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ORAL ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

The Mevalonate Pathway Is a Therapeutic Target in *TP53* Mutant Acute Myeloid Leukemia

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Introduction:
Acute myeloid leukemia (AML) with mutations in the *TP53* tumor suppressor gene is the most fatal of AMLs with a median overall survival of only six months due to chemotherapy resistance. To understand biologic differences in *TP53* mutant (MT) AML, as compared to wildtype (WT) AML, we performed transcriptomic analysis on sorted patient samples. Gene set enrichment analysis demonstrated significant upregulation of the cholesterol biosynthesis or mevalonate pathway in *TP53* MT AML. In *TP53* MT solid tumor models, the mevalonate pathway plays a key role in tumorigenesis and metastasis. We hypothesized that the mevalonate pathway is essential for chemotherapy resistance of *TP53* MT AML and represents a novel therapeutic target.
Methods:
We developed chemoresistant, isogenic *TP53* MT AML cell lines using CRISPR/Cas9 technology in the MOLM14 AML cell line. We used primary *TP53* MT AML patient samples to perform colony forming unit (CFU) assays, *in vitro* assays with a serum substitute and cytokines, and a patient derived xenograft (PDX) model of *TP53* MT AML.
Results:
We first sought to determine if *TP53* MT AML exhibits upregulation of mevalonate pathway activity in response to cytarabine (AraC), the backbone of AML therapy. We treated our isogenic *TP53* MT AML cell lines for 24 hours (h) with AraC and assessed mevalonate pathway gene expression and metabolites using qRT-PCR and liquid chromatography high resolution mass spectrometry (LC-HRMS), respectively. Only *TP53* MT cell lines respond to AraC with a significant upregulation of key mevalonate pathway genes and metabolites.
We then determined if *TP53* MT AML is sensitized to AraC by inhibition of the mevalonate pathway with a statin. We pretreated our isogenic cell lines with 24h rosuvastatin before 24h AraC and assessed cell viability with flow cytometry with AnnexinV/7AAD staining and XTT assays. We also performed CFU assays in *TP53* MT AML patient samples treated with the two drugs alone or in combination and assessed CFUs after 14 days. These studies revealed a synergistic reduction in cell viability and CFUs in *TP53* MT AML by rosuvastatin in combination with AraC.
We next addressed the mechanism by which a statin sensitizes *TP53* MT AML to AraC. Our group and others have demonstrated enhanced mitochondrial activity is associated with AML chemoresistance. We hypothesized that mevalonate pathway byproducts, known to be crucial for mitochondrial functions, contribute to the mitochondrial response to AraC. We treated *TP53* MT AML cell lines as above with rosuvastatin and AraC and used seahorse technology to measure oxidative phosphorylation (OXPHOS). We also used electron microscopy, flow cytometry of mitochondrial protein, TOM20, and qRT-PCR for mitochondrial DNA content to quantify and characterize mitochondria. These data demonstrate that *TP53* MT AML cell lines, compared to WT, exhibit a significant increase in OXPHOS after 24h that is due to an increase in total mitochondrial. These effects are abrogated by pretreatment with a statin. We also validated these findings in primary *TP53* MT AML patient samples *in vitro*. Importantly, co-treatment of the isogenic *TP53* MT AML cell lines with a soluble form of mevalonate or a downstream byproduct, geranylgeranyl pyrophosphate (GGPP), recovered OXPHOS and subsequent chemoresistance. Overall, this data

supports a model where *TP53* MT AML cells dynamically upregulate the mevalonate pathway to regulate OXPHOS and avoid DNA damage-induced cell death (Figure 1).
Finally, we studied a PDX model of *TP53* MT AML. Engrafted mice were treated with AraC for five days alone or in combination with high dose rosuvastatin. Mice were harvested on day eight with assessment of leukemic burden by flow, OXPHOS by seahorse and mevalonate byproducts by LC-HRMS. *TP53* MT AML leukemic burden was only significantly reduced by the combination therapy. Consistent with *in vitro* findings, mice sacrificed three days after completing AraC continue to demonstrate enhanced OXPHOS and increased mevalonate byproducts. This response is blunted by rosuvastatin.
Conclusions:
These results demonstrate that *TP53* MT AML requires the mevalonate pathway for chemotherapy resistance and targeting of this pathway in combination with chemotherapy may improve clinical responses. Analysis is ongoing to determine the role of GGPP in regulating the mitochondrial response to DNA damage in *TP53* MT cells in order to elucidate novel therapeutic approaches.

Disclosures Carroll: *Janssen Pharmaceuticals:* Consultancy; *Cartography Bioscences:* Membership on an entity's Board of Directors or advisory committees.

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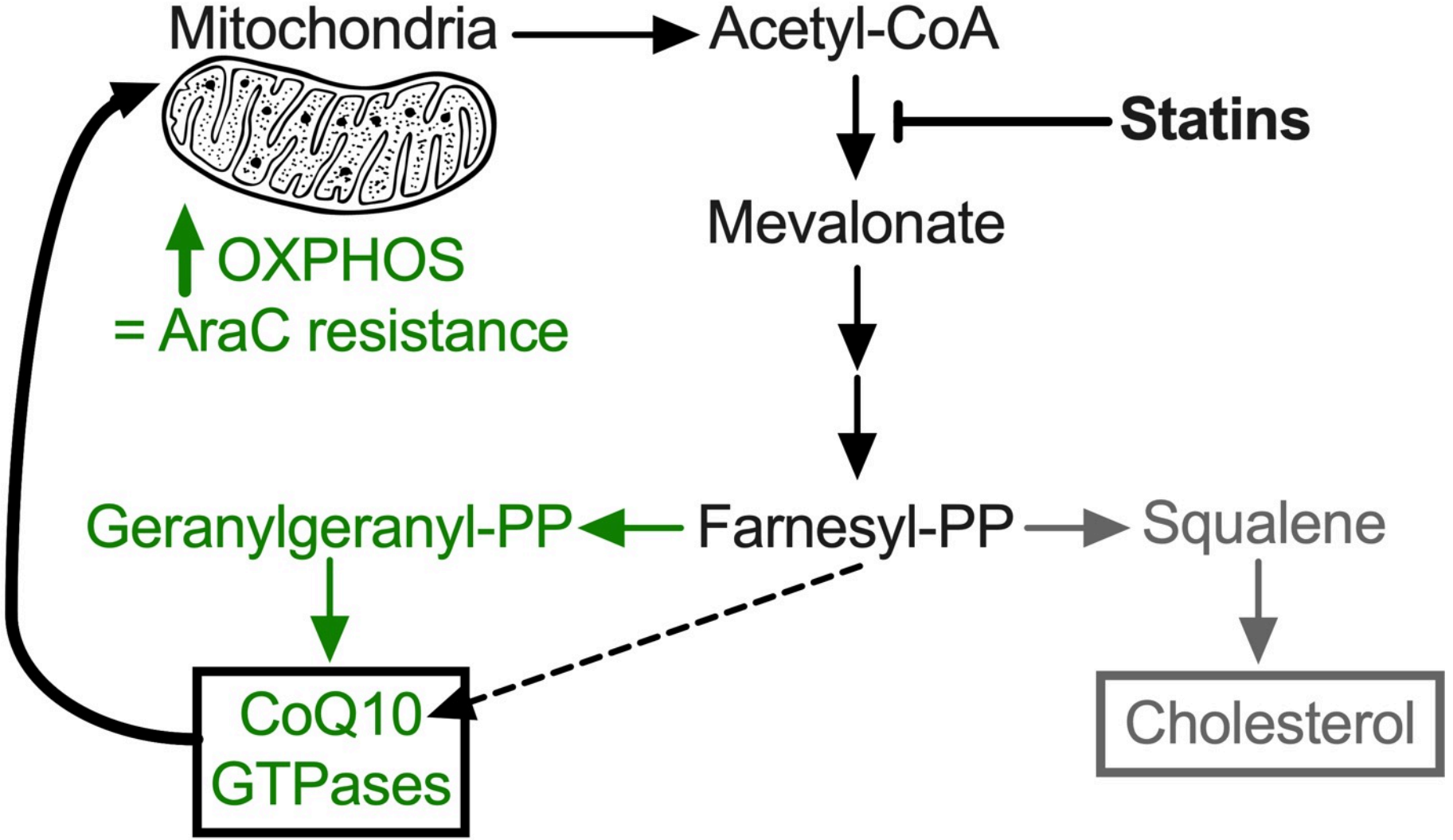

Figure 1. Proposed mevalonate pathway dependencies in chemoresistant *TP53* MT AML.

Figure 1