# Mathematical modelling of intra-clonal heterogeneity in multiple myeloma

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**RÉSUMÉ.** Cette étude est consacrée à la modélisation mathématique de l'hétérogénéité intra-clonale du myélome multiple (MM) et de sa résistance aux médicaments qui en résulte. Pour explorer les mécanismes inhérents qui régulent ce processus, nous développons un modèle hybride multi-échelles de la croissance des tumeurs MM dans la moelle. Les cellules malignes sont représentées par approche individuelle. L'action du traitement est introduite. La tumeur consiste en des clones en compétition. Le taux de division des cellules dans un clone dépend de sa compétition avec les autres. Nous étudions la dynamique de l'hétérogénéité intra-clonale dans le MM et nous décrivons son rôle dans l'émergence de phénotypes plus résistants au traitement.

**ABSTRACT.** This study is devoted to the mathematical modelling of multiple myeloma (MM) intra-clonal heterogeneity and the resulting drug resistance. To explore the underlying mechanisms of intra-clonal heterogeneity, we develop a multi-scale hybrid model of MM tumor growth in the bone marrow. Malignant plasma cells are represented by individual based approach. Drug action is introduced and its concentration inside each cell is described by an ordinary differential equation. The tumor consists of competing clones. The rate of cell division in each clone depends on the competition with the other clones. We study the dynamics of intra-clonal heterogeneity in MM and describe its role in the emergence of drug resisting phenotypes.

**MOTS-CLÉS :** myélome multiple; hétérogénéité intra-clonale; résistance aux médicaments, modélisation mathématique

**KEYWORDS :** multiple myeloma; intra-clonal heterogeneity; drug resistance; mathematical modelling

## 1. Introduction

Multiple myeloma (MM) is a malignancy characterized by the infiltration of cancerous plasma cells into the bone marrow. These cells form multiple tumors that expand and secrete apoptosis inducing cytokines which eliminate erythroid cells resulting in anemia. As in other cancers, MM cells undergo various mutations and the tumor is formed by different clones [1]. This feature is known as intra-clonal heterogeneity. It is related to the adaptation and natural selection of cancer cells. Malignant cells compete for limited nutrients, and more adapted cells survive and multiply. In addition to this selective pressure, cancer treatment can act as an additional factor which favors the survival of some clones more than others. While there are efficient treatment regimens of MM, drug resistance remains the major concern. In this regard, the resisting clones may be initially present in the first cells that infiltrate the bone marrow, but they can also emerge during treatment leading to relapse. The emergence of novel clones is due to the MM progression in branching pattern discussed below.

Mathematical models of cancer growth and intra-clonal heterogeneity falls in three main categories. The first one is continuous models. These are deterministic models that use partial differential equations to describe cancer development [7] and treatment [10]. Another type of models uses the discrete approach to describe cancer growth. These can be lattice [12] or off-lattice models [9]. The question of stress-induced drug resistance in tumors was also studied in some works [7]. Finally, hybrid models combine continuous and discrete approaches where cells are considered as individual objects, intracellular concentrations are described with ordinary differential equations and extracellular concentrations with partial differential equations [4].

Modelling methods previously developed to study hematopoiesis and blood diseases [5, 6] will be adapted in this work to study MM intra-clonal heterogeneity and drug resistance. In this approach, each cell is represented as an elastic sphere that can move due to the interaction with other cells. Cells can also divide or die by apoptosis. Each cell is characterized by its genotype which can change because of the mutations. When a cell divides, the daughter cells inherit the genotype of the mother cell with small random mutations. This leads to the emergence of new clones in the process of tumor growth. We use this approach to model the intra-clonal heterogeneity of MM. Furthermore, we apply it to study the emergence of drug resisting clones during chemotherapy.

## 2. The model

We consider a square computational domain with the side equal to 100 length units corresponding to 10 microns. Cells are represented by elastic spheres with initial diameters equal to one unit. They are removed from the domain when they reach its boundaries. We consider an initial tumor consisting of 208 malignant cells as initial condition with the same genotype. In the process of tumor growth, they can change their genotype due to mutations. Their rate of apoptosis depends on the competition between clones for resources.

### 2.1. Cells motion

We model cells as elastic spheres with an incompressible inner part and compressible outer part. Since cells divide, they push each other and can change their position. Cell motion is des-

cribed by Newton's second law for their centers. Let $x_i$ be the coordinate of the center of the ith cell (two-component vector). Then we have the following equation for its motion :

$$m\ddot{x}_i + m\mu\dot{x}_i - \sum_{j\neq i} f_{ij} = 0, \tag{1}$$

where

$$f_{ij} = \begin{cases} K\dfrac{h_0 - h_{ij}}{h_{ij} - (h_0 - h_1)}, & h_0 - h_1 < h_{ij} < h_0 \\ \\ 0, & h_{ij} \geq h_0 \end{cases}. \tag{2}$$

Here $f_{ij}$ is the force acting between cells $i$ and $j$, $h_{ij}$ is the distance between their centers, $h_0$ is the sum of their radii, K is a positive parameter and $h_1$ represents the incompressible part of each cell. The second term in Eq. (1) describes the friction by the surrounding medium. Cell radius increases in the process of cell division. More detailed description of the method can be found in [8].

## 2.2. Cells division and mutations

When the malignant cell reaches the end of its life cycle, it has two possible fates. Either it divides and self-renews giving rise to two daughter cells or it dies by apoptosis. The apoptosis probability is determined by cell genotype.

We characterize cell genotype by a real variable $z$. Let $z_m$ be a cell genotype before division. After cell division, the genotype of the daughter cells can take three values,$z_m$, $z_m + \epsilon$, $z_m - \epsilon$ where $\epsilon$ is a small positive number. The choice between these three values is random with equal probability. Thus, the genotype of the daughter cell can be the same as the genotype of the mother cell or it differs from it by $\epsilon$. This difference describes small random mutations after each division. If all cells have initially the same genotype $z_0$, then cell density distribution $u(z,t)$ with respect to the genotype becomes wider with time. The evolution of the function $u(z,t)$ can be described by the diffusion equation.

The probability of cell apoptosis depends on its genotype. We define viable cell clones by some intervals of genotype where apoptosis probability is less than the probablity of self-renewal. Consider the function $p(z)$ which determines the probability of apoptosis depending on the genotype. We set $p(z) = p_0$ for $z \in [a_i, b_i]$ and $p = p_1$ outside these intervals (Figure 1, a). Here $[a_i, b_i]$ with $i = 1..4$ are the intervals of genotype characterizing different clones, $p_0$ is the basic level of apoptosis of these clones. The ordering and distance between the clones in the function $p(z)$ mimic the moment of apparition of clones in experiments [13]. We consider the value $p_0$ sufficiently small in order for these cells to survive and multiply, $p_1$ is sufficiently close to 1. Then cell clones will survive while cells with different genotypes can appear due to mutations but they will mostly die after some time due to apoptosis.

Cell competition for resources increases their apoptosis. Hence apoptosis probability depends not only on cell genotype but also on the quantity of cells for different genotypes. We will specify this dependence below in the case of multiple myeloma.

In application to multiple myeloma, we will consider four cell mutations observed experimentally : ATM, FSIP2, GLMN, CLTC [13]. As a result, different clones emerge as shown in Figure 1, b. We denote these clones as $c_1$, $c_2$, $c_3$ and $c_4$. Clones $c_1$ and $c_2$ are sufficiently close to each other and they compete between themselves. Similarly, clones $c_3$ and $c_4$ are in competition

between each other [13]. We suppose that $c_1$ and $c_2$ do not compete with $c3$ and $c4$. We define the probability $p_i$ of cell apoptosis for each clone as follows :

$$p_1 = p_0+2\alpha(u_1+u_2),\ p_2 = p_0+\alpha(u_1+u_2),\ p_3 = p_0+\alpha(u_3+u_4),\ p_4 = p_0+\alpha(u_3+u_4). \quad (3)$$

Here $u_i$ are cell densities for each clone, $u_1 + u_1 + u_3 + u_4 = 1$, $p_0$ is the probability of cell apoptosis without competition for resources taken equal to 0.2, $\alpha$ is a positive number equal to 0.04. We note that apoptosis probability of the clone $c_1$ is greater than that of other clones. According to the biological data it is less adapted to the environment than the others. Apoptosis probabilities and the genotypes corresponding to different clones will be chosen in numerical simulations in order to fit the experimental data.

## 2.3. MM therapy and drug resistance

Multiple myeloma is treated by chemotherapy with myeloma specific drugs (thalidomide, lenalidomide and bortezomib), which kill malignant cells and do not influence other hematopoietic cells. Though chemotherapy treatment is efficient in reducing the number of MM cells, it does not eradicate them completely. In order to avoid relapse, chemotherapy is usually followed by bone marrow transplantation.

The intracellular drug concentration $q_i$ in the $i^{th}$ cell is described by the equation :

$$\frac{dq}{dt} = k_1 Q(t) - k_2 q, \quad (4)$$

where $Q(t)$ is the drug concentration in the bone marrow. We take it constant and equal to 0.7 for $t$ during the administration and 0 elsewhere. The treatment is administrated in the first two week of each cycle of 28 days during a four cycle protocol after 25 days of tumor development. It depends on time according to the treatment protocol and it is supposed to be equally distributed in space. The first term in the right-hand side of this equation describes drug influx and the second term its degradation and efflux. The coefficients $k_1$ and $k_2$ can be different for different clones. If the intracellular drug concentration reaches some critical value q*, then the cell dies. In numerical simulations dead cells are removed from the computational domain.

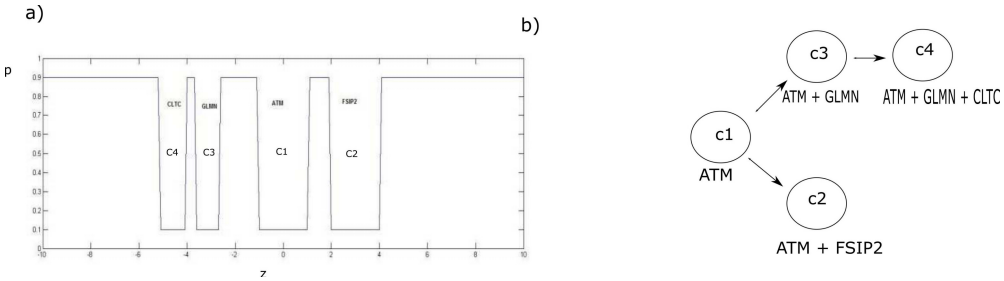


**Figure 1.** *(a) The apoptosis probability $p(z)$ as a function of genotype $z$. The four clones are shown. The values of their apoptosis probabilities (shown in dashed lines) are not fixed and depend on cell densities. (b) The branching pattern of multiple myeloma intra-clonal heterogeneity.*

# 3. Results

## 3.1. Intra-clonal heterogeneity and clones competition dynamics in multiple myeloma

MM is a genetically complex malignancy characterized by intra-clonal heterogeneity. Malignant myeloma cells undergo a number of mutations as the cancer progresses. We will compare here the results of our modeling with the biological data presented in [13]. In this work, MM intra-clonal heterogeneity and the presence of different coexisting clones were shown in the sequencing data. Furthermore, it was proven that more competitive clones emerge in the process of tumor growth. We use the genetic function model described in the previous section. We consider a population of malignant cells which initially belongs to clone $c_1$. As the simulation progresses, new clones emerge. The size of the clone $c_1$ population increases in the beginning. After some time, as clone $c_2$ emerges and starts expanding, clone $c_1$ declines since its apoptosis rate is greater than for clone $c_2$ (Figure 2, a). Clone $c_3$ emerges independently of clone $c_2$ and later than $c_2$

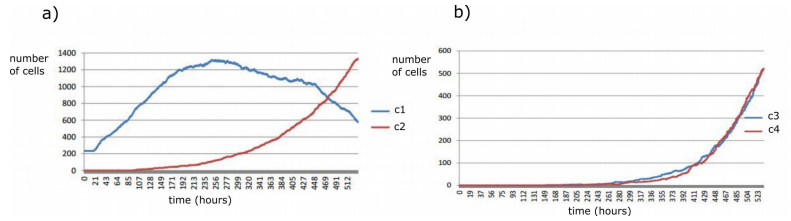


**Figure 2.** *Size of cell populations for clones $c_1$ and $c_2$ (a) and for clones $c_3$ and $c_4$ (b) over time.*

since its genetic distance from clone $c_1$ is larger. Clone $c_4$ appears from $c_3$ due to an additional mutation. As we discussed above, clones $c_1$ and $c_2$ compete with each other as well as clones $c_3$ and $c_4$. The numbers of cells in these clone in time are shown in Figure 2 and snapshots of growing tumor in Figure 3.

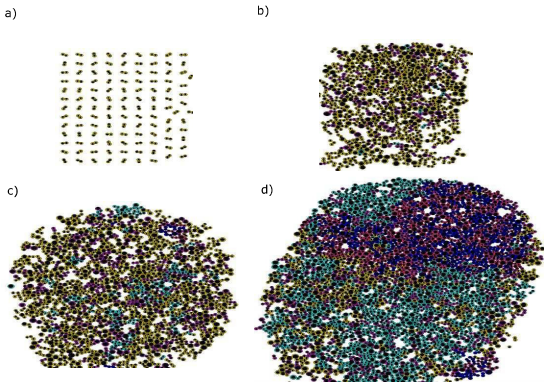


**Figure 3.** *Snapshots of the simulation with different stages of MM progression : (a) the initial cell population belongs to clone $c_1$ (yellow cells), (b) emergence of clone $c_2$ (cyan) followed by appearance of cells $c_3$ (magneta), (c) clones $c_2$ and $c_3$ form sub-populations across the tumor, (d) the tumor now consists primarily of clones $c_2$, $c_3$ and recently emerged clone $c_4$ (blue). The few cells that do not belong to any clone are also shown (purple).*

### 3.2. Intra-clonal heterogeneity role in MM drug resistance

To assess the tumor response to therapy, we suppose that the toxic effect of the drug on MM cells is different for each clone. Therefore the coefficients $k_1$ and $k_2$ in Eq. 4 depend on clone type. We suppose that the administrated drugs are more prone to eliminate the initial clone $c_1$ but are less efficient in eliminating the cells of $c_2,\ c_3,\ c_4$. We set $k_{c1,1} > k_{ci,1}, i = 2, 3, 4$. Treatment is administrated when tumor is formed and clone $c1$ is predominant while the other clones are only emerging. The overall population of malignant cells is compared with the population of clone $c_1$ in Figure 4.

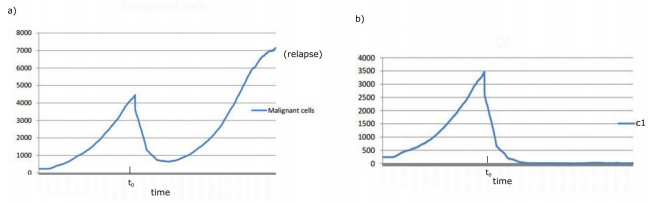


**Figure 4.** *The total population of malignant cells (left) and the population of the clone $c1$ cells (right) over time. Clone $c1$ disappears due to treatment while other more resistant clones emerge and multiply in spite of treatment.*

At the pre-treatment stage, the tumor grows with an exponential rate. Other clones have emerged from the initial cells and, thus, the tumor is no longer homogenous. By the end of the first cycle of therapy, the cells of the clone $c_1$ were completely eliminated while cells from the other clones have survived. The remaining cells form separate niches. Each niche consists of cells of the same clone. These cells take advantage of the rest period between chemotherapy cycles to divide and form independent tumors. These recently formed tumors are more resistant to treatment and they keep growing even after the beginning of the new cycle of therapy. After some time they form a single large tumor. Different stages of tumor grows are shown in Figure 5.

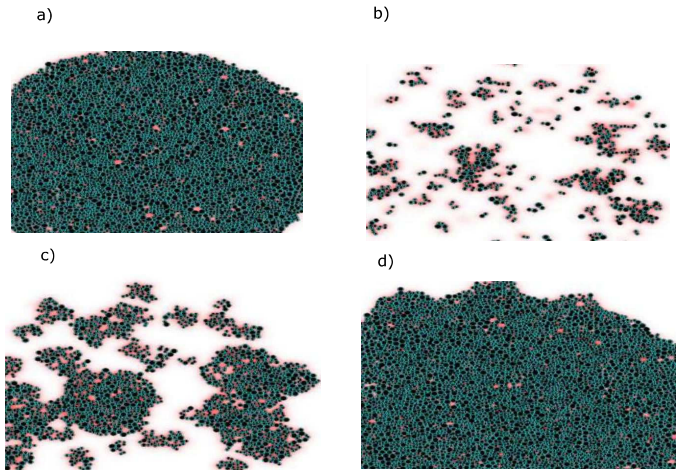


**Figure 5.** *Snapshots of a simulation of myeloma tumor growth under treatment : (a) the tumor reaches its maximal mass before the treatment, (b) the drugs eliminate the cells of clone $c_1$, the cells belonging to other clones survive and form separate niches, (c) the niches formed by the remaining cells consolidate and form independant tumors, (d) the tumors keep growing and join together in a one single tumor.*

## 4. Discussion

The heterogeneous nature of MM and drug resistance of the emerging clones represents a difficulty in the MM therapy. Different clones have different sensitivities to treatment and to the other components of the microenvironment. The heterogeneous property of MM usually leads to the relapse when treatment is finished. To understand the dynamics of clones competition and its impact on therapy resistance, we have developed a multi-scale model of myeloma tumor growth. We used this model to simulate the emergence of cell clones as observed in [13]. The model reproduces these phenomena not only qualitatively but also quantitatively. To quantify the results of the simulations and to compare them with the experiments, we introduce a mutation frequency variable ($m$) that corresponds to a scaling from 1 to 0 of the genetic variable $z$. It represents the inverse of the number of mutations undergone by the cell. We show the kernel density plot based on this variable in Figure 6. This plot allows the estimation of the general distribution of global mutational frequency in a population using a sample of cells. The results are in good agreement with the experimental data (Figure 4, b in [13]).

Biological observations show that cancer and mutations are reversible[11]. Hence the emergence of resistant clones is a reversible process. This property is taken into account in our model and it was observed in the simulations when new clones emerge. It can also be related to relapse when eliminated clones reemerge after the end of treatment. In order to prevent relapse, new therapeutical strategies were developed in MM treatment. In this context, sequential therapy was used as an induction followed by consolidation and maintenance [3]. In the induction phase, a part of the tumor is surgically removed to reduce its mass. Consolidation therapy is then used to eliminate cells belonging to all different clones. The remaining clonal cells are treated by maintenance therapy in which treatment is modified in order to eradicate the different clones.

The model presented here reproduces the main features of MM intra-clonal heterogeneity. More detailed intracellular and extracellular regulations and their influence on the emergence and competition of different clones will be studied in subsequent works.

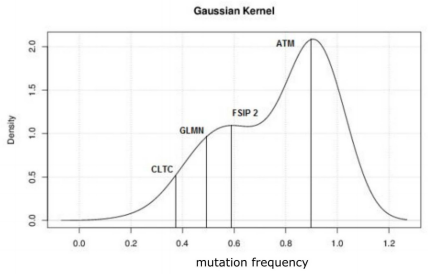


**Figure 6.** *Kernel density plot of heterogenous MM population at a certain moment of time during simulation. This distribution is similar to the experimentally observed distribution in [13].*

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