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Blood 142 (2023) 426–427

The 65th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

617.ACUTE MYELOID LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

Residual Mast Cells Can Explain Persistent Molecular Positivity in Difference from Normal Flow Cytometric-Defined MRD Negative Core Binding Factor AML

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Introduction: Core binding factor acute myeloid leukemia (CBF-AML) accounts for 30% of pediatric and 15% adult AMLs and is defined by fusions involving *RUNX1* or *CBFB*. RT-PCR is commonly used to monitor measurable residual disease (MRD) in patients with CBF-AML. However, it has been reported that these fusions can be detected in patients during complete remission (CR) or after hematopoietic cell transplant (HCT). This raises the question of if these patients should be considered MRD positive, given they appear otherwise disease free. We utilized flow cytometric cell sorting and fluorescent *in situ* hybridization (FISH) to determine the source of persistent CBF fusions.

Methods: Flow cytometry was used to isolate mast cells (CD117 bright/CD34 negative) from 13 patients with CBF-AML fusions (8 *RUNX1*:: *RUNX1T1* and 5 *CBFB*:: *MYH11*) alongside progenitor cells (CD34+/CD117 heterogeneous, 12 patients) and T cells (CD3+, 11 patients) as available for FISH analysis. These patients were fusion positive by RT-PCR but had no evidence of MRD by difference from normal multidimensional flow cytometry (ΔN), the gold standard for determination of residual disease in Children's Oncology Group-sponsored clinical trials for AML. Cells were sorted from 6 additional patients with a history of *RUNX1*:: *RUNX1T1* CBF-AML who were MRD negative by ΔN but without RT-PCR results available.

Results: In 11/13 patients, the mast cell fraction was positive for the CBF fusion but all other sorted cell fractions tested were negative. In the remaining two specimens, all cell fractions were negative, likely due to FISH sensitivity compared to RT-PCR. Both CBF fusions exhibited the same pattern, with fusions detected only in the mast cell fractions (7 *RUNX1*:: *RUNX1T1* and 4 *CBFB*:: *MYH11*). The additional 6 patients who were MRD negative by ΔN but without RT-PCR data followed the same pattern, with *RUNX1*:: *RUNX1T1* detected by FISH in the mast cells (6/6 patients) and not in other sorted populations (5/6 patients sorted for progenitor and T cells).

Two patients had a clinical history of CBF-AML and systemic mastocytosis (SM). ΔN revealed no evidence of residual AML but showed abnormal mast cells with expression of CD2 and CD25, consistent with abnormalities associated with SM. FISH analysis of sorted mast cells revealed the presence of the *RUNX1*:: *RUNX1T1* fusion, while progenitor and T cells were both negative. In all other patients the mast cells showed no phenotypic abnormalities, demonstrating that CBF fusions can be detected in both phenotypically normal and abnormal mast cells in patients who are free of residual AML.

Two patients with *CBFB*:: *MYH11* fusions had specimens evaluated by FISH on sorted cells over a month apart where the fusion persisted only in the mast cells. Two patients with *RUNX1*:: *RUNX1T1* fusions had serial specimens analyzed at 4 different timepoints. One patient had fusion positive mast cells for over 8 months after residual disease was last detected by ΔN. The other patient, who had previously undergone HCT, harbored fusion positive mast cells for 13 months. Both patients remained MRD negative by ΔN over the course of testing.

Discussion: These data demonstrate that in CBF-AML, fusions can be detected exclusively in the mast cells of otherwise leukemia-free patients, resulting in positive RT-PCR results in the absence of any other disease pathology. These mast cells can be normal or abnormal and can persist after successful HCT. Given that these patients are free of disease by other measures, these fusion positive mast cells do not appear to contribute to AML pathogenesis. Additionally, this post-HCT fusion positive persistence demonstrates a potential clinical implication: if patients such as reported here are assessed pre-transplant using RT-PCR on unsorted specimens alone, they may not be considered candidates for HCT even though the fusion is confined to terminally differentiated cells and successful transplant can be achieved. For these reasons, while RT-PCR is a specific and accurate assay, caution is required for CBF-AML patients. Flow cytometry and cell sorting in combination with genetic testing, in this case FISH, protect against these mast cell driven positive results and provide an accurate assessment of disease status.

These data highlight the importance of considering AML biology and combining multiple testing methods to monitor AML patients comprehensively and accurately.

Disclosures Cook: *Hematologics, Inc.:* Current Employment. **Lott:** *Hematologics, Inc.:* Current Employment. **Perry:** *Hematologics, Inc.:* Current Employment. **Nalla:** *Hematologics, Inc.:* Current Employment. **Xu:** *Hematologics, Inc.:* Current Employment, Current equity holder in private company. **Hudson:** *Hematologics, Inc.:* Current Employment. **Wells:** *Hematologics, Inc.:* Current Employment, Current equity holder in private company. **Loken:** *Hematologics, Inc.:* Current Employment, Current equity holder in private company. **Menssen:** *Hematologics, Inc.:* Current Employment.

https://doi.org/10.1182/blood-2023-180275