Editorial

Revealing the mutational landscape of acute promyelocytic leukemia

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Acute promyelocytic leukemia (APL) is a particular subtype of acute myeloid leukemia (AML), first described as a unique condition in 1957 (1). Right from the beginning, it became evident that APL had a peculiar morphology and distinctive clinical features to other forms of AML. Bone marrow of patients with APL showed a differentiation block resulting in the replacement of normal hematopoiesis by a neoplastic proliferation of cells with a promyelocyte phenotype. Clinically, it was a very aggressive disease characterized by high frequency of early bleeding and thrombosis, as well as a rapidly fatal outcome in the absence of treatment.

A major milestone in the understanding of APL biology was the discovery in 1977 of a genetic hallmark, a balanced reciprocal translocation between chromosomes 15 and 17 [t(15;17)(q24;q21)] in the leukemic cells of APL patients (2). In the early 90's, this chromosomal rearrangement was found to result, at the molecular level, in a chimeric oncoprotein containing the C terminus of the retinoic acid receptor-a (RARA) that fused to the N-terminus portion of the promyelocytic leukemia protein (PML) (3). The production of this fusion protein is considered to be the critical initiating step in the APL pathogenesis, acting as a transcriptional repressor of RARA and non-RARA target genes. This results in antagonizing the formation and function of PML nuclear bodies that regulate different signaling pathways, resulting both in an inhibition of cellular differentiation and aberrant self-renewal of APL cells (3,4). Experimental approaches in mouse models have demonstrated that PML-RARA induces a myeloproliferative syndrome and, with a variable penetrance and a relative long latency, is able to develop an acute transformation with morphological features that resemble human APL (5,6). The delayed onset is interpreted as an evidence for a multistep pathogenesis, suggesting that PML-RARA needs to cooperate with additional secondary genetic abnormalities for the development of the entire leukemic phenotype. In fact, PML-RARA is suspected to contribute to disease progression by promoting a 'mutator' phenotype inducing DNA damage and activating DNA repair in the pre-leukemic phase of the disease (7,8).

The recent advent of massively parallel sequencing technologies has permitted the discovery of a growing number of novel mutations in leukemia and other cancers. In APL, data available from preliminary whole genome/exome sequencing (WGS/WES) or targeted sequencing studies with different gene panels (9-15) show that APL has a distinct molecular mutation profile than other forms of AML.

Madan *et al.* (16), have recently reported a comprehensive mutational analysis of newly diagnosed and, for the first time, relapsed APLs, aiming to uncover somatic alterations which might cooperate with PML-RARA in the leukemogenic process. The study explored the mutational spectrum of 12 patients by WES (discovery cohort), followed by a targeted sequencing of the coding regions of 398 genes (109 selected from the discovery cohort and 289 previously described in hematological malignancies) in a frequency cohort of 200 individual APL samples. The study included 30 primary/relapse paired samples (8 WES/22 targeted analysis), 135 unpaired samples at diagnosis

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and 47 unpaired relapse samples. In addition to confirm a particular molecular signature of APL, this interesting study also provides new insights into the mutational profile of this disease at relapse. Among the most interesting findings the following are worth noting:

(I) Like AML, the exome mutational spectrum of both primary and relapse APL is dominated by C>T transitions (11,13,14,16). Spontaneous deamination of a high proportion of methylated cytosine nucleotides in coding regions could probably account for this association (11). Intriguingly, a previous report based on a limited series of AML patients found a significant increase in the transversion rate at relapse (i.e.: the substitution of a purine for a pyrimidine or vice versa) (17), suggesting a potential effect of chemotherapy on the mutational spectrum. Although Madan *et al.* do not address this issue in depth, their data suggest the lack of impact of the treatment on the types of relapse mutations. Whether this is a specific feature of APL or related to a less toxic treatment in APL is uncertain;

(II) The study showed an average of 8-10 non-silent somatic mutations per exome (including *PML-RARA*) in a variety of genes without a significant increase in mutation rate at relapse. However, most of these aberrations are rarely recurrent and unlikely play a significant role in leukemogenesis (11,13);

(III) Apart for *PML-RARA*, no other recurrent mutation is characteristic of APL. In fact, most of them are common in other myeloid leukemias, such as *FLT3*, *WT1*, *NRAS* and *KRAS*;

(IV) *FLT3* is by far the most frequent mutation in APL, affecting roughly one third of patients (18). These mutations involve both in-frame duplications within the juxtamembrane region (*FLT3*-ITD) and point mutations in the tyrosine kinase domain (*FLT3*-TKD). It has been speculated that these mutations may represent cooperating events with *PML-RARA* in driving the disease. In fact, in the study by Madan *et al.*, *FLT3* was the sole additional genetic aberration present in 25% of newly diagnosed APL cases. Nevertheless, longitudinal studies have demonstrated that *FLT3* mutations are late events in leukemogenesis and are confined to later proliferative clones (19). Moreover, they are unstable and may either appear or disappear at relapse (16). Therefore, the role of *FLT3* mutations in the pathogenesis of myeloid leukemias warrant further investigation;

(V) The mutational landscape of APL shows a virtual absence of mutations in *NPM1* and epigenetic modifiers such as *DNMT3A*, *TET2*, *IDH1/2* or *ASXL1*. It is supposed that the integrity of these genes is required for the development of the leukemic phenotype by the fusion protein (20). In fact, recurrent translocations in AML, including *PML-RARA*, are associated with a distinct DNA methylation signature. In this regard, it has been recently demonstrated in mice that a fully functional methyltransferase activity of DNMT3A is specifically required for PML-RARA to induce aberrant self-renewal in myeloid progenitor cells *ex vivo* and to initiate APL *in vivo* (20). These results highlight the role of DNA methylation in APL pathogenesis;

(VI) Interestingly, *DNMT3A*, *TET2*, and *ASXL1* mutations are known to be early lesions in leukemogenesis and have been recently documented to be associated with clonal hematopoiesis in a substantial proportion of elderly healthy individuals (19). This might explain, at least in part, why unlike other AML subtypes, APL or AML with some other specific rearrangements are less common in older individuals;

(VII) The study by Madan *et al.* revealed that other driver mutations distinct to *PML-RARA* are lacking up to 27% of APL patients (16). Therefore, they suggest that *PML-RARA* alone might be sufficient to drive the disease. In fact, it is suspected to act itself as a double-hit (3). However, this finding is more likely related to gaps in our molecular knowledge of cancer and also to methodological limitations. In this regard, mutations in non-coding regions or structural alterations not detected by conventional approaches, among others, may explain at least in part this finding. Additionally, recent evidence in AML has shown that differences in bioinformatic mutation calling leads to conflicting results (21). Future improvements in sequencing coverage and development of precise consensus guidelines in mutation calling will allow for an increase in sensitivity and a better understanding of the molecular pathogenesis of APL;

(VIII) Finally, the study by Madan *et al.* also reported the presence of loss-of-function mutations in genes

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ARID1A and *ARID1B*, involved on the SWI/SNF chromatin remodeling complex. Silencing of *ARID1B* had a marginal impact on cell growth, colony forming ability, and differentiation of NB4 cells in response to ATRA. So, the relevance of this finding awaits further studies. Surprisingly, although described in other types of cancer, mutations in *ARID1A* and *ARID1B* genes were previously undetected in APL (9-15). Without ruling out a selection bias, this finding emphasizes the need for consensus guidelines for bioinformatic analysis.

With regard to treatment, in the past three decades APL has evolved from the most fatal to the most curable form of acute leukemia. All-trans retinoic acid (ATRA) and arsenic trioxide (ATO) have revolutionized the treatment of APL (22). Both ATRA and ATO target PML-RARA fusion protein and trigger its degradation by non-overlapping mechanisms, leading to restoration of normal retinoid signaling (3). Treatment of APL with ATRA in combination with chemotherapy has resulted in complete remission rates >90% and long-term remission rates above 80%. Furthermore, the addition of ATO to ATRA without chemotherapy has shown to be safe and effective in frontline treatment for patients with low risk disease (23). However, despite the remarkable improvement in the treatment outcome in APL, a number of relapses have been observed in both ATRA- and ATO-treated patients, particularly in those with high-risk disease (WBC >10,000/μL) (24). Related to this, somatic mutations of *PML* and *RARA* genes associated with treatment secondary resistance have been reported in APL cases at relapse (25,26).

This study has also addressed the molecular signature of APL at relapse. Two important findings deserve to be highlighted: (I) the average number of mutations at relapse remained virtually unchanged as compared with the initial diagnosis; (II) relapse is rather characterized by emergence of frequent novel mutations in *PML* and *RARA* genes, that abrogates therapeutic response to ATO and ATRA inhibiting the correct binding to their corresponding targets. In this regard, a close relation was observed between the pattern of acquired *PML* and *RARA* mutations and the use of ATO or ATRA, respectively, at diagnosis. This drug resistance mechanism keeps close similarities with the emergence of point mutations within the BCR-ABL kinase domain in Chronic Myelogenous Leukemia (CML) because of the selective pressure exerted by tyrosine kinase inhibitors (27).

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Footnote

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