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Reviews

New Drug Targets and Therapies for Cancer

Guest Editor **S. Sebti**

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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.

Keywords: tyrosine kinase inhibitors; anti-tumor; anti-angiogenesis

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 that 4-anilinoquinazolines were potent (nM) inhibitors

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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: STI571 and SU101

STI571 (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 STI571. Originally disclosed by Novartis as a multitrophic tyrosine kinase inhibitor, STI571 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 STI571 (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 STI571 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). STI571 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 STI571 are ongoing in CML.

It is interesting to speculate as to the biochemical basis for both the efficacy and the toleration profile of STI571. Two other tyrosine kinases potently inhibited by STI571, 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 STI571 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 STI571 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). STI571 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 STI571, 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,

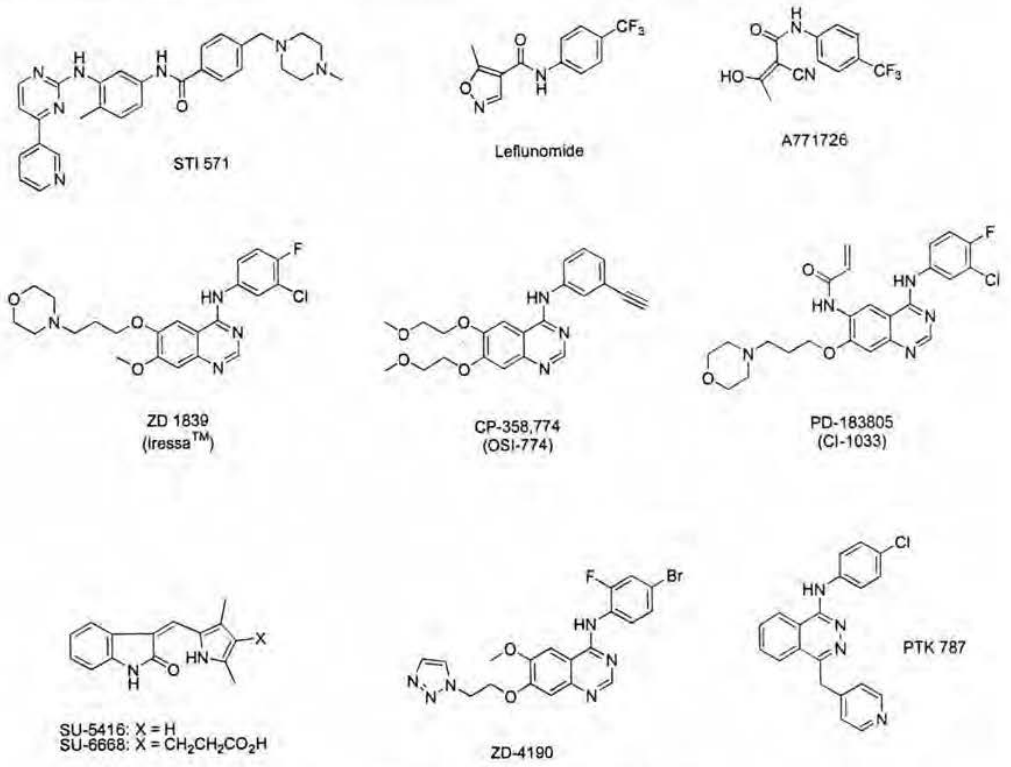

Figure 1 Structures of selected tyrosine kinase inhibitors in clinical development for cancer

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/m²/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

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