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POSTER ABSTRACTS

621.LYMPHOMAS: TRANSLATIONAL-MOLECULAR AND GENETIC

Circulating Tumor DNA (ctDNA) for Plasma Genotyping and Disease Monitoring in ALK-Positive Anaplastic Lymphoma (ALK+ALCL): A Proof of Concept Study

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Introduction

Circulating tumor DNA (ctDNA) detection has not been reported yet in ALK-positive anaplastic large cell lymphoma (ALK+ALCL), a rare aggressive non-Hodgkin lymphoma with a peak incidence in children, adolescents and young adults. In this study, we aimed to evaluate whether ctDNA can be detected, characterized and used to monitor minimal residual disease (MRD) in ALK+ALCL.

Patients and Methods

Plasma samples were collected from 23 French patients diagnosed with ALK+ALCL between November 2020 and March 2023. Based on cell-free DNA (cfDNA) extracted from plasma, we performed shallow whole genome sequencing (shWGS) and a comprehensive genomic profiling (CGP) of more than 500 genes covered at a median depth of over 3000 unique reads to assess ctDNA content. In this study, we defined samples as positive if they had a tumor fraction over 3%, evaluated by shWGS analysis, or if ALK rearrangements were identified using CGP. Additionally, *ALK* rearrangement genomic breakpoint were defined by CGP analysis. For each patient, specific digital droplet PCR (ddPCR) assays were designed based on the identified breakpoints to monitor ctDNA content during the ALK+ALCL disease follow-up.

Results

The median age of patients at the time of diagnosis was 13 years (range: 4-54). Sixteen patients, (69.6%) received at least one ALK tyrosine kinase inhibitor (TKI). A total of 80 plasma samples were collected at various time points from the 23 patients, with a median of 3 samples per patient (range: 1 - 20). Four samples failed for DNA extraction, resulting in 76 samples available for molecular analysis Of the 76 samples, 43 (56.6%) were analyzed using shWGS, 19 (25%) using CGP among which, 14 were analyzed using both techniques. CGP was found to be a more sensitive method for detecting ctDNA in ALK+ALCL compared to shWGS, with 12 out of 14 samples (85.7%) tested positive with CGP, compared to 5 out of 14 samples (35.7%) with shWGS ($p<0.01$).

Of the 19 samples tested with CGP, *ALK* rearrangement was identified in 14 samples (73.7%), with *NPM1*:: *ALK* fusion detected in 11 samples, and *ATIC*:: *ALK*, *TRAF1*:: *ALK* and *EEF1G*:: *ALK* detected in one sample each. The average tumor mutational burden was low, with 5.3 mutations per megabase (mut/Mb) (range: 0-14.5 mut/Mb). No microsatellite instability was detected. Amplification of *MDM4* and *MYC* genes was observed in three patients each. *ANKRD26* was the most frequently mutated gene in the study, with four patients affected. *LRP1B*, epigenetic modifiers such as *EP300* and *KMT2A*, and *TP53* were mutated in three patients each. Interestingly, CGP allowed the identification of the *ALK*:c.3520T>C;p.(Phe1174Leu) mutation in a plasma sample collected during disease progression while the patient was on long lasting crizotinib therapy.

Finally, we monitored MRD, based on ctDNA detection in 49 samples from 12 patients using ddPCR assays. Our results were compared with the established gold standard for MRD monitoring in ALK+ALCL, i.e. RT-PCR on circulating cells performed on the same samples. The ddPCR assay showed a 87% sensitivity, 100% specificity, 100% positive predictive value, and 83% negative predictive value. However, four samples had false-negative results due to pre-analytical sampling issues. These samples had been stored at room temperature without undergoing centrifugation for more than 24 hours.

Conclusion

To date, this study is the first to report on the feasibility and clinical value of ctDNA for the management of ALK+ALCL patients. CGP demonstrated high sensitivity in detecting ctDNA, identifying the genomic breakpoint within the *ALK* gene and, for the

first time in ALK+ALCL, detecting resistance mutations to ALK TKI. Additionally, our unique patient-specific ddPCR approach was proven to be a cost-effective method for MRD monitoring. Altogether, these findings serve as a proof-of-concept for the development of ctDNA techniques in the clinical management of ALK+ALCL.

Disclosures Sibon: *AbbVie:* Consultancy; *Janssen:* Consultancy; *Roche:* Consultancy; *Takeda:* Consultancy. **Minard-Colin:** *Roche:* Consultancy; *BMS:* Consultancy; *Adaptimmune Therapeutics plc:* Consultancy; *Aztra:* Consultancy.

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