

COUNTERCOVID analysis

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1. Data overview

- Exposure: imatinib vs. placebo.
- Survival outcome: 28-day survival.
- Mediator: IL6 on days 0, 2, 3, 5.

```
countercovid_long <- readRDS("./countercovid_long.rds")
countercovid_surv <- readRDS("./countercovid_surv.rds")
```

2. Descriptives

```
countercovid_wide <- countercovid_long%>% select(!c(endt, endt28)) %>%
  unite("biomarker_day", biomarker, day, sep = "_") %>%
  pivot_wider(names_from = biomarker_day, values_from = conc_log10) %>%
  mutate(IL6_0 = gsub("NULL", NA, IL6_0) %>% as.numeric(),
         IL6_2 = gsub("NULL", NA, IL6_2) %>% as.numeric(),
         IL6_3 = gsub("NULL", NA, IL6_3) %>% as.numeric(),
         IL6_5 = gsub("NULL", NA, IL6_5) %>% as.numeric())
```

```
tableone::CreateTableOne(countercovid_wide, strata = "randomized_group", vars = c("death_d28", "death_d90", "IL6_0", "IL6_2", "IL6_3", "IL6_5", "sex", "age", "bmi"))
```

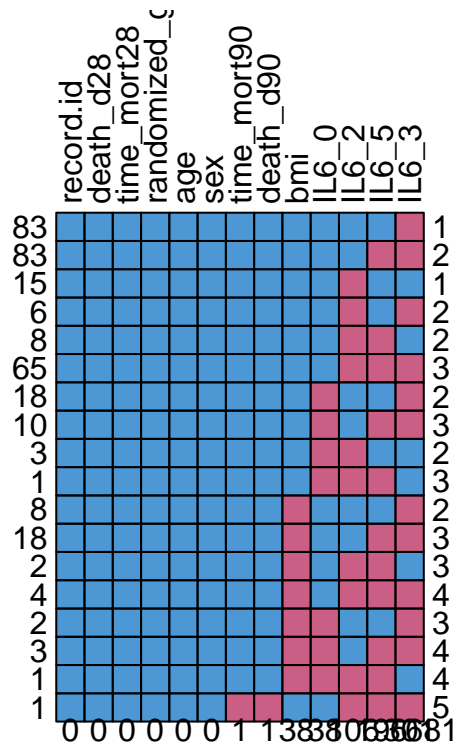
2.1. Table 1

Stratified by randomized_group				
	Imatinib	Placebo		
n	168	163		
death_d28 = 1 (%)	13 (7.7)	24 (14.7)		
death_d90 = 1 (%)	15 (8.9)	28 (17.3)		
IL6_0 (mean (SD))	1.03 (0.43)	1.12 (0.41)		
IL6_2 (mean (SD))	0.85 (0.49)	1.10 (0.46)		
IL6_3 (mean (SD))	1.04 (0.51)	1.14 (0.48)		
IL6_5 (mean (SD))	0.75 (0.59)	1.07 (0.54)		
sex = male (%)	126 (75.0)	108 (66.3)		
age (mean (SD))	64.45 (12.46)	63.76 (12.86)		
bmi (mean (SD))	28.58 (5.46)	30.03 (5.51)		

2.2. Missingness Of 386 patients, 55 have no IL-6 biomarker measures. They are not included in any of the analyses.

For the 331 subjects who do have IL6 measures, this is the pattern of missingness:

```
missing_counterccoid <- mice::md.pattern(countercovid_wide, rotate.names = TRUE)
```



```
# set the reference group
counterccoid_long$randomized_group <- counterccoid_long$randomized_group %>% as.factor()
counterccoid_long$randomized_group <- counterccoid_long$randomized_group %>% relevel(ref = "Placebo")
counterccoid_surv$randomized_group <- counterccoid_surv$randomized_group %>% as.factor()
```

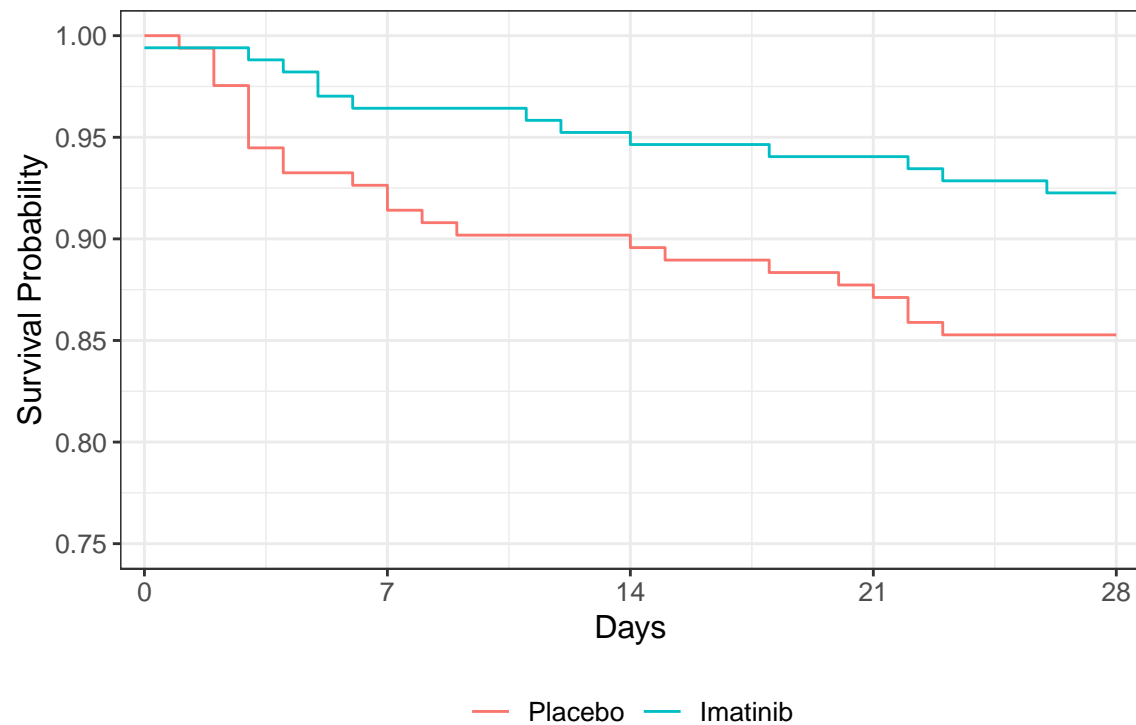
```

countercovid_surv$randomized_group <- countercovid_surv$randomized_group %>% relevel(ref = "Placebo")

class(countercovid_surv$death_d28) <- "integer"

survfit2(Surv(time_mort28, death_d28) ~ randomized_group, data = countercovid_surv) %>%
  ggsurvfit() + scale_ggsurvfit(x_scales= list(breaks = c(0, 7, 14, 21, 28))) +
  ylim(c(.75, 1))+
  xlab("Days")

```



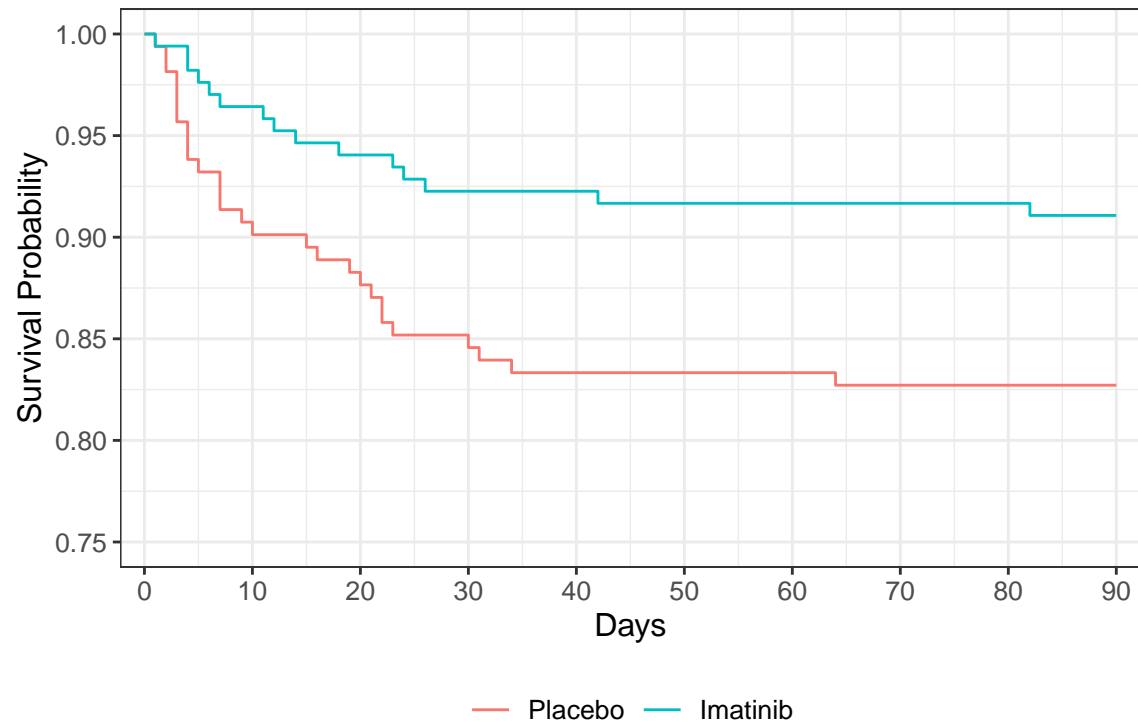
2.3. 28-day survival

```

class(countercovid_surv$death_d90) <- "integer"

survfit2(Surv(time_mort90, death_d90) ~ randomized_group, data = countercovid_surv) %>%
  ggsurvfit() + scale_ggsurvfit(x_scales= list(breaks = seq(0,90,10))) +
  ylim(c(.75, 1))+
  xlab("Days")

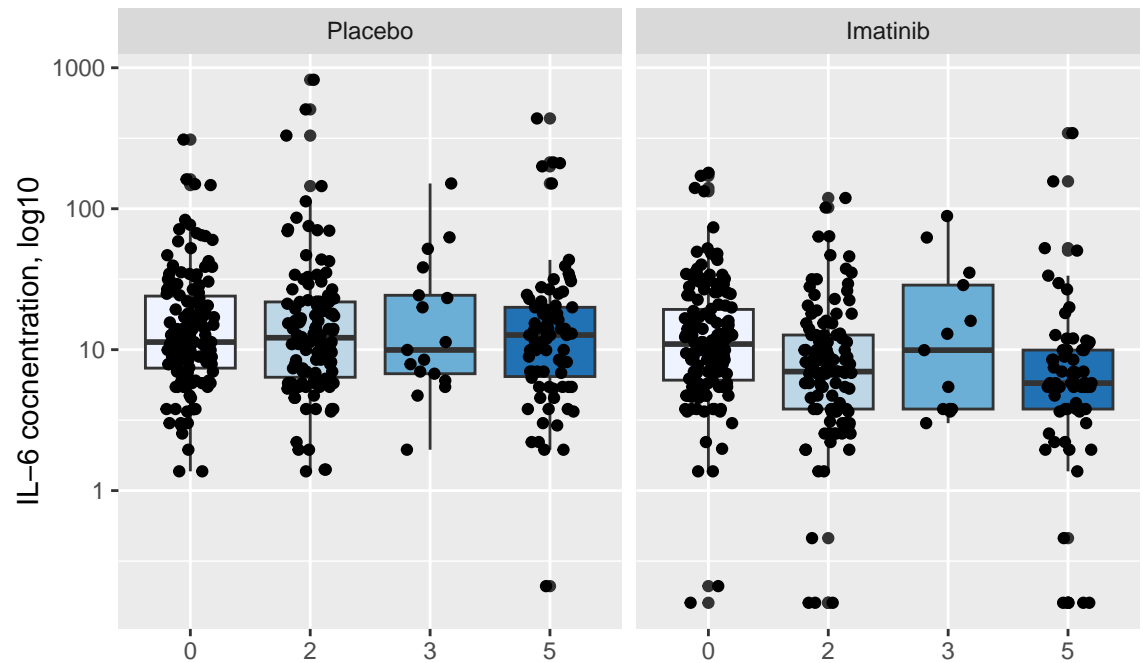
```



2.4. 90-day survival

```
countercovid_long %>% drop_na(conc_log10) %>%
  ggplot(aes(as.factor(day), 10^conc_log10, fill = as.factor(day)))+
  geom_boxplot() +
  geom_jitter(width = .2)+
  geom_smooth()+
  guides(fill = "none") +
  labs(x = "", y = "IL-6 concentration, log10")+
  scale_y_continuous(trans = "log10")+
  facet_grid(cols = vars(randomized_group))+
  ggtitle("IL-6 concentration on days 0, 2, 3 and 5")+
  scale_fill_brewer()
```

IL-6 concentration on days 0, 2, 3 and 5



2.5. IL6 over time

3. Models

3.1. Linear-mixed model for IL-6 over time Note: there is one patient with missing 90 day survival data. A separate lm is fit where we exclude this patient. This is necessary to have the same subjects in the longitudinal and survival submodels of the joint model.

```
## Fit linear mixed model for biomarker concentration
```

```
lmefit.countercovid <- lme(conc_log10~ day:randomized_group +day,
  random = ~ day | record.id,
  data = countercovid_long,
  control = lmeControl(opt = "optim"),
  na.action = na.omit)
```

```
summary(lmefit.countercovid)
```

```
## Linear mixed-effects model fit by REML
## Data: countercovid_long
##      AIC      BIC    logLik
## 821.9867 853.8542 -403.9933
##
## Random effects:
## Formula: ~day | record.id
## Structure: General positive-definite, Log-Cholesky parametrization
##           StdDev      Corr
## (Intercept) 0.33420336 (Intr)
## day          0.06973354 -0.018
## Residual    0.29178962
##
## Fixed effects: conc_log10 ~ day:randomized_group + day
```

```
##                               Value Std.Error DF t-value p-value
## (Intercept)                   1.0826867 0.02433411 371 44.49256 0.0000
## day                           -0.0147687 0.01137807 371 -1.29800 0.1951
## day:randomized_groupImatinib -0.0613266 0.01552601 371 -3.94992 0.0001
## Correlation:
##                               (Intr) day
## day                           -0.282
## day:randomized_groupImatinib 0.007 -0.677
##
## Standardized Within-Group Residuals:
##      Min      Q1      Med      Q3      Max
## -4.75444869 -0.39035017 -0.01291234 0.32388527 3.83083236
##
## Number of Observations: 704
## Number of Groups: 331
```

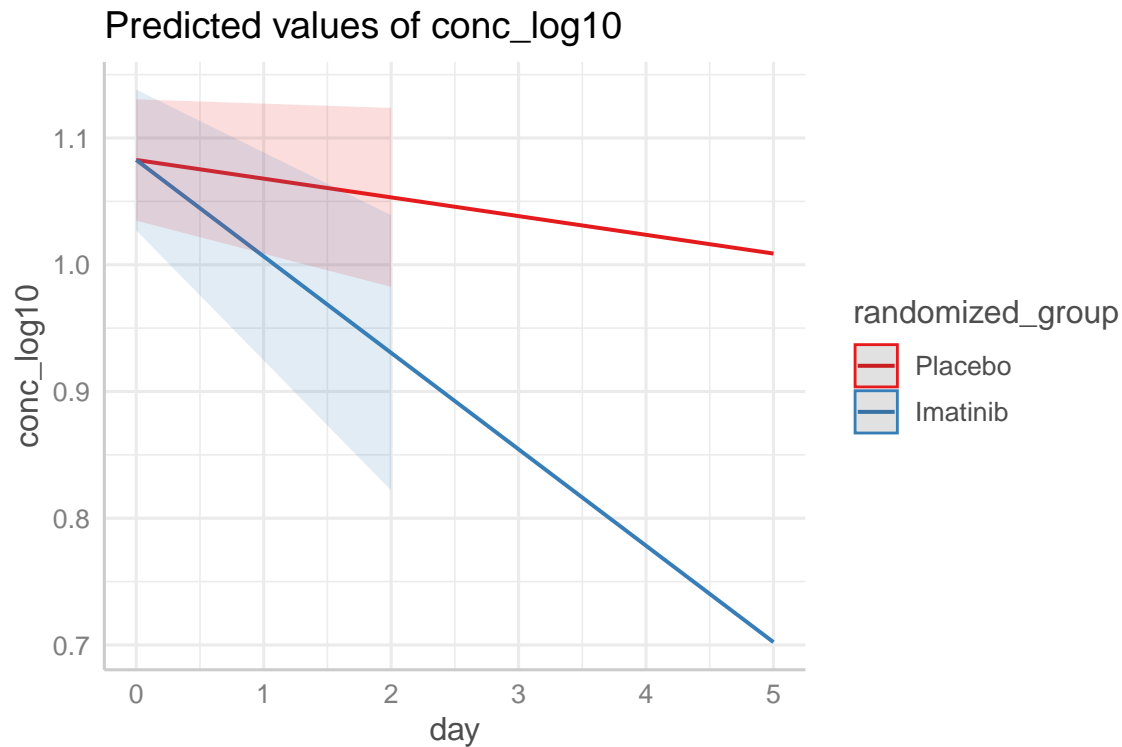
```
lmefit.countercovid_90 <- countercovid_long %>%
  filter(!is.na(death_d90)) %>%
  lme(conc_log10~ day:randomized_group,
      random = ~ day | record.id,
      data = .,
      control = lmeControl(opt = "optim"),
      na.action = na.omit)

summary(lmefit.countercovid_90)
```

```
## Linear mixed-effects model fit by REML
## Data: .
##      AIC      BIC    logLik
## 821.249 853.1065 -403.6245
##
## Random effects:
## Formula: ~day | record.id
## Structure: General positive-definite, Log-Cholesky parametrization
##           StdDev    Corr
## (Intercept) 0.33463777 (Intr)
## day          0.06974882 -0.019
## Residual     0.29179148
##
## Fixed effects: conc_log10 ~ day:randomized_group
##                               Value Std.Error DF t-value p-value
## (Intercept)                   1.0836557 0.02438946 371 44.43130 0.0000
## day:randomized_groupPlacebo    -0.0148995 0.01137977 371 -1.30930 0.1912
## day:randomized_groupImatinib  -0.0762125 0.01146541 371 -6.64717 0.0000
## Correlation:
##                               (Intr) dy:r_P
## day:randomized_groupPlacebo    -0.282
## day:randomized_groupImatinib  -0.270 0.076
##
## Standardized Within-Group Residuals:
##      Min      Q1      Med      Q3      Max
## -4.75467879 -0.39009716 -0.01326511 0.32357112 3.82916219
##
## Number of Observations: 703
## Number of Groups: 330
```

```
library(sjPlot)
library(sjmisc)
theme_set(theme_sjplot())

plot_model(lmefit.counterccovid, type = "int", terms = c("randomized_group", "day"))
```



```
# save the interaction estimate
a_res <- get_int(lmefit.counterccovid, "randomized_groupImatinib")

saveRDS(a_res, "COUNTERCOVID_beta_est.rds")
```

3.2. Cox proportional hazards model

```
## fit cox ph model
coxfit.counterccovid_28 <- coxph(Surv(time_mort28, death_d28) ~ randomized_group, data = counterccovid_surv)
summary(coxfits.counterccovid_28)
```

3.2.1. 28-day survival

```
## Call:
## coxph(formula = Surv(time_mort28, death_d28) ~ randomized_group,
##       data = counterccovid_surv, x = TRUE)
##
## n= 331, number of events= 37
##
##               coef exp(coef) se(coef)      z Pr(>|z|)
## randomized_groupImatinib -0.6907    0.5012  0.3444 -2.006  0.0449 *
```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##               exp(coef) exp(-coef) lower .95 upper .95
## randomized_groupImatinib    0.5012      1.995    0.2552    0.9844
##
## Concordance= 0.586  (se = 0.039 )
## Likelihood ratio test= 4.23  on 1 df,   p=0.04
## Wald test            = 4.02  on 1 df,   p=0.04
## Score (logrank) test = 4.18  on 1 df,   p=0.04
confint(coxfit.countercovid_28) %>% exp() %>% round(3)

##               2.5 % 97.5 %
## randomized_groupImatinib 0.255  0.984
```

```
## fit cox ph model
coxfit.countercovid_90 <- coxph(Surv(time_mort90, death_d90)~ randomized_group, data = countercovid_surv,
                                x=TRUE)

summary(coxfit.countercovid_90)
```

3.2.2. 90-day survival

```
## Call:
## coxph(formula = Surv(time_mort90, death_d90) ~ randomized_group,
##       data = countercovid_surv, x = TRUE)
##
##    n= 330, number of events= 43
##    (1 observation deleted due to missingness)
##
##               coef exp(coef) se(coef)      z Pr(>|z|)
## randomized_groupImatinib -0.7156    0.4889    0.3200 -2.236   0.0253 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##               exp(coef) exp(-coef) lower .95 upper .95
## randomized_groupImatinib    0.4889      2.045    0.2611    0.9154
##
## Concordance= 0.589  (se = 0.037 )
## Likelihood ratio test= 5.27  on 1 df,   p=0.02
## Wald test            = 5    on 1 df,   p=0.03
## Score (logrank) test = 5.22  on 1 df,   p=0.02
```

3.3. Joint model Using 28-day and 90-day survival as endpoints

```
set.seed(15)

## fit joint model
jointfit.countercovid_28<- JMBayes2::jm(coxfit.countercovid_28, lmefit.countercovid, time_var = "day",

summary(jointfit.countercovid_28)

saveRDS(jointfit.countercovid_28, "./jointfit_countercovid_28.rds")
```


3.3.1. 28-day endpoint

```
##
## Call:
## JMBayes2::jm(Surv_object = coxfit.counterccovid_28, Mixed_objects = lmefit.counterccovid,
##   time_var = "day", n_iter = 60000L, n_burnin = 5000L, n_chains = 2L,
##   n_thin = 5L, cores = 2)
##
## Data Descriptives:
## Number of Groups: 331          Number of events: 37 (11.2%)
## Number of Observations:
##   conc_log10: 704
##
##               DIC        WAIC        LPML
## marginal      1197.6815 1272.6692 -697.4384
## conditional   408.7553  300.0128 -454.1746
##
## Random-effects covariance matrix:
##
##      StdDev   Corr
## (Intr) 0.3198 (Intr)
## day    0.0502 0.3110
##
## Survival Outcome:
##               Mean StDev  2.5% 97.5%      P  Rhat
## randomized_groupImatinib 0.0125 0.5187 -1.013 1.0149 0.9814 1.0002
## value(conc_log10)        1.5326 0.5059  0.666 2.6977 0.0004 1.0026
##
## Longitudinal Outcome: conc_log10 (family = gaussian, link = identity)
##               Mean StDev  2.5% 97.5%      P  Rhat
## (Intercept)  1.0838 0.0241  1.0362 1.1308 0.0000 1.0007
## day          -0.0211 0.0105 -0.0417 -0.0004 0.0452 1.0023
## d:_I         -0.0485 0.0145 -0.0768 -0.0203 0.0007 1.0003
## sigma        0.3088 0.0136  0.2832  0.3362 0.0000 0.9999
##
## MCMC summary:
## chains: 2
## iterations per chain: 60000
## burn-in per chain: 5000
## thinning: 5
## time: 3.5 min
```

```
set.seed(15)
```

```
## fit joint model
```

```
jointfit.counterccovid_90<- JMBayes2::jm(coxfit.counterccovid_90, lmefit.counterccovid_90, time_var = "day"
```

```
summary(jointfit.counterccovid_90)
```

```
saveRDS(jointfit.counterccovid_90, "jointfit_counterccovid_90.rds")
```

3.3.2. 90-day endpoint

```
##
```

```
## Call:
## JMBayes2::jm(Surv_object = coxfit.counterccovid_90, Mixed_objects = lmefit.counterccovid_90,
##   time_var = "day", n_iter = 60000L, n_burnin = 5000L, n_chains = 2L,
##   n_thin = 5L, cores = 2)
##
## Data Descriptives:
## Number of Groups: 330          Number of events: 43 (13%)
## Number of Observations:
##   conc_log10: 703
##
##           DIC           WAIC          LPML
## marginal    1345.2180    1382.686    -786.7081
## conditional  687.2757 1408448.116 -283793.7446
##
## Random-effects covariance matrix:
##
##      StdDev   Corr
## (Intr) 0.3219 (Intr)
## day    0.0589 0.2081
##
## Survival Outcome:
##           Mean StDev   2.5%  97.5%      P   Rhat
## randomized_groupImatinib -0.1862 0.4778 -1.1184 0.7473 0.6943 1.0004
## value(conc_log10)        0.9843 0.2569  0.4949 1.4790 0.0000 1.0611
##
## Longitudinal Outcome: conc_log10 (family = gaussian, link = identity)
##           Mean StDev   2.5%  97.5%      P   Rhat
## (Intercept)  1.0853 0.0243  1.0382  1.1330 0.0000 1.0002
## day         -0.0245 0.0108 -0.0455 -0.0031 0.0233 1.0005
## d:_I        -0.0445 0.0145 -0.0736 -0.0166 0.0011 1.0008
## sigma       0.3030 0.0135  0.2773  0.3301 0.0000 1.0033
##
## MCMC summary:
## chains: 2
## iterations per chain: 60000
## burn-in per chain: 5000
## thinning: 5
## time: 4.1 min
```

4. Results

4.1. Indirect, direct, and total effects Of imatinib through IL6 on 28-day mortality

```
res28 <- get_effects(jointfit.counterccovid_28, coxfit.counterccovid_28, "randomized_groupImatinib") %>%
res28
```

```
##           effect      est  CI_lower  CI_upper  endpoint
## 1      direct 0.01250247 -1.0130430  1.01488399 28-day endpoint
## 2    indirect -0.07431096 -0.1345114 -0.02480730 28-day endpoint
## 3 total (Cox-PH) -0.69070841 -1.3657040 -0.01571283 28-day endpoint
## 4    total (JM) -0.06180850 -1.0664945  0.91792515 28-day endpoint
```

Of imatinib through IL6 on 28-day mortality

```

res90 <- get_effects(jointfit.countercovid_90, coxfit.countercovid_90, "randomized_groupImatinib")%>% c
res90

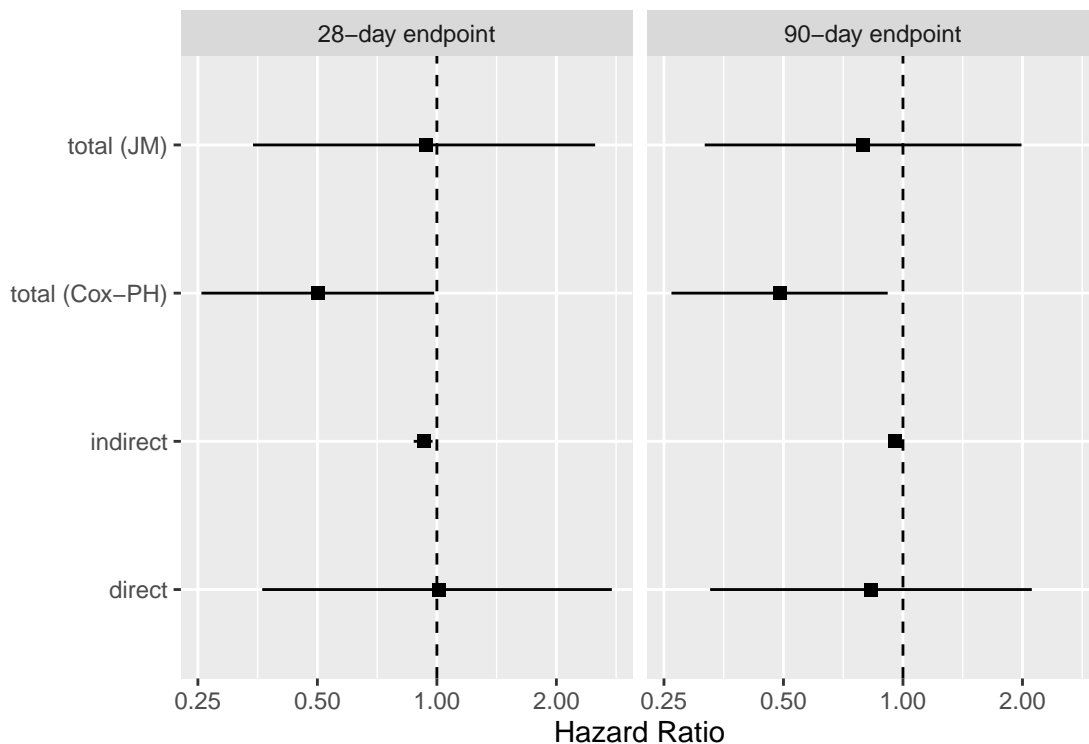
##          effect      est    CI_lower    CI_upper    endpoint
## 1      direct -0.18619161 -1.11843854  0.74734402 90-day endpoint
## 2    indirect -0.04380764 -0.07914674 -0.01483965 90-day endpoint
## 3 total (Cox-PH) -0.71560268 -1.34278903 -0.08841633 90-day endpoint
## 4    total (JM) -0.22999924 -1.14980734  0.68759550 90-day endpoint

res <- rbind(res28, res90)

saveRDS(res, "countercovid_res.rds")

res %>%
  ggplot(aes(y = effect)) +
  theme_grey() +
  geom_point(aes(x=exp(est)), shape=15, size=2) +
  geom_linerange(aes(xmin=exp(CI_lower), xmax=exp(CI_upper))) +
  geom_vline(xintercept = 1, linetype="dashed") +
  labs(x="Hazard Ratio", y="")+
  scale_x_continuous(trans = "log2") +
  facet_grid(~endpoint)

```



4.2. Association parameter Hazard ratio estimate and 95% CI for the association parameter α for a one unit increase (at any time point) of IL-6 and the hazard of death over 28 days.

```

print(paste0("HR alpha = ", round(exp(jointfit.countercovid_28$statistics$Mean$alphas),3), ", ", "95% CI",
  round(exp(quantile(unlist(jointfit.countercovid_28$mcmc$alphas), probs = c(0.025, 0.975))), 3)[2,

```

```
## [1] "HR alpha = 4.63, 95% CI = [1.946, 14.845]"
# save association estimates

alpha_28 <- get_alpha(jointfit.countercovid_28, "28-day endpoint")
alpha_90 <- get_alpha(jointfit.countercovid_90, "90-day endpoint")

alpha_est <- rbind(alpha_28, alpha_90)

saveRDS(alpha_est, "COUNTERCOVID_alpha_est.rds")
```

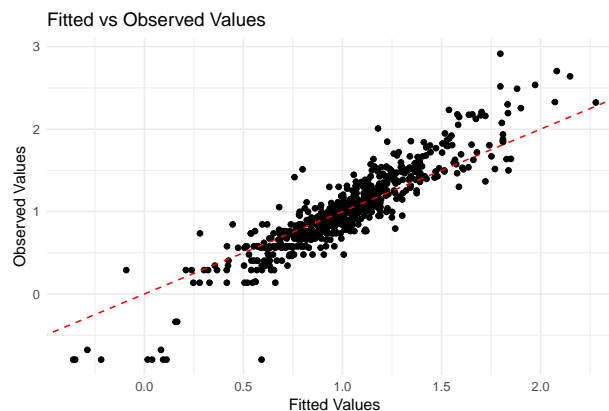
4.3. Conclusions

- From lme and JM we can conclude that there is an effect of imatinib over time on IL-6.
- From joint model we conclude that 1) there is no direct effect of imatinib on survival when controlling for IL-6, and 2) there is an association between IL-6 and survival.

5. Model checks

```
# get fitted values
fitted_values <- fitted(lmefit.countercovid)

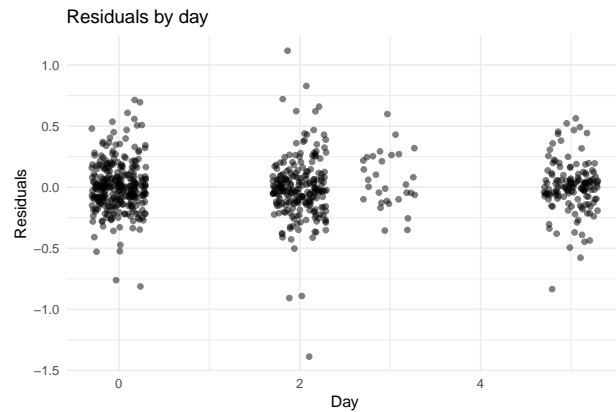
# plot observed vs fitted values
ggplot(data = countercovid_long, aes(x = fitted_values, y = conc_log10)) +
  geom_point() +
  geom_abline(slope = 1, intercept = 0, linetype = "dashed", color = "red") + # Line of perfect fit
  labs(x = "Fitted Values", y = "Observed Values") +
  ggtitle("Fitted vs Observed Values") +
  theme_minimal()
```



5.1. Longitudinal submodel

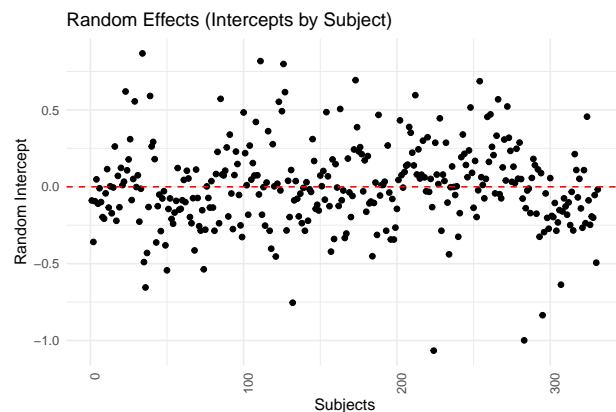
```
# get residuals
residuals_values <- resid(lmefit.countercovid)

# plot residuals vs time
ggplot(countercovid_long, aes(x = day, y = residuals_values)) +
  geom_jitter(width = 0.3, alpha = 0.5) +
  labs(x = "Day", y = "Residuals") +
  ggtitle("Residuals by day") +
  theme_minimal()
```

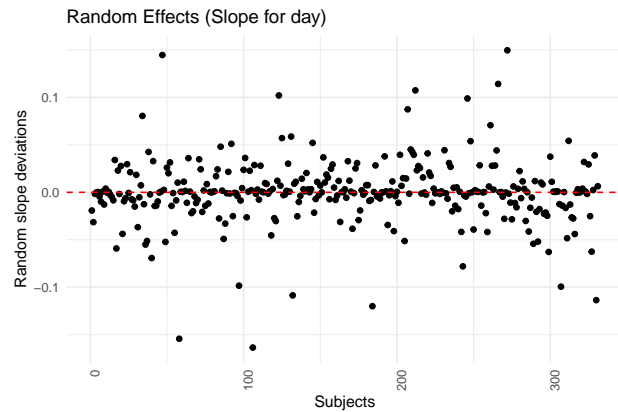


```
# get random effects
random_effects <- ranef(lmefit.countercovid)

#plot random effects
ggplot(random_effects, aes(x = c(1:nrow(countercovid_surv)), y = `(Intercept)`)) +
  geom_point() +
  geom_hline(yintercept = 0, linetype = "dashed", color = "red") +
  labs(x = "Subjects", y = "Random Intercept") +
  ggtitle("Random Effects (Intercepts by Subject)") +
  theme_minimal() +
  theme(axis.text.x = element_text(angle = 90, hjust = 1))
```

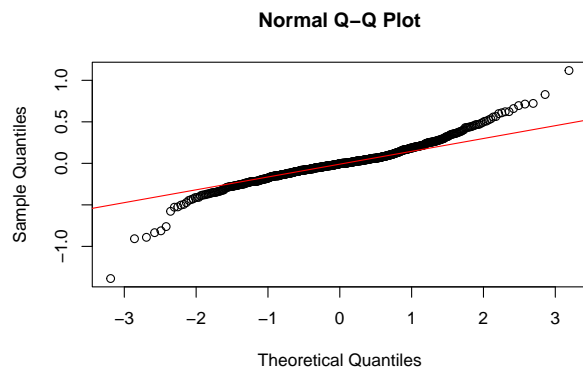


```
#plot random effects
ggplot(random_effects, aes(x = c(1:nrow(countercovid_surv)), y = `day`)) +
  geom_point() +
  geom_hline(yintercept = 0, linetype = "dashed", color = "red") +
  labs(x = "Subjects", y = "Random slope deviations") +
  ggtitle("Random Effects (Slope for day)") +
  theme_minimal() +
  theme(axis.text.x = element_text(angle = 90, hjust = 1))
```

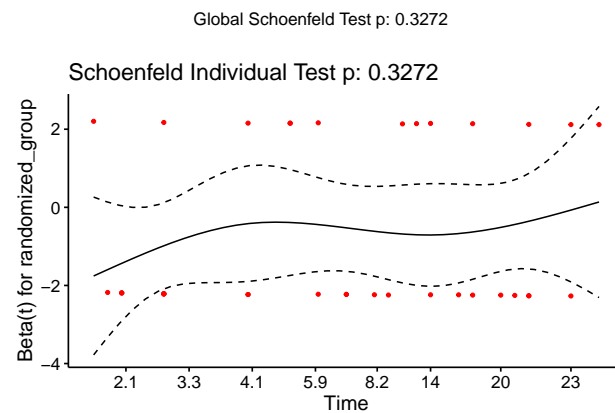


```
# qq plot for residuals
qqnorm(resid(lmefit.countercovid))

qqline(resid(lmefit.countercovid), col = "red")
```



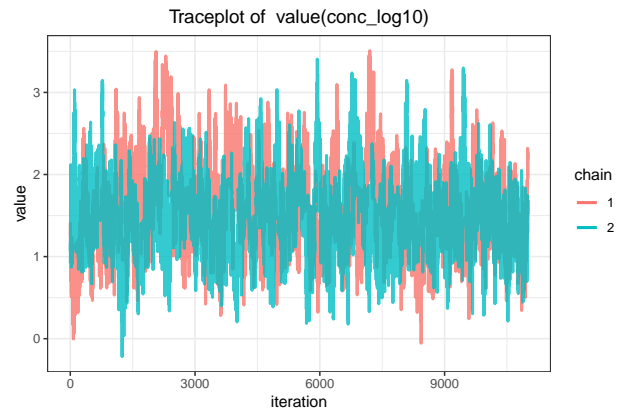
```
test.ph <- cox.zph(coxfit.countercovid_28)
survminer::ggcoxzph(test.ph)
```



5.2 Survival submodel

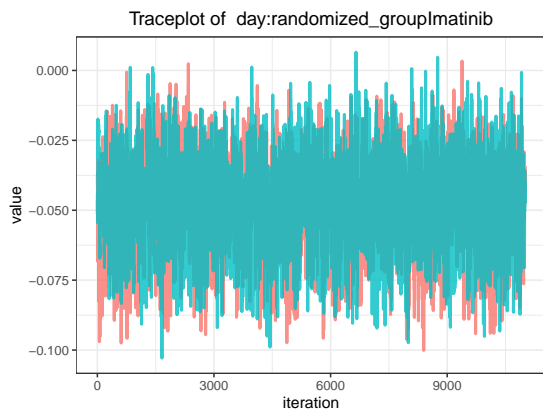
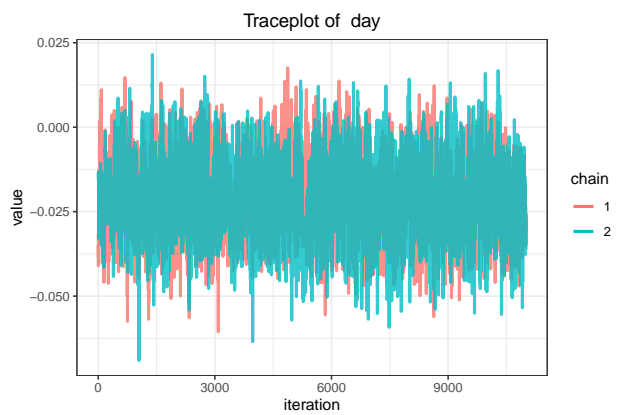
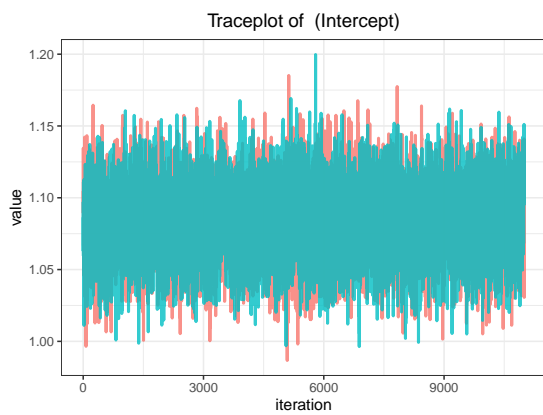
Proportional hazards assumption holds.

```
ggtraceplot(jointfit.countercovid_28, "alphas")
```

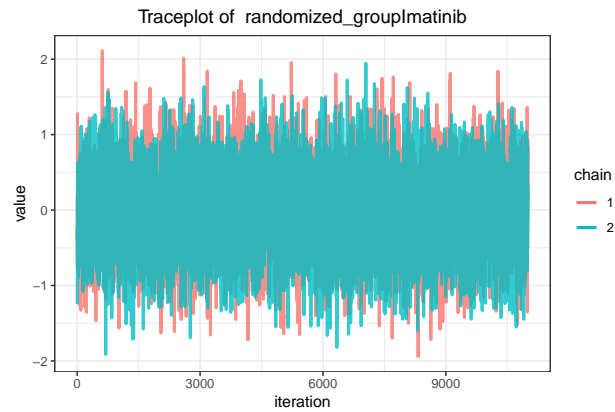


5.3 Traceplots joint model: 28-day endpoint

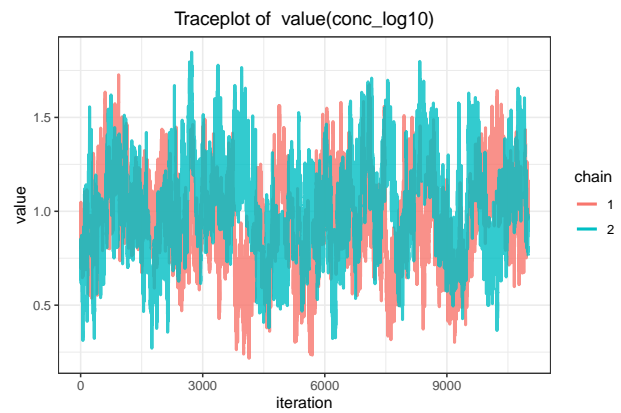
```
ggtraceplot(jointfit.countercovid_28, "betas")
```



```
ggtraceplot(jointfit.countercovid_28, "gammas")
```

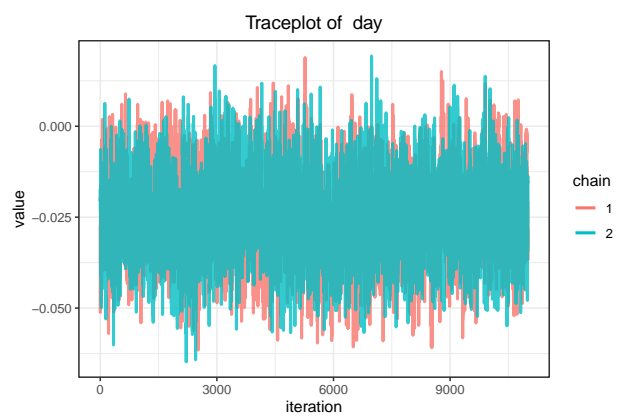
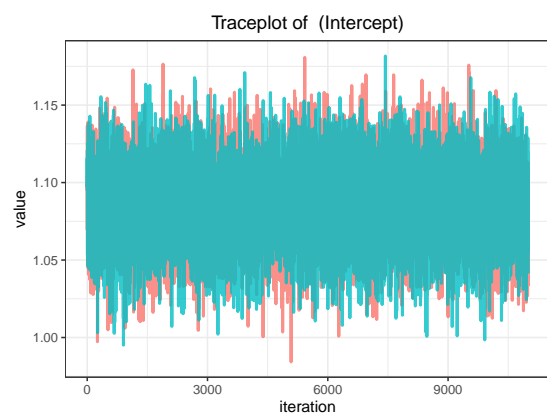


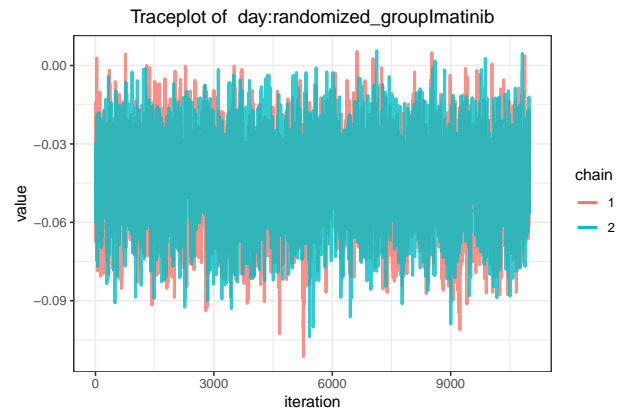
```
ggtraceplot(jointfit.countercovid_90, "alphas")
```



5.4 Traceplots joint model: 90-day endpoint

```
ggtraceplot(jointfit.countercovid_90, "betas")
```





```
ggtraceplot(jointfit.countercovid_90, "gammas")
```

