Ctdna monitoring in metastatic uveal melanoma patients treated with tebentafusp using mutation-agnostic multiplex ddpcr assays

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Abstract

Background & Objectives

Uveal melanoma (UM) is the most common primary intraocular tumor in adults with a low tumor mutational burden. In this study, we developed and validated mutation-agnostic multiplex droplet digital PCR (ddPCR) assays as a novel approach for circulating tumor DNA (ctDNA) monitoring in tebentafusp-treated metastatic UM (MUM) patients.

Methods

Two multiplex ddPCR assays were developed by combining three drop-off or simplex ddPCR assays into each multiplex. Multiplex 1 targeted clustered hotspot mutations in GNAQ and GNA11. Multiplex 2 targeted mutations in PLCB4, CYSLTR2, and SF3B1. Analytical sensitivity and specificity was evaluated using tumor-derived or synthetic DNA containing specific mutations (GNAQ 626 A>C, GNA11 626 A>T, GNA11 C>T for multiplex 1, and PLCB4 c.1888G>T, CYSLTR2 c.386 T>A, SF3B1 c.1874 G>A for multiplex 2) and genomic DNA from healthy donors. Validation was performed using plasma samples from a prospective study.

Results

The multiplex assays achieved detection limits of 0.06%–0.13%, comparable to simplex ddPCR assays. Detection of mutated sequences was successfully validated using tumor-derived or synthetic DNA. Additionally, ctDNA detection using the multiplex assays strongly correlated with simplex ddPCR results ($r = 0.99$, $p < 0.0001$ for multiplex 1, $N = 8$; $r = 0.96$, $p < 0.0001$ for multiplex 2, $N = 8$), confirming their reliability.

Conclusion

Mutation-agnostic multiplex ddPCR assays overcome the limitations of targeted NGS and simplex ddPCR, offering a cost-effective and reliable approach for ctDNA monitoring in MUM without requiring prior knowledge of target mutations. These assays provide valuable insights for treatment monitoring and have the potential to improve clinical management of patients treated with tebentafusp.

Do you have any conflicts of interest?

Yes, I have a conflict of interest.

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