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

Ultrasensitive Detection of SALL4 Oncoproteins in Peripheral Blood and Bone Marrow Aspirate: Companion Diagnostics and Therapeutic Monitoring

Sanjana Shah[1], *Jun Liu, MD PhD*[2,1], *Yasmeen Senussi, MBBS*[2,1], *Arek Melkonian, MD PhD*[1,2], *James Nuhfer*[1], *Scott Lovitch, MD PhD*[1], *Neal Lindeman, MD*[1], *Annette S. Kim, MD PhD*[1], *David R. Walt, PhD*[2,3], *Li Chai, MD*[2,1]

[1] Brigham and Women's Hospital, Boston, MA
[2] Harvard Medical School, Boston, MA
[3] Wyss Institute for Biologically Inspired Engineering, Boston, MA

Spalt-Like Transcription Factor 4 (SALL4), a member of the SALL family, is a regulator of embryonic stem cell development that plays a crucial role in cell renewal and proliferation. SALL4 is a tumor driver, as shown by gain-of-function and loss-of-function studies; high SALL4 expression invariably correlates with worse prognosis. These studies established SALL4 as a novel, druggable target, such as in liver, lung, and blood cancers. In myelodysplastic syndrome (MDS), SALL4 can be upregulated by hypomethylating agents (HMAs), correlating with worse patient outcomes. Despite its well-established function on oncogenesis, SALL4 targeting therapies (inhibitors and degraders) are under pre-clinical development. There are currently no FDA/CLIA approved SALL4 diagnostic tests beyond tissue immunohistochemistry. Therefore, there is outstanding clinical need for the development and validation of a less invasive and sensitive SALL4 expression assay via peripheral blood, which can be used for monitoring patients on HMA treatment and SALL4-targeting therapy such as molecular glue degraders.

We developed a protein based SALL4 assay, compatible with peripheral blood and bone marrow aspirates samples, using the SIMOA (Single Molecule Array) technology, a bead-based digital enzyme-linked immunosorbent assay (aka digital ELISA). Previously, circulating SALL4 oncoproteins have been detected in the peripheral blood of patients with liver cancer using a conventional ELISA kit, but it is only semi-quantitative with limited sensitivity.

We cross-tested 5 commercial SALL4 antibodies and 13 antibody pair combinations. After antibody screening, we used the best antibody pair to test the matrix effects of human plasma, buffy coats, and bone marrow aspirates. For quantitative assay development, we successfully expressed and purified full-length SALL4 for the first time, which was used to calibrate the standard curve and determine the assay's limit of quantification. The top bead and detector antibody pair could detect both SALL4A and SALL4B isoforms, with stronger signal detected with the SALL4A isoform in two different cell lysis buffers.

Assay sensitivity was then tested. The Simoa assay captured endogenous SALL4 expression with greater sensitivity compared to the Western blot. The Western blot's limit of detection for SALL4A protein was 0.08 µg/µL whereas the Simoa assay continued until 3 ng/µL.

Pre-analytic steps were then optimized, including our cell lysis protocol, isolation of buffy coats using density gradient separation, and sample collection method comparison. We assessed the effect of sample age for downstream processing and signal recovery as well as the possible matrix effect and interference from the buffy coat and plasma components of the peripheral blood and bone marrow samples. Our data showed no evidence of interference from the peripheral blood components, and interference from bone marrow aspirate can be further optimized.

Antibody validation was then conducted. The preliminary data showed that the SALL4 Simoa assay can be used as a proof of principle to monitor SALL4 upregulation on HMA treatment for blood cancers and for SALL4 targeting therapy with two cell lines (SNU398 and H661) for solid cancers.

To validate assay accuracy and precision, a retrospective cohort analysis is being conducted from patient peripheral blood samples. We obtained MGB Biobank samples for MDS, acute myeloid leukemia (AML), and liver cancer. We will use these samples to test for digital SALL4 protein detection. Further plans include expanding these analyses to breast and lung cancer. Additionally, we will conduct prospective studies to explore the role of the SALL4 protein biomarker in risk stratification and treatment monitoring, especially in MDS/AML patients undergoing HMA treatments.

In conclusion, we have developed an ultrasensitive SALL4 protein expression diagnostic assay for use in monitoring patient treatment response and for guiding targeted therapy by analyzing peripheral blood for both solid and liquid tumors. Future

steps include validating the diagnostic assay for specificity and sensitivity in a retrospective cohort analysis and conducting prospective studies to explore the role of the SALL4 protein biomarker for risk stratification, monitoring on HMA treatment, and guiding targeted therapy, ideally filing for approval as a laboratory developed test (LDT) in the near future

Disclosures No relevant conflicts of interest to declare.

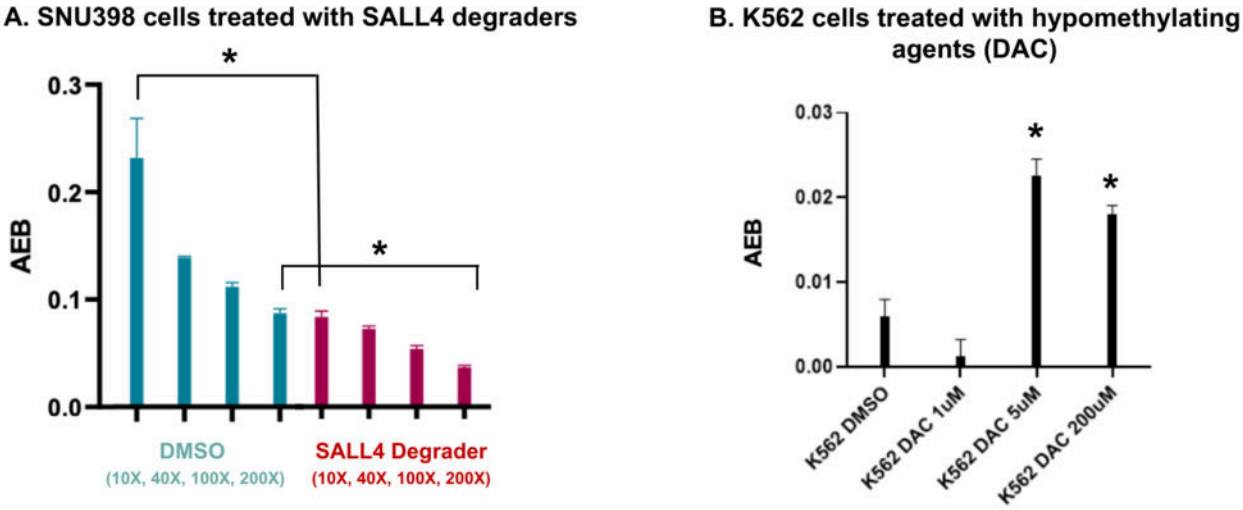

Figure 1. Serial dilutions of Simoa detection of SALL4 protein in SALL4 degrader-treated SNU398 (liver cancer) cell line (A) and K562 (leukemia) cell line upon HMA treatment (B)

Figure 1

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