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ARTICLE

Translational findings support regimen selection for first-in-human study of ubamatamab (MUC16 × CD3 bispecific antibody) in patients with recurrent ovarian cancer

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Abstract

Ubamatamab, a Mucin 16 (MUC16) × cluster of differentiation 3 (CD3) bispecific antibody that promotes T-cell-mediated cytotoxicity of MUC16-expressing cells, is being investigated for the treatment of ovarian cancer. Intravenous administration of ubamatamab, with or without the anti-programmed cell death-1 inhibitor cemiplimab, is being evaluated in a first-in-human study in patients with recurrent ovarian cancer. In vitro cytotoxicity and cytokine data and projected ubamatamab human pharmacokinetic (PK) profiles scaled with monkey PK parameters enabled starting-dose selection in humans. Mouse tumor regression studies identified ubamatamab effective concentrations. Preclinical and clinical PK, cytokine, safety, and efficacy data from dose escalation were integrated to determine expansion regimens. A starting dose of 0.1 mg was selected, which showed acceptable safety in patients. A step-up dosing approach was used to effectively manage cytokine release syndrome. Mouse tumor regression models suggested an ubamatamab efficacious concentration range of 0.4–50 mg/L, consistent with clinical activity observed at ubamatamab trough concentrations ≥5 mg/L. Integrating preclinical and clinical data determined a target trough concentration range of 5–30 mg/L, which supports evaluation of ubamatamab 250 mg with or without cemiplimab and 800 mg monotherapy once every 3 weeks in expansion cohorts. Preclinical data (cytokine release, tumor regression, monkey PK) had translational value in supporting regimen selection in dose escalation and subsequently in dose expansion after integration with patient data from dose escalation.

Clinical trial registration: NCT03564340.

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Ubamatamab, a MUC16 × CD3 bispecific antibody that promotes T-cell-mediated cytotoxicity of MUC16-expressing cells, is being evaluated in a first-in-human (FIH) study in patients with recurrent ovarian cancer. Preliminary clinical data indicate adequate safety and tolerability, and encouraging antitumor activity.

WHAT QUESTION DID THIS STUDY ADDRESS?

What is the translational value of ubamatamab preclinical data in determining an appropriate starting dose in patients, what step-up dosing should be selected for the management of acute immune reactions to T-cell-engaging therapy, and what expansion regimens should be chosen in clinical trials investigating bispecific antibodies for the treatment of solid tumors.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Data from in vitro cytotoxicity and cytokine release assays, mouse tumor regression models, and monkey pharmacokinetic experiments informed preclinical to clinical translation. These data enabled selection of starting, step-up, and treatment doses, and determination of an efficacious concentration range for expansion regimens even when an apparent exposure–response relationship could not be clearly established with data from dose escalation.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

Our findings demonstrate the value of ubamatamab preclinical data to aid efficient FIH study design. Some aspects may be generalized qualitatively to other bispecific antibodies for the treatment of solid tumors.

BACKGROUND

Ovarian cancer (OC) has an incidence of 300,000 cases/year and causes 200,000 deaths/year, worldwide.[1] Most cases are diagnosed at an advanced stage (stage III/IV),[2] where the current standard of care comprises surgical resection with adjuvant platinum-/taxane-based chemotherapy followed by maintenance therapy.[3] Recurrent OC is commonly treated with repeated platinum-based chemotherapy until the disease becomes platinum-resistant.[4] A high unmet need remains for patients with advanced OC: the 5-year overall survival rate is 30%–40%.[5]

T-cell-engaging bispecific antibodies (bsAbs) are a relatively new modality for cancer immunotherapy. Several T-cell-engaging bsAbs are approved for the treatment of hematologic malignancies (e.g., relapsed/refractory B-cell lymphoma and advanced multiple myeloma), while others are in clinical development for treating solid tumors (e.g., prostate cancer and OC).[6,7]

Ubamatamab (previously REGN4018) is a human hinge-stabilized immunoglobulin G4-based bsAb that binds to Mucin 16 (MUC16), a cell-surface antigen overexpressed in several epithelial cancers, including most OCs, and cluster of differentiation 3 (CD3), a subunit of the T-cell receptor complex.[8,9] Ubamatamab is designed to elicit MUC16-directed polyclonal T-cell-mediated killing and has reduced affinity for Fcγ receptors.[8] Preclinical experiments with ubamatamab have demonstrated induction of T-cell activation and T-cell-mediated MUC16-specific cytotoxicity in vitro, and potent T-cell-mediated antitumor activity in xenogenic and syngeneic mouse tumor models.[8] Combination with cemiplimab, an anti-programmed cell death-1 inhibitor, further enhanced antitumor effects.[8]

Preclinical findings supported ubamatamab clinical development in a first-in-human (FIH) study (NCT035640) to assess safety, efficacy, pharmacokinetics (PK), and pharmacodynamics of ubamatamab alone and ubamatamab + cemiplimab in patients with advanced, recurrent OC. Clinical data from the FIH study indicated that ubamatamab alone or with cemiplimab has an acceptable safety profile, along with evidence of antitumor activity in patients.[10,11]

Due to the risk of acute immune reactions with T-cell-engaging bsAbs, cytokine release syndrome (CRS) or other immune-related adverse events (AEs) are expected,[12,13] underscoring the importance of introducing step-up doses (i.e., lower dose[s] in early weeks) before administration of full doses.

Here, we evaluate ubamatamab preclinical and clinical PK, pharmacodynamics, and antitumor data, and identify the translational value of preclinical information in supporting FIH regimen selection. Although the translational value of preclinical information for supporting bsAb FIH

regimen has been recognized for hematologic malignancies,[12,14] to the best of our knowledge, translational work for solid tumors is limited, and translational analysis of bsAbs targeting MUC16 is not yet available.

METHODS

Cell surface binding of ubamatamab to human and monkey MUC16-expressing cell lines and human and monkey effector T cells in vitro

Binding of ubamatamab to MUC16 was evaluated by flow cytometry using human OC cell lines expressing endogenous human MUC16[15] (OVCAR-3, PEO1, and OVCA432), and an ID8 cell line engineered to express the membrane-proximal portion of human MUC16 (ID8/hMUC16) or cynomolgus monkey MUC16 (ID8/mfMUC16)[8] (Table S1; Supplementary Methods in Appendix S1). Binding of ubamatamab to human and monkey effector T cells was evaluated by flow cytometry as previously described.[8]

Characterization of ubamatamab cytotoxicity using a MUC16-specific T-cell-mediated in vitro assay

Ubamatamab cytotoxicity directed against fluorescent dye-labeled cell lines expressing human MUC16 was examined in the presence of unstimulated human effector T cells using flow cytometry as described previously.[8] Effector:target cell (E:T) ratio of 4:1 was used for in vitro studies, with an incubation time of 96 h. Cells were gated on live violet-labeled populations; the percentage of living cells was recorded and used for the calculation of survival.

In vitro characterization of cytokine release

The effect of ubamatamab (0.01–10 mg/L) on in vitro cytokine release from freshly isolated human peripheral blood mononuclear cells (PBMCs) from four donors was evaluated in the presence and absence of MUC16-expressing OVCAR-3 cells (Supplementary Methods in Appendix S1).

Antitumor effect of ubamatamab in a mouse xenograft ascites tumor model

Antitumor activity was assessed in non-obese diabetic mice with severe combined immunodeficiency and IL-2 receptor gamma chain knockout[15] (NOD SCID gamma [NSG]) engrafted with human PBMCs and injected with OVCAR-3/Luc cells (OC cell line transduced with luciferase) for evaluation of effective dose range and ubamatamab concentrations. Mice were dosed at 5, 1, and 0.1 mg/kg (i.v.) on day 4, day 7, day 11, and day 14 with blood samples collected for drug concentration determination 30 min after the first dose (day 4, C_{max}) and then approximately 4 days following the last dose (day 18, approximately C_{trough} for this regimen). This helped with understanding which concentration of ubamatamab is associated with antitumor effects (Table S1; Supplementary Methods in Appendix S1). In this study, 1 million OVCAR-3 ascites were transferred to NSG mice with five mice per dosing group.

Characterizing ubamatamab PK, immunogenicity, and cytokine release after i.v. dosing in cynomolgus monkeys

Ubamatamab PK was characterized following i.v. single (0.01–1 mg/kg) and multiple (0.01–1 mg/kg) doses in cynomolgus monkeys (Supplementary Methods in Appendix S1). The doses were selected to cover a 2-log range to maximize the understanding of the non-human primate PK parameters, and the doses selected for single-dose study matched with doses in the multiple-dose toxicology study to determine if the single-dose PK parameters predicted the multiple-dose parameters. No step-up regimen was needed in the non-human primate dosing scheme, and doses were delivered by a 30-min infusion for the multiple-dose toxicology study and a bolus administration for the single-dose PK study.

Development of antidrug antibodies post-ubamatamab administration was inferred from individual animal concentration–time profiles (i.e., precipitous decline in concentration compared with mean profiles). Antidrug antibody-impacted ubamatamab concentrations were excluded from all PK data analyses.

Cytokine concentrations in serum (IFN-γ, IL-2, IL-6, TNF-α) were monitored in the repeat-dose study before dosing and at 5 min, end of infusion, and 5 and 24 h after each weekly dose using validated assays (Supplementary Methods in Appendix S1).

Predicting ubamatamab exposure in humans

To estimate ubamatamab concentration in humans, a two-compartment PK model with linear elimination kinetics was used. Ubamatamab PK parameters in monkeys were scaled to derive human PK parameters using typical

allometric methods for a monoclonal antibody (details have been previously published).[16,17] Projected exposure metrics included maximum concentration of a dosing interval (C_{max}) and area under the concentration–time curve (AUC) at the proposed starting regimen and subsequent regimens of dose-escalation cohorts.

FIH study of ubamatamab alone and ubamatamab plus cemiplimab

Safety, tolerability, preliminary efficacy, and PK of ubamatamab alone or with cemiplimab are being investigated in the ongoing FIH, open-label, multicenter dose-escalation study (NCT03564340) with cohort expansion in female patients with recurrent OC (and other recurrent MUC16-positive cancers).[10,11] The observation period for dose-limiting toxicity in monotherapy cohorts was the first 4 weeks of cycle 1, and that for ubamatamab + cemiplimab combination was the first 3 weeks of cycle 2 (6 weeks per cycle).

Ubamatamab regimen selection for dose escalation

Ubamatamab dose escalation was designed to encompass a dose range of 0.1–800 mg via i.v. infusion once weekly (QW) as monotherapy or in combination with cemiplimab 350 mg every 3 weeks (Q3W). For monotherapy dose escalation, all cycles were 6 weeks. For dose escalation of the combination, initiation of cemiplimab required administration of two full doses of ubamatamab without ≥grade 2 CRS with the final dose. The first cycle varied between 4 and 6 weeks of ubamatamab monotherapy, depending on the time needed to reach a full dose without significant CRS. Subsequent cycles were 6 weeks. Due to the risk of CRS and MUC16 expression on epithelial tissue, a minimum anticipated biological effect level (MABEL)-based approach[12,18,19] was used to determine ubamatamab starting dose with a joint analysis using data from cytotoxicity, cytokine release, and binding assays, and predicted ubamatamab exposure in humans. In the monotherapy cohorts, ubamatamab regimens consisted of an initial dose in week 1, a transitional dose in week 2, and full doses from week 3 onwards in a series of dose-escalation cohorts. In the ubamatamab + cemiplimab cohorts, ubamatamab alone was administrated in cycle 1, and ubamatamab and cemiplimab were co-administered from cycle 2 onward. This treatment approach allowed patients to tolerate ubamatamab before combination with cemiplimab.[10] Selection of maximum dose was supported by exposure in the monkey repeat-dose toxicology study at the highest non-severely toxic dose.

Data analysis and measurement of drug and cytokine concentrations in humans

Blood samples were collected over time to measure ubamatamab, cemiplimab, and cytokine concentrations in serum (Supplementary Methods in Appendix S1). Ubamatamab, cemiplimab, and cytokine concentration data were analyzed with descriptive statistics by cohorts.

Ubamatamab dosing regimen selection with or without cemiplimab for the randomized expansion cohorts of the FIH study

Integrated analyses of clinical safety, PK, and efficacy data from dose escalation in conjunction with preclinical in vitro and in vivo activity of ubamatamab were performed for regimen selection in the expansion phase.

Ethics statement

All animal studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol (308) was approved by the Regeneron Pharmaceuticals Institutional Animal Care and Use Committee. Non-human primate studies were conducted by Covance Laboratories, Inc. (Covance) in accordance with the protocols (R4018-PK-16042 and R4018-TX-16014), and Covance standard operating procedures (SOPs). All procedures were in compliance with the Animal Welfare Act Regulations (9 CFR 3) and approved by Covance's IACUC. Human studies were conducted in accordance with the principles of the Declaration of Helsinki, International Council for Harmonisation Good Clinical Practice guidelines, and all applicable regulatory requirements. The research protocol was approved by the relevant institutional review boards or ethics committees at each site.

RESULTS

Cell surface binding of ubamatamab to human and monkey MUC16-expressing cell lines and effector T cells

We tested the activity of ubamatamab against multiple cell lines expressing a range of MUC16 levels (Table 1). This ensures activity against MUC16-expressing tumor cells with a range of MUC16 expression. The EC_{50} of ubamatamab for binding to different human OC cell lines endogenously

TABLE 1 In vitro studies used for MABEL estimation for starting dose and predicted vs. observed ubamatamab C_{max} at the proposed starting cohort in the FIH study.

Cell surface binding assay with ubamatamab				
	EC_{50}		EC_{90}	
Cell lines expressing human- and monkey (mf)-MUC16	nM	mg/L	nM	mg/L
OVCAR-3 (ABC 300,000)	2.03	0.30	15.6	2.34
PEO1 (ABC 40,000)	2.11	0.32	10.6	1.59
OVCA432 (ABC 9500)	1.15	0.17	6.71	1.007
ID8/hMUC16 (ABC 248,000)	2.15	0.32	8.77	1.32
ID8/mfMUC16 (ABC 203,000)	1.91	0.28	ND	ND

Cytotoxicity assay with ubamatamab in the presence of human T cells					
	EC_{50}		EC_{90}		
Cell lines expressing human-MUC16	pM	mg/L	pM	mg/L	% cytotoxicity
OVCAR-3 (ABC 300,000)	13.6	0.0020	16.0	0.00240	95
PEO1 (ABC 40,000)	108	0.0162	110.0	0.0165	50
OVCA432 (ABC 9500)	282	0.0423	800.0	0.120	10
ID8/hMUC16 (ABC 248,000)	14.6	0.00219	24.0	0.00360	ND
Mean	105	0.0157	237.5	0.0356	–

Predicted vs. observed ubamatamab C_{max} at 0.1/0.3 mg of starting cohort in the FIH study		
Dose	Predicted C_{max} (mg/L)	Observed C_{max} (mg/L)
0.1 mg i.v. (week 1)	0.027	0.021
0.3 mg i.v. (week 2)	0.089	0.057

Note: One experiment per assay.

Abbreviations: ABC, antibody binding capacity; C_{max}, maximum concentration of a dosing interval; EC_{50}, half maximal effective concentration; EC_{90}, 90% maximal effective concentration; FIH, first in human; i.v., intravenous; MABEL, minimum anticipated biological effect level; MUC16, Mucin 16; ND, not determined.

expressing human MUC16, and ID8/hMUC16 and ID8/mfMUC16 cell lines were similar (Table 1) although the measured antibody binding capacity (ABC) for these cell lines has a wide range of MUC16 expression on the cell surface, from 9500 (OVCA432) to 300,000 (OVCAR-3).

Binding of ubamatamab to human or monkey effector T cells was evaluated in separate experiments. Compared with human T cells, ubamatamab binding to monkey T cells was weaker[8] on non-activated (>20-fold weaker) and activated (≥70-fold weaker) T cells (data not shown).

Ubamatamab activity in T-cell-mediated cytotoxicity assay

Ubamatamab exhibited cytotoxic effects against human MUC16-expressing cell lines in the presence of human effector T cells, with EC_{50} values from 13.6 to 282 pM and percentage of cytotoxicity ranging from 10% to 95% (Table 1). The activity of ubamatamab was correlated to MUC16 expression and the most potent cytotoxicity was found with the cell line that had the highest MUC16 expression.

Cytokine release following stimulation of human PBMCs with ubamatamab

Ubamatamab induced the release of cytokines from PBMCs only in the presence of MUC16-expressing cells in vitro. Ubamatamab induced higher cytokine release versus control conditions (without ubamatamab) at concentrations ≥0.1 mg/L (IFN-γ, IL-2, IL-6) or ≥1 mg/L (TNF-α) (data not shown).

Antitumor effect in mouse tumor models and ubamatamab exposures

In the OC xenograft model using NSG mice engrafted with human PBMCs, significant antitumor activity was

observed in the 1 and 5 mg/kg but not in the 0.1 mg/kg ubamatamab dose groups (Figure 1a). Ubamatamab concentrations increased dose-dependently. In the active doses of 1 and 5 mg/kg, mean ubamatamab concentrations ranged from 0.5 to 43 mg/L from the first dose to 4 days after the last dose (Figure 1b).

Assessment of ubamatamab PK in monkeys

The 1 mg/kg dose was tolerated in cynomolgus monkeys. Concentration–time profiles of total ubamatamab in monkeys after single or repeat dosing were characterized by linear kinetics with an initial brief distribution phase followed by a single terminal elimination phase (Figure S1). Following a single i.v. dose PK study, and a five weekly i.v. doses toxicity study at 0.01–1 mg/kg doses, ubamatamab C_{max} and AUC_{inf} (AUC extrapolated to infinity after a single dose which equals AUC for a dosing interval at steady-state after repeat dosing) values increased in an approximately dose-proportional manner. The C_{max} values and AUC_{inf} following i.v. ubamatamab (0.01, 0.1, and 1 mg/kg) are described in Table 2; estimated clearance was independent of dose. Steady-state volume of distribution was similar across dose groups, and the estimated elimination half-life was ~10 days. Following five weekly doses, accumulation of ubamatamab concentration ranged from ~2-fold for the 0.1 and 1 mg/kg dose groups to ~3-fold for the 0.01 mg/kg dose group, as determined from trough concentration (C_{trough}) values after the first and the fifth dose. The total exposure to ubamatamab after five weekly doses determined by cumulative AUC was 8.25, 77.5, and 710 day•mg/L for the 0.01, 0.1, and 1 mg/kg i.v. dose groups, respectively. The highest non-severely toxic dose defined in the toxicology study was 0.1 mg/kg (C_{max} = 2.04 mg/L at the first dose and 3.2 mg/L at the fifth dose).

A two-compartment population PK model with linear elimination kinetics described the observed data well.

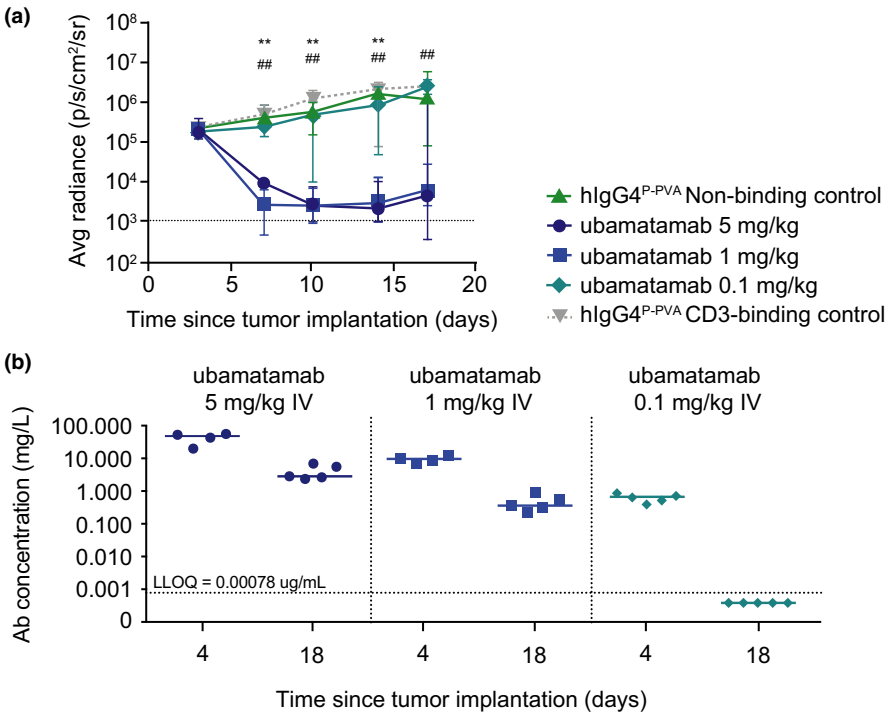

FIGURE 1 Dose–response study demonstrating antitumor activity of ubamatamab (a) and ubamatamab concentrations in serum (b) in NSG mice bearing human OVCAR-3/Luc xenografts and engrafted with human T cells. NSG mice were injected with OVCAR-3/Luc cells previously passaged in vivo (day 0) 21 days after engraftment with human PBMCs. Mice were administered i.v. ubamatamab 0.1, 1, or 5 mg/kg, or 5 mg/kg CD3-binding control, or 5 mg/kg non-binding control every 3–4 days, starting at day 4 after tumor implantation. Tumor burden was assessed by bioluminescence imaging and expressed as the group median with the associated confidence interval. Statistical significance was determined using unpaired nonparametric Mann–Whitney *t*-tests. Treatment with ubamatamab was compared with the $hIgG4^{P\text{-}PVA}$ non-binding control (**$p < 0.01$ ubamatamab 5 mg/kg i.v. compared with non-binding control, $^{\#\#}p < 0.01$ ubamatamab 1 mg/kg i.v. compared with non-binding control). Serum concentrations of ubamatamab below LLOQ are represented as LLOQ/2. Ab, antibody; Avg, average; CD3, cluster of differentiation 3; hIgG4, human immunoglobulin G4; i.v., intravenous; LLOQ, lower limit of quantification; NSG, NOD SCID gamma; PBMC, peripheral blood mononuclear cell.

TABLE 2 Ubamatamab mean (±SD) C_{max}, AUC values, total body clearance and steady-state volume of distribution following single or multiple i.v. administrations in cynomolgus monkeys.

	C_{max} and AUC_{inf} after a single dose		C_{max} and AUC_{tau} after five weekly doses	
	C_{max} (mg/L)	AUC_{inf} (day•mg/L)	C_{max} (mg/L)	AUC_{tau} (day•mg/L)
Dose	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0.01 mg/kg i.v.	0.241 ± 0.0297	1.66 ± 0.255	0.379 ± 0.0403	1.59 ± 0.141
0.1 mg/kg i.v.	2.56 ± 0.328	15.6 ± 3.25	3.20 ± 0.347	13.6 ± 1.84
1 mg/kg i.v.	23.1 ± 1.36	182 ± 18.9	33.4 ± 4.51	139 ± 20.3

	CL and V_{ss} after a single dose		C_{trough} after the first and fifth weekly doses	
	CL (mL/day/kg)	V_{ss} (mL/kg)	C_{trough} (mg/L) first	C_{trough} (mg/L) fifth
Dose	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0.01 mg/kg i.v.	5.10 ± 0.684	63.4 ± 1.77	0.0575 ± 0.0209	0.167 ± 0.0210
0.1 mg/kg i.v.	6.63 ± 1.41	79.6 ± 2.52	0.593 ± 0.0727	1.42 ± 0.263
1 mg/kg i.v.	5.55 ± 0.586	73.8 ± 7.54	6.52 ± 0.849	14.1 ± 3.92

Abbreviations: AUC_{inf}, area under the concentration–time curve extrapolated to infinity after a single dose; AUC_{tau}, AUC calculated for a dosing interval in week 5; CL, total body clearance; C_{max}, maximum concentration of a dosing interval; C_{trough}, the lowest concentration in a dosing interval; i.v., intravenous; SD, standard deviation; V_{ss}, volume of distribution at steady-state.

Observed and model-fitted concentration–time profiles of ubamatamab after 0.01, 0.1, or 1 mg/kg single or repeat dosing are shown in Figure S1. Model-predicted ubamatamab PK parameters in monkeys are presented in Table S2.

Cytokine release following ubamatamab i.v. administration in monkeys

In the weekly repeat-dosing study of ubamatamab in monkeys, IL-6 was elevated over baseline (median 1.10 pg/mL) within 24 h following the first dose of ubamatamab at 0.01, 0.1, and 1 mg/kg doses. In the 0.01, 0.1, and 1 mg/kg dose groups, median peak IL-6 (range) was 3.41 pg/mL (1.49–29.0 pg/mL), 8.6 pg/mL (0.990–124 pg/mL), and 9.60 pg/mL (1.12–335 pg/mL), respectively. Only sporadic increases in IL-6 were observed after subsequent weekly doses at all dose levels. IFN-γ, IL-2, and TNF-α showed no meaningful increase following ubamatamab administration in monkeys within the dose range examined.

Prediction of ubamatamab PK in humans and selection of starting dose of the FIH study

Ubamatamab exposure in humans was predicted with PK parameters scaled from the monkey PK model using allometric scaling (Table S2).

A MABEL-based approach was used to select the ubamatamab starting dose in humans using in vitro EC_{50} values of cytotoxicity. The average EC_{50} of ubamatamab in the T-cell-mediated cytotoxicity assay was 0.016 mg/L (Table 1), and cytokine release (IFN-γ, IL-2, IL-6) was observed at 0.1 mg/L in the presence of MUC16-expressing OVCAR-3 cells. Based on these in vitro results and the projected C_{max} of 0.027 mg/L in humans, a starting dose of ubamatamab 0.1 mg i.v. was selected. The projected C_{max} was within 2-fold of the ubamatamab EC_{50} in the cytotoxicity assay (Table 1) and below the concentration that triggered cytokine release in vitro.

The proposed dose at ≥week 2 was 0.3 mg in the first cohort if the 0.1 mg dose at week 1 was tolerated (0.1/0.3 mg step-up). The predicted human cumulative ubamatamab exposure following 0.1 mg in week 1 and 0.3 mg in weeks 2–5 (i.e., $AUC_{(0–35day)}$ of 2.92 day•mg/L) was calculated to be 17-fold lower than the cumulative exposure of tolerable dose of 0.1 mg/kg/week for 5 weeks ($AUC_{(0–35day)}$ of 49.8 day•mg/L) in monkeys to ensure a safe starting regimen in humans.

Ubamatamab and cemiplimab PK in the FIH study

From i.v. dose-escalation cohorts, ubamatamab concentration data were assessed with ≤800 mg QW monotherapy and ≤250 mg i.v. QW ubamatamab + cemiplimab 350 mg Q3W.

Proposed starting regimen of ubamatamab (0.1/0.3 mg) was well tolerated in patients. The observed and predicted C_{max} at week 1 was 0.021 and 0.027 mg/L with 0.1 mg, and 0.057 and 0.089 mg/L with 0.3 mg at week 2 (Table 1).

In monotherapy dose-escalation cohorts, ubamatamab i.v. regimens consisted of an initial dose in week 1, a transitional dose in week 2, and full doses at ≥week 3. At the completion of dose escalation, the tested initial doses ranged from 0.1 to 3 mg in week 1; transitional doses ranged from 0.3 to 20 mg (split over 2 days at ≥10 mg) in week 2; and full doses ranged from 0.3 to 800 mg weekly from week 3 onwards. The mean concentration–time profiles of ubamatamab from 0.1/0.3 mg to 1/20/800 mg ($n = 73$) in monotherapy cohorts during the first cycle are presented in Figure 2. The accumulation in ubamatamab exposure at full doses of ≥10 mg was ~2–3-fold under QW dosing regimen based on C_{max} after the first dose and at steady-state.

A preliminary population PK model of ubamatamab was developed using concentration data from monotherapy and in combination with cemiplimab. A two-compartment PK model with a linear first-order elimination described the ubamatamab observed concentration–time profiles reasonably well, thereby supporting an approximately dose-proportional and time-independent exposure of ubamatamab within the investigated dose range. The effect of cemiplimab co-administration on ubamatamab exposure was assessed as a covariate with the population PK model of ubamatamab. No effect of cemiplimab on ubamatamab exposure was identified.

In the combination cohorts of ubamatamab and cemiplimab, cemiplimab (350 mg i.v. Q3W) was introduced in cycle 2 after an ubamatamab monotherapy lead-in (cycle 1) period. PK analysis indicated that cemiplimab exposure was not affected by co-administration with ubamatamab. At steady-state of cemiplimab 350 mg i.v. Q3W, the mean (coefficient of variation %) C_{max} and C_{trough} of cemiplimab in serum was 164 mg/L (19%; $n = 11$) and 56.2 mg/L (31%; $n = 9$), respectively, consistent with steady-state cemiplimab concentrations reported in patients with other solid tumors following cemiplimab monotherapy.[20]

Cytokine release and CRS following ubamatamab monotherapy or in combination with cemiplimab

In the first cohort of ubamatamab at 0.1/0.3 mg ($n = 2$), no cytokine release was observed. Patients did not experience clinical symptoms of CRS, indicating that the regimen selected was safe and tolerable.

In the 1/3 mg and 3/10 mg (weeks 1/2+) cohorts, several inflammatory cytokines (IL-6, IFN-γ) were elevated

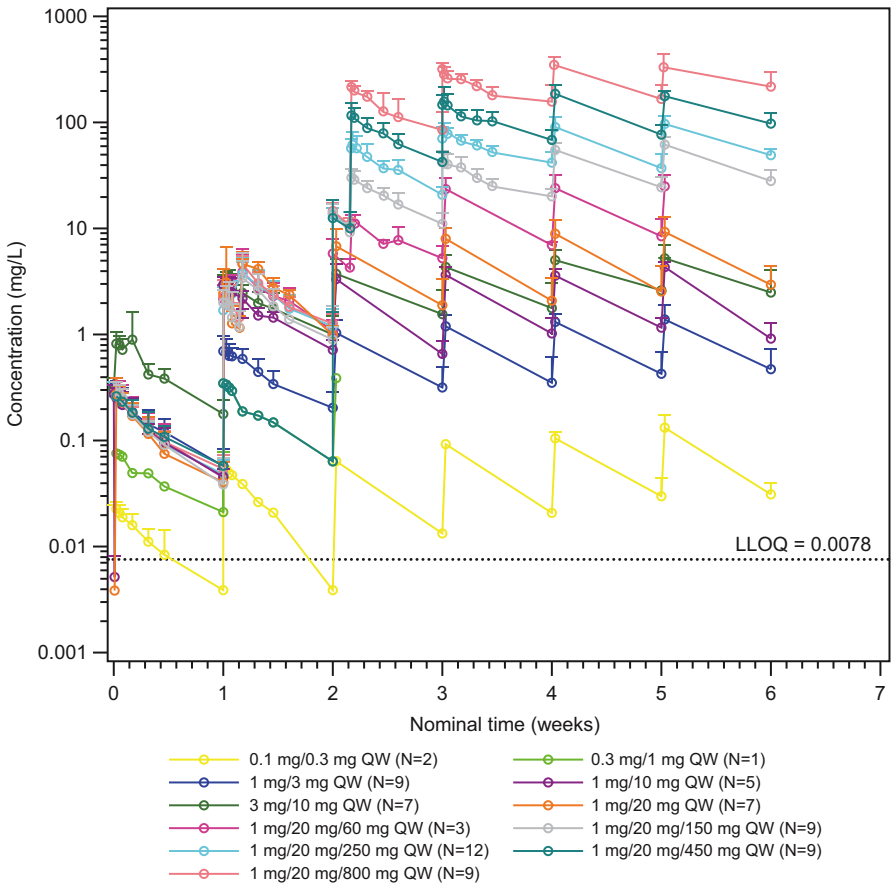

FIGURE 2 Observed mean (+SD) ubamatamab concentration–time profiles in cycle 1 following weekly i.v. doses in patients with ovarian cancer in the dose-escalation phase of the FIH study. FIH, first in human; i.v., intravenous; LLOQ, lower limit of quantification; QW, once weekly; SD, standard deviation.

over baseline in weeks 1 and 2. Over the dose range of 0.1–3 mg examined in week 1, there was a positive association between ubamatamab C_{max} and peak IL-6 or peak IFN-γ serum concentrations. The association, however, was not evident in weeks 2 or 3 when higher transitional and full doses of ubamatamab were administered, respectively (Figure 3).

Cytokines peaked predominantly during day 1 of weeks 1–2; transient cytokine elevation was not observed in most patients in subsequent weeks when full doses of ubamatamab (up to 800 mg) were administered. In combination cohorts, when cemiplimab was administered in cycle 2, cytokine elevation was negligible (Figure 4). CRS (grades 1–2) occurred in three patients (9%) following cemiplimab administration.

DISCUSSION

The translational value of ubamatamab preclinical data in support of dose selection in the FIH study is discussed here, with a focus on the selection of a starting dose in humans, assessment of cytokine release and determining appropriate step-up doses, prediction of ubamatamab exposure in humans, and integrated analyses for selecting dosing regimens in the expansion phase.

Selection of starting dose

Criteria for selecting an appropriate starting dose in humans may include that i) it does not trigger any severe treatment-emergent CRS or other AEs; ii) safety data from the first dose cohort can guide dose escalation to the next cohort; and iii) it can help to determine an appropriate step-up dosing regimen with a relatively small number of patients.

Multiple cell lines with low to high MUC16 expression were tested, representing a potential wide range of sensitivity to ubamatamab treatment in patients. The ubamatamab concentration that resulted in cytokine release (0.1 mg/L) in vitro with the most sensitive MUC16-expressing cells (OVCAR-3) was used to adjust the concentration (C_{max} 0.03 mg/L) of the starting dose of 0.1 mg in humans.

As there was no CRS observed in the 0.1/0.3 mg cohort, this information was used to guide dose escalation to the second and third cohorts (0.3/1 mg; 1/3 mg). With data from six patients in the first three cohorts, a 1 mg dose was determined as the initial step-up dose in week 1, and clinical safety assessment supports 1 mg/20 mg as step-up doses in weeks 1–2. The results reveal the translational value of preclinical data in selecting starting dose. This approach effectively prevented the occurrence of ≥grade 3 CRS and allowed more rapid dose escalation to pharmacologically active dose levels.[11]

Furthermore, a weekly regimen was initially selected to evaluate drug effect on safety post step-up dosing during the dose escalation, which could more effectively evaluate patients' adverse events under intensive exposure in a relatively small number of patients (3–6 patients in each escalation cohort). From a PK perspective, drug accumulation can be better assessed with weekly dosing versus less frequent regimens. Once preliminary results of safety and PK profiles from dose escalation are available, it can guide selecting less frequent dosing interval (e.g., Q3W regimen) in expansion cohorts and improve patient convenience.

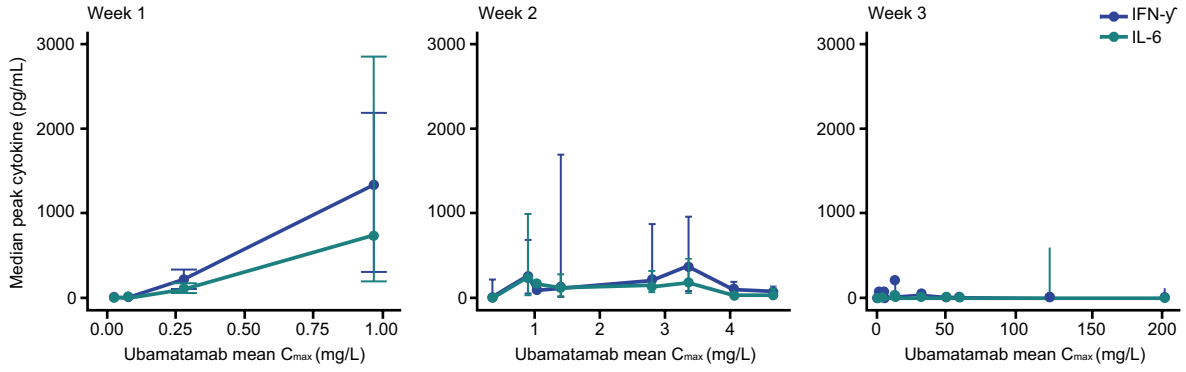

FIGURE 3 Peak concentrations of inflammatory cytokines in serum versus C_{max} of ubamatamab during the weekly step-up dosing of ubamatamab in cycle 1 of the dose-escalation phase of the FIH study. Once-weekly i.v. ubamatamab doses evaluated during dose escalation included 0.1–3 mg in week 1, 1–20 mg in week 2, and 1–800 mg in week 3. Doses ≥3 mg in week 2 and ≥10 mg in week 3 were generally split over 2 days. C_{max} of ubamatamab and peak cytokine levels in serum were calculated after each split dose within a given week where applicable. Data points are summary statistics from 2 to 79 patients at each dose level. Error bars indicate 95% confidence interval. Data from a single subject at any dose level are not included in the graphs. C_{max}, maximal concentration of a dosing interval; FIH, first in human; IFN-γ, interferon-γ; IL-6, interleukin-6; i.v., intravenous.

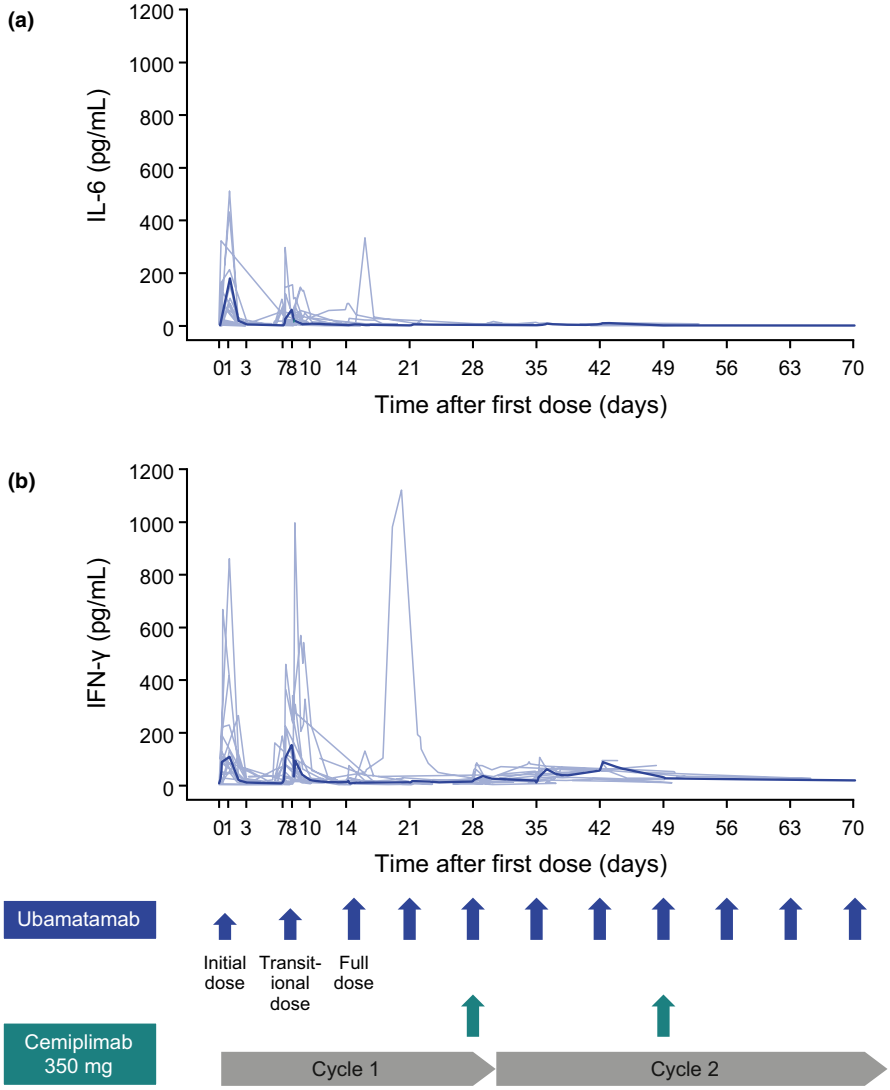

FIGURE 4 IL-6 (a) and IFN-γ (b) levels in serum versus time after treatment with once-weekly i.v. ubamatamab + cemiplimab in dose-escalation cohorts. Light blue lines represent cytokine levels in serum for individual patients versus actual time; dark blue line represents the median cytokine levels versus nominal time. Data are from 20 patients in the combination cohorts, who were assigned to an initial ubamatamab dose of 1 mg in week 1, transitional dose of 20 mg in week 2, and a weekly full dose of ≥20 mg from week 3 onward in cycle 1. i.v. cemiplimab 350 mg Q3W was added in cycle 2. IFN-γ, interferon-γ; IL-6, interleukin-6; i.v., intravenous; Q3W, every 3 weeks.

Cytokine release and selection of step-up doses

Cytokine elevation (IL-6, IFN-γ) was observed at ≥1 mg doses, mainly in weeks 1–2. The step-up dosing strategy effectively prevented ≥grade 3 CRS.[11]

As shown in Figure 3, IL-6 and IFN-γ showed a concentration-dependent elevation in week 1, but there was no clear association between cytokine levels and ubamatamab C_{max} in weeks 2–3. This finding suggests that week 1 is the most sensitive time for cytokine release, and the body can gradually adapt to the immune stimulation, allowing administration of a higher dose later without triggering significant cytokine release. Thus, for ubamatamab and other similar therapies, it is critical to determine step-up doses for mitigating ≥grade 3 CRS. The selection of ubamatamab 1 mg for the initial dose at week 1 was due to the occurrence of more acute AEs when 3 mg was dosed in week 1. Similarly, ubamatamab 3, 10, and 20 mg were tested in week 2, and a 20 mg dose was set at week 2 before administration of a full dose; this dose needed to be split over 2 days (e.g., 5/15 mg or 10/10 mg) to minimize cytokine release early in week 2.

In vitro cytokine assays showed IL-6 and IFN-γ levels of <100 and <1000 pg/mL, respectively, at an ubamatamab concentration of 0.1 mg/L. These values are close to IL-6 (<500 pg/mL) and IFN-γ (<1000 pg/mL) levels in patients who received ubamatamab 1 mg (C_{max} = ~0.3 mg/L, week 1), indicating that the in vitro cytokine assay with human PBMCs and OVCAR-3 cells has translational value in guiding selection of step-up doses in patients. By contrast, peak IL-6 concentration in monkeys after a 0.01 mg/kg dose (mean C_{max} = 0.241 mg/L) was 29 pg/mL, which was much lower than that observed in patients at a similar C_{max}. The relatively poor predictive value of cytokine release in monkeys for determining cytokine release in humans may be attributed to weaker binding of ubamatamab to non-activated monkey T cells than to human T cells, potentially lower in vivo potency, lower MUC16 expression in healthy monkeys, or to a

combination of these factors. Notably, ubamatamab potency in in vitro cytotoxicity assays was similar when using monkey (data not shown) or human T cells with OVCAR-3 cells, suggesting that in vitro ubamatamab cytotoxicity is unaffected by the lower binding affinity to monkey T cells.

To avoid higher cytokine release when dosing ubamatamab and cemiplimab in combination, cemiplimab was introduced in cycle 2 in combination cohorts after cytokine release diminished following the ubamatamab monotherapy period in cycle 1. This strategy appeared to be reasonable and successful for preventing any potential increased CRS incidence or severity following combination with cemiplimab. As shown in Figure 4, cytokine release in cycle 2 was minimal after ubamatamab and cemiplimab administration.

Prediction of human PK for ubamatamab

Ubamatamab concentration–time profiles at proposed dose cohorts in humans were simulated with scaled PK parameters from the parameters in monkeys. The observed clinical PK data showed that the observed C_{max} values in patients at the first two doses were within 2-fold of predicted values (Table 1), which allowed precise estimation of the starting dose from a safety perspective. Based on a preliminary assessment with a population PK model with human data, the clearance of ubamatamab in patients (0.581 L/day) appeared to be faster than the scaled clearance from the monkey PK (0.208 L/day), suggesting that disease may affect ubamatamab PK in patients. Consequently, the ubamatamab population PK model developed using clinical data was used to aid regimen selection for the expansion cohorts.

Regimen selection for expansion

Clinical safety data in the dose-escalation phase demonstrated tolerability of ubamatamab monotherapy ≤800 mg QW (highest tested dose).[11] For combination therapy, ubamatamab was tolerable at ≤250 mg QW in combination with cemiplimab. A maximum tolerated dose was not reached, as dose escalation was stopped due to a lack of a clear dose–safety relationship.[10] Clinical efficacy was observed at 20–800 mg QW doses of ubamatamab and at 10–250 mg QW doses of ubamatamab + cemiplimab 350 mg Q3W. Although ubamatamab showed dose-proportional increase in exposure, dose–response relationships were flat for safety and efficacy within the dose range evaluated. Therefore, the C_{trough} at steady-state ($C_{trough,ss}$) at 20 mg QW (5 mg/L) was considered as the minimum effective concentration in patients for selecting regimens for the expansion phase.

From an efficacy perspective, average EC_{50} (0.016 mg/L) and EC_{90} (0.0356 mg/L) derived from in vitro T-cell-mediated cytotoxicity assays appeared to be too low to show clinical efficacy. Considering the efficacious concentration range of 0.4–50 mg/L in mouse xenograft models at 1–5 mg/kg, and the minimal effective concentration in patients (5 mg/L), 30 mg/L was set as a reference $C_{trough,ss}$ for regimen selection for expansion cohorts. Model-based simulations determined that the ubamatamab 250 mg i.v. Q3W regimen with or without cemiplimab would yield a mean (SD) $C_{trough,ss}$ of 9.88 (10.1) mg/L, which is higher than the minimal efficacious $C_{trough,ss}$ of 5 mg/L, and that the ubamatamab 800 mg i.v. Q3W regimen would yield a mean (SD) $C_{trough,ss}$ of 31.6 (32.1) mg/L, similar to the reference concentration (30 mg/L; Figure 5).

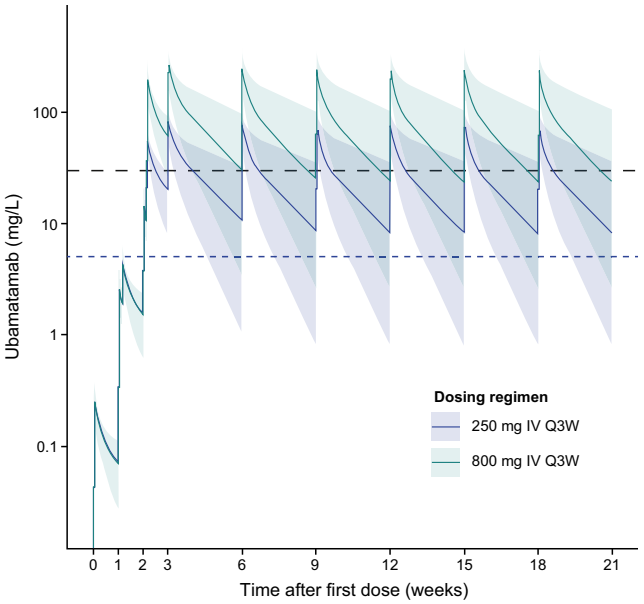

FIGURE 5 Model-based simulation of ubamatamab concentration–time profiles in patients with ovarian cancer following i.v. administration of ubamatamab using the dosing regimen selected for phase 2 dose expansion. Solid blue and green lines represent the predicted median PK profile of ubamatamab following 250 mg i.v. Q3W and 800 mg i.v. Q3W dosing regimen of ubamatamab after step-up, respectively. Step-up ubamatamab dosing consists of 1 mg i.v. in week 1 and 20 mg i.v. in week 2, split over 2 days. The first full dose of ubamatamab in week 3 is split over 2 days as 50 + 200 mg or 50 + 750 mg. The blue and green shaded areas represent the 90% prediction intervals for each dosing regimen, respectively. Horizontal long- and short-dashed lines represent the reference $C_{min,ss}$ of 30 mg/L set for dose expansion, and observed $C_{min,ss}$ in patients after the 20 mg i.v. QW dosing regimen in phase 1 dose escalation, which was the minimum effective dose. $C_{min,ss}$, trough concentration at steady-state; i.v., intravenous; PK, pharmacokinetics; Q3W, every 3 weeks, QW, once weekly.

As preclinical data showed cemiplimab increased the antitumor effect of ubamatamab,[8] the combination was evaluated clinically in dose escalation, and ubamatamab 250 mg Q3W in combination with cemiplimab was tested in dose expansion, mainly due to the observed flat dose–response of ubamatamab (10–250 mg QW) with cemiplimab, and an observed dose-limiting toxicity at ubamatamab 450 mg QW in combination with cemiplimab. Thus, ubamatamab ≥450 mg QW in combination with cemiplimab was not further tested.

Considering patient convenience and ease of drug combination (cemiplimab/chemotherapy), a Q3W dosing interval was selected for study, and efficacy of ubamatamab 250 mg with or without cemiplimab 350 mg will be compared. The goal of the expansion phase is to optimize ubamatamab dosing +/− cemiplimab and further testing dose effect (250 mg vs. 800 mg Q3W) in randomized cohorts to reduce confounding factors in regimen selection. Furthermore, available observed ubamatamab steady-state concentrations with Q3W regimen indicate that C_{trough} (mean ± SD; 13.8 ± 4.24 mg/L, $n = 6$, after 250 mg Q3W +/− cemiplimab, and 70.2 ± 28.1 mg/L, $n = 5$, after 800 mg Q3W) was above the reference concentration range of 5–30 mg/L supporting the regimen selection for the expansion cohorts. The ubamatamab population PK model will be further refined with data from Q3W regimens.

In summary, our work demonstrated the translational value of in vitro cytotoxicity and cytokine release assays, in vivo tumor regression mouse models, and monkey PK data for selecting starting dose, step-up doses, and treatment doses for the FIH study of ubamatamab in patients with recurrent OC. Using clinical data from the escalation phase, the rationale for regimen selection in expansion was established. This approach may be valuable for developing other bsAbs to treat solid tumors.

AUTHOR CONTRIBUTIONS

M.Z., P.M. wrote the manuscript; M.W.R., A.C., P.K., L.H. designed and performed the research; M.Z., T.S.U., J.B.-V., M.P., E.M. designed and performed the research; M.Z., P.M., A.C., J.B.-V., P.K., L.H., M.P., T.S.U., E.M., J.D.D., M.W.R. analyzed the data.

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CONFLICT OF INTEREST STATEMENT

Min Zhu, Alison Crawford, Jurriaan Brouwer-Visser, Pamela Krueger, Lauric Haber, Mary Peterman, Thomas S. Uldrick, Elizabeth Miller, John D. Davis, and Marc W. Retter are employees and shareholders of Regeneron Pharmaceuticals, Inc. Priyanka Madia was a former employee of Regeneron Pharmaceuticals, Inc.

DATA AVAILABILITY STATEMENT

All preclinical data associated with this study are present in the paper or the Supplementary Materials in Appendix S1. Regeneron materials described in this manuscript may be made available to qualified, academic, noncommercial researchers through a material transfer agreement upon request at https://regeneron.envisionpharma.com/ienv_research/. For questions about how Regeneron shares materials, use the email address (preclinical.collaborations@regeneron.com). For clinical data, qualified researchers may request access to study documents (e.g., study protocol with any amendments, blank case report form) that support the methods and findings reported in this manuscript. Individual anonymized participant data will be considered for sharing: (1) once the product and indication have been approved by major health authorities (e.g., FDA, EMA, PMDA, etc) or development of the product has been discontinued globally for all indications on or after April 2020 and there are no plans for future development; (2) if there is legal authority to share the data; and (3) if there is not a reasonable likelihood of participant re-identification. Submit requests to https://vivli.org/.

CONSENT TO PARTICIPATE

All patients provided written informed consent.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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