CLINICAL PROTEOMIC
TUMOR ANALYSIS CONSORTIUM

The Skyline software project for clinical proteomics: lessons learned

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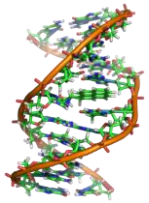
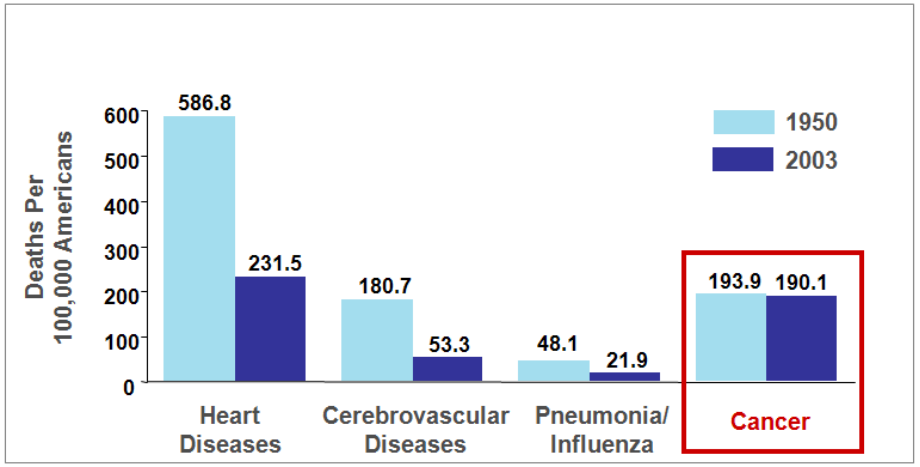
Outline

- Origin of CPTAC
- Origin of Verification Working Group
- Origin of Skyline
- Lessons learned

Origin of CPTAC - 2003

- Human genome is sequenced and available
- Proteomics shown to detect ovarian cancer via SELDI
- Despite advances in preventing and treating other diseases, mortality rate of cancer has not changed in 50 years
- NIH budget in the midst of doubling
- NCI committee recommends biomarker discovery program steeped in new technology





Source for 2006 deaths and diagnoses: American Cancer Society (ACS) 2006 Cancer Facts & Figures; Atlanta, Georgia
Source for 2003 age-adjusted death rate: National Center for Health Statistics, U.S. Department of Health and Human Services, NCHS Public-use file for 2003 deaths.

2005

- Questions have arisen about reproducibility of proteomic technologies
- Statisticians debunked some initial claims of biomarkers

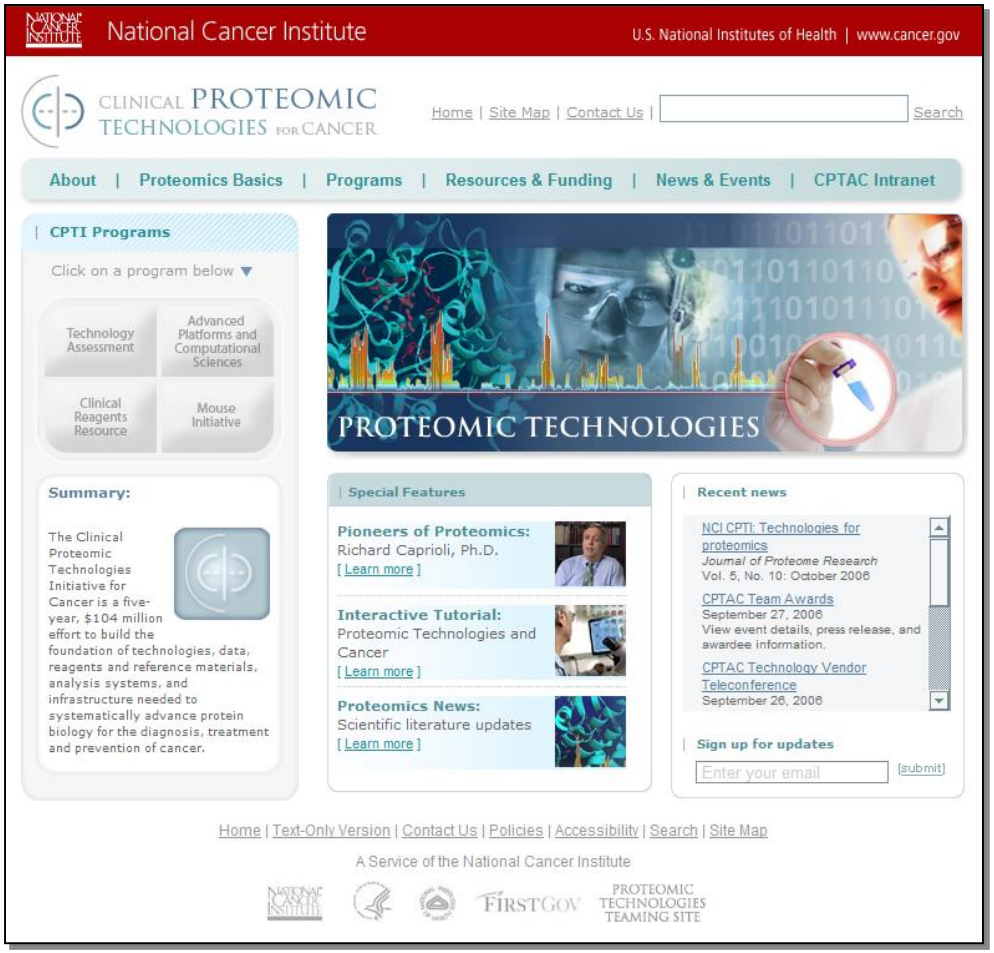
- BSA redirects NCI program toward technology assessment and standards

CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

CPTAC Goals

Integrated approach to address barriers in proteomic technologies, reagents and systems early in the "pipeline"

- Assess, enhance and develop proteomic technology measurement capabilities
- Build a foundation of technologies, data, reagents and standards, analysis systems, and infrastructure
- Systematically advance understanding of protein biology in cancer



http://proteomics.cancer.gov

CPTAC and MRM timeline

- Aug. 2006 – NBT Rifai, Carr, Gillette lays out biomarker pipeline with key MRM-verification step
- Sept. 2006 – CPTAC kickoff meeting
- Nov. 2006 – CPTAC adopts discovery/verification paradigm
- Dec. 2006 – CPTAC Verification WG established

"The mission of the Experimental Design and Statistics - Verification Studies Working Group is to develop approaches to and **define performance characteristics** of mass spectrometry-based proteomic measurement systems (platforms*) including incorporation of appropriate measurement assessment materials and reference standards; employ statistically designed approaches to experimentally **determine performance parameters and metrics** using biologically and clinically relevant samples; and actively propose and evaluate experimental design strategies and develop statistical protocols to effectively analyze the results within and among CPTAC teams"

Nature Biotechnology 24, 971 - 983 (2006)

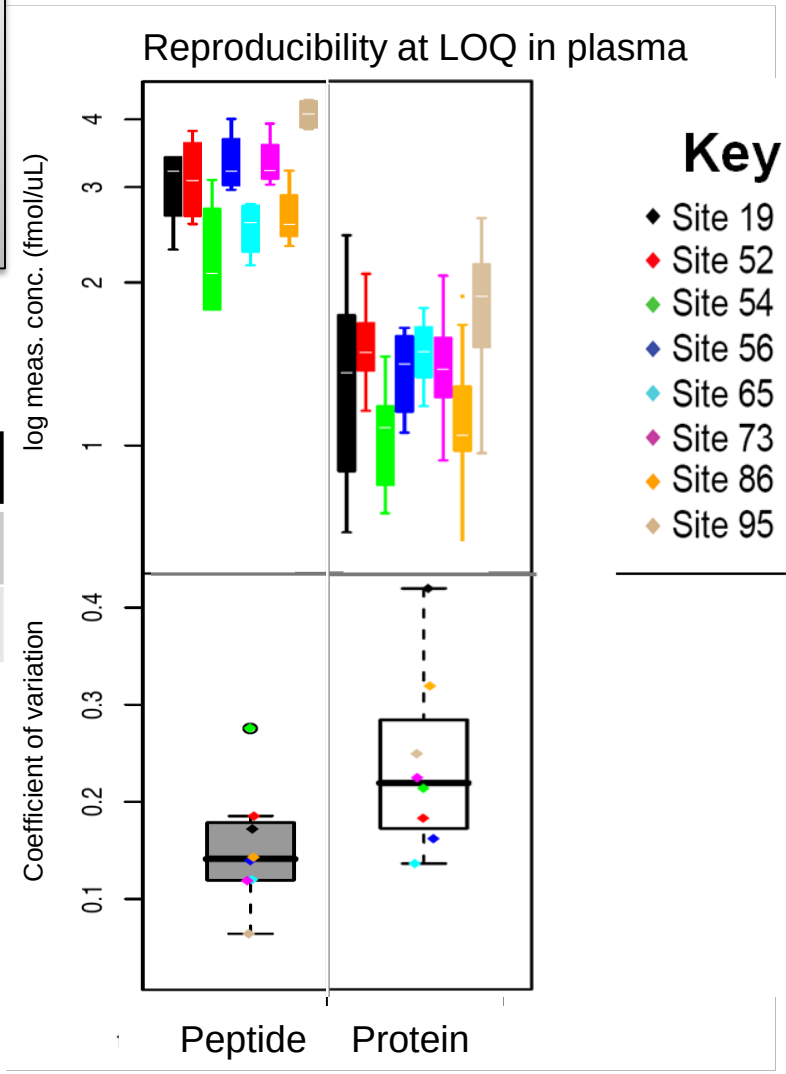
Inter-lab Reproducibility across 8 labs

Peptide: 10 light and heavy peptides spiked into digested plasma

Protein: 7 proteins spiked into plasma, then spiked with heavy peptides and digested.

	Peptide	Protein
CV:	6-13%	8-29%
Concentration	2.9 nmol/L	2.9 nmol/L

MRM is fit for verification



Addona et al., Nature Biotechnol., 2009, 613

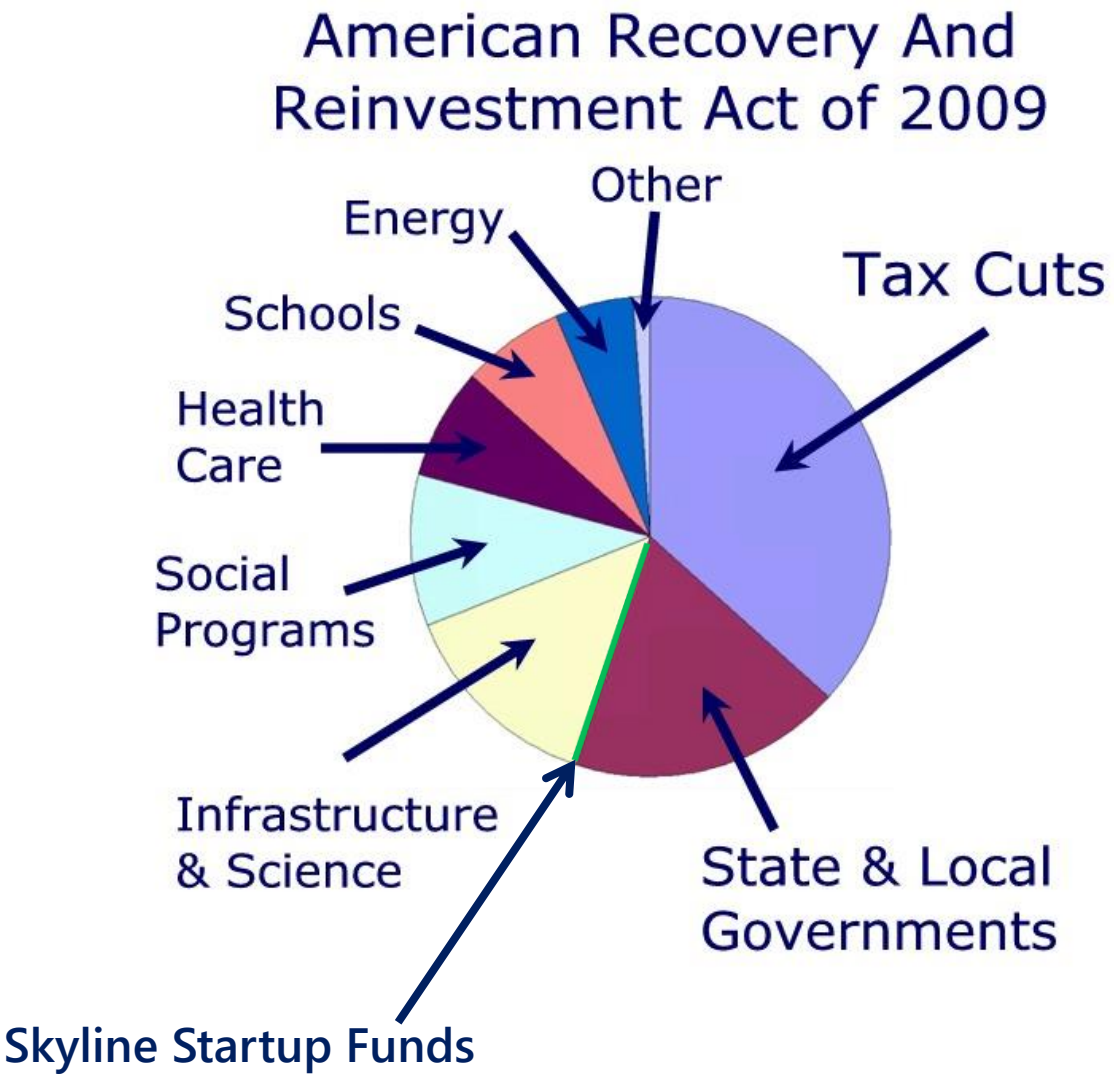
Data analysis for *NBT 2009, 16*

Terri A
Clifford
Simon
Michael
Eric B J
Trenton
Tony J
Mu Wa
Amanda

Causes of pain

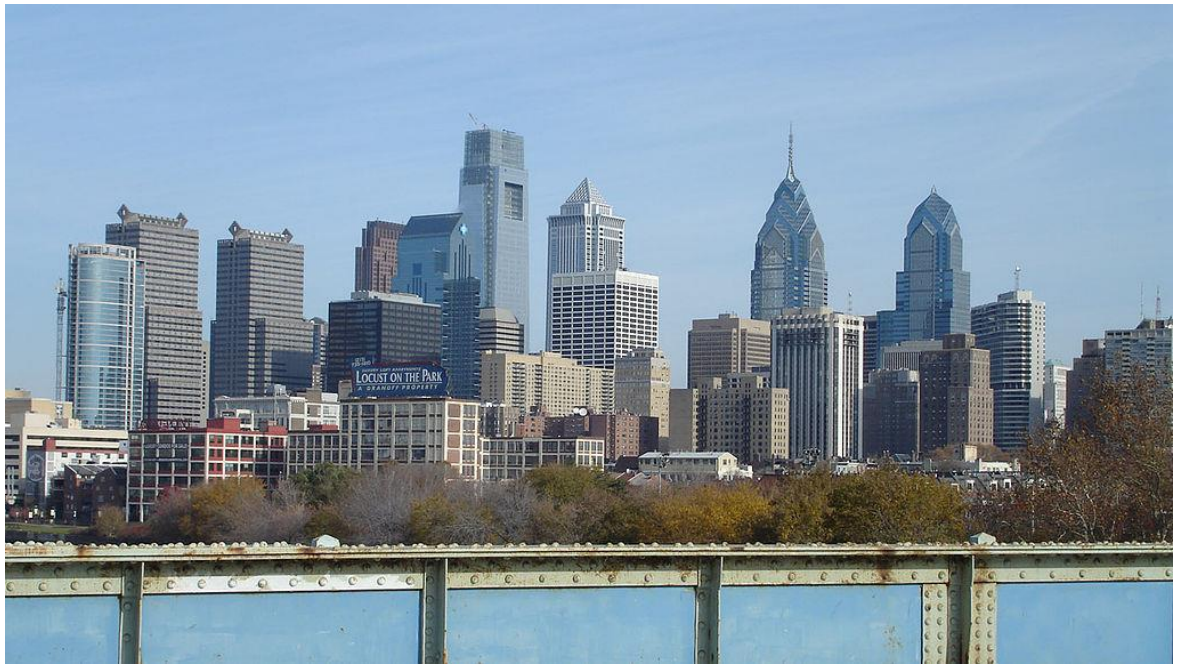
- Sample prep – scalability of digestion
- Different labs
- Different instruments
  - Different outputs
  - Different measurement methods
  - Data extraction from raw files was especially troublesome and time consuming for non-4000 QTRAP users
- Different analysis software
  - Different labs had different versions of analysis software
  - “software is needed to rapidly incorporate the established peptide retention times into the sMRM data acquisition method”
  - “Birgit has also devised a “brute force” protocol that extracts retention time information and that can generate metric plots from MultiQuant results tables”
  - Aggregating the data was done through Excel
  - “At some point, a bundled version of the statistical analysis software will need to be uploaded to the public website”
  - Dilution slope calculations
  - LOD/LOQ calculations
  - Log/log vs. log/linear plots
  - “First, a MultiQuant-like tool needs to created that will extract MRM data from TSQ Quantum raw files”
  - A tool needs to be devised that will translate MultiQuant output into a database format more versatile than Excel
  - Incorrect peak integration appears to explain many possible outliers
  - **“Long-term goals will encompass creating software tools for cross platform data analysis and quality control of peak integration results obtained from different manufacturers data analysis software packages” (3/11/2008)**

Stimulus to the Rescue



ASMS Philadelphia 2009

ASMS
Philadelphia
2009



Operators' workshop – June 2009

- Gathering of CPTAC triple-quad operators
- Group began using common data analysis software
- Real-time user feedback and developer solutions

Thank you email from Sue Abbatiello: "One major success (in my opinion) was the deployment of Skyline on the laptops of ~ 15 users in three separate sessions where people learned to build MRM methods, analyze data and further refine the MRM methods for maximum detection capabilities. "

System suitability

- **Design, Implementation and Multisite Evaluation of a System Suitability Protocol for the Quantitative Assessment of Instrument Performance in Liquid Chromatography-Multiple Reaction Monitoring-MS (LC-MRM-MS).**

Susan E. Abbatiello, D. R. Mani, Birgit Schilling, Brendan MacLean, Lisa J. Zimmerman, Xingdong Feng, Michael P. Cusack, Nell Sedransk, Steven C. Hall, Terri Addona, Simon Allen, Nathan G. Dodder, Mousumi Ghosh, Jason M. Held, Victoria Hedrick, H. Dorota Inerowicz, Angela Jackson, Hasmik Keshishian, Jong Won Kim, John S. Lyssand, C. Paige Riley, Paul Rudnick, Pawel Sadowski, Kent Shaddox, Derek Smith, Daniela Tomazela, Asa Wahlander, Sofia Waldemarson, Corbin A. Whitwell, Jinsam You, Shucha Zhang, Christopher R. Kinsinger, Mehdi Mesri, Henry Rodriguez, Christoph H. Borchers, Charles Buck, Susan J. Fisher, Bradford W. Gibson, Daniel Liebler, Michael MacCoss, Thomas A. Neubert, Amanda Paulovich, Fred Regnier, Steven J. Skates, Paul Tempst, Mu Wang, and Steven A. Carr

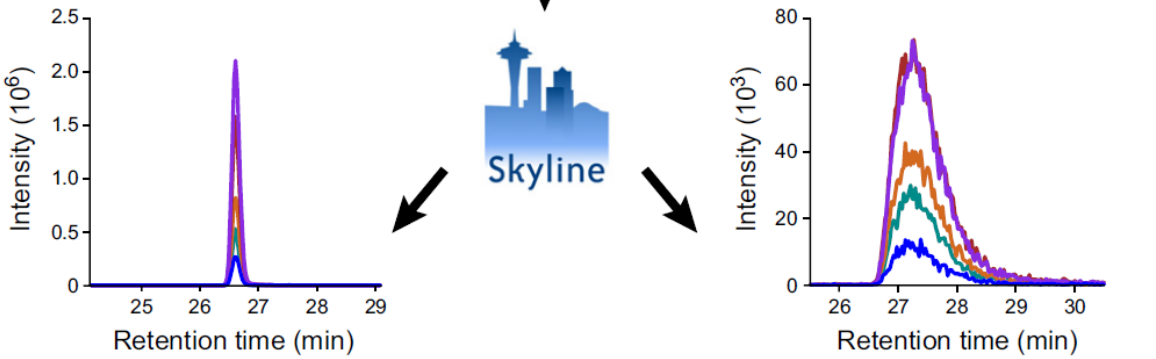
- **Facilitate performance evaluation of LC-SID-MRM-MS**
- **11 labs**
- **15 instruments**

Molecular & Cellular Proteomics 12.9, 2013, 2623

Performance assessment

B SSP evaluation and use

Final list of 9 peptides for System Suitability Study across all sites	
TAAYVNAIEK	TAA
GFCGLSQPK	GFC
DGGIDPLVR	DGG
VLDALDSIK	VLD
CAVVDVPFGGAK	CAV
LVNELTEFAK	LVN
DDGSWEVIEGYR	DDG
VGPLLACLLGR	VGP
FFVAPFPEVFGK	FFV

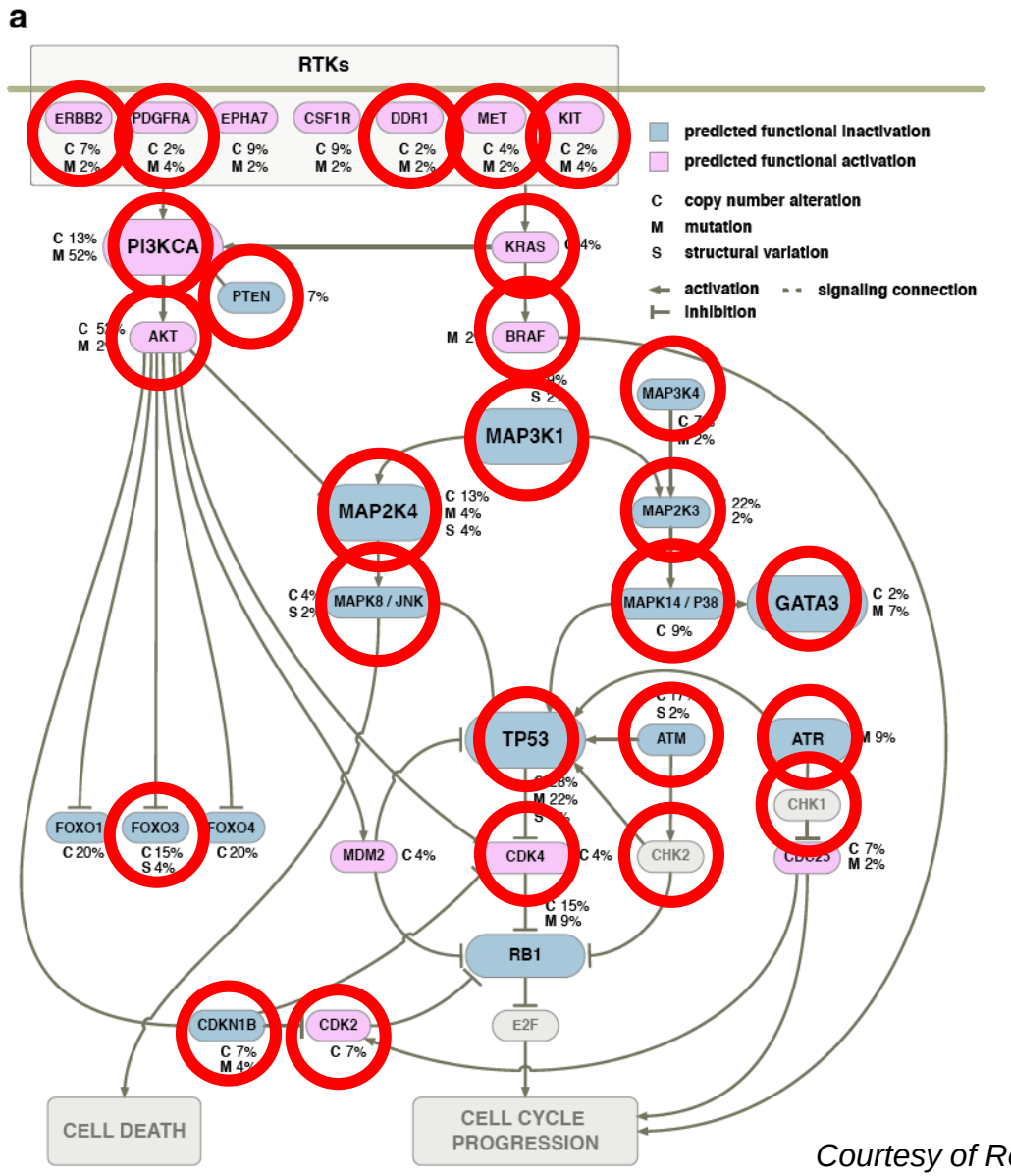


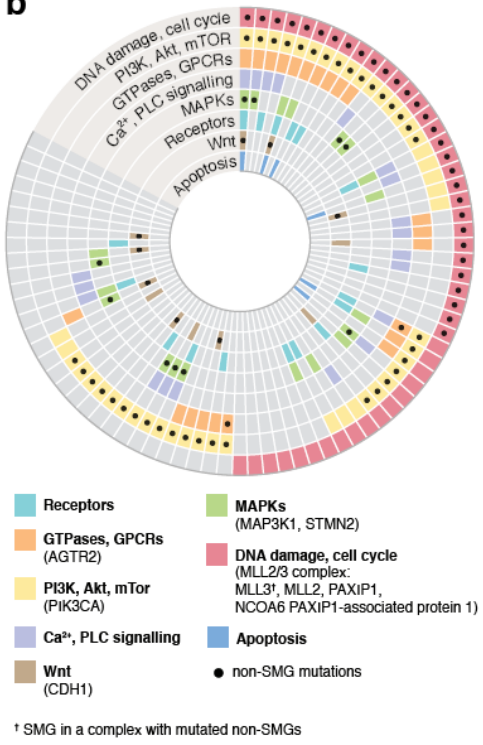
PASSED SSP: Proceed

FAILED SSP: Stop and Troubleshoot

- Peak area CV
- Peak width CV
- Retention time stdev
- Retention time drift

Targeted MRM Assay Panels
(Multiplexed, Thematic Panels)





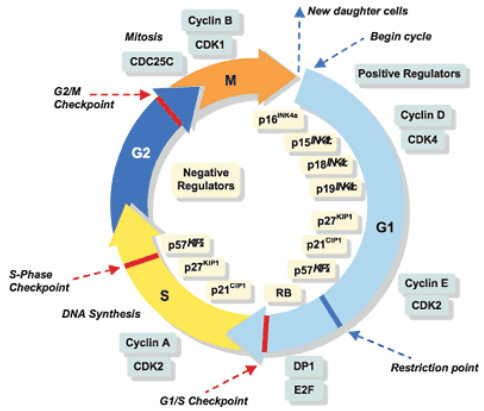
Receptor Tyrosine Kinase Signaling Cascade

Courtesy of Reid Townsend, Washington Univ of St. Louis

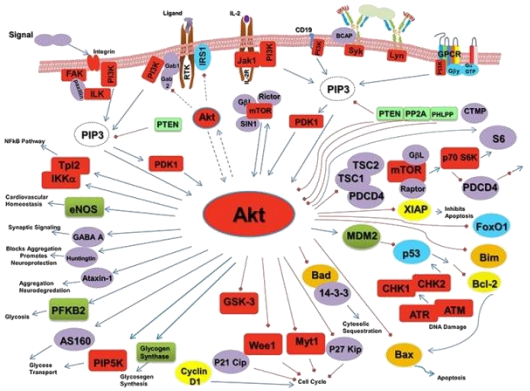
Other cell signaling pathways

http://assays.cancer.gov

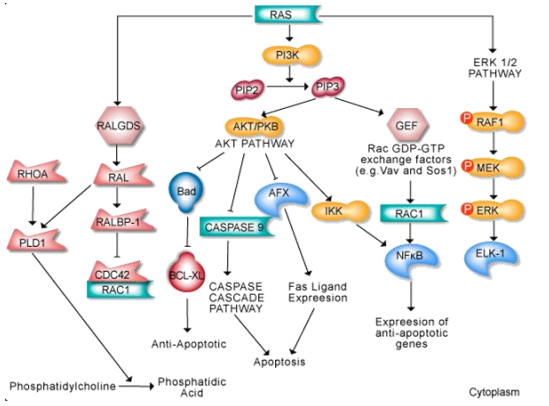
CDK



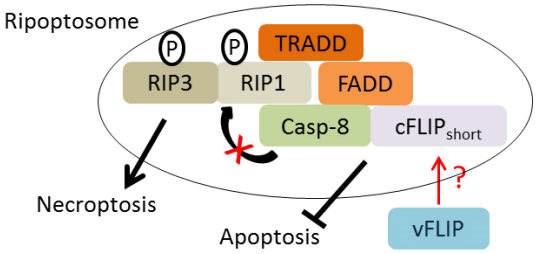
AKT



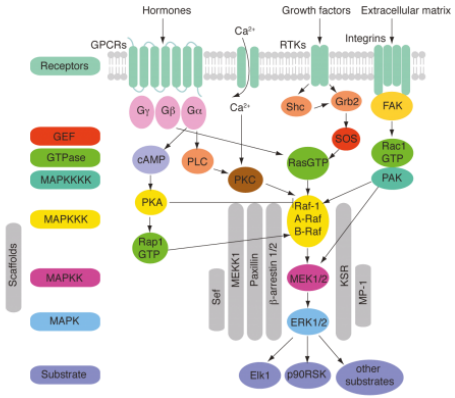
RAS



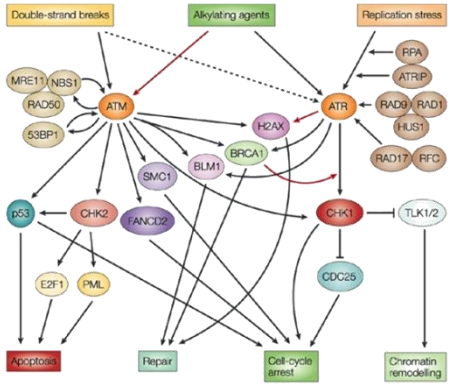
Ripoptosome



MAPK



DDR



Conclusions

- User group ready for a software solution
- Dedicated development/feedback loop rapidly led to robust product
- Quantitative proteomics has dramatically improved over 10 years
- CPTAC goals were achieved
- Doug Lowy (deputy director of NCI), quoting Al Jolson (The Jazz Singer) Nov. 13, 2013

“You ain’t heard nothing yet”

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