Temozolomide Followed by Combination With Low-Dose Ipilimumab and Nivolumab in Patients With Microsatellite-Stable, O6-Methylguanine–DNA Methyltransferase–Silenced Metastatic Colorectal Cancer: The MAYA Trial

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PURPOSE This is a multicenter, single-arm phase II trial evaluating the efficacy and safety of an immune-sensitizing strategy with temozolomide priming followed by a combination of low-dose ipilimumab and nivolumab in patients with microsatellite-stable (MSS) and O6-methylguanine–DNA methyltransferase (MGMT)–silenced metastatic colorectal cancer (mCRC).

PATIENTS AND METHODS Patients with pretreated mCRC were centrally prescreened for MSS status and MGMT silencing (ie, lack of MGMT expression by immunohistochemistry plus *MGMT* methylation by pyrosequencing). Eligible patients received two priming cycles of oral temozolomide 150 mg/sqm once daily, days 1-5, once every 4 weeks (first treatment part) followed, in absence of progression, by its combination with ipilimumab 1 mg/kg once every 8 weeks and nivolumab 480 mg once every 4 weeks (second treatment part). The primary end point was the 8-month progression-free survival (PFS) rate calculated from enrollment in patients who started the second treatment part, with ≥ 4 out of 27 subjects progression-free by the 8-month time point as decision rule.

RESULTS Among 716 prescreened patients, 204 (29%) were molecularly eligible and 135 started the first treatment part. Among these, 102 (76%) were discontinued because of death or disease progression on temozolomide priming, whereas 33 patients (24%) who achieved disease control started the second treatment part and represented the final study population. After a median follow-up of 23.1 months (interquartile range, 14.9-24.6 months), 8-month PFS rate was 36%. Median PFS and overall survival were 7.0 and 18.4 months, respectively, and overall response rate was 45%. Grade 3-4 immune-related adverse events were skin rash (6%), colitis (3%), and hypophysitis (3%). No unexpected adverse events or treatment-related deaths were reported.

CONCLUSION The MAYA study provided proof-of-concept that a sequence of temozolomide priming followed by a combination of low-dose ipilimumab and nivolumab may induce durable clinical benefit in MSS and MGMT-silenced mCRC.

INTRODUCTION

Immune checkpoint inhibitors (ICIs) provided unprecedented benefit in the small subgroup of patients with metastatic colorectal cancer (mCRC) and deficient in mismatch repair/microsatellite instability-high (dMMR/MSI-high) status,[1-5] which is found in 4% of cases. The biologic basis of such immune-sensitivity relies on the hypermutation related to deficiency of MMR machinery,[6] with increased load of clonal and immunogenic neoantigens and consequent immune escape via upregulation of several immune checkpoints in both cancer and microenvironment cells.[7] As a matter of fact, most patients with proficient mismatch repair/microsatellite-stable (pMMR/MSS) mCRC have

CONTEXT

Key Objective

The MAYA trial was designed to prospectively provide clinical proof-of-concept of the role of temozolomide as an immune-sensitizing agent because of induction of hypermutation in proficient mismatch repair/microsatellite-stable, O^6-methylguanine–DNA methyltransferase–silenced metastatic colorectal cancer.

Knowledge Generated

The MAYA strategy allowed to achieve durable disease control to low-dose ipilimumab and nivolumab combination in heavily pretreated patients, along with a manageable safety profile.

Relevance

The MAYA strategy is worth further investigation in properly selected patients with metastatic colorectal cancer and may be agnostically translated to other temozolomide-/dacarbazine-sensitive cancers.

immune-excluded tumors with intrinsic resistance to ICIs. Therefore, current efforts aim to investigate immune-sensitization strategies for these immune-cold tumors.

Temozolomide is an oral alkylating agent approved for patients with glioblastoma (GBM) and its efficacy in this disease is related to a validated predictive biomarker, ie, O^6-methylguanine–DNA methyltransferase (MGMT) promoter methylation.[8] Since MGMT is a key enzyme involved in the repair of DNA damage induced by alkylating agents, epigenetic MGMT silencing represents a mechanism of synthetic lethality after exposure to temozolomide. Although MGMT methylation is found in around 40% of colorectal cancers (CRCs), dacarbazine and its oral analog temozolomide yielded modest activity in selected patients with MGMT-methylated mCRC, with an overall response rate (ORR) < 10%.[9-14] Tumor responses to temozolomide are restricted to patients with concomitant lack of MGMT protein expression assessed by immunohistochemistry (IHC).[15-18] However, complete MGMT silencing at both gene and protein level, coupled with pMMR/MSS status, is necessary but not sufficient to predict response or clinical benefit to temozolomide.[19]

Acquired resistance to temozolomide may be associated with the onset of hypermutation, with a specific genomic scar characterized by C>T transitions (the Alexandrov's signature 11)[20,21] and frequent emergence of secondary mutations in MMR genes, especially MSH6.[22-26] Beyond the robust evidence progressively collected in hypermutated relapses of GBM,[22-26] this effect may be observed in virtually all temozolomide-sensitive tumors[27] and was demonstrated in CRC models and patients with mCRC.[18,28] Therefore, the induction of hypermutation by a temozolomide priming treatment provides the rationale for immune-sensitization of pMMR/MSS, MGMT-silenced mCRC. With the aim of clinically validating this hypothesis, the MAYA proof-of-concept trial was designed.

PATIENTS AND METHODS

Patient Eligibility

Eligible criteria were age ≥ 18 years; Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1; histologically confirmed metastatic and inoperable adenocarcinoma of the colon or rectum; measurable disease according to RECISTv1.1; progressive disease (PD) or contraindication to oxaliplatin, irinotecan, fluoropyrimidines, and anti–epidermal growth factor receptor agents (in RAS/BRAF wild-type tumors); centrally confirmed MSS status by multiplex polymerase chain reaction, MGMT promoter methylation by pyrosequencing, and MGMT absent expression by IHC. A full list of eligibility criteria is in the Protocol (online only).

Patients signed informed consents for both prescreening and study participation; the study was approved by ethical committees of all centers.

Molecular prescreening was centrally performed on formalin-fixed paraffin-embedded archival specimens. The adopted algorithm (Data Supplement, online only) started from pathologic review of tumor content and MGMT IHC: samples with any MGMT staining in tumor cells were prescreening failures, whereas those with lack of protein expression were submitted to pyrosequencing for detecting MGMT methylation using the 6% cutoff[29] and multiplex polymerase chain reaction to confirm MSS status.[30]

Study Procedures

As shown in Figure 1, eligible patients were enrolled and started the first treatment part with single-agent temozolomide at the oral dose of 150 mg/sqm once daily on days 1-5 once every 4 weeks, for two cycles. After radiologic reassessment by week 7 ± 5 days according to RECIST1.1 and blinded independent central review (BICR), patients with PD were out of study, whereas patients with complete response, partial response (PR), or stable disease (SD) started the second treatment part and received temozolomide at the previously adopted dose and schedule, in combination with nivolumab at the flat dose of 480 mg given intravenously once every 4 weeks plus low-dose ipilimumab at 1 mg/kg given intravenously once every 8 weeks. Imaging assessments were done at screening and every 8 weeks for up to 12 months, and then every 12 weeks.

In the first treatment part, patients received the study treatment until RECIST1.1 PD, unacceptable toxicity, consent withdrawal, or death, whichever occurred first. In the second treatment part, patients received the study treatments until RECIST1.1 PD, unacceptable toxicity, consent withdrawal, death, or immune-related RECIST (ir-RECIST) PD.[31] In fact, after discussion with the sponsor and signing a specific consent form, patients were allowed to continue the treatment beyond progression in case of investigator-assessed evidence of clinical benefit, treatment tolerance, absence of symptoms and signs indicating clinical progression, no decline in ECOG PS, and absence of any PD at critical sites (eg, leptomeningeal disease).

Safety assessments were done at each visit and included recording of the incidence, nature, and severity of adverse events (AEs), changes in vital signs, and laboratory abnormalities, graded as per National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), Version 4.0.

Health-related quality of life (QoL) was assessed every 8 weeks for up to 12 months, then every 12 weeks, through Patient-Reported Outcomes questionnaires.

Regarding exploratory end points, archival formalin-fixed paraffin-embedded tumor tissues used for the molecular prescreening were available. The collection of an optional tumor biopsy was possible before the third cycle of the second treatment part and/or at PD leading to treatment discontinuation, after signing a separate informed consent. Longitudinal blood samples (plasma and peripheral blood mononuclear cells) were collected at baseline, every 4 weeks until best response, every 8 weeks for up to 12 months, then every 12 weeks, and at PD.

Study End Points

The primary end point of the trial was investigator-assessed 8-month progression-free survival (PFS) rate in patients who started the second treatment part and was defined as the proportion of patients alive and progression-free by the 8-month time point from the start of the first treatment part.

The secondary end points were PFS, defined as the interval from the date of enrollment in the first treatment part to the date of PD by RECIST1.1 and ir-RECIST criteria, or death from any cause; overall survival (OS), defined as the interval from the date of enrollment in the first treatment part to the date of death from any cause or censored to the last follow-up for alive patients; ORR, defined as the proportion of patients achieving an objective response (complete response or PR) by RECIST1.1 and ir-RECIST criteria using the scan obtained before temozolomide monotherapy as baseline; duration of response (DoR); PFS, ORR, and DoR according to BICR; safety profile and AEs in each Treatment Part according to NCI CTCAE v4.0 and QoL as measured by EORTC QLQ-C30, EORTC QLQ-CR29, and EuroQoL EQ-5D questionnaires.

Statistical Analysis

The sample size was calculated on the basis of the primary end point of 8-month PFS rate. According to our previously published results,[11,12,15] the PFS of patients with MGMT-silenced mCRC and clinical benefit from single-agent temozolomide is almost always < 8 months. Therefore, we aimed to increase the 8-month PFS rate from 5% to 20% with the combination of temozolomide, nivolumab, and ipilimumab. According to a single-stage design and selecting p0 (8-month PFS in the null hypothesis) = 0.05, and p1 (8-month PFS in the alternative hypothesis) = 0.20, with 1-sided α- and β-error of 5% and 20%, respectively, a total of 27 patients were required in the second treatment part. The null hypothesis would have been rejected with ≥ 4 patients progression-free and alive by the 8-month time point.

PFS, OS, and DoR were assessed with the Kaplan-Meier method. The median follow-up was calculated by reverse Kaplan-Meier approach. For QoL analysis, mean scores (with standard deviation) at each time point were described, and compared with baseline scores by paired *t*-test.

RESULTS

Patient Characteristics

The CONSORT diagram of the study is depicted in Figure 2. Between March 22, 2019, and November 1, 2020, 716 patients were prescreened at 12 Italian Centers. Overall, 703 tumor samples were successfully analyzed, whereas the quality check failed in 13. A total of 204 patients (29%) were molecularly eligible and 135 of them were enrolled and started the first treatment part. Among these, 102 patients (76%) were discontinued because of death or disease progression on temozolomide priming, whereas 33 patients (24%) achieved disease control according to BICR and started the second treatment part. The Data Supplement shows the early response to the two priming cycles with temozolomide in the 112 patients who had the first postbaseline computed tomography scan. The Data Supplement shows PFS and OS in the 135 patients who started the first treatment part.

Table 1 shows the baseline characteristics of patients included in the first treatment part, in those with PD or death on temozolomide priming versus those in the second treatment part. Overall, the frequency of *RAS*-mutated tumors was high (72%), consistent with the *MGMT*-hypermethylated profile. Patients received ≥ 3 prior treatment lines in 55% of cases. When comparing patients with or without clinical benefit after temozolomide priming, no statistically significant differences were observed, except for lower median age and higher frequency of right-sidedness in patients who started the second treatment part. Among these 33 patients, no statistically significant differences for baseline characteristics were observed according to the 8-month PFS status (Data Supplement).
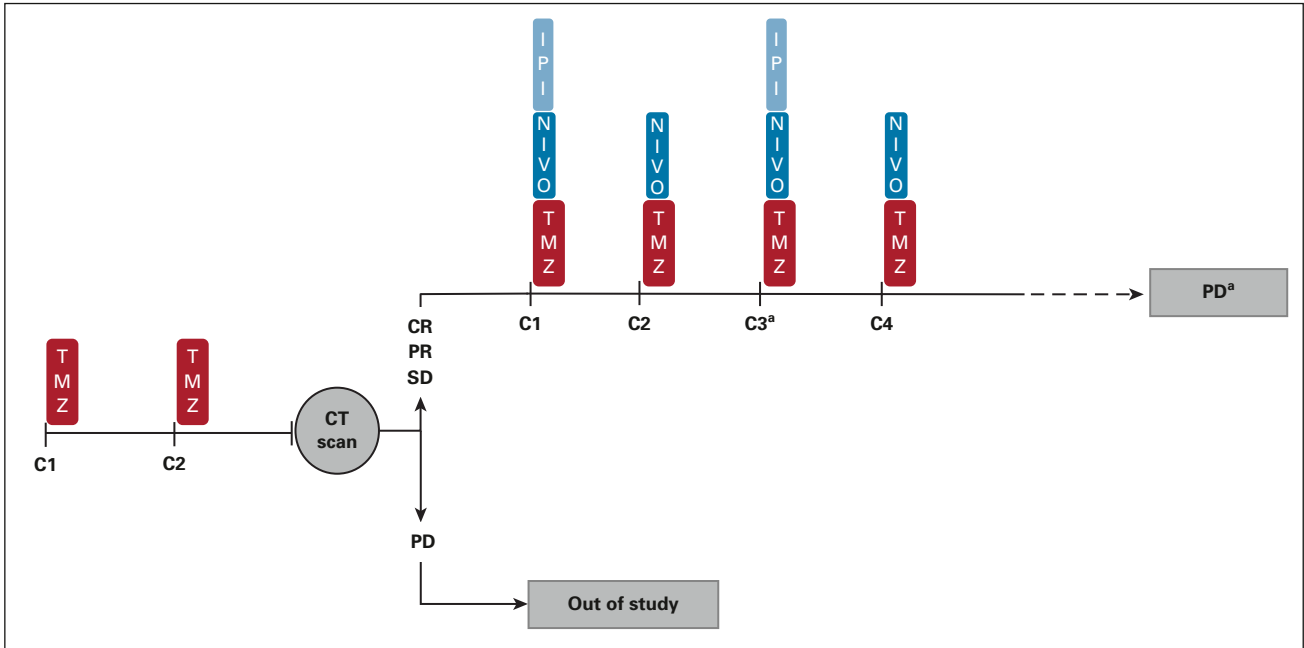
FIG 1. Trial Design. This figure depicts the design of the MAYA study, divided into the first treatment part with two cycles of temozolomide priming and the second treatment part, including combination of temozolomide and nivolumab plus low-dose ipilimumab. [a]Rebiopsy (optional). CT, computed tomography; CR, complete response; IPI, ipilimumab; NIVO, nivolumab; PD, progressive disease; PR, partial response; SD, stable disease; TMZ, temozolomide.
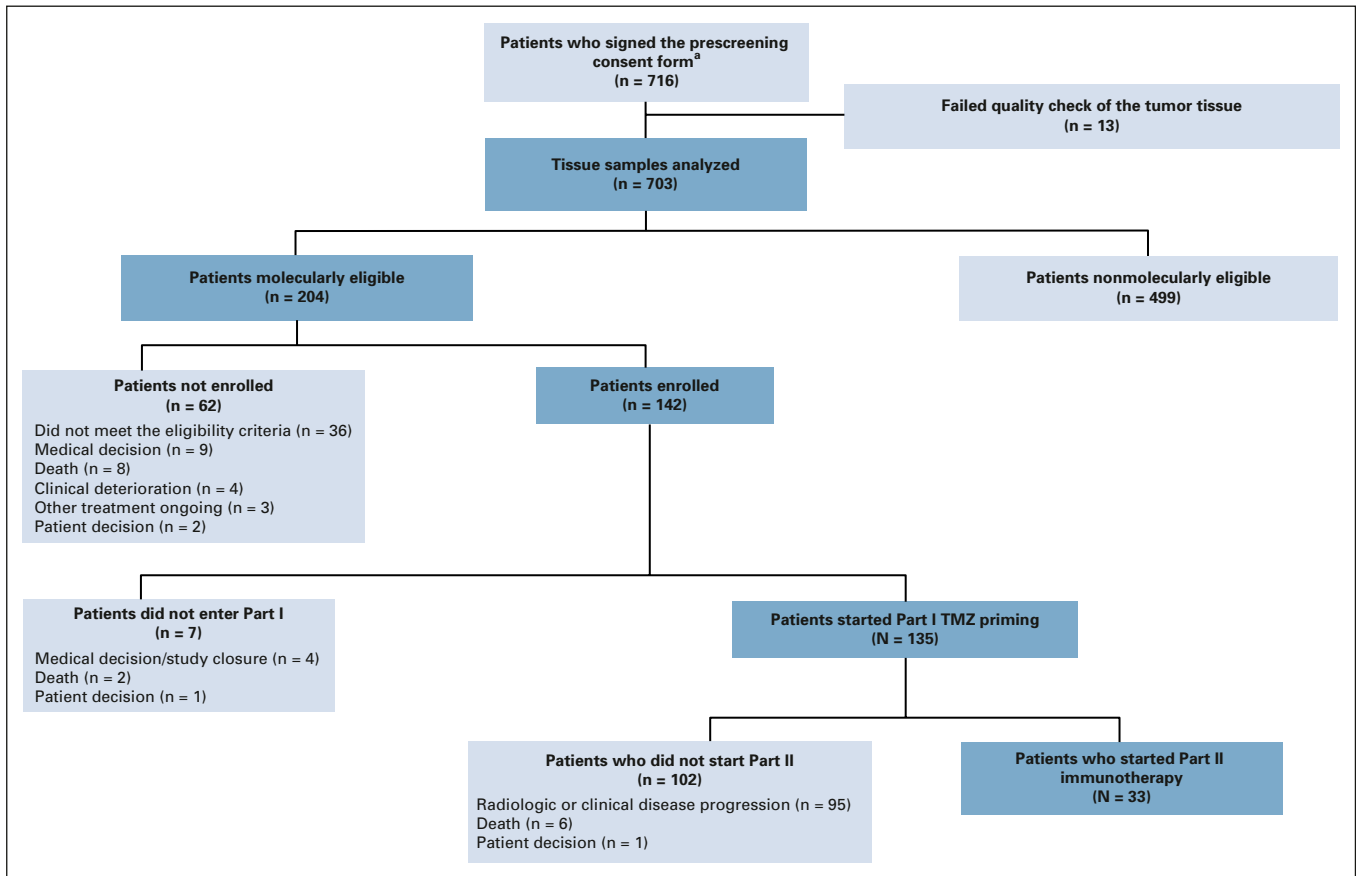
FIG 2. Flow diagram of the trial. [a]From March 2019 to November 2020 across 12 Italian sites. TMZ, temozolomide.

Efficacy and Activity of the Strategy

Regarding patients in the second treatment part, at the data cutoff date of December 17, 2021, the median follow-up was 23.1 months (interquartile range [IQR], 14.9-24.6 months). Overall, patients received a median of seven immunotherapy cycles (range, 1-26 cycles). A total number of 26 RECIST1.1 PFS events, 23 ir-RECIST PFS events, and 17 deaths occurred. The study met its primary end point: 12 out of 33 patients achieved a PFS > 8 months, with an 8-month PFS rate of 36% (95% CI, 23 to 57). The median PFS and OS were 7.0 months (95% CI, 5.5 to 8.3) and 18.4 months (95% CI, 14.9 to nonassessable), respectively (Fig 3); 12- and 18-month PFS were 24% (95% CI, 13 to -44) and 20% (95% CI, 10 to 41), respectively. The Data Supplement shows the PFS according to ir-RECIST. To reduce the impact of intrinsic selection biases, we exploratorily investigated the time-to-event outcomes calculated from the start of the second treatment part: the primary end point was still met (Data Supplement).

The ORR to the whole treatment strategy according to RECIST1.1 was 45% (95% CI, 29 to 62), with 15 PRs and 0 CRs. In 26 out of 33 cases (79%), a tumor shrinkage of any extent was observed (Fig 4A). As exploratory analysis, the ORR obtained exclusively in the second treatment part was 18% (95% CI, 9 to 34; Data Supplement). The median DoR according to RECIST1.1 was 4.8 months (95% CI, 3.8 to nonassessable). In details, eight PRs were observed at the first disease evaluation after the first treatment part, whereas the remaining seven PRs occurred during the Second Treatment Part, with five of them delayed after the 8-month time point (Fig 4B).

The investigators decided to continue the treatment beyond progression in 18 patients, of whom 15 (83%) had confirmed PD according to ir-RECIST, whereas three (17%) patients were still on treatment without the occurrence of a second PD. The median time from unconfirmed to confirmed PDs according to ir-RECIST was 2.1 months (IQR, 1.2-3.0 months; Fig 4C).

Safety and QoL

During the first treatment part, 53% of any grade and 3% of grade ≥ 3 temozolomide-related AEs were reported in the 135 patients. The most common any grade and grade ≥ 3 AEs reported were hematologic and GI (Table 2). During the second treatment part, the overall rate of any grade and grade ≥ 3 AEs was 91% and 21%, respectively. Immune-related AEs (irAEs) of any grade and grade ≥ 3 were mainly skin rash (18% and 6%), colitis (18% and 3%), hypothyroidism (21% and 0%), hyperthyroidism (9% and 0%), hypophysitis (6% and 3%), and adrenal insufficiency (3% and 0%; Table 2). No unexpected AEs or treatment-related deaths were reported.

Main QoL results (mean scores in global health status measured by EORTC QLQ C30 and EQ5D visual analog score) are reported in the Data Supplement. No relevant changes were evident during treatment, with a significant worsening at the time of PD.

Assessment of Tumor Mutational Burden

Baseline tumor mutational burden (TMB) was assessed by comprehensive genomic profiling with FoundationOne CDx and available for 27 out of 33 patients. For these, median TMB was 4.5 (IQR: 3.2-6.0). Four patients with individual PFS > 8 months had matched archival and on-treatment tumor rebiopsies available and successfully analyzed. A meaningful increase of TMB with retained MGMT-negative staining by IHC was observed in all cases, as detailed in the Data Supplement.

DISCUSSION

The MAYA trial met its primary end point, providing proof-of-concept evidence that a sequence of temozolomide priming followed by a combination of low-dose ipilimumab and nivolumab may induce a durable clinical benefit in patients with pMMR/MSS and MGMT-silenced mCRC. Our results represent the clinical translation of seminal efforts carried out in CRC models and patients[18,28] and in other temozolomide-sensitive cancers.[32]

Although these results should be interpreted with caution, given the nonrandomized nature of our trial, PFS and OS outcomes favorably compared with those achieved by the standard later line options available for patients with molecularly unselected mCRC.[33,34] Considering the poor prognosis of treatment-refractory disease, there is an unmet need for innovative treatment strategies and new immunotherapy-based combinations. In this scenario, the clinical importance of the MAYA study data relies on the opportunity to achieve long-term disease control, thanks to the immune-sensitization properties of temozolomide in a subset of pMMR/MSS cancers.

The study was designed with an initial priming part with temozolomide monotherapy followed, in patients with clinical benefit, by a second part with cytotoxic T-cell lymphocyte-4 (CTLA-4)/programmed cell death protein 1 dual blockade added to temozolomide backbone. The two-phase treatment strategy was necessary because (1) hypermutation is a mechanism of acquired resistance to temozolomide; thus, patients without evidence of treatment effect, ie, those with primary resistance and early disease progression at the first computed tomography scan, had to be excluded. (2) Despite initial molecular selection, only one out of four patients with complete MGMT silencing had radiologic evidence of an early disease control. Thus, temozolomide treatment–driven patients' selection remains crucial to enrich the patients' population targeted by this strategy, whereas a study design with upfront temozolomide plus ICIs would have likely failed; (3) Acquired resistance to temozolomide usually occurs rapidly within 8 months,[11,12,15] and only two priming cycles of temozolomide may not be sufficient to induce a meaningful

TABLE 1. Baseline Patients and Disease Characteristics, Overall and in Patients Who Started Only the First Treatment Part Versus Those in the Second Treatment Part

Characteristic	Overall Study Population (N = 135)	Patients With PD or Death After the First Treatment Part (n = 102)	Patients in the Second Treatment Part (N = 33)	P[a]
Median age, years (IQR)	63 (55-71)	65 (55-71)	58 (53-65)	.020
Sex, No. (%)				.843
Male	72 (53)	55 (53)	17 (52)	
Female	63 (47)	47 (46)	16 (48)	
ECOG PS, No. (%)				.835
0	86 (64)	64 (63)	22 (67)	
1	49 (36)	38 (37)	11 (33)	
Primary tumor resected, No. (%)				.356
Yes	119 (88)	88 (86)	31 (94)	
No	16 (12)	14 (14)	2 (6)	
Synchronous metastases, No. (%)				.313
Yes	77 (57)	61 (60)	16 (48)	
No	58 (43)	41 (40)	17 (52)	
No. of metastatic sites, No. (%)				.198
1	20 (15)	13 (13)	7 (21)	
2	57 (42)	41 (40)	16 (48)	
≥ 3	58 (43)	48 (47)	10 (31)	
Primary tumor location, No. (%)				.011
Right colon	40 (30)	25 (24)	15 (45)	
Left colon	72 (53)	57 (56)	15 (46)	
Rectum	23 (17)	20 (20)	3 (9)	
RAS status, No. (%)				.660
Wild-type	38 (28)	30 (29)	8 (24)	
Mutated	97 (72)	72 (71)	25 (76)	
BRAF status, No. (%)				> .999
Wild-type	127 (98)	95 (98)	33 (100)	
Mutated	2 (2)	2 (2)	0	
Not assessed	6	5	0	
No. of prior lines, No. (%)				.146
1	16 (12)	14 (14)	2 (6)	
2	44 (33)	29 (28)	15 (46)	
≥ 3	75 (55)	59 (58)	16 (48)	
Previous oxaliplatin, No. (%)				> .999
Yes	129 (96)	97 (95)	32 (97)	
No	6 (4)	5 (5)	1 (3)	
Previous irinotecan, No. (%)				.679
Yes	127 (94)	95 (91)	32 (97)	
No	8 (6)	7 (9)	1 (3)	

TABLE 1. Baseline Patients and Disease Characteristics, Overall and in Patients Who Started Only the First Treatment Part Versus Those in the Second Treatment Part (continued)

| Characteristic	Overall Study Population (N = 135)	Patients With PD or Death After the First Treatment Part (n = 102)	Patients in the Second Treatment Part (N = 33)	P[a]
Previous anti-VEGF agent, No. (%)				.770
Yes	117 (87)	89 (87)	28 (85)	
No	18 (13)	13 (13)	5 (15)	
Previous anti-EGFR agent, No. (%)				.660
Yes	38 (28)	30 (29)	8 (24)	
No	97 (72)	72 (71)	25 (76)	

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, Epidermal Growth Factor Receptor; IQR, interquartile range; PD, progressive disease; VEGF, Vascular Endothelial Growth Factor.

[a]P value using the Mann-Whitney test and the chi-square or Fisher exact test for numerical and categorical variables, respectively, to investigate the binary associations between patients and tumor characteristics of patients who entered the second treatment part and those with PD or death after the first treatment part.

increase of TMB. Therefore, the continuation of several courses of temozolomide during the second treatment part was necessary because of its potentially cumulative effects on neoantigens' renewal. In parallel, we chose an early combination with immunotherapy over a simple sequential approach because of (1) clinical reasons, ie, the high risk of rapid deterioration of patients' health status following progression to temozolomide, coupled with the possibility of delayed efficacy of immunotherapy; and (2) biologic reasons, ie, the rapid and early emergence of temozolomide-resistant clones under the selective pressure of treatment, which may be promptly targeted by the early addition of immunotherapy.

Notably, acquired resistance to temozolomide may emerge via two different mechanisms, ie, the expansion of MGMT-expressing cells versus secondary hypermutation, which were mostly mutually exclusive in patients with GBM[22-26] and in the small published series of patients with mCRC.[28] The former mechanism may be related to the horizontal heterogeneity and/or longitudinal changes of MGMT expression,[13] which may have been missed by the molecular prescreening on archival tumor specimens. On the basis of these considerations, although tumors with low and/or focal MGMT expression were excluded by MAYA trial, future studies should foresee pre-enrollment tumor biopsies to confirm molecular eligibility, especially in pretreated patients' populations. On the opposite, the increase of TMB during temozolomide may be preferentially observed in the subgroup of tumors with homogeneous lack of MGMT expression and high *MGMT* methylation percentage. As supported by the preliminary translational results reported here, the onset of hypermutation was coupled with retained MGMT-negative staining in patients with durable responses to immunotherapy. Remarkably, the plateau observed in the PFS curve and the occurrence of delayed responses after the 8-month time point in five patients suggest the efficacy of immunotherapy, at least in a patients' subgroup. The association of an anti–CTLA-4 agent to the anti–programmed cell death
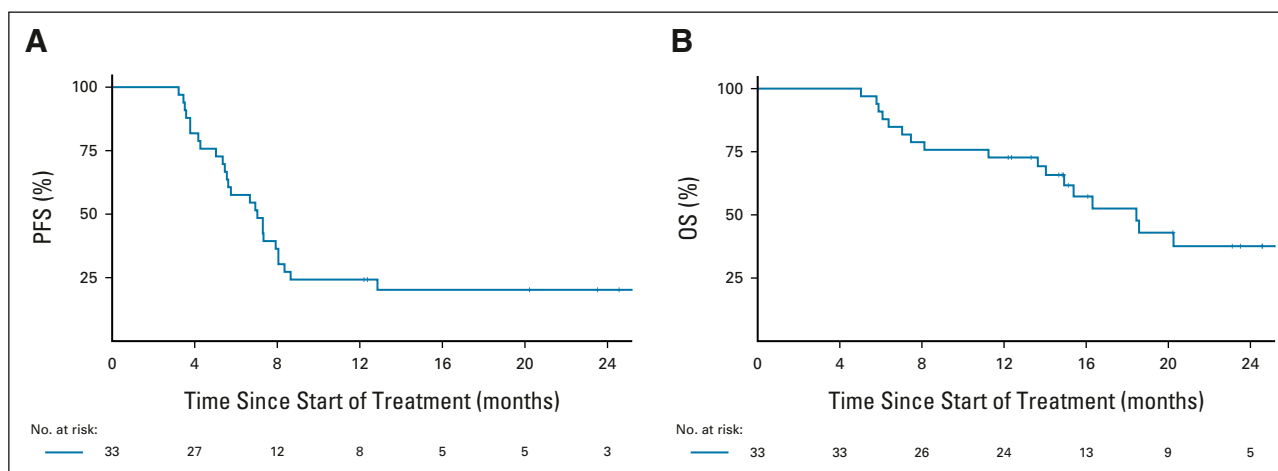
FIG 3. Kaplan-Meier curves for (A) PFS and (B) OS of patients in the second treatment part. OS, overall survival; PFS, progression-free survival.
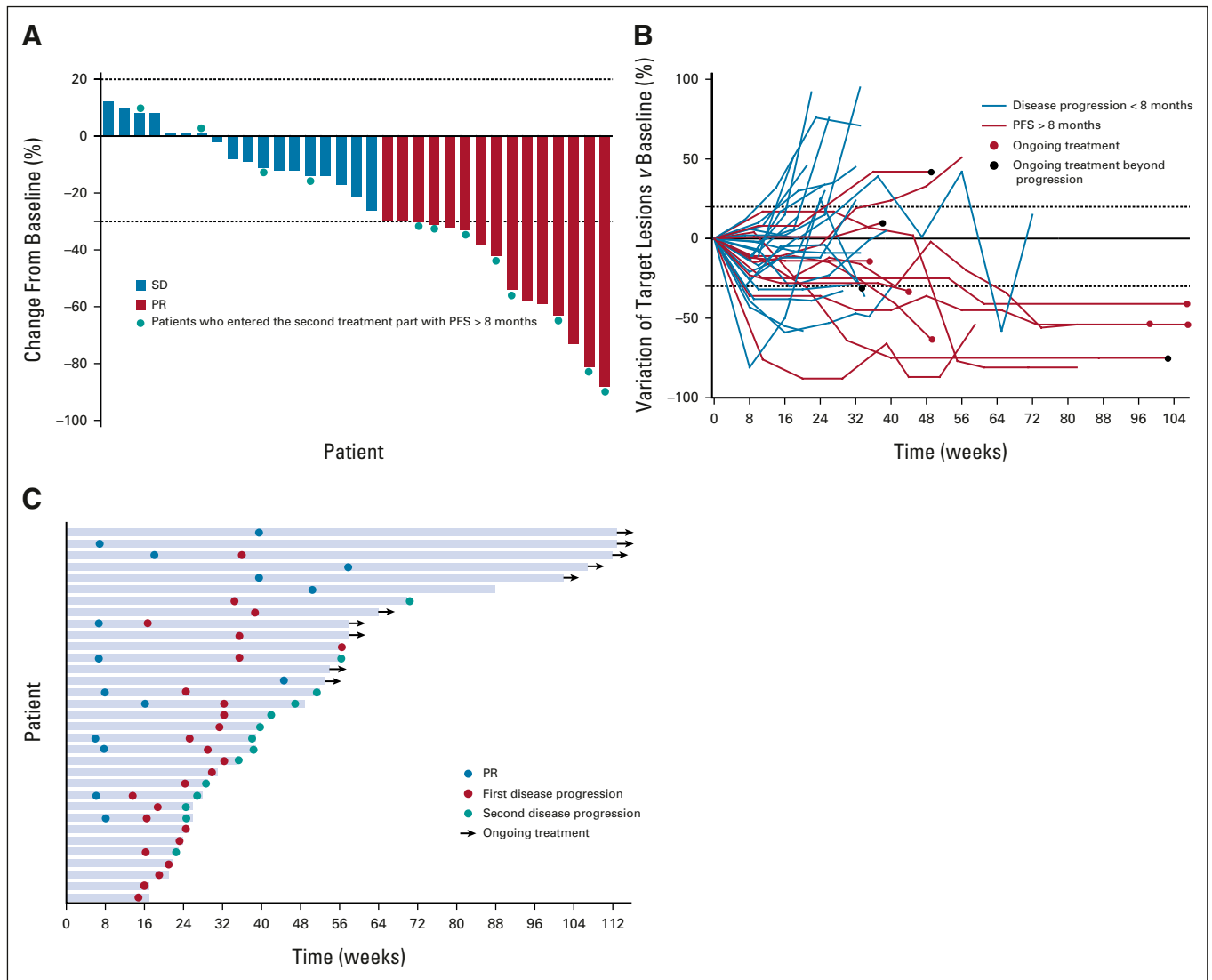
FIG 4. (A) Waterfall plot with the best tumor response achieved by each patient; (B) spider plot with dynamics of tumor response; (C) swimmer plot with treatment duration for each patient in the second treatment part, with the time points of occurrence of PR, first PD, and second PD. At the time of the data cutoff (December 17, 2021), 11 patients were still on study in the second treatment part: treatment was still ongoing in 10 patients (black arrows) without disease progression in six and beyond progression in four. One patient discontinued the treatment because of immune-related toxicity. Main reasons for study discontinuation in the 22 remaining patients were disease progression (n = 19) and death (n = 3, because of clinical progression in one and COVID-19 during the first outbreak in Italy in two). PFS, progression-free survival; PR, partial response; SD, stable disease.

protein 1 backbone in the MAYA study may have had an important role in boosting the antitumor immune responses. Despite all these observations, the high ORR to the whole strategy translated into a relatively short median DoR, in contrast with what usually occurs in dMMR/MSI-high cancers. Also, the response rate specifically observed only in the second treatment part was relatively lower. The reasons of these discrepancies may rely on the aforementioned heterogeneity of secondary resistance to temozolomide, as well as on the potential emergence of a majority of subclonal neoantigens.

The toxicity profile of the combination during the second treatment part was manageable, consistent with that of temozolomide monotherapy and ICIs, and in line with the literature data of temozolomide plus ICIs reported in patients with GBM,[35] with extremely low rate of treatment discontinuations because of toxicities. Notably, the low incidence of grade 3 or more irAEs is in line with the use of low-dose ipilimumab added to nivolumab, as reported in patients with MSI-high mCRC.[5] With the limitations of the small number of patients and the absence of a control arm, the lack of worsening of QoL during treatment may mirror the favorable safety profile associated with disease control.

The MAYA trial strategy is worth of being investigated by new clinical trials that should carefully choose the eligible patients' population, the optimal strategy, and its timing. We

TABLE 2. Treatment-Related Adverse Events During the First Treatment Part or in the Second Treatment Part

AEs	Safety Profile During the First Treatment Part (N = 135)		Safety Profile During the Second Treatment Part (N = 33)	
	Any Grade, No. (%)	Grade ≥ 3, No. (%)	Any Grade, No. (%)	Grade ≥ 3, No. (%)
All AEs	71 (53)	4 (3)	30 (91)	7 (21)
Anemia	8 (6)	1 (1)	7 (21)	1 (3)
Leukopenia	29 (21)	0	16 (48)	0
Neutropenia	6 (4)	2 (1)	1 (3)	0
Febrile neutropenia	0	0	0	0
Thrombocytopenia	27 (20)	3 (2)	12 (36)	2 (6)
Nausea	20 (15)	0	8 (24)	0
Vomiting	8 (6)	0	2 (6)	1 (3)
Lack of appetite	5 (4)	0	0	0
Diarrhea	2 (1)	0	—	—
Fatigue	18 (13)	0	8 (24)	0
Mucositis	4 (3)	1 (1)	1 (3)	0
Rash	—	—	6 (18)	2 (6)
Pruritus	—	—	8 (24)	0
Colitis	—	—	6 (18)	1 (3)
Arthritis	—	—	3 (9)	0
Infusion reaction	—	—	2 (6)	0
Hypothyroidism	—	—	7 (21)	0
Hyperthyroidism	—	—	3 (9)	0
Hypophysitis	—	—	2 (6)	1 (3)
Adrenal insufficiency	—	—	1 (3)	0
Interstitial pneumonia	—	—	1 (3)	0

Abbreviation: AE, adverse event.

acknowledge the relevant bottleneck effect observed in the trial because of both molecular and temozolomide treatment–driven selection. Consequently, only 5% of patients with mCRC may be eligible for this strategy, but these patients achieve highly promising long-term benefit, consistent with a personalized approach.

Given the promising results of the MAYA trial, ongoing translational analyses will be crucial to characterize the evolution of genomic and immune landscape during the treatment strategy. Moreover, a deeper understanding of the molecular mechanisms of response and resistance to temozolomide priming is important to reduce the failure rate of the priming treatment. On top of this, the enrichment of patients with right-sided primary tumor location and younger age among those with disease control on temozolomide confirms the need of ongoing research on the transcriptional profiles associated with treatment sensitivity and with specific clinical characteristics. In addition, we are performing analyses of serial liquid biopsies to track baseline plasma TMB and its evolution during the study treatment, thus consolidating the biologic rationale of the study in a larger patients' population. Also, the analysis of

liquid biopsies obtained during temozolomide priming by means of ultrasensitive assays[36] may allow for the early identification of the patients' subgroup with acquired resistance driven by hypermutation and mutations in MMR genes, thus potentially informing the selection of patients with long-lasting benefit from subsequent immunotherapy combination. Overall, the MAYA study will represent a translational platform with prospective collection of tumor and plasma samples to potentially provide answers to the still opened biologic questions regarding cancer and immune evolution under treatment.

Moving forward, the results of the MAYA trial open the way to further investigations in patients with pMMR/MSS and MGMT-silenced mCRC or with other temozolomide- or dacarbazine-sensitive tumors, even in the frame of agnostic basket studies. Regarding mCRC, our group is conducting a phase Ib trial on the FLIRT/BEV regimen with escalating doses of temozolomide plus fluorouracil, leucovorin, and irinotecan/bevacizumab (NCT04689347) in patients with previously untreated MSS, MGMT-silenced mCRC. The optimal dosing of this new triplet chemotherapy will potentially allow to investigate the role of maintenance

immunotherapy strategies in patients with disease control after FLIRT/BEV induction.

Our trial had several limitations. First, the study was not randomized and had a small sample size; therefore, the activity results are preliminary in nature. Second, most patients had acceptable ECOG PS despite being heavily pretreated, which suggests that only a selected population was enrolled in this study. Third, the selection of patients with clinical benefit from the 2-month priming with temozolomide may partly condition long-term outcomes, because of intrinsic biases such as the enrollment of patients with indolent and responsive disease. However, after calculating the time-to-event outcomes from the start of the second treatment part, the study still met its primary end point. These less biased results remained clinically meaningful compared with historical cohorts of patients with MGMT-silenced mCRC achieving disease control to temozolomide.[15] Fourth, although the choice of PFS rate end point over an activity end point may be questioned, the two-stage trial design makes challenging to discriminate the contribution of temozolomide versus immunotherapy to tumor responses; similarly, the 8-month cutoff was selected to exclude patients with potential benefit from temozolomide alone. Finally, the number of patients who started the second treatment part was slightly higher than the calculated sample size, because of logistical and ethical reasons. However, on the basis of the A'Hern calculation,[37] the hypotheses and type I-II errors remained substantially unchanged.

In conclusion, the MAYA study provided evidence on the role of temozolomide as an immune-sensitizing agent for MSS and immune-cold mCRCs selected by the presence of MGMT silencing and disease control on temozolomide priming. Further investigation is warranted to optimize the molecular and clinical selection of patients eligible for this therapeutic approach with the aim of maximizing its success rate.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Temozolomide Followed by Combination With Low-Dose Ipilimumab and Nivolumab in Patients With Microsatellite-Stable, O^6-Methylguanine–DNA Methyltransferase–Silenced Metastatic Colorectal Cancer: The MAYA Trial

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