A multi-scale hybrid model that integrates angiogenesis into tumor growth to study the effect of cytotoxic-T cells on cancer cells under anti-angiogenic drug treatment.

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Background research:

According to Our World in Data, cancer is the second leading cause of death after cardiovascular diseases, resulting in 10 million deaths per year. Among cancers, lung cancer is the leading cause of death, accounting for 2.02 million deaths annually as of 2021. Lung cancer's high mortality rate is largely due to its progression, invasion, and metastasis, which are facilitated by the blood vessels in the lung. One of the main hallmarks of cancer progression is angiogenesis. The ability of cancer cells to induce angiogenesis is a significant characteristic of cancer[1],[2]. Angiogenesis is the process of forming new blood vessels from existing ones. It is essential for growth, development, and wound healing. However, excessive, inefficient angiogenesis is linked to many diseases. For example, cancer cells cause excessive angiogenesis, while diabetic patients and Alzheimer's patients are often found to have inefficient angiogenesis [2].

When a person develops cancer and the tumor grows larger than 1-2 mm, it enters a hypoxic state due to insufficient nutrient and oxygen supply. In a hypoxic environment, cancer cells release molecules called angiogenic factors, such as Vascular Endothelial Growth Factors (VEGFs) and Transforming Growth Factors (TGFs). Additionally, cancer cells secrete Matrix Metalloproteases (MMPs), which degrade the extracellular matrix, allowing VEGFs to diffuse and move toward preexisting blood vessels and endothelial cells. Once VEGFs released by hypoxic cancer cells reach the endothelial cells (EC) in the preexisting blood vessels, they bind to receptors on the EC, such as tyrosine kinase receptors. This binding signals the EC to proliferate and migrate towards the VEGF gradient, which is directed towards the tumor cells. The endothelial cells use their long filopodia to move in the direction of the tumor [3]. These migratory cells are called tip cells. Tip cells signal to the cells behind them to proliferate and form a lengthened tube; these cells are called stalk cells. Stalk cells proliferate to create vessels and recruit pericytes, which stabilize and rigidify these blood vessels. Once pericytes attach to the stalk cells, they are referred to as phalanx cells [4].

This process occurs in several stages:

1. **Degradation**: Initially, the basement membrane of blood vessels is degraded by metalloproteases secreted by hypoxic cancer cells.
2. **Budding**: Tip cells start migrating towards the gradient of VEGF, a process called budding.
3. **Elongation**: Behind the tip cells, stalk cells proliferate, aiding in the elongation of the new blood vessel.
4. **Maturation**: Once the tube is formed, phalanx cells, which include pericytes, help in the maturation of the vessels, ensuring that blood does not leak out.

Several studies have shown that targeting proangiogenic factors such as VEGF can inhibit the angiogenesis process and slow down tumor growth and progression. There are two main strategies to achieve this and prevent cancer metastasis [5]:

1. **Inhibiting VEGFs**: This approach involves administering antibodies to the patient that bind to the VEGFs released by the cancer cells. When these antibodies conjugate with the VEGFs, the VEGFs can no longer bind to the receptors on endothelial cells (ECs), thereby halting proliferative signals, elongation, and migration. Example Aflibercept.

2. **Inhibiting VEGF Receptors**: Another approach is to directly inhibit the VEGF receptors on the membrane of the endothelial cells. Even if the VEGF ligands bind to the receptors, these receptors (tyrosine kinases) will not be functional due to the presence of tyrosine kinase inhibitors. As a result, the signal for cell proliferation is not transmitted, and angiogenesis is slowed down. Example Sunitinib.

Problem Statement:

After extensive research, we have formulated a problem statement to work on: Develop a multi-scale hybrid model that integrates angiogenesis into tumor growth to study the effect of cytotoxic T cells on cancer cells under anti-

angiogenic drug treatment. Using different FDA-approved drugs, we aim to analyze how these drugs alter the vascular structure of blood vessels forming towards the tumor.

Objective

1. **Simulate Tumor Growth and Immune Cell Interaction:** Model the interaction between tumor growth and immune cells, focusing on angiogenesis, under various drug treatments.
2. **Predict Tumor Responses to Drugs:** Predict in vivo tumor responses to drugs with different efficacies, specifically Sunitinib and Doxorubicin (Dox).
3. **Analyze Phenotypic Behavior and Dynamics**: Examine the phenotypic behavior and dynamics of tumor and endothelial cells with and without drug administration.

Methodology:

Modeling Approach

For modeling our objectives, I reviewed existing models and how researchers have approached this problem previously. Caleb M. et al., in 2020, and Paul Macklin et al., in 2012, developed similar models using a hybrid multiscale approach [6].

Caleb M. developed a model that integrates the continuum model and agent-based model into a single framework. The key components in his model are tumor cells and endothelial cells. The number of these cells, the forces between them, and their movements are modeled using agent-based modeling by defining specific rules for these components.

Gradients of VEGF secretion, consumption, nutrient availability, and hypoxic gradients are modeled using deterministic diffusion equations, known as the continuum model. This integrated approach allows for a comprehensive simulation of tumor growth and angiogenesis.
We are utilizing this model as the base model, and adding Immune cells as the key players in the Agent-based modeling approach and drug in the continuum model see Supplementary Fig S1.

Set the rules.
In the agent-based model, a set of rules is defined to simulate various cellular behaviors: endothelial cell migration towards VEGF gradients, vessel sprout branching, cytotoxic T-cell interactions with tumor cells, and tumor cell phenotypic switching. The phenotypic switching rules govern transitions between quiescence, apoptosis, and necrosis, as well as determining conditions for cell migration and proliferation. These rules help to capture the dynamic and complex interactions within the tumor microenvironment.
Phenotypic Switching of Tumor Cells

The phenotypic switching of tumor cells involves transitions between different states based on specific conditions and probabilistic functions see Fig (1 A) below.

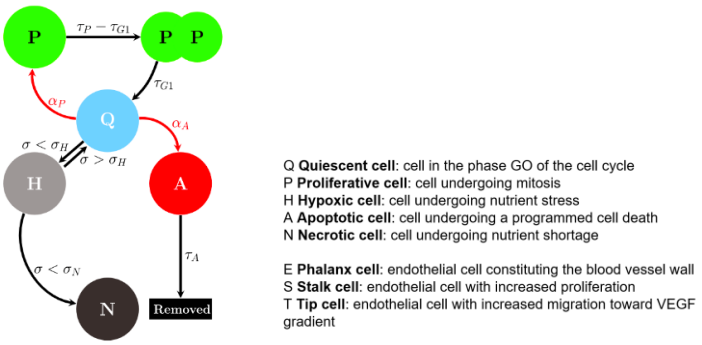
Quiescent Cells (Q Cells):

- Proliferative State: Quiescent cells can enter a proliferative state with a certain probability (α_p). Once in this state, the cell divides after a specific period. The daughter cells grow to their full radius and then return to the quiescent state.
- Apoptotic State: Quiescent cells can also transition to an apoptotic state, where they undergo programmed cell death. After a defined time (T_A), these cells die and are removed from the system.
- Hypoxic State: If the local availability of nutrients (σ) drops below a threshold (σ_h), quiescent cells enter a hypoxic state. In this state, they secrete VEGFs to induce angiogenesis.

Hypoxic Cells(H):

- Quiescent Reversion: Hypoxic cells, while secreting VEGFs, return to the quiescent state if nutrient levels rise above the threshold ($\sigma > \sigma_h$) due to newly formed blood vessels providing sufficient oxygen and nutrients.

- Necrotic State: If nutrient deficiency worsens, hypoxic cells transition to a necrotic state, leading to cell death. These necrotic cells cannot recover and are eventually removed from the system.



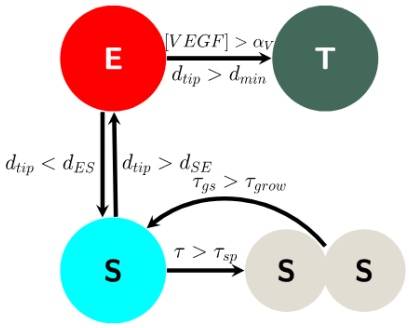


Fig 1A **Fig 1B**

Integration of Immune Cells into the Model (Added by OncoAngi team)

Adding immune cells in the above model, the following assumptions and rules are incorporated:

1. **Immune Cell Population Dynamics:**
   - The population of immune cells increases proportionally with the number of tumor cells.
2. **Immune Cell Migration:**
   - Immune cells migrate towards VEGF gradients, similar to the behavior of endothelial cells.
   - The migration is directed by the concentration of VEGFs secreted by hypoxic tumor cells.
3. **Immune Cell-Tumor Cell Interaction:**
   - Upon contacting tumor cells, immune cells induce immediate cell death in the tumor cells with some probability p.
   - The killed tumor cells are then removed from the system.

During the blood vessel formation, a few rules are defined for Endothelial cells also.

Endothelial Cell Dynamics: Tip Cells, Stalk Cells, and Phalanx Cells

Endothelial cells can exist in three states: tip cells (T), stalk cells (S), and phalanx cells (E). The transitions between these states are governed by the local concentration of VEGFs and the distances between different types of endothelial cells see Fig (1 B) above.

1. **Phalanx Cells to Tip Cells:**
   - Phalanx cells (E) can become tip cells (T) when the local concentration of VEGFs exceeds a threshold (VEGF > α_v) and the distance between the phalanx cell (E) and the tip cell (T) is greater than a minimum distance (d_{min}).
2. **Tip Cells to Stalk Cells:**
   - Tip cells (T) can switch to stalk cells (S) based on specific distance rules. If the distance between the phalanx cell (E) and the tip cell (T) is less than the distance between the phalanx cell (E) and the stalk cell (S), then the phalanx cell (E) switches to a stalk cell (S).
3. **Stalk Cells to Tip Cells:**
   - If the distance between the phalanx cell (E) and the tip cell (T) is greater than the distance between the phalanx cell (E) and the stalk cell (S), then the stalk cell (S) switches to a tip cell (T).
4. **Stalk Cell Proliferation:**
   - Only stalk cells (S) can divide and proliferate, contributing to the elongation of the blood vessel tube.

In the above model, we plan to include Drugs to inhibit the angiogenesis process. (Drug name= sunitinib)

Drug Inhibition: If the drug concentration is greater than a threshold ($D > D_0$), phalanx cells (E) do not switch to tip cells (T), thereby inhibiting the angiogenesis process.

Inhibit the Stalk cell proliferation: To inhibit stalk cell proliferation, we can reduce the proliferation rate by increasing the time it takes for the cells to divide. This can be achieved using a drug that delays spindle formation and arrests the cells in the mitotic phase for a longer duration [7].
Increasing the VEGF Threshold: Increasing the VEGF Threshold: We have also changed the VEGF threshold αv. By increasing this threshold, we have reduced the switch of Phalanx cells to Tip cells. With fewer Tip cells, cell migration is reduced, which is expected to hinder vessel formation and alter the angiogenesis structure. Biologically, this can be achieved by injecting an antibody against VEGF, which will reduce the local VEGF concentration because some of the VEGF will bind to the injected antibody and become inactive.

Results:

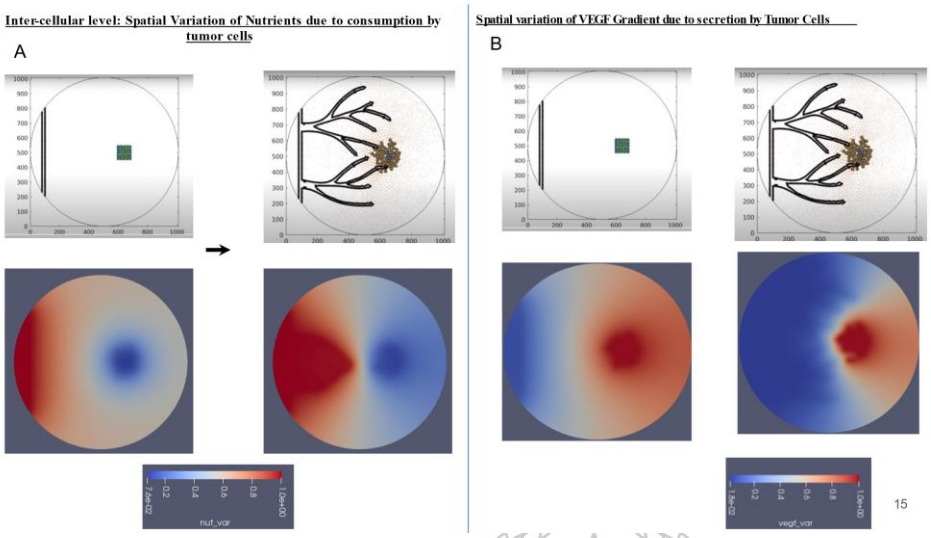


Fig 2.
Initially, we performed simulations without adding any complexity to the system, utilizing the already-developed modeling approach. The simulations were conducted using the Limbesh package in C++ code.

The simulation movie is given in the link.
This simulation shows how as soon as blood vessels reach to tumor quickly tumor progresses and increases in tumor size.
These are the results after making changes to the system. We have compared our results to the initial model.

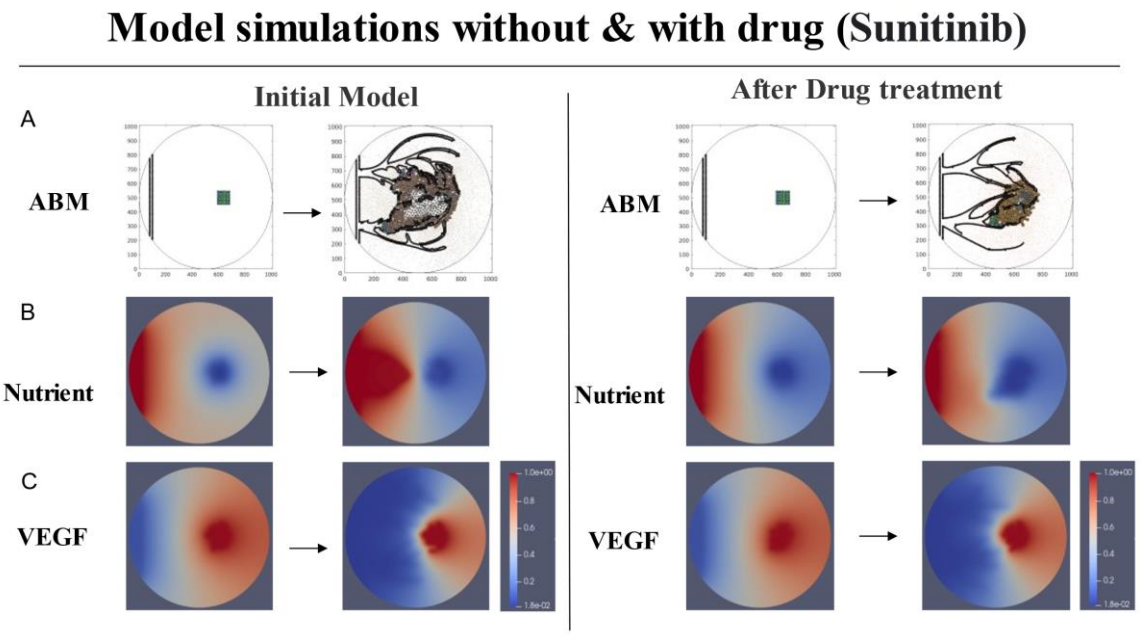


Fig 3. Representation of how vessel structure changes as angiogenesis is hindered by the drug: A) Showing that vessels are not formed properly, and fewer vessels have formed to some extent. Therefore, tumor cell number

and size have also decreased. **B)** As fewer vessels have formed there is a change in nutrient gradient also and nutrients have note reached to tumor site properly. **C)** There is not much change in the VEGF gradient because, in the model assumptions, only the Tip cells and Phalanx cells consume VEGF. Since these two cell types have not changed significantly, only the Stalk cells have been affected. Due to the changes in Stalk cell proliferation, the structure of the Stalk cells has been altered.

When a similar analysis was conducted with and without immune cells and compared to the initial model, a significant decrease was observed in the tumor size. This is because immune cells kill tumor cells based on a distance rule with some probability P. See Fig S1. and for the change in VEGF threshold see Fig S2.

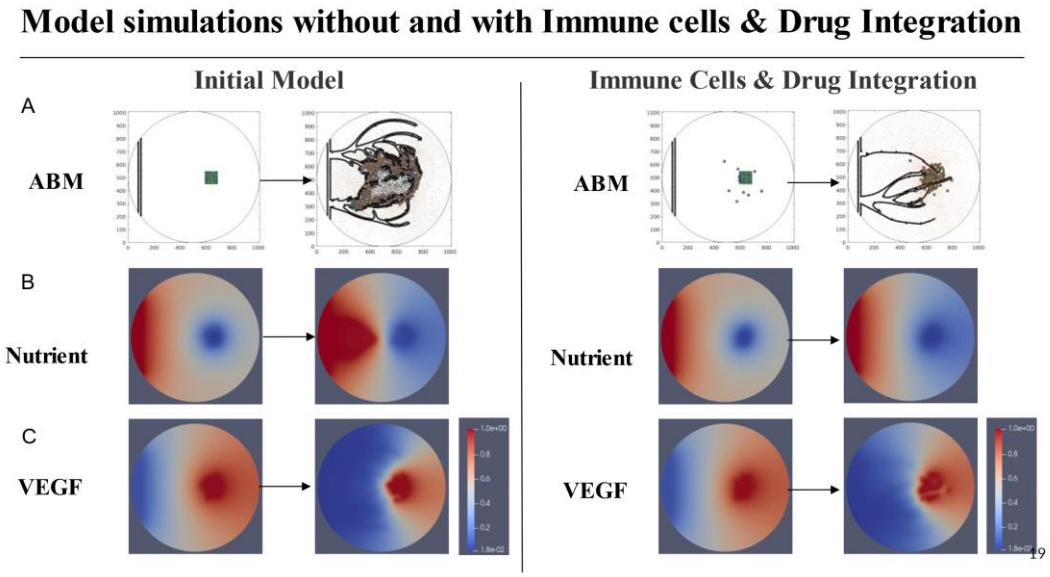


Fig 4. Representation of the change in tumor size and the gradient of nutrients and VEGF when both drug and immune therapy are integrated into the system. **A)** The tumor size has significantly reduced, indicating the effectiveness of the combined therapies. **B)** Fewer nutrients have reached the tumor site. **C)** The VEGF gradient has also been altered due to the change in tumor cell numbers.

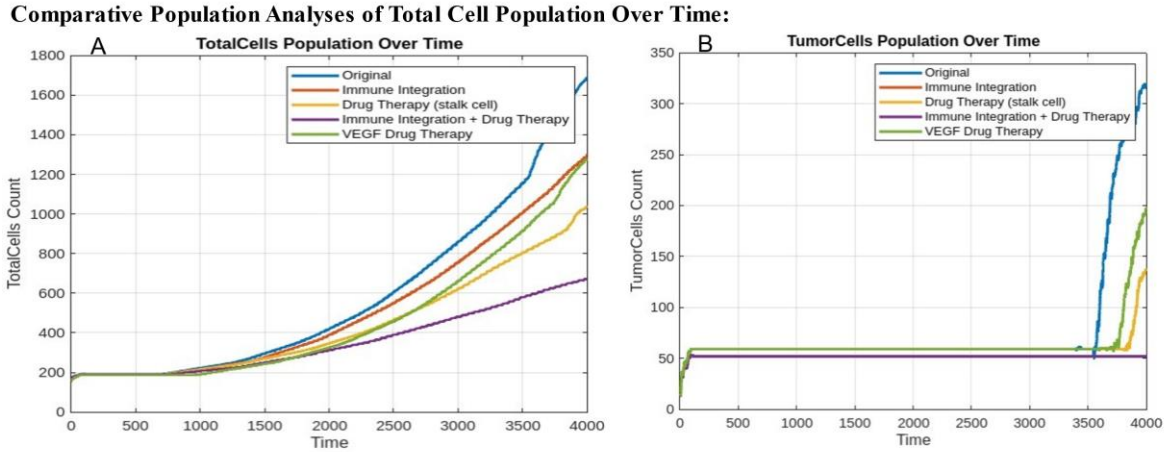


Fig 5 A) shows how the total cell population in the system changes with different kinds of therapy and drugs integrated into the system. In the initial time steps, not much change is seen, but just after the 2500 - time step, Drug Therapy, in which stalk cell proliferation time is altered, and VEGF Drug Therapy, where an antibody binds to VEGF secreted by tumor cells, both show almost the same kind of results and are better than Immune Therapy. However, the interesting part is when Drug Therapy and Immune Therapy are both administered together, yielding the best results by decreasing the tumor size and vessel formation, as indicated by the very low total cell count shown in Fig A. **B)** Similarly, for the tumor cell count, Immune Integration + Drug Therapy is found to be the most effective, as shown in Fig B.

A similar kind of analysis was performed for each cell type individually, including proliferative tumor cells, hypoxic tumor cells, and each endothelial cell type (stalk cells, tip cells, and phalanx cells). The same trends were observed as shown above. For more detailed information, please refer to the supplementary sheet.

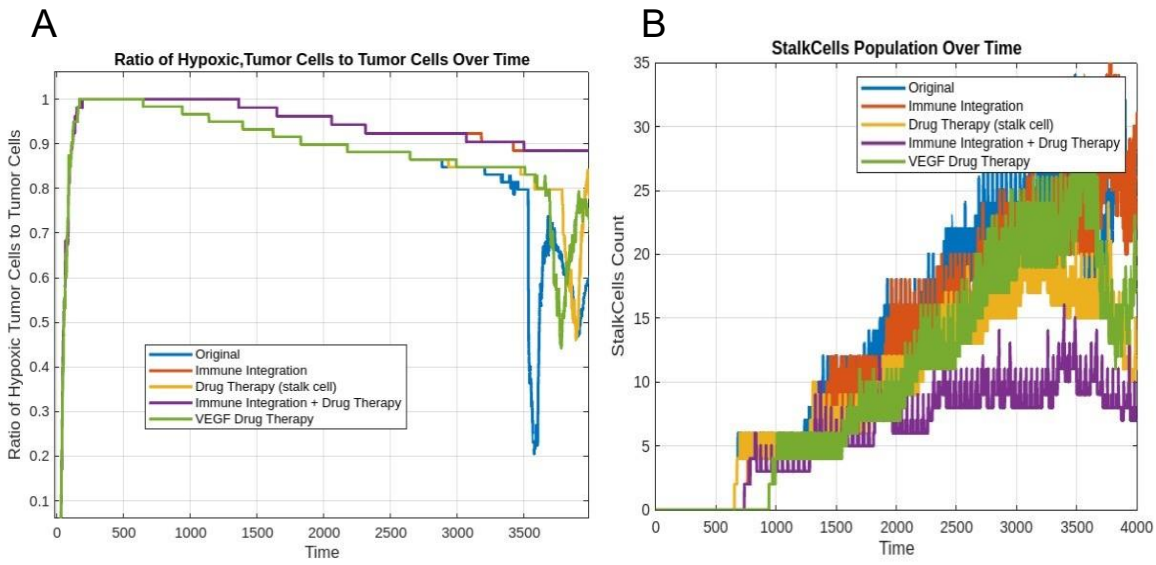
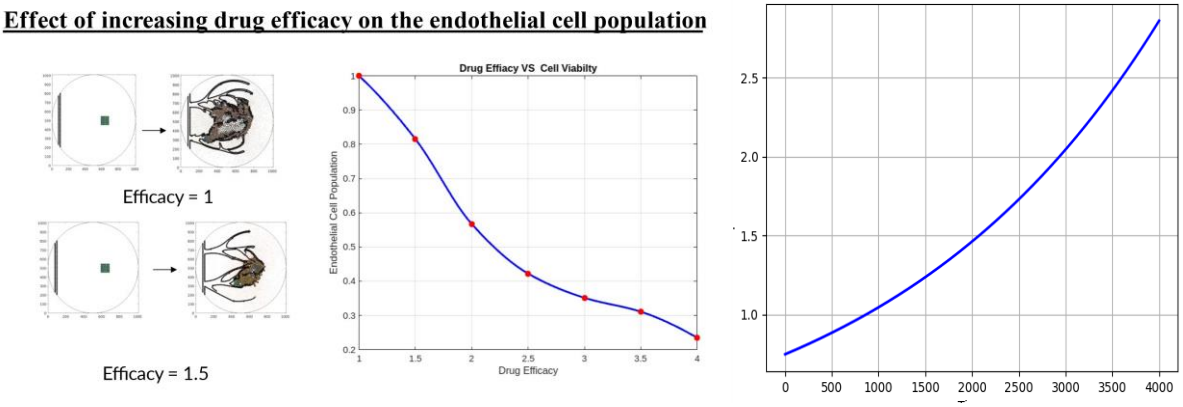


Fig 6.A) Initially, as cancer enters a hypoxic condition, the number of hypoxic cells increases suddenly because they consume all the nutrients provided. As the initial nutrients are depleted, the cell number starts decreasing. However, once the angiogenesis process begins and cells receive a full blood supply and nutrients, there is a sudden drop in hypoxic cells. As tumor proliferative cells divide rapidly and the cancer spreads, the core of the tumor becomes hypoxic again due to the sudden increase in tumor size. More blood vessels form, leading to another drop in hypoxic cells. Comparing the original model to the therapy and drug treatments, there is a delay in angiogenesis formation, shifting the peak to the right. The extent of angiogenesis is also reduced, resulting in a smaller drop in hypoxic cells. The most significant delay is observed with the combination of immune integration and drug therapy. By keeping the cells in a hypoxic condition for a longer time, more cells will die by switching to the necrosis state. B) The stalk cell count has not changed much in the case of immune integration combined with drug therapy. Since these cells are responsible for the length of the vessels, angiogenesis is greatly hindered.

Discussion



A) **B)**

Fig 7. A) This plot shows how different concentrations of a drug affect endothelial cells, which are part of vessels involved in the angiogenesis process. As the drug concentration increases, efficacy also increases, resulting in a greater reduction of endothelial cells to a greater extent. B) shows the effect of immune integration + Drug is more than both given individually.

As discussed earlier, the integration of immune cells with drug therapy leads to more effective killing of tumor cells and the greatest reduction in tumor size. This combination also significantly impedes angiogenic vessel structure and formation compared to when these two treatments are administered independently. We could this synergy by plotting the total cell count in each case treatment fitting the exponential curve into that finding the equation and putting those functions in the Loewe Additivity Model to prove the above synergy mathematically Fig 7 B. Fitting plots in Supplementary document. Total live cell population after treatment is more when drug and immune treatment is given independently compared to combined as shown in Fig 7B above.

$$S(t) = \frac{\text{ImmuneTherapy}(t) \cdot \text{DrugTherapy}(t)}{(\text{Immune \&DrugTherapy}(t))^2}$$

$$\text{SynEffect} = \frac{\int_0^{t=4000} S(t)dt}{t}$$

$$\text{SynEffect} = 1.76$$

Conclusion:

The study successfully demonstrates the significant inhibitory effects of combined drug and immune therapy on tumor angiogenesis and growth. Integrating immune cells with drug therapy resulted in the most substantial reduction in tumor size and vessel formation compared to treatments administered independently. This combination therapy not only delayed angiogenesis but also maintained hypoxic conditions longer, promoting tumor cell necrosis. Future directions include migrating cytotoxic T cells in the model, defining drug concentration gradients, and conducting parameter sensitivity analyses.

Limitation:
Migration of immune cells is not included in the system. Immune cells are already present around the tumor. The gradient of the drug is not included effect of the Drug is compensated by reducing the stalk cell proliferation and in another case of VEGF drug is by increasing the threshold value of VEGF for Phalanx cell to Tip cell switch.

Future Direction

Future research should focus on parameter sensitivity analysis, integrating mesenchymal phenotypes to analyze EMT and tumor metastasis, examining ECM heterogeneity's effect on angiogenesis, developing a 3D model for better spatial dynamics, and validating predicted synergies in vivo.

References:

[1] **Max Roser and Hannah Ritchie (2015) - "Cancer." Published online at OurWorldInData.org. Retrieved from: 'https://ourworldindata.org/cancer' [Our World in Data]**

[2] **Al-Ostoot, F.H.; Salah, S.; Khamees, H.A.; Khanum, S.A. "Tumor Angiogenesis: Current Challenges and Therapeutic Opportunities."** Cancer Treatment and Research Communications, Vol. 28, 2021, Article 100422. https://doi.org/10.1016/j.ctarc.2021.100422.

[3] **Lee, H.W.; Shin, J.H.; Simons, M. "Flow Goes Forward and Cells Step Backward: Endothelial Migration."** Experimental & Molecular Medicine, Vol. 54, pp. 711–719, 2022. https://doi.org/10.1038/s12276-022-00785-1.

[4] **Hida, K.; Maishi, N. et al. "Contribution of Tumor Endothelial Cells in Cancer Progression."** Int. J. Mol. Sci. 2018, 19(5), 1272. https://doi.org/10.3390/ijms19051272.

[5] **De Falco, S. "Anti-angiogenesis Therapy: An Update After the First Decade."** Korean J Intern Med, Vol. 29, No. 1, pp. 1-11, 2014. https://doi.org/10.3904/kjim.2014.29.1.1.

[6] **Phillips, C.M.; Lima, E.A.B.F. et al. "A Hybrid Model of Tumor Growth and Angiogenesis: In Silico Experiments."** PLOS ONE, Published: April 10, 2020. https://doi.org/10.1371/journal.pone.0231137.

[7] **Garrett, M.D.; Collins, I. "Anticancer Therapy with Checkpoint Inhibitors: What, Where and When?"** Trends in Pharmacological Sciences, Published: March 31, 2011. https://doi.org/10.1016/j.tips.2011.02.014.

[8] Code and data for simulation.

[9] Supplementary sheet.docx