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Systematic or Meta-analysis Studies

Distinct outcome patterns according to RAS and BRAF status in MSI-H/dMMR mCRC treated with immune checkpoint inhibitors: A systematic review and meta-analysis

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ABSTRACT

Background: Immune checkpoint inhibitors (ICIs) are superior to chemotherapy in metastatic colorectal cancer (mCRC) with MSI-H/dMMR. However, whether oncogenic driver alterations contribute to clinically meaningful heterogeneity in outcomes within this immunotherapy-sensitive population remains unclear. We conducted a systematic review and meta-analysis to evaluate the association between RAS and BRAF mutational status and outcomes in ICI-treated MSI-H/dMMR mCRC.

Methods: A systematic literature search identified studies reporting outcomes of ICI therapy according to RAS and/or BRAF status in MSI-H/dMMR mCRC. Study-level pooled analyses were conducted using random-effects models to estimate odds ratios (ORs) for objective response rate (ORR) and hazard ratios (HRs) for progression-free survival (PFS) and overall survival (OS).

Results: Nine studies were included, comprising a total of 13 treatment cohorts and 2564 patients were analysed. ORR did not significantly differ across molecular subgroups. Compared with wild-type tumours, RAS-mutated disease was associated with modestly longer PFS (HR 0.81, 95% CI 0.66–0.99), whereas BRAF-mutated tumours showed shorter PFS (HR 1.37, 95% CI 1.11–1.69). Overall survival was significantly worse in BRAF-mutated disease (HR 1.74, 95% CI 1.17–2.59), while no significant OS differences were observed for RAS-mutated tumours. Exploratory analyses suggested that dual checkpoint blockade may increase response rates particularly in BRAF-mutated and molecularly wild-type subgroups.

Conclusions: Within MSI-H/dMMR mCRC treated with ICIs, molecular subgroups show distinct survival patterns despite similar response rates. BRAF-mutated tumours retain an adverse prognostic impact, whereas RAS-mutated disease may exhibit more durable disease control. These findings support biological stratification within MSI-H/dMMR mCRC and provide a rationale for prospective biomarker-stratified studies of tailored immunotherapy strategies.

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Introduction

Microsatellite instability–high (MSI-H) or mismatch repair–deficient (dMMR) metastatic colorectal cancer (mCRC) is a distinct biological and clinical subtype, comprising about 4–5% of advanced CRC cases [1,2]. It features a hypermutated profile, increased neoantigen burden, and a highly inflamed tumour microenvironment, which explain its notable responsiveness to immune checkpoint inhibitors (ICIs) [3,4]. Over the last decade, both randomised trials and real-world data have shown that ICIs outperform traditional chemotherapy in MSI-H/dMMR mCRC, making anti–PD-1–based treatments the preferred first-line option [5–7].

Despite recent progress, MSI-H/dMMR tumours still exhibit significant molecular diversity [8,9]. Notably, RAS and BRAF mutations, common oncogenic drivers in colorectal cancer, can influence tumour immunobiology and affect responses to immune checkpoint inhibitors (ICIs). BRAF V600E mutations, found in about 20–25% of MSI-H metastatic colorectal cancers (mCRC), are linked with aggressive disease, worse outcomes, and specific transcriptional profiles, including immune exclusion features. On the other hand, KRAS mutations, while less frequent in MSI-H tumours than in microsatellite-stable CRC, have been variably associated with decreased T-cell infiltration, altered interferon signalling, and diminished response to immune therapies [10,11]. Whether these biological variations lead to meaningful clinical differences in ICI effectiveness is still uncertain.

So far, evidence regarding the predictive or prognostic significance of RAS and BRAF mutations in MSI-H/dMMR metastatic colorectal cancer (mCRC) has been limited to small subgroup analyses within clinical trials and diverse retrospective studies, which are often underpowered or show inconsistent results [5,6]. Some reports indicate worse outcomes for tumours with RAS mutations, while others highlight inferior survival rates in cases with BRAF mutations [11]. Nonetheless, a comprehensive review has not been conducted to determine the extent, direction, or clinical importance of these associations.

Given this evidence gap, we conducted a systematic review and meta-analysis to specifically examine the impact of RAS and BRAF mutations on clinical outcomes in patients with MSI-H/dMMR metastatic colorectal cancer treated with immune checkpoint inhibitors (ICIs). Using data derived from both clinical trials and real-world cohorts, we compared treatment efficacy across molecularly defined subgroups, focusing on response and survival outcomes. The main goal of this work was to investigate whether clinically meaningful differences in outcomes exist across molecularly defined subgroups of MSI-H/dMMR metastatic colorectal cancer treated with immune checkpoint inhibitors, and to explore the potential prognostic relevance of RAS and BRAF alterations within this immunotherapy-treated population.

Materials and methods

Study design and protocol registration.

This systematic review and meta-analysis followed the PRISMA guidelines. A detailed protocol was registered in PROSPERO (CRD420251180812; registered on 28 April 2026 and publicly available at https://www.crd.york.ac.uk/PROSPERO/view/CRD420251180812), outlining predefined objectives, eligibility criteria, and analytic strategies.

Search strategy

A comprehensive literature search was conducted across multiple electronic databases, including MEDLINE/PubMed, Embase, Embase.com, Cochrane CENTRAL, Science Citation Index, and Scopus, to identify eligible studies published between 1 January 2010 and 1 September 2025. Additional sources included major oncology conference abstracts (ASCO, ESMO, SITC), ClinicalTrials.gov, EU CTR, and backward citation searches of relevant reviews and included studies.

Only publications in English were included. The search combined controlled vocabulary and free-text terms, covering keywords such as "microsatellite instability," "mismatch repair deficiency," "colorectal cancer," "immune checkpoint inhibitors," "RAS mutation," and "BRAF mutation."

The complete reproducible search strategies for all databases are reported in Supplementary Appendix S1.

Eligibility criteria

Population

Eligible studies involved adult patients (≥18 years) with metastatic colorectal cancer (mCRC) that showed microsatellite instability–high (MSI-H) or mismatch repair deficiency (dMMR), and who were treated using immune checkpoint inhibitors (ICIs). Studies that did not report outcomes separately for MSI-H/dMMR patients were excluded.

Interventions

We included studies that evaluated: Anti–PD-1 agents (such as pembrolizumab and nivolumab); Anti–PD-L1 agents; and Anti–CTLA-4 agents (such as ipilimumab), either alone or in combination with PD-1/PD-L1 inhibitors. Studies focusing on vaccines, adoptive cell therapy, or immunomodulatory agents beyond ICIs were excluded.

Comparators

Outcomes were stratified based on RAS and/or BRAF mutational status. Comparisons included: BRAF-mutated versus BRAF wild-type; RAS-mutated versus RAS wild-type; and, when available, double wild-type (BRAF and RAS WT) subgroups.

Study designs

Eligible designs included: randomised controlled trials, prospective observational studies, and retrospective cohort studies. Excluded were case reports, case series, reviews, editorials, preclinical studies, and conference abstracts lacking extractable outcome data.

Screening and study selection

Two reviewers independently screened titles, abstracts, and full texts using a predefined selection algorithm; disagreements were resolved by consensus. Duplicate data from the same population were consolidated to avoid overlap. The study selection process is summarised in the PRISMA flow diagram (Supplementary Fig. S1).

Data extraction

Two reviewers independently extracted data using a standardised data collection form. Extracted variables included study design, year of publication, sample size, patient demographic and clinical characteristics, MSI-H/dMMR assessment methods, RAS and BRAF mutational status, and type of immunotherapy administered (monotherapy versus combination therapy). Data on clinical outcomes, including objective response rate (ORR), progression-free survival (PFS), and overall survival (OS), were collected when available. Discrepancies were resolved through discussion until consensus was reached. Missing data were not requested from study authors, in accordance with the predefined protocol.

Risk of bias assessment

Risk of bias was evaluated using the Cochrane Risk of Bias 2 (RoB 2)

tool for randomised controlled trials and the Newcastle–Ottawa Scale (NOS) for observational studies. Two reviewers independently assessed each study, and any discrepancies were resolved through discussion until consensus was achieved.

Outcomes

The primary endpoint was progression-free survival (PFS), assessed as pooled hazard ratios (HRs). Secondary endpoints included overall survival (OS), represented as HRs, and objective response rate (ORR), shown as odds ratios (ORs). Outcomes were analysed based on RAS and BRAF mutational status in patients with MSI-H/dMMR metastatic colorectal cancer treated with immune checkpoint inhibitors. Disease control rate (DCR), duration of response (DoR), and treatment-related adverse events (TRAEs) were prespecified secondary outcomes. However, these endpoints were inconsistently reported across the included studies and a quantitative synthesis was therefore not feasible.

Data synthesis and statistical analysis

A quantitative meta-analysis was performed using random-effects models (DerSimonian–Laird) to account for between-study heterogeneity. Time-to-event outcomes, including progression-free survival (PFS) and overall survival (OS), were pooled using hazard ratios (HRs) with 95% confidence intervals (CIs). Dichotomous outcomes, including objective response rate (ORR), were pooled using odds ratios (ORs) with 95% CIs.

Effect sizes were pooled on the logarithmic scale using the inverse-variance method. Heterogeneity across studies was assessed using the Cochran Q test and quantified with the I^2 statistic; values above 50% were considered indicative of substantial heterogeneity.

Predefined subgroup comparisons evaluated outcomes according to RAS-mutated versus RAS wild-type tumours, and BRAF-mutated versus double wild-type tumours (wild-type for both RAS and BRAF).

Sensitivity analyses were performed to assess the influence of individual studies on the pooled estimates. Potential publication bias was explored using funnel plots and Egger's regression test when at least ten studies were available for a given endpoint. All statistical analyses were conducted according to the predefined study protocol.

In addition to predefined molecular subgroup analyses, exploratory regimen-specific analyses were performed to investigate potential interactions between tumour genotype and immunotherapy strategy. Anti–PD-1/PD-L1 agents administered as single agents (e.g., pembrolizumab or nivolumab) were classified as monotherapy, whereas nivolumab plus ipilimumab was classified as dual checkpoint blockade.

Regimen-specific pooled comparisons were conducted at the study level for objective response rate when molecular subgroup-specific outcome data were available. Because treatment-regimen–specific survival outcomes were inconsistently reported across included studies, analogous pooled analyses for progression-free and overall survival were not feasible. These exploratory analyses were considered hypothesis-generating.

Certainty of evidence

The certainty of evidence for each outcome was assessed using the GRADE (Grading of Recommendations Assessment, Development and Evaluation) framework. The evaluation considered methodological limitations (risk of bias), consistency of results across studies, precision of the pooled estimates, indirectness of evidence, and potential publication bias. The overall certainty of evidence for each outcome was rated as high, moderate, low, or very low according to GRADE recommendations.

Results

Study selection and characteristics

Nine studies comprising 13 treatment cohorts met the eligibility criteria and were included in the quantitative synthesis, in accordance with the predefined protocol.

Overall, 2564 MSI-H/dMMR metastatic colorectal cancer patients treated with immune checkpoint inhibitors were included across all studies. Data were available for 539 patients in the objective response rate (ORR) analysis, 2100 patients in the progression-free survival (PFS) analysis, and 875 patients in the overall survival (OS) analysis. The number of patients contributing to each endpoint differed because subgroup-specific outcome reporting was inconsistent across studies (Table 1).

The analysis included data from both prospective clinical trials and real-world retrospective cohorts, with treatment regimens comprising anti–PD-1 monotherapies such as pembrolizumab and nivolumab, as well as combination immunotherapy such as nivolumab plus ipilimumab. All included studies reported outcomes according to RAS and/or BRAF mutational status, allowing comparisons across three molecular subgroups: RAS-mutated, BRAF-mutated, and RAS/BRAF wild-type tumours (Supplementary Table 1).

Objective response rate

Across the included studies, no significant differences in objective response rate (ORR) were observed among molecular subgroups. Compared with wild-type tumours, the pooled odds ratio for ORR was 0.93 (95% CI: 0.60–1.44; $I^2 = 0\%$; $k = 6$) for RAS-mutated tumours and 1.16 (95% CI: 0.73–1.83; $I^2 = 0\%$; $k = 6$) for BRAF-mutated tumours. Similarly, no significant difference in ORR was observed when comparing BRAF-mutated versus RAS-mutated tumours (OR 1.20; 95% CI: 0.75–1.93; $I^2 = 0\%$; $k = 6$). Although statistical heterogeneity was low, these findings should be interpreted cautiously, given the limited number of contributing studies. Overall, the available evidence suggests that oncogenic RAS or BRAF mutations do not materially influence the likelihood of achieving an objective response to immune checkpoint inhibitors in MSI-H/dMMR metastatic colorectal cancer (Fig. 1A–B).

Progression-free survival

In contrast to ORR, progression-free survival (PFS) differed significantly across molecular subgroups. Compared with wild-type tumours, patients with RAS-mutated MSI-H/dMMR mCRC showed significantly longer PFS (HR 0.81; 95% CI 0.66–0.99; $k = 8$; $I^2 = 0\%$). Conversely, BRAF-mutated tumours were associated with significantly shorter PFS compared with wild-type tumours (HR 1.37; 95% CI 1.11–1.69; $k = 9$; $I^2 = 0\%$). Direct comparison between the two molecular subgroups confirmed this pattern, with BRAF-mutated tumours exhibiting significantly worse PFS than RAS-mutated tumours (HR 1.66; 95% CI 1.16–2.37; $k = 8$; $I^2 = 16\%$). Taken together, these findings suggest a molecular gradient in PFS outcomes, with the most favourable estimates observed in RAS-mutated tumours and the poorest in BRAF-mutated disease (Fig. 2A–B).

Sensitivity analysis excluding selected real-world cohorts

To account for the potential for partial patient overlap across real-world datasets derived from similar institutional networks, a sensitivity analysis was performed, excluding the Nasca and Manca cohorts. In this restricted analysis, the association between RAS alterations and improved progression-free survival remained directionally consistent with the primary findings. Patients with RAS-mutated tumours continued to show numerically longer PFS compared with wild-type disease (HR $\approx$ 0.76; 95% CI 0.58–0.99; $k = 6$), supporting the overall

Table 1
Characteristics of studies included in the meta-analysis.

Study / cohort	Design	Phase	Setting	Regimen	N	Outcomes available
KEYNOTE-177	RCT	III	1 L	Pembrolizumab	153	ORR, PFS, OS
CheckMate-142	RCT	II	Multiple lines	Nivolumab + Ipilimumab	105	ORR, PFS
CheckMate-142 anti-LAG3	RCT	II	≥2 L	Pembrolizumab + anti-LAG3	44	ORR
CheckMate-8HW (Nivo+Ipi)	RCT	III	1 L	Nivolumab + Ipilimumab	296	ORR, PFS
CheckMate-8HW (Nivolumab)	RCT	III	1 L	Nivolumab	286	ORR, PFS
NIPICOL	Single-arm	II	≥2 L	Nivolumab + Ipilimumab	57	PFS
KEYNOTE-164 Cohort A	Single-arm	II	≥2 L	Pembrolizumab	61	ORR, PFS, OS
KEYNOTE-164 Cohort B	Single-arm	II	≥2 L	Pembrolizumab	63	ORR, PFS, OS
Nasca et al	RWD	–	1 L	Mixed	624	PFS
Taieb et al	RWD	–	1 L	Mixed	531	OS
Stemmer et al	RWD	–	1 L	Mixed	153	PFS
Manca et al	RWD	–	1 L	Mixed	110	PFS, OS
Eslinger et al	RWD	–	1 L	Mixed	81	OS

The number of patients contributing to each endpoint differed because subgroup-specific outcome reporting was inconsistent across studies.

pattern of more favourable outcomes observed in this molecular subgroup. Similarly, the adverse prognostic signal associated with BRAF mutations was maintained, with BRAF-mutated tumours exhibiting shorter PFS compared with wild-type disease. Overall, these findings were consistent with the primary analysis and support the presence of a molecular gradient in clinical outcomes across MSI-H/dMMR mCRC molecular subtypes, with numerically more favourable outcomes in RAS-mutated tumours and poorer outcomes in BRAF-mutated disease.

Overall survival

Overall survival (OS) also differed across molecular subgroups. Compared with wild-type tumours, RAS-mutated MSI-H/dMMR mCRC showed a trend toward improved OS, although this difference did not reach statistical significance (HR 0.71; 95% CI 0.45–1.10; k = 4; I^2 = 0%). In contrast, BRAF-mutated tumours were associated with significantly worse OS compared with wild-type tumours (HR 1.74; 95% CI 1.17–2.59; k = 5; I^2 = 0%). Direct comparison between molecular subgroups further confirmed this pattern, with BRAF-mutated tumours showing significantly poorer OS than RAS-mutated tumours (HR 2.16; 95% CI 1.12–4.15; k = 4; I^2 = 0%).

Although statistical heterogeneity was low, these findings should be interpreted cautiously given the limited number of contributing studies. Overall, the available evidence suggests that BRAF mutations retain an adverse prognostic association even in the context of immune checkpoint inhibitor therapy, whereas RAS mutations are not associated with inferior overall survival (Fig. 3A–B).

Exploratory regimen-specific molecular analysis

To further explore potential sources of heterogeneity in treatment outcomes, an exploratory analysis based on the immunotherapy regimen was conducted. Regimen-specific molecular subgroup analyses were feasible using response data from prospective trials that reported separate outcomes according to immunotherapy strategy. A pooled analysis involving approximately 566 patients showed that dual checkpoint blockade was associated with significantly higher objective response rates compared to anti–PD-1 monotherapy in BRAF-mutated tumours (OR 2.38, 95% CI 1.31–4.33) and in RAS/BRAF wild-type tumours (OR 2.33, 95% CI 1.25–4.34). However, a non-significant trend favouring dual therapy was observed in RAS-mutated disease (OR 1.39, 95% CI 0.75–2.57) (Fig. 4).

These findings are consistent with the direct comparison reported in the randomised CheckMate 8HW study, where nivolumab plus ipilimumab achieved higher objective response rates than nivolumab monotherapy across molecular subgroups (65% vs. 57% in RAS-mutated tumours, 76% vs. 58% in BRAF-mutated tumours, and 73% vs. 54% in wild-type tumours). High response rates were similarly observed in the dual-immunotherapy cohort of CheckMate 142. Overall, these exploratory findings suggest that the incremental benefit of dual checkpoint blockade in terms of objective response may be most evident in BRAF-mutated and RAS/BRAF wild-type disease.

Subgroup and sensitivity analyses

Subgroup analyses comparing molecular subgroups (RAS-mutated, BRAF-mutated, and wild-type tumours) were performed as predefined in the study protocol. Although regimen-specific survival analyses were not consistently feasible due to heterogeneous reporting across studies, exploratory analyses of treatment strategy were feasible for the objective response rate using molecular subgroup-specific data from selected prospective trials. Across all primary comparisons, statistical heterogeneity was low (I^2 close to 0% in most analyses), although relevant clinical and methodological heterogeneity remained. Sensitivity analyses excluding studies with mixed immunotherapy regimens did not materially alter the direction of the pooled response estimates.

Risk of bias and publication bias

Risk of bias and publication bias

Randomised clinical trials showed a low-to-moderate risk of bias according to the RoB 2 tool, whereas observational cohort studies achieved moderate-to-high quality scores based on the Newcastle–Ottawa Scale (NOS). Detailed domain-level RoB 2 assessments and NOS ratings are reported in Supplementary Tables S2 and S3, respectively.

Formal assessment of publication bias was limited because fewer than 10 studies contributed to several pooled analyses. These findings are consistent with the methodological plan specified in the registered PROSPERO protocol.

Certainty of evidence was assessed according to the GRADE framework. Overall, the certainty of evidence was rated as low for ORR and OS outcomes, mainly because of study-level limitations and imprecision related to the limited number of available studies. For PFS, certainty was considered moderate, owing to the consistency of findings across studies and the low statistical heterogeneity observed in pooled analyses. Detailed GRADE assessments are provided in Supplementary Table S4.

Certainty of evidence was downgraded for risk of bias because several pooled analyses included observational studies and post-hoc subgroup analyses. Imprecision was considered serious when the number of contributing studies was limited and/or confidence intervals were wide. Publication bias could not be formally assessed for most comparisons because fewer than 10 studies were available per outcome.

Discussion

In this systematic review and meta-analysis of 9 studies [12–24] involving 2564 patients with MSI-H/dMMR metastatic colorectal cancer

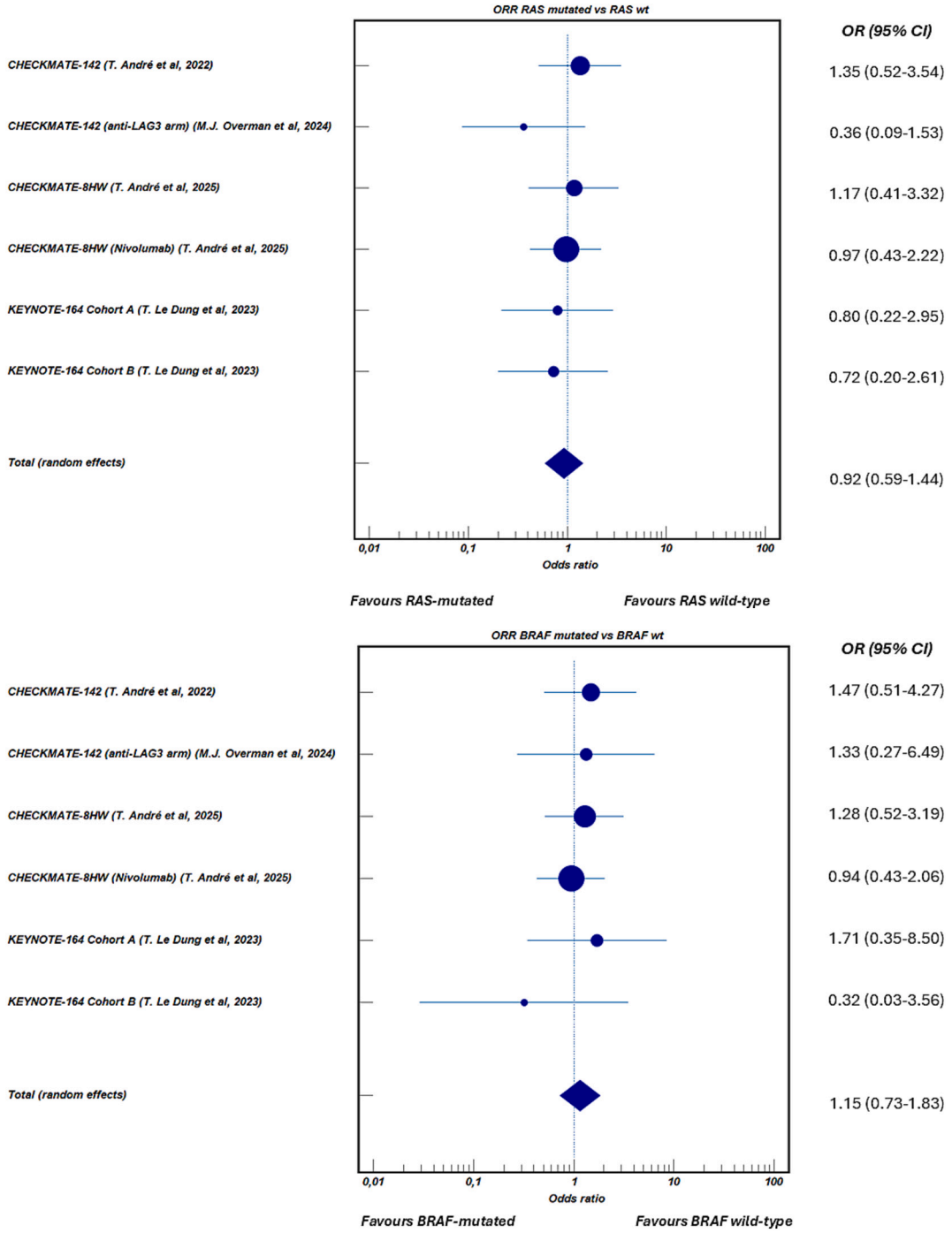

Fig. 1. Objective response rate according to RAS and BRAF mutational status in MSI-H/dMMR metastatic colorectal cancer treated with immune checkpoint inhibitors. (A) Forest plot comparing objective response rate (ORR) between RAS-mutated and wild-type tumours. (B) Forest plot comparing ORR between BRAF-mutated and wild-type tumours. Odds ratios (ORs) and 95% confidence intervals (CIs) were pooled using a random-effects model. Squares represent individual study estimates, with size proportional to study weight, and horizontal lines indicate 95% CIs. The diamond represents the pooled estimate. The vertical line indicates the null effect (OR = 1). Analyses were performed on a logarithmic scale.

treated with immune checkpoint inhibitors, we investigated whether RAS and BRAF mutations influence treatment outcomes. Although objective response rates were mostly comparable across molecular subgroups, differences appeared in survival outcomes, highlighting the clinical and biological diversity of MSI-H/dMMR disease.

Interestingly, the signal of improved progression-free survival observed in RAS-mutated tumours appears partially unexpected and not entirely consistent with subgroup analyses from pivotal trials. In the KEYNOTE-177 study [5], while pembrolizumab demonstrated a clear overall benefit, exploratory subgroup analyses suggested a potentially less favourable outcome among RAS-mutated patients, albeit with important limitations related to sample size and statistical power. Conversely, in the CHECKMATE-8HW trial [12,13], the benefit of nivolumab-based strategies appeared more consistent across molecular subgroups, including RAS-mutated tumours.

These discrepancies may reflect differences in study design, treatment strategies, and patient selection, as well as the inherent limitations of subgroup analyses. In this context, our pooled results should be

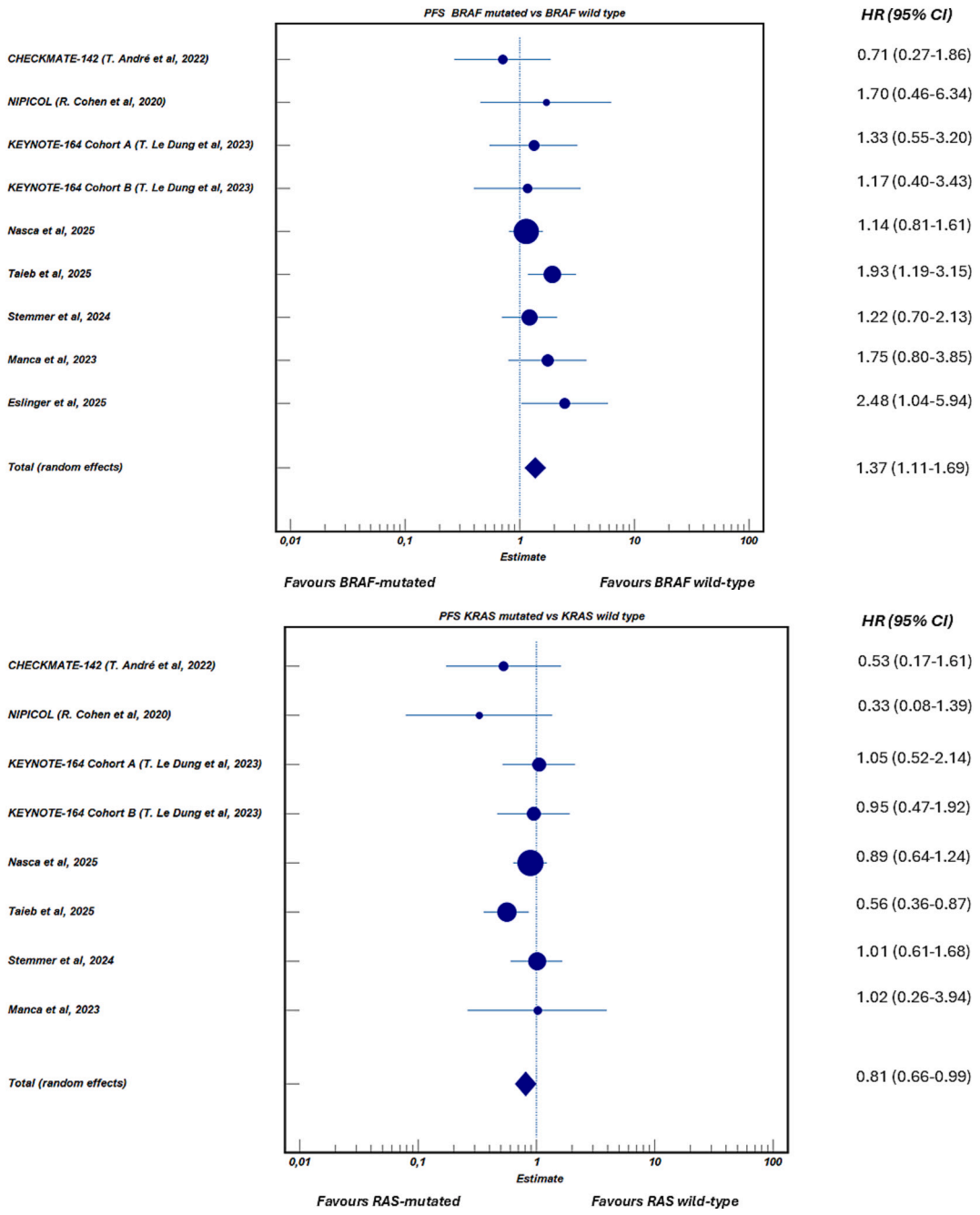

Fig. 2. Progression-free survival according to molecular subgroup in MSI-H/dMMR metastatic colorectal cancer treated with immune checkpoint inhibitors. (A) Forest plot comparing progression-free survival (PFS) between BRAF-mutated and wild-type tumours. (B) Forest plot comparing PFS between RAS-mutated and wild-type tumours. Hazard ratios (HRs) and 95% confidence intervals (CIs) were pooled using a random-effects model. Squares represent the effect estimates from individual studies, with size proportional to study weight, and horizontal lines indicate the corresponding 95% CIs. The diamond represents the pooled estimate. The vertical line indicates the null effect (HR = 1). Analyses were performed on a logarithmic scale.

interpreted cautiously, as they may capture broader trends that are not fully apparent within individual trial datasets. Notably, BRAF-mutated tumours were associated with shorter progression-free survival and worse overall survival compared with wild-type disease, supporting the clinical heterogeneity within MSI-H/dMMR colorectal cancer.Across the datasets examined, ORR showed no significant difference among BRAF-mutated, RAS-mutated, and RAS/BRAF wild-type tumours. This confirms that MSI-H/dMMR biology, characterised by hypermutation, high neoantigen load, and dense immune infiltration, confers inherent sensitivity to ICIs, regardless of oncogenic driver mutations. The finding aligns with the strong immunogenicity observed in MSI-H/dMMR cancers and emphasises that initial tumour reduction under PD-1 blockade is largely sustained even when RAS or BRAF mutations are present.

Survival outcomes, however, differed across molecular subgroups. In the pooled analysis, RAS-mutated tumours were associated with modestly longer progression-free survival compared with both wild-type and BRAF-mutated disease (HR 0.81, 95% CI 0.66–0.99), suggesting the possibility of more durable disease control despite similar objective response rates. However, this apparent PFS advantage in RAS-mutated tumours should be interpreted cautiously, as it is not fully consistent with signals from individual pivotal trials. In contrast, no statistically

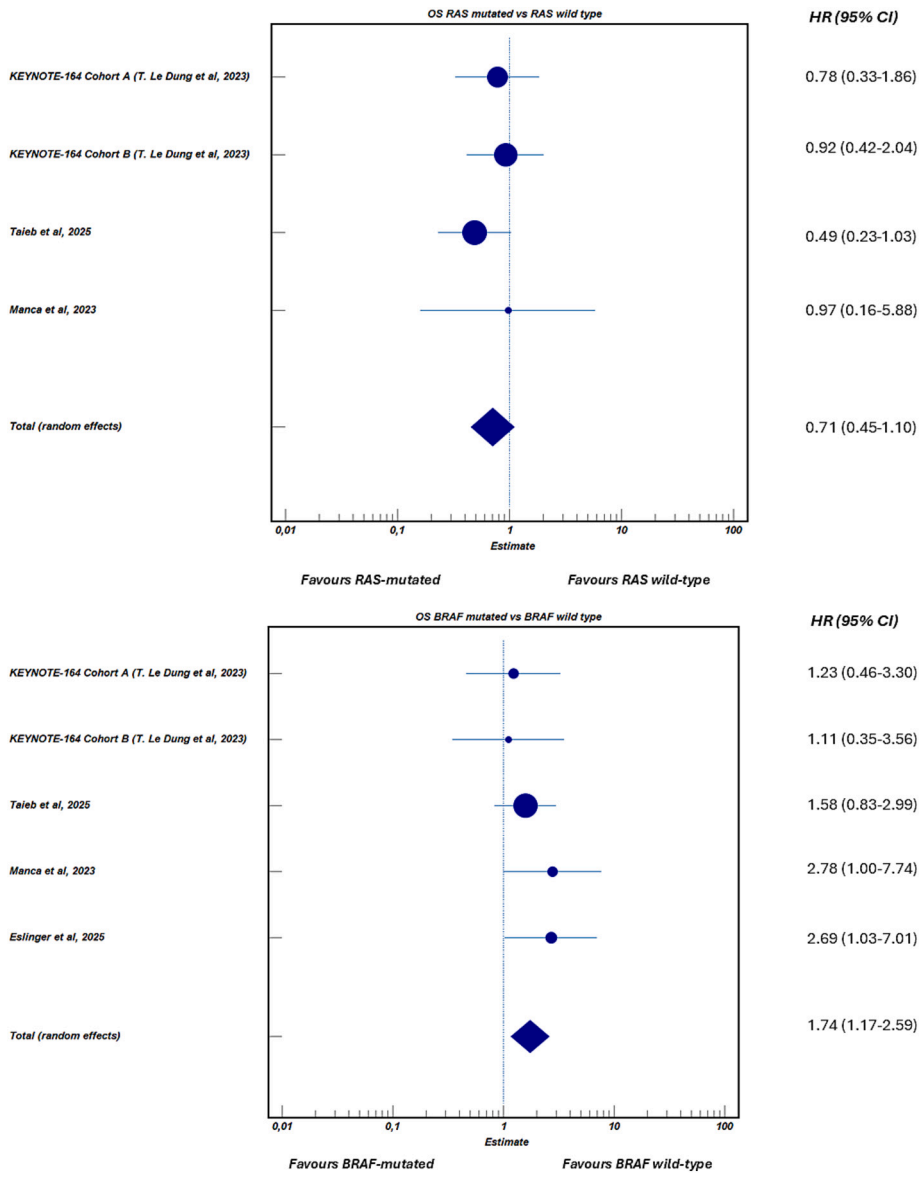

Fig. 3. Overall survival according to molecular subgroup in MSI-H/dMMR metastatic colorectal cancer treated with immune checkpoint inhibitors. (A) Forest plot comparing overall survival (OS) between RAS-mutated and wild-type tumours. (B) Forest plot comparing OS between BRAF-mutated and wild-type tumours. Hazard ratios (HRs) and 95% confidence intervals (CIs) were pooled using a random-effects model. Squares represent the effect estimates from individual studies, with size proportional to study weight, and horizontal lines indicate the corresponding 95% CIs. The diamond represents the pooled estimate. The vertical line indicates the null effect (HR = 1). Analyses were performed on a logarithmic scale.

significant differences in overall survival were observed between RAS-mutated and wild-type tumours.

The biological mechanisms underlying these findings remain uncertain. It is conceivable that oncogenic RAS signalling may interact with the highly immunogenic context of MSI-H disease in ways that influence patterns of immune-mediated tumour control or the timing of resistance emergence. However, given the modest magnitude of the pooled effect and the limited number of contributing studies, these observations should be interpreted cautiously and considered hypothesis-generating.

Preclinical evidence has suggested that oncogenic RAS signalling may modulate interferon pathways and PD-L1 expression, potentially influencing tumour–immune interactions in highly immunogenic settings [26]. Although these mechanisms remain speculative in the context of MSI-H/dMMR metastatic colorectal cancer, they provide a biologically plausible framework for the hypothesis that RAS-mutated tumours may experience more sustained disease control under immune checkpoint blockade [25,26]. These observations warrant further investigation in translational studies and individual patient-level analyses.

In contrast, BRAF-mutated MSI-H/dMMR mCRC was consistently associated with significantly worse overall survival compared with wild-type disease, despite largely comparable objective response rates, and with shorter progression-free survival. These findings suggest that BRAF mutation retains an adverse prognostic association even in the context of immune checkpoint inhibitor therapy, likely reflecting the intrinsically aggressive clinical biology of this subgroup rather than reduced sensitivity to immune checkpoint blockade.

Several mechanisms have been proposed to explain the unfavourable trajectory of BRAF-mutated MSI-H tumours, including distinct transcriptional programmes, enhanced stromal interactions, and immune evasion features that may limit the durability of response despite preserved initial sensitivity to immunotherapy. Importantly, exploratory regimen-specific analyses indicated that the incremental benefit of dual

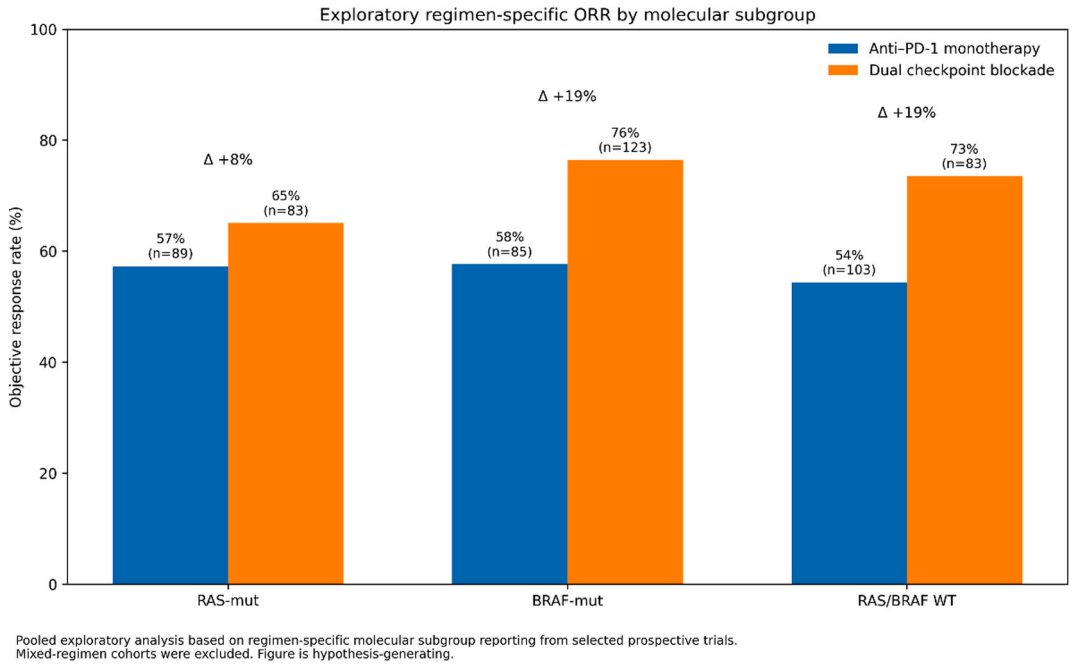

Fig. 4. Exploratory regimen-specific objective response rate according to molecular subgroup. Grouped bar plot showing pooled objective response rates in RAS-mutated, BRAF-mutated, and RAS/BRAF wild-type MSI-H/dMMR metastatic colorectal cancer treated with anti–PD-1 monotherapy versus dual checkpoint blockade. Data derive from regimen-specific molecular subgroup reporting in selected prospective trials; mixed-regimen cohorts were excluded. This analysis is exploratory and hypothesis-generating.

checkpoint blockade, in terms of objective response, may be more pronounced in BRAF-mutated and RAS/BRAF wild-type tumours than in RAS-mutated disease.

While these findings are hypothesis-generating, they suggest that treatment intensification strategies may be particularly relevant in biologically aggressive BRAF-mutated MSI-H disease and warrant prospective evaluation in biomarker-stratified clinical trials.

These findings may have prognostic implications. Molecular stratification within MSI-H/dMMR metastatic colorectal cancer could help refine risk assessment and generate hypotheses regarding biological heterogeneity within an otherwise immunotherapy-responsive disease. However, the present study was not designed to establish predictive biomarkers or guide treatment selection, and prospective biomarker-stratified studies are required before any clinical implications can be drawn. [27].

Given the retrospective nature of several included real-world studies and the partial overlap in participating institutional networks, a sensitivity analysis excluding the Nasca et al. [15] and Manca et al. [24] cohorts was performed. In this restricted analysis, the overall pattern of outcomes across molecular subgroups remained consistent, with RAS-mutated tumours showing numerically more favourable progression-free survival and BRAF-mutated tumours maintaining an association with poorer outcomes.

Although statistical precision decreased after excluding these cohorts, the overall direction and size of the pooled estimates remained generally consistent. These results support the robustness of the observed molecular gradient in clinical outcomes while emphasizing the need for confirmation in larger datasets and, ideally, individual patient-level meta-analyses.

This study has several limitations. First, the analysis was based on study-level aggregate data obtained from diverse sources, including both prospective clinical trials and retrospective real-world cohorts, which prevents uniform adjustment for potential confounders and consistent definition of comparator groups across studies. Importantly, the present analysis was intentionally limited to populations treated with immunotherapy, due to the well-established superiority of immune checkpoint inhibitors over chemotherapy in MSI-H/dMMR metastatic colorectal cancer. The primary aim was therefore not to compare treatment efficacies but to examine whether clinically significant differences in outcomes exist across molecular subgroups within an overall immunotherapy-responsive disease context. Accordingly, the observed associations should be seen as reflecting different prognoses within ICI-treated populations rather than conclusive evidence of treatment-specific predictive effects.

Furthermore, variability in treatment lines, immunotherapy regimens, and mutational testing methods may have contributed to ongoing clinical and methodological heterogeneity. Subgroup sizes were relatively small for some comparisons, especially in overall survival analyses, and potential partial overlap across institutional real-world datasets cannot be completely ruled out despite sensitivity analyses. Additionally, non-V600 BRAF alterations could not be assessed separately due to limited reporting.

Finally, regimen-specific analyses were exploratory, based on molecular subgroup reporting from a limited number of prospective studies, and should therefore be considered hypothesis-generating.

Despite these limitations, the current analysis represents the largest pooled dataset to date examining survival outcomes based on RAS and BRAF status in MSI-H/dMMR metastatic colorectal cancer treated with immune checkpoint inhibitors, offering a clinically relevant framework for future biomarker-stratified research.

In conclusion, this meta-analysis suggests that MSI-H/dMMR metastatic colorectal cancer treated with ICIs represents a biologically heterogeneous disease in which oncogenic driver alterations are associated with different clinical outcomes. BRAF-mutated disease retained a poor prognostic association despite mostly maintaining initial response rates, while RAS-mutated tumours showed slightly better progression-free survival. Exploratory analyses further suggested that intensified immune checkpoint blockade might enhance response depth in specific molecular subgroups, especially in BRAF-mutated tumours. Overall, these findings emphasise the importance of molecular stratification within MSI-H/dMMR disease and support the rationale for future biomarker-stratified studies assessing customised immunotherapy

approaches across biologically defined patient groups.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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