Practices and expectations on the use of circulating tumor DNA in colorectal cancer patients: A bi-national AGEO/AIOM/GERCOR/FFCD/FRENCH survey

Practices and expectations on the use of circulating tumor DNA in colorectal cancer patients: a bi-national AGEO/AIOM/GERCOR/FFCD/FRENCH survey.

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Abstract

Background: Increasing evidence showed that circulating tumor DNA (ctDNA) could be a promising tool in providing molecular, prognostic, predictive and dynamic information in colorectal cancer (CRC) patients. The present study aimed to provide a picture of knowledge, practice, attitudes and expectations about ctDNA in CRC patients.

Material and Methods: An online survey was distributed from November 2019 to January 2020 to French and Italian cooperative and scientific groups of Hepatogastro-enterologists (HGE), Medical Oncologists (MO), Radiotherapists (RT) and Digestive Surgeons (DS).

Results: 307 physicians completed the survey (57% Italian; 43% French). Most of them were MO (62%) and HGE (24%). They worked in University Hospital (48%), Cancer Centre (21%), General Hospital (21%) and Private Hospital (10%). Notably, half had access to ctDNA in their daily practice. Of them, 53% used ctDNA to assess RAS/BRAF status only, 46% RAS/BRAF with other mutations and 1% only other mutations.
MO and HGE identified quick RAS profiling (P=0.031) as the main interest of ctDNA. University Hospitals and Cancer Centres prescribed more ctDNA (P<0.001) and more often in their centre (P<0.001). The main future expectations concerning ctDNA use were: to guide therapeutic strategies in the metastatic (78%), and adjuvant (73%) settings, and to better/quicker profile disease at baseline (56%).

Conclusion: Half of participants could perform ctDNA in their daily practice. Molecular profiling of metastatic patients remains the main goal of ctDNA use to guide initial treatment or rechallenge. Therapeutic strategies based on ctDNA are an expectation for the future for both adjuvant and metastatic settings, but how to use it routinely remains to be defined.

1. Introduction

Colorectal cancer (CRC) represents the third leading cause of cancer worldwide. Despite recent breakthroughs gained in anticancer treatments, it remains the fourth leading cause of cancer-related death [1].

Current approaches to choose treatment regimens for CRC patients are primarily based on TNM staging and pathological assessment. In fact, tumor biopsy, capturing morphological variations in neoplastic tissues, is still recognized as the gold standard to get diagnostic, prognostic and predictive information in cancer patients [2].

However, during the last 15 years, growing data have established the pivotal role played by molecular biology and tumor heterogeneity, that have been considered as an important hallmarks of cancer prognosis and treatment [3]. Patients with the same TNM stage can show different clinical outcomes depending on their tumor molecular profile, reflecting the molecular heterogeneity and dynamicity of tumor [4,5]. Tissue biopsy, a static technique, spatially limited and hardly repeatable, is not able to capture molecular heterogeneity, clonal evolution and secondary resistances induced by anticancer treatments [6]. Moreover, it is an invasive procedure with several disadvantages related to hospitalizations, complications (eg. bleeding, organ perforation), not always accessible lesions or possibility to obtain adequate samples for molecular profiling in some cases [6].

Developing diagnostic, prognostic, and predictive molecular tools represent an urgent unmet need, greatly important to determine the optimal treatment strategy for each individual patient.

Liquid biopsy, based on circulating cancer-derived molecules sequencing, such as circulating tumor DNA (ctDNA), has earned attraction for its potential clinical utility in detecting early onset of cancer, minimal residual disease, molecular profiling together with treatment resistance[7–9].

In fact, because ctDNA can reflect the entire tumor genome, it could provide an accurate representation of disease biology and may be able to predict prognosis and treatment efficacy [10]. Since it is an easy blood sample collection, liquid biopsy is a minimally invasive, cost-effective and easily repeatable detection method that allows initial and dynamic assessment of each tumor genomic profile [11–13].

However, despite these interesting results, the dosage of ctDNA to guide our therapeutic decisions, whether in adjuvant or metastatic setting, is not yet validated by randomized phase III trials in CRC.

Therefore, the ctDNA use is still not widespread in clinical practice and is still very heterogeneous among experts and centers. Moreover, little is known about the specialists' expectations concerning this new biological tool. We therefore performed a survey to gain insights into the current landscape about ctDNA use in CRC patients. The present study aimed to provide a representative picture of the status of knowledge, practice, attitudes and expectations about ctDNA in CRC.

2. Material and Methods

2.1 Setting and Participants

The present study was carried out using an online survey addressed to national French and Italian cooperative groups of Hepato-gastroenterologists, Oncologists, Radiotherapists and Digestive Surgeons: GERCOR (Groupe Coopérateur Multidisciplinaire en Oncologie), AGEO (Association des gastro-

entérologues-oncologues), FFCD (Fédération Francophone de Cancérologie Digestive) and AIOM (Associazione Italiana di Oncologia Medica).

2.2 Survey Design and Procedures

The survey was sent to medical doctors working in participating groups between November 2019 and January 2020. Responses were collected in February 2020. Project staff emailed the groups leads instructions on questionnaire outreach, text about the survey and the survey hyperlink. It was performed in electronic form using the online platform, *SurveyMonkey*™. Three reminder emails were delivered approximately every 3 weeks after the initial outreach to encourage responses.

2.3 Questionnaire development

Based on the experience of the research team and the review of recent evidence, a 2-minutes survey was developed to allow a high participation rate. The survey was piloted at local center of Georges Pompidou European Hospital to test comprehension and length.

The questionnaire contained 15 questions, covering attitudes, current use and expectations about ctDNA use.

In particular, the first part of the survey had assessed demographic and professional characteristics of each survey recipients. The second part gathered experts' opinions about the role and the use of ctDNA in the current clinical practice for CRC patients. In the third part, based on the on-field experience, respondents were asked to describe their expectations about the use of ctDNA in the future and to report their possibility of accessing routine or exceptionally to molecular tumor board. It was allowed to skip questions that were judged not applicable. All responses were reported individually and anonymously.

2.4 Statistical analysis

Responders' characteristics were summarized through descriptive analysis. Categorical variables were described through frequency distribution. Differences across groups were compared through the Chi-square test, as appropriate. A value of $p < 0.05$ was considered statistically significant.

Statistical analysis was performed with STATA (StataCorp. (2015) Stata Statistical Software: Release 14.2. College Station, TX: StataCorp LP).

3. Results

3.1 Demographic and professional characteristics

A total of 307 responses were collected by survey recipients. Characteristics of respondents are displayed in Table 1. Approximately 57% were Italian (IT, 175) and 43% French (FR, 132). Most of them were Medical Oncologists (MO, 62%) and Hepato-gastro-enterologists (HE, 24%), while Digestive Surgeons (DS) and Radiotherapist (RT) were 10% and 4%, respectively. Of note, 53% visited more than 20 CRC per month. Moreover, approximately 48% worked primarily in University Hospital (UH), 21% in Cancer centre (CC), 21% in General Hospital (GH) and 10% in Private Hospital (PH).

3.2 Physicians' knowledge about ctDNA

Responses about expert's opinions on ctDNA are shown in table 2. As first answers, respondents were asked if they knew ctDNA and if they thought that ctDNA can help in daily practice in the future. Almost all respondents (98% and 99%, respectively) declared to know ctDNA and to consider it potentially useful for future clinical practice. Interestingly, 87% (268/307) of respondents considered interesting the use of ctDNA to detect residual disease in localized tumors, 80% (245/307) to detect secondary resistant mutations during targeted treatments and 63% (193/307) to discuss an anti-EGFR re-challenge. The majority of respondents also considered ctDNA use interesting to determine RAS status (60%), BRAF status (57%) and to bring prognostic information in the metastatic setting (52%).

3.3 Current applications of ctDNA use

Responses about expert's current use of ctDNA in daily practice are shown in table 2. Notably, 48% had access to ctDNA in their daily practice. Of them, 53% (26% of the whole cohort) used currently ctDNA to assess RAS/BRAF status only, 46% (22% of the entire population) RAS/BRAF with other mutations and 1% (0.6% of the all population) only other mutations. Most of them (39% of all population) were able to perform the analysis in their own centre (Table 2).

Interestingly, ctDNA was also used to evaluate the evolution of the mutational state during treatment to guide it (58%), for diseases in which it is difficult to obtain a biopsy to determine RAS/BRAF status (51%), followed by the analysis of the molecular profile when tumor tissue is not available in the treating centre (38%) and for prognostic assessment (29%). Notably, about 56% of French respondents had access to a molecular tumor board in their centre and 59% of them (33% of the entire population) can use it routinely (Table 3 and 4). The molecular tumor board question was not asked to Italian centers.

3.4 Future expectations

Responses about the main advantage and expectations of evaluating ctDNA are shown in table 2. Main advantages about ctDNA use for clinicians concerned the monitoring of the evolution of the tumor molecular profile (48%) and avoiding invasive biopsies (34%). Faster times to analyse RAS/BRAF status (13%), to avoid tumor sample requests from external centres (3%) and other, were considered less advantageous by physicians (Table 2). Furthermore, main expectations concerning ctDNA use were to guide therapeutic strategies by monitoring molecular profile (78%), to guide adjuvant therapy in non metastatic patients (73%) and to profile disease at baseline (56%). Fewer concerned prognostic (36%), diagnostic (21%), and screening aspects (27%).

3.5 Responses according to characteristics of physicians, centre and country

All the responses were compared according to characteristics of physicians, centre and country, in order to identify differences in the uses and expectations about ctDNA (Table 3). About current applications, Medical Oncologists and Hepato-gastro-enterologists find more interesting the ctDNA use to discuss about a re-challenge with anti-EGFR (P=0.031, HE 75.34%; MO 62.10%; DS 45.16%; RT 45.45%) compared to RT and DS, who, conversely considered more interesting its use for prognostic information in the metastatic setting (P=0.005, HE 53.42%; MO 45.78%; DS 80.64%; RT 54.54%; O 100.00%) (Table 3, Figure 1). The current ctDNA use among specialists are showed in Figure 2.

Noteworthy, University Hospital and Cancer Centre had more possibility to perform ctDNA (P<0.001, PH 19.23%; GH 29.68%; UH 51.36%; CC 54.54%) in their centre (P<0.001, CC 85.71%; UH 79.56%; GH 19.04%; PH 14.28%) than General and Private Hospitals. Intriguingly. University Hospital had more frequently access to a molecular tumor board (P<0.001, UH 66.17%; PH 36.36%; GH 26.92%; CC 8,75%) together with General Hospital and Private Hospital than Cancer Centre.

About the differences according to the country, compared to Italian, French physicians felt more interesting the ctDNA use to determine baseline RAS (P=0.001, FR=70.45%; IT=51.42%) and BRAF status (P=0.012, FR=65.15%; IT=50.85%), and before anti-EGFR re-challenge (p<0.001, 74.2 vs 54.3%) using it more frequently for molecular profiling in patients without biopsy (P=0.016, 65.3 vs 44%). Conversely, Italian doctors had a broader access to ctDNA (P=0.023, 48.6 vs 35.6%); currently using it more frequently for the detection of secondary resistance mutations (P=0.042, 54 vs 46%) and for prognostic purposes (P<0.001, 39 vs 5%), than French. No differences were observed about the future expectations among physicians and countries.

4. Discussion

Liquid biopsy is a reliable and non-invasive recently developed technique capable of detecting cancer-derived biomolecules, including DNA, vesicles, RNA and circulating tumor cells. This tool has the advantage of providing a real-time assessment of cancer heterogeneity and clonal evolution by the means of an ultrasensitive technology. The possible applications foreseen for ctDNA include early detection of cancer, determination of prognosis, progression and response to anti-cancer treatment [14–18].

Its use in oncology has become increasingly widespread over the last years. To date, ctDNA-based liquid biopsy for *EGFR* mutation testing (65.7% sensitivity and 99.8% specificity) is approved in routine clinical practice for patients with non-small-cell lung cancer [19].

Considering patients with CRC, Wang et al. have already reported, in 2004, that the detection of mutation of *APC*, *KRAS* and *TP53* in serum samples of CRC were associated to more frequent relapses after surgery [20].

Other recent data reported the utility of liquid biopsy in ctDNA quantification for post-operative surveillance, demonstrating that CRC patients had increased levels of ctDNA compared to healthy controls; the patients that demonstrated detectable ctDNA levels during follow-up also experienced more early recurrences. Moreover, it was shown that ctDNA correlates well with diseases burden [21,22].

Even though the use of ctDNA represents an evidence-based practice for lung cancer, this technique is still under evaluation for other tumor types such as CRC. Indeed, though some large prospective studies have shown the feasibility and reliability of ctDNA in the molecular characterization and monitoring of CRC (early stage as well as metastatic disease), no clear approval and reimbursement have been set up in this setting in France or Italy limiting ctDNA use in daily practice in many centers [12,15,21,23,24].

To best of our knowledge, this is the first study assessing experts' knowledge, practice, attitudes and expectations about ctDNA in CRC patients.

In our study, approximately half of the participants had access to ctDNA testing in their daily practice and used it mainly to assess *RAS/BRAF* status. Of note, most of them were able to perform the analysis in their own centre. Notably, clinicians currently use ctDNA even for obtaining RAS/BRAF status to treat with the correct targeted agent patients in which biopsies fail to obtain enough tissue for assessment or when tumor

tissue is not available in the treating centre. This is corroborated by the high concordance rate of *RAS* status demonstrated between plasma and tissue analysis procedures (89%) in the OncoBEAM trial [25].

The AGEO RASANC trial, evaluating the concordance of *RAS* and *BRAF* status between plasma and tumor tissue, showed an accuracy of 85.2% (95% CI, 81.4-88.5%) and 97.3% (95%CI 95.2-98.6%) for *RAS* and *BRAF*, respectively, and 94.8% (95%CI 91.9%-97.0%) and 98.6% (95% CI, 96.5-99.6) in patients with detectable ctDNA and liver metastases[26,27]. Moreover, Bettegowda et al. noticed a worse prognosis according to the level of *KRAS* mutant fragments [6]. In the CAPRI-GOIM trial, the detection of *RAS* mutation in the plasma had a concordance rate with tumor samples of 78.3% and demonstrated that the pejorative impact on clinical outcomes of having a RAS mutation was comparable whether detecting it in the blood or on tissue (median progression-free-survival: 7.8 versus 13.8 moths; P <0.001 for liquid biopsy and 7.9 versus 12.6 months, P =0.004 for tumor tissue) [28].

As for interest about ctDNA use, in our survey, most of the respondents considered interesting its use for detecting residual disease during post-surgery surveillance, for anticipating the presence of secondary resistance mutations in patients treated with targeted therapies and to discuss anti-EGFR re-challenge.

Accordingly, a recent analysis established the prognostic role played by ctDNA in early stage CRC [29–31]. Tie et al. reported higher recurrence rate (79%) among stage II CRC patients with post-operative detectable ctDNA [32]. Interestingly, the CIRCULATE trials ongoing in France and Germany, are currently evaluating the benefit of mFOLFOX6 adjuvant therapy compared observation in stage II CRC patients with post-operative detectable ctDNA [33].

In the IDEA France trial, dedicated to stage III colon cancer patients, 13.5% of the 1000 patients tested for ctDNA were positive after curative surgery and this finding led to worse disease-free survival (HR: 1.85; 95%CI 1.31 to 2.61; p < 0.001). Moreover, ctDNA-positive patients treated only for 3 months of adjuvant chemotherapy had poor clinical outcomes, regardless of being low or high-risk stage III [34]. Many trials are currently starting to test escalation and de-escalation strategies guided by ctDNA assessment in these patients.

In the metastatic setting, some studies showed that a rapid ctDNA decrease after treatment start predicts early response to treatment and prolonged PFS and OS (Garlan et al, PLACOL study, Clin Can Res 2017). Moreover, during targeted treatments, acquired resistance can occur due to genetic and epigenetic alterations [35]. Recent evidences highlighted that plasma *HER2* amplification changes over time predicting resistance to anti-EGFR when highly expressed [36]. In the CRICKET study, conducted on *RAS/BRAF* wild-type CRC patients with secondary resistance to first-line cetuximab based treatment, observed that 12 out of 28 patients were *RAS* mutant. None of them, showed a partial response to the re-challenge while it was possible to demonstrate an improved PFS for patients with *RAS* wt ctDNA (3.9 vs 1.9 month; HR: 0.48, 95%CI 0.20-0.98, p = 0.048) [37]. In our study, this feature had captured significantly more interest in MO and HE than RT and DS. Hence these results may have influenced the recipients of this survey for whom molecular profiling remained the main goal of current ctDNA use.

Major expectations for the future were directed towards its use for guiding initial treatment or re-challenge. In particular, *RAS/BRAF* detection, at baseline in the absence of a biopsy, and before anti-EGFR re-challenge, showed more interest and was more often used by French clinicians than Italian ones. Conversely, Italian physicians used ctDNA more frequently to look for secondary resistance mutations and for prognostic purposes than French ones.

All these could be advocated as possible future uses of ctDNA, even though some limitations should be pointed-out. Noteworthy, in our analysis University Hospitals and Cancer Centres had a significantly broader access to ctDNA use in their facilities than General and Private Hospital, probably due to the possibility of a greater number of reference laboratories in the first ones and financial supports from research grants. Moreover, University Hospital can consult more frequently a molecular tumor board than others. This last observation emerging from our survey takes to the spotlight the need for more homogenous access to ctDNA as necessary base for constructing the future diffusion of its use.

5. Conclusions

In the last years, the clinical utility of ctDNA in CRC patients has emerged. Many clincal tirals are currently using it to select or stratify the patients. Our survey showed that half of participants had access to ctDNA use in their daily practice, mainly in University Hospitals or Cancer Centres. Molecular profiling remains the main goal of ctDNA use to guide initial treatment or re-challenge. Interestingly, therapeutic strategies based on ctDNA analysis in early and late stage and to profile disease at baseline represented the main expectations for the future, but several practical issues remain to be defined.

In fact, it is necessary to set up standardized dosing techniques and analytical and pre-analytical methodologies, to define universal threshold values and to validate the role of ctDNA in large prospective clinical trials in order to assess its potential usefulness in early and advanced CRC patients.

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Table 1. Demographic and professional characteristics

Characteristics		N	%
Total		**307**	***100***
Country	France	132	***43.00***
	Italy	175	***57.00***
Role	Hepato-gastro-enterologist	73	***23.78***
	Medical Oncologist	190	***61.89***
	Digestive Surgeons	31	***10.10***
	Radiotherapist	11	***3.58***
	Other	2	***0.65***
Centre	General Hospital	64	***20.85***
	University Hospital	146	***47.56***
	Private Hospital	31	***10.10***
	Cancer Centre	66	***21.09***
Setting of practice	Hospital Doctor	123	***40.07***
	University Professor	50	***16.29***
	Specialist Assistant	76	***24.76***
	Doctor in Cancer Centre	32	***10.43***
	Liberal Doctor	26	***8.47***
Number of CRC patients seen per month	<5	33	**10.75**
	5-20	112	**36.48**
	>20	162	**52.77**

Table 2. Current applications of ctDNA use and future expectation

Questions	Answers	N	%
Total		307	100
Knowledge and Current Clinical Practice			
Do you know circulating tumor DNA?	• Yes	301	98.05
	• No	6	1.95
Do you think circulating tumor DNA can help in your daily practice in the future?	• Yes	303	98.70
	• No	4	1.30
If so, which applications do you find interesting? (various answers possible):	• Determination of RAS status *(Yes vs No)*	183	59.60
	• Determination BRAF status *(Yes vs No)*	175	57.00
	• Prognostic information in metastatic setting *(Yes vs No)*	159	51.79
	• Detection of residual disease after surgery in localized tumors *(Yes vs No)*	268	87.29
	• Detection of secondary resistant mutations to target treatments *(Yes vs No)*	245	79.80
	• Discussion about a re-challenge with anti-EGFR *(Yes vs No)*	193	62.86
	• Other	7	2.28
Do you have the opportunity to perform ctDNA analysis in current clinical practice?	• Yes	149	48.53
	• No	158	51.46
If yes, you can access it:	• In your Centre	120	39.08
	• Outside	71	23.12
If yes, in which situation (s) are you currently using it?	• Disease in which it is difficult to obtain a biopsy to know the status of RAS/BRAF (*Yes vs No)*	72	51.43
	• Tumor tissue not available in your center for the evaluation of the molecular profile of RAS/BRAF (*Yes vs No)*	53	38.41
	• Evolution of the mutational state during treatment to guide treatment (*Yes vs No)*	87	57.62
	• With prognostic value (*Yes vs No)*	36	28.80
If you use it, what are the mutations evaluated?	• RAS/BRAF only	79	25.73
	• RAS/BRAF and other mutations	68	22.14
	• Others	2	0.65
Do you have access to a tumor molecular board in your center to research rare molecular targets? (Only France)	• Yes	74	56.06
	• No	58	43.94
If so, how often do you use your tumor molecular board? (Only France)	• Routinely	44	33.33
	• Exceptionally	37	28.03
Expectations			
What do you think is the main advantage of evaluating circulating tumor DNA?	• To avoid an invasive biopsy	104	33.88
	• Faster times to get RAS / BRAF status	39	12.70
	• To avoid tumor sample requests from external centers	9	2.93
	• To monitor the evolution of the molecular profile during treatment	146	47.56
	• Other	9	2.93
What is your expectation (s) regarding circulating tumor DNA for the future? (various answers possible):	• For the purpose of colorectal cancer screening *(Yes vs No)*	82	26.71
	• For diagnostic purposes *(Yes vs No)*	66	21.49
	• For therapeutic purposes with the baseline molecular profile *(Yes vs No)*	171	55.70
	• For therapeutic purposes to guide adjuvant chemotherapy *(Yes vs No)*	224	72.96
	• For therapeutic purposes to monitor the molecular profile during treatment *(Yes vs No)*	241	78.50
	• For prognostic purposes	110	35.83

Table 3. Responses according to characteristics of physicians, centre and country

Current applications		
Expertise: • **Hepato-gastro-enterologist (HE)** • **Medical Oncologist (MO)** • **Digestive Surgeons (DS)** • **Radiotherapist (RT)** • **Other (O)**	**Which applications of ctDNA do you find interesting?** **(various answers possible):**	
	• Determination of RAS status *(Yes vs No)* (HE 72.60%; MO 57.89%; DS 45.16%; RT 45.45%; O 50.00%)	*P=0.060*
	• Determination BRAF status *(Yes vs No)* (HE 67.12%; MO 56.31%; DS 45.16%; RT 36.36%; O 0.00%)	*P=0.146*
	• Prognostic information in metastatic setting *(Yes vs No)* (HE 53.42%; MO 45.78%; DS 80.64%; RT 54.54%; O 100.00%)	***P=0.005***
	• Detection of residual disease after surgery in localized tumors *(Yes vs No)* (HE 72.60%; MO 87.89%; DS 93.54%; RT 72.72%; O 50.00%)	*P=0.212*
	• Detection of secondary resistant mutations to target treatments *(Yes vs No)* (HE 73.94%; MO 83.68%; DS 77.41%; RT 54.54%; O 100.00%)	*P=0.082*
	• Discussion about a re-challenge with anti-EGFR *(Yes vs No)* (HE 75.34%; MO 62.10%; DS 45.16%; RT 45.45%; O 50.00%)	***P=0.031***
	If you use ctDNA, in which situation do you currently use it? **(various answers possible):**	
	• Disease in which it is difficult to obtain a biopsy to know the status of RAS/BRAF *(Yes vs No)* (HE 65.51%; MO 62.10%; DS 50.00%; RT 25.00%; O 0.00%)	*P=0.083*
	• Tumor tissue not available in your center for the evaluation of the molecular profile of RAS/BRAF *(Yes vs No)* (HE 48.27%; MO 37.25%; DS 0.00%; RT 33.33%; O 0.00%)	*P=0.286*
	• Evolution of the mutational state during treatment to guide treatment *(Yes vs No)* (HE 42.85%; MO 61.06%; DS 50.00%; RT 66.66%; O 100.00%)	*P=0.403*
	• With prognostic value *(Yes vs No)* (HE 21.73%; MO 31.25%; DS 33.33%; RT 0.00%; O 0.00%)	*P=0.555*
	If you use it, what are the mutations evaluated? **(One answer allowed)**	
	• RAS/BRAF only (HE 50.00%; MO 53.63%; DS 50.00%; RT 50.00%; O 0.00%)	*P=0.875*
	• RAS/BRAF and other mutations (HE 50.00%; MO 44.54%; DS 50.00%; RT 50.00%; O 0.00%)	
	• Others (HE 6.45%; MO 1.18%; DS 0.00%; RT 0.00%; O 0.00%)	
Centre • **General Hospital (GH)** • **University Hospital (UH)** • **Private Hospital (PH)** • **Cancer Centre (CC)**	**Do you have the opportunity to perform ctDNA analysis in current clinical practice?** **(One answer allowed)**	***P<0.001***
	• Yes (GH 29.68%; UH 51.36%; PH 19.23%; CC 54.54%)	
	• No	
	If yes, you can access it: **(One answer allowed)**	***P<0.001***
	• In your Centre (GH 19.04%; UH 79.56%; PH 14.28%; CC 85.71%)	
	• Outside	
	If you use it, what are the mutations evaluated? **(One answer allowed)**	*P=0.646*
	• RAS/BRAF only (GH 43.75%; UH 56.16%; PH 50.00%; CC 44.73%)	
	• RAS/BRAF and other mutations (GH 50.00%; UH 41.09%; PH 50.00%; CC 45.94%)	
	• Others (GH 6.25%; UH 2.73%; PH 0.00%; CC 0.00%)	
	Do you have access to a tumor molecular board in your center to research rare molecular targets? (One answer allowed) (Only France)	***P<0.001***
	• Yes (GH 26.92%; UH 66.17%; PH 36.36%; CC 8,75%)	
	• No	
	If so, how often do you use your molecular laboratory? **(One answer allowed) (Only France)**	***P=0.011***
	• Routinely (GH 90.00%; UH 50.00%; PH 77.77%; CC 28.57%)	
	• Exceptionally	
Country • **France (FR)** • **Italy (IT)**	**Do you think circulating tumor DNA can help in your daily practice in the future?** **(One answer allowed)**	*P=0.464*
	• Yes (FR=99.24%; IT=98.28%)	
	• No	
	Which applications of ctDNA do you find interesting? **(various answers possible):**	
	• Determination of RAS status *(Yes vs No)* (FR=70.45%; IT=51.42%)	***P=0.001***
	• Determination BRAF status *(Yes vs No)* (FR=65.15%; IT=50.85%)	***P=0.012***
	• Prognostic information in metastatic setting *(Yes vs No)* (FR=53.78%; IT=50.28%)	*P=0.543*
	• Detection of residual disease after surgery in localized tumors *(Yes vs No)* (FR=90.15%; IT=85.14%)	*P=0.192*
	• Detection of secondary resistant mutations to target treatments *(Yes vs No)* (FR=75.51%; IT=82.28%)	*P=0.212*
	• Discussion about a re-challenge with anti-EGFR *(Yes vs No)* (FR=74.24%; IT=54.28%)	***P<0.001***
	Do you have the opportunity to perform ctDNA analysis in current clinical practice? **(One answer allowed)**	***P=0.023***
	• Yes (FR=35.60%; IT=48.57%)	
	• No	
	If yes, you can access it: **(One answer allowed)**	*P=0.276*
	• In your Centre (FR=15.15%; IT=29.14%)	
	• Outside	
	If you use ctDNA, in which situation do you currently use it?	

	(various answers possible):	
	• Disease in which it is difficult to obtain a biopsy to know the status of RAS/BRAF *(Yes vs No)* (FR=65.30%; IT=43.95%)	***P=0.016***
	• Tumor tissue not available in your center for the evaluation of the molecular profile of RAS/BRAF *(Yes vs No)* (FR=46.93%; IT=33.70%)	*P=0.126*
	• Evolution of the mutational status during treatment to guide treatment *(Yes vs No)* (FR=46.00%; IT=54.00%)	***P=0.042***
	• With prognostic value *(Yes vs No)* (FR=5.26%; IT=39.08%)	***P<0.001***
	If you use it, what are the mutations evaluated? **(One answer allowed)** • RAS/BRAF only (FR=54.90%; IT=51.00%) • RAS/BRAF and other mutations (FR=41.17%; IT=47.00%) • Others (FR=3.93%; IT=2.00%)	*P=0.663*
Expectations		
Expertise: • **Hepato-gastro-enterologist** • **Medical Oncologist** • **Digestive Surgeons** • **Radiotherapist** • **Other**	**What do you think is the main advantage of evaluating circulating tumor DNA?** **(One answer allowed)** • To avoid an invasive biopsy (HE 38.35%; MO 32.10%; DS 29.03%; RT 45.45%; O: 50.00%) • Faster times to get RAS / BRAF status (HE 13.69%; MO 12.10%; DS 16.12%; RT 0%; O: 50.00%) • To avoid tumor sample requests from external centers (HE 6.84%; MO 1.57%; DS 3.22%; RT 0%; O: 0.00%) • To monitor the evolution of the molecular profile during treatment (HE 38.35%; MO 52.10%; DS 45.16%; RT 45.45%; O: 0.00%) • Other (HE 2.73%; MO 2.10%; DS 6.45%; RT 9.00%; O: 0.00%)	*P=0.375*
	What is your expectation (s) regarding circulating tumor DNA for the future? (various answers possible):	
	• For the purpose of colorectal cancer screening *(Yes vs No)* (HE 26.02%; MO 25.78%; DS 29.03%; RT 36.36%; O: 50.00%)	*P=0.868*
	• For diagnostic purposes *(Yes vs No)* (HE 20.54%; MO 20.52%; DS 25.80%; RT 27.27%; O: 50.00%)	*P=0.797*
	• For therapeutic purposes with the baseline molecular profile *(Yes vs No)* (HE 67.12%; MO 51.05%; DS 54.83%; RT 14.63%; O: 100.00%)	*P=0.129*
	• For therapeutic purposes to guide adjuvant chemotherapy *(Yes vs No)* (HE 76.71%; MO 72.63%; DS 70.96%; RT 63.63%; O: 50.00%)	*P=0.806*
	• For therapeutic purposes to monitor the molecular profile during treatment *(Yes vs No)* (HE 86.30%; MO 76.84%; DS 77.41%; RT 63.63%; O: 50.00%)	*P=0.252*
	• For prognostic purposes *(Yes vs No)* (HE 34.24%; MO 32.63%; DS 54.83%; RT 45.45%; O: 50.00%)	*P=0.170*
Country • **France** • **Italy**	**What do you think is the main advantage of evaluating circulating tumor DNA?** **(One answer allowed)** • To avoid an invasive biopsy (FR=11.57%; IT=30.85%) • Faster times to get RAS / BRAF status (FR=15.15%; IT=10.85%) • To avoid tumor sample requests from external centers (FR=3.78%; IT=2.28%) • To monitor the evolution of the molecular profile during treatment (FR=38.63%; IT=54.28%) • Other (FR=4.54%; IT=1.71%)	*P=0.069*
	What is your expectation (s) regarding circulating tumor DNA for the future? (various answers possible):	
	• For the purpose of colorectal cancer screening *(Yes vs No)* (FR=26.51%; IT=26.85%)	*P=0.947*
	• For diagnostic purposes *(Yes vs No)* (FR=22.72%; IT=20.57%)	*P=0.649*
	• For therapeutic purposes with the baseline molecular profile *(Yes vs No)* (FR=65.90%; IT=48.00%)	***P=0.002***
	• For therapeutic purposes to guide adjuvant chemotherapy *(Yes vs No)* (FR=78.78%; IT=68.57%)	***P=0.046***
	• For therapeutic purposes to monitor the molecular profile during treatment *(Yes vs No)* (FR=81.81%; IT=76.00%)	*P=0.219*
	• For prognostic purposes *(Yes vs No)* (FR=35.60%; IT=36.00%)	*P=0.943*

Figure 1. Which applications of ctDNA do you find interesting?

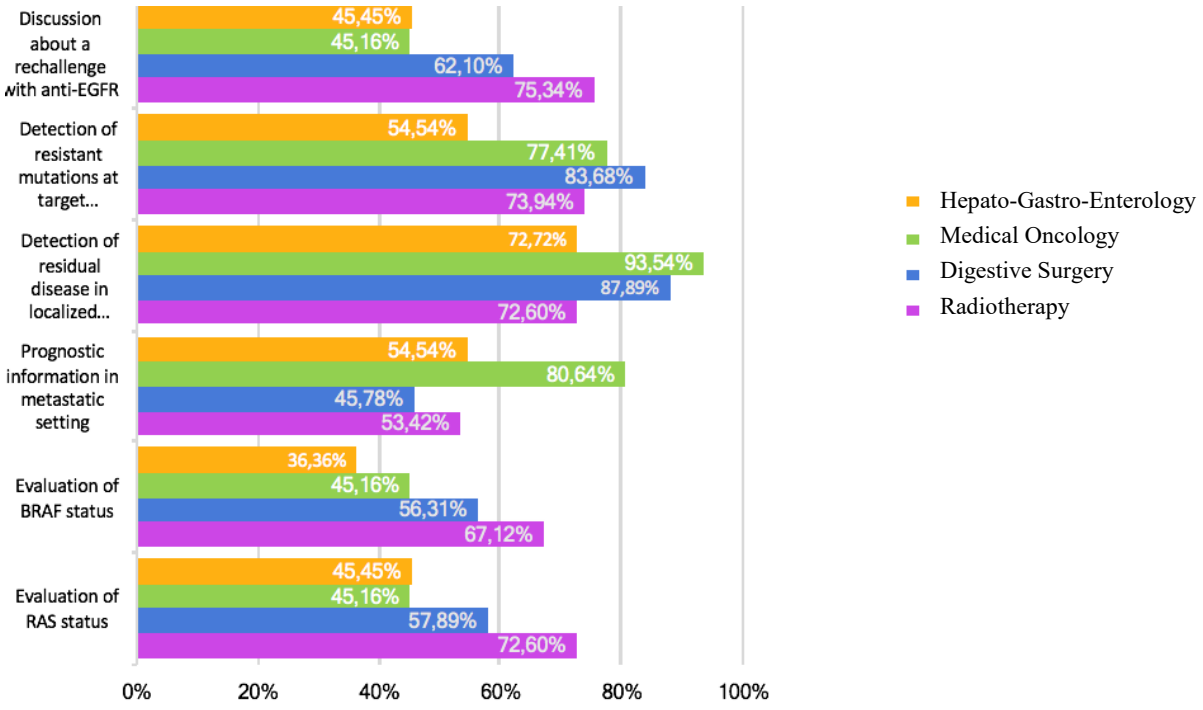

Figure 2. If you use ctDNA, in which situation do you currently use it?