Radiation Countermeasures: The Need for Predictive Biomarkers

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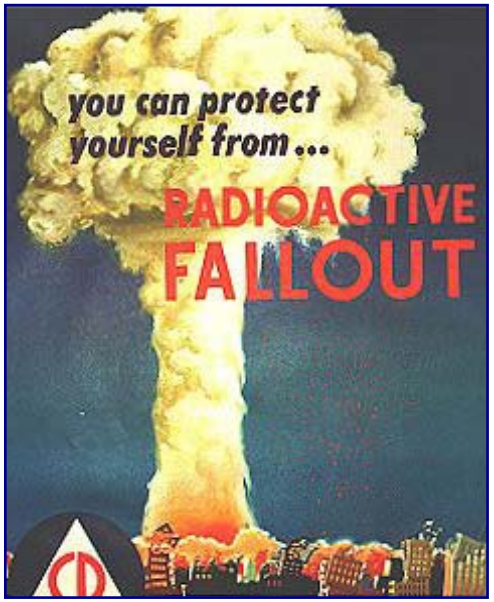
Richard J. Hatchett, MD
Associate Director for Radiation Countermeasures
Research and Emergency Preparedness
NIAID

Outline

- Threats
- Medical Countermeasures Enterprise
- Program
- Predictive Biomarkers

Radiological/Nuclear Threats

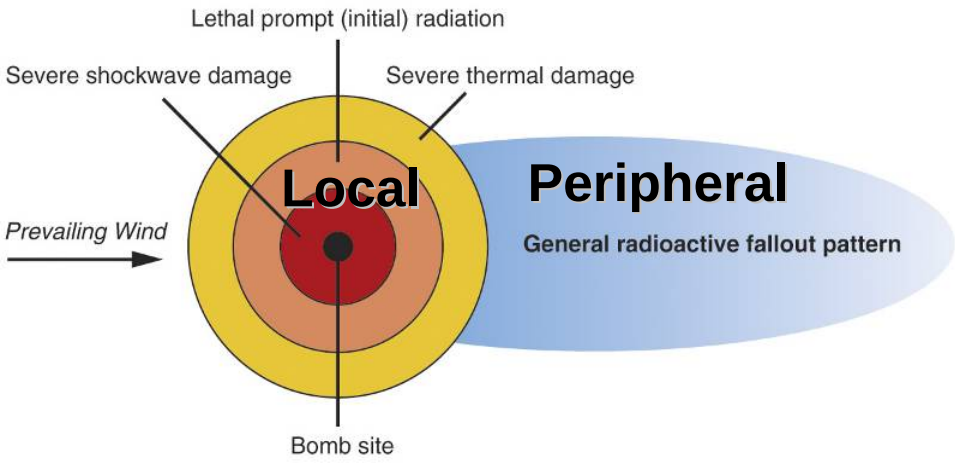
- Nuclear Detonation
- Radiologic dispersive devices (“dirty bombs”)
- Industrial and shipping accidents
  - Power plant releases
  - Food and medical irradiators
  - Sealed sources



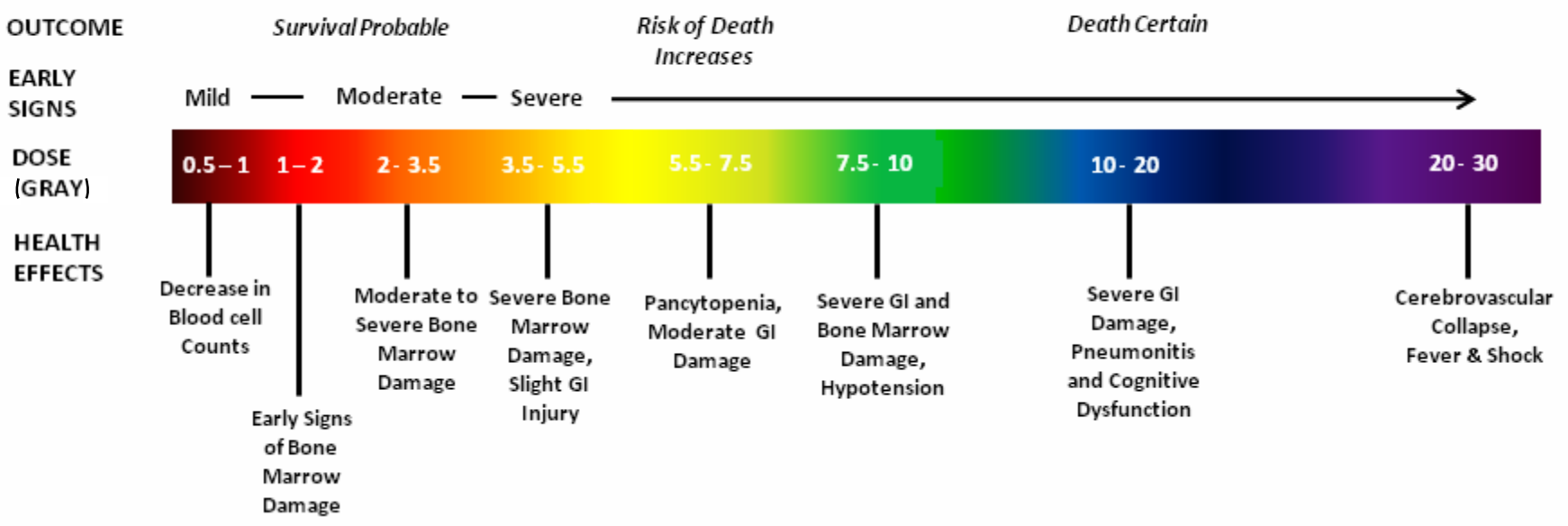
Improvised Nuclear Device (IND)

- If a 10 KT IND is detonated in a major U.S. city hundreds of thousands of victims will need treatment for effects of radiation exposure – and many times that many may request evaluation (“worried well”).

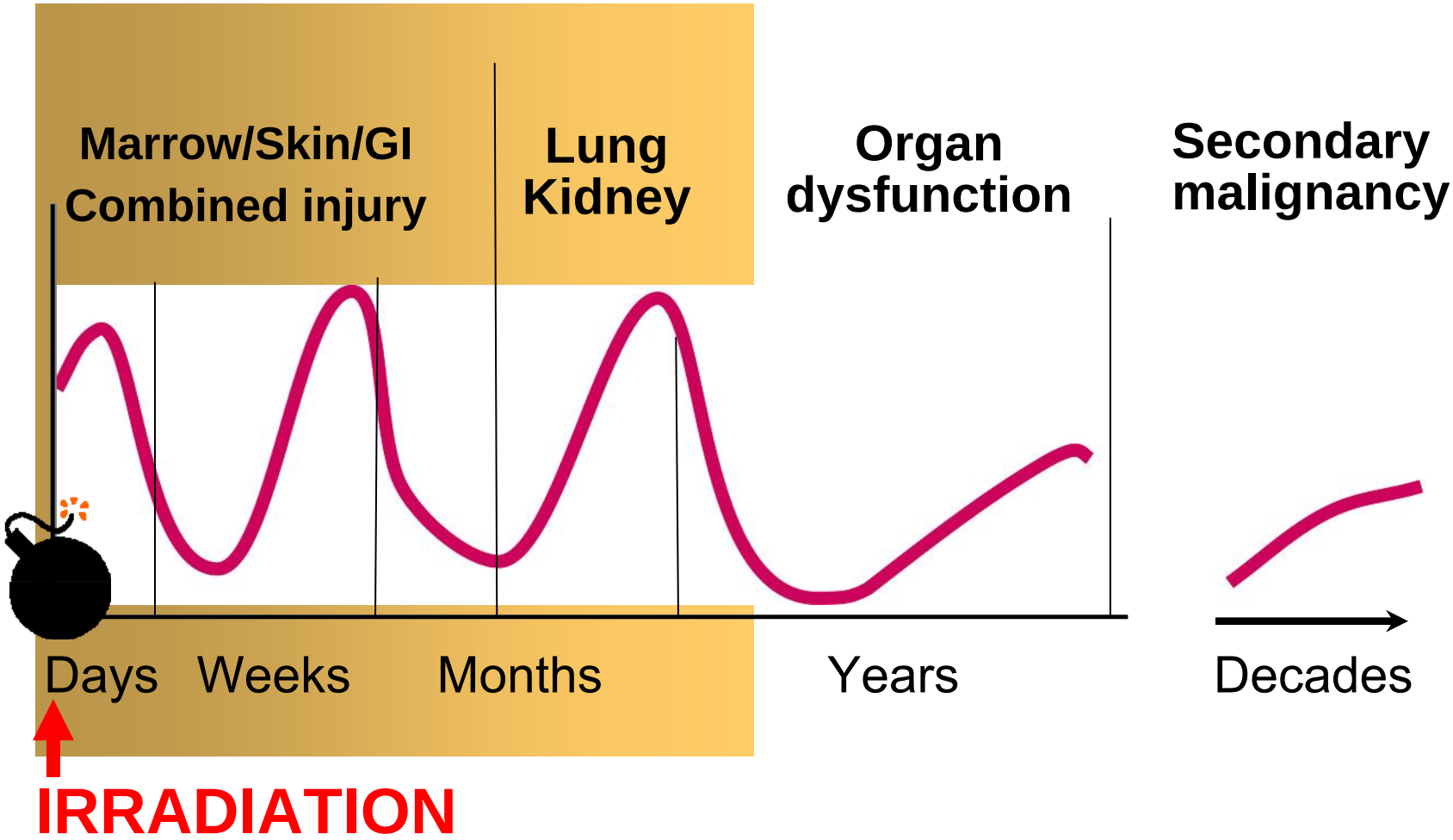
- 10 KT IND scenario
  - Medical consequences
    - Local Primary Event
      - ❖ Prompt radiation
      - ❖ Burn and blast
      - ❖ Combined injury
    - Peripheral Secondary Events
      - ❖ Fallout radiation



Spectrum of Radiation Health Effects



Syndromes Manifest Over Time



Radiation Countermeasure Mission Space

- ARS/DEARE
  - Hematopoietic ARS:
    - Neutropenia
    - Thrombocytopenia
    - Anemia
    - Lymphopenia
  - GI ARS
  - CNS Injury
  - Lung Injury
  - Kidney Injury
- Cutaneous Radiation Syndrome
- Combined Injury
- Radionuclide Threats
  - Co-60
  - Cs-137
  - Sr-90
  - I-131
  - Ir-192
  - Po-210
  - Ur-235
  - Pu-239
  - Am-241
- Carcinogenesis
- Cataractogenesis

HHS Public Health Preparedness: Complementary Roles

CDC

- Surveillance and Detection
- Train Local Response Teams
- Maintain Vaccine/Antimicrobial Stockpiles

NIH

- Conduct Basic Research
- Develop Medical Interventions
- Develop Research Infrastructure

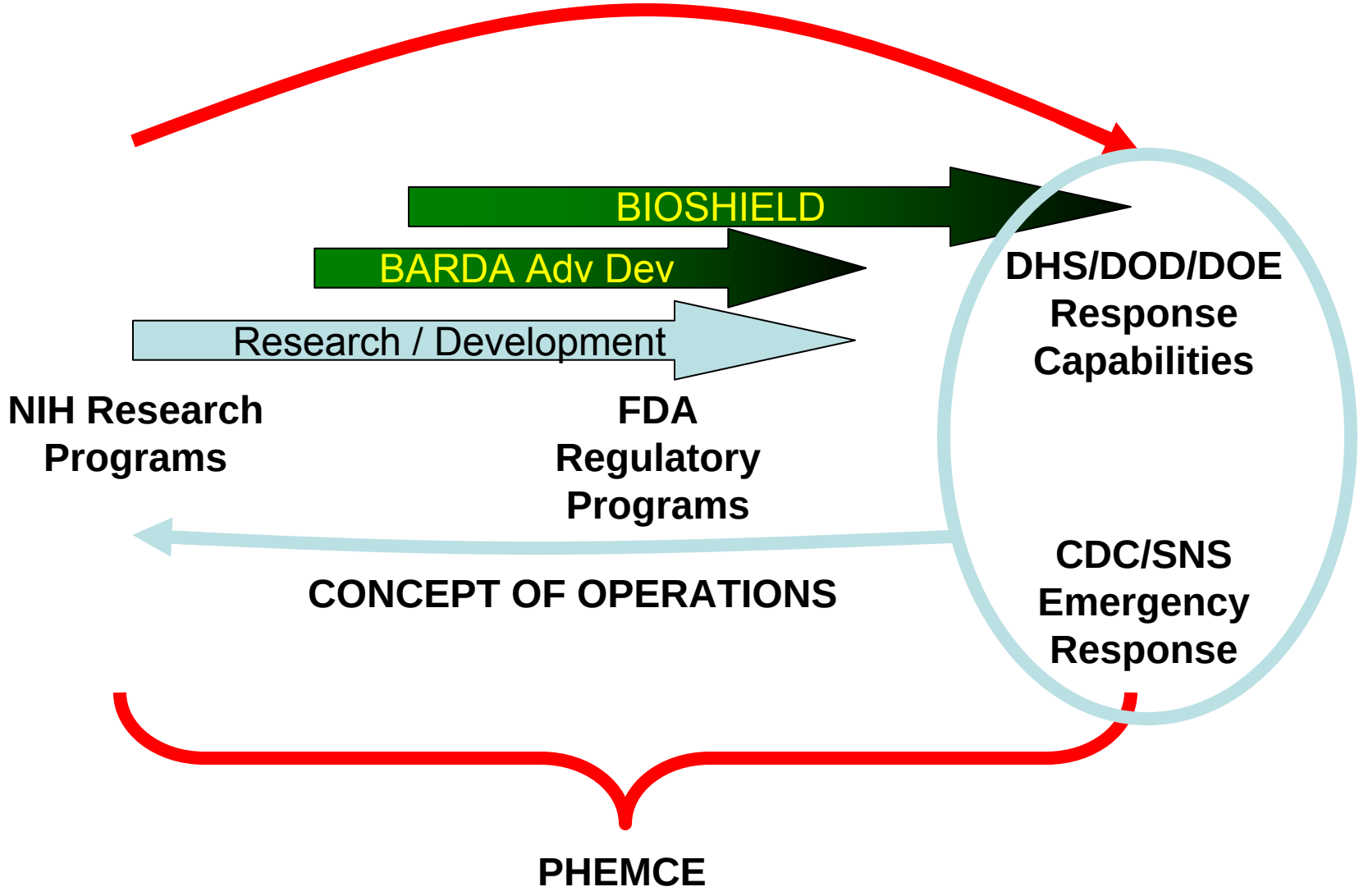
FDA

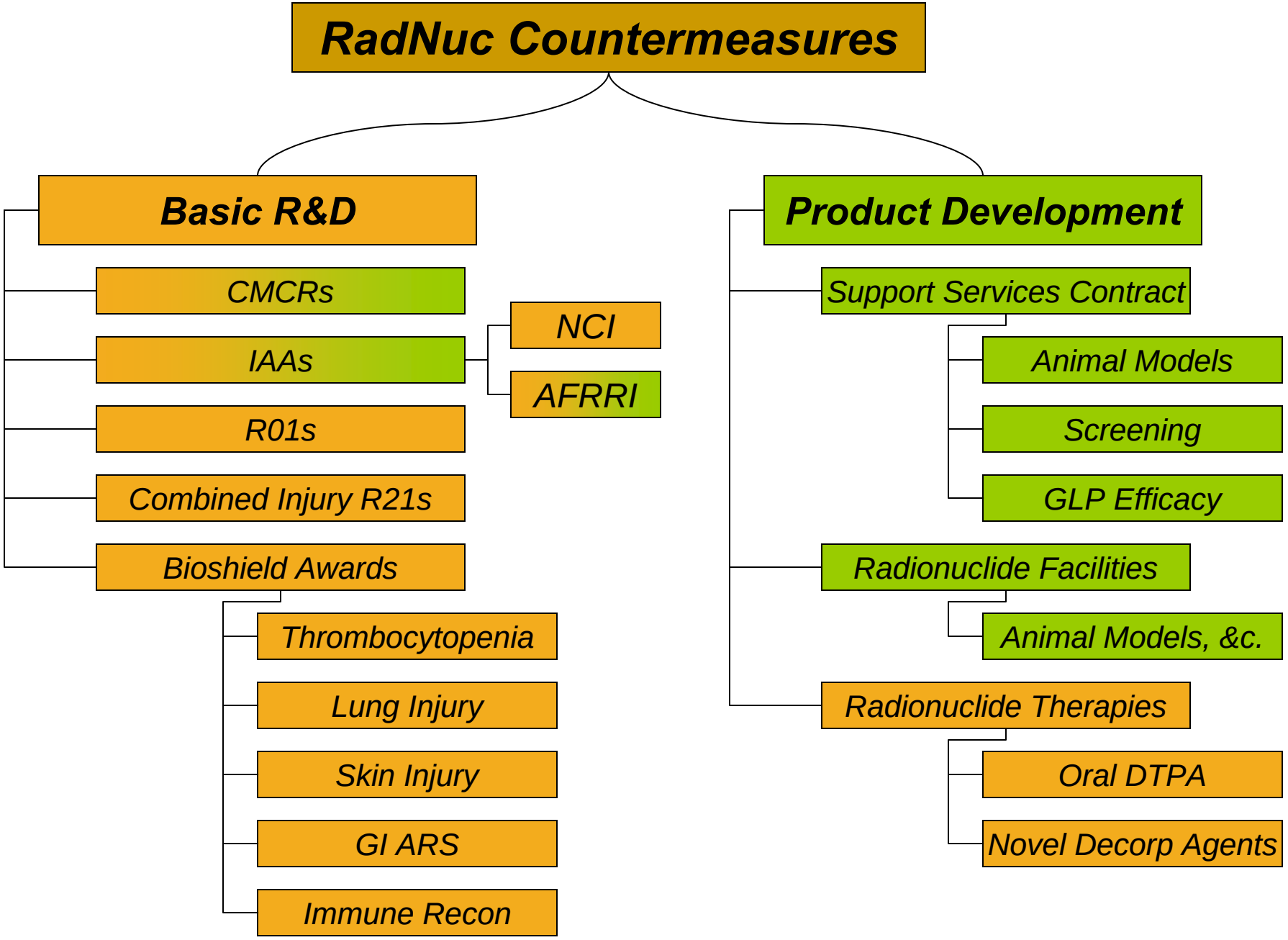
- Regulatory Approval
  - Vaccines
  - Therapeutics
  - Diagnostics

ASPR

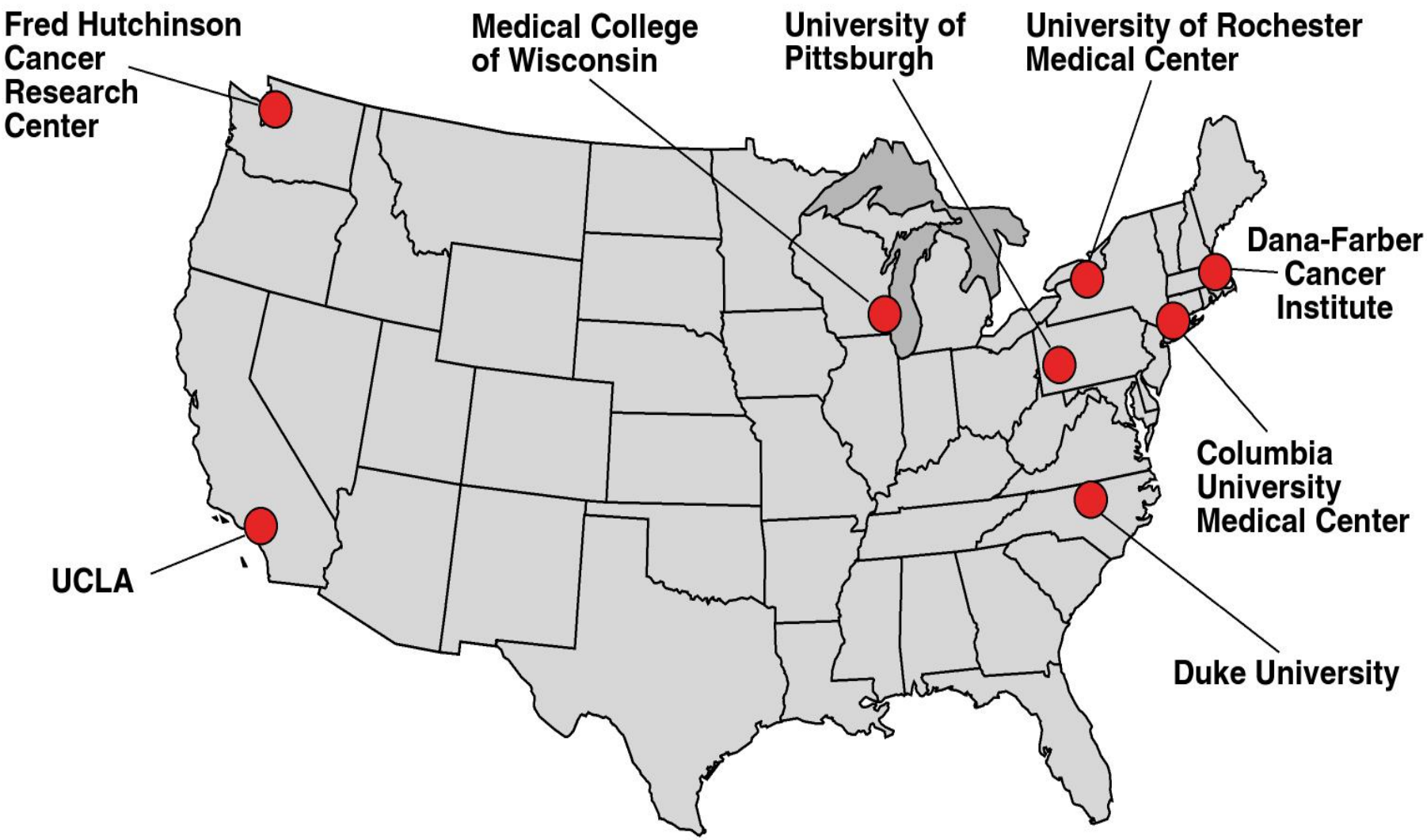
- HHS-Wide Coordination of Emergency Preparedness Activities

The Big Picture




RadNuc Countermeasures
Basic R&D
CMCRs
IAAs
NCI
AFRRI
R01s
Combined Injury R21s
Bioshield Awards
Thrombocytopenia
Lung Injury
Skin Injury
GI ARS
Immune Recon
Product Development
Support Services Contract
Animal Models
Screening
GLP Efficacy
Radionuclide Facilities
Animal Models, &c.
Radionuclide Therapies
Oral DTPA
Novel Decorp Agents

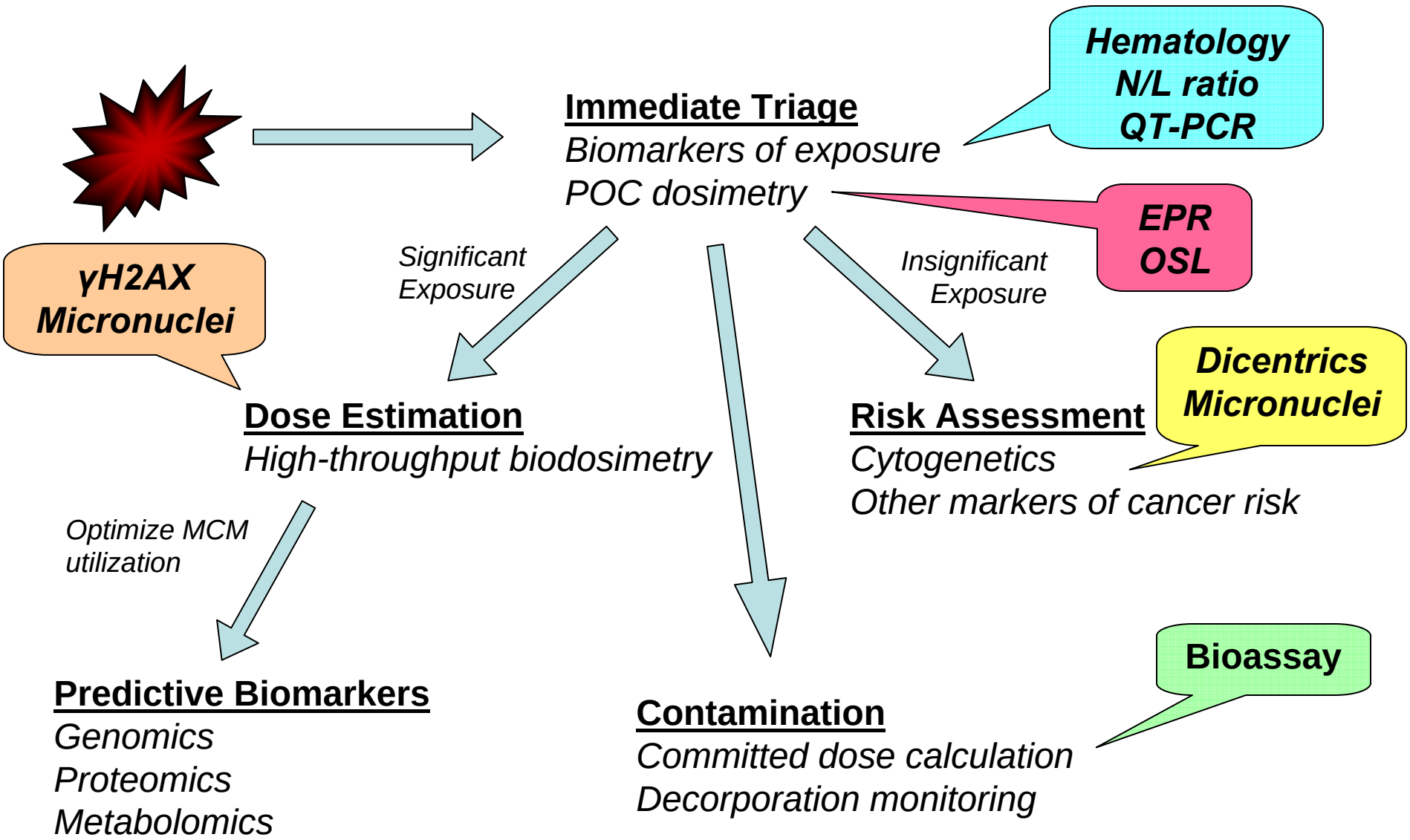
Centers for Medical Countermeasures Against Radiation ($28.0M in FY08)



Diagnostics Program

- **Technical Requirements of a Diagnostics Architecture**
  - — Capability for rapid screening of large populations
  - — Sufficiently accurate to guide clinical decision-making
  - — Sufficiently flexible to address different needs for different types
- **Medical / Operational Impact**
  - — Optimization of resource allocation
  - — Identification of patients requiring urgent medical assessment
  - — Reassurance for anxious individuals
  - — Improved risk assessment for delayed or late effects of radiation exposure
  - — Monitoring of therapy (bioassays)

Diagnostics architecture



NIAID Rad/Nuc Website

http://www3.niaid.nih.gov/research/topics/radnuc

Questions?

EPR Dosimetry

- **Current Status: in vivo calibrations for doses 100- 3000 cGy with a SEM 50 cGy completed**
- **Precision: In radiotherapy patients EPR measurements are within 10% of calculated dose**
- **Time to dose estimate: 10 min**
- **Signal stability: millennia**
- **Current research**
  - **Improving precision of dose estimates**
  - **Designing field-portable instruments**
  - **Development of capability to estimate absorbed dose from anterior teeth**
  - **Development of technique for measuring signal in fingernails (X-band EPR)**

EPR Dosimetry

Technical challenges:

- **Developing transportable magnets**
  - **Intraoral magnet**
  - **Helmet magnet**
  - **Flat permanent magnet**
- **Developing field-deployable electronics sufficiently robust for use by first-responders**
  - **Dartmouth instrument lacks necessary stability and ruggedness**
  - **Software for data acquisition and processing requires modification**
- **Validation under field conditions**
  - **Initial simulation planned for early 2008**
- **Understanding effects of partial body irradiation**

Time to fieldable assay:

- **Scientifically, probably 3-5 years, but likely longer to obtain full FDA approval**

Automated Cytogenetic Imaging

Fully-automated ultra high-throughput, robotics controlled image acquisition systems to analyze:

- **Micronuclei : 0.5-5 Gy**
  - **Lymphocytes: 2-3 days**
  - **Reticulocytes: 16 hours**
  - **Exfoliated buccal and urinary bladder cells: same day**
- **γ-H2AX foci: 0.1- >10Gy**
  - **Lymphocytes: 3 hours**

Automated Cytogenetic Imaging 1

- **Current Status (Phase I device – micronuclei only)**
  - — System design complete
  - — Biological assay optimized for high-throughput handling
  - — Subsystems designed; assembly and integration in progress
  - — Pre-IDE meeting with FDA took place in October 2007
  - — Scheduled for clinical trials in 2008, expected to be complete by 2009
- **Useful dose range**
  - — Micronuclei: 0.5-5 Gy
- **Time to dose estimate: 70 hours after samples received**
- **Signal stability: Years**
- **Technical challenges: Primary technical challenge is logistics of sample collection**

Automated Cytogenetic Imaging 1

- **Current Status (Phase II device – micronuclei and γ-H2AX foci)**
  - — System is in design stage (system will maintain duplicate many components of Phase I device, so development time is reduced)
  - — Optimization of γ-H2AX assay for high-throughput handling is underway
- **Useful dose range**
  - — Micronuclei: 0.5-5 Gy
  - — γ-H2AX: 0.1-10 Gy
- **Time to dose estimate**
  - — 3 hours for samples obtained up to 36 hours post-irradiation
  - — 70 hours for samples obtained thereafter
- **Signal stability: ~36 hours for γ-H2AX, years for micronuclei**
- **Technical challenges**
  - — Logistics of sample collection
  - — Increasing the throughput of cell harvesting – requires parallel processing
- **Timeline: Scientifically complete by 2010; field deployable device within 3-5 years**

Automated Cytogenetic Imaging 2

- **Current Status (micronucleated reticulocytes)**
  - — Baseline data for healthy adults obtained
  - — Initial studies in patients receiving partial body irradiation completed
  - — Human 3-D marrow culture being optimized for determination of human dose-response curve
  - — Still need to understand kinetics of appearance/disappearance of MN-RET in humans; determine useful dose range in humans; determine threshold doses for signal in humans
- **Useful dose range: 0.125-3 Gy**
- **Time to dose estimate**
  - — 16 hours (primarily for fixation; analysis takes 3 minutes)
- **Signal stability: ~48 hours**
- **Technical challenges**
  - — Saturation of response above a certain threshold
  - — Short-lived signal
- **Timeline: Scientifically complete in <3 years; fieldable assay in 3-5 years**

Gene Expression Signature 1

- **Current Status**
  - — **Identified a single 74-gene signature that distinguishes radiation doses between 0 and 8 Gy, separating pre- and post- exposure samples from cancer patients undergoing TBI**
  - — **Investigating possibility of differential response to radiation in smokers**
  - — **Preliminary analysis of a variety of inflammatory responses indicate limited overlap with non-radiation conditions.**
- **Useful dose range: 0 – 8 Gy**
- **Time to dose estimate: Hours**
- **Signal stability: 6 – 48 hours**
- **Technical challenges**
  - — **Extrapolation between ex vivo and in vivo results.**
  - — **Population variability and other potential confounding factors**
  - — **Understanding effects of partial body irradiation**
  - — **Exploitation of gene expression data to reveal individual differences in susceptibility and radiation injury**
- **Timeline: Scientifically complete in 3-5 years; fieldable assay in >5 years**

Gene Expression Signature 1 Device

- **Current Status**
  - 16-gene panel selected for qNPA
  - Integrated sample preparation front module and microarray detection module design and prototype completed
  - Small batch production of cartridges, now undergoing characterization and QC validation
  - Blood treatment protocols under development
  - Instrumentation recently redesigned and electronic circuitry transferred to microchip based electronics
- **Technical challenges**
  - Optimization of blood protocol
  - Minimizing population and other sources of variability
  - Validating the signatures
- **Timeline: Scientifically complete in 3-5 years; fieldable assay in >5 years**

Gene Expression Signature 2

- **Current Status**
  - — Refinement of 25-gene human peripheral blood radiation signature
  - — Development of a portable qRT-PCR radiation assay
  - — Initiation of large, prospective validation trial of human radiation signature in health individuals and irradiated patients
  - — Evaluation of time, gender, and genotype effects underway in mice
- **Useful dose range: 0.5, 2, 10 Gy**
- **Time to dose estimate: 24 hours**
- **Signal stability: Unique signals identified at 6 hr, 24 hr, 7 d**
- **Technical challenges**
  - — Development of a microarray-based assay with 24-hour turnaround
  - — Development of a fixed template of 25 radiation response genes v. a computer-based algorithm for analysis of samples
  - — Development of a portable qRT-PCR radiation assay
- **Timeline: Scientifically complete in 3-5 years; fieldable assay in >5 years**

Metabolomic Biomarkers

- **Current Status**
  - — **Consistent metabolomic markers identified in urine of mice**
  - — **Extending technique to saliva and serum**
  - — **Currently processing urine, blood, and saliva samples from patients receiving 1.5 Gy TBI**
- **Useful dose range: 3, 8 Gy**
- **Time to dose estimate: 24 hours**
- **Signal stability: Unknown**
- **Technical challenges**
  - — **Combination of differential ion-mobility spectrometry with a low-cost, portable, miniature mass spectrometer is required; current devices unsuitable**
  - — **Improvements in sample handling and electrospray procedures to improve stability and simplicity**
  - — **Detailed comparison of DMS-MS and LC-MS results needs to be performed**
- **Timeline: Scientifically complete in >5 years; fieldable assay in >5 years (within 3 years of identification and validation of target biomarkers)**

Proteomic Biomarkers

- **Current Status**
  - — Envision blood- or urine-based protein diagnostics similar to UPT
  - — 5 proteins (of 160 studied) with complimentary dosimetry profiles selected
  - — Rabbit monoclonal antibodies being developed
- **Useful dose range: 9 Gy**
- **Time to dose estimate: Minutes**
- **Signal stability: 24 hours**
- **Technical challenges**
  - — Generation of rabbit monoclonal cell lines
  - — Development of ELISA assays
  - — Generation of protein standards to normalize assays
  - — Validation of assays
  - — Conversion of assays to fieldable test strips
- **Timeline: Scientifically complete in 3-5 years; fieldable assay in >5 years**

qRT-PCR Dosimetry

- **Current Status**
  - 25 potential genes with radiation dose-dependent responses selected
  - *In vitro* validation of qRT-PCR assays for these genes in progress
  - Preliminary *in vivo* validation studies of candidate genes in progress
- **Useful dose range: 0.15 – 6 Gy**
- **Time to dose estimate: 6 hours**
- **Signal stability: Up to 24 hours demonstrated**
- **Technical challenges**
  - Development of large-scale whole-blood RNA extraction systems for use in mass casualty setting
  - Development of robotic systems to increase throughput (current technology may permit screening of 200-500 individuals every 5-6 hours)
  - Development of high-throughput qRT-PCR assays
- **Timeline: Scientifically complete in 3-5 years; fieldable assay in >5 years**

Hematopoietic Syndrome

- **Neutropenia**
  - — **Filgrastim** — **Licensed**
  - — **Pegfilgrastim** — **Licensed**
  - — **Sargramostim** — **Licensed**
  - — **Maxy-G34** — **Phase II Clinical Trial**
  - — **Human Growth Hormone** — **Licensed**
  - — **EA-230** — **Phase Ib Clinical Trial**
  - — **Endothelial Cell Transplantation** — **Preclinical**
  - — **Myeloid Progenitor Cell Transplantation** — **Preclinical**

Hematopoietic Syndrome (cont.)

- **Thrombocytopenia**
  - **AMG 531** — **Phase III Clinical Trial**
  - **AKR 501** — **Phase II Clinical Trial**
  - **Peg-TPOmp** — **Phase III Clinical Trial**
  - **Fab59** — **Preclinical, on hold**
  - **TPIAO** — **Licensed in China**
  - **NIP-004** — **Preclinical**

Gastrointestinal Syndrome

Protectan (CLBL 502)	Preclinical
FGF-20	Phase II Clinical Trial
R-spondin 1	Preclinical
SOM230	Phase II Clinical Trial
Mesenchymal Stem Cells	Phase III Clinical Trial

Cutaneous Radiation Syndrome

- **Ulceration/Necrosis**
  - — **Curcumin** — **Phase I/II Clinical Trial**
  - — **Eseculentoside A (EsA)** — **Preclinical**
  - — **Celecoxib** — **Licensed**
  - — **Mesenchymal Stem Cells** — **Phase III Clinical Trial**
- **Fibrosis**
  - — **Pentoxifylline (+ Vitamin E)** — **Licensed**
  - — **MnSOD** — **Phase I/II Clinical Trial**

Radiation-induced Lung Injury

- **Pneumonitis**
  - — Genestein — Nutraceutical
  - — KGF (palifermin) — Licensed
  - — Pentoxifylline — Licensed
  - — AEOL 10150 — Phase Ib Clinical Trial
  - — EUK-189 — Preclinical
  - — MnSOD Gene Therapy — Preclinical
- **Fibrosis**
  - — KGF (palifermin) — Licensed
  - — Pirfenidone — Phase III Clinical Trial
  - — AEOL 10150 — Phase Ib Clinical Trial
  - — Imatinib — Licensed

New Radionuclide Therapies

- **Oral DTPA**
  - — Prodrug — Preclinical
  - — Nanoparticles — Preclinical
  - — Enhanced Absorption — Preclinical
- **Novel Decorporating Agents**
  - — Biomaterials — Preclinical
  - — Nanoporous Sorbants — Preclinical
  - — Biomimetics (HOPO cmpounds) — Preclinical
  - — Desferrithiocin Analogues — Preclinical
  - — Amphipathic Oral Chelators — Preclinical

Strategy

Program Strategy

- Top priority: Developing treatments and diagnostics for ARS
- Focus on product development
  - Products with viable commercial markets
  - Products in late stages of development
  - Partners encouraged to identify commercial markets for their compounds and devices
- Emphasis on collaboration, transparency, and economy
  - Interagency: close coordination with RadNuc partners
  - NIH: improving interdisciplinary coordination (with, e.g., medical and radiation oncology, innate immunity, inflammation, biomedical imaging, mucosal biology programs)
  - RadNuc Program: collaborations and coordinated use of resources across CMCRs and between grant and contract programs

Example: Intestinal Side Effects of RT What a GI Countermeasure Can Do for Us

- Acute Intestinal Radiation Toxicity
  - >300,000 patients at risk per year (US)
  - >80% incidence of acute GI toxicity
  - Up to 20% treatment alteration due to acute GI toxicity
- Chronic Intestinal Radiation Toxicity
  - Up to 15% incidence of severe (grade 3-4) GI toxicity
  - 60-90% incidence of chronic GI dysfunction
  - >2.5 million cancer survivors with chronic radiation-induced GI dysfunction

Radiation Effects Research Foundation

- Successor to Atomic Bomb Casualty Commission (estab.1947)
- Facilities in Hiroshima and Nagasaki, Japan
- Jointly funded and managed by US and Japan
- Rich history of studying A-bomb survi
  - — Life Span Study Sample (120,000)
  - — Adult Health Study Sample (23,000)
  - — In Utero Sample (3600)
  - — F1 Sample (88,000)
- Staff of more than 280 scientists

Program

Biodosimetry