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Cancer Clones Revised

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Abstract

Cancers are hard to treat, and this is largely because cancer cells evolve and diversify through space and time, in patients. The study of clonal evolution relies on the study of cancer cell lineages, and the cutting of these lineages into clones, each clone representing cancer cells with distinctive properties relevant to cancer development and treatment. This notion of clone implies a (set of) simplification(s) that misrepresents the reality. The simplification has been useful and productive, but I argue that maintaining a critical awareness of what is done through this simplification can also be useful and productive. I distinguish three types of simplifications and show that each can offer a panel of therapeutic alternatives that may complement our arsenal of strategies in the battle against clones. The clinical challenge of better treating cancer partly relies on better defining (delineating) clones, but also partly on the more fundamental way we conceive clones. With or without changing the definition, changes in the way we conceive of clones induce changes in the way we treat clones.

Keywords Cancer · Cancer treatment · Clone evolution · Lineages

Introduction

Cancers are hard to treat. This is largely due to intra-tumor heterogeneity—the heterogeneity of the cancer cells that compose each tumor.¹ A treatment may work on some cancer cells of a tumor and not on others, at some time points and not at others (McGranahan and Swanton 2015, 2017; Goyette et al. 2023). A typical scenario of cancer treatment (for a treatment that does work) is a sharp initial reduction in the tumor burden, sometimes to the point that no tumor cells are left detectable, followed by a relapse. The relapse would

typically be different from the initial tumor and resistant to the initial drug. Patients may then undergo several lines of treatments, most of which will follow the same pattern, until no more treatment options are left available. This ability of cancer cells to adapt to our therapeutic arsenal has led to increasing interest in evolutionary theories of cancer. These evolutionary theories are regrouped under the banner of the clonal evolution model, illustrated in Fig. 1.

To make sense of the intra-tumor heterogeneity, the clonal evolution model involves regrouping cells into “clones” (as illustrated in Fig. 1). The concept of clone in oncology should not be confused with that of clone in reproductive cloning (e.g., Dolly the sheep) or in science fiction. In its colloquial sense, the clone refers to an organism being a copy of another organism, while in oncology (and more generally in cell biology), the concept refers to “a population of cells descendant from a single cell of origin” (Nowell 1976).² It thus primarily refers to a cell lineage. Because our cells reproduce by mitosis, cells of a same lineage should have the same genome. But this is not entirely true. Through time and successive cell generations, alterations occur. Thus, in oncology, there is more to the clone than cancer cells’ lineage relationships. Clones are considered as

¹ Intra-tumor heterogeneity can be genetic, epigenetic, transcriptomic, proteomic, phenotypic, and so on. In theory, intra-tumor heterogeneity could also be supra-cellular. For example, Lean and Plutynski (2016) have argued that metastasis can be viewed through the lens of multilevel selection, where metastases would be the units of selection. However, heterogeneity between metastases is most often rather considered as a case of inter-tumor heterogeneity, where each metastasis is considered as a tumor.

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² This quote comes from the most cited paper in the field of clonal evolution.

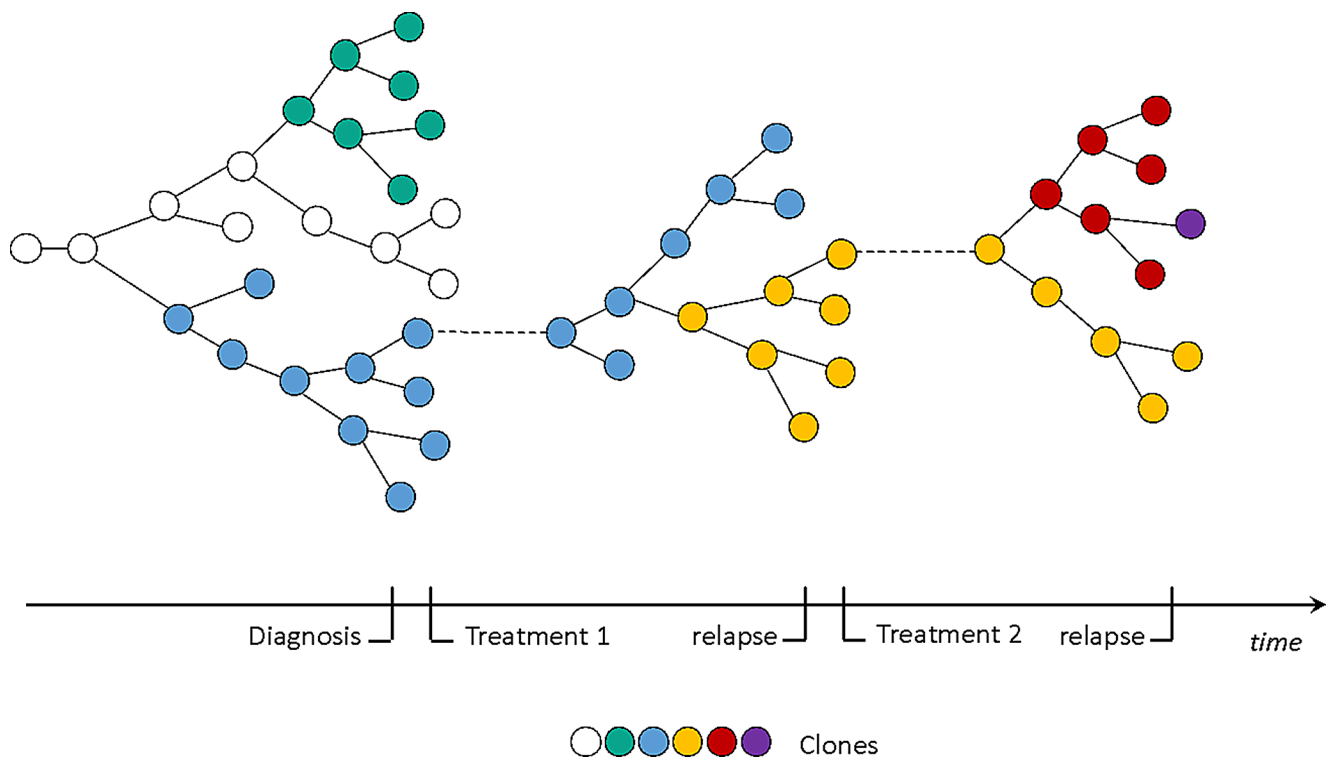


Fig. 1 Diagram of a typical representation of a fictitious case of clonal evolution in a patient’s tumor, from initiation to the various treatments and relapses

meaningful units that capture intra-tumoral heterogeneity. But this is where things get complicated:

A cancer “clone” remains a loosely defined entity, and its purest definition is “a group of cells within the tumor that share a common ancestor”. In phylogenetic terms, this would represent a monophyletic clade. However, this implies that any ancestor in the entire phylogenetic tree of a tumor can be identified as the founder of a distinct clone, even though it may show no biological difference from the rest of the cancer cells. This is why, in the field, we implicitly identify clones of interest, such as those that have growth/survival advantage (an ancestor under positive selection) or those that generate metastases (an ancestor that arrived and grew at a given metastatic site). (Caravagna et al. 2020, p. 898)

However, very few papers actually define clones with regard to phenotypic functional properties, for example: “A group of cells with the same phenotype, which have expressed that phenotype consistently since their most recent common ancestor” (Sottoriva et al. 2017). In practice, studies of clonal evolution based on functional properties are extremely rare (a notable exception is Acar et al. 2020). Most studies conceptually and experimentally define

clones on the basis of their genetic distinctiveness—for example, “Sets of cells that share an alteration of interest due to descent from a common ancestor cell” (Maley et al. 2017); “a set of cells that descend from a common ancestor and thus share genetic features” (Pogrebniak and Curtis 2018); “a genetically identical group of cells” (Steensma 2018)—and thus rely on the idea that genetic characteristics are informative of phenotypic/functional ones.³ Beyond the obvious difficulties associated with genetic reductionism, absence or presence of a mutation cannot per se distinguish the relevant clones:

Single cells within a neoplasm are likely to be genetically unique because of the relatively high mutation rate, and so each cell could be defined as a new clone. Because much of this genetic variation is non-functional (e.g. does not alter phenotype) clonal identity does not necessarily relate to phenotype, and so changes in clonal composition are unlikely to represent

³ It is to be noted that the concept of clone is very rarely defined in the scientific literature. Those who bother to make explicit their definition of clones are most often computational biologists, evolutionary biologists, or mathematicians. Definitions from biologists and clinicians are much rarer, and in general come with less precaution (e.g., the one from Steensma 2018).

changes in the evolutionary tempo (the rate of change of phenotype). (Cross et al. 2016, pp. 131–132)

Thus, in general clones are defined, conceptually tacitly but experimentally explicitly, as populations of cancer cells that have common driver mutations—mutations that are functionally involved in clonal expansion (e.g., Tokheim et al. 2016; for a philosophical analysis see Plutynski 2021)—inherited from a common ancestor. Scientists know this is an oversimplification. By focusing on sets of alterations, they may ignore alterations that end up mattering. But this simplification has proved its worth. For example, counting clones, or studying the organization of clones (relative clone size and clonal architecture) can allow patients stratification (Merlo et al. 2010; Itzykson et al. 2018; Cerrano et al. 2020). Noticeably, while adopting different definitions of clones (driver mutations, neutral mutations, microsatellites, or all of them) changed the metrics of intra-tumoral heterogeneity, Merlo et al. (2010) observed that the diversity measures remained robust biomarkers of risk of Barrett's esophagus transformation into esophageal adenocarcinoma in all instances (as far as the definition is consistently used for the analysis). The fruitfulness of this simplified notion of clone has led to its wide diffusion inside oncology, from fundamental research to clinics, to the point that the concept is now broadly used and rarely questioned.

Without denying the pragmatic value of the simplified concept of clone, I will argue that it is also pragmatically useful to maintain a critical awareness of what is done through the simplification, at minima because it can lead to new therapeutic solutions.

The first part highlights three clone simplifications that may lead to ignoring intra-clonal sources of relevant variations. First, the most ubiquitous agreement about the clone—the lineage—is an unquestioned oversimplification. Variation in resistance to treatments (as a result of cancer evolution) may not be driven by alterations in inherited properties bounded to the cancer cell lineage tree. Second, the problem with the current clone's focus on a specific set of driver alterations is not simply that we may choose the wrong set of alterations, but that the distinction between a driver (matters for resistance) and passenger (neutral) alteration is more context-dependent than is often recognized. Third, the very practice of determining a clone based on its inherited properties often depends on the commitment to a strict genotype–phenotype mapping that is undermined by significant phenotypic plasticity.

The second part features the relevance of this critical analysis of the clone. The problem of the definition of clone is most often taken as a technical challenge revolving around a unified goal to find the best possible definition of the clone, for example, “Translation of clonal diversity

measures to the clinic will require determination of which diversity measure should be used and, as new assays become available, what types of alterations should be used to define the clones” (Merlo et al. 2010, p. 1388). The second part will show that achieving theoretical clarity through deconvoluting the limitations of the clone simplification can help better define clones and has a high translational potential.

Deconvoluting the Clone Simplification

This section will show that the act of regrouping cancer cells into clones makes at list three types of simplification. They come as underlying assumptions hidden in the concept of clone: (1) lineages of cancer cells are considered inviolable; (2) clones are considered to accurately capture intra-tumor heterogeneity; (3) intra-tumor heterogeneity is considered to mostly rely on lineage's properties. Each of these assumptions face counterexamples indicating that the current definition of clone might ignore relevant sources of intra-tumoral heterogeneity, and therefore, might not be the best possible definition.

Lineages are Considered Inviolable

The clonal evolution model considers the phylogeny of cancer cells as a branching tree of cancer cells reproducing by mitosis exclusively. Inheritance is considered to be exclusively vertical (i.e., transmission goes from the mother cells to its daughter cells). Therefore, each lineage is separated, and that separation is deemed inviolable. This is the basis of the most shared and uncontested view of the clone: clones are populations of cells that come from a single common ancestor cell and therefore share the properties inherited from that cell. However, there are accumulating data suggesting that cancer cells lineages may not be as inviolable as suspected.

First, several studies have shown that cancer cells can exchange material through tunneling nanotubes (Pasquier et al. 2013; Caicedo et al. 2015; Tan et al. 2015; Moschoi et al. 2016; Burt et al. 2019). In particular, cells can exchange mitochondria. Such exchanges can occur between cancer cells as well as between cancer cells and normal cells. Transfer of mitochondria may not be random. Several studies observed more numerous, and sometime exclusive, transfers from stromal cells (mesenchymal stromal cells, endothelial cells, cancer associated fibroblasts) to cancer cells, in various solid and liquid cancers (Pasquier et al. 2013; Caicedo et al. 2015; Moschoi et al. 2016; Burt et al. 2019). Moschoi et al. observed an increase in mitochondria uptake by cancer cells when they are treated with chemotherapy, suggesting that cancer cells may actively uptake mitochondria from

normal cells. All these studies showed that the transfers from normal cells to cancer cells were beneficial to cancer cells. They increased the cancer cells' viability and resistance to chemotherapy (Pasquier et al. 2013; Moschoi et al. 2016; Burt et al. 2019), or increased their proliferative and migratory capacity (Caicedo et al. 2015; Tan et al. 2015).

Second, cancer cells can also exchange retrotransposons. Most retrotransposons are inactive, but some can still copy-paste (i.e., they can be transcribed into RNA, and reintegrate the genome at another location through reverse transcription). And some can produce virus-like particles that can infect other cells and be inserted in their DNA. Conversely to mitochondrial uptake, which is uncontroversial, retrotransposon horizontal transfers have been largely overlooked in oncology. Yet, a few studies have made direct or indirect observations indicating that such transfer are possible in human cancers (Bieda et al. 2001; Morgan and Brodsky 2004; Büscher et al. 2005; Dewannieux et al. 2006; Contreras-Galindo et al. 2015; Gibb et al. 2015). Given that the expression and/or retrotransposition of retrotransposons can change the fitness of the cancer cells, either positively or negatively (Lamoureux et al. forthcoming), their horizontal transfer may also affect the fitness of recipient cells.

Third, the recent attention to the microbiota and its roles in cancer has led to the observation that tumors are not sterile, and both microbes and fungi can in fact reside inside cancer cells (Bullman et al. 2017; Nejman et al. 2020; Kalaora et al. 2021; Narunsky-Haziza et al. 2022). Microbes can impact cancer cell fitness in various ways. For example, some microbes (*Mycoplasma*) are able to degrade some chemotherapies (Gemcitabine), allowing the cell to remain alive under treatment (Geller et al. 2017). Conversely, infected cells may present bacterial peptides at their surface that can be recognized by immune cells and result in the elimination of the infected cell (Kalaora et al. 2021). This has led colleagues and me to suggest reconsidering clonal evolution as a multispecies process, where several genomes, human and nonhuman, contribute to the evolution of cancer cells (Sepich-Poore et al. 2022). Microbes, like retrotransposons and mitochondria, contribute to horizontal transfers in cancers. They also have their own lineages, with their own horizontal gene transfers, and their own selective pressures, complicating the evolutionary history of cancer cells.

Trogocytosis is another fascinating process where cells can exchange part of their cell surface. In cancer, trogocytosis has received more attention recently with the development of a particular type of immunotherapy involving engineered immune cells called CAR, because trogocytosis can lead to therapy failure. The cancer cells exchange cell surface parts with the CAR cells with two consequences: (1) the cancer cells gain immune cell receptors and can thus hide from the immune system (being recognized as

immune cells rather than cancer cells); (2) the immune cells find themselves with cancer cell's proteins at their surface, and hence are recognized as cancer cells and killed by the other immune cells (Hamieh et al. 2019; Li et al. 2022). The heritability of these properties has not been studied. One can imagine it will be short, a handful of cell generations at most. Its relevance to clonal evolution might be limited. However, it is important to indicate that CAR treatments are done in one shot: the cells are engineered, then injected once to the patients. These treatments thus impose brutal changes in selective pressures that might not persist long, if trogocytosis leads to the elimination of the CAR cells. Short term inheritance might be sufficient to result in the positive selection of the lineages descending from the cancer cells that have undergone trogocytosis. More will be said on short-term inheritance in the next section.

Fourth, it is widely considered that cancer cells only reproduce by mitosis. Although this is the rule, there are exceptions, most notably, cell fusions. Cell fusions are rare but have been repeatedly reported in multiple cancers (references below, which are not exhaustive, concern breast, renal, colon, pancreatic cancers, and melanoma). Cell fusion can occur between cancer cells (Miller et al. 1989; Miroshnychenko et al. 2021), as well as between cancer cells and normal cells (Chakraborty et al. 2004; Yilmaz et al. 2005; Gast et al. 2018). Several studies have shown that not only can hybrid cells survive and divide, but they might also have a higher fitness. Several studies report faster time to division, and/or multidrug resistance (Miller et al. 1989; Gast et al. 2018; Miroshnychenko et al. 2021). Fusion is a way to acquire new properties through a single event. For example, fusion of solid cancer cells with hematopoietic cells such as macrophages can provide to the cancer cells the migratory ability of macrophages, enabling their metastasis (Rachkovsky et al. 1998; Pawelek and Chakraborty 2008). Fusion, like recombination, can contribute to the diversification of clones, and one single hybrid cell can lead to multiple clones as its descent undergoes ploidy reduction (Miroshnychenko et al. 2021). Other weird phenomena occur in cancers that could count as special cases of fusion, such as cell cannibalism. Cannibalism provides cancer cells the ability to survive in harsh conditions (Lugini et al. 2006; Bartosh et al. 2016). But it is unclear to what extent cell cannibalism can lead to genomic incorporation and give rise to hybrid cells.

All these cases show that the current focus on dividing cancer cells as the sole lineages in clonal evolution is neglecting events that may contribute to the evolutionary properties of the cancer cells. Various DNA-containing elements may follow different lineages. Retrotransposons, mitochondria, microorganisms, may form evolutionary trees that are only partially concordant with the cell tree. Given their contribution to cancer cell properties, it might

be the case that some cancer cells' properties of interest, such as treatment resistance, are lineages-related, without being tied to the cancer cell lineages themselves. Under which condition they might lead to the emergence of new clones depends on how one delineates clones, which will be the focus of the next section. But at minimum, these events can lead to introgression, that is, the introduction of genetic information from one lineage to another. Moreover, even the cancer cells' lineages themselves may contradict the assumption of lineage inviolability as cancer cells can fuse, or cannibalize other cells, producing hybrid cells able to generate new clones. All these cases of lineage violations can impact the fitness of the cancer cells and can represent evolutionary shortcuts for the acquisition of adaptive properties. One may ask how these lineage violations differ from mutations or karyotypic aberrations, which also genetically mess with lineages. I suggest restricting lineage violations to the cases where the genetic (or nongenetic in cases such as trophocytosis) innovation can be traced back, i.e., when there is a history prior to the event that involves already existing material. They may have similar consequences to mutations in that they may lead to the occurrence of new clones (and in this sense, they face the same issues that will be discussed in the next section). But Neto argued that unviolated and violated lineages should be conceived as ontologically different types of lineages because their distinct sources of heterogeneity come with distinct ways of evolving through time (Neto 2019). Moreover, in the case of cancer, what matters to my argument is that they may lead to very different therapeutic interventions, as will be discussed in the second part.

Nature Carved at its Joint: Clones are Considered Accurately Delineated

Pan-cancer sequencing projects have shown that there are abundant mutations in cancer cells, sometimes thousands of mutations. Cancer cells may well be each genetically unique. Thus, the clonal evolution model only focuses on a restricted set of alterations, most often the ones that are considered to play a role in the evolution of cancer cells.⁴ They are called driver alterations. Cancer cells lineages are cut into new clones when there are new driver alterations. Such identification of cancer clones is meant to capture the intra-tumoral heterogeneity that really matters to cancer

⁴ Note that which mutations or alterations are used has changed through time. In the first formalized version of the clonal evolution model, by Peter Nowell, the focus was on karyotypes as those were observable with *camera lucida* (Nowell 1976). With the rise of the oncogenes in the 1980s, and the development of sequencing technologies, the focus shifted toward driver mutations. Other sets of alterations can be and are sometimes used. But driver mutations are currently, by far, the most common traits used to identify clones.

evolution and leave aside all the noisy sources of diversity between cancer cells that are neutral. But this reasoning relies on the assumption that one can distinguish among all the alterations the ones that are drivers from the ones that are passengers. This assumption comes with two problems. One is related to the limited investigation of hereditary traits in cancer cells. The other is related to the nature of the driver/passenger dichotomy.

Traditionally, the clonal evolution model has focused on mutations and karyotypic alterations, a legacy of its origin from population genetics that largely dominates the field. In theory though, all heritable properties can contribute to cell fitness. And cells inherit much more than their genes. A growing interest on the role of the epigenetic in cancers is paving the way for a more inclusive characterization of cancer clones. Several studies have used epigenetic traits to reconstruct clonal evolution. Several have compared phylogenetic reconstruction based on genetic and epigenetic alterations and have shown good concordance (Brocks et al. 2014; Oakes et al. 2014; Mazor et al. 2015). However, some of these studies have also highlighted that epigenetic evolution can occur without genetic evolution (Li et al. 2016). This means that a genetic clone can contain several epigenetic clones (or vice-versa) (Sasca and Huntly 2016).

These studies illustrate that the clonal evolution model can support extending the concept of driver mutation to a more encompassing concept such as “driver alteration,” where the alterations could be genetic, or epigenetic. Notice, however, that the study of epigenetic clonal evolution has focused on methylation and chromatin. There might be more to investigate regarding cancer cell inheritance, such as maybe proteins with a longer life span than the duration of the cancer cell cycle. Some cancer may show an accelerated cell cycle. For example, in an experimental project on which I collaborate with Leïla Perié and Alessandro Donada (two biologists in Curie, Paris), we have observed that while the duration of a cell cycle in general is around 20 h for normal hematopoietic progenitor cells, it is around 10 h in leukemia, with some cases of exceptionally fast cycling cells (around 2 h). With such a short lifespan, many material elements of the cancer cells may be transmitted through several generations of cells.

Thus, current clone delineations, due to their focus on mutations, might not appropriately carve nature at its joint. They risk regrouping lineages that differ regarding traits that do matter for clonal evolution. This concern is acknowledged in the scientific community and might be progressively addressed with scientific progress. The wide diversification in technologies, in particular single-cell technologies, and the post-genomic area allowing more space for nongenetic investigations, should make possible the development of a more inclusive account of driver alterations, and thus

a better delineation of clone. Short timescale inheritance, however, remains overlooked in the community, a problem that will need to be addressed in the future, especially for cancer treatment-related research.

Even the most inclusive notion of driver alteration cannot escape a more conceptual issue related to the driver/passenger dichotomy. Passenger alterations are defined in opposition to driver alterations: they are alterations that happen to be there but play no role in cancer cells' fitness. Although the dichotomy is elegant and powerful, one must not forget that it relies on properties that are quantitative and context dependent (Pradeu et al. 2023). The effect of an alteration is quantitative, raising the question: what is the size effect necessary to call a mutation a driver mutation? It is also contextual. The same mutation can be driver in one cell type and not in another one (e.g., if the gene is not expressed, the mutation cannot drive anything). It can be driver at one time point and not another, depending on selective pressures. Targeted therapies provide a striking illustration. The mutations they target (e.g., *EGFR* in lung cancer, *KIT* in gastrointestinal stromal cancer, *IDH* in acute myeloid leukemia, and so on) are driver mutations before treatment, and lose their competitive advantage under treatment. Conversely, treatment can select cells with mutations that were passenger in the absence of treatment. For example, *PPMID*

drives hematopoietic clonal expansion in context of cytotoxic chemotherapies (Hsu et al. 2018). Infections, inflammation, smoking, aging, and many other factors can also change the fitness landscape and turn passenger mutations into driver mutations (Laconi et al. 2020; Florez et al. 2022). The environment, through the selection pressure it imposes on cancer cells, is a major factor of their fitness, and hence of which alteration may act as a driver. Thus, there are no absolute “driver” alterations. There are alterations that are driver in certain contexts, and to a certain amount. Consequently, which alterations should be used to identify clones is likely to fluctuate. But the ways in which clones are identified does not leave room for fluctuation: any given cell is considered to be a member of one and only one clone. Figure 2 illustrates this issue. At time t_1 , three sets of driver alterations are identified, resulting in the identification of three clones. Treatment introduces a new selective pressure, leading to the elimination of the green and white clones, and the selection of the light blue clone. Suppose the resistance comes from a mutation in cell *C* that was passenger prior to treatment and became driver under this new selective pressure. Given that the same mutation switched from neutral to driver, cell *C* and its descent should also switch from being part of the light blue clone (in the absence of treatment they exhibit similar properties to other light blue cells and thus

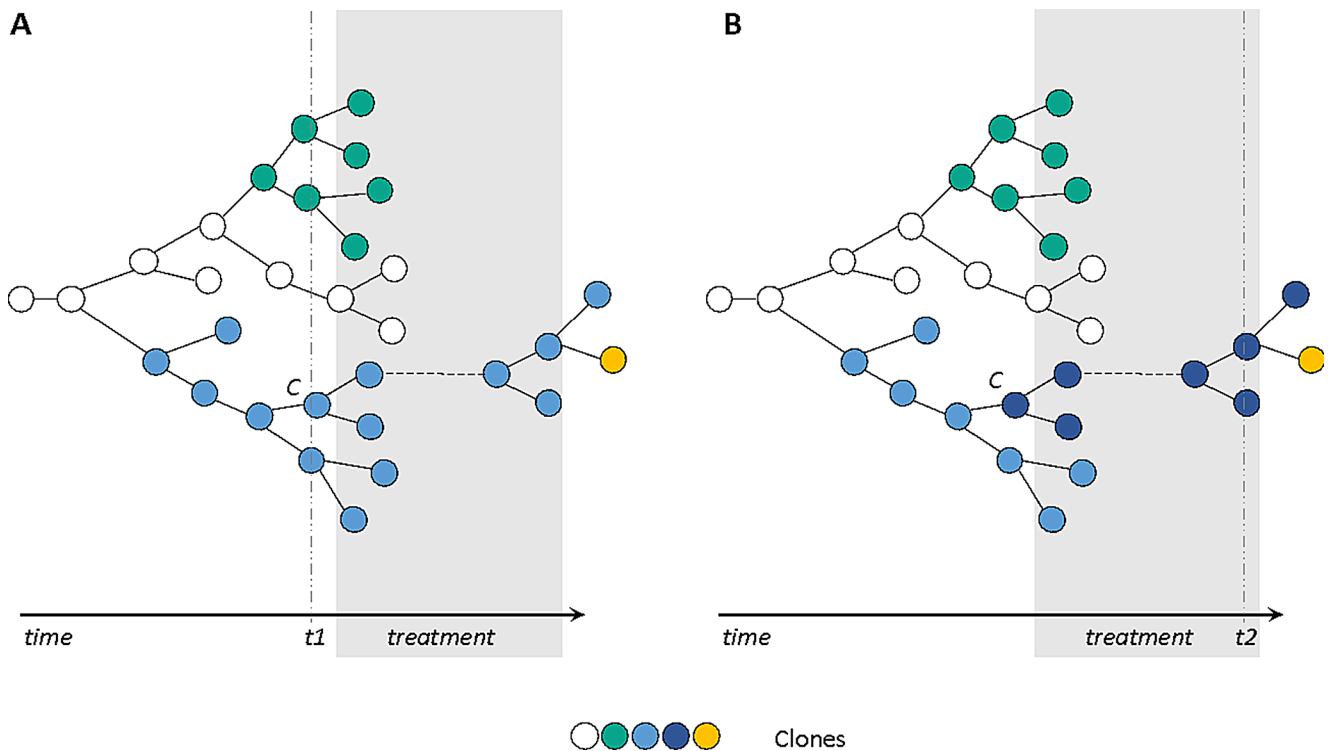


Fig. 2 Whether an alteration is driver or passenger depends on context. Thus, the same cell might be identified as belonging to two different clones in two different contexts. **(A)** Before treatment, cell *C* is considered a member of the light blue clone. **(B)** However, cell *C* had

a passenger mutation at time t_1 that became a driver mutation in its descents when they became exposed to treatment. Therefore, at time t_2 , the same cell *C* is considered as part of the dark blue clone

do not represent a distinct clone) to being part of a different clone, the dark blue one (in the presence of treatment they present different properties from the light blue cells and thus represent a distinct clone). The current uses of the clonal evolution model do not allow for such a fluctuating cut of the tree: either cell *C* is prospectively considered part of the light blue clone, and the model then poorly accounts for treatment resistance, or cell *C* is retrospectively considered as a member of the dark blue clone and the mutation responsible for treatment resistance is then considered as driver, even when it is not (at time *tI*).

Intra-Tumor Heterogeneity is Considered to Mostly Rely on Lineage's Properties

Regrouping cells into clones according to their genealogy assumes that whatever is inherited through cell lineages largely contributes to the phenotype of cancer cells, such that cells that belong to different lineages (and more precisely to different clones) have different phenotypic properties. But, in multicellular organisms, the cell phenotype is largely underdetermined by its genotype. Cells of a multicellular organism have different phenotypes despite their highly similar genotypes, and stem cell lineages can all give rise to multiple cell types inside a given tissue (Fagan 2017). How much lineages are relevant to cell phenotypes remains an open question. In oncology, especially in the context of cancer treatment, there are currently two views: treatment resistance may occur through evolutionary processes or through phenotypic plasticity (Marine et al. 2020). Note that those views are not mutually exclusive, but the debate is whether one plays a greater role than the other.

In this evolutionary framework, resistance to treatment selection pressures is a constitutive property that, when present, is transmitted to daughter cells. Thus, whether any given cancer cell is sensitive or resistant to a treatment largely depends on which lineage it belongs to. For example, in Fig. 2, resistance to treatment is attributed to a mutation present in cell *C*. As the mutation is inherited, cells that belong to the lineage initiated by cell *C* are resistant to treatment. The ever-growing list of treatment-resistant mutations supports this view. A famous case is *EGFR* mutation in lung cancers. *EGFR*^{T790M} provides resistance to EGFR first-generation inhibitors, while *EGFR*^{C797S} provides resistance to third-generation inhibitors. Cells belonging to lineages with those mutations escape those treatments. This does not mean that cells belonging to other lineages cannot be treatment resistant. Convergences are possible. Cells from different lineages may be resistant to a same inhibitor. Several evolutionary biologists have argued that convergence is common in cancer (Gatenby et al. 2011; Fortunato et al. 2017). To extend the example of EGFR-inhibitors, several

other mutations can converge toward the same resistance pathway (Bolan et al. 2020). The case of *EGFR* in lung cancer is not an exception, it is representative of a common limitation faced by targeted therapies. Another example would be BRAF-inhibitors in melanoma. They are very efficient in the case of *BRAF*^{V600E} mutations, but most patients relapse within a year through different resistance mutations that converge toward the reactivation of the MAPK pathway (Johnson et al. 2015). With or without convergence, according to the evolutionary view, the membership of a cell to a lineage plays a major causal role in its ability to resist treatment.

Conversely, in the case of phenotypic plasticity, some cancer cells can resist treatment for causes largely unrelated to lineages, such as the ability to switch phenotype (e.g., enter a quiescent state, returning to a cancer stem cell state, transdifferentiating, and so on) (Boumahdi and de Sauvage 2020; Marine et al. 2020). Interestingly, the plasticity view has also been advocated as a mechanism for treatment resistance to EGFR and BRAF inhibitors. Thus, the two views coexist to explain the same phenomenon. One striking observation considered in favor of plasticity is the cases of non-small-cell lung cancer relapsing after EGFR-inhibitors with a phenotype of small-cell lung cancer, associated in some of the patients with a regained sensitivity to the EGFR-inhibitor (Yano et al. 2005). The ability of cancer cells to escape treatment is often compared to bacteria. The concept of persisters is applied to cancer cells that can transiently survive lethal drug exposure. Again, arguments in favor of resistance through persistent cells have been collected in many cancers including the cases discussed above of lung cancer and melanoma. A key recurrent argument in favor of this view is the reversibility of the resistance that suggests a transient rather than long-term genetic heritability of the resistant trait (Sharma et al. 2010; Shaffer et al. 2017).

The theoretical relationships between the two views are not clear. Phenotypic plasticity can be partly heritable, and single-cell analysis of persistent cells are consistent with some forms of inheritance. For example, Turati et al. (2021) showed selection of cancer cells in a specific transcriptomic state inherited in persistent cells. Marsolier et al. (2022) showed selection of cancer cell lineages defined by a permissive histone state depleted in H3K27me3 which is pre-existing before treatment, stable in persistent cells, but may be lost in resistant cells, suggesting short-term inheritance. Moreover, the persistent/resistant dichotomy might be compatible with a Baldwin effect, where plasticity may buy time for acquisition of new mutations that may later be selected (Laplane and Maley 2024).

This first section shows that the current definition of the clone makes three types of oversimplifications, whose

cost is to ignore sources of variations that are relevant to cancer evolution and treatment. The first one concerns the way we conceive of lineages in cancer. We have highlighted multiple sources of lineage violations which can result in evolutionary shortcuts. This is the most original part of the first section, as the lineage component of the clone is considered unproblematic and almost never questioned. The second source of variation ignored by the current definition of clone pertains to the identification of driver alterations. This is a well-acknowledged challenge. However, the nature and extent of the challenge is not appropriately appreciated. Current efforts to improve the definition of clones are presbyopic to short-time inheritance and they ignore the labile nature of driverness. The third oversimplification is the assumption that relevant traits are heritable. This is a necessary assumption: the clone only captures lineage-related properties. Although not much can be done about this oversimplification, the next section will show that consciousness that clones can exhibit plasticity may impact the way we conceive their therapeutic management.

Clinical Implications

Without denying the pragmatic value of the simplified concept of clone, my aim is to show that critical awareness of what is done through the simplification has its own benefits. Here, I will mostly focus on the most pragmatic benefits for oncology—cancer treatment—and show that each type of simplification raises its own set of therapeutic solutions. Along the line, I will also call up additional theoretical benefits, when suggestions can be made regarding the challenge of better defining clones.

Lineage Violation

We have highlighted several potential breaches in lineages. They call for a revision of the current view of lineages (and therefore clones) in cancers. Boucher and Baptiste (2009) suggested distinguishing open and closed lineages. While closed lineages refer to the traditional view of lineages (sequences of biological organisms connected by reproduction and vertical inheritance of their genomic material), the concept of open lineages was introduced to account for the contribution of repeated gene transfers in the genetic variation of prokaryotes. A consequence of open lineages that would be of concern for clonal evolution is that when the contribution of extrinsic genetic material becomes higher than the contribution from intrinsic genetic material, the lineage can no longer be put on a tree.

There is no such thing as one last common ancestor for them, but rather populations of diverse ancestors, which contributed multiple genes and signals to the lineage over time. For these open lineages, long-term retrodiction is much harder: the biology of the hypothetical ancestor of a lineage had little to do with the biology of its actual members. (Boucher and Baptiste 2009, p. 534)

Given that breaches in cancer cell lineages have been largely overlooked, we hardly have any idea of their contribution to clonal evolution. It seems, however, safe to assume that even cumulated, they do not overload internal genetic variation. However, the importation of the concept of open lineage can help the clonal evolution model accommodate lineage violations caused by mitochondrial transfer, ERVs, microbes, fungi, trogocytosis, and cell fusion. More generally, while reticulation—a process by which a new lineage can form after lateral transfer or other merging of two ancestor lineages—is completely overlooked in cancer, it is highly studied in other areas of evolutionary biology, since the idea of a universal tree of life started to be criticized (Doolittle 1999). There is a rich area of theoretical and empirical research from which cancer research can take inspiration (Haber and Velasco 2022). How to empirically account for such reticulation events is outside the scope of the present article, but I would like to draw the consequences of reticulation for treatment.

The main way reticulations challenge cancer treatment is by allowing evolutionary shortcuts, most notably when they quickly introduce complex properties from outside the cancer cell lineage. The case of treatment resistance through uptake of mitochondria from surrounding noncancerous cells provides a clear example of this issue. If we want to avoid such evolutionary shortcuts, we need to develop treatments that can avoid reticulation events. In the case of mitochondria, they are transferred through tunneling nanotubes, which are targetable. For example, Bukoreshtliev et al. (2009) showed that cytochalasin B, which blocks filopodia, prevents the formation of tunneling nanotubes. In their experiment, it led to a drastic reduction of mitochondrial transfer. Burt et al. (2019) interfered with tunneling nanotube formation using vincristine, a microtubule inhibitor which prevented mitochondrial transfers and the acquisition of drug resistance by cancer cells.

Similarly, microbes can also provide shortcuts for the acquisition of drug-resistant phenotypes in cancer cells. One such example is the *Mycoplasma*, able to degrade gemcitabine (Geller et al. 2017). Microbes can be eliminated through antibiotics. Manipulating microbes in cancer has its own challenges, as different microbes may have different effects and which ones are beneficial or detrimental in

which context remains to be clarified (Sholl et al. 2022). Given active research done in the field of cancer microbiota, we can expect progress that may provide pharmaceutical tools to specifically target drug-degrading microbes.

Cell fusion also provides opportunities for evolutionary short-cuts. One example is the acquisition of migrating properties through fusion with macrophages (Pawelek and Chakraborty 2008). Fusion mechanisms are poorly studied in oncology but attract more attention in other areas of biology and medicine as fusion plays an essential role in several developmental and physiological processes (from fertilization to muscle and osteoclast formation). So far, very few fusogens (proteins implicated in fusion) have been clearly identified. The most well characterized is syncytin 1, an envelope protein of human endogenous retroviruses, known for its role in placenta formation. The involvement of syncytin in cancer cell fusion has been experimentally demonstrated in studies that also showed that treatment with syncytin inhibitory peptide was able to reduce fusions in vitro (Bjerregaard et al. 2006; Uygur et al. 2019). The knock-down of *ASCT2*, the syncytin receptor, has the same effect (Bjerregaard et al. 2006). Thus, syncytin and *ASCT2* inhibitors could provide therapeutic solutions. *ASCT2* has recently attracted attention as a potential target in cancer for other reasons⁵ (Ndaru et al. 2019), and several *ASCT2* inhibitors have shown promising results (Lyu et al. 2023; Qin et al. 2024). Their ability to block cell fusion remains to be tested. One advantage of targeting cell fusion through the syncytin/*ASCT2* axis is that it could also block some retrotransposon lateral transfers, potentially limiting two sources of evolutionary shortcuts at once.

Inadequate Delineation

We have seen that inadequate clone delineation might be partly reduced by a better understanding of cancer cell inheritance, but also that it might be a partly insoluble problem due to the inherent lability of driverness. These two observations may lead to different consequences for cancer treatment.

First, the best we can do regarding clone delineation is to adopt a more inclusive approach to the clone, and examine as many hereditary properties as possible, in as many contexts as possible. Research is very active in genetics and epigenetics, with much progress being done. This is encouraging. However, this research is still focused on long-term inheritance, while short-term inheritance remains nearly unexplored. Very little is known about what can be inherited on a few generations of cells (by few, I mean at least two). Yet, most cancer treatments impose high selective pressure

during short periods of time. Thus, properties inherited on only few generations of cells (maybe some proteins with long half-life, for example) could play determinant roles in treatment resistance. Exploring short inheritance could provide new directions and unanticipated therapeutic opportunities.

Second, the inherent lability of driverness challenges the fixed boundaries of the clone. Theoretically speaking, clones do not have to be thought of as having permanently fixed delineations. The same lineage of cells could well be labelled as belonging to different clones depending on context, as illustrated by Fig. 2. Kate MacCord makes a similar suggestion regarding the delineation of the germ cell lineage. A lineage can be considered as somatic, until a cell transdifferentiates into a germline cell, which then backward reassigned it and all its ascendants as germinal (MacCord, “Let’s Talk About Sex...Cell Lineages,” this issue). The pragmatic value of this theoretical suggestion is low as its application would be complex. But realizing how much clone delineation is context-dependent can reorientate the attention toward the intricate relationship between the cells and environmental selective pressures, which could provide new therapeutic opportunities. The therapeutic goal would be to make all heritable alterations passenger, or detrimental. The strategy would be to modulate driverness by acting on the context on which they rely. The tumor-microenvironment has become a growing focus of interest for cancer research, but the fuzziness and too restrictive understanding of the concept limits translation into therapeutic applications (Laplaine et al. 2018, 2019; Rondeau et al. 2019; Solary and Laplaine 2020). A difficulty one can anticipate is that driverness of each alteration depends on multiple factors and one factor could have opposite effects on different alterations. Yet, adaptive therapies offer a concrete example that partly overcomes this issue. Adaptive therapies are treatment protocols that are adjusted depending on tumor response (Gatenby et al. 2009). The goal of adaptive therapies is to avoid treatment escape. The strategy is to maintain cancer cells that are sensitive to available treatments by stopping treatment before the elimination of the sensitive clones. The assumption is that resistant cells have a lower fitness than sensitive cells in the absence of treatment. Thus, adaptive therapies aim at modulating the driverness of cancer cell alterations. Despite good preliminary results in a clinical trial (Zhang et al. 2022), the theory and practice of adaptive therapies faces some limitations (Mistry 2021; West et al. 2023), including the need to more largely account for factors that modulate cells’ fitness, such as spatial structures (Strobl et al. 2022).

⁵ It is involved in transporting glutamine which is highly consumed by rapidly growing cancer cells.

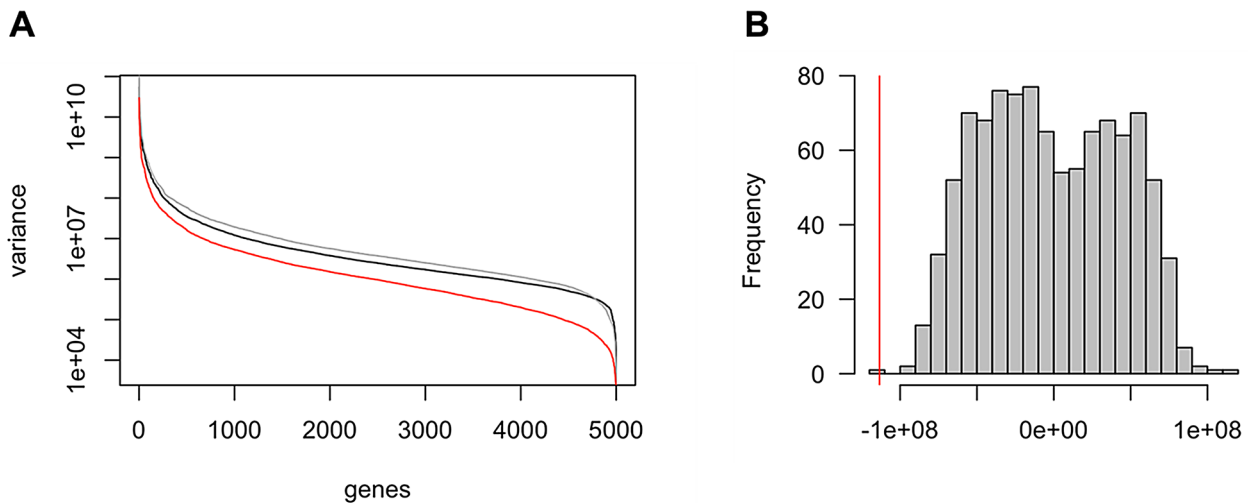


Fig. 3 Transcriptomic heterogeneity in control cells, *Srsf2* mutated cells, and *Jak2* mutated cells. **(A)** Measure of variance in gene expression. For each genotype (control in black, *Jak2* in red, *Srsf2* in grey), the 5000 most expressed genes were selected to measure their vari-

ance. The plot shows the variance (y axis) of each gene (x axis), in decreasing order. **(B)** Permutation test between *Srsf2* mutated and *Jak2* mutated cells (t test: 0.01)

Phenotypic Plasticity

With the assumptions that relevant properties are tied to lineages and that lineages with distinctive relevant properties can be distinguished into clones come a representation of clones that I will call “Batman-like.” Like Batman, cancer clones are considered as strong and powerful (each clone here should be considered as a singular Batman with its own strength). They do not follow the social rules, but they do have their own code of conduct. Crack that code, and you’ll have a cure. This view is the foundation of targeted therapies. Yet, acknowledging plasticity invites a different metaphor. Some cancer clones rather resemble the Joker. They are not that strong, but they follow no code of conduct, which makes them unpredictable. They have extensive sets of behaviors they can adopt in front of any varying context. There is no code of conduct, no logic, no reproducible strength or weakness to target.

Let me illustrate these two contrasting views of the clone with a concrete example. The *JAK2*^{V617F} mutation is famous for its involvement in myeloproliferative neoplasms, a type of chronic blood cancer. It is a typical driver mutation. Acquisition of a *SRSF2* mutation in these patients increases their risk of transformation into acute myeloid leukemia, with a drastically much worse prognosis. In my lab in Gustave Roussy, we worked on deciphering this clonal evolution. To our surprise, competition analyzes in mouse models showed a high fitness advantage of *Jak2* clones and impressively poor fitness of *Srsf2* clones (see Willekens et al. 2023; see its Fig. 2C). How can a clone with such a low fitness be so detrimental and difficult to manage in clinics? It was this

study that led Christophe Willekens (the first author of the paper) and me to the Batman/Joker metaphor. To dig further into this issue, I suggest taking advantage of the transcriptomic data we have and investigate how homogeneous or heterogeneous clones are in their gene expression. To this aim I measured the variance in gene expression in control, *Jak2* mutated, and *Srsf2* mutated cells. The results indicate a sharp contrast between the two types of clones: *Jak2* clones are more homogeneous (possibly on a narrow high adaptive pick?), while *Srsf2* clones are more heterogeneous (Fig. 3). This analysis illustrates the idea that there might be different types of clones: some with high fitness and homogeneous features, in line with the simplified definition of clones (Batman-like); others with lower fitness, regrouping cells exploring a wider phenotypic space (Joker-like).⁶

The dichotomy Batman/Joker types of clones invites thinking differently about how to treat Joker-type clones. A possibility would be to restrict the ability of the cells to explore a wide phenotypic space. Currently, works related to plasticity in cancers aim at identifying mechanisms, and targeting the various identified pathways (Labrie et al. 2022). However, there are many ways for a cancer cell to be plastic, limiting the efficacy of specific strategies (a similar issue is raised for the modulation of driveriness). Taking a step back on the process might be useful. The therapeutic goal would

⁶ This analysis should not be taken as more than illustrative. It suffers several limitations: (1) the data are bulk RNAseq of megakaryocytes, when for clonal evolution it would have been more adapted to investigate single cell RNAseq of stem cells. (2) The analysis relies on a very small sample size (transplantation in four mice). (3) The transcriptomic state is only one aspect of cell heterogeneity among many others.

be to avoid cancer cell resistance by plasticity. The assumption is that resistance occurs by change in cell state (plasticity), rather than through selection of a resistant cell state. Given this assumption, a more general strategy could be to block cancer cells state during treatment rather than targeting the multiple changes that might make them resistant. Therapeutic compounds that would make the genome inaccessible to translation, such as an hypermethylating agent, would drastically limit the ability of cells to adapt. Hypermethylating agents do not exist (in fact all clinical efforts are focused on the opposite strategy of hypomethylating the genome), and on a long period of time they would be very toxic to the cells (both healthy and cancerous). Whether short treatments could be supported by the cells, or whether small doses only partially hypermethylating the cells would be sufficient for clinical benefits, are empirical questions that remain to be addressed. But some experimental data indirectly support this strategy. For example, Marsolier et al. (2022) showed that the simultaneous use of an inhibitor of a histone demethylase with chemotherapy reduced the pool of persistent cells (the cells that do not die during treatment) *in vitro*, and delayed relapse and mortality *in vivo*. Thus, a minimal strategy could be to prevent demethylation by blocking demethylases. Cells would then keep access to genes they already have access to but would not be able to access those that are methylated. This would already restrict cell plasticity. A limit wired in this strategy of methylation modulation is the assumption that plasticity necessarily relies on gene expression. It is possible that other forms for cell reorganization may also allow resistance through plasticity. Those would require other strategies.

Conclusion

The heterogeneity of cancer cells is the biggest issue for cancer treatment. The clonal evolution model aims at providing evolutionary tools to deal with this problem. This model relies on the study of cancer cell lineages, and the cutting of lineages into clones, each clone representing cancer cells with distinctive properties relevant to cancer development and treatment. The concept of clone is making a simplification by regrouping cancer cells and assuming that these cells can be conceived as homogeneous, even if they may differ in some respects. Although this simplification has proved its usefulness, I showed that investigating what this simplification is really doing, i.e., how it misrepresents the reality, can also be useful and productive. I distinguished three types of simplifications and showed that each can offer a panel of therapeutic alternatives that may complement our arsenal of strategies in the battle against clones. The clinical challenge partly relies on the definition (delineation) of clones,

but also partly on the more fundamental way we conceive of clones. Thus, even without changing the definition, changes in the way we conceive clones induces changes in the way we treat clones.

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Data availability The transcriptomic data come from Willekens et al. Leukemia 2023, and are available in BioStudies under accession number: E-MTAB-11960.

Declarations

Conflicting Interests The author declares no conflicts of interest.

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