Direct targeting of STAT3 alone and in combination with immune checkpoint inhibitors to treat solid tumors

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Conflicts of interest

- Baylor College of Medicine hold patents on inventions made by David J. Tweardy (DJT), including methods of use of TTI-101 in cancer.
- The patents are licensed exclusively to Tvardi Therapeutics, Inc. (TTI).
- DJT is part-owner of TTI.
Objectives

- Rationale for targeting STAT3 in HCC, HNSCC, and NSLCC alone and in combination with immune checkpoint therapies
- Our strategy for developing small-molecule STAT3 inhibitors and success to date
- Results with danvatisen, STAT3 antisense oligonucleotide, alone in DLBCL, and in combination with durvalumab (anti-PD-L1) in HNSCC
Immune checkpoint inhibitor (ICI) therapy in advanced HCC, HNSCC, and NSCLC

- Hepatocellular carcinoma (HCC)
  - Anti-PD-1 (nivolumab/pembrolizumab) FDA approved as second-line based on objective response rates (ORR) = 15-20%.¹ ²

- Head and neck squamous cell carcinoma (HNSCC)
  - Nivolumab approved for second-line based on 13.3% ORR; pembrolizumab for first-line alone (if PD-1 score >1) and in combination with platinum-based therapy plus 5-fluorouracil based on overall survival (OS) benefit of 2-4 months.³ ⁴

- Non-small cell lung cancer (NSCLC)
  - Anti-PD-1 (nivolumab/pembrolizumab) and anti-PD-L1 (atezolizumab) approved for second-line based on ORR of 17-20% ⁴ ¹⁰ and 5-yr survival rates for nivolumab of 18% ¹¹

STAT3 promotes tumor cell growth and an immunosuppressive tumor microenvironment

Rationale for targeting STAT3 in HCC, HNSCC, and NSCLC

- STAT3 is activated (phosphorylated on tyrosine 705) in the majority of HCC (up to 100%), HNSCC (up to 75%), and NSCLC (up to 61%) tumors.\(^1-3\)

- STAT3 activation in HCC, HNSCC, and NSCLC correlates with increased tumor vascularity, size, and aggressiveness, as well as undifferentiated state, resistance to therapy, and reduced survival.\(^4-12\)

- STAT3 mediates tumor immune evasion, in part, through development and actions of myeloid derived suppressor cells (MDSCs), which contribute significantly to the ineffectiveness of ICI therapies.\(^13-18\)

Canonical STAT3 activation and its targeting with small molecules

Inflammation
Survival and cell cycling
Cachexia
Fibrosis
TTI-101 drug discovery summary

• **Virtual Ligand Screening:**¹
  - Computer docking of 920,000 drug-like compounds into the specific binding site of the pY-peptide binding pocket of the STAT3 SH2 domain, which was focused uniquely on residue E638.
  - Three hits (C3, C30, and C188) competitively inhibited STAT3 binding to immobilized pY-peptide ligand in a unique SPR assay and inhibited IL-6-mediated phosphotyrosylation and nuclear translocation of STAT3.
  - C188 was the most potent: Induced apoptosis of STAT3-dependent breast cancer cell line (MDA-MB-468) at 700 nM.

• **Hit-to-Lead Development:**²
  - 2D similarity screening of 490,000 drug-like compounds in the chemical structures data base using C188 as scaffold, which identified 202 candidates; used 3D pharmacophore analysis to rank them based on Gaussian similarity to C188.
  - Top 39 tested for inhibition of STAT3 binding by SPR assay; computational quantitative structure activity relation (QSAR) analysis identified TTI-101 as most active (6-fold more potent than C188).
  - Two rounds of medicinal chemistry-based SAR activity analysis and *in vitro* and *in vivo* pharmacokinetics established TTI-101 as lead.

TTI-101 binds to STAT3 with high-affinity and potently blocks STAT3 binding to pY-peptide ligand

Microscale thermophoresis (MST)

Surface Plasmon Resonance (SPR)

$K_D = 4.7 \text{nM}$

$K_i = 12.4 \text{nM}$

(Cheng-Prussoff equation)
TTI-101 potently inhibits binding of WT STAT3 and cancer-causing STAT3 GOF mutants to pY-peptide ligand

WT

IC50 = 0.66 uM

Y640F

IC50 = 1.78 uM

K658Y

IC50 = 0.82 uM

D661Y

IC50 = 3.13 uM

$K_i = 3-12$ nM (Cheng-Prussoff equation)
TTI-101 inhibits growth of STAT3−/− MEF cell clones stably expressing STAT3 WT and GOF mutation (D661Y)

Bharadwaj et al unpublished 2019
Additional key pre-clinical PK features of TTI-101

- PK studies in mice at 10 mg/kg demonstrated tumor levels 2.6 times those of simultaneously measured plasma levels.
- Pharmacokinetic (PK) studies in mice, rats, and dogs showed that TTI-101 administration provides excellent plasma exposures following oral administration.
TTI-101 inhibits HCC tumor growth, liver fibrosis and injury in the Hep$^{Pten^{-}}$ tumor model

**Reduced tumor volume**

- Placebo
- TTI-101 (100 mg/kg/d)

**Reduced fibrosis**

- Placebo
- TTI-188-9

**Normalized liver Injury**

- Wild Type Placebo C188-9

$P < 0.001$  
$P = 0.04$  
$\text{STAT3 targeting in solid tumors}$  
$P < 0.001$  
$P = 0.001$  

Jung et al Clin Cancer Res 2017
TTI-101 potently inhibits growth of radioresistant HNSCC cell line (UM-SCC-17B) in vitro and in vivo

Inhibition of cell growth in vitro

Inhibition of xenograft tumor growth (100 mg/kg/d ip)

Reduced tumor pY-STAT3 and pY-STAT1 levels

Bharadwaj et al Oncotarget 2015
TTI-101 inhibits growth of NSCLC cell line (A549) \textit{in vitro} and \textit{in vivo}

STAT3 shRNA and TTI-101 inhibit A549 growth \textit{in vitro}

TTI-101 inhibits A549 growth \textit{in vivo}

TTI-101 targets STAT3 in A549 tumors \textit{in vivo}

Lewis et al. Lung Cancer 2016
TTI-101 does not target STAT3’s mitochondrial function

Wegrzyn et al Science 2009; Kasembeli et al unpublished 2019
Selectivity of TTI-101 and lack of toxicity

- TTI-101 does not target tyrosine kinases upstream of STAT3
- TTI-101 only targeted STAT3 downstream of IL-6 in an analysis of 1,320 phosphoproteins in human muscles cells in vitro
- TTI-101 treatment (14 days) decreased Th17 cells and slightly increased Th1 cells in spleens of naïve and immunologically unchallenged mice.
- Minimal off-target toxicity or impairment of immunity expected
- Toxicity studies in rats and dogs revealed detectable gross, microscopic or clinical laboratory toxicity up to 200 and 100 mg/kg/d for 28 days, respectively.

Redell et al *Blood* 2011; Bharadwaj et al *Oncotarget* 2015; Bharadwaj et al unpublished 2018
Danvatirsen antisense STAT3 modified oligonucleotide

- Good activity as single agent in preclinical mouse models of cancer: NSCLC (PC-9 and H1975), colorectal cancer (CT26), breast (4T1), and lymphoma (A20)
- Improved responses to duvalumab (anti-PD-L1) in CT26 and A20 mouse models of cancer
- Demonstrated activity as monotherapy in patients with diffuse large B-cell lymphoma [DLBCL; 4 of 27 with partial (2) or complete (2) responses]; transaminitis in 40% and thrombocytopenia in 30%
- Anti-tumor activity in combination with duvalumab in patients with HNSCC

Hong et al *Science Translational Med* 2015; Reilley et al *J ImmunoTherapy Cancer* 2018; Cohen et al presented at *European Society for Medical Oncology Annual Meeting* Munich, Germany October 2018 (courtesy of Brett Monia Ph.D. COO Ionis Pharmaceutical, Inc.)
Danvatirsen (3 mg/kg) plus durvalumab doubles response rates vs. durvalumab alone

- 35 patients enrolled
  - 15 progressive disease
  - 10 stable disease
  - 7 partial response
  - 3 complete responses
- Danvatirsen uptake and STAT3 reduction shown in tumor biopsies

Cohen et al presented at European Society for Medical Oncology Annual Meeting Munich, Germany October 2018 (courtesy of Brett Monia Ph.D. COO Ionis Pharmaceutical, Inc.)
Summary and Conclusions

- ICIs have improved outcomes of patients with solid tumors including NSCLC.
- STAT3 contributes intrinsically to tumor cell growth and extrinsically to tumor immune evasion and is activated in most patients with HCC, HNSCC, and NSCLC.
- Antisense oligonucleotide and direct small-molecule STAT3 targeting strategies are progressing with evidence of single agent activity.
- Danvatirsen shows promise for improving responses to ICI alone in HNSCC.
Thank you!