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Reviews

New Drug Targets and Therapies for Cancer

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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 ligandcompetitive antibodies, intrabodies, antisense ribonucleotides, ribozymes, phosphatase inhibitors or SH2/SH3directed 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; antiangiogenesis

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 ATPbinding 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 Km for ATP in most kinase active sites is in the micromolar range. thus ensuring full-time saturation by ATP. ATPcompetitive 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 ATPdependent enzymes important to normal physiology, and so an indiscriminant ATP mimetic would likely have toxicities that were pharmacologically and medically unacceptable

This theoretical logiam 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, 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 he 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 biopharmaceufical 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

ST1571 (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 ber-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, her-abl and PDGFR autophosphorylation by the 2phenylaminopyrimidine 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 ber-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 ber-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 stromaprogenitor 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 IC₅₀ 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 nonhematopoietic 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/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 (IC₅₀ 25 nM) with 50-fold selectivity relative to closely homologous erbB family members (IC50 for erbB2 1-3 μM) and even greater selectivity for more divergent tyrosine kinases. It demonstrates good cellular potency (80 nM IC₅₀ for inhibition of EGFdependent 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 Iressam 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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