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# Effects of mutations and immunogenicity on outcomes of anti-cancer therapies for secondary lesions

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## Abstract

Cancer development is driven by mutations and selective forces, including the action of the immune system and interspecific competition. When administered to patients, anti-cancer therapies affect the development and dynamics of tumours, possibly with various degrees of resistance due to immunoediting and microenvironment. Tumours are able to express a variety of competing phenotypes with different attributes and thus respond differently to various anti-cancer therapies.

In this paper, a mathematical framework incorporating a system of delay differential equations for the immune system activation cycle and an agent-based approach for tumour-immune interaction is presented. The focus is on those metastatic, secondary solid lesions that are still undetected and non-vascularised.

By using available experimental data, we analyse the effects of combination therapies on these lesions and investigate the role of mutations on the rates of success of common treatments. Findings show that mutations, growth properties and immunoediting influence therapies' outcomes in nonlinear and complex ways, affecting cancer lesion morphologies, phenotypical compositions and overall proliferation patterns. Cascade effects

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on final outcomes for secondary lesions are also investigated, showing that actions on primary lesions could sometimes result in unexpected clearances of secondary tumours. This outcome is strongly dependent on the clonal composition of the primary and secondary masses and is shown to allow, in some cases, the control of the disease for years.

*Keywords:* secondary lesions, immune response, combination therapies, tumour morphology

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## 1. Introduction

Cancer is a generic definition of a disease that, among its typical features, is driven by dynamic alterations in the genome [1]. These microscopic changes not only give birth to a variety of different types of cancer at the macroscopic scale, but can also lead to heterogeneity within the same cancer tissue: tumour phenotypes undergo clonal expansion and genetic diversification, promoting natural selection mechanisms that favor cell clones with advantageous characteristics [2, 3].

Alterations in the DNA of the cell, such as inclusions of copy number aberrations and point mutations, occur early during the neoplastic transformation and usually before any possible clinical detection [4]. The step-wise accumulation of driver mutations may confer survival advantages in relation to the particular environment in which they are embedded and may be accelerated by so-called selective sweeps [5]. Furthermore, although the immune system routinely recognises and kills any dangerous host including cancer, mutations can provide cancer cells with the ability to avoid detection or immuno-suppress the environment, advantaging tumour progression or preventing eradication [1]. Processes involving mutations, cell growth and immune surveillance cumulatively result in the emergence of different cancer populations integrated in an environment made up of healthy tissue, immune cells and stroma [6, 7].

Understanding how these complex interactions shape and influence each other is one of the greatest challenges in current medical biosciences. For example, morphology is known to be strongly sensitive to tumour adaptation to the environment (e.g. the lack of nutrients, oxygen, space) and by the combined action of immune response and existing anti-cancer therapies [8] such as chemotherapy, radiotherapy, immune-boosting and so on. In the last quarter of century, a number of diverse contributions

29 have been proposed from the biomathematical community to shed light  
30 on some of these complex interaction mechanisms. Several mathemati-  
31 cal models have been advanced using the framework of population dy-  
32 namics, with tumour immune interactions considered, for example, in  
33 Ref. [9, 10] and cancer mutations in Ref. [11]. Other works have involved  
34 a discrete Cellular Potts approach [12] or different degrees of hybrid mod-  
35 elling [13, 14], with particular focus on tumour shape [8, 15]. The ef-  
36 fects of some of the currently available anti-tumour therapies have also  
37 been analysed in the context of evolutionary dynamics [16, 17], with im-  
38 munotherapy [18, 19, 20, 21] and, recently, using agent-based modelling  
39 in the context of virotherapies [22]. A number of reviews detailing the  
40 evolution and the contribution of these and other models also exist in the  
41 literature [23, 24, 25, 26].

42 The focus of the present work is on metastatic secondary solid lesions,  
43 with particular emphasis on the role of the immune system and mutations.  
44 Scope of the this work is the study of the effects of different combination  
45 therapies on secondary lesions in order to better understand the dynamics  
46 involved and the role of mutations on treatments' effectiveness. The rest of  
47 the paper is organised as follows. In the "Model" section, a description of  
48 the mathematical approach used to describe tumours, immune responses  
49 and anti-cancer therapies is given. Findings obtained via computational  
50 analysis are illustrated and analysed in the "Results" and "Discussion"  
51 sections. Finally, the "Conclusions" section terminates the paper.

## 52 **2. Model**

53 Let us consider the biological setting under study as follows: a primary,  
54 clinically detected cancer is present in a patient and it is scheduled to be  
55 treated with different therapeutic approaches, in an effort to improve the  
56 patients' clinical outlook. A secondary lesion is also growing, undetected  
57 and located away from the primary site, due to previous metastatic events  
58 and migration of tumour cells belonging to the first lesion. We are inter-  
59 ested in understanding how the secondary lesion is affected by strategies  
60 aimed at reducing the primary one. *Our approach is based on an existing*  
61 *mathematical model for tumour-immune interaction [27], which has been val-*  
62 *idated previously both from the point of view of biological appropriateness and*  
63 *sensitivity to model parameters. The phenomena at hand are inherently complex*  
64 *and there is a number of unknowns that still characterise these processes. Our*

65 *work is thus focussed on understanding the major trends and the typical out-*  
66 *comes that can emerge in treating secondary lesions, providing some quantitative*  
67 *data that can be tested experimentally.*

68 The dynamics between a heterogeneous, small, solid cancer lesion and  
69 the immune system is formulated using an hybrid agent-based model  
70 (ABM) coupled with a delay differential equation (DDE) system. An im-  
71 mune response to cancer cells that grow and mutate is simulated using a  
72 population of cytotoxic T lymphocytes (CTLs), which mature in a tumour-  
73 draining lymph node. The overall approach rests on an existing frame-  
74 work, originally discussing tumour cells endowed with only a unique, sin-  
75 gular phenotype. The novelty of the present formulation lies in considering  
76 more than one clone, with mutation processes strongly influencing and  
77 shaping tumour growth dynamics. For a full analysis and description of  
78 the model we refer the reader to Ref. [27], and only discuss the equations  
79 briefly in the following.

The system describing immune activation is given by:

$$\begin{cases} A_0'(t) = s_A - d_0 A_0(t) - \alpha T(t) A_0(t), \\ A_1'(t) = V_{\text{ratio}} \alpha T(t) A_0(t) - d_1 A_1(t), \\ C_0'(t) = r_C \left(1 - \frac{C_0(t)}{K}\right) C_0(t) - \mu A_1(t) C_0(t), \\ C_1'(t) = 2^m \mu A_1(t - \sigma) C_0(t - \sigma) - \mu A_1(t) C_1(t) + \\ \quad + 2\mu A_1(t - \rho) C_1(t - \rho) - \delta_1 C_1(t) - f C_1(t), \\ C_2'(t) = \frac{f C_1(t)}{V_{\text{ratio}}} - \delta_1 C_2(t), \end{cases} \quad (1)$$

80 where  $T$  is the total cancer cell population and  $A_0, A_1, C_0, C_1, C_2$  are the  
81 concentrations of antigen presenting cells (APC), mature APCs, memory  
82 CTLs, effector CTLs and CTLs, respectively. A sketch of the dynamics  
83 captured by the above equations is depicted in Fig.1.

84 The first two equations describe the transition from immature APCs  
85 circulating in the periphery to mature ones migrating to the lymph node  
86 as a response to tumour antigens.

87 The population of immature APCs is generated and dies at constant  
88 rates  $s_A$  and  $d_0$ , with the maximum value of  $A_0$  corresponding to the equi-  
89 librium level  $s_A/d_0$ . When tumour antigens are presented,  $A_0$  decreases  
90 proportionally to the antigenicity value  $\alpha$  and mature APCs ( $A_1$ ) begin  
91 entering the lymph node, with some dying at natural death rate  $d_1$ .

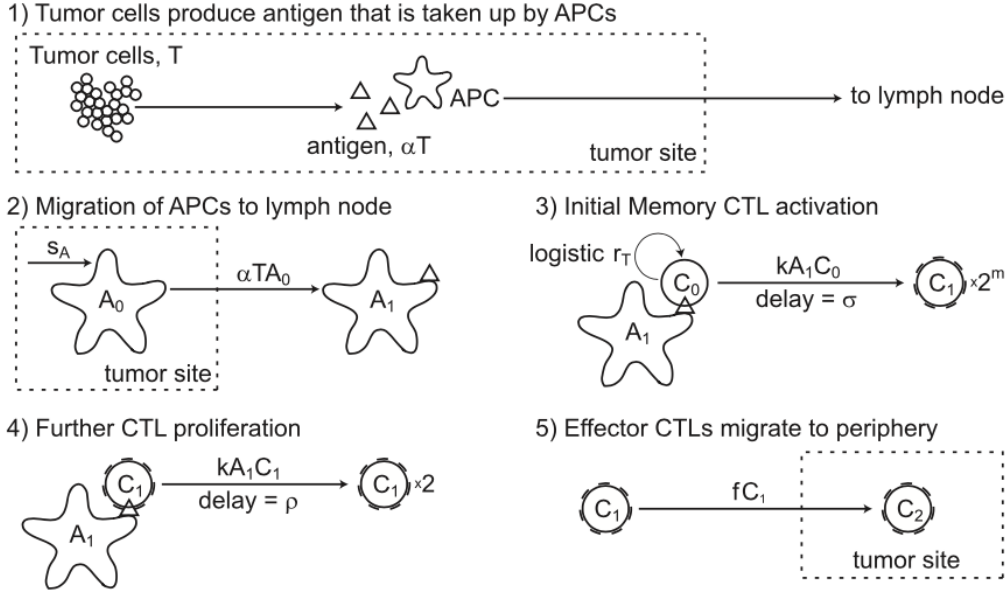


Figure 1: **Immune system activation cycle** as described by the system of equation (1). The behaviour of the immune system is modeled as [27]

92 The presence of mature, tumour-antigen-bearing APCs in the lymph  
 93 node causes memory CTLs to activate and mature into effector CTLs, with  
 94 a certain delay. Consequently, the effector CTLs proliferate and migrate to  
 95 the tumour site where the anti-tumour immune response starts. This process  
 96 is captured as follows. The third equation represents the stimulation  
 97 by the APCs of the memory CTLs ( $C_0$ ), with a logistic growth rate inde-  
 98 pendent of the external stimuli and a stimulation by mature APCs that  
 99 follows a mass action law. In the fourth equation, memory CTLs develop  
 100 a minimal division process, characterised by  $m$  times divisions, and evolve  
 101 in effector CTLs ( $C_1$ ) with a time delay  $\sigma$ . Then, effector CTLs divide again  
 102 in a time  $\rho$  and flow away of the lymph node or naturally die with a rate  
 103  $\delta_1$ . The last equation represents the concentration of CTLs ( $C_2$ ) in the pe-  
 104 riphery around the tumour and provides the concentration  $C_2$  used by the  
 105 ABM component of the model to generate the boundary conditions for the  
 106 tumour-site domain. Table 1 shows parameter values used in this system  
 107 of equations and their meanings. Subsection 2.1 reports the parameter  
 108 estimation and the related sensitivity analysis, which have been mostly  
 109 performed in previous works [27].

111 The ABM controls tumour growth dynamics and the interaction be-  
 112 tween tumour cells and CTLs at the tumour site, which follow specific  
 113 algorithmic rules. Our model does not consider healthy tissue around tu-  
 114 mours and other structures such as the stroma or the cells part of a vas-  
 115 cular network: it is assumed that tumour’s surroundings are effectively  
 116 healthy cells being “pushed away” by the growing tumour. Note also that  
 117 no vascularisation is present due to the limited size of the secondary tu-  
 118 mour lesion, which is considered to be small, solid and with no necrotic  
 119 core. Furthermore, other motility of metastatic processes from the sec-  
 120 ondary lesion are neglected. The overall assumption is that the secondary  
 121 tumour is trying to colonize the site and is in its early stages of prolifera-  
 122 tion. All cells partaking the dynamics are represented as spheres of radius  
 123  $r$  in 3D space, with no overlap. ABM is updated in discrete timesteps  $\Delta t$ .

124 **CTL agents.** The rules that govern CTLs cells via the ABM are three:  
 125 motion around the tumour, recruitment of other immune system cells and  
 126 killing of tumour cells. As mentioned, CTLs cells appear at a concentra-  
 127 tion  $C_2(t)$  at the border of the spherical domain representing the region  
 128 of interest where the tumour is growing. They then move into that re-  
 129 gion performing Brownian motion in 3D space until they either collide  
 130 with a cell or leave the domain. At each time step, the position of the  
 131 cells are given by independent random variables with normal distribution  
 132  $\mathcal{N}(0, \sigma^2 \Delta t)$ , where the variance is such that  $\sigma^2 = 2D$ , with  $D$  being the  
 133 diffusion rate of the CTLs. When an immune cell comes into contact with  
 134 a cancer cells three possibilities exist:

- 135 • A CTL clone can be recruited with a probability  $1 - e^{-\Delta t/C_{\text{recruit}}}$ , with  
 136  $C_{\text{recruit}}$  being the average recruitment time. Mathematically, CTL re-  
 137 cruitment is modeled similarly to Mallet et al. [38] with cellular  
 138 automata, and it is biologically validated as in [39, 40]. When the  
 139 first CTL cell engages a cancer cell and starts recruiting another CTL  
 140 clone, a second cell appears at a position adjacent to the first cell. The  
 141 direction of the new clone is chosen randomly among all directions  
 142 available.
- 143 • A cancer cell is not recognised with a probability  $1 - P_{i,\text{recog}} \cdot \Delta t$ ,  
 144 where  $P_{i,\text{recog}}$  is the probability of the  $i$ -th cancer phenotype (see be-  
 145 low) to be recognised by the immune system. The parameter  $P_{i,\text{recog}}$

146 has a value of one for cancer agents, expressing antigens completely  
 147 matching with the T-cell receptors and thus, that are always detected  
 148 by the immune system. A value of zero indicates that the antigens  
 149 of a phenotype are completely unrecognised. If the cancer cell is not  
 150 recognised, the CTL starts to move again choosing a new random di-  
 151 rection and accelerating up to the maximum unit standard deviation  
 152  $\sigma_{\max}$ . If  $C_{\text{acc}}$  is the time necessary to accelerate from the stationary  
 153 to the maximum diffusion rate, the CTL acceleration is computed as:  
 154  $\sigma(t) = \sigma_{\max} \cdot \min(t/C_{\text{acc}}, 1)$ . This approach aims at approximating  
 155 CTL chemotaxis along a chemokine gradient [41, 42].

- 156 • A cancer cell is recognised and killed with a probability  $1 - e^{-\Delta t/C_{\text{kill}}}$ ,  
 157 with  $C_{\text{kill}}$  being the average time for a CTL to eliminate a cancer cell.  
 158 The killing process is obtained by removing the agent. After the  
 159 agent is removed, the immune cell starts to move again as described  
 160 above.

161 If CTLs die naturally, then they are removed from the system. An expla-  
 162 nation of the ABM-parameters is reported in Table 2 whereas parameter  
 163 estimation and sensitivity analysis is discussed in Subsection 2.1.

164 **Cancer agents.** Tumour cells can proliferate, mutate or die, killed by  
 165 the immune system, and no migration is considered. This approximation  
 166 is motivated by the scope of the study, which is focused on the solid, grow-  
 167 ing secondary lesion after the colonisation of a new tissue. In this early  
 168 stage of implantation most of the cells are assumed to be in a proliferation  
 169 state and migration can be neglected [50]. Cellular division occurs with a  
 170 probability  $1 - e^{-\Delta t/T_{i,\text{div}}}$ ,  $i = 1, \dots, 5$ , where  $T_{i,\text{div}}$  is the average division  
 171 time of the  $i$ -th tumour phenotype. When a tumour cell divides, the posi-  
 172 tion of a new cell is chosen randomly on the mother cell's perimeter, such  
 173 that the daughter cell is tangent. If no space is available in the chosen posi-  
 174 tion, the division process fails and no new agent is created, mimicking the  
 175 contact-inhibition mechanism occurring in the early stages of metastasis  
 176 implantation [51].

177 To analyse the effect of mutations on cancer development and immune  
 178 response, we use five different cancer phenotypes that may emerge from  
 179 the mutation of an *original* clone, identified by different values of charac-  
 180 teristic parameters  $T_{\text{div},o} = T_o$  and  $P_{\text{recog},o} = P_o = 1$ . Mutations can occur  
 181 during cell duplication, with a probability  $P_{\text{mut}} \cdot \Delta t$  that aims to capture  
 182 the genetic instability of the system. Each mutated cell is then identified

183 by indices representing the level of expression of the two characteristic  
184 quantities  $T_{\text{div}}$  and  $P_{\text{recog}}$ . These values effectively classify the mutated  
185 clones and the following mutated phenotypes. Modeling few phenotypes  
186 of mutated cells is a simplification justified by several works showing that  
187 only a limited number of phenotypes are predominant in a tumour, see  
188 for example [8]. For the scope of our study, the five mutated clones are  
189 prototypical of a wide range of similar mutations. In Table 3 cancer clonal  
190 composition is considered. One of the assumptions is that only one class  
191 of CTLs is modeled and it is not antigen specific. Although different types  
192 of CTLs could take part in an immune response and act differently de-  
193 pending on the clone, our immune attacks are regulated only via  $P_{\text{recog},i}$ .

194 Using these different types of phenotypes, as we will see shortly, helps  
195 us to shed light on the role of mutations in determining the effectiveness of  
196 immune response and anti-cancer therapies. Different clonal compositions  
197 and reproductive and immunoediting advantages dramatically influence  
198 the outcomes of anti-cancer therapies.

#### 199 *Modeling therapies: chemotherapy, immune boosting and radiotherapy*

200 One of the typical features of secondary lesions is that they usually  
201 show cells with mutated functional characteristics respect to the original  
202 tumour, due to the genetic instability typical of metastatic masses they  
203 originate from. We reiterate that there is no analysis of the fate of the  
204 global cancer disease but only on such secondary lesions, which can show  
205 different dimensions, compositions, structures and biological characteris-  
206 tics from the primary neoplasia. Chemotherapy, immune boosting and  
207 radiotherapy are the strategies our modelling focuses on.

208 **Chemotherapy.** This treatment consists of cytotoxic drugs targeting a  
209 specific cellular phase of the cell cycle to induce cell death. The procedure  
210 acts against rapidly proliferating cells, independently from their nature  
211 [52]. This means that healthy cells and immune system cells are usually  
212 damaged along with cancer cells, and this leads to well-known side effects  
213 for the patients. In this work, only the primary killing effect against cancer  
214 cells and no direct effects on the immune system is assumed. This simpli-  
215 fication is motivated by two main points. First, the average CTL lifespan  
216 is 41 hours, whereas the tumour division rate is greater and the tumour  
217 death rate due to the therapy is slower. CTL cells are rapidly affected by  
218 the reduction of the tumour mass and no new CTL is recruited: the “old”  
219 cells tend to naturally die. Second, if on one side chemotherapy affects the

220 immune cells, on the other specific T-cell response is reinforced [53], and  
 221 the investigation of these secondary effects is not in the scope of the future  
 222 present work.

223 During a cycle of chemotherapy of duration  $Ch_{\text{time}}$ , the  $i$ -th cancer  
 224 phenotype can go through cellular death with probability  $1 - e^{-\Delta t/Ch_{\text{kill},i}}$ ,  
 225 where the average time for the drugs to induce cellular death is  $Ch_{\text{kill},i}$  and  
 226 depends from the proliferation potential of the phenotype.  $Ch_{\text{time}}$  takes  
 227 into account a single cycle of three injections every three days and rep-  
 228 represents the global time duration of the chemotherapy's effects. The drug  
 229 remains two days above a certain percentage level such that the cytotoxic  
 230 effects on tumour cells can be considered constant.

231 Different values of  $Ch_{\text{time}}$  have been explored as reported in Table 4,  
 232 supposing that the same total dose is inoculated in continuous cycles of  
 233 low metronomic doses. The effect of different  $Ch_{\text{time}}$  with the same total  
 234 dose is a faster or slower decrease of the cancer population with similar  
 235 qualitative dynamics. In particular, for clone  $(0, 1)$  (refer to Table 3 for no-  
 236 tation), i.e. the phenotype that grows slowly but is poorly immunogenic,  
 237  $Ch_{\text{kill},(0,1)} = Ch_{\text{time}}$ , namely a  $(0, 1)$ -death is very rare. Clone  $(0 + 0.25i, 1 -$   
 238  $0.25i)$ , with  $i = 1, \dots, 4$ , has  $Ch_{\text{kill},(0+0.25i,1-0.25i)} = Ch_{\text{time}} - i \cdot Ch_{\text{eff}} \cdot Ch_{\text{time}}$ ,  
 239 so that the tumour with higher proliferation rate has very high probability  
 240 to die due to the effect of the drug.

241 **Immune boosting.** We use this generic term to capture the number  
 242 of clinically available strategies that potentiate an immune response. For  
 243 example, a treatment that is increasingly used for cancer patients is the so-  
 244 called adoptive cell transfer (ACT), where patients' own immune cells are  
 245 stimulated and modified to treat their tumour. There are several types of  
 246 ACTs that go under different acronyms depending on the boosting strat-  
 247 egy employed, with the most used ones nowadays being TIL (Tumour  
 248 infiltrating lymphocytes), TCR (Tumour cell receptors) T-cell and CAR  
 249 (Chimeric antigen receptors) T-cells treatments [54].

250 We concentrate in particular on TIL therapy, where T-cells are extracted  
 251 from the patient's tumour, grown *in vitro* to boost their numbers and in-  
 252 jected back into the patient to contrast cancer progression. This strategy  
 253 appears to be, for example, one of the most effective treatment against  
 254 metastatic melanoma [54]. In our approach, TIL is modeled as a contin-  
 255 uous increase of the CTLs concentration in the cloud, depending on the  
 256 value of  $C_2$  at the starting time for the therapy. The net increase is mod-

257 eled by a number of  $B_{o_{\text{eff}}}$  cells for a short time  $B_{o_{\text{time}}}$ . For simplicity, in the  
258 following we refer to this treatment as immune boosting or simply boost.  
259 The parameters used to model boost and chemotherapy are explained and  
260 collated in Table 4.

261 **Radiotherapy.** Radiotherapy (RT) uses ionising radiation to induce cell  
262 death in a localised area under treatment. This therapy has several posi-  
263 tive and negative feedbacks on the immune system, modulating different  
264 compartments of the tumour microenvironment. In particular, tumour-  
265 specific antigens and immune-stimulatory signals are released by the dy-  
266 ing cancer cells.

267 Because of its contributing primarily to the original, metastatic neopla-  
268 sia, the effect of RT is here modelled as an indirect effect on the secondary  
269 lesion and is accounted for as as a restoring factor in the ability of CTL  
270 cells to recognise and kill various cancer phenotypes.

### 271 2.1. Parameter estimation and sensitivity analysis

272 The biological significance of parameters and processes that underpin  
273 the present model has been discussed at length elsewhere [27, 55]. In some  
274 cases, such as, for example, parameters used for tumour division time or  
275 cell radius, well-established values in the literature have been used [43,  
276 44, 45, 38]. In other cases, estimations from the available experimental and  
277 theoretical data have been carried out.

278 A sensitivity analysis has originally also been carried out for eight pa-  
279 rameters of the model:  $T_{\text{div}}$ ,  $\sigma_{\text{max}}$ ,  $C_{\text{acc}}$ ,  $C_{\text{recruit}}$ ,  $C_{\text{kill}}$ ,  $K$ ,  $\mu$ ,  $m$  and  $\alpha$ . Other  
280 parameters have not been considered because their role is known to be  
281 marginal. For instance, the replenishment rate for memory CTLs is known  
282 to be irrelevant, since only a very tiny fraction of memory CTLs ( 1%) is  
283 known to be affected by the tumour. Similarly, the duration of CTL divi-  
284 sion (time delay parameter  $\rho$  in the DDE) is too small to impact the CTL  
285 division program as a whole and does not influence final outcomes. Using  
286 Spearman’s rank-order correlations, tumour populations’ values and ex-  
287 tinction times, Kim et al. have concluded [27] that tumour division times  
288  $T_{\text{div}}$ , antigenicity  $\alpha$  and the number of divisions of memory CTLs upon  
289 activation  $m$  are the most sensitive parameters.

290 In this work, we use the same parameters proposed in the original pa-  
291 per, with the only difference of  $T_{\text{div}}$ , still chosen in the proposed interval  
292 but capturing a more aggressive tumour (i.e.  $T_{\text{div}} = 1 - 39$  days). The

293 effect on simulations is to shorten the proliferating phase, which occurs at  
294 a larger growth rate and allows for a quicker immune response.

295 The new probability coefficients introduced here, i.e.  $P_{\text{mut}}$  and  $P_{\text{recog}, i}$ ,  
296 have different effects. By using 5 different simulations with different initial  
297 random seeds and 10 different values of the parameters, we conclude that  
298  $P_{\text{mut}}$  has no effect on the final outcomes of the system, but only accelerates  
299 or delays the identical dynamics shown by the model.

300  $P_{\text{recog}, i}$  instead has a notable effect on the system. When  $P_{\text{recog}, i} = 0$  for  
301 a given  $i$ -th clone, the immune system is unable to eradicate that particular  
302 phenotype and, if no external therapy is present, the tumour endlessly  
303 grows.

304 As far as the values for therapies' parameters are concerned, i.e.  $Ch_{\text{time}}$ ,  
305  $Ch_{\text{eff}}$ ,  $Bo_{\text{time}}$ ,  $Bo_{\text{eff}}$ , they are chosen so that the dynamics between tumour,  
306 immune system and therapies display interesting behaviours and does not  
307 result in an immediate negative or positive outcome. In particular,  $Ch_{\text{eff}}$   
308 and  $Bo_{\text{time}}$  have been varied in a number of different instantiations of the  
309 model, with only the cases  $Ch_{\text{eff}} = 0.25$ ,  $Bo_{\text{time}} = 1000$  cells used in the  
310 discussion of results. Variations of those parameters do not alter in a sig-  
311 nificant way the prototypical dynamics that we will discuss shortly. Note  
312 that  $Ch_{\text{eff}} = 0.25$  has been chosen so that the cytotoxic drug targets fast  
313 proliferating cells.

## 314 2.2. Morphological and complexity measures

315 Three indices that capture the shape and cellular compositions of the  
316 tumour mass are introduced and monitored in our computational exper-  
317 iments. *Note that these indices can guide the evaluation of collective properties*  
318 *of the evolving tumours. They are useful to discriminate between different evo-*  
319 *lutions of the cancer masses and have also been validated in some in vitro experi-*  
320 *ments, as shown by other authors in previous works [56, 14].*

**Roughness.** Although random proliferation of a group of cells leads to an almost smooth and spherical object, a tumoural mass with diverse clonal families under the action of the immune system can present itself as a rough aggregate. To account for this, a measure of roughness  $M$  is introduced, as the ratio between the surface  $S$  and the volume  $V$  of the aggregate [56]. The minimum ratio is represented by a sphere  $S_s/V_s = (4\pi R_s^2)/(4/3\pi R_s^3) = 3/R_s$ , where the value has been non-dimensionalised as follows:  $M_{\text{min}} = \sqrt{S_s}/\sqrt[3]{V_s} = \sqrt{4\pi}/\sqrt[3]{(4/3)\pi}$ . The roughness index  $M$ ,

expressed in terms of the minimal ratio for a sphere, is given by:

$$M = \frac{\sqrt{S}}{\sqrt[3]{V}} \cdot \frac{1}{M_{\min}} = \frac{\sqrt{4\pi S}}{\sqrt[3]{3(4\pi)^2 V}}. \quad (2)$$

321 A compact, non-infiltrated, almost spherical tumour mass has an index  $M$   
 322 close to unity while a tumour with highly irregular borders, for instance  
 323 a solid tumour with fingers and clusters of invasive cells or a mass highly  
 324 infiltrated by the immune system, displays a higher value.

**Radius of gyration.** This value represents the radius of a sphere that contains the whole tumour aggregate and reads:

$$R_g = \sqrt{\frac{\sum_{i=1}^{N_c} (\mathbf{r}_i - \mathbf{r}_{cm})^2}{N_c}}, \quad (3)$$

325 where  $N_c$  is the total number of cancer cells and  $\mathbf{r}_i$  is the distance of each  
 326 clone from the center-of-mass of the tumour ( $\mathbf{r}_{cm}$ ) that can vary during the  
 327 tumour progression.

**Shannon Index.** This indicator is introduced to account for the presence of different phenotypes within a tumour, with regards to tumour heterogeneity and relative frequency of each clonal family ( $p_i$ ). The Shannon index  $H$  is thus defined as:

$$H = -\frac{\sum_{i=1}^s p_i \ln(p_i)}{\ln(s)} \quad (4)$$

328 where  $p_i$  is the relative abundance of the phenotype  $i$  and  $s$  is the total  
 329 number of different phenotypes (in our case  $s = 6$ ). For simplicity,  $H$  is  
 330 then normalised to the interval  $[0, 1]$ , where zero indicates a homogeneous  
 331 population with only one clonal family and unity represents a fully het-  
 332 erogeneous population where all phenotypes are equally present.

333

### 334 3. Results

335 The model outlined in the previous sections is the basis for *in silico* ex-  
 336 periments, where a different number of therapies and their combinations

337 are tried out for significant values of the parameter set. Depending on  
338 the initial conditions, the system exhibits three typical behaviours, namely  
339 eradication, sustained (irregular) oscillations or exponential, uncontrolled  
340 growth when an immune response to a growing tumour is present. We  
341 consider parameter values where the effect of clinical therapies are rel-  
342 evant. Cases where the tumour grows too fast or too slow, making the  
343 effects of therapies not noticeable, are excluded from our analysis. Sta-  
344 tionary behaviour has never been observed. Outcomes also depend on  
345 the characteristics of cellular phenotypes present in the growing mass,  
346 strongly influencing its speed of growth, its ability to counteract the ac-  
347 tion of T-cells with immunoediting and its morphological qualities, which  
348 can hinder the ability of the immune system to effectively erode the cancer.

349 Considering the dynamics observed in a number of computational ex-  
350 periments performed at biologically meaningful parameter values, tumour  
351 growth generally appears as exponential, with a consequent linear in-  
352 crease in the radius of gyration  $R_g$  with time, *as previously observed* [57].  
353 *The main reason, as explained in Ref. [14], is that the growth is driven by those*  
354 *cells that reside at the periphery of the mass.* The nearly spherical shape of  
355 the tumour when only a single clone is present changes significantly in  
356 the presence of mutations. The greatest contribution to asymmetry occurs  
357 when a new population with a faster proliferation rate than neighbouring  
358 cells is generated. In that case, this new population forms an evolutionary  
359 niche that can alter the sphericity of the tumour, until the new clones have  
360 proliferated enough to surround the slower cells and recreate a spherical  
361 appearance, as shown in Fig. 2. Note that, in our model, cells acquire  
362 a new phenotype upon mutation in a purely stochastic way and there is  
363 equal probability to mutate from the *original* phenotypes to all the others.

364 The heterogeneity of the mass increases with mutations until the faster  
365 clones are not outnumbering the other phenotypes. If this occurs, then  
366 the Shannon index  $H$  rapidly decreases with time in a way that it is in-  
367 versely proportional to the growth of the more proliferating clones, i.e.  
368 the faster they grow the faster  $H$  decreases. The action of the immune sys-  
369 tem usually tends to favor homogeneity over heterogeneity, rebalancing  
370 the distribution of phenotypes as long as the immune response is active.  
371 As T-cells erode the tumour, natural selection leads to an evolutionary bot-  
372 tleneck characterised by low  $H$ . It is interesting to note that the roughness  
373 of the tumour tends to be in the interval  $1 < M \leq 1.5$ , with signs of super-  
374 ficial infiltration by the immune system. The limited life span of the CTLs

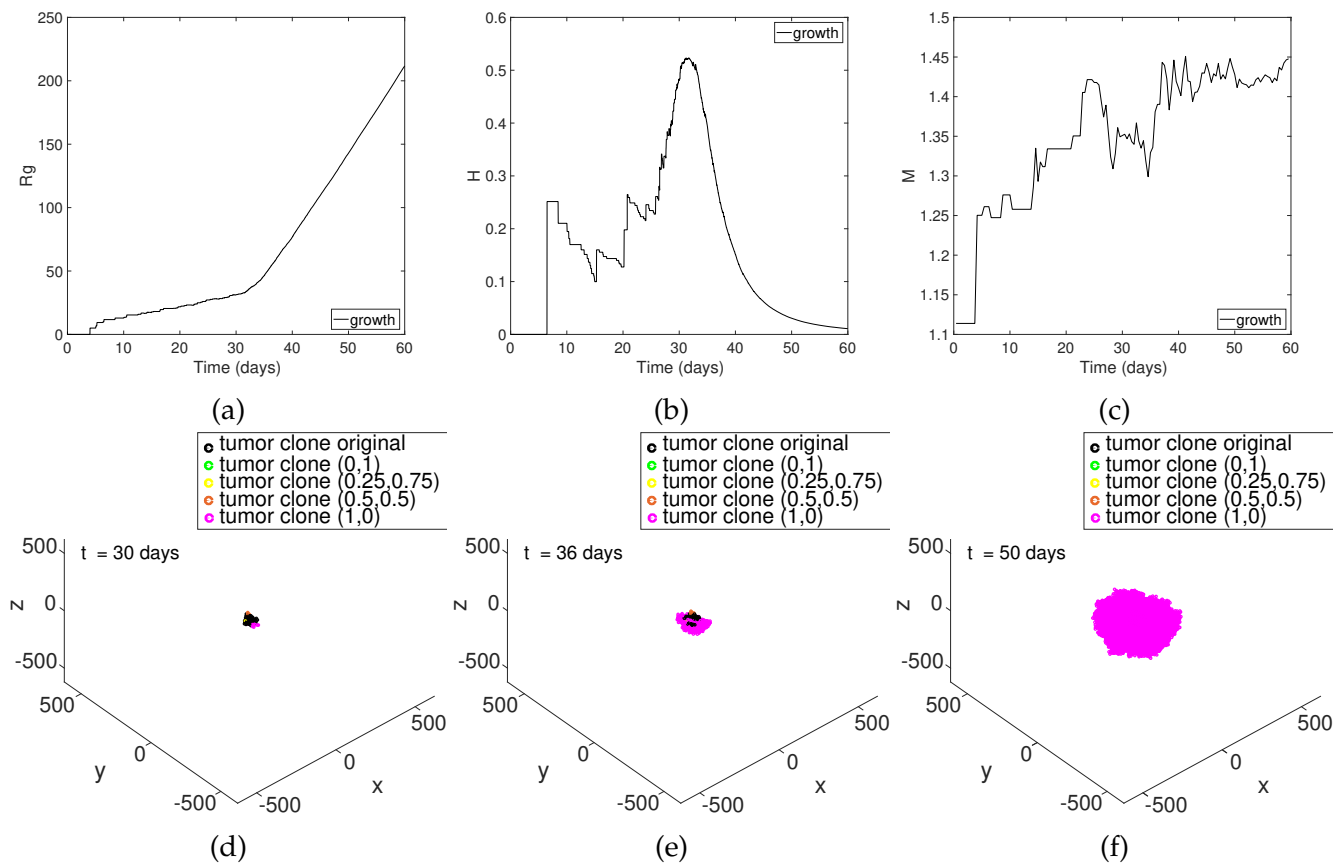


Figure 2: **Tumour growth dynamics** Panel (A)  $R_g$  as function of time, slow initial trend followed by a fast linear growth. Panel (B)  $H$  as function of time, increases with mutations and decreases when the fittest clonal population outnumbers the others. Panel (C)  $M$  as function of time. The roughness index increases with mutations, is higher when a new population with fast proliferation arises and reaches an almost stationary level when  $H$  is near zero. Panel (D), (E), (F) 3D-views of the tumour, respectively, just after the birth of the fittest clone ( $t=30$  days), when the fittest population starts to invade ( $t=36$  days), when the tumour returns to growing almost spherically with low grade of heterogeneity ( $t=50$  days).

375 used in our model tends to promote attacks that occur on the periphery  
376 and rarely result in deep infiltration, which, as we will show shortly, is  
377 instead present when therapies are activated.

### 378 *The effect of therapies on tumours*

379 In the following, all the parameters have been set as in Ref. [27] and  
380 are reported in Tables 1 and 2. The tumour mutation rate has been chosen  
381 following the principle that positive mutations, i.e. mutations that lead to  
382 an evolutionary benefit to the cells over therapies and immune response,  
383 are rare. As it is expected, the dominant phenotype population usually  
384 appears after a clonal expansion of few mutations. The range of variation  
385 of the proliferating, aggressive tumour has been set to ensure a biological  
386 meaning and a rate of growth that allows the cancer mass to escape  
387 the control of the immune system in a limited range of time. The main  
388 reason for this choice is that we are interested in modeling the impact of  
389 different therapies on cancers that will not be eradicated in the absence  
390 of anti-tumour therapies and that, at the same time, do not show growth  
391 rates that are unrealistic. Thanks to the probabilistic structure of the sys-  
392 tem, simulations can generate different outcomes also when parameters  
393 are kept fixed for the particular cancer studied. Among the different ex-  
394 periments, three paradigmatic dynamics emerge, which bear particular  
395 relevance and help understanding the typical scenarios that our model  
396 predicts. They are the result of stochastic variations on the evolution of  
397 initially identical tumours. These cases respectively correspond to a tu-  
398 mour mass with an initial slow growth and high heterogeneity (case A),  
399 and two fast growing tumours with either initial low (case B) or high (case  
400 C) heterogeneity.

### 401 *First single-therapy strategy: chemotherapy*

402 Our first choice is to simulate a cytotoxic chemotherapy that acts with  
403 more efficiency against the cancer cells that have the largest growth rate,  
404 starting at day 60 after the first tumour cell colonizes the site and for a total  
405 duration of 10 days. The probability of a cell to be killed by chemotherapy,  
406 with a total dose of drug fixed, is set independently from the time duration  
407 of the protocol in an effort to maximize the efficiency of the therapy.

408 Fig. 3 shows the evolution of the tumour cell population as a function  
409 of time, according to different phenotypic compositions. In the following,

410 *time is evaluated starting from the instant at which the original clone starts to col-*  
411 *onize the new organ. This time does not refer to the primary tumour or the history*  
412 *of his evolution.. In panel (A), the effect of chemotherapy on tumour case A,*  
413 *which is representative of those cancers with lower rates of proliferation*  
414 *but higher propensities to mutate.*

415 A too early start of the treatment results in a completely ineffective  
416 strategy, with a negative outcome. This is because chemotherapy reduces  
417 the more proliferating cells (in pink) at day 60 when those cells are still  
418 scarce and the tumour is too small to benefit from the action of the cyto-  
419 toxic drug. Once the treatment is over, the remaining cells restart to mu-  
420 tate and proliferate, with an exponential growth that the immune system  
421 alone cannot contain. As shown in the inset of panel (A), the number of  
422 cells belonging to the original phenotype (in black) remain almost constant  
423 throughout the procedure and do not change significantly for the duration  
424 of the experiment.

425 Panel (B) of Fig. 3 instead shows a complete eradication of case B,  
426 where tumour cells have initially a low heterogeneity but are reproduc-  
427 ing fast. The effect of the therapy is in this case to eliminate every cell  
428 belonging to the dominating, fast-reproducing phenotype before it is over,  
429 i.e. approximately around day 7 of its 10 days duration. Also, all cells of  
430 the original phenotype are eradicated by the end of the treatment, with the  
431 tumour completely cleared out by the effect of the cytotoxic drug and the  
432 immune response.

433 An initially fast reproducing tumour with high heterogeneity can in-  
434 stead lead to uncontrollable rebounds, with an overall negative outcome  
435 for the patient. In panel (C), the action of the chemotherapy is not suffi-  
436 cient to eliminate every single cell belonging to the mutated phenotype.  
437 According to our choice of parameters, it is enough that one original can-  
438 cer cell or one of the more proliferating clone survives after the chemother-  
439 apy that a fast, uncontrollable rebound can be expected.

440 Interestingly, these last two cases (i.e. B and C) do not show different  
441 evolutions of the radius of gyration  $R_g$  (*not shown in the Figure*) during the  
442 action of chemotherapy, since the treatment acts homogeneously on the  
443 cancer mass as a whole. This is associated directly to the limited dimen-  
444 sion of the tumour, leading to the drug acting on the aggregate with a high  
445 strength from all spatial directions. Roughness  $M$  instead show significant  
446 changes from case A and cases B and C. Tumour case A remains spherical  
447 and compact during the experiment, essentially because the treatment has

448 a very limited effect on the mass due to its premature start. Tumour case B  
449 and C, instead, reach high level of roughness during the treatment, show-  
450 ing, for the cases reported in the Figure 3, a maximal  $M$  of 3.77 and 3.45,  
451 respectively at day 64 and 65 for cases B and C. This clearly indicates that  
452 the tumour loses density and becomes morphologically inhomogeneous  
453 at around half of the treatment duration and is infiltrated to a relevant  
454 degree by the T-cells taking part in the immune response. The overall con-  
455 sideration from these results is that the correct timing of treatment, *here*  
456 *intended as the ideal treatment starting time and therapy duration to achieve op-*  
457 *timal patients benefit*, is a major variable for the outcome of the treatment  
458 and it is also strongly affected by phenotypical compositions.

459 *Second single-therapy strategy: immune boosting*

460 The overall effect of immune boosting is to increase the number of CTL  
461 cells circulating around the tumour site, which we simulate as an injection  
462 of cells starting at day 50 and occurring for a duration of 3 days. Erosion of  
463 cancer cells by the immune system proceeds from the periphery towards  
464 the center of the tumour mass, and is usually characterised by a linear de-  
465 crease of  $R_g$  during the first few days. Another typical characteristics of  
466 the dynamics that follows boosting is a clonal expansion of the CTL pop-  
467 ulation shortly after treatment. For the prototypical three cases A, B and  
468 C introduced above, all of our computational experiments indicate that  
469 boosting alone is not able to eradicate cancer: after an initial decrease in  
470 the tumour mass, two types of evolutions have been observed, both re-  
471 sulting in negative outcomes. Of particular relevance is case C, which,  
472 although not treatable by chemotherapy alone, shows a somewhat unex-  
473 pected and complicated morphology when subject to immune boosting.  
474 In fact, after an initial clonal selection of the less immunogenic phenotype,  
475 case C displays a clear deviation from sphericity in the mass, with a non-  
476 local spread of the tumour population in islands of different sizes, as re-  
477 ported in Fig. 4. After a decreasing phase due to an immune response that  
478 does not result in a complete eradication, the tumour population is even-  
479 tually subject to a faster, uncontrollable increase driven by disconnected,  
480 smaller masses. Overall, a selection of 5 parameter sets and 10 trials for  
481 different seeds give qualitatively similar results.

482 Panel (A) in Fig. 4, shows the number of cells for each phenotype as  
483 a function of time: an initially exponential growth is firstly slowed down  
484 then halted by immunotherapy, with a maximum cell population occur-

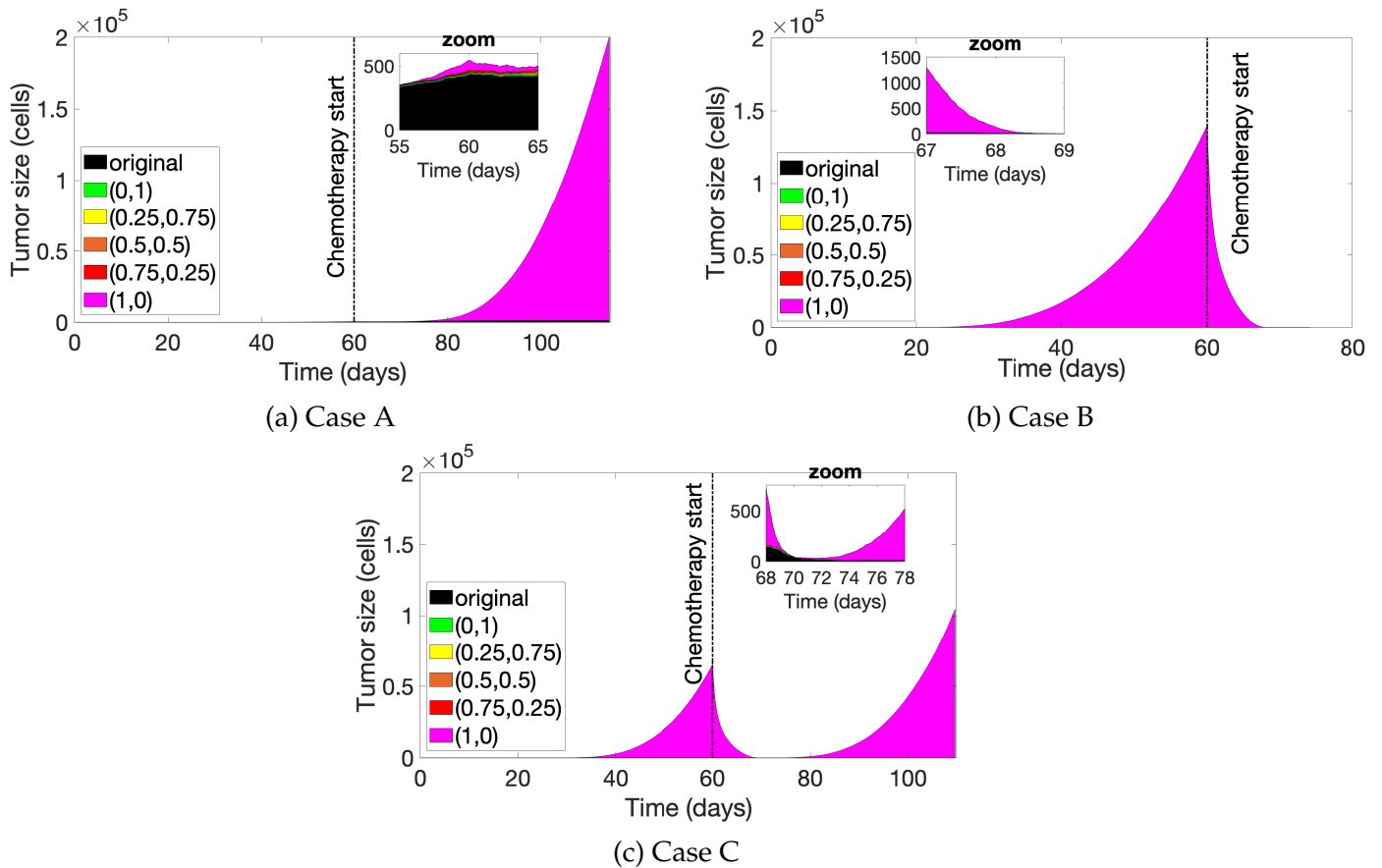


Figure 3: **Chemotherapy effect** Number of cancer cells and phenotypic composition as functions of time. Chemotherapy starts at day 60 and lasts for 10 days. Panel (A) shows an initial phenotype (in black) with slow reproductive rate (case A), eventually overtaken by a new phenotype that reproduces very fast (in pink). The inset shows cellular distribution during the last part of the treatment. Panel (B) shows an initially fast reproducing tumour with low heterogeneity (case B), with an inset of the last two days before complete eradication. Panel (C) depicts an initially fast reproducing tumour with high heterogeneity (case C), with the inset focusing on the dynamics at the end of the treatment and in the first days of the rebound phase.

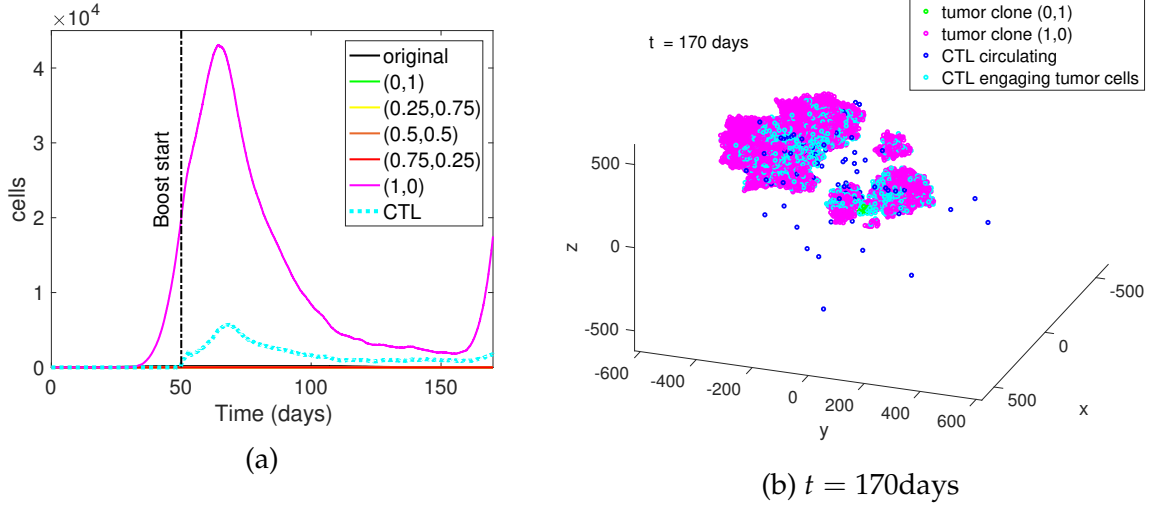


Figure 4: **Immunotherapy effect.** Results for the prototypical case C, a fast growing cancer with high phenotypical heterogeneity. Boost immunotherapy starts at day 50 and lasts a total of 3 days. Panel (A): time series of each cell population, panel (B): snapshot of the tumour mass at time  $t = 170$  days.

ring about 20 days after the beginning of the treatment. Around day 100, the evolution of the cell population changes. Frequent, local maxima in both tumour and T-cell curves represent the failed attempts made by the immune system to completely erode the tumour due to the increasing sparseness of the cancer. This behaviour seems to occur for a protracted period of time of about 60 days.

As the cells in the island's sizes begin to proliferate faster than the rate of killing of the T-cells, a rebound phase with a higher speed of growth than the *original* unbroken mass appears at day 180. Panel (B) provides an image of the scattered status of the tumour immediately before its exponential rebound. Let us remark that the model does not allow for migration of cancer cells and this picture is the result of the infiltration of T-cells coming from boosting and immune response.

As expected, morphology immediately before the rebound phase is characterised by a high value of roughness, with  $\max(M_{\text{CaseC}}) = 2.16$  at day 162. Also, there is almost a twofold increase in  $R_g$  compared to the value for identical number of cells in the first growth phase. For example, for  $10^4$  cells, we have  $R_{g\text{CaseC}} = 125$  at day 47 and  $R_{g\text{CaseC}} = 240$  at day 160.

503 *First example of synergistic therapy: chemotherapy and boosting*

504 Cancer heterogeneity has been invoked to explain one of the major as-  
505 pects of cancer development, namely acquired drug resistance, by which  
506 phases of remission are often followed by a rapid growth of tumour cells [3].  
507 One of the ways to overcome resistance is, for instance, to find more “evo-  
508 lutionarily enlightened” strategies that places malignant cells in an “evo-  
509 lutionary double bind” [17]. In cancer, a double blind could be obtained  
510 using the immune system as natural biological predator [58]. Clinical  
511 evidence shows that immunotherapy or oncolytic viruses alone are not  
512 effective, despite the possible theoretical advantages. Therefore, cancer  
513 treatment is adopting a multistep approach that combines biological and  
514 chemical/radioactive therapies using cytotoxic effects on one side and  
515 subsequent adaptation on the other side to limit tumour adaptive resis-  
516 tance [59, 60].

517 Guided by the poor outcomes displayed by immune boosting alone in  
518 the prototypical cases, we now consider the combination of chemotherapy  
519 and immune boosting, with the aim of discussing the major factors that  
520 maximize positive outcomes. The prototypical cases have been subjected  
521 to a protocol of an immune boosting injection at day 50 lasting three days,  
522 followed by a chemotherapy session at day 60. Results are displayed in  
523 Fig 5, with the insets displaying phenotypical composition over time. For  
524 cases A and B, the complete temporal range is shown, whereas for C the  
525 last 20 days are reported. Timing for these therapies has been chosen arbi-  
526 trarily. *For cases B and C the second lesion grows up to numbers of tumour cells*  
527 *that are close to the detectability threshold.*

528 The effects of this synergistic therapy in cases A and C are similar:  
529 chemotherapy preferentially kills those cells that are fast to reproduce,  
530 leaving the slowest reproducing phenotype unaffected. As a result, re-  
531 bounds occur once therapies end, with case A showing a negative outcome  
532 within the simulated time window and case C displaying a still moderate  
533 but uncontrollable growth at the end of the simulation. In other words, the  
534 effect of chemotherapy is to create an evolutionary bottleneck that selects  
535 the poorly immunogenic clones. *In particular, case C (see the inset of Panel*  
536 *(C)) shows a surviving tumour composed by only two clones: the clone (0, 1) and*  
537 *the clone (0.25, 0.75): these are the two families that are the slowest in proliferat-*  
538 *ing and have the lowest immunogenicity.* These clones have a strong immu-  
539 noediting ability and remain unnoticed by the immune system, resulting

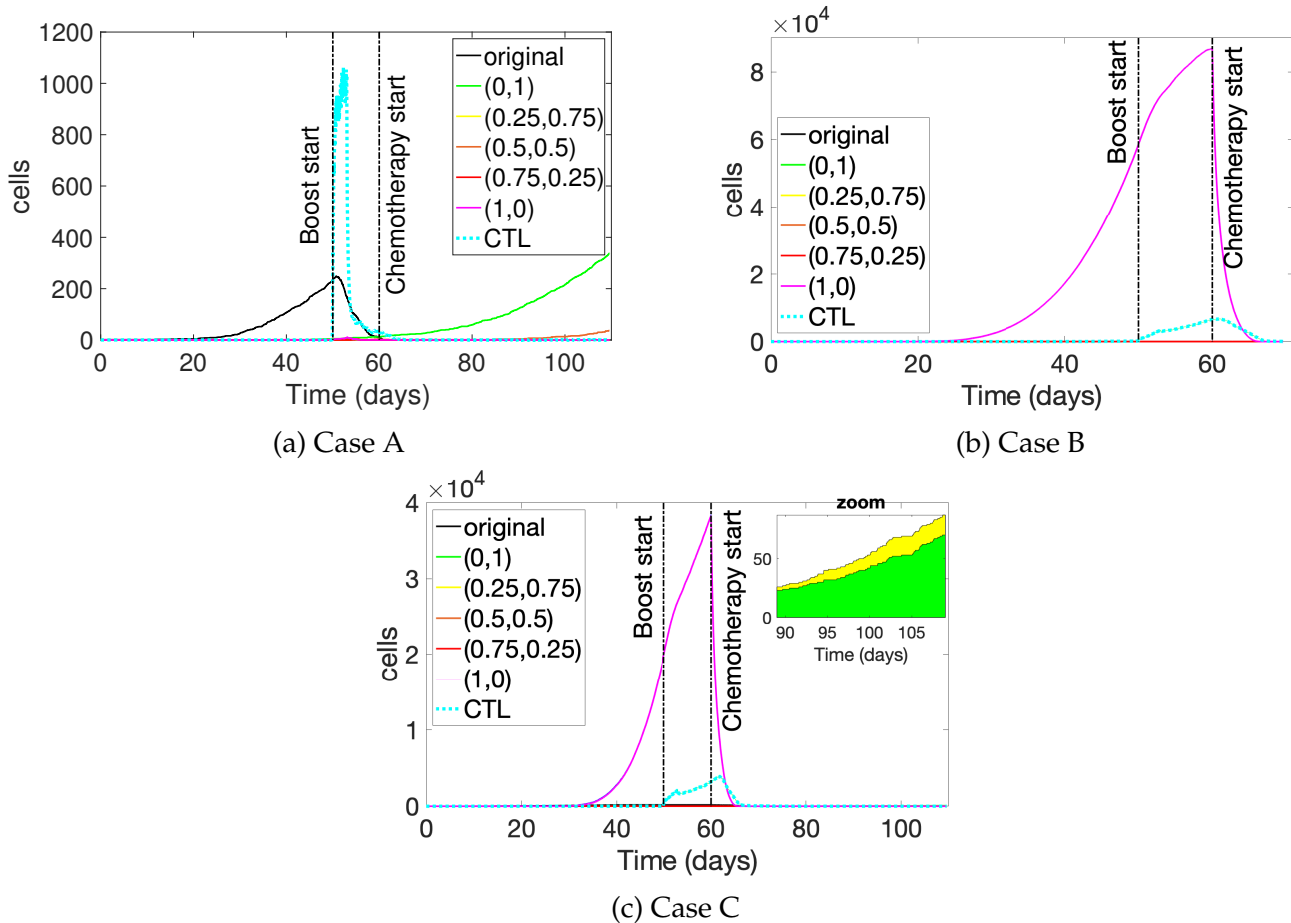


Figure 5: **First kind of synergistic therapy: chemotherapy and immune boosting.** Plots represent the time evolution of cellular populations, with insets showing phenotypical composition of the tumours with time. Immune boosting starts at day 50 and lasts 3 days, whereas chemotherapy starts at day 60 and has a duration of 10 days. Panel (A), (B) and (C) respectively show cases A (slowly growing tumour), B (quickly growing tumour, low heterogeneity) and C (quickly growing tumour, high heterogeneity). *Note that different scales have been used to allow for greater details of the dynamics. The inset in Panel (C) represents a close-up of the time range 90 – 110 days. The unit on the y- axes of every plot in the Figure is cells' numbers.*

540 in unending growth: *because of their ability to be elusive to both the immune*  
541 *response and chemotherapy, outcome C appears the worst of all.* Note that, for  
542 this reason, this phase is of a different nature than those previously re-  
543 ported for individual therapies (i.e. Figs. 3 and 4). Also, because of the  
544 low heterogeneity of case B, this combined therapy is instead successful in  
545 fully eradicating the tumour, which is eliminated during the administra-  
546 tion of the cytotoxic drug. On the other hand, case A, differently from the  
547 results obtained for chemotherapy alone, displays a selection of the poorly  
548 differentiated immunogenic clones.

549 From the morphological perspective, masses emerging from this syn-  
550 ergistic intervention appear to be low in roughness when the reproduc-  
551 tive rate is slow, with  $\max(M_{\text{CaseA}}) = 1.62$  at  $t = 81$  days. If the rate  
552 is instead fast, the level of heterogeneity usually determines the level of  
553 roughness, with low heterogeneity contributing high roughness during  
554 the chemotherapy phase, i.e.  $\max(M_{\text{CaseB}}) = 3.05$  at  $t = 64$  days and  
555  $\max(M_{\text{CaseC}}) = 2.64$  at  $t = 63$  days. Contrary to case B, case C shows a  
556 Shannon index of  $H > 0.5$  for most of the simulation, which results in an  
557 unsuccessful eradication.

558 Other time protocols and order of administration between boosting  
559 and chemotherapy are possible, and have been tested to some degree (re-  
560 sults not shown here). Although a study of optimisation of protocols is not  
561 within the scope of the present work, the overall insight from the simula-  
562 tions is that heterogeneity always plays an important role in the outcomes.  
563 For this combination therapy, high values of  $H$  are consistently associated  
564 with negative prognosis [61].

565 *Second example of synergistic therapy: radiotherapy, boosting and “abscopal”*  
566 *effect*

567 A second example of synergistic therapies that is currently used in clin-  
568 ical practice is the combination of an initial cycle of radiotherapy with an  
569 immune boosting protocol. Besides a better understanding of the param-  
570 eters that can trigger a positive outcome, our interest in testing such a  
571 combination resides in the occurrence of a somewhat rare and poorly un-  
572 derstood event, which is named “abscopal” effect. There are a number  
573 of clinical cases discussed in the medical literature where a reduction of  
574 a secondary tumour or an existing metastasis outside the primary, radi-  
575 ated lesion has been observed [62, 63, 64, 65, 66, 67]. Differently from  
576 chemotherapy, radiotherapy has a localised action on the region irradiated

577 and this makes the phenomenon, to some extent, counterintuitive. Some-  
578 times, the effect appears to affect a secondary lesion very distant from the  
579 region treated.

580 The complications inherent to the stimulation of such an event are due  
581 to the immune action apparently being as crucial as radiotherapy in trig-  
582 gering such a response.

583 The model allows us to test some hypotheses on the nature and causes  
584 of the effect of protocols introduced by Demaria et al. in Ref. [68], who  
585 have reported some interesting and positive outcomes. In particular, they  
586 have treated mice with a syngeneic mammary carcinoma in both flanks  
587 with immunotherapy and only one of the two tumours with radiother-  
588 apy. They use the non-irradiated lesion to monitor the insurgence of the  
589 abscopal effect. Biologically, a tumour-specific T-cells activation occurs af-  
590 ter inflammatory signals are introduced in the system as a consequence  
591 of the therapy. Dying cancer cells release tumour-specific antigens and  
592 immune-stimulatory signals that seem to induce an increased recognition  
593 of cancer cells with the same phenotypical characteristics in other areas  
594 of the body. Further, radiation modulates different compartments of the  
595 tumour microenvironment, resulting in exclusion-inhibition of effector T-  
596 cell and induction of de novo anti-tumour immune responses [69]. The  
597 protocol that we simulate is a radiotherapy (RT) on the primary tumour  
598 (not simulated or showed here) at day 1, followed by an immune boosting  
599 that lasts 10 days. As anticipated, RT is considered a restoring factor in  
600 the ability of CTL cells to recognise and kill all cancer phenotypes, with no  
601 exceptions.

602 The secondary lesion is composed of  $5 \times 10^4$  heterogeneous cells (the  
603 same number of cells injected in mice in the experiment by Demaria et al.),  
604 generated randomly with each clonal family representing at least 10% of  
605 the total population. We compare the action of two single therapies (RT  
606 and immune boosting), with a combination of the two and a control case  
607 where the second lesion remains untreated. Results are presented in Fig 6:  
608 *each panel represents the typical outcome from a single simulation. For each case,*  
609 *i.e. control (no treatment), combination, immunotherapy, and radiotherapy, we*  
610 *have performed three different simulation runs, with different initial conditions.*  
611 *The outcome of each simulation for any configuration consistently gives compa-*  
612 *parable results. Variation due to stochasticity are minimal and do not affect the*  
613 *outcomes.*

614 Firstly, no treatment or RT alone result in similar negative outcomes for

615 the secondary mass, not directly treated by RT, both from the perspective  
616 of surviving cancer cells (panel (A)) and the response of the CTLs of the  
617 immune system (panel (B)). An initial RT with no follow-up has the only  
618 effect of delaying an exponential rebound, not dissimilar to the behaviour  
619 of an untreated mass. Boosting alone does not impact the mass as much  
620 as when we combine boosting and RT, with the former giving rise to a  
621 tumour that after 30 days has less than half the mass than in the case of  
622 chemotherapy alone. Stimulated by the release of the antigens of the dead  
623 tumour cells of the primary lesion, both therapies show a maximum in the  
624 number of active CTLs, which occurs around day 7 and is then followed  
625 by a characteristic drop around day 10. Qualitatively the results of the  
626 model reproduce the experimental data in Ref. [69], with indications of a  
627 start of a remission for the secondary tumour.

628 Different strategies on the secondary mass also lead to different clonal  
629 compositions, which have an effect on the final outcome. One of the keys  
630 for the success of the strategy is to have no phenotype prevailing over  
631 the others, as shown in panel (C) for the combination of RT and immune  
632 boosting and, to a lesser extent, in panel (D) for immune boosting alone.  
633 *Note that the width of the coloured regions in panels (C)-(F) indicates the number*  
634 *of cells that belong to a specific clone population: the larger the width, the larger*  
635 *the population.* For example, combination therapy provides a very high  
636 Shannon index,  $H > 0.99$ , throughout the whole duration of the experi-  
637 ment. RT and no treatment show instead reduced indices, with values at  
638 day 30 of 0.43 and 0.55 respectively. Interestingly, the RT case appears to be  
639 less heterogeneous than the control case. Overall, it is important to stress  
640 that, for the case of the secondary lesion, high heterogeneity is not per se  
641 associated to a worse prognosis. The reason is that a successful action on  
642 the secondary mass reduces the fitness advantage of the phenotypes and  
643 makes the immune system able to recognise each clones equally. *Note that*  
644 *Panel (E) refers to the radiotherapy case made on primary tumour, showing the*  
645 *evolution of the secondary mass and the fact that the immunogenic effects induced*  
646 *by the treatment are not sufficient to contrast cancer growth. Panel (F) represents*  
647 *the control case, where no treatments are administered and the tumour is growing*  
648 *unchallenged as an aggressive breast cancer.*

649 We find that the relation between the phenotypical composition of the  
650 primary and secondary lesions plays a very important role in the dynam-  
651 ics of the so-called abscopal effect. The previous examples refer to a sec-  
652 ondary lesion that is antigenically related to the first tumour, but this is not

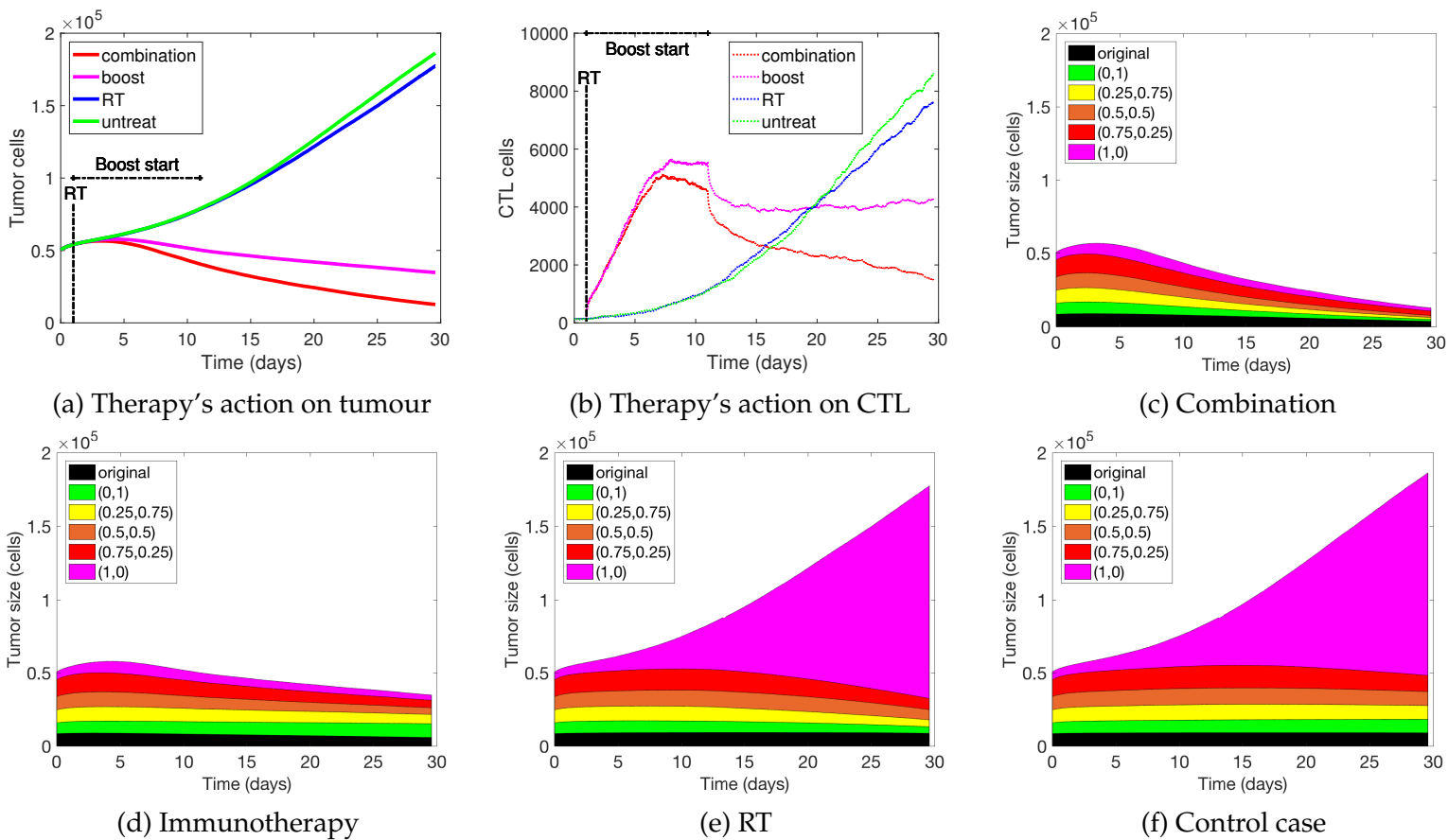


Figure 6: **Effects of anti-cancer treatments on a secondary tumour.** Panel (A and B): Cellular populations of a secondary tumour as functions of time, with cancer populations depicted by a continuous line (panel (A)) and CTLs population by a dashed line (panel (B)). Each color corresponds to a different treatment. *These curves represent a typical simulation run. Variability due to stochasticity is minimal.* Other panels show phenotypical composition of the secondary tumour for the curves shown in Panels (A)-(B)) and for the following strategies applied the primary tumour: combination therapy (panel (C)), immune boosting (panel (D)), radiotherapy (panel (E)), no treatment (panel (F)). Each color indicates a different cancer clone family. *Legends show labels for such families, whose properties are described in detail in Table 3.*

653 always the case in practice. Results vary considerably if the phenotypical  
654 compositions differ and this is important to stress.

655 For example, if the secondary tumour is characterised by clones that  
656 are not antigenically related to the first lesion, the final outcome of combi-  
657 nation therapy cannot be as positive as in the previously discussed cases.  
658 Given that radiotherapy affects phenotypes that are distributed in differ-  
659 ent ways in the first and second tumours, the immune system is not capa-  
660 ble of recognising specific tumour cells in the same successful way as in  
661 the previous examples. As a result, the therapy shows a worse outcome,  
662 as can be seen in Fig. 7(a). Furthermore, if the second tumour mass is in-  
663 stead implanted in an immune suppressed host (mathematically obtained  
664 by setting  $\alpha = 10^{-15}$ ), where the level of CTLs circulating around the tu-  
665 mour is lower than ordinary levels, the outcome is negative. As reported  
666 in Fig. 7(b), a reduced fitness of the immune system causes one pheno-  
667 type to prevail over the others and proliferate quickly out of control. It is  
668 reasonable to suppose that, if more cycles of therapies are repeated, the  
669 effectiveness of treatments is likely to be also reduced.

670

#### 671 *Mutation rates and eradication of secondary tumours*

672 A complete eradication of a secondary tumour as an indirect result  
673 of an anti-cancer therapy on a primary lesion is a relatively rare occur-  
674 rence. Also, it appears to be associated mainly with certain types of cancer,  
675 namely melanoma or breast cancer. This might be linked to the fact that  
676 generic metastatic events are characterised by a high genetic instability, of-  
677 ten making secondary lesions phenotypically unrelated to the first tumour.  
678 From this point of view, a possible speculation could be that the so-called  
679 abscopal effect is not a rare event per se, but it is an effect limited to sec-  
680 ondary tumours that have a phenotypical clone composition that is not  
681 too dissimilar from the originally treated lesion, and thus the effect only  
682 seldom changes the prognosis for secondary lesions. Indeed, extensive  
683 genetic and phenotypic variation are known to strongly influence thera-  
684 peutic outcomes [70].

685 To investigate how the rate of mutation of cancer cells affects outcomes  
686 on secondary lesions, we generate a tumour of  $5 \times 10^4$  cells with a full  
687 mutation capability and apply a combination therapy (RT and immune  
688 boosting) as per the previously introduced protocols. In other words, the  
689 complete secondary lesion before the start of treatments is composed by

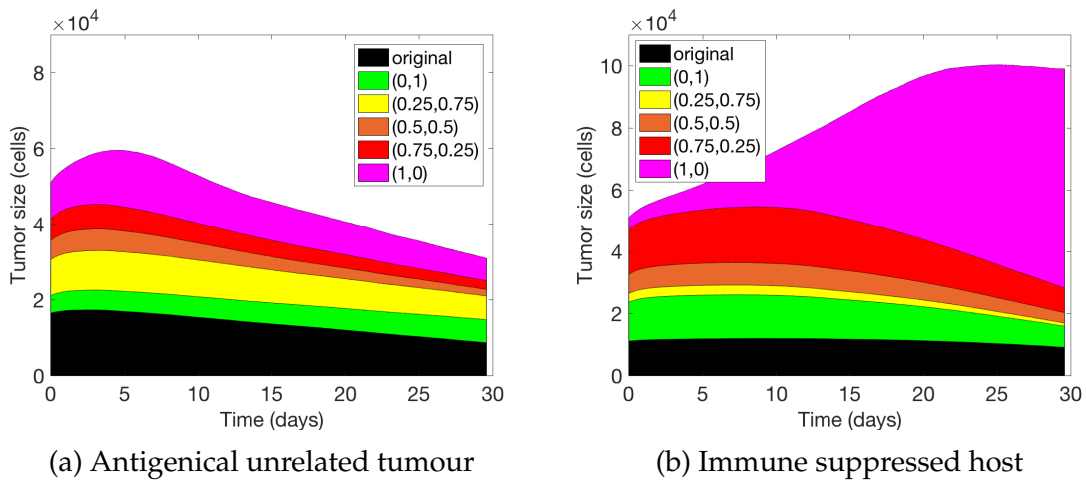
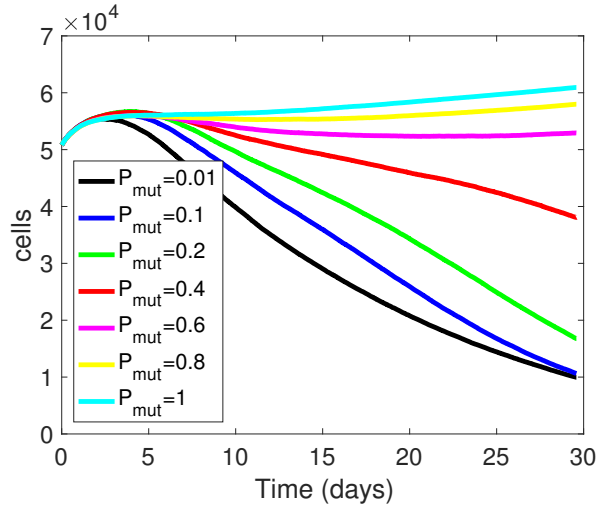


Figure 7: **Immune mediation on a secondary lesion.** Tumour size and composition as functions of time for three different secondary tumours, subjected to different anti-tumour strategies on the primary tumour. Panel (A) shows results for a secondary tumour antigenically unrelated to the primary and panel (B) shows the outcome for a secondary tumour that is antigenically related to the primary tumour but implanted in an immune-suppressed host. *Legends show labels for the tumour subpopulations emerging from the original population, via secondary mutations caused by the therapies. Properties of each clone family are described in detail in Table 3.*

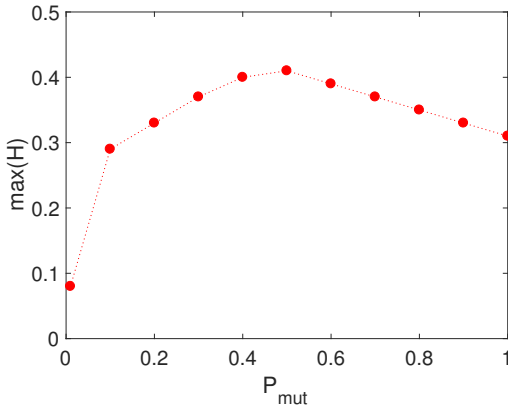
690 the *original* clone. Typical results are reported in Fig 8(a) and show that  
691 outcomes do not linearly depend on the rate of mutation. Interestingly,  
692 tumour reductions at day 30 are larger when the mutation rate is lower,  
693 but become negligible when the rate of mutation is approximately larger  
694 than  $P_{\text{mut}} = 0.6$ , with no relevant change in the overall outcome for higher  
695 rates (see purple, yellow and cyan lines). Also, for rates lower than  $P_{\text{mut}} =$   
696 0.1 (see blue and black lines), different dynamics of eradication can be  
697 present, with tumours having different cell counts after the treatment is  
698 administered, although the final result at day 30 appears almost identical.

699 Heterogeneity tends to increase with the mutation rate, but its max-  
700 imum value during therapy is not directly proportional to how fast the  
701 system can mutate. After a given rate of mutation, which is approximately  
702  $P_{\text{mut}} \approx 0.5 - 0.6$ , mutations do not increase the heterogeneity of the mass.  
703 This is because the fittest clone usually becomes dominant, its cells out-  
704 number the other phenotypes and heterogeneity reduces. Fig. 8(b) shows  
705 that the maximum values of  $H$  are a concave function of mutation proba-  
706 bility. Also, the overall distribution of phenotypes in the secondary mass  
707 is, as discussed previously for the case of a single cancer, strongly affected  
708 by the morphology.

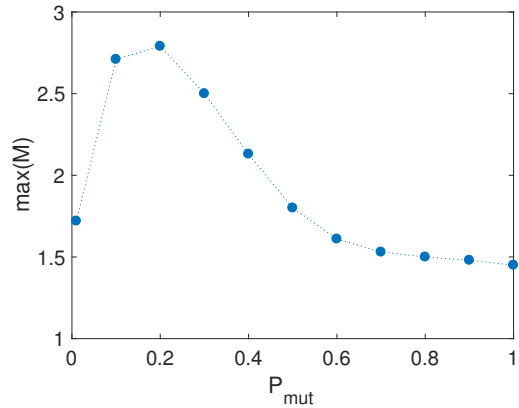
709 Different rates induce different roughness as shown in Fig. 8(c), where  
710 the maximum value of  $M$  reached by the tumour mass is plotted as a func-  
711 tion of mutation rate. This value increases for small rates and reaches a  
712 maximum for a rate  $P_{\text{mut}} = 0.2$ , followed by a sharp decrease. For rates  
713 larger than  $P_{\text{mut}} = 0.5$ , the value does not change significantly. Tumour  
714 morphology of the secondary lesion influences how the immune system  
715 progresses in its attack. In our simulations, we observe different dynamics  
716 of attack carried out by the immune system, with the tumour being eroded  
717 in different ways and often not in a homogeneous fashion. Nonetheless,  
718 a rougher tumour always appears more vulnerable to immune system at-  
719 tacks because of the degree of infiltration by CTLs it allows. Even in “ab-  
720 scopal” positive outcomes, infiltration plays a major role in the dynamics  
721 of erosion and high  $M$  correlates with better results. At the highest mu-  
722 tation rates, roughness is low because the immune system is not able to  
723 recognise phenotypes that are different from those of the primary mass  
724 and kill them. This results in a fast, unbounded growth of one or two  
725 phenotypes that increase the sphericity of the mass and quickly lower the  
726 roughness value approximately to unity, which corresponds to a spherical  
727 object. This is reflected in the plateau observed in Fig. 8 for  $P_{\text{mut}} > 0.5$ .



(a)



(b)  $\max(H(P_{mut}))$



(c)  $\max(M(P_{mut}))$

Figure 8: **Dependence of eradication of the secondary lesion from its mutation rate.** Results for secondary lesions with different mutation rates, when a combination therapy is administered to the first lesion. Panel (A): Total cancer population as function of time for different  $P_{mut}$ . At day 0, the secondary tumour mass is phenotypically identical to the primary lesion. Panel (B) and (C): Maximum values of the Shannon index  $H$  and of the roughness  $M$  as functions of the mutation rate. Points in the Figure represent results from single simulations. Maxima in panels (B) and (C) have been taken over time for each rate of mutation for the clones.

728 **4. Discussion**

729 Cancer and immune cells are complex systems with different charac-  
730 teristics that also depend on internal and external evolutionary pressures.  
731 In the last decades, improvements on the general knowledge of these pro-  
732 cesses have stimulated new therapeutic strategies which take into account  
733 to patients' particularities to some degree. The detailed model of immune  
734 interaction described here focuses on the salient traits of the dynamics and  
735 is able to reproduce the major features of a number of therapeutic inter-  
736 ventions.

737 An analysis of the effect of different drugs on three prototypical sec-  
738 ondary masses arising from a metastatic breast cancer (not modeled) has  
739 been proposed, showing a faithful representation of some of the main  
740 mechanisms of tumour-immune interactions present in literature [20, 9].  
741 In particular, we note a significant dependence of the outcomes on the  
742 heterogeneity of the tumour, with higher heterogeneity generally corre-  
743 lated with negative outcomes confirming biological evidence suggested in  
744 Ref. [70]. Indeed, therapies targeting heterogeneous cancer micro envi-  
745 ronments often show large rebounds of more resistant tumour cells, that  
746 are able to counteract the action of drugs or boosting in a consistent way.  
747 In particular, one original result from our modeling is that chemotherapy  
748 appears more efficient in a less phenotypically differentiated secondary le-  
749 sion independently of the rate of growth or the dimension of the mass. For  
750 the reproductive rates considered in this study, immune boosting alone is  
751 not sufficient to produce full eradication, but rather can trigger the spread  
752 of the more aggressive cells in the body by making the existing mass more  
753 sparse. A well-timed intervention with a combination of boosting and  
754 chemotherapy seems to be the safest of the protocols, allowing for a rele-  
755 vant reduction of the mass and preventing the unbounded growth of the  
756 most proliferating cells. Nevertheless, timing of intervention on the sec-  
757 ondary lesion can be critical.

758 One further result of this work is to uncover the importance of tumour  
759 morphology in evolution and fate of secondary lesions. The shape of sec-  
760 ondary masses conveys important information that could be an indica-  
761 tor of successful eradication. For instance, during or immediately after  
762 administration of chemotherapy, our modeling shows that a high infil-  
763 trated tumour is associated with the best outcome. At the same time, a  
764 harsh environment or a high selective pressure tend to generate a tumour

765 that has a greater roughness and the tendency to spread, as previously  
766 noted [8]. This often occurs and persists for many days after adminis-  
767 tration of chemotherapy. In particular, findings for case A suggest that  
768 clonal composition of surviving cells that originated from the beginning  
769 tumour colony, and are later influenced by the selected therapies, strongly  
770 affect the final outcomes of the metastasis. Similar dynamics is reported  
771 to be present in some types of tumours that are known to be particularly  
772 resistant to therapies [71].

773 Here we consider one cycle of treatment, but some extra *care* should  
774 be taken when multiple cycles are considered, since the immune system  
775 could be further weakened and respond less efficiently. On the other hand,  
776 there is evidence in the medical literature that a combination of radiother-  
777 apy and immunotherapy can provide a positive effect not only in the area  
778 affected by the radiation but also in other areas of the body. This seems  
779 to be due to the release of autologous neoantigens to the immune system  
780 [69], with the overall result of what it appears to be an individualised tu-  
781 mour vaccine. Our model captures the effects of this cascading action on  
782 a secondary metastatic mass and confirms that the immune system can  
783 act as a mediator for secondary attacks. In particular, there is evidence in  
784 our findings that immune-suppressed hosts or secondary lesions antigeni-  
785 cally unrelated to the treatment area do not show any abscopal effect, as  
786 experimentally noted.

787 There is a growing discussion in the community about the causes of  
788 this rare, positive occurrence on secondary masses. Currently, it seems  
789 that this is the result of a fragile balance between positive and negative  
790 signals activated with the radiation, dependent on the pre-existing envi-  
791 ronment and the immunogenicity of the tumour [69]. These biological  
792 elements, alongside a critical dependence on the dose and the interval be-  
793 tween radiation fractions, contribute to the low occurrence of this effect.  
794 Furthermore and interestingly, a dependence of the effect on the mutation  
795 rate of the cancer clones in the secondary site is apparent, suggesting that  
796 the role of genetic instability at that site should be investigated more.

797 Overall, our findings emphasises that the morphology of the secondary  
798 lesions before, during and after the treatment, bears some indications of  
799 the rate of success for the treatment. For the lesions under the detectability  
800 threshold, this work suggests that heterogeneity and roughness are both  
801 important quantities. Negative prognosis is linked to the selection of a  
802 poorly immunogenic clone and has been shown to lead to a large, un-

803 bounded regrowth of the tumour. It is thus vital to design a protocol that  
804 can minimize the immunoediting ability of cells surviving from therapies  
805 or improve the immune system ability to recognise and attacks such clones  
806 especially when we cannot detect individual, isolated lesions but only to-  
807 tal tumour burden. In regards to the latter, the activation of an “abscopal-  
808 like” response seems to be a strategy in re-calibrating the immune reaction  
809 to such cells. Results are still in their infancy and it is unknown whether  
810 such a response can be elicited and how. One of the ideas we suggest is to  
811 carefully consider the best tumour target to be irradiated: when possible,  
812 it could be advantageous to target a secondary metastasis antigenically re-  
813 lated to the more common lesions in the body, with a low grade of hypoxia  
814 and with a good grade of immunogenicity.

## 815 5. Conclusions

816 Cellular mutation constitutes one of the causes of negative outcomes  
817 in therapeutic strategies against cancer. Morphology, growth rates and  
818 the clonal composition of a tumour mass can, to some extent, be used as  
819 predictors of tumour resistance to a range of anti-cancer therapies and be  
820 analysed to combine treatments to maximize their impacts. In most of  
821 the commonly used protocols, the action of the immune system is cru-  
822 cial. Modern therapies elicit and enhance patient’s immune response, also  
823 because of its ability to adapt to, change and modify the tumour microen-  
824 vironment.

825 The mathematical model we have presented tries to capture the com-  
826 plexity of tumour-immune dynamics and discuss how therapies with dif-  
827 ferent scopes, doses and protocols can influence prognosis for small, solid,  
828 secondary tumoural lesions. *Even if these lesions can be small and not yet de-*  
829 *detectable, their role can be catastrophic for the patient if they are untreated or, as*  
830 *in some cases we have shown, the effect of therapies on the primary tumour can*  
831 *lead to the selection of more aggressive and resilient clones in the secondary le-*  
832 *sions. Ideally, individual, evidence-based modelling might provide a fast,*  
833 *reliable and patient-centred way to test and find optimum control of pro-*  
834 *ocols in vivo.*

835 Our findings suggest that the success of synergistic therapies is strongly  
836 influenced by the phenotypical composition of all the lesions, alongside  
837 their mutation rates and immunogenic properties. Effective strategies that  
838 can “normalise” the microenvironment [72] and will try to limit tumour

839 clonal mutation could be trialled to improve prognosis. Therapies that tar-  
840 get slowly proliferating and undifferentiated cells can also become viable  
841 in the future.

## 842 **6. Acknowledgements**

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844 the Australian Research Council Discovery Project DP180101512, Dynam-  
845 ical systems theory and mathematical modelling of viral infections.

## 846 **7. Code and Data availability**

847 The code used and the produced data for the simulations discussed in  
848 this work are available upon request.

## **References**

- [1] D. Hanahan, R. A. Weinberg, Hallmarks of cancer: the next generation, *Cell* 144 (2011) 646–674.
- [2] D. P. Tabassum, K. Polyak, Tumorigenesis: it takes a village, *Nature Reviews Cancer* 15 (2015) 473–483.
- [3] M. Gerlinger, C. Swanton, How Darwinian models inform therapeutic failure initiated by clonal heterogeneity in cancer medicine, *British Journal of Cancer* 103 (2010) 1139–1143.
- [4] A. Sottoriva, H. Kang, Z. Ma, T. A. Graham, M. P. Salomon, J. Zhao, P. Marjoram, K. Siegmund, M. F. Press, D. Shibata, et al., A big bang model of human colorectal tumor growth, *Nature Genetics* 47 (2015) 209.
- [5] A. Rübber, A. Araujo, Cancer heterogeneity: converting a limitation into a source of biologic information, *Journal of Translational Medicine* 15 (2017) 190.
- [6] J. M. Pacheco, F. C. Santos, D. Dingli, The ecology of cancer from an evolutionary game theory perspective, *Interface Focus* 4 (2014) 20140019.

- [7] T. Hillen, M. A. Lewis, *Mathematical ecology of cancer*, in: *Managing Complexity, Reducing Perplexity*, Springer, 2014, pp. 1–13.
- [8] A. R. Anderson, A. M. Weaver, P. T. Cummings, V. Quaranta, *Tumor morphology and phenotypic evolution driven by selective pressure from the microenvironment*, *Cell* 127 (2006) 905–915.
- [9] L. G. De Pillis, W. Gu, A. E. Radunskaya, *Mixed immunotherapy and chemotherapy of tumors: modeling, applications and biological interpretations*, *Journal of Theoretical Biology* 238 (2006) 841–862.
- [10] S. Wilson, D. Levy, *A mathematical model of the enhancement of tumor vaccine efficacy by immunotherapy*, *Bulletin of Mathematical Biology* 74 (2012) 1485–1500.
- [11] A. D. Asatryan, N. L. Komarova, *Evolution of genetic instability in heterogeneous tumors*, *Journal of theoretical biology* 396 (2016) 1–12.
- [12] A. Sottoriva, L. Vermeulen, S. Tavaré, *Modeling evolutionary dynamics of epigenetic mutations in hierarchically organized tumors*, *PLoS Computational Biology* 7 (2011) e1001132.
- [13] A. R. Anderson, V. Quaranta, *Integrative mathematical oncology*, *Nature Reviews Cancer* 8 (2008) 227.
- [14] J. Jeon, V. Quaranta, P. T. Cummings, *An off-lattice hybrid discrete-continuum model of tumor growth and invasion*, *Biophysical Journal* 98 (2010) 37–47.
- [15] Y. Jiao, S. Torquato, *Diversity of dynamics and morphologies of invasive solid tumors*, *AIP advances* 2 (2012) 011003.
- [16] R. A. Gatenby, A. S. Silva, R. J. Gillies, B. R. Frieden, *Adaptive therapy*, *Cancer Research* 69 (2009) 4894–4903.
- [17] R. A. Gatenby, J. Brown, T. Vincent, *Lessons from applied ecology: cancer control using an evolutionary double bind*, *Cancer research* 69 (2009) 7499–7502.
- [18] A. Eladdadi, A. Radunskaya, et al., *Modeling cancer-immune responses to therapy*, *Journal of pharmacokinetics and pharmacodynamics* 41 (2014) 461–478.

- [19] F. Frascoli, P. S. Kim, B. D. Hughes, K. A. Landman, A dynamical model of tumour immunotherapy, *Mathematical Biosciences* 253 (2014) 50–62.
- [20] E. Piretto, M. Delitala, M. Ferraro, Combination therapies and intratumoral competition: insights from mathematical modelling, *Journal of Theoretical Biology* 446 (2018) 149–159.
- [21] S. Bunimovich-Mendrazitsky, H. Byrne, L. Stone, Mathematical model of pulsed immunotherapy for superficial bladder cancer, *Bulletin of Mathematical Biology* 70 (2008) 2055–2076.
- [22] A. L. Jenner, C.-O. Yun, A. Yoon, A. C. Coster, P. S. Kim, Modelling combined virotherapy and immunotherapy: strengthening the anti-tumour immune response mediated by IL-12 and GM-CSF expression, *Letters in Biomathematics* (2018) 1–18.
- [23] N. Bellomo, N. Li, P. K. Maini, On the foundations of cancer modelling: selected topics, speculations, and perspectives, *Mathematical Models and Methods in Applied Sciences* 18 (2008) 593–646.
- [24] K. P. Wilkie, A review of mathematical models of cancer-immune interactions in the context of tumor dormancy, in: *Systems Biology of Tumor Dormancy*, Springer, 2013, pp. 201–234.
- [25] R. Eftimie, J. L. Bramson, D. J. Earn, Interactions between the immune system and cancer: a brief review of non-spatial mathematical models, *Bulletin of Mathematical Biology* 73 (2011) 2–32.
- [26] A. Eladdadi, P. Kim, D. Mallet, *Mathematical models of tumor-immune system dynamics*, Springer, 2014.
- [27] P. S. Kim, P. P. Lee, Modeling protective anti-tumor immunity via preventative cancer vaccines using a hybrid agent-based and delay differential equation approach, *PLoS Computational Biology* 8 (2012) e1002742.
- [28] D. M. Catron, A. A. Itano, K. A. Pape, D. L. Mueller, M. K. Jenkins, Visualizing the first 50 hr of the primary immune response to a soluble antigen, *Immunity* 21 (2004) 341–347.

- [29] H. Mohri, A. S. Perelson, K. Tung, R. M. Ribeiro, B. Ramratnam, M. Markowitz, R. Kost, L. Weinberger, D. Cesar, M. K. Hellerstein, et al., Increased turnover of t lymphocytes in hiv-1 infection and its reduction by antiretroviral therapy, *Journal of Experimental Medicine* 194 (2001) 1277–1288.
- [30] G. T. Belz, L. Zhang, M. D. Lay, F. Kupresanin, M. P. Davenport, Killer t cells regulate antigen presentation for early expansion of memory, but not naive, cd8+ t cell, *Proceedings of the National Academy of Sciences* 104 (2007) 6341–6346.
- [31] D. Wodarz, A. R. Thomsen, Effect of the ctl proliferation program on virus dynamics, *International Immunology* 17 (2005) 1269–1276.
- [32] R. J. De Boer, M. Oprea, R. Antia, K. Murali-Krishna, R. Ahmed, A. S. Perelson, Recruitment times, proliferation, and apoptosis rates during the cd8+ t-cell response to lymphocytic choriomeningitis virus, *Journal of Virology* 75 (2001) 10663–10669.
- [33] S. M. Kaech, R. Ahmed, Memory cd8+ t cell differentiation: initial antigen encounter triggers a developmental program in naive cells, *Nature Immunology* 2 (2001) 415.
- [34] R. J. De Boer, D. Homann, A. S. Perelson, Different dynamics of cd4+ and cd8+ t cell responses during and after acute lymphocytic choriomeningitis virus infection, *The Journal of Immunology* 171 (2003) 3928–3935.
- [35] C. A. Janeway, P. Travers, M. Walport, M. Shlomchik, et al., *Immunobiology: the immune system in health and disease*, volume 7, Current Biology London, 1996.
- [36] H. Veiga-Fernandes, U. Walter, C. Bourgeois, A. McLean, B. Rocha, Response of naive and memory cd8+ t cells to antigen stimulation in vivo, *Nature Immunology* 1 (2000) 47.
- [37] M. Ying, B. Pang, Three-dimensional ultrasound measurement of cervical lymph node volume, *The British journal of radiology* 82 (2009) 617–625.

- [38] D. G. Mallet, L. G. De Pillis, A cellular automata model of tumor-immune system interactions, *Journal of Theoretical Biology* 239 (2006) 334–350.
- [39] R. Soiffer, F. S. Hodi, F. Haluska, K. Jung, S. Gillessen, S. Singer, K. Tanabe, R. Duda, S. Mentzer, M. Jaklitsch, et al., Vaccination with irradiated, autologous melanoma cells engineered to secrete granulocyte-macrophage colony-stimulating factor by adenoviral-mediated gene transfer augments antitumor immunity in patients with metastatic melanoma, *Journal of Clinical Oncology* 21 (2003) 3343–3350.
- [40] R. Soiffer, T. Lynch, M. Mihm, K. Jung, C. Rhuda, J. C. Schmollinger, F. S. Hodi, L. Liebster, P. Lam, S. Mentzer, et al., Vaccination with irradiated autologous melanoma cells engineered to secrete human granulocyte-macrophage colony-stimulating factor generates potent antitumor immunity in patients with metastatic melanoma, *Proceedings of the National Academy of Sciences* 95 (1998) 13141–13146.
- [41] C. R. Mackay, Chemokine receptors and t cell chemotaxis., *Journal of Experimental Medicine* 184 (1996) 799–802.
- [42] M. Maurer, E. Von Stebut, Macrophage inflammatory protein-1, *The International Journal of Biochemistry & Cell Biology* 36 (2004) 1882–1886.
- [43] A. H. Lin, A model of tumor and lymphocyte interactions, *Discrete & Continuous Dynamical Systems-B* 4 (2004) 241–266.
- [44] T. Alarcón, H. M. Byrne, P. K. Maini, A cellular automaton model for tumour growth in inhomogeneous environment, *Journal of Theoretical Biology* 225 (2003) 257–274.
- [45] D. Kirschner, J. C. Panetta, Modeling immunotherapy of the tumor-immune interaction, *Journal of mathematical biology* 37 (1998) 235–252.
- [46] T. Kuroishi, S. Tominaga, T. Morimoto, H. Tashiro, S. Itoh, H. Watanabe, M. Fukuda, J. Ota, T. Horino, T. Ishida, et al., Tumor growth rate and prognosis of breast cancer mainly detected by mass screening, *Japanese Journal of Cancer Research* 81 (1990) 454–462.

- [47] J. Michaelson, S. Satija, R. Moore, G. Weber, E. Halpern, A. Garland, D. B. Kopans, Estimates of breast cancer growth rate and sojourn time from screening database information, *Journal of Womens Imaging* 5 (2003) 11–19.
- [48] P. Friedl, M. Gunzer, Interaction of t cells with apcs: the serial encounter model, *Trends in Immunology* 22 (2001) 187–191.
- [49] L. D. Wood, D. W. Parsons, S. Jones, J. Lin, T. Sjöblom, R. J. Leary, D. Shen, S. M. Boca, T. Barber, J. Ptak, et al., The genomic landscapes of human breast and colorectal cancers, *Science* 318 (2007) 1108–1113.
- [50] H. Hatzikirou, D. Basanta, M. Simon, K. Schaller, A. Deutsch, Go or grow: the key to the emergence of invasion in tumour progression?, *Mathematical Medicine and Biology: a Journal of the IMA* 29 (2012) 49–65.
- [51] A. M. Mendonsa, T.-Y. Na, B. M. Gumbiner, E-cadherin in contact inhibition and cancer, *Oncogene* (2018) 1.
- [52] L. L. Brunton, B. A. Chabner, B. C. Knollmann, *Therapeutics*, *MWateria Medica*, x887, 6ox (2011).
- [53] L. Zitvogel, L. Apetoh, F. Ghiringhelli, G. Kroemer, Immunological aspects of cancer chemotherapy, *Nature Reviews Immunology* 8 (2008) 59–73.
- [54] S. A. Rosenberg, N. P. Restifo, J. C. Yang, R. A. Morgan, M. E. Dudley, Adoptive cell transfer: a clinical path to effective cancer immunotherapy, *Nature Reviews Cancer* 8 (2008) 299.
- [55] F. Frascoli, E. Flood, P. Kim, A model of the effects of cancer cell motility and cellular adhesion properties on tumour-immune dynamics, *Mathematical Medicine and Biology : a journal of the IMA* 34 (2017) 215–240.
- [56] A. Sottoriva, J. J. Verhoeff, T. Borovski, S. K. McWeeney, L. Naumov, J. P. Medema, P. M. Slood, L. Vermeulen, Cancer stem cell tumor model reveals invasive morphology and increased phenotypical heterogeneity, *Cancer Research* 70 (2010) 46–56.

- [57] A. Bru, J. Pastor, I. Fernaud, I. Br, S. Melle, C. Berenguer, Super-rough dynamics on tumor growth, *Physical Review Letters* 81 (1998) 4008–4011.
- [58] D. Basanta, R. A. Gatenby, A. R. Anderson, Exploiting evolution to treat drug resistance: combination therapy and the double bind, *Molecular Pharmaceutics* 9 (2012) 914–921.
- [59] R. Ramakrishnan, D. Assudani, S. Nagaraj, T. Hunter, H.-I. Cho, S. Antonia, S. Altiok, E. Celis, D. I. Gabrilovich, Chemotherapy enhances tumor cell susceptibility to CTL-mediated killing during cancer immunotherapy in mice, *The Journal of Clinical Investigation* 120 (2010) 1111.
- [60] S. J. Antonia, N. Mirza, I. Fricke, A. Chiappori, P. Thompson, N. Williams, G. Bepler, G. Simon, W. Janssen, J.-H. Lee, et al., Combination of p53 cancer vaccine with chemotherapy in patients with extensive stage small cell lung cancer, *Clinical Cancer Research* 12 (2006) 878–887.
- [61] M. Greaves, C. C. Maley, Clonal evolution in cancer, *Nature* 481 (2012) 306–313.
- [62] E. A. Reits, J. W. Hodge, C. A. Herberts, T. A. Groothuis, M. Chakraborty, E. K. Wansley, K. Camphausen, R. M. Luiten, A. H. de Ru, J. Neijssen, et al., Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy, *Journal of Experimental Medicine* 203 (2006) 1259–1271.
- [63] A. A. Lugade, J. P. Moran, S. A. Gerber, R. C. Rose, J. G. Frelinger, E. M. Lord, Local radiation therapy of B16 melanoma tumors increases the generation of tumor antigen-specific effector cells that traffic to the tumor, *The Journal of Immunology* 174 (2005) 7516–7523.
- [64] S. E. Finkelstein, M. Fishman, Clinical opportunities in combining immunotherapy with radiation therapy, *Frontiers in Oncology* 2 (2012) 169.
- [65] S. E. Finkelstein, C. Iclozan, M. M. Bui, M. J. Cotter, R. Ramakrishnan, J. Ahmed, D. R. Noyes, D. Cheong, R. J. Gonzalez, R. V. Heysek,

- et al., Combination of external beam radiotherapy (EBRT) with intratumoral injection of dendritic cells as neo-adjuvant treatment of high-risk soft tissue sarcoma patients, *International Journal of Radiation Oncology Biology Physics* 82 (2012) 924–932.
- [66] S. E. Finkelstein, R. Timmerman, W. H. McBride, D. Schaefer, S. E. Hoffe, C. A. Mantz, G. D. Wilson, The confluence of stereotactic ablative radiotherapy and tumor immunology, *Clinical and Developmental Immunology* 2011 (2011).
- [67] R. E. Vatner, B. T. Cooper, C. Vanpouille-Box, S. Demaria, S. C. Formenti, Combinations of immunotherapy and radiation in cancer therapy, *Frontiers in Oncology* 4 (2014) 325.
- [68] S. Demaria, B. Ng, M. L. Devitt, J. S. Babb, N. Kawashima, L. Liebes, S. C. Formenti, Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated, *International Journal of Radiation Oncology Biology Physics* 58 (2004) 862–870.
- [69] S. Demaria, E. B. Golden, S. C. Formenti, Role of local radiation therapy in cancer immunotherapy, *JAMA oncology* 1 (2015) 1325–1332.
- [70] R. A. Burrell, N. McGranahan, J. Bartek, C. Swanton, The causes and consequences of genetic heterogeneity in cancer evolution, *Nature* 501 (2013) 338–345.
- [71] A. Kusoglu, C. Biray Avci, Cancer stem cells: A brief review of the current status, *Gene* 681 (2019) 80–85.
- [72] R. K. Jain, Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy, *Science* 307 (2005) 58–62.

Table 1: Parameters used in the DDE component of the model. For more details about parameter estimation refer to [27]

Par.	Description	Value (range)	Ref.	Notes/comments
$A_0(0)$	Initial concentration of immature APCs	0.01 k/mm <sup>3</sup>	[28]	same order of magnitude as the APCs in the lymph node
$d_0$	Death/turnover rate of immature APCs	0.03 day <sup>-1</sup>	[29]	similar to those of naïve T cells
$s_A$	Supply rate of immature APCs	0.3 k/mm <sup>3</sup> day <sup>-1</sup>	[29]	$s_A = A_0(0)d_0$
$d_1$	Death/turnover rate of mature APCs	0.8 day <sup>-1</sup>	[30]	using a half-life of 20 h: $d_1 = (\ln 2)/20 \text{ h}^{-1}$
$K$	Equilibrium concentration of memory CTLs	2% · 200 k/mm <sup>3</sup>	[28]	2% of the T-cells in a lymph node of radius 1 mm
$r$	Logistic growth rate of memory CTLs	log 2 day <sup>-1</sup>	[27]	minimum doubling time of 1 day
$m$	Minimal number of CTL divisions	10	[31]	range from 7 to 17 cell divisions [32, 33]
$\delta_1$	Death/turnover rate of effector CTLs	0.4 day <sup>-1</sup>	[34]	half-life during T-cell contraction of 41 h: $\delta_1 = (\ln 2)/41 \text{ h}^{-1}$
$\mu$	Mass-action coefficient	20 (k/mm <sup>3</sup> ) <sup>-1</sup> day <sup>-1</sup>	[28]	$\mu = 0.5\mu_0$ with $\mu_0 = 4.8\text{cell}^{-1} \text{ day}^{-1}$ and $V_{\text{lymph node}} = 8.4 \cdot 10^{-3} \text{ mm}^3$
$\rho$	Duration of one CTL division	8 h	[35]	T-cell doubles every 8 hours during expansion [34]
$\sigma$	Duration of CTL division program	$1 + (m - 1)\rho$	[36]	first division does not occur until 24 hours after stimulation
$\alpha$	Antigenicity of the tumour	$10^{-9} \text{ (k/mm}^3\text{)}^{-1}\text{day}^{-1}$	[27]	reciprocal of the rate of encountering of antigen from a tumor cell by APC
$f$	CTLs flow rate out of lymph node to tissue	0.7 day <sup>-1</sup>	[27]	effector CTLs emigrate at a half-life of 1 day: $f = \ln 2 \text{ day}^{-1}$
$V_{\text{ratio}}$	Ratio of volume of tissue to the lymph node	1000	[37]	lymph node compartment is $\sim 1 \text{ ml}$ and the breast tissue $\sim 1 \text{ l}$

Table 2: Parameters used in the ABM component of the model. For more details about parameter estimation refer to [27]

Par.	Description	Value (range)	Ref.	Notes / Comments
$\Delta t$	Time step	1 min	[27]	timescale of the fastest dynamic simulated in the model
$r$	Radius of cells	5 $\mu\text{m}$	[43]	[38, 44, 28]
$T_{\text{div},i}$	Avg. division time of i-th cancer phenotype	1-39 day	[45]	[38, 46, 47]
$T_0$	Avg. division time of <i>original</i> phenotype	7 day	[45]	[38, 46, 47]
$\sigma_{\text{max}}$	Max unit standard deviation of CTL diffusion	12 $\mu\text{m min}^{-1}$	[28]	[48]
$C_{\text{acc}}$	CTL acceleration time from 0 to $\sigma_{\text{max}}$	5 h	[27]	
$C_{\text{death}}$	Avg. CTL lifespan	41 h	[34]	
$C_{\text{recruit}}$	Avg. time fro CTL recruitment	22 h	[27]	[39, 40]
$C_{\text{kill}}$	Avg. time fro CTL to kill tumour cell	24 h	[27]	killing target cells may require a long recovery period
$R$	Radius of region of interest	620.4 $\mu\text{m}$	[27]	
$h$	Thickness of CTL cloud	$3\sigma_{\text{max}}\sqrt{\Delta t}$	[27]	probability that a CTL could pass from outside into the region of interest is 0.001
$P_{\text{mut}}$	Probability of mutation	0.01 $\text{min}^{-1/2}$	[49]	
$P_{\text{recog},i}$	Probability of recognition of i-th cancer phenotype	0-1		spans the entire probability range
$P_0$	Probability of recognition of <i>original</i> phenotype	1		the APC cell can always recognize the antigen released

Table 3: **Cancer clonal composition**

Name	Description	$P_{\text{recog}}$	$T_{\text{div}}$
<i>original</i>	First metastatic breast cancer clone that colonises the new tissue, with evolutionary potential of phenotypic mutations.	$1 \text{ min}^{-1}$	7 day
(0.5,0.5)	Clone proliferates at the same rate of the <i>original</i> clone, but has an increased ability to hide from the immune system.	$0.5 \text{ min}^{-1}$	7 day
(0,1)	Clone is not recognised by CTLs, but the evolutionary cost of its ability is paid in term of proliferation: this phenotype is the slowest to reproduce.	$0 \text{ min}^{-1}$	13 day
(0.25,0.75)	Clone has intermediate properties: strong ability to hide and slow proliferation	$0.25 \text{ min}^{-1}$	10 day
(0.75,0.25)	Clone has intermediate properties: weak ability to hide and fast proliferation	$0.75 \text{ min}^{-1}$	4 day
(1,0)	Clone has the ability to reach high number of cellular duplication, but is always recognised by the immune system.	$1 \text{ min}^{-1}$	1 day

Table 4: **Parameters used to model therapies.** Ranges indicate that different therapies (single and combined) are simulated with different values.

Parameter	Description	Value (range)
$Ch_{\text{time}}$	Duration of a chemotherapy cycle	10-50 day
$Ch_{\text{eff}}$	Effect of chemotherapy	$0 - 1/4$
$Bo_{\text{time}}$	Persistence time of boosting (TIL)	3 day
$Bo_{\text{eff}}$	Number of CTL cells injected	500-1000