Biomarker Research

CORRESPONDENCE Open Access

# Proteomics to predict relapse in patients with myelodysplastic neoplasms undergoing allogeneic hematopoietic cell transplantation

Guru Subramanian Guru Murthy[1*†], Tao Zhang[2†], Yung-Tsi Bolon[2], Stephen Spellman[2], Jing Dong[1], Paul Auer[3] and Wael Saber[4]

**Abstract**

Disease relapse remains a major barrier to success after allogeneic hematopoietic cell transplantation (allo-HCT) in myelodysplastic neoplasms (MDS). While certain high risk genomic alterations are associated with increased risk of relapse, there is a lack of clinically applicable tools to analyze the downstream cellular events that are associated with relapse. We hypothesized that unique proteomic signatures in MDS patients undergoing allo-HCT could serve as a tool to understand this aspect and predict relapse. Using the Center for International Blood and Marrow Transplant Research (CIBMTR) database, we identified 52 MDS patients who underwent allo-HCT and analyzed their proteomic profile from pretransplant blood samples in a matched case-control design. Twenty-six patients without disease relapse after allo-HCT (controls) were matched with 26 patients who experienced relapse (cases). Proteomics assessment was conducted using the Slow Off-rate Modified Aptamers (SOMAmer) based assay. In gene set enrichment analysis, we noted that expression in the hallmark complement, and hallmark allograft rejection pathways were statistically enriched among patients who had disease relapse post-transplant. In addition, correlation analyses showed that methylation array probes in *cis*- and transcription regulatory elements of immune pathway genes were modulated and differentially sensitize the immune response. These findings suggest that proteomic analysis could serve as a novel tool for prediction of relapse after allo-HCT in MDS.

**Keywords** Myelodysplasia, Stem cell transplantation, Allogeneic, Proteomics

## To the editor

Myelodysplastic neoplasms (MDS) are clonal hematopoietic disorders characterized by ineffective hematopoiesis and risk of transformation to acute myeloid leukemia. While allogeneic hematopoietic stem cell transplantation (allo-HCT) remains a potentially curative treatment, their long-term outcomes are suboptimal [1]. Disease relapse remains a major barrier and novel tools to predict relapse are urgently needed. Genomic abnormalities seen in MDS could translate into qualitative and quantitative alterations in downstream protein expression that may influence relapse [2, 3]. Utilization of proteomics could complement the insights gained through genomics and

†Guru Subramanian Guru Murthy and Tao Zhang are co-first authors and contributed equally to this work.

*Correspondence:
Guru Subramanian Guru Murthy
gmurthy@mcw.edu
[1]Division of Hematology & Oncology, Medical College of Wisconsin, Milwaukee, Wisconsin, USA
[2]National Marrow Donor Program/Be the Match, Minneapolis, Minnesota, USA
[3]Division of Biostatistics, Medical College of Wisconsin, Milwaukee, Wisconsin, USA
[4]CIBMTR® (Center for International Blood and Marrow Transplant Research), Department of Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

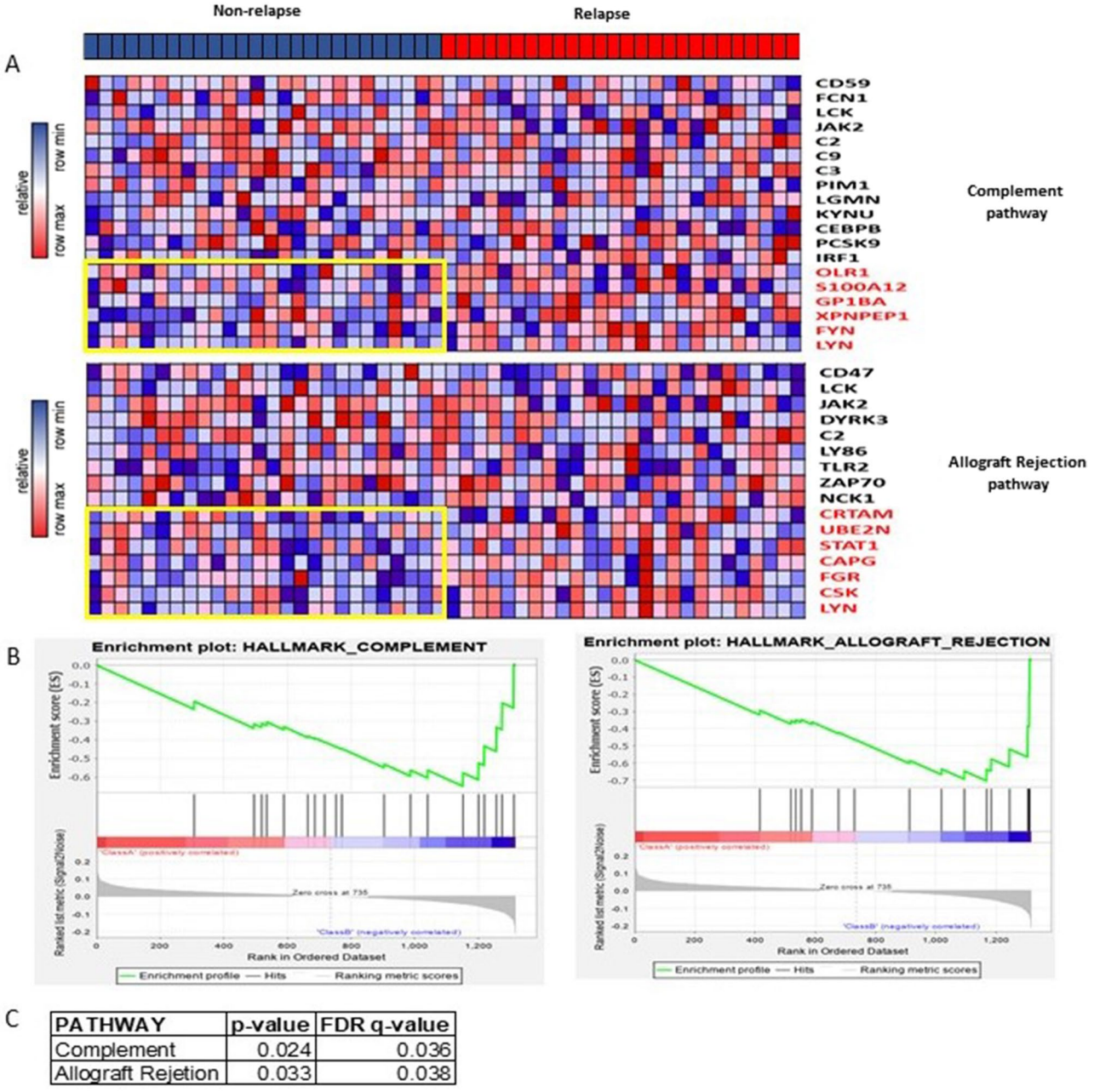
pave the way for an integrated multi-omics model to better understand relapse. Hence, we conducted a study to analyze the association between proteomics and relapse in MDS patients undergoing allo-HCT.

Using Center for International Blood and Marrow Transplant Research (CIBMTR) database, a retrospective matched case-control study was conducted in MDS patients who underwent allo-HCT from 2009 to 2014. Among 52 patients identified, 26 without relapse after allo-HCT (controls) were matched for 26 with relapse after allo-HCT (cases), based on age, gender, race/ethnicity, performance status, MDS therapy, IPSS-R, donor type, conditioning intensity, and transplant year (Figure S1, Supplement). Only patients with wild-type TP53, RAS pathway, and JAK2 genes were included to promote the discovery of novel risk factors. Relapse was defined as evidence of detectable MDS after allo-HCT, as reported by individual centers. Proteomic profiling was conducted with pre-transplant whole blood samples using Slow Off-rate Modified Aptamers (SOMAmer) based assay (details in Supplement) [4]. Pathway level differential expression analysis was conducted using gene set enrichment analysis (GSEA) [5]. DNA methylation signature in whole blood was investigated using Infinium MethylationEPIC array (Illumina). A predictive model with multi-omics was also constructed using iOmicsPASS approach (details in Supplement).

Baseline characteristics are included in Table S1 (Supplementary Material 1). In proteomic analysis, underexpression or overexpression of several pathway level proteins were seen in patients with or without relapse (Fig. 1). At a single protein level, a statistically significant candidate was not identified after adjusting for variables. However, GSEA showed that hallmark complement (P=0.024, FDR=0.036) and hallmark allograft rejection (P=0.033, FDR=0.038) pathways were significantly associated with relapse after investigation of several pathways included in GSEA (Supplementary Material 2). Of note, hallmark complement pathway includes a set of genes which encodes the complement system and hallmark allograft pathway includes genes upregulated during graft rejection [6]. Immune proteins such as OLR1, S100A12, GP1BA, XPNPEP1, FLY, LYN from hallmark complement pathway, and CRTAM, UBE2N, STAT1, CAPG, FGR, CSK, LYN from hallmark allograft rejection pathway were significantly upregulated in patients with relapse.

For correlation between proteomics and methylation, we found that methylation of probes on cis-regulatory elements in a subset of immune pathway genes were correlated with protein expression that could differentially sensitize the underlying immune response. Methylation in one of two TSS (transcription starting site) probes and four of nine gene-body probes in *OLR1* gene region were highly correlated to OLR1 protein expression, while methylation in one of four TSS probes in *S100A12* gene and two of eleven gene-body probes in *CRTAM* gene were highly correlated to their own protein expression (Table S2, Supplement). The methylation levels of transcription factors were also noted to be correlated, such as methylation of the TF (transcription factor) gene *PRMD16* and multiple immune-related proteins, including FYN, LYN and GP1BA (Table S3, Supplement). In iOmicsPASS analysis integrating a multi-omics approach, multiple subnetwork edges from TP53 and AKT pathways were enriched in patients with relapse. Most enriched subnetwork edges came from the proteomic data, indicating that proteomic signals might be the main contributor for relapse prediction. However, smaller sample size limits the overall accuracy of this model (60% with 5-fold cross-validation) (Figure S2, Supplement).

While limited studies have evaluated the role of proteomics in MDS, ours is the first to examine its association with relapse after allo-HCT and no prior studies have investigated GSEA pathways in MDS to our knowledge [7, 8]. Our observation noting an association between relapse with hallmark complement and hallmark allograft rejection pathways and methylation changes in several genes including PRMD16 highlight their potential role in regulating immune dysfunction in relapse [9, 10]. Given smaller sample size, further validation in an independent large cohort would be needed as our study serves to generate hypothesis and proof of concept.




C

| PATHWAY | p-value | FDR q-value |
|---|---|---|
| Complement | 0.024 | 0.036 |
| Allograft Rejetion | 0.033 | 0.038 |

**Fig. 1** Allograft rejection and complement pathways were enriched in MDS relapse group by proteomics gene set enrichment analysis. **A** Protein expression heatmap for two representative pathways: complement pathway and allograft rejection pathway; Differentially expressed proteins between non-relapse and relapse groups are highlighted by red color. **B** The profile of ES score and positions of DE gene candidates in the rank list from GSEA leading edge analysis. **C** the normal p value and FDR q value for complement and allograft rejection pathways

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40364-023-00550-0.

**Supplementary Material 1:** Supplement with methods and additional results including tables and figures

**Supplementary Material 2:** Gene set enrichment analysis pathways

### Author contributions

G.M., T.Z., W.S conceived and designed the study, collected and assembled the data, and wrote the manuscript; all authors performed data analysis and interpretation of data and provided final approval of the manuscript.

### Funding

The CIBMTR is supported primarily by Public Health Service U24CA076518 from the National Cancer Institute (NCI), the National Heart, Lung and Blood Institute (NHLBI) and the National Institute of Allergy and Infectious Diseases (NIAID); HHSH250201700006C from the Health Resources and Services Administration (HRSA); and N00014-20-1-2705 and N00014-20-1-2832 from the Office of Naval Research; Support is also provided by Be the Match

Foundation, the Medical College of Wisconsin, the National Marrow Donor Program, and from the following commercial entities: AbbVie; Accenture; Actinium Pharmaceuticals, Inc.; Adaptive Biotechnologies Corporation; Adienne SA; Allovir, Inc.; Amgen, Inc.; Astellas Pharma US; bluebird bio, inc.; Bristol Myers Squibb Co.; CareDx; CSL Behring; CytoSen Therapeutics, Inc.; Daiichi Sankyo Co., Ltd.; Eurofins Viracor; ExcellThera; Fate Therapeutics; Gamida-Cell, Ltd.; Genentech Inc; Gilead; GlaxoSmithKline; Incyte Corporation; Janssen/Johnson & Johnson; Jasper Therapeutics; Jazz Pharmaceuticals, Inc.; Karyopharm Therapeutics; Kiadis Pharma; Kite, a Gilead Company; Kyowa Kirin; Magenta Therapeutics; Medac GmbH; Merck & Co.; Millennium, the Takeda Oncology Co.; Miltenyi Biotec, Inc.; MorphoSys; Novartis Pharmaceuticals Corporation; Omeros Corporation; Oncopeptides, Inc.; Orca Biosystems, Inc.; Pfizer, Inc.; Pharmacyclics, LLC; Sanofi Genzyme; Seagen, Inc.; Stemcyte; Takeda Pharmaceuticals; Tscan; Vertex; Vor Biopharma; Xenikos BV.

**Data sharing**
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethics approval and consent to participate**
Patients provided written informed consent and the study was approved by the National Marrow Donor Program's Institutional Review Board.

**Consent for publication**
The authors agree to have this article published in the journal.

**Conflict of interest**
Dr. Guru Subramanian Guru Murthy reports the following outside the submitted work -other from Cardinal Health Honoraria, other from TG Therapeutics Honoraria, other from Cancerexpert now Consultancy, other from Qessential Consultancy, other from Techspert Consultancy, other from DAVA Oncology Honoraria, and other from Curio science Honoraria outside the submitted work.

**Role of the funder/sponsor**
The funding organizations had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

**Disclaimer**
The views expressed in this article do not reflect the official policy or position of the NIH, the Department of the Navy, the Department of Defense, the HRSA, or any other agency of the US Government.

Received: 9 October 2023 / Accepted: 19 December 2023
Published online: 25 January 2024

**References**

1. Platzbecker U, Kubasch AS, Homer-Bouthiette C, Prebet T, et al. Current challenges and unmet medical needs in myelodysplastic syndromes. Leukemia. 2021;35(8):2182–98.
2. Aivado M, Spentzos D, Germing U, Alterovitz G, Meng XY, Grall F, et al. Serum proteome profiling detects myelodysplastic syndromes and identifies CXC chemokine ligands 4 and 7 as markers for advanced Disease. Proc Natl Acad Sci U S A. 2007;104(4):1307–12.
3. Wang W, Auer P, Zhang T, Spellman S, Carlson K, Nazha A, et al. Impact of Epigenomic Hypermethylation at TP53 on allogeneic hematopoietic cell transplantation outcomes for myelodysplastic syndromes. Transpl Cell Ther. 2021;27(8):659e1–6.
4. Hensley P. *SOMAmers and SOMAscan– A protein Biomarker Discovery platform for Rapid Analysis of Sample Collections from Bench Top to the clinic*. J Biomol Tech, 2013. 24(S5).
5. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A. 2005;102(43):15545–50.
6. Liberzon A, Birger C, Thorvaldsdóttir H, Ghandi M, Mesirov JP, Tamayo P, et al. The Molecular signatures database (MSigDB) hallmark gene set collection. Cell Syst. 2015;1(6):417–25.
7. Majek P, Riedelova-Reicheltova Z, Suttnar J, Pecankova K, Cermak J, Dyr JE et al. *Proteome changes in the plasma of myelodysplastic syndrome patients with refractory anemia with excess blasts subtype 2* Dis Markers, 2014. 2014: p. 178709.
8. Romano A, Giallongo C, La Cava P, Parrinello NL, Chiechi A, Vetro C, et al. Proteomic analysis reveals Autophagy as Pro-survival Pathway elicited by long-term exposure with 5-Azacitidine in high-risk myelodysplasia. Front Pharmacol. 2017;8:204.
9. Corrigan DJ, Luchsinger LL, Justino de Almeida M, Williams LJ, Strikoudis A, Snoeck HW. PRDM16 isoforms differentially regulate normal and leukemic hematopoiesis and inflammatory gene signature. J Clin Invest. 2018;128(8):3250–64.
10. Zeiser R, Vago L. Mechanisms of immune Escape after allogeneic hematopoietic cell transplantation. Blood. 2019;133(12):1290–7.

## Publisher's Note