STAT3 inhibition in prostate and pancreatic cancer lines by STAT3 binding sequence oligonucleotides: differential activity between 5’ and 3’ ends

H. Dan Lewis,1 Ashley Winter,1 Thomas F. Murphy,1 Snehlata Tripathi,2 Virendra N. Pandey,2 and Beverly E. Barton1,3

Departments of 1Surgery, and 2Biochemistry and Molecular Biology, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, New Jersey and 3Veterans Administration-New Jersey Health Care System, East Orange, New Jersey

Abstract
Signal transducers and activators of transcription (STAT) were originally discovered as components of signal transduction pathways. Persistent aberrant activation of STAT3 is a feature of many malignancies including prostate cancer and pancreatic cancer. One consequence of persistently activated STAT3 in malignant cells is that they depend on it for survival; thus, STAT3 is an excellent molecular target for therapy. Previously, we reported that single-stranded oligonucleotides containing consensus STAT3 binding sequences (13410 and 13411) were more effective for inducing apoptosis in prostate cancer cells than antisense STAT3 oligonucleotides. Control oligonucleotides (scrambled sequences) had no effect. Here, we report that authentic STAT3 binding sequences, identified from published literature, were more effective for inducing apoptosis in prostate cancer cells and pancreatic cancer cells than was oligonucleotide 13410. Moreover, the authentic STAT3 binding sequences showed differing efficacies in the malignant cell lines depending on whether the canonical STAT3 binding sequence was truncated at the 5’ or the 3’ end. Finally, expression of one STAT3-regulated gene was decreased following treatment, suggesting that STAT3 may regulate the same set of genes in the two types of cancer. We conclude that truncating the 5’ end left intact enough of the canonical STAT3 binding site for effective hybridization to the genome, whereas truncation of the 3’ end, which is outside the canonical binding site, may have affected binding of required cofactors essential for STAT3 activity, thereby reducing the capacity of this modified oligonucleotide to induce apoptosis. Additional experiments to answer this hypothesis are under way. [Mol Cancer Ther 2008;7(6):1543–50]

Introduction
Prostate cancer is the most diagnosed adenocarcinoma in American men, with over 186,000 cases estimated for 2008 (1). Due at least in part to its intrinsic chemoresistant nature, prostate cancer is the second leading cause of cancer death for American men, accounting for over 27,000 deaths last year and predicted to cause at least 28,000 in 2008 (1). Recently, physicians documented that neither radical prostatectomy nor androgen-ablation therapy contributes to overall increased long-term survival (2, 3). The current selection of treatment options for prostate cancer patients is clearly not adequate: new therapeutic agents specifically designed to target prostate cancer are needed.

Pancreatic cancer is a deadly carcinoma, the incidence of which increases with age and correlates with gender and race. Most cases of pancreatic cancer are ductal adenocarcinoma. Pancreatic cancer is the most lethal adenocarcinoma in America, with ~36,000 cases estimated for 2008 that will ultimately lead to ~34,000 deaths (1). Due at least in part to its intrinsic chemoresistant nature and inherent difficulties in early prognosis, pancreatic cancer is at present the fourth leading cause of cancer death for Americans (4). Conventional therapy (chemotherapy, radiation surgery, or combinations of these modalities) has little effect on the course of pancreatic cancer (4). The current selection of treatment options for pancreatic cancer patients is clearly not adequate: new therapeutic agents for pancreatic cancer are urgently needed.

Transcription factors are latent proteins that bind to the genome on activation, either inducing or repressing gene expression. After activation, transcription factors bind to specific enhancer sequences on the genome upstream or near the promoter region of the gene regulated by the transcription factor. Signal transducers and activators of transcription (STAT) are part of the signal transduction pathway of many growth factors and cytokines and are activated by phosphorylation of tyrosine and serine residues by upstream kinases (5). For example, signaling by interleukin-6 generally induces phosphorylation of STAT3 (5). In benign cells, the signaling by STAT3 is under tight regulation, so that the signal is transient. However, aberrant signaling by STAT3 is found in many types of
malignancies: multiple myeloma, head and neck cancer, breast cancer, prostate cancer, etc. (6–12). Malignant cells expressing persistently activated STAT3 become dependent on it for survival; disruption of activation or expression of STAT3 results in apoptosis (12). STAT3 binds to two known sequences, HSIE and GAS (13–15), through which its antiapoptotic and oncogenic effects are directed (16, 17). These sites contain the canonical STAT3 binding motifs TTC(N)2-4GAA or TT(N)4-6AA (16, 17).

Previously, we showed that prostate cancer lines were sensitive to treatment with antisense STAT3 oligonucleotides; 48 h after treatment, most of the cells were apoptotic, but few cells treated with sense oligonucleotide were affected (12). However, antisense as a therapeutic category has shown nonspecific activation of the innate immune system primarily through unmethylated CpG motifs (18). Therefore, we created a new strategy for inhibiting STAT3 activity. Because STAT3 binds to discrete, known sequences on the genome, we synthesized two novel anticancer oligonucleotides based on the STAT3 consensus binding sequence, called 13410 and 13411 (19). We reported that oligonucleotides based on the STAT3 consensus binding sequence we obtained from the published literature. We found that two published human STAT3 binding sequences induced significant apoptosis in prostate cancer and pancreatic cancer cell lines. Furthermore, the two STAT3 binding sequences induced apoptosis differentially in both types of cancer depending on whether we truncated the sequences at the 3' end or the 5' end. We think our data reveal that genomic sequences past the 3' end of the canonical STAT3 binding sequence may contain cofactor binding sites necessary for STAT3 activity. Furthermore, our data show that the 5' end of the STAT3 canonical binding site may not be necessary for complete STAT3 activity.

**Materials and Methods**

**Oligonucleotides**

Oligonucleotides were synthesized using phosphorothioate chemistry by the Molecular Resources Facility at the University of Medicine and Dentistry-New Jersey Medical School. The ribose moieties for 5 bp at both 5' and 3' ends were modified with 2-O-methyl groups to increase stability of the oligonucleotides and to provide higher hybridization affinity (20, 21). For determinations of transfection efficiencies, a fluorescent oligonucleotide (13778a; Table 1) labeled with FITC was synthesized and included in every transfection experiment. Sequences of oligonucleotides used are given in Table 1. Two of the oligonucleotides, B and D, which spanned the 3' end of the STAT3 consensus binding sequence, were modified to span the 5' end of the consensus binding sequence (sequences BB and DD).

**Table 1. Putative STAT3 inhibiting oligonucleotide sequences**

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>13410a</td>
<td>5'-TCCCCGTAAATCCCTA-3'</td>
<td>(1); truncated 13410</td>
</tr>
<tr>
<td>13778a</td>
<td>5'-TATGATCTCTCCGT-3'</td>
<td>(1); truncated 13778</td>
</tr>
<tr>
<td>A</td>
<td>5'-TCCCCGTAATCCCT-3'</td>
<td>(2)</td>
</tr>
<tr>
<td>B</td>
<td>5'-TCCGGAAATCCCT-3'</td>
<td>(3)</td>
</tr>
<tr>
<td>BB</td>
<td>5'-TTCCGGAAATCCCT-3'</td>
<td>(3)</td>
</tr>
<tr>
<td>C</td>
<td>5'-TCTGGAATCTCCT-3'</td>
<td>(5)</td>
</tr>
<tr>
<td>D</td>
<td>5'-TCTGGAATCTCCT-3'</td>
<td>(6)</td>
</tr>
<tr>
<td>DD</td>
<td>5'-TTCTGGGAATTCCT-3'</td>
<td>(6)</td>
</tr>
</tbody>
</table>

NOTE: The sources and sequences for the oligonucleotides used in the experiments are listed.

**Cells**

DU145 cells were the gift of Dr. James Turkson (University of South Florida). They were grown in DMEM/Ham’s F12 (Invitrogen) plus 10% newborn bovine serum (Hyclone). LNCaP cells were obtained from the American Type Culture Collection; they were maintained in RPMI 1640 (Invitrogen) plus fetal bovine serum (Hyclone). PANc-1 cells were the gift of Dr. James Freeman (University of Texas Health Sciences Center; refs. 22, 23) and were grown in the same medium as DU-145 cells. Primary human dermal fibroblasts were obtained from LifeLine Cell Technology and grown in their proprietary medium. Cell viabilities were determined using fluorescein diacetate (Sigma) and a Universal RIII fluorescence microscope (Zeiss).

**Transfection of Oligonucleotides into Cells**

LipofectAMINE 2000 transfection reagent (Invitrogen) was used to transfect oligonucleotides into the cancer cell lines as described previously (19). Briefly, cells plated in six-well plates were grown to 50% confluence. Oligonucleotides were diluted in Opti-MEM I (Invitrogen) appropriately. Next, LipofectAMINE 2000 was diluted in Opti-MEM I (2 μL LipofectAMINE 2000 to 250 μL Opti-MEM I per well). The diluted LipofectAMINE 2000 was allowed to incubate for 5 min at room temperature; next, 250 μL/well LipofectAMINE was mixed with 250 μL diluted oligonucleotide. The liposome-oligonucleotide mixture remained at room temperature for 20 min before the 500 μL liposome-oligonucleotide mixture was added to each well of cells. Cells were incubated with the mixture for 6 h at 37°C; then, 1.5 mL of medium per well containing 30% serum was added to each well. Additional medium containing 10% serum was added as needed until the experiment was ended.

**Apoptosis Determinations**

FITC-Annexin V and propidium iodide staining (Abcam; Sigma) were used to measure the induction of apoptosis by oligonucleotides after incubation for 24 h. Harvested cells were washed twice in buffer; then, 5 × 10^6 cells in 1 mL buffer, containing at least 40 mmol/L Ca ^{2+}, were put into each tube (Falcon Plastics). Five microliters of FITC-Annexin V and either propidium iodide or 7-aminoactinomycin D (Sigma) were put into each tube as well. Fluorescence was quantified using a FACSscan flow...
cytometer (Becton Dickinson) on at least 10,000 events using CellQuest Pro software (Becton Dickinson) and an Apple Macintosh G4 dual coprocessor computer running OS X 10.3.9 (Apple). The JC-1 dye assay was used for measuring the reduction in mitochondrial transmembrane potential during apoptosis (24, 25). Briefly, 10⁶ cells in 1 mL were stained with 1 μL JC-1 (Molecular Probes) at 1 mg/mL in DMSO according to the manufacturer’s instructions at various times following treatment with active oligonucleotides or control oligonucleotides. After incubating for 15 min at 37°C, cells were analyzed for decreased orange fluorescence by flow cytometry.

CD46 Expression Assay
DU-145 or PANC-1 cells were transfected with oligonucleotides as described above. After 24 h, cells were harvested using trypsin, washed, and then stained with FITC-anti-human CD46 or matching isotype control (PharMingen) for 1 h using 1 μL antibody per 10⁶ cells. After thoroughly washing the cells, fluorescence was measured using a FACScan flow cytometer.

Statistical Analysis
The graphing program Kaleidagraph 4.1 (Synergy Software) and the statistical program InStat3 (GraphPad Software) were used for data analyses.

Results
Two Anti-STAT3 Sequences Induced Apoptosis in Pancreatic and Prostate Cancer Cell Lines
Our original STAT3-inhibiting oligonucleotide, 13410, was a phosphorothiorated 24-mer that was 2’-O-methoxylated at the 5 bases at each end (19). Because we are actively exploring the use of peptide nucleic acids (PNA) having STAT3 binding sequences as potential anticancer therapy, we truncated the sequence of 13410 to a 15-mer called 13410a. The reason it is necessary to truncate oligonucleotide sequences in this way is that the synthesis of PNA becomes very inefficient when the sequence is longer than 20 bases. Therefore, one of the original goals of these studies was to ascertain which sequence or sequences in oligonucleotide form (oligonucleotides being much easier to synthesize than PNA) might be superior to 13410a for inducing apoptosis in cancer cell lines. Therefore, all oligonucleotides used in these studies were 15-mers.

Figure 1 shows the comparison of the concentration response curves for the 15-mer oligonucleotides in DU-145 cells; 13778a induced very little apoptosis at 1,000 nmol/L, whereas 13410a and sequence D had IC₅₀ values of ~40 and 580 nmol/L, respectively. Extending these studies to PANC-1 cells, we found that 500 nmol/L 13410a induced apoptosis in ~43% of cells; however, the same concentration of sequence B induced more than 60% apoptosis (Fig. 2). We observed that 13778a induced only 18% apoptosis at 1,000 nmol/L, whereas 13410a and sequence B induced 40% and 65%, respectively (Fig. 2A). The IC₅₀ for sequence B on PANC-1 cells was calculated to be 427 nmol/L, whereas the IC₅₀ of sequence D was calculated to be 57 nmol/L (Fig. 2B). Sequence B, like sequence D, has

Figure 1. Effect of STAT3-inhibiting oligonucleotides on DU-145 cells. DU145 cells were transfected with oligonucleotides at the concentrations indicated. After 48 h, cells were harvested, washed, and stained with FITC-Annexin V and propidium iodide. Percent apoptosis was quantified by subtracting the percent viable population from the total. The concentration of oligonucleotide that induced 50% apoptosis (IC₅₀) is noted where appropriate. IC₅₀ values were obtained using the Identify tool in the Kaleidagraph software package. Average ± SD data from three experiments. A, comparison of active sequences 13410a and sequence D to control sequences E and 13778a. IC₅₀ values were calculated using the Identify tool from the Kaleidagraph software package. B, comparison of sequences D to DD, illustrating the importance of the purines at the 3’ end on STAT3-inhibiting efficacy. C, comparison of effects of sequences B and F to 13410a.
the same sequence at the 3' end as 13410a but differs from both sequence D and 13410a at the 5' end. To examine the importance of the 5' end of the STAT3 binding site, we synthesized oligonucleotides BB and DD, which retained the consensus STAT3 binding sequence on the 5' ends but were truncated at the 3' ends. We found that 500 nmol/L of either sequence BB or sequence DD induced much less apoptosis than the corresponding oligonucleotides B and D. Sequence BB induced only 17% apoptosis and sequence DD induced only 18.5% apoptosis compared with 51% for sequence B and 73% for sequence D at 500 nmol/L (Fig. 2). We conclude from these data that integrity of the oligonucleotide sequence extending beyond the 3' end of the canonical STAT3 binding sequence must be very important, if not required, for STAT3 activity.

To confirm the results presented above, we used the JC-1 dye to measure the inhibition of mitochondrial transmembrane potential (12). As shown in Fig. 3A and B, the orange fluorescence of aggregated (oxidized) JC-1 by DU-145 cells was significantly decreased by 80% in the presence of 500 nmol/L sequence D or by 30% in the presence of 500 nmol/L sequence B. Figure 3C and D shows the results in PANC-1 cells. The orange fluorescence of aggregated JC-1 was significantly decreased by ~45% in the presence

**Figure 2.** Effect of STAT3-inhibiting oligonucleotides on PANC-1 cells. PANC-1 cells were transfected with oligonucleotides at the concentrations indicated. After 48 h, cells were harvested, washed, and stained as in Fig. 1 legend. Percent apoptosis was quantified by subtracting the percent viable population from the total. The concentration of oligonucleotide that induced 50% apoptosis (IC50) is noted where appropriate. Average ± SD data from three experiments. **A,** comparison of effect of sequences B to BB on induction of apoptosis. Sequence B has an IC50 of ~270 nmol/L, whereas sequence BB has an IC50 calculated to be >4,400 nmol/L. **B,** comparison of effects of sequences D to DD on PANC-1 cells.
of 500 nmol/L sequence D or by 30% in the presence of 500 nmol/L sequence B. Scrambled sequence oligonucleotide 13778a had no effect on JC-1 oxidation even at 1,000 nmol/L (Fig. 3). Thus, by a measurement of the physiology of apoptosis, we noted that sequence D was more potent than sequence B in the 2 types of cancer cell lines used.

**Anti-STAT3 Oligonucleotides Did Not Induce Apoptosis in Primary Cells**

We postulated that targeting STAT3 for cancer treatment would spare benign cells the deleterious effects of most cancer treatments. To test this hypothesis, we previously published data indicating that benign immortalized prostate cell lines (BPH-1 and NRP-152) were not killed by oligonucleotide 13410 (12). However, benign immortalized cells, because they contain viral sequences, are not the same as the normal cells of the body (24, 26–28). Therefore, to examine the potential effect of STAT3-inhibiting oligonucleotides on bystander cells in the body, we used primary human dermal fibroblasts from LifeLine Cell Technology for the following series of experiments. Fibroblasts were transfected with oligonucleotides; apoptosis was determined by Annexin V/propidium iodide staining and flow cytometry 48 h later. The results shown in Table 2 indicate that concentrations of 13410a or sequence D that induced significant apoptosis in DU-145 or PANC-1 cells induced no apoptosis in human dermal fibroblasts. These data indicate that the oligonucleotides have specificity for the targeted binding sequence of STAT3 and do not kill bystander cells indiscriminately.

**Anti-STAT3 Oligonucleotides Inhibited Expression of a STAT3-Regulated Gene, CD46**

STAT3 is a known regulator of several antiapoptotic and cell cycling genes. Recently, it was shown to regulate the expression of CD46, a cell surface protein expressed by many tumors that helps tumor cells escape complement-mediated cytolysis (25, 29). Therefore, we followed up on this observation to see if CD46 was inhibited by the STAT3 inhibitors when given to PANC-1 and DU-145 cells. We found that our anti-STAT3 oligonucleotides inhibited CD46 expression; the results are presented in Fig. 4. Figure

![Figure 3](image-url)

**Figure 3.** Effect of STAT3-inhibiting oligonucleotides on JC-1 oxidation. **A,** effect of sequence D on DU-145 cells. Using JC-1, the IC₅₀ was calculated to be ~226 nmol/L. Differences among treated cells and untreated cells were highly significant \( (P = 0.0001, \text{ repeated ANOVA}) \). **B,** effect of sequence B on DU-145 cells. The IC₅₀ was calculated to be ~1,790 nmol/L. Differences among treated cells and untreated cells were less significant than for sequence D but still significant \( (P = 0.023, \text{ repeated ANOVA}) \). **C,** effect of sequence B on PANC-1 cells. The IC₅₀ was calculated to be ~841 nmol/L. Differences among values were very significant by repeated ANOVA \( (P = 0.0058) \). **D,** effect of sequence D on PANC-1 cells. The IC₅₀ was calculated to be ~1,011 nmol/L; the differences among values were not significant \( (P = 0.1225, \text{ repeated ANOVA}) \).
Table 2. Anti-STAT3 oligonucleotides did not induce apoptosis in normal human fibroblasts (average ± SD)

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>% Viable</th>
<th>Oligonucleotide</th>
<th>% Viable</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>92.9 ± 3.3</td>
<td>Sequence B</td>
<td>90.8 ± 3.5</td>
</tr>
<tr>
<td>13410a</td>
<td>92 ± 3</td>
<td>Sequence BB</td>
<td>90.5 ± 2.1</td>
</tr>
<tr>
<td>13778a</td>
<td>89.6 ± 2.1</td>
<td>Sequence D</td>
<td>89.8 ± 0.3</td>
</tr>
<tr>
<td>Sequence DD</td>
<td>91.8 ± 0.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Oligonucleotides were made as described in Materials and Methods. Oligonucleotides were transfected at 500 nmol/L into normal human dermal fibroblasts using LipofectAMINE 2000; apoptosis was determined at 48 h by flow cytometry after staining with FITC-Annexin V and propidium iodide.

4A and C shows that ~90% of untreated DU-145 cells expressed CD46. Treatment with 13410 significantly reduced expression by ~75% (Fig. 4B), whereas treatment with 13778 had no effect on CD46 expression. Transfection with the truncated form of 13410, 13410a, decreased CD46 expression from 91% to 45%, whereas sequence D inhibited CD46 expression on DU-145 cells by 50% (Fig. 4D).

Similar results were found when PANC-1 cells were transfected with 13410a or sequence D. PANC-1 cells expressed CD46 at somewhat lower levels than did DU-145 cells levels; ~67% of untreated PANC-1 cells expressed CD46 (Fig. 4E). Treatment with either 13410a or sequence D reduced expression of CD46 (Fig. 4F) from 67% to 35% and 33%, respectively. Once again, 13778a had no effect on CD46 expression (Fig. 4F). We concluded from these data that our STAT3-inhibiting oligonucleotides also inhibited STAT3-regulated genes other than those involved in apoptosis.

Discussion

STAT3 inhibitors merit extensive study in the context of cancer therapy because they have the potential to induce apoptosis selectively in cancer cells, sparing benign cells. Our previous data showed that a novel STAT3 inhibitor, 13410, spared benign immortalized prostate cells and hormone-sensitive prostate cancer cells while inducing apoptosis in hormone-refractory prostate cancer cells (19). Here, we extended those studies to evaluate the effect of related novel STAT3 inhibitors on prostate cancer cells and on benign primary cells. We observed that our novel STAT3 inhibitors did not induce apoptosis in primary fibroblasts (Table 2). We expect based on these data that bystander cells would not be affected adversely by anti-STAT3 treatment; thus, this treatment modality offers a tremendous advantage to the patient over conventional anticancer chemotherapy.

Figure 4. Effect of STAT3-inhibiting oligonucleotides on STAT3-regulated CD46 expression. Oligonucleotides were transfected as described in Materials and Methods. After 24 h, cells were harvested and then stained with FITC-anti-human CD46 or FITC-isotype control (both from PharMingen). Fluorescence was quantified on a FACScan flow cytometer. A, 24 h after transfection with 13410 (thick pink line), ~75% of the DU-145 cells stained less intensely with anti-CD46 and 22% of cells did not stain at all with anti-CD46. Red line, DU-145 cells treated with 13778; green line, isotype control. Approximately 90% of DU-145 cells stained positive for CD46 in these experiments (region defined by M1). B, untreated (red line) and 13778-treated (green line) DU-145 cells stained with anti-CD46 antibody exhibited the same fluorescence intensity. C, CD46 expression by untreated DU-145 cells in the second set of experiments; ~90% of cells expressed CD46. Green line, isotype control; red line, anti-CD46 Ab. D, green line, untreated DU-145 cells; pink line, 13778a; blue line, 13410a inhibited CD46 by ~45%; red line, sequence D inhibited CD46 by ~50%. E, CD46 expression by untreated PANC-1 cells. Abort 67% of PANC-1 cells expressed CD46 (red line); green line, isotype control. F, effect of 13410a and sequence D on CD46 expression in PANC-1 cells. Green line, untreated cells; pink, 13778a; blue, 13410a inhibited CD46 by ~40%; red, sequence D inhibited CD46 expression by ~50%.
Furthermore, we found that altering the sequence of 13410 by truncating it to the 15-mer 13410a decreased its efficacy somewhat, but that using an inhibitor with the sequence of an authentic human STAT3 binding sequence, sequence D, increased the efficacy (Figs. 1 and 2). The IC_{50} of 13410a was 410 nmol/L, but that of sequence D was 40 nmol/L, making sequence D 10-fold more potent for induction of apoptosis in DU-145 cells. At this juncture, we have no explanation for why the IC_{50} of sequence D measured by inhibition of JC-1 oxidation was observed to be 226 nmol/L (Fig. 3) other than to postulate that the two assays, Annexin V binding/pro-pidium iodide uptake and JC-1 oxidation, are fundamentally different enough that perhaps the only correlation one ought to expect is rank-order potency, which is what we observed.

Moreover, we showed that this set of novel STAT3 inhibitors decreased the expression of the STAT3-regulated gene product, CD46. CD46 is a cell-surface glycoprotein, one of those involved in protection of tumor cells against complement-mediated cytotoxicity. Recent work by Buettner et al. showed that CD46 is regulated by STAT3 (29). We used CD46 as a STAT3 target gene because of the ease with which it is detected and quantified. We found that at least 90% of DU-145 cells expressed CD46 but that the proportion of cells expressing CD46 decreased significantly by >50% when the cells were transfected with 13410, 13410a, or sequence D (Fig. 4A-D). Similar results were observed for PANC-1 cells, which normally express CD46 somewhat less than DU-145 cells (~67% of PANC-1 cells expressed CD46; Fig. 4E). When transfected with 13410a or sequence D, the level of expression was decreased by ~50% (Fig. 4F).

The most striking aspect of our data is the huge difference in efficacy observed when anti-STAT3 sequences were altered at the 3′ end. Apparently, the 5′ T of the canonical STAT3 binding sequence is not as important for anti-STAT3 activity as those bases beyond the 3′ end of the canonical STAT3 binding sequence (Fig. 1). In comparing sequences D to DD and B to BB, we observed >10-fold difference in IC_{50} between the pairs of oligonucleotides. We hypothesize that the sequences extending beyond the 3′ end of the STAT3 binding sites may be binding sites for cofactors required for STAT3 activity. Among the known STAT3 cofactors are CBP/p300 (30, 31), NcoA/SRC1a (32), MITF (33), cellular oncogenes such as c-jun and c-fos (34), and undoubtedly more besides the ones named. STAT1: STAT2 heterodimers are thought to recruit coactivators and undoubtedly more besides the ones named. STAT1: STAT3 cofactors are CBP/p300 (30, 31), NcoA/SRC1a (32), MITF (33), cellular oncogenes such as c-jun and c-fos (34), and undoubtedly more besides the ones named. STAT1: STAT2 heterodimers are thought to recruit coactivators and undoubtedly more besides the ones named. STAT1: STAT3 cofactors are CBP/p300 (30, 31), NcoA/SRC1a (32), MITF (33), cellular oncogenes such as c-jun and c-fos (34), and undoubtedly more besides the ones named. STAT1: STAT2 heterodimers are thought to recruit coactivators and undoubtedly more besides the ones named.

We are using oligonucleotides to test for optimum STAT3-inhibiting sequence, but in practice, we intend to develop a PNA STAT3 inhibitor. We believe that PNA are better because coupled to the correct cell-penetrating peptide, they offer more complete in vivo uptake than do oligonucleotides; they form stable triple helices, which are essential for inhibiting STAT3:genome binding; and their stability means little degradation (36–39). Thus, we are creating a test PNA for proof-of-principle to ascertain whether a PNA-bearing sequence D, the most potent sequence we have identified to date, could induce apoptosis in tumor cells. These data give us the rationale to continue to study STAT3 binding sequences as the basis for experimental inhibitors and to synthesize PNA for testing in the future in clinical trials.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
We thank the New Jersey Medical School Molecular Resources Facility, particularly Dr. Robert Donnelly.

References
Molecular Cancer Therapeutics

STAT3 inhibition in prostate and pancreatic cancer lines by STAT3 binding sequence oligonucleotides: differential activity between 5’ and 3’ ends

H. Dan Lewis, Ashley Winter, Thomas F. Murphy, et al.


Updated version  Access the most recent version of this article at: http://mct.aacrjournals.org/content/7/6/1543

Cited articles This article cites 37 articles, 14 of which you can access for free at: http://mct.aacrjournals.org/content/7/6/1543.full#ref-list-1

Citing articles This article has been cited by 7 HighWire-hosted articles. Access the articles at: http://mct.aacrjournals.org/content/7/6/1543.full#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link http://mct.aacrjournals.org/content/7/6/1543. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.