Peer Review File

Article Information: https://dx.doi.org/10.21037/jgo-24-272

Reviewer A

Comment 1: Tumors on the right side are characterized by being associated with mutations in the BRAF and RAS genes, as well as microsatellite instability and a methylated phenotype.
Please change "a methylated phenotype" to "increased hypermethylation of CpG islands".
Reference: Mukund K, Syulyukina N, Ramamoorthy S, Subramaniam S. Right and left-sided colon cancers - specificity of molecular mechanisms in tumorigenesis and progression. BMC Cancer. 2020 Apr 15;20(1):317. doi: 10.1186/s12885-020-06784-7.

- **Reply 1**: We changed "a methylated phenotype" to "increased hypermethylation of CpG islands" and we included the new reference.
- Changes in the text: see Page 1, line 29

Comment 2: Left-sided tumors also present more mutations in RAS, APC, and p53, HER1 and HER2 amplifications, as well as gene expression profiles that confer greater sensitivity to EGFR-targeted therapies.
Please remove RAS from the above paragraph since RAS mutation is more commonly seen in right-sided colon cancer.
Reference: Bylsma LC, Gillezeau C, Garawin TA, Kelsh MA, Fryzek JP, Sangaré L, Lowe KA. Prevalence of RAS and BRAF mutations in metastatic colorectal cancer patients by tumor sidedness: A systematic review and meta-analysis. Cancer Med. 2020 Feb;9(3):1044-1057. doi: 10.1002/cam4.2747.

- **Reply 2**: According to your comment we modified the paragraph " Left-sided tumors also present more mutations in APC and p53, HER1 and HER2 amplifications, as well as gene expression profiles that confer greater sensitivity to EGFR-targeted therapies." and we included the new reference.
- Changes in the text: see Page 1, line 32

Comment 3: Given the results of previous studies and the limited efficacy of anti-EGFR agents in this patient subgroup, guidelines now recommend combining an anti-VEGF with chemotherapy for RAS wild-type right-sided CRC patients.
Please change to "NCCN guideline now recommend in first-line treatment of advanced or metastatic CRC, combining an anti-VEGF agent with chemotherapy for tumor harboring pMMR/MSS and wild -type RAS and RAF; combining an anti-EGFR agent with chemotherapy for left-sided tumor harboring pMMR/MSS and wild -type RAS and RAF.
Reference:NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Colon Cancer Version 2.2024 — April 30, 2024

- **Reply 3:** According to your comment we included both NCCN and ESMO guidelines as follows: "NCCN and ESMO guidelines now recommend in first-

line treatment of advanced or metastatic CRC, combining an anti-VEGF agent with chemotherapy for tumor harboring pMMR/MSS and wild -type RAS and RAF; combining an anti-EGFR agent with chemotherapy for left-sided tumor harboring pMMR/MSS and wild -type RAS and RAF". Reference has been included.

- Changes in the text: see Page 2, line 53

Comment 4: Please discuss KRAS G12C mutation and its therapeutic implication.

- **Reply 4:** According to your comment we included the following paragraph: "40% of CRC harbor KRAS missense mutations in codons 12, 13, or 61 which confer a lack of response to anti-EGFR therapies [7]. $KRAS^{G12C}$ is detected in 2-4% of metastatic CRC and depicts an aggressive disease with a disappointing response to standard treatments[8]. Recent studies however, have evaluated de combination of small oral inhibitory molecules, as sotorasib or adagrasib, with anti-EGFR therapies achieving promising results."

  References:

  6. Koulouridi A, Karagianni M, Messaritakis I, et al. Prognostic Value of KRAS Mutations in Colorectal Cancer Patients. Cancers (Basel). 2022 Jul 7;14(14):3320.
  7. Ciardiello D, Maiorano BA, Martinelli E. Targeting $KRAS^{G12C}$ in colorectal cancer: the beginning of a new era. ESMO Open. 2023 Feb;8(1):100745. doi: 10.1016/j.esmoop.2022.100745. Epub 2022 Dec 20.
  8. Alese OB, Wu C, Chapin WJ, et al. Update on Emerging Therapies for Advanced Colorectal Cancer. Am Soc Clin Oncol Educ Book. 2023 May;43:e389574.

- Changes in the text: see Page 1, line 29

Reviewer B

Comment 5: The introduction was very informative, but it could be more concise.

- **Reply 5:** According to your comment we eliminated the following paragraph and references: ~~The primitive intestine derives from the endoderm which divides into three segments: anterior, middle, and caudal. The middle intestine forms the small intestine, ascending colon, and proximal transverse colon, while the caudal intestine gives rise to the distal transverse colon and the rectum.[2]~~
  - 2)Schoenwolf GC, Bleyl SB, Brauer PR, et al. Larsen's Human Embryology, 5th edition. Philadelphia, PA: Churchill Livingstone; 2015
  - 3)Shen H, Yang J, Huang Q, et al. Different treatment strategies and molecular features between right-sided and left-sided colon cancers. World J Gastroenterol. 2015 Jun 7;21(21):6470-8.
- Changes in the text: see Page 1, line 12 and references 2 and 3.

Comment 6: More detail could be provided for some relevant statements. For example, the statement on line 66-67; "Both the prognostic and predictive value of response to anti-EGFR drugs diminish with more advanced treatment lines, and location may not be

relevant in the advanced setting." It's quite relevant to the study, more explanation would provide readers a perspective of its complexicity in heavily pretreated paients with mCRC.

- **Reply 6:** According to your comment the following information has been modified:
    - Both the prognostic and predictive value of response to anti-EGFR drugs diminish with more advanced treatment lines, and location may not be relevant in the advanced setting due to the acquisition of new resistance mutations. Many trials, such as CORRECT or SUNLIGHT have reported significantly improved outcomes beyond second line in unselected patients. The use of multikinase inhibitors as regorafenib has been useful in this setting despite its limited clinical benefit[8]. Improvements in the understanding of underlying mechanisms of resistance, as *ERBB2* amplification or *PIK3CA*[22], will change this approach in the future."
- Changes in the text: see Page 2, line 67

Comment 7: Although, there was no statistically significant difference in OS and PFS between cetuximab and placebo groups, there was a trend toward longer survival in cetuximab group with clinically significant difference. It would be great to have some comment on this point.

- **Reply 7:** It was found a statistically significant difference when we focus on left-side colon cancer. The following sentence was modified according to these data as follows: "Patients with left-sided mCRC had superior outcomes with cetuximab compared with transverse for OS (median, 9.7 vs. 5.9 months; HR, 0.42; 95% CI, 0.27-0.67; P=0.0002) and PFS (median, 3.8 vs. 1.8 months; HR, 0,49; 95% CI, 0.31-0.76; P=0.001).

- Changes in the text: see Page 3, line 90