

Cross-Omics: Global Phosphoproteomics and Metabolomics Reveals a Connection Between Kinase Inhibition and RNA Processing in BCR-ABL Myeloma Cells

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6-18-2014

ASMS 2014 - Baltimore



Cancer Center

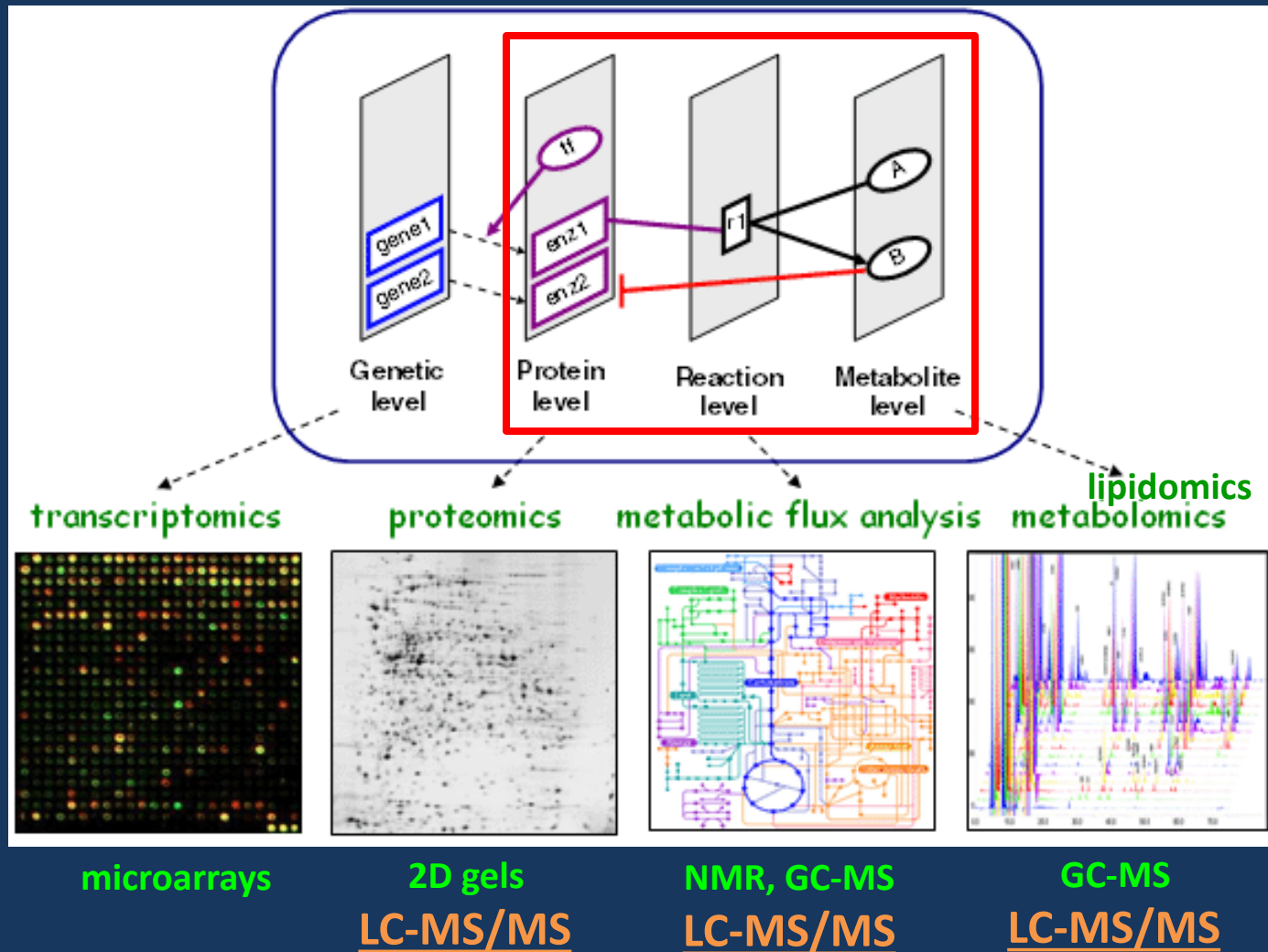


Beth Israel Deaconess
Medical Center



HARVARD MEDICAL SCHOOL
TEACHING HOSPITAL

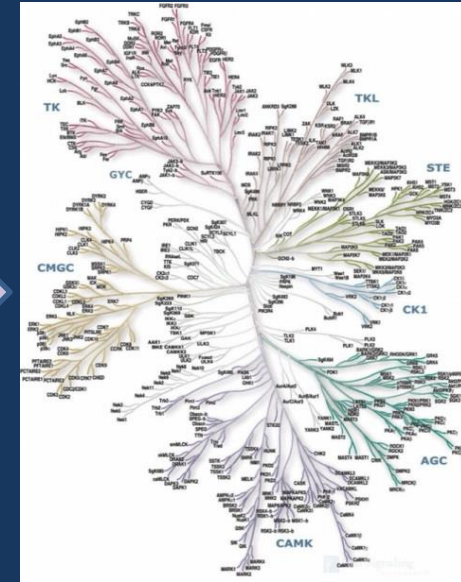
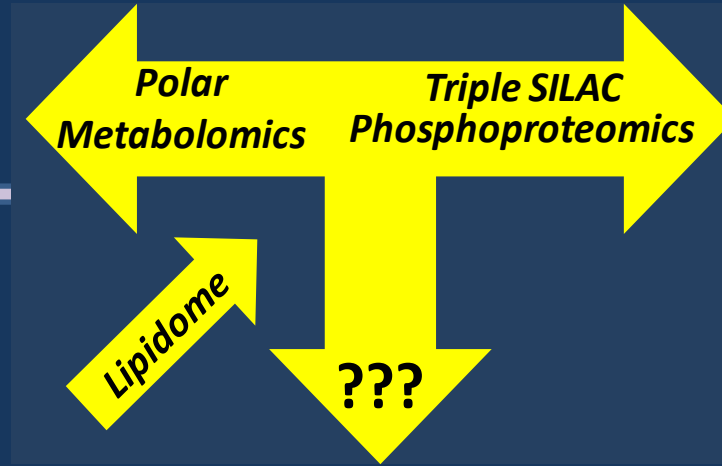
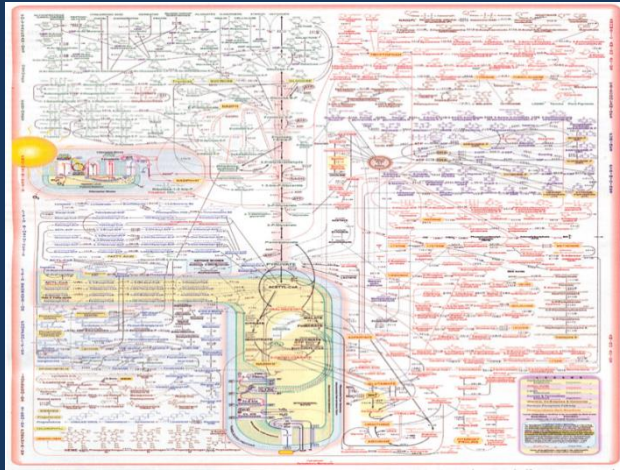
Integrating Different –Omics Approaches can Reveal insight into Biological Systems of Disease



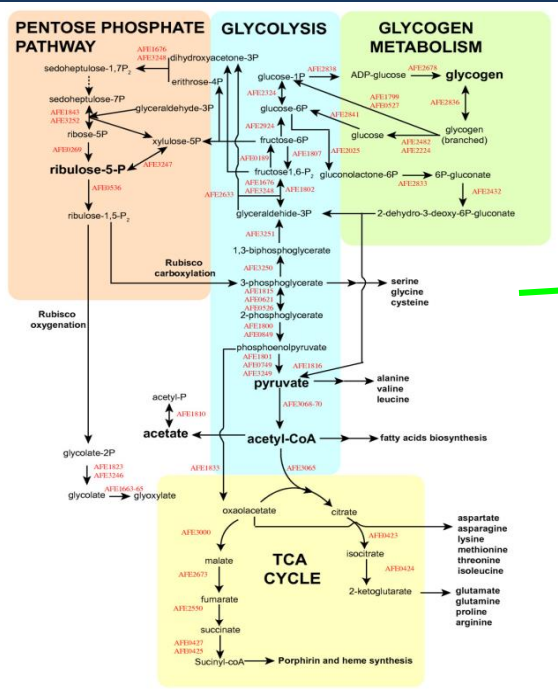
Cross-Omics Study in H929 Myeloma Cancer Cells

Human metabolome

Human kinome

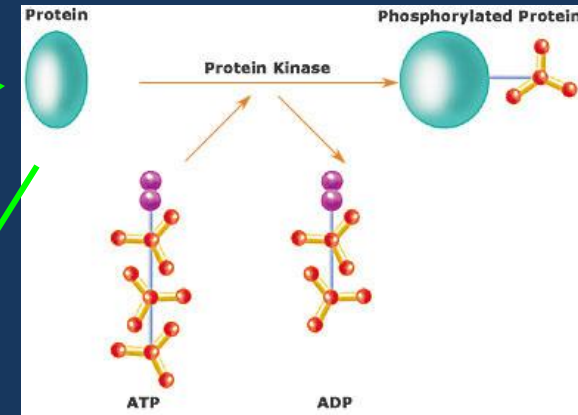


Central carbon metabolism

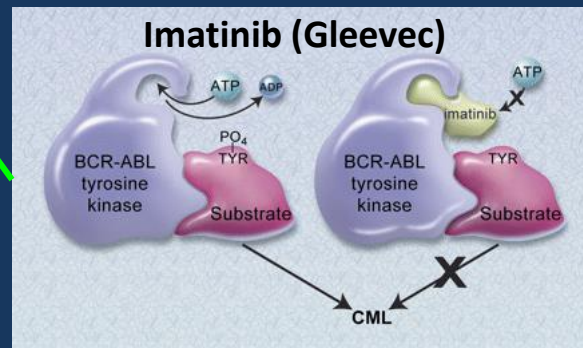


- Why do drugs fail in clinical trials?
- Do they have other affects?
- Patient selection?

Phosphorylation

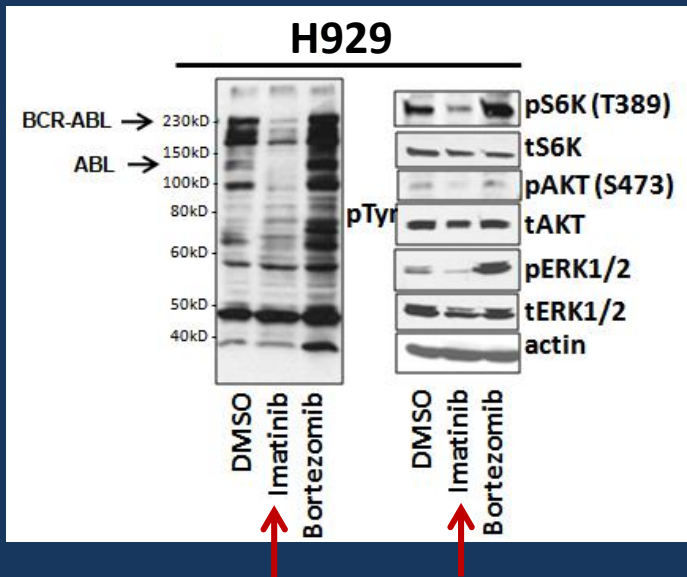


Kinase inhibition

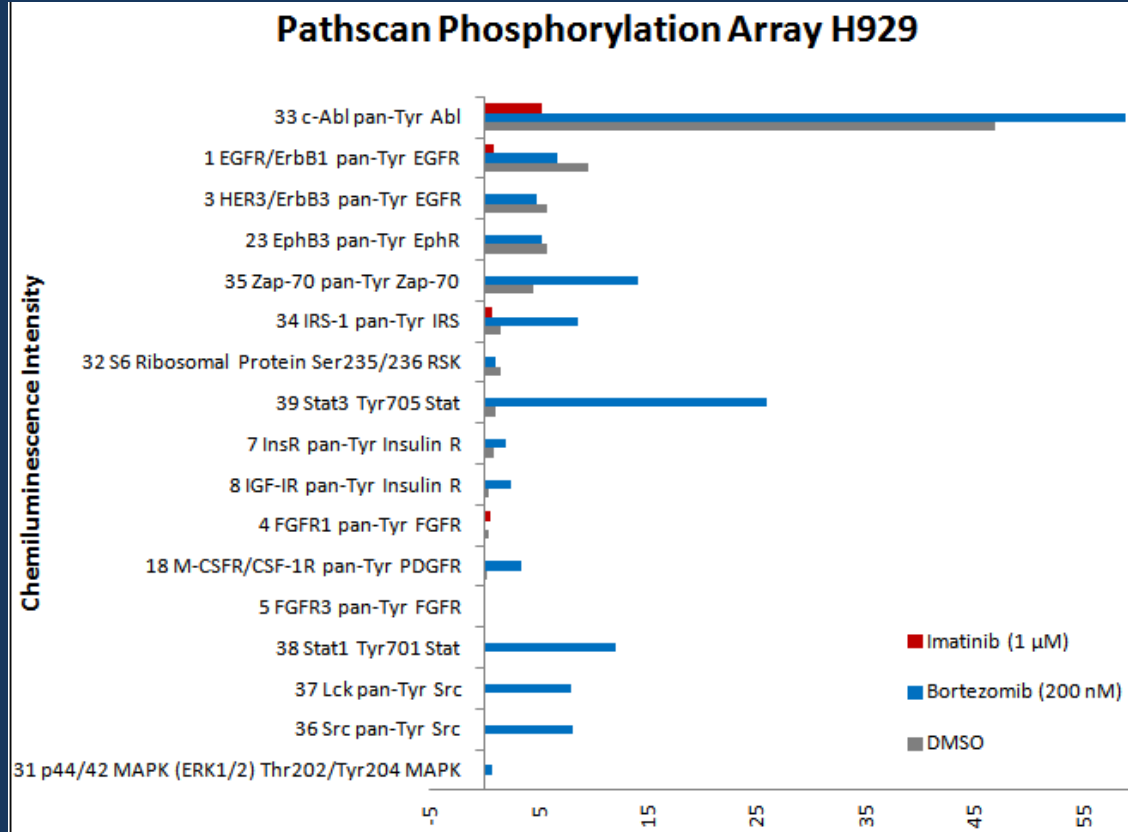
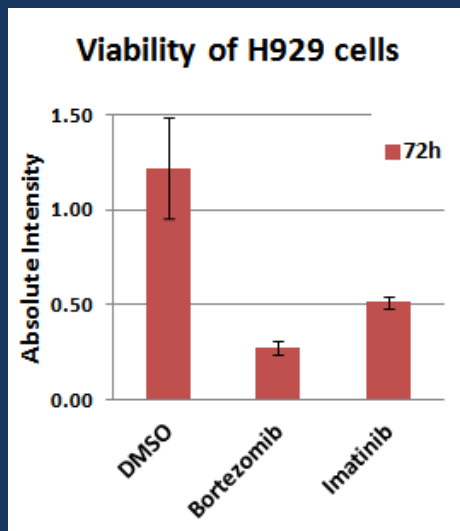


Signaling Overview of BCR-ABL H929 Multiple Myeloma Cells

Signaling blots



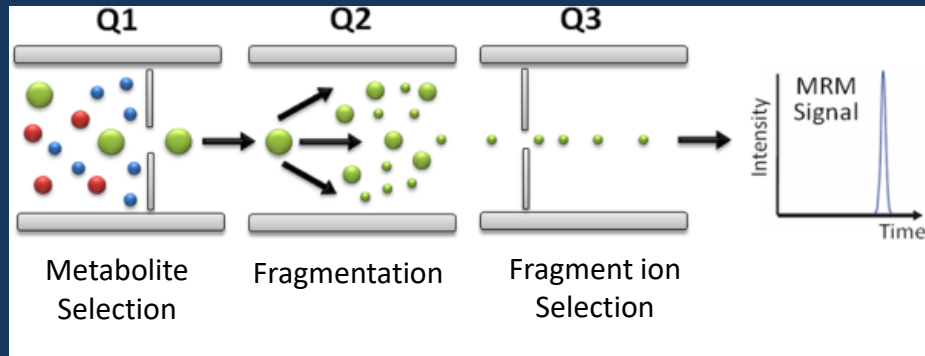
MTT assay



- Imatinib abrogates pTyr signaling while bortezomib enhances Tyr kinase signaling prior to apoptosis

Targeted Polar Metabolite Profiling Platform

Selected Reaction Monitoring (SRM) ~300 transitions (258 unique metabolites ⁻¹²C & ¹³C)



Mean R² = 0.978
 Mean CV = 0.12
 FWHH = ~9 seconds
 Cycle time = 1.67 sec
 3-4 msec dwell
 10-14 points per peaks

MultiQuant v2.0 Peak Area integration software

Sample Name	avg DMSO	avg BEZ	avg BKM	avg U0126
3-phosphoglycerate	1330385.962	871663.9395	1038599.88	943607.0569
3-phospho-serine	152487.2158	83097.6478	73986.5328	31000.0000
D.glyceraldehyde.3-phosphate	254477.5084	293208.1613	209570.2472	194280.8415
dihydroxy.acetone-phosphate	357217.2808	274197.6204	227350.7244	210859.1435
fructose.1,6-bisphosphate	1059370.361	808511.4636	1082381.874	682001.4833
fructose.6-phosphate	1471332.891	1019002.062	1046811.137	1054938.996
glucose.1-phosphate	761216.5713	605856.8664	815435.949	811274.948
glucose.6-phosphate	955670.737	704956.8497	635987.2986	741371.4211
hexose-phosphate	19302214.34	14375529.9	20558058.05	16548067.87
lactate	95442398.42	94957148.33	103044596.9	101662913.9
phosphoenolpyruvate	258107.2535	217613.2952	323347.9553	382350.6681

+/- switching

Amide XBridge
 HILIC - 1 column

4.6mm x 15cm

pH=9.0, NH₄⁺

400 μL/min

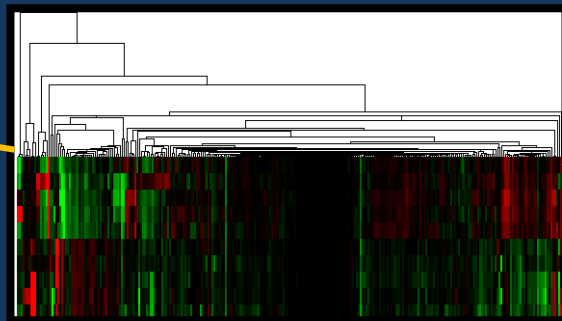
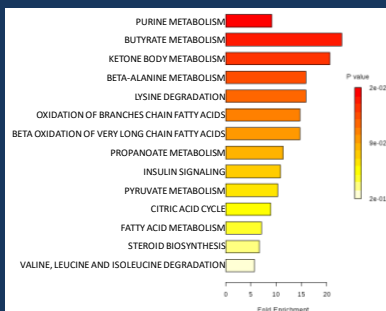
Extract metabolites with **80% methanol**
 From cells, tumor tissue, fluids, etc.



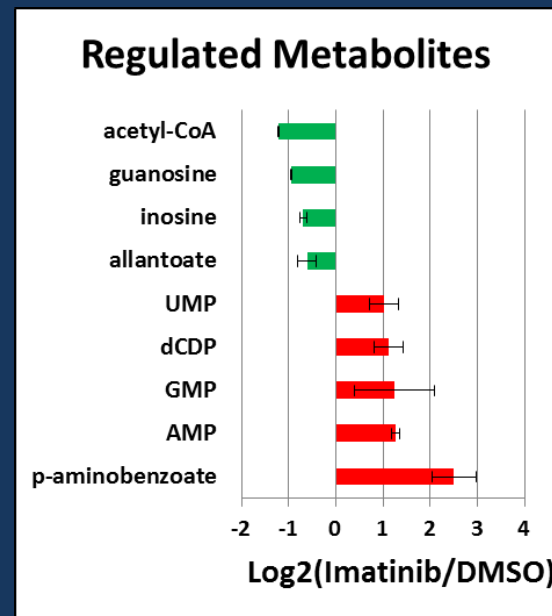
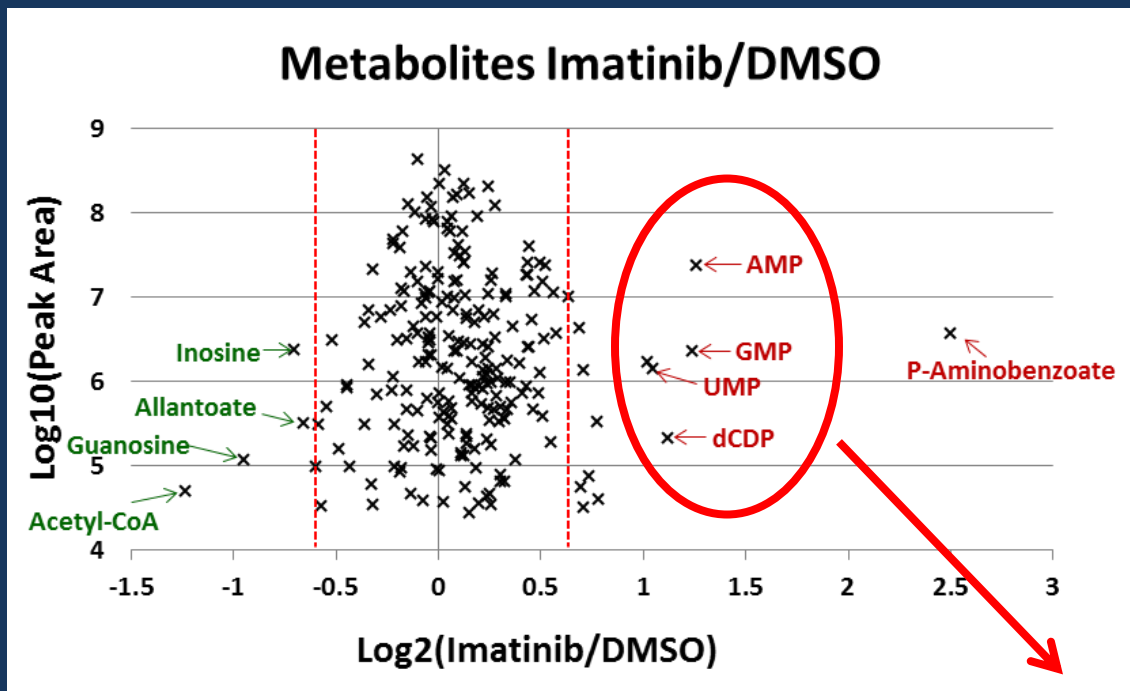
Cancer cells

Bioinformatics
 MarkerView
 Metaboanalyst.ca

Clustering (MatLab, Metaboanalyst.ca)
 KEGG pathway mapping

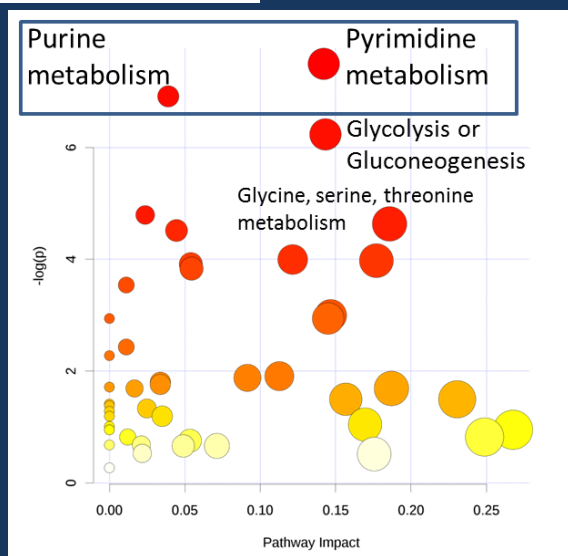


Distribution of Imatinib regulated polar metabolites



Nucleosides

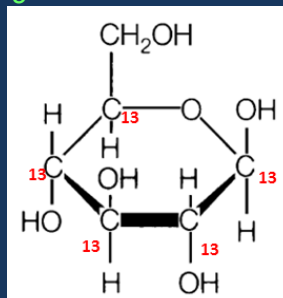
Nucleotides



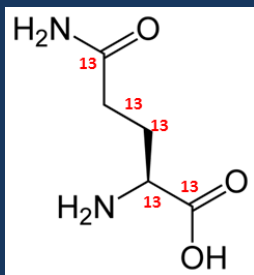
Steady-State Metabolic Flux Analysis:

"SILAC" version for metabolomics

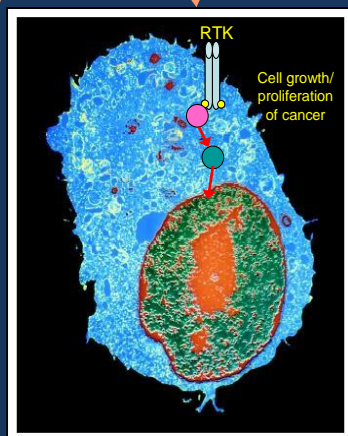
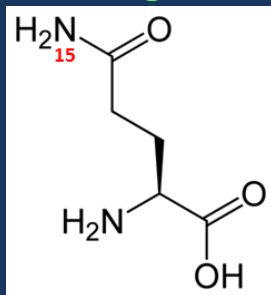
$^{13}\text{C}_6$ -labeled glucose



$^{13}\text{C}_5$ -labeled glutamine



^{15}N -labeled glutamine

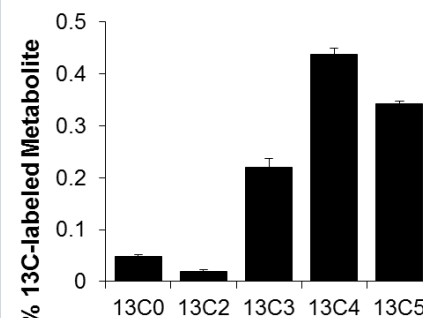


Cancer cell

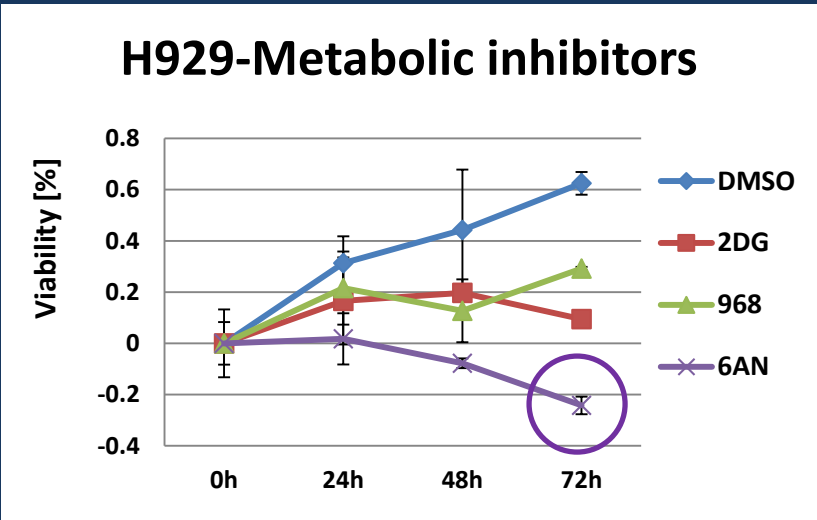


Q1	Q2	Dwell	Compound	CE
145	101	3	a-ketoglutarate	-12
146	101	3	a-ketoglutarate_13C1(2)	-12
146	102	3	a-ketoglutarate_13C1	-12
147	102	3	a-ketoglutarate_13C2(2)	-12
147	103	3	a-ketoglutarate_13C2	-12
148	103	3	a-ketoglutarate_13C3(2)	-12
148	104	3	a-ketoglutarate_13C3	-12
149	104	3	a-ketoglutarate_13C4(2)	-12
149	105	3	a-ketoglutarate_13C4	-12
150	105	3	a-ketoglutarate_13Q	-12

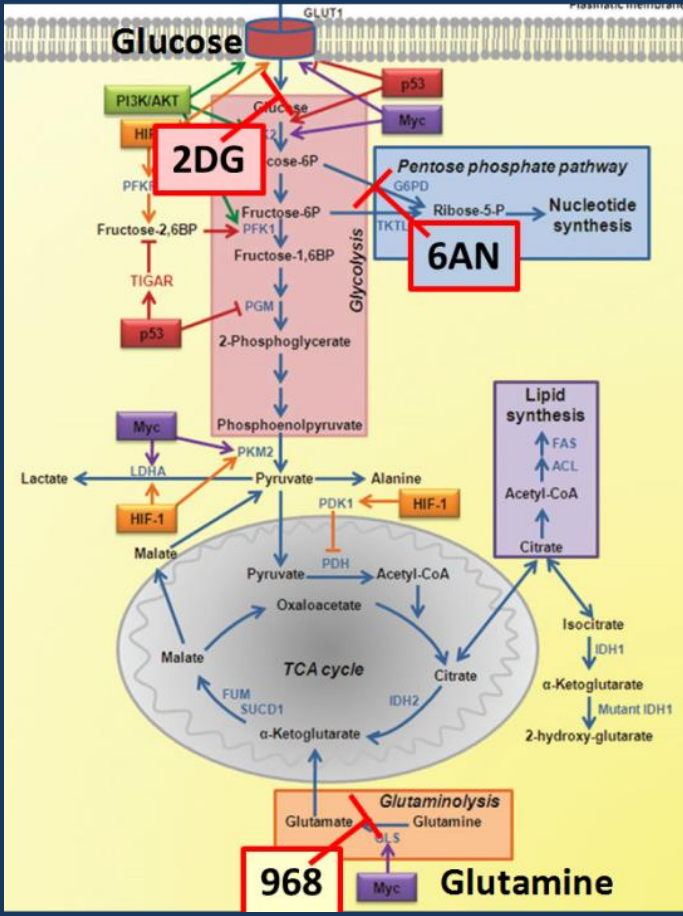
α -KG Isotopomer Analysis



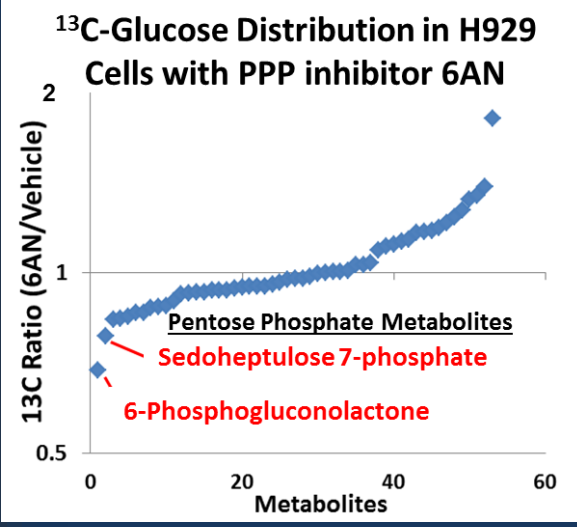
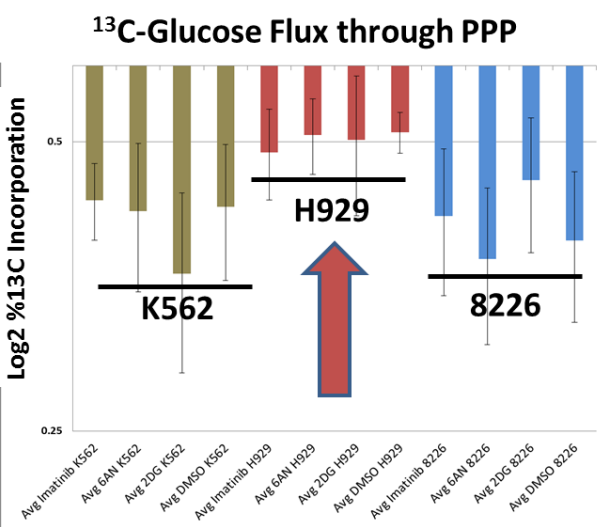
H929 cells are dependent upon glucose driving Pentose Phosphate Pathway



968 (Glutaminase, TCA inhibitor): 10 μ M
 2DG (Glycolysis inhibitor): 20 mM
 6AN (PPP inhibitor): 5 μ M



PPP is responsible for nucleotide synthesis



968 Myc Glutamine

Triple SILAC Phosphoproteomics Platform



Lys0, Arg0



Lys4, Arg6



Lys8, Arg10

Imatinib 1 μ M

Bortezomib 200 nM

DMSO control

Lysis

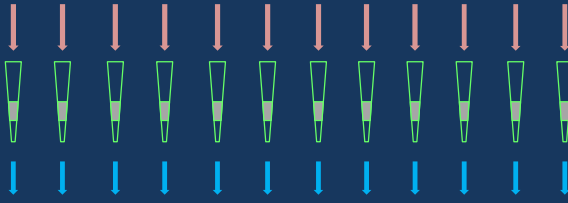
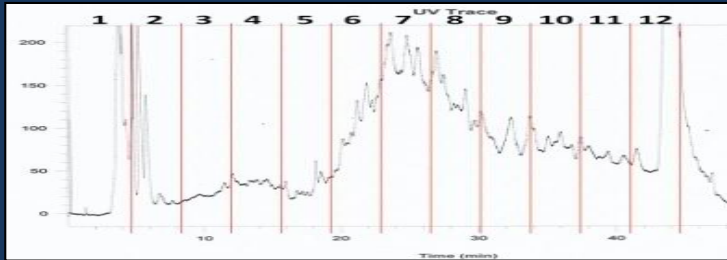
Lysis

Lysis

Mix 1:1:1

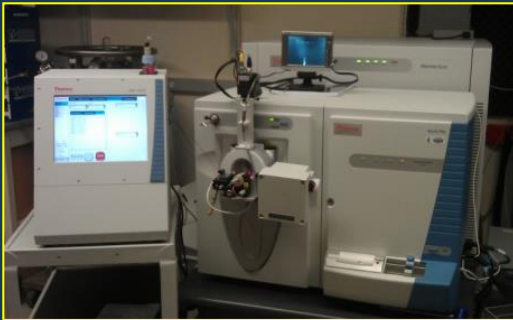
Trypsin digestion

Offline SCX chromatography



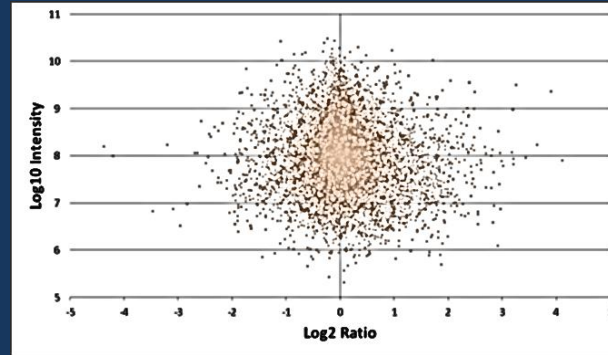
IMAC/ Fe^{3+}

2hr LC/MS/MS via HCD (Top 12) (2X) /
CID (Top 20) on Orbitrap Elite (1X)

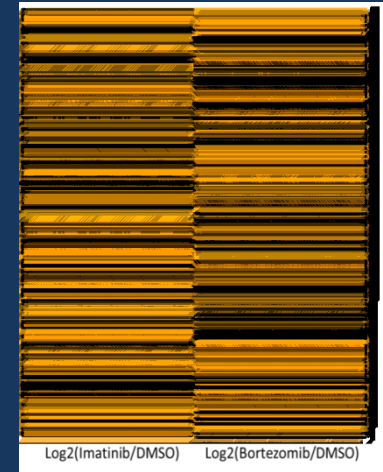


MaxQuant
identification/quantification

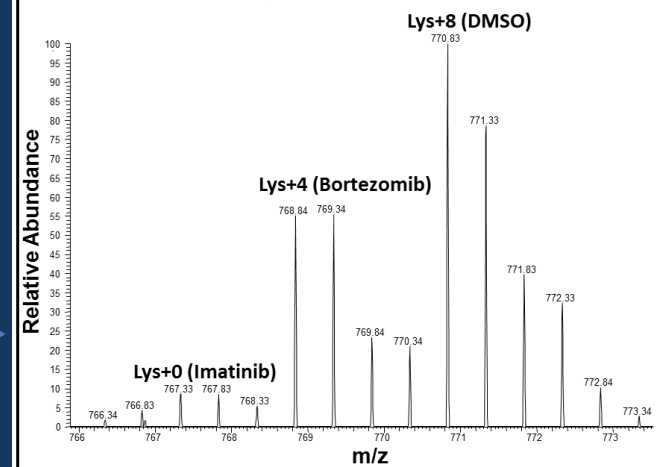
Peak distribution



Heat Map - Ratios



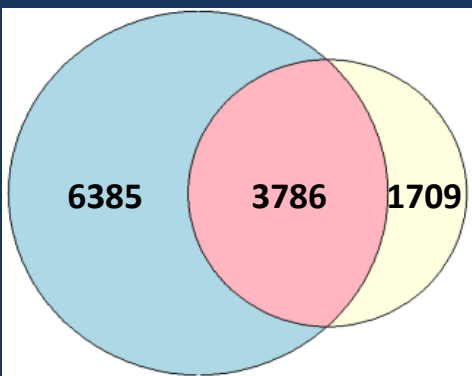
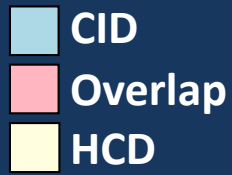
ABL2: LMTGDTpYTAHAGAK, m/z: 766.82, z: 2



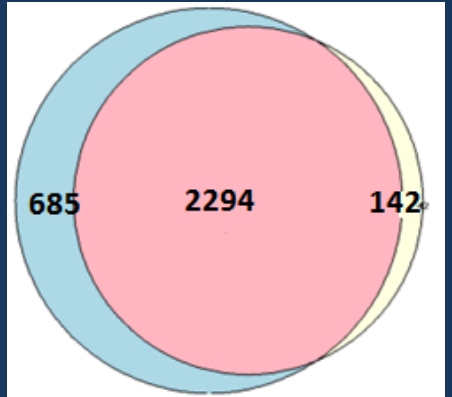
Triple SILAC

11,880 total unique phospho-STY sites

3,121 Total phosphoproteins

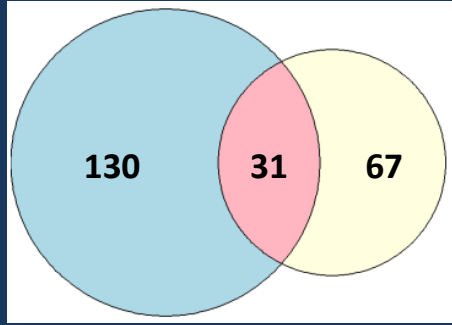


	Total	CID	HCD	Overlap
Y	202	162	51	11
T	1771	1384	788	401
S	9907	8625	4656	3374

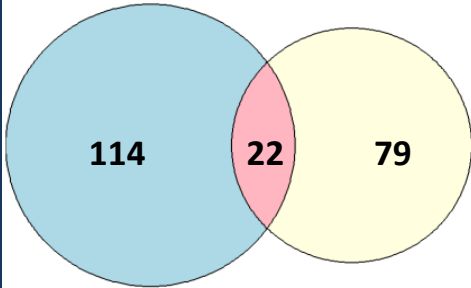


Down-regulated pSTY Imatinib

Up-regulated pSTY Imatinib



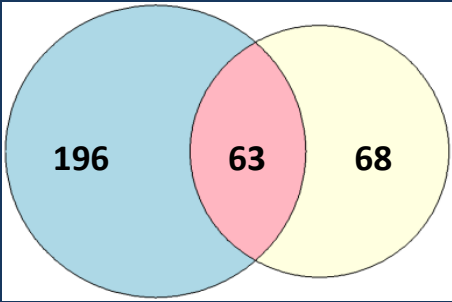
	CID	HCD	Overlap
Y	13	3	3
T	11	10	2
S	137	85	26



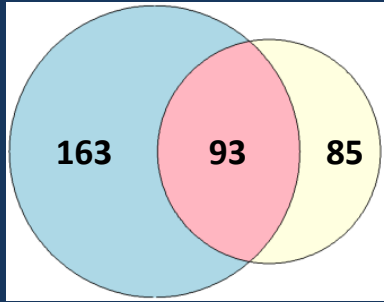
	CID	HCD	Overlap
Y	7	2	0
T	20	8	9
S	87	69	13

Down-regulated pSTY Bortezomib

Up-regulated pSTY Bortezomib



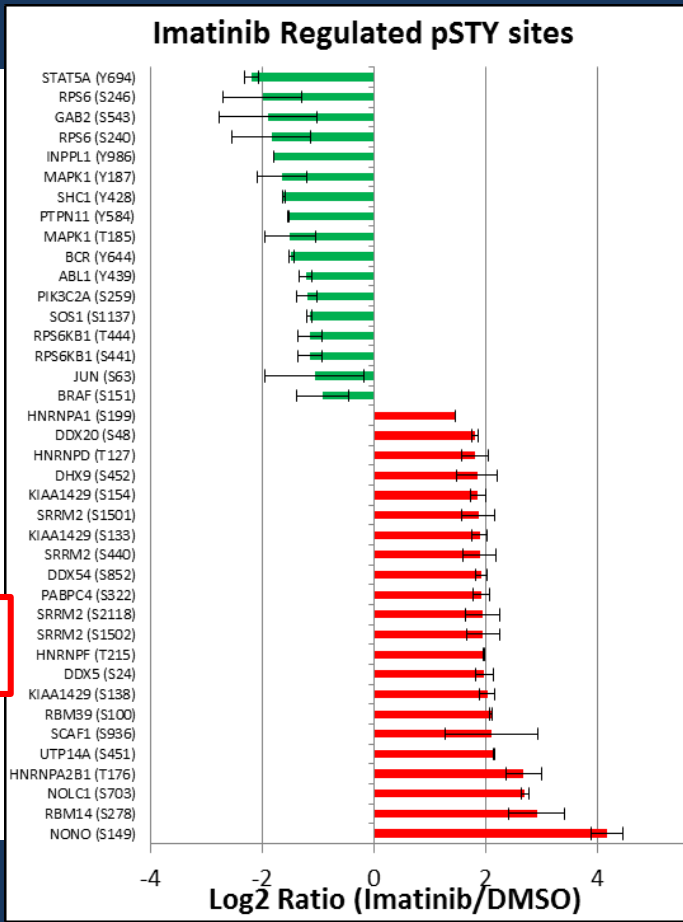
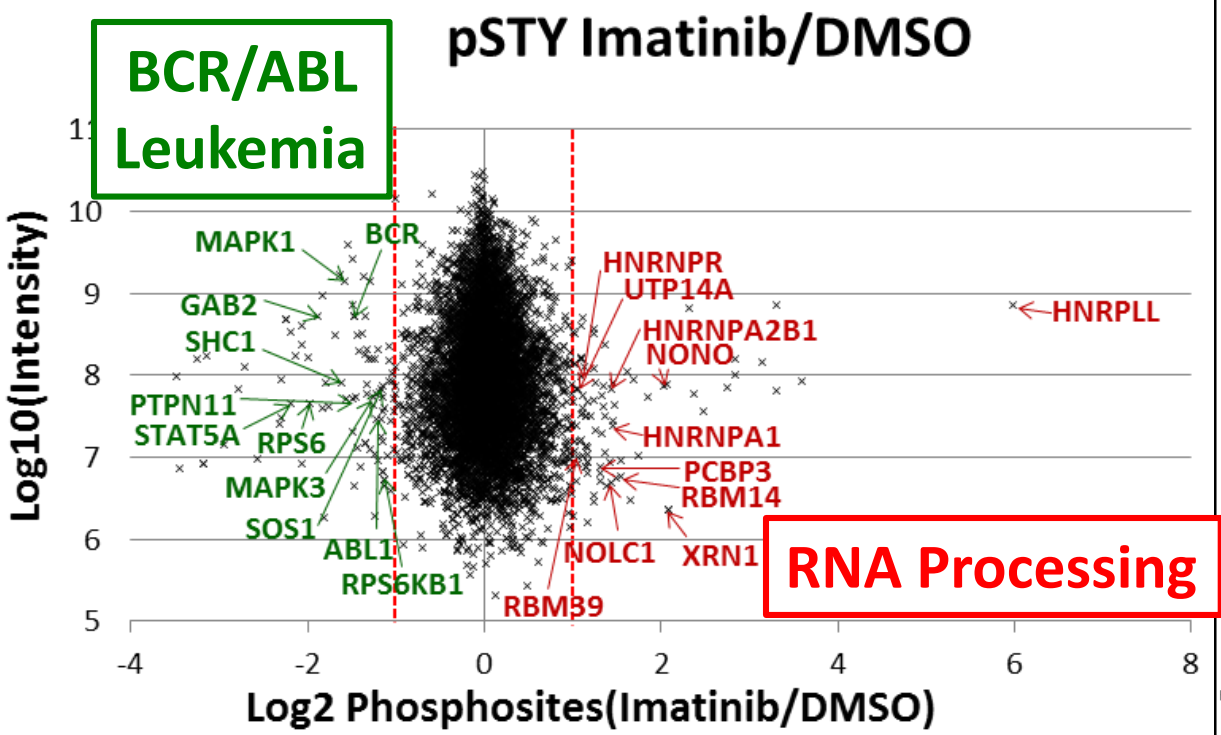
	CID	HCD	Overlap
Y	2	0	0
T	53	47	29
S	127	84	34



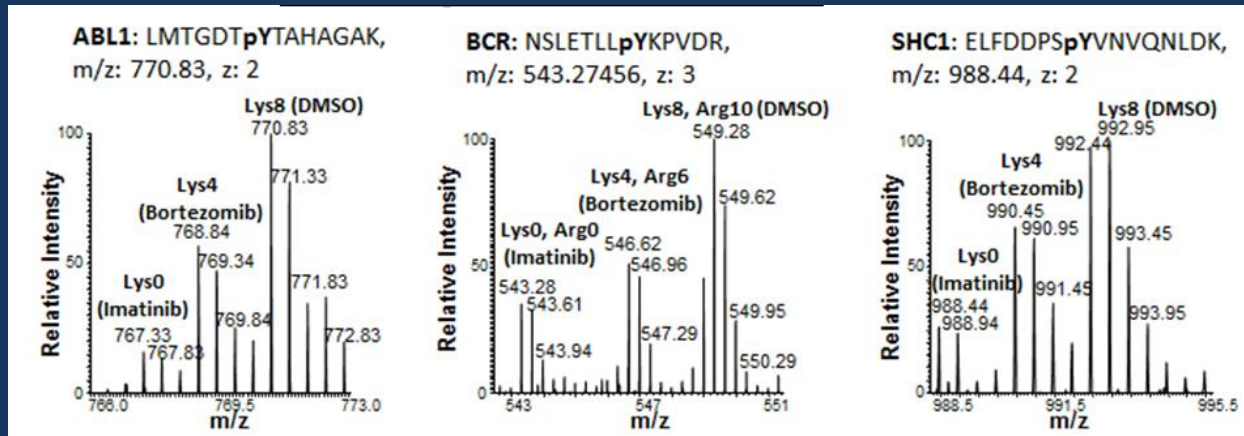
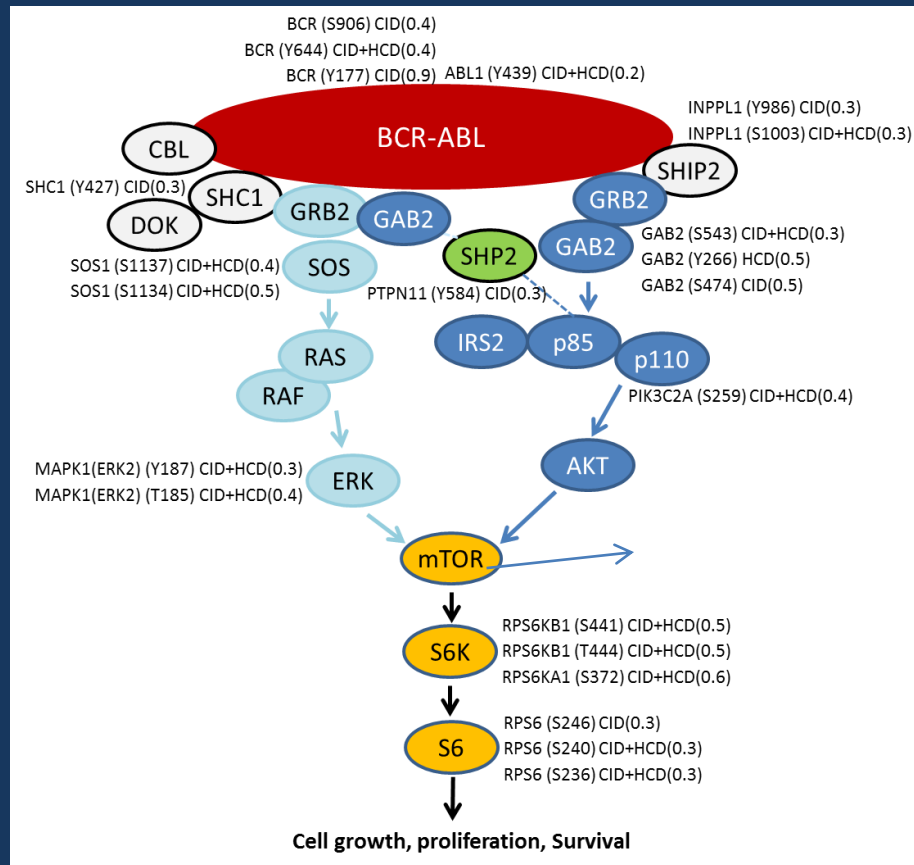
	CID	HCD	Overlap
Y	6	2	1
T	29	9	3
S	221	167	89

2 HCD biological replicates / 1 CID biological replicate

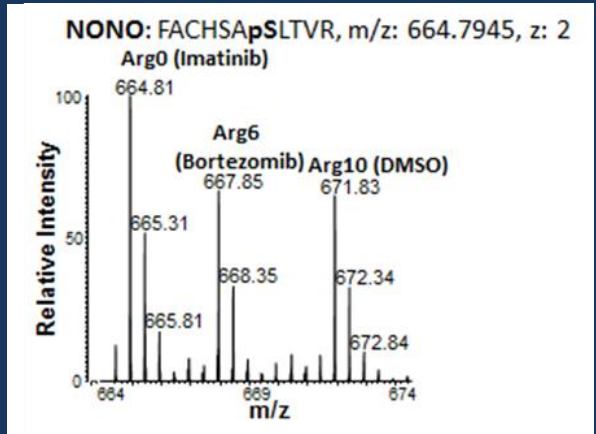
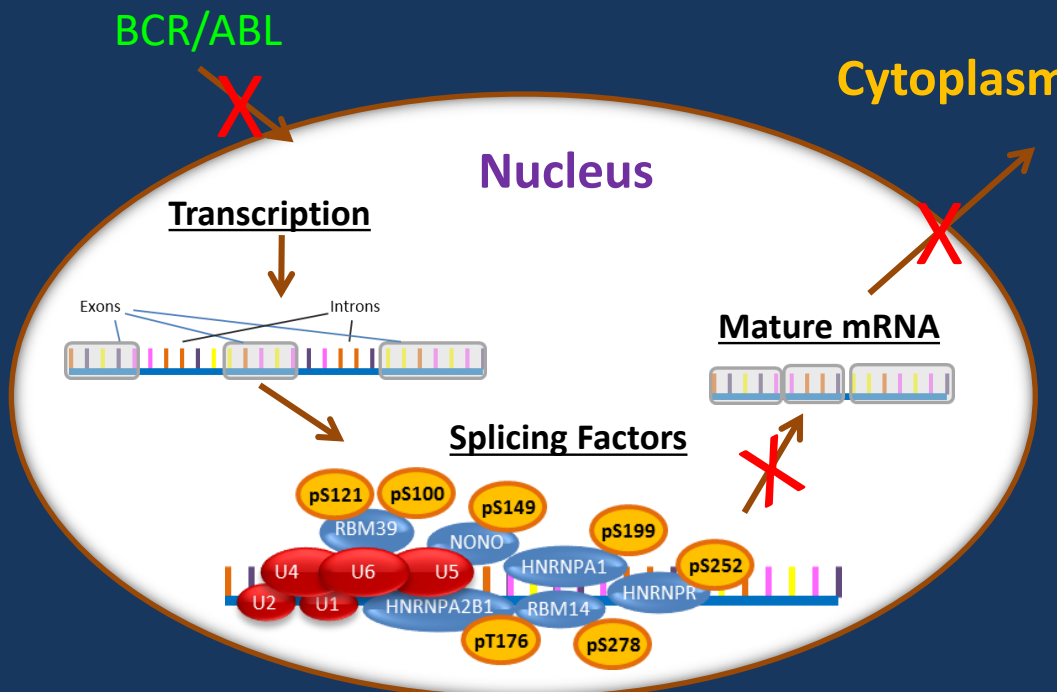
Distribution and Pathways of Regulated Class I Phosphorylation Sites (localization P >75%)



Imatinib Induced Down-Regulated Phosphosites in BCR-ABL Pathway

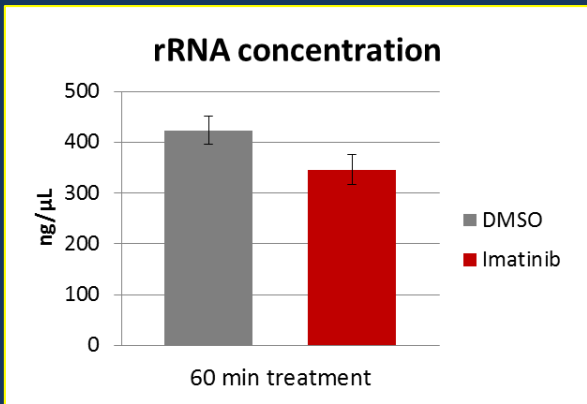
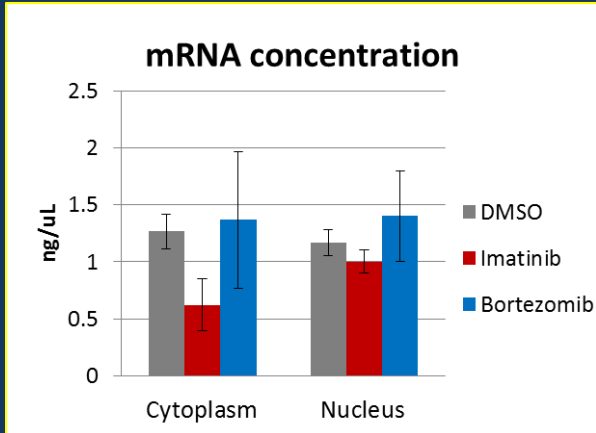


Imatinib Induced Up-Regulated Phosphosites inhibit RNA Transcription



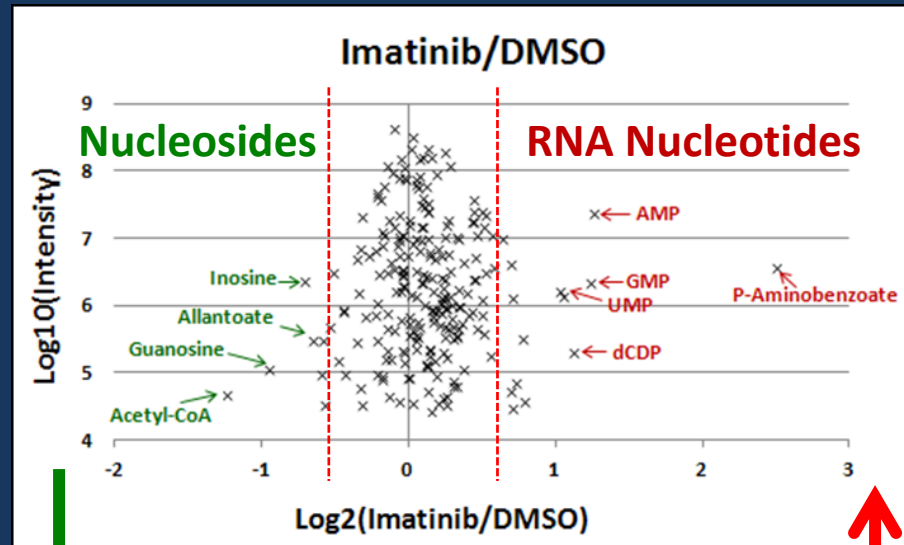
hnRNPA1 S199-p SQRGRSGpSGNFGGGR: *AKT site (shuttle in and out of nucleus)*
 - shown to be inhibitory to c-Myc ribosome entry

(Jo et al, 2008, JBC)

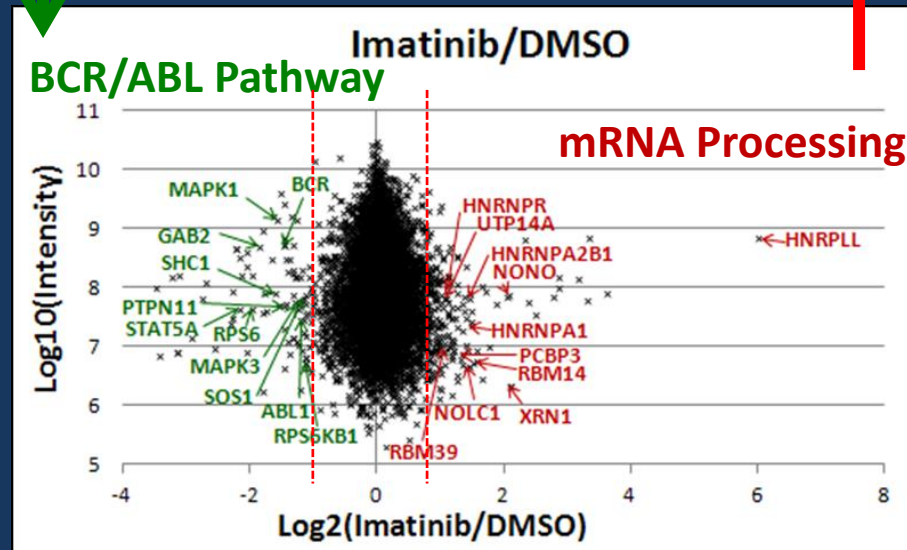


H929 Cells Respond to Imatinib through Nucleotide Accumulation and Inhibition of RNA Transcription

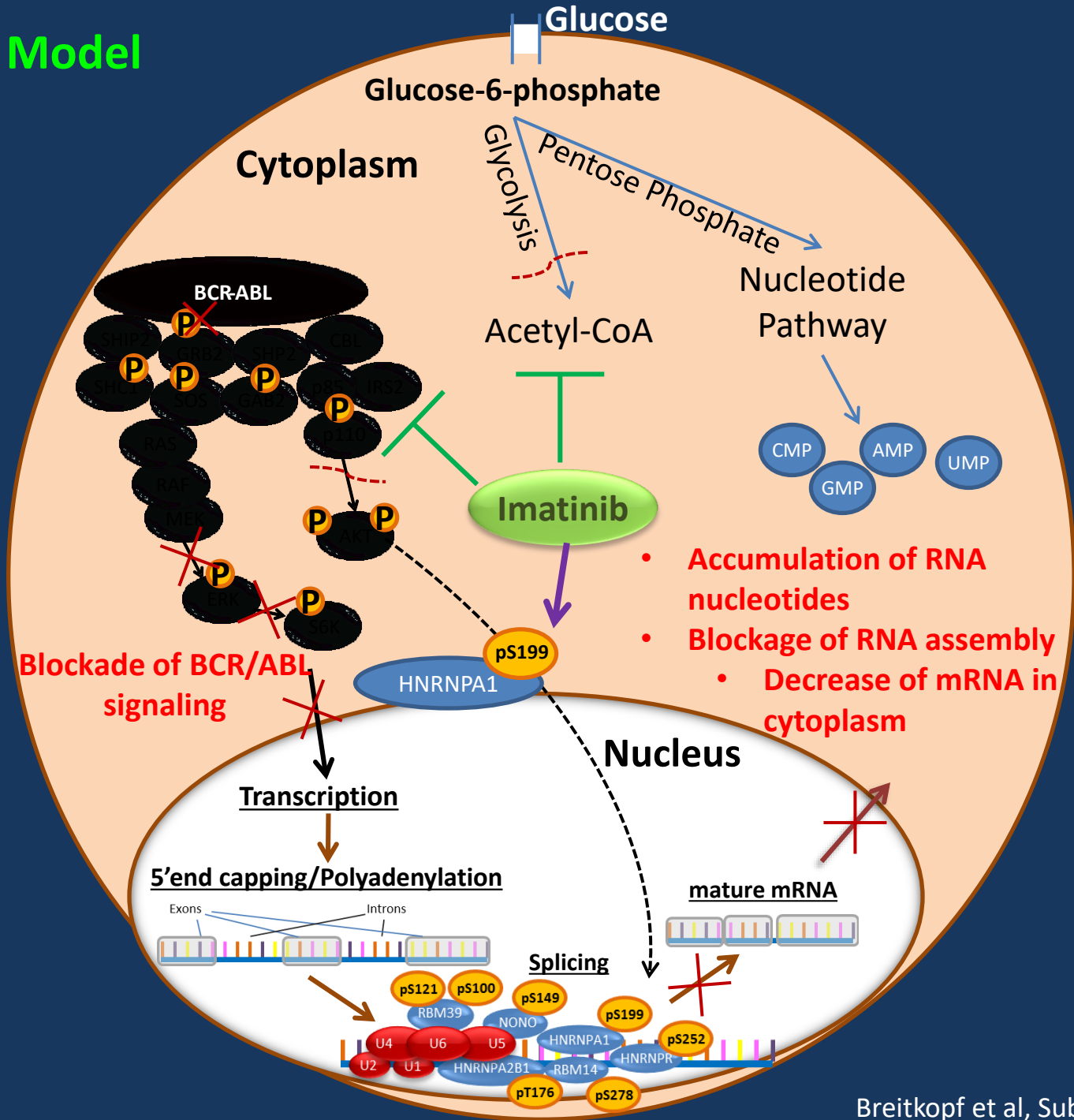
Polar Metabolomics



Triple SILAC Phosphoproteomics



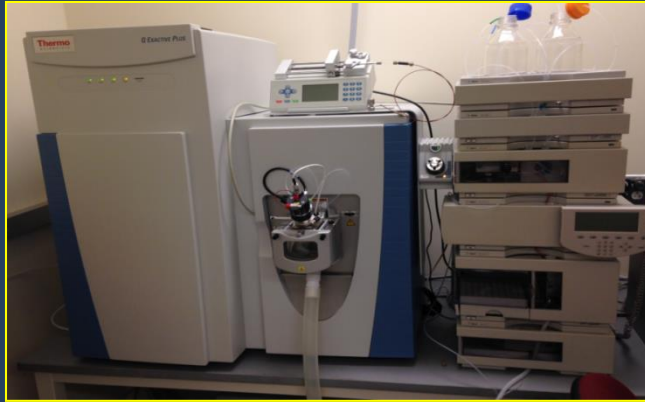
Biological Model



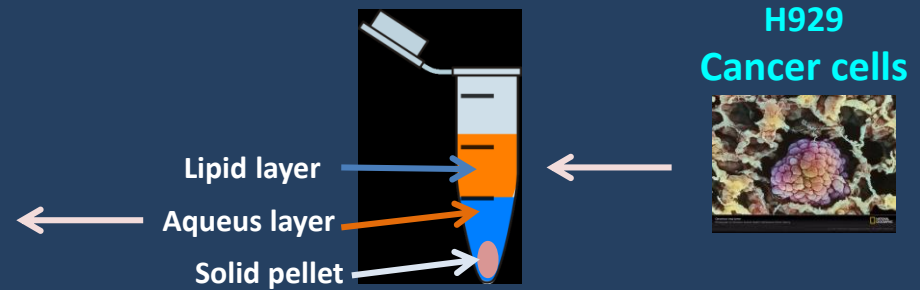
QExactive Plus Mass Spectrometer Platform for Lipidomics

Thermo QExactive Plus

Agilent 1100



methyl-tert-butyl ether (MTBE) or Chl:MeOH 2:1



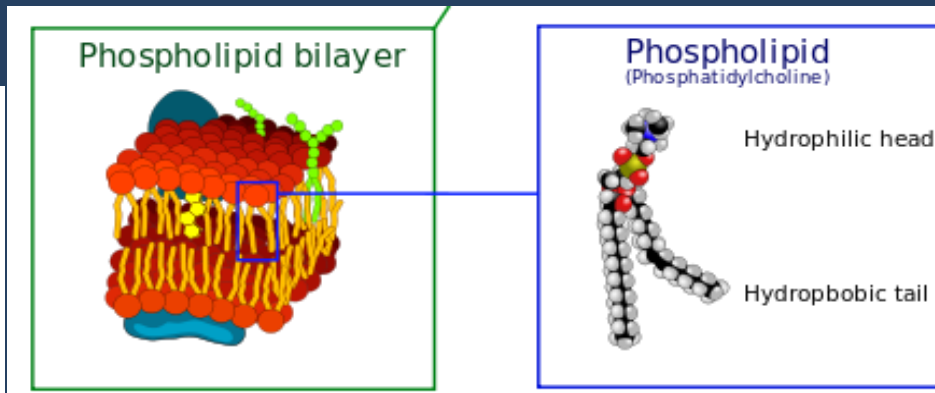
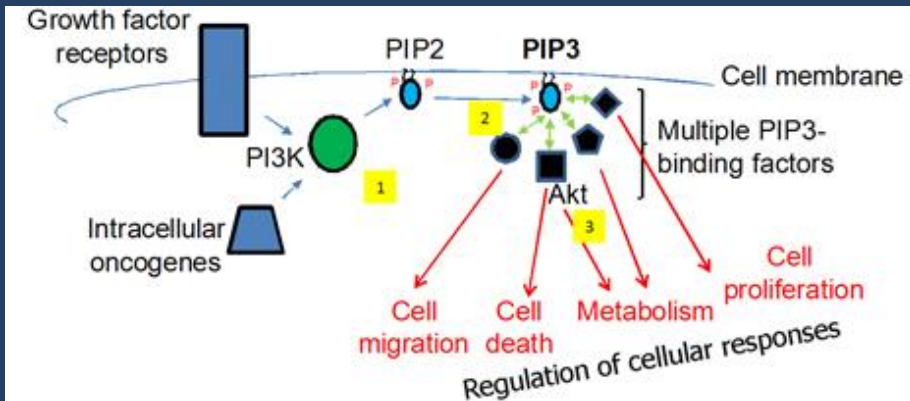
Matyash et al, J. Lipid Res., 2008.

4.6mm x 10cm Amide HILIC (high pH) or C₁₈ (low pH)
400 μL/min

Pos/-Neg polarity switching

DDA (Top 10) pos and neg mode (m/z 200-1500)

Lipids regulate cell growth/proliferation

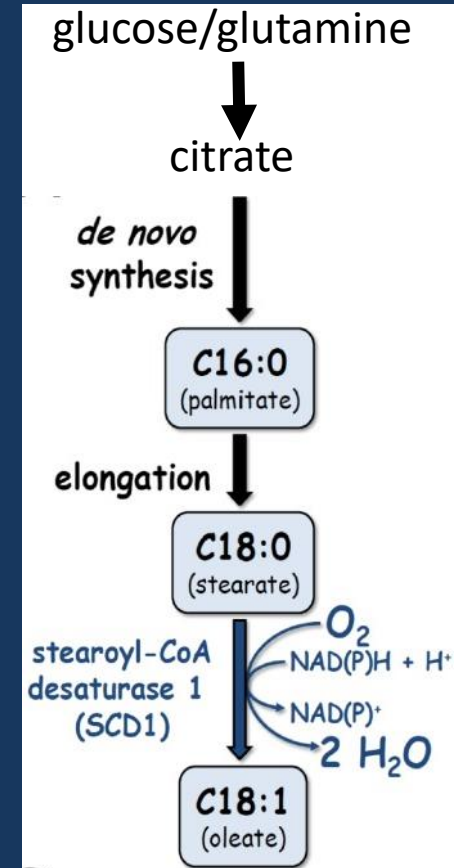


Fragmentation data is critical to the success of lipid (and metabolite) identification

De Novo Lipid Synthesis Pathway

SIEVE results form lipidomics MS1 data

(9E_13E)-9_13-Octadecadienoic acid	C18H32O2 (9E_13E)-9_13-Octadecadienoic acid	280.4455
8-Octadecynoic acid	C18H32O2 8-Octadecynoic acid	280.4455
2-octadecynoic acid	C18H32O2 2-octadecynoic acid	280.44548
3-Octadecynoic acid	C18H32O2 3-Octadecynoic acid	280.4455
4-Octadecynoic acid	C18H32O2 4-Octadecynoic acid	280.4455
5-Octadecynoic acid	C18H32O2 5-Octadecynoic acid	280.4455
10-Octadecynoic acid	C18H32O2 10-Octadecynoic acid	280.4455
11-Octadecynoic acid	C18H32O2 11-Octadecynoic acid	280.44548
14-Octadecynoic acid	C18H32O2 14-Octadecynoic acid	280.4455
15-Octadecynoic acid	C18H32O2 15-Octadecynoic acid	280.4455
16-Octadecynoic acid	C18H32O2 16-Octadecynoic acid	280.4455
10E_12Z-octadecadienoic acid	C18H32O2 10E_12Z-octadecadienoic acid	280.4455
9_15-octadecadienoic acid	C18H32O2 9_15-octadecadienoic acid	280.4455
Ethyl (2E_4Z)-2_4-hexadecadienoate	C18H32O2 Ethyl (2E_4Z)-2_4-hexadecadienoate	280.44548
11-Cycloheptylundecanoic acid	C18H34O2 11-Cycloheptylundecanoic acid	282.46136
Oleic acid	C18H34O2 Oleic acid	282.46136
(12Z)-12-Octadecenoic acid	C18H34O2 (12Z)-12-Octadecenoic acid	282.46136
Elaidic Acid	C18H34O2 Elaidic Acid	282.46136
cis-Petroselinic acid	C18H34O2 cis-Petroselinic acid	282.46136
trans-Vaccenic acid	C18H34O2 trans-Vaccenic acid	282.46136
3Z-octadecenoic acid	C18H34O2 3Z-octadecenoic acid	282.46136
octadecenoic acid	C18H34O2 octadecenoic acid	282.46136
(3E)-3-Octadecenoic acid	C18H34O2 (3E)-3-Octadecenoic acid	282.4614
(4E)-octadec-4-enoic acid	C18H34O2 (4E)-octadec-4-enoic acid	282.4614
5-octadecenoic acid	C18H34O2 5-octadecenoic acid	282.4614
(6E)-6-Octadecenoic acid	C18H34O2 (6E)-6-Octadecenoic acid	282.46136
(7Z)-Octadec-7-enoic acid	C18H34O2 (7Z)-Octadec-7-enoic acid	282.4614
(7E)-Octadec-7-enoic acid	C18H34O2 (7E)-Octadec-7-enoic acid	282.4614
(8Z)-octadec-8-enoic acid	C18H34O2 (8Z)-octadec-8-enoic acid	282.4614

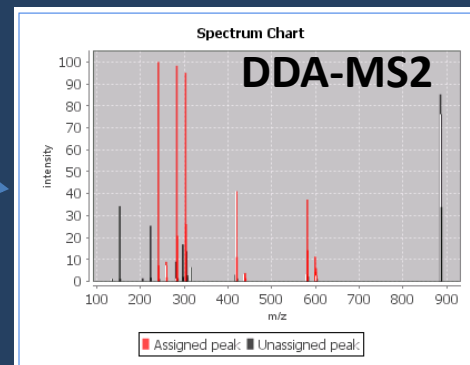
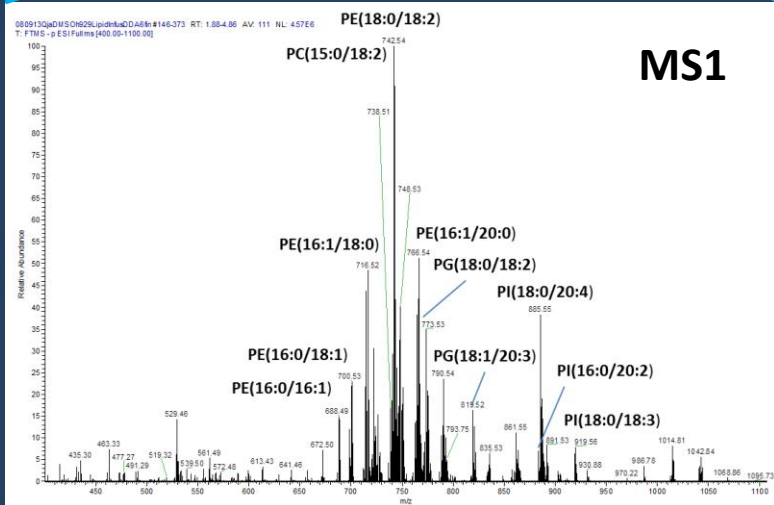
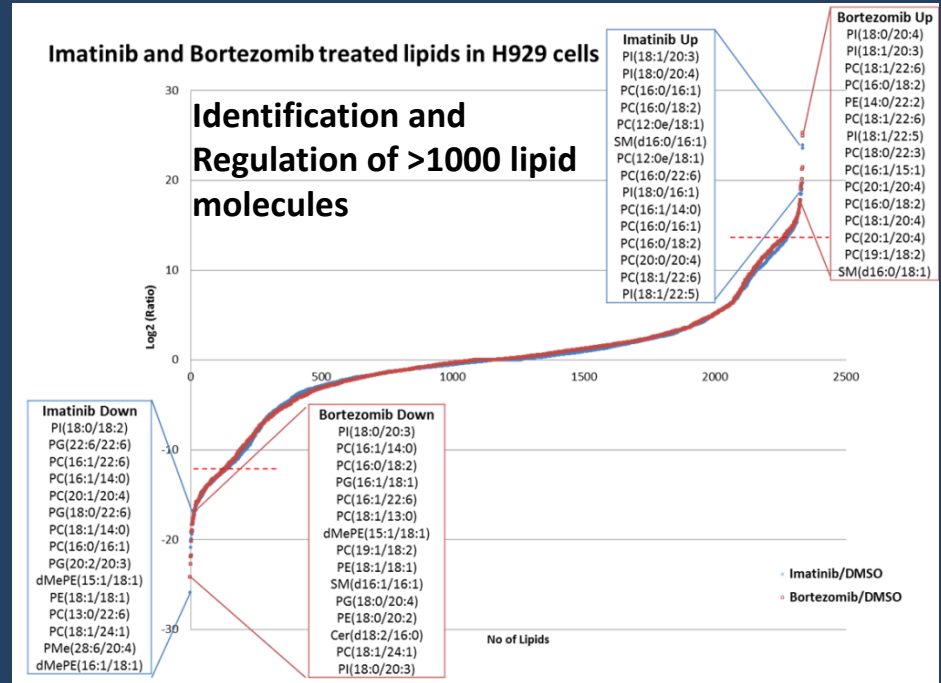
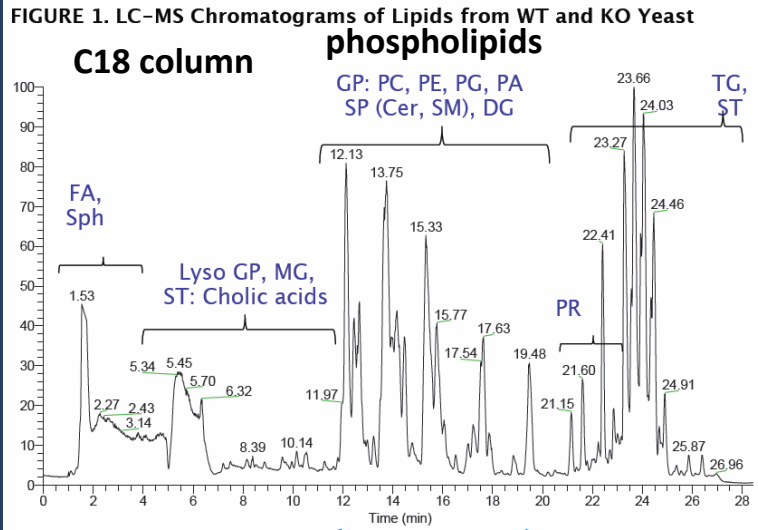


Kamphorst et al., PNAS 2013

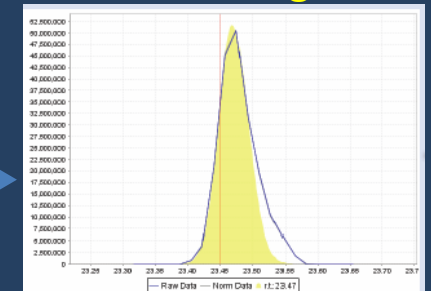
MS1-only produces too many possibilities, especially for lipids

LipidSearch Software (Thermo Scientific) for Lipid Identification and Quantification

Lipid Identification and Relative Quantification

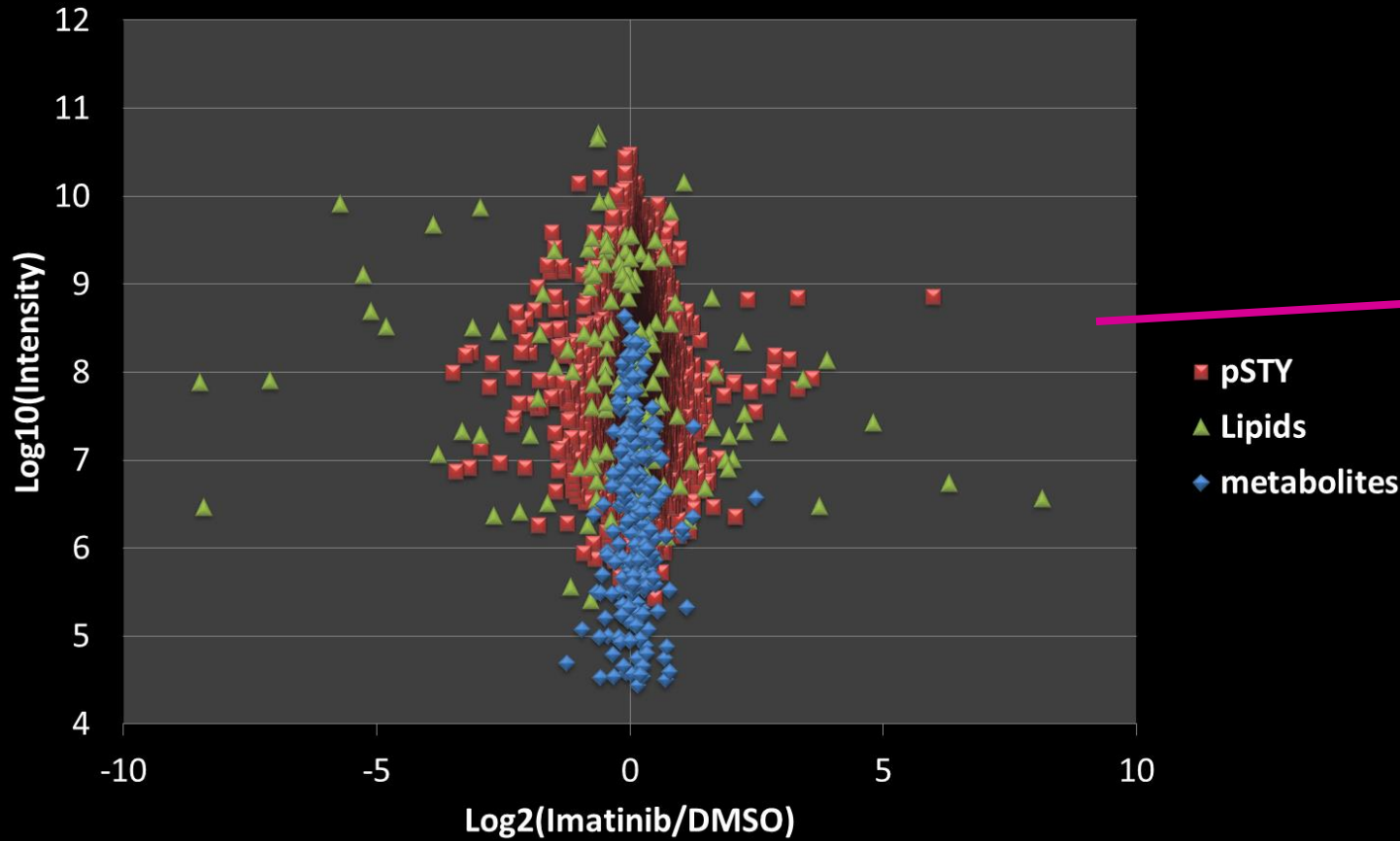


MS1 Peak integration



Merging the Phosphoproteome-Metabolome-Lipidome.....

Cross-Omics Distribution of Imatinib Treated Data



Biomarker(s)
Pathway(s)

Treatment Plan

CONCLUSIONS

We have an opportunity to merge/cross/combine different *-omics* technologies to reveal new biological insight in disease mechanisms

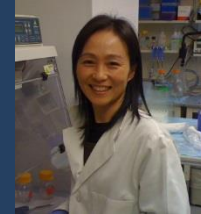
Revealed a novel mechanism for the TKI Imatinib in BCR/ABL myeloma cells using **global phosphoproteomics and **polar metabolomics**..... and **lipidomics****

Acknowledgements

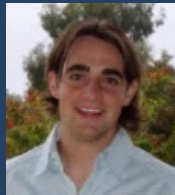
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