

# STAT3 inhibitors for cancer therapy

## Have all roads been explored?

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**Abbreviations:** IFNs, interferons; GAS, gamma-interferon-activated sequence; EMSA, electrophoretic migration shift assay; pY, phosphotyrosine

The signal transducer and activator of transcription STAT3 is a transcription factor which plays a key role in normal cell growth and is constitutively activated in about 70% of solid and hematological cancers. Activated STAT3 is phosphorylated on tyrosine and forms a dimer through phosphotyrosine/src homology 2 (SH2) domain interaction. The dimer enters the nucleus via interaction with importins and binds target genes. Inhibition of STAT3 results in the death of tumor cells, this indicates that it is a valuable target for anticancer strategies; a view that is corroborated by recent findings of activating mutations within the gene. Yet, there is still only a small number of STAT3 direct inhibitors; in addition, the high similarity of STAT3 with STAT1, another STAT family member mostly oriented toward apoptosis, cell death and defense against pathogens, requires that STAT3-inhibitors have no effect on STAT1. Specific STAT3 direct inhibitors consist of SH2 ligands, including G quartet oligodeoxynucleotides (ODN) and small molecules, they induce cell death in tumor cells in which STAT3 is activated. STAT3 can also be inhibited by decoy ODNs (dODN), which bind STAT3 and induce cell death. A specific STAT3 dODN which does not interfere with STAT1-mediated interferon-induced cell death has been designed pointing to the STAT3 DBD as a target for specific inhibition. Comprehensive analysis of this region is in progress in the laboratory to design DBD-targeting STAT3 inhibitors with STAT3/STAT1 discriminating ability.

### Central Role of STAT3 in Tumors

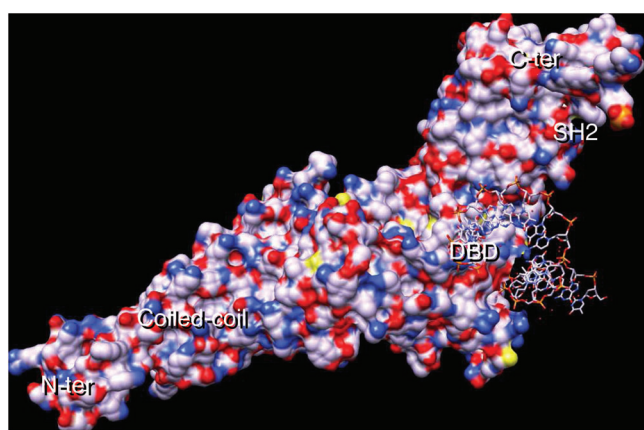
STAT3 belongs to a family of transcription factors (TFs) comprising STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B and STAT6.<sup>1</sup> Like STAT5, STAT3 was found to play an important role in cell growth,<sup>2</sup> and its activation has been

described in nearly 70% of solid and hematological tumors,<sup>3,4</sup> giving good reason for a search for specific direct inhibitors,<sup>5,6</sup> of which there are unfortunately only a few, and none in the clinic to this day. STAT3 comprises several distinct functional domains including: an N-terminal domain containing an oligomerization and a coiled-coil domain, a DNA binding domain (DBD), a linker domain, a Src homology 2 (SH2) domain involved in the interaction of two monomers via phosphotyrosine 705 resulting in dimerization and a C-terminal transactivation domain (see Fig. 1). STAT3 activation occurs following cytokine- or growth factor-receptor activation; it involves phosphorylation within the cytoplasm, dimerization and nuclear transfer<sup>7</sup> (Fig. 2). Nuclear transfer of STAT3 requires nuclear localization signals (NLS) which are in the coiled-coil domain (comprising arginines 214 and 215<sup>8</sup>) and in the dimer-dependent DBD (comprising arginines 414 and 417<sup>9</sup>). The NLSs interact with importin  $\alpha$ , yet which of the five importin  $\alpha$ s ( $\alpha 1$ ,  $\alpha 3$ ,  $\alpha 4$ ,  $\alpha 5$  or  $\alpha 7$ ) actually carries STAT3 is still debated,<sup>9,10</sup> the complex interacts with importin  $\beta$  and is carried through the nuclear pore complex (NPC) (Fig. 3). While arginines 214 and 215 appear to be the major importin-binding site, arginines 414 and 417 are thought to be required for STAT3 to adopt the proper conformation for importin binding.<sup>9</sup> Several studies have shown that STAT3 cycling is probably somewhat more complicated. Unphosphorylated forms of STAT3 can enter the nucleus and stimulate transcription of a subset of gene targets, apparently via interaction with the TF NF $\kappa$ B.<sup>11</sup> However, whether unphosphorylated STAT3 interacts on its own with importins for nuclear entry is not entirely clear: tyrosine 705-mutated STAT3 can shuttle to the nucleus<sup>12</sup> and phosphotyrosine 705/SH2-independent STAT3 dimers were shown to enter the nucleus (but more slowly than phosphorylated STAT3 dimers)<sup>13</sup> (Fig. 2). Interestingly, in the case of STAT1, unphosphorylated monomers enter the nucleus through direct interaction with the NPC proteins nucleoporins, not with importins<sup>14</sup> and unphosphorylated STAT1 dimers bind DNA with a 200-fold lower affinity than phosphorylated STAT1 dimers;<sup>15</sup> in fact, single-molecule imaging showed that interferon (IFN)- $\gamma$ -activated STAT1 has a

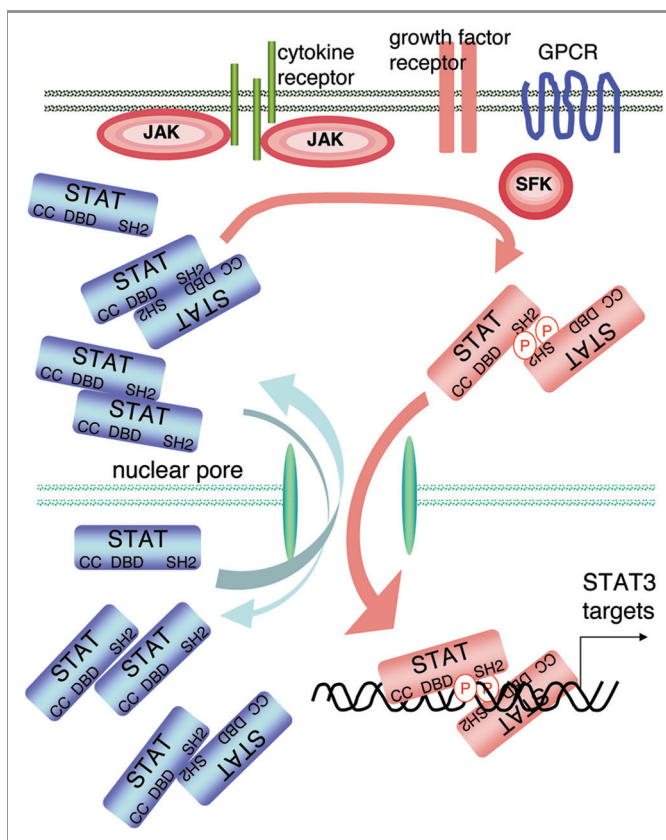
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reduced mobility and resides longer in the nucleus.<sup>16</sup> In any case, the nucleo-cytoplasmic shuttling of STAT3 is a major step of the activation process leading to increased transcriptional activity, suggesting that nuclear transfer of STAT3 per se can be a target for inhibition.

Constitutive activation of STAT3 in tumors can result from upstream activated signaling components, including increased cytokines (IL-6 and IL-10) production, activated receptor (cytokine receptors, VEGFR and EGFR) and non-receptor tyrosine kinases (including JAKs, Src and Abl). Recently, mutated hyperactive forms of STAT3 have been detected in tumors, interestingly most of the described mutations are located within STAT3's SH2<sup>17,18</sup> (see ref. 19). Due to the involvement of tyrosine protein kinases in the STAT3 activating pathways, tyrosine kinase inhibitors indirectly inhibit STAT3 resulting in anti-tumor activity. But STAT3 can also be activated when suppressors of the STAT signaling pathway are inactivated such as the SOCS (suppressor of cytokine signaling) the PIAS (protein inhibitor of activated STATs) and protein tyrosine phosphatases;<sup>20</sup> furthermore, in cells in which proliferation results from the inactivation of negative regulators of signaling, such as the inhibitor of the PI3Kinase pathway PTEN, or the inhibitor of the proapoptotic TF p53, MDM2, the inhibition of upstream signaling pathways may have little effect. Thus, when upstream STAT3 activators are not identified or do not have any known inhibitor, or when STAT3 is itself activated it becomes a relevant target for anti-tumor treatments. This reasoning, reinforced by STAT3's previously noted "signaling bottleneck" situation,<sup>21</sup> has been used by many authors searching for STAT3-specific direct inhibitors.



**Figure 1.** STAT3 with DNA-consensus sequence. STAT3 monomer showing the N-terminal coiled-coil domain, the DBD (half site), the SH2 domain and the C-terminal domain. Basic residues are blue and acid ones are red. The STAT3 crystal coordinates were downloaded from the protein data bank (PDB, file: 1BG1) and analyzed using the chimera program.<sup>73</sup> Note that the model shown comprises residues 136 to 716;<sup>74</sup> hence, the protein's N-terminal and C-terminal domains (comprising the transactivation domain) are missing; the coordinates corresponding to the cDNA strand were missing in the model and had to be reconstructed.<sup>65</sup>



**Figure 2.** STAT3 activation. The transcription factor STAT3 is present in a latent inactive non-phosphorylated form in the cytoplasm. Activated cytokine receptors activate the kinases JAK, which phosphorylate tyrosines located in the cytoplasmic portion of the cytokine receptors creating STAT-binding motifs. Once bound to these motifs, STAT3 becomes in turn phosphorylated by the JAKs. The phospho STAT3 dimers (colored in pink) enter the nuclei and bind STAT3 target genes; note that the DNA-bound dimer is drawn differently to indicate the STAT3 conformational change suggested by molecular dynamics simulations.<sup>22</sup> STAT3 can also be activated by tyrosine kinases of the Src family (SFK), the SFKs can themselves be activated by G protein-coupled receptors (GPCR) or growth factor receptors, the growth factor receptors (including EGF-receptors and VEGF-receptors) can also phosphorylate STAT3. The DBD is created by the phosphotyrosine 705/SH2-mediated dimer. There are also monomers, and dimers with non-phosphorylated STAT3, which can shuttle between the cytoplasm and the nucleus.

## The Peculiar STAT3-STAT1 Connection

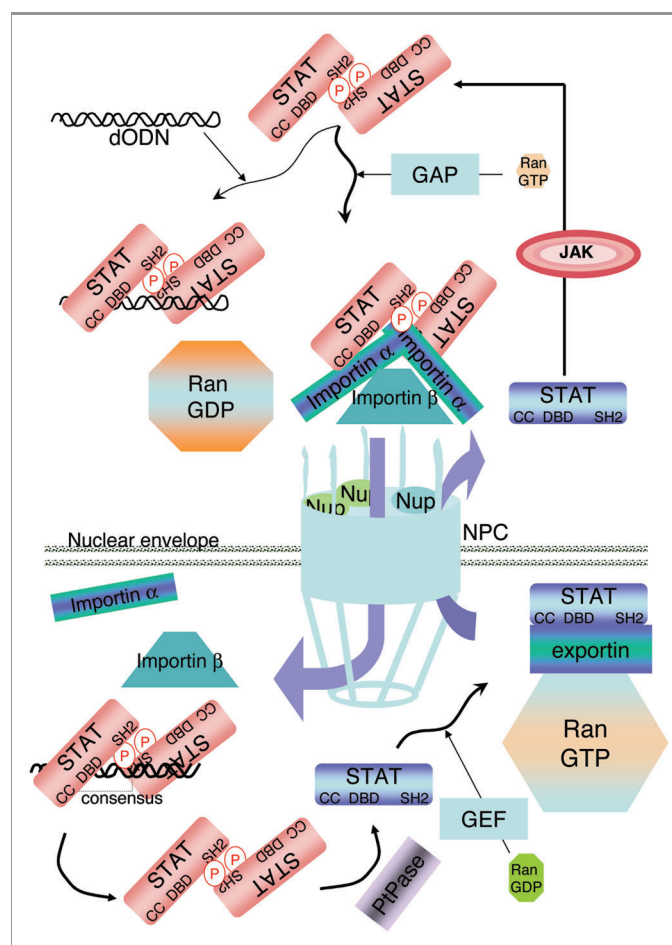
The high similarity between STAT3 and STAT1 is intriguing. Both TFs share a 50% amino-acid sequence homology<sup>22</sup> and share similar activating stimuli (types I and II IFNs, cytokines and growth factors); yet, their functions differ: STAT1 is mostly involved in immunity, host defense against pathogens and cell death (see refs. 23 and 24), with the notable exception that in certain contexts STAT1 exerts proliferative potential,<sup>25</sup> while STAT3 is mostly involved in cell growth and proliferation (see ref. 3). Their gene targets are mostly distinct: STAT3 stimulates the transcription of cell growth-associated genes, including cyclin-D1, survivin, Vegf, C-myc, Bcl-xL, Mcl-1, vascular endothelial growth factor, IL-10, transforming growth factor  $\beta$  and Bcl2<sup>3,26</sup> and

STAT1 stimulates the transcription of pro-inflammatory and anti-proliferative genes, including caspases, iNOS, Mdm2, p21waf/cip1 and p27kip1<sup>27,28</sup>; but, there is also an overlap of repertoires.<sup>23</sup> In reality, STAT3 and STAT1 recognize very similar DNA consensus sequences, based on a TTCNNN(T,G)AA motif<sup>29</sup> (see Table 1). In this motif, a 3N spacing (N representing any base) is most frequently present for STAT1 and STAT3, as shown by electrophoretic migration shift assay (EMSA).<sup>30</sup> Natural binding sites share this consensus with minor variations such as a preference for T at position -5 for STAT1, but there is greater diversity outside this consensus<sup>31</sup> (see Table 1), suggesting that target recognition *in vivo* requires additional co-factors. Thus, when attempting to inhibit STAT3 with the aim to kill tumor cell or block their growth, one must use substances that have no effect on STAT1, as has been pointed out<sup>32,33</sup> and shown in tumor cell lines with siRNA-suppressed STAT1.<sup>34,35</sup> Indeed, STAT1 is required for the antiproliferative effects of interferons  $\alpha$  and  $\gamma$ ,<sup>36</sup> apoptosis is defective in STAT1-null cells<sup>37</sup> and STAT1 is the major effector of IFN- $\gamma$ , a cytokine with antitumor and cancer immunosurveillance functions.<sup>38,39</sup>

### STAT3 Direct Inhibitors

The recognition that activated STAT3 is widely present in tumors and that its inhibition is a valuable anti-cancer strategy led to a search for STAT3-targeting compounds. Among those the DNA-alkylating platinum complexes were found to induce the death of tumor cells with activated STAT3 and were thought to act directly on STAT3;<sup>40</sup> other compounds target the SH2 of STAT3, including G quartet oligodeoxynucleotides and small molecules, some of which are highly specific for STAT3. STAT3 inhibitors also comprise the decoy oligodeoxynucleotides which target the DBD.

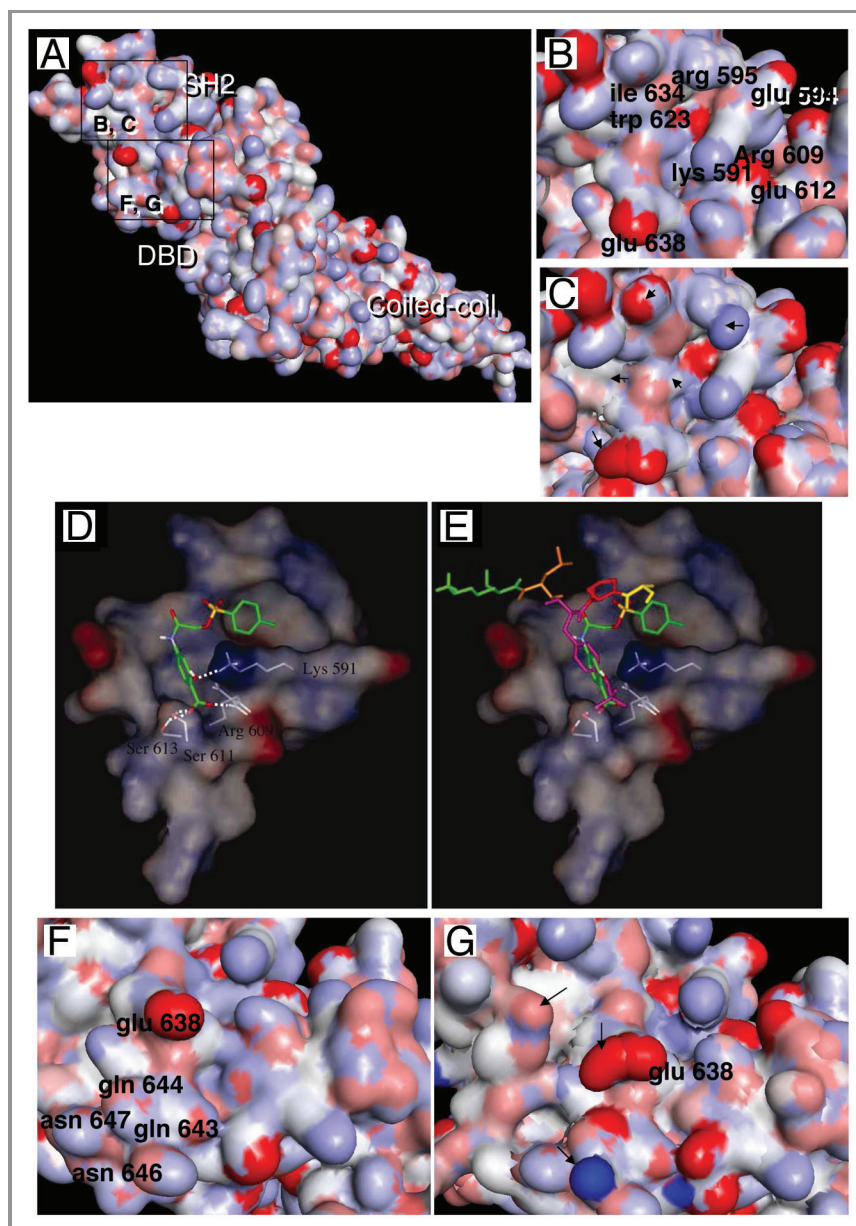
**Small molecules targeting the SH2 domain and inhibiting STAT3 dimerization.** The dimerization of STAT3 through reciprocal phosphotyrosine 705/SH2 interaction can be impaired by a phosphopeptide with the sequence PpYLKTK. This phosphopeptide inhibits STAT3 activity in tumor cell lines, induces cell death<sup>41</sup> and has a high affinity and specificity for STAT3: in particular it had no effect on the SH2-containing tyrosine kinase p56lck, and little effect on STAT1 as determined by EMSA. Despite their efficacy and specificity, the inefficient cell penetration of phosphopeptides led to a search for smaller equivalents using computational docking studies exploring the phosphotyrosine 705/SH2 interaction area (see Fig. 3A and B). These studies yielded several small molecules with high affinity and high specificity for STAT3, including STA-21,<sup>42</sup> Stattic,<sup>43</sup> S31-201<sup>44</sup> and recently BP-1-102, a compound with improved bioavailability and anti-cancer properties.<sup>45</sup> The mechanism of action of these compounds is thought to be based on their interaction with STAT3 SH2 (Fig. 4A and B), an area where the other monomer's phosphotyrosine 705 docks, thereby impairing the formation of the active dimer, as shown in<sup>44</sup> (see Fig. 4D and E, borrowed from ref. 44; the phosphotyrosine peptide is represented together with the inhibitor S31-201, both form H-bonds with the same residues, including lysine 591, serine 611,



**Figure 3.** STAT3 nuclear entry. The tyrosine phosphorylated STAT3 dimer interacts with the importins through its two NLSs [one within the coiled-coil (mL) domain: arginines 214 and 215, one within the DBD: arginines 414 and 417]. This complex is carried through the nuclear pore complex (NPC) by the Ran GDP which is formed in the cytoplasm by hydrolysis of Ran-bound GTP by GTPase activating protein (GAP). The NPC comprises nucleoporins (Nup). The importins release the dimer once it enters the nucleus. The high level of Ran-GTP in the nucleus is the result of a high GTP-exchange factor (GEF) activity in this compartment. The STAT3 dimer interacts with the STAT3 DNA consensus motif and then is released; once released from DNA the dimer is dephosphorylated by a nuclear phosphatase (Ptase). The dephosphorylated STAT3 is exported to the cytoplasm in combination with exportins and Ran-GTP.

serine 613 and arginine 609). Interestingly, STAT1-specific inhibitors have also been obtained using this peptidomimetic strategy.<sup>46</sup> STAT3 SH2-targeting compounds are efficacious STAT3 inhibitors, they inhibit STAT3 DNA binding, reduce STAT3-dependent cell proliferation and expression of biological targets. Yet, experimental demonstration of their interaction with STAT3 is missing, the actual data are based on computational studies, not on actual interaction measurements, as noted earlier.<sup>47</sup> This implies that a compound claimed to interact with SH2 might actually interfere with the binding of STAT3 to the receptor's phosphotyrosine site leading to biological effects similar to those of JAK-inhibitors. The compound might also interact with the DBD with just the same effect in the cell (reduced DNA binding, cell





**Figure 4.** A detailed view of STAT3 SH2 and DBD regions. (A) STAT3 surface obtained as in Figure 1. The areas in squares are the SH2 region where small molecules interact (B and C) and the SH2/DBD region where G quartets interact (D and E). (B) Close up view of the region of the SH2 of STAT3 that interacts with the small molecule inhibitors: the key aminoacids involved in interaction are labeled; the inhibitors interact particularly in the groove located between arginine 595 and lysine 591. (C) The same area as in (B) is shown but STAT3 and STAT1 are superimposed; this shows the overall great similarity between these two regions, yet some differences are present, accounting for the capacity of the inhibitors to discriminate between STAT3 and STAT1 (arrows); STAT1 crystal coordinates<sup>66</sup> used in (C) were from PDB file 1BF5. (D) Modeled interaction of inhibitor S31-201 with STAT3 SH2 domain, note the important role of lysine 591, serine 611 and arginine 609 in the interaction. (E) Same as (D), with added phosphotyrosine-peptide, showing its overlap with the STAT3 SH2/small molecule inhibitors binding area. (F) Detailed view of the region of STAT3 interacting with G quartets, this region includes the phosphotyrosine 705-interacting region of SH2 and a neighboring region including part of the DBD, including glutamic acid 638, glutamine 644, aspartic acid 647, glutamine 643 and asparagine 646. (G) Same region as in F is shown with the superimposition of STAT1, the major differences are indicated by arrows. [Panels (D and E) are reprinted from ref. 44 with permission; © 2007 National Academy of Sciences USA.]

death). Besides, compounds usually undergo modifications in a biological environment (cells or body fluids), these modifications can occur before the compounds reach their target. For instance, Stattic's inhibitory activity of STAT3 increases with time and is temperature- and dithiothreitol-sensitive, this suggests that it

interacts with a cysteine, such as cysteine 687 which is next to the phosphopeptide motif of STAT3 and faces the phosphopeptide-binding area of SH2.<sup>47</sup> While this point does not invalidate Stattic's specificity for STAT3, it suggests possible limitations for its in vivo utilization. Despite considerable progress and clear

**Table 1.** Comparison and alignment of the STAT3 and STAT1 specific DNA recognition sites and of the dODNs used to inhibit STAT3

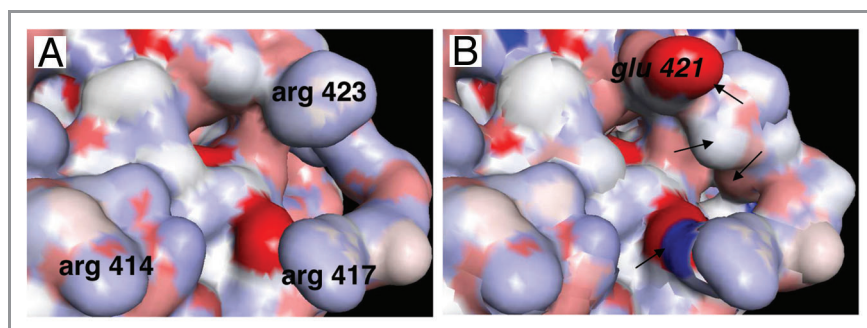
		-9	-8	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9						
STAT1																					References					
1				C	T	A	C	T	T	C	C	T	G	G	A	A	A	T	C	C	75					
2				C	G	C	T	T	T	C	C	C	C	T	A	A	A	T	G	G	76, 77					
3				T	G	A	T	T	T	C	C	C	C	G	A	A	T	G	A	C	78					
4				A	G	G	T	T	T	C	C	G	G	G	A	A	A	G	C	A	79					
5				A	C	T	C	T	T	C	C	T	T	G	A	A	A	C	G	C	80					
6				G	C	A	T	T	T	C	G	G	A	G	A	A	G	A	C	G	81					
7				C	C	A	C	T	T	C	T	G	A	T	A	A	A	G	C	A	82					
8				C	A	G	T	T	T	C	C	C	G	T	T	C	C	T	C	T	83					
9			T	G	T	G	A	A	T	T	A	C	C	G	G	A	A	G	T	G	84					
10					A	T	A	T	T	C	C	T	G	T	A	A	G	T	G		85, 86					
11	C	A	T	G	T	T	A	T	T	C	C	T	G	T	A	A	G	T	G		87					
12				G	G	C	G	T	T	C	T	T	G	G	A	A	A	T	G	C	G	C	C	88		
STAT3																										
13				C	A	T	T	T	C	C	C	G	T	A	A	A	T	C	G		57					
14				G	C	A	G	T	T	C	C	C	G	T	C	A	A	T	C	C	89					
15				C	T	C	C	T	T	C	C	C	G	G	C	A	G	C	A	T	90					
16				G	C	A	G	T	T	C	C	A	G	G	A	A	T	C	G	G	G	91				
17				C	T	G	C	T	T	C	C	C	G	A	A	C	G	T	G		30					
18				A	G	G	C	T	T	G	G	C	G	G	G	A	A	A	A	G	92					
19				A	T	C	C	T	T	C	T	G	G	G	A	A	T	T	C	T	A	93				
20				A	T	C	C	T	T	C	T	G	G	G	A	A	T	T	C	T	A	G	A	T	C	86, 94
STAT3 dODNs																										
21				C	A	T	T	T	C	C	C	G	T	A	A	A	T	C	G		57					
22				T	A	T	T	T	C	C	C	C	T	A	A	A	T	G	G		65					

The STAT1 and STAT3 consensus sequences are shown in bold.

anti-cancer efficacy, this family of compounds has not reached the clinic.

**G quartet oligodeoxynucleotides.** G quartets are G-rich oligodeoxynucleotides that form potassium-dependent four-stranded intramolecular G-quartet structures.<sup>48</sup> They inhibit STAT3 at micromolar concentrations and induce the death of several tumor cell lines, including head and neck cancer lines, they also arrest the development of breast tumor, prostate tumor or non-small cell lung cancer xenografts in nude mice.<sup>48,49</sup> These reagents are interesting in that their specificity for STAT3, demonstrated by EMSA showing high affinity binding to STAT3 and much lower affinity for STAT1,<sup>50</sup> is somewhat unexpected. A computational study of the interaction of the G quartet with STAT3 and STAT1 showed the following SH2 domain amino-acids to be involved in the binding: glutamic acid 638, glutamine 644, asparagine 647, glutamine 643 and asparagine 646<sup>51</sup> (Fig. 4F). Using a 3D analysis program (Accelrys) we compared STAT3 and STAT1 surfaces in the area where the G quartet interacts and found that the surfaces differ significantly (Fig. 4F and G). However, the use of G quartets is problematic due to their large size and potassium dependence, which limit cellular delivery.<sup>52,53</sup>

**Decoy oligodeoxynucleotides targeting the DNA binding domain.** Decoy oligodeoxynucleotides (dODNs) are short stretches of double stranded DNA containing a TF's consensus binding sequence. Once in the cells, dODNs inhibit the corresponding TF as shown for NFκB in animal models (cardiovascular disease<sup>54</sup> and ischemia-reperfusion injury<sup>55</sup>) and in cancer cells.<sup>56</sup> This property has been used for STAT3: in cells in which STAT3 is constitutively activated, STAT3 dODNs (Table 1) induce cell death.<sup>35,57,58</sup> While some studies suggested that dODNs must enter nuclei to exert their inhibitory action,<sup>59,60</sup> our studies of the subcellular location of STAT3 in dODN-treated cells have shown that the dODNs prevent nuclear translocation of STAT3.<sup>35,58</sup> The dODNs are thought to interact with STAT3's DBD within the cytoplasm and to prevent interaction with importins<sup>35</sup> which carry the STAT3 dimer within the nucleus.<sup>7</sup> Inhibition of importin binding by the dODN is thought to result from the masking of the DBD-located NLS (arginines 414 and 417) (see Figs. 1 and 5A) indirectly confirming its involvement in nuclear entry.<sup>9</sup> In fact, competition between DNA binding and importin binding was observed in vitro with STAT1<sup>61</sup> and within cells with STAT3.<sup>35</sup> Thus, despite the uncertainty regarding the two STAT3 NLSs' relative



**Figure 5.** Detailed view of the area of STAT3 interacting with DNA and importins. (A) Detail of the area of STAT3 interacting with the DNA consensus sequence. Arginines 414 and 417 play a key role in the interaction of STAT3 with importins and nuclear translocation, arginine 423 is involved in the interaction of DNA with STAT3.<sup>65</sup> (B) The same area as in (A) is shown, the corresponding area of STAT1 has been superimposed, showing the high level of homology between STAT3 and STAT1, except for glutamic acid 421 (in italic) of STAT1 whose interaction with DNA is very different from that of arginine 423 of STAT3 (see ref. 65), and other differences indicated by arrows.

requirement for nuclear transfer, it seems that the DBD-located NLS plays an important role for nuclear translocation since its masking is sufficient to impair it, confirming that it may be a good target for inhibition, as suggested by others.<sup>7,53,62</sup>

As discussed above, the opposed cellular functions of STAT1 and STAT3, in spite of their similarity, is puzzling. STAT1 and STAT3 can form heterodimers, whose function is not elucidated to this day, they recognize very similar DNA motifs (table), and they have targets in common and regulate one another. Indeed, in STAT3-deficient cells, STAT3-activators such as IL-6 trigger an IFN- $\gamma$ -like response through STAT1 activation<sup>63,64</sup>; and in STAT1-deficient cells, IFN- $\gamma$  and IFN- $\alpha$  trigger STAT3-dependent proliferative responses.<sup>23</sup> Thus the STAT1/STAT3 cross-regulation suggests that STAT3 inhibitors may work best in STAT1-expressing cells.<sup>28,35</sup> It is therefore essential to inhibit STAT3 without inhibiting STAT1 to keep cell death processes operational. In this regard, it should be noted that STAT3-dODNs inhibit STAT3<sup>57,58</sup> but also inhibit activated STAT1, thereby abolishing IFN- $\gamma$ -induced cell death<sup>35,58</sup> and reducing the dODNs anti-STAT3 efficacy. Computer analysis of the dODN's interaction with STAT1 and STAT3 DBDs showed that within the highly similar DNA sequences, there were subtle differences including a T at positions -7 and -5, a dC at position 0, a dA at position +5 (see Table 1, dODN #22),<sup>65</sup> which had been previously noted by computer analysis<sup>66</sup> and DNA-binding studies.<sup>30,31,67,68</sup> This allowed designing a dODN that could inhibit STAT3 without inhibiting STAT1, demonstrating that at this level specific interaction can be achieved.<sup>65</sup> Therapeutic use of the dODNs not only requires specific target recognition, but also stability in biological fluids. Modifications, including phosphothioate end modification and hairpin structures<sup>35,58,69,70</sup> considerably increased intracellular stability and efficacy. A recent improvement consisting of a cyclic STAT3 dODNs comprising a hairpin at both ends was designed and found to reduce xenograft tumors following intravenous administration.<sup>71</sup> Furthermore, a STAT3 dODN has been found to have few side effects when administered to primates,<sup>72</sup> suggesting interesting therapeutic perspectives.

Another possibility is to design smaller molecules mimicking the dODN's interaction with STAT3, similarly to what was achieved with the SH2 domain. The STAT3 area that interacts with the DNA target and the dODN (Fig. 5A) is of particular interest because it also interacts with importins. This area comprises the DBD-located NLS including arginines 414 and 417 that are located closely to arginine 423, involved in interaction with the dODN; these three arginines surround an opening in the surface in which small molecules could interact (Fig. 5A). Furthermore, superimposition of STAT3 and STAT1 showed that in spite of their striking overall similarity, these areas comprise in the same location glutamic acid 421 in STAT1 and arginine 423 in STAT3<sup>65</sup> (Fig. 5B), a difference that might be exploited for the design of a STAT3 small molecule inhibitor with STAT3/STAT1 discriminating capacity; studies are underway in the laboratory to try and design such a reagent.

## Conclusion

Because STAT3 is constitutively activated in nearly 70% of tumors, it provides a valuable target for anti-cancer therapy. The recent findings that tumors harbor activating mutations of STAT3 underlines the need for additional STAT3 inhibitors, and inhibitors that target other regions of STAT3 than its SH2, especially as most identified mutations are within this region. In addition, studies on cancer cell lines indicate that even when STAT3 direct targeting is insufficient to induce cell death; it can diminish the resistance to other anti-cancer compounds such as doxorubicin or EGFR inhibitors. Finally, STAT3 direct inhibitors are also probably less toxic than many others because inhibition of STAT3 in mature cells has minimal effects.

## Acknowledgments

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