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BRIEF REPORT

Risk assessment of drug–drug interaction potential for bintrafusp alfa with cytochrome P4503A4 substrates: A totality of evidence approach

Yulia Vugmeyster[1] | **George Locke**[1] | **Christoph Helwig**[2] | **P. Alexander Rolfe**[1] | **Jennifer Q. Dong**[1] | **Karthik Venkatakrishnan**[1]

[1]EMD Serono, Billerica, Massachusetts, USA

[2]The Healthcare Business of Merck KGaA, Darmstadt, Germany

Correspondence
Yulia Vugmeyster, EMD Serono, 45 Middlesex Turnpike, Billerica, MA 01821, USA.
Email: yulia.vugmeyster@emdserono.com

Abstract

Bintrafusp alfa, a first-in-class bifunctional fusion protein composed of the extracellular domain of TGF-βRII (a TGF-β "trap") fused to a human IgG1 mAb blocking PD-L1, is being evaluated for efficacy and safety in solid tumor indications as monotherapy and in combination with small-molecule drugs. We evaluated the perpetrator drug–drug interaction (DDI) potential of bintrafusp alfa via cytochrome P4503A4 (CYP3A4) enzyme modulation, which is responsible for the metabolism of a majority of drugs. The holistic approach included (1) evaluation of longitudinal profiles of cytokines implicated in CYP3A4 modulation and serum 4β-hydroxycholesterol, an endogenous marker of CYP3A4 activity, in a phase I clinical study, and (2) transcriptomics analysis of the CYP3A4 mRNA levels vs the *TGFB* gene expression signature in normal hepatic tissues. Bintrafusp alfa was confirmed not to cause relevant proinflammatory cytokine modulation or alterations in 4β-hydroxycholesterol serum concentrations in phase I studies. Transcriptomics analyses revealed no meaningful correlations between *TGFB* gene expression and CYP3A4 mRNA expression, supporting the conclusion that the risk of CYP3A4 enzyme modulation due to TGF-β neutralization by bintrafusp alfa is low. Thus, bintrafusp alfa is not expected to have DDI potential as a perpetrator with co-administered drugs metabolized by CYP3A4; this information is relevant to clinical evaluations of bintrafusp alfa in combination settings.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Bintrafusp alfa is a fusion protein targeting TGF-β and PD-L1 that is being evaluated for efficacy and safety in several cancer indications, both as monotherapy and in combination regimens with small-molecule drugs. A drug–drug interaction (DDI) risk assessment of bintrafusp alfa has not yet been described.

WHAT QUESTION DID THIS STUDY ADDRESS?

This study evaluated the CYP3A4-mediated perpetrator DDI potential of bintrafusp alfa.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

This study demonstrated that bintrafusp alfa lacks perpetrator DDI potential with drugs metabolized by CYP3A4. Based on available literature, transcriptomic analyses, and phase I data on cytokine and 4β-hydroxycholesterol, the risk of CYP3A4 enzyme modulation due to TGF-β neutralization following bintrafusp alfa treatment is inferred to be low.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

This study illustrates the application of a totality of evidence approach in addressing DDI risk assessment for therapeutic proteins with novel mechanisms. Our study provided valuable information for evaluation of efficacy and safety of bintrafusp alfa in combination settings.

INTRODUCTION

Therapeutic proteins are used to treat a variety of diseases and are often used in combination with small-molecule drugs.[1,2] Drug–drug interactions (DDIs) between therapeutic proteins and co-administered small-molecule drugs can alter the clearance of one or both agents.[2,3] DDIs with therapeutic proteins are unlikely to occur by direct interactions with small-molecule drugs and are often the result of indirect mechanisms.[1] Modulation of cytokines known to impact expression of transporters or drug-metabolizing enzymes is an established mechanism of perpetrator DDIs of therapeutic proteins with small-molecule drugs.[4] The expression of these cytokines can be impacted by disease conditions, such as cancer, inflammation, or infection.[5] The US Food and Drug Administration (FDA) recommends assessing potential DDIs for therapeutic proteins that can upregulate or downregulate the expression of cytochrome P450 (CYP).[6]

In this analysis, we evaluated the DDI potential of bintrafusp alfa, a first-in-class bifunctional fusion protein composed of the extracellular domain of the human TGF-β receptor II (TGF-βRII or TGF-β "trap") fused via a flexible linker to the C-terminus of each heavy chain of an IgG1 antibody blocking programmed death ligand 1 (anti-PD-L1), which has been explored as a potential treatment in a variety of cancer indications as a monotherapy and in combination with chemotherapies.[7]

Large therapeutic proteins, such as bintrafusp alfa, are thought to be primarily metabolized through proteolytic degradation.[8] Bintrafusp alfa does not show target-mediated drug disposition and is not associated with clinically relevant immunogenicity potential at therapeutic doses.[9] As a large fusion protein (177 kDa), bintrafusp alfa is not expected to act as a perpetrator affecting the pharmacokinetics (PKs) of concomitantly administered drugs by direct interactions with the molecular determinants of disposition of small-molecule drugs, nor is it expected to be affected as a victim of interactions with those drugs. However, the potential for bintrafusp alfa to modulate proinflammatory cytokines and, consequently, expression of CYPs in patients with cancer, has not been previously investigated and is difficult to predict. In patients with cancer, inflammation can increase proinflammatory cytokines and decrease CYP expression; these effects can be reversed by a successful treatment.[10] However, blockade of PD-L1 signaling by bintrafusp alfa is thought to enhance T-cell activation, resulting in the release of proinflammatory cytokines.[11] Studies of other therapeutic proteins have used profiling of proinflammatory cytokines to exclude potential perpetrator DDIs and eliminate the need for clinical DDI assessment.[12,13] Clinical safety data, together with population PK assessments, have indicated a low risk of victim DDI for bintrafusp alfa as monotherapy or in combination with chemotherapeutics, thus enabling the design of multiple combination phase II and III clinical trials NCT02517398, NCT04066491, and NCT03840915.[14,15]

Bintrafusp alfa has a unique mechanism of action and is a direct cytokine modulator via neutralization of TGF-β.[7] Preclinical studies showed that exogenous TGF-β can decrease mRNA levels of several CYP enzymes, including CYP3A4.[16] Thus, per the principles outlined in the FDA guidance for therapeutic protein-based DDIs, it was considered necessary to carry out a detailed assessment of the impact of TGF-β neutralization on expression of CYP enzymes involved in metabolism of small-molecule drugs.[3,6]

CYP3A4 enzyme is responsible for the metabolism of a majority of drugs.[17] Serum 4β-hydroxycholesterol is an endogenous biomarker of CYP3A4 activity. The 4β-hydroxycholesterol profiling is used to rule out strong CYP3A4 induction potential, including ruling out any increase in expression or activity of CYP3A4 upon TGF-β neutralization that may be expected to result in modulations of serum 4β-hydroxycholesterol concentrations. Treatment of patients with strong inducers of CYP3A4 enzyme has resulted in increases in serum concentrations of 4β-hydroxycholesterol.[18]

In this analysis, we used a holistic approach to assess the CYP3A4 modulation potential of bintrafusp alfa. We incorporated cytokine and 4β-hydroxycholesterol profiling in the design of phase I clinical trials to evaluate bintrafusp alfa perpetrator potential. We also evaluated the correlation between a *TGFB* gene expression signature and *CYP3A4* gene expression in normal liver samples to assess potential CYP3A4 expression modulatory DDI via bintrafusp alfa-mediated TGF-β neutralization.

METHODS

Clinical study data

We analyzed data from the cohorts of patients receiving bintrafusp alfa 1200 mg every 2 weeks, the recommended phase II dose (starting at day 1 and continuing until progression, unacceptable toxicity, death, or study withdrawal), in the open-label, phase I study NCT02517398. This study included patients with heavily pretreated solid tumors and had multiple expansion cohorts in specific tumor types.[14,15]

Cytokine modulation analysis

Serial blood samples for measurement of serum concentrations of a panel of cytokines comprising IL-1β, IL-6, IL-12, tumor necrosis factor α, and interferon γ (IFN- γ) were collected on days 1 (baseline), 2, 8, and 15 and at the 28-day safety follow-up in selected cohorts (see Tables S1 and S2). Serum concentrations of cytokines at baseline and on treatment were determined using a validated 10-plex immunoassay (Meso Scale Diagnostics, Rockville, MD) following manufacturer's instructions. If values were below the lower limit of quantification, they were imputed as 0.5 × lower limit of quantification for summary analysis. Fold change was calculated as the ratio of the postbaseline concentration and the baseline concentration for each patient per timepoint (N = 175, 168, and 167 for days 2, 8, and 15, respectively (Figure 1a) and n = 4 at the safety follow-up [not shown]).

4β-hydroxycholesterol profiling

Blood samples for measurement of serum 4β-hydroxycholesterol concentrations were collected at baseline and on days 43 and 85 in selected cohorts (n = 47 with available 4β-hydroxycholesterol data for all timepoints in selected cohorts [see Tables S1 and S3]). The assay was validated for use in serum, and sample testing was conducted at Q^2 Solutions (Durham, NC). Fold change was calculated as the ratio of the postbaseline and baseline concentrations for each patient.

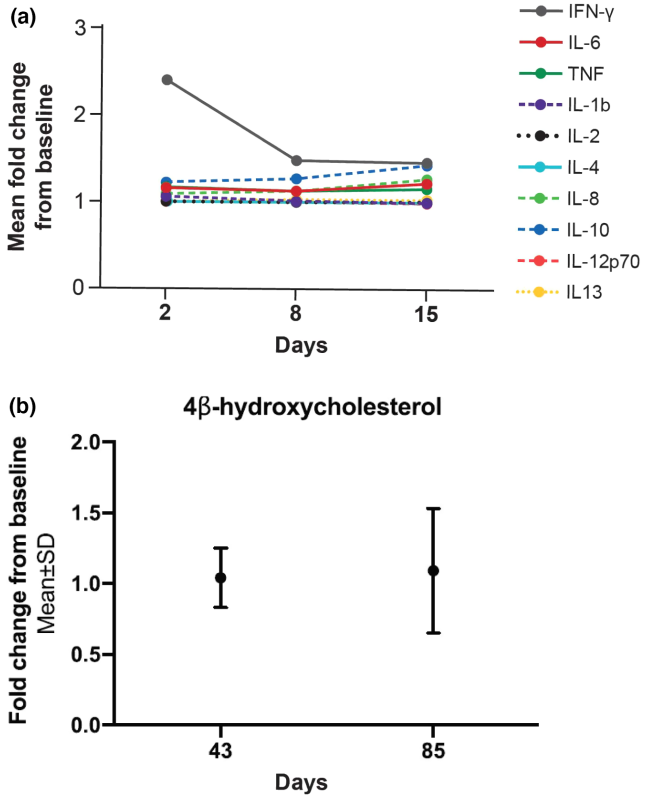

FIGURE 1 (a) Proinflammatory cytokines implicated in CYP enzyme modulation and (b) 4β-hydroxycholesterol in patients receiving bintrafusp alfa treatment. CYP, cytochrome P450; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor. Data from the pooled 1200-mg cohort in the phase 1 study (NCT02517398) in several tumor types (see Methods S1). Dosing was every 14 days starting on day 1. Figure parts a and b represent n = 175 and n = 47 at baseline, respectively.

Transcriptomic analyses

Three independent data sets (see Methods S1) consisting of gene expression data from normal liver samples were used to assess the correlations between a *TGFB* gene expression signature[19] and mRNA levels of CYP3A4 enzyme shown in Figure 2. The data sets included in this study were: The Cancer Genome Atlas Liver Hepatocellular Carcinoma (TCGA-LIHC) normal samples, consisting of nontumor areas from patients; the GTEx normal liver samples; and GSE24293, a non-cancer data set from patients undergoing gastric bypass surgery. The mRNA levels were previously determined with RNA sequencing (RNAseq) for all data sets except

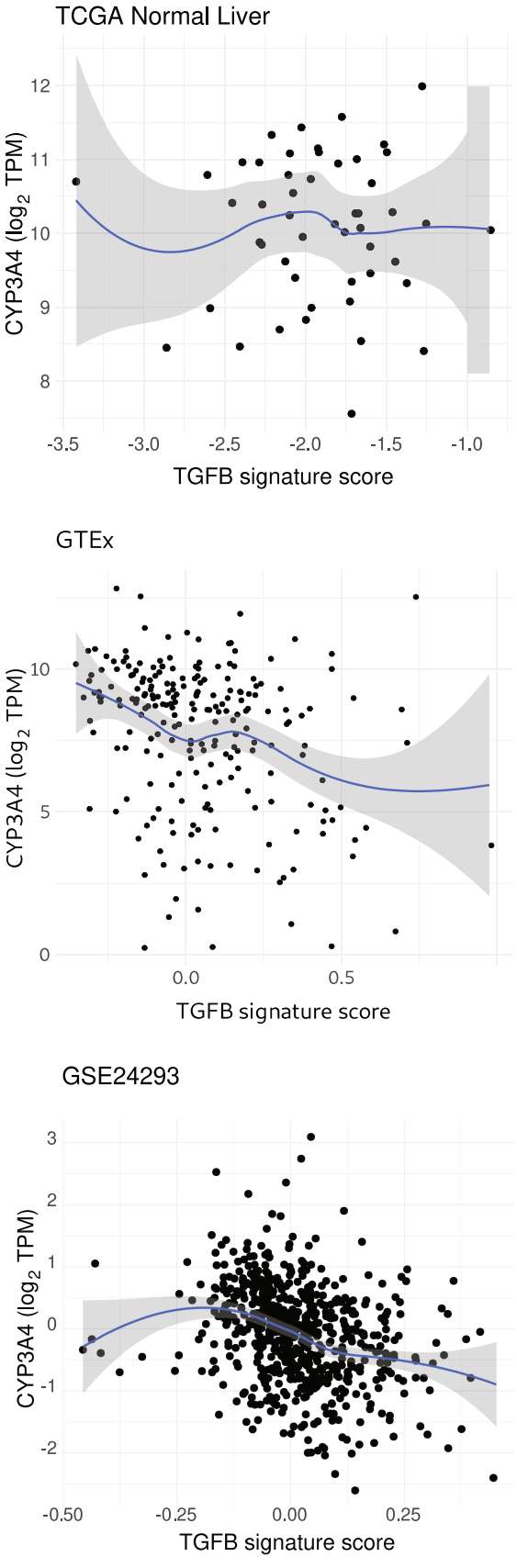

FIGURE 2 Transcriptomic analysis results evaluating the correlations between *TGFB* (*x* axis) and *CYP3A4* (*y* axis) gene expression in three normal liver data sets. The blue line shows LOESS regression; the gray area shows 95% confidence interval (CI). The data sets and statistical analyses are specified in Methods S1. CYP, cytochrome P450; LOESS, locally estimated scatterplot smoothing; rho, Spearman correlation coefficient; TGFB, transforming growth factor β; TPM, transcripts per million. TCGA normal liver, liver samples from gastric bypass (GSE24293), and normal liver samples from GTEx were used for these analyses, as described in Methods and Methods S1.

GSE24293. For GSE24293, microarray analysis was used, and gene expression values were computed as described in the Methods S1.

The relationship between *TGFB* (signature score) and *CYP* gene expression ($\log_2$ TPM) was assessed with the Spearman correlation coefficient (Spearman rho).

RESULTS

Bintrafusp alfa did not cause clinically relevant modulation of proinflammatory cytokines or 4β-hydroxycholesterol

Based on analyses conducted on samples from a phase I study in patients with metastatic or locally advanced solid tumors, bintrafusp alfa did not cause clinically relevant modulation of proinflammatory cytokines implicated in CYP3A4 enzyme modulation (Figure 1a). In this data set, baseline cytokine or 4β-hydroxycholesterol levels (Tables S2 and S3) appeared to overlap with the ranges reported in healthy patients.[13,20,21] The only cytokine with evidence of transient (2.4-fold) increase was IFN-γ, which had decreased toward the baseline by 2 weeks after the dose. No modulation of any other inflammatory cytokines was observed with bintrafusp alfa. Analyses conducted on samples from 47 patients in study 001 showed that bintrafusp alfa did not cause any relevant modulation in 4β-hydroxycholesterol concentrations (Figure 1b).

No meaningful correlation was found between TGFB and CYP3A4 expressions

Transcriptomic analyses of three normal liver data sets revealed no clinically meaningful correlation between *CYP3A4* mRNA levels and a *TGFB* gene expression signature that captures the downstream effects of TGF-β signaling (Figure 2), with the relatively low Spearman rho (absolute values 0.325 or less) in all data sets. Across all data sets, a relatively low fraction of interindividual variability in CYP3A4 expression could be explained by variation in *TGFB* gene expression, and no consistent relationships were discernible. Specifically, one data set showed no relationship (TCGA normal), the other showed

less than twofold difference in CYP3A4 mRNA across most of the range of the *TGFB* signature (GSE23293), whereas the third data set (GTEx), with a larger range in CYP3A4 mRNA levels over the larger range of *TGFB* signature scores, showed a relatively low Spearman rho of −0.305.

DISCUSSION

Bintrafusp alfa has previously shown efficacy and a manageable safety profile in patients with heavily pretreated advanced solid tumors.[9] The CYP3A4 perpetrator DDI potential of bintrafusp alfa by proinflammatory cytokine modulation or directly by TGF-β neutralization was investigated with consideration given to principles in the FDA guidance on DDI risk assessment for therapeutic proteins.[3,6,14] Proinflammatory cytokine data suggest that bintrafusp alfa does not cause relevant systemic inflammation or cytokine modulation in vivo. The largest increase was in IFN-ɣ, which showed transient increase to ≈2.4-fold but had decreased toward baseline by 2 weeks after treatment. Based on available literature, large changes in cytokine concentrations, such as IFN-ɣ, over ranges spanning several orders of magnitude are associated with clinically relevant modulation of CYP3A4 activity.[22]

The serum 4β-hydroxycholesterol profile served as an endogenous biomarker of CYP3A4. The lack of modulation of 4β-hydroxycholesterol indicated that bintrafusp alfa treatment lacks strong induction potential for CYP3A4. Both cytokine analysis and 4β-hydroxycholesterol profiling were performed on patients receiving the recommended phase II dose, 1200 mg every 2 weeks, which was selected to achieve maximal target inhibition of both PD-L1 and TGF-β for the duration of dosing.[14]

Additional supportive transcriptomics analyses were conducted to evaluate the extent of correlation between *TGFB* and *CYP3A4* gene expression in order to assess the potential impact of TGF-β neutralization. No meaningful correlations were observed between CYP3A4 mRNA levels and *TGFB* gene expression signature across the data sets examined.

The bintrafusp alfa DDI risk assessment presented in this report illustrates the application of a totality of evidence approach[23] supported by multidisciplinary collaboration on the DDI risk assessment for therapeutic proteins with novel mechanisms. We leveraged clinical pharmacology, biostatistics, biomarker sciences, and bioinformatics approaches to conclude that bintrafusp alfa does not have DDI potential as perpetrator of interactions with administered drugs metabolized by CYP3A4. Indeed, other studies have shown this approach to be successful in the assessment of potential DDIs.[12,13] The results of the DDI risk assessment have been valuable for clinical evaluations of bintrafusp alfa in combination settings.

AUTHOR CONTRIBUTIONS

Y.V., G.L., C.H., P.A.R., J.Q.D., and K.V. wrote the manuscript. Y.V., G.L., C.H., and P.A.R. designed the research. Y.V., G.L., C.H., P.A.R., J.Q.D., and K.V. performed the research. Y.V., G.L., C.H., and P.A.R. analyzed the data.

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CONFLICT OF INTEREST

Y.V., G.L., P.A.R., J.Q.D., and K.V. report employment with EMD Serono, Billerica, MA, USA. C.H. reports employment and stock ownership with the healthcare business of Merck KGaA, Darmstadt, Germany.

ORCID

Christoph Helwig https://orcid.org/0000-0003-4916-9918
P. Alexander Rolfe https://orcid.org/0000-0003-3540-1843
Karthik Venkatakrishnan https://orcid.org/0000-0003-4039-9813

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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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