Technical advances in plasma genomic biomarkers for mutation detection and monitoring in cancer patients

Sandra Fitzgerald[1], Cherie Blenkiron[1], Paula Shields[1], Annette Lasham[1] and Cristin Print[1,2]

[1] Faculty Medical and Health Sciences, University of Auckland,[2] Maurice Wilkins Centre, University of Auckland

Introduction

A sea-change is imminent for cancer medicine, due to the use of non-invasive genomic biomarkers in blood to inform screening, diagnosis and the selection of treatment. This technology may be used routinely in oncology within five years. Although numerous studies, including work in our laboratory, have shown that genomic analysis of blood can detect the presence and even the type of cancer, researchers have only scratched the surface of what this technology can do. There are still many technical challenges that need to be addressed before these biomarkers can be used routinely in the clinic. In our laboratory, we are generating new methods to improve the sensitivity and accuracy of non-invasive tests mutation detection in cancer patients.

Methods

Firstly, we are using a custom amplicon next generation sequencing panel – DNA QiaTarget, to screen for specific mutational hotspots or genes (Fig. 1 and 2). This strategy is useful when common mutations are absent, such as in Neuroendocrine Cancer, or to identify mutations in cancer types that have several genes mutated. Secondly, we are using Droplet Digital PCR (ddPCR) which allows the specific detection of a mutation of interest through a competitive probe assay. This method is particularly useful in cancers such as Melanoma, where discrete mutations such as *BRAF* V600E, are present in up to 40% of Melanomas. However, this requires the presence of each mutation to be screened for individually, and each assay requires optimisation (Figs 3 and 4).

Custom Amplicon NGS for identification of tumour mutations

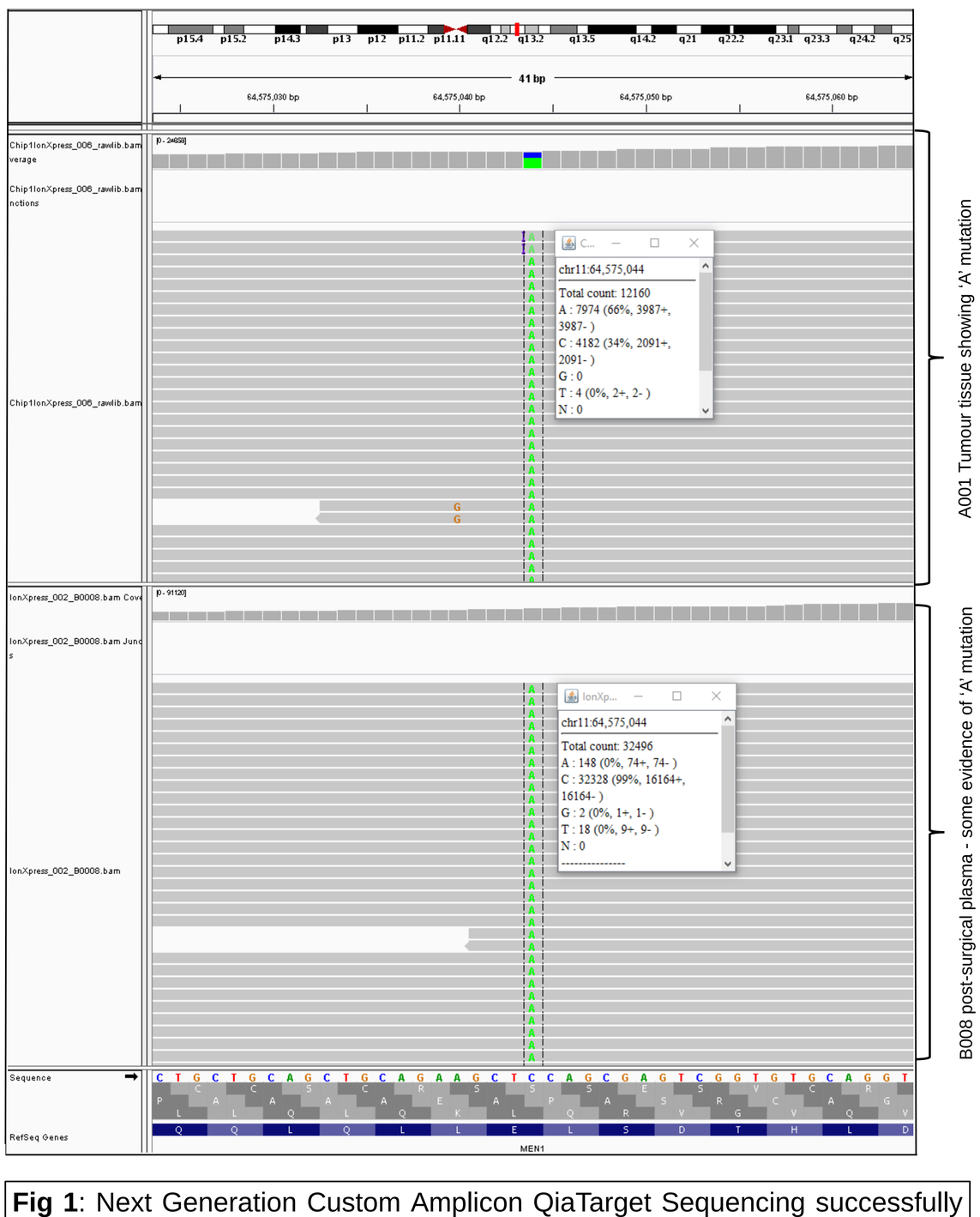

Fig 1: Next Generation Custom Amplicon QiaTarget Sequencing successfully identified *MEN1* mutation in the tumour of A001 Neuroendocrine patient (top panel). Sequencing of cell free DNA (cfDNA) extracted from plasma of this patient (bottom panel) also shows evidence of this mutation.

Fig 2: Neuroendocrine patient A0019 previously found to be aneuploidy for *MEN1*, is confirmed in the bottom panel by amplicon sequencing. Sequencing of cfDNA extracted from pre-surgical and post-surgical plasma from this patient (1st and 2rd panel) shows heterozygous SNP at this position

ddPCR assay optimisation for detection of *BRAF* V600E mutation – temperature gradient

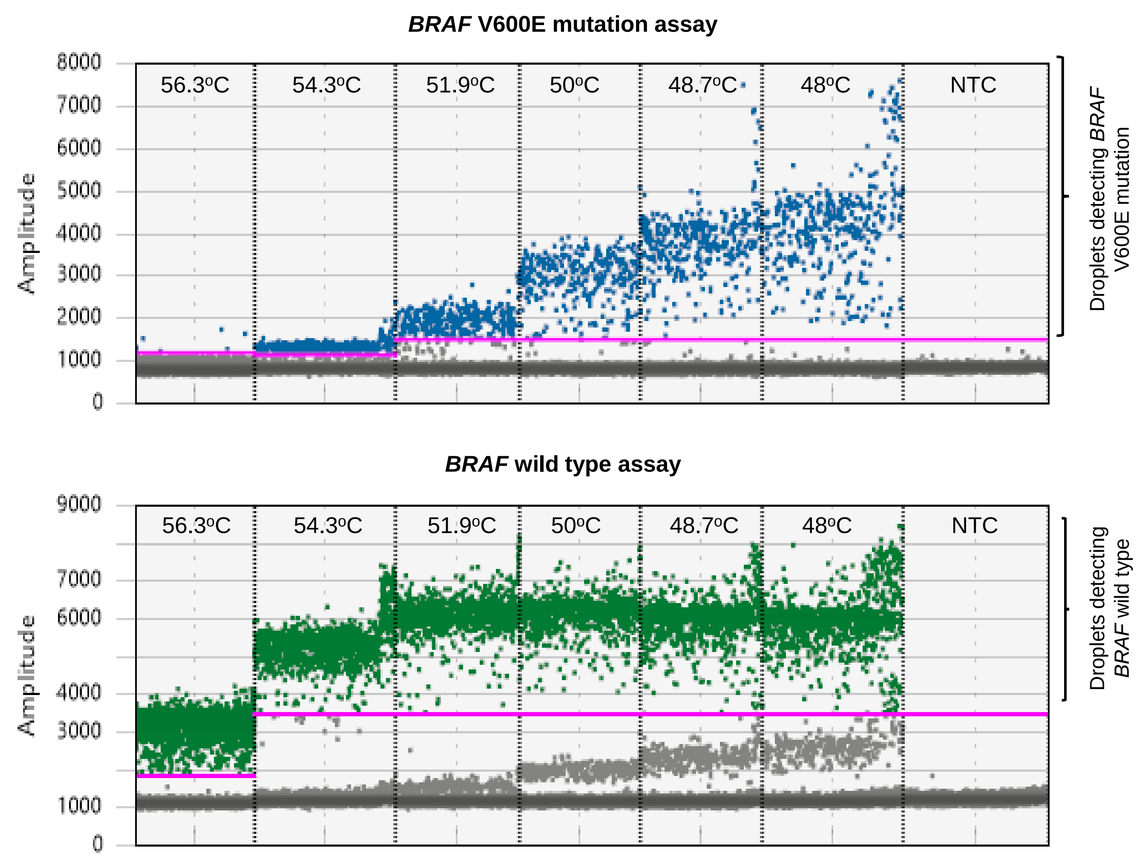

Fig 3: A temperature gradient has been used to determine maximum separation between positive and negative droplets for detection of *BRAF* V600E mutation

Sensitivity of ddPCR assays for detection of *BRAF* V600E melanoma mutation

Fig 4: Decreasing amounts of *BRAF* V600E positive cell line DNA has been used to determine levels of sensitivity - 1:2000 sensitivity as seen in the individual plots for the mutant (a), wildtype (b). The 2 dimension plot (c) shows clear separation between the two probe sets.

Future Directions

We will continue the development of non-invasive technologies in our laboratory to further investigate the limits of detection for diagnosis of mutations in cancer, and the roles these technologies may play in the monitoring for relapse in cancer patients.

Acknowledgements: Sandra would like to thank Genesis Oncology Trust and William Staunton Memorial Scholarship Fund for supporting this research and Translational Medicine Trust