Selection of the Recommended Phase 2 Dose for Bintrafusp Alfa, a Bifunctional Fusion Protein Targeting TGF-β and PD-L1

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Bintrafusp alfa, a first-in-class bifunctional fusion protein composed of the extracellular domain of the TGF-βRII receptor (TGF-β "trap") fused to a human IgG1-blocking PD-L1, showed a manageable safety profile and clinical activity in phase I studies in patients with heavily pretreated advanced solid tumors. The recommended phase 2 dose (RP2D) was selected based on integration of modeling, simulations, and all available data. A 1,200-mg every 2 weeks (q2w) dose was predicted to maintain serum trough concentration (C_{trough}) that inhibits all targets of bintrafusp alfa in circulation in > 95% of patients, and a 2,400-mg every 3 weeks (q3w) dose was predicted to have similar C_{trough}. A trend toward an association between exposure and efficacy variables and a relatively stronger inverse association between clearance and efficacy variables were observed. Exposure was either weakly or not correlated with probability of adverse events. The selected intravenous RP2D of bintrafusp alfa is 1,200 mg q2w or 2,400 mg q3w.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Recommended phase 2 dose (RP2D) selection for immune checkpoint inhibitors is performed on a case-by-case basis due to limited data and confounding factors in the interpretation of exposure-response and pharmacokinetic/pharmacodynamic analyses.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ Bintrafusp alfa showed clinical efficacy in various cancer types from early phase I studies; this study integrated available preclinical and clinical data to determine the RP2D for bintrafusp alfa.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ After integration of modeling and simulation approaches and careful consideration of confounding factors in the interpretation of the exposure-efficacy and exposure-safety relationships, 1,200 mg every 2 weeks and 2,400 mg every 3 weeks were selected as the RP2Ds for bintrafusp alfa. The study also highlighted the value of having more than one dose level for exposure-response analyses in support of dose selection for immune checkpoint inhibitors.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ The RP2D for bintrafusp alfa can be used for future monotherapy and combination trials; an integrative approach is crucial for dose selection for immune checkpoint inhibitors.

The advent of immune checkpoint inhibitors (ICIs) in the treatment of cancer was brought on by increased understanding of the role of the immune system in mediating an antitumor response. T-lymphocytes are primed and activated by interactions with T-cell receptors and antigen complexes on antigen-presenting cells.[1,2] These processes are regulated by immune checkpoint signaling, such as programmed death 1 (PD-1) binding its ligand and programmed death-ligand 1 (PD-L1), which results in inhibition of T-cell function as well as T-cell death.[3,4]

Upregulation of these inhibitory pathways in cancer leads to immunosuppression and cancer growth. Thus, inhibition of these pathways can reverse immunosuppression and stimulate the antitumor response, an effect that established immune checkpoints as important therapeutic targets in the management of cancer.[3,4] Numerous ICIs targeting both PD-1 and PD-L1 have been approved and are commonly prescribed for cancer treatment, including nivolumab, atezolizumab, durvalumab, pembrolizumab, and avelumab.[5]

Dose selection of checkpoint inhibitors for oncology indications is an evolving science. The recommended phase 2 dose (RP2D) for an ICI is selected using a case-by-case approach due to limited data and confounding factors in the interpretation of exposure-response and pharmacokinetic and pharmacodynamic (PK and PD) analyses. Maximum tolerated dose is often not reached for immunotherapies

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in phase I studies, and maximum dose tested may be higher than the fully efficacious dose. The challenges in interpretation of exposure-efficacy data, particularly for therapeutic proteins in cancer indications, have been described in the literature.[6–10] The discussion revolves around the interplay among PK, baseline disease factors (such as cachexia, inflammation status, tumor burden, and hypercatabolic state), and response, and how this interplay can affect the interpretation of exposure-efficacy and exposure-safety analyses. This confounding effect is most pronounced when exposure-response analyses are conducted using data from a single dose level, which is the common approach for design of the expansion phase of first-in-human studies for ICIs. The utility of PK-PD analyses is generally limited in oncology due to the lack of well-established PD markers linked to efficacy (other than tumor size).[11] Target engagement PD markers are used for PK-PD analyses. In some cases, peripheral target engagement PD markers do not provide meaningful demarcation for dose selection.[9] Tumor target engagement profiles are rarely assessed but can be modeled using standard assumptions on tissue distribution of therapeutic proteins.

In this report, we describe the selection of RP2D for bintrafusp alfa (M7824), a first-in-class bifunctional fusion protein composed of the extracellular domain of the TGF-βRII receptor (or TGF-β "trap") fused via a flexible linker to the C-terminus of each heavy chain of the immunoglobulin G1 antibody blocking PD-L1. The two components of bintrafusp alfa simultaneously block two pro-tumorigenic and immunosuppressive pathways, TGF-β and PD-L1, to inhibit tumor growth by potentially restoring and enhancing antitumor responses.[12] Preclinical data suggest that dual inhibition of TGF-β and PD-L1 signaling via a single bifunctional molecule (vs. 2 separate monotherapies) may facilitate localized and increased inhibition of TGF-β specifically in the tumor microenvironment.[12] Early phase I studies with bintrafusp alfa showed clinical activity in different cancer types, including but not limited to non-small cell lung cancer (NSCLC), biliary tract cancer, and HPV-associated cancers, as well as a manageable safety profile in patients with heavily pretreated advanced solid tumors.[13–17]

Due to the complexities outlined above, RP2D selection was based on integration of available preclinical and clinical data. Clinical data from phase I studies included safety and tolerability, PK and PD (PD-L1 target occupancy (TO) in peripheral blood mononuclear cells and TGF-β trapping in plasma), as well as efficacy in second-line (2L) NSCLC cohorts. The selection of the RP2D was also supported by modeling and simulation, such as population PK (PopPK), exposure efficacy, and exposure safety.

METHODS

Study design and patients

The objective of this report was to determine the RP2D of bintrafusp alfa. Patients with a wide range of solid tumor types from two phase I studies (ClinicalTrials.gov NCT02517398 (EudraCT 2015-004366-28) and NCT02699515) were included in the analyses; for the exposure-efficacy analysis, 80 patients with 2L NSCLC from NCT02517398 were included.[18] Both studies were conducted following international standards of good clinical practice consistent with the International Conference on Harmonisation Topic E6 Good Clinical Practice and the Declaration of Helsinki. Patients were enrolled in accordance with a protocol approved by the principal and coordinating investigators of the trial and relevant regulatory authorities. Further details on the designs of both trials have been reported previously[14,16] and are summarized in the **Supplementary Information**.

Exposure-efficacy and exposure-safety analyses

Exposure-efficacy and exposure-safety analyses were performed using R version 3.2.2. The adverse events (AEs) included in the exposure-safety analyses were treatment-emergent AEs (TEAEs), infusion-related reactions (IRRs), including drug hypersensitivity reactions, immune-related AEs (irAEs), skin AEs possibly related to PD-L1 (sPDAEs), and skin AEs possibly related to TGF-β (*s*TGAEs). The efficacy end points included best overall response (BOR) as assessed by investigator and progression-free survival (PFS). Definitions for BOR and PFS are given in the **Supplementary Information**.

Exposure metrics and clearance (CL) were derived using the previously described bintrafusp alfa PopPK model.[18] Metrics of exposure considered to be potential correlates of AEs included geometric mean trough concentrations at steady state ($C_{trough,ss}$), area under the curve at steady state (AUC_{ss}), C_{trough} after the first dose ($C_{trough,sd}$), and AUC after the first dose from 0 to 336 hours ($AUC_{0–336\,h}$). Concentration at the end of infusion at steady state ($C_{EOI,SS}$) and C_{EOI} after the first dose were also evaluated for IRR AEs. To mitigate the potential confounding impact of response and posttreatment effects on PK, the metrics of exposure $AUC_{0–336\,h}$ and $C_{trough,sd}$ were selected as potential covariates for BOR and PFS.[8]

The influence of exposure metrics or CL on BOR or probability of AEs was explored graphically, after which relationships were assessed using logistic regression. A Kaplan–Meier analysis by quartiles of exposure and Cox proportional hazards model were used to assess the relationship of PFS vs. bintrafusp alfa exposure or CL, as well as to explore the potential explanatory value of other covariates for this end point.

As a first step, we assessed each considered exposure metric or CL by a univariable analysis. Multivariable models were then fitted to assess the influence of exposure on the probability of response, PFS, or AEs adjusted for other covariates. A full model approach for covariate modeling was applied, in which all possible covariates of interest were included in the model simultaneously. Relationships between exposure metrics, covariates, and probability of response, PFS, or probability of AEs were explored graphically, and odds ratios (ORs) were reported. Discriminatory performance of the models was assessed using receiver operating characteristic curves. We performed no adjustment for multiplicity for the reported confidence intervals (CIs) corresponding to different efficacy or safety end points, exposure metrics, or covariates; all analyses were exploratory.

RESULTS

Efficacious concentration in a mouse tumor model and dose selection for phase I expansion cohorts

The efficacy and PK-PD profiles of bintrafusp alfa were assessed in EMT-6 tumor-bearing B cell–deficient homozygous Jh female mice (see **Supplementary Information**). PK-PD modeling based on mouse tumor models suggested that 95% tumor growth inhibition is associated with a mean bintrafusp alfa concentration of ~ 100 μg/mL (**Figure 1**). Therefore, for the selection of the expansion dose levels for dosing every 2 weeks (q2w) in the phase Ib study, a population average C_{trough} of 100 μg/mL was targeted. By integrating tumor growth inhibition simulations at the predicted human exposure (data not shown) and the initial PD data from the dose escalation,[13] a flat dose of 1,200 mg q2w was selected for the expansion cohorts in phase I studies. In

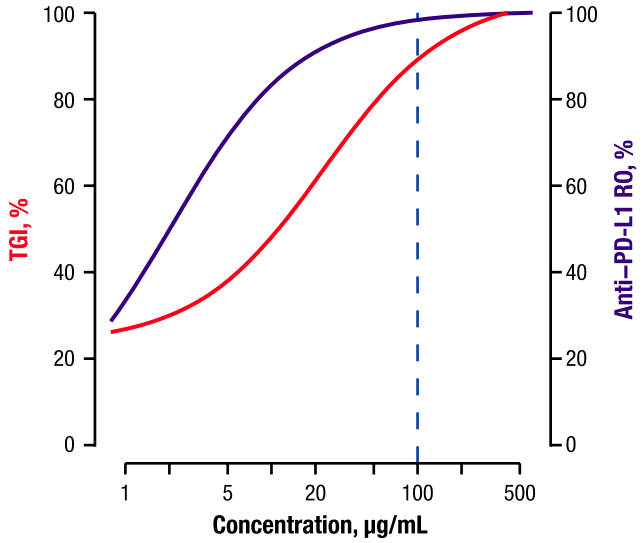

Figure 1 Tumor growth inhibition (% TGI) and anti–PD-L1 receptor occupancy (% RO) in the tumor vs. logarithmic bintrafusp alfa average concentration (μg/mL) modeling in preclinical mouse models. Preclinical pharmacokinetic-pharmacodynamic (PK-PD) modeling suggested that 95% TGI (red line) was achieved at an average concentration of ~ 100 μg/mL (dashed line), whereas 95% of anti–PD-L1 RO in tumor (purple line) was achieved at an average concentration of 40 μg/mL. The plot represents simulations following 3 weeks of treatment using dynamic PK-efficacy and PK-RO models.

the NSCLC tumor type expansion cohort, an additional dose of 500 mg q2w was chosen to support phase 2 dose selection, based on preclinical data that showed full PD-L1 inhibition in tumors and high % tumor growth inhibition at exposures associated with this dose in mice. Due to interpatient variability in PK, it was expected that the concentration required for maximal PD effect in humans would be < 100 μg/mL, such that with the population average C_{trough} of 100 μg/mL, the maximal PD effect will be achieved in most patients for the duration of dosing interval. This hypothesis was confirmed by additional PK-PD data from phase I studies, as described below.

PK and PK-PD profiles in phase I trials

Dose proportionality of PK profiles was assessed using dose-escalation data. In the dose-escalation phases of phase I trials NCT02517398 and NCT02699515,[13,15] patients were dosed with six different body weight-based dose levels (see the **Supplementary Information**). The observed first-dose PK profile indicated that an approximately dose-proportional increase in all exposures (AUC, maximum concentration (C_{max}), and C_{trough}) and approximately constant terminal half-life was achieved at doses > 3 mg/kg, suggesting that any target-mediated drug disposition was saturated at doses > 3 mg/kg.

The PK-PD data from trial NCT02517398 was used to estimate the bintrafusp alfa concentration that achieved maximal PD effect (in circulation) in the blood in all patients. Specifically, maximal PD-L1 TO in peripheral blood mononuclear cells and TGFs-β1, 2, and 3 trapping in circulation were observed in all patients when bintrafusp alfa serum concentrations were ≥50 μg/mL (**Figures 2** and **S1**), corresponding to doses of ≥10 mg/kg. Note that maximal PD-L1 TO and TGF-β1 and 3 TO in circulation were achieved in all patients at doses of ≥3 mg/kg (geometric mean (% coefficient of variation) first-dose C_{trough} of 11 μg/mL (33%)).

PopPK-based simulations indicated that the geometric mean (2.5th–97.5th percentiles) $C_{trough,ss}$ at the 500-mg and 1,200-mg q2w doses were 46.8 μg/mL (17.75–104.6 μg/mL) and 109.8 μg/mL (42.6–251.1 μg/mL), respectively. These simulations showed that 95% of patients dosed with 1,200 mg q2w were expected to have $C_{trough,ss}$ > 50 μg/mL, the concentration required for maximal PD effect in blood for all TGF-β isoforms and PD-L1. In addition, the geometric mean $C_{trough,ss}$ at 1,200 mg q2w in humans (~ 110 μg/mL) was similar to the mean efficacious concentration in mice (~ 100 μg/mL), associated with 95% tumor growth inhibition (**Figure 1**). Thus, PK-PD analyses of phase I data and PopPK-based simulations of $C_{trough,ss}$ distribution confirmed the selection of 1,200 mg q2w as the RP2D.

Exposure-efficacy analysis

Patients in the 2L NSCLC expansion cohorts of study NCT02517398 were randomized to receive 500 mg or 1,200 mg of bintrafusp alfa q2w (n = 40 per group) and were included in the exposure-efficacy analysis. At the data cutoff for exposure-efficacy analyses (see **Table S1**), a numerically higher investigator-assessed confirmed objective response rate (ORR) was observed with 1,200-mg q2w dosing (25% (95% CI, 12.7–41.2%)) compared with 500-mg q2w dosing (20% (95% CI, 9.1–35.6%)). Similarly, a trend of longer PFS was observed with 1,200-mg q2w dosing (median of 2.7 months; 95% CI: 1.4–8.2 months) compared with 500-mg dosing (median of 1.4 months; 95% CI: 1.3–2.7 months). The institutional review board–adjudicated efficacy data at a later data cutoff (July 23, 2018) confirmed earlier results, with median PFS of 1.4 months (95% CI, 1.3–4.2 months) and ORR of 17.5% with 500-mg dosing and median PFS of 4.0 months (95% CI, 1.3–9.5 months) and ORR of 25.0% with 1,200-mg q2w dosing.[14] Univariable logistic regression analyses of the probability of being a responder as a function of exposure (both $AUC_{0-336\ h}$ and $C_{trough,sd}$) showed a trend toward a positive association (**Figures 3a and S2**, **Table 1**), and 95% CIs for the OR overlapped 1. Because $AUC_{0-336\ h}$ and $C_{trough,sd}$ were highly correlated in this dataset, the results of these exposure-response analyses were similar between the two exposure metrics.

CL has recently been suggested to be a potential confounder for exposure-response analyses[19]; therefore, the relationship between CL to BOR was investigated. CL showed a stronger inverse association with BOR than any of the studied exposure metrics: larger OR, 95% CI model excluding 1 and smaller values, such as smaller Akaike information criterion (**Figure 3b**, **Table 1**). It is noted that the unit step for AUC (10,000 mg·hour/mL), C_{trough} (10 μg/mL), and CL (0.005 L/hour) for calculations of ORs corresponded to ~ 1 quartile of observed AUC, C_{trough}, and CL range, respectively, such that the ORs of exposure-BOR and CL-BOR univariable analyses could be compared.

Multivariable logistic regression models, including all covariates and each exposure metric separately, were also investigated,

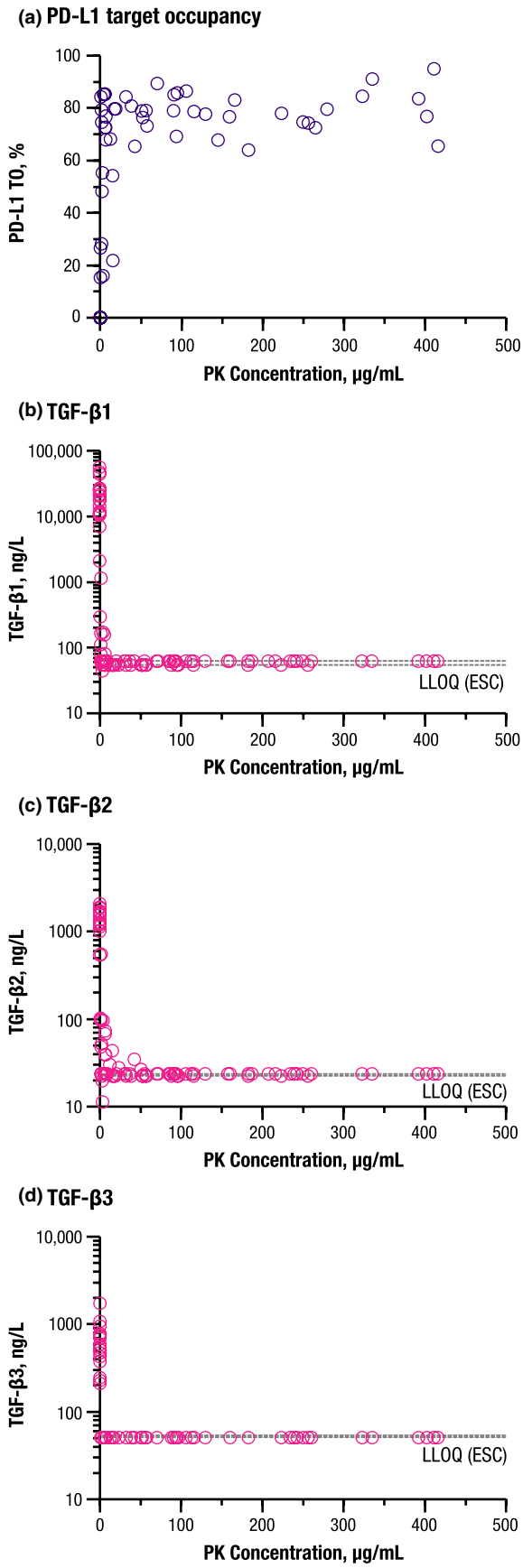

Figure 2 Pharmacokinetic-pharmacodynamic (PK-PD) profile of bintrafusp alfa in phase I studies. The relationships between bintrafusp alfa serum concentration (µg/mL) and PD-L1 target occupancy (% TO) (**a**) and between bintrafusp alfa serum concentration (µg/mL) and free TGF-β1/2/3 concentrations (ng/L) (**b–d**) are shown. Approximately maximal PD-L1 target occupancy in peripheral blood mononuclear cells and TGFs-β1, 2, and 3 trapping in circulation was observed in all patients at bintrafusp alfa concentrations ≥ 50 µg/mL.

as described in Methods. The covariates included in multivariable exposure-efficacy analyses, and results of these analyses, are shown in **Tables S2** and **S3**. The covariate effects were highly uncertain due to limited sample size, and 95% CI included the OR = 1 for most of the covariates, including exposure, but some trends were noted. Specifically, metastasis (classified per Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1), with 20% of patients having no metastasis at baseline) and high PD-L1 status on tumor cells (≥ 80%) showed a trend of association with response, with 95% CI excluding OR = 1.

In exposure-PFS analyses using univariable and multivariable models and in Kaplan–Meier analysis of PFS by exposure quartiles analysis, a lower risk for PFS events at higher exposure values was noted, with 95% CI excluding a hazard ratio = 1 for PFS (**Figures 3c and S2**, **Table 1**). In the univariable CL-PFS model, higher CL was associated with increasing risk for PFS events, with an apparently stronger association compared with that for exposure metrics in the univariable exposure-PFS models (**Table 1**) suggested by smaller hazard ratio (with unit step as described above for exposure-BOR) and smaller Akaike information criterion. In addition, metastases at baseline were associated with a higher risk for PFS events.

Thus, both the analyses of exposure-BOR and exposure-PFS consistently showed a trend toward an association between exposure variables and efficacy variables. However, the inverse association between CL and efficacy (BOR and PFS) seemed stronger than that between exposure and efficacy, which might be a manifestation of impact of disease status (e.g., cachexia) on both PK and efficacy, as expected for this class of immuno-oncology drugs.[6–10]

These exposure-efficacy and CL-efficacy analyses, together with a trend of improved ORR and PFS with 1,200-mg q2w dosing compared with 500-mg q2w dosing, suggested that exposures associated with the 1,200-mg q2w dose were associated with a better clinical outcome, supporting the selection of 1,200 mg q2w as an RP2D.

Exposure-safety analysis

A manageable safety profile was observed with bintrafusp alfa monotherapy, with a spectrum of irAEs consistent with other PD-(L)1 inhibitors, except potentially TGF-β–mediated skin lesions, which were observed in ~ 7% of the participants treated with bintrafusp alfa in phase I studies. The skin lesions mainly included hyperkeratosis, keratoacanthoma, and cutaneous squamous cell carcinoma and were most likely related to the TGF-β inhibition of bintrafusp alfa. These skin lesions are similar to what was reported with therapies targeting TGF-β blockade (e.g., fresolimumab).[20]

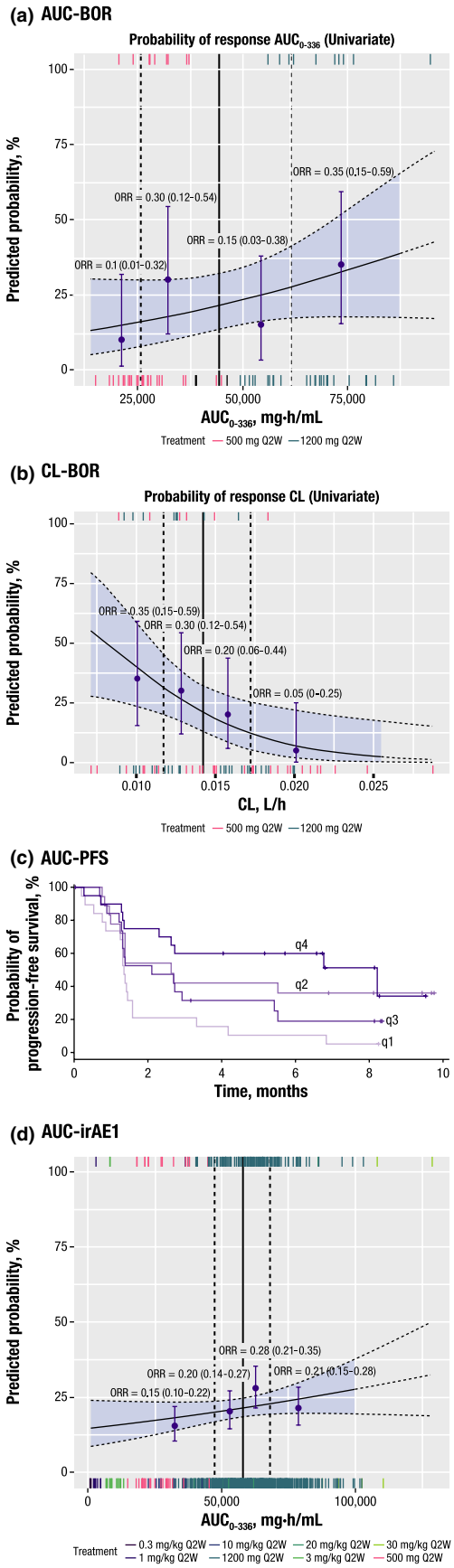

Figure 3 Evaluation of bintrafusp alfa exposure-response for efficacy and safety in phase I studies. (**a**, **b**) Univariable logistic regression analyses relating population pharmacokinetic (PopPK)-predicted bintrafusp alfa area under the curve (AUC) after first dose ($AUC_{0–336}$) or clearance (CL) to best overall response (BOR) in the 500-mg and 1,200-mg q2w 2L non-small cell lung cancer (NSCLC) cohorts are shown, respectively. (**c**) Kaplan–Meier analysis of progression-free survival (PFS) by exposure quartiles in the 500-mg and 1,200-mg q2w 2L NSCLC cohorts is shown. (**d**) Representative univariable exposure-safety analysis for $AUC_{0–336}$ vs. probability or immune-related adverse event of grade ≥ 1 (irAE1) in pooled dataset from the two phase I studies is shown. In **a** and **b**, points and error bars indicate objective response rate (ORR) and 95% confidence interval (CI) for estimated probability of response by $AUC_{0–336}$ or CL quartile (n = 20 per quartile); rugs show responders (above) and nonresponders (below). In **d**, points and error bars indicate means and 95% CIs for observed irAE1 probability by $AUC_{0–336}$ quartile; rugs above and below the represented distribution of $AUC_{0–336}$ for irAE1 (top) and none (bottom). In **a**, **b**, and **d**, solid vertical line is median exposure or CL; dashed vertical lines are 25th and 75th percentiles of exposure or CL, and the shaded area represents 95% CI to 99th percentile of exposure or CL (guide lines indicate predictions beyond this point).

Based on clinical observations, bintrafusp alfa was well-tolerated up to 30 mg/kg, and the maximum tolerated dose was not reached.[21–23] In addition, for the 2 dose levels evaluated in the 2L NSCLC cohorts of study NCT02517398 (500 and 1,200 mg i.v. q2w), overall safety findings were comparable and consistent with the observed safety profiles in studies NCT02517398 and NCT02699515. Exposure-safety analysis of bintrafusp alfa was based on safety data from 673 patients in the phase I studies, with most patients' doses at 1,200 mg q2w (**Table S4**). The AEs included in the analysis are shown in **Table 2** and the covariates are shown in **Tables S4 and S5**.

Overall, exposure-safety results for first dose and steady-state exposure metrics were similar, and results based on AUC and C_{trough} metrics were comparable. Logistic regression results in univariable and multivariable models for the first-cycle exposure metrics are summarized in **Table 2**. In the univariable models, positive exposure-safety association with 95% CI that excluded OR = 1 was observed for the following AEs: irAE incidence (grade ≥ 1; see **Figure 3d**), sPDAEs, and sTGAEs (**Figure S3**). Associations between exposure and above-listed AE incidence had ORs that were, in general, < 1.2 for an increase of 10 mg·hour/mL or 10 μg/mL in AUC or C_{trough}, respectively, and were considered relatively small given the range of exposures achieved. Bintrafusp alfa exposure was not associated with increased incidence of grade 3 irAEs (irAE3s), IRRs, grade 2 treatment-emergent AEs (TEAE2s), or grade 3 treatment-emergent AEs (TEAE3s) (**Table 2** and **Figure S3**). However, a negative association between exposure and TEAE incidence was observed. In addition, CL showed a positive correlation with incidence of irAE and TEAE2, which could be due to the confounding impact of disease status on exposure, although the effect size was relatively small.

The results for exposure metrics from the multivariable exposure-safety analyses were consistent with those obtained from univariate analysis (**Table 2**). Bintrafusp alfa exposure metrics

Table 1 Summary of univariable and multivariable (full) exposure-efficacy and CL-efficacy regression analyses

	Estimated odds ratio (95% CI) for BOR model		Estimated hazard ratio (95% CI) for PFS model	
Exposure metric or CL	Univariable model	Full model	Univariable model	Full model
$AUC_{0-336\ hours}$ (per 10,000 mg·hour/mL)	1.22 (0.945–1.58)	1.30 (0.899–1.97)	0.841 (0.732–0.966)	0.820 (0.692–0.972)
$C_{trough,sd}$ (per 10 μg/mL)	1.12 (0.947–1.32)	1.16 (0.914–1.51)	0.885 (0.804–0.973)	0.865 (0.772–0.970)
CL (per 0.005 L/hour)	0.341 (0.133–0.750)	—	1.956 (1.394–2.743)	—

AUC_{0-336h}, area under the concentration-time curve after the first dose; BOR, best overall response; CI, confidence interval; CL, clearance; $C_{trough,sd}$, serum trough concentration after the first dose; PFS, progression-free survival.
See **Table S1** for data extract dates and patient numbers.

Table 2 Summary of first-cycle exposure effects in univariable and multivariable exposure-safety regression analyses

	Univariable				Multivariable		
	AUC0–336h (per 10,000 mg·hour/mL)	Ctrough,sd (per 10 μg/mL)	CEOI,sd (per 10 μg/mL)	CL (per 0.005 L/hour)	AUC0–336h (per 10,000 mg·hour/mL)	Ctrough,sd (per 10 μg/mL)	CEOI,sd (per 10 μg/mL)
irAE1	1.084	1.075**	NE	0.6916***	1.157**	1.090**	NE
irAE3	1.020	1.048	NE	0.6853*	0.9333	0.9821	NE
IRR	1.083*	1.052**	1.012	0.7927**	1.072	1.038	1.013
sPDAE	1.146	1.100***	NE	0.6529***	1.263***	1.145***	NE
sTGAE	1.173	1.132***	NE	0.5664***	1.354***	1.181***	NE
TEAE1	NE	NE	NE	NE	NE	NE	NE
TEAE2	0.9939	0.9391	NE	1.733***	1.002	0.9503	NE
TEAE3	0.9250*	0.9223***	NE	1.584****	0.9234	0.9272**	NE

AUC_{0-336h}, area under the concentration-time curve after the first dose; $C_{EOI,sd}$, concentration at the end of infusion after the first dose; $C_{trough,sd}$, serum trough concentration after the first dose; CL, clearance; irAE1, grade 1 immune-related adverse event; irAE3, grade 3 immune-related adverse event; IRR, infusion-related reaction; NE, not evaluated; sPDAE, skin adverse event possibly related to PD-L1; sTGAE, skin adverse event possibly related to TGF-β; TEAE1, grade 1 treatment-emergent adverse event; TEAE2, grade 2 treatment-emergent adverse event; TEAE3, grade 3 treatment-emergent adverse event.
See **Table S1** for data extract dates and patient numbers.
*$P < 0.1$; **$P < 0.05$; ***$P < 0.01$; ****$P < 0.001$.

were generally weakly correlated or not correlated with AEs for all AEs. Exploration of other covariates was not the focus of this analysis because we observed an uneven distribution of covariates between the two phase I studies and dose levels that confounded the results (**Tables S4 and S5**). Overall, these exposure-safety results, together with the emerging safety profile of bintrafusp alfa at 1,200 mg q2w, supported the selection of 1,200 mg q2w as the RP2D.

Selection of RP2D for every 3 weeks (q3w) dosing

For concomitant administration of bintrafusp alfa with chemotherapies, which are frequently administered on a q3w schedule, 2,400 mg q3w of bintrafusp alfa was selected as the RP2D based on the analyses described below. For the selection of q3w dose, it was assumed that in order to achieve comparable efficacy, $C_{trough,ss}$ and time-averaged concentrations at steady state ($C_{avg,ss}$) should be similar to or higher than those achieved with 1,200 mg q2w dosing (monotherapy RP2D), such that PD effect is maintained in most patients for the duration of the dosing interval.

Specifically, based on PopPK modeling, the geometric mean $C_{trough,ss}$ achieved with 2,400-mg q3w dosing was 12% lower than that with 1,200-mg q2w dosing (96.8 vs. 110 μg/mL; **Figure 4a**). PopPK simulations also suggested that 88% of patients dosed with 2,400 mg q3w would have $C_{trough,ss}$ above 50 μg/mL (**Figure 4b**), which was the target $C_{trough,ss}$ based on PK-PD analyses. The $C_{avg,ss}$ over the dosing interval with 2,400 mg q3w dosing was expected to be ~ 33% higher than with 1,200-mg q2w dosing (328 vs. 246 μg/mL). Clinical assessment of the 2,400 mg q3w dose is currently ongoing.

DISCUSSION

Confounding factors in the interpretation of exposure-efficacy and exposure-safety data for therapeutic proteins in cancer indications include interplay among PK, baseline disease factors, and response.[6–10] These confounding factors are most pronounced when exposure-response analyses are conducted using data from a single dose level. Considering the potential confounders, results of the exposure-response modeling were interpreted in the context of all the available preclinical and clinical data.

First, clinical PK-PD profiles from the dose-escalation part of the phase I studies were used to establish a target serum concentration (50 μg/mL) that inhibited all four targets of bintrafusp alfa in circulation, specifically PD-L1 and TGFs-β1, 2, and 3 (**Figure 2**). This target concentration was mainly driven by the potency of bintrafusp alfa for neutralizing TGF-β2, which was the lowest among the four bintrafusp alfa targets, whereas maximal inhibition of the other three targets was achieved with

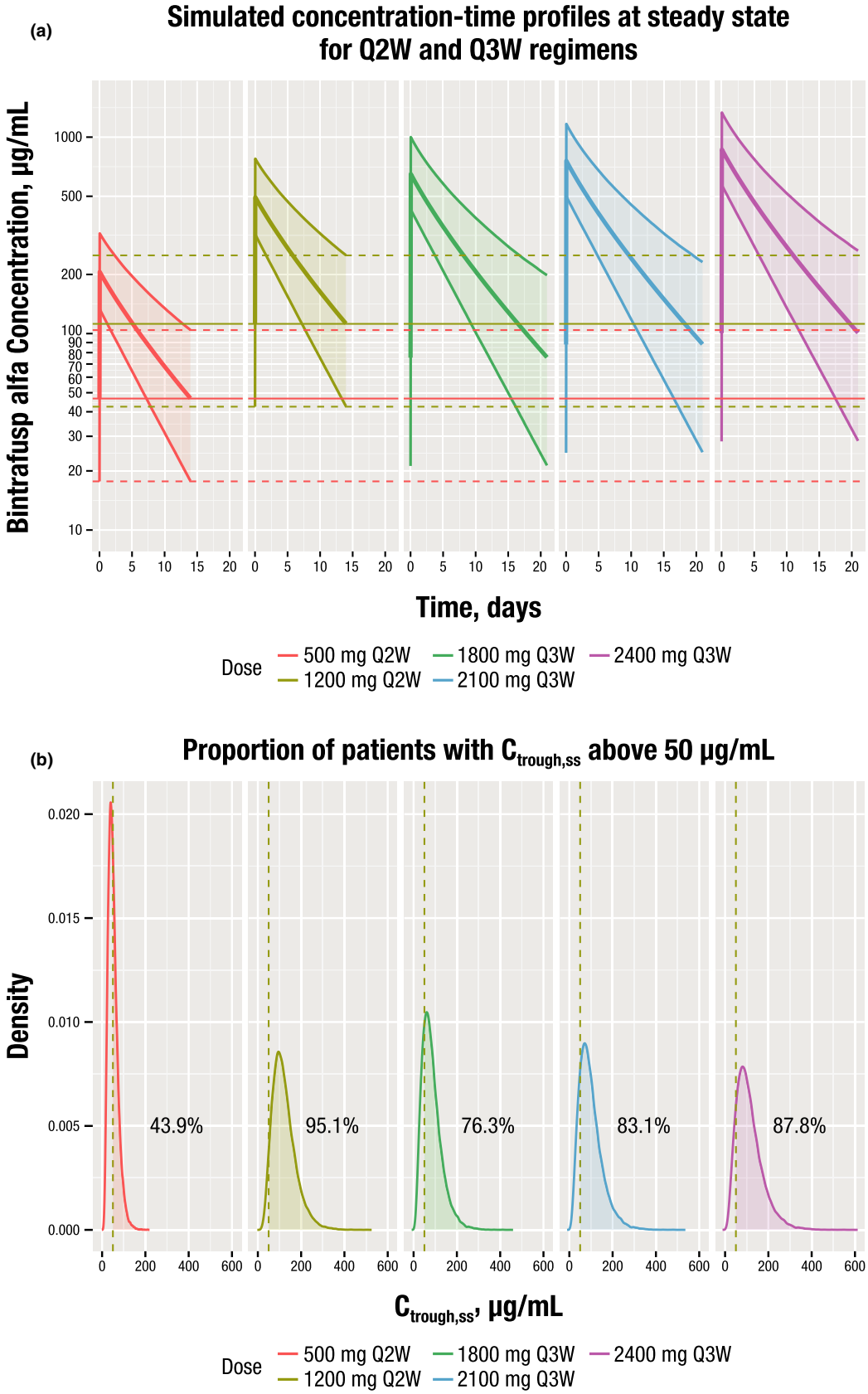

Figure 4 Simulated concentration-time profiles at steady state for q2w and q3w regimens (**a**) and proportions of patients above the target trough concentration (C_{trough}) of 50 µg/mL at steady state (**b**). Lines are medians. Shaded areas are 95% prediction intervals. Solid horizontal lines are median steady-state troughs for 500 mg q2w (orange) and 1,200 mg q2w (olive). Dashed horizontal lines are the 95% predicted range for steady-state troughs for 500 mg q2w (orange) and 1,200 mg q2w (olive).

the mean C_{trough} of ~ 11 μg/mL. The relative contribution of TGF-β isoforms as a driver of cancer pathogenesis remains to be fully established. However, considering that bintrafusp alfa is a large therapeutic protein and has limited tissue penetration,[24,25] higher concentrations of bintrafusp alfa are likely to be needed to inhibit PD-L1 and TGF-β1 in tumor tissues. The exact extent of tumor penetration of bintrafusp alfa in tissues (including tumors) is unknown, but assuming a typical tissue-to-blood ratio of 0.1 to 0.5 (based on reports for other monoclonal antibodies),[26] it is considered likely that 50 μg/mL of bintrafusp alfa in plasma will be associated with occupancy of PD-L1 and trapping of TGF-β1 in tissues. Accounting for interpatient variability in PK, it was predicted that the 1,200-mg q2w dose would maintain the target serum concentration of 50 μg/mL in > 95% of the patients.

Second, exposure-response and dose-response for efficacy were assessed in patients with the same tumor type (2L NSCLC) randomized into two dose levels: 500 mg q2w and 1,200 mg q2w. Overall, dose-efficacy and exposure-efficacy evaluations supported selection of 1,200 mg q2w as the RP2D for NSCLC participants. For all other indications explored in phase Ib, only a single dose level (1,200 mg) was evaluated. Therefore, due to confounding factors described above, exposure-efficacy analyses were not performed for indications other than NSCLC. Based on the mechanism of action of bintrafusp alfa, clinical experience with other checkpoint inhibitors, and the fact that there were no clinically relevant differences in bintrafusp alfa exposures across tumor types,[18] we found no evidence to suggest that the pharmacologically active or efficacious dose range would differ substantially among tumor types. However, the minimal effective dose may vary among tumor types due to differences in target expression and/or bintrafusp alfa penetration, further supporting the evaluation of 1,200 mg q2w instead of 500 mg q2w in multiple tumor types.

Third, exposure-safety analysis conducted on the integrated dataset from all patients treated with bintrafusp alfa across tumor types and indications also supported the selection of 1,200 mg q2w as the RP2D. At 1,200 mg q2w, the overall emerging safety profile of bintrafusp alfa is considered manageable and is consistent with targeted therapies in terms of the spectrum of irAEs seen with other checkpoint inhibitors and skin AEs observed with TGF-β inhibitors, such as fresolimumab.[20] Exposure was either weakly or not correlated with probability of AEs given the range of exposures achieved, and these correlations were not considered clinically meaningful. It is noted that the exposure-safety dataset was mostly composed of the 1,200-mg q2w cohorts (~ 85% of all patients), such that it was difficult to decouple the association of probability of an AE with exposure vs. that with baseline catabolic clearance. The extra layer of complexity for interpretation of exposure-safety modeling results was the finding that efficacy and safety were likely correlated for checkpoint inhibitors.[27] The mechanism of this interdependency between efficacy and safety was thought to be related to cross-reactivity between the tumor neoantigen and normal tissue antigens.[25] Overall, the emerging safety profile of bintrafusp alfa at the 1,200-mg q2w dose and the exposure-safety results support selection of 1,200 mg q2w as the RP2D of bintrafusp alfa. The selection of a flat dose vs. weight-based dosing approach is supported by modeling and simulations.[18]

Finally, for phase II and III studies in which bintrafusp alfa is administered in combination with chemotherapies, a modeling approach was used to select the q3w dose of bintrafusp alfa. Because most chemotherapies are administered q3w, the same dosing interval for bintrafusp alfa is preferred for convenience and compliance. 2,400 mg q3w is expected to achieve $C_{trough,ss}$ similar to that of 1,200-mg q2w dosing. The $C_{avg,ss}$ over the dosing interval is higher (33% increase) with 2,400-mg q3w dosing than 1,200-mg q2w dosing, an increase that is considered unlikely to have a clinically meaningful change in safety profile based on the exposure-safety profile described above. Similarly, considering a relatively flat concentration at the end of infusion (C_{EOI})-IRR relationship (**Table 2**), an increase in C_{EOI} with the 2,400-mg dose relative to that with the 1,200-mg q2w dose was not considered to significantly affect the benefit-risk ratio of the 2,400-mg q3w dosing regimen.

In summary, we describe the selection of q2w and q3w RP2D for bintrafusp alfa as 1,200 mg and 2,400 mg, respectively. This dose selection was based on integration of all available preclinical and clinical data from phase I studies. The modeling and simulation approaches, including PK-PD, PopPK, and exposure-response for efficacy and safety, were applied to support the selection of RP2D. The confounding factors for interpretation of exposure-efficacy and exposure-safety relationships were carefully considered, and the phase I study design included two randomized dose levels in the same indication (NSCLC) to partially decouple the impact of disease-related factors on both clinical outcome and PK. To further evaluate the clinical benefit of bintrafusp alfa in ongoing and future phase I through phase III studies in a variety of solid tumors, 1,200 mg q2w was selected for monotherapy and 2,400 mg q3w was selected for chemotherapy combination therapies.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

Supplementary Methods.
Tables S1–S5.
Figure S1.
Figure S2a.
Figure S2b.
Figure S3a.
Figure S3b.
Figure S3c.
Figure S3d.
Figure S3e.
Figure S3f.
Supplementary Figure Legends.

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CONFLICT OF INTEREST

A.K., S.E., L.K.-S., and A.K. are employees of Merck KGaA, Darmstadt, Germany. J.W. was employed as a consultant by Merck KGaA at the time the analysis was performed. Y.V., I.D., and L.S.O. are employees of EMD Serono Research & Development Institute, Inc., Billerica, MA, USA, a business of Merck KGaA. S.D. was an employee at EMD Serono Research & Development Institute, Inc., at the time the analysis was performed.

AUTHOR CONTRIBUTIONS

Y.V. and A.K. wrote the manuscript. All authors designed and performed the research. All authors analyzed the data.

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