Circulating tumour DNA (ctDNA) in metastatic melanoma, a systematic review and meta-analysis

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Circulating Tumour DNA (ctDNA) in Metastatic Melanoma, a systematic review and meta-analysis.

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Abstract

Introduction: ctDNA is an emerging biomarker in melanoma. We performed a systematic review and metanalysis to explore its clinical utility as a prognostic, pharmacodynamic and predictive biomarker.

Methods: A systematic search was conducted from Jan 2015 to April 2021, of the electronic databases PubMed, Cochrane Library and Ovid MEDLINE to identify studies. Studies were restricted to those published in English within the last 5 years, evaluating ctDNA in humans in ≥10 patients. Survival data was extracted for meta-analysis using pooled Treatment Effect (TE), i.e., log hazard ratios (HR) and corresponding standard error of TE for progression-free survival (PFS) or overall survival (OS) differences in patients who were ctDNA positive or negative. PRISMA statement guidelines were followed.

Results: A meta-analysis of 19 studies grouped according to methodology of ctDNA detection, revealed a combined estimate for HR of PFS (13 studies using ddPCR methodology (N=1002) of 2.10 (95%CI 1.71-2.59) revealing a poorer prognosis when ctDNA was detected. This result was confirmed in the smaller analysis of (non-ddPCR, N=347) 5 studies; HR 2.45 (95%CI 1.29-4.63). Similar findings were found in the overall survival analysis of 9 studies (ddPCR methodology, N=841) where the combined HR was 2.78 (95% CI 2.21-3.49), and of the 5 studies (non-ddPCR methodology, N=326) where the combined HR was 2.58 (95% CI 1.74-3.84). Serial ctDNA levels on treatment showed a PD role reflecting response or resistance earlier than radiological assessment

Conclusions: ctDNA is a predictive, prognostic and PD biomarker in melanoma. Technical standardisation of assays is required before clinical adoption.

Keywords:
Melanoma, biomarker, ctDNA, meta-analysis

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Introduction

The treatment for metastatic melanoma has significantly improved in the last decade[1] with the development of effective treatment options, namely targeted therapies against the RAF-MAPK pathway as well as immune checkpoint inhibitors (ICI). Both strategies are proven to increase overall survival but with important differences in response rate, durability of response and toxicity profiles[2, 3]. Targeted therapy is more likely to result in a rapid response, however, this is often short-lived due to development of treatment resistance. Conversely, although response rates are lower and associated with slower kinetics, Immune checkpoint inhibitors are more likely to result in long term survival. Thus, the choice of appropriate first line treatment and the optimal timing of sequencing of therapies is an urgent question in clinical practice, and one for which there is no existing biomarker to guide such management decisions.

Circulating tumour DNA (ctDNA) is fragmented DNA derived from tumours that is present in plasma. It is usually a minor constituent of the plasma cell-free DNA (cfDNA) alongside DNA not of tumour origin[4]. The presence of ctDNA fragments containing specific mutations can be used in diagnosis, as a "liquid biopsy" and to dynamically assess tumour burden and response to treatment through measuring the number of copies of ctDNA fragments. Assessment of ctDNA can also be used to identify known mechanisms of de novo or acquired resistance to therapy. Several studies have shown the biomarker utility of ctDNA in a variety of tumour types[4-6], yet beyond detection of EGFR mutations in non-small cell lung cancer, its use in routine clinical practise has been limited[7]. The refinement of technical assays for ctDNA detection has lead to a greater uniformity in ctDNA assessment and whilst not standardised, comparisons across studies are now easier.

We sought to evaluate the prognostic role of ctDNA in metastatic melanoma at baseline (pre-treatment). A biomarker in this setting may be used to select first line therapy, monitor response and initiate treatment changes if resistance emerges.

Materials and Methods

The aims of this study were to examine:

1) The prognostic value of ctDNA in unresectable stage III and stage IV melanoma by performing a meta-analysis of published studies on ctDNA's prognostic value in melanoma towards progression free (PFS) and overall survival (OS).
2) Review the literature regarding the use of ctDNA as a pharmacodynamic and possible predictive marker on treatment.
3) Explore through pooled analysis where possible the prognostic effect of ctDNA
    a. in different treatment groups (any treatment, immunotherapy or targeted therapy),
    b. in comparison to other known clinical characteristics
4) Explore associations between ctDNA detection and known clinical characteristics.

Literature search

A systematic search of literature was conducted by two researchers LG and LK in April 2021, covering the period Jan 2015 to April 2021. The electronic database PubMed was initially searched, followed by the Cochrane Library and Ovid MEDLINE to identify any additional literature.

The search terms were 'melanoma' AND ("cell free dna" OR "circulating dna'' OR "circulating free dna" OR "circulating cell free dna" OR "cfdna" OR "extracellular dna" OR "circulating tumor dna" OR "ctdna"). Studies were restricted to those published within the last 5 years, published in English, evaluating ctDNA and conducted in humans in a cohort of a minimum of

10 patients. The reference lists of the included studies and relevant reviews were also screened to identify further studies. Studies were independently screened for inclusion by two researchers (LG and LK). Any differences in opinion were discussed until concordance was reached. Full texts were then retrieved and studies were read to confirm inclusion criteria.

Data was independently extracted by two reviewers (LG and LK) and any discordance discussed and resolved. This systematic review was performed and reported according to the PRISMA statement guidelines[8].

Data extraction

Survival summary of PFS and OS (hazard ratio, median PFS/OS or any additional related statistic, and number of events data) was extracted and divided into 2 groups for comparison; where ctDNA was undetectable vs where ctDNA was detectable. The definition of ctDNA detectability or threshold for ctDNA detection was accepted as defined in the original paper. If survival data was not available according to this stratification, the lead author was contacted for the raw data so that hazard ratios could be calculated and pooled for the meta-analysis.

Clinical characteristics of gender, primary tumour ulceration, ECOG, stage, site of metastases, baseline LDH, tumour mutation, treatment, line of therapy and response to therapy were extracted from each study (if described and grouped according to ctDNA detection so that the data could be extracted for pooled analysis).

Statistical Analysis

ctDNA as a prognostic factor for PFS and OS survival.

The meta-analysis was performed using pooled Treatment Effect (TE), i.e. loghazard ratios (HR) and corresponding standard error of TE (seTE) for progression-free survival (PFS) or overall survival (OS) differences in patients who were ctDNA-positive or negative. For each

study, the TE and seTE were obtained either from (a) the original publication or; (b) estimation using the medians or the additional related statistic and number of events in each patient group as suggested by Allan Hackshaw[9] or lastly by (c) fitting the Cox proportional hazards model if the raw survival data was provided. Random-effect models were used when a significant heterogeneity was spotted with $p < 0.05$ or $I^2 > 50\%$. Otherwise, fixed-effect models were employed to calculate the pooled HR. The inverse variance method was used for HR pooling (using estimate of treatment effect or TE and their standard errors (seTE)). This data was used to generate forest plots. Chi-square test was used to explore the association of ctDNA detection with known clinical or disease characteristics where data was available to perform a pooled analysis.

Funnel plots were generated to visualize potential publication bias (i.e., all studies would not lie symmetrically around our pooled HR value if there was bias). All statistical analysis was done by using package meta in R environment (R Core Team 2015)[10, 11]

Results

Search Results

A total of 75 studies were retrieved from the initial literature search and screening. Forty-two studies were excluded, leaving 33 studies to be included in this review; 23 quantified ctDNA at baseline and evaluated prognosis, 13 of which also evaluated the pharmacodynamic value of ctDNA. A further 10 studies only evaluated the pharmacodynamic value of serial ctDNA monitoring on treatment. The phases of the review process of the literature search results are shown in figure 1.

Baseline ctDNA as a prognostic marker

Of the 23 studies evaluating the prognostic value of ctDNA, 19 studies[12-30] were included in the meta-analysis. For these 19 studies, either survival data for PFS and or OS could be

extracted from the publication or raw data was obtained from investigators (E.S Gray et al 2015[19], L Keller et al 2019[17], Sundahl et al 2019[22], Marsavela et al 2020[26] and Herbreteau et al 2021[28] respectively). An additional 4 studies could only be described. The 23 studies are detailed in table 1 including the mutations targeted, technical methodology for ctDNA detection and its limits of detection and if a cut off ctDNA level was used for survival analysis [12-34]. All studies bar 4 used RECIST 1.1 for radiological assessment; CT/PET and RECIST 1.0 were used in the other studies. Treatment if received on study is also detailed; targeted or immunotherapy or other.

Two of the 4 descriptive studies showed a prognostic value of ctDNA detection; Knol et al[31] detected ctDNA in 37 patients with metastatic disease and 1 patient with relapsed stage IIIc nodal disease, while a further 9 patients did not have detectable plasma ctDNA giving a prognostic value of ctDNA detection; p=0.02 (log rank) which was replicated when ctDNA levels were categorised according to high (N=14), low (N=15) and non-detectable (N=9), p=0.1 (log rank testing). Forschner et al[34] detected an OS survival advantage in patients with undetectable ctDNA (N=6) before starting targeted therapy compared to those with detectable ctDNA (N=13); p=0.008. However, in the study by Long-Mira et al[32] of 32 patients where 11 patients had detectable ctDNA, there was no significant OS prognostic value of ctDNA detection. Tang et al[33] limited survival analysis to patients treated with targeted therapy and did not find a prognostic value to ctDNA detection with a median PFS; 8.1 vs 6.7 months p=0.38 for patients where ctDNA was undetectable compared to patients with detectable levels.

The meta-analysis of the 19 studies was performed to evaluate progression free and overall survival with studies' data grouped according to methodology of ctDNA detection; digital droplet PCR (ddPCR) (13 studies) or any other methodology (5 studies); figures 2A-B show the

results for progression free survival and figures 3A-B show the results for overall survival. The studies were stratified according to analytical method to decrease heterogeneity. The combined estimate for hazard ratio for PFS from the 13 studies (using ddPCR methodology, patient numbers N=1002) confirmed a poorer outcome in patients with detectable ctDNA; HR 2.10 (95%CI 1.71-2.59). This result was also found for the smaller analysis of (non-ddPCR, N=347) 5 studies; HR 2.15 (95%CI 1.35--3.41). There were two outlier studies in the PFS analyses; Keller et al[17] was a study of 13 patients, 77% were ctDNA positive and all 13 patients had progressed with a median PFS of 3.2 months (5.2 months in patients that were ctDNA positive and 1.4 months in ctDNA negative patients). The second outlier was the Sundahl et al[22] study which evaluated ctDNA detection before and during treatment with ICI (nivolumab) and stereotactic body irradiation therapy in 20 patients; 40% of patients had detectable ctDNA and 90% were treated in the first line setting. Median PFS was 6.6. months (or 10.2 months in ctDNA positive compared to 5.2 months in ctDNA negative patients) and median OS was 30.9 months (or 35.3 months in ctDNA positive compared to 25.5 months in ctDNA negative patients), 55% patients had progressed and 55% had died at the time of analysis.

Similar results were found in the overall survival analysis of 9 studies (using ddPCR methodology, N=841) where the combined HR was 2.78 (95% CI 2.21-3.49), and of the 5 studies (using non-ddPCR methodology, N=326) where the combined HR was 2.58 (95% CI 1.74-3.84). The Sundahl et al study[22] did not show an OS prognostic significance for ctDNA detection. All or the majority of studies fell within the predicted 95% confidence interval in the survival forest plots. This result indicates that for a future observation, it would be highly likely to be contained within the prediction intervals described. Funnel plots for the studies included in each analysis described above showed symmetrical distribution supporting the

use of the forest plots for the meta-analysis and the lack of significant bias, supplementary figures 1A-D.

The prognostic value of ctDNA (irrespective of method of ctDNA detection) for PFS and OS was examined according to treatment received. Studies were grouped into any treatment (where it was not possible to analyse subgroups according to specific treatments); PFS (N=173) combined HR 2.5 (95% CI 1.74-3.59) and OS (N=141) combined HR 2.91 (95%CI 1.76-4.81) figures 4A and B respectively, immunotherapy (single agent or combination regimens); PFS (N=410) combined HR 2.36 (95% CI 1.82-3.05) and OS (N=360) combined HR 3.09 (95% CI 2.29-4.17) shown in figures 4C and D, or targeted therapy; PFS (N=656) combined HR 2.06(95% CI 1.60-2.67) and OS (N=666) combined HR 2.39 (95%CI 1.76-3.25) shown in figures 4E and F. Overall ctDNA was prognostic irrespective of treatment. There were two outlier studies in this analysis (as described in the initial prognostic analysis above), Sundahl et al (nivolumab plus radiotherapy given to all patients) and Keller et al (where patients received either pembrolizumab or nivolumab or ipilimumab or combination ipilimumab and nivolumab).

Lastly the prognostic value for PFS and OS of ctDNA detection in relation to known prognostic clinical characteristics was examined. Where LDH could be evaluated the combined HR for PFS was 1.84 (95% CI 1.41-2.4) with the ctDNA combined HR 1.97 (95% CI 1.47-2.64) for these same studies (N=342). For OS the combined HR for LDH was HR 3.38 (95% CI 2.24-5.1) and for ctDNA the combined HR was 3.23 (95% CI 2.07-5.05) (N=224), figures 5 A and B. Evaluating the prognostic value of ECOG showed a combined HR for PFS of 1.51 (95% CI 0.95-2.41) with a combined HR for PFS of ctDNA detection of 1.87 (95% CI 1.25-2.78) (N=137). For OS the combined HR for ECOG was 4.87 (95% CI 2.35-10.07) and for ctDNA detection the HR was 3.43

(95% CI 1.87-6.28) (N=137), figures 5C and D. There was therefore an added prognostic benefit of ctDNA detection compared to ECOG and LDH.

Association between ctDNA detection and clinical characteristics.

It was possible to pool data on several known clinical characteristics from studies where the data was categorised according to ctDNA detection or not; table 2. The clinical or disease characteristics that were associated with ctDNA detection were female gender, ECOG 0, Stage IV disease, stage M1C-D substage, the presence of lung, abdomen, cutaneous or bone metastases, higher baseline LDH, BRAF mutated tumour, single agent immunotherapy and lastly first line therapy.

ctDNA as a pharmacodynamic and predictive marker

The pharmacodynamic value of ctDNA was explored in a variety of treatment settings across the 23 studies we examined. All the studies recorded baseline and more than one ctDNA measurement across time in their patient cohorts but sampling times varied widely between days (2, 7 or 15 days) to weeks (3,4,6,8,9 or 12). Baseline levels were commonly associated with tumour burden [13, 18, 20, 30, 35] and could be indicative of aggressive disease course (and biology) or metabolic tumour burden as detectable levels (the higher the level of ctDNA the more significant) were associated with a poorer course of disease despite response on therapy[15, 18]. A minimal metabolic tumour burden ≥10cm^3 measured by PET-FDG may be needed for ctDNA detection, although patients could have negative values with higher tumour disease burdens[18]. Early sampling for ctDNA detection could show a flare effect if taken within days of starting therapy; levels increased at day 2 but then decreased on targeted therapy[12]. Patients on tumour infiltrating lymphocyte therapy showed an early peak in ctDNA levels (after conditioning chemotherapy followed by the cell infusion)[36] which then cleared or persisted. No durable remissions were seen in patients with no peak or

a peak that did not zero convert (i.e. become undetectable), the decrease in levels occurred between 7-87 days. Flare in ctDNA levels was also seen with radiotherapy in combination with ICI[22]. The greatest decrease in detectable levels in studies was seen with targeted therapy[12, 13, 16, 19, 23, 34].

All the 23 studies[12-23, 25, 26, 28-30, 34-44] with serial measurements showed a pharmacodynamic role for ctDNA measurements on both targeted and immunotherapies (ICI and adoptive cell therapy); levels on treatment fell in clinically and radiologically responding patients and either increased or were stable in the absence of such a response. The association between ctDNA and radiological response was poor in patients with brain only disease or patients that developed brain disease as their sole site of progression, on treatment or during monitoring[19, 25, 29, 42, 43]. There could also be progressive disease extracranially without ctDNA level increases[13, 18] where bone disease progressed in isolation. ctDNA detection could discriminate between real and pseudoprogression on ICI; when the levels were dropping on treatment despite radiological progression[22, 37].

There was a prognostic value of serial ctDNA measurements so that patients who had undetectable ctDNA at baseline and remained negative had the best survival, those with detectable levels that then zero converted had intermediate survival and those that had detectable ctDNA at baseline and remained positive had the poorest survival outcomes.[14, 16, 20, 23, 25, 37] This stratification may be more discriminatory for PFS differences as patients that seroconverted on treatment could have similar OS to patients who remained negative for ctDNA detection throughout.[14] The level of decrease was important in outcome so that patients that zero converted from a detectable level had the best overall survival. In patients that have significant decreases (x10 fold or higher) on treatment yet still have detectable levels, outcome varied from having a similar overall survival to patients that

remained undetectable from baseline,[37] to patients having similarly poor overall survival to patients that had detectable levels throughout.[26] It was not possible to perform pooled analyses on the prognostic value of ctDNA detection during treatment as extractable data on treatment was not available in the publications and studies differed in the timings of samples on treatment and the groupings of patients according to ctDNA status compared to baseline. Prognostic significance may differ depending on line of therapy (where this was reported in the study) so that significance of ctDNA levels is greatest on first line therapy.[14, 15, 24, 26] We were able to pool raw data from Marsavela et al[26], Varaljai et al[30], and Herbreteau et al 2021[28], studies (N=296), to produce a HR for prognostic value of ctDNA detection for PFS on first-line treatment of 2.78 (95% CI 1.83-4.22) compared to a HR on second-line treatment of 1.09 (95% CI 0.58-2.02). For OS we were able to pool raw data from Marsavela et al[26] and Herbreteau et al 2021[28] (N=200) to produce a HR of 3.13 (95% CI 1.75-5.62) on first-line treatment and 1.52 (95% CI 0.61-3.82) on second-line treatment. This suggests ctDNA may have prognostic value on first and second-line treatment but it is more strongly associated with prognosis for patients on first-line treatment. When compared with the protein biomarkers LDH and S100, ctDNA provided independent prognostic value[30, 34, 43]. In patients treated with combination ICI tumour mutation burden (TMB) and ctDNA measurements had independent prognostic value; patients with high TMB and low ctDNA had better prognosis than those with low TMB and high ctDNA at baseline. A high TMB and fall of more than 50% in ctDNA on treatment at first follow up was also associated with better outcome[44].

The association of ctDNA with progression on targeted therapy or ICI could indicate resistance earlier than radiological assessment[12, 14, 38, 41, 42]. In one study, this was used to change management by adding radiotherapy to systemic therapy leading to a decrease in ctDNA

suggesting the mitigation of developing resistance[19]. The molecular mechanisms of resistance or emergence of resistance clones could be detected by the emergence of NRAS ctDNA in BRAF mutated patients[19, 43]. Interestingly some patients had plasma NRAS ctDNA detected at baseline despite the tumour being negative indicating primary resistance to targeted therapy[30].

Telomerase reverse transcriptase (TERT) promoter mutation detection was used along with BRAF and NRAS detection in four of the studies reviewed. Marczynski[27] et al detected TERT promoter mutations in 74% of patients' tumours and 5 of these 14 patients had concurrent detectable plasma levels. This was comparable to the rates of BRAF ctDNA detection (5 of 12 patients with BRAF positive tumours). Forthun[21] et al detected TERT mutations in 79% of patients' tissue and 11 of these 15 patients had concurrent detectable levels in plasma. However, serial TERT promoter ctDNA monitoring did not predict treatment response and levels were not prognostic. Conversely Varaljai [30] et al found that TERT promoter ctDNA levels were prognostic. Furthermore, levels correlated with response to therapy so that increases predicted failure of therapy in 14 of 17 patients, on average 4 months before radiological scans revealed treatment failure. However, Pederson et al[40] only detected TERT promoter ctDNA in 1/16 patients limiting its utility.

Discussion

ctDNA is an emerging biomarker in solid tumours with a wide range of potential uses[45]. It is commonly used in cancer clinical trials as a pharmacodynamic marker for experimental therapeutics, and an indicator of minimal residual disease in the early disease stage setting, thus identifying patients for further experimental therapy[46]. The role of ctDNA to guide standard of care therapy in melanoma is undefined. The choice between first line ICI or targeted therapy in melanoma patients is clinically important. Long term outcomes are

superior with ICI but a minimal baseline patient fitness is required to mitigate toxicity and enable a response as this can be delayed. Strategies to switch treatments based on early indicators of efficacy may broaden access to immunotherapy and improve long term survival. Our study shows a prognostic role in melanoma patients of ctDNA for PFS and OS at baseline before targeted and immune therapies, and also on treatment when serial analysis was performed. The prognostic value compares favourably with other defined prognostic factors further suggesting that ctDNA utility as a biomarker to aide patient management. We compared undetectable vs detectable ctDNA in our meta-analysis. However a cut off level was used in some studies (based on ROC analysis amongst other methodology[19, 26]) to determine the optimal level that stratified patients prognostic groupings. The use of an optimal level may aid selection of first line therapy if ctDNA levels are confirmed to be indicative of metabolic activity.

Studies evaluating serial measurements on treatment show a pharmacodynamic role indicating efficacy earlier than radiological assessment,[12-23, 25, 26, 28-30, 34-44] and one that can direct further management choices. The optimal sampling time point to detect an early response is unclear from all the studies reviewed. Radiological assessment is commonly done at 12 weeks of standard of care (SOC) therapy. ctDNA detection levels became predictive at weeks 4 and 8 suggesting utility to detect outcomes before 12-week radiological assessment and thereby facilitate earlier management decisions. A possible flare in ctDNA levels within days of therapy appeared to settle by 7 days so that this could be the earliest time point to measure ctDNA to guide management. An optimal ctDNA level (as discussed above) may additionally aid in guidance of treatment decisions, for example, if a standard minimum threshold ctDNA level was possible to define such that levels on first-line targeted treatment fell below this, then the chances of safely initiating a planned switch to

immunotherapy could be maximised with the intention of optimising chances of long-term survival while avoiding an early rebound in disease in the event of a delayed response. The studies to date indicate zero conversion on treatment produces the best outcome but many patients do not achieve this. Furthermore, a 10-fold decrease or higher might not in itself be sufficient to improve survival outcomes in all patients. There are a number of ongoing clinical studies evaluating planned treatment switches. The CAcTUS - Circulating Tumour DNA Guided Switch (CAcTUS) (NCT03808441) is a phase II trial evaluating the role of ctDNA in guiding a switch from targeted therapy to ICI in advanced disease. The planned switch is initiated when there is a decrease in mutant BRAF variant allele frequency level of ≥80% from baseline. The primary outcome of the study is to assess if the 80% decrease is an appropriate trigger for the switch, survival outcomes are the secondary outcomes. The Sequential Combo Immuno and Target Therapy (SECOMBIT) Study (NCT02631447) is a three-arm study of 1) targeted therapy followed by combination ICI upon progression or 2) combination ICI followed by targeted therapy on progression or lastly 3) targeted therapy for 8 weeks followed by a planned switch to combination ICI until progression when targeted therapy will be restarted and continued until progression. The EBIN trial (NCT03235245) is 2 arm randomised study comparing combination ICI until progression followed by investigators choice of therapy, to induction with targeted therapy for 12 weeks followed by combination ICI until progression followed by targeted therapy until 2nd progression. These studies will help define the predictive biomarker role of ctDNA and the clinical outcomes of a planned switch to ICI after a short induction with targeted therapy.

Measurement of TERT promoter mutational status in ctDNA may be useful in patients negative for BRAF or NRAS mutations. Detection rate in melanoma tumours ranges between 20-65% and is prognostic[47]. However, detection rate in plasma is less well documented and

may be lower than for other mutations limiting its utility as a blood borne biomarker[48]. Furthermore technical challenges in detection (due to the high levels of GC bases) exist compared to BRAF or NRAS so that ddPCR may be more effective than NGS.[27]

Mechanisms of resistance were not explored in any of the studies beyond the testing for ctDNA NRAS in BRAF mutated tumours. The use of ctDNA and mRNA to look for splice variants and additional molecular mutations of resistance will require further study and may aid non-invasive tumour monitoring to define mechanisms of resistance on targeted therapy and treatments to combat these.

The studies reviewed here used a range of analytical methodologies to quantify plasma ctDNA via mutations in BRAF and also, in some studies, NRAS, TERT and/or KIT. The most common analytical method was ddPCR using allele-specific Taqman probes, most commonly performed on the Bio-Rad QX200 system. ddPCR is considered a reproducible and transferable assay[49] and allows an absolute quantification of mutated copies using an internal standard. However only two studies[15, 28] used internal standards and there was some variability across the reviewed studies in the number of positive partitions used as the threshold for ctDNA positivity. Most other studies were qPCR based but reported limits of detection similar to ddPCR. However, ddPCR has the advantage of directly measuring the number of copies of ctDNA per ml plasma whereas qPCR results are often reported as variant allele frequencies (i.e. ctDNA/total cfDNA). Using serial ctDNA measurements as a predictive or pharmacodynamic marker will require precise measurement of ctDNA; distinguished from the variations related to tumour progression and accounting for ctDNA concentration effects so that changes in levels from a starting concentration of a few copies per ml will affect precision differently to changes in levels from a few hundred per ml.

Standardising sample collection and processing, the amount of cfDNA analysed, the analytical method, data processing and reporting and using shared reference standards would make results more comparable and accelerate clinical uptake[50]. Furthermore BRAF and NRAS ctDNA detection is limited to approximately 50% of cutaneous tumours where the primary tumours are mutated[51], the additional use of TERT ctDNA could widen the clinical utility of ctDNA but will require additional validation. The development of tumour or patient specific sequencing panels may additionally widen ctDNA utility as a biomarker or liquid biopsy[52].

In summary ctDNA is an emerging biomarker with great potential clinical use. Technical considerations and standardisations as well as validation within clinical trials are needed before it can be used routinely in clinical settings.

Figure legends

Figure 1

Consort diagram of the flow of information through the phases of the literature search results and review process according to the PRISMA statement

Figure 2

Forrest plots depicting meta-analysis results for progression free survival analysis (PFS) (TE is the parameter estimate of HR in natural log scale and seTE is the standard error of TE)

2A Meta-analysis of studies evaluating progression free survival using ddPCR methodology for ctDNA detection.

2B Meta-analysis of studies evaluating progression free survival using non-ddPCR methodology for ctDNA detection (*Santiago-Walker analysis included data from BREAK-2 Dabrafenib, BREAK-3 DTIC, BREAK-MB Cohort B and METRIC chemotherapy arms)

Figure 3

Forrest plots depicting meta-analysis results for overall survival analysis (OS) (TE is the parameter estimate of HR in natural log scale and seTE is the standard error of TE)

3A Meta-analysis of studies evaluating overall survival using ddPCR methodology for ctDNA detection

3B Meta-analysis of studies evaluating overall survival using non-ddPCR methodology for ctDNA detection (**Santiago-Walker analysis included data from the BREAK-3 Dabrafenib study alone).

Figure 4

Forrest plots depicting meta-analysis results for progression free survival (PFS) and overall survival analysis (OS) for ctDNA detection according to treatment received (TE is the parameter estimate of HR in natural log scale and seTE is the standard error of TE),

4A Meta-analysis of studies for PFS of patients at the start of any treatment (any or mixture of targeted and Immunotherapy)

4B Meta-analysis of studies for OS of patients at the start of any treatment (any or mixture of targeted and Immunotherapy)

4C Meta-analysis of studies for PFS of patients at the start of Immunotherapy

4D Meta-analysis of studies for OS of patients at the start of Immunotherapy

4E Meta-analysis of studies for PFS of patients at the start of targeted therapy, (*Santiago-Walker analysis included data from BREAK-2 Dabrafenib and BREAK-MB Cohort B)

4F Meta-analysis of studies for OS of patients at the start of targeted therapy, (**Santiago-Walker analysis included data from BREAK-3 Dabrafenib alone)

Figure 5

Forrest plots depicting meta-analysis of studies comparing the prognostic value of LDH and ctDNA at baseline for PFS and OS ((TE is the parameter estimate of HR in natural log scale and seTE is the standard error of TE),

5A Meta-analysis of studies evaluating prognostic value of LDH and ctDNA at baseline for PFS

5B Meta-analysis of studies evaluating prognostic value of LDH and ctDNA at baseline for OS

5C Meta-analysis of studies evaluating prognostic value of ECOG and ctDNA at baseline for PFS

5D Meta-analysis of studies evaluating prognostic value of ECOG and ctDNA at baseline for OS

Supplementary Figure 1

Funnel plot analysis for heterogeneity of studies included in the meta-analysis for
1A studies using ddPCR methodology evaluating progression free survival prognostic significance
1B studies using non-ddPCR methodology evaluating progression free survival prognostic significance
1C studies using ddPCR methodology evaluating overall survival prognostic significance
1D studies using non-ddPCR methodology evaluating overall survival prognostic significance

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