Correlation of In Vitro and Human Drug Interaction Studies with Gemcabene

McShane M[1], Radulovic L[2], and Bisgaier CL[1]

Gemphire Therapeutics Inc.[1], Northville, MI and Drug Development Preclinical Services, LLC [2], Ann Arbor, MI

ABSTRACT

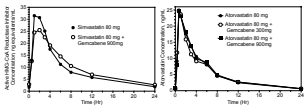
Background: Gemcabene is a novel lipid-regulating compound being developed as an adjunct to diet and statin therapy for the treatment of dyslipidemia. Patients with dyslipidemia typically take many medications often including multiple lipid-altering therapies such as statins. Therefore, it is essential to understand and mitigate the potential risk of drug-drug interaction (DDI). The current studies provide an analysis of potential drug interactions with gemcabene both *in vitro* and *in vivo* clinical studies.

Methods: Reaction phenotyping of gemcabene was performed with the 10 major cDNA-expressed human CYP P450 isoenzymes (CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4) and flavin monooxygenases (FMO-3). Gemcabene was also tested as a potential inhibitor/substrate of P-gp, BCRP, OAT1, OAT3, OATP1B1, OATP1B3, and OCT2 *in vitro*, as well as a potential inducer of CYP1A1/2 and CYP3A4 in isolated human hepatocytes and an inhibitor of the major drug-metabolizing CYP450 isozymes (CYP1A2, 2A6, 2C9, 2C19, 2D6, 2E1, and 3A4) *in vitro*. Clinical DDI studies were conducted with gemcabene and digoxin, atorvastatin, or simvastatin.

***In Vitro* Results:** Under the conditions tested, the *in vitro* studies showed: **1)** no evidence of gemcabene metabolism by cDNA-expressed human CYP450 isozyme or FMO-3 isoenzymes; **2)** low DDI potential for gemcabene with compounds metabolized by these CYP450 enzymes; **3)** no significant change in CYP1A1/2 activity and a moderate increase in CYP3A4-catalyzed testosterone 6β-hydroxylation; **4)** Gemcabene is not a substrate for P-gp, BCRP, OAT1, OAT3, OATP1B1, OATP1B3, OCT2; and **5)** Gemcabene is not an inhibitor of OCT2, is a borderline inhibitor of BCRP (expressed in MDCK cells only), and an inhibitor of P-gp, OAT1, OAT3, OATP1B1, and OATP1B3.

***In Vivo* Results:** In an open-label, multiple-dose study in 12 healthy subjects, gemcabene (900 mg) did not significantly affect the exposure (C_{max} and $AUC_{0\text{-}24}$) of digoxin (0.25 mg). Specifically, the 90% confidence interval for digoxin $AUC_{(0\text{-}24)}$ ratios were within the 80% to 125% range, thus confirming the in vitro results of no DDI with a P-gp substrate. In two open-label, multiple-dose studies in healthy volunteers, gemcabene (900 mg) did not significantly affect the exposure (C_{max} and $AUC_{0\text{-}24}$) of atorvastatin (80 mg) or simvastatin (80 mg) thus confirming the *in vitro* results of no DDI with CYP450 (see Figure below).

Pharmacokinetic Profiles of Statins Co-administered with Gemcabene



Conclusions: The results from *in vitro* and *in vivo* studies suggest co-administration of gemcabene is unlikely to result in an interaction with a variety of medications. The *in vitro* metabolism and drug transporter studies correlated well with the clinical P-gp interaction study, whereas, the atorvastatin and simvastatin clinical DDI studies confirmed the *in vitro* results of no metabolism-based interactions (i.e., CYP450) but dispelled the *in vitro* OATP1B1/OATP1B3 transporter results. An *in vivo* drug-drug interaction study is still needed to determine the potential for gemcabene to interact with an OAT1 or OAT3 substrate and possibly, BCRP since the extent of inhibition by gemcabene was borderline.

INTRODUCTION

Gemcabene is a novel lipid-regulating compound being developed as an adjunct to diet and statin therapy for the treatment of dyslipidemia. Patients with dyslipidemia typically take many medications often including multiple lipid-altering therapies such as statins; therefore, it is essential to understand the potential risk of drug-drug interaction (DDI) to minimize the risk of adverse drug reactions.

METHODS

Cytochrome P450 Studies: cDNA-expressed human CYP P450 isoenzymes, isolated human liver microsomes, and human hepatocytes were used to assess gemcabene as a potential substrate and/or inducer of the major drug-metabolizing CYP450 isozymes and FMO-3.

Transporter Studies: Gemcabene was tested as a potential inhibitor or substrate of P-gp, BCRP, OAT1, OAT3, OATP1B1, OATP1B3, and OCT2 (Absorption Systems) per FDA and EMA Guidelines[a,b]. Caco-2 cells were used to determine gemcabene potential as a substrate of P-gp and BCRP. Caco-2 cells and MDR1-MDCK cells were used to determine gemcabene inhibitor potential toward P-gp and BCRP. Human embryonic kidney (HEK293) epithelial cells transfected with either OAT1, OAT3, OATP1B1 or OATP1B3 were used to determine the substrate potential of gemcabene. HEK293 cells transfected with OCT2, OAT1, OAT3, OATP1B1 or OATP1B3 were also used to determine the inhibitor potential of gemcabene. Depending on the results of the initial in vitro studies at very high gemcabene concentrations, additional IC_{50} studies were conducted.

Human Drug-Drug Interactions Studies: Three *in vivo* drug-drug interactions studies were conducted with gemcabene. **Study 1027-011** was an open-label, multiple-dose study in 12 healthy subjects designed to evaluate the administration of gemcabene 900 mg orally for 10 days on the PK of digoxin 0.25 mg (a P-gp substrate) administered orally once daily for 20 days. **Study 1027-008**[c] was an open-label, multiple-dose, randomized, 2-way crossover study in 20 healthy subjects designed to evaluate the oral administration of gemcabene 900 mg QD for 15 days on the PK of simvastatin 80 mg administered QD orally. **Study A4141002**[d] was an open-label, multiple-dose, crossover study in 20 healthy subjects designed to evaluate the effect of gemcabene on the PK of atorvastatin 80 mg administered QD orally. Both atorvastatin and simvastatin are OATP1B1 and OATP1B3 substrates.

Lack of *in vivo* drug-drug interaction was defined as 90% CI for C_{max} and $AUC_{(0\text{-}24)}$ ratios within the 80% to 125% range.

ATVB 2016 Nashville, TN 05May 6:00-8:00 pm

Abstract Number: 466 Presentation Number: 229

IN VITRO RESULTS

Gemcabene Reaction Phenotyping: The purpose of this study was to determine which cDNA-expressed human CYP450 izoymes or Flavin monoxygenases (FMO-3) has the ability to metabolize gemcabene. The 10 major cDNA-expressed human CYP P450 isoenzymes (CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4) and flavin were individually incubated with 100 µg/mL gemcabene.

There was no evidence of gemcabene metabolism by CYP450 isozymes or FMO-3.

Effect of Gemcabene on Substrate Metabolism By Key Cytochrome P450 Enzymes in Human Liver Microsomes

Cytochrome	Reaction	Substrate	Gemcabene Effect (IC50)
CYP1A2	6-hydroxylation	(R)-Warfarin (0.5mM)	Not Significant IC50>1500µM
CYP2A6	7-hydroxylation	Coumarin (4µM)	Not Significant IC50>1500µM
CYP2C9	7-hydroxylation	(S)-Warfarin (4µM)	Not Significant IC50>1500µM
CYP2C19	4'-hydroxylation	(S)-Mephenytoin (50 µM)	Not Significant IC50>1500µM
CYP2D6	O-demethylation	Dextromethorphan (5µM)	Not Significant IC50>1500µM
CYP2E1	Formation of P-nitrocatechol	p-nitrophenol (40µM)	Not Significant IC50>1500µM
CYP3A4	10-hydroxylation	(R)-Warfarin (0.5mM)	Not Significant IC50>1500µM

Human CYP450 Inhibition: The potential for gemcabene (100, 300, and 1500 µM) to inhibit 7 major cytochrome P450 enzymes, which mediate drug and xenobiotic metabolism in humans, was investigated using isoform selective marker substrates and human liver microsomal preparations. As indicated in the table below, gemcabene was not a significant inhibition for any of the cytochrome CYP450 isozymes up to a concentration of 1500 µM (453 µg/mL). The *in vitro* results suggest a metabolically-based clinical interaction between gemcabene and other drugs whose clearance is dependent on one of the P450s tested is highly unlikely at therapeutic doses.

Effect of Gemcabene on CYP1A1/2 and CYP3A4 Activity in Primary Cultures of Human Hepatocytes

Test Compound	CYP1A1/2 Activity EXP A	EXP B	EXP C	Average	Fold Change
Control (0.1%DMSO)	0.06	0.14	0.11	0.10	**1.0**
β-Napthoflavone (50 µM)	1.10	2.89	3.68	2.56	**24.7**
Gemcabene (290 µM)	0.06	0.11	0.12	0.10	**0.9**
Gemcabene (870 µM)	0.06	0.10	0.15	0.10	**1.0**

Test Compound	CYP3A4 Activity EXP B	EXP C	EXP D	Average	Fold Change
Control (0.1%DMSO)	15.9	6.0	76.8	32.9	**1.0**
Rifampin (10 µM)	72.7	98.1	316.1	162.3	**4.9**
Gemcabene (290 µM)	36.0	14.2	141.8	64.0	**1.9**
Gemcabene (870 µM)	52.7	31.1	199.8	94.5	**2.9**

EXP A Human Hepatocytes 870, EXP B Human Hepatocytes 871
EXP C Human Hepatocytes 888, EXP D Human Hepatocytes 893

Human CYP450 Induction: Primary cultures of human hepatocytes used to assess the CYP1A- and CYP3A-induction potential of gemcabene, in comparison to the prototypical inducers rifampin and β naphthoflavone. Gemcabene, at concentrations as high as 870 µM (263 µg/mL) did not cause a significant change in CYP1A1/2 activity, suggesting it is not an inducer of CYP1A1/2. Gemcabene at 290 µM (81.5 µg/mL) and 870 µM (263 µg/mL) caused a moderate increase in CYP3A4-catalyzed testosterone 6β-hydroxylation in human hepatocytes suggesting there is a potential for drug interactions with gemcabene due to CYP3A4 enzyme induction.

Drug Transport: Gemcabene was initially evaluated at high concentrations. If the results did not meet criteria (indicated as green in table) defined in the regulatory guidance then no additional testing would be required. If the results met the criteria (indicated as orange in table) then additional testing to determine the IC_{50} would be conducted. The results from the *in vitro* drug transport studies indicate gemcabene:

- ✓ Is **not** a substrate for P-gp, BCRP, OAT1, OAT3, OATP1B1, OATP1B3, OCT2;
- ✓ Is **not** an inhibitor for P-gp or OCT2;
- ✓ May be an inhibitor of BCRP at doses exceeding 600 mg QD;
- ✓ Is an inhibitor of OATP1B1 and OATP1B3;
- ✓ Is an inhibitor of OAT1 and OAT3.

Effect of Gemcabene on P-gp, BCRP, OAT1, OAT3, OATP1B1, OATP1B3, and OCT2

Transporter	Substrate[1]	Inhibitor[2]	Additional *in Vitro* Testing[3]
P-GP	Caco-2	Caco-2	
	MDR1-MDCK	MDR1-MDCK	Not done
BCRP	Caco-2	Caco-2	Concentration-dependent inhibition toward BCRP with an IC_{50} of 10.2 mM
	MDR1-MDCK	MDR1-MDCK	IC_{50} gemcabene is not a significant inhibitor
OAT1	HEK293	HEK293	Concentration-dependent inhibition toward OAT1 with an IC_{50} of 0.431 mM
OAT3	HEK293	HEK293	Concentration-dependent inhibition toward OAT3 with an IC_{50} of 0.0944 mM
OATP1B1	HEK293	HEK293	Not done
OATP1B3	HEK293	HEK293	Not done
OCT2	NA	HEK293	

[1]Not a substrate for the transporter if the efflux ratios of gemcabene were less than 2.0.
[2]Not an inhibitor for the transporter if gemcabene percent inhibition were less than 50%.
[3]Additional *in vitro* testing was not conducted if an *in vivo* drug-drug interaction study had been conducted.

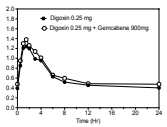
IN VIVO RESULTS (P-GP)

PK Profile of Digoxin Co-Administered with Gemcabene:

The 90% CI for digoxin C_{max} and $AUC_{0\text{-}24}$ ratios were within the 80% to 125% range, establishing absence of an effect of gemcabene on the PK of digoxin, a P-gp substrate.

These *in vivo* drug-drug interaction study results correlate well with the *in vitro* results for P-gp using Caco-2 cells but not for MDR1-MDCK.

Digoxin PK With and Without Gemcabene



Parameter	Least-Squares Mean Values Digoxin Alone (Reference)	Digoxin with CI-1027 (Test)	Ratio	90% Confidence Interval
N	12	12		
Cmax, ng/mL	1.32	1.43	108	97.6-120
tmax, hr	1.79	1.63	70.7	Not Applicable
AUC(0-24), ng/hr/mL	13.3	14.8	111	102-120
Cmin, ng/mL	0.373	0.446	120	107-134
t½, hr	45.0	40.1	89.0	42.4-136
CL/F, mL/min	327	294	90.1	81.7-98.4
Ae%	51.2	49.6	97.1	88.4-106
CLr, mL/min	165	141	86.5	77.3-97.8

Ratio = Ratio of treatment mean values, expressed as a percentage (100% × test/reference).
90% Confidence Interval = 90% confidence interval estimate for the ratio (test/reference) of treatment mean values, expressed as a percentage of the reference mean.

IN VIVO RESULTS (CYP450, OATP1B1, and OATP1B3)

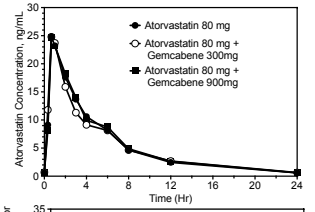
PK Profiles of Statins Co-administered with Gemcabene

The results of the two *in vivo* drug-drug interaction studies demonstrate that there is no clinically relevant effect of gemcabene on the PK of simvastatin or atorvastatin, both of which are CYP450 substrates.

ATORVASTATIN

Parameter	Least-Squares Mean Values Atorvastatin Alone (Reference)	Atorvastatin With Gemcabene (Test)	Ratio	90% Confidence Interval
	300 mg Gemcabene Once Daily			
Cmax, ng/mL	26.5	24.6	92.8	78.2 to 110
tmax, hr	1.03	0.752	72.7	Not Applicable
AUC(0-24), ng hr/mL	119	113	94.9	85.8 to 105
t½,* hr	6.44	6.23	96.7	82.9 to 111
	900 mg Gemcabene Once Daily			
Cmax, ng/mL	26.5	24.7	93.3	78.3 to 111
tmax, hr	1.03	1.07	103	Not Applicable
AUC(0-24), ng hr/mL	119	114	96.0	86.6 to 106
t½,* hr	6.44	6.33	98.2	84.1 to 112

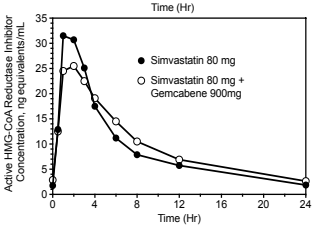
Ratio = Ratio of treatment mean values, expressed as a percentage (100% × test/reference).
90% Confidence Interval = 90% confidence interval estimate for the ratio (test/reference) of treatment mean values, expressed as a percentage of the reference mean.
* = Values for t½ are based on 0 to 24 hour data only and underestimate the true terminal half-life. Therefore, these values are substantially lower than those reported in previous studies.



SIMVASTATIN

Parameter	Least-Squares Mean Values Simvastatin Alone (Reference)	Simvastatin and CI-1027 (Test)	Ratio	90% Confidence Interval
Cmax, ng equi/mL	37.6	29.3	77.9	66.9 to 90.6
tmax, hr	1.55	1.95	126	Not Applicable
AUC(0-24), ng equi-hr/mL	211	219	104	93.9 to 116
t½, hr	8.15	8.73	107	96.6 to 118
Cmin, ng equi/mL	1.69	2.13	126	97.1 to 162

Parameters are described in Table 4.
Ratio = Ratio of treatment mean values, expressed as a percentage (100% × test/reference).
90% Confidence Interval = 90% confidence interval estimate for the ratio (test/reference) of treatment mean values, expressed as a percentage of the reference mean.



The *in vivo* drug-drug interaction study results correlate well with the *in vitro* CYP450 results; whereas, the *in vitro* transporter results suggesting gemcabene was an inhibitor of OATP1B1 and OATP1B3 did not correlate well with the clinical atorvastatin and simvastatin interaction studies.

CONCLUSIONS

The results from these *in vitro* and *in vivo* studies suggest gemcabene is unlikely to elicit metabolic (i.e., CYP450 or FMO3) or P-gp-, OATP1B1-, OATP1B3-, or OCT2-mediated drug interactions. The *in vitro* metabolism and drug transporter studies correlated well with the clinical P-gp interaction study, whereas, the atorvastatin and simvastatin clinical DDI studies confirmed the *in vitro* results of no metabolism-based interactions (i.e., CYP450) but were in contrast to and dispelled the *in vitro* OATP1B1/OATP1B3 transporter results.

An *in vivo* drug-drug interaction study is still needed to determine the potential for gemcabene to interact with an OAT1 or OAT3 substrate and possibly BCRP since the extent of inhibition by gemcabene was borderline.

CLINICAL IMPLICATIONS

Patients with dyslipidemia typically take many medications often including multiple lipid-altering therapies such as statins. Therefore, it is essential to understand and mitigate the potential risk of drug-drug interaction (DDI) to minimize the risk of adverse drug reactions. In the best circumstances, drugs entering the market are designed with the intent to minimize potential metabolic and transporter interactions when co-administered with commonly used drugs.

The clinical implications for a potential DDI for any medication may include dosage adjustment, requirement for additional safety monitoring, or a contraindication to concomitant use. A review of drug interactions associated with lipid-altering medications include: simvastatin drug interaction with strong CYP3A4 inhibitors or atorvastatin drug interaction with strong CYP3A inhibitors including fibrate products and niacin. In addition, the risk of myopathy and rhabdomyolysis is increased when statins (HMG-CoA reductase inhibitor therapy) are co-administered with Lopid®.

Results from the current *in vitro* and *in vivo* studies suggest co-administration of gemcabene is unlikely to result in an interaction with a variety of medications, including atorvastatin and simvastatin. The results suggest gemcabene could be formulated as a fixed-dose combination tablet with a statin, which may offer additional convenience and compliance to patients.

REFERENCES

[a] Food and Drug Administration (FDA) Guidance for Industry Drug Interaction Studies DRAFT (February 2012)
[b] European Medicines Agency (EMA) Guidance on the investigations of drug interactions (June 2012)
[c] 1027-008 - A Study to Evaluate the Effects of Gemcabene on the Steady-State Pharmacokinetics and Pharmacodynamics of Simvastatin in Healthy Volunteers (clinicaltrials.gov identifier: NCT02587390)
[d] A4141002 – A Study of the Effect of Oral, Multiple-Dose 300 mg and 900 mg Gemcabene (CI-1027) Administration on the Steady-State Pharmacokinetics of Atorvastatin 80 mg (clinicaltrials.gov identifier: NCT02587416)