SPATIAL TRANSCRIPTOMIC ANALYSIS OF BONE MARROW SAMPLES TO UNCOVER IMMUNE ESCAPE MECHANISMS UNDERLYING POST TRANSPLANTATION RELAPSE IN AML

Rao R.V.A.[1,3,4], **Zahler J.** [1,3,4] , **Sauerer T.** [1,3,4], **Filippini Velázquez G.** [1,3,4], **Xiao J.**[5] , **Reitsam N.**[2,3,4], **Farfán López F.** [2,3,4], **Märkl B.** [2,3,4], **Raue A.**[5], **Schmid C.** [1,3,4]

[1] Augsburg University Hospital, Department of Haematology and Oncology, Section for Stem Cell Transplantation, Augsburg, Germany
[2] University of Augsburg, Faculty of Medicine, Pathology, Augsburg, Germany
[3] Bavarian Cancer Research Center (BZKF), Augsburg, Germany
[4] Comprehensive Cancer Center, Augsburg (CCCA), Augsburg, Germany
[5] University of Augsburg, Faculty of Applied Computer Science, Institute of Computer Science, Chair of Modeling and Simulation of Biological Processes, Augsburg, Germany
[6] Augsburg University Hospital, Department of Haematology and Oncology, Translational Oncology, Augsburg, Germany

INTRODUCTION

- Acute myeloid leukaemia (AML) is an aggressive cancer caused by uncontrolled proliferation of myeloid progenitor cells
- Allogenic haematopoietic stem cell transplantation (allo-SCT) can be curative, post-transplant relapse occurs because leukaemic cells evade immune control through checkpoint upregulation, reduced HLA expression and an immunosuppressive bone marrow microenvironment
- Immune cells surrounding leukaemic blasts remain poorly characterised
- GeoMX™ digital spatial profiling enables transcriptomic analysis of defined regions in FFPE tissue while preserving cellular architecture, providing a way to investigate these local immune interactions and identify targets for personalised treatment

AIMS

1. Adapting the Bruker's GeoMx workflow for spatial transcriptomics in FFPE bone marrow tissue
2. Characterization of the spatial transcriptomic landscape of AML blasts and surrounding immune cells
3. Identification of changes in gene expression profiles at different clinical stages of AML (diagnosis, remission, relapse) and consequently identification of genes which might play a role in immune evasion of AML after allo-SCT

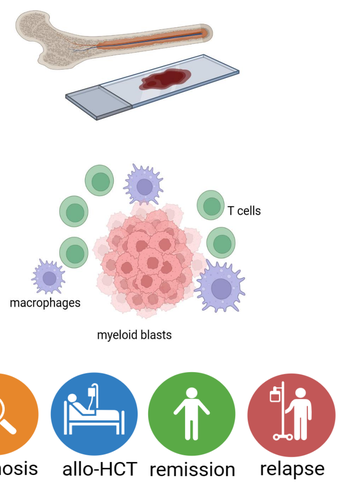

Fig 1: Aims of the study, Created in Biorender

EXPERIMENTAL WORKFLOW

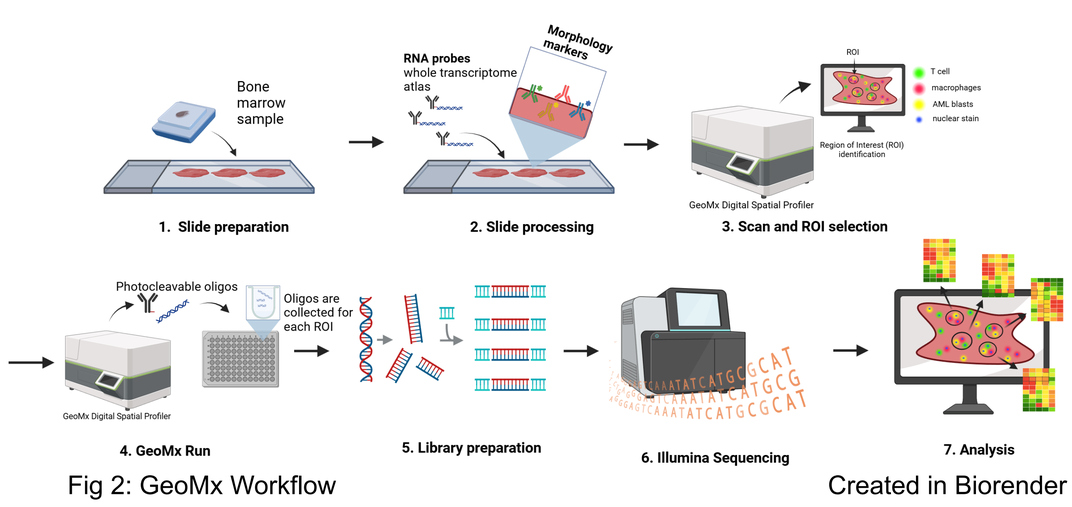

Fig 2: GeoMx Workflow

Created in Biorender

- Slides with tissue sections from FFPE bone marrow trephine biopsies from different clinical stages are processed with the modified GeoMx protocol developed in our lab.
- All clinical stages of one patient are taken on a single slide and examined simultaneously in one experimental run.
- User defined ROIs are selected.

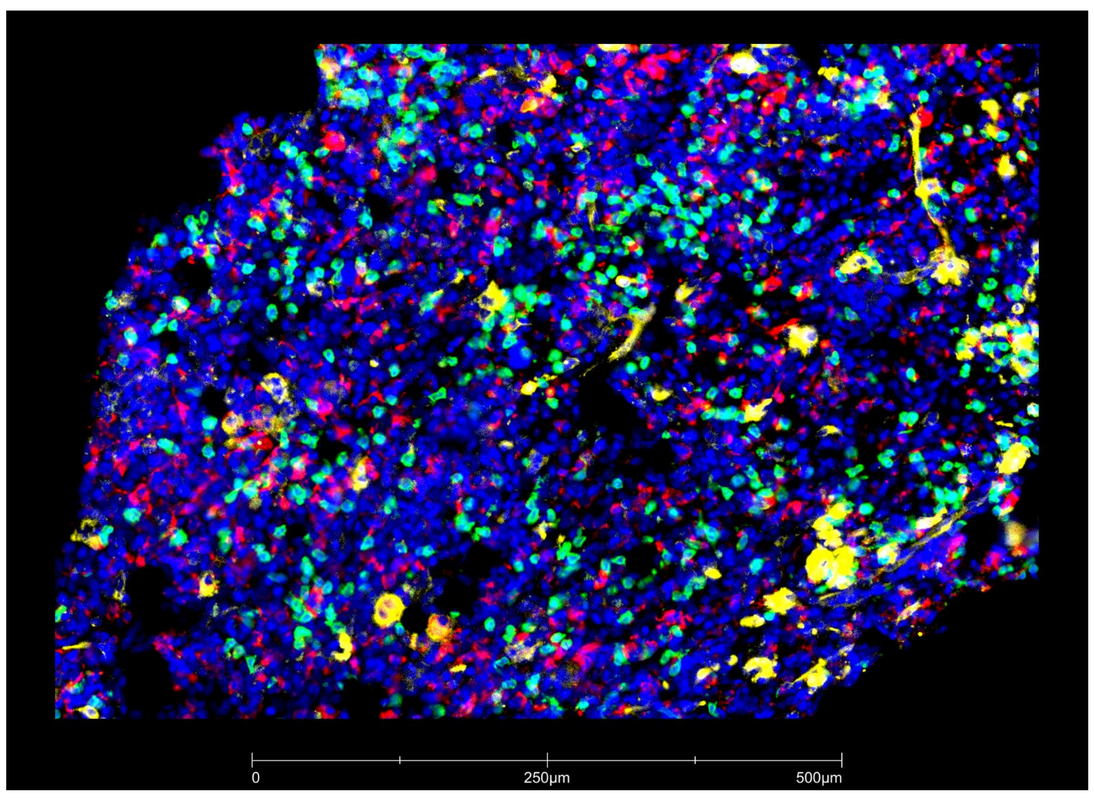

Fig 3: Stained FFPE BM section - GeoMX image using the modified protocol showing **Nucleus, CD3 (T-Cells), CD34 (Blasts), CD68 (Macrophages)**

RESULTS

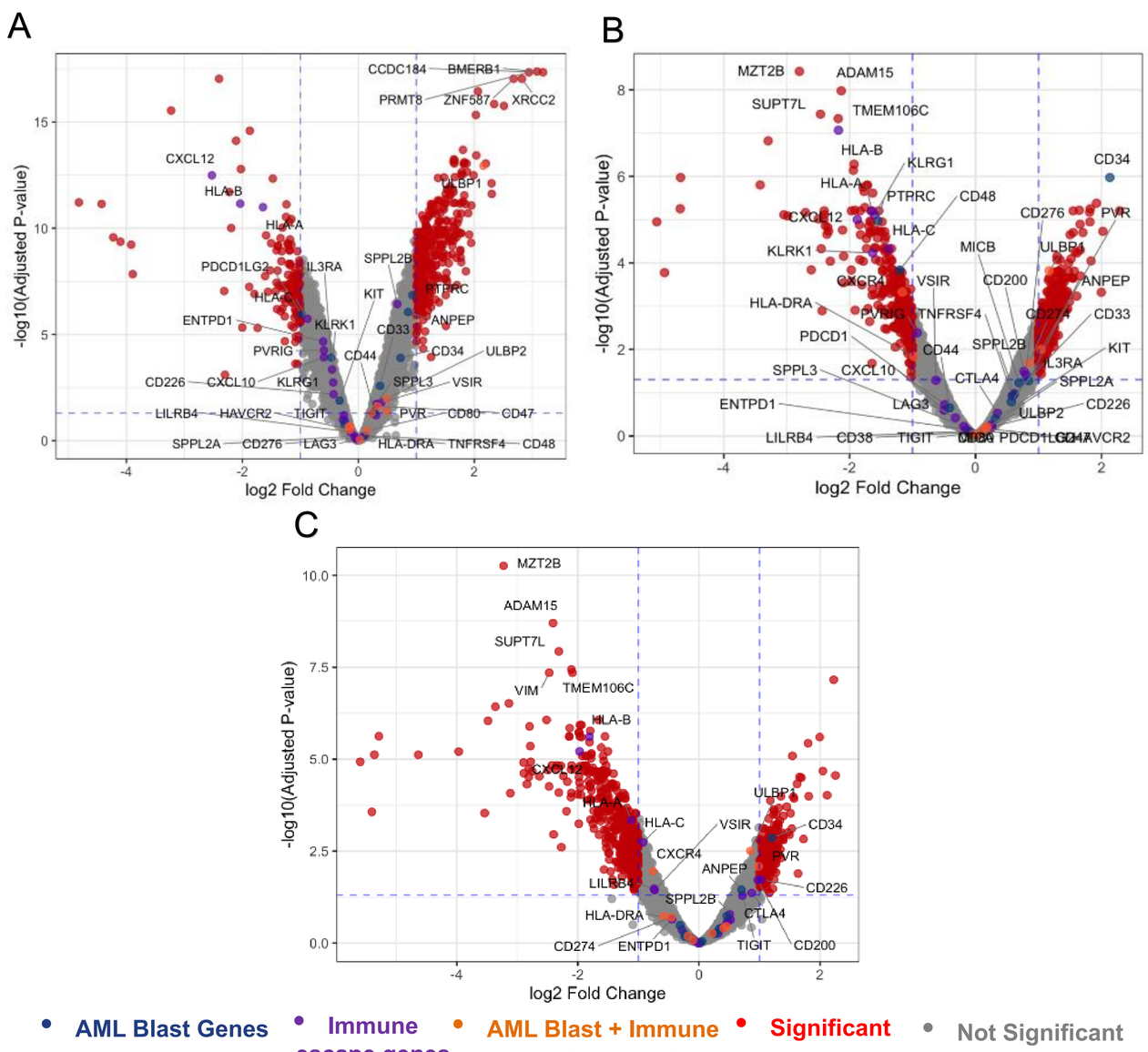

Fig 4: Gene expression pattern in the segments of AML blasts (A), T-Cells (B) and macrophages (C) between clinical stages diagnosis and relapse after allo-SCT.

Pathway Analysis:

- "Recognition and association of DNA Glycosylase with site containing an affected Purine/Pyrimidine" a common dysregulated pathway
- Pathway involved in base excision repair of DNA by DNA glycosylase
- Striking differential expression pattern of genes related to this pathway

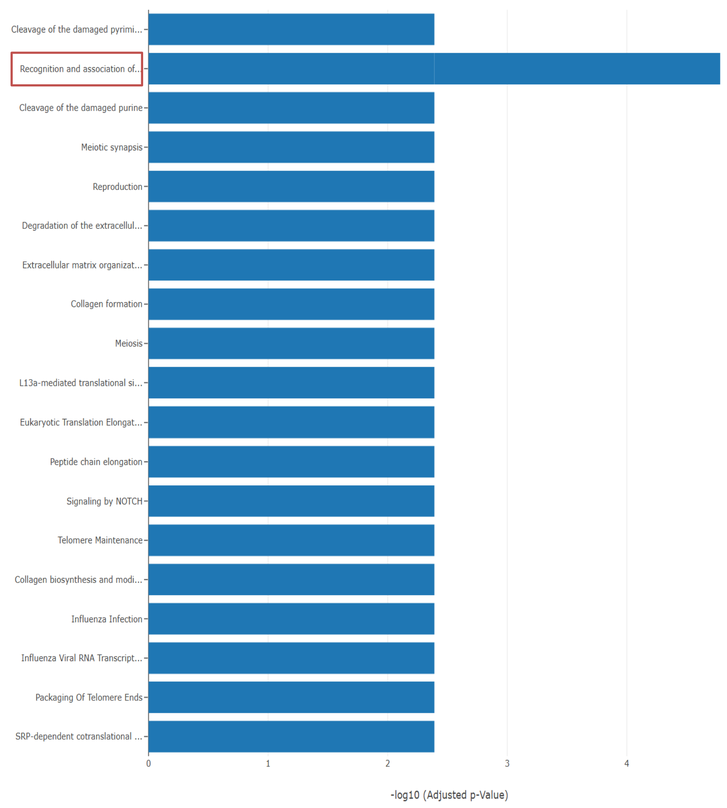

Fig 5: List of dysregulated pathways

SUMMARY

- Initial results exhibit the power of GeoMX to decipher the transcriptomic landscape of bone marrow in context of its cellular architecture
- Huge number of differentially expressed genes, involving many pathways could be detected with similarities but also significant differences between different clinical stages
- The insights into immune escape mechanisms gained through this study could help identify treatment targetable genes for AML relapse patients

CONTACT

Email: ranjitha.rao@uk-augsburg.de
Ph. No.: +49 821 400 16887