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 Introduction

The majority of deaths from cancer are due to metastasis of the disease [1]. It is thus of critical importance to understand better the progression from in situ to invasive disease. Underlying 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 shif 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

Here we briefly describe to core components of the model. Full details and equations are provided in the Supplement. 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).

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. 1).

2.1 Tumor Evolution

Associated with each tumor cells are two essential features: their mutational signature and their EMT score. We consider three idealized pathways that can be mutated: proliferation, when altered this increases the probability of the cell proliferating within each cell cycle; apoptosis, when altered this decreases the probability of a cell undergoing apoptosis; and immune evasion, when altered this decreases the probability that a mutated cell will be cleared by immune components.

2.2 Immune Cell Population Dynamics

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 Periodic 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 Epithelial-to-mesenchymal Transition

Each tissue cell has an EMT score between 0 and 1 with is set according to the concentration of TGF-β in the TME. Above a threshold, the cell acquires the phenotype of a mesenchymal tumor cell (MTC); otherwise, it is an epithelial tumor cell (ETC). For the purpose of simulation, ETCs are considered to be in the base state, and MTCs will have a subset of their parameters

updated. In modeling EMT this way, we are assuming that the same factor, TGF-β, drives EMT both at initiation and through progression of cancer.

Cells that have undergone EMT (i.e. MTCs) experience a reduction in proliferation, referred to as mesenchymal growth arrest (MGA), and a decrease in the likelihood that they will be cleared by immune cells (NKs or CTLs), referred to as mesenchymal immune evasion (MIE). Both these parameters lie within the range $[0, 1]$, thus we can sufficiently sample from their joint parameter space to explore it in depth with the need for informative priors to constrain their values.

2.5 Model Simulation

Initial conditions. Simulations are initialized with N_0 in situ tumor cells, determined by the choice of parameter values. A number of warmup cycles are run so that the model reaches steady state. During warmup, no mutations occur, and the only immune cells present are NKs. After warmup, mutations are permitted. Cells that do not mutate undergo an increase in their probability of mutation in a later cell cycle.

Tumor cell fate. During each cell cycle, the fate of each cell is assigned: proliferation, apoptosis, immune clearance, and rest in G_0, according the model rules. The probability of proliferation is affected by mutations to the proliferation pathway (increased) and my cells in a mesenchymal state (decreased). Probabilities of immune clearance are affected by the number of mutations harbored: cells with more mutations are assumed to be more immunogenic and have a higher probability of being cleared by the immune system, unless the cell has a mutation in the immune evasion pathway. Cells in a mesenchymal state can exhibit greater capacity to evade immune clearance.

Completing the Cell Cycle. Once all tumor cells have been updated and fates chosen accordingly, non-tumor model components are updated. Immune cell populations are updated in two steps. First, immune cell exhaustion is calculated based on the number of tumor cells cleared, e.g. clearance of one tumor cell by an NK cells results in the NK cell population decreasing by one. Second, all immune cells (NKs, CTLs, Tregs) are updated according to a system of coupled ordinary differential equations that govern their population dynamics. CTL and Treg recruitment rates are dependent on the number of tumor cells; in addition TGF-β enhances the recruitment rate of Tregs.

At the end of each cell cycle, new mutations can occur in cells that have undergone division, according to cell-specific probabilities that increase if no mutation occurs are reset to 0 in the event of a mutation. Finally, the concentration of TGF-β and the EMT score for each cell are updated. Tregs and (to a lesser extent) invasive tumor cells are the sources of TGF-β; the total concentration per cell cycle is divided randomly among tumor cells. EMT is then assessed, depending on the EMT score of the cell and the local concentration of TGF-β.

Mutational burden and progression to invasive disease. At the end of each cell cycle, the proportion of tumor cells that are invasive is calculated based on their mutational burden, and if it is above a certain threshold, the tumor is declared to have progressed to an invasive

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: *Description of key model parameters. Note that some are not constant as they can be affected by the inflammation state of the system.*

state and the simulation ends. The time to invasion is calculated as the time from the start of the simulation, minus the warmup period, until the invasive state is reached. Simulations run until either this occurs or until the maximum number of cell cycles has been reached.

2.6 Parameter Choice and Sensitivity Analysis

To study parameter sensitivity, we implemented Morris a one-step-at-a-time global sensitivity analysis. Parameters are varied one at a time from a set of sampled "base" points and the resulting simulations recorded [44,45]. For each run we simulated 1000 patients, and initialized the Morris sampling with 30 points in parameter space (at least 10 are recommended in [45]. Parameter sampling a choice of prior parameter distributions. For many parameters, such as for the immune population dynamics, measurements or estimates were available from literature [43]. For parameters such as MIE and MGA related to the mesenchymal phenotype,

little prior information was available, thus these was sampled across all possible values in $[0, 1]$. Tumor size in the model was scaled from cell numbers on the order of 10^9 cells [43] to the order of 10^2, and parameter values were scaled accordingly. Where parameter estimates existed, the prior for parameter θ_i is given as $\theta_i \sim \mathrm{N}(m_e, 2m_e)$, where m_e is the previous estimate and we take twice this value as the variance to obtain a range of samples that does not rely too heavily on previous work. The Morris algorithm computes the sensitivity, μ^*, as the average of the absolute change of the output, which in our model is the area under the survival curve (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 $n >= 1$ 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.

We apply this pipeline to several inflammation-associated cancers, denoted according to their annotations in TCGA, including “PAAD” for pancreatic cancer, “OV” for ovarian cancer, and “LIHC” for liver hepatocellular carcinoma.

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 assess the impact of different model components on the key measured outcomes: the probability of progression, and the time to invasion. Within the cell cycle, cell fate is determined by rules that are influenced by EMT and immune interactions (Fig. 1A), e.g. if a cell undergoes EMT, its probability of proliferation is reduced; if it gains a mutation in the apoptosis pathway, its probability of apoptosis is reduced. Meanwhile, NK cells and CTLs attempt to clear malignant tumor cells, and deactivate upon successful tumor cell clearance; Tregs inhibit this cytotoxic activity (1A Inset).

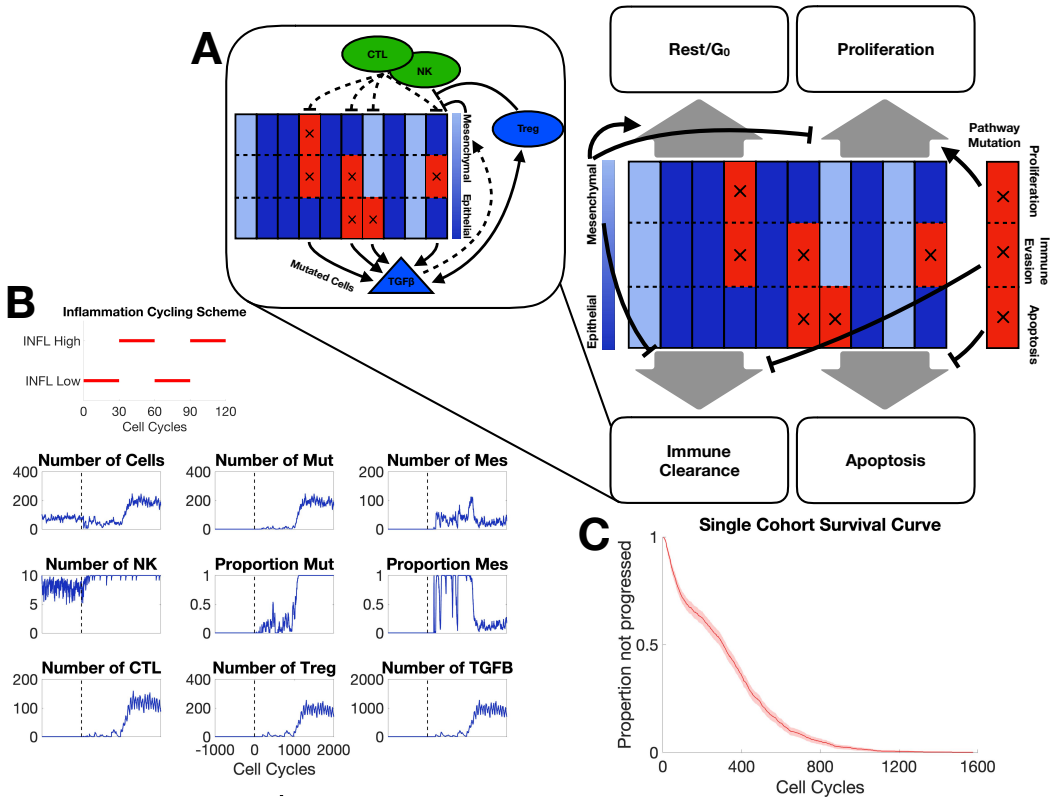

Figure 1: **A.** *Schematic depiction of agent-based model components; each of the 10 columns represents a single tumor cell divided into three compartments representing the state (altered or not) of the three pathways with mutagenic potential; red/blue denotes altered/unaltered pathways. Black arrows 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.*

The inflammation cycling scheme for a typical *in silico* patient consists of alternating high and low regimes with corresponding effects on the cell populations (Fig. 1B). For this patient, after warmup, mutations are observed at a rate low enough that they are cleared by cytotoxic cells for about 700 cell cycles, after which the mutated and thus invasive cell population begins to grow, leading to large recruitment of CTLs and Tregs and a peak in the concentration of TGF-β. After 841 cell cycles, the proportion of invasive cells reaches 50%: the threshold defining progression, thus this patient has a time to invasion of 841 cell cycles, or 631 days. Beyond this timepoint, we see a rapid increase in the number of invasive cells until it comprises 100% of the tumor population. Interesting EMT dynamics are also observed, the proportion of MTCs peaks shortly after the tumor becomes invasive, subsequently the majority of cells transition back to an epithelial state. We observe that while the NK population varies little over the simulation, CTLs and Tregs both undergo large expansions. CTLs and Tregs also appear to oscillate, however note that this is a direct result of the inflammation state, and is not immune cell-intrinsic.

In order to quantify patient dynamics and invasion-free survival as a population level, we simulate large cohorts of patients similar to the single patient shown in Fig. 1B. For a cohort of 500 patients, we simulate survival curves and see that a large number progress quickly to form invasive tumors, whereas a few lie in the tail of the distribution after the mutagenic event that a large number of tumors quickly progress while others takes some time before progressing Fig. 1C. By approximately 1200 cell cycles (2.5 years), all tumors have become invasive..

3.2 Identification of key model parameters via global sensitivity analysis

Exploring the parameter spaces of systems biology models *adequately* is – in general – a hard problem. Fitting parameters via (Bayesian) parameter inference is advisable wherever possible [46]. Here, despite a wealth of data on tumor growth dynamics, a lack of sufficient molecular measurements (i.e. immune cell dynamics) precludes 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 some components of the model studied previously can be constrained [8]. However, even here, new biological processes in the current system could push the model into new behavioral regimes. Thus to sample and characterize the parameter space of the model we use sensitivity analysis.

The results of Morris one-step-at-a-time sensitivity analysis on the 31 model parameters (Fig. 2) find a subset of parameters with much higher levels of sensitivity than others. The two most influential by this analysis are the recruitment rates of Tregs and CTLs in the low inflammation state. The parameters influencing EMT are also identified as influencing model outcomes. Since one goal of our analysis is to assess the specific effects of EMT on immune-cancer dynamics, parameters MIE and MGA are of particular interest. In addition, inflammation parameters controlling the periodic high/low inflammation states are of interest because they strongly influence model outcomes and are capable of being targeted by therapeutic treatments. For immune cell dynamics, the secretion of TGF-β by Tregs is found to be sensitive and thus will also be studied further below.

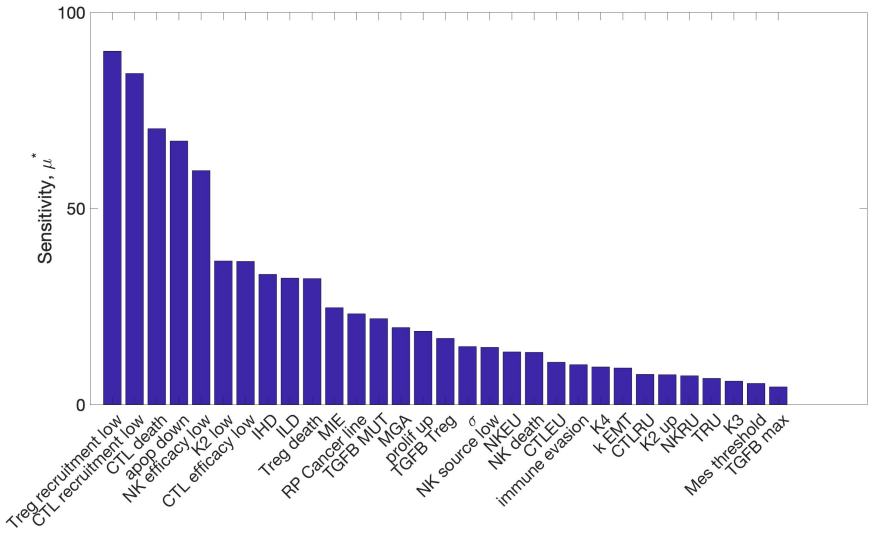

Figure 2: *Global sensitivity analysis of model parameters. The sensitivity (μ^*) denotes the average absolute change in the time to invasion over the range of variation of the parameter.*

3.3 Mesenchymal properties dramatically alter invasion-free survival times

Mesenchymal tumor cells (MTCs) are characterized by changes in two parameters: mesenchymal immune evasion (MIE) and mesenchymal growth arrest (MGA). Here we assess the effects of each, alongside the effects of TGF-β through its production by Tregs. As MIE increases, the invasion-free survival decreases (Fig. 3A) for all sets of parameters studied: as the subpopulation of invasive cells becomes more resistant to immune clearance, the tumor as a whole grows more resilient and thus can grow faster (Fig. 3D).

The relationship between MGA and invasion-free survival times displays a very different trend, and is non-monotonic with a local maximum appearing. For small values of MGA, increasing the MGA parameter results in increasing the invasion-free survival (Fig 3B, E). However for large values of MGA, invasion-free survival times decrease. This is explored further below.

TGF-β varies according to its production by tumor cells and its production by Tregs. Here we assess the effects of varying the production of TGF-β by Tregs on invasion-free survival (Fig 3B, E). We find, interestingly, that at lower production rates of TGF-β lead to more rapid invasion or if it survives to a certain time point, a longer time to invasion.

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

To investigate how competing interactions within the inflammatory tumor microenvironment affect EMT, we explored the effects of varying inflammation on invasion-free survival. Patient cohorts were simulated under different inflammation regimes: permanently low inflammation; permanently high inflammation; or variable (periodic high/low) inflammation. Compared to the other inflammation states, permanently high inflammation results in outcomes that vary

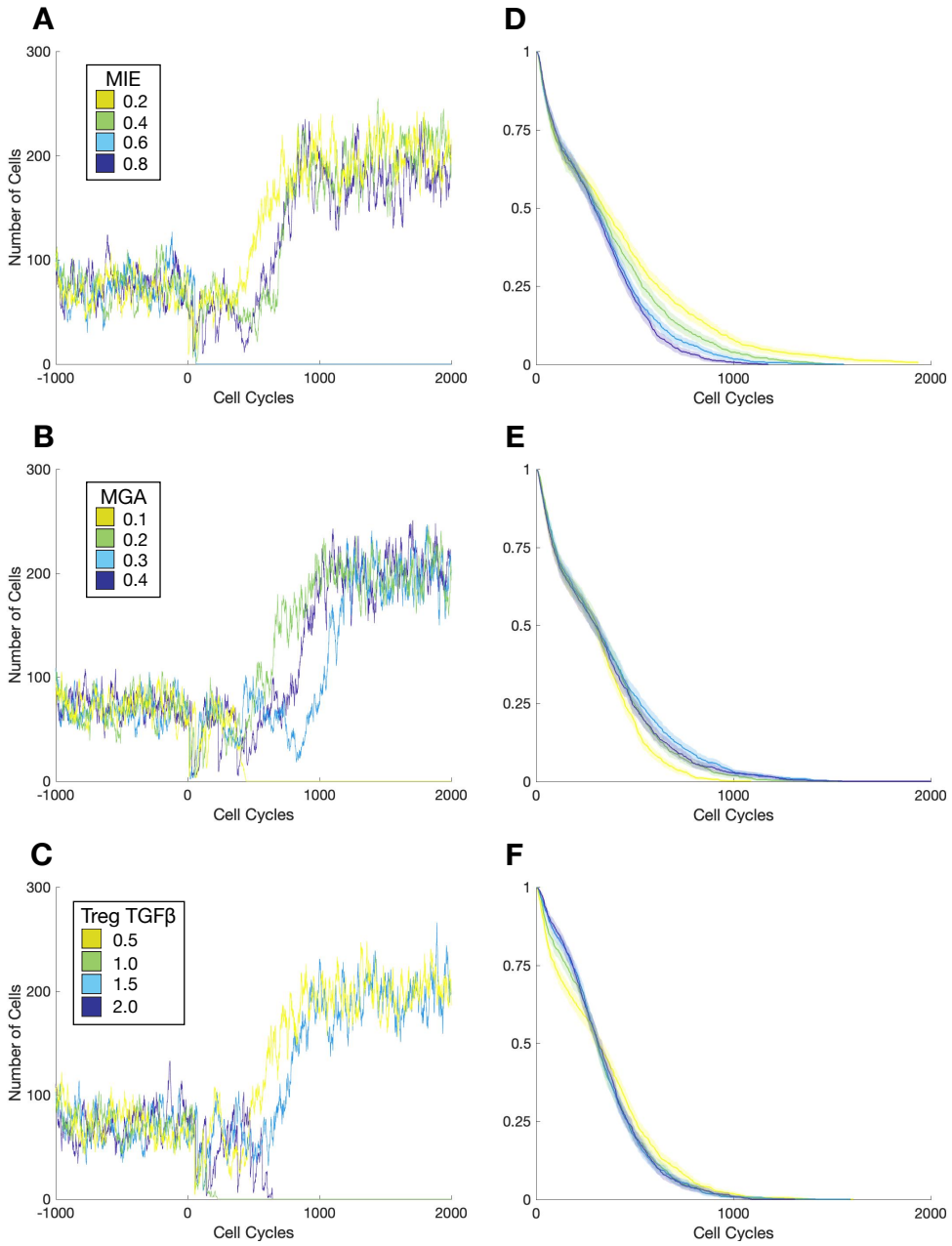

Figure 3: *Effects of mesenchymal tumor cell properties on the time to invasion. Trajectories of one patient per cohort including warmup and 2000 cell cycles for* **A.** *mesenchymal immune evasion (MIE);* **A.** *mesenchymal growth arrest (MGA);* **C.** *Production of TGF-β by Tregs.* **D.** *Survival curve corresponding to changes in the parameter MIE (A) for a patient cohort of 1000. Shaded region represents the 95% confidence interval over the cohort.* **E.** *Survival curve corresponding to changes in MGA.* **F.** *Survival curve corresponding to changes in Treg production of TGF-β.*

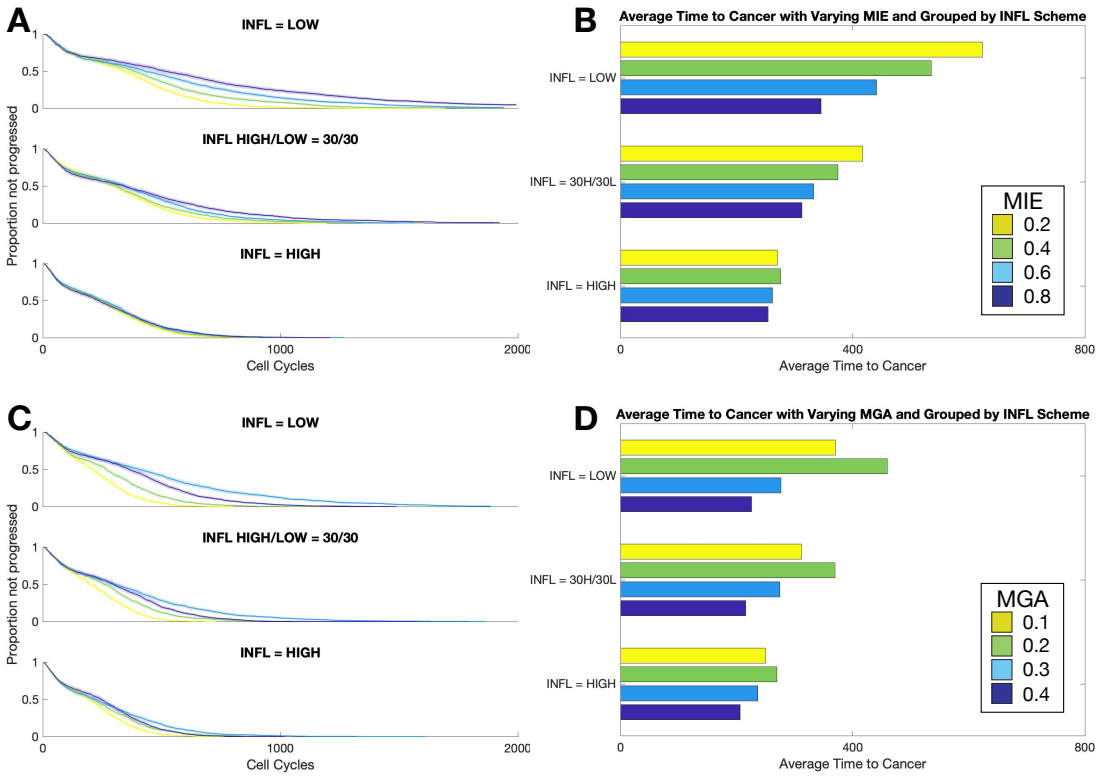

Figure 4: *Effects of inflammation on the time to invasion under different cycling schemes.* **A-B.** *As MIE varies, survival curves (each of 200 patients) and corresponding bar plots to summarize the mean Time to Cancer for each cohort are shown.* **C-D.** *As MGA varies, survival curves and corresponding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

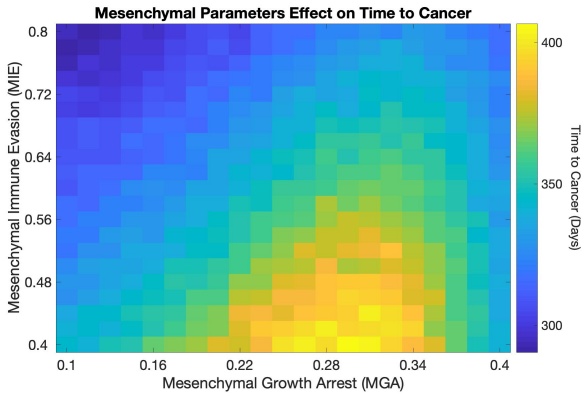

Figure 5: *Summary of the effects of MIE and MGA on invasion-free survival.*

more subtly with changes in the mesenchymal parameters (Fig. 4). When the inflammation state is either permanently or temporarily low, surprising trends emerge. In both these cases, invasion-free survival time is negatively correlated with MIE, and a local maximum for the invasion-free survival time is found with respect to MGA (close to $\Delta_{\text{MGA}} = 0.2$).

These differences in the mean invasion-free survival lead to striking variation in outcomes: tumors can be contained in situ for up to twice as long as they would otherwise be simply by varying the rates of mesenchymal growth arrest. These predictions point to intriguing therapeutic outcomes: a patient suffering intermittent high inflammatory attacks will benefit directly from EMT-directed therapies, however patients for whom a relatively high inflammation state is observed continuously will not obtain this benefit.

When MIE is varied under different inflammation cycling schemes, for all the periodic inflammation schemes studied, increasing MIE will decrease the invasion-free survival (i.e. worsen cancer progression and prognosis) (Fig. 4B). In the case of continuously high inflammation, the effects of MIE are minimal. Thus, under any inflammation regime with periods of low inflammation, as we might intuitively assume, any reduction in mesenchymal immune evasion will lead to improvements in patient outcomes.

3.5 Analysis of data from TCGA supports model predictions: EMT phenotypic properties worsen patient outcomes

The effects on cancer progression of mesenchymal phenotypic properties are summarized in Fig. 5. For all values of MGA, increasing MIE leads to a decrease in invasion-free survival times. For a given value of MIE however, there is an optimal value of MGA that maximizes invasion-free survival. Moreover, this optimum increases with increasing MIE.

To compare these model predictions with experimental studies, we analyzed data from The Cancer Genome Atlas (TCGA) [**?**]. We studied the effects of immune interactions and EMT on cancer prognosis, especially on tumors for which inflammation is known to play an important role, such as colonic or pancreatic [50, 51]. The TCGA Pan-Cancer Clinical Data Resource provides multiple computed clinical endpoints for pancreatic cancer (PAAD) [52]. Here, we focus on the disease-free interval (DFI) and the overall survival (OS). A tumor with a

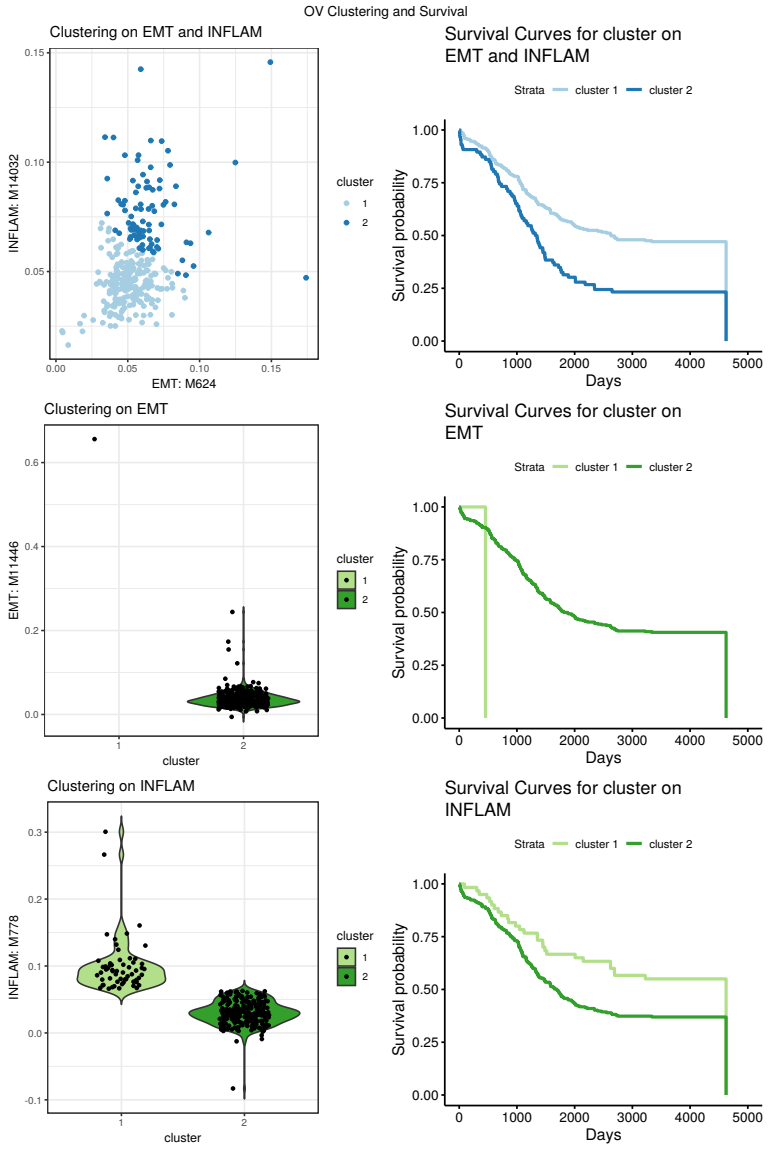

Figure 6: *A. K-means clustering of OV using gene ontology terms indicative of EMT and inflammation signatures ($k = 2$). B. Survival plots corresponding to the clustering on EMT and inflammation. C. K-means clustering of OV using gene ontology terms indicative of an EMT signature ($k = 2$). D. Survival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

short DFI will likely undergo rapid progression post-treatment. We thus expect the distance between DFI and OS to be small for a rapidly progressing tumor, following initial detection. We can therefore characterize tumors as either rapidly progressing or slowly progressing by defining a threshold for the ratio DFI/OS. We selected three tumors for study whose Clinical Data Resource profiles indicated slow progression (ovarian, OV; skin cutaneous melanoma, SKCM; liver hepatocellular carcinoma, LIHC) and two tumors whose profiles indicated rapid progression (pancreatic cancer, PAAD; lung adenocarcinoma, LUAD).

For each cohort of patients for which we have clinical and expression data, we cluster patients via k-means (n = 2) against the gene ontologies relating to either: EMT signature alone, inflammatory signature alone, or the combination of both signatures. We then plot the corresponding survival curves (using the OS from TCGA) for each of the two groups (Fig. 6 for ovarian cancer and Fig. 7 for pancreatic cancer). We see that for both these tumor types, survival is affected by the gene ontology signature, and the combination of both EMT and inflammatory signatures has a greater impact on survival than the effects of either EMT or inflammation alone. This suggests that in considering interactions between cancer and the immune system, is it of critical importance to consider the effects of EMT, as these can significant impact outcomes and should not be overlooked.

The model considered here studies tumor progression from in situ to invasive disease from a homogeneous initial point, whereas the data address how cancer may progress following treatment, thus comparisons between model and data should be made carefully. Nonetheless the core cellular tumor dynamics are at play both during the tumor progression addressed by the model, and post-treatment progression described in data from TCGA. Of particular note, 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, been a model developed that combines these three components. Here we studied cancer, the immune system, and EMT, during the progression from an in situ tumor to invasive disease. We saw this as a particularly pressing need given the shared factors influencing all these components, such as TGF-β. We used an individual cell-based model framework to describe the multiscale processes that can lead to cancer: DNA damage occurs during the cell cycle and this can lead to mutations in pathways that affect cell fitness, which in turn affects the cell population dynamics. Population dynamics are also influenced by the intrinsic state of the cell (through EMT), and extrinsic immune factors.

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

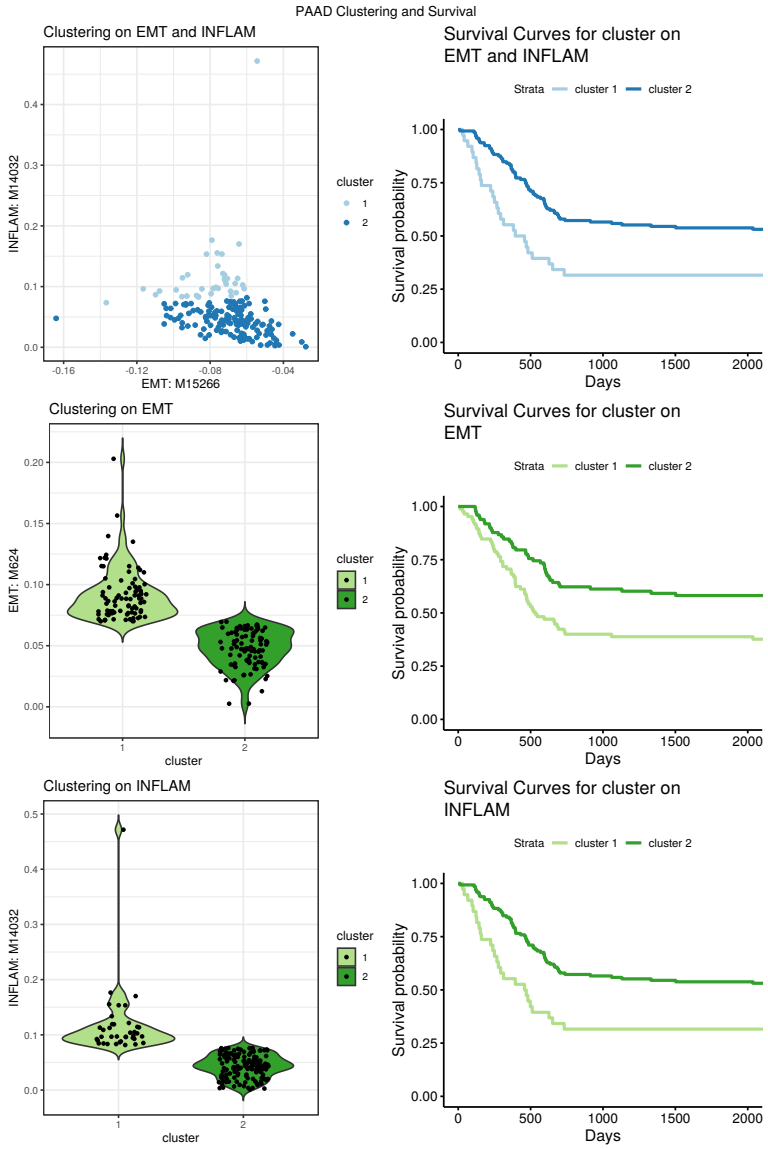

Figure 7: *A. K-means clustering of PAAD using gene ontology terms indicative of EMT and inflammation signatures ($k = 2$). B. Survival plots corresponding to the clustering on EMT and inflammation. C. K-means clustering of PAAD using gene ontology terms indicative of an EMT signature ($k = 2$). D. Survival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

we performed unsupervised analysis of pancreatic cancer data from The Cancer Genome Atlas, and looked at survival across groups with *rapidly progressing* or *slowly progressing* tumors. We found that combinatorial effects of EMT + inflammation increased the differences in survival between groups.

To capture the essential characteristics of the model, we summarized *in silico* patient studies with a single parameter: the invasion-free survival time (see Fig. 1B for a reminder of the full model heterogeneity). There are, of course, many trajectories that result in cancer progression. Analysis of the transient cell dynamics in cancer in situ and during progression is a pressing need to shed insight into cellular biomarkers of cancer.

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

The roles that TGF-β plays throughout the tumor and its microenvironment also warrant further investigation. We found that, below a certain threshold, reduction of TGF-β increases the Time to Cancer (Fig. 3E), thus reducing expression levels of TGF-β in the tumor microenvironment benefits survival. Intriguingly, recent experimental work demonstrated that TGF-β drives tumor suppression in pancreatic cancer by promoting EMT [54]. However, TGF-β is a master regulator implicated in numerous cellular signaling processes, and changing the concentrations of TGF-β even in a local tumor microenvironment could have large off-target effects. Indeed, 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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