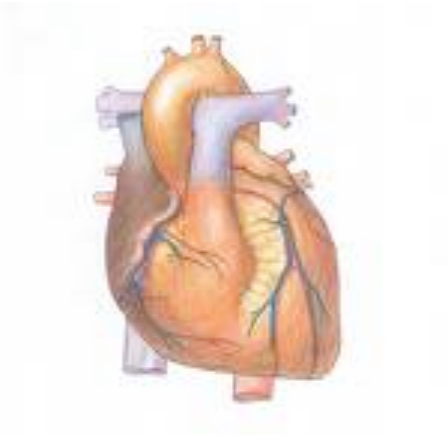
NTP
National Toxicology Program

Heart Breakout Group

Dr. Ben Van Houten, NIEHS



Characteristics of the "Ideal" Biomarker

- Method appropriate for the species being evaluated
- Sensitive, specific, predictive, efficient
- Bridges animal and human applications
- Additional attributes
  - Sampling procedure non-invasive or readily accessible (survival)
  - Assay easily and rapidly performed
  - Reliable
  - "Cost worthy"

Recommended biomarker #1: Troponin

- Is it applicable to rodents and humans? YES
- What is the disease process(es) evaluated (*e.g.*, tissue injury, altered function, inflammation, altered metabolism)? DEGENERATION, NECROSIS, MYOCYTE DAMAGE (Apoptosis?) Does it identify early or late events? EARLY
- Is it sensitive, specific, and/or predictive of the disease process? YES (indicative, not predictive) Could it be used to demonstrate a NOAEL? NOT YET (no reference ranges available)
- What type of specimen/measurement is needed? SERUM (<100 µL) Is obtaining it noninvasive and easily accessible? YES What is the appropriate time for sample or measurement collection? HOURS AND DAYS
- Are there other special considerations with including it in NTP studies (*e.g.*, extra animals needed)? TIME-DEPENDENT (6hr half-life) AND $50 PER TEST
- What technology is required? RIGHT ASSAY Is it accurate, reproducible, and cost effective? YES

Recommended biomarker #2: B-type Natriuretic Protein (BNP)

- Is it applicable to rodents and humans? YES
- What is the disease process(es) evaluated (*e.g.*, tissue injury, altered function, inflammation, altered metabolism)? MYOCARDIAL PRESSURE/VOLUME OVERLOAD
- Is it sensitive, specific, and/or predictive of the disease process? HIGH NEGATIVE PREDICTOR; POSITIVE LESS SPECIFIC, BUT PERSISTENT WITH PATHOLOGY (indicative, not predictive) Could it be used to demonstrate a NOAEL? UNKNOWN
- What type of specimen/measurement is needed? RNA Is obtaining it noninvasive and easily accessible? NO What is the appropriate time for sample or measurement collection? NECROPSY
- Are there other special considerations with including it in NTP studies (*e.g.*, extra animals needed)? RNA NEEDED; SERUM ASSAY COULD BE DEVELOPED; may want to parallel with ultrasound
- What technology is required? SEE ABOVE Is it accurate, reproducible, and cost effective? YES

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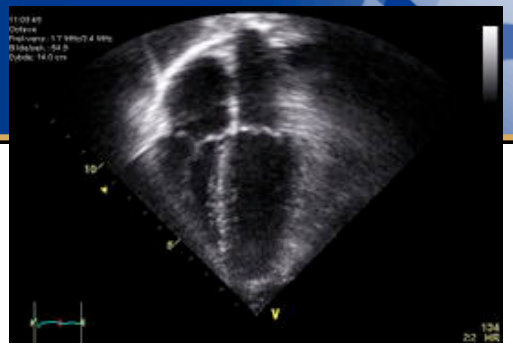
Recommended biomarker #3: Ultrasound



- Is it applicable to rodents and humans? YES
- What is the disease process(es) evaluated (e.g., tissue injury, altered function, inflammation, altered metabolism)? ALL THAT ALTER FUNCTION Does it identify early or late events? BOTH
- Is it sensitive, specific, and/or predictive of the disease process? INDICATIVE, NOT PREDICTIVE Could it be used to demonstrate a NOAEL? LIKELY
- What type of specimen/measurement is needed? LIVE ANIMAL (Isoflurane or Conscious) Is obtaining it noninvasive and easily accessible? YES What is the appropriate time for sample or measurement collection? END OF STUDY; AS NEEDED
- Are there other special considerations with including it in NTP studies (e.g., extra animals needed)? SKILLED OPERATOR NEEDED (can do 40-60 per day if conscious); STRESS vs. ANESTHESIA
- What technology is required? UPGRADE MACHINE Is it accurate, reproducible, and cost effective? YES (high throughput)

Recommended biomarker #4: α2-macroglobulin (Rat)

- Is it applicable to rodents and humans? ANALGOUS TO HUMAN CRP; MOUSE?
- What is the disease process(es) evaluated (e.g., tissue injury, altered function, inflammation, altered metabolism)? SYSTEMIC INFLAMMATION Does it identify early or late events? INTERMEDIATE
- Is it sensitive, specific, and/or predictive of the disease process? NEGATIVE PREDICTOR Could it be used to demonstrate a NOAEL? LIKELY
- What type of specimen/measurement is needed? SERUM Is obtaining it noninvasive and easily accessible? YES What is the appropriate time for sample or measurement collection? 48 HOURS POST-INJURY
- Are there other special considerations with including it in NTP studies (e.g., extra animals needed)? CAREFUL INTERPRETATION NEEDED (Non-specific); REFERENCE RANGES NEEDED
- What technology is required? ELISA Is it accurate, reproducible, and cost effective? YES

Additional biomarkers for future consideration

- Molecular Probes for Ultrasound/Near IR; micro-CT
- G protein-coupled receptor kinase-2 (heart & lymphocytes)
- Telemetry – expensive and time consuming
- Gene Expression – mechanism based
- Inbred Strains (NTP strain *vs.* JAX Lab)
  - Transgenics
  - Unpublished strain sensitivities
- EM - AZT
- mtDNA – atherosclerosis, dilated cardomyopathy, AZT
- Cell culture?

Decision Tree

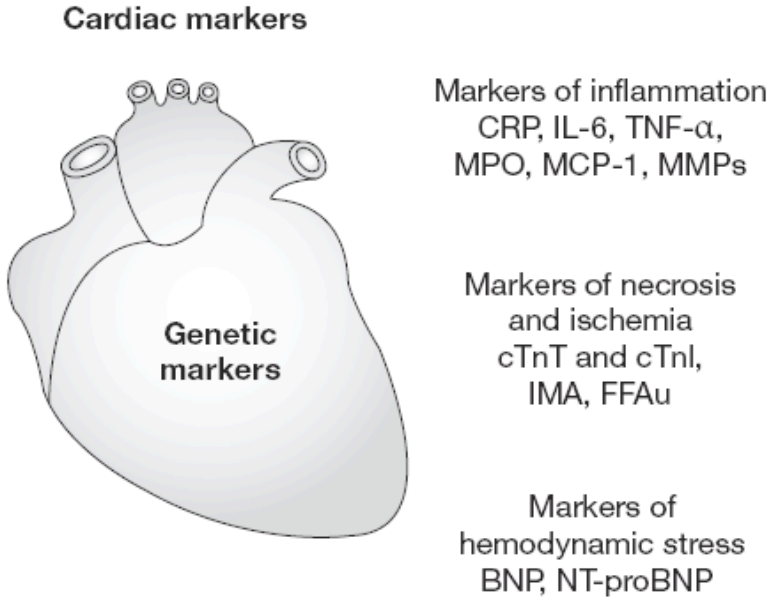
1. Standard Clinical Chemistry / Histopathology

Routine Studies:

1. Troponin
2. α2-macroglobulin (rat)
3. Serum BNP in conjunction with Ultrasound

Suspect Cardiotoxins:

1. Enhanced Imaging / Future



Breakout Group Participants

- **Ben Van Houten,** NIEHS **(Chair)**
- **Warren Lieuallen,** Pathology Associates **(Rappateur)**
- **Fred Apple,** Hennepin County Medical Center
- **Burns Blaxall,** University of Rochester Medical Center
- **John Bucher,** NIEHS
- **Mark Donahue,** Duke University
- **June Dunnick,** NIEHS
- **David Malarkey,** NIEHS
- **Pat Mastin,** NIEHS
- **Alex Merrick,** NIEHS
- **Abraham Nyska,** Integrated Laboratory Systems Inc.
- **Frank Sistare,** Merck & Co. Inc.