Abstract: P333

Title: SUBCLONAL AND CLONAL VARIANTS IN TP53 AND KRAS COMBINED WITH POOR TREATMENT RESPONSE IDENTIFY A SUBGROUP OF ULTRA-HIGH-RISK PATIENTS OF PEDIATRIC T-LYMPHOBLASTIC LEUKEMIA (T-ALL)

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Background:

Relapse of pediatric T-cell acute lymphoblastic leukemia (T-ALL) remains a substantial challenge in pediatric oncology. Patients who suffer relapse face a dismal prognosis caused by therapy resistance. There are no molecular biomarkers reliably predicting risk. In T-ALL relapse, inactivating variants in *TP53* were previously described to identify approximately 10% patients who all experienced fatal relapse (Hof et al. 2011; Richter-Pechanska et al. 2017). Variants in other genes were described to be either associated with a poor prognosis in relapse (*MSH6*, *USP7*, *IL7R*, *CNOT3*, *KRAS* and *NRAS* (Richter-Pechanska et al. 2017)) or to be characteristic for relapse (*CCDC88A* (Richter-Pechanska et al. 2017), *NT5C2* (Kunz et al. 2015; Meyer et al. 2013)).

Aims:

Considering that clonal evolution is a known mechanism that drives therapy resistance and relapse in cancer (Ding et al. 2012; O'Leary et al. 2018), we tested the hypothesis that subclonal or clonal variants that signify ultra-high-risk at the time of relapse may also be prognostic at the time of initial disease.

Methods:

Two cohorts of pediatric patients at initial diagnosis of T-ALL who were treated on ALL-BFM protocols were analyzed. Cohort-1 comprised a total of 160 samples that were selected based on a case-control design: 81 patients who later relapsed and 79 who reained in first complete remission for at least three years matched for treatment response/intensity, age and sex. In cohort-2, 226 samples of unselected consecutive T-ALL patients were analyzed. We performed targeted deep sequencing of 9 genes (*TP53, KRAS*, N*RAS, MSH6*, *USP7*, *IL7R*, *CNOT3*, *CCDC88A*, *NT5C2*) using the Agilent Haloplex High Sensitivity kit with unique molecular identifiers to enable the reliable detection of variants with very low allele frequencies (average read depth: 1 012 +/- 642 and 723 +/- 491 reads).

Results:

Overall, we identified 75 variants in 7/9 targeted genes in 54/160 (34%) T-ALL patients of cohort-1. The variants tended to be more common in relapsing than in non-relapsing patients (33 vs. 21; Chi^2, p=0.058). The mean allele frequency of the detected variants was 24.7% (SD±18; range 0.8-83). More than half of the variants (43/75) were found at allele frequencies <30% and were thus considered subclonal. Interestingly, *TP53* variants were identified exclusively in patients later developing a relapse (6/81; Chi^2, p=0.014). Variants of *KRAS* were also significantly more frequent in relapsing patients (9/81 vs. 2/79; Chi^2, p=0.032). All of these 14 patients with *TP53* and/or *KRAS* variants died in relapse, whereas 19/67 without such variants survived the relapse (p=0.023).

In the unselected patients of cohort-2, a total of 66 variants (subclonal: n=32, clonal: n=34) were found in 8/30 (27%) relapsing and 48/196 (25%) non-relapsing patients. *TP53* and *KRAS* variants were not enriched in patients who later relapsed, indicating that the presence of these variants *per se* does not predict the risk of relapse in unselected patients.

We thus extended our analysis by also considering treatment response. Of all the 386 analyzed patients, 188 were stratified into the high-risk arm because of poor treatment response. Of these 188 patients, 9 carried subclonal or clonal *TP53* and/or *KRAS* variants, of whom 8 suffered a relapse and subsequently died. Of these, 5 had not

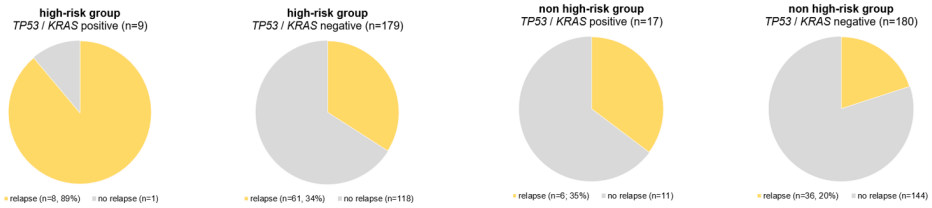
received stem cell transplantation.

Summary/Conclusion:

In conclusion, our findings indicate that the combination of *TP53* and/or *KRAS* variants with poor treatment response identifies a subgroup of patients with a dismal prognosis who might benefit from treatment intensification or experimental treatment approaches such as CD1a or CD7 CAR-T cell therapy.

Figure 1: The combination of poor treatment response with TP53 and/or KRAS variants identifies a subgroup of pediatric T-ALL patients at initial diagnosis with a dismal prognosis.

Of all analyzed patients (n=386), 188 patients were stratified to high-risk and 197 to non-high-risk based on their treatment status. Of one patient, the risk stratification was unknown.



Keywords: T-ALL, Acute lymphoblastic leukemia, High risk, Risk factor