Plasma circulating tumor DNA in pancreatic adenocarcinoma for screening, diagnosis, prognosis, treatment and follow-up: A systematic review

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ABSTRACT:

Background:
While no biomarker is currently recommended for the management of pancreatic adnocarcinoma (PA) Circulating tumor DNA (ctDNA) seems promising but little is known on how it may help to manage our patients in the near future.

Materials and methods:
This systematic review of literature was designed to explore the current knowledge on ctDNA as a screening, diagnostic, prognostic, predictive and theranostic biomarker in the management of PA.

Results:
We retrieved 82 publications, including 62 full-text articles, 3 meta-analyses, 2 clinical trials and 1 abstract. The results were categorized into sections about screening, diagnosis; prognosis and follow-up of localized and advanced PA together with possible theranostics applications. Although its specificity is excellent, the current sensitivity of ctDNA remains a limitation especially in patients without metastatic disease. Therefore, this biomarker cannot be currently used as a screening or diagnostic tool. Increasing evidence suggests that ctDNA is a relevant candidate biomarker to assess minimal residual disease after radical surgery, but also a strong independent biomarker linked to a poor prognosis in advanced PA. Some recent data also indicates that ctDNA is an attractive biomarker for longitudinal follow-up and possibly early treatment adaptation. Its role in tumor profiling in advanced disease to decide targeted treatments remains to be explored.

Conclusion:
CtDNA appears to be a reliable prognostic tool. Though romising results have been reported, further studies are needed to define exactly how ctDNA can help physicians in the screening, diagnosis and treatment, as PA is expected to become a major cause of cancer-related deaths in the forthcoming decade.

Keywords: *circulating tumor DNA, screening, diagnosis, prognosis, treatment monitoring, pancreatic adenocarcinoma*

HIGHLIGHTS:

- Detection of ctDNA is indicative of an unfavorable prognosis in patients with localized and advanced PA.
- The low sensitivity of ctDNA limits its interest for screening and diagnosis to date.
- Early ctDNA increase during the first month of chemotherapy could reflect treatment resistance.
- CtDNA for tumor molecular profiling in daily practice remains to be explored.

INTRODUCTION:

Pancreatic adenocarcinoma (PA) is the 7th leading cause of cancer-related death worldwide, with 458 918 deaths in 2018 (GLOBOCAN estimates). Its 5-year survival rate is one of the worst in the field of oncology and is <10% for all stages taken together[1]. GLOBOCAN predicts a trend towards increased PA incidence (+77.7%, with 356 358 new cases) and mortality (+79.9%, with 345 181 deaths) from 2018 to 2040 (http://gco.iarc.fr/tomorrow/home). PA may even become the 3rd leading cause of death from cancer in the European Union for men and women after lung and colorectal cancers by 2025[3].

Medical management of PA is challenging as around 80% to 90% of patients are diagnosed at an unresectable tumor stage. Moreover, PA diagnosis requires invasive procedures and may be difficult. Medical treatment consists of chemotherapy and radiotherapy depending on tumor stage. The treatment decision is not currently made according to tumor biology, unlike for many other cancers, with the exception of rare (1% to 5%) cases of PA with a micro-satellite instable (MSI) phenotype or a *BRCA1/2* germline mutation. During the last decade, significant therapeutic advances have been made, with an increase in effective combination chemotherapeutic regimens, first in the metastatic setting and more recently in the adjuvant setting. However, patient survival remains disappointing, with a median overall survival (OS) less than one year in metastatic patients[4] and a 3-year rate of relapse-free survival below 40% in resected PA treated with adjuvant mFOLFIRINOX[5].

We still lack reliable blood biomarkers for PA management. Carbohydrate antigen 19-9 (CA 19-9) is the most used biomarker for prognosis and monitoring, but has several limitations[6]. With a consensual cutoff of 37 IU/mL, its performance does not allow its use for diagnostic purposes. The sensitivity (Se) and specificity (Sp) of CA 19-9 for diagnosis in symptomatic patients is 79-81% and 82-90% respectively, but its positive predictive value (PPV) for screening purposes in asymptomatic populations remains insufficient[7,8]. Moreover, CA 19-9 assay frequently yields false-positive results in patients with cholestasis (whereas cholestasis has other frequent benign etiologies such as cholelithiasis), diabetes mellitus (which can also be induced by PA), cirrhosis, chronic pancreatitis (CP) and other gastrointestinal cancers. False negatives also represent a serious limitation since 5-10% of the Caucasian population of the Lewis-null phenotype are always negative for CA 19-9. Furthermore, there is controversial guidelines recommendation for the management of patients with advanced disease when invasive procedures fail to provide cytological confirmation of diagnosis. Some authors suggest to treat rapidly these patients with chemotherapy in case of poor general condition (ECOG-PS2) and CA 19-9 level higher than 10 times the upper limit of normal value (in patients without cholestasis), even without pathological proof[9].

For PA prognosis, CA 19-9 level at diagnosis seems to be associated with less R0 resection for localized PA if concentration is > 150 IU/mL, and with a metastatic disease if concentration is above 1000 IU/mL. In localized disease, normal preoperative CA 19-9 and clearance of CA 19-9 after resection reflect a good prognosis with prolonged survival[10–12]. In metastatic disease, the decreasing of CA 19-9 level during chemotherapy is associated with better oncological outcomes[13].

In the era of personalized medicine for patients with cancers, the identification of tissue biomarkers for diagnosis, prognosis and treatment is crucial but require invasive procedure at baseline but also during the follow-up. In this context, the translational research in PA is currently focused on the

promising non-invasive option of plasma biomarkers, within the so-called "liquid biopsy" field, including cell-free circulating tumor DNA (ctDNA), circulating tumor cells (CTC) and circulating microRNAs. The objective of our review is to understand to what extent ctDNA can help clinicians in the screening, diagnosis, prognosis, follow-up and prediction of PA.

MATERIALS AND METHODS:

Definition of the outcome

The aim is to evaluate the current clinical potential of ctDNA in PA management.

Data sources and search strategy

This systematic review was performed in January 2020 using several sources of human studies in the English language: PubMed database, unpublished data presented at international congresses as abstracts (American Society of Clinical Oncology ASCO and European Society of Medical Oncology ESMO) and ongoing clinical trials available on EudraCT (EU), clinicaltrial.gov (USA) and anzctr.org (Australia and New Zealand). Inclusion criteria included ctDNA measurement in plasma of patients at all PA stages and its correlation with screening, diagnosis, treatment response or oncological outcomes. Articles on patients with pancreatic cancers other than adenocarcinoma were excluded.

RESULTS

We screened 295 articles for inclusion in the review: 172 in PubMed using the following MeSH in January 2020: ("liquid biopsy" OR "cell free tumor dna" OR "cell free dna" OR "circulating tumor dna") AND ("pancreas/cancer" OR "adenocarcinoma/pdac" OR "pancreas/adenocarcinoma" OR "pancreatic/tumor" OR "pdac" OR “pancreatic cancer”); 29 in EudraCT, 41 in Clinicaltrial.gov, and 53 in anzctr.org. Finally, we excluded 24 records dealing exclusively with CTC, exosomes or miRNA. Eleven studies concerned biomarkers not assayed in peripheral blood (pancreatic juice, tissue, portal-vein blood samples). Thirty-five articles involved biomarkers other than ctDNA, and 17 studies focused on tumors other than PA (including upper gastrointestinal cancers and benign tumors such as intraductal papillary mucinous neoplasms (IPMN)). Four case reports, 2 editorials, 2 trials including < 15 patients and 8 reviews were not taken into account. Eighty-two records were finally eligible and included in the present review: 3 meta-analyses, 2 published trials, 62 full-text articles on translational studies, 1 ASCO abstract, 1 recommendation and 13 ongoing ctDNA clinical trials.

We organized our results into categories according to the potential clinical role of ctDNA in PA: screening, diagnosis and differential diagnosis, prognosis and prediction of treatment response in localized and advanced disease, and finally the theranostic impact. These data are summarized in Tables 1-3 and Table 4 presents ongoing trials.

Technical considerations:

Circulating cell-free (cfDNA) is present in plasma, urine and other bodily fluids. cfDNA may be detected in patients with cancers but also increase in benign conditions such as infections or inflammatory process. For patients with cancer, a fraction of this cfDNA contains specific tumor alterations (mostly point mutations) and is called circulating tumor DNA (ctDNA) [15]. In PA, ctDNA is widely detected by codon 12 *KRAS* mutations (*KRAS*^{mut}) (G12V and G12D), which are found in more than 90% of PA tissues[16]. Less frequently, ctDNA is detected by means of other *KRAS* mutations (G12R, G12C, G12S, G12A, G13D, codons 59, 60 and 61) or mutations in other genes (mainly *TP53, CDKN2A, SMAD4, NRAS, PIK3CA, STK11* and *ERBB4*). However, other methods have been developed for the ctDNA detection based on epigenetics modifications through the

methylation profile of gene promoters. Hypermethylation of CpG islands in tumor suppressor gene promoters leads to gene silencing.

As mentioned previously, the identification of ctDNA may be difficult because its potential dilution among total cfDNA. According to the tumor stage, ctDNA can represent less than 1% of the total cfDNA (especially in earlier tumor stage) and its detection therefore requires highly sensitive techniques[17]. The most common techniques are schematically divided into targeted, such as digital droplet-based PCR (ddPCR), or non-targeted, such as Next-Generation Sequencing (NGS), with a good correlation between these 2 techniques[18,19]. NGS offers the possibility to amplify a large number of mutations through a gene panel, whereas ddPCR often limits the detection to a few mutations in parallel. The ddPCR is usually more sensitive than NGS (0.001% vs 2%), even if recent NGS-based techniques such as Base-Position Error Rate analysis have enhanced ctDNA detection[20–22].

These innovative detection techniques for the detection of ctDNA in a noninvasive way could provide rapidly informations regarding the genetic profil of cancer and also explore the torah molecular heterogeneity between different locations such as primary and metastatic site. These strategies based on ctDNA detection and analysis could have some potential clinical application, including screening, diagnosis and prediction of tumor response or prognosis.

ctDNA as a biomarker for screening and early detection (Table 1)

There is currently no consensual validated screening program in PA, even for high-risk patients (hereditary pancreatitis, family history of PA), highlighted the urgent need for a tool able to screen or detect early this tumor[23]. Nowadays, the best preventive strategy relies on reduction in PA risk factors (tobacco, alcohol and unhealthy diet)[1].

The carcinogenesis of PA has a long infra clinical period during which ctDNA detection could be used to screen for PA. It is estimated that the first mutational event initiating preneoplasic lesion (PanIN) occurs more than 10 years before the appearance of symptoms[24]. The diagnosis and treatment of PA at an early asymptomatic stage is associated with dramatically improved outcomes[4,5]. In this context, ctDNA brings several advantages of PA screening, including high reproducibility, noninvasiveness and acceptability as a simple blood test. *KRAS*^{mut} blood detection seemed to be the best candidate as *KRAS* gene alterations are early molecular events in PA carcinogenesis[25]. The high Sp (>96%) of *KRAS*^{mut} ctDNA seems to be a promising marker for PA screening in asymptomatic patients[26, 27]. However, the low Se of ctDNA has limited its use in screening and early diagnosis among asymptomatic patients, as a negative result may not exclude early PA. *KRAS*^{mut} ctDNA detection rates range between 10% and 45% for localized PA, with similar results reported with NGS and ddPCR[26–31]. Higher detection rates (45%) were reported by Allenson *et al.*[30] but were obtained at the price of lower Sp with up to 15% of false positives in healthy controls. However, the definition of ctDNA positivity according to a cutoff of the mutant allelic fraction (MAF) could allow optimal Se and Sp to be reached. Increasing plasma sample size could also improve *KRAS*^{mut} detection Se (43% with 4 mL of plasma[31] vs 35% with 2 mL using the same technique[29]). However, the best performances observed to date are 45% for Se and 99.5% for Sp, which are insufficient for screening. Besides, as *KRAS*^{mut} occurs in the oncogenesis of other cancers (colorectal[32], lung[33]), its detection is not specific to PA.

The combination of several biomarkers, detection of other mutations and different protocols have been studied in an attempt to improve the low Se of ctDNA. Combining *KRAS*^{mut} ctDNA detection with 4 protein biomarkers (CA 19-9, carcinoembryonic antigen, hepatocyte growth factor and osteopontin) increased Se for early PA detection to 64%[27], but other target mutations (e.g.*TP53*) did not. Adding NGS-base gene panel for *SMAD4*, *CDKN2A* and *TP53* mutations to *KRAS*^{mut} led to a higher ctDNA detection in PA[34]. Finally, methylation-based biomarkers such as promoter methylation of *ADAMTS1* and *BNC1* were respectively associated with an Se of 87% and 64%, and a comparable Sp of around 94% in detection of resectable PA[35]. While the combination with CA 19-9 did not improve Se, combining *ADAMTS1* and *BNC1* methylation status led to promising

performances (Se 94.8%, Sp 91.6%). These promising strategies of combined biomarkers to improve ctDNA-based screening accuracy have now to be confirmed in larger series and compared to find the ideal strategy to move forward to screening programs.

ctDNA as a diagnostic tool (Table 1)

PA diagnosis remains invasive as histological samples are collected by surgical resection, endoscopic ultrasound-guided fine needle aspiration/biopsy (EUS-FNA/B) or biopsy of accessible metastases. These procedures are linked to the risks of tumor dissemination, acute pancreatitis and bleeding which may delay the treatment and therefore impact the clinical outcomes[36]. Moreover, EUS-FNA remains inconclusive in approximately 20% of cases[37]. PA is responsible for desmoplastic reaction and most pancreatic material after EUS-FNA is composed of stromal cells that contribute to these false-negative results.

In this context , liquid biopsy for diagnosis using ctDNA has been evaluated in a recent meta-analysis of 369 patients from 14 studies. ctDNA detection was correlated with molecular genetic alterations of PA tissue samples (from EUS-FNA or surgical resection). Patients without PA (healthy volunteers, chronic pancreatitis, IPMN) were excluded. Overall Se and Sp were 70% and 86%, respectively. Considering the 11 studies of this meta-analysis evaluating *KRAS*^{mut} only, Se was lower (65%) but Sp was higher (91%) than with NGS gene panel methods. Here again, a better Se is needed to move to ctDNA as a routine practice tool for PA diagnosis.

Factors influencing ctDNA diagnostic performances. ctDNA Se and Sp for PA diagnosis have been related to tumor stage. ctDNA detection has better Se in advanced cancers than in early-stage cancers, possibly due to higher tumor burden in the bloodstream. Among 410 malignancies including 155 PA, Bettegowda *et al.*[39] detected ctDNA in 48% and 75% of patients with localized and metastatic cancers, respectively. ctDNA detection rate and concentration similarly increased from stages I to IV. Studies focusing on PA confirmed this trend[19,26,27,40] with a *KRAS*^{mut} ctDNA detection rate of 10%, 17.5% and 33% for resectable, locally advanced and metastatic tumor stages, respectively[26]. The mean number of genetic alterations was higher in advanced than in resectable PA[41]. A high *KRAS*^{mut} ctDNA detection rate was correlated with undifferentiated tumor [19].

Beyond ctDNA detection, its quantification was also correlated with tumor stage[42–44]. Plasma *KRAS*^{mut} concentrations and mutant allele fractions (MAF, proportion of mutated alleles among all alleles of a gene) were higher in metastatic than in locally advanced[18] and resectable PA[41,44,45]. The *KRAS*^{mut} MAF also increased with tumor size (sum of lesions' maximal diameters)[42] and 3D-measured tumor volume[18].

Interestingly, ctDNA detection rates differed according to the location of metastases as reported in other cancers, such as colorectal cancer in the RASANC study[46]. *KRAS*^{mut} ctDNA MAF were significantly higher in case of liver metastases compared to lung and peritoneal metastases[18], and for "liver or lung" compared to "peritoneal metastases" in another study[47]. Furthermore, the capacity of ctDNA detection may be affected by previous anti-cancer treatments. In resectable PA, neoadjuvant chemotherapy was associated with a lower preoperative ctDNA detection rates than chemo-naïve resectable PA (21% vs 69%)[48]. Another study described similarly low ctDNA detection rate (29%) in patients with advanced PA previously treated by chemotherapy[49]. To our knowledge, there is no study evaluating the impact of radiotherapy on ctDNA detection rate.

How to improve ctDNA performance. ctDNA false-positive results for healthy patients would have disastrous psychological, medical and economic consequences, and conversely, ctDNA false-negative results would mean that patients will not benefit from adequate treatment in due time. In this context, some strategies have been developed in order to increase the ctDNA performance for diagnosis by using (i) combination markers, (ii) methylated marker and/or (ii) new advanced techniques. Some studies have shown that adding a cancer-associated target genes (including notably *TP53*, *CDKN2A, ROS1* or *SMAD4*) to *KRAS*^{mut} detection was associated with a better Se for

detection of ctDNA for the diagnosis of patients with PA[51-52]. Other techniques such as ultrasensitive assays (able to detect degraded DNA with lower molecular weight assays and/or combining PCR and NGS) provided high detection rates (86-94%)[43,53,54]. The main limitation consisted of a 30% false-positive rate in healthy controls[53].

ctDNA in differential diagnosis with benign pancreatic diseases

When *KRAS*^{mut} ctDNA positivity provided high Sp to distinguish PA from healthy controls, it was not reliably accurate in differentiating benign pancreatic disease (pancreatitis, pancreatic cysts, benign tumors) from PA. Up to 20% of chronic pancreatitis (CP)[55,56], 8% of benign pancreatic tumors[26], 16% of pancreatic cysts and 17% of non-neoplastic pancreatic masses[42] had detectable *KRAS*^{mut} ctDNA. Among undetermined solid pancreatic tumors referred for EUS-FNA, CA 19-9 had even better Se (79% vs 65%) and Sp (93% vs 75%) than *KRAS*^{mut} ctDNA for PA diagnosis[57].

Quantitative ctDNA assessments combining biomarkers and methylation techniques enhanced the modest Sp of qualitative analysis of ctDNA and discriminated benign from malignant pancreatic diseases. ctDNA concentrations were lower in side-branch IPMN (without worrying features) and CP than in PA[58]. *KRAS*^{mut} MAF was also lower in benign pancreatic tumors (pseudocyst, IPMN, serous and mucinous cystadenoma)[45] and in CP[26] than in PA. Combining other biomarkers with ctDNA was more controversial. Cumulating high levels for at least 2 biomarkers (between CA 19-9, ctDNA and CTC) led to better Sp for patients with cholestasis (100% vs 86% for patients without cholestasis)[57], while combining ctDNA (> 16 ng/mL) with CA 19-9 and thrombospondin-2 increased Se for early-stage PA detection from 70% to 87%, and Sp from xx to 92% in differentiating PA from CP and IPMN[58]. Nevertheless, the combination of *KRAS*^{mut} ctDNA and CA 19-9 did not perform better than CA 19-9 alone[26,45.] Methylation biomarkers gave interesting results and were able to distinguish PA from benign pancreatic disorders, but not from healthy controls[50]. The number (8 vs 5) of hypermethylated genes and their integration into a clinical and laboratory diagnostic model (AUC=0.86, Se 76%, Sp 83%) reliably differentiated PA from benign pancreatic diseases (chronic and acute pancreatitis, cystadenomas) independently of the stage of PA (unlike ctDNA alone)[59]. Plasma methylation profile of 14 gene promoters significantly differed between CP and PA (Se 91.2%, Sp 90.8%, $p<0.01$)[60].

ctDNA use in patients with resectable PA (Table 2)

Beyond ctDNA screening and diagnostic use, which is currently not recommended in clinical practice because of insufficient accuracy, the prognostic value of ctDNA before and after surgery, but also before and after (neo)adjuvant treatments, has been studied in resectable PA.

Preoperative ctDNA detection is a strong negative prognostic biomarker in resectable PA. Preoperative ctDNA positivity was associated with poorer OS (HR=2.3, 95% CI: 1.1-4.6), and with a non-significant trend to higher recurrence risk (HR=1.96, 95% CI: 0.65-5.9) in a meta-analysis including 375 patents with resectable PA from 5 retrospective studies[61], which is consistent with a prospective study involving 39 resected patients[62].

Postoperative ctDNA was clinically relevant as a surrogate for "minimal residual disease" (MRD) in PA, as described in hematologic malignancies. If ctDNA levels fall after surgery[48], immediate postoperative ctDNA *KRAS*^{mut} positivity has been associated to poorer recurrence-free survival (RFS) and OS[19,48,63], which is consistent with a recent meta-analysis (OS: HR=3.7, 95% CI: 1.45-9.3, and RFS: HR=2.2, 95% CI: 0.99-4.9)[61]. The shift from preoperative *KRAS*^{mut} negativity to postoperative *KRAS*^{mut} positivity assessed by ctDNA was also an important independent poor prognostic factor for OS (HR=9.4; 95% CI: 2–44; $p=0.004$) in another study with 45 patients[64].

Postoperative ctDNA monitoring could also help clinicians to detect and treat early PA relapse. Prospective postoperative emergence of *KRAS*^{mut} ctDNA during monitoring (including at least 3 serial liquid biopsies) significantly predicted worse OS (HR=54, 95% CI: 6.6-447, p<0.001) in 39 cases of PA[62]. Moreover, ctDNA follow-up predicted relapse 84 days (Se 90%, Sp 88%)[48] to 6.5 months[31] earlier than CT imaging. However, it is at present unknown if this early detection could increase OS through early treatment approaches. The clinical utility of ctDNA to guide our treatment strategies needs to be clarified as data are currently scarce. In one study, *KRAS* MAF variation after neoadjuvant therapy did not help clinicians to indicate surgery[42]. Adjuvant treatment with doublet chemotherapy seemed better than gemcitabine alone in a postoperative ctDNA-positive cohort of 13 patients[63]. Another study (DYNAMIC-Pancreas) is evaluating adjuvant strategies according to ctDNA testing. Resected patients are randomized after surgery to a biomarker-driven arm (de-escalation treatment if "ctDNA-negative" and escalation chemotherapy with FOLFIRINOX or gemcitabine Nab-paclitaxel if "ctDNA-positive"), or to a standard-of-care adjuvant chemotherapy. However, the impressive results of adjuvant FOLFIRINOX published last year seriously compromised the DYNAMIC-Pancreas relevance.

ctDNA use in advanced PA

Baseline ctDNA as a prognostic biomarker in advanced PA (Table 3)

The prognostic role of ctDNA in locally advanced and metastatic PA has also been largely studied. Pre-chemotherapy *KRAS*^{mut} detection clearly predicted worse progression-free survival (PFS) and 2- to 4-fold worse OS compared to ctDNA-negative patients in all studies focusing on advanced PA[18,19,65,66], which is consistent with prospective phase I/II studies results in advanced PA[68,69] and with a meta-analysis of 18 articles (PFS: HR=2.31 [1.47, 3.64], OS: HR=2.57 [1.95, 3.38]) with 1243 patients (also including non-metastatic patients)[67]. Among *KRAS* mutations, G12V *KRAS*^{mut} seemed particularly associated with poorer OS compared to the other *KRAS*^{mut} (HR=2.62 [1.32, 5.20])[40,55,67,71]. Cumulating plasma *KRAS* copy number gain and *KRAS*^{mut} predicted worse outcomes compared to *KRAS*^{mut} only[72]. Studies testing ctDNA detection as a prognostic biomarker in PA are summarized in Table 3.

KRAS^{mut} ctDNA quantitative detection approaches are also related to prognosis in advanced PA[73], but are still subject to controversy, unlike *KRAS*^{mut} ctDNA qualitative detection. OS significantly decreased in patients with the highest *KRAS*^{mut} MAF[19,72], *KRAS*^{mut} concentrations[44], and ctDNA levels (including other mutations such as *TP53*)[41]. Combined with CT scan tumor volume, ctDNA MAF has also been reported to be an independant predictor for OS [18]. In contrast, other studies didn't find any correlation between *KRAS*^{mut} median MAF [42], and *KRAS*^{mut} fraction abundance[44] with PFS and/or OS in metastatic patients.

Biomarkers other than *KRAS*^{mut} have also been described as unfavorable prognostic biomarkers in advanced PA, such as total cell-free plasma DNA levels[74], *ERBB2* exon 17 mutation[71], and methylated ctDNA (less expensive and providing more rapid results than NGS)[75–77].

ctDNA as an early predictive marker for treatment response in advanced PA

ctDNA is a consensual prognostic biomarker, but is also a promising treatment monitoring tool, even if the literature provides less evidence for PA than for other tumors.
On the top of baseline ctDNA prognostic value, the issue for unresectable patients consists of early adaptation of toxic and futile systemic treatment without waiting 1 or 2 months for confirmation of

radiologic progression. Moreover, interpretation of imaging can be difficult after administration of chemotherapy, as CT scans cannot easily differentiate viable carcinoma and fibrosis. In patients with a discordant response (mixed response and progression in different tumor lesions, or progression on CT contrasting with a clinical and biological response or conversely), ctDNA could help to better identify a real efficacy (or not) to treatment.
In order to overcome CT-scan follow-up limits, concomitant *KRAS*^{mut} ctDNA was evaluated every 8 weeks. ctDNA detection rate reliably coincided with radiological treatment response for 10 of 13 metastatic patients in a first study[71]. The emergence of ctDNA positivity predicted earlier progression under treatment[71]. Similar results for PFS and OS were found in a prospective study of 39 unresectable patients[62]. Conversely, *KRAS*^{mut} ctDNA clearance during chemotherapy provided better PFS than remaining positive *KRAS*^{mut} ctDNA[47]. Nevertheless, ctDNA monitoring failed to predict disease progression in another study[42].
Early *KRAS*^{mut} ctDNA level variations were also associated with tumor response to chemotherapy in patients with advanced disease. An increase of *KRAS*^{mut} ctDNA between baseline and day 14 was significantly associated with worse PFS and OS [78] and was able to detect progressive disease with an Se of 83% and an Sp of 100%[79]. Another study[70] found significant association between *KRAS*^{mut} ctDNA slope (ΔctDNA/Δtime) and OS with a sharp and deep decline correlated with longer OS. In contrast, the kinetics of other protein-based tumor markers (CA 19-9, CEA and CYFRA 21-1) had no predictive value for treatment response[79].
Mutations other than *KRAS* have finally been tested in 2 studies and seem to reliably reflect chemotherapeutic efficacy. In a study of 15 patients[52], *KRAS*^{mut} allele frequency was significantly higher in progressive patients but also for other target genes. Combined *KRAS* and *TP53* MAF levels also significantly decreased during treatment and increased at progression, contrary to CA 19-9 analyses[51].

These results could in the future open the way to the early adaptation of chemotherapy in patients with early ctDNA increase. However, if the interest of ctDNA for treatment monitoring seems thus promising, it needs further validation in large prospective cohorts to be used in clinical practice.

ctDNA as a promising tool for personalized treatment
Beyond prognostic and treatment monitoring concerns, ctDNA may also be interesting for molecular screening of specific subtypes of PA in the future. ctDNA can indeed be complementary to tissue biomarkers in exploring PA genetic profiling in order to screen actionable molecular alterations for targeted treatments. Luchini *et al.*[38] in their meta-analysis reported 32% concordance between all the mutations detected with NGS multi-gene panels in tissue specimens vs blood samples. In this work, 38% of mutations were detected in tissue specimens only, while 30% were detected in liquid biopsy only. These results can probably be explained by intratumor heterogeneity and by the emergence of different clones in the primary tumor and the metastases. Another study even concluded that ctDNA was more representative of tumor molecular heterogeneity than tissue specimens in colon and pancreatic cancers[80]. Indeed, 78% of mutations detected in ctDNA were not detected in primary tumor samples, and new mutations were detected in blood compared to previous ctDNA when the tumor progressed.

Actionable molecular alterations have already been described in PA tissue with current clinical therapeutic applications. For example, approximately 1% of PA have mismatch repair deficiency (dMMR, MSI) and could be eligible for checkpoint inhibitor trials[81,82]. Four to 7% of PA harbor tissue germline *BRCA*1/2 mutation and may benefit from PARP inhibitor treatment[83]. More recently, tissue *NRG1* gene fusions were described in up to 6% of PA, which are targetable by afatinib with promising clinical outcomes [84]. Plasma MSI and *BRCA2* mutation detection are feasible and were respectively reported to be 50% in resected[85] and 12% in metastatic PA[71], when no *NRG1* ctDNA assessment seems available to date. Other tumor tissue targetable biomarkers have also been

identified in plasma in up to 73% of PA such as *ATM*, *PALB2* (indicating sensitivity to PARP inhibitors or platinum agents), *CCND2, CDK4* and *CDK6* (CDK inhibitors), *AKT1, AKT2, ARID1A, PIK3CA PIK3CG* (PI3K/mTOR inhibitors) and tyrosine kinase receptors *AXL, EGFR, FGFR1, FLT3* and *PDGFRA* (tyrosine kinase inhibitors)[41,49].
Hence, ctDNA therapeutic screening seems feasible in clinical practice and could in the future represent a promising way to screen for targeted therapies, as obtaining sufficient tissue samples remains challenging in many patients. Nevertheless, ctDNA has currently no therapeutic application in PA because of the lack of consensual optimal technique, high costs and lack of prospective clinical trials evaluating the allocation of targeted therapies according to plasma mutation detection.

Conclusion:

ctDNA detection appears already to be a strong unfavorable prognostic biomarker of RFS/PFS and OS in resected and advanced PA. The high specificity of ctDNA could provide significant help in PA diagnosis if ctDNA is detected in advanced disease patients, to avoid repeated invasive procedures and treatment delay, but its low sensitivity prevents for now its screening and diagnostic use in clinical practice.
Developing ultrasensitive techniques, detecting other mutations, methylation-specific profiles, or combination with other plasma biomarkers could enhance the diagnostic and screening performances of ctDNA in the future.
ctDNA could also be combined with other liquid biopsy techniques such as exoDNA (additional source of plasma DNA in the form of microvesicles) to improve their complementary diagnostic and monitoring performances[30,42].
Early ctDNA increase during chemotherapy seems to be relevant to assess early treatment efficacy, but needs further validation as studies were conducted in a very limited number of patients.
Finally, less invasive procedures could even be developed in the future, as Terasawa *et al.*[86] recently found similar *KRAS*^{mut} detection rates in plasma and urine.
To conclude, ctDNA is a promising biomarker for PA management. In the forthcoming years we will have to agree on consensual standardized protocols for the techniques used, the biomarker or combination chosen, and to validate them prospectively on large patients series. This, together with dedicated prospective trials accurately designed for each clinical situation, is the only way to potentially allow the use of ctDNA for daily practice PA patients' management in the future.

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