Analysis of tumour growth in mice

S. Colin, M. Colomb, M. Hirari, A. Yazdani and B. Zaffora

Mixed Linear Models – Dr. D. Couturier (fall semester 2019/2020)

1. Introduction

This report investigates the efficacy of a new drug in reducing or stopping the growth of tumour in mice, before the new drug is tested on humans. To this end, researchers enrolled 33 mice when their tumour size was close to 100 mm^3 and divided them into three groups, two control groups and one treatment group. The new drug was administered to the treatment group while no drugs were administered to the mice in the control groups.

The aim of the present analysis is therefore to understand if the new drug is efficacious in stopping the tumour growth, based on the longitudinal data provided. Namely, we are interested in testing if there is a sensible difference in tumour growth between treatment and control groups and if this difference is statistically significant.

This report consists of three main parts. In the first part, we present summary statistics and exploratory analysis of the data provided. The second part describes the logic behind the models used to answer the scientific question of interest. In particular, we describe the model that we think better captures the characteristic of the data, we illustrate how robust analysis could allow for a better control of potential outlying values and we present the results of the tests made when building the final model. The last part of this report presents our findings as well as directions for further investigation. Finally, the appendix shows comprehensive results from the statistical analysis performed as well as relevant plots.

2. Summary statistics and exploratory data analysis

The data provided consists of repeated observations over time (longitudinal data) of the tumour size on mice. In particular, we have 263 observations of which 120 were made on the treatment group. For the needs of the analysis, we created a new variable called "time_from_enrolment" to account for the time passed from the enrolment of each mouse. The observations are regularly spaced over time as the measurements were performed on Mondays and Fridays. Figure 1 in Appendix shows a statistical summary of the data.

The data shows potential heteroscedasticity (see Figure 2 and Figure 3 in Appendix) hinting at the need for a transformation of the dependent variable "volume". The initial exploratory analysis suggests as well that the mean size of tumours is different between the control groups and the treatment group (see Figure 4 to Figure 6 in Appendix). In addition, Figure 5 and Figure 6 in Appendix suggest a potential interaction between group and time from enrolment, as the tumour size shows a marked dependence on time. Finally, the tumour growth rate might be different from mouse to mouse (random slope) (see Figure 7 in Appendix) and, by design, the tumour size at enrolment can be different (random intercept).

Based on the previous exploratory analysis, we start our model investigation considering a mixed effect model in which the factor "group" and the numeric variable "time_from_enrolment" are the fixed effect (with interaction). To take into account the variable initial size of tumour for each mouse, as well as different growth rates of the tumour, we allow for random effects on both the intercept and the slope.

3. Model analysis and diagnostic

3.1 Mixed linear model

The first model that we consider in our analysis was described at the end of the previous section and its mathematical expression is given in Equation 1.

$$volume_{i,j} = \beta_0 + \beta_1 control1_i + \beta_2 control2_i + s_i + (\beta_3 + r_i) \times time\_from\_enrolment_{i,j} + \beta_4 control1_i \times time\_from\_enrolment_{i,j} + \beta_5 \times control2_i \times time\_from\_enrolment_{i,j} + \varepsilon_{i,j}$$

Equation 1. A first model with random intercept and slope.

In Equation 1, $volume_{i,j}$ is the tumour volume of mouse i at time j, $control1_i$ is a dummy variable taking value 1 if mouse i belongs to group control 1 and 0 otherwise, $control2_i$ is a dummy variable taking value 1 if the mouse belongs to group control 2 and 0 otherwise, $time\_from\_enrolment_{i,j}$ gives the number of days after enrolment, s_i is the random intercept specific to mouse i, r_i is the random slope associated with the time from enrolment and epsilon is the residual.

Figure 8 in Appendix shows the estimates of the parameters of this model as well as complementary useful statistics. Figure 9 shows the residual plot (Pearson residuals against fitted value) of the model. As expected, this model is characterized by heteroscedasticity in its residuals and the figure indicates the presence of a number of potential outliers. To address this issue we log-transform the variable "volume". The results of the new fitted model are presented in Figure 10 to Figure 14 of the Appendix. We can easily recognize that the trend in the residuals disappears indicating that the transformation of the variable "volume" was effective.

We want now test for the significance of the interaction between "group" and "time_from_enrolment" as well as for the significance of the random terms. For the interaction term, we perform an Anova analysis in which we compare two models with and without interaction. The results are shown in Figure 15 of the Appendix. The model with the interaction (Fmod.ml.1_lme) is indeed significantly different from the model without interaction (p-value < 0.0001). Similarly, we test for the significance of the random terms (slope); the results of the Anova analysis is given in Figure 16 of Appendix. We can see that the model with and without random slope are significantly different (p-value = 0.0028) and that the model including random slope (Rmod.ml.1_lme) is better (in capturing the variability of the data) based on the AIC criterion.

In addition, we performed a Wald F-test ("Anova of type III") with Satterthwaite's method to correct the degrees of freedom of the fixed effect part (see Figure 17 in Appendix). We can easily see that the conclusions of all the tests performed agree, suggesting that all parameters are significant at 5% with the exception of the factor group.

In conclusion, the model that we retain at this stage consists of the log-transformed output variable ("volume"), an interaction term between the factor "group" and the numeric variable "time_from_enrolment" as well as random slope and intercept to account for the individual variability of the tumour size for each mouse. Its mathematical formulation is presented in Equation 2.

$$log\,(volume)_{i,j} = \beta_0 + \beta_1 control1_i + \beta_2 control2_i + s_i + (\beta_3 + r_i) \times time\_from\_enrolment_{i,j}$$
$$+ \beta_4 control1_i \times time\_from\_enrolment_{i,j} + \beta_5 \times control2_i \times time\_from\_enrolment_{i,j} + \varepsilon_{i,j}$$

Equation 2. New model accounting for the log-transformation of the dependent variable.

3.2 Robust and outliers analyses

Figure 12 in the Appendix shows that some residuals deviate from normality. Therefore, we perform a robust fit of the model in Equation 2 (see Figure 18 in Appendix). Although the fitting algorithm converges, the estimating equations are not satisfied. Some diagnostic plots of this model are presented in Figure 19 to Figure 21. We can observe that the outlying values are detected and downweighted, but that the model estimates does not change much compared with the ones presented in Figure 10.

Another way to evaluate the impact of influential points is to fit a model to the data without outliers and analyse whether the fit changes significantly. The new fit and the residuals of the new model are presented in Figure 22 and Figure 23 of the Appendix. Despite the improvement on the residuals, the lack of scientific reasons (such as evidence for measurement or transcription errors) to remove the "statistical" outliers as well as the fact that the new estimates do not change sensibly from the model obtained when considering all the data direct us towards considering the model obtained with the full data set as final model.

4. Conclusion

From the analysis performed, we conclude that there is statistical evidence of the effectiveness of the new drug in reducing tumour growth. Indeed, the tumour volume of the treated mice is significantly smaller than the tumour volume of mice in the control groups over time. The model suggests as well that the difference between the control groups is not significant, which can be interpreted as a random assignment of mice in the two controls. In fact, the two control groups can be merged for an even higher power of the designed experiment.

Finally, we noticed that some mice of the treatment group lived longer when compared with the mice in the control groups. However, this might be because of the suppression of the mice when their tumour became too big, or if all mice of the control groups died on average earlier. Additional information in this direction would be necessary to ensure a better understanding of both the experimental design and the conclusions of the study.

Appendix

```
summary(tumour)

volume             date                 mouse              group             time_from_enrolment
Min.   :  49.43    Min.   :2018-10-15   33233(547): 14     Treatment :120    Min.   : 0.00
1st Qu.: 207.82    1st Qu.:2018-11-02   33217(581): 11     Control I : 54    1st Qu.: 7.00
Median : 398.67    Median :2018-11-09   33221(550): 11     Control II: 89    Median :14.00
Mean   : 489.12    Mean   :2018-11-09   33235(548): 11                       Mean   :14.78
3rd Qu.: 647.59    3rd Qu.:2018-11-19   33238(451): 10                       3rd Qu.:21.00
Max.   :2002.77    Max.   :2018-12-03   33287(499): 10                       Max.   :49.00
                                        (Other)   :196
```

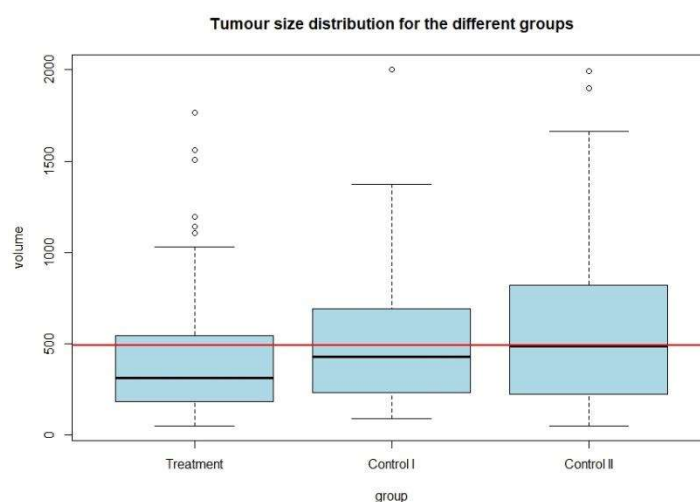Figure 1. Summary statistics of the data.

Figure 2. Distribution of tumour size by group. The red line indicates the average tumour size over all groups.
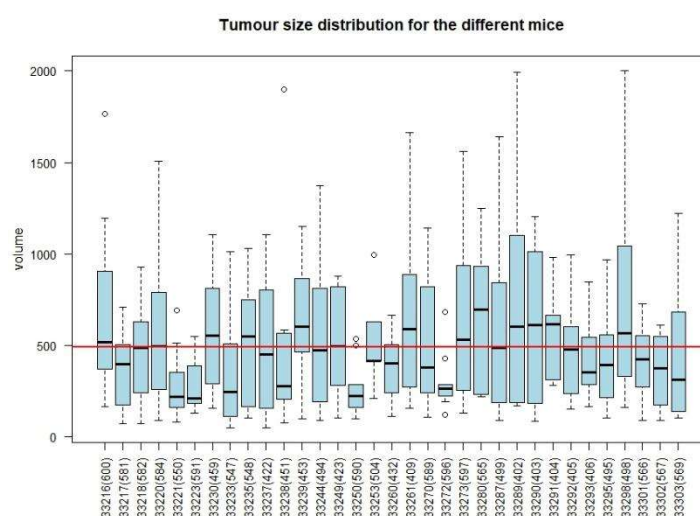

Figure 3. Distribution of tumour size by mouse. The red line indicates the average tumour size.
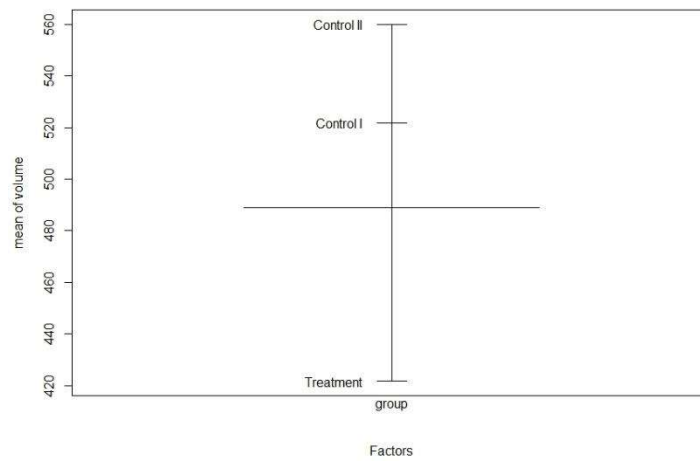
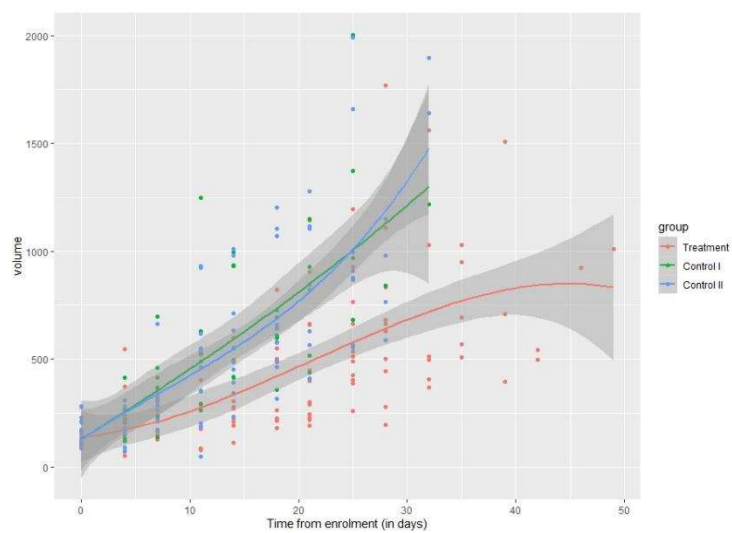Figure 4. Plot design considering groups as factors.

Figure 5. Evolution of tumour size with time (from enrolment) by group (smoothing via local polynomial regression).
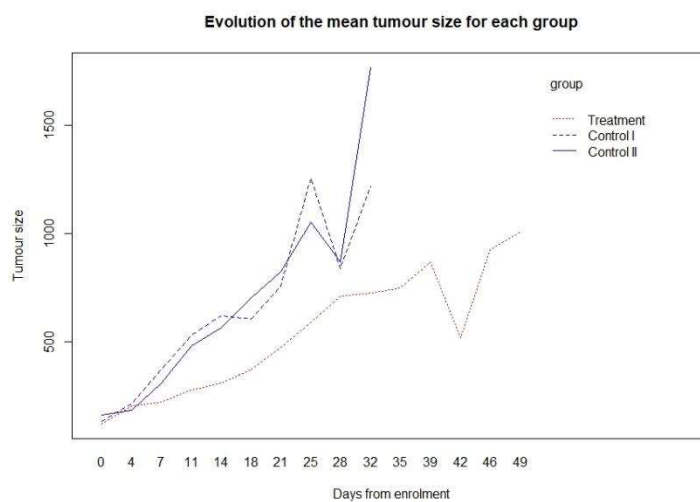

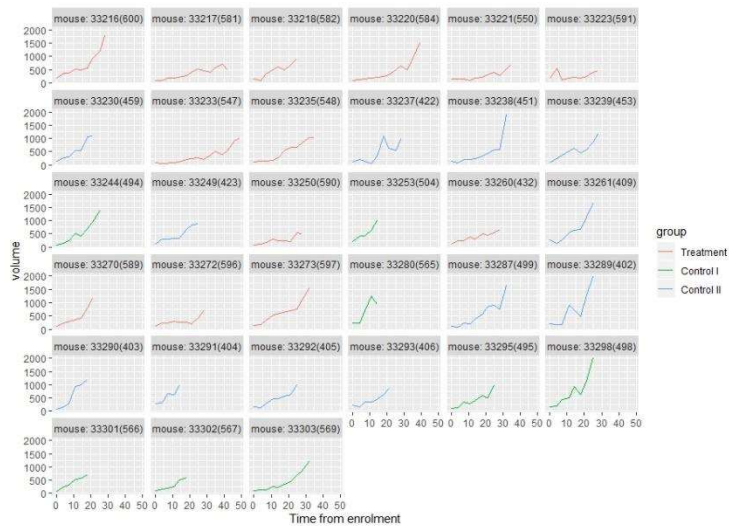Figure 6. Evolution of mean tumour size by group.

Figure 7. Evolution of tumour growth for individual mice.

```
mod.reml.0 <- lmer(volume ~ time_from_enrolment*group + (time_from_enrolment | mouse), data = tumour)
summary(mod.reml.0)

Linear mixed model fit by REML. t-tests use Satterthwaite's method [lmerModLmerTest]
Formula: volume ~ time_from_enrolment * group + (time_from_enrolment | mouse)
Data: tumour

REML criterion at convergence: 3507.2

Scaled residuals:
    Min      1Q  Median      3Q     Max
-3.1861 -0.5276 -0.0521  0.4176  4.9010

Random effects:
 Groups   Name                Variance Std.Dev. Corr
 mouse    (Intercept)           438.5   20.94
          time_from_enrolment   138.7   11.78   1.00
 Residual                     31946.6  178.74
Number of obs: 263, groups:  mouse, 33

Fixed effects:
                                   Estimate Std. Error      df t value   Pr(>|t|)
(Intercept)                          49.416     31.115 149.596   1.588    0.11436
time_from_enrolment                  24.022      3.654  21.480   6.574   1.47e-06 ***
groupControl I                       -1.055     54.297 172.256  -0.019    0.98451
groupControl II                     -22.126     47.219 160.555  -0.469    0.64001
time_from_enrolment:groupControl I   20.439      6.588  30.609   3.102    0.00411 **
time_from_enrolment:groupControl II  19.124      5.551  25.790   3.445    0.00196 **

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
            (Intr) tm_fr_ grpCnI grpCII tm__:CI
tm_frm_nrlm -0.198
groupCntrlI -0.573  0.113
gropCntrlII -0.659  0.130  0.378
tm_frm_n:CI  0.110 -0.555 -0.328 -0.072
tm_frm_:CII  0.130 -0.658 -0.075 -0.274  0.365
```

Figure 8. First model considered. The tumour volume is a function of the group and the time from enrolment (fixed effects) and we allow for random intercept and slope to take into account the differences between mice.
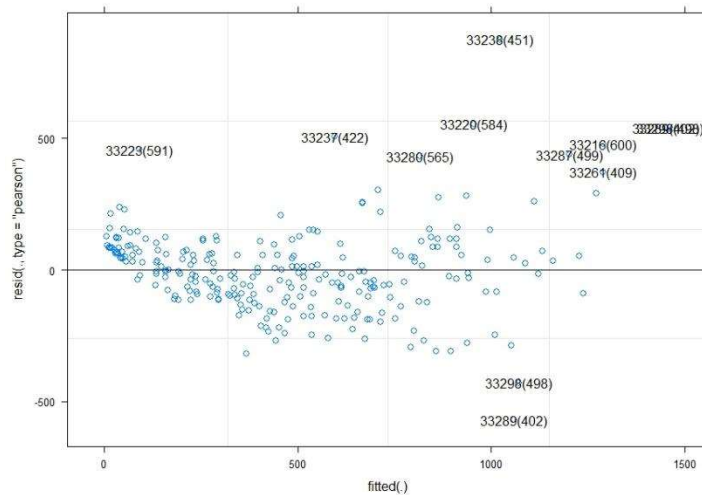
Figure 9. Diagnostic plot of the model mod.reml.0 given in the previous figure.

```
mod.reml.1 = lme(log(volume)~time_from_enrolment*group,
                 data = tumour, random =~time_from_enrolment | mouse, method = "REML")
summary(mod.reml.1)

Linear mixed-effects model fit by REML
 Data: tumour
       AIC       BIC     logLik
  288.8793 324.3701 -134.4397

Random effects:
 Formula: ~time_from_enrolment | mouse
 Structure: General positive-definite, Log-Cholesky parametrization
                   StdDev    Corr
(Intercept)        0.2830857 (Intr)
time_from_enrolment 0.0104285 0.68
Residual           0.3223225

Fixed effects: log(volume) ~ time_from_enrolment * group
                                   Value Std.Error  DF  t-value    p-value
(Intercept)                     4.817073 0.09584989 227 50.25643   0.0000
time_from_enrolment             0.059584 0.00407062 227 14.63756   0.0000
groupControl I                  0.128993 0.15909346  30  0.81080   0.4239
groupControl II                 0.117568 0.14081021  30  0.83494   0.4104
time_from_enrolment:groupControl I  0.036459 0.00814453 227  4.47649   0.0000
time_from_enrolment:groupControl II 0.028463 0.00658440 227  4.32277   0.0000

 Correlation:
                                (Intr) tm_fr_ grpCnI grpCII tm__:CI
time_from_enrolment              0.061
groupControl I                  -0.602 -0.037
groupControl II                 -0.681 -0.042  0.410
time_from_enrolment:groupControl I  -0.031 -0.500 -0.080  0.021
time_from_enrolment:groupControl II -0.038 -0.618  0.023 -0.030  0.309

Standardized Within-Group Residuals:
      Min         Q1        Med         Q3        Max
-5.0020217 -0.4834757  0.0123504  0.5444057  4.1583357

Number of Observations: 263
Number of Groups: 33
```

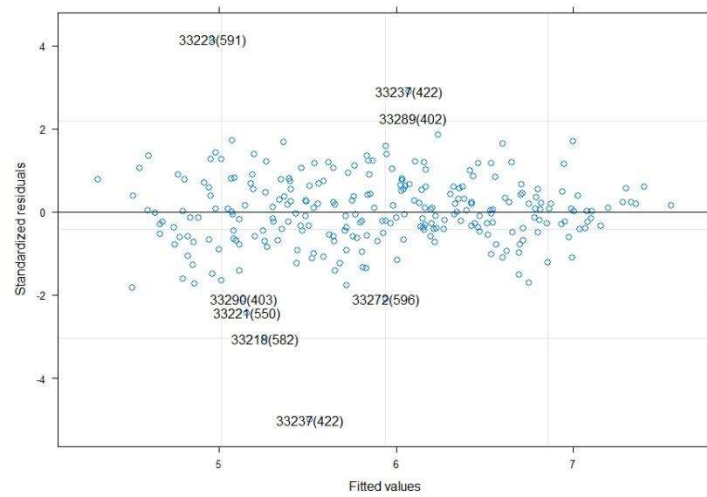Figure 10. Model with log-transformed volume to address heteroscedasticity.

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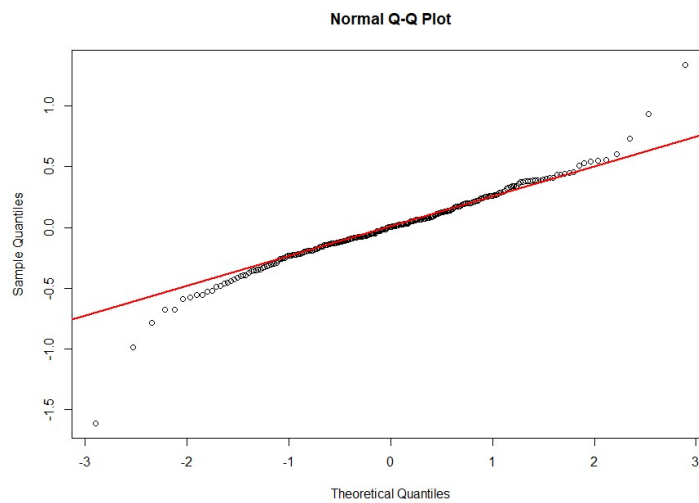Figure 11. Residual vs fitted values plot of the model with log-transformed output.

Figure 12.Q-Q plot of the residuals obtained from the model with log-transformed output.
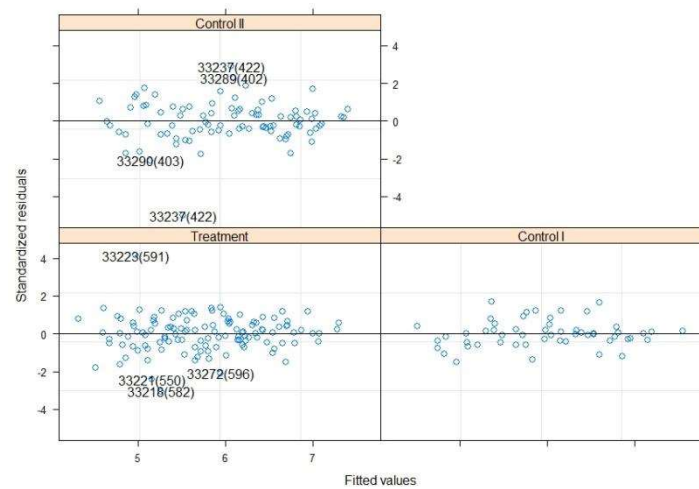

Figure 13. Residual vs fitted values plot of the model with log-transformed output by group.
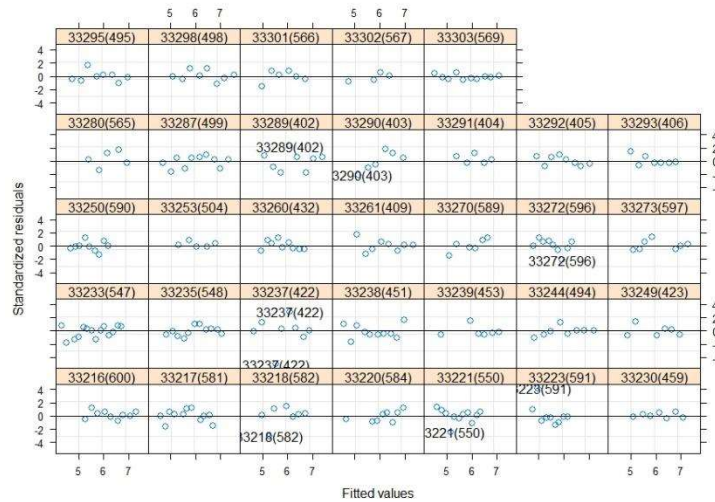
Figure 14. Residual vs fitted values plot of the model with log-transformed output by mouse.

```
Fmod.ml.1_lme = lme(log(volume)~time_from_enrolment*group, data = tumour, random =~time_from_enrolment
| mouse, method = "ML")

Fmod.ml.2_lme = lme(log(volume)~time_from_enrolment+group, data = tumour, random =~time_from_enrolment
| mouse, method = "ML")

anova(Fmod.ml.2_lme,Fmod.ml.1_lme)

              Model df      AIC      BIC    logLik   Test  L.Ratio p-value
Fmod.ml.2_lme     1  8 272.9675 301.5447 -128.4838
Fmod.ml.1_lme     2 10 254.7336 290.4551 -117.3668 1 vs 2 22.23392  <.0001
```

Figure 15. Test of significance of the interaction between group and time.

```
Rmod.ml.1_lme = lme(log(volume)~time_from_enrolment*group, data = tumour, random =~time_from_enrolment
| mouse, method = "ML")

Rmod.ml.2_lme = lme(log(volume)~time_from_enrolment*group, data = tumour, random =~1 | mouse, method =
"ML")

anova(Rmod.ml.2_lme,Rmod.ml.1_lme)

              Model df      AIC      BIC    logLik   Test  L.Ratio p-value
Rmod.ml.2_lme     1  8 262.4962 291.0734 -123.2481
Rmod.ml.1_lme     2 10 254.7336 290.4551 -117.3668 1 vs 2 11.76258  0.0028
```

Figure 16. Test of significance of the random slope.

```
anova(mod.reml.1, type="III", dff="Satterthwaite")

Type III Analysis of Variance Table with Satterthwaite's method
                            Sum Sq Mean Sq NumDF   DenDF  F value    Pr(>F)
time_from_enrolment         66.247  66.247     1  26.206 637.6551 < 2.2e-16
group                        0.100   0.050     2  32.374   0.4804    0.6229
time_from_enrolment:group    3.074   1.537     2  24.070  14.7962 6.449e-05

time_from_enrolment         ***
group
time_from_enrolment:group   ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Figure 17. F-test with Satterthwaite's correction.

```
mod.robust <- rlmer(log(volume) ~ time_from_enrolment*group + (time_from_enrolment | mouse),
                data = tumour, control=lmerControl(optimizer = "Nelder_Mead",
optCtrl=list(maxfun=1e6)), method = "DASvar")

Warning in rlmer.fit.DAS.nondiag(lobj, verbose, max.iter, rel.tol):
algorithm converged, but estimating equations are not satisfied.

summary(mod.robust)

Robust linear mixed model fit by DASvar
Formula: log(volume) ~ time_from_enrolment * group + (time_from_enrolment | mouse)
   Data: tumour
Control: lmerControl(optimizer = "Nelder_Mead", optCtrl = list(maxfun = 1e+06))
Scaled residuals:
    Min      1Q  Median      3Q     Max
-6.0921 -0.5328  0.0001  0.6223  5.3578

Random effects:
 Groups    Name                 Variance  Std.Dev. Corr
 mouse     (Intercept)          8.558e-02 0.292538
           time_from_enrolment  8.558e-05 0.009251 1.00
 Residual                       7.700e-02 0.277481
Number of obs: 263, groups: mouse, 33
Fixed effects:
                                   Estimate Std. Error t value
(Intercept)                        4.818427   0.096711   49.82
time_from_enrolment                0.060148   0.003625   16.59
groupControl I                     0.124186   0.159656    0.78
groupControl II                    0.144216   0.141568    1.02
time_from_enrolment:groupControl I  0.036185   0.007197    5.03
time_from_enrolment:groupControl II 0.025787   0.005855    4.40

Correlation of Fixed Effects:
            (Intr) tm_fr_ grpCnI grpCII tm__:CI
tm_frm_nrlm  0.353
groupCntrlI -0.606 -0.214
gropCntrlII -0.683 -0.241  0.414
tm_frm_n:CI -0.178 -0.504  0.172  0.122
tm_frm_:CII -0.219 -0.619  0.132  0.237  0.312

Robustness weights for the residuals:
 208 weights are ~= 1. The remaining 55 ones are summarized as
  Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
 0.221   0.655   0.819   0.775   0.949   0.993

Robustness weights for the random effects:
 52 weights are ~= 1. The remaining 14 ones are summarized as
  Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
 0.820   0.848   0.895   0.899   0.957   0.996

Rho functions used for fitting:
  Residuals:
    eff: smoothed Huber (k = 1.345, s = 10)
    sig: smoothed Huber, Proposal II (k = 1.345, s = 10)
  Random Effects, variance component 1 (mouse):
    eff: smoothed Huber (k = 1.345, s = 10)
    vcp: smoothed Huber (k = 1.345, s = 10)
```
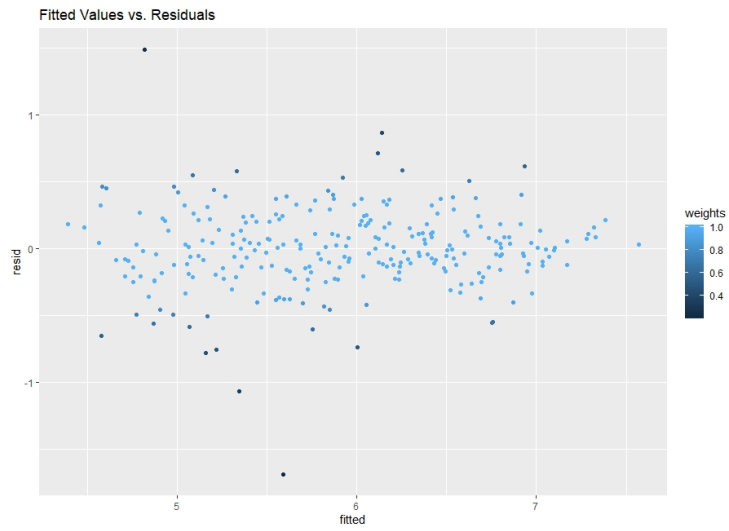
Figure 18. Robust model.

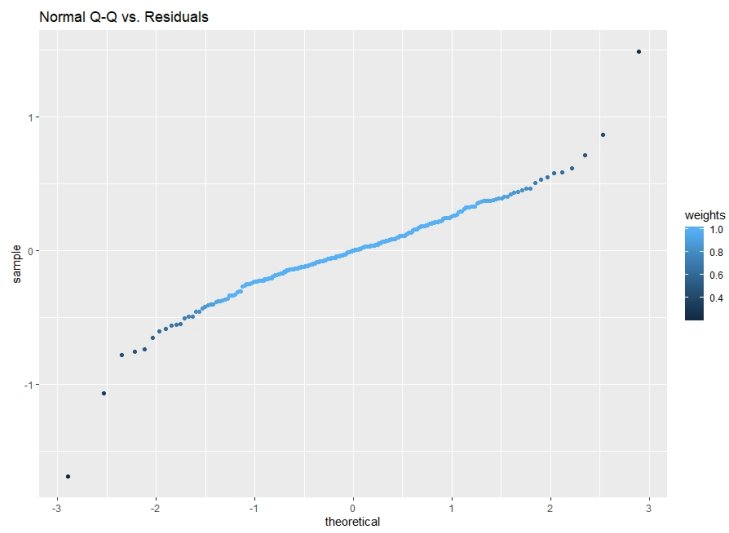Figure 19. Residual vs fitted values for the robust model.

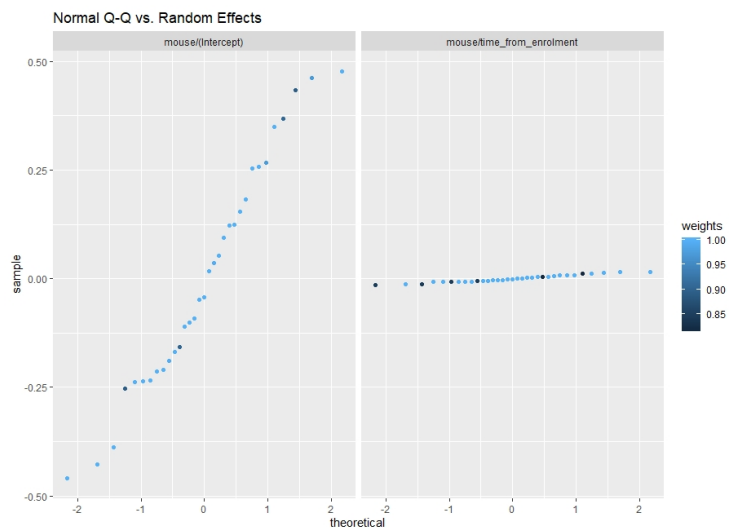Figure 20. Q-Q plot of the residuals for the robust model.

Figure 21. Q-Q plot of the residuals for the random terms in the robust model.

```
mod.reml.without_outliers = lme(log(volume)~time_from_enrolment*group,
              data = tumour_without_outliers, random =~time_from_enrolment | mouse, method = "REML")

summary(mod.reml.without_outliers)

Linear mixed-effects model fit by REML
 Data: tumour_without_outliers
       AIC       BIC    logLik
  186.3284 221.5029 -83.16418

Random effects:
 Formula: ~time_from_enrolment | mouse
 Structure: General positive-definite, Log-Cholesky parametrization
                  StdDev      Corr
(Intercept)       0.300358702 (Intr)
time_from_enrolment 0.009883128 0.694
Residual          0.256877480

Fixed effects: log(volume) ~ time_from_enrolment * group
                                   Value  Std.Error  DF  t-value
(Intercept)                        4.820526 0.09470069 219 50.90276
time_from_enrolment                0.060127 0.00359984 219 16.70271
groupControl I                     0.120557 0.15539147  30  0.77583
groupControl II                    0.155579 0.13840610  30  1.12408
time_from_enrolment:groupControl I  0.036568 0.00696758 219  5.24832
time_from_enrolment:groupControl II 0.025554 0.00572584 219  4.46292
                                   p-value
(Intercept)                         0.0000
time_from_enrolment                 0.0000
groupControl I                      0.4439
groupControl II                     0.2699
time_from_enrolment:groupControl I   0.0000
time_from_enrolment:groupControl II  0.0000
 Correlation:
                                   (Intr) tm_fr_ grpCnI grpCII tm__:CI
time_from_enrolment                 0.209
groupControl I                     -0.609 -0.127
groupControl II                    -0.684 -0.143  0.417
time_from_enrolment:groupControl I  -0.108 -0.517  0.079  0.074
time_from_enrolment:groupControl II -0.131 -0.629  0.080  0.122  0.325

Standardized Within-Group Residuals:
       Min          Q1          Med          Q3          Max
-2.155289560 -0.600548920 -0.009889969  0.660688297  2.251633303

Number of Observations: 255
Number of Groups: 33
```

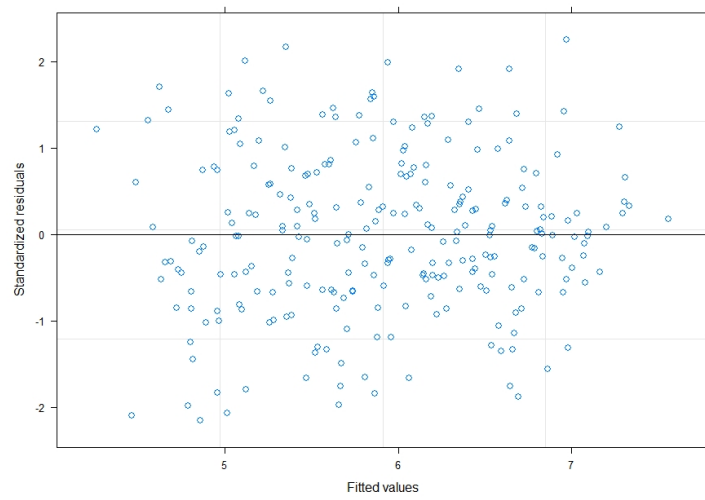Figure 22. Summary of the model without outliers.

Figure 23. Diagnostic plot (residuals vs fitted values) for the model without outliers.