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Tumour neoantigen heterogeneity thresholds provide a time window for combination immunotherapy

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Following the advent of cancer immunotherapy, increasing insight has been gained on the role of mutational load and neoantigens as key ingredients in T cell recognition of malignancies. However, not all highly mutational tumours react to immune therapies, and initial success is often followed by eventual relapse. Heterogeneity in the neoantigen landscape of a tumour might be key in the failure of immune surveillance. In this work, we present a mathematical framework to describe how neoantigen distributions shape the immune response. The model predicts the existence of an antigen diversity threshold level beyond which T cells fail at controlling heterogeneous tumours. Incorporating this diversity marker adds predictive value to antigen load for two cohorts of anti-CTLA-4 treated melanoma patients. Furthermore, our analytical approach indicates rapid increases in epitope heterogeneity in early malignancy growth following immune escape. We propose a combination therapy scheme that takes advantage of preexisting resistance to a targeted agent. The model indicates that the selective sweep for a resistant subclone reduces neoantigen heterogeneity, and we postulate the existence of a time window before tumour relapse where checkpoint blockade immunotherapy can become more effective.

1. Introduction

The mechanisms that make cancer a major cause of human death are deeply rooted in the principles of evolution [1]. Through depletion of early mutations affecting mostly multicellular regulation and tissue homeostasis, cancer cells can overcome multiple selection barriers, making the human genome a pool for the evolution of a myriad possible phenotypes [2]. Highly diverse rogue populations within a tumour include cells that resist or evolve resistance to a vast array of therapy schemes [3,4].

Among other selection barriers, cancer cells often evolve the ability to evade immune surveillance [5] required to prevent the outgrowth of transformed cells [6] (figure 1a). Recent decades have seen a rapidly growing insight into the molecular details of immune silencing and tolerance [7], leading to the advent of checkpoint inhibition therapies able to target specific immune-evasion mechanisms in malignancies [8]. Once the immune system is back in place, the role of neoantigens, i.e. mutated surface proteins that elicit T cell recognition, has proven to be crucial, making genetic instability and mutational load valuable markers of eventual prognosis [9–13]. However, recent research highlights that not only the amount of neoantigens, but also their heterogeneity and distribution across the tumour determines the eventual fate of immune therapies [14,15]. In particular, it has been found that a high mutational burden might not be enough for immune recognition, as only clonal antigens elicit sufficient lymphocyte response [14].

Recent modelling efforts have explicitly introduced cancer neoantigens as key ingredients in the interaction between cancer and the immune system

Figure 1. Mathematical framework for the cancer-immune ecology in heterogeneous antigen landscapes. T cell recognition of antigens a_i (a) induces a selective pressure towards immune evasion. In the lack of surveillance, neoantigens proliferate and evolve neutrally (b). After checkpoint blockade immunotherapy, subclonal death in our model is a function of neoantigen load α_i .

[16,17]. However, the exact role of neoantigen heterogeneity on the tempo and mode of the immune response is not fully understood. In this paper, we seek to understand, using theoretical models, how neoantigen heterogeneity evolves during immune evasion, and its effects on T cell recognition when lymphocyte function is back in place.

In this work, we introduce a mathematical framework (§2) that captures the effects of both neoantigen load and heterogeneity on immune surveillance. To this goal, two scenarios are considered, namely neutral antigen evolution in cancers that have evaded the immune system (figure 1b) and T cell recognition of heterogeneous tumours after immune reactivation (figure 1a). The model brings together knowledge from search processes in the immune response [18] and measures of epitope immunogenicity [17] to obtain a novel description of T cell reaction to heterogeneity. As shown below (§3), we predict that neoantigen diversity shapes an all-or-none transition separating growing from immune-controlled tumours. Moreover, to understand how diversity arises during malignancy progression, we infer the evolutionary pace of average antigen clonality in a growing tumour along with the evolution of the antigen-cold and -hot fractions of the malignancy.

Beyond heterogeneity, several evolutionary mechanisms are known to drive resistance to immunotherapy [19] providing novel opportunities for combination approaches to render immunotherapy more effective [20]. This includes the study of the possible timings of combining targeted agents with checkpoint blockade [21,22]. In this context, our model presents a qualitative scheduling approach that takes into account the evolutionary dynamics of neoantigen distributions when combination therapy is at work.

2. Mathematical framework

2.1. Neoantigen heterogeneity and the cancer-immune ecology

Here, we develop our approach to the ecology of tumour subclonal dynamics where each subclone comprises those cells harbouring the same epitope composition (figure 1b). Our toy model seeks to capture the minimal ecological dynamics of cancer subclones under immune surveillance, where each clone has a particular neoantigen set-up $\{a_i\}$ (figure 1b). Although several cell types participate in the immune

response [23], the model focuses on those that react to antigen composition in order to capture the specific effect of neoantigen heterogeneity. Subclonal dynamics will be described by a set of coupled differential equations, namely

$$\frac{dc_i}{dt} = r_i c_i \left(1 - b \sum_j c_j \right) - (k D_i E_i) \frac{c_i}{g + \sum_j c_j}. \quad (2.1)$$

The first term on the right-hand side is the logistic growth of each subclone c_i growing at rate r_i and with b indicating the inverse carrying capacity [24]. Malignant cells die at a rate (second term on the right-hand side) depending on their subclonal antigen composition, which implicitly introduces the adaptive immune system dynamics [16,17]. Here, k is the rate of killing by T cells once a target cell is recognized [25]. Subclonal death rate depends upon the dominance D_i of the immunogenic antigen in the subclone [26,27] and the efficiency E_i of the immune system to detect the antigen depending on its frequency. The most relevant novelty of model (1) is that it explicitly captures the effects of subclonal neoantigen composition by obtaining mathematical expressions for both D and E .

The model is built by considering several experimental sources. The first is that there is a causal correlation between tumour antigen load and immune response [12]. We know from studies on non-self antigens that only a few elicit immunological response [26]. Recent evidence on the overall lack of negative selection in tumours further supports that most antigens are not activating an immune attack [28]. We postulate that a higher antigen load increases the probability of presenting more immunogenic epitopes, thus increasing subclonal dominance D . Additionally, it has been shown that even highly recognizable antigens fail at inducing a T cell response if they are not present in a sufficient fraction of the tumour [14,15]. Following research on immune search mechanisms [18,29,30], we hypothesize that increased epitope heterogeneity leading to more private antigens will result in a loss of T cell efficiency E .

Finally, reduced T cell circulation [31], penetration into the solid tumour [32,33] and increased immunosuppressive factor production [34] are known to relate tumour size with reduced immune effectiveness. A Michaelis–Menten saturation term (last term in equation (2.1)) is introduced following standard models of cancer–immune system dynamics [31]. The value of the Michaelis constant g indicates the cancer

Figure 2. Correlation between subclonal neoantigen load α_i and dominance D . (a) Simulations show a linear increase $D_i = \rho \alpha_i$, as neoantigens are drawn and more dominant ones presented. Here, $\rho \simeq 0.06$ from our simulations on random neoantigen presentation. (b) Data from [17] also indicate a (weak) linear trend, with considerable data scattering.

population at which death is half of what it would be without size-related effects.

Finding the neoantigen composition $\{a_i\}$ in each subclone and estimating its immunogenicity and frequency relies on bulk data processing with sampling biases due to localized biopsies [35] and imperfect antigen prediction [36]. Mathematical techniques can bring up a simpler description to better understand how neoantigen landscapes affect prognosis by studying the underlying dynamics relating D and E to antigen composition. Below, we explain how the nature of these two key components of equation (2.1) can be determined.

2.1.1. Neoantigen dominance D

We aim at understanding the possible correlation between the immunogenicity of a subclone D_i and its antigen load α_i . Recent computational methods have been able to estimate the immunogenic capacity of cancer neoantigens [17]. The associated distribution is highly skewed: only a small subset of cancer epitopes elicits a T cell response. This result is consistent with the concept of *immunodominance* [26,27] and the overall lack of negative selection in the cancer genome [28]. Here, we assume that neoantigen load correlates with immune attack because a higher antigen burden increases the overall likelihood that a dominant one is presented. A novel and parameter-free stochastic framework is built to assess the hypothesis. In it, we measure the most dominant antigen of a cancer population as new mutations accumulate (see electronic supplementary material).

Simulations indicate that recognition potential of the most dominant antigen increases linearly with the total neoantigens of subclone α_i (figure 2a). This result is consistent with the notion that there is no known mechanism by which the probability of finding a more immunogenic antigen increases as more antigens are being found [12]. Since antigens are produced randomly, the probability that the next one is dominant remains stable, resulting in a linear relation $D_i \sim \alpha_i$. For larger antigen loads, maximum immunogenicity saturates as the specific epitope database is finite. This might be only due to limitations in sequencing depth [36,37]. In figure 2b, our simulation results can be compared with existing data on subclonal antigen burden and recognition potential from [17]. A similar trend can be obtained,

although considerable scattering of the available data is at work (see electronic supplementary material for detailed descriptions of simulations and data analysis)

The previous results thus suggest approximating subclonal immunogenicity as a linear function of the antigen burden, namely

$$D_i = \rho \alpha_i. \quad (2.2)$$

Current research points to evidence for underestimation of mutational loads during next-generation sequencing [37,38], resulting in detection errors for antigens present in less than 5% of the tumour. This would imply a decrease in the steepness ρ in figure 2b (more mutations are actually accumulating to find the given antigens), but not a change in the linearity of the neutral accumulation dynamics, which actually governs the qualitative results of the present work.

2.1.2. Immune search efficiency E

The second part of our model definition requires a mechanistic description of the search efficiency term E in (1). The mechanism of T cell clonal selection is another key component in the immune response to cancers. Upon presentation of a given antigen by dendritic cells, helper T cells with the matching TCR replicate and release cytokines that eventually result in the expansion of a cytotoxic T cell clone [39]. This ensures efficient surveillance and further memory of previous antigenic encounters. Several search and migration processes underlie an efficient cascade [18].

The complete process is initiated by dendritic cells that recognize tumour antigens and present them to naive T cells in the lymph node [18]. Consequently, T cells become activated and migrate to the tumour site where they search for cancer cells expressing the same antigens [18]. We hypothesize here that there is a relation between neoantigen concentration in a tumour and the efficiency of the immune search processes involved. This might be key in the observed role of antigen heterogeneity in immunotherapy prognosis [14,15].

In order to make these relations explicit, we study a spatial simulation framework of a tumour surface built upon previous research [30] that minimizes the estimated parameters at play (see electronic supplementary material). In it, dendritic and T cell agents search for cancer antigens

Figure 3. Modelling the effect of neoantigen frequency on immune search efficiency. (a) Neoantigens are distributed randomly on a two-dimensional grid according to their frequency. We sum the average times for a migrating dendritic cell and its activated T cell counterpart to find their cognate antigen distributed with density γ . (b) The efficiency of the immune search process scales linearly with antigen frequency γ_i for low antigen concentrations (c) as those in [17]. Following migration data from [29], simulations of Lévy-migrating T cells are presented for comparison, consistent with results indicating that Lévy strategies are more efficient than Brownian search [40]. The overall linear trend $E \sim \gamma$ is maintained across search strategies.

distributed according to their frequency γ (figure 3a). We explicitly compute the dependencies of search time and efficiency E (the inverse of search time) on neoantigen frequency (see electronic supplementary material).

This novel approach separates those steps that participate in the activation-and-killing process from the two searches directly related to neoantigen concentrations on the tumour surface: the motion of dendritic cells to find tumour antigens and the search of effector T cells that have migrated to the tumour site once activated.

The complete immune response process builds upon many layers of complexity. However, even for clonal antigens, the timescale of the search processes involved seems to be longer than those of immune cell activation or cancer cell removal (see electronic supplementary material), supporting the notion that the frequency-dependent processes play a determining role in the average speed of the immune response.

Efficiency diverges in the simulations when an antigen is found in most of the cells of the tumour surface. However, clinical antigen frequencies are much smaller, and a linear trend can be found for small γ levels similar to those found in tumours (figure 3c, [17]). This translates into immune efficiency E being a linear function of the frequency of the dominant antigen in the subclone, $E_i = s\gamma_i$. Here, s is the slope of the linear correlation, related to other molecular or non-antigenic processes that affect the timing of the search, such as immune cell activation or cancer cell removal [18], which we find to be rate-limited by the timescale of the antigen-search processes at play (see electronic supplementary material). By rewriting equation (2.1), we now map subclonal death rate with antigen load α_i and dominant epitope concentration γ_i

$$\frac{dc_i}{dt} = r_i c_i \left(1 - b \sum_j c_j \right) - k(\rho \alpha_i s \gamma_i) \frac{c_i}{g + \sum_j c_j}. \quad (2.3)$$

In model (2.3), subclones will die at rates corresponding to their number of antigens (as more antigens increase the probability of presenting a dominant one). The frequency of their most dominant antigen will also increase the efficiency of dendritic search and T cell surveillance. Is it possible to gain insight from (2.3) into how T cells react at neoantigen distributions? Are there common patterns separating responding from non-responding patients, and what can we learn from them? In the Results section, we study the relation between this model and the experimentally observed correlation between neoantigen heterogeneity and poor prognosis.

2.2. Neutral evolution of neoantigen distributions

So far our mathematical framework introduces cancer cell death under a correctly functioning T cell cohort. However, the evolution of mechanisms to avoid T and B lymphocyte attack is recognized as a hallmark of cancer cells [6] and needs to be introduced too. The lack of an efficient immune response ensures that neoantigens are no longer negatively selected [28,41], making for tumours where neoantigen load and heterogeneity respond to neutral evolutionary dynamics [42]. What epitope landscape $\{\alpha_i, \gamma_i\}$ will T cells find when they are activated and equation (2.3) is back in place?

The role played by neutral evolution in cancer has seen increasing attention [28,37,42]. In a recent work, Lakatos and colleagues developed a computational approach for the evolutionary dynamics of neoantigens, and indicators of effectively neutral evolution are found in colorectal cancer exome sequencing data [16] consistent with the overall lack of negative selection across the cancer genome [28]. We present a coherent analytical approach to understand how neoantigen load and concentration evolve in the absence of T cell surveillance. Further analytical methods are provided to estimate the fraction of antigen-cold cells in a given tumour. Stochastic simulations of neoantigen evolution in

growing tumours are built to verify our analytical estimates (see electronic supplementary material for the mathematical and computational methods).

2.3. The effect of combination therapy on neoantigen landscapes

The evolution and composition of the neoantigen landscape is key to model and understand the interaction between cancer and the immune system and needs to be taken into account within our modelling approach. Recent research is focusing on the use of combination therapy to render checkpoint blockade more effective [20–22]. We here study the effects of combination therapy on neoantigen heterogeneity and propose a novel therapeutic approach able to modulate antigen landscapes.

Interdisciplinary approaches have already considered taking advantage of evolution to explore novel therapeutic designs. Mathematical models have studied the use of drug sequencing to avoid the evolution of cross-resistance [43] or the notion of an *evolutionary double bind* [44] to take advantage of its metabolic cost [45]. In this context, experimental evidence indicates that alkylating agents causing DNA damage can increase subclonal neoantigen burdens [14,46]. This results in a more heterogeneous neoantigen landscape. On the other hand, immunotherapy often results in selective pressures towards immune evasion through silencing of clonal antigens [19,47]. We here study other therapeutic combinations that do not increase neoantigen heterogeneity or reduce epitope presentation.

In particular, molecular-targeted therapies such as BRAF and MEK inhibitors for melanoma are being combined with immune checkpoint blockade in the search for optimal drug sequencing [20–22]. Knowing that resistance to targeted therapies mediated by preexisting or acquired mutations is common [48,49], we study the therapeutic designs that could take advantage of it. The stochastic simulations previously described are modified to include a generalistic targeted therapy and mutations driving resistance to it (see electronic supplementary material). We follow the dynamics of neoantigen distributions to understand the effects of drug resistance on tumour and epitope heterogeneity, and study which scenarios are better suited for checkpoint blockade efficiency.

3. Results

3.1. Before checkpoint blockade immunotherapy

The amount and distribution of neoantigens is key to predict the response of tumours to immunotherapy. However, early selective pressure following T cell recognition is known to drive immune evasion [41]. Until checkpoint blockade immunotherapy reactivates lymphocyte attack, neoantigen distributions evolve neutrally [28,42].

To address this problem, we use our model to study the evolution of average antigen load and clonality in growing tumours. Tumour growth and neoantigen production are known to slow down along progression. We can characterize the fastest neoantigen dynamics by studying the exponential phases of tumour growth. This will also be useful for the proposed combination therapy design that takes into account small tumours and distant metastases.

In particular (see the electronic supplementary material for details), we find that the amount of antigenic mutations

Figure 4. Evolution of antigen frequency during tumour growth. The analytical result (3.3) is compared with simulations of exponential growth, logistic growth under extreme spatial constraints ($K = 10^4$) and increased cell death ($d/b = 0.1$).

in the tumour, M_α will follow (see [42])

$$M_\alpha(t) = 2\mu p_\alpha \frac{b}{b-d} c_0 (e^{(b-d)t} - 1). \quad (3.1)$$

where the tumour grows at an average rate $r = b - d$, μ stands for the overall mutational probability and $p_\alpha \sim 0.13$ is the approximate portion of mutations predicted to bind MHC1 indicating possible immunogenicity [32]. The two factor stems from the fact that both daughter cells can undergo errors at birth at rate $b\mu p_\alpha$. The result is consistent with logistically growing tumours, where slowing down of neoantigen production is only significant for very small carrying capacities (see electronic supplementary material). Moreover, another key element in therapy prognosis is the existence of cells that do not harbour antigens. It can be shown (see electronic supplementary material) that analytical results and simulations predict an exponential decay of the antigen-cold fraction of the tumour

$$c_{\alpha^-}(t) = e^{-2r\mu p_\alpha t}. \quad (3.2)$$

This result is indicative of the rapid dynamics at which microsatellite-unstable tumours become populated by antigen-hot cells, consistent with experimental insight [13]. The model seems to support the idea that, provided sufficient mutational load [37,46], it could be the excess of heterogeneity and not the lack of antigens that drives checkpoint blockade therapy failure [14,15].

We also study the dynamics of average neoantigen clonality as tumours grow (see electronic supplementary material). We find that the average clonality decays in time as

$$\langle \gamma(t) \rangle = \frac{1}{c_0(e^{(b-d)t} - 1)} [\psi^{(0)}(\theta e^{(b-d)t} + 1) - \psi^{(0)}(\theta + 1)] \quad (3.3)$$

where $\theta = 2\mu p_\alpha b c_0 / (b - d)$ and $\psi^{(0)(x)}$ is the digamma function [50]. This result can be compared to simulations (figure 4a) and is a first analytical measure of how heterogeneity increases in the neoantigen landscape.

The predicted antigen clonality decay results from exponential growth in early tumours or small metastatic burdens, interesting for the therapeutic scheme proposed later on. However, tumour growth is known to slow down at later stages. Simulations indicate that logistic growth has a small effect on heterogeneity dynamics (figure 4), even

for very small carrying capacities of $K = 10^4$. This is because initial exponential growth ensures a very fast decay of average clonality (equation (3.3)) that remains low on later tumour growth phases. Increased cancer death (figure 4) or finite epitope landscape scenarios (see electronic supplementary material) are also consistent with the analytical estimate.

Neoantigen heterogeneity increases rapidly in growing tumours. This could be indicative of a decay in the efficiency of T cell surveillance, giving an explanation for the role of epitope clonality observed in immunotherapy prognosis [14,15]. Can our mathematical framework capture this effect?

3.2. After checkpoint blockade immunotherapy

These results predict the evolution of the neoantigen landscapes during immune evasion. After checkpoint blockade immunotherapy, T cell attack is back in place and equation (2.3) holds. The model is solvable for specific subclones. However, we aim to understand whole-tumour dynamics when immunotherapy is administered, so that several approximations are introduced to reduce the set of parameters at play.

On the one hand, neoantigen presentation does not affect the rate of cell division r_i [17]. Therefore, subclones characterized by any neoantigen composition are considered to replicate at an average rate $\langle r \rangle$. The final result stems from whole-tumour dynamics, depending only on $\langle r \rangle$ which can be measured from patient data [17].

Another similar assumption regarding antigen neutrality can be performed. Provided that neoantigens were isolated from immune attack prior to therapy [41], dominant antigens at the time of therapy are not negatively selected [28] and, on average, are as common as the rest. This allows us to write $\gamma_i \sim \langle \gamma \rangle$.

Inspired in the analysis of antigenic diversity thresholds in the evolution of HIV [51], we want to understand the conditions under which all subclonal growth is controlled by the immune surveillance mechanisms. This will occur provided that the following inequality:

$$\frac{dc_i}{dt} < 0 \quad (3.4)$$

holds for all $i = 1, \dots, S$. A necessary condition for this to be true is that the sum of subclonal dynamics (2.3) over all S clones is negative

$$\sum_i^S \frac{dc_i}{dt} = \sum_i^S c_i \left[\langle r \rangle \left(1 - b \sum_j^S c_j \right) - \frac{k\rho\alpha_i s}{g + \sum_j^S c_j} \langle \gamma \rangle \right] < 0. \quad (3.5)$$

Even if (3.5) is not a sufficient condition (since a single clone could grow despite overall negative replication) it is required for immune surveillance to succeed: if the overall sum of growth rates is not negative, at least one of the clones will outgrow immune barriers. Since all clone populations c_i are either zero or positive, the sum of terms inside parentheses must be negative.

The number of elements in the sum S accounts for the number of subclones harbouring identical epitope configurations (figure 1b). Summing over (3.5) we find the following inequality:

$$\frac{\langle r \rangle}{k\rho s} \left(1 - b \sum_j^S c_j \right) \left(g + \sum_j^S c_j \right) < \langle \gamma \rangle \frac{\sum_i^S \alpha_i}{S}. \quad (3.6)$$

This result indicates the possibility of a threshold condition separating tumour growth from immune surveillance provided that tumour size $\sum_j c_j$ can be estimated. The left-hand side is the replication/predation ratio, that has to be larger than the tumour immunogenicity term of the right-hand side. Furthermore, the product of the logistic and Michaelis-Menten terms on the left-hand side defines to which extent the spatial constraints affect more tumour growth or else immune circulation. It can be seen that the $(1 - b \sum_j^S c_j)(g + \sum_j^S c_j)$ term can become large only for large carrying capacities and tumour sizes (see electronic supplementary material).

We are also interested in understanding (3.5) in the particular scenario of small tumours. On the one hand, we want to study the effects of a targeted therapy reducing tumour bulk prior to immunotherapy, so that the immune system will most probably face a small resistant subclone or distant metastases. On the other hand, because of the competitive release of resistant cells after therapy [45], we are looking at modelling a time window of fastest antigen production when tumour growth is unbounded, which happens when $\sum_j c_j$ is away from its carrying capacity and the growth dynamics are exponential. In this scenario, the non-linear effects of large tumour masses can be neglected. Similar models considering unbounded growth in subclones are already in place [43], acknowledging that the effects of extracellular matrix barriers [33] or immunosuppressive factor production [34] are reduced. Now the threshold condition contains exclusively tumour-averaged parameters: average antigen frequency and average subclonal neoantigen load should outgrow the growth/recognition ratio

$$\langle \gamma \rangle \frac{\sum_i^S \alpha_i}{S} > \frac{\langle r \rangle g}{k\rho s}. \quad (3.7)$$

The existence a catastrophic shift separating tumour growth from extinction as a function of the average neoantigen clonality resembles recent clinical studies [14,15]. The parameters on the right-hand side of equation (3.7) are dependent on tumour type and microenvironment specificities. Producing a quantitative prediction for given cancer types and measures is away from the scope of the article. However, the threshold depends on a tumour immunogenicity value that we can study using existing data. To which extent does the immunogenicity marker of equations (3.6) and (3.7) correlate with patient prognosis after checkpoint blockade immunotherapy?

Using available neoantigen estimates from the database in [17] (see electronic supplementary material), we can study the correlation between our result and months of survival in anti-PD-1-treated patients with lung cancer [9] and anti-CTLA-4 treated patients with melanoma [10,11]. Without accessible data on tumour size $\sum_j c_j$, Kaplan–Meier curves and cumulative hazard ratios of overall survival of anti-CTLA-4 treated melanoma patients from [10,11] seem to indicate that our measure extracted from equation (3.7) could be a better biomarker for immunotherapy prognosis than total neoantigen load (figure 5), consistent with experimental results [14].

Modelling, simulations and data seem to consistently indicate that neoantigen heterogeneity might shape a threshold condition separating tumour growth from cure. Average antigen frequency decays rapidly as tumours grow, and advanced malignancies will probably be highly heterogeneous and hard to target by immune activation. Could we design a

Figure 5. Correlation of patient biomarkers with months of survival after checkpoint inhibition therapy. The Kaplan–Meier curves for decay of survival probability are depicted, with 166 anti-CTLA-4 treated melanoma patients from [10,11] separated in two groups by the median of either neoantigen load (a, log-rank test p -value 0.0179) and our threshold value $\gamma \sum \alpha_i/S$ (b, log-rank test p -value 0.0002). Cumulative hazard ratios (c,d) also support that including average neoantigen clonality adds predictive capacity to the well-accepted neoantigen load marker. This suggests $\gamma \sum \alpha_i/S$ as a possible complementary biomarker for immunotherapy prognosis in melanoma. Including a cohort of 30 anti-PDL-1 treated lung cancer patients from [9] does not improve results (see electronic supplementary material, figure S6).

therapeutic scheme able to steer tumour evolution towards a more homogeneous neoantigen landscape?

3.3. Combination therapy could reduce neoantigen heterogeneity

The mathematical model predicts an heterogeneity threshold beyond which lymphocytes fail to reduce tumour growth, consistent with recent research [14,15]. Results also indicate that neoantigen frequency decreases rapidly during growth, meaning that therapy reactivating the immune system will not necessarily succeed. Now we study the (simulated) effect of administering a molecular-targeted therapy in the case that resistance is already present, following existing experimental efforts [20,22].

As therapy is administered, the death rate of sensitive cells increases, leading to a general decay in tumour cell number (figure 6a) that is commonly seen in combination approaches targeting tumour burden [20]. This decrease in cell size comes with a halt in the production of novel antigens, while total present antigens are reduced as some may go extinct (figure 6b).

When therapy is administered, the overall reduction of cell number produces a rapid increase in average antigen frequency. Knowing that resistance mutations are usually in

place [37,49], can it be used in our favour? In spite of many antigens going extinct, those that belong to a surviving lineage (of cells that are resistant to therapy) increase their overall frequency (figure 6c).

Based on our *in silico* models, we postulate that resistance to therapy produces a selective sweep to a more homogeneous epitope landscape, which is easier to target by a reactivated immune system. Furthermore, previous results indicated that, along the growth process of the tumour, the fraction of antigen-cold cells decays rapidly. This is not altered by the targeted agent provided it has no alkylating effect, so that antigen accumulation continues until checkpoint blockade is administered. Until the resistant clone has grown into a full-size tumour, we observe a time window during which the average frequency of neoantigens is higher than when the cancer was of detectable size (figure 6c). This represents a first qualitative effort to understand combination therapy scheduling [20] when resistance mutations are in place.

4. Discussion

Immunological approaches to cancer therapy have seen remarkable advances in recent years [5,8,13]. However, not all

Figure 6. Evolutionary dynamics of neoantigens during therapy. (a) Cell number decreases after therapy until a resistant subclone repopulates the tumour. (b) Production of novel antigens slows down after therapy (black line) while total present antigens are reduced (dashed line). (c) Tumour size decay produces an increase in clonality of remaining antigens. Before complete relapse, increased average clonality creates a time window where the tumour is more neoantigen-homogeneous and T cells increase their efficiency (timescale in days). (d) A scheme of the population dynamics shows subclonal outgrowth after maximum tolerated dose (MTD) targeted treatment. We postulate that these processes elicit homogenization of the neoantigen landscape where checkpoint blockade immunotherapy (CBIT) can be more effective.

underlying mechanisms of immune surveillance and resistance are understood, and relapse is a common outcome [5,19]. A critical aspect of immune efficiency lies in the incidence of high neoantigen heterogeneity in poor therapy response [14–17]. To address some of these problems, in this paper, we have built a minimal model of heterogeneous cancer populations explicitly introducing their antigenic composition. In particular, neoantigen-related subclonal death takes into account antigen load and frequency across the tumour while keeping the number of model parameters small. Our framework contemplates computational methods and data in both epitope immunogenicity [17] and immune search efficiency [18,29,30] to translate previous evidence into a mathematical model able to produce insight into the underlying subclonal dynamics. Analytical results of the new model indicate that a heterogeneity threshold separates cancer growth from immune control, so that highly heterogeneous neoantigen landscapes might impair immune efficiency.

By incorporating our results into survival studies, predictive power could be added to immunotherapy prognosis in

melanoma patients, providing a novel therapy biomarker consistent with experimental results [14,15]. To understand why tumours have heterogeneous neoantigen landscapes, the model studies the evolutionary dynamics of antigenic mutations. As shown by the model, the antigen-cold fraction of the tumour becomes rapidly smaller, meaning that the growing tumour is rapidly populated by antigens. However, analytical estimates and simulations indicate a fast decay of average antigen clonality during immune evasion phases of tumour growth. This is consistent with the correlation between increased antigen heterogeneity and poor response to checkpoint blockade immunotherapy.

Further layers of tumour complexity that do not depend on neoantigen composition have been taken out of the system to produce a treatable model, particularly in the immune search modelling. With this, we can compare the qualitative results of our approach with experimental evidence while keeping only with a minimal set of measurable parameters. Furthermore, we have studied specific scenarios where the model simplifies, such as immune activity in small tumours and

metastases, where nonlinear effects of tumour size can be neglected as in other therapeutic designs [43]. This also allows the use of existing data on neoantigen distributions where population counts are not available. Further non-antigenic events in the search and cytotoxic processes could be introduced to obtain a more quantitative approach, together with including extrinsic noise in the cancer–immune system interaction [52].

Mathematical approaches have shown how evolutionary dynamics can be used to obtain novel insight into the sequencing [43] or timing [45] of combination therapies. Ongoing research is currently exploring the possibility of combining targeted therapy with checkpoint blockade [21,22]. Our model highlights how evolutionary dynamics underlying resistance to a targeted agent can increase neoantigen homogenization. Although a refractory drug is not of interest in principle, resistance is so common that we propose a combination approach that takes advantage of it.

Simulations indicate that positive selection for a drug-resistant clone results in increased antigen clonality. Before complete relapse, a second hit with immunotherapy could be more

effective as reactivated lymphocytes face a more homogeneous tumour. Continuation of immune attack after checkpoint blockade is known to drive selective pressure towards epigenetic silencing of targeted neoantigens [47]. Further analysis could result in more complex combination schemes that enhance neoantigen generation after prolonged T cell attack.

Data accessibility. This article has no additional data.

Authors' contributions. Both authors have contributed equally in this work.

Competing interests. We declare we have no competing interests.

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References

- Greaves M, Maley CC. 2012 Clonal evolution in cancer. *Nature* **481**, 306–313. (doi:10.1038/nature10762)
- Negrini S, Gorgoulis VG, Halazonetis TD. 2010 Genomic instability—an evolving hallmark of cancer. *Nat. Rev. Mol. Cell Biol.* **11**, 220–228. (doi:10.1038/nrm2858)
- Holohan C, Schaeybroeck SV, Daniel B, Longley DB, Johnston PG. 2013 Cancer drug resistance: an evolving paradigm. *Nat. Rev. Cancer* **13**, 714–726. (doi:10.1038/nrc3599)
- Burrell RA, Swanton C. 2014 Tumour heterogeneity and the evolution of polyclonal drug resistance. *Mol. Oncol.* **8**, 1095–1111. (doi:10.1016/j.molonc.2014.06.005)
- Ribas A. 2015 Adaptive immune resistance: how cancer protects from immune attack. *Cancer Disc.* **5**, 915–919. (doi:10.1158/2159-8290.CD-15-0563)
- Hanahan D, Weinberg RA. 2011 Hallmarks of cancer: the next generation. *Cell* **144**, 646–74. (doi:10.1016/j.cell.2011.02.013)
- Xing Y, Hongquist KA. 2012 T-cell tolerance: central and peripheral. *Cold Spring Harb. Perspect. Biol.* **4**, 6. (doi:10.1101/cshperspect.a006957)
- Ribas A, Wolchok JD. 2018 Cancer immunotherapy using checkpoint blockade. *Science* **359**, 1350–1355. (doi:10.1126/science.aar4060)
- Rizvi NA *et al.* 2015 Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* **348**, 124–128. (doi:10.1126/science.aaa1348)
- Snyder A *et al.* 2014 Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N. Engl. J. Med.* **371**, 2189–2199. (doi:10.1056/NEJMoa1406498)
- Van Allen EM *et al.* 2015 Genomic correlates of response to CTLA4 blockade in metastatic melanoma. *Science* **350**, aad0095. (doi:10.1126/science.aad0095)
- Schumacher TN, Schreiber RD. 2015 Neoantigens in cancer immunotherapy. *Science* **348**, 69–74. (doi:10.1126/science.aaa4971)
- Xiao Y, Freeman GJ. 2015 The microsatellite instable subset of colorectal cancer is a particularly good candidate for checkpoint blockade immunotherapy. *Cancer Disc.* **5**, 16–18. (doi:10.1158/2159-8290.CD-14-1397)
- McGranahan N *et al.* 2016 Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. *Science* **351**, 1463–1469. (doi:10.1126/science.aaf1490)
- Wolf Y *et al.* 2019 UVB-induced tumor heterogeneity diminishes immune response in melanoma. *Cell* **179**, 219–235. (doi:10.1016/j.cell.2019.08.032)
- Lakatos E *et al.* 2019 Evolutionary dynamics of neoantigens in growing tumours. *BioRxiv* 209:536433. (doi:10.1101/536433)
- Luksha M *et al.* 2017 A neoantigen fitness model predicts tumour response to checkpoint blockade immunotherapy. *Nature* **551**, 517–20. (doi:10.1038/nature24473)
- Krummel MF, Bartumeus F, Gérard A. 2016 T cell migration, search strategies and mechanisms. *Nat. Rev. Immunol.* **16**, 193. (doi:10.1038/nri.2015.16)
- Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. 2017 Primary, adaptive, and acquired resistance to cancer immunotherapy. *Cell* **168**, 707–723. (doi:10.1016/j.cell.2017.01.017)
- Zappasodi R, Merghoub T, Wolchok JD. 2018 Emerging concepts for immune checkpoint blockade-based combination therapies. *Cancer Cell* **33**, 581–598. (doi:10.1016/j.ccell.2018.03.005)
- Ribas A *et al.* 2015 Phase I study combining anti-PD-L1 (MEDI4736) with BRAF (dabrafenib) and/or MEK (trametinib) inhibitors in advanced melanoma. *J. Clin. Oncol.* **33**, 3003. (doi:10.1200/jco.2015.33.15_suppl.3003)
- Bendell JC *et al.* 2016 Clinical activity and safety of cobimetinib (cobi) and atezolizumab in colorectal cancer (CRC). *J. Clin. Oncol.* **34**, 3502. (doi:10.1200/JCO.2016.34.15_suppl.3502)
- Bindea G *et al.* 2013 Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. *Immunity* **39**, 782–795. (doi:10.1016/j.immuni.2013.10.003)
- Gatenby RA, Gillies RJ. 2008 A microenvironmental model of carcinogenesis. *Nat. Rev. Can.* **8**, 56–61. (doi:10.1038/nrc2255)
- Budhu S *et al.* 2010 CD⁸⁺ T cell concentration determines their efficiency in killing cognate antigen-expressing syngeneic mammalian cells *in vitro* and in mouse tissues. *J. Exp. Med.* **207**, 223–235. (doi:10.1084/jem.20091279)
- Yewdell JW, Bennink JR. 1999 Immunodominance in major histocompatibility complex class I-restricted T lymphocyte responses. *Annu. Rev. Immunol.* **17**, 51–88. (doi:10.1146/annurev.immunol.17.1.51)
- Schreiber H, Wu TH, Nachman J, Kast WM. 2002 Immunodominance and tumor escape. *Semin. Cancer Biol.* **12**, 25–31.
- Martincorena I *et al.* 2017 Universal patterns of selection in cancer and somatic tissues. *Cell* **171**, 1029–1041. (doi:10.1016/j.cell.2017.09.042)
- Harris TH *et al.* 2012 Generalized Lévy walks and the role of chemokines in migration of effector CD⁸⁺ T cells. *Nature* **486**, 545–548. (doi:10.1038/nature11098)
- Macfarlane FR, Chaplain MA, Lorenzi T. 2019 A stochastic individual-based model to explore the role of spatial interactions and antigen recognition

- in the immune response against solid tumours. *J. Theor. Biol.* **480**, 43–55. (doi:10.1016/j.jtbi.2019.07.019)
31. Kuznetsov VA, Makalkin IA, Taylor MA, Perelson AS. 1994 Nonlinear dynamics of immunogenic tumors: parameter estimation and global bifurcation analysis. *Bull. Math. Biol.* **56**, 295–321. (doi:10.1016/S0092-8240(05)80260-5)
  32. Matzavinos A, Chaplain MA, Kuznetsov VA. 2004 Mathematical modelling of the spatio-temporal response of cytotoxic T-lymphocytes to a solid tumour. *Math. Med. Biol.* **21**, 1–34. (doi:10.1093/imammb/21.1.1)
  33. Salmon H *et al.* 2012 Matrix architecture defines the preferential localization and migration of T cells into the stroma of human lung tumors. *J. Clin. Invest.* **122**, 899–910. (doi:10.1172/JCI45817)
  34. Wahl SM *et al.* 1988 Transforming growth factor-beta is a potent immunosuppressive agent that inhibits IL-1-dependent lymphocyte proliferation. *J. Immunol.* **140**, 3026–3032.
  35. Stead LF, Sutton KM, Taylor GR, Quirke P, Rabitts P. 2013 Accurately identifying low-allelic fraction variants in single samples with next-generation sequencing: applications in tumor subclone resolution. *Hum. Mutat.* **34**, 1432–1438. (doi:10.1002/humu.22365)
  36. Yadav M *et al.* 2014 Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing. *Nature* **515**, 572–576. (doi:10.1038/nature14001)
  37. Loeb LA *et al.* 2019 Extensive subclonal mutational diversity in human colorectal cancer and its significance. *Proc. Natl Acad. Sci. USA* **116**, 26 863–26 872. (doi:10.1073/pnas.1910301116)
  38. Roerink SF *et al.* 2018 Intra-tumour diversification in colorectal cancer at the single-cell level. *Nature* **556**, 457–462. (doi:10.1038/s41586-018-0024-3)
  39. Starr TK, Jameson SC, Hogquist KA. 2003 Positive and negative selection of T cells. *Annu. Rev. Immunol.* **21**, 139–176. (doi:10.1146/annurev.immunol.21.120601.141107)
  40. Viswanathan GM, Buldyrev SV, Havlin S, Raposo EP, Stanley HE. 1999 Optimizing the success of random searches. *Nature* **401**, 911–914. (doi:10.1038/44831)
  41. DuPage M, Mazumdar C, Schmidt LM, Cheung AF, Jacks T. 2012 Expression of tumour-specific antigens underlies cancer immunoediting. *Nature* **482**, 405–409. (doi:10.1038/nature10803)
  42. Williams MJ, Werner B, Barnes CP, Graham TA, Sottoriva A. 2016 Identification of neutral tumor evolution across cancer types. *Nat. Gen.* **48**, 238–244. (doi:10.1038/ng.3489)
  43. Beckman RA, Schemmann GS, Yeang CH. 2012 Impact of genetic dynamics and single-cell heterogeneity on development of nonstandard personalized medicine strategies for cancer. *Proc. Natl Acad. Sci. USA* **109**, 14 586–14 591. (doi:10.1073/pnas.1203559109)
  44. Gatenby RA, Brown J, Vincent T. 2009 Lessons from applied ecology: cancer control using an evolutionary double bind. *Cancer Res.* **69**, 7499–7502. (doi:10.1158/0008-5472.CAN-09-1354)
  45. Gatenby RA, Silva AS, Gillies RJ, Frieden BR. 2009 Adaptive therapy. *Cancer Res.* **69**, 4894–4903. (doi:10.1158/0008-5472.CAN-08-3658)
  46. Campbell BB *et al.* 2017 Comprehensive analysis of hypermutation in human cancer. *Cell* **171**, 1042–1056. (doi:10.1016/j.cell.2017.09.048)
  47. Anagnostou V *et al.* 2017 Evolution of neoantigen landscape during immune checkpoint blockade in non-small cell lung cancer. *Cancer Disc.* **7**, 264–276. (doi:10.1158/2159-8290.CD-16-0828)
  48. Corcoran RB *et al.* 2012 EGFR-mediated reactivation of MAPK signaling contributes to insensitivity of BRAF-mutant colorectal cancers to RAF inhibition with vemurafenib. *Cancer Disc.* **2**, 227–235. (doi:10.1158/2159-8290.CD-11-0341)
  49. Diaz Jr LA *et al.* 2012 The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. *Nature* **486**, 537–540. (doi:10.1038/nature11219)
  50. Abramowitz M, Stegun IA (eds). 1972 6.3 psi (Digamma) Function. In *Handbook of mathematical functions with formulas, graphs, and mathematical tables*, pp. 258–259. New York, NY: Dover.
  51. Nowak MA, May RM, Anderson RM. 1990 The evolutionary dynamics of HIV-1 quasispecies and the development of immunodeficiency disease. *Aids* **4**, 1095–1103. (doi:10.1097/00002030-199011000-00007)
  52. d’Onofrio A. 2010 Bounded-noise-induced transitions in a tumor–immune system interplay. *Phys. Rev. E* **81**, 021923. (doi:10.1103/PhysRevE.81.021923)