IMPACT OF TUMOR OXYGENATION ON DRUG RESISTANCE EVOLUTION UNDER HYPOXIA-ACTIVATED PRODRUG THERAPY

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SUMMARY
Hypoxia-activated prodrugs (HAPs) have been designed to target low-oxygen regions within tumors, where drug-resistant clones may thrive. We demonstrated in previous mathematical modeling work that, when combined with standard tyrosine kinase inhibitor therapy, they may have the potential to significantly improve treatment outcomes for patients with EGFR-mutant non-small cell lung cancer (NSCLC). Here, we extend this work to investigate how varying tumor oxygenation may be used to alter response to the combination therapy. Our framework can be used to develop optimal therapeutic regimens that may further reduce tumor burden and probability of resistance in patients with NSCLC.

Key words: drug resistance, tumor hypoxia, cancer evolution

1 INTRODUCTION
The heterogeneity of the tumor microenvironment (TME) has been shown to play a crucial role in the emergence and evolution of drug resistance [1]. Specifically, solid tumor vasculature is notoriously disorganized [2], leading to spatial variation in the concentrations of drug, nutrients, and oxygen. This can lead to sustained cell proliferation in low-drug regions, which then promotes the production of drug-resistant mutants. These regions of low drug concentration often coincide with hypoxic (low oxygen) conditions since both drugs and oxygen diffuse into tumors via the blood vessels. Hypoxia-activated prodrugs (HAPs) such as evofosfamide have been designed to specifically target these oxygen-deprived portions of the TME [3]. Under normoxic (normal oxygen) conditions, evofosfamide penetrates effectively through tumor tissue; it only metabolizes into an active drug upon entry into a hypoxic region [4]. This novel action allows evofosfamide to target cells in a region of the TME often unreachable by standard therapies, whose range is usually confined to more well-vascularized regions.

Although HAPs have failed to show a survival benefit in several recent clinical trials, our previous work has demonstrated the importance of determining the correct dosing sequence and timing in order to maximize therapeutic benefit [5]. One standard therapy currently used to treat EGFR-mutant NSCLC is erlotinib, a tyrosine kinase inhibitor that binds to EGFR. Although most patients are initially responsive to erlotinib, patients eventually develop resistance within 12-18 months on average [6]. Therefore, novel strategies designed to prevent or delay the onset of resistance to erlotinib are of great clinical importance.

In [7], we developed a stochastic mathematical model, informed by experimental and clinical data, to predict the evolutionary dynamics of a population of cancer cells within a heterogeneous TME during treatment with erlotinib and evofosfamide. We optimized over the space of tolerated combination dosing schedules to find maximally effective strategies. Our model predicts that the dosing schedules with the greatest potential to minimize tumor burden and probability of resistance are those that sequentially alternate between erlotinib and evofosfamide doses while minimizing the treatment break after a dose of evofosfamide and before the next dose of erlotinib.

Here, we extend our model from this previous work to study the effect of changing the TME on response to treatment with erlotinib and evofosfamide. Since erlotinib response is microenvironment-dependent and evofosfamide is hypoxia-activated, it may be possible to improve predicted treatment...
outcomes even further by manipulating the state of the TME. We compare predicted treatment outcomes using a standard TME with those using TMEs where the overall oxygen concentration throughout the tumor has been either increased or decreased. This comparison provides insight into how alterations to the TME could be leveraged to maximize the therapeutic benefit of these drugs. The addition of this work contributes to a more complete understanding of the interactions between cancer cells and their microenvironments as well as provides a potentially improved strategy for optimal tumor control in NSCLC patients.

2 METHODOLOGY

To capture the effect of microenvironmental heterogeneity on the cancer cell population dynamics, we use a pseudo-spatial compartment-based model of the TME in which cells are distributed amongst a weighted series of environmental habitats with varying concentrations of oxygen and drug [1]. Each environmental compartment has some inherent oxygen concentration so that the total collection of compartments is representative of the range of oxygen concentrations observed in solid tumor physiology. Oxygen decays spatially away from the nearest blood vessel, and we parameterize this decay rate using estimates of the half-length away from the vessel. This decay rate, together with the oxygen concentration in each compartment, allows us to define (for each compartment) a distance away from the nearest blood vessel at which that volume of cells is located. The relative contribution of each of these compartments to the TME as a whole is determined based on experimental data capturing the relative frequencies of oxygen partial pressures throughout solid tumors [8]. A schematic of this model is shown in Figure 1.

Within each compartment, we model the population of cancer cells during treatment using a two-type continuous-time birth-death process. For simplicity, we assume the evolutionary dynamics within each compartment are independent. In compartment $i$, let $X_i(t)$ represent the number of erlotinib-sensitive cancer cells at time $t$, and let $Y_i(t)$ denote the number of erlotinib-resistant cells at time $t$. The sensitive cells in compartment $i$ proliferate and die with rates $\lambda_{X,i}(t)$ and $\mu_{X,i}(t)$, respectively, and the resistant cells proliferate and die with rates $\lambda_{Y,i}(t)$ and $\mu_{Y,i}(t)$. These time-dependent birth and death rates reflect the effect of treatment on the population of cancer cells in compartment $i$ and therefore depend on the concentrations of both oxygen and drug found in that compartment. Each time a sensitive cell divides, a mutation may arise with probability $u = 10^{-7}$, giving rise to a new resistant cell. We start with an initial population of $M = 1.6 \cdot 10^6$ sensitive cells and no resistant cells. The number of sensitive cells $M_i$ initially in compartment $i$ is calculated using the relative compartment weights.

The evolutionary dynamics of this population of cancer cells can be described using analytic approximations for the probability of resistance and means of the sensitive and resistant cells. Specifically, the mean of the sensitive cells in compartment $i$ is

$$\mathbb{E}[X_i(t)] = M_i \exp \left[ \int_0^t (\lambda_{X,i}(\tau) - \mu_{X,i}(\tau)) \, d\tau \right] .$$

The mean of the resistant cells is

$$\mathbb{E}[Y_i(t)] = \int_0^t b_i(\tau) \exp \left[ \int_0^\tau (\lambda_{Y,i}(\eta + \eta) - \mu_{Y,i}(\tau + \eta)) \, d\eta \right] \, d\tau ,$$

where $b_i(t) = M_i \exp \left[ \int_0^t (\lambda_{X,i}(\tau) - \mu_{X,i}(\tau)) \, d\tau \right] \lambda_{X,i}(t)u$. Lastly, the probability of resistance
in compartment $i$ is $P[Y_i(t) > 0] = 1 - \exp\left[\int_{0}^{T} -b_i(T) \left(1 - P_{i}^{ext}(T, t)\right) dT\right]$, where $P_{i}^{ext}(T, t) = \frac{\int_{0}^{T-T} \mu_{Y,i}(\tau+T)\omega_i(\tau, T) d\tau}{1+\int_{0}^{T-T} \mu_{Y,i}(\tau+T)\omega_i(\tau, T) d\tau}$ and $\omega_i(\tau, T) = \exp\left[\int_{0}^{\tau} \left(\mu_{Y,i}(\eta + T) - \lambda_{Y,i}(\eta + T)\right) d\eta\right]$. Detailed derivations of these equations are outlined in [9].

As previously stated, the birth and death rates of sensitive and resistant cells in compartment $i$ depend on both the oxygen concentration in compartment $i$ and the concentrations of erlotinib and evofosfamide, which vary over time, depending on dosing schedule. To estimate the temporal effects of these drugs on the growth kinetics in each compartment, we use (i) experimentally derived birth and death rates under a spectrum of environmental perturbations of oxygen and erlotinib concentration, (ii) experimental results on cell viability in response to evofosfamide therapy, and (iii) pharmacokinetic data mapping erlotinib and evofosfamide dose to plasma concentration. For a detailed description of how this model is parameterized, see [7].

3 RESULTS AND CONCLUSIONS

In [7], we used the model to examine the evolutionary dynamics of a tumor undergoing a wide variety of therapies with erlotinib and evofosfamide, and found that the dosing schedules with the highest potential to minimize probability of resistance and tumor burden are those that alternate sequentially between erlotinib and evofosfamide. Here we investigate the possibility of further improving treatment outcomes during combination therapy with these drugs by altering tumor oxygenation levels. We predict the evolutionary dynamics of three different tumors (one well-oxygenated, one poorly-oxygenated, and one whose oxygen profile mirrors standard tumor physiologic data) undergoing therapy with two separate combination dosing regimens. For each of these tumors and each of the two therapies, the predicted mean tumor size and probability of resistance up to recurrence time (the time at which the cancer cell population reaches its initial size once again) are plotted in Figure 2. The blue curves show the evolutionary dynamics of a tumor with a 13.16% oxygen concentration at the blood vessels, which matches tumor physiologic data [8]. The red curves show the dynamics of a more well-oxygenated tumor (26.32% at the blood vessels) and the yellow curves show the dynamics of a more poorly-oxygenated tumor (6.58% at the blood vessels). Predictions for a dosing schedule in which the patient is given 150 mg erlotinib daily on the first 5 days of each week and 575 mg/m² evofosfamide on the last day of each week are shown in Figures 2A and B. Predictions for a dosing schedule in which the patient receives one dose of 150 mg erlotinib and one dose of 248 mg/m² evofosfamide in every 36-hour period (the optimal dosing schedule identified in [7]) are shown in Figures 2C and D.

These results suggest that treatment with a combination of erlotinib and evofosfamide may be more effective on poorly-oxygenated tumors and less effective on well-oxygenated tumors. Although we observe a benefit to poorly-oxygenated tumors during therapy with each drug since cellular growth rates are reduced as oxygen levels are lowered, changes in tumor oxygenation play a larger role in response to evofosfamide than to erlotinib. This is because evofosfamide is hypoxia-activated, and hence more effective at lower oxygen concentrations. Thus tumor oxygenation differences lead to a more dramatic difference in response during the dosing schedule used in Figure 2C vs. the dosing schedule used in Figure 2A. In light of these observations, we conclude that the optimal treatment strategies proposed in [7] may be improved further by decreasing the oxygen throughout the tumor and maximizing the number of evofosfamide doses.

The addition of this work suggests another approach with the potential to further improve treatment outcomes for patients with NSCLC. In [7], our model predicted that, while the emergence of resistance is inevitable during monotherapy with erlotinib, the addition of evofosfamide may result in tumor eradication for a significant fraction of patients. Here we have shown that this combination strategy may be improved upon even further with the addition of therapy aimed at decreasing tumor oxygenation. Given the correct timing and dosing sequence of erlotinib and evofosfamide, the combination of these two drugs, together with additional therapy to decrease oxygen concentration throughout tumors, may have the potential to significantly improve treatment outcomes for NSCLC patients.
Figure 2: Comparison of evolutionary dynamics over time for tumors with varying oxygen profiles. Mean tumor size (A) and probability of resistance (B) are calculated up to recurrence time for a dosing schedule consisting of 150 mg erlotinib daily for days 1–5 and 575 mg/m² evofosfamide given on day 7 of every week. Mean tumor size (C) and probability of resistance (D) are plotted for a dosing schedule consisting of one dose of 150 mg erlotinib and one dose of 248 mg/m² evofosfamide given every 36 hours. Results are shown for a tumor with a concentration of 13.16% oxygen at the blood vessels in blue, 26.32% oxygen in red, and 6.58% oxygen in yellow.

REFERENCES


