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Emergent properties of a computational model of tumour growth

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While there have been enormous advances in our understanding of the genetic drivers and molecular pathways involved in cancer in recent decades, there also remain key areas of dispute with respect to fundamental theories of cancer. The accumulation of vast new datasets from genomics and other fields, in addition to detailed descriptions of molecular pathways, cloud the issues and lead to ever greater complexity. One strategy in dealing with such complexity is to develop models to replicate salient features of the system and therefore to generate hypotheses which reflect on the real system. A simple tumour growth model is outlined which displays emergent behaviours that correspond to a number of clinically relevant phenomena including tumour growth, intra-tumour heterogeneity, growth arrest and accelerated repopulation following cytotoxic insult. Analysis of model data suggests that the processes of cell competition and apoptosis are key drivers of these emergent behaviours. Questions are raised as to the role of cell competition and cell death in physical cancer growth and the relevance that these have to cancer research in general is discussed.

1 **Emergent Properties of a Computational Model of Tumour Growth**

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6 Abstract

7 While there have been enormous advances in our understanding of the genetic drivers and
8 molecular pathways involved in cancer in recent decades, there also remain key areas of dispute
9 with respect to fundamental theories of cancer. The accumulation of vast new datasets from
10 genomics and other fields, in addition to detailed descriptions of molecular pathways, cloud the
11 issues and lead to ever greater complexity. One strategy in dealing with such complexity is to
12 develop models to replicate salient features of the system and therefore to generate hypotheses
13 which reflect on the real system. A simple tumour growth model is outlined which displays
14 emergent behaviours that correspond to a number of clinically relevant phenomena including
15 tumour growth, intra-tumour heterogeneity, growth arrest and accelerated repopulation following
16 cytotoxic insult. Analysis of model data suggests that the processes of cell competition and
17 apoptosis are key drivers of these emergent behaviours. Questions are raised as to the role of cell
18 competition and cell death in physical cancer growth and the relevance that these have to cancer
19 research in general is discussed.

20 Introduction

21 Tumour growth is a complex process characterised by multi-scale phenomena involving both
22 cancer and non-cancer cell populations. Where previously our focus was directed primarily at the
23 activities of the cancer cell populations, once conceptualised as a single homogeneous mass, our
24 increased understanding of cancer biology now incorporates a more nuanced evolutionary or
25 ecological view of cancer growth (Gatenby, Gillies & Brown, 2011; Kareva, 2011). Key
26 elements of this view of cancer as an evolutionary system are a focus on the genetic
27 heterogeneity of tumour cell populations (Fisher, Pusztai & Swanton, 2013; De Sousa E Melo et
28 al., 2013), the importance of the tumour microenvironment and the cross-talk between cancer
29 and non-cancer cell populations (Allen & Louise Jones, 2011; Hanahan & Coussens, 2012; Quail
30 & Joyce, 2013). A concern among some investigators is that in the absence of an evolutionary
31 understanding of population dynamics in cancer, therapeutic interventions may be doomed to
32 failure (Silva & Gatenby, 2010; Tian et al., 2011; Gillies, Verduzco & Gatenby, 2012). In other
33 cases there is interest in understanding the role of the microenvironment in the process of cancer
34 initiation (Pantziarka, 2015) or the metastatic cascade (Psaila et al., 2007; Barcellos-Hoff, Lyden
35 & Wang, 2013).

36 More fundamentally, there are also competing theoretical views of cancer at the most basic level.
37 The predominant view of cancer – termed the somatic mutation theory (SMT) – is that it is a
38 disease caused, and then driven, by genetic mutations in cells. An alternative view – termed the
39 tissue-organisation field theory (TOFT) – views cancer as a disease caused by tissue dysfunction,
40 development gone astray, with genetic changes not as the drivers but as a consequence of the
41 disease. A number of recent publications outline these competing views of cancer (Baker, 2014;
42 Bizzarri & Cucina, 2014; Sonnenschein et al., 2014).

43 A challenge to all fundamental theories of cancer is to incorporate the vast array of new data that
44 molecular biology has afforded to the researcher. The literature expands exponentially as we
45 develop the tools to probe ever deeper into cellular structures, signalling pathways and the large
46 data volumes generated by the various ‘omics. Against this backdrop of ever greater detail it is
47 becoming harder to integrate the data into a coherent ‘big picture’. Robert Weinberg makes the
48 point that we are going full circle – from an initially complex picture of disjointed
49 phenomenological facts to simplifying models arising from the revolution in molecular biology

50 and back to a picture of endless complexity again (Weinberg, 2014). The impacts of this lack of
51 progress are ultimately felt in the clinic, where, with a few significant exceptions, progress in
52 developing treatments has significantly slowed in recent years (Jalali, Mittra & Badwe, 2016).

53 Computational models can provide ideal platforms for developing conceptual understanding of
54 complex biological systems (Saetzler, Sonnenschein & Soto, 2011; Janes & Lauffenburger,
55 2013). A range of techniques are available to build such software models of cancer growth
56 specifically to explore evolutionary or ecological hypotheses at an abstract and non-
57 physiological level, including techniques from evolutionary game theory (Basanta et al., 2008;
58 Krzeslak & Swierniak, 2014) and machine learning (Gerlee, Basanta & Anderson, 2011).

59 For example Ribba et al created a hybrid cellular automaton model which aimed to replicate
60 some features of CHOP therapy for Non-Hodgkin's Lymphoma (NHL) (Ribba et al., 2004). The
61 model was calibrated in such a way as to make specific predictions as to the response of NHL
62 cells to treatment with the chemotherapeutic drug doxorubicin. Gerlee and Anderson developed
63 an evolutionary hybrid cellular automaton model of solid tumour growth to investigate the
64 impact of tissue oxygen concentration on the growth and evolutionary dynamics of a tumour
65 (Gerlee & Anderson, 2007). A key aspect of this model was the calibration of parameters with
66 physically relevant data in terms of oxygen and glucose consumption rates, time estimates for
67 cellular proliferation and so on. Enderling and colleagues have developed a series of hybrid
68 cellular automaton models which include both qualitative and quantitative results related to
69 cancer stem cell theory and tumour growth (Enderling, Hlatky & Hahnfeldt, 2009; Enderling &
70 Hahnfeldt, 2011; Poleszczuk & Enderling, 2016). Closer in intent to this work was the genetic
71 algorithm model developed by Gerlee et al to investigate the evolution of homeostatic tissue in a
72 two-dimensional monolayer system (Gerlee, Basanta & Anderson, 2011).

73 NEATG (Non-physiological Evolutionary Algorithm for Tumour Growth) is a simple software
74 model of tumour growth which models cell-to-cell and tissue-level interactions and population
75 dynamics under different evolutionary scenarios. Furthermore the platform is structured such that
76 anti-tumour interventions can also be modelled within these different scenarios. A number of
77 scenarios are explored in this paper, including the simulation of cellular response to homeostasis,
78 stress conditions, nutrient deprivation and cytotoxic intervention. The approach in this work is
79 primarily qualitative rather than quantitative and does not depend on calibration to physical
80 tumour growth models

81 While computational models enable the construction of *in silico* experiments involving
82 biological systems, they differ from traditional mathematical models (differential and other
83 equation-based systems) in that the model itself is encoded in computer code, input/output file
84 formats, configuration files etc. Therefore, it is important in reporting on such a model that there
85 is exposition not just of the algorithmic details but also an exploration of how the model behaves
86 at different stages, of results with differing inputs, the modelling of different scenarios and so on.
87 Therefore the Results section of this work presents a significant level of detail in the hope that
88 we can lessen the degree of opacity.

89 **Methods**

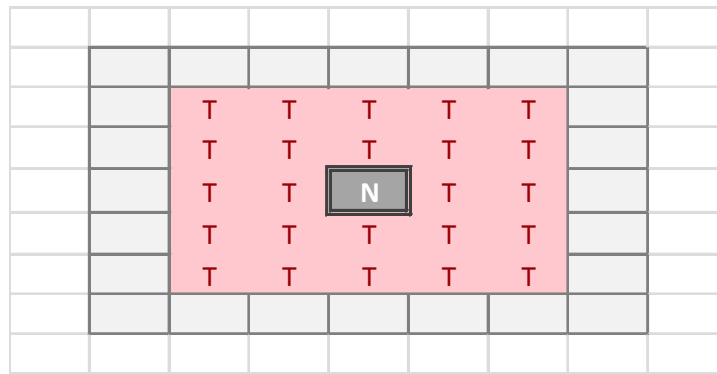
90 NEATG is implemented as a hybrid model incorporating elements from both genetic algorithms
91 and cellular automata. It is dual scale, non-deterministic and represents both cell-level and tissue-
92 level behaviour. It is coded in the Java programming language.

93 Grid or Tissue-Level

94 The tissue-level is represented as a rectangular grid, with each grid element containing a set of
 95 modelled cells, which may be Malignant or Normal. The relative proportion of Normal and
 96 Malignant cells in a grid element determines the state of that grid element. These states are:

$$97 \quad E = \{\text{Normal, Majority Normal, Majority Malignant, Tumour, Necrotic}\}$$

98 Transition of a grid element from one state to another takes place at every clock tick (generation)
 99 and is determined by the proportions of different cell populations within that element, but also by
 100 the state of neighbouring grid elements. Grid elements which are in the Tumour state, that is they
 101 do not have any Normal cells within them, can transition to a Necrotic state if they are
 102 surrounded by an extended neighbourhood which consists exclusively of other Tumour grid
 103 elements. By default this is a Moore neighbourhood of radius 2 (see Figure 1), though this is a
 104 configurable model parameter. This Necrotic state is designed to model cellular compartments
 105 within solid tumours in which a high rate of hypoxia and a low level of nutrient availability lead
 106 to high levels of cellular necrosis.



107

108

Figure 1 - Moore Neighbourhood of radius 2

109 Grid elements in the Necrotic state are suspended and do not take part in further computational
 110 activity unless the neighbouring grid population changes, in which case the Necrotic state reverts
 111 to Tumour.

112 Each grid element is populated with an initial, optimum population of Normal cells. The size of
 113 this optimum population is a model input parameter. The size of the population can vary over
 114 time and can increase to a defined maximum value, termed the carrying capacity, after which
 115 cellular competition takes place (as described below).

116 Each grid element receives as input a Nutrient, represented as an integer value, and a set of Gene
 117 Factors, represented as real values. The number of Gene Factors is equal to the number of genes
 118 in the cell structure. The Nutrient score can be loosely interpreted as a combination of oxygen
 119 and cellular nutrients (e.g. glucose), while the Gene Factors may be viewed as generic growth
 120 factors required for cellular growth and survival.

121 The grid element has a distribution function to compute the share of Nutrient (DN) assigned to
 122 each cell in its population of P cells based on the relative demand represented by the Nutrient
 123 Target values T for each cell:

$$DN_i = \frac{T_i}{\sum_{p=1}^P T_p} \quad (1)$$

124

125 Similarly the Gene Factor values which are inputs into each grid element are distributed to each
126 cell according to the transfer function based on the Gene Targets (G):

$$DG_i = \frac{G_i}{\sum_{p=1}^P G_p} \quad (2)$$

127

128 The distribution functions in equations 1 and 2 are designed to share resources within a given
129 grid element based on relative demand in order to reflect the levels of avidity in individual cells.
130 In real cells this avidity is controlled via the glucose transporter proteins Glut1 – Glut3, for
131 example.

132

133 Cell Level

134 There are two types of cell in this model, Normal and Malignant, with the same internal structure
135 regardless of type. While the structure is the same the behaviour is type-dependent during cell
136 division, as will be shown later. Crucially, the Malignant cell has non-zero values for Mutation
137 and Invasion rates, whereas for the Normal cells these values are set to zero.

138 Each cell is a data structure that encodes a Genome and an internal clock. The internal clock,
139 implemented as an integer value, counts down from a maximum value, known as the Lifetime, to
140 zero. Cell division is initiated when the clock reaches zero – Lifetime is therefore analogous to
141 the length of time for the cell cycle. When the system is first instantiated each cell is initialised
142 with an internal clock value that is equal to a random integer between the Lifetime and zero. In
143 the experiments that follow a Lifetime of 100 generations has been used as this simplifies the
144 numerical analysis and empirical testing showed that it produced robust results in different
145 scenarios. Shorter Lifetimes accelerated rates of cell proliferation, and longer values slowed
146 growth rates however the qualitative results were similar in all cases. At initiation all cells have
147 the same Lifetime, though this is heritable and mutable and therefore subject to evolutionary
148 adaptation over time.

149 The Genome is a set of N genes, which are defined by a Target and a Tolerance, both represented
150 as real numbers. The Genome is defined as:

$$151 \quad G = \{(Target_0, Gene\ Tolerance_0) \dots (Target_N, Tolerance_N)\}$$

152 While the size of the genome is configurable, the default value of N is 3 in the experiments
153 presented in this work. Empirical testing indicated that three genes were sufficient to illustrate
154 genetic evolution and diversity. Increasing the value of N increased the run-time of the system
155 but did not otherwise produce much change in the major output characteristics such as growth in

156 Malignant cell numbers, measures of genetic heterogeneity etc. Decreasing N improved
157 performance somewhat but with reduced scope for genetic evolution to take place.

158 The Gene Target is the optimum level of the corresponding Gene Factor that exists in the grid
159 environment, and the Tolerance defines a band of tolerable values on either side of the Target
160 that is the healthy range for that gene. Real cells are able to survive variations in nutrients,
161 growth factors and so on; the Target and Tolerance mechanism is therefore a mechanism to
162 allow modelled cells to similarly survive in varying conditions. Gene health is therefore defined
163 as a Boolean value which evaluates as True when the Gene Factor is within the desired range, or
164 False if the Gene Factor is above or below the tolerable range:

$$\text{Health} = (\text{Gene Factor} < (\text{Gene Target} + \text{Gene Tolerance})) \& (\text{Gene Factor} > (\text{Gene Target} - \text{Gene Tolerance})) \quad (3)$$

165 In addition to flagging health status, Genes are also used as a mechanism for the cell to influence
166 the local grid environment. This is a simple feedback mechanism by which each cell attempts to
167 alter the local environment in order to achieve the level of Gene Factor required for its own
168 optimum health. The expression function is:

$$E = 1 - e^{-(T-F)} \quad (4)$$

169

170 Where T is the Gene Target value and F exogenously supplied Factor.

171 The actual level of Gene Factor available in each Grid Element is calculated as the sum of the
172 exogenously supplied Factor, which is an input parameter in the model, and the sum of the
173 expression values from each cell in that grid element.

174 Additional components of the cell are the Nutrient Target and a Nutrient Rate, which represent
175 the demand for nutrient and the rate at which nutrient is consumed respectively. Nutrient which
176 is not consumed is stored in the Nutrient Store. Each cell also has a Mutation Rate and an
177 Invasion Rate, which are used when cell division is necessitated for Malignant cells.

178 Cells can exist in a number of states:

$$179 \quad S = \{\text{HEALTHY, DIVIDING, APOPTOTIC, TO_BE_CLEARED, NECROTIC}\}$$

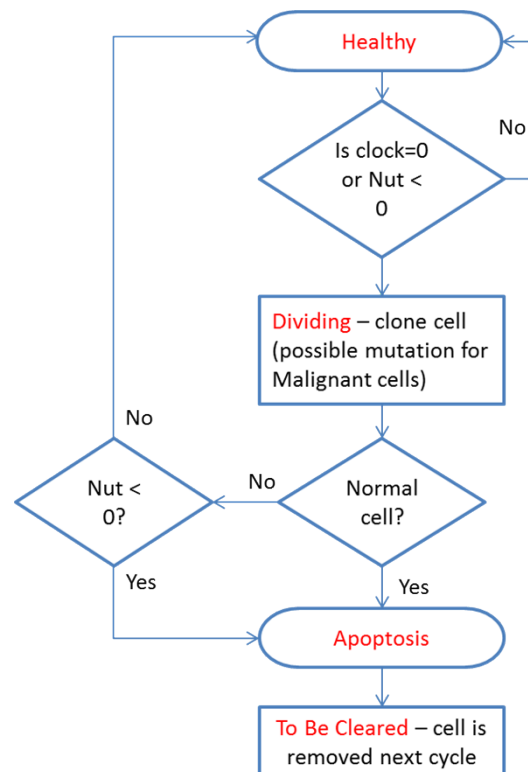
180 Note that the cell state of Healthy implies viability, rather than whether a cell is Normal or
181 Malignant.

182 At every clock tick the health status of each cell is assessed and the cell clock decremented
183 according to the state of health. A healthy cell, with adequate Nutrient and Gene Factors, will
184 decrease the cell clock by 1. Each unhealthy gene will also decrement the cell clock by one. A
185 cell that has a value of zero for Nutrient store will have the cell clock set to zero because it is
186 unable to meet its metabolic requirements and must therefore transition from a Healthy state.

187 All cells undergo a similar cell cycle. A cell starts as Healthy and undergoes a number of
188 iterations (clock ticks) in which nutrient and gene factors are processed, the cell clock decreases
189 at rates that depend on how well the cell is adapted to the local grid environment defined by the

190 available Nutrient and Gene Factors. When the cell clock or nutrient store reaches zero the cell
 191 changes state according to the following cycle:

192 Healthy > Dividing > Apoptotic > To Be Cleared



193

194

Figure 2 - Cell Cycle

195 The cell cycle algorithm is shown in Figure 2 (note that cell states are indicated in red). Cells that
 196 are flagged as To Be Cleared are removed from the grid element. Dividing cells undergo cell
 197 division during which a new daughter cell is generated and enters the local population. When the
 198 grid element contains fewer than the carrying capacity of the grid element a new cell is cloned
 199 from the dividing cell. At this point the difference between Malignant and Normal cells is
 200 apparent in that Normal cells have Mutation and Invasion rates fixed at zero, whereas Malignant
 201 cells have non-zero values. In the case of Malignant cells therefore cloning can also incur a
 202 mutation in which one of the elements of the cell can change value, for example the Nutrient
 203 Target, a Gene Tolerance value or the cell Lifetime itself may undergo an increase or decrease.
 204 Note that the rate of mutation events is controlled by the Mutation Rate, which is itself mutable
 205 and can increase or decrease.

206 If the grid element is already at carrying capacity then the cell division process is more complex.
 207 In addition to undergoing a chance of mutation, Malignant cells may also undergo a migration
 208 event in which the cell moves into a randomly selected adjacent grid element. The rate of such
 209 migration events is controlled by the Invasion Rate, which, like the Mutation Rate, is mutable.
 210 Cells which are not selected for migration are added to the local population. To preserve the
 211 carrying capacity of the grid element, all cells are then ranked according to fitness and the least

212 fit cells are removed. This ranked selection algorithm is not biased by cell type, and both
213 Malignant and Normal cells are included in the process.

214 The fitness function, F , is designed to penalise cells which are poorly adapted to the *local* grid
215 environment rather than being a global function across the entire population of cells. It is defined
216 as:

$$F = \frac{1}{G} \sum_{g=1}^G e^{-\left(\frac{|T_g - A_g|}{T_g}\right)} \quad (5)$$

217

218 where T is the Gene Target and A is the Gene Factor value for each Gene in the Genome G . The
219 fitness function yields a value in the range $[0, 1]$, with a perfect fitness equal to 1, and is used as
220 the ranking value when selecting the least fit cells for elimination. The rationale is that at any
221 given point the cell that has Nutrient and Gene Factor demands which correspond most closely to
222 the available levels in the local grid is the most well-adapted and hence the fittest. Note that this
223 is evaluated at every clock tick, and therefore fitness changes over time as the local conditions
224 change.

225 Evolutionary Strategies

226 The processing of Nutrient and Gene Factors is controlled by the treatment strategy object active
227 during that clock tick. This software component, coded in Java, enables the NEATG system to
228 model multiple evolutionary strategies, each of which can implement different algorithms in
229 terms of controlling the rate of cellular attrition, ageing and division. Strategies are coded as Java
230 components which extend an AbstractTreatmentStrategy class, which in turn implements an
231 IStrategy Java interface. Dependency injection is used to load the required strategy, specified in a
232 run-time configuration file, when it is required. Treatment strategies become active at specific
233 time points, either by activation at a specified generation or at a specified level of tumour
234 growth. Once triggered a treatment strategy can remain active until the final generation or for a
235 specified number of generations. There is also a default 'no treatment' strategy during the
236 iterations before and after the 'active' strategy is in operation.

237 Results

238 Homeostasis

239 Before exploring the results for different tumour growth scenarios it is important to validate the
240 behaviour of the system during homeostatic and non-tumour scenarios. Cells in this scenario are
241 supplied with optimal Nutrient and Gene Factor values, ensuring that they are unstressed and in
242 'good health'. In the absence of tumour cells we would expect that the system will display
243 homeostatic behaviour characterised by regular cellular turn-over as cells age and die, and that
244 cell population size will fluctuate but remain relatively constant.

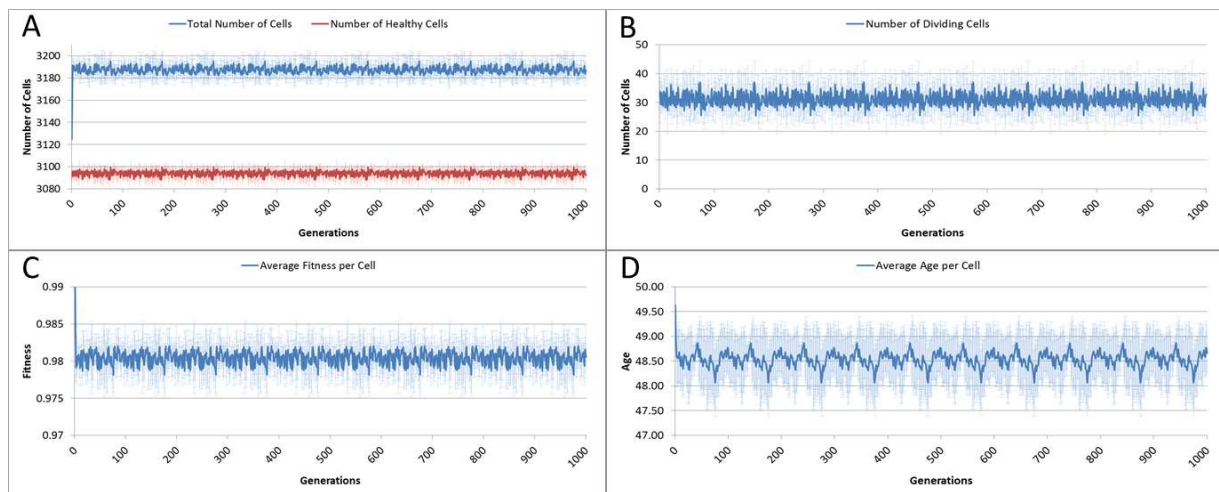
245 To represent this scenario a series of experiments were run using a 25 x 25 grid. Empirical
246 testing had shown that a grid of this size was sufficient to provide illustrative results in a wide
247 range of scenarios, including tumour growth scenarios in addition to homeostasis and other non-
248 tumour growth scenarios. The optimum cell population for each grid was set at 5, with a
249 population of 10 cells as the maximum carrying capacity. The Nutrient Target used was 10, with

250 a Nutrient Rate of 1. The Nutrient input to each grid element was also set at 10, ensuring that at
 251 optimum population level each cell would receive a Nutrient input of $10 / 5 = 2$. A genome of
 252 three identical genes was used:

$$253 \quad G = \{(5.0, 1.0), (5.0, 1.0), (5.0, 1.0)\}$$

254 The Gene Factor supplied to each grid element was set at $\{25.0, 25.0, 25.0\}$, to ensure that each
 255 cell received the Target value of 5.0.

256 The system was run five times, with 1000 iterations per run, and the results averaged for this
 257 analysis. Given our input parameters for a grid of 625 elements (25 x 25), and an optimum cell
 258 density of 5 cells per grid element, we would expect a total cell count of 3125. However, not all
 259 of these cells will be healthy, some will be dividing or being cleared. Figure 3A shows the
 260 overall population of cells over time.



261 A. Total and healthy cell counts over time. B. Number of dividing cells over time. C. Average cell fitness over. D.
 262 Average cell age over time. (All mean \pm SD).

262 **Figure 3 - Cell change over time**

263 The number of dividing cells over time is shown in Figure 3B. Note that the average over the
 264 1000 iterations is 31.25. This is as we would expect given that the Lifetime for the cells is 100,
 265 so that at any one time 1% of cells is dividing.

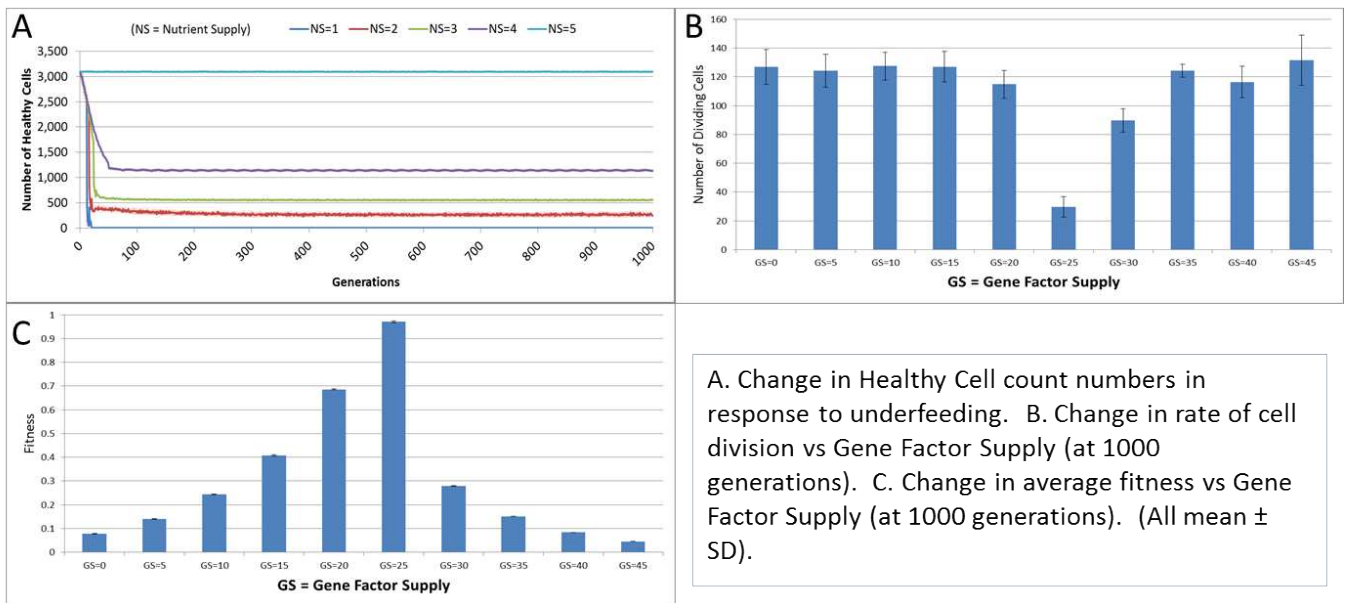
266 The average fitness, Figure 3C, is high, fluctuating just below the maximum possible value of
 267 1.0. And the average age, Figure 3D, fluctuates just below a value of 50. These latter two figures
 268 display more clearly a pronounced periodicity which is also evident in the population density.
 269 This is due to the random distribution of ages in the initial cell population. In the absence of
 270 stress or environmental perturbation the population of cells ages and divides in a uniform manner
 271 that preserves the distribution of ages from the initial population.

272 **Stress Conditions**

273 In the next experiments we assess the behaviour of NEATG when homeostasis is disturbed. In
 274 particular we are interested in the responses to changes in Nutrient and Gene Factors as these

275 both have an influence on cell ageing and survival. Again this series of experiments does not
 276 include Malignant cells as we are primarily interested in exploring the behaviour of the system in
 277 non-tumour scenarios. For both of the following experiments the same basic parameters as in the
 278 previous experiment are used.

279 The first stress experiment varies the Nutrient input from 1 to 15, in integer steps. Given that the
 280 Nutrient Rate is set at a value of 1 and the optimum cell population is set to 5, we would expect
 281 that if the Nutrient Supply to each grid element falls below a value of 5 each cell in the grid
 282 would consume more nutrient than it receives as input and eventually deplete the value in its
 283 Nutrient Store (which was set to an initial value of 10). Figure 4A shows the number of healthy
 284 cells for different Nutrient Supply values. There is a decline in cell numbers over time for
 285 Nutrient Supply values below 5 but none for greater values (data not shown). Cell populations
 286 are therefore shown to be sensitive to the supply of Nutrient such that under-feeding can deplete
 287 numbers and in some cases ‘starvation’ reduces cell numbers to zero.



288

289 **Figure 4 - Changes during Stress Conditions**

290 The supply of Gene Factors is the other external input to each grid element. These are analogous
 291 to generic growth and survival factors and are used to assess the health or otherwise of each cell
 292 in a grid element. In this experiment the same parameters are used as before, but the Gene Factor
 293 Supply is varied from $\{0.0, 0.0, 0.0\}$ to $\{45.0, 45.0, 45.0\}$, in increments of 5.0.

294 There was little variation in cell counts in response to changes in Gene Factor Supply (data not
 295 shown), however, Gene Factors did have an influence on cell turnover, such that it was lowest
 296 for optimum values of Gene Factor Supply and increased by a factor of four as the deviations
 297 from the optimum values increased, as shown in Figure 4B. The number of dividing cells at the
 298 optimal Gene Factor Supply value is around 1% of the total cell count, whereas for non-optimal
 299 Supply values there is an increased rate of cell division. This is as we would expect given that
 300 ‘unhealthy’ genes cause an increased rate of cell aging.

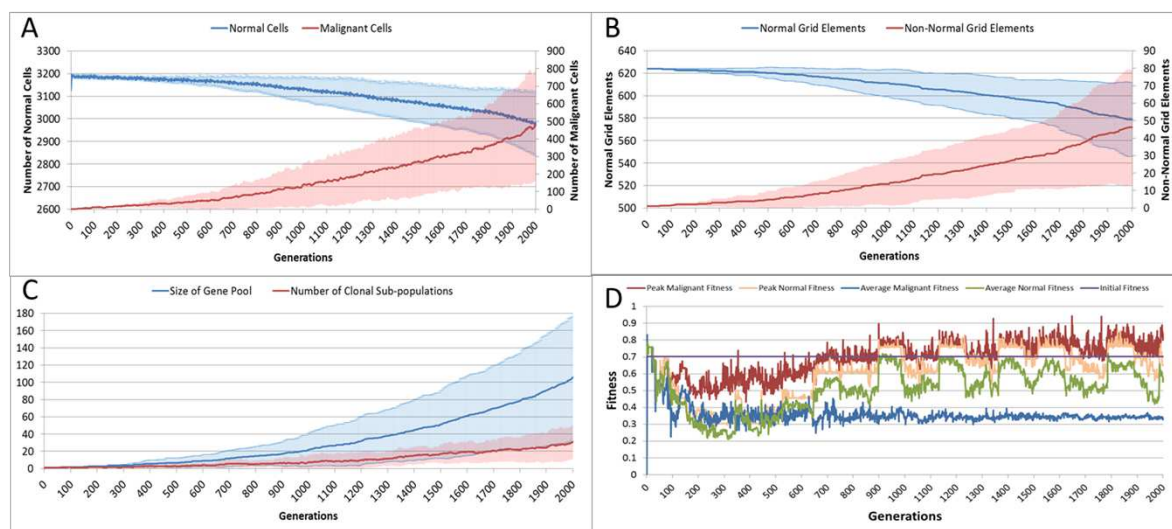
301 In addition to being a factor in the cellular aging process, the Genes are also used in calculations
 302 of cell fitness. Fitness is used in the rank selection process to identify the least fit cells when the
 303 population density in a grid element exceeds the maximum capacity. In this experiment no
 304 Malignant cells are present therefore the rank selection procedure is not active; however we can
 305 still assess the influence of the Gene Factor Supply on cell fitness, (which is defined in the range
 306 $[0, 1]$), as shown in Figure 4C.

307 Tumour Growth - No Treatment

308 Having established the behaviour of the system under homeostatic and non-tumour stress
 309 scenarios, we can now begin to introduce Malignant cells. Initially we will explore the behaviour
 310 of NEATG in the absence of any treatment scenarios.

311 In this first series of experiments we will continue to use the same parameters as before, although
 312 the iteration period is increased to 2000 to allow greater time for the evolution of appreciable
 313 number of tumour cells. Tumour growth is initiated by the insertion of a single Malignant cell
 314 into the grid element in the centre of our 25 x 25 grid. The only difference between this
 315 Malignant cell and the Normal cells is that the cell type is set to Malignant, and that it has a
 316 mutation rate of 5% and an invasion rate of 10%. Estimates of mutation rates in higher
 317 eukaryotes, including human cells, vary considerably. These initial values were derived from
 318 empirical testing of NEATG and were selected as they generated consistent tumour growth. In
 319 subsequent experiments these values will be varied so that we can see how tumour growth
 320 patterns are affected. Drake et al report a mutation rate per effective genome per replication of
 321 0.004 in humans, which is an order of magnitude lower than the rate used here [30].

322 With the introduction of Malignant cells we can view results both in terms of the changes in cell
 323 populations across the whole system and also in the evolution of the grid elements. The change
 324 in the global population counts cells is shown in Figure 5A and grid elements in Figure 5B.



A. Change in Normal and Malignant cell counts (mean \pm SD). B. Change in Normal and Non-Normal Grid Element Counts (mean \pm SD). C. Change in Gene Pool and Clonal Populations Over Time (mean \pm SD). D. Change In Fitness Over Time (mean)

325

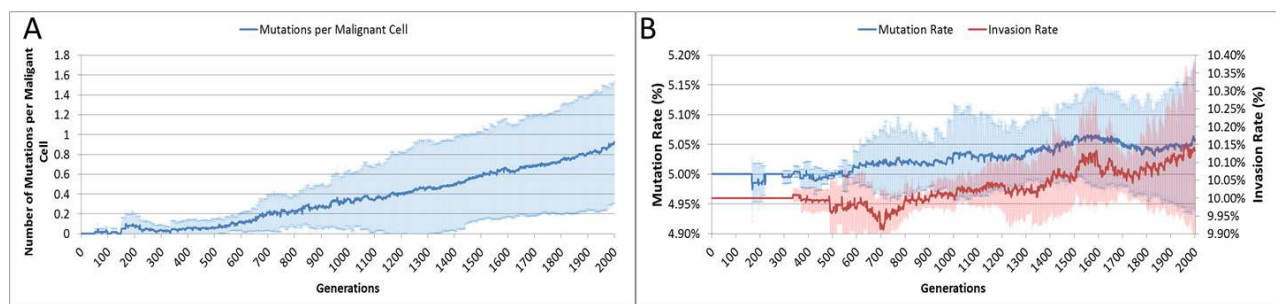
326 **Figure 5 - Tumour growth - no treatment**

327 Using Figure 5A we can see that the doubling time for Malignant cells is approximately 500
 328 generations. The doubling time for the NCI-60 human cancer cell lines range from 14.4 hours
 329 (HCT-116 colon cancer) to 79.5 hours (HOP-92 non-small cell lung cancer), with a median value
 330 of 33.25 hours (NCI). If we use this median value then each generation equates to 4 minutes, and
 331 the entire run of 2000 generations is equivalent to around 5.5 days of in vitro growth.

332 Changes in grid elements and cell populations are not the only metrics of interest. Also of
 333 interest is the process of evolutionary change in the Malignant cell populations. In the initial
 334 population there is only a single genotype but as shown in Figure 5C the rate of change of the
 335 gene pool – the cumulative total of all clonal sub-populations which have been generated,
 336 including extinct populations in addition to existing ones – rises over time, increasing in line
 337 with the Malignant cell counts. Also shown in Figure 5C is the rise in the number of clonal sub-
 338 populations, reflecting the growth of different active Malignant cell sub-populations.

339 The evolution of fitness is shown in Figure 5D. The first Malignant cell has the same fitness as
 340 the Normal cells in the grid element into which it is inserted, however as the number of cells
 341 increases, the number of mutations rises, Malignant cells proliferate into neighbouring grid
 342 elements and competition for Nutrient and Gene Factors takes place. The noisy signals indicate a
 343 good deal of change and adaptation taking place over time. The initial high fitness value is
 344 degraded once the cell populations start to increase and competition takes place. It is also clear
 345 that the Normal cell population retains an average fitness that is higher than the average of the
 346 Malignant cell population. One plausible explanation is that many of the mutations are
 347 deleterious and do not lead to improved survival for those cells. However, if we look at the
 348 maximum values for the Malignant cells we can see that there are indeed some cells which do
 349 achieve a higher fitness than maximum of the Normal cells.

350 The average number of mutations per Malignant cell is shown in Figure 6A. As can be seen for
 351 the first 100 generations or so there are no mutations, which accords with Figure 5D.



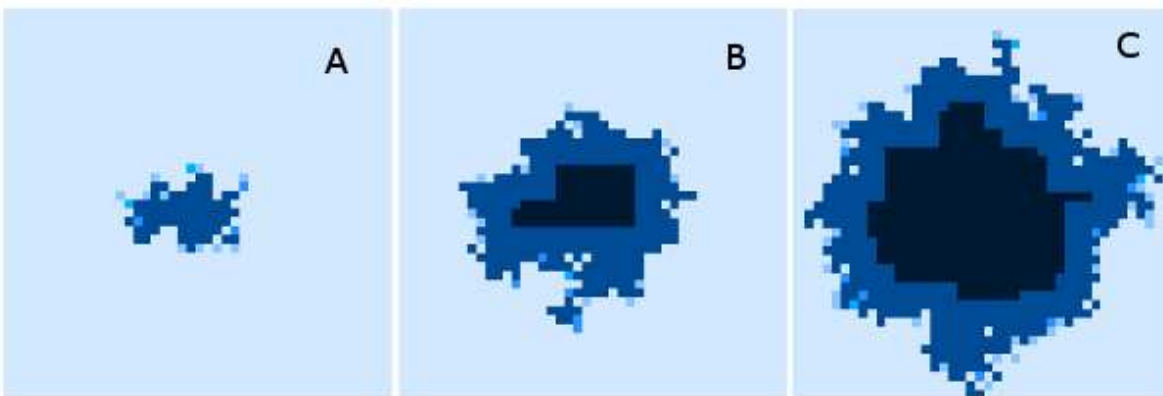
A. Mutations per Malignant cell over time (mean \pm SD) . B. Change in Mutation and Invasion Rates over time (mean \pm SD)

352

353 **Figure 6 - Mutation rates over time**

354 The mutation rate and the invasion rate, which are both mutable characteristics show some
 355 change over time, as shown in Figure 6B. While initially there is little change, indeed both rates
 356 dip below the starting values, both rates show an increasing trend over time.

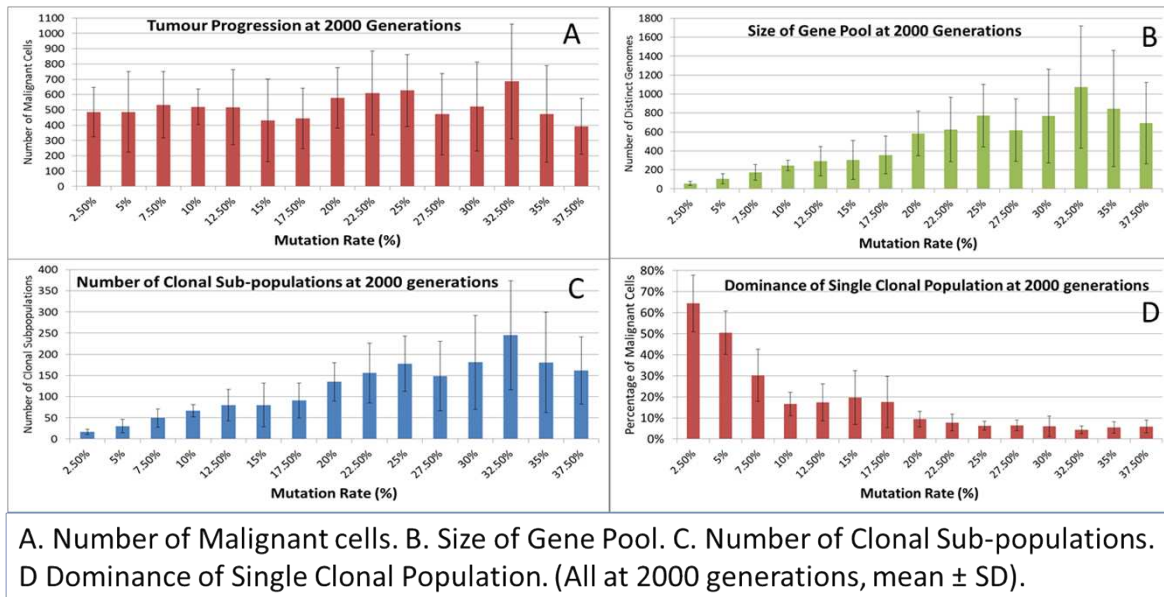
357 Finally, while we have explored the rates of change at the cellular and grid element levels, we
358 have not explored the spatial distribution of the spread of Malignant cells. A representative
359 example of the 'no treatment' scenario is shown in Figure 7, an extended run of 6000 generations
360 and a grid size of 45 x 45 has been used to illustrate more fully the development of the tumour
361 over time. Figure 7A shows 'tendrils' of cancer cells infiltrating into healthy tissue (light
362 coloured background representing Normal cells) from the centre of the dark blue tumour mass, in
363 Figure 7B the tumour mass has expanded considerably and shows a black inner necrotic core and
364 a perimeter of tumour cells with tendrils extending into the healthy cells. Finally Figure 7C
365 shows continued expansion, including an expanding area of necrosis. If allowed to continue
366 expanding the tumour eventually dominates the grid completely until further growth is
367 impossible and the mass becomes mainly necrotic.



368 Evolving tumour mass at A: 2000 generations, B: 4000 generations, C: 6000 generations. Note
369 that black areas are necrotic grid elements.

Figure 7 - Spatial distribution of tumour growth

370 We can vary the Mutation and Invasion rates to understand the impact they have on tumour
371 growth. First we vary the Mutation Rate from 2.5% to 30% in increments of 2.5%, all other
372 settings are as before. Note that while figures are shown for the final time point of 2000
373 generations, these values are representative of the trends apparent at earlier time points. Whether
374 we look at tumour progression in terms of grid elements or in terms of Malignant Cell counts, as
375 in Figure 8A, there is no direct relationship between mutation rate and tumour progression.

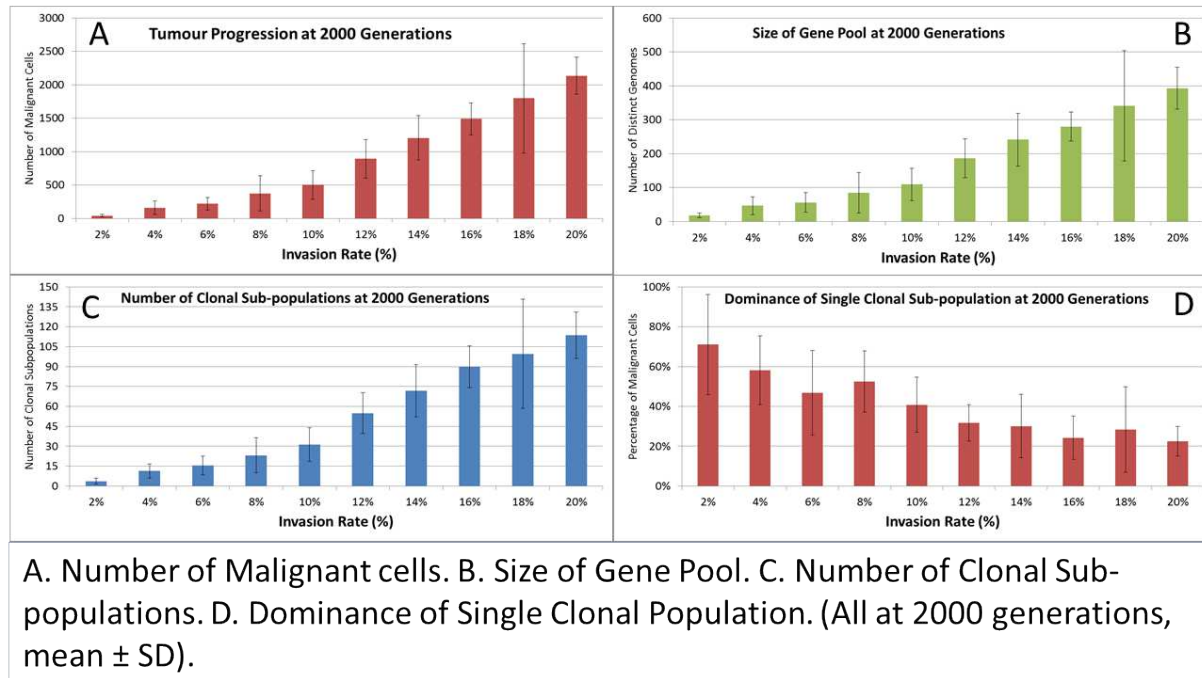


376

377 **Figure 8 - Mutation rates and clonal sub-populations**

378 We would expect to see a correlation between the mutation rate and the size of the Gene Pool,
 379 Figure 8B, though even here the relationship is not completely monotonous as a mutation rate of
 380 32.5% generated a larger gene pool than a mutation rate of 37.5%. Similarly, if we look at the
 381 number of currently existing clonal sub-populations, Figure 8C, there is a correlation with the
 382 mutation rate, but again this is not linear. Another interesting metric is the degree of dominance
 383 of the largest of the clonal sub-populations, Figure 8D. This shows the percentage of the total
 384 number of Malignant cells which belong to the largest clonal sub-population and shows that a
 385 lower mutation rate yields a greater degree of dominance by a single clonal sub-population.

386 We also vary the Invasion Rate to see what impact this has on the degree of tumour growth and
 387 the size of the gene pool. In this experiment the Invasion Rate is varied from 2% to 20% in 2%
 388 increments, the Mutation Rate of 5% is used; all other settings are as before. Clearly, as shown in
 389 Figure 9A, there is a direct relationship between the Invasion Rate and the rate of tumour growth.
 390 More migration events correlate closely with increased tumour spread.



391

392 **Figure 9 - Invasion rates and clonal sub-populations**

393 This increased rate of tumour growth also leads to an increase in the size of the Gene Pool and
 394 the number of clonal sub-populations, Figure 9B and Figure 9C. However, when compared to the
 395 scale of the increase of the Gene Pool with a rising Mutation Rate (Figure 8B) it is clearly lower
 396 and indicates a less heterogeneous Malignant cell population. In terms of the dominance of a
 397 single clonal population, Figure 9D, a lower Invasion rate is associated with an increased
 398 dominance by a single clonal sub-population, but even at a high Invasion Rate of 20% the degree
 399 of dominance is much higher than that associated with a high Mutation Rate (Figure 8D).

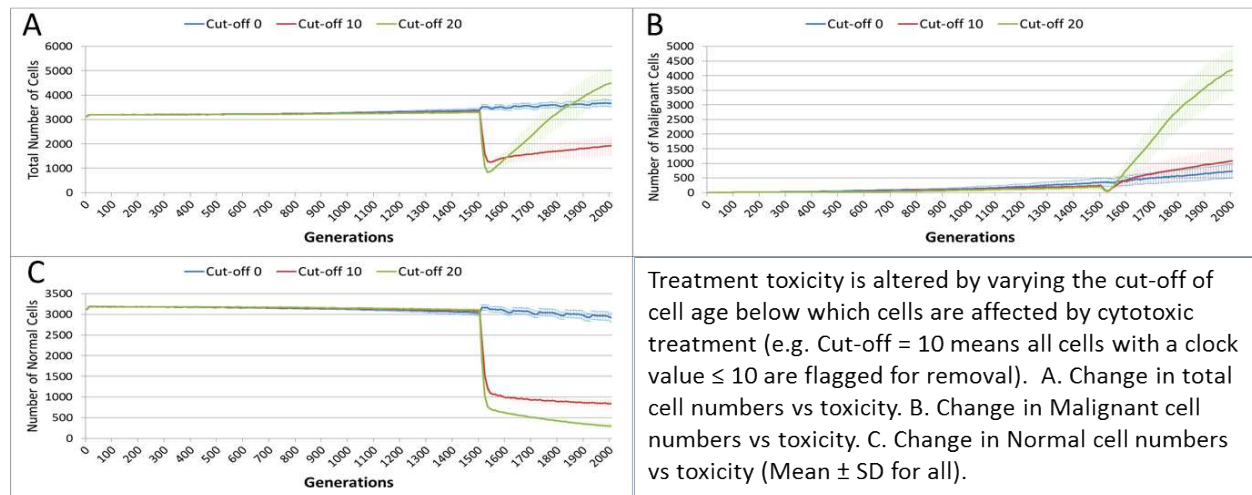
400 **Tumour Growth - With Treatment**

401 The previous experiments have shown that in the absence of any interventions the number of
 402 Malignant cells and Tumour grid elements increase over time. In the next series of experiments
 403 we investigate the impact on these growth patterns of a number of interventions using an active
 404 treatment strategy. This is loosely based on the example of high-dose cytotoxic chemotherapy.
 405 Just as with cytotoxic chemotherapy this is not a targeted therapy – it is applied to both Normal
 406 and Malignant cells. Where real chemotherapy causes apoptotic or necrotic cell death in rapidly
 407 dividing cells, the treatment strategy in this model flags cells above a specified age with the cell
 408 state of TO_BE_CLEARED. The arbitrary age cut-off is based on the value of a cell's clock and
 409 this value is a configurable parameter. By adjusting the cut-off value we can approximately
 410 control the 'toxicity' of the treatment, the higher the cut-off value the more toxic the treatment as
 411 more cells will be flagged for disposal. The system also allows a degree of specificity in that we
 412 can make Malignant cells more susceptible to the treatment than Normal cells.

413 In this experiment the same parameters will be used as in the No Treatment scenario. The
 414 treatment will commence at generation 1500 (of 2000), and will be applied for 25 generations.
 415 Three different toxicity values are assessed, with both Malignant and Normal having the same
 416 cut-off values. The values used are 0, 10 and 20, which means that any cell with a clock value \leq

417 the cut-off is 'treated'. Note that the zero cut-off value does not trigger cell division as in the
 418 default scenario, but triggers apoptosis and cell clearance. It represents the least toxic scenario
 419 and is therefore close to the 'no treatment' scenario.

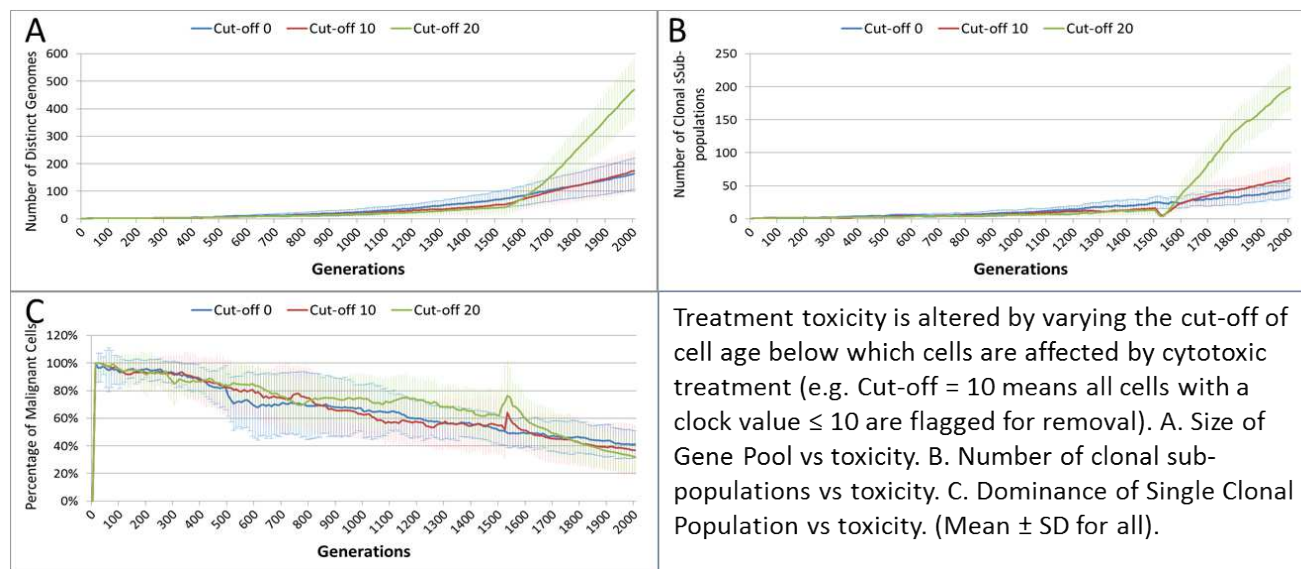
420 The effect of treatment on the total cell count, Figure 10A, is dramatic. In the case of the more
 421 toxic treatments, there is a sharp decline in total cell numbers followed by a recovery, and in the
 422 case of the highest cut-off value of 20 cell growth accelerates above the pre-treatment trend.



423

424 **Figure 10 - Tumour response to treatment toxicity**

425 This growth trajectory is also reflected in the Malignant cell view of tumour growth, Figure 10B.
 426 This shows that the slow rise in number is briefly interrupted when treatment begins but then
 427 accelerates sharply after the completion of treatment. Furthermore in both figures the more
 428 aggressive treatment is related to an increased tumour growth rate following the cessation of
 429 treatment. The change in the Normal Cell population is shown in Figure 10C. The treatment
 430 induces a sharp reduction in cell numbers that continues even after the cessation of treatment,
 431 though not at the same rate. We can assume that the decline in Normal cell numbers has provided
 432 the conditions in which Malignant cells can expand rapidly in number. Supporting evidence is
 433 provided by the Gene Pool trends, shown in Figure 11A. Here we can see that following
 434 treatment there is an increase in the size of the Gene Pool, indicating a post-treatment burst of
 435 clonal evolution.



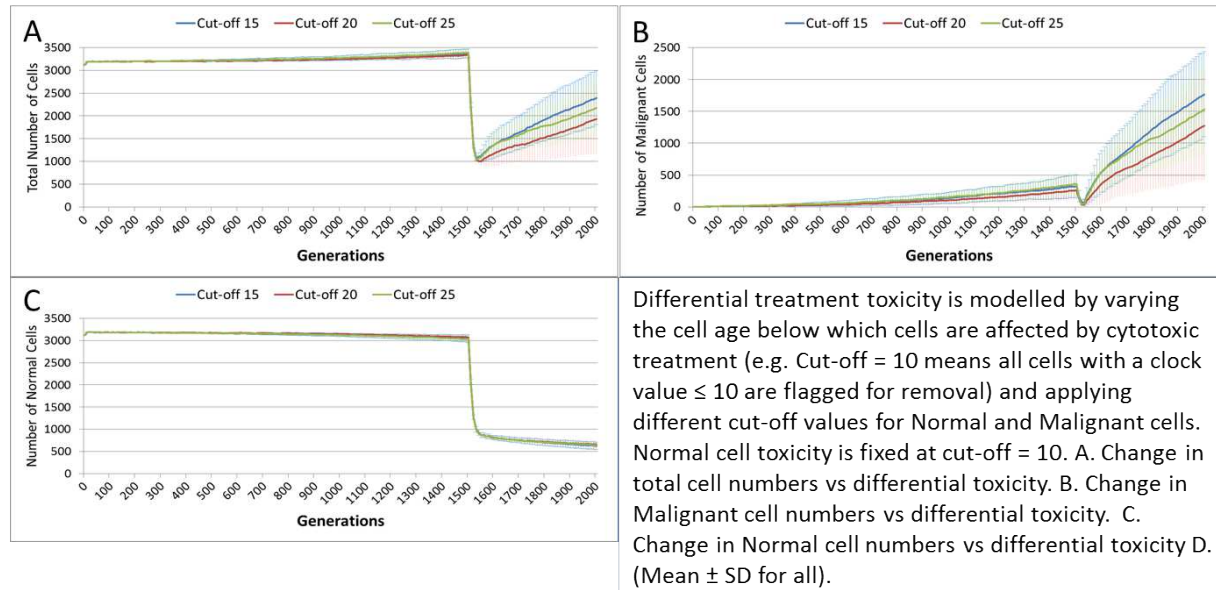
436

437 **Figure 11 - Treatment toxicity and clonal sub-populations**

438 The number of active clonal subpopulations, Figure 11B, shows a similar trend – a slow increase
 439 until treatment commences at which point there is a dip in numbers followed by a post-treatment
 440 evolutionary explosion. Another view of this evolutionary burst, Figure 11C, shows that the
 441 process of tumour growth leads to an increase in genetic heterogeneity, as measured by the
 442 decreasing proportion of the Malignant cell population belonging to the largest sub-population.
 443 The increasing heterogeneity is interrupted when treatment begins and there is a spike which
 444 shows that the largest sub-population increases as a proportion of the total, from which we can
 445 infer that a number of clonal sub-populations have been exterminated completely, in line with
 446 Figure 11B.

447 In clinical practice maximum tolerated dose (MTD) chemotherapy does not cause equal levels of
 448 damage to all cell populations. Because it impacts rapidly proliferating cells the ‘collateral
 449 damage’ to non-tumour cells is restricted to certain populations of non-cancer cells in the
 450 immune system, gut and other tissues associated with the side effects of treatment (Chen et al.,
 451 2007). We can model this differential impact in the NEATG system by setting a lower cut-off
 452 value for Normal cells compared to Malignant cells, thus causing fewer Normal cells to be
 453 affected. In the following experiment the cut-off for the Normal cells is set to 10, and for the
 454 Malignant cells it is set to 15, 20 and 25 in three different scenarios. All other parameters are the
 455 same as in the previous experiment.

456 In terms of the total cell counts, Figure 12A, there is a similar pattern to the previous experiment,
 457 although the rate of recovery is much lower than in Figure 10A. The lower sensitivity of the
 458 Normal cells means that even when the cut-off for the Malignant cells matches the previous
 459 values, the recovery of cell populations is lower. The pattern of increased tumour growth and
 460 evolutionary change following the cessation of treatment also occurs, Figure 12B.



461

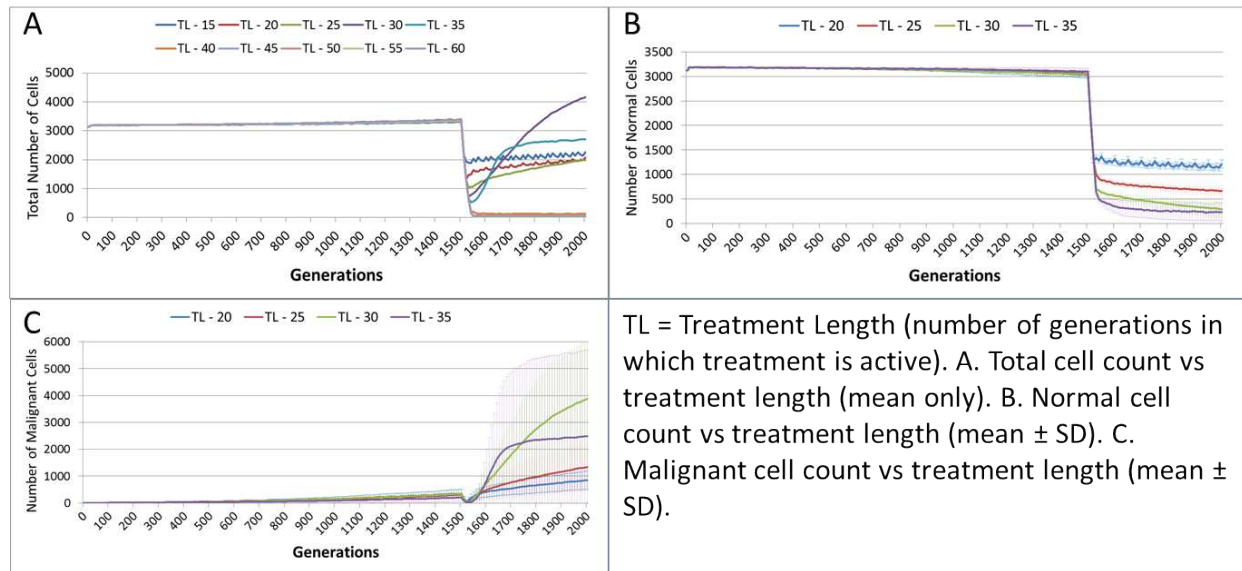
462 **Figure 12 - Tumour response to differential treatment toxicity**

463 The lower sensitivity of the Normal cells does not mean that they are immune from effects of
 464 treatment. Figure 12C shows a marked decline when treatment commences, followed by a
 465 continued decline after treatment ends. Note there is no difference in the three scenarios shown,
 466 indicating that the Normal cells are not affected directly by the higher sensitivity of the
 467 Malignant cells. The values shown here are a close match to those shown for the Cut-off 10
 468 scenario illustrated in Figure 10C.

469 Two rather obvious questions arise from this data. The first is what happens if the period of
 470 treatment is extended? It is clear that for the duration of treatment the number of Malignant cells,
 471 tumour grid elements and clonal populations decrease. Is it possible to extend the treatment
 472 period so that the entire Malignant cell population is destroyed? Secondly, it is clear that the
 473 treatment damages Normal cells and that this coincides with increased cancer growth following
 474 the cessation of treatment. Therefore we can ask what happens in the case when the differential
 475 toxicity is such that there is *no* damage to the Normal cells – in other words what would happen
 476 in the case of a ‘magic bullet’ which has toxic effects only on Malignant cells? These questions
 477 are addressed in turn in the next two of experiments.

478 In the following experiment the treatment duration which was varied from 15 – 60 generations,
 479 in increments of 5. A differential toxicity was used, with a Malignant cut-off value of 20 and a
 480 Normal value of 10, all other settings are unchanged.

481 Figure 13A, shows a relationship between the treatment length and the size of the total cell
 482 population. The relationship is complex and non-linear, but it is apparent that treatment duration
 483 longer than 40 generations causes significant reductions in the total population. This result was
 484 robust to repeated runs of the system and there was essentially no difference between results for
 485 any treatment length above this level. Furthermore, this upper cut-off figure for treatment length
 486 was related to the length of the cell Lifetime (which is 100 in these experiments). In order to
 487 simplify the exposition, the rest of the results in this experiment will focus on treatment lengths
 488 of 20 – 35.

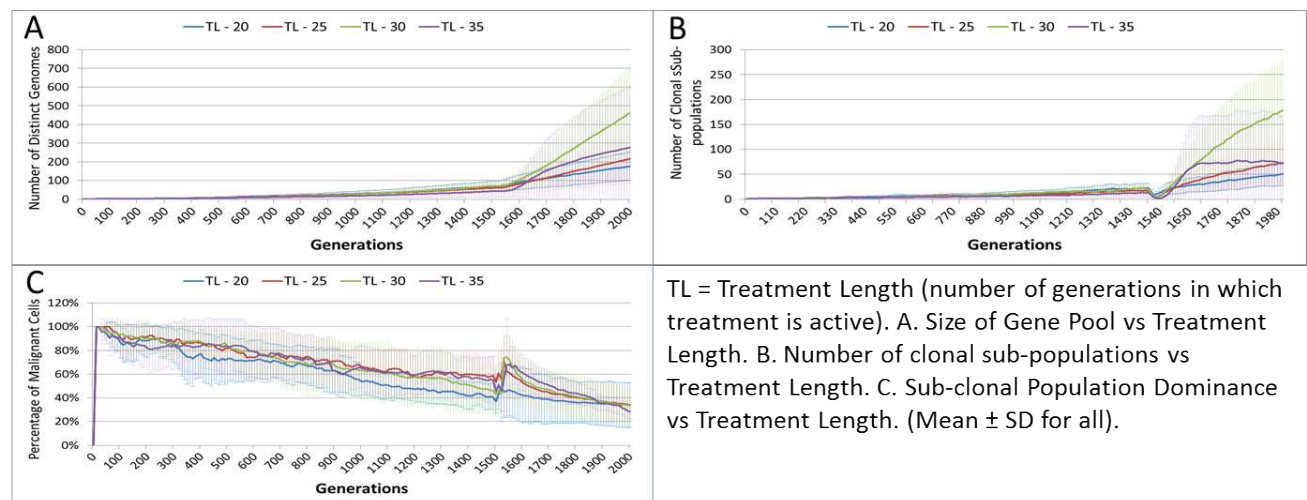


489

490 **Figure 13 - Tumour response to treatment length**

491 The effect of treatment length on the Normal and Malignant cell populations is shown in Figure
 492 13B and Figure 13C respectively. In the case of the Normal cell populations increasing treatment
 493 length is strongly associated with the scale of the decline in cell numbers. However, in the case
 494 of the Malignant cells, the treatment length is also associated with the rate of recovery. Figure
 495 13C shows that longer treatment length can sometimes lead to an accelerated increase in
 496 Malignant cell numbers, though for treatment lengths beyond 40 (data not shown), there is no
 497 recovery in cell numbers, (as should be clear from the collapse in total cell counts in Figure
 498 13A). The somewhat surprising result is that in some cases a more aggressive treatment (longer
 499 treatment period) can lead to an unexpected acceleration in tumour growth.

500 Length of treatment is also associated with an increase in the size of the Gene Pool, Figure 14A,
 501 and acts as a spur to clonal evolution, as shown in Figure 14B.

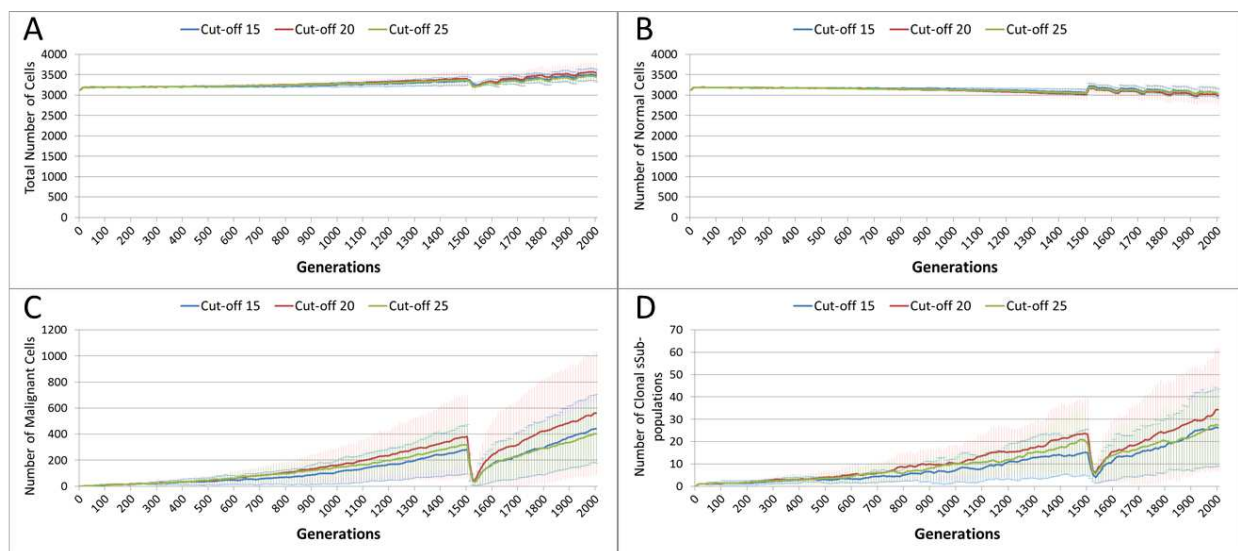


502

503 **Figure 14 - Treatment length and clonal sub-populations**

504 A further indication of the effect of treatment length on clonal evolution is shown in Figure 14C,
 505 which charts the percentage of the total Malignant population in the most populous clonal sub-
 506 population. It is clear that longer treatment increases dominance as cells from less popular
 507 genotypes are removed, whereas for the short treatment of 20 generations there is no such spike
 508 in dominance.

509 In the final experiment in this section we investigate a ‘magic bullet’ scenario where treatment is
 510 applied only to Malignant cells. In this experiment three different toxicity levels are applied to
 511 the Malignant cells, representing cut-off values of 15, 20 and 25. In stark contrast to Figure 10A
 512 and Figure 12A, treatment does not lead to a sharp decline in total cell numbers, as shown in
 513 Figure 15A. This is confirmed by the Normal cell numbers, Figure 15B, where there is a slow
 514 decline prior to the commencement of treatment followed by a recovery in numbers and then a
 515 slow decline again.



Treatment toxicity is altered by varying the cut-off of cell age below which cells are affected by cytotoxic treatment (e.g. Cut-off = 10 means all cells with a clock value ≤ 10 are flagged for removal). Here we model ‘no collateral damage’ and toxicity only applies to Malignant cells. A. Total cell count vs no collateral damage. B. Normal cell count vs no collateral damage. C. Malignant cell count vs no collateral damage. D. Clonal sub-populations vs no collateral damage. (Mean \pm SD for all).

516

517 **Figure 15 - Tumour response to no collateral damage**

518 The impact of treatment on Malignant cells, Figure 15C, shows that the increase in cell numbers
 519 is reversed sharply by the treatment but is then followed by a recovery and a resumption of
 520 tumour growth. However, note that while the pattern is similar to previous experiments, the
 521 absolute number of Malignant cells is markedly lower than in Figure 10B and Figure 12B.

522 In terms of the impact on clonal evolution, Figure 15D, while there is a pause during the
 523 treatment period, it continues at a similar rate to the pre-treatment trend afterwards. Again, while

524 this pattern is familiar, the number of clonal sub-populations is lower than in previous
525 experiments, as shown by Figure 11B and Figure 14B.

526 Discussion

527 The results outlined above display a range of behaviours and phenomena which are indicative of
528 real tumour growth. In the first instance the model is capable of reproducing homeostatic
529 behaviour. In optimal conditions the model displays a steady turnover of cells, which age and
530 divide in such a manner that the target cell population is preserved. However, under stress
531 conditions, such as a restriction in the Nutrient supply or a reduction in Gene Factors, we see a
532 change in behaviour. In the case of underfeeding or starvation we see that cell numbers are
533 markedly reduced, however over-feeding does not lead to an increase in cell populations.

534 For Gene Factors, we see that under or over-supply does not impact cell numbers to the same
535 extent, though both scenarios lead to a small reduction in total cell numbers. The variations in
536 Gene Factor supply do however impact on cell turnover, with an increase in rates of cell division
537 in both under and over-supply situations. In this respect we may view the impact of deviations
538 from the Gene Factor target values acting as mitogenic factors. There is also a marked impact on
539 the calculation of cell fitness, with deviations from the optimal values fitness. We may conclude,
540 therefore, that variations in the Gene Factor supply are deleterious to some extent, but do not
541 cause the same level of cellular damage as restriction in the supply of Nutrient.

542 Tumour Growth

543 Once tumour growth is initiated the proliferation of cancer cells, (also reflected in the number of
544 affected Grid Elements), increases in the absence of any counter-measures (i.e. left untreated).
545 As each Grid Element can support a number of cells over and above the optimum level, this
546 initial increase in numbers does not displace or replace non-cancer cells. However, once the
547 carrying capacity of the Grid Element has been reached there is a competition between cells in
548 which ultimately the Malignant cells out-compete the Normal cells. The influence of carrying
549 capacity on Malignant cell growth is illustrated in Figure 17B, which shows that changing the
550 trigger point for competition by varying the optimum cell count has an impact on the rate of
551 tumour growth. Over time the number of Malignant cells increases and the rate of invasion
552 increases, while there is a corresponding decrease in Normal cell numbers. As with the
553 homeostatic case, this behaviour is not pre-programmed but emerges from the interactions
554 between the cells, between neighbouring Grid Elements and the operation of a few simple rules.
555 Additionally, there is a consistent increase in the number of clonal sub-populations as growth
556 continues – mirroring the genetic heterogeneity which is a hall-mark of real tumour growth (Sun
557 & Yu, 2015). The system also shows that in the face of changing conditions there is an increase
558 in the number of clonal sub-populations and a decrease in the dominance of the most populous
559 sub-clone over time, again, reflecting real tumour genetic heterogeneity (Jamal-Hanjani et al.,
560 2015).

561 We should note that in the first instance the seeded Malignant cell has the same genomic
562 structure as the Normal cell population in these experiments. That is the Malignant cell is not
563 conferred any genetic advantage over the rest of the non-Malignant cell population. The single
564 difference between the Malignant cell and the Normal cell is that the Malignant cell is flagged as
565 such and that it has an ability to mutate and undergo repeated division. It may be assumed that
566 the increasing success of the Malignant cells in outcompeting Normal cells may be due to an

567 increasing evolutionary fitness that arises through a succession of mutational events occurring
568 during cell division. However the data does not support this assumption.

569 Evolutionary fitness is not defined in absolute or global terms in NEATG. Instead it is a local
570 function that reflects cellular adaptation to the changing conditions *in each Grid Element*. Thus it
571 is clear from the data, as shown in Figure 5D, that in general the fitness of many Malignant cells
572 is lower than the initial fitness of the Normal cells, and that it often decreases as a result of intra-
573 Grid Element competition between cells. Furthermore, many mutations are actually deleterious
574 and do not confer evolutionary advantage over competing cells, Normal or Malignant. Some
575 Malignant cells do experience mutations which provide an advantage, and these are the cells
576 which manage to survive and expand in number. However, a cell with a positive advantage in
577 one Grid Element may migrate to an adjacent Grid Element and find that it is less fit and
578 therefore does not survive. This view of evolutionary fitness as locally responsive to the
579 environment and therefore having an impact on the success, or otherwise, of genetic mutations is
580 in line with more recent theoretical models of evolutionary processes in cancer (Rozhok &
581 DeGregori, 2015).

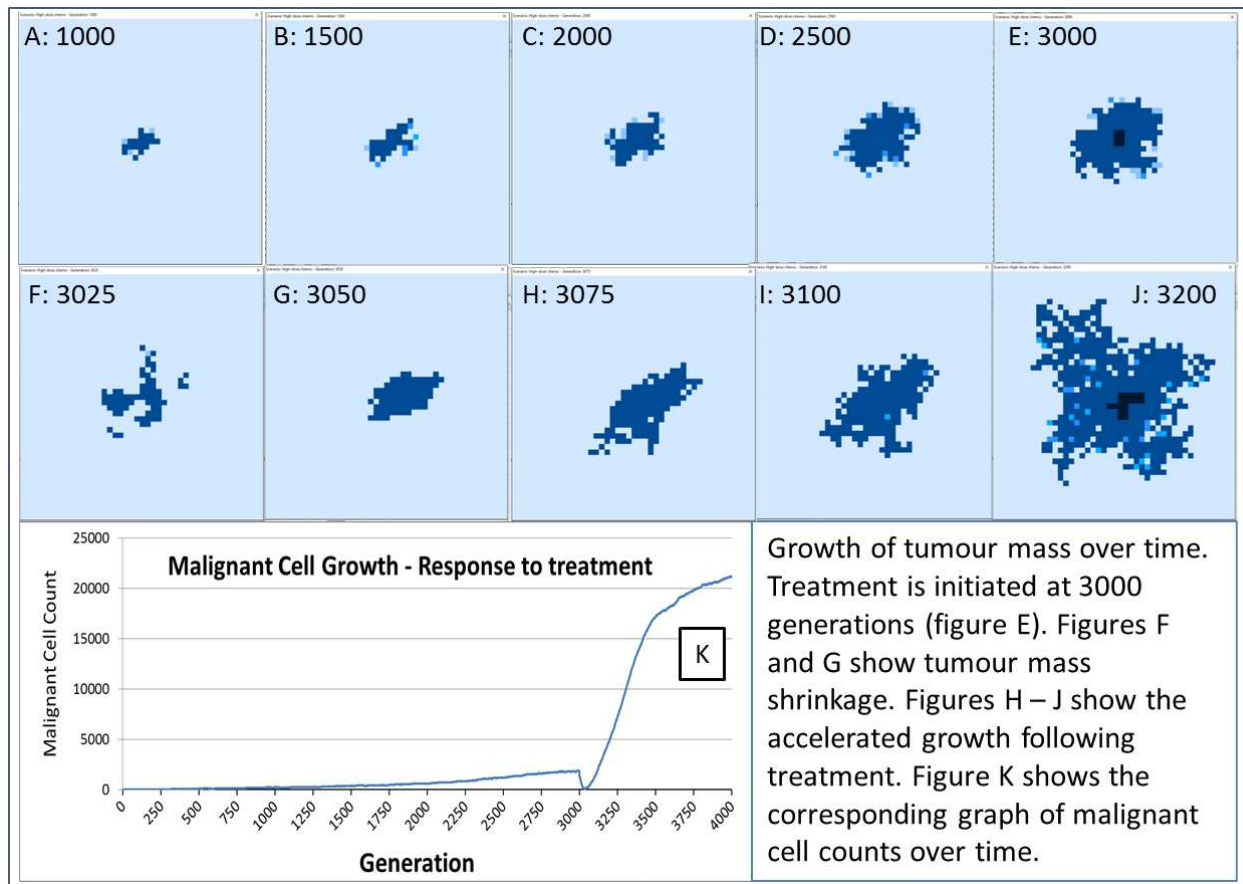
582 The rate of evolutionary change is initially set by the Mutation Rate, which is heritable and
583 mutable. It may be thought that the Mutation Rate would be an important driver in the rate of
584 cancer growth; however our data show that in this model it has a weak influence on the rate of
585 growth of cancer. It does however directly influence the size of the Gene Pool and the number of
586 clonal sub-populations. More influential in terms of driving growth is the Invasion Rate, which
587 represents the probability that a dividing Malignant cell in an overcrowded Grid Element can
588 migrate to a neighbouring Grid Element. The data show that this is a very strong driver of growth
589 rates, but it does not lead to the same increase in the size of the Gene Pool or the number of
590 clonal sub-populations. These observations are in line with results reported by Enderling et al
591 also using an agent-based model of tumour growth (Enderling, Hlatky & Hahnfeldt, 2009).

592 In terms of modelling interventions against tumour growth we have explored the use of a
593 treatment option that loosely mimics maximum tolerated dose chemotherapy in two key respects.
594 Firstly the treatment is not genetically targeted – it applies to both Normal and Malignant cells,
595 though we can confer an increased sensitivity to Malignant cells if required. Secondly the
596 treatment induces cell death in affected cells, analogous to the apoptotic or necrotic cell death
597 induced by chemotherapy. And finally cells are affected depending on where they are in the cell
598 cycle – which is modelled in this instance by the reading of the cell clock.

599 **Tumour Regrowth**

600 One of the most interesting emergent behaviours exhibited by the NEATG system is the response
601 of the modelled tumour mass to a treatment that mimics aspects of chemotherapy treatment.

602 The response to this treatment, which we have varied in intensity and duration, is consistent in
603 our experiments. There is an initial response marked by massive tumour kill followed by a
604 resumption of tumour growth, which is often characterised by an accelerated and aggressive
605 tumour expansion, as shown in Figure 16.



606

607 **Figure 16 - Growth/Regrowth of Tumour Mass**

608 This response to treatment bears some resemblance to real cancer treatment, where an initial
 609 reduction in tumour growth, characterised as complete or partial remission, is followed by
 610 renewed tumour growth or the appearance of metastatic disease. Clinically this phenomenon is
 611 sometimes termed accelerated repopulation (Davis & Tannock, 2000; Kurtova et al., 2015; Yom,
 612 2015). While the mechanisms of treatment resistance in real tumours are complex and
 613 multifactorial it is assumed that tumour heterogeneity is an important factor; a tumour may
 614 harbour clonal subpopulations which are resistant to treatment and which therefore benefit from
 615 reduced competition after chemo-sensitive populations have been destroyed by treatment (von
 616 Manstein et al., 2013; Gottesman et al., 2016).

617 In the NEATG model treatment resistance is not related to drug efflux or other mechanisms of
 618 acquired resistance. Instead the phenomenon is associated with a pool of cells which survive due
 619 to their age (i.e. they are above the treatment cut-off age) and which are therefore faced with a
 620 decreased level of competition for resources and a lower population density of cells in each Grid
 621 Element. This is a finding in line with the Norton-Simon hypothesis in which a proportion of
 622 cancer cells in tumour are resistant not due to biochemical factors but due to the growth kinetics
 623 of the tumour (Norton & Simon, 1977).

624 Increasing the intensity or duration of treatment as a strategy to improve response is shown to be
 625 problematic in that it can cause reductions in Normal cell numbers which do not recover and
 626 therefore this strategy is assumed to be deleterious. Again, there is a clear parallel to clinical

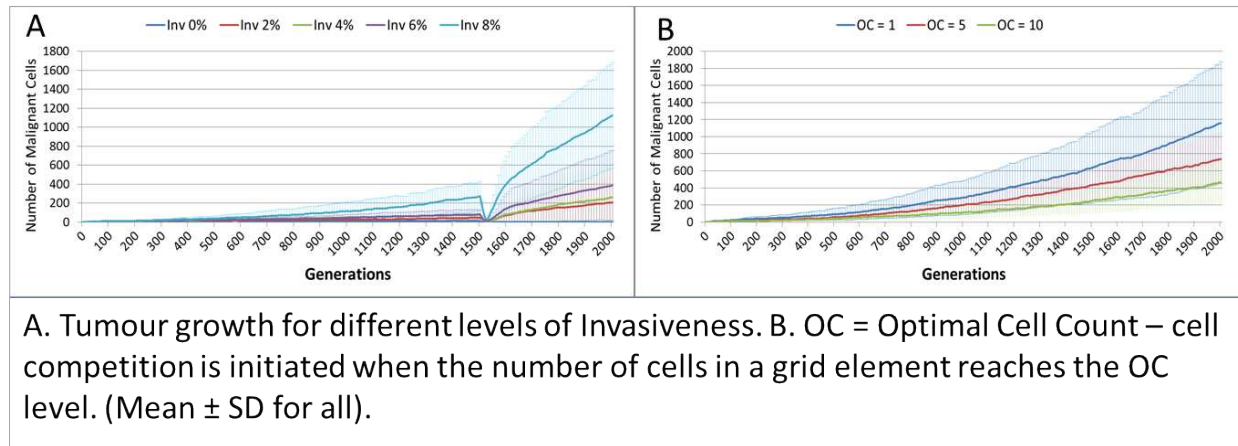
627 experience in which increased toxicity causes excess morbidity without necessarily leading to
628 improved outcomes.

629 **The Role of Mutations**

630 The rule of genetic mutation is a central concern in oncology, both in terms of fundamental
631 theories and increasingly at a clinical level in terms of targeted treatments. At a simplistic level
632 the SMT places the delinquent cell at the centre of cancer development, whereas the TOFT
633 places the poor neighbourhood central to the story (Baker, 2014; Sonnenschein et al., 2014). A
634 key difference between these competing theories is the role of cellular proliferation. The SMT
635 suggests that in the non-transformed state cells are non-proliferative by default. Mutations in
636 genes associated with cell cycle control mean cells become proliferative and malignant. In
637 contrast the TOFT posits that cells are proliferative by default and that this proliferative ability is
638 kept in check at the tissue level. A disordered tissue results in the removal of the proliferative
639 blocks and the cell can multiply without control.

640 In our model both cell and tissue (Grid Element) level structures are featured. The process of
641 cancer initiation consists of seeding a transformed cell into a grid element and letting it
642 proliferate. The model does not have anything to say about how the initial cell is transformed, it
643 is taken as a given. The initial cell has the same parameters as the untransformed cells, the only
644 difference is that proliferative blocks have been removed. The transformed cell, and its progeny,
645 is able to accumulate mutations during cell division and replication. Some of these mutations
646 will be deleterious and some will be advantageous, we would expect therefore that the average
647 fitness of the Malignant population will increase and that these advantageous mutations will
648 drive further evolutionary change – particularly mutations that increase the Invasion rate.
649 However this does not appear to occur. Indeed, a surprising result is that neither the Mutation
650 Rate nor the Invasion Rate, which are both heritable and mutable, appears to undergo significant
651 increase during the process of tumour growth. In fact, as shown in Figure 6B, both show
652 marginal rates of change, and can rise and fall rather than rising monotonically and driving
653 malignant growth. While some mutations may provide evolutionary advantage, it is clear that the
654 majority of mutations are passenger mutations rather than driver mutations. This is another
655 instance where the NEATG model parallels biological systems, as it has become increasingly
656 clear that the majority of somatic mutations in human tumours are also passenger mutations,
657 many of which are actively deleterious to the cancer cell (Greenman et al., 2007; McFarland et
658 al., 2013; McFarland, Mirny & Korolev, 2014).

659 The question arises then as to whether mutational change is a necessary precondition for cancer
660 growth in this model. To investigate this question an additional series of experiments was
661 performed in which the Mutation Rate was set at zero, and the Invasion Rate varied from zero to
662 8% in increments of 2%, with all other settings as in the previous set of experiments. The results
663 show that Malignant cell growth can occur even with a zero Mutation rate, which was verified by
664 confirming that the Gene Pool retained a constant value of 1 (data not shown). This may be
665 viewed as analogous to tissue hyperplasia where non-transformed cells proliferate at an increased
666 rate. The rate of growth in this model, as shown in Figure 17A, depends on the Invasion Rate, as
667 one would expect, but even at the lowest non-zero rate tumour growth occurs, and furthermore
668 the growth rate accelerates after treatment.



669

670 **Figure 17 - Invasion Rate and Optimal Cell Count**

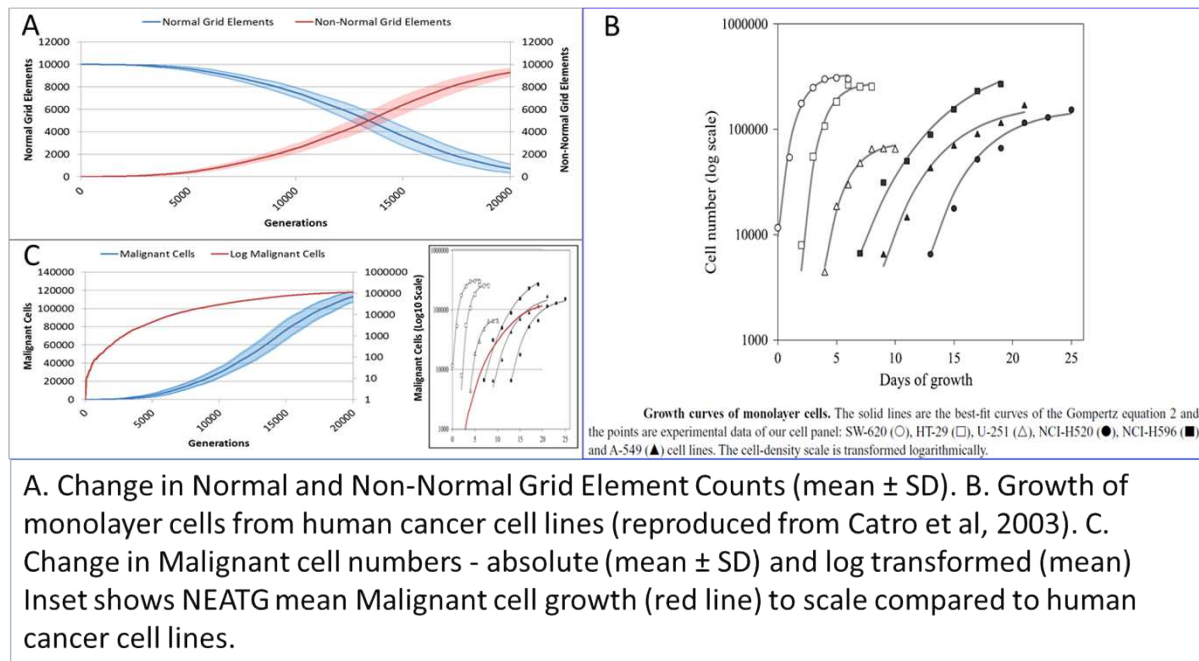
671 What is more, the data shows that with a zero rate of Invasion and Mutation there is growth in
 672 Malignant cell numbers to the maximum possible in the Grid Element where seeding occurred,
 673 but that without an Invasion Rate there is no possibility of a Malignant cell migrating to a
 674 neighbouring Grid Element. One implication of this result is that in the NEATG model cancer
 675 growth is not driven primarily by somatic mutation and is primarily dependent on proliferation
 676 and invasiveness.

677 **Reflecting on Real Tumour Growth**

678 Clearly this is a very simple model that does not incorporate many biologically relevant
 679 oncogenic mechanisms – the model was deliberately designed to be as parsimonious as possible.
 680 Validation of the growth curves produced by this model in comparison to biologically relevant
 681 systems is not straightforward but we can make some preliminary assessments. As NEATG is a
 682 two-dimensional grid model the most appropriate place to look for validation is *in vitro*
 683 monolayer systems using a range of different cancer cell lines. To fully assess that this is the case
 684 with the NEATG model a series of additional experiments were performed in which the model
 685 grid was scaled up and the run-time, in terms of the number of generations, was significantly
 686 extended by a factor of 10. Running NEATG with a grid size of 100 x 100 and for 20000
 687 generations allowed for a more realistic generation of cell numbers and tumour growth, (albeit at
 688 the cost of increased computation time).

689 The first thing to note is that the growth of the tumour area more clearly follows a sigmoidal
 690 curve, as shown by looking at the growth curves in Figure 18A. These results can be compared
 691 to the log growth curves in cell numbers for a range of human colon carcinoma, malignant
 692 glioma and non-small cell lung carcinoma cell lines reported by Castro et al (Castro et al., 2003),
 693 and reproduced in Figure 18B. We can compare these results to the log transformed growth
 694 curve of Malignant cells, Figure 18C, and verify that there is a similar pattern of growth, and that
 695 the asymptotic cell numbers are of roughly the same order of magnitude. Furthermore we can
 696 compare the x-axes and make an informal estimate that in these experiments 1000 generations in
 697 NEATG roughly corresponds to one day of growth in the monolayer systems used by Castro et al
 698 in their experiments. In doing so we can scale the NEATG Malignant cell growth line
 699 appropriately and compare to the human cancer cell lines growth, as shown in the inset in Figure
 700 18C.

701 While this falls short of fully validating the growth patterns produced by NEATG, they do
 702 indicate that the growth rates that emerge from model share some quantitative and qualitative
 703 features of the growth of human cancer cell lines in vitro.



704

705 **Figure 18 - Comparison to real tumour growth**

706 Yet, given the limited physiology modelled by the system it has reproduced a series of emergent
 707 phenomena which are analogous to biologically relevant phenomena– tumour growth, intra-
 708 tumour genetic heterogeneity, response to virtual cytotoxic intervention and accelerated
 709 repopulation.

710 By definition this is an evolutionary model, ‘descent with modification’ is a given, but as we
 711 have seen it is also possible to run the model with a zero mutation rate and still generate a
 712 growing population of Malignant cells. One notes that although they harbour no mutations and
 713 may be considered Normal cells with a hyperplastic phenotype, there are also rare instances of
 714 cancers in which no genetic mutations or epigenetic drivers are present (Versteeg, 2014). We
 715 have also defined Malignant cells as those with the ability to mutate and to move into
 716 neighbouring Grid Elements. How these abilities arise is not a question we are investigating in
 717 the model. What then are the key drivers of tumour growth and accelerated repopulation? The
 718 detailed analysis of the behaviours outlined in the Results suggests that there are two key drivers:

- 719 • Cell competition
- 720 • Cell death

721

722 Competition occurs in the NEATG model within each Grid Element when the population density
 723 reaches a set level (the optimum cell count). As can be seen in Figure 17B, when competition
 724 begins earlier (when the optimum cell count is 1), the rate of tumour growth is much higher. As
 725 one would expect, competition also spurs growth of the gene pool (data not shown). Competition

726 for resources leads to cell death when the number of cells exceeds the carrying capacity of the
727 Grid Element. A ranked selection algorithm means that the least fit (within that Grid Element)
728 cells are removed. Importantly, this competitive process takes place entirely within a Grid
729 Element and is a process that involves both cell-to-cell (cell-autonomous) and tissue-level (non-
730 cell-autonomous, defined by the optimum cell count for the Grid Element) factors.

731 Cell death arises both from the competition between cells within each Grid Element and also
732 exogenously via ‘treatment’ – in this work loosely modelled on maximum tolerated dose
733 chemotherapy. It is clear from the data that increasing the rate of cell death, both in Normal and
734 Malignant cells, leads to accelerated repopulation and more aggressive tumour growth.

735 So, while both cell competition and cell death are both integral components of the model, the
736 *impact* that these have is not pre-programmed. These are unexpected key drivers of the emergent
737 behaviours that the model displays and the major findings of this work.

738 Many of the *core* findings from molecular biology are *not* included in this model. For example
739 the NEATG model does not explicitly make use of the cancer stem cell hypothesis. Cancer stem
740 cells (CSC), also known as tumour-initiating or cancer-initiating cells, are functionally
741 characterised as a small fraction of tumour cell populations with the ability to self-renew,
742 differentiate into multiple cell types and to generate new tumours when transplanted (Reya et al.,
743 2001; Jordan, Guzman & Noble, 2006; Bozorgi, Khazaei & Khazaei, 2015). Crucially, CSC are
744 assumed to generate the non-CSC cells which make up the major population of malignant cells
745 in a tumour. In addition to being characterised by a range of cell markers (CD44+, CD133+,
746 ALDH1 etc), CSC are theorised to be relatively chemo- and radio-resistant and a key factor in
747 resistance to treatment (Yang & Rycaj, 2015).

748 However, the CSC hypothesis is increasingly being challenged as evidence emerges that rather
749 than being a distinct cell population there is a set of properties which together define ‘stemness’
750 (Lewis, 2008; Antoniou et al., 2013; Wang et al., 2015). In particular the claim that tumour
751 growth is mainly attributable to the rapid proliferation of CSC populations rather than the non-
752 CSC fraction is open to some dispute (Adams & Strasser, 2008; Hegde et al., 2012). Additionally
753 there is evidence that cancer cells display a significant degree of plasticity such that ‘stemness’
754 traits can be acquired by non-CSC cells (Chaffer et al., 2011; Cabrera, Hollingsworth & Hurt,
755 2015). Indeed some recent work suggests that non-CSC cells acquire stem-like properties in
756 response to therapeutic challenge with chemotherapy (Hu et al., 2012; Martins-Neves et al.,
757 2016).

758 NEATG, therefore, does not explicitly model CSC and non-CSC populations but makes the
759 simplifying assumption that all Malignant cells are proliferative. The key point is that the
760 existence of CSC, whether as a separate population of cells or a collection of cellular traits, is
761 immaterial to the operation of the model and the ability to reproduce tumour cell growth. At this
762 level of abstraction the behaviour of the model would be the same regardless of the underlying
763 complexities of the CSC hypothesis.

764 Similarly the model does not include oncogenes, specific molecular pathways, a realistic cell
765 cycle, a vascular or lymphatic system, immune responses, different cell types, tumour stroma and
766 many more biologically important aspects of real disease. However, the model does propose that
767 cell competition and cell death have an important, and perhaps underestimated, role in patterns of

768 tumour growth and response to treatment. Given that induction of cell death, particularly via the
769 apoptotic pathway, is central to the most common forms of cancer treatment this would be of
770 some clinical significance if confirmed in the laboratory. Based on these results it is therefore
771 hypothesised that cell competition and cell death are major drivers of tumour growth.

772 There are some indications that these two aspects of cancer biology are of biological
773 significance.

774 A number of investigators have looked at the question of the role of cell competition in cancer,
775 for example Baker and Li (Baker & Li, 2008), and Moreno (Moreno, 2008), both referring to
776 results from research in *Drosophila melanogaster* which outlined the process whereby cells of
777 differing genotype *within* a given compartment engage in competition such that *locally* less fit
778 cells undergo apoptosis and are replaced with *locally* fitter cells. Very recent work by
779 Suijkerbuijk et al has described the process whereby cell competition between APC^{-/-} intestinal
780 adenoma cells and normal host cells in *Drosophila melanogaster* leads to cell death in normal
781 cells, host tissue attrition and the invasion of more rapidly proliferating adenoma cells
782 (Suijkerbuijk et al., 2016). Eichenlaub and colleagues have also investigated cell competition in
783 the same animal model (Eichenlaub, Cohen & Herranz, 2016). They report that EGFR over-
784 expression in wing imaginal disc cells leads to benign tissue hyperplasia and subsequent
785 epithelial tumour formation.

786 We should note that there are different cell types, molecular drivers and pathways active in the
787 latter two studies, yet both groups report that blocking the apoptotic process blocks tumour
788 development. This prompts the conclusion that targeting cell competition itself may be a valid
789 strategy in cancer therapy (Gil & Rodriguez, 2016).

790 While cell competition may be a necessary pre-condition of cancer development, it is not
791 sufficient, and our model clearly indicates that cell death is also required. This poses the question
792 as to the role of cell death, particularly apoptosis, in tumour growth. One of the hallmarks of
793 cancer is defined as ‘resistance to apoptosis’ (Hanahan & Weinberg, 2011), yet it is known that
794 tumours show a high rate of apoptosis, and at least in some cancer types high apoptosis rates are
795 a negative prognostic factor (Nishimura et al., 1999). A number of recent studies have outlined
796 the much more complex relationship between cancer and apoptosis than has been assumed in the
797 past (Gregory & Pound, 2011; Wang et al., 2013; Labi & Erlacher, 2015; Lauber & Herrmann,
798 2015; Ford et al., 2015). While these studies outline numerous mechanistic explanations as to
799 why increased apoptosis may lead to increased tumour growth, it is clear that there are
800 underlying phenomena which may have important clinical implications in terms of treatment
801 strategies.

802 One rather obvious conclusion is that rather than aiming at maximum tumour kill using
803 traditional cytotoxic chemotherapy perhaps, other treatment strategies which produce lower
804 levels of cancer cell death may be more beneficial. For example, using metronomic
805 chemotherapy, in which chemotherapeutic drugs are administered at non-cytotoxic doses and
806 with no treatment breaks is one such strategy (Scharovsky, Mainetti & Rozados, 2009; Kareva,
807 Waxman & Klement, 2014; André, Carré & Pasquier, 2014). Another example is the concept of
808 ‘adaptive therapy’, in which chemotherapy is used to maintain a population of tumour cells
809 rather than aiming to maximise tumour kill (Gatenby et al., 2009; Enriquez-Navas et al., 2016).

810 While it is clear that the NEATG system does not provide us with mechanistic explanations for
811 the pro-tumour growth effects of cell competition and apoptosis, it does direct our attention to
812 these areas of current, active but not yet mainstream research. Having directed our attention to
813 the role of cell competition and cell death there is ample scope for continuing to use the model to
814 explore the processes at work and, perhaps, to suggest relevant laboratory experiments in light of
815 further model results.

816 **Conclusion**

817 Having identified cell competition and apoptosis as key concerns we may look to incorporate
818 additional aspects of this in more detail. For example the onset of cell competition is triggered
819 when the optimum cell count is reached. In part this is a function of the carrying capacity of the
820 Grid Element – when this level is exceeded Malignant cells are able to migrate to a randomly
821 selected neighbouring Grid Element (a stochastic process depending on the Invasion Rate). In
822 some respects this is analogous to tissue stiffness or rigidity in that Grid Elements can be made
823 more or less ‘stiff’ by increasing or decreasing the carrying capacity. Tissue stiffness is also a
824 current concern in oncology (Wei & Yang, 2016) and may be amenable to additional
825 investigation by extending this model.

826 NEATG has been designed as a platform for investigating different interventions and how they
827 impact the growth of Malignant cells and tumour Grid Elements. In the experiments described in
828 this paper only one strategy, loosely based on maximum tolerated dose chemotherapy, has been
829 explored. Clearly there is scope for additional interventions to be modelled, for example
830 combinations of Nutrient restriction and chemotherapy, a treatment strategy of some clinical
831 interest (Raffaghello et al., 2008; Safdie et al., 2009; Lee et al., 2012), may be modelled in
832 NEATG. Similarly the use of metronomic chemotherapy, chemo-switch strategies, targeted
833 therapies and the use of different treatment schedules are also amenable to modelling using the
834 NEATG system.

835 The value of agent-based evolutionary models is that they can generate biologically relevant
836 behaviour through algorithmic means, which may in turn shed new light on the underlying
837 biological systems. Obviously increasing the complexity of the model so that additional features
838 are included may be of some value. Here we have generated hypotheses as to the role that cell
839 competition and cell death have in cancer, suggesting that these relatively under-researched
840 processes may have much greater important than has hitherto been accepted. At the same time
841 the model has not required the implementation of cancer stem cell populations, specific
842 oncogenic pathways and has shown a limited role for genetic mutation – all of which are
843 currently predominant concerns in much cancer research.

844

845

846 **References**

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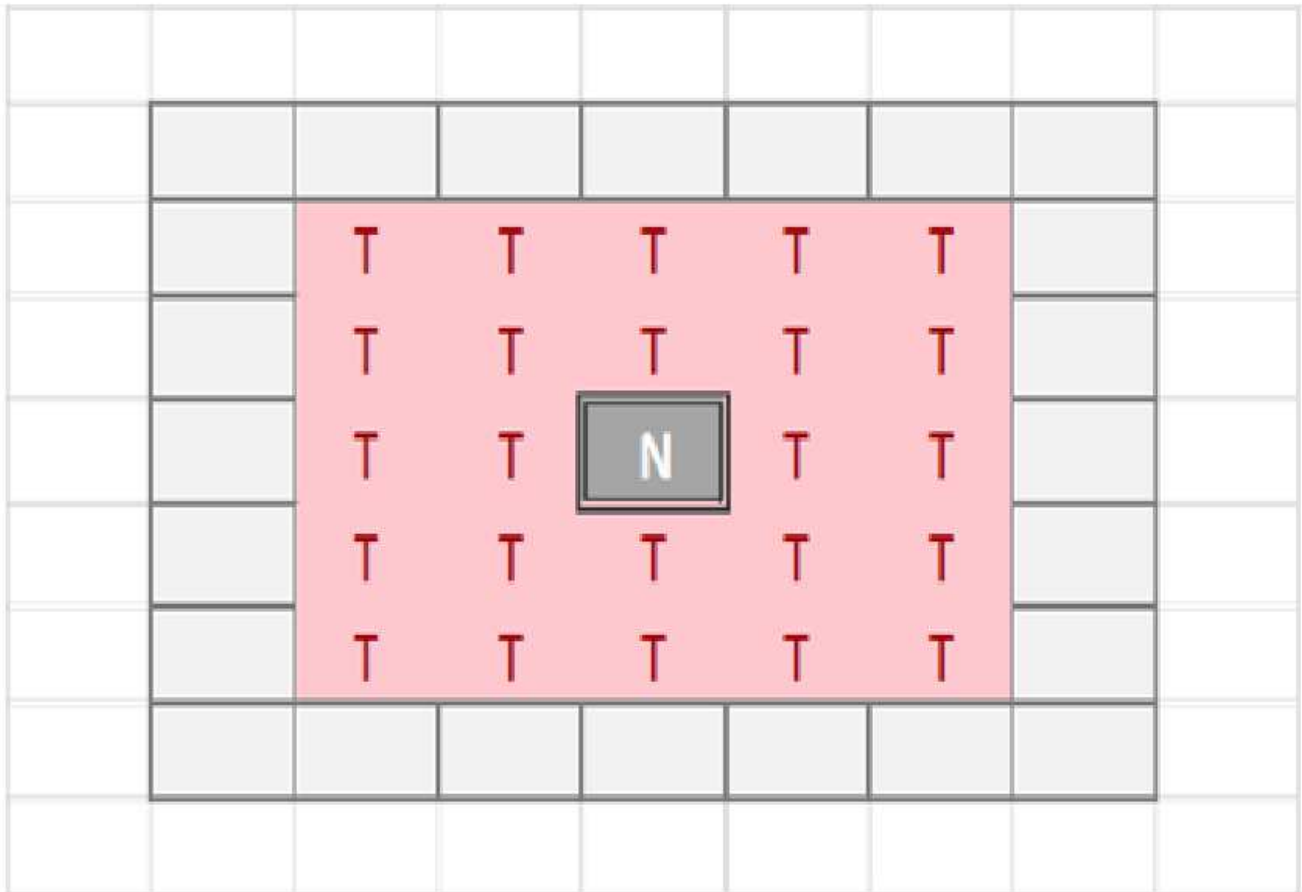
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1

Moore Neighbourhood of radius 2

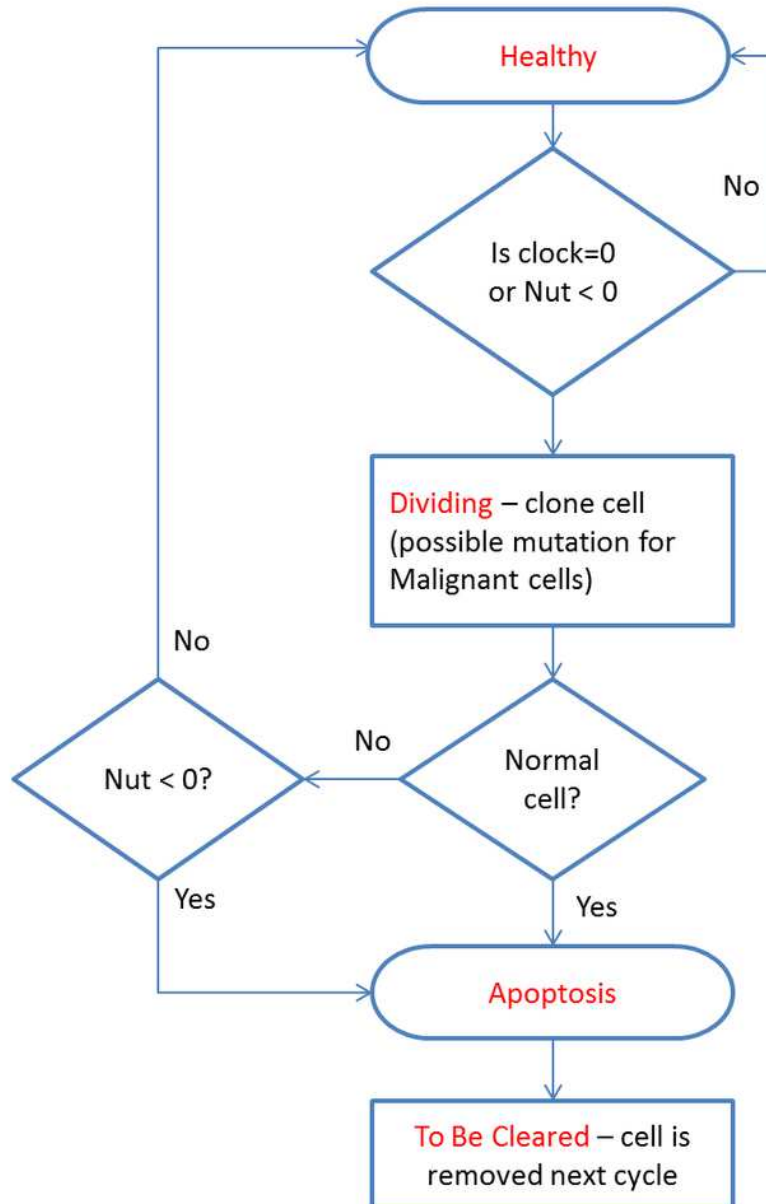
Figure 1. Moore Neighbourhood of radius 2



2

Cell cycle algorithm

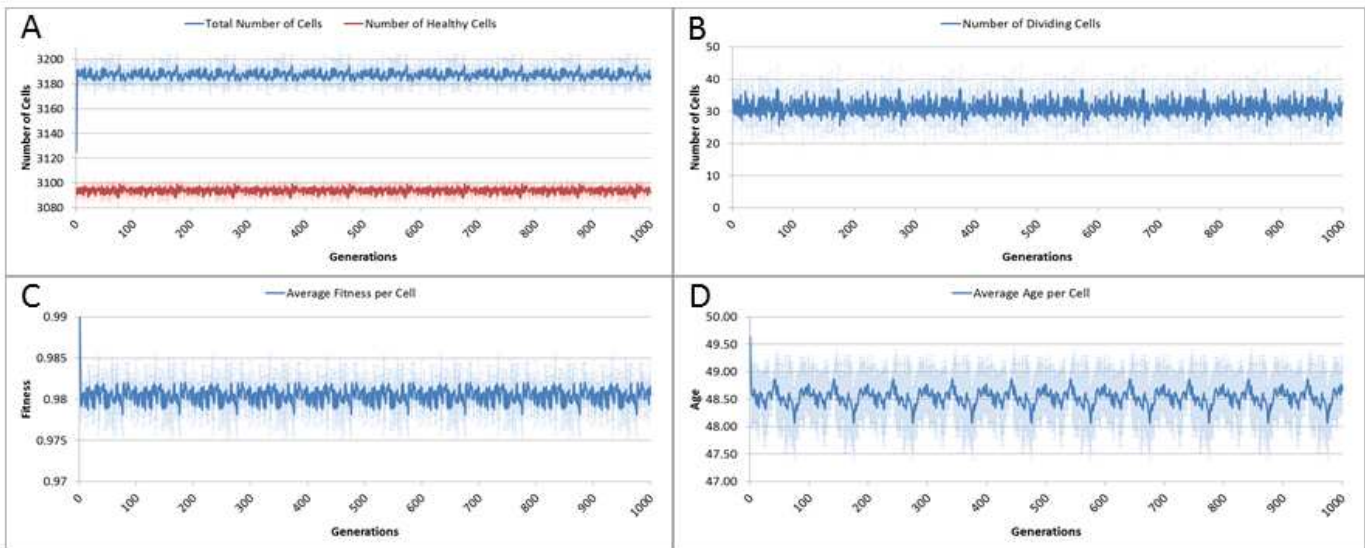
Figure 2. Cell cycle algorithm



3

Cell change over time

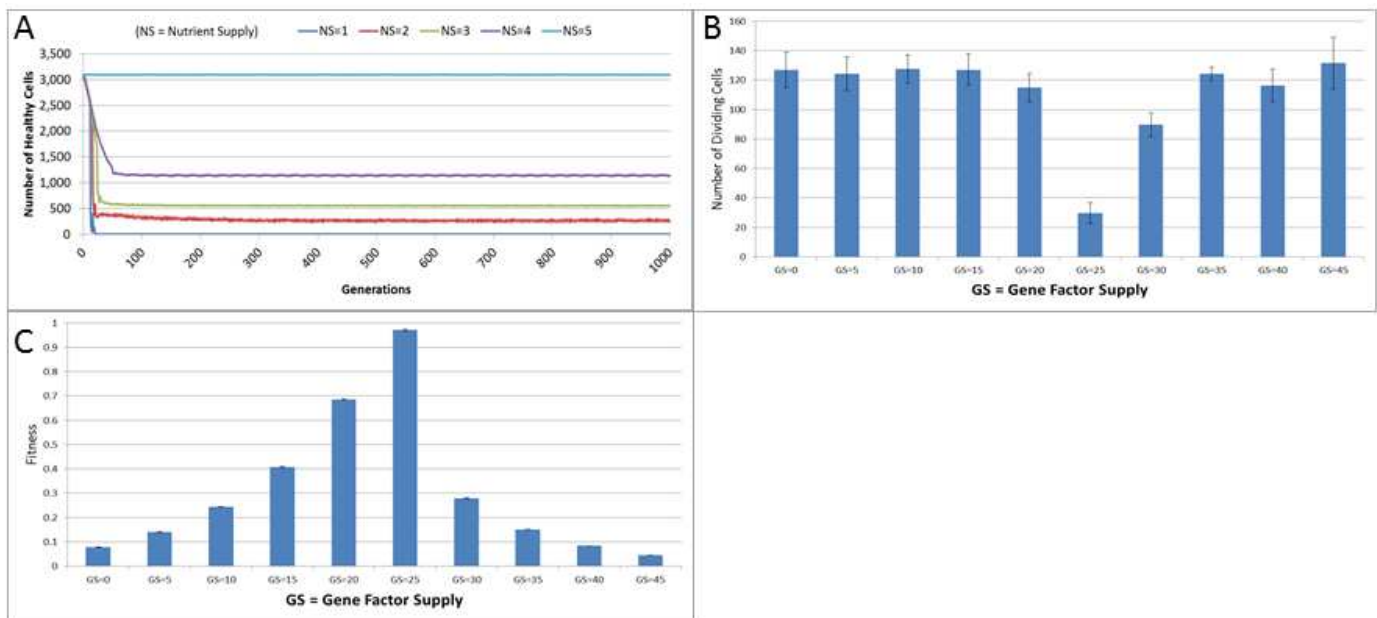
Figure 3. A. Total and healthy cell counts over time. B. Number of dividing cells over time. C. Average cell fitness over time. D. Average cell age over time. (All mean \pm SD)



4

Changes during stress conditions

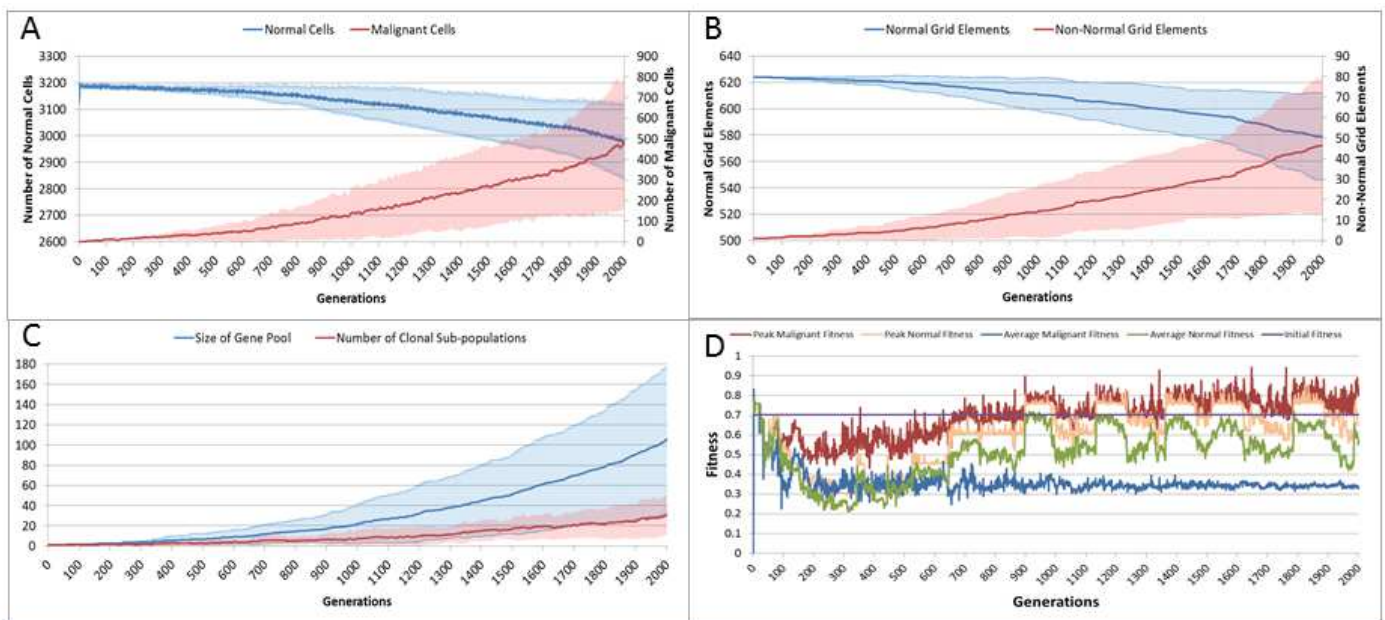
Figure 4. A. Change in Healthy Cell count numbers in response to underfeeding. B. Change in rate of cell division vs Gene Factor Supply (at 1000 generations). C. Change in average fitness vs Gene Factor Supply (at 1000 generations). (All mean \pm SD)



5

Tumour growth - no treatment

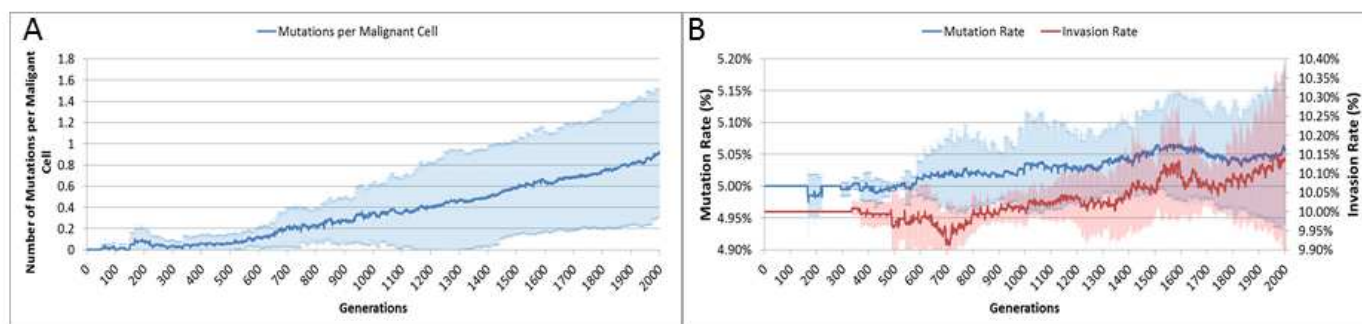
Figure 5. A. Change in Normal and Malignant cell counts (Mean \pm SD). B. Change in Normal and Non-Normal Grid Element Counts (All mean \pm SD). C. Change in Gene Pool and Clonal Populations Over Time (All mean \pm SD). D. Change In Fitness Over Time (Mean).



6

Mutation rates over time

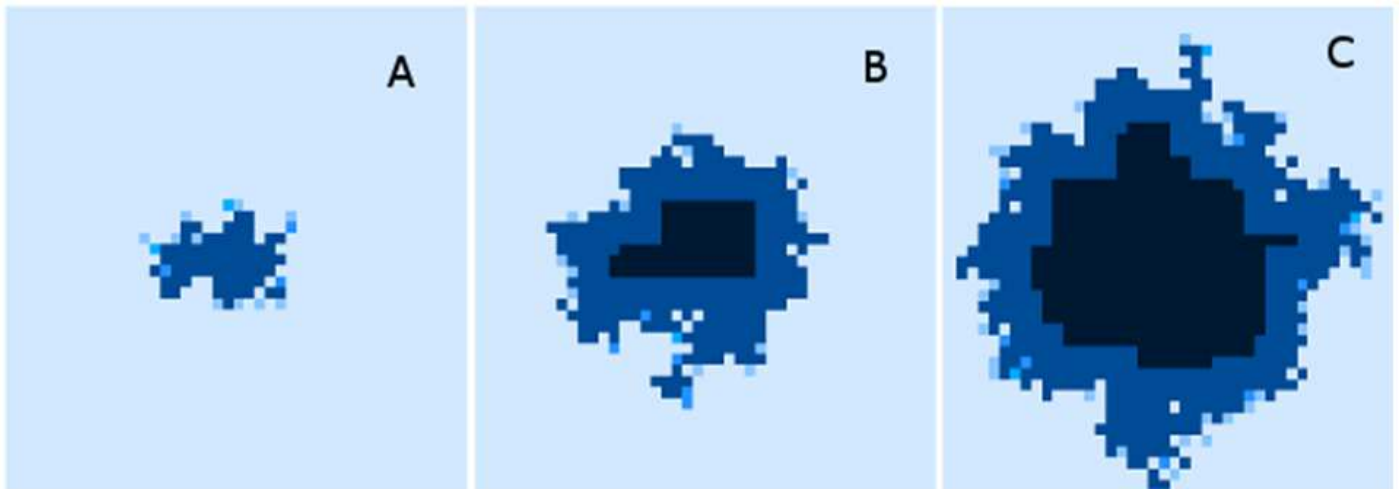
Figure 6. A. Mutations per Malignant cell over time. B. Change in Mutation and Invasion Rates over time. (All mean \pm SD)



7

Spatial distribution of tumour growth

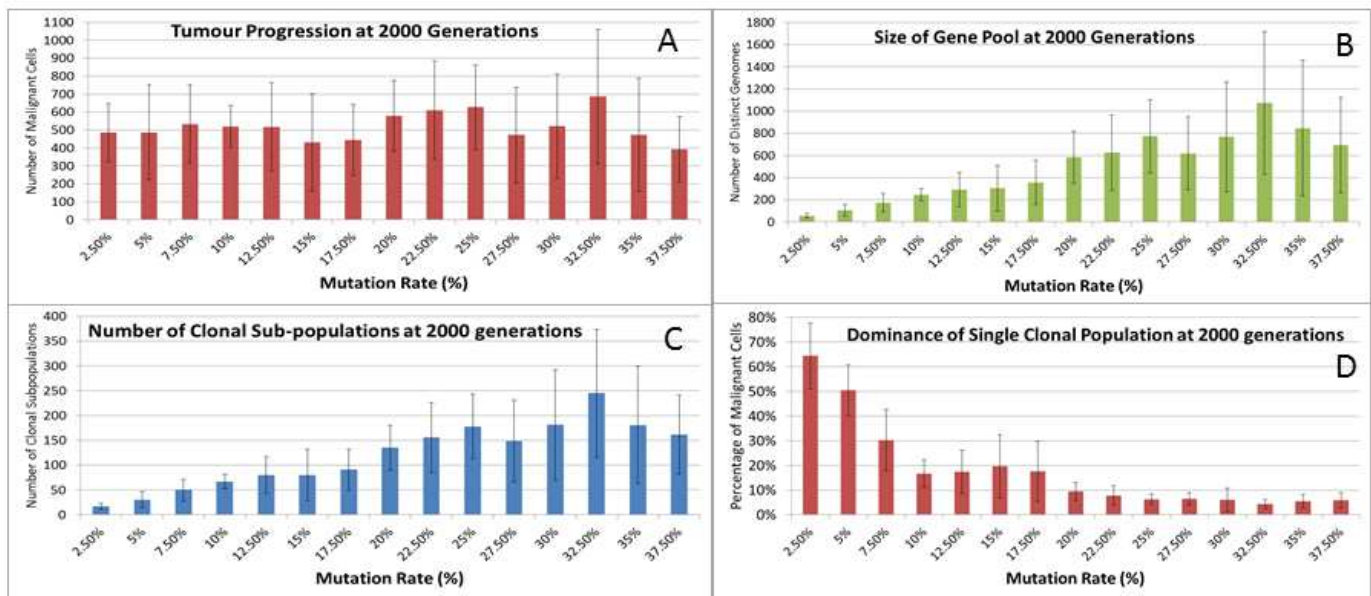
Figure 7. Evolving tumour mass at A: 2000 generations. B: 4000 generations. C: 6000 generations. Note that black areas are necrotic grid elements.



8

Mutation rates and clonal sub-populations

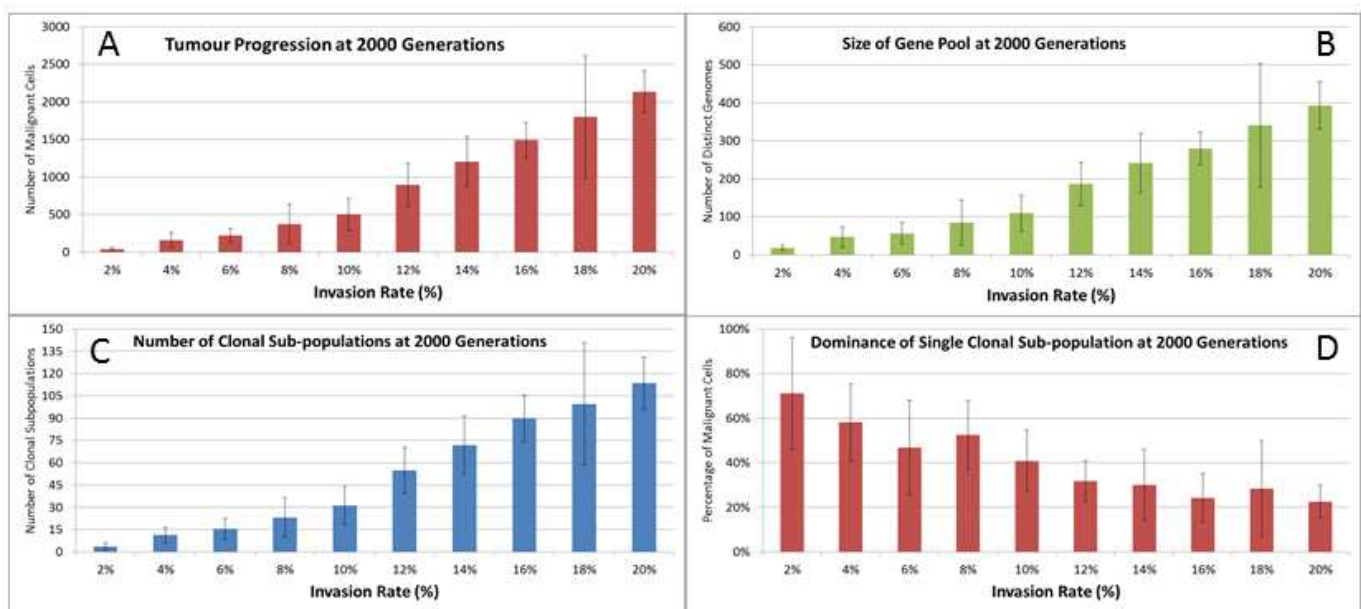
Figure 8. A. Number of Malignant Cells. B. Size of Gene Pool. C. Number of Clonal Sub-populations. D Dominance of Single Clonal Population. (All at 2000 generations, mean \pm SD).



9

Invasion rates and clonal sub-populations

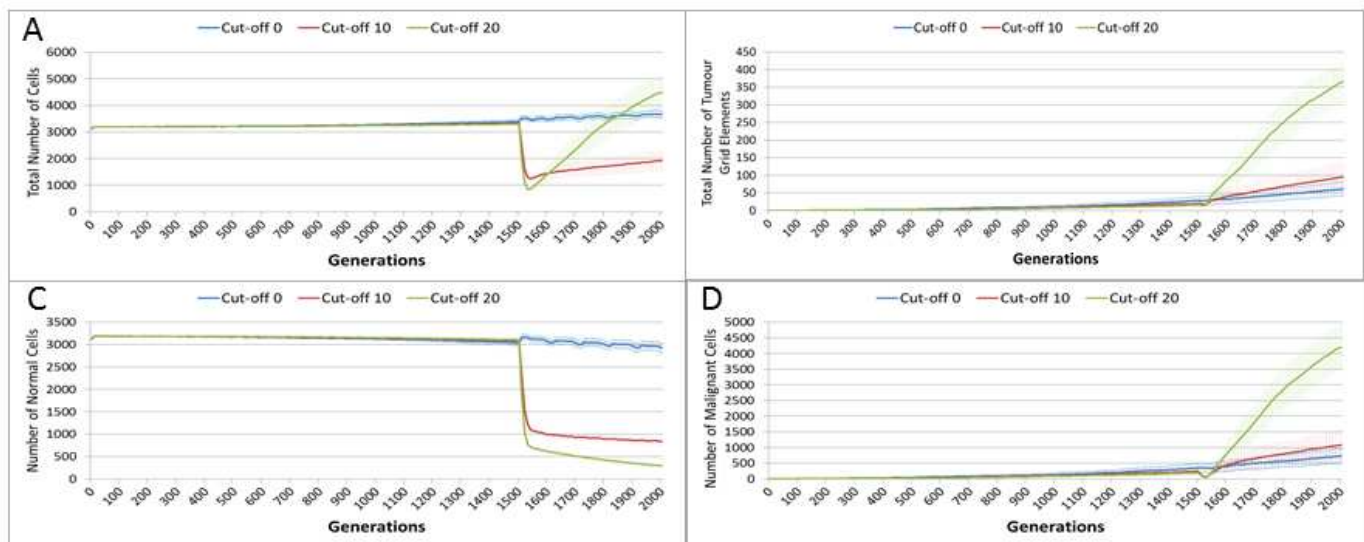
Figure 9. A. Number of Malignant cells. B. Size of Gene Pool. C. Number of Clonal Sub-populations. D. Dominance of Single Clonal Populations. (All at 2000 generations, mean \pm SD)



10

Tumour response to treatment toxicity

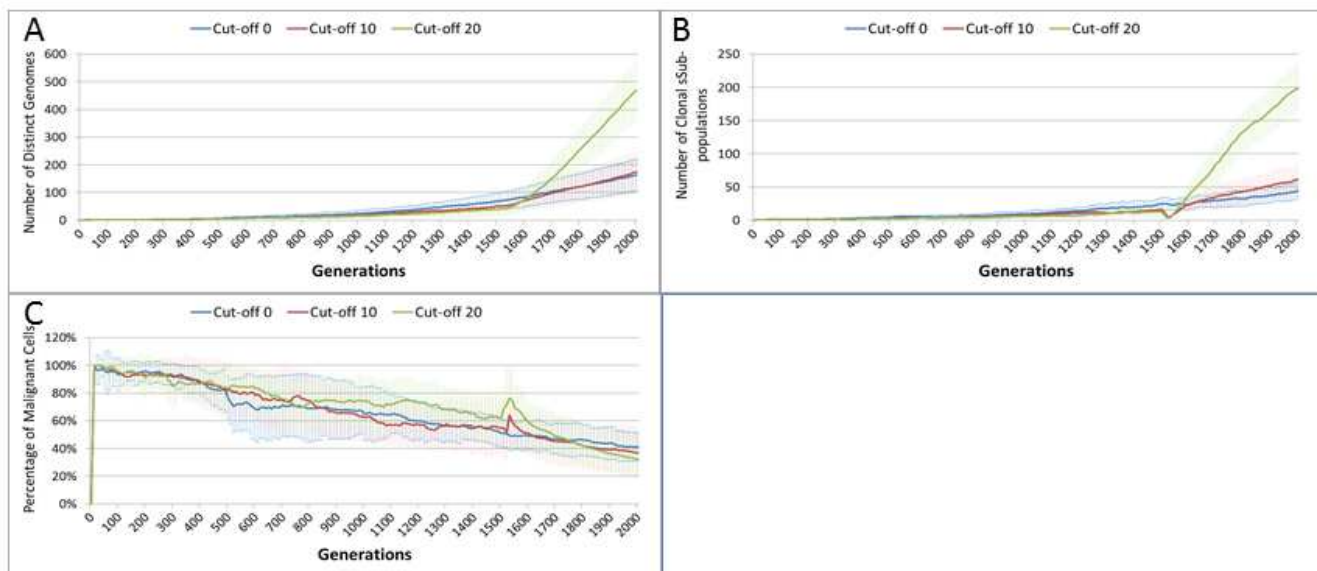
Figure 10. Treatment toxicity is altered by varying the cut-off of cell age below which cells are affected by cytotoxic treatment (e.g. Cut-off = 10 means all cells with a clock value ≤ 10 are flagged for removal). A. Change in total cell numbers vs toxicity. B. Change in Malignant cell numbers vs toxicity. C. Change in Normal cell numbers vs toxicity (Mean \pm SD for all).



11

Treatment toxicity and clonal sub-populations

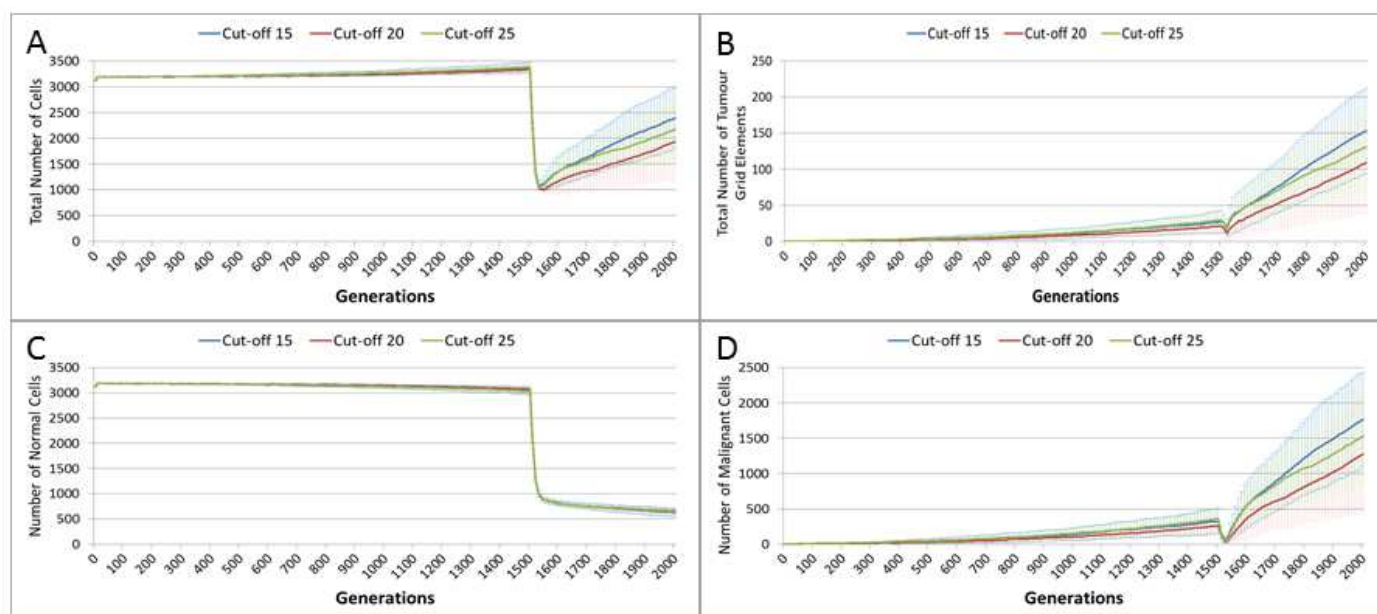
Figure 11. Treatment toxicity is altered by varying the cut-off of cell age below which cells are affected by cytotoxic treatment (e.g. Cut-off = 10 means all cells with a clock value ≤ 10 are flagged for removal). A. Change in total cell numbers vs toxicity. B. Change in Malignant cell numbers vs toxicity. C. Change in Normal cell numbers vs toxicity (Mean \pm SD for all).



12

Tumour response to differential treatment toxicity

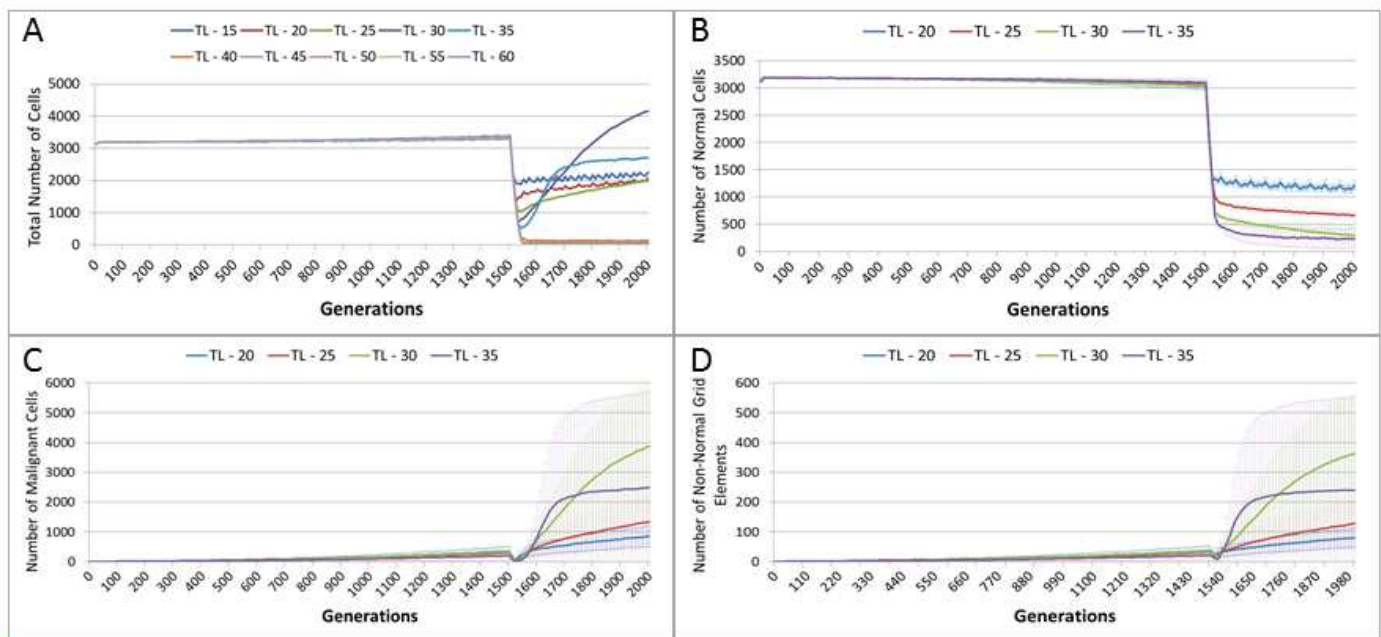
Figure 12. Differential treatment toxicity is modelled by varying the cell age below which cells are affected by cytotoxic treatment (e.g. Cut-off = 10 means all cells with a clock value ≤ 10 are flagged for removal) and applying different cut-off values for Normal and Malignant cells. Normal cell toxicity is fixed at cut-off = 10. A. Change in total cell numbers vs differential toxicity. B. Change in Malignant cell numbers vs differential toxicity. C. Change in Normal cell numbers vs differential toxicity. (Mean \pm SD for all).



13

Tumour response to treatment length

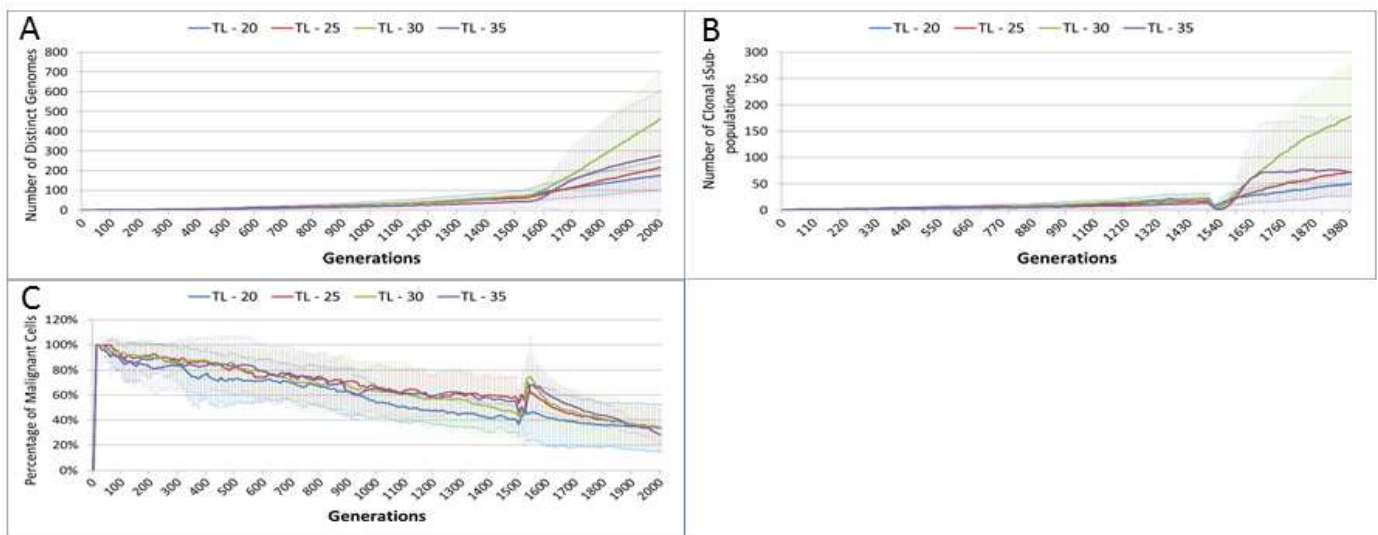
Figure 13. TL = Treatment Length (number of generations in which treatment is active). A. Total cell count vs treatment length (mean only). B. Normal cell count vs treatment length (mean \pm SD). C. Malignant cell count vs treatment length (mean \pm SD).



14

Treatment length and clonal sub-populations

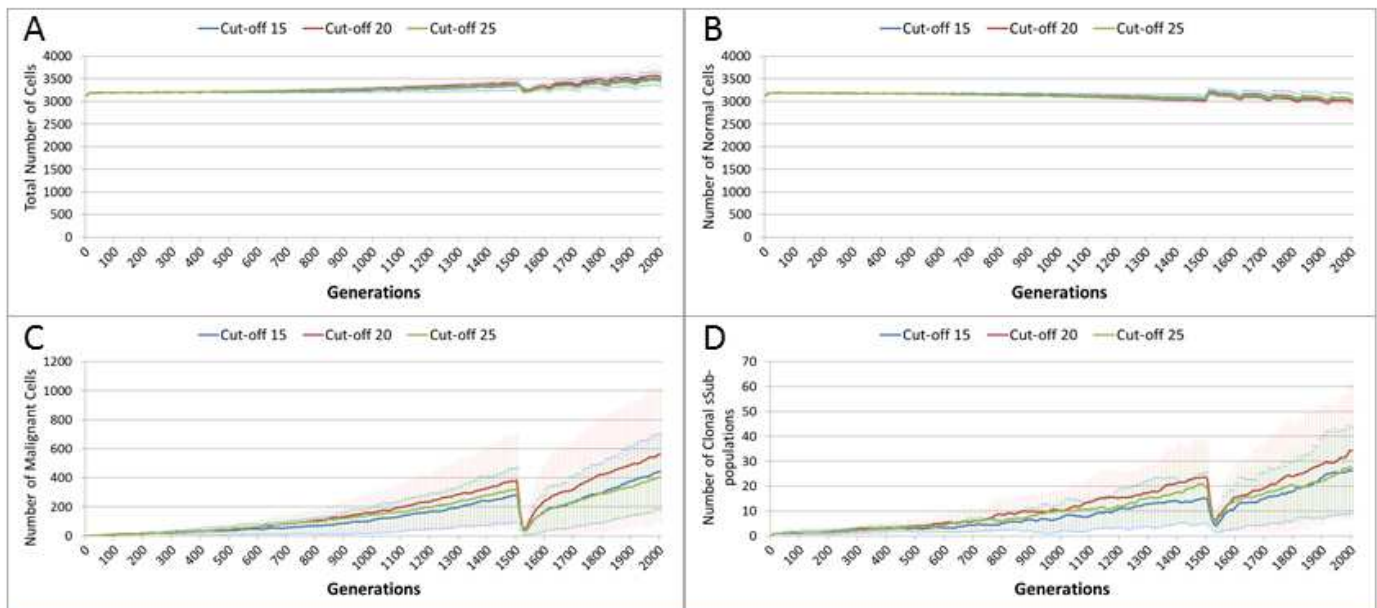
Figure 14. TL = Treatment Length (number of generations in which treatment is active). A. Size of Gene Pool vs Treatment Length. B. Number of clonal sub-populations vs Treatment Length. C. Sub-clonal Population Dominance vs Treatment Length. (Mean \pm SD for all).



15

Tumour response to 'magic bullet'

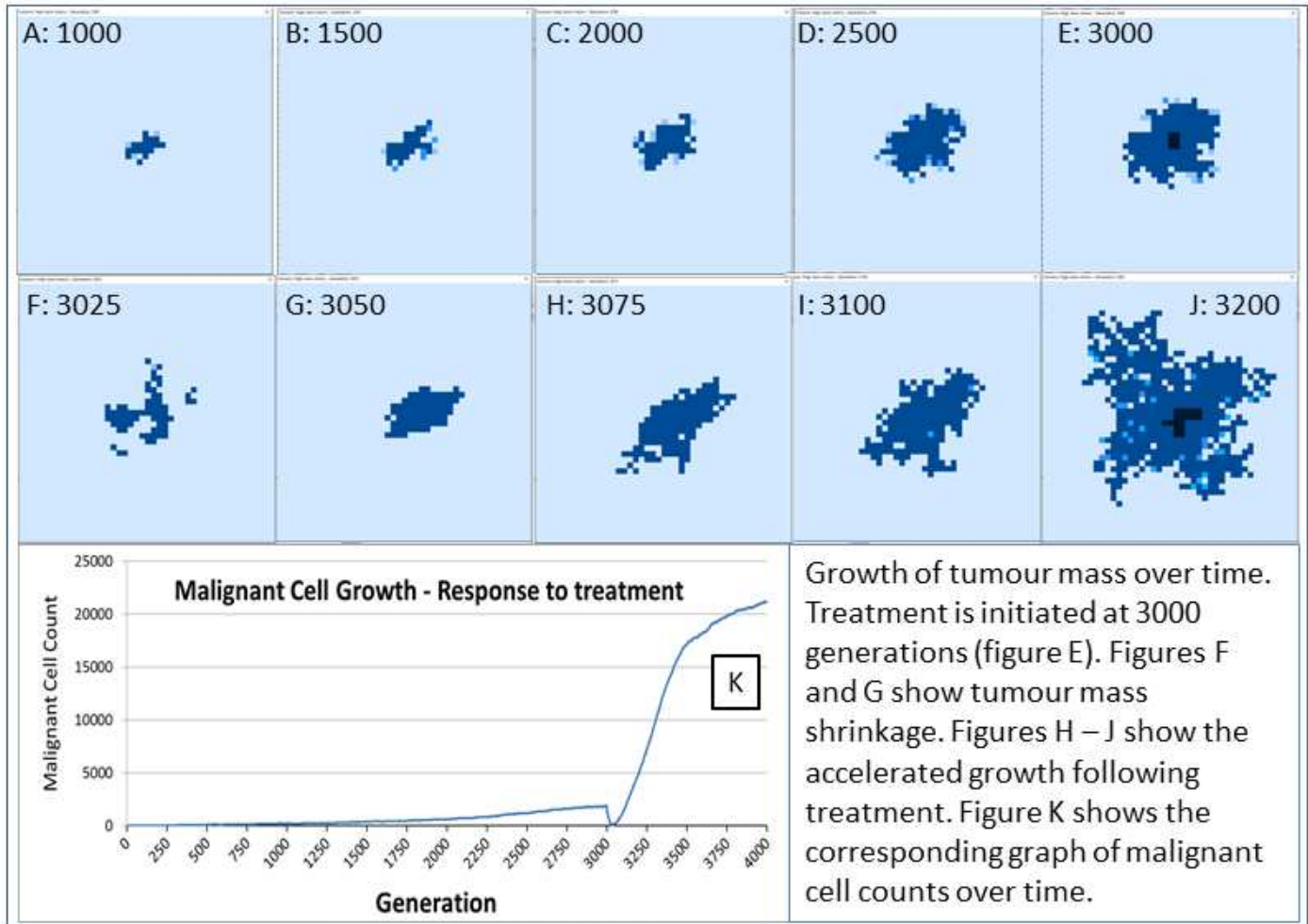
Figure 15. Treatment toxicity is altered by varying the cut-off of cell age below which cells are affected by cytotoxic treatment (e.g. Cut-off = 10 means all cells with a clock value ≤ 10 are flagged for removal). Here we model 'no collateral damage' and toxicity only applies to Malignant cells. A. Total cell count vs no collateral damage. B. Normal cell count vs no collateral damage. C. Malignant cell count vs no collateral damage. D. Clonal sub-populations vs no collateral damage. (Mean \pm SD for all).



16

Growth/Regrowth of Tumour Mass

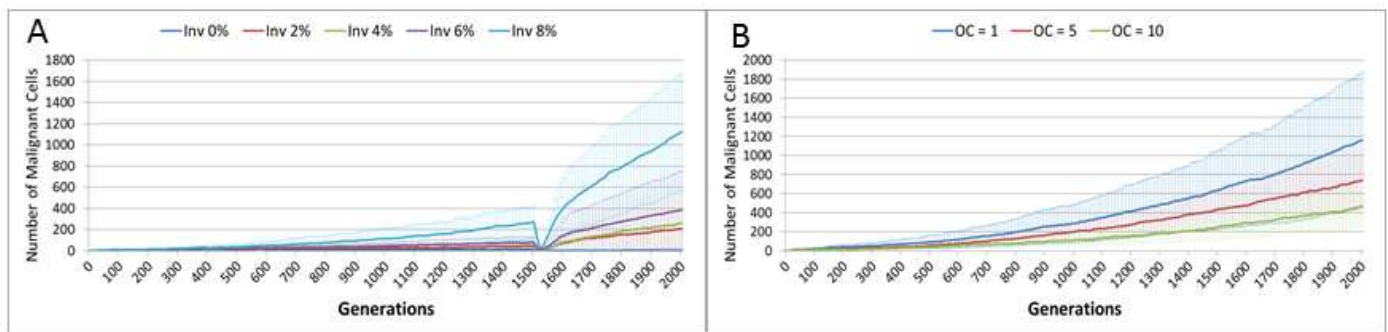
Figure 16. Accelerated repopulation of tumour mass after treatment



17

Invasion Rate and Optimal Cell Count

Figure 17. A. Tumour growth for different levels of Invasiveness. B. OC = Optimal Cell Count - cell competition is initiated when the number of cells in a grid element reaches the OC level. (Mean \pm SD for all).



18

Comparison to real tumour growth

Figure 18. A. Change in Normal and Non-Normal Grid Element Counts (mean \pm SD). B. Growth of monolayer cells from human cancer cell lines (reproduced from Castro et al, 2003). C. Change in Malignant cell numbers - absolute (mean \pm SD) and log transformed (mean) Inset shows NEATG mean Malignant cell growth (red line) to scale compared to human cancer cell lines.

