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ORAL ABSTRACTS

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

Acute Myeloid Leukemia Differentiation State and Genotype Influence Anti-Apoptotic Protein Expression, Venetoclax Sensitivity, and Survival in AML

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Background:
Somatic mutations (mut) and acute myeloid leukemia (AML) differentiation state (phenotype) influence sensitivity and response to treatment. However the interaction between genotype and phenotype with respect to sensitivity or resistance to venetoclax (VEN) based therapy has yet to be fully evaluated.

Methods:
Samples from patients (pts.) with newly diagnosed (ND) AML enrolled in Beat AML with available bulk RNA sequencing (RNAseq), whole exome sequencing, and *ex vivo* drug sensitivity testing (N=255) were analyzed for correlation between AML phenotype (defined using RNAseq [van Galen et. al. Cell 2019] and surface immunophenotype via multiparameter flow cytometry [MFC]), genotype, and VEN sensitivity. A monocytic score corresponding to monocytic gene expression and myeloid differentiation for each sample was included as previously defined (Bottomly et. al. Cancer Cell 2022).
Clinical outcomes were assessed in an independent retrospective patient cohort (N=97) treated with frontline hypomethylating agents (HMA)+VEN at Oregon Health & Science University. Categorical and continuous variables were analyzed using Fisher's or Wilcoxon rank sum testing. Time to event outcomes utilized the log-rank method with cox multivariable regression modeling.

Results:
Median patient (pt) age at diagnosis in the surveyed cohort was 61 years (range 20-88). AML phenotype determined using proportional deconvolution of RNAseq data was HSC/progenitor (primitive) in 31% (N=78), mixed/intermediate in 31% (N=79), cDC/monocytic (mature) in 29% (N=73), and unknown in 10% (N=25) of pts.
Muts frequent in primitive vs. mature AML samples included transcription factor (*RUNX1, CEBPA, GATA2, GATA1, ETV6, IKZF1*; 30% vs. 15%, p=0.050), tumor suppressor (*TP53, PHF6, WT1*; 21% vs. 7%, p=0.019), and *IDH1/2* (27% vs. 12%, p=0.027). Active signaling mut (*FLT3*-ITD/TKD, *K/NRAS*, *PTPN11*, *BRAF*) were more frequent in mature vs. primitive samples (78% vs. 44%, $p < 0.0001$), underscoring the correlation between genotype and myeloid differentiation (Fig.A).
Expression of anti-apoptotic protein and myeloid differentiation markers measured using RNAseq varied by AML genotype and phenotype and corresponded to VEN resistance. The ratios of *BCL2A1* (pearson R=0.62, $p < 0.0001$), *CD11b* (R=0.65, p

< 0.0001), *CD14* (R=0.73, p < 0.0001), and *CLEC7A* (R=0.72, p < 0.0001) to *BCL2* positively correlated with VEN resistance (i.e., increasing area under the curve [AUC]).

While *IDH1/2* and *NPM1* mutated samples demonstrated the highest *BCL2* expression and lowest *BCL2A1*: *BCL2* ratio and remained largely sensitive to VEN, myeloid differentiation (in particular monocytic differentiation) increased VEN resistance irrespective of genotype. For instance, classification of a primitive vs. mature phenotype further stratified *ex vivo* VEN sensitivity in pts with *IDH1* (median AUC: 61 vs. 195, p: 0.004), *IDH2* (median AUC: 61 vs. 185, p: 0.002), or *NPM1* (median AUC: 60 vs. 220, p < 0.0001) mut (Fig.B).

Overall survival (OS) following HMA+VEN was evaluated based on the presence or absence of surface immunophenotypic markers measured via MFC that correlated with myeloid differentiation. CD117+ AML (HR: 0.30 95% CI: 0.14 - 0.65, p=0.002) associated with improved OS, while inferior OS was observed with CD11b+ (HR: 2.03 95% CI: 1.07 - 3.86, p=0.031) and CD64+ AML (HR:1.86 95% CI: 0.90 - 3.85, p=0.095).

When assessing the combined influence of genotype and immunophenotype on OS in CD11b+ or CD64+ AML, *IDH1/2* mut (N=8) numerically improved OS compared to *IDH1/2* wild-type cases (N=17) (median 12.3 vs. 5.8 months, p: 0.17). In multivariate analysis adjusted for CD11b or CD64 positivity, age, *IDH1/2* mut, and secondary or therapy-related AML, CD11b or CD64 positivity (HR: 1.98 95% CI: 1.08-3.66, p=0.028) retained the strongest impact on OS.

Conclusions:

Genotype and phenotype both modulate VEN sensitivity in AML. Myeloid differentiation is associated with increased alternative anti-apoptotic protein expression and VEN resistance irrespective of genotype; however certain mutations (i.e., *IDH1/2*) may retain prognostic significance independent of myeloid differentiation. Given both factors influence VEN sensitivity, prognostic models incorporating AML phenotype and genotype may further improve risk stratification and predict response to VEN based therapy in AML.

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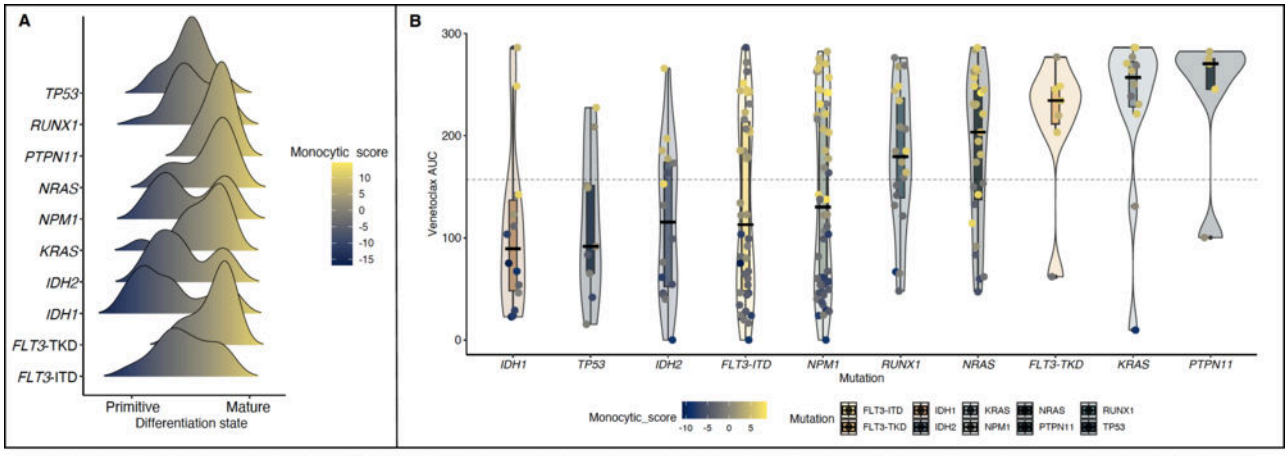

Figure 1: When evaluating mutations with known clinical sensitivity or resistance to venetoclax (VEN), certain somatic mutations (i.e., genotype) correlated with differing myeloid differentiation states (i.e., phenotype) assessed using a monocytic gene expression score from deconvoluted bulk RNA sequencing (A). Integration of AML genotype and phenotype further stratified VEN sensitivity when measured using area under the curve (AUC) in a 72-hour *ex vivo* drug sensitivity assay.

Figure 1

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