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ORAL ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

N/KRAS-Mutant AML LSCs Originate from Committed Myelomonocytic Progenitors and Drive Clinical Resistance to Venetoclax

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Driver mutations in acute myeloid leukemia (AML) often exhibit distinct temporal acquisition patterns, but the biological basis for this, if any, remains unknown. RAS mutations occur invariably late in AML, upon progression or relapsed/refractory disease, in contrast to their function as early drivers in solid tumors.

We developed synthetic leukemogenesis models in human induced pluripotent stem cell (iPSC)- and primary cord blood (CB)-derived human hematopoietic stem/progenitor cells (HSPCs) by introducing the prototypical *NRAS* G12D mutation alone or with other mutational combinations, using CRISPR, and engraftment of a transplantable leukemia into NSG mice as the read-out. RAS mutations alone were not sufficient for leukemogenesis, but required specific cooperating mutations. Subsequently, by creating temporally controlled engineered models, we found that RAS mutations are obligatory late events that can only drive leukemic transformation when acquired after, but not before, cooperating mutations.

We provide the mechanistic explanation for this in a requirement for mutant RAS to specifically transform committed granulocyte-monocyte progenitors (GMPs) harboring previously acquired driver mutations, through sorting and transplantation and multi-omics experiments. In contrast, acquisition of RAS mutations in earlier progenitors (common myeloid progenitors, CMP), with or without cooperating mutations, blocked the emergence of GMPs and abolished engraftment or gave rise to a myeloproliferative neoplasm. These results indicate that advanced RAS-mutant (MT) leukemic clones have a different cell type of origin from the ancestral clone.

Next, using single-cell transcriptomics in cells isolated from xenografts of two AML-iPSC lines generated from an AML patient with a subclonal *KRAS* G12D mutation, one capturing the RAS WT major clone and one the *KRAS* G12D subclone, we show that *KRAS* G12D leukemia stem cells (LSCs) give rise to leukemia with a more monocytic immunophenotype, while the ancestral clone within the same patient generates more leukemic cells with primitive and neutrophil markers. These results were corroborated by Genotyping of Transcriptomes (GoT) in another AML patient with subclonal *NRAS* G12D mutation.

Recent studies have linked monocytic AML and, independently, RAS pathway mutations to poor responses to Venetoclax (VEN)-containing regiments. In view of the causal link we found between RAS mutations and monocytic disease, we evaluated the associations between monocytic AML, RAS mutations and response to VEN. We reanalyzed data from a cohort of 118 older/unfit newly diagnosed AML patients treated on a prospective clinical trial with VEN and decitabine (DEC) (NCT03404193). All outcomes were comparable between monocytic and non-monocytic groups, including overall response rate, duration of response (DOR) and overall survival. On the other hand, patients with *N/KRAS* mutations had significantly shorter DOR. These results support the presence of *N/KRAS* mutations and not the monocytic stage as predictors of inferior responses to VEN regimens.

To definitely test this, we treated defined cell types generated from RAS WT and RAS MT iPSC lines - CD34+ LSC-like and CD11b+/CD68+/CD14+ monocytic cells - with VEN. In agreement with previous studies, monocytes were resistant and RAS WT LSCs were sensitive to VEN. However, strikingly RAS MT LSCs were VEN-resistant. Consistent with these, single-cell transcriptomics in patient-derived AML-iPSC cells in vivo, GoT in primary AML cells and bulk transcriptomics in synthetic iPSC-AML cells showed downregulation of BCL2 and upregulation of MCL1 in the RAS MT LSCs.
This study provides the first example of a mechanistic explanation of why the timing of mutational acquisition in AML is so strongly biased. It shows for the first time that interaction between an oncogenic driver and the non-genetic developmental hematopoietic hierarchy imposes a specific LSC cell-of-origin restriction and in turn shapes the hierarchical structure of the resulting AML and critically determines therapeutic outcomes in patients. Importantly, our results have direct implications for clinical practice, as they support that RAS MT LSCs drive clinical resistance to VEN and that treatment with VEN in patients with RAS mutations can accelerate disease progression by selection of RAS MT LSCs (Figure).

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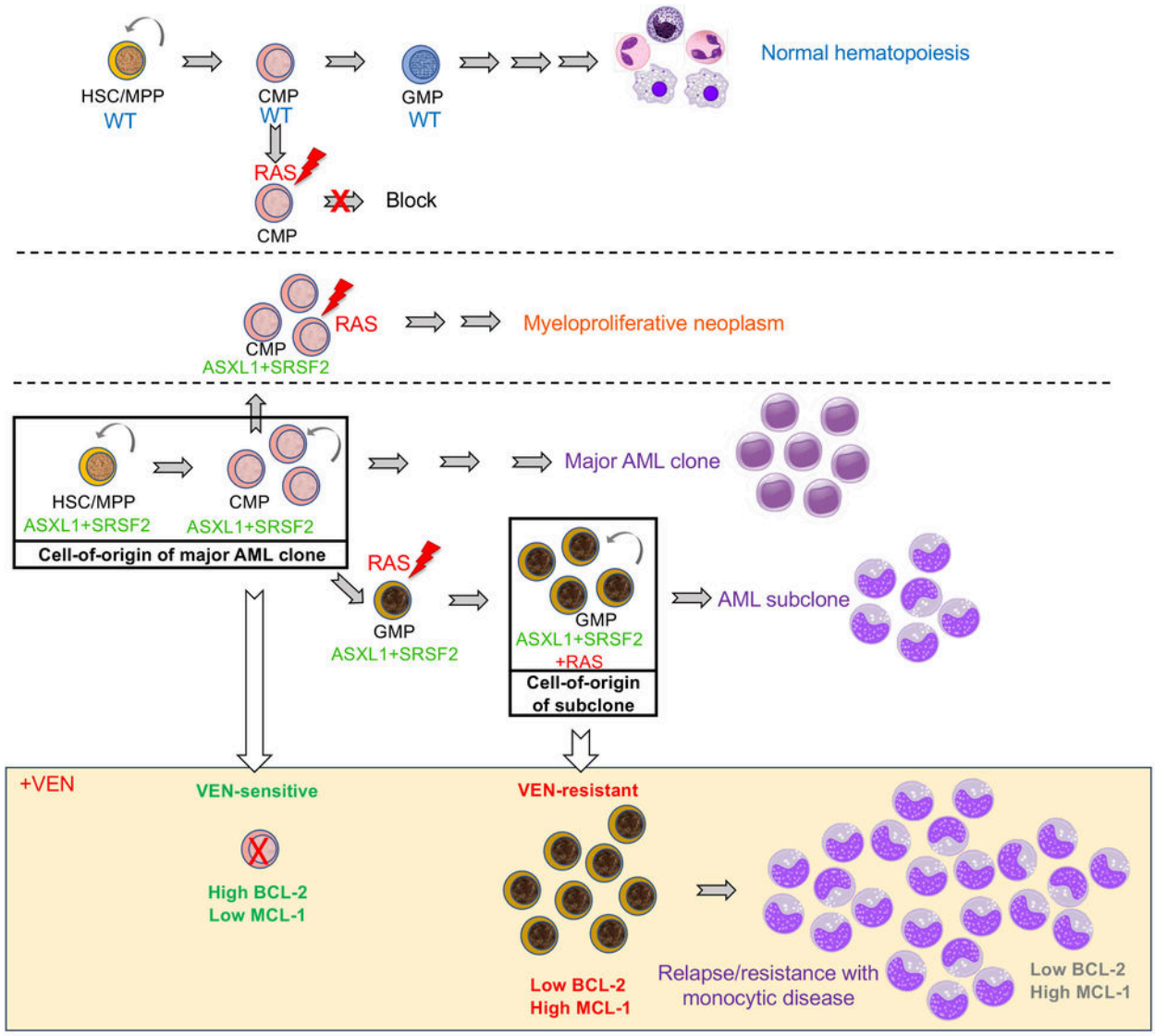

Figure: Proposed model for how RAS mutations drive disease progression and VEN resistance in AML through a LSC cell-of-origin restriction. RAS mutations occurring in WT CMPs (and possibly earlier uncommitted stem/progenitor cells) cause arrest at the CMP stage and fail to promote leukemia. If they occur in CMPs with preexisting mutations they can promote myeloproliferative disease. Only when RAS mutations occur in a GMP harboring previously acquired driver mutations (here *ASXL1* and *SRSF2*) can they generate an LSC. Thus, the LSC of the RAS-mutant subclone originates from a different type of cell in the hematopoietic hierarchy than the LSC of the major clone. RAS-mutant LSCs give rise to leukemic cells with mature monocytic immunophenotype, whereas the major AML clone LSCs without RAS mutations give rise to leukemic cells with more immature features. RAS WT LSCs express high levels of BCL-2 and are the targets of VEN therapy, whose elimination translates into a clinical response. (In contrast, monocytic blasts, regardless of genotype, are uniformly VEN-resistant, as they lack expression of BCL-2 and instead rely on MCL-1 expression for survival. However, resistance of the monocytes has no impact on the clinical response, which is instead dependent on the elimination of LSCs – the cells with self-renewal potential that can maintain and regenerate the leukemia.) Critically, RAS MT LSCs downregulate BCL-2 and upregulate MCL-1 (and possibly BCL-xL) and thus are resistant to VEN. Treatment with VEN imposes selection pressure at the level of the LSCs, selecting for RAS MT LSCs, as these are resistant to VEN – in contrast to RAS WT LSCs, which are VEN-sensitive. Because RAS MT LSCs produce more monocytic blasts than RAS WT LSCs, expansion of the RAS MT LSC compartment is also accompanied by an increase in the fraction of monocytic blasts. However, it is the LSCs and not the monocytic cells that mediate clinical resistance and relapse, with the increase in monocytic cells being a byproduct of RAS MT LSC expansion without relevance to the clinical outcome.

Figure 1

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