Actions from Peer Review of Draft NTP Approach to Genomic Dose-Response Modeling
October 23–25, 2017

NTP convened an expert panel ("the Panel") on October 23–25, 2017, to peer review the *Draft NTP Approach to Genomic Dose-Response Modeling* (available at https://ntp.niehs.nih.gov/go/750911). NTP developed the draft approach using BMDExpress software to perform gene and pathway-level genomic dose-response modeling as part of Tox21 Phase 3 and *in vivo* screening-level studies. NTP's draft approach considers a number of factors including methods accepted in the peer-review literature, ease of translation to risk assessment, and ease of understanding for the variety of potential end users that may not necessarily be experts in mathematical and systems modeling. The Panel provided NTP scientific input on its proposed approach at the public meeting. Prior to the meeting, NTP hosted four webinars that presented different approaches to genomic dose-response modeling. When completed, the updated approach will be published on the NTP website (https://ntp.niehs.nih.gov/go/750911).

Overall Approach

NTP proposed an approach with the following features:

- Implement filtering
- Perform benchmark dose (BMD) modeling
- Define gene sets
- Report potency

The Panel voted unanimously (8 yes, 0 no, 0 abstentions) to recommend the following revisions to NTP's proposed overall approach:

- Scope:
  - Clarify the scope of the objectives to include use of BMD approaches to:
    - Model the dose-response behavior of genes and gene sets
    - Identify a dose below which biological and toxicological effects are unlikely to occur
    - The design is sufficient at this time to consider its future application to risk assessment
- Out of scope:
  - Limit the toxicological interpretation of effects
- Context of use:
  - Screening and prioritization
  - Interim point of departure (POD)
- Time points:
  - Specify how the approach will consider changes in dose-response relationships across different time points and how it will accommodate bioaccumulative substances
- Other:
  - Add examples to document to illustrate the method and test approach on existing datasets
  - Include more details about objectives to discern objectives of *in vivo* and *in vitro* studies in approach

Filtering Measured Features

NTP proposed the following approach for filtering of measured features:

- Empirical approach maximizing permissiveness, noise reduction, and reproducibility. Details:
  - ANOVA p-value <0.05
  - Fold change >1.5
  - No multiple testing correction

The Panel voted unanimously (8 yes, 0 no, 0 abstentions) to recommend the following revisions to NTP's proposed approach for filtering of measured features:

- Do not use proposed approach. Instead, customize specific filter parameters and tests for different platforms or experiments, with the goal to enhance reproducibility of results

- Begin to introduce nonparametric tests

Fitting Features to Dose-Response Curves

NTP's proposed the following approach for fitting features to dose-response curves:

- Features are fit to 9 parametric continuous models
- Benchmark response (BMR) = 1.349 x SD of controls
- 2-step process for best model selection [nested chi square and Akaike information criterion (AIC)]
- From the best fitting model, BMD, BMD_L, and BMD_U are determined (BMD_L = BMD lower confidence limit and BMD_U = BMD upper bound)

The Panel voted unanimously (8 yes, 0 no, 0 abstentions) to recommend the following revisions to NTP's proposed approach for fitting features to dose-response curves:

- Use the parametric models proposed; consider additional parametric models when available
- Introduce nonparametric models into BMDExpress to build confidence and experience
- Constrain parameters of polynomial models to eliminate multiple direction changes
- Specify explicitly whether the model-fitting approach uses dose or log-dose and investigate the effects of each
- Consider using model averaging to take into account model uncertainty as approach moves toward risk assessment

Gene Set-Level Potencies

NTP proposed the following approach for determining gene set-level potencies:

- Fit p-value threshold >0.0001
- BMD_U/BMD_L ratio threshold of <40
- Threshold for "active" gene sets
    - 3 genes, 5% populated, and Fisher Exact Test p-value <0.05
- Determining potency of a gene set: median and mean BMD

The Panel voted unanimously (8 yes, 0 no, 0 abstentions) to recommend the following revisions to NTP's proposed approach for determining gene set-level potencies:

- Eliminate use of Fisher Exact Test and investigate other methods, such as resampling, to perform enrichment testing
- When estimating gene set potency, use weighted average instead of median of individual gene BMDs to capture variability
- Consider higher curve fit p-value >0.0001
    - Alternative: Use R^2 value instead of or in addition to a global goodness-of-fit p-value
- Investigate the use of bootstrapping to determine confidence intervals on gene set

Study Design

NTP proposed the following approach for addressing study design:

- BMD-centric design
    - *In vivo* parameters
        - Male Sprague Dawley rats, 6-8 weeks of age
        - 5-day repeat dose
        - Liver and other expert-selected organs
        - Use of a 5-day maximum tolerated dose (MTD)
    - *In vitro* parameters
        - Human cell lines, sex based on availability
        - Expert-determination of duration
        - Organotypic culture
        - Top dose selection: LC20 (20% reduction in cell viability relative to control)
- 10 to 12 dose levels, 3 replicates/dose group

The Panel voted unanimously (8 yes, 0 no, 0 abstentions) to recommend the following revisions to NTP's proposed approach to study design:

- Consider study design as 1st phase of a larger effort to inform genomic-based risk assessment
- Include an earlier time point to the 5-day study design as a pilot for application to risk assessment
- Use pharmacokinetic predictions to determine steady-state timescale for duration determination and time point selection
- Consider including additional replicates in the control group
- Use most sensitive sex in *in vivo* studies
    - Range-finding studies can be used to find differences between sexes
- Expand organ collection list beyond liver to top 3 endpoints [kidney toxicity, lung toxicity (inhalation), neurotoxicity] for future testing
- Incorporate metabolic considerations in study design in both *in vivo* and *in vitro* studies

Biological Interpretation

NTP proposed the following approach for addressing biological interpretation:

- Expand and curate hallmark data sets to provide a toxicological and mechanistic interpretation that is species and organ/tissue specific. Expand:
    - Mine the GEO database to identify co-regulated gene sets not currently captured in the Hallmark gene sets
    - Mine existing phenotypic-anchored signatures such as those contained in the DrugMatrix database and those from the published literature
    - Remine MSigDB and CPDB in a manner similar to what was done to create the Hallmark gene sets to identify additional sets that may have been overlooked

The Panel voted (7 yes, 1 no, 0 abstentions) to recommend the following revisions to NTP's proposed approach to biological interpretation:

- Do not use the proposed approach at this time
- Use an existing curated data set to produce a functioning pipeline
- Focus proposal on identifying biologically responsive dose and not hazards
- With release of data, include a statement that this is a screening assessment
- Report the lowest gene set and its name; list the bottom 5-10 gene sets; do not interpret further
    - Release all data publically
- Consider proposed approach at a later time with evaluation and comparison with more traditional gene sets

The panelist who voted "no" did not agree with the existing proposed approach and recommended that a simpler, alternate approach should be proposed.