

Chapitre 5 - Annexe A : Robustness in the estimation of Δ

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Contenu de l'annexe

Dans cette annexe, nous étudions comment la violation de certaines hypothèses de notre modèle - concernant le fait que le paramètre Δ serait constant tout au long de la vie ou encore le fait que le nombre de HSC WT soit constant - influencent les résultats de notre estimation de notre paramètre Δ .

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1 Context

In our model-based study of the development of MPN, we have some assumptions :

- A constant value for Δ over the lifetime
- A constant number of WT HSCs

We will illustrate in the following how violations of these assumptions might impact our estimation for Δ .

To simplify, we will consider the case of a mutation acquired in fetal life (that is, $T_0 = 0$, or equivalently, $\lambda = 0$) and study the dynamics of the clonal expansion described by our deterministic approximation, that is :

$$N(t) = \exp(\alpha\Delta t)$$

We set $\alpha = 1/30$ [/days] and $\Delta = 0.02$.

2 Different values for Δ over lifetime

An expanding malignant clone (with one initial driver mutation) can acquire an associated mutation over the lifetime, resulting in two subclones : one without the associated mutation and one with it. This latter subclone would be supposed to have a greater proliferative advantage. Thus, if we only look at the driver mutation, that is, disregarding whether or not the associated mutation is present, we might overestimate the proliferative advantage of the driver mutation since our observation also includes the information about an associated mutation. We illustrate this point in the following example.

We consider a malignant clone (with the initial driver mutation) that expands at a rate of $\alpha\Delta$. We consider that one mutated cell of this clone acquires an associated mutation at time $t = 20$ years, resulting in two subclones : one with only the driver mutation that continues to expand at a rate $\alpha\Delta$, the second with the associated mutation that will develop at a growth rate $\alpha\Delta(1+z)$ with $z > 0$. Here, we choose $z = 1$ (Fig. 1).

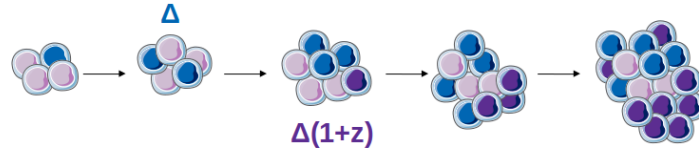


FIGURE 1 – Schematic representation of the clonal expansion of a malignant clone with one driver mutation (blue cells), whose proliferative advantage is Δ . At some time, one mutated cells acquires an associated mutation (purple cell), that confers to it an higher proliferative advantage $\Delta(1+z)$. Then, two subclones expand in parallel, with different growth rates.

Figure 2 (top panel) shows the number of mutated cells according to whether or not the (sub)clone presents the associated mutation. In the first years following the associated mutation, the subclone having only the driver mutation remains the main one : the subclone with the associated mutation would be barely perceptible during this period (Fig. 2, middle panel). Then, there is a period during which both subclones coexist, each with non-negligible CF. Eventually, the subclone with the associated mutation will take over.

Without the information about the associated mutation, we would measure the CF of both subclones together. From this observation, we would infer the value for Δ (Fig. 2 bottom panel). In this illustrative example, when the associated mutation is barely perceptible, the estimated value for Δ would be the one of the initial clone. Then, when both subclones coexist, we slightly overestimate this parameter. Yet, even when one subclone take over, we are far from estimating the proliferative advantage of the subclone with the associated mutation.

This qualitative study shows that the proliferative advantage of the driver mutation (either $JAK2^{V617F}$ or $CALR^m$) could be slightly overestimated because of patients having associated

mutations. However, the acquisition of mutations in "WT" cells (which should not be considered WT anymore) could also compensate for the effect. We study this latter point in the next paragraph.

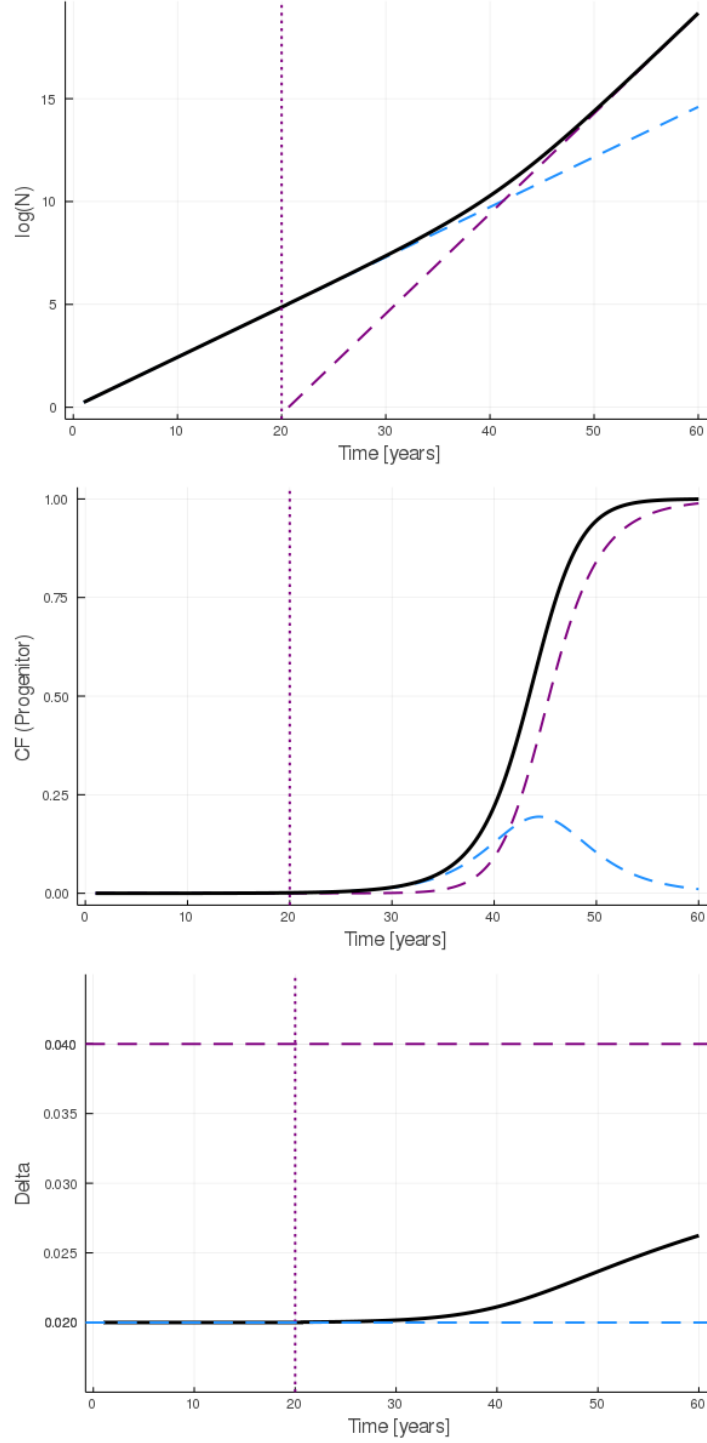


FIGURE 2 – Dynamics of two subclones : the first one (blue line) having only the driver mutation (with a proliferative advantage $\Delta = 0.02$), and the second one appeared at $t = 20$ years (vertical purple dashed line) having an associated mutation which conferred to him a higher proliferative advantage $\Delta(1 + z) = 0.04$. The top panel shows the clonal expansion (log number of HSCs) of each subclone (blue and purple dashed lines) when the solid black line represents the number of both mutated cells (that is, mutated cells with the driver mutation, whether or not they also have the associated mutations). When, experimentally, we only search for the driver mutation, it corresponds to having the information for the black line (at a given time). The middle panel represents the CF that would be measured among progenitor cells. The bottom panel's black curve indicates what would be the theoretical estimated value of Δ when there are two subclones but that we only assume a clonal expansion of one clone. The objective would be to accurately estimate the proliferative advantage of the driver mutation ($\Delta = 0.02$, blue line). Still, because of the acquisition of an associated mutation, the value of Δ might be overestimated.

3 A varying number of WT HSCs over life

Our model assumes a constant number of WT HSCs equal to 100,000 [1]. Yet, the number of WT HSCs is not necessarily constant over the lifetime. WT cells can also acquire mutations in the *TET2* or *DNMT3A* genes, for example, so that they would not be really WT anymore. So, to be more precise, we should say that we study the expansion of a malignant clone with *JAK2*^{V617F} or *CALR*^m driver mutations parallel to HSCs that do not have this mutation. In our model, we assume this latter pool to be of constant size when it might actually also expand because of mutation acquisitions.

To illustrate that point, we consider a clonal expansion as described in previous paragraph ; that is, we set $\Delta = 0.02$. Then, we consider that the number of WT HSCs (we continue to define them a bit abusively as WT ; it would be more precise to consider them as not *JAK2*^{V617F} or not *CALR*^m) increases, from an initial number of $N_{WT}=100,000$, at a rate $\alpha\Delta x$ with $x \ll 1$. We illustrate this dynamics in Fig. 3 (top panel) with different values for x : 0.0 (as in the main model), 0.05, 0.1, and 0.2.

The expansion of the WT HSC pool will result in a reduced CF among progenitor cells $\bar{\eta}$, compared to the situation with a constant number of HSC (η when $x = 0$), as illustrated in Fig. 3 (middle panel) :

$$\bar{\eta}(t) = \frac{1 - \Delta}{1 - \Delta + N_{WT} \exp(\alpha\Delta(x - 1)t)} < \eta(t)$$

Then, if the previous equation describes the actual dynamics but we still model the WT HSC pool as being of constant size, we would estimate a reduced value $\bar{\Delta} \approx \Delta(1 - x)$, as illustrated in Fig. 3 (bottom panel). If the malignant clone of interest has a much higher proliferative advantage than the ones for the mutations that occurred in WT cells (i.e, $x \ll 1$), we would only slightly underestimate the value Δ of the mutation.

The number of WT HSCs could also vary because of other factors, such as ageing. Lee-Six et al. inferred, based on phylodynamics methods and the data of a 59 years old healthy individual, the evolution of the HSC population size over life [1]. We report their findings (mean value) in Fig. 4 (top panel) and now study how our estimation of Δ would be impacted if, instead of considering that $\log(N_{WT}(t))$ linearly increases over life, we consider the same evolution for $N_{WT}(t)$ as reported by Lee-Six et al. [1]. Results of this study are displayed in Fig. 4 (bottom panel). Above 30 years, when the presence of mutated cells becomes perceptible in our illustrative example, sometimes we overestimate, sometimes we underestimate the true proliferative advantage $\Delta = 0.02$, depending on the evolution of the WT HSC pool size.

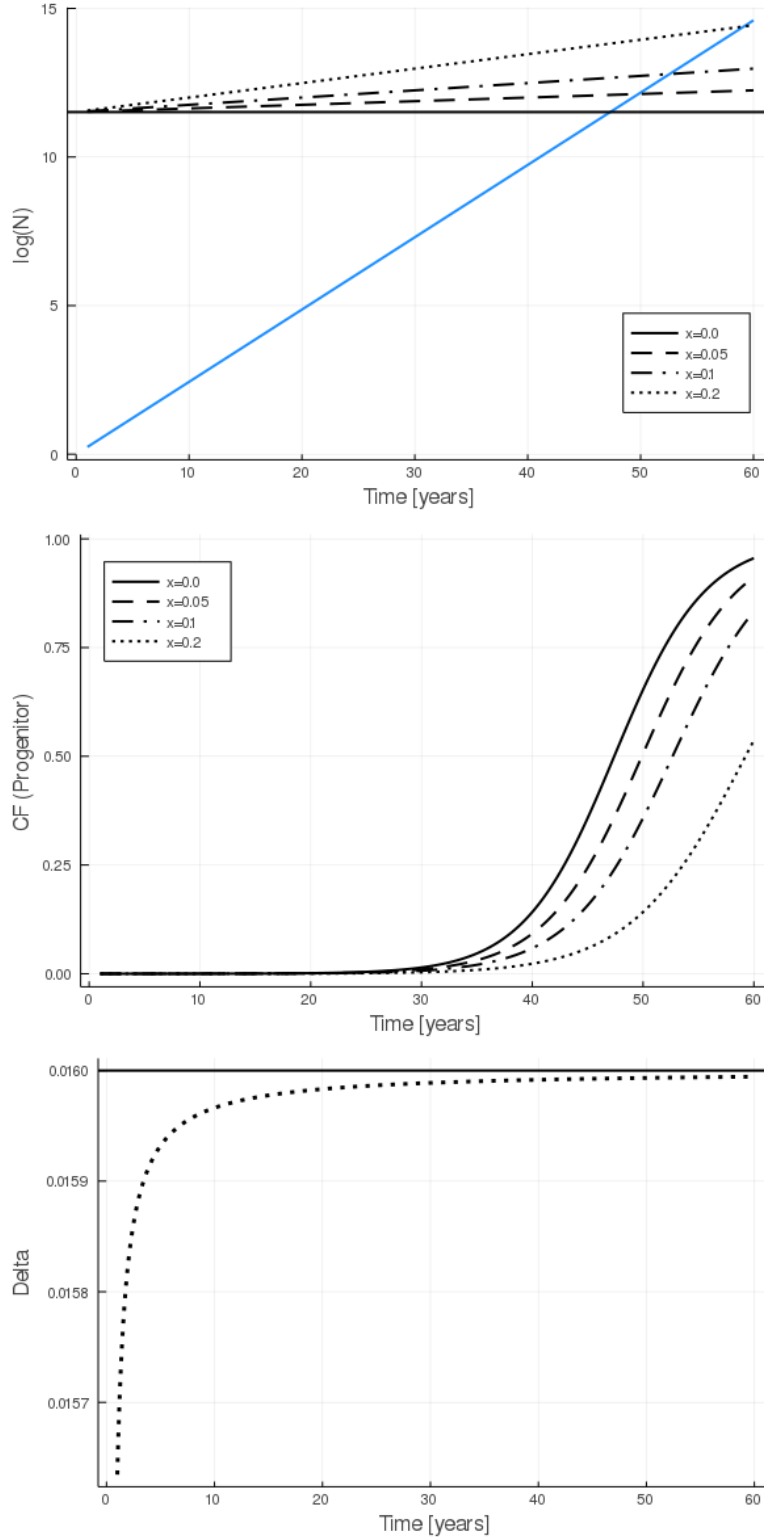


FIGURE 3 – Dynamics of the clonal expansion of a malignant clone parallel to WT HSCs that also expand. The top panel displays the increasing log-number of mutated HSCs (blue line) and the log-number of WT HSCs for different x -values (black lines). $x = 0$ corresponds to our model, with N_{WT} being constant. To these dynamics in the HSC pool correspond evolutions of CF among progenitor cells, as displayed in the middle panel. An expansion of the WT HSC pool will result in reduced values for the CF of mutated cells, and, thus, underestimating the proliferative advantage Δ of the driver mutation. The bottom panel shows what would be the estimation of Δ (dotted line) if assuming a constant number of WT HSCs, when, in reality their pool would expand with $x = 0.2$. The horizontal line corresponds to $\Delta(1 - x)$. When mutated cells are perceptible, we will underestimate Δ by a factor $(1 - x)$.

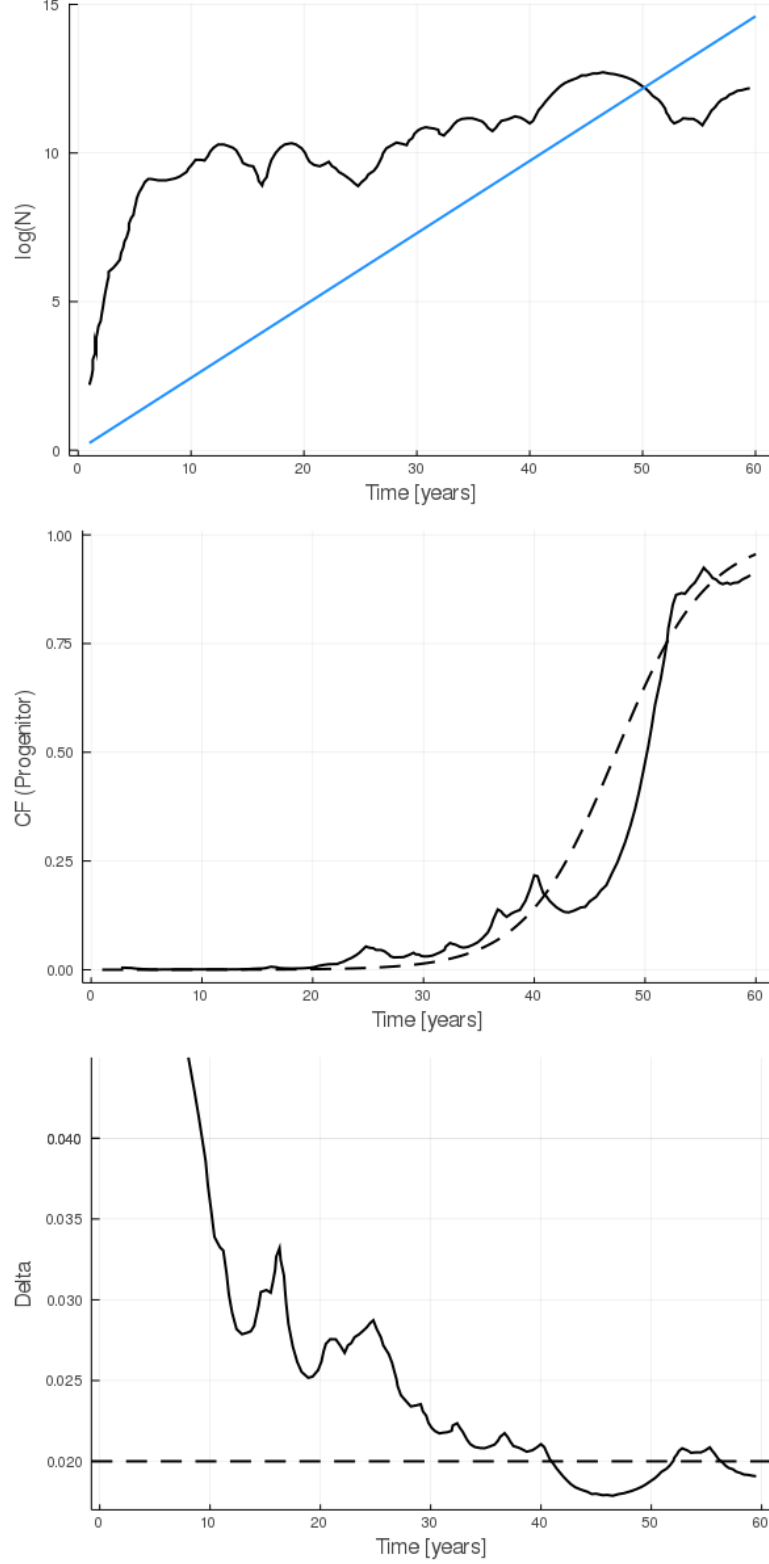


FIGURE 4 – The top panel presents the dynamics of the clonal expansion of a malignant clone (blue line) parallel to WT HSCs whose number vary over life according to Lee-Six et al. [1] (black line). y-axis are in a log-scale. The middle panel shows the evolution of the corresponding CF among progenitor cells (solid line) compared to the one that would be obtained from our model, assuming a constant number of WT HSCs equal to 100,000 (dashed line). The bottom panel shows what would be the estimation of Δ (solid line) if assuming a constant number of WT HSCs, when, in reality their pool expands according to the dynamics reported in [1].

4 Conclusion of this study

As a conclusion of this qualitative study, based only on limited examples, we see how more complex models that account for the acquisition of associated mutations in the malignant clone, acquisition of mutations in WT HSCs, or evolution of the WT HSC pool size over life, can impact our estimations of the proliferative advantage Δ of the driver mutation of interest (either $JAK2^{V617F}$ or $CALR^m$). When the acquisition of associated mutations in a patient might result in overestimating Δ , the increasing size of the WT HSC pool will have the reverse effect. If both occur, they might compensate in some ways. We should then consider that our estimation of parameter Δ also accounts for various effects over a lifetime that are impossible to enumerate in detail, less alone quantify.

Our results are therefore valid under the assumptions that these effects do not predominate over the expansion of the mutated clone, that they do not all lead to an overestimation (or, respectively, underestimation) of Δ (that is, they compensate), and that they would have the same order of magnitude for patients having the mutation $JAK2^{V617F}$ and those having the mutation $CALR^m$ (such that we could compare both populations). To note that, to respect the first assumption, we excluded patients with homozygous mutated cells. Indeed, we could not reasonably assume that homozygous subclones have the same proliferative advantage than the heterozygous ones.

We should also highlight that parameter Δ is estimated at a population level, not for a given patient. When looking at the mutations in the population of $JAK2^{V617F}$ or $CALR^m$ patients, only some of them were found with associated mutations (note that patients with Essential Thrombocythemia display very few associated mutations); patients with more than one driver mutation might not bias our estimations to a too large extent.

Références

- [1] Henry Lee-Six, Nina Friesgaard Øbro, Mairi S Shepherd, Sebastian Grossmann, Kevin Dawson, Miriam Belmonte, Robert J Osborne, Brian JP Huntly, Inigo Martincorena, Elizabeth Anderson, et al. Population dynamics of normal human blood inferred from somatic mutations. *Nature*, 561(7724) :473–478, 2018.