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Abstract: Background: The management of locally advanced rectal cancer (RC) is an evolving clinical field where the multidisciplinary approach can reach its best and liquid biopsy for obtaining tumor-derived component such as circulating tumor DNA (ctDNA) might provide complementary informations.

Methods: A systematic review of studies available in literature of liquid biopsy in non-metastatic RC has been performed according to PRISMA criteria to assess the role of ctDNA as a diagnostic, predictive and prognostic biomarker in this setting.

Results: Twenty-five publications have been retrieved, of which 8 full-text articles, 7 abstracts and 10 clinical trials. Results have been categorized into three groups: diagnostic, predictive and prognostic. Few but promising data are available about the use of liquid biopsy for early diagnosis of RC, with the main limitation of sensitivity due to low concentrations of ctDNA in this setting. In terms of prediction of response to chemoradiation, still inconclusive data are available about the utility of a pre-treatment liquid biopsy, whereas some studies report a positive correlation with a dynamic (pre/post-treatment) monitoring. The presence of minimal residual disease by ctDNA was consistently associated with worse prognosis across studies.

Conclusions: The use of liquid biopsy for monitoring response to chemoradiation and assess the risk of disease recurrence are the most advanced potential applications for liquid biopsy in RC, with implications also in the context of non-operative management strategies.

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Editors-in-Chief, *Cancer Treatment Reviews*

Dear Professors Pavlidis and Stahel,

We would like to submit to your attention the manuscript entitled “Liquid biopsy for rectal cancer: a systematic review” for publication in *Cancer Treatment Reviews.*

The management of locally advanced rectal cancer is an evolving clinical field where the multidisciplinary approach can reach its best and liquid biopsy for obtaining tumor-derived component such as circulating tumor DNA (ctDNA) might provide important complementary pieces of information. While there are many reviews available in the literature concerning the broad applications of liquid biopsy in cancer treatment, including a few about colorectal cancer as a whole, no systematic review has been performed with the focus of this specific tumor type, that has distinctive clinical features and different treatment modalities as compared to colon cancer.

We performed a systematic review categorizing results into three groups: diagnostic, predictive and prognostic applications of liquid biopsy for rectal cancer. We found interesting potential application of this diagnostic tool with implications also in the context of the emerging approach of non-operative management strategies, making overall the topic of very up to date according to the latest developments in this type of cancer. We therefore would like to submit our systematic review to the Journal for consideration since we feel that *Cancer Treatment Reviews* can offer proper dissemination to pathologists and oncologists.

Thank You very much indeed for your attention and collaboration. Kindest regards,

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***Conflict of Interest Statement**

CONFLICT OF INTEREST

A.S-B. has acted as a consultant/advisory member for Amgen, Bayer, Lilly and Merck-Serono. S.S is advisory board member for Amgen, Bayer, BMS, Celgene, Incyte, Merck, Novartis, Roche, Seattle Genetics. A.A. is advisory board member for Amgen and Bayer.

Liquid biopsy for rectal cancer: a systematic review

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Abstract

Background: The management of locally advanced rectal cancer (RC) is an evolving clinical field where the multidisciplinary approach can reach its best and liquid biopsy for obtaining tumor-derived component such as circulating tumor DNA (ctDNA) might provide complementary informations.

Methods: A systematic review of studies available in literature of liquid biopsy in non-metastatic RC has been performed according to PRISMA criteria to assess the role of ctDNA as a diagnostic, predictive and prognostic biomarker in this setting.

Results: Twenty-five publications have been retrieved, of which 8 full-text articles, 7 abstracts and 10 clinical trials. Results have been categorized into three groups: diagnostic, predictive and prognostic. Few but promising data are available about the use of liquid biopsy for early diagnosis of RC, with the main limitation of sensitivity due to low concentrations of ctDNA in this setting. In terms of prediction of response to chemoradiation, still inconclusive data are available about the utility of a pre-treatment liquid biopsy, whereas some studies report a positive correlation with a dynamic (pre/post-treatment) monitoring. The presence of minimal residual disease by ctDNA was consistently associated with worse prognosis across studies.

Conclusions: The use of liquid biopsy for monitoring response to chemoradiation and assess the risk of disease recurrence are the most advanced potential applications for liquid biopsy in RC, with implications also in the context of non-operative management strategies.

Key Words: rectal cancer, liquid biopsy, ctDNA, non-operative management

BACKGROUND

Colorectal cancer (CRC) is one of the most diagnosed cancers worldwide, with 1.84 million estimated new cases in 2018 [1]. About 25-30% of all colorectal cancer diagnoses accounts for rectal cancer (RC) [2]. In the European Union, the incidence of RC is ~125000 per year, i.e. ~35% of the total colorectal cancer incidence, reflecting 15–25 cases/100 000 population per year and is predicted to further increase in both genders. The mortality is 4–10/100 000 population per year, with a median age at diagnosis of ~70 years [3].

The rectum and colon have a different embryological origin, anatomy and function [4]. RC has thus distinctive clinical features as compared to colon cancer, with an increased risk of local spread and recurrence. As a consequence, the treatments for primary rectal and colon cancer are different [5]. The incidence of RC has been decreasing as the increasingly spread use of screening allows for identification and endoscopic removal of premalignant lesions [6]; however, several recent studies have shown an increase in incidence of rectal cancers among young people [7].

The treatment landscape in RC paralleled that of colon cancer and has evolved over the last decade following the approval of several targeted therapies for the advanced disease, leading to improvements in tumor response rates and patient survival [8,9]. However, progresses in medical treatment in the metastatic setting have been mainly incremental despite considerable advances in the knowledge of tumor biology [10]. In this regard, primary tumor location (right-sided or left-sided of the colorectum) has been identified as a surrogate marker for underlying molecular classification, with differences in a continuum spectrum between colon and rectal carcinomas [11].

In the non-metastatic setting, operative approaches such as transanal endoscopic microsurgery, open and laparoscopic proctectomy [12] are effective in earlier stages, while a trimodality treatment (pre-operative chemoradiation therapy (CRT), surgery with total

mesorectal excision (TME) followed by adjuvant chemotherapy) is the standard of care for locally advanced RC patients. A significant risk of distal recurrence is present in rectal tumors radically operated in particular within the first 5 years for stages II and III of Dukes at around 30% and 50%, respectively, probably caused by the presence of micrometastatic spread [6]. A pooled analysis of five European randomized controlled trials demonstrated that the 5-year distant metastasis rate was 30.8% in 2.759 recruited patients [3]. An increasing number of reports suggested that a non-operative management (NOM)**,** consisting of close surveillance of patients with clinical complete response (cCR) after chemoradiotherapy, could be an acceptable alternative to rectal surgery (proctectomy). Led by small prospective series published since the late 90's by Habr-Gama and colleagues [13,14], several international series have reported similar oncologic outcomes in cCR patients followed by close active surveillance (the so-called watch-and-wait (W&W) or NOM approach) compared to those treated with radical surgery [15,16]. More recently, the International Watch & Wait Database (IWWD) described the outcome of the W&W strategy in a large-scale registry of more than 1000 patients, reporting excellent survival and small risk of local unsalvageable disease recurrence [17]. Despite the body of retrospective literature is greatly increasing, key knowledge gaps limiting widespread use of W&W/NOM remain, and clinical studies aimed at identifying patients who are good candidates for this approach are ongoing [18].

Follow-up supported by clinical examinations, imaging and endoscopies aims to improve prognosis by early detection of local or distant recurrence. Isolated carcinoembryonic antigen (CEA) monitoring is insufficiently sensitive. The analysis of serum protein levels, such as CEA, allows a fast and cost-effective method to quantify cancer progress, but it's distorted by limited sensitivity and specificity, in particular during treatment courses due to inflammation and discharge of protein in the bloodstream. Moreover, a portion of patients

with metastatic RC does not show visible plasmatic CEA levels during the disease [19,20].

The management of RC is an evolving clinical field where the multidisciplinary approach can reach its best and the ability to discern patients at low risk from those at high risk of recurrence is the prerequisite for the most appropriate treatment choice. With this regard, liquid biopsy for obtaining tumor-derived component such as circulating tumor DNA (ctDNA) or circulating tumor cells (CTCs) might provide crucial complementary information at the diagnosis of RC and in different moments during its treatment. Liquid biopsies may indeed represent a precious basin of new generation biomarkers [21,22] and are being evaluated also in RC for diagnosis, treatment choice, monitoring disease response, tracking acquired mutations linked to targeted therapy resistance, and detecting minimal residual disease.

The aim of this systematic review is to assess the role of ctDNA as a diagnostic, predictive and prognostic biomarker in non-metastatic RC.

METHODS

Definition of the Outcome

The purpose is to evaluate the current clinical potential of liquid biopsy, in particular cell-free DNA (cfDNA)/ circulating tumor DNA (ctDNA), in non-metastatic RC patients.

Data Source and Search Strategy

A systematic literature review was performed according to PRISMA Statement Criteria [23,24] in June 2019. The PubMed database was systematically reviewed as of June 11th, 2019 and all retrieved studies were manually screened for relevant references missed in the primary search. Unpublished data presented as abstract in relevant international

congresses [American Society of Clinical Oncology (ASCO), European Society of Medical Oncology (ESMO)] were also systematically searched for. Furthermore, ongoing clinical trial exploring the value of liquid biopsy in non-metastatic rectal cancer were searched on clinicaltrial.gov. The decision to include a study for review was made by consensus between two authors (EGP and DM). The research criteria were limited to human studies published only in English language. The Medical Subject Heading terms used for the search were ("rectal" or "rectum" or "LARC") and ("liquid biopsy" or "ctDNA" or "cfDNA" or "circulating tumor DNA" or "circulating free DNA" or "methylated DNA" or "DNA methylation").

Main study inclusion criteria:

- Involved the measurement of cfDNA/ctDNA in plasma/serum in patients with RC;
- Diagnosis, treatment response and/or survival data collected and correlated with cf/ctDNA.

Study exclusion criteria:

- Involved patients with metastatic RC;
- Involved patients with CRC without mention about the tumor location.

RESULTS

A total of 838 records were screened to be included in the systematic review (**Figure 1**). We identified 8 records found through database searching (PUBMED) and 17 additional records identified through other sources (ESMO, ASCO, Clinicaltrial.gov).

As a result, 26 records were eligible and included in the systematic review: 8 full-text articles studies, 7 abstracts presented at international congresses, and 10 ongoing ctDNA clinical trials.

We subdivided our results into 3 categories according to the investigated role of ctDNA/cfDNA or methylated DNA: diagnostic, predictive of treatment response and

prognostic (in terms of disease recurrence or survival) (**Figure 2**). Finally, we provide a summary of published or presented works (**Table 1**) and of ongoing trials (**Table 2**).

cfDNA and ctDNA as diagnostic tool

In 2011 an Italian group evaluated the ability of cfDNA to discriminate healthy patients (plasma samples collected after a negative colonoscopy) from patients with RC. Through quantitative PCR (using Alu 115, Alu 147 and β-globin gene), they found that the baseline level of cfDNA was significantly higher in RC patients than in healthy individuals [25].

A Chinese group also observed higher concentration of cfDNA in RC than in healthy individuals, where mutated *KRAS* and methylated *MGMT* were not detected. Moreover, the ratio of 400-/100-bp DNA fragments (an index of cfDNA integrity) was higher in RC patients than in healthy controls, in which cfDNA is considered to originate mainly from apoptotic process of normal cells [26].

Shalaby et al. highlighted the capacity of *MGMT* and *ERCC1* methylation status to distinguish benign and malignant rectal tumors. The study was performed in blood and tissue of 43 benign and 50 malignant rectal tumors patients. They observed a significant higher frequency of *MGMT* and *ERCC1* methylation in RC patients than in cases with benign tumors, both in tissue and blood samples (sensitivity around 60% and specificity of 93-95% for each gene on plasma). The combination of *MGMT* and *ERCC1* methylation reached a specificity for differentiation between benign and malignant rectum tumor of 100% in blood samples, with a sensitivity of 32% [27].

More recently, Zhang et al. observed that both colon and RC could be detected by ctDNA, with the latter having lower median plasma cfDNA concentrations in plasma than colon cancer patients (14.2 ng/ml vs. 8.94 ng/ml). The study was conducted on 29 patients, including 10 with RC. For each patient, a freshly frozen tissue sample was collected during

surgery and the plasma obtained was analyzed using an 85-gene panel, with a mutation concordance rate between ctDNA and tissue of 70% in all patients, lower in the subgroup of RC patients (5/10, 50%) [28].

At the present time, one clinical trial is exploring the feasibility of ctDNA as a detection index for RC (**Table 2**).

cfDNA and ctDNA as tool to predict treatment response

Different groups analyzed the role of cf/ctDNA as a tool to monitor treatment response after CRT in locally-advanced rectal cancer (LARC). In 2008, Zitt and colleagues [29] observed for the first time a correlation between the concentration of cfDNA and clinical response after surgery (not after neoadjuvant CRT) in 26 patients: responders exhibited a reduction in cfDNA level, while non-responders showed an incremented cfDNA [29].

Agostini et al. analyzed cfDNA on 67 LARC patients before and after neoadjuvant CRT, before surgery. Levels of longer fragments of cfDNA were reduced in responsive patients (tumor regression grade (TRG) 1-2, according to the Mandard score) compared to non-responsive (TRG 3-5). In particular the post-CRT cfDNA integrity (the ratio of 400-/100-bp DNA fragments) was associated with response ($P = 0.0009$), confirming that cfDNA long fragments are more tumor-specific than short fragments. Baseline levels of cfDNA were not correlated with tumor response [25].

The relationship between cfDNA concentration and TRG score was also observed by a Chinese group who focused also on different aspects of cfDNA: the 400-/100-bp ratio of DNA fragments, the methylation status of *MGMT* and the mutational status of *KRAS*. They treated 34 LARC patients with CRT followed by surgery. The good response group of patients had a significantly higher baseline 400-bp plasma cfDNA levels and showed a significant decrease of these fragments in plasma after CRT. On the contrary, no difference was observed regard the level of 100-bp fragments before and after CRT both

in responders and non-responders. In addition, the rate of *MGMT* promoter methylation at baseline was higher in responders, with no reduction after treatment, while the rate of *KRAS* mutation decreased in both groups after CRT [26].

Shalaby et al. described the same correlation between methylated status of the promoter of *MGMT* and *ERCC1* genes with response to CRT. A higher methylation status was associated with a better tumor response after preoperative CRT [27].

A Danish group quantified the total level of cfDNA by fluorescence assay, using 40 µL of plasma of 123 LARC patients. They observed no differences either between baseline and post-treatment (CRT preceded by induction chemotherapy in 42% of cases) levels of cfDNA or between patients achieved a pathological complete response (pCR) and poor responders [30].

Carpinetti et al. firstly observed a decrease in ctDNA (detected due to tumor-specific chromosomal rearrangements) in 4 patients that achieved a response to CRT. By the way, cfDNA was negative both in patients with partial and complete pathological response [31].

Tie et al. analyzed tissue and multiple plasma samples of 159 LARC patients treated with neoadjuvant CRT through next generation sequencing (NGS). ctDNA was detectable in 77% of plasma samples before treatment, 8% during CRT and 12% at a postoperative stage. Detectable ctDNA after surgery was associated with known high risk pathological features (i.e. ypT3-4, node positive), but there was no statistically meaningful association between reduction or negativization of ctDNA after CRT and pCR [32].

Li et al. observed in a small study of 30 patients that ctDNA level variations (somatic mutations identified by NGS of 61-gene panels) can predict pathological response to neoadjuvant CRT, better than classic markers as CEA or CA 19.9 [33].

McDuff et al. reported a higher rate of R0-node negative resections after CRT among 17 patients with undetectable preoperative ctDNA, compared to 10 patients with detectable ctDNA. The former group had a higher pCR rate (24% vs. 10%) [34].

Chen et al. identified ctDNA analyzing the methylation status of *BCAT1* or *IKZF1* gene through qPCR assay in 9 LARC patients. Five patients showed positivity for one or both of methylated genes before CRT, four of them exhibited a decrease in detection after treatment, consistent with partial or complete responses [35].

Two Chinese prospective cohort studies, presented at 2019 ASCO meeting, recruited 180 LARC patients, overall, with serial plasma collection analyzed through NGS gene panels to detect mutations in ctDNA [36,37]. Yang et al. reported a negative correlation between presence of *TP53* and *APC* gene in pre-treatment samples and response to nCRT, and detectability of pre-treatment mutations during nCRT significantly decreased from TRG3 to TRG0 group [37]. Zhou et al. observed a significant predictive role of pre-surgery ctDNA levels, where its persistency was linked with pathological N+, while an undetectable preoperative ctDNA correlated with pathological downstaging [36].

At the present time, six clinical trials are exploring the possibility to use ctDNA as a predictive tool (**Table 2**).

cfDNA and ctDNA as a prognostic tool for disease recurrence or survival

In a Danish study of 123 LARC patients, a solid association of baseline cfDNA level (measured through a fluorescence assay) with disease free survival (DFS) was found. High levels of cfDNA were correlated with higher risk of local or distant recurrence and with shorter time to recurrence. A non-statistically significative trend for overall survival (OS) was also observed [30].

At a median follow up of 24 months, Tie and colleagues noticed an increased risk of recurrence in patients with ctDNA persistence after CRT or surgery. This risk of recurrence was irrespective either of pathological risk level (ypT3-4N+ vs ypT1-2N0 vs pCR) or of adjuvant therapy, with an estimate 3y RFS of 33% vs 87%. Post-operative ctDNA

detection was a stronger prognostic biomarker than CEA levels. Moreover, 74% of patients recurred within 12 months after surgery, 9/19 had persistent ctDNA in plasma [38].

As already said, Carpinetti et al, analysed in 4 LARC patients the use of ctDNA to monitor disease response and recurrence. Two patients with persistent positive level of ctDNA, during their follow up, developed liver metastasis concomitantly with an incremental in ctDNA level. Other 2 patients showed a drop in ctDNA levels after CRT, with negative follow up for recurrence and no more evidence of ctDNA in plasma [31].

Four studies, presented at last ASCO and ESMO congresses, have explored the use of ctDNA as a tool to assess response and predict surgical outcome in LARC [34,35,39,40]. McDuff et al., among 22 patients treated with preoperative CRT, reported a shorter recurrence free survival in cases with detectable post-operative ctDNA [34]. Khakoo showed that persistence of ctDNA at mid CRT or detection of ctDNA at the end of CRT were associated with development of metastasis [40]. In the work presented by Chen, one patient that showed persistent high level of methylated genes after CRT, recurred in two months after surgery [35].

Conversely, in a study performed on 97 LARC patients receiving induction chemotherapy with CAPOX followed by CRT and then adjuvant CAPOX with or without cetuximab (EXPERT-C trial), Sclafani et al. did not found a significant association between ctDNA positivity/negativity and progression free survival (PFS) or OS using qPCR [41] .

At the 2019 ASCO meeting, Yang et al. described a significant association between persistence of pre-treatment mutations in ctDNA after completion of CRT and worse DFS [37].

Eight clinical trials are exploring the possibility to use cf/ctDNA as a prognostic tool (**Table 2**).

DISCUSSION

In present review several studies supported the use of liquid biopsy in RC as an innovative, minimally invasive procedure that could assist either the diagnostic-staging process and the assessment of treatment response. The limitations of data retrieved are mostly related to the relatively small sample size of the studies, heterogeneity of techniques used for liquid biopsy and timing of plasma samples (eg. after CRT or after surgery), and differences in treatment courses (e.g. induction chemotherapy or not) for patients with different stages of non-metastatic RC.

We found 4 studies (overall 204 patients) which explored the role of liquid biopsy as a diagnostic tool in RC. Measurement of cfDNA levels was performed with different techniques: ALU-based quantitative-PCR, tracking mutations of *KRAS,* or, more recently, tracking of several gene with NGS and assessing the methylation status of *MGMT* and *ERCC1* in ctDNA. These reports, although heterogeneous, suggest that measuring cfDNA levels or detecting ctDNA might discriminate RC patients from healthy controls and from individuals harbouring rectal adenoma. In particular, a high specificity in discriminating RC was reported for *MGMT* and *ERCC1* methylation or *KRAS* mutation detection.

These data are consistent with results obtained in colorectal cancer patients [42–44]. However, it should be taken into account that in this setting of early detection of cancer, the sensibility of liquid biopsy is limited by low concentrations of circulating DNA in this setting, that have been reported to be even lower in RC [28].

Contrasting data have been retrieved about the reliability of circulant DNA as a tool to predict treatment response in RC. The clinical value of baseline levels of cf/ctDNA is not clear. Only one small study showed a correlation between higher levels of longer cfDNA fragments (index of DNA integrity) and response [26], but these data were not consistent with results of a previous wider study [25]. Nevertheless, a strong methylation of *MGMT* or *ERCC1* genes at baseline might better predict a tumor response after preoperative CRT [26,27]; on the contrary, detection of *TP53* and *APC* gene in ctDNA of pre-treatment

samples has been negatively associated with response to CRT [37]. Notably, in a previous presentation of the same study, Yang et al. reported no difference in baseline ctDNA levels between responders and non-responders [39].

A noteworthy evidence, observed by most groups with different assays, consists in the reduction in cf/ctDNA levels in responders, while non-responders can show an incremented circulant DNA [25,26,29,31–33,35–37,45]. The timing of plasma collection represents a crucial aspect in this setting: at baseline, after induction chemotherapy, after neoadjuvant CRT, after surgery. Zitt et al. observed a reduction of DNA levels after CRT in all 26 patients but it was not predictive of pathological downstaging. A significant difference between responders and non-responders was found only in plasma samples collected after surgery [29].

In this same setting, a DNA integrity index (a ratio between long and short DNA fragments) has been proposed to be a useful guide to discriminate responding and non-responding patients even with plasma analysis conducted after neoadjuvant CRT [25,26].

More recent studies, adopting NGS assays, have demonstrated the potential of post-CRT ctDNA samples to predict tumor response, enhancing the confidence in ctDNA as a tool to guide patient selection for watch and wait strategy. Different groups observed a correlation between undetectable preoperative ctDNA status and pathological downstage [33,36–38,45]. A weaker methylation of *BCAT1* or *IKZF1* has been observed after CRT in good responders by Chen et al. [35].

Finally, we found inconclusive data about the association between reduction or total clearance of circulant DNA after CRT and pCR. Likely, circulant DNA has no sufficient sensitivity to rule out the presence of minimal residual disease [38].

In terms of survival and disease recurrence, almost all studies have shown a correlation between persistence of ctDNA after treatment and disease recurrence during follow up

[30,32,35–37,40,45]. One group also observed an association between high baseline cfDNA level and local or distant recurrence, with a trend for shorter OS [30].

Tie et al., in a recent prospective study of LARC patient, detected ctDNA in 77%, 8,3% and 12% of pretreatment, postchemoradiotherapy and post-surgery plasma samples, respectively. On the basis of ctDNA levels, they were able to stratify patients at very high risk of recurrence (ctDNA detectable after CRT (HR 6,6) or after surgery (HR 13,0)), estimating a 3-year recurrence-free survival of 33% vs 87% in positive/negative ctDNA patients. Postoperative ctDNA status remained an independent predictor of RFS irrespective of clinicopathological risk factor or adjuvant chemotherapy [38]

These results are in line with the conclusions of studies conducted in the setting of resected colorectal cancer, where evidence of ctDNA after surgery or after adjuvant chemotherapy were linked with shorter recurrence-free survival [46,47].

In contrast, among patients treated in the EXPERT-C trial (induction CAPOX, CRT, surgery, adjuvant CAPOX +/- cetuximab) a significant association between ctDNA positivity/negativity and PFS or OS was not observed. However, the plasma sample in this study was collected before surgery and all patients received both neoadjuvant and adjuvant chemotherapy [48].

The potential role of liquid biopsy in RC is also currently being explored as translational endpoints in numerous clinical trials and can find an important application in the setting of NOM. The ongoing No-Cut study, a phase 2 clinical trial, will assess whether an oxaliplatin-enhanced neoadjuvant CRT, followed by an imaging-intensive, liquid biopsy-enriched surveillance, can spare stage II-III rectal cancers from undergoing up-front demolitive radical surgery with a clinically acceptable rate of distant relapse. The translational component of the study could establish, by retrospective correlative analysis of contextual imaging and blood molecular findings, whether circulating mutated and/or

methylated tumoral DNA is a predictive marker for residual disease, and whether there is a correlation between ctDNA and cancer relapse (NCT03565029) (**Figure 3**).

Ethical approval and consent to participate

Not applicable because the article is a review.

Contributors

• Daniela Massihnia: conception and design of the review, acquisition, analysis and interpretation of data, drafting of the article.

• Elio Gregory Pizzutilo: conception and design of the review, acquisition, analysis and interpretation of data, drafting of the article.

• Alessio Amatu: acquisition and interpretation of data, critical revision of the draft.

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• Angelo Vanzulli: interpretation of data, critical revision of the draft.

• Salvatore Siena: conception and design of the review, analysis and interpretation of data, critical revision of the draft, final approval of the version to be submitted.

• Andrea Sartore-Bianchi: conception and design of the review, analysis and interpretation of data, drafting of the article, critical revision of the draft, final approval of the version to be submitted.

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Declaration of interests

A.S-B. has acted as a consultant/advisory member for Amgen, Bayer, Lilly and Merck-Serono. S.S is advisory board member for Amgen, Bayer, BMS, Celgene, Incyte, Merck, Novartis, Roche, Seattle Genetics. A.A. is advisory board member for Amgen and Bayer.

REFERENCES

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394–424. doi:10.3322/caac.21492.

[2] Wolpin BM, Meyerhardt JA, Mamon HJ, Mayer RJ. Adjuvant treatment of colorectal cancer. CA Cancer J Clin 2007;57:168–85.

[3] Glynne-Jones R, Wyrwicz L, Tiret E, Brown G, Rödel C, Cervantes A, et al. Rectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol Off J Eur Soc Med Oncol 2017;28:iv22–40. doi:10.1093/annonc/mdx224.
[4] Heald RJ, Moran BJ. Embryology and anatomy of the rectum. Semin Surg Oncol 1998;15:66–71.
[5] Tamas K, Walenkamp AME, de Vries EGE, van Vugt M a. TM, Beets-Tan RG, van Etten B, et al. Rectal and colon cancer: Not just a different anatomic site. Cancer Treat Rev 2015;41:671–9. doi:10.1016/j.ctrv.2015.06.007.
[6] Lange MM, Martz JE, Ramdeen B, Brooks V, Boachie-Adjei K, van de Velde CJH, et al. Long-term results of rectal cancer surgery with a systematical operative approach. Ann Surg Oncol 2013;20:1806–15. doi:10.1245/s10434-012-2832-2.
[7] Mauri G, Sartore-Bianchi A, Russo A-G, Marsoni S, Bardelli A, Siena S. Early-onset colorectal cancer in young individuals. Mol Oncol 2019;13:109–31. doi:10.1002/1878-0261.12417.
[8] Bekaii-Saab T, Kim R, Kim TW, O'Connor JM, Strickler JH, Malka D, et al. Third- or Later-line Therapy for Metastatic Colorectal Cancer: Reviewing Best Practice. Clin Colorectal Cancer 2018. doi:10.1016/j.clcc.2018.11.002.
[9] Modest DP, Pant S, Sartore-Bianchi A. Treatment sequencing in metastatic colorectal cancer. Eur J Cancer Oxf Engl 1990 2019;109:70–83. doi:10.1016/j.ejca.2018.12.019.
[10] Taieb J, Jung A, Sartore-Bianchi A, Peeters M, Seligmann J, Zaanan A, et al. The Evolving Biomarker Landscape for Treatment Selection in Metastatic Colorectal Cancer. Drugs 2019. doi:10.1007/s40265-019-01165-2.
[11] Baek SK. Laterality: Right-Sided and Left-Sided Colon Cancer. Ann Coloproctology 2017;33:205–6. doi:10.3393/ac.2017.33.6.205.
[12] Toda S, Kuroyanagi H. Laparoscopic surgery for rectal cancer: Current status and future perspective. Asian J Endosc Surg 2014;7:2–10. doi:10.1111/ases.12074.
[13] Habr-Gama A, de Souza PM, Ribeiro U, Nadalin W, Gansl R, Sousa AH, et al. Low rectal cancer: impact of radiation and chemotherapy on surgical treatment. Dis Colon Rectum 1998;41:1087–96.
[14] Habr-Gama A, São Julião GP, Vailati BB, Castro I, Raffaele D. Management of the Complete Clinical Response. Clin Colon Rectal Surg 2017;30:387–94. doi:10.1055/s-0037-1606116.
[15] Appelt AL, Pløen J, Harling H, Jensen FS, Jensen LH, Jørgensen JCR, et al. High-dose chemoradiotherapy and watchful waiting for distal rectal cancer: a prospective observational study. Lancet Oncol 2015;16:919–27. doi:10.1016/S1470-2045(15)00120-5.
[16] Renehan AG, Malcomson L, Emsley R, Gollins S, Maw A, Myint AS, et al. Watch-and-wait approach versus surgical resection after chemoradiotherapy for patients with rectal cancer (the OnCoRe project): a propensity-score matched cohort analysis. Lancet Oncol 2016;17:174–83. doi:10.1016/S1470-2045(15)00467-2.
[17] Valk MJM van der, Hilling DE, Bastiaannet E, Kranenbarg EM-K, Beets GL, Figueiredo NL, et al. Long-term outcomes of clinical complete responders after neoadjuvant treatment for rectal cancer in the International Watch & Wait Database (IWWD): an international multicentre registry study. The Lancet 2018;391:2537–45. doi:10.1016/S0140-6736(18)31078-X.
[18] Dossa F, Moore SE, Baxter NN. Looking Forward, Not Backward, on Watch and Wait for Rectal Cancer. JAMA Oncol 2019. doi:10.1001/jamaoncol.2019.1867.
[19] Tarantino I, Warschkow R, Schmied BM, Güller U, Mieth M, Cerny T, et al. Predictive Value of CEA for Survival in Stage I Rectal Cancer: a Population-Based Propensity Score-Matched Analysis. J Gastrointest Surg Off J Soc Surg Aliment Tract 2016;20:1213–22. doi:10.1007/s11605-016-3137-8.

[20] Sun X, Huang T, Cheng F, Huang K, Liu M, He W, et al. Monitoring colorectal cancer following surgery using plasma circulating tumor DNA. Oncol Lett 2018;15:4365–75. doi:10.3892/ol.2018.7837.
[21] Jin CE, Koo B, Lee TY, Han K, Lim SB, Park IJ, et al. Simple and Low-Cost Sampling of Cell-Free Nucleic Acids from Blood Plasma for Rapid and Sensitive Detection of Circulating Tumor DNA. Adv Sci Weinh Baden-Wurtt Ger 2018;5:1800614. doi:10.1002/advs.201800614.
[22] Nikolic A, Vlajnic M, Ristanovic M, Petrovic J, Dimitrijevic I, Krivokapic Z, et al. Cell-free DNA as biomarker and source for mutation detection in primary colorectal cancer. J BUON Off J Balk Union Oncol 2017;22:178–83.
[23] Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, et al. The PRISMA Statement for Reporting Systematic Reviews and Meta-Analyses of Studies That Evaluate Health Care Interventions: Explanation and Elaboration. PLOS Med 2009;6:e1000100. doi:10.1371/journal.pmed.1000100.
[24] Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. J Clin Epidemiol 2009;62:1006–12. doi:10.1016/j.jclinepi.2009.06.005.
[25] Agostini M, Pucciarelli S, Enzo MV, Del Bianco P, Briarava M, Bedin C, et al. Circulating cell-free DNA: a promising marker of pathologic tumor response in rectal cancer patients receiving preoperative chemoradiotherapy. Ann Surg Oncol 2011;18:2461–8. doi:10.1245/s10434-011-1638-y.
[26] Sun W, Sun Y, Zhu M, Wang Z, Zhang H, Xin Y, et al. The role of plasma cell-free DNA detection in predicting preoperative chemoradiotherapy response in rectal cancer patients. Oncol Rep 2014;31:1466–72. doi:10.3892/or.2013.2949.
[27] Shalaby SM, El-Shal AS, Abdelaziz LA, Abd-Elbary E, Khairy MM. Promoter methylation and expression of DNA repair genes MGMT and ERCC1 in tissue and blood of rectal cancer patients. Gene 2018;644:66–73. doi:10.1016/j.gene.2017.10.056.
[28] Zhang X, Xia L, Xu M, Qu H, Wen M, Melançon CE, et al. ctDNA sequencing for early detection of colorectal cancers: Comparative analysis of colon cancer and rectal cancer data. J Clin Oncol 36 2018 Suppl Abstr E15653 n.d.
[29] Zitt M, Müller HM, Rochel M, Schwendinger V, Zitt M, Goebel G, et al. Circulating cell-free DNA in plasma of locally advanced rectal cancer patients undergoing preoperative chemoradiation: a potential diagnostic tool for therapy monitoring. Dis Markers 2008;25:159–65.
[30] Schou JV, Larsen FO, Sørensen BS, Abrantes R, Boysen AK, Johansen JS, et al. Circulating cell-free DNA as predictor of treatment failure after neoadjuvant chemo-radiotherapy before surgery in patients with locally advanced rectal cancer. Ann Oncol Off J Eur Soc Med Oncol 2018;29:610–5. doi:10.1093/annonc/mdx778.
[31] Carpinetti P, Donnard E, Bettoni F, Asprino P, Koyama F, Rozanski A, et al. The use of personalized biomarkers and liquid biopsies to monitor treatment response and disease recurrence in locally advanced rectal cancer after neoadjuvant chemoradiation. Oncotarget 2015;6:38360–71. doi:10.18632/oncotarget.5256.
[32] Tie J, Cohen JD, Wang Y, Li L, Christie M, Simons K, et al. Serial circulating tumour DNA analysis during multimodality treatment of locally advanced rectal cancer: a prospective biomarker study. Gut 2019;68:663–71. doi:10.1136/gutjnl-2017-315852.
[33] Li M, Xiao W, Zhao GQ, Guo Z, Yang X, Lin S, et al. Predictive value of circulating tumor DNA in locally advanced rectal cancer patients receiving neoadjuvant radiochemotherapy. J Clin Oncol 2017;35, (suppl; abstr e15125).
[34] McDuff S, Parikh AR, Hazar-Rethinam M, Zheng H, Van Seventer E, Nadres B, et al. Using circulating tumor DNA (ctDNA) to predict surgical outcome after neoadjuvant

chemoradiation for locally advanced pancreatic cancer (LAPC). J Clin Oncol 2018;36:272–272. doi:10.1200/JCO.2018.36.4_suppl.272.
[35] Chen C-T, Tufts L, Aggarwal P, Heller F, Weiser MR. Detection of methylated BCAT1 and IKZF1 in stage II/III rectal cancer receiving chemoradiation. J Clin Oncol 2019;37:602–602. doi:10.1200/JCO.2019.37.4_suppl.602.
[36] Zhou J, Lin G, Gong Y, Zhang Y, Guan Y-F, Xia X, et al. Serial ctDNA analysis as a real-time indicator of neoadjuvant chemoradiotherapy in rectal cancer. J Clin Oncol 2019;37:3569–3569. doi:10.1200/JCO.2019.37.15_suppl.3569.
[37] Yang L, Wang Y, Bao H, Wan J, Fan X, Bao H, et al. ctDNA as a potential prognostic marker for locally advanced rectal cancer patients with 'watch and wait' approach. J Clin Oncol 2019;37:3544–3544. doi:10.1200/JCO.2019.37.15_suppl.3544.
[38] Tie J, Cohen JD, Wang Y, Li L, Christie M, Simons K, et al. Serial circulating tumour DNA analysis during multimodality treatment of locally advanced rectal cancer: a prospective biomarker study. Gut 2018. doi:10.1136/gutjnl-2017-315852.
[39] Yang L, Wang Y, Shen L, Wan J, Deng W, Zhu J, et al. Predicting treatment outcome of rectal cancer patients underwent neoadjuvant chemoradiotherapy by ctDNA: The potential use of ctDNA monitoring as organ-sparing approach. J Clin Oncol 2018;36, (suppl; abstr 3608).
[40] Khakoo S, Carter P, Valeri N, Shaikh R, Jones T, Begum R, et al. 1867PCirculating tumour DNA (ctDNA) as a tool to assess response and guide therapy adaptation in rectal cancer. Ann Oncol 2018;29. doi:10.1093/annonc/mdy303.037.
[41] Sclafani F, Chau I, Cunningham D, Hahne JC, Vlachogiannis G, Eltahir Z, et al. KRAS and BRAF mutations in circulating tumour DNA from locally advanced rectal cancer. Sci Rep 2018;8:1445. doi:10.1038/s41598-018-19212-5.
[42] Mead R, Duku M, Bhandari P, Cree IA. Circulating tumour markers can define patients with normal colons, benign polyps, and cancers. Br J Cancer 2011;105:239–45. doi:10.1038/bjc.2011.230.
[43] da Silva Filho BF, Gurgel APAD, Neto MÁ de FL, de Azevedo DA, de Freitas AC, Silva Neto J da C, et al. Circulating cell-free DNA in serum as a biomarker of colorectal cancer. J Clin Pathol 2013;66:775–8. doi:10.1136/jclinpath-2013-201521.
[44] Hao TB, Shi W, Shen XJ, Qi J, Wu XH, Wu Y, et al. Circulating cell-free DNA in serum as a biomarker for diagnosis and prognostic prediction of colorectal cancer. Br J Cancer 2014;111:1482–9. doi:10.1038/bjc.2014.470.
[45] McDuff SGR. Using circulating tumor DNA (ctDNA) to predict surgical outcome and postoperative recurrence following neoadjuvant chemoradiation (CRT) for borderline resectable/locally advanced rectal cancer (LARC). J Clin Oncol 37 2019 Suppl 4 Abstr 562 2019.
[46] Tie J, Wang Y, Tomasetti C, Li L, Springer S, Kinde I, et al. Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. Sci Transl Med 2016;8:346ra92. doi:10.1126/scitranslmed.aaf6219.
[47] Schøler LV, Reinert T, Ørntoft M-BW, Kassentoft CG, Árnadóttir SS, Vang S, et al. Clinical Implications of Monitoring Circulating Tumor DNA in Patients with Colorectal Cancer. Clin Cancer Res Off J Am Assoc Cancer Res 2017;23:5437–45. doi:10.1158/1078-0432.CCR-17-0510.
[48] Sclafani F, Chau I, Cunningham D, Hahne JC, Vlachogiannis G, Eltahir Z, et al. KRAS and BRAF mutations in circulating tumour DNA from locally advanced rectal cancer. Sci Rep 2018;8:1445. doi:10.1038/s41598-018-19212-5.

Figure Legends

Figure 1: Flow diagram representing the systematic review process performed according to PRISMA Statement.

Figure 2: Euler-Venn diagram representing the results of our systematical research divided according to the role of ct/cf DNA

Figure 3: Study design of NO-CUT trial and the potential role of liquid biopsy in non-operative management of rectal cancer.

Table 1: Studies exploring the role of liquid biopsy in non-metastatic rectal cancer
* Only as an abstract

Reference	Year	N. patients	Country	Assay	Main findings
			Diagnostic		
Agostini et al.	2011	67	Italy	qPCR	cfDNA levels (using Alu 115, 247 and β globin gene) were higher in RC than in healthy group (P < 0.0001).
Sun et al.	2014	34	China	qPCR	Concentrations of 100 bp and 400 bp fragments and the ratio of 400-/100-bp DNA were higher in RC than in healthy group (p<0.01). Mutated *KRAS* and methylated *MGMT* were not found in cfDNA of healthy controls.
Shalaby et al.	2017	93	Egypt	PCR	*MGMT* or *ERCC1* were methylated for 4.7% and 7% in the blood of patients with benign lesions and for 58% and 60% in RC patients (p < 0.001).
Zhang et al.*	2019	10	China/USA	NGS	Mutation concordance rate among ctDNA and tissue was 50% in RC patients.
			Predict/Monitor Treatment Response		
Zitt. et al.	2008	26	Austria	qPCR	Post surgery cfDNA responders: 2.2 ng/ml; cfDNA non responders: 5.1 ng/ml (p = 0.006).
Agostini et al.	2011	67	Italy	qPCR	Baseline cfDNA levels not correlated with tumor response. In responders, the median levels of Alu 247 and the cfDNA integrity index (the ratio of 400-/100-bp DNA fragments) were significantly lower after CRT compared to baseline (p = 0.0048 and 0.0005, respectively).
Sun et al.	2014	34	China	PCR	The good response group had significantly higher baseline 400-bp DNA levels and DNA integrity index. Good response group had lower cfDNA integrity after CRT compared before CRT. MGMT promoter methylation at baseline was higher in responders, with no reduction after treatment, while the rate of KRAS mutation decreased in both groups after CRT.
Shalaby et al.	2017	93	Egypt	PCR	Significant correlation between baseline MGMT and ERCC1 methylation and response to CRT.
Schou et al.	2018	123	Denmark	Fluorescence	No differences in cfDNA levels between before and after CRT.
Carpinetti et al.	2015	4	Brazil	Whole genome sequencing	ctDNA levels decreased in RC achieving response to CRT.
Tie et al.	2018	159	Australia	NGS	No association between post-CRT ctDNA status and pCR. Postoperative ctDNA detection was associated with high-risk pathological factors such as ypT3-4 and ypN1-2 stage.

Li et al.* 1	2017	30	China	NGS	ctDNA predicts change in tumor burden better than CEA.
McDuff et al.* 1	2019	31	USA	NGS	The rate of R0-NN resection was higher among pts with undetectable preoperative ctDNA compared to pts with a detectable ctDNA.
Chen et al.* 1	2019	9	USA	qPCR	Methylated *BCAT1* or *IKZF1* genes were found in 5/9 patients. Correlation between decrease of methylation and partial/complete response.
Zhou et al.* 1	2019	61	China	NGS	Correlation between undetectable preoperative ctDNA status and pathological downstage (p=0.02). Correlation between preoperative ctDNA positivity and the persistently involved lymph node (p = 0.02).
Yang et al.* 1	2019	119	China	NGS	TP53 and APC gene in pre-treatment samples negatively correlated with response to nCRT. Detection of pre-treatment mutations in any time points during nCRT was significantly (P = 0.03) decreased from TRG3 to TRG0 group.
Predicting disease recurrence					
Tie et al. 1	2018	159	Australia	NGS	Worse RFS if ctDNA was detectable after CRT or after surgery (estimated 3-year recurrence-free survival was 33% for postoperative ctDNA-positive patients vs 87% for ctDNA-negative).
Schou et al. 1	2018	123	Denmark	Fluorescence	High risk of recurrence pts with baseline cfDNA levels above 75th quartile (HR=2.48, 95% P=0.007).
Carpinetti et al. 1	2015	4	Brazil	Whole genome sequencing	Changes of ctDNA levels after surgery predict tumour recurrence.
McDuff et al* 1	2019	31	USA	NGS	Patients with detectable postoperative ctDNA had worse RFS.
Khakoo et al.* 1	2018	47	UK	Sequencing	ctDNA level was higher in pts who showed metastases (64%) related to pts that did not (8.3% P = 0.0005).
Chen et al.* 1	2019	9	USA	qPCR	Patients with high levels of methylated *IKZF1* and *BCAT1* in post-treatment ctDNA recurred 2 months after surgery.
Sclafani et al. 0	2018	97	UK	ddPCR	No difference in outcome between patients with or without detectable ctDNA after CRT.
Yang et al.* 1	2019	119	China	NGS	Detection of pre-treatment mutations in ctDNA after completion of nCRT was significantly associated with worse DFS.

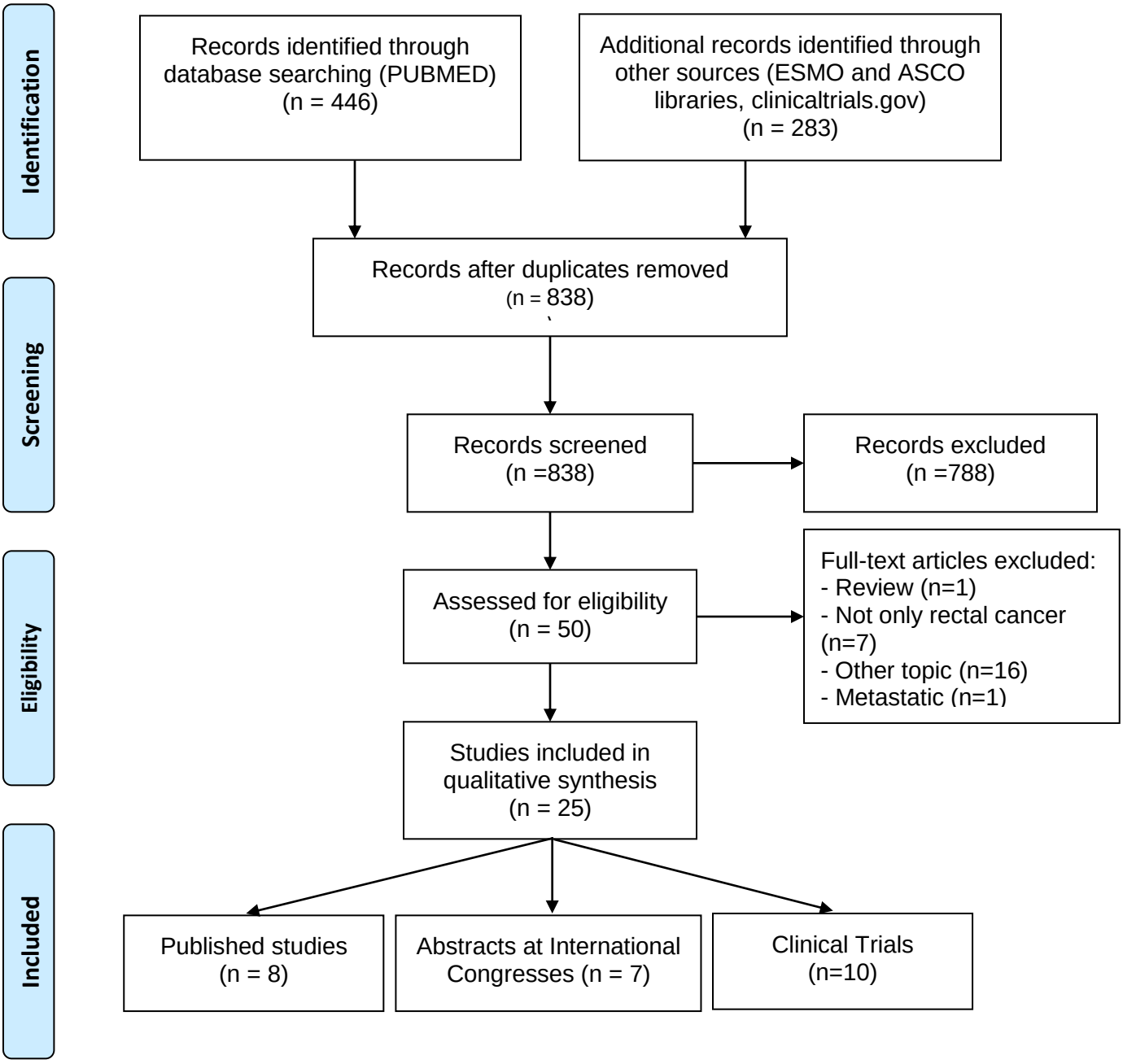
cfDNA: circulating free DNA; RC: rectal cancer; ctDNA: circulating tumor DNA; CRT: chemoradiotherapy; pCR: pathologic complete response; NN: node negative; TRG: tumor regression grade; RFS: relapse-free survival; DFS: disease-free survival.

Table 2. Ongoing studies investigating the role of cf/ct-DNA in non-metastatic rectal cancer.

Study (Study ID) STATUS	Location	Phase	Pts	cf/ct DNA related outcomes
Total Neoadjuvant Treatment Without Surgery For Locally Advanced Rectal Cancer: Prospective Clinical Trial To Assess Tumor Complete Response, Circulating Tumor Genetic And Epigenetic Biomarkers, And Stromal Transcriptome To Interpret Clinical Outcome (NO-CUT) (NCT03565029)	Italy	II	180	**PROGNOSTIC**: local a/o relapse free survival
Circulating Tumour DNA (ctDNA) Rectal Cancer and the Relationship to Extramural Venous Invasion (NCT02579278)	UK	Observational, Prospective	40	**PREDICTIVE**: presence or absence of ctDNA post CRT in EMVI-positive rectal cancer
Application of Circulating Tumor DNA Test in the Diagnosis and Treatment of Patients With Advanced Rectal Cancer (NCT03615170)	China	Observational, Prospective	200	**DIAGNOSTIC**: explore the feasibility of ctDNA as a detection index for rectal cancer **PREDICTIVE**: evaluation of preoperative concurrent chemoradiotherapy, so as to provide guidance for subsequent treatment **PROGNOSTIC**: search for possible recurrence related mutations
Observational Study on Rectal Cancer to Verify if Response After Chemo-radiotherapy Can be Predicted With a Particular Blood Test. (LiBReCa) (NCT03699410)	Switzerland	Observational, Prospective	35	**PREDICTIVE**: negative prognostic value of ctDNA drawn from the mesenteric and peripheral blood to investigate if can predict the response after chemo-radiotherapy and before surgery
A Study of the Role of Circulating Tumor DNA in Predicting the Likelihood of Organ Preservation After Clinical Complete Response to Neoadjuvant Therapy for Rectal Cancer (NCT03749083)	USA	Observational, Prospective	55	**PROGNOSTIC**: local recurrence rate
Investigation of the Value of ctDNA in Diagnosis, Treatment, and Surveillance of Surgically Resectable Colorectal Cancer – *Cohorts for T1-2N0 rectal cancer who undergo local or radical resection* (NCT03038217)	China	Observational, Prospective	300	**PROGNOSTIC**: disease free survival, local recurrence rate, overall survival
MRI Simulation-guided Boost in Short-course Preoperative Radiotherapy for Unresectable Rectal Cancer (SUNRISE) (NCT03714490)	China	Phase II	200	**PREDICTIVE**: predicting of treatment response **PROGNOSTIC**: survival
Multicenter, Prospective, RCT：Investigation of Combined Modality Therapy for Locally Advanced Mid/Low Rect (NCT03042000)	China	Prospective, observational	1200	**PREDICTIVE**: predicting the therapeutic effects of NCRT **PROGNOSTIC**: disease free survival
Totally Neoadjuvant FOLFOXIRI + Short-course Radiation + XELOX in Patients With Locally Advanced Rectal Cancer (NCT03484221)	China	Phase II	30	**PROGNOSTIC**: survival
Preoperative Chemoradiotherapy With Raltitrexed for Intermediate or Locally Advanced Rectal Cancer in the Fit Elderly (NCT02992886)	China	Phase II	68	**PREDICTIVE**: predictive treatment response **PROGNOSTIC**: disease free survival

Figure 1

PRISMA 2009 Flow Diagram



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit www.prisma-statement.org.

Figure 2

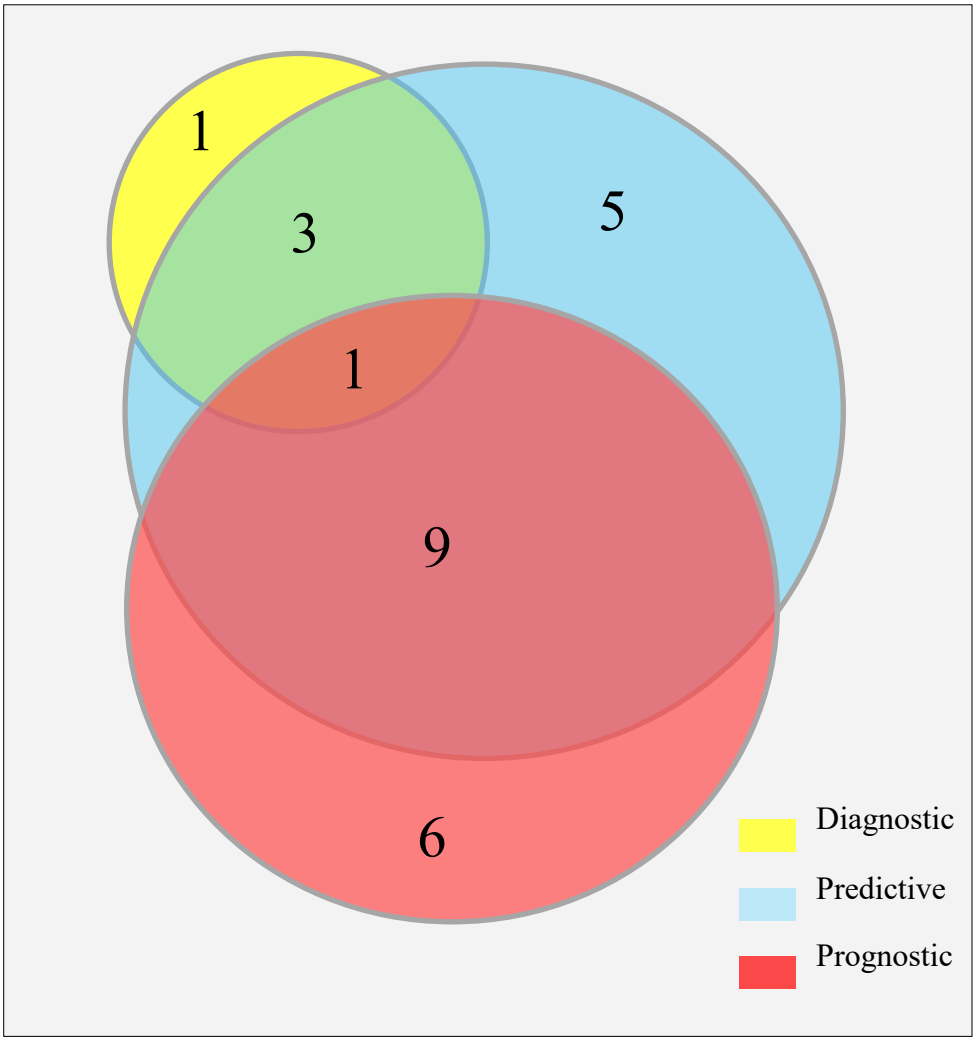
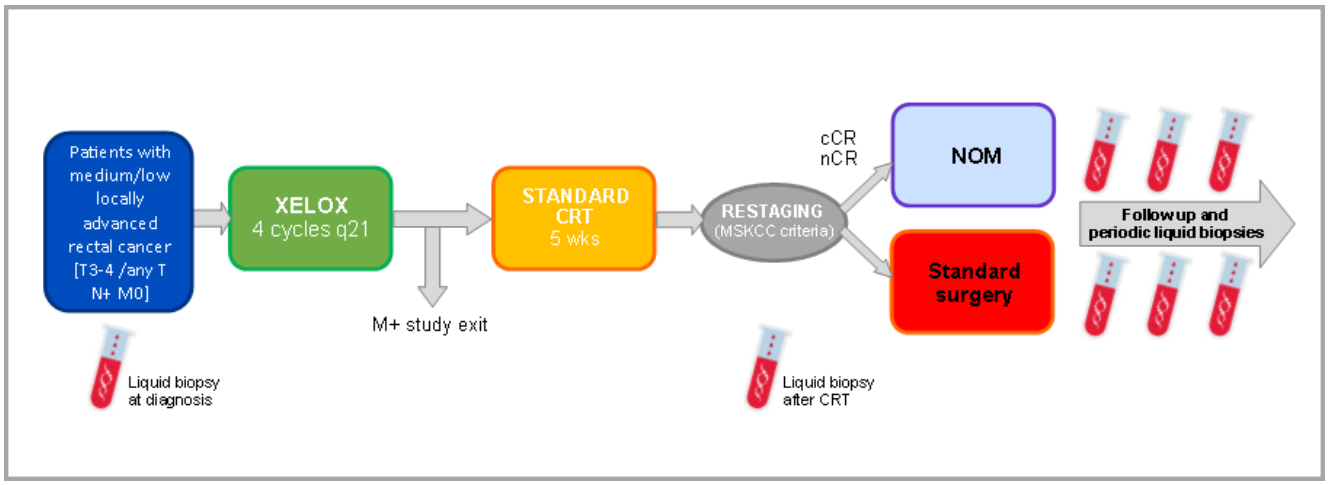
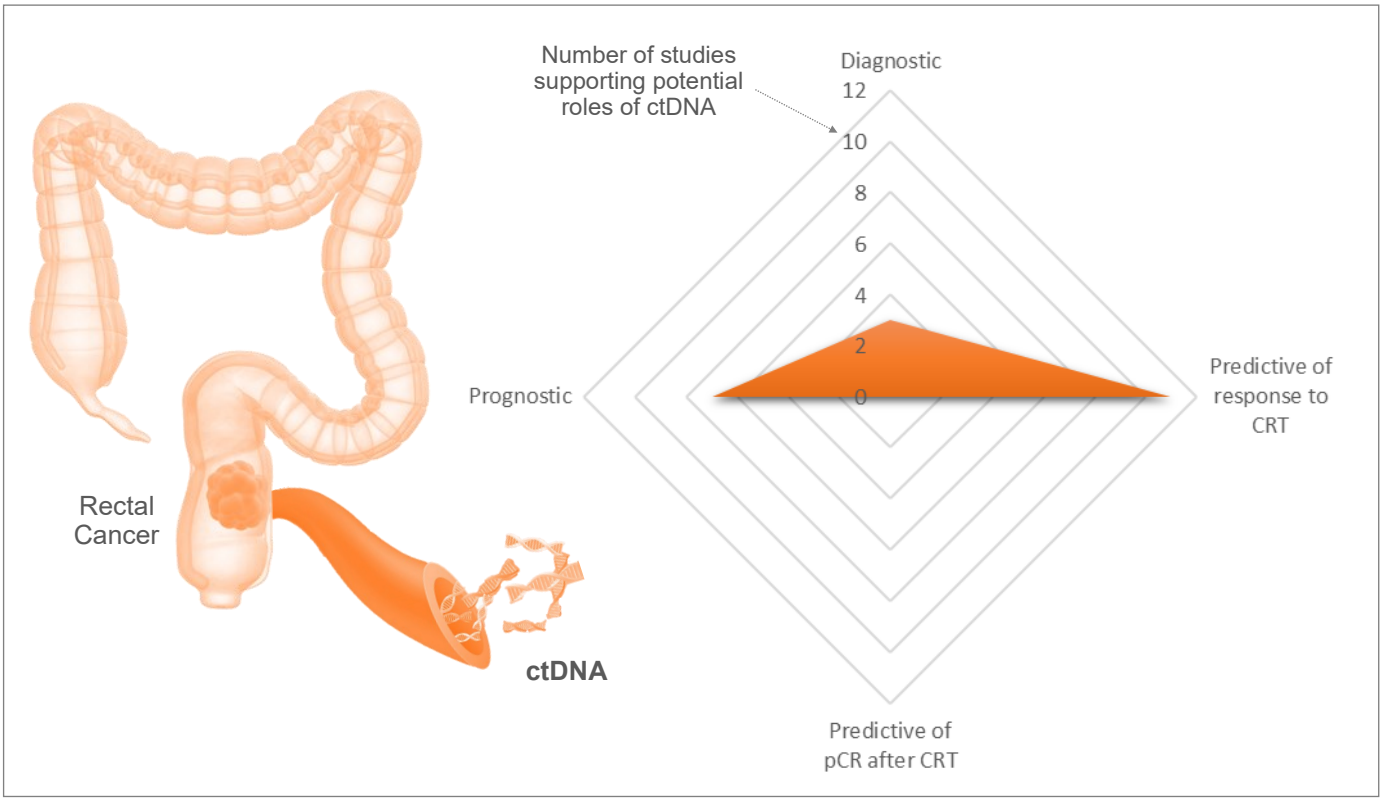


Figure 3


Patients with medium/low locally advanced rectal cancer [T3-4 /any T N+ M0]
XELOX
4 cycles q21
M+ study exit
STANDARD CRT
5 wks
RESTAGING
(MSKCC criteria)
cCR
nCR
NOM
Standard surgery
Follow up and periodic liquid biopsies
Liquid biopsy at diagnosis
Liquid biopsy after CRT

Graphical Abstract (for review)



***Highlights (for review)**

- Liquid biopsy for obtaining tumor-derived component such as circulating tumor DNA (ctDNA) might be used as a biomarker for improving rectal cancer management
- ctDNA is under study in rectal cancer as for diagnostic, predictive and prognostic utility
- Monitoring response to chemoradiation and assessing the risk of disease recurrence are the most advanced potential applications for liquid biopsy in rectal cancer