Growth of mixed tumor cell population – *in silico* the size matters
Supplementary materials

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1. Description of the model

The block diagram (Fig. S1) explains the main program operations. Implementation of GoL rules for cells of different sizes was performed by lattice transformation that occurs at each step of model iteration (Fig. S2c). The iteration of Line1 was executed on the "virtual" bigger cell lattice, and the iteration of Line2 occurred on the smaller cell lattice. Both operations together formed a single iteration step of the model. The program output contains the graphs for each iteration step visualizing the lattice and the growth curve (as in Fig. S7). The CSV files update the number of Line1, Line2 cells, and the percentage of lattice filling per iteration.
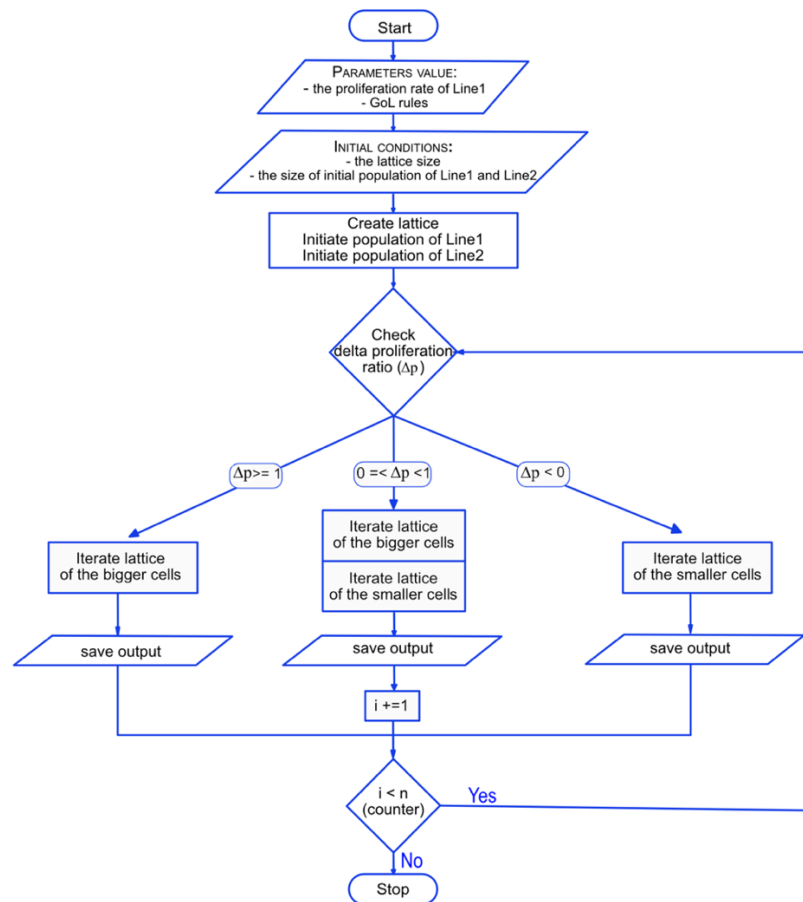

Fig. S1. The block diagram of the different cell sizes in the mixed culture program. The delta proliferation ratio ($\triangle p$) results from the subtraction of the proliferation rates of Line2 from Line1 and "n" is the number of iterations.
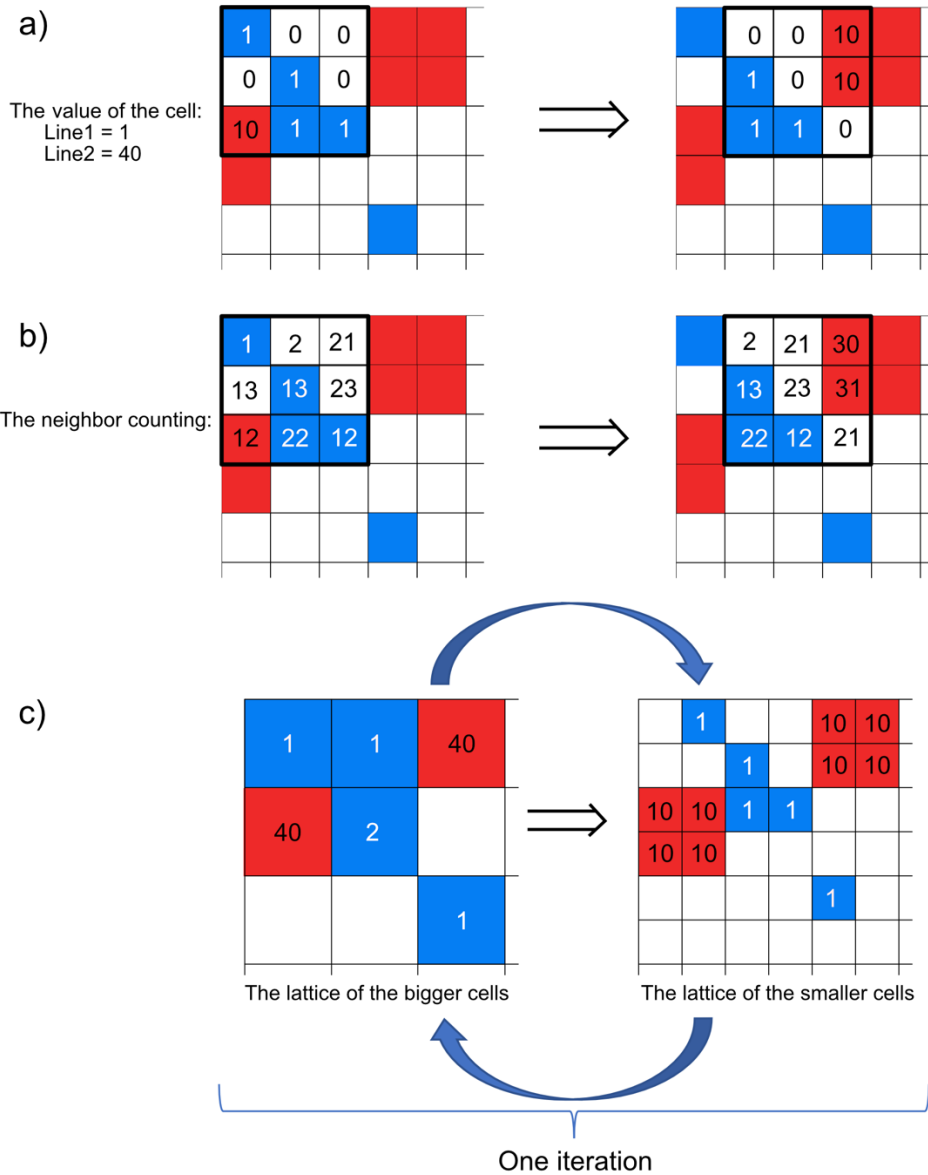
Fig. S2. Iteration of the different cell size mixed culture. (**a**) The lattice with cells of Line1 (red) and Line2 (blue) with its value. (**b**) The neighbor counting process. The frame is moving along the lattice. For each site, the program is counting the value of neighbor sites (i.e., adds 1 if the neighbor site is occupied by a Line2 cell and adds 10 when the neighbor site is occupied by a cell of Line1). In this way, the value of the current site is determined. In turn, this value will determine the future of that site, according to the implemented game of life rules. (**c**) The iteration step for lines of different cell sizes. The iteration of Line1 occurs on the "virtual" lattice of the bigger cells (red); next, the same lattice is converted into the lattice of the smaller cells (blue), and the iteration of Line2 is performed. Both of these operations form one iteration step of the mixed culture.

2

2. The initial placement of the cells

(a)
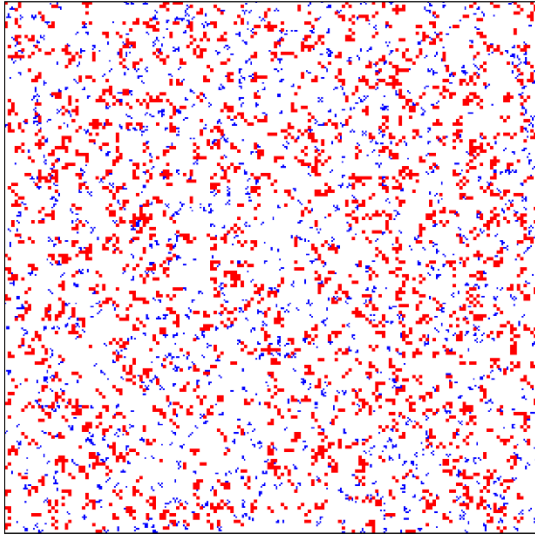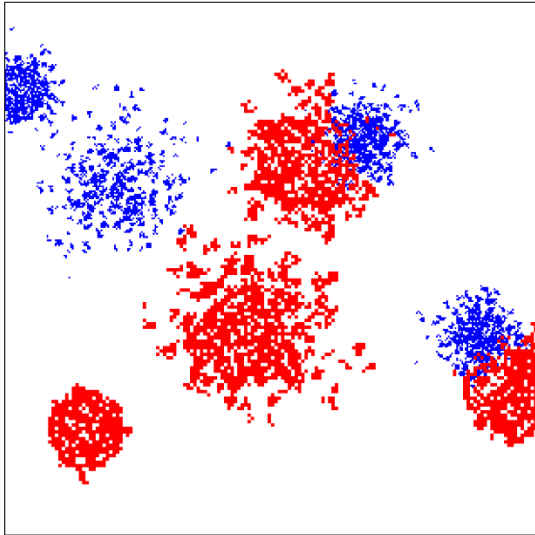
Fig. S3. Initial cells placement. The mixed cancer culture model offers four main distributions of cells in the initial population. (**a**) Mixed tumor (the random uniform distributions). (**b**) Collision tumor (4 clusters per line). (**c**) Collision tumor (1 cluster per line). (**d, e**) The cases of tumor-to-tumor metastasis, d - the scenario where the smaller cells of Line2 (blue) invade the bigger cells (red), e - the scenario where the bigger cells of Line1 (red) invade the smaller cells - (blue). All of the presented simulations are performed on the 100k lattice. The size of the initial population for figure (a), (b), (c) is 2.5k cells evenly distributed between lines. In (d), there are 1250 of the bigger cells (red ones) and 500 of the smaller cells. The opposite is true for (e) with 500 of the bigger cells and 1250 for the smaller ones.

(b)
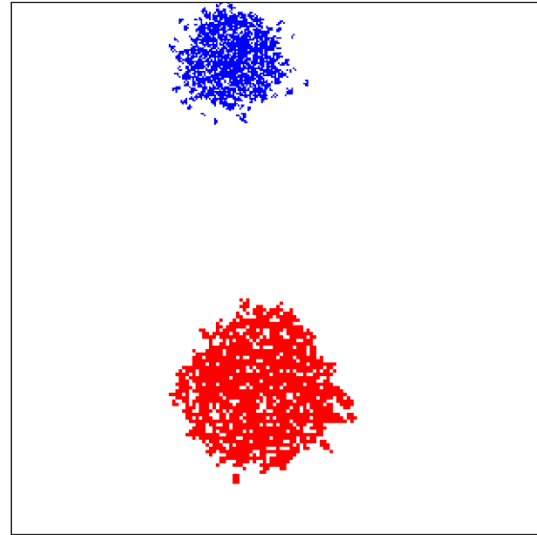
(c)
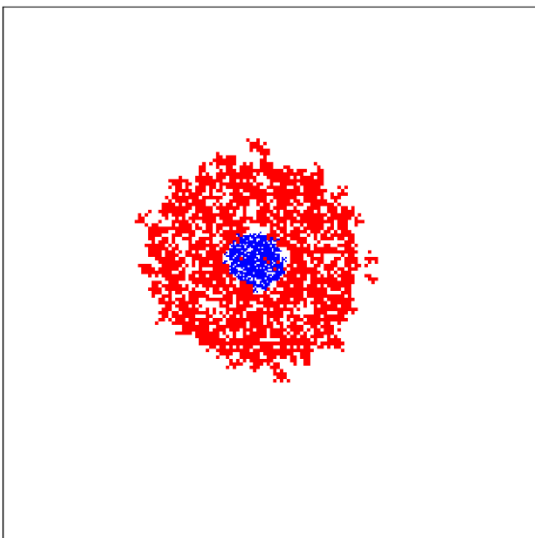
(d)
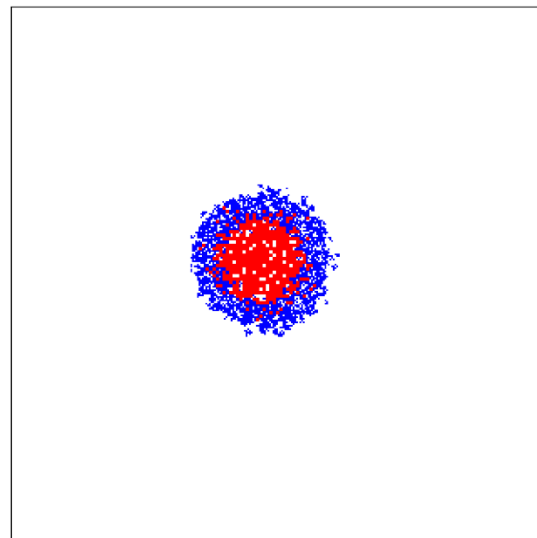
(e)

3. The lattice size
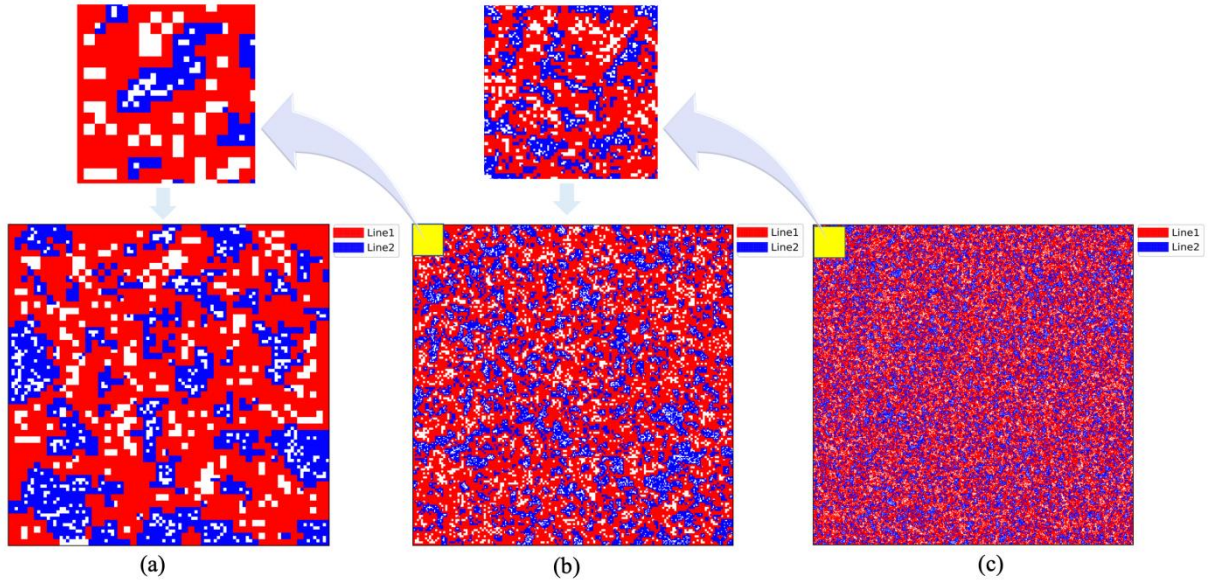
a. Visual estimation

Fig S4. Visual representation of simulation run on lattices of sizes: (**a**) 10k, (**b**) 100k, (**c**) 1000k. Visually the same pattern (at various magnifications) is observed for three different lattice sizes.

Figure S4 represents simulations performed under identical conditions with the size of the initial population appropriately scaled to the lattice (i.e., 0.25k for lattice 10k, 2.5k for 100k lattice, and 250k for 1000k lattice). Cutting 1:100 piece of the bigger lattice and zooming it in reveals no significant differences between analyzed extracts and the pattern of the smaller lattice. Visually the difference in pattern formation cannot be concluded. However, the smallest lattice considered (10k) seems to be most sensitive to the random distribution of the initial population, which could impact the final result. In bigger lattices (100k, 1000k), any such initial bifurcations are statistically dissipated.

b. Comparison of the parameters of Gompertz fit
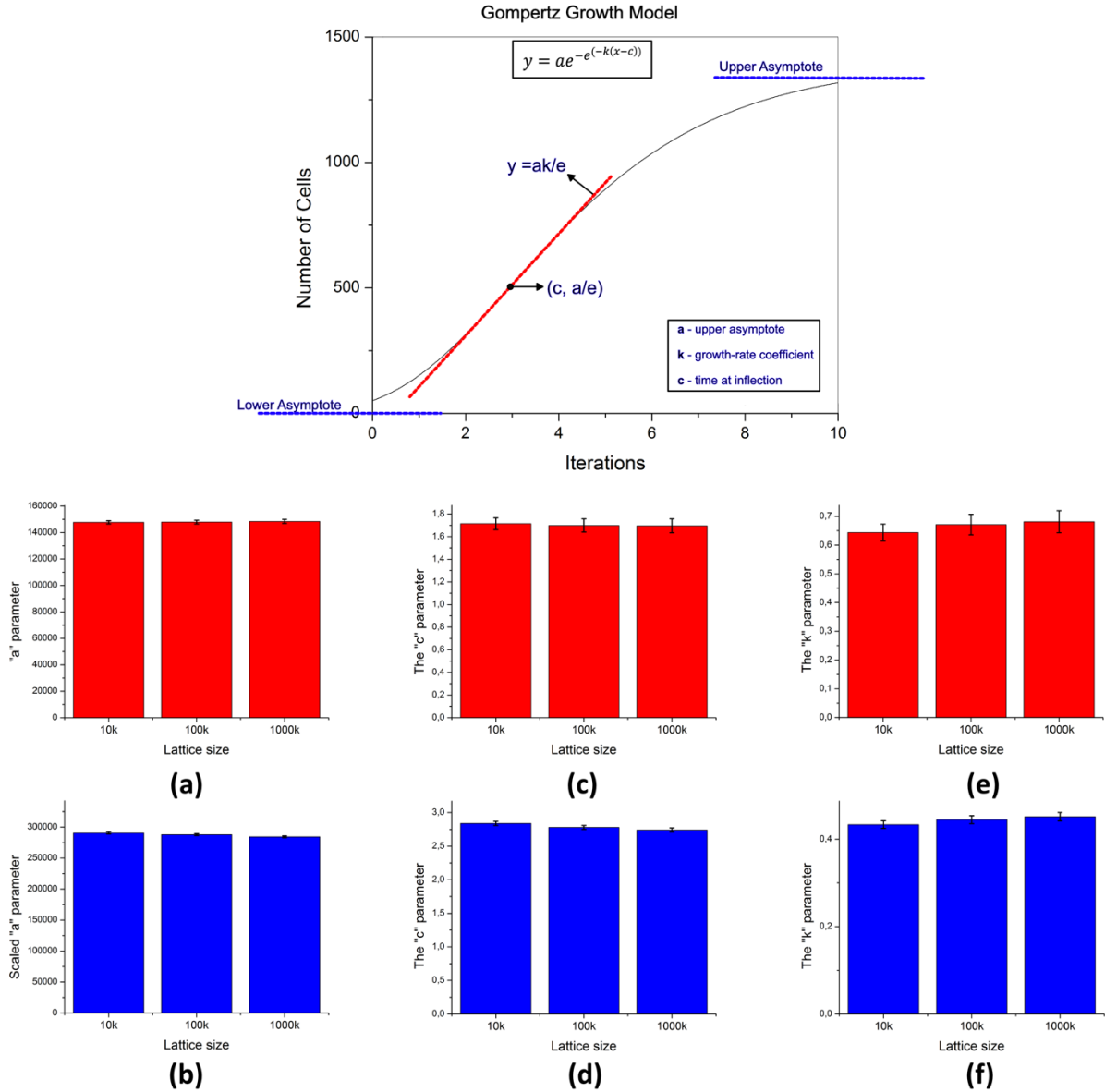
Fig. S5. Comparison of the Gompertz fit parameters. The upper panel visually explains the Gompertz function parameters. The lower panel shows the comparison of the parameters obtained by fitting the simulations run on three lattice sizes (10k, 100k, 1000k). Line1 is in red, Line2 is in blue. For the "*a*" parameters see figures (**a**) and (**b**); for the "*c*" parameters see (**c**) and (**d**); for the "*k*" parameters see (**e**) and (**f**). (Note that the "*a*" parameters were appropriately scaled). The lower panel shows that the Gompertz function parameters stay the same for different scales within the error limit.

Table S1. The table contains parameters obtained by fitting simulations performed under standard conditions with the size of the initial population of 0.25k for lattice 10k; 2.5k for 100k lattice; and 250k for 1000k. The cells of both lines were equally present in the initial population. The table shows that the Gompertz function parameters stay the same for different scales within the error limit.

| Line1	*a*	*a*(Err)	*a* scaled	*a*(Err) scaled	*c*	*c*(Err)	*k*	*k*(Err)	Adj. R-Square
10k	1477,31	12,47	147730,75	1247,09	1,714	0,052	0,644	0,029	0,997
100k	14769,60	140,72	147909,02	1409,26	1,699	0,058	0,671	0,036	0,996
1000k	148441,83	1485,39	148441,83	1485,39	1,696	0,061	0,681	0,038	0,995
Line2	*a*	*a*(Err)	*a* scaled	*a*(Err) scaled	*c*	c(Err)	*k*	*k*(Err)	Adj. R-Square
10k	2905,32	16,40	290531,73	1640,32	2,840	0,030	0,434	0,009	0,999
100k	28744,80	158,83	287862,55	1590,61	2,780	0,030	0,445	0,009	0,999
1000k	284402,57	1626,98	284402,57	1626,98	2,742	0,031	0,452	0,010	0,999
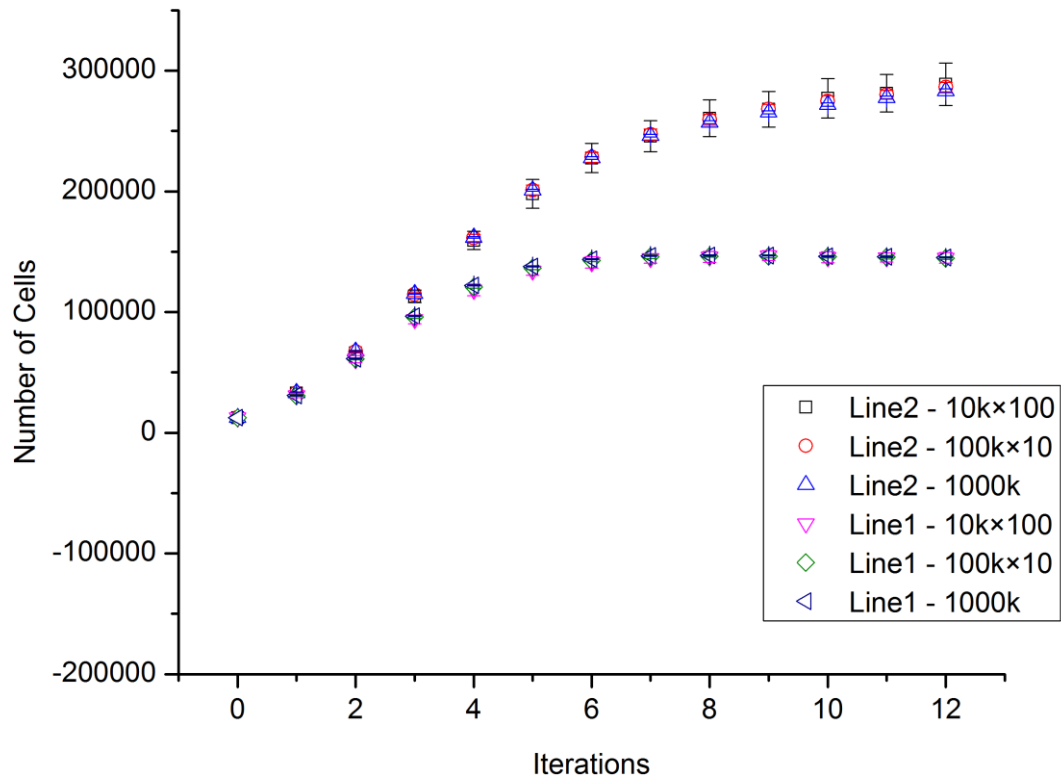
Fig. S6. The data scaled for different lattice sizes. The number of cells at each step of the iteration is appropriately scaled accordingly to the lattice size (10k×100, 100k×10). The graph shows the linear scalability of this model.
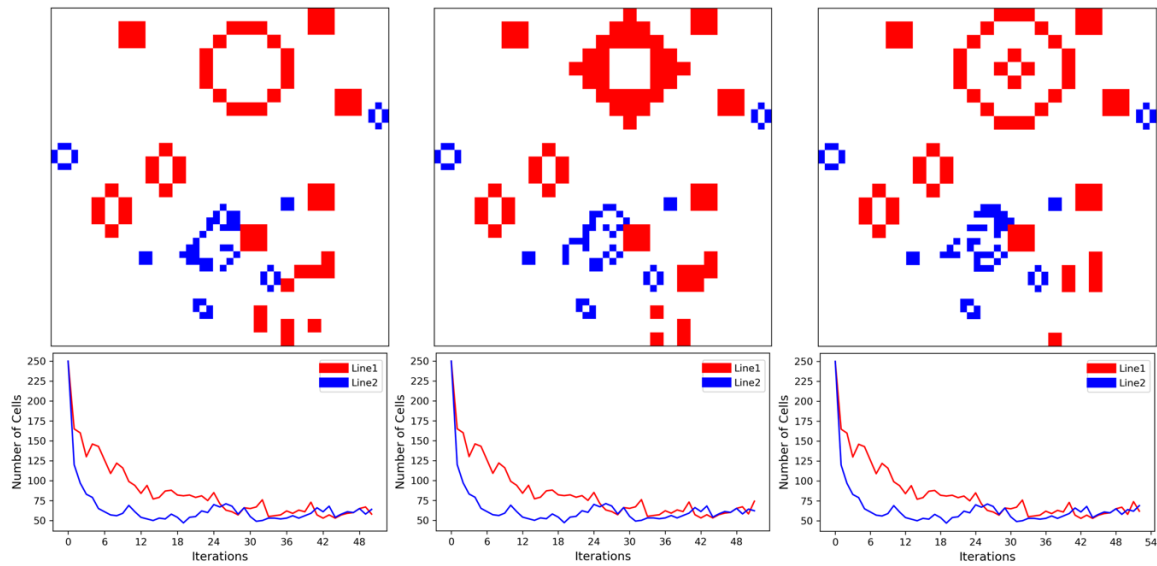
4. The rules of the game of life

Fig. S7. The Conways GoL rules. The figures show three iteration steps in a cellular automata simulation based on the Conway's rules. From a certain moment, the size of the population is stabilizing, partly because of the appearance of the oscillators that do not increase the number of cells.
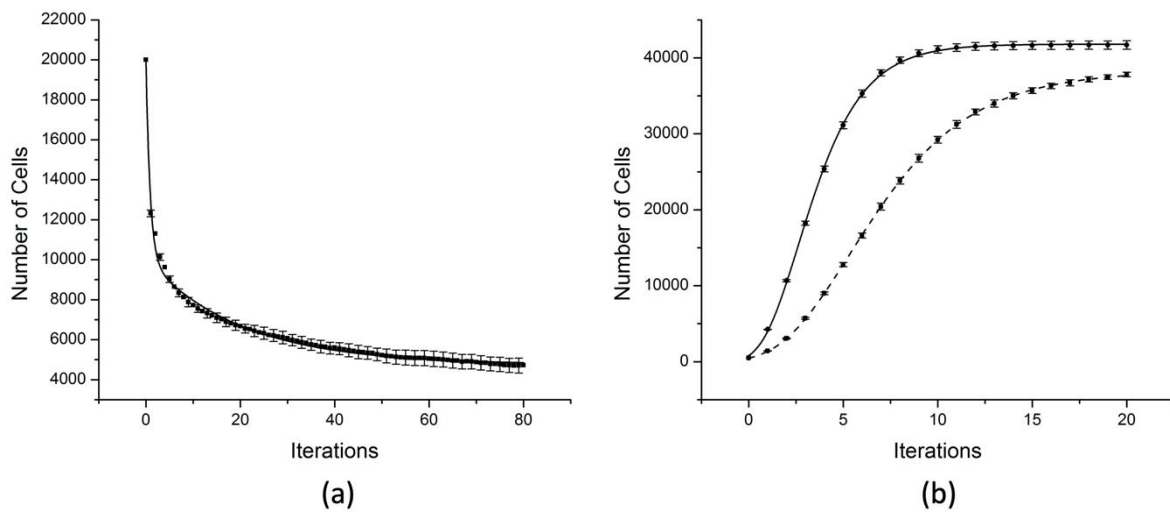

Fig. S8. The game of life rules comparison. (**a**) The Conway's GoL rules. A large initial population has to be established to apply Conway's rules (20k is set). The outcome data are fitted with exponential decay function, with R-Squared of 0.994 accuracies. (**b**) The standard and maximum growth GoL rules. The simulation run under standard GoL rules is visualized with dashed lines, whereas the maximum growth simulation is shown as a solid line. The standard and maximum growth laws allow for an initial population of any size (0.5k is set). Data are fitted with the Gompertz function, with R-Squared of 0.999 accuracies for both standard and maximum growth rules. The figure exposes the uselessness of the Conway's GoL rules for modeling cancer growth vs. the standard and maximum growth GoL rules. Each point represents the mean of 10 repetitions +/- SD.

Table S2. Fitting functions for simulations run in accordance with (**a**) Conway's, and (**b**) standard and maximum growth game of life rules. For (a) the best fit was the two-phase exponential decay, in other cases, the Gompertz growth model performed optimal fit. The size of the initial population was 2.5k equally divided between Line1 and Line2, and the lattice size was 100k.

(a) The Conway's game of life rules

Model	Two-phase Exponential Decay	
Equation	$y = A_1 e^{\left(\frac{-x}{t_1}\right)} + A_2 e^{\left(\frac{-x}{t_2}\right)} + y_o$	
Reduced Chi-Sqr	29499,71	
Adj. R-Square	0,994	
	Value	**Standard Error**
y_o	4755,79	51,40
A_1	5298,90	110,53
t_1	19,99	0,92
A_2	9821,45	209,32
t_2	0,89	0,046

(b) The standard and maximum growth game of life rules

Model	Gompertz Growth Model		
Equation	$y = ae^{-e^{(-k(x-x_c))}}$		
Reduced Chi-Sqr	5584,46 (standard)	39696,07 (max. growth)	
Adj. R-Square	0,999 (standard)	0,999 (max. growth)	
		Value	**Standard Error**
Standard	a	38296,64	42,053
	c	5,33	0,009
	k	0,28	0,001
Max. growth	a	41808,97	61,244
	c	2,63	0,014
	k	0,52	0,005

5. The global sensitivity analysis of the Gompertz model

The Sobol method of the variance-based sensitivity analysis was used for the Gompertz model investigation. The calculation was performed with help of SALib (v. 1.3) python package for four parameters (no_iter - number of iteration, a, k, c) that was tested in following bounders: $no\_iter = [1, 20]$, $a = [1, 40000]$, $k = [0.01, 2]$, $c = [0.01, 4]$. The results are shown in Table S3.

Table S3. The Sobol sensitivity analysis of Gompertz function. S_i1 (the first-order indices) measure the contribution of a single parameter to the output variance. S_i2 (the second-order indices) return the contribution to the output variance from the interaction of two parameters. S_iT (total-order indices) summarize the contribution of both the first and the second-order interactions to the output variance. S_i_conf store the corresponding confidence intervals. Positive values of S_i1 indicate that all determinants exhibit first-order sensitivity, however, with a clear dominance of the "a" parameter. Small values of S_i2 indicate that there is no higher-order interaction between parameters, or their interconnections are very weak.

Parameter	S_i1	S_i1_conf	S_iT	S_iT_conf
no_iter	0,107377	0,040207	0,176998	0,037896
a	0,780041	0,074201	0,828228	0,05732
k	0,027238	0,018304	0,056272	0,011603
c	0,005481	0,02329	0,045612	0,016092

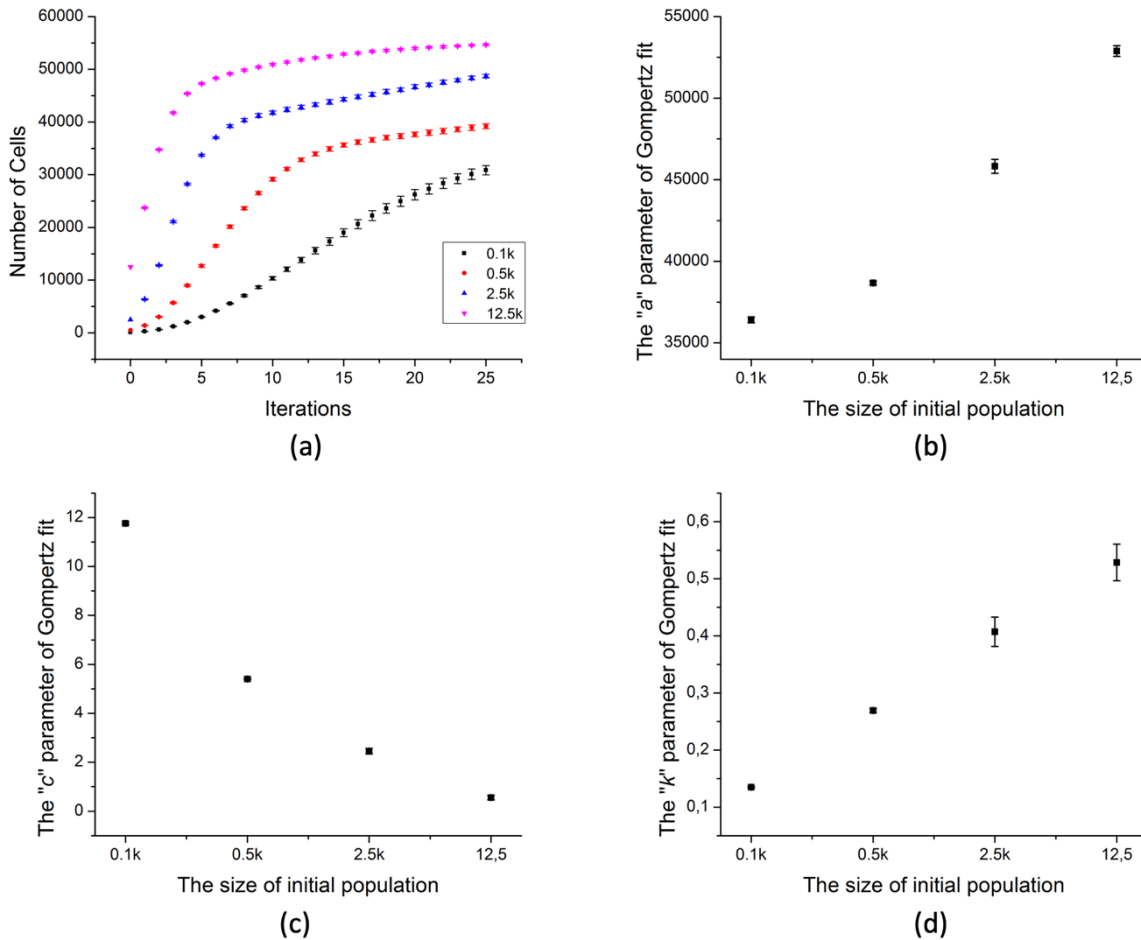| Parameter_1	Parameter_2	S_i2	S_i2_conf
no_iter	*a*	0,018579	0,052771
no_iter	*k*	0,000929	0,043736
no_iter	*c*	0,028544	0,046981
a	*k*	0,004372	0,084212
a	*c*	0,003776	0,082608
k	*c*	0,003805	0,027948
a	*k*	0,004372	0,084212
a	*c*	0,003776	0,082608
k	*c*	0,003805	0,027948

6. The size of the initial population

Fig. S9. (**a**) The dependency of a mixed culture growth curve on the initial population size. The comparison of the Gompertz function parameters: (**b**) the "*a*"parameter, (**c**) the "*c*"parameter, (**d**) the "*k*" parameter for different initial population sizes of 0.1k, 0.5k, 2.5k, 12.5k. The figures show that there is no linear relationship between the model kinetics and the size of the initial population. Each point represents the mean of 10 repetitions +/- SD.

7. Supplementary video

The supplementary video shows: (**part 1**) Initial placement of the cells: (**part 1-A**) mixed tumor; (**part 1-B**) collision tumor (4 clusters per line); (**part 1-C**) collision tumor (1 cluster per line); (**part 1-D, E**) tumor-to tumor metastasis; (part 1-D tumor 2 to tumor 1, and E – tumor 1 to tumor 2). (**part 2**) The Conway's game of life rules. The simulation performed under Conway's game of life rules on the lattices size of 2.5k and initial population of 0.5k equally distributed between Line1 and Line2. (**part 3**) The formation of a lobular meshwork pattern for the mixed culture of identical cell size. Simulation is performed under the standard game of life rules on lattices size of 100k and initial population of 2.5k equally distributed between Line1 and Line2. (**part 4**) The formation of a lobular meshwork pattern for the mixed culture of different cell size under the same conditions. (**part 5**) The simulation in overcrowding conditions run under standard GoL rules at 1000k lattice and initial population of 1k equally distributed between Line1 and Line2.