Simulation of SynergyFinder

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```
library(synergyfinder)
```

#INTRODUCTION

CCancer treatment is often reliant on drug combinations. Typically, the clinical development of this drug combination is achieved through trial-and-error. Screening and exploration of synergistic, additive, and antagonistic drug combination may be conducted.

SynergyFinder is a R package software to analyze pre-clinical drug combination with the advantage of conducting assay chemical compounds for promising drug pair. SynergyFinder allows quantification of drug combination synergy using scoring models, including HSA, Loewe, Bliss, and ZIP.

In this simulation, the potential therapeutic combinations of therapy for the treatment of diffuse large B-cell lymphoma (DLBCL). The combinations of the BTK inhibitor ibrutinib with canertinib was evaluated for potential synergistic activity based on the percentage viability of the TMD8 cell line.

#Load screening data or screening result

```
data("mathews_screening_data")
head(mathews_screening_data)
```

```
##   block_id  drug_row  drug_col conc_r  conc_c  response conc_r_unit conc_c_unit
## 1        1 ispinesib ibrutinib   2500 50.0000  7.802637          nM          nM
## 2        1 ispinesib ibrutinib   2500 12.5000  6.831317          nM          nM
## 3        1 ispinesib ibrutinib   2500  3.1250 15.089589          nM          nM
## 4        1 ispinesib ibrutinib   2500  0.7812 24.503885          nM          nM
## 5        1 ispinesib ibrutinib   2500  0.1954 38.043076          nM          nM
## 6        1 ispinesib ibrutinib   2500  0.0000 45.790634          nM          nM
```

#Reshape data

The drug combination dataset then reshaped into a dataframe for the input, that following the format of block_id, drug1, drug2, conc1, conc2, response, and conc_unit.

```
res <- ReshapeData(
  data = mathews_screening_data,
  data_type = "viability",
  impute = TRUE,
  impute_method = NULL,
  noise = TRUE,
  seed = 1)
```

```r
str(res)
```

```
## List of 2
##  $ drug_pairs: tibble [2 x 7] (S3: tbl_df/tbl/data.frame)
##   ..$ block_id  : int [1:2] 1 2
##   ..$ drug1     : chr [1:2] "ispinesib" "canertinib"
##   ..$ drug2     : chr [1:2] "ibrutinib" "ibrutinib"
##   ..$ conc_unit1: chr [1:2] "nM" "nM"
##   ..$ conc_unit2: chr [1:2] "nM" "nM"
##   ..$ input_type: chr [1:2] "viability" "viability"
##   ..$ replicate : logi [1:2] FALSE FALSE
##  $ response  : tibble [72 x 5] (S3: tbl_df/tbl/data.frame)
##   ..$ block_id       : int [1:72] 1 1 1 1 1 1 1 1 1 1 ...
##   ..$ conc1          : num [1:72] 2500 2500 2500 2500 2500 2500 625 625 625 625 ...
##   ..$ conc2          : num [1:72] 50 12.5 3.125 0.781 0.195 ...
##   ..$ response       : num [1:72] 92.2 93.2 84.9 75.5 62 ...
##   ..$ response_origin: num [1:72] 92.2 93.2 84.9 75.5 62 ...
```

```r
head(res)
```

```
## $drug_pairs
## # A tibble: 2 x 7
##   block_id drug1      drug2     conc_unit1 conc_unit2 input_type replicate
##      <int> <chr>      <chr>     <chr>      <chr>      <chr>      <lgl>
## 1        1 ispinesib  ibrutinib nM         nM         viability  FALSE
## 2        2 canertinib ibrutinib nM         nM         viability  FALSE
##
## $response
## # A tibble: 72 x 5
##    block_id conc1  conc2 response response_origin
##       <int> <dbl>  <dbl>    <dbl>           <dbl>
##  1        1  2500 50         92.2            92.2
##  2        1  2500 12.5       93.2            93.2
##  3        1  2500  3.12      84.9            84.9
##  4        1  2500  0.781     75.5            75.5
##  5        1  2500  0.195     62.0            62.0
##  6        1  2500  0         54.2            54.2
##  7        1   625 50         94.1            94.1
##  8        1   625 12.5       93.4            93.4
##  9        1   625  3.12      85.9            85.9
## 10        1   625  0.781     76.7            76.7
## # i 62 more rows
```

The output was two tibbles consisted of drug pairs and response

```r
res$drug_pairs
```

```
## # A tibble: 2 x 7
##   block_id drug1      drug2     conc_unit1 conc_unit2 input_type replicate
##      <int> <chr>      <chr>     <chr>      <chr>      <chr>      <lgl>
## 1        1 ispinesib  ibrutinib nM         nM         viability  FALSE
## 2        2 canertinib ibrutinib nM         nM         viability  FALSE
```

```
res$response
```

```
## # A tibble: 72 x 5
##    block_id conc1  conc2 response response_origin
##       <int> <dbl>  <dbl>    <dbl>           <dbl>
##  1        1  2500 50         92.2            92.2
##  2        1  2500 12.5       93.2            93.2
##  3        1  2500  3.12      84.9            84.9
##  4        1  2500  0.781     75.5            75.5
##  5        1  2500  0.195     62.0            62.0
##  6        1  2500  0         54.2            54.2
##  7        1   625 50         94.1            94.1
##  8        1   625 12.5       93.4            93.4
##  9        1   625  3.12      85.9            85.9
## 10        1   625  0.781     76.7            76.7
## # i 62 more rows
```

#Calculation of synergy and sensitivity analysis

1. Calculation of synergy scores

```
res <- CalculateSynergy(
  data = res,
  method = c("ZIP", "HSA", "Bliss", "Loewe"),
  Emin = NA,
  Emax = NA,
  correct_baseline = "non")
```

```
## Calculating synergy score(s) for block 1...

## Warning: There were 2 warnings in `dplyr::mutate()`.
## The first warning was:
## i In argument: `pred = furrr::future_map(...)`.
## Caused by warning:
## ! package 'future' was built under R version 4.4.3
## i Run `dplyr::last_dplyr_warnings()` to see the 1 remaining warning.

## Calculating synergy score(s) for block 2...
```

```
head(res$synergy_scores)
```

```
## # A tibble: 6 x 13
##   block_id conc1  conc2 ZIP_fit ZIP_ref ZIP_synergy HSA_ref HSA_synergy
##      <int> <dbl>  <dbl>   <dbl>   <dbl>       <dbl>   <dbl>       <dbl>
## 1        1  2500 50        93.3    79.4        13.9    71.3       20.9
## 2        1  2500 12.5      92.3    79.4        12.9    54.2       39.0
## 3        1  2500  3.12     82.7    67.9        14.8    54.2       30.7
## 4        1  2500  0.781    75.2    37.2        38.1    54.2       21.3
## 5        1  2500  0.195    57.3    37.2        20.1    54.2        7.75
## 6        1  2500  0        54.2    54.2         0      54.2        0
## # i 5 more variables: Bliss_ref <dbl>, Bliss_synergy <dbl>, Loewe_ref <dbl>,
## #   Loewe_synergy <dbl>, Loewe_ci <dbl>
```

2. Sensitivity scoring

Calculation of 3 sensitive scores : - relative IC50 - relative inhibition (Ri) for single drug treatment - combination of sensitivity score (CSS) for drug combinations

```
res <- CalculateSensitivity(
  data = res,
  correct_baseline = "non"
)
```

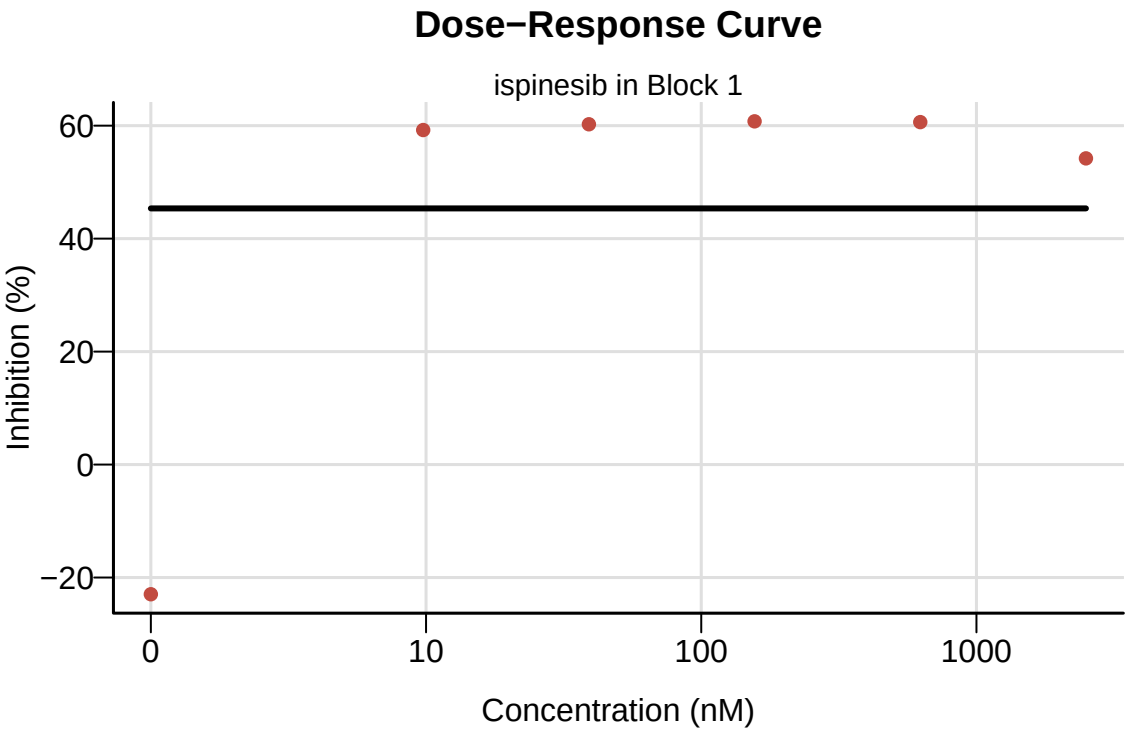
```
## Calculating sensitivity scores for block 1 ...

## Calculating sensitivity scores for block 2 ...
```

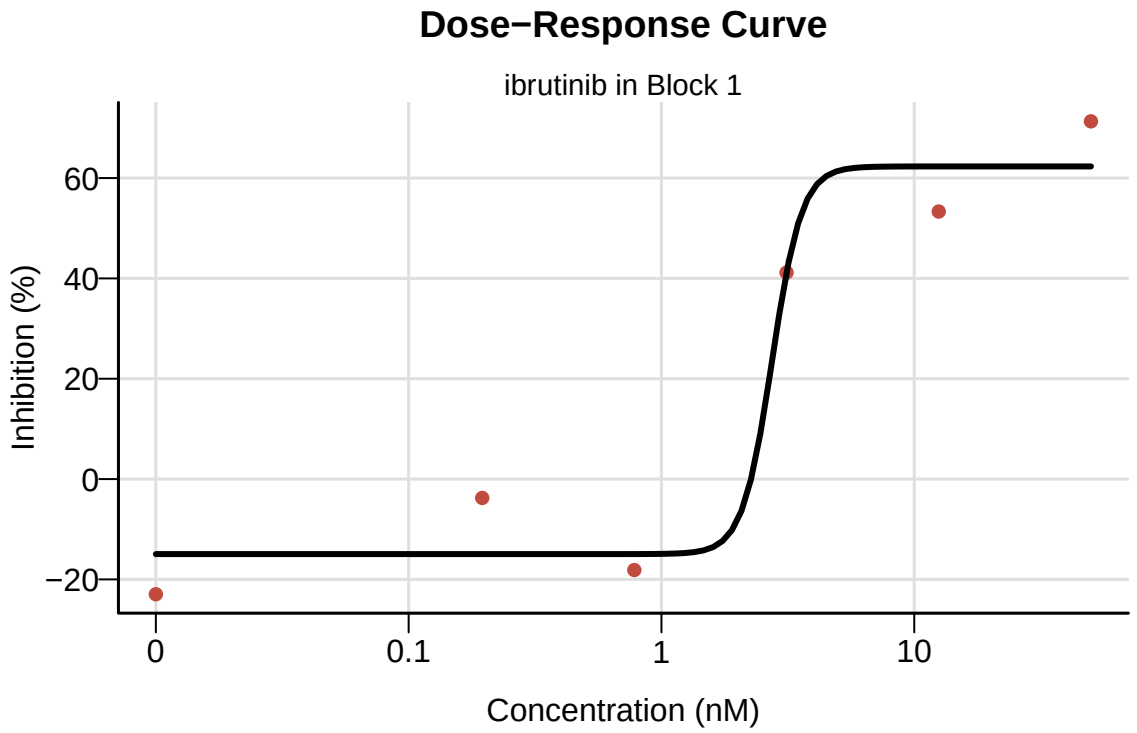
```
sensitive_columns <- c(
  "block_id", "drug1", "drug2",
  "ic50_1", "ic50_2",
  "ri_1", "ri_2",
  "css1_ic502", "css2_ic501", "css")
res$drug_pairs[, sensitive_columns]
```

```
## # A tibble: 2 x 10
##   block_id drug1     drug2 ic50_1 ic50_2  ri_1  ri_2 css1_ic502 css2_ic501   css
##      <int> <chr>     <chr>  <dbl>  <dbl> <dbl> <dbl>      <dbl>      <dbl> <dbl>
## 1        1 ispinesib ibru~  2500    2.74  60.0  27.0       85.9       82.8   84.3
## 2        2 canertin~ ibru~   973.   1.44 -48.0  45.2      -35.9       -6.99 -21.4
```

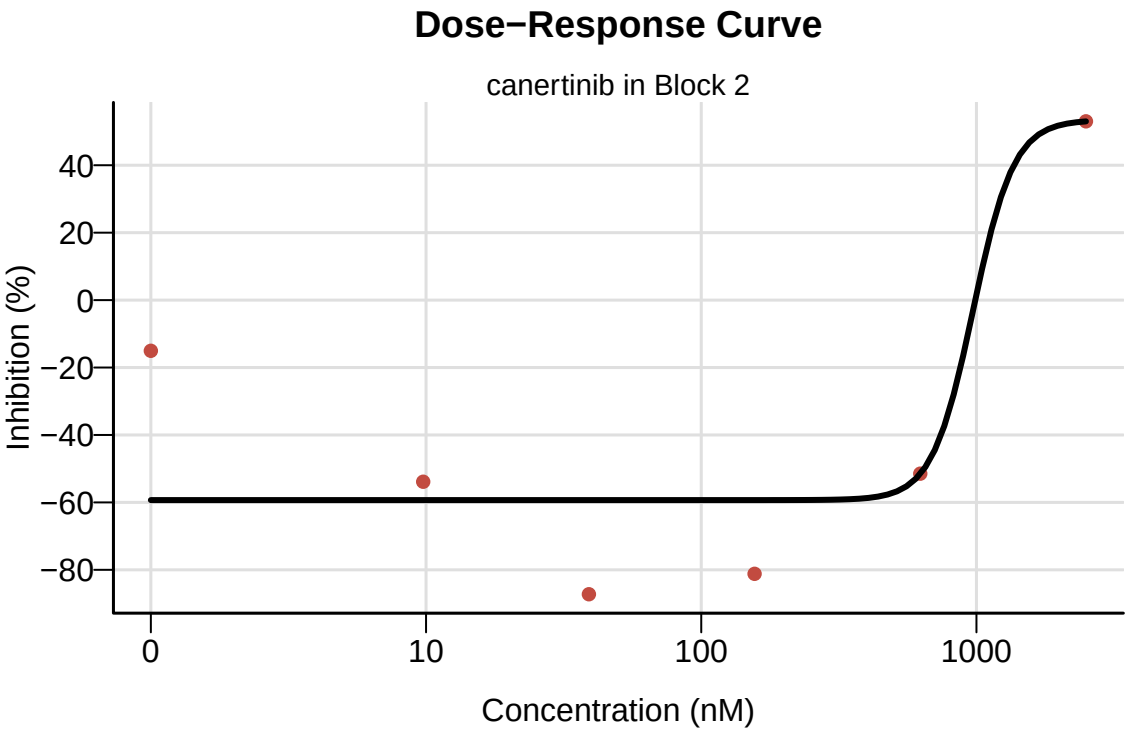
#Dose-response curve and dose response matrix

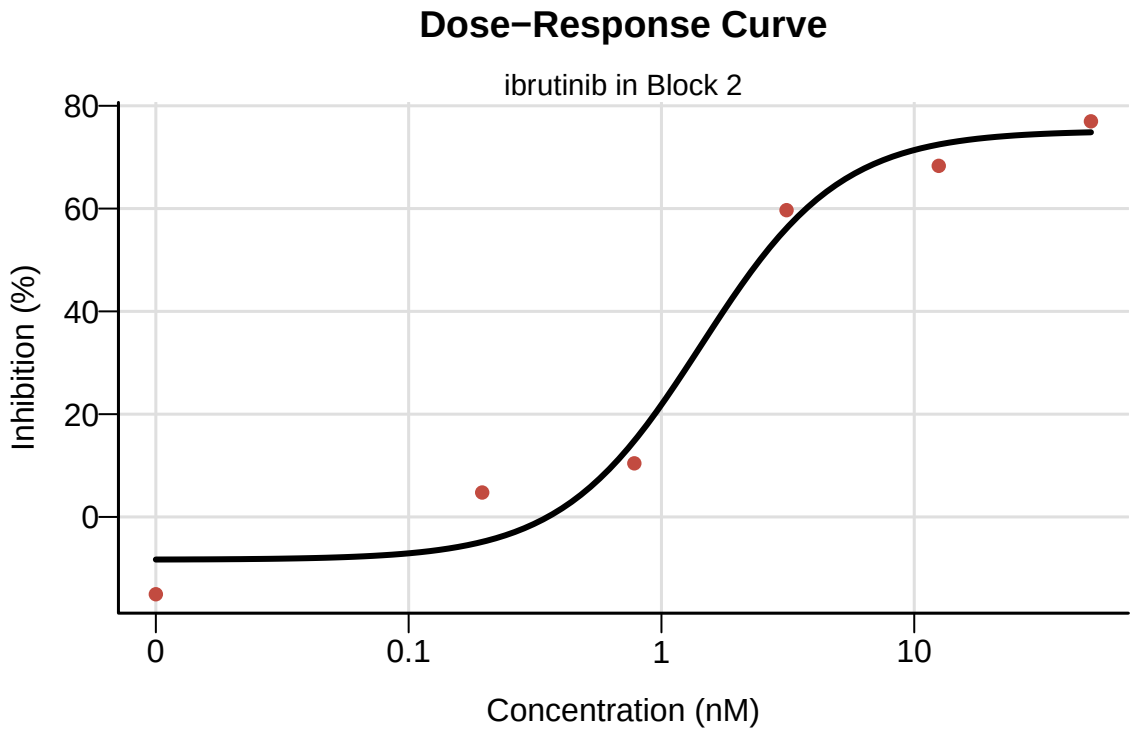
```
for (i in c(1, 2)){
  PlotDoseResponseCurve(
    data = res,
    plot_block = 1,
    drug_index = i,
    plot_new = FALSE,
    record_plot = FALSE
  )
}
```


Dose−Response Curve
ispinesib in Block 1
Inhibition (%)
60
40
20
0
−20
0
10
100
1000
Concentration (nM)

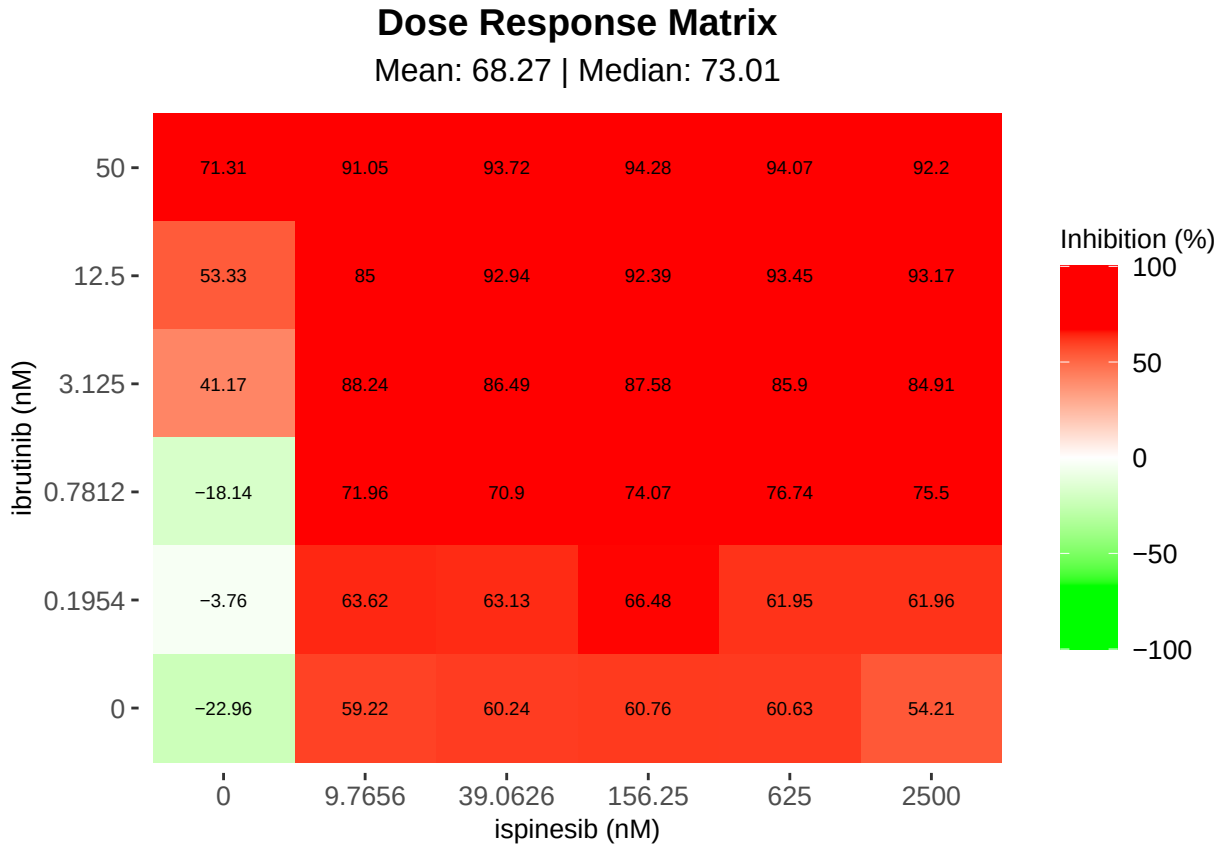



```
for (i in c(1, 2)){
  PlotDoseResponseCurve(
    data = res,
    plot_block = 2,
    drug_index = i,
    plot_new = FALSE,
    record_plot = FALSE
  )
}
```

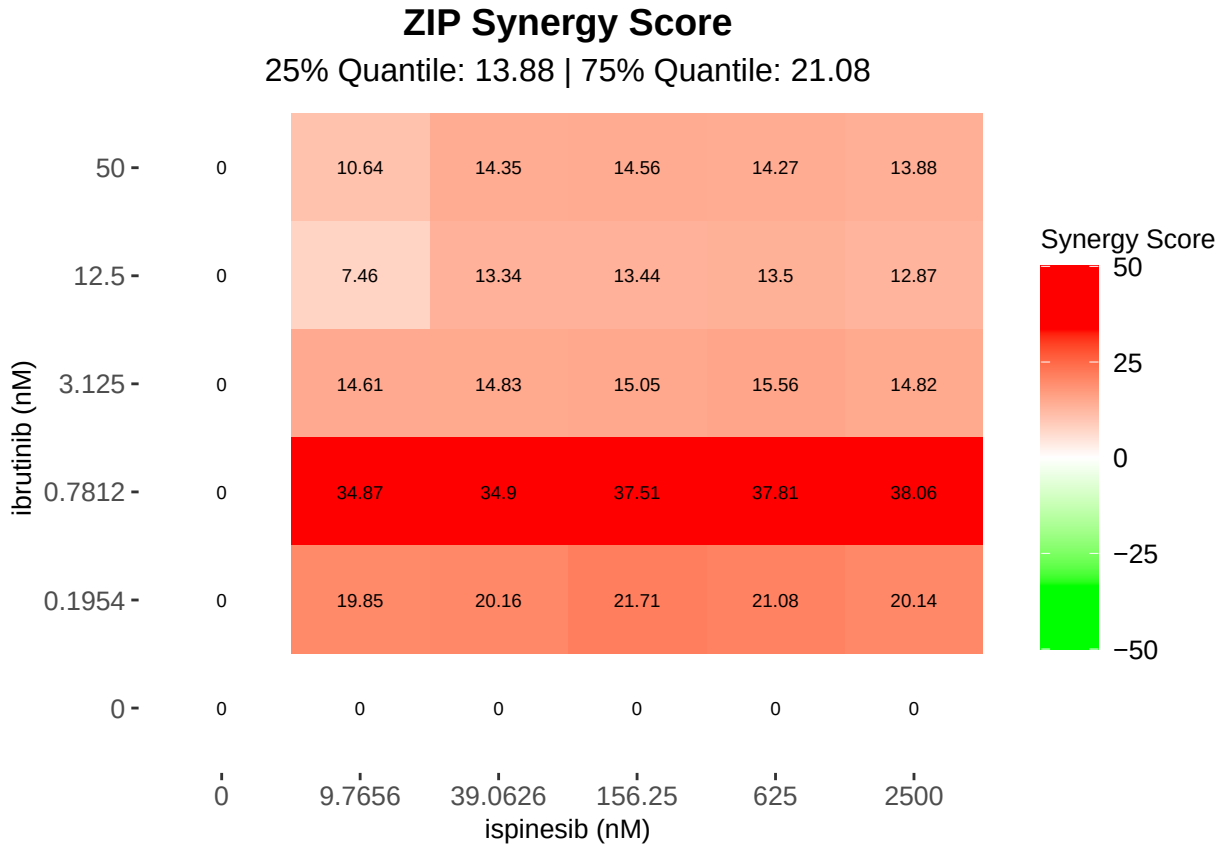

Dose−Response Curve
canertinib in Block 2
Inhibition (%)
40
20
0
−20
−40
−60
−80
0
10
100
1000
Concentration (nM)




```
Plot2DrugHeatmap(
  data = res,
  plot_block = 1,
  drugs = c(1, 2),
  plot_value = "response",
  dynamic = FALSE,
  summary_statistic = c("mean",  "median")
)
```

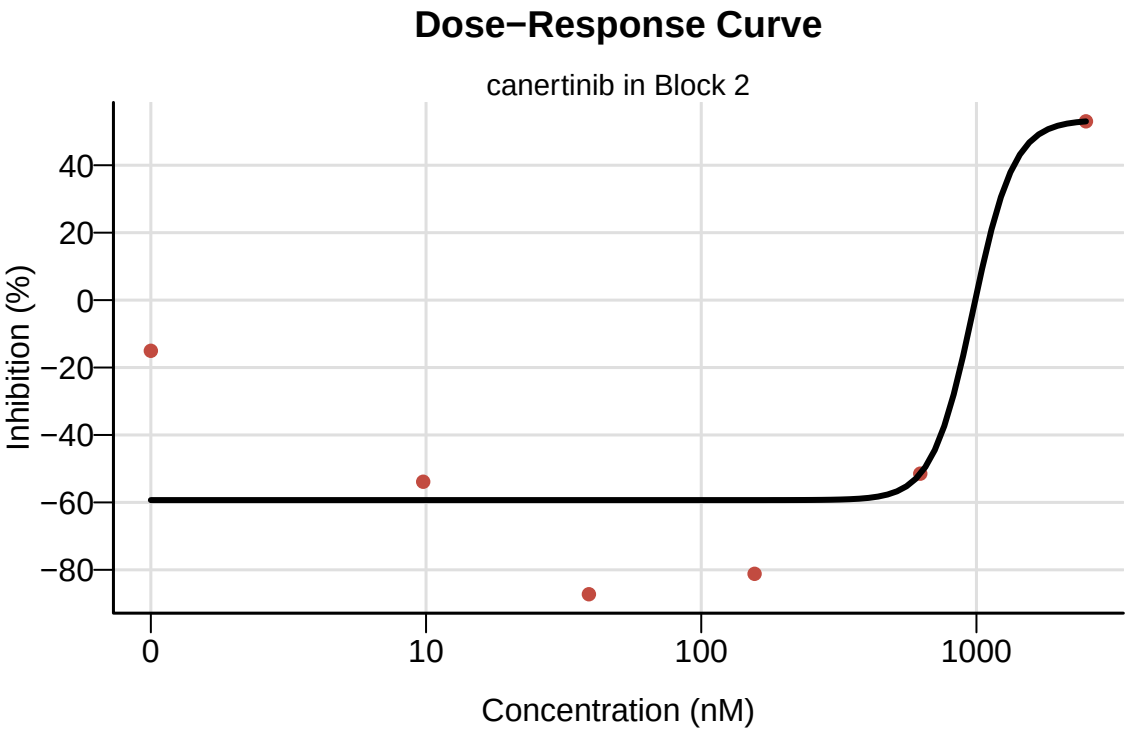


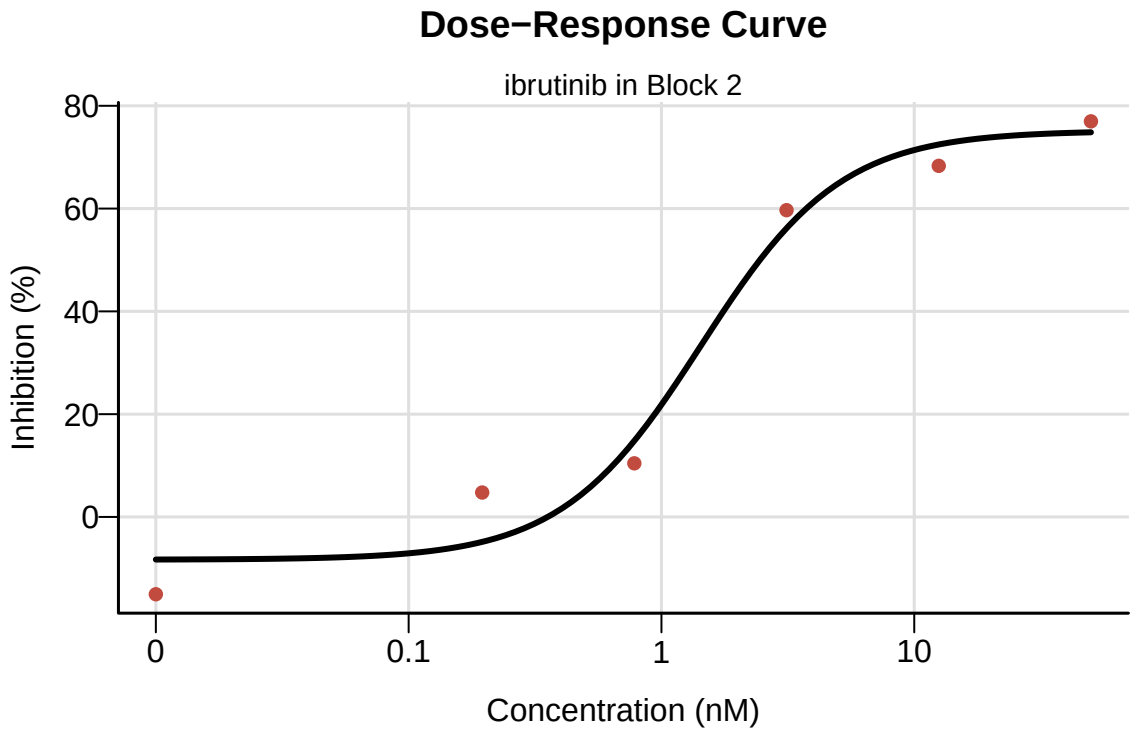

```
Plot2DrugHeatmap(
  data = res,
  plot_block = 1,
  drugs = c(1, 2),
  plot_value = "ZIP_synergy",
  dynamic = FALSE,
  summary_statistic = c( "quantile_25", "quantile_75")
)
```



Combination of ibrutinib & canertinib

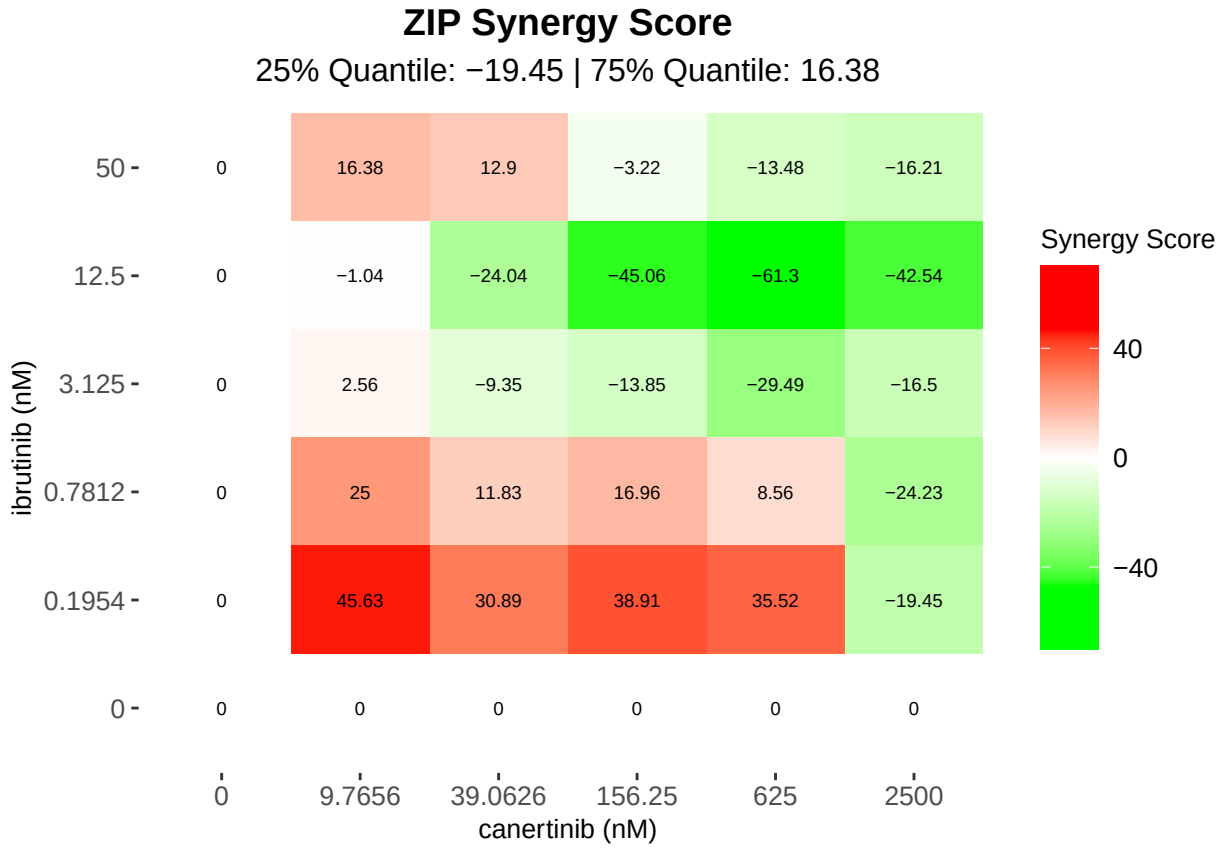
```
for (i in c(1, 2)){
  PlotDoseResponseCurve(
    data = res,
    plot_block = 2,
    drug_index = i,
    plot_new = FALSE,
    record_plot = FALSE
  )
}
```


Dose−Response Curve
canertinib in Block 2
Inhibition (%)
40
20
0
−20
−40
−60
−80
0
10
100
1000
Concentration (nM)




```
Plot2DrugHeatmap(
  data = res,
  plot_block = 2,
  drugs = c(1, 2),
  plot_value = "response",
  dynamic = FALSE,
  summary_statistic = c("mean",  "median")
)
```


```
Plot2DrugHeatmap(
  data = res,
  plot_block = 2,
  drugs = c(1, 2),
  plot_value = "ZIP_synergy",
  dynamic = FALSE,
  summary_statistic = c( "quantile_25", "quantile_75")
)
```



Analysis of Individual Drug Dose-Response Curves

##Block 2 ###Canertinib Dose-Response Curve 1) At concentration of 0 nM, the baseline inhibition was around -60% which was a normal biological variability 2) The dose-response curve of canertinib steeply increased approximately around 800 nM to 1200 nM.The IC50% was observed at concentration over 1000 nM which indicates that canertinib requires a higher concentration to achieve significant inhibition. 3) The maximum inhibition (Emax) is approximately 45-50%, which indicates that inhibition of nacertinib is limited.

###Ibrutinib Dose-Response Curve 1) The curve for ibrutinib shows a sigmoidal dose-response relationship, with the steepest part of the curve between 1 nM and 10 nM. 2) The IC50 of ibrutinib is around 3 nM which was significantly lower than that of canertinib. This indicates that ibrutinib is much more potent than canertinib, providing IC50 at lower concentration. 3) The maximum inhibiton of ibrutinib is approximately 75%, suggesting ibrutinib as a more effectif inhibitor of TMD8 cell line compared to canertinib. Efficacy: The maximum inhibition (E max

Analysis of the Dose Response Matrix ibrutinib and canertinib

In the first column, ibrutinib alone shows increasing inhibition with increasing concentration, from -15.04% at 0 nM to 70.97% at 50 nM. Meanwhile, canertinib alone (first row) exhibits increase of inhibition reaching 53.02% at 2500 nM.

Combination Effects: 1. Potential of high antagonism showed by the combination of ibrutinib and canertinib particularly at moderate concentrations. For an example, at 0.7812 nM ibrutinib and 156.25 nM canertinib, the inhibition is a highly negative -56.94%. This means that at those concentrations, the combination shows

a pro-proliferative effect. 2. The antagonism is concentrated in a triangular region of the matrix, with the lowest point of inhibition being at 0.1954 nM ibrutinib and 156.25 nM canertinib, where the inhibition is -78.45%. 3. Synergy/Additivity shows at high concentrations (50 nM ibrutinib and 2500 nM canertinib) with the inhibition reaches 80.71%. This may indicate a transition from antagonism to an additive or even weakly synergistic effect at the highest concentrations.