
Modeling the competing effects of the immune system and EMT on epithelial cancers

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During progression from carcinoma in situ to an invasive tumor, the immune system is engaged in complex sets of interactions with various tumor cells. Moreover, tumor cell plasticity can significantly alter disease trajectories via epithelial-to-mesenchymal transition (EMT). Several of the same pathways that regulate EMT are involved in tumor-immune interactions, yet little is known about the mechanisms and consequences of these regulatory processes. Here we introduce a multiscale evolutionary model to describe the interplay between tumor-immune-EMT interactions and their impact on epithelial cancers. Through in silico analysis of large patient cohorts, we find controllable regimes that maximize invasion-free survival. We identify that delaying tumor progression depends crucially on properties of the mesenchymal tumor cell phenotype: its growth rate and its immune-evasiveness. Through analysis of pancreatic and ovarian cancers from The Cancer Genome Atlas to assess model predictions against clinical measurements, we find that association with EMT significantly worsened the invasion-free survival probabilities of inflammation-associated cancer. These results offer novel means to delay disease progression by regulating properties of EMT, and demonstrate the importance of studying cancer-immune interactions in light of EMT.

¹ 1 Introduction

² The majority of deaths from cancer are due to metastasis of the disease [1]. It is thus of critical
³ importance to understand better the progression from in situ to invasive disease. Underlying
⁴ this progression are genetic and epigenetic events, including mutations in pathways critical to

the success of the cancer cell (driver mutations) [2]. These pathways include cell proliferation, apoptosis, and immunogenicity.

Cancer and the immune system interact in myriad ways. The immune system modulates the tumor microenvironment (TME), since immune signals that affect the tumor can be amplified or repressed through feedback in response to local inflammatory signals. This complex cell signaling occurs alongside the targeting (and potential eradication) of the tumor by immune cells [3].

The effects of the immune system on a tumor can be broadly summarized into two branches. The cytotoxic branch of the immune system, such as natural killer cells (NKs) and cytotoxic T cells (CTLs), seek out and lyse tumor cells. Upon carrying out their effector functions, these cytotoxic cells lose efficacy or deactivate [4]. The regulatory branch of the immune system (Tregs, and other factors), inhibits the effective functioning of the cytotoxic branch [5]. Inflammation can increase the probability of cancer incidence and progression, with some of the most pronounced effects seen for tumors originating in gastrointestinal and pancreatic tissues [6, 7]. Recent work has shown, contrary to the typical effects of inflammation on cancer, that under certain conditions inflammation may not be oncogenic but rather onco-protective [8].

Immunotherapies are beginning to realize their potential, with significant impacts on patient health and survival [9, 10], and may even provide a cure for certain hematopoietic cancers via anti-CD19 CAR-T cells [?]. The presentation of antigens on tumor cells is recognized by innate immune cells that are transported to lymph nodes where T cells (and other components) can be activated [11]. The tumor also engages in processes that can indirectly modify the TME, for example by releasing transforming growth factor beta (TGF- β), which can shift the TME towards a tumor-supportive environment by enhancing immunosuppression via activation of Tregs [11].

Epithelial-to-mesenchymal transition (EMT) describes a reversible process by which cells displaying an epithelial phenotype transition into cells with a mesenchymal phenotype. Epithelial cells are – in part – defined by tight cell-cell adhesion. Mesenchymal cells exhibit less adhesion, greater ranges of motility, and may possess stem-like properties [12], although controversy regarding ‘stemness’ and EMT remains [13, 14]. Recent work has shown that – rather than being a binary process – at least two stable intermediate EMT states exist [15, 16]. Ongoing investigations into the plasticity and stability of EMT overlap with discussions elsewhere, e.g. of discrete vs. continuous processes during cell differentiation [17]. Intermediate states have emerged as a central mechanism by which cell fates (and the noise inherent within them) can be controlled [18–20].

Two features of the mesenchymal phenotype are of particular relevance in the context of cancer-immune interactions. i) mesenchymal tumor cells proliferate less than epithelial cells, we refer to this as mesenchymal growth arrest (MGA), and can be considered related to (in the sense of quiescence) the “stemness” phenotype of the mesenchymal tumor cells [21]. ii) mesenchymal cells are less susceptible to immune clearance [22]. As a cell is targeted by cytotoxic immune cells for clearance, a physical connection between the two cells must be established. This immunological synapse – mediated in part by T-cell receptors bound to antigens and the major histocompatibility complex on the target cell – is down-regulated in mesenchymal cells, thus inhibiting formation of the synapse [22]. We refer to this phenotype as mesenchymal immune evasion (MIE).

In addition to the prominent role it plays in metastasis, EMT has more recently been shown

to also regulate other aspects of tumor progression [12, 23]. TGF- β , a master regulator of EMT [24], is at once implicated heavily in tumor-mediated immune responses, since Tregs release TGF- β upon arriving at the tumor site [22]. In hepatocellular carcinoma, for example, there is direct evidence linking Treg-secreted TGF- β with EMT [25]. Thus, even by considering only the TGF- β pathway, we find compelling evidence that these three core components (the tumor, the immune system, and EMT) all interact. It therefore strikes us as a priority to develop models to understand how the interactions between each of these three components affect cancer incidence and progression.

Mathematical oncology, that is, mathematical models of cancer incidence, progression, and treatment, has become a well-developed field; many models have offered insight into the cellular interactions underlying cancer and its interplay with the immune system, including older [26–28] and more recent works [29–42]. These studies have increased our understanding of how tumors grow in the presence of various immune components, and how treatment regimes can be designed to maximize the efficacy of cytotoxicity while minimizing risks to the patient. However, to our knowledge no models have addressed how the effects of EMT alter interactions between the immune system and cancer, and the subsequent implications for treatment.

Here we develop a model with the goal of studying interactions between the tumor, the immune system and EMT. We seek to describe a set of crucial molecular and cellular interactions in epithelial tumor cells, including effects due to DNA damage and mutation, to investigate the probability that in situ tumors will progress and, if so, when. A recent model of cancer-immune interactions [8] described the effects of the TME on the risk of cancer, and we build on the core cell cycle component of this model, adding significant new interactions to the immune component of the model (which was previously modeled by a single interaction), as well as adding the effects of EMT. In doing so, we shift the focus of the previous model from cancer initiation to cancer progression. We do this to reflect the fact that cancer progression hinges on escape from the immune system and the fact that EMT has a more well-defined role during progression and metastasis. We seek to understand whether this more complex immune module will change our understanding of inflammatory effects on the tumor, and how the epithelial-mesenchymal axis influences these.

In the next section we develop the model, explaining the intuition behind each of its components. We go on to analyze its behavior: global “one-at-a-time” sensitivity analysis identifies parameters that are crucial for progression. We study these in more depth, focusing on the competing effects of EMT and of the immune system on progression, and discover that EMT intricately regulates progression: under certain regimes a careful balance of EMT- and immune-driven processes can significantly prolong invasion-free survival. To test these predictions, we analyze data from the Cancer Genome Atlas (TCGA), and find evidence for the synergistic effects of inflammation and EMT predicted by the model for patients with pancreatic or ovarian cancer.

Quick Guide to Equations and Assumptions

To become invasive, An in situ tumor relies on mutations to alter cellular signaling pathways that enable cancer progression. The immune system simultaneously responds to the tumor upon recognition of neoantigens, and shapes the TME through dynamic inflammatory and regulatory signals.

To capture these dynamics, we developed a non-spatial agent-based model. Tumor cells are modeled individually as agents and immune cell populations are described homogeneously by differential equations. We consider two tumor cell types: epithelial tumor cells (ETCs) and mesenchymal tumor cells (MTCs). Time is treated discretely in 18 hour steps; approximately the time of one cell cycle. During each cell cycle, tumor cells can either undergo division, apoptosis, immune clearance, or can rest. The likelihood that a cell will proliferate depends on the tumor size (competition for resources) and on cell-intrinsic factors. The likelihood that a cell will undergo apoptosis is constant but varies between cell types (ETC and MTC). The likelihood of immune clearance depends on the number and type of mutated cells in the tumor, cytotoxicity, regulatory cells, and cell-intrinsic factors. As tumor cells proliferate, DNA damage can occur, and over time they become increasingly likely to acquire pathway mutations that change their propensities for proliferation or cell death. Natural killer (NK) cells identify and clear tumor cells, a process which results in neoantigens priming and activating T cells in local lymph nodes. T cells can subsequently infiltrate the TME. At the tumor site, cytotoxic T cells (CTLs) lyse tumor cells, and T regulatory cells (Tregs) suppress cytotoxic activity. The following equation determine the per-cell cycle probability that a tumor cell will be lysed by a CTL:

$$\rho_{\text{CTL}} = \delta_{\text{MUT}} \frac{N_{\text{CTL}}}{N_C/K_1 + N_{\text{CTL}}} \frac{E_{\text{CTL}}}{1 + N_{\text{Treg}}/K_2} (1 - \delta_{\text{IE}}\Delta_{\text{IE}})(1 - \zeta\Delta_{\text{MIE}})$$

Here, δ_{MUT} is 1 or 0 depending on whether or not the cell has a mutation. The second term is a hill function modeled after [43] and the third term describes onco-protective effects of Tregs. The second-to-last term describes the increased immune-evasiveness that can occur following mutation in the immune evasion pathway, and the last term quantifies the additional immune evasiveness of associated with MTCs. The inflammatory state of the TME thus impacts (through multiple factors) immune recruitment and cytotoxicity at the tumor site.

EMT impacts tumor cells through their proliferation and potential to evade the immune system change. MTCs have reduced proliferation and increased immune evasiveness TGF- β , an activator of EMT, is produced both by Tregs and tumor cells, thus connecting tumor-immune interactions with EMT, and as a result plays an important role in shaping tumor outcomes. EMT depends on TGF- β concentration through a Hill-type function that determines the likelihood of EMT (or the reverse MET) through its magnitude relative to τ_{i-1} . This is described by the equation:

$$\tau_i = \frac{\tau_{\text{max}}}{N_C} \frac{\tau/K_3}{1 + \tau/K_3} + X_i, \quad X_i \sim N(0, \sigma^2)$$

Here, τ is the concentration of TGF- β in the TME and τ_{max} represents a limit on the amount of TGF- β that can be absorbed by all cells, with Gaussian noise added.

Our goal is to determine when the cancer becomes invasive, determined through the proportion of tumor cells harboring mutations in pathways that permit escape from in situ, relative to the total tumor cell population.

2 Methods

We develop an agent-based model to describe the relationships between cancer, the immune system, and EMT, building on the cell-cycle and tissue-cell components described in [8]. The agents in the model are the cells that have already formed an in situ tumor yet lack key pathway mutations to become invasive. In the process of the simulation, these cells can acquire mutations altering any of three key pathways (Fig. 1A). If the pathway is altered, the component is colored red and is marked with 'X'.

We model immune cells as continuous variables, i.e. we assume that the tumor microenvironment is well-mixed with regards to the infiltrating immune cells. The cytokine TGF- β is also assumed to be well-mixed in the tumor microenvironment. Tumor cells can take on either epithelial or mesenchymal phenotypes in a plastic manner: these phenotypes depend on both the TME and cell-intrinsic factors. While the EMT score is continuous, a threshold determines if a given cell is labeled as epithelial or mesenchymal (Fig. 1A).

2.1 Tumor Evolution

Associated with each tumor cells are two important quantities: their particular mutational signature and their EMT score. We consider three idealized pathways that can be mutated: a proliferation pathway that when altered increases the probability of the cell proliferating; an apoptosis pathway that when altered decreases the probability of the cell undergoing apoptosis; and an immune evasion pathway that when altered decreases the probability that an immune cell can effectively clear the mutated cell.

2.2 Dynamics of the Immune Response

The immune system is modeled by three immune cell types: NKs, CTLs, and Tregs. The NKs and CTLs act on the system by recognizing invasive cells and clearing them. Upon clearance, they are deactivated and removed from the immune population. Tregs suppress the function of NKs and CTLs (reduce tumor cell clearance), and in addition, release TGF- β which further shapes the TME by pushing tissues cells more towards a mesenchymal phenotype.

2.3 Inflammation

Inflammation is modeled as a cycling scheme between low and high inflammatory states, with varying on/off durations and intensities. For the purpose of simulation we consider the default state to be low inflammation, and update the immune activity parameters whenever a switch to the high state occurs. In the SI (Table S2) we give full details of parameter settings during low and high inflammation.

2.4 EMT

Each tissue cell has an EMT score between 0 and 1. Above a threshold, the cell is a mesenchymal tumor cell (MTC). Otherwise, it is an epithelial tumor cell (ETC). MTCs notable have increased rates of immune evasion and decreased rates of proliferation.

In modeling EMT this way, we are assuming that the same factor, TGF- β , drives EMT both at initiation and through progression of cancer. In addition, we choose to ignore other factors that are known to induce EMT such as hypoxia as well as the typical spatial organization of epithelial and mesenchymal cells as this pertains to the invasive front.

For the cells that have undergone EMT and are currently labeled in the model as mesenchymal, they experience two modifications to their governing parameters. The first is a reduction in proliferation, which we call mesenchymal growth arrest (MGA). This causes mesenchymal cells to be less likely to undergo proliferation and more likely to rest during a cell cycle. The second is a decrease to their likelihood of being cleared by the immune system, which we call mesenchymal immune evasion (MIE). This decreases the likelihood of mesenchymal cells being cleared by both NKs and CTLs during a cell cycle. Though we have not found clinical data on which to base parameter values for either of these, by their very nature they are proportions on the interval $[0, 1]$. Thus, it is possible to sample from the entire parameter and in fact we do present results from a large subset of this parameter space and our sensitivity analysis fully explores this space.

2.5 Model Simulation

2.5.1 Initial conditions

Every model run starts with a number of in situ tumor cells, N_0 , determined by the set of parameters used for the run. A set of warmup cycles are run to ensure arrival to this steady state. During these warmup cycles, no mutations are allowed to occur the only immune cells present are NKs. After the warmup cycles are complete, a mutagenic event is simulated in which all the tumor cells experience a random increase in their probability to mutate. From then on, a cell which proliferates also either mutates or increases its probability of mutating later on.

2.5.2 Tumor cell fate

During each cell cycle, every cell is randomly assigned one of four fates: proliferation, apoptosis, immune clearance, and rest in G_0 . Depending on both cell intrinsic, e.g. EMT and mutational state, and cell extrinsic, e.g. immune infiltrate and total number of tumor cells, each of the four options is given a probability of occurring for each cell. For proliferation, we assume the total number of tumor cells exerts a negative feedback on the proliferation probability for each cell. Also, cells that have a driver mutation in the proliferation pathway have an increased probability of proliferating. Meanwhile, mesenchymal cells have a decreased probability of proliferating and an increased probability of resting. For apoptosis, the probability is constant except for those cells with a driver mutation in the apoptosis pathway which have a decreased probability of apoptosis. For immune clearance, the malignant tumor cells are assumed more immunogenic and thus have a higher probability of being cleared by the immune system. This probability can be decreased by a cell having a driver mutation in the immune evasion pathway or by a cell being mesenchymal. Further details and equations are given in the supplement.

2.5.3 Completing the Cell Cycle

Once all the cell fates are carried out, the rest of the components of the model update to account for these changes. First, the immune populations are updated in two steps. In step one, immune cells are exhausted based on the number of malignant cells that were cleared by their type. So, each clearance by an NK cell results in the NK population decreasing by one, and similarly for CTLs. In step two, all three immune populations are updated deterministically based on a simple ODE model of their dynamics. NKs have a constant source and death rate while CTLs and Tregs have a source rate that is dependent on the number of malignant cells cleared that cycle and a constant death rate. In addition, the concentration of TGF- β mediates recruitment of Tregs with higher concentrations of TGF- β resulting in a greater influx of Tregs.

Also at this time, new driver mutations have a chance to occur. The cells that proliferated are the only ones that have a probability of acquiring a new driver mutation. This probability is cell-specific and is increased if no mutation occurs and reset to 0 if a mutation does occur.

Finally, the concentration of TGF- β is updated as well as the EMT score for each cell is updated. Malignant cells and Tregs are the two sources of TGF- β each producing a constant amount. Tregs are assumed to produce much more TGF- β than malignant cells. The resulting quantity of TGF- β is divided up randomly among the tumor cells. This quantity is combined with the EMT score of each cell and the resulting quantity determines which cells undergo EMT and which undergo MET. The equations governing these processes can be found in the supplement.

2.5.4 Mutation and progression

At the end of each cell cycle, the proportion of tumor cells that are invasive is calculated, and if it is above a certain threshold, the simulation ends and we declare the tumor to have progressed. The Time to Cancer is given as the time from the start of the simulation, not including warmup, until this timepoint. Simulations run until either a this occurs or until the predetermined maximum number of cycles has been reached.

2.6 Parameter Sensitivity Analysis

To study parameter sensitivity, we implemented the Morris method, a one-step-at-a-time global sensitivity analysis algorithm. The algorithm runs the model numerous times by picking several base points and then varying one parameter at a time from each base point and tracking the difference [44, 45]. Each model run involved 1000 patients and the value returned was the area under the survival curve. We ran the algorithm by sampling at 30 points in parameter space and then changing each parameter one time at each point. In the implementation by Sohier, they recommend using at least 10 points [45].

To determine which points in parameter space to sample at, we had to create distributions on all parameter values. To do this, we derived many parameters from existing literature and the rest we made reasoned predictions. For example, many previous studies have established the parameters of immune population dynamics such as recruitment and death rates [43]. Because our model only works with on the order of 10^2 tumor cells whereas an actual tumor would have on the order of 10^9 cells [43], we scaled the parameters so the immune populations

Name	Description
p	proliferation rate of tumor cells
d_C	death rate of tumor cells
Δ_{MIE}	mesenchymal immune evasion
Δ_{MGA}	mesenchymal growth arrest
Δ_A	decrease in apoptosis rate in cells with driver mutation in apoptosis pathway
Δ_{IE}	increase in immune evasion in cells with driver mutation in immune evasion pathway
Δ_P	increase in proliferation in cells with driver mutation in proliferation pathway
K_0	EC50 term for negative feedback of tumor cells on own proliferation
K_1	EC50 term for probability of NK cell finding mutant cell
K_2	EC50 term for Treg inhibition of cytotoxic functions
K_3	EC50 term for how much TGF- β each cell has
K_4	EC50 term for TGF- β activation of Tregs
E_{NK}	rate of NKs clearing mutants
E_{CTL}	rate of CTLs clearing mutants
σ_{NK}	NK source rate
σ_{CTL}	CTL source rate per cleared malignant cell
σ_{Treg}	Treg source rate per cleared malignant cell
d_{NK}	NK death rate
d_{CTL}	CTL death rate
d_{Treg}	Treg death rate
k_{EMT}	EMT/MET rate
σ	standard deviation of noise in TGF- β each cell receives
τ_{max}	max amount of TGF- β any cell can receive
τ_{MUT}	rate of TGF- β production by mutant cells
τ_{Treg}	rate of TGF- β production by Treg

Table 1: The model parameter names and descriptions. Note that some of these values are affected by the inflammation state of the system.

would also be proportionately reduced. That is, for instance, how we ended up with NK cells having a steady state population of 10 cells in the model.

Once we had these parameter values, we created Gaussian distributions centered at these values with large variances and truncated at 0. Some of the parameters were also truncated to the right because they represented a proportion, such as MIE and MGA, or because we simply wanted to keep the values within a reasonable range, such as the inflammation low and high duration parameters. The standard deviations were chosen as twice the mean to get a large spread of values.

The algorithm computes the average of the absolute change of the output, which in our model is the area under the survival curve. This output of the algorithm, μ^* , is the sensitivity plotted in Fig. 2.

2.7 Analysis of patient survival data from TCGA database

We obtain primary tumor bulk mRNA sequencing and censored survival data for all individuals monitored in the relevant project from the Cancer Genome Atlas (TCGA) from the Genomic Data Commons portal in R, using the package TCGABiolinks. Given $n \geq 1$ gene set keywords (e.g. "inflammatory" and "emt"), the symbolic names of all msigdb gene sets are searched for matches to these keywords, and matched gene sets are grouped by keyword. For each element in the product S of all keyword groups, the following analysis is performed.

1. The first principle component of the expression across the gene set is obtained for each element of the n -tuple of gene sets.
2. Two clusters of n -dimensional patient vectors are obtained by k -means clustering.
3. The patients are separated by their cluster identity and Kaplan-Meier curves are fit to their corresponding survival data.
4. Under the null hypothesis that there is no difference between the survival of the two groups, a log-rank test is performed.
5. The log-rank test p -value for each element in S is placed in an ordered list, the lowest of which defines the element of S whose composite gene sets are most predictive of patient survival in the given TCGA project or tumor type.

Below we analyze data gathered for pancreatic cancer, denoted by "PAAD" in TCGA.

3 Results

3.1 A multiscale agent-based model of EMT-immune-tumor cell interactions to study tumor progression

We begin by investigating general features of the model to establish baseline conditions and to assess the impact of various model components on the key measured outcomes: the probability of progression, and the Time to Cancer. Within the cell cycle, cell fate is determined via a set of rules that are influenced by EMT and immune interactions (Fig. 1A). For example, if a cell undergoes EMT, the probability that it will proliferate is reduced; if a cell gains a driver mutation in the apoptosis pathway, the probability that it undergoes apoptosis is greatly reduced. The different means by which the immune system acts on tumor cells are also shown (1A Inset).

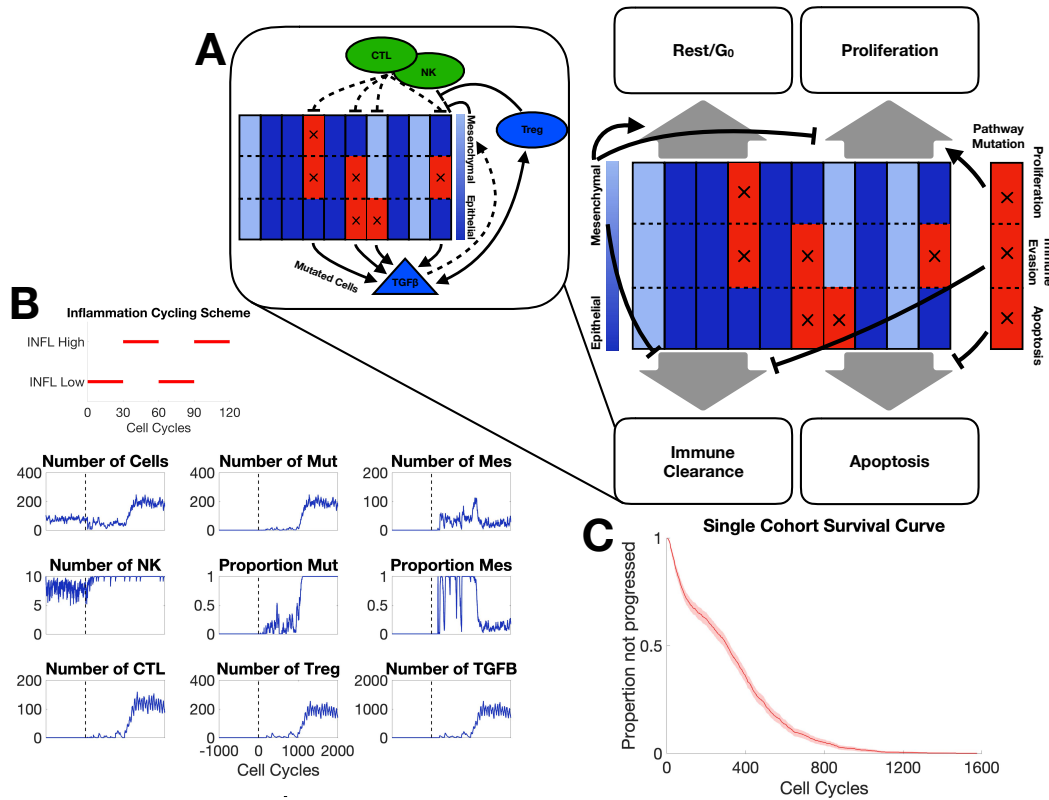


Figure 1: A. Schematic depiction of agent-based model components; each of the 10 columns represents a single tumor cell divided into three compartments representing the state (altered or not) of the three pathways with mutagenic potential; red/blue denotes altered/unaltered pathways. Black arrows depict regulation of the cell fate in each cell cycle. Inset depicts major interactions between the immune system and tumor cells. B. A representative simulation of one patient. The model parameter values used can be found in Table S2. The inflammation cycling scheme is represented above the patient dynamics. The vertical dashed line denotes the end of the warmup period. Mut: malignant cells; Mes: mesenchymal cells. C. Survival curve for one cohort of patients with the parameter values given in Table S2.

NK cells and CTLs attempt to clear malignant tumor cells, and deactivate upon successfully carrying out their cytotoxic function. Tregs inhibit cytotoxic activity. Tregs also release TGF- β which promotes the recruitment of Tregs, as well as increasing the rate of EMT.

The inflammation cycling scheme for a typical *in silico* patient consists of alternating high and low regimes (Fig. 1B); the inflammation schemes modeled will be discussed in detail below. For this patient, after the warmup period, cell mutations are observed at a rate low enough that they are cleared by cytotoxic cells before being able to establish a tumor. After about 700 cell cycles, the malignant cell population begins to grow steadily. At this point, large numbers of cells from the adaptive immune system (CTLs and Tregs) are being quickly recruited into the TME, and a peak in TGF- β expression is observed. After 841 cell cycles, the proportion of malignant cells reaches 50%: the threshold defining progression. Thus this patient has a Time to Cancer of 841 cell cycles, or 631 days. For this patient, we let the simulation go beyond this timepoint, and we see that the number of malignant cells grows rapidly and soon makes up 100% of the cell population. A peak in number of mesenchymal cells is observed shortly after this threshold is reached, and following this most cells transition back to epithelial.

Considering the immune system dynamics, we see that the NK population is approximately constant, while the adaptive populations (CTL and Treg cells) grow quickly and dwarf NK cells in number following the accumulation of driver mutations. The adaptive immune populations also appear to exhibit oscillatory behavior, however note that this is not due to intrinsic dynamics but rather due to the inflammation scheme that the patient is undergoing: alternating between 30 cell cycles of high inflammation and 30 cell cycles of low inflammation. Within each of these periods, adaptive immune populations increase or decrease rapidly in accordance with the inflammation state.

The *in silico* patient described here is given for the purpose of illustrating features of the model. Given the multiple sources of noise in the model, in order to quantify patient dynamics and cancer-free survival rates, below we will simulate large cohorts of patients. In Fig. 1C we simulate the survival curve for a cohort of 500 patients: we see that after the mutagenic event that a large number of tumors quickly progress while others takes some time before progressing. By about 1200 cell cycles, all tumors have progressed.

3.2 Identifying regulatory parameters via Morris global sensitivity analysis

Exploring the parameter spaces of models in systems biology is – in general – a hard problem. Performing Bayesian parameter inference to inform parameter values is advisable wherever possible [46]. Here, a lack of detailed molecular measurements (i.e. data for tumor growth dynamics abound, but simultaneous data on the immune dynamics are lacking) preclude inference of the full model. In addition, while inference schemes for agent-based models are developing [47, 48], simulation times remain a hurdle [49]. Parameters for aspects of this model that were studied previously can be constrained [8], however even for these, the new additions to the model could push it into new behavioral regimes. Thus to adequately sample the parameter space of the model and identify those areas of parameter space that exert the most control, we use sensitivity analysis.

To assess the sensitivity of the model parameters and thus identify those that are most important in determining progression-free survival times, we performed Morris one-step-at-a-time (OAT) sensitivity analysis (see Methods). The results of Morris OAT on the 31 model

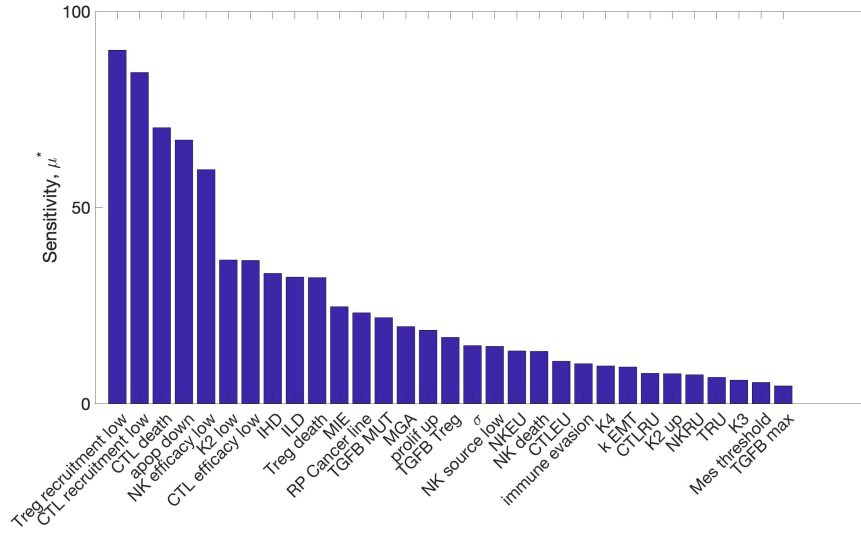


Figure 2: Sensitivity analysis of model parameters, determined via the global Morris one-step-at-a-time method. μ^* denotes the average absolute change in Time to Cancer when the parameter is varied.

parameters are shown in Figure 2. We see that a subset of parameters demonstrate much higher levels of sensitivity than others. The two most influential according to this analysis are the recruitment rates of Tregs and CTLs under low inflammation. We also note that the parameters related to EMT have a moderate effect on Time to Cancer.

Many parameters in the model change depending on the inflammation state. For the sake of a naming convention, any parameter which includes “low” in the name represents the parameter value when inflammation is low. For when the inflammation is high, these “low” parameters get scaled by parameters which include “up” in the name. The ILD and IHD parameters determine the duration of low and high inflammation, respectively. A cell with altered pathways will be affected by the Δ_A , Δ_P , and Δ_{IE} parameters, depending on whether the apoptosis, proliferation, or immune evasion pathways are altered. The mesenchymal cells are influenced by the MGA and MIE parameters. Finally, σ , k_{EMT} , τ_{max} , and K_3 all control how cells transition between the epithelial and mesenchymal states.

Since one goal of our analysis is to assess the specific effects of EMT on immune-cancer dynamics, the parameters MIE and MGA are of particular interest. In addition, inflammation parameters dictating the cycling scheme are of interest because they are both highly influential on Time to Cancer and capable of being targeted by therapeutic treatments. In terms of Treg cells, their secretion of TGF- β is highly significant and we will also study it further.

3.3 Mesenchymal phenotypic properties dramatically alter invasion-free survival times

When a cell transitions from an epithelial to a mesenchymal state, two phenotypic cell characteristics change: mesenchymal immune evasion (MIE) and mesenchymal growth arrest (MGA). Both parameters are defined proportionally and hence lie in $[0, 1]$, where higher values indicate more mesenchymal-like properties. The parameter Δ_{MIE} is the proportional reduction in the

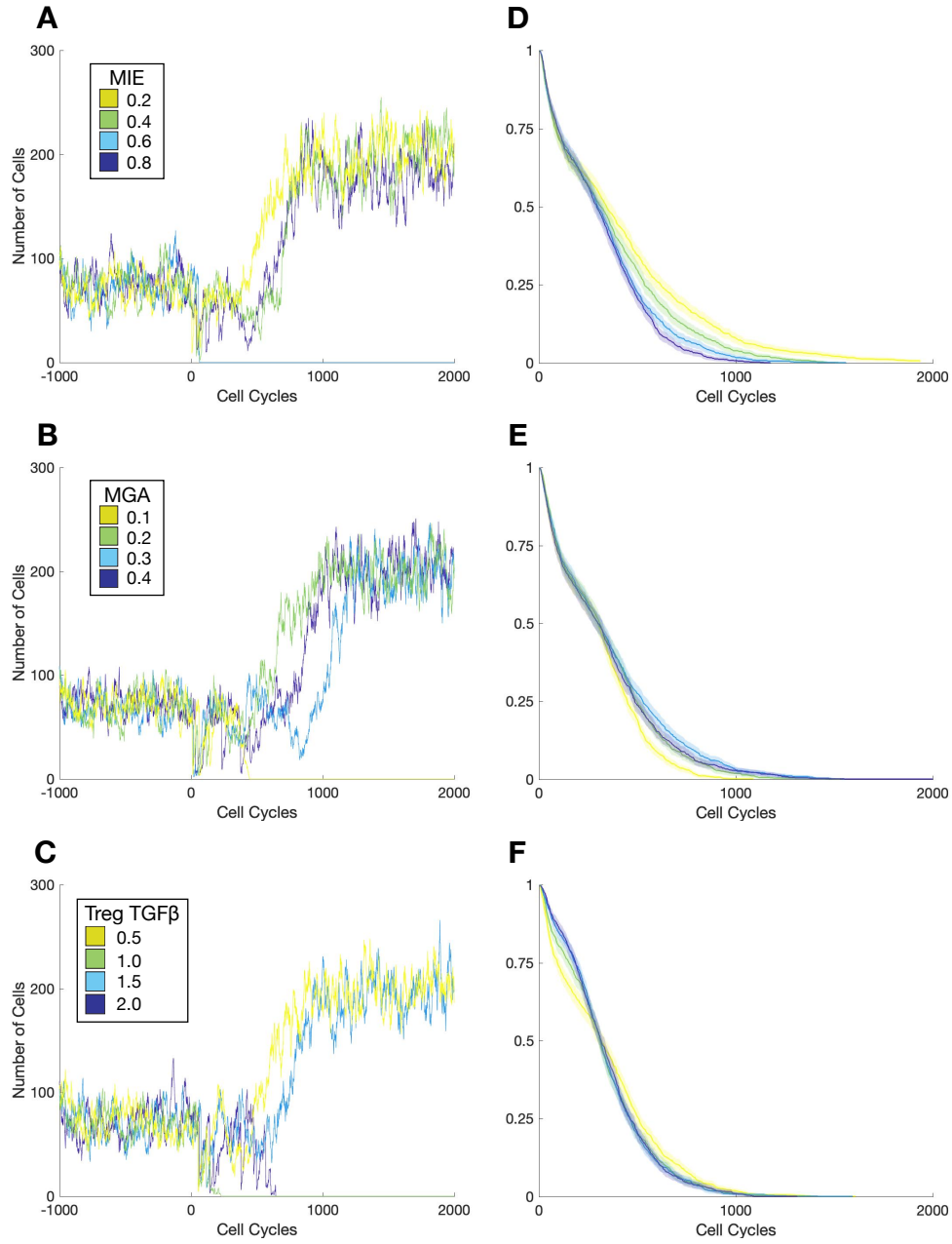


Figure 3: Effects of mesenchymal tumor cell properties on the Time to Cancer. Trajectories of one patient per cohort from warmup period $[-1000, 0]$ to 2000 cell cycles, for Δ_{MIE} (A); Δ_{MGA} (B); and τ_{Treg} (C). D. Survival curves corresponding to changes in MIE (A) for a patient cohort of size 1000; shaded region represents the 95% confidence interval for the evaluated function. E. Survival curves for patient cohort corresponding to changes in MGA (B). F. Survival curves for patient cohort corresponding to changes in Treg $TGF-\beta$ (C).

probability that an invasive mesenchymal cell will be subject to immune clearance in a given cycle. The parameter Δ_{MGA} is the proportional reduction in the probability that a mesenchymal cell will proliferate in a given cell cycle, thus is equivalent (strictly in terms of its impact on the cell cycle) to an increased proportion of time spent resting in the G_0 phase. The cytokine TGF- β is also involved in the EMT process (by increasing the probability of EMT). All three of these parameters were found to have large effects on the model by the Morris OAT analysis presented in Section 3.2.

As MIE increases, the invasion-free survival decreases (Fig. 3A) under all sets of parameters studied: as this subpopulation of invasive cells becomes more resistant to immune clearance, the tumor as a whole grows more resilient and thus will grow faster. These results are summarized by Fig. 3D.

The relationship between MGA and invasion-free survival is non-monotonic. For smaller values of MGA, an increase in MGA results in increased invasion-free survival (Fig 3E). This result is explored in Section 3.4.

Third, TGF- β can be varied in two ways: the production by malignant cells and the production by Treg cells. In Fig 3F, the results of varying Treg TGF- β production are shown, indicating that an increased Treg TGF- β does not have much effect on invasion-free survival time. The effect we do see is that lower Treg TGF- β results in more rapid invasion or if it survives to a certain time point, a longer time to invasion. Here, we choose to focus on the results of the effect of MIE and MGA as the more direct link to the mesenchymal phenotype.

3.4 A key EMT regime maximizes cancer-free survival time under chronic inflammation

We explored the effects of varying the inflammation state of the patient on invasion-free survival, to investigate competing interactions within the tumor microenvironment and their effect on EMT. Patient cohorts were simulated under different inflammation regimes: permanently low inflammation; permanently high inflammation; or variable inflammation. Compared to the other inflammation regimes, a permanently high inflammation state results in tumors that are relatively resistant to changes in mesenchymal parameters (Fig. 4). However, when the inflammation either has temporary or permanent periods of low inflammation, different relationships emerge: invasion-free survival time becomes negatively correlated with MIE, and a local maximum for the invasion-free survival time is found with respect to MGA ($\Delta_{\text{MGA}} = 0.2$).

These striking differences in the mean invasion-free survival – that can be extended by up to double compared to other MGA values– have clear therapeutic implications. Our model predicts that a patient suffering intermittent inflammatory attacks at the tumor site will benefit from controlling the proliferation of the mesenchymal cells.

In contrast, when MIE is varied for different inflammation cycling schemes, regardless of the cycling scheme, increasing the MIE will decrease the invasion-free survival (worsen cancer progression and prognosis) or have minimal effect. Thus under any inflammation regime with periods of low inflammation, reductions of any size in the rate of mesenchymal immune evasion lead to improved patient outcomes.

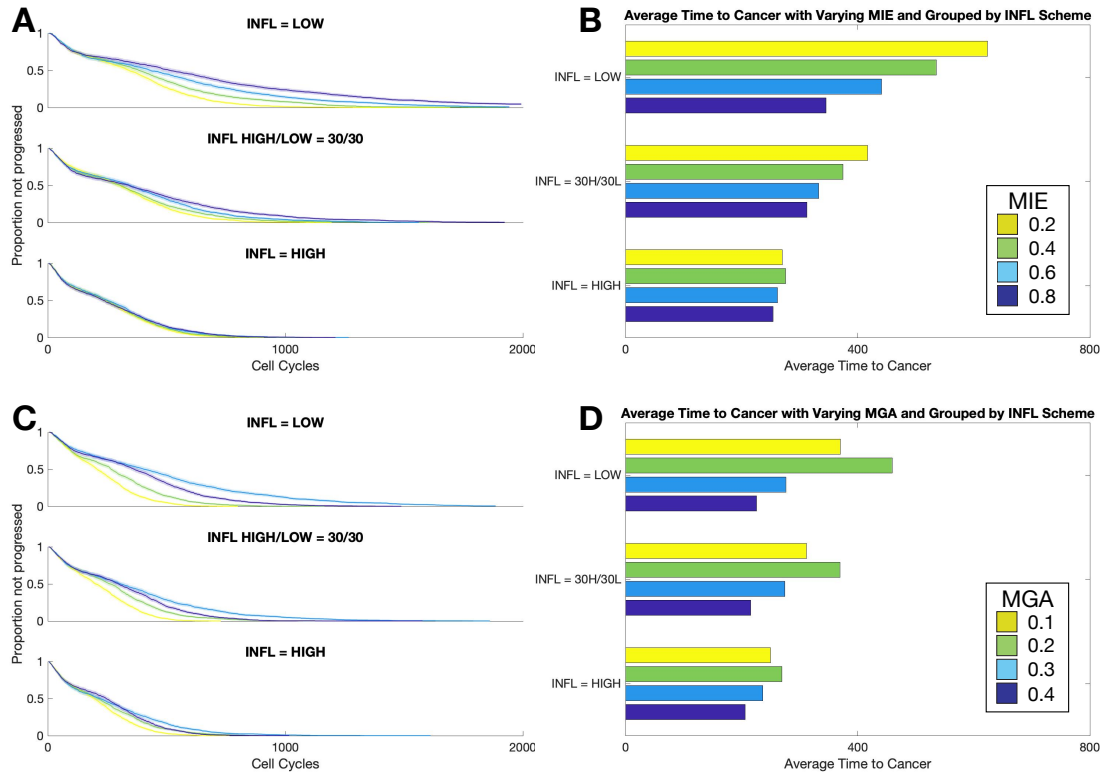


Figure 4: Effects of inflammation on the Time to Cancer under different cycling schemes. A-B. As MIE varies, survival curves (each of 200 patients) and corresponding bar plots to summarize the mean Time to Cancer for each cohort are shown. C-D. As MGA varies, survival curves and corresponding bar plots to summarize the mean Time to Cancer for each cohort are shown.

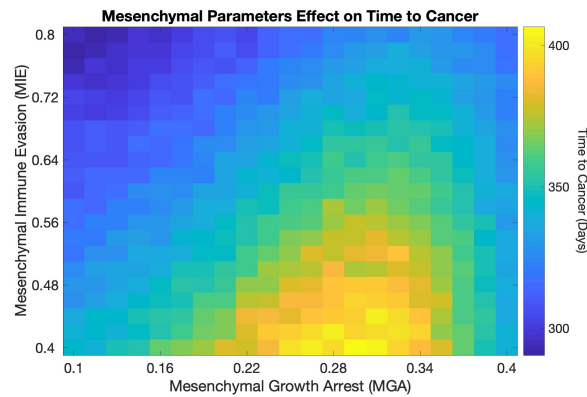


Figure 5: Summary of the contrasting effects of MIE and MGA on the Time to Cancer.

3.5 TCGA data analysis supports model predictions by showing that mesenchymal phenotypic properties reduce invasion-free survival

The effects that mesenchymal phenotypic properties have on progression can also be assessed by studying mesenchymal immune evasion (Δ_{MIE}) and mesenchymal growth arrest (Δ_{MGA}) on a heatmap (Fig. 5). We found that over the full range of values of Δ_{MGA} considered, increasing Δ_{MIE} decreases the invasion-free survival time. However, for any given value of mesenchymal immune evasion, there is a value of mesenchymal growth arrest that maximizes the invasion-free survival. Moreover, this optimal value increases with increasing MIE. Together these results show that while reducing the ability of a mutated cell to evade the immune system by any amount will improve the probability of invasion-free survival, an optimal value of MGA will prolong the tumor remaining in situ.

To compare these model predictions with experimental studies, we analyzed data from The Cancer Genome Atlas (TCGA) database to study the effects of immune and EMT interactions on prognosis of cancers for which inflammation is known to play an important role, such as colonic or pancreatic cancers [50, 51]. The TCGA Pan-Cancer Clinical Data Resource (TCGA-CDR) provides multiple computed clinical endpoints for PAAD [52]. Here, we focus on the disease-free interval (DFI) and the overall survival (OS) endpoints. In essence, a tumor where the DFI is short will undergo rapid (post-treatment) initiation. In turn, we expect the distance between DFI and OS to be relatively small for a tumor undergoing rapid progression following initial (post-treatment) detection. Therefore, recurrent tumors may be roughly categorized as either rapidly progressing or slowly progressing by defining a threshold for the DFI/OS divergence. We selected three tumor types whose TCGA-CDR profile indicated slow progression (OV, SKCM, LIHC) and two tumor types whose profile indicated rapid progression (PAAD, LUAD). See Figures 6 and 7 for OV and PAAD. The others are in the supplement.

For each cohort of cancer patients, we cluster the patients via k-means ($n = 2$) on gene ontologies relating to only EMT (Figures 6C, 7C), only inflammation (Figures 6E, 7E), and both EMT and inflammation (Figures 6A, 7A). We also plot the corresponding survival curves for each of the clusters obtained (utilizing the originally reported OS endpoint). We see that in both cases, survival is affected by the gene ontology signature, but the presence of both EMT and inflammatory signatures has a greater impact on survival than the effects of EMT alone. This suggests that when studying interactions between cancer and the immune system, it is important to consider EMT; the effects of which may impact the dynamics and should not be overlooked.

We note that the comparison between simulation and data here is indirect since the model studies the progression of cancer from an arbitrary starting point, while the data address the progression of cancer following treatment, but analogous processes are at play during both the progression of clonal dynamics addressed by the model, and the progression of disease after cancer treatment. In particular, the plasticity of tumor cells allows them to evade treatment by undergoing post-treatment processes resembling the de-novo appearance of cancer [53].

4 Discussion

Despite the intense research focus on interactions between cancer and the immune system, and well as on the effects of EMT on cancer, there has not previously, to the best of our knowledge,

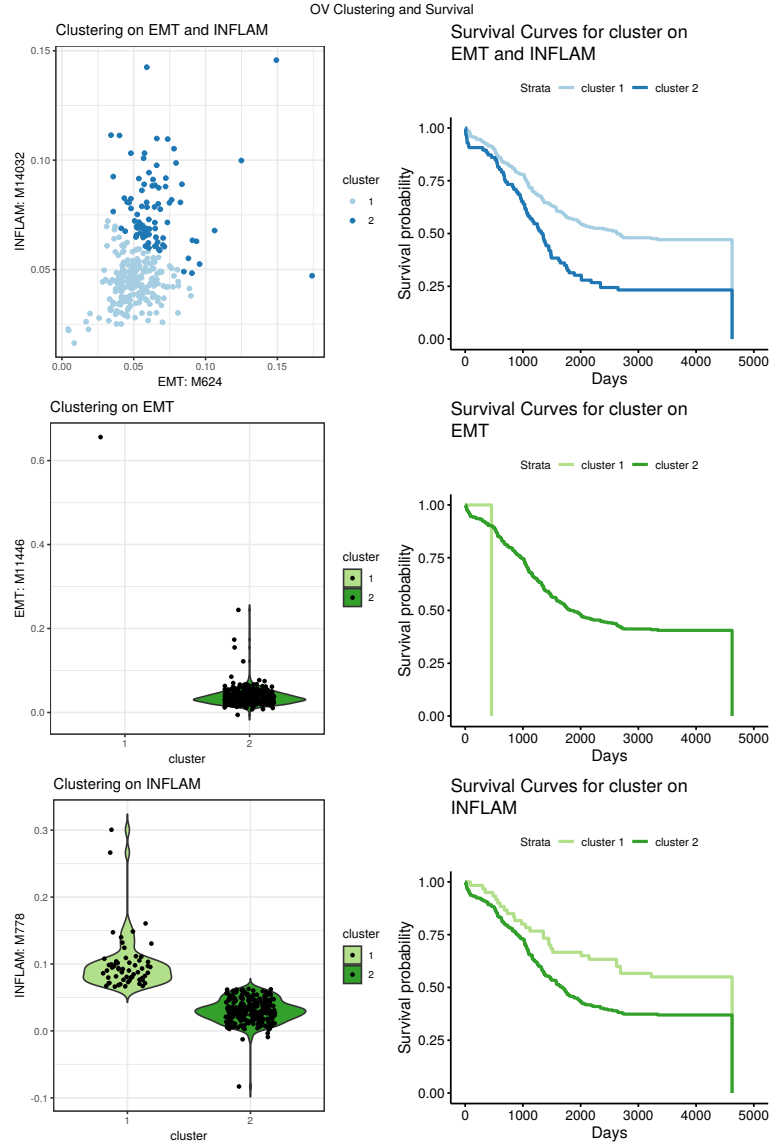


Figure 6: A. K-means clustering of OV using gene ontology terms indicative of EMT and inflammation signatures ($k = 2$). B. Survival plots corresponding to the clustering on EMT and inflammation. C. K-means clustering of OV using gene ontology terms indicative of an EMT signature ($k = 2$). D. Survival plots corresponding to the clustering on EMT. E. K-means clustering of OV using gene ontology terms indicative of inflammation ($k = 2$). F. Survival plots corresponding to the clustering on inflammation.

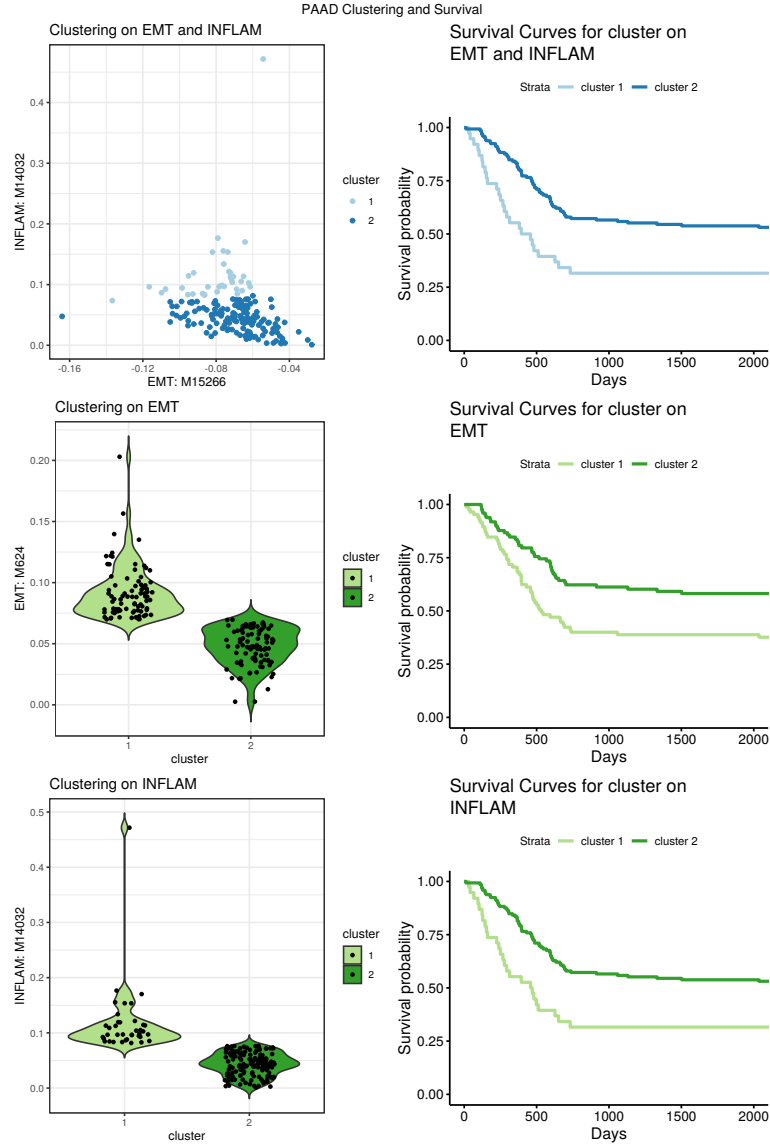


Figure 7: A. K-means clustering of PAAD using gene ontology terms indicative of EMT and inflammation signatures ($k = 2$). B. Survival plots corresponding to the clustering on EMT and inflammation. C. K-means clustering of PAAD using gene ontology terms indicative of an EMT signature ($k = 2$). D. Survival plots corresponding to the clustering on EMT. E. K-means clustering of PAAD using gene ontology terms indicative of inflammation ($k = 2$). F. Survival plots corresponding to the clustering on inflammation.

390 been a model developed that combines these three components. Here we studied cancer, the
391 immune system, and EMT, during the progression from an in situ tumor to invasive disease.
392 We saw this as an even more pressing need given the shared factors that influence all of these,
393 such as TGF- β . We used an individual cell-based model framework to describe the multiscale
394 processes that can lead to cancer: DNA damage occurs during the cell cycle and this can lead
395 to driver mutations in pathways that affect the fitness of the cell, which in turn affects the cell
396 population dynamics, which are also influenced by the intrinsic state of the cell (EMT), and
397 extrinsic immune factors (inflammation, cytotoxic cells, etc).

398 We found that this model recapitulated invasion-free survival dynamics, and via global
399 parameter sensitivity analysis, we identified parameters exerting key control over model behav-
400 ior. Focusing on these led us to identify that: increasing mesenchymal immune evasion and
401 increasing Treg TGF- β production both lead to shorter invasion-free survival times. However,
402 varying the level of inflammation led to paradoxical effects with regards to mesenchymal growth
403 arrest: under regimes with periods of low inflammation, an optimal level of mesenchymal
404 growth arrest can improve outcomes and maximize the invasion-free survival.

405 To test these predictions, we performed unsupervised analysis of pancreatic cancer data
406 from The Cancer Genome Atlas, and looked at survival across two groups: we found that the
407 presence of an EMT signature increased differences in survival between groups in combination
408 with inflammation. We chose to summarize *in silico* patient simulations with a single parameter:
409 the invasion-free survival time, which we found captured the essential characteristics of the
410 model. There are, of course, many tissue trajectories that all result in cancer, and it is our
411 opinion that analysis of the transient cell dynamics in premalignant tissues is a pressing need
412 to shed insight into cellular biomarkers of cancer.

413 These results represent promising steps in understanding the competing roles of the immune
414 system and EMT during progression of epithelial cancers, yet much remains to be done. Further
415 development of the inflammation module of this model is important given the large and
416 sometimes paradoxical roles that the inflammatory state exerts on the epithelia and cancer-free
417 survival (Figs. 2 and 4B, D). Currently, inflammation is modeled as independently cycling
418 between high and low schemes, however many of the agents considered in the model actively
419 contribute to the inflammatory state, thus as an extension these components could be coupled,
420 for example by assuming that the level of inflammation depends on the number of and the
421 degree of mutations that cells in the tumor harbor). Another layer of complexity is revealed
422 by the natural anti-inflammatory role of Tregs. One consequence of the current model is
423 that decreasing the number of Tregs increases the cancer-free survival. Clearly, there exists
424 a trade-off to be accounted for, and adding to the model the main effector function of Tregs
425 could remedy this and add depth to our understanding of the various roles that Tregs play in
426 and around the tumor.

427 The roles that TGF- β plays throughout the tumor and its microenvironment also warrant
428 further investigation. We found that, below a certain threshold, reduction of TGF- β increases the
429 Time to Cancer (Fig. 3E), thus reducing expression levels of TGF- β in the tumor microenviron-
430 ment benefits survival. Intriguingly, recent experimental work demonstrated that TGF- β drives
431 tumor suppression in pancreatic cancer by promoting EMT [54]. However, TGF- β is a master
432 regulator implicated in numerous cellular signaling processes, and changing the concentrations
433 of TGF- β even in a local tumor microenvironment could have large off-target effects. Indeed,
434 it has been shown that TGF- β promotes invasion and heterogeneity (although suppresses cell

proliferation) in squamous cell carcinoma [55]. Future work thus ought to consider the effects of targeting signaling factors downstream of TGF- β that still have the ability to modulate epithelial cell dynamics. Towards this end, we are currently developing a larger TGF- β signaling pathway module with appropriate crosstalks to epithelial/mesenchymal/immune cell functions to be incorporated into the model.

A further goal for future work is to explore (and exploit) the heterogeneity of tumor evolution in greater depth: this heterogeneity aids the evasion of the tumor from immune effects. Studying the consequences of decanalization [56] during cancer progression is too-often sidelined, despite evidence supporting its prominence [57–59]. Yet despite these challenges, for which the complexity of the disease may be often in large part responsible, great progress has been and continues to be made. As we approach a new generation of immunotherapies, it is these very complexities that we must better understand in order to control or eradicate the disease.

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References

- [1] Dillekås H, Rogers MS, Straume O. Are 90% of deaths from cancer caused by metastases? *Cancer Medicine*. 2019;.
- [2] Ryan BM, Faupel-Badger JM. The hallmarks of premalignant conditions: a molecular basis for cancer prevention. In: *Seminars in oncology*. vol. 43. Elsevier; 2016. p. 22–35.
- [3] De Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. *Nature Reviews Cancer*. 2006;6(1):24.
- [4] Finn OJ. Immuno-oncology: understanding the function and dysfunction of the immune system in cancer. *Annals of Oncology*. 2012;23(Suppl 8):viii6–viii9. doi:10.1093/annonc/mds256.
- [5] Ruffell B, DeNardo DG, Affara NI, Coussens LM. Lymphocytes in cancer development: polarization towards pro-tumor immunity. *Cytokine & Growth Factor Reviews*. 2010;21(1):3–10.
- [6] Hu B, Elinav E, Huber S, Booth CJ, Strowig T, Jin C, et al. Inflammation-induced tumorigenesis in the colon is regulated by caspase-1 and NLRC4. *Proceedings of the National Academy of Sciences*. 2010;107(50):21635–21640. doi:10.1073/pnas.1016814108.

- 471 [7] Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *The Lancet*.
472 2001;357(9255):539–545. doi:10.1016/S0140-6736(00)04046-0.
- 473 [8] Guo Y, Nie Q, MacLean AL, Li Y, Lei J, Li S. Multiscale Modeling of Inflammation-Induced
474 Tumorigenesis Reveals Competing Oncogenic and Oncoprotective Roles for Inflammation.
475 *Cancer Research*. 2017;77(22):6429–6441. doi:10.1158/0008-5472.CAN-17-1662.
- 476 [9] Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nature*
477 *Reviews Cancer*. 2012;12(4):252.
- 478 [10] Restifo NP, Dudley ME, Rosenberg SA. Adoptive immunotherapy for cancer: harnessing
479 the T cell response. *Nature Reviews Immunology*. 2012;12(4):269.
- 480 [11] Schreiber RD, Old LJ, Smyth MJ. Cancer Immunoediting: Integrating Immunity's
481 Roles in Cancer Suppression and Promotion. *Science*. 2011;331(6024):1565–1570.
482 doi:10.1126/science.1203486.
- 483 [12] Nieto MA, Huang RYJ, Jackson RA, Thiery JP. EMT: 2016. *Cell*. 2016;166(1):21–45.
- 484 [13] Nie Q. Stem cells: a window of opportunity in low-dimensional EMT space. *Oncotarget*.
485 2018;9(61). doi:10.18632/oncotarget.25852.
- 486 [14] Sha Y, Haensel D, Gutierrez G, Du H, Dai X, Nie Q. Intermediate cell states in epithelial-
487 to-mesenchymal transition. *Physical Biology*. 2019;16(2):021001. doi:10.1088/1478-
488 3975/aaf928.
- 489 [15] Hong T, Watanabe K, Ta CH, Villarreal-Ponce A, Nie Q, Dai X. An Ovol2-Zeb1 mutual
490 inhibitory circuit governs bidirectional and multi-step transition between epithelial and
491 mesenchymal states. *PLoS Computational Biology*. 2015;11(11):e1004569.
- 492 [16] Jolly MK, Jia D, Boareto M, Mani SA, Pienta KJ, Ben-Jacob E, et al. Coupling the modules of
493 EMT and stemness: A tunable 'stemness window' model. *Oncotarget*. 2015;6(28):25161–
494 25174.
- 495 [17] Moris N, Pina C, Martinez Arias A. Transition states and cell fate decisions in epigenetic
496 landscapes. *Nature Reviews Genetics*. 2016;17(11):693–703. doi:10.1038/nrg.2016.98.
- 497 [18] MacLean AL, Hong T, Nie Q. Exploring intermediate cell states through
498 the lens of single cells. *Current Opinion in Systems Biology*. 2018;9:32–41.
499 doi:10.1016/j.coisb.2018.02.009.
- 500 [19] Ta CH, Nie Q, Hong T. Controlling stochasticity in epithelial-mesenchymal transition
501 through multiple intermediate cellular states. *Discrete and Continuous Dynamical Systems*
502 *- Series B*. 2016;21(7):2275–2291. doi:10.3934/dcdsb.2016047.
- 503 [20] Rackauckas C, Schilling TF, Nie Q. Mean-Independent Noise Control of Cell Fates via
504 Intermediate States. *iScience*. 2018;0(0). doi:10.1016/j.isci.2018.04.002.
- 505 [21] Woods K, Pasam A, Jayachandran A, Andrews MC, Cebon J. Effects of epithelial to
506 mesenchymal transition on T cell targeting of melanoma cells. *Frontiers in Oncology*.
507 2014;4:367.

- [22] Terry S, Savagner P, Ortiz-Cuaran S, Mahjoubi L, Saintigny P, Thiery JP, et al. New insights into the role of EMT in tumor immune escape. *Molecular Oncology*. 2017;11(7):824–846.
- [23] Peinado H, Olmeda D, Cano A. Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? *Nature Reviews Cancer*. 2007;7:415 EP –.
- [24] Lim J, Thiery JP. Epithelial-mesenchymal transitions: insights from development. *Development*. 2012;139(19):3471–3486.
- [25] Shi C, Chen Y, Chen Y, Yang Y, Bing W, Qi J. CD4+ CD25+ regulatory T cells promote hepatocellular carcinoma invasion via TGF- β 1-induced epithelial–mesenchymal transition. *OncoTargets and therapy*. 2019;12:279.
- [26] Anderson ARA, Chaplain MAJ. Continuous and discrete mathematical models of tumor-induced angiogenesis. *Bulletin of Mathematical Biology*. 1998;60(5):857–899.
- [27] Sherratt Jonathan A , Nowak Martin A . Oncogenes, anti-oncogenes and the immune response to cancer : a mathematical model. *Proceedings of the Royal Society of London Series B: Biological Sciences*. 1992;248(1323):261–271. doi:10.1098/rspb.1992.0071.
- [28] Pillis LGd, Radunskaya AE, Wiseman CL. A Validated Mathematical Model of Cell-Mediated Immune Response to Tumor Growth. *Cancer Research*. 2005;65(17):7950–7958. doi:10.1158/0008-5472.CAN-05-0564.
- [29] Kim E, Kim JY, Smith MA, Haura EB, Anderson ARA. Cell signaling heterogeneity is modulated by both cell-intrinsic and -extrinsic mechanisms: An integrated approach to understanding targeted therapy. *PLOS Biology*. 2018;16(3):e2002930. doi:10.1371/journal.pbio.2002930.
- [30] Gallaher J, Babu A, Plevritis S, Anderson ARA. Bridging Population and Tissue Scale Tumor Dynamics: A New Paradigm for Understanding Differences in Tumor Growth and Metastatic Disease. *Cancer Research*. 2014;74(2):426–435. doi:10.1158/0008-5472.CAN-13-0759.
- [31] Gallaher JA, Enriquez-Navas PM, Luddy KA, Gatenby RA, Anderson ARA. Spatial Heterogeneity and Evolutionary Dynamics Modulate Time to Recurrence in Continuous and Adaptive Cancer Therapies. *Cancer Research*. 2018;78(8):2127–2139. doi:10.1158/0008-5472.CAN-17-2649.
- [32] An G, Kulkarni S. An agent-based modeling framework linking inflammation and cancer using evolutionary principles: Description of a generative hierarchy for the hallmarks of cancer and developing a bridge between mechanism and epidemiological data. *Mathematical Biosciences*. 2015;260:16–24. doi:10.1016/j.mbs.2014.07.009.
- [33] Serre R, Benzekry S, Padovani L, Meille C, André N, Ciccolini J, et al. Mathematical Modeling of Cancer Immunotherapy and Its Synergy with Radiotherapy. *Cancer Research*. 2016;76(17):4931–4940. doi:10.1158/0008-5472.CAN-15-3567.

- 544 [34] Louzoun Y, Xue C, Lesinski GB, Friedman A. A mathematical model for pancre-
545 atic cancer growth and treatments. *Journal of Theoretical Biology*. 2014;351:74–82.
546 doi:10.1016/j.jtbi.2014.02.028.
- 547 [35] Briones-Orta MA, Levy L, Madsen CD, Das D, Erker Y, Sahai E, et al. Arkadia regulates tu-
548 mor metastasis by modulation of the TGF- β pathway. *Cancer Research*. 2013;73(6):1800–
549 1810. doi:10.1158/0008-5472.CAN-12-1916.
- 550 [36] Lavi O, Greene JM, Levy D, Gottesman MM. The Role of Cell Density and Intratumoral Het-
551 erogeneity in Multidrug Resistance. *Cancer Research*. 2013;doi:10.1158/0008-5472.CAN-
552 13-1768.
- 553 [37] Greene JM, Levy D, Fung KL, Souza PS, Gottesman MM, Lavi O. Modeling intrinsic
554 heterogeneity and growth of cancer cells. *Journal of Theoretical Biology*. 2015;367:262–
555 277. doi:10.1016/j.jtbi.2014.11.017.
- 556 [38] Greene JM, Levy D, Herrada SP, Gottesman MM, Lavi O. Mathematical Modeling
557 Reveals That Changes to Local Cell Density Dynamically Modulate Baseline Varia-
558 tions in Cell Growth and Drug Response. *Cancer Research*. 2016;76(10):2882–2890.
559 doi:10.1158/0008-5472.CAN-15-3232.
- 560 [39] Cho H, Levy D. Modeling the Dynamics of Heterogeneity of Solid Tumors in Re-
561 sponse to Chemotherapy. *Bulletin of Mathematical Biology*. 2017;79(12):2986–3012.
562 doi:10.1007/s11538-017-0359-1.
- 563 [40] Benzekry S, Lamont C, Barbolosi D, Hlatky L, Hahnfeldt P. Mathematical Modeling of
564 Tumor–Tumor Distant Interactions Supports a Systemic Control of Tumor Growth. *Cancer*
565 *Research*. 2017;77(18):5183–5193. doi:10.1158/0008-5472.CAN-17-0564.
- 566 [41] Owen MR, Stamper IJ, Muthana M, Richardson GW, Dobson J, Lewis CE, et al. Mathemat-
567 ical Modeling Predicts Synergistic Antitumor Effects of Combining a Macrophage-Based,
568 Hypoxia-Targeted Gene Therapy with Chemotherapy. *Cancer Research*. 2011;71(8):2826–
569 2837. doi:10.1158/0008-5472.CAN-10-2834.
- 570 [42] West J, You L, Brown J, Newton PK, Anderson AR. Towards multi-drug adaptive therapy.
571 *bioRxiv*. 2018; p. 476507. doi:10.1101/476507.
- 572 [43] de Pillis LG, Radunskaya AE. Modeling tumor–immune dynamics. In: *Mathematical*
573 *Models of Tumor-Immune System Dynamics*. Springer; 2014. p. 59–108.
- 574 [44] Morris MD. Factorial sampling plans for preliminary computational experiments. *Techno-*
575 *metrics*. 1991;33(2):161–174.
- 576 [45] Sohier H, Farges JL, Piet-Lahanier H. Improvement of the Representativity of the Morris
577 Method for Air-Launch-to-Orbit Separation. *IFAC Proceedings Volumes*. 2014;47(3):7954–
578 7959.
- 579 [46] Kirk PDW, Thorne T, Stumpf MPH. Model selection in systems and synthetic biology. *Cur-*
580 *rent Opinion in Biotechnology*. 2013;24(4):767–774. doi:10.1016/j.copbio.2013.03.012.

- 581 [47] Gallaher J, Hawkins-Daarud A, Massey SC, Swanson K, Anderson ARA. Hybrid approach
582 for parameter estimation in agent-based models. *bioRxiv*. 2017;doi:10.1101/175661.
- 583 [48] Warne D, Baker RE, Simpson MJ. Simulation and inference algorithms for stochastic
584 biochemical reaction networks: from basic concepts to state-of-the-art. *Journal of The*
585 *Royal Society Interface*. 2019;16(151):20180943. doi:10.1098/rsif.2018.0943.
- 586 [49] Lambert B, MacLean AL, Fletcher AG, Combes AN, Little MH, Byrne HM. Bayesian
587 inference of agent-based models: a tool for studying kidney branching morphogenesis.
588 *Journal of Mathematical Biology*. 2018;10(12):106. doi:10.1007/s00285-018-1208-z.
- 589 [50] Greten FR, Grivennikov SI. Inflammation and Cancer: Triggers, Mechanisms, and Conse-
590 quences. *Immunity*. 2019;51(1):27–41.
- 591 [51] Hu B, Elinav E, Huber S, Booth CJ, Strowig T, Jin C, et al. Inflammation-induced tumori-
592 genesis in the colon is regulated by caspase-1 and NLRC4. *Proceedings of the National*
593 *Academy of Sciences*. 2010;107(50):21635–21640.
- 594 [52] Liu J, Lichtenberg T, Hoadley KA, Poisson LM, Lazar AJ, Cherniack AD, et al. An integrated
595 TCGA pan-cancer clinical data resource to drive high-quality survival outcome analytics.
596 *Cell*. 2018;173(2):400–416.
- 597 [53] Sánchez-Danés A, Larsimont JC, Liagre M, Muñoz-Couselo E, Lapouge G, Brisebarre A,
598 et al. A slow-cycling LGR5 tumour population mediates basal cell carcinoma relapse after
599 therapy. *Nature*. 2018;562(7727):434.
- 600 [54] David C, Huang YH, Chen M, Su J, Zou Y, Bardeesy N, et al. TGF- β Tumor Suppression
601 through a Lethal EMT. *Cell*. 2016;164(5):1015–1030. doi:10.1016/j.cell.2016.01.009.
- 602 [55] Oshimori N, Oristian D, Fuchs E. TGF- β Promotes Heterogeneity and Drug Resistance in
603 Squamous Cell Carcinoma. *Cell*. 2015;160(5):963–976. doi:10.1016/j.cell.2015.01.043.
- 604 [56] Gibson G. Decanalization and the origin of complex disease. *Nature Reviews Genetics*.
605 2009;10(2):134–140. doi:10.1038/nrg2502.
- 606 [57] Cyll K, Ersvær E, Vlatkovic L, Pradhan M, Kildal W, Kjær MA, et al. Tumour heterogeneity
607 poses a significant challenge to cancer biomarker research. *British Journal of Cancer*.
608 2017;117(3):367–375. doi:10.1038/bjc.2017.171.
- 609 [58] Punt CJA, Koopman M, Vermeulen L. From tumour heterogeneity to advances in precision
610 treatment of colorectal cancer. *Nature Reviews Clinical Oncology*. 2017;14(4):235–246.
611 doi:10.1038/nrclinonc.2016.171.
- 612 [59] Dagogo-Jack I, Shaw AT. Tumour heterogeneity and resistance to cancer therapies. *Nature*
613 *Reviews Clinical Oncology*. 2018;15(2):81–94. doi:10.1038/nrclinonc.2017.166.