

Drug quantification in single 3D organoids reveals correlation to reported in vivo brain uptake and demonstrates translational relevance for prediction of blood-brain barrier permeability

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1 Overview

This document provides all code required to reproduce the final published regression analysis examining the relationship between organoid Uptake ratios and literature-derived brain-to-plasma ratios.

The published dataset accompanies this document and contains all variables required for reproducing the final model.

2 Reproducibility Information

```
knitr::opts_chunk$set(  
  echo = TRUE,  
  warning = FALSE,  
  message = FALSE  
)  
  
library(tidyverse)  
library(here)  
library(deming)  
library(boot)  
library(broom)
```

3 Load Data

The published dataset is supplied as an `.rda` file.

```
datatable_df <- read.csv2(here("datatable_df.csv"))
datatable_df <- datatable_df %>%
  mutate(logvalue_organoid_incubation = as.numeric(str_replace_all(logvalue_
    _organoid_incubation, "\\,", ".")),
    logvalue_comparison = as.numeric(str_replace_all(logvalue_
    _comparison, "\\,", ".")))
glimpse(datatable_df)
## Rows: 72
## Columns: 9
## $ Drug <chr> "Lorlatinib", "Larotrectinib", "Sel
itrect...
## $ value_organoid_incubation <dbl> 6.30, 4.50, 1.20, 2.70, 2.10, 425.2
0, 0.0...
## $ SD_organoid_incubation <dbl> 0.9837564, 0.6210207, 1.0759492, 0.
173205...
## $ logvalue_organoid_incubation <dbl> 1.8405496, 1.5040774, 0.1823216, 0.
993251...
## $ logSD_organoid_incubation <dbl> 0.20743261, 0.12756919, 0.50085314,
0.060...
## $ comparison <chr> "Brain_Plasma_Lit", "Brain_Plasma_L
it", "...
## $ value_comparison <dbl> 0.63, 0.06, 0.04, 0.17, 0.03, 15.40
, 0.00...
## $ SD_comparison <dbl> 0.084, 0.012, 0.014, 0.050, 0.008,
4.390,...
## $ logvalue_comparison <dbl> -0.46203546, -2.81341072, -3.218875
83, -1...
```

4 Variable Description

Variable	Description
Drug	Drug name
value_organoid_incubation	Raw Organoid Uptake ratio
SD_organoid_incubation	Standard deviation of Organoid Uptake
logvalue_organoid_incubation	Natural logarithm of Organoid Uptake
logSD_organoid_incubation	Standard deviation on ln scale
comparison	Comparison category
value_comparison	Literature brain-to-plasma ratio
SD_comparison	Standard deviation of literature value
logvalue_comparison	Natural logarithm of literature brain-to-plasma ratio

5 Preparation of Final Analysis Dataset

The final published model was fitted using ln-transformed organoid Uptake ratio and ln-transformed brain-to-plasma ratios.

Octreotide was excluded as specified in the manuscript.

6 Estimation of Log-Scale Standard Deviations

For organoid Uptake ratios, replicate-level observations were available. Therefore, the standard deviation on the logarithmic scale was calculated directly from the ln-transformed replicate values:

$$SD_{\ln(y)} = SD(\ln(y))$$

For literature-derived brain-to-plasma, mainly published summary statistics (mean and standard deviation) were available. Consequently, direct calculation of the standard deviation on the logarithmic scale was not possible.

To estimate the uncertainty after ln transformation, the first-order Taylor approximation (delta method) was applied:

$$\text{Var}(\ln(x)) \approx (d\ln(x)/dx)^2 \text{Var}(x)$$

Since

$$d\ln(x)/dx = 1/x,$$

the standard deviation on the ln scale can be approximated as

$$SD_{\ln(x)} \approx SD_x / x$$

where:

- x is the reported literature mean brain-to-plasma ratio,
- SD_x is the reported literature standard deviation.

Accordingly, the ln-scale standard deviation used in the Deming regression was estimated as:

```
analysis_df <- datatable_df %>%
  filter(Drug != "Octreotide") %>%
  filter(
    value_organoid_incubation > 0,
    value_comparison > 0
  ) %>%
  group_by(Drug) %>%
  summarise(
    SD_organoid_incubation =
      first(SD_organoid_incubation),

    logSD_organoid_incubation =
      first(logSD_organoid_incubation),

    organoid_incubation =
      mean(value_organoid_incubation),
```

```

log_organoid_incubation =
  mean(logvalue_organoid_incubation),

brain_plasma =
  first(value_comparison),

log_brain_plasma =
  first(logvalue_comparison),

SD_brain_plasma =
  first(SD_comparison),

.groups = "drop"
) %>%
mutate(
  logSD_brain_plasma =
    SD_brain_plasma / brain_plasma
) %>%
filter(
  !is.na(logSD_organoid_incubation),
  logSD_organoid_incubation > 0
)

analysis_df
## # A tibble: 12 × 9
##   Drug          SD_organoid_incubation logSD_organoid_incub...1 organoid_in
cubation
##   <chr>          <dbl>          <dbl>
<dbl>
##  1 Fluoxetine      116.          0.285
423.
##  2 Futibatinib      0.173          0.0608
2.8
##  3 Larotrecti...    0.621          0.128
4.82
##  4 Lorlatinib       0.984          0.207
4.73
##  5 Olaparib        1.25          0.462
2.49
##  6 Paroxetine      331.          0.766
584.
##  7 Repotrecti...    2.03          0.317
6.23
##  8 Selinexor       15.4          0.501
32.8
##  9 Selitrecti...    1.08          0.501
1.82
## 10 Senicapoc        5.00          0.204
24.3
## 11 Temsirolim...   10.5          0.494
23.9
## 12 Ulixertinib      2.37          0.0924
26.3
## # [i] abbreviated name: 1logSD_organoid_incubation
## # [i] 5 more variables: log_organoid_incubation <dbl>, brain_plasma <dbl>
,
## #   log_brain_plasma <dbl>, SD_brain_plasma <dbl>, logSD_brain_plasma <d
bl>

```

7 Deming Regression Function

A bootstrap procedure was used to generate 95% confidence intervals around the fitted regression line.

```
boot_deming_ci <- function(
  data,
  formula,
  xstd_col,
  ystd_col,
  x_col,
  nboot = 1000
){

  data$.xstd <- data[[xstd_col]]
  data$.ystd <- data[[ystd_col]]

  x_vals <- data[[x_col]]
  y_col <- as.character(formula[[2]])

  fit <- deming(
    formula,
    data = data,
    xstd = .xstd,
    ystd = .ystd,
    conf = 0.95
  )

  intercept <- fit$coefficients[1]
  slope <- fit$coefficients[2]

  boot_fn <- function(d, indices){

    subset_data <- d[indices, ]

    coeffs <- tryCatch(
      deming(
        formula,
        data = subset_data,
        xstd = .xstd,
        ystd = .ystd
      )$coefficients,
      error = function(e) c(NA, NA)
    )

    coeffs[1] + coeffs[2] * x_vals
  }

  set.seed(123)

  boot_out <- boot(
    data,
    boot_fn,
    R = nboot
  )

  valid_rows <- complete.cases(boot_out$t)

  boot_matrix <- boot_out$t[valid_rows, ]

  band <- data.frame(
```

```

x = x_vals,
fit = intercept + slope * x_vals,
lwr = apply(
  boot_matrix,
  2,
  quantile,
  probs = 0.025
),
upr = apply(
  boot_matrix,
  2,
  quantile,
  probs = 0.975
)
)

r2 <- cor(
  data[[y_col]],
  band$fit
)^2

list(
  fit = fit,
  band = band,
  r2 = r2
)
}

```

8 Final Published Regression Model

Model:

$$\ln(\text{Organoid Uptake ratio}) = \beta_0 + \beta_1 \times \ln(\text{brain-to-plasma ratio})$$

```

final_model <- boot_deming_ci(
  analysis_df,
  formula =
    log_organoid_incubation ~
    log_brain_plasma,
  xstd_col =
    "logSD_brain_plasma",
  ystd_col =
    "logSD_organoid_incubation",
  x_col =
    "log_brain_plasma"
)

```

9 Regression Output

```

final_model$fit
##
## Call:
## deming(formula = formula, data = data, xstd = .xstd, ystd = .ystd, c
onf = 0.95)
##
## n= 12
##
##          Coef    se(coef) lower 0.95 upper 0.95

```

```
## Intercept 3.0237239 0.6130224 1.8222221 4.225226
## Slope      0.8173857 0.2694950 0.2891853 1.345586
##
##      Scale= 2.518724
```

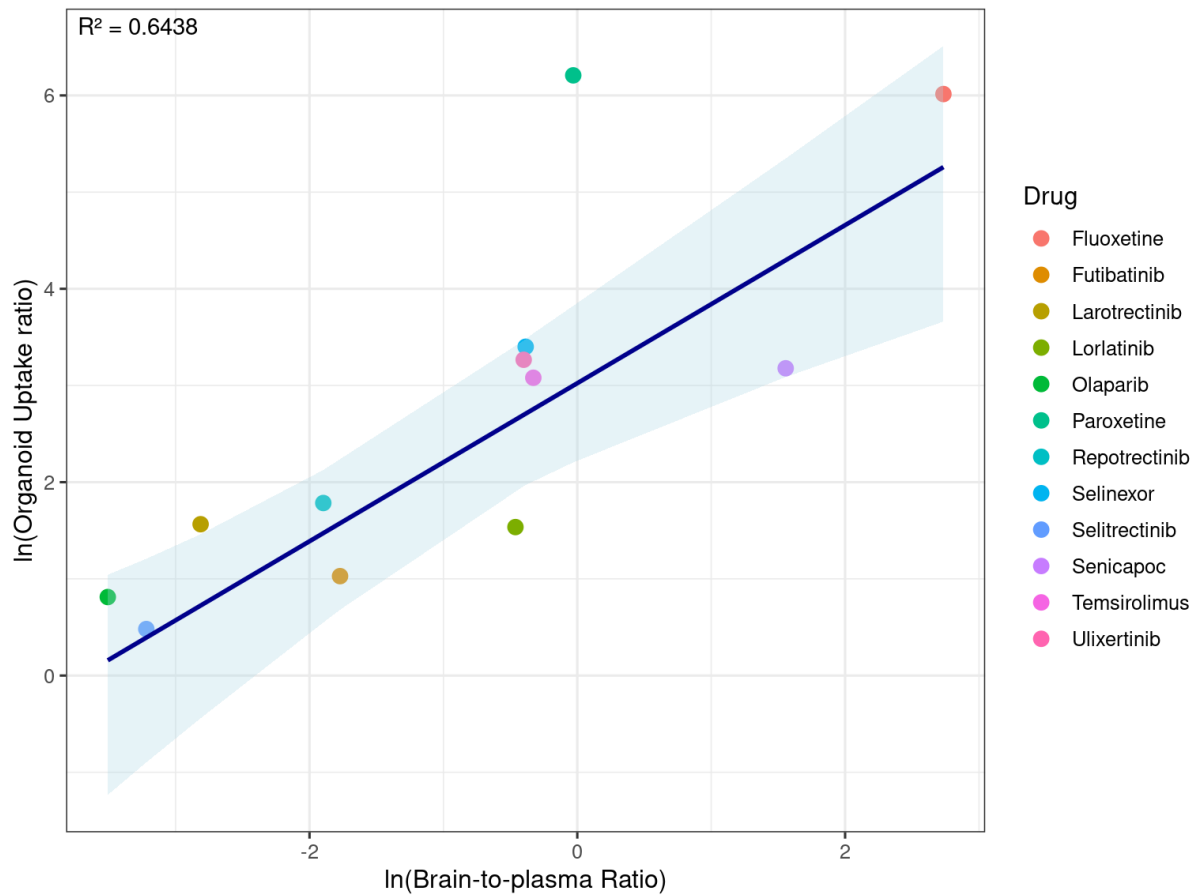
9.1 Model Statistics

```
cat(
  "R-squared:",
  round(final_model$r2, 4)
)
## R-squared: 0.6438
```

10 Regression Figure

```
ggplot(
  analysis_df,
  aes(
    x = log_brain_plasma,
    y = log_organoid_incubation,
    color = Drug
  )
) +
  geom_point(size = 3) +
  geom_ribbon(
    data = final_model$band,
    aes(
      x = x,
      ymin = lwr,
      ymax = upr
    ),
    inherit.aes = FALSE,
    alpha = 0.3,
    fill = "lightblue"
  ) +
  geom_line(
    data = final_model$band,
    aes(
      x = x,
      y = fit
    ),
    inherit.aes = FALSE,
    linewidth = 1,
    color = "darkblue"
  ) +
  annotate(
    "text",
    x = -Inf,
    y = Inf,
    hjust = -0.1,
    vjust = 1.5,
    label = paste0(
      "R2 = ",
      round(final_model$r2, 4)
    )
  ) +
  labs(
```

```
x = "ln(Brain-to-plasma Ratio)",
y = "ln(Organoid Uptake ratio)",
color = "Drug"
) +
theme_bw(base_size = 12)
```



11 Export Figure

```
ggsave(
  here("Figure_Final_Deming_Regression.png"),
  width = 8,
  height = 6,
  dpi = 300
)
```

12 Session Information

```
sessionInfo()
## R version 4.3.1 (2023-06-16)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 20.04.6 LTS
##
## Matrix products: default
## BLAS: /usr/lib/x86_64-linux-gnu/blas/libblas.so.3.9.0
## LAPACK: /usr/lib/x86_64-linux-gnu/lapack/liblapack.so.3.9.0
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8      LC_NUMERIC=C
##  [3] LC_TIME=en_US.UTF-8      LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=en_US.UTF-8  LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=en_US.UTF-8     LC_NAME=C
##  [9] LC_ADDRESS=C             LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
##
## time zone: Europe/Berlin
## tzcode source: system (glibc)
##
## attached base packages:
## [1] stats      graphics  grDevices  utils      datasets  methods   base
##
## other attached packages:
##  [1] broom_1.0.8      boot_1.3-28      deming_1.4-1     here_1.0.1
##  [5] lubridate_1.9.4  forcats_1.0.0    stringr_1.5.1    dplyr_1.1.4
##  [9] purrr_1.0.4      readr_2.1.5      tidyr_1.3.1      tibble_3.3.0
## [13] ggplot2_3.5.2    tidyverse_2.0.0
##
## loaded via a namespace (and not attached):
##  [1] gtable_0.3.6      jsonlite_2.0.0    compiler_4.3.1    tidyselect
##  [5] jquerylib_0.1.4    scales_1.4.0      yaml_2.3.10       fastmap_1.
##  [9] R6_2.6.1          labeling_0.4.3    generics_0.1.4    knitr_1.50
## [13] backports_1.5.0    rprojroot_2.0.4    bslib_0.9.0       pillar_1.1
## [17] RColorBrewer_1.1-3 tzdb_0.5.0        rlang_1.1.6       utf8_1.2.6
## [21] cachem_1.1.0      stringi_1.8.7     xfun_0.52         sass_0.4.1
## [25] timechange_0.3.0   cli_3.6.5         withr_3.0.2       magrittr_2
## [29] digest_0.6.37      grid_4.3.1        rstudioapi_0.17.1 hms_1.1.3
## [33] lifecycle_1.0.4    vctrs_0.6.5       evaluate_1.0.3    glue_1.8.0
## [37] farver_2.1.2       rmarkdown_2.29    tools_4.3.1       pkgconfig_
## [41] htmltools_0.5.8.1
```