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Influence of somatic mutations and pretransplant strategies in patients allografted for myelodysplastic syndrome or secondary acute myeloid leukemia

To the Editor:

Somatic mutations and pretransplant strategy both impact the outcome of patients with myelodysplastic syndromes (MDS), and acute myeloid leukemia derived from MDS (sAML) after allogeneic hematopoietic stem cell transplantation (allo-HSCT). While the prognostic influence of several somatic mutations, especially *TP53*, as a disease-related variable is established,[1] the optimal pretransplant strategy is less well defined due to the lack of prospective trials. In a recent analysis we showed that outcome after direct, so called upfront transplantation is at least not inferior compared to pretransplant cytoreduction, with AML-like induction chemotherapy (CTX) or hypomethylating agents (HMA).[2] In the current analysis we aimed to comprehensively investigate the interplay of mutations and pretransplant strategy on outcome after allo-HSCT within one analysis. For this purpose, we examined pretransplant DNA samples from 128 of the 165 previously published patients with MDS (n = 97, 76%), sAML (n = 20, 15%) or chronic myelomonocytic leukemia (n = 11, 9%) for somatic mutations in 54 genes using the TruSight Myeloid panel (Illumina, San Diego, CA). Patients' characteristics, sequencing analysis and statistics are given in Tables S1-S4. Of these, 73 patients (57%) were transplanted without prior cytoreduction (upfront group), whereas 55 (43%, treatment group) had received either anthracycline-containing induction (n = 37, 29%, CTX group) or a median of four cycles (range: one to eight cycles) of Azacitidine (Aza, n = 18 14%, Aza group) prior transplant (Figure S1). Even though there was a higher frequency of sAML in the CTX group and a lower BM blast count in the upfront group at diagnosis, progression to advanced disease or even sAML between diagnosis and transplantation occurred in 14 (19%) and 7 (10%) patients within the upfront group (median 6.4 months; Tables S1 and S3). Consequently, at the time of cytoreductive treatment there was no statistically significant difference regarding the frequency of sAML between the upfront and treated group (15% vs 29%). With a median follow-up of 71 months estimated 5-year OS, RFS, CIR, and non-relapse mortality (NRM) probabilities of the entire cohort were 56%, 42%, 40% and 18%, respectively (Figure S2).

First, we performed amplicon-based sequencing to adress the prognostic impact of somatic mutations. Hereby, we identified 285 mutations which affected 36 of the 54 investigated genes in 111 of 128 patients (87%, median two mutations per patient, range, zero to six) and reflected the clinical high-risk characteristics with *RUNX1*, *TET2*, *ASXL1*, *TP53*, *SRSF2* and *DNMT3A* representing the most commonly mutated genes (Figures S3-S7; Table S5). With exception for *RUNX1*, *TET2* and *ASXL1*, the mutation profile did not differ between treatment groups, even when focusing only on MDS patients (Figure S6, S8, S9). In those 17 genes mutated in ≥5% of patients we identified mutations in four individual genes (*TP53*, *SF3B1*, *NRAS* and *DNMT3A*), which negatively impacted OS and RFS (Figure 1A; Table S6, Figures S10-S12). Mutations in *TP53* and *SF3B1* were also associated with higher relapse incidence, while *NRAS* and *SF3B1* mutations negatively influenced NRM (Table S7; Figure S13-S15). Consequently, mutations in these four genes, which were mutually exclusive to each other in three of four genes (*TP53*, *NRAS*, *SF3B1*), were summarized as poor-risk mutations for further analyses (Table S6-S8; Figures S16-S17). Acknowledging the negative prognostic impact of complex karyotype (CK, n = 25, Figure S18; Table S9) and the overlap between CK and poor-risk mutations (Figure S16), we analyzed their prognostic

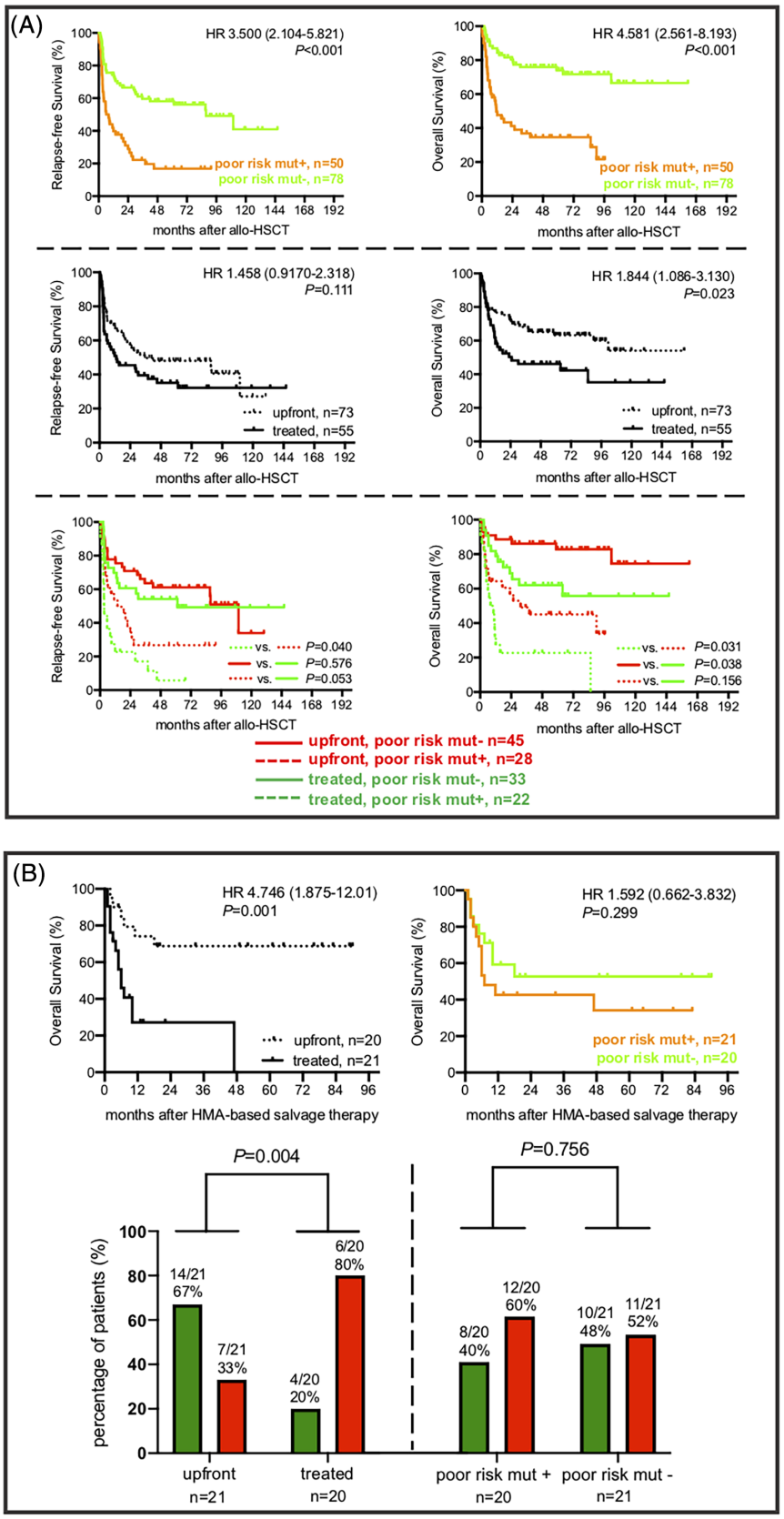

FIGURE 1 Effects of "poor risk mutations" and pretransplant strategy on posttransplant outcome. A, Illustrates posttransplant relapse-free and overall survival depending on "poor risk mutation" status and pretransplant strategy as well as the interplay between both parameters. B, Illustrates effects of "poor risk mutation" status and pretransplant strategy on outcome after HMA-based salvage therapy for posttransplant relapse in terms of complete remission rate and overall survival in 41 relapsed patients. "Poor-risk mutation" status and pretransplant strategy are depicted in indicated colors and line pattern respectively. Hazard ratios are relative to those patients without any poor risk mutation and relative to those receiving upfront transplantation, respectively. Allo-HSCT, allogeneic hematopoietic stem cell transplantation; CR, complete remission; HMA, hypomethylating agents; HR, hazard ratio; mut, mutation

interaction. In accordance with recently reported findings[3,4] we show that the presence of poor risk mutations facilitates refinement of the prognostic information of CK with a dismal prognosis in patients carrying both CK and poor-risk mutations (Figure S19).

Focusing in a next step on other disease-related, patient-associated and transplant-related factors (Tables S9,S10; Figures S20-S23) we found that pretransplant strategy was the most prominent factor significantly influencing OS. Patients, who were transplanted

without prior cytoreduction, had longer survival compared to patients who received pretransplant cytoreduction (Figure 1A, Figure S15). In addition, patients undergoing upfront transplant had a trend towards a lower relapse rate (Figure 1A). Except for age (OS and RFS) and CK (RFS) no other factor impacted OS, RFS, relapse incidence or NRM (Tables S9-S10; Figures S20-S23).

In multivariate analyses the presence of poor-risk mutations as well as pretransplant strategy confirmed their prognostic role with poor-risk mutation carriers and pretreated patients having inferior survival and higher risk of relapse. Additionally, *BCOR* and *EZH2* mutations were associated with relapse and *NRAS* and *SF3B1* mutations with NRM (Tables S11-S13).

Based on this, we addressed the hypothesis that there might be an interaction between these two disease-related and procedure-related factors and re-analyzed our cohort by combining the information about mutation status of these four genes and pretransplant strategy (Figure 1A). Indeed, the outcome of patients with poor-risk mutations, who had received pretransplant therapy, was poor with 5-year OS and RFS of 23% and 5.7% and significantly inferior compared to patients with poor-risk mutations in the upfront group (5-year OS: 45%, 5-year RFS: 27%). In contrast, among patients without poor-risk mutations the upfront group had a favorable 5-year OS of 83% compared to 62% in pretreated patients, while 5-year RFS was comparable (5-year RFS 61% vs 54%). These data were confirmed by a separate analysis of patients with MDS (Figure S24).

Relapse, mainly driven by poor-risk mutations and pretransplant strategy (Table S12), was the major cause of treatment failure (Figure S2) with 62% of deaths being attributable to relapse. Therefore, we finally asked whether these two variables also influenced response to and survival following salvage therapy with HMA (Aza n = 40, decitabine n = 1) and donor lymphocytes infusions (DLI, Figure S25; Table S14). The negative prognostic impact of poor-risk mutations was indeed abrogated after salvage therapy, as indicated by a comparable CR rate (43% vs 53%) and survival (2-year OS 43% vs 53%) in mutation carriers and patients without poor-risk mutations. In contrast, the CR rate was significantly higher (73% vs 21%) and survival (2-year OS 69% vs 27%) significantly longer in the upfront group compared to pretreated patients (Figure 1B, Figure S26).

A major criticism of our previous analysis, where we adressed the role of upfront transplantation in 165 patients with MDS and sAML,[2] is a possible selection bias where poor-risk patients receive pretransplant cytoreduction whereas „better-risk "patients may be scheduled for upfront transplantation. To overcome this we now focused on the underlying disease biology in terms of somatic mutations in 128 of the previous cohort,[2] which were selected based on DNA availability and exhibited comparable pretransplant CR rate and survival like the entire cohort (data not shown). We identified mutations in four genes (*TP53*, *DNMT3A*, *NRAS* and *SF3B1*) that were associated with poor post-transplant outcome primarily related to a higher relapse incidence. Our data regarding *TP53*, the *NRAS/RAS* pathway and in parts *DNMT3A* mutations are consistent with results from six other retrospective analyses in 62 to 1514 patients,[1,3-5] with discrepancies between the analyses probably related to imbalances regarding patient characteristics, treatment state, sampling and sequencing. The second major finding of our current, even though retrospective analysis, which also proved true when considering only the 97 MDS patients, is that upfront transplantation resulted in a significantly better survival. This is in line with previous retrospective analyses suggesting at least non-inferiority of upfront transplantation compared to pretransplant cytoreduction.[2] By integrating molecular information and pretransplant strategy, we here show that patients with similar disease biology, namely poor-risk mutations have dismal outcome after pretransplant cytoreduction and seem to benefit from direct transplantation. It remains unclarified at this point, whether dose-intensification of conditioning regimen as another procedure-related, per-se modifiable variable may influence the prognosis associated with individual mutations such as *RAS* or *TP53* mutations, as suggested by one,[5] but not confirmed in our (Figure S27) and another analysis.[3] Furthermore, whether pretransplant implementation of novel compounds like CPX-351, venetoclax or APR-246 may overcome chemoresistance implicated by the four poor-risk mutations will be subject of future investigations.

Besides the trend towards a lower relapse rate the advantage of upfront transplantation in the entire, as well as in the molecularly defined poor-risk group, appears to result particularly from a higher efficacy of salvage therapy with HMA and DLI. A hypothesis explaining this phenomenon may be a Darwinian mechanism which selects resistant leukemic clones during pretransplant cytoreduction resulting in resistant relapse after transplant,[6] but requires prospective confirmation by longitudinal sampling. In addition to disease burden and diagnosis of MDS previously reported as predictors for response and survival after post-transplant therapy with Aza, we here show for the first time that the pretransplant strategy, but not the four poor-risk mutations (Figure 1B, Figure S26 and S28) predict response and survival after HMA-based salvage therapy. We conclude that an individualized allografting concept for patients with MDS should incorporate molecular profiling at the time of decision for allo-SCT, and planning of pretransplant strategy. If feasible, upfront transplantation appears to be associated with favorable outcome particularly in patients with high-risk molecular characteristics and may be augmented by post-transplant HMA-based salvage therapy in case of relapse. These data build the hypothesis and rationale for confirmatory testing within a prospective study.

CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

Christina Rautenberg, Guido Kobbe and Thomas Schroeder designed the research study.

Christina Rautenberg, Stefanie Stepanow, Karl Köhrer, and Thomas Schroeder performed the research.

Christina Rautenberg, Ulrich Germing, Paul S. Jäger, Stefanie Geyh, Guido Kobbe and Thomas Schroeder contributed essential data.

Christina Rautenberg, Ulrich Germing, Michael Lauseker, Min Fan and Thomas Schroeder analyzed the data.

Christina Rautenberg, Ulrich Germing, Guido Kobbe and Thomas Schroeder wrote the paper.

All authors critically revised the manuscript.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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An evaluation of no-treatment decisions in patients with newly diagnosed acute myeloid leukemia

To the Editor:

The standard treatment for acute myeloid leukemia (AML) consists of induction chemotherapy, followed by consolidation chemotherapy ± allogeneic hematopoietic stem cell transplantation (HSCT). The median age of AML is 68 to 70 years. Most older patients are unfit for such intensive treatments, owing to co-morbidities and frailty. For such patients, palliative chemotherapy with hypomethylating agents (HMA) or low-dose cytarabine (LDAC) have usually been offered.

There has been increasing interest in real-world data in AML which reflect treatments and outcomes in unselected patient populations. Recent real-world registry data from Europe have reported that a significant proportion of older patients do not receive any anti-leukemic therapy at the time of diagnosis, other than best supportive case (BSC).[1-3] However, there has been a paucity of reported analysis on why such patients do not receive treatment. In