could have significant single agent efficacy, as measured by objective tumor regressions, in patients with advanced disease. The clinical observations have therefore recapitulated the pre-clinical data showing that Iressa™ increased apoptosis and regressions in human tumor xenograft models (Ciardello *et al*., 2000).

The Iressa™ data indicate that the efficacy of these agents can be measured using more classically defined clinical endpoints. There will undoubtedly be significant value in the use of pharmacodynamic and surrogate endpoints to guide dose-intensification or to pre-select patients for whom other tyrosine kinase inhibitors might represent the most promising treatment option. Pharmacodynamic endpoints have not played a major role in the early development of EGFR tyrosine kinase inhibitors, despite the fact that several reasonable options exist, including both invasive techniques (direct measurement of tumor-derived or normal tissue-derived EGFR phosphotyrosine, phosphorylation of down-stream signaling molecules; apoptosis markers) and non-invasive techniques such as PET imaging of metabolically modulated tumors

(Pollack *et al*., 1999; Goss *et al*., 2000; Allen *et al*., 2000). Given the overall safety and toleration profile of Iressa™, AstraZeneca has committed to an aggressive development strategy, which includes two large Phase III studies to assess the use of Iressa™ in combination with cis- or carbo-platinum plus a taxane or gemcitabine in first-line therapy for NSCLC (trials 14 and 17), as well as a Phase II trial (trial 16) to confirm the single agent activity of Iressa™ in patients with advanced NSCLC (Kelly *et al*., 2000). It is important to note that these trials do not call for a prospective selection for patients with tumors with some pre-defined level of EGFR over-expression. All epithelial tumors express some EGFR, and in the disease target here, NSCLC, tumors often present with a high proportion of EGFR over-expression (up to 80–90% in advanced disease). The strategy is also consistent with pre-clinical data suggesting that efficacy in drug combinations may not be determined in large part by the level of EGFR over-expression in tumors (Sirotnak *et al*., 2000). Results are expected from these pivotal trials in a late-2001 or early-2002 timeframe.

OSI-774 (CP-358,774) CP-358,774 is also a potent and selective quinazoline-based inhibitor of the EGFR function (Figure 1). This compound is a reversible, ATP-competitive inhibitor (IC_{50} of 2 nM) of the EGFR tyrosine kinase, with greater than 500-fold selectivity against other tyrosine kinases, such as the closely related *erb*B2 kinase, as well as v-*src*, c-*abl* and the insulin and IGF-1 receptors, (Moyer *et al*., 1997). CP-358,774 inhibits the autophosphorylation of the EGF receptor in a variety of EGFR over-expressing tumor cells (IC_{50} = 20 nM), and produces cell cycle arrest and apoptosis in multiple cell types (Moyer *et al*., 1997; Barbacci *et al*., 1997; Iwata *et al*., 1997). *In vivo*, CP-358,774 effectively inhibits EGFR-specific tyrosine phosphorylation in human tumor xenografts (ED_{50} of 10 mg/kg p.o. when given as a single dose) with significant duration of action; daily dosing produces substantial growth inhibition and regressions in human tumor xenografts (Pollack *et al*., 1999). Moreover, the dose-response for tumor growth inhibition shows good agreement with the dose-response for inhibition of EGFR-phosphotyrosine in tumors from treated animals. As with Iressa™, CP-358,774 was found to generate additive anti-tumor activity when used in combination with cis-platinum and other cytotoxic agents, without exacerbating the toxicities of the other chemotherapeutants (Pollack *et al*., 1999).

Clinical studies with CP-358,774 have revealed that the agent is well-tolerated at oral doses that achieve plasma concentrations projected to be required for anti-tumor efficacy in humans (400–500 ng/ml). In one study, escalating doses were administered orally once every week (Karp *et al*., 1999). Eighteen patients with advanced solid tumors were treated at five doses (100–1000 mg) for a maximum period of 24 weeks. Toxicities were observed only at doses higher than 200 mg/week, and included mild fatigue, Grade 2 maculopapular (acneiform) rash, Grade 2 nausea, and Grade 2 diarrhea. Like Iressa™, CP-358,774 exhibited intra- and inter-subject variability in exposure, but dose-proportional increases in exposure were observed throughout the 100–1000 mg weekly dose range. During the first 24 h following a single dose, the C

(0.9–4.8 mg/ml for 100–1000 mg doses, respectively) was some two- to 10-fold above the projected efficacious plasma concentration. No maximally tolerated dose or dose-limiting toxicity was discerned in this study. In a second Phase I study (Siu *et al*., 1999), patients were given CP-358,774 tablets in a variety of dose schedules, culminating in daily dosing at the maximally tolerated dose. The target C_{avg} of 400–500 ng/ml was achievable at doses at and above 100 mg/day on a well-tolerated schedule (C_{avg} values following continuous daily dosing at the 50, 100 and 200 mg/day levels were 432, 973 and 2120 ng/ml, respectively). Dose-limiting diarrhea was encountered at the 200 mg/day level. An intermediate dose of 150 mg/day was subsequently defined as the maximally tolerated dose (two of three patients had Grade 1 diarrhea with loperamide support).

Siu and co-workers also made efforts to understand the 'characteristic' Grade 1–2 acneiform rash seen in patients treated with CP-358,774, which was limited to regions of the upper body where adolescent acne is usually manifest (face, back and scalp). Histopathology of skin biopsies showed subepidermal neutrophilic infiltration and epidermal hyperproliferation (Siu *et al*., 1999). While the precise cytopathic basis for the acneiform rash has not yet been determined, the consistent clinical observations with three different agents targeting EGFR function (CP-358,774, Iressa™ and Imclone's C-225 antibody) suggest that this is a mechanism-based finding (Siu *et al*., 1999; Ferry *et al*., 2000; Cohen *et al*., 2000b). Skin changes are consistently noted in preclinical studies with rodents exposed to CP-358,774 for extended dosing periods, and these toxicological results are analogous to the skin changes seen in the waved-2 mouse, which has a mutated and marginally functional EGFR tyrosine kinase (Luetteke *et al*., 1994).

Early efficacy readouts from ongoing Phase II clinical trials with CP-358,774 have been compelling. The agent appears to have a broad potential to treat a variety of human solid tumors, including NSCLC, breast, ovarian and squamous head and neck tumors (Bonomi *et al*., 2000; Allen *et al*., 2000; Siu *et al*., 2000; Hammond *et al*., 2000). For example, in 34 NSCLC patients who had failed prior chemotherapy, daily oral doses of 150 mg CP-358,774 were well-tolerated, with a maculopapular (acneiform) rash being the most common adverse event reported. In 56 total patients evaluable for tumor response, there have been six partial responses in the lung and/or liver at 8 weeks and several patients with stable disease (Bonomi *et al*., 2000). In 71 patients with refractory squamous carcinomas of the head and neck, CP-358,774 was again found to cause a reversible acneiform rash and Grade 1–2 diarrhea. Of 78 patients evaluable for response, there have been at least eight confirmed partial responses and 23 patients with stable disease (Siu *et al*., 2000). These preliminary results indicate that CP-358,774 is generally well-tolerated and demonstrates evidence of single agent anti-tumor activity in patients with recurrent head and neck cancer, as well as in treatment-refractory NSCLC.

Due to significant interests in both CP-358,774 and CI-1033, Pfizer was directed to divest one of these two agents as a condition of their acquisition of Warner Lambert in 2000. As such, Oncogene Science (OSIP) has taken over complete responsibility for the development of CP-358,774, which is now formally referred to as OSI-774.

CI-1033 (PD183805) As described above, the selective and reversible inhibitors of the EGFR tyrosine kinase appear to offer the promise of therapeutic efficacy coupled to reasonable tolerability. It is important to note, however, that the therapeutic index of neither Iressa™ nor CP-358,774 has yet to be fully elaborated, and that there may be significant proximity between the maximally tolerated doses and the efficacious doses for both agents. Moreover, the efficacy of neither agent has yet to be established in a blinded, placebo controlled study. As such, there continues to be an opportunity to discover and develop distinctly different EGFR tyrosine kinase inhibitors with even greater potential for efficacy and a broader spectrum of activity. CI-1033 is one such distinctly different development candidate. As recently reviewed by David Fry of the former Warner Lambert organization, signaling through the *erb*B family of tyrosine kinase receptors often involves complex cross-talk among the members of that receptor family (Fry, 2000). The four family members (EGFR or *erb*B; *erb*B2, *erb*B3 and *erb*B4) are known to intensify their kinase-dependent transforming signals via the formation of heterodimers with each other (Tzahar *et al*., 1996). There is, therefore, a compelling rationale to consider the potential utility of nonspecific but selective inhibitors that effectively block the function of the *erb*B family but do not inhibit more structurally diverse tyrosine kinases.

There is also a strong rationale to consider irreversible tyrosine kinase inhibitors. The reversible inhibitors have apparently generated clinical efficacy with dosing regimens designed to maintain plasma concentrations at fairly high levels for extended periods of time. The optimal dosing paradigm for an irreversible inhibitor would be less likely to require prolonged exposure. Moreover, the 'absolute finality' (Fry, 2000) of the irreversible inhibitors could conceivably provide significant advantages in terms of antitumor efficacy. To be balanced, a multi-tropic and irreversible inhibitor would also have the potential to generate a toxicity profile that was different and, perhaps, without advantages relative to the more selective, reversible inhibitors. Preclinical data suggest that irreversible EGFR tyrosine kinase inhibitors can generate significant efficacy with good toleration (Vincent *et al*., 1999), but the ultimate utility of these agents can only be determined in clinical trials.

Homology modeling of ATP binding to the pocket of EGFR suggested that the thiol of cys773 would be a key potential site for attack by a rationally designed irreversible ATP-mimetic. One compound containing an acrylamide functionality at the six position of the 4-anilinoquinazoline nucleus (Figure 1) was found to have a profoundly rapid onset and long-lasting inhibition of both EGFR and *erb*B2 in tumor cells, and to be selective relative to non-*erb*B tyrosine kinases (Fry *et al*., 1998). When compared to very closely related reversible analogs (in which the acrylamide double bond was reduced), the 6-substituted irreversible analogs were more potent *in vitro* and had significantly greater efficacy *in vivo*. Further improve-

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