

Response to reviewers

Title: Emerging Role of Liquid Biopsy in RAS Mutated Metastatic Colorectal Cancer After Treatment: a Case Series of Dynamic Monitoring and Treatment Tailoring in NeoRAS wild-type

Manuscript Number: 87164

Corresponding Author: João Gramaç

Dear Sirs,

We sincerely thank the opportunity to revise our manuscript “**Emerging Role of Liquid Biopsy in RAS Mutated Metastatic Colorectal Cancer After Treatment: a Case Series of Dynamic Monitoring and Treatment Tailoring in NeoRAS wild-type**” (871640).

We thank the editors and reviewers for their insightful and constructive comments and their effort and time to review the manuscript, which has helped us to improve it.

We have carefully addressed all comments and suggestions point-by-point and reviewed our manuscript accordingly. All the changes are highlighted in yellow. Please find below a point-by-point response to the reviewers’ comments and a summary of the changes.

We look forward to hearing from you regarding our submission and remain available to respond to any further questions and comments.

Kind Regards,

João Gramaça

Reviewer #1

1. Please list the abbreviation in a new section for readers.

Reply: As requested, we inserted an abbreviation list in the manuscript

Now in the text:

8) Abbreviations

1L: first line

2L: second line

3L: third line

4L: fourth line

cfDNA: cell-free DNA

CRC: colorectal cancer

CT: chemotherapy

EGFR: Epidermal growth factor receptor

ESMO: European Society for Medical Oncology

LB: liquid biopsy

mCRC: metastatic colorectal cancer

OS: overall survival

PFS: progression-free survival

RAS: Rat sarcoma virus gene

WHO: World Health Organization

2. Please do not use abbreviation in core tip.

Reply: All the abbreviations in the Abstract, Keywords and Core Tip were detailed

Now in the text:

Background: In patients with metastatic colorectal cancer (mCRC), the treatment

options are limited and proved to be affected by Rat sarcoma virus gene (RAS) mutational status. The combination of anti-epidermal growth factor receptor (EGFR) monoclonal antibodies with chemotherapy (CT) is more effective than CT

alone, in RAS wild-type (wt) patients. On the other hand, RAS-mutated patients are not eligible for treatment with anti-EGFR antibodies.

Case Summary: Eleven patients with initially RAS-mutated mCRC were followed since diagnosis to May 2022. At the moment of cell-free DNA (cfDNA) determination, five patients had undergone one CT line, five patients had undergone two CT lines, and one patient had undergone three CT lines (all in combination with bevacizumab). At second and third lines (2L, 3L), patients with Neo-RAS wt received a combination of CT and cetuximab.

Our results showed a significant increase in 2L and 3L progression-free survival (PFS) in Neo-RAS wt patients treated with anti-EGFR (14.5 months, p=0.119 and

3.9 months, p=0.882, respectively). Regarding 2L overall survival (OS), we registered a slight increase in Neo-RAS wt patients treated with anti-EGFR (33.6 months vs 32.4 months, p=0.385). At data cut-off, two patients were still alive: a RAS-mutated patient undergoing 3L treatment and a neo-RAS wt patient who received 2L treatment with anti-EGFR (ongoing).

Conclusions: Our case series evidenced that monitoring RAS mutations in mCRC

by liquid biopsy may provide an additional treatment line for Neo-RAS wt patients.

Keywords: metastatic colorectal cancer, RAS mutational status, liquid biopsy, RAS wild-type, Neo-RAS wild-type, anti-epidermal growth factor receptor (EGFR) therapy.”

Core Tip:

“In patients with metastatic colorectal cancer, the treatment options are limited and proved to be affected by RAS mutational status. This manuscript describes a

series of 11 RAS-mutated metastatic colorectal cancer patients who were treated with a combination of chemotherapy and bevacizumab at first line. Four patients became neo-RAS wt after first or second line treatment and were treated with cetuximab, with advantages in terms of survival and disease progression.”

3. It is suggested to supply more details of liquid biopsy in MM section.

Reply: More details about liquid biopsy can be found in the added reference of the assay.

Now in the text:

“The search for mutations in the KRAS (NM_033360.3) (codons 12, 13, 59 and 61), NRAS (NM_002524.4) (codons 12, 13, 59 and 61) and BRAF (MN_004333.4)

(codon 600) genes was performed through next-generation sequencing using the

"*Oncomine Lung cfDNA assay*" (Ion Torrent™ Oncomine™ assays – Thermo Fisher Scientific)."

4. Please replace 'wild type' with 'wild-type'.

Reply: The requested change was performed in the title and throughout the text.

Now in the text:

Title

"Emerging Role of Liquid Biopsy in RAS Mutated Metastatic Colorectal Cancer After Treatment: a Case Series of Dynamic Monitoring and Treatment Tailoring in NeoRAS wild-type"

Keywords

"metastatic colorectal cancer, RAS mutational status, liquid biopsy, RAS wild-type,
Neo-RAS wild-type, anti-epidermal growth factor receptor (EGFR) therapy."

Abstract

"The combination of anti-epidermal growth factor receptor (EGFR) monoclonal antibodies with chemotherapy (CT) is more effective than CT alone, in RAS wild-type (wt) patients"

Introduction

"Current clinical and research evidence demonstrate that the combination of anti epidermal growth factor receptor (EGFR) monoclonal antibodies, like cetuximab and panitumumab, with chemotherapy (CT) is more effective than CT alone, in RAS wild-type (wt) mCRC patients, which are non-mutated at disease presentation^[6]."

Reviewer #2

1. In the sixth line of the first paragraph. Even though, the patient was kept on maintenance CT and showed a very long and sustained response. Case 2 had a right-sided relapsed mCCR with liver metastasis. There is a spelling mistake. Write "mCRC"incorrectly as " mCCR ".

Reply: The mistake was corrected.

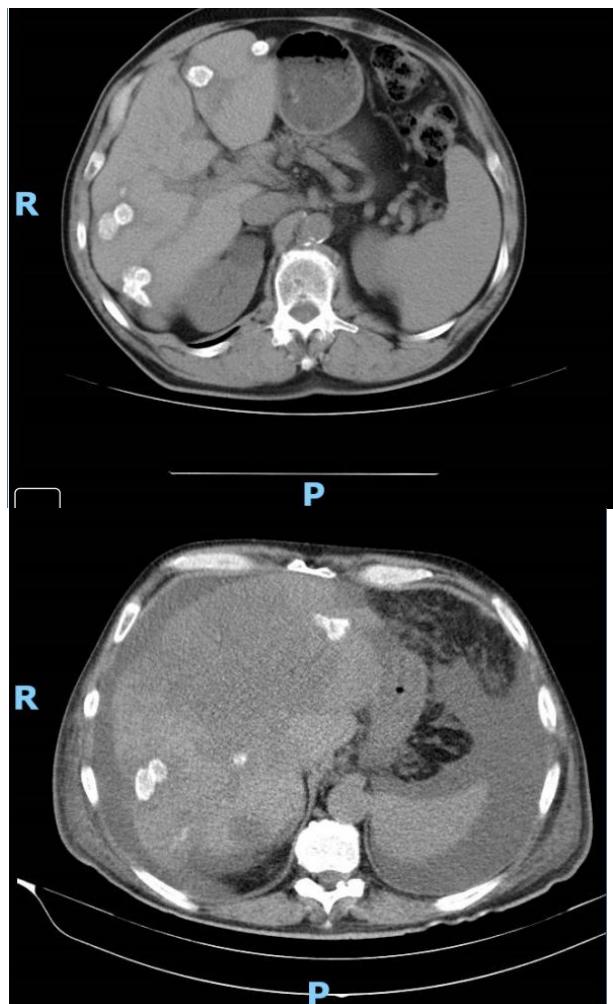
Now in the text:

"Case 2 had a right-sided relapsed mCRC with liver metastasis."

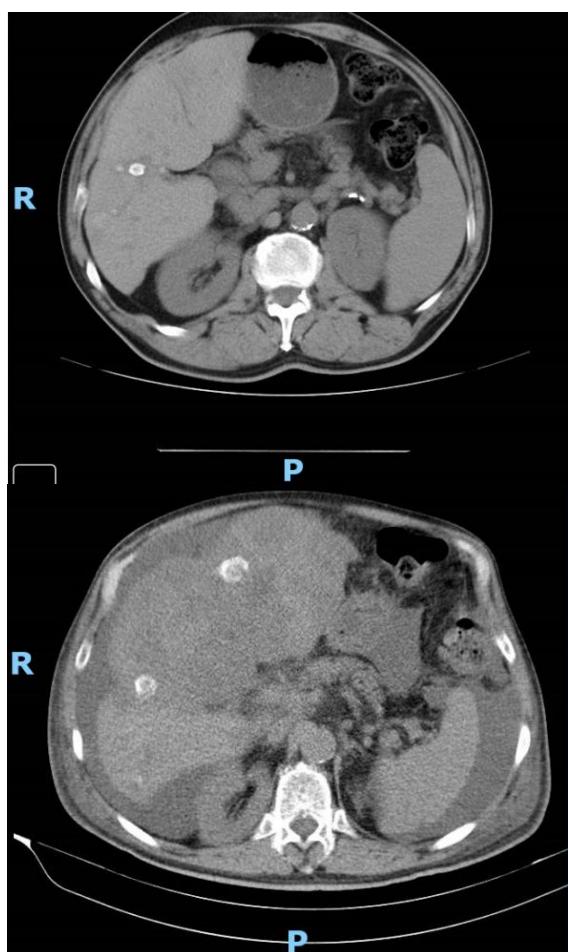
2. In Figure 1. Imaging evaluation of Case [11. in](#) picture A, three months later, the calcified intrahepatic metastases appear to be significantly increased, in addition, for liver metastases on CT scan, the lack of imaging parameters, this seems to be a flat scan, is it accurate?

Reply: We thank you the Reviewer for this observation. In fact, the order of the pictures is the other way around. Figure 1 was corrected accordingly.

Now in the text:



A.



B.

Figure 1. Imaging evaluation of Case 11.

- A. Imaging evaluation at baseline (July 2021, *on the left*) and at 3 months (October 2021, *on the right*), upper axial plane, (*flat CT scan with IV contrast*).
B. Imaging evaluation at baseline (July 2021, *on the left*) and at 3 months (October 2021, *on the right*), lower axial plane, (*flat CT scan with IV contrast*).

3. The detection threshold of RAS gene mutation by tissue biopsy and ctDNA is different. It is suggested that the authors should supplement the value of Ras mutation abundance before and after treatment, which is convenient for readers to study and discuss.

Reply: Even though we fully agree with the Reviewer, some reports are older and do not include that information. When discussing re-testing of the original biopsies, we were concerned about the data quality of the older biopsies

concerning RASmut abundance. In more recent patients, these data are being considered.