

Supplemental Digital Content 2

CALGB 80303 ANC Paper

May 21, 2018

C80303 GWAS data

We start with de-identified preprocessed data.

```
gdatfname <- "/data1/workspace/CALGB80303/GWAS/ANC/Proc/C80303_ANC_GWAS_DI.RData"  
tools::md5sum(gdatfname)  
  
## /data1/workspace/CALGB80303/GWAS/ANC/Proc/C80303_ANC_GWAS_DI.RData  
## "978062242bf7784d046d7b84d5bcaa42"  
  
attach(gdatfname)
```

Summary of GWAS Data

The data for the survival and progression dates outcomes were obtained from the clinical data set published by Kindler(2010).The high-grade (grade 3 or higher) treatment-related neutropenia data were obtained from updated clinical data quired on Aug 18th, 2014.

OStime test by treatment arm

```
gwa294DI@phdata$TREAT_ASSIGNED <- c("Bevacizumab","Placebo")[factor(gwa294DI@phdata$arm)]
survfit(Surv(ostime,osevent)~1,data=gwa294DI@phdata)

## Call: survfit(formula = Surv(ostime, osevent) ~ 1, data = gwa294DI@phdata)
##
##           n events median 0.95LCL 0.95UCL
## 294.00 291.00   5.95   5.36   6.97

survfit(Surv(ostime,osevent)~TREAT_ASSIGNED,data=gwa294DI@phdata)

## Call: survfit(formula = Surv(ostime, osevent) ~ TREAT_ASSIGNED, data = gwa294DI@phdata)
##
##           n events median 0.95LCL 0.95UCL
## TREAT_ASSIGNED=Bevacizumab 154 152 5.83 4.90 7.06
## TREAT_ASSIGNED=Placebo     140 139 6.31 5.06 7.95

summary(coxph(Surv(ostime,osevent)~TREAT_ASSIGNED,data=gwa294DI@phdata))

## Call:
## coxph(formula = Surv(ostime, osevent) ~ TREAT_ASSIGNED, data = gwa294DI@phdata)
##
## n= 294, number of events= 291
##
##              coef exp(coef) se(coef)      z Pr(>|z|)
## TREAT_ASSIGNEDPlacebo -0.008086 0.991946 0.117669 -0.069 0.945
##
##              exp(coef) exp(-coef) lower .95 upper .95
## TREAT_ASSIGNEDPlacebo 0.9919 1.008 0.7876 1.249
##
## Concordance= 0.506 (se = 0.017 )
## Rsquare= 0 (max possible= 1 )
## Likelihood ratio test= 0 on 1 df, p=0.9452
## Wald test = 0 on 1 df, p=0.9452
## Score (logrank) test= 0 on 1 df, p=0.9452
```

PFStime test by treatment arm

```
survfit(Surv(pfs,pd)~TREAT_ASSIGNED,data=gwa294DI@phdata)

## Call: survfit(formula = Surv(pfs, pd) ~ TREAT_ASSIGNED, data = gwa294DI@phdata)
##
##              n events median 0.95LCL 0.95UCL
## TREAT_ASSIGNED=Bevacizumab 154     96  5.88   4.44   7.49
## TREAT_ASSIGNED=Placebo    140    100  4.21   2.92   5.49

summary(coxph(Surv(pfs,pd)~TREAT_ASSIGNED,data=gwa294DI@phdata))

## Call:
## coxph(formula = Surv(pfs, pd) ~ TREAT_ASSIGNED, data = gwa294DI@phdata)
##
## n= 294, number of events= 196
##
##              coef exp(coef) se(coef)    z Pr(>|z|)
## TREAT_ASSIGNEDPlacebo 0.2665    1.3054  0.1433 1.86  0.0629 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##              exp(coef) exp(-coef) lower .95 upper .95
## TREAT_ASSIGNEDPlacebo  1.305    0.766   0.9857   1.729
##
## Concordance= 0.549 (se = 0.02 )
## Rsquare= 0.012 (max possible= 0.998 )
## Likelihood ratio test= 3.45 on 1 df,  p=0.0631
## Wald test = 3.46 on 1 df,  p=0.06293
## Score (logrank) test = 3.48 on 1 df,  p=0.06217
```

Summary of Adverse event

The time to event was calculated as time from treatment start date to date of high-grade neutropenic event or treatment termination, whichever occurred first. Patients lost to follow-up prior to the occurrence of neutropenia or treatment termination were considered censored at date of last follow-up.

Neut3Code from the raw data are decoded as N3Code3,N3CodeBIN:

N3Code3 : 0=control 1=ANC3,2=death/prog, 3=TTAE, 4=Unknown.

N3CodeBIN : 0=all other reason than ANC3,1=ANC3.

N3Code3CENS5WEEK:

if Neut3time<=35 days, N3Code3CENS5WEEK==N3Code3; otherwise N3Code3CENS5WEEK==0.

N3Code3CENS5WEEKBIN:

if N3Code3CENS5WEEK==1,N3Code3CENS5WEEKBIN==1; otherwise N3Code3CENS5WEEKBIN==0.

Neut3Code was defined as the following:

- ▶ 1: Neutropenia.
- ▶ 2: Death.
- ▶ 3: Progression.
- ▶ 5: Hypertension.
- ▶ 6: Proteinurea.
- ▶ 7: Other ADR.
- ▶ 8: W/o consent or Withdrew from study (not related to ADR).*
- ▶ 9: Other/unknown/error/incomplete info.**
- ▶ 10: Error.
- ▶ 11: Thrombosis.
- ▶ 12: Incomplete.***

*For code=8, we decided to code them as "4" which means "unknown" since they are un-informative censoring data.

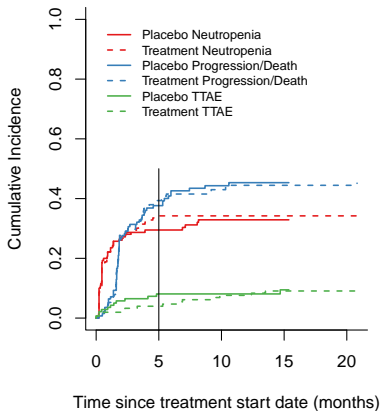
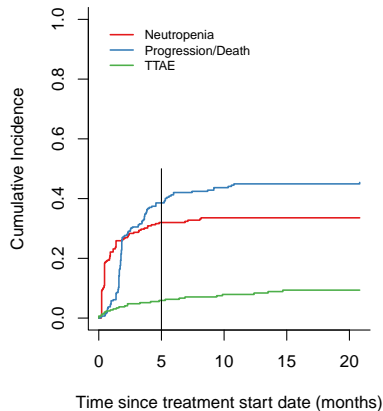
**For code=9, we decided to code them as "0".

***For code=12, we decided to code them as "0" since the date indicates the last date of AE free.

```
table(gwa294DI0phdata$N3Code3CENS5WEEKBIN,gwa294DI0phdata$arm,exclude=NULL)
```

```
##
##      1  2
##  0 119 107
##  1  35  33
```

cumulative incidence curve



CDA SNP association with early ANC

rs2072671

```
SURV <- Surv(gwa294DI0phdata[["Neut3timeCENS5WEEK"]],gwa294DI0phdata[["N3Code3CENS5WEEKBIN"]])
TREATMENTARM <- gwa294DI0phdata[["arm"]]
SNP <- as.numeric(gwa294DI0gtdata[,"rs2072671"])
summary(coxph(SURV~SNP))$ctest

##          test          df      pvalue
## 6.55850724  1.00000000  0.01043839

summary(coxph(SURV~SNP+TREATMENTARM))$coef

##                coef exp(coef) se(coef)      z
## SNP                -0.50259210  0.6049605  0.1974028 -2.5460227
## TREATMENTARMTreatment -0.07343684  0.9291948  0.2447122 -0.3000947
##                Pr(>|z|)
## SNP                0.01089581
## TREATMENTARMTreatment 0.76410494

summary(coxph(SURV~dominant(SNP)))$coef

##                coef exp(coef) se(coef)      z Pr(>|z|)
## dominant(SNP) 1-2 -0.7400645  0.4770832  0.2466657 -3.000273  0.002697375

summary(coxph(SURV~dominant(SNP)))$conf

##                exp(coef) exp(-coef) lower .95 upper .95
## dominant(SNP) 1-2  0.4770832    2.096071  0.2941927  0.773671
```


Linkage Disequilibrium of CDA SNPs

rs2072671 and rs471760

```
#LD(makeGenotypes(as.character(gwa294DI0gtdata[,c("rs2072671", "rs471760"]))))
SNP1 <- snp(as.character(gwa294DI0gtdata[,c("rs2072671")]), sep="/")
SNP2 <- snp(as.character(gwa294DI0gtdata[,c("rs471760")]), sep="/")
LD(SNP1, SNP2)

##
## Pairwise LD
## -----
##           D           D'       Corr
## Estimates: 0.2028357 0.9137059 0.887693
##
##           X^2 P-value  N
## LD Test: 390.8475      0 248
```

rs3825876

```

SURV <- Surv(gwa294DI0phdata[["Neut3timeCENS5WEEK"]],gwa294DI0phdata[["N3Code3CENS5WEEKBIN"]])
TREATMENTARM <- gwa294DI0phdata[["arm"]]
SNP <- as.numeric(gwa294DI0gtdata[, "rs3825876"])
summary(coxph(SURV~SNP))$sctest

##      test      df    pvalue
## 5.1472362 1.0000000 0.0232834

summary(coxph(SURV~SNP+TREATMENTARM))$coef

##              coef exp(coef) se(coef)      z
## SNP          0.40995349 1.5067477 0.1836157  2.23267108
## TREATMENTARMTreatment -0.01717601 0.9829707 0.2445002 -0.07024949
##              Pr(>|z|)
## SNP          0.02557064
## TREATMENTARMTreatment 0.94399508

summary(coxph(SURV~recessive(SNP)))$coef

##              coef exp(coef) se(coef)      z Pr(>|z|)
## recessive(SNP)2 0.8371565  2.30979 0.2664656  3.141706 0.001679665

summary(coxph(SURV~recessive(SNP)))$conf

##              exp(coef) exp(-coef) lower .95 upper .95
## recessive(SNP)2  2.30979  0.4329398  1.370114  3.893932

```

Linkage Disequilibrium of SLC28A1 SNPs

rs3825876 and rs12148896

```
SNP1 <- snp(as.character(gwa294DI0gtdata[,c("rs3825876")]),sep="/")
SNP2 <- snp(as.character(gwa294DI0gtdata[,c("rs12148896")]),sep="/")
LD(SNP1,SNP2)
```

```
##
## Pairwise LD
## -----
##           D           D'          Corr
## Estimates: 0.1669254 0.7178159 0.6749239
##
##           X^2 P-value   N
## LD Test: 162.1659      0 178
```

Luciferase Assay rs11118109 in PANC1

```
summary(lmer(log(Totalnorm)~geno+(1|clone)+(1|day),data=rs11118109PANC1[rs11118109PANC1$strand=="fwd",]))$coef

##           Estimate Std. Error      df  t value Pr(>|t|)
## (Intercept) 0.60431067  0.1415819  3.040459  4.2682750 0.0229731
## genoT      0.09420621  0.1298151 12.000001  0.7256952 0.4819395

summary(lmer(log(Totalnorm)~geno+(1|clone)+(1|day),data=rs11118109PANC1[rs11118109PANC1$strand=="rev",]))$coef

##           Estimate Std. Error      df  t value  Pr(>|t|)
## (Intercept) -1.0554217  0.1044947  3.620732 -10.10024 0.0008821785
## genoC      0.3388925  0.1096715 12.000000  3.09007 0.0093611081
```

Luciferase Assay rs11118109 in K562

```
summary(lmer(log(Totalnorm)~geno+(1|clone)+(1|day),data=rs11118109K562[rs11118109K562$strand=="fwd",]))$coef
```

##	Estimate	Std. Error	df	t value	Pr(> t)
## (Intercept)	1.58218189	0.1481514	3.982278	10.6794934	0.0004457727
## genoT	0.08416625	0.1063070	12.000000	0.7917279	0.4438884199

```
summary(lmer(log(Totalnorm)~geno+(1|clone)+(1|day),data=rs11118109K562[rs11118109K562$strand=="rev",]))$coef
```

##	Estimate	Std. Error	df	t value	Pr(> t)
## (Intercept)	-0.2896085	0.1231277	2.227256	-2.352099	0.130050864
## genoC	0.1708443	0.0564768	14.000000	3.025036	0.009088969

Luciferase Assay rs2799090 in PANC1

```
summary(lmer(log(Totalnorm)~geno+(1|clone)+(1|day),data=rs2799090PANC1[rs2799090PANC1$strand=="fwd",]))$coef

##           Estimate Std. Error df    t value    Pr(>|t|)
## (Intercept)  0.3842142 0.06165161 16    6.232022 1.199036e-05
## genoG       -0.1198291 0.08718855 16   -1.374367 1.882739e-01

summary(lmer(log(Totalnorm)~geno+(1|clone)+(1|day),data=rs2799090PANC1[rs2799090PANC1$strand=="rev",]))$coef

##           Estimate Std. Error      df    t value    Pr(>|t|)
## (Intercept) -0.06714687 0.06288305   2.86528 -1.067806 0.36721013
## genoT        0.15172611 0.05138191 14.00000   2.952909 0.01048534
```

Luciferase Assay rs2799090 in K562

```
summary(lmer(log(Totalnorm)~geno+(1|clone)+(1|day),data=rs2799090K562[rs2799090K562$strand=="fwd",]))$coef
```

```
##           Estimate Std. Error      df    t value Pr(>|t|)
## (Intercept) -0.02467147 0.11226702  3.656803  -0.2197570  0.8377748
## genoG       -0.03454999 0.09848932 17.000000  -0.3507994  0.7300483
```

```
summary(lmer(log(Totalnorm)~geno+(1|clone)+(1|day),data=rs2799090K562[rs2799090K562$strand=="rev",]))$coef
```

```
##           Estimate Std. Error      df    t value Pr(>|t|)
## (Intercept) -0.06414295 0.10702331  2.842017  -0.5993362  0.5933437
## genoT        0.08578950 0.08633689 20.000000   0.9936598  0.3322592
```

Luciferase Assay rs2799083 in PANC1

```
summary(lmer(log(Totalnorm)~geno+(1|clone)+(1|day),data=rs2799083PANC1[rs2799083PANC1$strand=="fwd",]))$coef
```

```
##           Estimate Std. Error      df    t value      Pr(>|t|)
## (Intercept) -0.3818125 0.06183839   3.847219 -6.174359 0.0039694689
## genoT       0.1800304 0.04098823  12.000000  4.392245 0.0008772446
```

```
summary(lmer(log(Totalnorm)~geno+(1|clone)+(1|day),data=rs2799083PANC1[rs2799083PANC1$strand=="rev",]))$coef
```

```
##           Estimate Std. Error      df    t value      Pr(>|t|)
## (Intercept) -0.3214002 0.08249701   3.524781 -3.895901 0.022329709
## genoG       0.1607851 0.04698717  12.000000  3.421894 0.005060844
```


Luciferase Assay rs2799083 in K562

```
summary(lmer(log(Totalnorm)~geno+(1|clone)+(1|day),data=rs2799083K562[rs2799083K562$strand=="fwd",]))$coef
```

```
##           Estimate Std. Error      df    t value  Pr(>|t|)  
## (Intercept) -0.48984852  0.1773680  3.035838 -2.7617645  0.06912025  
## genoT       0.07093129  0.1552646 14.000000  0.4568412  0.65479303
```

```
summary(lmer(log(Totalnorm)~geno+(1|clone)+(1|day),data=rs2799083K562[rs2799083K562$strand=="rev",]))$coef
```

```
##           Estimate Std. Error      df    t value  Pr(>|t|)  
## (Intercept) -0.5586893  0.1529707  2.976693 -3.652264  0.03589806  
## genoG       0.2532778  0.1309645 14.000000  1.933942  0.07359613
```

Session Information

- ▶ **R version 3.4.4 (2018-03-15)**, x86_64-pc-linux-gnu
- ▶ **Running under:** Ubuntu 18.04 LTS
- ▶ **Matrix products:** default
- ▶ **BLAS:** /usr/lib/x86_64-linux-gnu/openblas/libblas.so.3
- ▶ **LAPACK:** /usr/lib/x86_64-linux-gnu/libopenblas-r0.2.20.so
- ▶ **Base packages:** base, datasets, graphics, grDevices, methods, parallel, stats, tcltk, utils
- ▶ **Other packages:** asbio 1.4-2, cmprsk 2.2-7, dplyr 0.7.4, gdata 2.18.0, GenABEL 1.8-0, GenABEL.data 1.0.0, haplo.stats 1.7.9, knitr 1.20, lme4 1.1-17, lmerTest 3.0-1, MASS 7.3-49, Matrix 1.2-12, mvtnorm 1.0-7, qvalue 2.10.0, RColorBrewer 1.1-2, readxl 1.1.0, SNPAssoc 1.9-2, survival 2.41-3
- ▶ **Loaded via a namespace (and not attached):** acepack 1.4.1, assertthat 0.2.0, backports 1.1.2, base64enc 0.1-3, bindr 0.1.1, bindrcpp 0.2.2, cellranger 1.1.0, checkmate 1.8.5, cluster 2.0.6, codetools 0.2-15, colorspace 1.3-2, compiler 3.4.4, data.table 1.11.2, deSolve 1.20, digest 0.6.15, evaluate 0.10.1, foreign 0.8-69, Formula 1.2-3, ggplot2 2.2.1, glue 1.2.0, grid 3.4.4, gridExtra 2.3, gtable 0.2.0, gtools 3.5.0, highr 0.6, Hmisc 4.1-1, htmlTable 1.11.2, htmltools 0.3.6, htmlwidgets 1.2, lattice 0.20-35, latticeExtra 0.6-28, lazyeval 0.2.1, magrittr 1.5, MatrixModels 0.4-1, minqa 1.2.4, multcomp 1.4-8, multcompView 0.1-7, munsell 0.4.3, nlme 3.1-131, nloptr 1.0.4, nnet 7.3-12, numDeriv 2016.8-1, pillar 1.2.2, pixmap 0.4-11, pkgconfig 2.0.1, plotrix 3.6-6, plyr 1.8.4, polyspline 1.1.12, quantreg 5.35, R6 2.2.2, Rcpp 0.12.16, reshape2 1.4.3, rlang 0.2.0, rms 5.1-2, rpart 4.1-13, rstudioapi 0.7, sandwich 2.4-0, scales 0.5.0, scatterplot3d 0.3-41, SparseM 1.77, splines 3.4.4, stringi 1.2.2, stringr 1.3.1, TH.data 1.0-8, tibble 1.4.2, tools 3.4.4, zoo 1.8-1