PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	BESPOKE study protocol: A multicenter, prospective observational study to evaluate the impact of circulating tumor DNA guided therapy on patients with colorectal cancer
AUTHORS	Kasi, Pashtoon; Sawyer, Sarah; Guilford, Jessica; Munro, Michelle; Ellers, Sascha; Wulff, Jacob; Hook, Nicole; Krinshpun, Shifra; Koyen Malashevich, Allyson; Malhotra, Meenakshi; Rodriguez, Angel; Moshkevich, Solomon; Grothey, Axel; Kopetz, Scott; Billings, Paul; Aleshin, Alexey

VERSION 1 – REVIEW

REVIEWER	Cunningham, D Department of Medicine, Royal Marsden Hospital, Sutton , Surrey, United Kingdom
REVIEW RETURNED	29-Jan-2021

GENERAL COMMENTS	We would like to congratulate the authors on designing an observational study of ctDNA dynamics in patients with stage II/III colorectal cancer (CRC) who undergo curative study to aid real-time decision making of adjuvant chemotherapy by physicians in the real-world setting. This is an interesting approach to address a clinically pertinent question and adds to the growing portfolio of studies world-wide, addressing the clinical utility of circulating tumour DNA (ctDNA) as a surrogate of molecular or minimal residual disease (MRD) after curative surgery to guide adjuvant chemotherapy decisions in patients with stage II/III CRC. The BESPOKE study, an academic and industry collaboration, is a multicentre, prospective, observational study which plans to recruit a total of 1,000 patients from 200 U.S. sites. It is well designed for an observational study and statistically powered to address a clinically pertinent question. The use of a validated, commercially available, tumour informed personalised assay for ctDNA detection is one the main strengths of the study as is the use of Hospital Anxiety and Depression Scale (HADS) and Fear of Recurrence (FCR-4) questionnaires for patient related outcomes. Please can you address the following questions as part of the review process? 1. You have rightly highlighted the main limitation of the study is the lack of randomisation. Once the study is complete, the results are likely to generate level 2 evidence. How do you see the data impact clinical utility and update by physicians given the level of evidence? 2. The study also does not dictate if and which type of intervention should be selected. By incorporating real-time ctDNA test results into the study which can impact treatment decisions, there is

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	bound to be individual variation in decision making which will introduce bias and heterogeneity making the data difficult to interpret. Do you have an a priori statistical analysis plan to address this issue and can this be included? 3. Is a follow-up period of 2 years sufficient for this group of patients? Along the same lines, one of the secondary end-points include survival of MRD negative patients treated with adjuvant chemotherapy versus no adjuvant chemotherapy. Is this 2-year disease free survival (DFS)? Three-year DFS is considered surrogate for overall survival in patients with early CRC, do you think this will impact on the uptake of this approach by clinicians given the shorter follow-up period. 4. The control arm is ~300 matched patients enrolled retrospectively with 2-year clinical follow-up. How will authors account for the impact of change in clinical practice due to non-ctDNA related factors, e.g., the recently published overall survival data of the IDEA collaboration, when analysing the results of the BESPOKE study? Would a contemporaneous control group mitigate these issues? 5. Inclusion of patients with locally advanced rectal cancer treated with neoadjuvant chemoradiotherapy is an important factor of this study to study ctDNA dynamics in the adjuvant setting. What proportion of patients with LARC are you planning to/ anticipate recruiting? 6. In the sample size calculations, what is the 20% attrition rate based on? Do you have any data supporting this? More details regarding sample size calculation will strengthen this publication. 7. A lack of Patient and Public Involvement in the study design has been highlighted by the authors. Are discussions or surveys with patient focus groups or clinicians for their views on the study planned/ in progress? 8. Please can you provide information on the timelines for recruitment and when you plan on opening this study? 9. Can you please clarify the number of scans patients have as part of the routine clinical follow-up in Table 2 schedule of events? It currently suggests patients need to have up to 4 scans within week 20 after surgery. We hope these points will strengthen the study and this publication.

REVIEWER	Rumpold , Holger Ordensklinikum Linz GmbH
REVIEW RETURNED	16-Mar-2021

GENERAL COMMENTS	Congratulations to the trial!

REVIEWER	Ebi, Hiromichi Aichi Cancer Center Research Institute, Molecular Therapeutics
REVIEW RETURNED	04-Jun-2021

GENERAL COMMENTS	Currently, all patients with stage III colorectal cancer receive adjuvant chemotherapy even though about 60% of patients does not recur without it. This Bespoke study tries to address whether ctDNA analysis helps to avoid non necessarily adjuvant chemotherapy. The clinical question is worth to pursue, and the results may change current clinical practice. Minor comment,

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	1. Modification of adjuvant chemotherapy following results of ctDNA analysis could affect the recurrence rate of colorectal cancer. Will this study analyze association between escalation/de-escalation of chemotherapy and the recurrence rate among these patients? Please describe detail how the author will analyze the percentage of patients who recur. 2. Brief description regarding technical advances of the custom ctDNA analysis in comparison to pre-designed ctDNA panels is helpful. 3. In Figure 1, asterisk was marked on future research, however, the meaning was not described in the Figure legend. 4. In the reference, a recent protocol paper describing similar study can be added (Taniguchi et al. https://doi.org/10.1111/cas.14926).

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Prof. D Cunningham, Department of Medicine, Royal Marsden Hospital, Sutton, Surrey, United Kingdom

Comments to the Author:

We would like to congratulate the authors on designing an observational study of ctDNA dynamics in patients with stage II/III colorectal cancer (CRC) who undergo curative study to aid real-time decision making of adjuvant chemotherapy by physicians in the real-world setting. This is an interesting approach to address a clinically pertinent question and adds to the growing portfolio of studies world-wide, addressing the clinical utility of circulating tumour DNA (ctDNA) as a surrogate of molecular or minimal residual disease (MRD) after curative surgery to guide adjuvant chemotherapy decisions in patients with stage II/III CRC.

The BESPOKE study, an academic and industry collaboration, is a multicentre, prospective, observational study which plans to recruit a total of 1,000 patients from 200 U.S. sites. It is well designed for an observational study and statistically powered to address a clinically pertinent question. The use of a validated, commercially available, tumour informed personalised assay for ctDNA detection is one the main strengths of the study as is the use of Hospital Anxiety and Depression Scale (HADS) and Fear of Recurrence (FCR-4) questionnaires for patient related outcomes.

Please can you address the following questions as part of the review process?

We thank the reviewer for their encouraging feedback and have answered all the questions below. We have also made appropriate changes to the manuscript as suggested. Please note that our study has now been expanded to include Stage I to IV CRC patients and the amendment was recently approved by the Advarra IRB on June 10, 20121. We have made appropriate modifications reflecting this change in the manuscript.

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1. You have rightly highlighted the main limitation of the study is the lack of randomisation. Once the study is complete, the results are likely to generate level 2 evidence. How do you see the data impact clinical utility and update by physicians given the level of evidence?

Answer: The reviewers correctly point out that our observational study will generate level 2 evidence for use of ctDNA in aiding physician decision making. The clinical utility of ctDNA in this study is in the monitoring setting. We note that our study will provide critically important evidence regarding the percentage of times the physician changes their management, as well as assess how it changes the physician and patient perception of MRD testing. The importance of real-world evidence (RWE) is increasingly being recognized for its added value to clinical research. Our study is one of the largest prospective studies being conducted in this space today. It will provide insight into distinct aspects of treatment and patient outcomes. Together with results from clinical trials, RWE can help illustrate a more complete picture of the tolerability, effectiveness, and impact of a drug (Webster et al. 2019 Clinical Therapeutics).

2. The study also does not dictate if and which type of intervention should be selected. By incorporating real-time ctDNA test results into the study which can impact treatment decisions, there is bound to be individual variation in decision making which will introduce bias and heterogeneity making the data difficult to interpret. Do you have an a priori statistical analysis plan to address this issue and can this be included?

Answer: We acknowledge the point made by the reviewer. Changes in the percentage of patients prescribed post-surgical systemic therapy can be assessed by comparing the treatment arm to the control arm as described in our answer to the reviewers concern #4 below. Our interest is in the percentage change in patients receiving post-surgical systemic therapy, if the systemic therapy is decided, the treatment regimen will be at the discretion of the healthcare provider per routine practice. A list of standard ACT regimens can be found in the National Comprehensive Cancer Network (NCCN) guidelines. We have now added a new section on “post-operative systemic therapy” on page 9 of the revised manuscript.

3. Is a follow-up period of 2 years sufficient for this group of patients? Along the same lines, one of the secondary end-points include survival of MRD negative patients treated with adjuvant chemotherapy versus no adjuvant chemotherapy. Is this 2-year disease free survival (DFS)? Three-year DFS is considered surrogate for overall survival in patients with early CRC, do you think this will impact on the uptake of this approach by clinicians given the shorter follow-up period.

Answer: This is a valid question. While a longer follow-up period will provide a more complete picture, we think that a two-year follow-up is sufficient to address the main goal of our study, which is to observe the impact of MRD testing on adjuvant treatment decisions post-surgery. In addition to the

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2-year DFS, we will also identify an additional percentage of patients who are determined to have detectable ctDNA in their blood but are asymptomatic, i.e. with no evidence of clinical recurrence yet. This could be a surrogate to identifying patients, who would potentially recur in the following year (3rd year). Future amendments to the protocol may include later follow-up time points to address long-term patient survival outcomes.

4. The control arm is ~300 matched patients enrolled retrospectively with 2-year clinical follow-up. How will authors account for the impact of change in clinical practice due to non-ctDNA related factors, e.g., the recently published overall survival data of the IDEA collaboration, when analysing the results of the BESPOKE study? Would a contemporaneous control group mitigate these issues?

Answer: We acknowledge the point made by the reviewer. Our protocol has now been amended to enroll a total of 600 historical control subjects at an approximate ratio of 3:1. Each participating site will contribute retrospectively collected control patients in accordance with the site-specific study contract. Comparisons between the prospectively treated patients and the retrospective control will be conducted using inverse probability weighted data for baseline covariate adjustment. The inverse probability weights will be derived using a propensity score model that includes but is not limited to: 1) stage, 2) age, and 3) gender. The retrospective controls will be patients from a similar time period such that treatment options are similar. The controls will meet all study inclusion/exclusion criteria.

This detail has now been added on page 8-9 of the revised manuscript.

5. Inclusion of patients with locally advanced rectal cancer treated with neoadjuvant chemoradiotherapy is an important factor of this study to study ctDNA dynamics in the adjuvant setting. What proportion of patients with LARC are you planning to/ anticipate recruiting?

Answer: We appreciate the reviewers bringing this point to our attention. Given the real world nature of this study, the exact proportion of LARC patients is difficult to define. However, our study is targeting an enrollment of 10-20% of rectal cancer patients. This is consistent with the overall distribution of patients with colon versus rectal cancer who are eligible for adjuvant therapy.

6. In the sample size calculations, what is the 20% attrition rate based on? Do you have any data supporting this? More details regarding sample size calculation will strengthen this publication.

Answer: A 20% attrition rate was assumed based on factors such as, patients who are lost to follow-up, patients with non-evaluable Signatera™ results, etc. We have now added additional detail around the statistical considerations and sample size justification. Please see pages 14-16 in the revised manuscript.

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7. A lack of Patient and Public Involvement in the study design has been highlighted by the authors. Are discussions or surveys with patient focus groups or clinicians for their views on the study planned/ in progress?

Answer: The protocol was designed and discussed with the patient advocacy group and academic community (GI oncology) and underwent multiple revisions based on their input. This information has now been added in the patient and public involvement section of the revised manuscript on page 16.

8. Please can you provide information on the timelines for recruitment and when you plan on opening this study?

Answer: The study started in May 2020 and is open for recruitment until October 2022. This detail has now been added on page 8, under the overall study design section in the revised manuscript.

9. Can you please clarify the number of scans patients have as part of the routine clinical follow-up in Table 2 schedule of events? It currently suggests patients need to have up to 4 scans within week 20 after surgery.

Answer: The scanning protocol is based on the recommended guidelines from NCCN. However, the nature and frequency of scan is left up to the individual investigators/physicians.

We hope these points will strengthen the study and this publication.

We agree with the reviewer and believe that these are great suggestions. Likewise we have incorporated the feedback into the manuscript.

Reviewer: 2

Dr. Holger Rumpold , Ordensklinikum Linz GmbH

Comments to the Author:

Congratulations to the trial!

We thank the reviewer for their feedback.

Reviewer: 3

Dr. Hiromichi Ebi, Aichi Cancer Center Research Institute

Comments to the Author:

Currently, all patients with stage III colorectal cancer receive adjuvant chemotherapy even though about 60% of patients does not recur without it. This Bespoke study tries to address whether ctDNA

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analysis helps to avoid non necessarily adjuvant chemotherapy. The clinical question is worth to pursue, and the results may change current clinical practice.

We thank the reviewer for their valuable feedback. Below we have addressed all the questions and have made appropriate changes to the manuscript.

Minor comment,

1. Modification of adjuvant chemotherapy following results of ctDNA analysis could affect the recurrence rate of colorectal cancer. Will this study analyze the association between escalation/de-escalation of chemotherapy and the recurrence rate among these patients? Please describe in detail how the author will analyze the percentage of patients who recur.

Answer: We thank the reviewer for their feedback. In our study, we will be looking at it as an exploratory endpoint. We understand that treatment may be de-escalated for patients receiving ctDNA negative tests. As such, we will compare disease-free survival (DFS) between the prospectively treated patients and controls. Thus, if recurrence has increased due to treatment de-escalation among the prospectively treated patients, DFS will be inferior among this cohort as compared to the controls. The DFS comparison will be accomplished by fitting a Cox proportional hazards regression model to the inverse probability weighted data where the inverse-probabilities are derived from fitting the propensity-score model described in our response to Concern #4 of Reviewer 1. The appropriateness/validity of the Cox proportional hazards regression model will be assessed using a Schoenfeld residual test.

2. Brief description regarding technical advances of the custom ctDNA analysis in comparison to pre-designed ctDNA panels is helpful.

Answer: We thank the reviewer for their valuable input. We have now added the description below in the revised manuscript.

"Unlike pre-designed ctDNA static panels, a personalized, tumor-informed assay like Signatera is technically advanced as it relies on the prior knowledge of the mutational status of the patient's tumor. Having the patient tumor tissue allows whole exome sequencing to be performed, in order to understand all of the somatic variants and select the clonal variants that are present in that patient's tumor. By identifying and tracking clonal variants, which are expected to be present in every cancer cell from the patient, the tumor informed approach ensures that residual disease can be detected with both a high sensitivity and high specificity, reliably detecting variants down to 0.01% VAF. The tumor informed method also significantly reduces the false-positive rates by filtering out clonal hematopoiesis of indeterminate potential (CHIP) and germline-derived variants from analysis."

3. In Figure 1, asterisk was marked on future research, however, the meaning was not described in the Figure legend.

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Answer: Thank you for bringing this to our attention. The asterisk meant to indicate: "optional" (patients have to opt-in to provide the sample, it isn't a required blood draw for the study). We have now added this description in the revised manuscript.

4. In the reference, a recent protocol paper describing a similar study can be added (Taniguchi et al. https://doi.org/10.1111/cas.14926).

Answer: We thank the reviewer for their suggestion, we have now added the suggested reference in the revised manuscript.

Reviewer: 1

Competing interests of Reviewer: For Prof. Cunningham: Amgen, Sanofi, Merrimack, AstraZeneca, Celgene, Medimmune, Bayer, 4SC, Clovis, Eli Lilly, Janssen, Merck, Ovibio

For Dr. Anandappa: None declared

Reviewer: 2

Competing interests of Reviewer: none declared

Reviewer: 3

Competing interests of Reviewer: Honoraria from Taiho, Chugai, Kyowa-kirin, AstraZeneca, Takeda.

VERSION 2 – REVIEW

REVIEWER	Cunningham, D Department of Medicine, Royal Marsden Hospital, Sutton , Surrey, United Kingdom
REVIEW RETURNED	18-Aug-2021

GENERAL COMMENTS	Excellent trial

REVIEWER	Ebi, Hiromichi Aichi Cancer Center Research Institute, Molecular Therapeutics
REVIEW RETURNED	23-Jul-2021

GENERAL COMMENTS	The author appropriately addressed the points raised by the reviewer.

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