

# Targeting Src family kinases for anticancer therapy

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## 1 . Src family kinases as targets

cSrc was the first protein kinase to be identified as selective for tyrosine residues. Its tyrosine kinase activity was initially discovered by studying the 60kDa protein encoded by the vSrc gene in the chicken Rous sarcoma virus (1;2). The presence of a similar protein in vertebrate cells had already been described (3) and the cellular chicken gene was soon characterised (4). A large number of publications have described the effects of introducing vSrc or equivalent mutants into mammalian cells. However, since its discovery, mammalian cSrc is very rarely been found mutated in tumours. In one case, 12% of analysed advanced colon carcinomas presented a Gln to stop mutation at codon 531 (5). Since this publication, several groups around the world have tested tumour samples with little success (6-10).

Evidence of a role of cSrc in tumour cell growth and survival comes from expression of anti-sense and dominant-negative constructs in colon carcinoma cells. In colon carcinoma HCT15 cells expression of a dominant-negative cSrc construct inhibits migration of cells in vitro and strongly inhibits peritoneal dissemination in SCID mice. The anti-migration effect of DN-Src was shown by a reduced invasion of peritoneal tumours obtained into surrounding tissue and their higher fibrotic nature (11). The same group also showed that treatment of mice with PP2, a selective SFK inhibitor, after intrasplenic injection of HT29 cells reduced the size of liver metastasis obtained (12). Also in colon carcinoma cells, antisense constructs directed to cSrc reduce the ability of these cells to survive to anoikis in vitro (13). Recently, Gallick et al used cSrc shRNA expression in the metastatic L3.6pl pancreatic tumour cell line to reduce endogenous Src levels. In this context, Src knock-down had a clear effect on the metastasis potential of these cells when implanted orthotopically but had no impact on growth of the primary tumour. In contrast, treatment of L3.6pl tumours with Dasatinib had an effect both on primary tumour growth and on metastasis (see below) (14). This suggests that in the context of this particular pancreatic cell line, inhibition of cSrc alone is sufficient to stop metastasis, but it may not be sufficient in vivo to trigger tumour regression. Finally, a recent paper reports the effect of inducible overexpressing dominant-negative cSrc in mammary cancer cells. This resulted in a reduction of migration and proliferation rate with an accumulation of cells in G1 and tumour growth in vivo (15).

## 2. Src inhibitors

### 2.1. Historical overview

One of the first compounds to be reported were Pfizer's pyrazolo-pyrimidines PP1 and related compound PP2. These compounds were synthesised with the aim of inhibiting T-cell activation via Src family kinases Lck and FynT. PP1 and PP2 showed good potency against Lck (IC<sub>50</sub> of 4 nM) and a surprising selectivity for the Src family (IC<sub>50</sub> 170 nM) (16). PP2 is one of the most selective Src inhibitors available and has been used in vivo by intraperitoneal injection (12). In the late nineties, PD173955 and later PD173956 were shown to inhibit cSrc with an IC<sub>50</sub> of 20 nM on pure enzyme assays and at 5 µM in cells. Its selectivity was lower than that of PP2, since these compounds are inhibitors of Abl, Csk, PDGFR and EGFR. Used at 5µM, PD173955 was shown to block several cell lines in the G2/M phase of the cell cycle, although an effect on apoptosis was not clearly demonstrated (17). Later, a different effect of PD173955 on the cell cycle was demonstrated at 1 µM, a dose sufficient to inhibit cSrc in cells and induce accumulation of cells in G1 and increased the pro-apoptotic effect of

detaching cells from matrix (13). So far, no data has been published on in vivo studies using PD173955 or PD173956. At around the same time, Novartis developed pyrrolo-pyrimidine (ex. CGP77675) and olomoucine-derived (ex. NVPAAK980) compounds as Src inhibitors targeted both to cancer and osteoporosis (18). Amongst these compounds, CGP77675 showed activity in vivo in bone resorption models. CGP76030, a second compound of the same type, was later shown to inhibit migration and DNA synthesis of PC3 prostate cancer cells in vitro (19). Very recently, CGP76030 has been shown to be active in vivo, orally in a bone metastasis mouse model using MDA-231 breast cancer cells (20). In this paper, no effect of CGP76030 on primary tumour growth was reported, although expression of dominant-negative Src in the same cellular context led to reduced primary growth and bone metastasis. In 2000, Sugen published SU6656, an indolinone typical of Sugen's library, showing high selectivity but relatively low potency against cSrc (280 nM) (21). This was followed closely by two successors SU11333 and SU11336, which showed target hitting in vivo in breast and colon tumour xenografts after intra-peritoneal injection (22). Since 2001 a third generation of Src inhibitors has appeared. This time, molecules are characterised by their higher potency in enzyme assays and their dual inhibition of cAbl and a variety of Glivec-resistant cAbl mutations. The strong activity against cAbl accompanying potent anti-Src activity can be explained by the strong structural similarity of the ATP binding sites in both kinases (23;24). This dual selectivity has allowed some of the pharmaceutical companies behind these compounds to take advantage of the anti-cAbl activity to accelerate the development of their anti-Src compounds, using very sensitive cell and animal models driven by the BCR-Abl translocation.

## **2.2. Molecules in clinical trials**

### **SKI-606 and its successors (WYETH)**

Wyeth-Ayers reported a quinoline-carbonitrile series in 2002 which included SKI-606 (AACR 2002). This compound is a dual Src-Abl inhibitor with an IC<sub>50</sub> of 1.2 nM against Src. It can inhibit Src in cultured cancer cells at 1 µM doses and in vivo, oral administration of this compound at 100 mg/kg to nude mice leads to complete inhibition of Src in implanted colon carcinoma tumours (25). Due to its anti-cAbl activity, SKI606 causes regression of K562 tumours grown subcutaneously in nude mice (26). However, and despite this efficient target hitting activity, oral treatment with SKI-606 results only in a delay in growth of colon carcinoma tumours at 100 mg/kg, its maximal tolerated dose. As a result, based on its activity against Abl, Wyeth initiated CML clinical trials but no results have been reported. A phase I on patients carrying solid tumours was also announced in 2005 but no reports have been done. Very recently (AACR 2006), a new compound, SKI785, was presented as a potential successor of SKI606 for clinical trials (poster AACR 2006).

### **AZD0530 (ASTRAZENECA)**

This anilino-quinazoline and its related compounds are also mixed Src/Abl compounds with potency against Src of IC<sub>50</sub> 10 nM. Published data on the in vivo activity of AZD0530 is sketchy. Most of the preclinical data published relates to another compound AZM475271, which showed an antimetastatic effect on a L3.6pl pancreatic tumour model (27). AZD0530 is orally active and was shown to delay growth of NBTII tumours and growth of Src-transformed fibroblast in nude mice (Abstract EORTC 2005). Inhibition of Src in implanted tumours has not been shown. AstraZeneca initiated phase I clinical trials on healthy volunteers to evaluate toxicity, PK and the use of the bone resorption marker CTX. In these studies, 70 % inhibition of CTX accumulation was observed at the dose of 180 mg/day which is the maximal tolerated dose in man. Currently, a phase I/II is in progress on pancreatic cancer patients in combination with gemcitabine. Whether sufficiently high plasma levels can

be achieved in man for Src inhibition in tumours remains to be seen, given the dose limiting toxicity observed in phase I.

### **BMS-354825**

BMS-453825 (Dasatinib) is the most potent Src inhibitor in clinical development. It's IC<sub>50</sub> is 0.6 nM and it can inhibit cSrc efficiently in cultured cells at doses between 0.1-0.3 μM. This compound emerged from a series of Lck inhibitors originally synthesised as anti-inflammatory compounds. As a consequence, Dasatinib is also a potent inhibitor of Lck with a IC<sub>50</sub> of approximately 4 nM. As SKI606 and AZD0530, BMS-354825 is potent against cAbl and BCR-Abl, inhibiting the pure enzyme with an IC<sub>50</sub> of 0.05 nM. Dasatinib is also active against EGFR, PDGFR, cKit, EphA2 and Csk with IC<sub>50</sub>s below 200 nM. Due to this activity against BCR-Abl but especially due to its activity against mutant forms of BCR-ABL that resist to Glivec treatment, Bristol-Myers developed this compound as therapy against CML and ALL in pre-clinical and clinical trials. As shown in vitro, Dastinib was effective on patients harboring mutations resistant to Glivec but not in those patients whose CML cells contained mutation T315I. This mutant proved to be resistant to Dasatinib in vitro. These results led to FDA approval for Glivec-resistant CML in June 2006 at a recommended dose for CML. Despite this success on CML, the possibility of using Dasatinib as therapy on solid tumours, in which cAbl is not a target and where BCR-Abl does not exist, is unclear. Unlike CML, which is a clonal blood disease based on the activity of BCR-Abl, metastasising solid tumours present a more complex challenge, with less clearly defined targets and higher barriers. Although dasatinib is active against growth of many cancer cell types in vitro, very little data is available on the activity of BMS-354825 on tumour models other than the CML cell line K562. One recent poster presentation by BMS described growth delay of PC-3 tumours in nude mice at the dose of 30 mg/kg (AACR 2005). Gallick et al recently described a 50% growth delay of pancreatic L3pl tumours grown orthotopically and a complete inhibition of metastasis following Dasatinib treatment (14).

## **3. Conclusions**

Inhibition of Src family kinases in solid tumours is likely to lead to objective responses in a subset of patients. In these patients, signalling defects may have generated both Src deregulation and dependence on its activity for tumour survival. Identifying these patients by the use of reliable selection biomarkers is a necessity. A more general effect on reducing the metastatic potential of many if not all tumours will be observed. However, this beneficial effect may be difficult to measure in classical clinical trials. Mixed Src/Abl compounds such as Dasatinib are likely to show impressive activity against CML. However, the success of such drugs on CML should not distract us from the more ambitious challenge of inhibiting Src in solid tumours sufficiently to obtain a clinical response.

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