Extended Follow-Up of Botensilimab Plus Balstilimab in an Expanded Cohort of Microsatellite-Stable Metastatic Colorectal Cancer Without Active Liver Metastases

Benjamin L. Schlechter[1,a,b], Marwan G. Fakih[2,b], Apostolia M. Tsimberidou[3,b], Andrea J. Bullock[4], Agustin Pimentel[5], Sunil Sharma[6], Heinz-Josef Lenz[7], Michael S. Gordon[6], Ghassan K. Abou-Alfa[8–10], Thomas U. Marron[11], Robert W. Lentz[12], Wells Messersmith[12], Ian Chau[13], Bruno Bockorny[4], Dhan Chand[14], Manushak Avagyan[14], Wei Wu[14], Benny Johnson[14], Joseph E. Grossman[14], Steven J. O'Day[14], Anthony B. El-Khoueiry[7,c], Neil H. Segal[8,9,c]

[1]Dana-Farber Cancer Institute, Boston, MA, USA; [2]City of Hope Comprehensive Cancer Center, Duarte, CA, USA; [3]University of Texas, MD Anderson Cancer Center, Houston, TX, USA; [4]Beth Israel Deaconess Medical Center, Boston, MA, USA; [5]University of Miami, Sylvester Comprehensive Cancer Center, Miami, FL, USA; [6]HonorHealth Research Institute, Scottsdale, AZ, USA; [7]University of Southern California, Norris Comprehensive Cancer Center, Los Angeles, CA, USA; [8]Memorial Sloan Kettering Cancer Center, New York, NY, USA; [9]Weill Cornell Medical College, New York, NY, USA; [10]Trinity College Dublin, Dublin, Ireland; [11]Mount Sinai, Tisch Cancer Center, New York, NY, USA; [12]University of Colorado Cancer Center, Aurora, CO, USA; [13]The Royal Marsden Hospital, London, UK; [14]Agenus Inc, Lexington, MA, USA

[a]Corresponding author.
[b]Joint-first authorship.
[c]Joint-last authorship.

Running Title: Botensilimab Plus Balstilimab in MSS mCRC NLM

Corresponding Author:
Benjamin L. Schlechter
Dana-Farber Cancer Institute
450 Brookline Ave
Boston, MA 02215
benjamin_schlechter@dfci.harvard.edu

Conflict of Interest Statement: BLS reports participation on an advisory board with Agenus, Janssen, Lyell Immunopharma, AstraZeneca, and Larkspur. **MGF** reports institutional grant funding from Agenus, Genentech/imCORE, and Verastem; consulting fees from AbbVie, Adagene, Astellas, Bayer Corporation, Bristol Myers Squibb, Delcath Systems, HUYABIO International, Iterion Therapeutics, Merck, Microbial Machines, Mirati Therapeutics, Pfizer, Revolution Medicines, Summit Therapeutics, Taiho Oncology, and Totus Medicines; and participation on a Data Safety Monitoring Board or advisory board with Exelixis, Janssen Research & Development, Merus NV, Mirati Therapeutics, Roche/Genentech, Tempus, and Xilio Therapeutics. **AMT** reports receiving institutional funding from OBI Pharma, Agenus, IMMATICS, Tachyron, Novocure, Parker Institute for Cancer Immunotherapy, Orionis, Tempus, and Tvardi; an advisory role with BrYet, NEX-I, OBI Pharmaceuticals, and Integra Connect; and participation as a coordinating PI for Anaveon and AbbVie. **AJB** reports participation on a Data Safety Monitoring Board or advisory board with Agenus. **AP** reports nothing to disclose. **SS** reports payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Tisch Cancer Institute; support for attending meetings and/or travel from Cure CRC Summit (air and hotel); participation on a Data Safety Monitoring Board or

advisory board with Iterion, Tisch Cancer Institute, and Mirati Therapeutics (BMS); and a leadership or fiduciary role with Black Canyon Bio, Stingray Therapeutics, and Bonneville Bio. **HJL** reports institutional payments from NCI Moonshot U2C and NCI UG1, UM1; consulting fees from BMS, Merck KG, Bayer, Merck, Isofol, Oncocyte, Invitae, Affini-T, 3T Bioscience, Repimmune, G1 Therapeutics, Jazz Therapeutics, Adagene, and Fulgent; payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from BMS and Merck; support for attending meetings and/or travel from BMS (ASCO); participation on a Data Safety Monitoring Board or advisory board with 3T Bioscience and Adagene; and stock or stock options with Biobreak and Fulgent. **MSG** reports stock and other ownership interests at Sphinx Health Solutions (previously CAREMISSION); a consulting or advisory role with Imaging Endpoints, Morphic Therapeutic, Viracta Therapeutics, Qualigen Therapeutics, OnQuality Pharmaceuticals, SpringWorks Therapeutics, Remepy, and Deciphera; institutional research funding from Genentech/Roche, GlaxoSmithKline, AbbVie, Merck Serono, Pfizer, Plexxikon, Deciphera, Corcept Therapeutics, Syndax, Tolero Pharmaceuticals, ImaginAb, Arcus Biosciences, Agenus Inc, Novartis, Revolution Medicines, IgM Biosciences, Veru, Forma Therapeutics, DynamiCure Biotechnology, Fore Biotherapeutics, NiKang Therapeutics, Nimbus Therapeutics, OncoResponse, Pionyr, Riboscience, Sirnaomics, Theseus Pharmaceuticals, SQZ Biotech, Shenzen Ionova, and Pyxis; and patents, royalties, or other intellectual property (Application Serial No.: PCT/US24/27766 Filing Date: May 3, 2024 Title: Planet atmosphere gases enwrapped into composite nanomaterials with medical treatment applications). **GKA** reports institutional research support from AbbVie, Agenus, Amgen, Arcus, AstraZeneca, Atara, Beigene, BioNtech, BMS, Coherus, Digestive Care, Elicio, Genentech/Roche, Helsinn, Incyte, J-Pharma, Revolution Medicines, Tango Therapeutics, and Yiviva; consulting fees from AbbVie, Ability Pharma, Agenus, Alligator Biosciences, Amgen, Astellas, Arcus, AstraZeneca, Autem, Berry Genomics, BioNtech, BMS, Boehringer Ingelheim, Fibrogen, Genentech/Roche, Ikena, Immuneering, Ipsen, J-Pharma, Merck, Merus, Moma Therapeutics, Neogene, Novartis, Pfizer, Regeneron, Revolution Medicines, Servier, Syros, Tango Therapeutics, Tempus, Vector, and Yiviva; and payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Servier (ESMO). **TUM** reports grants or contracts from Regeneron, Genentech, Bristol-Myers Squibb, Merck, and Boehringer Ingelheim; consulting fees from Boehringer Ingelheim, Geneos, Astellas, AbbVie, Merck, EMD Serono, Avammune, Storm, Arrowhead, Tubulis, EvolveImmune, Natures Toolbox, AN2 Therapeutics, Tatum, Caris, and GE Healthcare; and participation on a Data Safety Monitoring Board or advisory board with AbbVie. **RWL** reports receiving institutional research funding from ALX Oncology, Merck, Lilly, Guardant, EDDC, Boehringer Ingelheim, and AstraZeneca; institutional honoraria from Agenus, Boehringer Ingelheim, Merck, Natera, Kahr, and EDDC; and stock or stock options with Natera. **WM** reports participation as a local PI of a clinical trial for ALX Oncology, Pfizer, Agenus, Rascal Therapeutics, Revolution Medicine, Nurix, Accent Therapeutics, and EDDC; participation as a coordinating PI of a clinical trial for Exelixis; participation in an advisory board with ALX Oncology; participation on a Data Safety Monitoring Committee with Amgen; and directorship with Criterium Inc. **IC** reports participation on an advisory board with Astella, AstraZeneca, BeiGene, BioNtech RNA Pharmaceuticals GMBH, Daiichi Sankyo, Elevation Oncology, Gilead, GSK, Jazz Pharmaceuticals, Merck Serono, Revolution Medicine, and Takeda; participation as an invited speaker with Astellas, AstraZeneca, BMS, Daiichi Sankyo, and Servier; conference coverage for Jazz Pharmaceuticals; and participation as a coordinating PI for Eli Lilly. **BB** reports grants or contracts from Agenus; consulting fees from BioLine Rx, Boxer, and N1X10; payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Total Health; payment for expert testimony from Foster & Eldridge, LLC; N1X10; and Phelps Dunbar, LLP; a patent planned, issued, or pending (US20220291222A1); and participation on a Data Safety Monitoring Board or advisory board with Enlivex. **DC**, **MA**, **WW**, **BJ**, **JEG**, and **SJO** report employment and stock options with

Agenus Inc. **ABE** reports participation on an advisory board with Exelixis, AstraZeneca, Genentech, Agenus, Tallac, APL Bio, Senti Biosciences, Qurient, Terumo, and Elevar; receiving institutional funding from Fulgent, Astex, and AstraZeneca; serving as a steering committee member for Merck; participation as a coordinating PI for Genentech and Auransa; and serving as a PI for Agenus, Affimed, and Bayer. **NHS** reports participation on an advisory board with 3T Biosciences, Regeneron Pharmaceuticals, Pfizer, Astellas, Agenus, Boehringer Ingelheim, and Oncolytics; and participation as a local PI for Roche/Genentech, Pfizer, Merck, BMS, AstraZeneca, Regeneron Pharmaceuticals, and Agenus.

Funding: Study was funded by Agenus Inc.

Word Count: 4198/5000

Figures/Tables: 6/6

Translational Relevance

We report extended safety and efficacy follow-up of botensilimab (BOT; multifunctional Fc-enhanced anti–CTLA-4) plus balstilimab (BAL; anti–PD-1) in a fully-enrolled phase 1b cohort of patients with microsatellite-stable metastatic colorectal cancer and no active liver metastases (MSS mCRC NLM), including a late-line–exposed subgroup. BOT+BAL induced deep/durable responses in heavily pretreated MSS mCRC NLM, a population in which durable survival >18 months remains uncommon. With a confirmed ORR of 21%, sustained survival beyond 2 and 3 years, consistent activity in late-line–exposed patients, and a manageable safety profile, this regimen represents a potentially important advance in the treatment of MSS mCRC NLM. Clinical activity occurred independent of PD-L1 or tumor mutational burden status supporting BOT's FcγR-dependent mechanism. These findings underscore the potential of BOT+BAL to generate meaningful antitumor activity in immunologically "cold" and immune checkpoint inhibitor–refractory tumors and support phase 3 evaluation.

Abstract

Purpose: Microsatellite-stable metastatic colorectal cancer (MSS mCRC) has limited treatment options and responds poorly to conventional immunotherapy. We report extended follow-up results from a fully-enrolled, phase 1b cohort evaluating botensilimab (BOT; Fc-enhanced anti–CTLA-4) plus balstilimab (BAL; anti–PD-1) in patients with MSS mCRC and no active liver metastases (NLM).

Patients and Methods: Patients received BOT 1 or 2 mg/kg every 6 weeks (Q6W) plus BAL 3 mg/kg Q2W up to 2 years or until progression or unacceptable toxicity. Primary objective was safety/tolerability. Efficacy endpoints included objective response rate (ORR), duration of response (DOR), and progression-free survival (PFS); overall survival (OS) was exploratory. Patients with prior regorafenib, trifluridine/tipiracil±bevacizumab, or fruquintinib ("late-line exposed") were analyzed post hoc.

Results: As of December-13-2025, 123 patients were treated (median follow-up, 20.2 months [range, 0.7–62.3]). Median prior lines was 3 (range, 1–10; 67% had ≥3 prior lines). Most common treatment-related adverse events were diarrhea (39%; grade ≥3, 8%) and fatigue (37%; grade ≥3, 2%). ORR was 21% (95% CI, 14–29). Median DOR was not reached (NR; 95% CI, 7.3–NR), median PFS was 4.0 months (95% CI, 2.8–4.1), and median OS was 21.2 months (95% CI, 16.2–23.8; 36-month OS, 33% [95% CI, 24–43]). Efficacy was consistent in late-line–exposed patients (n=37; ORR, 22% [95% CI, 10–38]; median OS, 16.2 months [95% CI, 9.7–31.3]).

Conclusions: BOT+BAL demonstrated durable responses, long-term survival, and manageable safety in previously-treated MSS mCRC NLM, including in late-line–exposed patients. These results support further evaluation.

ClinicalTrials.gov Identifier: NCT03860272 (C-800-01 Trial)

Introduction

Colorectal cancer (CRC) is the third most common type of cancer worldwide, with approximately 1.9 million new diagnoses in 2022 (1). In the US, approximately 154,300 people were diagnosed in 2025, with 52,900 estimated deaths from the disease (2). Despite declining mortality for most major cancers in individuals aged <50 years in the US, early-onset CRC is now the leading cause of cancer-related death in this age group (3). Nearly 95% of patients with advanced CRC have microsatellite-stable (MSS)/mismatch repair–proficient (pMMR) disease, which is associated with limited effective treatment options and substantial unmet need (4,5).

Survival rates in metastatic CRC (mCRC) have increased modestly with the use of combination chemotherapies plus treatments targeting vascular endothelial growth factor (VEGF) or epidermal growth factor receptor (EGFR) (6-10). After failure of such therapies, standard later-line salvage therapies include regorafenib, trifluridine/tipiracil ± bevacizumab, and fruquintinib, which have demonstrated limited clinical benefit in randomized phase 3 trials (predominantly MSS populations), with objective response rates (ORRs) of 1%–6% and median overall survival (OS) of approximately 6–11 months (11-16). Even when limiting these study populations to patients with no active liver metastases (NLM), ORR remains poor (3%–8%) as does median OS (approximately 12–14 months) (17-19). This highlights that although patients with NLM have better prognoses than patients with active liver metastases (19), patients with NLM remain a population with high unmet need. Altogether, durable survival in MSS mCRC remains uncommon, with 18-month survival observed in a minority of late-line patients and ≥24-month survival rarely reported.

Immune checkpoint inhibitors (ICI) targeting cytotoxic T-lymphocyte associated protein 4 (CTLA-4) or programmed cell death (ligand) 1 (PD-[L]1) have demonstrated notable efficacy in several cancers, with durable survival plateaus and event-free survival observed in a proportion of patients (20,21). In mCRC, however, meaningful clinical benefit from ICI has historically been limited to the small subset of patients (about 5%) with microsatellite instability-high (MSI-H)/mismatch repair–deficient (dMMR) disease (4,22,23). In contrast, MSS mCRC has historically been refractory to ICI. Dual PD-(L)1 and CTLA-4 checkpoint blockade in MSS mCRC has demonstrated ORRs of 1% (durvalumab + tremelimumab) and 5% (nivolumab + ipilimumab) (24,25). Thus, no ICI regimen has yet achieved meaningful clinical benefit in MSS mCRC.

The limited efficacy with conventional ICI observed in MSS mCRC likely reflects its poor immunogenicity and “cold” tumor microenvironment, defined by low $CD8^+$ T-cell infiltration, minimal immune activation, and the presence of suppressive myeloid and regulatory T cell populations, which are all hallmarks of dysfunctional adaptive T-cell immunity (26). Botensilimab (BOT) is a multifunctional Fc-enhanced anti–CTLA-4 antibody that, unlike conventional anti–CTLA-4 agents such as ipilimumab and tremelimumab, was designed to activate antigen-presenting cells, enhance T-cell priming and memory, and selectively reduce intratumoral regulatory T cells, thereby remodeling the tumor microenvironment and enabling activity in immunologically “cold” tumors (27,28). Additionally, the Fc domain of BOT was engineered to mitigate complement C1q binding, thereby reducing complement-mediated immune toxicities associated with conventional anti–CTLA-4 agents (27). When combined with balstilimab (BAL), an anti–PD-1 antibody pharmacologically comparable to approved PD-1 inhibitors (29,30), BOT+BAL has demonstrated antitumor immunity with deep and durable tumor responses across tumors classified as “cold” or treatment refractory (31), including MSS mCRC (as previously published) (32), sarcomas (33), ovarian cancer (34), and ICI-refractory hepatocellular carcinoma (35) and melanoma (36).

We now report extended follow-up data from the C-800-01 trial on 123 patients with MSS mCRC NLM treated with BOT+BAL, including 37 patients previously exposed to available late-line therapies. Given that active liver metastases are associated with a uniquely immunosuppressive tumor microenvironment that can abrogate systemic antitumor immunity in an already “cold” disease (37,38), we prospectively defined a cohort of patients with MSS mCRC who lacked active liver metastases to evaluate BOT+BAL safety and efficacy. In this analysis, no active liver metastases (“NLM”) was defined as patients with definitively treated liver metastases or no history of liver metastases. Importantly, and as previously mentioned, patients with NLM have better prognoses than patients with active liver metastases (19). In this report, we provide extended follow-up of safety and efficacy data, including long-term survival outcomes, in an MSS mCRC NLM population.

Patients and Methods

Trial Design and Participants

C-800-01 (NCT03860272) was an open-label, phase 1b, multicenter trial evaluating the safety, tolerability, efficacy, and pharmacokinetic/pharmacodynamic profiles of BOT±BAL. Patients with MSS mCRC NLM were enrolled and treated between May 11, 2020, and September 29, 2023 at

15 sites across the United States and the United Kingdom (now closed to enrollment). The trial design, dose escalation, and interim results from the MSS mCRC cohort have been previously reported (32). The prior report included 77 evaluable patients with MSS mCRC NLM at an earlier data cut off; additional patients with MSS mCRC NLM have since been treated, comprising the total cohort with NLM, which is the subject of this report.

Comprehensive eligibility criteria are in the **Supplemental Methods**. Eligible patients were ≥18 years of age with measurable disease per Response Evaluation Criteria in Solid Tumors (RECIST) v1.1, an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1, adequate organ function, and confirmed metastatic or locally advanced disease. Previous anti–PD-(L)1±CTLA-4 was permitted, with no limit on prior lines of therapy. This report only included patients with NLM, defined as those with definitively treated liver metastases or no history of liver metastases. MSS/pMMR was tested per approved local standard testing (immunohistochemistry, polymerase chain reaction, and/or next generation sequencing [NGS]). The trial was conducted in compliance with the Declaration of Helsinki and International Conference on Harmonisation Guidelines for Good Clinical Practice and was approved by the Institutional Review Board at each institution, with all patients providing written informed consent.

Procedures

Patients enrolled in the dose expansion phase received BOT 1 or 2 mg/kg intravenously (IV) once every 6 weeks (Q6W) + BAL 3 mg/kg IV Q2W, both for up to 2 years or until disease progression or unacceptable toxicity. Patients were assigned to available open treatment cohorts through the study's operational slot-allocation and enrollment process, with cohort availability determined by Safety Monitoring Committee–reviewed dose decisions.

Endpoints

The primary objective/endpoint was to assess safety and toxicity, including the occurrence of dose-limiting toxicities (DLTs), during dose escalation. As previously reported, a maximum tolerated dose was not reached (NR), and no DLTs occurred in the MSS mCRC cohort (32). Secondary endpoints included assessment of the frequency, severity, and duration of treatment-emergent adverse events (TEAEs) according to the National Cancer Institute's Common Terminology Criteria for Adverse Events v5.0 (NCI CTCAE v5.0). Immune-mediated adverse events (imAEs) were also assessed; imAEs were medically adjudicated and defined as related to treatment, thought to have an immune mechanism, determined by the investigator to be

immune-related, and/or treated with systemic steroids (of any dose) and/or other immunosuppressants or with thyroid replacement hormones. imAEs were identified from a prespecified list of preferred terms. Secondary efficacy endpoints included objective response rate (ORR), duration of response (DOR), disease control rate (DCR; defined as best response of complete response [CR], partial response [PR], or stable disease [SD] at ≥6 weeks), and progression-free survival (PFS), per RECIST v1.1 based on investigator assessment. OS was an exploratory endpoint. Clinical benefit rate (CBR; defined as best response of CR, PR, or SD at ≥24 weeks) was also assessed. An exploratory, post hoc subgroup analysis was conducted, which included patients previously exposed to available later-line therapies for MSS mCRC prior to enrollment (deemed as the "late-line–exposed" subgroup; defined as ≥1 prior regimen of regorafenib, trifluridine/tipiracil ± bevacizumab, or fruquintinib). Patients who have progressed following these regimens have limited remaining treatment options and represent a population with historically poor outcomes. As such, this subgroup analysis was intended to evaluate a population with high unmet need in MSS mCRC and whether BOT+BAL had clinical activity in patients with highly treatment-refractory disease. Imaging was performed Q6W (±3 days) from the first dose.

Statistical Analysis

The safety population (in which all analyses were conducted) included all patients who received ≥1 dose of study drug. Safety endpoints were tabulated by dose level using descriptive statistics, and the severity of adverse events (AEs) was graded using NCI CTCAE v5.0 whenever possible. Descriptive statistics were also used to summarize trial results (means, medians, ranges, and variability). Categorical variables were summarized by counts and percentages. For patients with a CR or PR, DOR was summarized using Kaplan-Meier methods, as were PFS and OS for all patients. Median time to event(s) and 95% confidence interval (CI) for the median(s) were also provided. Missing efficacy or safety data was not imputed unless otherwise specified in the statistical analysis plan (SAP). Statistical analyses were performed with SAS software [RRID: SCR_008567] (v9.4; SAS Institute Inc., Cary, NC). The protocol and SAP can be found among the **Supplemental Materials**.

Correlative Studies

Correlative analyses were exploratory in nature and evaluated associations between PD-L1 expression (centrally assessed), tumor mutational burden (TMB), and treatment response. Detailed methodology is in the **Supplemental Methods**.

Data Availability

Individual patient clinical data will be de-identified in addition to molecular data supporting the findings presented herein, all of which will be made available for academic use upon reasonable written request, subject to informed consent constraints. Any researchers interested in accessing data from this manuscript should submit a request to the corresponding author (Benjamin Schlechter; benjamin_schlechter@dfci.harvard.edu), in addition to a signed data transfer agreement. Access will be granted only if terms are agreed upon in the data transfer agreement, including provisions that data can only be used for research purposes, must maintain patient confidentiality, and other information, all for the duration of the agreement. All materials, data, and protocols described in the manuscript will be made available upon request, if the request is made within 6 years of publication of this article. Each request will be reviewed individually, with responses provided within a maximum of 2 weeks.

Results

Patients

The prior interim report of this cohort included 77 evaluable patients with MSS mCRC NLM (prior data cut off: November 29, 2023) (32). Here, we report findings from an additional 46 patients (N=123) with extended follow-up, mature survival landmarks, and treatment-free survival characterization. As of the data cutoff for this report (December 13, 2025), 123 patients with MSS mCRC NLM were treated with BOT 1 mg/kg (n=62) or 2 mg/kg (n=61) Q6W plus BAL 3 mg/kg Q2W (**Figure S1**). The median number of BOT and BAL doses received was 2 (range, 1–17) and 6 (range, 1–52), respectively. Median follow-up was 20.2 months (range, 0.7–62.3). Median follow-up estimate via reverse Kaplan-Meier was 34.8 months (95% CI, 28.2–38.1); reverse Kaplan-Meier follow-up treats deaths as censored observations and provides an estimate of the potential follow-up time available for assessing long-term outcomes.

Demographics and baseline disease characteristics are in **Table 1**. Median age was 56 years (range, 25–82). Patients had received a median of 3 prior lines of therapy (range, 1–10; 67% [n=82] had ≥3 prior lines); 16% (n=20) had previously treated liver metastases, 84% (n=103) had no history of liver metastases, and 15% (n=18) received prior anti–PD-(L)1±CTLA-4. Fifty-six patients (46%) had an ECOG PS of 0 and 67 (54%) had a score of 1. The median time from initial diagnosis and metastatic disease diagnosis to study treatment was 46.0 months (range, 6.1–208.1) and 33.9 months (range, 0.2–179.4), respectively. Patients had a high metastatic burden, with 69% having metastases at ≥2 sites and frequent lung (78%), lymph node (44%), and peritoneal (39%) involvement. Thirty-seven patients (30%) received ≥1 regimen of

regorafenib, trifluridine/tipiracil ± bevacizumab, or fruquintinib. In patients with TMB data available by local clinical test (n=77), 8% (n=6) had TMB >10 mutations/megabase (Mut/Mb), and none had TMB >25 Mut/Mb. Of 113 patients with *RAS* data available, 67% had tumors with *RAS* mutation. A study representativeness table is in **Table S1**.

Safety

No new safety signals were observed with extended follow-up (N=123; safety population). All patients experienced any TEAE (grade ≥3, 73%), and 113 patients (92%) experienced any treatment-related AE (TRAE; grade ≥3, 41%). The most common TRAEs (by preferred term) were diarrhea (39%; grade ≥3, 8%), fatigue (37%; grade ≥3, 2%), and pyrexia (24%; grade ≥3, 3%) (**Table 2**). Overall, 21 patients (17%) had TRAEs (related to both BOT and BAL) leading to discontinuation of both BOT and BAL (reflects patients who discontinued all study treatment). There were no treatment-related deaths (no grade 5 TRAEs).

imAEs, which were treatment-related, defined using criteria described in the methods, and analyzed using grouped preferred terms unless otherwise stated (see **Table S2**), occurred in 71 patients (58%; grade 3, 29%; grade 4, 1%; no grade 5). The most common was immune-mediated diarrhea/colitis (imDC; grouped term that included immune-mediated enterocolitis, colitis, diarrhea, and autoimmune colitis) (n=51, 41%; grade ≥3, 15%). Other imAEs occurred in ≤10% of patients and included thyroid disorders (7%; grade ≥3, 0%), pulmonary events (5%; grade ≥3, 2%), and hepatitis (5%; grade ≥3, 2%). There was only 1 grade 4 imAE (imDC; details in the supplement), which occurred in the BOT 2 mg/kg dose group. Hypophysitis occurred in 1 patient (grade 3; details in the supplement). imAEs were dose-dependent; both any-grade imAEs and any-grade imDC occurred in fewer patients receiving BOT 1 mg/kg + BAL compared with BOT 2 mg/kg + BAL (**Table S2**). Due to imAEs, 33 patients (27%) temporarily interrupted and 42 patients (34%) discontinued one or both study drugs (BOT and/or BAL; patients may have discontinued one drug while continuing the other).

Tumor necrosis factor–α (TNF-α) inhibitors (e.g., infliximab) were used for imDC management, including in steroid-sensitive patients, to accelerate symptom resolution, reduce cumulative corticosteroid exposure, and enable safe retreatment (39). Per protocol, TNF-α inhibition was recommended within 3 days of imDC symptom onset of any grade, including grade 1. Among the 51 patients who experienced imDC, 63% (n=32/51) received combination systemic steroids and TNF-α inhibition, 25% (n=13/51) received systemic steroids alone, and 10% (n=5/51) received TNF-α inhibition alone; 1 patient (2%; n=1/51) did not receive immunosuppressive

agents. Overall, 98% (n=50/51) of imDC events resolved; median time to resolution from imDC onset was 14 days (interquartile range, 7–27).

Efficacy

In this updated analysis (prior data cut off: November 29, 2023; current data cut off: December 13, 2025) with more patients (N=123) and extended follow-up as previously noted, additional responders were identified; the confirmed ORR was 21% (26/123; 95% CI, 14–29), including 3 CRs and 23 PRs (**Figure 1A-B**; **Table 3**) (32,40). Across each BOT dose, efficacy was consistent (BOT 1 mg/kg + BAL: ORR, 21% [13/62]; BOT 2 mg/kg + BAL: ORR, 21% [13/61]) (**Table 3**; **Figure S2**). Among responders (n=26), the median number of botensilimab doses was 3 (range, 1–17), and the median number of balstilimab doses was 23 (range, 2–49). The median DOR was NR (95% CI, 7.3–NR) (**Figure 1C**), and DORs ranged from 1.9 to ≥37.4 months, with 10 responses ongoing at data cutoff. DCR was 69% (85/123; 95% CI, 60–77), and CBR was 28% (34/123; 95% CI, 20–36). Median PFS was 4.0 months (95% CI, 2.8–4.1), 12-month PFS was 23% (95% CI, 15–31), and 18-month PFS was 18% (95% CI, 11–26) (**Figure 2A**; **Table 3**). Median OS was 21.2 months (95% CI, 16.2–23.8), with 12-month, 24-month, and 36-month OS rates of 65% (95% CI, 55–72), 41% (95% CI, 32–50), and 33% (95% CI, 24–43), respectively (**Figure 2B**; **Table 3**). At last follow-up, 21 patients (17%) were alive and off all therapy (**Figure S3A**), of whom 13 were responders. Treatment-free survival is shown in **Figure S3B**.

Efficacy in the exploratory, post hoc subgroup of patients previously exposed to available late-line therapies (n=37; demographics in **Table S3**) was similar to the overall population (**Figure 3A-B**; **Table S4**); confirmed ORR was 22% (8/37; 95% CI, 10–38; including 1 CR and 7 PRs). Median DOR was 16.6 months (95% CI, 1.9–NR) (**Figure 3C**); DORs ranged from 1.9 to ≥24.3 months, with 2 responses ongoing at data cutoff. DCR was 70% (26/37; 95% CI, 53–84), and CBR was 27% (10/37; 95% CI, 14–44). Median PFS was 4.1 months (95% CI, 2.8–6.4), 12-month PFS was 20% (95% CI, 9–36), and 18-month PFS was 17% (95% CI, 6–32) (**Figure S4A**; **Table S4**). Median OS was 16.2 months (95% CI, 9.7–31.3), with 12-month, 24-month, and 36-month OS rates of 54% (95% CI, 37–68), 35% (95% CI, 19–51), and 30% (95% CI, 15–46), respectively (**Figure S4B**; **Table S4**).

Correlative Biomarker Studies

Responses to BOT+BAL were observed in tumors with low TMB and PD-L1 combined positive score (CPS) of 0 points (**Table S5**). There was no significant difference in TMB between

responders (patients with CR/PR) and non-responders (patients with SD/PD) (p=0.94), and TMB did not correlate with best percent change in target lesions from baseline (R^2=0.02, p=0.46) (**Figure S5A-B**). PD-L1 CPS did not differ significantly between responders and non-responders (p=0.99), nor did it correlate with best percent change in target lesions from baseline (R^2=0.02, p=0.39) (**Figure S5C-D**). Of the responders with clinical tissue-based NGS data available (n=18), none had a TMB >13 nor a *POLE* mutation associated with hypermutation. Thus, these conventional ICI biomarkers did not reliably differentiate responders from non-responders to BOT+BAL.

Discussion

With mature follow-up, BOT+BAL resulted in durable and clinically meaningful outcomes among this expanded cohort of 123 patients with treatment-refractory MSS mCRC NLM, including 21% ORR, 28% 24-week CBR, and a median OS of 21.2 months. Two-thirds of patients had received ≥3 prior therapy lines, 15% had prior exposure to anti–PD-(L)1±CTLA-4, and many had longstanding metastatic disease with a substantial burden of metastatic involvement at study entry. Additionally, 30% of patients had prior exposure to available late-line therapies (≥1 regimen of regorafenib, trifluridine/tipiracil ± bevacizumab, or fruquintinib), and clinical activity in this exploratory, post hoc subgroup was comparable to the overall population. The sustained activity in this exploratory, post hoc subgroup exposed to available late-line therapies is particularly notable, as the efficacy of conventional cytotoxic and targeted therapies typically diminishes with subsequent lines of treatment, in part because more effective options are used earlier (41). These long-term outcomes are consistent with the prior NLM cohort analysis (ORR, 20%; median OS, 20.9 months) (40) and, with extended follow-up, further substantiate the durable and sustained clinical benefit with BOT+BAL. No new safety signals emerged with extended follow-up, and findings were consistent with previous reports (32,40).

These results with BOT+BAL are clinically meaningful given the substantial unmet need for patients with MSS mCRC, particularly after progression on doublet/triplet chemotherapy with anti-VEGF or anti-EGFR biologics (6-8,10). Additionally, although patients with NLM have better prognoses than patients with active liver metastases, clinical benefit with currently available salvage therapies remains limited with NLM (19). In the salvage setting among patients with NLM, regorafenib, trifluridine/tipiracil ± bevacizumab, and fruquintinib evaluated in phase 3 studies have demonstrated ORRs of 3%–8% and median OS of approximately 12–14 months (17-19). Further, although recent, pivotal phase 3 results with zanzalintinib (multi-targeted tyrosine kinase inhibitor) + atezolizumab (anti–PD-L1) demonstrated a statistically significant

survival improvement vs regorafenib in patients with NLM (interim analysis: median OS, 15.9 vs 12.7 months), the survival improvement was clinically modest (42). Among allcomers, there was no meaningful increase in ORR (4% vs 1%) and no evidence of a meaningful and durable survival plateau with zanzalintinib + atezolizumab (42). Contextualizing these data, durable disease control and long-term survival remain rare after standard therapies in patients with NLM, highlighting the persistent unmet need for more effective novel treatments. Importantly, none of these therapies involve ICI alone, and all standard lines of treatment include chemotherapy or targeted therapy. Thus, BOT+BAL stands out as a mature, ICI-only treatment paradigm in MSS mCRC NLM.

Against this historical backdrop of limited survival in treatment-refractory MSS mCRC NLM, BOT+BAL demonstrated sustained long-term benefit, with 24-month and 36-month OS rates of 41% and 33%, respectively. The maturity of the dataset, reflected by a reverse Kaplan-Meier median follow-up estimate of 34.8 months (a measure of potential follow-up that is less influenced by early deaths) and continued patient observation beyond 24 and 36 months, supports the robustness of these long-term survival findings and the characterization of a durable survival tail. Durable survival in MSS mCRC NLM remains uncommon with currently available therapies, as Kaplan-Meier curves show that few patients remain alive at 18 months with very few at risk by 24 months (17-19). Although the median PFS in this study was modest (4.0 months), landmark PFS rates provide additional context regarding the disease control demonstrated among the subset of patients who remained progression-free beyond 1 year, rather than reflecting the experience of the overall study population. Notably, the 12-month and 18-month PFS rates with BOT+BAL were 23% and 18%, respectively, indicating that approximately 20% of patients remained progression-free beyond 1 year despite many receiving therapy in the ≥3-line setting. With regorafenib, trifluridine/tipiracil, and fruquintinib, few patients with NLM remain progression-free beyond 1 year, with 18-month PFS rates uniformly approaching zero (17-19). Importantly, cross-trial comparisons should be interpreted with caution given differences in study design and patient populations; thus, these observations should be viewed as hypothesis-generating rather than definitive.

The safety profile of BOT+BAL remained consistent with prior reports (32,40), with no new safety signals with extended follow-up. imAEs were dose-dependent, with lower rates of any-grade imAEs and imDC observed at the BOT 1 mg/kg + BAL dose, supporting its selection for phase 3 investigation. Consistent with evolving best practices for ICI-related imDC management (39,43,44), early integration of steroid-sparing agents, including TNF-α inhibitors, was

incorporated in steroid-responsive cases. This approach minimized prolonged high-dose corticosteroid exposure and contributed to a manageable, largely resolvable (98% in this cohort) imDC toxicity profile. Severe immune-related toxicity was infrequent, with only a single grade 4 imAE (imDC that resolved completely) and no treatment-related deaths.

Per trial design, patients could receive BOT (administered every 6 weeks) for up to 2 years; however, the median number of BOT doses administered was 2 (range, 1–17). This is consistent with clinical precedent from first-generation/conventional anti–CTLA-4 therapies combined with anti–PD-(L)1 agents, including ipilimumab (historically administered as a short induction course) and tremelimumab (administered as a single priming dose in the STRIDE regimen), which demonstrated that prolonged CTLA-4 blockade is not required to generate durable anticancer immunity (20,45,46). These observations support the hypothesis that CTLA-4 blockade primarily drives early immune priming and expansion of antitumor T-cell responses, whereas continued PD-(L)1 blockade supports maintenance of long-term antitumor immunity (47). Consistent with this mechanism, durable responses and prolonged survival were observed despite limited BOT exposure (median of 2 BOT doses), with 41% and 33% of patients alive at 2 and 3 years, respectively.

Critically, extended follow-up continued to demonstrate consistent efficacy across both BOT doses (ORRs of 21% with both BOT 1 and 2 mg/kg), with lower rates of immune-mediated toxicity at BOT 1 mg/kg + BAL. These findings are also consistent with results from a randomized phase 2 trial in MSS mCRC NLM, in which BOT 75 mg + BAL demonstrated a confirmed ORR of 19% with similar safety observations (48). Together, these data support an improved benefit–risk profile without loss of efficacy at the lower BOT dose, justifying selection of BOT 75 mg as the fixed dose for further evaluation in future clinical trials.

BOT+BAL demonstrated clinical activity independent of standard immunotherapy biomarkers, including TMB and PD-L1 expression, supporting the FcγR-dependent mechanism involving myeloid reprogramming and T-cell priming rather than tumor-intrinsic immunogenicity (28). This suggests that BOT+BAL activity extends beyond conventional ICI biomarker-selected subpopulations in MSS mCRC and that TMB and PD-L1 do not reliably distinguish responders from non-responders. Accordingly, predictive biomarker development will require integrative translational approaches that capture tumor microenvironment architecture and systemic immune context rather than tumor-intrinsic features alone. Exploratory analyses in other BOT+BAL–treated populations, including ovarian cancer, sarcomas, and a large pan-tumor

cohort (n=341), have begun to identify predictive features and provide further mechanistic insight (33,34,49).

This study had several limitations. This was a nonrandomized, open-label phase 1b cohort without a control arm, limiting cross-trial comparisons and definitive efficacy conclusions. Restriction to patients with NLM, while biologically justified, may have enriched for patients with more favorable prognoses and limited generalizability to those with active liver metastases. In addition, the exploratory, post hoc subgroup analysis was not powered for formal comparison and should be considered descriptive. Biomarker analyses were limited by data availability. Ongoing randomized studies are needed to confirm clinical benefit and better define patients most likely to derive durable benefit from BOT+BAL.

In summary, BOT+BAL demonstrated clinically meaningful and durable antitumor activity in heavily pretreated patients with MSS mCRC NLM, a population that has a more favorable prognosis than patients with active liver metastases but in whom durable survival beyond 18 months remains uncommon. With a confirmed ORR of 21%, sustained survival beyond 2 and 3 years, consistent activity in heavily pretreated patients, and a manageable safety profile, this regimen represents a potentially important advance in the treatment of immunologically “cold” MSS mCRC NLM. These findings underscore the potential of BOT, an Fc-enhanced anti–CTLA-4 antibody, combined with PD-1 blockade (BOT+BAL), to generate meaningful antitumor activity in immunologically “cold” and ICI-refractory tumors and supports phase 3 evaluation.

Acknowledgments

The authors are grateful to the patients who participated in this trial and their families. The authors also thank Amanda Agazio, PhD, CMPP and Ellie Manca, MPH (Agenus Inc) for editorial assistance in the preparation of this manuscript; Cara Constance, PhD, BSN, RN (Agenus Inc) for interpretation and acquisition of clinical data; Chloe Delepine, PhD; Sean Bourdelais, MS; and Bishnu Joshi, PhD (Agenus Inc) for interpretation and acquisition of biomarker data; Tyler Curiel, MD, MPH for his insights when developing the C-800-01 trial; and Richard Goldberg, MD for clinical insights and contributions to interpretation of the study findings. Agenus Inc supplied the drugs and partook in the design, conduct, analysis, and reporting of the trial. This study was funded by Agenus Inc.

References

1. American Cancer Society. Global Cancer Facts & Figures (5th Edition). Atlanta, GA, USA 2024.
2. Siegel RL, Kratzer TB, Giaquinto AN, Sung H, Jemal A. Cancer statistics, 2025. *CA Cancer J Clin* 2025;**75**(1):10-45 doi https://doi.org/10.3322/caac.21871.
3. Siegel RL, Wagle NS, Jemal A. Leading Cancer Deaths in People Younger Than 50 Years. *JAMA* 2026;**335**(7):632-4 doi 10.1001/jama.2025.25467.
4. Buchler T. Microsatellite Instability and Metastatic Colorectal Cancer - A Clinical Perspective. *Front Oncol* 2022;**12**:888181 doi 10.3389/fonc.2022.888181.
5. Guven DC, Kavgaci G, Erul E, Syed MP, Magge T, Saeed A*, et al.* The Efficacy of Immune Checkpoint Inhibitors in Microsatellite Stable Colorectal Cancer: A Systematic Review. *Oncologist* 2024;**29**(5):e580-e600 doi 10.1093/oncolo/oyae013.
6. Hoang T, Sohn DK, Kim BC, Cha Y, Kim J. Efficacy and Safety of Systemic Treatments Among Colorectal Cancer Patients: A Network Meta-Analysis of Randomized Controlled Trials. *Front Oncol* 2021;**11**:756214 doi 10.3389/fonc.2021.756214.
7. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W*, et al.* Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004;**350**(23):2335-42 doi 10.1056/NEJMoa032691.
8. Loupakis F, Cremolini C, Masi G, Lonardi S, Zagonel V, Salvatore L*, et al.* Initial therapy with FOLFOXIRI and bevacizumab for metastatic colorectal cancer. *N Engl J Med* 2014;**371**(17):1609-18 doi 10.1056/NEJMoa1403108.
9. Saltz LB, Cox JV, Blanke C, Rosen LS, Fehrenbacher L, Moore MJ*, et al.* Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. *N Engl J Med* 2000;**343**(13):905-14 doi 10.1056/nejm200009283431302.
10. Van Cutsem E, Köhne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A*, et al.* Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 2009;**360**(14):1408-17 doi 10.1056/NEJMoa0805019.
11. Dasari A, Lonardi S, Garcia-Carbonero R, Elez E, Yoshino T, Sobrero A*, et al.* Fruquintinib versus placebo in patients with refractory metastatic colorectal cancer (FRESCO-2): an international, multicentre, randomised, double-blind, phase 3 study. *Lancet* 2023;**402**(10395):41-53 doi 10.1016/s0140-6736(23)00772-9.
12. Grothey A, Van Cutsem E, Sobrero A, Siena S, Falcone A, Ychou M*, et al.* Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet* 2013;**381**(9863):303-12 doi 10.1016/s0140-6736(12)61900-x.
13. Mayer RJ, Van Cutsem E, Falcone A, Yoshino T, Garcia-Carbonero R, Mizunuma N*, et al.* Randomized trial of TAS-102 for refractory metastatic colorectal cancer. *N Engl J Med* 2015;**372**(20):1909-19 doi 10.1056/NEJMoa1414325.
14. Prager GW, Taieb J, Fakih M, Ciardiello F, Van Cutsem E, Elez E*, et al.* Trifluridine-Tipiracil and Bevacizumab in Refractory Metastatic Colorectal Cancer. *N Engl J Med* 2023;**388**(18):1657-67 doi 10.1056/NEJMoa2214963.
15. Li J, Qin S, Xu R, Yau TC, Ma B, Pan H*, et al.* Regorafenib plus best supportive care versus placebo plus best supportive care in Asian patients with previously treated

metastatic colorectal cancer (CONCUR): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* 2015;**16**(6):619-29 doi 10.1016/s1470-2045(15)70156-7.

16. Li J, Qin S, Xu RH, Shen L, Xu J, Bai Y*, et al.* Effect of Fruquintinib vs Placebo on Overall Survival in Patients With Previously Treated Metastatic Colorectal Cancer: The FRESCO Randomized Clinical Trial. *JAMA* 2018;**319**(24):2486-96 doi 10.1001/jama.2018.7855.

17. Garcia-Carbonero R, Dasari A, Eng C, Tabernero J, Yoshino T, Hochster H*, et al.* Efficacy and safety of fruquintinib in patients with refractory metastatic colorectal cancer with and without liver metastasis: A subgroup analysis of the phase 3 FRESCO-2 trial. Poster presented at: European Society for Medical Oncology 2024 Congress Poster 520P Barcelona, Spain.

18. Tabernero J, Prager GW, Taieb J, Calleja E, Liu M, Amellal N*, et al.* Impact of colorectal liver metastases (CRLM) in patients with metastatic colorectal cancer (mCRC) receiving trifluridine/tipiracil (FTD/TPI) with or without bevacizumab (BEV) in the phase 3 SUNLIGHT trial. Poster Presented at: American Society for Clinical Oncology Annual Meeting 2024 Poster 3584. Chicago, IL, US.

19. Cohen R, Raeisi M, Chibaudel B, Shi Q, Yoshino T, Zalcberg JR*, et al.* Prognostic value of liver metastases in colorectal cancer treated by systemic therapy: An ARCAD pooled analysis. *Eur J Cancer* 2024;**207** doi 10.1016/j.ejca.2024.114160.

20. Wolchok JD, Chiarion-Sileni V, Rutkowski P, Cowey CL, Schadendorf D, Wagstaff J*, et al.* Final, 10-Year Outcomes with Nivolumab plus Ipilimumab in Advanced Melanoma. *N Engl J Med* 2025;**392**(1):11-22 doi 10.1056/NEJMoa2407417.

21. Blank CU, Lucas MW, Scolyer RA, van de Wiel BA, Menzies AM, Lopez-Yurda M*, et al.* Neoadjuvant Nivolumab and Ipilimumab in Resectable Stage III Melanoma. *N Engl J Med* 2024;**391**(18):1696-708 doi 10.1056/NEJMoa2402604.

22. André T, Elez E, Lenz HJ, Jensen LH, Touchefeu Y, Van Cutsem E*, et al.* Nivolumab plus ipilimumab versus nivolumab in microsatellite instability-high metastatic colorectal cancer (CheckMate 8HW): a randomised, open-label, phase 3 trial. *Lancet* 2025;**405**(10476):383-95 doi 10.1016/s0140-6736(24)02848-4.

23. André T, Shiu KK, Kim TW, Jensen BV, Jensen LH, Punt C*, et al.* Pembrolizumab in Microsatellite-Instability-High Advanced Colorectal Cancer. *N Engl J Med* 2020;**383**(23):2207-18 doi 10.1056/NEJMoa2017699.

24. Overman MJ, Kopetz S, McDermott RS, Leach J, Leonardi S, Lenz HJ*, et al.* Nivolumab ± ipilimumab in treatment (tx) of patients (pts) with metastatic colorectal cancer (mCRC) with and without high microsatellite instability (MSI-H): CheckMate-142 interim results. Oral presentation at the 2016 American Society of Clinical Oncology Presentation #3501. Chicago, IL, US.

25. Chen EX, Jonker DJ, Loree JM, Kennecke HF, Berry SR, Couture F*, et al.* Effect of Combined Immune Checkpoint Inhibition vs Best Supportive Care Alone in Patients With Advanced Colorectal Cancer: The Canadian Cancer Trials Group CO.26 Study. *JAMA Oncol* 2020;**6**(6):831-8 doi 10.1001/jamaoncol.2020.0910.

26. Binnewies M, Roberts EW, Kersten K, Chan V, Fearon DF, Merad M*, et al.* Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nat Med* 2018;**24**(5):541-50 doi 10.1038/s41591-018-0014-x.

27. Chand D, Savitsky DA, Krishnan S, Mednick G, Delepine C, Garcia-Broncano P*, et al.* Botensilimab, an Fc-Enhanced Anti-CTLA-4 Antibody, Is Effective against Tumors Poorly Responsive to Conventional Immunotherapy. *Cancer Discov* 2024;**14**(12):2407-29 doi 10.1158/2159-8290.Cd-24-0190.

28. Waight JD, Chand D, Dietrich S, Gombos R, Horn T, Gonzalez AM*, et al.* Selective FcγR Co-engagement on APCs Modulates the Activity of Therapeutic Antibodies Targeting T Cell Antigens. *Cancer Cell* 2018;**33**(6):1033-47.e5 doi 10.1016/j.ccell.2018.05.005.

29. O'Malley DM, Oaknin A, Monk BJ, Selle F, Rojas C, Gladieff L*, et al.* Phase II study of the safety and efficacy of the anti-PD-1 antibody balstilimab in patients with recurrent and/or metastatic cervical cancer. *Gynecol Oncol* 2021;**163**(2):274-80 doi 10.1016/j.ygyno.2021.08.018.

30. O'Malley DM, Neffa M, Monk BJ, Melkadze T, Huang M, Kryzhanivska A*, et al.* Dual PD-1 and CTLA-4 Checkpoint Blockade Using Balstilimab and Zalifrelimab Combination as Second-Line Treatment for Advanced Cervical Cancer: An Open-Label Phase II Study. *J Clin Oncol* 2022;**40**(7):762-71 doi 10.1200/jco.21.02067.

31. Gordon MS, Tsimberidou AM, Schlechter BL, Wilky BA, Fakih MG, Bullock AJ*, et al.* Emerging survival plateaus with botensilimab and balstilimab: Pan tumor data from a large phase 1b trial of advanced solid tumors. Oral Presentation at the European Society for Medical Oncology 2025 Annual Meeting Presentation #1517MO. Berlin, Germany.

32. Bullock AJ, Schlechter BL, Fakih MG, Tsimberidou AM, Grossman JE, Gordon MS*, et al.* Botensilimab plus balstilimab in relapsed/refractory microsatellite stable metastatic colorectal cancer: a phase 1 trial. *Nat Med* 2024;**30**(9):2558-67 doi 10.1038/s41591-024-03083-7.

33. Wilky BA, Schwartz GK, Gordon MS, El-Khoueiry AB, Bullock AJ, Henick B*, et al.* Botensilimab (Fc-enhanced anti-cytotoxic lymphocyte-association protein-4 antibody) Plus Balstilimab (anti-PD-1 antibody) in Patients With Relapsed/Refractory Metastatic Sarcomas. *J Clin Oncol* 2025;**43**(11):JCO2402524 doi 10.1200/JCO-24-02524.

34. Porter R, Bockorny B, Corr BR, Mahadevan D, Wilky BA, El-Khoueiry AB*, et al.* Botensilimab (Fc-enhanced anti–CTLA-4 antibody) plus balstilimab (anti–PD-1 antibody) in patients with treatment-refractory ovarian cancer. *J Immunother Cancer* 2025;**13**(12) doi 10.1136/jitc-2025-013222.

35. El-Khoueiry AB, Lieu CH, Abou-Alfa GK, Tsimberidou AM, Mahadevan D, Hanna DL*, et al.* A phase 1b study of botensilimab and balstilimab in treatment-refractory hepatocellular carcinoma. *Liver Cancer* 2026;Online ahead of publication. doi 10.1159/000551630.

36. Atkins MB, Azambuja AA, Robert C, Dummer R, Lebbé C, Nathan PD*, et al.* Botensilimab ± Balstilimab in Patients with Advanced Cutaneous Melanoma Refractory/Resistant to Anti–PD-(L)1 ± CTLA-4: A Phase 2 Trial. Poster #259. Poster presentation at the 2026 American Society of Clinical Oncology. Chicago, IL, US.

37. Sathe A, Mason K, Grimes SM, Zhou Z, Lau BT, Bai X*, et al.* Colorectal Cancer Metastases in the Liver Establish Immunosuppressive Spatial Networking between Tumor-Associated SPP1+ Macrophages and Fibroblasts. *Clin Cancer Res* 2023;**29**(1):244-60 doi 10.1158/1078-0432.Ccr-22-2041.

38. Liu S, Zhang Z, Wang Z, Liu C, Liang G, Xu T*, et al.* SPP1 Drives Colorectal Cancer Liver Metastasis and Immunotherapy Resistance by Stimulating CXCL12 Production in

Cancer-Associated Fibroblasts. *Cancer Res* 2026;**86**(1):58-79 doi 10.1158/0008-5472.Can-24-4916.

39. Faleck DM, Dougan M, Tello M, Grossman JE, Moss AC, Postow MA. Accelerating the Evolution of Immune-Related Enterocolitis Management. *J Clin Oncol* 2023;**41**(17):3110-5 doi 10.1200/jco.22.02914.

40. Schlechter BL, Fakih MG, Tsimberidou AM, Bullock AJ, Pimentel A, Sharma S*, et al.* Botensilimab plus balstilimab in an expanded cohort of 123 patients with metastatic microsatellite stable colorectal cancer and no active liver metastases. Poster presented at the European Society for Medical Oncology Gastrointestinal Cancers Symposium 2025 Poster 8P. Barcelona, Spain.

41. Hammond WA, Swaika A, Mody K. Pharmacologic resistance in colorectal cancer: a review. *Ther Adv Med Oncol* 2016;**8**(1):57-84 doi 10.1177/1758834015614530.

42. Hecht JR, Park YS, Tabernero J, Lee MA, Lee S, Virgili AC*, et al.* Zanzalintinib plus atezolizumab versus regorafenib in refractory colorectal cancer (STELLAR-303): a randomised, open-label, phase 3 trial. *Lancet* 2025;**406**(10517):2360-70 doi 10.1016/s0140-6736(25)02025-2.

43. Abu-Sbeih H, Ali FS, Wang X, Mallepally N, Chen E, Altan M*, et al.* Early introduction of selective immunosuppressive therapy associated with favorable clinical outcomes in patients with immune checkpoint inhibitor-induced colitis. *J Immunother Cancer* 2019;**7**(1):93 doi 10.1186/s40425-019-0577-1.

44. Johnson DH, Zobniw CM, Trinh VA, Ma J, Bassett RL, Jr., Abdel-Wahab N*, et al.* Infliximab associated with faster symptom resolution compared with corticosteroids alone for the management of immune-related enterocolitis. *J Immunother Cancer* 2018;**6**(1):103 doi 10.1186/s40425-018-0412-0.

45. Rimassa L, Chan SL, Sangro B, Lau G, Kudo M, Reig M*, et al.* Five-year overall survival update from the HIMALAYA study of tremelimumab plus durvalumab in unresectable HCC. *J Hepatol* 2025;**83**(4):899-908 doi 10.1016/j.jhep.2025.03.033.

46. Schadendorf D, Hodi FS, Robert C, Weber JS, Margolin K, Hamid O*, et al.* Pooled Analysis of Long-Term Survival Data From Phase II and Phase III Trials of Ipilimumab in Unresectable or Metastatic Melanoma. *J Clin Oncol* 2015;**33**(17):1889-94 doi 10.1200/jco.2014.56.2736.

47. Wei SC, Duffy CR, Allison JP. Fundamental Mechanisms of Immune Checkpoint Blockade Therapy. *Cancer Discov* 2018;**8**(9):1069-86 doi 10.1158/2159-8290.Cd-18-0367.

48. Fakih MG, Segal NH, Schlechter BL, André T, Pietrantonio F, Johnson B*, et al.* Preliminary results from a randomized, open-label, phase 2 study of botensilimab with or without balstilimab in refractory microsatellite stable metastatic colorectal cancer with no liver metastases. Oral Presentation at the 2025 American Society of Clinical Oncology Gastrointestinal Cancers Symposium Abstract 23. San Francisco, CA, US.

49. Delepine C, Bourdelais S, Mednick G, Keith JG, Driscoll E, Rozen A*, et al.* Systemic and tumor-microenvironment inflammation shape outcomes in patients with immunologically cold, treatment-refractory tumors treated with fc-enhanced anti–CTLA-4 botensilimab Poster presented at the Annual American Association for Cancer Research Immuno-Oncology Conference Poster B036. Los Angeles, CA, US.

Table 1. Demographics and baseline disease characteristics in all patients with MSS mCRC NLM (N=123)

	Overall N=123	BOT 1 mg/kg + BAL n=62	BOT 2 mg/kg + BAL n=61
Age, median (range)	56 (25–82)	56 (35–81)	57 (25–82)
Sex, n (%)			
Male	63 (51)	32 (52)	31 (51)
ECOG PS, n (%)			
0	56 (46)	33 (53)	23 (38)
1	67 (54)	29 (47)	38 (62)
Primary site, n (%)			
Colon	81 (66)	37 (60)	44 (72)
Rectal	42 (34)	25 (40)	17 (28)
Time from initial cancer diagnosis to first dose of study drug, months, median (range)	46.0 (6.1–208.1)	41.5 (6.1–208.1)	52.2 (9.5–181.2)
Time from metastatic disease to first dose of study drug, months, median (range)	33.9 (0.2–179.4)	28.5 (0.2–104.6)	39.9 (5.4–179.4)
Target lesions SOD, mm, median (range)	61 (10–220)	71 (10–220)	53 (10–181)
Sites of metastases, n (%)			
≥2 metastatic sites	85 (69)	44 (71)	41 (67)
Lung metastases	96 (78)	50 (81)	46 (75)
Lymph node metastases	54 (44)	27 (44)	27 (44)
Peritoneal metastases	48 (39)	23 (37)	25 (41)
Liver metastases status, n (%)[a]			
Treated liver metastases	20 (16)	3 (5)	17 (30)
No liver metastases	103 (84)	59 (95)	44 (72)
Number of prior lines of therapy			
Median (range)	3 (1–10)	3 (1–8)	4 (1–10)
≥3, n (%)	82 (67)	34 (55)	48 (79)
≥4, n (%)	57 (46)	20 (32)	37 (61)
≥5, n (%)	34 (28)	9 (15)	25 (41)
≥6, n (%)	21 (17)	6 (10)	15 (25)
Prior types of therapy, n (%)			
Anti–PD-(L)1±CTLA-4	18 (15)	6 (10)	12 (20)
≥1 regimen of regorafenib, trifluridine/tipiracil ± bevacizumab, or fruquintinib	37 (30)	12 (19)	25 (41)
Other characteristics			
MSS/pMMR, n (%)	123 (100)	62 (100)	61 (100)
TMB >10, Mut/Mb, n/N (%)[b,c]	6/77 (8)	4/41 (10)	2/36 (6)
TMB >25, Mut/Mb, n/N (%)[b,c]	0/77 (0)	0/41 (0)	0/36 (0)
RAS mutation, n/N (%)[c]	76/113 (67)	44/59 (75)	32/54 (59)
BRAF V600E mutation, n/N (%)[c]	1/60 (2)	1/23 (4)	0/37 (0)

BAL, balstilimab; BOT, botensilimab; *BRAF*, V-Raf murine sarcoma viral oncogene homolog b; CTLA-4, cytotoxic T-lymphocyte associated protein 4; ECOG, Eastern Cooperative Oncology Group; mCRC, metastatic colorectal cancer; MSS, microsatellite stable; Mut/Mb, mutations/megabase; NLM, no active liver metastases; PD-(L)1, programmed cell death (ligand) 1; pMMR, mismatch repair proficient; PS, performance status; *RAS*, rat sarcoma virus; SOD, sum of the diameter; TMB, tumor mutational burden.

Data cut off: December 13, 2025.

[a]All patients had no active liver metastases, which was defined as patients with definitively treated liver metastases or no history of liver metastases.

[b]TMB per tumor-based local clinical test.

683 [c]N represents the number of patients with available data from local clinical test.

Table 2. Safety overview, including the most common treatment-related imAEs and AEs (occurred in ≥10% of patients with MSS mCRC NLM) (N=123)

n (%)	Overall N=123		BOT 1 mg/kg + BAL n=62		BOT 2 mg/kg + BAL n=61	
Any TRAE	113 (92)		55 (89)		58 (95)	
Grade ≥3	51 (41)		22 (35)		29 (48)	
Leading to discontinuation of both BOT and BAL[a]	21 (17)		7 (11)		14 (23)	
Leading to death	0		0		0	
	Any Grade	**Grade ≥3**	**Any Grade**	**Grade ≥3**	**Any Grade**	**Grade ≥3**
Any treatment-related imAE[b]	72 (59)	37 (30)	28 (45)	15 (24)	44 (72)	22 (36)
Most common (≥10%)						
Diarrhea/colitis[c]	51 (41)	19 (15)	17 (27)	6 (10)	34 (56)	13 (21)
Most common (≥10%) TRAEs[d]	**Any Grade**	**Grade ≥3**	**Any Grade**	**Grade ≥3**	**Any Grade**	**Grade ≥3[e]**
Diarrhea	48 (39)	10 (8)	27 (44)	3 (5)	21 (34)	7 (12)
Fatigue	46 (37)	2 (2)	23 (37)	1 (2)	23 (38)	1 (2)
Pyrexia	30 (24)	4 (3)	16 (26)	2 (3)	14 (23)	2 (3)
Decreased appetite	27 (22)	0	16 (26)	0	11 (18)	0
Rash maculo-papular	27 (22)	2 (2)	14 (23)	1 (2)	13 (21)	1 (2)
Chills	26 (21)	0	9 (15)	0	17 (28)	0
Pruritus	26 (21)	0	13 (21)	0	13 (21)	0
Immune-mediated enterocolitis	24 (20)	10 (8)	5 (8)	3 (5)	19 (31)	7 (12)
Nausea	23 (19)	0	10 (16)	0	13 (21)	0
Colitis	22 (18)	6 (5)	10 (16)	3 (5)	12 (20)	3 (5)
ALT increased	21 (17)	3 (2)	12 (19)	2 (3)	9 (15)	1 (2)
Arthralgia	21 (17)	0	11 (18)	0	10 (16)	0
Anemia	18 (15)	1 (1)	9 (15)	1 (2)	9 (15)	0
AST increased	16 (13)	2 (2)	10 (16)	1 (2)	6 (10)	1 (2)
Headache	15 (12)	1 (1)	9 (15)	0	6 (10)	1 (2)
Myalgia	14 (11)	0	8 (13)	0	6 (10)	0
Rash	13 (11)	0	4 (7)	0	9 (15)	0
Blood ALP increased	11 (9)	0	4 (7)	0	7 (12)	0
Stomatitis	11 (9)	0	6 (10)	0	5 (8)	0

AEs, adverse events; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BAL, balstilimab; BOT, botensilimab; imAE, immune-mediated adverse event; mCRC, metastatic colorectal cancer; MSS, microsatellite stable; NLM, no active liver metastases; TRAE, treatment-related adverse event.

Data cut off: December 13, 2025.

[a]Related to both BOT and BAL.

[b]imAEs were medically adjudicated and defined as related to treatment, thought to have an immune mechanism, determined by the investigator to be immune-related, and/or treated with systemic steroids (of any dose) and/or other immunosuppressants or with thyroid replacement hormones. imAEs were identified from a prespecified list of preferred terms.

[c]Identified events of “diarrhea/colitis” (grouped term) included the preferred terms of immune-mediated enterocolitis, colitis, diarrhea, and autoimmune colitis.

[d]Preferred terms. Multiple preferred terms may have occurred in 1 patient during a given event.

[e]Among the TRAEs in this table, there was only 1 grade 4 event (1 patient with grade 4 colitis in the BOT 2 mg/kg + BAL arm).

Table 3. Efficacy overview in all patients with MSS mCRC NLM (N=123)

	Overall N=123	BOT 1 mg/kg + BAL n=62	BOT 2 mg/kg + BAL n=61
ORR, n (%) 95% CI	26 (21) 14–29	13 (21) 12–33	13 (21) 12–34
BOR, n (%)			
CR	3 (2)	0	3 (5)
PR	23 (19)	13 (21)	10 (16)
SD	59 (48)	26 (42)	33 (54)
PD	34 (28)	21 (34)	13 (21)
NE	4 (3)	2 (3)	2 (3)
Median DOR, months (95% CI)	NR (7.3–NR)	12.9 (3.2–NR)	NR (7.3–NR)
DCR at ≥6 weeks, n (%) 95% CI	85 (69) 60–77	39 (63) 50–75	46 (75) 63–86
CBR at ≥24 weeks, n (%) 95% CI	34 (28) 20–36	19 (31) 20–44	15 (25) 15–37
Median PFS, months (95% CI)	4.0 (2.8–4.1)	3.3 (2.7–4.4)	4.1 (2.8–5.1)
12-month PFS, % (95% CI)	23 (15–31)	24 (14–36)	21 (11–33)
18-month PFS, % (95% CI)	18 (11–26)	20 (10–31)	17 (8–29)
Median OS, months (95% CI)	21.2 (16.2–23.8)	20.2 (11.5–24.4)	21.2 (13.3–31.3)
18-month OS, % (95% CI)	57 (48–65)	55 (42–66)	59 (46–71)
24-month OS, % (95% CI)	41 (32–50)	39 (26–51)	43 (30–56)
30-month OS, % (95% CI)	37 (28–46)	37 (24–49)	38 (25–51)
36-month OS, % (95% CI)	33 (24–43)	37 (24–49)	32 (19–46)
Median follow-up, months (range)	20.2 (0.7–62.3)	19.8 (0.7–62.3)	20.7 (2.3–50.0)
Median follow-up (reverse KM), months (95% CI)	34.8 (28.2–38.1)	28.8 (27.6–36.2)	41.4 (28.4–43.2)

BAL, balstilimab; BOR, best overall response; BOT, botensilimab; CBR, clinical benefit rate; CR, complete response; DCR, disease control rate; DOR, duration of response; mCRC, metastatic colorectal cancer; MSS, microsatellite stable; NE, not evaluable; NLM, no active liver metastases; NR, not reached; ORR, objective response rate; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease.

Data cut off: December 13, 2025.

Figure Legends

Figure 1. (A) Best overall response, (B) response over time, and (C) duration of response in the overall population of patients with MSS mCRC NLM receiving either dose of BOT+BAL. Data cut off: December 13, 2025.

[a]n=121 patients with percent change from baseline in tumor measurement. [b]Response data for this patient were corrected due to documented database error identified after database lock; correction reflects validated source data.

Figure 2. (A) Progression-free survival and (B) overall survival in the overall population of patients with MSS mCRC NLM receiving either dose of BOT+BAL. Data cut off: December 13, 2025.

Figure 3. (A) Best overall response, (B) response over time, and (C) duration of response in patients with MSS mCRC NLM previously exposed to available late-line therapies[a] who received either dose of BOT+BAL. Data cut off: December 13, 2025.

[a]Received ≥1 prior regimen of regorafenib, trifluridine/tipiracil ± bevacizumab, or fruquintinib. This was an exploratory, post hoc subgroup analysis.

Figure 1

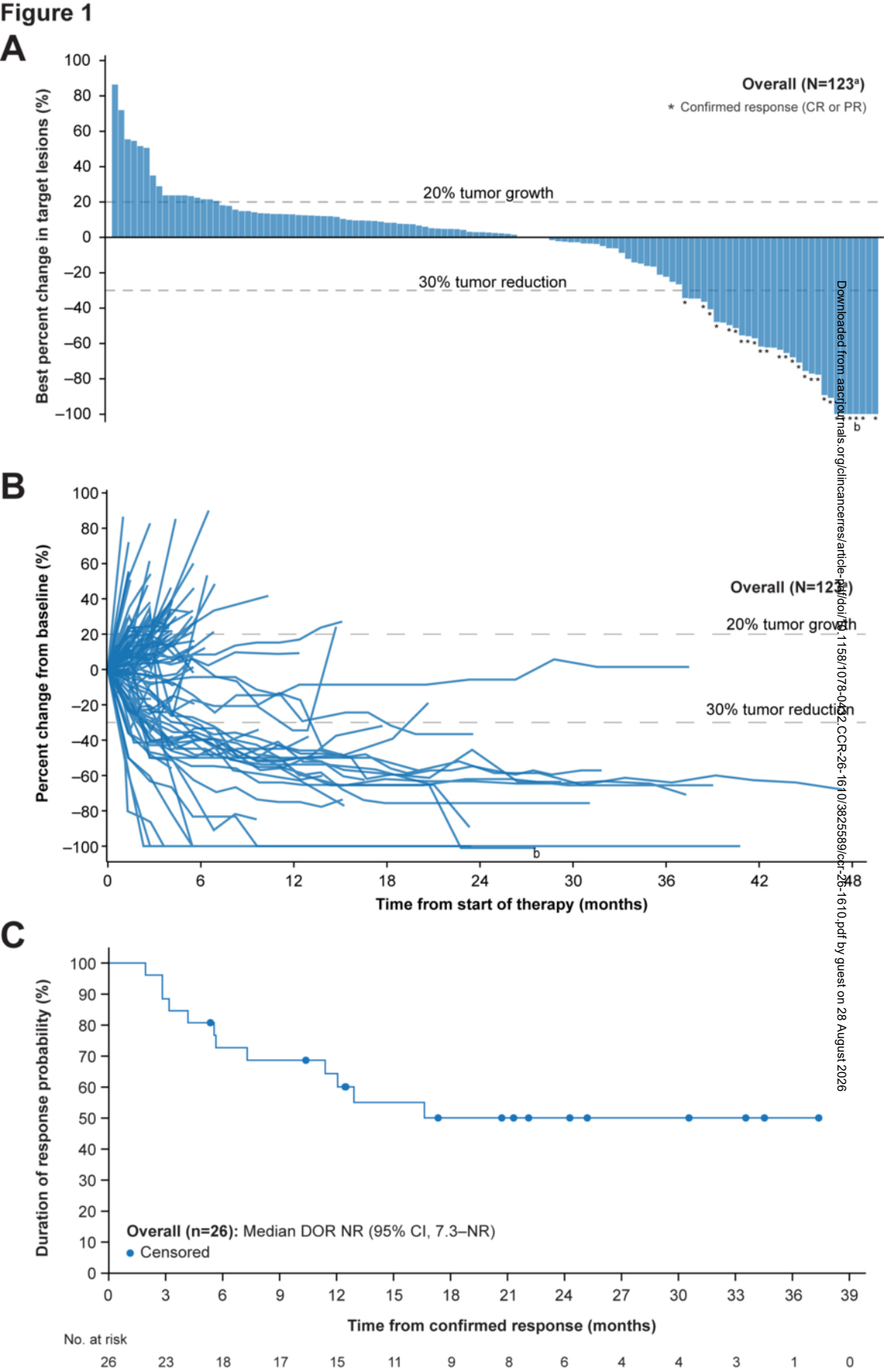
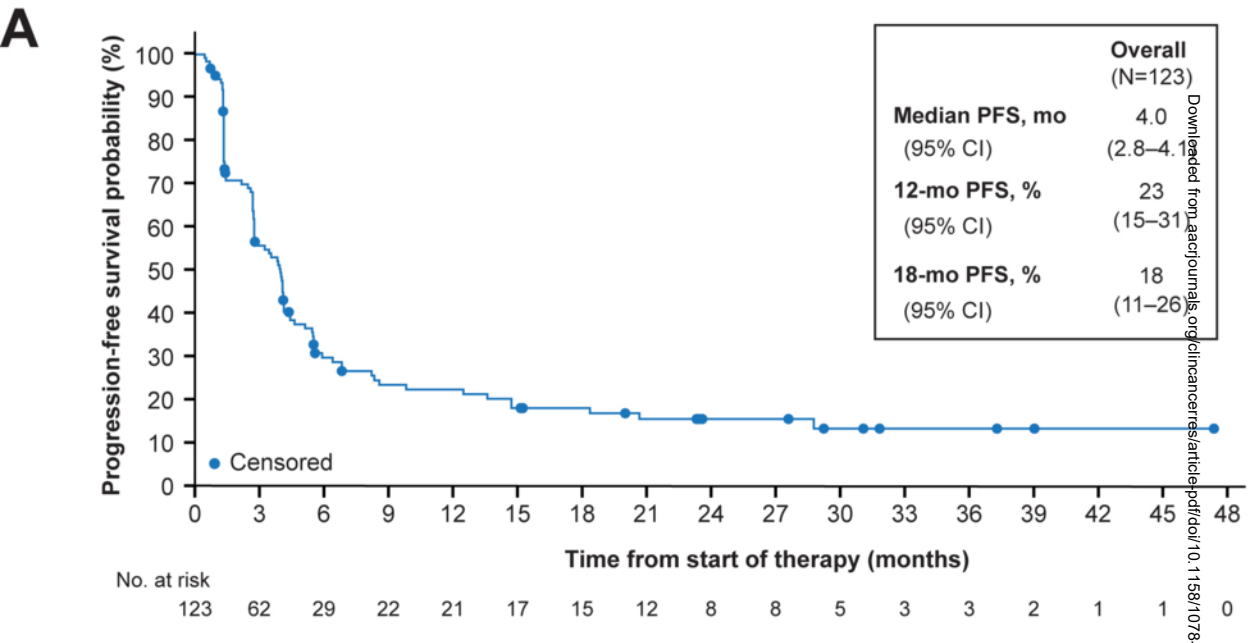


Figure 2



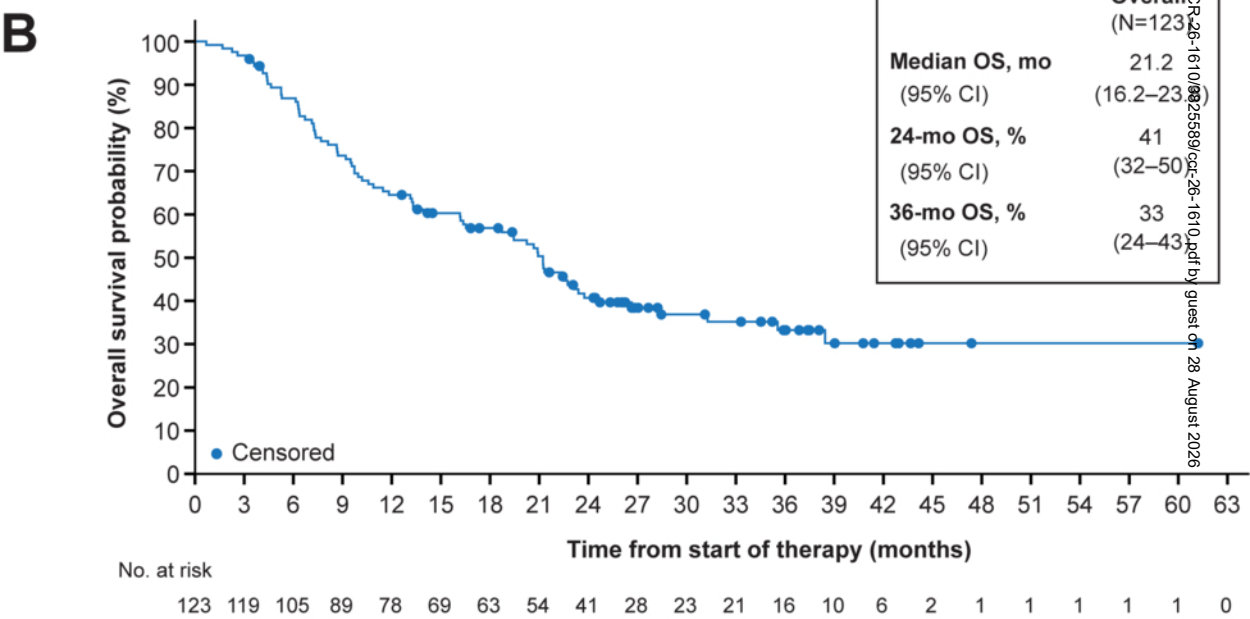


Figure 3