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The use of cell free DNA (cfDNA) for mutational screening of multiple myeloma

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A B S T R A C T

Multiple myeloma (MM) is an incurable haematological malignancy which relies heavily on bone marrow biopsies for disease monitoring and prediction of treatment response. In recent years, liquid biopsy derived cell-free DNA (cfDNA) has emerged as alternative for invasive biopsies. This pilot study aimed to evaluate the feasibility of using cfDNA for the detection of oncogenic mutations in the mitogen-activated protein kinase (MAPK) pathway genes NRAS, KRAS, and BRAF in MM patients. Matched peripheral blood and bone marrow aspirates were collected from thirteen MM patients at various disease stages. cfDNA was isolated using the Qiagen Circulating Nucleic Acid Kit while bone marrow DNA was extracted using the Maxwell Promega platform. The presence of NRAS, KRAS, and BRAF mutations was analysed by ddPCR and compared between the cfDNA and gDNA samples. Although our data come from a small patient cohort, mutations were detected, which supports cfDNA utility for mutational screening and prognostication in MM.

Multiple myeloma (MM) is an incurable haematological malignancy with an average survival of 5 years. The gold standard in MM diagnosis is bone marrow biopsy which limits the frequency of follow-up sampling for disease monitoring, prediction of drug response and assessment of clonal evolution. In addition, the patchy distribution of myeloma cells challenges the accurate estimation of disease burden via bone marrow biopsy. Liquid biopsies, which involve the analysis of cell free DNA (cfDNA) in blood may be a non-invasive alternative as blood collection is already a standard procedure in clinical practice. There is evidence that cfDNA contains a proportion of circulating tumour DNA (ctDNA), which is more representative of cancer heterogeneity than traditional biopsies [1,2]. Gene mutation screening using cfDNA isolated from peripheral blood and other body fluids is emerging in diagnostic laboratories as a non-invasive, less painful alternative for cancer patients. This is of special interest for those cases where the tumour cannot be easily accessed such as brain tumours [3] or in haematological malignancies where bone marrow biopsies are associated with risks and discomfort [4].

The mutational landscape of MM has been well-characterised with mutations in the mitogen activated protein kinase (MAPK) pathway in oncogenes such as NRAS, KRAS and BRAF genes presenting at a frequency of 20, 36 and 4 % respectively. Importantly, these mutations are reported to be predictive of treatment response and outcome [5]. Here, we present a pilot study, testing the presence of oncogenic mutations in these genes in peripheral cfDNA compared to biopsy DNA isolated from patient matched bone marrow aspirates. Plasma was separated and cfDNA was isolated using the Qiagen Circulating Nucleic Acid Kit (Qiagen, Australia) as per manufacturers' instructions and biopsy DNA was extracted on a Maxwell Promega platform (Promega, Australia) as per manufacturers' instructions. Detection of the $NRAS^{Q61R}$, $BRAF^{V600E}$, $KRAS^{Q61H}$ variants was performed using the QX200 Droplet Digital PCR platform with in-house developed assays (Bio-Rad, Australia).

Our cohort consisted of 13 MM patients of varying stages of disease (Table 1). There were 7 newly diagnosed multiple myeloma (NDMM), 4 smouldering MM (SMM) and 2 MM patients with progressive disease according to the International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma [6]. No $BRAF^{V600E}$ or $KRAS^{Q61H}$ variants were detected in our cohort in either cfDNA or bone marrow biopsy. The $NRAS^{Q61R}$ variant was detected in both the cfDNA and biopsy DNA samples of patient 6. This patient had progressive disease, stage II R-IPSS with high risk

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Table 1
Paired Bone Marrow and Peripheral Blood samples.

Patient Id	Sex	Disease status at collection	Lytic lesions	R-IPSS*	fluorescent in situ hybridisation (FISH) characteristics	Prior Lines of therapy	gDNA isolated from bone marrow			cfDNA isolated from peripheral blood		
							BRAF	NRAS	KRAS	BRAF	NRAS	KRAS
1	F	NDMM	Yes	II	Standard	0	wt	wt	wt	wt	wt	wt
2	M	SMM	No	I	Standard	0	wt	wt	wt	wt	wt	not tested
3	M	NDMM	Yes	II	Standard	0	wt	mut	wt	wt	wt	not tested
4	F	PD	Yes	II	Standard	3	wt	wt	wt	wt	wt	wt
5	F	NDMM	Yes	II	Standard	0	wt	wt	wt	wt	wt	wt
6	M	PD	Yes	II	High	9	wt	mut	wt	wt	mut	wt
7	M	NDMM	No	I	High	0	wt	wt	wt	wt	wt	wt
8	F	SMM	No	I	High	0	wt	wt	wt	wt	wt	wt
9	M	SMM	No	I	High	0	wt	wt	wt	wt	wt	wt
10	M	NDMM	Yes	II	Standard	0	wt	wt	wt	wt	wt	wt
11	F	NDMM	Yes	III	High	0	wt	wt	wt	wt	wt	wt
12	M	NDMM	Yes	II	Standard	0	wt	wt	wt	Wt	wt	Wt
13	F	SMM	No	I	Standard	0	wt	wt	wt	wt	wt	wt

NDMM, newly diagnosed multiple myeloma; SMM, smouldering multiple myeloma; PD, progressive disease; wt: wildtype mut: mutant.
* R-IPSS Revised International Staging System for Multiple Myeloma [13].

fluorescent in situ hybridisation (FISH) aberrations and lytic bone lesions. He received 9 prior lines of treatment and survived 5 months. It is of note that this patient had no extramedullary disease at the time of sample collection. The $NRAS^{Q61R}$ variant was also detected in the bone marrow sample of patient 3 but not in the matching cfDNA. This patient was stage II R-IPSS with standard risk FISH aberrations and survived 15 months. Amongst the 7 NDMM patients, only patient 3 harboured the $NRAS^{Q61R}$ variant in his biopsy DNA.

In NDMM, mutations in the RAS and BRAF genes occur in ~ 20–50 % [7] and ~4 % [8] of cases respectively while the NRAS and KRAS mutations occur in 45–80 % of relapsed/refractory cases. The lower incidence of variants in our cohort is possibly due to the small cohort size with presence of SMM and PD patients (Table 1). Furthermore, only 3 variants of the RAS and BRAF genes were screened for in this study whereas other variants in these genes have been reported in MM [7].

Our results align with previous studies investigating the concordance between paired cfDNA and tumour samples in solid tumours [9,10]. The observed discordance in these studies, as well as ours, could be due to technical accuracy, tumour heterogeneity, varying tumour burden and stage of disease at the time of sample collection and processing. On the other hand, in a study of tumours located in the central nervous system, Bale et al. reported that cfDNA isolated from cerebrospinal fluid (CSF) was superior for mutation detection compared to biopsy DNA [11]. In MM, a previous study [12] in asymptomatic myeloma showed a weak correlation between tumour mass and ctDNA while a whole exome sequencing study demonstrated high concordance between blood and bone marrow samples.

In addition to the use of cfDNA for cancer-associated mutation analysis, several studies have highlighted another potential value of using cfDNA in prognostication and disease monitoring. The concentration of cfDNA varies between healthy individuals and between unhealthy individuals and can increase during cancer progression. Spindler et al. [14] reported colorectal cancer patients could be distinguished from healthy controls based on their respective cfDNA levels. This study also reported a shorter survival after treatment in cancer patients with high cfDNA levels compared to patients with lower cfDNA levels. In some haematological cases, cfDNA allowed the detection of biomarkers that were undetectable in DNA extracted from bone marrow samples[15], further strengthening the usefulness of cfDNA.

We acknowledge that the small sample size has resulted in a lower rate of variant detection in the genes of the MAPK pathway. However, these preliminary results support further evaluation of cfDNA for mutational screening and prognostication in MM.

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Declaration of Competing Interest

None.

References

[1] B. Pereira, C.T. Chen, L. Goyal, et al., Cell-free DNA captures tumor heterogeneity and driver alterations in rapid autopsies with pre-treated metastatic cancer, Nat. Commun. 12 (1) (2021) 3199.
[2] M. Russano, A. Napolitano, G. Ribelli, et al., Liquid biopsy and tumor heterogeneity in metastatic solid tumors: the potentiality of blood samples, J. Exp. Clin. Cancer Res. 39 (1) (2020) 95.
[3] R. Mair, F. Mouliere, Cell-free DNA technologies for the analysis of brain cancer, Br. J. Cancer 126 (3) (2022) 371–378.
[4] S. Hibbs, This Is Going to Hurt: revisiting the Patient Experience of Bone Marrow Biopsies, Hemasphere 6 (4) (2022) e710.
[5] S. Mithraprabhu, J. Reynolds, R. Turner, et al., Circulating tumour DNA analysis predicts relapse and improves risk stratification in primary refractory multiple myeloma, Blood Cancer J. 13 (1) (2023) 25.
[6] S. Kumar, B. Paiva, K.C. Anderson, et al., International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma, Lancet Oncol. 17 (8) (2016) e328–e346.
[7] S. Bezieau, M.C. Devilder, H. Avet-Loiseau, et al., High incidence of N and K-Ras activating mutations in multiple myeloma and primary plasma cell leukemia at diagnosis, Hum. Mutat. 18 (3) (2001) 212–224.
[8] E. O'Donnell, N.S. Raje, Targeting BRAF in multiple myeloma, Cancer Discov. 3 (8) (2013) 840–842.
[9] J. Grasselli, E. Elez, G. Caratu, et al., Concordance of blood- and tumor-based detection of RAS mutations to guide anti-EGFR therapy in metastatic colorectal cancer, Ann. Oncol. 28 (6) (2017) 1294–1301.
[10] P.N. Ding, T.M. Becker, V.J. Bray, et al., The predictive and prognostic significance of liquid biopsy in advanced epidermal growth factor receptor-mutated non-small cell lung cancer: a prospective study, Lung Cancer 134 (2019) 187–193.
[11] T.A. Bale, S.R. Yang, J.P. Solomon, et al., Clinical experience of cerebrospinal fluid-based liquid biopsy demonstrates superiority of cell-free DNA over cell pellet genomic DNA for molecular profiling, J. Mol. Diagn. 23 (6) (2021) 742–752.
[12] M. Manzoni, A. Pompa, S. Fabris, et al., Limits and applications of genomic analysis of circulating tumor DNA as a liquid biopsy in asymptomatic forms of multiple myeloma, Hemasphere 4 (4) (2020) e402.

[13] A. Palumbo, H. Avet-Loiseau, S. Oliva, et al., Revised international staging system for multiple myeloma: a report from international myeloma working group, J. Clin. Oncol. 33 (26) (2015) 2863–2869.
[14] K.L. Spindler, A.L. Appelt, N. Pallisgaard, R.F. Andersen, I. Brandslund, A. Jakobsen, Cell-free DNA in healthy individuals, noncancerous disease and strong prognostic value in colorectal cancer, Int. J. Cancer 135 (12) (2014) 2984–2991.
[15] N.J. Short, K.P. Patel, M. Albitar, et al., Targeted next-generation sequencing of circulating cell-free DNA vs bone marrow in patients with acute myeloid leukemia, Blood Adv. 4 (8) (2020) 1670–1677.