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# POSTER ABSTRACTS

## 602.MYELOID ONCOGENESIS: BASIC

### AML1-ETO and CCND2 Overexpression Cooperate to Drive AML Initiation and Progression

*Junli Mou*[1], *Yu Liu*[1], *Xiaoyu Liu*[1], *Runxia Gu, MD*[1], *Yingxi Xu, PhD*[1], *Kejing Tang*[1], *Zheng Tian*[1], *Haiyan Xing*[1], *Qing Rao, MS*[1], *Min Wang*[2], *Shaowei Qiu, MD*[3], *Jianxiang Wang, MD*[2]

[1] State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin, China
[2] State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Haihe Laboratory of Cell Ecosystem, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences &Peking Union Medical College, Tianjin, China
[3] National Clinical Research Center for Blood Diseases, State Key Laboratory of Experimental Hematology, Haihe Laboratory of Cell Ecosystem, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences &Peking Union Medical College, Tianjin, China

**Objectives**: The translocation between chromosome 8 and 21, resulting in the AML1-ETO fusion gene, is one of the most common cytogenetic abnormalities observed in acute myeloid leukemia (AML). However, AML1-ETO alone is insufficient to cause leukemia, additional secondary events are required for leukemogenesis. CCND2 gene, a key member of the CyclinD2-CDK4/6 complex, plays a crucial role as a cell cycle regulator.
In hematopoietic malignancies, CCND2 mutations (particularly with a conserved mutated hotspot on Thr280) have been found in AML, particularly in core binding factor leukemias. The mutated CCND2 protein exhibits greater stability and resistance to proteasomal degradation. Previous research had shown that CCND2 was a transcriptional target of AML1-ETO, indicating that AML1-ETO can promote CCND2 expression. However, it remains unclear whether mutated CCND2 can cooperate with the AML1-ETO to drive leukemia initiation and progression.
**Methods**: In our previous study, a conditional AML1-ETO knock-in mouse model was developed, in which the AML1-ETO fusion gene was tagged with m-Cherry. To investigate the role of CCND2 in this model, the coding sequences of both CCND2 $^{wt}$ (wild-type) and CCND2 $^{mut}$ (mutant with Thr280Ala substitution) were cloned into the retroviral vector MSCV-IRES-EGFP. Subsequently, the fetal liver cells (embryonic day=12.5) from AML1-ETO mice were infected with the corresponding retroviruses.
**Results**: Four months post AML1-ETO induction, the percentage of m-Cherry $^{+}$ GFP $^{+}$ cells of the mice in CCND2 $^{wt}$ and CCND2 $^{mut}$ groups began to increase. The results revealed a substantial upregulation of CCND2 mRNA and protein expression in these groups compared to that of the Vec group. Additionally, the CCND2 $^{mut}$ group exhibited even higher CCND2 protein expression due to impaired protein degradation. Moreover, the shortened survival time and pathological findings in mice from the CCND2 $^{wt}$ and CCND2 $^{mut}$ groups confirmed that they were succumbed to leukemia infiltration. To assess the stemness of leukemia stem cells (LSCs), extreme limiting dilution analysis (ELDA) was conducted, and it showed a significant increase in LSC frequency in the CCND2 $^{wt}$ and CCND2 $^{mut}$ groups compared to that of the Vec group. Notably, transplanting AML1-ETO cells alone didn't confer any competitive advantage in sublethally irradiated mice, while the overexpression of CCND2 provided them with the ability to survive.
Transcriptome analysis indicated that compared to the Vec group, CCND2 $^{wt}$ and CCND2 $^{mut}$ groups exhibited notable enrichment in OXPHOS, E2F, and G2M gene sets. Interestingly, the mTORC1 signaling pathway was particularly enriched in the CCND2 $^{mut}$ group compared to the other two groups. To verify this speculation, phosphorylated S6 and total S6 protein levels were assessed through Western blotting. The results showed that compared to Vec group, the p-S6/total S6 ratio show a gradual increase in the CCND2 $^{wt}$ and CCND2 $^{mut}$ groups, indicating activation of the mTOR pathway by CCND2 protein.
Everolimus, a selective inhibitor of mTOR, was administered to CCND2 $^{wt}$ and CCND2 $^{mut}$ mice at a dose of 5mg/kg via oral gavage (three times a week) for three consecutive weeks. The Everolimus-treated mice exhibited reduced leukemic burden and fewer number of LSCs compared to that of the vehicle-treated group. Additionally, Everolimus restored the non-leukemic cells and extended their survival time in CCND2 $^{wt}$ and CCND2 $^{mut}$ group mice.

**Conclusion**: In our study, a leukemia mouse model with co-expression of AML1-ETO and CCND2 mutation was successfully established, which demonstrated that the introduction of the CCND2 gene into the existing AML1-ETO model can trigger the development of leukemia. It was also confirmed that CCND2 overexpression results in the upregulation of the mTOR pathway, which drove the progression of leukemia. Everolimus effectively improved the outcome of mice with CCND2 $^{wt}$ or CCND2 $^{mut}$, suggesting its potential as a therapeutic agent for this subtype of leukemia.

**Disclosures** No relevant conflicts of interest to declare.

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