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POSTER ABSTRACTS

618.ACUTE LYMPHOBLASTIC LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

Novel Insights in TKI Resistance in *BCR*::*ABL1*-Positive B-Cell Acute Lymphoblastic Leukemia Beyond Kinase Domain Mutations

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Background and Aim

Despite the introduction of tyrosine kinase inhibitors (TKIs), approximately 30% of newly diagnosed pediatric and adult *BCR*::*ABL1*-positive B-cell acute lymphoblastic leukemia (B-ALL) patients experience relapsed or refractory disease (Biondi et al, Lancet Haematology 2018; Bassan et al, Journal of Clinical Oncology 2010). ABL1 kinase domain mutations, hindering TKI binding, explain part of the relapses and are acquired during TKI therapy but are rarely detected in newly diagnosed *BCR*::*ABL1*-positive B-ALL patients (Baer et al, Haematologica 2022). Intrinsic TKI resistance mechanisms in *BCR*::*ABL1*-positive B-ALL present prior to TKI treatment are less well documented. Therefore, we aimed to identify potential biomarkers for TKI sensitivity at diagnosis using primary patient samples and newly generated cell lines made from patient-derived xenografts (PDX cell lines).

Patients and Methods

Leukemic cells from diagnosis of *BCR*::*ABL1*-positive B-ALL patients without *ABL1*-kinase domain mutations were used to measure cell survival in an *ex vivo* co-culture assay after imatinib (1.95-125 μM), dasatinib (1.95-125 nM), or bosutinib (0.016-2 μM) exposure. TKI sensitivity was assessed using the area under the dose-response curve (AUC), with <80 arbitrary units (AU) classified as imatinib-sensitive, 80-120 AU as intermediate, and >120 AU as resistant. Multiplex ligation-dependent probe amplification, whole exome sequencing, and RNA sequencing were performed on diagnostic material if available. ABL1 phosphorylation and expression levels were measured using (phospho)flow cytometry in patient-derived xenografts from diagnostic samples and (PDX) cell lines.

Results

We determined *ex vivo* sensitivity to imatinib, dasatinib, and bosutinib in 32 pediatric and 19 adult *BCR*::*ABL1*-positive B-ALL patient samples. We observed a substantial variability in *ex vivo* imatinib response in pediatric and adult patient samples (median AUC: 98 AU; range: 16 to 281 AU). The sensitivity towards imatinib strongly correlated with the sensitivity towards second-generation TKIs dasatinib and bosutinib (rho = 0.84; $p < 0.001$ and rho= 0.89; $p < 0.001$). Moreover, imatinib's AUC of samples derived from newly diagnosed patients who relapsed after TKI treatment was higher compared with those derived from patients who did not suffer from a relapse (median AUC 123 vs 84 AU; $p = 0.03$).

We observed that an increase in *ex vivo* imatinib resistance was correlated with a decreased amount of phosphorylated ABL1 protein (rho=-0.92; p=0.0013) and total ABL1 protein (rho=-0.82; p=0.01) in untreated, newly diagnosed samples. *Ex vivo* exposure to imatinib reduced the ABL1 phosphorylation levels in both sensitive and resistant samples but resistant cells exhibited lower levels of (p)ABL1 protein, indicating reduced dependence on BCR-ABL1 signaling.

The *ex vivo* imatinib resistance in samples carrying deletions in B-cell development genes *IKZF1* and/or *PAX5* was higher than in samples without these deletions (median AUC: 122 versus 80 AU; p=0.01). In addition, one imatinib-resistant patient had a p.His1038Arg mutation in *ZEB2*, which is recurrent in B-ALL and this gene has also been described as B-cell development gene (Studd et al, Blood Cancer Journal 2021). Interestingly, another resistant patient had a mutation in *SETD2*,a gene previously associated with *ex vivo* TKI resistance in chronic myeloid leukemia cell lines (Sheng et al, Cell Proliferation 2019).

Other secondary lesions included *SH2B3* inactivation lesions and *CRLF2* rearrangements. These lesions were present in intermediately sensitive and resistant *BCR*:: *ABL1*-positive samples and are expected to lead to increased JAK/STAT signaling, potentially serving as a resistance mechanism for TKI.

Conclusion

Our study revealed that the amount of total and phosphorylated ABL1 protein was correlated with *ex vivo* TKI sensitivity. Newly diagnosed TKI-resistant *BCR*:: *ABL1* samples were further characterized by somatic lesions in B-cell development genes including *IKZF1*. These results link the known poor prognostic value of *IKZF1* deletions in *BCR*:: *ABL1*-positive B-ALL patients (van der Veer et al. Blood 2014; Fedullo et al, Haematologica 2019) with intrinsic mechanisms of TKI resistance other than tyrosine kinase domain mutations.

Disclosures Rijneveld: *BMS:* Honoraria; *Servier:* Honoraria.

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