

Multiplex Liquid Biopsy Assays to Predict Melanoma Recurrence



Suzanah C. Boyd^{1,2}, Ashleigh Stewart^{1,2}, Wei Yen Chan^{1,2}, Jenny Lee^{1,3}, Russell J. Diefenbach^{1,2}, Helen Rizos^{1,2}

Affiliations: ¹Macquarie University, ²Melanoma Institute of Australia, ³Chris O'Brien Lifehouse



Aim

To develop multiple multiplex droplet digital PCR (ddPCR) solutions for the rapid detection of predictive circulating biomarkers in melanoma patients.

Background

Targeted therapies and immune checkpoint inhibitors have improved melanoma outcomes, but there is a crucial need for biomarkers to predict treatment response, resistance, and facilitate patient monitoring¹.

ddPCR allows for sensitive analysis of circulating tumour DNA (ctDNA) to monitor response. However, further optimisation is needed to simultaneously detect multiple key biomarkers to expand applicability.

ddPCR Limitations

- Requires prior knowledge of tumour-specific mutations.
- Limited multiplex capacity.
- High reagent costs.
- Needs improved sensitivity².

Assay Design

1. Primer and Probe Design

- Locked Nucleic Acids (LNAs)³
- Determine homology using NCBI Primer-BLAST
- Assess primer-probe interactions (IDT oligo analyzer)

2. ddPCR Optimisation

- PCR conditions
- PCR enhancers
- Restriction enzymes

3. ddPCR Efficiency

- Specificity (limit of blank)
- Sensitivity (limit of detection)

4. Validate ddPCR

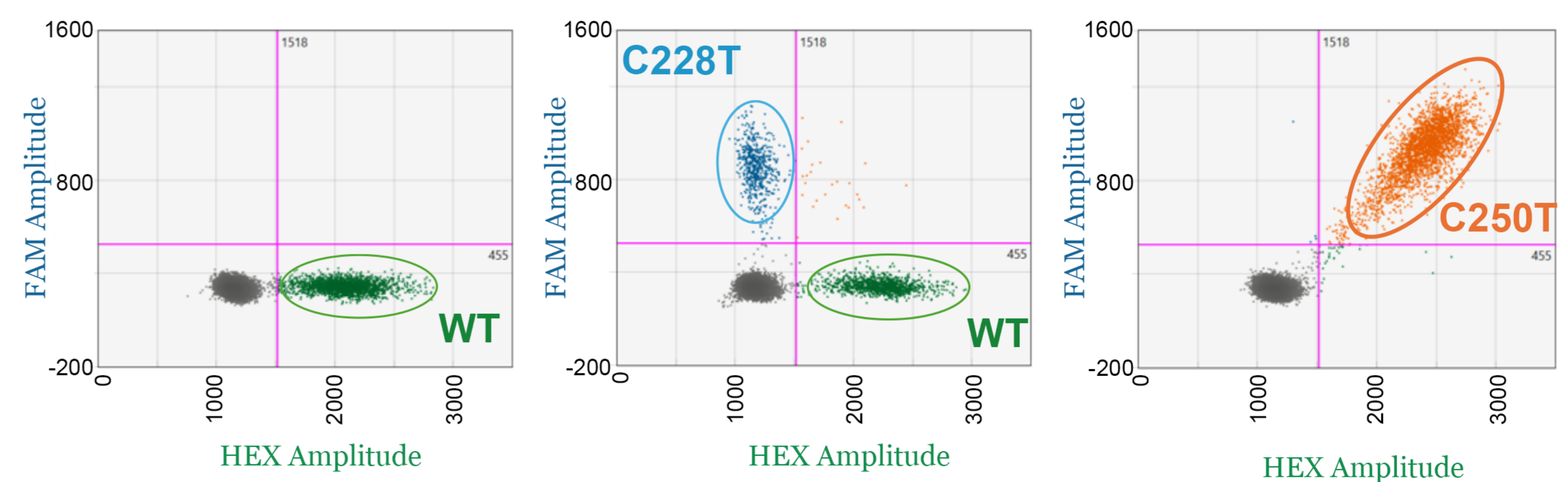
- Perform ddPCR on patient cfDNA
- Correlate with treatment response and disease progression

Acknowledgements

This research has been supported by Macquarie University Research Excellence Scholarships, Emma Betts Foundation, Melanoma Institute of Australia, CLEARbridge Foundation, Medical Research Future Fund and US Department of Defence

Results

Multiplex ddPCR assays provide solutions for real-time surveillance of melanoma patients



TERT mutation detection ddPCR – multiplex detection of TERT mutations C228T and C250T

Additional ddPCR Solutions in Progress

- Developing multiplex ddPCR assays for the simultaneous detection of common mutations in melanoma, specifically, *BRAF* (*BRAFV600E/K/R*) and *NRAS* (*NRASQ61R/K/L*), along with TERT assay, to detect 80% of melanoma cases⁴.
- Testing methylation ddPCR assays targeting melanoma lineage specific methylation sites to be used for patients with mutation agnostic melanoma.

Conclusion

The development and implementation of these highly specific, reproducible, and sensitive multiplex ddPCR assays will enable rapid and cost-effective longitudinal detection of melanoma-associated ctDNA in patients, enhancing personalised patient management. This will improve diagnostic accuracy, improve disease monitoring and broaden applicability.

References

1. Reinhart JP *et al.* Incidence and mortality trends of primary cutaneous melanoma: A 50-year Rochester Epidemiologic Project study. *JAAD Int* 2024, 16:144-154.
2. You Y *et al.* Design of LNA probes that improve mismatch discrimination. *Nucleic Acids Res* 2006, 34:e60.
3. Corné J *et al.* Development of multiplex digital PCR assays for the detection of PIK3CA mutations in the plasma of metastatic breast cancer patients. *Sci Rep* 2021, 11:17316.
4. Mutation Analysis using C-Biportal: <https://www.cbiportal.org/>

