Proteomic strategies for identifying resistance mechanisms and therapeutic targets in lymphoma

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Joint (HUP and CHOP)

GENERAL SESSION 3

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Perelman School of Medicine University of Pennsylvania PATHOLOGY AND LABORATORY MEDICINE

Abramson Cancer Center Penn Medicine

The Children's Hospital of Philadelphia

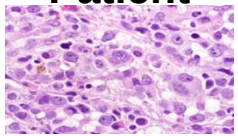
Disclosure

- No relevant items to disclose

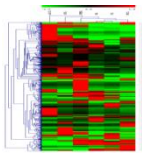
- GENOMENON: Co-Founder and Advisor

Paradigm for Research

Patient



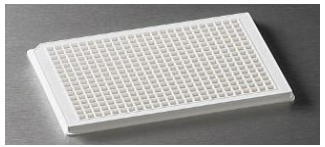
Genome Sequencing | Gene Expression | Proteome Profiling | Metabolome Profiling



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

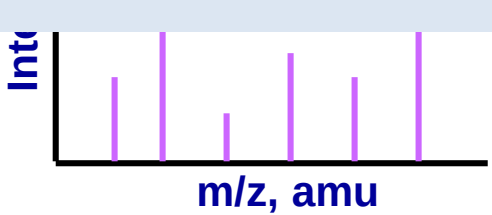
- **Unambiguously** identify proteins
- Femtomolar sensitivity
- **Unbiased**
- Identify the **precise** site of a **post-translational modification**

MS scan

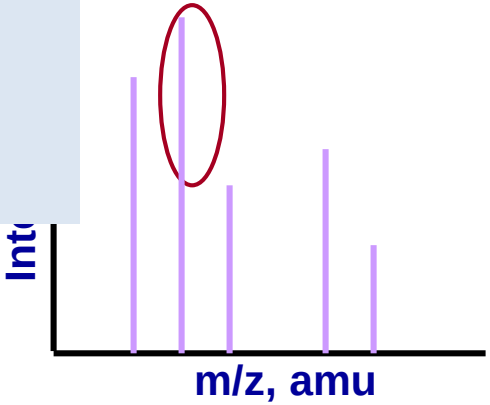
Parent ion selected

Bioinformatics

Protein ID



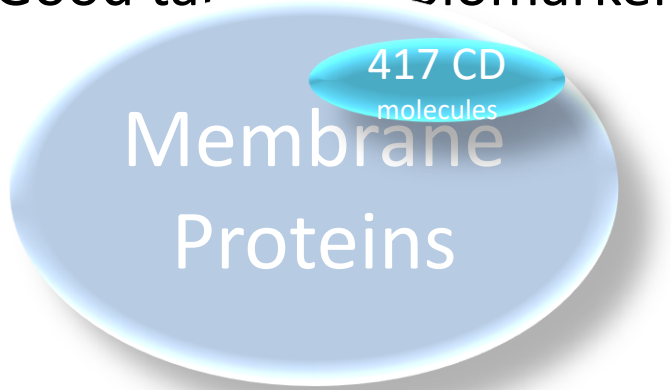
MS/MS



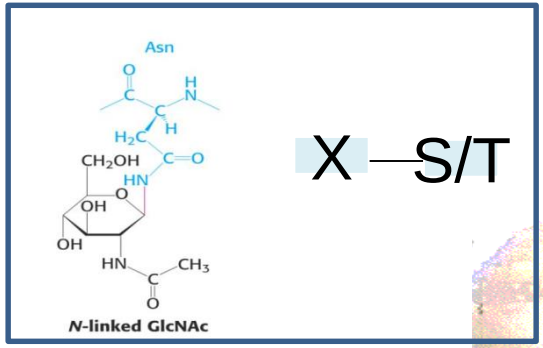
N-glycoproteomic signatures of lymphoma

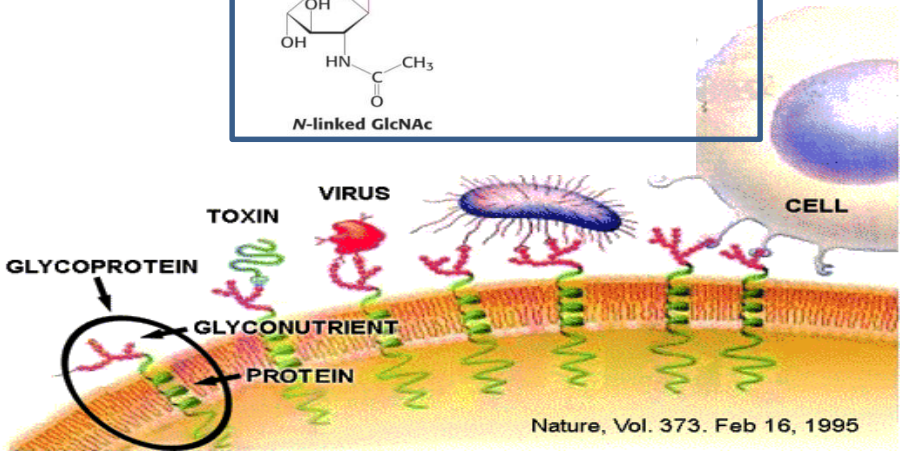
N-Glycoproteins are excellent lymphoma biomarkers

- 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



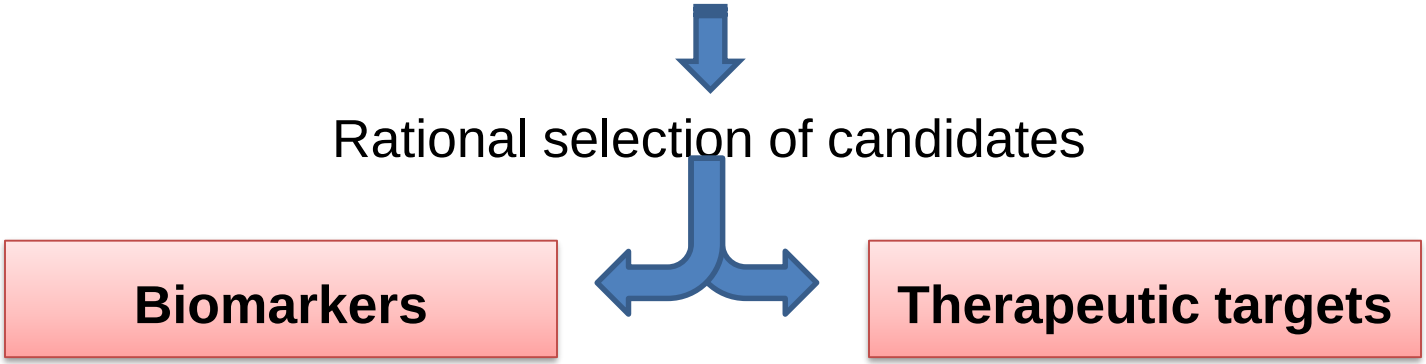
13,000 predicted TM proteins
3100 membrane glycoproteins UniProt





Hypothesis

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



Aims

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

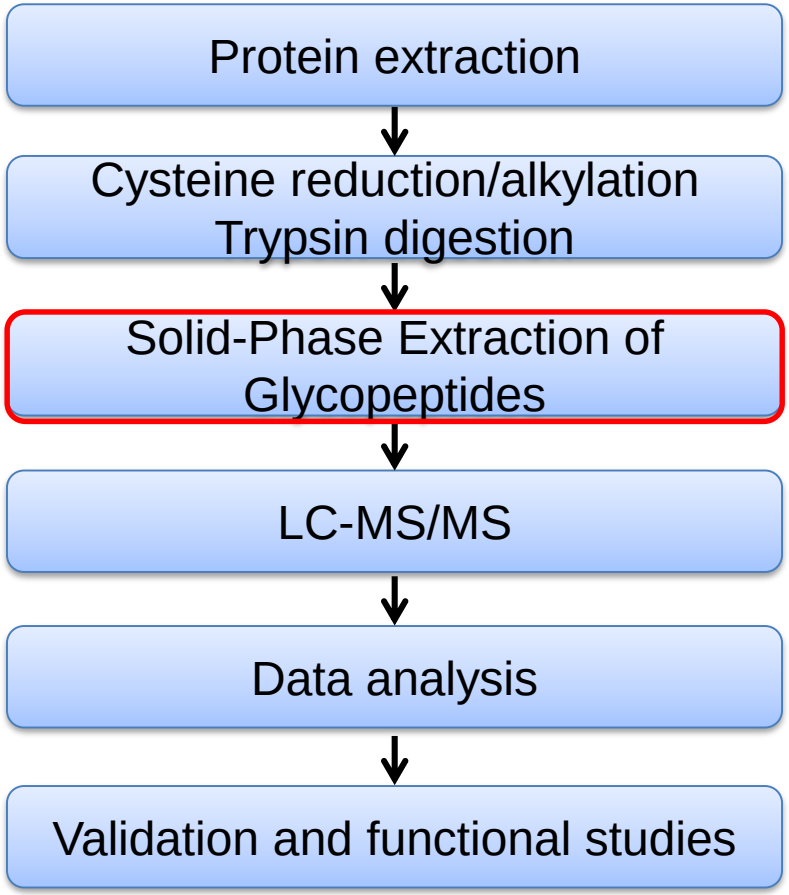
- Functional study of candidate glycoproteins

Unbiased N-glycoproteomics of lymphoid neoplasia

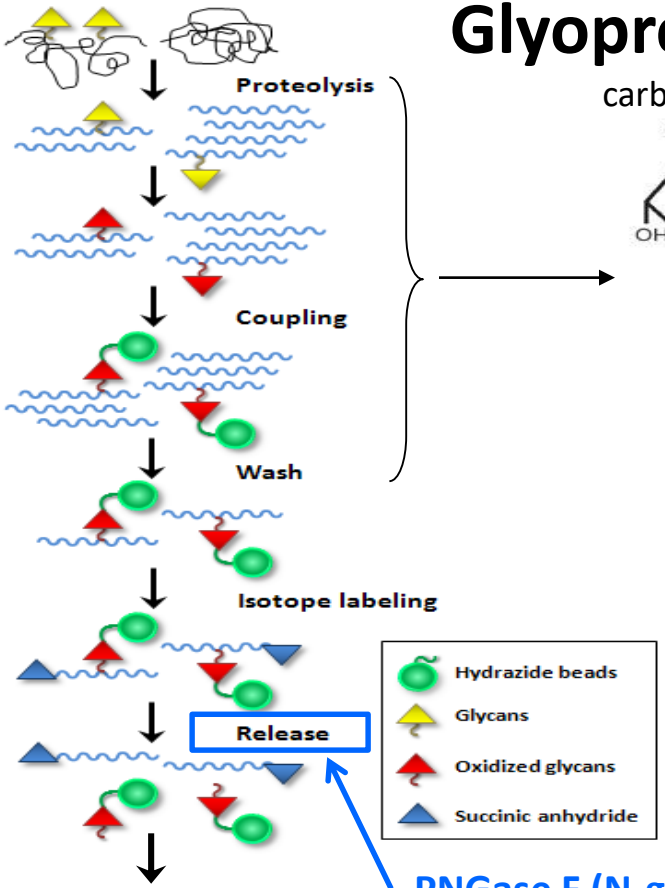
36 well-characterized human cell lines

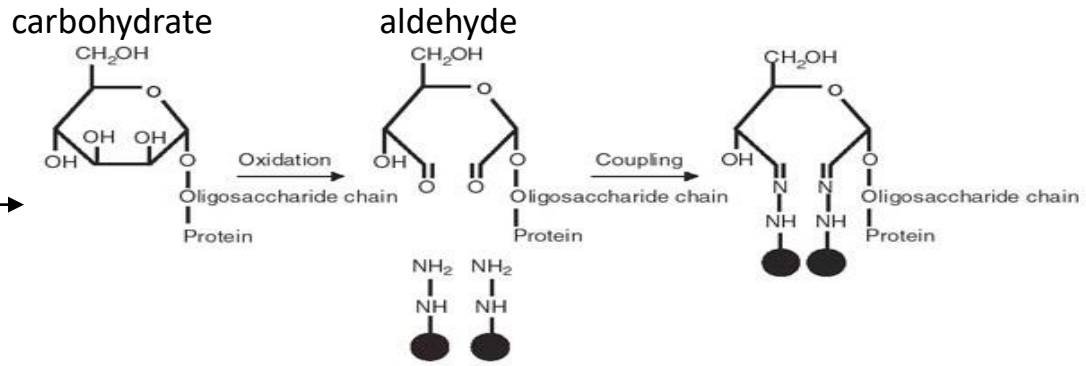
14 subtypes of lymphoid neoplasia

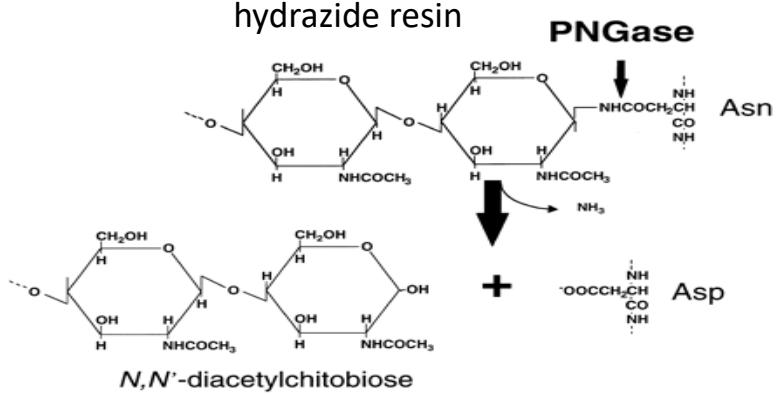
WHO entities	Lineage	Origin	N
T-ALL	T	Precursor T	1
ALCL, ALK +	T	Mature T	5
ALCL, ALK -	T	Mature T	2
MF	T	Mature T	1
Sézary syndrome	T	Mature T	1
Aggressive NK-cell leukemia	NK	Mature NK	3
MCL	B	Pre-GC	3
BL	B	GC	3
DLBCL	B	GC	1
PMBL	B	GC	2
FL	B	GC	6
Classical HL	B	GC	3
NLPHL	B	GC	1
Myeloma	B	Post-GC	4



Glycoproteomic Profiling By Solid Phase Extraction of Glyoproteins (SPEG)







PNGase F (N-glycosidase) : N-glycopeptides

Alkaline β-elimination : O-glycopeptides

Modified from Nat Biotech 2003 & Nat Protocol 2007

Consensus N-glycosylation motif analysis

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

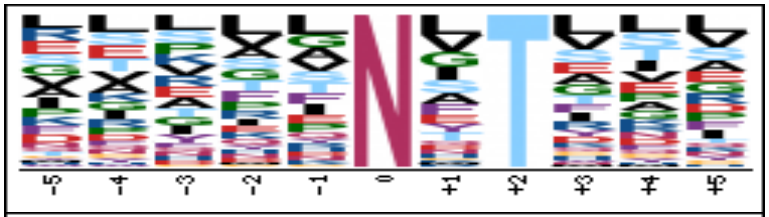
Motif #	Count	Fold Inc.*
1	1080	8.88
2	59	25.87
3	703	10.37
4	24	19.89

Fold Inc. = Fold Increase over background sequence data

xxxmotif-xxxx

Schwartz et al. (2005). Nature Biotech. v23(11):1391-1398.

1



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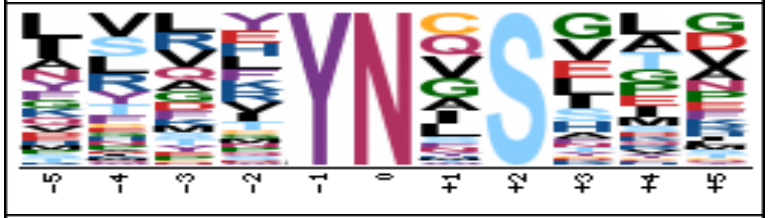
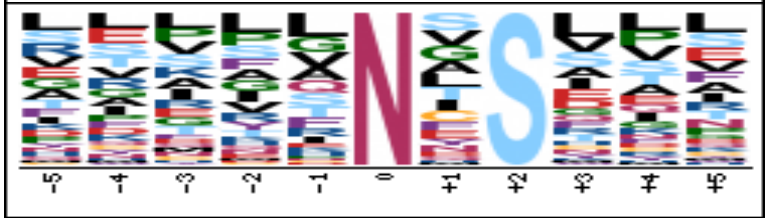
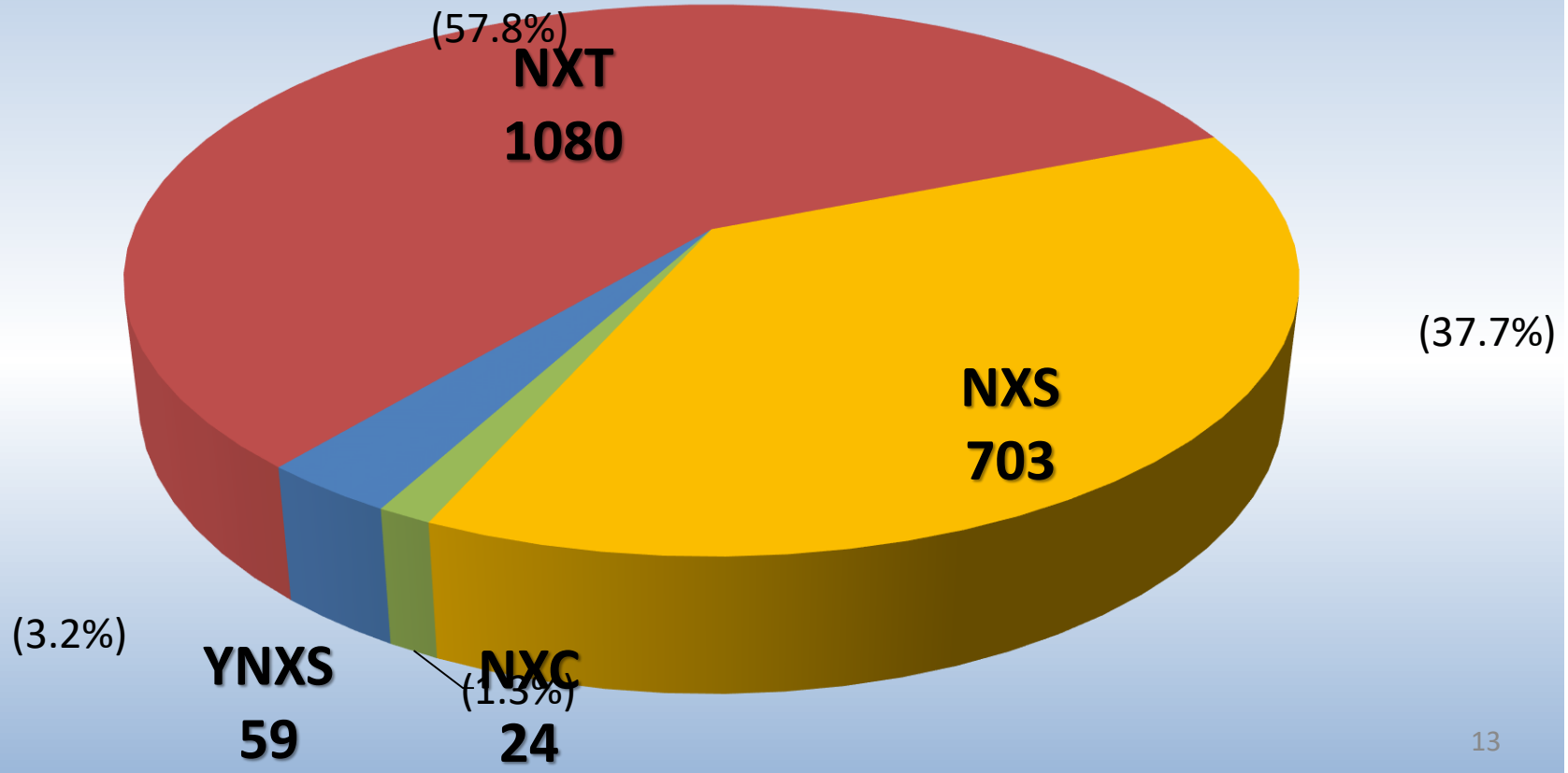


3

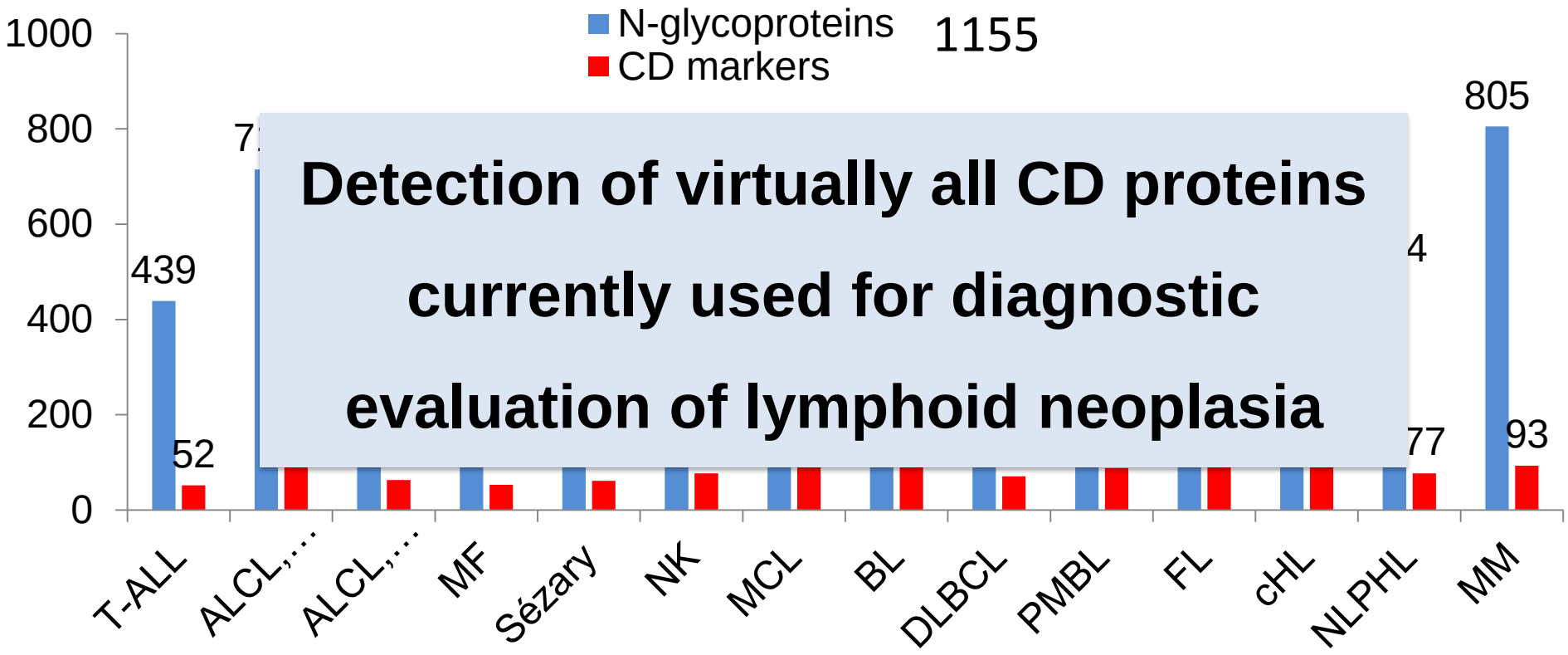


4

Consensus N-glycosylation motif analysis

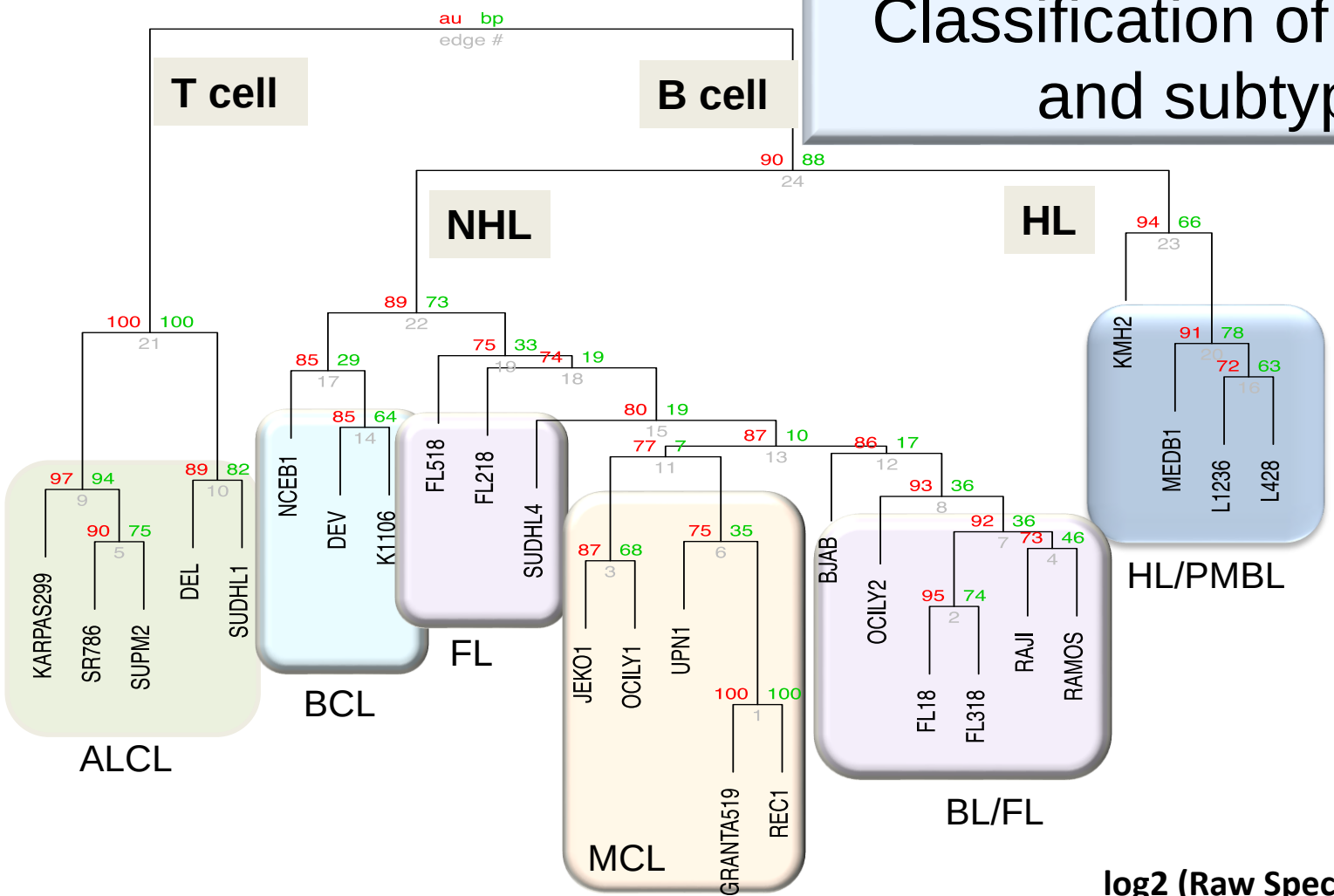


N-glycoproteins identified in 36 cell lines



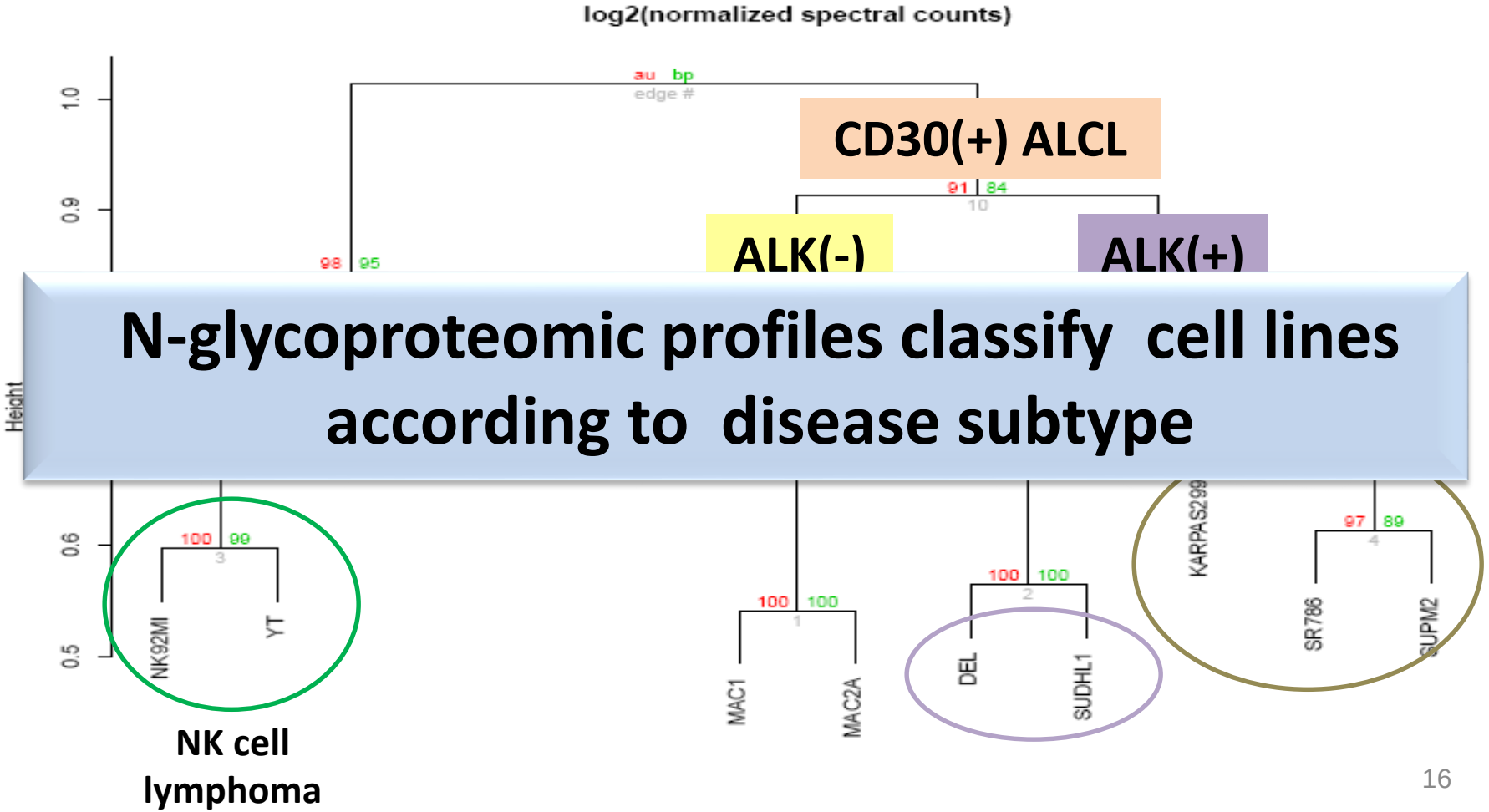
Detection of virtually all CD proteins currently used for diagnostic evaluation of lymphoid neoplasia

Classification of lineage and subtype



log2 (Raw Spectral Counts +1)

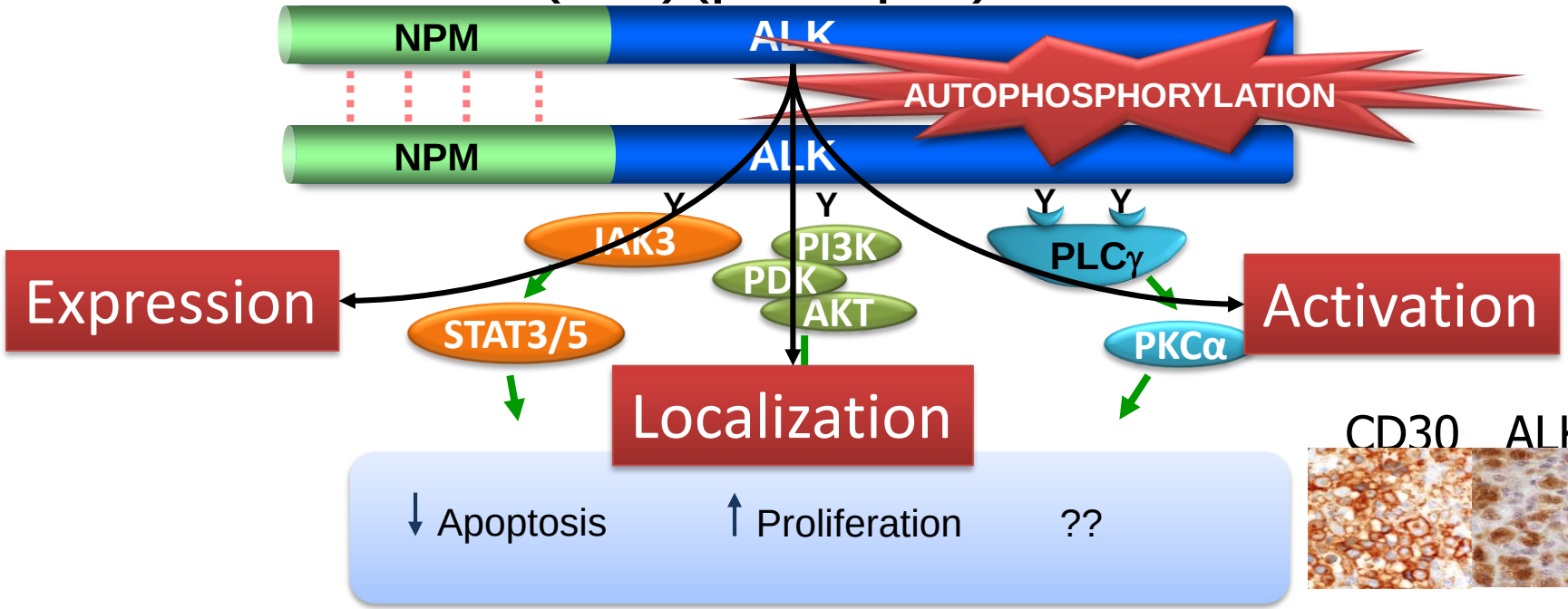
T/NK cell lymphoma cell lines



N-glycoproteomic profiles classify cell lines according to disease subtype

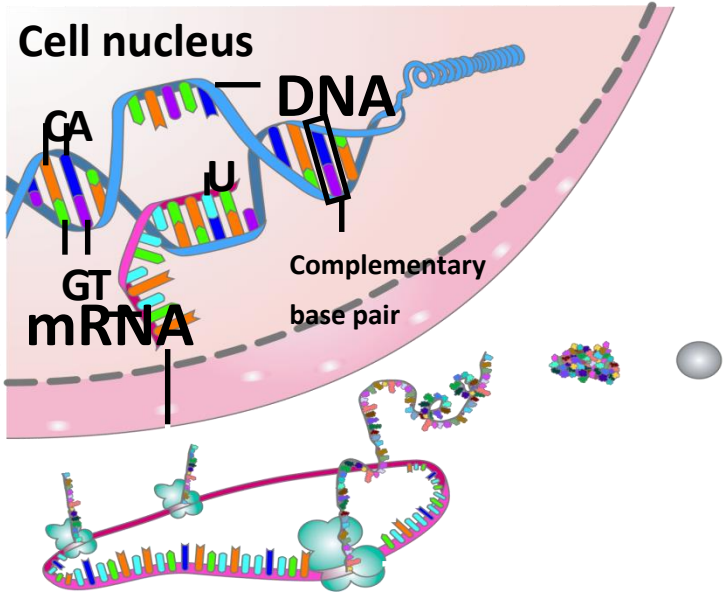
NPM-ALK+ ALCL as a biologic tumor model for functional studies

t(2;5)(p23;q35)



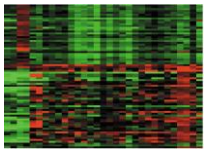
Leverage Integrative Large-Scale Data

Transcriptome and N-Glycoproteome



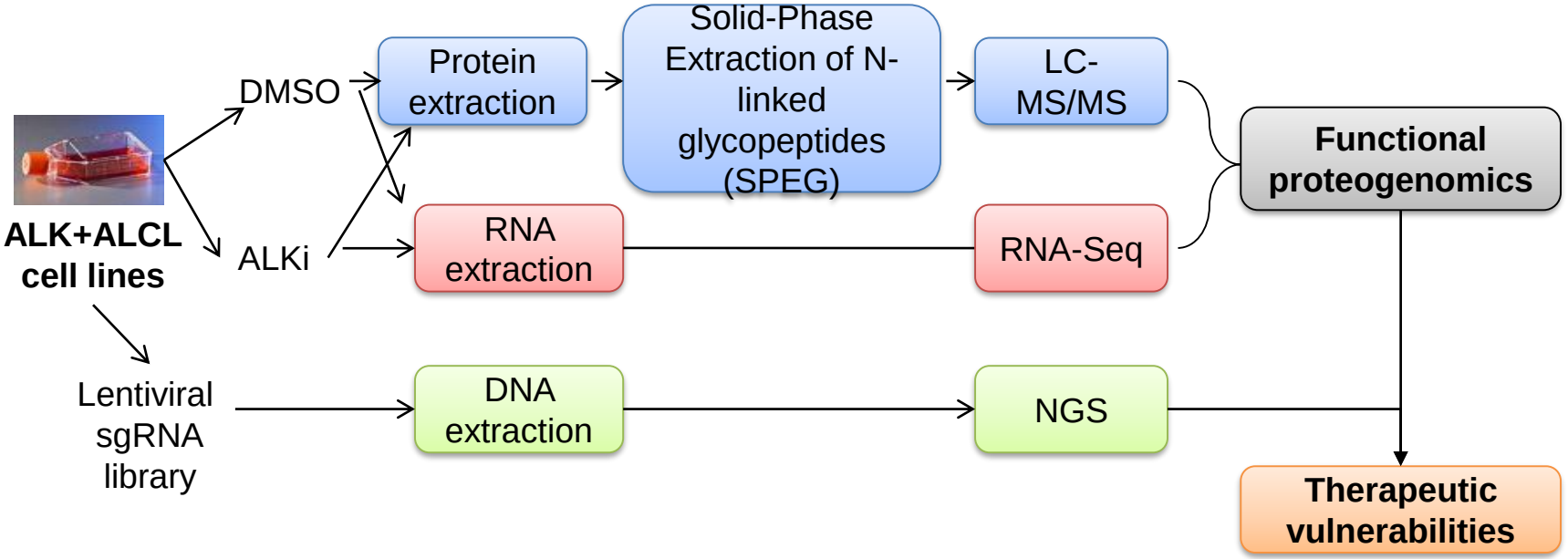
Genomics
(24,000)

Transcriptomics
(100,000)

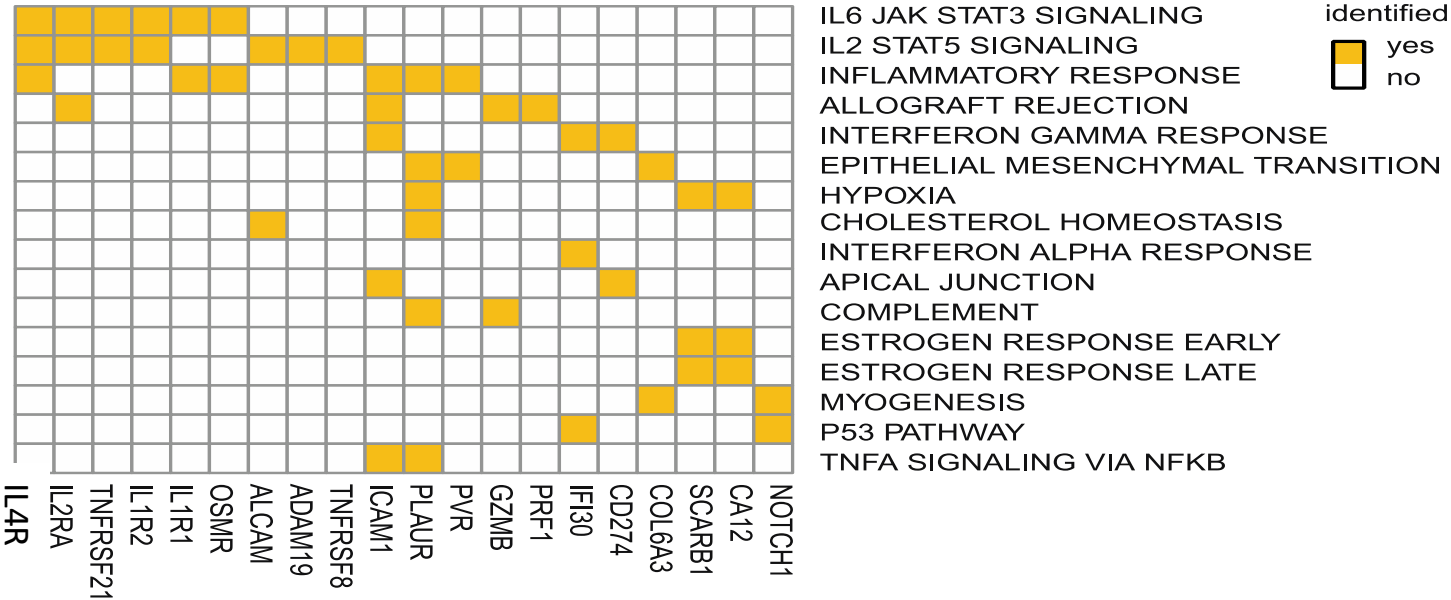


Proteomics
(1,000,000)

Investigation of ALK “regulome” by integrating N-glycoproteomics and functional genomics

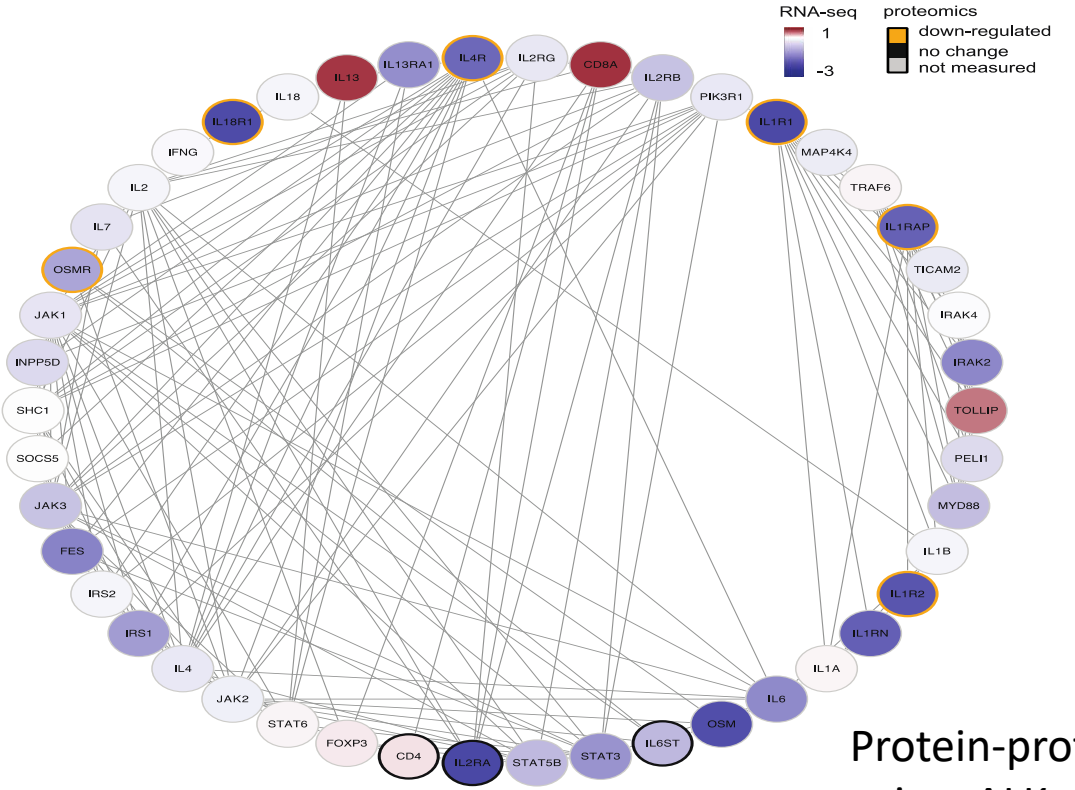


Cytokine/receptor signaling pathways are regulated by ALK activity in ALK+ALCL



Integrated N-glycoproteomic and transcriptomic data

A distinct cytokine-mediated protein network regulated by ALK



seed:

IL31Rβ

IL18R1

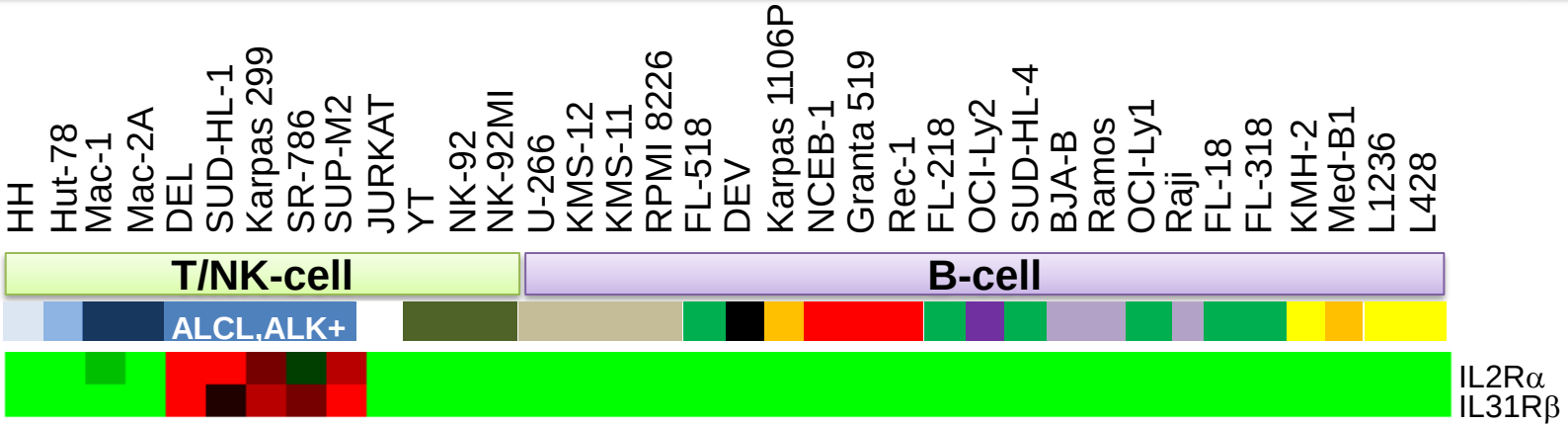
IL1R1

IL1R2

IL2RA

Protein-protein interaction networks using ALK-dependent cytokine receptors

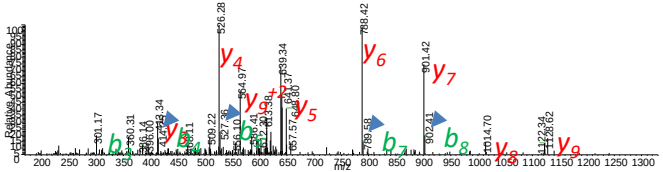
Validation: A distinct cytokine signature is characteristic of ALK+ ALCL



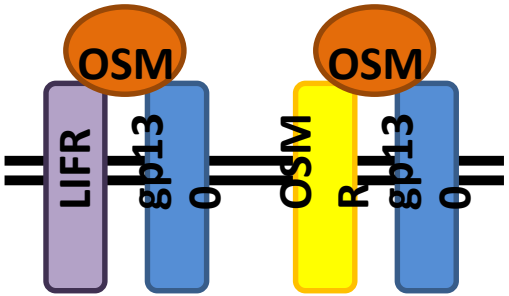
- IL2Rα (CD25)
- IL31Rβ (Oncostatin M receptor)

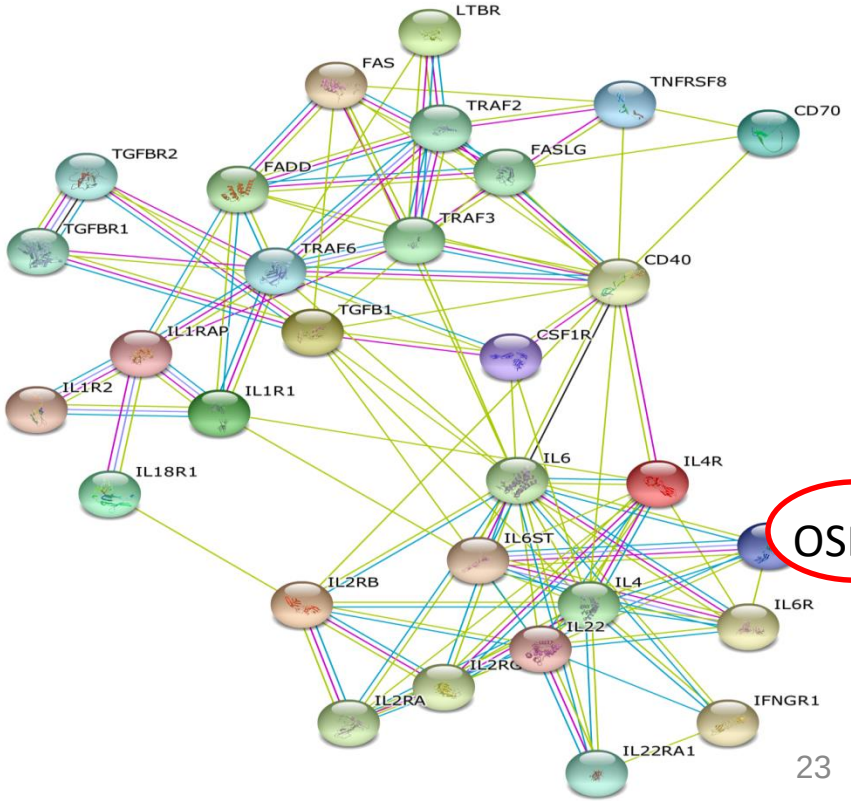
Potential novel biomarkers

Oncostatin M Receptor (IL31Rβ) in ALK+ ALCL



Position	Sequence
176	NIQNN*VSCYLEGK
326	SVNILFN*LTHR
380	MMQYN*VSIK
491	ILFYNVVVENLDKPSSSELHSIPAPAN*STK
580	NVGPN*TTSTVISTDAFRPGVR

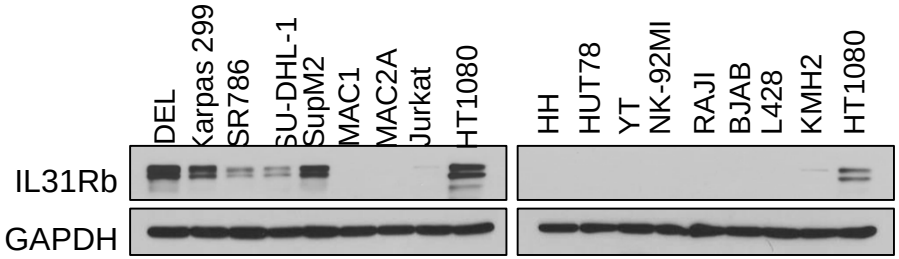


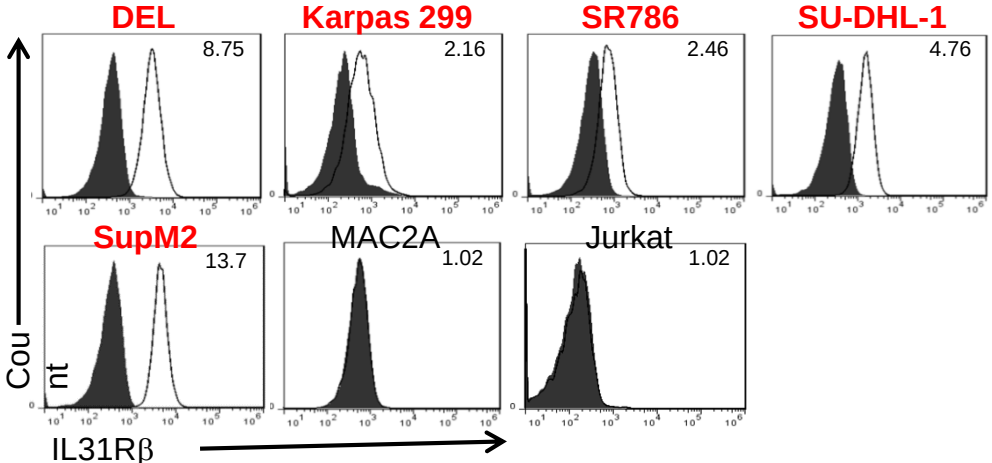


IL31Rβ is expressed in ALK+ALCL

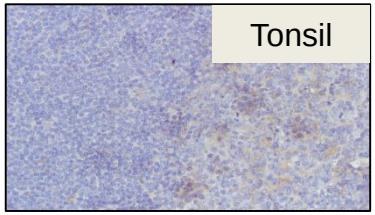
Cell lines

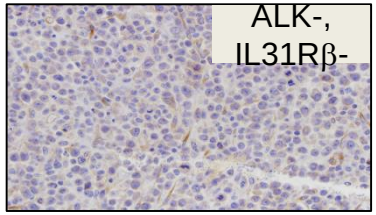
ALCL, ALK+

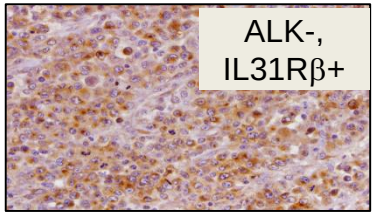


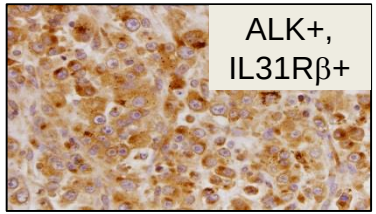


56 primary biopsies of ALCL







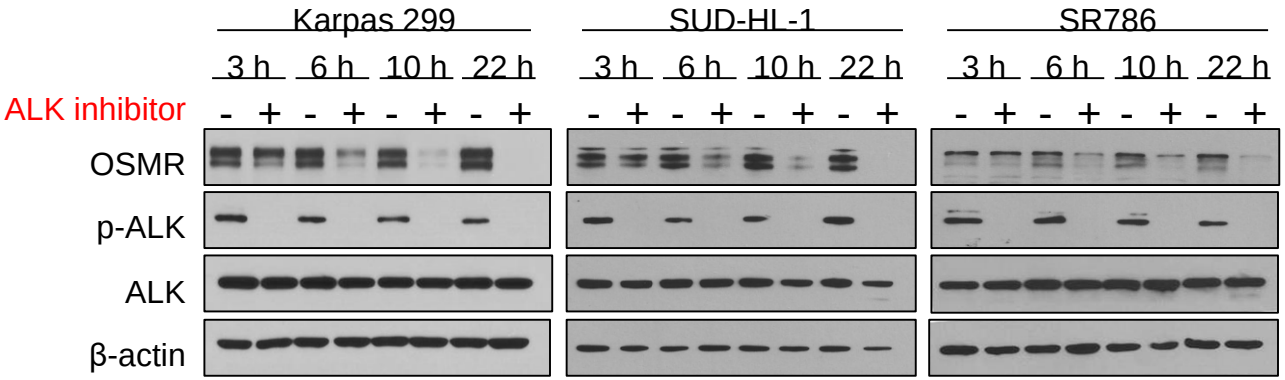


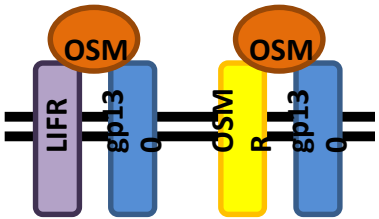
	IL31Rβ +	IL31Rβ -
ALK +	21	0
ALK -	14	21

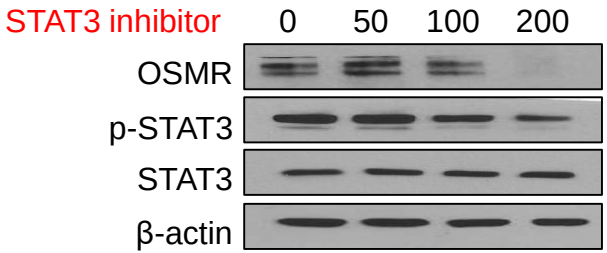
$X^2 = 20.16$

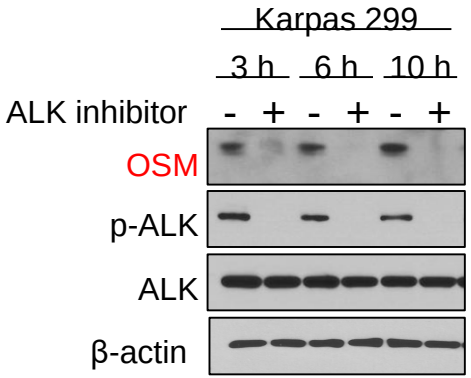
$p<0.001$

IL31Rβ and OSM expression is ALK-dependent and mediated via STAT3



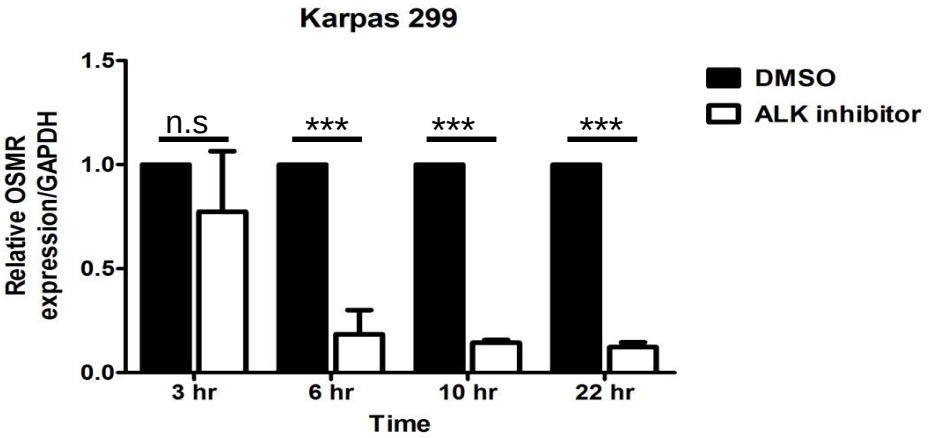


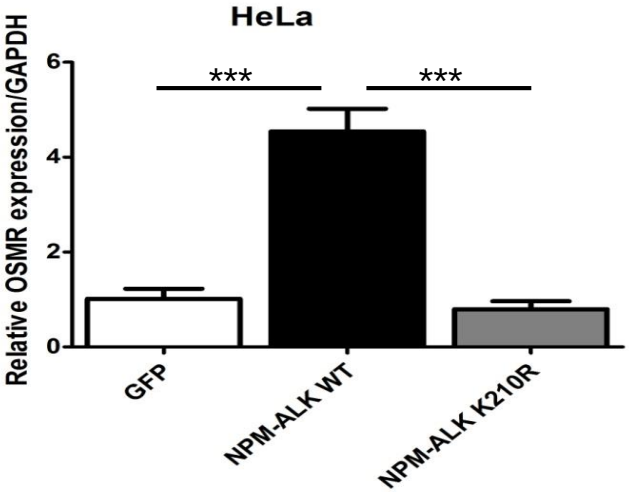




NPM-ALK regulates IL31Rβ in a kinase dependent manner

Real time RT-PCR





**** P<0.001 by student T-test*

CRISPR-Cas9 sgRNA genome-wide vulnerability

Weinstock D, Ngo S, Root, D

14, 250 sgRNAs

PCR & Cloning

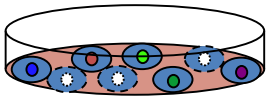
Lentiviral Vector

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

Barcoded Pooled Plasmid sgRNA Library

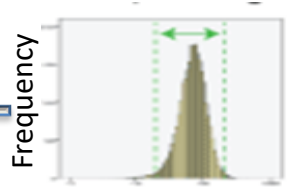
Packaged Pooled Lentiviral sgRNA Library

Target Cell Transduction



Transduced Target Cells

Barcoded sgRNA Amplification

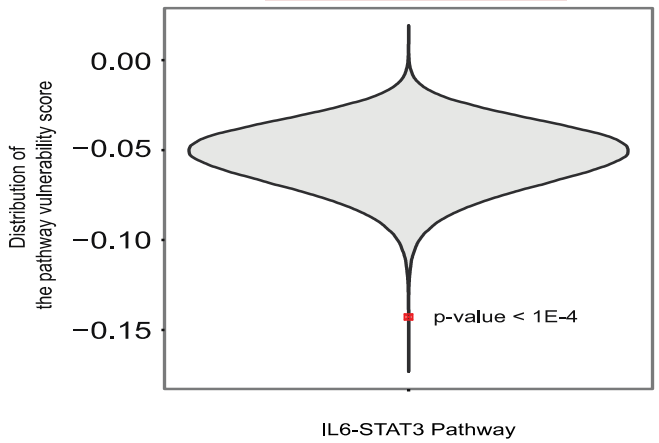


80-90% of Sequences Within 1 Order of Magnitude

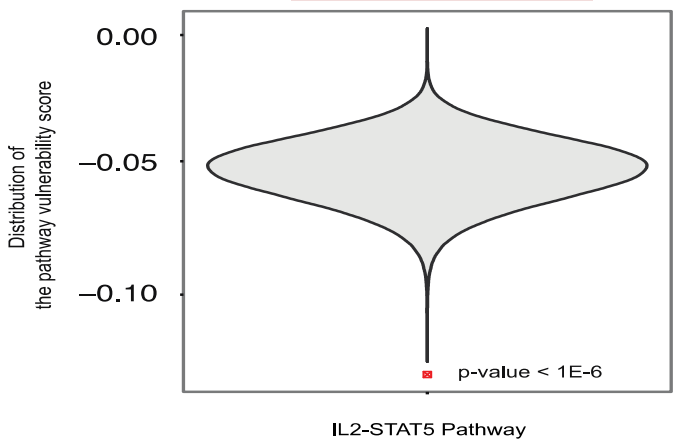
Quantitative Identification of Enriched or Depleted sgRNA Corresponding to Gene Targets

Cytokine receptor pathways are exquisite vulnerability targets in ALK+ALCL

IL6-STAT3

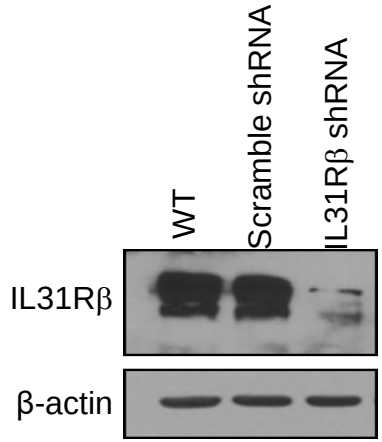


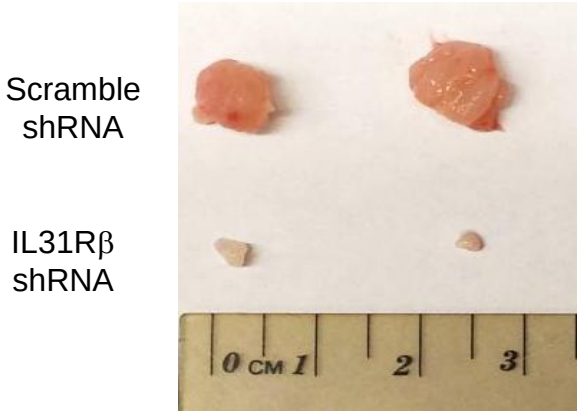
IL2-STAT5

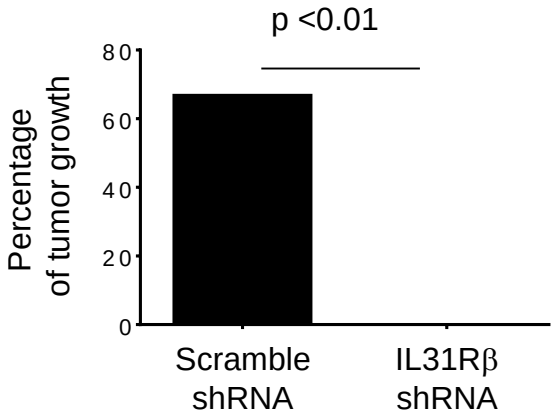


Markov Chain Monte Carlo Simulation

IL31Rβ contributes to oncogenesis in ALK+ALCL







IL31Rβ knockdown abrogates tumor growth in ALK+ALCL xenotransplants

Delphine Rolland

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:

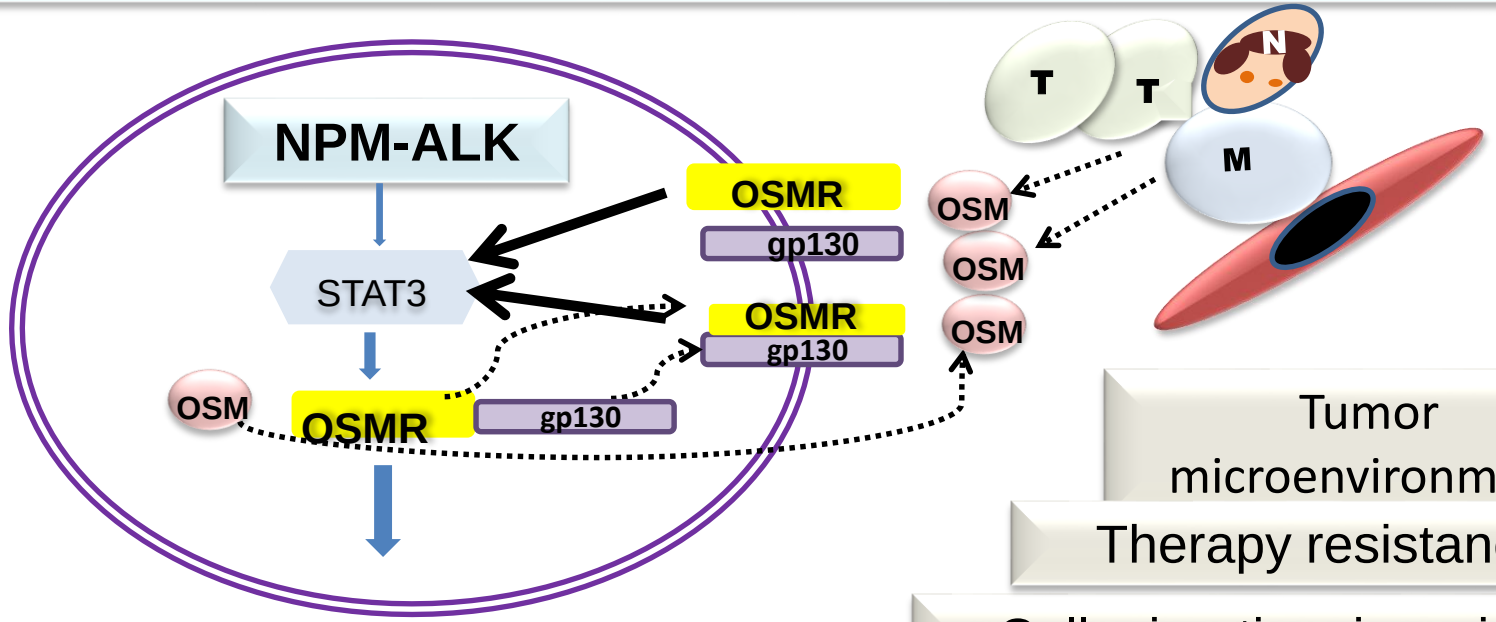
  Lineage, Cell of origin, WHO subtypes

- 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



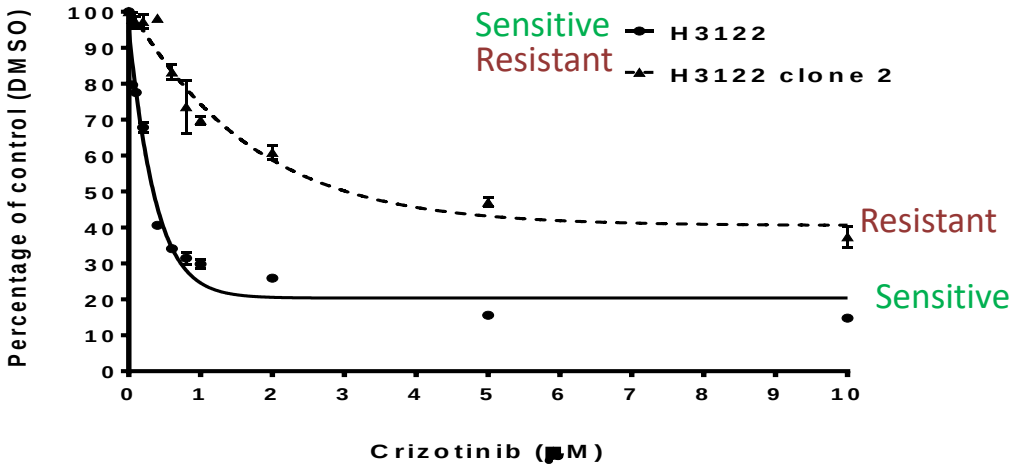
Tumor microenvironment

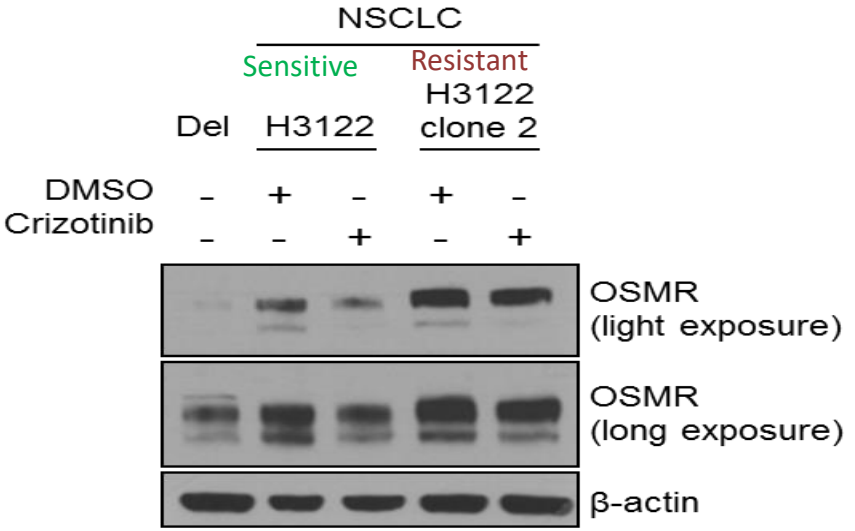
Therapy resistance

Cell migration, invasion

Autocrine and paracrine promotion of cell survival, proliferation

OSMR is regulated by ALK in EML4-ALK+ lung cancer and upregulated in acquired resistance

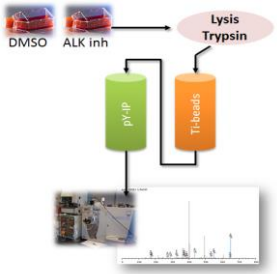




Future Directions

Mechanisms and biomarkers of CAR-T therapy resistance

Phosphoproteome



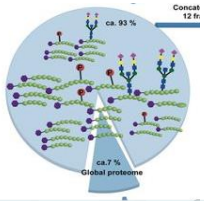
Cell Culture
ALCL cells treated +/- ALK inhibitor

Peptide Preparation
Trypsin-mediated cleavage

MOAC
Ti-bead chromatography for phosphopeptide enrichment (S/T/Y)

Immunoprecipitation
Three pY specific antibodies

LC-MS/MS
Identification and quantification of phosphopeptides

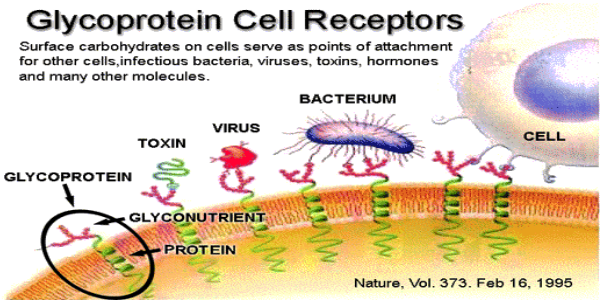


5000-6000 proteins

35000 phosphopeptides

2500 phosphoproteins

N-Glycoproteome



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