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POSTER ABSTRACTS

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

Secondary Lesions and Sensitivity to Signaling Inhibitors of Pediatric iAMP21 B-Cell Precursor Acute Lymphoblastic Leukemia

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Introduction

Intrachromosomal amplification of chromosome 21 (iAMP21) B-cell precursor acute lymphoblastic leukemia (BCP-ALL) in children is a high-risk subtype for which targeted drugs are lacking. In this study we aimed to determine the frequency of secondary lesions and investigated the cellular sensitivity for candidate targeted drugs.

Methods

We performed total RNA sequencing on 28 iAMP21 and 28 B-other (negative for sentinel fusion genes) pediatric samples to determine the frequency of secondary lesions in newly diagnosed patients. A panel of 18 patient derived xenografts (PDX) of 8 primary iAMP21 ALL samples was generated, and secondary lesions were validated by PCR, RT-PCR, and whole exome sequencing. To test sensitivity, primary or PDX cells were exposed *ex vivo* to a concentration range of gilteritinib (FLT3 inhibitor), trametinib (MEK1/2 inhibitor), or ruxolitinib (JAK1/2 inhibitor).

Results

Secondary lesions in the cytokine receptor gene *FLT3* were enriched in iAMP21 compared with B-other ALL including internal tandem duplications (ITD) and other activating lesions (50.0% vs. 10.7%, p=0.003). Lesions in genes encoding cytokine receptors *CRLF2* and *IL7R* had a similar frequency between iAMP21 and B-other cases (25% vs. 17.9%, p=0.75 and 7.1% vs. 0%, p=0.49 respectively). Inactivating lesions in *SH2B3*, the downstream negative regulator of JAK/STAT and FLT3 signalling, were more frequent in iAMP21 cases vs. B-other (46.4% vs. 7.1%, p=0.002), while the frequency of *JAK1* and *JAK2* mutations did not differ (3.6 vs. 0% and 10.7 vs. 10.7% p = 1 for both). All *SH2B3*, *CRLF2* and *JAK* lesions were retained in PDX samples, whereas in contrast *FLT3*-ITD was retained in only 2 of 5 PDX.

Gilteritinib sensitivity did not differ between iAMP21 and B-other cases (median LC50 1.24 μM, range 0.33 to 4.85 μM vs. median LC50 1.21 μM, range 1.20 to 1.3 μM, p=0.95). Grouping iAMP21 cases by *FLT3* and *SH2B3* status, samples with both *FLT3*-ITD and *SH2B3* lesion had the highest sensitivity to gilteritinib (median LC50 0.39 μM, range 0.35 to 0.43 μM). Samples with only *FLT3* or *SH2B3* lesion did not show increased sensitivity compared to those without a lesion (p>0.5 for both). Median ruxolitinib sensitivity did not statistically differ between iAMP21 and B-other cases (median LC50 8.38 μM, range 1.00 to >10 μM, vs. median LC50 0.48, range 0.21 to >10 μM; p=1) although extreme resistance to ruxolitinib seemed more frequent in iAMP21 cases (6 out of 12 cases) and was not related to *FLT3* or *SH2B3* status. *CRLF2*-rearranged (*CRLF2*r) iAMP21 cases were slightly more sensitive to ruxolitinib than those lacking *CRLF2*r, although this difference did not reach significance (median LC50 4.72 μM, range 1.0-4.85 vs. median LC50 >10 μM, range 2.66 to >10 μM; p=0.08). The highest sensitivity was found in the only case with both a *CRLF2*r and a *JAK1* mutation (LC50 of 1.00 μM). A large variation was present in trametinib sensitivity amongst both iAMP21 and B-other cases (median LC50 0.11 μM, range 0.017 to >5 μM vs. median LC50 0.18, range 0.018 to >5 μM; p=0.72). More than half of iAMP21 cases were sensitive irrespective of RAS-pathway lesion status.

Conclusion

iAMP21 leukemias are enriched in *FLT3* and in *SH2B3* lesions which, when co-occurring, affect sensitivity to FLT3 inhibition by gilteritinib but do not affect JAK-inhibition by ruxolitinib. This suggests these lesions act synergistically and might bypass downstream JAK/STAT signalling. This might also explain the observed sensitivity to RAS-pathway inhibition irrespective of

secondary lesions. These results suggest that further research into FLT3 and RAS signalling inhibitors might lead to better treatment options for pediatric iAMP21 BCP-ALL.

Disclosures No relevant conflicts of interest to declare.

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