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# Pooled analysis of 2 clinical trials of first-line chemoimmunotherapy for metastatic microsatellite stable colorectal cancer MEDITREME and METIMMOX studies

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**ABSTRACT**

**Background:** Colorectal cancer with microsatellite stable (MSS) status is intrinsically resistant to immune checkpoint inhibitor monotherapy targeting PD-1 or PD-L1. However, emerging evidence suggests that chemotherapies may overcome this resistance when combined with immunotherapy.

**Methods:** We conducted a pooled analysis of individual patient data from two phase II trials evaluating oxaliplatin-based chemotherapy combined with checkpoint inhibitors as first-line treatment for MSS metastatic colorectal cancer. The MEDITREME trial is a single-arm study assessing durvalumab and tremelimumab with oxaliplatin-based chemotherapy, while the METIMMOX trial is a randomized study comparing standard oxaliplatin-based chemotherapy with an alternating regimen of oxaliplatin-based chemotherapy and nivolumab. We compared overall survival (OS), progression-free survival (PFS), and response rates between patients treated with chemotherapy alone *versus* chemoimmunotherapy, and evaluated the association between these outcomes and transcriptomic and immunoscore data.

**Results:** A total of 130 patients were included: 38 who received chemotherapy alone and 92 chemoimmunotherapy. The median OS was significantly improved in the chemoimmunotherapy group compared with chemotherapy alone (not reached *vs*. 15.3 months; HR = 0.58; 95% CI, 0.36–0.96; $p = 0.03$). The complete response rates were higher with chemoimmunotherapy (14% *vs*. 5%). No significant differences were observed in PFS. High baseline $CD8^+$ T-cell infiltration was associated with improved outcomes in patients receiving chemoimmunotherapy but not in those treated with chemotherapy alone.

**Conclusions:** This pooled analysis provides compelling evidence supporting the clinical benefit of first-line chemoimmunotherapy in a subset of patients with MSS mCRC. Baseline $CD8^+$ T-cell infiltration may serve as a predictive biomarker for identifying patients most likely to benefit from this treatment approach.

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## Background

Colorectal cancer (CRC) is a heterogeneous disease that can be broadly divided into two major clinical groups: tumors with deficient mismatch repair (dMMR) or microsatellite instable (MSI), and tumors with proficient mismatch repair (pMMR) or microsatellite stable (MSS) status. Both MSS and MSI tumors are infiltrated by immune cells,[1] and high levels of $CD3^+$ or $CD8^+$ T-cell infiltration are associated with improved survival in patients with either localized or metastatic disease across both tumor types.[2] Despite

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the established prognostic role of immune infiltration in CRC, MSI and MSS tumors differ markedly in their responsiveness to immune checkpoint inhibitors (ICIs), such as PD-1/PD-L1 or CTLA-4 blockade.[3] MSI tumors are highly sensitive to drugs targeting PD-1/PD-L1, and a phase III trial demonstrated that pembrolizumab outperforms chemotherapy in this population.[4] Similarly, combinations of anti-PD-1 and anti-CTLA-4 antibodies are highly effective in MSI CRC, both in localized and metastatic settings, with superiority over either chemotherapy or PD-1 inhibition alone, while anti-CTLA-4 demonstrated superiority over chemotherapy or anti-PD-1 alone.[5-7] In contrast, anti-PD-1, or a combination of anti-PD-L1 and anti-CTLA-4 used alone, has shown no clinical efficacy in MSS patients.[3,8] This absence of efficacy of immunotherapy in MSS tumors is related to their biological differences compared to MSI tumors. In fact, MSS tumors are considered cold tumors, usually characterized by low levels of TILs compared with their MSI counterpart. MSI tumors harbor a higher number of genomic mutations, leading to increased neoantigen formation and enhanced immunogenicity.[9] Conversely, MSS tumors often exhibit constitutive activation of the Wnt/β-catenin pathway, which reduces tumor infiltration by dendritic cells (DCs) and T cells, through inhibition of CCL4 expression.[10,11] In addition, MSS tumors frequently carry activating *RAS* mutations, which promote activation of the MAPK pathway, and contribute to reduced expression of MHC molecules, thereby impairing antigen presentation.[12]

The microsatellite instability phenotype is observed in approximately 20% of stage II colorectal cancers, 9%–10% of stage III cases, and 4%–5% of stage IV cases. These percentages highlight the importance of developing new strategies for the MSS population, which represents the vast majority of patients.

Several clinical trials have tested chemotherapy combined with ICIs in the first-line MSS mCRC setting, showing preliminary signals of efficacy but no definitive conclusions can be drawn from these trials (ATEZOTRIBE, METIMMOX, CHECKMATE 9X8).[13-15] Our group recently conducted the MEDITREME clinical trial (NCT03202758), which evaluated first-line FOLFOX plus durvalumab and tremelimumab in MSS mCRC.[16] This single-arm study met its primary endpoint, achieving a 3-month PFS rate of 90.7% (95% CI: 79.2–96%), and demonstrated that high $CD8^+$ T-cell infiltration was associated with improved outcomes, suggesting that chemoimmunotherapy may benefit MSS patients with immune-infiltrated tumors.

In parallel, the METIMMOX (NCT03388190) trial compared standard oxaliplatin-based chemotherapy (control arm) to an alternating regimen of chemotherapy and nivolumab (experimental arm), showing a non-significant trend toward improved OS with chemoimmunotherapy, likely limited by statistical power:[17] control arm: median 14.6 months (95% CI, 10.6–23.2); experimental arm: median 20.7 months (95% CI, 15.9–24.9), $p$-value = 0.68. PFS was comparable – control group: median 9.2 months (95% confidence interval (CI), 6.3–12.7); experimental group: median 9.2 months (95% CI, 4.5–15.0).

Building on these findings, we performed a pooled analysis of the MEDITREME and METIMMOX trials to assess whether chemoimmunotherapy provides clinical benefit over chemotherapy alone, and to evaluate whether patterns of immune infiltration could serve as predictive biomarkers for treatment response.

## Materials and methods

### *Patients and study design*

This pooled analysis includes individual patient data from the MEDITREME study (Evaluation of the Safety and the Tolerability of Durvalumab Plus Tremelimumab Combined with FOLFOX in mCRC, NCT03202758, EudraCT: 2016-005006-19) and the METIMMOX study (alternating short-course oxaliplatin-based chemotherapy and nivolumab as first-line treatment of patients with abdominal metastases from microsatellite-stable colorectal cancer; NCT03388190, EudraCT: 2017-001845-29). The protocols and clinical results of the individual studies have previously been published.[16,17] Both studies were conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonisation Good Clinical Practice guidelines, and the CONSORT 2010 statement. All patients provided written informed consent prior to study enrollment.

The secondary analysis presented in this work was done in 2025. Sequencing for MEDITREME was done from 2017 to 2021 through the inclusions, and in 2025 for METIMMOX.

### Immunoscore

For immunoscore-testing, PD-L1 and CD8, we collected biopsies from patients with chemoimmunotherapy treatment. A single tumor tissue section was processed by immunohistochemistry for PD-L1 and CD8 staining. The stained slides were scanned with an Evident VS200 scanner to obtain 20 × digital images. For analysis, the densities of PD-L1 + cells and cytotoxic $CD8^+$ T cells in the tumor core and invasive margin were quantified. The PD-L1 tumor proportion score (TPS) and combined positive score (CPS) were optically determined by the pathologist blinded to clinical outcomes.

### RNA-sequencing

For the MEDITREME study, RNA depleted of ribosomal RNA was used for library preparation with a NEBNext Ultra II RNA Directional Library Prep Kit for Illumina according to the manufacturer's instructions (New England Biolabs). RNA-seq was performed on a NextSeq 500 device (Illumina). The libraries were sequenced with 76-bp paired-end reads.

For the METIMMOX study, by using the Maxwell RNA FFPE Kit (Promega), fifty nanograms of total RNA were ribodepleted with the RiboCop rRNA Depletion Kit (Lexogen) following the manufacturer's protocol, and then purified using the NEB Kit following the manufacturer's instructions.

### RNA-seq data analysis

Raw FASTQ data were pseudo-aligned, and gene counts and transcripts per kilobase million (TPM) values were quantified using Kallisto software[18] based on the GRCh37 assembly Ensembl. Gene-level count and transcript matrices were then obtained with the DESeq2 package. Low-count genes were pre-filtered by removing genes with too few reads. Genes that were differentially expressed were selected using the same package.[19] Gene set enrichment analysis (GSEA)[20] was performed on the resulting differential genes using hallmarks of cancer gene sets from the Broad Institute and the fgsea R package.[21]

Tumor microenvironment (TME)-associated transcriptomic elements were quantified using MCP-counter. The MCP-counter[22] method allows the robust quantification of the absolute abundance of eight immune and two stromal cell populations. Six transcriptomic signatures were also computed as a metagene by taking the mean expression of the corresponding genes. Three signatures are related to IFN pathways or checkpoint inhibitors: IFNy, extended immune gene signature (EIG), and T cell-inflamed gene expression profile (GEP), and three are related to T cell immune infiltrate: cytotoxicity (CYTOX), the TH1 orientation (TH1), and cytotoxic lymphocytes (CTL). Genes lists are described here: (1) T-cell inflamed gene expression profile (GEP): CCL5, CD27, CD274 (PD-L1), CD276, (B7-H3), CD8A, CMKLR1, CXCL9, CXCR6, HLA-DQA1, HLA-DRB1, HLAE, IDO1, LAG3, NKG7, PDCD1, LG2, (PDL2), PSMB10, STAT1, and TIGIT; (2) Interferon gamma (IFN-$\gamma$): IDO1, CXCL10, CXCL9, HLA.DRA, STAT1, and IFNG; (3) Extended immune signature (EIG): CD3D, IDO1, CIITA, CD3E, CCL5, GZMK, CD2, HLA-DRA, CXCL13, IL2RG, NKG7, HLA.E, CXCR6, LAG3, TAGAP, CXCL10, STAT1, and GZMB; (4) Cytotoxicity (CYTOX): GZMA/B/K/H, GLNY, PRF1; (5) Th1 orientation (TH1): TBX21, IFNG; (6) Cytotoxic lymphocytes (CTL): CD8A, CD3D/E/G, PTPRC.

### Tumor mutational burden (TMB)

In the MEDITREME study, TMB was assessed using whole-exome sequencing, whereas in the METIMMOX trial, TMB was determined using the TruSight Oncology 500 panel (Illumina).

### Statistical analyses

Clinical and molecular baseline characteristics were compared among subgroups using the chi-square, Fisher's exact, or Mann–Whitney tests, as appropriate. PFS was defined as the time from randomization or inclusion, respectively, for the MEDITREME and METIMMOX trials to the first evidence of disease progression or death, whichever occurred first, and overall survival (OS) was defined as the time from randomization to death. The objective response rate (ORR) was defined as the percentage of patients who

achieved partial or complete response according to RECIST (Responsethe Response Evaluation Criteria in Solid Tumors) V.1.1 criteria. For the METIMMOX trial, tumor assessments were performed by means of CT scans repeated every 8 weeks throughout the study participation. As for the MEDITREME trial, tumor assessments were repeated every 12 weeks. The disease control rate (DCR) was defined as the percentage of patients who achieved stable disease or partial or complete response according to the RECIST V.1.1 criteria. Survival curves were estimated by the Kaplan–Meier method and compared with the log-rank test. Hazard ratios (HRs) with 95% confidence intervals (CIs) were estimated with a Cox proportional hazards model. When needed, continuous variables were dichotomized into two groups (low and high) using the best cut-off strategy based on survival information (maxstat R library).[23] Statistical significance was set at a $p$-value < 0.05 for a bilateral test. All analyses were performed with R (version 4.2.2) software, and graphics were drawn using Graphpad Prism PRISM (version 9.4.1).

### Data availability

RNAseq data from the Meditreme and Metimmox trials are available under accession numbers GSE235919 and GSE313035, respectively.

## Results

### Patient characteristics

MEDITREME involved 57 patients between August 2017 and December 2019. We excluded 3 patients with MSI status from the analysis. METIMMOX enrolled 80 subjects between May 2018 and October 2021, of which 4 were excluded because they did not receive at least one treatment cycle. In total, 130 patients were retained for this analysis: 38 patients were treated by chemotherapy, and 92 patients were treated by chemoimmunotherapy. In the whole cohort, the median age was 64 y (range, 28–81); 66 (51%) patients were female; 92 (73%) patients had left-sided CRC; 107 (82%) patients had liver metastases; 53 (41%) patients had lung metastases; 45 (35%) patients had lymph node metastases; and 88 (68%) patients had *RAS* mutated status (Table 1). A comparison of the baseline demographic characteristics between the chemoimmunotherapy and chemotherapy groups showed no differences in the main prognostic clinical characteristics (Tables 2 and 3). The same observations were made when comparing the two chemoimmunotherapy cohorts (Supplementary Table 1).

### Efficacy analysis

Treatment efficacy was evaluated. Overall, 13 out of 92 patients (15%) experienced a complete response in the chemoimmunotherapy group, versus 2 out of 38 (6%) in the chemotherapy group. The complete response was thus numerically higher in the chemoimmunotherapy group, but this observation did not reach statistical significance. We did not observe any difference in terms of disease response rate or overall control rate between the chemotherapy and chemoimmunotherapy groups (Figure 1A). For PFS, chemotherapy appears to slightly outperform the immune-based arm, with a median PFS of 9.2 months (95% CI, 7.3–14.7) in the chemotherapy group and 8.44 months (95% CI, 7.2–13) in the chemoimmunotherapy group. However, the survival curves overlap, with a non-significant survival difference between the two groups (HR = 0.96 [0.58; 4.58]; $p = 0.87$; Figure 1B). For OS, in contrast, we observed improved OS in the chemoimmunotherapy group, with a median not reached (NR), *versus* 15.1 months (95% CI, 10.9-NR) (HR = 0.60; 95% CI, 0.37–0.98 $p = 0.04$) in the chemotherapy group (Figure 1C). In the subgroup analysis, only the MEDITREME group presented significantly improved OS compared to the METIMMOX chemotherapy group (HR = 0.50 [0.28;0.87]; $p = 0.01$). No difference was observed between the experimental group and the METIMMOX chemotherapy group (HR = 0.77 [0.44; 1.36]; $p = 0.36$) (Figure 1D). MEDITREME median follow-up times were 39.20 months (IQR 28.37; 59.13). METIMMOX median follow-up times were 46.00 months (IQR 40.70; 48.70) for the control group and not reached (NR) (IQR 39.40; NR) for the experimental group.

**Table 1.** Summary of clinical characteristics of the pooled cohort.

| Characteristic | $N = 130$[a] |
|---|---|
| Age | 64 (57, 72) |
| Sex | 64 (49%) |
| Sidedness | |
| Left | 68 (54%) |
| Rectum | 24 (19%) |
| Unknown | 4 |
| Lymphocytes (10^9/L) | 1.59 (1.10, 2.10) |
| NLR | 3.04 (1.87, 4.67) |
| LDH | 216 (180, 366) |
| Unknown | 2 |
| Metastases | |
| Bone | 4 (3.1%) |
| Lung | 53 (41%) |
| Lymph node | 45 (35%) |
| Liver | 107 (82%) |
| Cohort | |
| Meditreme (FOLFOX-durvalumab–tremelimumab) | 54 (42%) |
| Metimmox control arm (FLOX) | 38 (29%) |
| Metimmox experimental arm (FLOX–nivolumab) | 38 (29%) |
| Treatment | |
| Chemotherapy | 38 (29%) |
| Chemoimmunotherapy | 92 (71%) |
| RECIST | |
| CR | 15 (12%) |
| PR | 58 (47%) |
| SD | 37 (30%) |
| PD | 13 (11%) |
| Objective Response | |
| Non-Responder | 57 (44%) |
| Responder | 73 (56%) |
| TMB | 7.0 (5.0, 9.0) |
| Unknown | 28 |
| *KRAS* | |
| Mutated | 88 (68%) |
| Wild-type | 42 (32%) |
| *BRAF* | |
| Mutated | 14 (11%) |
| Wild-type | 116 (89%) |

[a] Median (IQR); *n* (%).

NLR: Neutrophil to lymphocyte ratio; LDH: Lactate Dehydrogenase. CR: Complete Response, PR: Partial Response, SD: Stable Disease, and PD: Partial Disease.

In the chemoimmunotherapy group, we observed that *BRAF* mutated status, high baseline lymphocyte count, right-sided CRC, and high TMB were associated with better PFS. For OS, a low baseline blood lymphocyte count and the presence of lung metastasis were associated with poorer OS (Figure 1E).

## *Immunoscore analysis*

Histological slides were available for 107 patients for the determination of CD8 and PD-L1 infiltration. No patient had a PD-L1-positive TPS (Figure 2A). When PD-L1 was assessed by the CPS, 30% of patients had a CPS score < 1, 43% had a CPS between 1 and 4, 23% between 5 and 9, and 4% had a CPS ≥ 10. CD8 infiltration was also evaluated using semi-quantitative analysis, and 6% of patients had absent or weak CD8 infiltration, 36% had low CD8 infiltration, 42% had moderate CD8 infiltration, and 16% of patients had high CD8 infiltration. PD-L1 expression was mostly found in the area richly infiltrated with CD8 T cells (Figure 2B). The PD-L1 CPS expression was not related to PFS or OS, either in patients treated with chemotherapy or in those treated with chemoimmunotherapy (Figure 2C). In contrast, high CD8 infiltration was associated with better PFS and OS in the chemoimmunotherapy group, and with OS in the control arm (Figure 2C). The presence of PD-L1 + cells in close interaction with CD8 + cells was determined optically as present or absent. No significant difference in $PD\text{-}L1^{+}/CD8^{+}$ cell interactions was observed between patients receiving chemoimmunotherapy and those receiving chemotherapy alone.

**Table 2.** Comparison of clinical characteristics between patients with chemotherapy alone or chemoimmunotherapy treatment.

| Characteristic | Chemotherapy $N = 38$[a] | Chemo-immunotherapy $N = 92$[a] | $p$-value[b] |
|---|---|---|---|
| Age | 65 (58, 73) | 62 (56, 72) | 0.2 |
| Sex | 23 (61%) | 41 (45%) | 0.10 |
| Sidedness | | | 0.001 |
| Right | 11 (29%) | 23 (26%) | |
| Left | 13 (34%) | 55 (62%) | |
| Rectum | 14 (37%) | 10 (11%) | |
| Unknown | 0 | 4 | |
| Lymphocytes (10^9/L) | 1.60 (1.10, 2.10) | 1.56 (1.12, 2.10) | >0.9 |
| NLR | 3.16 (2.10, 5.13) | 2.97 (1.78, 4.54) | 0.4 |
| LDH | 208 (188, 353) | 220 (180, 368) | 0.8 |
| Unknown | 0 | 2 | |
| Metastases | | | |
| Bone | 1 (2.6%) | 3 (3.3%) | >0.9 |
| Lung | 14 (37%) | 39 (42%) | 0.6 |
| Lymph node | 22 (58%) | 23 (25%) | <0.001 |
| Liver | 32 (84%) | 75 (82%) | 0.7 |
| TMB | 7.0 (5.0, 9.0) | 6.5 (5.0, 9.0) | 0.8 |
| Unknown | 5 | 23 | |
| *KRAS* | | | <0.001 |
| Mutated | 15 (39%) | 73 (79%) | |
| Wild-type | 23 (61%) | 19 (21%) | |
| *BRAF* | | | <0.001 |
| Mutated | 10 (26%) | 4 (4.3%) | |
| Wild-type | 28 (74%) | 88 (96%) | |
| Responder | 23 (61%) | 50 (54%) | |

[a]Median (IQR); *n* (%).
[b]Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test. NLR: Neutrophil to lymphocyte ratio; LDH: Lactate Dehydrogenase.

**Table 3.** Comparison of response characteristics between patients with chemotherapy alone or chemoimmunotherapy treatment.

| Characteristic | Chemotherapy $N = 38$[a] | Chemo-immunotherapy, $N = 92$[a] | $p$-value[b] |
|---|---|---|---|
| RECIST | | | 0.2 |
| CR | 2 (5.9%) | 13 (15%) | |
| PD | 3 (8.8%) | 10 (11%) | |
| PR | 21 (62%) | 37 (42%) | |
| SD | 8 (24%) | 29 (33%) | |
| Unknown | 4 | 3 | |
| Objective response | | | 0.5 |
| Non-responder | 15 (39%) | 42 (46%) | |
| Responder | 23 (61%) | 50 (54%) | |
| Progression-free survival | 9.2 (7.3–14.7) | 8.44 (7.2–13) | |
| Overall Survival | NR | 15.1 (10.9–NR) | |

[a]Median (95% CI); *n* (%).
[b]Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test. CR: Complete Response, PR: Partial Response, SD: Stable Disease, and PD: Partial Disease.

### *Transcriptomic data*

RNA sequencing analysis of baseline samples could be generated in 104 patients from the MEDITREME and METIMMOX experimental ($n = 36$) and control ($n = 31$) arms. The results for the MEDITREME cohort were previously described in Thibaudin et al.[16] In the METIMMOX cohort, RNAseq analysis revealed distinct patterns across treatment groups. GSEA hallmark of cancer analysis underlined that an inflammatory signal (Allograft rejection, IL2/STAT5 signaling, IL6/JAK/STAT3 signaling, and Interferon alpha response) was associated with a poor response (PD and SD patients) in chemoimmunotherapy-treated patients (Figure 3A), while KRAS signaling was associated with a benefit (CR and PR patients). Conversely, in chemotherapy-only patients, oncogenic pathways were related to the response to chemotherapy (beta-catenin pathways, P53 pathway, G2M checkpoint, hypoxia, and mitotic spindle) (Figure 3B).

MCP-counter analysis demonstrated that T-cell, NK cell, and B-cell infiltration was associated with better response rates in chemoimmunotherapy arms, but not in chemotherapy-only patients (Figure 3C).

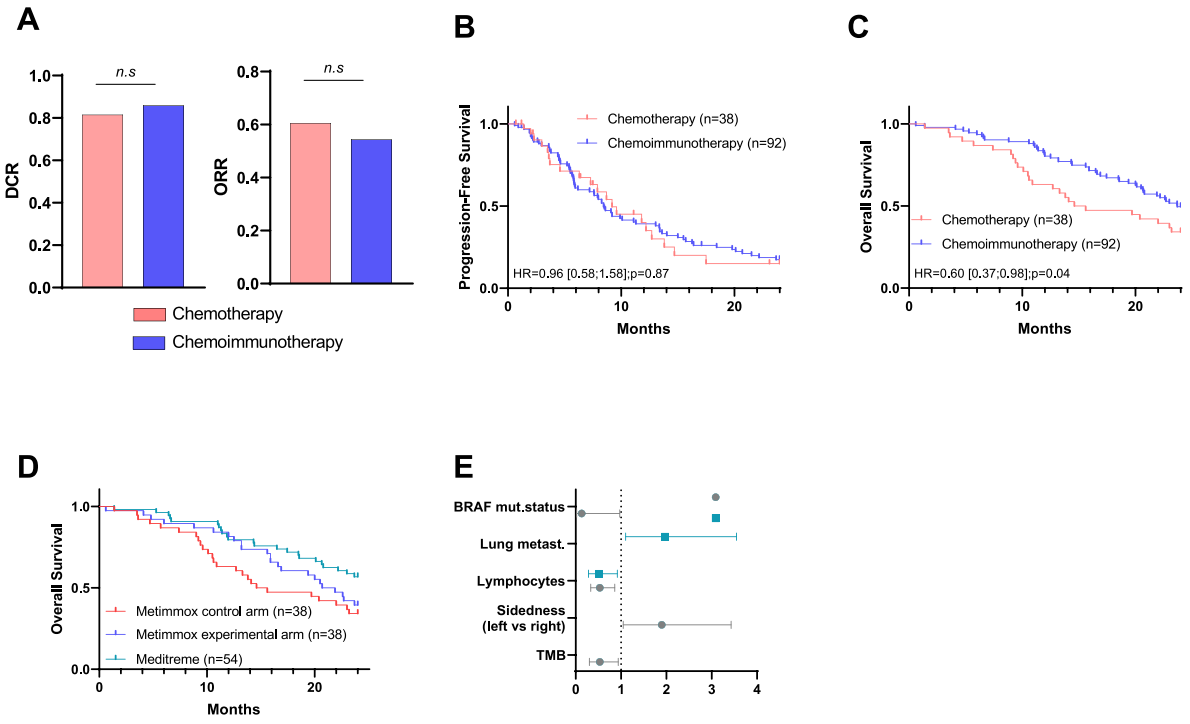


**Figure 1.** (A) Bar plot showing the disease control rate (DCR) and objective response rate (ORR) for chemotherapy-only and chemoimmunotherapy patients. (B, C) Kaplan–Meier curves according to treatment (chemotherapy alone vs chemoimmunotherapy) for progression-free (B) and overall (C) survival. (D) Kaplan–Meier curves according to cohort and treatment for overall survival. (E) Forest plots representing hazard ratios and confidence intervals for Cox models for progression-free (gray dots) and overall (blue squares) survival.

Transcriptomic signatures associated with the immunotherapy response (T-score, Th1 signature) were different in the chemoimmunotherapy arm, but not in the chemotherapy alone arm (Figure 3C).

Finally, we found that CTLA4 gene expression was associated with better PFS and OS in both the MEDITREME and METIMMOX experimental arms (Figure 3D). CTLA-4 gene expression was not associated with outcome in the METIMMOX control arm. Furthermore, we performed CTLA-4 RNAscope in patients from the MEDITREME and METIMMOX trials. This analysis was coupled with CD8A staining using mAb in 5 good responders with more than 1 y of PFS, and 5 non-responders with progression after 3 months of treatment. We observed that CTLA-4 was expressed by cancer cells, but also CD8 T cells and other immune cells, probably CD4 or regulatory T cells (Supplementary Figure 1). We observed that CTLA-4 expression in CD8 cells seemed to be higher in responders than in non-responders (Figure 3E); this difference was not significant, likely due to a lack of power. These data strongly suggest the predictive role of CTLA-4 expression in patients treated with chemoimmunotherapy.

## Discussion

This pooled analysis of patients with MSS mCRC from the MEDITREME and METIMMOX clinical trials provides further evidence that chemoimmunotherapy may offer clinical benefit compared with chemotherapy alone in the first-line setting. Our findings also support baseline $CD8^+$ T-cell infiltration as a potential biomarker to identify patients most likely to benefit from chemoimmunotherapy.

The most striking observation from our analysis is the increased complete response rate observed with chemoimmunotherapy compared to chemotherapy alone (14% *vs.* 5%). For reference, the phase III Intergroup N9741 trial, which compared FOLFOX with IROX in 696 patients, reported a 6% complete response rate in the FOLFOX arm,[24] which closely matches the rate observed in our chemotherapy control group.

However, direct comparison between our chemoimmunotherapy data and historical FOLFOX results should be interpreted cautiously, as standard-of-care chemotherapy is now commonly combined with biologic agents. In the PARADIGM trial, for instance, response rates reached approximately 80% when

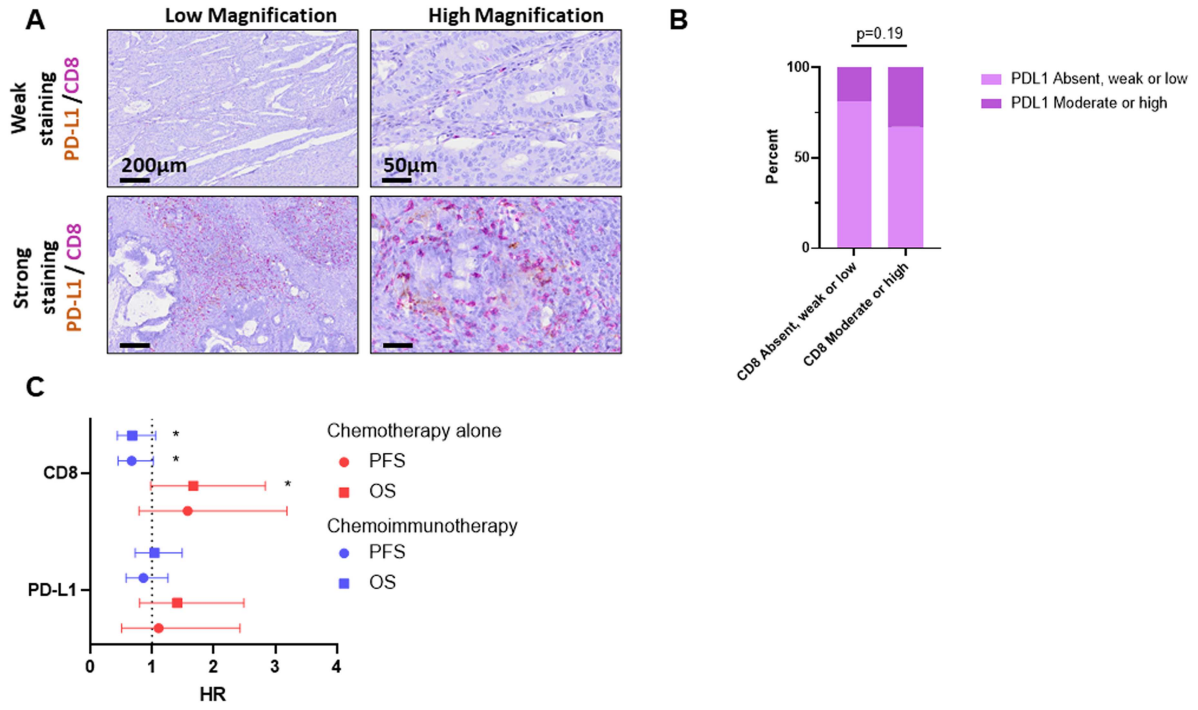


**Figure 2.** (A) Representative data of PD-L1/CD8 dual chromogenic staining. Upper panel: Sample displaying a weak stain for PD-L1 (brown) and CD8 (pink) at a low or high magnification. Lower panel: Sample displaying a strong stain for PD-L1 (brown) and CD8 (pink) at a low or high magnification. The scale bars used for both samples are identical. (B) Bar plot showing repartition of PDL1 and CD8 levels of enrichment. (C) Forest plots representing hazard ratios and confidence intervals for Cox models for progression-free (dots) and overall (squares) survival for patients treated with chemotherapy alone (red) or chemoimmunotherapy (blue).

FOLFOX was combined with an anti-EGFR antibody and 68% with anti-VEGF therapy. However, such reference studies include patients with RAS wild-type tumors, who generally have a better prognosis and higher response rates than the MSS RAS-mutant population analyzed here. Considering these data, the higher CR rate observed with chemoimmunotherapy remains noteworthy, but the comparison with historical FOLFOX arms should not be overinterpreted. Such an effect is consistent with the ability of immunotherapy to generate anti-tumor immune memory, potentially leading to the eradication of microscopic residual disease. Supporting this hypothesis, the MEDITREME trial previously demonstrated that chemoimmunotherapy can trigger immune responses against shared antigens and tumor neoantigens in patients who derived clinical benefit. It should be underlined that, in the METIMMOX trial, overall survival for the control arm did not exceed 15.3 months, possibly due to an overrepresentation of *BRAF*-mutant cases, who did not receive anti-EGFR therapy, highlighting the unfavorable prognosis of this subgroup. Interestingly, all *BRAF*-mutant patients receiving chemoimmunotherapy in our studies achieved a complete response, a result in line with recent work suggesting improved outcomes for intermediate/high TMB and *BRAF* phenotypes, especially among older female patients.[25] BRAF-mutant tumors are known to be more immunologically active than MSS BRAF wild-type tumors based on the CMS classification.[26,27] This observation also raises the hypothesis that gender-specific or autoimmune susceptibility pathways may interact with BRAF signaling to confer benefit. This has already been discussed, in particular by Nasca et al., who were the first to demonstrate that mCRC BRAF tumors show different responses according to sex.[28]

Several trials have investigated chemoimmunotherapy as a first-line treatment for MSS mCRC. This strategy is biologically plausible, as chemotherapy plus immune checkpoint blockade has shown positive results in phase III across other cancers, including non-small-cell lung cancer (NSCLC),[29] gastric cancer,[30] and triple-negative breast cancer.[31] Chemotherapeutic agents commonly used in CRC may also boost anti-tumor immune response: fluoropyrimidine can eliminate myeloid-derived suppressor cells, while

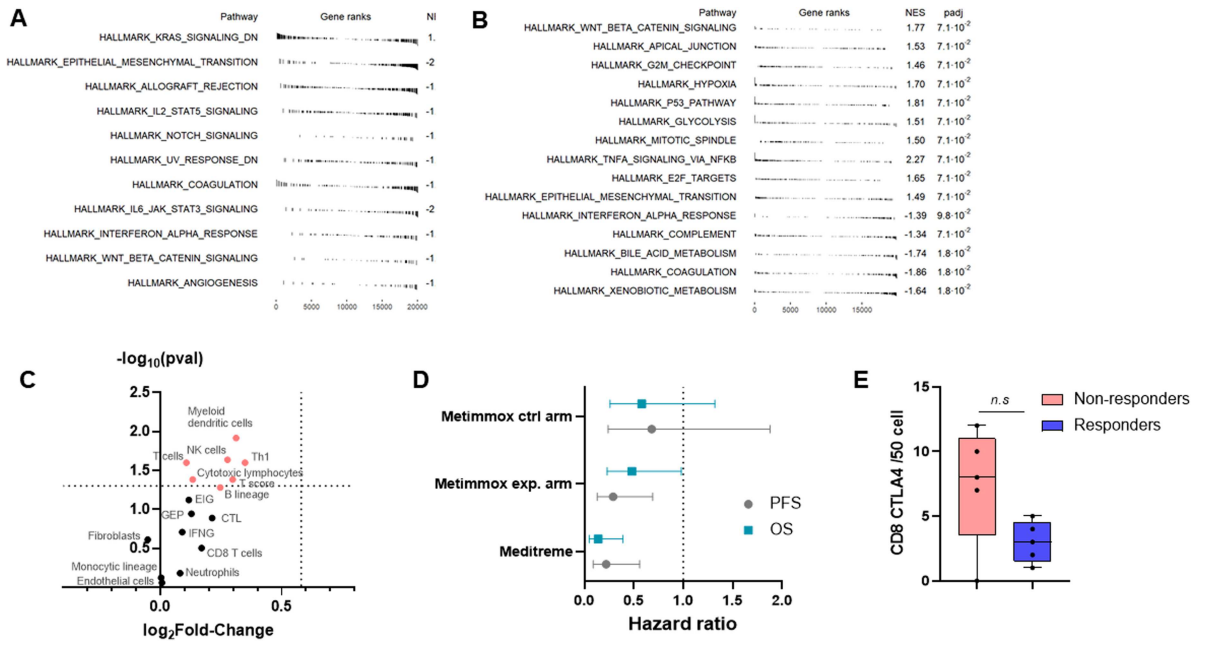


**Figure 3.** (A, B) Enrichment barcode plots for significant Hallmark gene sets when considering differential genes between responders and non-responders for patients treated by chemoimmunotherapy (A) or chemotherapy alone (B). NES: normalized enrichment score. (C) Volcano plot describing differential analysis performed on MCP-counter populations and immune signatures for the Metimmox experimental arm between responders and non-responders. Each dot represents one variable. Red dots correspond to significant *p*-values. (D) Forest plots representing hazard ratios and confidence intervals from Cox models for progression-free (gray dots) and overall (blue squares) survival estimated using CTLA4 gene expression derived from RNAseq data, in each of the 3 cohorts. (E) Boxplot showing the expression of CD8 CTLA4 per 50 cells for chemoimmunotherapy patients with good ($n = 5$) or bad ($n = 5$) response.

trifluridine/tipiracil targets type 2 immunosuppressive macrophages,[32,33] and oxaliplatin can induce immunogenic cell death, leading to T-cell priming and immune recruitment.[34] Preclinical data therefore support the hypothesis that fluoropyrimidines combined with oxaliplatin enhance immune checkpoint inhibitor efficacy,[35] an effect that may be further amplified by VEGF inhibition with bevacizumab.[36] However, clinical results have been mixed, and the first association of fluoropyrimidine alone with anti-PD-1/PD-L1 failed to show clinical efficacy in patients previously treated with chemotherapy.[37-39]

The overall survival benefit observed in the chemoimmunotherapy group in our pooled analysis (HR = 0.58; $p = 0.03$) is consistent with results from other recent trials evaluating chemoimmunotherapy in MSS mCRC. The CheckMate 9 × 8 study, which compared FOLFOX plus bevacizumab with or without nivolumab, did not meet its primary PFS endpoint; however, post hoc analysis underlined that patients receiving nivolumab achieved a signal of better response and improved overall survival,[40] but with non-significant results. The AtezoTRIBE trial showed that adding atezolizumab to FOLFIRINOX plus bevacizumab led to modest PFS improvements and a trend toward longer overall survival.[13,41] Together with our findings, these results provide converging arguments for a better efficacy of chemoimmunotherapy compared with chemotherapy alone in MSS CRC.

An important point across these studies is the consistent improvement in response rates and overall survival, despite limited or absent PFS benefit. This raises questions about the appropriateness of PFS as a primary endpoint in these chemoimmunotherapy trials. The dissociation between PFS and OS likely reflects the delayed but durable nature of immune-mediated responses. Nevertheless, the magnitude of clinical benefit conferred by chemoimmunotherapy remains modest, underscoring the need for predictive biomarkers to optimize patient selection.

Our data further point strongly to $CD8^+$ T-cell infiltration as the most informative biomarker for both survival and patient selection, corroborating the POCHI trial (selecting patients based on CD8 status).[42] Similarly, the AtezoTRIBE trial demonstrated that higher immunoscore values and higher TMB were

associated with greater benefit from immunotherapy in pMMR CRC.[43] Our study supports these findings, highlighting the role of baseline $CD8^+$ T-cell infiltration assessment to select patients for future trials. Importantly, $CD8^+$ infiltration was predictive only in patients receiving chemoimmunotherapy, and not in those treated with chemotherapy alone, strengthening its role as a predictive rather than purely prognostic biomarker.

Transcriptomic analyses provided further mechanistic insights into treatment response. In patients receiving chemoimmunotherapy, inflammatory signatures (allograft rejection, IL-2/STAT5 signaling, IL-6/JAK/STAT3 signaling, and interferon-$\alpha$ response) were paradoxically associated with poor outcomes, while KRAS signaling was associated with a benefit. Similar results were recently observed in a trial testing encorafenib, cetuximab, and nivolumab for MSS *BRAF* mutant colon cancer.[44] In contrast, in patients treated with chemotherapy alone, oncogenic pathways ($\beta$-catenin, p53, the G2M checkpoint, hypoxia, and the mitotic spindle) were linked to the response. These findings suggest that the mechanisms of response differ fundamentally between chemoimmunotherapy and chemotherapy, with moderate – rather than excessive – immune activation potentially favoring chemoimmunotherapy efficacy.[45] Interestingly, we also identified CTLA-4 expression on $CD8^+$ T cells as a potential biomarker of the response to anti-CTLA-4 therapy.

At last, the presence of liver metastases substantially influences the efficacy of immunotherapy in colorectal cancer. Liver involvement is a well-established negative predictive factor for immune checkpoint inhibitors (ICIs), largely due to the organ's tolerogenic microenvironment, which induces T-cell apoptosis and regulatory T-cell expansion, thereby suppressing systemic antitumor immunity.[46,47] In MSS metastatic colorectal cancer (mCRC), where ICIs already show limited activity, this effect is even more pronounced.

In the phase 1 trial of botensilimab plus balstilimab, patients with liver metastases had lower response rates than those without. Similarly, the regorafenib–ipilimumab–nivolumab trial in MSS CRC reported reduced efficacy in patients with hepatic disease. Emerging data suggest that combining ICIs with chemotherapy may mitigate this negative impact by inducing immunogenic cell death and modulating the hepatic immune milieu.[48,49] These findings emphasize the importance of stratifying future immunotherapy trials in CRC by liver metastasis status. However, in our studies, this observation was not performed. A hypothesis could be that, in a first-line setting, the immunoregulatory role of liver metastases is less important.

The main limitation of our study is the relatively small sample size and the retrospective, non-randomized design, pooling data from two prospective clinical trials. As such, inherent biases cannot be excluded. Larger, randomized studies are needed to validate these findings.

## Conclusions

First-line chemoimmunotherapy with oxaliplatin-based regimens appears capable of achieving higher complete response rates and prolonging overall survival in patients with MSS mCRC. $CD8^+$ T-cell infiltration emerges as a valuable predictive biomarker to identify patients most likely to benefit from this approach. These findings strongly support the initiation of phase III randomized trials evaluating chemoimmunotherapy in MSS mCRC patients with high $CD8^+$ tumor infiltration.

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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## Author contributions

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## Data availability statement

RNAseq data from the Meditreme and Metimmox trials are available under accession numbers GSE235919 and GSE313035, respectively. Other data and materials supporting the results will be made available upon reasonable request to the corresponding author.

## Ethics approval statement

All patients provided written informed consent before enrolment. Approval from the European Union Drug Regulating Authorities Clinical Trials Database was obtained for both trials (EudraCT MEDITREME: 2016-005006-19; METIMMOX: 2017-001845-29). The trials and present study were conducted in accordance with the principles of the Declaration of Helsinki and registered with ClinicalTrials.gov (MEDITREME: NCT03202758; METIMMOX: NCT03388190). The MEDITREME protocol was approved by the Ethics Committee CPP TOURS – Région Centre – Ouest 1 on 27 March 2017 under the number 2017T1-03 and was registered with the French National Health Products Agency (ANSM). The METIMMOX Protocol was approved by the Regional Committee for Medical and Health Research Ethics of South-East Norway Reference Number: 2017/1850.

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