THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOMEDICAL ENGINEERING

Investigation of Dual-Switch Combination Therapy's Efficacy in Drug Resistant Cancer Cells

ZEYU YANG
SPRING 2021

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Biomedical Engineering
with honors in Biomedical Engineering

Reviewed and approved* by the following:

Justin R. Prichard*
Associate Professor of Biomedical Engineering
Thesis Supervisor

Jian Yang*
Professor of Biomedical Engineering
Honors Adviser

* Electronic approvals are on file.

ABSTRACT

Without joining the competition between newly developed target therapy drugs against cancer and acquired resistance for cancer cells, we here address a forward engineering approach that uses the available drugs to create a high dose environment locally in the tumor that solves the drug resistance problem. With proper evolutionary pressure achieved by the target therapy drug, an engineered cell population grows to be the dominating population of a tumor and then self-destructs themselves as well as the surroundings non-discriminatively by turning prodrugs into drugs. In the thesis, three experiments' results have been shown as evidence supporting the efficacy of the strategy.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

When I was a sophomore disturbing the teaching assistants that I met in BME 201, I definitely could not imagine the passion that burst out the first time hearing about the elegant and innovative ways of cancer therapies. Luckily enough, I got into Penn State and the Prichard Lab, which are the best places for me to get important guidance as a hesitative undergraduate student.

It was indeed a hard time for me to do research on this thesis topic due to the pandemic and campus closure, but the lab as a whole did not leave me alone. I first want to express my deep gratitude to Mr. Scott M. Leighow, which is a Ph.D. student in the Prichard Lab. As a direct instructor, he offered extensive help for me to overcome the initial barriers in academic research, from benchwork technique to cancer biology, from experiment design to scientific awareness. Even for this specific thesis, I should definitely give him credit for his great intellectual work that is the founding base of the gene-drive project, as well as the massive experiments he did before I joined the project. It's a great fortune for a noob researcher to always find a person who knows all you want to learn and is willing to deliver his experience in the simplest words.

I also want to sincerely thank Dr. Justin R. Prichard, the beloved PI of the Prichard Lab, one of the most popular instructors for BME 201, and the person who truly offered delightful guidance during my aimless time. His precise understanding of me and genuine trust allowed me to knock on the door of science. More importantly, he showed me the potential of taking a career in the science and engineering field, enjoying the plain life with a wild heart that chases around science sparks. His affable communication style does not mean any compromise on science. He is also the major intellectual founder of the project which includes my thesis. Besides the scope of the project, his broad knowledge backup is always helpful whenever I feel confused.

At last, special thanks to my family, namely my parents Jie Yang and Lixia Zhang, and the people who offered their kindness that supports me to be the man I am today. Thanks to Penn State that bringing me these memorable and meaningful years. WE ARE still together in the future.

Chapter 1

Introduction

Problem Statement and Background

Currently, one of the most popular treatments against cancer is target therapy. Compared to chemotherapy and radiotherapy which have a massive unspecified-killing effect on a wide category of cells, target therapy drugs precisely aim at the cancer cells based on their unique biological features[1–4]. However, for the past decades, more and more evidence has shown that during or after the target therapy treatment, mutated cancer cells grew out in patients which leaves the accurate drugs no longer being useful. In other words, those cells are resistant to target therapy. *Figure 1* is an example of a target therapy drug treatment (Crizotinib is an FDA-approved drug for ALK+ lung cancer) that does not have a fully satisfying outcome. After 20 months, only less than 30% of the patients are in the state progression-free. Although there are multiple reasons accounting for this sharp decrease over time, drug resistance is the major cause of relapse.

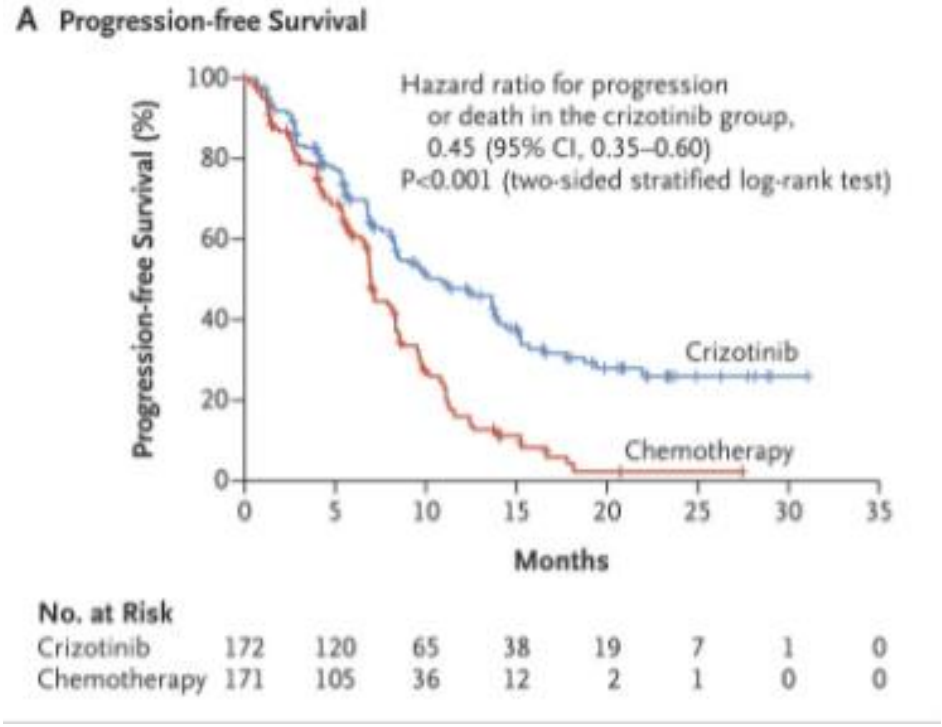


Figure 1: Progression-free survival vs. Time for ALK+ lung cancer[5].

The blue line represents data from patients taken Crizotinib, which is a target therapy. The orange line represents data from patients who received only chemotherapy. There is an overall superiority for the target therapy for all time points. However, even target therapy performs better than chemotherapy, its high progression-free survival rate still could not be conserved over time.

This problem is not going to be simply solved by designing better drugs that "prevent" mutations because of the heterogeneity of cancer. There has been one observed cancer cell type showing that the mutation causing drug resistance is preexisting[6], where the treatment becomes an evolutionary pressure that helps the resistant cells to win in the competition with other cells. From this perspective, we may still describe such resistance as an "acquired" resistance from the treatment for convenience, although this term may not be accurate.

One solution to the drug resistance problem is to continue the drug vs. resistance competition: once recognizing a common mutation acquired from the treatment, scientists develop a new drug against it. For example, for Non-Small-Cell Lung Cancer (NSCLC), there are three generations of tyrosine kinase inhibitors (TKI) being approved by FDA[7]. TKIs are

designed to stop the proliferation by binding to the kinase domain of the EGFRs, which block the proliferation signal. The first generation (e.g. Erlotinib) works well for some wild-type NSCLC cells (e.g. L858R and ex19del in EGFR gene, EGFR stands for epidermal growth factor receptor), and after treatments, multiple kinds of resistance arise. The second generation (e.g. Afatinib) has a broader inhibitory profile, but it still could not avoid the generation of T790M mutation, which is a common resistant mutation to EGFR TKIs. Third generation drug, namely Osimertinib, works for wild-type cancer cells and those with T790M mutation, but after the treatment, the resistant C797S mutation arises. *Figure 2* is a plot of the generalized procedure mentioned above. This endless competition has made great achievements, but drug development always costs extremely high expenditure and a long time. No one can guarantee the future of such a costly competition.

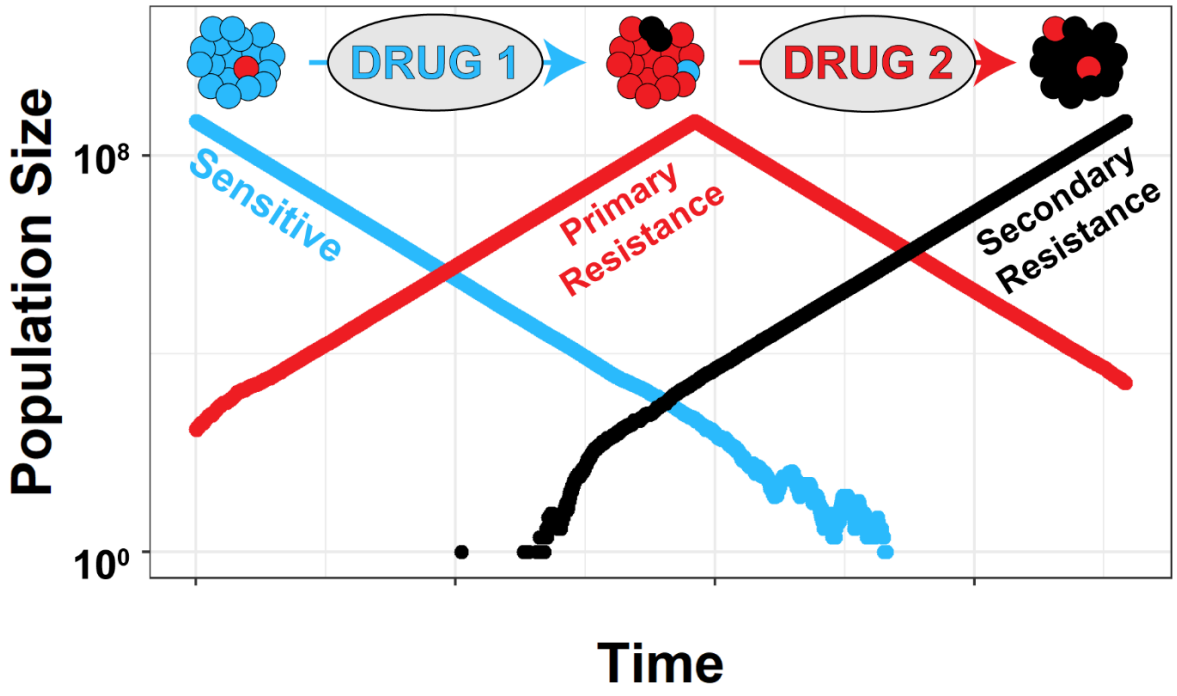


Figure 2: Schematic diagram of the development of drug resistance in cancer cells.

In the beginning, blue cells are sensitive to drug 1, but one red cell is not. After drug 1 kills most of the blue population, red cells grow out and become the dominant population. The same procedure happens again, when drug 2 is targeted for the red population, and the black resistant population ended up being the dominant population.

Another popular approach is to combine therapeutic agents together, usually target therapy drugs. With this combination therapy (or sometimes called systemic therapy), multiple drugs that target cancer cells differently will create a lower possibility of the cancer cells gaining both mutations[8]. However, one problem in this therapy is that all therapeutic agent doses need to be decreased compared to single-agent therapies[9]. Otherwise, there is a risk of overdosage[10,11]. The perfect therapeutic window that can kill cancers cells without serious harm to the human body is hard to hit in such a way. Target therapy combined with chemotherapy will face a similar situation, where chemotherapy drugs cannot be distributed to the tumor at a high enough dose that eliminates the possibility of resistant mutation.

Outside the direction of traditional drug-directed therapies, one interesting drug-directed therapy called "suicide gene therapy" is currently under testing. The guiding principle is to transform some of the tumor cells into engineered cells that can kill the surroundings as well as themselves[12–15]. The mechanism is to allow the engineered cells to take a bioinert prodrug and transform them into a cytotoxic drug. However, the data shows that this therapy, although managed to deliver a high enough dose of drug locally, the treatment's efficacy is significantly limited by the efficiency of vector[15]. Without a large enough amount cell being transformed into the engineered population, the treatment is not taking its full advantage.

Dual-Switch Combination Therapy Introduction

Based on understanding the resistance problem and dosing problem, members in the Prichard Lab come out with a biological solution with a forward engineering technique. The approach is to create a kind of artificial cells that have (1) the ability to quickly divide as cancer cells, (2) resistance to target

therapy drugs while under control, and (3) self-destruct as well as killing the surrounding cells once receiving a signal. With functions 1 and 2, the engineered population will be able to grow out in the presence of TKIs (switch 1). With function 3, the engineered cells release a cytotoxic substance that affects themselves and the surrounding cells, which we described as the "by-stander killing effect" (switch 2). The expected result would look like the graph below in *Figure 3*.

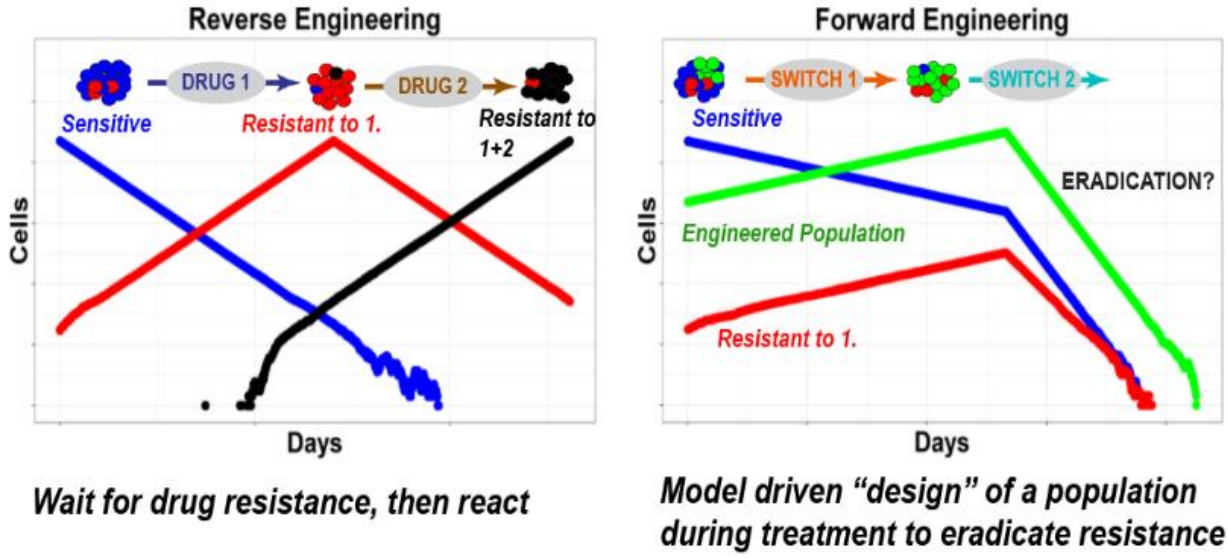


Figure 3: (Left) Schematic diagram of conventional target therapy. (Right) Schematic diagram of the forward engineering therapy.

In the beginning, the sensitive population is the dominant population. Once treated with TKIs, the resistant population will grow out like the case on the left, but the engineered population, with a larger initial population and artificial resistance to the TKI being given to the system, is going to be the dominating population. Once switch 2 is turned on, the engineered population is going to release cytotoxic substances, which will be the origin of the by-stander killing effect. All diagrams above are also generated from experimental data, so it's not purely a schematic plot only for description purposes.

Previously, Scott M. Leighow in the Prichard lab has created a growth tracking model *in silico* and carried out multiple experiments *in vitro* to validate the idea and find out practical approaches to achieve the design criteria above (see *Figure 4*). For example, for normal cells with EGFR, the EGF is a dimerizer that triggers the proliferation of cells. In the engineered population, we designed receptors that only accept a specific kind of dimerizer (D) and trigger the proliferation of cells, which are also genetically coded to be resistant to the target therapy drug (T). Also, in an engineered population, there is genetic information for the cells to create enzymes that turn prodrug (P), which is bioinert, into a drug

(P*), which is cytotoxic to a broad range of cells. With this dual-switch system, which we call the gene-drive construct, a local high dose of a non-discriminative drug is being delivered.

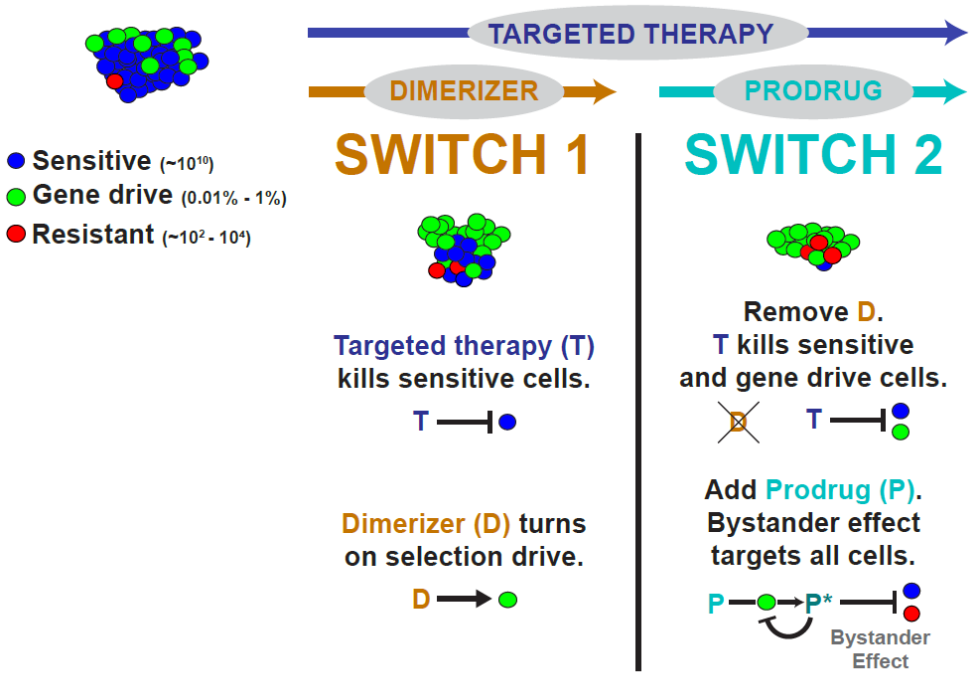
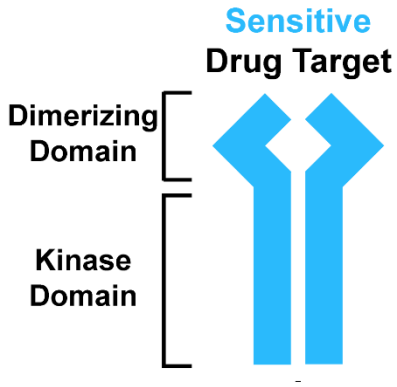
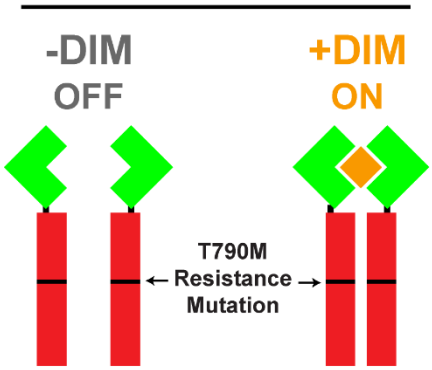
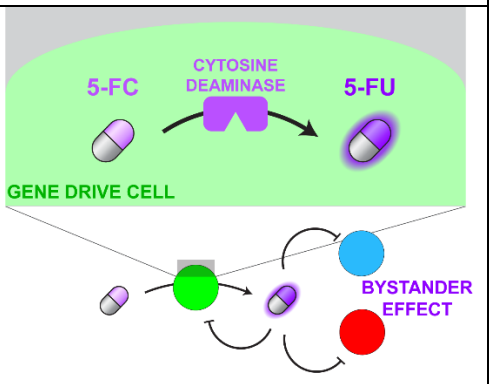


Figure 4: Schematic diagram of the design of engineered cancer cells for the dual-switch combination therapy.

In the proof-of-concept experiment (done by Scott M. Leighow), we have chosen the BaF3 cell line, a pro-B murine cell line that is dependent on interleukin-3 (IL-3) for growth. Advantages of using this cell line are (1) it's easy to modify with lentiviral infection technique and (2) the dependence of IL-3 makes the selection easier. The sensitive population is BaF3 cells with EGFR-L858R mutation. The resistant population contains EGFR-L858R-T790M mutation, which is resistant to erlotinib. The engineered population contains genes that are shown in *Figure* 5 and explained in *Table 1*. The experiment turned out to be successful: in *Figure 3*, the plots are real data that are generated in this experiment. The dual-switch therapy is proved to be worth exploring.

Table 1: Approaches used in BaF3 proof-of-concept experiment for desired functions.

Function Needed in the BaF3 Experiment	Approach	Schematic Diagram
Quick division that under signal regulation	Homodimerizer (HM) receptor domain receives rimiducid as a growth factor; the other part is the EGFR kinase domain that triggers the proliferation signal pathway	
Resistance to TKI	A T790M mutation in the EGFR gene	
Self-destruction and by-stander Killing effect	Cytosine deaminase (CD) converts flucytosine (5-FC, bioinert substance) into fluorouracil (5-FU, chemotherapy drug)	
The ability to be trackable	Green Fluorescence Protein	

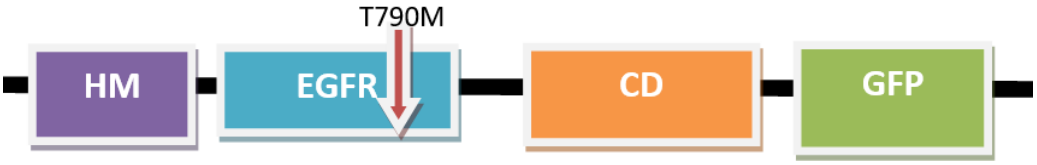


Figure 5: Gene being added into BaF3 cells in the proof-of-concept experiment

Although the success was encouraging, this prototype is still far away from the real-world scenario. On one hand, the previous design, shown in *Figure 5*, does not contain an oncogene (e.g. L858R). This means that the survival of the engineered population is also depending on the dimerizer regardless of the presence of TKIs. In an actual clinical scenario, a local high dose of dimerizer in the tumor that allows the engineered to compete with other cell lines may not be always easy to be achieved. Besides the difficulty of dimerizer delivery problem, the possibility of locating and transforming normal cells in a tumor is low due to the abundance of cancer cells. This means that in reality, there are very few cells that can have this construct being integrated with a normal genome (we call it incomplete). Most of the cases will be cancer cells that have oncogene being transformed, containing the oncogene and the gene-drive construct (we call complete). At this moment, whether the complete dual-switch system works well is remaining unknown. One step further from the previous statement, *in vitro* data may not be as convincing as *in vivo* data, so animal experiments need to be carried out to verify the efficacy of this system.

In this study, we designed experiments to give answers to the previous questions. A complete gene-drive construct that carries an ontogenetic mutation that makes the engineered cells' survival no longer dependent on dimerizer is tested in a growth tracking experiment. Also, we test the dual-switch system in a PC9 cell line, which is a human-origin NSCLC cell line that has EGFR-ex19del mutation. A mouse-based *in vivo* experiment is also carried out to verify the effect of a dual-switch system in mammals.

Chapter 2

Method

BaF3 Complete Gene-Drive Construct Experiment

Preparation of cells

To allow the engineered population to grow without the presence of dimerizers, ontogenetic modification like EGFR-L858R is designed to be integrated into the BaF3 genome (*Figure 6*). Due to the large amount of genetic information that needs to be carried, a single-step infection is not capable of our purpose. We designed a three-step infection method. Three plasmids are used in the procedure. The first one contains green fluorescence protein (GFP) and puromycin genes. The second one contains the gene-drive constructs, which are the genetic coding for switch 1 and switch 2 proteins. The third one contains a normal EGFR gene with L858R mutation.

All plasmids are generated by GenScript Biotech. For the first round, the plasmid DNA (GFP + puromycin) is added to the culture of *Escherichia coli*. After 18 hours of culture, the maxiprep procedure is carried out to harvest the desired DNA. Transfection-grade DNA vectors are then added to HEK293T cells with other necessary components of the lentiviral genome. After 18 hours, the viral supernatant is collected and given to BaF3 cultures. The infected BaF3 cells are removed from the virus and washed after 24 hours. In order to get a pure engineered population, the selection of GFP-positive cells is needed and achieved by puromycin.

The second and third infection cycle is the same as the first round. The only difference is that the selection markers are different. For the second cycle, cells are selected based on IL-3, dimerizer, and erlotinib. With the IL-3 and dimerizer, the engineered population will be able to survive under erlotinib, while the population that does not have a full gene-drive construct will not be able to get resistance and survive. There is a possibility of natural resistance coming up. But due to our cell population size in total is not large, there is very little possibility of a large natural resistant population to grow out in the competition with a large group of an engineered resistant population. The third-round infection is selected with IL-3, since the difference between a successful infection and failed infection is the dependence of survival on IL-3.

The resistant population and sensitive population are also needed to be modified since the BaF3 cell line itself is not a cancer cell line. Compared to the engineered population, the preparation of these two is relatively easy. The sensitive population only contains the EGFR-L858R gene, and the resistant population only contains the EGFR-L858R-T790M + MCherry (a kind of reddish fluorescence protein) gene, which is used for experimental tracking. All these genes are integrated into BaF3 in the same manner in only one infection cycle.

Figure 6: Gene being added into BaF3 cells for a complete system.

With L858R mutation, even without the presence of dimerizer, the cell will be able to divide like normal sensitive cancer cells. Puromycin and other necessary components for transcription to be carried well like promotor and IRES genes are ignored for simplicity.

Design of experiment

A mix-population growth tracking experiment is carried out. Prepared cells are placed in a 12-well plate. There are three conditions, each with three replicates. The first group contains 98% sensitive

cells and 2% resistant cells. The second and third groups both have 88% sensitive cells, 2% resistant cells, and 10% engineered cells. Each well contains roughly 1.5M cells at day 0. All these numbers are measured with flow cytometry.

There is an initial drug given to the cell mixture at day 0. For condition 1 and 2, after adding the initial drug (including dimerizer) combination, the concentrations of drugs in the wells are 250nM erlotinib and 10 nM dimerizer. For condition 3, the concentrations are is 250 nM erlotinib and 10 mM 5-FC. The drug concentration comes from the dose-response experiment result (*Figure 7*), which showed that the therapeutic window for this experiment should be around 10^2 to $10^{2.5}$ nM. All wells have a total volume of 2 ml liquid that contains RPMI, cell mixtures, and drugs.

On day 3, another set of drugs is added. For condition 1 and 2, another drug combination of 400 μl is added (including RPMI), which has drug concentrations of 250 nM erlotinib and 25mM 5-FC. For condition 3, the 400 μl drug mixture contains 250 nM erlotinib and 10 mM 5-FC. This volume is because the evaporation volume for three days is about 300~400 μl. With this addition of drugs, the concentration of drugs will be conserved at the desired level even if some of them bind to cells or degrade over time.

On day 6, a 300 μl drug mixture is added to all three conditions, each with 250 nM erlotinib and 10 mM 5-FC, for the same purpose.

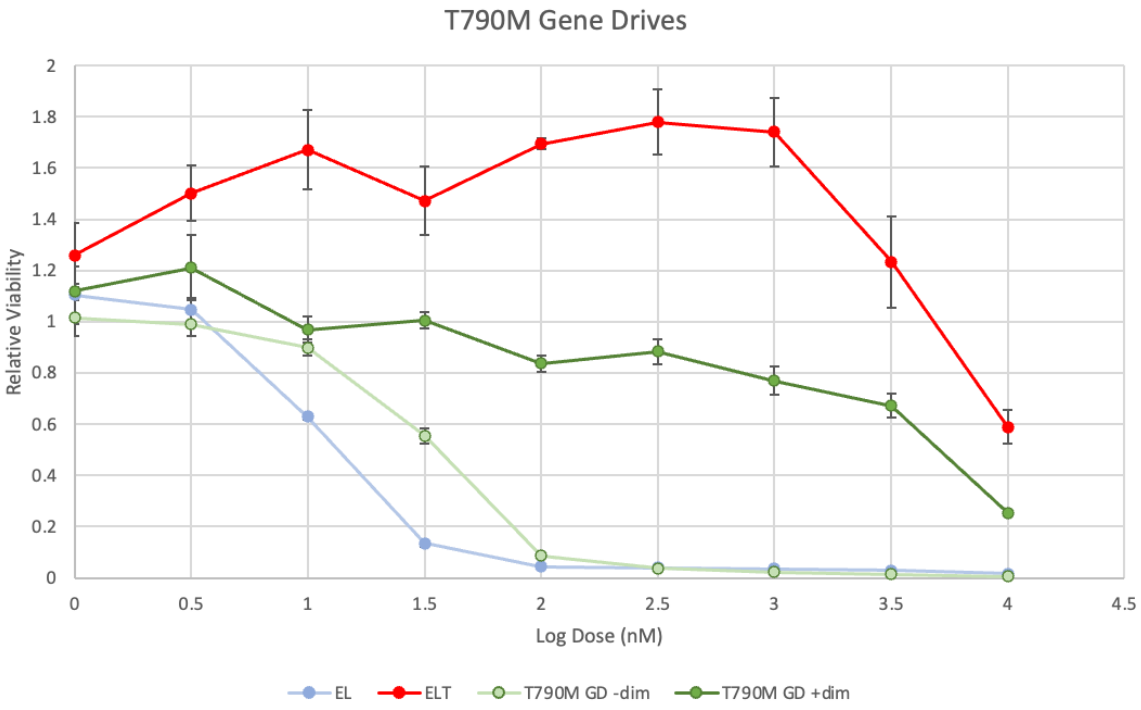


Figure 7: IC50 dose-response curve for erlotinib and the prepared cells in complete gene-drive construct experiment.

EL stands for EGFR-L858R; ELT stands for EGFR-L858R-T790M; T790M GD is the population with the complete gene-driven construct. + and – dim stands for giving or not the dimerizer which is necessary for the resistance to exist.

PC9 Complete Gene-Drive Construct Experiment

Preparation of cells

Like the BaF3 experiment, the PC9 experiment needs three populations: the sensitive, the resistant, and the engineered. Two special characteristics of PC9 make the preparation different: (1) PC9 does not depend on any molecules like IL-3 for BaF3 to survive, and (2) itself contains EGFR-ex19del, which is an oncogene. Since PC9 is a cancer cell line, the sensitive population does not need to be manually modified. For the resistant population, we chose to add another EGFR-L858R-C797S mutation. This is a substitute that has the same experimental and clinical effect of directly giving the EGFR-ex19del a resistant mutation[16]. The infection of this resistant population is an exact mirror of the procedure described previously for the resistant population in BaF3. For the engineered population, we used a two-

step split GFP procedure to guarantee the purity of the final population after selection. With the split genetic coding of a complete GFP gene into two pieces, GFP_{1-10} and GFP_{11}, only the cells that get both pieces can assemble GFP inside the cell membrane and show fluorescence[17]. For the first round, we create a virus that contains GFP_{1-10} + puromycin genetic information and select BaF3 cells with puromycin. For the second round, we create a virus that contains GFP11, and the rest gene drive constructs and do the infection. The selection is carried out by fluorescence-activating cell sorting (FACS) technology. In this way, we set an orthogonal selection marker that does not interface with the parameter that we want to test, which is survival under drugs. The final construct of the engineered PC9 population is shown in *Figure 8.*

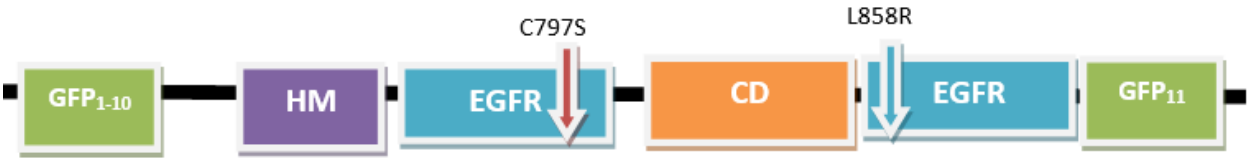


Figure 8: Gene being added into PC9 cells for a complete system.

Design of experiment

Two drug response experiments have been carried out with the cells mentioned above. For the osimertinib response experiment, four conditions are being tested with three populations (the engineered population is tested with and without the presence dimerizer). The drug concentration range is 1~10000 nM on a logarithmic scale. The 5-FC response experiment is carried out to verify the efficacy of switch 2, so 6 groups are being tested. Two are pure sensitive/engineered populations, and the other four are mixed populations of sensitive and engineered from 1:1 (1/2) to 1:15 (1/16). The dose of 5-FC varied on a logarithmic scale from 1 to 10000 μM.

Also, a mixed-population growth tracking experiment is carried out. All experimental settings for the growth tracking experiment are similar to the previous BaF3 experiment. One change is made in the choice of TKI. Since this time the resistance mutation is C797S, we choose the third-generation TKI osimertinib as a replacement for erlotinib.

BaF3 Gene-Drive Construct In Vivo Experiment

Design of experiment

Before validating the whole system, the verification of both switches' functions is necessary. Two dose-response experiments have been carried out. The first one is the dose-response of dimerizer *in vivo,* which is an experiment that verifies the effect of switch 1 in mice. The second one is done for the verification of the self-destruction function, which is the verification of switch 2. For the switch 1 verification experiment, we inject 4 groups of mice (each arm contains 3 mice, so a total of 12) with incomplete BaF3 cells under their skin. The 4 arms are given 0, 0.1, 1, 10 mg/kg dimerizer per day, and the growth of tumors is measured by volume after 12 days. For the switch 2 verification experiment, 10 mice are injected with complete gene-drive cells, and 5 are injected with EGFR-L858R BaF3 cells (which means the cells are sensitive to TKIs). For 5 mice with gene-drive cells and all mice with sensitive cells, they received daily intraperitoneal injections of 5-FC. The other 5 gene-drive mice are given PBS of the same volume daily. Measurements of tumor size come out on days 2, 4, and 6. All cells used in this experiment come from the BaF3 Incomplete Gene-Drive Experiment and the BaF3 Complete Gene-Drive Experiment.

Chapter 3

Result and Discussion

BaF3 Complete Gene-Drive Construct Experiment

For the BaF3 complete gene-drive experiment with the EGFR-T790M system, the pure sensitive-resistant population composition (condition 1) shows that without other treatments, the resistant population will grow out, which matches what has been observed numerous times in the clinical field.

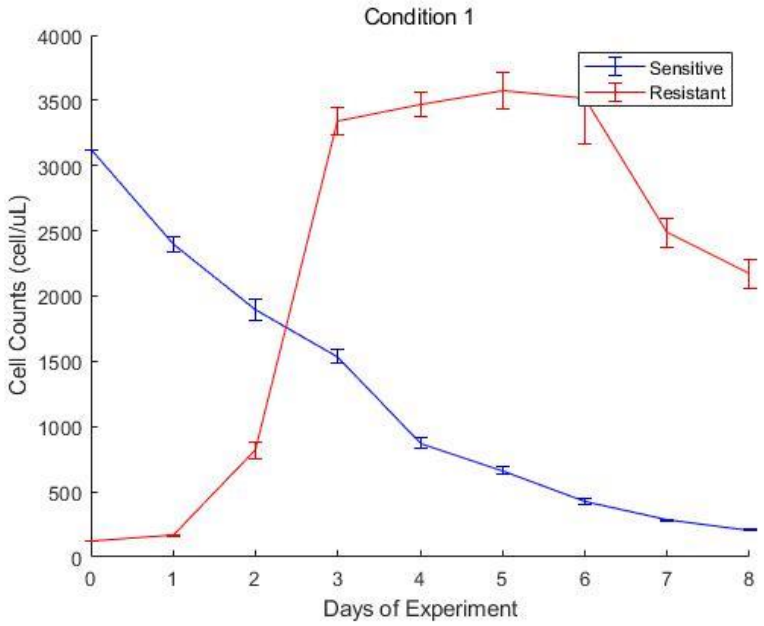


Figure 9: BaF3 Complete Gene-Drive Construct growth tracking experiment condition 1 result.

Condition 1 is composed of an initial population of 98% sensitive cells and 2% resistant cells. The drug combination is 250nM erlotinib,10 nM dimerizer, and 10 mM 5-FC (after day 3).

But with the gene-drive cells, in the presence of proper drug combinations, all populations end up being gone. In condition 2 result, before the 5-FC was added, both resistant and engineered cells, which have dimerizers for the resistance, kept increasing their population in the presence of erlotinib. But once the 5-FC is added, the cytosine deaminase turned it to be a locally high-dose 5-FU, which is the direct cause of the population drop. After the addition of 5-FC, the engineered population drops more rapidly than the resistant population, which may be worrying. But one understanding is that the death of

engineered cells causes a more violent release of 5-FU to the surrounding, which definitely strengthens the by-stander killing effect.

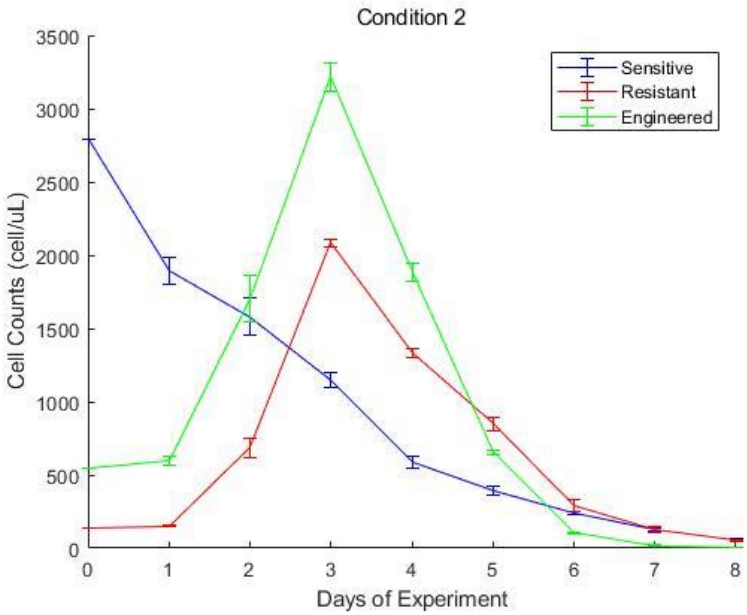


Figure 10: BaF3 Complete Gene-Drive Construct growth tracking experiment condition 2 result.

Condition 2 is composed of an initial population of 88% sensitive cells, 2% resistant cells, and 10% engineered cells. The drug combination is 250nM erlotinib,10 nM dimerizer, and 10 mM 5-FC (after day 3).

For condition 3 result, the drug combination is different from condition 2. There is an initial 5-FC given to the same system, which is a mimic of the current suicide-gene therapy. We can also see that the therapy works well, which partially supports the efficacy of that therapy. However, like what has been introduced before, suicide gene therapy is limited by the special composition of the population. Our experiment is a well-mixed 2D scenario, where an engineered population is widely spread in the whole culture, releasing 5-FU and spreading death. It would be reasonable to doubt the efficacy of this therapy in a 3D culture experiment or *in vivo* experiment with current evidence.

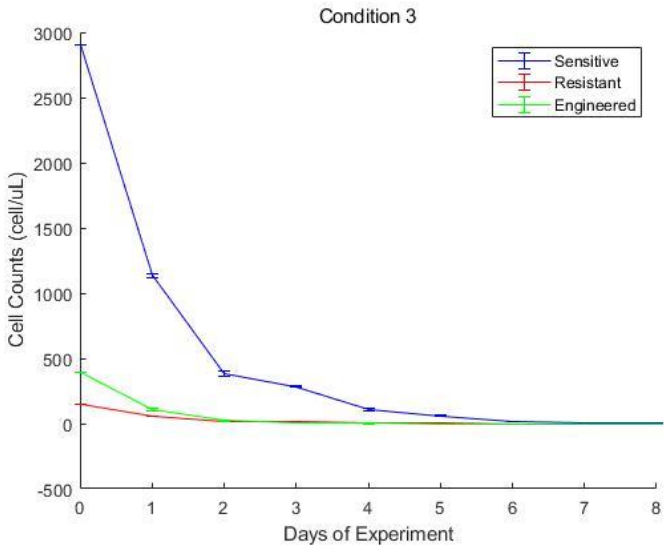


Figure 11: BaF3 Complete Gene-Drive Construct growth tracking experiment condition 3 result.

Condition 3 is composed of an initial population of 88% sensitive cells, 2% resistant cells, and 10% engineered cells. The drug combination is 250nM erlotinib and 10 mM 5-FC (since day 0).

PC9 Complete Gene-Drive Construct Experiment

Figures 12 and *13* are dose-response experiment results for the following growth tracking experiment. Figure 12 is the osimertinib response curve, but the gray curve is indeed confusing at the first glance. In theory, without a dimerizer, the gene-drive cells are sensitive to TKIs, but in the dose-response experiment, the GD-Dim curve dropped even later than the resistant population. From another way of understanding, the resistant cells are not as resistant as the gene-drive cells with dimerizers, which does not make sense. Even with this big problem, the plot is still quantitively good for the initial purpose, which is looking for a reasonable therapeutic window for the osimertinib dose in a follow-up experiment (45 nM). For *Figure 13*, the experiment of 5-FC dose-response as well as a simplified model of switch 2 verification, the data end up being laid out nicely in order.

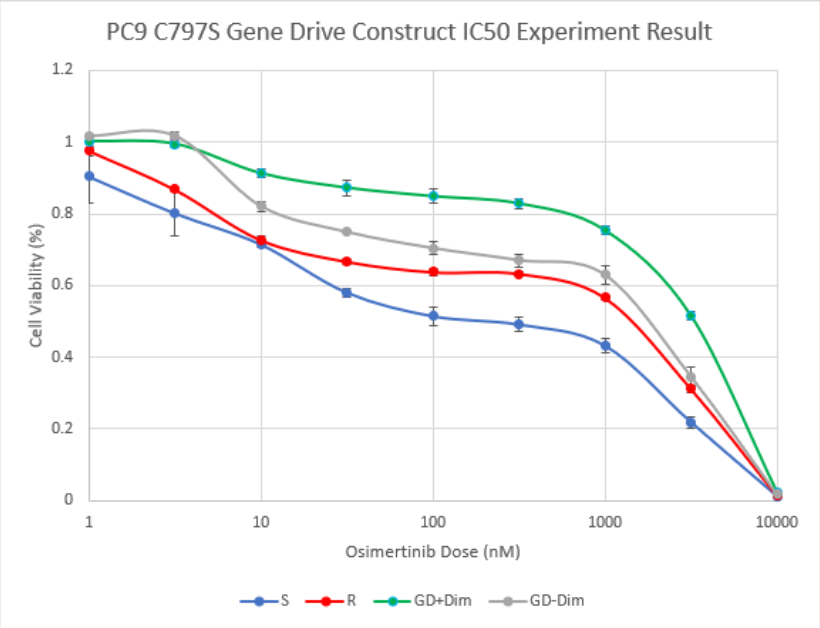


Figure 12: PC9 Complete Gene-Drive Construct osimertinib drug response curve.

The blue curve is for the sensitive population (wild-type PC9); the red curve is for the resistant population (EGFR-L858R being integrated into WT PC9); green and grey curves are all engineered populations: one with the dimerizer and one without. Cell viability data is collected on day 3.

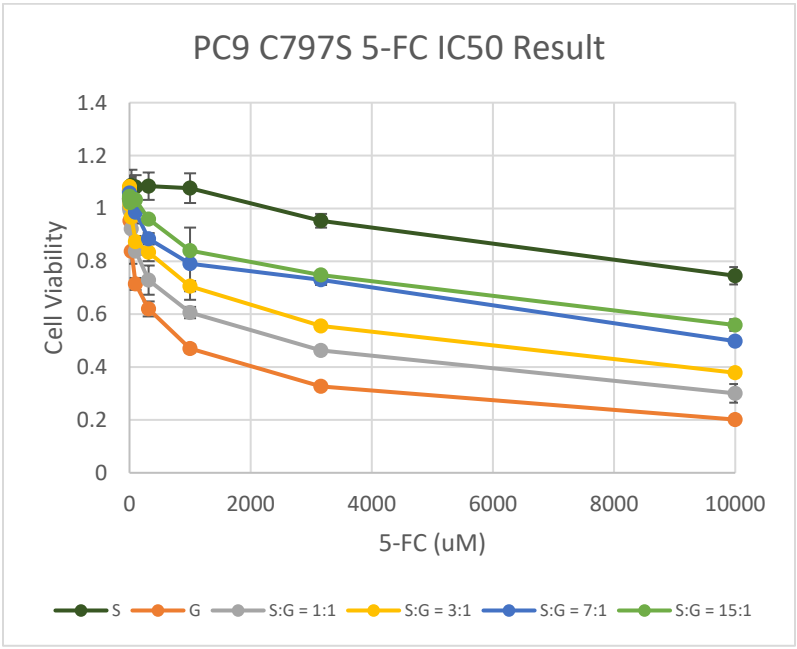


Figure 13: PC9 Complete Gene-Drive Construct 5-FC drug response curve.

The legend S and G represent sensitive and engineered populations. The ratio is the mixture composition ratio of the sensitive vs. engineered population. Cell viability data is collected on day 3.

For the growth tracking part of the PC9 experiment, again, the strange thing happened for at least two conditions, where the resistant population growth did not outpace the sensitive population even in the presence of osimertinib. Comparing the growth of the three populations in condition 1 and condition 2, a decent doubt might come to the osimertinib' s quality. If the drug's quality issue is true, this can explain what has been observed in the dose-response curve (resistant and non-resistant populations getting too close to each other) and the slow growth of resistant cells. The switch 1 verification can be viewed as failed for this experiment. However, switch 2 can still be tested. In condition 2, after day 8, which is longer than the BaF3 experiment's setting for a larger engineered population ratio, 5-FC was added and a significant drop appeared. In condition 3, with an initial 5-FC, all populations died much faster than in the two other conditions. This is a good sign of showing the efficacy of switch 2.

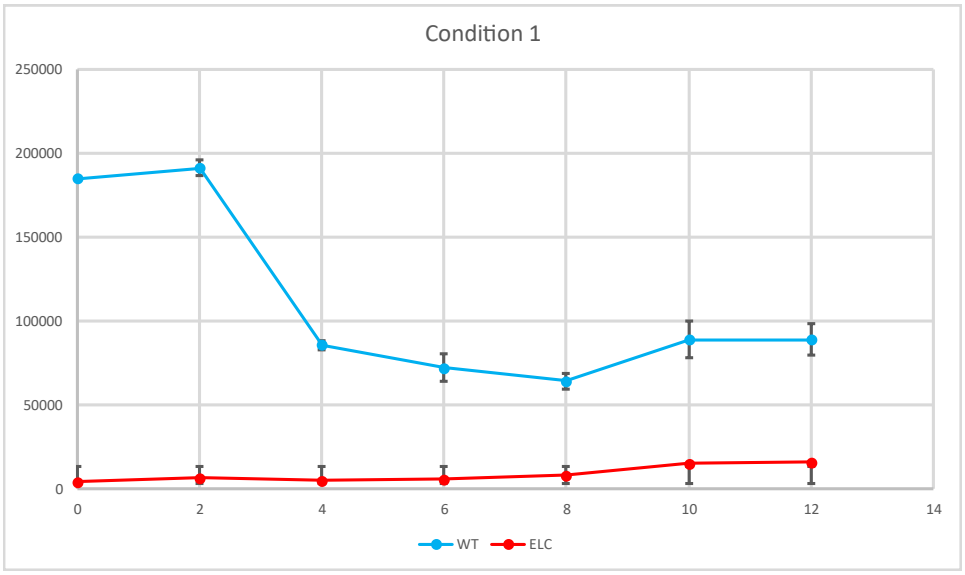


Figure 14: PC9 Complete Gene-Drive Construct growth tracking experiment condition 1 result.

Condition 1 is composed of an initial population of 98% sensitive cells and 2% resistant cells. The drug combination is 45nM erlotinib,10 nM dimerizer, and 10 mM 5-FC (after day 8).

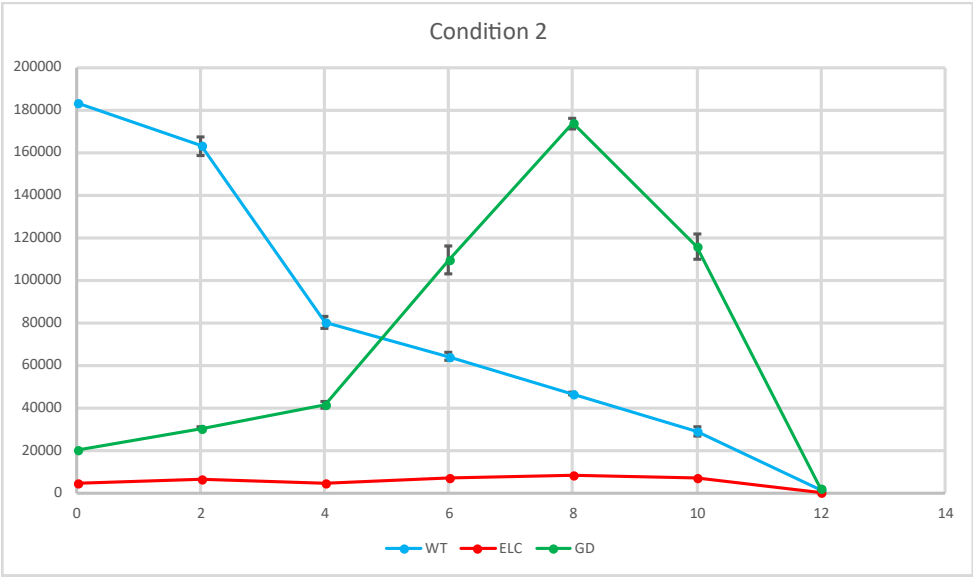


Figure 15: PC9 Complete Gene-Drive Construct growth tracking experiment condition 2 result.

Condition 2 is composed of an initial population of 88% sensitive cells, 2% resistant cells, and 10% engineered cells. The drug combination is 45nM erlotinib,10 nM dimerizer, and 10 mM 5-FC (after day 8).

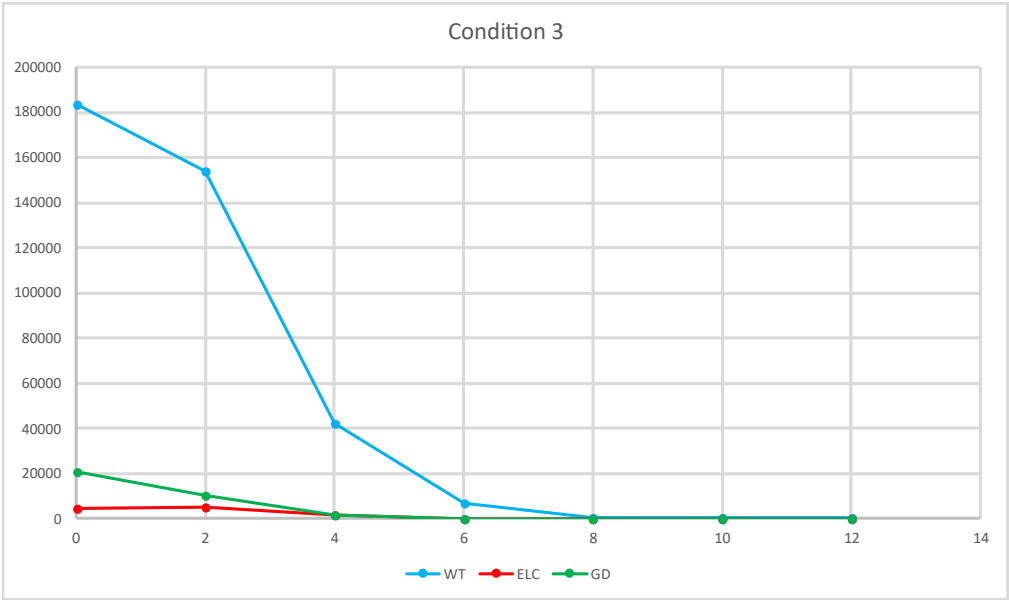


Figure 16: PC9 Complete Gene-Drive Construct growth tracking experiment condition 3 result.

Condition 3 is composed of an initial population of 88% sensitive cells, 2% resistant cells, and 10% engineered cells. The drug combination is 45nM erlotinib and 10 mM 5-FC (since day 0).

BaF3 Gene-Drive Construct In Vivo Experiment

The result for the *in vivo* switch 1 verification is count-intuitive since the previous verifications are both growth trackings of cells under TKIs. In the *in vivo* case, we tested switch 1's efficacy by verifying the dependence of the dimerizer for engineered BaF3 cells. Since the construct used here is an incomplete gene-drive construct, which leads the engineered BaF3 cells to be addicted to the oncogene and dimerizer, the absence of dimerizer will lead to cell death, which is the first column in *Figure 17.* With more dimerizers, the cells proliferate more in a non-linear relationship. This result might be potentially risky to be used as the evidence supporting the efficacy of resistant function, but with the previous *in vitro* BaF3 incomplete gene-drive experiment, which showed there is very little chance for the engineered population to be sensitive as well as being addicted to dimerizer.

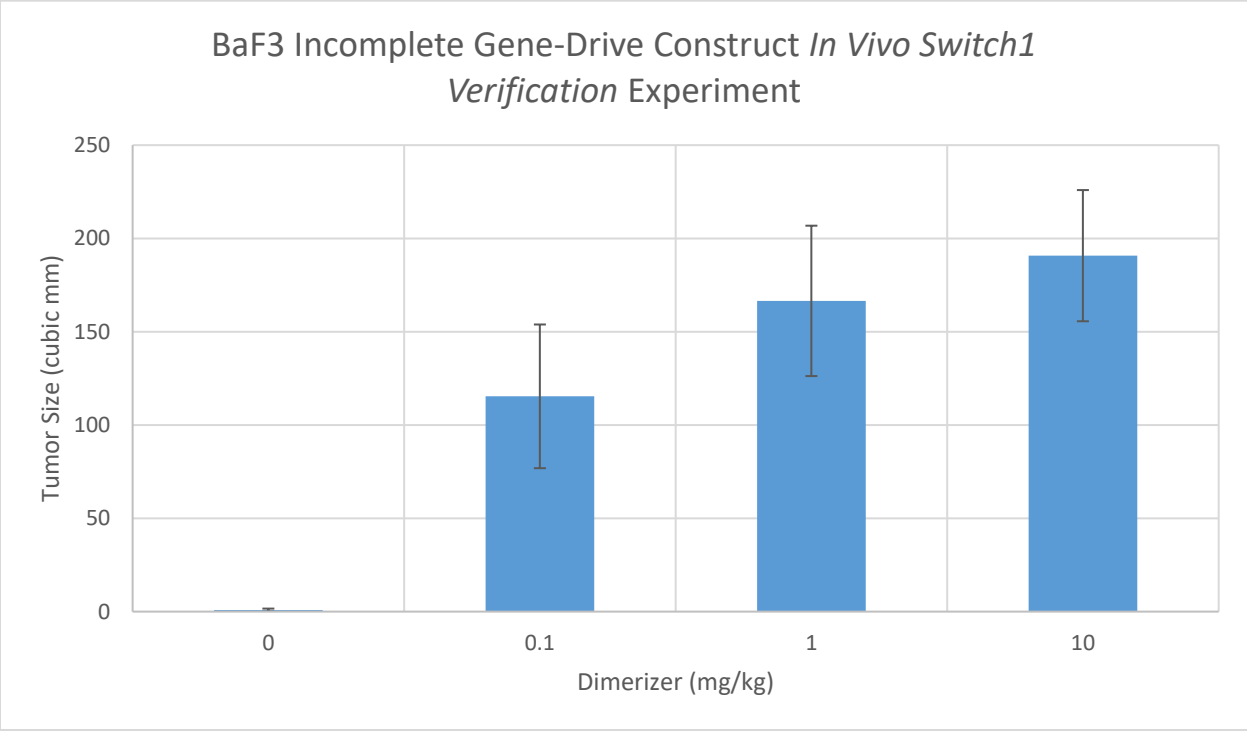


Figure 17: BaF3 Gene-Drive Construct *In Vivo* experiment switch 1 verification result.

The tumor sizes are counted at day 12 for all arms. The cells used here are an incomplete gene-drive construct.

At last, *Figure 18* showed that sensitive BaF3 cells are not disturbed by the injection of 5-FC, while the population of engineered cells is greatly affected. This result is consistent with the *in vitro*

experiments. Although there is a larger error bar in this result compared to the previous ones, the conclusion should still work well since the difference is clear enough.

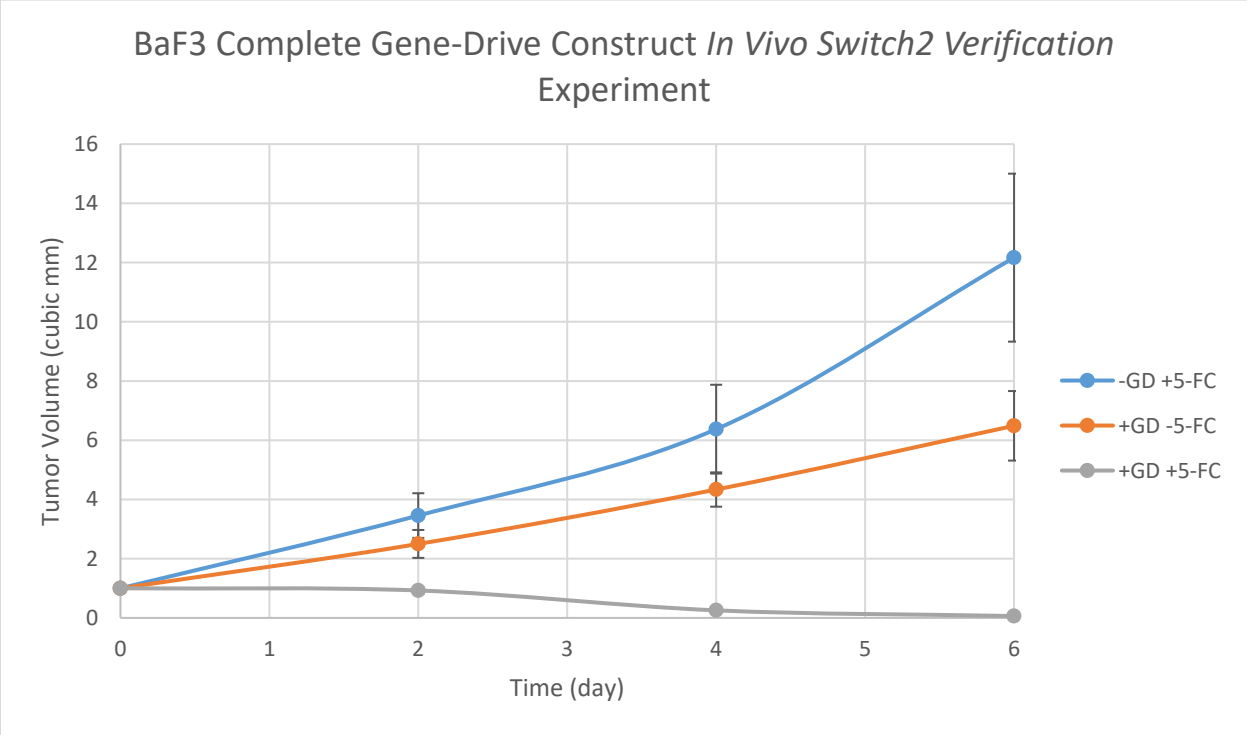


Figure 18: BaF3 Gene-Drive Construct *In Vivo* experiment switch 2 verification result.

The cells used here are a complete gene-drive construct. 5-FC is injected into the tumor daily.

In the previous three experiments, results showed that the idealized scenario where the evolutionary force driving model functions well as the initial design. In the BaF3 cells, the experiment result matches nearly perfectly with the model estimation. However, for PC9, due to the drug quality issue, the result was not that elegant, but the second switch was still verified to be functioning. And for the *in vivo* experiment, the result showed that both switches functions in mammals. In general, the progress so far is a fairly solid base for the later big plans.

Chapter 4

Future Work

There is a clear outline of our future work. In this study, we showed that in the PC9 cell line, which has EGFR mutation, the dual-switch selection combination therapy works in vitro. But this conclusion shall not be generalized with current evidence. Our next step is to try the other two cell lines, which are the TPC1 cell line (RET+) and H3122 cell line (ALK+). In the previous BaF3 complete system, Scott M. Leighow has verified that the RET mutation is still under the control of the dual-switch system, and I tested the ALK mutation. The ALK result did not work well due to high noise, so this experiment may be redone in the future.

Another weak point so far in the frame of experiments is that the mutation in resistant population is always known, while in reality, the heterogeneity of cancer is significant. One way of testing this is to make a library of random EGFR mutations, including the known resistant ones (T790M, C797S, etc.). After infecting the cells with the virus from the library, doing a growth tracking experiment will show the efficacy of this system in a complex environment.

The last question within the current frame that we are interested in investigating is the difference between 2D and 3D culture. *In vivo* study partially replied to this question that whether the BaF3 grows in 3D, but this experiment is hard to achieve when the cell lines are of human origin unless using special mouse models. A 3D culture *in vitro* experiment may be carried out in the future.

Beyond the current frame, there is another interesting direction that this project can move toward, which is immunotherapy. There has been a paper showing that immunotherapy also has the by-stander-killing effect which works differently in mechanism than the 5-FC system we are using now[18]. In the future, testing the fitness of the dual-switch system with immunotherapy has great potential.

BIBLIOGRAPHY

1. Gore L, DeGregori J, Porter CC. Targeting developmental pathways in children with cancer: what price success? *Lancet Oncol*. 2013;14(2). doi:10.1016/S1470-2045(12)70530-2
2. Cai J, Ma H, Huang F, et al. Correlation of bevacizumab-induced hypertension and outcomes of metastatic colorectal cancer patients treated with bevacizumab: a systematic review and meta-analysis. *World J Surg Oncol*. 2013;11. doi:10.1186/1477-7819-11-306
3. Petrelli F, Borgonovo K, Cabiddu M, Lonati V, Barni S. Relationship between skin rash and outcome in non-small-cell lung cancer patients treated with anti-EGFR tyrosine kinase inhibitors: a literature-based meta-analysis of 24 trials. *Lung Cancer*. 2012;78(1):8-15. doi:10.1016/J.LUNGCAN.2012.06.009
4. Flaherty KT, Infante JR, Daud A, et al. Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. *N Engl J Med*. 2012;367(18):1694-1703. doi:10.1056/NEJMOA1210093
5. Solomon BJ, Mok T, Kim DW, et al. First-Line Crizotinib versus Chemotherapy in ALK -Positive Lung Cancer . *New England Journal of Medicine*. 2014;371(23):2167-2177. doi:10.1056/NEJMOA1408440/SUPPL_FILE/NEJMOA1408440_DISCLOSURES.PDF
6. Bhang HEC, Ruddy DA, Radhakrishna VK, et al. Studying clonal dynamics in response to cancer therapy using high-complexity barcoding. *Nature Medicine*. 2015;21(5):440-448. doi:10.1038/nm.3841
7. Wang S, Li J. Second-generation EGFR and ErbB tyrosine kinase inhibitors as first-line treatments for non-small cell lung cancer its effectiveness. Data from patients receiving afatinib in. Published online 2019. doi:10.2147/OTT.S198945
8. Mokhtari RB, Homayouni TS, Baluch N, et al. Combination therapy in combating cancer. *Oncotarget*. 2017;8(23):38022-38043. doi:10.18632/ONCOTARGET.16723

9. Principles of Dose, Schedule, and Combination Therapy - Holland-Frei Cancer Medicine - NCBI Bookshelf. Accessed April 3, 2022. https://www.ncbi.nlm.nih.gov/books/NBK12635/

10. Spigel DR, Edelman MJ, O'Byrne K, et al. Results from the Phase III Randomized Trial of Onartuzumab plus Erlotinib Versus Erlotinib in Previously Treated Stage IIIB or IV Non–Small-Cell Lung Cancer: METLung. *Journal of Clinical Oncology*. 2017;35(4). doi:10.1200/JCO.2016.69.2160

11. Mok T, Wu YL, Siu-Kie Au J, et al. Efficacy and Safety of Erlotinib in 1242 East/South-East Asian Patients with Advanced Non-small Cell Lung Cancer. Published online 2010. doi:10.1097/JTO.0b013e3181e15d55

12. P Z, K D, A S, et al. Suicide Gene Therapy for Cancer - Current Strategies. *J Genet Syndr Gene Ther*. 2013;4(04). doi:10.4172/2157-7412.1000139

13. Tamura R, Miyoshi H, Yoshida K, Okano H, Toda M. Recent progress in the research of suicide gene therapy for malignant glioma. *Neurosurgical Review 2019 44:1*. 2019;44(1):29-49. doi:10.1007/S10143-019-01203-3

14. Duarte S, Carle G, Faneca H, Lima MCP de, Pierrefite-Carle V. Suicide gene therapy in cancer: Where do we stand now? *Cancer Letters*. 2012;324(2):160-170. doi:10.1016/J.CANLET.2012.05.023

15. Hossain JA, Marchini A, Fehse B, Bjerkvig R, Miletic H. Suicide gene therapy for the treatment of high-grade glioma: past lessons, present trends, and future prospects. *Neuro-Oncology Advances*. 2020;2(1):1-12. doi:10.1093/noajnl/vdaa013

16. Hong W, Wu Q, Zhang J, Zhou Y. Prognostic value of EGFR 19-del and 21-L858R mutations in patients with non-small cell lung cancer. *Oncology Letters*. 2019;18(4):3887. doi:10.3892/OL.2019.10715

17. Kamiyama D, Sekine S, Barsi-Rhyne B, et al. Versatile protein tagging in cells with split fluorescent protein. *Nature Communications 2016 7:1*. 2016;7(1):1-9. doi:10.1038/ncomms11046

18. Upadhyay R, Boiarsky JA, Pantsulaia G, et al. A Critical Role for Fas-Mediated Off-Target Tumor Killing in T-cell Immunotherapy. *Cancer Discov*. 2021;11(3):599-613. doi:10.1158/2159-8290.CD-20-0756

ACADEMIC VITA

Zeyu YANG

zxy5149@psu.edu

EDUCATION

Pennsylvania State University University Park & Abington, PA Sep 2018 – Present

Bachelor of Science in Biomedical Engineering – Biochemical Option

Honors: Pennsylvania State University Schreyer Honors Scholar (2020–Present); Dean's List (2018–Present); President's Freshman Award, Penn State Abington (2019)

RESEARCH

The Pritchard Lab | Penn State Huck Institute & BME Department

University Park, PA

Individual Undergraduate researcher | Mentor: Scott Leighow; Advisor: Prof. Justin Pritchard

Stochastic Models to study the Interplay between Evolutionary Forces across Broad Contexts of Drug Resistance in Cancer Nov 2020 – Aug 2021

- Focused on a more accurate modeling of drug resistance evolution under the clinic diversity in non-small cell lung cancer (NSCLC)
- Constructed well-mixed dynamic models (with Matlab) by considering the evolution of genetic mutations and microenvironmental factors to study the drug resistance in NSCLC
- Improved the models by changing the logistic model to stochastic model, and introducing spatial constraints with increasing parameters to consider more microenvironmental factors

Development of a Novel Dual-Switch Cancer Therapy – a Combination of Target Therapy and Suicide Gene Therapy Jul 2021 – Present

- Aim to develop a two-switch cancer therapy to solve the heterogeneity problem in cancer relapse
- Trying to expand the previous one-drug/one-cell line research to more anti-cancer drugs in more cell lines to generalize the therapy method

Dr. Mukul Talaty's Lab | Penn State Abington

Abington, PA

Collaborative undergraduate researcher | Advisor: Prof. Mukul Talaty

Fit and Strength Testing of 3D Printed Lower Extremity Prosthetic Sockets Sep 2018 – May 2019

- Focused on the material property testing of 3D printed prosthetic sockets (a better alternative to conventional method)
- Designed and developed a novel fixture to expand the strength-testing from one (vertical) direction to various angulations (to mimic real walking)
- Established the testing standards of quality of fit by both patient comfort assessment and mechanical measurements
- Presented our results as posters in two workshops

PROFESSIONAL EXPERIENCES

Microport CardioFlow Medtech Cooperation Jun – Jul 2021

Intern, Marketing Department

- Wrote and published medical paper reviews on the Wechat official account to improve company's public impacts
- Performed functional tests on the MOOC website under construction and reported bugs to the outsourcing software company
- Assisted in producing and managing marketing materials and coordinating video productions between various parties

Student Achievement Center, Penn State Abington Sep 2019 – Jan 2020

- Volunteered as part-time tutor to help freshmen and sophomores with their studies in chemistry, physics, calculus and algebra