NTP’s Proposed Approach to Estimating Gene Set Level Potencies

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Populating Gene Sets

- For a feature to be considered its best model must:
  - Have convergent BMD, $BMD_L$ and $BMD_U$ values
    - Indicates model parameters are optimized
    - Ensures complete representation of the uncertainty around the BMD
  - Not map to more than one gene
    - Removes features with uncertain gene association
  - Not have a BMD> highest dose
    - Avoids model extrapolation
  - Have a nominal global goodness of fit p-value >0.0001
    - Higher values indicate better fit
    - Ensures a minimum (albeit liberal) fit of the model to the data
  - $BMD_U / BMD_L < 40$
    - Removes features with highly uncertain BMDs
• EPA Guidance
  – Prior model hypothesis, fit p-value > 0.05
  – No prior hypothesis, multiple models, fit p-value > 0.1

• Justification for the lower threshold fit p-value
  – A number of orthogonal filters for removing non-responsive or noisy data are included in the analysis pipeline
    – Fold change and ANOVA
    – BMDU/BMDL ratio <40
    – Gene set level filters - 3 genes, 5% populated, Fisher Exact test p<0.05
  – We use agglomerative estimates of potency
  – Loss of critical information particularly for moderate signal test articles
At least 3 genes
- Ensure that small gene sets are minimally populated
- Assuming no prior knowledge, minimum number of genes required to indicate a pathway or gene set is responding to treatment
- Minimum number of genes from which you can identify a median value

At least 5% populated
- Ensure larger gene sets require more than 3 genes

Fisher Exact Test (p<0.05)
- Nominal Statistical Filter

Identifying Active Gene Sets and Potency

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>BMD</th>
<th>BMD&lt;sub&gt;L&lt;/sub&gt;</th>
<th>BMD&lt;sub&gt;U&lt;/sub&gt;</th>
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<tbody>
<tr>
<td>Gene 1</td>
<td>10</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>Gene 2</td>
<td>50</td>
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<td>Gene 15</td>
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</tr>
</tbody>
</table>

= Median value = Gene Set BMD, BMD<sub>L</sub>, BMD<sub>U</sub>
Points to Consider

- Fit p-value threshold >0.0001
- BMDU/BMDL ratio threshold of <40
- Threshold for “active” gene sets
  - 3 genes, 5% populated and Fisher Exact P-value < 0.05
- Determining potency of a gene set
  - Median and Mean BMD

- Other variables to consider
  - GSEA-based approach
  - Bayesian alternative to enrichment
  - Focus only on use of select biomarker genes from AOPNs
  - What if only 2 biomarker genes are active (e.g., p21 and Ccng1)? Ignore?
  - Data supporting use of thresholds of “3 genes, 5% populated and Fisher Exact P-value < 0.05”
  - Pathway agnostic agglomerative BMD
    - Median BMD of the 20 most differentially expressed genes