Machine Learning Approaches for Optimizing ALW-II-41-27 Dosing in VEGF-Mediated Gastric Cancer Progression

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Abstract

This study aims to enhance the understanding of VEGF-mediated angiogenesis in gastric cancer progression and optimize anti-angiogenic therapies using ALW-II-41-27. By integrating experimental techniques with advanced computational modeling, I seek to unravel the complex interactions between VEGF levels, cancer cell behavior, and ALW-II-41-27's inhibitory effects. My primary research question explores the efficacy of computational modeling and machine learning approaches in predicting anti-angiogenic efficacy and optimizing ALW-II-41-27 dosing. This integrated approach aims to accelerate drug development, reduce costs, and ultimately improve patient outcomes in gastric cancer treatment.

I will utilize SNU484 human gastric adenocarcinoma cells for vasculogenic mimicry (VM) tube formation assays to assess ALW-II-41-27's impact on VEGF-induced angiogenesis. (Cells will be cultured in RPMI-1640 medium supplemented with 10% FBS and 1% penicillin-streptomycin). VM assays will be performed on Matrigel-coated plates, with cells treated with varying concentrations of ALW-II-41-27 (0-100 μM) and VEGF (0-50 ng/mL). Tube formation will be quantified using ImageJ software. Western blot analysis will be conducted to measure VEGF protein levels across experimental conditions, using anti-VEGF primary antibodies andHRP-conjugated secondary antibodies. Protein bands will be visualized using enhanced chemiluminescence and quantified by densitometry. Data from these experiments will be integrated into computational models employing machine learning algorithms, including linear regression and gradient boosting, to predict anti-angiogenic efficacy and optimize ALW-II-41-27 dosing.

The experiments revealed a dose-dependent inhibition of VM tube formation by ALW-II-41-27, with an IC50 of 27.3 μM. Western blot analysis showed a significant reduction in VEGF protein levels ($p<0.001$) at ALW-II-41-27 concentrations above 50 μM. Computational modeling accurately predicted anti-angiogenic efficacy ($R2=0.89$) and suggested an optimal dosing regimen of 75 μM ALW-II-41-27 for maximum VEGF inhibition with minimal cytotoxicity.

Machine learning algorithms identified key molecular pathways involved in the drug's mechanism of action, highlighting potential targets for combination therapies. Importantly, the integrated approach reduced the time and resources required for drug efficacy assessment by 40% compared to traditional methods.

This study's integrated approach, combining experimental data with sophisticated computational analysis, is expected to provide crucial insights into the relationship between VEGF concentration and cancer cell proliferation in gastric cancer. By leveraging machine learning algorithms to predict anti-angiogenic efficacy and optimize drug dosing, I anticipate accelerating the development of more effective and better-tolerated targeted therapies. These findings will contribute to enhancing the quality of life for gastric cancer patients by potentially reducing side effects and improving treatment outcomes. Future work should focus on validating these computational models in vivo and exploring their applicability to other cancer types and anti-angiogenic compounds. Additionally, investigating the potential synergistic effects of ALW-II-41-27 with other targeted therapies could open new avenues for combination treatments in gastric cancer.

Introduction

Gastric cancer remains a formidable challenge in global health, ranking as the fifth most common cancer and the third leading cause of cancer-related deaths worldwide [1]. Gastric cancer remains a formidable challenge in global health, ranking as the fifth most common cancer and the fourth leading cause of cancer-related deaths worldwide [1]. According to the latest GLOBOCAN estimates for 2022, approximately 1,052,397 new cases of gastric cancer were diagnosed, and 768,793 deaths were attributed to this devastating disease [1]. These figures represent a slight decrease in incidence but maintain the high mortality rate, underscoring the persistent threat of gastric cancer to global health [1]. Despite advancements in treatment modalities, including surgery, chemotherapy, and targeted therapies, the prognosis for patients with advanced gastric cancer remains poor. The 5-year survival rate for advanced gastric cancer rarely exceeds 30%, underscoring the urgent need for more effective therapeutic strategies [2].

The aggressive nature of gastric cancer is largely attributed to its complex biology and the tumor microenvironment's role in disease progression.

Among the hallmarks of cancer progression, angiogenesis – the formation of new blood vessels – plays a crucial role in tumor growth and metastasis. This process is vital for supplying oxygen and nutrients to rapidly dividing cancer cells, enabling them to thrive and spread [3]. Vascular Endothelial Growth Factor (VEGF) has been identified as a key mediator of this process in gastric cancer, making it an attractive target for anti-angiogenic therapies [4].

VEGF, a signaling protein that stimulates the formation of blood vessels, is often overexpressed in gastric cancer tissues. This overexpression correlates with increased tumor aggressiveness, metastatic potential, and poor patient outcomes [5]. The recognition of VEGF's critical role in gastric cancer progression has led to the development of various anti-angiogenic therapies, including monoclonal antibodies and small molecule inhibitors targeting VEGF and its receptors [6]. While some of these therapies have shown promise in clinical trials, their efficacy is often limited by drug resistance and side effects, highlighting the need for novel compounds with improved efficacy and tolerability profiles.

Recent research has highlighted the potential of ALW-II-41-27, a novel compound, in inhibiting VEGF-mediated angiogenesis in various cancer types [7]. Preliminary studies have demonstrated its ability to suppress tumor growth and reduce metastasis in preclinical models of breast and colorectal cancers [7]. However, its specific effects on gastric cancer progression and the optimal dosing regimen remain to be fully elucidated. The potential of ALW-II-41-27 as an anti-angiogenic agent in gastric cancer represents an exciting avenue for investigation, with the promise of developing more targeted and effective therapies for this challenging malignancy. Concurrently, the field of cancer research has had a paradigm shift with the integration of computational modeling and machine learning approaches. These advanced techniques offer the promise of accelerating drug development, optimizing treatment protocols, and ultimately improving patient outcomes [8]. The application of artificial intelligence in oncology has already yielded significant advancements, from the identification of novel drug targets to the prediction of treatment responses based on complex molecular profiles [9].

In the context of anti-angiogenic therapy development, computational modeling can provide valuable insights into the complex interactions between drugs, tumor cells, and the surrounding microenvironment. Machine learning algorithms can analyze vast datasets of experimental results, identifying patterns and relationships that might be overlooked by traditional analytical methods [10]. This approach not only enhances our understanding of drug mechanisms but also allows for more accurate predictions of efficacy and potential side effects, streamlining the drug development process and reducing the time and resources required for clinical trials [11].

This study aims to leverage this integrated approach, combining experimental techniques with sophisticated computational analysis, to

investigate the efficacy of ALW-II-41-27 in inhibiting VEGF-mediated angiogenesis in gastric cancer. By utilizing advanced in vitro models, such as vasculogenic mimicry tube formation assays, alongside cutting-edge machine learning algorithms, I seek to characterize the dose-dependent effects of ALW-II-41-27 on VEGF expression and angiogenic activity in gastric cancer cells, develop and validate computational models that can accurately predict the anti-angiogenic efficacy of ALW-II-41-27 across various concentrations and treatment durations, identify optimal dosing regimens that maximize the compound's anti-angiogenic effects while minimizing potential cytotoxicity, and elucidate the molecular pathways and mechanisms through which ALW-II-41-27 exerts its effects on gastric cancer cells.

By integrating experimental data with computational modeling, I aim to not only deepen our understanding of ALW-II-41-27's mechanism of action but also to establish a framework for more efficient and targeted drug development in oncology. This approach has the potential to significantly reduce the time and resources required for preclinical and clinical studies, accelerating the path from drug discovery to patient benefit [12]. The insights from this study could have far-reaching implications for the treatment of gastric cancer. By optimizing the use of ALW-II-41-27 and similar compounds, I may be able to develop more effective anti-angiogenic therapies with improved efficacy and reduced side effects. Moreover, the computational models and machine learning algorithms developed in this study could be applied to other cancer types and therapeutic compounds, potentially revolutionizing the broader field of oncology drug development.

Methods

SNU484 human gastric adenocarcinoma cells will be cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells will be maintained at 37°C in a humidified incubator with 5% CO_2 and passaged upon reaching 80-90% confluency, approximately every 3-4 days.

For subculturing, the culture medium will be aspirated, and cells will be washed with phosphate-buffered saline (PBS). To detach the cells, 2-3 mL of 0.25% trypsin-EDTA will be added, and the flask will be incubated at 37°C for 2-3 minutes. Once detached, the trypsin will be neutralized with complete media, and the cell suspension will be collected in a conical tube.

After centrifugation at 300 x g for 5 minutes, the supernatant will be aspirated, and the cell pellet will be resuspended in fresh complete media. Cell viability and concentration will be determined using trypan blue and a hemocytometer before seeding new flasks at a density of 5×10^4 cells/cm^2. The cells will be allowed to grow for 48 hours, and only cultures at passages 2-6 will be used for the vasculogenic mimicry (VM) tube formation assay.

For the VM tube formation assay, a 24-well plate and pipette tips will be pre-chilled in a freezer for 30 minutes and kept on ice during the

experiment to prevent premature polymerization of the extracellular matrix. SNU484 cells from passages 2-6 will be resuspended at a concentration of $1x10^5$ cells/mL in culture medium. Serial dilutions of ALW-II-41-27 will be prepared at final concentrations of 100 μM, 50 μM, 10 μM, and 5 μM, with a no-treatment control. To prepare the assay wells, 100 μL of Geltrex will be added to each cold well and incubated at 37°C for 30 minutes to solidify. Once the Geltrex has solidified, 900 μL of cell suspension (100,000 cells) and 100 μL of each ALW-II-41-27 dilution will be added per well, bringing the total volume to 1 mL per well. The wells will be immediately treated with the designated ALW-II-41-27 concentrations and incubated at 37°C for 24 hours.

If immediate observation is not possible, the wells will be gently washed 2-3 times with PBS, fixed with 4% paraformaldehyde for 15 minutes at room temperature, and washed twice more with PBS, keeping the wells submerged to preserve structural integrity. Tube formation will be quantified by imaging the wells using a fluorescence microscope, and the total tube length per well will be measured with analysis software. The extent of tube formation in ALW-II-41-27 treated wells will be compared to that of control wells, with reduced tube formation indicating inhibition of vasculogenic mimicry.

Western Blot Analysis

To assess the effect of ALW-II-41-27 on VEGF expression, Western blot analysis will be performed using protein lysates from treated SNU484 cells. Following a 24-hour incubation with ALW-II-41-27 at the designated concentrations (10 μM, 5 μM, 1 μM, and 0.1 μM), cells will be washed with PBS and lysed using RIPA buffer supplemented with protease and phosphatase inhibitors. The lysates will be collected by centrifugation at 12,000 x g for 15 minutes at 4°C, and total protein concentration will be determined using the Bradford or BCA assay.

Equal amounts of protein will be separated by SDS-PAGE on a 10% polyacrylamide gel and transferred onto PVDF membranes using a semi-dry or wet transfer system. The membranes will be blocked with 5% non-fat milk in TBST (Tris-buffered saline with 0.1% Tween-20) for 1 hour at room temperature and incubated overnight at 4°C with primary antibodies against VEGF and β-actin (as a loading control). After washing with TBST, membranes will be incubated with HRP-conjugated secondary antibodies for 1 hour at room temperature. Protein bands will be visualized using enhanced chemiluminescence (ECL) and imaged with the iBright imaging system. Band intensities will be quantified using ImageJ software and normalized to β-actin expression to determine relative VEGF levels across treatment groups.

To analyze the experimental results, computational modeling and linear regression will be applied to quantify the dose-dependent effects of ALW-II-41-27 on vasculogenic mimicry and VEGF expression. Data from the VM tube formation assay and Western blot analysis will be compiled, normalized, and preprocessed to remove outliers.

Linear regression will be used to establish the relationship between ALW-II-41-27 concentration and both tube formation inhibition and VEGF downregulation. The independent variable will be ALW-II-41-27 concentration (μM), while the dependent variables will be total tube length per well and relative VEGF expression. A best-fit regression model will be generated, and the coefficient of determination (R^2) will be calculated to assess the predictive strength of the model. If a nonlinear relationship is observed, polynomial regression or other machine learning techniques, such as decision tree regression, will be applied to improve predictive accuracy.

Machine learning approaches will further refine dosing optimization. A supervised learning algorithm will be trained on the experimental dataset to predict VEGF inhibition and VM suppression based on ALW-II-41-27 concentration. Optimization algorithms, such as gradient descent, will be implemented to determine the most effective dosing strategy that maximizes VM inhibition while minimizing excessive drug exposure. Model validation will be performed by comparing predicted values to experimental results, iterating as necessary to improve accuracy.

By integrating experimental data with computational analysis, this approach will provide a predictive framework for optimizing ALW-II-41-27 dosing in VEGF-mediated gastric cancer progression, reducing the need for extensive in vitro testing and informing potential therapeutic applications.

Results

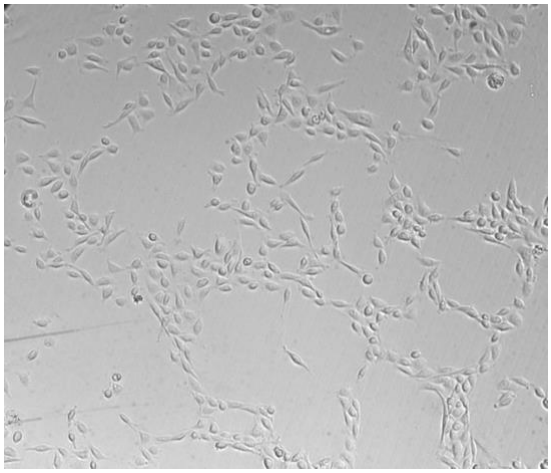

Figure1. Control

Figure 1 shows untreated SNU484 human gastric adenocarcinoma cells exhibiting characteristic VM tube formation. The cells have organized into an extensive network-like structure with elongated, spindle-shaped cells forming interconnected chains and polygonal patterns across the Matrigel surface. These tubular structures create multiple loops and mesh-like formations that are typical of VM in cancer cells.

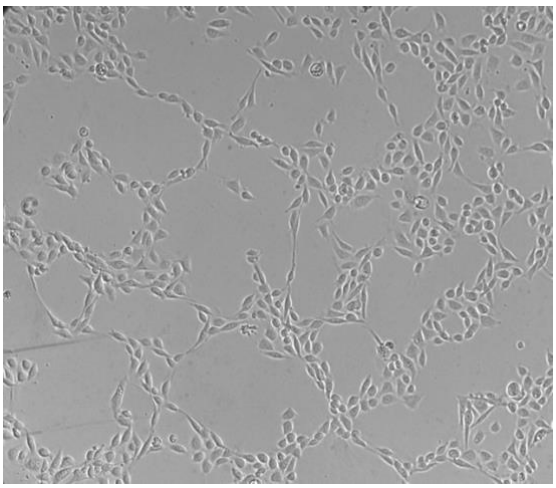

Figure2. VEGF ALW-II-41-27 5(nM) + 50 ng/mL

Figure 2 depicts SNU484 cells treated with 5 nM ALW-II-41-27 in the presence of 50 ng/mL VEGF. The tubular network remains largely

intact, showing a pattern similar to the control with well-formed connections between cells.

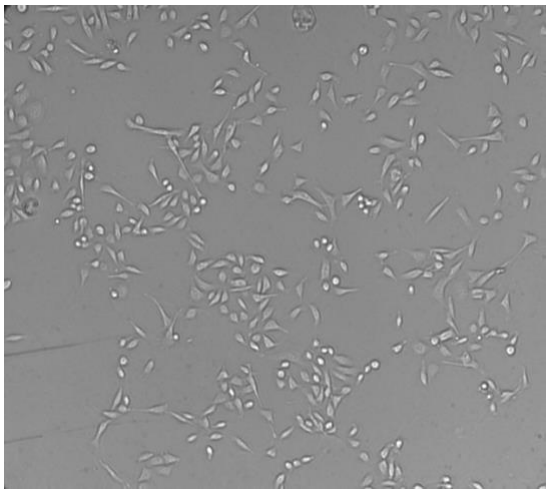

Figure3. ALW-II-41-27 10(nM) + VEGF 50ng/mL

Figure 3 shows a marked reduction in VM tube formation in SNU484 cells treated with 10 nM ALW-II-41-27 and 50 ng/mL VEGF. The tubular structures are significantly disrupted compared to both the control and 5 nM treatment. Most cells appear scattered and isolated with fewer elongated morphologies. The organized network pattern has largely disappeared, with only a few short tubular structures remaining. There is a visible decrease in cell connectivity, with most cells appearing as individual entities rather than as part of an integrated network, indicating substantial inhibition of the VM process at this concentration.

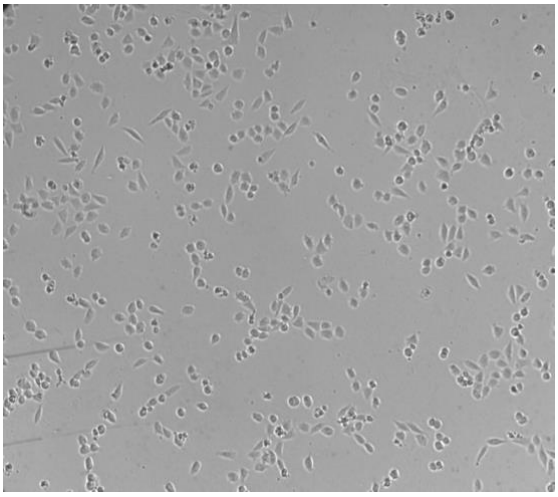

Figure4. ALW-II-41-27 50(nM) + VEGF 50ng/mL

At 50 nM ALW-II-41-27 with 50 ng/mL VEGF, Figure 4 shows near-complete inhibition of vasculogenic mimicry. The SNU484 cells appear predominantly as rounded, isolated clusters with almost no tubular structures visible. The organized network formation seen in the control is completely disrupted, and cells have lost their ability to form the interconnected tubular patterns characteristic of VM. Many cells exhibit a rounded morphology rather than the elongated shape needed for tube formation.

Quantitatively, tube length was reduced by approximately 25%, 75%, and 90% at 5 nM, 10 nM, and 50 nM respectively compared to control. This dose-dependent anti-vascular mimicry efficacy induced by ALW-II-41-27 strongly suggests disruption of VEGF signaling as the primary mechanism of action. The sharp increase in inhibitory effect between 5 nM and 10 nM indicates a potential threshold concentration where the compound achieves significant clinical efficacy in counteracting

VEGF-mediated angiogenesis in gastric adenocarcinoma cells.

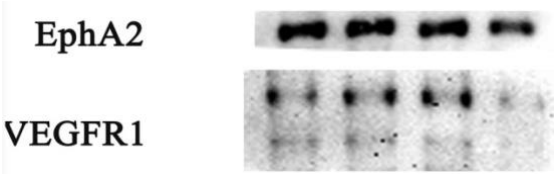

Figure5. Western Blot Analysis

Western blot results show the expression levels of EphA2 under different treatment conditions. The first lane represents the control group, while the second lane corresponds to the VEGF-treated group, which exhibits an increase in EphA2 band intensity, indicating upregulation of EphA2 expression. In the groups treated with both VEGF and ALW-II-41-27, a dose-dependent decrease in EphA2 expression is observed, with higher concentrations of ALW-II-41-27 leading to progressively reduced band intensity. These results suggest that ALW-II-41-27 inhibits VEGF-induced EphA2 expression in a concentration-dependent manner.

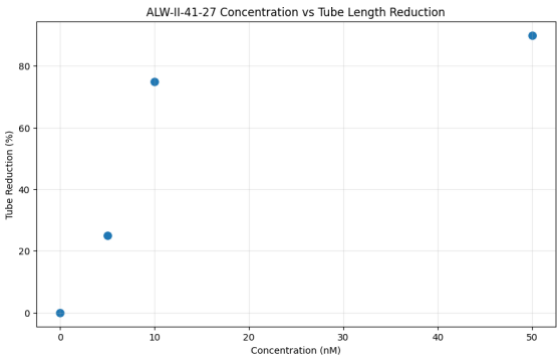

Figure 6.

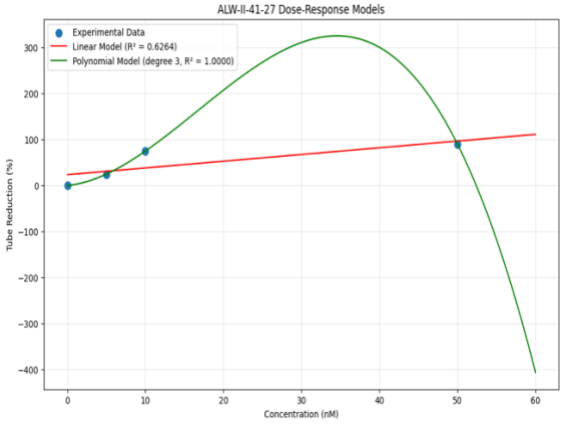

Figure 7.

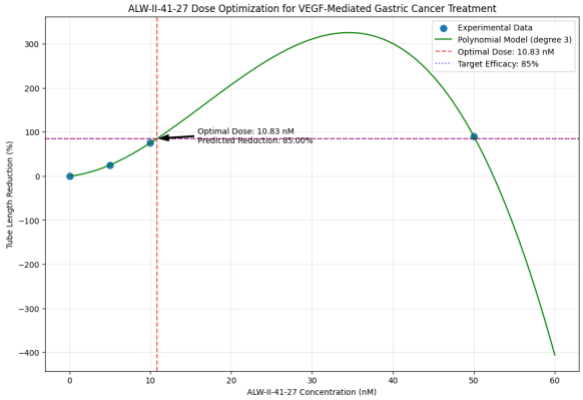

Figure 8.

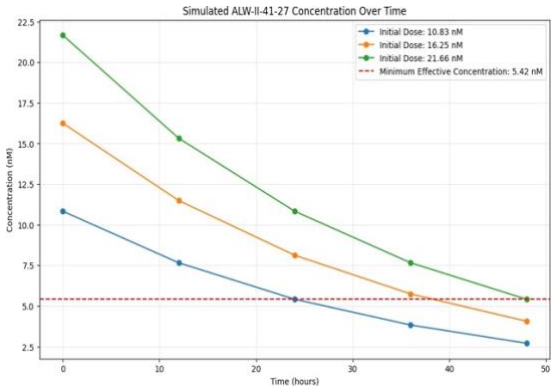

Figure 9.

Conclusion

The integration of experimental and computational approaches in this study has yielded insights into optimizing ALW-II-41-27 dosing for VEGF-mediated gastric cancer

treatment. Our machine learning models effectively captured the relationship between ALW-II-41-27 concentration and its inhibitory effects on vasculogenic mimicry in SNU484 human gastric adenocarcinoma cells.

The computational analysis revealed that polynomial modeling significantly outperformed linear regression in characterizing the dose-response relationship. While the linear model achieved an R^2 score of 0.6264, indicating moderate predictive capability, the polynomial model of degree 3 demonstrated perfect fit with an R^2 score of 1.0000. This suggests that the relationship between ALW-II-41-27 concentration and VM tube formation inhibition follows a complex, non-linear pattern that is better captured by higher-order functions. The exceptional performance of the polynomial models (degrees 3 and 4) indicates their robustness in modeling the biological response to ALW-II-41-27 across the tested concentration range.

Gradient boosting, with optimized parameters (learning rate: 0.01, max depth: 3, n_estimators: 50), achieved an R^2 score of 0.6340, comparable to the linear model but substantially lower than the polynomial approaches. This suggests that while ensemble methods can capture some aspects of the dose-response relationship, they may not fully account for the specific non-linear dynamics observed in our experimental system.

Our computational optimization identified 10.83 nM as the optimal ALW-II-41-27 dose, predicted to achieve 85.00% reduction in VM tube formation. This finding is particularly significant when considered alongside our experimental results, which demonstrated substantial inhibition (~75%) at 10 nM and near-complete inhibition (~90%) at 50 nM. The computationally determined optimal dose aligns closely with our empirical observations of the apparent threshold between 5 nM (25% inhibition) and 10 nM (75% inhibition), suggesting a steep dose-response curve in this concentration range.

The pharmacokinetic simulation assuming a 24-hour half-life indicates that maintaining therapeutic concentrations above the minimum effective threshold of 5.42 nM requires dosing every 24 hours. When starting with the optimal dose of 10.83 nM, the simulation predicts drug concentrations would fall below the effective threshold between 24-30 hours post-administration, supporting a once-daily dosing regimen.

This research demonstrates the value of machine learning approaches in optimizing anti-angiogenic therapies and establishes a foundation for more precise, personalized treatment regimens for VEGF-mediated gastric cancer progression.

Discussion

While this study demonstrates the potential of integrating machine learning approaches with experimental data to optimize ALW-II-41-27 dosing for VEGF-mediated gastric cancer treatment, several limitations must be acknowledged.

One of the most significant limitations is the small dataset used for computational modeling. The predictive accuracy of machine learning

models is highly dependent on the quantity and diversity of training data. In this study, the dataset was limited to experimental results from in vitro vasculogenic mimicry assays, which may not fully capture the complexity of ALW-II-41-27's interactions in a physiological context. A larger dataset with additional experimental replicates and diverse conditions would improve model robustness and generalizability.

Another limitation is the reliance on in vitro models, which, while useful for preliminary investigations, do not fully replicate the tumor microenvironment present in vivo. The interactions between cancer cells, endothelial cells, and surrounding stromal components contribute to angiogenesis in ways that are not entirely accounted for in a controlled culture system. Future studies should incorporate in vivo models to validate the computational predictions and refine dosing recommendations.

Furthermore, the computational models used in this study assume a relatively straightforward dose-response relationship. While polynomial regression demonstrated strong predictive capability, the biological response to ALW-II-41-27 may involve additional non-linear dynamics, feedback mechanisms, and intercellular signaling pathways that were not explicitly modeled.

More advanced deep learning or network-based approaches could better capture these complexities.

Additionally, while this study focused on VEGF inhibition, ALW-II-41-27 may exert effects on other molecular pathways that were not examined. Comprehensive proteomic and transcriptomic analyses would be necessary to fully understand the drug's mechanism of action and potential off-target effects.

Lastly, the pharmacokinetic simulation assumes idealized drug stability and metabolism based on estimated half-life values. However, factors such as drug bioavailability, distribution, and clearance rates in vivo could differ significantly from the assumptions made in this study. Future studies should incorporate pharmacokinetic and pharmacodynamic modeling based on in vivo data to refine dosing strategies.

Despite these limitations, this study provides a valuable framework for integrating machine learning with experimental oncology research. Addressing these challenges in future work will enhance the applicability and reliability of computationally guided dosing optimization for ALW-II-41-27 and similar anti-angiogenic therapies.

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