The most common genetic aberration in AML is a gain-of-function mutation in the FMS-like tyrosine kinase 3 (FLT3) receptor, which is present in about 30% of CN-AML and confers a poor prognosis (1). FLT3 encodes a receptor tyrosine kinase expressed on hematopoietic progenitor cells involved in stem cell differentiation and proliferation (2). FLT3 activating mutations such as internal tandem duplication (ITD) lead to constitutive, ligand-independent activation of this receptor, conferring a growth and survival advantage. The mutation itself has not been shown to independently drive leukemic transformation in vivo (1,3). Rather, FLT3-ITD must collaborate with additional oncogenic mutations to trigger hematopoietic malignancy (3). Despite extensive research throughout the last decades, only Midastaurin has been recently approved by the FDA as a first line treatment in combination with chemotherapy (4) highlighting the difficulties for establishing targeted therapies. Therefore, novel approaches such as targeting downstream effectors of FLT3-ITD signaling are relevant to explore new therapeutic targets.

MicroRNAs (miRNAs) are small noncoding RNAs that control post-transcriptional gene expression in various biological and pathological processes. MiRNA expression has been shown to be highly dysregulated in AML. Specifically, miR-155 is the most significantly overexpressed miRNA in FLT3-ITD mutated AML (5-7). MiR-155 is a known oncogene that accelerates formation of lymphomas and when overexpressed in HSPCs leads to a myeloproliferative disorder as previously shown by O’Connell et al. (8,9). Our group recently linked miR-155 upregulation to MLL-rearranged AML (10), a subgroup with high FLT3 levels (2) and not only, as previously reported, to FLT3-ITD positive AML (5,7,11) or FAB M4/5 AML (12), implying a broader role for this miRNA in AML. This finding is in line with previous reports in B-cell lymphomas, where miR-155 overexpression was detected in all screened subtypes, regardless of cytogenetics (13). Additionally, high miR-155 expression levels were associated with an inferior overall survival in CN-AML (14) and were found to be part of a leukemic stem cell miRNA signature (15). Enforced expression of miR-155 in myeloid cells has been shown to have both oncogenic and tumor suppressor functions in AML. Palma et al. proposed an anti-leukemic role for miR-155 through the induction of apoptosis and myeloid differentiation in the AML cell line OCI-AML3 (11), whereas several groups have reported miR-155 as an oncogene in leukemia (14,16-19) and therefore a potential therapeutic target (16). Based on the published data, it appears that the function of miR-155 is context-dependent. Narayan et al. recently proposed a novel dose-dependent role for miR-155 in the regulation of AML, which might partially explain the observed discrepancy (20). Considering that miR-155 has hundreds of predicted targets, determining its regulated downstream pathways is highly context-dependent and thus challenging. Of note, although miR-155 is highly expressed in murine hematopoietic stem and progenitor cells (HSPCs), miR-155−/− mice did not show impaired myeloid differentiation or any perturbations in the HSPC compartment (21).
Wallace et al. now uncovered the downstream mechanism of miR-155 in FLT3-ITD AML (22). Mice homozygous for FLT3-ITD (FLT3-ITD+/−) develop a myeloproliferative disease (MPD) defined by a myeloid-specific cell expansion, however, the mutation itself does not drive leukemic transformation. Interestingly, a comparable phenotype was observed through engineered overexpression of miR-155 in HSPCs. Through crossing homozygous FLT3-ITD (FLT3-ITD+/−) mice with miR-155 knock out (miR-155−/−) mice generating FLT3-ITD+/−/miR-155−/− mice, Wallace et al. showed that deletion of miR-155 in FLT3-ITD homozygous mice weakens the MPD phenotype, suggesting a functional relationship. More importantly, this direct genetic approach resolved previous controversies about the functional relationship of miR-155 and FLT3-ITD in AML cells.

FLT3-ITD+/−/miR-155−/− mice had a decreased myeloid progenitor compartment compared to FLT3-ITD+/− mice as well as reduction in proliferation of LSK and myeloid progenitor cell populations, indicating a role for miR-155 in promoting myeloid progenitor expansion in the pre-malignant context of FLT3-ITD mediated MPD. Using an RNA sequencing approach of sorted LSK cells, the authors identified the interferon pathway as highly enriched in FLT3-ITD+/−/miR-155−/− vs. FLT3 ITD+/− LSK cells. This was further confirmed by Western blot analysis of STAT1, a master regulator of interferon responses, which showed highly increased STAT1 protein levels in FLT3-ITD+/−/miR-155−/− cells. Based on this finding, the authors concluded that miR-155 promotes proliferation of myeloid progenitor cells by reducing the anti-proliferative effects of interferon signaling in FLT3-ITD+/− leukemia. By mining their hypothesis with the TCGA-LAML dataset, the authors confirmed that IFN-α and IFN-γ were significantly downregulated in FLT3-ITD AML compared with FLT3-WT AML. Subsequent genetic depletion through CRISPR/ Cas9 of miR-155 in human AML cell lines showed elevated Interferon signaling and STAT1 levels. To identify the relevant targets of miR-155 in the context of FLT3-ITD mutated AML, the authors showed the upregulation of established miR-155 targets including Ship1, Pu.1 and Cebp in FLT3-ITD+/−/miR-155−/− compared to FLT3-ITD+/− mice and further confirmed the expression levels of these targets in the human TCGA-LAML dataset. CEBPβ is a known interferon regulator whereas Ship1 is an inhibitor of AKT signaling, demonstrating that miR-155 works through several targets to modulate multiple signaling pathways and responses in FLT3-ITD mutated AML. Finally, Wallace et al. translated their findings to primary human FLT3-ITD mutated AML cells, in which they could demonstrate reduced survival and increased apoptosis after treatment with a miR-155 inhibitor in vitro.

The findings of Wallace et al. highlight the relevance of miR-155 in FLT3-ITD driven AMLs and open a new path to the possibility that the depletion or inhibition of miR-155 may provide a therapeutic angle. While much has been learned about miR-155 biology, there are still many unanswered questions that add complexity to its role in AML and it is yet to be determined if inhibition of this miRNA in vivo is ready for prime time and will eventually lead to a better outcome for AML patients.

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Footnote

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