**國 立 成 功 大 學**

**生 物 醫 學 工 程 研 究 所**

**碩 士 論 文**

基於表型響應面的轉移性乳腺癌藥物篩選優化

**Phenotypic Response Surface Based Optimization of Metastatic Breast Cancer Drug Screening**

研究生 : 陳躬約Yosua Septianto Santoso

指導教授 : 涂庭源 Tu Ting Yuan

中華民國112年7月10日

# ABSTRACT

Traditional method to developing drugs for complex disease like cancer require tremendous resources and time. Incorporating drug repurposing into phenotypic assay will help reduce the development time immensely and induce the possibility to discover novel method to treat cancer. Triple negative breast cancer is one of the most common case and death cause among cancer patients. The genetic heterogeneity and adaptive nature of cancer makes the treatment for each patient need to be personalized. With the rising of targeted therapy and drug combination therapy, the optimization method for metastatic cancer become attractive and interesting issue.

Currently second order linear polynomial already has been established as optimizing model due to the nature of phenotypic surface response of the drug combination in biological assays has smooth landscape. This finding encourages second order linear polynomial as alternative predictive model to optimize the drug combination for metastatic breast cancer. In addition, this model also can be used to identify the interaction between drugs in the drug combinations. An experimental design which termed as Orthogonal Array Composite Design (OACD) also has been developed to minimized the required number of experiment to be fitted with the second order linear polynomial.

Low toxicity antimetastatic drug is one of the important strategies for cancer therapy since most cause of cancer death is metastases. Compared to the antiproliferative drug, the research in this field is still lacking and currently no study about migration in breast cancer by using this model. This study aims to develop second order polynomial model to optimize low toxicity anti metastatic drug for breast cancer.

Twelve drugs already been identified as suitable for drug combination study and Chou-Talalay method in migration assay for MDA-MB-231. Those drugs are A-83-01, Gefitinib (ZD1839), PD168393, BIO, Panobinostat (LBH589), Rocilinostat (ACY-1215), BMS-536924, Orantinib (SU6668), CP-673451, GW9662, Saracatinib (AZD0530), Cabozantinib malate (XL184). The iteration already been performed in 4 times which reducing the total number of drugs from 12 drugs to 4 drugs.

Multiple objective optimization was performed in 8 drug combination and suggesting two set of 4-drug combination for further optimization. These 2 set are PD168393 + Saracatinib + Rocilinostat + GW9662 and BIO + Gefitinib + GW9662 + Cabozantinib. However these set of combination might changed due the model still lack enough experimental data.

The synergistic effect from the model also already been validated with Chou-Talalay method. However, there are discrepancy between the result which may be caused due biased model or the different drug ratio which can influence the synergistic between drugs.

**Keywords:**

**Drug screening, Migration assay, Proliferation assay, MDA-MB-231, Phenotypic surface response**

# ACKNOWLEDGEMENTS

I would like to show my sincerest gratitude to:

God, as my Shepherd who leads and shepherd me all this time,

Professor Tu Ting Yuan 涂庭源, as my advisor, whose support and encouragement help me to get through this topic,

IMBSL members, especially 阮氏金梅 Nguyen Thi Kim Mai, 蔡雅竹, 蘇冠琳, 毛彬旭, 黃寶賢Christina who taught me basic knowledge in cell culture and experiments, 張柏禹, 張崴宥, and 陳宜汝 who taught me in lab tasks, 吳氏金銀Ngo Thi Kim Ngan as my fellow master degree’s fighter whose knowledge and sharing helped me a lot in research and life, 林鄒全 who cooperate with me well in maintaining our laboratory financial report, 楊睿濬 who helped me in ordering my experiment consumables and apparatus,

My whole family, who keep pray and encourage me through this study,

The church in Tainan, who keep remind me in your prayer and shepherd me with abundant grace and blessings,

And everyone whose name I can’t mentioned one by one, without whom I would not be able to finish my master journey. Thank you for all your support and encouragement.

Yosua Septianto Santoso

# TABLE OF CONTENTS

[ABSTRACT I](#_Toc139883746)

[ACKNOWLEDGEMENTS III](#_Toc139883747)

[TABLE OF CONTENTS IV](#_Toc139883748)

[LIST OF TABLES VII](#_Toc139883749)

[LIST OF FIGURES X](#_Toc139883750)

[LIST OF ABBREVIATIONS XIV](#_Toc139883751)

[CHAPTER 1 INTRODUCTION 1](#_Toc139883752)

[1.1 Background 1](#_Toc139883753)

[1.1.1 Drug development 1](#_Toc139883754)

[1.1.2 Breast cancer and treatment 2](#_Toc139883755)

[1.1.3 Metastases and EMT in breast cancer 4](#_Toc139883756)

[1.1.4 Synergistic effect and validation model 6](#_Toc139883757)

[1.1.5 Drug optimization model – second order linear regression 13](#_Toc139883758)

[1.1.6 Drug optimization model – feedback system control 20](#_Toc139883759)

[1.1.7 Drug optimization model – Orthogonal Array Composite Design 20](#_Toc139883760)

[1.2 Critical Issues and Specific Aims 21](#_Toc139883761)

[1.2.1 The Critical Issues 21](#_Toc139883762)

[1.2.2 The Specific Aims 22](#_Toc139883763)

[CHAPTER 2 MATERIALS AND METHODS 23](#_Toc139883764)

[2.1 Experimental workflows 23](#_Toc139883765)

[2.2 Drug candidates 24](#_Toc139883766)

[2.3 Cell culture 24](#_Toc139883767)

[2.4 Spot migration assay 25](#_Toc139883768)

[2.5 Single drug dose response 26](#_Toc139883769)

[2.6 Initial screening: 2-level fractional factorial design 27](#_Toc139883770)

[2.7 Orthogonal Array Composite Design (OACD) 27](#_Toc139883771)

[2.8 Proliferation assay 27](#_Toc139883772)

[2.9 Image analysis 28](#_Toc139883773)

[2.10 Second order linear regression analysis 29](#_Toc139883774)

[2.11 Combination index of Chou-Talalay method 30](#_Toc139883775)

[2.12 Statistical analysis 30](#_Toc139883776)

[CHAPTER 3 RESULTS 32](#_Toc139883777)

[3.1 Single drug dose response 32](#_Toc139883778)

[3.2 1st iteration: 12 drug 2-level migration assay 34](#_Toc139883779)

[3.3 2nd iteration: 8 drug 3-level migration assay 36](#_Toc139883780)

[3.4 3rd iteration: 8 drugs 2-level migration and proliferation assay 38](#_Toc139883781)

[3.4.1 8 drugs 2-level migration assay 38](#_Toc139883782)

[3.4.2 8 drug 2-level proliferation assay 41](#_Toc139883783)

[3.5 4th iteration: 4 drug 3-level migration and proliferation assay 44](#_Toc139883784)

[3.5.1 4 drug 3-level migration assay 44](#_Toc139883785)

[3.5.2 4 drug 3-level proliferation assay 45](#_Toc139883786)

[3.6 Combination Index validation method for migration assay 47](#_Toc139883787)

[CHAPTER 4 DISCUSSION 48](#_Toc139883788)

[4.1 Single drug dose response 48](#_Toc139883789)

[4.2 1st iteration: 12 drug 2-level migration assay 49](#_Toc139883790)

[4.3 2nd iteration: 8 drug 3-level migration assay 49](#_Toc139883791)

[4.4 3rd iteration: 8 drug 2-level migration and proliferation assay 49](#_Toc139883792)

[4.6 4th iteration: 4 drug 3-level migration and proliferation assay 51](#_Toc139883793)

[4.6 The linear regression analysis 51](#_Toc139883794)

[4.7 Combination Index validation method 52](#_Toc139883795)

[CHAPTER 5 CONCLUSION 53](#_Toc139883796)

[REFERENCES 54](#_Toc139883797)

# LIST OF TABLES

[**Table 1.1 The percentage for metastatic research funding in various organization** [11]**.** 3](#_Toc139883856)

[**Table 1.2 OACD for 3-10 factors** 21](#_Toc139883857)

[**Table 4.1 The summary of single effect and 2-drug interaction effect of migration assay and proliferation assay of 3rd iteration**. 50](#_Toc139883858)

[**Table S1 The result of single drug dose response** 67](#_Toc139883859)

[**Table S2 The result of 1st iteration of 128 combinations with OACD for 12 drugs at 2 levels (0 and 1).** The 12 drugs consist of Gefitinib / ZD1839 (Gef), A83-01 (A83), BIO, Saracatinib / AZD0530 (Sara), Rocilinostat / ACY-1215 (Roci), PD168393 (PD), CP-673451 (CP), BMS-536924 (BMS), Orantinib / SU6668 (Oran), GW9662 (GW), SCH79797 (SCH), and Cabozantinib malate / XL184 (Cabo). Code 0 and 1 indicate no drugs and IC10 for each drugs respectively. 70](#_Toc139883860)

[**Table S3 Drug concentrations used in 1st iteration for 12 drug combinations at 2 levels (0 and 1).** 76](#_Toc139883861)

[**Table S4 Estimate and significance of standardized second order polynomial linear regression coefficients of 1st iteration for 12 drugs at 2 levels.** ns, \*, \*\*, \*\*\*, and \*\*\*\* indicates p > 0.05, p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, and p ≤ 0.0001, respectively. Number of observations: 129. Root Mean Squared Error: 0.341. R-squared: 0.903. Adjusted R-Squared: 0.884. 77](#_Toc139883862)

[**Table S5 The result of 2nd iteration of 90 combinations with OACD for 8 drugs at 3 levels (0, 1, and 2).** The 8 drugs consist of Gefitinib / ZD1839 (Gef), BIO, Saracatinib / AZD0530 (Sara), Rocilinostat / ACY-1215 (Roci), PD168393 (PD), BMS-536924 (BMS), GW9662 (GW), and Cabozantinib malate / XL184 (Cabo). Code 0, 1, and 2 indicate no drugs, half of IC15, and IC15 for each drugs respectively. 78](#_Toc139883863)

[**Table S6 Drug concentrations used in 2nd iteration for 8 drug combinations at 3 levels (0, 1, and 2).** 81](#_Toc139883864)

[**Table S7 Estimate and significance of standardized second order polynomial linear regression coefficients of 2nd iteration for 8 drugs at 3 levels.** ns, \*, \*\*, \*\*\*, and \*\*\*\* indicates p > 0.05, p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, and p ≤ 0.0001, respectively. Number of observations: 91. Root Mean Squared Error: 0.39. R-squared: 0.877. Adjusted R-Squared: 0.848. 81](#_Toc139883865)

[**Table S8 The result of 3rd iteration of 36 combinations for 8 drugs at 2 levels (0 and 1).** The 8 drugs consist of Gefitinib / ZD1839 (Gef), BIO, Saracatinib / AZD0530 (Sara), Rocilinostat / ACY-1215 (Roci), PD168393 (PD), BMS-536924 (BMS), GW9662 (GW), and Cabozantinib malate / XL184 (Cabo). Code 0 and 1 indicate no drugs and IC15 for each drugs respectively. 82](#_Toc139883866)

[**Table S9 Drug concentrations used in 3rd iteration for 8 drug combinations at 2 levels (0 and 2).** 83](#_Toc139883867)

[**Table S10 Estimate and significance of standardized second order polynomial linear regression coefficients of migration assay of 3rd iteration for 8 drugs at 2 levels.** ns, \*, \*\*, \*\*\*, and \*\*\*\* indicates p > 0.05, p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, and p ≤ 0.0001, respectively. Number of observations: 37. Root Mean Squared Error: 0.318. R-squared: 0.938. Adjusted R-Squared: 0.899. 84](#_Toc139883868)

[**Table S11 Estimate and significance of standardized second order polynomial linear regression coefficients of proliferation assay of 3rd iteration for 8 drugs at 2 levels.** ns, \*, \*\*, \*\*\*, and \*\*\*\* indicates p > 0.05, p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, and p ≤ 0.0001, respectively. Number of observations: 37. Root Mean Squared Error: 0.344. R-squared: 0.951. Adjusted R-Squared: 0.882. 84](#_Toc139883869)

[**Table S12 The result of 4th iteration of 23 combinations for 4 drugs at 3 levels (0, 1, and 2).** The 4 drugs consist of Saracatinib / AZD0530 (Sara), Rocilinostat / ACY-1215 (Roci), PD168393 (PD), and GW9662 (GW). Code 0 and 1 indicate no drugs and IC15 for each drugs respectively. 85](#_Toc139883870)

[**Table S13 Drug concentrations used in 4th iteration for 4 drug combinations at 3 levels (0, 1, and 2).** 86](#_Toc139883871)

[**Table S14 Estimate and significance of standardized second order polynomial linear regression coefficients of migration assay of 4th iteration for 4 drugs at 3 levels.** ns, \*, \*\*, \*\*\*, and \*\*\*\* indicates p > 0.05, p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, and p ≤ 0.0001, respectively. Number of observations: 24. Root Mean Squared Error: 0.385. R-squared: 0.884. Adjusted R-Squared: 0.852. 87](#_Toc139883872)

[**Table S15 Estimate and significance of standardized second order polynomial linear regression coefficients of proliferation assay of 4th iteration for 4 drugs at 3 levels.** ns, \*, \*\*, \*\*\*, and \*\*\*\* indicates p > 0.05, p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, and p ≤ 0.0001, respectively. Number of observations: 24. Root Mean Squared Error: 0.322. R-squared: 0.928. Adjusted R-Squared: 0.896. 87](#_Toc139883873)

[**Table S16 The result of CI validation for 4 drugs at 5 levels (0, 1, 2, 3, 4).** The 4 drugs consist of Saracatinib / AZD0530 (Sara), Rocilinostat / ACY-1215 (Roci), PD168393 (PD), and GW9662 (GW). Code 0, 1, 2, 3, 4, and 5 indicate no drugs, 0.25x, 0.5x, 0.75x, and 1x IC25 for each drugs respectively. 88](#_Toc139883874)

# LIST OF FIGURES

[**Figure 1.1 Economical comparison in drug development** [1]. (A) Molecular target-based approach. (B) Phenotype-based approach. (C) Drug repurpsong with phenotype-based approach. Copyright 2013 The Authors, licensed under a Creative Commons Attribution (CC BY) license. 2](#_Toc139883939)

[**Figure 1.3 Illustration of metastatic cascade** [12]. Copyright 2019 The Authors, licensed under a Creative Commons Attribution (CC BY) license. 5](#_Toc139883940)

[**Figure 1.4 The change of cell markers and phenotype in EMT** [13]. Copyright 2010 The Authors, licensed under a Creative Commons Attribution (CC BY) license. 6](#_Toc139883941)

[**Figure 1.5 Signaling pathway of TNBC** [14]. Copyright 2018 The Authors, licensed under a Creative Commons Attribution (CC BY) license. 6](#_Toc139883942)

[**Figure 1.6 Illustration of interaction between drugs in drug combinations**. Red color, blue color, and purple color represent the response of drug A, drug B, and combination of drug A and drug B in system, respectively. 7](#_Toc139883943)

[**Figure 1.7 Sigmoidal dose response curve be fitted with Hill model**. Adapted from [18]. Copyright 2014 The Authors, licensed under a Creative Commons Attribution (CC BY) license. 8](#_Toc139883944)

[**Figure 1.8 The example result of Chou-Talalay method of synergistic effect analysis** [19]. (A) Fa-CI plot / Chou-Talalay plot. CI < 1, CI = 1, CI > 1 represents antagonistic, additive, and synergistic effect, respectively. (B) Classic isobologram for constant ratio. (C) Normalized isobologram for non-constant ratio. The lower left area and the upper right of represents synergistic and antagonistic effect. (D) Fa-DRI plot / Chou-Martin plot. Higher fold between the dose of single drug compared the dose of drug in drug combination at same response levels represent more favorable dose reduction. (E) Polygonogram of two drug interactions in 5-drug combinations. The straight line and dashed line represent synergistic and antagonistic effect respectively. The thicker the line represent the stronger effect compared to other interaction. The strength of the effect represent the range of combination index for each interaction as explained by the table on the right side. Copyright 2011 The Authors, licensed under a Creative Commons Attribution (CC BY) license. 13](#_Toc139883945)

[**Figure 1.9 The histogram of residuals with six distribution shapes** [24]**.** 16](#_Toc139883946)

[**Figure 1.10 The q-q plot six distribution shapes** [24]**.** 17](#_Toc139883947)

[**Figure 1.11 The spread level plot and scatter plot for four different datasets** [24]**.** 18](#_Toc139883948)

[**Figure 2.2 Experiment workflow** 23](#_Toc139883949)

[**Figure 2.3 Migration Assay.** (A) The procedure. (B) The position of DI water in 96 wells to minimize evaporation. 26](#_Toc139883950)

[**Figure 2.4 Migration and proliferation assay.** The cells need to be washed with medium without serum after completed Hoechst staining and before Hoechst staining after 1 day of drug treatment. 28](#_Toc139883951)

[**Figure 2.5 The result of image analysis of negative control and drug treated cells.** (A)Segmentation of cell migration. (B) Segmentation of cell proliferation. 29](#_Toc139883952)

[**Figure 3.2 Single drug-dose response curve.** The experimental single drug dose-response curve of fifteen drugs used in the study. The data was used to identify the drug concentrations to be used in drug combination study. 33](#_Toc139883953)

[**Figure 3.3 Regression analysis of 1st iteration.** (A) The observed and predicted plot. (B) Residual plot. (C) Normal Q-Q plot. (D) Histogram of residual. 35](#_Toc139883954)

[**Figure 3**.**4 Standardized linear regression coefficients of 1st iteration.** Based on the result of 128 combinations for 12 drug combinations, the estimate and significance of each coefficient were calculated. (A) Coefficients are sorted according to smallest value and significance; (B) Coefficients are grouped according to each individual drug. ns, \*, \*\*, \*\*\*, and \*\*\*\* indicates p > 0.05, p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, and p ≤ 0.0001, respectively. The 12 drugs consist of Gefitinib / ZD1839 (Gef), A-83-01 (A83), BIO, Saracatinib / AZD0530 (Sara), Rocilinostat / ACY-1215 (Roci), PD168393 (PD), CP-673451 (CP), BMS-536924 (BMS), Orantinib / SU6668 (Oran), GW9662 (GW), SCH79797 (SCH), and Cabozantinib malate / XL184 (Cabo). 36](#_Toc139883955)

[**Figure 3.5 Regression analysis of 2nd iteration.** (A) The observed and predicted plot. (B) Residual plot. (C) Normal Q-Q plot. (D) Histogram of residual. 37](#_Toc139883956)

[**Figure 3**.**6 Standardized linear regression coefficients of 2nd iteration.** Based on the result of 90 combinations for 8 drug combinations, the estimate and significance of each coefficient were calculated. Coefficients are sorted according to smallest value and significance. ns, \*, \*\*, \*\*\*, and \*\*\*\* indicates p > 0.05, p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, and p ≤ 0.0001, respectively. The 8 drugs consist of Gefitinib / ZD1839 (Gef), BIO, Saracatinib / AZD0530 (Sara), Rocilinostat / ACY-1215 (Roci), PD168393 (PD), BMS-536924 (BMS), GW9662 (GW), and Cabozantinib malate / XL184 (Cabo). 38](#_Toc139883957)

[**Figure 3.7 Regression analysis of migration assay of 3rd iteration.** (A) The observed and predicted plot. (B) Residual plot. (C) Normal Q-Q plot. (D) Histogram of residual. 39](#_Toc139883958)

[**Figure 3**.**8 Standardized linear regression coefficients of migration assay of 3rd iteration.** Based on the result of 36 combinations for 8 drug combinations, the estimate and significance of each coefficient were calculated. (A) Coefficients are sorted according to smallest value and significance; (B) Coefficients are grouped according to each individual drug. ns, \*, \*\*, \*\*\*, and \*\*\*\* indicates p > 0.05, p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, and p ≤ 0.0001, respectively. The 8 drugs consist of Gefitinib / ZD1839 (Gef), BIO, Saracatinib / AZD0530 (Sara), Rocilinostat / ACY-1215 (Roci), PD168393 (PD), BMS-536924 (BMS), GW9662 (GW), and Cabozantinib malate / XL184 (Cabo). 40](#_Toc139883959)

[**Figure 3.9 Regression analysis of proliferation assay of 3rd iteration.** (A) The observed and predicted plot. (B) Residual plot. (C) Normal Q-Q plot. (D) Histogram of residual. 41](#_Toc139883960)

[**Figure 3.10 Regression analysis of proliferation assay of 3rd iteration after boxcox transformation.** (A) The observed and predicted plot. (B) Residual plot. (C) Normal Q-Q plot. (D) Histogram of residual. 42](#_Toc139883961)

[**Figure 3**.**11 Standardized linear regression coefficients of proliferation assay of 3rd iteration.** Based on the result of 36 combinations for 8 drug combinations, the estimate and significance of each coefficient were calculated. (A) Coefficients are sorted according to smallest value and significance; (B) Coefficients are grouped according to each individual drug. ns, \*, \*\*, \*\*\*, and \*\*\*\* indicates p > 0.05, p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, and p ≤ 0.0001, respectively. The 8 drugs consist of Gefitinib / ZD1839 (Gef), BIO, Saracatinib / AZD0530 (Sara), Rocilinostat / ACY-1215 (Roci), PD168393 (PD), BMS-536924 (BMS), GW9662 (GW), and Cabozantinib malate / XL184 (Cabo). 43](#_Toc139883962)

[**Figure 3.12 Regression analysis of migration assay of 4th iteration.** (A) The observed and predicted plot. (B) Residual plot. (C) Normal Q-Q plot. (D) Histogram of residual. 44](#_Toc139883963)

[**Figure 3**.**13 Standardized linear regression coefficients of migration assay of 4th iteration.** Based on the result of 23 combinations for 4 drug combinations, the estimate and significance of each coefficient were calculated. Coefficients are sorted according to smallest value and significance. ns, \*, \*\*, \*\*\*, and \*\*\*\* indicates p > 0.05, p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, and p ≤ 0.0001, respectively. The 4 drugs consist of Saracatinib / AZD0530 (Sara), Rocilinostat / ACY-1215 (Roci), PD168393 (PD), and GW9662 (GW). 45](#_Toc139883964)

[**Figure 3.14 Regression analysis of proliferation assay of 4th iteration.** (A) The observed and predicted plot. (B) Residual plot. (C) Normal Q-Q plot. (D) Histogram of residual. 46](#_Toc139883965)

[**Figure 3**.**15 Standardized linear regression coefficients of proliferation assay of 4th iteration.** Based on the result of 23 combinations for 4 drug combinations, the estimate and significance of each coefficient were calculated. Coefficients are sorted according to smallest value and significance. ns, \*, \*\*, \*\*\*, and \*\*\*\* indicates p > 0.05, p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, and p ≤ 0.0001, respectively. The 4 drugs consist of Saracatinib / AZD0530 (Sara), Rocilinostat / ACY-1215 (Roci), PD168393 (PD), and GW9662 (GW). 47](#_Toc139883966)

[**Figure 3**.**16 Fa-CI plot and polygonogram of migration assay.** (A) The normal scale for Fa-CI plot. (B) The logarithmic scale for CI axis in Fa-CI plot. (C) Polygonogram. The 4 drugs consist of Saracatinib / AZD0530 (SA), Rocilinostat / ACY-1215 (RO), PD168393 (PD), and GW9662 (GW). 48](#_Toc139883967)

# LIST OF ABBREVIATIONS

2D Two-dimensional

3D Three-dimensional

CI Combination Index

DE Differential Evolution

DRI Dose Reduction Index

EMT Epithelial-Mesenchymal Transition

FBS Fetal Bovine Serum

FSC Feedback System Control

h Hour

HCl Hydrochloric Acid

HG-DMEM High Glucose Dulbecco's Modified Eagle's Medium

NaHCO3 Sodium Bicarbonate

OACD Orthogonal Array Composite Design

P/S Penicillin-Streptomycin

PBS Phosphate Buffered Saline

TNBC Triple Negative Breast Cancer

# LIST OF EQUATIONS

[**Equation 1 Hill model in dose-response curve** [18] 7](#_Toc139883968)

[**Equation 2 Combination Index in Loewe additivity model** 7](#_Toc139883969)

[**Equation 3 Median-effect equation** 8](#_Toc139883970)

[**Equation 4 Combination Index in Chou-Talalay method** 9](#_Toc139883971)

[**Equation 5 Dose Reduction Index (DRI)** 9](#_Toc139883972)

[**Equation 6 Second order linear polynomial** 12](#_Toc139883973)

[**Equation 7 Z-score transformation** 12](#_Toc139883974)

[**Equation 8 Boxcox transformation** 17](#_Toc139883975)

[**Equation 9 Cooks distance** 17](#_Toc139883976)

[**Equation 10 Cell enumeration** 23](#_Toc139883977)

[**Equation 11 Cell seeding** 23](#_Toc139883978)

[**Equation 12 Cell migration** 26](#_Toc139883979)

[**Equation 13 Cell proliferation** 26](#_Toc139883980)

# CHAPTER 1 INTRODUCTION

## 1.1 Background

### 1.1.1 Drug development

The common approach for drug development is by using molecular target-based approach. This approach starts by identifying potential targets like gene, enzyme, receptor and molecular mechanism and selecting molecule or compound which affect our targets. Another approach is based on phenotype-based perspective, focusing on observable characteristic of organism. Some examples of phenotype assay are cell viability, cell secretion, cell infection, cell motility, cytoskeleton arrangement, and neurite outgrowth. Phenotype-based approach starts by developing phenotypic assay which can be translated to human disease and screening potential lead. After the lead was identified by these two approaches, leads need to be optimized and be validated in vivo, in vitro studies, and clinical trial [1].

Compared to target-based approach, phenotypic-based approach has higher predictive ability for translating results from in vivo studies into clinical trials. However, in oncology field the strong transability is difficult to establish due the complex interaction at molecular or organism level. Moreover, late stage cancer are highly heterogeneous and adaptive which possesses big challenge to only rely on single molecular target. Phenotypic-based approach also provided an opportunity of discovering novel target or mechanism of action [2]. In addition to phenotypic-based approach, drug repurposing is an promising option to lowering the development time of drug discovery. **Figure 1.1** show the estimation of combining these two approaches can reduces development time and cost by 75% and 99%, respectively [1]. Drug repurposing use the existing drug to treat disease which currently lack any effective treatments. This approach is useful since the safe dose range, route of administration, pharmacokinetics, and contraindications already been known [3].

A diagram of a procedure

Description automatically generated

**Figure 1.1 Economical comparison in drug development** [1]. (A) Molecular target-based approach. (B) Phenotype-based approach. (C) Drug repurpsong with phenotype-based approach. Copyright 2013 The Authors, licensed under a Creative Commons Attribution (CC BY) license.

### 1.1.2 Breast cancer and treatment

Among many types of cancer, breast cancer is one of the most common types of cancer and leading cause of cancer-induced death. According to GLOBOCAN 2020, it is estimated that there are over 2,000,000 new cases and over 600,000 deaths in 2020 [4]. The main cause of cancer deaths is metastases. The severity of cancer is determined by how far cancer already spread. According to National Cancer Institute, the 5-year survival rate for localized stage, regional stage, and distant stage are 99%, 86%, and 29% respectively [5]. Among all breast cancer types, triple negative breast cancer (TNBC) is the aggressive type which lack of expression of estrogen (ER), progesterone (PR), and human epidermal growth factor receptor-2 (HER2). It accounts of 15-20% of all breast cancer patients under 40 years old and approximately 46% of patients have distant metastasis. TNBC has 40% mortality rate within first 5 year after diagnosis, has recurrence rate after surgery as high as 25%, the mortality rate within 3 months after recurrence as high as 75% [6]. One of the TNBC cell line that commonly used for experiment is MDA-MB-231. MDA-MB-231 was classified as TNBC Basal B which among breast cancer cell line was well-known for its poor prognosis and aggressive nature [7]. Currently, combination chemotherapy regimens which are commonly used to treat TNBC was using taxane (taxel/docetaxel), anthracycline, cyclophosphamide, cisplatin, fluorouracil, adriamycin, epirubicin, and methotrexate. Up to 4 combinations are preferred adjuvant regimens for TNBC [6].

Chemotherapy treatment has several drawbacks: visible side effects like nausea, vomiting, constipation, diarrhea, skin rashes, alopecia, or mucositis; declining in blood cell due to bone marrow injury; gradual loss of cognitive function; severe damage or failure of multiple organ due to long term accumulated toxicity; and poor efficacy may lead to tumor recurrence [6], [8]. To reduce toxicity and improve therapy effectiveness, targeted therapy has already been developed as another option for cancer treatment. Targeted therapy will specifically targeting protein kinase and its receptor, growth factor and its receptor, hormone and its receptor, or immune checkpoints which related with cancer cell growth, survival, or progression [9]. Anti-metastatic / migrastatic drug is one of the cancer drug development strategies which target migration related factor. Cytostatic drug is another strategy which target proliferation related factor. Current trend for drug developing agents are focusing on antiproliferative drug since tumor shrinkage is one of the requirement for regulation. However, the main cause of cancer death comes from metastates, therefore focusing on migrastatic drug is arguably more preferred. The lack of research for migrastatic drug can also be inferred from as average percentage of metastases research in cancer research funding only occupy 5% in average. With the development of proteomic analysis of blood and high resolution imaging technique, the focus of cancer treatment will shifted into the early stage treatment, which will have more benefit with the development of anti-metastatic drug. Another concern related with anti-metastatic drug is the higher frequency of administration of anti-metastatic drug which lead to the need of low toxicity of metastatic drug [10], [11].

***Table 1.1 The percentage for metastatic research funding in various organization*** *[11]****.***

|  |  |  |  |
| --- | --- | --- | --- |
| **Organization and country** | **Period** | **Cancer research funding** | **% Metastasis funding** |
| Deutsche Krebshilfe (German Cancer Aid) | 2007 | 73,150,000 EUR | 4.3 |
| National Cancer Research Institute Partners, UK | 2002 | £ 257,000,000 | 2.9 |
| National Cancer Research Institute Partners, UK | 2006 | £ 391,000,000 | 5.1 |
| Oncosuisse, Swiss Cancer League and Foundation Cancer Research, Switzerland | 2006–2008 | 43,943,750 CHF | 11.6 |
| European Union (FP6) | 2002–2006 | 485,000,000 EUR | 5,0 |
| American Cancer Society, USA | 2009 | 485,000,000 $ | 2.3 |
| Canadian Cancer Research, Alliance | 2006 | 390,200,000 CAD | 6.5 |

### 1.1.3 Metastases and EMT in breast cancer

The processes of metastatic cascade can be divided into 5 major steps as shown in **Figure 1.2**: (1) invasion of basement membrane and cell migration, (2) intravasation into surrounding blood circulation or lymphatic system, (3) survival in circulation as circulating tumor cell to reach distant organ, (4) extravasation into secondary tissue through endothelial barrier, and (5) colonization in metastatic target organ[12]. Among these steps, Epithelial-Mesenchymal Transition (EMT) is one of the important phenomenon in early step of migration.



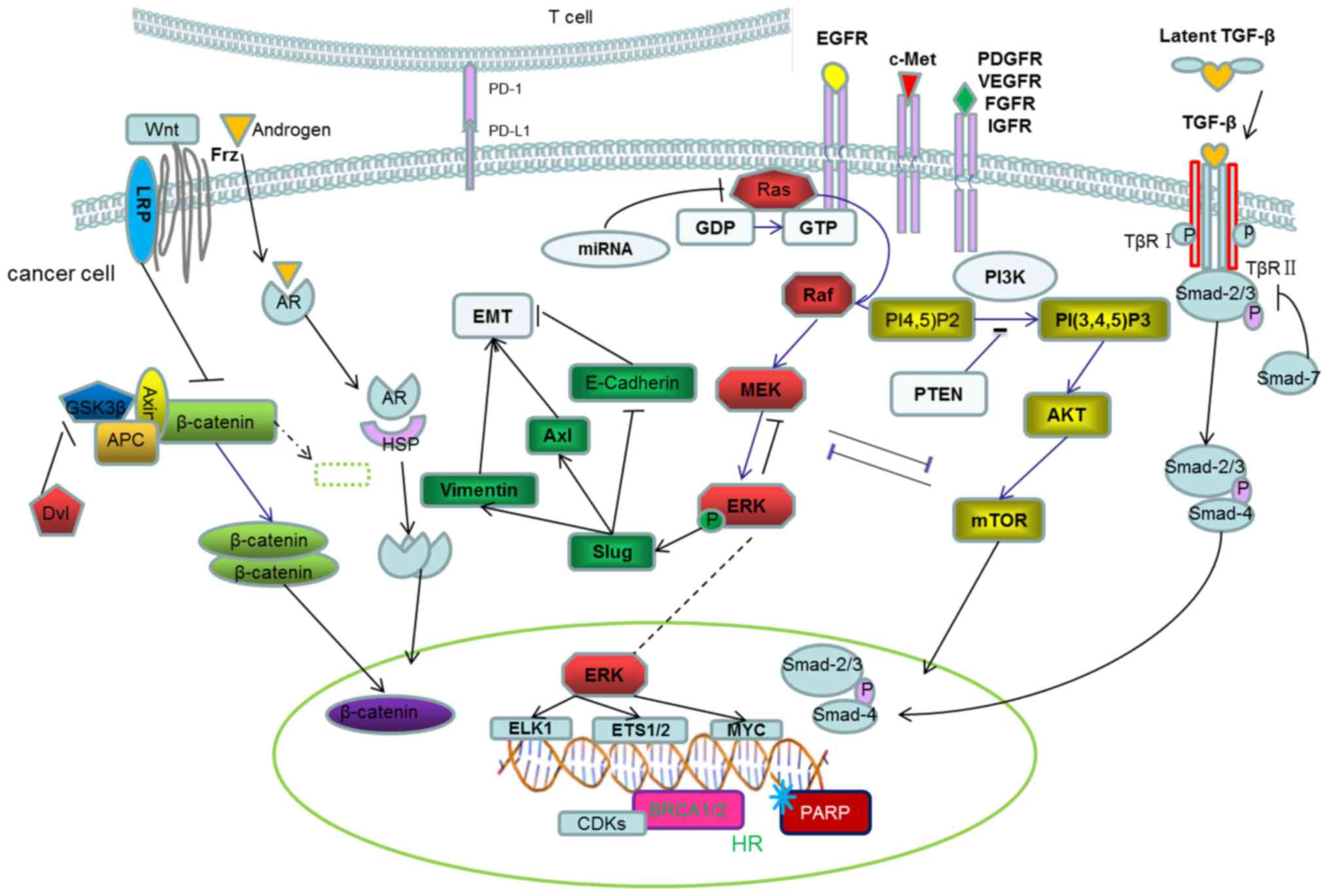
**Figure 1.3 Illustration of metastatic cascade** [12]. Copyright 2019 The Authors, licensed under a Creative Commons Attribution (CC BY) license.

When cells undergo EMT, it will lose epithelial features and acquire mesenchymal feature as shown in **Figure 1.3**. This transition will enhance its migratory capacity, invasiveness, elevated resistance to apoptosis, and greatly increased production of ECM component [13]. This transition is the result of molecular interaction within the cell as the response of extracellular signal which is termed as signaling pathway. There are some signaling pathways related to EMT in triple negative breast cancer. Some receptor tyrosine kinase (RTK) like EGFR, IG1FR, hepactocyte growth factor receptor, c-Met, non-RTK like Src, embryonic transcription factor like Twist and Slug, other signaling pathway like MAPKs, PI3K, nuclear factor κB, Notch, and Wnt/β-catenin. The summary of signaling pathway of TNBC can be seen in **Figure 1.4** [14].

A diagram of a cell

Description automatically generated

**Figure 1.4 The change of cell markers and phenotype in EMT** [13]. Copyright 2010 The Authors, licensed under a Creative Commons Attribution (CC BY) license.



**Figure 1.5 Signaling pathway of TNBC** [14]. Copyright 2018 The Authors, licensed under a Creative Commons Attribution (CC BY) license.

### 1.1.4 Synergistic effect and validation model

Even though in preclinical experiments some targeted drugs show promising result, however a large number of targeted drugs could not provide reproducible improvements in patients when used as single agents. Some tumors might develop resistance by mutation while others might become resistant due having compensatory signaling. Therefore, combining multiple drugs become attractive due more proteins can be targeted at one time and the optimal combination can reduce drug concentration of individual drugs to achieve the same result. This lower concentration can reduce the possibility for cancer to develop resistance and generate lower toxicity. The effect of combining drugs can be classified into synergistic, additive, and antagonistic as illustrated in **Figure 1.5** below. Synergistic effect where drugs as single agents have some antitumor activity, will have greater effect than sum of its parts in combination. Additive effect where combined drugs will have effect equal to sum of effect of individual drugs. Antagonistic effect where combined drugs have lower effect compared to sum of effect of individual drugs [15], [16].

A group of colorful squares

Description automatically generated

**Figure 1.6 Illustration of interaction between drugs in drug combinations**. Red color, blue color, and purple color represent the response of drug A, drug B, and combination of drug A and drug B in system, respectively.

The synergism between drugs can be quantitatively evaluated with several models. There are two common models to validated this synergistic effect which are Bliss independence model and Loewe additivity model. Dose response curve usually has sigmoid shape which can be fit according to Hill model by four parameter logistic nonlinear regression as shown in **Equation 1 and Figure 1.6**. By this reason, Loewe additivity model will be preferred due to the Bliss independence model only be limited to exponential curve.

The Loewe additivity model assumes that dose equivalence principle and sham combination principle. At any response, certain dose of drug A is equivalent with certain dose of drug B which give same effect and these doses can be added together to achieve additivity effect. **Equation 2** describe the relationship between two drugs and calculation for combination index (CI).The model will be assessed by the value of combination index (CI) which resulting from the given equation of each model. The value of CI < 1, CI = 1, CI > 1 represent synergistic, additivity, and antagonistic effect of drugs. This model only focus on the concentration of drug with effect while disregarding dose response curve of single drug and mechanism of interaction between drugs [17].



**Figure 1.7 Sigmoidal dose response curve be fitted with Hill model**. Adapted from [18]. Copyright 2014 The Authors, licensed under a Creative Commons Attribution (CC BY) license.

**Equation 1 Hill model in dose-response curve** [18]

Note:

= dose of the drug

= effect of the drug

*=* the dose of the drug at 50% response

= maximum asymptote of the response (100% after normalization)

= minimum asymptote of the response (0% after normalization)

= the Hill coefficient

**Equation 2 Combination Index in Loewe additivity model**

Note:

= dose of drug A in drug combination

= dose of drug B in drug combination

= dose equivalent of single drug A which have same effect x% as drug combination

*=* dose equivalent of single drug B which have same effect x% as drug combination

Another synergistic analysis also be developed by combining median-effect equation with Loewe additivity model. Median-effect equation as shown in **Equation 3** is the simplest form as part of unified form for the mass-action law in biochemistry and biophysics. From this equation, we can derive Henderson-Hasselbatch equation for PH ionization, Michaelis-Menten equation for substrate saturation, Scatchard equation for receptor binding, and Hill equation for higher order of ligand occupancy. By substituting **Equation 3** to Combination Index in Loewe additivity model (**Equation 2)**, we will get Combination Index in Chou-Talalay method (**Equation 4).** Dose Reduction Index (DRI) is another parameter by measuring how many fold of drug dose is needed to achieve same effect by only using single drug and drug combinations which be expressed in **Equation 5**. Polygonogram are visualization method to see the interaction between two drugs and all drugs in our drug combination study. The range of combination index will be used as criteria to deciding the strength of this interaction. By using this method, there are four plots in total which can be generated as result: Fa-CI plot / Chou-Talalay plot, isobologram, Fa-DRI plot / Chou-Martin plot, and polygonogram. Some example of these result can be seen in **Figure 1.7**.

To facilitate the usage of this method, CompuSyn software already been developed. The drug dose (D) and response (fa) for single drug and drug combinations are required as input. For drug combination, the combination can be done in constant ratio or non-constant ratio. Classic isobologram can be generated for constant ratio while normalized isobologram will be generated for non-constant ratio. The minimum data point that is required for input is two since **Equation 3** can be made into linear in their logarithmic form. This small number of required experiment is useful for the development of low-cost approach of drug development approach [19]**.**

**Equation 3 Median-effect equation**

Note:

= fraction affected by dose

= 1 – = fraction unaffected by dose

= the drug dose

= median effect dose like IC50 and ED50

= the Hill coefficient (m < 1, m = 1, m > 1 represent flat sigmoidal, hyperbolic, and sigmoidal dose response curve)

**Equation 4 Combination Index in Chou-Talalay method**

Note:

= fraction affected by dose

= the dose of drug A in drug combination

= the dose of drug B in drug combination

= the median effect dose of drug A (IC50)

= the median effect dose of drug B (IC50)

= the Hill coefficient of drug A

= the Hill coefficient of drug B

**Equation 5 Dose Reduction Index (DRI)**

Note:

= dose equivalent of single drug A which have same effect x% as drug combination

= dose equivalent of single drug B which have same effect x% as drug combination

= dose of drug A in drug combination

= dose of drug B in drug combination

A collage of graphs and diagrams

Description automatically generated

**Figure 1.8 The example result of Chou-Talalay method of synergistic effect analysis** [19]. (A) Fa-CI plot / Chou-Talalay plot. CI < 1, CI = 1, CI > 1 represents antagonistic, additive, and synergistic effect, respectively. (B) Classic isobologram for constant ratio. (C) Normalized isobologram for non-constant ratio. The lower left area and the upper right of represents synergistic and antagonistic effect. (D) Fa-DRI plot / Chou-Martin plot. Higher fold between the dose of single drug compared the dose of drug in drug combination at same response levels represent more favorable dose reduction. (E) Polygonogram of two drug interactions in 5-drug combinations. The straight line and dashed line represent synergistic and antagonistic effect respectively. The thicker the line represent the stronger effect compared to other interaction. The strength of the effect represent the range of combination index for each interaction as explained by the table on the right side. Copyright 2011 The Authors, licensed under a Creative Commons Attribution (CC BY) license.

### 1.1.5 Drug optimization model – second order linear regression

Chou-Talalay method already been widely used for analyzing synergistic effect. However to determining the optimum drug dose still poses different challenge. The possibility of combination can be calculated by powering the number of dose levels for each drug by the number of drugs. For example, we have 5 drugs with 6 dose levels for each drug, then there’s 65 = 7,776 possible combinations [20].

The team by Al-Shyoukh, et. al. discover that two order of polynomial regression is sufficient enough to model the viability of A549 cells which treated with 3-drug combinations in 8 dose levels and AG02603 cells which treated with 4-drug combinations in 7 dose levels with only testing 6-15% of all possible combinations [21]. Further studies have demonstrated that drug-dose combination versus drug efficacy landscape surface is smooth. All data points can be further analyzed by using second order of regression model which can be translated into interaction between two drugs [22]. Another confirmation also comes from the team by Ding, et. al. which analyze the result of four different experiments. The R2 value of the experiments are 0.74 - 0.96. The value as low as 0.74 for biological assay arguably still acceptable due the large variance in experimental batch-to-batch. They introduce the elimination of drug candidates by analyzing the regression coefficients. From initial 9 drug candidates, they identified the optimum 3-drugs combination and their synergistic effect to inhibit proliferation of ECRF24 [23].

**Equation 6** shows the model of second order polynomial. Before regression was performed independent and dependent variables need to be standardized because our independent variable which is the drug concentration has different concentration range for each drug. To compare each variable in same scale, the input need to be transformed in similar scale which has mean of 0 and standard deviation of 1 by following **Equation 7** [24]. The positive value of a linear coefficient contributes to the maxima value of the outcome while the negative value of a linear coefficient contributes to the minima value of the outcome.

The multiple regression model can be built from various input variables through series of steps. There are three common steps which have been used are forward selection, backward elimination, and bidirectional elimination. A significance level is set beforehand which usually is 0.05. Before we start the procedure, the correlations between input variables and response variables were calculated by t-test. In forward elimination, the starting model with start with zero coefficient then a regression coefficient which has highest correlation with response variable will be added. In case the regression coefficient has high significance, the coefficient will remain, and the second highest coefficient will be added into model. This process will stop until there are no more statistically significance variables left in the model. In backward selection, all regression coefficients were put into one model and the coefficient with lowest correlation with response variable will be removed. The process will stop until the model only contains statistically significance coefficient. In bidirectional stepwise procedure, the procedure begins with no coefficients in starting model like forward elimination and starting to add the coefficient which has highest correlation with response. The procedure also might consider dropping variables that were previously included. A variable maybe added, removed, and added again in different steps. The process will stop after all variables have been evaluated or there are no more variable which meet the inclusion criteria [25]–[27]. For this combination study, we choose bidirectional elimination as correlations between variables are expected in the model.

**Equation 6 Second order linear polynomial**

Note:

*β*0 = coefficients of the intercept

*βi* = coefficients of the linear (single drug linear effects)

*βii =* coefficients of the quadratic (single drug quadratic effects)

*βij* = coefficients of the bilinear (2-drugs bilinear effect)

*y* = response variable (output)

*xi* , *xj* = independent variables (inputs like drugs dose level)

*ɛ* = error term with a mean close to zero

**Equation 7 Z-score transformation**

Note:

= z-score of

= independent variable

= sample mean of

= sample standard deviation of

The linear regression model has several assumptions to be hold. First, there is the linear relationship between predictor and outcome. Second, the errors follow normal distribution since many statistical analyses rely on assumption of this normality and ensure the confidence limit otherwise will lead to unreliable predictions. Third, homoscedasticity of error which errors in any predictor variable shaving the same variances. Fourth, independence and randomness of error which ensure no relationship between the cause of the error. Fifth, no error for the measurement of predictor. Sixth, there is no extreme influential factor which change the relationship between predictor and outcome.

To hold assumption of normality of errors, the standard residuals need to follow normal standard distribution. There are several shapes of histogram of residual which can be seen in **Figure 1.8.** The normally distributed residual has symmetric bell-shaped curve. In addition to histogram, quantile-quantile plot (q-q plot) also can be used to observe the distribution of residuals. There are several shapes of q-q plots which can be seen in **Figure 1.9**. The normally distributed residual in q-q plot will following the straight line. For identifying homoscedasticity, spread-level plots and scatterplots can be used. Some different shapes of scatterplots can be seen in **Figure 1.10.** The normally distributed residual should have equal distribution in positive and negative residual which focused into the center of the line [24]**.**

A group of gray and black graphs

Description automatically generated

***Figure 1.9 The histogram of residuals with six distribution shapes*** [24]***.***

A graph of different types of lines

Description automatically generated

***Figure 1.10 The q-q plot six distribution shapes*** [24]***.***

A group of black dots with white text

Description automatically generated

***Figure 1.11 The spread level plot and scatter plot for four different datasets*** [24]***.***

If residuals are found to not be distributed normally, another model of regression can be chosen or transforming the variables. Boxcox is one of transformation method that can be used on non-normally distributed data to an approximately normal distribution. Boxcox transformation will be performed on the outcome. **Equation 8** show the calculation of Boxcox transformation. λ is the parameter that determine the type and strength of the transformation. It can be obtain by using maximum likelihood estimation (MLE) as measurement how well transformed data fit to the distribution assumption. Nowadays the value of λ can be automatically computed when using boxcox transformation in statistical software [28].

**Equation 8 Boxcox transformation**

The outlier between the data points can be identified by using Cook’s distance method which can be seen in **Equation 9**. Cook’s distance method will estimate the influence of each observation on the fitted response values. The estimation is related with the sum of the squared differences between predictions made with all original observations and predictions made with excluding the observation in question [29].

**Equation 9 Cooks distance**

Note:

= Cook’s distance

= the fitted response value for observation *j*th

= the fitted response value for observation *j*th, where *i*th observation was excluded

= the number of coefficients in the regression model

= mean squared error of the regression model

### 1.1.6 Drug optimization model – feedback system control

There are two methods which be developed to be used in the same conjunction with second order polynomial linear regression. The first one is Feedback System Control Method which using differential evolution (DE) algorithm as refining algorithm. Differential evolution is a popular population-based, parallel search, stochastic algorithm that already been used to solve problem in engineering field. This random behavior will prevent algorithm to be trapped in a local optimum, increasing the chance to obtain global optimum, avoid uncertainty or variability, and fit to be used on non-linear functions. It requires only three control variables which is number of population (Np), scaling factor (F), and crossover rate (C), easy to implement, robust, and can converge in high accuracy and speed. Like genetic algorithms, it also been inspired by natural evolution which adding mutation, cross over, and selection in its algorithm [30]–[32]. DE algorithm already been used in cell viability assay, migration assay, apoptosis assay, in vivo tumor inhibition assay [33], antiviral assay [34], luciferase assay [35] and osteogenic differentiation [36] . The research by Weiss, et. al., show that synergistic effect can be observed in 3-drug combinations in viability test [33]. The research by Ding, et. al., showed that the drug combination can lower required dose of each drug to achieve the same result [34].

### 1.1.7 Drug optimization model – Orthogonal Array Composite Design

Even though FSC already able to reduce the number of experiments to obtain optimal drug combinations, long time of iteration and repeated combinations still be able to be optimized. **Table 1.2** contains the experimental design which proposed by Xu, et. al. to overcome this challenge. This design called as “Orthogonal Array Composite Design” (OACD) as it is composed of 3-level fractional factorial and 2-level Orthogonal Array (OA) with minimum aberration criterion for maximum resolution. The OACD can be used in single experiment or sequential experiments. The first two level factorial can be used for factor screening to identify the factor with most influence with response. The next three level OA can be used for surface response modeling to optimize the relationship between factors and response [37]. The OACD already been used on antiviral assay [38], [39] and cell viability [40], [41].

**Table 1.2 OACD for 3-10 factors**

A picture containing text, screenshot, number, font

Description automatically generated

## 1.2 Critical Issues and Specific Aims

### 1.2.1 The Critical Issues

There is smaller interest toward antimigration drug compared to cytostatic drug in drug development. Since the main cause of cancer death is metastases, the study about migration should be one of the main focus on cancer research [10]. With the high number of cases and deaths of breast cancer, it become interesting topic to be focused on [4]. More over, many targeted therapy agents already been identified as an option to limited treatment for TNBC [42]. Second order linear polynomial is one of the promising model to optimize the need of personalized breast cancer treatment which become an interesting topic to begin with [23].

The current research regarding second order linear regression are focus on single objective, for example cell viability [40], [41]. With the need of low toxicity anti-metastatic drug, we want to try increasing the number of objective of the optimization into two which focus on cell migration and cell proliferation [10].

In addition to optimizing drug doses, second order linear regression also provide us the information related with synergistic effect between drugs. Chou-Talalay method is one of the most common method for anaylizing interation between drugs [19]. Since there are no validation for synergistic effect has been used in previous research regarding second linear polynomial model, therefore Chou-Talalay method will be added as comparison and validation to interaction.

### 1.2.2 The Specific Aims

There are research team which successfully identifying 11 EMT inhibitor from a pool of 1300 small inhibitors on MDA-MB-231, a highly invasive metastatic breast cancer, based on the percentage of cell be transformed from mesenchymal into epithelial [43]. This result provide us an opportunity to find the best drug combination for inhibiting EMT in MDA-MB-231. In addition to this pool, 4 EMT inhibitor with different target also be added into initial drug candidates pool for combination study [44]. The first aim of this study is to identify whether the selected drugs are suitable for drug combination study and Chou-Talalay method.

The second aim of this study is to see whether second order linear regression can optimize multiple objective functions. The inhibition of metastases will be the primary goal with minimizing toxicity is the secondary goal.

The third aim of this study is to compare the analysis of synergistic effect of second order linear regression with the analysis of synergistic effect of Chou-Talalay method. Since the second order polynomial only has two-drug interaction, the Chou-Talalay method only be focused on two drug interactions.

# CHAPTER 2 MATERIALS AND METHODS

## 2.1 Experimental workflows

The experiment is carried out by following the workflow which is explained in **Figure 2.1** below.

A black background with blue arrows

Description automatically generated

**Figure 2.2 Experiment workflow**

## 2.2 Drug candidates

There are 15 drugs were used in this experiment. All drugs were dissolved in DMSO in their highest solubility according to manufacture. All drugs, their target, and maximum solubility in DMSO are listed in **Table 2.1**. All drugs with exception of SCH79797 and CP-673451 were purchased from Selleckchem, USA. SCH79797 and CP-673451 were purchased from Chemscene, USA. DMSO was purchased from Sigma-Aldrich, USA.

**Table 2.1 Fifteen drugs candidate**

|  |  |  |  |
| --- | --- | --- | --- |
| **No** | **Name** | **Target** | **Maximum solubility (mM)** |
| 1 | A-83-01 | ALK5-TD, ALK4-TD, ALK7-TD | 49.8 |
| 2 | Gefitinib (ZD1839) | EGFR | 100.7 |
| 3 | PD168393 | EGFR | 197.7 |
| 4 | BIO | GSK3 | 199.3 |
| 5 | Panobinostat (LBH589) | HDAC | 200.3 |
| 6 | Trichostatin A (TSA) | HDAC | 198.4 |
| 7 | CUDC-101 | HDAC, EGFR, HER2 | 92.1 |
| 8 | Rocilinostat (ACY-1215) | HDAC6 | 200.7 |
| 9 | BMS-536924 | IGF-1R/IR | 200.0 |
| 10 | SCH79797 | PAR1 | 134.6 |
| 11 | Orantinib (SU6668) | PDGFR | 199.8 |
| 12 | CP-673451 | PDGFRα/β | 239.5 |
| 13 | GW9662 | PPARγ | 198.8 |
| 14 | Saracatinib (AZD0530) | Src | 184.5 |
| 15 | Cabozantinib malate (XL184) | VEGFR2, Axl | 157.3 |

## 2.3 Cell culture

MDA-MB-231 breast cancer cells were maintained in T-75 cell culture flask with High Glucose Dulbecco's Modified Eagle Medium (HG-DMEM) which supplemented with 10% Fetal Bovine Serum (FBS) and 1% Penicillin-Streptomycin (P/S) 100X Solution. Sodium bicarbonate (NaHCO3) and [Hydrochloric acid](https://www.epa.gov/sites/default/files/2016-09/documents/hydrochloric-acid.pdf) (HCl) were added into medium to adjust the pH of medium at 7.22-7.24. The medium was changed for every 2 days. The cells were incubated in 37oC with 5% CO2.

After cell reached 80-90% confluency, the medium was removed and the cells were washed with 6 mL 1X Phosphate Buffered Saline (PBS). After PBS was removed, 3 mL 0.25% Trypsin was added and the cells were incubated at 37oC for 3 minutes. Then 12 mL HG-DMEM was added to stop trypsinization process and was centrifuged at 1000 rpm in 3 minutes. The supernatant was removed and the 3 mL medium is added into remaining cells.

50 μL of cell suspension was stained with 50 μL Trypan Blue (Corning, USA). Then 10 μL of mixture was added into single chamber of hemocytometer. The number of cells were counted under 4x microscope objective with CKX53 Inverted Microscope (Olympus, Japan). The number of cell in cell suspension can be calculated by using **Equation 10**.

**Equation 10 Cell enumeration**

After cell enumeration, the required cell to seed for the next experiment can be calculated by following **Equation 11**.

**Equation 11 Cell seeding**

MDA-MB-231 was purchased from Bioresource Collection and Research Center (BCRC, Hsinchu, Taiwan). HG-DMEM, and FBS were purchased from Gibco, USA. Trypsin 2.5% and P/S were purchased from Corning, USA. NaHCO3 was purchased from Sigma-Aldrich, USA. HCl was purchased from Honeywell, Germany. PBS 10X was purchased from GeneDireX, USA.

## 2.4 Spot migration assay

For every first and last for each row and column and every gap between each well of 96 well plate need to be filled with 100 μL deionized water to reduce the water evaporation of cell droplets. In one plate, there will be at least one negative control, which is our drug vehicle (0.1% DMSO in medium). For each parameter, the experiment is done triplicate. One μL droplet of cell suspension was dispensed in the center for each well in 96 well plates, then the cells were incubated for 1 hour. After that, 100 μL medium was added into well and the cells were further incubated for next 4 hours. Next, the medium got replaced with the drug solution and negative control. After medium replacement with drug solution and negative control, the image of cell colony for each well was obtained under 4x microscope objective with IX83 Inverted Microscope (Olympus, Japan) at 0 and 24 hours (**Figure 2.2**).

A screenshot of a game

Description automatically generated with medium confidence

**Figure 2.3 Migration Assay.** (A) The procedure. (B) The position of DI water in 96 wells to minimize evaporation.

## 2.5 Single drug dose response

The migration assay for MDA-MB-231 usually be carried out with 0.1% DMSO [45], [46]. For single drug dose response curve, first all drugs were diluted with HG-DMEM 1,000 times, so the concentration of DMSO reach 0.1%. This concentration was set to be the highest concentration that will be used in initial screening. Then 10-fold serial dilution was performed for four times for each drug, which in total all drugs have five data points. With each dilution, the drug solution was vortexed at least 10 seconds to ensure well mixing. In the case of narrow region of slope or unobservable inhibition response, the concentrations were readjusted again accordingly. When the observed single drug dose response curve showed a different shape than sigmoidal shape, more data points will be added within the concentration range to show higher accuracy of the shape of the mentioned drug.

The result was plotted and analyzed by using software GraphPad Prism 8. The result was fitted according to Hill model. The result of drug treatment was normalized with the result of negative control, with minimum value = 1 (indicates no change from 0h) as 0% and maximum value = the result of negative control as 100%. The IC values for each drug were obtained from migration assay.

## 2.6 Initial screening: 2-level fractional factorial design

The purpose of the initial screening of drug combinations was to rapidly reduce number of drugs, therefore the two level orthogonal array was adequate for drug combinations [47]. The design for two level factorial fractional design with minimum aberration can be seen in this reference [48]. The fractional design of experiment was generated by using software Minitab 18.

## 2.7 Orthogonal Array Composite Design (OACD)

The design for OACD can be seen in **Table 1.2** [37]. The design of 2-level fractional factorial portion experiment was generated by using software Minitab 18.

## 2.8 Proliferation assay

Hoechst staining (Invitrogen, USA) was used to stain the nucleus of cell in the cell colony. The staining was conducted while doing spot migration assay. Prior to medium replacement with drug solution and negative control, the medium will be replaced with 100 μL of 0.1 μg/mL Hoechst in medium with serum was added into each well, then the plate was incubated at 37oC in 1 hour. After incubation, the cell was washed with medium without serum briefly three times, and finally medium without serum was dispensed into each well. Since the imaging for the whole 60 wells requires two hours, medium without serum will minimize the cell migration and proliferation due the lack of serum. When the imaging was completed, the medium will be replaced with the drug solution or negative control. For Hoechst staining, DAPI filter was used with exposure time less than 1 second. The signal was imaged under 20x microscope objective with IX83 Inverted Microscope (Olympus, Japan). After 1 day of incubation, the cells will be washed with medium without serum briefly three times and incubated with Hoechst again for 1 hour in medium without serum for the same reason. Then the cells will be washed with medium without serum briefly three times, and medium without serum was dispensed into each well for imaging. The procedure of the proliferation assay can be seen in **Figure 2.3**.

A screenshot of a video game

Description automatically generated with medium confidence

**Figure 2.4 Migration and proliferation assay.** The cells need to be washed with medium without serum after completed Hoechst staining and before Hoechst staining after 1 day of drug treatment.

## 2.9 Image analysis

The image of migration assay and immunofluorescence area of cell will be analyzed by using software Fiji 2.9.0 / Image J 1.53t. Prior the analysis, the measurement need to be rescaled with the real scale in the image. The macro for calculating area of cell colony was adapted from [49] and can be seen in **Code 1**. The macro for cell segmentation and enumeration can be seen in **Code 2**. The result of image analysis can be seen in **Figure 2.4**.

A picture containing text, screenshot

Description automatically generated

**Figure 2.5 The result of image analysis of negative control and drug treated cells.** (A)Segmentation of cell migration. (B) Segmentation of cell proliferation.

The result of analysis was expressed as Cell Migration and Cell Proliferation, which was calculated by using **Equation 12** and **Equation 13**, respectively.

**Equation 12 Cell migration**

**Equation 13 Cell proliferation**

## 2.10 Second order linear regression analysis

The algorithm for linear regression analysis is obtained from [22] and can be seen in **Code 3**. The dose of each drug and the response of the assay was used as input. Prior to regression, both input and output will be standardized by following **Equation 7**. The estimate and significance of each regression coefficient was obtained as the result of linear regression analysis. The regression will be carried out in stepwise procedure.

## 2.11 Combination index of Chou-Talalay method

The experiment for determining combination index according to Chou-Talalay method was done in constant ratio. The IC50 for each drug was required before starting combining drugs. Since the second order polynomial only has the two drugs interaction, only two drug combination is performed for Chou-Talalay method. The drug combination design for two drugs in constant ratio can be seen in **Table 2.3** [50]. Since the data of single drug dose response already been obtained and constrained of time, only two drug combination are performed in this section. The analysis was done by inputing dose and response into software CompuSyn. The Fa-CI plot, Fa-DRI plot, isobologram and polygonogram will be generated as the result of analysis.

**Table 2.3 Drug combinations for combination index of two drugs**

A table with text on it

Description automatically generated

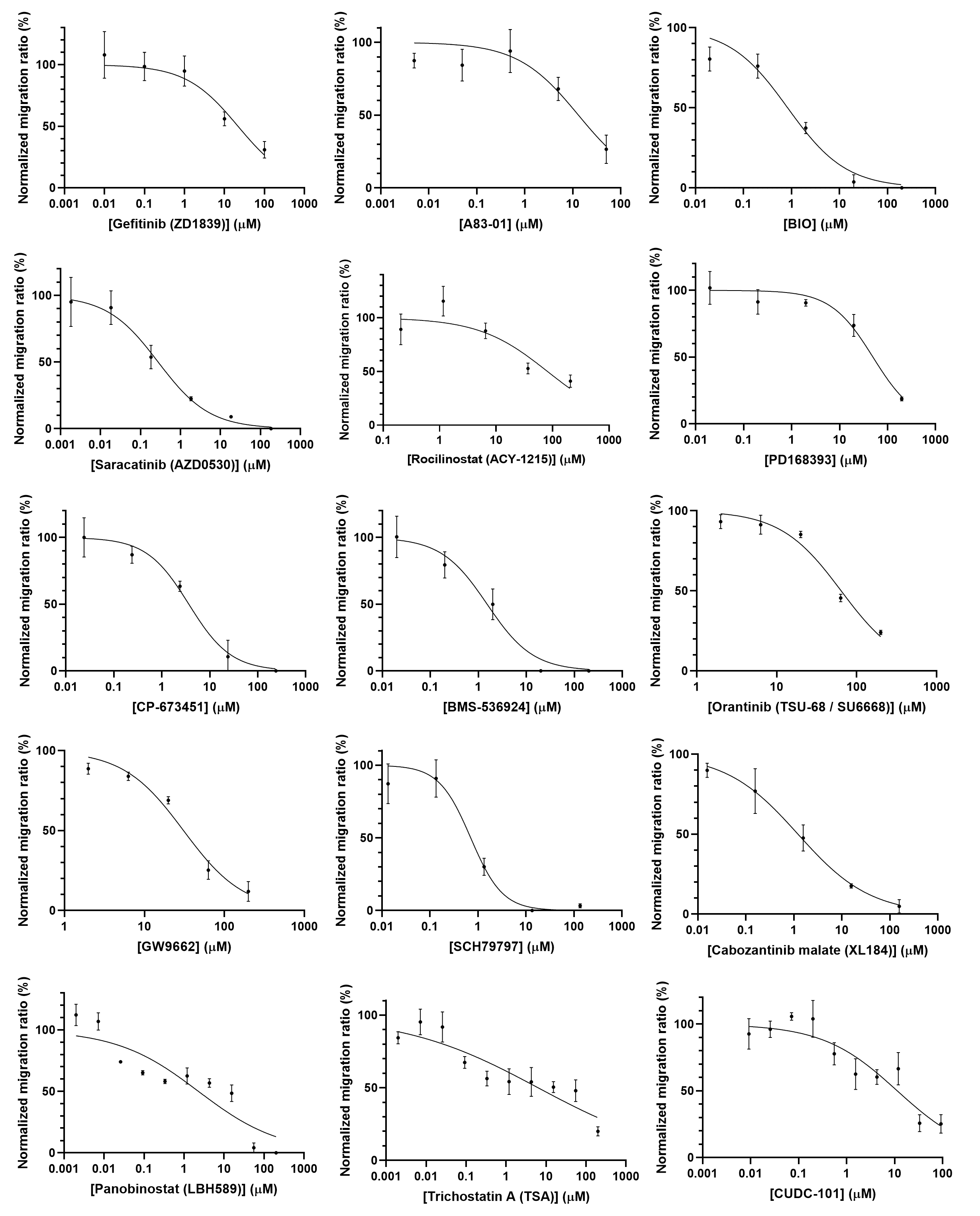
## 2.12 Statistical analysis

The experiment is done in triplicate. One out of three result may be removed if be judged as outlier. The plotted data was presented with mean and standard error of mean. The statistical significance is tested using Tukey’s comparison test and two-sided student’s t-test. The mark of ns, \*, \*\*, \*\*\*,\*\*\*\* indicates the significance level p > 0.05, 0.05 ≥ p ≥ 0.01, 0.01 ≥ p ≥ 0.001, 0.001 ≥ p ≥ 0.0001, 0.0001 ≥ p, respectively. In case of non-normal distribution residual, Boxcox transformation can be applied into outcome by using Matlab software.

# CHAPTER 3 RESULTS

## 3.1 Single drug dose response

The single drug dose response for 15 drugs can be seen in **Figure 3.1** and **Table S1**. Gefitinib, A83-01, BIO, Saracatinib, PD168393, CP-673451, BMS-536924, Orantinib, GW9662, SCH79797, and Cabozantinib malate show the sigmoidal dose-response curve. Rocilinostat shows hormetic dose-response curve. Panobinostat, Trichostatin A, and CUDC-101 show two plateau region in dose-response curve. The IC values for remaining 15 drugs were calculated and summarized in **Table 3.1**.



***Figure 3.2 Single drug-dose response curve.*** *The experimental single drug dose-response curve of fifteen drugs used in the study. The data was used to identify the drug concentrations to be used in drug combination study.*

***Table 3.1 The summary of IC value for 15 drugs****. The single drug response curve data were plotted according to Hill model. The IC values for all drugs were calculated by using GraphPad software.*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Drug** | **Concentration (μM)** | | | |
| **IC10** | **IC15** | **IC25** | **IC50** |
| Gefitinib (ZD1839) | 0.81 | 1.63 | 4.22 | 21.91 |
| A-83-01 | 0.54 | 1.05 | 2.65 | 13.12 |
| BIO | 0.04 | 0.07 | 0.18 | 0.83 |
| Saracatinib (AZD0530) | 0.01 | 0.02 | 0.05 | 0.28 |
| Rocilinostat (ACY-1215) | 3.79 | 7.23 | 17.58 | 81.51 |
| PD168393 | 5.34 | 8.54 | 16.27 | 49.6 |
| CP-673451 | 0.5 | 0.77 | 1.4 | 3.96 |
| BMS-536924 | 0.14 | 0.23 | 0.46 | 1.53 |
| Orantinib (SU6668) | 9.43 | 14.12 | 24.59 | 64.07 |
| GW9662 | 4.59 | 6.86 | 11.9 | 30.87 |
| SCH79797 | 0.13 | 0.19 | 0.31 | 0.71 |
| Cabozantinib malate (XL184) | 0.03 | 0.06 | 0.18 | 1.22 |
| Panobinostat (LBH589) | 0.01 | 0.04 | 0.17 | 2.30 |
| Trichostatin A (TSA) | 0.001 | 0.007 | 0.08 | 6.34 |
| CUDC-101 | 0.23 | 0.52 | 1.61 | 11.36 |

## 3.2 1st iteration: 12 drug 2-level migration assay

For first screening of 12 drug combinations, two level factorial design was chosen with minimum aberration, which are with H = ADE, J = ABCF, K = BDFG, L = CEFG, M = ABCDEG [48]. The drug combinations and the result can be seen in **Table S2**. The fitted value and observed value plot (**Figure 3.2 A**) follow the straight line which imply the fitted model can represent the experimental data. The residual vs fitted plot (**Figure 3.2 B**) was distributed equally in positive and negative value which show the model is not biased. The Q-Q plot (**Figure 3.2 C**) and histogram of residual (**Figure 3.2 D**) also show the residual was distributed normally.

A picture containing text, diagram, line, plot

Description automatically generated

***Figure 3.3 Regression analysis of 1st iteration.*** *(A) The observed and predicted plot. (B) Residual plot. (C) Normal Q-Q plot. (D) Histogram of residual.*

**Figure 3.3** shows the linear regression coefficients from the result sorted with their value and significance. All drugs have negative value for their single drug effect. For the two-drug interaction effect, all of them with exception of PD168393:SCH79797 show the antagonism effect as indicated by their positive value.

A picture containing screenshot, black, design

Description automatically generated

***Figure 3****.****4 Standardized linear regression coefficients of 1st iteration.*** *Based on the result of 128 combinations for 12 drug combinations, the estimate and significance of each coefficient were calculated. (A) Coefficients are sorted according to smallest value and significance; (B) Coefficients are grouped according to each individual drug. ns, \*, \*\*, \*\*\*, and \*\*\*\* indicates p > 0.05, p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, and p ≤ 0.0001, respectively. The 12 drugs consist of Gefitinib / ZD1839 (Gef), A-83-01 (A83), BIO, Saracatinib / AZD0530 (Sara), Rocilinostat / ACY-1215 (Roci), PD168393 (PD), CP-673451 (CP), BMS-536924 (BMS), Orantinib / SU6668 (Oran), GW9662 (GW), SCH79797 (SCH), and Cabozantinib malate / XL184 (Cabo).*

## 3.3 2nd iteration: 8 drug 3-level migration assay

For second screening of 8 drugs, OACD design with resolution V were chosen. The design and generators for 2-level factorial are with G = ABCDE, H = ABCF. For 3-level OA, OA (27) with column 1-8 was chosen [37]. The drug combinations and the result can be seen in **Table S5**. The fitted value and observed value plot (**Figure 3.4 A**) show the fitted model can represent the experimental data. The residual vs fitted plot (**Figure 3.4 B**) also show the model is not biased. The Q-Q plot (**Figure 3.4 C**) and histogram of residual (**Figure 3.4 D**) also show the residual was distributed normally.

A picture containing text, diagram, line, plot

Description automatically generated

***Figure 3.5 Regression analysis of 2nd iteration.*** *(A) The observed and predicted plot. (B) Residual plot. (C) Normal Q-Q plot. (D) Histogram of residual.*

**Figure 3.5** shows the linear regression coefficients from the result sorted with their value and significance. All drugs have negative value for their single linear effect. Cabozantinib, GW9662, BIO, Gefitinib, and Rocilinostat also show their negative value for their single quadratic effect. BIO and Saracatinib have antagonism interaction.



***Figure 3****.****6 Standardized linear regression coefficients of 2nd iteration.*** *Based on the result of 90 combinations for 8 drug combinations, the estimate and significance of each coefficient were calculated. Coefficients are sorted according to smallest value and significance. ns, \*, \*\*, \*\*\*, and \*\*\*\* indicates p > 0.05, p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, and p ≤ 0.0001, respectively. The 8 drugs consist of Gefitinib / ZD1839 (Gef), BIO, Saracatinib / AZD0530 (Sara), Rocilinostat / ACY-1215 (Roci), PD168393 (PD), BMS-536924 (BMS), GW9662 (GW), and Cabozantinib malate / XL184 (Cabo).*

## 3.4 3rd iteration: 8 drugs 2-level migration and proliferation assay

## 3.4.1 8 drugs 2-level migration assay

For third screening of 8 drugs, based on the result of 2nd screening of 8 drugs combination, top 36 drug combination was chosen for migration and proliferation assay. The drug combinations and the result of migration assay can be seen in **Table S8**. The fitted value and observed value plot (**Figure 3.6 A**) show the fitted model can represent the experimental data. The residual vs fitted plot (**Figure 3.6 B**) also show the model is not biased. The Q-Q plot (**Figure 3.6 C**) and histogram of residual (**Figure 3.6 D**) also show the residual was distributed normally.

A collage of graphs and diagrams

Description automatically generated

***Figure 3.7 Regression analysis of migration assay of 3rd iteration.*** *(A) The observed and predicted plot. (B) Residual plot. (C) Normal Q-Q plot. (D) Histogram of residual.*

**Figure 3.7** shows the linear regression coefficients from the result sorted with their value and significance. Rocilinostat, PD168393, BIO, and GW9662 have high significance for their single linear effect. PD168393 show good synergism effect with Saracatinib and Rocilinostat. Gefitinib also show good synergism with BIO. GW9662 show antagonism effect with BIO and Saracatinib.

***Figure 3****.****8 Standardized linear regression coefficients of migration assay of 3rd iteration.*** *Based on the result of 36 combinations for 8 drug combinations, the estimate and significance of each coefficient were calculated. (A) Coefficients are sorted according to smallest value and significance; (B) Coefficients are grouped according to each individual drug. ns, \*, \*\*, \*\*\*, and \*\*\*\* indicates p > 0.05, p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, and p ≤ 0.0001, respectively. The 8 drugs consist of Gefitinib / ZD1839 (Gef), BIO, Saracatinib / AZD0530 (Sara), Rocilinostat / ACY-1215 (Roci), PD168393 (PD), BMS-536924 (BMS), GW9662 (GW), and Cabozantinib malate / XL184 (Cabo).*



**A**

**B**

## 3.4.2 8 drug 2-level proliferation assay

The result of proliferation assay of 3rd iteration can be seen in **Table S8**. The fitted value and observed value plot (**Figure 3.8 A**) show the fitted model can represent the experimental data. The residual vs fitted plot (**Figure 3.8 B**) also show the model is not biased. However the Q-Q plot (**Figure 3.8 C**) and histogram of residual (**Figure 3.8 D**) also show that residual wasn’t distributed normally.

A collage of graphs

Description automatically generated

***Figure 3.9 Regression analysis of proliferation assay of 3rd iteration.*** *(A) The observed and predicted plot. (B) Residual plot. (C) Normal Q-Q plot. (D) Histogram of residual.*

Facing the non-normal distribution of residuals as shown in histogram of residuals. The outcome of proliferation assay will undergo Boxcox transformation before another linear regression again. The fitted value and observed value plot (**Figure 3.9 A**) show the fitted model can represent the experimental data. The residual vs fitted plot (**Figure 3.9 B**) also show the model is not biased. After Boxcox transformation, the Q-Q plot (**Figure 3.9 C**) and histogram of residual (**Figure 3.9 D**) show that residual was distributed normally. The R2 also increased from 0.947 to 0.951 after Boxcox transformation.

A close-up of graphs

Description automatically generated

***Figure 3.10 Regression analysis of proliferation assay of 3rd iteration after boxcox transformation.*** *(A) The observed and predicted plot. (B) Residual plot. (C) Normal Q-Q plot. (D) Histogram of residual.*

**Figure 3.10** shows the linear regression coefficients from the result sorted with their value and significance. Rocilinostat, PD168393, and GW9662 have high significance for their single linear effect. PD168393 show antagonistic effect with BIO, Saracatinib, Rocilinostat, and BMS-536924. GW9662 show synergistic effect with Gefitinib, BIO, Saracatinib, and Rocilinostat. BMS-536924 show antagonistic effect with BIO, Saracatinib, and PD168393. Cabozantinib show antagonistic effect with Gefitinib and BIO.

A black background with white and gray squares

Description automatically generated

***Figure 3****.****11 Standardized linear regression coefficients of proliferation assay of 3rd iteration.*** *Based on the result of 36 combinations for 8 drug combinations, the estimate and significance of each coefficient were calculated. (A) Coefficients are sorted according to smallest value and significance; (B) Coefficients are grouped according to each individual drug. ns, \*, \*\*, \*\*\*, and \*\*\*\* indicates p > 0.05, p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, and p ≤ 0.0001, respectively. The 8 drugs consist of Gefitinib / ZD1839 (Gef), BIO, Saracatinib / AZD0530 (Sara), Rocilinostat / ACY-1215 (Roci), PD168393 (PD), BMS-536924 (BMS), GW9662 (GW), and Cabozantinib malate / XL184 (Cabo).*

## 3.5 4th iteration: 4 drug 3-level migration and proliferation assay

## 3.5.1 4 drug 3-level migration assay

For 4-drug combinations, full factorial design for 2-level and OA (9) with column (1-4) for 3-level was chosen [37]. The drug combinations and the result of migration assay can be seen in **Table S12**. The fitted value and observed value plot (**Figure 3.11 A**) show the fitted model can represent the experimental data. The residual vs fitted plot (**Figure 3.11 B**) also show the model is not biased. The Q-Q plot (**Figure 3.11 C**) and histogram of residual (**Figure 3.11 D**) also show the residual was distributed normally.

A collage of graphs

Description automatically generated

***Figure 3.12 Regression analysis of migration assay of 4th iteration.*** *(A) The observed and predicted plot. (B) Residual plot. (C) Normal Q-Q plot. (D) Histogram of residual.*

**Figure 3.12** shows the linear regression coefficients from the result of migration assay which sorted with their value and significance. PD168393, Rocilinostat, and GW9662 have high significance in their single inhibiting effect. In addition, Rocilinostat has single quadratic effect which stimulate the cell migration.



***Figure 3****.****13 Standardized linear regression coefficients of migration assay of 4th iteration.*** *Based on the result of 23 combinations for 4 drug combinations, the estimate and significance of each coefficient were calculated. Coefficients are sorted according to smallest value and significance. ns, \*, \*\*, \*\*\*, and \*\*\*\* indicates p > 0.05, p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, and p ≤ 0.0001, respectively. The 4 drugs consist of Saracatinib / AZD0530 (Sara), Rocilinostat / ACY-1215 (Roci), PD168393 (PD), and GW9662 (GW).*

## 3.5.2 4 drug 3-level proliferation assay

The result of proliferation assay can be seen in **Table S12**. The fitted value and observed value plot (**Figure 3.12 A**) show the fitted model can represent the experimental data. The residual vs fitted plot (**Figure 3.12 B**) also show the model is not biased. The Q-Q plot (**Figure 3.12 C**) and histogram of residual (**Figure 3.12 D**) also show the residual was distributed normally.

A close-up of several graphs

Description automatically generated

***Figure 3.14 Regression analysis of proliferation assay of 4th iteration.*** *(A) The observed and predicted plot. (B) Residual plot. (C) Normal Q-Q plot. (D) Histogram of residual.*

**Figure 3.13** shows the linear regression coefficients from the result of proliferation assay which sorted with their value and significance. PD168393 and GW9662 have high significance in their single effect. Rocilinostat show the single stimulating linear effect but single inhibiting quadratic effect. There is also the antagonistic effect between PD168393 and Rocilinostat.



***Figure 3****.****15 Standardized linear regression coefficients of proliferation assay of 4th iteration.*** *Based on the result of 23 combinations for 4 drug combinations, the estimate and significance of each coefficient were calculated. Coefficients are sorted according to smallest value and significance. ns, \*, \*\*, \*\*\*, and \*\*\*\* indicates p > 0.05, p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, and p ≤ 0.0001, respectively. The 4 drugs consist of Saracatinib / AZD0530 (Sara), Rocilinostat / ACY-1215 (Roci), PD168393 (PD), and GW9662 (GW).*

## 3.6 Combination Index validation method for migration assay

To investigate 2-drugs interaction between 4 drugs, there are 6 pairwise combination in this experiment which are PD168393 + Rocilinostat, PD168393 + GW9662, PD168393 + Saracatinib, Rocilinostat + GW9662, Rocilinostat + Saracatinib, and GW9662 + Saracatinib. The result of drug study following Chou-Talalay method can be seen in **Table S16**. The dose and response then be added into Compusyn software for calculating Combination Index (CI). The Fa-CI plot and polygonogram can be seen in **Figure 3.15**. Only PD168393 + GW9662 and Rocilinostat + GW9662 has CI < 1 which indicates synergism. Based on polygonogram, only the interaction of GW9662 with Rocilinostat and PD168393 are synergistic effect while other two drug interaction are antagonistic.

A diagram of a graph

Description automatically generated

***Figure 3****.****16 Fa-CI plot and polygonogram of migration assay.*** *(A) The normal scale for Fa-CI plot. (B) The logarithmic scale for CI axis in Fa-CI plot. (C) Polygonogram. The 4 drugs consist of Saracatinib / AZD0530 (SA), Rocilinostat / ACY-1215 (RO), PD168393 (PD), and GW9662 (GW).*

# 

# CHAPTER 4 DISCUSSION

## 4.1 Single drug dose response

For drug combination study, we need particular IC value for each drug. For example in combination of 10 drugs in migration assay, the response of maximum concentration for each drugs need to have 10% inhibition and in 5 drugs combination, the maximum concentration for each drugs should have 20% inhibition. Hence the drug dose response which have plateau region is less suitable for this study. In addition, Chou-Talalay method also require the single drug response to have sigmoidal or hyperbolic curve. By following these reasons, the drugs which have two plateau region dose-response curve will be removed from our next iteration which are Panobinostat, Trichostatin A, and CUDC-101.

## 4.2 1st iteration: 12 drug 2-level migration assay

From the result of the analysis in **Figure 3.3**, A-83-01 can be removed due to its effect is not significant and do not showing any interaction with any drugs. Orantinib can be removed from our list since both its single drug effect is not significant and its interaction with GW9662 showing antagonism effect. CP-673451 can be removed due to antagonism effect with BIO, Gefitinib, SCH79797, and PD168393. SCH79797 even though has synergism effect with PD168393 however it also similar antagonism effect with Rocilinostat, therefore it also can be removed. BIO, GW9662, Cabozantinib, and Gefitinib even though have stimulating effect by interacting with the remaining drugs, but their single drug effect has higher inhibiting effect. With the removal of 4 drugs, PD168393 and Rocilinostat only have single inhibiting effect. Saracatinib and BMS-536924 only show their single inhibiting effect. In summary, 4 drugs (A-83-01, Orantinib, CP-673451, and SCH79797) were removed for next iteration.

## 4.3 2nd iteration: 8 drug 3-level migration assay

From the result from the analysis in **Figure 3.5**, the only observed interaction between drugs is antagonism interaction between BIO and Saracatinib. Since in migration aspect, no longer further elimination can be done by using regression coefficient. The next iteration will include the proliferation aspect to be explored in 8 drug combinations.

## 4.4 3rd iteration: 8 drug 2-level migration and proliferation assay

In this study, the interactions between drugs are the main focus. Since the synergistic effect will suggest lower required dose to get similar response which can also lead to lower toxicity. The summary of the single and bilinear effect from both migration assay and proliferation assay in **Figure 3.7** and **Figure 3.10**, canbe seen in **Table 4.1**. By focusing on the synergistic effect, some potential drug combinations that we identified are PD168393 + Saracatinib + Rocilinostat + GW9662 and BIO + Gefitinib + GW9662 + Cabozantinib. In the 4th iteration, we are going to explore the first 4 set of combinations.

When we try to compare the result of regression of migration assay in 2nd iteration (**Figure 3.5**) and 3rd iteration (**Figure 3.7**), some interaction between drugs are observable in the 3rd iteration while only one interaction are observable in the 2nd iteration. This difference maybe caused by the difference of dose level that is used in experiment. In 3rd iteration 2 dose level was used and in 2nd iteration 3 dose level was used. When we using 3 level, we can use model with single linear (), bilinear (), and single quadratic effect () but in 2 level, we only has model with single linear () and bilinear () effect [37]. The limitation of regression coefficients in the model maybe the cause that single quadratic effect (Cabozantinib^2, GW9662^2, BIO^2, Gefitinib^2, Rocilinostat^2) more observable in 2nd iteration which use 3 level and bilinear effect (Saracatinib:PD168393, Rocilinostat: PD168393, Gefitinib:BIO, BIO:GW9662, Saracatinib:GW9662) more observable in 3rd iteration which use 2 level.

**Table 4.1 The summary of single effect and 2-drug interaction effect of migration assay and proliferation assay of 3rd iteration**.

|  |  |  |
| --- | --- | --- |
| **Regression coefficient** | **Single linear and bilinear effect** | |
| **Migration Assay** | **Proliferation Assay** |
| BIO | + |  |
| BIO:Gefitinib | + |  |
| PD168393 | + | - |
| PD168393:BIO |  | - |
| PD168393: Saracatinib | + | - |
| PD168393:Rocilinostat | + | - |
| PD168393:BMS-536924 |  | - |
| GW9662 | + | - |
| GW9662: Gefitinib |  | + |
| GW9662:BIO | - | + |
| GW9662: Saracatinib | - | + |
| GW9662: Rocilinostat |  | + |
| Cabozantinib:Gefitinib |  | + |
| Cabozantinib:BIO |  | + |
| BMS-536924:BIO |  | - |
| BMS-536924:Saracatinib |  | - |
| BMS-536924: PD168393 |  | - |
| Rocilinostat | + | - |

**Note**: + and – indicates synergistic and antagonistic effect

## 4.6 4th iteration: 4 drug 3-level migration and proliferation assay

Most of interaction effect which was observed in 8-drugs combination is not observed in 4-drugs combination. Only interaction between PD168393 and Rocilinostat was observed in 4-drugs combination. The gap of the observation may be caused by lack of data in 8 drug combinations, since only 36 of 90 combinations were performed. The second possible reason is that the concentration range from 4 drug combination and 8 drug combination also different. In 8 drug combination, the dose that is used are for each drug are its IC15 and half of IC15 while in 4 drug combination, the dose that is used for each drug are its IC25 and half of IC25. This difference of ratio between 2 drugs may influence the synergistic effect between 2 drugs. There is a research group who found that by combining Doxorubicin and Paclitaxel by 5:1 and 3:3 ratio showing the synergistic effect while 1:5 ratio showing antagonistic effect in cytoxicity assay with B16 and 4T1 tumor cells [51].

## 4.6 The linear regression analysis

R2 value for each model that is generated in result is in range from 0.877 to 0.951 which suggest that our model can be used to reflect the result of our experiment. Due to high variation between experimental batch-to-batch and various controlled factor in biological assay, 0.7-0.8 still considered as acceptable range for R2 [23].

## 4.7 Combination Index validation method

The result of combination index suggesting that GW9662 has synergistic effect with Rocilinostat and PD168393. However these interaction is not observable in the model of 8 drug combination nor the model of 4 drug combination. There several possible reasons are might cause this problem. The first reason is single drug dose response for each drug also need to be performed while in this study we only use the result from prior experiment as the replacement. The second reason are due the lack of datapoints to have proper 8 drug combination. The third reason are the different ratio for synergistic effect.

# CHAPTER 5 CONCLUSION

The current experiment already identify twelve drugs which are suitable for drug combination study and Chou-Talalay method in migration assay for MDA-MB-231: A-83-01, Gefitinib (ZD1839), PD168393, BIO, Panobinostat (LBH589), Rocilinostat (ACY-1215), BMS-536924, Orantinib (SU6668), CP-673451, GW9662, Saracatinib (AZD0530), Cabozantinib malate (XL184). The selection of the drugs was based on the shape of the single drug response curve which most of them have sigmoidal shape.

Multiple objective optimization already been performed for maximizing inhibition and minimizing toxicity. Currently there are 2 set candidate for 4-drug combination based on multiple objective optimization: PD168393 + Saracatinib + Rocilinostat + GW9662 and BIO + Gefitinib + GW9662 + Cabozantinib. However the model that been used still not adequate enough due the lack of data.

The analysis from Chou-Talalay method is different with the analysis from linear regression which maybe cause that synergistic effect was induced by different drug ratio.

# REFERENCES

[1] W. Zheng, N. Thorne, and J. C. McKew, “Phenotypic screens as a renewed approach for drug discovery.,” *Drug Discov. Today*, vol. 18, no. 21–22, pp. 1067–1073, Nov. 2013, doi: 10.1016/j.drudis.2013.07.001.

[2] J. G. Moffat, F. Vincent, J. A. Lee, J. Eder, and M. Prunotto, “Opportunities and challenges in phenotypic drug discovery: an industry perspective,” *Nat. Rev. Drug Discov.*, vol. 16, no. 8, pp. 531–543, Aug. 2017, doi: 10.1038/nrd.2017.111.

[3] K. Nishimura and K. Takata, “Combination of Drugs and Cell Transplantation: More Beneficial Stem Cell-Based Regenerative Therapies Targeting Neurological Disorders.,” *Int. J. Mol. Sci.*, vol. 22, no. 16, Aug. 2021, doi: 10.3390/ijms22169047.

[4] H. Sung *et al.*, “Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries,” *CA. Cancer J. Clin.*, vol. 71, no. 3, pp. 209–249, May 2021, doi: 10.3322/caac.21660.

[5] National Cancer Institute, “SEER\*Explorer Application: Surveillance, Epidemiology, and End Result Program,” *National Cancer Institute*. https://seer.cancer.gov/statistics-network/explorer/application.html (accessed Jun. 26, 2022).

[6] L. Yin, J.-J. Duan, X.-W. Bian, and S. Yu, “Triple-negative breast cancer molecular subtyping and treatment progress,” *Breast Cancer Res.*, vol. 22, no. 1, p. 61, Jun. 2020, doi: 10.1186/s13058-020-01296-5.

[7] X. Dai, H. Cheng, Z. Bai, and J. Li, “Breast Cancer Cell Line Classification and Its Relevance with Breast Tumor Subtyping.,” *J. Cancer*, vol. 8, no. 16, pp. 3131–3141, 2017, doi: 10.7150/jca.18457.

[8] U. Anand *et al.*, “Cancer chemotherapy and beyond: Current status, drug candidates, associated risks and progress in targeted therapeutics,” *Genes Dis.*, vol. 10, no. 4, pp. 1367–1401, Jul. 2023, doi: 10.1016/j.gendis.2022.02.007.

[9] F. J. Esteva, “Monoclonal Antibodies, Small Molecules, and Vaccines in the Treatment of Breast Cancer,” *The Oncologist*, vol. 9, no. S3, pp. 4–9, Jun. 2004, doi: 10.1634/theoncologist.9-suppl\_3-4.

[10] A. Gandalovičová *et al.*, “Migrastatics-Anti-metastatic and Anti-invasion Drugs: Promises and Challenges.,” *Trends Cancer*, vol. 3, no. 6, pp. 391–406, Jun. 2017, doi: 10.1016/j.trecan.2017.04.008.

[11] J. Sleeman and P. S. Steeg, “Cancer metastasis as a therapeutic target.,” *Eur. J. Cancer Oxf. Engl. 1990*, vol. 46, no. 7, pp. 1177–1180, May 2010, doi: 10.1016/j.ejca.2010.02.039.

[12] L. A. Hapach, J. A. Mosier, W. Wang, and C. A. Reinhart-King, “Engineered models to parse apart the metastatic cascade,” *Npj Precis. Oncol.*, vol. 3, no. 1, p. 20, Aug. 2019, doi: 10.1038/s41698-019-0092-3.

[13] R. Kalluri and R. A. Weinberg, “The basics of epithelial-mesenchymal transition,” *J. Clin. Invest.*, vol. 119, no. 6, pp. 1420–1428, Jun. 2009, doi: 10.1172/JCI39104.

[14] N. Wu *et al.*, “Precision medicine based on tumorigenic signaling pathways for triple‑negative breast cancer (Review),” *Oncol. Lett.*, vol. 16, no. 4, pp. 4984–4996, Oct. 2018, doi: 10.3892/ol.2018.9290.

[15] W. Sun, P. E. Sanderson, and W. Zheng, “Drug combination therapy increases successful drug repositioning,” *Drug Discov. Today*, vol. 21, no. 7, pp. 1189–1195, Jul. 2016, doi: 10.1016/j.drudis.2016.05.015.

[16] J. S. Lopez and U. Banerji, “Combine and conquer: challenges for targeted therapy combinations in early phase trials,” *Nat. Rev. Clin. Oncol.*, vol. 14, no. 1, pp. 57–66, Jan. 2017, doi: 10.1038/nrclinonc.2016.96.

[17] D. Duarte and N. Vale, “Evaluation of synergism in drug combinations and reference models for future orientations in oncology,” *Curr. Res. Pharmacol. Drug Discov.*, vol. 3, p. 100110, Jan. 2022, doi: 10.1016/j.crphar.2022.100110.

[18] A. Beam and A. Motsinger-Reif, “Beyond IC50s: Towards Robust Statistical Methods for in vitro Association Studies,” *J. Pharmacogenomics Pharmacoproteomics*, vol. 5, p. 1000121, Mar. 2014, doi: 10.4172/2153-0645.1000121.

[19] T.-C. Chou, “The mass-action law based algorithm for cost-effective approach for cancer drug discovery and development.,” *Am. J. Cancer Res.*, vol. 1, no. 7, pp. 925–954, 2011.

[20] A. Weiss and P. Nowak-Sliwinska, “Current Trends in Multidrug Optimization: An Alley of Future Successful Treatment of Complex Disorders.,” *SLAS Technol.*, vol. 22, no. 3, pp. 254–275, Jun. 2017, doi: 10.1177/2472630316682338.

[21] I. Al-Shyoukh *et al.*, “Systematic quantitative characterization of cellular responses induced by multiple signals,” *BMC Syst. Biol.*, vol. 5, pp. 88–88, May 2011, doi: 10.1186/1752-0509-5-88.

[22] P. Nowak-Sliwinska *et al.*, “Optimization of drug combinations using Feedback System Control,” *Nat. Protoc.*, vol. 11, no. 2, pp. 302–315, Feb. 2016, doi: 10.1038/nprot.2016.017.

[23] X. Ding *et al.*, “Discovery of a low order drug-cell response surface for applications in personalized medicine,” *Phys. Biol.*, vol. 11, no. 6, p. 065003, Nov. 2014, doi: 10.1088/1478-3975/11/6/065003.

[24] P. Martin, *LINEAR REGRESSION: AN INTRODUCTION TO STATISTICAL MODELS*. SAGE Publications Ltd, 2021.

[25] J. P. Hoffmann, *Linear Regression Models: Application in R*. in Statistics in the Social and Behavorial Science Series. CRC Press, 2022.

[26] S. Chatterjee and A. S. Hadi, *Regression Analysis by Example*, 5th ed. in Wiley Series in Probability and Statistics. USA: John Wiley & Sons, Inc, 2012.

[27] G. Smith, “Step away from stepwise,” *J. Big Data*, vol. 5, no. 1, p. 32, Sep. 2018, doi: 10.1186/s40537-018-0143-6.

[28] R. Sakia, “The Box-Cox Transformation Technique: A Review,” *The Statistician*, vol. 41, Jan. 1992, doi: 10.2307/2348250.

[29] K. J. Keen, *Graphics for Statistics and Data Analysis with R*. in Texts in Statistical Science. USA: Taylor and Francis Group, LLC, 2010.

[30] C.-P. Sun *et al.*, “Integrative systems control approach for reactivating Kaposi’s sarcoma-associated herpesvirus (KSHV) with combinatory drugs,” *Integr. Biol. Quant. Biosci. Nano Macro*, vol. 1, no. 1, pp. 123–130, Jan. 2009, doi: 10.1039/b815225j.

[31] B.-J. Yoon, “Enhanced stochastic optimization algorithm for finding effective multi-target therapeutics,” *BMC Bioinformatics*, vol. 12 Suppl 1, no. Suppl 1, pp. S18–S18, Feb. 2011, doi: 10.1186/1471-2105-12-S1-S18.

[32] J. Ronkkonen, S. Kukkonen, and K. V. Price, “Real-parameter optimization with differential evolution,” in *2005 IEEE Congress on Evolutionary Computation, IEEE CEC 2005. Proceedings*, Oct. 2005, pp. 506-513 Vol.1. doi: 10.1109/CEC.2005.1554725.

[33] A. Weiss *et al.*, “Rapid optimization of drug combinations for the optimal angiostatic treatment of cancer,” *Angiogenesis*, vol. 18, no. 3, pp. 233–244, Jul. 2015, doi: 10.1007/s10456-015-9462-9.

[34] X. Ding, D. J. Sanchez, A. Shahangian, I. Al-Shyoukh, G. Cheng, and C.-M. Ho, “Cascade search for HSV-1 combinatorial drugs with high antiviral efficacy and low toxicity,” *Int. J. Nanomedicine*, vol. 7, pp. 2281–2292, 2012, doi: 10.2147/IJN.S27540.

[35] H. Yu *et al.*, “Optimizing combinations of flavonoids deriving from astragali radix in activating the regulatory element of erythropoietin by a feedback system control scheme,” *Evid.-Based Complement. Altern. Med. ECAM*, vol. 2013, pp. 541436–541436, 2013, doi: 10.1155/2013/541436.

[36] Y. Honda, X. Ding, F. Mussano, A. Wiberg, C. Ho, and I. Nishimura, “Guiding the osteogenic fate of mouse and human mesenchymal stem cells through feedback system control,” *Sci. Rep.*, vol. 3, no. 1, p. 3420, Dec. 2013, doi: 10.1038/srep03420.

[37] H. Xu, J. Jaynes, and X. Ding, “COMBINING TWO-LEVEL AND THREE-LEVEL ORTHOGONAL ARRAYS FOR FACTOR SCREENING AND RESPONSE SURFACE EXPLORATION,” *Stat. Sin.*, vol. 24, no. 1, pp. 269–289, 2014.

[38] X. Ding, H. Xu, C. Hopper, J. Yang, and C.-M. Ho, “Use of Fractional Factorial Designs in Antiviral Drug Studies,” *Qual. Reliab. Eng. Int.*, vol. 29, no. 2, pp. 299–304, Mar. 2013, doi: 10.1002/qre.1308.

[39] A. Silva *et al.*, “Output-driven feedback system control platform optimizes combinatorial therapy of tuberculosis using a macrophage cell culture model.,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 113, no. 15, pp. E2172-2179, Apr. 2016, doi: 10.1073/pnas.1600812113.

[40] M. B. Mohd Abdul Rashid *et al.*, “Identification and Optimization of Combinatorial Glucose Metabolism Inhibitors in Hepatocellular Carcinomas.,” *J. Lab. Autom.*, vol. 20, no. 4, pp. 423–437, Aug. 2015, doi: 10.1177/2211068215579612.

[41] M. B. M. A. Rashid *et al.*, “Optimizing drug combinations against multiple myeloma using a quadratic phenotypic optimization platform (QPOP),” *Sci. Transl. Med.*, vol. 10, no. 453, p. eaan0941, Aug. 2018, doi: 10.1126/scitranslmed.aan0941.

[42] Y. Li, Z. Zhan, X. Yin, S. Fu, and X. Deng, “Targeted Therapeutic Strategies for Triple-Negative Breast Cancer.,” *Front. Oncol.*, vol. 11, p. 731535, 2021, doi: 10.3389/fonc.2021.731535.

[43] G. V. Vijay *et al.*, “GSK3β regulates epithelial-mesenchymal transition and cancer stem cell properties in triple-negative breast cancer,” *Breast Cancer Res.*, vol. 21, no. 1, p. 37, Mar. 2019, doi: 10.1186/s13058-019-1125-0.

[44] K.-N. Chua, W.-J. Sim, V. Racine, S.-Y. Lee, B. C. Goh, and J. P. Thiery, “A cell-based small molecule screening method for identifying inhibitors of epithelial-mesenchymal transition in carcinoma,” *PloS One*, vol. 7, no. 3, pp. e33183–e33183, 2012, doi: 10.1371/journal.pone.0033183.

[45] S. Liu, Y. Dong, Y. Wang, P. Hu, J. Wang, and R. YL. Wang, “Pristimerin exerts antitumor activity against MDA-MB-231 triple-negative breast cancer cells by reversing of epithelial-mesenchymal transition via downregulation of integrin β3,” *Biomed. J.*, vol. 44, no. 6, Supplement 1, pp. S84–S92, Dec. 2021, doi: 10.1016/j.bj.2020.07.004.

[46] C. Luo, Y. Wang, C. Wei, Y. Chen, and Z. Ji, “The anti-migration and anti-invasion effects of Bruceine D in human triple-negative breast cancer MDA-MB-231 cells.,” *Exp. Ther. Med.*, vol. 19, no. 1, pp. 273–279, Jan. 2020, doi: 10.3892/etm.2019.8187.

[47] A. M. Silva Vite, “Feedback System Control: optimizing drug combinations for tuberculosis treatment,” UCLA, 2014. [Online]. Available: https://escholarship.org/uc/item/7123k296

[48] C. F. J. Wu and M. S. Hamada, *Experiments: Planning, Analysis, and Optimization, 3rd Edition*, Third Edition. in Wiley Series in Probability and Statistics. USA: John Wiley & Sons, Inc., 2021.

[49] J. P. Silva Nunes and A. A. Martins Dias, “ImageJ macros for the user-friendly analysis of soft-agar and wound-healing assays,” *BioTechniques*, vol. 62, no. 4, pp. 175–179, 2017, doi: 10.2144/000114535.

[50] A. Elwakeel *et al.*, “Implementation of the Chou-Talalay method for studying the in vitro pharmacodynamic interactions of binary and ternary drug combinations on MDA-MB-231 triple negative breast cancer cells,” *Synergy*, vol. 8, p. 100047, 2019, doi: https://doi.org/10.1016/j.synres.2019.100047.

[51] Y. Liu, J. Fang, Y.-J. Kim, M. K. Wong, and P. Wang, “Codelivery of doxorubicin and paclitaxel by cross-linked multilamellar liposome enables synergistic antitumor activity.,” *Mol. Pharm.*, vol. 11, no. 5, pp. 1651–1661, May 2014, doi: 10.1021/mp5000373.

**Supplementary Tables and Figures**

**Code 1 Cell segmentation of cell migration**

open("C:/Users/yosua/Desktop/Area Calc/B2.jpg");

run("8-bit");

run("Sharpen");

run("Find Edges");

setAutoThreshold("Default dark");

run("Threshold...");

setThreshold(30, 255);

run("Convert to Mask");

// post-processing to fill holes in each cell

run("Options...", "iterations=100 count=5 do=Open stack");

run("Options...", "iterations=5 count=5 do=Fill Holes stack");

//standard attributes to calculate the area of the colony. May vary according the size (0-infinity) of image

run("Analyze Particles...", "size=100000-Infinity show=Outlines display summarize add stack");

//save the result

saveAs("Results", "C:/Users/yosua/Desktop/Area Calc/TEB2 Calc.csv");

saveAs("Jpeg", "C:/Users/yosua/Desktop/Area Calc/TIB2 Calc.jpg");

open("C:/Users/yosua/Desktop/Area Calc/B2.jpg");

run("Sharpen", "stack");

run("From ROI Manager");

run("Overlay Options...", "stroke=yellow width=3 fill=none apply set");

saveAs("Jpeg", "C:/Users/yosua/Desktop/Area Calc/0B2.jpg");

roiManager("Delete");

Table.deleteRows(0, 1000000);

**Code 2 Cell segmentation of cell proliferation**

//initial post-processing, for threshold can be adjust to 3-7 depends on the brightness of picture

open("C:/Users/yosua/Desktop/DAPI/B2.jpg");

run("8-bit");

run("Subtract Background...", "rolling=50");

run("Enhance Contrast...", "saturated=1");

saveAs("Jpeg", "C:/Users/yosua/Desktop/DAPI/0B2.jpg");

setThreshold(3, 255);

setOption("BlackBackground", false);

run("Convert to Mask");

// post-processing to segment cells

run("Watershed");

saveAs("Jpeg", "C:/Users/yosua/Desktop/DAPI/1B2.jpg");

//standard attributes to calculate the area of the colony. May vary according the size (0-infinity) of image

run("Analyze Particles...", "size=50-Infinity show=Outlines display summarize add");

saveAs("Jpeg", "C:/Users/yosua/Desktop/DAPI/2B2.jpg");

**Code 3 Linear Regression**

% Andrea Weiss

% May 2015

%

% Code to performs stepwise linear regression analysis on data

%

% filename: RegressionAnalysis

% datafile: regressionanalysis.mat

% figures: real vs pred\_model1

% regressionanalysis\_alldata

% real vs pred\_model2

% regressionanalysis\_outliersremoved

%

%

% Variables

% x - indepdent variables for the regression model - represents the tested drug combinations

% y - dependent variables for the regression model - represents the response corresponding to each drug combination provided in the matrix x

% N - represents the number of drug combinations tested (rows of 'x')

% D - represents the number of drugs per drug combinations (columns of 'y')

%

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

clear all;

clc;

%Define drug combinations as an MxN matrix

x = input('Input drug combinations tested by pasting them as an NxD matrix enclosed by brackets, []:')

y = input('Input response to drug combinations tested by pasting them as an Nx1 matrix enclosed by brackets, []:')

%%%%%% Zscored the input and output for regression analysis

[xtrans, mi, si] = zscore(x);

[ytrans, mo\_p, so\_p] = zscore(y);

model = LinearModel.stepwise(xtrans, ytrans, 'quadratic')

%%%%%%%%%%%%%%%%%%%%%%%%%%% model anaylsis %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

ypred = predict(model,xtrans);

scatter(ytrans, ypred)

title('observed vs fitted')

saveas(figure, 'real vs pred\_model1','jpg');

subplot(2,2,1)

plotResiduals(model, 'fitted')

title('fitted values vs residuals')

subplot(2,2,2)

plotDiagnostics(model, 'cookd')

title('cooks distance')

subplot (2,2,3)

plotResiduals(model, 'probability')

title('normal probability plot');

subplot (2,2,4)

plotResiduals(model, 'histogram')

title('histogram');

xlabel('residual');

ylabel('relative frequency')

model1ANOVA = anova(model)

%save figure

saveas(figure, 'regressionanalysis\_alldata','jpg');

%%%%%%%%%%%%%%%%%%%%%%%%%%%% remove outliers %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%Defines outlier based on cook's distance - can select to remove all

%outliers with cooks distance greater than three times the average cook's

%distance OR to remove only the data point with the largest cook's

%distance. Defaults setting to remove only the largest outlier.

%defined as value with cook's distance more than 3 times greater than average

outlier = find(model.Diagnostics.CooksDistance > 3\*mean(model.Diagnostics.CooksDistance));

%OR

%Remove only largest outlier based on cooks distance

%outlier = find(model.Diagnostics.CooksDistance == max(model.Diagnostics.CooksDistance));

%make new regression model with outlier(s) removed

model2 = LinearModel.stepwise(xtrans, ytrans, 'quadratic','exclude',outlier)

%%%%%%%%%%%%%%%%%%%%%%%%%%% model anaylsis %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

ypred = predict(model2,xtrans);

scatter(ytrans, ypred)

title('observed vs fitted')

saveas(figure, 'real vs pred\_model2','jpg');

subplot(2,2,1)

plotResiduals(model2, 'fitted')

title('fitted values vs residuals')

subplot(2,2,2)

plotDiagnostics(model2, 'cookd')

title('cooks distance')

subplot (2,2,3)

plotResiduals(model2, 'probability')

title('normal probability plot');

subplot (2,2,4)

plotResiduals(model2, 'histogram')

title('histogram');

xlabel('residual');

ylabel('relative frequency')

model2ANOVA = anova(model2)

%save figure

saveas(figure, 'regressionanalysis\_outliersremoved','jpg');

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% save matlab file %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%save file

save regressionanalysis.mat;

**Table S1 The result of single drug dose response**

|  |  |  |
| --- | --- | --- |
| **Drugs** | **Concentration (μM)** | **Cell migration (fold)** |
| BMS-536924 | 0.02 | 1.523 ± 0.081 |
| BMS-536924 | 0.2 | 1.414 ± 0.051 |
| BMS-536924 | 2 | 1.26 ± 0.06 |
| BMS-536924 | 20 | 0.993 ± 0.002 |
| BMS-536924 | 200 | 0.927 ± 0.044 |
| Rocilinostat (ACY-1215) | 0.21 | 1.464 ± 0.074 |
| Rocilinostat (ACY-1215) | 1.2 | 1.602 ± 0.072 |
| Rocilinostat (ACY-1215) | 6.5 | 1.457 ± 0.038 |
| Rocilinostat (ACY-1215) | 37 | 1.275 ± 0.026 |
| Rocilinostat (ACY-1215) | 207 | 1.213 ± 0.03 |
| Neg | 0.1% DMSO | 1.521 ± 0.035 |
| A-83-01 | 0.005 | 1.5 ± 0.029 |
| A-83-01 | 0.05 | 1.483 ± 0.063 |
| A-83-01 | 0.5 | 1.538 ± 0.085 |
| A-83-01 | 5 | 1.389 ± 0.046 |
| A-83-01 | 50 | 1.152 ± 0.056 |
| SCH79797 | 0.0135 | 1.499 ± 0.078 |
| SCH79797 | 0.135 | 1.52 ± 0.074 |
| SCH79797 | 1.35 | 1.172 ± 0.034 |
| SCH79797 | 13.5 | 0.971 ± 0.013 |
| SCH79797 | 135 | 1.018 ± 0.008 |
| Cabozantinib malate (XL184) | 0.0157 | 1.515 ± 0.025 |
| Cabozantinib malate (XL184) | 0.157 | 1.44 ± 0.08 |
| Cabozantinib malate (XL184) | 1.57 | 1.273 ± 0.047 |
| Cabozantinib malate (XL184) | 15.7 | 1.101 ± 0.008 |
| Cabozantinib malate (XL184) | 157 | 1.022 ± 0.034 |
| Neg | 0.1% DMSO | 1.572 ± 0.049 |
| BIO | 0.02 | 1.397 ± 0.111 |
| BIO | 0.2 | 1.433 ± 0.043 |
| BIO | 2 | 1.213 ± 0.02 |
| BIO | 20 | 1.021 ± 0.026 |
| BIO | 199 | 0.985 ± 0.018 |
| CP-673451 | 0.024 | 1.476 ± 0.199 |
| CP-673451 | 0.24 | 1.509 ± 0.038 |
| CP-673451 | 2.4 | 1.371 ± 0.022 |
| CP-673451 | 24 | 1.036 ± 0.11 |
| CP-673451 | 240 | 0.892 ± 0.035 |
| Neg | 0.1% DMSO | 1.57 ± 0.052 |
| Panobinostat (LBH589) | 0.002 | 1.611 ± 0.047 |
| Panobinostat (LBH589) | 0.007 | 1.582 ± 0.038 |
| Panobinostat (LBH589) | 0.026 | 1.403 ± 0.005 |
| Panobinostat (LBH589) | 0.093 | 1.355 ± 0.01 |
| Panobinostat (LBH589) | 0.334 | 1.316 ± 0.01 |
| Panobinostat (LBH589) | 1.2 | 1.341 ± 0.035 |
| Panobinostat (LBH589) | 4.32 | 1.31 ± 0.019 |
| Panobinostat (LBH589) | 15.5 | 1.264 ± 0.037 |
| Panobinostat (LBH589) | 55.7 | 1.016 ± 0.033 |
| Panobinostat (LBH589) | 200 | 0.93 ± 0.01 |
| Saracatinib (AZD0530) | 0.002 | 1.518 ± 0.1 |
| Saracatinib (AZD0530) | 0.018 | 1.495 ± 0.068 |
| Saracatinib (AZD0530) | 0.184 | 1.293 ± 0.048 |
| Saracatinib (AZD0530) | 1.84 | 1.123 ± 0.008 |
| Saracatinib (AZD0530) | 18.4 | 1.049 ± 0.004 |
| Saracatinib (AZD0530) | 184 | 0.892 ± 0.062 |
| Neg | 0.1% DMSO | 1.545 ± 0.026 |
| Trichostatin A (TSA) | 0.002 | 1.501 ± 0.025 |
| Trichostatin A (TSA) | 0.007 | 1.565 ± 0.053 |
| Trichostatin A (TSA) | 0.026 | 1.544 ± 0.062 |
| Trichostatin A (TSA) | 0.092 | 1.4 ± 0.024 |
| Trichostatin A (TSA) | 0.331 | 1.334 ± 0.03 |
| Trichostatin A (TSA) | 1.19 | 1.321 ± 0.052 |
| Trichostatin A (TSA) | 4.28 | 1.321 ± 0.059 |
| Trichostatin A (TSA) | 15.4 | 1.299 ± 0.023 |
| Trichostatin A (TSA) | 55.2 | 1.285 ± 0.044 |
| Trichostatin A (TSA) | 198 | 1.119 ± 0.018 |
| Neg | 0.1% DMSO | 1.593 ± 0.082 |
| CUDC-101 | 0.009 | 1.488 ± 0.06 |
| CUDC-101 | 0.026 | 1.506 ± 0.032 |
| CUDC-101 | 0.071 | 1.557 ± 0.015 |
| CUDC-101 | 0.199 | 1.547 ± 0.073 |
| CUDC-101 | 0.552 | 1.409 ± 0.043 |
| CUDC-101 | 1.54 | 1.329 ± 0.061 |
| CUDC-101 | 4.28 | 1.318 ± 0.029 |
| CUDC-101 | 11.9 | 1.35 ± 0.064 |
| CUDC-101 | 33.1 | 1.135 ± 0.033 |
| CUDC-101 | 92.1 | 1.133 ± 0.036 |
| Orantinib (SU6668) | 2 | 1.491 ± 0.023 |
| Orantinib (SU6668) | 6.3 | 1.48 ± 0.031 |
| Orantinib (SU6668) | 20 | 1.448 ± 0.011 |
| Orantinib (SU6668) | 63 | 1.24 ± 0.013 |
| Orantinib (SU6668) | 200 | 1.126 ± 0.007 |
| Neg | 0.1% DMSO | 1.526 ± 0.046 |
| Gefitinib (ZD1839) | 0.01 | 1.581 ± 0.102 |
| Gefitinib (ZD1839) | 0.101 | 1.53 ± 0.062 |
| Gefitinib (ZD1839) | 1.01 | 1.51 ± 0.066 |
| Gefitinib (ZD1839) | 10.1 | 1.302 ± 0.031 |
| Gefitinib (ZD1839) | 101 | 1.166 ± 0.036 |
| PD168393 | 0.02 | 1.548 ± 0.066 |
| PD168393 | 0.198 | 1.491 ± 0.05 |
| PD168393 | 1.98 | 1.488 ± 0.013 |
| PD168393 | 19.8 | 1.397 ± 0.044 |
| PD168393 | 198 | 1.101 ± 0.008 |
| GW9662 | 2 | 1.477 ± 0.019 |
| GW9662 | 6.3 | 1.452 ± 0.014 |
| GW9662 | 20 | 1.371 ± 0.012 |
| GW9662 | 63 | 1.136 ± 0.031 |
| GW9662 | 199 | 1.064 ± 0.033 |
| Neg | 0.1% DMSO | 1.538 ± 0.049 |

**Table S2 The result of 1st iteration of 128 combinations with OACD for 12 drugs at 2 levels (0 and 1).** The 12 drugs consist of Gefitinib / ZD1839 (Gef), A83-01 (A83), BIO, Saracatinib / AZD0530 (Sara), Rocilinostat / ACY-1215 (Roci), PD168393 (PD), CP-673451 (CP), BMS-536924 (BMS), Orantinib / SU6668 (Oran), GW9662 (GW), SCH79797 (SCH), and Cabozantinib malate / XL184 (Cabo). Code 0 and 1 indicate no drugs and IC10 for each drugs respectively.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Combo** | **Gef** | **A83** | **BIO** | **Sara** | **Roci** | **PD** | **CP** | **BMS** | **Oran** | **GW** | **SCH** | **Cabo** | **Response** |
| 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1.301 ± 0.058 |
| 2 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1.298 ± 0.064 |
| 3 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1.172 ± 0.032 |
| 4 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1.208 ± 0.041 |
| 5 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1.279 ± 0.036 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1.45 ± 0.054 |
| 7 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1.272 ± 0.05 |
| 8 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1.353 ± 0.045 |
| 9 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1.179 ± 0.049 |
| 10 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1.308 ± 0.014 |
| 11 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1.118 ± 0.007 |
| 12 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1.318 ± 0.035 |
| 13 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1.353 ± 0.015 |
| 14 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1.319 ± 0.008 |
| 15 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1.205 ± 0.016 |
| 16 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1.191 ± 0.003 |
| 17 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1.35 ± 0.044 |
| 18 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1.214 ± 0.022 |
| 19 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1.428 ± 0.023 |
| 20 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1.359 ± 0.057 |
| 21 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1.232 ± 0.005 |
| 22 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1.283 ± 0.03 |
| 23 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1.409 ± 0.066 |
| 24 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1.212 ± 0.041 |
| 25 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1.287 ± 0.042 |
| 26 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.146 ± 0.024 |
| 27 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1.235 ± 0.037 |
| 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1.454 ± 0.075 |
| 29 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1.287 ± 0.062 |
| 30 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1.25 ± 0.025 |
| 31 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1.222 ± 0.008 |
| 32 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1.538 ± 0.053 |
| 33 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1.379 ± 0.038 |
| 34 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1.22 ± 0.018 |
| 35 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1.348 ± 0.025 |
| 36 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1.273 ± 0.058 |
| 37 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1.338 ± 0.052 |
| 38 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1.324 ± 0.042 |
| 39 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1.208 ± 0.036 |
| 40 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1.236 ± 0.075 |
| 41 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1.237 ± 0.027 |
| 42 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1.191 ± 0.033 |
| 43 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1.205 ± 0.02 |
| 44 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1.173 ± 0.007 |
| 45 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1.246 ± 0.028 |
| 46 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1.319 ± 0.069 |
| 47 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1.179 ± 0.033 |
| 48 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1.315 ± 0.017 |
| 49 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1.131 ± 0.023 |
| 50 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1.186 ± 0.025 |
| 51 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1.334 ± 0.054 |
| 52 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1.14 ± 0.019 |
| 53 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1.135 ± 0.023 |
| 54 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1.321 ± 0.018 |
| 55 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1.223 ± 0.029 |
| 56 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1.265 ± 0.015 |
| 57 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1.277 ± 0.006 |
| 58 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1.29 ± 0.002 |
| 59 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1.306 ± 0.044 |
| 60 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1.344 ± 0.038 |
| 61 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1.442 ± 0.05 |
| 62 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1.317 ± 0.071 |
| 63 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1.213 ± 0.028 |
| 64 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1.163 ± 0.019 |
| 65 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1.243 ± 0.037 |
| 66 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1.17 ± 0.01 |
| 67 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1.293 ± 0.02 |
| 68 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1.319 ± 0.051 |
| 69 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1.362 ± 0.026 |
| 70 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1.225 ± 0.01 |
| 71 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1.246 ± 0.024 |
| 72 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1.169 ± 0.009 |
| 73 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1.143 ± 0.025 |
| 74 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1.175 ± 0.019 |
| 75 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1.171 ± 0.033 |
| 76 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1.233 ± 0.033 |
| 77 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1.302 ± 0.048 |
| 78 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1.217 ± 0.051 |
| 79 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1.159 ± 0.043 |
| 80 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1.101 ± 0.035 |
| 81 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1.264 ± 0.038 |
| 82 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1.208 ± 0.041 |
| 83 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1.234 ± 0.063 |
| 84 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1.365 ± 0.031 |
| 85 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1.332 ± 0.02 |
| 86 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1.105 ± 0.005 |
| 87 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1.127 ± 0.021 |
| 88 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1.157 ± 0.024 |
| 89 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1.139 ± 0.005 |
| 90 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1.185 ± 0.016 |
| 91 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1.164 ± 0.025 |
| 92 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1.147 ± 0.022 |
| 93 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1.304 ± 0.036 |
| 94 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1.349 ± 0.041 |
| 95 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1.251 ± 0.02 |
| 96 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1.273 ± 0.018 |
| 97 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1.245 ± 0.034 |
| 98 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1.261 ± 0.024 |
| 99 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1.417 ± 0.063 |
| 100 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1.306 ± 0.101 |
| 101 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1.229 ± 0.058 |
| 102 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1.18 ± 0.027 |
| 103 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1.309 ± 0.034 |
| 104 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1.158 ± 0.027 |
| 105 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1.301 ± 0.044 |
| 106 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1.257 ± 0.014 |
| 107 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1.145 ± 0.006 |
| 108 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1.285 ± 0.018 |
| 109 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1.371 ± 0.014 |
| 110 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1.2 ± 0.012 |
| 111 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1.269 ± 0.012 |
| 112 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1.109 ± 0.011 |
| 113 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1.309 ± 0.01 |
| 114 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1.356 ± 0.025 |
| 115 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1.285 ± 0.024 |
| 116 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1.14 ± 0.006 |
| 117 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1.341 ± 0.023 |
| 118 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1.372 ± 0.035 |
| 119 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1.437 ± 0.09 |
| 120 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1.255 ± 0.012 |
| 121 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1.269 ± 0.019 |
| 122 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1.202 ± 0.014 |
| 123 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1.285 ± 0.021 |
| 124 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1.116 ± 0.022 |
| 125 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1.429 ± 0.057 |
| 126 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1.288 ± 0.046 |
| 127 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1.147 ± 0.033 |
| 128 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1.171 ± 0.033 |
| 129 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.521 ± 0.044 |

***Table S3 Drug concentrations used in 1st iteration for 12 drug combinations at 2 levels (0 and 1).***

|  |  |  |
| --- | --- | --- |
| **Drugs** | **0** | **1** |
| **No drugs** | **IC10 (µM)** |
| Gefitinib (ZD1839) | 0 | 0.81 |
| A-83-01 | 0 | 0.54 |
| BIO | 0 | 0.04 |
| Saracatinib (AZD0530) | 0 | 0.01 |
| Rocilinostat (ACY-1215) | 0 | 3.79 |
| PD168393 | 0 | 5.34 |
| CP-673451 | 0 | 0.50 |
| BMS-536924 | 0 | 0.14 |
| Orantinib (TSU-68 / SU6668) | 0 | 9.43 |
| GW9662 | 0 | 4.59 |
| SCH79797 | 0 | 0.13 |
| Cabozantinib malate (XL184) | 0 | 0.03 |

***Table S4 Estimate and significance of standardized second order polynomial linear regression coefficients of 1st iteration for 12 drugs at 2 levels.*** *ns, \*, \*\*, \*\*\*, and \*\*\*\* indicates p > 0.05, p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, and p ≤ 0.0001, respectively. Number of observations: 129. Root Mean Squared Error: 0.341. R-squared: 0.903. Adjusted R-Squared: 0.884.*

|  |  |  |
| --- | --- | --- |
| **Coefficients** | **Estimate** | ***p* value** |
| (Intercept) | -0.005 | 0.8804 |
| Gefitinib | -0.1 | 0.0012 |
| A-83-01 | -0.055 | 0.0734 |
| BIO | -0.56 | < 0.0001 |
| Saracatinib | -0.152 | < 0.0001 |
| Rocilinostat | -0.388 | < 0.0001 |
| PD168393 | -0.461 | < 0.0001 |
| CP-673451 | -0.068 | 0.0251 |
| BMS-536924 | -0.127 | 0.0001 |
| Orantinib | -0.032 | 0.2864 |
| GW9662 | -0.168 | < 0.0001 |
| SCH79797 | -0.054 | 0.0755 |
| Cabozantinib | -0.12 | 0.0001 |
| Gefitinib:CP-673451 | 0.093 | 0.0027 |
| BIO:CP-673451 | 0.15 | < 0.0001 |
| BIO:GW9662 | 0.102 | 0.0011 |
| BIO:Cabozantinib | 0.064 | 0.0355 |
| Rocilinostat:SCH79797 | 0.074 | 0.0165 |
| PD168393:CP-673451 | 0.072 | 0.0194 |
| PD168393:SCH79797 | -0.084 | 0.0068 |
| CP-673451:SCH79797 | 0.061 | 0.0467 |
| Orantinib:GW9662 | 0.061 | 0.0465 |

***Table S5 The result of 2nd iteration of 90 combinations with OACD for 8 drugs at 3 levels (0, 1, and 2).*** *The 8 drugs consist of Gefitinib / ZD1839 (Gef), BIO, Saracatinib / AZD0530 (Sara), Rocilinostat / ACY-1215 (Roci), PD168393 (PD), BMS-536924 (BMS), GW9662 (GW), and Cabozantinib malate / XL184 (Cabo). Code 0, 1, and 2 indicate no drugs, half of IC15, and IC15 for each drugs respectively.*

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Combo** | **Gef** | **BIO** | **Sara** | **Roci** | **PD** | **BMS** | **GW** | **Cabo** | **Response** |
| 1 | 2 | 2 | 0 | 0 | 2 | 0 | 2 | 2 | 1.215 ± 0.026 |
| 2 | 2 | 2 | 2 | 0 | 0 | 0 | 2 | 0 | 1.199 ± 0.014 |
| 3 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 1.425 ± 0.02 |
| 4 | 0 | 0 | 0 | 0 | 2 | 2 | 2 | 0 | 1.364 ± 0.012 |
| 5 | 2 | 2 | 2 | 0 | 2 | 0 | 0 | 0 | 1.163 ± 0.003 |
| 6 | 2 | 0 | 0 | 0 | 2 | 2 | 0 | 2 | 1.257 ± 0.008 |
| 7 | 2 | 0 | 2 | 2 | 0 | 2 | 2 | 0 | 1.156 ± 0.01 |
| 8 | 2 | 2 | 2 | 2 | 2 | 0 | 2 | 0 | 1.082 ± 0.016 |
| 9 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 1.345 ± 0.023 |
| 10 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1.068 ± 0.012 |
| 11 | 2 | 2 | 0 | 2 | 2 | 0 | 0 | 2 | 1.159 ± 0.016 |
| 12 | 2 | 2 | 2 | 0 | 0 | 2 | 2 | 2 | 1.129 ± 0.009 |
| 13 | 0 | 0 | 0 | 2 | 2 | 2 | 0 | 0 | 1.314 ± 0.018 |
| 14 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 1.255 ± 0.018 |
| 15 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 1.26 ± 0.02 |
| 16 | 0 | 0 | 0 | 2 | 0 | 2 | 2 | 0 | 1.303 ± 0.043 |
| 17 | 0 | 2 | 0 | 0 | 0 | 2 | 2 | 2 | 1.224 ± 0.02 |
| 18 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 1.347 ± 0.028 |
| 19 | 0 | 0 | 2 | 2 | 2 | 0 | 2 | 0 | 1.216 ± 0.019 |
| 20 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 2 | 1.203 ± 0.026 |
| 21 | 0 | 2 | 2 | 0 | 0 | 2 | 0 | 0 | 1.203 ± 0.046 |
| 22 | 0 | 2 | 0 | 0 | 2 | 2 | 0 | 2 | 1.212 ± 0.022 |
| 23 | 0 | 0 | 2 | 0 | 0 | 2 | 2 | 2 | 1.245 ± 0.032 |
| 24 | 2 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 1.288 ± 0.059 |
| 25 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 1.304 ± 0.007 |
| 26 | 2 | 2 | 2 | 0 | 2 | 2 | 0 | 2 | 1.108 ± 0.005 |
| 27 | 2 | 2 | 0 | 2 | 0 | 2 | 2 | 0 | 1.121 ± 0.022 |
| 28 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 2 | 1.326 ± 0.049 |
| 29 | 2 | 2 | 0 | 0 | 2 | 2 | 2 | 0 | 1.161 ± 0.052 |
| 30 | 2 | 0 | 0 | 2 | 2 | 2 | 2 | 2 | 1.168 ± 0.037 |
| 31 | 0 | 0 | 2 | 0 | 2 | 2 | 0 | 2 | 1.211 ± 0.004 |
| 32 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 1.422 ± 0.049 |
| 33 | 2 | 0 | 2 | 0 | 2 | 2 | 2 | 0 | 1.174 ± 0.041 |
| 34 | 2 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 1.141 ± 0.041 |
| 35 | 0 | 2 | 0 | 2 | 2 | 0 | 2 | 0 | 1.173 ± 0.033 |
| 36 | 2 | 2 | 0 | 2 | 0 | 0 | 2 | 2 | 1.141 ± 0.052 |
| 37 | 2 | 0 | 2 | 2 | 2 | 0 | 0 | 2 | 1.138 ± 0.016 |
| 38 | 2 | 2 | 0 | 2 | 2 | 2 | 0 | 0 | 1.103 ± 0.022 |
| 39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1.386 ± 0.018 |
| 40 | 0 | 2 | 2 | 0 | 2 | 2 | 2 | 0 | 1.136 ± 0.02 |
| 41 | 0 | 0 | 2 | 2 | 0 | 2 | 0 | 2 | 1.176 ± 0.034 |
| 42 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 1.228 ± 0.027 |
| 43 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 1.194 ± 0.01 |
| 44 | 0 | 2 | 0 | 2 | 2 | 2 | 2 | 2 | 1.096 ± 0.034 |
| 45 | 0 | 2 | 2 | 0 | 2 | 0 | 2 | 2 | 1.173 ± 0.022 |
| 46 | 0 | 2 | 2 | 2 | 2 | 0 | 0 | 2 | 1.134 ± 0.026 |
| 47 | 0 | 2 | 0 | 2 | 0 | 2 | 0 | 2 | 1.148 ± 0.026 |
| 48 | 2 | 0 | 2 | 0 | 2 | 0 | 2 | 2 | 1.191 ± 0.011 |
| 49 | 2 | 0 | 0 | 2 | 0 | 2 | 0 | 2 | 1.191 ± 0.026 |
| 50 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 1.318 ± 0.035 |
| 51 | 2 | 0 | 2 | 2 | 0 | 0 | 2 | 2 | 1.139 ± 0.014 |
| 52 | 2 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 1.158 ± 0.035 |
| 53 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 2 | 1.317 ± 0.029 |
| 54 | 2 | 2 | 2 | 2 | 0 | 2 | 0 | 2 | 1.072 ± 0.005 |
| 55 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 2 | 1.307 ± 0.043 |
| 56 | 0 | 2 | 2 | 2 | 2 | 2 | 0 | 0 | 1.121 ± 0.045 |
| 57 | 0 | 0 | 2 | 2 | 2 | 2 | 2 | 2 | 1.139 ± 0.005 |
| 58 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1.387 ± 0.017 |
| 59 | 0 | 2 | 2 | 2 | 0 | 0 | 2 | 2 | 1.111 ± 0.005 |
| 60 | 0 | 2 | 2 | 2 | 0 | 2 | 2 | 0 | 1.097 ± 0.004 |
| 61 | 2 | 0 | 0 | 0 | 0 | 2 | 2 | 2 | 1.273 ± 0.045 |
| 62 | 0 | 0 | 2 | 0 | 0 | 0 | 2 | 0 | 1.301 ± 0.017 |
| 63 | 2 | 0 | 0 | 2 | 2 | 0 | 2 | 0 | 1.247 ± 0.018 |
| 64 | 2 | 0 | 2 | 2 | 2 | 2 | 0 | 0 | 1.147 ± 0.011 |
| 65 | 0 | 0 | 1 | 1 | 0 | 2 | 1 | 2 | 1.443 ± 0.036 |
| 66 | 0 | 0 | 2 | 2 | 0 | 1 | 2 | 1 | 1.395 ± 0.029 |
| 67 | 0 | 1 | 0 | 1 | 2 | 1 | 0 | 1 | 1.323 ± 0.056 |
| 68 | 0 | 1 | 1 | 2 | 2 | 0 | 1 | 0 | 1.26 ± 0.027 |
| 69 | 0 | 1 | 2 | 0 | 2 | 2 | 2 | 2 | 1.25 ± 0.033 |
| 70 | 0 | 2 | 0 | 2 | 1 | 2 | 0 | 2 | 1.199 ± 0.023 |
| 71 | 0 | 2 | 1 | 0 | 1 | 1 | 1 | 1 | 1.305 ± 0.064 |
| 72 | 0 | 2 | 2 | 1 | 1 | 0 | 2 | 0 | 1.232 ± 0.012 |
| 73 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1.428 ± 0.043 |
| 74 | 1 | 0 | 1 | 2 | 1 | 0 | 2 | 2 | 1.308 ± 0.061 |
| 75 | 1 | 0 | 2 | 0 | 1 | 2 | 0 | 1 | 1.411 ± 0.037 |
| 76 | 1 | 1 | 0 | 2 | 0 | 2 | 1 | 1 | 1.234 ± 0.05 |
| 77 | 1 | 1 | 1 | 0 | 0 | 1 | 2 | 0 | 1.334 ± 0.045 |
| 78 | 1 | 1 | 2 | 1 | 0 | 0 | 0 | 2 | 1.294 ± 0.043 |
| 79 | 1 | 2 | 0 | 0 | 2 | 0 | 1 | 2 | 1.299 ± 0.055 |
| 80 | 1 | 2 | 1 | 1 | 2 | 2 | 2 | 1 | 1.153 ± 0.011 |
| 81 | 1 | 2 | 2 | 2 | 2 | 1 | 0 | 0 | 1.184 ± 0.029 |
| 82 | 2 | 0 | 0 | 2 | 2 | 2 | 2 | 0 | 1.283 ± 0.059 |
| 83 | 2 | 0 | 1 | 0 | 2 | 1 | 0 | 2 | 1.39 ± 0.06 |
| 84 | 2 | 0 | 2 | 1 | 2 | 0 | 1 | 1 | 1.377 ± 0.085 |
| 85 | 2 | 1 | 0 | 0 | 1 | 0 | 2 | 1 | 1.328 ± 0.043 |
| 86 | 2 | 1 | 1 | 1 | 1 | 2 | 0 | 0 | 1.275 ± 0.063 |
| 87 | 2 | 1 | 2 | 2 | 1 | 1 | 1 | 2 | 1.182 ± 0.027 |
| 88 | 2 | 2 | 0 | 1 | 0 | 1 | 2 | 2 | 1.181 ± 0.028 |
| 89 | 2 | 2 | 1 | 2 | 0 | 0 | 0 | 1 | 1.231 ± 0.065 |
| 90 | 2 | 2 | 2 | 0 | 0 | 2 | 1 | 0 | 1.263 ± 0.022 |
| 91 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.542 ± 0.057 |

***Table S6 Drug concentrations used in 2nd iteration for 8 drug combinations at 3 levels (0, 1, and 2).***

|  |  |  |  |
| --- | --- | --- | --- |
| **Drugs** | **0** | **1** | **2** |
| **No drugs** | **Half of IC15 (µM)** | **IC15 (µM)** |
| Gefitinib (ZD1839) | 0 | 0.81 | 1.63 |
| BIO | 0 | 0.04 | 0.07 |
| Saracatinib (AZD0530) | 0 | 0.01 | 0.02 |
| Rocilinostat (ACY-1215) | 0 | 3.62 | 7.23 |
| PD168393 | 0 | 4.27 | 8.54 |
| BMS-536924 | 0 | 0.11 | 0.23 |
| GW9662 | 0 | 3.43 | 6.86 |
| Cabozantinib malate (XL184) | 0 | 0.03 | 0.06 |

***Table S7 Estimate and significance of standardized second order polynomial linear regression coefficients of 2nd iteration for 8 drugs at 3 levels.*** *ns, \*, \*\*, \*\*\*, and \*\*\*\* indicates p > 0.05, p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, and p ≤ 0.0001, respectively. Number of observations: 91. Root Mean Squared Error: 0.39. R-squared: 0.877. Adjusted R-Squared: 0.848.*

|  |  |  |
| --- | --- | --- |
| **Coefficients** | **Estimate** | **p value** |
| Intercept | 1.68 | < 0.0001 |
| Gefitinib | -0.235 | < 0.0001 |
| BIO | -0.555 | < 0.0001 |
| Saracatinib | -0.308 | < 0.0001 |
| Rocilinostat | -0.35 | < 0.0001 |
| PD168393 | -0.154 | 0.0004 |
| BMS-536924 | -0.222 | < 0.0001 |
| GW9662 | -0.124 | 0.0035 |
| Cabozantinib | -0.176 | < 0.0001 |
| Gefitinib | 0.075 | 0.0725 |
| BIO:Saracatinib | 0.109 | 0.0106 |
| BIO:Cabozantinib | 0.078 | 0.0644 |
| Saracatinib:BMS-536924 | 0.081 | 0.0556 |
| Gefitinib^2 | -0.298 | 0.0326 |
| BIO^2 | -0.311 | 0.0260 |
| Rocilinostat^2 | -0.293 | 0.0340 |
| GW9662^2 | -0.397 | 0.0045 |
| Cabozantinib^2 | -0.4 | 0.0046 |

***Table S8 The result of 3rd iteration of 36 combinations for 8 drugs at 2 levels (0 and 1).*** *The 8 drugs consist of Gefitinib / ZD1839 (Gef), BIO, Saracatinib / AZD0530 (Sara), Rocilinostat / ACY-1215 (Roci), PD168393 (PD), BMS-536924 (BMS), GW9662 (GW), and Cabozantinib malate / XL184 (Cabo). Code 0 and 1 indicate no drugs and IC15 for each drugs respectively.*

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Combo** | **Gef** | **BIO** | **Sara** | **Roci** | **PD** | **BMS** | **GW** | **Cabo** | **Migration Ratio** | **Proliferation Ratio** |
| 1 | 2 | 2 | 0 | 0 | 2 | 0 | 2 | 2 | 1.065 ± 0.009 | 1.157 ± 0.124 |
| 2 | 2 | 2 | 2 | 0 | 0 | 0 | 2 | 0 | 1.17 ± 0.012 | 1.493 ± 0.054 |
| 3 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 1.102 ± 0.019 | 1.407 ± 0.092 |
| 4 | 0 | 0 | 0 | 0 | 2 | 2 | 2 | 0 | 1.142 ± 0.033 | 1.408 ± 0.252 |
| 5 | 2 | 2 | 2 | 0 | 2 | 0 | 0 | 0 | 0.759 ± 0.067 | 0.895 ± 0.108 |
| 6 | 2 | 0 | 0 | 0 | 2 | 2 | 0 | 2 | 0.851 ± 0.05 | 0.809 ± 0.144 |
| 7 | 2 | 0 | 2 | 2 | 0 | 2 | 2 | 0 | 0.976 ± 0.06 | 1.222 ± 0.086 |
| 8 | 2 | 2 | 2 | 2 | 2 | 0 | 2 | 0 | 1.126 ± 0.018 | 1.62 ± 0.018 |
| 9 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 1.18 ± 0.011 | 1.94 ± 0.125 |
| 10 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 0.993 ± 0.014 | 1.061 ± 0.134 |
| 11 | 2 | 2 | 0 | 2 | 2 | 0 | 0 | 2 | 0.939 ± 0.006 | 0.994 ± 0.052 |
| 12 | 2 | 2 | 2 | 0 | 0 | 2 | 2 | 2 | 1.047 ± 0.011 | 1.068 ± 0.158 |
| 13 | 0 | 0 | 0 | 2 | 2 | 2 | 0 | 0 | 1.027 ± 0.023 | 1.194 ± 0.108 |
| 14 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 1.169 ± 0.011 | 1.157 ± 0.052 |
| 15 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0.99 ± 0.023 | 1.409 ± 0.375 |
| 16 | 0 | 0 | 0 | 2 | 0 | 2 | 2 | 0 | 0.874 ± 0.071 | 0.898 ± 0.1 |
| 17 | 0 | 2 | 0 | 0 | 0 | 2 | 2 | 2 | 0.971 ± 0.023 | 1.197 ± 0.111 |
| 18 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 1.016 ± 0.036 | 1.071 ± 0.083 |
| 19 | 0 | 0 | 2 | 2 | 2 | 0 | 2 | 0 | 0.897 ± 0.06 | 0.887 ± 0.068 |
| 20 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 2 | 1.076 ± 0.018 | 0.943 ± 0.131 |
| 21 | 0 | 2 | 2 | 0 | 0 | 2 | 0 | 0 | 1.202 ± 0.049 | 1.387 ± 0.024 |
| 22 | 0 | 2 | 0 | 0 | 2 | 2 | 0 | 2 | 1.177 ± 0.013 | 1.691 ± 0.157 |
| 23 | 0 | 0 | 2 | 0 | 0 | 2 | 2 | 2 | 0.8 ± 0.053 | 0.694 ± 0.054 |
| 24 | 2 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 1.106 ± 0.018 | 1.283 ± 0.018 |
| 25 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 0.814 ± 0.033 | 0.758 ± 0.012 |
| 26 | 2 | 2 | 2 | 0 | 2 | 2 | 0 | 2 | 1.081 ± 0.026 | 1.443 ± 0.068 |
| 27 | 2 | 2 | 0 | 2 | 0 | 2 | 2 | 0 | 1.16 ± 0.023 | 1.093 ± 0.118 |
| 28 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 2 | 1.16 ± 0.032 | 1.313 ± 0.067 |
| 29 | 2 | 2 | 0 | 0 | 2 | 2 | 2 | 0 | 1.086 ± 0.04 | 1.116 ± 0.119 |
| 30 | 2 | 0 | 0 | 2 | 2 | 2 | 2 | 2 | 1.164 ± 0.012 | 1.655 ± 0.106 |
| 31 | 0 | 0 | 2 | 0 | 2 | 2 | 0 | 2 | 0.988 ± 0.011 | 1.276 ± 0.302 |
| 32 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0.931 ± 0.046 | 0.743 ± 0.13 |
| 33 | 2 | 0 | 2 | 0 | 2 | 2 | 2 | 0 | 0.775 ± 0.085 | 0.648 ± 0.146 |
| 34 | 2 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 1.038 ± 0.035 | 1.286 ± 0.055 |
| 35 | 0 | 2 | 0 | 2 | 2 | 0 | 2 | 0 | 1.078 ± 0.021 | 1.608 ± 0.025 |
| 36 | 2 | 2 | 0 | 2 | 0 | 0 | 2 | 2 | 1.016 ± 0.019 | 1.106 ± 0.042 |
| 37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.415 ± 0.042 | 1.64 ± 0.274 |

***Table S9 Drug concentrations used in 3rd iteration for 8 drug combinations at 2 levels (0 and 2).***

|  |  |  |
| --- | --- | --- |
| **Drugs** | **0** | **2** |
| **No drugs** | **IC15 (µM)** |
| Gefitinib (ZD1839) | 0 | 1.63 |
| BIO | 0 | 0.07 |
| Saracatinib (AZD0530) | 0 | 0.02 |
| Rocilinostat (ACY-1215) | 0 | 7.23 |
| PD168393 | 0 | 8.54 |
| BMS-536924 | 0 | 0.23 |
| GW9662 | 0 | 6.86 |
| Cabozantinib malate (XL184) | 0 | 0.06 |

***Table S10 Estimate and significance of standardized second order polynomial linear regression coefficients of migration assay of 3rd iteration for 8 drugs at 2 levels.*** *ns, \*, \*\*, \*\*\*, and \*\*\*\* indicates p > 0.05, p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, and p ≤ 0.0001, respectively. Number of observations: 37. Root Mean Squared Error: 0.318. R-squared: 0.938. Adjusted R-Squared: 0.899.*

|  |  |  |
| --- | --- | --- |
| **Coefficients** | **Estimate** | **p value** |
| Intercept | -0.014 | 0.7939 |
| Gefitinib | -0.071 | 0.2189 |
| BIO | -0.416 | < 0.0001 |
| Saracatinib | -0.094 | 0.1094 |
| Rocilinostat | -0.695 | < 0.0001 |
| PD168393 | -0.457 | < 0.0001 |
| GW9662 | -0.273 | 0.0001 |
| Cabozantinib | -0.076 | 0.1767 |
| Gefitinib:BIO | -0.148 | 0.0229 |
| Gefitinib:GW9662 | 0.123 | 0.0515 |
| Gefitinib:Cabozantinib | 0.113 | 0.0569 |
| BIO:GW9662 | 0.157 | 0.0146 |
| Saracatinib:PD168393 | -0.187 | 0.0051 |
| Saracatinib:GW9662 | 0.165 | 0.017 |
| Rocilinostat:PD168393 | -0.179 | 0.0065 |

***Table S11 Estimate and significance of standardized second order polynomial linear regression coefficients of proliferation assay of 3rd iteration for 8 drugs at 2 levels.*** *ns, \*, \*\*, \*\*\*, and \*\*\*\* indicates p > 0.05, p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, and p ≤ 0.0001, respectively. Number of observations: 37. Root Mean Squared Error: 0.344. R-squared: 0.951. Adjusted R-Squared: 0.882.*

|  |  |  |
| --- | --- | --- |
| **Coefficients** | **Estimate** | **p value** |
| (Intercept) | -0.013 | 0.8249 |
| Gefitinib | -0.03 | 0.6479 |
| BIO | -0.029 | 0.6697 |
| Saracatinib | -0.015 | 0.827 |
| Rocilinostat | -0.421 | < 0.0001 |
| PD168393 | -0.452 | < 0.0001 |
| BMS-536924 | -0.122 | 0.0881 |
| GW9662 | -0.403 | < 0.0001 |
| Cabozantinib | -0.101 | 0.1492 |
| Gefitinib:GW9662 | 0.241 | 0.0138 |
| Gefitinib:Cabozantinib | 0.311 | 0.0084 |
| BIO:Rocilinostat | -0.185 | 0.0652 |
| BIO:PD168393 | -0.426 | 0.0002 |
| BIO:BMS-536924 | -0.218 | 0.0211 |
| BIO:GW9662 | 0.221 | 0.0143 |
| BIO:Cabozantinib | 0.223 | 0.019 |
| Saracatinib:PD168393 | -0.432 | < 0.0001 |
| Saracatinib:BMS-536924 | -0.219 | 0.0483 |
| Saracatinib:GW9662 | 0.354 | 0.0016 |
| Rocilinostat:PD168393 | -0.273 | 0.0078 |
| Rocilinostat:GW9662 | 0.305 | 0.0025 |
| PD168393:BMS-536924 | -0.182 | 0.0114 |

***Table S12 The result of 4th iteration of 23 combinations for 4 drugs at 3 levels (0, 1, and 2).*** *The 4 drugs consist of Saracatinib / AZD0530 (Sara), Rocilinostat / ACY-1215 (Roci), PD168393 (PD), and GW9662 (GW). Code 0 and 1 indicate no drugs and IC15 for each drugs respectively.*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Combo** | **PD** | **BMS** | **GW** | **Cabo** | **Migration Ratio** | **Proliferation Ratio** |
| 1 | 2 | 2 | 2 | 2 | 0.255 ± 0.017 | 0.116 ± 0.036 |
| 2 | 2 | 2 | 2 | 0 | 0.481 ± 0.209 | 0.252 ± 0.105 |
| 3 | 2 | 2 | 0 | 2 | 1.145 ± 0.109 | 1.27 ± 0.227 |
| 4 | 2 | 2 | 0 | 0 | 1.215 ± 0.044 | 1.322 ± 0.091 |
| 5 | 2 | 0 | 2 | 2 | 0.968 ± 0.102 | 0.927 ± 0.131 |
| 6 | 2 | 0 | 2 | 0 | 1.022 ± 0.07 | 0.916 ± 0.044 |
| 7 | 2 | 0 | 0 | 2 | 1.397 ± 0.025 | 1.535 ± 0.161 |
| 8 | 2 | 0 | 0 | 0 | 1.42 ± 0.073 | 1.471 ± 0.022 |
| 9 | 0 | 2 | 2 | 2 | 0.581 ± 0.096 | 0.391 ± 0.094 |
| 10 | 0 | 2 | 2 | 0 | 0.703 ± 0.011 | 0.498 ± 0.043 |
| 11 | 0 | 2 | 0 | 2 | 1.206 ± 0.032 | 1.282 ± 0.194 |
| 12 | 0 | 2 | 0 | 0 | 1.175 ± 0.086 | 1.117 ± 0.145 |
| 13 | 0 | 0 | 2 | 2 | 1.051 ± 0.065 | 0.978 ± 0.087 |
| 14 | 0 | 0 | 2 | 0 | 1.202 ± 0.061 | 1.055 ± 0.054 |
| 15 | 0 | 0 | 0 | 2 | 1.562 ± 0.028 | 1.639 ± 0.119 |
| 16 | 0 | 1 | 1 | 2 | 1.031 ± 0.124 | 0.889 ± 0.171 |
| 17 | 0 | 2 | 2 | 1 | 0.845 ± 0.055 | 0.666 ± 0.012 |
| 18 | 1 | 0 | 1 | 1 | 1.288 ± 0.007 | 1.273 ± 0.054 |
| 19 | 1 | 1 | 2 | 0 | 1.063 ± 0.025 | 0.871 ± 0.091 |
| 20 | 1 | 2 | 0 | 2 | 1.105 ± 0.145 | 1.124 ± 0.017 |
| 21 | 2 | 0 | 2 | 2 | 1.105 ± 0.096 | 1.181 ± 0.089 |
| 22 | 2 | 1 | 0 | 1 | 1.145 ± 0.093 | 1.28 ± 0.13 |
| 23 | 2 | 2 | 1 | 0 | 0.233 ± 0.255 | 0.176 ± 0.187 |
| 24 | 0 | 0 | 0 | 0 | 1.549 ± 0.077 | 1.668 ± 0.287 |

***Table S13 Drug concentrations used in 4th iteration for 4 drug combinations at 3 levels (0, 1, and 2).***

|  |  |  |  |
| --- | --- | --- | --- |
| **Drugs** | **0** | **1** | **2** |
| **No drugs** | **Half of IC25 (µM)** | **IC25 (µM)** |
| GW9662 | 0 | 5.96 | 11.91 |
| PD168393 | 0 | 8.14 | 16.27 |
| Rocilinostat (ACY-1215) | 0 | 8.79 | 17.58 |
| Saracatinib (AZD0530) | 0 | 0.03 | 0.05 |

***Table S14 Estimate and significance of standardized second order polynomial linear regression coefficients of migration assay of 4th iteration for 4 drugs at 3 levels.*** *ns, \*, \*\*, \*\*\*, and \*\*\*\* indicates p > 0.05, p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, and p ≤ 0.0001, respectively. Number of observations: 24. Root Mean Squared Error: 0.385. R-squared: 0.884. Adjusted R-Squared: 0.852.*

|  |  |  |
| --- | --- | --- |
| Coefficients | Estimate | p value |
| (Intercept) | -0.242 | 0.3906 |
| GW9662 | -0.302 | 0.0018 |
| PD168393 | -0.639 | < 0.0001 |
| Rocilinostat | -0.58 | < 0.0001 |
| GW9662^2 | -0.449 | 0.0596 |
| Rocilinostat^2 | 0.702 | 0.0056 |

***Table S15 Estimate and significance of standardized second order polynomial linear regression coefficients of proliferation assay of 4th iteration for 4 drugs at 3 levels.*** *ns, \*, \*\*, \*\*\*, and \*\*\*\* indicates p > 0.05, p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, and p ≤ 0.0001, respectively. Number of observations: 24. Root Mean Squared Error: 0.322. R-squared: 0.928. Adjusted R-Squared: 0.896.*

|  |  |  |
| --- | --- | --- |
| Coefficients | Estimate | *p* value |
| (Intercept) | -0.298 | 0.2163 |
| GW9662 | -0.157 | 0.0338 |
| PD168393 | -0.594 | < 0.0001 |
| Rocilinostat | -0.677 | < 0.0001 |
| Saracatinib | -0.021 | 0.7722 |
| PD168393:Rocilinostat | -0.169 | 0.0265 |
| Rocilinostat^2 | 0.641 | 0.0034 |
| Saracatinib^2 | -0.339 | 0.0926 |

***Table S16 The result of CI validation for 4 drugs at 5 levels (0, 1, 2, 3, 4).*** *The 4 drugs consist of Saracatinib / AZD0530 (Sara), Rocilinostat / ACY-1215 (Roci), PD168393 (PD), and GW9662 (GW). Code 0, 1, 2, 3, 4, and 5 indicate no drugs, 0.25x, 0.5x, 0.75x, and 1x IC25 for each drugs respectively.*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Combo** | **PD** | **BMS** | **GW** | **Cabo** | **Migration Ratio** | **Proliferation Ratio** |
| 1 | 1 | 1 | 0 | 0 | 1.244 ± 0.018 | 1.019 ± 0.067 |
| 2 | 2 | 2 | 0 | 0 | 1.098 ± 0.065 | 0.832 ± 0.044 |
| 3 | 3 | 3 | 0 | 0 | 0.909 ± 0.188 | 0.769 ± 0.094 |
| 4 | 4 | 4 | 0 | 0 | 0.795 ± 0.183 | 0.623 ± 0.157 |
| 5 | 1 | 0 | 1 | 0 | 1.135 ± 0.071 | 0.977 ± 0.069 |
| 6 | 2 | 0 | 2 | 0 | 0.937 ± 0.148 | 0.841 ± 0.005 |
| 7 | 3 | 0 | 3 | 0 | 0.664 ± 0.122 | 0.606 ± 0.162 |
| 8 | 4 | 0 | 4 | 0 | 0.303 ± 0.093 | 0.301 ± 0.092 |
| 9 | 1 | 0 | 0 | 1 | 1.415 ± 0.051 | 1.678 ± 0.246 |
| 10 | 2 | 0 | 0 | 2 | 1.373 ± 0.087 | 1.363 ± 0.209 |
| 11 | 3 | 0 | 0 | 3 | 1.353 ± 0.047 | 1.359 ± 0.117 |
| 12 | 4 | 0 | 0 | 4 | 1.281 ± 0.056 | 1.308 ± 0.029 |
| 13 | 0 | 1 | 1 | 0 | 0.618 ± 0.093 | 0.456 ± 0.129 |
| 14 | 0 | 2 | 2 | 0 | 0 ± 0 | 0.032 ± 0.03 |
| 15 | 0 | 3 | 3 | 0 | 0 ± 0 | 0.014 ± 0.012 |
| 16 | 0 | 4 | 4 | 0 | 0 ± 0 | 0.029 ± 0.026 |
| 17 | 0 | 1 | 0 | 1 | 1.245 ± 0.031 | 1.566 ± 0.11 |
| 18 | 0 | 2 | 0 | 2 | 1.099 ± 0.07 | 1.178 ± 0.041 |
| 19 | 0 | 3 | 0 | 3 | 0.982 ± 0.029 | 1.024 ± 0.095 |
| 20 | 0 | 4 | 0 | 4 | 0.904 ± 0.072 | 1.086 ± 0.048 |
| 21 | 0 | 0 | 1 | 1 | 1.216 ± 0.074 | 1.571 ± 0.112 |
| 22 | 0 | 0 | 2 | 2 | 1.112 ± 0.052 | 1.418 ± 0.172 |
| 23 | 0 | 0 | 3 | 3 | 1.01 ± 0.091 | 1.153 ± 0.113 |
| 24 | 0 | 0 | 4 | 4 | 0.953 ± 0.064 | 0.961 ± 0.105 |
| 25 | 0 | 0 | 0 | 0 | 1.539 ± 0.064 | 1.731 ± 0.219 |