



## The 65th ASH Annual Meeting Abstracts

## POSTER ABSTRACTS

## 602.MYELOID ONCOGENESIS: BASIC

**Serum Amyloid A1 (SAA1) Secreted By the Stromal Microenvironment Drives Malignant Clonal Proliferation in Myelodysplastic Syndromes (MDS) and Acute Myeloid Leukemia (AML)**

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Drivers of malignant hematopoietic stem cell clonal proliferation from age-related clonal hematopoiesis (ARCH) to Myelodysplastic Syndromes (MDS) and Acute Myeloid Leukemia (AML) are not well understood. However, a pro-inflammatory and immune tolerant aging bone marrow (BM) microenvironment is thought to contribute to disease development. Serum Amyloid A1 (SAA1) has been identified as a novel pro-inflammatory oncoprotein the levels of which progressively increase with MDS and AML progression. SAA1 also selectively promotes growth of patient-derived MDS and AML cells independent of cytogenetic or mutational profile. We aimed to characterize the mechanism by which SAA1 drives oncogenesis within the microenvironment.

Herein we further stratified the disease marking properties of SAA1 by clarifying the association of its BM levels with disease progression in MDS and AML. SAA1 levels were significantly elevated in BM aspirates from MDS and AML patients as compared to those from healthy age-matched subjects ( $p=0.0002$  and  $p=0.0052$ , respectively).

The effects of SAA1 on mutated hematopoietic cells were assessed in a model of age-related clonal hematopoiesis (ARCH). Utilizing a CRISPR-Cas9 system, ASXL1-mutant CD34<sup>+</sup> hematopoietic stem cells (HSCs) were generated; the replating capacity of both mutated and healthy CD34<sup>+</sup> cells were assessed in the presence or absence of SAA1. Notably, SAA1 increased the replating capability assessed by an increase in the number of colonies formed on serial replating of ASXL1-mutant CD34<sup>+</sup> cells, but not those of SAA1-treated healthy CD34<sup>+</sup> or vehicle treated ASXL1-mutant CD34<sup>+</sup> cells, suggesting that the presence of SAA1 favors stemness of mutated HSCs, and implicating SAA1 as a driver of malignant clonality.

To investigate the mechanism of action of SAA1 BM mononuclear cells from AML patients or healthy age-matched subjects were treated with SAA1 or vehicle overnight and single-cell RNA sequencing analysis was performed. Seurat and cell cluster annotation analysis showed that AML samples treated with SAA1 had increased numbers of hematopoietic stem and progenitor cells (HSPCs) and plasmacytoid dendritic cells as compared to matched untreated AML samples. Further, Enrichr analysis showed that pathways upregulated by SAA1 in AML samples included pro-inflammatory molecules, anti-apoptotic, and antiviral defense mechanisms ( $p<0.0001$ ). Shared genes upregulated in these pathways in HSPCs include interferon-induced signals including JAK2, STAT1, and OAS3, and genes implicated in resistance to chemotherapy, including CD44 and CD36, as well as the anti-phagocytosis signal CD47.

Lastly, we initiated the development of a blocking monoclonal antibody against SAA1 as a means of inhibiting its potent oncogenic effects on malignant HSCs and examining its potential therapeutic activity. Utilizing a RAW 264.7 NF $\kappa$ B-luciferase reporter cell line, we screened 51 clones for their ability to selectively block SAA1-mediated NF $\kappa$ B activation. 7 optimal candidates were identified which demonstrated dose-dependent reduction in NF $\kappa$ B activity upon SAA1 challenge. Importantly, these candidate clones were specific for SAA1 and did not affect NF $\kappa$ B activation mediated by lipopolysaccharide (LPS)-

mediated activation. We observed up to an 85% reduction of SAA1-mediated activity using supernatants from further sub-cloning of previously selected candidates. Moreover, cell proliferation of a human AML cell line (OCI-AML3) stimulated by SAA1 was markedly reduced in the presence of candidate antibodies, suggesting anti-proliferative activity. Our findings show that SAA1 levels correlates with disease severity in MDS and AML progression, selectively promotes stemness and proliferative potential of ASXL1-mutant cells, and demonstrates unique immune-related mechanisms selecting for malignant clonality. Further development and purification of a novel inhibitory antibody against SAA1 for future in vivo and ex vivo testing is underway as a promising novel target to reduce SAA1-mediated, niche-driven effects with broad implications in the treatment of hematological malignancies.

**Disclosures Galan-Diez:** Regeneron Pharmaceuticals: Current Employment. **Ali:** TFC Therapeutics: Consultancy, Current equity holder in private company; VOR Biopharma: Consultancy, Patents & Royalties, Research Funding; **Brahma Therapeutics:** Patents & Royalties; **Actinium Pharmaceuticals:** Research Funding. **Koehnke:** TenSixteen Bio: Consultancy. **Majeti:** MyeloGene: Current equity holder in private company; **Pheast Therapeutics:** Current equity holder in private company; **858 Therapeutics:** Membership on an entity's Board of Directors or advisory committees; **Orbital Therapeutics:** Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees; **kodikaz Therapeutic Solutions:** Membership on an entity's Board of Directors or advisory committees. **Carroll:** Cartography Biosciences: Membership on an entity's Board of Directors or advisory committees; **Janssen Pharmaceuticals:** Consultancy. **Raza:** TFC Therapeutics: Consultancy, Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees, Patents & Royalties; **Grail:** Membership on an entity's Board of Directors or advisory committees, Other: Travel, Accommodations, Expenses, Research Funding; **Epizyme:** Honoraria; **Taiho Oncology:** Honoraria; **Rarecells:** Other: Travel, Accommodations, Expenses, Research Funding; **Regeneron:** Research Funding; **Immuneel Therapeutics:** Research Funding; **Genzyme:** Consultancy, Membership on an entity's Board of Directors or advisory committees; **Takeda:** Consultancy, Membership on an entity's Board of Directors or advisory committees; **Janssen:** Consultancy, Membership on an entity's Board of Directors or advisory committees. **Kousteni:** Celgene: Research Funding.

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