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Circulating tumor DNA-guided adjuvant therapy in locally advanced colon cancer: the randomized phase 2/3 DYNAMIC-III trial

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Adjuvant chemotherapy in stage III colon cancer provides uncertain benefit at the individual level. Circulating tumor DNA (ctDNA) may help refine risk-adjusted treatment selection. In this multicenter, randomized, phase 2/3 trial, patients with stage III colon cancer underwent ctDNA testing 5–6 weeks after surgery and were assigned (1:1) to ctDNA-guided or standard management. In the ctDNA-guided arm, patients negative for ctDNA received de-escalated therapy, whereas ctDNA-positive patients received escalated therapy. Clinicians prespecified the standard regimen. Primary endpoints were 3-year recurrence-free survival (RFS) for ctDNA-negative patients and 2-year RFS for ctDNA-positive patients. Secondary endpoints included treatment-related hospitalization and ctDNA clearance. Among 968 evaluable patients, 702 (72.5%) were ctDNA negative. With a median follow-up of 47 months, ctDNA-negative patients experienced significantly fewer recurrences than ctDNA-positive patients (3-year RFS 87% versus 49%; $P < 0.001$). In ctDNA-negative patients, de-escalation reduced oxaliplatin use (34.8% versus 88.6%) and hospitalizations (8.5% versus 13.2%) but yielded slightly lower RFS than standard management (85.3% versus 88.1%), not meeting the non-inferiority margin. In ctDNA-positive patients, higher ctDNA burden correlated with recurrence risk (3-year RFS 77% to 23% across quartiles; $P < 0.001$). Escalated therapy did not improve outcomes over standard management (2-year RFS 51% versus 61%). There was no unexpected toxicity. Persistent ctDNA after treatment predicted markedly worse prognosis (3-year RFS 14% versus 79%). ctDNA is validated as a strong prognostic classifier. ctDNA-guided de-escalation reduced oxaliplatin exposure and adverse events with outcomes approaching standard of care, whereas exploratory chemotherapy intensification conferred no RFS benefit, suggesting a need for novel strategies in ctDNA-positive disease. Australian New Zealand Clinical Trials Registry Identifier: ACTRN12617001566325.

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Oxaliplatin-based chemotherapy is standard of care for patients with stage III colon cancer[1]. In routine care, older or frailer patients may receive a fluoropyrimidine monotherapy[2,3]. For all patients with stage III disease, current clinicopathological risk stratification lacks precision in predicting an individual patient's benefit from adjuvant chemotherapy[4]. New prognostic markers could better inform treatment selection and duration. Prospective data across all stages of colorectal cancer have confirmed that ctDNA, as a direct indicator of minimal residual disease, is a powerful prognostic marker[5–10]. For stage III colon cancer specifically, an initial prospective series reported 3-year RFS of 47% and 76% for ctDNA-positive versus ctDNA-negative patients (hazard ratio =3.8; $P < 0.001$)[6].

In the DYNAMIC study enrolling stage II colon cancer, patients in the ctDNA-informed arm received adjuvant therapy only if they were ctDNA positive, resulting in less chemotherapy use without compromising RFS; among those treated, oxaliplatin-containing regimens were used more frequently under ctDNA guidance[11]. DYNAMIC also demonstrated that post-surgery ctDNA quantification provided further risk stratification[12]. Here we report the primary results of a randomized trial of ctDNA-informed versus clinician-defined standard treatment for stage III colon cancer, with treatment in the intervention arm escalated (if ctDNA positive) or de-escalated (if ctDNA negative). Secondary endpoints of treatment-related hospitalization, high-grade toxicity of special interest and ctDNA clearance, as well as prespecified exploratory analysis of ctDNA molecular burden and sites of recurrence, are also reported. Additional planned secondary endpoints not reported in this Article are overall survival and end-of-treatment ctDNA status for ctDNA-negative patients.

Results

Patient characteristics

From 18 October 2017 through 29 March 2023, 1,040 patients were enrolled and 1,002 were randomized (Fig. 1). Of 502 patients assigned to ctDNA-guided management, 482 (96%) were included in the intention-to-treat analysis (353 ctDNA negative, 129 ctDNA positive). Of 500 patients assigned to standard management, 479 (96%) were included (349 ctDNA negative, 130 ctDNA positive). ctDNA analysis was successful in 982 of 992 (99%) patients tested. The median follow-up time from randomization to database lock for analysis (29 July 2025) was 45 months for ctDNA-negative and 49 months for ctDNA-positive cohorts. The percentage of complete 3-year follow-up information for RFS was greater than 90%, with no observed differences in censoring rate at 3 years between the study arms[13].

Table 1 shows key patient characteristics. Median patient age was 61.7 years, and 99% had an Eastern Cooperative Oncology Group (ECOG) performance status score of 0 or 1. There was a higher proportion of clinical high-risk disease and extramural tumor deposits among ctDNA-positive patients in ctDNA-guided than standard management. Post hoc analysis showed that fewer ctDNA-negative patients than ctDNA-positive patients had clinical high-risk disease (33.3% versus 56.6%; relative risk: 0.58; 95% confidence interval: 0.50–0.68) or extramural tumor deposits (20.5% versus 35.3%; relative risk: 0.58; 95% confidence interval: 0.47–0.72).

Treatment delivery and adherence

Treatment delivery and adherence by ctDNA status are shown in Table 2. In the ctDNA-guided arm, 90.4% of ctDNA-negative patients and 89.1% of ctDNA-positive patients received per-protocol de-escalation or escalation, respectively (details in Extended Data Table 1). Among ctDNA-negative patients, oxaliplatin-based doublet chemotherapy was administered less frequently (34.8% versus 88.6%; relative risk: 0.41; 95% confidence interval: 0.35–0.47; $P < 0.001$), and treatment duration was shorter (mean 101 days (s.d. 43.4) versus 118 days (s.d. 48.5); $P < 0.001$) with ctDNA guidance. Among ctDNA-positive patients in the ctDNA-guided arm, 50% received FOLFOXIRI and 43% received 6 months of oxaliplatin-based doublet. In the standard management arm, 45% and 41% received 3 months and 6 months of oxaliplatin-based doublet, respectively.

Efficacy according to management arm

At the time of analysis, 108 of 702 (15.4%) ctDNA-negative patients and 135 of 259 (52.1%) ctDNA-positive patients had recurred or died. Non-inferiority of ctDNA-guided treatment de-escalation versus standard management for ctDNA-negative patients was not confirmed for 3-year RFS (85.3% versus 88.1%; absolute difference: −2.8%; 97.5% lower confidence interval: −8.0%; Fig. 2a,b). Prespecified subgroup analysis for differences in 3-year RFS (Fig. 2b and Extended Data Table 2) suggests that non-inferiority may be observed in patients with clinical low-risk (T1–3N1) tumors, as this subgroup met the prespecified margin for non-inferiority (91.0% versus 93.2%; absolute difference: −2.2%; 97.5% lower confidence interval: −7.2; Extended Data Fig. 1). This observation is hypothesis generating only, as the test of interaction between these risk groups was not statistically significant. Three-year RFS for high-risk (T4 and/or N2) disease was 72.8% versus 78.6% (absolute difference: −5.8%; 97.5% lower confidence interval: −17.0; Extended Data Fig. 1) and, for MMR-deficient tumors, was 88.4% versus 96.4% (absolute difference: −8.1%; 97.5% lower confidence interval: −19.8; Extended Data Table 2).

For ctDNA-positive patients, RFS for ctDNA-guided escalation compared to standard management did not meet the prespecified threshold for superiority (2-year RFS 51% versus 61%; hazard ratio = 1.16; 95% confidence interval: 0.87–1.53; Fig. 2c). Analysis of the treatment effect on RFS according to key subgroups did not reveal any that potentially benefited from chemotherapy escalation (Fig. 2d). Given the baseline imbalances in high-risk features, to address potential confounding we performed sensitivity analyses in the ctDNA-positive cohort using Cox proportional hazard models adjusting separately for key covariates (age, clinical risk, sex, MMR status, N stage, tumor location, tumor deposits and T stage). Across models, the adjusted treatment effect hazard ratios ranged from 1.11 to 1.20 with 95% confidence intervals spanning 1 and P values 0.29–0.55 (Extended Data Table 3), mirroring the primary analysis.

Safety

Treatment-related hospitalization and grade 3 or 4 acute adverse events of special interest (AESIs) are shown in Table 3. Among ctDNA-negative patients, a lower rate of treatment-related hospitalization (8.5% versus 13.2%; relative risk = 0.64; 95% confidence interval: 0.42–1.00; $P = 0.047$) and high-grade AESI (6.2% versus 10.6%; relative risk = 0.59; 95% confidence interval: 0.35–0.98; $P = 0.037$) was observed with ctDNA guidance than standard management. No difference in treatment-related hospilization or high-grade AESI was observed between treatment escalation and standard management for ctDNA-positive patients.

RFS according to post-surgery ctDNA status and burden

Patients who were ctDNA negative recurred less frequently than patients who were ctDNA positive at 5–6 weeks after surgery (3-year RFS 87% versus 49%; $P < 0.001$; Extended Data Fig. 2). Among all post-surgery ctDNA-positive cases, ctDNA molecular burden was measured as the number of tumor-derived mutant molecules (TDMM) per milliliter of plasma. Recurrence risk progressively increased with ascending quartiles of ctDNA burden, with 3-year RFS rates of 77%, 60%, 37% and 23%, respectively (hazard ratio = 5.9 for TDMM highest quartile versus lowest quartile; 95% confidence interval: 3.7–9.1; $P < 0.001$; Fig. 3a). Even in patients with the lowest quartile of ctDNA positivity, 3-year RFS was worse than in ctDNA-negative patients (77% versus 87%; $P = 0.043$; Fig. 3a).

For the ctDNA-positive cohort, prespecified subgroup analyses of RFS (Fig. 2d) suggested a treatment-by-T-stage interaction

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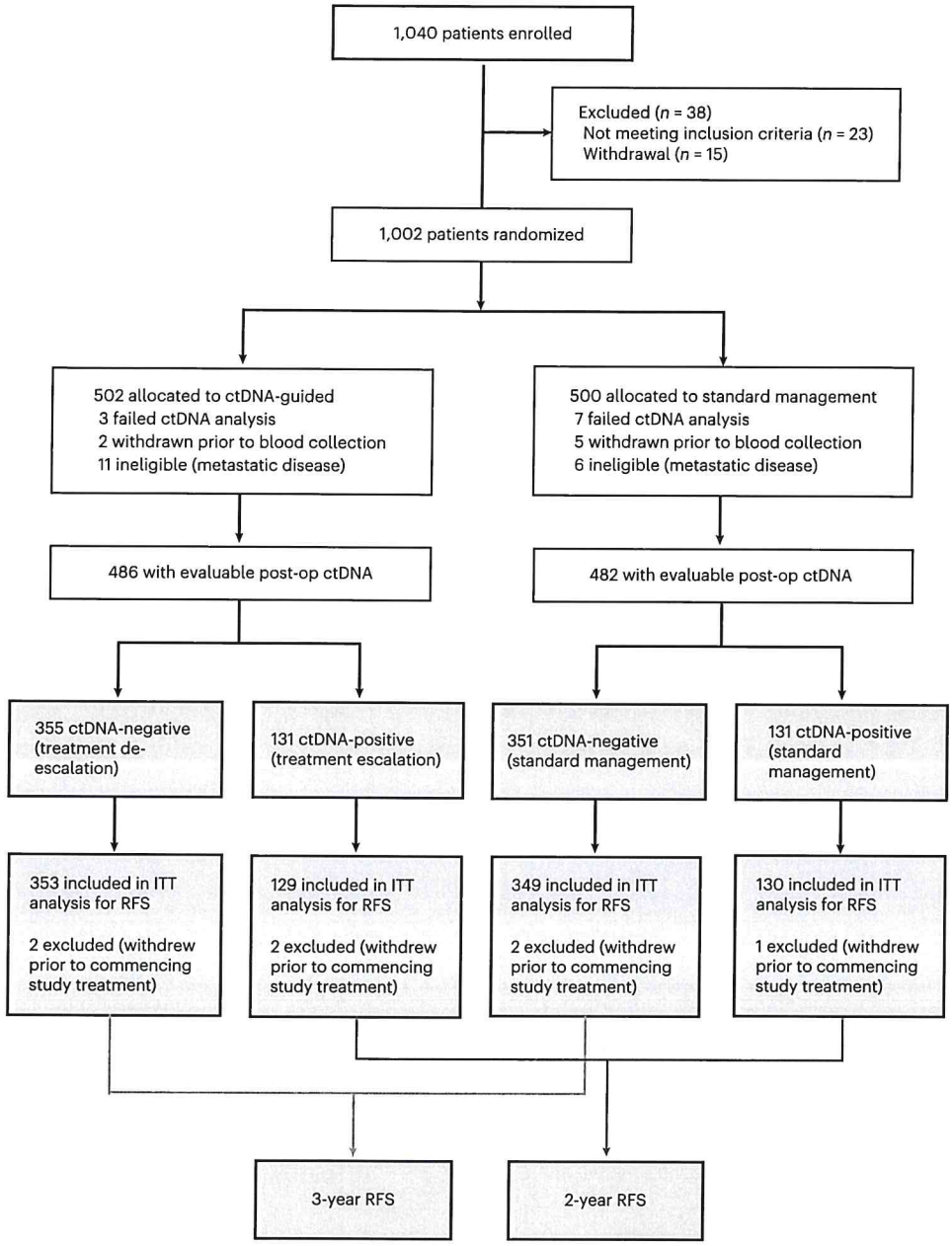

Fig. 1 | Patient enrollment, randomization and follow-up. Patients with resected stage III colon cancer were randomly assigned to ctDNA-guided management or standard management based on clinicopathologic criteria. Standard adjuvant regimens were prespecified by treating clinicians prior to randomization. In the ctDNA-guided arm, ctDNA-negative patients received de-escalated therapy, whereas ctDNA-positive patients received escalated therapy. The primary analysis compared 3-year RFS between ctDNA-negative patients in the guided and standard arms (non-inferiority) and 2-year RFS between ctDNA-positive patients in the two arms (superiority). ITT, intention-to-treat; post-op, postoperative.

($P_{interaction}$ = 0.0627), with T4 disease appearing to favor standard management. Given the strong prognostic value of ctDNA molecular burden observed in the DYNAMIC stage II study, we examined baseline ctDNA levels within the T4 subgroup and found a significant imbalance, with lower ctDNA burden in the standard management arm (median TDMM per milliliter: 0.15 versus 0.79; P = 0.025). This imbalance likely underlies the more favorable outcomes observed in T4 patients assigned to standard management. Additionally, we also observed a numerically higher molecular burden in patients treated with FOLFOXIRI than those treated with oxaliplatin-based doublet (median TDMM per milliliter: 0.28 versus 0.15; P = 0.236).

End-of-treatment ctDNA

We analyzed end-of-treatment plasma samples from 136 post-surgery ctDNA-positive patients (98 ctDNA guided, 38 standard management) with the v96 assay, where up to 96 candidate tumor-specific mutations per participant were selected for tracking based on whole-genome sequencing (WGS) of a patient's tumor tissue. The median number of variants tracked was 93 (interquartile range: 91–94). ctDNA clearance (ctDNA positive to ctDNA negative) was observed in 82 (60%) patients with similar clearance rates for FOLFOXIRI and oxaliplatin-based doublet chemotherapy (Fig. 3b). RFS for patients failing to clear their ctDNA was substantially worse than for patients whose ctDNA was cleared,

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Table 1 | Patient characteristics at baseline in the intention-to-treat population

Characteristics	ctDNA negative		ctDNA positive	
	Standard management (*N*=349)	ctDNA-guided management (*N*=353)	Standard management (*N*=130)	ctDNA-guided management (*N*=129)
Male sex – number (%)	187 (53.6)	180 (51.0)	80 (61.5)	82 (63.6)
Median age (range), years	62 (26, 84)	61 (27, 87)	61 (30, 90)	64 (30, 87)
ECOG status – number (%)				
0 or 1	345 (98.9)	352 (99.7)	128 (98.5)	126 (97.7)
2	4 (1.1)	1 (0.3)	2 (1.5)	3 (2.3)
Primary tumor site[a] – number (%)				
Right-sided colon	152 (43.6)	169 (47.9)	53 (40.8)	63 (48.9)
Left-sided colon	160 (45.8)	149 (42.2)	64 (49.2)	55 (42.6)
Rectal	37 (10.6)	35 (9.9)	13 (10.0)	11 (8.5)
Tumor stage – number (%)				
T1 or T2	76 (21.8)	70 (19.8)	18 (13.8)	18 (14.0)
T3	192 (55.0)	224 (62.5)	63 (48.5)	63 (48.8)
T4	81 (23.2)	59 (16.7)	49 (37.7)	48 (37.2)
Nodal stage – number (%)				
N1	272 (77.9)	282 (79.9)	85 (65.4)	75 (58.1)
N2	77 (22.1)	71 (20.1)	45 (34.6)	54 (41.9)
Number of lymph nodes examined, median (range)	21 (7, 80)	21 (7, 84)	20 (5, 90)	22 (9, 52)
Presence of extramural tumor deposits – number (%)	79 (22.6)	65 (18.4)	40 (30.8)	51 (39.5)
Clinical risk group – number (%)				
Low (T1–3N1)	227 (65.0)	242 (68.6)	61 (46.9)	52 (40.3)
High (T4, N2 or both)	122 (35.0)	111 (31.4)	69 (53.1)	77 (59.7)
MMR status – number (%)				
Proficient	321 (92.0)	308 (87.2)	118 (90.8)	115 (89.1)
Deficient	28 (8.0)	43 (12.2)	12 (9.2)	14 (10.9)
Missing data	0 (0)	2 (0.6)	0	0
KRAS mutation – number (%)	148 (42.4)	138 (39.1)	63 (48.5)	56 (43.4)
BRAF V600E mutation – number (%)	55 (15.8)	48 (13.6)	17 (13.1)	24 (18.6)
Median follow-up time – months	47.3	43.4	47.9	48.2

[a] A right-sided tumor was defined as arising from the caecum to the transverse colon; a left-sided tumor was defined as arising from the splenic flexure to the rectosigmoid junction.

with landmark 3-year RFS of 14% versus 79% (hazard ratio = 8.33; 95% confidence interval: 5.3–14.3; $P < 0.001$; Fig. 3c). Consistent with the worsening of RFS with increasing ctDNA burden, ctDNA clearance rate with chemotherapy also decreased with increasing quartiles of ctDNA burden (83%, 64%, 61% and 28%; $P < 0.001$; Fig. 3d).

Sites of recurrence according to post-surgery ctDNA status

Among 224 patients with recurrence, 129 (58%) were ctDNA positive and 95 (42%) were ctDNA negative after surgery. The patterns of spread differed by post-surgery ctDNA status (Extended Data Table 4). Distant recurrence was numerically more common in the ctDNA-positive group (77% versus 64%; $P = 0.052$), whereas locoregional recurrence was less frequent (39% versus 49%; $P = 0.133$). Liver involvement was markedly enriched in ctDNA-positive patients (53% versus 24%; $P < 0.001$), whereas lung (21% versus 39%; $P = 0.004$) and peritoneal disease (20% versus 34%; $P = 0.030$) were more frequent in ctDNA-negative patients. Distant-only recurrences were numerically more common with ctDNA positivity (61% versus 51%; $P = 0.133$), driven by a higher proportion with liver-only metastasis (37% versus 16%; $P = 0.001$), whereas lung-only recurrences were enriched among ctDNA-negative patients (25% versus 9%; $P = 0.001$). Mixed distant and locoregional patterns were similar between groups (16% versus 14%).

Sites of relapse according to study arm are shown in Extended Data Table 5. Curative-intent salvage surgery or ablation was undertaken in 77 of 224 (34%) recurrences overall, with similar rates across arms (standard management: ctDNA negative 12/40 (30.0%), ctDNA positive 21/62 (33.9%); ctDNA guided: ctDNA negative 22/55 (40.0%), ctDNA positive 22/67 (32.8%)).

Discussion

Current treatment for stage III colon cancer lacks personalization, with approximately 30% of patients experiencing recurrence despite standard adjuvant therapy, whereas 40–50% are cured by surgery alone[1,4,14]. The present study explored a risk-adjusted approach, informed by post-surgery ctDNA analysis. For ctDNA-negative patients, a ctDNA-informed approach resulted in less oxaliplatin use and fewer adverse events, and the non-inferiority comparison of 3-year RFS narrowly missed the prespecified margin. The signal-seeking randomized phase 2 analysis of ctDNA-positive patients showed no statistically significant reduction in recurrence risk, but results should be interpreted

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Table 2 | Treatment delivery and adherence according to study arm for the intention-to-treat population

	ctDNA negative		ctDNA positive	
	Standard management (*N* = 349)	ctDNA-guided de-escalation (*N* = 353)	Standard management (*N* = 130)	ctDNA-guided escalation (*N* = 129)
Treatment characteristic				
Planned standard treatment – number (%)				
No adjuvant treatment	7 (2.0)	3 (0.9)	2 (1.6)	3 (2.3)
Single-agent fluoropyrimidine	32 (9.2)	51 (14.4)	13 (10.0)	20 (15.5)
3 months FOLFOX/CAPOX	167 (47.8)	168 (47.6)	45 (34.6)	37 (28.7)
6 months FOLFOX/CAPOX	143 (41.0)	131 (37.1)	70 (53.8)	69 (53.5)
Commenced per protocol treatment – number (%)	347 (99.4)	319 (90.4)	126 (96.9)	115 (89.1)
Chemotherapy regimen received – number (%)				
No chemotherapy	8 (2.3)	26 (7.4)	4 (3.1)	4 (3.1)
3 months single-agent fluoropyrimidine	1 (0.3)	119 (33.7)	0 (0.0)	0 (0.0)
6 months single-agent fluoropyrimidine	31 (8.9)	85 (24.1)	14 (10.8)	3 (2.3)
3 months oxaliplatin-based doublet	166 (47.6)	117 (33.1)	59 (45.4)	1 (0.8)
6 months oxaliplatin-based doublet	143 (41.0)	6 (1.7)	53 (40.8)	56 (43.4)
≥3 months FOLFOXIRI	Not applicable	Not applicable	0 (0.0)	65 (50.4)
Time from surgery to commencing chemotherapy (days)				
Median (interquartile range)	53 (48, 59)	56 (51, 63)	53 (49, 61)	59 (52, 68)
Treatment duration (days)				
Mean (s.d.)	118 (48)	101 (43.4)	120 (53)	149 (40)
Completion of planned treatment – number (%)[a]				
Completed planned treatment cycles	282 (82.7)	294 (89.9)	89 (70.6)	95 (76.0)
Did not complete planned treatment cycles	59 (17.3)	33 (10.1)	37 (29.4)	30 (24.0)

[a] Patients who did not receive chemotherapy were excluded from this calculation.

with caution pending definitive phase 3 confirmation. The ability of post-surgery ctDNA quantification to further refine the recurrence risk of ctDNA-positive patients was confirmed in our study, as was the markedly elevated recurrence risk associated with persistent ctDNA detection at chemotherapy completion.

Overall, baseline characteristics for patients randomized to ctDNA-guided and standard management were well balanced. Clinicopathologic factors associated with elevated recurrence risk, including N2 disease, were, however, more prevalent in ctDNA-positive patients randomized to the ctDNA-guided arm. As shown in Table 2, planned treatment ranged from 6 months of oxaliplatin-based chemotherapy to no treatment, reflecting approaches pursued in routine clinical care. The dominant population, 86% of patients, was planned for an oxaliplatin-based doublet.

The dominant ctDNA-informed de-escalation strategy for ctDNA-negative patients was from an oxaliplatin-based doublet to a fluoropyrimidine alone. With reduced oxaliplatin use compared to patients receiving standard management (89% to 35%), severe adverse events and treatment-related hospitalization were reduced. Reduced oxaliplatin-related neuropathy would also be anticipated. The recurrence rate for ctDNA-negative patients in both arms was low (3-year RFS of 88.1% and 85.3%), with an absolute difference of −2.8%. Because clinical acceptability of non-inferiority margins varies, we prespecified a 7.5% margin to approximate the plausible absolute oxaliplatin benefit while balancing toxicity tradeoffs. Using the prespecified, conversative one-sided 97.5% confidence interval (two-sided 95%), the lower bound was −8.0%, narrowly crossing the −7.5% non-inferiority margin; this borderline result is best interpreted as statistical uncertainty rather than definitive evidence against non-inferiority. Because non-inferiority inference depends on the chosen margin and confidence level, a more conventional one-sided 95% confidence interval (two-sided 90%) yields a lower bound of −7.1, which would meet the −7.5% margin, whereas tighter margins (for example, 5%) are not met. Overall, the point estimate and confidence interval are compatible with, at most, a small decrement in efficacy that should be weighed against the substantial reduction in chemotherapy exposure and toxicity achieved with ctDNA-guided management. Prespecified subgroup analyses suggested that de-escalation may be non-inferior in patients with clinically low-risk disease (where 3-year RFS exceeded 90%). However, findings from subgroup analysis should be considered exploratory and require confirmation in adequately powered, prospectively stratified studies.

Among 702 post-surgery ctDNA-negative patients, 95 (13.5%) recurred, with a predominance of lung (39%) and peritoneal (34%) relapses, sites recognized to shed less ctDNA[8,15]. This pattern suggests that a proportion of 'ctDNA-negative' cases may reflect assay false negatives rather than biological cure, underscoring the need to enhance assay sensitivity for low-shedding disease. We previously showed that sensitivity increases when more tumor-specific variants are tracked[12]; additional approaches (for example, higher input, reduced cell-free DNA clearance and serial testing) may further improve detection[16]. If 'false-negative' results after surgery can be reduced, this could attenuate the small observed decrement in RFS with treatment de-escalation and potentially influence non-inferiority conclusions. These hypotheses require prospective evaluation.

Three previous randomized studies of adjuvant irinotecan in early-stage colon cancer did not demonstrate survival gains versus fluoropyrimidine alone, suggesting no activity in this setting[17–19]. Adding irinotecan to oxaliplatin-based therapy (triplet therapy) in the adjuvant setting is currently being tested in the IROCAS trial but does improve overall survival versus doublet therapy in the metastatic setting[20]. In the present study, escalation to FOLFOXIRI occurred in 50.4% of ctDNA-positive patients in the ctDNA-informed arm. Because of

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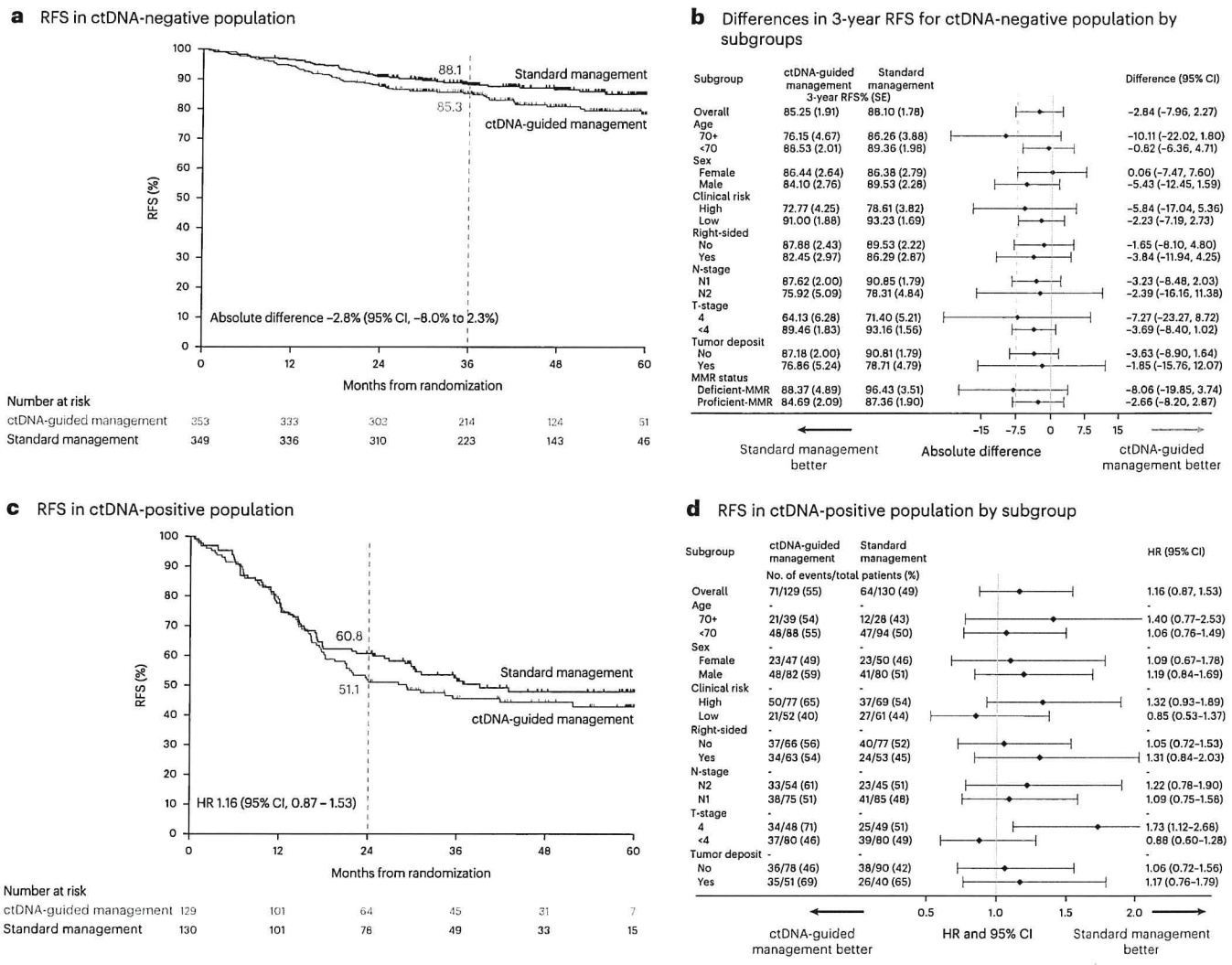

Fig. 2 | RFS with ctDNA-guided versus standard-of-care adjuvant therapy. **a**, Kaplan–Meier estimates of RFS according to the assigned management group for ctDNA-negative patients. At 3 years, 85.3% of the patients in the ctDNA-guided group and 88.1% of patients in the standard management group were alive without disease recurrence. **b**, Absolute differences in 3-year RFS with 97.5% lower confidence intervals for all ctDNA-negative patients and predefined subgroups. The dashed line indicates the −7.5% non-inferiority margin; non-inferiority was not met (lower confidence interval: −8.0%) for the overall ctDNA-negative population. **c**, Kaplan–Meier estimates of RFS for ctDNA-positive patients, with 2-year RFS of 51% in the ctDNA-guided group and 61% with standard management (hazard ratio = 1.16, above the superiority threshold of 0.746). **d**, Subgroup hazard ratios and 95% confidence intervals for ctDNA-positive patients comparing ctDNA-guided and standard management. CI, confidence interval; HR, hazard ratio; SE, standard error.

the known additional toxicity of FOLFOXIRI, the protocol mandated a 3-month minimum with clinician-directed extension to 6 months based on tolerability; in our cohort, 69% received ≥4 months and 48% completed 6 months. Outcomes were poor for all ctDNA-positive patients, 51% and 61% of ctDNA-informed and standard management patients being recurrence free at 2 years. It is unlikely that awareness of relapse risk in the ctDNA-informed arm affected RFS, as both arms followed an identical, protocol-defined surveillance schedule. Although we did not observe a statistically significant difference in hospitalization or prespecified grade 3 or higher toxicities between the escalation and standard management arms, an analysis likely underpowered due to sample size, treatment escalation would be expected to increase overall toxicity, particularly low-grade events not captured by our endpoints. The escalation comparison in ctDNA-positive patients was a randomized phase 2, signal-seeking analysis and is interpreted as exploratory. Although treatment escalation in ctDNA-positive patients did not meet our prespecified superiority threshold of hazard ratio < 0.746, the sample size is modest, lacking power to detect differences in outcome that can be achieved only in adequately powered phase 3 trials, with overrepresentation of high-risk disease in the ctDNA-informed arm. Sensitivity analyses using Cox regression with covariate adjustment including key risk factors yielded treatment effect estimates similar to the primary analysis (adjusted hazard ratios: 1.11–1.20; all 95% confidence intervals crossing 1). Accordingly, these findings should be viewed as hypothesis generating, with formal testing of escalation from doublet to triplet therapy in ctDNA-positive patients underway in ongoing phase 3 studies, such as CIRCULATE-North America (NCT05174169).

DYNAMIC-III was intentionally designed as a pragmatic randomized strategy trial, comparing ctDNA-guided de-escalation/escalation with standard management, accommodating the varied real-world prescribing pattern of adjuvant chemotherapy. In the standard management arm, chemotherapy choices were guideline-concordant standard of care declared a priori; in the ctDNA-guided arm, both de-escalation and escalation options were protocol-defined experimental strategies delivered at clinician discretion. Randomization at the strategy level and intention-to-treat analyses preserve internal validity for the

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Table 3 | Treatment-related toxicity according to study arm

	ctDNA negative			ctDNA positive		
	Standard management (*N*=349)	ctDNA-guided de-escalation (*N*=353)	*P* value	Standard management (*N*=130)	ctDNA-guided escalation (*N*=129)	*P* value
Treatment-related hospitalization – number (%)	46 (13.2)	30 (8.5)	0.047	18 (13.8)	21 (16.3)	0.584
Any grade 3 or 4 treatment-related AESI – number (%)[a]	37 (10.6)	22 (6.2)	0.037	13 (10.0)	17 (13.2)	0.444
Febrile neutropenia	5 (1.4)	3 (0.8)		3 (2.3)	3 (2.3)	
Diarrhea	30 (8.6)	19 (5.4)		10 (7.7)	14 (10.9)	
Oral mucositis	6 (1.6)	2 (0.6)		1 (0.8)	2 (1.6)	
Nausea	15 (4.3)	11 (3.1)		5 (3.8)	3 (2.3)	
Vomiting	8 (2.3)	8 (2.3)		3 (2.3)	2 (1.6)	

[a] Six patients had grade 5 events; only high-grade AESIs were collected in the study given.

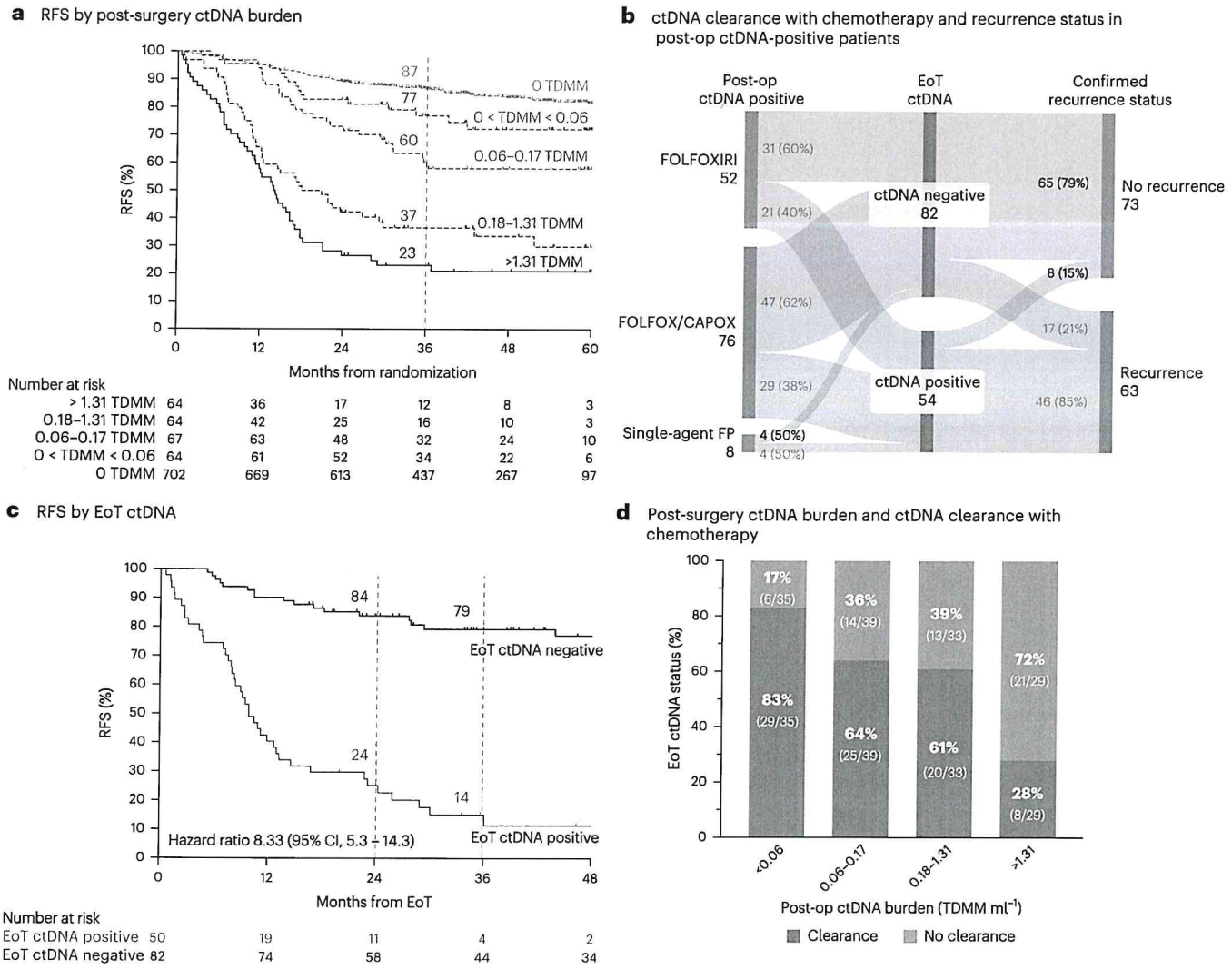

Fig. 3 | ctDNA burden, end-of-treatment ctDNA and RFS for ctDNA-positive patients. a, RFS for ctDNA-negative patients (0 TDMM) and ctDNA-positive patients stratified by quartiles of post-surgery ctDNA molecular burden (TDMM per milliliter). **b**, Sankey plot of end-of-treatment (EoT) ctDNA and confirmed recurrence status after FOLFOXIRI, oxaliplatin-based doublet or single-agent fluoropyrimidine (FP) adjuvant chemotherapy in 136 ctDNA-positive patients with available EoT samples; one EoT ctDNA-positive patient had an unconfirmed recurrence just prior to death. **c**, Kaplan–Meier estimates of RFS by EoT ctDNA status using a tumor WGS-guided assay; 3-year RFS was 14% in patients remaining ctDNA positive and 79% in those converted to ctDNA negative after chemotherapy. Four EoT-positive cases were excluded from landmark analysis due to EoT blood draw occurring after documented recurrence. **d**, ctDNA clearance rates after treatment across quartiles of ctDNA burden.

primary estimand—the effect of assignment to ctDNA-guided management under usual-care conditions—and allowance for regimen choice enhances external validity. Because the experimental regimens were not individually controlled, the trial cannot isolate the efficacy of any single regimen; our inferences pertain to the clinical utility of the ctDNA-guided strategy as implemented. Surveillance modalities and timing were identical across arms, minimizing detection bias related to risk awareness. We report regimen distributions and adherence to support interpretation and acknowledge these design features as both a strength for implementation relevance and a limitation for regimen-specific conclusions.

Beyond a binary positive versus negative ctDNA result, the quantification of detectable ctDNA further refines recurrence risk estimates, with markedly different 3-year RFS for patients in the lowest (77%) versus highest (23%) quartiles. These data suggest that quantification of post-surgery ctDNA should be considered a stratification factor for future adjuvant colorectal cancer trials. Patients with a high ctDNA burden remain a particular group in need of novel treatment strategies, with ctDNA clearance in only 28%, indicating that few are cured by current chemotherapy strategies.

The dynamic nature of ctDNA as a marker of minimal residual disease adds further potential utility beyond the initial result obtained after surgery. Analyses during adjuvant therapy, at completion of therapy and during surveillance all potentially could inform further therapy. Here we present the end-of-treatment data, which, along with data from stage II patients in the DYNAMIC study, demonstrate that very few patients (eight of 54 (15%)) with a positive end-of-treatment ctDNA remained disease free (Fig. 3b), noting that one patient had imaging findings suspicious of recurrence (unconfirmed recurrence) just prior to death. Whether they represented false positives from the assay or had low levels of residual disease that were later eradicated immunologically or by prolonged antineoplastic effects[21,22] could be elucidated using serial ctDNA analyses in the future. By contrast, most (65 of 82 (79%)) of the patients with a negative end-of-treatment ctDNA did not relapse. In other words, using the v96 assay, end-of-treatment ctDNA is positive in 54 of the 63 patients who subsequently relapsed, providing a clinical sensitivity of 86%.

Data on how best to manage end-of-treatment ctDNA-positive, high-risk patients are starting to emerge from interventional trials of novel therapies. In the ALTAIR study, patients with colorectal cancer with detectable ctDNA after standard curative-intent therapy, including adjuvant chemotherapy, were randomized to receive trifluridine/tipiracil or placebo[23]. No improvement in ctDNA clearance or reduction in disease-free survival was observed with trifluridine/tipiracil compared to placebo. Multiple ongoing studies are exploring alternative strategies (for example, temozolomide and irinotecan in MGMT-silenced tumor (NCT05031975) and autogene cevumeran (NCT04486378)). Given the established efficacy of biomarker-directed therapies in metastatic colorectal cancer, such as encorafenib–cetuximab-based regimens for $BRAF^{V600E}$ mutated tumors, HER2-targeted combinations for HER-2 amplified disease and PD-1 blockade for MMR deficient tumors, an important approach is instituting early molecularly guided treatment for patients who remain ctDNA positive after chemotherapy. This concept is being prospectively evaluated in the SU2C ACT3 trial (NCT03803553), which assigns ctDNA-positive patients after adjuvant therapy to biomarker-matched targeted/immunotherapy when actionable alterations are present or to FOLFIRI when not[24].

Our results confirm that ctDNA-guided treatment de-escalation and escalation in stage III colon cancer was operationally feasible with high protocol compliance. ctDNA is a powerful prognostic marker, particularly when ctDNA burden is considered. Although current evidence does not support the routine adoption of ctDNA-guided approaches to adjuvant therapy selection in stage III colon cancer, our findings may help guide future prospective studies and could potentially inform individualized risk–benefit discussions. Strategies beyond chemotherapy escalation are required to achieve survival gains in ctDNA-positive patients, and novel therapies in combination with standard chemotherapy should be explored. Patients with a high burden of ctDNA after surgery and those with detectable ctDNA after adjuvant treatment are in urgent need of new treatment approaches.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-025-04030-w.

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Methods

Ethics approval and consent

The DYNAMIC-III study was conducted in accordance with International Ethical Guidelines for Biomedical Research Involving Human Subjects, Good Clinical Practice guidelines and the principles of the Declaration of Helsinki. The study protocol and amendments were approved by the ethics committees of the following hospitals or institutions: Western Health, Peter MacCallum Cancer Centre, Eastern Health, Northern Health, St. Vincent's Hospital Fitzroy, Melbourne Private Hospital, Bendigo Health, Western Private Hospital, Barwon Health, South West Healthcare, Peninsula Health, Peninsula Private Hospital, Grampians Health, Warringal Private Hospital, St. Vincent's Private Hospital–Werribee, Epworth Health, Newcastle Private Hospital, Wollongong Hospital, Port Macquarie Base Hospital, Campbelltown Hospital, Concord Cancer Centre, Liverpool Hospital, Genesis Care, Goulburn Valley Health, Tweed Hospital, Tamworth Hospital, Gosford & Wyong Hospital, Nepean Cancer Care Centre, St. Vincent's Hospital–Sydney, Macquarie University Hospital, Border Medical Oncology, Flinders Medical Centre, Queen Elizabeth Hospital, Royal Brisbane & Women's Hospital, Toowoomba Hospital, Royal Darwin & Katherine Hospitals, Fiona Stanley Hospital, Royal Hobart Hospital, Christchurch Hospital, Vancouver Cancer Centre, Niagara Health Centre, Cambridge Memorial Hospital, North York General Hospital, Ottawa Hospital Research Institute, Allan Blair Cancer Centre, Stronach Regional Health Centre, Juravinski Cancer Centre, London Regional Cancer Program, Royal Victoria Regional Health Centre, Grand Reiver Regional Cancer Centre, University Health Network–Princess Margaret Cancer Centre, CIUSSS NIM–Hospital du Sacre Couer de Montreal, Centre Integre de Sante et de Services Sociaux de la Monteregie, Cancer Care Manitoba, CIUSSS de l'Est-de-l'Ile-de-Montreal Hopital Maissonneuve-Rosemont, Tom Baker Cancer Centre, Vancouver Island Cancer Centre, Trillium Health Partners–Credit Valley Hospital, Saskatoon Cancer Centre, St. John Regional Hospital, Lakeridge Health Oshawa, Oak Valley Health, Dr. H. Bliss Murphy Cancer Centre, Sinai Health System, the Jewish General Hospital, the Walter and Eliza Hall Institute of Medical Research and Johns Hopkins Medical Institutions. All patients provided written informed consent. Participants received no financial compensation.

Trial design and participants

The DYNAMIC-III study (ACTRN12617001566325) is an investigator-initiated, randomized phase 2/3 trial of ctDNA-informed adjuvant therapy, conducted across 66 centers in Australasia and Canada within the Australasian Gastro-Intestinal Trials Group (AGITG) and the Canadian Cancer Trials Group networks. The trial was registered on 21 November 2017 at https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=373948. The trial evaluated a de-escalation or escalation adjuvant treatment strategy in stage III colon cancer informed by ctDNA status compared to standard management. Patients were randomized using an interactive voice response system in a 1:1 ratio to be managed according to ctDNA results (ctDNA-guided management) or to clinican-defined standard management. Randomization was conducted centrally with the minimization method, stratified by treating center and clinical risk group (low risk = T1–3N1; high risk = T4 and/or N2).

Eligible patients had resected, histologically confirmed, stage III (any T, N1–2, M0) colon or rectal adenocarcinoma with negative resection margins and had to be medically fit for at least adjuvant fluoropyrimidine monotherapy. Exclusion criteria included another primary cancer within the last 3 years, multiple primary colorectal cancers or treatment with neoadjuvant chemotherapy and/or radiotherapy. Patients were enrolled up to 6 weeks after surgery, with tumor samples to be provided for mutation analysis within 1 week. Sex was not specifically considered in the study design; rather, sequential patients with stage III colon cancer were enrolled. Sex of participants was determined based on self-report.

Study interventions

Plasma samples for ctDNA analysis were collected at weeks 5–6 after surgery. Randomization followed confirmation of adequate tumor tissue and successful blood draw. Clinicians nominated their standard-of-care treatment, based on conventional criteria, prior to randomization. Standard-of-care options included 6 months of oxaliplatin doublet, 3 months of oxaliplatin doublet, 6 months of fluoropyrimidine or no adjuvant treatment. Dose modifications to chemotherapy were per local standards.

For patients randomized to ctDNA-guided management, ctDNA results were provided to the treating clinician 8–12 weeks after surgery. Clinicians could opt to initiate one cycle of standard-of-care chemotherapy before results were available, after which treatment would de-escalate or escalate (Extended Data Table 1), with the bridging cycle included in the total treatment duration.

Endpoints and assessments

The primary efficacy endpoints were 3-year RFS for the ctDNA-negative cohort and 2-year RFS for the ctDNA-positive cohort. RFS time was calculated from the date of randomization to the date of recurrence confirmation or death from any cause or censored at the last date of follow-up. Secondary endpoints included compliance with de-escalation strategy, ctDNA clearance, overall survival, treatment-related hospitalization and selected high-grade toxicities. Exploratory endpoints included ctDNA burden, ctDNA kinetics during treatment and follow-up, tumor mutation profile, sites of recurrence and cost-effectiveness.

All patients were to be followed for 5 years with carcinoembryonic antigen measured every 3 months for 24 months and then every 6 months for 36 months. Contrast-enhanced computed tomography of chest, abdomen and pelvis was performed every 6 months for 24 months and at 36 months. An end-of-treatment plasma sample was collected from ctDNA-guided patients 4–8 weeks after final treatment; this collection was optional for the standard management group. A subsequent protocol amendment on 25 November 2020 added follow-up blood collections at 12 months, at 18 months and at recurrence. Safety analyses focused on treatment-related hospitalizations and selected grade 3–4 adverse events (febrile neutropenia, diarrhea, oral mucositis, nausea and vomiting). Toxicity was graded according to Common Terminology Criteria for Adverse Events version 5.0 of the National Cancer Institute. Intensity and dose adjustments for administered chemotherapy were recorded.

Trial oversight

The AGITG led the trial, and the Walter and Eliza Institute of Medical Research (WEHI) coordinated the study. All study samples were analyzed at the Ludwig Center at Johns Hopkins, using the SaferSeqS tumor-informed personalized ctDNA assay[15,25]. Trial monitoring was by an independent data and safety monitoring committee (IDSMC). Interim monitoring did not include efficacy. The AGITG IDSMC reviewed safety biannually and advised on a single feasibility/futility interim focused on treatment compliance (de-escalation rate) in the ctDNA-negative cohort. A statistical analysis plan was written before the database lock. Study data were collected and managed using REDCap electronic data capture tools hosted by the WEHI[26,27]. All authors vouch for the accuracy of the data and analyses reported and adherence to the trial protocol. No one who is not an author contributed to writing the paper.

ctDNA analysis method

We used a tumor-informed personalized approach for ctDNA analysis, where somatic mutations were first identified by sequencing of each patient's tumor tissue, and the presence of the same mutation(s) was then assessed in the plasma samples. For the primary aim of the DYNAMIC study, which was to select patients for adjuvant therapy, targeted sequencing of commonly mutated colorectal cancer genes

was performed to identify somatic variants for plasma analysis. For a secondary aim, to assess minimal residual disease after adjuvant therapy (ctDNA clearance), we used an assay (v96) to track more variants selected from WGS. In particular, v96 was performed in the 136 cases where end-of-treatment plasma samples were available. All tumor tissue mutation and ctDNA analyses were performed by the study scientists who were blinded to the clinical outcome (J.D.C., K.L., Y.W., J.P., N.S., L.D., M.P. and B.V.).

Targeted tumor sequencing. Formalin-fixed, paraffin-embedded (FFPE) tumor tissue from the primary tumor was analyzed for somatic mutations in 15 genes recurrently mutated in colorectal cancer (*SMAD4*, *TP53*, *AKT1*, *APC*, *BRAF*, *CTNNB1*, *ERBB3*, *FBXW7*, *HRAS*, *KRAS*, *NRAS*, *PIK3CA*, *PPP2R1A*, *RNF43* and *POLE*). Tumor sections were macro-dissected under a dissecting microscope to enrich neoplastic cell content. DNA was purified with a Qiagen FFPE Kit (Qiagen, 56494). Primers were designed and sequencing results analyzed as previously described[5].

Whole-genome tumor sequencing. WGS was performed on an Illumina NovaSeq 6000 instrument or a Complete Genomics T7 instrument to a depth of approximatyely 30× for the primary tumor and matched normal leukocytes. FASTQ files were generated using Illumina's bcl2fastq or by Complete Genomics' Ztron Lite Server. Adapter sequences were removed with Cutadapt[28]. The trimmed sequences were then aligned to hg38 reference genome with BWA-MEM with default settings[29]. Duplicate sequencing clusters were removed with Picard (http://broadinstitute.github.io/picard). Variants in the plasma sample were called using Strelka2 (Illumina) using the same participant's leukocytes as the matched normal. Any mutation present in the matched leukocytes was excluded from further analysis.

Up to 96 candidate tumor-specific mutations per participant were selected as previously described[12,30]. In brief, mutations in repetitive regions, regions with difficult alignments to the reference genome (hg38), regions that were difficult to amplify efficiently and regions containing single-nucleotide polymorphisms or transitions at CpG sites were excluded. These exclusions were informed through previous analysis of samples from individuals without cancer, using whole-genome or targeted sequencing. The candidate mutations described above were then used to design a personalized assay (v96).

Plasma sample collection. Next, 30–60 ml of blood samples was collected in Streck tubes or K2-EDTA tubes and processed within 3 hours by double centrifugation; buffy coat was collected after the first centrifugation. All samples were stored at −80 °C prior to extraction and analysis. At least 10 ml of plasma was purified from each patient using the QIAamp Circulating Nucleic Acid Kit (Qiagen, 55114).

ctDNA analysis with mutations identified by targeted sequencing of tumor. SaferSeqS assays[25] were designed for each mutation chosen from targeted sequencing. All mutations per patient were tested in one multiplex polymerase chain reaction (PCR) using KAPA HiFi HotStart ReadyMix (Roche). Mutation analysis was done as previously described[25,30]. After sequencing on a NovaSeq 6000 or Complete Genomics T7 instrument, the data were evaluated as described[25,30]. A sample was considered positive if any mutant molecule was detected, as defined by the presence of the mutation in both the Watson and Crick strands of the same molecule.

The number of tumor molecules in plasma was estimated based on the number of distinct molecular barcodes or unique identifiers (UIDs) that tagged each DNA fragment in the plasma sample[31]. When multiple mutations are tracked for the patient, the maximum number of UIDs for any mutation was used as a conservative estimation of the number of haploid genome equivalents in the sample. The number of TDMM per milliliter of plasma was then estimated by the ratio of the number of tumor molecules in the sample and the volume (ml) of plasma represented by the sample.

ctDNA analysis with mutations identified from WGS of tumor (v96). SaferSeqS assays[25] were designed for each mutation chosen from WGS. All mutations per patient were tested in one multiplex PCR using KAPA HiFi HotStart ReadyMix (Roche). Mutation analysis was done as previously described[25,30]. Only mutant positions that could be uniquely mapped to the reference genome, at all positions except the mutant one, were evaluated. For evaluating plasma, a mutation found in the tumor through WGS had first to be validated to be present in the tumor using the multiplex PCR test described above. The mutation also had to be absent in both DNA from the matched normal white blood cells from the same patient and in an unmatched plasma sample from a healthy control. All mutations were assessed in a high number of molecules from matched white blood cells to exclude clonal hematopoeisis of indeterminate potential (CHIP). After sequencing on a NovaSeq 6000 or Complete Genomics T7 instrument, the data were evaluated as described[25,30]. A sample was considered positive if more than one mutant molecule was detected or if the mutant allele frequency exceeded 0.0001%. All analyses were performed in a blinded fashion.

Statistical analysis

DYNAMIC-III was designed as a platform to assess the efficacy of de-escalation/escalation treatment strategies independently in the ctDNA-negative and ctDNA-positive cohorts. Sample size calculations were performed for each cohort based on the premise that approximately 25% of evaluable patients would have a positive ctDNA and approximately 75% would have a negative ctDNA. The ctDNA-negative cohort comprised a phase 2/3 design: the phase 2 de-escalation compliance rate in initial ctDNA-negative patients was used to determine progression to phase 3, which tested non-inferiority of treatment de-escalation in ctDNA-negative patients and efficacy of treatment escalation in ctDNA-positive patients against standard management. For phase 2, 100 ctDNA-negative patients managed by ctDNA guidance would provide more than 90% power at a 95% confidence level to exclude an unacceptably low de-escalation rate of 65% in favor of a target rate of 80% (Simon optimal design). For phase 3, we estimated that 750 ctDNA-negative patients would provide 80% power to demonstrate non-inferiority in 3-year RFS from 75% to 67.5%. A predefined non-inferiority margin of 7.5% was chosen to conclude non-inferiority between standard management and ctDNA-guided de-escalation, with a one-sided 97.5% confidence level. This choice of a 7.5% margin was based on previously documented benefit attributable to adjuvant oxaliplatin[32,33]. Assuming that 75% of patients will be ctDNA negative, a total of 1,000 patients were to be enrolled.

The ctDNA-positive cohort was designed as a randomized phase 2 study to explore the efficacy of treatment escalation rather than as a formal superiority trial. In the anticipated 250 ctDNA-positive patients, clinical efficacy was evaluated by estimating the hazard ratio for 2-year RFS with ctDNA-guided escalation versus standard management, with a prespecified precision defined by a 90% confidence interval bounded by (hazard ratio / 1.341 to hazard ratio × 1.341), corresponding to 63 recurrence events per arm. If the upper limit of this predefined confidence interval is less than 1 (corresponding to hazard ratio < 0.746), then we would conclude that ctDNA-guided escalation is superior to standard management[34].

The preplanned phase 2 analysis of 100 ctDNA-negative patients in the ctDNA-guided arm showed a de-escalation rate of 95%, enabling progression to the phase 3 study. Primary efficacy was assessed in all eligible patients who were randomized and had post-surgical blood draws and was analyzed according to the intention-to-treat principle. Non-inferiority of ctDNA-guided de-escalation over standard management was declared if the lower bound of the one-sided 97.5% confidence interval (two-sided 95% confidence interval) for

the estimated difference in the 3-year RFS rates did not cross −7.5%. RFS rates at 2-year and 3-year landmark points were obtained from the Kaplan–Meier survival curves together with the associated 95% confidence interval. Differences in adjuvant chemotherapy use were assessed using proportions in each arm. All statistical analyses were performed using R version 3.6.1 (R Core Team) and SAS (SAS/STAT User's Guide, version 9.4; SAS Institute). Archived R and SAS analysis scripts can be accessed by contacting the study chair (corresponding author). The study protocol and statistical analysis plan are available in the Supplementary Information.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Qualified external researchers may request access to anonymized individual patient-level clinical data based on submitted curriculum vitae and reflecting non-conflict of interest. The request proposal must also include a statistician. Data requests should be sent to AGITG (the sponsor) study chair at jeanne.tie@petermac.org. Approval of such requests is at AGITG and the trial steering committee's discretion and is dependent on the nature of the request, the merit of the research proposed, the availability of the data and the intended use of the data. We will attempt to respond to data requests within 2 months, but this timeframe may vary depending on the requester's availability to respond to comments. Once approved, a data transfer agreement will be required prior to any data transfer.

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Author contributions

J.T., B.V. and P.G. conceived and designed the study. J.T., Y.W., D.E., J.D.C., N.P., K.W.K. and B.V. acquired and analyzed the data. V.G. and D.E. performed the statistical analyses. All authors contributed to data interpretation as well as development, writing and approval of the paper.

Competing interests

J.T. has served as an advisor/consultant for Haystack Oncology, Amgen, Novartis, AstraZeneca, Merck Serono, Merck Sharp & Dohme, BeiGene, Pierre Fabre, Bristol Myers Squibb, Gilead, Roche, Takeda and Daiichi-Sankyo and reports funding to their institution from Pfizer, Roche, Grail, Pierre Fabre, Daiichi-Sankyo, Zentalis, AstraZeneca and GlaxoSmithKline. Y.W. is a consultant to Belay Diagnostics and Haystack Oncology. J.L. consults for Taiho, Ipsen, Pfizer, Amgen, Merck, GlaxoSmithKline and Novartis and received research funding from Ipsen, Amgen, AstraZeneca, Foundation Medicine, Bayer, Personalis Inc., Guardant and Agenus. B.V. and K.W.K. are founders of Exact Sciences. K.W.K. and N.P. are advisors to, and hold equity in, Exact Sciences. B.V., K.W.K. and N.P. are founders of, and hold equity in, Clasp Therapeutics and Haystack Oncology, a Quest Diagnostics company. K.W.K., B.V. and N.P. are consultants to, and hold equity in, CAGE Pharma. B.V. is a consultant to, and holds equity in, Catalio Capital Management. C.B. is a consultant to Depuy-Synthes, Bionaut Labs, Haystack Oncology and Galectin Therapeutics. C.B. is also a co-founder of OrisDx and a co-founder of Belay Diagnostics. M.P. is a consultant to Haystack Oncology. L.D. is an employee of Haystack Oncology. The companies named above, as well as other companies, have licensed previously described technologies related to the work described in this paper from Johns Hopkins University. B.V., K.W.K., N.P. and C.B. are inventors on some of these technologies. Licenses to these technologies are or will be associated with equity or royalty payments to the inventors as well as to Johns Hopkins University. Patent applications on the work described in this paper may be filed by Johns Hopkins University. The terms of all these arrangements are being managed by Johns Hopkins University in accordance with its conflict of interest policies. D.B. has received honoraria for consultancies from AstraZeneca, Janssen, BeiGene, Boehringer Ingelheim, Bayer, Guardant Health and Amgen and speaking fees from Merck, AstraZeneca and Roche. P.G. is a consultant to Haystack Oncology. The remaining authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41591-025-04030-w.

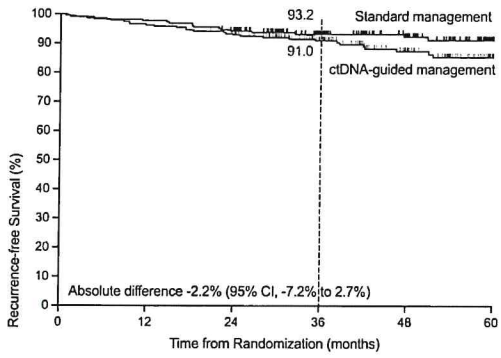
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41591-025-04030-w.

Correspondence and requests for materials should be addressed to Jeanne Tie.

Peer review information *Nature Medicine* thanks Elena Elez, Arndt Stahler, Frank Konietschke and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Primary Handling Editor: Ulrike Harjes, in collaboration with the *Nature Medicine* team.

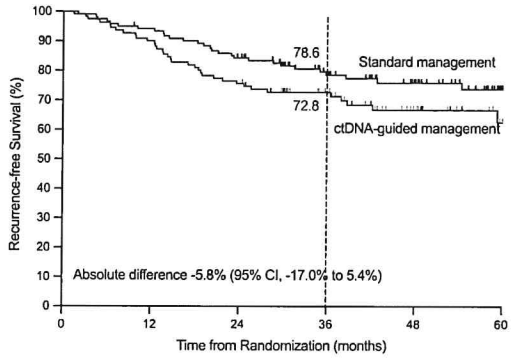
https://doi.org/10.1038/s41591-025-04030-w

A Clinical Low-Risk (T1-3N1)



No. at Risk						
ctDNA-guided management	242	233	219	160	93	39
Standard management	227	222	209	152	95	32

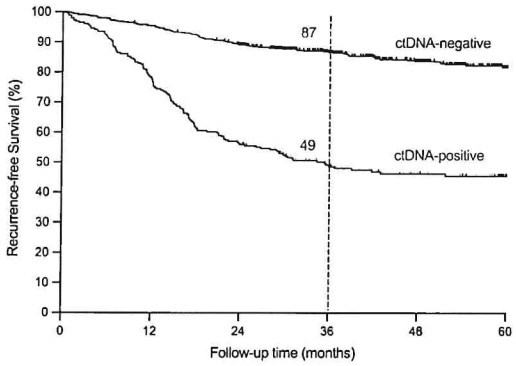
B Clinical High-Risk (T4 and/or N2)



No. at Risk						
ctDNA-guided management	111	100	84	54	31	12
Standard management	122	114	101	71	48	14

Extended Data Fig. 1 | Recurrence-Free Survival with ctDNA-Guided versus Standard-of-Care Adjuvant Therapy for ctDNA-Negative Patients, According to Clinical Risk. a. Clinical Low-Risk (T1-3N1) **b.** Clinical High-Risk (T4 and/or N2).

https://doi.org/10.1038/s41591-025-04030-w



No. at Risk						
Post-surgery ctDNA-negative	702	669	614	437	267	97
Post-surgery ctDNA-positive	259	202	142	94	64	22

Extended Data Fig. 2 | Recurrence-Free Survival According to Post-Surgery ctDNA Status for the Entire Study Population. Patients with a negative ctDNA 5-6 weeks after surgery had a significantly better outcome than those with a positive ctDNA, with 3-year RFS 87% versus 49%; $P < 0.001$.

https://doi.org/10.1038/s41591-025-04030-w

Extended Data Table 1 | ctDNA-Guided Treatment Strategies and Per Protocol Treatment Exposure Details

ctDNA-Negative Cohort (319 patients commenced per protocol de-escalation)	
Pre-specified standard regimen → Per protocol de-escalated regimen	**N (%)**
No chemotherapy → No chemotherapy	3 (1)
6 months fluoropyrimidine (FP) → No chemotherapy or 3 months of FP	
No chemotherapy	12 (4)
3 months of FP	38 (12)
3 months of oxaliplatin-based doublet → 3 or 6 months of FP	
3 months of FP	76 (24)
6 months of FP	70 (22)
6 months of oxaliplatin-based doublet → 6 months of FP or 3 months of oxaliplatin-based doublet	
6 months of FP	14 (4)
3 months of oxaliplatin-based doublet	106 (33)
ctDNA-Positive Cohort (115 patients commenced per protocol escalation)	
Pre-specified standard regimen → Per protocol escalated regimen	**N (%)**
No chemotherapy → 6 months of fluoropyrimidine	1 (1)
6 months fluoropyrimidine → 6 months of oxaliplatin-based doublet	18 (16)
3 months of oxaliplatin-based doublet → 6 months of oxaliplatin-based doublet or at least 3 months of FOLFOXIRI	
6 months of oxaliplatin-based doublet	31 (27)
At least 3 months of FOLFOXIRI	3 (2)
6 months of oxaliplatin-based doublet → at least 3 months of FOLFOXIRI	62 (54)

https://doi.org/10.1038/s41591-025-04030-w

Extended Data Table 2 | Differences in 3-Year Recurrence-Free Survival (RFS) with ctDNA-Guided versus Standard-of-Care Adjuvant Therapy for ctDNA-Negative Patients, According to Pre-Specified Subgroups

		ctDNA-Guided			Standard			Difference			
Variable	Subgroup	N	3-year RFS	SE	N	3-year RFS	SE	3-year RFS	SE	Lower CI	Upper CI
Clinical risk	High	111	**0.728**	0.042	122	**0.786**	0.038	**-0.058**	0.057	-0.170	0.054
	Low	242	**0.910**	0.019	227	**0.932**	0.017	**-0.022**	0.025	-0.072	0.027
Age	70+	86	**0.762**	0.047	83	**0.863**	0.039	**-0.101**	0.061	-0.220	0.018
	<70	258	**0.885**	0.020	253	**0.894**	0.020	**-0.008**	0.028	-0.064	0.047
Gender	Female	173	**0.864**	0.026	162	**0.864**	0.028	**0.001**	0.038	-0.075	0.076
	Male	180	**0.841**	0.028	187	**0.895**	0.023	**-0.054**	0.036	-0.124	0.016
MMR status	deficient-MMR	43	**0.884**	0.049	28	**0.964**	0.035	**-0.081**	0.060	-0.198	0.037
	proficient-MMR	308	**0.847**	0.021	321	**0.874**	0.019	**-0.027**	0.028	-0.082	0.029
N stage	N1	282	**0.876**	0.020	272	**0.908**	0.018	**-0.032**	0.027	-0.085	0.020
	N2	71	**0.759**	0.051	77	**0.783**	0.048	**-0.024**	0.070	-0.162	0.114
Primary	Left/Rectal	184	**0.879**	0.024	197	**0.895**	0.022	**-0.017**	0.033	-0.081	0.048
	Right	169	**0.825**	0.030	152	**0.863**	0.029	**-0.038**	0.041	-0.119	0.043
T stage	4	59	**0.641**	0.063	81	**0.714**	0.052	**-0.073**	0.082	-0.233	0.087
	<4	293	**0.895**	0.018	268	**0.932**	0.016	**-0.037**	0.024	-0.084	0.010
TD	No	288	**0.872**	0.020	270	**0.908**	0.018	**-0.036**	0.027	-0.089	0.016
	Yes	65	**0.769**	0.052	79	**0.787**	0.048	**-0.018**	0.071	-0.158	0.121

TD = tumor deposit, SE = standard error

https://doi.org/10.1038/s41591-025-04030-w

Extended Data Table 3 | ctDNA-Positive Cohort: Cox Proportional Regression Recurrence Free Survival Analysis for Treatment Effect Adjusted for Key Covariates

Adjusted for	HR (95% CI) for treatment effect	P-value
Age	1.15 (0.81, 1.63)	0.4330
Clinical risk	1.14 (0.81, 1.60)	0.4548
Sex	1.15 (0.82, 1.62)	0.4095
MMR-status	1.15 (0.82, 1.62)	0.4040
N-stage	1.14 (0.81, 1.60)	0.4602
Right sided	1.16 (0.83, 1.63)	0.3804
Tumour-deposit	1.11 (0.79, 1.56)	0.5457
T-stage	1.20 (0.85, 1.68)	0.2923

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Extended Data Table 4 | Sites of Disease Recurrence According to Post-Surgery ctDNA Status

Recurrence Site	ctDNA-Positive N = 129	(%)	ctDNA-Negative N = 95	(%)
Distant only	**79**	**61**	**48**	**51**
Liver only	48	37	15	16
Lung only	11	9	24	25
Distant lymph nodes only	5	4	3	3
> 1 distant site	15	12	6	6
Locoregional only	**30**	**23**	**34**	**36**
Peritoneal/omental/ovary only	13	10	18	19
Regional nodes only	5	4	2	2
Anastomosis only	2	2	0	0
Local soft tissue only	5	4	4	4
> 1 locoregional sites	5	4	10	11
Distant and Locoregional	**20**	**16**	**13**	**14**
Liver + locoregional	11	9	3	3
Lung + locoregional	2	2	4	4
Distant lymph nodes + locoregional	4	3	3	3
Other	3	2	3	3
Any distant	99	77	61	64
Any locoregional	50	39	47	49
Any liver	69	53	23	24
Any lung	27	21	37	39
Any peritoneal/omental/ovary	26	20	32	34
Any nodes	32	25	24	25

https://doi.org/10.1038/s41591-025-04030-w

Extended Data Table 5 | Sites of Disease Recurrence and Curative Intent Salvage Surgery or Ablation According to Treatment Arm

	Standard Management				ctDNA-Guided Management			
	ctDNA-negative N=349	%	ctDNA-positive N=130	%	ctDNA-negative N=353	%	ctDNA-positive N=129	%
Recurrence	40	11.5	62	47.7	55	15.6	67	51.9
Liver only	7/40	17.5	24/62	38.7	8/55	14.5	24/67	35.8
Lung only	12/40	30.0	4/62	6.5	12/55	21.8	7/67	10.4
Locoregional only	15/40	37.5	17/62	27.4	19/55	34.5	13/67	19.4
Curative intent salvage surgery/ablation	12/40	30.0	21/62	33.9	22/55	40.0	22/67	32.8

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Last updated by author(s): Sep 24, 2025

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
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☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Study data were collected and managed using REDCap electronic data capture tools (15.7.2) hosted at the Walter and Eliza Hall Institute
Data analysis	All statistical analyses were performed using R version 3.6.1 (R Core Team, Vienna, Austria) and SAS (SAS/STAT User's guide, Verions 9.4; SAS Institute, Cary, NC)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

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Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

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Qualified external researchers may request access to anonymized individual patient-level clinical data based on submitted curriculum vitae and r
eflecting non-conflict of interest. The request proposal must also include a statistician. Data requests should be sent to AGITG (the sponsor) study
chair at jeanne.tie@petermac.org. Approval of such requests is at AGITG and the trial steering committee's discretion and is dependent on the nature o

f the request, the merit of the research proposed, the availability of the data and the intended use of the data. We will attempt to respond to data requests within 2 months, but this timeframe may vary depending on the requesters availability to respond to comments. Once approved, a data transfer agreement will be required prior to any data transfer.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Biological sex was determined based on self-report. Aggregated sex data reported in baseline characteristics table and as a co-variate for subgroup analysis. Sex was not specifically considered in the study design, rather sequential patient with stage III colon cancer were enrolled.
Reporting on race, ethnicity, or other socially relevant groupings	no race/ethinicity or socially relevant groups were collected in this study
Population characteristics	Sex and age, and tumour pathological features were used as co-variates
Recruitment	Sequential stage III colon canccer patiets were recruited by oncologists from clinic
Ethics oversight	Melbourne Health human ethics committees is the lead IRB approving the master protocol. IRB from all the participating centers also approved the protocol

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	DYNAMIC-III was designed as a platform to assess the efficacy of de-escalation/escalation treatment strategies independently in the ctDNA-negative and ctDNA-positive cohorts. Sample size calculations were performed for each cohort based on the premise that ~25% of evaluable patients would have a positive ctDNA and ~75% to have a negative ctDNA. we estimated 750 ctDNA-negative patients would provide 80% power to demonstrate non-inferiority in 3-year RFS from 75% to 67.5%. A pre-defined non-inferiority margin of 7.5% was chosen to conclude non-inferiority between standard-management and ctDNA-guided de-escalation, with a one-sided 97.5% confidence level.The ctDNA-positive cohort was designed as a randomized phase II study to explore the efficacy of treatment escalation, rather than as a formal superiority trial. In the anticipated 250 ctDNA-positive patients, clinical efficacy was evaluated by estimating the hazard ratio for 2-year RFS with ctDNA-guided escalation versus standard-management, with a prespecified precision defined by a 90% confidence interval (CI) bounded by [HR/1.341 to HR×1.341], corresponding to 63 recurrence events per arm. If the upper limit of this pre-defined CI is less than 1 (corresponding to HR<0.746), then we would conclude ctDNA-guided escalation is superior to standard-management.
Data exclusions	Patients who withdrew consent, who did not have post-op blood collections or found to have stage IV disease post enrolment were excluded
Replication	Not applicable for randomized trial
Randomization	Patients were randomized in a 1:1 ratio to be managed according to ctDNA results (ctDNA-guided management) or to clinican defined standard-management. Randomization was conducted centrally with the minimization method, stratified by treating centre and clinical risk group (low risk = T1-3N1; high risk = T4 and/or N2).
Blinding	Investigators were not blinded to group allocation but was blinded to the ctDNA results if allocated to the control group.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

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n/a	Involved in the study
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☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
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☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	ACTRN12617001566325
Study protocol	Full trial protocol can be accessed by emailing the corresponding author
Data collection	From 18th October 2017 through 29th March 2023, 1040 patients were enrolled from hospitals in Australia, New Zealand and Canada. Source data was collected at participating hospitals
Outcomes	The primary efficacy endpoints were 3-year recurrence-free survival (RFS) for the ctDNA-negative cohort and 2-year RFS for the ctDNA-positive cohort. RFS time was calculated from the date of randomization to the date of recurrence confirmation or death from any cause, or censored at the last date of follow-up. Secondary endpoints included compliance with de-escalation strategy, ctDNA clearance, overall survival, treatment-related hospitalization and selected high-grade toxicities. Exploratory endpoints included ctDNA burden, ctDNA kinetics during treatment and follow-up, tumor mutation profile, sites of recurrence and cost-effectiveness.

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
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Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.*

LBA285

Oral Abstract Session

Zanidatamab + chemotherapy (CT) ± tislelizumab for first-line (1L) HER2-positive (HER2+) locally advanced, unresectable, or metastatic gastroesophageal adenocarcinoma (mGEA): Primary analysis from HERIZON-GEA-01.

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Background: HERIZON-GEA-01 (NCT05152147) is a global, open-label, phase 3 trial of zanidatamab (dual HER2-targeted bispecific antibody) + CT ± tislelizumab (anti–PD-1) vs trastuzumab (tras) + CT in 1L HER2+ mGEA. **Methods:** Eligible patients (pts) with previously untreated HER2+ mGEA, regardless of PD-L1 status, were randomized (1:1:1) to zanidatamab (1800 mg [<70 kg] / 2400 mg [≥70 kg] IV Q3W) + tislelizumab (200 mg IV Q3W) + capecitabine/oxaliplatin (CAPOX) or 5-FU/cisplatin (FP); zanidatamab + CAPOX or FP; or tras + CAPOX or FP. Dual primary endpoints were progression-free survival (PFS) by blinded independent central review and overall survival (OS). **Results:** 914 pts were randomized (Dec 2021 to Feb 2025). Demographics and baseline disease characteristics were balanced. At data cutoff (Oct 2025), median follow-up was 26 mo. Compared with tras + CT, PFS was significantly prolonged in zanidatamab-containing arms (Table). A statistically significant OS benefit was observed with zanidatamab + tislelizumab + CT (Table). OS for zanidatamab + CT was not significant at the first interim analysis, although a strong trend favoring zanidatamab + CT was observed. Improvements in PFS and OS occurred across major subgroups, including by region and PD-L1 TAP score. Grade ≥3 treatment-related AEs (TRAEs) occurred in 71.8% of pts with zanidatamab + tislelizumab + CT, 59.0% with zanidatamab + CT, and 59.6% with tras + CT. Grade ≥3 TRAEs occurring in >10% of pts in either zanidatamab-containing arm were diarrhea, hypokalemia, and anemia; the tras + CT arm were diarrhea, anemia, neutrophil count decreased, and platelet count decreased. HER2-targeted therapy was discontinued for related AEs in 11.9% of pts with zanidatamab + tislelizumab + CT, 8.5% with zanidatamab + CT, and 2.3% with tras + CT. **Conclusions:** Both zanidatamab-containing regimens demonstrated a clinically meaningful and statistically significant prolongation of PFS (mPFS >12 mo) vs tras + CT. Zanidatamab + tislelizumab + CT also provided a statistically significant and clinically meaningful OS benefit (mOS >26 mo). The trial is ongoing with additional OS analyses planned for zanidatamab + CT. No new safety signals were observed for zanidatamab or tislelizumab. These results support zanidatamab as a new standard in HER2-targeting agents, potentially replacing tras, as well as the use of tislelizumab in 1L HER2+ mGEA. Clinical trial information: NCT05152147. Research Sponsor: Jazz Pharmaceuticals; BeOne Medicines Ltd.

	Tras + CT (n = 308)	Zanidatamab + CT (n = 304)	Zanidatamab + Tislelizumab + CT (n = 302)
mPFS (95% CI), mo	8.1 (7.0, 8.9)	12.4 (9.8, 14.5)	12.4 (9.8, 18.5)
Hazard ratio (95% CI)	–	0.65 (0.52, 0.81); P <0.0001	0.63 (0.51, 0.78); P <0.0001
18-mo PFS, %	20.9	38.0	43.9
mOS (95% CI), mo	19.2 (16.8, 21.8)	24.4 (20.4, 30.0)	26.4 (21.5, 30.3)
Hazard ratio (95% CI)	–	0.80 (0.64, 1.01) [Interim] P = 0.0564	0.72 (0.57, 0.90) P= 0.0043
24-mo OS, %	38.8	50.3	54.3

Abstract data current as of December 22, 2025.

Zanzalintinib plus atezolizumab versus regorafenib in refractory colorectal cancer (STELLAR-303): a randomised, open-label, phase 3 trial

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The STELLAR-303 study investigators are listed in the appendix (pp 2–10)
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Summary

Background Zanzalintinib is a multitargeted tyrosine-kinase inhibitor that, when combined with atezolizumab, showed promising antitumour activity and manageable toxicity in a phase 1 study. We aimed to compare the efficacy and safety of zanzalintinib–atezolizumab versus regorafenib in patients with previously treated metastatic colorectal cancer.

Methods STELLAR-303 is a global, randomised, open-label, phase 3 trial done at 121 centres (including hospitals, academic medical centres, and specialised cancer research facilities) in 16 countries. Patients aged 18 years and older with confirmed metastatic adenocarcinoma of the colon or rectum, who had previously received standard-of-care therapy, and did not have microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) tumours were randomly assigned (1:1) in blocks of four to oral zanzalintinib (100 mg daily) plus intravenous atezolizumab (1200 mg every 3 weeks) or oral regorafenib (160 mg daily on days 1–21 of each 28-day cycle) using an interactive response technology system, stratified by geographical region, *RAS* status, and presence of liver metastases. Dual primary endpoints were overall survival in the intention-to-treat (ITT) population and in the subset of patients without liver metastases. Safety was assessed in all patients who received at least one dose of study drug. This report is based on a planned overall survival analysis (data cutoff April 30, 2025); the trial is active but not recruiting, and continues to the final overall survival analysis in the subset of patients without liver metastases. This trial is registered with ClinicalTrials.gov (NCT05425940).

Findings 1325 patients were screened for eligibility; between Sept 7, 2022, and July 15, 2024, 901 patients were randomly assigned to zanzalintinib–atezolizumab (n=451) or regorafenib (n=450). 528 (59%) patients were male and 373 (41%) were female; 485 (54%) were White, 338 (38%) were Asian, 18 (2%) were Black, 24 (3%) were other races, and 36 (4%) had race not reported. At a median follow-up of 18·0 months (IQR 14·6–21·5), zanzalintinib–atezolizumab showed a significant overall survival benefit versus regorafenib in the ITT population (stratified hazard ratio [HR] 0·80 [95% CI 0·69–0·93]; p=0·0045) with a median overall survival of 10·9 months (95% CI 9·9–12·1) versus 9·4 months (8·5–10·2). At the interim analysis of overall survival in the subset of patients without liver metastases, the stratified HR for zanzalintinib–atezolizumab versus regorafenib was 0·79 (95% CI 0·61–1·03); p=0·087 (median overall survival 15·9 months [95% CI 13·5–17·6] *vs* 12·7 months [10·9–15·5]). Grade 3 or worse treatment-related adverse events occurred in 268 (60%) of 446 patients receiving zanzalintinib–atezolizumab and 161 (37%) of 434 patients receiving regorafenib. There were five (1%) treatment-related deaths in the zanzalintinib–atezolizumab group and one (<1%) in the regorafenib group.

Interpretation STELLAR-303 is the first phase 3 trial to show a significant improvement in overall survival with an immunotherapy-based regimen, zanzalintinib–atezolizumab, in patients with relapsed or refractory metastatic colorectal cancer that is not MSI-H or dMMR. This combination represents a chemotherapy-free treatment option with a novel mechanism of action for heavily pretreated patients in need of improved therapies.

Funding Exelixis.

Introduction

Outcomes for metastatic colorectal cancer remain poor, with a 5-year relative survival rate of approximately 15%.[1] Immune checkpoint inhibitors improve outcomes for patients with microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) tumours and are standard of care in this setting;[2–6] however, only 5% of metastatic colorectal cancers are MSI-H or dMMR.[7] For the majority of patients with metastatic colorectal cancer, improved therapies in the salvage setting are needed. Although anti-VEGF agents and chemotherapy are integral to metastatic colorectal cancer treatment, overall survival with third-line monotherapy agents, including regorafenib, trifluridine–tipiracil, and

Research in context

Evidence before this study

We searched PubMed from Oct 1, 2014 to March 23, 2022, for clinical trials published in all languages that evaluated refractory metastatic colorectal cancer treatments using the search terms "colorectal cancer" AND ("metastatic" OR "advanced") AND ("refractory" OR "previously treated" OR "previously-treated") AND ("immunotherapy" OR "immune checkpoint inhibitor"). We also searched major oncology congress websites for relevant abstracts published between May 1, 2013, and March 23, 2022. From our searches, we found the phase 3 IMblaze370 trial, which evaluated atezolizumab with or without cobimetinib versus regorafenib and did not show an overall survival benefit in either atezolizumab group in patients with previously treated metastatic colorectal cancer that was not microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR), which represents 95% of patients with metastatic colorectal cancer. We also identified CAMILLA and other phase 1/2 trials of immune checkpoint inhibitor plus VEGF receptor tyrosine-kinase inhibitor combinations that showed higher response rates and overall survival outcomes than expected with either treatment alone in this population. During the conduct of the phase 3 STELLAR-303 study, data were presented from the phase 1 STELLAR-001 study showing that addition of atezolizumab to the multitargeted tyrosine-kinase inhibitor, zanzalintinib, improved overall survival, progression-free survival, and objective response rate compared with zanzalintinib alone with manageable toxicity. In addition, results from the phase 3 LEAP-017 trial were reported, showing that the immune checkpoint inhibitor plus VEGF receptor tyrosine-kinase inhibitor combination pembrolizumab–lenvatinib did not prolong survival versus regorafenib or trifluridine–tipiracil.

Added value of this study

To our knowledge, STELLAR-303 is the first phase 3 trial to show a significant improvement in overall survival with an immunotherapy-based regimen, zanzalintinib–atezolizumab, in patients with relapsed or refractory metastatic colorectal cancer that is not MSI-H or dMMR. A consistent overall survival benefit was observed across key subgroups, including liver involvement, *RAS* status, geographical region, and previous anti-VEGF therapy. The zanzalintinib–atezolizumab safety profile was generally consistent with that previously reported for this and similar immune checkpoint inhibitor plus tyrosine-kinase inhibitor combinations, although there was a higher rate of treatment-related deaths with the combination compared with regorafenib in the current trial.

Implications of all the available evidence

The results from STELLAR-303 show an overall survival benefit for zanzalintinib–atezolizumab compared with regorafenib in patients with previously treated metastatic colorectal cancer, regardless of liver metastasis status. These data, together with previous data from early phase trials, support that the zanzalintinib–atezolizumab combination represents a chemotherapy-free treatment option with a novel mechanism of action for heavily pretreated patients in need of improved therapies.

fruquintinib, is suboptimal, with median overall survival outcomes of less than 7·5 months in patients unselected for microsatellite instability status.[8–10]

The limited benefit from monotherapies prompted exploration of combination strategies. Adding bevacizumab to trifluridine–tipiracil prolonged survival over trifluridine–tipiracil monotherapy; however, the benefit was greater in bevacizumab-naive versus bevacizumab-pretreated patients.[11] In addition, immune checkpoint inhibitor-based combinations have been explored. The phase 3 IMblaze370 trial did not show an overall survival benefit of an immune checkpoint inhibitor and MEK inhibitor combination.[12] Conversely, CAMILLA and other phase 1/2 trials of immune checkpoint inhibitor plus VEGF receptor tyrosine-kinase inhibitor combinations showed higher response rates and overall survival outcomes than expected with either treatment alone, particularly in patients without active liver metastases.[13,14] However, to date, no phase 3 trial of an immune checkpoint inhibitor-based combination has shown improvement in overall survival in metastatic colorectal cancer that is not MSI-H or dMMR. The absence of benefit might be driven by several factors, including tumour location, vascular composition, and an immunosuppressive tumour microenvironment,[12,15–17] the latter prompting investigation into mechanisms to increase responsiveness to immune checkpoint inhibitors in this setting.

Zanzalintinib is a novel, small molecule inhibitor of multiple kinases, including the TAM kinases (TYRO3, AXL, and MER), MET, and VEGF receptors.[18] The TAM family receptors are negative immune regulators,[19] and the zanzalintinib kinase inhibition profile might help reprogramme the tumour microenvironment to support immune activation, thereby improving responsiveness to immune checkpoint inhibitors. Combining zanzalintinib with immune checkpoint inhibitors enhanced tumour growth inhibition in preclinical models[18] and, in the randomised metastatic colorectal cancer cohort of the phase 1 STELLAR-001 study, adding atezolizumab to zanzalintinib improved overall survival, progression-free survival, and objective response rate compared with zanzalintinib alone, with manageable toxicity.[20] Given the encouraging outcomes of early phase trials with immune checkpoint inhibitor plus VEGF receptor tyrosine-kinase inhibitor combinations as well as the zanzalintinib target profile, we aimed to evaluate the efficacy and safety of zanzalintinib–atezolizumab compared with regorafenib in patients with previously treated metastatic colorectal cancer.

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See Online for appendix

Methods

Study design and patients

STELLAR-303 is a global, randomised, open-label, phase 3 trial done at 121 centres (including hospitals, academic medical centres, and specialised cancer research facilities) in 16 countries (appendix pp 2–10). Eligible patients were aged 18 years or older with confirmed metastatic adenocarcinoma of the colon or rectum that radiographically progressed on or was refractory or intolerant to previous therapy with a fluoropyrimidine, irinotecan, and oxaliplatin, with or without an anti-VEGF monoclonal antibody; an anti-EGFR monoclonal antibody (if *RAS* wild-type); and a BRAF inhibitor (if known $BRAF^{V600E}$ [ie, Val600Glu] mutation). Eligibility also required that patients were documented not to have MSI-H or dMMR status. Measurable disease, determined by investigators using Response Evaluation Criteria in Solid Tumours (RECIST) version 1.1, and an Eastern Cooperative Oncology Group performance status of 0–1 were required. Previous treatment with zanzalintinib, regorafenib, trifluridine–tipiracil, or PD-L1 and PD-1 targeting immune checkpoint inhibitors was not allowed. Documented *RAS* status and tumour tissue (archival or fresh biopsy) were required. Full eligibility criteria are provided in the trial protocol (appendix p 24).

This trial was approved by Advarra (Columbia, MD, USA) as well as the institutional review board or ethics committee at each site (appendix pp 2–10) and conducted in accordance with requirements of the regulatory authorities of each country and provisions of the Declaration of Helsinki and Good Clinical Practice guidelines of the International Council for Harmonisation. The trial investigators are listed in the appendix (pp 2–10). Independent patient representatives and advocacy groups were not involved in the design or conduct of the study or in writing the manuscript. Patients provided written informed consent. All unmasked efficacy and safety data were reviewed as planned by an independent data monitoring committee and according to charter.

This trial is registered with ClinicalTrials.gov (NCT05425940) and is active but not recruiting, and continues to the final overall survival analysis in the subset of randomly assigned patients without liver metastases (nlmITT population).

Randomisation and masking

Patients were randomly assigned (1:1) to zanzalintinib–atezolizumab or regorafenib monotherapy with stratification by geographical region (Asia *vs* rest of the world), *RAS* status (wild-type *vs* mutant), and presence of liver metastases (yes *vs* no).

Stratified permuted block randomisation (with block sizes of four) was performed centrally by an interactive response technology system. Although STELLAR-303 was an open-label study, unmasking of study treatment and sponsor review of aggregated data by treatment group was not permitted until after the primary analysis of overall survival, to maintain study data integrity and avoid bias during the conduct of the study.

Procedures

Zanzalintinib was administered at 100 mg orally (daily) and atezolizumab at 1200 mg intravenously every 3 weeks. Regorafenib was given at 160 mg orally (daily) on days 1–21 of each 28-day cycle. Patients continued study treatment in each group if they continued to have clinical benefit in the opinion of the investigator, or until unacceptable toxicity, the need for alternative anticancer treatment, or other reasons for treatment discontinuation. Treatment beyond radiographic progression was allowed per investigator discretion. Dose reductions were permitted for zanzalintinib and regorafenib, but not atezolizumab. Dose delays for adverse events were permitted for all agents. Either component of the combination could be continued if the other was discontinued. Tumour imaging was performed at baseline, then every 8 weeks through to week 49, and every 12 weeks thereafter, until disease progression. Tumour imaging consisted of CT of the chest, abdomen, and pelvis or CT of the chest and MRI of the abdomen and pelvis; MRI or CT of the brain was performed in patients with known or suspected brain metastasis at screening. The schedule of assessments is provided in the protocol (appendix p 24). Information on sex, race, and ethnicity was self-reported and entered into the case report form by study personnel.

Outcomes

The dual primary endpoints were overall survival among all randomly assigned patients (intention-to-treat [ITT] population) and in the nlmITT population. Secondary endpoints were progression-free survival (in the ITT, nlmITT, and the subset of patients with liver metastases [lmITT] populations), objective response rate, including sum of target lesion diameters and disease control rate (in the ITT and nlmITT populations), and duration of response (in the ITT and nlmITT populations) according to RECIST version 1.1 as well as overall survival in the lmITT population, plasma concentration of zanzalintinib, serum concentration of atezolizumab, and incidence of antidrug antibody response against atezolizumab. Overall survival was defined as the time from randomisation to death due to any cause. Progression-free survival was defined as the time from randomisation to either the date of radiographic progression or death (due to any cause), whichever was earlier. The best overall response was defined as the best tumour assessment category as determined by RECIST version 1.1. Duration of objective response was defined as the time from the first documentation of an objective response that was subsequently confirmed at a visit that was at least 28 days after the response was first observed to radiographic

disease progression or death due to any cause, whichever occurred first. Safety was assessed in patients who received at least one dose of any assigned treatment and included evaluation of laboratory parameters and vital signs as an exploratory endpoint. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0.

Overall survival and progression-free survival outcomes were additionally evaluated in prespecified subgroups (sex, age group, race, ethnicity, geographical region, presence of liver metastases, *RAS* status, Eastern Cooperative Oncology Group performance status at baseline, location of primary tumour, location of colon tumour, single metastatic site *vs* multiple metastatic sites of disease, liver metastasis only *vs* liver metastasis plus at least one additional metastatic site of disease *vs* no liver metastatic site, peritoneal carcinomatosis, previous systemic therapy for metastatic colorectal cancer, time since diagnosis of metastatic colorectal cancer, previous anti-VEGF antibody treatment, previous anti-EGFR antibody treatment, and *BRAF* status).

The following secondary endpoints are not reported in this Article: progression-free survival in the lmITT population, objective response rate in the nlmITT population, duration of response in the ITT and nlmITT populations, overall survival in the lmITT population, pharmacokinetics, and immunogenicity. The following exploratory endpoints are not reported in this Article: patient-reported outcomes, health-care resource utilisation, biomarker analyses of tumour markers and circulating tumour DNA, and correlation of biomarker analyses with clinical outcomes in the ITT population. Analyses of these endpoints are in progress and will be considered for future publications.

Statistical analysis

The primary overall survival analysis in the ITT population was planned for when approximately 616 deaths had occurred to provide approximately 87% power to detect a hazard ratio (HR) of 0·75 using the log-rank test and a two-sided α of 0·015. For overall survival in the nlmITT population, it was estimated that 280 deaths would provide approximately 93% power to detect an HR of 0·65 at a two-sided α of 0·035, accounting for a prespecified interim analysis using Lan-DeMets O'Brien–Fleming α-spending function. One interim overall survival analysis in the nlmITT population was planned to coincide with the primary overall survival analysis in the ITT population. All secondary endpoints were analysed at the same timepoint as the primary overall survival analysis of the ITT population using a hierarchical approach; statistical significance of secondary endpoints cannot be claimed until superiority of overall survival in both the ITT and nlmITT populations has been shown in the final analysis.

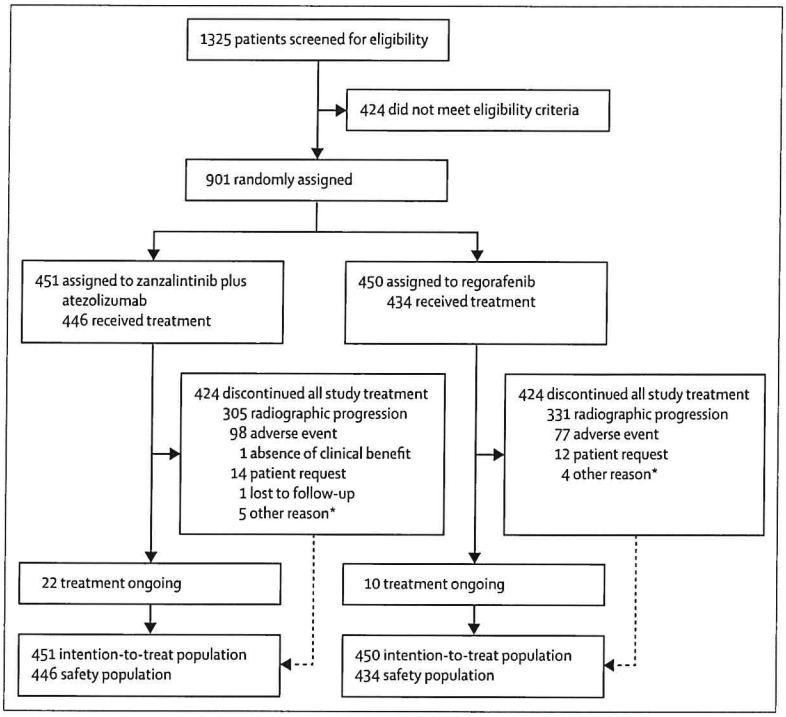


Figure 1: **Trial profile**
*Other reasons included investigator decision (n=1), clinical progression (n=3), and multiple reasons (clinical progression and patient decision, n=1) for the zanzalintinib–atezolizumab group and investigator decision (n=3) and clinical progression (n=1) for the regorafenib group.

The type I error rate associated with the dual primary and secondary endpoint analyses was controlled by a modified Bonferroni procedure and graphic approach.[21] The study-wise two-sided α of 0·05 was divided between the dual primary overall survival endpoints in the ITT population (0·015) and nlmITT population (0·035). The secondary endpoints of progression-free survival in both the ITT and nlmITT populations, overall survival in the lmITT population, and progression-free survival in the lmITT population were planned to be tested sequentially only after overall survival was significant in both the ITT and nlmITT populations.

Overall survival and progression-free survival were estimated using the Kaplan–Meier method and compared between treatment groups with the use of a two-sided log-rank test and stratification factors as collected at the time of randomisation. Estimates of the HR with corresponding two-sided 95% CIs were calculated using a stratified Cox proportional hazards model. Two-sided 95% exact CIs were used to describe objective response and disease control in each treatment group. Two-sided 95% CIs for between-group difference were calculated with the use of the normal approximation to the binomial distribution. Unstratified analysis results are reported for subgroup analyses of those endpoints.

This report is based on a planned overall survival analysis (data cutoff April 30, 2025). The trial continues

	Zanzalintinib–atezolizumab (n=451)	Regorafenib (n=450)
Median age, years	60 (53–68)	60 (51–67)
Age group, years		
<65	293 (65%)	300 (67%)
≥65	158 (35%)	150 (33%)
Sex		
Male	260 (58%)	268 (60%)
Female	191 (42%)	182 (40%)
Race		
White	248 (55%)	237 (53%)
Asian	163 (36%)	175 (39%)
Black	10 (2%)	8 (2%)
Other	15 (3%)	9 (2%)
Not reported	15 (3%)	21 (5%)
Eastern Cooperative Oncology Group performance status score		
0	210 (47%)	200 (44%)
1	239 (53%)	249 (55%)
Location of the primary tumour		
Rectum	167 (37%)	154 (34%)
Colon	284 (63%)	296 (66%)
Left side	184 (41%)	186 (41%)
Right side*	100 (22%)	110 (24%)
Number of metastatic sites		
Single site	88 (20%)	86 (19%)
Multiple sites	362 (80%)	363 (81%)
Presence of liver metastasis		
Yes	264 (59%)	254 (56%)
No	187 (41%)	196 (44%)
Non-MSI-high or non-dMMR†	451 (100%)	450 (100%)
BRAF and *RAS* mutation status		
BRAF mutant	15 (3%)	17 (4%)
RAS mutant	268 (59%)	268 (60%)
Previous systemic therapies for metastatic colorectal cancer		
Median previous systemic therapies	2 (2–3)	2 (2–3)
Previous fluoropyrimidine, irinotecan, and oxaliplatin	449 (>99%)‡	450 (100%)
Any previous anti-VEGF antibody	363 (80%)	375 (83%)
Any previous anti-EGFR antibody	184 (41%)	190 (42%)

Data are median (IQR) or n (%). dMMR=deficient mismatch repair. MSI=microsatellite instability. *Includes the transverse colon. †All patients were tested for MSI status, MMR status, or both. ‡Of the two patients who did not receive all three agents, one received a fluoropyrimidine, oxaliplatin, and bevacizumab without having received irinotecan and the other received two fluoropyrimidines, irinotecan, bevacizumab, and cetuximab without having received oxaliplatin.

Table 1: **Demographic and clinical characteristics of the intention-to-treat population at baseline**

to the final overall survival analysis in the nlmITT population. Statistical analyses were performed using SAS version 9.4. The statistical analysis plan can be found in the appendix (p 24).

Role of the funding source

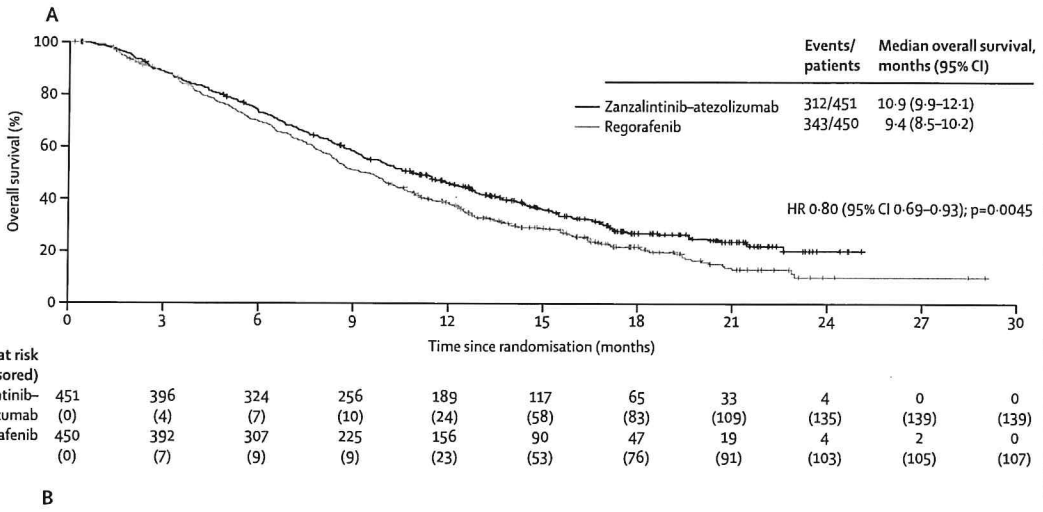
The trial was designed by the sponsor in collaboration with the investigators with input from Roche, the atezolizumab manufacturer. The sponsor, in collaboration with the authors, was also involved in data analysis and data interpretation. The sponsor had no role in data collection. The authors vouch for the completeness and accuracy of the data and fidelity of the trial to the protocol. All authors, including those employed by the sponsor (CSH, GW, and RS), contributed to writing of the report and provided final approval of the manuscript. As part of the site agreement, investigators agreed to keep all aspects and outcomes of the trial confidential. Medical writers employed by the sponsor assisted with manuscript preparation.

Results

1325 patients were screened for eligibility; between Sept 7, 2022, and July 15, 2024, 901 patients were randomly assigned to zanzalintinib–atezolizumab (n=451) or regorafenib (n=450; figure 1). The primary reason for discontinuing treatment was radiographic progression (305 [68%] of 451 patients in the zanzalintinib–atezolizumab group and 331 [74%] of 450 patients in the regorafenib group). Important protocol deviations are described in the appendix (p 11). Baseline characteristics were representative of patients with previously treated metastatic colorectal cancer and well balanced between the groups (table 1). 528 (59%) patients were male and 373 (41%) were female; 485 (54%) were White, 338 (38%) were Asian, 18 (2%) were Black, 24 (3%) were other races, and 36 (4%) had race not reported. 738 (82%) patients received a previous anti-VEGF antibody and 374 (42%) received a previous anti-EGFR antibody.

At a median follow-up of 18·0 months (IQR 14·6–21·5), zanzalintinib–atezolizumab showed a significant overall survival benefit over regorafenib in the ITT population (stratified HR 0·80 [95% CI 0·69–0·93]; p=0·0045; figure 2A). Median overall survival was 10·9 months (95% CI 9·9–12·1) with zanzalintinib–atezolizumab and 9·4 months (8·5–10·2) with regorafenib. Separation of the Kaplan–Meier curves occurred early and consistently favoured zanzalintinib–atezolizumab thereafter. The 12-month overall survival estimates were 46% (95% CI 41–51) for zanzalintinib–atezolizumab and 38% (34–43) for regorafenib; the 24-month overall survival estimates were 20% (15–26) for zanzalintinib–atezolizumab and 10% (6–16) for regorafenib.

At the interim analysis of overall survival in the nlmITT population, the stratified HR for zanzalintinib–atezolizumab versus regorafenib was 0·79 (95% CI 0·61–1·03); p=0·087 (median overall survival 15·9 months [95% CI 13·5–17·6] *vs* 12·7 months [10·9–15·5]). The overall survival benefit of zanzalintinib–atezolizumab over regorafenib was consistent across subgroups for the ITT population, including geographical region, *RAS* status, presence of liver metastases, and previous anti-VEGF antibody treatment (figure 2B).



B

	Zanzalintinib–atezolizumab		Regorafenib		HR (95% CI)
	Events/ patients	Median, months (95% CI)	Events/ patients	Median, months (95% CI)	
Sex					
Male	177/260	11·5 (9·8–12·8)	213/268	9·7 (8·3–10·4)	0·73 (0·60–0·90)
Female	135/191	10·3 (8·7–12·3)	130/182	9·2 (8·1–11·0)	0·91 (0·71–1·15)
Age, years					
<65	201/293	11·0 (9·9–12·5)	222/300	10·0 (8·8–10·9)	0·84 (0·69–1·01)
≥65	111/158	10·3 (8·4–13·4)	121/150	8·3 (7·7–9·9)	0·73 (0·56–0·94)
Geographical region					
Asia	103/158	11·5 (9·2–13·7)	120/159	8·8 (7·8–10·4)	0·77 (0·59–1·00)
Rest of the world	209/293	10·9 (9·3–12·3)	223/291	9·8 (8·3–10·9)	0·82 (0·68–0·99)
***RAS* status**					
Wild type	117/183	12·0 (10·1–14·6)	133/182	10·4 (8·7–12·3)	0·79 (0·61–1·01)
Mutant	195/268	10·3 (9·0–11·9)	210/268	8·7 (8·0–9·8)	0·80 (0·66–0·98)
Liver metastases					
Yes	214/264	8·9 (8·0–9·9)	224/254	7·7 (6·5–8·5)	0·78 (0·65–0·94)
No	98/187	15·9 (13·5–17·6)	119/196	12·7 (10·9–15·5)	0·77 (0·59–1·01)
ECOG performance status					
0	135/210	12·0 (10·8–14·4)	140/200	10·7 (9·2–12·7)	0·85 (0·67–1·07)
≥1	175/239	9·3 (8·3–11·6)	203/250	8·3 (7·4–9·6)	0·76 (0·62–0·93)
Primary tumour location					
Rectum	106/167	13·1 (10·6–15·5)	121/154	9·8 (8·5–10·6)	0·63 (0·48–0·82)
Colon	206/284	9·9 (8·7–11·3)	222/296	8·8 (8·0–10·6)	0·92 (0·76–1·11)
Time since diagnosis of mCRC					
<18 months	67/85	8·4 (5·9–10·0)	92/110	7·5 (5·3–8·5)	0·80 (0·58–1·09)
≥18 months	245/366	11·6 (10·3–13·2)	251/340	10·2 (8·9–11·1)	0·82 (0·69–0·98)
Previous anti-VEGF antibody					
Yes	252/363	10·5 (9·3–12·5)	285/375	8·8 (8·3–9·9)	0·80 (0·68–0·95)
No	60/88	11·5 (8·7–13·5)	58/75	11·1 (9·5–12·6)	0·80 (0·56–1·15)
Previous anti-EGFR antibody					
Yes	121/184	11·5 (9·3–14·4)	138/190	10·3 (8·5–12·3)	0·81 (0·64–1·04)
No	191/267	10·3 (9·1–12·1)	205/260	8·7 (8·0–9·8)	0·78 (0·64–0·95)
***BRAF* status**					
Wild type	236/338	10·9 (9·5–12·5)	276/349	8·9 (8·3–10·0)	0·75 (0·63–0·89)
Mutant	10/15	8·7 (4·0–NE)	12/17	11·3 (3·3–19·5)	0·96 (0·42–2·18)
Overall	**312/451**	**10·9 (9·9–12·1)**	**343/450**	**9·4 (8·5–10·2)**	**0·80 (0·69–0·93)**

Figure 2: **Overall survival in the intention-to-treat population and according to subgroup**
(A) Overall survival in the intention-to-treat population. (B) Overall survival by stratification factors and clinical and molecular subgroups in the intention-to-treat population. ECOG=Eastern Cooperative Oncology Group. HR=hazard ratio. mCRC=metastatic colorectal cancer. NE=not estimable.

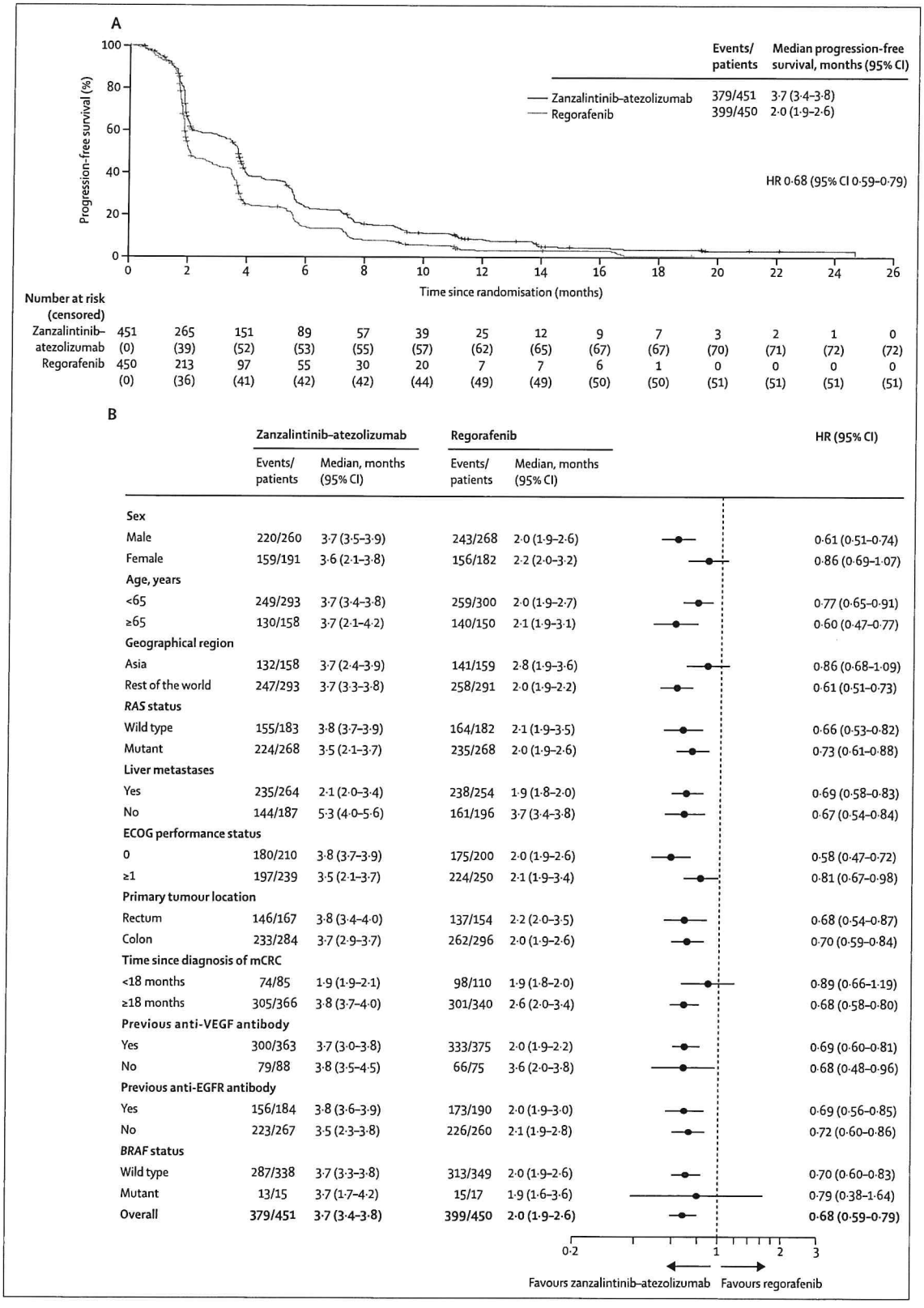


Figure 3: **Progression-free survival in the intention-to-treat population and according to subgroup**
(A) Progression-free survival in the intention-to-treat population. (B) Progression-free survival according to stratification factors and clinical and molecular subgroups in the intention-to-treat population. Progression-free survival assessed according to Response Evaluation Criteria in Solid Tumours version 1.1. ECOG=Eastern Cooperative Oncology Group. HR=hazard ratio. mCRC=metastatic colorectal cancer.

For progression-free survival in the ITT population, the stratified HR for zanzalintinib–atezolizumab versus regorafenib was 0·68 (95% CI 0·59–0·79; median 3·7 months [95% CI 3·4–3·8] *vs* 2·0 months [1·9–2·6]; figure 3A); in the nlmITT population, the stratified HR was 0·66 (0·52–0·83; median 5·3 months [4·0–5·6] *vs* 3·7 months [3·4–3·8]). Statistical superiority for progression-free survival cannot be claimed at this time due to the prespecified hierarchical testing strategy. Progression-free survival was consistent across subgroups (figure 3B). Subsequent anticancer therapy was well balanced between the groups (appendix p 12).

In the ITT population, an objective response was observed in 16 (4% [95% CI 2–6]) of 451 patients in the zanzalintinib–atezolizumab group versus five (1% [0–3]) of 450 patients in the regorafenib group (appendix p 13). Any reduction in sum of target lesion diameters was observed in 168 (42%) of 401 evaluable patients in the zanzalintinib–atezolizumab group versus 102 (27%) of 382 evaluable patients in the regorafenib group; disease control was observed in 242 (54%) of 451 patients versus 183 (41%) of 450 patients.

446 patients in the zanzalintinib–atezolizumab group and 434 in the regorafenib group received at least one dose of assigned treatment and were included in the safety population. Exposure data are summarised in the appendix (p 11). Any-grade treatment-related adverse events occurred in 423 (95%) of 446 patients in the zanzalintinib–atezolizumab group and 399 (92%) of 434 patients in the regorafenib group. Grade 3 or worse treatment-related adverse events occurred in 268 (60%) of 446 patients receiving zanzalintinib–atezolizumab and 161 (37%) of 434 patients receiving regorafenib; grade 3 treatment-related adverse events occurred in 248 (56%) patients in the zanzalintinib–atezolizumab group and 143 (33%) patients in the regorafenib group and grade 4 treatment-related adverse events occurred in 15 (3%) patients in the zanzalintinib–atezolizumab group and 17 (4%) patients in the regorafenib group. The most common grade 3 or 4 treatment-related adverse events were hypertension (65 [15%] of 446 patients in the zanzalintinib–atezolizumab group *vs* 38 [9%] of 434 patients in the regorafenib group), proteinuria (26 [6%] *vs* seven [2%]), fatigue (25 [6%] *vs* eight [2%]), and diarrhoea (25 [6%] *vs* eight [2%]; table 2; appendix pp 14–22). A lower rate of treatment-related palmar–plantar erythrodysesthesia occurred in the zanzalintinib–atezolizumab group than in the regorafenib group (any grade, 71 [16%] of 446 patients *vs* 216 [50%] of 434 patients; grade 3, 12 [3%] *vs* 41 [9%]). Serious adverse events occurred in 255 (57%) of 446 patients in the zanzalintinib–atezolizumab group and 184 (42%) of 434 patients in the regorafenib group (appendix p 23); serious treatment-related adverse events occurred in 118 (26%) patients in the zanzalintinib–atezolizumab group and 45 (10%) in the regorafenib group.

	Zanzalintinib–atezolizumab (n=446)		Regorafenib (n=434)	
	Any grade	Grade ≥3	Any grade	Grade ≥3
At least one adverse event	423 (95%)	268 (60%)	399 (92%)	161 (37%)
Serious adverse event	118 (26%)	96 (22%)	45 (10%)	41 (9%)
Event occurring in ≥10% of patients in either group				
Diarrhoea	221 (50%)	25 (6%)	104 (24%)	8 (2%)
Hypertension	153 (34%)	65 (15%)	114 (26%)	38 (9%)
Fatigue	146 (33%)	25 (6%)	88 (20%)	8 (2%)
Nausea	139 (31%)	12 (3%)	56 (13%)	4 (1%)
Decreased appetite	134 (30%)	10 (2%)	85 (20%)	6 (1%)
Vomiting	95 (21%)	10 (2%)	35 (8%)	1 (<1%)
Rash	84 (19%)	12 (3%)	27 (6%)	5 (1%)
Hypothyroidism	79 (18%)	0	11 (3%)	0
Aspartate aminotransferase increase	77 (17%)	11 (2%)	26 (6%)	9 (2%)
Proteinuria	75 (17%)	26 (6%)	29 (7%)	7 (2%)
Alanine aminotransferase increase	74 (17%)	10 (2%)	19 (4%)	4 (1%)
Palmar–plantar erythrodysesthesia syndrome	71 (16%)	12 (3%)	216 (50%)	41 (9%)
Stomatitis	68 (15%)	7 (2%)	46 (11%)	1 (<1%)
Platelet count decreased	65 (15%)	16 (4%)	16 (4%)	3 (1%)
Pyrexia	64 (14%)	4 (1%)	21 (5%)	0
Asthenia	62 (14%)	11 (2%)	68 (16%)	11 (3%)
Blood bilirubin increased	47 (11%)	10 (2%)	27 (6%)	4 (1%)
Thrombocytopenia	47 (11%)	12 (3%)	12 (3%)	0
Arthralgia	45 (10%)	4 (1%)	10 (2%)	0
Dysphonia	28 (6%)	0	54 (12%)	0

Data are n (%). Treatment-related adverse events that occurred in 10% or more of patients in either group are shown. The full list of treatment-related adverse events is in the appendix (pp 14–22). Preferred terms of disease progression under study in the adverse events database, as per medical review, are excluded. Events are listed by decreasing frequency in the any grade zanzalintinib–atezolizumab group. Adverse events are classified according to the Medical Dictionary for Regulatory Activities, version 28.0.

Table 2: **Treatment-related adverse events in the safety population**

Adverse events leading to discontinuation of the zanzalintinib–atezolizumab regimen occurred in 82 (18%) of 446 patients; 64 (15%) of 434 patients discontinued regorafenib due to adverse events. The most frequent adverse events leading to discontinuation of the zanzalintinib–atezolizumab regimen were abdominal pain, asthenia, and general physical health deterioration (four [1%] patients each of 446 patients).

Deaths considered related to treatment by investigators were due to intestinal perforation (n=2) for zanzalintinib, pneumonitis (n=1) and renal failure (n=1) for atezolizumab, altered state of consciousness (n=1) for zanzalintinib–atezolizumab, and jejunal perforation (n=1) for regorafenib.

Discussion

Zanzalintinib–atezolizumab significantly prolonged overall survival compared with regorafenib in patients with previously treated metastatic colorectal cancer. Robust and consistent survival benefits were observed across key subgroups, including liver involvement, *RAS* status, geographical region, and previous anti-VEGF

therapy, suggesting that the survival benefit in the ITT population was not driven by enrichment for a single subset of patients. The zanzalintinib–atezolizumab safety profile was generally consistent with that previously reported for this combination and other similar immune checkpoint inhibitor plus tyrosine-kinase inhibitor combinations,[20,22] although there was a higher rate of treatment-related deaths with the combination compared with regorafenib in the current trial.

To our knowledge, STELLAR-303 is the first phase 3 trial to show that an immunotherapy-containing regimen, zanzalintinib–atezolizumab, improved overall survival compared with standard of care in metastatic colorectal cancer that is not MSI-H or dMMR. In the phase 3 LEAP-017 trial,[22] lenvatinib–pembrolizumab did not prolong overall survival versus regorafenib or trifluridine–tipiracil. The differences between these trials might be explained by the variation in kinase inhibition profiles between lenvatinib and zanzalintinib—in addition to VEGF receptors, zanzalintinib targets the TAM kinases and MET.[18] TAM kinases contribute to tumour immune evasion by promoting an immunosuppressive macrophage phenotype and facilitating efferocytosis, thereby dampening proinflammatory responses.[19] Inhibiting these kinases with zanzalintinib might improve responsiveness to immune checkpoint inhibitors. Although the contribution of adding atezolizumab to zanzalintinib cannot be ascertained from this phase 3 trial, previous data from a randomised cohort in STELLAR-001 comparing zanzalintinib with zanzalintinib–atezolizumab in a similar population of patients with *RAS* wild-type metastatic colorectal cancer provide supportive evidence for the atezolizumab contribution across all key efficacy parameters tested in the ITT population.[20] The benefit of adding zanzalintinib to atezolizumab is supported by the phase 3 IMblaze370 trial,[12] in which atezolizumab monotherapy yielded an inferior overall survival outcome to regorafenib and did not improve progression-free survival or objective response rate. Collectively, these results suggest that targeting angiogenesis and immunosuppressive signalling, via multikinase inhibition, is needed for optimal outcomes with immune checkpoint inhibitor plus tyrosine-kinase inhibitor combinations in this setting.

In STELLAR-303 subgroup analyses, the overall survival benefit with zanzalintinib–atezolizumab was consistent in patients with liver metastases and without liver metastases. The nlmITT population results were from an interim analysis; patients will continue to be followed up to the planned final analysis. The activity in our patients with liver metastases is notable as LEAP-017 and early phase studies of immune checkpoint inhibitor plus VEGF receptor tyrosine-kinase inhibitor combinations showed a lack of benefit in this population.[22,23] The limited activity of immunotherapy combinations in these studies might be due to an immunosuppressive tumour microenvironment in the liver.[24–26] The benefit observed with zanzalintinib–atezolizumab in this population might result from immunostimulatory activity stemming from the different kinase inhibition profile with zanzalintinib.[18]

The overall survival HR of 0·80 in STELLAR-303 reflected the comparison with an active treatment. At the time this trial was initiated, guideline-directed, standard-of-care options in third-line or later-line metastatic colorectal cancer were regorafenib and trifluridine–tipiracil. Phase 3 trials with regorafenib and trifluridine–tipiracil were placebo-controlled, with overall survival HRs of 0·77 (95% CI 0·64–0·94) and 0·68 (0·58–0·81), respectively.[8,10] The median overall survival observed with these agents were similar at 6·4 months (IQR 3·6–11·8; regorafenib) and 7·1 months (95% CI 6·5–7·8; trifluridine–tipiracil).[8,10] In addition, regorafenib was associated with a lower grade 3 and 4 adverse event rate than trifluridine–tipiracil (270 [54%] of 500 *vs* 370 [69%] of 533, respectively). Given these data, regorafenib was selected as the control for STELLAR-303. In STELLAR-303, the median overall survival with regorafenib was 9·4 months, greater than the 6·4 months (IQR 3·6–11·8) observed in the phase 3 CORRECT trial[8] and the 8·8 months (95% CI 7·3–9·8) observed in the phase 3 Asian CONCUR trial.[27] Survival outcomes can differ in trials over time as a result of evolving patient management approaches, as well as differences in patient characteristics. Notably, the median progression-free survival associated with regorafenib in STELLAR-303 is consistent with that reported previously,[8] suggesting that progression-free survival outcomes with regorafenib have not substantially changed over time and access to additional life-prolonging therapies has affected overall survival. The median progression-free survival difference between groups in STELLAR-303 of 1·7 months is consistent with the median overall survival benefit of 1·5 months, suggesting that patients benefitted equally from subsequent anticancer therapies regardless of treatment assignment.

Additional standard-of-care regimens in refractory metastatic colorectal cancer include fruquintinib monotherapy and trifluridine–tipiracil–bevacizumab. Fruquintinib has shown a median overall survival of 7·4 months (95% CI 6·7–8·2), consistent with other single-agents and reflecting its selective inhibition of VEGF receptors.[9] In SUNLIGHT,[11] the median overall survival with trifluridine–tipiracil-bevacizumab was 10·8 months (95% CI 9·4–11·8) overall but was 9·0 months (8·3–10·8) among patients previously treated with bevacizumab, an established standard of care. Acknowledging the caveats associated with cross-trial comparisons and drawing conclusions from subgroup analyses, in STELLAR-303, the median overall survival with zanzalintinib–atezolizumab was 10·5 months among patients who received previous anti-VEGF antibody treatment. These findings suggest a potential survival

benefit in a heavily pretreated population, including those previously exposed to bevacizumab. Importantly, this combination offers a non-chemotherapy-based regimen and was associated with a lower incidence of haematological adverse events than the chemotherapy-based SUNLIGHT regimen.

The objective response rate of 4% observed in our trial is consistent with that observed in other trials in the salvage metastatic colorectal cancer setting, despite improvements in overall survival.[9,11] These data suggest that a benefit of agents in this setting might instead be manifested through measures of disease control.

Adverse events were consistent with known class effects and no new safety signals emerged. Most adverse events were grade 1–3. The most common any-grade and grade 3 or worse adverse events (eg, diarrhoea, hypertension, and fatigue) were more frequent with zanzalintinib–atezolizumab than with regorafenib. In the case of palmar–plantar erythrodysesthesia, the rate with zanzalintinib–atezolizumab (16% any grade, 3% grade 3 or worse) was lower than that with regorafenib (50% any grade, 9% grade 3 or worse) despite the longer treatment duration with the combination. No serious adverse events occurred in more than 5% of patients in either group. The safety profile of zanzalintinib–atezolizumab was consistent with that previously reported for this regimen and similar combinations in metastatic colorectal cancer[20,22] as well as other immune checkpoint inhibitor plus tyrosine-kinase inhibitor regimens in different tumour types.[28–30] The adverse events observed with zanzalintinib–atezolizumab are well known and manageable by dose reductions or supportive care treatment strategies familiar to oncologists. A higher rate of treatment-related deaths was observed with the combination compared with regorafenib in the current trial. Ultimately, the safety profile must be balanced with the efficacy outcomes observed in this advanced setting.

Strengths of this trial include its size and the stratification by liver metastasis status, permitting hypothesis testing in these relevant subgroups, and its international scope and active-controlled design. A limitation was the open-label design, which might have influenced patient management at the investigator level. In addition, since the study was initiated, the trifluridine–tipiracil–bevacizumab regimen has become a standard-of-care treatment in refractory metastatic colorectal cancer. A randomised clinical trial comparing that regimen with zanzalintinib–atezolizumab is an unmet need in the metastatic colorectal cancer treatment landscape at this time.

In summary, STELLAR-303 is the first phase 3 trial to show a significant improvement in overall survival with an immunotherapy-based regimen, zanzalintinib–atezolizumab, in patients with relapsed or refractory metastatic colorectal cancer that is not MSI-H or dMMR. This combination represents a chemotherapy-free treatment option with a novel mechanism of action for heavily pretreated patients in need of improved therapies.

Contributors

All authors participated in drafting and critically revising the manuscript. The study sponsor along with ASa, JRH, YSP, JT, and CE were responsible for the conceptualisation and design of the study. JRH, YSP, M-AL, SL, ACV, MVdE, EF, MF, GA, JS, ASt, OD, LB, CE, and ASa participated in enrolling patients, supervising the trial, and collecting data. CSH, GW, and RS were involved in data analysis, and all authors were involved in data interpretation. ASa, JRH, and CSH accessed and verified the raw data. All authors had full access to the study data, vouch for the completeness and accuracy of the data and for the fidelity of the trial, and had final responsibility for the decision to submit for publication.

Declaration of interests

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Data sharing

Individual patient data will not be shared. The study protocol is available online as part of the appendix (p 24).

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