

# Drug prediction tutorial

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## Introduction

The goal of this tutorial is to show an example where dynamic logic model is used to predict drug response.

The tutorial is based on the paper

Eduati et al (2017) Drug resistance mechanisms in colorectal cancer dissected with cell type-specific dynamic logic models. *Cancer Research*. DOI: 10.1158/0008-5472.CAN-17-0078

We investigate here the drug-response of colorectal cancer cell lines. For this, we use drug response data and a signaling dataset.

The Genomics of Drug Sensitivity in Cancer (GDSC), <https://www.cancerrxgene.org/> offers drug response data for more than a 1000 human cancer cell lines, for hundreds of drugs. A small part of these data is can be found in `"./data/IC50_GDSC.csv"`.

The perturbation dataset contains the short time signaling response of 14 colorectal cancer cell lines, where 14 phosphoproteins are measured under 43 perturbation conditions (5 stimuli, 7 inhibitors).

First, we construct signaling models based on the perturbation data to the cell lines, here we use the CNORode modelling package. In the next step, we will associate model features to drug response to see why certain cell lines respond to certain drugs and others do not. Here we use a linear modeling framework.

## CNORode

CNORode is a member of the CellNOptR logic based tool family. It can translate the network to ordinary differential equation (ODE) model, fit the model parameters to data and make predictions.

## Dependencies

These should be already installed from previous tutorial.

```
# installs devtools package if not already installed
if(!require("devtools")) install.packages('devtools')

# installs CellNOptR and CNORode from GitHub:
if(!require("CellNOptR")) devtools::install_github('saezlab/CellNOptR')
if(!require("CNORode")) devtools::install_github('saezlab/CNORode')

if(!require("dplyr")) install.packages('dplyr')
if(!require("readr")) install.packages('readr')
if(!require("tidyr")) install.packages('tidyr')
```

If you don't have devtools and cannot install it, then

1. please visit the <https://github.com/saezlab/CellNOptR> and <https://github.com/saezlab/CNORode> websites,
2. download the toolboxes by clicking "Clone or download" then "Download Zip"
3. Unzip the files
4. In RStudio run:
 

```
install.packages("../CellNOptR-master", repos = NULL, type = "source")
install.packages("../CNORode-master", repos = NULL, type = "source")
```

---

Make sure to import the libraries

```
library(CellNOptR)
library(CNORode)
library(MEIGOR)
```

```
## Loading required package: Rsolnp
## Loading required package: snowfall
## Loading required package: snow
##
## Attaching package: 'snow'

## The following objects are masked from 'package:BiocGenerics':
##
##   clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
##   clusterExport, clusterMap, clusterSplit, parApply, parCapply,
##   parLapply, parRapply, parSapply
##
## The following objects are masked from 'package:parallel':
##
##   clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
##   clusterExport, clusterMap, clusterSplit, makeCluster,
##   parApply, parCapply, parLapply, parRapply, parSapply,
##   splitIndices, stopCluster

## Loading required package: deSolve

library(dplyr)
library(tidyr)
library(ggplot2)
```

## PART I: DRUG response exploration

```
IC50 <- readr::read_csv("./data/tutorial_3/IC50_GDSC.csv") %>% rename("cell_line" = "X1")
```

```
## Warning: Missing column names filled in: 'X1' [1]
```

```
## Parsed with column specification:
```

```
## cols(  
##   .default = col_double(),  
##   X1 = col_character()  
## )
```

```
## See spec(...) for full column specifications.
```

```
print(IC50)
```

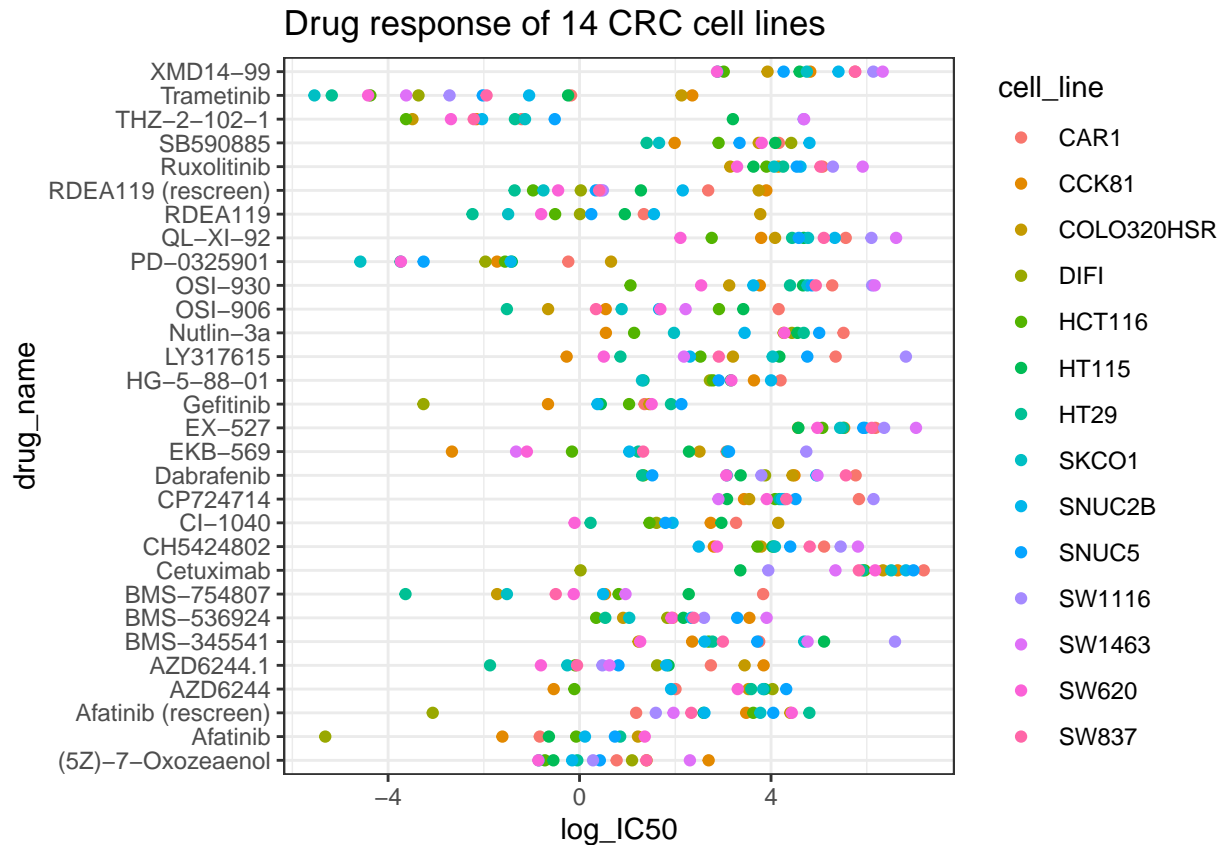
```
## # A tibble: 14 x 31
```

```
##   cell_line Gefitinib  RDEA119 `CI-1040` Afatinib `Nutlin-3a` `PD-0325901`  
##   <chr>         <dbl>    <dbl>    <dbl>    <dbl>    <dbl>    <dbl>  
## 1 CAR1          1.36    1.34      3.27   -0.831    5.51   -0.235  
## 2 CCK81        -0.659   NA        2.74   -1.61    0.551   -1.72  
## 3 COLO320H~    1.46    3.78      4.15    1.22    4.26    0.658  
## 4 DIFI         -3.26    0.00908   1.61   -5.31    4.44   -1.97  
## 5 HCT116        1.03   -0.510    1.46   -0.0704   1.14   -1.56  
## 6 HT115         0.444    0.946    2.96   -0.640    4.55   -1.41  
## 7 HT29          1.91   -2.24     0.230   0.849    4.68   -3.74  
## 8 SKCO1         NA     -1.49     NA      NA      1.97   -4.58  
## 9 SNUC2B        0.375    1.55     1.94    0.113    3.45   -1.44  
## 10 SNUC5        2.13    0.246    1.79    0.739    5.01   -3.26  
## 11 SW1116       NA      NA      NA      NA      NA      NA  
## 12 SW1463       NA      NA      NA      NA      NA      NA  
## 13 SW620        1.51   -0.802   -0.104   1.36    4.28   -3.73  
## 14 SW837       NA      NA      NA      NA      NA      NA
```

```
## # ... with 24 more variables: SB590885 <dbl>, AZD6244 <dbl>,  
## #   `BMS-536924` <dbl>, Cetuximab <dbl>, `HG-5-88-01` <dbl>,  
## #   `(5Z)-7-Oxozeaenol` <dbl>, Trametinib <dbl>, Dabrafenib <dbl>,  
## #   `Afatinib (rescreen)` <dbl>, AZD6244.1 <dbl>, `RDEA119  
## #   (rescreen)` <dbl>, `BMS-754807` <dbl>, `OSI-906` <dbl>,  
## #   `BMS-345541` <dbl>, Ruxolitinib <dbl>, LY317615 <dbl>,  
## #   `XMD14-99` <dbl>, CP724714 <dbl>, CH5424802 <dbl>, `EKB-569` <dbl>,  
## #   `OSI-930` <dbl>, `QL-XI-92` <dbl>, `EX-527` <dbl>, `THZ-2-102-1` <dbl>
```

```
IC50 %>% gather(drug_name, log_IC50, -cell_line) %>%  
  ggplot() +  
  geom_point(aes(drug_name, log_IC50, col=cell_line)) +  
  coord_flip() +  
  theme_bw() +  
  ggtitle("Drug response of 14 CRC cell lines")
```

```
## Warning: Removed 49 rows containing missing values (geom_point).
```



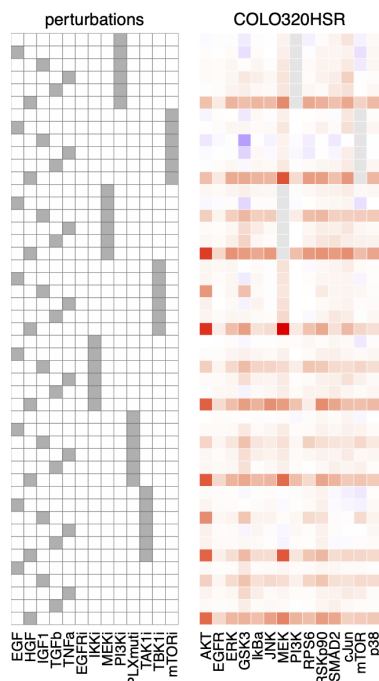
From the raw IC<sub>50</sub> values we can see that there are some drugs that are more effective (Trametinib) than others, like XMD14-99. There are also cell-line differences, for example, DIFI shows stronger sensitivity to Afatinib than any other cell lines. What could be the reason for this?

## PART II: cell-line models

The goal of part II is to build a cell-line specific model from the perturbation data

### Perturbation data

The following heatmap shows an overview on the perturbation data. The first block outlines the combinations of treatment. Then each other block represents the response of a cell line. Different columns within a block shows the different phosphoprotein markers.



In the tutorial we make a single model for the first cell line *COLO320HSR*.

## Model of a single cell line

```
# load Prior Knowledge Network (PKN)
pknmodel<-readSIF("./data/tutorial_3/PKN.sif")

# load normalised perturbation data
# select MIDAS file for the desired cell line
MIDASfile <- "./data/tutorial_3/processed/MIDAS/MD-COLO320HSR_Ktuned_v1_n4_all_noEGFRi_CNORode.csv"

Mydata<-readMIDAS(MIDASfile=MIDASfile)

## [1] "Your data set comprises 86 conditions (i.e. combinations of time point and treatment)"
## [1] "Your data comprises only a DA:ALL column; all readouts are assumed to have been acquired at the same time point"
## [1] "Your data set comprises measurements on 25 different species"
## [1] "Your data set comprises 13 stimuli/inhibitors and 1 cell line(s) ( COLO320HSR )"
## [1] "Please be aware that CNO only handles measurements on one cell line at this time."
## [1] "Your data file contained 'NaN'. We have assumed that these were missing values and replaced them with 0."

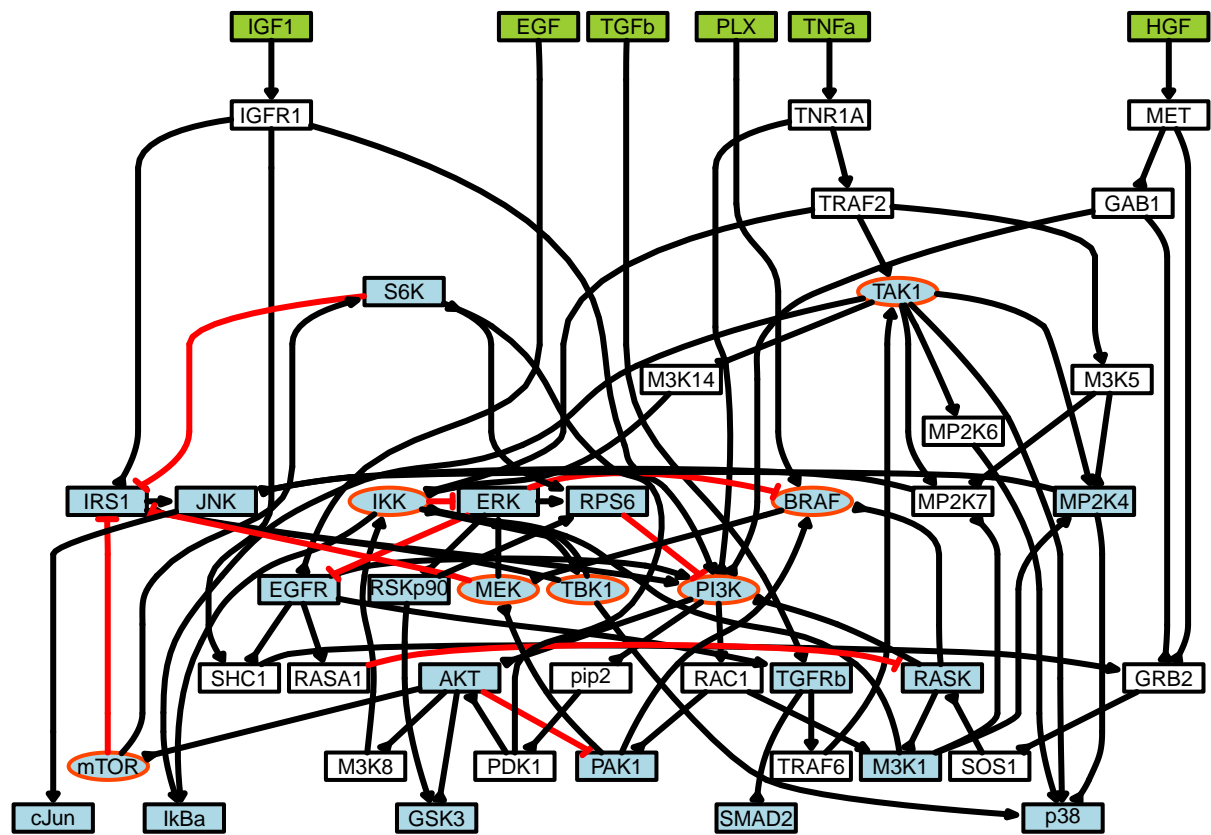
cnolist<-makeCNolist(Mydata, subfield=F)

## [1] "Please be aware that if you only have some conditions at time zero (e.g. only inhibitor/no inhibitor) then the value of the stimulus should be set to 0.5"

cnolist$valueStimuli[cnolist$valueStimuli==0]=0.5

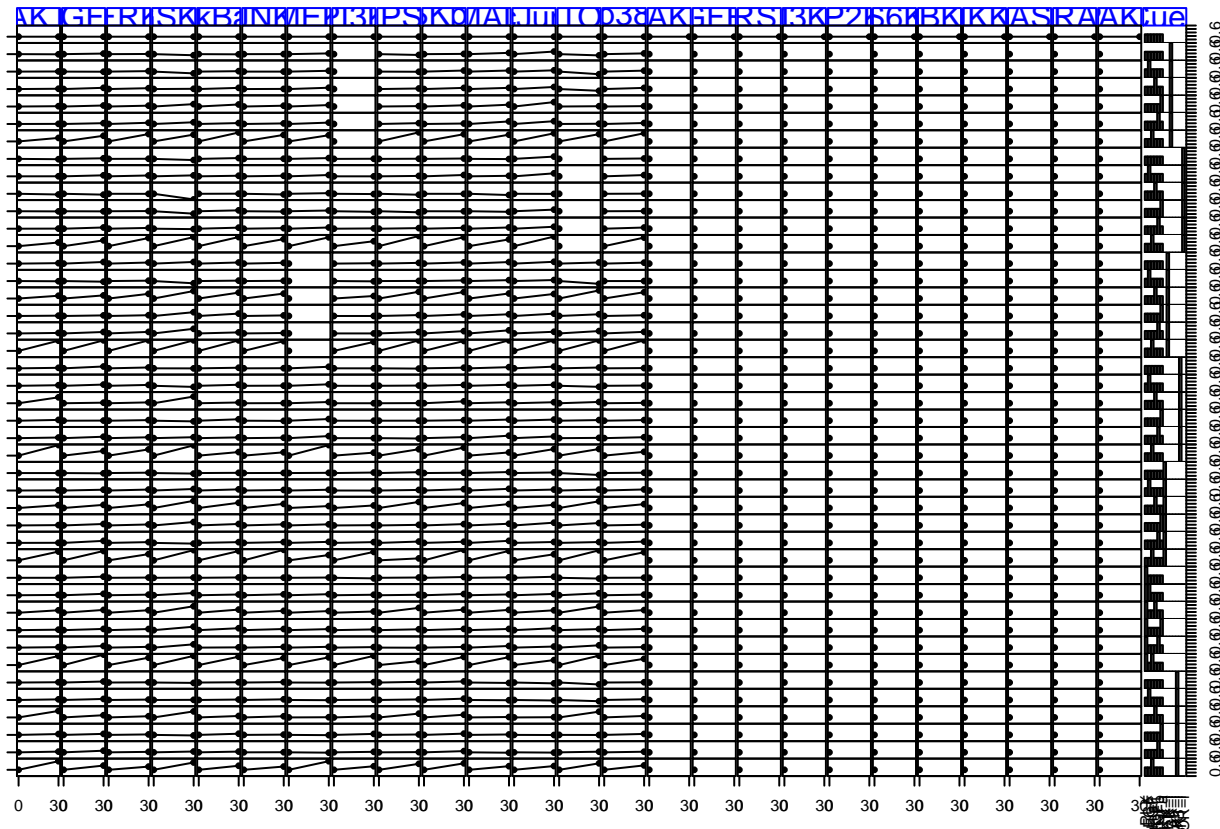
Show the network first

plotModel(pknmodel,cnolist)
```



Then the data in CellNOpt format:

```
plotCN0list(cnolist)
```



```
# compress the network (no expansion, only OR gates are considered)
model<-preprocessing(data=cnolist, model=pknmodel, compression=TRUE, expansion=FALSE)

## [1] "The following species are measured: AKT, EGFR, ERK, GSK3, Ikb, JNK, MEK, PI3K, RPS6, RSKp90, S
## [1] "The following species are stimulated: PLX, EGF, HGF, IGF1, TGFb, TNFa"
## [1] "The following species are inhibited: IKK, MEK, PI3K, BRAF, TAK1, TBK1, mTOR"
## [1] "The following species are not observable and/or not controllable: "

# set initial parameters (here parameters 'k' and 'tau' are optimised and 'n' fixed to 3)
ode_parameters <- createLNodeContPars(model,
    LB_n = 1, LB_k = 0, LB_tau = 0,
    UB_n = 3, UB_k = 1, UB_tau = 1,
    default_n = 3,
    default_k = 0.5,
    default_tau = 0.01,
    opt_n = FALSE, opt_k = TRUE, opt_tau = TRUE,
    random = TRUE)

# PLX -> BRAF is an artificial regulation used to model paradoxical effect of PLX4720,
# which works as selective BRAF inhibitor in cell-lines where BRAF is mutated in V600E (i.e. HT29 and S
# but induces a paradoxical activation of wild type BRAF cells (modeled as stimulus on those cell lines)
ode_parameters$parValues[which(ode_parameters$parNames=="PLX_k_BRAF")]<-0.5
ode_parameters$index_opt_pars<-setdiff(ode_parameters$index_opt_pars, which(ode_parameters$parNames=="P

## Parameter Optimization
# essm
paramsSSm=defaultParametersSSm()
paramsSSm$local_solver = "DHC"
```

```
paramsSSm$maxtime = 30; #36000;
paramsSSm$transfer_function = 4;
```

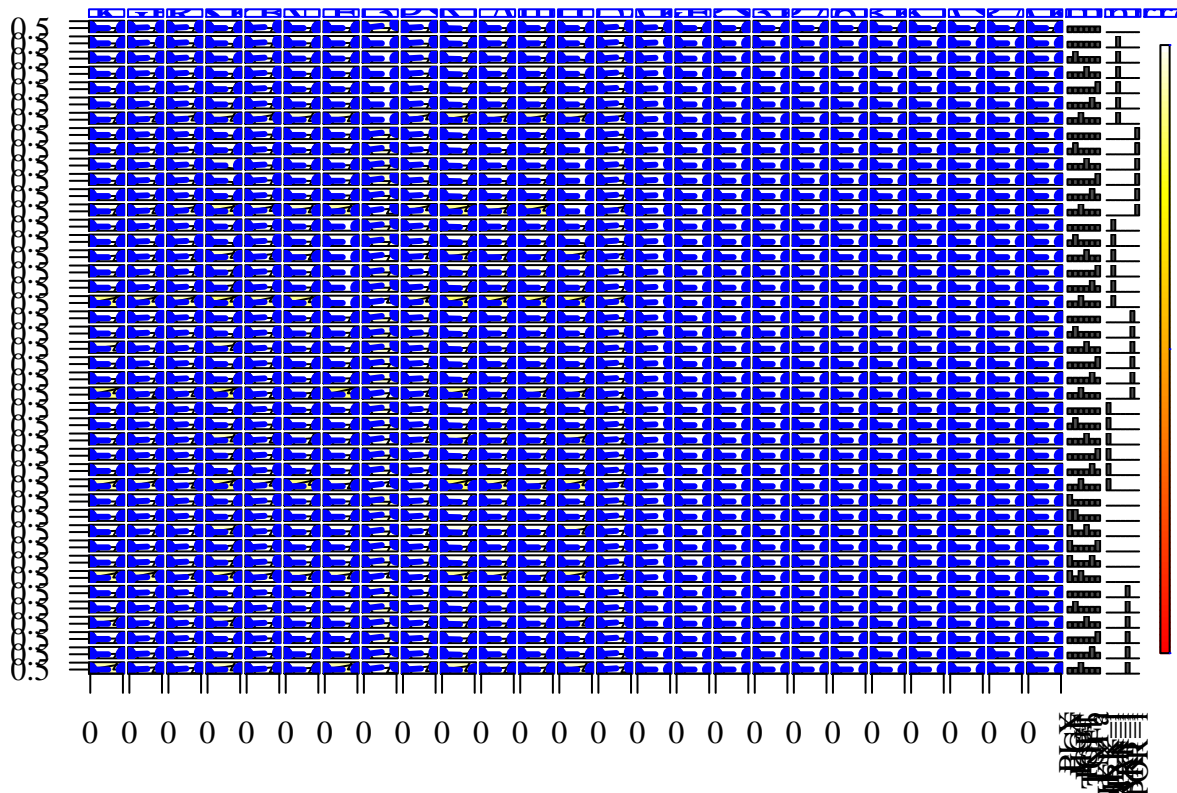
The actual optimisation takes around 10 mins, instead of the 30 sec. So, instead of running it here, we just load the results:

```
opt_pars = parEstimationLNode(cnolist, model, method="essm",
                             ode_parameters=ode_parameters,
                             paramsSSm=paramsSSm)
#write_rds(opt_pars,"data/tutorial_3/opt_pars_30sec.RDS")
```

```
opt_pars <- read_rds("data/tutorial_3/opt_pars_30sec.RDS")
```

Plot the fit of the model:

```
sim_res <- CNORode::plotLNodeFitness(cnolist,model,ode_parameters = opt_pars)
```







The fit is a bit better than a random model, but these optimisations should be run for around 10 hours.

The optimised parameters for this cell line

```
opt_par_values <- opt_pars$parValues
names(opt_par_values) <- opt_pars$parNames
opt_par_values
```

```
##  TGFb_n_TAK1  TGFb_k_TAK1  TNFa_n_TAK1  TNFa_k_TAK1  tau_TAK1
##  3.000000e+00  6.290662e-01  3.000000e+00  0.000000e+00  7.787088e-03
##  TGFb_n_TGFRb  TGFb_k_TGFRb  EGFR_n_TGFRb  EGFR_k_TGFRb  tau_TGFRb
##  3.000000e+00  2.802733e-01  3.000000e+00  1.542701e-01  0.000000e+00
##  EGF_n_EGFR    EGF_k_EGFR    ERK_n_EGFR    ERK_k_EGFR    tau_EGFR
##  3.000000e+00  0.000000e+00  3.000000e+00  2.007754e-07  7.015971e-03
##  S6K_n_IRS1    S6K_k_IRS1    TBK1_n_IRS1   TBK1_k_IRS1   mTOR_n_IRS1
##  3.000000e+00  0.000000e+00  3.000000e+00  0.000000e+00  3.000000e+00
##  mTOR_k_IRS1    IGF1_n_IRS1    IGF1_k_IRS1   MEK_n_IRS1    MEK_k_IRS1
##  0.000000e+00  3.000000e+00  0.000000e+00  3.000000e+00  4.060043e-07
##  tau_IRS1       PI3K_n_AKT     PI3K_k_AKT     tau_AKT       PI3K_n_M3K1
##  0.000000e+00  3.000000e+00  4.100319e-02  0.000000e+00  3.000000e+00
##  PI3K_k_M3K1    RASK_n_M3K1    RASK_k_M3K1    tau_M3K1      ERK_n_RPS6
##  4.432509e-01  3.000000e+00  5.075054e-08  1.568557e-09  3.000000e+00
##  ERK_k_RPS6     S6K_n_RPS6     S6K_k_RPS6     RSKp90_n_RPS6  RSKp90_k_RPS6
##  2.498247e-01  3.000000e+00  0.000000e+00  3.000000e+00  8.162135e-01
##  tau_RPS6       IKK_n_ERK      IKK_k_ERK      MEK_n_ERK     MEK_k_ERK
##  1.027507e-02  3.000000e+00  3.065270e-01  3.000000e+00  1.271568e-01
##  tau_ERK        TAK1_n_MP2K4    TAK1_k_MP2K4    M3K1_n_MP2K4   M3K1_k_MP2K4
##  9.911941e-03  3.000000e+00  1.000000e+00  3.000000e+00  0.000000e+00
##  TNFa_n_MP2K4    TNFa_k_MP2K4    tau_MP2K4      mTOR_n_S6K     mTOR_k_S6K
##  3.000000e+00  0.000000e+00  0.000000e+00  3.000000e+00  5.503381e-01
##  PI3K_n_S6K     PI3K_k_S6K      tau_S6K        IKK_n_TBK1     IKK_k_TBK1
##  3.000000e+00  0.000000e+00  1.462364e-05  3.000000e+00  6.452658e-01
##  tau_TBK1       AKT_n_mTOR      AKT_k_mTOR      tau_mTOR       TAK1_n_IKK
##  9.931725e-03  3.000000e+00  0.000000e+00  0.000000e+00  3.000000e+00
##  TAK1_k_IKK     AKT_n_IKK       AKT_k_IKK       M3K1_n_IKK     M3K1_k_IKK
##  8.246211e-01  3.000000e+00  0.000000e+00  3.000000e+00  0.000000e+00
##  TNFa_n_IKK     TNFa_k_IKK      TBK1_n_IKK      TBK1_k_IKK     tau_IKK
```

```
## 3.000000e+00 0.000000e+00 3.000000e+00 8.707054e-05 0.000000e+00
## EGFR_n_P13K EGFR_k_P13K IRS1_n_P13K IRS1_k_P13K RPS6_n_P13K
## 3.000000e+00 1.599331e-01 3.000000e+00 0.000000e+00 3.000000e+00
## RPS6_k_P13K TNFa_n_P13K TNFa_k_P13K IGF1_n_P13K IGF1_k_P13K
## 1.000000e+00 3.000000e+00 0.000000e+00 3.000000e+00 0.000000e+00
## HGF_n_P13K HGF_k_P13K RASK_n_P13K RASK_k_P13K tau_P13K
## 3.000000e+00 4.751120e-01 3.000000e+00 3.009077e-02 3.042961e-02
## EGFR_n_RASK EGFR_k_RASK IGF1_n_RASK IGF1_k_RASK HGF_n_RASK
## 3.000000e+00 0.000000e+00 3.000000e+00 0.000000e+00 3.000000e+00
## HGF_k_RASK tau_RASK BRAF_n_MEK BRAF_k_MEK PAK1_n_MEK
## 5.156353e-07 0.000000e+00 3.000000e+00 7.505019e-03 3.000000e+00
## PAK1_k_MEK tau_MEK ERK_n_BRAF ERK_k_BRAF RASK_n_BRAF
## 0.000000e+00 0.000000e+00 3.000000e+00 5.244096e-02 3.000000e+00
## RASK_k_BRAF PAK1_n_BRAF PAK1_k_BRAF PLX_n_BRAF PLX_k_BRAF
## 0.000000e+00 3.000000e+00 9.269049e-02 3.000000e+00 3.603077e-06
## tau_BRAF ERK_n_RSKp90 ERK_k_RSKp90 tau_RSKp90 TAK1_n_JNK
## 0.000000e+00 3.000000e+00 9.381897e-01 1.471951e-02 3.000000e+00
## TAK1_k_JNK IRS1_n_JNK IRS1_k_JNK M3K1_n_JNK M3K1_k_JNK
## 0.000000e+00 3.000000e+00 3.617738e-05 3.000000e+00 0.000000e+00
## TNFa_n_JNK TNFa_k_JNK MP2K4_n_JNK MP2K4_k_JNK tau_JNK
## 3.000000e+00 8.352255e-02 3.000000e+00 0.000000e+00 0.000000e+00
## AKT_n_PAK1 AKT_k_PAK1 PI3K_n_PAK1 PI3K_k_PAK1 tau_PAK1
## 3.000000e+00 5.496838e-01 3.000000e+00 0.000000e+00 0.000000e+00
## TAK1_n_p38 TAK1_k_p38 MP2K4_n_p38 MP2K4_k_p38 TBK1_n_p38
## 3.000000e+00 2.582269e-01 3.000000e+00 0.000000e+00 3.000000e+00
## TBK1_k_p38 tau_p38 TGFrb_n_SMAD2 TGFrb_k_SMAD2 tau_SMAD2
## 8.704423e-01 1.299676e-02 3.000000e+00 0.000000e+00 0.000000e+00
## AKT_n_GSK3 AKT_k_GSK3 RSKp90_n_GSK3 RSKp90_k_GSK3 tau_GSK3
## 3.000000e+00 0.000000e+00 3.000000e+00 0.000000e+00 0.000000e+00
## TAK1_n_IkBa TAK1_k_IkBa IKK_n_IkBa IKK_k_IkBa tau_IkBa
## 3.000000e+00 4.080694e-01 3.000000e+00 7.350341e-01 1.539245e-02
## JNK_n_cJun JNK_k_cJun tau_cJun
## 3.000000e+00 0.000000e+00 0.000000e+00
```

Similar to the above cell-line, we can build a model for each of the cell lines. This is very time consuming, therefore we just load the optimised parameters from the paper.

```
optimised_parameters <- read_delim("./data/tutorial_3/allModelsParameters.txt",delim = "\t")
```

```
## Parsed with column specification:
## cols(
##   .default = col_double(),
##   cell_line = col_character()
## )
## See spec(...) for full column specifications.
```

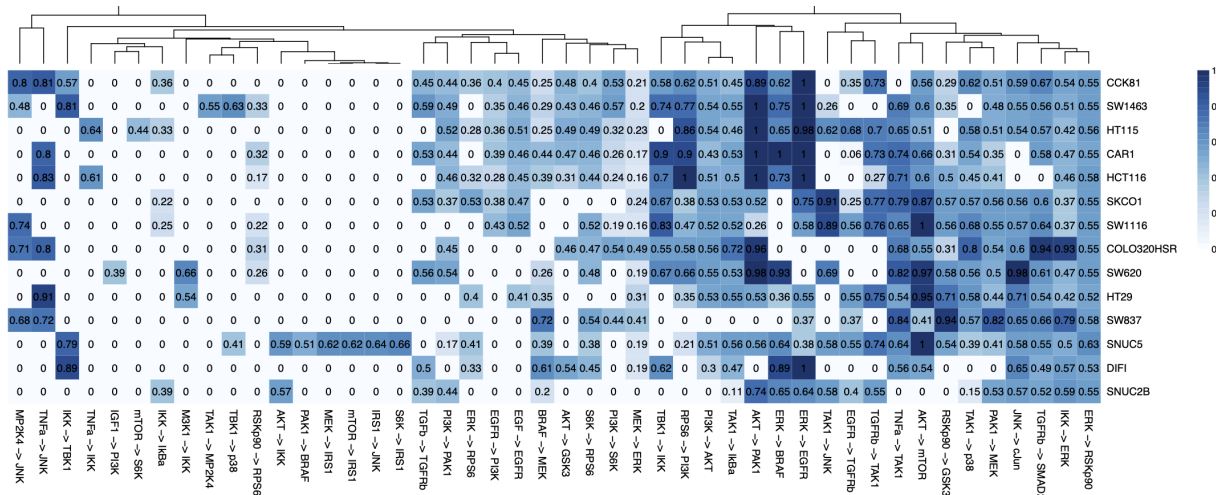
Let's check edge and node parameters.

```
edge_id <- grep("_k_",colnames(optimised_parameters))
edge_parameters_HM <- optimised_parameters[edge_id]%>% as.matrix()
rownames(edge_parameters_HM) <- optimised_parameters$Cell_line
```

```
## Warning: Unknown or uninitialised column: 'Cell_line'.
```

```
# heatmap(edge_parameters_HM, main = "Cell line edge parameters")
```

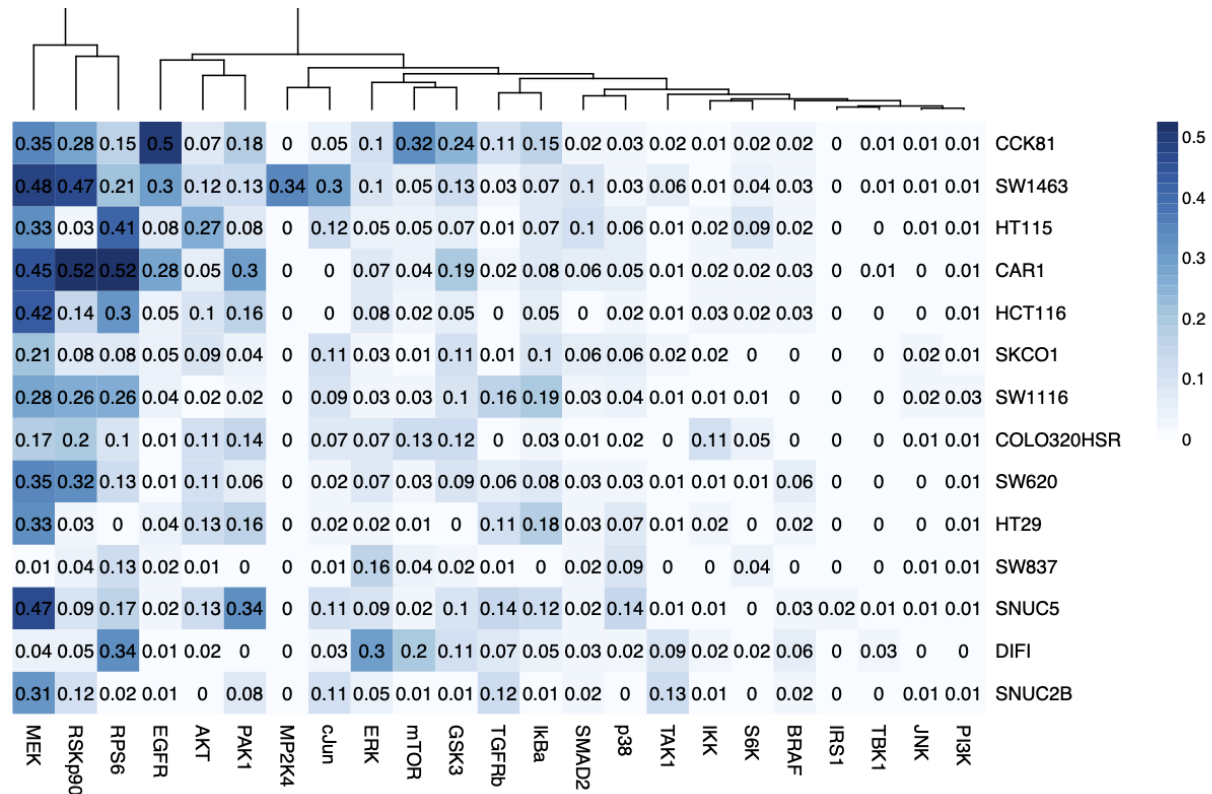
```
knitr::include_graphics("../data/tutorial_3/parHeatmap_k.png")
```



```
node_id <- grep("tau_", colnames(optimised_parameters))
node_parameters_HM <- optimised_parameters[node_id] %>% as.matrix()
rownames(node_parameters_HM) <- optimised_parameters$Cell_line
```

```
## Warning: Unknown or uninitialised column: 'Cell_line'.
```

```
# heatmap(node_parameters_HM, main = "Cell line node parameters")
knitr::include_graphics("../data/tutorial_3/parHeatmap_tau.png")
```



The level of edge and node parameters differs across the cell-lines.

## PART III: Associate model parameters and drug response

Which model parameters correlates with drug response IC50 ?

```
# first we need to remove the parameters, that are zero across all the models
zero_pars <- names(which(colMeans(optimised_parameters[,-1]) == 0))

# join the IC50 data and network model parameters based on cell_lines
drug_model_data <- optimised_parameters %>% select(-zero_pars) %>%
  gather(parameter,par_value,-cell_line) %>%
  left_join(IC50 %>% gather(drug, IC50,-cell_line),by = "cell_line")

# for each drug and each parameter compute the correlation coefficient

corr_data <- drug_model_data %>% group_by(drug, parameter) %>%
  summarise(corr_par_drug = cor(par_value,IC50,use = "complete.obs"))
```

Let's show some correlation

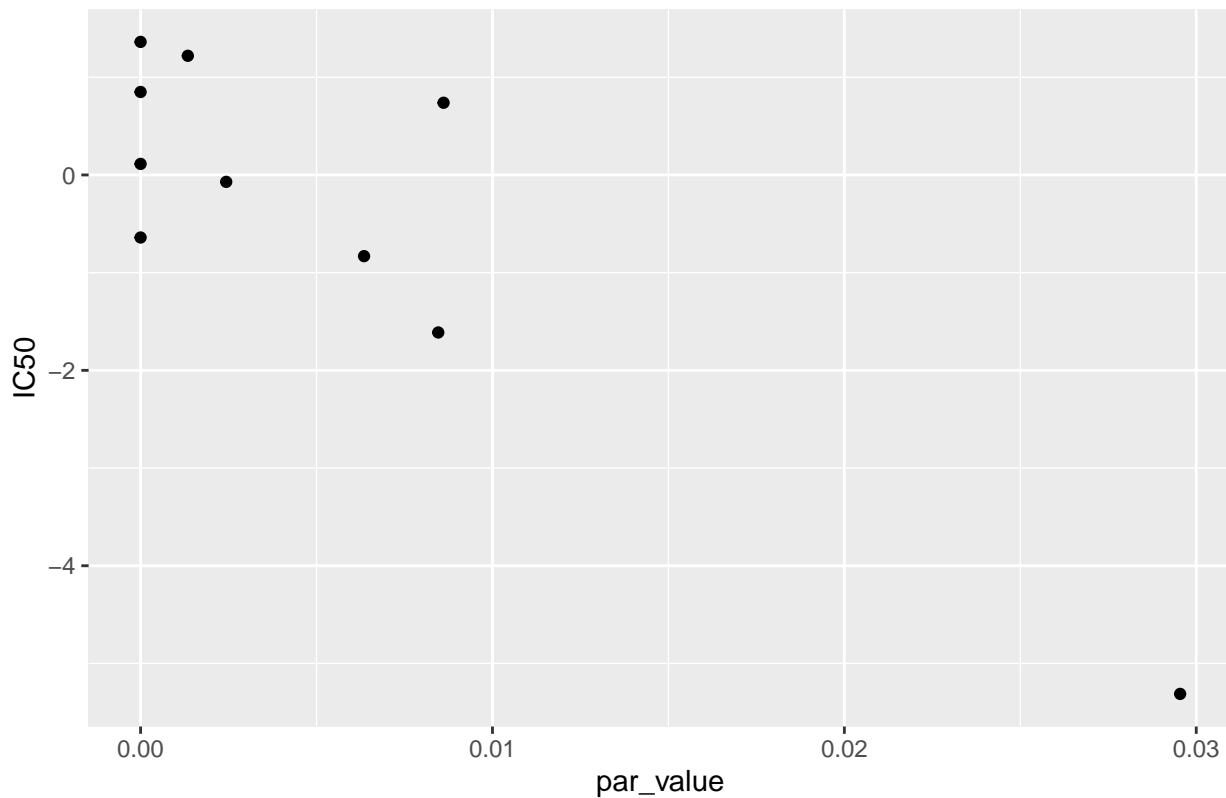
```
corr_data %>% arrange(desc(abs(corr_par_drug))) %>% print(.,n=25)
```

```
## # A tibble: 2,070 x 3
## # Groups:   drug [30]
##   drug                parameter    corr_par_drug
##   <chr>                <chr>          <dbl>
## 1 Afatinib            tau_TBK1          -0.896
## 2 Afatinib            tau_ERK           -0.875
## 3 BMS-754807          tau_RPS6           0.861
## 4 Gefitinib           tau_ERK           -0.859
## 5 OSI-906             tau_RPS6           0.846
## 6 Afatinib            PAK1_k_MEK         0.834
## 7 RDEA119             IKK_k_ERK          0.818
## 8 Gefitinib           tau_TBK1          -0.813
## 9 SB590885            tau_IkBa          -0.799
## 10 Afatinib (rescreen) tau_TBK1          -0.784
## 11 CI-1040             PI3K_k_S6K         0.777
## 12 CI-1040             M3K1_k_IKK        -0.776
## 13 Afatinib (rescreen) tau_ERK           -0.762
## 14 HG-5-88-01          ERK_k_BRAF         0.756
## 15 HG-5-88-01          ERK_k_RPS6        -0.754
## 16 PD-0325901          AKT_k_GSK3         0.732
## 17 RDEA119             PI3K_k_S6K         0.731
## 18 PD-0325901          PI3K_k_S6K         0.721
## 19 SB590885            RSKp90_k_GSK3     -0.717
## 20 (5Z)-7-Oxozeaenol  PI3K_k_S6K         0.717
## 21 RDEA119             MP2K4_k_JNK        0.712
## 22 OSI-906            ERK_k_BRAF         0.708
## 23 Gefitinib           tau_MEK            0.708
## 24 (5Z)-7-Oxozeaenol  MP2K4_k_JNK        0.707
## 25 THZ-2-102-1        tau_SMAD2          0.706
## # ... with 2,045 more rows
```

```
drug_model_data %>% filter(drug=="Afatinib",parameter=="tau_TBK1") %>%
  ggplot() + geom_point(aes(par_value,IC50)) +
  ggtitle("DRUG: Afatinib; parameter: tau_TBK1")
```

```
## Warning: Removed 4 rows containing missing values (geom_point).
```

DRUG: Afatinib; parameter: tau\_TBK1



Think what the problem might be here?

We have only 14 cell-lines, therefore each of the correlations between model parameter and drug IC50 is based on 14 data points. There are 31 drugs and 89 model parameters, which results in  $31 \times 89 = 2759$  tests.

Also this is only a single parameter - single drug association. It is possible, that the existence of multiple edges makes a cell-line sensitive/resistant. Therefore (Eduati et al) derived linear models, that funds multiple parameters at the same time.

```
knitr::include_graphics("../data/tutorial_3/Eduatietal_Figure5.png")
```

Figure 5

A

| Drug              | Associated model parameter | Targets                                   | Associated genomic alterations | Weight |
|-------------------|----------------------------|---|--------------------------------|--------|
| OSI-906           | k ERK, RSKp90              | IGF1R                                     | MYC->PDK1, SMAD2               | 0.5    |
| OSI-906           | k MEK, ERK                 | IGF1R                                     | MYC->PDK1, SMAD2               | 0.5    |
| BMS-345541        | t cJun                     | IKKB                                      | PARK2-IcJun; MYC->PDK1, SMAD2  | 0.5    |
| HG-5-88-01        | k IKK, ERK                 | EGFR                                      |                                | 0.5    |
| OSI-906           | t RPS6                     | IGF1R                                     | MYC->PDK1, SMAD2               | 0.5    |
| BMS-345541        | k EGFR, PI3K               | IKKB                                      | PARK2-IcJun; MYC->PDK1, SMAD2  | 0.5    |
| SB590885          | t IkBa                     | BRAF                                      |                                | 0.5    |
| AZD6244           | t GSK3                     | MEK                                       |                                | 0.5    |
| HG-5-88-01        | k MEK, ERK                 | EGFR                                      |                                | 0.5    |
| PD-0325901        | k IKK, ERK                 | MEK                                       |                                | 0.5    |
| HG-5-88-01        | k ERK, RSKp90              | EGFR                                      |                                | 0.5    |
| BMS-754807        | t RPS6                     | IGF1R                                     | MYC->PDK1, SMAD2               | 0.5    |
| SB590885          | k ERK, RPS6                | BRAF                                      |                                | 0.5    |
| (5Z)-7-Oxozeaenol | k IKK, TBK1                | MAP3K7 (TAK1)                             |                                | 0.5    |
| BMS-345541        | k PI3K, PAK1               | IKKB                                      | PARK2-IcJun; MYC->PDK1, SMAD2  | 0.5    |
| AZD6244           | k IKK, ERK                 | MEK                                       |                                | 0.5    |
| AZD6244           | k RSKp90, GSK3             | MEK                                       |                                | 0.5    |
| (5Z)-7-Oxozeaenol | k TGFRb, SMAD2             | MAP3K7 (TAK1)                             |                                | 0.5    |
| RDEA119           | k IKK, ERK                 | MEK                                       |                                | 0.5    |
| RDEA119           | k TGFRb, SMAD2             | MEK                                       |                                | 0.5    |
| (5Z)-7-Oxozeaenol | k MP2K4, JNK               | MAP3K7 (TAK1)                             |                                | 0.5    |
| Dabrafenib        | k ERK, RPS6                | BRAF                                      |                                | 0.5    |
| THZ-2-102-1       | t cJun                     | CDK7->MEK, PAK1                           | MYC->PDK1, SMAD2               | 0.5    |
| RDEA119           | t GSK3                     | MEK                                       |                                | 0.5    |
| HG-5-88-01        | k ERK, RPS6                | EGFR                                      |                                | 0.5    |
| SB590885          | k RSKp90, GSK3             | BRAF                                      |                                | 0.5    |
| Atatinib          | t ERK                      | EGFR; ERBB2->EGFR, SHC1, PI3K, GRB2, PAK1 | MYC->PDK1, SMAD2               | 0.5    |
| (5Z)-7-Oxozeaenol | k PI3K, S6K                | MAP3K7 (TAK1)                             |                                | 0.5    |
| Dabrafenib        | k TGFRb, SMAD2             | BRAF                                      |                                | 0.5    |
| BMS-345541        | t mTOR                     | IKKB                                      | PARK2-IcJun; MYC->PDK1, SMAD2  | 0.5    |
| BMS-345541        | k EGFR, TGFRb              | IKKB                                      | PARK2-IcJun; MYC->PDK1, SMAD2  | 0.5    |
| PD-0325901        | k ERK, RPS6                | MEK                                       |                                | 0.5    |
| BMS-345541        | t RPS6                     | IKKB                                      | PARK2-IcJun; MYC->PDK1, SMAD2  | 0.5    |
| (5Z)-7-Oxozeaenol | t EGFR                     | MAP3K7 (TAK1)                             |                                | 0.5    |
| PD-0325901        | k AKT, GSK3                | MEK                                       |                                | 0.5    |
| CI-1040           | k PI3K, S6K                | MEK                                       |                                | 0.5    |
| HG-5-88-01        | k ERK, BRAF                | EGFR                                      |                                | 0.5    |
| (5Z)-7-Oxozeaenol | k TAK1, JNK                | MAP3K7 (TAK1)                             |                                | 0.5    |
| Atatinib          | t RPS6                     | EGFR; ERBB2->EGFR, SHC1, PI3K, GRB2, PAK1 | MYC->PDK1, SMAD2               | 0.5    |
| PD-0325901        | k PI3K, S6K                | MEK                                       |                                | 0.5    |
| Dabrafenib        | k PI3K, AKT                | BRAF                                      |                                | 0.5    |
| BMS-345541        | k EGF, EGFR                | IKKB                                      | PARK2-IcJun; MYC->PDK1, SMAD2  | 0.5    |
| Trametinib        | k IKK, ERK                 | MEK                                       |                                | 0.5    |
| RDEA119           | k IKK, IkBa                | MEK                                       |                                | 0.5    |
| BMS-345541        | k AKT, PAK1                | IKKB                                      | PARK2-IcJun; MYC->PDK1, SMAD2  | 0.5    |
| AZD6244           | k AKT, GSK3                | MEK                                       |                                | 0.5    |
| AZD6244           | t mTOR                     | MEK                                       |                                | 0.5    |
| SB590885          | k ERK, BRAF                | BRAF                                      |                                | 0.5    |
| PD-0325901        | t IkBa                     | MEK                                       |                                | 0.5    |
| Trametinib        | k TGFRb, SMAD2             | MEK                                       |                                | 0.5    |

B

