A phase 1b/2 study of the anti-CD47 antibody magrolimab with cetuximab in patients with colorectal cancer and other solid tumors

Submission to Targeted Oncology

Authors:
Cathy Eng,[1*] Nehal J. Lakhani,[2] Philip A. Philip,[3] Charles Schneider,[4] Benny Johnson,[5] Adel Kardosh,[6†] Mark P. Chao,[7] Amita Patnaik,[8] Fadi Shihadeh,[9] Yeonju Lee,[9] Kai Song,[9] Denise Jin,[9] Yanan Huo,[9] Michael Howland,[9] George A. Fisher,[6] J. Randolph Hecht[10]

[1]Division of Hematology/Oncology, Department of Internal Medicine, Vanderbilt University Medical Center, Nashville, TN, United States
[2]The START Center for Cancer Research, Grand Rapids, MI, United States
[3]Division of Hematology-Oncology, Department of Medicine, Karmanos Cancer Institute, Wayne State University, Detroit, MI, United States
[4]Abramson Cancer Center, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, Unites States
[5]Department of Gastrointestinal Medical Oncology, University of Texas MD Anderson Cancer Center, Houston, TX, United States
[6]Department of Medicine, Division of Medical Oncology, Stanford University School of Medicine, Stanford, CA, United States
[7]Forty Seven, Inc, Menlo Park, CA, United States
[8]The START Center for Cancer Research, San Antonio, TX, United States
[9]Gilead Sciences, Inc, Foster City, CA, United States
[10]Division of Hematology/Oncology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, Unites States

*Corresponding Author: cathy.eng@vumc.org
†Current Affiliation: Department of Medicine, Division of Medical Oncology, Oregon Health & Science University, Portland, OR, United States

Supplementary File 1 Supplementary methods

Adverse events excluded from the dose-limiting toxicity definition

The following adverse events were not considered dose-limiting toxicities (DLTs):

- Grade 3 anemia; however, Grade 3 hemolytic anemia that was medically significant, required hospitalization, prolonged an existing hospitalization, disabling, or limited selfcare activities of daily life was considered a DLT.
- Grade 3 indirect/unconjugated hyperbilirubinemia that resolved to Grade ≤2 with supportive care within 1 week and was not associated with other clinically significant consequences.
- Isolated Grade 3 electrolyte abnormalities that resolved to Grade ≤2 with supportive care within 1 week and were not associated with other clinically significant consequences.
- Grade 3 elevation in alanine aminotransferase, aspartate aminotransferase, or alkaline phosphatase that resolved to Grade ≤2 with supportive care within 1 week and was not associated with other clinically significant consequences.
- Grade 3 nausea, vomiting, or diarrhea that resolved to Grade ≤2 with supportive care within 72 hours.
- Grade 3 fatigue that resolved to Grade ≤2 within 2 weeks on study.
- Grade 3 magrolimab- or cetuximab-related infusion reactions in the absence of an optimal pretreatment regimen, which was defined as acetaminophen or a comparable nonsteroidal anti-inflammatory agent, plus an antihistamine and corticosteroids.
- Grade 3 tumor lysis syndrome or electrolyte disturbances (hyperkalemia, hypophosphatemia, or hyperuricemia) that resolved to Grade ≤2 or baseline within 1 week.
- Grade 3 hypomagnesemia that resolved to Grade ≤2 within 1 week.
- Grade 3 or 4 lymphopenia or leukopenia.

Pharmacokinetics and immunogenicity analyses

Blood samples for assessment of serum magrolimab concentrations were collected from patients according to the schedule in **Supplementary Table S6**. A validated sandwich enzyme-linked immunosorbent assay (ELISA) was used to measure serum concentrations of magrolimab. The range of quantitation of the method was 250 to 2500 ng/mL.

Peripheral blood for immunogenicity assessment was collected on C1D1, C1D8, and on Day 1 of each subsequent cycle, at the end-of-treatment visit, and at the safety follow-up visit. Blood samples were collected before administration of treatment dose if sample collection and treatment occurred on the same day. The presence of ADAs against magrolimab was determined by a validated electrochemiluminescent (ECL) assay.

Samples were analyzed using a multi-tiered approach. In an initial screening assay, samples with signal less than the screening cut-off were regarded as negative and not subjected to further analyses. Positive samples were retested in the presence of excess magrolimab. Samples with percent inhibition levels at or above the confirmatory cut point were defined as confirmed positive and evaluated in a titer assay. For patients who tested positive for ADAs, the impact of ADAs on PK, safety, and biologic activity was assessed. Neutralizing antibodies to magrolimab were also assessed for patients who tested positive for ADAs.

Tumor biopsies in the immunohistochemistry (IHC) analysis set

Tumor biopsies were obtained during both the phase 1b and 2 parts of the study. Participants signed the Institutional Review Board/Research Ethics Committee (IRB/REC)–approved informed

consent form (ICF) prior to participation in any study-specific procedure. In the phase 1b part, tumor biopsies were optional. Tumor biopsies were mandatory for phase 2 unless the Investigator determined otherwise.

A tumor biopsy was obtained prior to treatment (within 2 weeks before the first dose of drug) and on Cycle 2 Day 8 (±2 weeks). Patients were excluded from this analysis due to small tumor size that failed quality control (4 patients), missing screening biopsy (1 patient), and no available response data (1 patient).

IHC assays

Duplex chromogenic IHC assay of CD3 (Clone 2GV6, Roche Diagnostics) and CD8 (Clone SP57, Roche Diagnostics) was performed on the DISCOVERY ULTRA autostainer (Ventana Medical System) by sequentially applying primary antibodies with specific heat deactivation steps between for elution. Nuclei were visualized with hematoxylin II counterstain (Cat # 790-2208, Roche Diagnostics). CD3 and CD8 stains were detected using DISCOVERY Purple (Cat # 07053983001, Roche Diagnostics) and DISCOVERY Yellow (Cat # 08502641001, Roche Diagnostics), respectively. The CD68 (Clone PGM1, Abcam) IHC staining was performed using Leica BOND RX (Leica Biosystems). CD68 staining was visualized using 3,3-diaminobenzidine (DAB) as brown chromogen and nuclei using hematoxylin counterstain (Bond polymer refine detection; Cat # DS9800, Leica Biosystems). Staining results were qualitatively assessed by a pathologist.

Endpoint definitions

ORR was defined as the proportion of patients with a confirmed best response of complete remission (CR) or partial remission (PR) and was calculated in patients with measurable disease at baseline. DCR was defined as the percentage of patients with a confirmed best response of CR, PR, or stable disease (SD). DOR was defined for confirmed responders as the duration of time from the date when the first (objective) response was met until the first date of objectively documented progressive disease (PD).

PFS was defined as the duration of time from dose initiation until the first date of objectively documented PD per RECIST, version 1.1, or death due to any cause, whichever occurred first. OS was defined as the duration of time from dose initiation to the date of death due to any cause. Patients who did not die were censored at the date they were last known to be alive.

Treatment-emergent adverse events were defined as adverse events that started from the date of the first dose of any study drug up to 30 days after the date of the last dose of any study drug. Treatment-related adverse events were defined as any treatment-emergent adverse event assessed by the investigator to be related to a study drug.

Supplementary Table S1 Patient dose levels and schedule.

	Dose Cohort	Dose Levels in Cycle 1			Magrolimab Schedule in Cycle ≥2[a]
		Day 1 (Magrolimab Priming Dose)	Day 8 (Magrolimab Maintenance Dose and Cetuximab Loading Dose)	Day 15, 22 (Maintenance Dose)	
Phase 1b	1	Magrolimab 1 mg/kg	Magrolimab 10 mg/kg + cetuximab 300 mg/m^2	Magrolimab 10 mg/kg +cetuximab 200 mg/m^2	Every week
	2		Magrolimab 10 mg/kg + cetuximab 400 mg/m^2	Magrolimab 10 mg/kg + cetuximab 250 mg/m^2	
	3		Magrolimab 20 mg/kg + cetuximab 400 mg/m^2	Magrolimab 20 mg/kg + cetuximab 250 mg/m^2	
	4		Magrolimab 30 mg/kg + cetuximab 400 mg/m^2	Magrolimab 30 mg/kg + cetuximab 250 mg/m^2	
	5[b]		Magrolimab 45 mg/kg + cetuximab 400 mg/m^2	Magrolimab 45 mg/kg + cetuximab 250 mg/m^2	Cycle 2: every week Cycle ≥3: every 2 weeks
Phase 2	1: KRASwt	Magrolimab 1 mg/kg	Magrolimab 30 mg/kg + cetuximab 400 mg/m^2	Magrolimab 30 mg/kg + cetuximab 250 mg/m^2	Every 2 weeks
	2: KRASmt				
	3: KRASmt[b]		Magrolimab 45 mg/kg + cetuximab 400 mg/m^2	Magrolimab 45 mg/kg + cetuximab 250 mg/m^2	Cycle 2: every week Cycle ≥3: every 2 weeks

KRASmt, *KRAS* mutant; KRASwt, *KRAS* wild-type.

[a]Cetuximab was administered on a weekly schedule.

[b]A loading dose of magrolimab 45 mg/kg was administered on Cycle 1 Day 11.

Supplementary Table S2 Dose-limiting toxicities.

Phase	Cohort	Magrolimab Dose[a]	Cetuximab Dose[b]	No. of Patients, n (%)	No. of DLT-Evaluable Patients[c]	No. of Patients With DLTs
1b	1	10 mg/kg	200 mg/m^2	6	6	1 (16.7)
1b	2	10 mg/kg	250 mg/m^2	3	3	0
1b	3	20 mg/kg	250 mg/m^2	9	9	0
1b	4	30 mg/kg	250 mg/m^2	8	7	1 (14.3)
1b	5	45 mg/kg	250 mg/m^2	6	6	0
2	Safety run-In	30 mg/kg	250 mg/m^2	11	9	0

DLT, dose-limiting toxicity.

[a]Includes a priming dose of 1 mg/kg.

[b]Includes a loading dose of 400 mg/m^2 (patients in Phase1b Cohort 1 received a cetuximab loading dose of 300 mg/m^2).

[c]The DLT-evaluable analysis set included patients who received at least 1 dose of any study treatment and either experienced a DLT any time during the DLT assessment period (the first 4 weeks of treatment) or completed at least 4 infusions of magrolimab and 2 infusions of cetuximab at the assigned dose level.

Supplementary Table S3 Summary of magrolimab pharmacokinetic parameters by dose level.

Maintenance Dose	Dose Day	Pharmacokinetic Parameter			
		T_{max}, Day	**C_{max}, µg/mL**	**AUC_{last}, Day µg/mL**	**AUC_{τ}, Day µg/mL**
10 mg/kg (n = 9)	1 (Cycle 1 Day 1)	NA	1.02 ± 0.706 (69.5)[a] 0.629 ± 0.437 (69.5)[b]	NA	NA
	8 (Cycle 1 Day 8)	0.10 (0.09-0.13)	216 ± 71.2 (33.0)	676 ± 75.3 (11.1)	676 ± 75.3 (11.1)
	29 (Cycle 2 Day 1)	0.13 (0.10-1.09)	327 ± 101 (30.7)	1470 ± 586 (39.7)	1600 ± 491 (30.6)
	Accumulation ratio[c]	NA	1.51	2.17	2.37
20 mg/kg (n = 10)	1 (Cycle 1 Day 1)	NA	0.662 ± 0.374 (56.6)[b]	NA	NA
	8 (Cycle 1 Day 8)	0.12 (0.12-6.94)	455 ± 105 (23.0)	1530 ± 513 (33.4)	1630 ± 649 (39.9)
	29 (Cycle 2 Day 1)	0.13 (0.12-0.96)	728 ± 207 (28.5)	3330 ± 1270 (38.2)	3630 ± 1040 (28.7)
	Accumulation ratio[c]	NA	1.60	2.18	2.23
30 mg/kg (n = 38)	1 (Cycle 1 Day 1)	NA	0.496 ± 0.202 (40.7)[b]	NA	NA
	8 (Cycle 1 Day 8)	0.13 (0.08-6.82)	571 ± 143 (25.0)	2110 ± 668 (31.6)	2110 ± 668 (31.6)
	29 (Cycle 2 Day 1)	0.14 (0.10-0.15)	923 ± 185 (20.1)	4480 ± 1220 (27.2)	4480 ± 1220 (27.2)
	Accumulation ratio[c]	NA	1.62	2.12	2.12
45 mg/kg (n = 21)	1 (Cycle 1 Day 1)	NA	0.522 ± 0.292 (55.9)[b]	NA	NA
	8 (Cycle 1 Day 8)	0.12 (0.11-6.99)	970 ± 214 (22.1)	2920 ± 600 (20.6)	NC
	29 (Cycle 2 Day 1)	0.12 (0.12-0.13)	2050 ± 525 (25.6)	7340 ± 2380 (32.4)	8770 ± 1120 (12.8)
	Accumulation ratio[c]	NA	2.11	2.51	NC

Parameters are presented as arithmetic mean ± standard deviation (CV%), except T_{max}, which is presented as median (range). AUC_{last}, area under the concentration time curve from time 0 to the last on observed concentration; AUC_{τ}, area under the concentration time curve from time 0 to τ; C_{max}, the maximum observed concentration; N, total number of patients in the dose cohort; NA, not applicable; NC, not calculable; T_{max}, time of maximum observed concentration.

[a]C_{max} for Cycle 1 Day 1 was taken 15 minutes post-infusion timepoint after the 1 mg/kg priming dose on Cycle 1 Day 1.

[b]C_{max} for Cycle 1 Day 1 was taken 1 hour post infusion timepoint after the 1 mg/kg priming dose on Cycle 1 Day 1.

[c]Accumulation ratio was calculated from the Day 29 mean parameter value / Day 8 mean parameter value.

Supplementary Table S4 Anti-drug antibody prevalence and incidence by treatment cohort and dose level.

Dose Cohort	Magrolimab Maintenance Dose	ADA Prevalence		ADA Incidence					
		Patients Evaluated, n	Prevalence, n (%)	Patients Evaluated, n	Pre-Existing, n (%)[a]	Treatment-Boosted, n (%)	Treatment-Induced, n (%)	Persistent, n (%)	Transient, n (%)
Phase 1b Cohort 1	10 mg/kg	6	2 (33.3)	6	0	0	2 (33.3)	2 (33.3)	0
Phase 1b Cohort 2	10 mg/kg	3	0	3	0	0	0	0	0
Phase 1b Cohort 3	20 mg/kg	10	0	10	0	0	0	0	0
Phase 1b Cohort 4	30 mg/kg	7	1 (14.3)	7	1 (14.3)	0	0	0	0
Phase 1b Cohort 5	45 mg/kg	6	0	6	0	0	0	0	0
Phase 2 Cohort 1	30 mg/kg	16	2 (12.5)	16	1 (6.3)	0	1 (6.3)	0	1 (6.3)
Phase 2 Cohort 2	30 mg/kg	15	2 (13.3)	15	0	0	2 (13.3)	1 (6.7)	1 (6.7)
Phase 2 Cohort 3	45 mg/kg	15	0	14[b]	0	0	0	0	0

ADA, anti-drug antibody.

[a]1 patient was positive at baseline only; the other at baseline and at 1 or more post-treatment visits without treatment-boosted ADAs.

[b]1 patient was excluded from ADA prevalence evaluation due to missing baseline ADA result.

Supplementary Table S5 Patient disposition in the immunohistochemistry analysis.

	Cohort	No. of Patients Enrolled	IHC Analysis Set			
			No. of Patients IHC Performed	No. of Patients Included in IHC Analysis	BOR	
					SD	PD
Phase 1b[a]	5	6	1[b]	1[b]	0	1[b]
Phase 2	1: KRASwt	16	2	2	0	1[c]
	2: KRASmt	15	7	6[d]	2	4
	3: KRASmt	15	9	6[d]	2	4

BOR, best overall response; IHC, immunohistochemistry; KRASmt, *KRAS* mutant; KRASwt, *KRAS* wild-type; PD, progressive disease; SD, stable disease.

[a]No IHC analysis were performed on patients from Phase 1b Cohort 1–4.

[b]Patient with *KRAS* mutant tumor.

[c]No response was available from 1 patient.

[d]IHC quality control failed in samples from 4 patients due to small tumor area during biopsy.

Supplementary Table S6 Schedule of blood sampling for the pharmacokinetic analyses.

	C1						C2						C3		C4-C5	≥ C7	EOT	SFU
Day	**1**	**8**	**9**	**11**	**15**	**22**	**1**	**2**	**4**	**8**	**15**	**22**	**1**	**8**	**1**	**1**	**—**	**—**
Phase 1b																		
Before cetuximab infusion (within 12 h)		X			X	X	X						X	X	X[a]		X	X
Before magrolimab infusion (within 12 h)	X	X[b]			X[b]	X[b]	X[b]			X[b]	X[b]	X[b]	X[b]	X[b]	X[ab]	X[a]	X	X
1 h (±15 min) after magrolimab infusion	X	X			X	X	X			X			X	X	X[a]	X[a]		
24 h (±8 h) after magrolimab infusion (C1 Day 8 or C2 Day 1)			X					X										
72 h (±12 h) after magrolimab infusion (C1 Day 8 or C2 Day 1)				X[c]					X									
1 h (±15 min) after cetuximab infusion		X					X						X		X[a]			
Phase 2																		
Before cetuximab infusion (within 12 h)		X					X				X		X		X[d]			
1 h (±15 min) after cetuximab infusion		X			X		X						X		X[d]			
Before magrolimab infusion (within 12 h)	X	X[e]			X[e]		X[e]				X		X[e]		X[de]			
1 h (±15 min) after magrolimab infusion	X	X			X		X				X		X		X[d]			

C, cycle; EOT, end of treatment visit; PK, pharmacokinetic; SFU, safety follow-up visit.

[a]Starting with C5, the samples were collected every other cycle.

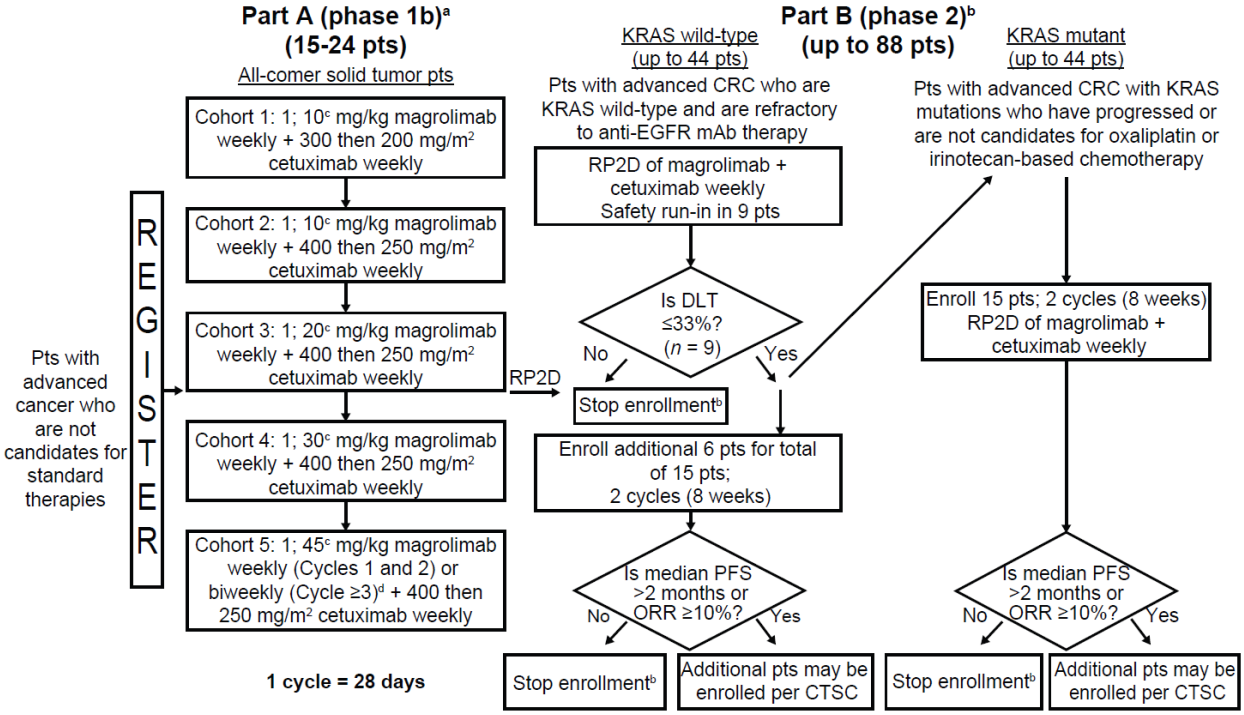
[b]The magrolimab sample may have been collected before the cetuximab infusion at same time as the "pre-cetuximab infusion" PK timepoint.

[c]The sample was collected before the magrolimab dose if the cohort utilized a magrolimab loading dose (ie, Phase 1b Cohort 5).

[d]Starting with C5, the samples were collected every fourth cycle (eg, C5, C9, C13, EOT, and SFU).

[e]The magrolimab sample was collected before the cetuximab infusion at same time as the "pre-cetuximab infusion" PK timepoint.

Supplementary Fig. S1 Study design diagram.



CRC, colorectal cancer; CTSC, clinical study steering committee; DLT, dose-limiting toxicity; EGFR, epidermal growth factor receptor; mAb, monoclonal antibody; ORR, objective response rate; PFS, progression-free survival; pts, patients; RP2D, recommended phase 2 dose.

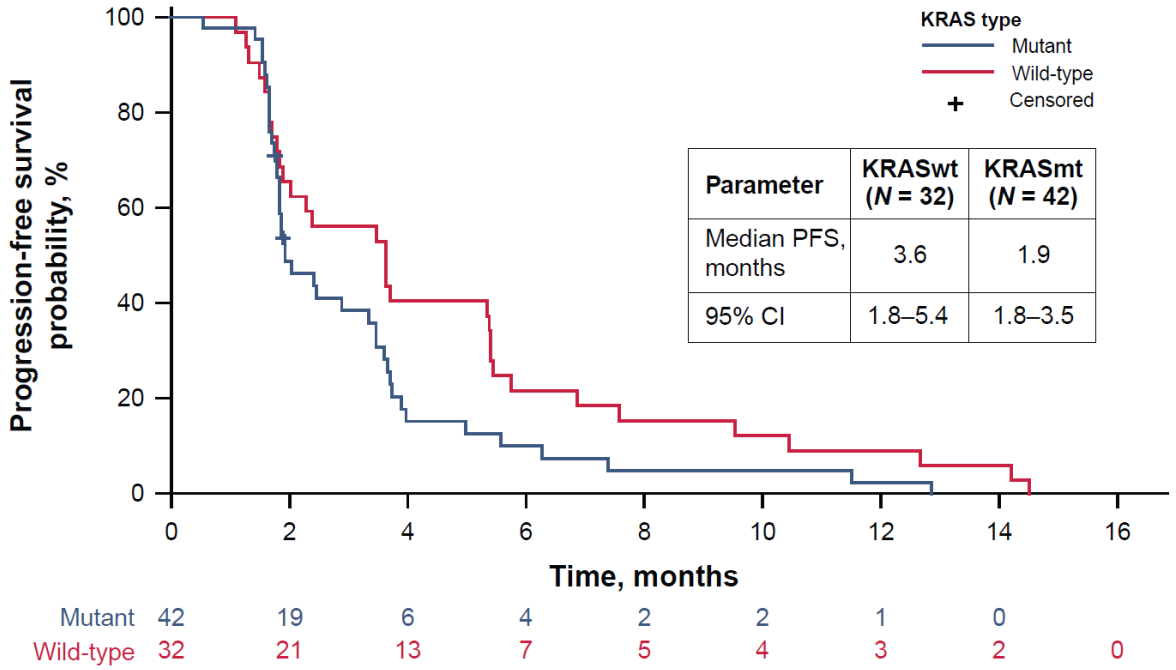
[a]A 3 + 3 dose escalation design.

[b]An alternative dose regimen may be evaluated in additional patients as determined by the CTSC. Once the initial safety run-in in 9 patients with *KRAS* wild-type cancer is completed and declared to be well-tolerated, a run-in phase with 9 patients with *KRAS* wild-type tumors will no longer be required if alternative phase 2 dose regimens are going to be tested.

[c]1, 10 mg/kg represents a first priming dose of 1 mg/kg followed by a maintenance dose of 10 mg/kg of magrolimab weekly thereafter, similarly for 1, 20 mg/kg, 1, 30 mg/kg, and 1, 45 mg/kg.

[d]Cohort 5 magrolimab dosing regimen will consist of 1 mg/kg priming dose on Day 1, followed by two 45 mg/kg doses in Cycle 1 Week 2 (Day 8 and loading dose Day 11), and then weekly maintenance doses of 45 mg/kg for the remainder of Cycle 1 and Cycle 2, then biweekly (Days 1 and 15) maintenance doses for Cycle 3 and beyond.

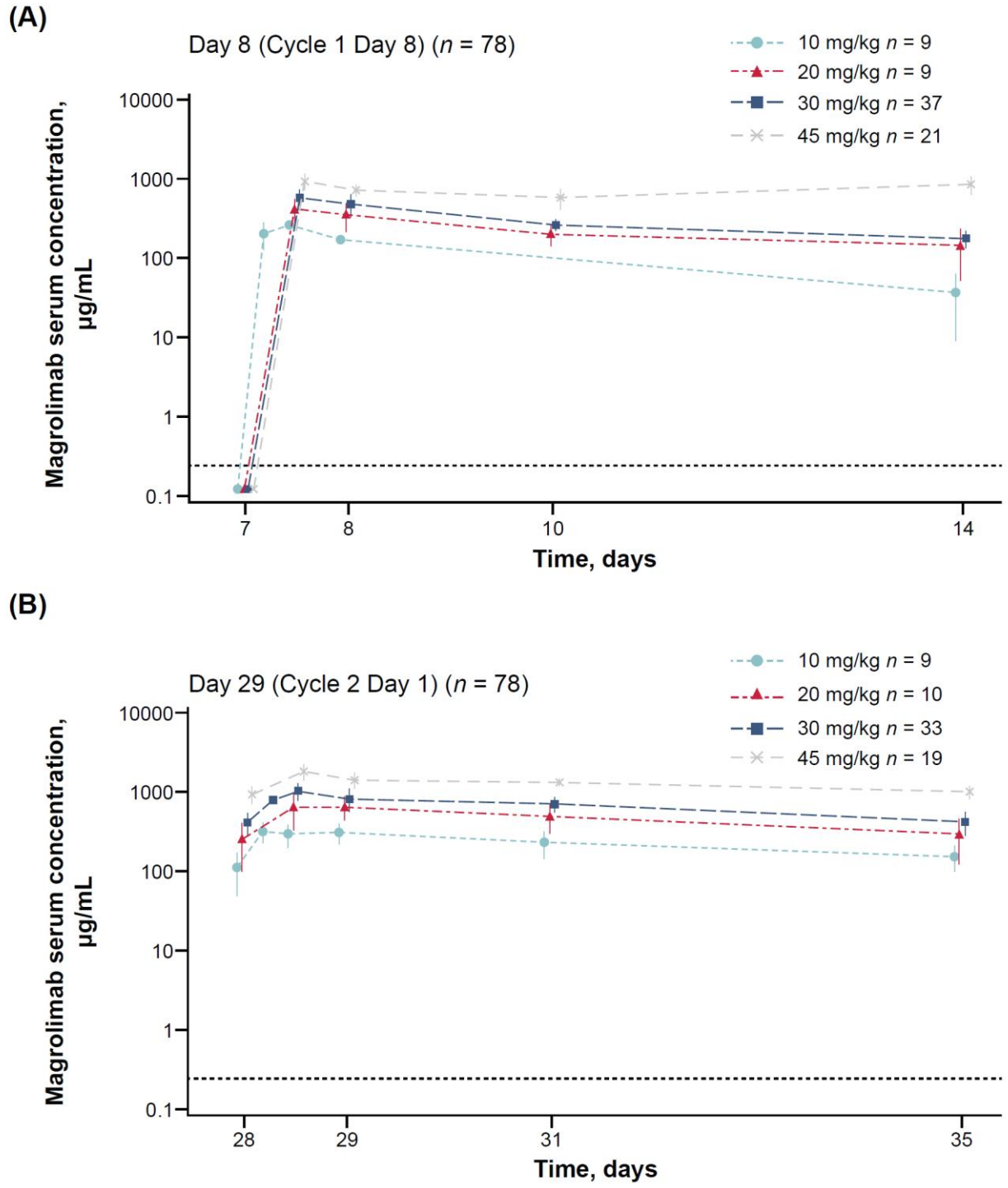
Supplementary Fig. S2 Kaplan-Meier curves for progression-free survival by *KRAS* type.



Parameter	KRASwt (*N* = 32)	KRASmt (*N* = 42)
Median PFS, months	3.6	1.9
95% CI	1.8–5.4	1.8–3.5

CI, confidence interval; KRASmt, *KRAS* mutant; KRASwt, *KRAS* wild-type; PFS, progression-free survival.

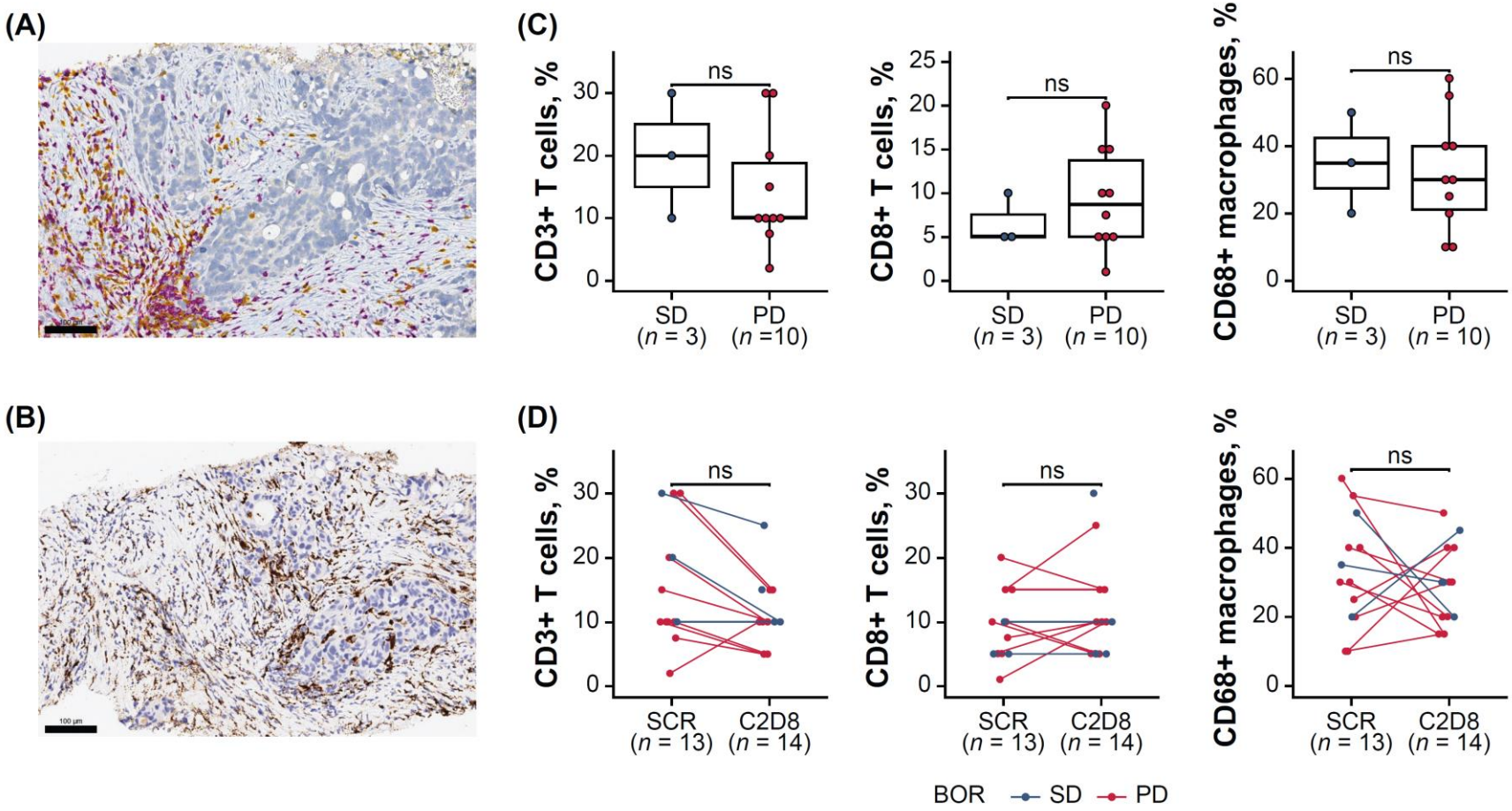
174 **Supplementary Fig. S3** Magrolimab serum concentration over time after weekly IV infusion of (A) Cycle
175 1 and (B) Cycle 2.



176
177 The black dashed horizon line represents the LLOQ value. Data below LLOQ (0.25 µg/mL) are plotted at
178 half of LLOQ for illustrative purposes only. LLOQ, lower limit of quantification; SD, standard deviation.

179

Supplementary Fig. S4 IHC analyses of metastatic site biopsies. (A) Representative IHC image of a biopsy from a metastatic site (abdominal lymph node) stained for CD3 (purple) and CD8 (yellow) T cells and nuclei (blue). (B) Representative IHC image of a biopsy from a metastatic site (abdominal lymph node) stained for CD68 (brown) macrophages and nuclei (blue). (C) Baseline immune cell infiltration (CD3, CD8, and CD68) in the tumor microenvironment stratified by treatment response. (D) Immune cell infiltration at screening and at C2D8 of treatment with magrolimab + cetuximab treatment.



The scale bars in (A) and (B) indicate 100 µm; nuclei were stained using hematoxylin. BOR, best overall response; C2D8, Cycle 2 Day 8; ns, not significant; PD, progressive disease; SCR, screening; SD, stable disease.