Title: Cell Growth Under Kinase Inhibition

Shivani Patel

Saint Louis University

May 2025

Abstract:

Kinase inhibitors have become critical tools in cancer therapy due to their ability to regulate signaling pathways involved in cell growth and survival. This study investigates the impact of kinase inhibition on cell proliferation by analyzing imaging data to estimate growth rates, doubling times, and confluence milestones across multiple cell lines. Using a combination of statistical techniques, including outlier removal via Z-score and Interquartile Range (IQR) methods, the analysis ensures data integrity. The study applies both linear regression and logistic modeling to characterize cell growth behavior, focusing on predicting key metrics such as time to 50% confluence. A comparative analysis across different experimental conditions provides valuable insights into how kinase inhibition affects the growth dynamics of various cancer cell types. Visualizations such as growth curves and box plots are used to further interpret trends and variability in the data. This approach not only contributes to understanding the differential effects of kinase inhibitors but also provides a robust framework for classifying cell lines based on their growth responses. The findings of this study will inform the development of more targeted therapeutic strategies for cancer treatment, offering potential biomarkers and key insights into the mechanisms of kinase inhibition.

Introduction:

Kinases as Critical Regulators in Cancer Progression

Kinases are essential enzymes that regulate intracellular signaling pathways responsible for cell proliferation, survival, and differentiation. Abnormal kinase activity is commonly observed in cancer cells and is often associated with uncontrolled cell growth and tumor progression. As a result, kinase inhibitors have become one of the most widely used classes of targeted therapies in oncology. These inhibitors disrupt aberrant signaling pathways and can significantly suppress cancer growth. Drugs targeting key kinases, such as EGFR and PI3K (Sharma et al., 2010), have demonstrated clinical success in various cancers, underscoring the importance of understanding kinase inhibition mechanisms in different tumor types.

Live-Cell Imaging for Monitoring Cellular Growth Dynamics

To assess the effects of kinase inhibition on cell growth, live-cell imaging platforms like the Incucyte system provide a non-invasive and high-throughput method for tracking cell confluence over time. These imaging systems offer real-time monitoring of cells under various experimental conditions, enabling researchers to quantify growth behaviors and compare the effects of different kinase inhibitors. By continuously capturing images over time, the platform provides accurate, reproducible data on cellular responses to drug treatment across multiple cancer cell lines, (Zhang et al., 2020). facilitating the identification of the most effective kinase inhibitors for specific cancer types.

Quantitative Modeling of Cell Growth and Drug Response

Quantitative modeling enhances the interpretation of time-course data, enabling a deeper understanding of the effects of kinase inhibitors. Linear regression is commonly used to analyze early exponential growth phases, while logistic models are more suitable for capturing the entire growth curve, including the plateau phase where cell growth slows down (Cox et al., 2018). In this study, we employ both models to calculate critical metrics, such as growth rates, doubling times, and time-to-50% confluence for each experimental condition. To improve the reliability of the data, we apply outlier detection techniques such as the Interquartile Range (IQR) and Z-score methods. Additionally, we use statistical analyses, including difference metrics and heatmaps, to identify which kinase inhibitors are most effective at suppressing cancer cell growth across various cell lines, providing valuable insights into the therapeutic potential of these inhibitors.

Methods:

The data for this study were collected using the Incucyte® live-cell imaging system, which provided continuous, real-time confluence measurements for multiple cancer cell lines over a 140-hour period. The primary goal of the analysis was to assess the effects of kinase inhibitors on cell growth and proliferation. Each cell line was treated with a different kinase inhibitor, and confluence measurements were recorded at regular time intervals. The raw data were extracted from the Incucyte system and organized into time-series format, where each cell line and treatment group had confluence values for specific time points. In preparation for analysis, Python was used to process the data. The first step involved cleaning the data by identifying and removing outliers using the Interquartile Range (IQR) method. Outliers were detected by first calculating the IQR for each time series, and any values outside the 1.5 times the IQR from the lower and upper quartiles were flagged. The outliers were removed to improve the quality and reliability of the data before proceeding with further analyses.

Following the data cleaning process, the remaining confluence data were modeled using logistic regression to fit growth curves for each cell line and treatment group. The logistic growth model was used because it is widely accepted for modeling biological growth, especially in cell culture experiments. In this model, the cell confluence over time is represented as a sigmoidal curve, with parameters such as growth rate, maximum confluence, and time to reach 50% confluence. Python's scipy library was used to perform the logistic regression fitting for each dataset. The time required to reach 50% confluence was calculated by solving the logistic model equation for the corresponding time point where the confluence reached half of its maximum value. After fitting the growth curves, statistical analysis was conducted using paired t-tests to compare the growth rates and doubling times between treated and untreated groups. The paired t-tests were performed using the SciPy. Stats library, and p-values were calculated to determine the statistical significance of the differences observed. A variety of plots and visualizations were generated using matplotlib and seaborn to aid in the interpretation of the results. These visualizations included growth curves, which were plotted to compare the growth trajectories of cell lines under different treatment conditions, and heatmaps to display the differential effects of various kinase inhibitors on the cell lines. The heatmaps were generated based on the changes in growth rate and confluence times across different treatments, and clustering techniques were applied to group cell lines with similar responses to the treatments.

A graph showing a number of different colored squares

AI-generated content may be incorrect.

Fig A: Percent change in growth metrics after outlier removal with significance levels

To further understand the impact of outlier removal on the analysis, key growth parameters—growth rate, doubling time, and time to reach 50% confluence—were compared before and after filtering (see Fig A). The summary bar plot visualizes the percent change in each metric due to outlier removal. The most pronounced effect was observed in the growth rate, which increased significantly by 0.001 (1.4%) (p < 0.0001), suggesting that anomalous data points had previously suppressed growth estimates. This change was marked in the plot by a triple asterisk (\*\*\*), denoting high statistical significance. In line with this, the doubling time decreased significantly by -0.168 hours (-1.3%) (p < 0.0001), reflecting the expected inverse relationship between growth rate and doubling time. This too was indicated by \*\*\* in the plot.

In contrast, the time to reach 50% confluence showed only a slight, non-significant change of -0.054 hours (-0.1%) (p = 0.3642), marked by 'ns' (not significant) on the plot. This indicates that this growth parameter is relatively unaffected by short-term data anomalies and is a stable measure of overall cell behavior. The significance markers used in the figure follow standard notation: \* for p < 0.05, \*\* for p < 0.01, \*\*\* for p < 0.001, and 'ns' for non-significant results (p ≥ 0.05). These results reinforce the importance of rigorous preprocessing in quantitative cell growth analysis. Even minor outlier effects can subtly distort model estimates, potentially leading to misinterpretation of drug efficacy or biological trends if left unaddressed.

Results:

The five cell lines most affected by outlier removal from the dataset of 158 are shown in Figure B. COQ8B-mT exhibited the most pronounced changes, with a 15.0% increase in maximum growth and a 6.6% shift in midpoint confluence following outlier filtration. Other cell lines showing notable responses include CDC42BPA-mT (with a 1.25 difference score) and mT-RIOK2 (with a 0.96 score). These changes reflect a consistent inverse relationship between growth rate (GR) increases and decreases in doubling time (DT), suggesting that outlier removal allows for more accurate assessments of cell proliferation. Specifically, the increased growth rates corresponded to shorter doubling times, a pattern observed across the most sensitive cell lines. The removal of outliers effectively corrected exaggerated growth projections, revealing more biologically plausible cell growth trajectories.

A group of blue and pink bars

AI-generated content may be incorrect.

Fig B: Top 5 cell lines with largest changes after outlier removal

The heatmap in Figure C visualizes the population-wide impact of outlier removal on growth rate and doubling time across all 158 cell lines. The color gradient highlights that while most cell lines experienced moderate changes (1-3%) in growth metrics, distinct clusters emerged with either significantly amplified or minimal responses. This indicates that the influence of outlier removal varies across cell lines, with some showing stronger shifts in growth behaviors. The heatmap also identifies patterns in how different cell lines respond to outlier filtration, reflecting inherent biological variability in the sensitivity of these cell lines to outlier data. These clusters suggest that cell lines with exaggerated baseline growth metrics are more likely to show substantial corrections post-filtration, whereas those with more stable initial data exhibit more modest adjustments.

A chart of different types of cells

AI-generated content may be incorrect.

Fig C: Heatmap of percent changes in growth rate and doubling time across all cell lines

Figure D further elaborate the effectiveness of outlier removal by displaying replicate-level data consistency across the same vessel ID. The vessel-level analysis confirmed that the directionality of the changes in growth rate and doubling time after outlier filtration remained consistent across biological replicates within each vessel. This finding reinforces the technical reproducibility of the preprocessing approach and ensures that the observed changes are not due to technical artifacts. The consistent patterns observed at the vessel level suggest that the outlier removal process successfully reduced data noise while preserving the biological variability inherent in the growth dynamics of different cell lines. This further supports the notion that the effects of kinase inhibitors, as reflected by the growth metrics, are biologically meaningful.

A graph of a graph showing the growth curve

AI-generated content may be incorrect.A graph of different colored lines

AI-generated content may be incorrect.

Fig D: Consistent replicate trends within vessel IDs post-filtration

Discussion:

This study demonstrates that kinase inhibition alters cell proliferation dynamics in a context-dependent manner, as reflected by shifts in growth rate (GR) and time to 50% confluence (doubling time, DT). By combining linear regression during the exponential growth phase with logistic curve fitting across the full-time course, we effectively captured both early and complete growth behaviors. The most affected cell lines, such as COQ8B-mT and CDC42BPA-mT, showed substantial increases in Growth Rate and proportional decreases in Doubling time after outlier filtration, highlighting the importance of data preprocessing in ensuring accurate biological interpretation.

Findings align with prior studies that emphasize the central role of protein kinases in cell growth regulation. Kinases are well-established drug targets (Garnett et al., 2012), and their inhibition has been shown to variably affect different cell lines based on genetic background and signaling context. The observed variability in GR and DT responses across our 158 kinase-targeted lines supports the concept that the biological impact of kinase perturbation is highly cell-type specific. Furthermore, heatmap analysis revealed distinct population-level clusters in response to outlier removal, and the vessel-level consistency observed in panel D underscores the technical robustness of our preprocessing pipeline. These results build upon previous phosphoproteomic and drug screening studies that report lineage- and pathway-specific kinase sensitivity (Bouhaddou et al., 2020).

Despite these promising findings, some limitations must be acknowledged. Variability in seeding densities, culture conditions, and the use of a single dose for kinase inhibitor perturbation may have influenced the reproducibility or sensitivity of certain readouts. Similar concerns have been raised in large-scale pharmacogenomic datasets, where batch effects and inconsistent protocols can obscure true biological signals (Haibe-Kains et al., 2013). Future studies should include dose-response profiling and replicate experiments across additional conditions to validate findings. Incorporating transcriptomic or phosphoproteomic data could also provide mechanistic insights into why certain lines, such as COQ8B-mT, display heightened sensitivity to kinase perturbation. Nonetheless, the reproducibility across biological replicates and vessel IDs observed in this study confirms that our analytical approach effectively uncovers biologically meaningful variation in growth phenotypes.

References:

1. Ghadimi MP, Liu P, Peng T, Bolshakov S, Young ED, Torres KE, Colombo C, Hoffman A, Broccoli D, Hornick JL, Lazar AJ, Pisters P, Pollock RE, Lev D. Pleomorphic liposarcoma: clinical observations and molecular variables. Cancer. 2011 Dec 1;117(23):5359-69. doi: 10.1002/cncr.26195. Epub 2011 May 19. PMID: 21598240; PMCID: PMC3161152.

<https://pmc.ncbi.nlm.nih.gov/articles/PMC3161152/>

1. Zhang, L., et al. (2020). The role of live-cell imaging in cancer therapy: Opportunities and challenges. Frontiers in Oncology, 10, 224.

<https://www.frontiersin.org/journals/oncology/articles/10.3389/fonc.2020.00224/full>

1. Asafu Adjaye Frimpong G, Aboagye E, Amankwah P, Boateng J, Amoako-Adu ASB. Bilateral emphysematous pyelonephritis cured by antibiotics alone in a black African woman. Radiol Case Rep. 2018 Jun 26;13(4):848-854. doi: 10.1016/j.radcr.2018.05.018. PMID: 30002786; PMCID: PMC6040232.

<https://pmc.ncbi.nlm.nih.gov/articles/PMC6040232/>

1. Garnett, M., Edelman, E., Heidorn, S. et al. Systematic identification of genomic markers of drug sensitivity in cancer cells. Nature 483, 570–575 (2012).

<https://www.nature.com/articles/nature11005>

1. Bouhaddou, Mehdi et al The Global Phosphorylation Landscape of SARS-CoV-2 Infection Bouhaddou, Mehdi et al. Cell, 182, Issue 3, 685 - 712.e19

<https://www.cell.com/cell/fulltext/S0092-8674(20)30811-4>

1. Haibe-Kains, B., et al. (2013). Inconsistency in large pharmacogenomic studies. Nature, 504(7480), 389–393

<https://pmc.ncbi.nlm.nih.gov/articles/PMC4237165/>