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Julien Taieb, John Souglakos, Ioannis Boukovinas, Antoine Falcoz, Franck Pages, et al.. Combined Analyses of Circulating Tumor DNA and Immunoscore in Patients With Stage III Colon Cancer: A Post Hoc Analysis of the PRODIGE-GERCOR IDEA-France/HORG-IDEA-Greece Trials. *Journal of Clinical Oncology*, 2025, 10.1200/jco.24.00648 . hal-04955618

HAL Id: hal-04955618

<https://hal.science/hal-04955618v1>

Submitted on 21 Feb 2025












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Combined Analyses of Circulating Tumor DNA and Immunoscore in Patients With Stage III Colon Cancer: A Post Hoc Analysis of the PRODIGE-GERCOR IDEA-France/HORG-IDEA-Greece Trials

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DOI <https://doi.org/10.1200/JCO.24.00648>

ABSTRACT

PURPOSE Immunoscore (IS) and circulating tumor DNA (ctDNA) are two emerging technologies in improving prognostication and tailoring adjuvant treatments in patients resected from a stage III colon cancer (CC). Here, we analyzed the prognostic value of the two biomarkers in patients who participated in the randomized phase III IDEA-France and HORG trials.

METHODS Plasma samples were collected after surgery and before adjuvant chemotherapy. ctDNA analysis was performed using a clinically validated, personalized, tumor-informed 16-plex protein chain reaction assay. Multivariable analyses for time to recurrence (TTR; patients without recurrence or death due to CC) and overall survival (OS) were performed using ctDNA and IS results, along with other parameters including treatment duration and disease risk group.

RESULTS Of the 554 patients with available ctDNA results, 445 were ctDNA-negative (80.3%) and 109 were ctDNA-positive (19.7%); baseline characteristics showed more T4/N2 and venous embolism/lymphatic invasion/perineural invasion+ in ctDNA-positive patients. With a median follow-up of 6.7 years, the 2-year TTR rate was 43.5% (95% CI, 34.1 to 52.6) for ctDNA-positive patients and 88.1% (95% CI, 84.7 to 90.8) for ctDNA-negative patients ($P < .0001$). ctDNA was confirmed as an independent prognostic marker for both TTR (adjusted hazard ratio [adjHR], 5.21 [95% CI, 3.59 to 7.58]; $P < .001$) and OS (adjHR, 4.84 [95% CI, 3.40 to 6.89]; $P < .001$). ctDNA remained the most significant prognostic factor irrespective of disease stage, treatment duration, and IS results. IS was not prognostic in ctDNA-positive patients but remained a significant prognostic tool for ctDNA-negative patients.

CONCLUSION In this combined analysis of two adjuvant trials dedicated to patients with stage III CC after surgery, ctDNA was detectable in 19.7% of the patients and was confirmed as a major independent prognostic biomarker. IS seems to bring additional prognostic information in the 80.3% of patients who are ctDNA-negative.

ACCOMPANYING CONTENT

 Appendix
 Data Sharing Statement

Accepted December 2, 2024

Published February 4, 2025

J Clin Oncol 00:1-14

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INTRODUCTION

After surgical resection of stage III colon cancer (CC), 6 months of fluoropyrimidine-based chemotherapy was the standard adjuvant treatment¹ and decreases the risk of death by 10%-15% when administered as a single agent and by 20%-22% when combined with oxaliplatin.² More recently

the option of administering only 3 months of adjuvant treatment has been validated for low risk stage III patients (defined as T1-3 and N1).³ Indeed, the IDEA collaboration found a difference of less than 0.4% in the 5-year overall survival (OS) rates observed in patients treated for 3 or 6 months. Discontinuing oxaliplatin after a minimum of 3 months of doublet chemotherapy has also emerged as an

CONTEXT

Key Objective

Postsurgery circulating tumor DNA (ctDNA) to predict disease recurrence has been assessed in cohorts with limited follow-up, catching only partially long-term disease recurrences. In addition, ctDNA primarily measures the presence of viable tumor cells but does not account for essential information regarding the tumor microenvironment.

Knowledge Generated

This study shows, with a median follow-up of more than 80 months, allowing full assessment of disease recurrence, that ctDNA is an independent prognosticator that can help decide the duration of treatment for low-risk stage III patients, and that assessment of the tumor immune infiltrate with the Immunoscore adds prognostic information in ctDNA-negative patients.

Relevance (E.M. O'Reilly)

This combined analysis from two prospective adjuvant trials provides additional support for the strong prognostic impact of detectable ctDNA following potentially curative surgery in colon cancer. The key challenge for now and the future is to translate this prognostic signal into an intervention that enhances cure.*

*Relevance section written by JCO Associate Editor Eileen M. O'Reilly, MD.

option when a treatment of 6 months' duration is decided in multidisciplinary meetings.⁴ Nonetheless, many patients are cured by the surgical removal of their tumor, and some experience recurrence despite receiving adjuvant chemotherapy. In this context, the management of stage III CC has been the subject of debate for over 30 years, and personalized assessment of each patient's risk of recurrence is currently the most important challenge in this setting. Various clinicopathologic and molecular prognostic factors have been reported since the 80s and are helping us in our day-to-day decisions to administer adjuvant treatment.^{2,5} More recently, there has been an acceleration in the development of prognostic tools to better individualize treatment decisions. These new tools were mainly based on tracking residual disease after curative-intent cancer surgery,^{6,7} or scores that consider not only the cancer itself but also its microenvironment. Among these tools, the Immunoscore (IS) and circulating tumor DNA (ctDNA) testing have generated the most significant number of scientific publications.⁸⁻¹²

IS, assessing tumor-infiltrating lymphocytes, with a validated standardized digital pathology-based assay, provides a reliable estimate of the risk of recurrence in patients resected from a stage I-III CC. This score, prospectively validated in an international consortium of 14 centers in 13 countries, showed a risk of recurrence ranging from 8% in patients with a high IS to 32% in patients with a low IS (hazard ratio [HR], 0.2 [95% CI, 0.10 to 0.36]).⁸ More recently, testing ctDNA after CC resection showed the best performance to predict disease recurrence in exploratory cohorts as in phase III clinical trials.¹⁰⁻¹³ The HR for DFS between positive and negative ctDNA patients, after surgery, was found in a recent meta-analysis to be 6.92 (95% CI, 4.49 to 10.64).¹⁴

Here, we evaluate the independent prognostic value of tumor-informed ctDNA testing and IS in patients treated in the PRODIGE-GERCOR-IDEA-France¹⁵ and HORG¹⁶-IDEA-Greece prospective phase III trials and assess their respective prognostic capacities alone or in combination.

METHODS

Study Design and Patients

IDEA-France and IDEA-Greece trials were two multicenter, two-arm, open-label, randomized phase III trials conducted at 129 French and 24 Greek centers, respectively. Eligible patients were age 18 years and older, with stage III histologically confirmed CC, curative-intent surgery no more than 8 weeks before random assignment, an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1, and postoperative carcinoembryonic antigen (CEA) level <10 ng/mL. The study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Approval of the protocol and translational research program was obtained from two independent ethics committees.

Between April 2009 and May 2014, a total of 2010 stage III patients were recruited in France and 707 patients were recruited in Greece. Treatment was randomly assigned (1:1) to 3 or 6 months of adjuvant chemotherapy (modified FOLFOX6 or capecitabine plus oxaliplatin per the physician's choice), with 74% of patients receiving infusional and bolus 5-fluorouracil, leucovorin and oxaliplatin (FOLFOX).¹¹ Results reveal superiority of 6 months of adjuvant chemotherapy over 3 months, especially in the high-risk T4 and/or N2 subgroups.^{15,16} However, these results should be considered alongside the international IDEA collaboration data.¹⁷

All patients enrolled in this study signed the study-specific translational research informed consent.

Assessment of ctDNA and IS

Plasma samples were collected from patients after surgery and after the study consent was signed and before the start of chemotherapy. Plasma samples that were processed after the first cycle of chemotherapy were not analyzed. Furthermore, for a subset of patients from IDEA-Greece ($n = 71$), who did not have matched normal tissue available, whole-exome sequencing (WES) data were generated using plasma WES approach.

Retrospective analysis was performed on plasma samples using a clinically validated, personalized, tumor-informed 16-plex protein chain reaction next-generation sequencing (NGS) assay for molecular residual disease (MRD) detection (Natera, Inc). Briefly, WES was performed on formalin-fixed, paraffin-embedded (FFPE) tumor tissue samples from surgical resection to target 16 patient-specific, somatic single-nucleotide variants, as previously described.¹¹ Personalized modified-PCR-NGS ctDNA assays, consisting of these somatic variants, were designed for each study participant. Cell-free DNA was extracted from patient plasma (median, 2.1 mL; range, 0.5–10 mL) at a prespecified time point. The personalized ctDNA assays were used to detect the presence of ctDNA for the associated plasma samples accordingly. Plasma samples with at least two of 16 patient-specific somatic variants above the detection confidence threshold were defined as ctDNA-positive.¹¹

For a subset of patients from IDEA-Greece ($N = 71$), who did not have matched normal DNA available, WES data were generated using a plasma WES approach that showed similar performances to that obtained from WBC DNA variations in characterizing germline variations.¹⁸

IS testing was performed on tissue samples as previously described.⁹ In brief, sections of FFPE tissues were incubated with rabbit monoclonal antihuman CD3⁺ (clone HDx2; HalioDx, Marseille, France) and mouse monoclonal antihuman CD8⁺ (clone HDx1; HalioDx). Counterstained slides were digitalized at $\times 10$ magnification and 0.45 $\mu\text{m}/\text{pixel}$ resolution (NanoZoomer-XR, Hamamatsu, Japan), and CD3⁺ and CD8⁺ stained cells were quantified with the Image analysis software (Immunoscore Analyzer; HalioDx).

Statistical Analysis

Baseline characteristics were described with mean (standard deviation), median (IQR), and frequencies (percentage) for continuous and categorical variables, respectively, and compared between patients with and without ctDNA analysis results and between those with the ctDNA-positive and ctDNA-negative profiles using Wilcoxon test and chi-square test (or Fisher's exact test, if appropriate).

The primary outcome was time to recurrence (TTR), measured from the date of random assignment to the first

documented radiologic recurrence or death linked to CC, whichever occurred first. Patients without event of recurrence or death related to CC were censored at the last follow-up visit. Secondary outcome was OS, defined as the time from random assignment to the date of death from any cause. Patients alive were censored at the last follow-up visit.

TTR and OS curves were estimated with the Kaplan-Meier method, described with median or rate at specific time points with 95% CI and compared with the log-rank test. HR with 95% CI were provided by Cox proportional hazard models. Median follow-up and its IQR were estimated with the reverse Kaplan-Meier method.

Associations of baseline parameters with TTR and OS were first examined using univariable Cox proportional hazard models and then parameters with a $P < .1$ were entered in a Cox regression multivariable model (full multivariable model) and then a stepwise selection procedure was performed to obtain a final multivariable model with only parameters significantly independently associated with the outcome (final parsimonious multivariable model). Parameters with more than 20% of missing data were not included in the multivariable analyses.

Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc, Cary, NC) and R software version 4.3.1 (R Development Core Team, Vienna, Austria). $P < .05$ were considered statistically significant and given on exploratory purposes because of the post hoc context of the analysis and the absence of correction for multiple testing; all tests were two-sided.

RESULTS

Of 2,717 patients enrolled in IDEA-France and IDEA-Greece, 2,052 signed informed consent for the optional translational studies and 554 (132 from IDEA-Greece and 422 from IDEA-France) had sufficient plasma left to assess ctDNA (Fig 1). No major differences were observed between patients included ($n = 554$) and those excluded from this analysis ($n = 2,163$); only more ECOG PS of 0 (79.0% v 74.7%; $P = .04$) and more venous embolism/lymphatic invasion/perineural invasion+ (VELIPI+; 58.4% v 50.9%; $P < .01$) were observed in the analyzed patients (Appendix Table A1, online only). There was no statistical difference in TTR between these two groups ($P = .58$; Appendix Fig A1). Upon evaluating the impact of treatment duration, 6 months of therapy improved TTR (patients without disease recurrence or death due to their CC) compared with 3 months in the overall population ($n = 2,717$) of both trials (Appendix Fig A2A; HR, 1.18 [95% CI, 1.03 to 1.36]; $P = .021$). This difference was even greater in the 554 patients available for the present ctDNA analysis (Appendix Fig A2B; HR, 1.39 [95% CI, 1.02 to 1.90]; $P = .038$). Median time from the date of surgery to postsurgical blood collection was 39 days (IQR, 34–50; $n = 554$). Median time from surgery to chemotherapy initiation was 44 days (IQR, 37–51; $n = 554$).

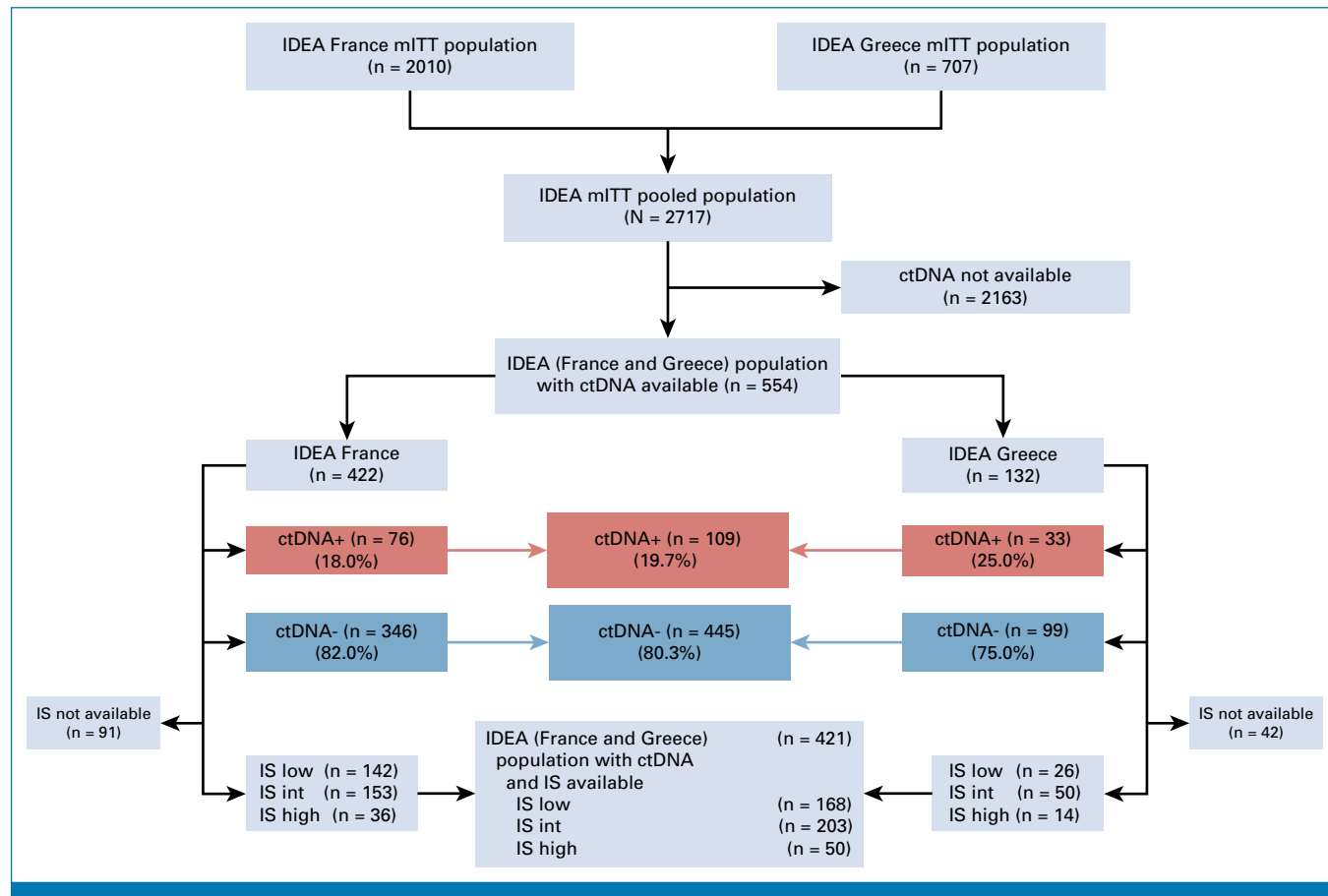


FIG 1. Flowchart. ctDNA, circulating tumor DNA; int, intermediate; IS, Immunoscore; mITT, modified intention-to-treat population.

Appendix Table A2 shows differences in baseline characteristics between the French and Greek cohorts. It shows that bowel obstruction (14.2% v 3.8%; $P < .01$), high-grade tumors (7.2% v 0.8%; $P < .01$), presence of VELIPI (64.3% v 58.3%; $P < .01$), and low IS (42.9% v 28.9%; $P = .02$) were more frequently observed in the French cohort compared with the Greek cohort.

Overall, 445 patients were ctDNA-negative and 109 ctDNA-positive (Fig 1), leading to a postoperative, prechemotherapy positivity rate of 19.7% (95% CI, 16.4 to 23.2) using the personalized, tumor-informed assay. Rate of ctDNA-positive patients was not different for patients sampled before 6 weeks (16.9%), between 6 and 8 weeks (17.3%), or after 8 weeks (24.1%) after surgery. Baseline characteristics were similar in both ctDNA-positive and ctDNA-negative groups (Table 1) except for T4 and/or N2 tumors (57.8% v 37.7%; $P < .01$), VELIPI+ tumors (74.5% v 54.3%; $P < .01$), and postoperative plasma CEA levels >5 ng/mL (12.3% v 2.7%; $P < .01$) that were more frequent in the ctDNA-positive patient group.

With the median follow-up of 6.7 years (IQR, 5.6-7.6), the 2-year rate of patients without recurrence or death due to CC

was 43.5% (95% CI, 34.1 to 52.6) for ctDNA-positive patients and 88.1% (95% CI, 84.7 to 90.8) for ctDNA-negative patients (HR, 5.75 [95% CI, 4.20 to 7.87]; $P < .001$; Fig 2A). This rate was 33.1% (95% CI, 24.4 to 42.1) versus 81.0% (95% CI, 76.9 to 84.4) at 5 years. Similar results were observed with OS with 5-year OS rates of 52.7% (95% CI, 42.6 to 61.8) versus 89.4% (95% CI, 86.1 to 92.0) for ctDNA-positive and ctDNA-negative patients, respectively (HR, 5.31 [95% CI, 3.75 to 7.51]; $P < .0001$; Fig 2B).

Overall, ctDNA-negative patients had better outcomes compared with ctDNA-positive patients.

Among ctDNA-positive and ctDNA-negative groups, patients receiving 6 months of therapy recurred less frequently compared with those receiving 3 months (Fig 3A). The same results were observed for OS (Appendix Fig A3A).

Similarly, among ctDNA-positive and ctDNA-negative groups, patients with low-risk stage III (pT1-3 and N1) tumors recurred less frequently (Fig 3B) compared with those with high-risk stage III (pT4 and/or N2) tumors (Fig 3B). The same results were observed for OS (Appendix Fig A3B).

TABLE 1. Baseline Characteristics in ctDNA+ and ctDNA- Patients

Characteristic	Pooled Population (N = 554)	ctDNA- (n = 445)	ctDNA+ (n = 109)	P ^a
Age, years				.91
Mean (SD)	63.9 (9.4)	63.8 (9.7)	64.2 (8.3)	
Median (Q1-Q3)	65.0 (58.0-71.0)	65.3 (58.0-71.0)	65.3 (58.0-71.0)	
Age, years, No. (%)				.98
≤70	396 (71.5)	318 (71.5)	78 (71.6)	
>70	158 (28.5)	127 (28.5)	31 (28.4)	
Sex, No. (%)				.89
Male	322 (58.1)	258 (58.0)	64 (58.7)	
Female	232 (41.9)	187 (42.0)	45 (41.3)	
ECOG PS, No. (%)				.44
0	399 (79.0)	322 (78.4)	77 (81.9)	
1-2	106 (21.0)	89 (21.6)	17 (18.1)	
Missing	49	34	15	
Tumor and node stage, No. (%)				<.01
T1-3 and N1	323 (58.3)	277 (62.3)	46 (42.2)	
T4 and/or N2	231 (41.7)	168 (37.7)	63 (57.8)	
Chemotherapy duration, No. (%)				.33
3 months	272 (49.1)	223 (50.1)	49 (45.0)	
3 months of FOLFOX	217 (79.8)	178 (79.8)	39 (79.6)	
3 months of CAPOX	55 (20.2)	45 (20.2)	10 (20.4)	
6 months	282 (50.9)	222 (49.9)	60 (55.0)	
6 months of FOLFOX	217 (77.0)	177 (79.7)	40 (66.7)	
6 months of CAPOX	65 (23.0)	45 (20.3)	20 (33.3)	
Obstruction, No. (%)				.35
Yes	65 (11.7)	55 (12.4)	10 (9.2)	
No	489 (88.3)	390 (87.6)	99 (90.8)	
Perforation, No. (%)				.27
Yes	29 (5.2)	21 (4.7)	8 (7.3)	
No	525 (94.8)	424 (95.3)	101 (92.7)	
Colon, No. (%)				.34
Left	278 (56.9)	222 (55.9)	56 (60.9)	
Right	209 (42.7)	174 (43.8)	35 (38.0)	
Both	2 (0.4)	1 (0.3)	1 (1.1)	
Missing	65	48	17	
Histologic grade, No. (%)				.78
Well or moderately diff	507 (94.4)	412 (94.3)	95 (95.0)	
Slightly or not diff	30 (5.6)	25 (5.7)	5 (5.0)	
Missing	17	8	9	
Immunoscore, No. (%)				.97
Low	168 (39.9)	137 (39.9)	31 (39.7)	
Intermediate/high	253 (60.1)	206 (60.1)	47 (60.3)	
Missing	133	102	31	
No. (%)				.22
Low	168 (39.9)	137 (39.9)	31 (39.7)	
Intermediate	203 (48.2)	161 (46.9)	42 (53.9)	
High	50 (11.9)	45 (13.1)	5 (6.4)	
Missing	133	102	31	

(continued on following page)

TABLE 1. Baseline Characteristics in ctDNA+ and ctDNA- Patients (continued)

Characteristic	Pooled Population (N = 554)	ctDNA- (n = 445)	ctDNA+ (n = 109)	<i>P</i> ^a
CEA, No. (%)				<.01
<5	262 (95.3)	212 (97.3)	50 (87.7)	
≥5	13 (4.7)	6 (2.7)	7 (12.3)	
Missing	279	227	52	
VELIPI, No. (%)				<.01
Yes	295 (58.4)	219 (54.3)	76 (74.5)	
No	210 (41.6)	184 (45.7)	26 (25.5)	
Missing	49	42	7	

NOTE. Bold entries indicate a significant $P < .1$.

Abbreviations: CAPOX, capecitabine plus oxaliplatin; CEA, carcinoembryonic antigen; ctDNA, circulating tumor DNA; ECOG PS, Eastern Cooperative Oncology Group performance status; FOLFOX, infusional fluorouracil, leucovorin, and oxaliplatin; SD, standard deviation; VELIPI, venous embolism/lymphatic invasion/perineural invasion.

^a*P* value comparing population with ctDNA- versus population with ctDNA+.

When combining ctDNA status, risk group, and chemotherapy duration, patients with positive ctDNA belonging to the low-risk group and treated with 3 months of chemotherapy had similar prognosis when compared with those with ctDNA-positive status belonging to the high-risk group treated with 3 or 6 months of chemotherapy with a 2-year survival rate without recurrence of 31.6% (95% CI, 12.9 to 52.2), 33.3% (95% CI, 17.5 to 50.0), and 40.6% (95% CI, 23.8 to 56.8), respectively. In addition, ctDNA-positive low-risk patients treated with 6 months of adjuvant chemotherapy experienced a 2-year survival rate without recurrence of 66.7% (95% CI, 45.7 to 81.1; Appendix Fig A4).

Finally, as shown in Figure 1, 421 patients had available data for both ctDNA and IS. There was no correlation between ctDNA and IS, with similar percentages of IS-high/IS-intermediate/IS-low in ctDNA-positive and ctDNA-negative patients (Table 1). Among ctDNA-negative patients in this overlapping cohort, IS-high remained prognostic for TTR compared with IS-intermediate and IS-low subgroups (Fig 3C). Two-year rates of patients without recurrence in ctDNA-negative patients were 86.0% (95% CI, 78.9 to 90.8), 89.9% (95% CI, 84.0 to 93.7), and 100% (95% CI, 100.0 to 100.0) for IS-low, IS-intermediate, and IS-high groups, respectively, but with a difference statistically significant only between IS-high and IS-low (HR, 3.67 [95% CI, 1.12 to 12.01]; $P = .02$).

In univariable analysis, ctDNA status, clinical risk group (low v high), IS (low v intermediate v high), plasma CEA levels, VELIPI status, and treatment duration were significantly associated with TTR (Table 2). ctDNA status, age (>70 or <70), clinical risk group (low v high), plasma CEA levels, and VELIPI status were significantly associated with OS (Table 2). For OS, a trend was also observed for IS, tumor perforation, and treatment duration without reaching significance.

In multivariable analysis, ctDNA status using the personalized, tumor-informed assay remained an independent prognostic marker for both TTR (adjusted HR [adjHR], 5.21 [95% CI, 3.59

to 7.58]; $P < .001$) and OS (adjHR, 4.84 [95% CI, 3.40 to 6.89]; $P < .001$), and had the strongest independent association with both outcomes. Other prognostic factors for TTR were risk groups and treatment duration, IS being nonsignificant (HR, 2.12 [95% CI, 0.92 to 4.93]; $P = .079$). Other prognostic factors for OS were age and risk group (Table 2).

Similar results were found for all these analyses when excluding the 71 Greek patients with blood-based WES analysis (data not shown).

DISCUSSION

Both ctDNA and IS have been largely investigated in the past decade to improve prognostication in nonmetastatic CC.^{6-12,20} Although extensive literature on ctDNA has emerged in the past few years, additional studies with longer follow-up are needed to reinforce the utility of these tests. Indeed, a minimal follow-up of 5-6 years remains necessary to have a full picture of disease recurrences and deaths in a CC population after surgical resection of the primary tumor.^{2,21} In addition, ctDNA and IS have been very rarely analyzed together in the same patient population to determine the respective prognostic value of each test²² and never in stage III disease patients, to our knowledge. Herein, for the first time to our knowledge, this study provides results combining both tests in a large population of patients resected from a stage III CC with a median follow-up of more than 6 years.

Our findings suggest that personalized, tumor-informed ctDNA testing was strongly associated with patient outcomes such as TTR and OS. In multivariable analyses, ctDNA remained the strongest prognostic factor associated with both TTR and OS, outperforming disease stage and other prognostic factors, including IS.

We previously reported ctDNA results for 1,010 patients enrolled in the IDEA-France trial, using a tumor-agnostic

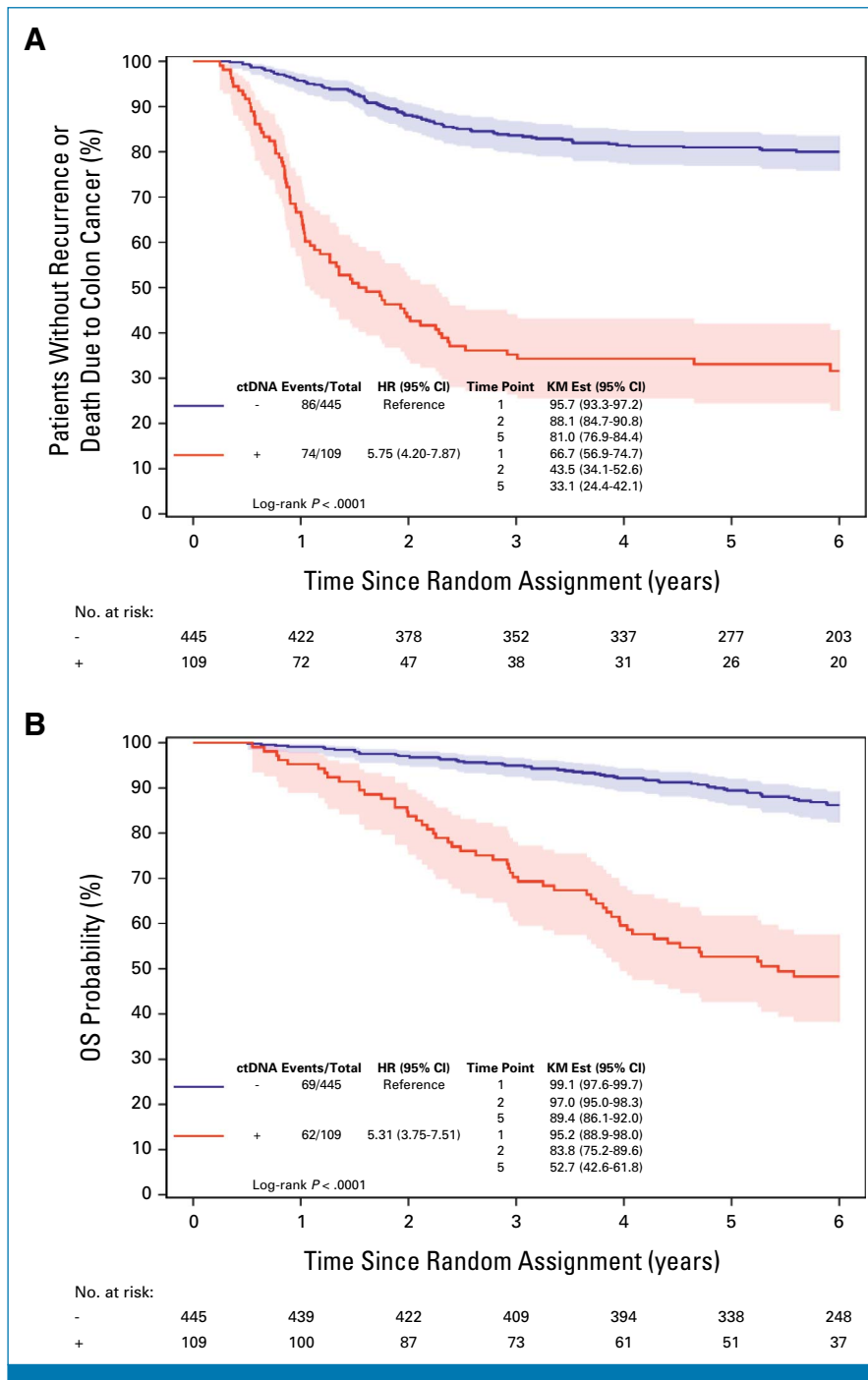


FIG 2. Kaplan-Meier estimate of (A) TTR in ctDNA-positive and ctDNA-negative patients and (B) OS in ctDNA-positive and ctDNA-negative patients. ctDNA, circulating tumor DNA; HR, hazard ratio; KM Est, Kaplan-Meier estimates; OS, overall survival; TTR, time to recurrence.

methylation test showing the independent prognostic value of this test on TTR and OS.²³ However, the results obtained in the present work suggest that using a personalized, tumor-informed approach leads to better prognostication capabilities than the previous academic tumor-agnostic methylation test. Recently, the COBRA NRG005 trial, dedicated to stage II CC after surgery, has been stopped because of an unexpected false-positive rate, using a

tumor-agnostic test (Guardant LUNAR-01), underlining the possibility that current tumor-agnostic tests may be suboptimal in identifying MRD after surgical resection in patients with CC, although these agnostic tests may bring more relevant information in the metastatic setting.²⁴ In this analysis of IDEA-France samples, we hypothesized whether 6 months of therapy would be beneficial over 3 months in ctDNA-positive patients only. However, because

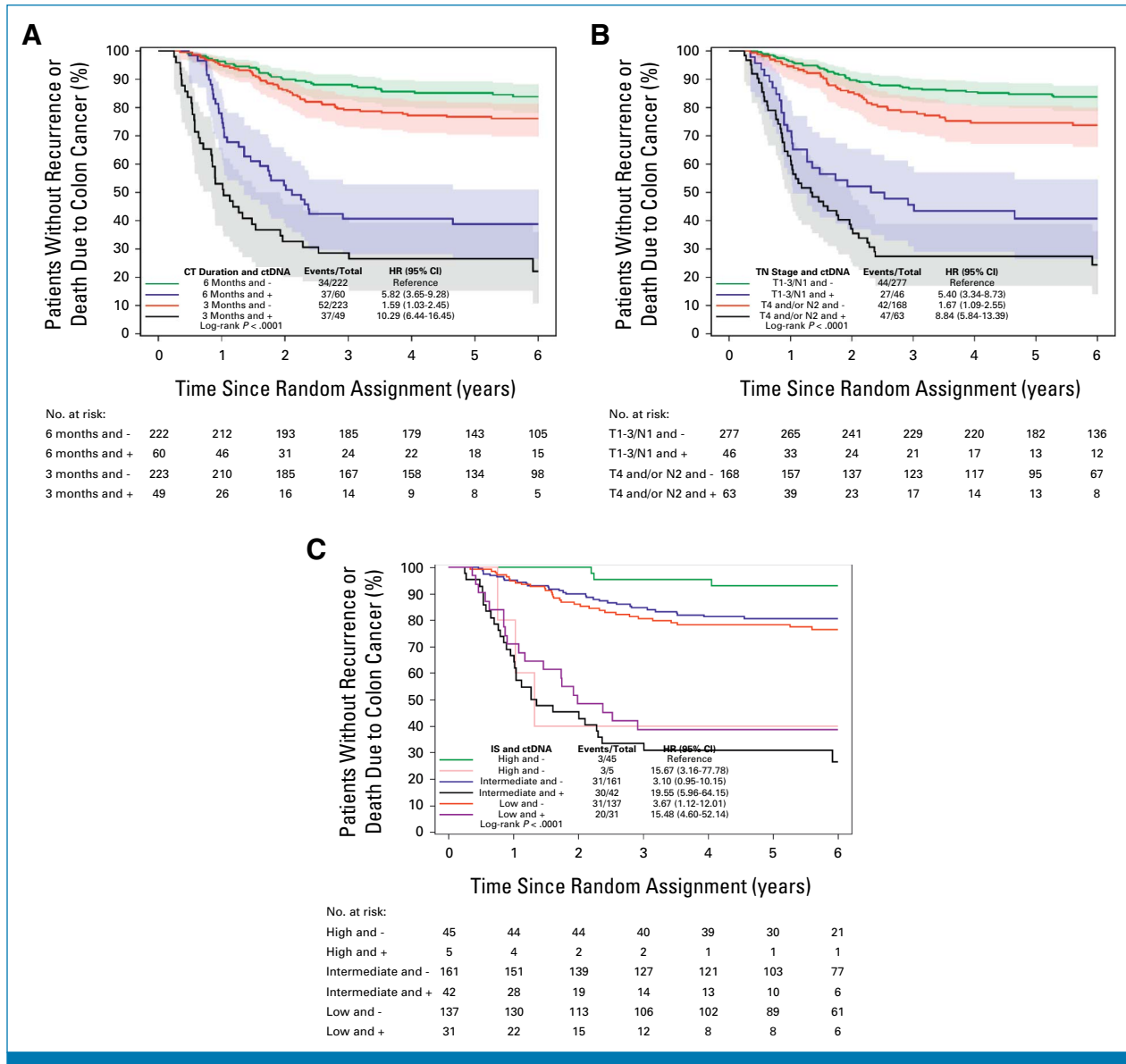


FIG 3. Kaplan-Meier estimate of TTR according to (A) ctDNA status and treatment duration (3 v 6 months; A) and (B) risk group (T1-3/N1 v T4 and/or N2; B), and (C) Immunoscore classification (low v intermediate v high; C). CT, computed tomography; ctDNA, circulating tumor DNA; HR, hazard ratio; IS, Immunoscore; TN, tumor and node; TTR, time to recurrence.

of an inherent bias of 6 months of therapy being beneficial over 3 months (HR, 0.72; $P = .038$; Appendix Fig A2) irrespective of the ctDNA status in the subset of the ctDNA evaluable cohort ($n = 554$), we were not able to confirm this interaction. However, the worse prognostic groups included ctDNA-positive patients who were treated only for 3 months, with 2-year TTR rates of 31.6% and 33.3%, for high-risk and low-risk stage III patients, respectively. Interestingly, current guidelines recommend to treat low-risk stage III patients with 3 months of adjuvant treatment. But we observed that, in ctDNA-positive low-risk patients, 6 months of treatment was associated with a 2-year survival without recurrence rate of 66.7% compared with

31.6% in those treated for 3 months as shown in Appendix Figure A4. This result may suggest that a longer treatment duration may be beneficial in ctDNA-positive low-risk patients, although it should be interpreted cautiously owing to the limited number of patients in these subgroups.

In this study, we observe that 19% of patients who were ctDNA-negative experienced disease recurrence and/or death due to their CC within 5 years of surgery, which is higher than reported in the GALAXY prospective observational cohort of CIRCULATE-Japan (7.5%).²⁵ This highlights that, although ctDNA is an excellent prognostic marker, its negativity is still associated with a significant number of

TABLE 2. TTR and OS According to Clinicopathologic Variables and Postsurgical ctDNA Status

End Point	Univariable Analysis				Multivariable Analysis			
	No.	Event	HR (95% CI)	P	No.	Event	HR (95% CI)	P
TTR								
ctDNA status	554	160			421	118		
ctDNA–			1				1	
ctDNA+			5.75 (4.20 to 7.87)	<.001			5.21 (3.59 to 7.58)	<.001
IS	421	118			421	118		
ISCC high			1				1	
ISCC intermediate			2.81 (1.21 to 6.49)	.016			2.12 (0.92 to 4.93)	.079
ISCC low			2.77 (1.19 to 6.47)	.018			2.00 (0.85 to 4.69)	.110
Sex	554	160						
Female			1					
Male			1.16 (0.84 to 1.60)	.4				
Age > 70 years old	554	160						
No			1					
Yes			1.01 (0.72 to 1.43)	>.9				
Performance status	505	141						
0			1					
1-2			0.85 (0.56 to 1.31)	.5				
Obstruction	554	160						
No			1					
Yes			1.04 (0.65 to 1.67)	.9				
Perforation	554	160						
No			1					
Yes			1.30 (0.69 to 2.48)	.4				
Sidedness	489	138						
Distal			1					
Distal and proximal			2.62 (0.36 to 18.9)	.3				
Proximal			1.00 (0.71 to 1.40)	>.9				
Risk on the basis of T and N	554	160			421	118		
pT1-T3 and pN1			1				1	
pT4 and/or pN2			2.00 (1.47 to 2.74)	<.001			1.94 (1.33 to 2.84)	<.001
Grade	537	152						
G1-2			1					
G3-4			0.66 (0.29 to 1.50)	.3				

(continued on following page)

TABLE 2. TTR and OS According to Clinicopathologic Variables and Postsurgical ctDNA Status (continued)

End Point	Univariable Analysis				Multivariable Analysis			
	No.	Event	HR (95% CI)	<i>P</i>	No.	Event	HR (95% CI)	<i>P</i>
Chemotherapy duration	554	160			421	118		
3 months			1				1	
6 months			0.72 (0.53 to 0.98)	.038			0.60 (0.41 to 0.86)	.006
CEA	275	83						
<5			1					
≥5			3.39 (1.63 to 7.04)	.001				
VELIPI	505	148						
No			1					
Yes			2.62 (1.80 to 3.81)	<.001				
OS								
ctDNA status	554	131			554	131		
ctDNA-			1				1	
ctDNA+			5.31 (3.75 to 7.52)	<.001			4.84 (3.40 to 6.89)	<.001
IS	421	93						
ISCC high			1					
ISCC intermediate			1.96 (0.84 to 4.60)	.12				
ISCC low			1.97 (0.83 to 4.64)	.12				
Sex	554	131						
Female			1					
Male			0.98 (0.69 to 1.39)	>.9				
Age >70 years	554	131			554	131		
No			1				1	
Yes			1.47 (1.02 to 2.11)	.037			1.61 (1.12 to 2.32)	.010
ECOG performance status	505	114						
0			1					
1-2			1.23 (0.79 to 1.90)	.4				
Obstruction	554	131						
No			1					
Yes			0.83 (0.47 to 1.48)	.5				
Perforation	554	131						
No			1					
Yes			1.75 (0.94 to 3.25)	.075				

(continued on following page)

TABLE 2. TTR and OS According to Clinicopathologic Variables and Postsurgical ctDNA Status (continued)

End Point	Univariable Analysis				Multivariable Analysis			
	No.	Event	HR (95% CI)	<i>P</i>	No.	Event	HR (95% CI)	<i>P</i>
Sideness	489	112						
Distal			1					
Distal and proximal			3.55 (0.49 to 25.7)	.2				
Proximal			1.35 (0.93 to 1.96)	.12				
Risk on the basis of T and N	554	131			554	131		
pT1-T3 and pN1			1				1	
pT4 or pN2			2.22 (1.57 to 3.15)	<.001			1.85 (1.30 to 2.63)	<.001
Grade	537	125						
G1-2			1					
G3-4			0.55 (0.20 to 1.49)	.2				
Chemotherapy duration	554	131						
3 months			1					
6 months			0.75 (0.53 to 1.07)	.11				
CEA	275	74						
<5			1					
≥5			2.48 (1.07 to 5.73)	.03				
VELIPI	505	124						
No			1					
Yes			2.09 (1.41 to 3.08)	<.001				

NOTE. Risk groups: high-risk, T4 and/or N2 stage; low-risk, T1-3 and N1; grade 1-2, well and moderately differentiated; grade 3-4, poorly or undifferentiated. Bold entries indicate a significant result with *P* < .05.

Abbreviations: CEA, carcinoembryonic antigen; ctDNA, circulating tumor DNA; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; IS, Immunoscore; ISCC, immunoscore colon cancer; OS, overall survival; TTR, time to recurrence; VELIPI, venous embolism/lymphatic invasion/perineural invasion.

events and cannot completely predict cure of the disease. This result also highlights the need for long follow-up to accurately assess the performances of a ctDNA test. Moreover, other classical prognosticators such as T and N stages remain valuable in addition to ctDNA. As shown in [Figure 3B](#), patients with a T4 and/or N2 tumor had 5-year TTR rates of around 10% lower than those with T1-3 and N1 tumors, irrespective of ctDNA result. This is particularly important for ctDNA-negative patients with high-risk tumors. In fact, the 5-year recurrence rate around 25% in these patients cannot be considered sufficiently satisfactory for oxaliplatin-based chemotherapy to remain an unquestionable standard of adjuvant treatment in this indication. There is therefore plenty of scope for further therapeutic research in this area.

We previously reported IS results for 1,300 patients enrolled in the IDEA-France trial, showing the independent prognostic value of this score on oncologic outcomes.⁹ Indeed, IS-low and IS-intermediate identified patients at higher risk of relapse or death compared with IS-high (HR, 2.42 and 1.69, respectively; $P = .0001$). In this study, a low or intermediate IS was also associated with a higher risk of relapse or death from CC (HR, 2.77 and 2.81; $P < .05$ in both cases) without reaching significance in the multivariable analysis (HR, 2.12 and 2.0; $P = .08$ and $.11$) after adjustment for other prognostic factors, including ctDNA. Number attrition, with only 421 patients for whom both ctDNA and IS were available, may be responsible for not reaching statistical significance. However, in the 80% of patients who are ctDNA-negative, IS appears to provide additional clinically relevant prognostic value with 5-year TTR rates of 93.0% (95% CI, 79.8 to 97.7) in IS-high patients compared with 80.6% (95% CI, 73.4 to 86.0) and 78.3% (95% CI, 70.3 to 84.4) in IS-intermediate and IS-low patients, respectively. A recent paper tested IS and ctDNA (using a tumor informed test) in patients with stage II CC after surgery and showed among the 49 patients

with postoperative ctDNA data that IS-high patients had the lowest ctDNA positivity rate, suggesting that they were most eligible for chemotherapy-free treatment.²² In the current large stage III CC patient cohort, we were not able to confirm this association between ctDNA positivity and IS-high. However, we agree with the authors' conclusion that IS and ctDNA may jointly optimize adjuvant treatment strategies for early-stage CRC in the future. Overall, results seen within the Greek and French cohorts were very similar and in line with previously reported outcomes using the same test in the same patient population (adjHR >5 for TTR).^{6,7,10-12,25}

Our study strengths are large sample size, from two randomized, phase III prospective trials with long follow-up and well-annotated clinical and pathologic variables.

Our study, however, has several limitations, mainly attrition of many participants and suboptimal preanalytical conditions, as ctDNA was not an option when the IDEA trials were designed. Second, there was a deviation in the protocol wherein a subset of patients ($n = 71$) were analyzed using a WES-based ctDNA assay where plasma WES was used as a matched normal source (nonvalidated approach).

In conclusion, we show in this translational work, derived from two randomized phase III trials with long follow-up, that ctDNA assessed with a clinically validated, personalized, tumor-informed assay is the most powerful independent prognostic marker after resection of stage III CC. However, ctDNA primarily measures the presence of viable tumor cells but does not account for essential information regarding the tumor microenvironment and can be improved by combined analyses with the IS that provides additional prognostic information in ctDNA-negative patients, who represented approximately 80% of our study cohort.

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SUPPORT

Supported by French INCa and Ministry of Health (PHRC 2009: ref AOM09 202) and Fondation ARC pour la recherche sur le Cancer. HaliDx provided Immunoscore tests and logistical support. NATERA provided Singatera tests and logistical support. The IDEA-France clinical trial was supported by the French INCA (ref 2012-218), French Ministry of Health (PHRC 2009: ref AOM09 202); the study sponsor was GERCOR.

CLINICAL TRIAL INFORMATION

[NCT00958737](https://clinicaltrials.gov/ct2/show/study/NCT00958737) and [NCT01308066](https://clinicaltrials.gov/ct2/show/study/NCT01308066)

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at DOI <https://doi.org/10.1200/JCO.24.00648>.

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DATA SHARING STATEMENT

A data sharing statement provided by the authors is available with this article at DOI <https://doi.org/10.1200/JCO.24.00648>. This is a post hoc analysis from a previously published study.¹⁵

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ACKNOWLEDGMENT

The authors thank Natera, Inc and HaliDx for having supported this study by performing the tests and the GERCOR cooperative group for the continuous support.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Combined Analyses of Circulating Tumor DNA and Immunoscore in Patients With Stage III Colon Cancer: A Post Hoc Analysis of the PRODIGE-GERCOR IDEA-France/HORG-IDEA-Greece Trials

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/jco/authors/author-center.

Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians ([Open Payments](#)).

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No other potential conflicts of interest were reported.

APPENDIX

TABLE A1. Baseline Characteristics in Patients With and Without ctDNA Available

Characteristic	Pooled Population (N = 2,717)	ctDNA Available (n = 554)	ctDNA Not Available (n = 2,163)	P ^a
Age, years				.71
Mean (SD)	63.9 (9.5)	63.9 (9.4)	63.9 (9.6)	
Median (Q1-Q3)	65.1 (58.1-71.2)	65.0 (58.0-71.0)	65.1 (58.1-71.3)	
Age, years, No. (%)				.42
≤70	1,904 (70.1)	396 (71.5)	1,508 (69.7)	
>70	813 (29.9)	158 (28.5)	655 (30.3)	
Sex, No. (%)				.45
Male	1,541 (56.7)	322 (58.1)	1,219 (56.4)	
Female	1,176 (43.3)	232 (41.9)	944 (43.6)	
ECOG PS, No. (%)				.04
0	1,979 (75.5)	399 (79.0)	1,580 (74.7)	
1-2	642 (24.5)	106 (21.0)	536 (25.3)	
Missing	96	49	47	
Tumor and node stage, No. (%)				.11
T1-3 and N1	1,664 (61.2)	323 (58.3)	1,341 (62.0)	
T4 and/or N2	1,053 (38.8)	231 (41.7)	822 (38.0)	
Chemotherapy duration, No. (%)				.71
3 months	1,353 (49.8)	272 (49.1)	1,081 (50.0)	
3 months of FOLFOX	1,043 (77.1)	217 (79.8)	826 (76.4)	
3 months of CAPOX	310 (22.9)	55 (20.2)	255 (23.6)	
6 months	1,364 (50.2)	282 (50.9)	1,082 (50.0)	
6 months of FOLFOX	1,061 (77.8)	217 (77.0)	844 (78.0)	
6 months of CAPOX	303 (22.2)	65 (23.0)	238 (22.0)	
Obstruction, No. (%)				.82
Yes	326 (12.0)	65 (11.7)	261 (12.1)	
No	2,389 (88.0)	489 (88.3)	1,900 (87.9)	
Missing	2	0	2	
Perforation, No. (%)				.71
Yes	134 (4.9)	29 (5.2)	105 (4.9)	
No	2,582 (95.1)	525 (94.8)	2,057 (95.1)	
Missing	1	0	1	
Colon, No. (%)				.36
Left	1,495 (59.1)	278 (56.9)	1,217 (59.6)	
Right	1,019 (40.3)	209 (42.7)	810 (39.7)	
Both	17 (0.7)	2 (0.4)	15 (0.7)	
Missing	186	65	121	
Histologic grade, No. (%)				.35
Well or moderately diff	2,459 (93.5)	507 (94.4)	1,952 (93.3)	
Slightly or not diff	170 (6.5)	30 (5.6)	140 (6.7)	
Missing	88	17	71	
Immunoscore, No. (%)				.81
Low	600 (40.4)	168 (39.9)	432 (40.6)	
Intermediate/high	885 (59.6)	253 (60.1)	632 (59.4)	
Missing	1,232	133	1,099	

(continued on following page)

TABLE A1. Baseline Characteristics in Patients With and Without ctDNA Available (continued)

Characteristic	Pooled Population (N = 2,717)	ctDNA Available (n = 554)	ctDNA Not Available (n = 2,163)	<i>P</i> ^a
CEA, No. (%)				.13
<5	1,354 (96.7)	262 (95.3)	1,092 (97.1)	
≥5	46 (3.3)	13 (4.7)	33 (2.9)	
Missing	1,317	279	1,038	
VELIPI, No. (%)				<.01
Yes	1,295 (52.5)	295 (58.4)	1,000 (50.9)	
No	1,173 (47.5)	210 (41.6)	963 (49.1)	
Missing	249	49	200	

NOTE. Bold entries indicate a significant result with $P < .05$.

Abbreviations: CAPOX, capecitabine plus oxaliplatin; CEA, carcinoembryonic antigen; ctDNA, circulating tumor DNA; ECOG PS, Eastern Cooperative Oncology Group performance status; FOLFOX, infusional fluorouracil, leucovorin, and oxaliplatin; SD, standard deviation; VELIPI, venous embolism/lymphatic invasion/perineural invasion.

^a*P* value comparing population with ctDNA available versus population without ctDNA available.

TABLE A2. Baseline Characteristics of French and Greek Patients Included in the ctDNA Analysis

Characteristic	Pooled Population (N = 554)	France Population (n = 422)	Greece Population (n = 132)	P ^a
Age, years				.85
Mean (SD)	63.9 (9.4)	64.0 (9.3)	63.6 (9.8)	
Median (Q1-Q3)	65.0 (58.0-71.0)	64.8 (58.1-70.6)	67.0 (58.0-72.0)	
Age, years, No. (%)				.34
≤70	396 (71.5)	306 (72.5)	90 (68.2)	
>70	158 (28.5)	116 (27.5)	42 (31.8)	
Sex, No. (%)				.96
Male	322 (58.1)	245 (58.1)	77 (58.3)	
Female	232 (41.9)	177 (41.9)	55 (41.7)	
ECOG PS, No. (%)				.19
0	399 (79.0)	329 (78.0)	70 (84.3)	
1-2	106 (21.0)	93 (22.0)	13 (15.7)	
Missing	49	0	49	
Tumor and node stage, No. (%)				.69
T1-3 and N1	323 (58.3)	248 (58.8)	75 (56.8)	
T4 and/or N2	231 (41.7)	174 (41.2)	57 (43.2)	
Chemotherapy duration, No. (%)				.52
3 months	272 (49.1)	204 (48.3)	68 (51.5)	
3 months of FOLFOX	217 (79.8)	183 (89.7)	34 (50.0)	
3 months of CAPOX	55 (20.2)	21 (10.3)	34 (50.0)	
6 months	282 (50.9)	218 (51.7)	64 (48.5)	
6 months of FOLFOX	217 (77.0)	193 (88.5)	24 (37.5)	
6 months of CAPOX	65 (23.0)	25 (11.5)	40 (62.5)	
Obstruction, No. (%)				<.01
Yes	65 (11.7)	60 (14.2)	5 (3.8)	
No	489 (88.3)	362 (85.8)	127 (96.2)	
Perforation, No. (%)				.97
Yes	29 (5.2)	22 (5.2)	7 (5.3)	
No	525 (94.8)	400 (94.8)	125 (94.7)	
Colon, No. (%)				.54
Left	278 (56.9)	235 (57.9)	43 (51.8)	
Right	209 (42.7)	169 (41.6)	40 (48.2)	
Both	2 (0.4)	2 (0.5)	0	
Missing	65	16	49	
Histologic grade, No. (%)				<.01
Well or moderately diff	507 (94.4)	376 (92.8)	131 (99.2)	
Slightly or not diff	30 (5.6)	29 (7.2)	1 (0.8)	
Missing	17	17	0	
Immunoscore, No. (%)				.02
Low	168 (39.9)	142 (42.9)	26 (28.9)	
Intermediate	203 (48.2)	153 (46.2)	50 (55.6)	
High	50 (11.9)	36 (10.9)	14 (15.6)	
Missing	133	91	42	
ctDNA, No. (%)				.08
ctDNA-	445 (80.3)	346 (82.0)	99 (75.0)	
ctDNA+	109 (19.7)	76 (18.0)	33 (25.0)	

(continued on following page)

TABLE A2. Baseline Characteristics of French and Greek Patients Included in the ctDNA Analysis (continued)

Characteristic	Pooled Population (N = 554)	France Population (n = 422)	Greece Population (n = 132)	P ^a
CEA, No. (%)				.48
<5	262 (95.3)	135 (94.4)	127 (96.2)	
≥5	13 (4.7)	8 (5.6)	5 (3.8)	
Missing	279	279	0	
VELIPI, No. (%)				<.01
Yes	295 (58.4)	240 (64.3)	77 (58.3)	
No	210 (41.6)	133 (35.7)	55 (41.7)	
Missing	49	49	0	

NOTE. Bold entries indicate a significant result with $P < .05$.

Abbreviations: CAPOX, capecitabine plus oxaliplatin; CEA, carcinoembryonic antigen; ctDNA, circulating tumor DNA; ECOG PS, Eastern Cooperative Oncology Group performance status; FOLFOX, infusional fluorouracil, leucovorin, and oxaliplatin; SD, standard deviation; VELIPI, venous embolism/lymphatic invasion/perineural invasion.

^aP value comparing France population versus Greek population.

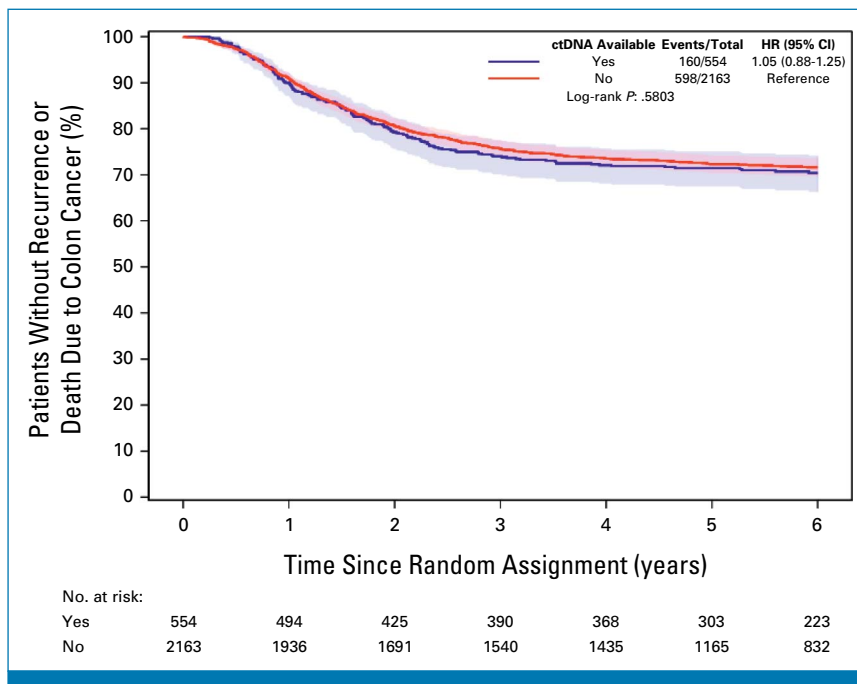


FIG A1. Kaplan-Meier estimates of TTR from random assignment according to ctDNA information available or not. ctDNA, circulating tumor DNA; HR, hazard ratio; TTR, time to recurrence.

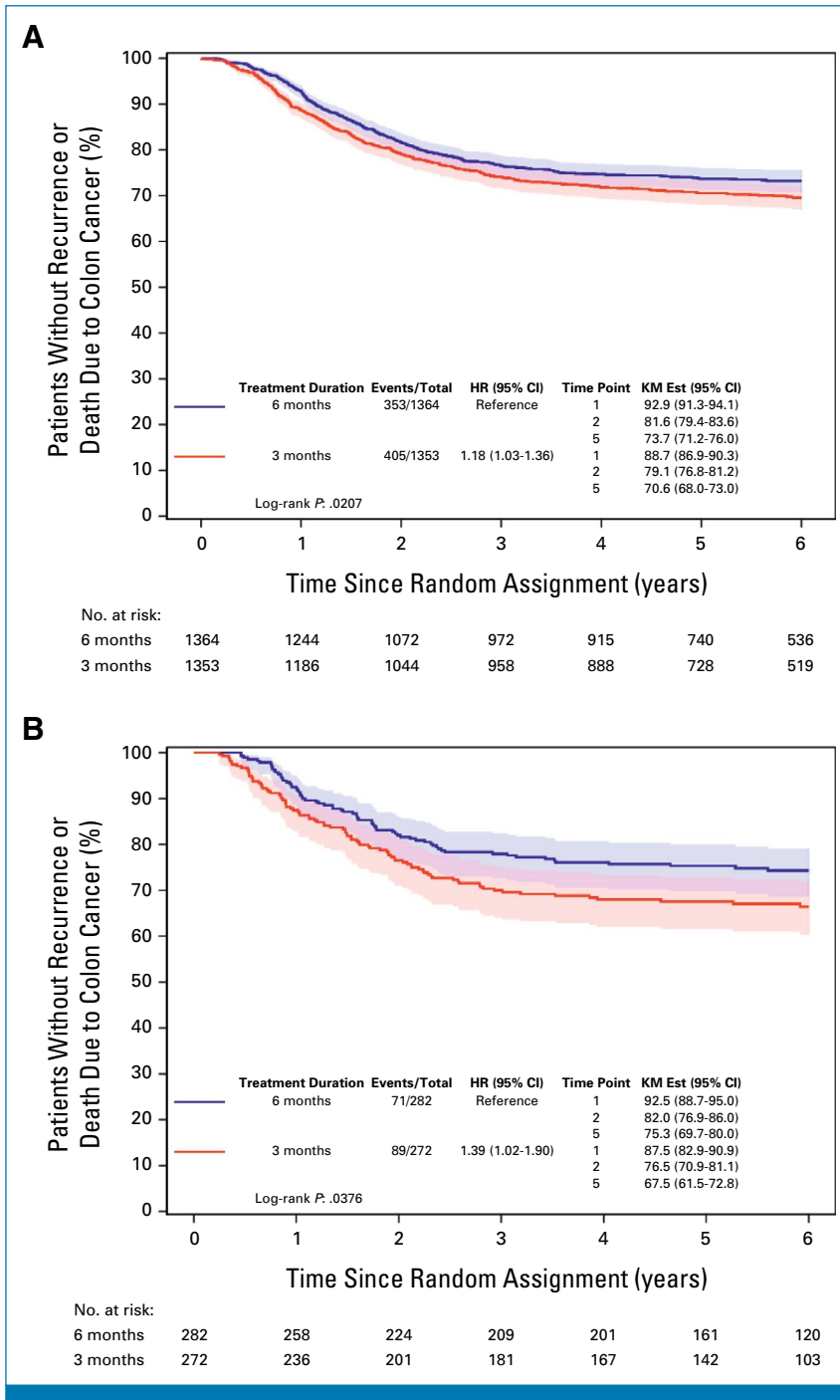


FIG A2. Kaplan-Meier estimates of TTR from random assignment according to treatment duration in (A) the mITT population (n = 2,717) and (B) patients with ctDNA available (n = 554). ctDNA, circulating tumor DNA; HR, hazard ratio; KM Est, Kaplan-Meier estimates; mITT, modified intention-to-treat population; TTR, time to recurrence.

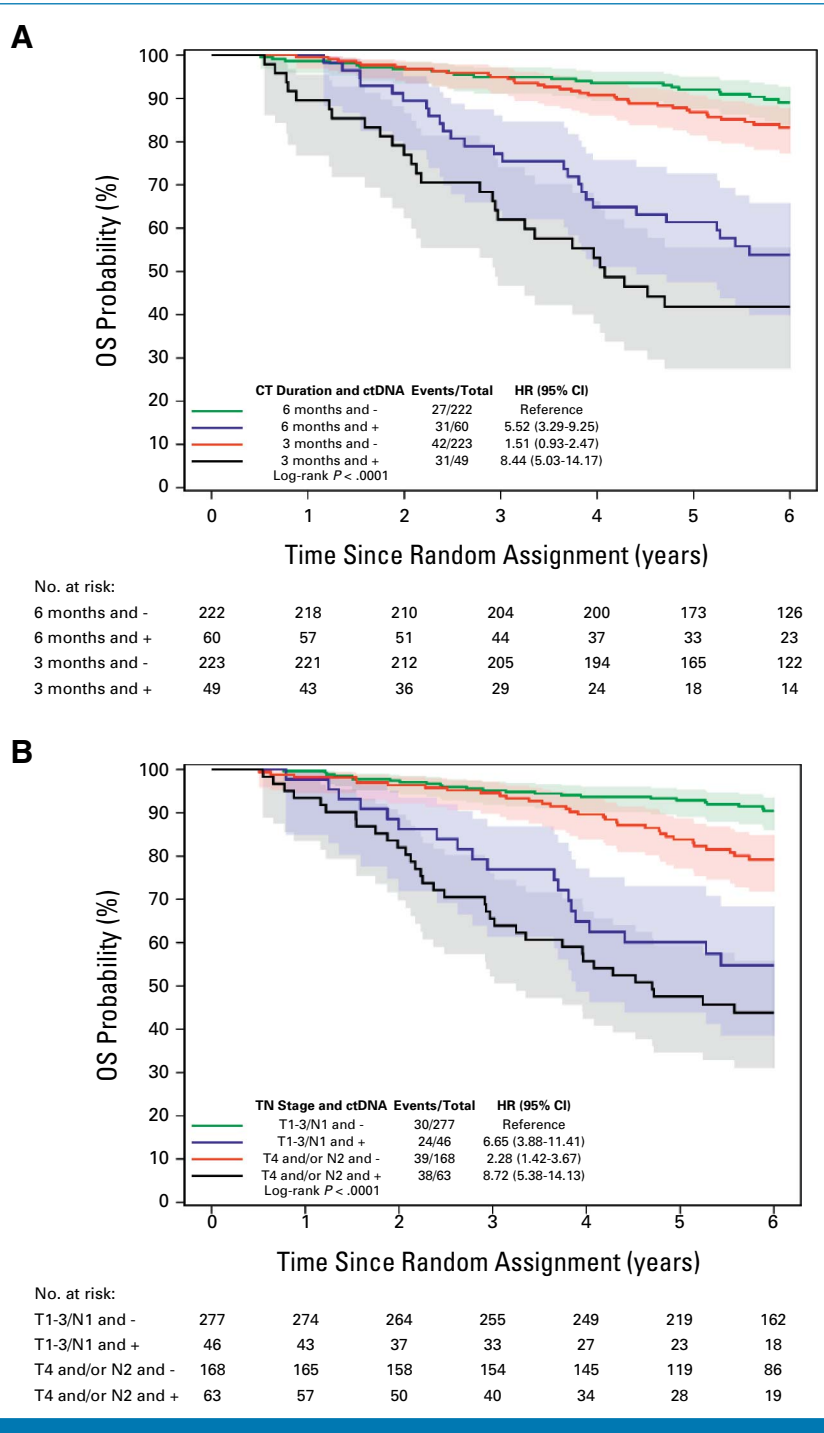


FIG A3. Kaplan-Meier estimates of OS from random assignment according to ctDNA and (A) treatment duration or (B) disease stage. CT, computed tomography; ctDNA, circulating tumor DNA; HR, hazard ratio; OS, overall survival; TN, tumor and node.

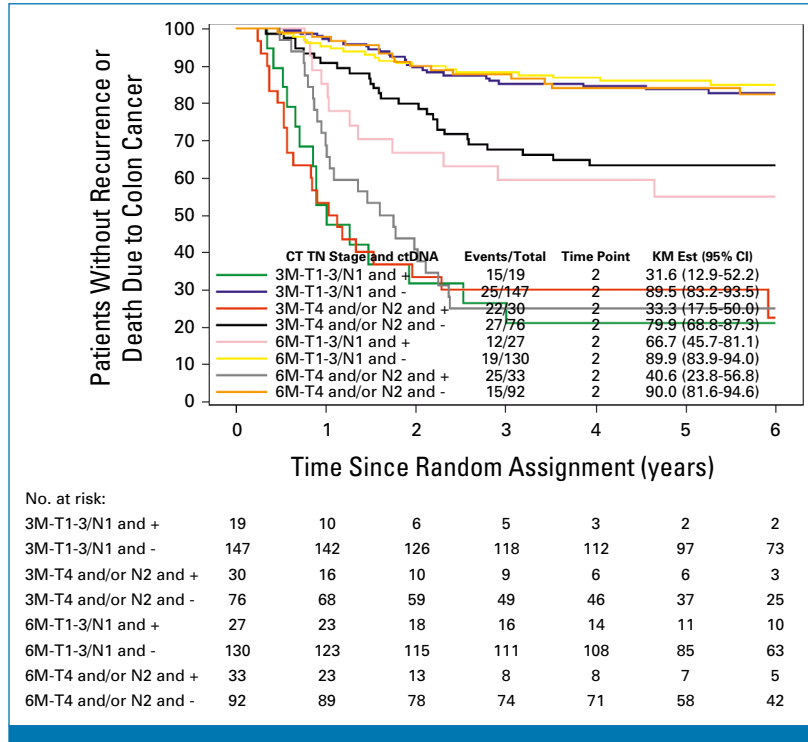


FIG A4. Kaplan-Meier estimates of TTR from random assignment according to treatment duration, disease stage, and ctDNA status. CT, computed tomography; ctDNA, circulating tumor DNA; KM Est, Kaplan-Meier estimates; TN, tumor and node; TTR, time to recurrence.