The Challenges of Identifying Possible Biomarkers to take forward into further research from a subgroup analysis.

Caro-Anne Badcock (Statistical Revelations)

Renal specialist David Johnson, PhD Student Jeoungjee Cho (assays)

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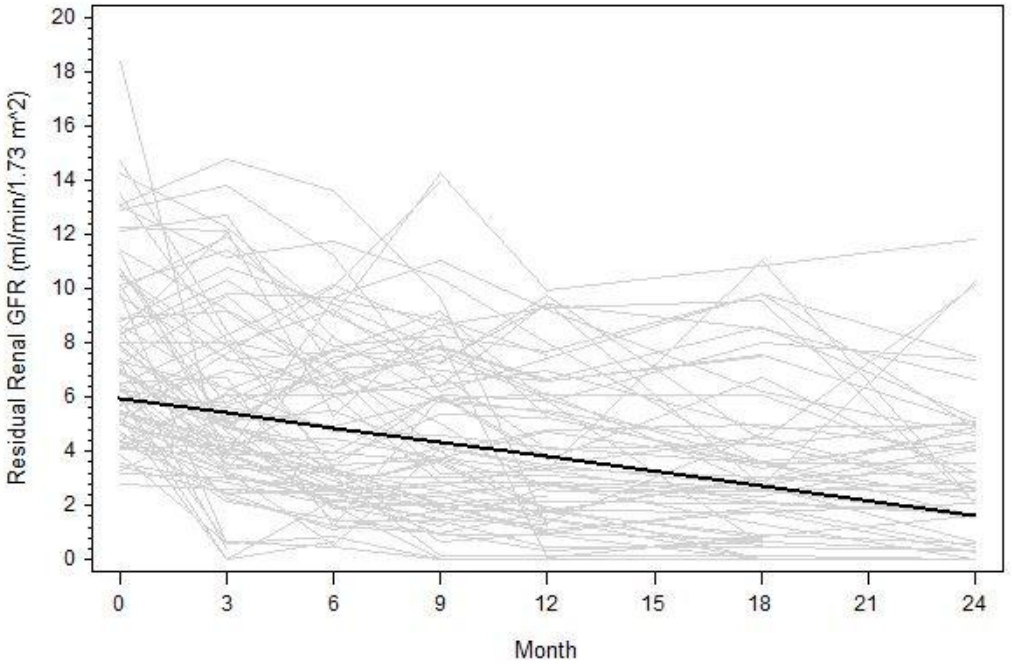
- Main study
- Sub-study
- Challenges/questions
- Original plan
- Actual approach
- Results
- Questions
- Conclusions

Main Study

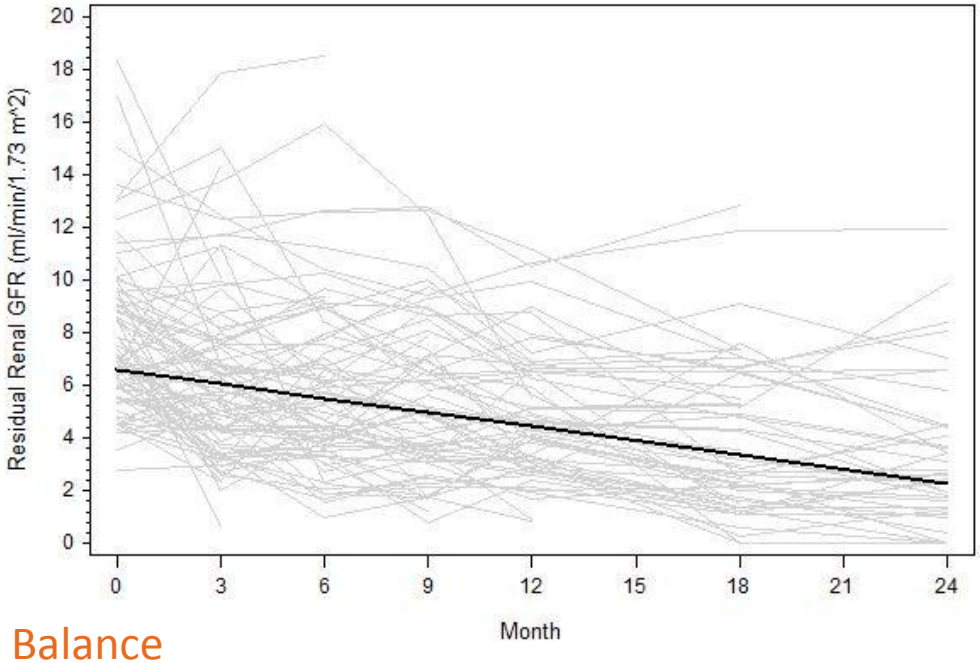
- Compare the rate of decline in residual renal function in peritoneal dialysis patients over 24 months using Balance solution compared with the conventional Stay.Safe solution.
- Secondary endpoints:
  - Time to anuria
  - Peritonitis infection rates
  - Ultrafiltration (adjusted for total glucose exposure)
  - Urine volume
  - 4 hr D/P creatinine and D/D0 glucose
- Total sample size 167

Linear slopes

Rate of change same for both treatments (-0.18),
12 mths difference Balance to SS 0.65 (95% CI=-0.17, 1.47)
24 mths 0.64 (95% CI=-0.54 to 1.82)



Stay safe/ Sleep safe



Balance

Medics questioned differences between first and second years
Dropouts – possibly informative
Time to anuria highly significant

Sub-study

- biomarker measurements: baseline, 3, 6, 12, 18 and 24 months.
- 19 kidney panel biomarkers:
  albumin, beta-2-microglobulin, cystatin, NGAL, osteopontin, uromodulin, EGF, α GST, calbindin, clusterin, KIM-1/TIM-1, osteoactivin, TFF3, VEGF, IP-10, MIG, MIF, TIMP-1, piGST, RBP4
- 1 peritoneal solute biomarker: IL-6
- Urine clinical outcomes: GFR, urine volume
- Peritoneal solute clinical outcomes: peritoneal ultrafiltration, peritoneal D:P Cr and D/D0 glucose, peritonitis infection rates
- Phase I
  - n=25 urine samples from each treatment arm (i.e N=50)
  - Times: baseline, 12 and 24 months
  - Patients must have completed the study
- Phase II
  - Expansion to all patients who completed the study (49 Stay.Safe, 42 Balance)

Challenges/ questions

- Different baselines (month 0, 1 or 3)
- Multiple biomarkers with multiple outcomes
- Different sets of biomarkers with different outcomes (kidney panel with urine outcomes, peritoneal biomarker with peritoneal outcomes)
- Should the baseline biomarker be used to predict outcome at 24 months?
- Should biomarkers measured over time be used to predict outcome?
- Transformation of biomarkers - logged
- Adjustment of biomarkers – adjusted for urine creatinine
- Does treatment need to be taken into account?
- Maybe treatment affects the biomarker?
- As sample has completed study maybe dilutes effect of biomarker?
- Desire to explore vs multiplicity
- Power to detect real effects for taking into further research.

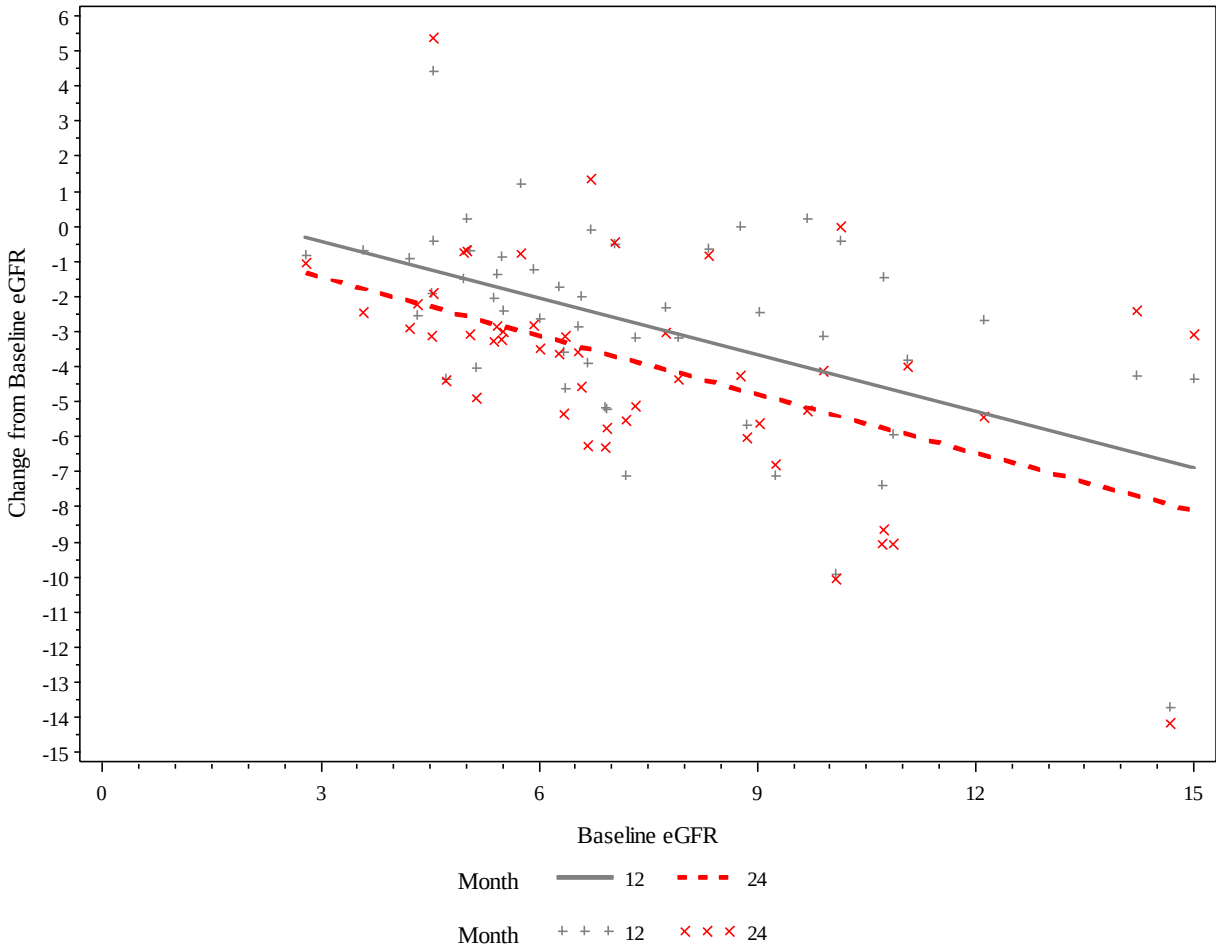
Original Plan

- Biomarker selection in the face of multiplicity – lots of research (gene expression)
- Baseline biomarkers
  - Compute variable importance measure (VIM) for each biomarker using targeted maximum likelihood estimation (TMLE)
    - Susan Gruber and Mark van der Laan, University of California, Berkeley (R program, binary point treatment on binary or continuous outcomes at one timepoint.
- Biomarkers over time
  - Compute 2 marginal VIMs for each biomarker using longitudinal TMLE (Bosny J, 2010)
    - Time slope through the origin. Biomarkers with a positive coeff will have positive effect
    - Absolute area under the LOESS curve (to capture a curvilinear effect over time if necessary)
- Estimate confidence intervals and p-values (using bootstrap)
- Adjust p-values for multiplicity to control false discovery rate using Benjamini and Yekutieli false discovery rate.
- Rank p-values to get index
- This and clinical interpretation to decide biomarkers to take forward.

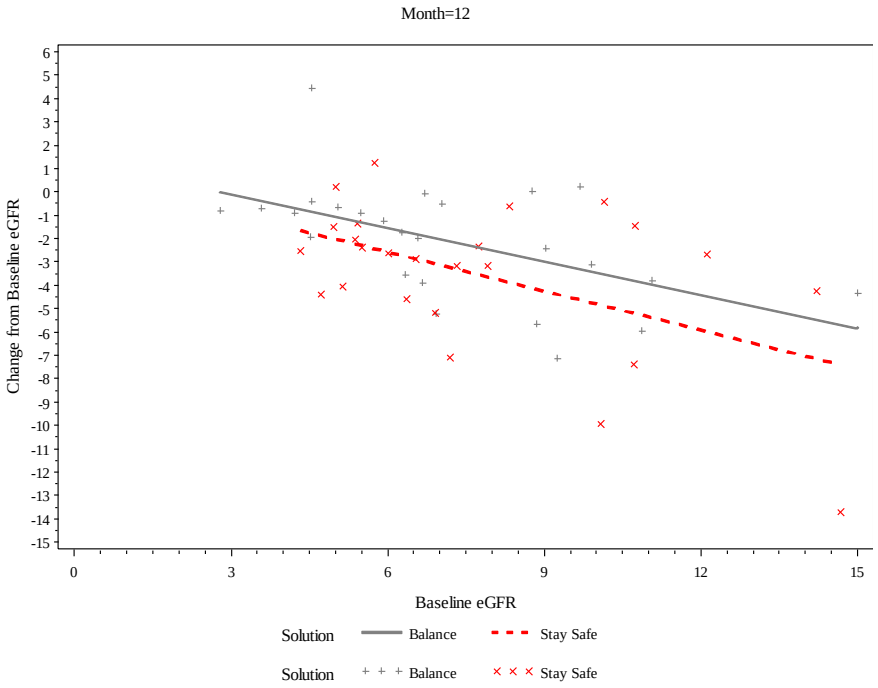
Actual Approach

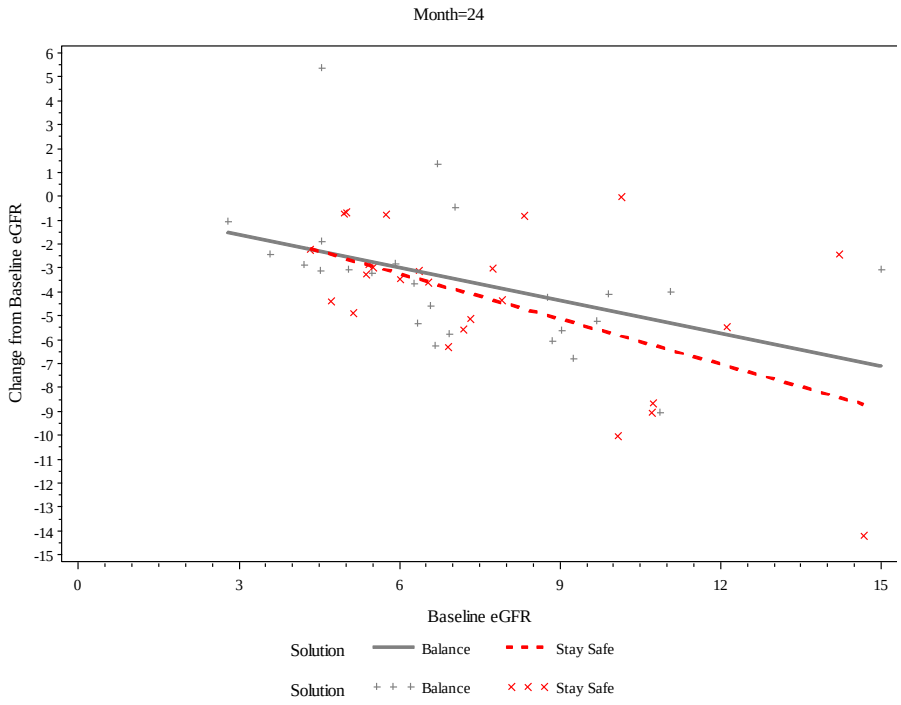
- Separated urine and peritoneal investigations
- For each biomarker
  - Analysed as change from baseline for 12 and 24 months
    - Adjusted for baseline biomarker only
    - Fitting baseline biomarker and treatment group
- For each clinical outcome (GFR, urine volume)
  - Same model as main study to confirm similar results (only 3 timepoints, 25 pats/gp)
  - Analysed as change from baseline to give similar format as for biomarkers
  - Analysed as change from baseline for 12, 24 months,
    - adjusted for baseline GFR only
    - fitting baseline GFR and treatment group
    - Fitting baseline GFR and urine urine albumin: creatinine ratio (extra variable added much later,)
    - fitting baseline GFR and separately for each biomarker
    - Fitting baseline GFR and treatment group and separately for each biomarker
    - Fitting baseline GFR and urine ACR and separately for each biomarker
    - Fitting baseline GFR and treatment group and urine ACR and separately for each biomarker

GFR

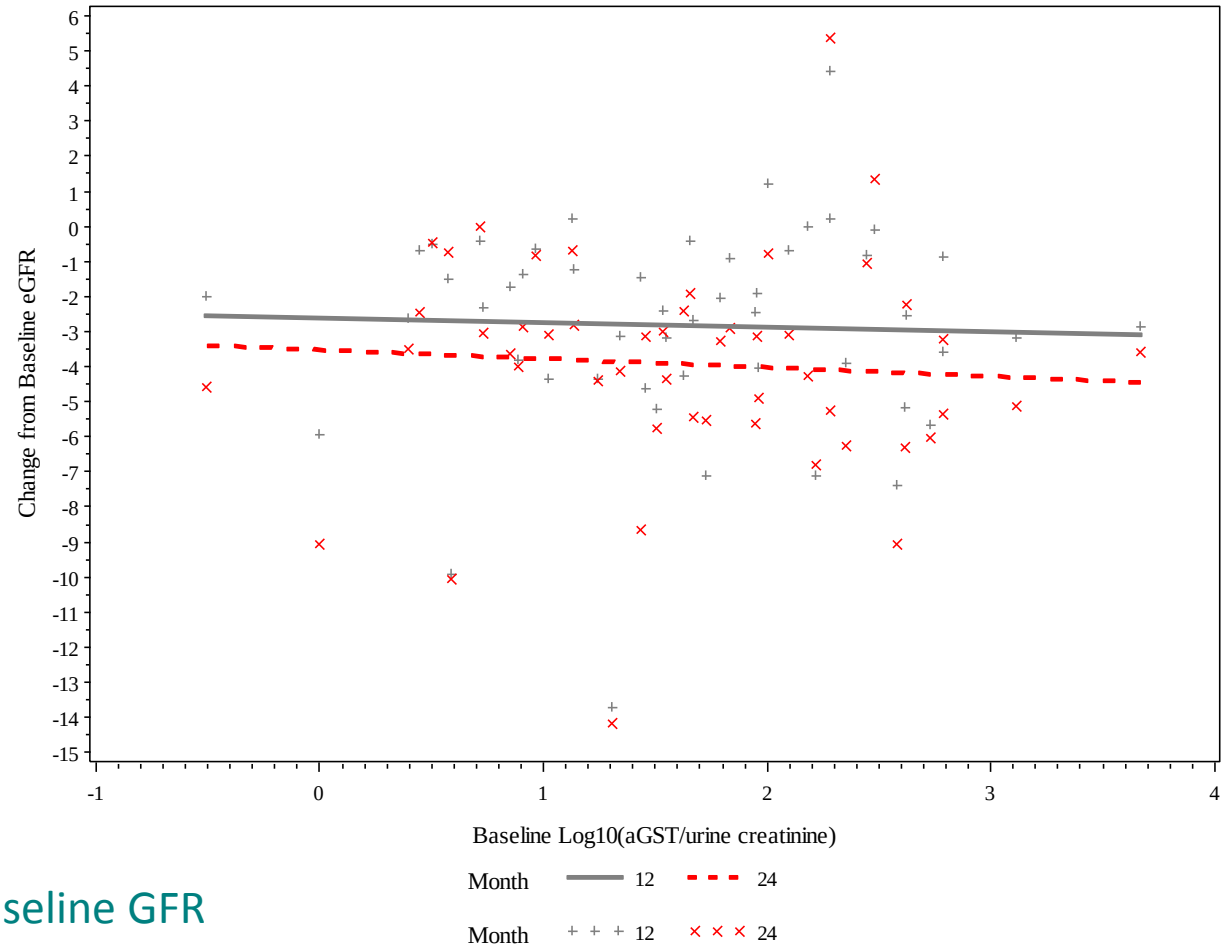

Change from Baseline eGFR
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
-11
-12
-13
-14
-15
0
3
6
9
12
15
Baseline eGFR
Month
12
24
Month
12
24

GFR by treatment



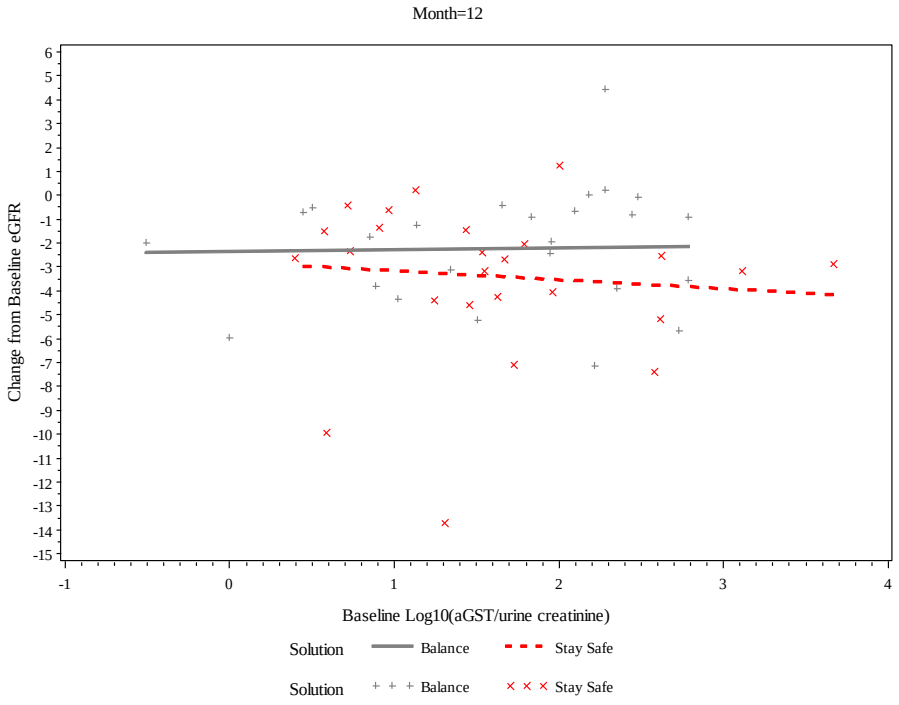


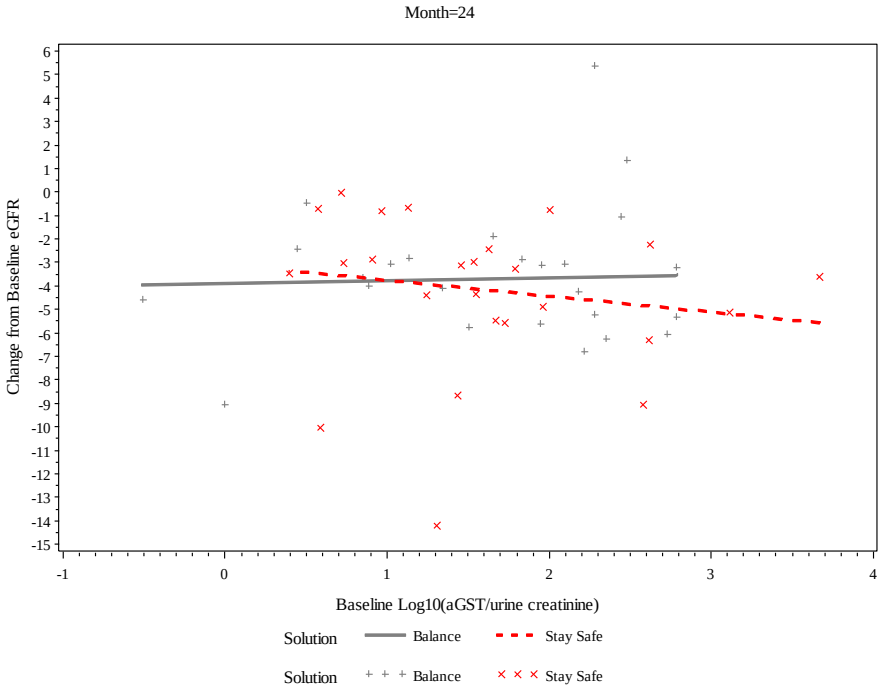
αGST



At mean baseline GFR

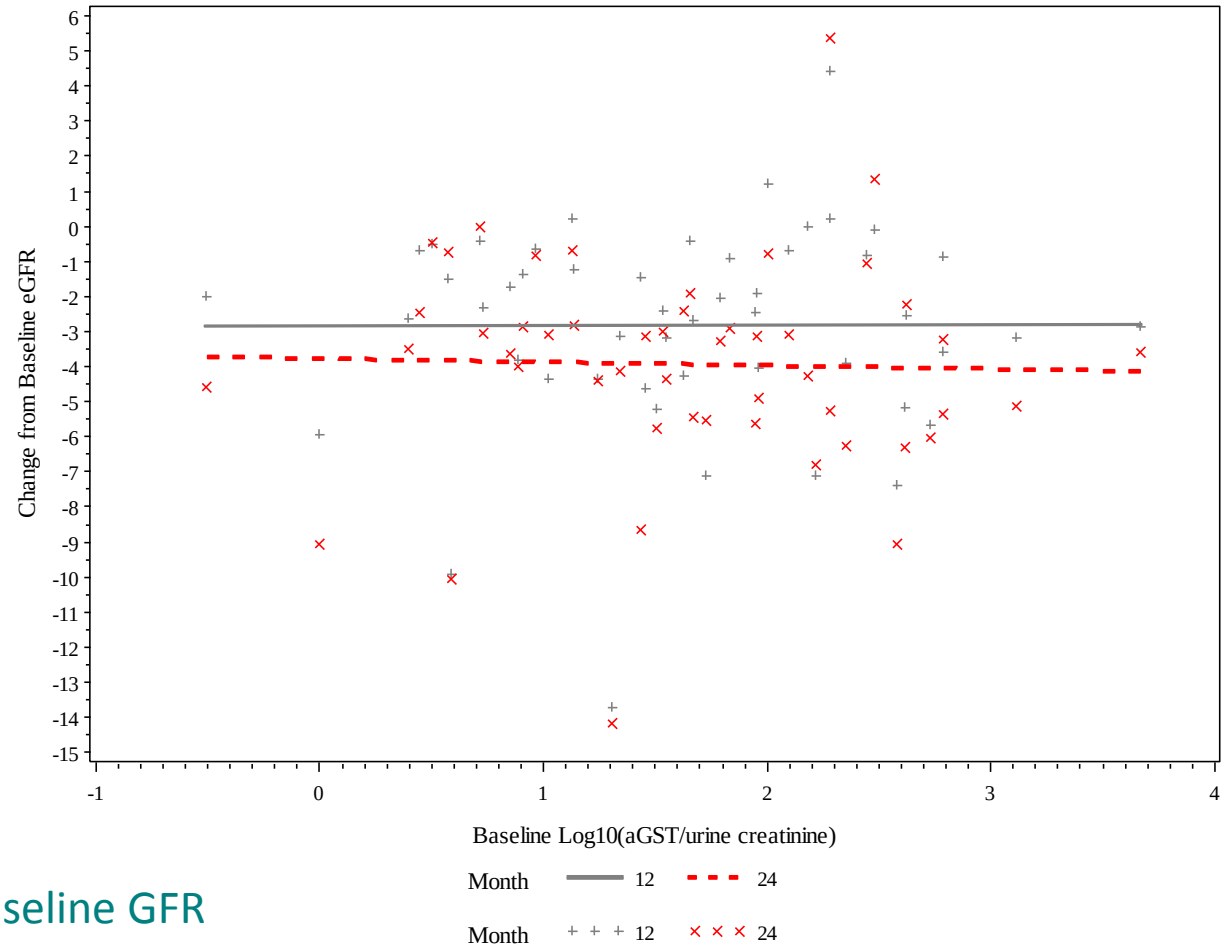
αGST by treatment





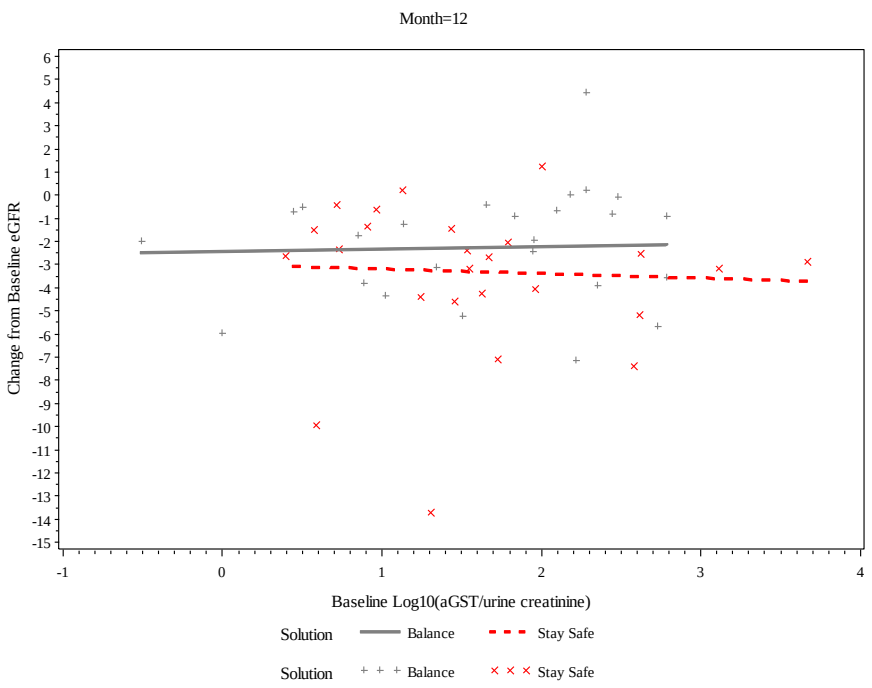
At mean baseline GFR

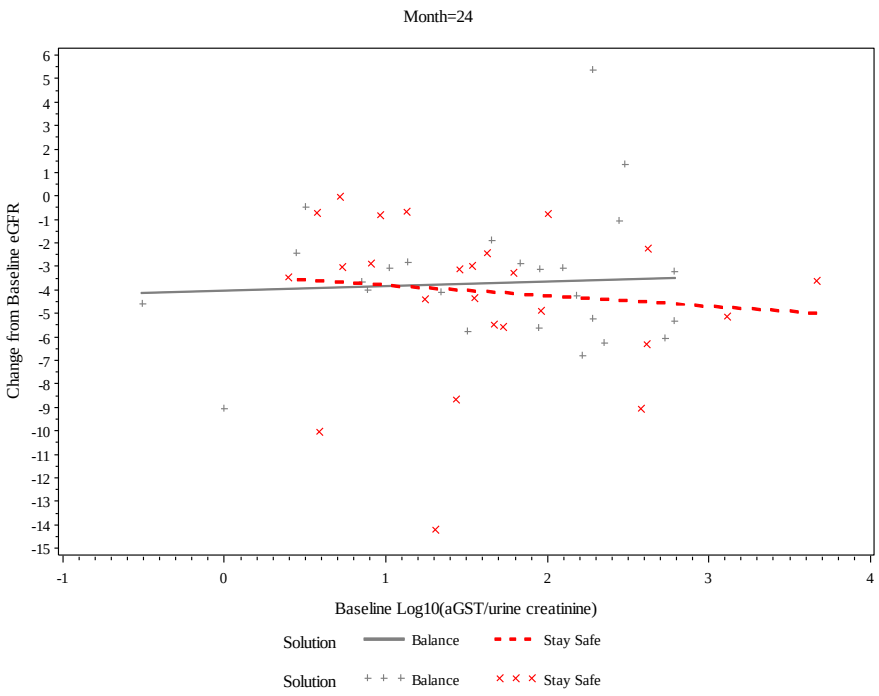
αGST adjusted for baseline urine ACR



At mean baseline GFR

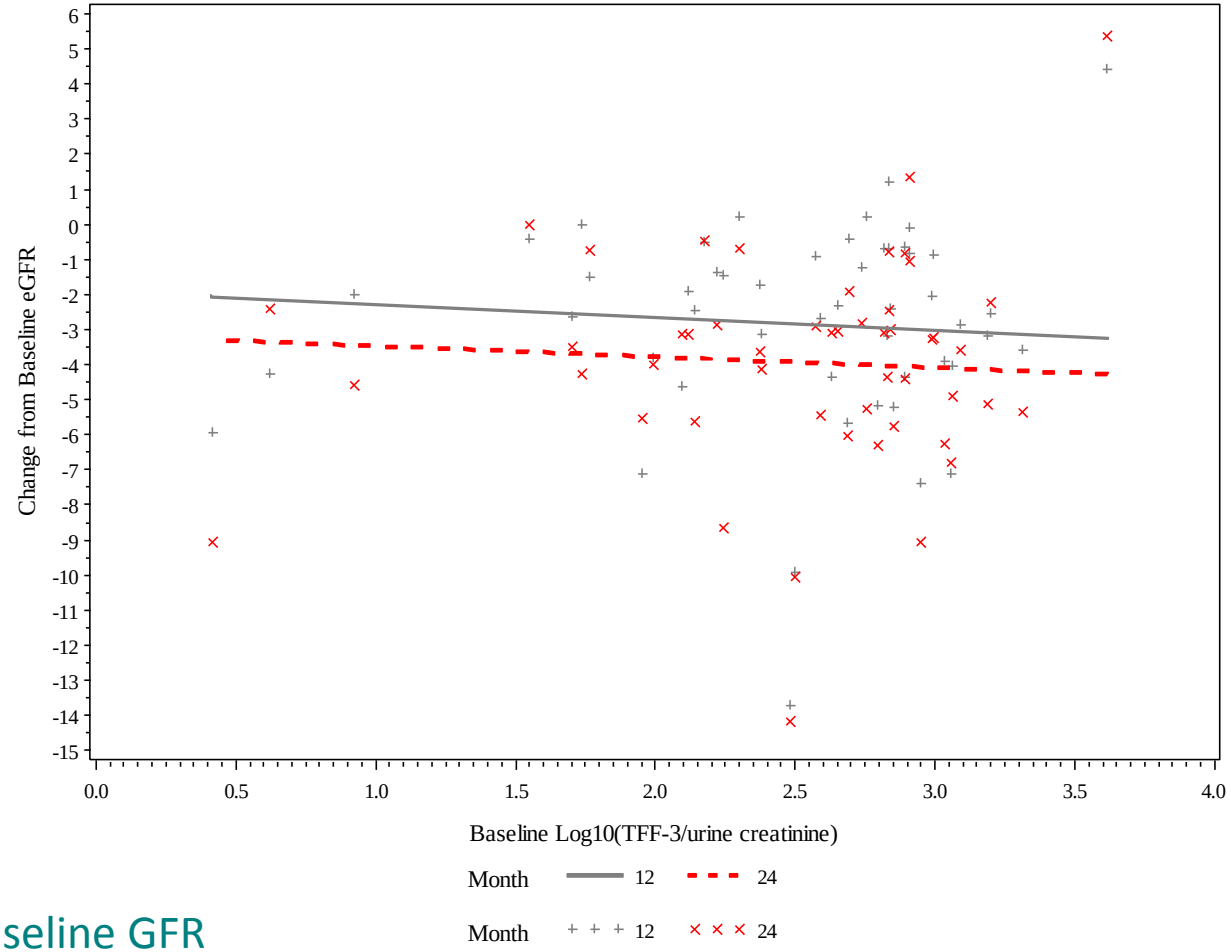
αGST adjusted for baseline urine ACR and treatment





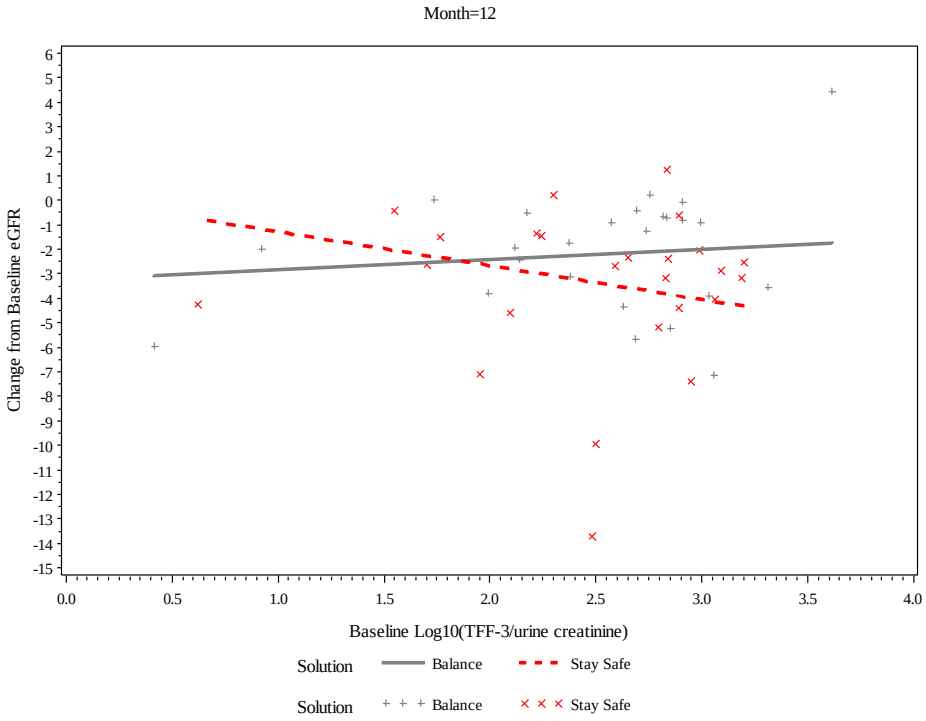
At mean baseline GFR

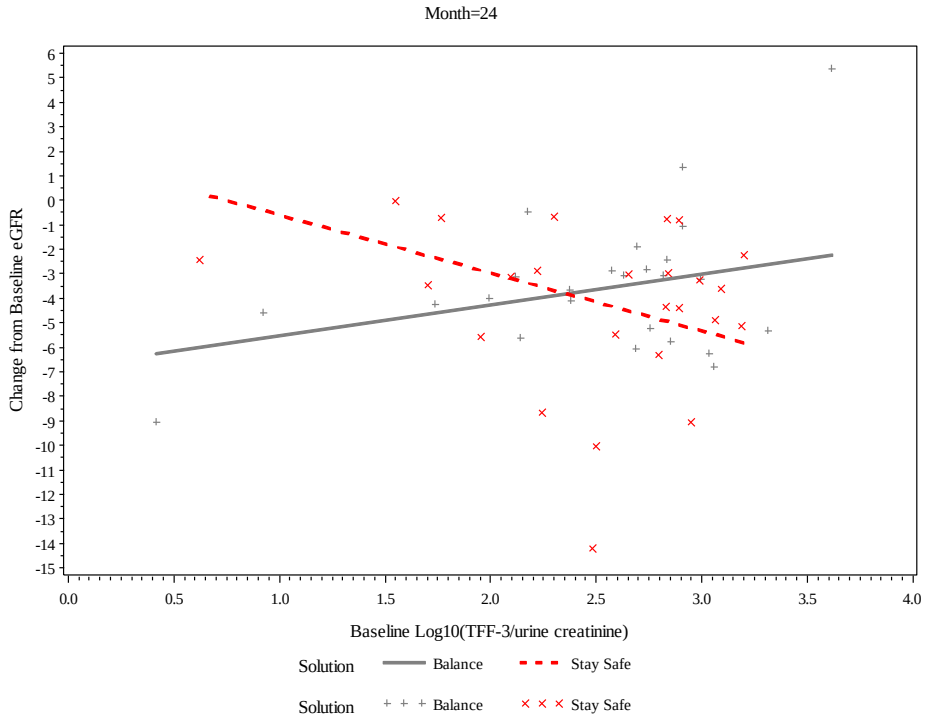
TFF-3



At mean baseline GFR

TFF-3 by treatment

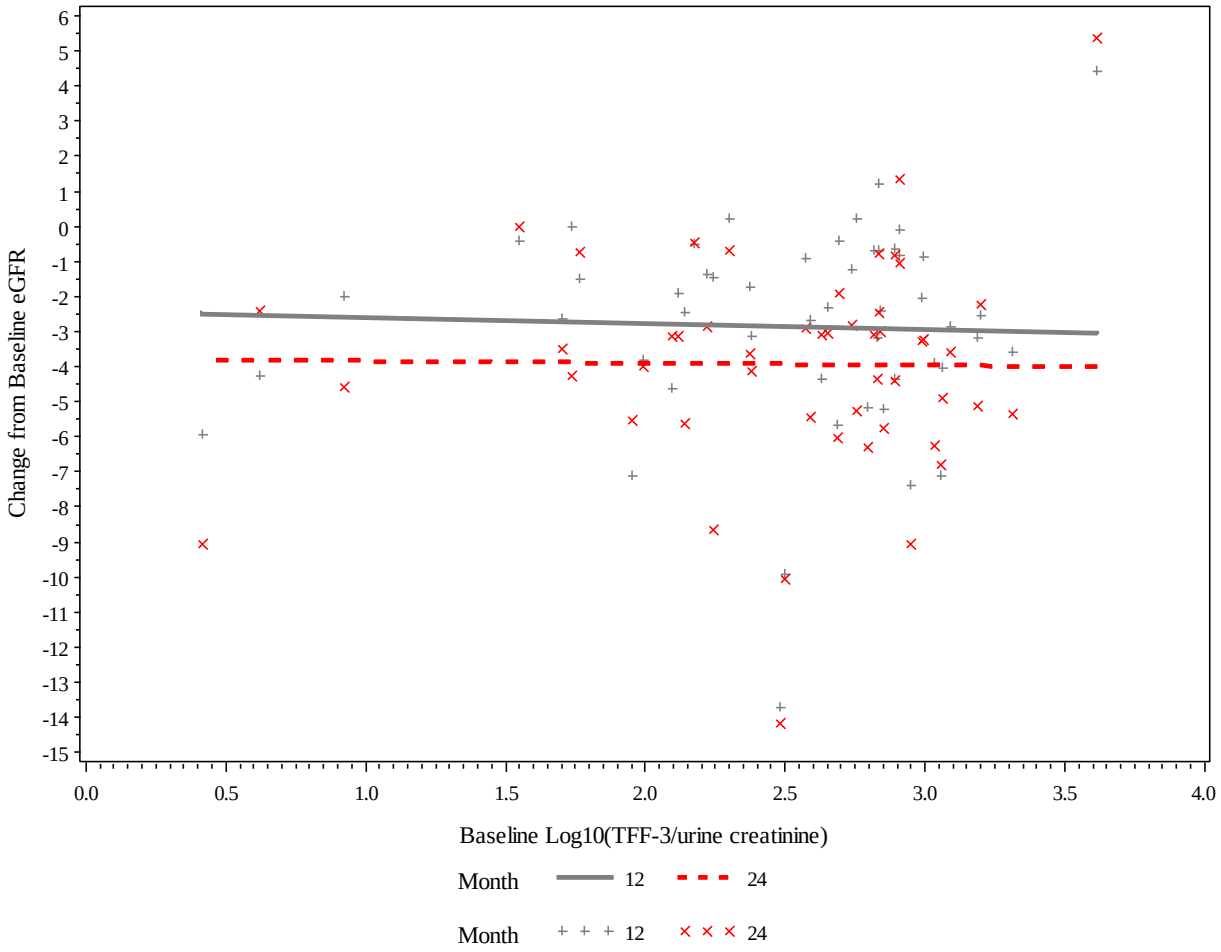




At mean baseline GFR

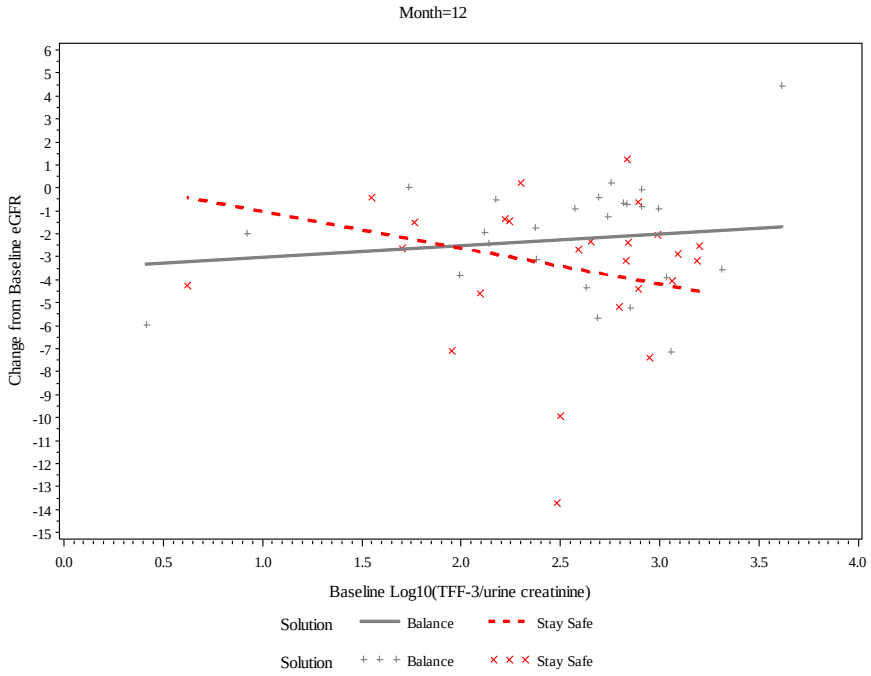
TFF3 x treatment x time p=0.02

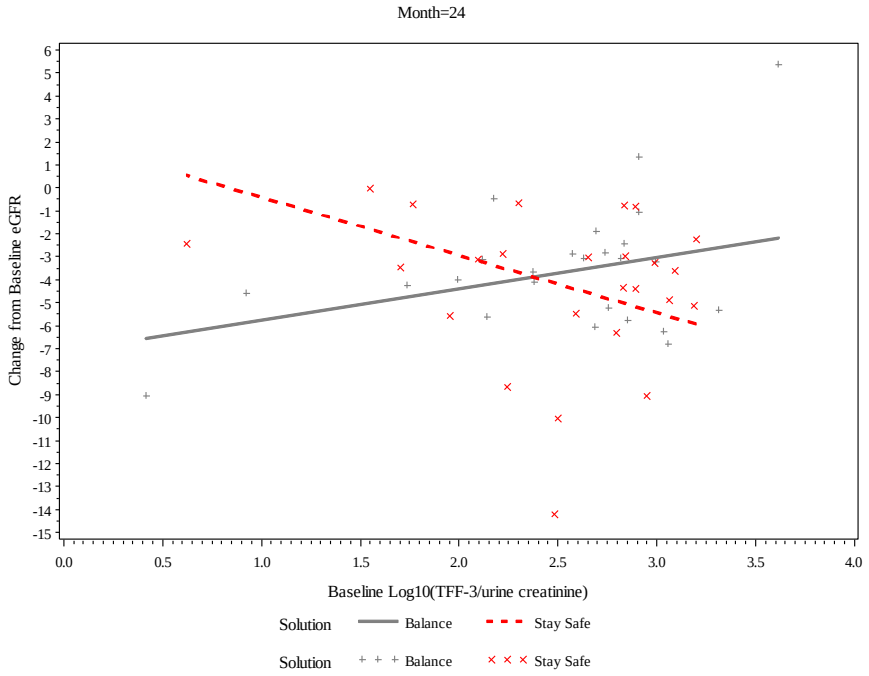
TFF3 adjusted for baseline urine ACR



At mean baseline GFR and ACR

TFF3 adjusted for baseline urine ACR and treatment





At mean baseline GFR and ACR

TFF3 x treatment x time p=0.02

Urine outcomes: questions

- p-values
  - importance
  - Adjustment of p-values for multiplicity
  - how many analyses to adjust for
- Interactions
  - Real?
  - Should baseline biomarker x treatment be fitted as both prior to randomisation
  - Cross over effect with baseline (eg Balance over all baseline TFF3 improves GFR but higher baseline TFF 3 with Stay.Safe)
- Interpretation of relationships
  - Transformation of outcomes (in this case none)
  - Transformation of biomarkers (originally logged only, then biomarker:urine creatinine ratio logged)

Conclusions

- Accept the need to explore all possible combinations
- Write up in exploratory framework
- Attempt to reduce reliance on p-values
  - May have no significant p-values given sub-group but might have interesting pattern to explore in further research