


## ORIGINAL ARTICLE

# Direct and cytokine-mediated effects of albumin-fused growth hormone, TV-1106, on CYP enzyme expression in human hepatocytes in vitro

Maciej Czerwiński<sup>1</sup>  | Immaculate Amunom<sup>1</sup> | Victor Piryatinsky<sup>2</sup> | Hussein Hallak<sup>2</sup> | Yousif Sahly<sup>2</sup> | Oren Bar-Ilan<sup>2</sup> | Paul Bolliger<sup>1,3</sup> | Merav Bassan<sup>2</sup>

<sup>1</sup>Sekisui XenoTech, LLC, Kansas City, KS, USA

<sup>2</sup>Teva Pharmaceutical Industries Ltd, Petach Tikva, Israel

<sup>3</sup>Current affiliation: KCAS, Shawnee, KS, USA

## Correspondence

Maciej Czerwiński, Sekisui XenoTech, LLC, Lenexa, KS, USA.

Email: mczerwinski@xenotechllc.com

## Abstract

Some biologics can modulate cytokines that may lead to changes in expression of drug-metabolizing enzymes and cause drug-drug interactions (DDI). DDI potential of TV-1106—an albumin-fused growth hormone (GH)—was investigated. In this study, human blood was exposed to recombinant human growth hormone (rhGH) or TV-1106, followed by isolation of the plasma and its application to human hepatocytes. While the treatment of blood with rhGH increased multiple cytokines, treatment of blood with TV-1106 had no effect on any of the nine cytokines tested. The interleukin (IL)-6 concentration was higher in the rhGH than in the TV-1106-treated plasma ( $P < .05$ ). While rhGH had little or no effect on CYP1A2 or CYP2C19 mRNA but increased CYP3A4 mRNA twofold, TV-1106 had little or no effect on cytochrome P450 (CYP) mRNAs in hepatocytes. Although the plasma from rhGH-treated blood lowered CYP1A2 activity, the TV-1106 plasma had no effect on CYP activities. The CYP1A2 activity was lower in the rhGH- then in the TV-1106-plasma treated hepatocytes ( $P < .05$ ). The results indicated that fusing GH with albumin made TV-1106 an unlikely participant of CYP1A2, CYP2C19 or CYP3A4-facilitated, direct or cytokine-driven DDI.

## KEYWORDS

Carrier protein, CYP suppression, cytokine release, drug-drug interaction, growth hormone

## 1 | INTRODUCTION

The ability to predict drug interactions concerning biotherapeutics that can modify cytokines and potentially impact cytochrome P450

(CYP) enzymes, has been of growing interest to pharmaceutical companies and regulatory agencies. The 2012 Food and Drug Administration (FDA) guidance on drug interactions recommended assessment of therapeutic proteins that are cytokines or cytokine modulators (that have recognized effects on CYPs), are to be used concurrently with small molecule drugs, or cases where mechanisms other than biologics' influence on CYPs or transporters were identified.<sup>1</sup> The mechanism of therapeutic protein drug-drug interaction (DDI) implicates cytokine-mediated suppression of xenobiotic metabolizing enzymes. Multiple human investigations have documented

**Abbreviations:** CYP, cytochrome P450; DDI, drug-drug interaction; DMSO, dimethyl sulfoxide; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GH, growth hormone; GM-CSF, granulocytes monocyte-colony stimulating factor; IFN, interferon; IGF, insulin-like growth factor; IL, interleukin; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LPS, lipopolysaccharide; MCM+, modified Chee's medium; rhA, recombinant human albumin; rhGH, recombinant human growth hormone; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ .

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the effect of cytokines and their modulators on CYPs and transporters.<sup>2</sup>

TV-1106 is recombinant human growth hormone (rhGH) genetically fused to recombinant human albumin (rhA). Fusion of rhGH and rhA—a carrier protein without hormone activity, but with a long plasma half-life—is expected to extend systemic circulation of rhGH and preserve its activity.<sup>3,4</sup> TV-1106 is being developed for the treatment of GH deficiency to provide a sustained exposure that can reduce the frequency of injections and consequently improve compliance and quality of life for patients.<sup>3</sup> Phase 1 clinical trial demonstrated that the TV-1106 is well tolerated, has a prolonged plasma half-life, and is hormonally active in GH-deficient adult patients.<sup>5</sup> Side effects of GH therapy are rare and rhGH was shown to have a favorable overall safety profile.<sup>6</sup> But it is noted that children with Turner syndrome who were treated with rhGH exhibited a higher risk for targeted adverse effects (intracranial hypertension, slipped capital femoral epiphysis, scoliosis, and pancreatitis).<sup>7</sup> GH also increased synthesis of inflammatory cytokines interleukin-1- $\alpha$  (IL-1- $\alpha$ ), IL-6 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) by lipopolysaccharide (LPS)-activated monocytes in whole blood; and its administration at high doses to critically ill adults was associated with an increase in morbidity and mortality.<sup>8</sup>

Morgan et al<sup>9</sup> demonstrated that GH was a central regulator of CYP expression in rat livers. A separate investigation revealed induction of CYP3A4 in cultured human hepatocytes after exposure to GH.<sup>10</sup> Studies in GH-deficient children and adults have shown that the peptide affects the half-life of CYP-metabolized drugs.<sup>11–14</sup> The GH induced CYP1A2 and inhibited CYP2C19, while the hormone did not change CYP2D6 and CYP3A4 enzymes in healthy elderly men.<sup>15</sup> An evaluation of TV-1106 effects on plasma cytokines and on biotransformation of small molecules in the liver is warranted since rhGH also modulated plasma cytokines in GH-deficient and normal children of short stature.<sup>16,17</sup> We developed a method for evaluation of biologics as direct and cytokine-mediated determinants of hepatic biotransformation of xenobiotics. The assay is based on a treatment of whole blood with the biologic drug, followed by application of the resulted stimulated plasma to cultured hepatocytes.<sup>18</sup> The CYPs selected for this study dominate xenobiotic metabolism in human liver and are suppressed by IL-6 in vitro. We examined the ability of TV-1106 or rhGH to initiate cytokine release in whole blood and the effect of the drugs themselves or the stimulated plasma on CYPs 1A2, 2C19, and 3A4 expression in human hepatocytes.

## 2 | MATERIALS AND METHODS

### 2.1 | Chemicals and reagents

TV-1106, rhGH and a sterile drug vehicle were (10 mM sodium phosphate, 200 mM mannitol, 60 mM trehalose dehydrate, 0.08% (w/v) polysorbate 80, pH 7.2) provided by Teva Pharmaceutical Industries Ltd. *Escherichia coli* LPS was purchased from Sigma-Aldrich (St. Louis, MO). Blood was donated by healthy volunteers who gave informed consent to participate in the study. IL-6 and TRIZOL were purchased from EMD Biosciences (La Jolla, CA) and Invitrogen (Grand Island, NY),

respectively. The sources of the other reagents used in this study have been described elsewhere.<sup>19</sup>

### 2.2 | Cytokine release assay

Blood from four donors (three male, one female, 25–53 years old) was drawn into multiple sterile glass 10 mL vacutainers containing sodium heparin (Becton-Dickinson, Franklin Lakes, NJ), transferred into sterile 50 mL polypropylene tubes (BD Biosciences, San Diego, CA) and aliquoted into sterile polypropylene micro tubes (Sarstedt, Newton, NC). The concentration of rhGH applied to whole blood was about 15 times higher than  $C_{max}$  (~80  $\mu\text{g/L}$ ) measured in GH-deficient children dosed with 0.75 or 1.5 mg/kg of Nutropin Depot (somatropin).<sup>20</sup> The drug vehicle (1% v/v), LPS (50  $\mu\text{g/mL}$ ), rhGH (1.25  $\mu\text{g/mL}$ ), or TV-1106 (5  $\mu\text{g/mL}$ , concentration equimolar to rhGH) were added to blood, gently mixed and incubated at 37°C for 24 hours. Plasma was separated from whole blood by centrifugation at 600g for 10 minutes, aliquoted and stored at –80°C. Interferon (IFN)- $\gamma$ , interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-2, IL-6, IL-8, IL-10, IL-12p70, TNF- $\alpha$  and granulocytes monocyte-colony stimulating factor (GM-CSF) were measured in the plasma by sandwich immunoassay with electrochemiluminescence detection (Human Proinflammatory 9-Plex Ultra-Sensitive Kit, Meso Scale Discovery, Gaithersburg, MD) according to the manufacturer's instructions. The measurements were conducted by Viracor-IBT Laboratories (Lee's Summit, MO) with Meso Scale Discovery Sector Imager 2400. The lower and upper limits of cytokine quantitation were 0.6 and 2500 pg/mL, respectively.

### 2.3 | Hepatocyte culture and treatments

Hepatocytes were isolated from six nontransplantable livers by a two-step collagenase perfusion method and plated on collagen-coated 24-well plates, as described previously.<sup>21</sup> This isolation procedure routinely resulted in a coculture of hepatocytes and liver macrophages and the cells were treated according to published protocols.<sup>19,22</sup> Briefly, the confluent cocultures were achieved with  $\sim 4 \times 10^5$  cells per well, overlaid with Matrigel 2–3 hours after seeding and were adapted to culture conditions for 2–3 days with daily changes of medium. Following the adaptation period, the cells were treated once daily for 3 consecutive days with modified Chee's medium (MCM+), containing 0.1% v/v dimethyl sulfoxide (DMSO) (vehicle, negative control), IL-6 (10 ng/mL, positive control), 0.5% v/v drug vehicle, TV1106 (2.5  $\mu\text{g/mL}$ ) or 1 of 2 known human CYP enzyme inducers, namely, omeprazole (100  $\mu\text{mol/L}$ ) and rifampin (10  $\mu\text{mol/L}$ ). The MCM+ was supplemented with 0.98  $\mu\text{mol/L}$  dexamethasone. In addition, the hepatocytes were treated with plasma from drug vehicle-, LPS-, rhGH-, or TV-1106-treated blood. Aliquots of pooled plasma were thawed on the day of treatment. The plasmas were added to cultured cells at 10%, 20%, or 50%, v/v. Following last treatment, the cell culture medium was removed and the cells were washed with 1X phosphate buffered saline. Fresh incubation medium without plasma was added containing phenacetin (100  $\mu\text{mol/L}$ ), S-mephenytoin (400  $\mu\text{mol/L}$ ) or midazolam (30  $\mu\text{mol/L}$ ). After 30-minutes incubation with these probe substrates, 150  $\mu\text{L}$  of the medium was mixed with an equal volume of acetonitrile containing an appropriate

internal standard, as described in Paris et al.<sup>19</sup> Metabolite formation was analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the formation of acetaminophen (CYP1A2), 4'-hydroxymephenytoin (CYP2C19) or 1'-hydroxymidazolam (CYP3A). Details of the analytical methods are given in Table S1. Additional cells were lysed with TRIzol for isolation of mRNA.

## 2.4 | Cytotoxicity assessment

The leakage of lactate dehydrogenase in hepatocyte cultures was determined by the method provided by Roche Diagnostics (Catalog # 116447930010). Daily light microscopic examinations of the cultures throughout the culturing period were conducted.

## 2.5 | mRNA analysis

Total RNA was phase extracted with TRIzol followed by purification with an RNeasy Mini Kit (Qiagen, Valencia, CA). Purified RNA was reverse transcribed to cDNA with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA) and the Applied Biosystems 7300 real-time PCR System (AB7300). Quantitative PCR was performed with the AB7300 with the Applied Biosystems Universal Master Mix and TaqMan<sup>®</sup> Gene Expression Assays. CYP mRNA levels were normalized to the levels of glyceraldehyde 3-phosphate dehydrogenase mRNA, which were assumed to remain unaffected by the in vitro treatments.

## 2.6 | Data processing and statistical analysis

All data were processed and graphed with Microsoft Excel software (Office 2007; Microsoft, Redmond, WA). One-way analysis of the effects of rhGH and TV-1106 on the cytokine release in the whole blood was conducted with the nonparametric Wilcoxon test (Rank Sums). This type of statistical analysis was selected following the recognition of the large variability in the data, meaning the normality

of the data cannot be assumed. Two-way analysis of variance on the effects of drug vehicle, TV-1106 or rhGH plasma on CYPs mRNA and enzyme activity was conducted using the Wilcoxon test, for similar reasons as stated earlier. All statistical analysis was done with a software package—JMP Statistical Discovery Ver. 12.0.1, SAS Institute Inc., Cary, NC.

## 3 | RESULTS

### 3.1 | Effects of rhGH and TV-1106 on cytokine release in human whole blood in vitro

The effects of drug vehicle, LPS, rhGH or TV-1106 on the release of IFN- $\gamma$ , IL-1 $\beta$ , IL-2, IL-6, IL-8, IL-10, IL-12p70, TNF- $\alpha$ , and GM-CSF in whole blood are presented in Table 1. Statistical analysis of the data is shown in Table S2. Treatment of whole blood with LPS, a positive control, caused the expected release of cytokines. Treatment of whole blood with rhGH increased all but IL-12p70 cytokine, between 2.29- and 27.8-fold above the vehicle control. However, the increases of the cytokines were not statistically significant. Treatment of whole blood with TV-1106 changed cytokines concentration between 0.51- and 4.3-fold of vehicle control. The nonparametric Wilcoxon test (Rank Sums) did not reveal significant differences in the cytokine concentrations between the TV-1106 and vehicle control (analysis not shown). Statistical tests revealed a significant drop of IL-6 in the TV-1106 group as compared to the rhGH group ( $P < .05$ ).

### 3.2 | Cytotoxicity assessment

Lactate dehydrogenase released into the cell culture medium at 24, 48, and 72 hours was lower in the cultures treated with TV-1106 than rhGH for all 3 hepatocyte donors (data not shown). Daily light microscopic examinations of the cells throughout the culturing period did not reveal signs of toxicity.

**TABLE 1** The effects of drug vehicle, LPS, rhGH, or TV-1106 on cytokine release in whole human blood ex vivo

|                                 | Vehicle (1% v/v)                | LPS (50 $\mu$ g/mL)     | rhGH (1.25 $\mu$ g/mL)               | TV-1106 (5 $\mu$ g/mL)               |
|---------------------------------|---------------------------------|-------------------------|--------------------------------------|--------------------------------------|
| Interferon- $\gamma$            | 15.3 $\pm$ 15.1 <sup>a, b</sup> | 2990 $\pm$ 4180 (196)   | 39.6 $\pm$ 55.5 <sup>c</sup> (2.59)  | 7.73 $\pm$ 13.6 (0.506)              |
| Interleukin-1 $\beta$           | 4.64 $\pm$ 2.92                 | 13 200 $\pm$ 364 (2850) | 90.1 $\pm$ 67.8 (19.4)               | 20.1 $\pm$ 17.8 (4.32)               |
| Interleukin-2                   | 32.9 $\pm$ 41.1                 | 317 $\pm$ 315 (9.64)    | 213 $\pm$ 175 (6.47)                 | 61.3 $\pm$ 53.9 (1.87)               |
| Interleukin-6                   | 31.7 $\pm$ 42.2                 | 15 100 $\pm$ 3190 (476) | 879 $\pm$ 856* (27.8)                | 78.4 $\pm$ 99.1 (2.48)               |
| Interleukin-8                   | 2540 $\pm$ 1960                 | 10 900 $\pm$ 310 (4.29) | 5810 $\pm$ 3150 (2.29)               | 3510 $\pm$ 2640 (1.38)               |
| Interleukin-10                  | 8.81 $\pm$ 4.65                 | 1200 $\pm$ 652 (136)    | 27.5 $\pm$ 22.7 (3.13)               | 5.12 $\pm$ 4.50 (0.581)              |
| Interleukin-12p70               | 23.7 $\pm$ 20.3 <sup>b</sup>    | 46.0 $\pm$ 43.8 (1.94)  | 5.25 $\pm$ 4.64 <sup>b</sup> (0.222) | 12.7 $\pm$ 7.49 <sup>c</sup> (0.536) |
| Tumor necrosis factor- $\alpha$ | 19.5 $\pm$ 15.9                 | 8500 $\pm$ 4200 (435)   | 134 $\pm$ 144 (6.88)                 | 26.8 $\pm$ 15.2 (1.37)               |
| Granulocyte monocyte-CSF        | 81.7 $\pm$ 50.2                 | 470 $\pm$ 330 (5.75)    | 306 $\pm$ 320 (3.75)                 | 225 $\pm$ 215 (2.75)                 |

LPS, lipopolysaccharide; rhGH, recombinant human growth hormone; CSF, colony-stimulating factor; BLQ, below level of quantitation.

<sup>a</sup>Values are mean  $\pm$  standard deviation in pg/mL (fold change from vehicle) of duplicate determinations in blood from four donors.

<sup>b</sup>Values from two donors in this group were BLQ.

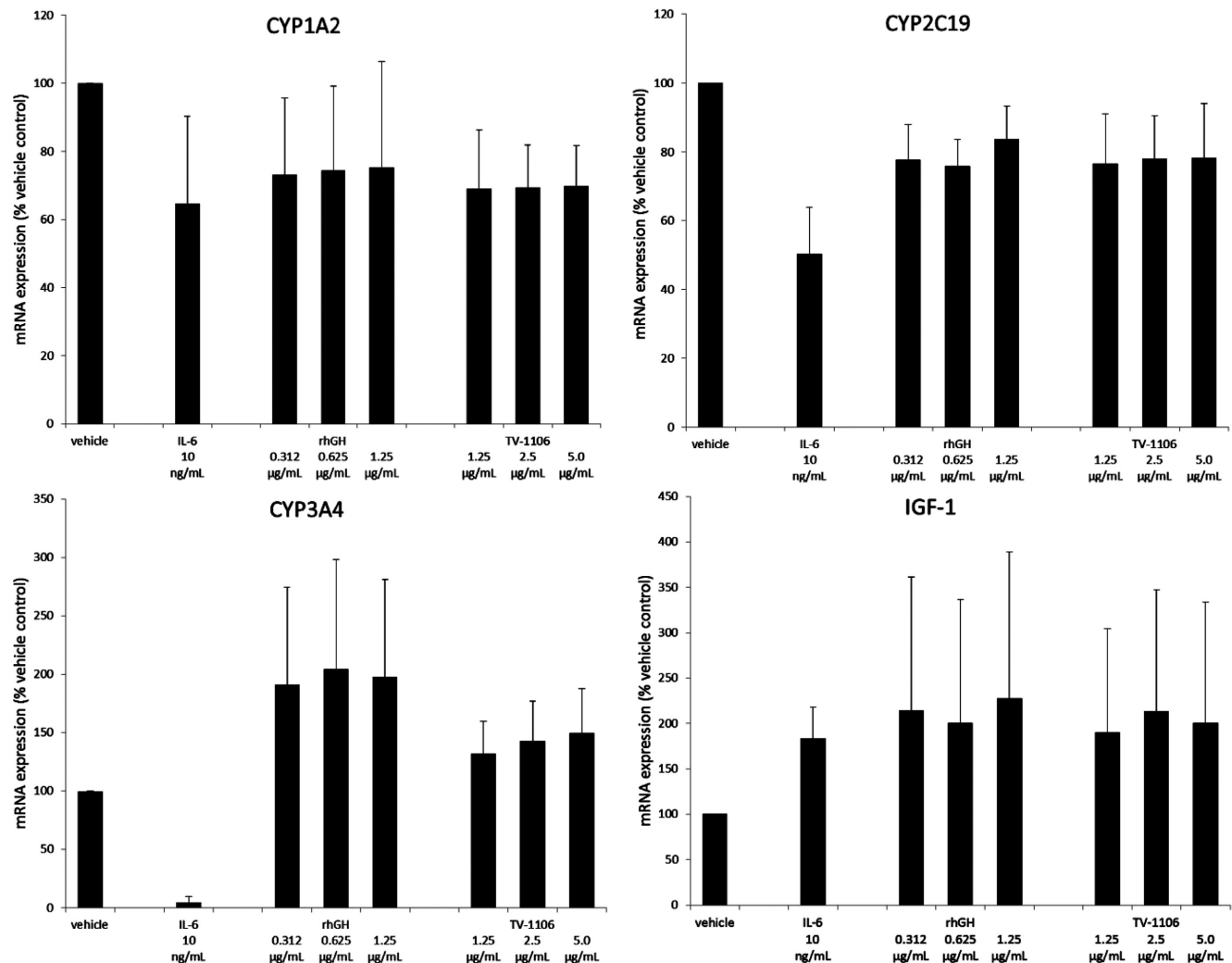
<sup>c</sup>Value from one donor in this group was BLQ.

\*IL-6 concentration was statistically higher in rhGH than in the TV-1106 group, nonparametric Wilcoxon tests (Rank Sums)  $P < .05$  (Table S2).

### 3.3 | Effects of rhGH and TV-1106 on CYP and IGF-1 mRNAs in human hepatocytes in vitro

To establish an in vitro equivalence of TV-1106 and rhGH, human hepatocytes (donors H1270, H1271, and H1272) were treated with vehicle, IL-6, rhGH or TV-1106. The TV-1106 was at equimolar concentrations to rhGH with regard to the hormone portion of the fused molecule. The effects of the treatments on CYPs 1A2, 2C19, 3A4 and insulin-like growth factor-1 (IGF-1) mRNAs are shown in Figure 1. Treatment of hepatocytes with IL-6 resulted in the expected suppression of CYP mRNAs and a twofold increase in IGF-1 mRNA. The CYPs and IGF-1 mRNA responses to equimolar

concentrations of rhGH and TV-1106 were not statistically different in three cultures of hepatocytes (*t* test: paired two sample for means,  $\alpha = 0.05$ , Microsoft Excel 2010, data not shown). The effects of treating additional cultures of hepatocytes with 0.1% DMSO, omeprazole, phenobarbital or rifampin on the CYP mRNAs and enzyme activities are presented in Table S3. As expected, treatment of hepatocytes with omeprazole caused, on average, a 79.2-fold increase in CYP1A2 mRNA levels. Treatment of hepatocytes with rifampin caused, on average, a 3.3- and 63.1-fold increase in CYP2C19 and CYP3A4 mRNA levels, respectively. Omeprazole, rifampin and phenobarbital caused, on average, about 50% reduction in IGF-1 mRNA levels.

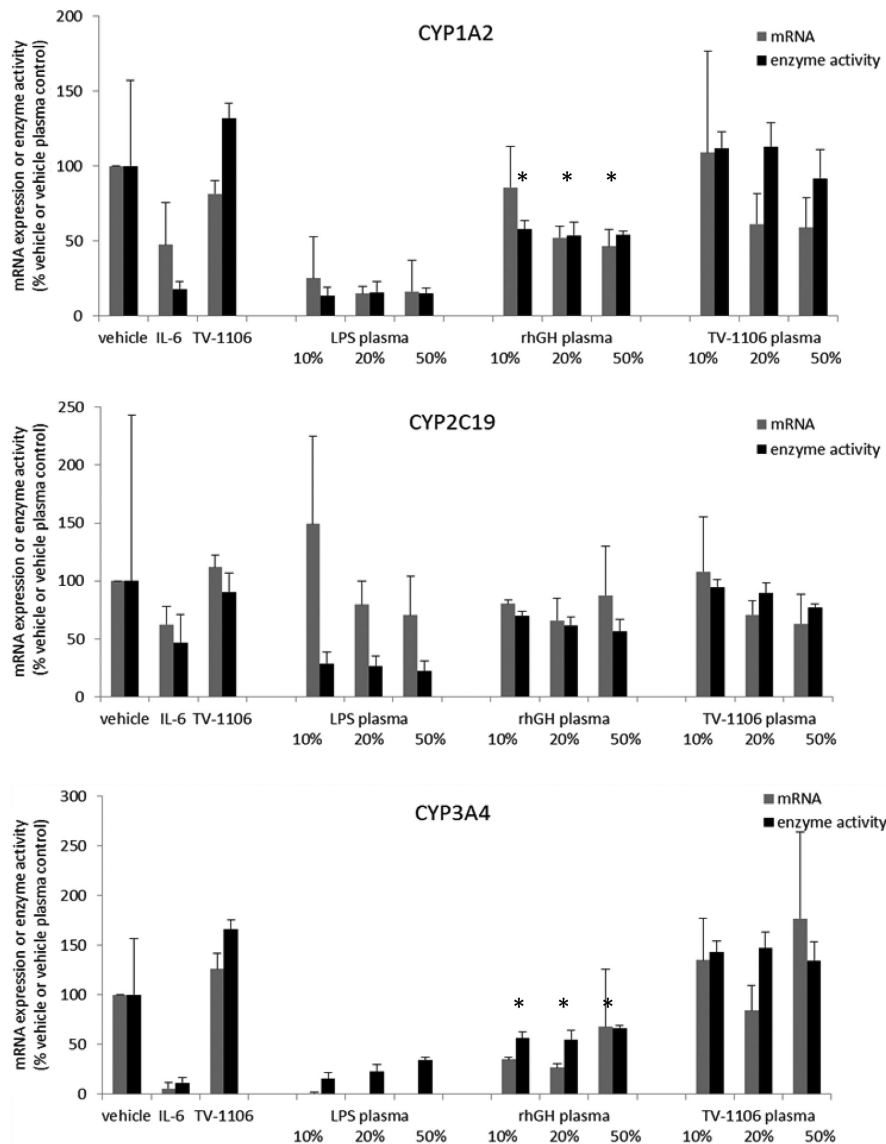


**FIGURE 1** Effects of incubating human hepatocytes with equimolar, with respect to the hormone portion of the molecule, concentrations of rhGH and TV-1106 on CYPs 1A2, 2C19, 3A4, and IGF-1 mRNA. Primary human hepatocytes (donors H1270, H1271, and H1272) were incubated for 72 hours with medium containing vehicle (0.5% v/v), IL-6 (10 ng/mL), rhGH (0.312, 0.625, or 1.25 µg/mL) or TV-1106 (1.25, 2.5, or 5.0 µg/mL). The mRNA levels were measured by quantitative PCR and normalized first to the levels of GAPDH mRNA and then to the levels of CYPs 1A2, 2C19, 3A4, and IGF-1 mRNA in hepatocytes incubated with cell culture medium containing drug vehicle. The mRNA values are the mean and standard deviation of measurements in 3 cultures. Differences in the rhGH and TV-1106 levels of the mRNAs were not significant (*t* test: paired two sample for means,  $\alpha = 0.05$ , Microsoft Excel 2010). rhGH, recombinant human growth hormone; CYPs, cytochrome P450; IGF-1, insulin-like growth factor-1; IL, interleukin; GAPDH, glyceraldehyde 3-phosphate dehydrogenase

### 3.4 | Effects of TV-1106 on CYP mRNAs and enzyme activities in human hepatocytes in vitro

The effects of treating cultured human hepatocytes (donors H1091, H1097, and H1108) with vehicle, TV-1106, or IL-6 on CYP mRNAs expression and enzyme activity are shown in Figure 2. The effects

of treating hepatocytes with 0.1% DMSO, omeprazole or rifampin caused expected changes in CYP mRNA levels and corresponding enzymatic activities (Table S3). The mRNA levels in these cultures were comparable to those in the donors H1270, H1271, and H1272 (Table S3). Treatment of human hepatocytes with IL-6 caused, on average, 53%, 38%, and 94% suppression and 82%, 53%, and 86%



**FIGURE 2** Effects of incubating human hepatocytes with TV-1106 or plasma prepared from whole human blood treated with drug vehicle, LPS, rhGH or TV1106 on CYPs 1A2, 2C19 and 3A4 mRNA and enzyme activity. Primary human hepatocyte cultures were incubated for 72 hours with medium containing vehicle (0.5% v/v), IL-6 (10 ng/mL), TV-1106 (2.5 µg/mL) or zero, 10%, 20%, or 50% plasma prepared from whole human blood treated with drug vehicle (1% v/v), LPS (50 ng/mL), rhGH (1.25 µg/mL) or TV-1106 (2.5 µg/mL). Plasma was pooled from four blood donors. The levels CYPs 1A2, 2C19, and 3A4 mRNA were measured by quantitative PCR and normalized first to the levels of GAPDH mRNA and then to the levels of CYPs 1A2, 2C19, and 3A4 mRNA in hepatocytes incubated with cell culture medium containing drug vehicle or plasma from blood treated with drug vehicle alone. The enzymatic activity of CYPs 1A2, 2C19, and 3A4 was determined in situ with 30-minute incubations with phenacetin, *S*-mephenytoin or midazolam, respectively, as described in Materials and Methods. Values are the mean and standard deviation of measurements in three cultures of hepatocytes (Table S4). \* - CYP1A2 mRNA in the rhGH plasma group (10%) was significantly lower than TV-1106,  $p < .05$ . CYP1A2 enzyme activity in the rhGH plasma groups (10 – 50%) was significantly lower than TV-1106,  $p < .005$ . CYP3A4 mRNA and enzyme activity in the rhGH were significantly lower than TV-1106,  $p < .01$  (Supplemental Table 2). LPS, lipopolysaccharide; rhGH, recombinant human growth hormone; CYPs, cytochrome P450; IL-6, interleukin-6; GAPDH, glyceraldehyde 3-phosphate dehydrogenase

reduction in CYPs 1A2, 2C19, and 3A4 mRNA and enzyme activity, respectively. Treatment of human hepatocytes with TV-1106 caused little or no change in CYP1A2 and CYP2C19 mRNA levels or enzyme activity. Treatment of human hepatocyte with TV-1106 caused little or no change in CYP3A4 mRNA levels (26% increase) or in CYP3A activity (66% increase); however, in 1 donor, this treatment had 34% of the rifampin effect at inducing CYP3A activity.

### 3.5 | Effects of plasma from drug vehicle-, LPS-, rhGH, or TV-1106-treated blood on CYP mRNAs and enzyme activities in human hepatocytes in vitro

Data from hepatocytes (donors H1091, H1097, and H1108) incubated with 10%, 20% or 50% plasma from LPS-, rhGH-, or TV-1106-treated blood were normalized to cultures from drug vehicle-treated plasma cultures at corresponding plasma concentrations (Figure 2, Table S4). Statistical analysis of the data is presented in Table S2. As expected, treatment of human hepatocytes with plasma from LPS-treated blood caused up to an 85% suppression of CYP1A2 mRNA levels and a reduction of up to 87% of phenacetin O-dealkylation activity. Treatment of hepatocytes with plasma from rhGH-treated blood caused a concentration-dependent suppression of up to 54% of CYP1A2 mRNA levels and a reduction of up to 42% in CYP1A2 activity. Treatment of hepatocytes with plasma from TV-1106-treated blood caused a concentration-dependent suppression of up to 41% of CYP1A2 mRNA levels, but had little or no effect on CYP1A2 activity (8% decrease to 13% increase in 3 cultures). The TV-1106 or rhGH plasma, as compared to control, did not have a significant effects on CYP1A2 mRNA. The rhGH plasma decreased CYP1A2 mRNA as compared with the TV-1106-plasma ( $P < .05$ ), and it dose-dependently reduced CYP1A2 enzyme activity, as compared to the TV-1106-plasma ( $P < .005$ ).

Treatment of human hepatocytes with 10% plasma from LPS-treated blood had little effect on CYP2C19 mRNA expression (a 49% increase), while treatment with 20% or 50% of this plasma decreased CYP2C19 mRNA levels up to 30%. Treatment of hepatocytes with plasma from LPS-treated blood caused up to a 78% decrease in 5-mephenytoin 4'-hydroxylation. Treatment of hepatocytes with plasma from rhGH- or TV-1106-treated blood caused decreases of up to 34% and 37% in CYP2C19 mRNA levels, respectively, and reductions of 44% and 23% in CYP2C19 activity, respectively. The TV-1106-plasma, as compared to the rhGH-plasma, did not have a significant effect on CYP2C19 mRNA or enzyme activity.

Treatment of cultured human hepatocytes with plasma from LPS-treated blood caused the suppression of CYP3A4 mRNA levels in 2 hepatocyte cultures (donors H1091 and H1108), but caused an unexpected increase in CYP3A4 mRNA levels in the third hepatocyte culture (up to 6.43-fold, donor H1097). Treatment of hepatocytes with 10%, 20%, or 50% plasma from LPS-treated blood caused, on average, 85%, 77%, and 66% reduction in midazolam 1'-hydroxylase, respectively. Treatment of hepatocytes with up to 50% plasma from rhGH-treated blood caused a decrease of up to 73% in CYP3A4 mRNA levels and a corresponding decrease of up to 45% in CYP3A

activity. Treatment of hepatocytes with up to 50% plasma from TV-1106-treated blood caused little change in CYP3A4 mRNA levels (up to a 77% increase) and a corresponding increase (up to 48%) in CYP3A activity. The rhGH plasma, as compared to TV-1106 plasma, significantly decreased CYP3A4 mRNA and enzyme activity ( $P < .01$ ).

## 4 | DISCUSSION

We compared the ability of TV-1106, an albumin-fused GH, and rhGH to stimulate cytokine release in whole human blood and the effects of the plurality of the released cytokines on hepatic drug metabolism in vitro. We found that genetically fusing GH to albumin attenuated the peptide cytokine response in whole blood and its effects on CYPs 1A2, 2C19, and 3A4 mRNA and enzyme activity in hepatocytes from healthy donors. The cytokine and CYP in vitro response to TV-1106 demonstrated weak-to-no DDI potential of the drug compared to rhGH. The essential findings of this investigation are in agreement with earlier studies. Liddle et al<sup>10</sup> observed an increase in CYP3A4 mRNA in hepatocytes treated with rhGH or dexamethasone while CYP1A2 mRNA was decreased on day 4 of the culture but increased on day 6, as compared to the vehicle. Similarly, we did not observe an effect of rhGH on CYP1A2 mRNA, while the increase in CYP3A4 mRNA was, as expected, lower than that reported by Liddle since we cultured the cells in MCM+ containing dexamethasone. Effects of GH, dexamethasone, or both compounds together on CYP mRNAs were studied in human hepatocytes.<sup>23</sup> Authors observed an inductive effect of dexamethasone on CYP1A2 mRNA and protein level. The GH treatment suppressed CYP1A2 in male hepatocytes but had neither an inductive nor suppressive effect on the CYP1A2 mRNA in cultures from female donors. In agreement with our culture medium containing dexamethasone, the authors stated that a combination of constant GH and dexamethasone was more inductive for CYP3A4 mRNA than GH alone.

Pagani et al<sup>17</sup> reported significant increases of TNF- $\alpha$  and IL-6, but not in IL-4 or IL-12, in GH-deficient children during substitutive GH therapy. Bozzola et al<sup>16</sup> found that in normal short stature children application of GH increased plasma concentration of IGF-1, IFN- $\gamma$ , IL-1 $\beta$ , IL-2, IL-12, and TNF- $\alpha$ . In our study GH increased IL-1 $\beta$ , IL-2, and IL-6 and it had little or no effect on IL-12p70 in blood from healthy adults, but these changes were not statistically significant. In our study, the TV-1106 cytokine response, as compared with GH, was significantly lower only for IL-6, and it can be attributed to steric hindrance of the peptide interaction with its molecular partners due to close proximity of the albumin.

Human hepatocytes cultured on Matrigel-coated culture dishes have been shown to maintain GH-responsiveness, as demonstrated by an increase in IGF-1 and CYP3A4 mRNAs and CYP3A enzyme activity.<sup>10</sup> We hypothesize that the lack of a direct effect of albumin-fused GH on CYPs in our system is due to steric hindrance of the peptide by albumin.

Indirect, cytokine-mediated, suppression of mRNA and reduction in enzyme activity of CYP1A2 and CYP3A4 by plasma from blood treated with LPS were expected.<sup>18,24</sup> In our study the cytokine-mediated effect of albumin-fused rhGH is attenuated, likely due to reduced cytokine response to TV-1106 as compared to rhGH.

We recognize that this study has a limitation, one of which is that only four blood donors we used in the cytokine release assay. This experimental design is therefore the simplest form of repeated measures, however, the correlation between pairs of the observations was not very consistent so analysis of repeated measures can be neglected. This limitation has a bearing on assigning causality to the observed limited responsiveness of hepatic CYPs in TV-1106- as compared to the rhGH-treated cells. Despite the low number of the observations in the cytokine release assay, Wilcoxon test supported a finding that rhGH treatment of the blood, but not that with TV-1106, resulted in increased level of IL-6. Other limitation of the study is that potential cytokine release from macrophages at the site of drug injection and lymphatic and blood vessel endothelial cells is not accounted for in vitro.

Study of human GH noncovalently attached to albumin did not identify safety concerns with the molecule, which was intended for a once weekly dosing.<sup>25</sup> Also, a recombinant fusion protein of albumin and IFN did not increase adverse respiratory events in patients with chronic hepatitis C virus, as compared to pegylated IFN, which indicated that modifying IFN with albumin was as safe as pegylation of the cytokine.<sup>26</sup>

Taken together, our data and investigations of other albumin-linked peptides identify albumin as a safe carrier protein for a delivery of sustained dose of GH. Since TV-1106 appeared to have reduced ability to stimulate cytokine release and to exert cytokine-mediated effects on hepatic CYPs 1A2, 2C19 and 3A4, we conclude that DDIs mediated by these enzymes are unlikely.

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## DISCLOSURES

None declared.

## ORCID

Maciej Czerwiński  <http://orcid.org/0000-0003-0047-7979>

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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