Biomarker Analysis Strategies: Some Case Studies

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Pharmacodynamic Analysis

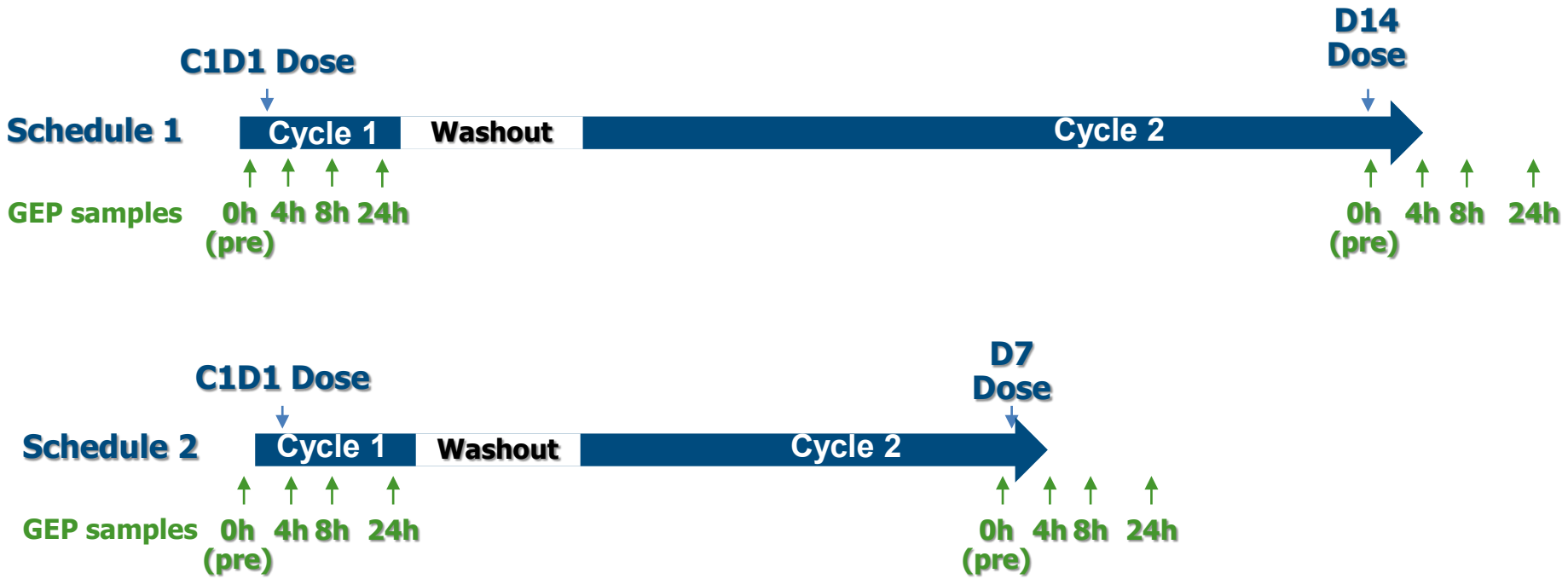
Analysis Goals

- Analyze blood RNA-Seq data (141 samples from 18 patients with selected advanced solid tumors and haematological malignancies) for 18,591 genes to help inform dose and schedule selection.

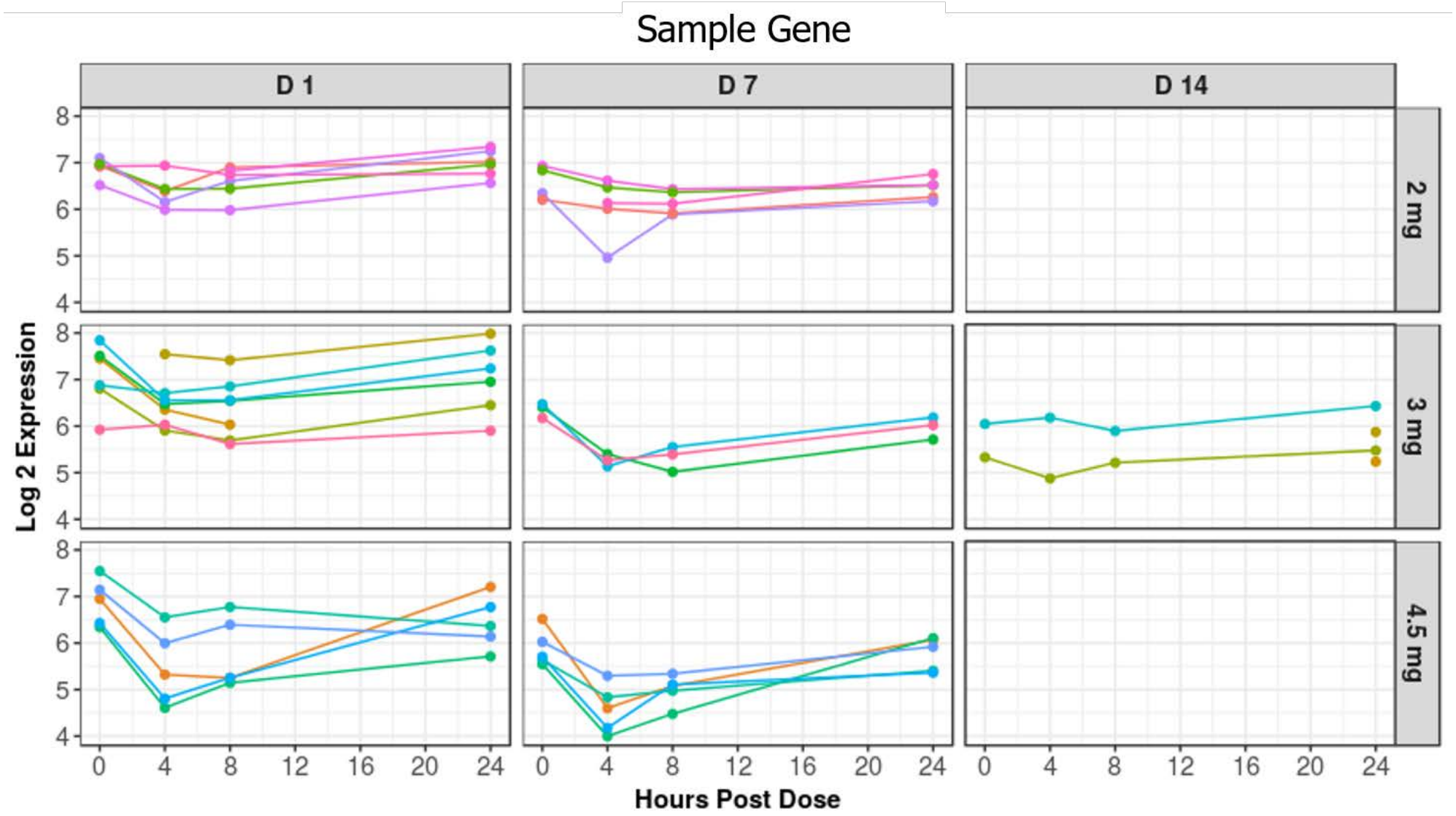
- Identify top genes with changes in expression over time that are consistent across dosing schedule

Sample Collections

Subjects and doses split over multiple batches – needed to account for subject and batch variation in model



Sample Collections

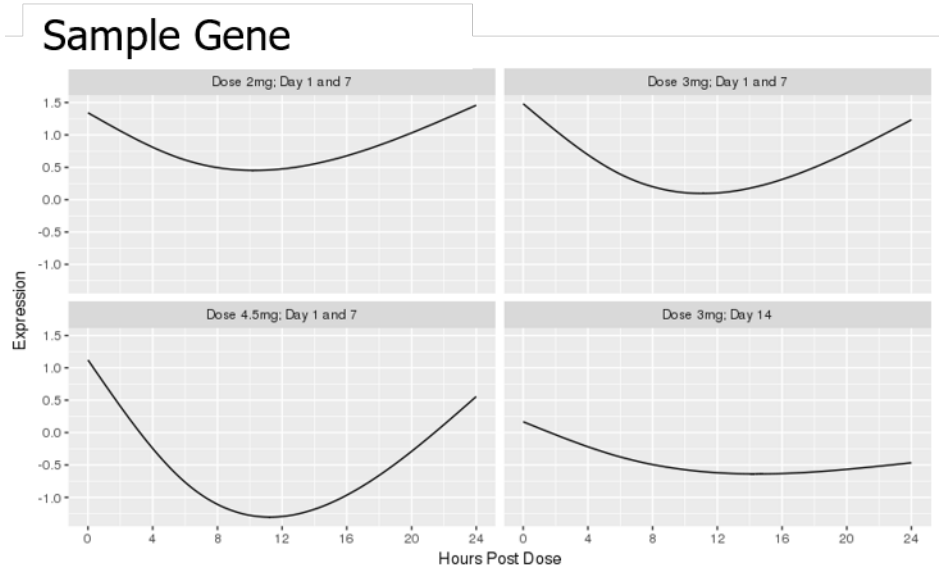


Analysis Challenges

- Batch Effects
  - Subject-subject and batch variability
  - Bridge samples
- Dose*Day (schedule)*Time (hours post-dose) interaction
- Anticipated non-linear relationships between gene expression and time
- Over 4,500 genes were found to have Benjamini-Hochberg adjusted p-values < 0.05
- Looking for expression patterns that are consistent across dose levels and cycle day

Gene Selection

- Ran Linear Mixed Models to test for and estimate the relationship between gene expression and time
  - Averaged time effects across C1D1 and C2D7 for each dose level to identify genes with changes consistent across days
    - Did not test for relationship in C2D14 samples due to small number of samples
- Removed genes that had a B-H adjusted p-value > 0.05
  - ~ 4,500 genes remained, out of 18,591 total



Gene Expression Model

- Linear mixed effects model (using R lmer function)
- Response variable: Log2(gene expression)
- Fixed Effects Portion of Model
  - Expression = Factor + CS + Factor*CS
    - Factor Levels (combination of Dose and Day):
      - 2mg C1D1 & C2D7
      - 3mg C1D1 & C2D7
      - 4.5mg C1D1 & C2D7
      - 3mg C2D14
    - CS represents two cubic spline basis functions for hours post dose, with one knot at 6 hours post dose.
- Random Effects Portion of Model
  - Batch: Random intercept
  - Subject: Random intercept and random slope based on time as a continuous variable across both cycles, which imposed a decreasing correlation over time within each subject

Hypothesis Testing

- H_0: Coefficient for each CS function (main effect or any interaction, excluding the interaction with curve representing C2D14) = 0
  - Interpretation: No time effect at any dose
- H_1: Time effect at one or more doses
- Likelihood Ratio Test, using bootstrap to get empirical p-values (via "pbkrtest" package in R)

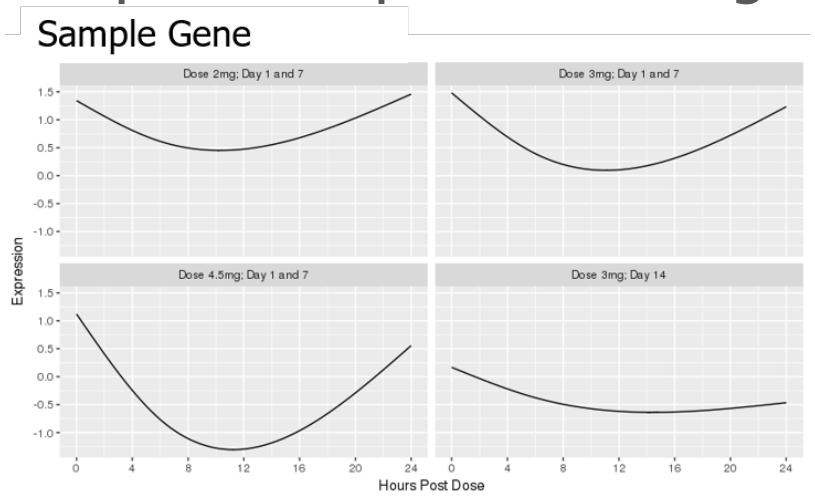
Models

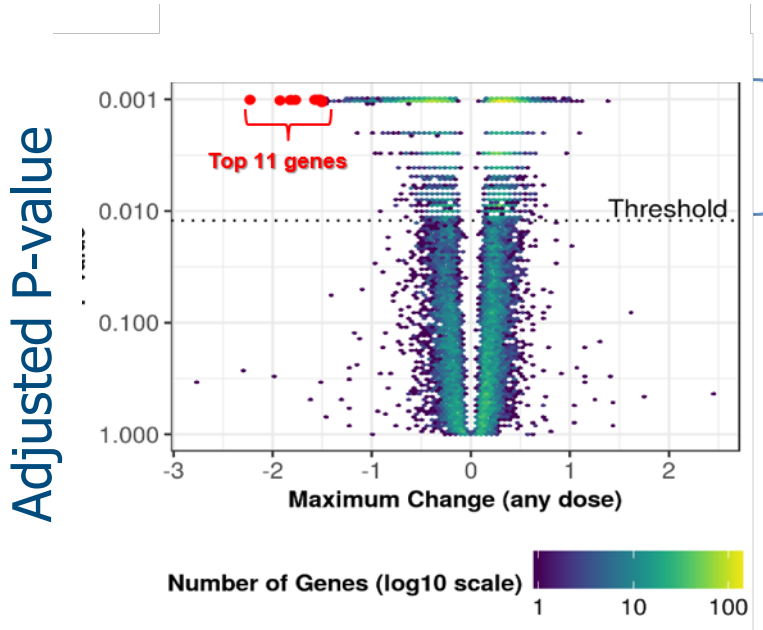
	Fixed Effects	
Factor	Full Model	Reduced Model
2mg C1D1 & C2D7	$\mu + \tau_1 + \tau_2$	μ
3mg C1D1 & C2D7	$\mu + \beta_2 + \tau_1 + \tau_2 + i_{21} + i_{22}$	$\mu + \beta_2$
4.5mg C1D1 & C2D7	$\mu + \beta_3 + \tau_1 + \tau_2 + i_{31} + i_{32}$	$\mu + \beta_3$
3mg C2D14	$\mu + \beta_4 + \tau_1 + \tau_2 + i_{41} + i_{42}$	$\mu + \beta_4 + i_{41} + i_{42}$

μ: Intercept
β_i: Main effect factor coefficient
τ_i: Main effect basis function for cubic spline coefficient
i_{ij}: Interaction coefficient between basis function and factor

Gene Rankings

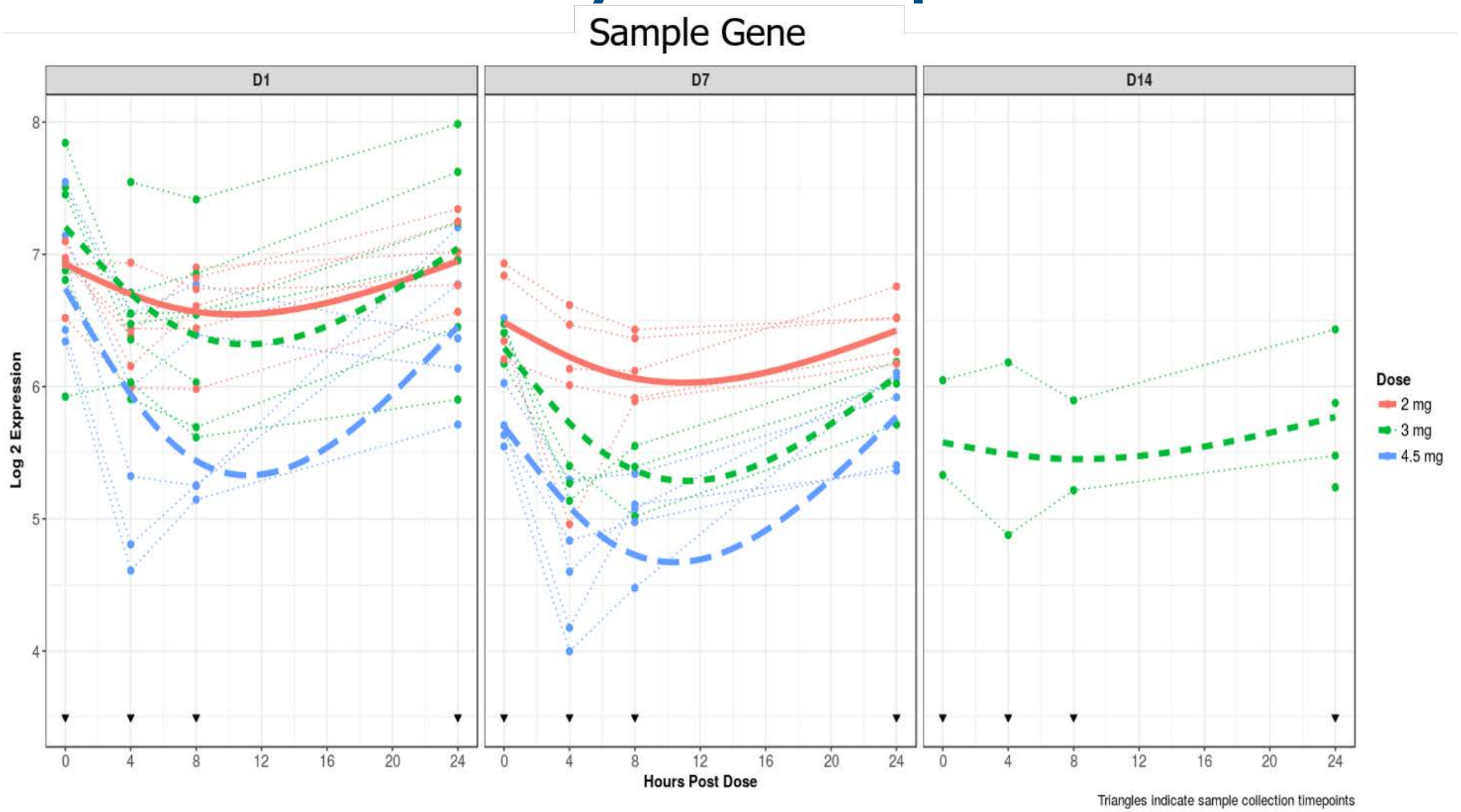
- Calculated maximum change from baseline for each of the four curves described above within each gene
- Selected curve with maximum absolute change (excluding the curve representing Cycle Day 14) for each gene
- Ranked genes according to their respective selected maximum absolute change
- Reported top 11 ranked genes





~4,500 **of ~18,500** genes had B-H adjusted p-value < .05

Visual Summary of Example Gene



Composite Biomarker Analysis

Summary of Analysis Methods

- **Efficacy measures:** BOR (Best Overall Response), PFS, OS
- **Predictors:** 3 continuous biomarkers (A, B, and C) and treatment arm
- **Analysis population:** 183 subjects
- **Model-building approaches:**
  - **2 machine learning (ML) methods:** MARS (earth package) and Grouped Lasso (glinternet package)
    - Both methods allow for possible interactions among predictor variables
    - MARS allows also for potential non-linear effects of predictor variables
  - **Generalized Linear Models (GLM)**, with interaction terms and all effects linear
    - Logistic Regression for BOR
    - Cox Proportional-Hazards Models for PFS and OS
- **Model performance assessment:**
  - Performance metrics: ROC, C-index, PPV and NPV
  - Metrics estimated by repeated, nested (for ML methods) cross-validation
  - Visualization by effect-estimate plots

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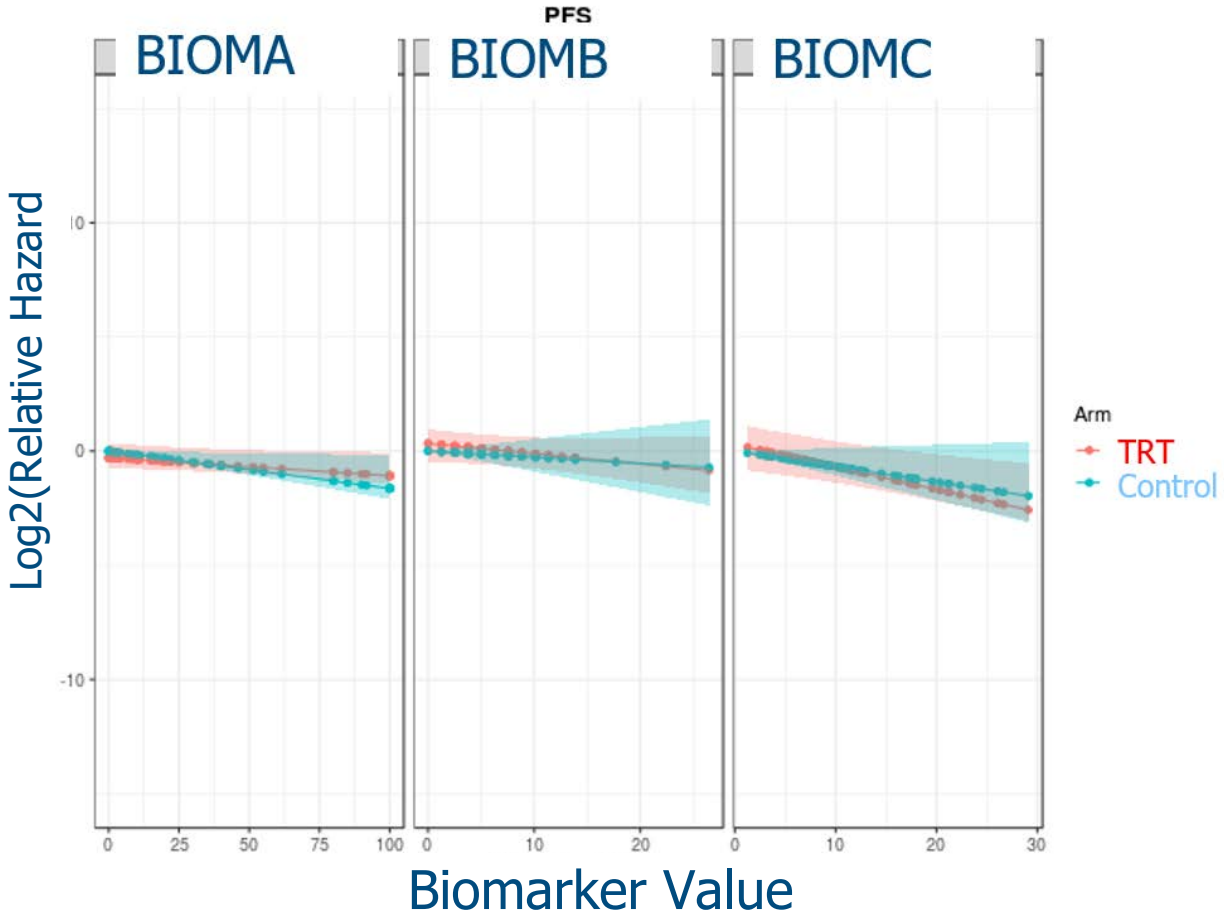
Outline

- Investigated 7 models
  - 3 Single Biomarker models
  - 3 Pairwise Biomarker models
  - 1 3-way Biomarker model
- Model Assessments
  - Effect Size Estimates
  - Prediction Matrix Plots
  - Performance Metrics
  - Hypothesis Testing

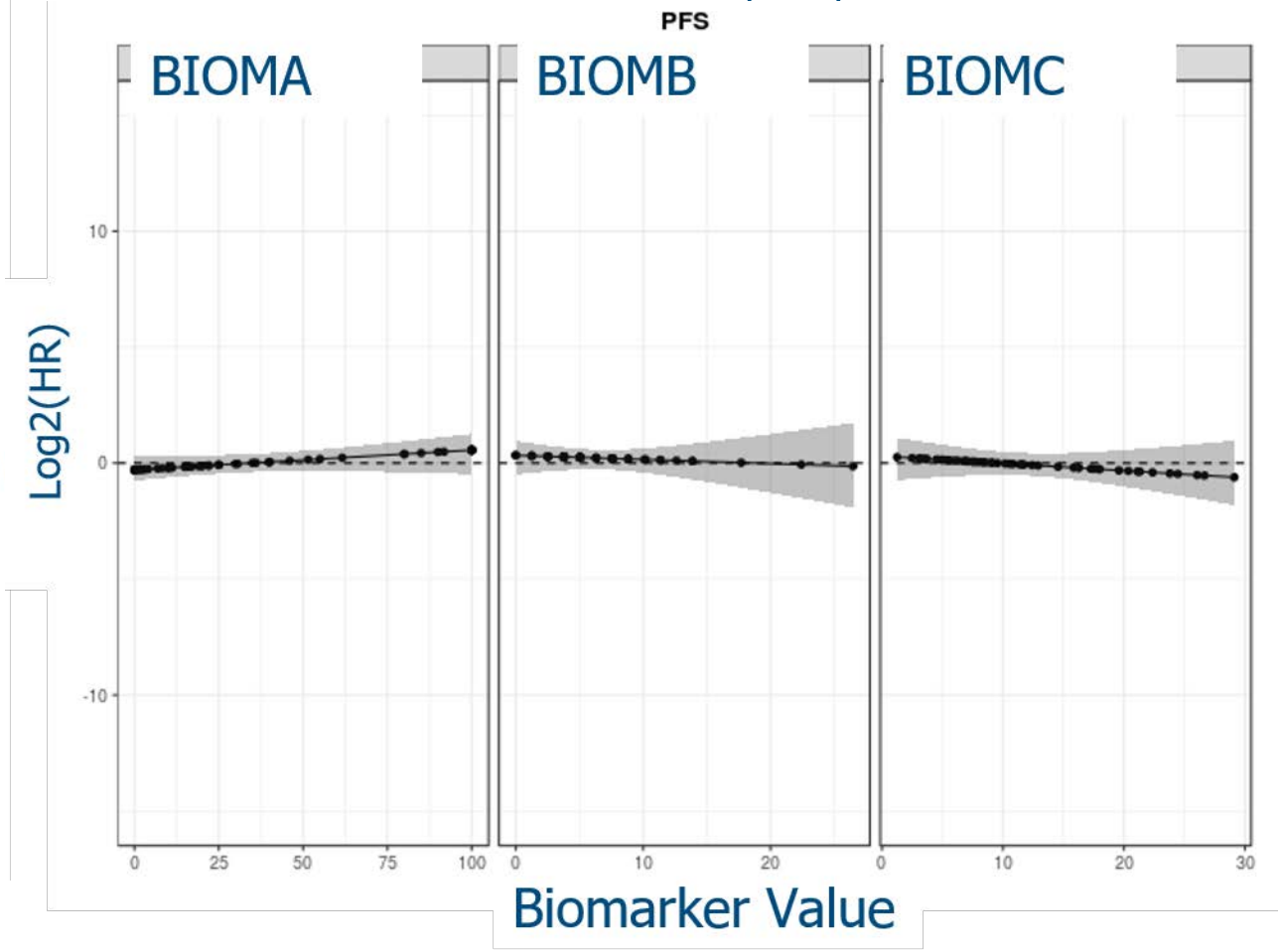
PFS, Individual Biomarkers (BM)

No noticeable interaction with treatment

Effect of BM on Relative Hazard

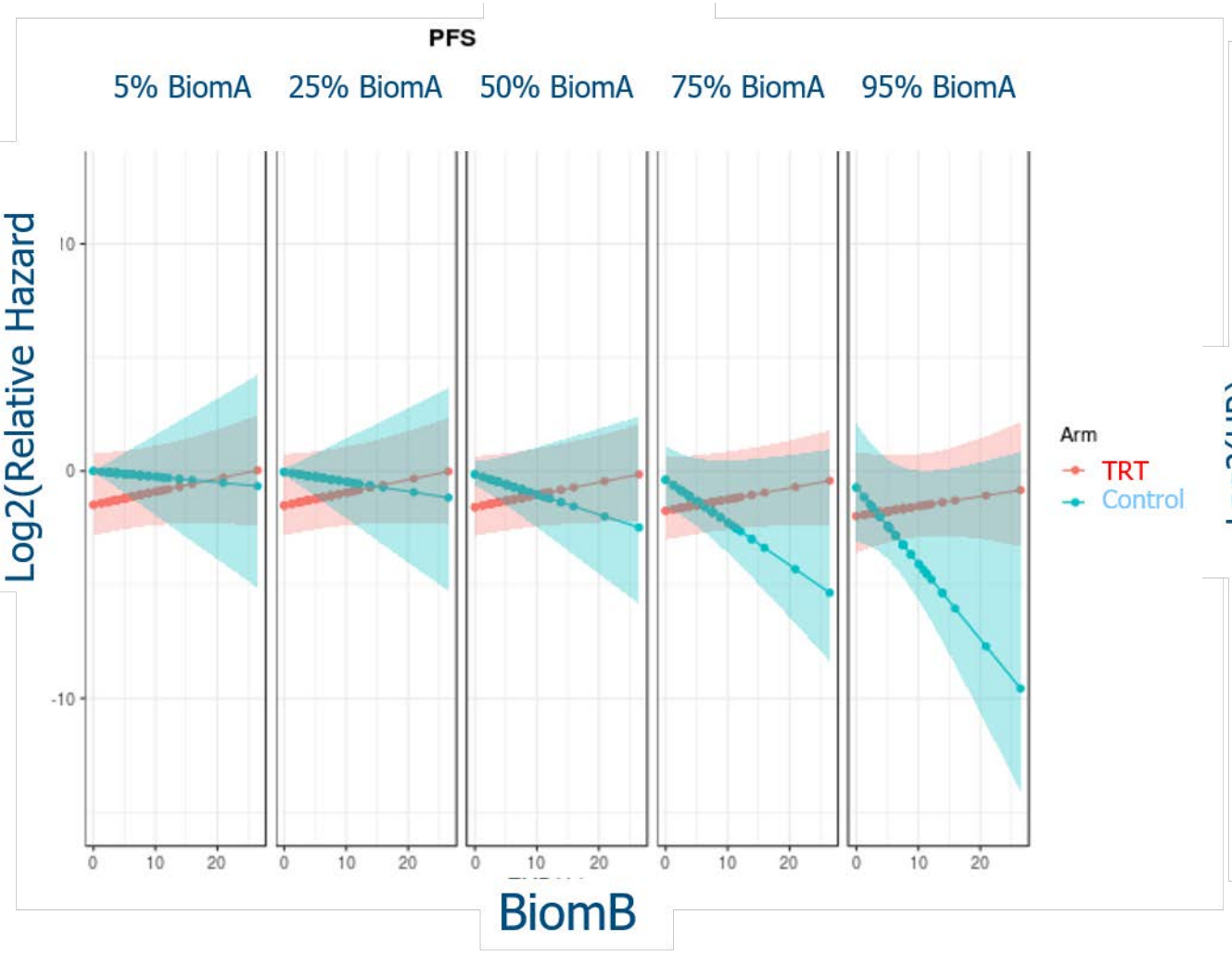


Effect of BM on Hazard Ratio (HR) of TRT vs Control

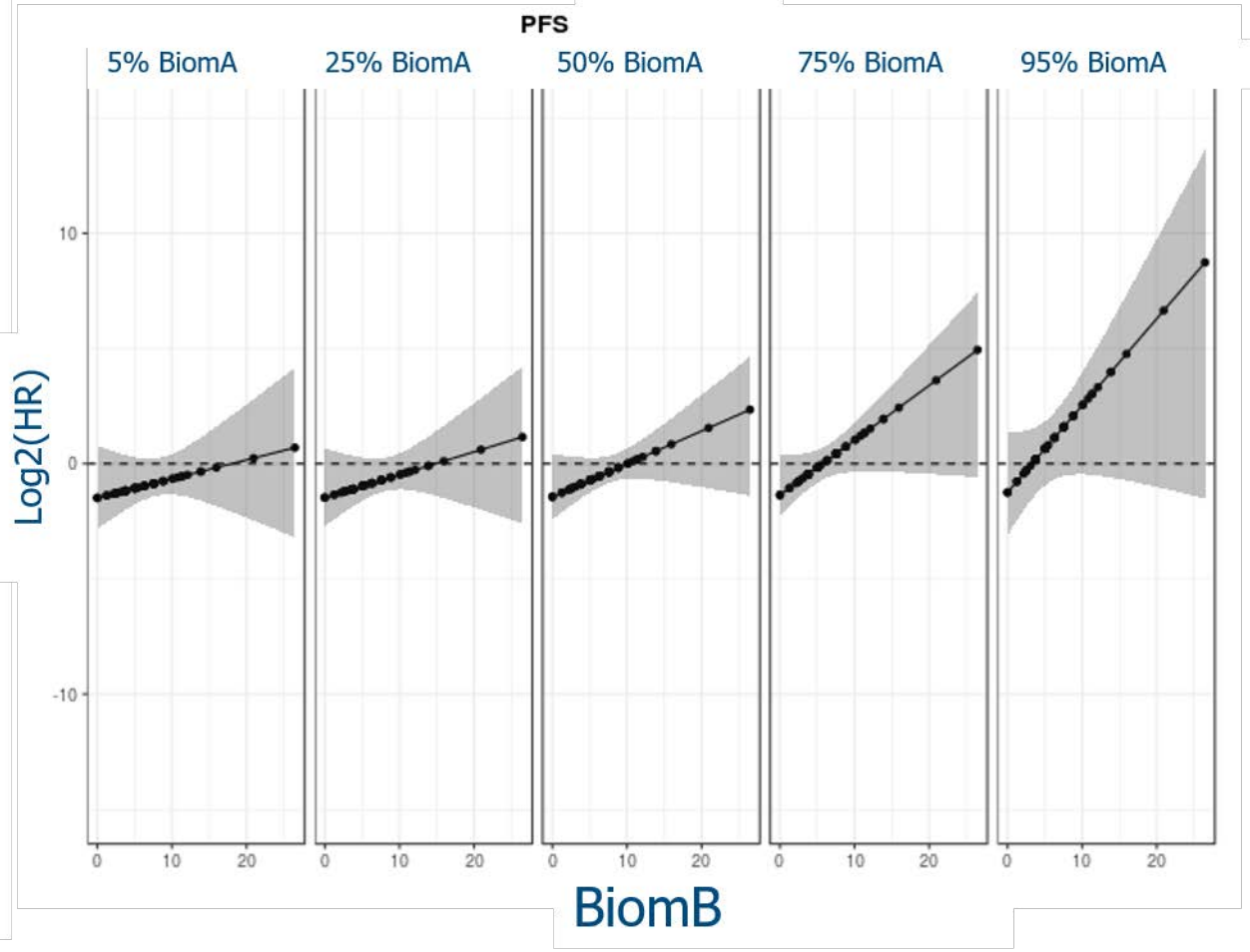


PFS, BiomA+BiomB: Effect of BM on HR of TRT vs Control

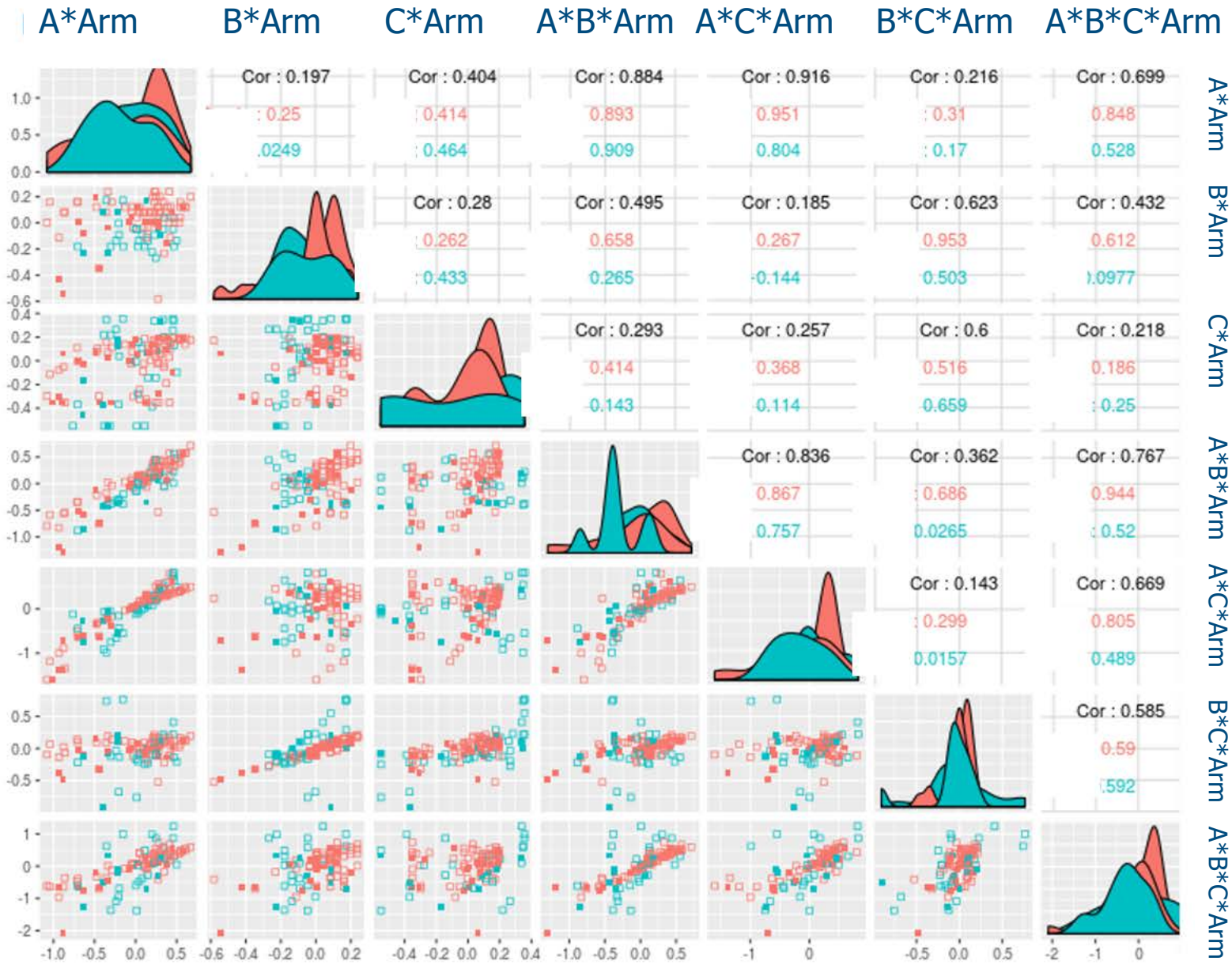
Effect of BiomB for fixed BiomA



Log2(HR) for TRT vs Control



PFS: Predicted Relative Hazard

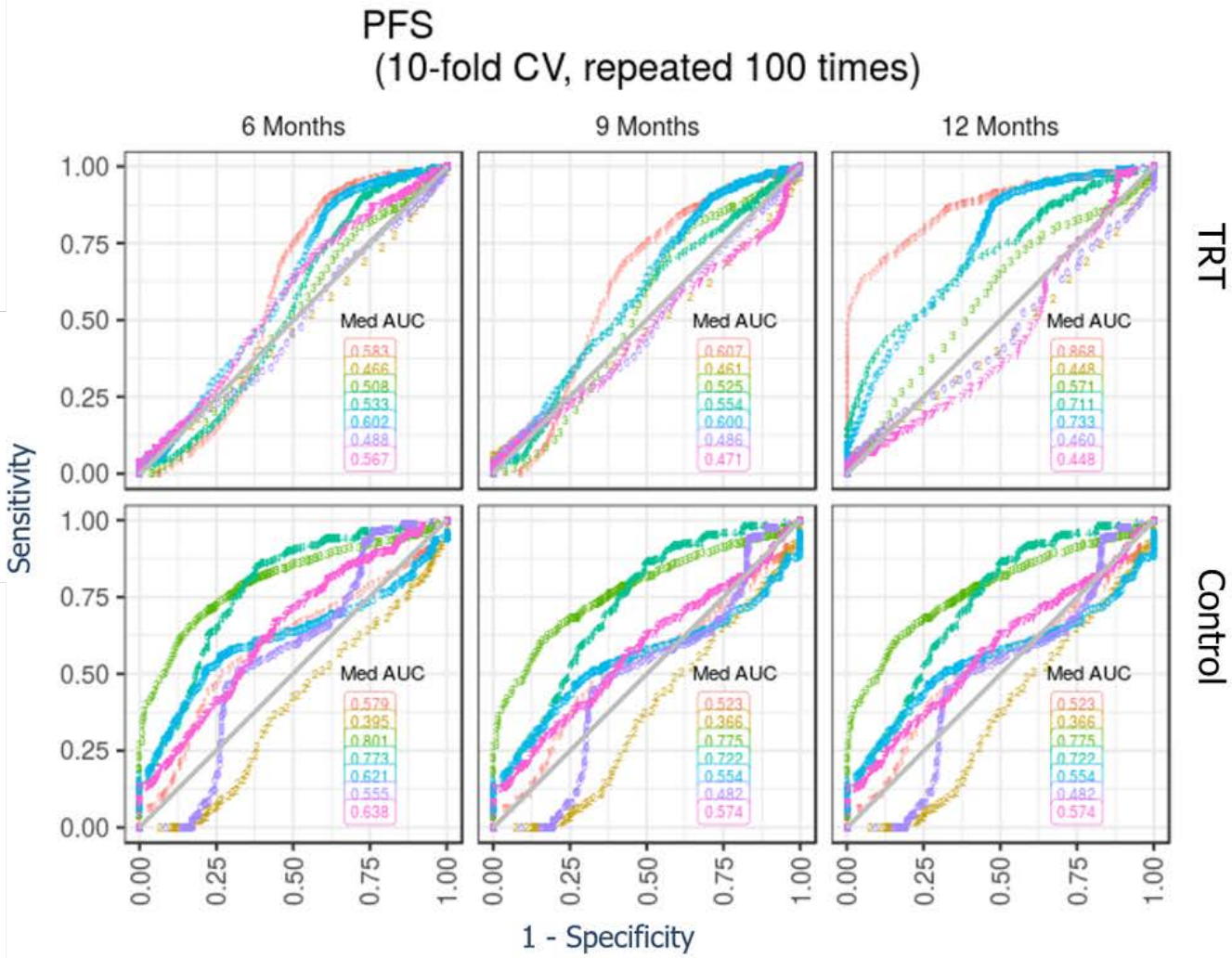


Legend:
A = BiomA
B = BiomB
C = BiomC

BOR
PD+SD+NE
CR+PR

ARM
TRT
Control

PFS - ROC



TRT

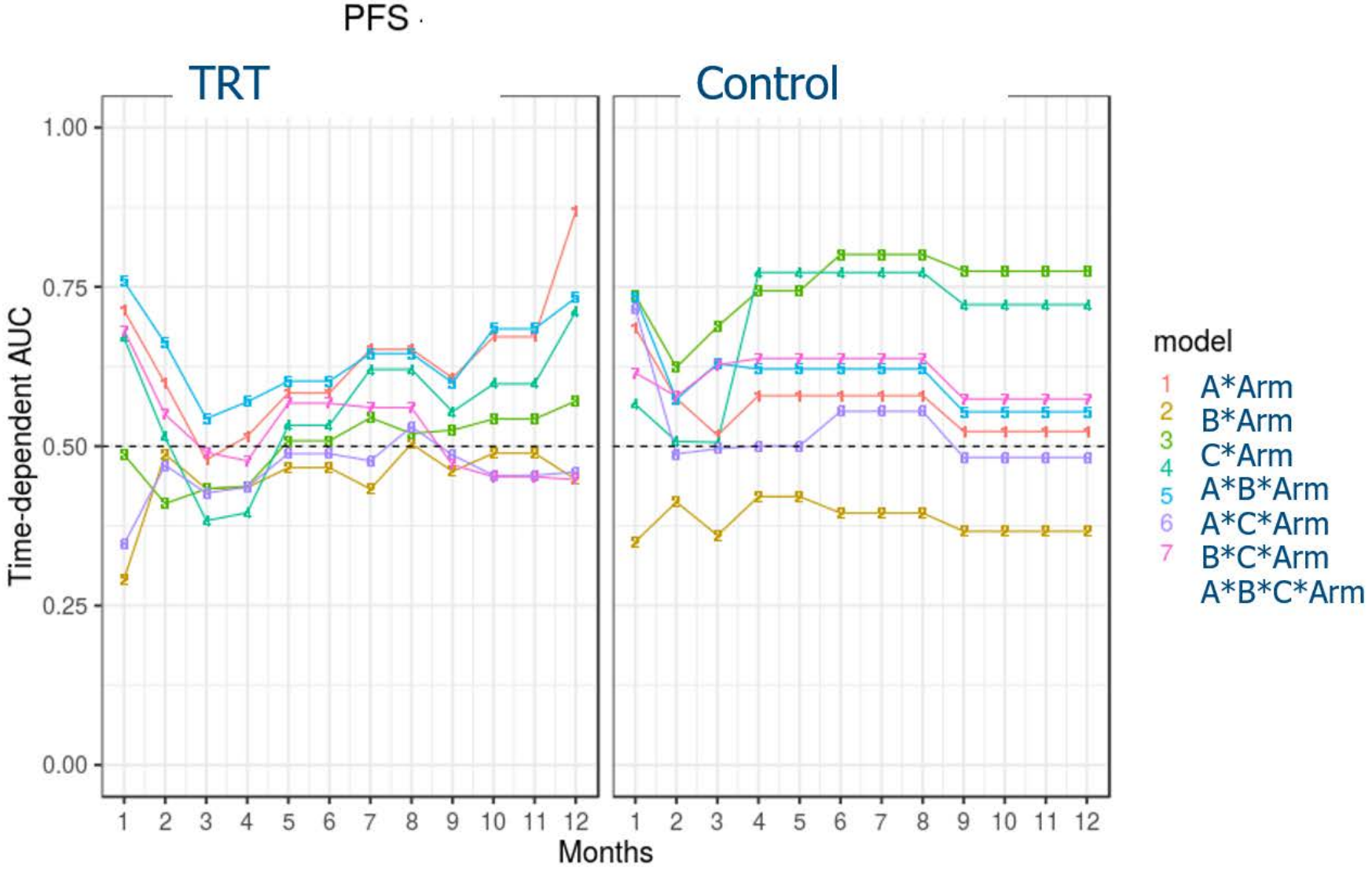
model	Cindex
A*Arm	0.57
B*Arm	0.45
C*Arm	0.48
A*B*Arm	0.50
A*C*Arm	0.60
B*C*Arm	0.44
A*B*C*Arm	0.55

model
1 A*Arm
2 B*Arm
3 C*Arm
4 A*B*Arm
5 A*C*Arm
6 B*C*Arm
7 A*B*C*Arm

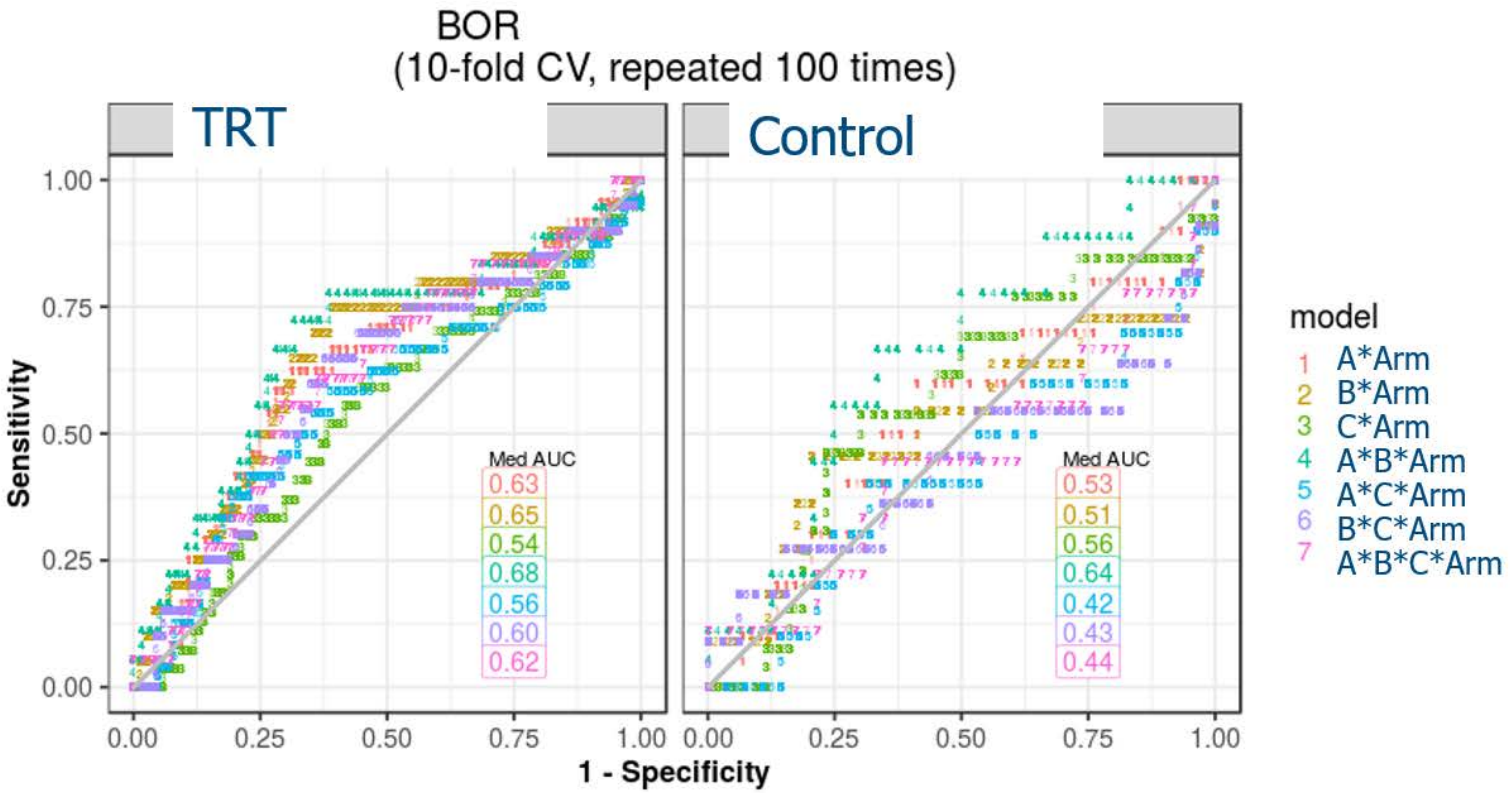
Control

model	Cindex
A*Arm	0.52
B*Arm	0.40
C*Arm	0.60
A*B*Arm	0.56
A*C*Arm	0.54
B*C*Arm	0.57
A*B*C*Arm	0.59

PFS - AUC


PFS
TRT
Control
Time-dependent AUC
1.00
0.75
0.50
0.25
0.00
1 2 3 4 5 6 7 8 9 10 11 12
Months
model
1 A*Arm
2 B*Arm
3 C*Arm
4 A*B*Arm
5 A*C*Arm
6 B*C*Arm
7 A*B*C*Arm

BOR - ROC


BOR
(10-fold CV, repeated 100 times)
TRT
Control
Sensitivity
1 - Specificity
0.00
0.25
0.50
0.75
1.00
Med AUC
0.63
0.65
0.54
0.68
0.56
0.60
0.62
Med AUC
0.53
0.51
0.56
0.64
0.42
0.43
0.44
model
1 A*Arm
2 B*Arm
3 C*Arm
4 A*B*Arm
5 A*C*Arm
6 B*C*Arm
7 A*B*C*Arm

Acknowledgements

- Scott Chasalow
- Donald Jackson

Backup

GLM Likelihood Ratio Tests (LRTs)

- A saturated model includes all main effects and interactions for four variables – BiomA, BiomB, BiomC, and Treatment Arm – with biomarker effects assumed linear

1. Test contribution of all biomarkers as a collective whole
   - Compare saturated model to reduced model containing only treatment effect
   - 1 Chi-Square Test with 14 Degrees of Freedom
2. Test contribution of individual biomarkers to saturated model
   - Compare saturated model to reduced model which excludes one of the biomarkers
   - 3 Chi-Square Tests with 8 Degrees of Freedom each
3. Test contribution of interactions with treatment arm to saturated model
   - Compare saturated model to reduced model which excludes all interactions containing treatment arm
   - 1 Chi-Square Test with 7 Degrees of Freedom
4. Test contribution of each pair of biomarkers to a saturated model that excludes the third biomarker
   - Repeat Test 1 after excluding one biomarker at a time from saturated model
   - 3 Chi-Square Tests with 6 Degrees of Freedom each
5. Test contribution of each pair of biomarkers to saturated model
   - Compare saturated model to reduced model which excludes each pair of the biomarkers
   - 3 Chi-Square Tests with 12 Degrees of Freedom each

GLM Summary

			OR		PFS		OS	
Hypothesis Test	Biomarker (BM)	N	P-value	FDR	P-value	FDR	P-value	FDR
1. Contribution of all BM	All	105	**0.08**	0.44	0.18	0.50	0.32	0.50
2. Individual BM contribution to saturated model	BiomA	105	0.36	0.50	0.20	0.50	0.35	0.50
	BiomB	105	0.70	0.70	0.56	0.64	0.68	0.70
	BiomC	105	**0.04**	0.44	0.30	0.50	0.59	0.65
3. Treatment-arm interactions	All	105	0.35	0.50	0.70	0.70	0.26	0.50
4. Contribution of BM pairs	A & B	124	0.30	0.50	**0.01**	0.18	0.26	0.50
	A & C	108	**0.01**	0.18	**0.06**	0.44	**0.13**	0.45
	B & C	132	**0.10**	0.45	0.30	0.50	0.32	0.50
5. Pairwise BM contribution to saturated model	A & B	105	0.52	0.64	0.27	0.50	0.41	0.54
	A & C	105	**0.07**	0.44	0.14	0.45	0.30	0.50
	B & C	105	**0.14**	0.45	0.55	0.64	0.53	0.64