# Improving Study Designs for Quantifying Biological Potency with Genomics Data

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**Peer Review of the Draft NTP Approach to Genomic Dose-Response Modeling**
**October 23-25, 2017**

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# Roadmap

- What comprises “Design”
- Special features of genomic concentration/dose – response (DR henceforth), and constraints on design
- Tools for evaluating an experimental design
- Classical toxicological design: BMD changed all that
- Classical Optimal Design for DR
- Injecting Realism
- Conclusions

# What Do I Mean by Design?

- Number of dose (concentration) groups
- What concentrations to use (e.g., control + 1, 10, 100 mg/kg in *in vivo* study)?
- How to distribute replicates among doses?

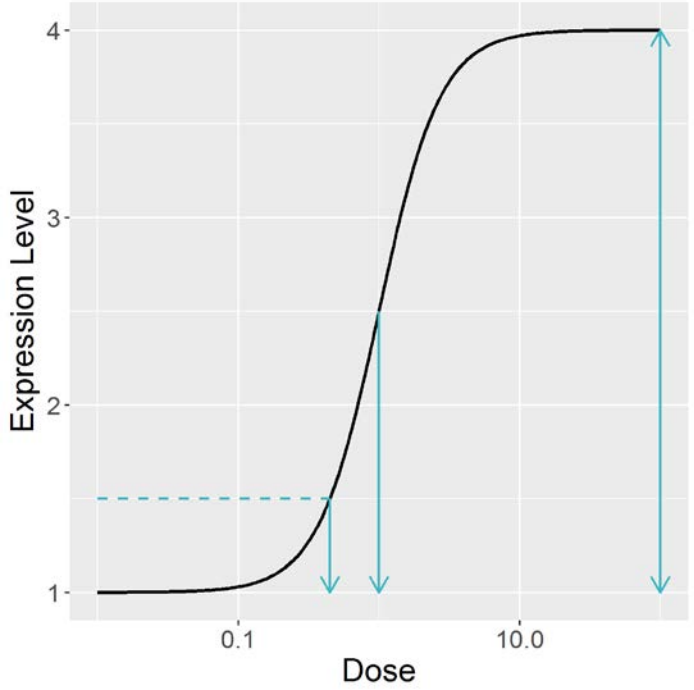
Resource and structural constraints will limit some or all of these.

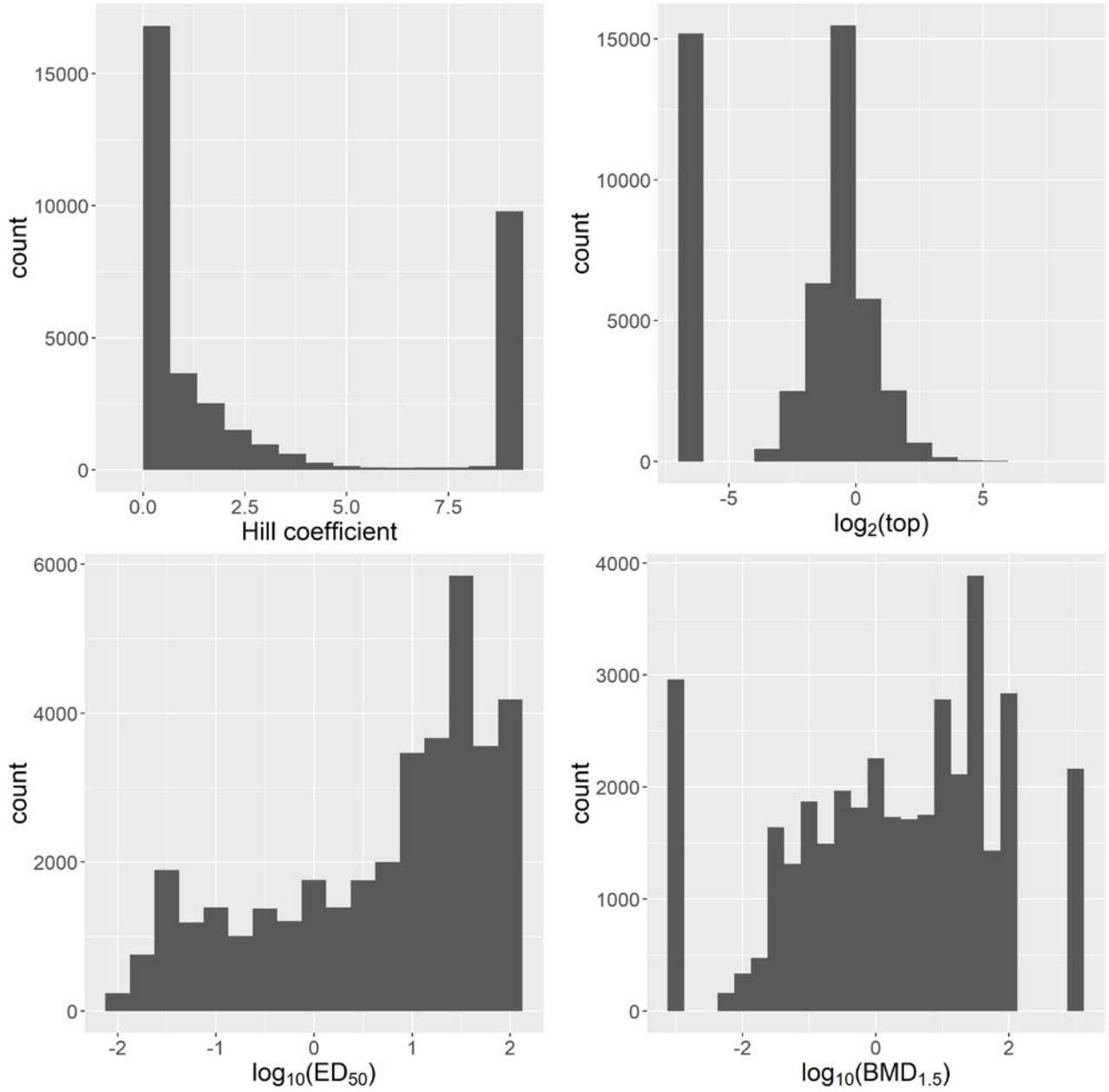
*E.g.*, it may not be feasible in a high throughput *in vitro* study to have unequal replication.

# Design Considerations of Features of Genomic Dose Response

- Most curves are likely to be sigmoid (approximated by a Hill model), but can be nonmonotonic, mainly at high doses.
- Thousands of endpoints (genes) – much worse than chronic bioassay!
- For a chemical, the design should function well over the full range of:
  - gene-specific potencies (*e.g.*, BMDs).
  - gene-specific DR shapes (*e.g.* power parameter, limiting fold-change).

- 44 chemicals, TempO-Seq whole genome, gene expression in MCF7 cells.
- DR: 8 half-log doses, 0.03 - 100 µM + vehicle control
- 3 biological reps – separate cultures, 1/plate
- Hill model fits



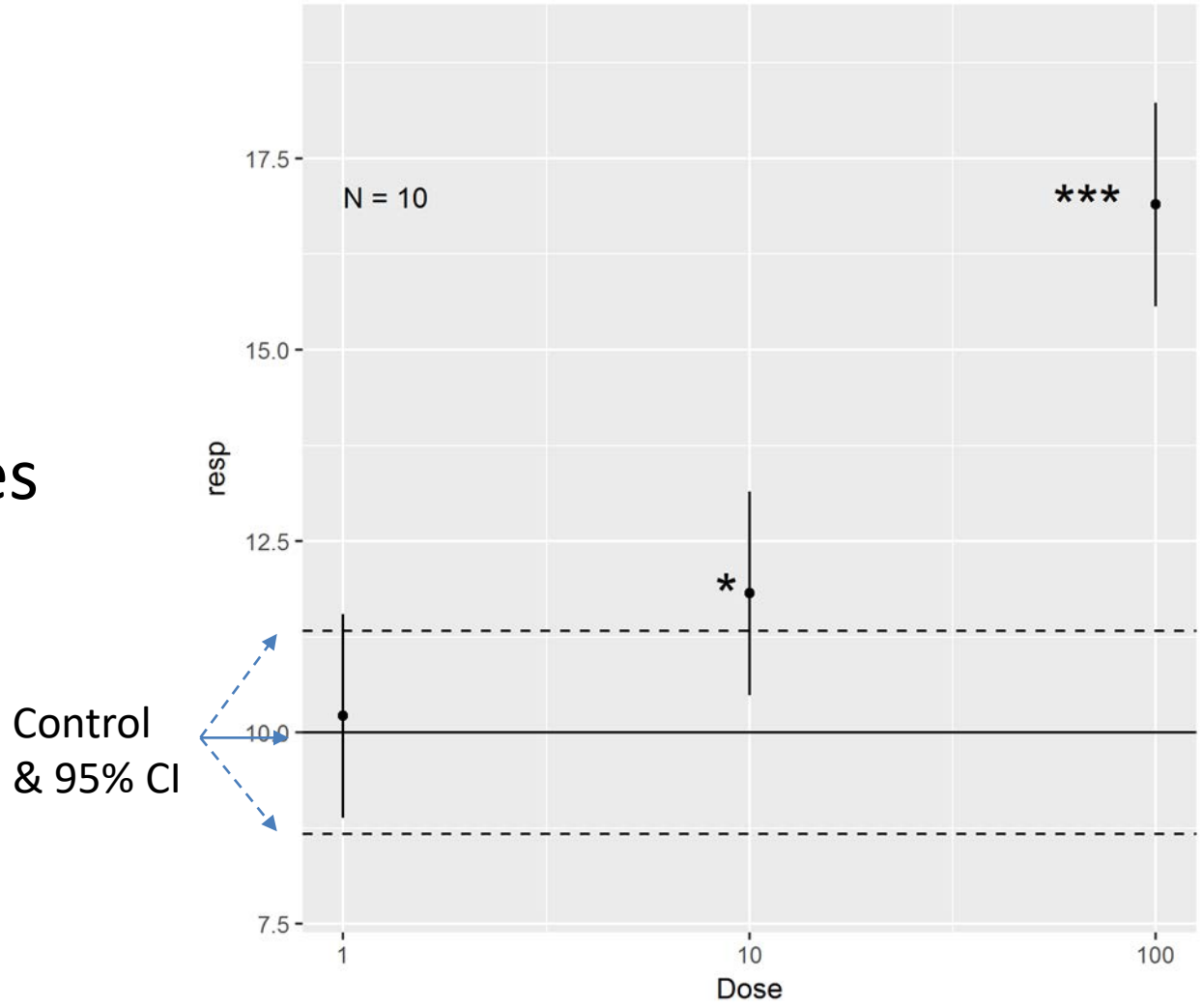



# Conceptual Tools for Evaluating Experimental Design

- From Statistical Theory:
  - Requires a statistical model:
    - specific (though maybe very flexible) DR model (*e.g.*, Hill (or Emax), spline) +
    - error model (*e.g.*, data are normal, lognormal, negative binomial, *etc.*)
    - usually assumes the true model up to parameter values is known.
  - Select a criterion to characterize the design:
    - The general variance of all model parameters: the determinant of the asymptotic covariance matrix of the parameter estimates
    - variance of a function of model parameters, *e.g.*, the asymptotic variance of the log BMD.
    - …
  - Explore the effect of different designs on the selected criteria.
  - Computationally (relatively) straightforward
  - Relies on asymptotic results
- Simulation
  - Simulate replicate data sets using different designs, and estimate model parameters for the simulated data
  - Use variances among replicate fits to characterize the performance of different designs
  - Computationally challenging for large scale evaluations
  - Captures the effects of finite, small sample sizes

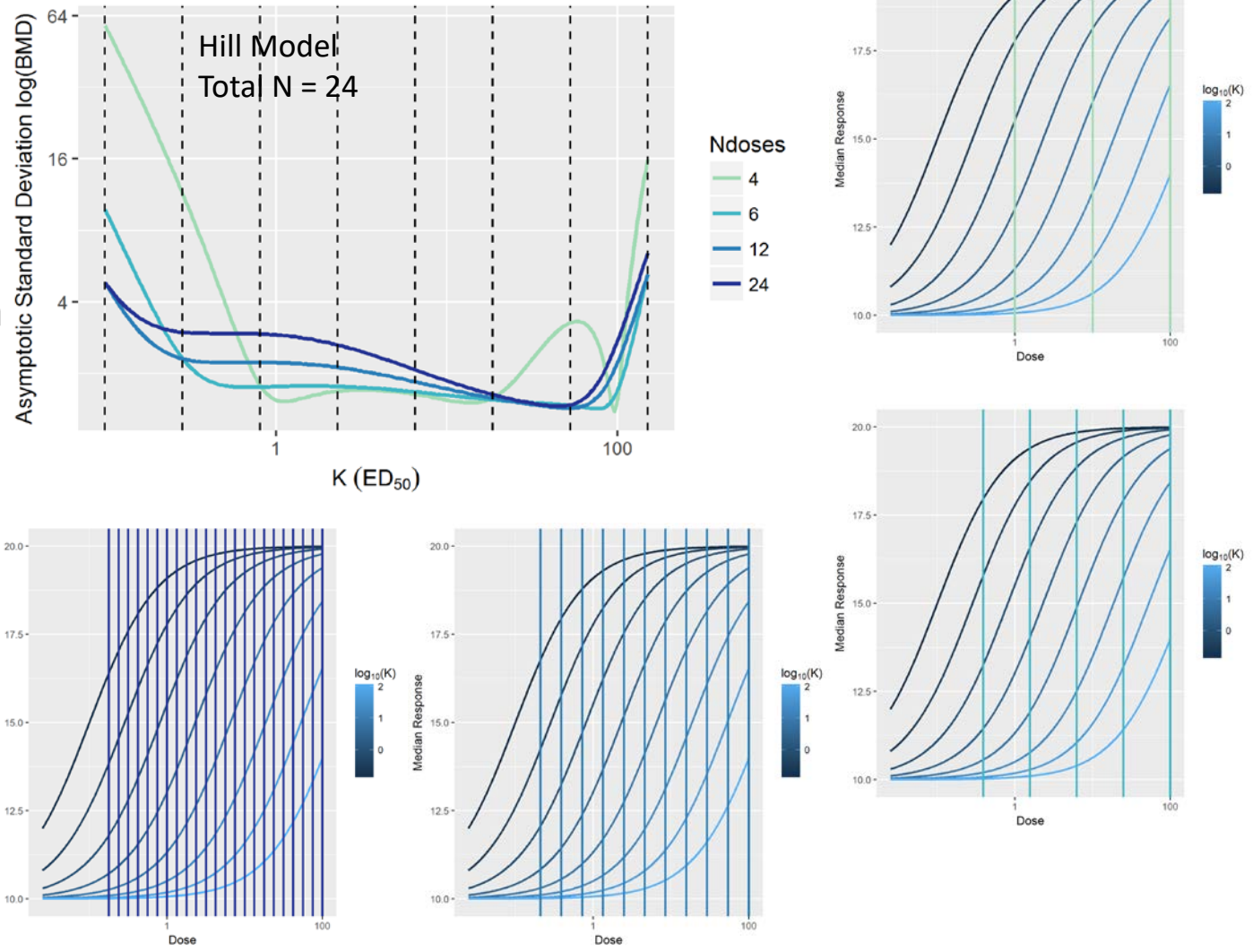
# Classical Toxicology Design

- Goal: provide sufficient power to identify a dose where the response was “different enough” from background - POD
- Few doses, multiple replicates per dose.
- Analyzed with sequential tests against control
- Later, analyzed with BMD



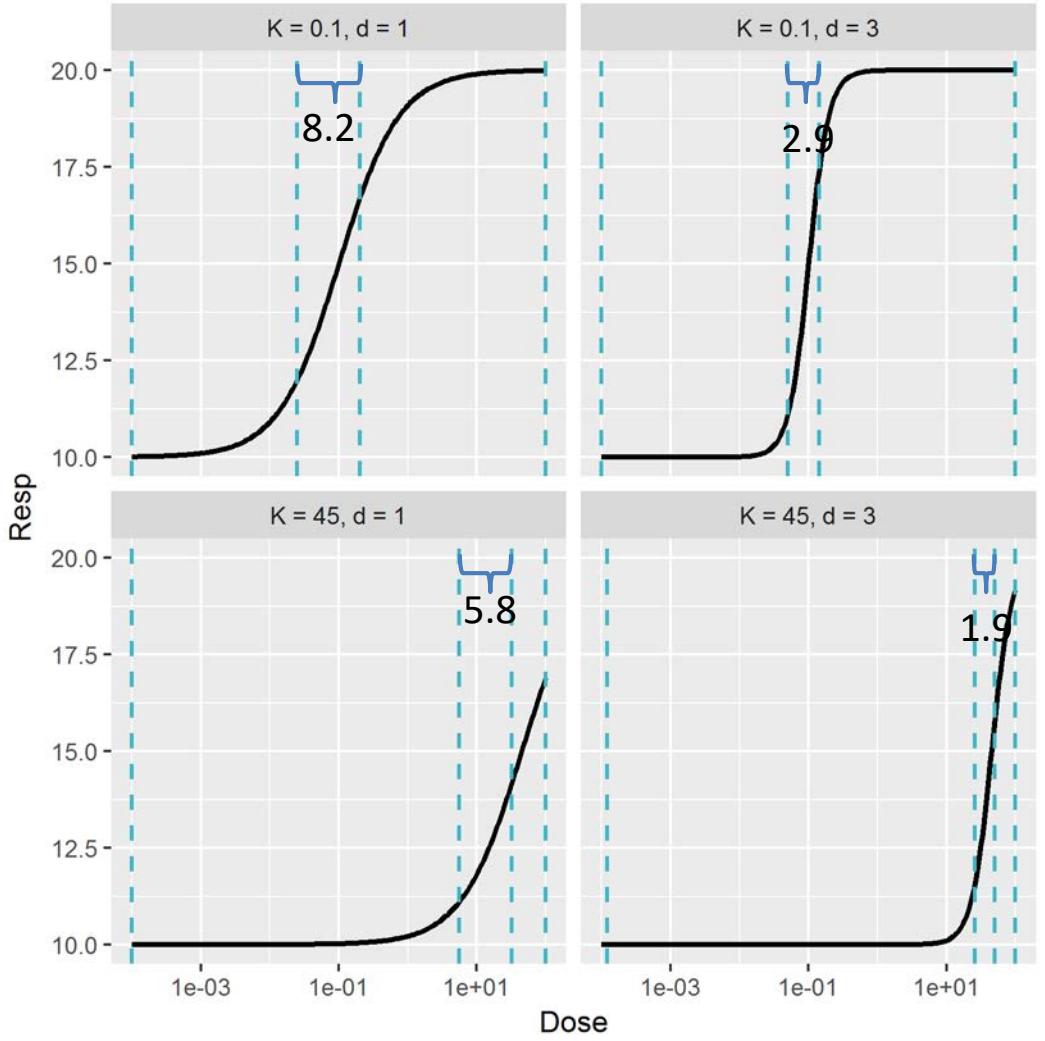
# Modifications for Dose-Response and BMD Estimation

- Kavlock et al (1996): For BMD estimation, it does not hurt to decrease reps per dose and increase doses, and the increased number of doses help. Disposition of doses matters.
- Slob et al (2005): Performance of design depends on total number of subjects, regardless of number of doses. Dose placement is crucial – including more doses improves the chances of good dose placement.
- Why Increase number of doses?
  - Robustness against range of DR curves
  - Robustness against extra, dose-group level 'noise' (e.g., Slob & Setzer, 2014)



# Optimal Design for Hill Dose-Response

- "Optimal Design" – design that minimizes the performance criterion, *e.g.*:
  - D-optimal design: minimizes the determinant of the asymptotic parameter covariance matrix
  - c-optimal design: minimizes the variance of a function of model parameters, *e.g.* the variance of the log(BMD).
- Optimal design depends on model parameter values: You have to know the truth to see it.
- For Hill w/lognormal error, D-optimal design has:
  - 4 doses: control, max dose, 2 bracketing the $ED_{50}$.
  - Equal weights
  - The spacing between the 2 bracketing doses decreases as the power ("hill coefficient") increases.
- There has been a lot of literature on this recently (see *References*).



# Adding Realism

- In reality, we have to design to be able to estimate models over a pretty wide range of DRs. No single optimal design will do.
  <u>Theoretical Alternatives:</u>
  - Multi-stage design – alternate experiment and optimization to close in on the best parameter estimates. –*not practical for genomics DR*
  - Find the design that minimizes the maximum variance over the range of uncertain parameters: make a design in which the worst-fit DR is fit well enough
  - Find the design that minimizes the criterion *on average* over a prior distribution of parameter values – Bayesian optimal design: make a design that does pretty well on average.
    - Both tend to add dose levels to the design.
- Determinants of practical designs:
  - the top and bottom doses
  - the dose spacing required to cover the range of DR steepnesses (steeper curves require closer spacing) (may not be regular!)
  - Replication both reduces variance, also protects against 'outliers'
  - Consider alternative DR models (including splines)
  - Incorporate noise, and use simulation to evaluate proposed designs

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# Conclusions

- Switching from the classical tox approach to DR to benchmark dose-like considerations leads to designs with more dose levels and fewer replicates per dose
- Classical optimal design considerations: “To see the truth, you have to know it first” – a design is optimal only for a single DR curve. Still, provides useful information about DR shape and dose spacing.
- Practical designs will have multiple dose levels, log-spaced, evenly weighted.
- Dose spacing should depend on the range of steepnesses of the curves.
- The lower end of the dose-range is probably the most interesting – there will be tension between dose spacing, achieving low enough doses, and cost.
- Both simulation and theory jointly should inform designs used.

# References and Additional Reading


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