**Peer Review File**

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**Reviewer A Comments**

This editorial by Navid and Biegel is well written and discussing the evolving role of ctDNA in the prognosis of IR-RMS and how this new technology could potentially be used clinically to guide therapy.

While it is well written and the authors provide a brief overview of the limitations and applications for ctDNA in RMS, it isn't clear what this editorial adds to the published work from Abbou et al. (2023) that the authors cited.

***Response:*** *The intent of this invited commentary is to highlight the work of Abbou et al. as the first to evaluate the use of ctDNA in a large cohort of patients as a biomarker in RMS and the potential limitations and future directions of the work.*

**Reviewer B Comments**

The present editorial commentary of the article by Abbou S. et al. "Circulating Tumor DNA Is Prognostic in Intermediate-Risk Rhabdomyosarcoma: A Report From the Children's Oncology Group" published on Journal of Clinical Oncology, doi: 10.1200/JCO.22.00409, well summarized the key aspects of the original study, underlying the potential role of evaluating tumor circulating DNA at the diagnosis of patients with intermediate risk rhabdomyosarcoma, in order to better stratifying the therapeutic strategy and evaluate the efficacy of treatment. The commentary is overall clear and well written; in my opinion it would need a minor revision. Hereafter are listed some remark:

I would suggest adding a brief description of demographic characteristic of patients, and, if available, of therapies conduced.

***Response:*** *Detailed demographic and treatment data are available in the paper by Abbou et al. We did not feel it was necessary to duplicate the data in this commentary.*

• I would recommend using either SNVs or SNAs, as they are synonyms.
***Response:*** *Text has been revised to only use the term single nucleotide variants (SNVs)*

• Lines 92-94: I would recommend correcting FP-RMS with FN-RMS in the following sentence: "… the detection of pretreatment ctDNA, at least in the IR FP-RMS, may be a useful biomarker to augment therapy for this group of patients."
***Response:*** *Text has been corrected.*

• Lines 103-105: I would suggest rephrasing this sentence, avoiding the use of a question form: "Would the addition of methylation status, the presence of circulating tumor cells or assays based on RNA or protein extracted from extracellular vesicles provide better biomarkers from liquid biopsies?".
***Response:*** *Sentence has been rephrased as suggested.*

• Lines 126-127: I would suggest rephrasing this sentence, avoiding the use of a question form: "Is the sensitivity and specificity of the results comparable across platforms?"
***Response:*** *Sentence has been rephrased as suggested.*

**Reviewer C Comments**

This is an analysis of the recent publication of COG about ctDNA in Intermediate risk group of Rhabdomyosarcoma. This important topic should be explored.
For Editorial Commentary is a good topic
***Response:*** *Thank you for your comments.*

**Reviewer D Comments**

This is a good summary of the recent study by Abbou et al and a commentary on the potential for ctDNA assessment of rhabdomyosarcomas.

Line 60 "Tumor tissue testing is relevant for studies evaluating ctDNA because in general, if the tumor does not have CNAs then ULP-WGS is uninformative. Similarly, if the specific SNVs or translocations assessed by Rhabdo-seq are not present in the tumor, the assay is uninformative."
The explanation here does not clearly explain why tissue is relevant to identifying ctDNA – especially as it is followed by the following statement
"As with the current study, there are circumstances when ctDNA may be detectable when the tumor was not informative. This situation is likely due to the heterogeneity of the tumor."
Needs clarification
***Response:*** *Clarification has been provided in the text that the area of tumor sampled for*

*molecular testing may not be represented of the entire tumor and may be the reason for not detecting a molecular abnormality in the tumor tissue.*

Paragraphs starting with Line 67 and 79: Useful to mention the limits of detection by the different techniques and the ctDNA quantification -how does this fit with the findings described?
***Response:*** *Please see lines 52-54 for the limits of detection for the two assays used in the Abbou et al. study.*

Line 87 – can the authors speculate why FP-data is not an independent prognostic indicator?
***Response:*** *Speculation, smaller sample size and higher sensitivity of assay to detect fusion, as to why ctDNA is not an independent prognostic factor is added to the text.*

Line 90 and 126 Other reports re assessing ctDNA should also be refered to PMID: 36265118; PMID: 31543384PMID: 33287361; PMID: 34794856 PMID: 37189699; PMID: 30739374.
***Response:***

- *PMID: 36265118 (Ruhen et al) – Report added to references. 18 RMS patient samples from diagnosis were included in this report.*
- *PMID: 31543384 (George et al.) – Primarily focused on NGS panel for pediatric solid tumors with a small subset of plasma samples for ctDNA in 12 patients, only 2 of which had RMS, both samples at relapse.*
- *PMID: 33287361(McConnell et al)-Not included because paper describing a feasibility of a NGS panel in detecting abnormalities in various sarcomas. Only 2 samples from RMS patients while on therapy. No circulating DNA detected.*
- *PMID: 34794856 (Van Paemel et al) Pan pediatric solid tumor assessment of ctDNA compared to tumor tissue. Report added to references to support that various assays and platforms for detection of ctDNA*
- *PMID: 37189699 (Ruas et al) – Not included because analysis of total of 35 patients using digital PCR with only 4 patients with RMS in cohort.*
- *PMID: 30739374 – Not included because single Case Report*

Line 114 -122 Whilst this specific discussion of trials is of interest "the goal of estimating the frequency of patients with detectable ctDNA at diagnosis and subsequent time-points" does not seem very meaningful. May be a broader goal of learning from assessment in trials how to use which analytical approach and when in the patient pathway most optimally to improve management and outcomes?

***Response:*** *We agree with the reviewer that additional applications of ctDNA detection should be to optimize how we use the assays to improve management and outcome of the patients. However, the stated objective in the protocol of the ARST1431 clinical trial for collection of*

*ctDNA is detection rate of ctDNA at diagnosis and various time-points. Our understanding is that the study team does have plans to perform secondary analysis, including correlation with outcome. This data will be useful in planning future studies. No revisions were made in the text.*

**Reviewer E Comments**

The authors provide an accurate summary of the article by Abbou et al. The authors provide a background of RMS subtypes and definition of the risk groups and their associated outcome. The editorial impact can be increased if the authors comment on whether detection of ctDNA from liquid biopsies could inform treatment strategies or early detection/monitoring of tumor progression.
***Response:*** *See text line 93-94 and line 108-110.*

And how ctDNA detection compares to alternate methodologies. The authors can also better explain the two strategies (ULP-WGS and Rhabdo-Seq) that the paper employed, and their reasoning of using both strategies to come to their conclusion.
***Response:*** *See text line 48-54 for rationale for using 2 assays to detect ctDNA in RMS.*

The authors do a good job pointing out limitations of using ctDNA as a biomarker and efforts towards standardization of obtaining ctDNA in clinical practice.
***Response:*** *Thank you!*

**Reviewer F Comments**

Overall, I think this is a clear, concise, well-written commentary on an important article. I only have a few minor points:

1. Line 59 refers to tumor tissue being evaluated from 30 FP-RMS patients, but Abbou et al. evaluated tissue from 30 FP-RMS tumors via Rhabdo-Seq and from 35 FP-RMS tumors via WGS due to material limitations. I think it would be worth it to clarify that in line 59, as the later tissue number discrepancy (on lines 74-75) when discussing percentages might be confusing to readers.
***Response:*** *The text on line 59 has been revised to correct the total number of tumor tissue samples available for evaluation by either method from 30 to 35.*

2. Line 93: Do the authors means that ctDNA pretreatment detection might be useful in fusion

*negative* RMS to 'augment therapy', since that is the subgroup in which ctDNA demonstrated significance as an independent prognostic factor? If not, and I'm misunderstanding their train of thought regarding its utility for FP-RMS, I think it would be helpful if they expanded on that point.

***Response:*** *The text has been corrected. The comment refers to fusion negative RMS.*

**Reviewer G Comments:**

A thorough review. Would be helpful to include some of the data in table form.

***Response:*** *We appreciate this comment by the reviewer; however, a table would not add any additional information and would be repetitive.*