

ORIGINAL ARTICLE

Efficacy and safety of atezolizumab plus bevacizumab in MSI-like metastatic colorectal cancer: a multicenter, single-arm, phase II, open-label clinical trial

E. Elez¹, S. Siena², A. Sartore-Bianchi², J. Ros¹, F. Ciardello³, E. Martinelli³, N. Steeghs^{4,5}, S. Huijberts^{6,7}, A. Cervantes⁸, S. Roselló⁸, S. Tejpar^{9,10}, B. Wilmser^{6,7}, R. Salazar¹¹, C. Santos¹¹, G. de Schaetzen¹², S. Muñoz¹, R. Bernards^{9,10}, V. Moreno^{11,13,14,15}, E. García-Galera¹⁶, R. Alemany¹¹, A. Gros¹, A. Bardelli^{17,18}, S. Arena^{17,19}, A. Piris¹, E. Chavarría¹, R. Dienstmann^{16,20} & J. Tabernero^{1*}

¹Vall d'Hebron Institute of Oncology (VHIO), Vall d'Hebron University Hospital, Barcelona, Spain; ²Università degli Studi di Milano, Niguarda Cancer Center and Department of Medical Oncology, ASST Grande Ospedale Metropolitano Niguarda, Milan; ³Università degli Studi della Campania "Luigi Vanvitelli", Naples, Italy; ⁴The Netherlands Cancer Institute, Amsterdam; ⁵University Medical Center Utrecht, Utrecht, The Netherlands; ⁶KU Leuven, Leuven, Belgium; ⁷Amsterdam UMC, Amsterdam, The Netherlands; ⁸Department of Medical Oncology, Hospital Clínico Universitario, INCLIVA Biomedical Research Institute, University of Valencia, Valencia, Spain; ⁹UZ Gasthuisberg, Katholieke University Leuven, Leuven, Belgium; ¹⁰The Netherlands Cancer Institute, Amsterdam, The Netherlands; ¹¹Catalan Institute of Oncology, Oncobell Program (IDIBELL), University of Barcelona (Campus Bellvitge), CIBERONC, L'Hospitalet de Llobregat, Barcelona, Spain; ¹²EORTC Headquarters, Brussels, Belgium; ¹³Colorectal Cancer Group, ONCOBELL Program, Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Barcelona; ¹⁴Department of Clinical Sciences, Faculty of Medicine and Health Sciences and University of Barcelona Institute of Complex Systems (UBICS), University of Barcelona, L'Hospitalet de Llobregat, Barcelona; ¹⁵Consortium for Biomedical Research in Epidemiology and Public Health (CIBERESP), Madrid; ¹⁶Oncology Data Science (ODysSey) Group, Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain; ¹⁷Department of Oncology, University of Torino, Torino; ¹⁸IFOM ETS — The AIRC Institute of Molecular Oncology, Milan; ¹⁹Candiolo Cancer Institute, FPO-IRCS, Candiolo, Torino, Italy; ²⁰University of Vic — Central University of Catalonia, Vic, Spain



Available online xxx

Background: The use of immune checkpoint inhibitors (ICIs) in metastatic colorectal cancer (mCRC) remains limited to tumors harboring microsatellite instability (MSI); however, a subset of microsatellite stable (MSS) tumors features an MSI-like phenotype that could predict responses to ICIs combined with anti-angiogenesis agents.

Patients and methods: In this single-arm phase II trial, 45 mCRC patients with a positive tumoral MSI-like gene expression signature (GES) progressing to at least one chemotherapy regimen were recruited from seven European sites within the MoTriColor framework. Of these, 24 and 21 were MSI and MSS, respectively, by standard assays. Patients received intravenous atezolizumab (1200 mg) plus bevacizumab (7.5 mg/kg) infusions in 21-day cycles until progression, unacceptable toxicity, or consent withdrawal. The main outcome measure was the objective response rate (ORR, RECIST 1.1).

Results: The median (interquartile range) age of participants was 63 (58-73) years, 51.1% were male, 60.0% had right-sided tumors, and 31.1% had liver metastases. The ORR in the whole (MSI-like) sample was 38.6% [95% confidence interval (CI) 24.4% to 54.5%]. Among patients with MSI and MSS tumors, the ORR was 65.2% (95% CI 42.7% to 83.6%) and 9.5% (95% CI 1.2% to 30.4%), respectively. In the MSS subgroup without liver metastasis, the ORR was 15.4% (95% CI 1.9% to 45.4%). Overall median progression-free survival was 6.4 (95% CI 4.1-21.2) months (23.2 and 4.0 months in patients with MSI and MSS tumors, respectively). Grade ≥ 3 adverse events related to atezolizumab and bevacizumab occurred in 5 (11.1%) and 10 patients (22.2%), respectively. There were two grade 5 adverse events, of which one (colonic hemorrhage) was related to bevacizumab.

Conclusions: MSI-like GES does not identify a population with higher sensitivity to immune checkpoint plus angiogenesis inhibition. However, responses are promising in patients with MSI tumors and, to a lesser extent, in patients without liver metastasis regardless of MSI status.

Key words: angiogenesis inhibitors, immune checkpoint inhibitors, immunotherapy, intestinal neoplasms, microsatellite instability, precision medicine

*Correspondence to: Prof. Josep Tabernero, Vall d'Hebron Institute of Oncology (VHIO), Centro Cellex, Carrer de Natzaret, 115-117, Barcelona, Spain. Tel: +34-932543450

E-mail: jtabernero@vhio.net (J. Tabernero).

2059-7029/© 2025 The Author(s). Published by Elsevier Ltd on behalf of European Society for Medical Oncology. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

INTRODUCTION

The tumor microenvironment (TME) plays a major role in colorectal cancer (CRC) progression and response to therapy.¹ A subset of CRCs (~15%) harbors a DNA mismatch repair-deficient (dMMR) system as the dominant genomic feature,

giving rise to hypermutation, microsatellite instability (MSI), persistent neoantigen generation, and a strong immune and lymphocytic infiltration of the TME.²⁻⁵ These highly immunogenic tumors overexpress programmed death-ligand 1 (PD-L1), which leads to tumor immune evasion.⁶ In any case, this property makes dMMR/MSI mCRCs highly sensitive to immune checkpoint inhibitor (ICI) therapy.⁷ Alternatively, MMR-proficient (pMMR) or microsatellite stable (MSS) CRCs characteristically display far fewer (up to 100-fold) somatic mutations and feature an immune-excluded TME,⁸ with T cells being absent or concentrated at the invasive margin.¹ The absence of T cells in the tumor core may blunt the efficacy of ICIs in pMMR/MSS mCRCs.⁹ Nonetheless, a subset of pMMR/MSS tumors with higher mutational burdens displays an 'MSI-like' phenotype by gene expression profiling.¹⁰ In fact, the gene expression-based consensus molecular subtypes (CMS groups) of CRC display distinct molecular phenotypes that only partially overlap with dMMR/MSI status.^{11,12} Although most CMS1 'immune' tumors test positive for MSI, the remaining could also prove sensitive to immunotherapy despite being MSS.¹¹ Detection of such an MSI-like phenotype may help select a population of patients with immune-rich tumors that responds to novel combination approaches. Angiogenesis is critical for tumor growth,¹³ and its key regulator, the vascular endothelial growth factor (VEGF), fosters an immune-suppressive TME.¹⁴⁻¹⁶ Indeed, combined inhibition of the programmed cell death protein 1 (PD-1)/PD-L1 and VEGF axes has resulted in therapeutic activity in different tumors.¹⁷⁻²⁰ This study aimed to evaluate the efficacy and safety of combining atezolizumab—an anti-PD-L1 monoclonal antibody—with bevacizumab—an anti-VEGF monoclonal antibody—in chemotherapy-resistant mCRC patients with a positive MSI-like gene expression signature (GES) as a whole, and whether efficacy was limited to those with confirmed dMMR/MSI by standard testing.

PATIENTS AND METHODS

Study design and treatment

This multicenter, single-arm, phase II, open-label trial (NCT0298269; EudraCT: 2016-002001-19) assessed the efficacy and safety of atezolizumab and bevacizumab combination therapy in patients with chemotherapy-refractory mCRC and classified as MSI-like by a GES in formalin-fixed paraffin-embedded (FFPE) primary tissue samples (Agendia® microarray analysis), which was developed to accurately identify MSI tumors based on microarray data.^{21,22} Patients either MSS or MSI (henceforth MSI-real) by standard diagnostic assays [i.e. PCR and immunohistochemistry (IHC)] were enrolled at seven European sites (three in Spain, two in Italy, one in the Netherlands, and one in Belgium) within the framework of the European Commission-funded Molecularly-guided Trials for advanced Colorectal cancer (MoTriColor) Project.

Patients received atezolizumab (1200 mg every 21 days) plus bevacizumab (7.5 mg/kg every 21 days) until progression, unacceptable toxicity, or consent withdrawal.

The protocol was reviewed and approved by the relevant institutional review boards at each center. All patients provided written informed consent before enrollment. This study was conducted in accordance with the terms of the Declaration of Helsinki, Good Clinical Practice (GCP) guidelines, and all applicable regulatory requirements. The manuscript followed the Consolidated Standards of Reporting Trials (CONSORT) and Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

Participants

Eligible patients were aged ≥ 18 years and had histological proof of CRC, unresectable disease with at least one measurable lesion according to RECIST version 1.1, disease progression or relapse after at least one line of chemotherapy in the metastatic setting, positive MSI-like GES in tumor tissue samples regardless of MSI status, and adequate hematologic and end-organ function with a life expectancy exceeding 12 weeks and World Health Organization performance status of one or less. Patients previously treated with CD-137 agonists, anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), anti-PD-1, or anti-PD-L1 therapeutic antibodies or immune-related pathway-targeting agents were excluded. A full list with the inclusion and exclusion criteria is provided in the [Supplementary Table S1](https://doi.org/10.1016/j.esmoop.2025.105892), available at <https://doi.org/10.1016/j.esmoop.2025.105892>.

Study endpoints

The primary endpoint was the investigator-assessed objective response rate (ORR; the best of complete or partial responses) assessed using RECIST 1.1, assuming a minimum response rate of 25% and an overall target efficacy of 45%. Secondary endpoints included progression-free survival (PFS); overall survival (OS); time to response (TTR, among patients who responded); and duration of response (DOR, among patients who responded), all assessed with RECIST 1.1; and the safety and tolerability of the study treatments, assessed through the accumulated incidence and severity of adverse events (AEs) according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. RECIST evaluations were scheduled every 9 weeks. Also, a *post hoc* exploratory analysis was carried out to evaluate the clinical impact of liver metastasis (LM) on treatment response.

Statistical analysis

All collected data were described using appropriate descriptive statistics, including means (standard deviations), medians, and quartiles for continuous variables, and numbers and frequencies for categorical variables. All descriptions were carried out for the overall MSI-like set as well as for the 'MSI-real' and 'MSS' subgroups. All collected data were described on the safety analysis set (defined as those patients who received at least one dose of the study treatment), which was also used for all safety endpoints. An intention-to-treat

(ITT) analysis set was defined as those patients who received at least one dose of the study treatment and had at least one efficacy endpoint available; it was used for all efficacy analyses. Categorical efficacy endpoints (such as the ORR and response components) were analyzed as binomial proportions. Interval inferences of population parameters were done using exact 95% two-sided binomial confidence intervals (CIs). In addition, two-sided exact *P* values were calculated by binomial enumeration for testing two null hypotheses that the observed ORRs represented true population values of 25% (minimum response rate) and 45% (overall efficacy). They were also compared between MSI-real and MSS subgroups by means of Pearson's χ^2 or Fisher's exact tests. Time-to-event endpoints (such as TTR, PFS, DOR, or OS) were described using the Kaplan–Meier non-parametric method. Interval inferences of population parameters were done using asymptotic pointwise two-sided 95% CIs obtained with the Greenwood formula with logarithmic bounding. Time-scale (horizontal) median CIs were obtained using the Brookmeyer–Crowley method. Log-rank tests were used to compare survival curves between the MSI-real and MSS subgroups. Safety endpoints were analyzed as categorical efficacy endpoints.

A sample size of 36 patients is required to reject one null hypothesis that the response rate is $\leq 25\%$ in a one-sided binomial test at a significance level of 0.05 when the observed response is 45%. Assuming a drop-out rate of 10% and supposing that the MSI-real subgroup would represent 70% of the sample, the recruitment target was set at 58 patients to ensure enough statistical power within this subgroup.

The planned study duration was 3 years. However, when this deadline was reached in October 2020, recruitment had not been completed. It was then decided that an interim analysis of the ORR should be carried out because the response rate seemed low in the MSS subgroup. For this purpose, a protocol amendment that did not include sample size adjustments but introduced an early stopping rule for futility based on conditional power ($< 20\%$ probability of rejecting the null hypothesis at the end of the study given the data that emerged so far) was passed. The ORR was tested among patients who had received at least one restaging procedure by 15 August 2020 or had been diagnosed with progressive disease. Since the stopping rule was met within the MSS subgroup, recruitment was closed and a post-trial access program was initiated to enable trial treatment continuation in patients still experiencing benefit.

All statistical analyses were carried out using R software version 4.3 (R Project for Statistical Computing, Vienna, Austria). Two-sided *P* values of ≤ 0.05 indicated statistical significance.

RESULTS

Patients

Between November 2017 and October 2020, a total of 46 MSI-like mCRC patients were included at seven European

participating sites. Of these, tumors were MSI-real per standard diagnostic assays (PCR/IHC) in 24 (52.2%) patients, while they were MSS in 22 (47.8%) patients. One patient was a screening failure and was excluded before receiving any dose (Figure 1); thus, all analyses were carried out on the remaining 45 patients (ITT set), except for response-related endpoints (i.e. ORR and PFS) that excluded another patient who did not have any valid post-baseline RECIST assessment available.

Patients had a median (interquartile range) age of 63 (58–73) years and 23 (51.1%) of them were men. The median (interquartile range) follow-up was 48.8 (42.6–56.2) months. Overall, the median (interquartile range) time from diagnosis to study entry was 24.0 (15.6–33.8) months (Table 1). MSI-real patients had a higher proportion of high-grade disease at diagnosis than MSS patients (47.6% versus 22.2%). Most patients had been exposed to 5-fluorouracil-based chemotherapy with oxaliplatin (Supplementary Figure S1, available at <https://doi.org/10.1016/j.esmoop.2025.105892>). Previous exposure to two or more chemotherapy lines was less frequent among MSI-real patients (25.0%) than among MSS patients (76.2%). Fourteen (31.1%) had metastases in the liver, this proportion being higher among MSS patients (8 out of 21, 38.1%) than MSI-real patients (6 of 24, 25.0%).

Efficacy

Efficacy results were analyzed in the ITT analysis set. An ORR was observed in 38.6% (95% CI 24.4% to 54.5%) of the patients ($P = 0.053$ assuming that the true value in the population was 25%); however, the ORR among patients with MSI-real tumors (65.2%, 95% CI 42.7% to 83.6%) was significantly higher than in patients with MSS tumors (9.5%, 95% CI 1.2% to 30.4%, $P = 0.001$) (Table 2 and Supplementary Figure S2, available at <https://doi.org/10.1016/j.esmoop.2025.105892>). Presence of LMs undermined treatment responses considerably. Overall, ORR was observed in 50.0% (95% CI 31.3% to 68.7%) of the patients with no LMs (NLMs) and in 14.3% (95% CI 1.8% to 42.8%) of those with LMs (Table 2 and Supplementary Figure S2, available at <https://doi.org/10.1016/j.esmoop.2025.105892>). Furthermore, the ORR of MSI-real patients was substantially higher than that of their MSS counterparts within each stratum (i.e. LMs and NLMs) (Table 2). Interestingly, within the NLM stratum, the ORR in the subgroup of patients with MSS tumors was 15.4% (95% CI 1.9% to 45.4%) (Table 2).

The median PFS was 6.4 (95% CI 4.1–21.2) months. Nonetheless, MSI-real patients had a significantly ($P < 0.001$) longer median PFS (23.2 months, 95% CI 8.1 months–not estimable) than the MSS patients (4.0 months, 95% CI 2.0–8.1 months), as shown in Figure 2A. Median OS was 26.1 [95% CI 9.1–not estimable (NE)] months, but MSI-real patients lived significantly longer ($P < 0.001$) than their MSS peers (Figure 2B). Unsurprisingly, LM patients had a much-reduced median PFS and OS than NLM patients in

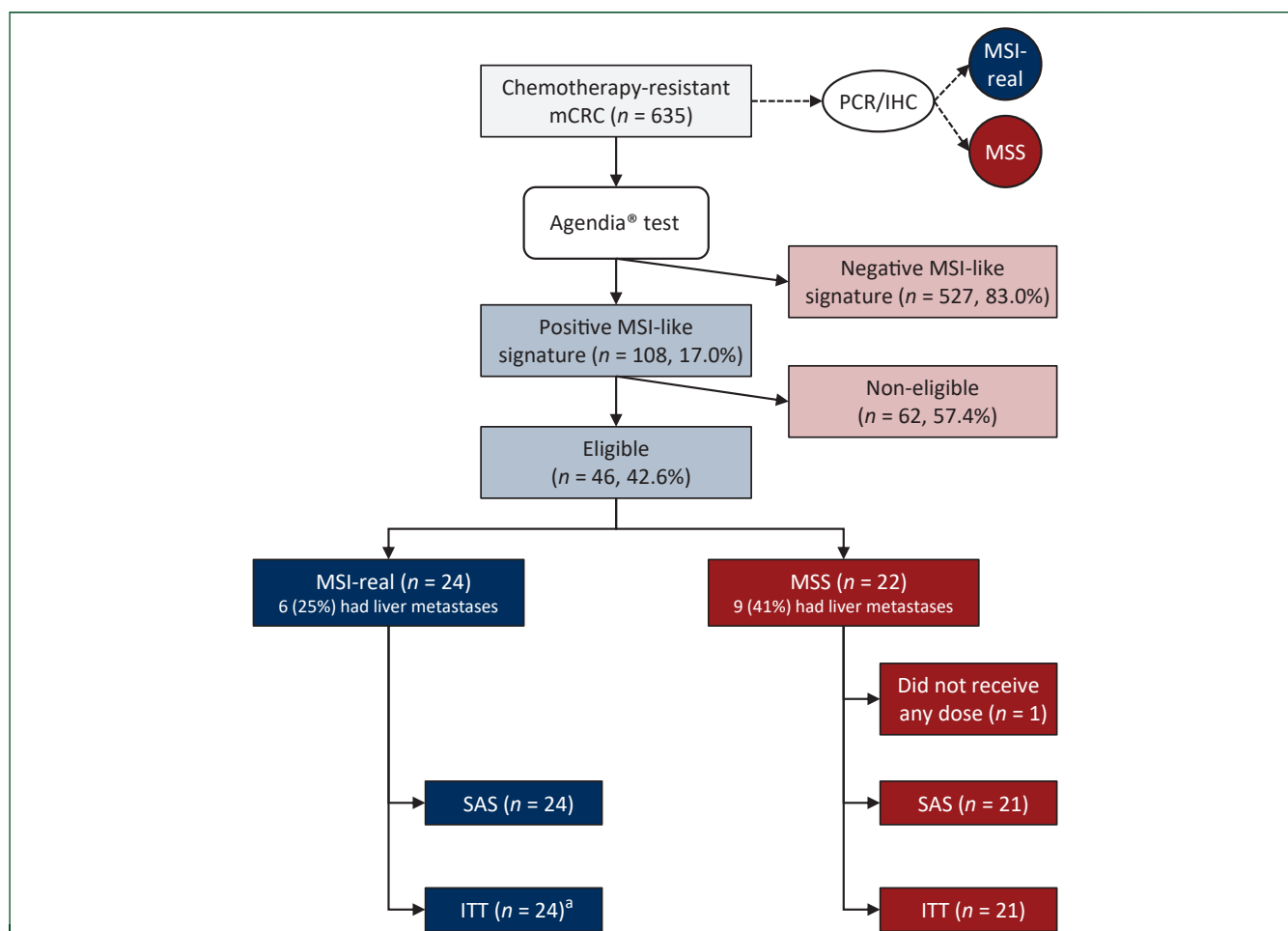


Figure 1. Study flowchart.

^aOne patient did not have any post-baseline RECIST assessment available and was excluded from response-related analyses (ORR, PFS) but not from other analyses (OS, safety, etc.).

ITT, intention-to-treat; mCRC, metastatic colorectal cancer; MSI, microsatellite instability; MSS, microsatellite stable; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; SAS, safety analysis set.

Table 1. Demographics and baseline characteristics

Characteristic	MSI-real (n = 24)	MSS (n = 21)	Total (MSI-like) (n = 45)
Age, median (IQR), years	64.0 (57.3-73.0)	62.0 (59.0-73.0)	63.0 (58.0-73.0)
Sex, n (%)			
Male	12 (50.0)	11 (52.4)	23 (51.1)
Female	12 (50.0)	10 (47.6)	22 (48.9)
Height, median (IQR), cm	167.0 (161.0-177.5)	168.0 (158.0-175.0)	167.5 (159.5-176.3)
Missing	1	0	1
Months from diagnosis to study entry, median (IQR)	18.2 (14.2-31.0)	26.1 (23.9-39.1)	24.0 (15.6-33.8)
Site of the primary tumor, n (%)			
Right colon	17 (70.8)	10 (47.6)	27 (60.0)
Transverse colon	1 (4.2)	0 (0)	1 (2.2)
Left colon	3 (12.5)	4 (19.1)	7 (15.6)
Rectum and/or sigmoid	3 (12.5)	7 (33.3)	10 (22.2)
Histological grade, n (%)			
Low grade (G1/G2)	10 (47.6)	11 (61.1)	21 (53.9)
High grade (G3/G4)	10 (47.6)	4 (22.2)	14 (35.9)
Not evaluable	1 (4.8)	3 (16.7)	4 (10.3)
Missing	3	3	6
Metastasis in the liver, n (%)	6 (25.0)	8 (38.1)	14 (31.1)
Previous chemotherapy lines, n (%)			
None	3 (12.5)	0 (0)	3 (6.7)
One line	15 (62.5)	5 (23.8)	20 (44.4)
Two or more lines	6 (25.0)	16 (76.2)	22 (48.9)
Previous bevacizumab therapy, n (%)	13 (54.2)	17 (81.0)	30 (66.7)

IQR, interquartile range; MSI, microsatellite instability; MSS, microsatellite stable.

Table 2. Objective response rate stratified by the presence of liver metastases

Status	n	CR + PR	ORR (95% CI), %	P value ^a	
				ORR 25%	ORR 45%
MSI-real (LM + NLM)	23	15	65.2 (42.7-83.6)	<0.001	0.060
MSI-real (LM)	6	2	33.3 (4.3-77.7)	0.644	0.697
MSI-real (NLM only)	17	13	76.5 (50.1-93.2)	<0.001	0.013
MSS (LM + NLM)	21	2	9.5 (1.2-30.4)	0.131	<0.001
MSS (LM)	8	0	—	—	—
MSS (NLM only)	13	2	15.4 (1.9-45.4)	0.539	0.047
Total (MSI-like) (LM + NLM)	44	17	38.6 (24.4-54.5)	0.053	0.450
Total (LM)	14	2	14.3 (1.8-42.8)	0.540	0.028
Total (NLM only)	30	15	50.0 (31.3-68.7)	0.005	0.588

CI, confidence interval; CR, complete response; CR + PR, number of patients achieving either CR or PR; LM, liver metastases; MSI, microsatellite instability; MSS, microsatellite stable; NLM, no liver metastases; ORR, objective response rate; PR, partial response.

^aP values corresponding to the null hypothesis that the proportions in the population from which the study sample was drawn are the ones specified. These P values are two-sided and have been calculated via binomial enumeration.

both the MSI-real subgroup and the MSS subgroup (Figure 2C-F).

The median TTR in the MSI-real subgroup was 2.1 (95% CI 2.0-5.1) months and 9.8 (95% CI 2.2-NE) months in patients with MSS tumors (Supplementary Table S2 and Figure S3, available at <https://doi.org/10.1016/j.esmoop.2025.105892>). Consistently, the DOR lasted longer among MSI-real patients (median not reached) than among MSS patients (5.9 months, 95% CI 5.9 months-NE) (Supplementary Table S3 and Figure S4, available at <https://doi.org/10.1016/j.esmoop.2025.105892>). Nevertheless, these differences between MSI-real and MSS patients were not significant for both the TTR ($P = 0.200$) and DOR ($P = 0.400$).

Safety

Almost all patients (97.8%) experienced some kind of AEs. The most common AE was fatigue (48.9%), followed by hypertension (28.9%), abdominal pain (24.4%), and diarrhea (22.2%) (Supplementary Table S4, available at <https://doi.org/10.1016/j.esmoop.2025.105892>). Grade ≥ 3 AEs occurred in 22 (48.9%) patients. In 5 patients (11.1%) they were related to atezolizumab and in 10 (22.2%) to bevacizumab. Two patients (4.4%) suffered grade 5 AEs: one MSI-real patient died of colonic hemorrhage possibly related to bevacizumab and one MSS patient died of severe acute respiratory distress (unrelated to either study drug).

DISCUSSION

As expected, the response to atezolizumab immunotherapy combined with the anti-VEGF agent bevacizumab in patients with mCRC harboring MSI ascertained by conventional PCR- or IHC-based methods was satisfactory. However, this benefit did not transpose to a broader set of patients ascertained by a gene expression classifier that identifies tumors harboring downstream consequences of

deficient DNA mismatch repair mechanisms. The response within the subgroup of MSS CRCs was small and comparable to that reported in a recent systematic review.²³

With immunotherapy becoming a pillar of cancer care, there is great interest in expanding its benefits to the greatest possible number of CRC patients and in detecting those most likely to respond.²⁴ Although several studies have confirmed diverging response profiles to ICIs between MSI and MSS CRCs,²⁵⁻²⁸ still about one-third of patients with MSI mCRC will experience progression after starting ICI therapy.¹² In turn, some recent studies have suggested that combined chemotherapy with anti-angiogenesis agents may render a small subset of pMMR/MSS mCRCs sensitive to ICI therapy.²⁹⁻³⁵ This encourages further research to identify susceptible patients and delve deeper into their mechanisms of action and relative contributions, in analogy to the different tumors mentioned in the Introduction section.¹⁷⁻²⁰ In this regard, transcriptomic classifiers are being developed to gain a deeper insight into the underlying biology of tumors and uncover potential biomarkers of immunotherapy response beyond MSI status.^{11,12,36} The MoTriColor Project contributed to these efforts by matching CRC molecular transcriptomic signatures to selected therapies, including the combination evaluated in the present clinical trial. However, contrary to what was hypothesized, the MSI-like signature failed to detect immune-sensitive CRCs beyond those classified as MSI by conventional testing. Nevertheless, two findings worth mentioning have emerged: (i) the ORR in patients with MSI-real tumors exceeded other reports and was comparable to those attained with anti-PD-L1/CTLA-4 checkpoint inhibition combinations,³⁷ and (ii) the modest ORR among patients with MSS tumors was restricted to those without metastases in the liver, suggesting that some of them may derive benefit from ICI/anti-VEGF combinations. Due to differences in prognosis, reporting for the subgroup of patients without LM has recently been recommended.²³ Our results are aligned with recent phase I clinical trials that show responses with combination ICIs (anti-PD-1 plus anti-CTLA-4) with or without multi-kinase angiogenesis inhibitor regorafenib in mCRC patients with MSS tumors without LMs.³³⁻³⁵

The main reason for the failure of the GES classifier in this study may derive from the fact that it was developed for diagnostic purposes (MSI-like) rather than for predicting therapeutic responses. There are subtle differences between the diagnostic and predictive settings. Although CRCs are thought to harbor most mutations from an early stage, downstream gene expression and pathway activation patterns can actually evolve during disease progression or as a result of (sub)clonal selection induced by treatment pressure.³ Although the MSI-like GES was tested in primary tumor samples, the signature status in metastatic lesions after progression to standard-of-care chemotherapies was not assessed. In addition, although MSI-like tumors overall may display a relatively immune-active TME, immune evasion mechanisms other than PD-L1 overexpression may also be in place. The poorer outcomes observed in patients with metastases in the liver, an immune-privileged organ

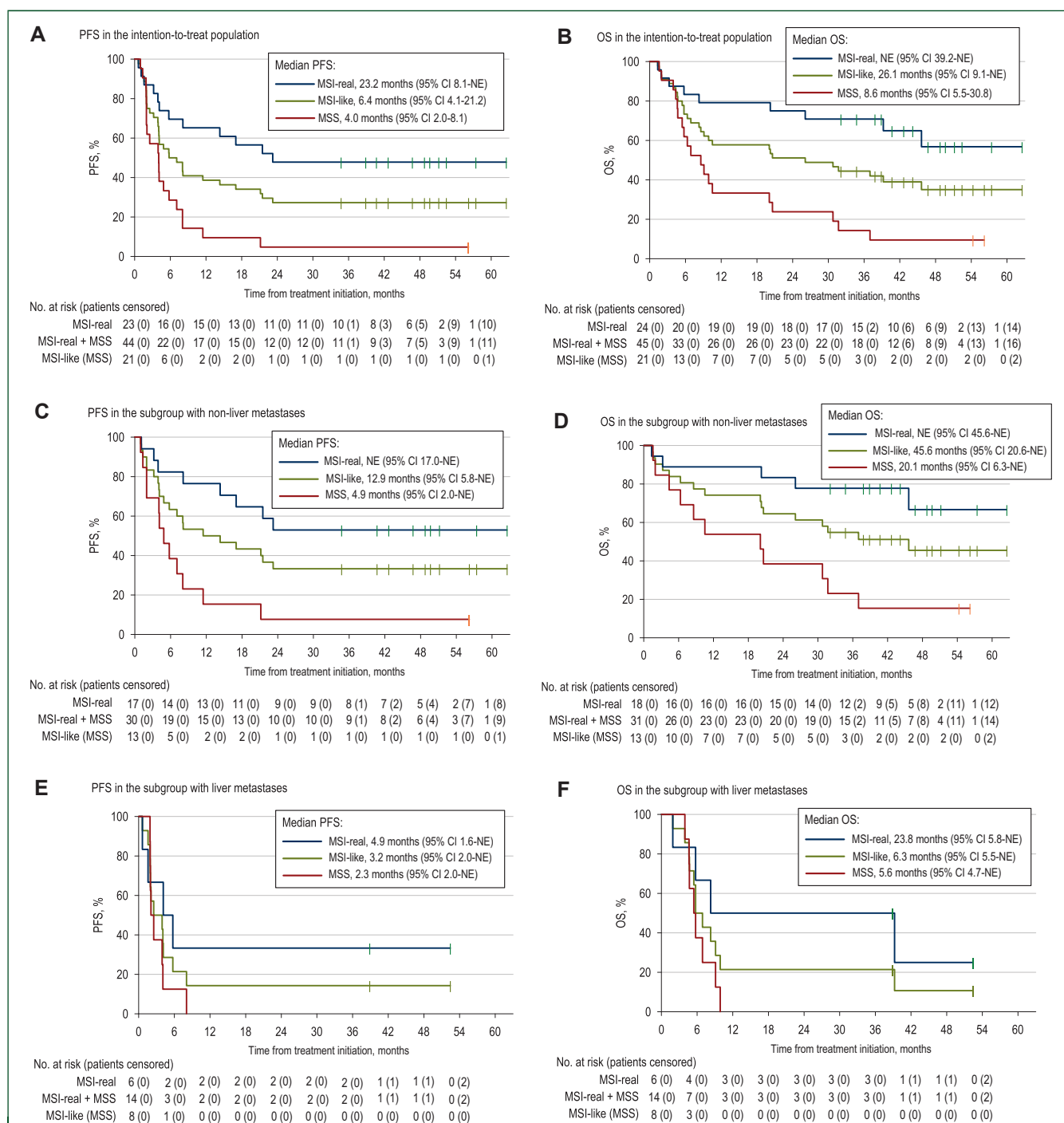


Figure 2. Kaplan–Meier curves of PFS and OS. PFS (A) and OS (B) for each subgroup (MSI-real and MSS) and total sample in the intention-to-treat population. PFS (C) and OS (D) for each subgroup (MSI-real and MSS) and total sample in the stratum with no liver metastases. PFS (E) and OS (F) for each subgroup (MSI-real and MSS) and total sample in the stratum with liver metastases.

CI, confidence interval; MSI, microsatellite instability; MSS, microsatellite stable; NE, not estimable; OS, overall survival; PFS, progression-free survival.

that may hamper the effectiveness of immunotherapy,^{24,38} further support this notion. Interestingly, animal models investigating the mechanisms by which viral infections can perpetuate in the liver have shown an expansion of liver-primed CD8⁺ T-regulatory immunosuppressive cells that occurred independently from PD-L1 expression.³⁹ Another nuance between diagnostic and predictive settings relates to the fact that, while most MSI tumors are known to

cluster in CMS1, up to 30% of CMS3 tumors that also present with MSI, hypermutation, and intermediate levels of hypermethylation of MMR-related genes strikingly display lower levels of immune infiltration.^{3,11} These and other reasons may help understand divergences between transcriptomic and (functional) immune subtyping of CRC.

A note of caution is warranted regarding the prognostic value of liver involvement: patients with confirmed MSI

and LMs did not respond satisfactorily to atezolizumab plus bevacizumab (ORR 33.3%). In addition to the aforementioned relative immunosuppression,⁴⁰ the transforming growth factor- β pathway, a main proliferative and pro-invasive driver in CRC that can also enable immune evasion,⁴¹ has been shown to be prominent in LMs.²³

Limitations

One limitation concerns statistical power. Since the interim analysis was not planned in the original protocol, sample size calculations did not account for the inherent multiplicity, which would have required the use of error spending methods to establish formal stopping boundaries. Otherwise, a rule based on conditional power within the MSS subgroup was used. As a result, the power to reject the null hypothesis in the MSI-real subgroup turned out to be insufficient, hence the marginal results. Nevertheless, given other highly significant results obtained within the latter subgroup, it is reasonable to conclude that multiplicity adjustments would not have affected the study conclusions. Further research into potential predictive biomarkers correlating with lack of response, such as investigating the TME features of the MSS subgroup or microbiome signatures that associate with upfront resistance to immunotherapy combinations, could have shed more light on the reasons for the negative result, but these were outside of the scope of this study.

Conclusions

A microarray-based MSI-like gene expression classifier applied to FFPE primary tumor samples was not able to detect a subset of patients with MSS mCRC sensitive to immunotherapy in combination with anti-VEGF therapy beyond those ascertained by conventional PCR- or IHC-based MSI assays. Nonetheless, high responses to the combination of atezolizumab with bevacizumab were attained in patients with MSI-real tumors. Although the classifier used in this study may not merit further clinical testing, it is worth noting that some patients without LM who test MSS by conventional methods may respond to ICIs in combination with antiangiogenic agents.

FUNDING

This work was supported by the European Commission's Horizon 2020 Research and Innovation Program [grant number 635342]; the European Research Council (ERC) under the European Union's Horizon 2020 Research and Innovation Program (TARGET) [grant number 101020342] to AB; the AIRC under 5 per Mille 2018 program for principal investigator AB [grant number 21091]; the FPRC under 5 per Mille Ministero della Salute 2022 CARESS for SA; the IMI contract ID: 101007937 PERSIST-SEQ (to AB); the AIRC under IG 2023 for principal investigator AB [grant number 28922] and SA [grant number 29286]; the Italian Ministry of Health—Ricerca Corrente 2025 (to SA); and PRIN 2022—Prot. 2022CHB9BA financed by the European Union—Next Generation EU (to AB). In addition, F.

Hoffmann-La Roche Ltd. (no grant number) provided the study medication and partial funding for logistical issues. The funders did not have any direct corporate role in the design, analysis, interpretation of results, or preparation of the manuscript.

DISCLOSURE

EM reports collaborations with Merck KGaA, Bayer, Roche, Takeda, Servier, and Pierre Fabre as invited speaker; participation in advisory boards for Bayer, Merck KGaA, Servier, and Pierre Fabre; and receipt of travel expenses from AstraZeneca and Pierre Fabre; is a member of ESMO and AIOM. AG reports membership of advisory boards at Achilles Therapeutics plc., SingulaBIO, RoothPath Inc, and BioNTech SE, and participation as consultant advisor for Bio AG; is co-inventor of licensed patents (E-059-2013/0 E-085-2013/0, E-149-2015/0) and receives royalties from Intima Bioscience Inc., Intellia Therapeutics Inc., Tailored Therapeutics LLC, Cellular Biomedicine Group Inc., and Geneius Biotechnology Inc. AB reports receipt of grants/research support from Neophore, AstraZeneca, Boehringer Ingelheim and honoraria/consultation fees from Guardant Health; is stock shareholder of Neophore and Kither Biotech; is a member of advisory boards for Neophore. All other authors have declared no conflicts of interest.

REFERENCES

- Ghiringhelli F, Fumet JD. Is there a place for immunotherapy for metastatic microsatellite stable colorectal cancer? *Front Immunol*. 2019;10:1816.
- Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. *Nature*. 2012;487(7407):330-337.
- Dienstmann R, Vermeulen L, Guinney J, Kopetz S, Tejpar S, Tabernero J. Consensus molecular subtypes and the evolution of precision medicine in colorectal cancer. *Nat Rev Cancer*. 2017;17(2):79-92.
- Germano G, Lamba S, Rospo G, et al. Inactivation of DNA repair triggers neoantigen generation and impairs tumour growth. *Nature*. 2017;552(7683):116-120.
- Llosa NJ, Cruise M, Tam A, et al. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. *Cancer Discov*. 2015;5(1):43-51.
- Blank C, Gajewski TF, Mackensen A. Interaction of PD-L1 on tumor cells with PD-1 on tumor-specific T cells as a mechanism of immune evasion: implications for tumor immunotherapy. *Cancer Immunol Immunother*. 2005;54(4):307-314.
- Borelli B, Antoniotti C, Carullo M, Germani MM, Conca V, Masi G. Immune-checkpoint inhibitors (ICIs) in metastatic colorectal cancer (mCRC) patients beyond microsatellite instability. *Cancers (Basel)*. 2022;14(20):4974.
- Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med*. 2015;372(26):2509-2520.
- Yoon HH, Shi Q, Heying EN, et al. Intertumoral heterogeneity of CD3+ and CD8+ T-cell densities in the microenvironment of DNA mismatch-repair-deficient colon cancers: implications for prognosis. *Clin Cancer Res*. 2019;25(1):125-133.
- Saller J, Qin D, Felder S, Coppola D. Microsatellite stable colorectal cancer with an immunogenic phenotype: challenges in diagnosis and treatment. *Clin Colorectal Cancer*. 2020;19(2):123-131.
- Guinney J, Dienstmann R, Wang X, et al. The consensus molecular subtypes of colorectal cancer. *Nat Med*. 2015;21(11):1350-1356.

12. Gallois C, Landi M, Taieb J, et al. Transcriptomic signatures of MSI-high metastatic colorectal cancer predict efficacy of immune checkpoint inhibitors. *Clin Cancer Res*. 2023;29(18):3771-3778.
13. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med*. 2003;9(6):669-676.
14. Saeed A, Park R, Sun W. The integration of immune checkpoint inhibitors with VEGF targeted agents in advanced gastric and gastroesophageal adenocarcinoma: a review on the rationale and results of early phase trials. *J Hematol Oncol*. 2021;14(1):13.
15. Kusmartsev S, Eruslanov E, Kubler H, et al. Oxidative stress regulates expression of VEGFR1 in myeloid cells: link to tumor-induced immune suppression in renal cell carcinoma. *J Immunol*. 2008;181(1):346-353.
16. Manning EA, Ullman JG, Leatherman JM, et al. A vascular endothelial growth factor receptor-2 inhibitor enhances antitumor immunity through an immune-based mechanism. *Clin Cancer Res*. 2007;13(13):3951-3959.
17. Finn RS, Qin S, Ikeda M, et al. Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. *N Engl J Med*. 2020;382(20):1894-1905.
18. Rini BI, Plimack ER, Stus V, et al. Pembrolizumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. *N Engl J Med*. 2019;380(12):1116-1127.
19. Rini BI, Powles T, Atkins MB, et al. Atezolizumab plus bevacizumab versus sunitinib in patients with previously untreated metastatic renal cell carcinoma (IMmotion151): a multicentre, open-label, phase 3, randomised controlled trial. *Lancet*. 2019;393(10189):2404-2415.
20. Socinski MA, Jotte RM, Cappuzzo F, et al. Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. *N Engl J Med*. 2018;378(24):2288-2301.
21. Tian S, Roepman P, Popovici V, et al. A robust genomic signature for the detection of colorectal cancer patients with microsatellite instability phenotype and high mutation frequency. *J Pathol*. 2012;228(4):586-595.
22. Jorissen RN, Lipton L, Gibbs P, et al. DNA copy-number alterations underlie gene expression differences between microsatellite stable and unstable colorectal cancers. *Clin Cancer Res*. 2008;14(24):8061-8069.
23. Guven DC, Kavgaci G, Erul E, et al. The efficacy of immune checkpoint inhibitors in microsatellite stable colorectal cancer: a systematic review. *Oncologist*. 2024;29(5):e580-e600.
24. Bilen MA, Shabto JM, Martini DJ, et al. Sites of metastasis and association with clinical outcome in advanced stage cancer patients treated with immunotherapy. *BMC Cancer*. 2019;19(1):857.
25. Diaz LA Jr, Shiu KK, Kim TW, et al. Pembrolizumab versus chemotherapy for microsatellite instability-high or mismatch repair-deficient metastatic colorectal cancer (KEYNOTE-177): final analysis of a randomised, open-label, phase 3 study. *Lancet Oncol*. 2022;23(5):659-670.
26. Eng C, Kim TW, Bendell J, et al. Atezolizumab with or without cobimetinib versus regorafenib in previously treated metastatic colorectal cancer (IMblaze370): a multicentre, open-label, phase 3, randomised, controlled trial. *Lancet Oncol*. 2019;20(6):849-861.
27. Kawazoe A, Xu RH, Garcia-Alfonso P, et al. Lenvatinib plus pembrolizumab versus standard of care for previously treated metastatic colorectal cancer: final analysis of the randomized, open-label, phase III LEAP-017 study. *J Clin Oncol*. 2024;42(24):2918-2927.
28. Lenz H-J, Lonardi S, Elez E, et al. Nivolumab (NIVO) plus ipilimumab (IPI) vs chemotherapy (chemo) as first-line (1L) treatment for microsatellite instability-high/mismatch repair-deficient (MSI-H/dMMR) metastatic colorectal cancer (mCRC): expanded efficacy analysis from CheckMate 8HW. *J Clin Oncol*. 2024;42(suppl 16):3503.
29. Antoniotti C, Rossini D, Pietrantonio F, et al. Upfront FOLFOXIRI plus bevacizumab with or without atezolizumab in the treatment of patients with metastatic colorectal cancer (AtezoTRIBE): a multicentre, open-label, randomised, controlled, phase 2 trial. *Lancet Oncol*. 2022;23(7):876-887.
30. Herting CJ, Farren MR, Tong Y, et al. A multi-center, single-arm, phase Ib study of pembrolizumab (MK-3475) in combination with chemotherapy for patients with advanced colorectal cancer: HCRN G14-186. *Cancer Immunol Immunother*. 2021;70(11):3337-3348.
31. Lenz HJ, Parikh A, Spigel DR, et al. Modified FOLFOX6 plus bevacizumab with and without nivolumab for first-line treatment of metastatic colorectal cancer: phase 2 results from the CheckMate 9X8 randomized clinical trial. *J Immunother Cancer*. 2024;12(3):e008409.
32. Tabernero J, Grothey A, Arnold D, et al. MODUL cohort 2: an adaptable, randomized, signal-seeking trial of fluoropyrimidine plus bevacizumab with or without atezolizumab maintenance therapy for BRAF(wt) metastatic colorectal cancer. *ESMO Open*. 2022;7(5):100559.
33. Bullock AJ, Schlechter BL, Fakih MG, et al. Botensilimab plus balstilimab in relapsed/refractory microsatellite stable metastatic colorectal cancer: a phase 1 trial. *Nat Med*. 2024;30(9):2558-2567.
34. Fakih M, Sandhu J, Lim D, Li X, Li S, Wang C. Regorafenib, ipilimumab, and nivolumab for patients with microsatellite stable colorectal cancer and disease progression with prior chemotherapy: a phase 1 nonrandomized clinical trial. *JAMA Oncol*. 2023;9(5):627-634.
35. Wu H, Deng M, Xue D, et al. PD-1/PD-L1 inhibitors for early and middle stage microsatellite high-instability and stable colorectal cancer: a review. *Int J Colorectal Dis*. 2024;39(1):83.
36. Takei S, Tanaka Y, Lin YT, et al. Multiomic molecular characterization of the response to combination immunotherapy in MSS/pMMR metastatic colorectal cancer. *J Immunother Cancer*. 2024;12(2):e008210.
37. Lenz HJ, Van Cutsem E, Luisa Limon M, et al. First-line nivolumab plus low-dose ipilimumab for microsatellite instability-high/mismatch repair-deficient metastatic colorectal cancer: the phase II CheckMate 142 study. *J Clin Oncol*. 2022;40(2):161-170.
38. Mazzolini G, Ochoa MC, Morales-Kastresana A, Sanmamed MF, Melero I. The liver, liver metastasis and liver cancer: a special case for immunotherapy with cytokines and immunostimulatory monoclonal antibodies. *Immunotherapy*. 2012;4(11):1081-1085.
39. Dolina JS, Braciale TJ, Hahn YS. Liver-primed CD8+ T cells suppress antiviral adaptive immunity through galectin-9-independent T-cell immunoglobulin and mucin 3 engagement of high-mobility group box 1 in mice. *Hepatology*. 2014;59(4):1351-1365.
40. He K, Barsoumian HB, Bertolet G, et al. Novel use of low-dose radiotherapy to modulate the tumor microenvironment of liver metastases. *Front Immunol*. 2021;12:812210.
41. Ghahremanifard P, Chanda A, Bonni S, Bose P. TGF-beta mediated immune evasion in cancer-spotlight on cancer-associated fibroblasts. *Cancers (Basel)*. 2020;12(12):3650.