Proteomic strategies for identifying resistance mechanisms and therapeutic targets in lymphoma

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Professor, Director of Hematopathology
Joint (HUP and CHOP)

GENERAL SESSION 3
May 10, 2019
Disclosure

• No relevant items to disclose

• GENOMENON: Co-Founder and Advisor
Paradigm for Research

Patient

Genome Sequencing  Gene Expression  Proteome Profiling  Metabolome Profiling

Pathobiologic events

Animal Model  Functional Screens  Biomarkers
Outline

• **Discovery** of novel targetable ALK-regulated cytokine network through integration of N-glycoproteomic and functional genomics

• Functional validation of **novel target (IL31Rβ)** in ALCL

• Conclusions and broad applications for identifying novel CAR-T targets in de novo disease and resistance
**LC-MS/MS-based proteomics**

- **Unambiguously** identify proteins
- Femtomolar sensitivity
- **Unbiased**
- Identify the **precise** site of a **post-translational modification**

**Bioinformatics**
- Protein ID

**MS scan**
- Parent ion selected

**m/z, amu**
- Intensity

**MS/MS**
- Intensity
- m/z, amu
N-glycoproteomic signatures of lymphoma
N-Glycoproteins are excellent lymphoma biomarkers

- Glycosylation is a common post translational modification
- Glycoproteins are secreted or expressed in the cell surface
- Most CD markers recognize glycoproteins
- Good target for biomarker discovery

Membrane Proteins

13,000 predicted TM proteins
3100 membrane glycoproteins UniProt
Hypothesis

Glycoproteins can be used as biomarkers for early disease detection, diagnosis, monitoring and harnessed as a therapeutic target in lymphoma

Rational selection of candidates

Biomarkers

Therapeutic targets
Aims

• Compendia of glycoproteomic profiles for distinct lymphoma cell lines using LC-MS/MS

• Functional study of candidate glycoproteins
### Unbiased N-glycoproteomics of lymphoid neoplasia

**36** well-characterized human cell lines

**14** subtypes of lymphoid neoplasia

<table>
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<tr>
<th>WHO entities</th>
<th>Lineage</th>
<th>Origin</th>
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<tr>
<td>Myeloma</td>
<td>B</td>
<td>Post-GC</td>
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**Protein extraction**

- Cysteine reduction/alkylation
- Trypsin digestion

**Solid-Phase Extraction of Glycopeptides**

**LC-MS/MS**

**Data analysis**

**Validation and functional studies**
Glycoproteomic Profiling By Solid Phase Extraction of Glycoproteins (SPEG)

**Proteolysis**
- Proteolysis

**Coupling**
- Coupling to hydrazide resin

**Wash**
- Washing

**Isotope labeling**
- Isotope labeling

**Release**
- Release

**LC-MS/MS analysis**
- Liquid Chromatography-Mass Spectrometry

**PNGase F (N-glycosidase)**: N-glycopeptides

**Alkaline β-elimination**: O-glycopeptides

Consensus N-glycosylation motif analysis

- 1905 unique 11mers
- N[115] in the center

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Fold Inc. = Fold Increase over background sequence data

Consensus N-glycosylation motif analysis

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<td>YNXS</td>
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<td>19.89</td>
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Fold Inc. = Fold Increase over background sequence data

Consensus N-glycosylation motif analysis

- **NXT** (1080 occurrences, 57.8%)
- **NXS** (703 occurrences, 37.7%)
- **YNXS** (59 occurrences, 3.2%)
- **NXC** (24 occurrences, 1.3%)
N-glycoproteins identified in 36 cell lines

Detection of virtually all CD proteins currently used for diagnostic evaluation of lymphoid neoplasia
Classification of lineage and subtype

log2 (Raw Spectral Counts +1)
N-glycoproteomic profiles classify cell lines according to disease subtype
NPM-ALK+ ALCL as a biologic tumor model for functional studies

t(2;5)(p23;q35)
Leverage Integrative Large-Scale Data
Transcriptome and N-Glycoproteome

Genomics
(24,000)

Transcriptomics
(100,000)

Proteomics
(1,000,000)
Investigation of ALK “regulome” by integrating N-glycoproteomics and functional genomics

ALK+ALCL cell lines → DMSO → Protein extraction → Solid-Phase Extraction of N-linked glycopeptides (SPEG) → LC-MS/MS

ALKi → RNA extraction → RNA-Seq

Lentiviral sgRNA library → DNA extraction → NGS

Functional proteogenomics → Therapeutic vulnerabilities
Cytokine/receptor signaling pathways are regulated by ALK activity in ALK+ALCL

Integrated N-glycoproteomic and transcriptomic data
A distinct cytokine-mediated protein network regulated by ALK

Protein-protein interaction networks using ALK-dependent cytokine receptors
**Validation: A distinct cytokine signature is characteristic of ALK+ ALCL**

- **IL2Rα (CD25)**
- **IL31Rβ (Oncostatin M receptor)**

**Potential novel biomarkers**
Oncostatin M Receptor (IL31Rβ) in ALK+ ALCL

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<th>Position</th>
<th>Sequence</th>
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<tr>
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<td>MMQYN*VSIK</td>
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<tr>
<td>580</td>
<td>NVGPN*TSTVISTDAFPGVR</td>
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**IL31Rβ is expressed in ALK+ALCL**

### ALCL, ALK+

**Cell lines**

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<tr>
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<th>DEL</th>
<th>Karpas 299</th>
<th>SR786</th>
<th>SU-DHL-1</th>
<th>MAC2A</th>
<th>Jurkat</th>
<th>HT1080</th>
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**GAPDH**

#### 56 primary biopsies of ALCL

- **Tonsil**
- **ALK-, IL31Rβ-**
- **ALK-, IL31Rβ+**
- **ALK+, IL31Rβ+**

#### IL31Rβ expression

- **DEL**
- **Karpas 299**
- **SR786**
- **SU-DHL-1**

- **SupM2**
- **MAC2A**
- **Jurkat**

#### ALK status

<table>
<thead>
<tr>
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<th>ALK-</th>
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<tbody>
<tr>
<td>IL31Rβ+</td>
<td>21</td>
<td>14</td>
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<tr>
<td>IL31Rβ-</td>
<td>0</td>
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\[ X^2 = 20.16 \]

\[ p < 0.001 \]
IL31Rβ and OSM expression is ALK-dependent and mediated via STAT3

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<th>SR786</th>
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<tr>
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<td>10 h</td>
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<tr>
<td>ALK inhibitor</td>
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<td>+</td>
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<tr>
<td>OSMR</td>
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<tr>
<td>p-ALK</td>
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<td>ALK</td>
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<td>β-actin</td>
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<table>
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NPM-ALK regulates IL31Rβ in a kinase dependent manner

**Real time RT-PCR**

**Karpas 299**

- **DMSO**
- **ALK inhibitor**

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<th>n.s</th>
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<tr>
<td>22 hr</td>
<td></td>
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**HeLa**

- **GFP**
- **NPM-ALK WT**
- **NPM-ALK K210R**

***P<0.001 by student T-test***
**CRISPR-Cas9 sgRNA genome-wide vulnerability**

Weinstock D, Ngo S, Root, D

14, 250 sgRNAs

- Lentiviral Vector
- Packaged Pooled Lentiviral sgRNA Library
- Barcoded Pooled Plasmid sgRNA Library
- Transduced Target Cells

Quantitative Identification of Enriched or Depleted sgRNA Corresponding to Gene Targets

80-90% of Sequences Within 1 Order of Magnitude

- Designs: sgRNA
- sgRNA expression: U6, U6-Tet, H1 or H1-Tet
- Markers: GFP, RFP, PuroR,
- Promoters: UbiC, EF1a, CMV

PCR & Cloning

Barcoded sgRNA Amplification

Transduced Target Cells

Target Cell Transduction
Cytokine receptor pathways are exquisite vulnerability targets in ALK+ALCL

Markov Chain Monte Carlo Simulation
IL31Rβ contributes to oncogenesis in ALK+ALCL

IL31Rβ knockdown abrogates tumor growth in ALK+ALCL xenotransplants
Conclusions and Implications

- Largest compendium of N-glycoproteins in lymphoma
  1,115 glycoproteins, including 198 CD markers

- N-glycoprotein signatures classify lymphoid neoplasia according to:
  Lineage, Cell of origin, WHO subtypes

- Integrated N-glycoproteomics and transcriptomics are complementary

- A distinctive cytokine/receptor-JAK-STAT signaling network regulated by ALK
  IL31Rβ are pathogenetically-relevant vulnerable targets

Rolland D et al., Proc Natl Acad Sci, 2017
Model of OSM-OSMR signaling in ALCL and acquired resistance

NPM-ALK

STAT3

OSM

OSMR

gp130

Autocrine and paracrine promotion of cell survival, proliferation

Tumor microenvironment

Therapy resistance

Cell migration, invasion
OSMR is regulated by ALK in EML4-ALK+ lung cancer and upregulated in acquired resistance.
Future Directions
Mechanisms and biomarkers of CAR-T therapy resistance

**Phosphoproteome**

- Cell Culture
  - ALCL-treated +/- ALK inhibitor
- Peptide Preparation
  - Trypsin-mediated cleavage
- MOAC
  - Ti-bead chromatography for phosphopeptide enrichment
- Immunoprecipitation
  - Three pY specific antibodies
- LC-MS/MS
  - Identification and quantification of phosphopeptides

- 5000-6000 proteins
- 35000 phosphopeptides
- 2500 phosphoproteins

**N-Glycoproteome**

*Glycoprotein Cell Receptors*
Surface carbohydrates on cells serve as points of attachment for other cells, infectious bacteria, viruses, toxins, hormones and many other molecules.
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