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December 20, 2020

To: Lesley Inker, MD, MS
Tom Greene, PhD
Hocine Tighiouart, MS
Jian Ying, PhD

From: Edward F. Vonesh, PhD

Topic: Amendment to Technical report summarizing different methods for determining and characterizing the potential timing of acute effects based on select CKD clinical trials.

1. Introduction

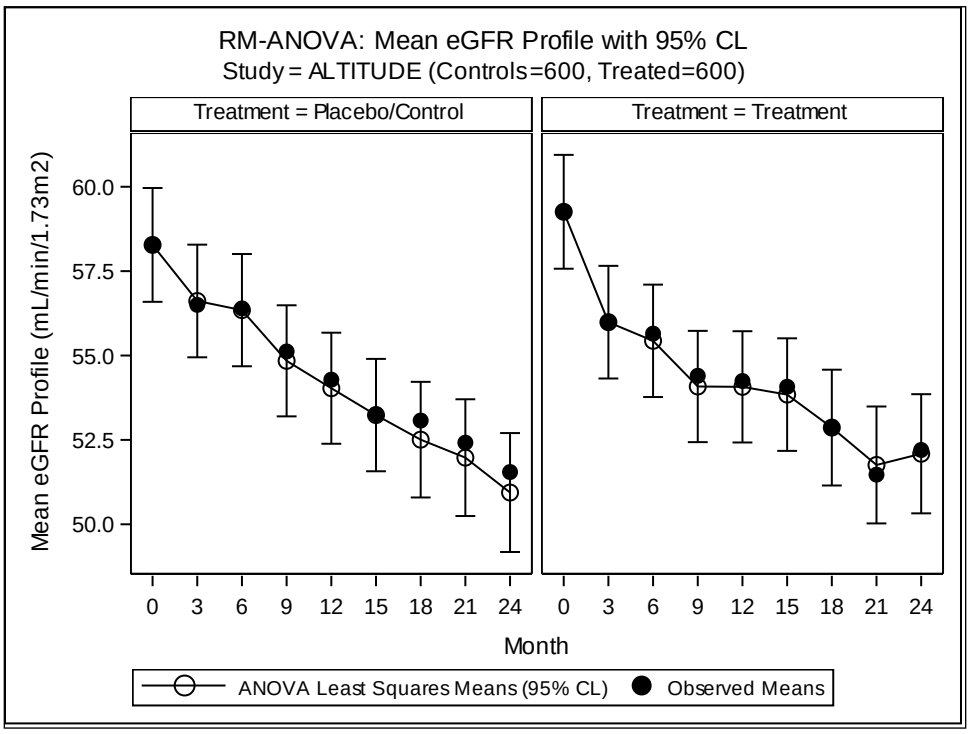
This amendment to the initial report gives results for the RM-ANOVA and RM-ANCOVA analyses in which a weighted linear spline regression model was fit to the least squares means with weights given by the squared inverse of the RM-ANOVA (or RM-ANCOVA) standard errors. This provides a best linear unbiased estimate of the linear spline parameters based on the estimated variances of the RM-ANOVA and RM-ANCOVA least square means. The results are graphically displayed in the attached Appendices 1a – 9a corresponding to the 9 selected studies. While the regression parameters from the weighted linear spline regression analysis differ slightly from those given by the unweighted linear spline regression analysis, there are no perceptible differences in the prediction lines (see attached graphs) nor has there been any change in the knot selection or in the estimated acute treatment effects which are based on the least squares mean differences and their standard errors.

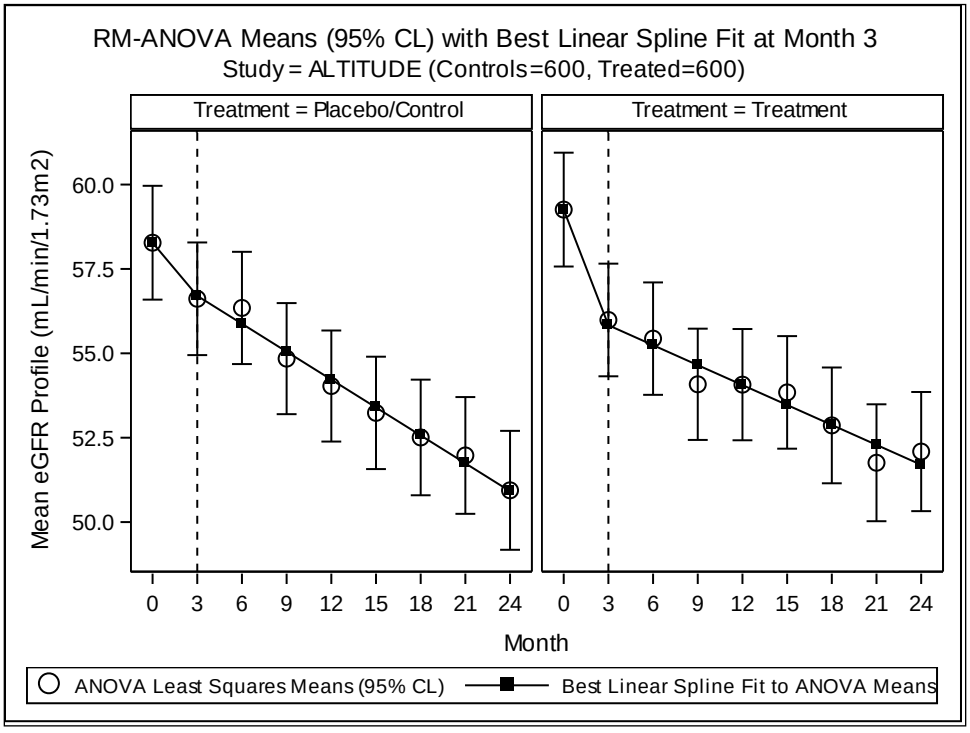
2. Conclusions

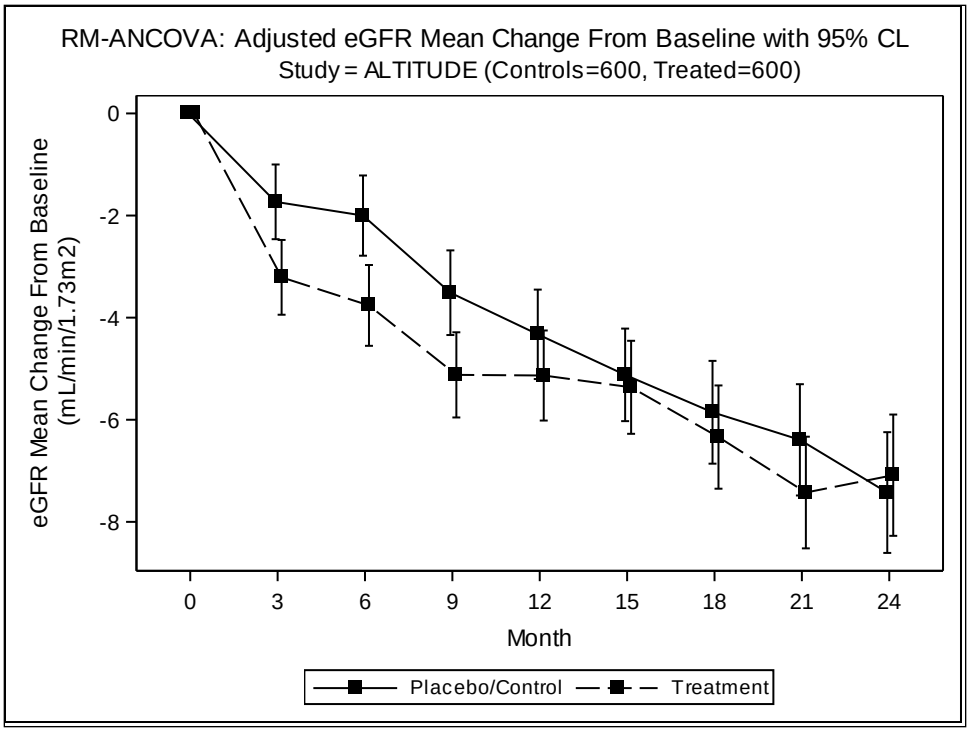
This new analysis supports the original findings as it pertains to the two-step repeated measures profile analysis using either a RM-ANOVA model or a RM-ANCOVA model. Results for the linear spline-mixed-effects analyses remain unchanged from the original report.

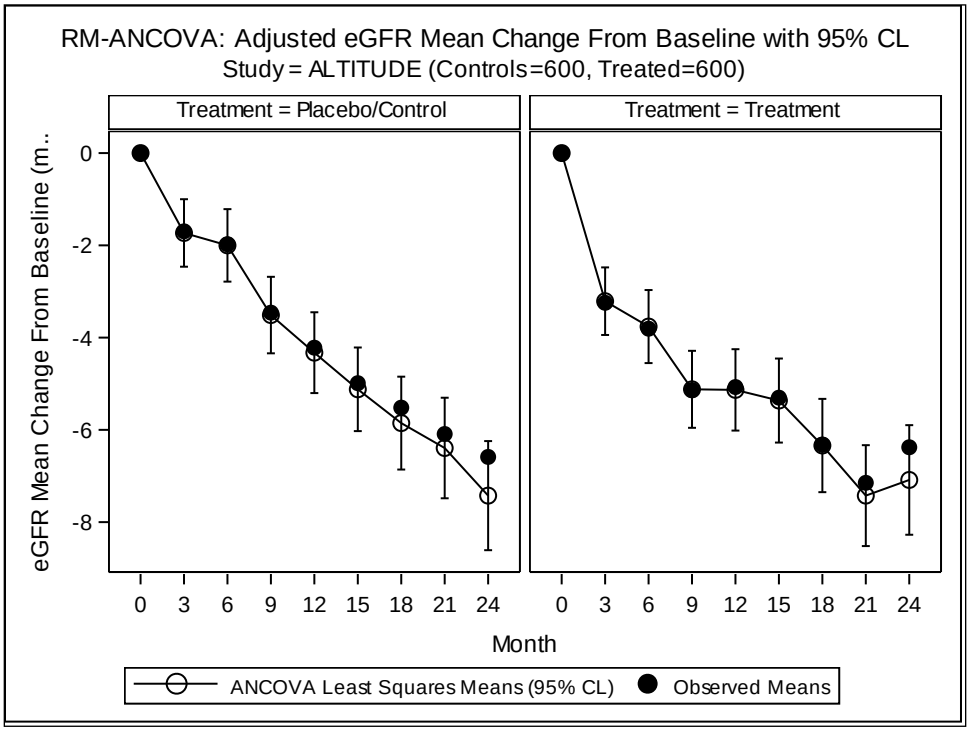
Appendix 1a: ALTITUDE results

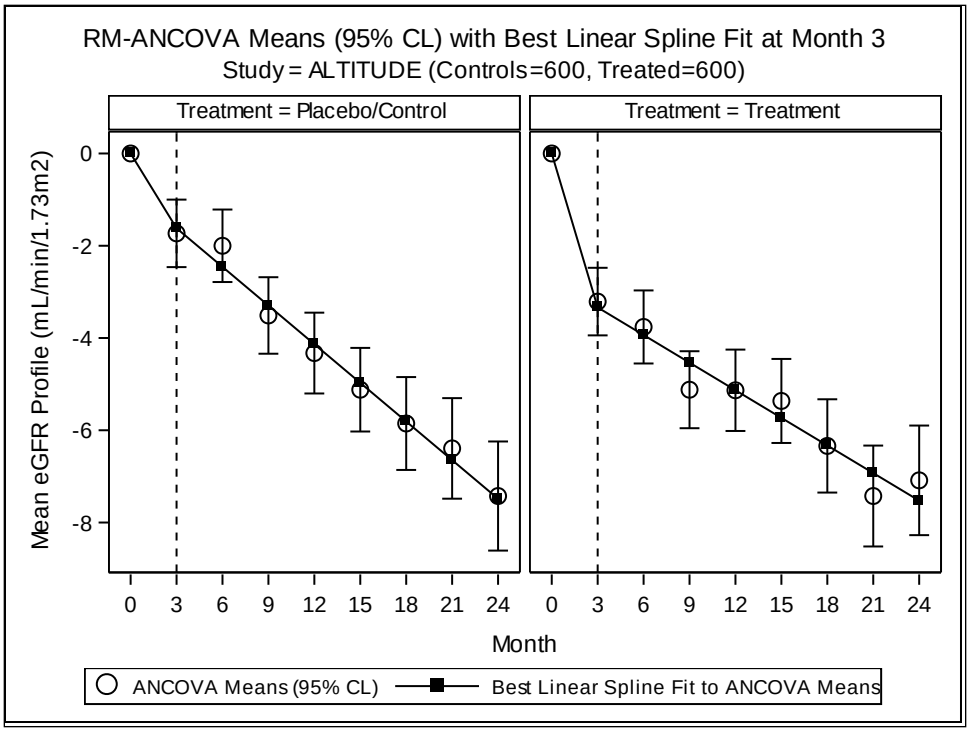
Case 1: Sample Size=600 per Treatment group

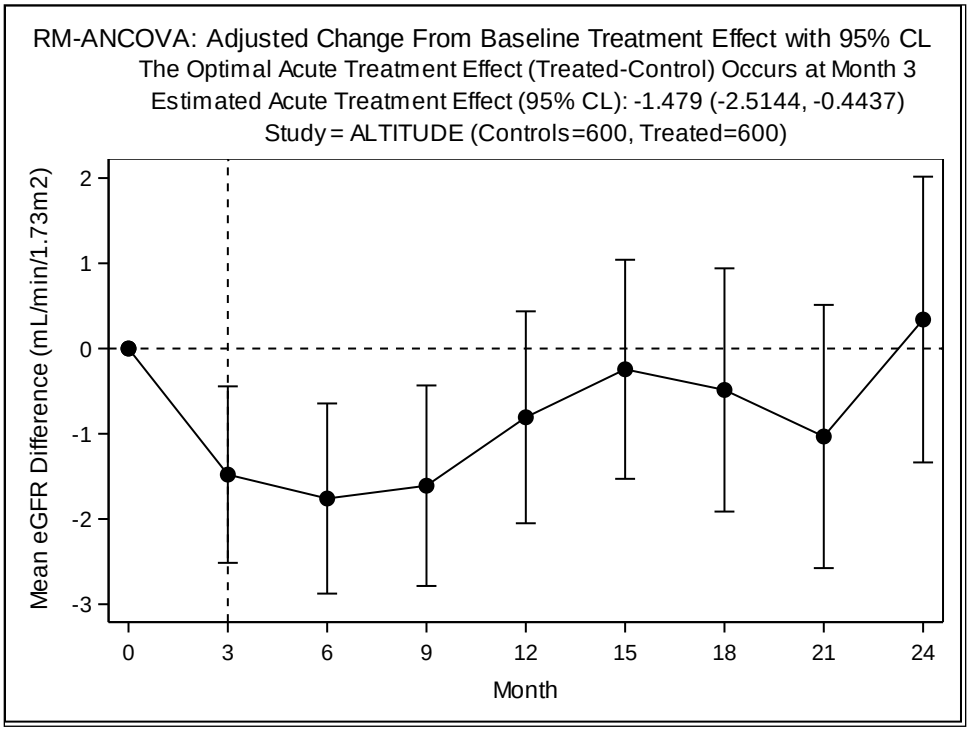




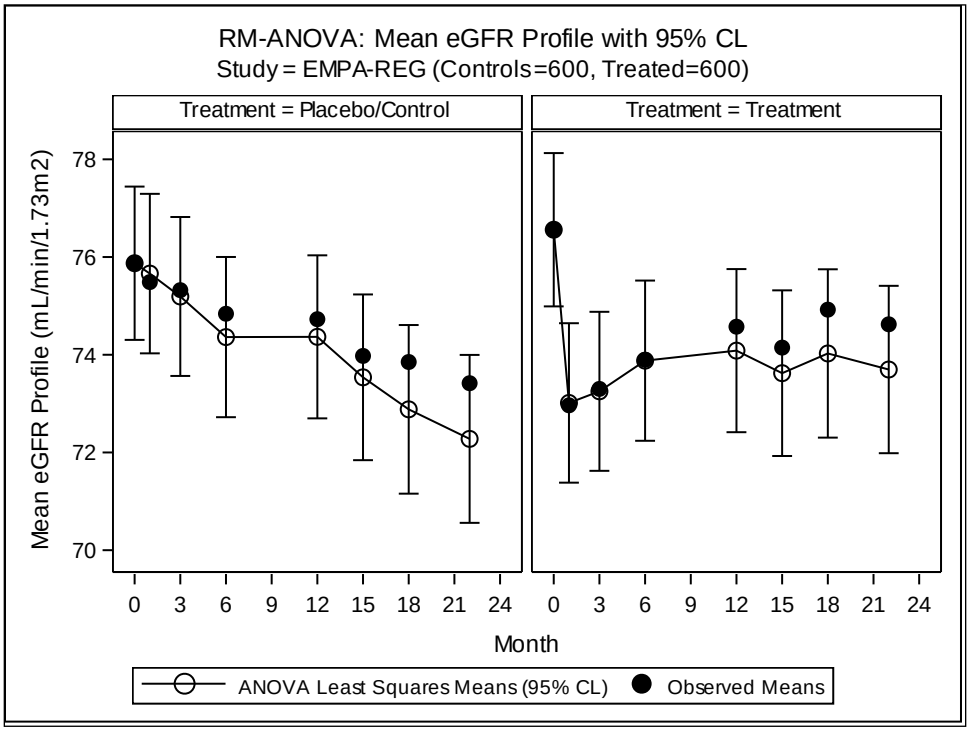

RM-ANCOVA: Adjusted eGFR Mean Change From Baseline with 95% CL
Study = ALTITUDE (Controls=600, Treated=600)
eGFR Mean Change From Baseline (mL/min/1.73m2)
0
-2
-4
-6
-8
0
3
6
9
12
15
18
21
24
Month
Placebo/Control
Treatment

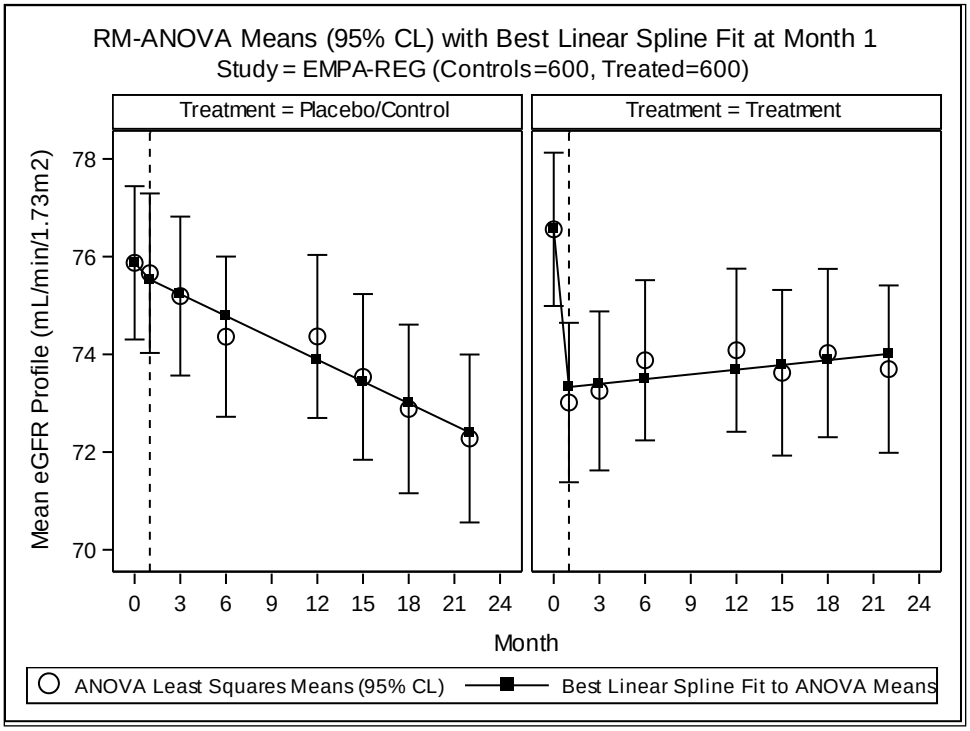

RM-ANCOVA: Adjusted eGFR Mean Change From Baseline with 95% CL
Study = ALTITUDE (Controls=600, Treated=600)
Treatment = Placebo/Control
Treatment = Treatment
eGFR Mean Change From Baseline (m...
0
-2
-4
-6
-8
0
3
6
9
12
15
18
21
24
Month
ANCOVA Least Squares Means (95% CL)
Observed Means

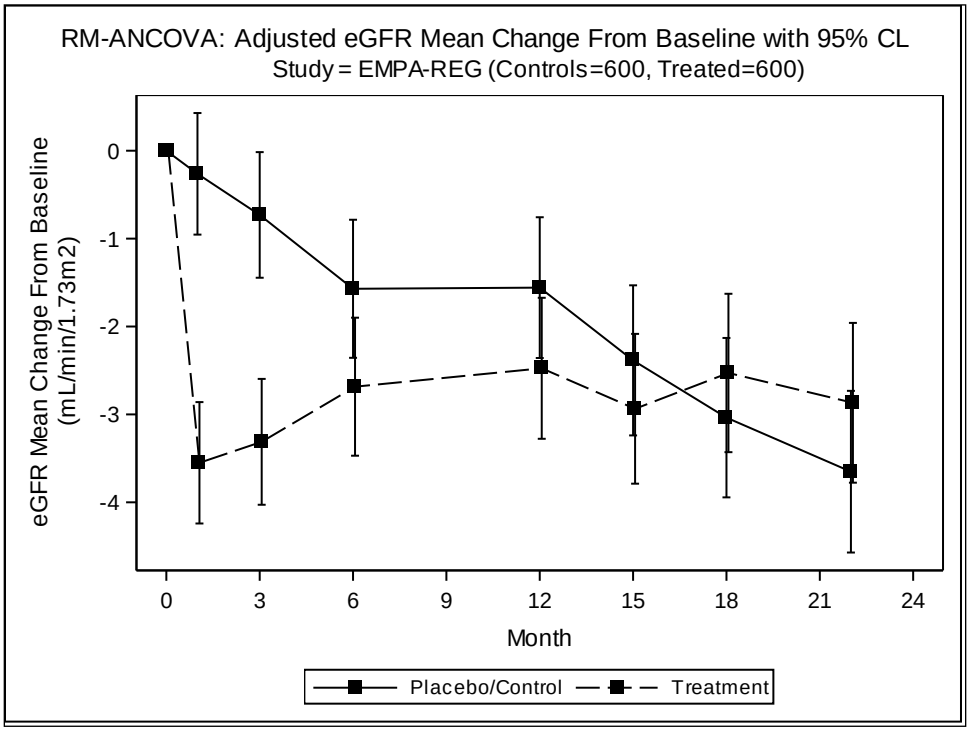

RM-ANCOVA Means (95% CL) with Best Linear Spline Fit at Month 3
Study = ALTITUDE (Controls=600, Treated=600)
Treatment = Placebo/Control
Treatment = Treatment
Mean eGFR Profile (mL/min/1.73m2)
0
-2
-4
-6
-8
0 3 6 9 12 15 18 21 24
Month
ANCOVA Means (95% CL)
Best Linear Spline Fit to ANCOVA Means

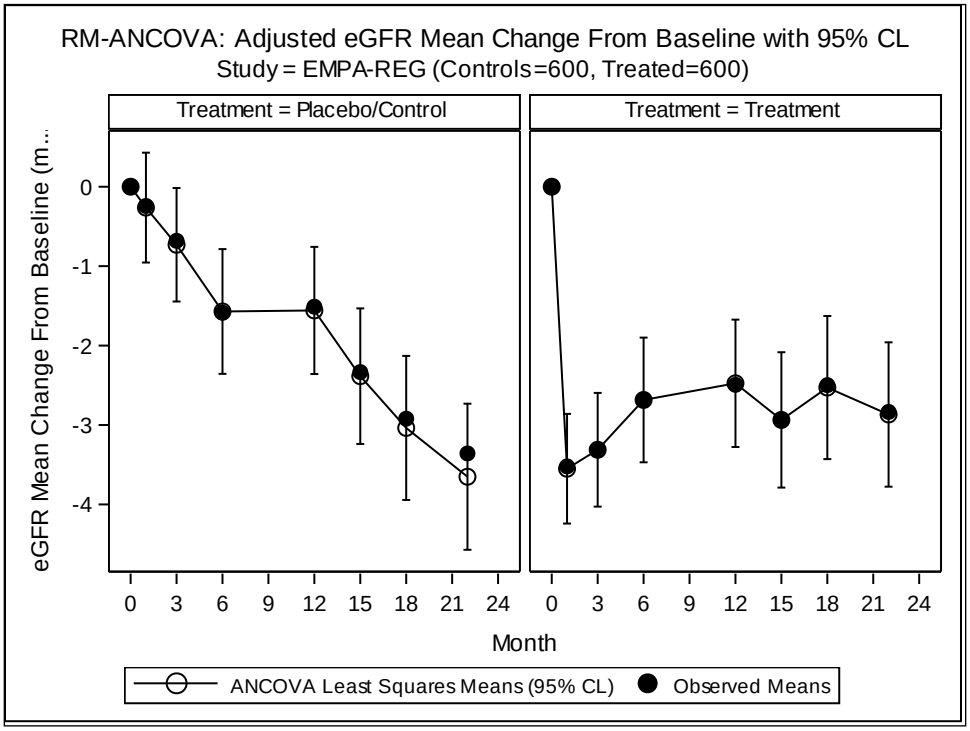

RM-ANCOVA: Adjusted Change From Baseline Treatment Effect with 95% CL
The Optimal Acute Treatment Effect (Treated-Control) Occurs at Month 3
Estimated Acute Treatment Effect (95% CL): -1.479 (-2.5144, -0.4437)
Study = ALTITUDE (Controls=600, Treated=600)
Mean eGFR Difference (mL/min/1.73m2)
2
1
0
-1
-2
-3
0 3 6 9 12 15 18 21 24
Month

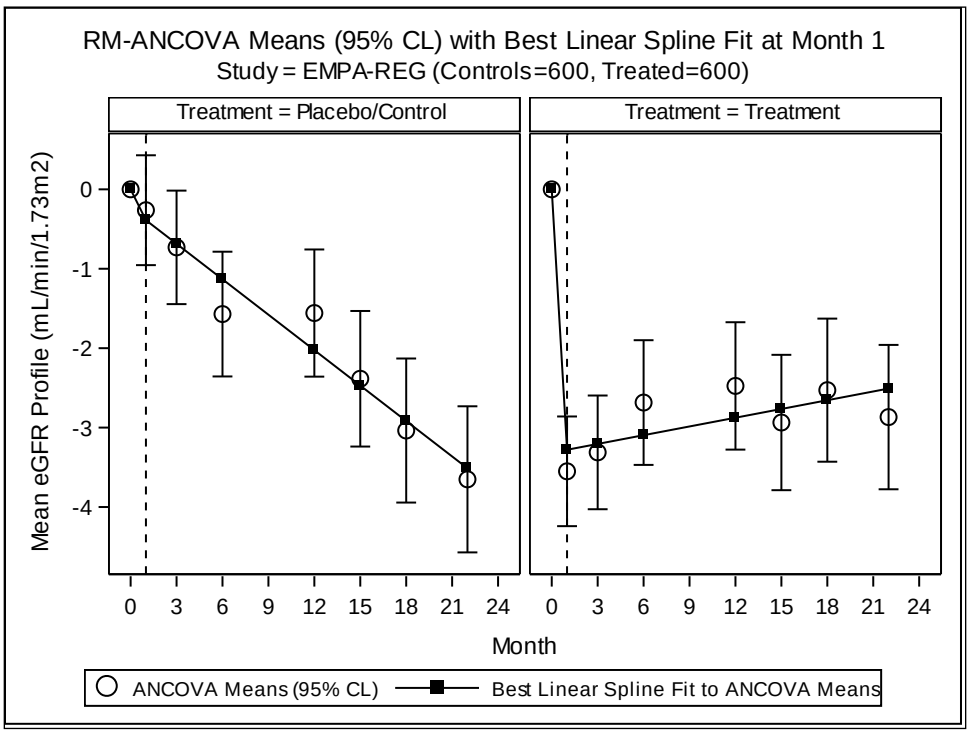
Appendix 2a: EMPA-REG results

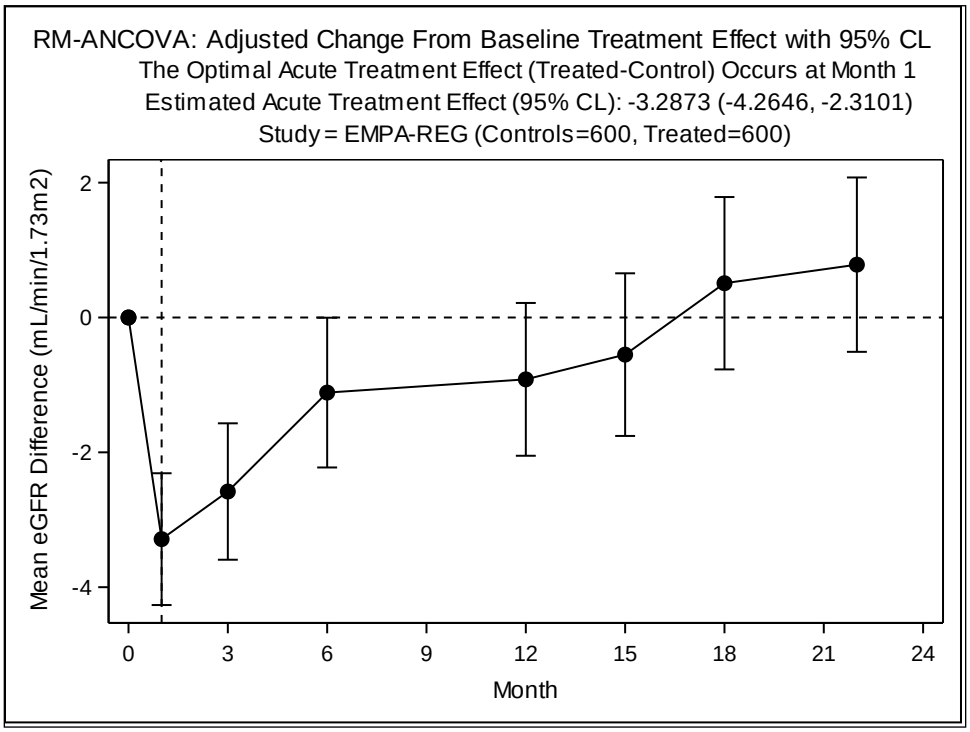




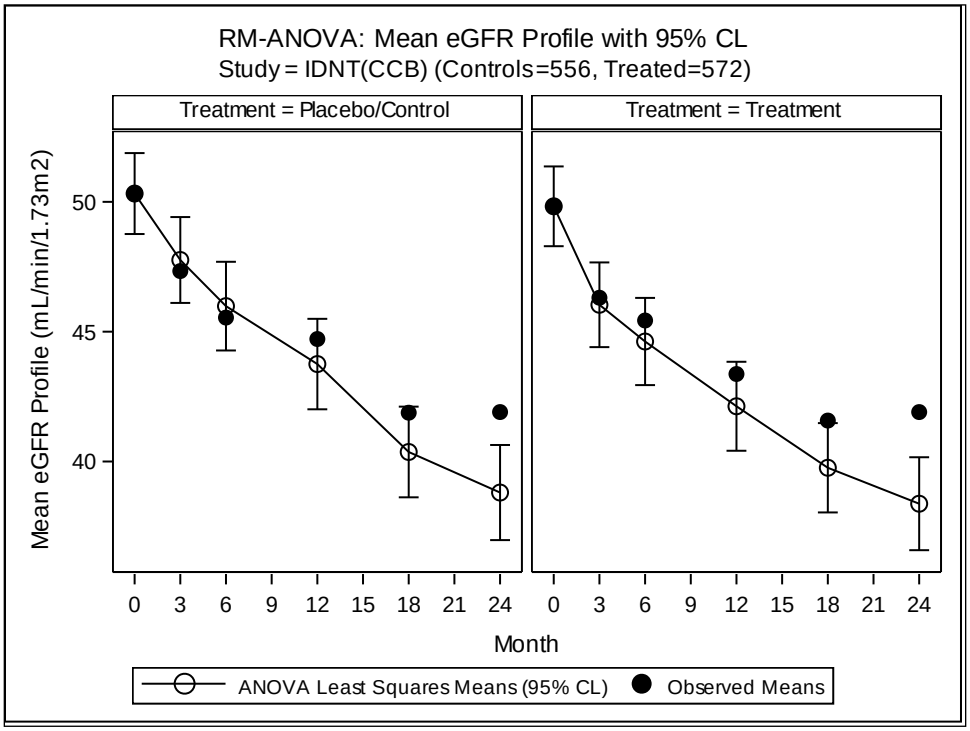

RM-ANCOVA: Adjusted eGFR Mean Change From Baseline with 95% CL
Study = EMPA-REG (Controls=600, Treated=600)
eGFR Mean Change From Baseline (mL/min/1.73m2)
0
-1
-2
-3
-4
0
3
6
9
12
15
18
21
24
Month
Placebo/Control
Treatment

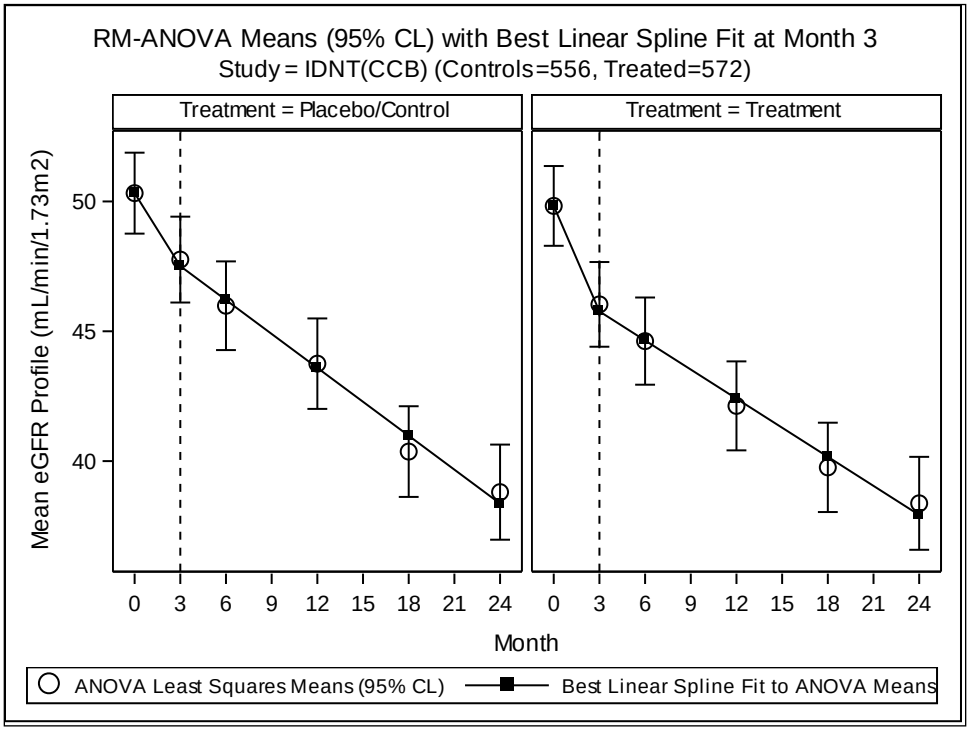

RM-ANCOVA: Adjusted eGFR Mean Change From Baseline with 95% CL
Study = EMPA-REG (Controls=600, Treated=600)
Treatment = Placebo/Control
Treatment = Treatment
eGFR Mean Change From Baseline (m...
0
-1
-2
-3
-4
0
3
6
9
12
15
18
21
24
Month
ANCOVA Least Squares Means (95% CL)
Observed Means

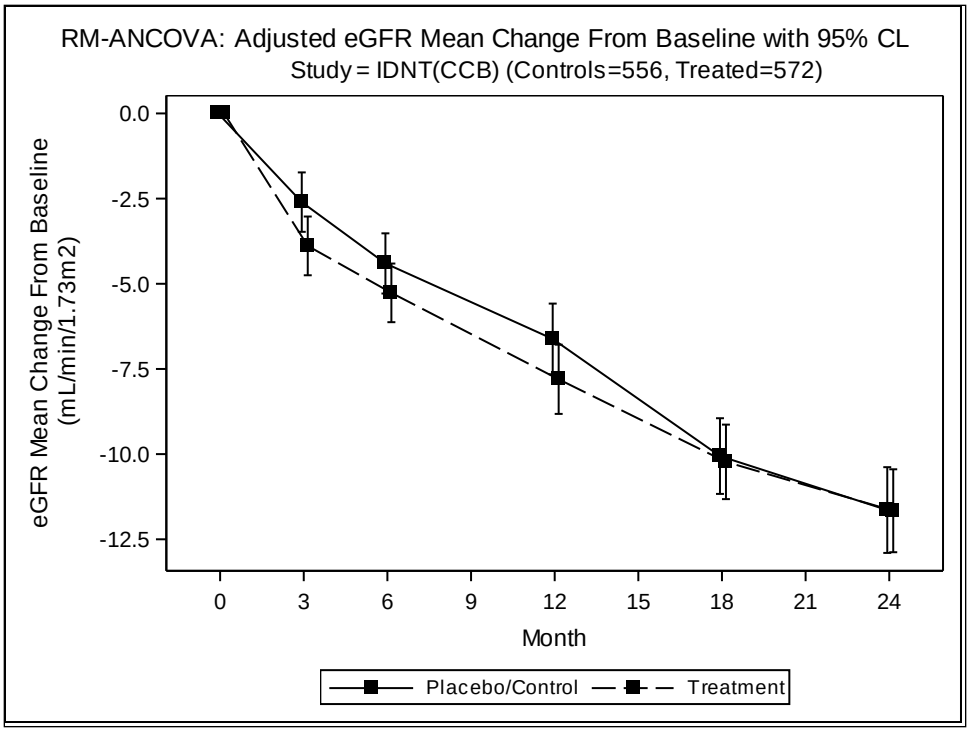

RM-ANCOVA Means (95% CL) with Best Linear Spline Fit at Month 1
Study = EMPA-REG (Controls=600, Treated=600)
Treatment = Placebo/Control
Treatment = Treatment
Mean eGFR Profile (mL/min/1.73m2)
0
-1
-2
-3
-4
0 3 6 9 12 15 18 21 24
Month
ANCOVA Means (95% CL)
Best Linear Spline Fit to ANCOVA Means

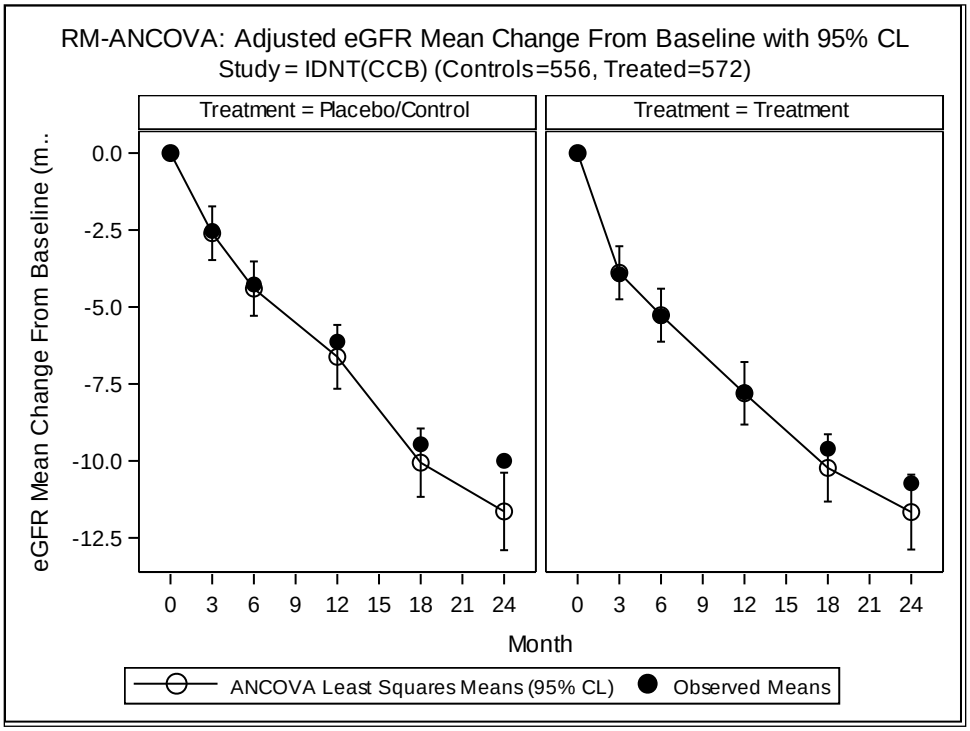

RM-ANCOVA: Adjusted Change From Baseline Treatment Effect with 95% CL
The Optimal Acute Treatment Effect (Treated-Control) Occurs at Month 1
Estimated Acute Treatment Effect (95% CL): -3.2873 (-4.2646, -2.3101)
Study = EMPA-REG (Controls=600, Treated=600)
Mean eGFR Difference (mL/min/1.73m2)
2
0
-2
-4
0 3 6 9 12 15 18 21 24
Month

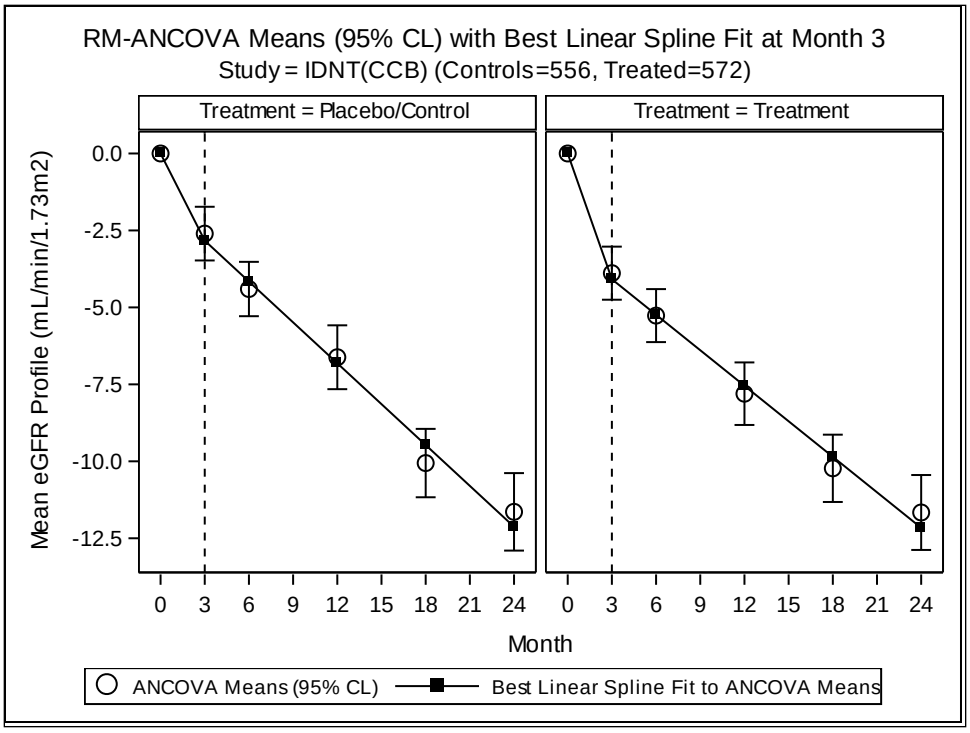
Appendix 3a: IDNT(CCB) results

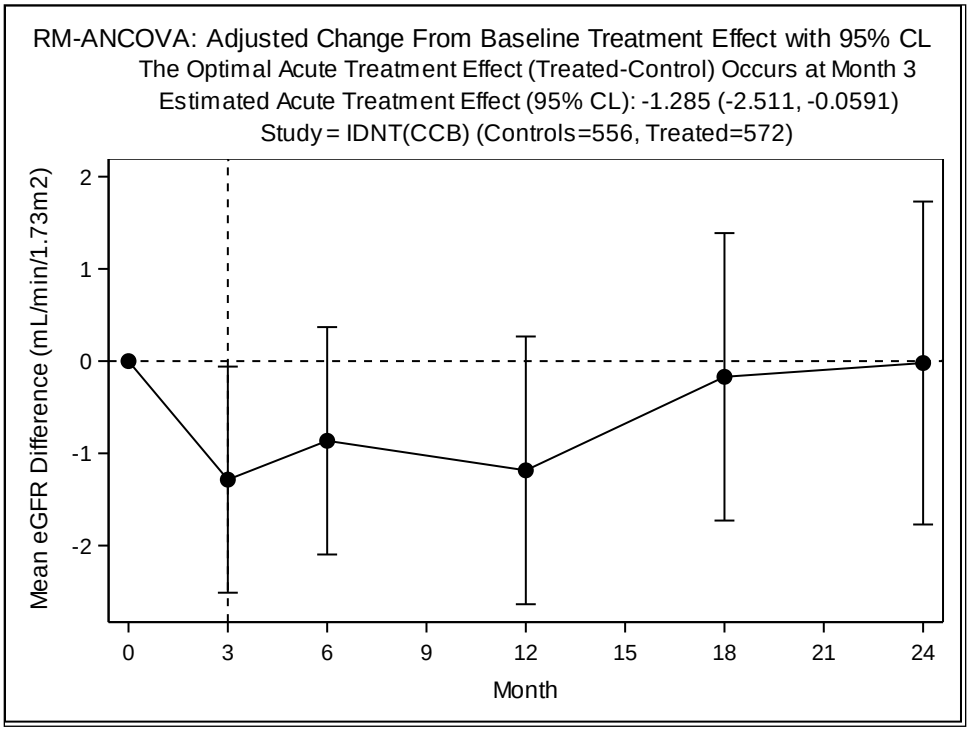




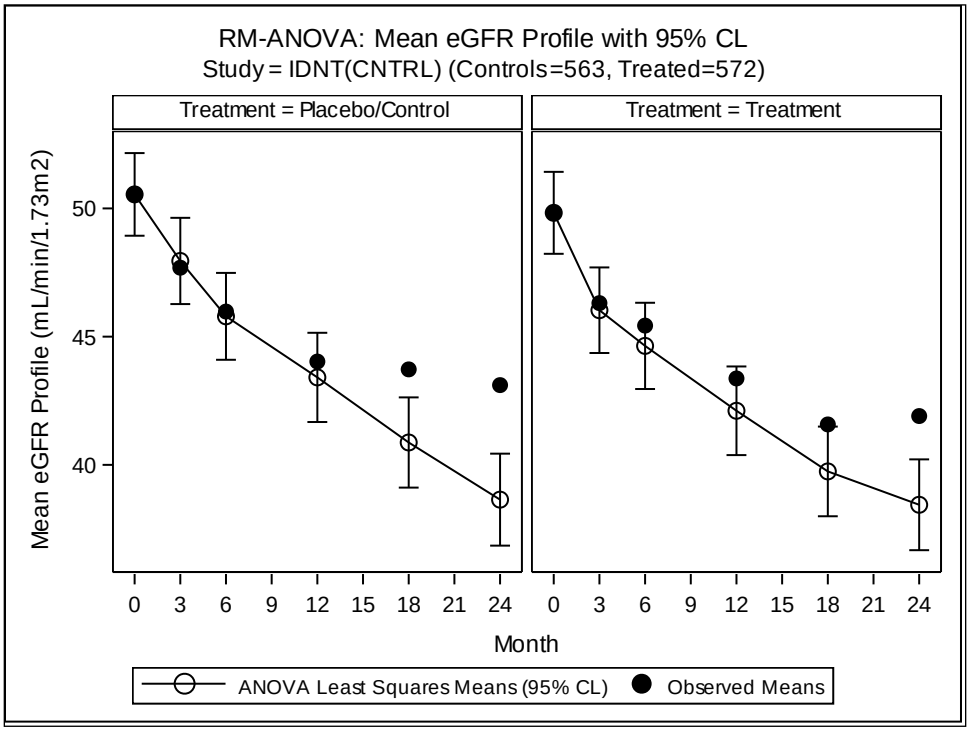

RM-ANCOVA: Adjusted eGFR Mean Change From Baseline with 95% CL
Study = IDNT(CCB) (Controls=556, Treated=572)
eGFR Mean Change From Baseline (mL/min/1.73m2)
0.0
-2.5
-5.0
-7.5
-10.0
-12.5
0
3
6
9
12
15
18
21
24
Month
Placebo/Control
Treatment

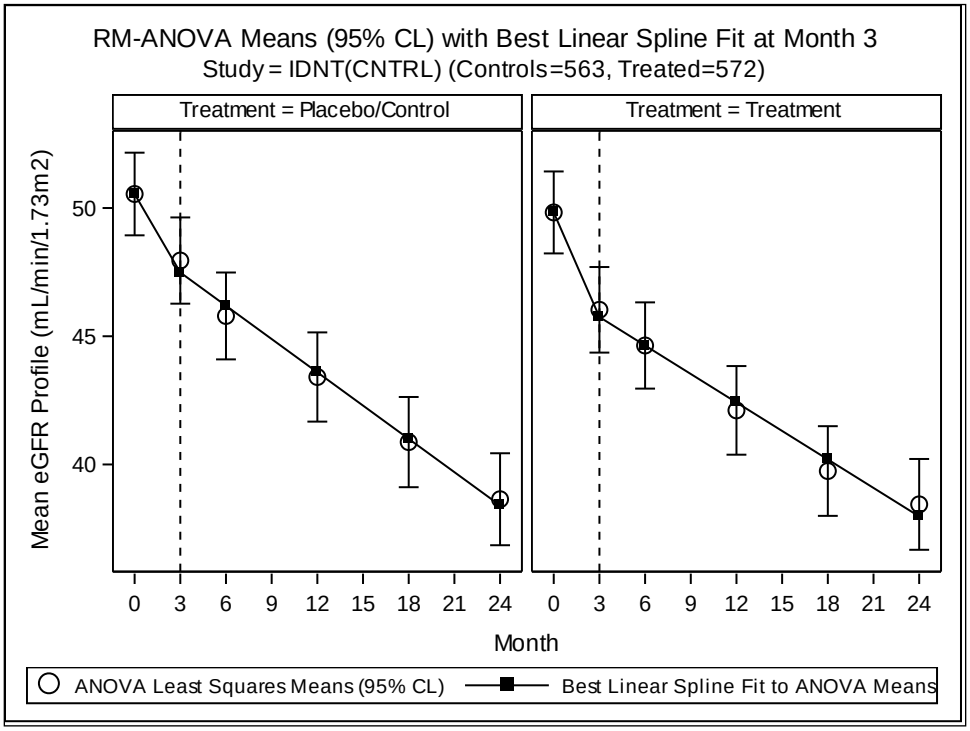

RM-ANCOVA: Adjusted eGFR Mean Change From Baseline with 95% CL
Study = IDNT(CCB) (Controls=556, Treated=572)
Treatment = Placebo/Control
Treatment = Treatment
eGFR Mean Change From Baseline (m...
0.0
-2.5
-5.0
-7.5
-10.0
-12.5
0
3
6
9
12
15
18
21
24
Month
ANCOVA Least Squares Means (95% CL)
Observed Means

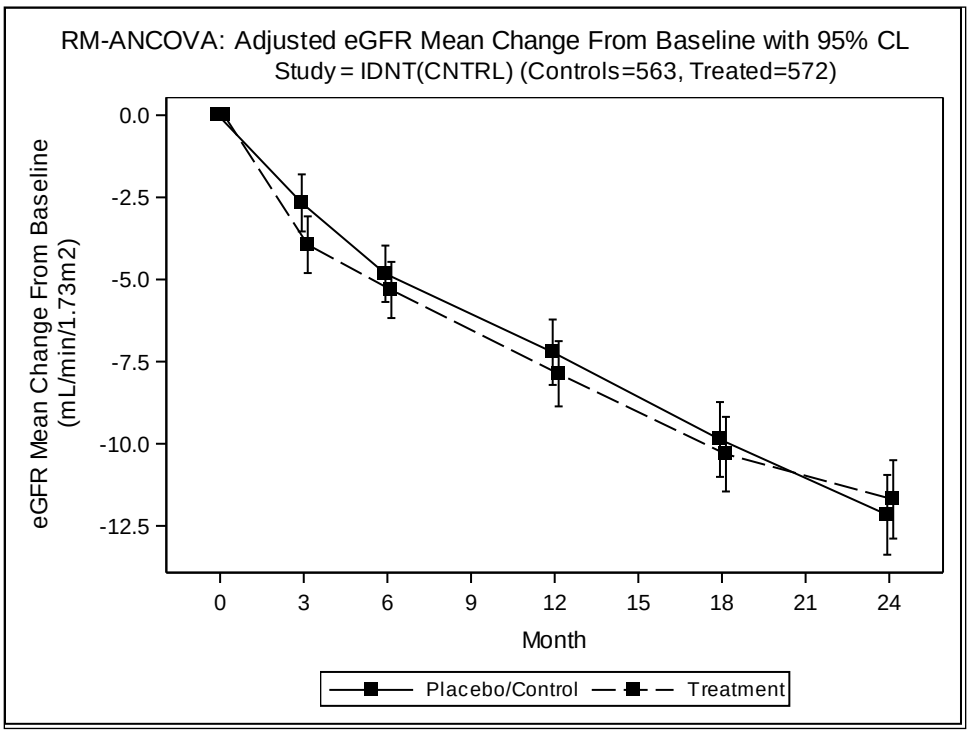

RM-ANCOVA Means (95% CL) with Best Linear Spline Fit at Month 3
Study = IDNT(CCB) (Controls=556, Treated=572)
Treatment = Placebo/Control
Treatment = Treatment
Mean eGFR Profile (mL/min/1.73m2)
0.0
-2.5
-5.0
-7.5
-10.0
-12.5
0 3 6 9 12 15 18 21 24
Month
ANCOVA Means (95% CL)
Best Linear Spline Fit to ANCOVA Means

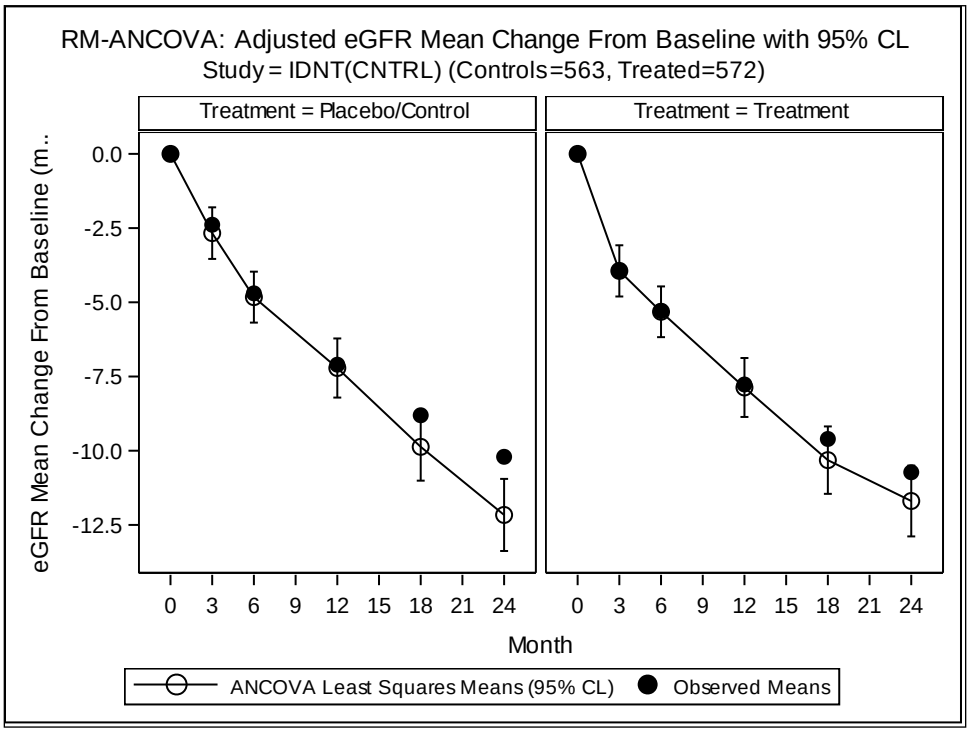

RM-ANCOVA: Adjusted Change From Baseline Treatment Effect with 95% CL
The Optimal Acute Treatment Effect (Treated-Control) Occurs at Month 3
Estimated Acute Treatment Effect (95% CL): -1.285 (-2.511, -0.0591)
Study = IDNT(CCB) (Controls=556, Treated=572)
Mean eGFR Difference (mL/min/1.73m2)
2
1
0
-1
-2
0 3 6 9 12 15 18 21 24
Month

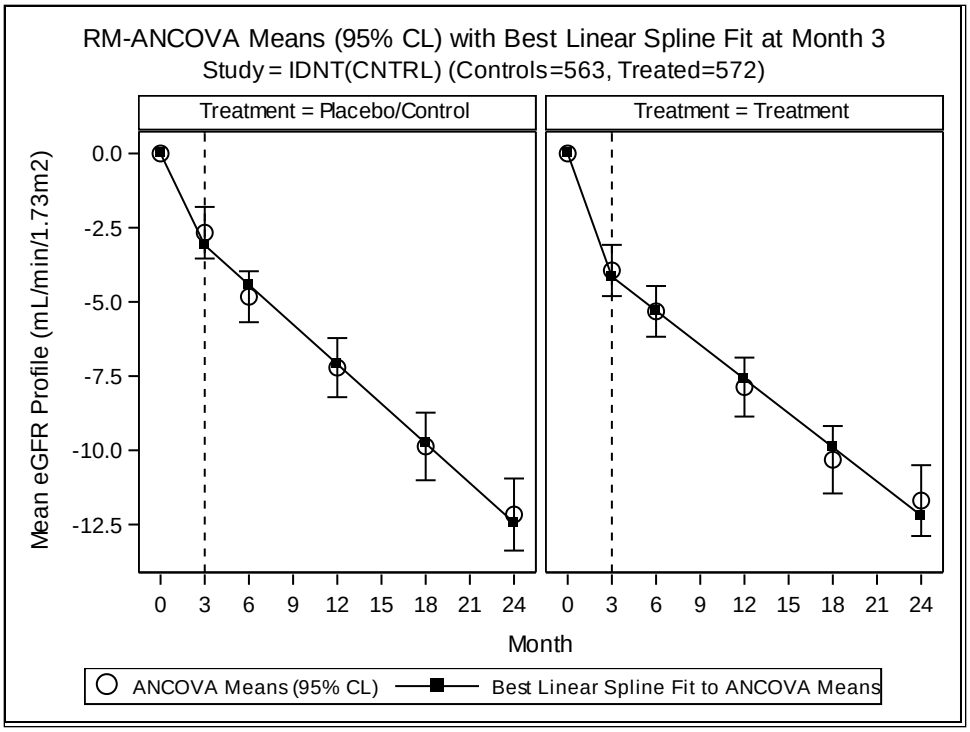
Appendix 4a: IDNT(CNTRL) results

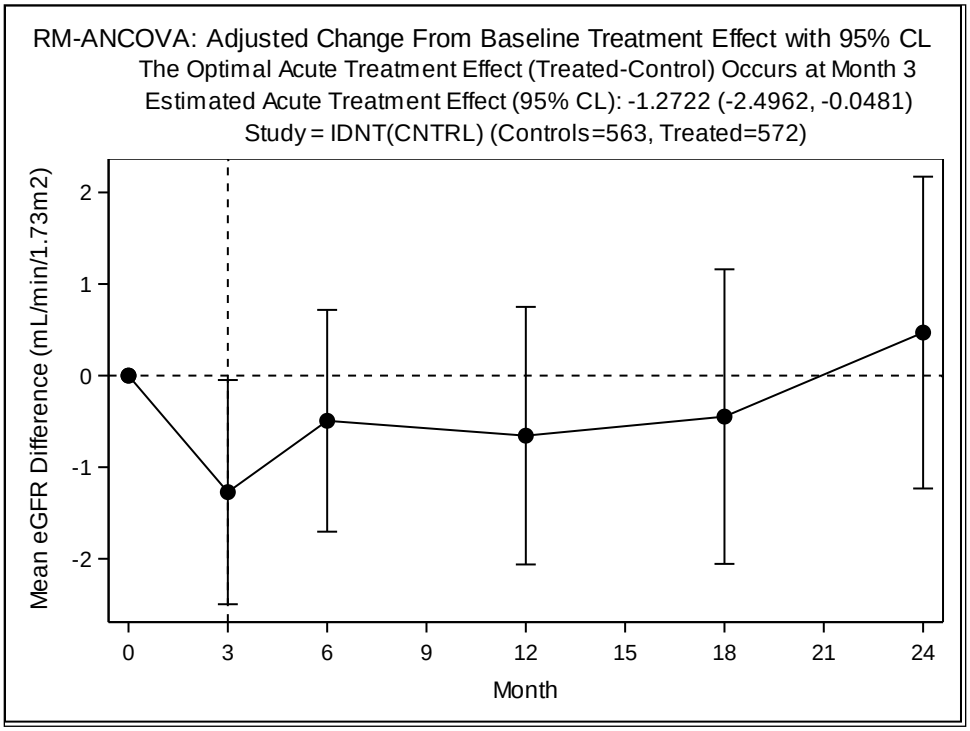




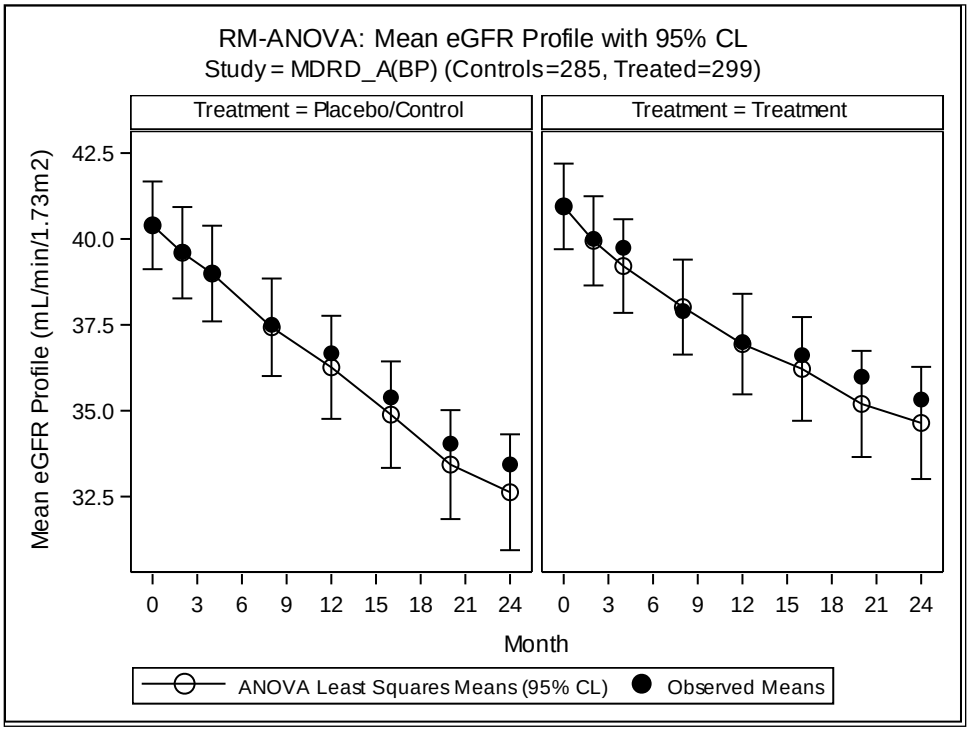

RM-ANCOVA: Adjusted eGFR Mean Change From Baseline with 95% CL
Study = IDNT(CNTRL) (Controls=563, Treated=572)
eGFR Mean Change From Baseline (mL/min/1.73m2)
0.0
-2.5
-5.0
-7.5
-10.0
-12.5
0
3
6
9
12
15
18
21
24
Month
Placebo/Control
Treatment

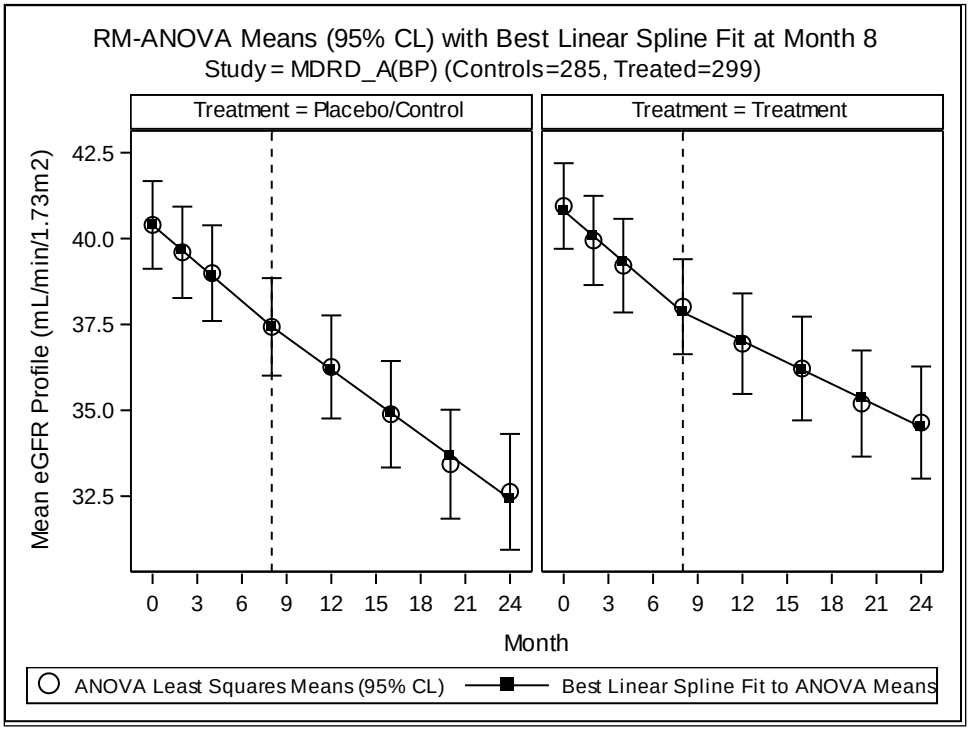

RM-ANCOVA: Adjusted eGFR Mean Change From Baseline with 95% CL
Study = IDNT(CNTRL) (Controls=563, Treated=572)
Treatment = Placebo/Control
Treatment = Treatment
eGFR Mean Change From Baseline (m...
0.0
-2.5
-5.0
-7.5
-10.0
-12.5
0
3
6
9
12
15
18
21
24
Month
ANCOVA Least Squares Means (95% CL)
Observed Means

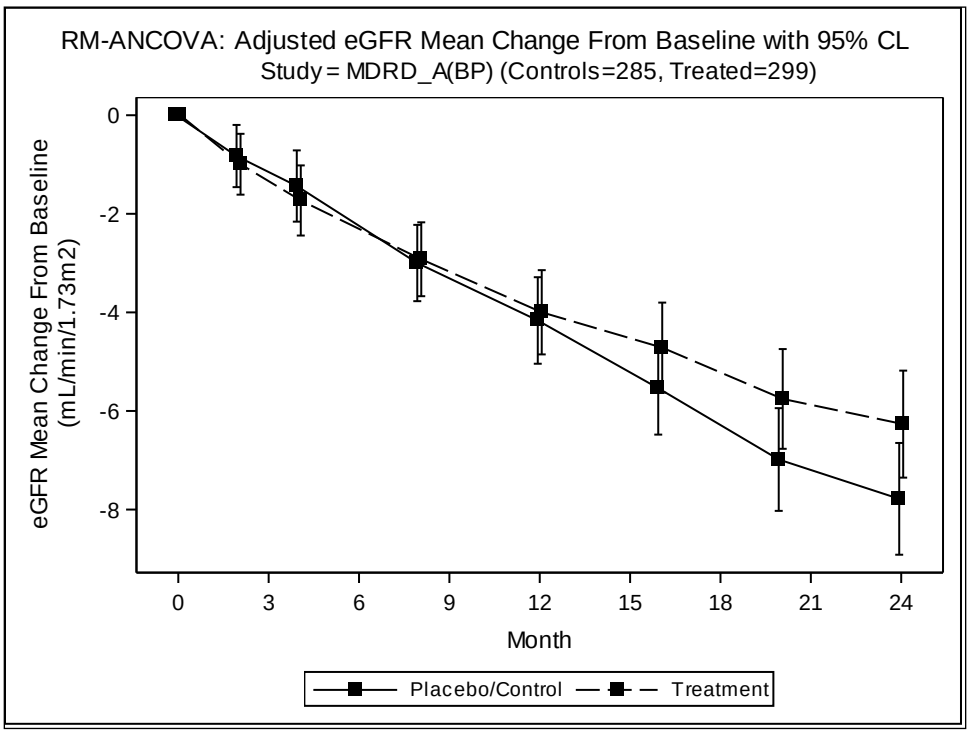

RM-ANCOVA Means (95% CL) with Best Linear Spline Fit at Month 3
Study = IDNT(CNTRL) (Controls=563, Treated=572)
Treatment = Placebo/Control
Treatment = Treatment
Mean eGFR Profile (mL/min/1.73m2)
0.0
-2.5
-5.0
-7.5
-10.0
-12.5
0 3 6 9 12 15 18 21 24
Month
ANCOVA Means (95% CL)
Best Linear Spline Fit to ANCOVA Means

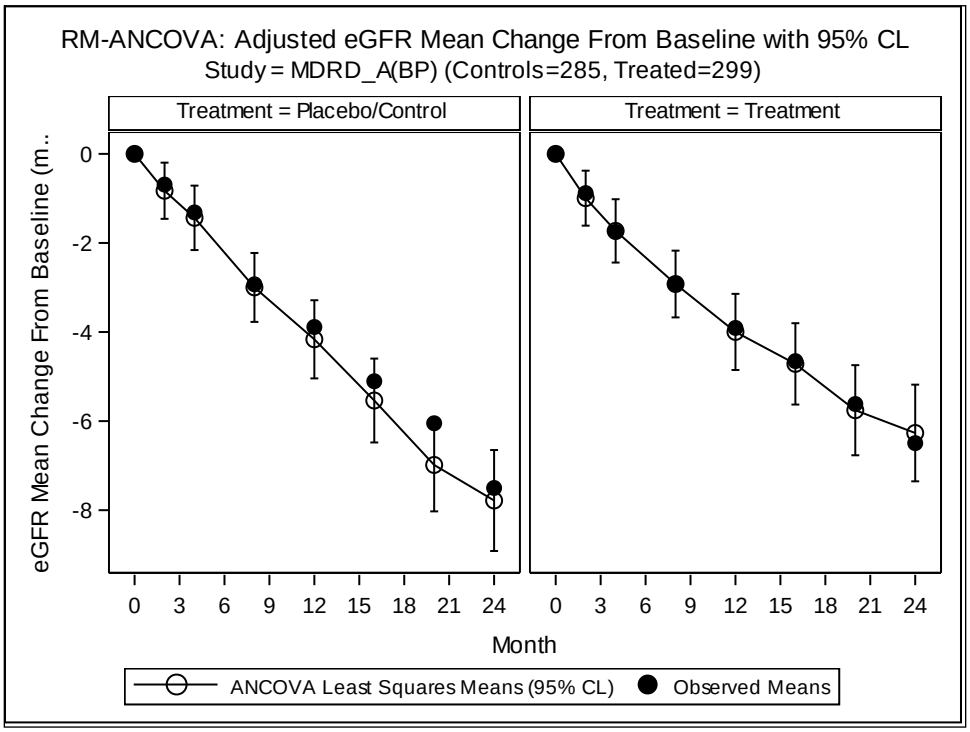

RM-ANCOVA: Adjusted Change From Baseline Treatment Effect with 95% CL
The Optimal Acute Treatment Effect (Treated-Control) Occurs at Month 3
Estimated Acute Treatment Effect (95% CL): -1.2722 (-2.4962, -0.0481)
Study = IDNT(CNTRL) (Controls=563, Treated=572)
Mean eGFR Difference (mL/min/1.73m2)
2
1
0
-1
-2
0 3 6 9 12 15 18 21 24
Month

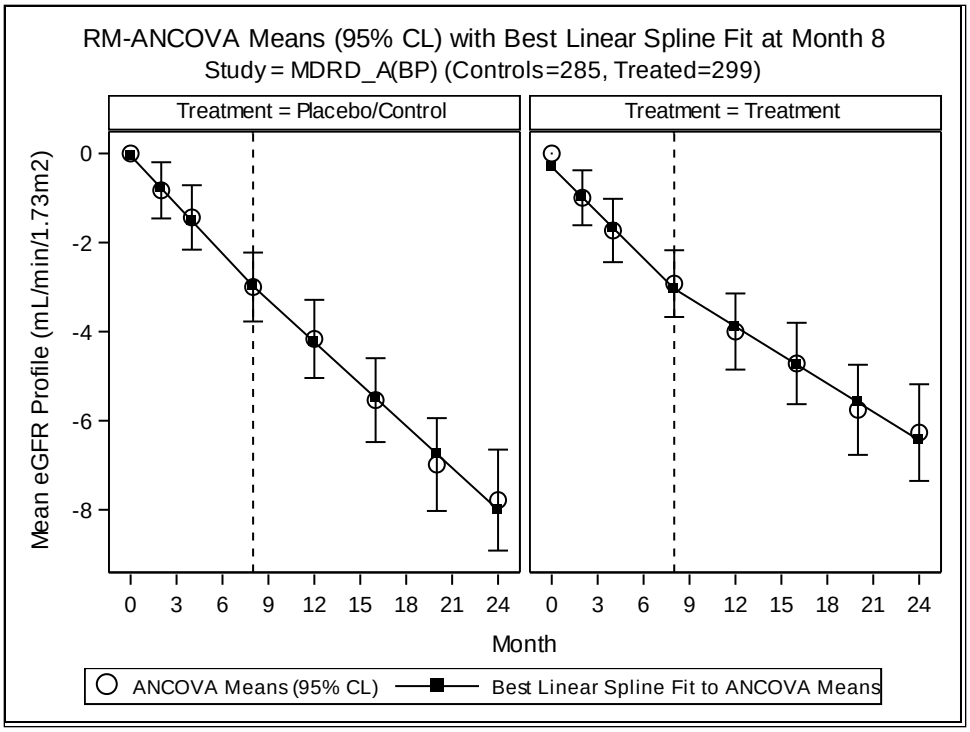
Appendix 5a: MDRD-A(BP) results

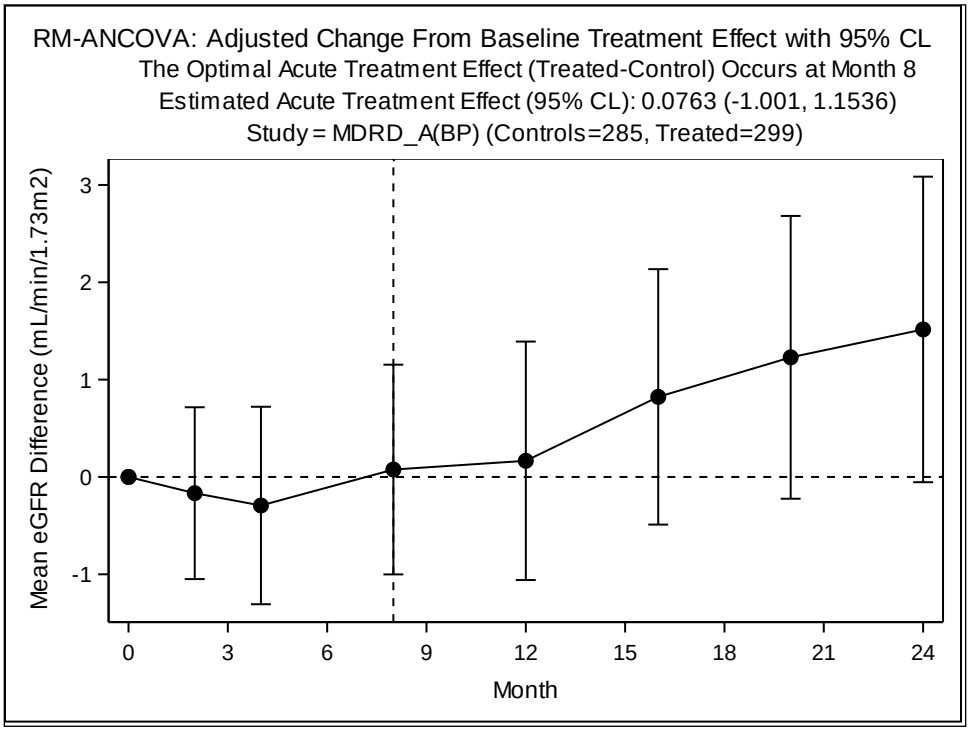




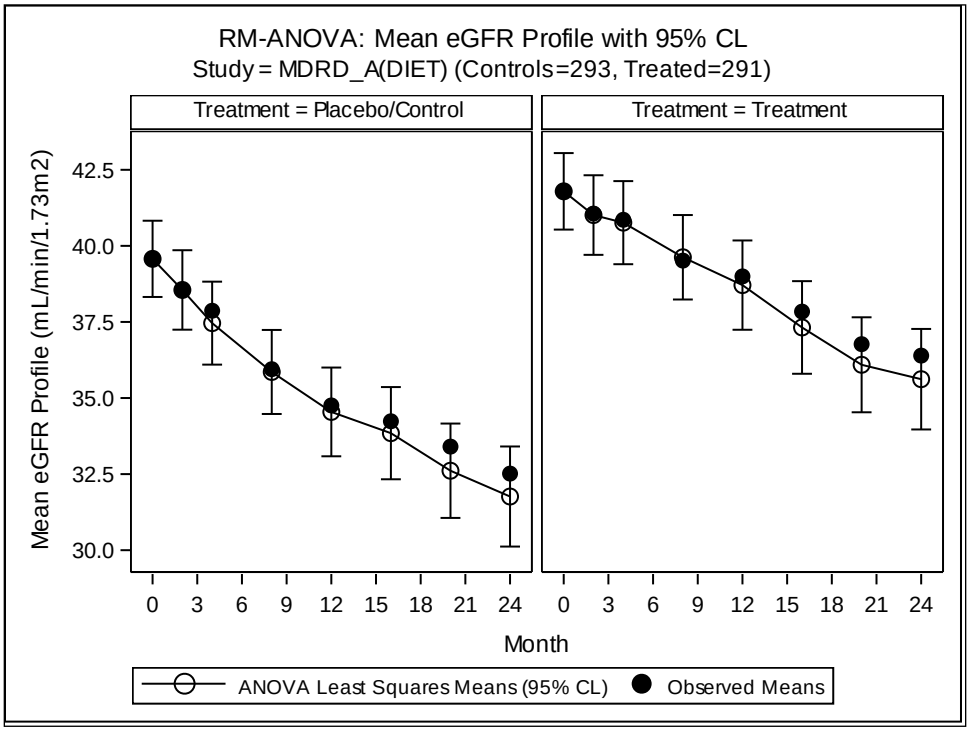

RM-ANCOVA: Adjusted eGFR Mean Change From Baseline with 95% CL
Study = MDRD_A(BP) (Controls=285, Treated=299)
eGFR Mean Change From Baseline (mL/min/1.73m2)
0
-2
-4
-6
-8
0
3
6
9
12
15
18
21
24
Month
Placebo/Control
Treatment

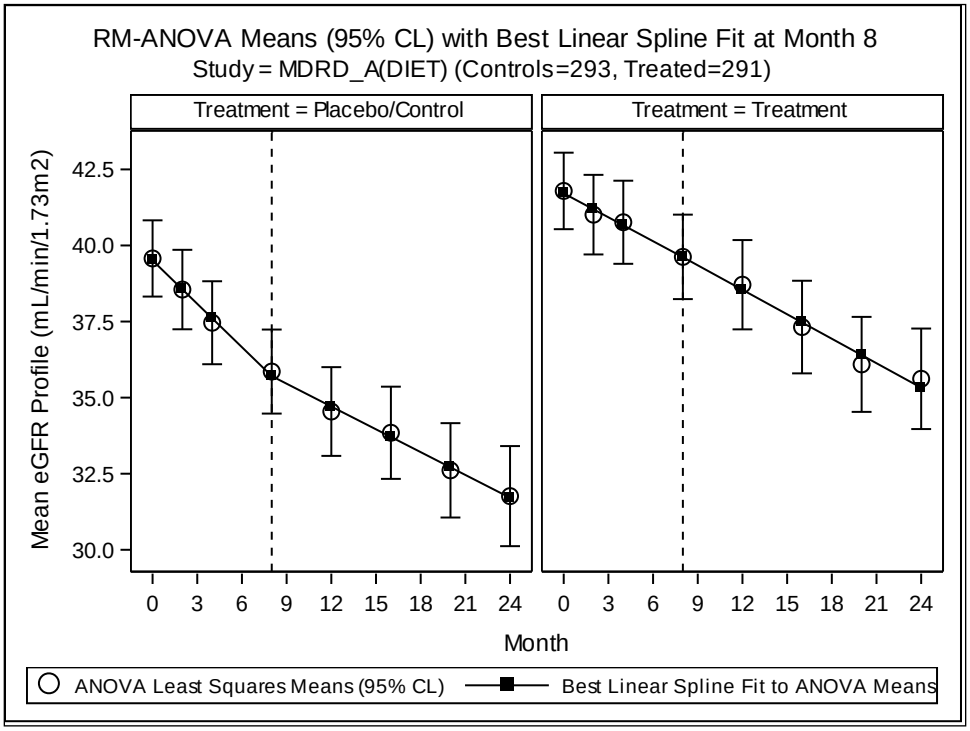

RM-ANCOVA: Adjusted eGFR Mean Change From Baseline with 95% CL
Study = MDRD_A(BP) (Controls=285, Treated=299)
Treatment = Placebo/Control
Treatment = Treatment
eGFR Mean Change From Baseline (m...
0
-2
-4
-6
-8
0
3
6
9
12
15
18
21
24
Month
ANCOVA Least Squares Means (95% CL)
Observed Means

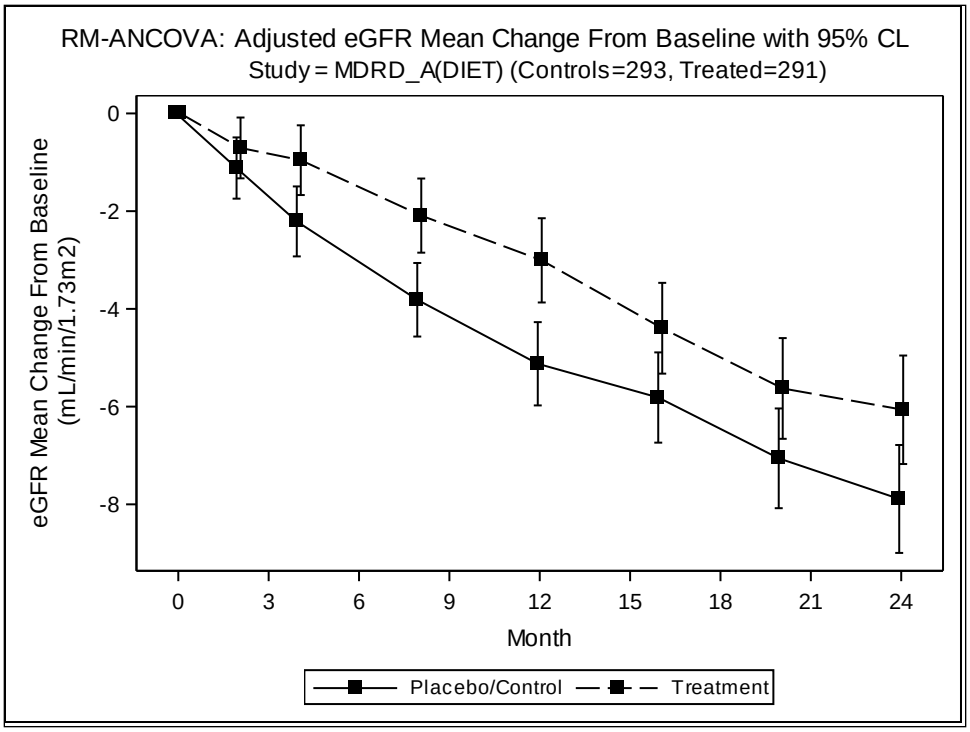

RM-ANCOVA Means (95% CL) with Best Linear Spline Fit at Month 8
Study = MDRD_A(BP) (Controls=285, Treated=299)
Treatment = Placebo/Control
Treatment = Treatment
Mean eGFR Profile (mL/min/1.73m2)
0
-2
-4
-6
-8
0 3 6 9 12 15 18 21 24
Month
ANCOVA Means (95% CL)
Best Linear Spline Fit to ANCOVA Means

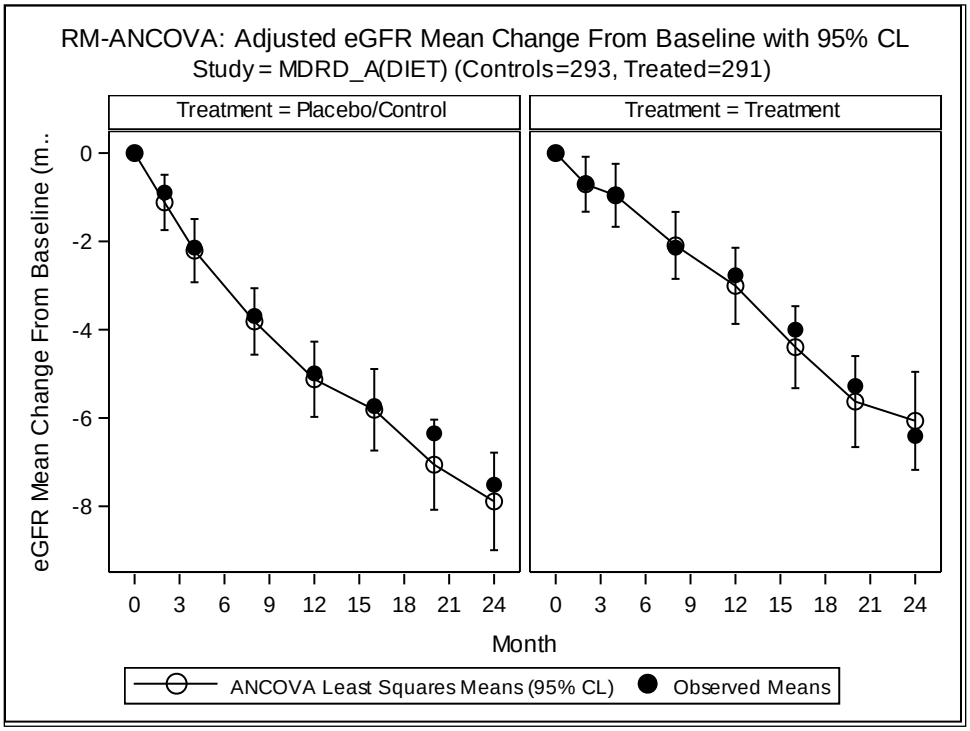

RM-ANCOVA: Adjusted Change From Baseline Treatment Effect with 95% CL
The Optimal Acute Treatment Effect (Treated-Control) Occurs at Month 8
Estimated Acute Treatment Effect (95% CL): 0.0763 (-1.001, 1.1536)
Study = MDRD_A(BP) (Controls=285, Treated=299)
Mean eGFR Difference (mL/min/1.73m2)
3
2
1
0
-1
0 3 6 9 12 15 18 21 24
Month

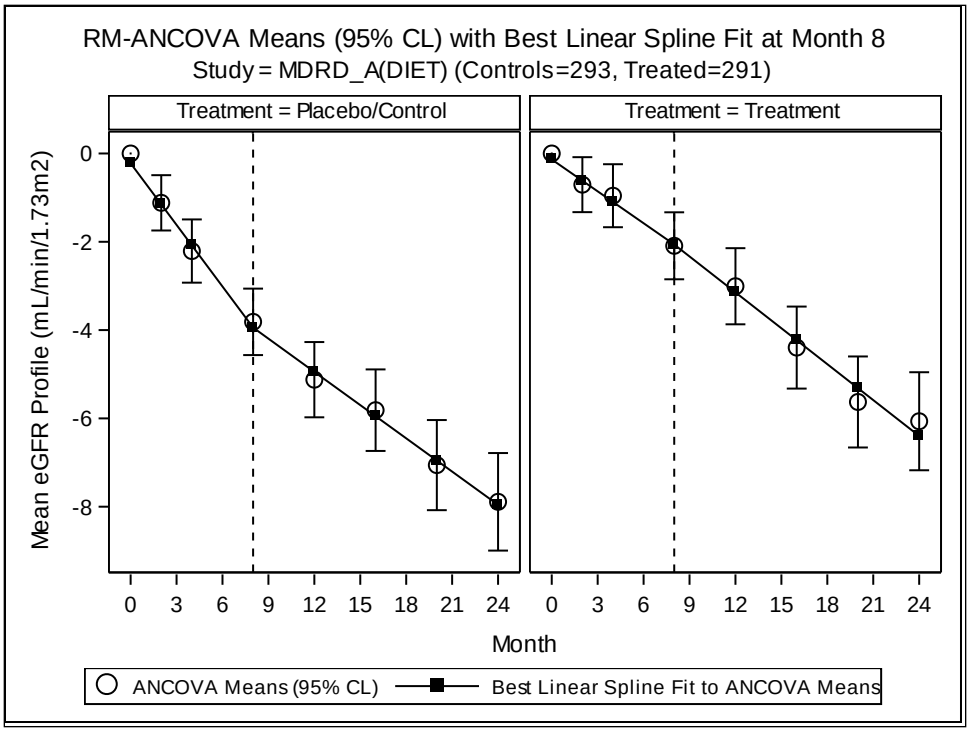
Appendix 6a: MDRD-A(DIET) results

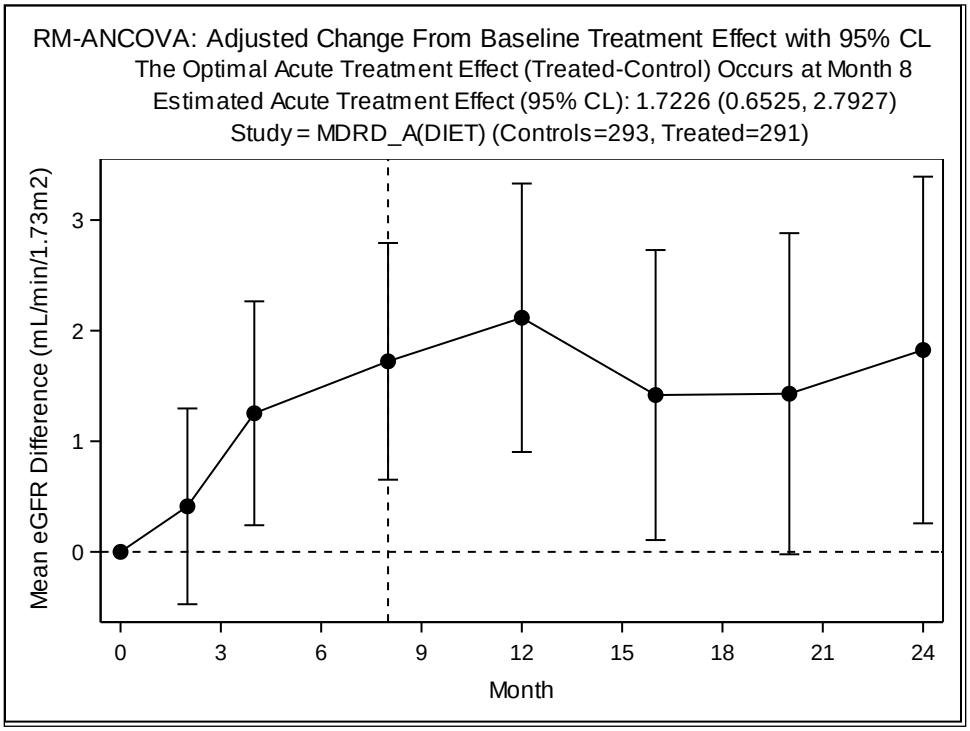




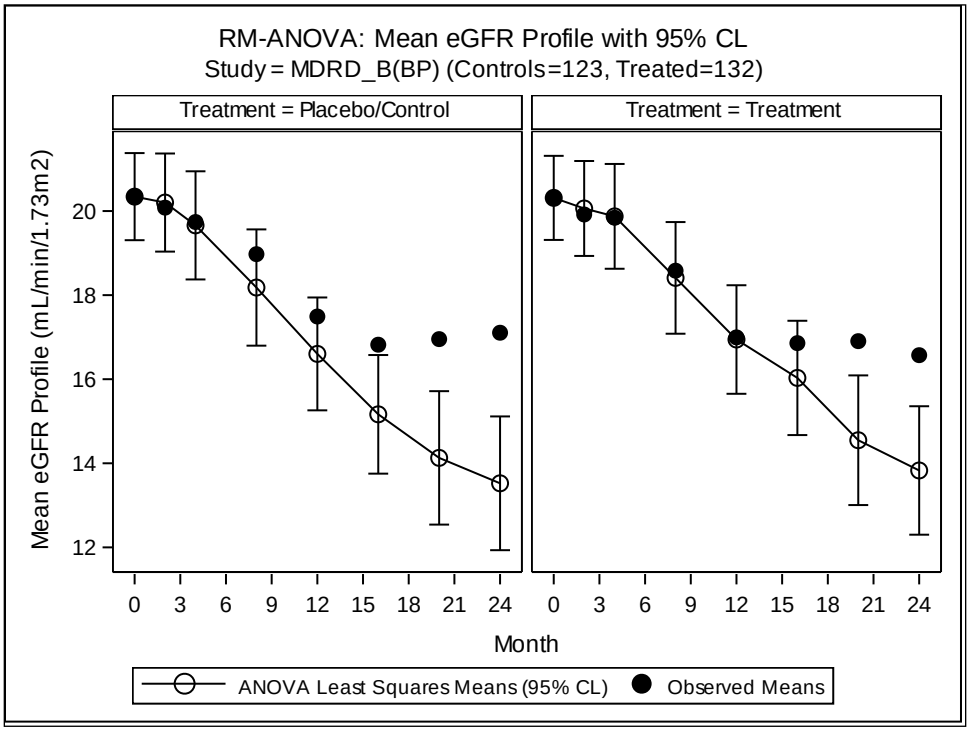

RM-ANCOVA: Adjusted eGFR Mean Change From Baseline with 95% CL
Study = MDRD_A(DIET) (Controls=293, Treated=291)
eGFR Mean Change From Baseline (mL/min/1.73m2)
0
-2
-4
-6
-8
0 3 6 9 12 15 18 21 24
Month
Placebo/Control
Treatment

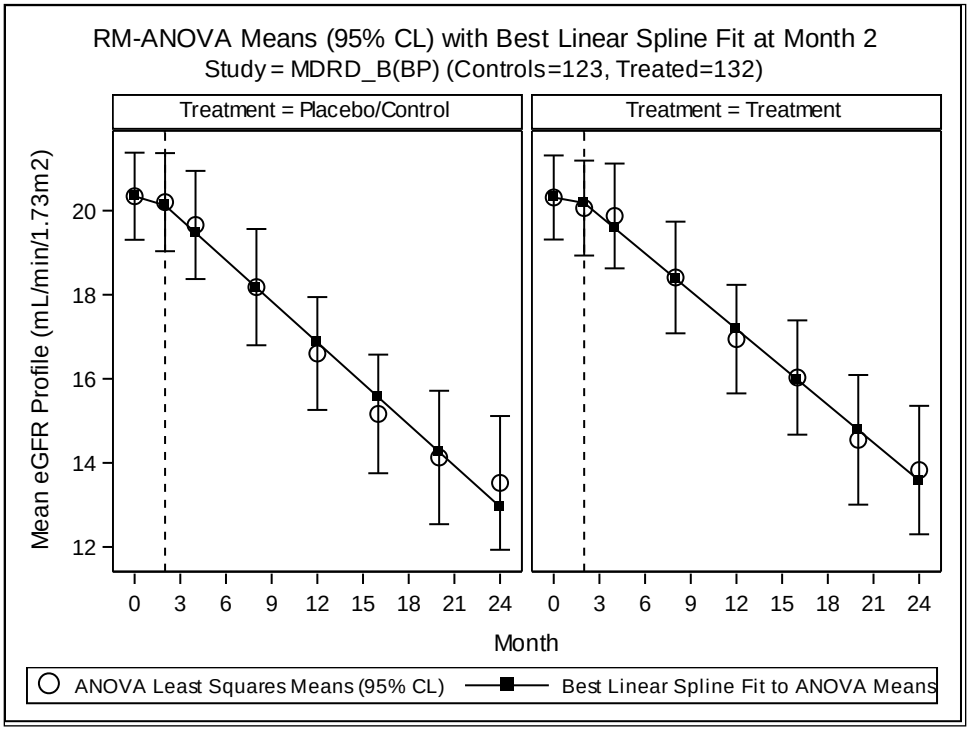

RM-ANCOVA: Adjusted eGFR Mean Change From Baseline with 95% CL
Study = MDRD_A(DIET) (Controls=293, Treated=291)
Treatment = Placebo/Control
Treatment = Treatment
eGFR Mean Change From Baseline (m...
0
-2
-4
-6
-8
0 3 6 9 12 15 18 21 24
Month
ANCOVA Least Squares Means (95% CL)
Observed Means

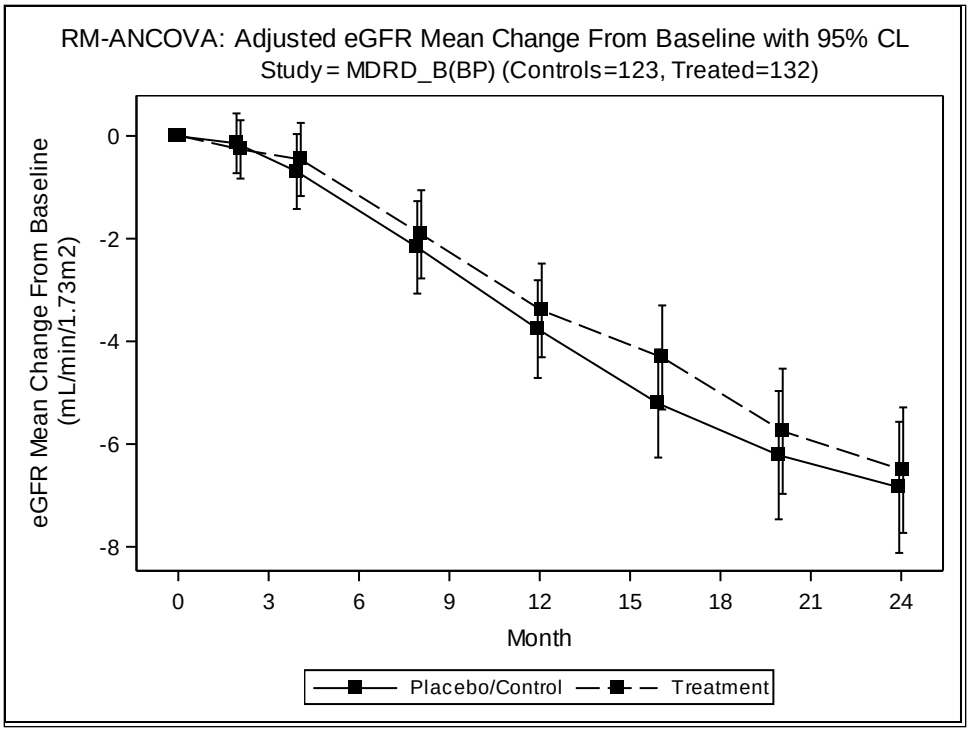

RM-ANCOVA Means (95% CL) with Best Linear Spline Fit at Month 8
Study = MDRD_A(DIET) (Controls=293, Treated=291)
Treatment = Placebo/Control
Treatment = Treatment
Mean eGFR Profile (mL/min/1.73m2)
0
-2
-4
-6
-8
0 3 6 9 12 15 18 21 24
Month
ANCOVA Means (95% CL)
Best Linear Spline Fit to ANCOVA Means

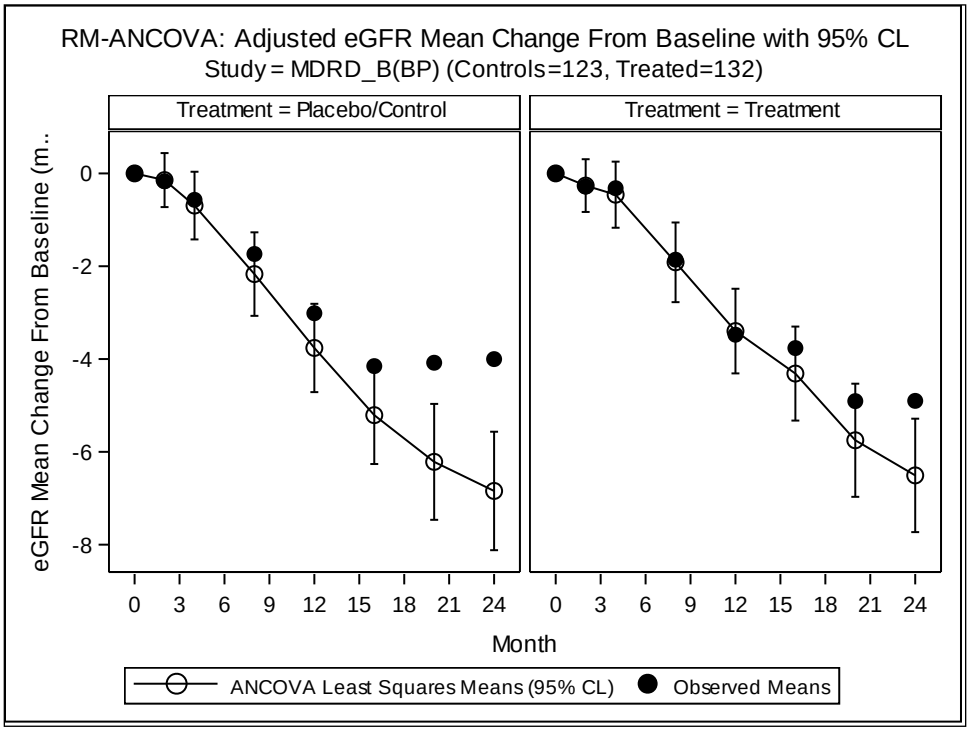

RM-ANCOVA: Adjusted Change From Baseline Treatment Effect with 95% CL
The Optimal Acute Treatment Effect (Treated-Control) Occurs at Month 8
Estimated Acute Treatment Effect (95% CL): 1.7226 (0.6525, 2.7927)
Study = MDRD_A(DIET) (Controls=293, Treated=291)
Mean eGFR Difference (mL/min/1.73m2)
3
2
1
0
0 3 6 9 12 15 18 21 24
Month

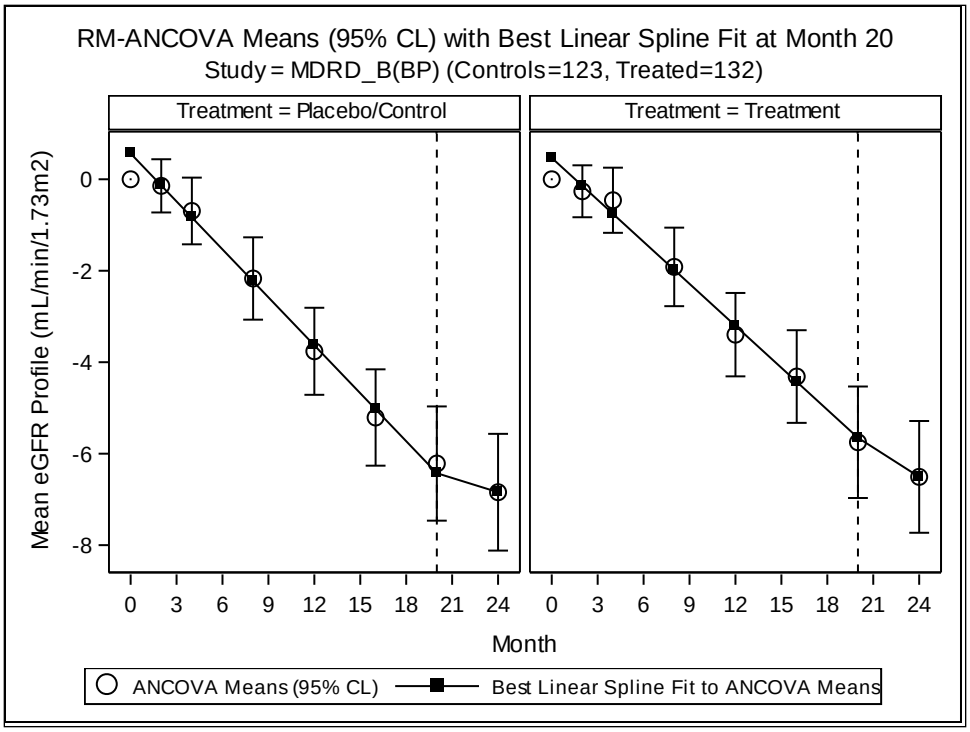
Appendix 7a: MDRD-B(BP) results

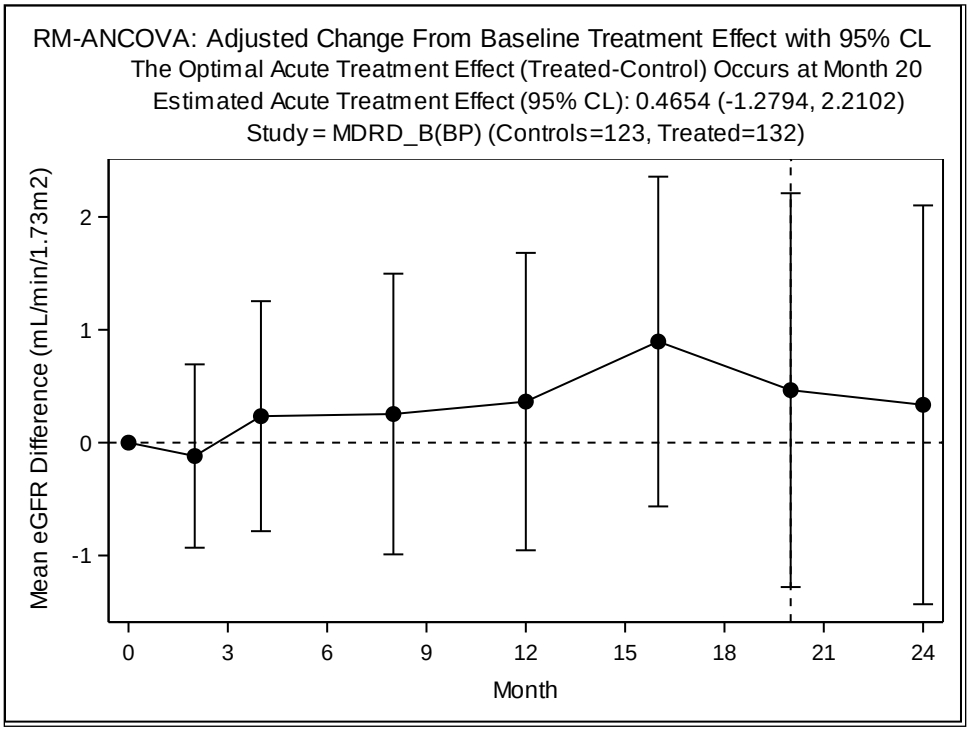




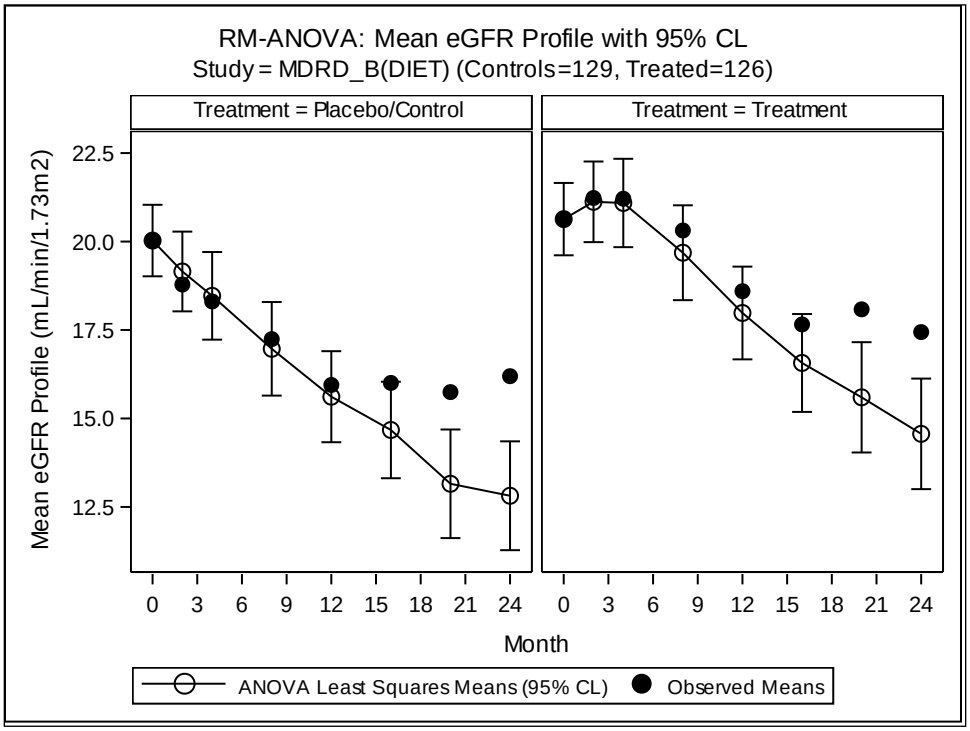

RM-ANCOVA: Adjusted eGFR Mean Change From Baseline with 95% CL
Study = MDRD_B(BP) (Controls=123, Treated=132)
eGFR Mean Change From Baseline (mL/min/1.73m2)
0
-2
-4
-6
-8
0
3
6
9
12
15
18
21
24
Month
Placebo/Control
Treatment

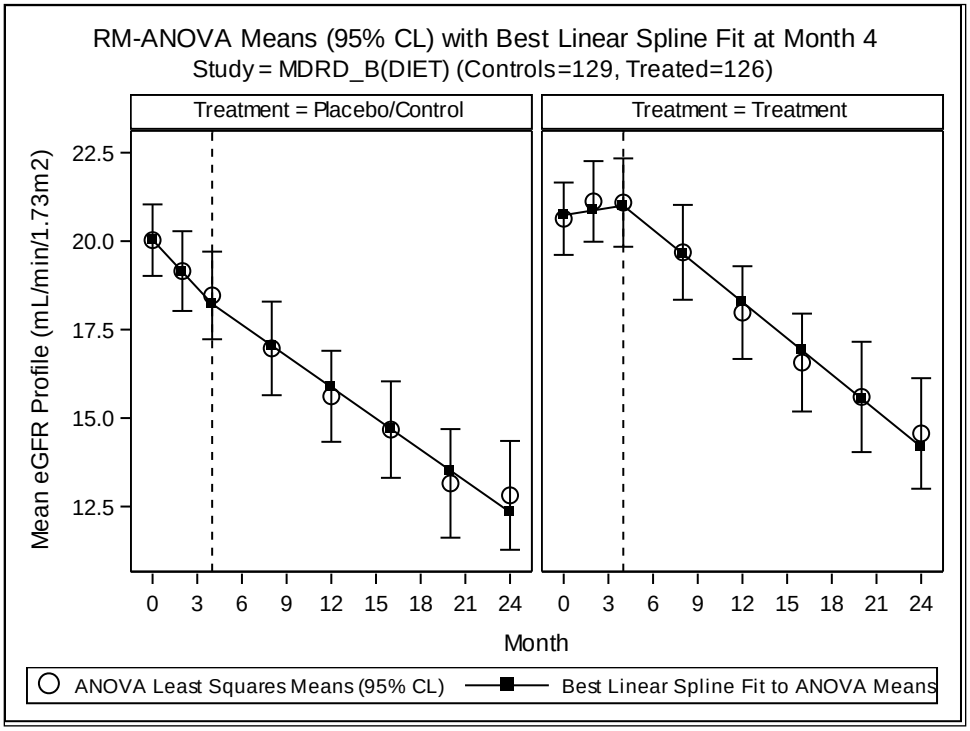

RM-ANCOVA: Adjusted eGFR Mean Change From Baseline with 95% CL
Study = MDRD_B(BP) (Controls=123, Treated=132)
Treatment = Placebo/Control
Treatment = Treatment
eGFR Mean Change From Baseline (m...
0
-2
-4
-6
-8
0
3
6
9
12
15
18
21
24
Month
ANCOVA Least Squares Means (95% CL)
Observed Means

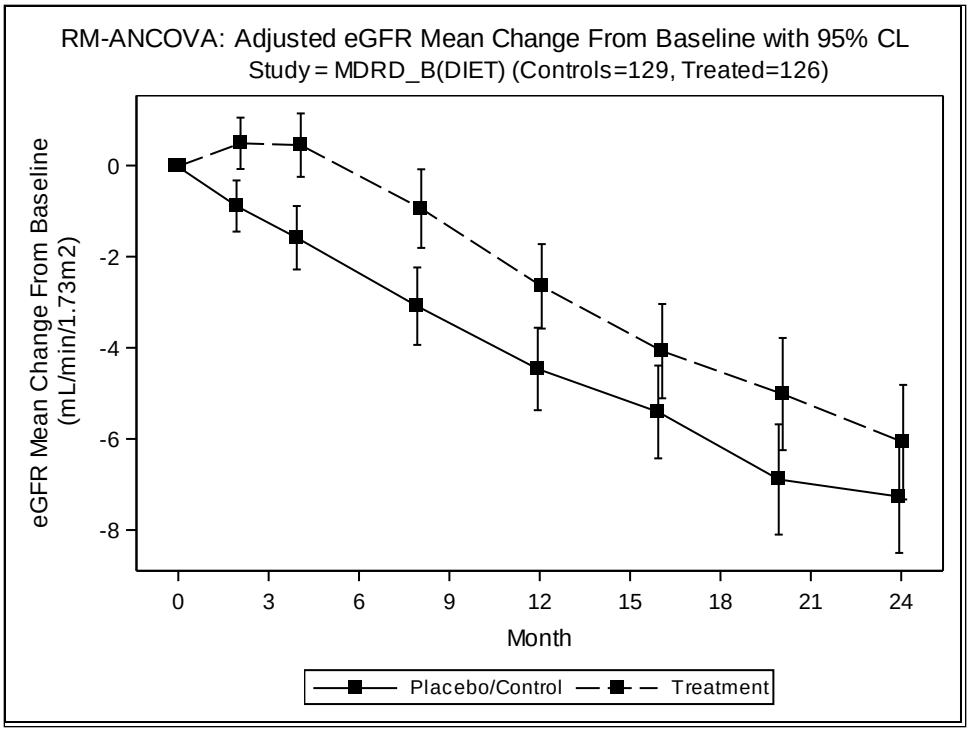

RM-ANCOVA Means (95% CL) with Best Linear Spline Fit at Month 20
Study = MDRD_B(BP) (Controls=123, Treated=132)
Treatment = Placebo/Control
Treatment = Treatment
Mean eGFR Profile (mL/min/1.73m2)
0
-2
-4
-6
-8
0 3 6 9 12 15 18 21 24
Month
ANCOVA Means (95% CL)
Best Linear Spline Fit to ANCOVA Means

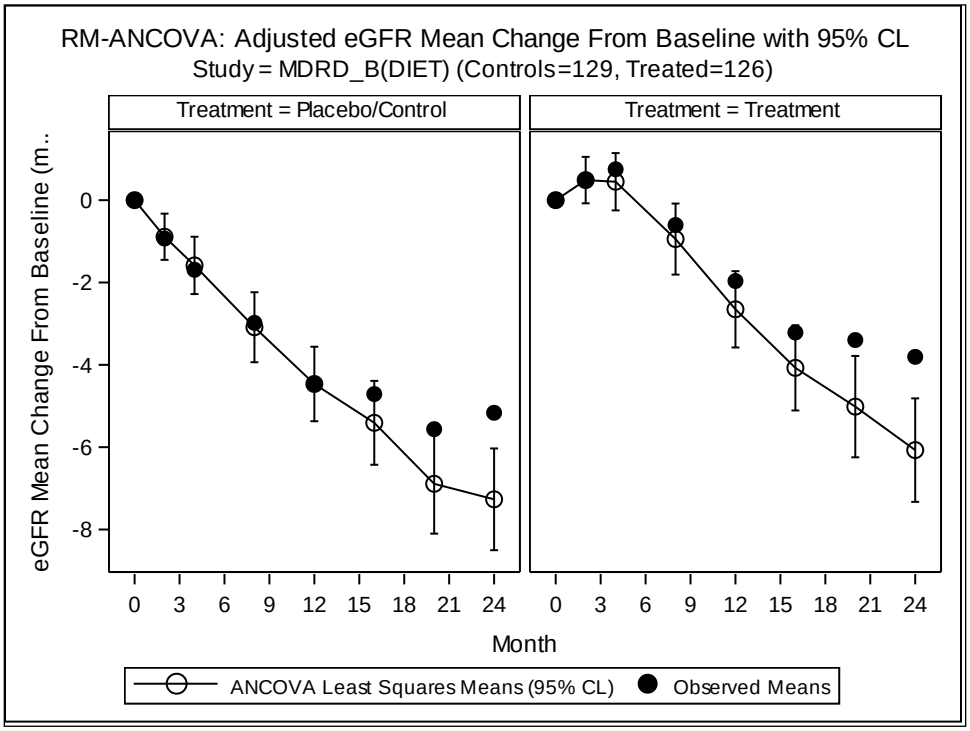

RM-ANCOVA: Adjusted Change From Baseline Treatment Effect with 95% CL
The Optimal Acute Treatment Effect (Treated-Control) Occurs at Month 20
Estimated Acute Treatment Effect (95% CL): 0.4654 (-1.2794, 2.2102)
Study = MDRD_B(BP) (Controls=123, Treated=132)
Mean eGFR Difference (mL/min/1.73m2)
2
1
0
-1
0 3 6 9 12 15 18 21 24
Month

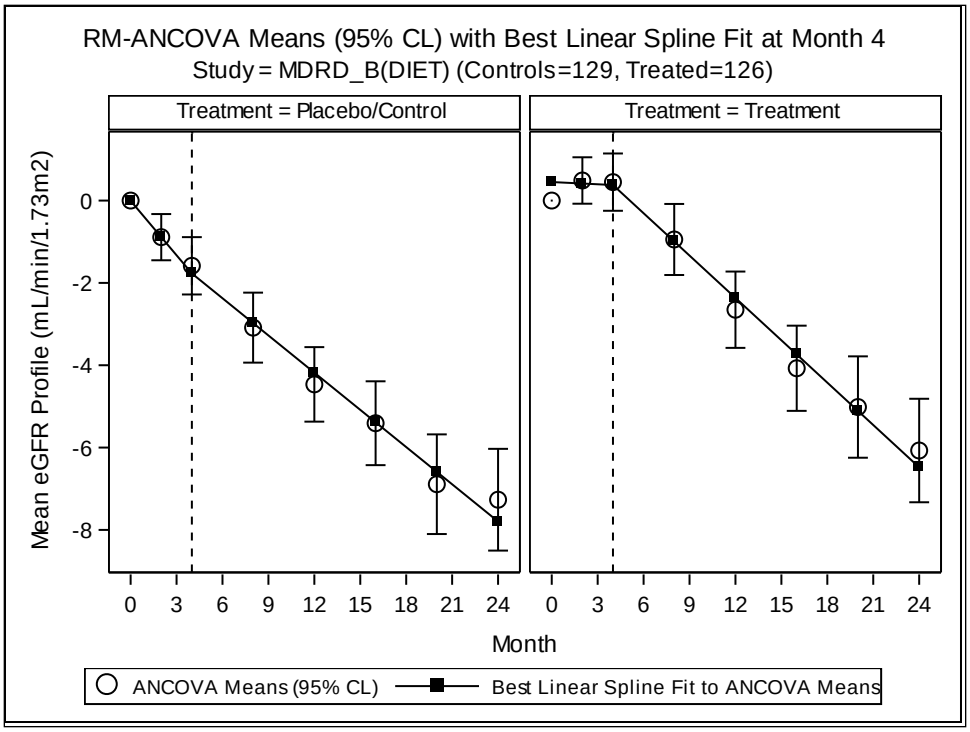
Appendix 8a: MDRD-B(DIET) results

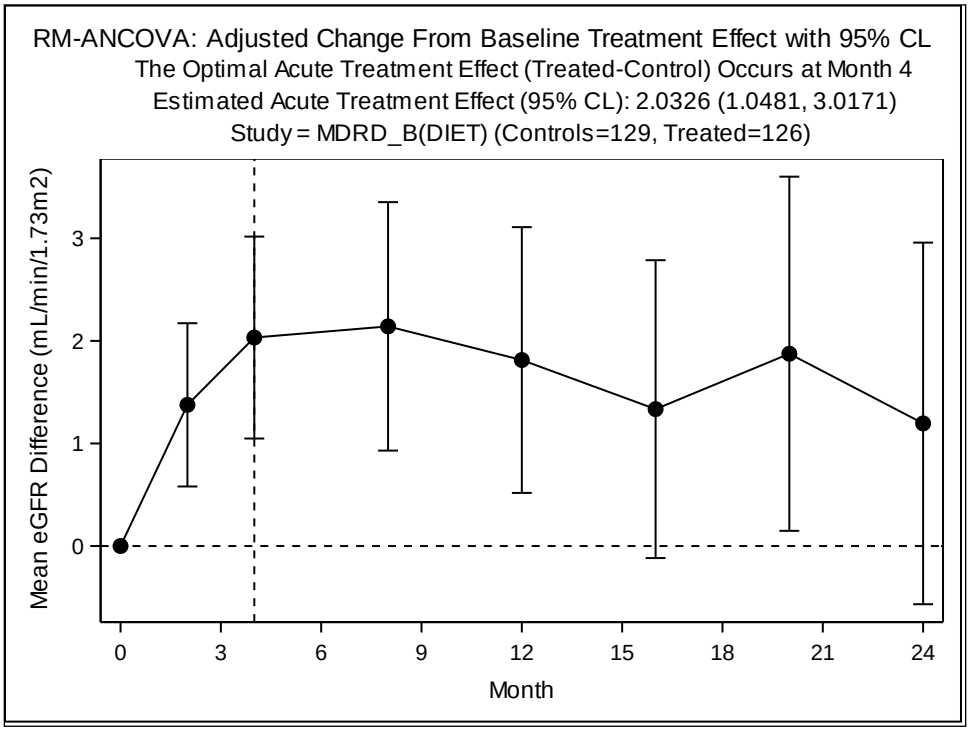




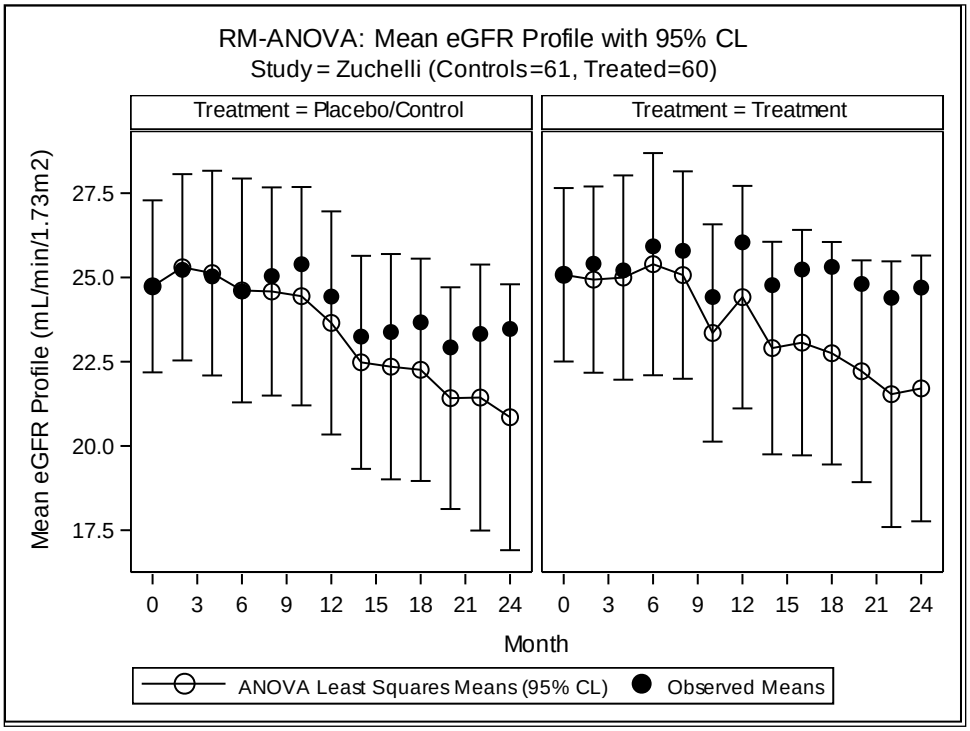

RM-ANCOVA: Adjusted eGFR Mean Change From Baseline with 95% CL
Study = MDRD_B(DIET) (Controls=129, Treated=126)
eGFR Mean Change From Baseline (mL/min/1.73m2)
0
-2
-4
-6
-8
0
3
6
9
12
15
18
21
24
Month
Placebo/Control
Treatment

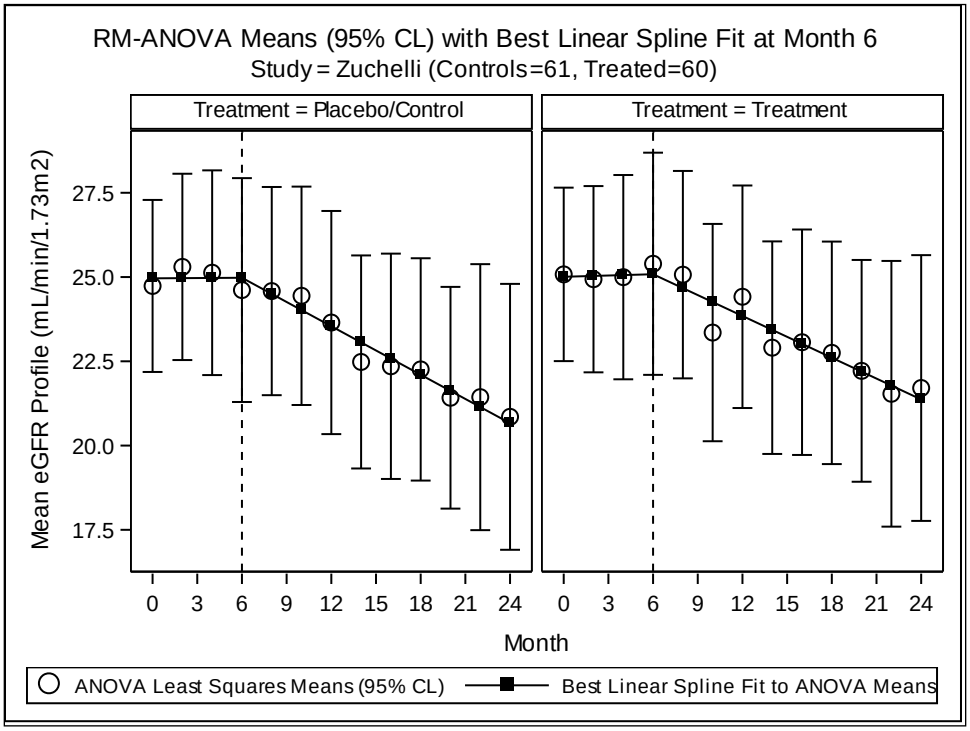

RM-ANCOVA: Adjusted eGFR Mean Change From Baseline with 95% CL
Study = MDRD_B(DIET) (Controls=129, Treated=126)
Treatment = Placebo/Control
Treatment = Treatment
eGFR Mean Change From Baseline (m...
0
-2
-4
-6
-8
0
3
6
9
12
15
18
21
24
Month
ANCOVA Least Squares Means (95% CL)
Observed Means

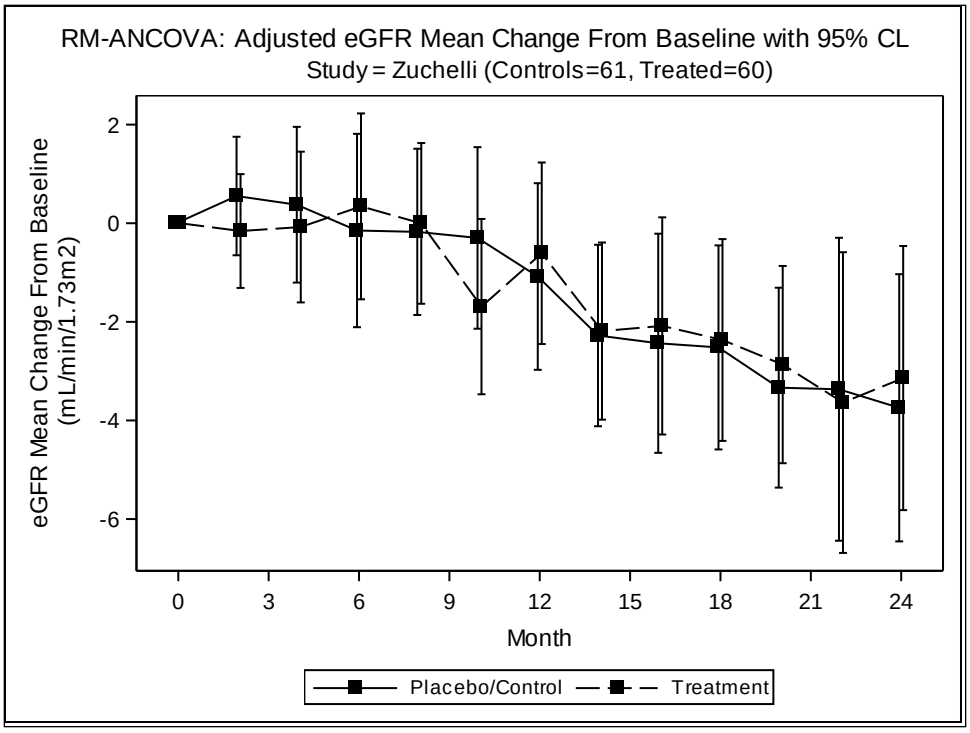

RM-ANCOVA Means (95% CL) with Best Linear Spline Fit at Month 4
Study = MDRD_B(DIET) (Controls=129, Treated=126)
Treatment = Placebo/Control
Treatment = Treatment
Mean eGFR Profile (mL/min/1.73m2)
0
-2
-4
-6
-8
0 3 6 9 12 15 18 21 24
Month
ANCOVA Means (95% CL)
Best Linear Spline Fit to ANCOVA Means

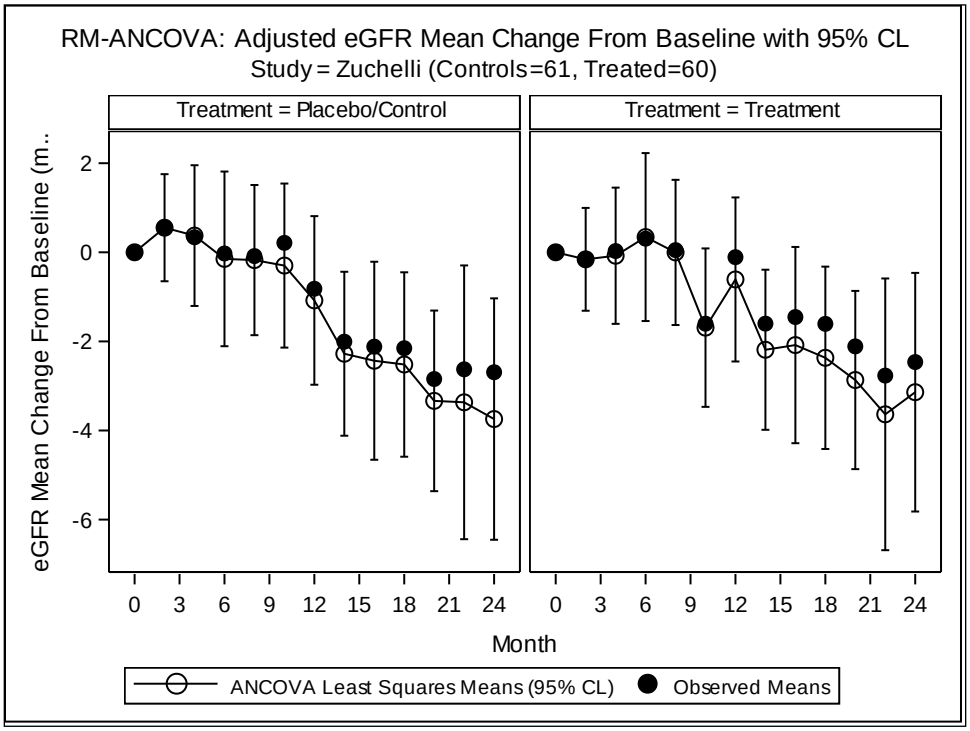

RM-ANCOVA: Adjusted Change From Baseline Treatment Effect with 95% CL
The Optimal Acute Treatment Effect (Treated-Control) Occurs at Month 4
Estimated Acute Treatment Effect (95% CL): 2.0326 (1.0481, 3.0171)
Study = MDRD_B(DIET) (Controls=129, Treated=126)
Mean eGFR Difference (mL/min/1.73m2)
3
2
1
0
0 3 6 9 12 15 18 21 24
Month

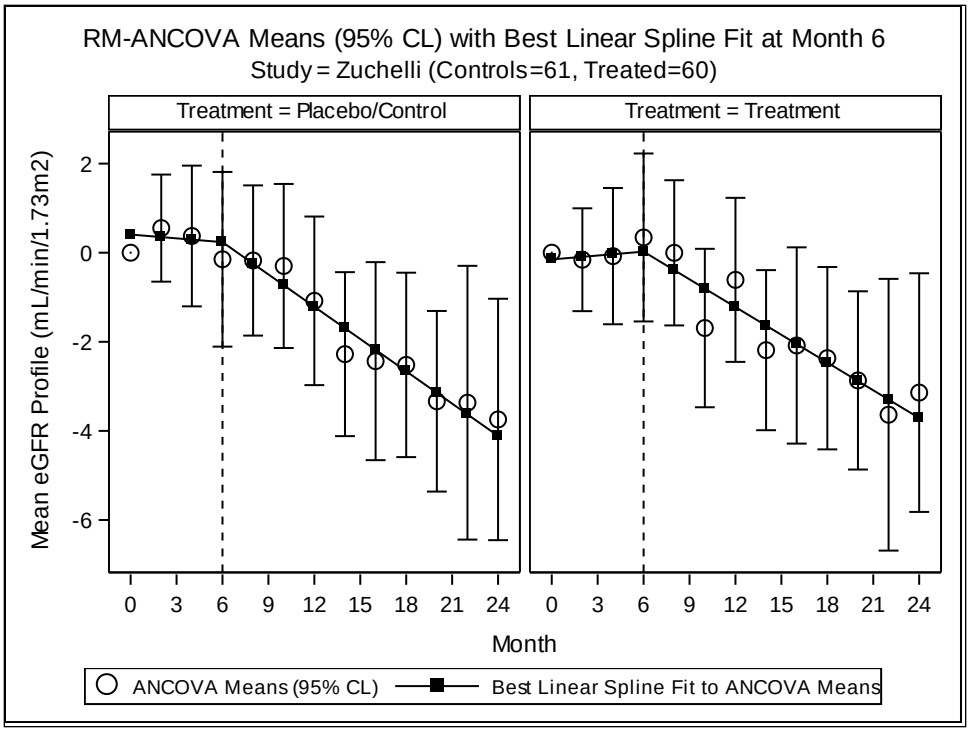
Appendix 9a: Zuchelli results

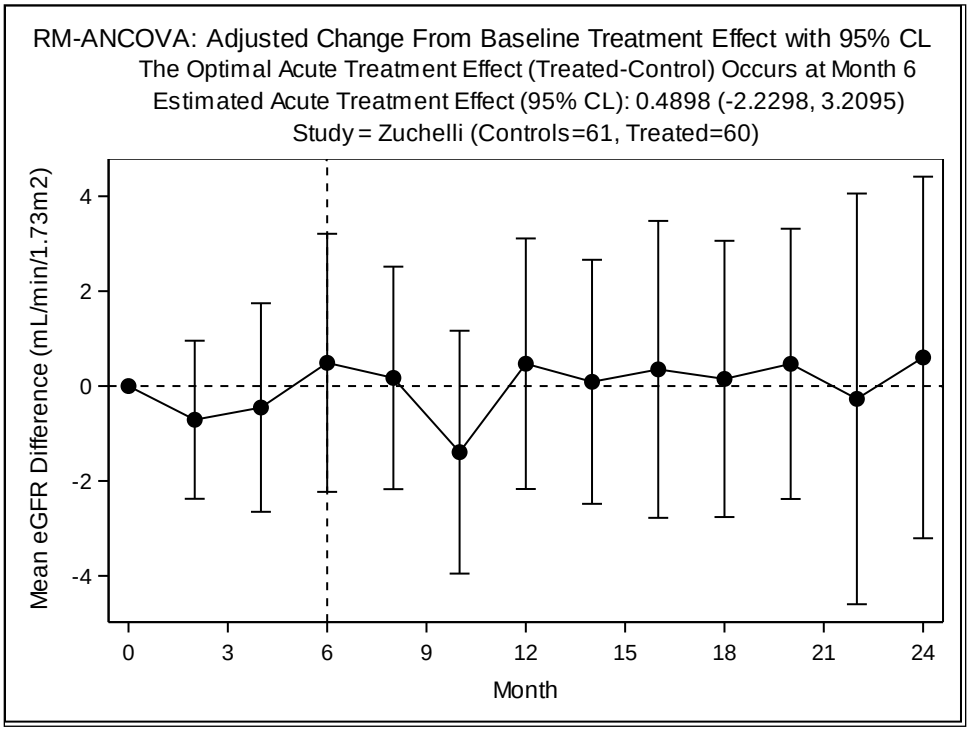





RM-ANCOVA: Adjusted eGFR Mean Change From Baseline with 95% CL
Study = Zuchelli (Controls=61, Treated=60)
eGFR Mean Change From Baseline (mL/min/1.73m2)
2
0
-2
-4
-6
0
3
6
9
12
15
18
21
24
Month
Placebo/Control
Treatment


RM-ANCOVA: Adjusted eGFR Mean Change From Baseline with 95% CL
Study = Zuchelli (Controls=61, Treated=60)
Treatment = Placebo/Control
Treatment = Treatment
eGFR Mean Change From Baseline (m...
2
0
-2
-4
-6
0
3
6
9
12
15
18
21
24
Month
ANCOVA Least Squares Means (95% CL)
Observed Means


RM-ANCOVA Means (95% CL) with Best Linear Spline Fit at Month 6
Study = Zuchelli (Controls=61, Treated=60)
Treatment = Placebo/Control
Treatment = Treatment
Mean eGFR Profile (mL/min/1.73m2)
2
0
-2
-4
-6
0 3 6 9 12 15 18 21 24
Month
ANCOVA Means (95% CL)
Best Linear Spline Fit to ANCOVA Means


RM-ANCOVA: Adjusted Change From Baseline Treatment Effect with 95% CL
The Optimal Acute Treatment Effect (Treated-Control) Occurs at Month 6
Estimated Acute Treatment Effect (95% CL): 0.4898 (-2.2298, 3.2095)
Study = Zuchelli (Controls=61, Treated=60)
Mean eGFR Difference (mL/min/1.73m2)
4
2
0
-2
-4
0 3 6 9 12 15 18 21 24
Month