NTP
National Toxicology Program

Lung Breakout Group

Breakout Group Participants

- **Steven Kleeberger,** NIEHS **(Chair)**
- **Mike Jokinen,** Pathology Associates **(Rappateur)**
- **James Bonner,** Chemical Industry Institute of Toxicology
- **Rajendra Chhabra,** NIEHS
- **Daniel Costa,** U.S. Environmental Protection Agency
- **Angela King-Herbert,** NIEHS
- **George Leikauf,** University of Cincinnati
- **Robert Maronpot,** NIEHS
- **Joe Mauderly,** Lovelace Respiratory Research Institute
- **Daniel Morgan,** NIEHS
- **Brooke Mossman,** University of Vermont
- **Edward Postlethwait,** University of Alabama at Birmingham
- **Sally Tinkle,** NIEHS

Recommendation #1: Bronchoalveolar Lavage Analysis

- Is it applicable to rodents and humans? Yes. If not currently applied to rodents could it be? Yes.
- What is the disease process(es) evaluated (e.g., tissue injury, altered function, altered metabolism)? Especially useful for markers of injury, inflammation, and other tissue response indicators. Does it identify early or late events? Largely useful for early events, but may be a good indicator of selected late events as well.
- Is it sensitive, specific, and/or predictive of the disease process? It could be all of these, depending on the panel of indicators chosen for investigation. Could it be used to demonstrate a NOAEL? Probably not.
- What type of specimen/measurement is needed? Whole lung or partial lung lavages are doable. Is obtaining it noninvasive and easily accessible? It is largely invasive in small animals, but easily accessible. What is the appropriate time for sample or measurement collection? Likely most informative at early time points.
- Are there other special considerations with including it in NTP studies (e.g., extra animals needed)? Depending on the number of other biomarkers assessed, extra animals may be necessary.
- What technology is required? Trivial. Is it accurate, reproducible, and cost effective? It can be. We recommend strongly that a SOP be developed to maximize these outcomes.

Bronchoalveolar Lavage Fluid Analysis – other considerations

- Especially useful for cell counts and differentials
- Propose that panels of molecular markers (chemokines, cytokines, antioxidant, albumin, etc) should be considered instead of measuring a specific few of these.
  - marker panels could be designed to exaluated processes such as innate immunity, acquired immunity, and inflammation.
  - cost-effective methods have been developed that make this approach reasonable

Recommendation #2: Enhanced Histopathology

- Is it applicable to rodents and humans? Yes. If not currently applied to rodents could it be?
- What is the disease process(es) evaluated (e.g., tissue injury, altered function, altered metabolism)? Tissue injury, inflammation, apoptosis, repair, etc. Does it identify early or late events? Early and late.
- Is it sensitive, specific, and/or predictive of the disease process? Yes for all. Could it be used to demonstrate a NOAEL? Perhaps.
- What type of specimen/measurement is needed? Appropriately fixed lung or lung lobe(s). Is obtaining it noninvasive and easily accessible? Invasive and easily accessible. What is the appropriate time for sample or measurement collection? Investigator defined – likely appropriate at all times.
- Are there other special considerations with including it in NTP studies (e.g., extra animals needed)? Depending on other biomarkers assessed in the lung, extra animals may be needed.
- What technology is required? Good pathologists… Is it accurate, reproducible, and cost effective?

Enhanced Histopathology – other considerations

- Investigation/development of fixation techniques to allow for morphometric analysis (shrinkage of tissue)
- Immunohistochemistry for proteins, particularly those that may be found in BAL analysis. Emphasis on cross-platform confirmation
- Perform trichrome and PAS stains
- Stain for Ki67 protein for cell proliferation

Recommendation #3: Gene Expression Analysis

- Is it applicable to rodents and humans? Yes. If not currently applied to rodents could it be?
- What is the disease process(es) evaluated (e.g., tissue injury, altered function, inflammation, altered metabolism)? It may be useful for multiple disease processes. Informatics have developed or will be developed to better understand process. Does it identify early or late events? Early and late.
- Is it sensitive, specific, and/or predictive of the disease process? It may be very sensitive, specific, and predictive. Could it be used to demonstrate a NOAEL? Uncertain.
- What type of specimen/measurement is needed? High quality RNA. Is obtaining it noninvasive and easily accessible? It could be noninvasive, and is easily accessible. What is the appropriate time for sample or measurement collection? Investigation/question-defined.
- Are there other special considerations with including it in NTP studies (e.g., extra animals needed)? May require additional animals.
- What technology is required? Multiple microarray platforms. Is it accurate, reproducible, and cost effective? Answers to these questions are forthcoming.

Gene Expression Analysis – other considerations

- Analyses will identify some genes that are part of disease and some part of the process
- Probably not for routine use – however, save tissue for future evaluation if warranted
- Cost for large microarray chips coming down so can make evaluation of very large numbers of genes feasible in near future
- Need to have phenotypic changes (anchoring) to correlate with genotypic changes

Additional biomarkers for consideration (up to 5)

- #1
  - What is the disease process(es) evaluated?
  - Why is it recommended with a lower priority?
- #2
  - What is the disease process(es) evaluated?
  - Why is it recommended with a lower priority?
- #3
  - What is the disease process(es) evaluated?
  - Why is it recommended with a lower priority?

Biomarkers that offer promise, but need more work

- Imaging
  - What is the disease process evaluated? Many. A particular advantage is the value-added that imaging may represent. Multiple organ systems may be evaluated simultaneously.
  - Could it provide information not otherwise obtainable? Perhaps, but real strength is that it may be non-invasive, and repeated measures could be possible in the same animal.
  - Would it be applicable to rodents and humans? Yes
  - What type of development is needed to make it useful?
    - What is the roadblock(s) to it being developed? None – progress is ongoing to refine techniques
    - If resources were available, how long would it take? Projected 1 yr before ready for prime-time.

Other recommendations or comments

- Investigate routinely freezing tissues for future evaluation
- Investigate use of imaging techniques
  - Need to define information desired from imaging
- Suggest that the NTP conducts internal validation work with a known chemical to determine if these biomarkers are useful.

Conclusions

- Plethora of available biomarkers
- Recommend utilizing bronchoalveolar lavage and enhanced histopathology to develop suites or clusters of biomarkers that may be used and applied to developing systems analyses
- Gene expression may be a very useful biomarker, with caveats
- We recommend that plans should be made to appropriately archive multiple tissues for biomarker application using methods not currently ready for implementation, but will be available shortly

Other questions, time permitting

- Gene Chip Technology: to investigate patterns of gene expression
  - How could this tool be used? Should NTP put resources into its development? Why or why not?
    - Is there evidence that this methodology is appropriate for use in preclinical rodent studies?
    - Is there a specific list of genes that should be targeted?
    - How frequently should the list of targeted genes be reviewed?
    - What is the potential for identifying new gene targets?
- Proteomic Profiling Technology: to investigate patterns of protein expression
  - How could this tool be used? Should NTP put resources into its development? Why or why not?
    - Is there evidence that this methodology is appropriate for use in preclinical rodent studies?
    - What is the potential for identifying new protein targets?