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Detection of clinically relevant early genomic lesions in B-cell malignancies from circulating tumour DNA using a single hybridisation-based next generation sequencing assay

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Genomic characterisation to guide the diagnosis, prognosis and treatment of haematological malignancy is typically performed on tumour specimens (e.g. lymph node biopsy or bone marrow aspirate) however there is increasing interest in the detection of genomic abnormalities of DNA shed from tumour cells that can be detected in the plasma known as “circulating tumour DNA” (ctDNA). In B-cell malignancies, the characterisation of genomic lesions from ctDNA to date has been mostly limited to simple sequence variant detection. However, recurrent translocations involving the immunoglobulin heavy chain locus (*IGH*) and copy number changes are particularly important in guiding diagnostic categorisation and treatment decisions in these disorders. Detection of these larger genomic changes in B-cell lymphoproliferative neoplasms in ctDNA is of potential significant clinical utility but remains relatively undescribed.

We describe a novel approach to two cases of B-cell malignancies (Burkitt lymphoma and multiple myeloma [MM]) where comprehensive genomic characterisation of sequence variants, genome-wide copy number changes and *IGH* translocations was performed from ctDNA using a single targeted hybridisation-based next generation sequencing (NGS) assay. We also demonstrate that this technique is of particular utility for detecting early and clinically relevant genomic lesions in B-cell malignancies.

Details of sample processing, library preparation and assay description are detailed in *Supplementary Methods;* however, briefly, a hybridisation-based NGS assay and a suite of

bioinformatics tools were designed and optimised for variant, copy number and translocation detection (Peter MacCallum Cancer Centre (PMCC) "PanHaem" Panel).

Case 1 - a 65-year-old woman - presented with progressive back pain. Bone marrow aspirate showed 51% morphologically abnormal plasma cells and kappa light chains of >16500 mg/l. Targeted amplicon sequencing on CD138+ selected plasma cells from the bone marrow aspirate was performed as well as PMCC PanHaem hybridisation-based NGS of unselected aspirate and ctDNA (Table I).

Sequencing of the ctDNA demonstrated reads mapped to chromosome 11 in the intergenic region upstream of *CCND1* with read pair mates mapping to *IGH* on chromosome 14, representing sequencing evidence of the t(11;14) that was confirmed by fluorescence in situ hydridisation (FISH) in the bone marrow compartment. Monosomy 13 was detected by sequencing in both the unselected aspirate and ctDNA compartments. Of note, Monosomy 13 was only equivocally detected in the unselected bone marrow aspirate compartment by sequencing whereas it could be confidently called in the ctDNA by sequencing (Figure 1).

Given the limit of detection of the PMCC PanHaem hybridisation NGS assay, it is notable that both the t(11;14) and monosomy 13 were easily detectable in the ctDNA compartment whereas a dominant *KRAS* Gly12Asp mutation observed in the CD138+ selected tumour sample (at 40% variant allele frequency [VAF]) was only present at 7% VAF in the ctDNA. This observation is consistent with a greater representation of the t(11;14) and monosomy 13 in ctDNA compared to the *KRAS* mutations (despite similar frequency of monosomy 13 and *KRAS* mutations in the CD138+ compartment). Spatial heterogeneity exists with regard to variant detection in MM (Mithraprabhu*, et al* 2017), however as observed in this case, the t(11;14) and monosomy 13 would be predicted to be less affected by this phenomenon given they arise early in myeloma pathogenesis(Avet-Loiseau*, et al* 1999).

Whilst variant detection in ctDNA from MM patients has been previously described(Mithraprabhu*, et al* 2017, Rustad*, et al* 2017), clinical decision-making is currently not primarily driven by the detection of sequence variants. Rather, copy number changes (e.g. del 17p) and *IGH* translocations are of greater therapeutic relevance and are integrated into current risk stratification guidelines which guide clinical decision-making(Palumbo*, et al* 2015) highlighting the clinical utility of this type of assay.

Case 2 was a 28-year-old man who presented with increasing shortness of breath, acute kidney injury and a markedly elevated lactate dehydrogenase. A bone marrow biopsy showed extensive

involvement by primitive-appearing CD10+, CD20+ cells with surface lambda light chain expression. FISH demonstrated dual *MYC*/*IGH* signals in keeping with the presence of the t(8;14)(q24;q32). A diagnosis of Burkitt lymphoma (BL) was made. A diagnostic lumbar puncture showed extensive involvement by BL. No DNA sample could be obtained from the bone marrow sample; instead, assessment using the PMCC PanHaem hybridisation-based NGS assay was performed on his cerebrospinal fluid (CSF) specimen in addition to ctDNA.

The genomic lesions detected in both the CSF and ctDNA samples are compared in Table I. Reads were detected within intron 1 of *MYC,* with read pair-mates mapping to *IGH* on chromosome 14 representing the canonical t(8;14)(q24;q32) observed in BL (Figure 1). A typical mutation profile of BL was observed in both samples (Table I). Multiple *MYC* mutations were observed consistent with aberrant somatic hypermutation (aSHM). Of note, a significantly different profile of *MYC* mutations was seen in the CSF compared to the ctDNA, with multiple mutations detectable in the CSF DNA that were either not detectable, or detectable at low VAF in the ctDNA. This observation is consistent with aSHM-induced *MYC* mutations being a late (and ongoing) clonal event in BL(Gutierrez*, et al* 1997). Moreover, malignancies in the central nervous system may be poorly represented in plasma ctDNA(De Mattos-Arruda*, et al* 2015). A 1q gain was noted in the CSF tumour DNA that was not detectable in the ctDNA (or in the original bone marrow biopsy by FISH), consistent with it being a secondary change in BL, which may occur later in pathogenesis(de Souza*, et al* 2011).

In summary, we have described the utility of a single targeted hybridisation-based NGS assay to characterise the full spectrum of clinically relevant genomic lesions (including mutation, copy number variation and translocations) in two contrasting cases of B-cell lymphoproliferative disorders in the diagnostic laboratory. Our observations re-emphasise the potential discrepancy between the genomic profile of ctDNA and the assessed cellular tumour compartment but with the important demonstration of the ability of ctDNA to detect genomic lesions that occur early in lymphomagenesis. Given the relevance of these early lesions in therapeutic decision-making and diagnostic categorisation, this technique represents a novel, efficient and clinically useful application of ctDNA NGS in B-cell lymphoproliferative disorders.

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Author contributions

PB designed the study, analysed data and wrote the paper. GR performed experiments, analysed data and wrote the paper, JM/DC/AP created bioinformatics tools and analysed data, KJ/JG performed experiments, MW/ET/DC analysed data, HMP/MD/DW wrote the paper.

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Table I – Comparison of genetic lesions detected in one case each of multiple myeloma and Burkitt lymphoma

	Category of genomic change	**Tumour** compartment (VAF)**	**ctDNA compartment (VAF)**
Case 1 – Multiple myeloma			
KRAS c.35G>A, p.Gly12Asp	Mutation	DETECTED CD138+ - 37.9%, Unselected – 6.8%	DETECTED* (7.0%)
KRAS c. c.35G>T, p.Gly12Val	Mutation	DETECTED CD138+ - 5.1%, Unselected – 1.2%	DETECTED* (5.6%)
TP53 c.638G>C, p.Arg213Pro	Mutation	DETECTED CD138+ - 3.4%, Unselected – 2.4%	DETECTED* (1.9%)
Monosomy 13	CNV	EQUIVOCAL*** (confirmed by FISH)	DETECTED
IGH-CCND1	Translocation	DETECTED	DETECTED
Case 2 – Burkitt lymphoma			
ID3 c.211C>T, p.Gln71*	Mutation	DETECTED (97.3%)	DETECTED (44.7%)
CCND3 c.851C>T, p.Pro284Leu	Mutation	DETECTED (48.1%)	DETECTED (37.9%)
MYC c.214C>A, p.Pro72Thr	Mutation	DETECTED (53.4%)	DETECTED (1.4%)
MYC c.654C>G, p.Ser218Arg	Mutation	DETECTED (44.2%)	NOT DETECTED
MYC c.1152G>C, p.Glu384Asp	Mutation	DETECTED (53.1%)	DETECTED (26.1%)
MYC c.577C>T, p.(=)	Mutation	DETECTED (10.1%)	NOT DETECTED

MYC c.340C>T, p.(=)	Mutation	DETECTED (51.25%)	DETECTED (5.6%)
MYC c.211C>T, p.(=)	Mutation	DETECTED (52.53%)	NOT DETECTED
CDKN2A ($p14^{ARF}$) c.14_17dupTCTT, p.Leu6Phefs*58	Mutation	DETECTED (53.8%)	DETECTED (33.8%)
1p copy number loss (1 copy)	CNV	DETECTED	DETECTED
1q copy number gain (3 copies)	CNV	DETECTED	NOT DETECTED
2p copy number loss (1 copy)	CNV	DETECTED	NOT DETECTED
IGH-MYC	Translocation	DETECTED	DETECTED

* On manual inspection of sequencing reads

** Bone marrow aspirate for Case 1 and cerebrospinal fluid for Case 2

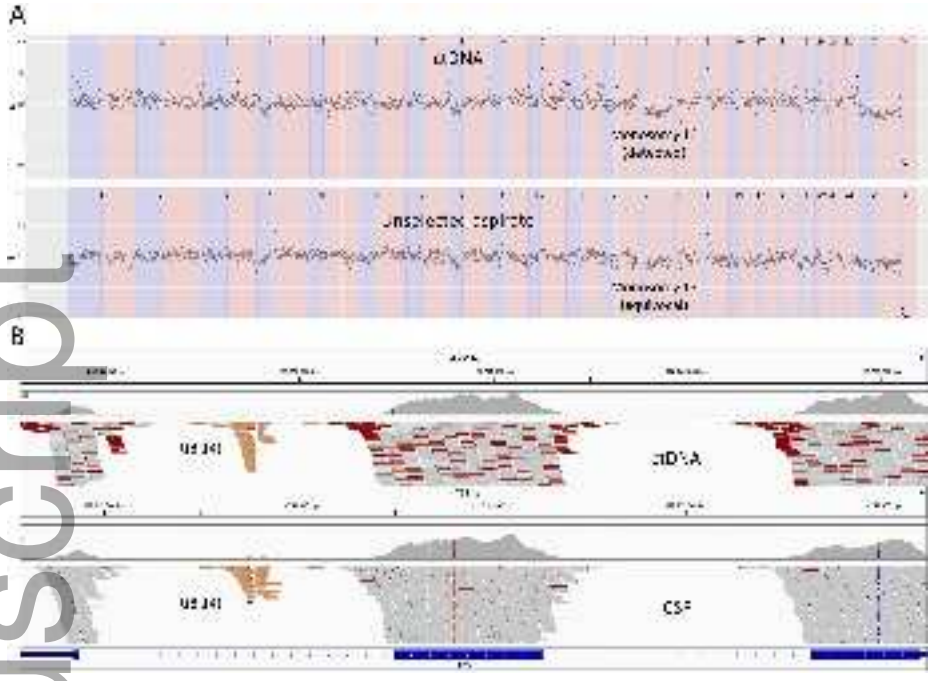
***Equivocal by PanHaem sequencing, detected in 43% of plasma cells by FISH

ID3 (NM_002167.4), *CCND3* (NM_001760.3), *MYC* (NM_002467.4), *CDKN2A* (NM_058195.3), *KRAS* (NM_033360.2), *TP53* (NM_000546.5)

CNV: copy number variation; ctDNA: circulating tumour DNA; FISH: fluorescence *in situ* hybridisation; VAF: variant allele frequency.

Figure 1 – Comparison of genomic findings from ctDNA and tumour specimens. (A) Genome-wide copy number detection comparison between ctDNA and bone marrow aspirate (51% plasma cells) from patient with multiple myeloma demonstrating monosomy 13 in both compartments (B) Integrated genome viewer visualisation of reads mapped to chromosome 8 with mates mapped to *IGH* on chromosome 14 representing t(8;14)

CSF: cerebrospinal fluid; ctDNA: circulating tumour DNA; MYC: *MYC* gene



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