



United States Department of  
**Health & Human Services**  
Office of the Assistant Secretary for Preparedness and Response



# **Rapid, Sensitive Dose Assessment Using Mass Spectrometry as a Tool to Identify Markers for Public Health Emergencies**

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**Biomedical Advanced Research and Development Authority**

**April 19, 2012**  
**44<sup>th</sup> Annual Oak Ridge Conference**  
***Emerging Technologies for 21<sup>st</sup> Century Diagnostics***  
**San José, California**

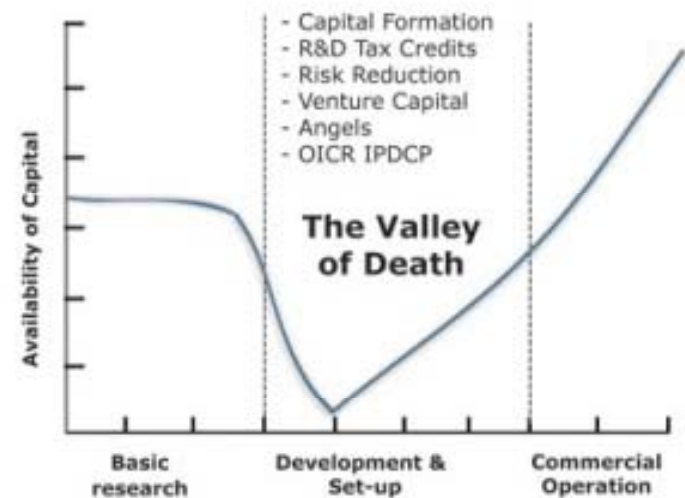


# Biomedical Advanced Research and Development Authority (BARDA) Mission



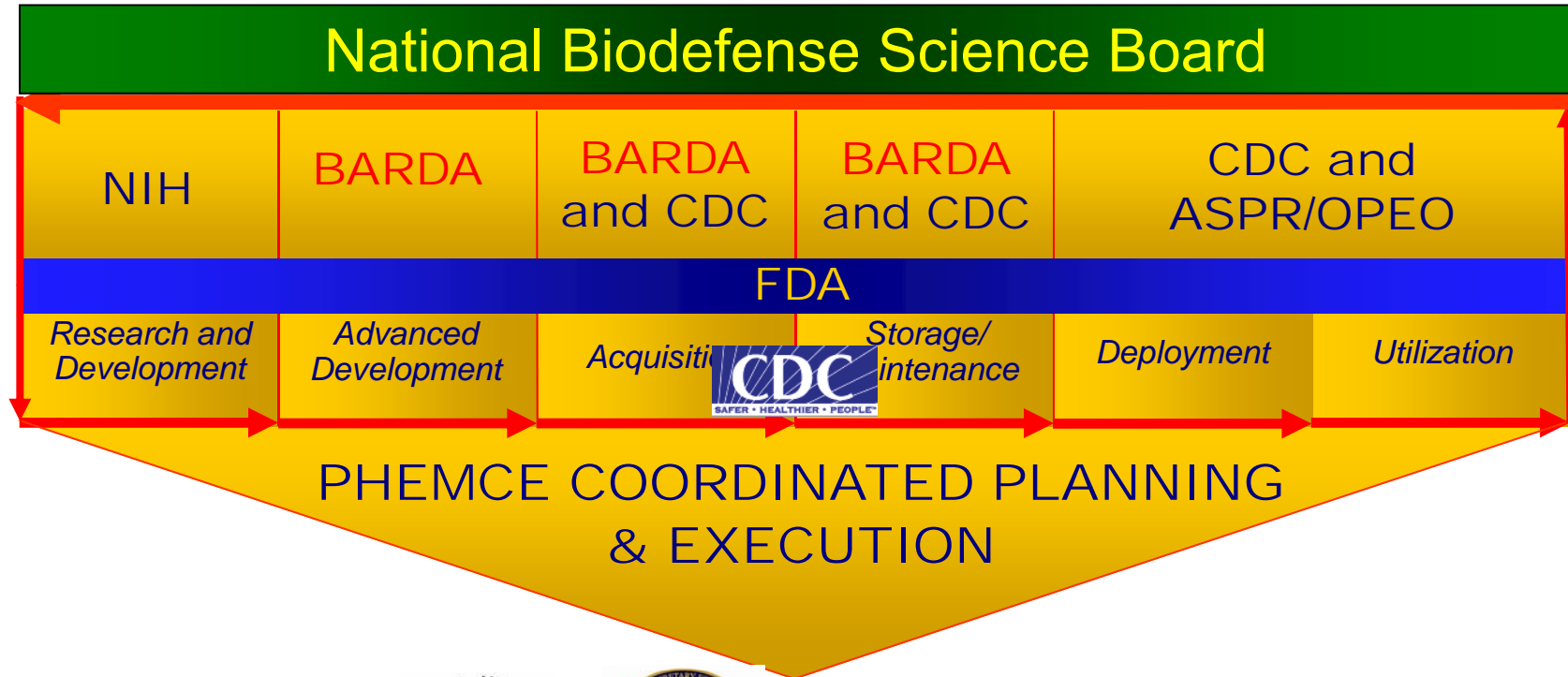
## ***Ensuring the availability of medical countermeasures and diagnostic tools to address public health emergencies***

- Three threat areas: Chem/Bio/Rad/Nuc, Pandemic Influenza, Emerging Infectious Diseases
- Comprehensive portfolio approach to development and acquisition of products
- Unique niche in USG biomedical R&D
  - Bridge the “Valley of Death”
  - Mid- to late-stage product development
  - Work with industry to progress product candidates through the pipeline





# Public Health Emergency Medical Countermeasures Enterprise



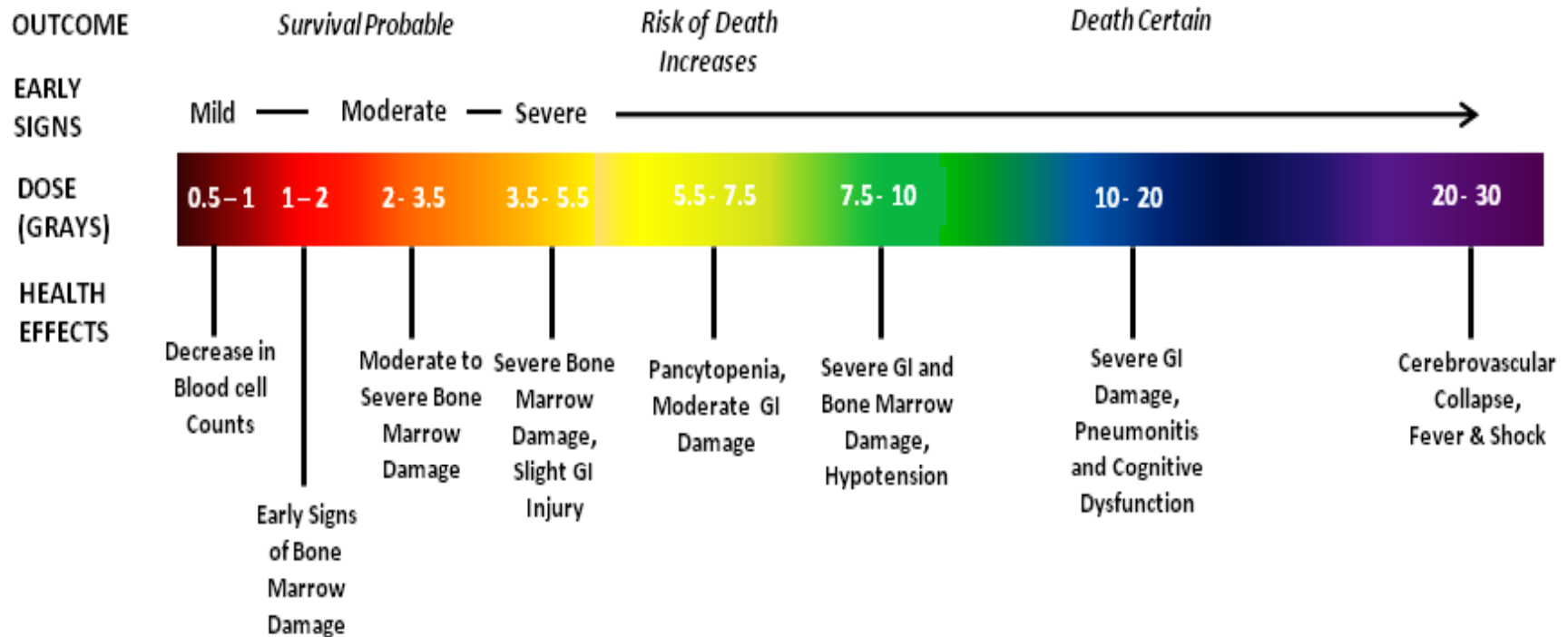
Ex Officio Members:



*ASPR: Resilient People. Healthy Communities. A Nation Prepared.*

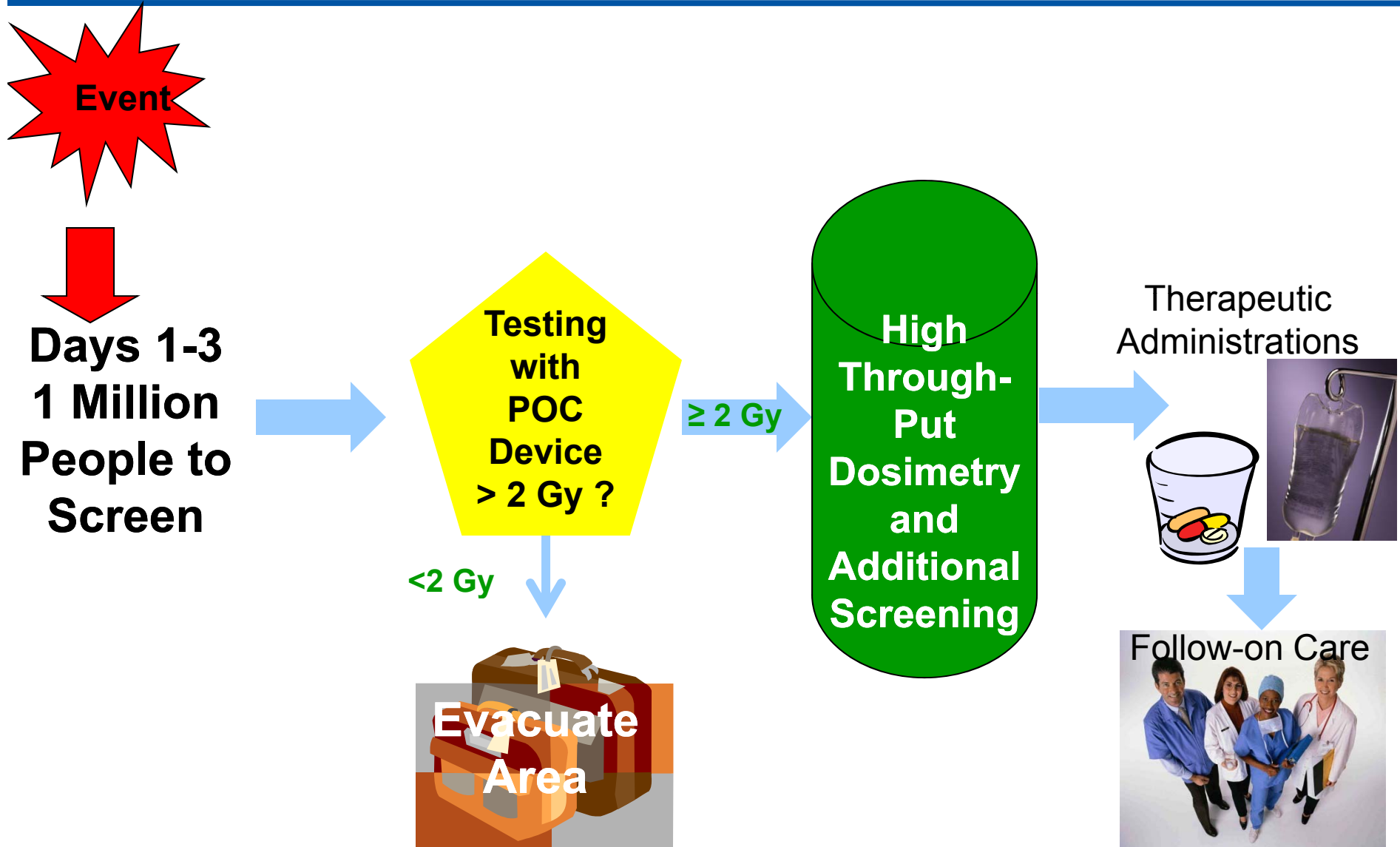


# Spectrum of Radiation Health Effects





# Biodosimetry Concepts of Operation





# Current Biodosimetry Capabilities



Radiation Dose Assessment Tools	Deficiency
Victim's location relative to incident	Provides only a rough estimate
Signs and Symptoms	↑ false positive rate
Blood Cell Counts	Requires two blood draws separated by days and the ability to connect data
Laboratory-based Cytogenetics	Dicentric Chromosome Assay takes 2-3 days for result and few laboratories offer the test



# Current BARDA Biodosimetry Program



Contractors	Point of Care (POC) or High Through-put (HT)	TECHNOLOGY					Electron Paramagnetic Resonance
		Protein	DNA	mRNA	Volatile Organic Compounds	Ocular	
Contractor 1	Both	✓					
Contractor 2	HT			✓			
Contractor 3	POC	✓					
Contractor 4	HT		✓				
Contractor 5	HT			✓			
Contractor 6	POC					✓	
Contractor 7	POC				✓		
Contractor 8	POC						✓

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## Example of One Contractor's Approach

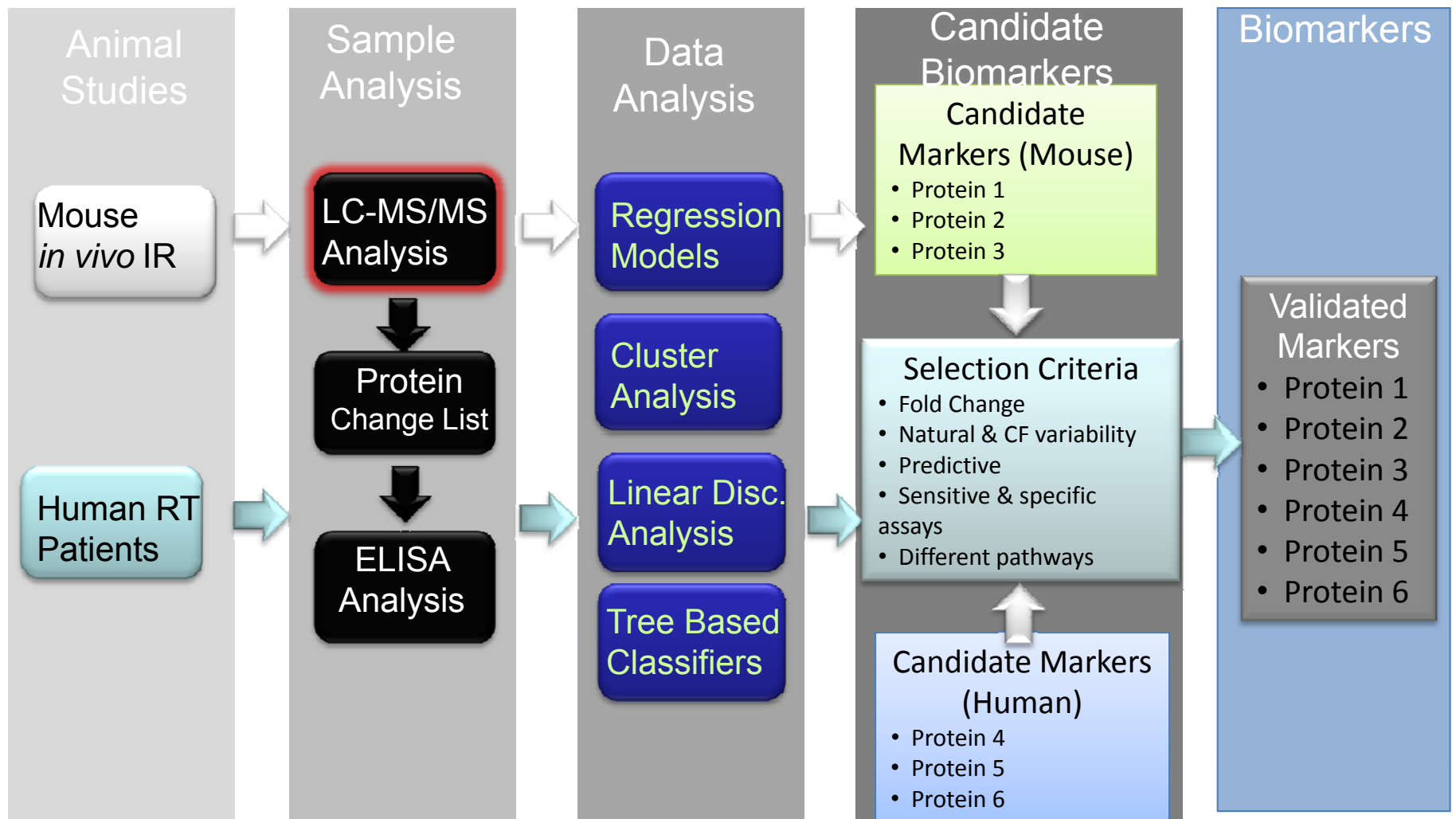


# Using Mass Spectrometry as a Tool for Identifying Biodosimetry Markers of Interest



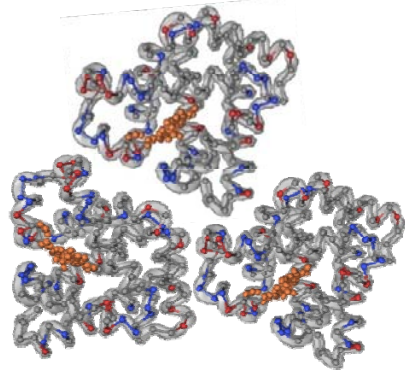


# Example Project Methodology



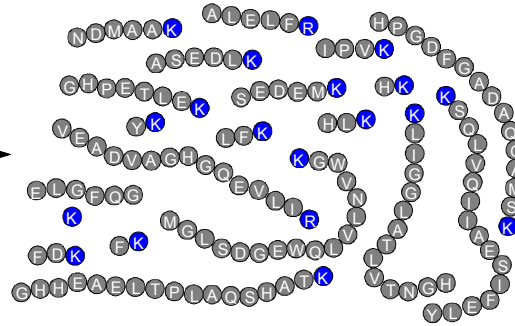


# Example LC-MS/MS Approach for Protein Identification



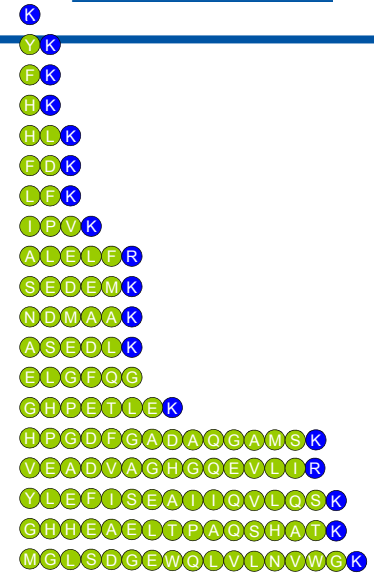
Complex Mixture of Proteins

**digestion**

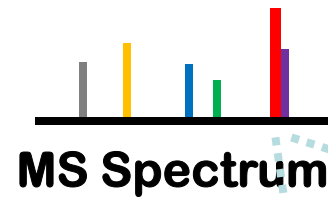


Peptide digest

**Separation by HPLC**



**On-line analysis by OrbiTrap**



MS Spectrum



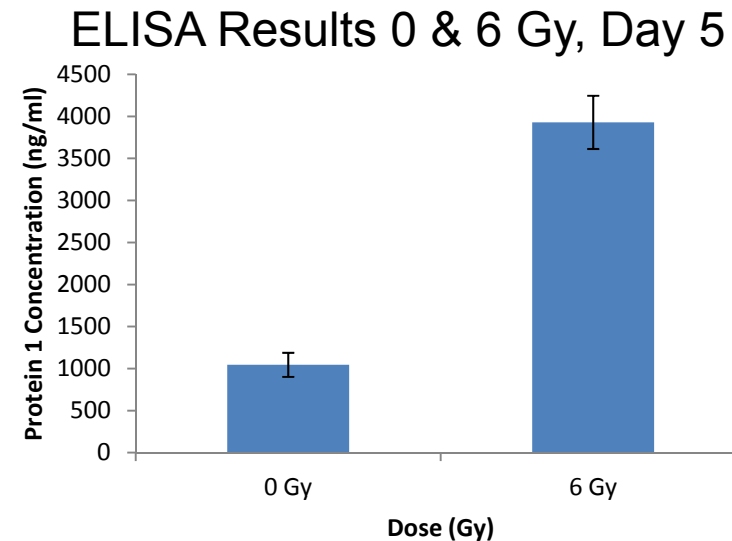
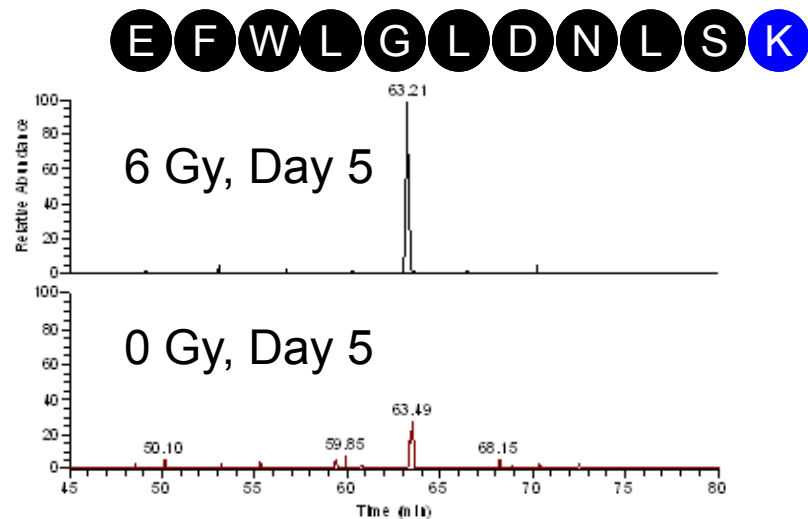
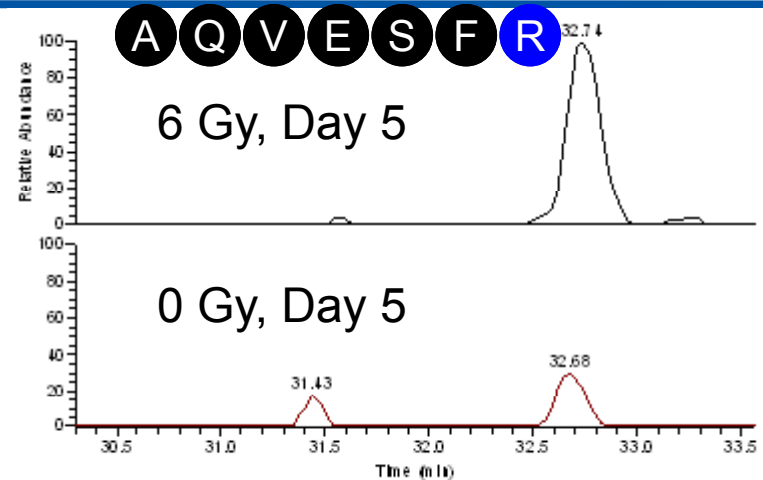
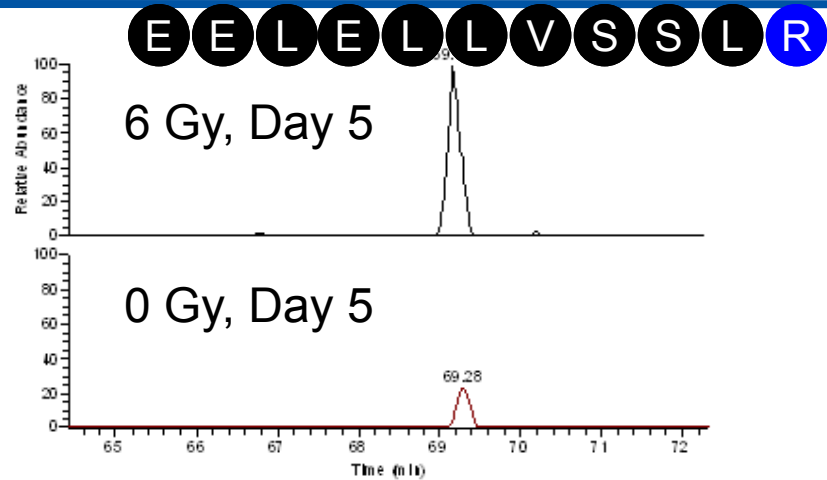
**Database search  
Protein ID**



**MS/MS Spectrum**



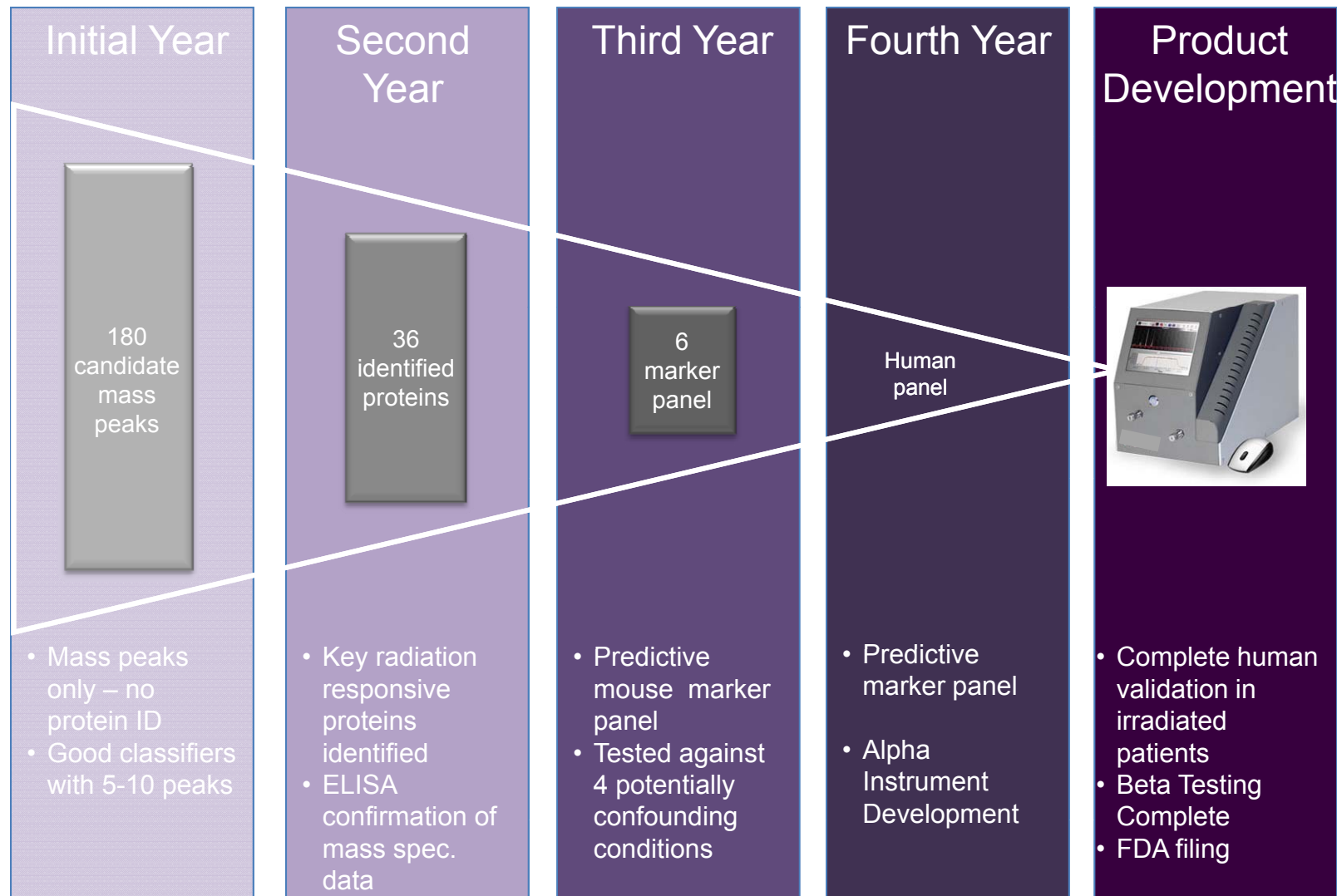
# Protein 1 LC-MS/MS Results



Demonstrated Correlation Between LC-MS/MS Analysis on Pooled Samples and ELISA Analysis on Individual Samples

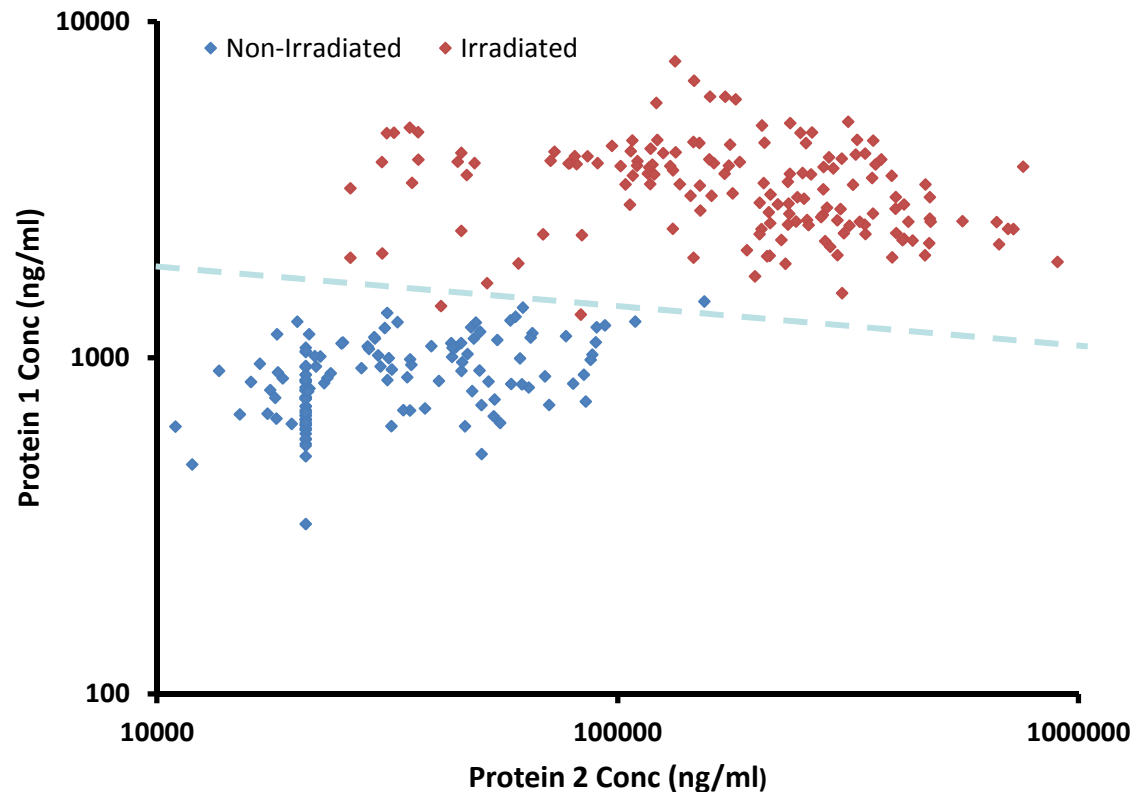


# Example Project Plan – Focused on Product Development





# Scatter Plots to Separate Irradiated versus Non-irradiated Mice

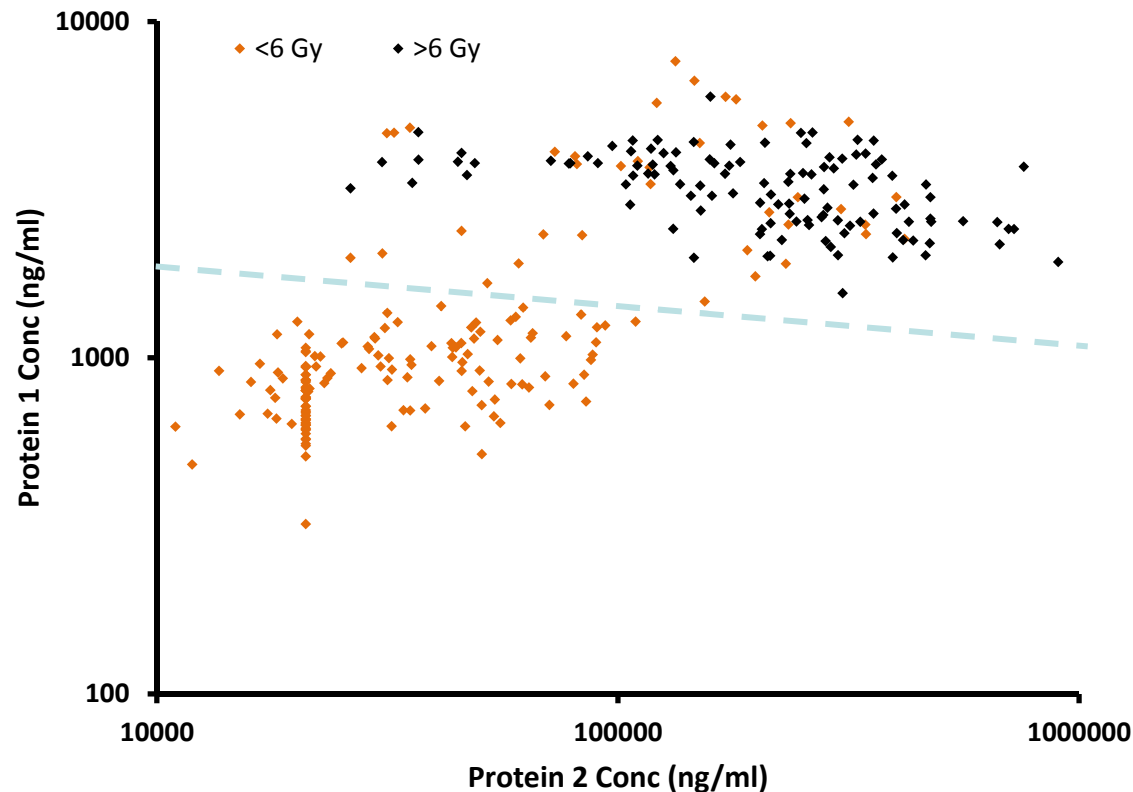


- 2 false negatives out of 298 samples (0.67% Fn)
- 1 false positive out of 298 samples (0.34% Fp)

Protein 1 alone results in 99% Correct classification of non-irradiated from irradiated



# Additional Proteins are Necessary for < and $\geq$ 6 Gy in Mice



- Scatter plot of Protein 1 and Protein 2 results in 87% correct classification <6 Gy from  $\geq$  6 Gy
- 0 false negatives out of 298 samples (0.0% Fn)
- 38 false positive out of 298 samples (12.8% Fp)
- All false positive samples are 4 Gy classified as  $\geq$  6Gy

Additional Proteins and More Sophisticated Models are Needed to Separate  
 $\geq$  and < 6 Gy

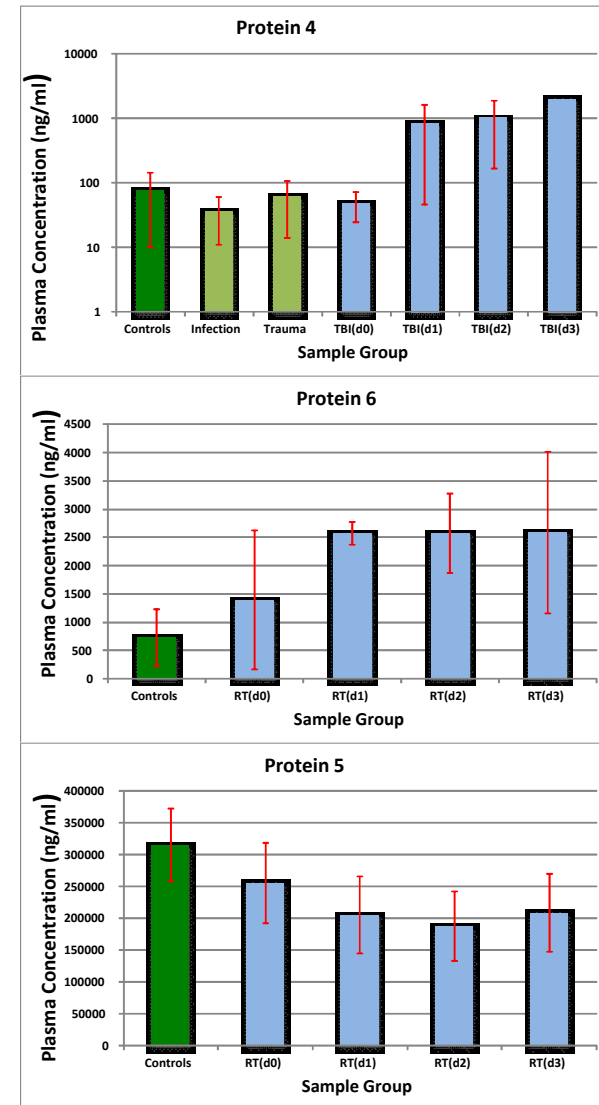
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# Human Radiotherapy Patient Panel

- The three most important radiation responsive proteins in the panel include
  - Protein 4 (up-regulated)
  - Protein 5 (down-regulated)
  - Protein 6 (up-regulated in TBI patients)
- Two of these proteins are used in the mouse panel
- Tree classifiers using these three proteins can distinguish irradiated patients from controls with 96% accuracy

Data provides positive indication that plasma protein biodosimetry is feasible in humans – requires NHP study to expand panel and confirm





# Example Summary and Conclusions



- **Using LC-MS/MS techniques identified 180 mass peaks for interigation**
- **The LC-MS/MS Approach identified 36 Radiation-responsive proteins in the mouse model**
  - A subset of these proteins have been confirmed in mouse plasma using ELISA
- **Using 6 proteins from this panel correctly classifies all large-scale mouse data sets**
  - Two groups ( $< 6$  Gy or  $\geq 6$  Gy) with low false negative (FN) and false positive (FP) rates
- **The LC-MS/MS Approach identified 8 Radiation-responsive proteins in human radiotherapy patient samples**
  - Preliminary panels of 3 proteins can easily distinguish between irradiated patients and controls





# Areas of Interest for Contractors in Diagnostic Development



A pre-proposal conference is planned to on Thursday, May 3, 2012, from 2:00pm to 4:00pm provide details regarding the following potential Broad Agency Announcements (BAAs):

- BARDA-CBRN-BAA-12-100-SOL-00011, entitled “Advanced Research and Development of Chemical, Biological, Radiological, and Nuclear Medical Countermeasures”;
- BARDA-SST-BAA-12-100-SOL-00013, entitled “Science and Technology Platforms Applied to Medical Countermeasure (MCM) Development”, and;
- BARDA-FLU-BAA-12-100-00018, entitled “Advanced Development of Medical Countermeasures for Pandemic Influenza”.

To register for the conference, please email the name of your company, as well as the names and \*citizenship of registrants to [AMCG-Opportunities@hhs.gov](mailto:AMCG-Opportunities@hhs.gov) by 3:00 PM EDT Tuesday, May 1, 2012.



# Interfacing with BARDA



- [www.medicalcountermeasures.gov](http://www.medicalcountermeasures.gov)
  - Portal to BARDA
  - Register, request a meeting
  - Tech Watch presentation
- [www.fedbizopps.gov](http://www.fedbizopps.gov)
  - Official announcements and detailed information about all government contract solicitations
  - See presolicitation announcement:  
<https://www.fbo.gov/index?s=opportunity&mode=form&id=495eaf1a1eaed051a0223ffd180b5cbe&tab=core&cvview=1>