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ORAL ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

The TFG-ROS1 Fusion Is an Oncogenic Driver of Human Myeloid Leukemia

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Fusion genes play a critical role in the pathogenesis of human leukemia. Characterization of oncogenic fusions such as BCR-ABL and PML-RARA have led to curing therapies for CML and APL patients. Despite these milestone advances proving the importance of understanding and targeting leukemogenic fusions, many rare fusion genes remain to be discovered and targeted. Here we report the first case of TFG-ROS1 gene fusion in de novo myeloid leukemia, the mechanism by which TFG-ROS1 drives leukemogenesis, and finally propose a targeted therapy effectively eradicating the disease in both mouse models and a patient with TFG-ROS1 fusion.

We first describe the case of a 39-year-old male with refractory chronic myelomonocytic leukemia (CMML) who did not respond to multiple lines of therapies including Venetoclax plus Azacitidine (VA) and progressed to acute myeloid leukemia (AML) six months later. He failed to respond to induction chemotherapy based on Cladribine and Cytarabine and then received salvage hematopoietic stem cell transplantation (HSCT), but relapsed only half a month after a transient remission post-HSCT. Next generation sequencing (NGS) and chromosomal karyotype analysis identified the presence of the TFG-ROS1 fusion gene, with no other myeloid mutations or fusion genes detected. The TFG-ROS1 fusion consists of a rearrangement of exon 4 of the TFG gene and exon 35 of the ROS1 gene, retaining the tyrosine kinase domain of ROS1. Interestingly, targeted single-cell RNA-seq analysis revealed the presence of the TFG-ROS1 fusion in multiple lineages. Immunophenotyping of bone marrow (BM) also revealed 49% of monocytes, 4% of T cells and 1% of pDC cells with aberrant immunophenotype, indicating a multilineage disease possibly originated from early hematopoietic stem cells. We next systematically examined ROS1 fusion in RNA-seq data of 8,608 leukemia patients (including AML and ALL). We found 3 cases of ROS1 fusion with different partners, representing about 0.035% of all cases, indicating that ROS1 fusion is a rare but recurrent event in human leukemia. Notably, in all fusion events, the ROS1 kinase domain remained intact, suggesting a common underlying oncogenic mechanism.

Next, we investigated the oncogenic transforming potential of TFG-ROS1. To this end, we transduced Vector, TFG-ROS1, and TFG-ROS1-KD (a kinase dead variant of ROS1: TFG-ROS1.pK1980E) into Ba/F3 cells and normal C57 donor mouse BM cells. TFG-ROS1 uniquely enabled Ba/F3 cells with a strong proliferation capacity independent of growth factor mIL-3 and significantly enhanced the colony-forming ability of mouse BM cells comparing to Vector and TFG-ROS1-KD. In syngeneic mouse models, about six weeks after transplantation, mice carrying TFG-ROS1 but not TFG-ROS1-KD exhibited clear leukemia characteristics, including pale BM and hepatosplenomegaly. Immunophenotyping of BM and peripheral blood (PB) showed a rapid outgrowth of a dominant Gr-1+/Mac-1+ blast population, indicating the occurrence of AML. Consequently, the overall

survival of the TFG-ROS1 group was significantly shorter than the Vector or TFG-ROS1-KD controls. Mechanistically, RNA-seq and biochemical analyses found that TFG-ROS1 drives strong activation of the MEK/ERK pathway, suggesting the use of ROS1 kinase inhibitor for their treatment.

Finally, we evaluated treatment strategies for this newly discovered fusion gene. Our in vitro drug toxicity assays demonstrated that ALK/ROS1 inhibitors Ceritinib and Crizotinib were the most effective in treating primary samples of the patient comparing to chemotherapy and VA. In PDX models established by the patient's sample, Ceritinib treatment significantly reduced leukemia burden in the BM, PB, and spleen. Meanwhile, VA was not effective. Most importantly, based on these laboratory findings, the patient harboring the TFG-ROS1 fusion received Ceritinib and showed dramatic response with symptoms improving rapidly. The patient achieved complete remission, subsequently received a second HSCT and has remained in continuous remission for 9.5 months of follow-up to date.

In summary, to our knowledge, this is the first characterization of TFG-ROS1 fusion as a novel oncogenic driver of myeloid leukemia. Our analysis elucidate that TFG-ROS1 fusion is a rare but recurrent oncogenic driver of human leukemia through the MEK/ERK signaling axis, and this fusion protein can be effectively targeted by ALK/ROS1 inhibitors.

Disclosures No relevant conflicts of interest to declare.

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