Quantitative Analysis of Tyrosine Kinase Inhibitors Uptake in Cancer Cells Using Stimulated Raman Scattering Microscopy

Thomas Hanpyo Ro

A thesis

submitted in partial fulfillment of the

requirements for the degree of

Master of Science

University of Washington

2022

Committee:

Dan Fu

Ashleigh Theberge

Program Authorized to Offer Degree:

Applied Chemical Science and Technology

University of Washington

Abstract

Quantitative Analysis of Tyrosine Kinase Inhibitors Uptake in Cancer Cells Using Stimulated Raman Scattering Microscopy

Thomas Hanpyo Ro

Chair of the Supervisory Committee:

Dr. Dan Fu

Multidrug resistance remains a challenging problem in anti-cancer treatment. Transporters that regulate drug uptake and excretion are widely known to cause multidrug resistance in cancer cells. Tyrosine kinase inhibitor, a small molecule that binds to the ATP-binding site of the tyrosine kinase domain of epidermal growth factor receptors, is emerging as an alternative anti-cancer treatment drug or supplementary to chemotherapy. However, even the treatment with tyrosine kinase inhibitors faces multidrug resistance. Although some studies show interactions between several tyrosine kinase inhibitors and different membrane transporters, much is left to be learned. Here, three different tyrosine kinase inhibitors, lapatinib, afatinib, and osimertinib, are examined

with six drugs that inhibit solute carrier transporters using stimulated Raman scattering microscopy.

ACKNOWLEDGEMENT

I would like to thank my group members who taught me and helped me throughout my graduate studies. Dr. Andrew T. Francis and Dr. Bryce A. Manifold, thank you for teaching me and providing guidance. Rachel Hu, thank you for your hard work in cell culturing. Your care and dedication to keeping the cells in good condition were all I could ask for. Fiona Epsilons, Zach Ellsworth, Robert Espinoza, Brian Wong, Erin Dunny, and Emily Rathbone, thank you for your friendship and assistance in helping me through many experiments and analyses. Thank you for always being there when I needed your assistance. Colburn Cobb-Bruno, Harrison Hu, and Christine Wu, thank you for being a great help as undergraduate colleagues. Your dedication and eagerness to learn motivated me a lot.

I would also like to thank those who assisted me outside of Fu Group. Dr. Sarah L. Keller, thank you for being a great professor and a mentor that gave me the confidence and courage to pursue the degree. I also appreciate your help in some of our collaborations.

I thank my committee, Dr. Ashleigh Theberge, who also encouraged me and gave me confidence in my work.

Finally, Dr. Dan Fu, thank you for the great opportunities you have given me. Your knowledge and guidance gave me a sense of fulfillment and confidence that I never had before. My journey as a chemical scientist started all because of you. It was indeed an honor working with you.

DEDICATION

To Kyuhyon, Eunha, and Hannah, who bless me with their unconditional love.

To my dearest Alyssa, my heart belongs to you until the light exists no more.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Chapter 1. Introduction

1.1 Drug Resistance in Cancer Cells

Chemotherapy is one of the most relied-upon methods for treating most types of cancer, but the drug-resistant tendency of cancer cells hinders the effectiveness of chemotherapeutic drugs. According to the National Cancer Institute, the number of patients with cancer in the United States has been decreasing slowly since the early 1990s, and the number of cancer survivors has increased [1]. However, as risk factors for cancer, such as obesity and longer life expectancy rise, so too will the demand for cancer treatment. Cancer chemotherapy is estimated to reach a market value of 74 billion dollars by 2027 [2]. Characterizing drug resistance in cancer cells remains a critical field of study in pursuing increasingly efficient drug treatment.

The ability of tumor cells to resist various anti-cancer drugs is called multidrug resistance (MDR). There are two general categories of multidrug resistance: intrinsic and acquired (figure 1.1). Intrinsic resistance refers to the disposition of resistance factors within tumor cells before chemotherapy, making the treatment ineffective. Acquired resistance can occur during chemotherapy through mutations. Drugs introduced into tumor cells trigger adaptive responses, leading to a higher expression of defensive genes against chemotherapeutic drugs. A significant consequence of this effect is cancer relapse amongst patients receiving chemotherapy. Mutations in membrane transporters and changes in drug metabolism cause cells to develop resistance to a non-specific, wide range of drugs [4].

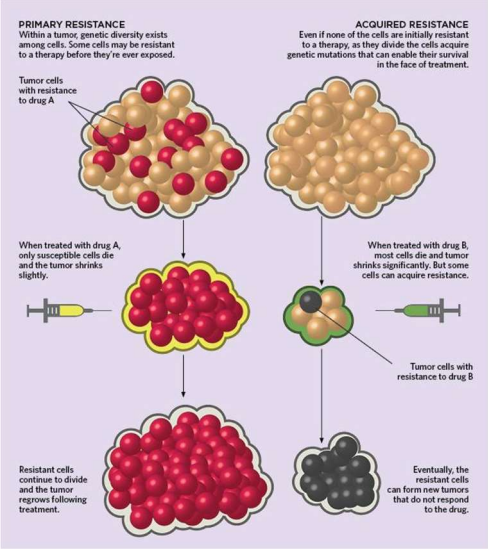

Figure 1.1 Intrinsic (Primary) vs. Acquired Resistance
Intrinsic - when cells are resistant to treatment drug before the treatment. Acquired - when cells develop resistance during the treatment, resulting in relapse *Figure is adopted from [3]*

1.2 Epidermal Growth Factor Receptors and Tyrosine Kinase Inhibitors

Epidermal Growth Factor Receptor (EGFR) is a transmembrane protein consisting of three domains: an extracellular receptor domain, a transmembrane domain, and an intracellular kinase domain (figure 1.2) [5]. EGFR, first isolated and characterized by Nobel Prize laureate Stanley Cohen, is known for its role in cell growth and differentiation. When the receptor of the extracellular domain is bound to the ligand Epidermal Growth Factor (EGF), EGFR undergoes a conformational change that opens the ATP-binding site on the kinase domain. The binding of ATP on this site results in phosphorylation near the C-terminal tyrosines, acting as a "docking" site for various activating kinases such as Atk. This triggers a signaling cascade that leads to cell proliferation and suppression of apoptosis (figure 1.3) [6].

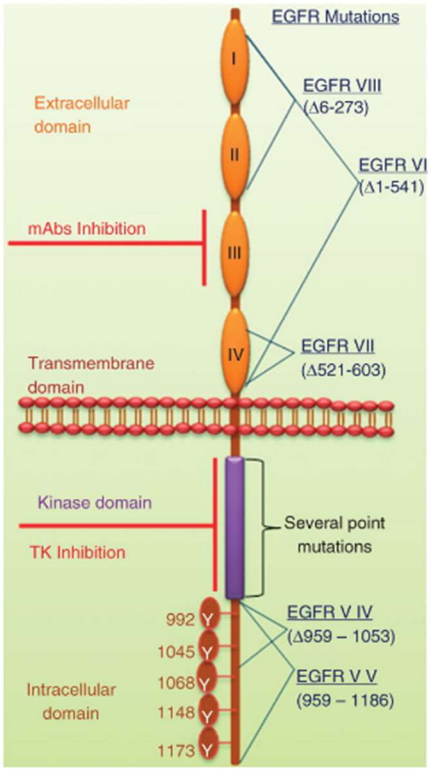

Figure 1.2 Structure of an Epidermal Growth Factor Receptor
Epidermal growth factor receptor, EGFR, is dived into three parts: extracellular domain, where receptors for epidermal growth factor (EGF) can bind, transmembrane domain, and kinase domain, where ATP-binding site is located. Subdivision of domains are shown. *Figure is adopted from [5]*

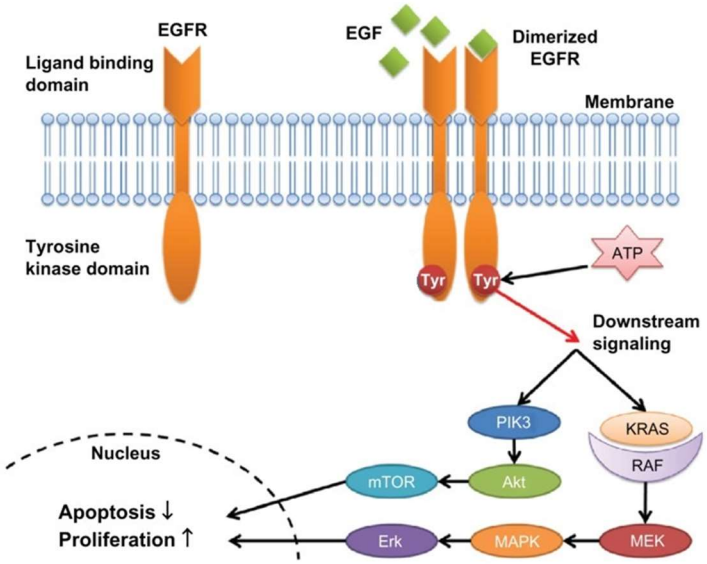

Figure 1.3 Mechanism of Epidermal Growth Factor Receptor
When EGFR ligand EGF binds, conformational changes on EGFR allows ATP binding on tyrosine kinase domain. Tyrosines near C-terminal phosphorylates when ATP is bound to the ATP-binding site, serving as "docking" site for other activating kinases that leads to cell proliferation. *Figure is adopted from [6]*

Overexpression of EGFR genes in tumor cells has directed treatment plans toward inhibiting the EGFR signaling pathway to stop the uncontrolled cell growth and division [5]. Anti-EGFR-targeted therapies have been developed and are currently employed (figure 1.4) [5]. These types of therapies have two different targets: extracellular and intracellular domains. Antibodies are used to bind to extracellular receptors of EGFR in replacement of EGF, preventing the ligand from binding and impeding subsequent signaling pathways. Small molecules called Tyrosine Kinase Inhibitors (TKIs) can bind to the intracellular ATP-binding site of the kinase domain and block phosphorylation. On-going studies show the effectiveness of targeting EGFR transcription at the gene level [5]. This study focuses on TKIs.

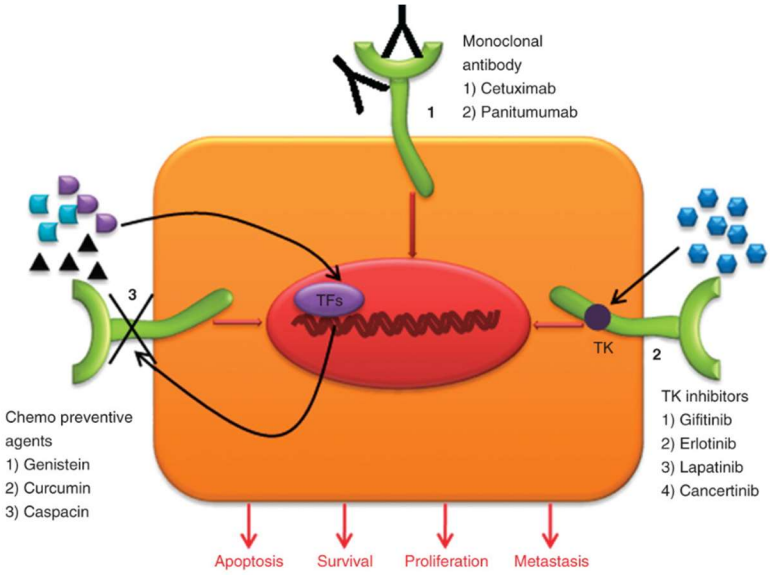

Figure 1.4 Multidimensional Targeting of EGFR
1. Monoclonal antibodies is used to target receptor domain of EGFR 2. Tyrosine kinase inhibitors are used to bind to ATP binding site of kinase domain. Both 1 and 2 are used to stop phosphorylation of tyrosines on kinase domain. 3. Chemo preventive agents are used to inhibit transcription of EGFR. *Figure is adopted from [5]*

Chapter 2. Quantitative Analysis of Tyrosine Kinase Inhibitors Inside Living Cells Using Stimulated Raman Scattering Microscopy

2.1 Motivation and overview

While the effectiveness of TKIs in suppressing tumor growth is proven, patients receiving TKI treatment often experience relapse [7, 21]. As chemotherapeutic drugs become less effective over time, tumor cells can become resistant to TKIs [8]. Multiple studies have shown how ATP-binding cassette (ABC) subfamily membrane transporters are involved in the efflux of TKIs [9-12,22]. ABC transporters are known to be overexpressed in tumor cells [9]. Solute carriers (SLC) family membrane transporters are responsible for uptaking small molecules into cells. However, little is known about influx transporters that regulate drug uptake. This study aims to expand the knowledge on influx transporters and selected TKIs. Isolating influx transporters interacting with

TKIs will provide insight into drug resistance, enhanced treatment plans, and guidance for future experiments.

2.2 SRS Microscopy

There is yet to be any study on TKI-SLC transporter interactions on live cells non-invasively. Here, stimulated Raman scattering (SRS) microscopy was used to collect real-time images of live cells without modifications to any drug or biomolecules. Fluorescence microscopy, though widely used in biophotonics, has its limitations. Attaching fluorescent molecules could easily alter drug properties: widely used fluorescence dyes are often similar in size to TKIs, which may interrupt drug transportation through transmembrane transporters [13-15]. Photobleaching of fluorescent dyes can also interfere with the accurate quantification of drugs inside the cells. Infrared (IR) microscopy, in which photons are absorbed to excite molecules to their vibrational state, can be used as label-free imaging. However, a significant drawback of IR is the strong absorption of water molecules, which makes IR microscopy difficult for biological samples [13,15]

Raman scattering can overcome some shortcomings of fluorescence and IR microscopy in biophotonics. Raman scattering occurs when the energy released from a molecule in a virtual excited state matches its vibrational state [16]. Spontaneous Raman scattering utilizes these emission properties. However, the probability of spontaneous Raman emission is minuscule. The time it takes to collect spontaneous Raman emissions from forging meaningful microscopic images within the biologically relevant laser powers and wavelength would be intolerably long, especially for living cells [13,15-16].

Stimulated Raman scattering (SRS) and Coherent anti-Stokes Raman Scattering (CARS) microscopy are third-order, nonlinear optical processes [13-16]. Energy level diagrams for IR, spontaneous, and SRS are shown in figure 2.1. CARS was widely used in biophotonics before the demonstration of SRS by Freudiger et al. [14]. CARS has heterodyne contributions that can lead to anti-stoke shifted signals that are off-resonant, resulting in a distorted spectrum. SRS is restricted to two pathways that can only happen if the molecule has $\Omega = \omega_{pump} - \omega_{Stokes}$. One significant advantage of SRS is that it is free from the nonresonant background observed in CARS [13,15-18]. Also, the SRS signal is linearly proportional to concentration [17,18]. Since the introduction of SRS, it has been a powerful alternative to fluorescence and CARS microscopy in biophotonics.

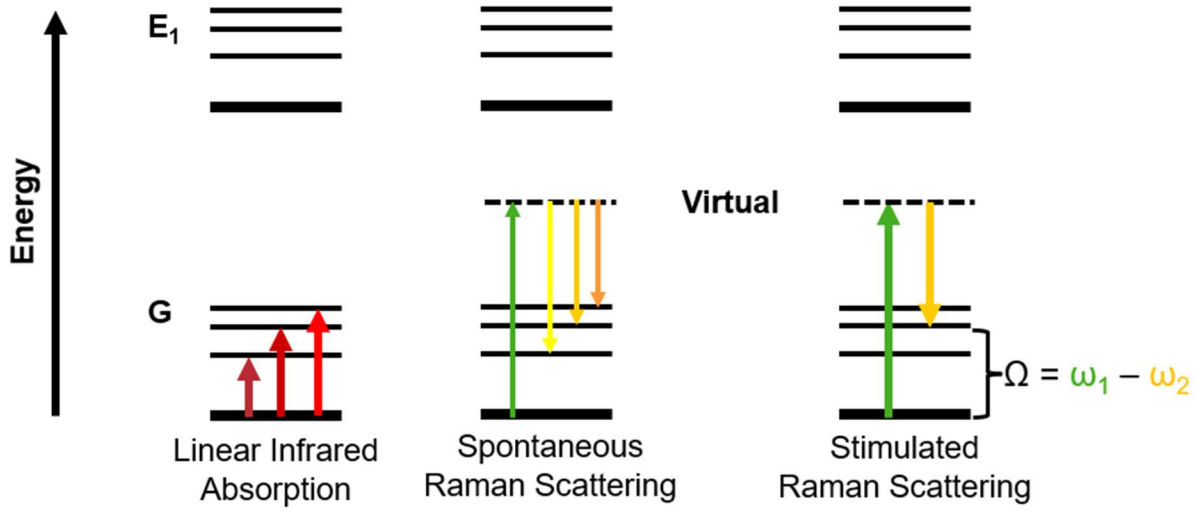

Figure 2.1 Energy Level Diagram of IR Absorption, Spontaneous & Stimulated Raman Scattering

2.3 Transporter Inhibitors and Tyrosine Kinase Inhibitors

Tyrosine Kinase Inhibitors lapatinib (GW-572016, SelleckChem), afatinib (BIBW2992, SelleckChem), and osimertinib (AZD9291, SelleckChem) were investigated. Human organic

cation transporter (hOCT1, SLC22A1 gene), hOCT2 (SLC22A2 gene), hOCT3 (SLC22A3 gene), multidrug and toxin extrusion 1 (MATE1, SLC47A1 gene) were treated with amantadine, corticosterone, procainamide, prazosin, nicotinamide, and pyrimethamine. Each drug inhibits one or two transporters; the details are described in table 2.1.

2.4 Cell Preparation

A459 cells were cultured in F12K media and 5% CO2 atmosphere. Cells were preincubated with 5 μM of one of the transporter Inhibitors described previously for 24 hours. After, 2 μM of one of the TKIs was added for 24 hours until the cells were fixed on the coverslip for the imaging.

2.5 SRS Imaging

SRS images were acquired using a femtosecond dual-output laser (Spectra-Physics Insight DS+) which emits a tunable beam (680-1300 nm) and fixed beam (1040 nm) pulse trains, known as the pump and Stokes beams, respectively. The pump beam was fixed at 912 nm to collect fingerprint SRS images of live A549 cells. The pump and Stokes beams were chirped using high refractive index glass rods. Images were collected from 1300 cm^{-1} to 1500 cm^{-1} with a spectral resolution of 11 cm^{-1}. Stokes beam was broadened by coupling with Ytterbium-doped single mode double-clad fiber (YB1200-6/125 DC-PM, Thorlabs) [17]. The microscope is a Nikon Eclipse FN1 with a 40x/1.15 NA water immersion objective. A schematic diagram of the setup is shown in figure 2.2. Pump beam power was held constant at 40 mW at focus, and Stokes power was held at 60~100mW at focus. All images collected were 512 × 512 pixels.

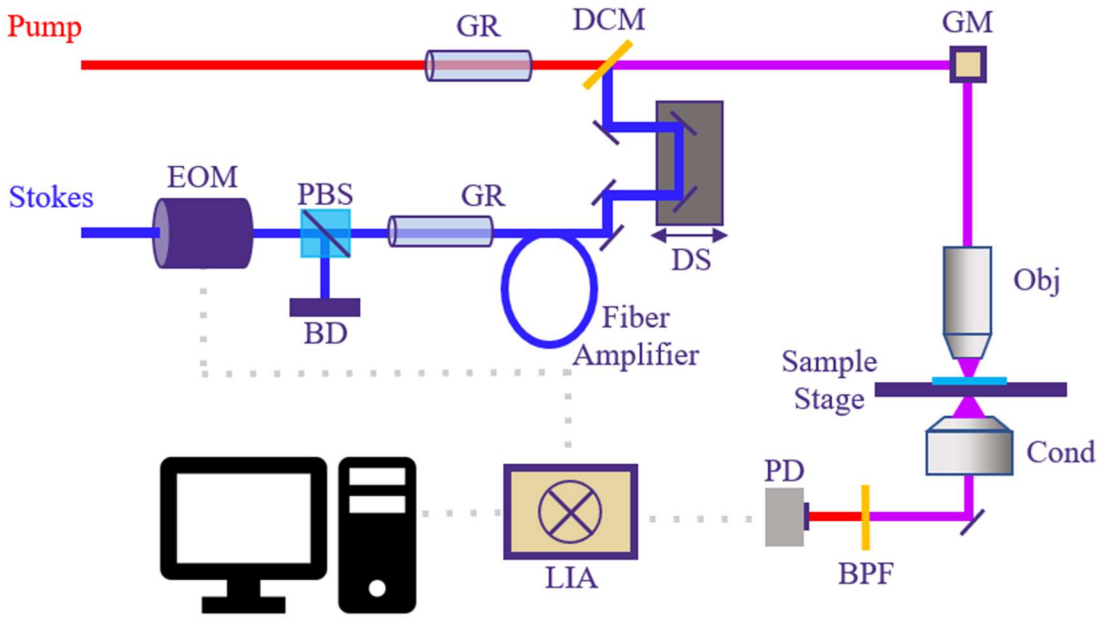

Figure 2.2 A Schematic Diagram of SRS Microscope Setup
DS = Delay stage; DCM = dichroic mirror; GM = galvo mirrors; EOM = electro-optic modulator; PBS = polarizing beam splitter; PD = photodiode; GR = highly dispersive dense glass rod; BD = beam dump; BPF = bandpass filter; LIA = lock-in amplifier.

2.6 Data Analysis

A data analysis pipeline for hyperspectral images of live cells was developed, and the procedure is as follows (figure 2.3). The background signal was subtracted, and the hyperspectral images were denoised using block-matching and 3-D filtering (BM3D) algorithms customized to denoise hyperspectral data (figure 2.3 A). As mentioned briefly, the SRS signal is linearly proportional to the chemical concentration. A simple, built-in MATLAB least linear square fitting algorithm was used to unmix the spectrum at each pixel of images to four different components: TKI, cytoplasm, nucleus, and background (figure 2.3 C). TKI concentrations inside the cell were averaged per field of view to generate the total average concentration.

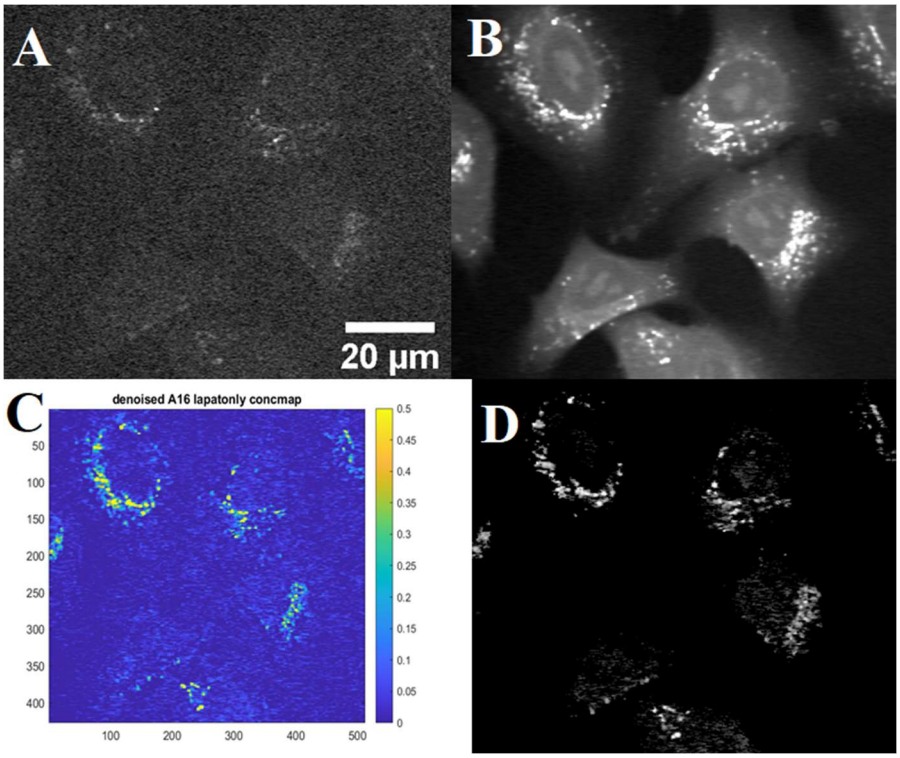

Figure 2.3 Data Analysis Procedure Showing One of Lapatinib-Dosed Images
A. Original hyperspectral data. Background signal is subtracted and denoised using BM3D algorithm to create B. Least linear curve fitting with a non-negative constraint using a calibration matrix consisting of four pure spectra (TKI, cytoplasm, nucleus, and background) generated a concentration map shown in C. Binary mask was made by thresholding B so that only cell features were remaining. Combining B and C resulted in D, which was used to produce graphs on figure 2.3. A16 denotes the image number. Concmap = concentration map

2.7 Results

A total of 18 TKI-influx inhibitor combinations were examined, with three tyrosine kinase inhibitors and six different SLC inhibitors (Table 2.1). Note that multidrug and toxin extrusion 1 (MATE1) protein, although widely known for regulating the efflux of toxic chemicals out of the cell, is included in the study [23,24]. Some reports show that MATE1 regulates cellular uptake of imatinib (not examined in this study) [19]. The results of the examinations are listed in Figure 2.4.

Lapatinib concentration inside cells increased when OCT2 and OCT3 were inhibited. Inhibition of MATE1 also increased lapatinib concentration. An increase in concentration could mean that the transporters that were inhibited are likely to regulate drug efflux. Although studies show that MATE1 regulates the efflux of cytotoxic drugs, there is little to no evidence that organic cation transporters are responsible for drug extrusion[23]. Osimertinib concentration increased when both OCT1 and OCT2 were inhibited simultaneously by procainamide, and its concentration was increased when OCT3 was explicitly inhibited by corticosterone. Afatinib concentration decreased when OCT1 and OCT3 were inhibited; the concentration also decreased when MATE1 was inhibited. Controls, which have no tyrosine kinase inhibitors, showed that at least 1 mM of TKIs are present. This is due to the data analysis process in which a non-negative constraints algorithm was used with linear curve fitting.

SLC Inhibitors	Transporter(s)
Procainamide	OCT1 & OCT2
Amantadine	OCT1 & OCT2
Prazosin	OCT1 & OCT3
Corticosterone	OCT3
Methylnicotinamide	OCT2
Pyrimethamine	MATE1

Tyrosine Kinase Inhibitors
Lapatinib
Osimertinib
Afatinib

Table 2.1 List of Tyrosine Kinase Inhibitors and Solute Carrier Inhibitors with Their Corresponding Target(s)

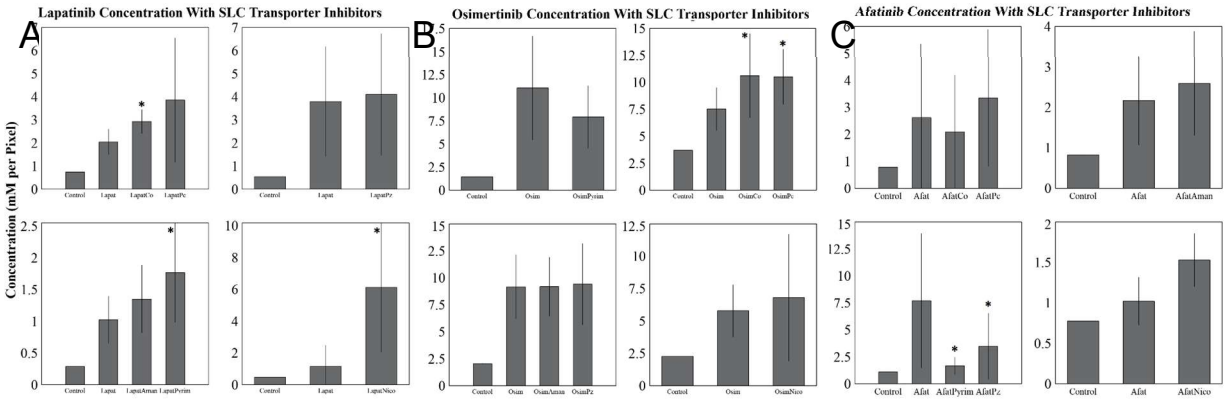

Figure 2.4 Tyrosine Kinase Inhibitor Concentrations with Different SLC Inhibitors
Each graph was collected on different days. Control = DMSO-dosed A549 cells (no drug). A: lapatinib concentrations. Lapat = lapatinib. B: Osimertinib concentrations. Osim = osimertinib. C: Afatinib concentrations. Afat = afatinib. For all graphs, Co = corticosterone, Pc = procainamide, Aman = amantadine, Pz = prazosin, Nico = N-Methylnicotinamide, Pyrim = pyrimethamine. The bar graphs show total average of all field of views. Standard deviations of average are shown as error bars. * = p-value < 0.05 compared to TKI-only dosed sample. One pixel = 0.03815 μm^2

2.8 Discussion and Future Work

Here, preliminary results of interactions between tyrosine kinase inhibitors and solute carrier transporters were presented. Although the study showed that the inhibition of MATE1 increased the concentration of lapatinib, other findings suggest that organic cation transporters could regulate drug efflux, which cannot be found in other studies. Some results also exhibit inconsistency. Procainamide and amantadine inhibit the same organic cation transporters, OCT1 and OCT2. However, osimertinib concentration increased with procainamide but not when amantadine was pre-dosed. Indeed, the drugs used to inhibit various solute carriers in this study could affect different parts of the cell that change its influx and efflux behavior. Affinity differences between transporter inhibitors and TKIs could also be examined more carefully to increase the validity of the results.

Multiple studies have shown that tyrosine kinase inhibitors are substrates and inhibitors of transporters [8]. Developing chemotherapy plans include using TKIs as efflux transporter inhibitors instead of traditional inhibitors to increase the cytotoxic activity of chemotherapeutic drugs while decreasing the activity of EGFR [5,21]. Little is known about the substrate/inhibitor relationship between solute carriers and TKIs used in this study (lapatinib, osimertinib, afatinib). Investigating these relationships could help further the interpretation of the results.

One of the biggest challenges in this study is reproducibility. It is challenging to produce similar results with varying electronics to optical processes and cell conditions when the experiments are done over multiple days and weeks. These variances prevent collecting reliable data that can be compared from one day to another. TKI concentrations on TKI-only dosed cells vary from one experiment to another (figure 2.4). Scrutinizing these random variables to produce more reliable and consistent data is required to validate the results.

Finally, on-going studies in the group, such as membrane permeability of TKIs and examining TKI-resistant cell lines, will be used along with this study to investigate TKI uptake and efflux in cancer cells. Further experiments on efflux transporters and 3D cancer cell spheroids will be conducted to enhance our knowledge of drug movement and multidrug resistance.

Bibliography

1. "Cancer Chemotherapy Market - Global Industry Analysis, Market Size, Opportunities and Forecast 2020 - 2027." *Acumen Research and Consulting*, https://www.acumenresearchandconsulting.com/cancer-chemotherapy-market.
2. Islami F, Ward EM, Sung H, et al. Annual report to the nation on the status of cancer, Part 1: National cancer statistics. *JNCI*. July 8, 2021. DOI: 10.1093/jnci/djab131.
3. Azvolinsky, Anna "Infographic: Mechanisms of Resistance." *The Scientist Magazine®*, 31 Mar. 2017, https://www.the-scientist.com/multimedia/infographic-mechanisms-of-resistance-31747.
4. Holohan, C., Van Schaeybroeck, S., Longley, D. *et al.* "Cancer drug resistance: an evolving paradigm." *Nat Rev Cancer* 13, 714–726 (2013). https://doi.org/10.1038/nrc3599
5. Seshacharyulu, Parthasarathy, et al. "Targeting the EGFR Signaling Pathway in Cancer Therapy." *Expert Opinion on Therapeutic Targets*, vol. 16, no. 1, 2012, pp. 15–31., https://doi.org/10.1517/14728222.2011.648617.
6. Araki, Takuya, et al. "Review of the Treatment of Non-Small Cell Lung Cancer with Gefitinib." *Clinical Medicine Insights: Oncology*, vol. 6, 2012, https://doi.org/10.4137/cmo.s7340.
7. Arora, Amit, and Eric M. Scholar. "Role of Tyrosine Kinase Inhibitors in Cancer Therapy." *Journal of Pharmacology and Experimental Therapeutics*, vol. 315, no. 3, 2005, pp. 971–979., https://doi.org/10.1124/jpet.105.084145.
8. Krchniakova, Maria, et al. "Repurposing Tyrosine Kinase Inhibitors to Overcome Multidrug Resistance in Cancer: A Focus on Transporters and Lysosomal Sequestration." *International Journal of Molecular Sciences*, vol. 21, no. 9, 2020, p. 3157., https://doi.org/10.3390/ijms21093157.
9. Chen, Zhen, et al. "Osimertinib (AZD9291) Enhanced the Efficacy of Chemotherapeutic Agents in ABCB1- and ABCG2-Overexpressing Cells *in Vitro, in Vivo*, and *Ex Vivo*." *Molecular Cancer Therapeutics*, vol. 15, no. 8, 2016, pp. 1845–1858., https://doi.org/10.1158/1535-7163.mct-15-0939.
10. Zhang, Yan, et al. "Afatinib Decreases p-Glycoprotein Expression to Promote Adriamycin Toxicity of A549T Cells." *Journal of Cellular Biochemistry*, vol. 119, no. 1, 2017, pp. 414–423., https://doi.org/10.1002/jcb.26194.
11. Dai, Chun-ling, et al. "Lapatinib (Tykerb, GW572016) Reverses Multidrug Resistance in Cancer Cells by Inhibiting the Activity of ATP-Binding Cassette Subfamily B Member 1 and G Member 2." *Cancer Research*, vol. 68, no. 19, 2008, pp. 7905–7914., https://doi.org/10.1158/0008-5472.can-08-0499.
12. Radic-Sarikas, B., Halasz, M., Huber, K.V.M. *et al.* "Lapatinib potentiates cytotoxicity of YM155 in neuroblastoma via inhibition of the ABCB1 efflux transporter." *Sci Rep* 7, 3091 (2017). https://doi.org/10.1038/s41598-017-03129-6
13. Freudiger, Christian W., et al. "Label-Free Biomedical Imaging with High Sensitivity by Stimulated Raman Scattering Microscopy." *Science*, vol. 322, no. 5909, 2008, pp. 1857–1861., https://doi.org/10.1126/science.1165758.

14. Freudiger, Christian W., et al. "Label-Free Biomedical Imaging with High Sensitivity by Stimulated Raman Scattering Microscopy." *Science*, vol. 322, no. 5909, 2008, pp. 1857–1861., https://doi.org/10.1126/science.1165758.
15. Manifold, Bryce, and Dan Fu. "Quantitative Stimulated Raman Scattering Microscopy: Promises and Pitfalls." *Annual Review of Analytical Chemistry*, vol. 15, no. 1, 2022, pp. 269–289., https://doi.org/10.1146/annurev-anchem-061020-015110.
16. Cheng, Ji-Xin, et al. *Stimulated Raman Scattering Microscopy: Techniques and Applications*. Elsevier, 2022.
17. Benjamin Figueroa, Walter Fu, Tai Nguyen, Kseniya Shin, Bryce Manifold, Frank Wise, and Dan Fu, "Broadband hyperspectral stimulated Raman scattering microscopy with a parabolic fiber amplifier source," Biomed. Opt. Express 9, 6116-6131 (2018)
18. Fu, Dan, et al. "Hyperspectral Imaging with Stimulated Raman Scattering by Chirped Femtosecond Lasers." *The Journal of Physical Chemistry B*, vol. 117, no. 16, 2013, pp. 4634–4640., https://doi.org/10.1021/jp308938t.
19. Minematsu, Tsuyoshi, and Kathleen M. Giacomini. "Interactions of Tyrosine Kinase Inhibitors with Organic Cation Transporters and Multidrug and Toxic Compound Extrusion Proteins." *Molecular Cancer Therapeutics*, vol. 10, no. 3, 2011, pp. 531–539., https://doi.org/10.1158/1535-7163.mct-10-0731.
20. Thomas, Julia, et al. "Active Transport of Imatinib into and out of Cells: Implications for Drug Resistance." *Blood*, vol. 104, no. 12, 2004, pp. 3739–3745., https://doi.org/10.1182/blood-2003-12-4276.
21. Chang YS, Choi CM, Lee JC. "Mechanisms of Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor Resistance and Strategies to Overcome Resistance in Lung Adenocarcinoma." Tuberc Respir Dis (Seoul). 2016 Oct;79(4):248-256. doi: 10.4046/trd.2016.79.4.248. Epub 2016 Oct 5. PMID: 27790276; PMCID: PMC5077728.
22. Gottesman MM. "Mechanisms of cancer drug resistance." Annu Rev Med. 2002;53:615-27. doi: 10.1146/annurev.med.53.082901.103929. PMID: 11818492.
23. Nies, A.T., Koepsell, H., Damme, K., Schwab, M. (2011). "Organic Cation Transporters (OCTs, MATEs), In Vitro and In Vivo Evidence for the Importance in Drug Therapy. In: Fromm, M., Kim, R. (eds) Drug Transporters. Handbook of Experimental Pharmacology, vol 201. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-14541-4_3
24. Yao, X., Watkins, N.H., Brown-Harding, H. *et al.* "A membrane transporter determines the spectrum of activity of a potent platinum–acridine hybrid anti-cancer agent." *Sci Rep* 10, 15201 (2020). https://doi.org/10.1038/s41598-020-72099-z