

Toward a Deeper Understanding of Clonal Evolution in Acute Myeloid Leukemia: Translational and Clinical Impacts

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Abstract

To understand disease biology of Acute Myeloid Leukemia requires an appreciation of initial clonal heterogeneity of the disease, as well as the selective pressures on these clones and the resulting change in cytomolecular profiles over time. Elucidating these underpinnings of leukemogenesis is critical for attempts to enhance therapeutic efficacy. Here, we review key findings derived from a retrospective analysis of a diverse acute myeloid leukemia cohort of 207 patients and discuss biological mechanisms underlying these trends.

Keywords: Acute Myeloid Leukemia, Clonal Evolution, FLT-3, Leukemogenesis, TP53

Commentary

Tumor genetic heterogeneity is widely understood to represent a key pathophysiologic mechanism for the development of cancer, progression, metastasis, and therapeutic resistance in nearly all malignancies [1]. Elucidating common patterns of clonal evolution in Acute Myeloid Leukemia (AML), and how this knowledge can be harnessed to develop effective treatment paradigms to eradicate all clones has recently garnered much attention [2]. In fact, AML provides one of the simpler models for understanding such heterogeneity, as AML typically has fewer genetic mutations in individual patients, compared to many other tumor types [3]. Particular patterns of clonal evolution based on host factors, disease biology, bone marrow microenvironment, and therapeutic drivers of clonal evolution in AML remain poorly understood. In this regard, understanding temporal aspects of clonal heterogeneity and clonal evolution over time in AML represents a key consideration for increased understanding of the disease biology of this malignancy.

From its earliest onset, AML is a heterogeneous disease [4]. It is understood that there are some discernable patterns of somatic mutation acquisition in leukemogenesis [5]. For example, this often starts with acquisition of myeloid clonal hematopoiesis of indeterminant potential (M-CHIP)-type mutations in epigenetic regulators such as DNA methyltransferase 3 alpha (DNMT3A), Ten-eleven translocation methylcytosine dioxygenase 2 (TET2), and Additional sex combs like 1 (ASXL1). M-CHIP mutations are somatic mutations known to be involved in myeloid malignancies, present at a variant allele frequency (VAF) of >2%, and may or may not represent a pre-malignant state [6,7]. In fact, most of these patients will never develop myelodysplastic syndrome nor AML. At least one of three genes is mutated in 87% of all M-CHIP cases [8]. From here, a complex interplay between host and environmental factors which may include immune dysregulation and various other epigenetic stressors, a multitude of cellular pathways, bone marrow microenvironment, time, and aging allow for expansion of the abnormal clone via selective pressure [9,10]. Work is ongoing to understand these interactions and how

they may be therapeutically targeted in our lab and many others; a full review is outside the scope of this commentary [11-14]. Chemotherapy and targeted therapies themselves represent selective pressures, thus, understanding which patterns of clonal progression may emerge from targeting particular pathways with therapeutic intent may ultimately inform treatment options and ultimately help to prevent such progression.

We recently published a retrospective analysis of 207 consecutive adult patients with either refractory or relapsed acute myeloid leukemia treated over a decade at an urban comprehensive cancer center, with respect to changes in cytomolecular profiling over time obtained from clinical bone marrow biopsies/aspirates. Importantly, this report describes a cohort diagnosed between 2013 and 2023, an era which saw next-generation sequencing panels become more commonplace, and ultimately standard practice in the diagnosis, characterization, and prognosis of AML [15]. This trend ushered in a treatment paradigm shift toward “less intensive” hypomethylating (HMA)/venetoclax (VEN)-based treatment options for more patients. The demographics of this cohort was also unique in an urban cancer center with a diverse and high-proportion underserved population which may contribute to an increase in epigenetic stressors that in turn exert differential effects on tumor biology and response to various therapies [16,17].

Our work highlighted several key findings, three of which will be expanded upon here: 1. Clonal evolution becomes more common over time – that is, a patient with relapsed disease is more likely to relapse with a different clone than a patient with primary refractory disease. 2. Certain cytogenetic and molecular profiles seem more prone to the development of clonal evolution, namely, aberrations in Chromosome 17, tumor protein 53 (TP53) mutations, Kirsten rat sarcoma viral oncogene homolog (KRAS) mutations, and FMS-like tyrosine kinase 3 (FLT3) mutations. 3. Isocitrate dehydrogenase (IDH)1/2 mutations and nucleophosmin 1 (NPM1) mutations may predict disease that is less likely to undergo clonal evolution.

Our first key finding sheds light on the pace of clonal evolution in AML. Biologically, we understand that this is often initially a decades-long process that ultimately proceeds in a logarithmic fashion commencing in overt clinical disease. Moreover, it argues that refractory disease is likely more a factor of primary disease biology and not a sub-clonal issue. In contrast, at time of relapse, AML is more likely to be phenotypically distinct from the original diagnosis. This has both diagnostic and therapeutic implications in clinical practice. First, it highlights the need for detection of small sub-clones with respect to their unique cytomolecular profile – which might be missed on traditional next-generation-sequencing platforms with greater breadth but much less depth than PCR-based platforms. It also suggests the need for

multi-agent treatment backbones (when tolerated) to target these distinct subclones for optimized clinical outcomes.

It is likely no surprise to the reader that aberrations in Chromosome 17, to include TP53 mutations, is associated with higher rates of clonal evolution in AML. TP53 is the most commonly mutated gene among all cancers and is characterized by genetic instability [18]. It is also understood that not all TP53 alterations operate in the same fashion – various missense mutations, truncating mutations, and loss of the TP53 loci exert differential biological effects both within and between different malignancies [18]. We, and other investigators have characterized these aberrations with respect to clinical phenotype in AML [19-21]. In general, this mutation is characterized by a dismal prognosis and refractoriness to both novel and conventional therapies [21-23]. Unfortunately, the retrospective nature of this work and small sample size was not able to detect potential effects of co-occurring mutational profiling within a given sample – however, further prospective work with both next-generation bulk tumor sequencing and single-cell DNA sequencing is under way to further elucidate patterns of evolution with respect to complete cytomolecular profiling.

Mutations in FLT3 occur in about 30% of adult AML patients, with KRAS mutations in approximately 5% [24,25]. In clinical practice, we recognize that signaling pathway activators, including FLT3 and KRAS tend to be hyperproliferative and carry an inferior prognosis [25-27]. It follows, then, that a rapidly dividing disease may be more prone to ongoing accumulation of genetic mutations over time. FLT3 is of particular interest here, due to both its relatively later appearance in the process of leukemogenesis, and resulting inherent subclonality in many cases, as well as its targetability which may directly drive further clonal evolution. Indeed- previous work has found selection of resistant ITD (Internal Tandem Duplicate) clones, loss of FLT-3 ITD, and acquisition in other signaling pathway mutations as important factors in relapsed or refractory disease under the selective pressure of midostaurin (a FLT-3 inhibitor) and intensive chemotherapy [28]. Similar patterns were seen with gilteritinib, a second-generation FLT3 inhibitor FDA approved as a single-agent with relapsed/refractory FLT3-ITD+ and TKD+ AML, where clonal selection for NRAS or KRAS was frequently noted at 36%. Interestingly, BCR:ABL1 fusions were also observed, as well as subclonal expansion in genes including IDH2 and SF3B1 [29]. **Figure 1** demonstrates a case-example of this clonal evolution paradigm seen in our cohort. The serial acquisition of DNMT3A, followed by NPM1, then FLT3-ITD acquisition is the best understood pattern of clonal evolution to-date [30]. Most others are much less understood. It is tempting to devise rational combinatorial therapies that target these relatively predictable patterns of resistance or evolution if sufficient understanding of these pathways could be ascertained. This is a particularly worthwhile goal with the field of AML rapidly moving toward combinatorial

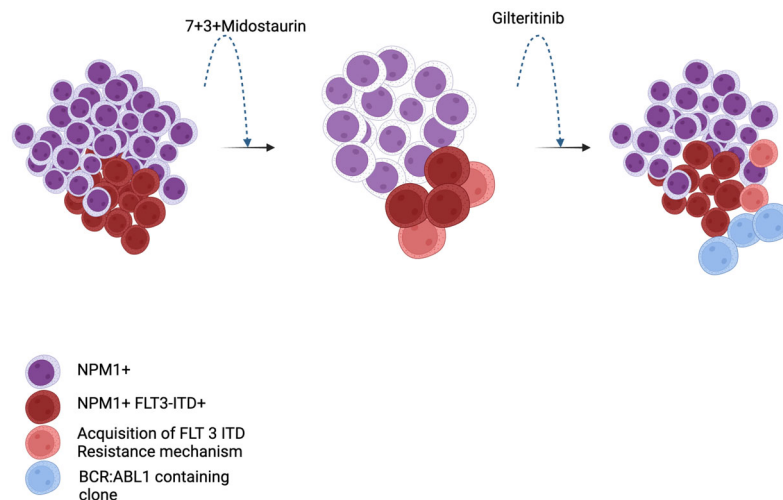


Figure 1: Schematic of a linear pattern of clonal evolution in a case example in our cohort, originally diagnosed with FLT3-ITD+ NPM1+ AML, treated with 1st line 7+3+Midostaurin. Ultimately, had relapsed disease, and after treatment with Gilteritinib developed a BCR:ABL1-containing clone. She was treated with HMA/Venetoclax/Quizartinib/Ponatinib, and achieved transient response but ultimately succumbed to her disease.

targeted therapeutics which will exert yet-unknown selective pressures on the primary tumor. Targeted therapies are now widely commercially available not only FLT-3 ITD and TKD mutations, but, NPM1mut & KMT2A rearrangements, and IDH1 & 2 mutations [31-33]. Many of these aberrations have more than one commercially available target, with slightly different pharmacology which may exert differential selective pressures as well as different co-administered drugs, often in combination with a hypomethylating agent with or without a BH3 mimetic. Of course, this calls into the question of tolerability of multi-agent backbones, as well as the issue of emergent clones based upon these now new/different selective pressures. Nonetheless, further prospective study in of this in well-designed clinical trials is warranted.

Lastly, key mutations were identified that are relatively less likely to undergo clonal evolution – NPM1 (without FLT3) and IDH1/2 (mutations). NPM1 (without FLT3 mutations) has been long recognized as a relatively favorable-risk mutation in AML [22,34]. Likewise, in the modern era with increasing reliance upon HMA/Ven based therapies, IDH1/2 mutations without co-occurring signaling pathway mutations are now considered favorable risk for patients receiving these less-intensive therapies [35]. This favorable-risk clearly speaks to the underlying disease biology, which seems to have an impact on clonality (which varies depending upon co-mutational profiles). NPM1mutation is considered a foundational AML mutation and this mutation often remains upon relapse. In contrast, IDH1/2 mutations are often pre-leukemic events and can also be seen in other myeloid malignancies [36]. The relative genomic stability we noted in patients with these two mutations likely arises from this earlier acquisition and

without the hyperproliferative effects seen in mutations likely to lead to clonal evolution.

The process of clonal evolution in AML is as heterogeneous as the disease itself, and varies temporally within a given patient, with respect to chosen therapeutics, and with different co-mutational profiling. While understanding, and being able to effectively therapeutically target, leukemic stem cells in totality would obviate the process of clonal evolution, to-date, this has been an elusive goal. Consequently, understanding the selective pressures of the disease itself, as well as those that we place on the disease with therapeutic interventions, can help to inform treatment decisions. Our current understanding of leukemic clonal evolution and therapeutic resistance is largely based in bulk-tumor next generation sequencing across a panel of genes, and analyzing variant allele frequencies (VAFs) with the general assumption that mutations with a lower VAFs are more likely to be from a smaller clone and/or occur later in leukemogenesis compared to the converse with higher VAFs [37]. This basic understanding itself may be flawed as there may be partial elimination of earlier subclones which would convolute this natural history. We are currently moving into yet another new era in the detection, description, and characterization AML – that of single-cell sequencing as a method of better understanding of the clonality of the disease [30,38]. This will allow us to understand clonal evolution in a fuller light, and, rather than simple linear patterns which has been the most common conceptualization to-date, will allow the detection of convergent, divergent, and branching patterns of clonal evolution [38,39]. This work is currently underway in our lab, and others.

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