Development of selective STAT3 targeted inhibitors for Head & Neck Squamous Cell Carcinoma (HNSCC) therapy

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8/3/12
Head & Neck Squamous Cell Carcinoma (HNSCC)

- HNSCC 6th most common malignancy in the world.
  - 600,000 cases per year and 50% mortality rate.
- HNSCC Risk Factors: Smoking, EtOH, age and HPV 16 (OP).
- Areodigestive Tract: non-keratinizing squamous epithelium
  - oral cavity; oropharynx, supraglottis, hypopharynx, glottis
- Signs & Symptoms: otalgia, dysphagia, dysphonia, sore throat
  - Late signs & symptoms: dyspnea, non-painful neck mass
- Multidisciplinary Treatment: TNM staging
  - Early stage (T1/2N0M0): single modality; surgery or external radiation
  - Late stage (T3/4N+M0): multi-modality; surgery + radiation ± chemotherapy
Total Laryngectomy for Advanced Stage Glottic HNSCC

Intra-operative picture of removal of voice box

Stoma after total laryngectomy = breathing hole

Intra-operative pictures were taken by Dr. Naib Tabr
HNSCC Chemotherapy Agents

- **Alkylating Agents:**
  - Cisplatin
  - Carboplatin

- **Antimetabolites:**
  - Methotrexate (MTX)
  - 5-Flurouracil

- **Microtubule Stabilizer:**
  - Paclitaxel

**Side effects:** Non selectivity, hair loss, bone pain, immunosuppression.

- **Epidermal Growth Factor Receptor (EGFR) inhibitor:**
  - Cetuximab (monoclonal antibody) – Selective, but only 30% patients respond.
We Need New Chemotherapy Agents!

- Chemotherapy (cisplatin) saved this patient’s life!

- Our dreaming new chemotherapy agents:
  - Great selectivity of HNSCC cells over normal cells.
  - Greater efficacy.
  - Great tolerability.

*Ultimately, our goal is to increase the overall survival!*

Pictures were taken by Dr. Naib Tabr
STAT3 Signaling Pathway as a Therapeutic Target in Cancer

Table 1. STAT3 in the Context of Various Cancers: Validation as an Anticancer Target

<table>
<thead>
<tr>
<th>Cancers Characterized by Elevated STAT3 Expression or Activity</th>
<th>Poor Prognosis Linked to High STAT3 Levels</th>
<th>Upstream/Downstream Abnormalities of STAT3 Signaling</th>
<th>Xenograft Models Responsive to Inhibition of STAT3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemia</td>
<td>Renal cell carcinoma</td>
<td>Elevated EGFR expression</td>
<td>Head and neck squamous cell carcinoma</td>
</tr>
<tr>
<td>Lymphomas</td>
<td>Colorectal cancer</td>
<td>Constitutively activated EGFR-RTK</td>
<td>Glioblastoma</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>Ovarian carcinoma</td>
<td>Overexpression of SFKs</td>
<td>Myeloproliferative neoplasms</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Gastric carcinoma</td>
<td>Hyperactivated JAKs</td>
<td>Renal cell carcinoma</td>
</tr>
<tr>
<td>Prostate carcinoma</td>
<td>Intestinal-type gastric adenocarcinoma</td>
<td>Elevated TGFα/IL-6</td>
<td>Breast cancer</td>
</tr>
<tr>
<td>Lung cancer (non-small-cell)</td>
<td>Cervical squamous-cell carcinoma</td>
<td></td>
<td>Lung adenocarcinoma</td>
</tr>
<tr>
<td>Renal cell carcinoma lung cancer</td>
<td>Osteosarcoma</td>
<td></td>
<td>Acute lymphoblastic leukemia</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>Epithelial ovarian carcinoma</td>
<td></td>
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<tr>
<td>Cholangiocarcinoma</td>
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<tr>
<td>Ovarian carcinoma</td>
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<td></td>
<td></td>
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<tr>
<td>Pancreatic adenocarcinoma</td>
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<td></td>
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<tr>
<td>Melanoma</td>
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</tbody>
</table>

*Head and neck squamous cell carcinoma

What is STAT3?

- Signal Transducer and Activator of Transcription 3 (STAT3).
- STAT3 is a proto-oncogene.
- Constitutively activated STAT3 (STAT3C) mediates cellular transformation.
- STAT3 up-regulates.
  - Apoptosis inhibitor genes (Bcl-xl, Mcl-1, & Survivin).
  - Cell-cycle regulators (cyclin D1, pim-1 and c-Myc).
- Activated STAT3 present in many cancers.
  - Directs tumor cells toward proliferation and survival.
  - Induces angiogenesis.
  - Alters the tumor microenvironment.
  - Promotes tumor metastases through its effect on cell migration and invasion.
  - In antigen presenting cells leads to dendritic cell anergy which triggers T cell tolerance and suppresses the anti-tumor immune response.
Signal Transducers and Activators of Transcription

- **Common STAT domain structure**
  - Crystal structure of N- and C-terminally truncated STAT1 molecule bound to DNA

- **Conserved tyrosine residue-**
  - Y701, Y705 or Y695

- **Seven members:**
  - STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b & STAT6
  - Cytoplasmic transcription factors regulating cytokine gene expression
    - Interferons, IFNα/β and IFNγ (prototypic activators of STAT1 & STAT2)
    - Hematopoietic cytokines, Growth hormone, Receptor tyrosine kinases (EGF, PDGF, TGFα, & Insulin)
    - Src & Tec family kinases
  - STATs 1, 3 & 5 are each activated by a large number of cytokines
  - STATs 2, 4 & 6 are activated by relatively few
Degraded by the ubiquitin proteasome pathway. The Src homology domain-containing tyrosine phosphatases 1/2 (SHP-1/2) interact with the intracellular domain of cytokine receptors and dephosphorylate and inactivate JAK, Stat3, and the receptor. In addition, active Stat3 binds to the promoter regions of suppressor of cytokine signaling (SOCS) genes. Similar to SHP-1/2, SOCS-1 and SOCS-3 interact with the kinase domain of various JAK proteins or the phosphotyrosine residues in the intracellular portion of the respective receptor, leading to the reduction of Stat3 activation. This is another negative feedback mechanism of the Stat3 signaling pathway and its upstream kinases. However, SOCS-3 does not affect SRC-induced Stat3 activation. Besides SHPs and SOCSs, protein inhibitor of activated Stat3 (PIAS3) specifically inhibits the binding of dimerized phosphorylated Stat3 to DNA, thus abolishing the transcription of Stat3 target genes. Recently, the protein tyrosine phosphatase receptor T (PTPRT) has been shown to specifically dephosphorylate Stat3 at Tyr705 and thereby regulates its cellular localization as well as Stat3 target gene expression.

4. STAT3 AND CANCER

Accumulating evidence in the past few years strongly implicates the critical role of aberrant Stat3 activation in malignant transformation and tumorigenesis. In contrast to the transient nature of Stat3 activation in normal cells, overexpression and/or persistent activation of Stat3 has been reported in most human solid and hematological tumors, including ovarian, endometrial, cervical, breast, colon, pancreatic, lung, brain, renal, and prostate cancers, head and neck squamous cell carcinoma (HNSCC), glioma, melanoma, lymphomas, and leukemias. The expression and activation levels of Stat3 in select cancer and normal tissues as well as cell lines are shown in Figure 3. Stat3 is significantly expressed in some normal tissues including pericardium, neutrophils of bone marrow, leukocytes of peripheral blood, peritoneum, and mammary gland (Figure 3a) and in systems including digestive tract, kidney, bladder, hematopoietic, lung, and peripheral nervous system (PNS) (Figure 3b), indicating that Stat3 plays an important role in the physiological processes in these tissues.
Strategies and Challenges to Therapeutic Intervention in STAT3 Signaling

Table 2. Strategies and Challenges to Therapeutic Intervention into STAT3 Signaling

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Targets</th>
<th>Examples</th>
<th>Challenges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibit phosphorylation/activation of STAT3</td>
<td>EGFR agonism</td>
<td>Cetuximab, panitumumab</td>
<td>Modest efficacy; development of resistance; myelosuppression, GI toxicity, and adverse events; kinase selectivity and cardiovascular toxicity</td>
</tr>
<tr>
<td></td>
<td>TKR activity</td>
<td>Gefitinib, erlotinib, lapatinib</td>
<td></td>
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<tr>
<td></td>
<td>JAK activity</td>
<td>AG490, LS-104, ICNB1824, CEP-701</td>
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<tr>
<td></td>
<td>SFK activity</td>
<td>Dasatinib, AZD0530, bosutinib</td>
<td></td>
</tr>
<tr>
<td>Inhibit intermolecular interactions that involve STAT3</td>
<td>STAT3 SH-2 domains</td>
<td>Oligopeptides designed from EGFR, gp130, and other receptor or pY-containing peptides; peptide aptamers; G-quartet oligonucleotides; small-molecule peptidomimetics</td>
<td>Poor cell permeability and efficacy; poor metabolic stability; poor selectivity for specific SH2 domains; potential for adverse events</td>
</tr>
<tr>
<td>Inhibit nuclear import/export of STAT3</td>
<td>Importins α3, α 5, α 7 Importin β Exportin 1</td>
<td>Karyostatin 1A (effect on STAT3 undetermined) Leptomycin B and Ratjadone A</td>
<td>Multicomponent nature of nuclear pore and translocation not fully determined; specificity for translocated proteins problematic</td>
</tr>
<tr>
<td>Inhibit STAT3-mediated transcription</td>
<td>DNA binding site of STAT3</td>
<td>dsODN decoys; peptide aptamers</td>
<td>Poor cell permeability without effective and specific delivery systems; poor metabolic stability</td>
</tr>
<tr>
<td>Natural products</td>
<td>Unspecified</td>
<td>Guggulsterone, honokiol, curcumin, resveratrol, flavopiridol, cucurbitacin</td>
<td>Specificity, potency, and efficacy, mechanism of action unknown</td>
</tr>
</tbody>
</table>

Poor cellular activity and lack selectivity killed a lot STAT3 inhibitors identified by target based approaches such as high-throughput screening or virtual screening.

The STAT3 oncoprotein promotes:
- Cell Proliferation
- Cell survival
- Angiogenesis
- Migration
- Metastasis
- Evasion of immunity

The STAT1 tumor suppressor favors:
- Cell cycle arrest
- Apoptosis
- Anti-tumor immunity

STAT3 and STAT1 have divergent roles in tumorigenesis.
## STAT3 inhibitors in Clinical Trials

<table>
<thead>
<tr>
<th>Agent</th>
<th>Structure</th>
<th>Trial phase</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>STA-21</td>
<td><img src="image" alt="STA-21 Structure" /></td>
<td>Phase I/II</td>
<td>Psoriasis</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td><img src="image" alt="Pyrimethamine Structure" /></td>
<td>Phase I/II</td>
<td>Chronic lymphocytic leukemia / Small lymphocytic lymphoma</td>
</tr>
<tr>
<td>OPB-31121</td>
<td>Structure not disclosed</td>
<td>Phase I</td>
<td>Advanced solid tumor</td>
</tr>
<tr>
<td>RTA 402</td>
<td><img src="image" alt="RTA 402 Structure" /></td>
<td>Phase I/II</td>
<td>Pancreatic cancer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phase II</td>
<td>Solid tumors and lymphoid malignacies</td>
</tr>
</tbody>
</table>

1st Hypothesis

An inhibitor that selective targets STAT3, without affecting STAT1 signaling pathway, will act as ideal cancer therapeutic &

pSTAT3 HCS and pSTAT1 selectivity assays in HNSCC cells will identify the selective STAT3 pathway inhibitors with improved therapeutic potential
High Content Screening (HCS) Assay
STAT3 HCS Plate Map

- 32 minimum controls 0.2% DMSO
- 32 maximum controls IL-6 in 0.2% DMSO

% Inhibition Heat Map

Ranges:
- min 83.07671452459
- median 5.524834463383
- max 111.6701012189
**UPDDI 2 × ImageXpress Ultra’s (IXUs)**

- **Automated point scanning confocal imager**
  - Adjustable pinhole
- **4 laser lines**
  - 405, 488, 561 & 635 nm
- **4 objective turret**
  - Selectable in software
  - 4×, 10×, 20×, 40×, 60× & 100×
- **4 PMT detectors**
  - Sequential / parallel acquisition
- **CRS Catalyst express plate loader**
- **MetaXpress & AcuityXpress**
- **MDCStore database**
- **PowerCore**
- **10 Image Analysis Modules**
pSTAT3-Y705 HCS Image Acquisition

- 20× 0.45 NA ELWD objective
- IR Laser autofocus
- Laser excitation
  - 405, 488, 561 & 635 nm
- Quad filter cube 405/488/561/635
  - 417-477 nm, 496-580 nm, 553-613 nm, & 645-725 nm
- 2 fluorescent channels acquired sequentially
  - Hoechst channel laser autofocus Z-offset -6.98 µM, 405 laser 10% power, PMT gain 550
  - pSTAT3-Y705 FITC channel Z-offset from W1 12.96 µM, 488 laser 10% power, PMT gain 625
- 2 images per channel per well
- Time to scan 384-well plate ~ 90 min
ImageXpress Ultra 20× 0.45NA Objective

<table>
<thead>
<tr>
<th>Media</th>
<th>Hoechst Ch1</th>
<th>pSTAT3-Y705 Ch2</th>
<th>Composite</th>
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</thead>
<tbody>
<tr>
<td></td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
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<tr>
<td>IL-6</td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
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*Slide by Dr. Paul A. Johnston*
Acknowledgement

- Dr. Peter Wipf.
- Committee members:
  - Dr. Donna Huryn, Dr. Barry Gold, Dr. Jelena Janjic.
  - Dr. Paul A Johnston (HCS bioassay), Dr. Jennifer Grandis (Kinase profile), Dr. James Jaber, Dr. Lynn Resnick, Dr. Matthew G LaPorte, Dr. Erin Skoda, Mr. Pete Chambers (ELS, LC-MS).
- Wipf group members past & present.
- Funding: NCI/SAIC–Frederick 29XS127.