CHAPTER

7

STAT3 as a Major Contributor to Chemoresistance

David S. Lee, Jennifer R. Grandis, Daniel E. Johnson

University of California at San Francisco, San Francisco, CA, USA

Abstract

Signal transducer and activator of transcription 3 (STAT3) is a well-validated anticancer target that promotes tumorigenesis. Although it has historically been considered "undruggable", multiple classes of direct STAT3 inhibitors have recently been discovered that successfully abrogate STAT3 activation or DNA-binding activity, ultimately generating selective cytotoxicity against cancerous cells. Considering the growing body of evidence implicating STAT3 in the development of treatment resistance, direct STAT3 inhibitors have promising potential to augment the antitumor effects of conventional chemotherapy drugs, radiation, and molecular targeting agents, leading to improved patient outcomes. This chapter focuses on the progress made in developing direct STAT3 inhibitors and their evaluation in preclinical models and clinical trials.

ABBREVIATIONS

DBDI	DNA binding domain inhibitor
DN	Dominant-negative
EGFR	Epidermal growth factor receptor
FDA	Food and Drug Administration
FGFR	Fibroblast growth factor receptor
HNSCC	Head and neck squamous cell carcinoma
hSIE	High affinity serum-inducible element
IL-6	Interleukin-6
IL-6R	Interleukin-6 receptor
JAK	Janus kinase
mts	Membrane-translocation sequence
NDI	N-terminal domain inhibitor
NMR	Nuclear magnetic resonance
NSCLC	Non-small cell lung cancer
NTD	N-terminal domain
pY	Phosphotyrosine
SDI	SH2 domain inhibitor
SH2	SRC-homology 2
STAT	Signal transducer and activator of transcription
VEGF	Vascular endothelial growth factor

Targeting Cell Survival Pathways to Enhance Response to Chemotherapy. http://dx.doi.org/10.1016/B978-0-12-813753-6.00007-X

7.1 INTRODUCTION

The family of signal transducer and activator of transcription (STAT) proteins facilitates a wide variety of physiological functions essential to cell survival, including proliferation, differentiation, and inflammation [1,2]. In total, there are seven members of the STAT family (STAT1-4, STAT5a, STAT5b, and STAT6) [3,4]. Their structures consist of an N-terminal domain, coiled-coil region, DNA-binding sequence, and SRC-homology 2 (SH2) domain. The N-terminal domain facilitates STAT3 oligomerization, a poorly understood process that is important for enhanceosome stability and association with regulatory proteins. Similarly, the coiled-coil region serves as a loading site for regulatory proteins. The DNA-binding domain recognizes a specific DNA sequence motif, enabling direct interactions with response elements in the promoter regions of STAT target genes. The SH2 domain binds to phosphotyrosine (pY) residues present on activated tyrosine kinases, leading to subsequent phosphorylation of the STAT protein by the bound kinase. The SH2 domain also facilitates STAT dimerization via reciprocal associations with a key pY residue (pY705 in STAT3) of another monomer [1]. This dimerization plays an essential role in the binding of the STAT protein to DNA. Transient and highly regulated activation of STATs leads to expression of a variety of target genes, encoding proteins such as Bcl-X_L, c-Myc, cyclin D1, and VEGF [1,5].

STAT activation can be achieved through multiple mechanisms (Fig. 7.1). In response to stimulation by growth factors and cytokines, cell-surface receptors dimerize, leading to activation of an intrinsic receptor tyrosine kinase activity or the activation of receptor-associated kinases like Janus kinases (JAKs). These activated kinases then facilitate recruitment of inactive STAT monomers by phosphorylating tyrosine residues in the cytoplasmic region of the receptor protein. The receptor pY residues serve as docking sites for the SH2 domain of STAT monomers within the cytoplasm. Once recruited, STAT monomers undergo receptor- or JAK-mediated tyrosine phosphorylation, followed by dimerization via interactions between the SH2 domain of one STAT monomer with a pY residue on another monomer. The STAT dimers translocate into the nucleus where they act to modulate the expression of STAT target genes [1]. Well-studied signaling pathways, including those activated by interferon-gamma, interleukin-6 (IL-6), epidermal growth factor, and platelet-derived growth factor, employ STAT activation as described [6–9]. In addition, nonreceptor tyrosine kinases, such as Abelson leukemia protein and SRC-related kinases, have been shown to phosphorylate/activate STATs in the absence of an extracellular stimulus [10–12]. Cell confluence, leading to cadherin–cadherin stimulation, has been proposed as another mechanism of nonreceptor-mediated STAT activation, although the role of cellular microenvironment in STAT activation is less well characterized [13–15].

Consistent with the multifaceted role of STAT in cellular proliferation and survival, a growing body of evidence supports the idea that dysregulated STAT activity—STAT3, in particular—plays a direct role in oncogenesis [16,17]. Constitutive STAT3 activation has been observed in multiple cancers and is associated with poor clinical prognoses [18–20]. *In vitro* studies have demonstrated that inhibition of STAT3 in cancer cell lines with aberrantly activated STAT3 results in decreased proliferation and increased apoptosis [21–23]. However, inhibition of STAT3 does not appear to result in cytotoxicity in models of normal adult tissue, suggesting pathway redundancy under physiological circumstances and oncogenic dependency in the context of cancer [1,24]. With numerous studies establishing additional roles for

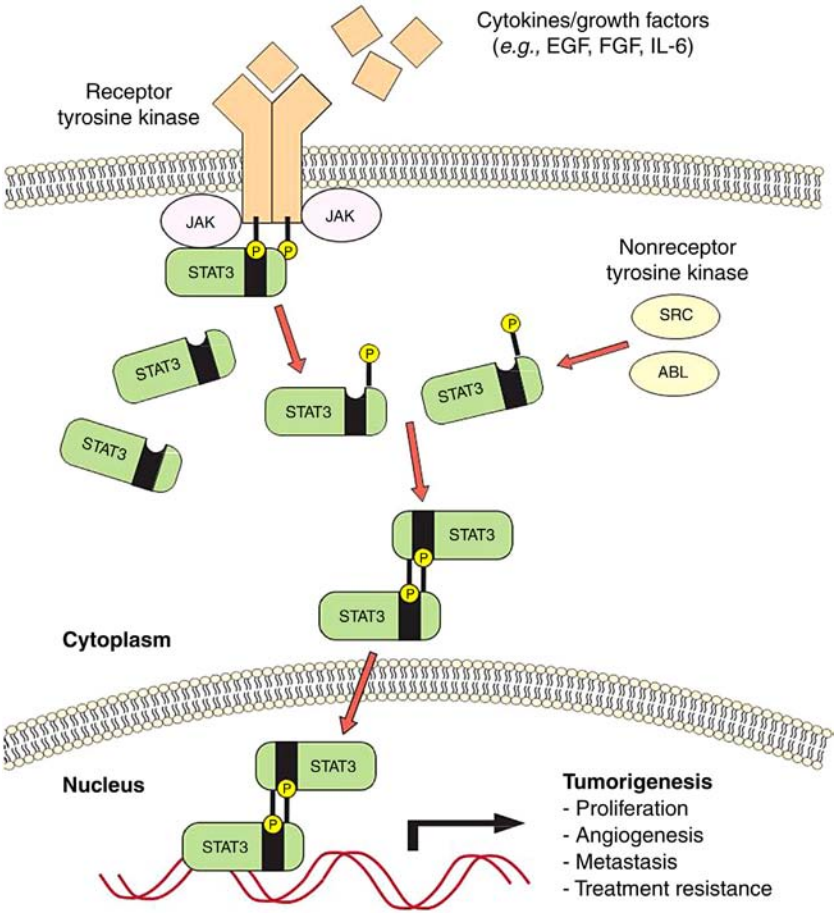


FIG. 7.1 **STAT3 activation and signal transduction pathway.** Ligand-activated receptor tyrosine kinases and activated nonreceptor tyrosine kinases phosphorylate STAT3 on pY705. The SH2 domain, indicated by the black region on STAT3, serves as a binding site for the pY705 residue of another activated monomer, facilitating reciprocal interactions essential to STAT3 dimerization. STAT3 dimers translocate to the nucleus for subsequent target gene expression.

STAT3 in metastasis, angiogenesis, treatment resistance, and suppression of antitumor immunity, a large body of evidence identifies STAT3 inhibition as a promising therapeutic strategy with a low toxicity profile [1,25,26].

Four main classes of drugs have been developed to directly inhibit the activity of STAT3: SH2 domain inhibitors (SDI), DNA-binding domain inhibitors (DBDI), N-terminal domain inhibitors (NDIs), and STAT3 antisense (Figs. 7.2 and 7.3) [27]. Each of the first three classes utilizes a different aspect of the STAT3 domain structure to abrogate dimerization, protein–DNA interactions, or formation of protein complexes necessary for transcription, while antisense inhibitors act to prevent expression of the protein. Collectively, direct inhibitors of

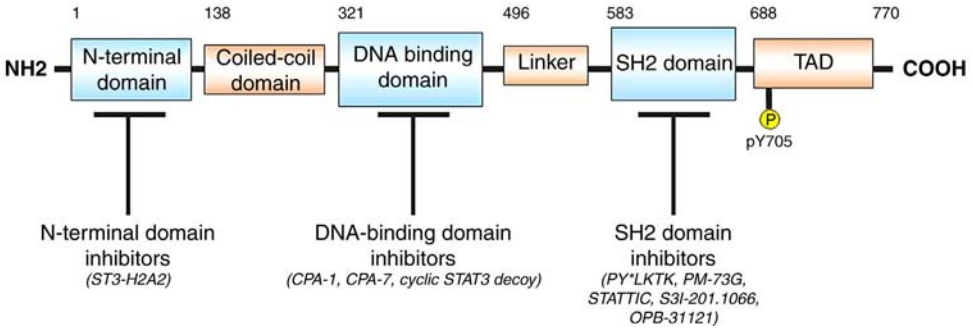


FIG. 7.2 **Schematic of STAT3 domain structure and corresponding target sites for direct STAT3 inhibitors.**

STAT3 face the multiple challenges of intracellular drug delivery, including cell membrane permeability, selectivity, and systemic toxicities. Although a fifth class of indirect STAT3 inhibitors exists in the form of upstream receptor/receptor-associated tyrosine kinase inhibitors, this chapter will focus on depicting current progress related to direct STAT3 inhibitors and their potential use as standalone or adjuvant therapies for cancer.

7.2 ROLE OF STAT3 IN TREATMENT RESISTANCE

A growing body of literature has identified a connection between STAT3 activation and treatment resistance through experiments employing STAT3 dominant-negative (DN) mutants, RNA interference, or treatment with STAT3 inhibitors. For example, exogenous expression of DN-STAT3(Y705F) markedly enhances radiation sensitivity in treatment-resistant glioblastoma cancer cells (U87) that harbor constitutively activated STAT3 [28]. Similarly, siRNA-mediated silencing of STAT3 in chemoradioresistant colorectal carcinoma results in increased sensitivity to 5-fluorouracil and radiation [29,30]. Indeed, a broad number of investigations have reported sensitization to chemotherapy and/or radiation in multiple solid tumors, including breast, prostate, and esophageal cancers, following STAT3 inhibition or downregulation [26,31–33].

Mechanistic studies have characterized the role of STAT3 in generating resistance to radiation, chemotherapy, and immunotherapy. Investigation of non-small-cell lung cancer (NSCLC), melanoma, and colon cancer models resistant to epidermal growth factor receptor (EGFR) inhibitor have suggested that development of resistance to small-molecule inhibitors is associated with autocrine stimulation of interleukin-6 receptor (IL-6R) and fibroblast growth factor receptor (FGFR), ultimately leading to downstream STAT3 activation [15,34,35]. The link between disruption of EGFR signaling pathways and activation of STAT3 has also been shown to be conserved across multiple cancer types, indicating that inhibition of STAT3 may provide a general avenue for overcoming treatment resistance [36]. Similar autocrine feedback loops have been proposed to explain STAT3-mediated resistance to conventional chemotherapy drugs and radiation [26,37–39].

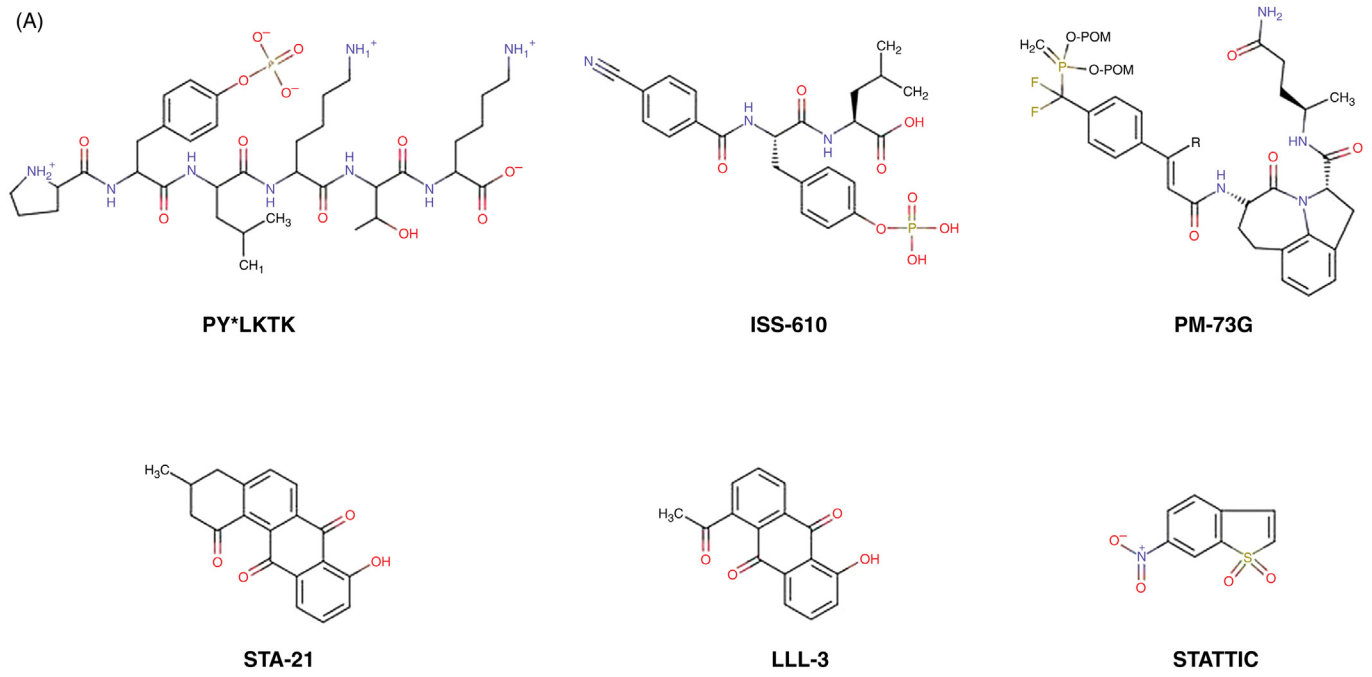
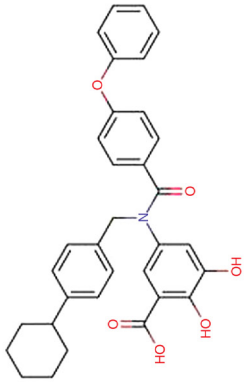
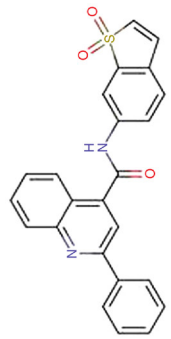


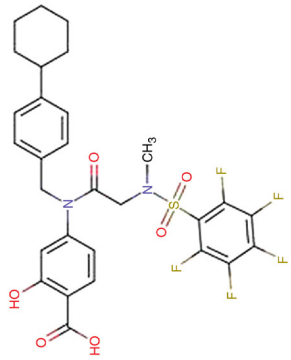
FIG. 7.3 Chemical structures of STAT3 inhibitors. (A) SDIs. (B) DBDIs. (C) Antisense inhibitor. Lowercase nucleotides in the STAT3 decoy molecules indicate phosphorothioate bases. Lowercase nucleotides in AZD9150 indicate bases modified with cET. Chemical structures were drawn using Marvin Sketch. Marvin 17.11.0, 2017, ChemAxon (http://www.chemaxon.com).



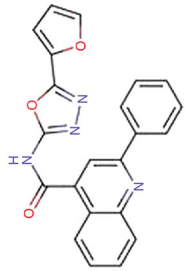
S3I-201.1066



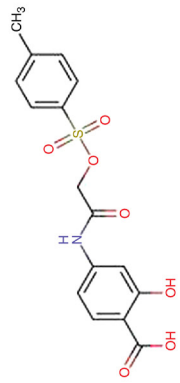
HJC0123



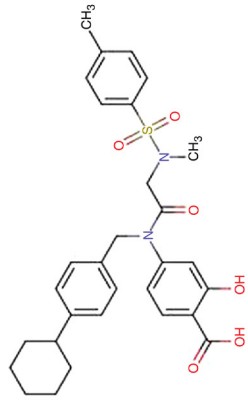
BP-1-102



STX-0119



S3I-201



S3I-1757

FIG. 7.3 *(cont.)*

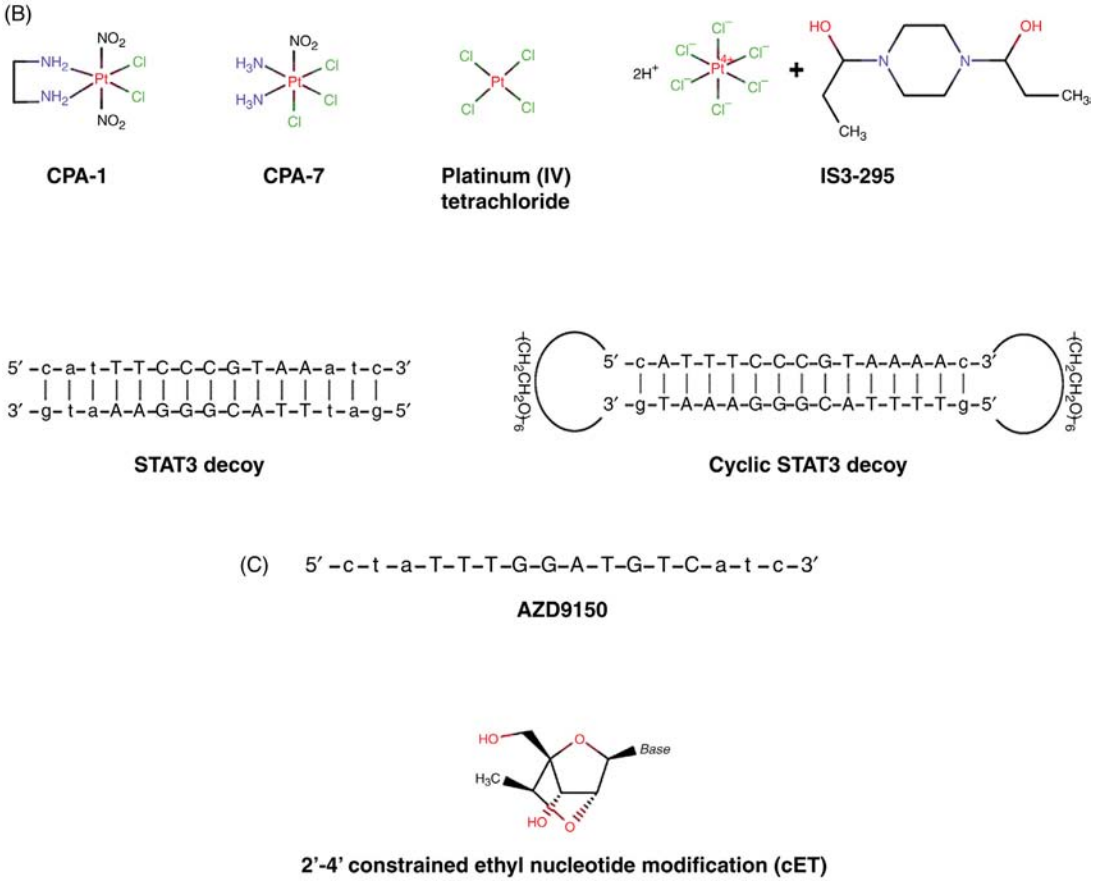


FIG. 7.3 *(cont.)*

Considering that multimodal therapy has become the standard approach to managing patients with solid tumors, targeting STAT3 may provide a promising method for enhancing the antitumor effects of conventional therapies, as well as novel molecular targeting and immunomodulatory agents. Although direct STAT3 inhibitors have not yet been translated to the clinic for standard of care use, concerted efforts to discover and evaluate drugs leading to STAT3 abrogation have generated multiple promising leads.

7.3 DIRECT INHIBITORS OF STAT3

7.3.1 SH2 Domain Inhibitors

The SH2 domain is essential to STAT3 activation. It facilitates association with receptor kinases at pY residues in the receptor cytoplasmic region. Ultimately, this physical association leads to STAT3 phosphorylation by either the receptor or receptor-associated kinases (e.g.,

JAKs). The SH2 domain also stabilizes reciprocal pY/SH2 interactions necessary for homo- or heterodimerization with other STAT monomers. Disruption of SH2-mediated interactions can abrogate STAT3 function and prevent the downstream expression of target genes, leading to inhibition of STAT3-mediated tumor growth. SH2 domains typically span approximately 100 residues and recognize and bind pY residues that occur within specific sequence contexts [40]. The consensus SH2 recognition site for STAT3 has been reported as PY*LKTK (Y* indicates the pY residue) [41]. While nuclear magnetic resonance (NMR) and X-ray crystallography studies of phosphopeptide/SH2 domain complexes have been reported, including the SH2 domains of Src, Lck, and Grb2, the conformational structure of STAT3 binding to various receptors remains elusive [42]. Nonetheless, several different approaches, including phosphopeptide library screening, computer-assisted virtual screening, modification of naturally occurring STAT3 inhibitors, and fragment-based drug design have led to the discovery of two subcategories of SDIs: peptides/peptidomimetics and nonpeptidic small-molecule inhibitors (Table 7.1, Fig. 7.3A) [43,44].

The first proof-of-concept study for phosphopeptide and peptidomimetic STAT3 inhibitors was reported in 2001 [41]. In these studies, the phosphopeptide PY*LKTK demonstrated an *in vitro* dose-dependent inhibition of STAT3 DNA-binding activity in nuclear extracts. It was proposed that PY*LKTK binds to the SH2 domain of inactive STAT3 monomers and competitively inhibits their homodimerization, preventing activation and target gene expression. However, the suppression of STAT3 activity by PY*LKTK was only observed at high concentrations, with an IC_{50} of 235 μM. With respect to selectivity, DNA-binding assays illustrated that PY*LKTK did not appreciably affect STAT5 activity, but did modestly inhibit STAT1 activity [41]. To overcome the obstacle of transporting a phosphorylated peptide across the cell membrane, a hydrophobic membrane-translocating sequence (mts) was linked to PY*LKTK peptide at its C-terminus. Treatment of NIH 3T3/v-Src fibroblasts exhibiting hyperactivation of STAT3 with the PY*LKTK-mts peptide decreased STAT3 DNA-binding activity relative to treatment with an unphosphorylated control peptide (PYLKTK-mts). The decreased STAT3 DNA-binding activity was accompanied by increased apoptosis and reduced malignant transformation [41]. The concentration required to abrogate STAT3-mediated gene transcription was 1 mM, highlighting a need for greater potency to achieve clinical translation [41,89].

Although specificity, stability, and intracellular transport will continue to challenge the clinical translation of peptide inhibitors, some peptidic agents have yielded noteworthy preclinical results. ISS-610, a tripeptide phosphopeptidomimetic based on the original PY*LKTK peptide, demonstrated increased potency with an *in vitro* IC_{50} of 42 μM for suppression of STAT3 DNA-binding activity [45]. It also suppressed the growth and survival of multiple cell lines with constitutively active STAT3, including Src-transformed fibroblasts and NSCLC and breast carcinoma cells.

In an additional attempt to overcome the poor cell permeability and stability associated with phosphopeptides, Mandal et al. [47] designed PM-73G, a novel pro-drug. PM-73G contains a phosphonodifluoromethyl group to prevent dephosphorylation via phosphatases and esterase-labile pivaloyloxymethyl groups to hide the negative phosphate charges. In breast cancer cells, PM-73G disrupted both constitutively activated and IL-6-induced STAT3 DNA-binding activity with an IC_{50} of 100–500 nM [47]. *In vivo* PM-73G also abrogated tumor growth and angiogenesis in mice harboring MDA-MB-468 breast cancer xenografts, although no

TABLE 7.1 Direct STAT3 Inhibitors

	Drug candidate	Target domain	Proposed mechanism of action	Preclinical/Clinical evidence	References
Short peptides	PY*LKTK	SH2	Inhibits homodimerization	*In vitro*	[41]
	ISS-610	SH2	Inhibits homodimerization	*In vitro*	[45]
	PM-73G	SH2	Inhibits STAT3 phosphorylation	*In vitro, in vivo*	[46,47]
Peptidomimetics	STA-21	SH2	Inhibits homodimerization	*In vitro*	[48,49]
	LLL-3	SH2	Inhibits homodimerization	*In vitro, in vivo*	[50,51]
	STATTIC	SH2	Inhibits STAT3 phosphorylation	*In vitro, in vivo*	[52–54]
Nonpeptidic small-molecule inhibitors	BP-1-102	SH2	Inhibits homodimerization	*In vitro, in vivo*	[55]
	S3I-201.1066	SH2	Inhibits homodimerization	*In vitro, in vivo*	[56]
	S3I-1757	SH2	Inhibits homodimerization	*In vitro*	[23]
	STX-0119	SH2	Inhibits homodimerization	*In vitro, in vivo*	[57,58]
	HJC0123	SH2	Inhibits homodimerization	*In vitro, in vivo*	[59]
	OPB-31121	SH2	Inhibits homodimerization	*In vitro, in vivo,* Phase I (advanced solid tumors)	[60–64]
	OPB-51602	SH2	Inhibits homodimerization	*In vitro, in vivo,* Phase I (advanced solid tumors)	[62,65,66]
Platinum-based compounds	CPA-1	DNA binding domain	Inhibits STAT3 DNA binding	*In vitro*	[67]
	CPA-7	DNA binding domain	Inhibits STAT3 DNA binding	*In vitro, in vivo*	[67–69]
	Platinum (IV) tetrachloride	DNA binding domain	Inhibits STAT3 DNA binding	*In vitro*	[67]
	IS3 295	DNA binding domain	Inhibits STAT3 DNA binding domain	*In vitro*	[70]

(*Continued*)

TABLE 7.1 Direct STAT3 inhibitors (*cont.*)

	Drug candidate	Target domain	Proposed mechanism of action	Preclinical/Clinical evidence	References
Double-stranded oligonucleotides	STAT3 decoy	DNA binding domain	Competitively inhibits STAT3 DNA binding	*In vitro, in vivo,* Phase 0 (HNSCC)	[71–79]
	Cyclic STAT3 decoy	DNA binding domain	Competitively inhibits STAT3 DNA binding	*In vitro, in vivo*	[5,74]
Antisense oligonucleotides	2′-*O*-methylethyl antisense oligonucleotide	STAT3 mRNA	Binds STAT3 mRNA for degradation	*In vitro, in vivo*	[80–82]
	AZD9150	STAT3 mRNA	Binds STAT3 mRNA for degradation	*In vitro, in vivo,* Phase 1 (lymphoma and NSCLC)	[83–86]
N-terminal helices	ST3-H2A2	N-terminal domain	Inhibition of downstream suppressors of pro-apoptosis	*In vitro*	[87,88]

impact on apoptosis or cyclin D1 was detected [46]. Subsequent studies of PM-73G in other tumor models have not been reported.

Collectively, peptide and peptidomimetic inhibitors of the SH2 domain have sufficiently overcome the challenges of poor intracellular transport and stability in preclinical studies to warrant their evaluation in clinical trials. To date, PY*LKTK has primarily been employed as a research tool compound to identify and characterize STAT3-dependent processes, including activation of STAT3 by cell confluence, innate and adaptive immune responses of bone marrow progenitor cells, and STAT3-mediated resistance to apoptosis in polyamine-depleted cells [89–91]. Despite their current lack of clinical utility, peptide and peptidomimetic inhibitors that bind to the STAT3 SH2 domain have validated the concept that phosphopeptides can successfully disrupt or prevent STAT3 homodimerization. Further optimization may lead to the discovery of clinically viable agents in this class of compounds.

The search for STAT3 inhibitors has primarily shifted away from peptides to nonpeptidic small molecules because of the latters increased potency, stability, and cell membrane penetration. Computational docking studies and molecular modeling have led to the rational design of multiple promising compounds (Fig. 7.3A) [44,49,54,55]. STA-21 is a small-molecule inhibitor derived from a naturally occurring deoxytetrangomycin that binds to pY*705 residues of STAT3 and prevents dimerization [49]. In human breast carcinoma, rhabdomyosarcoma, and osteosarcoma cell lines that overexpress active STAT3, inhibition of STAT3 DNA binding and induction of apoptosis was observed with STA-21 concentrations of 20–30 μM [48,49]. Furthermore, STA-21 exhibited minimal cytotoxicity against human skin fibroblasts and breast carcinoma cells that lack overexpression of active STAT3 [49]. LLL-3 is an analog

of STA-21 and was found to induce apoptosis of glioblastoma cell lines with hyperactivation of STAT3 at concentrations in the range 10–40 μM [50]. *In vivo* administration of LLL-3 to mice with intracranial glioblastoma tumors resulted in suppression of tumor growth and increased survival, relative to control treatment [50]. In view of evidence suggesting that disruption of STAT3 activity improves therapy response in malignant glioma cells, LLL-3 or related analogs hold potential for clinical application as a chemoradiotherapy adjunct in this devastatingly lethal malignancy [36,50,92,93]. Additional studies have shown that LLL-3 enhances the activity of imatinib against the chronic myelogenous leukemia cell line K562 [51]. However, despite promising preclinical data, neither LLL-3 nor any of its derivatives have been evaluated in human trials.

STAT3 inhibitory compound (STATTIC) is another SDI of STAT3. STATTIC was identified via fluorescence polarization-based assays that screened more than 17,000 compounds for their ability to dissociate phosphopeptide binding to STAT3 SH2 domain; subsequent electrophoretic mobility shift assays confirmed that STATTIC inhibited STAT3 DNA-binding activity [53,54]. *In vivo* studies have shown that STATTIC inhibits tumor growth and promotes apoptosis in breast cancer and head and neck squamous cell carcinoma (HNSCC) xenograft tumors [54]. Interestingly, STATTIC was demonstrated to sensitize nasopharyngeal carcinoma cell lines to treatment with cisplatin and radiation [52]. Nasopharyngeal carcinomas are frequently characterized by constitutively activated STAT3, modest response to chemoradiation, and poor clinical prognosis [94]. However, no further investigations have been performed with STATTIC in multimodal treatment for this carcinoma, leaving its clinical utility largely unexplored.

A few nonpeptidic SDIs have demonstrated efficacy as orally bioavailable compounds. BP-1-102, a derivative of the lead compound S3I-201, has been shown to inhibit STAT3 DNA-binding activity at an IC_{50} of 6.8 μM [55]. Oral administration of BP-1-102 to mice harboring breast cancer and NSCLC xenografts resulted in inhibition of tumor cell proliferation and invasiveness [55]. These data are consistent with results obtained with other nonoral analogs of S3I-201, including S3I-201.1066 and S3I-1757 [23,56]. Pharmacokinetic profiling in tumor-bearing mice showed that steady-state BP-1-102 plasma levels can be achieved that exceed the *in vitro* IC_{50} for STAT3 inhibition. Uptake and accumulation within tumor tissues also reached therapeutic levels, supporting the clinical potential of this orally bioavailable drug [55].

STX-0119 is an orally bioavailable inhibitor that has been shown to inhibit the growth of human lymphomas in mouse models. In these studies, STX-0119 treatment was not associated with any observable nonspecific toxicities to the mice [57,58]. HJC0123, another orally bioavailable STAT3 inhibitor, was generated by fusing together STATTIC and STX-0119 [59]. HJC0123 has been reported to inhibit the growth of breast and pancreatic cancer cell lines with IC_{50} values (0.1–1.25 μM) lower than that can be achieved by either STATTIC or STX-0119 components alone. Oral administration of HJC0123 inhibited the growth of estrogen receptor-negative breast cancer xenograft tumors in mice without evidence of adverse toxicities [59].

OPB-31121 and OPB-51602 are the only two orally administered nonpeptidic SH2 domain antagonists to reach clinical trials to date. Computational docking and molecular dynamics simulation suggest that these antagonists bind with high affinity (K_d 10 nM) to the STAT3 SH2 domain at a binding pocket distinct from the binding sites of other STAT3 SH2 domain antagonists [61,62]. Preclinical data for OPB-31121 demonstrate inhibition of STAT3 DNA

binding across multiple cell lines and against xenograft tumors expressing constitutively active STAT3 [61,63,64]. Furthermore, OPB-31121 has been shown to induce apoptosis in models of drug-resistant leukemia, while exhibiting synergistic antitumor effects in gastric cancer models when combined with 5-fluorouracil [63,64]. These findings indicate the potential of OPB-31121 to act as a monotherapy and to sensitize cancer cells to conventional chemotherapy. However, the first-in-human phase I clinical trial of OPB-31121 in patients with advanced solid tumors demonstrated dose-limiting toxicities (nausea/vomiting, diarrhea, and fatigue) at drug concentrations significantly lower than needed to achieve target inhibition, ultimately resulting in failure to halt or reverse tumor progression [60]. In preclinical studies, OPB-51602 demonstrated similar anti-tumor effects as OPB-31121 [65]. A phase I dose-escalation trial in patients with treatment-refractory solid tumors found that OPB-51602 initially exhibited tolerable toxicities (nausea/vomiting, diarrhea, peripheral neuropathy, and lactic acidosis) at therapeutic concentrations, although side effects increased after multiple treatment cycles and led to eventual treatment discontinuation [66]. Following administration of OPB-51602, phosphorylated STAT3 levels were decreased in peripheral monocytes, and tumor regression was observed in two NSCLC patients (5% of overall cohort; one patient had complete resolution of target lesions after 10 cycles of OPB-51602 and a 6.9-month progression-free interval; the other patient had a 41% reduction of measurable tumor burden, but stopped OPB-51602 treatment after five cycles due to intolerable peripheral neuropathy) [66]. Both patients with tumor regression had known treatment resistance against EGFR tyrosine kinase inhibitors and had received at least one round of chemotherapy. However, post-treatment biopsies were not performed to establish changes in activated STAT3 concentrations [66]. A subsequent phase I trial has examined OPB-51602 in refractory hematologic cancers [65].

As a class, the landscape of STAT3 SDIs has steadily improved since their initial conception in 2001. Optimization of lead molecules through innovative methods of structure-based design has led to improved potency as evidenced by IC_{50} levels in the nanomolar range, increased intracellular uptake, enhanced antitumor effects *in vivo*, and greater specificity for STAT3. In spite of this progress, systemic toxicities and modest bioavailabilities continue to limit their translation to the clinic.

7.3.2 DNA-Binding Domain Inhibitors

As a transcription factor, STAT3 contains a unique DNA-binding region that enables recognition and association with corresponding response elements in the promoters of target genes. Targeting this STAT3:DNA association offers another approach to mitigating aberrant STAT3-mediated cellular processes that facilitate tumor growth and survival. STAT3 DBDIs have largely been derived from two distinct classes of compounds: platinum-based molecules and decoy oligonucleotides (Table 7.1, Fig. 7.3B). Although the DBDIs have not received as much research focus as SDIs, a few stand out as potentially viable therapeutic agents.

In 2004, a screen of platinum-based compounds by Turkson et al. [67] identified CPA-1, CPA-7, and platinum (IV) tetrachloride, as molecules capable of disrupting STAT3:DNA associations (Fig. 7.3B). Although platinum (II)-based compounds such as cisplatin are known to produce antitumor effects by cross-linking to purines, platinum (IV)-based compounds were found to exert cytotoxicities by downmodulating expression of STAT3-dependent genes [67,95]. The effects of CPA-1, CPA-7, and platinum (IV) tetrachloride have been studied in

multiple cancer cell lines with constitutively activated STAT3, including v-Src-transformed mouse fibroblasts (NIH 3T3/v-Src), human breast carcinoma (MDA-MB-231, MDA-MB-435, and MDA-MB-468), colon cancer (CT26), and melanoma (M2 and Cl10) cells [67]. Treatment with these platinum-based compounds resulted in lower levels of activated STAT3, decreased STAT3 DNA binding activity, and induction of apoptosis (IC_{50} values $\leq$5.0 μM). Additionally, these novel compounds exhibited 2.5- to 4-fold greater selectivity for STAT3 over STAT1 [67]. Overall, CPA-1, CPA-7, and platinum (IV) tetrachloride demonstrate potency and selectivity comparable to the previously mentioned SDIs.

Despite promising *in vitro* findings with the platinum-based compounds, *in vivo* studies have only been performed with CPA-7. Intravenous treatment of mice harboring colon carcinoma xenografts with CPA-7 (5 mg/kg) resulted in decreased STAT3 activity and induction of complete or partial tumor regression relative to treatment with vehicle control [67]. Additional investigations on human melanoma xenograft tumors confirmed inhibition of angiogenesis and induction of tumor regression following intravenous administration of CPA-7 [69]. However, CPA-7 failed to promote tumor regression in mice harboring intracranial melanoma and glioblastoma xenograft tumors, suggesting poor central nervous system penetration [68].

Another platinum (IV)-based compound, IS3-295, exhibits similar antitumor effects as CPA-7 [70]. IS3-295 was originally discovered by screening the NCI 2000 diversity set for compounds that decrease STAT3 DNA binding activity. Although its exact mechanism has not fully been elucidated, IS3-295 likely binds to the STAT3 DNA-binding domain [70]. Treatment of breast carcinoma cell lines with IS3-295 resulted in G_o/G_1-phase cell-cycle arrest. Furthermore, TUNEL staining suggested that malignant cell lines harboring constitutively activated STAT3, including human breast cancer (MDA-MD-435), NSCLC (A549), prostate carcinoma (DU145), multiple myeloma (U266), and pancreatic carcinoma (Panc1), exhibited greater apoptosis after treatment with IS3-295 compared to control groups. Although IS3-295 exhibits potent inhibitory activity against STAT3 and reduces STAT3-mediated expression of cyclin D1 and Bcl-X_L *in vitro*, no *in vivo* studies have been reported to date [70]. As a subclass of STAT3 DBDIs, platinum (IV)-based compounds have demonstrated intriguing therapeutic potential. However, their translation is currently limited by lack of toxicity and pharmacology studies and they remain untested in treatment-resistant models.

Double-stranded "decoy" oligonucleotides offer an alternative approach to disrupting the STAT3:DNA-binding interaction. Decoy oligonucleotides contain double-stranded DNA sequences that mimic the binding site of transcription factors, leading to competitive inhibition of authentic interactions between the transcription factors and their target genes [96]. Previous studies on arterial wall gene therapy and STAT6-mediated T helper 2 cell activity demonstrated that adequate intracellular concentrations of decoy lead to successful inhibition of transcription factor DNA-binding activity and decreased expression of target genes [97,98]. Coupled with evidence that antisense oligonucleotides and dominant-negative STAT3 mutants induce apoptosis in multiple cancer cell lines [99–102], these studies provided the conceptual framework to pursue the design of a double-stranded decoy oligonucleotide that modulates STAT3-mediated gene expression.

In 2003, Leong et al. [72] synthesized and evaluated the first STAT3 double-stranded decoy oligonucleotide (Fig. 7.3B). The decoy consisted of the sequence 5′-CATTTCCCGTAAATC-3′ according to the STAT3 DNA-binding sequence located in the c-*fos* gene promoter (high-affinity serum-inducible element; hSIE) [41,103]. Confocal microscopy demonstrated cellular

uptake and nuclear and cytoplasmic localization of the STAT3 decoy following treatment of cancerous and noncancerous cells for 6 h. Dose-dependent inhibition of the proliferation of HNSCC cell lines was seen using STAT3 decoy concentrations ranging from 0.0125 to 25 μM. By contrast, a point mutant control decoy exerted only minimal effects on proliferation. Treatment of HNSCC cells harboring an hSIE-luciferase construct with the STAT3 decoy resulted in decreased luciferase activity relative to mutant control decoy; similarly, reduced expression was also seen for antiapoptotic Bcl-X_L—the product of a STAT3 target gene. Importantly, normal human oral keratinocytes demonstrated no cytotoxic effects after incubation with STAT3 decoy, maintaining growth at rates similar to keratinocytes incubated with mutant control decoy or no treatment. Altogether, *in vitro* studies suggest that STAT3 decoy achieves intracellular transport, leads to enhanced apoptosis, and decreases expression of STAT3 target genes, highlighting its potential as a therapy [72].

Follow-up *in vivo* studies on the antitumor efficacy of STAT3 decoy provided promising results in terms of both efficacy and specificity. In one study, athymic nude mice were xenografted with two HNSCC tumors each [77]. One tumor received daily intratumoral injections of STAT3 decoy and the other received mutant control decoy. A 3.25-fold increase in cell death was observed in tumors treated with STAT3 decoy vs mutant control decoy. Decreased tumor cell expression of cyclin D1 and Bcl-X_L was also seen [77]. STAT5 activation was unaffected by treatment with STAT3 decoy, highlighting the specificity of the decoy. Similar inhibition of tumor growth and reduction in STAT3 activity has been observed in other tumor xenograft models following intratumoral injection of STAT3 decoy, including human glioblastoma multiforme (U251), lung carcinoma (A549), and ovarian epithelial cancer (SKOV3) models [76,78,79]. The *in vivo* evidence suggests that STAT3 decoy facilitates tumor growth inhibition via STAT3 blockade and is at least partially due to increased apoptosis.

STAT3 decoy therapy may be particularly useful in the case of cancers resistant to EGFR inhibitors [104–107]. Administration of the STAT3 decoy to multiple HNSCC cell lines with resistance to erlotinib, a Food and Drug Administration (FDA)-approved EGFR inhibitor, downmodulated STAT3 target gene expression and decreased cell proliferation compared to treatment with mutant control decoy (EC_{50} values ranged from 5.2 to 11.1 nM) [73]. Similar *in vitro* results were observed in bladder cancers resistant to cetuximab, an FDA-approved EGFR monoclonal antibody [73]. Studies of cetuximab-sensitive and cetuximab-resistant HNSCC xenograft tumors illustrated that STAT3 decoy augmented the antitumor effects of cetuximab in both the sensitive and resistant models compared to treatment with cetuximab plus mutant control decoy [73]. Since treatment with cetuximab and radiation has been shown to modestly improve survival in HNSCC, relative to radiation alone, the addition of STAT3 decoy to this regimen may yield a more efficacious approach [108].

Synergistic activity of STAT3 decoy in combination with bortezomib has been observed in HNSCC cell lines [71]. These data are particularly encouraging in light of clinical evidence that show sustained decreases in tumor burden and disease stabilization among patients with recurrent HNSCC after treatment with bortezomib and radiation, suggesting that further optimization of bortezomib/radiation therapy has the potential to improve patient outcomes [109,110].

Treatment with STAT3 decoy has also demonstrated success in sensitizing tumors to chemotherapy. Combination of STAT3 decoy and the chemotherapy drug cisplatin resulted in a

2.6-fold increase in apoptosis when compared to STAT3 decoy monotherapy in HNSCC cell lines [77,111]. *In vivo* studies using an HNSCC xenograft model confirmed that STAT3 decoy exhibited an additive effects with cisplatin, as evidenced by decreased tumor cell expression of VEGF, Bcl-X_L, cyclin D1, and PCNA in comparison to treatment with the decoy alone [77]. In ovarian cancer cells (SKOV3 and OVCAR3), treatment with STAT3 decoy was able to render the cells more sensitive to paclitaxel [112]. Collectively, these preclinical data support further investigation of STAT3 decoy in combination with conventional chemotherapy drugs in multiple tumor models.

Toxicology studies with the STAT3 decoy have revealed no evidence of systemic or local toxicities [75]. Based on these favorable results, a phase 0 clinical trial was conducted to assess the safety profile and pharmacodynamic effects of STAT3 decoy in patients with resectable HNSCC [74]. STAT3 decoy and normal saline (control) were injected directly into the tumors at doses ranging from 250 μg to 1 mg per injection. No toxicities were reported in either study arm and the maximum tolerated dose remains unknown. Downmodulation of cyclin D1 and Bcl-X_L was observed in tumor biopsies treated with STAT3 decoy compared to those treated with saline injections, demonstrating pharmacodynamic activity of the decoy in the target tissue [74].

Additional studies have assessed the antitumor effects of STAT3 decoy after intravenous administration. In an HNSCC xenograft model, systemic administration of STAT3 decoy failed to abrogate tumor growth, presumably due to nucleolytic degradation and/or thermal denaturation of the oligonucleotide free ends [74]. To overcome this barrier, Sen et al. [74] generated a cyclic version of the decoy with hexaethylene glycol linkages replacing the free ends (Fig. 7.3B). The cyclic STAT3 decoy demonstrated markedly enhanced thermal stability and resistance to nucleases. Importantly, intravenous administration of the cyclic STAT3 decoy inhibited the growth of HNSCC xenograft tumors, relative to treatment with cyclic mutant STAT3 decoy [74]. Subsequent toxicity studies in mice demonstrated that systemically administered cyclic STAT3 decoy did not lead to organ damage, significant changes in weight, or abnormal serum chemistries over a 2-week study period (no-observable-adverse-effect-level was 100 mg/kg) [5]. Thus, the cyclic STAT3 decoy exhibits antitumor activity following systemic administration has a favorable safety profile and warrants further evaluation in clinical trials. Overall, STAT3 DBDIs demonstrate promising potential for translation to clinical settings. In particular, these inhibitors of STAT3 may be well suited for enhancing the antitumor effects of conventional chemotherapy drugs, radiation, and molecular targeting agents. Cyclic STAT3 decoy exhibits notable potential due to a high degree of specificity for STAT3, lack of systemic toxicities, capacity for systemic delivery, and demonstrated efficacy in cancers resistant to multimodal therapy.

7.3.3 N-Terminal Domain Inhibitors

The N-terminal domain (NTD) of STAT3 provides another target site for inhibition of STAT3 activity. NMR and X-ray crystallography data suggest that the STAT3 NTD is comprised of 130 amino acids and 8 alpha helices and reflects a well-conserved structural region across STAT family members [87,113]. The STAT3 NTD facilitates tetramerization of activated STAT3 dimers; this tetramerization enables interactions with proteins that

stabilize enhanceosomes [114,115]. Presumably, these interactions increase the range of STAT3-mediated gene expression to include suppressors of pro-apoptotic genes, although further studies need to be performed to define the complete set of target genes regulated by the STAT3 NTD [88,113]. Other functions of the NTD include a role in chromatin remodeling and transnuclear shuttling of both phosphorylated and unphosphorylated STAT3, suggesting additional ancillary roles to gene regulation [88,116,117].

In 2007, Timofeeva et al. [87] designed and evaluated a library of short peptide NTD inhibitors. The library was designed based on the second and eighth helices of the STAT3 NTD. Diffusion of the peptides across the cell membrane was augmented by covalently attaching Penetratin, a peptide transduction motif, to the C-termini [87]. *In vitro* studies have shown that ST3-H2A2, an optimized NTD inhibitor, exhibits IC_{50} values in the low micromolar range against breast cancer cells harboring constitutively active STAT3 (MDA-MB-231, MDA-MB-435, T47D, AND MCF-7) [87]. Similar effects of ST3-H2A2 on growth suppression and induction of apoptosis were observed in prostate cancer cells with high basal levels of phosphorylated STAT3 (LNCaP, PC3, and DU145) [88]. Although the precise mechanism of action is not completely understood, data from ChIP assays and human promoter arrays suggest that ST3-H2A2 decreases the number of genes bound by STAT3, possibly disrupting expression of pro-apoptotic suppressors [88,113]. Importantly, the cytotoxic effects of ST3-H2A2 were not observed in normal mouse embryonic fibroblasts, suggesting the possibility of acceptable treatment toxicities [87].

Although preclinical data suggest therapeutic potential for NTD inhibitors, it is likely that the same issues of stability, specificity, and intracellular transportation that have challenged other peptide inhibitors of STAT3 will be encountered. In particular, cell membrane penetration will pose an obstacle for *in vivo* studies of ST3-H2A2 since the Penetratin motif exhibits low serum stability, limiting drug delivery to its site of action [87]. Moreover, peptide inhibitors are likely to generate an immune response, necessitating the need to develop peptidomimetic anologs. Nonetheless, NTD inhibitors provide a novel alternative approach for abrogating STAT3-mediated cancer proliferation. Considering the nascent history of NTD inhibitors, future areas of research may include characterizing the mechanism of NTD inhibitor action, defining the full complement of response genes modulated by the STAT3 NTD and NTD inhibitors and development of additional NTD inhibitor molecules.

7.3.4 STAT3 Antisense

Antisense oligonucleotides designed to bind and promote the destruction of STAT3 mRNA molecules provide another option for abrogating STAT3-mediated cell proliferation and tumor growth. Studies have been performed using a STAT3 antisense oligonucleotide comprised of the sequence 5′-GCTCCAGCATCTGCTGCTTC-3′, capped with 2′-*O*-methylethyl groups to prevent degradation of free ends (Table 7.1) [80,82]. Treatment with STAT3 antisense reduced the expression of STAT3 response genes in human melanoma, breast carcinoma, and prostate cancer cell lines containing elevated levels of phosphorylated STAT3 [80,118,119]. Treatment with antisense STAT3 led to significant reductions in angiogenesis and tumor size in mice harboring prostate and hepatocellular carcinoma xenograft tumors, including androgen-resistant prostate cancer models and models of hepatocellular lung metastases [80,81]. Considering the high recurrence rate of treatment-resistant prostate cancers and the aggressive nature of

hepatocellular carcinomas, these results raise the prospect of integrating STAT3 antisense oligonucleotides into current treatment regimens for these malignancies [120–122].

Although preclinical studies suggest the potential for clinical application of antisense oligonucleotides, AZD9150 (ISIS 481464) is the only STAT3 antisense that has been evaluated in human clinical trials (Fig. 7.3C). This second-generation STAT3 antisense oligonucleotide was optimized by replacing the 2′-*O*-methylethyl groups of previous iterations with 2′-4′ constrained ethyl-modified residues to increase stability [84]. *In vitro* experiments performed in lymphoma (KARPAS299 and SUP-M2) and neuroblastoma (IMR 32) cell lines showed that AZD9150 treatment led to reductions in total STAT3 protein and downstream targets [84,86]. Moreover, systemic administration of AZD9150 promoted antitumor effects against xenograft models of lymphoma, lung carcinoma, and neuroblastoma [84,86]. Interestingly, pretreatment with AZD9150 sensitized neuroblastoma tumors to cisplatin [86]. Toxicity studies with systemically administered AZD9150 have revealed a lack of end-organ damage and adverse toxicities [83]. These promising preclinical data provided the impetus for a phase I clinical trial (NCT01563302) in patients with advanced lymphoma and treatment-refractory solid tumors [85]. Intravenous administration of AZD9150 was well tolerated at doses of 2 and 4 mg/kg; notable toxicities included a 70% reduction in platelet counts from baseline and one instance of treatment-related thrombotic microangiopathy at 4 mg/kg [85]. One patient in the 15-patient cohort experienced more than a 55% reduction in tumor size and another patient achieved sufficient partial response to undergo successful autologous stem-cell transplantation [85]. The favorable safety profile and promising initial results of AZD9150 offer compelling evidence for further optimization and clinical trials.

7.4 CONCLUSIONS

In the last 30 years, improved understanding of the role of STAT3 in cancer biology has led to great strides in the discovery, optimization, and evaluation of anti-STAT3 agents. Collectively, preclinical experiments have progressed well beyond proof-of-concept studies and should encourage optimism for the eventual realization of novel therapies that directly inhibit STAT3, although adequate specificity, potency, stability, and drug delivery persist as challenging issues. Despite these advances, patient response to treatment will ultimately determine the clinical utility of STAT3 inhibitors, and given the paucity of randomized controlled trials that have been conducted, research efforts should continue to pursue clinical validation. Possible future directions after clinical validation include identifying tumor biomarkers that stratify patients according to STAT3 inhibitor sensitivity and investigating other disease processes that depend upon aberrant expression of targetable transcription factors. Overall, therapeutic inhibition of STAT3 has the potential to be another crucial turning point in the management of cancer, and the rapidly expanding base of knowledge surrounding STAT3 suggests that this promise may reach fruition within the upcoming decade.

Acknowledgments

This work was supported by the National Institutes of Health grants R01 DE24728 (D.E.J.), P50CA097190 (D.E.J. and J.R.G.), and R01 DE023685 (J.R.G.), and American Cancer Society grant CRP-13-308-06-COUN (J.R.G.).

Conflict of Interest: No potential conflicts of interest were disclosed.

References

[1] Yu H, Jove R. The STATs of cancer—new molecular targets come of age. Nat Rev Cancer 2004;4:97–105.
[2] Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. Nat Rev Cancer 2009;9:798–809. Available: http://www.ncbi.nlm.nih.gov/pubmed/19851315.
[3] Darnell JE. STATs and gene regulation. Science 1997;277:1630–5.
[4] Darnell JE, Kerr IM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 1994;264:1415–21.
[5] Sen M, Paul K, Freilino ML, Li H, Li C, Johnson DE, et al. Systemic administration of a cyclic signal transducer and activator of transcription 3 (STAT3) decoy oligonucleotide inhibits tumor growth without inducing toxicological effects. Mol Med 2014;20:46–56. Available: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3951464&tool=pmcentrez&rendertype=abstract.
[6] Hirano T, Ishihara K, Hibi M. Roles of STAT3 in mediating the cell growth, differentiation and survival signals relayed through the IL-6 family of cytokine receptors. Oncogene 2000;19:2548–56. Available: http://www.nature.com/onc/journal/v19/n21/full/1203551a.html.
[7] Silvennoinen O, Schindler C, Schlessinger J, Levy DE. Ras-independent growth factor signaling by transcription factor tyrosine phosphorylation. Science 1993;261:1736–9. Available: http://www.ncbi.nlm.nih.gov/pubmed/8378775.
[8] Stark GR, Kerr IM, Williams BR, Silverman RH, Schreiber RD. How cells respond to interferons. Annu Rev Biochem 1998;67:227–64. Available: http://www.ncbi.nlm.nih.gov/pubmed/9759489.
[9] Zhong Z, Wen Z, Darnell JE. Stat3: a STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. Science 1994;264:95–8. Available: http://www.ncbi.nlm.nih.gov/pubmed/8140422.
[10] Bowman T, Garcia R, Turkson J, Jove R. STATs in oncogenesis. Oncogene 2000;19:2474–88.
[11] Danial NN, Rothman P. JAK-STAT signaling activated by Abl oncogenes. Oncogene 2000;19:2523–31.
[12] Irby RB, Yeatman TJ. Role of Src expression and activation in human cancer. Oncogene 2000;19:5636–42.
[13] Arulanandam R, Vultur A, Cao J, Carefoot E, Elliott BE, Truesdell PF, et al. Cadherin-cadherin engagement promotes cell survival via Rac1/Cdc42 and signal transducer and activator of transcription-3. Mol Cancer Res 2009;7:1310–27.
[14] Stairs DB, Bayne LJ, Rhoades B, Vega ME, Waldron TJ, Kalabis J, et al. Deletion of p120-catenin results in a tumor microenvironment with inflammation and cancer that establishes it as a tumor suppressor gene. Cancer Cell 2011;19:470–83.
[15] Vultur A, Villanueva J, Krepler C, Rajan G, Chen Q, Xiao M, et al. MEK inhibition affects STAT3 signaling and invasion in human melanoma cell lines. Oncogene 2014;33:1850–61. Available: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3769503&tool=pmcentrez&rendertype=abstract.
[16] Darnell JE. Validating Stat3 in cancer therapy. Nat Med 2005;11:595–6.
[17] Germain D, Frank D a. Targeting the cytoplasmic and nuclear functions of signal transducers and activators of transcription 3 for cancer therapy. Clin Cancer Res 2007;13:5665–9. Available: http://www.ncbi.nlm.nih.gov/pubmed/17908954.
[18] Chen CL, Cen L, Kohout J, Hutzen B, Chan C, Hsieh FC, et al. Signal transducer and activator of transcription 3 activation is associated with bladder cancer cell growth and survival. Mol Cancer 2008;7:78.
[19] Takemoto S, Ushijima K, Kawano K, Yamaguchi T, Terada A, Fujiyoshi N, et al. Expression of activated signal transducer and activator of transcription-3 predicts poor prognosis in cervical squamous-cell carcinoma. Br J Cancer 2009;101:967–72.
[20] Wen W, Wu J, Liu L, Tian Y, Buettner R, Hsieh M-Y, et al. Synergistic anti-tumor effect of combined inhibition of EGFR and JAK/STAT3 pathways in human ovarian cancer. Mol Cancer 2015;14:100. Available: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4437681&tool=pmcentrez&rendertype=abstract.
[21] Alas S, Bonavida B. Inhibition of constitutive STAT3 activity sensitizes resistant non-Hodgkin's lymphoma and multiple myeloma to chemotherapeutic drug-mediated apoptosis. Clin Cancer Res 2003;9:316–26.
[22] Rivat C, De Wever O, Bruyneel E, Mareel M, Gespach C, Attoub S. Disruption of STAT3 signaling leads to tumor cell invasion through alterations of homotypic cell-cell adhesion complexes. Oncogene 2004;23:3317–27. Available: http://www.ncbi.nlm.nih.gov/pubmed/15077188.
[23] Zhang X, Sun Y, Pireddu R, Yang H, Urlam MK, Lawrence HR, et al. A novel inhibitor of STAT3 homodimerization selectively suppresses STAT3 activity and malignant transformation. Cancer Res 2013;73:1922–33.

[24] Schlessinger K, Levy DE. Malignant transformation but not normal cell growth depends on signal transducer and activator of transcription 3. Cancer Res 2005;65:5828–34.
[25] Fukuda A, Wang SC, Morris JP, Folias AE, Liou A, Kim GE, et al. Stat3 and MMP7 contribute to pancreatic ductal adenocarcinoma initiation and progression. Cancer Cell 2011;19:441–55.
[26] Spitzner M, Ebner R, Wolff HA, Michael Ghadimi B, Wienands J, Grade M. STAT3: a novel molecular mediator of resistance to chemoradiotherapy. Cancers (Basel) 2014;6:1986–2011.
[27] Yue P, Turkson J. Targeting STAT3 in cancer: how successful are we? Expert Opin Invest Drugs 2009;18:45–56. Available: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2610472&tool=pmcentrez&rendertype=abstract.
[28] Zhou H, Miki R, Eeva M, Fike FM, Seligson D, Yang L, et al. Reciprocal regulation of SOCS 1 and SOCS3 enhances resistance to ionizing radiation in glioblastoma multiforme. Clin Cancer Res 2007;13:2344–53. Available: http://www.ncbi.nlm.nih.gov/pubmed/17438093.
[29] Spitzner M, Emons G, Kramer F, Gaedcke J, Rave-Fränk M, Scharf JG, et al. A gene expression signature for chemoradiosensitivity of colorectal cancer cells. Int J Radiat Oncol Biol Phys 2010;78:1184–92.
[30] Spitzner M, Roesler B, Bielfeld C, Emons G, Gaedcke J, Wolff HA, et al. STAT3 inhibition sensitizes colorectal cancer to chemoradiotherapy *in vitro* and *in vivo*. Int J Cancer 2014;134:997–1007.
[31] Chen M-F, Chen P-T, Lu MS, Lin PY, Chen W-C, Lee K-D. IL-6 expression predicts treatment response and outcome in squamous cell carcinoma of the esophagus. Mol Cancer 2013;12:26. Available: http://www.ncbi.nlm.nih.gov/pubmed/23561329%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3667147.
[32] Kim KW, Mutter RW, Cao C, Albert JM, Shinohara ET, Sekhar KR, et al. Inhibition of signal transducer and activator of transcription 3 activity results in down-regulation of Survivin following irradiation. Mol Cancer Ther 2006;5:2659–65.
[33] Wu C-T, Chen M-F, Chen W-C, Hsieh C-C. The role of IL-6 in the radiation response of prostate cancer. Radiat Oncol 2013;8:159. Available: http://ro-journal.biomedcentral.com/articles/10.1186/1748-717X-8-159.
[34] Kim SM, Kwon O-J, Hong YK, Kim JH, Solca F, Ha S-J, et al. Activation of IL-6R/JAK1/STAT3 signaling induces de novo resistance to irreversible EGFR inhibitors in non-small cell lung cancer with T790M resistance mutation. Mol Cancer Ther 2012;11:2254–64. Available: http://www.ncbi.nlm.nih.gov/pubmed/22891040.
[35] Prahallad A, Sun C, Huang S, Di Nicolantonio F, Salazar R, Zecchin D, et al. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. Nature 2012;483:100–3. Available: http://www.nature.com/doifinder/10.1038/nature10868%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/22281684.
[36] Lee HJ, Zhuang G, Cao Y, Du P, Kim HJ, Settleman J. Drug resistance via feedback activation of stat3 in oncogene-addicted cancer cells. Cancer Cell 2014;26:207–21.
[37] Kong J, Kong F, Gao J, Zhang Q, Dong S, Gu F, et al. YC-1 enhances the anti-tumor activity of sorafenib through inhibition of signal transducer and activator of transcription 3 (STAT3) in hepatocellular carcinoma. Mol Cancer 2014;13:7. Available: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3895679&tool=pmcentrez&rendertype=abstract.
[38] Yin ZJ, Jin FG, Liu TG, Fu EQ, Xie YH, Sun RL. Overexpression of STAT3 potentiates growth, survival, and radioresistance of non-small-cell lung cancer (NSCLC) cells. J Surg Res 2011;171:675–83.
[39] You S, Li R, Park D, Xie M, Sica GL, Cao Y, et al. Disruption of STAT3 by niclosamide reverses radioresistance of human lung cancer. Mol Cancer Ther 2014;13:606–16.
[40] Pawson T. Protein modules and signalling networks. Nature 1995;373:573–80.
[41] Turkson J, Ryan D, Kim JS, Zhang Y, Chen Z, Haura E, et al. Phosphotyrosyl peptides block Stat3-mediated DNA binding activity, gene regulation, and cell transformation. J Biol Chem 2001;276:45443–55. Available: http://www.ncbi.nlm.nih.gov/pubmed/11579100.
[42] Makriyannis A, Biegel D. Drug discovery strategies methods. New York: Marcel Dekker; 2004.
[43] Li H, Liu A, Zhao Z, Xu Y, Lin J, Jou D, et al. Fragment-based drug design and drug repositioning using multiple ligang simultaneous docking (MLSD): identifying celecoxib and template compounds as novel inhibitors of signal transducer and activator of transcription 3 (STAT3). J Med Chem 2011;54:5592–6.
[44] Siddiquee K, Zhang S, Guida WC, Blaskovich MA, Greedy B, Lawrence HR, et al. Selective chemical probe inhibitor of Stat3, identified through structure-based virtual screening, induces antitumor activity. Proc Natl Acad Sci USA, 104. p. 7391–6.
[45] Turkson J, Kim JS, Zhang S, Yuan J, Huang M, Glenn M, et al. Novel peptidomimetic inhibitors of signal transducer and activator of transcription 3 dimerization and biological activity. Mol Cancer Ther 2004;3:261–9.

[46] Auzenne EJ, Klostergaard J, Mandal PK, Liao WS, Lu Z, Gao F, et al. A phosphopeptide mimetic prodrug targeting the SH2 domain of Stat3 inhibits tumor growth and angiogenesis. J Exp Ther Oncol 2012;10:155–62. Available: http://www.ncbi.nlm.nih.gov/pubmed/23350355.

[47] Mandal PK, Gao F, Lu Z, Ren Z, Ramesh R, Birtwistle JS, et al. Potent and selective phosphopeptide mimetic prodrugs targeted to the Src homology 2 (SH2) domain of signal transducer and activator of transcription 3. J Med Chem 2011;54:3549–63.

[48] Chen CL, Loy A, Cen L, Chan C, Hsieh FC, Cheng G, et al. Signal transducer and activator of transcription 3 is involved in cell growth and survival of human rhabdomyosarcoma and osteosarcoma cells. BMC Cancer 2007;7:111. Available: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17598902.

[49] Song H, Wang R, Wang S, Lin J. A low-molecular-weight compound discovered through virtual database screening inhibits Stat3 function in breast cancer cells. Proc Natl Acad Sci USA, 102. p. 4700–5. Available: http://www.ncbi.nlm.nih.gov/pubmed/15781862%5Cnhttp://www.ncbi.nlm.nih.gov/pmc/articles/PMC555708/pdf/pnas-0409894102.pdf.

[50] Fuh B, Sobo M, Cen L, Josiah D, Hutzen B, Cisek K, et al. LLL-3 inhibits STAT3 activity, suppresses glioblastoma cell growth and prolongs survival in a mouse glioblastoma model. Br J Cancer 2009;100:106–12.

[51] Mencalha AL, Rocher BD, Salles D, Binato R, Abdelhay E. LLL-3, a STAT3 inhibitor, represses BCR-ABL-positive cell proliferation, activates apoptosis and improves the effects of Imatinib mesylate. Cancer Chemother Pharmacol 2010;65:1039–46.

[52] Pan Y, Zhou F, Zhang R, Claret FX. Stat3 inhibitor static exhibits potent antitumor activity and induces chemo- and radio-sensitivity in nasopharyngeal carcinoma. PLoS ONE 2013;8. e54565.

[53] Schust J, Berg T. A high-throughput fluorescence polarization assay for signal transducer and activator of transcription 3. Anal Biochem 2004;330:114–8. Available: http://www.ncbi.nlm.nih.gov/pubmed/15183768.

[54] Schust J, Sperl B, Hollis A, Mayer TU, Berg T. Stattic: a small-molecule inhibitor of STAT3 activation and dimerization. Chem Biol 2006;13:1235–42.

[55] Zhang X, Yue P, Page BDG, Li T, Zhao W, Namanja AT, et al. Orally bioavailable small-molecule inhibitor of transcription factor Stat3 regresses human breast and lung cancer xenografts. Proc Natl Acad Sci, 109. p. 9623–8.

[56] Zhang X, Yue P, Fletcher S, Zhao W, Gunning PT, Turkson J. A novel small-molecule disrupts Stat3 SH2 domain-phosphotyrosine interactions and Stat3-dependent tumor processes. Biochem Pharmacol 2010;79:1398–409.

[57] Ashizawa T, Miyata H, Ishii H, Oshita C, Matsuno K, Masuda Y, et al. Antitumor activity of a novel small molecule STAT3 inhibitor against a human lymphoma cell line with high STAT3 activation. Int J Oncol 2011;38:1245–52.

[58] Matsuno K, Masuda Y, Uehara Y, Sato H, Muroya A, Takahashi O, et al. Identification of a new series of STAT3 inhibitors by virtual screening. ACS Med Chem Lett 2010;1:371–5.

[59] Chen H, Yang Z, Ding C, Chu L, Zhang Y, Terry K, et al. Fragment-based drug design and identification of HJC0123, a novel orally bioavailable STAT3 inhibitor for cancer therapy. Eur J Med Chem 2013;62:498–507.

[60] Bendell JC, Hong DS, Burris HA, Naing A, Jones SF, Falchook G, et al. Phase 1, open-label, dose-escalation, and pharmacokinetic study of STAT3 inhibitor OPB-31121 in subjects with advanced solid tumors. Cancer Chemother Pharmacol 2014;74:125–30.

[61] Brambilla L, Genini D, Laurini E, Merulla J, Perez L, Fermeglia M, et al. Hitting the right spot: mechanism of action of OPB-31121, a novel and potent inhibitor of the signal transducer and activator of transcription 3 (STAT3). Mol Oncol 2015;9:1194–206.

[62] Genini D, Brambilla L, Laurini E, Civenni G, Pinton S, Sarti M, et al. CVC: novel inhibitors of signal transducer and activator of transcription 3 (STAT3) show potent activity in cell cultures and tumor xenografts. 105 Annu Meet Am Assoc Cancer Res 2014;.

[63] Hayakawa F, Sugimoto K, Harada Y, Hashimoto N, Ohi N, Kurahashi S, et al. A novel STAT inhibitor, OPB-31121, has a significant antitumor effect on leukemia with STAT-addictive oncokinases. Blood Cancer J 2013;3:e166. Available: http://www.mendeley.com/research/hallmarks-cancer-next-generation-3/.

[64] Kim MJ, Nam HJ, Kim HP, Han SW, Im SA, Kim TY, et al. OPB-31121, a novel small molecular inhibitor, disrupts the JAK2/STAT3 pathway and exhibits an antitumor activity in gastric cancer cells. Cancer Lett 2013;335:145–52.

[65] Ogura M, Uchida T, Terui Y, Hayakawa F, Kobayashi Y, Taniwaki M, et al. Phase I study of OPB-51602, an oral inhibitor of STAT3, in patients with relapsed/refractory hematological malignancies. Cancer Sci 2015;106:896–901. Available: http://www.ncbi.nlm.nih.gov/pubmed/25912076.

[66] Wong AL, Soo RA, Tan DS, Lee SC, Lim JS, Marban PC, et al. Phase I and biomarker study of OPB-51602, a novel signal transducer and activator of transcription (STAT) 3 inhibitor, in patients with refractory solid malignancies. Ann Oncol 2015;26:998–1005.
[67] Turkson J, Zhang S, Palmer J, Kay H, Stanko J, Mora LB, et al. Inhibition of constitutive signal transducer and activator of transcription 3 activation by novel platinum complexes with potent antitumor activity. Mol Cancer Ther 2004;3:1533–42. Available: http://www.ncbi.nlm.nih.gov/pubmed/15634646.
[68] Assi HH, Paran C, VanderVeen N, Savakus J, Doherty R, Petruzzella E, et al. Preclinical characterization of signal transducer and activator of transcription 3 small molecule inhibitors for primary and metastatic brain cancer therapy. J Pharmacol Exp Ther 2014;349:458–69. Available: http://www.ncbi.nlm.nih.gov/pubmed/24696041.
[69] Xu Q, Briggs J, Park S, Niu G, Kortylewski M, Zhang S, et al. Targeting Stat3 blocks both HIF-1 and VEGF expression induced by multiple oncogenic growth signaling pathways. Oncogene 2005;24:5552–60. Available: http://dx.doi.org/10.1038/sj.onc.1208719.
[70] Turkson J, Zhang S, Mora LB, Burns A, Sebti S, Jove R. A novel platinum compound inhibits constitutive Stat3 signaling and induces cell cycle arrest and apoptosis of malignant cells. J Biol Chem 2005;280:32979–88.
[71] Changyou L, Zang Y, Sen M, Leeman-Neill RJ, Man DS, Grandis JR, et al. Bortezomib up-regulates activated signal transducer and activator of transcription-3 and synergizes with inhibitors of signal transducer and activator of transcription-3 to promote head and neck squamous cell carcinoma cell death. Mol Cancer Ther 2009;8:2211–20.
[72] Leong PL, Andrews GA, Johnson DE, Dyer KF, Xi S, Mai JC, et al. Targeted inhibition of Stat3 with a decoy oligonucleotide abrogates head and neck cancer cell growth. Proc Natl Acad Sci USA, 100. p. 4138–43.
[73] Sen M, Joyce S, Panahandeh M, Li C, Thomas SM, Maxwell J, et al. Targeting Stat3 abrogates EGFR inhibitor resistance in cancer. Clin Cancer Res 2012;18:4986–96.
[74] Sen M, Thomas SM, Kim S, Yeh JI, Ferris RL, Johnson JT, et al. First-in-human trial of a STAT3 decoy oligonucleotide in head and neck tumors: Implications for cancer therapy. Cancer Discov 2012;2:694–705.
[75] Sen M, Tosca PJ, Zwayer C, Ryan MJ, Johnson JD, Knostman KAB, et al. Lack of toxicity of a STAT3 decoy oligonucleotide. Cancer Chemother Pharmacol 2009;63:983–95.
[76] Shen J, Li R, Li G. Inhibitory effects of decoy-ODN targeting activated STAT3 on human glioma growth *in vivo*. In Vivo (Brooklyn) 2009;23:237–44.
[77] Xi S, Gooding WE, Grandis JR. *In vivo* antitumor efficacy of STAT3 blockade using a transcription factor decoy approach: implications for cancer therapy. Oncogene 2005;24:970–9.
[78] Zhang X, Liu P, Zhang B, Mao H, Shen L, Ma Y. Inhibitory effects of STAT3 decoy oligodeoxynucleotides on human epithelial ovarian cancer cell growth *in vivo*. Int J Mol Med 2013;32:623–8.
[79] Zhang X, Zhang J, Wang L, Wei H, Tian Z. Therapeutic effects of STAT3 decoy oligodeoxynucleotide on human lung cancer in xenograft mice. BMC Cancer 2007;7:149. Available: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1988829&tool=pmcentrez&rendertype=abstract.
[80] Barton BE, Murphy TF, Shu P, Huang HF, Meyenhofer M, Barton A. Novel single-stranded oligonucleotides that inhibit signal transducer and activator of transcription 3 induce apoptosis in vitro and in vivo in prostate cancer cell lines. Mol Cancer Ther 2004;3:1183–91.
[81] Li W-C, Ye S-L, Sun R-X, Liu Y-K, Tang Z-Y, Kim Y, et al. Inhibition of growth and metastasis of human hepatocellular carcinoma by antisense oligonucleotide targeting signal transducer and activator of transcription 3. Clin Cancer Res 2006;12:7140–8.
[82] Mora LB, Buettner R, Seigne J, Diaz J, Ahmad N, Garcia R, et al. Constitutive activation of Stat3 in human prostate tumors and cell lines: Direct inhibition of Stat3 signaling induces apoptosis of prostate cancer cells. Cancer Res 2002;62:6659–66.
[83] Burel SA, Han S-R, Lee H-S, Norris DA, Lee B-S, Machemer T, et al. Preclinical evaluation of the toxicological effects of a novel constrained ethyl modified antisense compound targeting signal transducer and activator of transcription 3 in mice and cynomolgus monkeys. Nucleic Acid Ther 2013;23:213–27. Available: http://www.ncbi.nlm.nih.gov/pubmed/23692080.
[84] Hong D, Kurzrock R, Kim Y, Woessner R, Younes A, Nemunaitis J, et al. AZD9150, a next-generation antisense oligonucleotide inhibitor of STAT3 with early evidence of clinical activity in lymphoma and lung cancer. Sci Transl Med 2015;7:314ra185. Available: http://stm.sciencemag.org/cgi/doi/10.1126/scitranslmed.aac5272.
[85] Hong DS, et al. A phase I study of ISIS 481464 (AZD9150), a first-in-human, first in-class, antisense oligonucleotide inhibitor of STAT3, in patients with advanced cancers. ASCO 2013;. Abstract No. 8523. Available: http://abstracts2.asco.org/AbstView_132_112636.html.

[86] Odate S, Veschi V, Yan S, Lam N, Woessner R, Thiele C. Inhibition of STAT3 with the generation 2.5 antisense oligonucleotide, AZD9150, decreases neuroblastoma tumorigenicity and increases chemosensitivity. Clin Cancer Res 2017;23:1771–84.
[87] Timofeeva OA, Gaponenko V, Lockett SJ, Tarasov SG, Jiang S, Michejda CJ, et al. Rationally designed inhibitors identify STAT3 N-domain as a promising anticancer drug target. ACS Chem Biol 2007;2:799–809.
[88] Timofeeva OA, Tarasova NI, Zhang X, Chasovskikh S, Cheema AK, Wang H, et al. STAT3 suppresses transcription of proapoptotic genes in cancer cells with the involvement of its N-terminal domain. Proc Natl Acad Sci, 110. p. 1267–72. Available: http://www.pnas.org/lookup/doi/10.1073/pnas.1211805110.
[89] Vultur A, Cao J, Arulanandam R, Turkson J, Jove R, Greer P, et al. Cell-to-cell adhesion modulates Stat3 activity in normal and breast carcinoma cells. Oncogene 2004;23:2600–16. Available: http://www.ncbi.nlm.nih.gov/pubmed/15007380.
[90] Bhattacharya S, Ray RM, Johnson LR. STAT3-mediated transcription of Bcl-2, Mcl-1 and c-IAP2 prevents apoptosis in polyamine-depleted cells. Biochem J 2005;392:335–44.
[91] Wang T, Niu G, Kortylewski M, Burdelya L, Shain K, Zhang S, et al. Regulation of the innate and adaptive immune responses by Stat-3 signaling in tumor cells. Nat Med 2004;10:48–54.
[92] Hussain SF, Kong L-Y, Jordan J, Conrad C, Madden T, Fokt I, et al. A novel small molecule inhibitor of signal transducers and activators of transcription 3 reverses immune tolerance in malignant glioma patients. Cancer Res 2007;67:9630–6.
[93] Rosenberg S a, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. Nat Med 2004;10:909–15.
[94] Lui VWY, Wong EYL, Ho Y, Hong B, Wong SCC, Tao Q, et al. STAT3 activation contributes directly to Epstein–Barr virus-mediated invasiveness of nasopharyngeal cancer cells *in vitro*. Int J Cancer 2009;125:1884–93.
[95] Siddik ZH. Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene 2003;22:7265–79. Available: http://www.ncbi.nlm.nih.gov/pubmed/14576837.
[96] Bielinska A, Shivdasani RA, Zhang LQ, Nabel GJ. Regulation of gene expression with double-stranded phosphorothioate oligonucleotides. Science 1990;250:997–1000. Available: http://www.ncbi.nlm.nih.gov/pubmed/2237444.
[97] Nabel EG, Plautz G, Nabel GJ. Site-specific gene expression *in vivo* by direct gene transfer into the arterial wall. Science 1990;249:1285–8.
[98] Wang LH, Yang XY, Kirken RA, Resau JH, Farrar WL. Targeted disruption of stat6 DNA binding activity by an oligonucleotide decoy blocks IL-4-driven T(H)2 cell response. Blood 2000;95:1249–57. Available: http://www.ncbi.nlm.nih.gov/pubmed/10666197.
[99] Catlett-Falcone R, Landowski TH, Oshiro MM, Turkson J, Levitzki A, Savino R, et al. Constitutive activation of Stat3 signaling confers resistance to apoptosis in human U266 myeloma cells. Immunity 1999;10:105–15. Available: http://www.ncbi.nlm.nih.gov/pubmed/10023775.
[100] Grandis JR, Drenning SD, Zeng Q, Watkins SC, Melhem MF, Endo S, et al. Constitutive activation of Stat3 signaling abrogates apoptosis in squamous cell carcinogenesis *in vivo*. Proc Natl Acad Sci USA 2000;97:4227–32.
[101] Nielsen M, Kstel C, Eriksen K, Woetmann A, Stokkedal T, Kaltoft K, et al. Inhibition of constitutively activated Stat3 correlates with altered Bcl-2/Bax expression and induction of apoptosis in mycosis fungoides tumor cells. Leukemia 1999;13:735–8. Available: http://www.stockton-press.co.uk/leu.
[102] Niu G, Heller R, Catlett-Falcone R, Coppola D, Jaroszeski M, Dalton W, et al. Gene therapy with dominant-negative Stat3 suppresses growth of the murine melanoma B16 tumor *in vivo*. Cancer Res 1999;59:5059–63.
[103] Sadowski HB, Shuai K, Darnell JE, Gilman MZ. A common nuclear signal transduction pathway activated by growth factor and cytokine receptors. Science 1993;261:1739–44. Available: http://www.ncbi.nlm.nih.gov/pubmed/8397445.
[104] Ciardiello F, Tortora G, Magrassi SF, Lanzara A. EGFR antagonists in cancer treatment. N Engl J Med 2008;358:1160–74.
[105] Harandi A, Zaidi AS, Stocker AM, Laber DA. Clinical efficacy and toxicity of anti-EGFR therapy in common cancers. J Oncol 2009;2009. 567486.
[106] Thatcher N, Chang A, Parikh P, Rodrigues Pereira J, Ciuleanu T, von Pawel J, et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). Lancet 2005;366:1527–37. Available: http://www.sciencedirect.com/science?_ob=MImg&_imagekey=B6T1B-4HDY-FM3-R-7&_cdi=4886&_user=1029205&_pii=S0140673605676258&_origin=&_coverDate=11/04/2005&_sk=996330503&view=c&wchp=dGLbVlz-zSkWB&md5=d67f3c6c2d4256106eb755f18ef91a18&ie=/sdarticle.pdf.

[107] Yamatodani T, Ekblad L, Kjellen E, Johnsson A, Mineta H, Wennerberg J. Epidermal growth factor receptor status and persistent activation of Akt and p44/42 MAPK pathways correlate with the effect of cetuximab in head and neck and colon cancer cell lines. J Cancer Res Clin Oncol 2009;135:395–402.

[108] Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med 2006;354:567–78. Available: http://www.ncbi.nlm.nih.gov/pubmed/19567214.

[109] Allen C, Saigal K, Nottingham L, Arun P, Chen Z, Van Waes C. Bortezomib-induced apoptosis with limited clinical response is accompanied by inhibition of canonical but not alternative nuclear factor-kB subunits in head and neck cancer. Clin Cancer Res 2008;14:4175–85.

[110] Van Waes C, Chang A a, Lebowitz PF, Druzgal CH, Chen Z, Elsayed YA, et al. Inhibition of nuclear factor-kappaB and target genes during combined therapy with proteasome inhibitor bortezomib and reirradiation in patients with recurrent head-and-neck squamous cell carcinoma. Int J Radiat Oncol Biol Phys 2005;63:1400–12.

[111] Mayer F, Honecker F, Looijenga LHJ, Bokemeyer C. Towards an understanding of the biological basis of response to cisplatin-based chemotherapy in germ-cell tumors. Ann Oncol 2003;14:825–32.

[112] Zhang X, Liu P, Zhang B, Wang A, Yang M. Role of STAT3 decoy oligodeoxynucleotides on cell invasion and chemosensitivity in human epithelial ovarian cancer cells. Cancer Genet Cytogenet 2010;197:46–53.

[113] Hu T, Yeh JE, Pinello L, Jacob J, Chakravarthy S, Yuan G-C, et al. Impact of the N-terminal domain of STAT3 in STAT3-dependent transcriptional activity. Mol Cell Biol 2015;35:3284–300. Available: http://mcb.asm.org/lookup/doi/10.1128/MCB.00060-15.

[114] Lerner L, Henriksen MA, Zhang X, Darnell JE. STAT3-dependent enhanceosome assembly and disassembly: synergy with GR for full transcriptional increase of the a2-macroglobulin gene. Genes Dev 2003;17:2564–77.

[115] Zhang X, Darnell JE. Functional importance of Stat3 tetramerization in activation of the a2-macroglobulin gene. J Biol Chem 2001;276:33576–81.

[116] Pranada AL, Metz S, Herrmann A, Heinrich PC, Mu G, Müller-Newen G. Real time analysis of STAT3 nucleocytoplasmic shuttling. J Biol Chem 2004;279:15114–23. Available: http://www.ncbi.nlm.nih.gov/pubmed/14701810.

[117] Vogt M, Domoszlai T, Kleshchanok D, Lehmann S, Schmitt A, Poli V, et al. The role of the N-terminal domain in dimerization and nucleocytoplasmic shuttling of latent STAT3. J Cell Sci 2011;124:900–9. Available: http://jcs.biologists.org/cgi/doi/10.1242/jcs.072520.

[118] Barton BE, Karras JG, Murphy TF, Barton A, Huang HF-S. Signal transducer and activator of transcription 3 (STAT3) activation in prostate cancer: Direct STAT3 inhibition induces apoptosis in prostate cancer lines. Mol Cancer Ther 2004;3:11–20.

[119] Niu G, Wright KL, Huang M, Song L, Haura E, Turkson J, et al. Constitutive Stat3 activity up-regulates VEGF expression and tumor angiogenesis. Oncogene 2002;21:2000–8. Available: http://www.nature.com/doifinder/10.1038/sj.onc.1205260.

[120] Brawer MK. Prostate cancer: where we have been, where we are, where we are going. World J Surg 2000;24:1200–4.

[121] Denmeade SR, Lin XS, Isaacs JT. Role of programmed (apoptotic) cell death during the progression and therapy for prostate cancer. Prostate 1996;28:251–65.

[122] Tang ZY, Ye SL, Liu YK, Qin LX, Sun HC, Ye QH, et al. A decade's studies on metastasis of hepatocellular carcinoma. J Cancer Res Clin Oncol 2004;130:187–96.