European Journal of Cancer 207 (2024) 114159

Contents lists available at ScienceDirect

European Journal of Cancer

journal homepage: www.ejcancer.com

Review

A review of trials investigating ctDNA-guided adjuvant treatment of solid tumors: The importance of trial design

Noortje Verschoor *, Manouk K. Bos, Esther Oomen-de Hoop, John W.M. Martens, Stefan Sleijfer, Agnes Jager, Nick Beije

Department of Medical Oncology, Erasmus MC Cancer Institute, Erasmus University Medical Center, the Netherlands

ARTICLE INFO

Keywords:
CtDNA
Cell-free DNA
Solid tumors
Adjuvant treatment

ABSTRACT

Circulating tumor DNA (ctDNA) holds promise as a biomarker for guiding adjuvant treatment decisions in solid tumors. This review systematically assembles ongoing and published trials investigating ctDNA-directed adjuvant treatment strategies. A total of 57 phase II/III trials focusing on ctDNA in minimal residual disease (MRD) detection were identified, with a notable increase in initiation over recent years. Most trials target stage II or III colon/colorectal cancer, followed by breast cancer and non-small cell lung cancer. Trial methodologies vary, with some randomizing ctDNA-positive patients between standard-of-care (SoC) treatment and intensified regimens, while others aim to de-escalate therapy in ctDNA-negative patients. Challenges in trial design include the need for randomized controlled trials to establish clinical utility for ctDNA, ensuring adherence to standard treatment in control arms, and addressing the ethical dilemma of withholding treatment in high-risk ctDNA-positive patients. Longitudinal ctDNA surveillance emerges as a strategy to improve sensitivity for recurrence, particularly in less proliferative tumor types. However, ctDNA as longitudinal marker is often not validated yet. Ultimately, designing effective ctDNA interventional trials requires careful consideration of feasibility, meaningful outcomes, and potential impact on patient care.

1. Background

A large proportion of patients with solid tumors is surgically treated with curative intent in early-stage disease. In a subset of those patients, a limited number of tumor cells has spread through the bloodstream to form micro-metastatic disease in the absence of measurable disease: so-called minimal residual disease (MRD). Adjuvant therapies such as radiation, chemo-, targeted-, and immunotherapies are intended to eradicate MRD after curative intent treatment of the primary lesion. The overall aim of adjuvant treatment is to achieve a clinically meaningful improvement in overall survival (OS) without unacceptable long-term diminishment of quality of life (QoL). Whether an intervention is clinically meaningful is defined by the European Society of Medical Oncology as >5 % improvement of survival at ≥ 3 years of follow-up or for studies without mature survival data, an improvement in disease-free survival (DFS) with an HR < 0.65 (lower limit of the 95 % confidence interval). Non-inferior OS or DFS with reduced treatment toxicity or improved QoL can also qualify as clinically meaningful [1]. Hence, adjuvant treatment should be withheld in patients in whom it does not improve OS,DFS or QoL meeting the criteria as mentioned. In most tumor types, the added value of adjuvant treatment has been demonstrated in patients with disease characteristics that are associated with a higher risk of relapse [2–5]. Therefore, those clinicopathological factors currently play an important role in the decision whether to start adjuvant treatment. Although clinicopathological characteristics currently provide the strongest prognostic information, a proportion of patients with high-risk tumors is still being overtreated, causing unnecessary toxicity. The latter poses the question whether additional biomarkers could provide further additional prognostic information to better guide adjuvant treatment choices.

Liquid biopsies are currently the most convenient biomarkers to serve such purpose. Liquid biopsies refer to tumor-derived materials, such as cell or DNA fragments, that circulate in a patients' blood plasma. Of those liquid biopsies, circulating tumor DNA (ctDNA) is the most studied entity, probably because methods to detect ctDNA are most straight forward [6]. As such, ctDNA could be used a marker for MRD after curative treatment [7–11]. Clinical trials have been designed to evaluate the clinical utility of ctDNA detection as an early marker of

* Correspondence to: Postbus 2040, Room Be-414a, 3000 CA, Rotterdam, the Netherlands.
E-mail address: n.verschoor@erasmusmc.nl (N. Verschoor).

https://doi.org/10.1016/j.ejca.2024.114159
Received 16 April 2024; Received in revised form 24 May 2024; Accepted 30 May 2024
Available online 6 June 2024

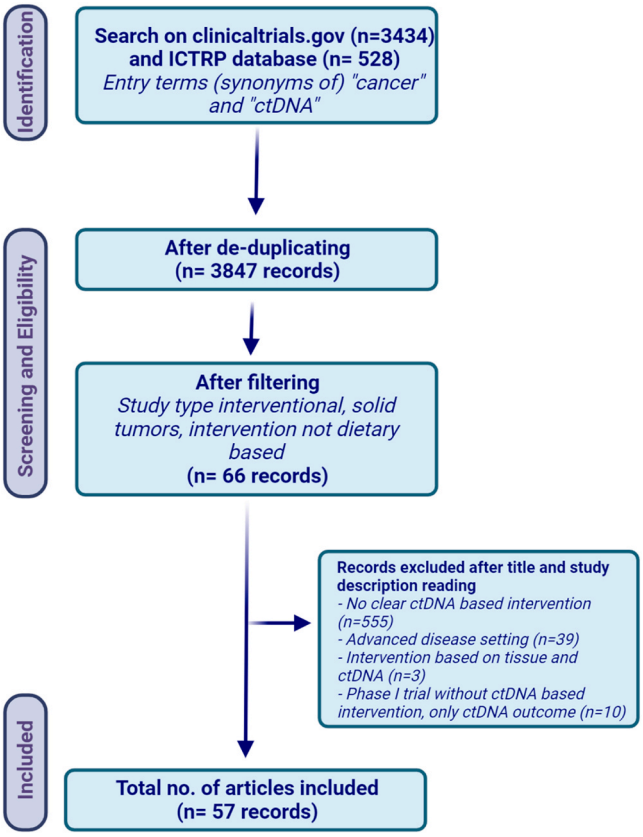

Fig. 1. Flowchart of entry selection.

recurrence, with the ultimate goal to apply adjuvant treatment strategies more precisely [12,13]. The general hypothesis is that patients with MRD detected by ctDNA should be treated with adjuvant treatment, or more intensive adjuvant treatment when this is already the standard of care, to eradicate remaining micro-metastatic disease and improve overall survival. On the other hand, ctDNA might also be of help to identify those patients in whom adjuvant treatment can be safely withheld.

There is an ongoing discussion on the appropriate design of clinical studies involving ctDNA in the adjuvant setting [14,15]. Since it has already been widely established that patients with detectable ctDNA usually have a very high risk of relapse [7,8,10], trials designed to investigate additional treatment in patients with detectable ctDNA in a randomized manner are sometimes deemed unethical, especially if treatment is not escalated in these patients. For certain tumor types or stages however, adjuvant therapy has never been demonstrated to improve OS or DFS and is therefore not the standard of care (SoC) in the first place, rendering randomization as a necessary step to draw meaningful conclusions. This emphasizes the importance of proper trial design that is fitted for the clinical question that is being posed. In this review, we systematically assemble a comprehensive list of ongoing and published studies investigating ctDNA-directed adjuvant treatment, describe utilized trial designs and discuss their benefits and pitfalls.

2. Methods

2.1. Clinical database search

We set out to summarize all running phase II and III interventional trials on ctDNA in the MRD setting in solid tumors. We searched trial databases *clinicaltrials.gov* and the WHO International Clinical Trials Registry Platform (ICTRP) on the 1st of September 2023 with (synonyms of) "cancer" and "circulating tumor DNA" or "minimal residual disease" and subsequently filtered on study type 'interventional', selected solid tumors in 'conditions' and excluded dietary interventions (Fig. 1). Then, we independently (MB and NV) selected all currently published, running or upcoming trials which allocated an intervention according to ctDNA status. We excluded phase I trials that merely used ctDNA levels as a surrogate marker for response and study records that were in non-English, limiting their interpretation.

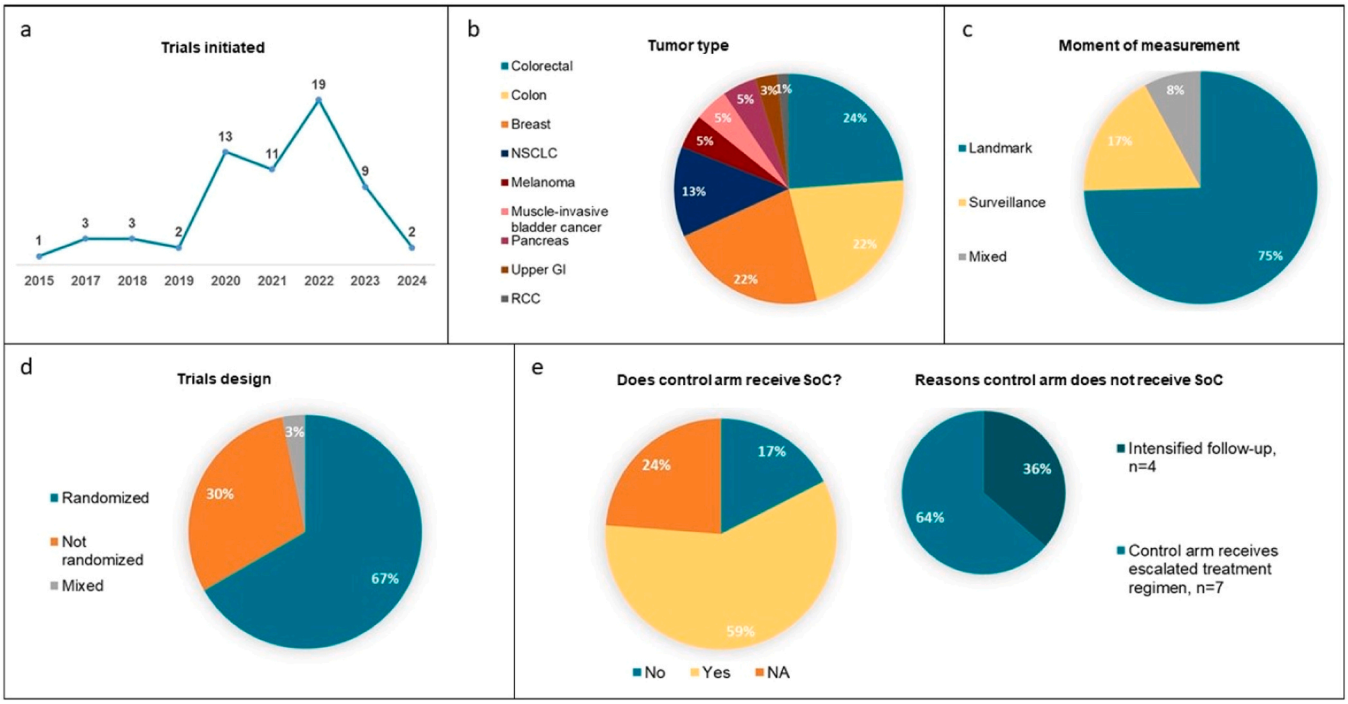

Fig. 2. Overview of trial characteristics.

2.2. Data extraction

We searched for detailed trial information on clinicaltrials.gov, the WHO ICTRP database, or in published trial designs. Trial information that we collected was trial number, trial name, initiation year (i.e., actual or estimated start of inclusion), target accrual, tumor type, ctDNA detection moment (landmark meaning single observation, surveillance meaning repeated measurement), intervention, clinical management of the control group, primary outcome and whether patients will be randomized.

All the above was performed by two authors independently (MB and NV) and in case of discordance, this was discussed until agreement was achieved. Furthermore, we registered for each individual study whether the control arm received SoC treatment. SoC treatment was defined as treatment or follow-up approaches according to NCCN, ASCO or ESMO guidelines.

3. Results

3.1. Characteristics of ongoing trials

We found 57 records of phase II/III trials which reported ctDNA-based interventional trials related to MRD detection. As shown in Fig. 2, ctDNA MRD trials were more often initiated in the last years. Most MRD interventional trials are being performed in stage II or stage III colon/colorectal cancer (n = 26, 46 %). Furthermore, tumor types include breast cancer (n = 12), non-small cell lung cancer (n = 8), melanoma (n = 3), muscle-invasive bladder cancer (n = 3), pancreatic cancer (n = 3) and esophageal/GEJ cancer (n = 2) (Figure 2b). The utilization of ctDNA as a surveillance marker where multiple sampling points are incorporated into the follow-up scheme and where the intervention is triggered if a patient becomes ctDNA positive at a given time during follow-up, is used in 18 % (n = 10) as a main strategy (Figure 2c). Randomization was performed in 68 % (n = 39) of trials (Figure 2d). In most trials, the control arm received treatment according to the current standard of care, being no treatment or a less intensive treatment regimen than in the experimental arm (Figure 2e). Notably, of these ten trials, six are being performed in breast cancer. This is presumably triggered by the fact that especially in hormone receptor positive breast cancer the recurrence risk remains high for years [16], and this is why endocrine treatment is administered for up to ten years in the high-risk group. Most trials used landmark ctDNA analysis at one fixed time point, usually post-operative. It should however be realized that increasing the number of time points for ctDNA analyses mightl result in a better overall sensitivity of the test [17,18].

Supplementary tables 1a-b provide a detailed overview per study record. Two studies, the DYNAMIC-study in colorectal cancer and the c-TRAK-TN trial in breast cancer, were published in a peer-reviewed journal [19,20]. To our knowledge, there are no other published trials in the adjuvant treatment setting that based their intervention on ctDNA detection. The chosen primary outcome measures were mostly recurrence free survival (RFS) or disease-free survival (DFS) (n = 31) or ctDNA dynamics, i.e., ctDNA clearance or drop (n = 20). Two trials use overall survival (OS) as the primary outcome measure and three trials use other outcome measures, for example 'number of evaluable patients enrolled'. Lastly, three trials focus on imaging or intensified follow-up, primary outcome measures are therefore related to sensitivity and specificity of ctDNA detection.

3.2. Trials performed in a setting in which adjuvant treatment is SoC

In tumor types where adjuvant treatment is the SoC (Supplementary Table 1a), the addition of adjuvant treatment has supposedly been proven to improve OS with acceptable QoL. In these tumor types, the decision whether to start adjuvant treatment and the type of adjuvant treatment is mostly dependent on the stage of the primary tumor and other clinicopathological factors associated with a high risk of recurrence. In these settings, determining whether ctDNA is detectable in blood might identify patients who will or will not benefit from adjuvant treatment. Of the identified trials, all but one are aiming to escalate SoC therapy on the basis of ctDNA detection. Most studies in this category randomize only ctDNA positive patients between SoC and an intensified regimen using a landmark ctDNA analysis, such as the APOLLO trial in stage II-III triple negative breast cancer (TNBC; NCT04501523), the MERMAID trials in stage II-III non-small cell lung cancer (NSCLC, NCT04385368, NCT04642469) and the AFFORD (NCT05427669) trial in high-risk stage II or stage III colorectal cancer. The APOLLO trial investigates the addition of the Programmed Cell Death Protein 1 (PD1)-inhibitor tislelizumab to SoC capecitabine chemotherapy; taking into account that SoC chemotherapy is given to patients without evidence of pathological complete response (pCR) after neoadjuvant chemotherapy. Additionally, it investigates capecitabine in the ctDNA positive patients who achieved pCR. As such, patients with detectable ctDNA after neoadjuvant chemotherapy are randomized between tislelizumab and capecitabine in case of non-pCR, or capecitabine or observation in case of pCR. Also using a post-resection landmark analysis, the MERMAID-trials in NSCLC include ctDNA positivity to evaluate the efficiency of durvalumab in ctDNA-positive patients. Other studies, which are mainly performed in hormone-receptor positive early breast cancer, perform longitudinal ctDNA analyses and randomize patients between a new or intensified treatment regimen and SoC endocrine treatment when ctDNA becomes detectable, such as the LEADER trial (NCT03285412), the DARE trial (NCT04567420), the TRAK-ER trial (NCT04985266) and the TRAIL study (ACTRN12618001766202). In those studies, ctDNA negative patients receive treatment and follow-up according to SoC.

One study aims to not only escalate treatment in ctDNA positive patients, but also to de-escalate SoC when patients are ctDNA negative. In this CIRCULATE-US study (NCT05174169), stage II and stage III colon cancer patients, but not patients with T4 tumors, who are ctDNA negative are randomized between CAPOX or FOLFOX chemotherapy, which is SoC for highrisk stage II and stage III colon cancer patients, and ctDNA surveillance. In case ctDNA is detected and there are no radiological signs of metastatic disease, patients start with chemotherapy and are randomized between CAPOX and FOLFOXIRI, the latter being an escalated treatment regimen. As such, the CIRCULATE-US trial aims to investigate both escalation and de-escalation of treatment in patients with colorectal cancer.

Additionally, the VEGA-trial, performed in colon cancer, has the aim of de-escalating therapy in patients with undetectable ctDNA. In this trial, that is part of the CIRCULATE-Japan trial platform aiming to refine adjuvant therapy in colorectal cancer patients, high-risk stage II or low-risk stage III colon cancer patients in whom no ctDNA is detected, are randomized between observation and capecitabine. Patients in the observation group are prospectively monitored with serial ctDNA measurements and in case of a positive result, patients are included in the ALTAIR-trial, where they are randomized between trifluridine and tipiracil or placebo after they received SoC adjuvant chemotherapy.

The studies mentioned before, applied the approach of randomizing patients based on ctDNA presence. Another approach is to randomize patients between adjuvant treatment guided by standard clinicopathologic features and treatment guided by ctDNA, such as applied by the DYNAMIC series. In the DYNAMIC study, including stage II colon cancer, patients who were randomized to the ctDNA-directed group had ctDNA analyzed at week 4 and week 7 after surgery [19]. Patients with a positive ctDNA result at either week 4 or week 7 received adjuvant single-agent fluoropyrimidine or oxaliplatin-based chemotherapy, with the treatment regimen chosen at the clinician's discretion. Patients with negative ctDNA results at both week 4 and week 7 were subjected to observation. This design ultimately aimed for treatment de-escalation and was powered to detect non-inferior 2-year disease free survival in the ctDNA-directed arm with the overall hypothesis that a smaller proportion of patients would receive treatment in this arm.

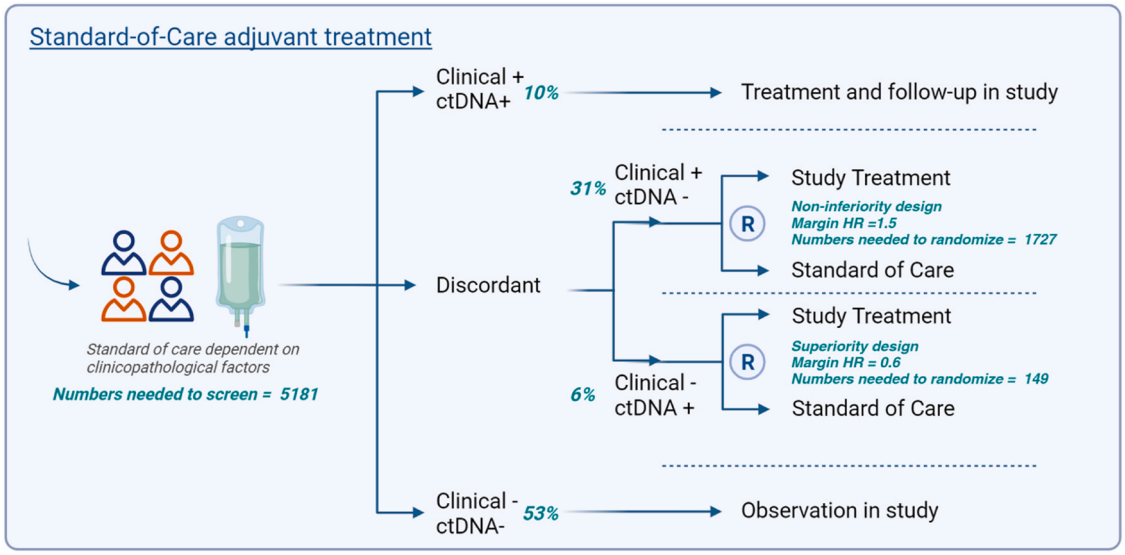

Fig. 3. Trial design displayed for stage II colorectal cancer in which current clinicopathological risk assessment is taken into account. The objective is to test the additional effect of the MRD-test using ctDNA over the current standard; if escalation is needed if MRD is detected, and if de-escalation is safe when MRD is not detected.

3.3. Trial methodology of studies in settings in which adjuvant treatment is SoC

When we revisit the objective of trials in the abovementioned group, the main research question should be *"Can ctDNA improve current clinical stratification to select patients who benefit from adjuvant treatment?"*, i.e., to prevent patients from being undertreated or to spare patients from overtreatment. We believe that the gold standard to establish the predictive value of ctDNA is a ctDNA interventional trial, randomizing ctDNA positive patients as well as ctDNA negative patients between treatment and control [21,22], before accepting this as standard practice. This is also justified given that the used technique comes with high costs (personalized assays are estimated around $1750 per patient) and complex logistics. When reviewing the trials listed in Supplementary Table 1a, it becomes evident that it is often challenging to adhere to the abovementioned design.

The trial methodology of currently running studies listed in Supplementary table 1a that address this issue have several pitfalls. First, ctDNA-positive patients are frequently included in single-arm trials without randomization. This is most likely triggered by cumulative evidence from observational studies which report a very high recurrence risk in patients with detectable ctDNA. Some retrospective data suggests that ctDNA-positive patients have inferior outcome compared to ctDNA-negative patients despite standard of care chemotherapy [23]. As a result, withholding the study intervention from ctDNA-positive patients is ethically challenging. However, it has often not been proven yet that intensifying treatment in the ctDNA-positive subgroup improves outcome, as ctDNA might be a mere prognostic marker. Randomization is necessary to provide the much-wanted answer if more treatment is indeed leading to improved outcome, outweighing the associated toxicities from a more intensified treatment. Cost-effectiveness analyses of such strategies are imperative, since this would lead to a major increase in costs in clinical care.

Second, in trials where the ctDNA positive subgroup is randomized, patients in the control arm receive treatment that is not SoC, but an escalated version of current SoC. In five studies, the treatment or follow-up in the control arm of the ctDNA positive group is escalated. For example, in colon cancer trials (CIRCULATE-US and CIRCULATE-SPAIN-01), all stage II colon cancer patients receive an oxaliplatin-based schedule that is not standard treatment in all stage 2 colon cancer patients according to current guidelines [24]. Also, three studies performed in colon cancer escalate follow-up through intensifying the number of imaging scans in ctDNA positive patients. The control arm should however always adhere to the current standard of care, or it will be impossible to evaluate the added value of ctDNA testing.

Third, ctDNA-negative cases usually do not get included in the trial. Ultimately, this will not answer the question whether de-escalation in a selected group of patients is justified. A seemingly straightforward solution to include ctDNA-negative patients in the study design is to randomize patients between a clinically guided arm and a ctDNA-guided arm, like in the DYNAMIC trial. However, the design of the DYNAMIC study has been under debate [25–28]. Although the authors from the study concluded that a ctDNA-based approach resulted in less chemotherapy without the loss of efficacy, it is not very likely that clinicians will omit adjuvant chemotherapy in clinically high-risk stage II colon cancer patients who are ctDNA negative. The study was not powered to demonstrate non-inferiority in this subgroup, but only for the ctDNA directed strategy as a whole. From the observational GALAXY cohort of the CIRCULATE trial, it is strongly suggested that ctDNA positivity is predictive for the benefit of adjuvant chemotherapy in high-risk stage II patients, adjusted for clinicopathological factors [23]. Importantly, in the DYNAMIC ctDNA-negative group there was a recurrence rate of 6 % after 3 years, as opposed to the 14 % in the ctDNA positive group. But numerically, recurrence occurred three times more often in the untreated ctDNA negative patients than in the treated ctDNA positive patients. These recurrences occurred almost exclusively in clinically high risk patients. Therefore, introducing the ctDNA strategy in clinical practice will likely lead to more expensive testing without the intended treatment de-escalation for the group as a whole, because clinicians will be hesitant to withhold treatment in the clinically high risk group.

To deal with the described issues, a design like used in the MINDACT trial might provide a solution. The MINDACT trial was specifically designed to prove the clinical utility of the addition of the 70-gene signature to standard clinical-pathological criteria in selecting breast cancer patients for adjuvant chemotherapy. To this end, the investigators specifically randomized the patients who had discordant clinical and gene signature results. When we apply this design on stage II colorectal cancer, numbers are displayed in Fig. 3. In this trial design only the discordant cases are randomized, while the truly high risk patients are being treated to reflect current clinical practice. This trial design also helps to focus more on de-escalation of therapy in truly low risk patients, since sparing patients from overtreatment should be a

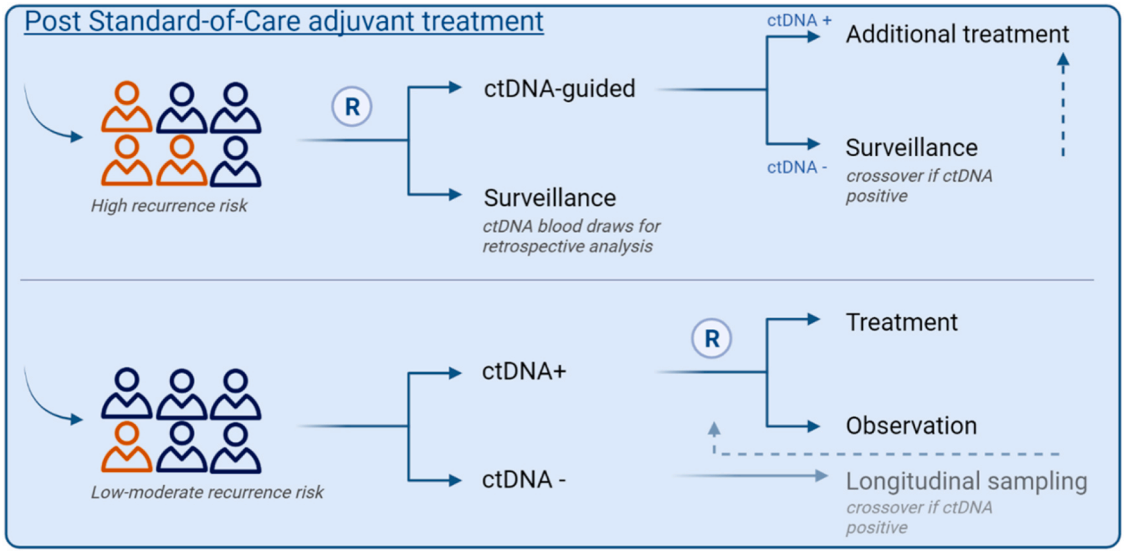

Fig. 4. Trial design displayed for post standard-of-care settings.

main goal of trials performed in adjuvant care. In the non-inferior part of the trial, which is aimed at safe de-escalation of therapy in ctDNA-negative patients, consensus will have to be reached for what an acceptable goal is in the specific context. To illustrate, we performed a hypothetical power calculation in stage II colorectal cancer (Fig. 3). We calculated the number of patients that need to be included with a primary endpoint of recurrence free survival, with a non-inferiority margin of 4.2 % for the analysis of 3-year RFS. The proposed trial design asks for a high rate of patient inclusion, but with 520.000 new cases of colorectal cancer across Europe yearly, of which 30 % is estimated stage II, this should be feasible in a collaborative effort [29]. Incorporating a surveillance strategy that has proven to increase the sensitivity for disease recurrence in most tumor types [7] is also possible in this trial design, although it might introduce difference in surveillance times and increase lead time bias.

3.4. Trials performed in a setting in which adjuvant treatment is not SoC

The majority of trials (56 %) aim to identify patients in whom micrometastatic disease was not eradicated after completion of SoC adjuvant therapy, as defined by measurable ctDNA (Supplementary Table 1b). The main research question and primary goal in this setting is *"Is it possible to identify those patients who were not cured with standard of care and to improve outcome in these patients?"* The finding that most ongoing trials are designed to escalate treatment when a patient is MRD positive after completion of SoC is, therefore, not surprising. Given the previously mentioned high risk of relapse in ctDNA positive patients, this design is considered straightforward. Generally, two approaches are used in this setting. Most studies test all patients and randomize ctDNA-positive patients between additional treatment and observation according to SoC, whilst one study (COBRA, NCT04068103) randomizes patients upfront between a ctDNA-directed approach and SoC active surveillance, with ctDNA stored for analysis in hindsight which allows for retrospective analysis of the true predictive value of ctDNA. In addition, some phase II trials investigate the efficacy of tebentafusp, an immune-mobilizing monoclonal T-cell receptor against cancer, which has shown significant benefit for metastatic uveal, but not cutaneous melanoma [30], in patients with detectable ctDNA in a non-randomized design, for example the TebeMRD (NCT05315258) study. Here, 850 patients with high risk (stage not defined) melanoma (600 cuteaneous and 250 uveal melanoma) are prospectively screened for detectable ctDNA and if ctDNA is detected, treated with tebentafusp after SoC immunotherapy. Indeed, testing a strategy that proved to be effective in metastatic patients may be effective as adjuvant treatment in the ctDNA-positive subgroup. In this case, the recurrence rate in the experimental group will have to be compared to the untreated ctDNA positive subgroup from observational cohorts, which is not ideal.

Most trials start treatment that is already established in metastatic disease, like in the TOMBOLA study (NCT04138628) in muscle-invasive bladder cancer, where ctDNA-positive patients will be treated with atezolizumab. Interestingly, the Imvigor011 trial (NCT04660344) is evaluating efficacy of atezolizumab in ctDNA-positive patients in the same patient group, but in a randomized and placebo-controlled manner. In this tumor type and stage, ctDNA was previously identified as a possible predictive marker in a retrospective analysis of the Imvigor010 study, in which patients with muscle invasive bladder cancer were randomized between atezolizumab or observation after surgery [21,31]. Therefore, the prospective Imvigor011 study will provide important insights into whether ctDNA-guided administration of atezolizumab will improve outcome. This emphasizes the importance of well-designed, preferably randomized, trials following biomarker analysis from currently running unselected adjuvant trials.

The c-TRAK TN trial investigated the utility of ctDNA testing post SoC and the efficacy of pembrolizumab in high-risk stage II-III breast cancer patients by randomizing ctDNA-positive patients to pembrolizumab or observation [20]. This was the first ctDNA-driven MRD intervention study in breast cancer. Unfortunately, about 70 % of the patients in the treatment arm appeared to have metastatic disease found by staging at the time of ctDNA detection, leading to high drop-out rates in the treatment arm. Interestingly, the observation arm of the study was prematurely closed due to the high relapse rate and short lead time of patients in whom ctDNA was detected. As a result, all ctDNA positive patients crossed over to the treatment arm. Additionally the ZEST trial, evaluating TNBC patients, was terminated early because of the high rate of patient with overt metastatic disease upon a positive ctDNA result.

3.5. Trial methodology of studies in settings in which adjuvant treatment is not SoC

Longitudinal ctDNA surveillance is increasingly performed as a strategy to identify those patients who are at high risk of recurrence. Cohort studies have shown that this method of sampling is associated with a higher accuracy for predicting disease recurrence than a single post-operative landmark analysis [7]. But this is also dependent on the inherent risk of recurrence associated with the tumor biology and the time frame in which this recurrence occurs, e.g. hormone receptor

positive breast cancer is a slow growing disease, resulting in low ctDNA shedding. Apparently, the risk of recurrence is an important caveat in the design of these trials, given the fact that the control group of the TRAK-TN study was halted for the high amount of recurrences in the ctDNA positive group. This reconfirms previous findings that ctDNA positivity is associated with worse prognosis and may pose an ethical dilemma in randomizing ctDNA positive patients to observation, especially in those patient groups which have high recurrence risks. However, a high recurrence risk should not justify exposing ctDNA positive patients to a treatment that has no proven effectiveness in that patient group. Moreover, these findings should not be generalized to all tumor types. The c-TRAK TN trial included patients with TNBC which is known to be a highly proliferative disease with half of the high-risk patients relapsing after initial treatment. This is also evidenced by the observed lead-time between ctDNA detection and disease recurrence at only 1.6 months. However, for less proliferative tumor types such as ER-positive/HER2-negative breast cancer or low stages of colorectal cancer median lead times are much longer, so allocating ctDNA positive patients to observation remains reasonable [15,32]. Therefore, randomizing patients between treatment and placebo or observation in disease settings with moderate to low risk of recurrence will be preferable, as visualized in Fig. 4. The risk of recurrence may also be estimated by the lead time, investigated in cohort studies, or by longitudinal ctDNA analysis, enabling risk assessment by ctDNA doubling time [33]. Accordingly, in these less proliferative tumor types SoC treatment eradicated most micro-metastases and residual disease may grow out slowly, therefore a landmark analysis alone may not be sufficient. Several authors have shown that longitudinal sampling, i.e., MRD surveillance, increases sensitivity for recurrence without losing specificity [34–36]. Longitudinal tracking and cross over to treatment if ctDNA becomes positive probably increases the treatment effect in the post SoC setting. Alternatively, if the risk of recurrence is found to be unacceptably high, alike the c-TRAK-TN study, investigators might consider a design like the COBRA study, in which patients are randomized between SoC, being surveillance, and a ctDNA directed approach (Fig. 4). As such, all patients who are prospectively tested positive for ctDNA will be allocated to treatment whilst the ctDNA status remains unknown in the patients randomized to surveillance. This approach was also discussed in the previous paragraph where we discussed the setting in which adjuvant treatment is SoC. In that setting, this approach would not suffice as current strategies also include clinicopathologic factors that could not be ignored. The retrospective analysis in the surveillance arm will serve as control arm with the interpretation of results in the ctDNA guided arm.

Concluding, in post standard-of-care settings the trials in patient groups with a high recurrence risk are most feasible in terms of inclusions needed because of the higher event rates. However, when the recurrence risk becomes too high, because of a high proliferating tumor, ctDNA analysis might not be of additional value for patient stratification. Patient groups with lower recurrence risks will require larger trials, but will answer the important question if ctDNA is able to pre-select patients who will gain the most benefit from treatment.

3.6. General methodology considerations

Some trials use ctDNA conversion or ctDNA clearance as their primary endpoint to draw conclusion about treatment efficacy. Although it is of interest to investigate ctDNA dynamics in an exploratory manner, its use as a surrogate marker has not yet been sufficiently validated. Furthermore, ctDNA assessment methods are heterogeneous as some studies are looking for a 30 % drop in ctDNA load whilst others use complete ctDNA clearance as an endpoint. Importantly, complete ctDNA clearance is dependent from the LOD of the analytical assay that is used. A well-performed study correlated ctDNA clearance to response rate and overall survival in data from retrospective studies in metastatic malignancies [37]. The authors found that ctDNA response rate had a high correlation with median survival and was even superior to overall response rate according to RECIST as a surrogate end-point. CtDNA dynamics for the early setting are yet to be established as response marker, something that the currently running trials might be suitable for. Nonetheless, the FDA recently suggested in a draft guidance that such data should come from randomized trials and including a patient population representative of the population in which the endpoint ultimately will be used [38]. As such, trials that collect ctDNA data before and after drug treatment should also collect long term outcome data to characterize the association between ctDNA clearance and outcome.

Table 1
Challenges and considerations for liquid biopsy based trials.

Challenge	Consideration
Validation of liquid biopsies as a stratification marker	The assays used for detection should have well-described analytical and clinical validity, including sensitivity, specificity and median lead time for the specific clinical subgroup.
Validation of liquid biopsy dynamics as a surrogate endpoint in current clinical trials	Dynamics as a surrogate endpoint require further validation. Understanding the correlation between changes in liquid biopsy levels and clinical outcomes is essential for establishing its reliability as an endpoint.
Randomizing patients with a positive assay	Randomization is justifiable, especially in more slowly proliferation tumors. If not, results are not interpretable and thus will lead to overtreatment and overuse of expensive diagnostics. Independently blinding patients who are assay-negative and who are assay-positive but not allocated to the treatment group is necessary.
Incorporation of current stratification based on clinicopathological factors	As the sensitivity of liquid biopsies assays is inferior to the specificity, it cannot replace current clinical practice as a standalone biomarker. The additional value of liquid biopsy-based risk stratification can only be evaluated in a trial that incorporates current risk stratification methods.
Superiority and non-inferiority	Given the significant efforts and costs associated with liquid biopsy assays, the superiority of incorporating liquid biopsies as a stratification marker should be demonstrated. Non-inferiority is only justifiable if the objective of the trial is the de-escalation of treatment.
Representation of de-escalation trials	An important research goal in the adjuvant treatment setting is to identify those who benefit and those who do not. As a guideline: if it has been proven from well-powered, preferably randomized data that liquid biopsy stratification is associated with ◦ A $>$ 5 % improvement in overall survival ◦ The lower limit of the CI of the hazard ratio is $\leq$ 0.65 in DFS without mature survival data then there is sufficient reason to omit randomization in the liquid biopsy positive arm (aligned with the ESMO-MCBS). Wide consensus has to be reached for the appropriate non-inferiority margin.

4. Conclusions

Circulating tumor DNA (ctDNA) is a promising liquid biopsy based biomarker currently undergoing testing as a stratification marker for adjuvant treatment in multiple trials. In general: there is a scarcity of retrospective ctDNA data from randomized controlled trials which

defined the current standard of care. Prospective, interventional trials are necessary to evaluate the additional value of these tests. However, the appropriate clinical trial design for ctDNA and other liquid biopsies as a biomarker poses a challenge due to the complex balance between trial feasibility in terms of patient inclusions and costs, and the need for meaningful outcomes. Table 1 summarizes the current challenges that were discussed in this review, including considerations for future interventional trials with liquid biopsies.

Overall, rethinking the clinical trial design for ctDNA interventional trials is vital to maximize their potential in affecting clinical decision-making for adjuvant treatment. It is essential to address these considerations and strike a balance between trial feasibility, meaningful outcomes, and the potential impact on patient care.

Funding

None declared.

CRediT authorship contribution statement

N. Verschoor: conceptualization, methodology, data curation, writing – original draft; visualization; **M.K. Bos:** conceptualization, methodology, data curation, writing – review & editing; **E. Oomen-de Hoop:** methodology, writing – review & editing; **J.W.M. Martens:** supervision, writing - review & editing; **S. Sleijfer:** supervision, writing - review & editing; **A. Jager:** supervision, writing - review & editing; **N. Beije:** supervision, methodology, writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2024.114159.

References

[1] Cherny NI, et al. ESMO-Magnitude of Clinical Benefit Scale version 1.1. Ann Oncol 2017;28:2340–66. https://doi.org/10.1093/annonc/mdx310.
[2] Cardoso F, et al. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Ann Oncol 2019;30:1194–220.
[3] Argilés G, et al. Localised colon cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up†. Ann Oncol 2020;31:1291–305. https://doi.org/10.1016/j.annonc.2020.06.022.
[4] Postmus PE, et al. Early and locally advanced non-small-cell lung cancer (NSCLC): ESMO clinical practice guidelines for diagnosis, treatment and follow-up†. Ann Oncol 2017;28:iv1–21. https://doi.org/10.1093/annonc/mdx222.
[5] Michielin O, van Akkooi ACJ, Ascierto PA, Dummer R, Keilholz U. Cutaneous melanoma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up††Approved by the ESMO Guidelines Committee: February 2002, last update September 2019. Ann Oncol 2019;30:1884–901. https://doi.org/10.1093/annonc/mdz411.
[6] Heitzer E, Haque IS, Roberts CES, Speicher MR. Current and future perspectives of liquid biopsies in genomics-driven oncology. Nat Rev Genet 2019;20:71–88. https://doi.org/10.1038/s41576-018-0071-5.
[7] Moding EJ, Nabet BY, Alizadeh AA, Diehn M. Detecting liquid remnants of solid tumors: circulating tumor DNA minimal residual disease. Cancer Discov 2021;11:2968–86. https://doi.org/10.1158/2159-8290.Cd-21-0634.
[8] Faulkner LG, Howells LM, Pepper C, Shaw JA, Thomas AL. The utility of ctDNA in detecting minimal residual disease following curative surgery in colorectal cancer: a systematic review and meta-analysis. Br J Cancer 2022. https://doi.org/10.1038/s41416-022-02017-9.
[9] Wang B, et al. Prognostic potential of circulating tumor DNA detection at different time periods in resectable non-small cell lung cancer: Evidence from a meta-analysis. Crit Rev Oncol Hematol 2022;177:103771.
[10] Cullinane C, et al. Association of circulating tumor DNA With disease-free survival in breast cancer: a systematic review and meta-analysis. JAMA Netw Open 2020;3:e2026921. https://doi.org/10.1001/jamanetworkopen.2020.26921.
[11] Lee RJ, et al. Circulating tumor DNA predicts survival in patients with resected high-risk stage II/III melanoma. Ann Oncol 2018;29:490–6.
[12] Hayes DF. Defining clinical utility of tumor biomarker tests: a clinician's viewpoint. J Clin Oncol 2020;39:238–48. https://doi.org/10.1200/jco.20.01572.
[13] Pascual J, et al. ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group. Ann Oncol 2022;33:750–68. https://doi.org/10.1016/j.annonc.2022.05.520.
[14] Andersen CL, Heitzer E. ctDNA-guided adjuvant chemotherapy for colorectal cancer—ready for prime time? Cancer Cell 2022. https://doi.org/10.1016/j.ccell.2022.08.017.
[15] LoRusso PM, Freidlin B. Improving precision oncology through better designs and reporting of biomarker-driven randomized clinical trials. JNCI: J Natl Cancer Inst 2022:djac212. https://doi.org/10.1093/jnci/djac212.
[16] Pan H, et al. 20-Year risks of breast-cancer recurrence after stopping endocrine therapy at 5 years. N Engl J Med 2017;377:1836–46. https://doi.org/10.1056/NEJMoa1701830.
[17] Henriksen TV, et al. Unraveling the potential clinical utility of circulating tumor DNA detection in colorectal cancer—evaluation in a nationwide Danish cohort. Ann Oncol 2024;35:229–39. https://doi.org/10.1016/j.annonc.2023.11.009.
[18] Pellini B, Chaudhuri AA. Circulating tumor DNA minimal residual disease detection of non-small-cell lung cancer treated with curative intent. J Clin Oncol 2022;40:567–75.
[19] Tie J, et al. Circulating tumor DNA analysis guiding adjuvant therapy in stage II colon cancer. N Engl J Med 2022;386:2261–72. https://doi.org/10.1056/NEJMoa2200075.
[20] Turner NC, et al. Results of the c-TRAK TN trial: a clinical trial utilising ctDNA mutation tracking to detect molecular residual disease and trigger intervention in patients with moderate and high-risk early stage triple negative breast cancer. Ann Oncol 2022. https://doi.org/10.1016/j.annonc.2022.11.005.
[21] Sumithra JM, Daniel JS. Clinical trial designs for predictive biomarker validation: theoretical considerations and practical challenges. J Clin Oncol 2009;27:4027–34. https://doi.org/10.1200/jco.2009.22.3701.
[22] Janes H, Pepe MS, Bossuyt PM, Barlow WE. Measuring the performance of markers for guiding treatment decisions. Ann Intern Med 2011;154:253–9.
[23] Kotani D, et al. Molecular residual disease and efficacy of adjuvant chemotherapy in patients with colorectal cancer. Nat Med 2023;29:127–34. https://doi.org/10.1038/s41591-022-02115-4.
[24] Baxter NN, et al. Adjuvant therapy for stage II colon cancer: asco guideline update. J Clin Oncol 2021;40:892–910. https://doi.org/10.1200/jco.21.02538.
[25] Andersen CL, Heitzer E. ctDNA-guided adjuvant chemotherapy for colorectal cancer—ready for prime time? Cancer Cell 2022;40:911–3. https://doi.org/10.1016/j.ccell.2022.08.017.
[26] Morris VK, George TJ. Using circulating tumor DNA for colon cancer adjuvant therapy: to be or not to be? Clin Cancer Res 2022;28:438–40. https://doi.org/10.1158/1078-0432.Ccr-21-3564.
[27] Olivier T, Prasad V. Molecular testing to deliver personalized chemotherapy recommendations: risking over and undertreatment. BMC Med 2022;20:392. https://doi.org/10.1186/s12916-022-02589-6.
[28] Olivier T, Haslam A, Prasad V. Additional considerations before using a ctDNA-guided approach for informing adjuvant chemotherapy in colorectal cancer. BMC Med 2023;21:344.
[29] Cardoso R, et al. Colorectal cancer incidence, mortality, and stage distribution in European countries in the colorectal cancer screening era: an international population-based study. Lancet Oncol 2021;22:1002–13. https://doi.org/10.1016/S1470-2045(21)00199-6.
[30] Nathan P, et al. Overall survival benefit with tebentafusp in metastatic uveal melanoma. N Engl J Med 2021;385:1196–206.
[31] Powles T, et al. Updated overall survival by circulating tumor DNA status from the phase 3 IMvigor010 trial: adjuvant atezolizumab versus observation in muscle-invasive urothelial carcinoma. Eur Urol 2023.
[32] Jacqueline AS, et al. Serial postoperative circulating Tumor DNA assessment has strong prognostic value during long-term follow-up in patients with breast cancer. JCO Precis Oncol 2024:e2300456. https://doi.org/10.1200/po.23.00456.
[33] Henriksen TV, et al. Circulating Tumor DNA in Stage III Colorectal Cancer, beyond Minimal Residual Disease Detection, toward Assessment of Adjuvant Therapy Efficacy and Clinical Behavior of Recurrences. Clin Cancer Res 2022;28:507–17. https://doi.org/10.1158/1078-0432.Ccr-21-2404.
[34] Parikh AR, et al. Minimal residual disease detection using a plasma-only circulating tumor DNA assay in patients with colorectal cancer. Clin Cancer Res 2021;27:5586–94. https://doi.org/10.1158/1078-0432.Ccr-21-0410.
[35] Garcia-Murillas I, et al. Assessment of molecular relapse detection in early-stage breast cancer. JAMA Oncol 2019;5:1473–8.
[36] Lipsyc-Sharf M, et al. Circulating tumor DNA and late recurrence in high-risk hormone receptor–positive, human epidermal growth factor receptor 2–negative breast cancer. J Clin Oncol 2022;40:2408–19. https://doi.org/10.1200/jco.22.00908.
[37] Jakobsen AKM, Spindler KG. ctDNA-Response evaluation criteria in solid tumors - a new measure in medical oncology. Eur J Cancer 2023;180:180–3.
[38] FDA. Use of Circulating Tumor Deoxyribonucleic Acid for Early-Stage Solid Tumor Drug Development; Draft Guidance for Industry., <⟨https://www.fda.gov/regulatory-information/search-fda-guidance-documents/use-circulating-tumor-deoxyribonucleic-acid-early-stage-solid-tumor-drug-development-draft-guidance⟩> (2022).