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Original Research

Plasma total cell-free DNA (cfDNA) is a surrogate biomarker for tumour burden and a prognostic biomarker for survival in metastatic melanoma patients

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Melanoma;
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Abstract ***Introduction:*** Tumour burden is a prognostic biomarker in metastatic melanoma. However, tumour burden is difficult to measure and there are currently no reliable surrogate biomarkers to easily and reliably determine it. The aim of this study was to assess the potential of plasma total cell free DNA as biomarker of tumour burden and prognosis in metastatic melanoma patients.

Materials and methods: A prospective biomarker cohort study for total plasma circulating cell-free DNA (cfDNA) concentration was performed in 43 metastatic melanoma patients. For 38 patients, paired blood collections and scan assessments were available before treatment and at first response evaluation. Tumour burden was calculated as the sum of volumes from three-dimensional radiological measurements of all metastatic lesions in individual patients.

Results: Baseline cfDNA concentration correlated with pre-treatment tumour burden ($\rho = 0.52$, $P < 0.001$). Baseline cfDNA levels correlated significantly with hazard of death and overall survival, and a cut off value of 89 pg/μl identified two distinct prognostic groups (HR = 2.22 for high cfDNA, $P = 0.004$). Patients with cfDNA ≥89 pg/μl had shorter OS (10.0 versus 22.7 months, $P = 0.009$; HR = 2.22 for high cfDNA, $P = 0.004$) and the significance was maintained when compared with lactic dehydrogenase (LDH) in a multivariate analysis. We also found a correlation between the changes of cfDNA and treatment-related changes in tumour burden ($\rho = 0.49$, $P = 0.002$). In addition, the ratio between baseline

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cfDNA and tumour burden was prognostic (HR = 2.7 for cfDNA/tumour volume ≥8 pg/(μl*cm^3), $P = 0.024$).

Conclusions: We have demonstrated that cfDNA is a surrogate marker of tumour burden in metastatic melanoma patients, and that it is prognostic for overall survival.

Key Message: Plasma cfDNA level correlates to tumour volume and is a surrogate biomarker for tumour burden and a prognostic marker for survival in metastatic melanoma patients.

1. Introduction

Circulating cell-free DNA (cfDNA) is short fragment (usually 130–180 base pairs) double stranded DNA that is present in blood and other body fluids [1–4]. Its origin is thought to be mainly from apoptotic or necrotic cell death, although active release mechanisms have also been suggested [5,6]. Increased levels of cfDNA in the blood are frequently observed in cancer patients and in some settings increased cfDNA is an adverse prognostic factor [7]. However, tumours are not the sole source of cfDNA, and increased levels are also linked to impaired renal clearance and production of white blood cells (WBC) [8,9]. Moreover, the mechanisms of cfDNA release are poorly understood, and their prognostic value and relationship to tumour burden are controversial [10]. In particular, the correlation between tumour volume and cfDNA is still under study [11].

One explanation for the current lack of evidence to directly correlate tumour burden and cfDNA levels is that precise evaluations of tumour burden are not routinely performed. This is because assessing tumour burden in individual patients is demanding and requires time-consuming procedures to measure all metastatic lesions. Metastatic load in melanoma is considered an important prognostic and predictive factor in melanoma and surrogate biomarkers are currently used for clinical purposes, including the number of metastatic sites and Response Evaluation Criteria in Solid Tumours (RECIST) marker lesion measurement [12,13]. However, the current version (RECIST 1.1) relies on mono-dimensional measurements of no more than 2 lesions per organ and a maximum of 5 lesions selected at the discretion of the radiologist, therefore is subject to interpretation bias as demonstrated by the significant differences often observed between investigators' and central review's assessments in clinical trials [14–16]. As a consequence, RECIST 1.1 is a poor tool with which to investigate the relationship between cfDNA and tumour burden.

One of the most powerful uses of cfDNA is related to the identification of tumour-specific mutations that are derived from the cancer cells. The analysis of this circulating tumour DNA (ctDNA) allows application of liquid biopsies for personalised strategies [17–20]. Critically however, a clear link between cfDNA or ct DNA and tumour burden has not been established, and routine analysis of ctDNA is often unfeasiblebecause it requires information on the mutational landscape of the tumour.

In the present study, we examined the relationship between cfDNA, ctDNA and tumour burden in patients with metastatic melanoma. We used computed tomography (CT) and/or magnetic resonance imaging (MRI) scans to determine the total tumour burden in the patients and then compared this to cfDNA levels. Intriguingly, we did not find a correlation between ctDNA and cfDNA, but did find a correlation between cfDNA and tumour burden. Our study shows the potential of cfDNA as biomarker of tumour burden in metastatic melanoma patients, and we show that cfDNA is a biomarker for prognosis and response to treatment.

2. Materials and methods

2.1. Patients

A prospective longitudinal biomarker cohort study was performed in collaboration between Cancer Research UK Manchester Institute and The Christie NHS Foundation Trust. Ethical approval was granted by the Manchester Cancer Research Centre (MCRC) Biobank Access Committee (Protocol number 13RIMA01). All patients gave written informed consent. Inclusion criteria were the diagnosis of metastatic melanoma, patients naïve for systemic oncological treatments or with an interval from therapy (in the adjuvant or metastatic setting) of at least 2 years, to be longitudinally followed up during treatment. Patients were studied with paired blood collections and scan assessments performed before treatment initiation and at treatment response evaluation (at 12–16 weeks).

2.2. Tumour burden and response assessment

Tumour burden estimation was performed with computed tomography (CT), magnetic resonance imaging (MRI) or positron-emission tomography coupled with CT (PET-CT) (slide thickness 3 mm). Scans were evaluated by a radiologist as per RECIST 1.1 and then independently reviewed for metastases volume analysis; Picture Archiving and Communication System