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SUPPLEMENTAL MATERIAL

A phase I, open-label, multicenter, first-in-human study to evaluate safety, pharmacokinetics, and efficacy of AMG 404, a PD-1 inhibitor, in patients with advanced solid tumors

FIGURE LEGENDS

Supplemental figure S1. Study design and treatment schema

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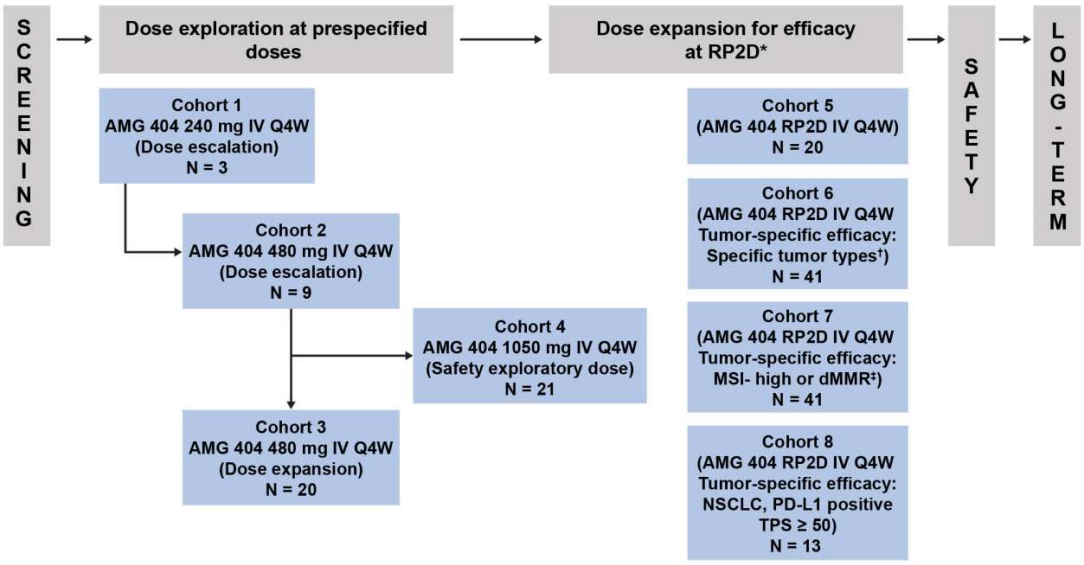
A phase I, open-label, multicenter, first-in-human study to evaluate safety, pharmacokinetics, and efficacy of AMG 404, a PD-1 inhibitor, in patients with advanced solid tumors



Per the study protocol, Cohort 5 was a China/Taiwan/Hong Kong-specific expansion cohort with a safety lead-in at one dose below the RP2D followed by evaluation at RP2D, as appropriate. Cohort 5 was excluded from this analysis and remaining cohorts were renumbered for clarity. Hence, the cohort numbers herein differ from those in the protocol.

One patient in the AMG 404 240 mg cohort had intra-patient dose escalation to 480 mg after cycle 4.

Price T, *et al. BMJ Open* 2025; 15:e088578. doi: 10.1136/bmjopen-2024-088578

Safety follow-up was 30 (+ 3) days and 140 days (± 7 days) after the last dose. Long-term follow-up was at 6 months (± 1 week) after the last dose to record data on survival, start of new therapies, and disease status.

*480 mg Q4W was selected as the RP2D.

†Cohort 6 included specific tumor types: melanoma; small cell lung cancer; NSCLC, PD-L1 positive; HNSCC, PD-L1 positive; urothelial, PD-L1 positive; gastric or GEJ adenocarcinoma, PD-L1 positive; esophageal, squamous, PD-L1 positive; cervical, PD-L1 positive; hepatocellular carcinoma; MCC; squamous cell carcinoma of the skin; RCC, clear cell; subtypes of sarcoma: undifferentiated pleiomorphic/malignant, fibrous histiocytoma, poorly differentiated and/or dedifferentiated, liposarcoma, alveolar soft tissue sarcoma, and angiosarcoma; thymic carcinoma; nasopharyngeal carcinoma (EBV positive); and mesothelioma.

‡Cohort 7 included MSI-H or dMMR: approximately 20 patients with mCRC.

dMMR = DNA mismatch repair–deficient; EBV = Epstein–Barr virus; GEJ = gastroesophageal junction; HNSCC = head and neck squamous cell carcinoma; IV = intravenous; MCC = Merkel cell carcinoma; mCRC = metastatic colorectal cancer; MSI-H = microsatellite instability–high; NSCLC = non–small cell lung cancer; PD-L1 = programmed death–ligand 1; Q4W = every 4 weeks; RCC = renal cell carcinoma; RP2D = recommended phase II dose; TPS = tumor proportion score.

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Supplemental figure S2. Association between TMB, TIS, and IFNγ biomarkers and clinical response

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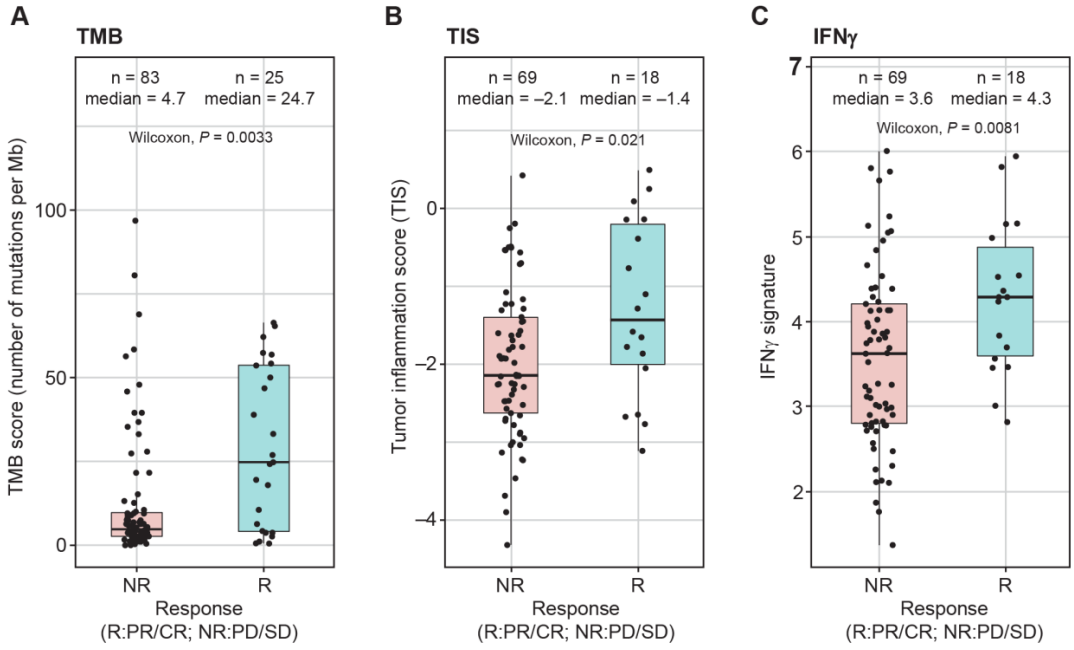

CR = complete response, IFNγ = interferon gamma; Mb = megabase; NR = non-responders; PD = progressive disease; PR = partial response, R = responders; SD = stable disease; TIS = tumor inflammation score; TMB = tumor mutational burden.

Supplemental table S1. List of Institutional Review Board (IRB) or Independent Ethics Committee (IEC) for all sites that enrolled patients

Official site name	IRB/IEC name	IRB/IEC address
The Queen Elizabeth Hospital	HREC: Central Adelaide Local Health Network Human Research Ethics Committee RGO: Central Adelaide Local Health Network (CALHN) Research Governance	HREC: Central Adelaide Local Health Network Human Research Ethics Committee Level 3, Roma Mitchell Building, 136 North Terrace, Adelaide, SA, 5000 RGO: CALHN Research Services Level 3, Roma Mitchell Building, 136 North Terrace, Adelaide, SA 5000
Chris O'Brien Lifehouse	HREC: Hunter New England Local Health District RGO: Chris O'Brien Lifehouse Research Governance	HREC: Hunter New England Local Health District Board Lookout Road, New Lambton NSW 2305 Australia RGO: Chris OBrien Lifehouse 119-143 Missenden Road, Camperdown NSW 2050 Australia
Universitair Ziekenhuis Antwerpen	Committee for Medical Ethics of UZA-UA	Drie Eikenstraat 655 2650 Edegem, Belgium
Hospital de Base de Sao Jose do Rio Preto	Comite de Etica em Pesquisa em Seres Humanos da Faculdade de Medicina de Sao Jose do Rio Preto	Avenida Brigadeiro Faria Lima 5512, Sao Pedro, Sao Jose do Rio Preto, Sao Paulo, 15090 000, Brazil
Sociedade Beneficente de Senhoras Hospital Sirio Libanes	Comite de Etica em Pesquisa da Sociedade Beneficiente de Senhoras Hospital Sirio Libanes	Rua Dona Adma Jafet 91, Bela Vista, 11º andar, salas 1117, 1118, Sao Paulo, Sao Paulo - 01308-050, Brazil
Hospital Sao Lucas da Pontificia Universidade Catolica do Rio Grande do Sul	Comitê de Ética em Pesquisa da Pontificia Universidade Catolica do Rio Grande do Sul	Avenida Ipiranga 6690, 3ºAndar, Sala 314, Jardim Botânico, Porto Alegre, Rio Grande do Sul, 90610-000, Brazil
Instituto Coi	Comite de Etica em Seres Humanos do Pesquisa do Hospital Pro Cardiaco	Rua General Polidoro,192 – Botafogo – Rio de Janeiro-RJ – CEP 22290-003
Nucleo de Oncologia da	Comite de Etica do Hospital	Avenida Bonfim 161, Largo

Official site name	IRB/IEC name	IRB/IEC address
Bahia	Santo Antonio Obras Sociais Irma Dulce	de Roma, Salvador, Bahia, 40420-000, Brazil
Tom Baker Cancer Centre	Health Research Ethics Board of Alberta The Cancer Committee	10104 103 Avenue Northwest, Suite 1500, Edmonton, AB, T5J 0H8, Canada
National Hospital Organization Shikoku Cancer Center	National Hospital Organization Shikoku Cancer Center Institutional Review Board	160 Kou minamiumemotomachi, Matsuyama-shi, Ehime, 791-0280, Japan
Wakayama Medical University Hospital	Wakayama Medical University Institutional Review Board	811-1 Kimiidera, Wakayama-shi, Wakayama, 641-8510, Japan
National Cancer Center Hospital East	National Cancer Ctr IRB 2-J	5-1-1 Tsukiji, Chuo-ku, Tokyo, 104-0045, Japan
Narodowy Instytut Onkologii im Marii Skłodowskiej-Curie - Państwowy Instytut Badawczy	Komisja Bioetyczna przy Narodowym Instytucie Onkologii im. Marii Skłodowskiej-Curie – Państwowy Instytut Badawczy w Warszawie	ul. W. K. Roentgena 5, 02-781, Warszawa, Poland
Medical University of Gdansk	Komisja Bioetyczna przy Narodowym Instytucie Onkologii im. Marii Skłodowskiej-Curie – Państwowy Instytut Badawczy w Warszawie	ul. W. K. Roentgena 5, 02-781, Warszawa, Poland
National University Hospital	Domain Specific Review Board (DSRB)	c/o National Healthcare Group, NHG Group Research, Nexus @One-North (South Tower), No.3 Fusionopolis Link, #03-08, Singapore 138543
National Cancer Centre Singapore	Domain Specific Review Board (DSRB)	c/o National Healthcare Group, NHG Group Research, Nexus @One-North (South Tower), No.3 Fusionopolis Link, #03-08, Singapore 138543
Severance Hospital Yonsei University Health System	Severance Hospital, Institutional Review Board	2nd Floor, JeJung Building, 50-1, Yonsei-ro, Seodaemun-gu, Seoul, Republic of Korea
Samsung Medical Center	Samsung Medical Center Institutional Review Board	81, Irwon-ro, Gangnam-gu, Seoul, Republic of Korea

Official site name	IRB/IEC name	IRB/IEC address
Asan Medical Center	Asan Medical Center Institutional Review Board	Asan Medical Center Institutional Review Board, Convergence Innovation Bldg. 88, Olympic-ro 43-gil, Songpa-gu, Seoul, Republic of Korea
Hospital Universitari Vall d Hebron	CEIm del Hospital Universitario la Paz	
Hospital Universitario La Paz	CEIm del Hospital Universitario la Paz	Pº Castellana 261. 28046 Madrid- España
Linkou Chang Gung Memorial Hospital of Chang Gung Medical Foundation	Chang Gung Medical Foundation institutional review board	No. 5, Fuxing Street, Gueishan District, Taoyuan 33305, Taiwan
Doktor Abdurrahman Yurtaslan Ankara Onkoloji Egitim ve Arastirma Hastanesi	Ege University Faculty of Medicine Clinical Research Ethics Committee	Ege Universitesi Tip Fakultesi Dekanligi, 2.Kat Erzene Ankara Caddesi, Bornova, 35100, Izmir, Turkiye
Sarah Cannon Research Institute UK	South Central – Oxford B Research Ethics Committee	South Central – Oxford B Research Ethics Committee Health Research Authority Level 3, Block B, Whitefriars Lewins Mead Bristol BS1 2NT
University of Texas MD Anderson Cancer Center	The University of Texas MD Anderson Cancer Center Institutional Review Board	7007 Bertner Avenue, Unit 1637, Houston, TX, 77030, USA
Sarah Cannon Research Institute at HealthONE	IntegReview IRB	3815 South Capital of Texas Highway, Suite 320, Austin, TX, 78704, USA
Wake Forest University Health Sciences	Wake Forest University Health Sciences IRB	Medical Center Boulevard, Institutional Review Board, The Office of Research and Development, Winston-Salem, NC, 27157, USA
Sarcoma Oncology Research Center LLC	WCG, Western Institutional Review Board Copernicus Group, IRB	1019 39th Avenue Southeast, Suite 120, Puyallup, WA, 98374, USA

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Supplemental table S2. Details and numbering of cohorts

Cohort details	Protocol and ClinicalTrails.gov cohort number	Manuscript cohort number
AMG 404 240 mg IV Q4W dose escalation	1	1
AMG 404 480 mg IV Q4W dose escalation	2	2
AMG 404 480 mg IV Q4W dose expansion	3	3
AMG 404 1050 mg IV Q4W safety exploratory dose	4	4
AMG 404 RP2D dose expansion in China/Taiwan/Hong Kong	5	Not included
AMG 404 RP2D dose expansion	6	5
AMG 404 RP2D dose expansion in specific tumor types	7	6
AMG 404 RP2D dose expansion in MSI-H or dMMR tumors	8	7
AMG 404 RP2D dose expansion in NSCLC, PD-L1 positive, TPS ≥ 50%	9	8

dMMR = DNA mismatch repair–deficient; IV = intravenous; MSI-H = microsatellite instability–high; NSCLC = non–small cell lung cancer; PD-L1 = programmed death–ligand 1; Q4W = every 4 weeks; RP2D = recommended phase II dose; TPS = tumor proportion score.

Supplemental table S3. Summary of TEAEs regardless of attribution

	AMG 404 240 mg (n = 3)	AMG 404 480 mg (n = 144)	AMG 404 1050 mg (n = 21)	All dose cohorts (N = 168)
All TEAEs	3 (100.0)	143 (99.3)	21 (100.0)	167 (99.4)
Grade 1	0	9 (6.3)	1 (4.8)	10 (6.0)
Grade 2	1 (33.3)	60 (41.7)	5 (238)	66 (39.3)
Grade 3	0	37 (25.7)	4 (19.0)	41 (24.4)
Grade 4	1 (33.3)	3 (2.1)	3 (14.3)	7 (4.2)
Fatal	1 (33.3)	34 (23.6)	8 (38.1)	43 (25.6)
Serious	2 (66.7)	75 (52.1)	15 (71.4)	92 (54.8)
Leading to discontinuation of AMG 404	0	10 (6.9)	2 (9.5)	12 (7.1)
TEAEs in ≥ 10% of patients in any cohort				
Fatigue	2 (66.7)	49 (34.0)	7 (33.3)	58 (34.5)
Nausea	2 (66.7)	41 (28.5)	5 (23.8)	48 (28.6)
Decreased appetite	1 (33.3)	34 (23.6)	8 (38.1)	43 (25.6)
Constipation	1 (33.3)	25 (17.4)	4 (19.0)	30 (17.9)
Diarrhea	0	28 (19.4)	2 (9.5)	30 (17.9)
Pyrexia	0	26 (18.1)	4 (19.0)	30 (17.9)
Anaemia	1 (33.3)	24 (16.7)	3 (14.3)	28 (16.7)
Vomiting	1 (33.3)	24 (16.7)	2 (9.5)	27 (16.1)
Arthralgia	1 (33.3)	21 (14.6)	3 (14.3)	25 (14.9)
Pruritus	1 (33.3)	18 (12.5)	4 (19.0)	23 (13.7)
Rash	0 (0.0)	20 (13.9)	3 (14.3)	23 (13.7)
Abdominal pain	1 (33.3)	16 (11.1)	4 (19.0)	21 (12.5)
Dyspnea	0	20 (13.9)	1 (4.8)	21 (12.5)

	AMG 404 240 mg (n = 3)	AMG 404 480 mg (n = 144)	AMG 404 1050 mg (n = 21)	All dose cohorts (N = 168)
Back pain	0	17 (11.8)	3 (14.3)	20 (11.9)
Hypothyroidism	0	14 (9.7)	3 (14.3)	17 (10.1)
Insomnia	0	14 (9.7)	3 (14.3)	17 (10.1)
AST increased	0	16 (11.1)	0	16 (9.5)
COVID-19	0	16 (11.1)	0	16 (9.5)
Headache	0	12 (8.3)	3 (14.3)	15 (8.9)
Cough	1 (33.3)	12 (8.3)	1 (4.8)	14 (8.3)
Dizziness	0	13 (9.0)	1 (4.8)	14 (8.3)
Myalgia	0	11 (7.6)	3 (14.3)	14 (8.3)
Urinary tract infection	0	13 (9.0)	1 (4.8)	14 (8.3)
Upper respiratory tract infection	0	10 (6.9)	2 (9.5)	12 (7.1)
ALT increased	0	11 (7.6)	0	11 (6.5)
Weight decreased	0	9 (6.3)	2 (9.5)	11 (6.5)
Abdominal pain upper	0	7 (4.9)	3 (14.3)	10 (6.0)
Edema peripheral	1 (33.3)	8 (5.6)	1 (4.8)	10 (6.0)
Malaise	0	7 (4.9)	2 (9.5)	9 (5.4)
Abdominal distension	1 (33.3)	7 (4.9)	0	8 (4.8)
Chest pain	0	8 (5.6)	0	8 (4.8)
Pleural effusion	1 (33.3)	5 (3.5)	2 (9.5)	8 (4.8)
Ascites	1 (33.3)	4 (2.8)	0	5 (3.0)
Fall	1 (33.3)	4 (2.8)	0	5 (3.0)
Sepsis	0 (0.0)	2 (1.4)	3 (14.3)	5 (3.0)
Confusional state	1 (33.3)	3 (2.1)	0	4 (2.4)
Hyperhidrosis	1 (33.3)	3 (2.1)	0	4 (2.4)
Bone pain	1 (33.3)	2 (1.4)	0	3 (1.8)

	AMG 404 240 mg (n = 3)	AMG 404 480 mg (n = 144)	AMG 404 1050 mg (n = 21)	All dose cohorts (N = 168)
Hypoxia	1 (33.3)	1 (0.7)	1 (4.8)	3 (1.8)
Hiccups	1 (33.3)	1 (0.7)	0	2 (1.2)
Angioedema	1 (33.3)	0	0	1 (0.6)
Death	1 (33.3)	0	0	1 (0.6)
Photophobia	1 (33.3)	0	0	1 (0.6)

Data are presented as number (%) of patients.

The safety analysis set comprised all patients who received ≥ 1 dose of AMG 404.

One patient in the AMG 404 240 mg cohort had intra-patient dose escalation to 480 mg after cycle 4.

A TEAE was defined as starting on or after the first administration of AMG 404 and up to and including 140 days after the last dose or end of the study, whichever occurred earlier.

For patients with multiple events under the same category, only the worst grade is reported.

AEs were coded using MedDRA v25.0.

AE = adverse event; ALT = alanine aminotransferase; AST = aspartate aminotransferase; COVID-19 = coronavirus disease 2019; MedDRA = Medical Dictionary for Regulatory Activities; TEAE = treatment-emergent AE.

Supplemental table S4. Tumor response to AMG 404 per modified RECIST v1.1 in solid tumors by tumor type

	Cohorts 1–5 Any tumor type (n = 73)	Cohort 6 Selected tumor types[a] (n = 41)	Cohort 7 MSI-H tumors[b] (n = 41)	Cohort 8 NSCLC, PDL1-H (n = 13)	All cohorts (N = 168)
Complete response (CR)	2 (2.7)	0	2 (4.9)	0	4 (2.4)
Partial response (PR)	5 (6.8)	7 (17.1)	13 (31.7)	4 (30.8)	29 (17.3)
Stable disease (SD)	26 (35.6)	13 (31.7)	14 (34.1)	6 (46.2)	59 (35.1)
Progressive disease (PD)	35 (47.9)	18 (43.9)	7 (17.1)	1 (7.7)	61 (36.3)
Not evaluable (NE)	2 (2.7)	1 (2.4)	0	0	3 (1.8)
Not done	3 (4.1)	2 (4.9)	5 (12.2)	2 (15.4)	12 (7.1)
Complete response (CR) rate	2 (2.7)	0	2 (4.9)	0	4 (2.4)
80% CI[c]	(0.7–7.1)	(0–5.5)	(1.3–12.5)	(0–16.2)	(1.0–4.7)
Objective response (CR/PR) rate	7 (9.6)	7 (17.1)	15 (36.6)	4 (30.8)	33 (19.6)
80% CI[c]	(5.4–15.6)	(9.8–27.0)	(26.4–47.8)	(14.2–52.3)	(15.7–24.1)
Disease control rate (CR/PR/SD)	33 (45.2)	20 (48.8)	29 (70.7)	10 (76.9)	92 (54.8)
80% CI[c]	(37.2–53.4)	(37.8–59.9)	(59.8–80.1)	(55.6–91.2)	(49.5–59.9)
PFS, median months	2.8	3.1	14.8	4.4	3.7
80% CI[d]	(1.9–3.7)	(1.9–4.3)	(9.0–NE)	(2.2–9.7)	(3.5–4.5)

Data are presented as number (%) of patients, unless indicated otherwise.

One patient in the AMG 404 240 mg cohort had intra-patient dose escalation to 480 mg after cycle 4.

[a]Selected tumor types in Cohort 6 included melanoma; small cell lung cancer; NSCLC, PD-L1 positive; HNSCC, PD-L1 positive; urothelial, PD-L1 positive; gastric or GEJ adenocarcinoma, PD-L1 positive; esophageal, squamous, PD-L1 positive; cervical, PD-L1 positive; hepatocellular carcinoma; MCC; squamous cell carcinoma of the skin; RCC, clear cell; subtypes of sarcoma: undifferentiated pleiomorphic/malignant, fibrous histiocytoma, poorly differentiated and/or dedifferentiated, liposarcoma, alveolar soft tissue sarcoma, and angiosarcoma; thymic carcinoma; nasopharyngeal carcinoma (EBV positive); and mesothelioma.

[b]MSI-H or dMMR cohort included approximately 20 patients with mCRC.

[c]Binomial proportion with exact 80% CI.

[d]Calculated using the Brookmeyer and Crowley method.

DCR was defined as the proportion of patients in whom ORR (CR or PR) or SD was determined as per modified RECIST v1.1.

ORR was defined as a tumor response assessment of either CR or PR measured by PET/CT, CT, or MRI and assessed per modified RECIST v1.1.

The safety analysis set comprised all patients who received ≥ 1 dose of AMG 404.

CI = confidence interval; CT = computed tomography; DCR = disease control rate; dMMR = DNA mismatch repair–deficient; EBV = Epstein–Barr virus; GEJ = gastroesophageal junction; HNSCC = head and neck squamous cell carcinoma; MCC = Merkel cell carcinoma; mCRC = metastatic colorectal cancer; MRI = magnetic resonance imaging; MSI-H = microsatellite instability–high; NE = not evaluable; NSCLC = non–small cell lung cancer; ORR = objective response rate; PD-L1 = programmed death–ligand 1; PET = positron emission tomography; PFS = progression-free survival; RCC = renal cell carcinoma; RECIST = Response Evaluation Criteria in Solid Tumours.

Supplementary Table S5. Biomarker ascertainment by cohort

Cohort	Dosage	PD-L1 TC	MSI	TIS	IFNγ	TMB
Cohort 1	240 mg	1	3	3	3	3
Cohort 2	480 mg	8	7	5	5	7
Cohort 3	480 mg	15	14	10	10	14
Cohort 4	1050 mg	10	10	10	10	11
Cohort 5	480 mg	12	14	11	11	11
Cohort 6	480 mg	14	34	28	28	32
Cohort 7	480 mg	26	41	21	21	34
Cohort 8	480 mg	11	9	4	4	9

Data are presented as the number of patients, with individual biomarker result reported in each cohort.

IFNγ = interferon gamma; MSI = microsatellite instability; PD-L1 = programmed death–ligand 1; TC = tumor cell; TIS = tumor inflammation score; TMB = tumor mutational burden.

SUPPLEMENTAL METHODS AND STUDY OVERSIGHT

Study design and treatment

The study comprised a screening period of ≤ 28 days, a treatment period of ≤ 2 years, and a follow-up period of 6 months after treatment completion. The study duration for an individual patient was approximately ≤ 2.5 years. Patients were not dosed at the current dose level or higher when the modified toxicity probability interval (mTPI) model recommended stopping enrollment at the current dose level, and patients were not dosed at an escalated dose level when the mTPI recommended staying at the current dose level. The Dose Level Review Team could recommend escalation to the next planned dose, escalation to an intermediate dose (a dose lower than the next planned dose), continuation or delay in dosing, repetition or expansion of a cohort, de-escalation to a lower dose, or termination of the study. If treatment was completed or discontinued for reasons other than progression, tumor evaluations continued until progression or the start of a new treatment regimen. Patients could continue treatment beyond initial progressive disease (PD) if the conditions for continuation were met. If a patient required corticosteroid dosing of > 10 mg prednisone daily (or equivalent) as supportive care, AMG 404 dosing was withheld until the corticosteroid dose was reduced to < 10 mg prednisone daily (or equivalent). If treatment was discontinued before PD (e.g., due to unacceptable toxicity) per modified Response Evaluation Criteria in Solid Tumours (RECIST) v1.1, tumor assessments continued until PD as determined by the investigator, consent withdrawal, or start of another anticancer therapy.

Patients

Inclusion criteria

Patients were eligible to participate in the study only if all of the following criteria applied:

- Patient had provided informed consent prior to initiation of any study-specific activities/procedures.
- Age ≥ 18 years at the time of signing informed consent.
- Life expectancy of > 3 months, in the opinion of the investigator.

- Patient must have histologically or cytologically confirmed metastatic or locally advanced solid tumors not amenable to curative treatment with surgery or radiation. Additionally, for:
    - Cohort 6: Patients must have a tumor as specified in the Study Schema.
    - Cohort 7: Patient must be microsatellite instability–high (MSI-H) or DNA mismatch repair–deficient (dMMR).
    - Cohort 8: Patient must have non-small cell lung cancer (NSCLC), PD-L1 positive, tumor proportion score ≥ 50%; not have *EGFR*, *ALK*, or *ROS1* genomic tumor aberrations and may not have received prior systemic treatment for the advanced disease (prior neoadjuvant, adjuvant, or concurrent chemoradiation was allowed).
- At least 1 measurable lesion as defined by modified RECIST v1.1, which had not undergone biopsy within 3 months of the screening scan. This lesion was not permitted to be biopsied at any time during the study. If there was only one lesion available for biopsy and radiographic assessment, it could be permitted to be biopsied after discussion with sponsor.
- Patients with treated brain metastases were eligible provided they met the following criteria:
    - Definitive therapy was completed at least 2 weeks prior to enrollment.
    - No evidence of radiographic central nervous system (CNS) progression or CNS disease following definitive therapy and by the time of study screening. Patients manifesting progression in lesions previously treated with stereotactic radiosurgery may still be eligible if pseudoprogression was demonstrated by appropriate means and after discussion with the medical monitor.
    - Any CNS disease that was asymptomatic, any neurologic symptoms due to CNS disease that returned to baseline or was deemed irreversible, the patient was off steroids for at least 7 days (physiologic doses of steroids were permitted), and the patient was off or on stable doses of antiepileptic drugs for malignant CNS disease.

- Eastern Cooperative Oncology Group performance status of ≤ 2.
- Hematologic function, as follows without growth factor support within 2 weeks prior to study day 1: absolute neutrophil count ≥ 1.0 x 10^9/L; platelet count ≥ 75 x 10^9/L; hemoglobin ≥ 9 g/dL (90 g/L).
- Adequate renal laboratory assessments, as follows: estimated glomerular filtration rate based on MDRD (Modification of Diet in Renal Disease) calculation ≥ 60 ml/min/1.73 m^2 for Cohorts 1, 2, and 4. Estimated glomerular filtration rate based on MDRD calculation ≥ 45 mL/min/1.73 m^2 for Cohorts 3, 5, 6, 7 and 8.
- Hepatic function, as follows: total bilirubin less than or equal to 1.5 x upper limit of normal (ULN) or less than or equal to 3 x ULN for patients with liver metastasis; aspartate aminotransferase (AST) less than or equal to 3 x ULN or less than or equal to 5 x ULN for patients with liver metastasis; alanine aminotransferase (ALT) less than or equal to 3 x ULN or less than or equal to 5 x ULN for patients with liver metastasis; alkaline phosphatase less than or equal to 2.5 x ULN or less than or equal to 5 x ULN for patients with liver metastasis (Note: elevated alkaline phosphatase was acceptable if it was due to non-hepatic associated pathology [e.g., bone disease]).
- Patients enrolled to Cohorts 6-8 were required to submit tumor tissue sample. Fresh tumor biopsies could be performed if the patient had a readily accessible tumor lesion and who provided consent to the biopsies. If fresh biopsies could not be obtained, archival tumor samples were acceptable. Prior to enrollment it was required to determine that there was enough tumor tissue available to be sent to the central laboratory. Cohorts 6 and 8: archival tissue collected up to 12 months prior to screening date was permitted. Biopsies collected between 12-18 months prior to screening were allowed upon discussion with the medical monitor. Patients with EBV-associated nasopharyngeal carcinoma could submit biopsy with EBV test results within 36 months prior to screening; Cohort 7: archival tissue with MSI-high/dMMR test results collected up to 36 months prior to screening was permitted.

Exclusion criteria

Patients were excluded from the study if any of the following criteria applied:

- Disease related:
    - Primary brain tumor, untreated or symptomatic brain metastases and leptomeningeal disease (exception: benign asymptomatic tumors were permitted).
- Other medical conditions:
    - History of other malignancy within the past 2 years, with the following exception(s): malignancy treated with curative intent and with no known active disease present for ≥ 2 years before enrollment and felt to be at low risk for recurrence by the treating physician; adequately treated non-melanoma skin cancer or lentigo maligna without evidence of disease; adequately treated cervical carcinoma in situ without evidence of disease; adequately treated breast ductal carcinoma in situ without evidence of disease; prostatic intraepithelial neoplasia without evidence of prostate cancer; adequately treated urothelial papillary non-invasive carcinoma or carcinoma in situ; other malignancies which do not require systemic therapy, may be considered upon discussion with the medical monitor.
    - History of solid organ transplantation.
    - Major surgery within 28 days of study day 1.
- Prior/concomitant therapy
    - Prior treatment with anti-programmed death 1 (PD-1), anti-PD-L1, CTLA-4 or other checkpoint inhibitors
    - Antitumor therapy (radiotherapy, chemotherapy, antibody therapy, molecular targeted therapy, or investigational agent) within 21 days before study day 1. Palliative radiotherapy was permitted.
    - Live vaccine therapy within 4 weeks prior to study drug administration.
    - Current treatment or within 14 days of day 1 with immunosuppressive corticosteroid defined as > 10 mg prednisone daily or equivalent. Corticosteroids

with no or minimal systemic effect (such as topical or inhalation) were permitted. Corticosteroids > 10 mg prednisone used for management of contrast allergy for study scans was allowed.

- Prior/concurrent clinical study experience
    - Currently receiving treatment in another investigational device or drug study, or less than 21 days prior to study day 1 since ending treatment on another investigational device or drug studies.
- Diagnostic assessments
    - Evidence of interstitial lung disease or active, non-infectious pneumonitis.
    - History of any immune-related colitis. Infectious colitis was allowed if evidence of adequate treatment and clinical recovery existed and at least 3 months interval observed since diagnosis of colitis.
    - History of allergic reactions or acute hypersensitivity reaction to antibody therapies.
    - Positive/non-negative test for human immunodeficiency virus (HIV).
    - Known active hepatitis B (e.g., hepatitis B antigen [HBsAg] reactive) or hepatitis C (e.g., HCV RNA [qualitative] is detected).
    - Currently active infection requiring systemic therapy.
    - Active or history of any autoimmune disease or immunodeficiencies. Patients with type I diabetes, vitiligo, psoriasis, hypo- or hyper-thyroid disease not requiring immunosuppressive treatment were permitted.
    - Myocardial infarction within 6 months of study day 1, symptomatic congestive heart failure (New York Heart Association > class II), unstable angina, or cardiac arrhythmia requiring antiarrhythmic medication.
    - Unresolved toxicities from prior antitumor therapy, defined as not having resolved to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 grade 1, or were stable and well controlled with minimal, local, or non-invasive

intervention and there was agreement to allow by both the investigator and the sponsor medical monitor.

- Other exclusions
    - Males and females of reproductive potential who were unwilling to practice highly effective methods of birth control while on study through 6 months (females) and 8 months (males) after receiving the last dose of AMG 404
    - Females with a positive pregnancy test.
    - Females who were lactating/breast feeding or planning to breastfeed while on study through 6 months after receiving the last dose of study drug.
    - Females planning to become pregnant while on study through 6 months after receiving the last dose of the study drug.
    - Males unwilling to abstain from donating sperm during treatment and for an additional 8 months after the last dose of AMG 404
    - Known sensitivity to any of the products or components to be administered during dosing.
    - Patient likely to not be available to complete all protocol-required study visits or procedures, and/or to comply with all required study procedures to the best of the patient and investigator's knowledge.
    - History or evidence of any other clinically significant disorder, condition, or disease (with the exception of those outlined above) that, in the opinion of the investigator or sponsor physician, if consulted, would pose a risk to patient safety, or interfere with the study evaluation, procedures or completion.
    - Known sensitivity to any of the products to be administered during the study.

Dose-limiting toxicity (DLT) evaluation and dose escalation

Patients were considered DLT-evaluable if they had received ≥ 90% of the planned dose and were followed up for ≥ 1 cycle or had experienced a DLT during the DLT window. Intra-

patient dose escalations were permitted. The Dose Level Review Team reviewed the safety data and provided recommendations on dose modifications based on all enrolled patients.

Assessments

A DLT was defined as any of the following toxicities occurring during the DLT evaluation period: any treatment-related grade 5 toxicity; grade 4 neutropenia or thrombocytopenia > 7 days in duration; febrile neutropenia; grade 4 anemia; grade 3 or 4 non-hematologic toxicity with prespecified exemptions; recurrent grade 2 pneumonitis; delay in cycle 2 treatment for > 14 days due to an adverse event (AE) in the dose escalation portion of the study due to AMG 404–related toxicity; or any other toxicity requiring permanent discontinuation of AMG 404.

Treatment-emergent (TEAE), treatment-related (TRAE), fatal, and serious (SAE) AEs, as well as those leading to treatment discontinuation, were assessed.

For ADA assessments, the ECL-based immunoassay method uses the Meso Scale Discovery (MSD) platform and follows a 2-tiered assay approach consisting of a screening assay and a confirmatory assay. The screening assay is performed on samples to detect the presence of antibodies capable of binding to AMG 404. Samples with a signal-to-noise (S/N) value greater than the assay cut point are then tested to confirm the specificity of the response. Drug-treated samples tested in the confirmatory assay which show a reduction of S/N in the presence of excess soluble drug are reported as positive for the presence of anti-AMG 404 antibodies. As a neutralizing antibody assay was not validated for this study, anti–AMG 404 binding antibody–positive samples were not tested for their neutralizing activity.

Duration of stable disease (SD) was measured by computed tomography/magnetic resonance imaging. In select patients with readily accessible tumor lesions, fresh tumor biopsies were collected for biomarker analysis at screening, week 8 (end of cycle 2), and the end of the study.

Statistical analysis

The primary analysis was performed when all patients had the opportunity to complete 6 months in the study or withdrew from the study.

Continuous data were summarized using descriptive statistics, including mean, median, standard deviation, and range, while categorical data were summarized using frequency counts and percentages. The pharmacokinetic (PK) analysis set included patients who received ≥ 1 dose of AMG 404 and had ≥ 1 sample for PK analysis collected. Patients were not evaluable for PK analysis if the number of data points required for the analysis was inadequate, significant protocol deviations had affected the data, or key dosing or sampling information was missing.

The incidence of ADAs and percentage of patients who developed ADAs were summarized overall and by dose level. The proportion of patients with an objective tumor response and disease control rate (DCR) with the corresponding exact 80% confidence interval (CI) were tabulated, as were the proportion of patients and 80% CI for the 1-year duration of overall response, progression-free survival (PFS), and SD.

Kaplan–Meier (KM) curves were provided for the duration of overall response, progression-free survival, and duration of SD, with estimates for rate and 80% CI at selected weeks if data allowed. KM methods were planned to estimate landmarks for time-to-event endpoints with the Greenwood formula used to estimate the standard error used in CI calculation.

Futility analyses was planned for some cohorts. If the observed rate of responses was consistent with a response rate of < 15%, enrollment and treatment at the recommended phase II dose could be terminated due to futility. For purposes of assessing futility, a response was defined as an objective response per modified RECIST v1.1.

Study oversight

This study was sponsored by Amgen Inc. Protocols and subsequent amendments were approved by the Institutional Review Boards (IRBs) or ethics committees at each participating site. The clinical trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines of the International Council for Harmonisation.

Written informed consent was provided by all patients. The trial was designed by employees of the sponsor in collaboration with the primary investigators. Data were collected by investigators and analyzed by statisticians employed by the sponsor. The Dose Level Review Team, comprising trial investigators and representatives of the Amgen study teams, reviewed safety and efficacy data until the data snapshot for the primary analysis to ensure no avoidable increased risk of harm to patients. All authors contributed to the interpretation of the data and review and editing of the manuscript.