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Review

Clinical significance of Src expression and activity in human neoplasia

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Summary. Src, a 60 kDa non-receptor tyrosine kinase, is the product of normal *c-src* of the human genome and member of the Src protein tyrosine kinases family (SFK). As described by Martin and Rous, a genetic recombination between *c-src* and the RSV oncogene of Rous sarcoma virus results in a modified Src protein, with increased intrinsic activity and transforming potential in animal and human tissues. Several *in vitro* and *in vivo* studies supported this theory providing insight in the signalling pathways involved. Accumulating evidence from studies on clinical samples supported the role of Src in the process of carcinogenesis and disease progression in several human malignancies. Some studies have further reinforced the significance of the kinase in malignacy by correlating its expression and/or activity with important clinicopathological parameters, such as tumour stage, histopathological grade, proliferative capacity and most importantly patient's survival. This review is a comprehensive report of the published evidence on the expression and clinical significance of Src in human malignancy, which constitutes the background of the current studies and clinical trials on the use of Src inhibitors as novel potent antineoplastic strategy.

Key words: Src, Expression, Activity, Cancer, Malignancy

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Introduction

Rous sarcoma virus (RSV) was first described as a virus with oncogenic properties in chicken (Rous, 1911a,b), with the definitive confirmation coming later with the identification of the *v-src* as an oncogene (Martin, 1970). *v-src* is the product of recombination between the *RSV* and the normal *c-src* of the human genome that codes the 60 kDa non-receptor tyrosine kinase Src protein (Collett and Erikson, 1978; Levinson et al., 1978; Hunter and Sefton, 1980), a member of the Src protein tyrosine kinase family (SFK). *v-src* differs from the normal *c-src* in carboxy-terminal deletions and point mutations throughout the gene sequence (Takeya and Hanafusa, 1982). These structural differences are held responsible for the higher levels of intrinsic activity and transforming potential of the v-Src protein (Jove and Hanafusa, 1987; Parsons and Weber, 1989).

Since Rous and Martin, science has progressed in

Abbrevations: Arg: arginine, Csk: c-Src tyrosine kinase, EGFR: Epidermal Growth Factor Receptor, ER: estrogen receptor, FAK: Focal Adhesion Kinase, FAs: focal adhesions, FGFR: Fibroblast Growth Factor Receptor, HGF: Hepatocyte Growth Factor, HCC: hepatocellular carcinoma, IHC: immunohistochemistry, NSCLC: non-small cell lung carcinoma, p130cas: CRK-associated substrate, PCNA: Proliferating Cell Nuclear Antigen, PCR: Polymerase Chain Reaction, PDGFR: Platelet-derived Growth Factor Receptor, PI3K: phosphoinositide 3-kinase, PR: progesterone receptor, pSrc: phosphorylated Src, pSrc: phsophorylated Src, PTP1B: protein tyrosine phosphatase 1B, PTPa: protein tyrosine phosphatase a, RCC: renal cell carcinoma, RSV: Rous sarcoma virus, SCC: squamous cell carcinoma, SFK: Src protein tyrosine kinases family, SH: Src homology, SHP1: SH2-containing phosphatase 1, SHP2: SH2-containing phosphatase 2, STAT: signal transducers and activators of transcription, tSrc: total Src, Tyr: Tyrosine.

the understanding of the role of Src in human malignancy. Accumulated evidence supported the role of the kinase in the promotion and regulation of malignant cellular characteristics in both animals and humans. Based on these observations, concurrent studies evaluated targeting Src in *in vitro* and *in vivo* animal studies, with promising results (Aleshin and Finn, 2010). Consequently, factors that inhibit the enzyme's kinase activity have recently entered clinical trials as chemotherapeutic agents (Kopetz et al., 2007; Aleshin and Finn, 2010). The purpose of this review is a comprehensive analysis of the existing data on Src expression and clinical significance in human malignancy, which constitute the background of the Src inhibitors use in cancer management.

Molecular structure, activation and regulation of the human c-Src

The c-Src protein is composed of a carboxy-terminal tail, four Src homology (SH) domains and a unique amino-terminal domain (Brown and Cooper, 1996) (Fig. 1). The carboxy tail of the molecule contains the negative regulatory tyrosine (Tyr) residue 530. The SH1 kinase domain contains Tyr419, the autophosphorylation site of the protein. The SH2 domain binds phosphotyrosine polypeptide segments based on significant specificity particularly for three residues (Mayer et al., 1991; Songyang et al., 1993; Waksman et al., 1993). It was found to interact with the negative regulatory domain and also to bind the Platelet-derived Growth Factor Receptor (PDGFR) (Mori et al., 1993) and Her2 at Tyr215, resulting in increased Src activity (Stover et al., 1996). The arginine (Arg) 175 residue has been described as an important site for the recruitment of the molecule to the focal adhesions (FAs) and the phosphorylation of Focal Adhesion Kinase (FAK) at Tyr397 (Yeo et al., 2006). The SH3 domain promotes intramolecular contact with the inactivated kinase domain and intermolecular contact via proline-rich sequences (Krueger et al., 1982; Ren et al., 1993). The amino-terminal side of the molecule is comprised by a region of unique residues for each SFK family member and the SH4 domain, a site important for the localisation of the molecule on the cellular membrane (Krueger et al., 1982; Xu et al., 1997). The functions of the amino-terminal region have not been clearly defined, but it has been shown that changes in the amino acid sequence in this area reduced the transforming potential of v-Src in chicken embryo fibroblast cultures (Krueger et al., 1982).

A closed configuration of the molecule in its inactive state is responsible for the covering of the kinase domain and the subsequent failure to interact with its substrates (Yamaguchi and Hendrickson, 1996). This is accomplished by the interactions between the carboxy-terminus and SH2, as well as the SH3 and kinase domains. The activation of the protein is dependent upon the status of the carboxy-terminus. When this is phosphrylated on the residue Tyr530 by the c-Src tyrosine kinase (Csk) the protein is inactive (Cooper et al., 1986; Masaki et al., 1999). When Tyr530 is dephosphorylated by the action of phosphatases, such as protein tyrosine phosphatase a (PTPa) (Zheng et al., 1992), protein tyrosine phosphatase 1B (PTP1B) (Bjorge et al., 2000), SH2-containing phosphatase 1 (SHP1) and 2 (SHP2) (Jung and Kim, 2002) Src is activated. An alternative mode of Src activation is by interaction with the activated FAK (Schaller et al., 1994) or its substrate CRK-associated substrate (p130cas) (Thomas et al., 1998) via the SH2 and SH3 domains. Finally, Src can also be activated by various receptors, such as Epidermal Growth Factor Receptor (EGFR) (Luttrell et al., 1994; Mao et al., 1997; Tice et al., 1999), PDGFR (Courtneidge et al., 1993; DeMali et al., 1999; Bowman et al., 2001), Her2/neu (Luttrell et al., 1994; Muthuswamy et al., 1994), Fibroblast Growth Factor Receptor (FGFR) (Landgren et al., 1995; LaVallee et al., 1998), Hepatocyte Growth Factor (HGF) receptor c-Met (Rahimi et al., 1998), nitric oxide signaling (Akhand et al., 1999) or by naturally occurring mutations that truncate the carboxy-terminal region proximal to Tyr530 (Irby et al., 1999; Sugimura et al., 2000).

Inactivated Src is localised in the perinuclear region,

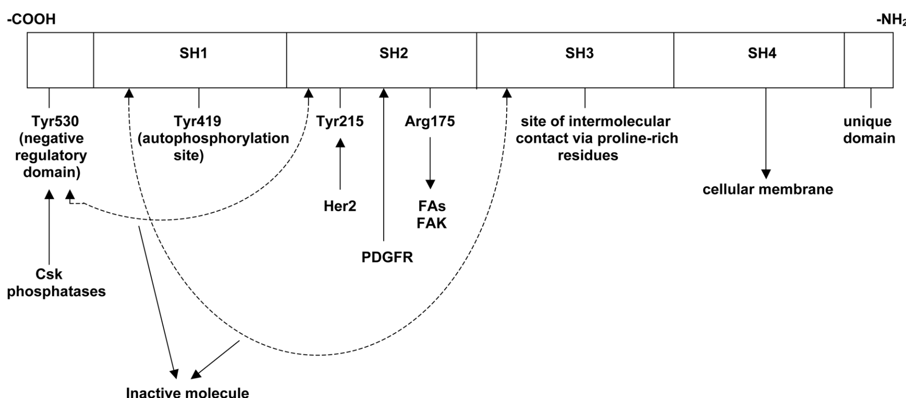

Fig. 1. Schematic presentation of Src protein.

but when activated its SH3 domain becomes indirectly associated with actin and the protein attaches to the cellular membrane via its SH4 domain (Sefton et al., 1982). This localisation seems to be regulated by the G-proteins RHOA, Rac1 and CDC42 (Timpson et al., 2001).

Src signalling in malignancy

In malignancy, increased Src activity has been attributed to either increased expression and/or increased intrinsic kinase activity. Several theories have been proposed about the sequence of events for the increased intrinsic Src activity observed (Summy and Gallick, 2003; Yeatman, 2004). Low levels of the negative Src regulator Csk have been suggested to play a role in the activation of the kinase in cancer (Masaki et al., 1999), while high levels have been shown to suppress metastasis in mouse models (Nakagawa et al., 2000). Similarly, hyperactivity of the kinase might be achieved by high levels of the phosphatases responsible for its activation, such as PTP1B in human breast cancer cell lines (Bjorge et al., 2000). Finally, point mutations that truncate the protein at a point proximal to the carboxy-terminal regulatory part have been shown to lead to activation in colon and endometrial carcinomas (Yamaguchi and Hendrickson, 1996; Irby et al., 1999; Sugimura et al., 2000).

Src exerts its effects on cellular behaviour through a variety of mechanisms and signalling cascades. Substrates and interacting molecules in these signalling cascades include FAK and its substrates, p190Rho-GAP, signal transducers and activators of transcription (STAT) factors, connexin-43, p120-catenin and cortactin (Frame, 2002, 2004; Chatzizacharias et al., 2008; Guarino, 2010). Deregulation of these signalling pathways due to uncontrolled Src signalling has been implicated in the process of carcinogenesis, by altering several primary cellular functions, such as attachment to the extracellular matrix, cellular proliferation, epithelial-to-mesenchymal transition, cellular invasion, migration and metastasis, angiogenesis (Frame, 2002, 2004; Summy and Gallick, 2003; Yeatman, 2004; Guarino, 2010) and mechanisms providing resistance to chemotherapeutic agents (Vigneron et al., 2005; Chen et al., 2005; George et al., 2005; Riggins et al., 2006; Hiscox et al., 2006, 2007; Planas-Silva and Hamilton, 2007) and radiation (Contessa et al., 2006).

Src expression and activity in malignancy

Based on the evidence of studies showing that Src promotes cellular malignant characteristics *in vitro*, various studies were conducted evaluating the significance of the molecule's expression and activity in human malignancies, as well as its correlation with clinicopathological parameters of the disease. A comprehensive presentation of such data will follow (Table 1).

Neurological neoplasia

The evidence on the role of Src in neurological malignancies is still scarce. Src was detected by immunohistochemistry (IHC) in the cytoplasm of 61.4% of the astrocytomas studied, while normal brain tissue and other tumour types, including oligodendroglioma, choroid plexus papilloma, ependymoma, medulloblastoma, neurinoma and haemangioblastoma, did not express the kinase (Takenaka et al., 1985). However, most of the published data are related to neuroblastoma. Src RNA levels (Northern blot) in differentiated neuroblastomas were similar to controls, while they varied among undifferentiated tumours (Matsunaga et al., 1991). Furthermore, the alternatively spliced neuronal Src mRNA (SrcN2) variant was significantly more common in infantile and localised disease rather than metastatic, as detected by RNA-Polymerase Chain Reaction (PCR) (Matsunaga et al., 1998). High Src mRNA levels (Northern blot) were associated with good clinical course and low with poor outcome (Matsunaga et al., 1991), while high SrcN2 to total Src ratio correlated with longer event-free survival (Matsunaga et al., 1998). Similar was the evidence on childhood neuroblastomas (and retinoblastomas), where higher expression of the c-Src splice variant than total Src (tSrc) (immunoblotting) also correlated with good prognosis (Bjelfman et al., 1990). Additionally, this expression pattern might be indicative of a higher degree of differentiation or differentiation potential and could be used to separate these tumours from others with signs of neuronal differentiation, such as neuroepitheliomas (Bjelfman et al., 1990).

tSrc expression has also been studied in meningiomas of different histological types, including fibroplastic, syncytial, transitional, atypical and malignant, and was found to be absent (IHC) (Takenaka et al., 1985) or of very low level (Northern blot) without correlation to cellular proliferation (Detta et al., 1993).

Head and neck neoplasia

Initial studies on human tissue specimens revealed tSrc to be overexpressed in hyperproliferating regions of squamous cell carcinomas (SCCs), dysplasias, papillomas and inflamed normal tissues (Van Oijen et al., 1998). Most recently, immunohistochemical analysis of phosphorylated Src (pSrc) expression (Tyr416) in tongue cancer specimens showed positive staining in 38% of cases, while adjacent normal tissue was pSrc negative (Ben-Izhak et al., 2010). Positive expression was correlated with increased apoptotic rate (TUNEL test) and c-erbB2 levels of immunostaining. pSrc (Tyr416) expression and strong intensity of immunostaining were significantly correlated with tumour size, stage, T and N substages. No correlation was identified between the staining characteristics and patients' sex, age or tumour histopathological grade. Survival analysis identified significantly reduced

Table 1. Correlation of Src expression with basic clinicopathological parameters in various human malignancies.

FAK expression in tumors *in vivo*	Method	Sample size	Grade	Stage	pT	pN	pM	Proliferation (mitotic index or Ki67)	Survival/ Prognosis
Neuroblastomas									
Matsunaga et al., 1991	NB (mRNA)	22							+
Head and neck carcinomas									
Ben-Izhak et al., 2010	IHC (pSrc)	39	-	+	+	+			+
Thyroid cancer									
Michailidi et al., 2010	IHC (Src)	108				-		-	
Lung and pleural cancer									
Mazurenko et al., 1992	IHC (Src)	33	+						
Leung et al., 2009	IBT (pSrc)		+						
	IBT (pSrc)	187		-		-			
Masaki et al., 2003	KA (activity)	30	-	-					
Tsao et al., 2007	IHC (Src)	46							-
	IHC (pSrc Tyr419)			+			+		-
	IHC (pSrc Tyr530)					+			-
Esophageal cancer									
Iravani et al., 2003	KA (activity)	34							-
Colon cancer									
de Heer et al., 2008	IHC (Src)	104	-	-					+
Han et al., 1996	IBT (pSrc)	31	-						-
Weber et al., 1992	IBT (pSrc)	9	+						
Aligayer et al., 2002	KA (activity)	45							+
Hepatocellular carcinoma									
Lau et al., 2009	IHC (Src and pSrc)	64	-					-	-
Ito et al., 2001	IHC (pSrc)	87	+					+	
Masaki et al., 1998	IBT, WB (pSrc)	20	+						
Cholangiocarcinoma									
Ito et al., 2001	IHC (pSrc)	19	-	-					
Pancreatic cancer									
Chatzizacharias et al., 2010	IHC (Src)	65	-	+	-	-	-	-	+
Hakam et al., 2003	IHC (Src)	47	-	-					-
Nagaraj et al., 2010	IHC (Src)	68	+						+
Morton et al., 2010	IHC (Src)	114	+	-		+			-
	IHC (pSrc)		-	-		-			
Pancreatoblastoma									
Di Florio et al., 2007	IHC, WB (Src)	27	-						
Prostate cancer									
Paronetto et al., 2004	WB (pSrc)	23	+						
Tatarov et al., 2009	IHC, WB (pSrc)	50	-						+
Endometrial cancer									
Desouki and Rowan, 2004	WB (Src)	33	-						
Chatzizacharias et al., 2010	IHC (Src)	43	- *	-				-	-
	IHC (pSrc)		- *	-				-	-
Breast cancer									
Hennipman et al., 1989	KA (activity)	70				-			+
	IHC, rtPCR (Src)								+
Campbell et al., 2008	IHC, WB (cytoplasmic pSrc)	262	-			-		-	
	IHC, WB (nuclear pSrc)		-			+		-	+
Wilson et al., 2006	IHC (pSrc)	129						+	-
Verbeek et al., 1996	IHC (Src)	27	-					-	
Ito et al., 2002	IHC (Src)	73	+	+		+		+	
Elsberger et al., 2010	IHC (Src)	314	+					+	-
	IHC (pSrc Tyr215)		+						+
	IHC (pSrc Tyr219)		+						
Morgan et al., 2009	IHC (Src)	75							+
	IHC (pSrc)								+
Zhang et al., 2009	CGE (src gene expression)	615				+		+	
Melanomas									
Homsi et al., 2009	IHC (pSrc)	35	-						+

*: pSrc expression was correlated with the grade of differentiation in the endometrioid type; +: correlation (directly or inverrsely); -: no correlation; KA: kinase assay; IHC: immunohistochemistry; CGE: cytokine gene expression; IBT: immunoblotting; rtPCR: reverse transcriptase PCR; WB: Western blot; NB: Northern blot

survival in patients with pSrc (Tyr416) positive tumours (Ben-Izhak et al., 2010).

The role of Src in human thyroid disease was evaluated by IHC in patients operated for benign (hyperplastic nodules and Hashimoto thyroiditis) and malignant (papillary, medullary, follicular and anaplastic carcinomas) lesions, while normal thyroid tissue specimens, negative for the kinase, were used as controls (Michailidi et al., 2010). A higher incidence of tSrc positivity was noted in malignant compared to benign lesions, which was significant only between papillary carcinomas and hyperplastic nodules. The kinase was expressed in 54.6% of the study sample, exerting both cytoplasmic and membranous pattern of immunostaining. More specifically, 69% of the papillary, 12% of the medullary, 40% of the follicular, none of the anaplastic, 51% of the hyperplastic nodules and 56% of the Hashimoto thyroiditis specimens were tSrc positive (Michailidi et al., 2010). tSrc positivity was significantly lower in patients with larger tumour size, while no correlation was identified with patients' age, sex, tumour's proliferative capacity (Ki-67 labelling index), lymph node involvement and capsular, lymphatic or vascular invasion. Similarly, pSrc (Tyr418) expression was significantly higher in malignant compared to benign lesions (Michailidi et al., 2010).

Lung and pleural neoplasia

The clinical significance of Src in lung carcinomas has been approached by several studies. tSrc and pSrc expression (use of mouse monoclonal antibody Mab327 against phsophotyrosine) (IHC, Western blot and immunoblotting) was detected in most lung cancer specimens (Mazurenko et al., 1992; Masaki et al., 2003), including 60-80% of the adenocarcinomas, 50% of the SCCs and 100% of the small cell carcinomas, but rarely (0-21%) in normal tissue (Mazurenko et al., 1992). In another study, increased levels of phosphorylation (Tyr416) (immunoblotting) were found in 28.3% of the non-small cell lung carcinoma (NSCLC) cases examined (Leung et al., 2009). Src activity, measured by protein kinase assays, was significantly higher in both lung adenocarcinoma and SCCs compared to normal controls (Masaki et al., 2003). Further analysis showed that the increase in Src activity in SCCs was due to an increase in the protein levels, while in adenocarcinomas it was caused by both an increase in the protein levels and the specific kinase activity (Masaki et al., 2003).

With regards to the clinicopathological parameters of the patient and the disease, tSrc and pSrc expression (use of Mab327) (IHC and immunoblotting) was correlated with poor histopathological grade of differentiation overall and within the squamous type subcategory (Mazurenko et al., 1992). No association was identified between pSrc (Tyr416) expression (immunoblotting) and patients' age, sex, smoking history, tumour stage and lymph node involvement in NSCLCs (Leung et al., 2009). In the adenocarcinomas, Src activity (protein kinase assay) was correlated with increased tumour size, but not with the disease stage or tumour histopathological grade of differentiation, even though there was a tendency for higher levels in poorly differentiated tumours (Masaki et al., 2003). In SCCs, it was not correlated with the tumour size, the stage or the grade. Interestingly, a retrospective evaluation of *src* gene expression (microarray/gene expression signatures) in NSCLC specimens, showed that Src pathways were more likely to be activated in patients younger than 70 years of age and with the shortest recurrence-free survival (Mostertz et al., 2010).

Src expression was also studied in human malignant pleural mesothelioma tissue specimens, including tumours with epithelioid, sarcomatoid and biphasic histology (Tsao et al., 2007). A mainly membranous and cytoplasmic pattern of tSrc, pSrc (Tyr419) and pSrc (Tyr530) expression (IHC) was noted. Stage was correlated with membranous pSrc (Tyr419) expression. The presence of organ metastasis was correlated with membranous and cytoplasmic pSrc (Tyr419) expression, while the presence of lymph node metastasis was inversely correlated with membranous pSrc (Tyr530) expression. A trend was identified between asbestos exposure, as well as a heavy smoking history with decreased cytoplasmic pSrc (Tyr419) expression. No association was revealed between Src and pSrc (Tyr419 and Tyr530) expression and disease relapse, site of relapse or overall patients' survival (Tsao et al., 2007).

Oesophagogastric neoplasia

The role of Src has also been studied in the process of oesophageal neoplasia and more specifically in the malignant transformation of Barrett's oesophageal epithelium to adenocarcinoma. tSrc protein expression (IHC) increased with the progression of normal epithelium to Barrett's and cancerous, with Src intensity of staining being mainly moderate in cancer specimens and weak in non-cancerous ones (Jankowski et al., 1992). Interestingly, opposing results have also been published suggesting no change in pSrc protein levels (use of Mab327) (immunoblotting) between normal, Barrett and malignant epithelia (Kumble et al., 1997). Nonetheless, higher Src activity using the same methodology was noticed in adenocarcinomas (6-fold) and in Barrett's oesophagus (3-4-fold) compared to normal controls (Kumble et al., 1997).

Furthermore, it has been suggested that the role of Src in the process of oesophageal malignant exchange is an early event, as strong and diffuse cytoplasmic tSrc staining was observed more frequently in high grade dysplasias (93%) than in adenocarcinomas (85%) and low grade dysplasias (72%) or Barrett's specimens (41%) (Iravani et al., 2003). Src activity (kinase assay) was 2-4-fold higher in low grade dysplasias than in metaplasias and 2-3-fold higher in adenocarcinomas than low grade dysplasias. However, no correlation was established with patients' survival (Iravani et al., 2003).

In gastric neoplasia, elevated Src kinase activity was detected in 50% of gastric cancer tissue specimens compared to normal mucosa, with activity levels not necessarily correlating with phosphorylated protein expression levels as detected with Western blot analysis (Takekura et al., 1990). This observation indicated that post-translational changes might be responsible for the increased activity.

Colorectal neoplasia

Several studies have implicated Src activity in the process of human colon cancer development. More specifically, Src activity (immunoblotting and tyrosine kinase assay) was increased in colorectal carcinomas (between 2- and 57-fold) (Cartwright et al., 1989; Weber et al., 1992; Talamonti et al., 1993; Han et al., 1996; Aligayer et al., 2002) and liver metastases (between 0.3- and 390-fold) (Termuhlen et al., 1993; Talamonti et al., 1993; Han et al., 1996) compared to normal colonic mucosa, while phosphorylated protein levels (immunoblotting) were found to be either increased (Cartwright et al., 1989; Termuhlen et al., 1993; Talamonti et al., 1993; Licato and Brenner, 1998) or unchanged (Han et al., 1996). Similarly, extrahepatic metastases also presented increased activity (between 0.2- and 159-fold) (Termuhlen et al., 1993). Investigating the process of malignant transformation of polyps, adenomatous polyps presented elevated pSrc expression (use of Mab327) and activity (immunoblotting and tyrosine kinase assay) compared to normal colonic mucosa, with those with the highest malignant potential, large (d>2cm), villous and dysplastic, presenting the highest levels of activity (Cartwright et al., 1990; Talamonti et al., 1993). pSrc expression levels (use of Mab327) were not increased concomitantly with the observed increase in kinase activity, suggesting a specific increase in the intrinsic enzymatic activity at the early stages of polyp malignant transformation (Talamonti et al., 1993). Src kinase activity was also gradually elevated in the progress of premalignant ulcerative colitis lesions towards carcinomas, as detected by immunoblot analysis, in patients suffering from the disease for more than 10 years. A 2.5-fold increase in Src activity was noticed in severe quiescent lesions compared to mild, a 10-fold increase in low grade dysplasia and a 20-fold increase in cases with high grade dysplasia and carcinoma (Cartwright et al., 1994).

On the contrary, other studies suggested that early stages of colonic cancer progression exhibit higher levels of Src activity (Sakai et al., 1998; Iravani et al., 1998). Activated Src (use of antibody Clone 28, which exclusively recognises active form) intensity of immunostaining was weak in 36% of the hyperplastic polyps studied (Sakai et al., 1998). Adenomas exhibited mostly (92%) moderate or strong tSrc expression (immunoblotting, immune complex assays and Western blot) (Iravani et al., 1998) and intensity of pSrc immunohistochemical staining (use of Clone 28) (Sakai et al., 1998), while normal colonic mucosa did not express the kinase (Sakai et al., 1998) or exhibited weak and diffuse staining (immunoblotting, immune complex assays and Western blot) (Iravani et al., 1998). Notably, only 25% of the adenocarcinoma specimens presented weak and mainly nuclear or perinuclear pSrc staining (use of Clone 28) (Sakai et al., 1998) of significantly decreased intensity compared to the adenomatous mucosa (Iravani et al., 1998), while no significant increase in tSrc levels (IHC, immunoblotting, immune complex assays and Western blot) was observed with progression to either lymph node or liver metastasis (Iravani et al., 1998; de Heer et al., 2008). Such results suggested that increased Src activity possibly represents an early event of colonic malignant transformation.

With regards to the clinical aspect of the disease, tSrc expression (IHC) failed to correlate with patients' sex, age and tumour stage (de Heer et al., 2008). Tumours with high tSrc expression (IHC) were significantly more often localised distally to the splenic flexure (de Heer et al., 2008). Similarly, activated Src (pSrc) expression (use of Mab327) (immonoblotting) was not correlated with the extent of liver metastases, presence of extrahepatic metastasis, Karnofsky's performance status and pre-or post-operative chemotherapy (Han et al., 1996). With respect to the level of differentiation, tSrc (IHC) (de Heer et al., 2008) and pSrc (use of Mab327) (immunoblotting) (Han et al., 1996) expression were not correlated with tumour histopathological grade. On the contrary, Src activity (immunoblotting) was found to be significantly higher in moderately and poorly differentiated tumours compared to normal mucosa and highly differentiated ones (Weber et al., 1992). In one study, patients' survival was not affected by activated (phosphorylated) Src expression (use of Mab327) (immunoblotting) (Han et al., 1996), while another associated high tSrc expression (IHC) with shorter time to recurrence, either combined with increased total FAK (tFAK) expression (IHC) (tFAK and tSrc expressions were intercorrelated), or independently after multivariate analysis, along with age and tumour stage, imposing a 2.98-fold higher risk (de Heer et al., 2008). Finally, more than 2-fold increased Src activity (kinase assay) in colorectal adenocarcinoma has been identified as an independent prognostic factor for poor overall survival, imposing a risk increase of 3.54 times, poor survival in patients without distant metastasis, imposing a risk increase of 20.82 times, and decreased disease-free interval, imposing a risk increase of 11.53 times (Aligayer et al., 2002).

Liver neoplasia

Studies on hepatocellular carcinoma (HCC) specimens confirmed increased tSrc expression (IHC) and activity (IHC, Western blot and immunoblotting) in these tumours compared to normal liver tissue and non-malignant disease specimens used as controls (Masaki et al., 1998; Ito et al., 2001; Lau et al., 2009). The

increased Src activity was attributed to both increased levels and intrinsic activity of the enzyme (Masaki et al., 1998). Once again, opposing data has also been published. Src gene expression was found by RNA Dot Hybridization to be similar between a series of Morris hepatomas (transplantable tumours that provide models for the study of liver tumour biology) and normal liver tissue specimens (Cote and Chiu, 1987), as well as between resected hepatomas and normal tissue (Zhang et al., 1987).

Evaluation of the clinical role of Src in HCC presented various results. Total and pSrc (use of Clone 28 and rabbit antibody against pSrc) expression (IHC) was not correlated with the patients' age or gender, history of viral infection, presence of liver cirrhosis (Ito et al., 2001; Lau et al., 2009), tumour cell polymorphism, mitotic figures, acini formation, presence of clear and giant cells, different size of tumour cells, presence of apoptotic features, expression of Proliferating Cell Nuclear Antigen (PCNA), p53, Transforming Growth Factor-α, cholangioepithelial markers (CK-19 and AE1-AE3) and most importantly patients' survival (Lau et al., 2009). Alpha fetoprotein expression has been described to correlate directly with both total and active Src (use of rabbit antibody) immunohistochemical expression (Lau et al., 2009) or indirectly with activated immunohistochemical Src expression (use of Clone 28) (Ito et al., 2001).

With respect to tumour grade of differentiation and proliferative capacity different data have been described. Higher pSrc expression (use of Clone 28 and Mab327) (IHC, immunoblotting and Wesern blot) was correlated both with higher (Ito et al., 2001) and lower (Masaki et al., 1998) degree of differentiation, while data indicating that there was no correlation also exist (use of rabbit antibody) (Lau et al., 2009). Similarly, cellular proliferation, reflected by Ki-67 labelling index, was not correlated with tSrc and pSrc (use of rabbit antibody) immunohistochemical expression (Lau et al., 2009) or to be independently (in multivariate analysis) inversely correlated with higher pSrc expression (use of Clone 28) (IHC) (Ito et al., 2001). Finally, higher pSrc expression (use of Clone 28) (IHC) was detected more frequently in tumours without intrahepatic metastases (Ito et al., 2001).

The evidence on Src expression in cholangiocarcinoma is limited and suggests no correlation of the kinase (IHC expression of pSrc with the use of Clone 28) with either the grade or the stage of the tumour (Ito et al., 2001). Finally, one study revealed pSrc expression (use of Mab327) (immunoblotting) in childhood hepatoblastoma (Bjelfman et al., 1990).

Pancreatic neoplasia

Several studies have approached the clinical significance of Src in human pancreatic adenocarcinoma. Increased cytoplasmic tSrc expression (IHC) and pSrc (Tyr418) (IHC) was noted in the majority of the malignant specimens compared to the normal controls (Hakam et al., 2003; Morton et al., 2010; Nagaraj et al., 2010; Chatzizacharias et al., 2010). More specifically tSrc levels were found to increase in the cytoplasm and the nucleus of cells in progression from normal tissue to inflammation (pancreatitis) and cancer (Nagaraj et al., 2010).

Tumour stage was found to correlate with tSrc immunohistochemical expression (Chatzizacharias et al., 2010), while the opposite has also been described (Hakam et al., 2003; Morton et al., 2010). Additionally, pSrc (Tyr418) expression (IHC) was not correlated with stage (Morton et al., 2010). tSrc expression (IHC) has also been correlated with the presence of lymph node metastasis and vascular invasion, while pSrc (Tyr418) failed to correlate (Morton et al., 2010). Opposite data also exist, suggesting no correlation between tSrc expression (IHC) or any of the T, N and M substages of the disease (Chatzizacharias et al., 2010). With respect to tumour histological grade, different results have been published, with some suggesting its correlation with increased tSrc expression (IHC) (Morton et al., 2010; Nagaraj et al., 2010) and others that the two parameters are not associated (Hakam et al., 2003; Chatzizacharias et al., 2010). pSrc (Tyr418) expression (IHC) was not correlated with grade (Morton et al., 2010). No association was found between tSrc and pSrc (Tyr418) expression (IHC) and the size and marginal status of the tumour (Morton et al., 2010) or between tSrc (IHC) expression and the proliferative capacity (Ki-67 expression) of the tumours or the age of the patients (Chatzizacharias et al., 2010). Opposing evidence has also been published on the role of Src in patients' survival. tSrc expression (IHC) has been shown not to correlate with survival (Hakam et al., 2003; Morton et al., 2010), while high membranous tSrc expression (IHC) correlated with decreased overall survival (Nagaraj et al., 2010) and cytoplasmic with improved survival (Chatzizacharias et al., 2010; Nagaraj et al., 2010), being also an independent prognostic factor in multivariate analysis (Chatzizacharias et al., 2010). In one study, the combination of grade and tSrc expression (IHC) identified a group with significantly poor survival, while tumours with regions of very high pSrc (Tyr418) expression had a significantly poorer outcome after pancreaticoduodenectomy (Morton et al., 2010).

Finally, one study found pSrc (use of Mab327) to be expressed in childhood pancreatoblastoma (Bjelfman et al., 1990), while another evaluated the role of Src in human pancreatic endocrine tumours (Di Florio et al., 2007). tSrc expression (IHC and Western blot) was diffusely cytoplasmic and of clearly stronger intensity compared to normal cells, while pSrc (Tyr416) expression (IHC) was significantly higher in neoplastic compared to normal specimens. tSrc expression in primary tumours was significantly higher than in metastatic lesions. No significant correlation was found

with tumour differentiation (Di Florio et al., 2007).

Renal neoplasia

The product of the src gene has been detected (immunoblotting) in Wilm's tumour (Bjelfman et al., 1990), while the carcinogenic effect of the v-src oncogene in human renal cells has been described *in vitro* (Nanus et al., 1991). Normal cells transfected with the oncogene developed clonal karyotypic abnormalities, including, invariably, the deletion of the 3p14-23 region on chromosome 3p and often others on chromosomes 5, 7, 17, 18 and Y, which are frequently seen in renal cell carcinoma (RCC). Phenotypically the cells developed a well-defined epithelial morphology typical of the RCC cells, decreased doubling time, loss of contact inhibition, anchorage independence, and immortality, while in an *in vivo* mouse model caused tumourogenecity (Nanus et al., 1991).

Bladder neoplasia

Elevated tSrc and pSrc (use of Mab327) expression was identified by Western blot analysis in human bladder carcinomas compared to normal tissue specimens (Fanning et al., 1992). Maximum Src activity, expressed by pSrc (use of Mab327) (Western blot), was observed in T1 stage invasive carcinomas, suggesting its role in early invasion (Benistant et al., 2000). However, such activation was observed also in advanced tumours. Increased Src activity was observed along all grades of differentiation (Benistant et al., 2000), but primarily (>80%) in low-grade (grade 1 and 2) tumours (Fanning et al., 1992).

Prostatic neoplasia

Activated Src levels (pSrc Tyr416) (Western blot) have been found to be increased in 75% of the more advanced (Gleason 7-9) prostate carcinoma cases, while the activated kinase was not expressed in the less advanced (Gleason 4-6) tumours (Paronetto et al., 2004). Activated Src expression has also been studied in prostate cancer with respect to the acquisition of androgen independence (Tatarov et al., 2009). pSrc (Tyr419) levels (IHC and Western blot) were significantly higher in specimens from androgen-independent tumours, with more intense staining in the cytoplasm and the membrane. The staining characteristics were also compared between paired samples (androgen-dependent tumours that acquired independence) and an increase in both pSrc (Tyr419) cytoplasmic (26%) and membranous (28%) expression was observed. In this study, pSrc (Tyr419) staining characteristics were not correlated with either the Gleason score at diagnosis or the PSA levels at relapse. However, samples from patients with confirmed bone metastasis presented more intense membranous, but not cytoplasmic, pSrc (Tyr419) staining. Survival analysis revealed that patients with increased pSrc (Tyr419) expression in the membrane at the transition from androgen-dependent to independent cancer presented significantly lower time to relapse and time to death after relapse, resulting in significantly lower overall survival. Multivariate analysis rendered pSrc (Tyr419) expression as an independent prognostic factor for overall survival and time from relapse to death (Tatarov et al., 2009).

Endometrial and cervical neoplasia

Total and activated Src (pSrc Tyr418) expression evaluated by immunohistochemistry and Western blot analysis was found to be elevated in endometrial adenocarcinomas, including endometrioid, papillary serous and clear cell carcinomas, compared to benign controls (Desouki and Rowan, 2004; Chatzizacharias et al., 2011). No difference was detected in the distribution of tSrc and pSrc (Tyr418) between proliferative and secretory phases of the endometrium in benign tissues (Desouki and Rowan, 2004) or between the different histological types of the malignant specimens (Desouki and Rowan, 2004). Furthermore, no correlation was found between tSrc and pSrc (Tyr418) expression, overexpression and intensity of immunostaining (IHC) and patient's age, tumour type, grade, FIGO stage and Ki-67 proliferative index (Chatzizacharias et al., 2011). However, a trend was noticed between patients' survival and tSrc expression and overexpression (IHC) and pSrc (Tyr418) expression and staining intensity (IHC) in these tumours (Chatzizacharias et al., 2011). Furthermore, tSrc expression was also evaluated separately (IHC) in the two most common types of the disease, the endometriod and the papillary serous (Chatzizacharias et al., 2011). In the first one, tSrc expression and overepxression were significantly correlated with the histological type, with the adenocarcinoma cases with squamous elements showing enhanced expression, and pSrc expression with the grade of differentiation. No significant correlations were identified in the second subgroup (Chatzizacharias et al., 2011).

Similarly, pSrc (Tyr416) immunohistochemical expression was significantly increased in HPV-positive cervical cancer tissue specimens compared to normal controls, presenting cytoplasmic and membranous pattern of immunostaining (Kong et al., 2011).

Ovarian neoplasia

Published evidence supported the significance of Src in metastatic ovarian cancer (Wiener et al., 2003). Immunohistochemical tSrc expression was significantly higher in these tumours compared to normal ovarian epithelium, with more than half of them presenting moderate to strong staining intensity. Evaluation of the specimens for pSrc (use of Mab327) followed a similar pattern, with almost all samples with high tSrc staining intensity also expressing pSrc (IHC). tSrc expression

was not correlated with the type of the tumour (papillary serous or serous) (Wiener et al., 2003).

Breast neoplasia

Over the years, several studies using different methods (IHC, immunoprecipitation, immunoblotting, Western blot and tyrosine kinase assays) presented data indicating increased cytoplasmic tSrc expression and/or activity in human breast cancer compared to normal controls or benign breast disease specimens (Jacobs and Rubsamen, 1983; Hennipman et al., 1989; Koster et al., 1991; Ottenhoff-Kalff et al., 1992; Verbeek et al., 1996; Reissig et al., 2001; Belsches-Jablonski et al., 2001; Ito et al., 2002; Wilson et al., 2006; Madan et al., 2006; Campbell et al., 2008; Zou et al., 2009; Morgan et al., 2009). tSrc expression and pSrc (use of Clone 28) staining intensity (IHC) increased with tumour invasive status (Zou et al., 2009), while interestingly, data supporting the opposite also exist, that is, no difference in cytoplasmic pSrc (Ty416) (IHC) between invasive and *in situ* lesions (Madan et al., 2006). Nuclear activated Src levels (pSrc Tyr416) (IHC and Western blot) were found to be both significantly lower (Morgan et al., 2009) and higher (Campbell et al., 2008) in cancer specimens than normal controls. Opposing data have also been published, suggesting no difference in Src expression (reverse transcriptase PCR) between malignant and normal breast tissue specimens (Elsberger et al., 2010).

The evaluation of possible associations of Src expression and activity with clinicopathological parameters revealed controversial results. No correlation was found between Src kinase activity (tyrosine kinase assay) and patients' age (Hennipman et al., 1989). With respect to tumour size the published evidence is contradictory, with some supporting that it is correlated with membranous tSrc and pSrc (Tyr419) expression (IHC) (Elsberger et al., 2009, 2010) and others that there is no correlation (IHC, Western blot analysis and tyrosine kinase assay) (Hennipman et al., 1989; Wilson et al., 2006; Campbell et al., 2008). No correlation was found between Src activity (kinase assay) and lymph node metastasis (Hennipman et al., 1989), while nuclear activated Src (pSrc Tyr416) expression (IHC and Western blot) was correlated with lymph node negativity (Campbell et al., 2008). Levels of pSrc (Clone 28 antibody) (IHC and Western blot) were associated with the presence of comedo necrosis and increased proliferation of ductal *in situ* tumour cells represented by the nuclear grade and high Ki-67 labelling index (Wilson et al., 2006). Additionally, nuclear tSrc expression (IHC) was associated with Ki-67 proliferation index (Elsberger et al., 2010). On the other hand, data also exist for the opposite, that is, no correlation between immunohistochemical tSrc or pSrc (Tyr416 and use of Mab327) expression and Ki-67 levels (Verbeek et al., 1996; Campbell et al., 2008). Some published evidence also supported the correlation between cytoplasmic tSrc, nuclear pSrc (Tyr215) and membranous pSrc (Tyr419) expression (IHC) and grade of tumour differentiation (Elsberger et al., 2009, 2010), while others argue for the opposite (Verbeek et al., 1996; Campbell et al., 2008). Interestingly, one study suggested that activated immunohistochemical tSrc expression was correlated with smaller tumour size, lower grade of differentiation, decreased Ki-67 labelling index, early TNM stage and absence of lymph node involvement, supporting the role of Src in the early stages of breast tumourogenesis (Ito et al., 2002).

The significance of the oncogene products Her2, EGFR/Her1 and p53 in breast cancer is well established. Several studies evaluated possible associations between the expression of these molecules with Src expression and activity that would signify the presence of clinically important molecular interactions. In one study, the expression of pSrc (use of Mab327) and Her2 was correlated only in 23% of the cases, suggesting that Src may or may not function independently from Her2 in breast carcinogenesis (Belsches-Jablonski et al., 2001). Other studies presented evidence that cytoplasmic, nuclear and membranous tSrc and cytoplasmic and membranous pSrc (Tyr419 and Tyr215) expression (IHC and Western blot) (Wilson et al., 2006; Elsberger et al., 2009, 2010) were correlated with Her2 positivity, supporting a possible interaction of the two molecules. With regards to EGFR/Her1 expression, no correlation has been established with pSrc (use of Clone 28) levels (IHC and Western blot) (Wilson et al., 2006), while no correlation has been identified between pSrc (use of Mab327) (IHC) and p53 expression either (Verbeek et al., 1996).

The presence of estrogen (ER) and/or progesterone receptors (PR) is a prognostic factor for breast cancer and affects management decisions. The published evidence on the possible correlation of Src expression with these hormone receptors is controversial. Tumours expressing PR have been found to display higher cytoplasmic and membranous tSrc expression (IHC) (Elsberger et al., 2010). With regards to ER status, studies presented data revealing no correlation between ER positivity and pSrc (use of Clone 28) expression or actvity (IHC, Western blot and tyrosine kinase assay) (Hennipman et al., 1989; Wilson et al., 2006). Not surprisingly, evidence supporting a correlation with tSrc cytoplasmic and membranous expression (IHC) (Elsberger et al., 2010) and nuclear pSrc (Tyr2125) (IHC) (Elsberger et al., 2009) and an inverse correlation with cytoplasmic tSrc and membranous tSrc and pSrc (Tyr419) (IHC) (Elsberger et al., 2009) has also been published.

Many studies have also approached the potential implication of Src in disease prognosis with controversial results. Studies showed that cytoplasmic Src activity (IHC, kinase assay, reverse transcriptase PCR) was associated with early systemic disease relapse (Hennipman et al., 1989) and reduced patients' survival in ER positive tumours (Morgan et al., 2009; Elsberger

et al., 2010), while elevated nuclear expression of activated Src (pSrc Tyr416) (IHC) was correlated with improved hormonal response (Morgan et al., 2009), low Nottingham prognostic index, improved survival and decreased recurrence in ER and PR positive tamoxifen-treated patients (Campbell et al., 2008). This last correlation failed to be an independent prognostic factor after multivariate analysis, while in patients with PR negative tumours the significance was lost. Another study suggested that pSrc (use of Clone 28) levels (IHC and Western blot) failed to become an independent prognostic factor of 5-year disease recurrence after multivariate analysis, even though they were identified as a significant predictor with univariate analysis (along with nuclear grade, Ki-67, Her2 status, age and margin status) (Wilson et al., 2006). Similar results were recently presented by Elsberger et al. (2010), showing that tSrc expression (IHC) failed to become an independent prognostic factor of patients' survival after multivariate analysis. A study from the same group identified cytoplasmic pSrc (Tyr215) as an independent prognostic factor for disease-specific survival; cytoplasmic pSrc (Tyr215) in ER negative, ER/Her2 negative and EE/Her2/PR negative patients; and nuclear pSrc (Tyr215) in ER positive patients (Elsberger et al., 2009). The role of Src in breast cancer disease prognosis was also evaluated with respect to the patient's age by evaluating the gene expression signature (Anders et al., 2008). In women younger than 45 years of age, a low probability of Src deregulation (along with a low probability of E2F and a high probability of phosphoinositide 3-kinase [PI3K], Myc and ß-catenin) conferred a 4.15-fold worse prognosis. On the contrary, in women older than 65 years of age, worse prognosis (2.7-fold) was associated with a high probability of Src deregulation (along with a high probability of the transcription factor E2F and a low probability of PI3K, Myc and ß-catenin). Another interesting study in this field suggested that src gene expression (cytokine gene expression analysis) was associated with late onset (>5 years after diagnosis) metastatic disease, being an independent prognostic factor particularly for bone metastasis (Zhang et al., 2009). This evidence was further supported by the overexpression of osteopontin by tumour cells from invasive tumours with increased Src activity (Zou et al., 2009). On the contrary, no correlation was made between src expression and lung or brain relapse, while an association was made with liver relapse, but only in ER positive patients (Zhang et al., 2009).

Haematological neoplasia

The role of Src in human haematologic malignancies has been supported by studies in various leukaemia and lymphoma cell lines. Some, such as K562, Namalva and U937, expressed higher tSrc levels (IHC) compared to normal mononuclear cells used as controls, while others, such as MOLT4, BALL-1 and Daudi, did not (Irino et al., 1988). The use of immunoprecipitation and Western blot analysis detected constitutively activated SFKs, including pSrc (Tyr416), in various B lymphoma cell lines (Ke et al., 2009). Similarly, the 12-fold increase in Src activity in human GM-CSF-dependent leukemia cell line TF-1 was attributed, at least in part, to the overexpression of Src on the RNA and protein levels (immunoprecipitation and Northern blot respectively) (Horn et al., 2003). The use of techniques inhibiting Src activity proved the role of the kinase in malignant cells. Src antisense recombinant plasmid treatment of human K562 leukemia cells resulted in reduced cellular proliferation and inhibited erythropoietin-induced haemoglobin synthesis (Kitanaka et al., 1994), while similar treatment decreased cellular proliferation in human U937 monoblastoid leukemia cells (Waki et al., 1994). Src tyrosine kinase inhibitors resulted in inhibition of B lymphoma cellular growth *in vitro* and tumour growth, demonstrated by smaller sized spleens with fewer blasts, in an *in vivo* mouse model (Ke et al., 2009).

However, the clinical significance of Src in haematological neoplasia is still obscure. In samples from acute myelogenous and lymphocytic leukaemia patients, Src expression was undetectable by quantitative total RNA analysis with the use of dot blot hybridization (Mavilio et al., 1986). Other published evidence supported that there was no difference in the immunohistochemical expression patterns of tSrc levels between normal and malignant or T- and B-lineage lymphoid cells (Punt et al., 1992) or a variable immunohistochemical expression, suggesting no correlation between tSrc expression and the type or the French-American-British classification of the disease (Irino et al., 1988).

Soft tissue and bone neoplasia

The evidence on the clinical significance of Src in human soft tissue malignancies remains limited. In an effort to quantify Src activity in spontaneous human sarcomas, kinase activity, detected with the use of an autoradiograph IgG protein kinase assay in each sarcoma specimen was compared to paired normal tissues and fibroblasts (Jacobs and Rubsamen, 1983). In about 33% of the sample a 4-20-fold increase in Src activity was demonstrated. Furthermore, pSrc (Tyr419) expression (IHC) was considered minimal in the metastatic sites of human osteosarcoma cells in an *in vivo* mouse model, indicating the possibility that Src may not have an essential role in this type of tumour (Hingorani et al., 2009). Finally, pSrc (use of Mab327) has been detected (immunoblotting) in malignant cells from rhabdomyosarcoma tumour of the childhood (Bjelfman et al., 1990).

Skin neoplasia

Early evaluation of Src expression with the use of

immunoprecipitation and Western blot analysis showed no difference between melanomas and normal skin samples (Loganzo et al., 1993). On the contrary, a more recent study suggested that malignant skin lesions express tSrc and pSrc (mouse antibody for pSrc) (Western blot) at levels significantly higher than normal tissue (Lee et al., 2010). Immunohistochemical analysis of the three most common types of skin tumours, basal cell carcinoma, SCC and melanoma, revealed moderate Src expression in SCCs and melanomas, while only mild in basal cell tumours (Lee et al., 2010). Similar were the results from another study, suggesting mainly weak pSrc (Tyr416) immunohistochemical expression in cutaneous, mucosal and metastatic melanomas (Homsi et al., 2009). This study also evaluated the clinical significance of pSrc (Tyr416) expression and identified a significantly worse survival in cases which strongly stained for the molecule, while no association was established between pSrc (Tyr416) expression and patients' age and sex, tumour histopathological type and location (Homsi et al.,

Table 2. Clinical trials for the use of Src inhibitors as chemotherapeutic agents.

Src inhibitor	Identification code	Phase of clinical trial
Dasatinib	NCT00858403	Phase 2 (lung cancer)
	NCT00564876	Phase 2 (non small cell carcinoma of the lung)
	NCT00787267	Phase 2 (non small cell carcinoma of the lung)
	NCT00936975	Phase 2 (prostate and metastatic cancer)
	NCT00546104	Phase 2 (stage III and IV breast cancer)
	NCT00859937	Phase 2 (head and neck cancer)
	NCT00507767	Phase 2 (head and neck cancer)
	NCT00563290	Phase 2 (skin cancer except melanoma)
	NCT00624585	Phase 2 (myelodysplastic syndromes)
	NCT00306202	Phase 1 (leukaemia)
	NCT00978731	Phase 1 (leukaemia)
	NCT00316953	Phase 1 (leukaemia and childhood tumours of unknown origin)
	NCT00509041	Phase 2 (pleural mesothelioma)
	NCT00652574	Phase 1 (pleural mesothelioma)
	NCT00835679	Phase 1 (colon cancer and metastatic cancer)
Dasatinib, Bevacizumab	NCT00792545	Phase 1 (breast, lung, renal, ovarian, prostate cancer, melanoma and gastrointestinal stromal tumours)
Dasatinib, Bevacizumab, Paclitaxel	NCT01015222	Phase 1 (advanced stage cancers)
Dasatinib, Dacarbazine	NCT00597038	Phase 1 and 2 (metastatic melanoma)
Dasatinib, Paclitaxel	NCT00820170	Phase 1 and 2 (breast cancer)
Dasatinib, Paclitaxel, Carboplatin	NCT00672295	Phase 1 (ovarian cancer and peritoneal metastases)
Dasatinib, Cetuximab	NCT00388427	Phase 1 (advanced stage cancers)
Dasatinib, Cetuximab, Leucovorin, Oxaliplatin, 5-FU	NCT00501410	Phase 1 (colon cancer)
Dasatinib, Erlotinib	NCT00444015	Phase 1 (non small cell carcinoma of the lung)
Dasatinib, Imatinib	NCT00982488	Phase 2 (leukemia)
Dasatinib, Vandetanib	NCT00996723	Phase 1 (glioma of the pons)
Dasatinib, all-trans retinoic acid	NCT00892190	Phase 1 (acute myeloid leukemia)
Dasatinib, [a]elphalan, Prednisone	NCT01116128	Phase 2 (myeloma)
Dasatinib, Temozolomide, radiotherapy	NCT00895960	Phase 1 and 2 (glioblastoma)
Saracatinib	NCT00528645	Phase 2 (lung cancer and metastatic cancer)
(AZD0530)	NCT00752206	Phase 2 (osteosarcoma)
Saracatinib, Paclitaxel	NCT01196741	Phase 2 και 3 (ovarian cancer and peritoneal metastases)
Saracatinib, Paclitaxel, Carboplatin	NCT00610714	Phase 2 (ovarian cancer)
	NCT00496028	Phase 1 (neoplasms)
	NCT01000896	Phase 1 (non small cell carcinoma of the lung and ovarian cancer)
Imatinib mesylate	NCT00041197	Phase 3 (gastrointestinal stromal tumours)
XL999	NCT00277303	Phase 2 (colon cancer)
	NCT00277290	Phase 2 (ovarian cancer)
	NCT00277329	Phase 2 (non small cell carcinoma of the lung)
	NCT00491699	Phase 1 (non small cell carcinoma of the lung)
	NCT00277316	Phase 2 (renal cancer)
	NCT00322673	Phase 2 (acute myeloid leukemia)
XL228	NCT00526838	Phase 1 (lymphoma)
KX2-391	NCT00646139	Phase 1 (lymphoma, lymphoproliferative disorders, small bowel cancer)

2009). An effort to identify potential differences in Src activity between primary melanomas, including superficial spreading, nodular and acrolentiginious types, and their metastases was performed by Barnekow et al. (1987). Varying kinase activity, detected by immunoprecipitation, was determined among different patients and among metastatic sites in the same patient, while no correlation was found with the type of the tumour. Nonetheless, Src activity was 4-20 times higher in melanomas compared to normal skin (Barnekow et al., 1987).

Conclusion

There is a large body of evidence providing a clear insight into the role of Src in malignant transformation and regulation of cellular behaviour *in vitro* and *in vivo*. However, results from studies on human cancer histopathological specimens are controversial, with different studies presenting different data for malignancies of the same or different organs. Several groups identified increased Src activity, due to increased protein expression and/or intrinsic kinase activity, in different types of malignancies, including astrocytomas, neuroblastomas, thyroid and tongue cancer, lung tumours and malignant mesotheliomas, oesophago-gastric, colorectal, liver and pancreatic tumours, malignancies of the male and female urogenital tract, breast cancer, sarcomas, haematological and skin neoplasias. In some of these cases, such as in neuroblastomas, tongue cancers, lung tumours and malignant mesotheliomas, colonic, hepatic and pancreatic neoplasms, prostate, breast cancer and melanomas, increased Src expression and/or activity was associated with important clinicopathological characteristics of the disease. Nonetheless, opposing data have also been published, for almost everyone of the malignancies mentioned earlier, arguing that Src expression and activity is not altered during the process of carcinogenesis or, even if it is, these changes are clinically and pathologically unimportant. The described differences in the literature could be explained by the use of different methods (IHC, immunoblotting, PCR, kinase asssays, Western and Northern blot analysis) and antibodies detecting different forms of the kinase (total Src, pSrc etc.) at the various cellular locations and stages of the disease process, or even by the different definitions of "increased Src expression" used in the various study designs and the variability in the samples' size. Therefore, all published evidence should be approached and interpreted carefully, as different forms of expression and different localisations of these forms may have a different impact in cellular behaviour.

Given the diversity in the possible roles of Src in malignant transformation and disease progression in humans, substances that inhibit its enzymatic activity (dasatinib, bosutinib, AZD0530) or the binding with its substrates (KXO1) have been tested preclinically, *in vitro* and *in vivo*, for their effects on cancer cellular physiology and behaviour, with promising results (Kopetz et al., 2007; Aleshin and Finn, 2010). More recently, several agents have entered clinical trials (mostly phase I and II) for possible use, alone or in combination with currently established chemo-therapeutic agents, in the treatment of a variety of haematological and solid malignancies, including breast, colon, lung, ovarian, prostate and pancreatic ones (Table 2) (retrieved 09/04/2011, from http://www.clinicaltrials.gov and http://www.cancer.gov/clinicaltrials).

A large body of evidence has identified Src as an important modulator and possible therapeutic target in human malignancy. However, our understanding of its actions, substrates and signalling pathways, as well as its role and clinical significance, is far from complete. Future studies should concentrate on providing answers and new insight into these unresolved questions with an aim of translating the scientific knowledge to everyday medical practice.

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