

**ADVANCES IN THE STRUCTURAL BIOLOGY, DESIGN AND
CLINICAL DEVELOPMENT OF VEGF-R KINASE INHIBITORS FOR
THE TREATMENT OF ANGIOGENESIS**

PAUL WILLIAM MANLEY, GUIDO BOLD, GABRIELLE FENDRICH,
PASCAL FURET, JÜRGEN MESTAN, THOMAS MEYER, BERND
MEYHACK, WILHELM STARK, ANDRE STRAUSS
and JEANETTE WOOD
Novartis Pharma, CH-4002 Basel, Switzerland

Vascular endothelial cells are central to the process of angiogenesis and targeting the vascular endothelial growth factor (VEGF) receptor on these cells is a widely accepted strategy for the modulation of angiogenesis. This is a particularly attractive approach for cancer therapy since, in contrast to cancer cells, endothelial cells are genetically stable and their proliferation is tightly controlled and regulated. Furthermore, VEGF receptor (VEGFR) inhibitors, and VEGFR-2 (KDR) inhibitors in particular, preferentially target the tumour vasculature and promise to be safe enough for chronic administration to patients. Support for these hypotheses has come from findings with anti-VEGF-neutralising monoclonal-antibodies, such as bevacizumab, which has been well tolerated when administered as a single agent in clinical trials and, has shown some signs of efficacy, both as a single agent and in combination with cytotoxic agents.

The initial results in clinical trials with prototype VEGFR-2 kinase inhibitors, such as SU5416 (Sugen), were disappointing and possibly the result of suboptimal dosing coupled with adverse effects resulting from a lack of selectivity. However, other VEGFR-2 kinase inhibitors, such as ZD6474 (AstraZeneca) and PTK787/ZK222584 (Novartis/Schering) are now proceeding into large clinical studies and these compounds are looking more promising, both in terms of efficacy as well as tolerability. For example, PTK787/ZK222584 is well tolerated at once-daily, oral doses up to 2000 mg and, in Phase II studies, has demonstrated clinical responses and stable disease in both colorectal cancer and renal cell carcinoma.

The design of second generation, small molecular weight molecules to specifically target VEGFR-2, from among the 518 protein kinases which modulate intracellular signalling in mammalian cells, and thereby obtain safe, well-tolerated drugs, continues to represent a challenge. Structural biology studies of complexes between inhibitors and the catalytic domain of kinases clarify the manner in which inhibitors bind within the ATP-binding site of the protein and are of great value in the design of more selective agents.

We have studied the three-dimensional structure of a complex between the anthranilamide, 2-[(4-pyridyl)methyl]amino-N-[3-(trifluoromethyl)phenyl] benzamide (AAL993/ZK260255; Novartis/Schering; VEGFR-2 kinase $IC_{50} = 23 \pm 6$ nM) and the kinase domain of VEGFR-2 by X-ray crystallography.

Recombinant protein, corresponding to the human VEGFR-2 kinase domain (amino acids M806-K939/ Δ 50/E990V/A991-D1171), but lacking the insert loop (residues T940-E989) and with a E990V mutation, was produced using baculovirus-infected Sf21 insect cells. Fermentation in the presence of PTK787/ZK222584 (to increase the protein titre), followed by anion-exchange purification and staurosporin-affinity chromatography (eluent ATP/MgCl₂), led to the isolation of autophosphorylated protein. Co-crystallisation, followed by data collection using synchrotron radiation provided a high resolution (2.5 Å) molecular structure of a complex between the anthranilamide and the diphosphorylated (Y1054, Y1059) protein. This structure reveals that the ligand binds within the ATP-binding site of a catalytically inactive conformation of the kinase. Analogous binding modes have recently been observed in crystal structures of complexes between Glivec and Abl and between BIRB796 and p38 MAP kinase. Binding involves three hydrogen-bond interactions involving the pyridine-N with the back-bone NH of C919 in the hinge-region of the protein, the anthranilamide-C=O with the backbone-NH of D1046 of the DGF-loop and the anthranilamide-NH with the side-chain of E885 of helix-C. The phenyl ring of the anthranilamide moiety is sandwiched between the hydrophobic side-chains of V916 and K868. In addition, the trifluoromethylphenyl moiety of the inhibitor adopts a close topological fit with a lipophilic pocket defined by protein residues I888, L889, I892, V898, V899, L1019 and I1044. These structural studies are helping to rationalise the structure-activity findings within the anthranilamide and phthalazine classes of VEGFR-2 kinase inhibitors and facilitating the design of further potent and selective molecules.

Building upon what has been learned from the prototype compounds, a new generation of VEGFR-2 kinase inhibitors, exemplified by AAL993 / ZD26055, CEP-7055 (Cephalon), CP-547,632 (Pfizer) and SU11248 (Sugen), based upon a variety of chemical scaffolds are now emerging. With such an armoury of targeted anti-angiogenic agents the potential of VEGFR-2 kinase inhibitors in the treatment of both solid tumours and leukaemias will soon be clarified and this might provide a spring-board for the exploration of anti-angiogenic agents in other non-oncological diseases.