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

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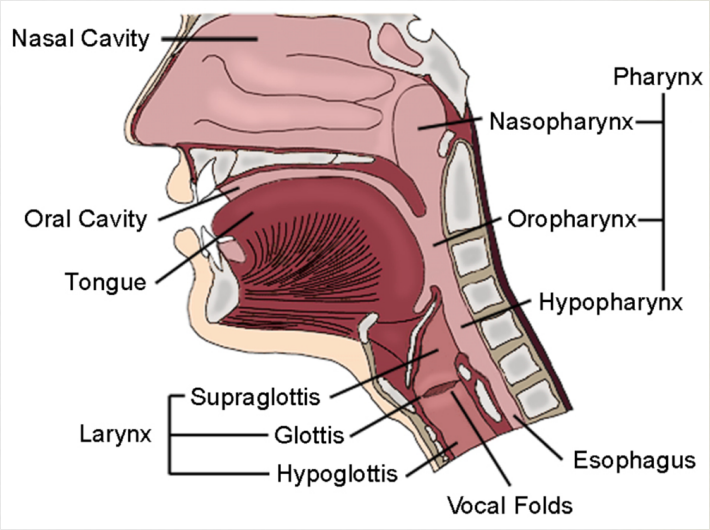
8/3/12

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

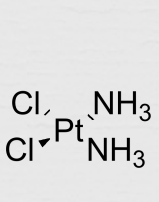
Intra-operative picture of removal of voice box

Stoma after total larygectomy = breathing hole

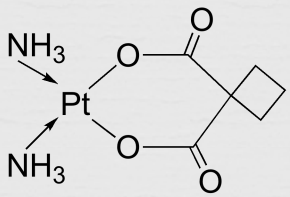
Intra-operative pictures were taken by Dr. Naib Tabr

HNSCC Chemotherapy Agents

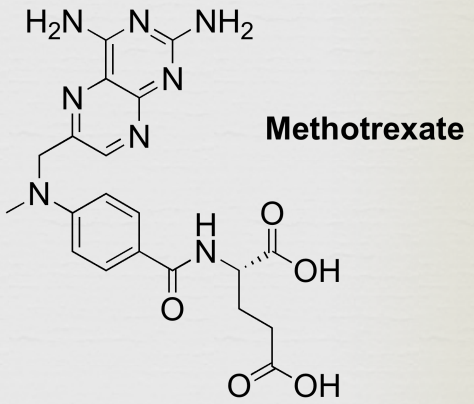
- **Alkylating Agents:**
  - Cisplatin
  - Carboplatin
- **Antimetabolites:**
  - Methotrexate (MTX)
  - 5- Flurouracil
- **Microtubule Stabilizer:**
  - Paclitaxel



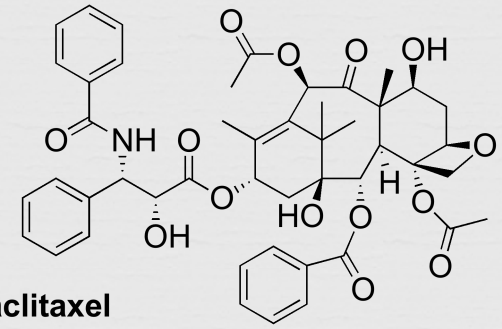
Cisplatin



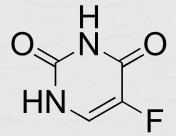
Carboplatin



Methotrexate



Paclitaxel



Fluorouracil

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

- **Epidermal Growth Factor Receptor (EGFR) inhibitor:**
  - Cetuximab (monoclonal antibody) – Selective, but only 30% patients respond it.

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.***
  - ***Great 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

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

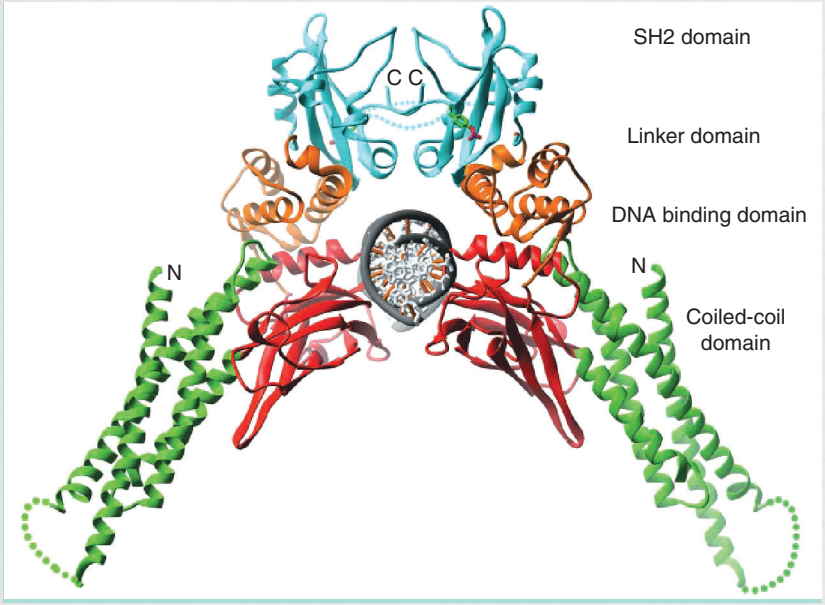
*Johnston PA, Grandis JR, Mol Interv. **2011** Feb; 11 (1): 18-26*

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**



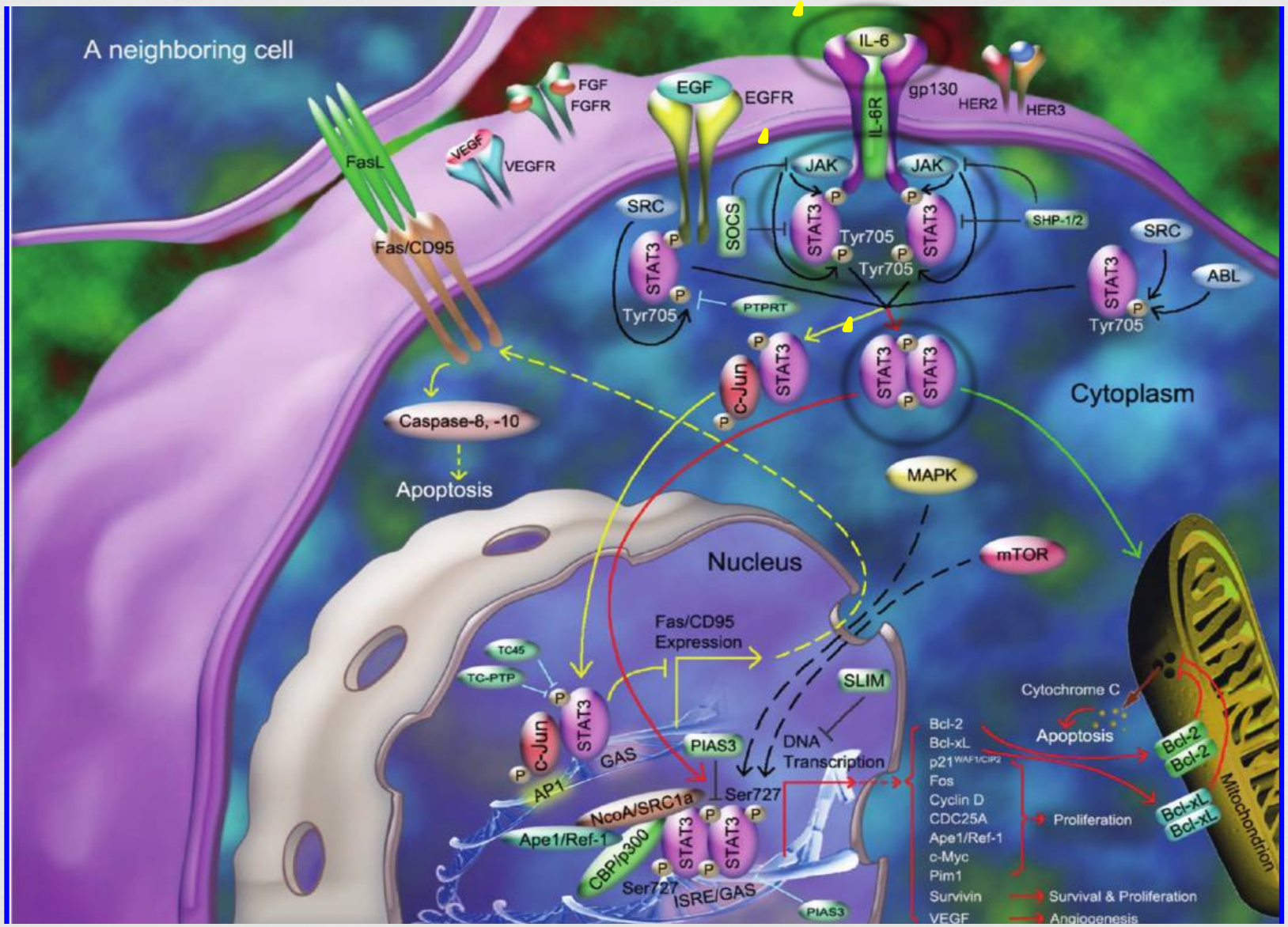
Crystal structure of N- and C-terminally truncated STAT1 molecule bound to DNA

Future Med. Chem. (2011) 3(5), 567-597

- **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

STAT3 signaling pathway



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Strategies and Challenges to Therapeutic Intervention in STAT3 Signaling

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

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

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

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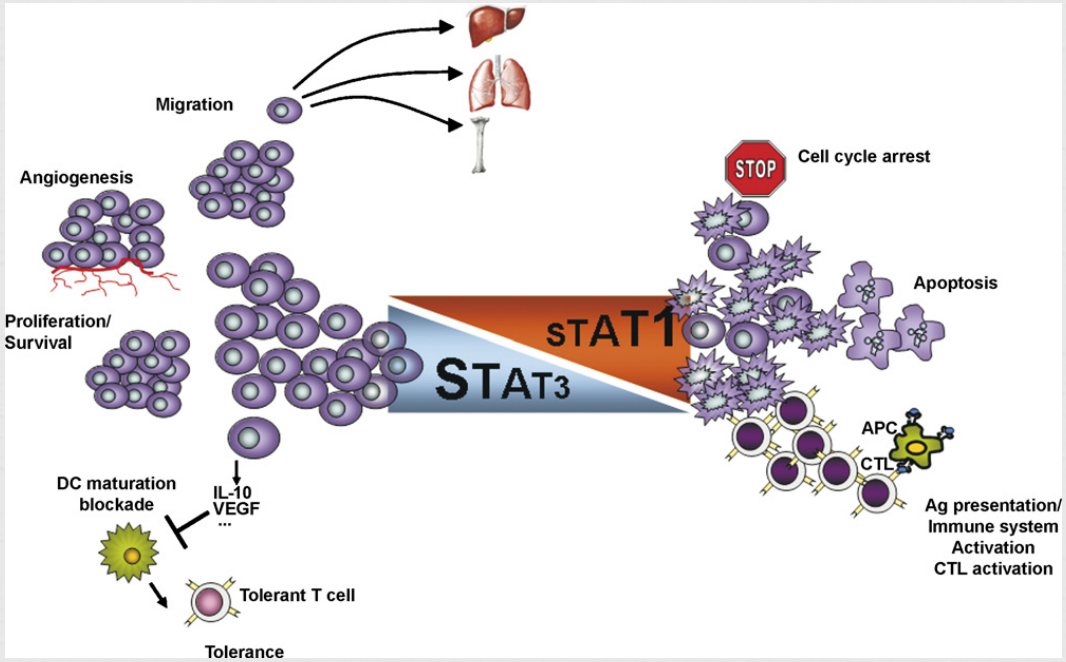
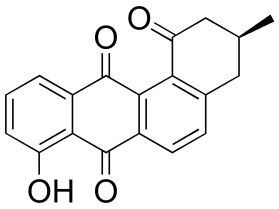
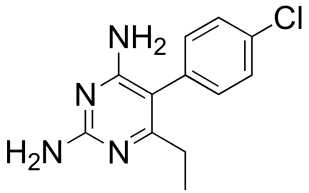
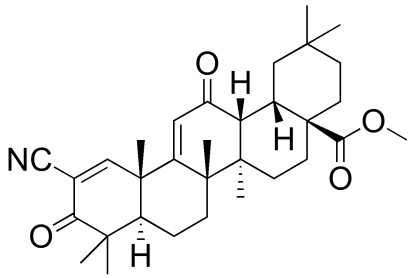
STAT3 and STAT1 have divergent roles in tumogenesis



- ***The STAT3 oncogene promotes:***
  - ***Cell Proliferation***
  - ***Cell survival***
  - ***Angiogenesis***
  - ***Migration***
  - ***Metastasis***
  - ***Evasion of immunity***
- ***The STAT1 tumor suppressor favors:***
  - ***Cell cycle arrest***
  - ***Apoptosis***
  - ***Anti-tumor immunity***

G. Regis et al. / Seminars in Cell & Dev. Biology 19 (2008) 351–359

STAT3 inhibitors in Clinical Trials

Agent	Structure	Trial phase	Indication
STA-21		Phase I/II	Psoriasis
Pyrimethamine		Phase I/II	Chroniv lymphocytic leukemia / Small lymphocytic lymphoma
OPB-31121	Structure not disclosed	Phase I	Advanced solid tumor
RTA 402		Phase I/II Phase II	Pancreatic cancer Solid tumors and lymphoid malignacies

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1st Hypothesis

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

&

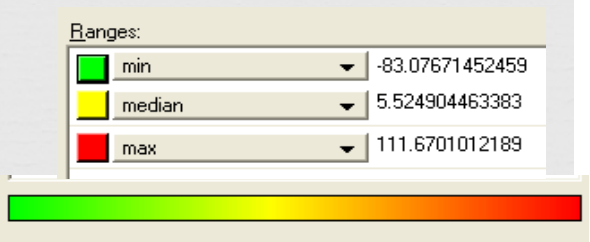
pSTAT3 HCS and pSTAT1 selectivity assays in HNSCC cells will identify the selective STAT3 pathway inhibitors with improved therapeutic potential

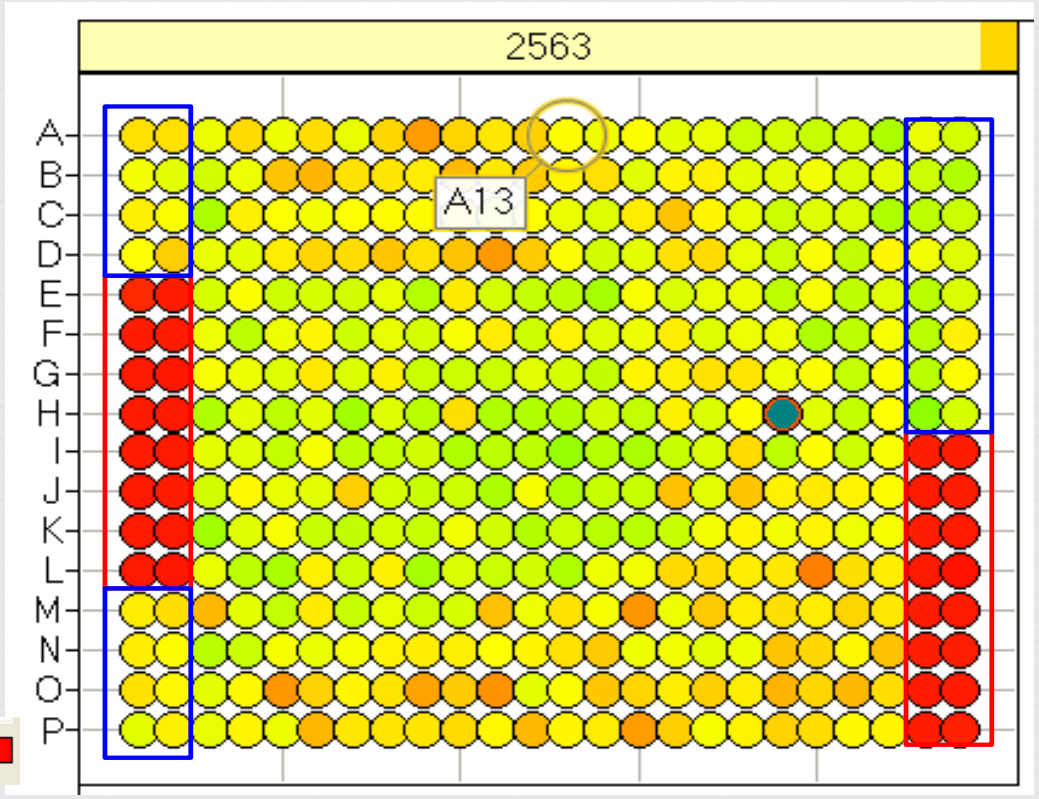
High Content Screening (HCS) Assay

STAT3 HCS Plate Map

32 minimum controls
0.2% DMSO

32 maximum controls
IL-6 in 0.2% DMSO





Slide by Dr. Paul A. Johnston

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

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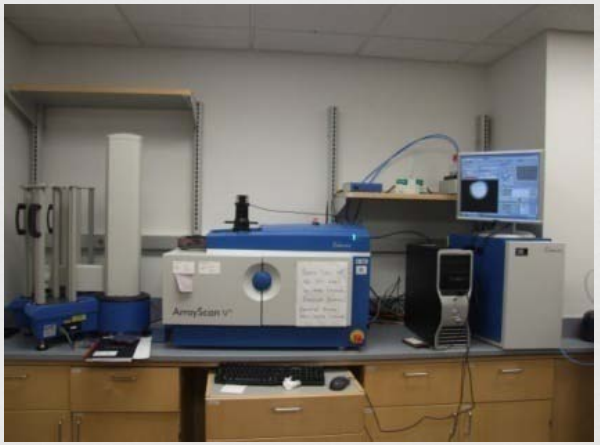
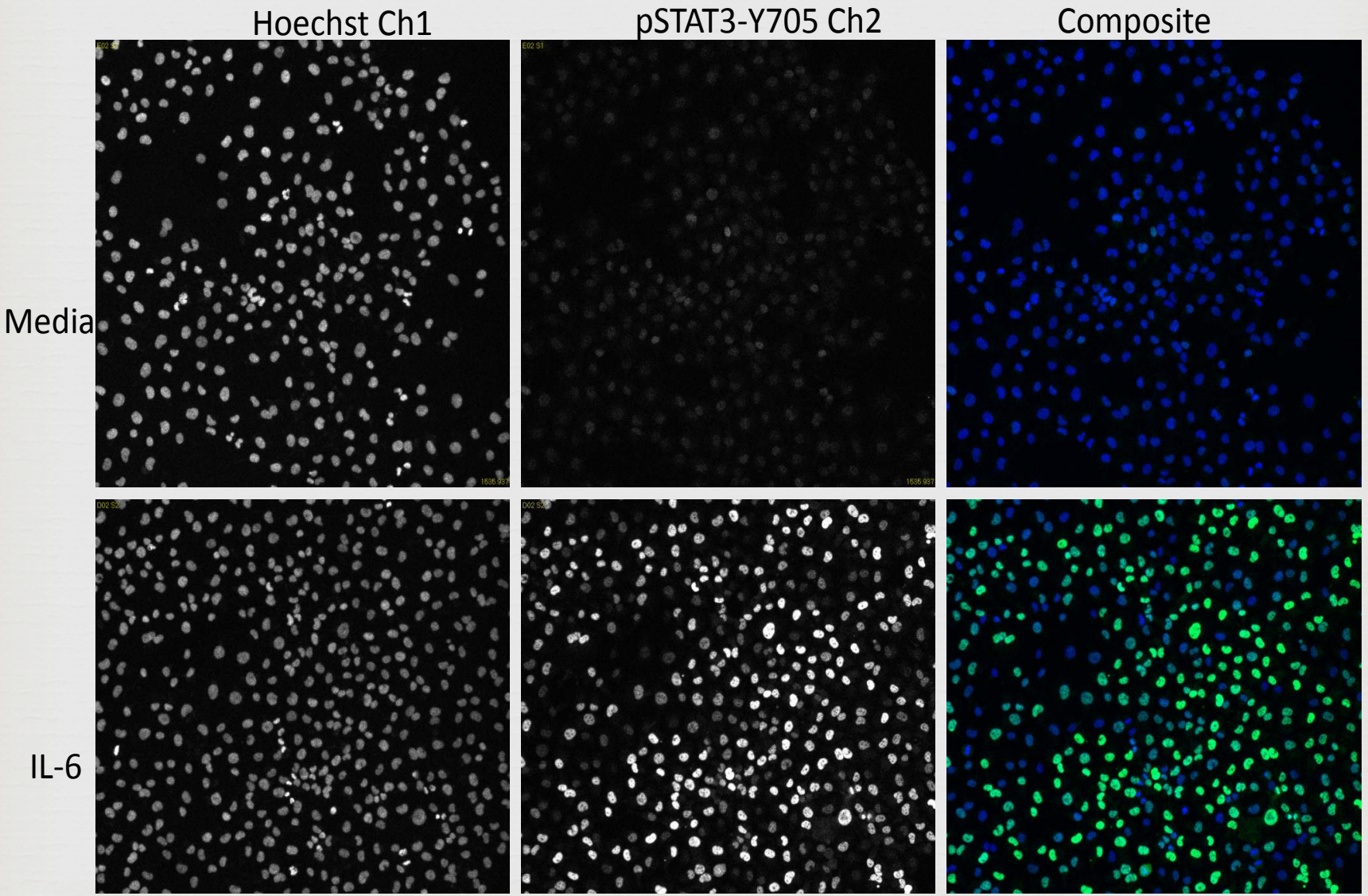
pSTAT3-Y705 HCS Image Acquisition



- 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

Slide by Dr. Paul A. Johnston

ImageXpress Ultra 20× 0.45NA Objective



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