Immunological signatures of the bone marrow niche in acute myeloid leukemia patients treated with the demethylating agent azacytidine

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Background

Demethylating agents like azacytidine (AZA) are licensed for the treatment of acute myeloid leukemia (AML) in elderly patients ineligible for intensive chemotherapy. Yet, AML is biologically heterogeneous and reliable early response-predictive biomarkers supporting personalized treatment strategies are needed.

There is emerging evidence that AZA exhibits immunomodulatory functions that range from derepression of tumor antigen expression, induction of CD8+ T cell response to broadening of the T cell repertoire. Further insight into these mechanisms may help to identify immunological signatures predicting response to AZA and guide treatment decisions in elderly AML patients.

Aims

1. To establish a workflow for T and B cell repertoire analyses in bone marrow samples from AML patients.
2. To sequence the bone marrow T and B cell space in pre-treatment and day 15 samples of AML patients treated with AZA and correlate the derived immune metrics with clinical data to assess response-predictive signatures.

Methods

In the pilot phase, next-generation sequencing (NGS) of the T cell receptor beta (TRB) was performed in baseline bone marrow samples of AML patients (n=7). Broad immune metrics were assessed and compared to previously sequenced bone marrow samples of patients with myelodysplastic syndrome (MDS).

Calculation of immune metrics

- Diversity: represented by the Shannon Index (H)

$$H = \sum_{i=1}^{S} p_i \log_2 p_i$$

S – richness
p – proportion of each clone in the repertoire

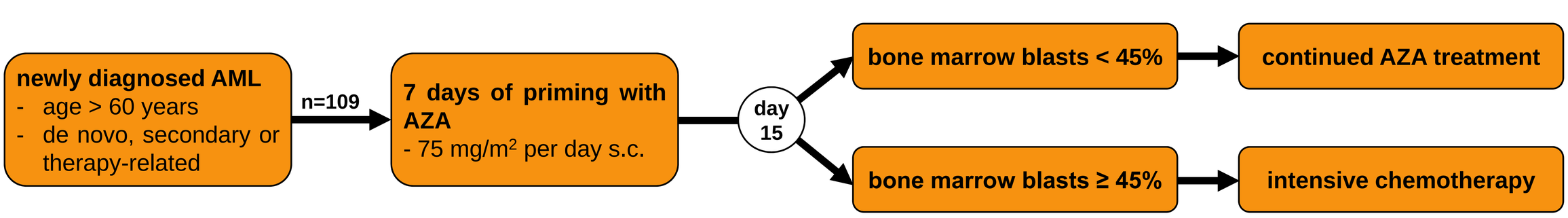
- Richness = number of different clones in the repertoire
- Eveness = $\frac{H}{H_{max}}$

H_{max} – maximal possible H, if every clone in the repertoire was present at the same frequency

- Clonality = 1 – eveness

The RAS-AZIC Trial

The RAS-AZIC study (DRKS00004519) is an investigator initiated multicenter trial in which AZA was integrated with intensive chemotherapy in a response-based sequential approach.



Results

All of the sequenced pre-treatment AML bone marrow samples showed adequate read depth.

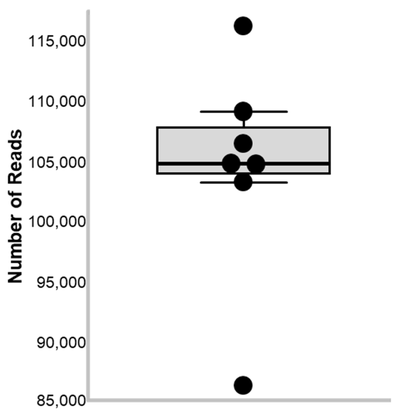


Figure 1. Total read counts of the pre-treatment AML bone marrow samples sequenced in the pilot phase.

The immune metrics of the AML T cell space was comparable to previously sequenced bone marrow samples of MDS patients.

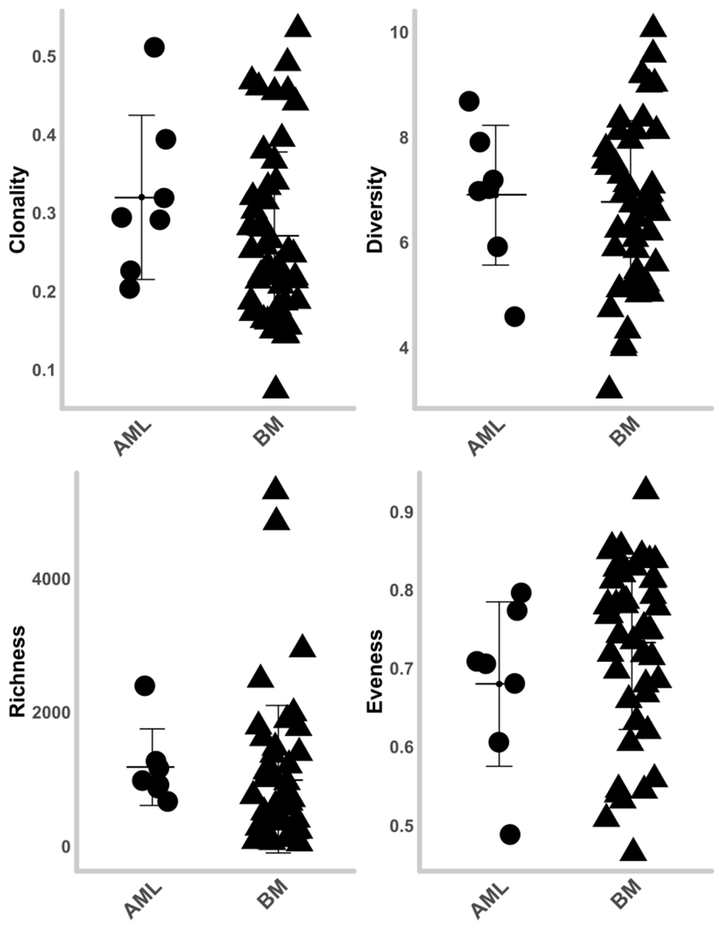


Figure 2. Comparison of broad T cell space immune metrics of the pilot phase AML bone marroiw samples to MDS bone marrow samples.

Conclusion and Outlook

The pilot phase demonstrated that the applied NGS workflow is feasible to analyze the T cell repertoire in AML patients treated within the RAS-AZIC trial. Applying this workflow to the pre-treatment and day 15 samples of the RAS-AZIC cohort will help to gain insights in the response-predictive value of immunological signatures in AML patients receiving AZA.

42 AML patients treated within the RAS-AZIC trial
- matched bone marrow samples pre-treatment and at day 15

analyses of TRB and IGH locus

identification of response-predictive immune signatures

- broad immune metrics
- T cell cluster analysis
- analysis of immunodynamics after 15 days of AZA

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