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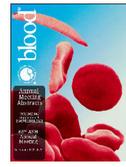

The 65th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

Primary *U2AF1* ^{S34F} Mutated Hematopoietic Cells Are Sensitive to Nonsense-Mediated RNA Decay Disruption *In Vivo*

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Expression of mutant splicing factors alters RNA splicing in myeloid malignancies, including increased production of nonsense transcripts that rely on nonsense-mediated RNA decay (NMD) for clearance. Cell lines expressing spliceosome mutants are more sensitive to NMD inhibition than wild-type cells, and the differential sensitivity is partially dependent on R-loop formation. However, the impact of NMD disruption on the viability of primary hematopoietic cells with splicing factor mutations has not been tested *in vivo*.

We engineered mouse MLL-AF9 acute myeloid leukemia (AML) cells to inducibly express a wild type (*U2AF1* ^{WT}) or mutant (*U2AF1* ^{S34F}) transgene of the U2AF1 splicing factor, and tested the effects of inhibiting the NMD pathway protein kinase, SMG1, *in vivo*. We injected *U2AF1* ^{WT} and *U2AF1* ^{S34F} mouse AML cells (B6, CD45.2) into congenic, irradiated secondary recipient mice (B6, CD45.1), induced transgene expression, and then treated the mice for 2 weeks with either a SMG1 inhibitor (SMG1i) at 60 mg/kg/day or vehicle. SMG1i treatment increased the overall survival (OS) of mice bearing *U2AF1*S34F AML cells (median OS of 53 days compared to 42 days for mice treated with vehicle; p =0.0003), but did not impact the OS of mice with *U2AF1*WT AML cells (median OS of 33 days for SMG1i-treated mice compared to median OS of 32 days for vehicle-treated mice, p=0.360). We extended these findings to a second cohort of mice, where we transplanted one primary *U2AF1* ^{S34F} banked mouse AML sample into 48 secondary recipient mice. To allow direct comparison of isogenic cells with or without *U2AF1* ^{S34F} expression, half the mice were treated with doxycycline to induce *U2AF1* ^{S34F} expression, and half of the mice did not receive doxycycline. SMG1i treatment increased the OS of mice expressing the *U2AF1*S34F mutant allele (median OS of 66 days, compared to median OS of 54 days for mice treated with vehicle; p<0.0001), but had no impact on survival of mice bearing AML cells where *U2AF1* ^{S34F} was not expressed (median OS of 50 days, compared to median OS of 52 days for mice treated with vehicle; p=0.95).

To explore the underlying mechanism for the sensitivity of spliceosome mutant cells to NMD inhibition, we first examined the effects of SMG1i treatment on cell cycle progression and cell death in primary cells. We observed that primary *U2AF1* ^{S34F}-expressing AML cells treated with SMG1i *ex vivo* (24 hours) had an increased fraction of cells in G2/M phase compared to *U2AF1* ^{WT}-treated cells (mean 48% vs. 11% at 100nM, respectively, p<0.001). In addition, the fraction of Annexin V $^{+}$ cells was increased in *U2AF1* ^{S34F}-expressing cells treated with SMG1i *ex vivo* compared to *U2AF1* ^{WT} treated cells (mean fold-change at 500nM relative to DMSO 4.0 vs. 2.3, respectively, p=0.027). We then isolated primary mouse AML cells after 7 days of *in vivo* SMG1i treatment and analyzed R-loops (S9.6 antibody) and DNA damage (gH2AX) by immunofluorescence. SMG1i-treated *U2AF1* ^{S34F} cells had a marked increase in R-loop levels compared to *U2AF1* ^{WT}-treated cells (mean S9.6 nuclear intensity, 306.9 vs 182.6, respectively, p<0.0001). In addition, H2AX phosphorylation was increased in SMG1i-treated *U2AF1* ^{S34F} compared to *U2AF1* ^{WT} cells (mean gH2AX nuclear intensity, 136.7 vs 87.8, respectively p<0.0001).Thus, disrupting NMD in splicing factor mutant AML cells alters cell cycle progression and causes R-loop accumulation, DNA damage, and apoptotic cell death.

The NMD pathway has been implicated in the regulation of the unfolded protein response (UPR) that controls protein homeostasis and impacts cell viability. We generated K562 cell lines expressing doxycycline-inducible *U2AF1* ^{S34F} or *U2AF1* ^{WT}

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constructs and confirmed increased sensitivity of *U2AF1* ^{S34F} cells treated with SMG1i(IC50 128.1nM vs 578.5nM at 72 hours, respectively, $p<0.0001$). Next, we performed RNA-sequencing on *U2AF1* ^{WT} and *U2AF1* ^{S34F} cells that were treated with vehicle or SMG1i for 24 hours (n=3) and observed upregulation of endoplasmic reticulum and UPR pathway genes in SMG1i-treated *U2AF1* ^{S34F} compared to *U2AF1* ^{WT} cells (GSEA; FDR<0.01). Validation of these findings using primary mouse AML cells is ongoing, including the analysis of key regulators and protein biomarkers of the UPR pathway.
The vulnerability of primary hematopoietic cancer cells with spliceosome mutations to NMD inhibition suggests the possibility for therapeutic targeting of NMD to treat myeloid malignancies with aberrant splicing.

Disclosures Bailis: *Amgen, Inc:* Current Employment, Other: Current holder of individual stocks.

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