CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-161

PHARMACOLOGY REVIEW(S)

MEMORANDUM

LEXISCAN (regadenoson injection)

Date: March 27, 2008
To: File for NDA #22-161
From: John K. Leighton, PhD, DABT
Associate Director for Pharmacology
Office of Oncology Drug Products

I have examined the labeling and pharmacology/toxicology supporting reviews and memoranda provided by Drs Biade and Laniyonu and concur with their conclusions that Lexiscan may be approved. No additional pharmacology/toxicology studies are necessary.

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/s/

John Leighton
3/27/2008 11:52:37 AM
PHARMACOLOGIST

Supervisory Pharmacologist Memo

NDA: **22-161**
Drug: **Lexiscan™ (Regadenoson)**
Sponsor: **CV Therapeutics**

Regadenoson (adenosine, 2-[4-[(methylamino)carbonyl]-1H-pyrazol-1-yl]-, monohydrate) is an adenosine analogue proposed as a pharmacologic stress agent for use with radionuclide myocardial perfusion imaging (MPI) in patients unable to exercise adequately for MPI. Regadenoson is a selective, low affinity agonist for adenosine A_{2A} receptor subtype mediating adenosine-induced coronary and systemic vasodilation. Its affinity at the A_1 adenosine receptor is at least 10 fold lower, and it possesses weak affinity for A_{2B} and A_3 adenosine receptors. Regadenoson-induced increase in coronary blood flow was demonstrated in several preclinical pharmacology studies. The proposed dose is 0.4 mg in 5 mL by rapid intravenous injection, followed immediately by saline flush and radiopharmaceutical.

Dr. Siham Biade reviewed the preclinical Pharmacology and Toxicology section of NDA 22-161. She concluded that the studies conducted support safety and efficacy from preclinical Pharmacology/Toxicology perspectives, and recommended approval. This secondary review was based on Dr. Biade's review; please see Dr. Biade's review for details.

Cardiovascular safety evaluations including QT_c, hERG potassium channels, and cardiac action potential assessments were adequate. There were no significant effects on hERG potassium channels and cardiac action potential. Regadenoson (3.0-2400 µg/kg; 0.2-160 times the maximum recommended human dose (MRHD)) administered intravenously to dogs caused dose dependent changes in T-wave polarity and prominent elevation and arch of the ST segment. Dose-dependent decreases in mean arterial blood pressure, non-dose-dependent increases in heart rate, and RR interval reduction were noted. For CNS safety evaluation, 1/5 rats developed moderate catalepsy at 200 µg/kg that was not observed in another study at up to 400µg/kg. Dr. Biade did not identify safety issues that would require the conduct of additional preclinical studies.

Elimination $t_{1/2}$ was 18-24 min in rats, 16-32 min in dogs, and 37-60 min in rabbits following a single intravenous dose of regadenoson. 90% of the drug was eliminated in 24 hours (rats) or 48 hours (dogs). No accumulation was observed in dogs or rats following repeated administration for up to 28 days.

Definitive toxicology (acute and repeat-dose) studies were conducted in rats and dogs. These studies were adequate. Of note was the increased incidence of minimal cardiomyopathy observed in rats on day 2 but not in rats sacrificed on day 15 in the single dose toxicity bridging study. The cardiomyopathy was characterized by scattered foci of lymphocytes and macrophages associated with few or no necrotic myocytes. The Division requested a consult on the significance of these findings from the Division of Cardio-renal Drug Products. Dr. Albert Defelice in his consult memo opined that the

minimal cardiomyopathy is irrelevant in a pharmaco-therapeutic context, and expected to be without important sequelae based on chronic animal studies with other vasodilators.

A full battery of genetic toxicology studies was conducted. Regadenoson was negative in these studies.

Reproductive studies in rats showed that regadenoson produced significant ossification delays at maternal toxic doses (10-20 X MRHD). Skeletal variation occurred in all treated groups. The no effect dose level for maternal toxicity is 0.1 mg/kg/day (2 X MRHD). In rabbits, there were no teratogenic effects in offspring at regadenoson doses 4 times the MRHD, although signs of maternal toxicity occurred at this dose. At regadenoson doses equivalent to 12 and 20 times the MRHD, maternal toxicity occurred along with increased embryo-fetal loss and fetal malformations. It is not clear whether malformations that occurred at maternally toxic doses of regadenoson in both animal species were due to fetal drug effects or only to the maternal toxic effects.

Dr. Biade concluded that the preclinical package of regadenoson was complete, and that the studies conducted support the safety and efficacy of regadenoson from preclinical pharmacology/toxicology perspectives. She recommends approval of the NDA and suggested changes in the label that would more appropriately reflect findings from preclinical studies.

I concur with Dr. Biade's recommendations.

Adebayo Laniyonu, Ph.D.

Supervisory Pharmacologist

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/s/

Adebayo Laniyonu
3/24/2008 12:46:05 PM
PHARMACOLOGIST

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-161**
SERIAL NUMBER:	**N000**
DATE RECEIVED BY CENTER:	**05/14/07**
PRODUCT:	**Lexiscan**
INTENDED CLINICAL POPULATION:	**Patients unable to exercise adequately for myocardial perfusion imaging (MPI)**
SPONSOR:	**CV Therapeutics**
DOCUMENTS REVIEWED:	**Electronic submission (eCTD)**
REVIEW DIVISION:	**Division of Medical Imaging and Hematology Products (HFD-160)**
PHARM/TOX REVIEWER:	**Siham Biade, Ph.D.**
PHARM/TOX SUPERVISOR:	**Adebayo Laniyonu, Ph.D.**
DIVISION DIRECTOR:	**Rafael Dwaine Rieves, MD**
PROJECT MANAGER:	**Tiffany Brown, MPH**

Date of review submission to Division File System (DFS):

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EXECUTIVE SUMMARY

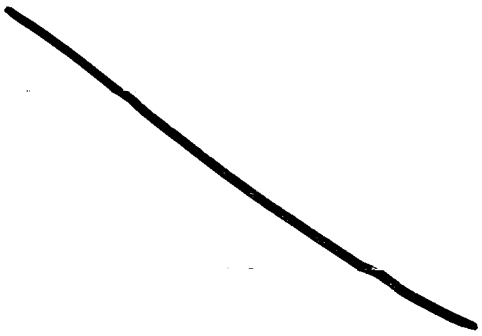
I. Recommendations:

A. Recommendation on approvability: From a pharmacology/toxicology perspective, Lexiscan is recommended for approval

B. Recommendation for nonclinical studies: None

C. Recommendations on labeling: The proposed labeling by the sponsor was edited as follows: the nonclinical pharmacology and toxicology edits are underlined or in ~~strikethrough~~.

Pregnancy Category C:



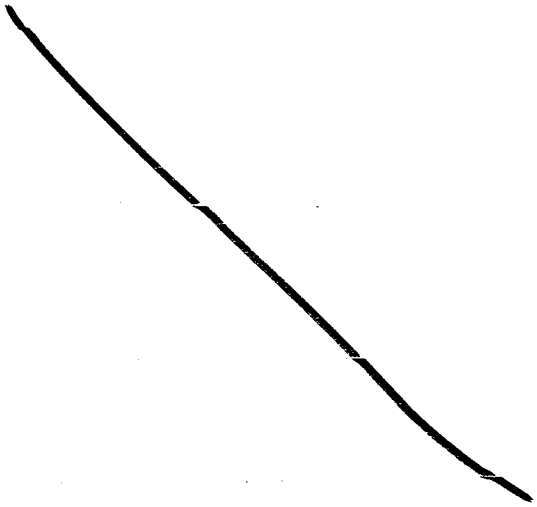
1 Page(s) Withheld

_____ Trade Secret / Confidential

✓ Draft Labeling

_____ Deliberative Process

Withheld Track Number: Pharm/Tox- 4



II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Safety pharmacology

Cardiovascular safety pharmacology:
In transfected HEK293 cells, regadenoson had no effect on hERG tail current at the single dose of 5 μM in which it was tested. In isolated canine Purkinje fibers, the effects of up to 10 μM regadenoson on action potential parameters were isolated, and non dose-dependent and therefore considered not drug related. In isolated canine left ventricle myocytes, regadenoson (10 μM) caused ~25% reduction of I_{Kr} tail current. In rabbit isolated hearts, regadenoson (0.01-30 μM) caused coronary vasodilatation (increase in coronary conductance) but did not prolong the ventricular action potential duration.

In conscious dogs, regadenoson (2.5–10 μg/kg, 0.16-0.7 XHD) caused a dose-dependent shortening of the QT interval and an increase in HR. QT interval was shortened by 14, 24, and 27 ms and RR interval by 150, 212, and 251 ms after 2.5, 5, and 10 μg/kg regadenoson, respectively. In another study conducted in conscious dogs, regadenoson (5 μg/kg) caused a significant increase in CBF, lasting ~ 10 min, with a peak of ~191

mL/min from a baseline of ~34 mL/min. Regadenoson caused a decrease in MAP (15%) and LVSP (9%), and an increase in HR (114%) and in LV dP/dt_{Max} (65%).

Cardiovascular safety was monitored as part of two single dose and two repeat dose toxicity studies in dogs. Single intravenous administration of bolus doses of 3.0-2400 µg/kg regadenoson (0.2-160 XHD) to dogs caused dose dependent changes in T-wave polarity usually involving a change from a positive T-wave in Lead II to a negative T-wave lasting for the entire 15-min post-dose period (longest observation period); prominent elevation and arch of the ST segment was also noted. At doses ≥ 20µg/kg, regadenoson caused dose-dependent decreases (up to 60%) in mean arterial blood pressure, non-dose-dependent increases in heart rate, and RR interval reduction. QTc interval prolongation of ~6 ms (in 2/4 dogs) and some atrial premature depolarizations (APD) were noted in a single dose escalating study in dogs using 3-20 µg/kg regadenoson (0.2-1.3 XHD): the latter effects were not observed in other studies. In repeated i.v. dose toxicity studies in dogs treated for 7 or 28 days of treatment at doses of 2, 20 or 200 µg/kg/day, ECG recording showed changes in T-wave morphology (T-wave inversion) and slight elevation and arch of the T-segment in high dose males and females at one hour post dosing (longest monitoring time) in the 28 day study and to a lesser extent in the 7 day study.

Central nervous and respiratory systems:
Intravenous administration of 2 and 200 µg/kg regadenoson to rats induced a transient decrease in spontaneous activity including a single occurrence of slight to moderate catalepsy at 200 µg/kg (1/5 rats), and reduction in body temperature. In another CNS study in rats, regadenoson (40-400 µg/kg) caused a dose-dependent decrease in body temperature (-1% to -7.2%) in all treated groups and at 400 µg/kg, caused reduced activity and abdominal activity. Catalepsy was not observed in this study. In a respiratory safety study in rats, i.v. administration of 80 and 200 µg/kg regadenoson increased the respiratory rate by 12 and 28% respectively.

TOXICOLOGY

Pharmacokinetics/ADME

Following a single i.v. dose administration of regadenoson, elimination $T_{1/2}$ was 18-24 min in rats, 16-32 min in dogs, and 37-60 min in rabbits. Plasma protein binding was 10-17% in rats and dogs. In rats, 90% of the drug was eliminated in 24 hours (55% in feces, 37% in urine). In dogs, 90% of the drug was eliminated in 48 hours. Following 7 or 28 day repeated dose i.v. administration, the toxicokinetics in rats and dogs were similar with peak at 2 min post dose, plasma levels proportional to administered dose and comparable between males and females. No accumulation of regadenoson was observed in rats or dogs following daily doses of up to 200 µg/kg/day for up to 28 days.

In a biodistribution study conducted in albino and pigmented rats, the highest concentrations of regadenoson were observed at 30 min post-dose in urinary bladder, bile

duct, kidneys, and intestines and in addition in dorsal nerve roots in pigmented rats. In albino rats, significant concentrations were found in the skin up to 72 hr and in the lungs up to 120 h post dose, whereas in pigmented rats, radioactivity remained quantifiable up to 120 h post dose in the lungs, uveal tract and pigmented skin. The calculated half $life_{(24\text{-}120\ h)}$ of total radioactivity in the pigmented eye was approximately 7 days but was not provided for albino eye. Absorbance spectrum analysis of regadenoson identified 3 absorbance maxima below the UV-visible range; however, some absorbance was noted above 290 nm (290-320nm), which may potentially cause adverse photoeffects. However, regadenoson is intended for single administration, and no further evaluation is needed.

The metabolism of regadenoson was evaluated in plasma, urine, and bile in rats and dogs in vivo. No metabolites were detected in dogs, whereas in rats, three minor metabolites were found in urine (12% activity), which each represented 2% of total activity.

Single dose toxicity

In studies conducted in rats and dogs using a methylboronic acid formulation, administration of single i.v. doses of up to 1500 µg/kg to rats (30 XHD) and up to 2400 µg/kg to dogs (160 XHD) elicited no mortality and no clinical signs of toxicity. Single i.v. doses of 20 to 2400 µg/kg administered to dogs caused dose-dependent decreases in MABP, non-dose-dependent increases in HR and changes in T-wave polarity.

Since most of the preclinical studies were conducted using a methylboronic acid (MBA) formulation, a single dose bridging toxicity study was conducted in rats to evaluate the safety of the propylene glycol (PG) clinical formulation intended for clinical use. Four groups of rats received i.v. administration of vehicle, 0.08, 0.2, or 0.8 mg/kg PG regadenoson (1.6, 4, and 16 XHD). As a comparator, an additional group was given 0.2 mg/kg regadenoson in MBA formulation. Red discolored urine was observed with vehicle and drug treated animals in the PG formulation groups. Most of the clinical, hematology, and clinical chemistry changes occurred on day 2 in the group treated with the MBA formulation. One female in the PG vehicle group and one female in the 0.8 mg/kg PG group had similar, chronic, focal liver lesions consisting of mineralization and fibrosis. However, in the 0.8 mg/kg group female, these changes were associated with coagulative necrosis. Increased incidence of minimal cardiomyopathy characterized by scattered foci of lymphocytes and macrophages associated with few or no necrotic myocytes was observed on day 2 in males at doses of 0.08, 0.2 and 0.8 mg/kg (1/5, 2/5, and 5/5 rats) and in females (2/5) at 0.8 mg/kg. Cardiomyopathy was also seen in males (3/5) administered 0.2 mg/kg of MBA regadenoson. No cardiomyopathy was noted on day 15 in any of the groups: one control female and one low dose male in the PG group had minimal myocardial vacuolation, and one female treated with 0.2 mg/kg MBA regadenoson had a focal myocardial chronic inflammation. The cardiomyopathy was determined to be regadenoson related.

Repeated dose toxicity

Studies in rats and dogs of 7 and 28 days duration at doses of 2, 20 or 200 µg/kg/day (0.04, 0.4, and 4 XHD, and 0.13, 1.3, and 13 XHD for rats and dogs respectively) were

conducted to evaluate the toxicity from repeated administration of regadenoson in MBA formulation.

In rats, no adverse effects on survival and body weight were seen at these dose levels for up to 28 days. Decreased MCH (35%) was seen in treated females, and higher than control serum levels (up to 3 fold) of creatine kinase and lactate dehydrogenase as well as higher bilirubin, potassium and phosphorus were seen in high dose female rats after 7 days, but not after 28 days of treatment. Gross and microscopic findings were limited to minimal inflammation at the site of intravenous injection and lymphoid hyperplasia of mandibular or mesenteric lymph nodes in the high dose groups. In view of the observed clinical chemistry changes, NOAEL in rats was considered to be 20 µg/kg/day (0.4 XHD).

In dogs, no adverse effect on survival or body weight was seen after 7 or 28 days of treatment. ECG recording showed changes in T-wave morphology (T-wave inversion) and slight elevation and arch of the T-segment in high dose males and females at one hour post dosing in the 28 day and (to a lesser extent) 7 day studies. Gross and microscopic lesions were limited to hemorrhage and inflammation at the site of injection in both treated and control animals and did not appear to be related to treatment. The NOAEL in dogs was considered to be 20 µg/kg/day (1.3 XHD).

Genetic toxicology

Regadenoson was negative in the standard battery of genotoxicity tests. Three impurities found in the drug substance were also evaluated: 1) [redacted] was positive in the bacterial reverse mutation assay, however specification of NMT 10 ppm in the drug product are acceptable levels. 2) A drug formulation spiked with [redacted] each of the other [redacted] impurities [redacted], was negative in a bacterial mutation assay; the same formulation induced a statistical increase in the percentage of cells with numerical aberrations in a chromosomal aberration assay in CHO cells, in the non-activated 4-hour exposure group; however, because the percentage was within the historical solvent control, the results were considered negative. .

Reproductive toxicology

Fertility and pre- and post-natal development were not evaluated. Embryofetal development was evaluated in rats and rabbits using a formulation containing 8% propylene glycol.

Reproduction studies were conducted in rabbits and rats using doses of LEXISCAN that were 2 to 20 times (rats) and 4 to 20 times (rabbits) the maximum recommended human dose (MRHD), based on body surface area comparison.

When administered to rabbits during organogenesis, regadenoson caused maternal toxicity including tachypnea, soft, liquid or scant feces, and localized alopecia in all treated groups, and caused reduction in body weight and feed consumption at 0.3 and 0.5 mg/kg/day (12 and 20 X MRHD, respectively). At regadenoson doses equivalent to 12 and 20 times the MRHD, maternal toxicity occurred along with decreased number of live

fetuses, reduced fetal body weight, and occurrence of fetal variations and malformations. At regadenoson doses equivalent to 20 times the MRHD, resorptions were increased and fetal body weights reduced. Fetal malformations included microphtalmia (1/116 at 20 X MHRD), interrelated vertebrae/rib alterations (2/145 and 2/116 each at 12 and 20 X MHRD), and misaligned caudal vertebrae (3/145 at 12 X MHRD). Fetal toxicity was only observed at maternally toxic doses. The no effect dose level for fetal toxicity is 0.1 mg/kg (4 X MRHD). A no effect dose level was not identified for maternal toxicity.

When regadenoson was administered to pregnant rats during the period of major organogenesis, 4/25 rats from the 1.0 mg/kg/day group (20 X MRHD) and 1/25 rats from the 0.8 mg/kg (16 X MRHD) group died immediately following the first dose of regadenoson. All dams had decreased motor activity and one was gasping post-dosing. At doses ≥ 0.5 mg/kg (10 X MRHD), maternal toxicity included decreased motor activity, increased limb extension, excess salivation, and reduction in body weight and feed consumption. At doses ≥ 0.5 mg/kg, fetal body weights were significantly reduced and significant ossification delays were observed in fore- and hindlimb phalanges and metatarsals. Skeletal malformations included delayed ossification of the skull (1/167), and hemivertebra present at a thoracic vertebra (1/167), observed at 16-20 X MHRD, and small arches of a lumbar and sacral vertebrae (1/174) observed at 2 X MRHD. The no effect dose level for maternal toxicity is 0.1 mg/kg/day (2 X MRHD).

Local tolerance

Intravenous administration of Lexiscan to rabbits resulted in perivascular hemorrhage, vein vascultis, inflammation, thrombosis and necrosis, with signs of reversibility except for the inflammation an thrombosis, which persisted through day 8 (last observation day)
Perivascular administration of Lexiscan to rabbits resulted in hemorrhage, inflammation (acute or histiocytic), pustule formation and epidermal hyperplasia, which persisted through day 8 except for the hemorrhage which resolved.
Subcutaneous administration of Lexiscan to rabbits resulted in hemorrhage, and acute inflammation, and on day 8 in fiber regeneration.

B. Pharmacologic activity

Activation of the A_{2A}-Adenosine receptor (A_{2A}-AdoR) by regadenoson causes coronary vasodilation and increases coronary blood flow (CBF), which is the basis for its use in radionuclide myocardial perfusion imaging (MPI).

Pharmacodynamics

Regadenoson is a selective A_{2A} adenosine receptor agonist. In a receptor screen assay, regadenoson was selective for Adenosine A_{2A} binding sites. The affinity of regadenoson for A_{2A}-AdoR was about 10-fold higher than for the human A_1-AdoR (Ki 1.3 μM vs. Ki > 16.5 μM), and it elicited very weak activity at the A_{2B}- and A_3-AdoR. Regadenoson activated the endogenous A_{2A} receptor in PC12 cells and caused cAMP accumulation, but

did not induce cAMP accumulation in HEK-293 cells that express adenosine A_{2B} receptors.

In isolated perfused rat and guinea pig hearts, regadenoson elicited a concentration-dependent increase in coronary arterial conductance with EC_{50} of 6.4 nM and 6.7-18.6 nM respectively (Adenosine: 59.2 nM and 86.0 nM). At concentrations of regadenoson that increased coronary conductance in isolated rat hearts (0.1 nM to 30 uM), there was no negative dromotropic effect, whereas in guinea pig hearts, regadenoson caused second degree A-V block at concentrations $\geq$ 3.0 µM with an EC_{50} of 4.0 µM. Thus, regadenoson was at least 4,600 and 215 times more selective for causing coronary vasodilation than for a negative dromotropic effect in rats and pigs respectively.

Regadenoson causes a short lasting, large, reversible, and dose-dependent increase in coronary blood flow (CBF) in rodents, pigs, dogs, and humans. In dogs, regadenoson (1-10 µg/kg) caused dose-dependent increases in average peak velocity of CBF, increase in HR and MAP decrease. Infusion (10 min) of 20 mg/kg aminophylline, a phosphodiesterase inhibitor, attenuated the effects of regadenoson on CBF. In rats, regadenoson (10 µg/kg) administration was associated with increased plasma norepinephrine levels. In anesthetized dogs, regadenoson causes a more pronounced vasodilation in coronary artery than in limb, pulmonary, and brain vascular beds.

Regadenoson induces cAMP stimulation in human platelets and neutrophils (EC_{50} of 472 nM and 406 nM), and inhibits human platelet aggregation, intracellular calcium, and oxygen production (IC_{50} of 437, 108, and 328 nM).

Intravenous administration of 1 to 10 mg/kg caffeine (up to 52 µM), a non selective adenosine receptor antagonist, to dogs did not significantly reduce the regadenoson-induced peak increases in CBF but caused a dose-dependent decrease in the duration of CBF increase, and attenuated regadenoson–induced sinus tachycardia and hypotension.

C. Nonclinical safety issues relevant to clinical use:

The sponsor conducted the required safety pharmacology and toxicological evaluation for regadenoson. There were no unresolved toxicology issues in this NDA. With regard to the minimal cardiomyopathy observed in the single dose bridging toxicity study conducted in rats, a consult was requested from the Division of Cardio-renal for assistance in interpreting the findings. Dr Albert F. Defelice's review of the data can be found in DFS (2/29/08).

Dr. Defelice considered the minimal focal cardiomyopathy (CM) to be irrelevant in a pharmaco-therapeutic context, whether veterinary or clinical. He further opined that the CM is expected to be without important sequelae based on chronic animal studies with other vasodilators, and his understanding that such lesions have not been observed at autopsy of patients treated with minoxidil - the prototype vasodilator for provoking such lesions (as well as coronary arteriopathy) in animals.

The cardiomyopathy findings have been incorporated into the label.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22,161
Review number: 1

Sequence number	*Type of submission*	*Date of submission*
N000	Original submission	5/14/07
N008	Nonclinical amendment	12/12/07
N010	Nonclinical amendment	12/12/07
N012	Nonclinical amendment	2/19/08
N014	Nonclinical amendment	2/28/08

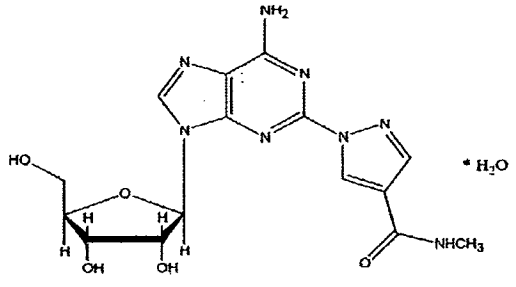
Information to sponsor: Yes (x) No ( )
Sponsor and/or agent: CV Therapeutics,
3172 Porter Drive, Palo Alto, CA 94304

Manufacturer for drug substance: [redacted]

Reviewer name: Siham Biade, Ph.D.
Division name: Medical Imaging & Hematology Products
HFD #: 160
Review completion date: 03/21/08

Drug:

Trade name: Lexiscan™ (regadenoson injection)
Generic name: Regadenoson
Code name: CVT-3146
Chemical name: 2-[N-1-(4-N-methylcarboxamidopyrazolyl)] adenosine
CAS registry number: 875148-45-1
Molecular formula/molecular weight: $C_{15}H_{18}N_8O_5 \cdot H20/408.37$
Structure:



Relevant INDs/NDAs/DMFs: 62,862

Drug class: Pharmacologic stress agent for use with radionuclide myocardial perfusion imaging (MPI)

Intended clinical population: Patients unable to exercise adequately for myocardial perfusion imaging (MPI)

Clinical formulation:
Two formulations were used during the development: (1) a formulation containing methylboronic acid formulated in bicarbonate buffer used in most of the nonclinical and early clinical studies; and (2) a formulation containing propylene glycol (PG) formulated in phosphate buffer used in some nonclinical, clinical and registration stability studies.

The drug product (DP), Regadenoson Injection, contains 0.08 mg/mL regadenoson in a clear, colorless, sterile, non-pyrogenic, preservative-free solution intended for intravenous (i.v.) injection.

Table 1: Unit dose composition

Ingredient	*Reference to Quality Standard*	*Function*	*Quantity (mg/mL)*
Regadenoson	In-house standard	Active	0.08[a]
Dibasic Sodium Phosphate, Dihydrate[b]	USP	[redacted]	10.9
Monobasic Sodium Phosphate, Monohydrate	USP		5.4
Propylene glycol	USP		150.0
Edetate Disodium Dihydrate	USP		1.0
Water for Injection	USP		q.s.[c]

[a]Concentration of regadenoson on anhydrous basis
[b]Alternatively, an equivalent amount (8.7 mg/mL) of Dibasic Sodium Phosphate, Anhydrous may be used in place of Dibasic Sodium Phosphate, Dihydrate
[c]q.s: quantity sufficient

Route of administration:
The dose will be administered as a rapid bolus intravenous injection lasting approximately 10 seconds, to be followed immediately by a 5 mL saline flush. The radiopharmaceutical agent is to be administered 10-20 seconds after the saline flush.

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed in this submission

Study number	**STUDY TITLE**
	Primary pharmacodynamics
1002510	Combination screen
CVT3146.014-N[*]	*Pharmacological and functional characterization of novel adenosine receptor agonist by radioligand binding and cAMP assays*
CVT3146.015-N[*]	*Coronary vasodilation in rat and guinea pig, isolated perfused hearts by A_{2A} adenosine receptor agonists*
CVT3146.016-N[*]	*The A_{2A} adenosine receptor agonists, CVT-3146 and CVT-3033 are more potent in*

	causing coronary vasodilation than depressing AV nodal conduction in rat and guinea pig isolated hearts
*CVT3146.030-P**	*Effects of CVT-3146 on coronary blood flow velocity, systemic arterial blood pressure and heart rate in anesthetized dogs*
*CVT3146.031-P**	*Selective A_{2A} adenosine receptor agonist, for pharmacological stress test during myocardial perfusion imaging*
*CVT3146.033-P**	*Differential effects of CVT-3146 and adenosine on blood flow velocity in coronary and peripheral arteries, systemic arterial blood pressure and heart rate in anesthetized dogs*
CVT3146.055-P	Cardiovascular effects of CVT-3146 in the anesthetized dog
CVT3146.132-P	A2A-Adenosine receptor reserve of CVT-3146 for coronary vasodilation in Guinea pig isolated hearts
	Secondary pharmacodynamics
CVT3146.053-P	Effect of the adenosine A_{2A} receptor agonist, CVT-3146, on I_{Kr} and I_{Ks} in isolated canine left ventricle myocytes
CVT3146.112-P	Effect of CVT-3146 on hypotension induced vasoconstriction in mouse isolated heart
CVT-3146.124-P	Effect of CVT-3146 on the attenuation of blood flow reserve associated with coronary artery stunning in dogs
CVT3146.125-P	Effect of CVT3146, A Selective A_{2A} Adenosine Receptor Agonist, on Ventricular Action Potential Duration and Coronary Perfusion Pressure in Female Rabbit Isolated Hearts
CVT3146.128-P	The effects of CVT-3146 on human platelet and neutrophil function
CVT3146.129-P	Tachycardia caused by regadenoson is mediated by direct sympathoexcitation in awake rats
CVT3146.134-P	Effects of regadenoson (CVT-3146), a novel A_{2A} adenosine receptor agonist, on QT interval
	Safety Pharmacology
1491-CVT-01-B	Effects of CVT-3146 (2 and 200 µg/kg, i.v.) on behaviour and physiological state as assessed by the Irwin test and on body temperature in rats
CVT3146.117-P	Effects of CVT-3146 on HERG Tail Current Recorded from Stably Transfected HEK293 Cells
CVT3146.118-P	CVT-3146: Effects on Cardiac Action Potential Parameters in Isolated Canine Purkinje Fibers.
CVT3146.122-P	Pulmonary assessment of CVT-3146 in the anesthetized rat
	Pharmacodynamics Drug Interaction
CVT3146.130-P	Effects of caffeine on regadenoson-induced coronary vasodilation and changes in hemodynamics in conscious dogs
	Absorption
CVT3146.026-R	Pharmacokinetics of CVT-1346 in female rabbits after a single intravenous dose of CVT-3146 at 100, 300, or 500 µg/kg
CVT3146.032-P	Pharmacokinetics of CVT-1346 in male rats after a single intravenous dose of CVT-3146 at 2, 20, or 200 µg/kg
	Distribution
CVT3146.001-N	Binding of CVT-3146 to rat and dog plasma in vitro
CVT314.016-R	The disposition and tissue distribution of total radioactivity in the rat following intravenous administration of [^{14}C]-CVT3146
	Metabolism

CVT3146.009-MET	Metabolic profiles of CVT-3146 following intravenous administration of a single 250 µg/kg dose of [^{14}C]CVT-3146 to intact and bile duct-cannulated rats
CVT3146.011-MET	Metabolic profiles of CVT-3146 following intravenous administration of a single 200 µg/kg dose of [^{14}C]CVT-3146 to intact and bile duct-cannulated dogs
	Excretion
CVT3146-017-r	The biliary elimination of total radioactivity in the rat following intravenous administration of [^{14}C]-CVT-3146
CVT3146.024-R	Disposition of [^{14}C]-CVT-3146 in male Beagle dogs after intravenous administration
CVT3146.025-R	Disposition of [^{14}C]-CVT-3146 in male bile duct-cannulated Beagle dogs after intravenous administration
	Single dose toxicity
*124-001**	*Single dose (MTD) study of CVT-1346 administered intravenously to Sprague-Dawley rats*
*124-002**	*Single dose escalating (MTD) study of CVT-1346 administered intravenously to beagle dogs*
*124-009**	*Single dose escalating (MTD) study of CVT-1346 administered intravenously to Beagle dogs*
	Repeat-dose toxicity
*124-003**	*Seven-day repeated dose toxicity study of CVT-3146 administered via intravenous administration to Sprague-Dawley rats*
*124-004**	*Seven-day repeated dose toxicity study of CVT-3146 administered intravenously to Beagle dogs*
*124-011**	*28-day repeated dose toxicity study of CVT-3146 administered via intravenous injection to Sprague-Dawley rats*
*124-012**	*28-day repeated dose toxicity study of CVT-3146 administered intravenously to Beagle dogs*
*CVT3146.014-T**	*Toxicokinetics of CVT-3146 on days 1 and 7 during a seven-day repeated intravenous administration toxicology study in Sprague-Dawley rats*
*CVT3146.015-T**	*Pharmacokinetics of CVT-3146 in dogs after single or multiple intravenous administration of CVT-3146 at 2, 20, or 200 µg/kg*
*CVT3146.017-T**	*Toxicokinetics of CVT-3146 on days 1 and 28 during a twenty eight-day repeat intravenous administration toxicology study in Sprague-Dawley rats*
*CVT3146.018-T**	*Pharmacokinetics of CVT-3146 in dogs after single or twenty five repeat daily intravenous administration of CVT-3146 at 2, 20, or 200 µg/kg*
	Genotoxicity
*20608-0-422SC**	*Mutagenicity test with CVT-3146 in the Salmonella-Escherichia coli/Mammalian-Microsome reverse mutation screening assay (Ames test) preincubation method*
*20608-0-437OECD**	*Chromosomal aberrations test of CVT-3146 in Chinese Hamster Ovary (CHO) cells**
20608-1-422	*Mutagenicity test with CVT-3146 in the Salmonella-Escherichia coli/Mammalian-Microsome reverse mutation assay preincubation method*
*20608-0-455OECD**	*In vivo mouse micronucleus assay with CVT3146*
	Reproductive Toxicology
3003-004	Intravenous developmental toxicity study of CVT-3146 in rabbits
3003-004P	Intravenous Dosage-Range Developmental Toxicity Study of CVT-3146 in rabbits
3003-005	Intravenous developmental toxicity study of CVT-3146 in rats.
3003-005P	Intravenous dosage-range developmental toxicity study of CVT-3146 in rats

	Local Tolerance
124-021	Acute intravenous irritation study in rabbits with CVT-3146 in 15% propylene glycol with a saline flush
124-022	Acute intravenous irritation study in rabbits with CVT-3146 in 15% propylene glycol
124-023	Acute intravenous irritation study in rabbits with CVT-3146 in 20% propylene glycol
[redacted]	
	Other Toxicity Studies
6892-108	Salmonella-Escherichia coli/Mammalian- Microsome Reverse Mutation Assay [redacted]
CVT3146.046-T	Bacterial Reverse Mutation Assay with CVT-3146 Containing Impurities [redacted]
CVT3146.047-T	In vitro mammalian chromosome aberration test with CVT-3146 containing impurities [redacted]
CVTTOX 04-004	Bacterial reverse mutation assay
CVT3146.046N	Absorbance spectrum of CVT-3146

In italic, studies reviewed by Dr. Anthony Proakis

Studies not reviewed within this submission:

	Primary pharmacodynamics
[redacted]	

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Pharmacology

Regadenoson is an adenosine analogue chemically described as <adenosine, 2-[4-[(methylamino)carbonyl]-1H-pyrazol-1-yl]-, monohydrate>. Adenosine is the natural ligand for the adenosine receptor and activates all four known receptor subtypes, designated A_1, A_{2A}, A_{2B} and A_3. Activation of adenosine A_1 receptors depresses cardiac activity through negative chronotropic and dromotropic effects, and attenuates the cardiac stimulatory effects of catecholamines. The adenosine A_{2A} receptor mediates coronary and systemic vasodilation caused by adenosine. In addition, stimulation of the A_{2A}

receptors on afferent nerve endings in the heart, skeletal muscle, and kidney and in the carotid bodies produces a sympatho-excitatory effect which can result in increases in heart rate, systolic blood pressure and ventilation. The functions of the A_{2B} and A_3 receptors are not as clearly understood, although activation of the A_{2B} receptor may play a role in the systemic vasodilatory response to adenosine. Both adenosine A_{2B} and A_3 receptors have been implicated in the bronchoconstriction that may be provoked by adenosine in patients with asthma or chronic obstructive pulmonary disease.

Pharmacodynamics:

Regadenoson is a selective A_{2A} adenosine receptor agonist. In a receptor screen assay, regadenoson was selective for adenosine A_{2A} binding sites. The affinity of regadenoson for A_{2A}-AdoR was about 10-fold higher than for the human A_1-AdoR (Ki 1.3 μM vs. Ki > 16.5 μM), and it elicited very weak activity at the A_{2B}- and A_3-AdoR. Regadenoson activated the endogenous A_{2A} receptor in PC12 cells and caused cAMP accumulation, but did not induce cAMP accumulation in HEK-293 cells that express adenosine A_{2B} receptors.

In isolated perfused rat and guinea pig hearts, regadenoson elicited a concentration-dependent increase in coronary arterial conductance with EC_{50} of 6.4 nM and 6.7-18.6 nM respectively (Adenosine: 59.2 nM and 86.0 nM). At concentrations of regadenoson that increased coronary conductance in isolated rat hearts (0.1 nM to 30 uM), there was no negative dromotropic effect, whereas in guinea pig hearts, regadenoson caused second degree A-V block at concentrations ≥ 3.0 μM with an EC_{50} of 4.0 μM. Thus, regadenoson was at least 4,600 and 215 times more selective for causing coronary vasodilation than for a negative dromotropic effect in rats and pigs respectively.

Regadenoson causes a short lasting, large, reversible, and dose-dependent increase in coronary blood flow (CBF) in rodents, pigs, dogs, and humans. In dogs, regadenoson (1-10 μg/kg) caused dose-dependent increases in average peak velocity of CBF, increase in HR and MAP decrease. Infusion (10 min) of 20 mg/kg aminophylline, a phosphodiesterase inhibitor, attenuated the effects of regadenoson on CBF. In rats, regadenoson (10 μg/kg) administration was associated with increased plasma norepinephrine levels. In anesthetized dogs, regadenoson causes a more pronounced vasodilation in coronary artery than in limb, pulmonary, and brain vascular beds.

Regadenoson induces cAMP stimulation in human platelets and neutrophils (EC_{50} of 472 nM and 406 nM), and inhibits human platelet aggregation, intracellular calcium, and oxygen production (IC_{50} of 437, 108, and 328 nM).

Intravenous administration of 1 to 10 mg/kg caffeine (up to 52 μM), a non selective adenosine receptor antagonist, to dogs did not significantly reduce the regadenoson-induced peak increases in CBF but caused a dose-dependent decrease in the duration of CBF increase, and attenuated regadenoson–induced sinus tachycardia and hypotension.

Safety pharmacology:

Cardiovascular safety pharmacology:
In transfected HEK293 cells, regadenoson had no effect on hERG tail current at the single dose of 5 μM in which it was tested. In isolated canine Purkinje fibers, the effects of up to 10 μM regadenoson on action potential parameters were isolated, and non dose-dependent and therefore considered not drug related. In isolated canine left ventricle myocytes, regadenoson (10 μM) caused ~25% reduction of I_{Kr} tail current. In rabbit isolated hearts, regadenoson (0.01-30 μM) caused coronary vasodilatation (increase in coronary conductance), but did not prolong the ventricular action potential duration.

In conscious dogs, regadenoson (2.5–10 μg/kg, 0.16-0.7 XHD) caused a dose-dependent shortening of the QT interval and an increase in HR. QT interval was shortened by 14, 24, and 27 ms and RR interval by 150, 212, and 251 ms after 2.5, 5, and 10 μg/kg regadenoson, respectively.

In a study conducted in conscious dogs, regadenoson (5 μg/kg) caused a significant increase in CBF, lasting ~ 10 min, with a peak of ~191 mL/min from a baseline of ~34 mL/min. Regadenoson caused a decrease in MAP (15%) and LVSP (9%), and an increase in HR (114%) and in LV dP/dt_{Max} (65%).

Cardiovascular safety was monitored as part of two single dose and two repeat dose toxicity studies in dogs. Single intravenous administration of bolus doses of 3.0-2400 μg/kg regadenoson (0.2-160 XHD) to dogs caused dose dependent changes in T-wave polarity usually involving a change from a positive T-wave in Lead II to a negative T-wave lasting for the entire 15-min post-dose period (longest observation period); prominent elevation and arch of the ST segment was also noted. At doses > 20μg/kg, regadenoson caused dose-dependent decreases (up to 60%) in mean arterial blood pressure, non-dose-dependent increases in heart rate, and RR interval reduction. QTc interval prolongation of ~6 ms (in 2/4 dogs) and some atrial premature depolarizations (APD) were noted in a single dose escalating study in dogs using 3-20 μg/kg regadenoson (0.2-1.3 XHD): the latter effects were not observed in other studies. In repeated i.v. dose toxicity studies in dogs treated for 7 or 28 days of treatment at doses of 2, 20 or 200 μg/kg/day, ECG recording showed changes in T-wave morphology (T-wave inversion) and slight elevation and arch of the T-segment in high dose males and females at one hour post dosing (longest monitoring time) in the 28 day study and to a lesser extent in the 7 day study.

Central nervous and respiratory systems:
Intravenous administration of 2 and 200 μg/kg regadenoson to rats induced a transient decrease in spontaneous activity including a single occurrence of slight to moderate catalepsy at 200 μg/kg (1/5 rats), and reduction in body temperature. In another CNS study in rats, regadenoson (40-400 μg/kg) caused a dose-dependent decrease in body temperature (-1% to -7.2%) in all treated groups and at 400 μg/kg, caused reduced activity and abdominal activity. Catalepsy was not reported for this study. In a

respiratory safety study in rats, i.v. administration of 80 and 200 µg/kg regadenoson increased the respiratory rate by 12 and 28% respectively.

2.6.2.2 Primary pharmacodynamics

Mechanism of action:
Regadenoson is a selective A_{2A} adenosine receptor agonist. Activation of the A_{2A}-AdoR by regadenoson causes coronary vasodilation and increases coronary blood flow (CBF). The increase CBF duration is short and is therefore suitable for investigation of myocardial perfusion defects using radionuclide imaging in the clinic.

Drug activity related to proposed indication:
The coronary vasodilator effect of regadenoson is the basis for its use, in conjunction with radionuclide myocardial perfusion imaging (MPI), to detect underperfused areas of myocardium. Adenosine and dipyramidole are currently used in MPI. Adenosine is the naturally occurring nucleoside agonist for the adenosine receptor while dipyridamole, a nucleoside transport inhibitor, acts by limiting cellular re-uptake of adenosine thereby increasing plasma and tissue levels of adenosine. Both adenosine directly, and dipyridamole indirectly, activate all four known AdoR subtypes, designated A_1, A_{2A}, A_{2B}, and A_3, which mediate a variety of responses in different tissues. Because a number of the side effects caused by adenosine and dipyridamole appear to be mediated by receptor subtypes other than the A_{2A}-AdoR, the sponsor believes that regadenoson, a selective agonist of the A_{2A} receptor has the potential to cause fewer undesirable effects than the approved adenosine and dipyridamole when used in MPI.

Study 1002510: Combination screen data report ([redacted] Aug 99)

This study evaluated the activity of CV-11 (regadenoson) in a [redacted] and additional selected assays including enzyme, ion channel, and radioligand binding assays. Regadenoson was evaluated at a single dose of 10 µM; significant activity (≥50%) was observed only for displacement of radioligand from Adenosine A_{2A} binding sites with 97% inhibition. A summary of results obtained from a few other receptors is presented in the following table.

TARGET	% Inhibition
Adenosine $_{A1}$	16
Adenosine $_{2A}$	97
Adenosine $_{A2B}$	0
Adrenergic α_{1A}, α_{1B}, α_{1D}, β_1	18, 19, 21, -18
Dopamine D_1, D_{2L}, $D_{4.2}$, D_5	11, 17, 14, -19
Gaba A Benzodiazepine Central	18
Gaba A Chloride Channel	13
Glucocorticoid	36
Glutamate NMDA, Agonist	31
Glycine, Strychnine Sensitive	-29
Muscarinic M_1	24
Muscarinic M_2	26

Nicotinic Actetylcholine, Central	29
Potassium Channel [K_V]	22
Serotonin 5-HT_{2A},5-HT_4, 5-HT_{5A}, 5-HT_7	-20, 24, 17, -22

Drug-Receptor Binding to Adenosine Receptor (Study CVT3146.014-N)
Pharmacological and functional characterization of novel adenosine receptor agonist by radioligand binding and cAMP assays)
(Reviewed by Dr. Anthony Proakis)

CVT-3146 was tested for its affinity for the adenosine receptor by radioligand binding techniques. Its affinity for the adenosine A_{2A} receptor was compared to that of the high affinity A_{2A} adenosine agonists, CGS21680 and WRC0470, a non-selective adenosine agonist (NECA) and a selective adenosine A_1 agonist (R-PIA). The affinities for the adenosine receptors were determined by competitive binding to cell membranes from human embryonic kidney (HEK-293) cells that express the recombinant human adenosine A_{2A} receptor and the Chinese hamster ovary (CHO-K1) cells that express the recombinant human adenosine A_1 receptor.

CVT-3146 was shown to be selective for the adenosine A_{2A} receptor relative to the A_1 receptor. The selectivity for the A_{2A} receptor by CVT-3146 was similar to that displayed by the two adenosine A_{2A} receptor agonists, CGS21680 and WRC0470 (Table 1).

Table 1. Affinities of Various Adenosine Receptor Agonists for the Adenosine A_{2A} and A_1 Receptors.

	K_i (nM)	
Molecule	**HEK – hA_{2A} receptor**	**CHO – hA_1 receptor**
CVT-3146	1269	> 16460
CGS21680	609	> 3540
WRC0470	272	7278
NECA	360	328
R-PIA	1656	477

The affinity of CVT-3146 for adenosine A_{2B} and A_3 receptor sites was assessed by its potency to compete for these binding sites on membranes derived from human embryonic kidney cells (HEK-293, A_{2B} receptor) and Chinese hamster ovary cells (A_3 receptor). At 10 uM, CVT-3146 displaced less than 22% of radioligand specific binding at either of these 2 receptor sites.

CVT-3146 activated the endogenous A_{2A} receptor in PC12 cells and caused cAMP accumulation, but did not induce cAMP accumulation in HEK-293 cells that express adenosine A_{2B} receptors.

These studies indicate that CVT-3146 has an affinity for the adenosine A_{2A} receptor and acts as an A_{2A} receptor agonist to stimulate cAMP accumulation; it has very weak if any interaction with adenosine A_{2B} or A_3 receptors.

Coronary Vasodilation in Isolated Rat and Guinea Pig Hearts (Study CVT3146.015-N: Coronary vasodilation in rat and guinea pig, isolated perfused hearts by A_{2A} adenosine receptor agonists).
(Reviewed by Dr. Anthony Proakis)

The coronary vasodilator effect of CVT-3146 was assessed in isolated rat and guinea pig perfused hearts and compared to responses produced by adenosine and by the positive control adenosine A_{2A} receptor agonists, CGS21680 and WRC0470. The hearts were perfused at a constant flow (10 ml/min), paced at a constant atrial cycle length (340 msec, approx. 175 bpm) and instrumented for measurements of coronary artery perfusion pressure from which coronary artery conductance was calculated (conductance = measure of amount of blood that can pass through a vessel in a given time for a given pressure gradient; i.e., ml/sec/mmHg).

CVT-3146, like the positive controls, produced concentration-dependent vasodilation that was reversible upon washout. The EC_{50} values for increasing coronary conductance by CVT-3146 and the other agonists are summarized in Table 2. The EC_{50} values for CVT-3146 for increased coronary conductance in rat and guinea pig hearts were 6.4 nM and 18.6 nM, respectively. CVT- 3146 was more potent than adenosine in this response in both rat and guinea pig hearts.

Table 2. Effect of CVT-4136 and Other Adenosine Agonists on Coronary Vasodilation (Conductance Increase) in Isolated Perfused Rat and Guinea Pig Hearts.

Agonist	n	Potency (EC_{50}, nM)	
		Rat	Guinea Pig
CVT-3146	4	6.4 ± 1.2	18.6 ± 6.0
Adenosine	4	59.2 ± 6.4	86.0 ± 0.5
CGS21680	4	0.5 ± 0.1	1.7 ± 0.4
WRC0470	3	0.6 ± 0.2	2.4 ± 1.1

The increase in coronary conductance caused by CVT-3146 was abolished by the adenosine A_{2A} receptor antagonist ZM241835 but not by the A_1 receptor antagonist CPX. The coronary vasodilation caused by either continuous or repeated exposures to CVT-3146 did not desensitize and/or develop tachyphylaxis.

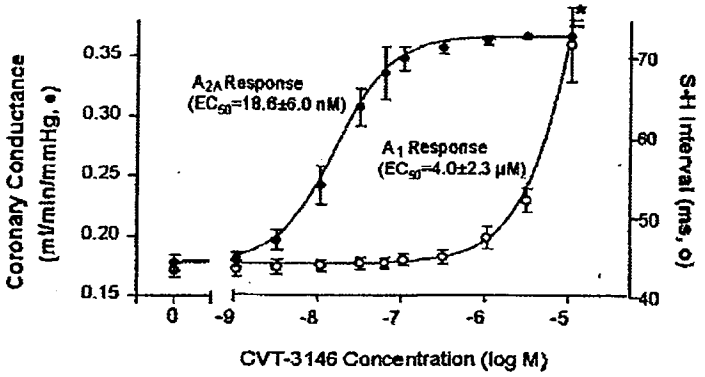
Effect of CVT-3146 on Coronary Vasodilation and Negative Dromotropic Activity in Isolated Rat and Guinea Pig Hearts (Study CVT3146.016-N: the A_{2A} adenosine receptor agonists, CVT-3146 and CVT-3033 are more potent in causing coronary

vasodilation than depressing AV nodal conduction in rat and guinea pig isolated hearts) **(Reviewed by Dr. Anthony Proakis)**

The negative dromotropic (adenosine A_1 mediated response) effects of CVT-4146 were studied in rat and guinea pig isolated perfused hearts.

At concentrations (0.1 nM to 30 uM) of CVT-3146 that increased coronary conductance in isolated rat hearts, CVT-3146 had no negative dromotropic effect. However, in guinea pig hearts, CVT-3146 prolonged the stimulus-to-His bundle interval; the EC_{50} for this response is 4.0 uM. For comparison, the observed EC_{50} for increasing coronary conductance in guinea pig hearts was 18.6 nM. Thus CVT-3146 is at least 215X more selective for causing coronary vasodilation than for a negative dromotropic effect in the isolated guinea pig heart (Fig 1).

Fig 1. Functional Selectivity of CVT-3146 in Guinea Pig Isolated Perfused Hearts for Producing Coronary Vasodilation Relative to the Negative Dromotropic Effect



Error bars indicate means ± SEM of single determinations from each of 4 hearts.
* All 4 hearts developed second-degree AV block at a CVT-3146 concentration of ≥ 3 µM.

Effect on Coronary Blood Flow Velocity, Systemic Arterial Blood Pressure and Heart Rate in Anesthetized Dogs (Study CVT3146.030-P: Effects of CVT-3146 on coronary blood flow velocity, systemic arterial blood pressure and heart rate in anesthetized dogs. **Study CVT3146.033-P:** Differential effects of CVT-3146 and adenosine on blood flow velocity in coronary and peripheral arteries, systemic arterial blood pressure and heart rate in anesthetized dogs)
(Reviewed by Dr. Anthony Proakis)

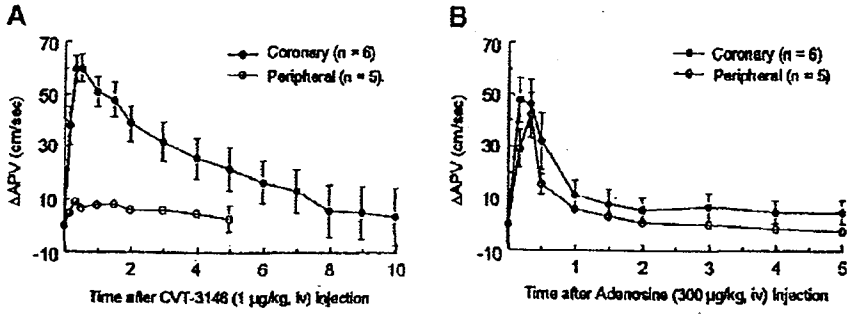
CVT-3146 was assessed for effects on coronary blood flow velocity, systemic arterial blood pressure and heart rate in anesthetized mongrel dogs. The dogs were instrumented with a Doppler transducer-tipped wire in the left anterior descending or the circumflex coronary artery (for measurement of average peak flow velocity), a pressure transducer positioned in the descending aorta (measurement of systemic blood pressure) and

electrocardiographic limb leads (measurement of heart rate). In one group of 10 dogs, increasing doses of CVT-3146 (1, 3, 6, 9 and 10 ug/kg) were administered via a peripheral vein. In a second group of 6 dogs, CVT-3146 was administered as a single dose of 1 ug/kg i.v. in a peripheral vein.

CVT-3136 caused a dose-dependent increase in coronary average peak velocity (APV); the peak occurred 20 to 30 seconds after IV injection. At a dose of 1 ug/kg IV, CVT-3146 caused a 3.3- fold increase in APV above baseline that was associated with a transient maximal drop in MABP of 17 mmHg and a maximal increase in heart rate of 21 bpm. The duration of increased APV by CVT- 3146 was short (= 120 sec), and all values for APV, MABP and HR measured at 10 min post-injection were not statistically different from baseline.

In a second group of 6 anesthetized dogs, CVT-3146 was administered as a single dose (1 ug/kg i.v.) and the effects on APV, MABP and HR were compared to responses produced by a single dose of adenosine (300 ug/kg i.v.). CVT-3146 produced a 2.6-fold increase in coronary arterial APV above baseline and a 1.1-fold increase in APV in a peripheral (cranial circumflex humeral) artery. CVT-3146 was also associated with a transient maximal decrease in MABP of 18 mmHg and a maximal increase in HR of 19 bpm. Adenosine (300 ug/kg i.v.) caused a 2.5-fold increase in coronary APV and a 2.0-fold increase in peripheral arterial APV. This dose of adenosine was associated with a transient maximal decrease in MABP of 36 mmHg and a maximal increase in HR of 5 bpm. These results indicate that CVT-3146 elicits a greater selectivity for coronary artery vasodilation than that seen with adenosine (Fig. 2).

Figure 2. Changes in Coronary and Peripheral Blood Flow Velocity Caused by CVT-3146 and Adenosine in Anesthetized Dogs.



Time course of changes in coronary (●) and peripheral (○) artery average peak flow velocity (APV) after an iv bolus injection of CVT-3146 (1 µg/kg; Panel A) or adenosine (300 µg/kg; Panel B) to anesthetized closed chest dogs. APV values (in cm/sec) are the changes in average peak flow velocity (ΔAPV) above baseline. Each point is the mean ±SEM of single determinations from 6 dogs for coronary and 5 dogs for peripheral artery APV.

Effect of CVT-3146 on Coronary Blood Flow in Conscious Dogs (Study CVT3146.031-P: Selective A_{2A} adenosine receptor agonist, for pharmacological stress test during myocardial perfusion imaging)
(Reviewed by Dr. Anthony Proakis)

The effect of CVT-3146 on coronary blood flow and other cardiovascular parameters were assessed in conscious dogs that were surgically instrumented to obtain measurements of coronary blood flow (CBF), left ventricular systolic pressure (LVSP), rate of rise of left ventricular pressure (LV dp/dt), mean arterial blood pressure (MABP), mean coronary resistance (CR) and heart rate (HR).

CVT-3146 (0.1-5 ug/kg IV) caused a dose-dependent increase in CBF with an ED_{50} of 0.34 ug/kg IV. The duration of the CBF increase (at least a 2-fold increase) also increased with increasing doses of CVT-3146 (Table 3). After the IV bolus of 2.5 ug/kg of CVT-3146, the peak CBF was attained in 17 sec and remained at least 2-fold above baseline for 97 seconds.

Table 3. Duration of 2-Fold Increase in Mean CBF after a 10-sec IV Bolus of CVT-3146 to Conscious Dogs.

CVT-3146 (µg/kg)	Duration of 2-fold increase in mean CBF (seconds) (± SEM)	*N*
0.1	2-fold increase not observed	6
0.175	2-fold increase not observed	5
0.25	8 ± 3	4
0.5	15 ± 6	6
1.0	22 ± 5	6
2.5	97 ± 14	6
5.0	247 ± 39	6

No tachyphylaxis of the effect of CVT-3146 on CBF was observed after three consecutive 1 ug/kg IV boluses administered 5 to 10 min apart. At 2.5 ug/kg, the peak effect of CVT-3146 on CBF was associated with a transient (20 sec) increase in HR of 81% above baseline and a transient decrease in MABP of 12 mmHg. Of the 5 dogs that had evaluable ECG recordings, one dog experienced a transient T-wave inversion after receiving CVT-3146. This effect on T-waves has also been observed with adenosine in dogs and in humans.

Study CVT3146.055-P: Cardiovascular effects of CVT-3146 in the anesthetized dog

Sponsor study no.: CVT3146.055-P

Conducting laboratory: [redacted]

Study date:	Jan-Jul, 2002
GLP compliance:	Yes () No (x)
QA report:	No
Drug, lot#, and % purity:	CVT-3146, lots#315-53 & #4P9002, purity not provided

The hypotension caused by regadenoson may be due to a peripheral vasodilatory action of the drug probably mediated by A_2AdoR in the peripheral resistive vessels. It was hypothetized that A_2AdoR reserve in the peripheral vessels is less than that of the coronary vasculature and this differential receptor reserve may account for the apparent selectivity of CVT-3146 for the coronary arteries over peripheral vasculature. The purpose of this study was to determine the differential selectivity of the vasodilatory action of CVT-3146 for the coronary vasculature (left circumflex coronary artery) vs. other vasculature beds (brain arteries, right forelimb artery, pulmonary artery).

Methods:
Mongrel dogs of either sex (n=37, 17-20 kg) were anesthetized with 30 mg/kg sodium pentobarbital and artificially ventilated (endotracheal tubing) with room air. Doses of 0.01-1.0 µg/kg of CVT-3146 were i.v. injected when the Doppler catheter was positioned in the left circumflex coronary artery (CFX) and doses of 1.0-3.0 µg/kg were given when the catheter was positioned in the brain arteries (BA), forelimb artery (FA), or pulmonary artery (PA). All arteries were of comparable diameter as determined by angiography. Baseline values were measured following a stabilization period of 20 minutes.

Results:
Administration of 0.01 and 1.0 µg/kg CVT-3146 to dogs (n=30) caused a 1.4 fold and a 3.1 fold increase in CFX average peak velocity (APV) respectively. This effect reached a peak within 30 sec post dose and began dissipating thereafter. The effect was associated with a transient drop in MAP of 2 and 14 mmHg, and an increase in heart rate from 139 to 144 bpm, and from 150 to 170 bpm respectively. Five minutes post-dose, APV values returned to baseline for the lowest doses, but a residual effect was noted at 0.1, 0.5, and 1 µg/kg (~1.3-1.5 fold)

Administration of 1 and 3.0 µg/kg CVT-3146 caused an increase in blood flow velocity in the FA (1.2 fold), PA (1.1 fold), and BA (1.5 fold), associated with increase in heart rate and decrease in blood pressure. These effects were noted during the 2 min observation post dose.

In summary, an i.v. bolus injection of 1.0 µg/kg CVT-3146 transiently enhances blood flow by 3.1-, 1.4-, 1.2-, and 1.1-fold in the CFX, BA, FA, and PA respectively.

Reviewer's comments:
Baseline values for heart rate and MAP were higher with increasing doses, due to the short time allowed between two consecutive drug administrations. The A_2AdoR reserve

in the peripheral vessels is presumably less than that of the coronary vasculature and this differential receptor reserve may account for the apparent selectivity of CVT-3146 for the coronary arteries over peripheral vasculature. More studies are needed to support the proposed hypothesis, *i.e.* to demonstrate that the differential effect observed is directly due to a difference in the receptor reserve in the vessels evaluated.

Study CVT3146.132-P: A2A-Adenosine receptor reserve of CVT-3146 for coronary vasodilation in Guinea pig isolated hearts

Study objective:
The EC_{50} values for increased coronary conductance in rat and guinea pigs isolated hearts by regadenoson were previously determined to be 6.4 nM and 6.7-18.6 nM. On the other hand, the affinity, as indicated by Ki values in radioligand binding assays for the A_2AdoR was 1.3 μM. The purpose of this study was to test the hypothesis that the relatively high increase in coronary conductance is due to a large A_2AdoR reserve for regadenoson.

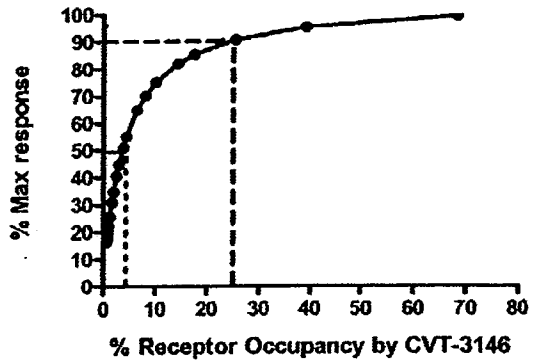
Study design:
This study was conducted in the guinea pig heart and used an irreversible antagonist of A_{2A}-AdoR (FSPTP) to inactivate receptors and reduce the response to agonist. Coronary perfusion pressure and ECG were monitored continuously. Following control recordings, 6 isolated hearts were perfused with adenosine (10-3000 nM) or regadenoson (1-300 nM) for 10 min in a cumulative manner. In another series of experiments, 6 hearts were perfused with FSPTP at 500 nM for 25 min followed by a 25 min washout; adenosine or regadenoson was then added at similar concentrations to the above.

The equilibrium dissociation constant (K_A) for binding to the A_2AdoR, measured by increase in coronary conductance, was determined as well as the fraction of functional receptors remaining after exposure to the irreversible A_2AdoR antagonist FSPTP. The extent of receptor reserve for an agonist to increase coronary conductance was estimated at near-maximal effect and at 50% of maximal effect. For comparison purposes, pairs of concentrations of agonist that caused equal increases of coronary conductance before and after inactivation of a fraction of A_2AdoRs with FSPTP were selected.

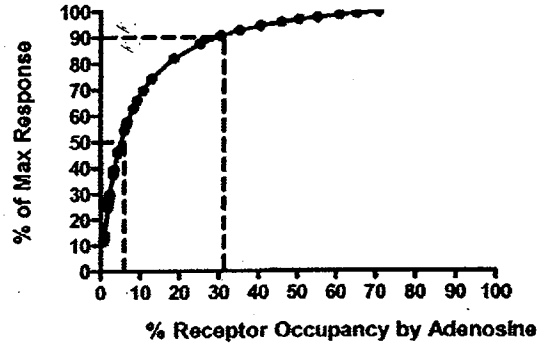
Results:
Activation of ~4% of A_{2A}-AdoR by CVT-3146 (6% for adenosine) caused half of the maximal increase of coronary flow that could be achieved by the drug. Activation of ~25% of A_{2A}-AdoR by CVT-3146 (32 % for adenosine) caused an increase of coronary flow that was 90% of the maximal increase of coronary flow caused by CVT-3146 (Figure 1)

Figure 1: Occupancy-Response Relationships for Coronary Vasodilation for Regadenoson and Adenosine.

A)



B)



Report's conclusions:
Receptor reserves for A_{2A}-AdoR-mediated coronary vasodilation for both adenosine and CVT-3146 were large. Activation of 4% and 6%, respectively, of A_{2A}-AdoRs by CVT-3146 and adenosine was sufficient to elicit a half-maximal coronary vasodilation response. The receptor reserve measured for adenosine compares reasonably well with the previously reported value. According to the sponsor, these large receptor reserves for increases of coronary conductance caused by CVT-3146 and adenosine can explain why the concentrations of these A_{2A}-AdoR agonists needed to cause coronary vasodilation are lower than the concentrations needed to displace the binding of a radioligand to an A_{2A}-AdoR.

Reviewer's comments:
Based on the results of this study, regadenoson appears to be very similar to adenosine with respect to receptors reserve.

2.6.2.3 Secondary pharmacodynamics

Study title: Effect of the adenosine A2A receptor agonist, CVT-3146, on I_{Kr} and I_{Ks} in isolated canine left ventricle myocytes.

Sponsor study no.: CVT3146.053-P
Conducting laboratory: [redacted]

Study date: September 2002
GLP compliance: Yes ( ) No (x)
Drug, lot#, and % purity: No information provided

Study objective: To determine the effects of CVT-3146 on the delayed rectifier(s), I_{Kr} and I_{Ks} in isolated canine left ventricle myocytes.

Test article/drugs:
CVT-3146 was tested at final concentrations of 10 nM, 100 nM, 1 μM, and 10 μM. Chromanol, 30 μM, an inhibitor of I_{Ks}, was used in external solution for I_{Kr} recordings. E-4031, 5 μM, an inhibitor of I_{Kr}, was added in external solution for I_{Ks} recordings. $CdCl_2$, 300 μM, was added to the external solution to inhibit L-type Ca^{2+} current.

Methods:
Myocytes excised from the epicardial region of the left ventricle of dogs were isolated by enzymatic dissociation. Whole cell patch clamp techniques were used to record I_{Kr} and I_{Ks}. Whole cell currents were acquired at 10 kHz, filtered at 4 kHz, and stored on a computer. Inclusion of K-Aspartate caused a liquid junction potential of about -10 mV. I_{Kr} was measured as the time-dependent tail current measured at -30 mV following a short 250 ms depolarizing pulse to 40 mV from a holding potential of -50 mV. I_{Ks} was measured as the time-dependent tail current measured at -30 mV following a 2 sec depolarizing pulse to 40 mV from a holding potential of -50 mV. Tail currents were initially measured 3 minutes after membrane rupture. The voltage clamp protocol was repeated 4 times before drug, 4 times during drug (beginning 3 minutes after exposure of the cell to the drug) and 4 times after washout of the drug (beginning 3 minutes after initiation of washout). Averages for each of the 4 runs were compared using a one way repeated measured analysis of variance.

Results:
Peak I_{Kr}, tail current was reduced by about 25% at 10 μM whereas peak I_{Ks}, tail current was unaffected. No effect was observed at the lower concentrations tested. It was concluded that there was minimal activity of CVT-3146 to block I_{Kr} and I_{Ks}.

Reviewer's comments:
Individual data were not provided. Drug nominal concentrations were not verified post recording. The sponsor did not test the ability of positive control E-4031 to inhibit the K channel in an adequate condition, *i.e.* no I_{Kr} recordings were performed in presence of I_{Kr} inhibtor, E-4031, which was used during I_{Ks} recordings only. The number of cells was not provided. The results were expressed as values corresponding to the average of 3-6 experiments. Overall, it is not clear from the report whether the means used to generate the figure were of the repeat measurements during one experiment, or from different experiments, or even if multiple cells were used by concentration. In view of the

availability of similar results from other better designed in vitro studies evaluating the effect of regadenoson on hERG channels, this study does not need to be repeated.

Study CVT3146.112-P: Effect of CVT-3146 on hypotension induced vasoconstriction in mouse isolated heart (Conducted by [redacted], CVT-3146 lot#011175044)

This study was designed to determine whether CVT-3146 can prevent or modify coronary microcirculatory vasoconstriction.

Methods & Results:

Mouse isolated hearts (n = 60) were perfused with Langendorff solution, and coronary vascular resistance (CVR) was continuously monitored for 70 min during various interventions in 3 protocols.
Protocol 1: Untreated hearts were perfused at 65 mmHg for 70 min, CVR remained stable.
Protocol 2: After 20 min at physiological pressure of 65 mmHg, followed by a 30 min decrease of perfusion pressure to 30 mmHg, the physiological pressure was restored for the remaining 30 min. In untreated hearts, CVR increased up to 191% above baseline, whereas in CVT-3146 treated hearts, CVR increased by 131% at 3 nM, and the increase was abolished at 10 and 30 nM. In untreated hearts, restoring CPP to 65 mmHg caused a sudden but incomplete vasodilation resulting in a lower CVR that was 155% above baseline. This response was significantly reduced to 117% by 3 nM CVT-3146 and abolished by 10 and 30 nM.

Protocol 3: In untreated hearts, the sustained lowering of CPP to 30 mmHg over an extended period of 50 min was accompanied by a progressive rise in CVR that reached a maximum of 241% of control. In CVT-3146 treated hearts, 10 nM to the perfusate, either at 10 or 30 min after lowering CPP, resulted in a rapid and progressive vasodilation bringing CVR to baseline values within 10 min.

Report's conclusion:
In summary, the isolated heart appears to react to lowering of CPP with a progressive and severe vasoconstriction that can be prevented by pretreatment with an A_{2A} agonist. The sponsor believes that activation of A_{2A}, adenosine receptors during the vasoconstriction can blunt and reverse this microvascular reaction, and hence, improve myocardial perfusion and reduce the severity of ischemia.

Reviewer's comments:
The effect of regadenoson on the coronary vascular resistance seemed to reach a plateau at 10 nM. This study suggests that treatment of mouse isolated hearts with regadenoson can mitigate/prevent the vasoconstriction induced by lowering of physiological pressure. The sponsor claims that ischemia would thereby be prevented; however, no histological confirmation of the presence of ischemia was provided to support this assumption.

Study CVT-3146.124-P: Effect of CVT-3146 on the attenuation of blood flow reserve associated with coronary artery stunning in dogs.

Sponsor study no.: CVT3146.124-P
Conducting laboratory: [redacted]

Study date: Oct 2004-July 2005
GLP compliance: Yes () No (x)
Drug, lot#, and % purity: CVT-3146, lot#4P9002, purity not provided

The purpose of this study was to evaluate the effect of intracoronary CVT-3146 on the coronary vasculature injury associated with acute transient myocardial ischemia in dogs.

Methods:
Anesthetized Mongrel dogs (n=19, 25-35 kg) were artificially ventilated by endotracheal tubing with room air. An endovascular dilation catheter was used for imposing coronary occlusion and injection. Blood flow velocity in the coronary arteries was measured using Doppler transducer-tipped guide wires. Endothelium-dependent microvascular reactivity was determined using intracoronary acetylcholine and a maximal hyperemic response following a stabilization period. To determine the maximal hyperemic response, the intra-coronary balloon was inflated to completely occlude the left anterior descending coronary artery (LAD); the balloon was deflated after 30 sec and in a subsequent intervention the balloon was inflated for 60 sec. All parameters were allowed to return to baseline between two successive interventions. This protocol was repeated after 60 min of complete LAD occlusion followed by 30 min reperfusion. Reperfusion was done gradually and lidocaine was administered as necessary to avoid lethal reperfusion arrhythmias. In group I, intra-coronary saline was given (10 µL/kg/min) 5 min prior to reperfusion and during 30 minutes of reperfusion. In group II, CVT-3146 (0.1 µg/kg/min) was given instead of saline.

Results:
Intracoronary acetylcholine and brief occlusion of the LAD caused respectively a dose and time dependent vasodilation. Acetylcholine and transient ischemia-dependent vasodilation quantified by maximum average peak coronary blood flow velocity was markedly attenuated following 60 min occlusion and 30 min of reperfusion in Group I and II. There were no significant changes in heart rate or blood pressure in either group. In group II, there was a trend for intracoronary CVT-3146 to protect the microvasculature from reperfusion injury while preserving vascular reactivity. Thus, occlusion/reperfusion injury reduced vascular flow-reserve recruitment, and CVT-3146 treatment seemed to reverse this outcome. The low number of animals completing the study precluded rigorous statistical analysis.

Reviewer's comments:

The purpose of this study was to evaluate whether intracoronary CVT-3146 can attenuate the injury on the coronary vasculature associated with acute transient myocardial ischemia in dogs. However, as was the case in the mouse study, no histological confirmation of the presence of ischemia was provided to support this assumption. In light of these results (as well as those obtained in the above mouse study), the sponsor believes that CVT-3146 may be used as an adjuvant cardioprotective therapy during coronary reperfusion interventions, i.e. facilitated interventions. It was not stated whether this hypothesis was explored in clinical studies.

Study CVT3146.125-P: Effect of CVT3146, A Selective A_{2A} Adenosine Receptor Agonist, on Ventricular Action Potential Duration and Coronary Perfusion Pressure in Female Rabbit Isolated Hearts

Sponsor study no.: CVT3146.125-P
Conducting laboratory: [redacted]

Study initiation date: January 14, 2005
GLP compliance: Yes () No (x)
Drug, lot#, and % purity: CVT-3146, lot#011175044, purity not provided

The objective of this study was to evaluate the potential of CVT1346 to cause QT interval prolongation, by determining the effects of the drug on the duration of left ventricular monophasic action potential (MAPD) and coronary perfusion pressure in rabbit isolated perfused hearts.

Test article/Reference substance: Stock solutions of CVT-3146 (100 mM) and positive control E-4031 (1 mM) were prepared in DMSO and saline, respectively and diluted in saline for use in experiments. Final concentration of DMSO in saline during experiments was $\leq 0.1\%$.

Methods: Hearts isolated from anesthetized female rabbits were perfused with modified Krebs-Henseleit solution, and instrumented for recording of the left ventricular monophasic action potential (MAP), and the electrocardiogram (ECG). To facilitate the recording of MAPs, atrioventricular conduction was blocked by thermoablation and the left ventricle was paced at a rate of 1 Hz. After initiation of the pacing, a 30-50 min period was allowed for heart rhythm and perfusion pressure to achieve a steady state. Cumulative concentrations of CVT-3146 were infused for ~5-10 min, to achieve final coronary drug concentrations of 0.01, 0.1, 1, 10 and 30 μM. After washout of CVT-3146 (15-20 min) and after MAPD stabilization, E-4031 (100 nM) was infused to the same heart for 15 min or until MAP duration reached a steady-state, and then E-4031 was washed out (20 min). After washout of drug, the post-drug control MAPD and CPP were determined. All data were reported as means ± SEM.

Results:

LVMAPD$_{90}$: In absence of drug, the control values of epicardial and endocardial MAPD$_{90}$ of the left ventricle were 183±6 and 205±5 ms, respectively. CVT-3619 (0.01-30 µM) did not significantly increase either the epicardial or the endocardial LV MAPD$_{90}$. At 30 µM, LV MAPD$_{90}$ was 191±6 and 202±9 ms respectively. E-4031 (100 nM) significantly and reversibly prolonged both epicardial and endocardial MAPD$_{90}$ by 41% and 49% from 185±4 and 206±9 ms to 261±8 and 307±17 ms, respectively. CVT-3146 caused neither early after-depolarization nor ventricular arrhythmia at the concentrations tested. In contrast, E-4031 (100 nM) caused early-after depolarization (EAD), frequent ectopic ventricular beats or polymorphic ventricular tachycardia in 4 out of 5 hearts.

Coronary conductance: CVT-3146 (0.01-30 µM) increased coronary conductance by up to ~35% (from 0.65±0.05 to 0.88±0.08 mL/min/mmHg) in a concentration-dependent manner. After washout of CVT-3146, coronary conductance decreased to 0.78±0.07. E-4031 (100 nM) caused no significant change in coronary conductance.

Report's conclusions:
CVT-3146 caused coronary vasodilatation, i.e. an increase in coronary conductance, but did not prolong the ventricular action potential duration. The sponsor suggested that it is unlikely that CVT-3146 would prolong the QT interval or cause proarrhythmic activity.

Reviewer's comments:
This study indicates that in rabbit isolated perfused rats, 5-10 min infusion with regadenoson (0.01-30 µM) does not prolong the ventricular action potential duration. At the same dose, coronary conductance was increased by 35%. This increase in conductance is lower than the increase observed with the clinical dose, and thus the concentration at which regadenoson was tested in the present study may not have been sufficiently high to better mimick clinical situations. However, drugs eliciting no effect at 30 µM are believed to have a low probability of inducing QT interval prolongation.

Study CVT3146.128-P: The effects of CVT-3146 on human platelet and neutrophil function

Through A_{2A} receptors activation, adenosine may be involved in platelet anti-aggregatory effects, neutrophil anti-inflammatory responses as well as in modulation of immune cell function.
This study evaluated the affinity of regadenoson for A_{2A}-AdoR on membranes prepared from human platelets and neutrophils, or from rat striatum and CHO cells transfected with human A_{2A} receptors. Binding experiments were conducted using a radiolabeled agonist ([^{3}H] CGS 21680) and a radiolabeled antagonist ([^{3}H] ZM 241385) (Table 1). cAMP levels, aggregation and cytoplasmic free Ca^{++} concentration were determined in human platelet and cAMP levels and superoxide anion production were determined in human neutrophils. EC_{50}s were determined for cAMP on platelets, neutrophils, CHO cells transfected with human A_{2A} adenosine receptors (Table 2).

Table 1: Affinity values (Ki, nM) of GSS 21680 and regadenoson for A_{2A}-AdoR (Table provided by sponsor)

Agonist	Human Platelets	Human Neutrophils	Rat Striatum	CHO – hA_{2A}
Radioligand - [^{3}H] ZM 241385				
CGS 21680	430	243	276	234
Regadenoson	534	327	347	318
Radioligand - [^{3}H] CGS 21680				
CGS 21680	ND	ND	22	19
Regadenoson	ND	ND	50	43

ND = Not done

Table 2: Regadenoson and CSG 21680 effects on cAMP production and platelet and neutrophil function (Table provided by sponsor)

Assay	CGS 21680 (nM)	Regadenoson (nM)
cAMP Stimulation (EC_{50})		
Human Platelets	389	472
Human Neutrophils	320	406
CHO – hA_{2A}	30	56
Human Platelet Aggregation (IC_{50})	158	437
Intracellular Calcium Inhibition in Human Platelets (IC_{50})	60	108
Oxygen Production in Human Neutrophils (IC_{50})	219	328

Conclusions:
CGS 21680 and regadenoson had slightly lower affinity (< 2-fold) for the A_{2A}-AdoR in human platelets relative to those in human neutrophils, rat striatum, and CHO cells transfected with human A_{2A}-AdoR. CGS 21680 affinity was higher (up to ~2-fold) in all tissues than the affinity of regadenoson.

CGS 21680 had higher potency (up to ~2-fold) than regadenoson in all assays. The highest potencies (30–100 nM) for both agonists were observed for stimulation of cAMP production in CHO cells and for inhibition of a thrombin-induced increase of calcium concentration in human platelets.

In summary, the receptor binding and functional activities of regadenoson and of the prototypical adenosine A_{2A}-AdoR agonist CGS 21680 were similar in assays of human platelets and neutrophils and were consistent with the identification of regadenoson as a selective A_{2A}-AdoR agonist.

Study CVT3146.129-P: Tachycardia Caused by Regadenoson is Mediated by Direct Sympathoexcitation in Awake Rats (CVT Dept of Pharmacology, Oct02-Jan03)

Adenosine has been shown to elicit a direct positive chronotropic effect and to cause stimulation of the carotid body chemoreceptors, possibly by A_{2A}-R activation. The objective of this study was to determine the role of A_{2A} receptor subtype in regadenoson-induced tachycardia.

Awake male Sprague-dawley rats (n=3-8) were given i.v. injections of regadenoson at doses of 0.3, 1.0, 3, 10, 30, and 50 μg/kg, up to 2 doses each with 30-min intervals or longer at the higher doses. A separate group of animals was used for measurement of norepinephrine levels. Regadenoson given at 0.3-50 μg/kg caused a dose-dependent 10-30% increase in heart rate, whereas mean arterial pressure slightly increased at lower doses (0.3-1.0 μg/kg) and decreased by up to ~20% at higher doses (≥10 μg/kg). Tachycardia and MAP decrease lasted for up to 30 min at the higher doses. When rats were pretreated with ZM241385, an A_{2A} receptor antagonist, the decrease in MAP and increase in HR caused by regadenoson were both attenuated. When rats were pretreated with metoprolol, a beta-blocker, the increase in HR was attenuated whereas no effect was noted on the hypotension caused by regadenoson. In the presence of hexamethonium, a ganglionic blocker, the tachycardia was completely prevented even though MAP was further reduced. Ten μg/kg regadenoson increased plasma norepinephrine levels by almost 2-fold above baseline, with a peak increase at 1-5 min, and return to baseline values by 60 min post-injection.

It was concluded that the tachycardia caused by regadenoson is independent of the decrease in MAP and may not entirely be baroreflex mediated, which suggests that regadenoson may cause a direct stimulation of the sympathetic nervous system (SNS) via activation of A_{2A} adenosine receptors.

Reviewer's comments:
Direct activation of endothelial and vascular smooth muscle cells A_{2A} receptors by adenosine causes vasodilation, resulting in a decrease in arterial pressure, a baroreflex-mediated activation of the sympathetic nervous system, and thereby an increase in heart rate. The sponsor concludes that the results of this study suggest that regadenoson-induced tachycardia in awake rats may not entirely be due to a baroreceptor reflex, and that increased circulating NE levels are indicative of sympathoexcitation as well. The report also referenced a clinical pharmacokinetic study with regadenoson suggesting a similar potential direct mechanism.

Study CVT3146.134-P: Effects of regadenoson (CVT-3146), a novel A_{2A}, adenosine receptor agonist, on QT interval

Sponsor study no.:	CVT3146.134-P
Conducting laboratory:	[redacted]
Study initiation date:	May 9, 2006
GLP compliance:	Yes () No (x)

Drug, lot#, and % purity: CVT-3146, lot#803604, purity not provided

Methods:
Male mongrel dogs (n = 16, 20-28kg) were instrumented for measurements of mean arterial pressure (MAP), heart rate (HR), and ECG (limb Lead II or III). A pair of electrodes was sutured onto the surface of the right atrium for electrical pacing. Blood pressure (BP), HR and ECG were recorded continuously on conscious dogs. When MAP and HR were stable (~20-30 min), the heart was atrially paced at 135, 150 and 165 bpm for ~5 min at each rate. Ten minutes after discontinuation of pacing, the dog received iv bolus injections of regadenoson at doses of 1, 2.5, 5, and 10 µg/kg with interval between doses of 15, 20, 30, and 45 min respectively (0.07, 0.17, 0.33, and 0.7 XHD respectively).

At least 45 min after the last regadenoson injection, the heart was atrially paced at 165 bpm for 10 min, and the injections of 5 and 10 µg/kg regadenoson were repeated while the heart was paced at 165 bpm. Afterwards, the dog received propranolol (1 mg/kg, iv) and atropine (0.1 mg/kg, iv) followed by injections of regadenoson (5 and 10 µg/kg, iv). Propranolol was administered again prior to the administration of 10 µg/kg regadenoson.

On another day, some dogs received hexamethonium, (20 to 25 mg/kg, iv, over 30 to 35 min) followed by an iv injection of 5 µg/kg regadenoson.

Sotalol (4 mg/kg) was infused intravenously over 10 min to 7 dogs, and ECG, BP, and HR were recorded for 1 h after the completion of the sotalol infusion.

Results:
Atrial pacing at 135, 150, and 165 bpm shortened the QT interval from a baseline value of 224 ms, in a frequency-dependent manner by 15, 22, and 39 ms respectively. QT intervals were significantly correlated with R-R intervals. No change in MAP was noted.

Effects of regadenoson alone: Regadenoson caused a dose-dependent shortening in the QT interval (maximum effect at 2 min) associated with an increase in HR, i.e. shortening in R-R interval (maximum effect between 0.5 and 2 min). The QT interval was shortened by 14, 24, and 27 ms and the R-R interval by 150, 212, and 251 ms after injections of 2.5, 5, and 10 µg/kg regadenoson respectively. QT intervals were significantly correlated with R-R intervals and MAP was slightly decreased at these dose levels (7-13 mmHg).

Effects of regadenoson following atrial pacing at 165 bmp with a steady state baseline QT of 189 ms: regadenoson at 5 and 10 µg/kg did not cause a significant change in either the QT or the R-R intervals. There was a decrease in MAP which was statistically significant only at 10 µg/kg.

Effects of regadenoson after combination of β-adrenergic and muscarinic-cholinergic blockade: Injection of propranolol and atropine was followed by an increase in HR from a baseline value of 88 to 146 bpm and a shortening in QT interval from 240 to 211 ms. Subsequent administration of 5 and 10 µg/kg regadenoson did not cause a significant

change in the QT interval but at 3 min post dose, a 10 µg/kg regadenoson caused a slight shortening of the R-R interval (5%). The regadenoson-induced changes in HR/RR interval were relatively small (<5% for 5 µg/kg and <8% for 10 µg/kg regadenoson) and significant increase/decrease (5-8%) in HR/R-R interval. Regadenoson at 5 and 10 µg/kg caused a decrease in MAP (11 and 19 mmHg respectively) after treatment with propranolol and atropine.

Effects of regadenoson after ganglionic blockade: Regadenoson (5 µg/kg) did not induce significant changes in QT and R-R intervals after treatment with Hexamethonium but caused a greater MAP decrease.

Sotalol (4mg/kg) infusion (10 min) induced more than one hour QT interval prolongation, and increase in the R-R interval (or decrease in HR), and did not cause a statistically significant change in MAP.

Report's conclusions:
Regadenoson causes a dose-dependent shortening in the QT interval associated with an increase in HR; however it does not shorten the QT interval when atrial pacing or pharmacological treatment with either propranolol and atropine or hexamethonium procedures were used to prevent changes in HR. Sotalol causes a sustained prolongation of the QT interval that was not directly correlated with a decrease in HR. The findings indicate that regadenoson does not have a direct effect on the QT interval in the conscious dog. Rather, the effect of regadenoson to shorten the QT interval is a response to an increase of HR that may be mediated by activation of the sympathetic nervous system or/and the withdrawal of vagal tone.

Reviewer's comments:
Regadenoson causes a dose-dependent shortening in the QT interval associated with an increase in HR, and decrease in MAP. These effects were observed at 2.5, 5, and 10 µg/kg regadenoson corresponding to 0.07, 0.17, 0.33, and 0.7 X the clinical anticipated dose of 400 µg/administration. Irrespective of the causative mechanism, this effect on QT interval is causally related to regadenoson and has been conveyed to the clinical reviewer.

2.6.2.4 Safety pharmacology

Neurological effects:

Study 1491-CVT-01-B: Effects of CVT-3146 (2 and 200 µg/kg, i.v.) on behaviour and physiological state as assessed by the Irwin test and on body temperature in rats.

Sponsor study no.: 1491-CVT-01-B
Conducting laboratory: [redacted]

Date of initiation: 25 Jan 2002
GLP compliance: Yes (x) No ( ) (Compliance with Swiss Ordinance stated to be accepted by the USFDA GLP Part58)
QA report: Yes
Drug, lot#, and % purity: CVT-3146, lot#4P9002, [redacted]

Animal species/strain/sex per dose/weight/age: Rat/Sprague-Dawley/5males/dose/185-230 g/Not provided
Doses/vehicle: Drug was at 2 or 200 µg/kg, in a volume of 2 mL/kg. Vehicle was 5.5 mg/mL sodium chloride, 4.2 mg/mL sodium bicarbonate, and 1 mg/mL methylboronic acid, pH 9.2.
Reference item: Haloperidol
Duration/route: Slow bolus injection over 30 sec/Intravenous, approximately 5 minutes before the beginning of the test

Methods:
Animals were assigned to 4 groups so that the mean rectal temperature was not statistically different in the 4 groups. Animals received an intravenous bolus injection of the vehicle or CVT-3146 as follows:

Group	*Treatment*	*Dose, Route*	*Volume*	*HDM**
E. Vehicle	Vehicle	i.v.	2 mL/kg	-
F. Dose 1	CVT-3146	2 µg/kg, i.v.		0.04X
G. Dose 2	CVT-3146	200 µg/kg, i.v.		4X
H. Reference	Haloperidol	1 mg/kg, i.p.		-

*HDM: human dose multiple based on a body surface area comparison for a 50 kg adult to receive a clinical dose of 400 µg, equivalent to 300 µg/m^2

Vehicle or CVT-3146 was administered as a slow i.v. bolus over 30 sec approximately 5 minutes before the beginning of the test. Haloperidol was i.p. administered, ~30 min before the beginning of the test. Rectal temperature was recorded 5, 15, 30 min, 1 hr, 4 hr, and 24 hr post dose for regadenoson and vehicle, and 30 min, 1 hr, 4 hr, and 24 hr post dose for haloperidol. Irwin test or Functional Observational Battery (FOB) was performed before each temperature recording The animal was then placed in an open field and observed during approximately 3 minutes and the number of rears (supported and un-supported) and grooming episodes were counted, and the gait characteristic and arousal level were ranked. The presence of convulsions or tremors and palpebral closure were noted again. At the end of the 3 min, the number of fecal boluses and pools of urine were recorded and testing of animal's reflex, consisting in recording the animal's response to the approach of a pencil, a touch to the rump, finger snap and tail pinch was performed. Righting reflex, catalepsy and grip strength were rated. Animals were euthanized at the end of the test.

Results
Mortality
No animal died spontaneously during the study.

Effects of CVT-3146 and haloperidol in the Irwin test
•At 200 µg/kg, number of rears and arousal state were decreased and home cage posture was affected from 5 to 30 min post-dose, with statistical significance at 5 and 15 min for number of rears and posture modification.
•At 2 and 200 µg/kg, the reactivity of animals to being handled was decreased from 5 to 30 min. This effect was significant at 200 µg/kg compared to vehicle group at 30 min.
•Sensory reactivity was slightly decreased from 5 and up to 30 min after 200 µg/kg, and 5 min after 2 µg/kg: response to the approach, touch and tail pinch were slightly decreased.
•At 200 µg/kg, one animal out of 5 showed a slight to moderate catalepsy 15 and 30 min after administration.

These effects were transient and from 1 to 24 hours, CVT-3146 (2 and 200 µg/kg, i.v.) did not significantly modify behavioral/physiological signs.

Haloperidol at 1 mg/kg induced significant behavioral alterations 30 min to 24 hrs post i.p administration. The changes included significant decrease in spontaneous activity, occurrence of tremors and stereotypies, palpebral closure and piloerection, gait alteration, significantly modified sensorimotry reactivity. Catalepsy was observed at 30 min and up to 24 hours.

Effects of CVT-3146 and haloperidol on body temperature
Hypothermia was observed in a dose- and time- dependent manner from 5 min up to 1 hour after 200 µg/kg CVT-3146 administration. This effect was statistically significant when compared to the vehicle-treated group.
Haloperidol (1 mg/kg, i.p.) did not cause any significant change on body temperature.

Report's conclusions:
Rats given CVT-3146 at a dose of 200 µg/kg exhibited a decrease in spontaneous activity and hypothermia. According to the sponsor, these effects were transient and all rats showed a typical behavior by 1 hour post-dose.

Reviewer's comments:
CVT-3146 at 2 and 200 µg/kg induced modification in various parameters in the Irwin test. These changes and hypothermia were statistically significant at 200 µg/kg. No NOAEL was established in rats for CNS effects.

Study 3146.149-P: Neuropharmacological profile (NPP) of CVT-3146 in rats.

Sponsor study no.:	3146.149-P
Conducting laboratory:	[redacted]
Date of initiation:	19 Oct 2007
GLP compliance:	Yes (x) No ( )
QA report:	Yes

Drug, lot#, and % purity: CVT-3146, lot#902438 (Package) and lot#902624 [redacted]

Animal species/strain/sex per dose/weight/age: Rat [redacted] CD(SD)/10males/dose/213-245 g/7 weeks
Test article/vehicle: The test article was formulated at 0.08 mg/mL; vehicle formulation was as follows: 15% w/w propylene glycol, 0.1% w/w edentate disodium dehydrate, 10.9 mg/mL dibasic sodium phosphate dihydrate, and 5.4 mg/mL monobasic sodium phosphate monohydrate, pH 6.3-7.7.
Duration/route: Intravenous administration as a slow push, over 30 sec (Groups 2 and 3) to 2 min (Groups 1, 4, and 5) to each rat.

Methods:
Animals were dosed as follows:

Group (n=10)	*Treatment*	*Dose (mg/kg)*	*Volume (mL/kg)*	*HDM**
1	Vehicle	0	5	
2	CVT-3146	0.04	0.5	0.8X
3	CVT-3146	0.08	1.0	1.6X
4	CVT-3146	0.2	2.5	4X
5	CVT-3146	0.4	5.0	8X

*HDM: human dose multiple based on a body surface area comparison for a 50 kg adult to receive a clinical dose of 400 µg (300 µg/m^2)

Rats were observed for signs of pharmacological activity/toxicity at 2-5, 15, 30, and 45 minutes, 1, 2, 3, 4, and 24 hours following treatment with the results recorded. Observations were made at ± 5-15 minutes of the designated times. The following symptoms were observed: seizures/convulsions, body tremors, ataxia, abnormal posture, excretion, awareness reaction, motor activity, piloerection, stereotypy, decreased respiration. Abdominal tone was assessed by feeling the abdominal muscles. Body temperatures (rectal) were taken at 60 min (+5 min) post-dose.

Animals were euthanized at the end of the experiment.

Statistical analysis:
No statistical analysis was performed for behavioral tests as according to the sponsor, the data generated is not quantitative enough to allow for meaningful comparison.

Results

No animal died spontaneously during the study.

Results are summarized in the following table

Intravenous Treatment	Dose (mg/kg)	60 min Mean Body Temperature[a] (°C)	Signs Observed								
			2-5 min	15 min	30 min	45 min	1 hr	2 hr	3 hr	4 hr	24 hr
Vehicle	0	38.8 ± 0.10	No signs								
CVT-3146	0.04	38.4 ± 0.12 (-1.0 %)[b]	No signs								
CVT-3146	0.08	38.0 ± 0.14* (-2.1 %)[b]	No signs								
CVT-3146	0.20	36.9 ± 0.12* (-4.9%)[b]	No signs								
CVT-3146	0.40	36.0 ± 0.14* (-7.2%)[b]	Decreased abdominal tone 2-5 minutes to 1 hour 10/10 Decreased abdominal tone 2 hour 2/10 Decreased activity 15 to 45 minutes 10/10 Decreased activity 1 hour 1/10 No signs 3 to 24 hours 10/10								

[a] Data are presented as Mean ± SEM.
[b] Percent change calculated from the vehicle group
* = Statistically significant ($p \leq 0.05$) change when compared to the vehicle group - ANOVA/Tukey HSD Multiple Comparison Test

Intravenous administration of vehicle at 5 mL/kg and CVT-3146 at 0.04, 0.08, and 0.20 mg/kg did not produce any pharmacological or toxicological signs.

Decreased abdominal tone was observed in all the animals treated with 0.40 mg/kg dose of CVT-3146 at 2-5, 15, 30, 45, and 1 hour observations. At 2-hour observation, 2 out of 10 animals in this group showed decreased abdominal tone. Decreased activity was observed in all animals administered 0.4 mg/kg dose of CVT-3146 at 15, 30, and 45 minutes post-dose. At the 1 hour observation, 1 animal out of 10 in this group had decreased activity.

Statistically significant decreases in body temperature were observed in the groups receiving CVT-1346 at 0.08, 0.2, and 0.4 mg/kg (-2.1%, -4.9%, and -7.2% respectively), compared to the vehicle group.

Report's conclusions:
The intravenous administration of CVT-3146 at 0.04, 0.08 or 0.2 mg/kg did not produce any apparent neuropharmacological or toxicological effects in rats. At the 0.4 mg/kg dose level, decreased abdominal tone and/or decreased activity were observed. Statistically significant decrease in body temperature was observed with 0.08, 0.20 and 0.40 mg/kg doses of CVT-3146.

Reviewer's comments:
There was a dose-dependent decrease in body temperature. The body temperature was recorded at 60 minutes post administration only. Additional recording would have been helpful in determining whether the effect was reversible. However, the decrease was minimal at clinical equivalent dose of 0.04 and 0.08 mg/kg (-1 and -2.1% respectively).

Contrary to previous results obtained in the CNS Study #1491-CVT-01-B, a dose of 0.2 mg/kg had no effect on any of the neuropharmacological parameters measured. The decrease in body temperature is consistent with the previous study.

Cardiovascular effects:

Study CVT3146.117-P: Effects of CVT-3146 on HERG Tail Current Recorded from Stably Transfected HEK293 Cells.

Sponsor study no.: CVT3146.117-P
Conducting laboratory: [redacted]
Study initiation date: August 22, 2005
GLP compliance: Yes (x) No ()
QA report: Yes
Drug, lot#, and % purity: CVT-3146 monohydrate, lot#0406CV301, [redacted]

The purpose of this study was to evaluate the effects on CVT-1346 on a human HERG channel tail current recorded from HEK293 cells.

Test article: CVT-3146 was formulated in bath solution to achieve a final perfusion concentration of 3 μM. Analysis of samples taken from the perfusion baths following 15 minutes exposure showed that cells were exposed to 5 μM CVT-3146.
Vehicle: Bath solution was used as vehicle and consisted of (mM): NaCl 137; KCl 4; $CaCl_2$ 1.8; $MgCl_2$ 1.0; D-glucose 10; HEPES 10; pH 7.4 with 1 N NaOH.
Reference substance: E-4031 was prepared in 100% deionized water. Aliquots were added to the bath solution to achieve a final perfusion concentration of 100 nM.

Methods:
The data were collected from a total of 8 cells. Groups of cells were treated with vehicle, reference substance, or the test article, as follows:

Vehicle group:	Bath solution, n=4
Test article group:	5 μM CVT-3146, n=4
Reference group:	100 nM E-4031, n=8 (the 4 vehicle cells and the 4 test article cells)

The membrane voltage was depolarized from a holding voltage of -80 mV to a test voltage of +20 mV for 4.8 s, repolarized to -50 mV for 5 s and then back to the holding voltage. After the voltage protocol was run for a minimum of 10 times at a 15-s interval, CVT-3146 was perfused through the bath and allowed to equilibrate for ~15 min. Preliminary results showed that a 15-min exposure to CVT-3146 at 5 μM produced an effect similar to that produced in the vehicle-treated group. The reference substance (E-4031, 100 nM) was applied after CVT-3146 treatment to verify that the current generated could be inhibited. The no-effect result for CVT-1346 was obtained in four cells. The effect of the vehicle was examined in four cells. The reference substance, E-4031, was applied to the 4 test article-treated cells as well as the 4 vehicle-treated cells and the hERG currents were recorded for ~10 min.

Results:
In vehicle-treated cells, perfusion with bath solution for ~15 min produced a mean decrease of 17.2% in tail current, which according to the sponsor, was in the range as expected for current rundown. Exposure of cells to 5 μM CVT-3146 for ~15 min produced a mean decrease in hERG tail current of 15.5%. Statistical analysis showed that there was no significant difference between the vehicle- and test CVT-1346-treated groups. E-4031 at 100 nM inhibited hERG tail current; with a mean decrease of 93.3% in all cells tested.

Report's conclusions:
Treatment with 5 μM CVT-3146 for ~15 min produced no inhibition of HERG tail current compared with the vehicle-treated group, in HEK293 cells stably transfected with hERG cDNA. The reference substance, E-4031, inhibited hERG tail current consistent with its expected pharmacological action.

Reviewer's comments:
Only one concentration of the test item was tested. At least 3 concentration levels should have been tested. In addition, 4 cells were used for the test item and the positive control: 6 cells per concentration are usually recommended. IC_{50} was not determined. Based on the results of this study, the IC_{50} for CVT-3146 is higher than 5 μM.

Study CVT3146.118-P: CVT-3146: Effects on Cardiac Action Potential Parameters in Isolated Canine Purkinje Fibers.

Sponsor study no.:	CVT3146.118-P
Conducting laboratory:	[redacted]
Study initiation date:	August 24, 2005
GLP compliance:	Yes (x) No ()
QA report:	Yes
Drug, lot#, and % purity:	CVT-3146 monohydrate, lot#0406CV301, [redacted]

The effects of various concentrations of CVT-3146 on cardiac action potential were investigated after tissue exposure for 20 minutes or until steady state at each concentration

Test article: CVT-3146 was dissolved in physiological salt solution (PSS) to achieve a starting solution of 10 μM, which was further diluted in PSS to give additional solutions of 0.05, 0.1, and 1 μM.
Reference substance: E-4031 in deionized water

Methods:
The Purkinje fibers from male Beagle dogs (n=6) were electrically stimulated at 1 Hz and impaled with 3 M KCl-filled glass microelectrodes (resistance range: 20-55MΩ) to record action potential.

The fibers were exposed sequentially to each concentration of treatment (or vehicle) for ~20 min or until steady-state, starting with the lowest concentration in a time-matched manner. At the highest concentration of test article, the effects on maximum rate of depolarization (MRD) were also investigated at 3 Hz. At the end of each experiment, the fibers were exposed to 30 nM E-4031 for 30 min (steady-state), to confirm the sensitivity of the test system.

The following parameters were measured at 0.5 and 1 Hz: action potential duration at 60% and 90% repolarization (APD_{60} and APD_{90}), triangulation (APD_{40} to APD_{60} duration), maximum rate of depolarization (MRD), upstroke amplitude (UA), and resting membrane potential (RMP).

Statistical analysis was carried out in two different ways: 1) comparison between the baseline values in each treatment group; and 2) comparison between treatment groups (vehicle and CVT-3146) in a time-matched fashion.

Results:
Baseline action potential parameters were similar between 0.5 and 1 Hz. When compared to their respective baseline values, CVT-3146 and vehicle did not affect the action potential parameters.

- At 1 Hz, exposure to concentrations of 0.1, 1, and 10 μM CVT-3146 had no effect on action potential parameters compared to vehicle. The only effect seen was a slight 4% prolongation of APD_{60} at 0.05 μM.
- At 0.5 Hz, exposure to 0.05-10 μM CVT-3146 had no effect on action potential parameters compared to vehicle, with the exception of a 7.6% APD_{60} prolongation, and 4.7% APD_{90} prolongation at 1 μM.
- When stimulation frequency was increased from 1 to 3 Hz, a decrease in MRD was observed in vehicle (5.4%) and in 10 μM (1.9%) groups but percentage changes were not significantly different between the two groups. According to the sponsor, this finding suggests that CVT-3146, at concentrations up to 10 μM, does not block cardiac sodium channels.
- CVT-3146 had no significant effect on triangulation (APD_{40} to APD_{90} duration).

The effects of positive control E-4031 are summarized in the following table: the effect was evaluated at the end of each experiment evaluating vehicle (column #2) and CVT3146 (column #3).

		% Increase Vehicle treated fibers	% Increase CVT3146 treated fibers
ADP_{60}	**1 Hz**	34.3	31.8
	0.5 Hz	42.7	46.4
ADP_{90}	**1 Hz**	33.3	30.8
	0.5 Hz	41.6	45.5
Triangulation	**1 Hz**	56.4	44.7
	0.5 Hz	72.0	68.3

The expected inverse frequency-dependence and the effect of E-4031 on triangulation indicate that all fibers were responsive to I_{Kr} blockers.

Conclusions
No significant test article-related effects on action potential parameters measured (RMP, UA, MRD, APD_{60}, APD_{90}, and triangulation) were observed in the presence of 0.05, 0.1, 1, and 10 µM concentrations of CVT-3146.

Reviewer's comments:
Based on the results of this study, concentrations of 0.05, 0.1, 1, and 10 µM CVT-3146 have no significant effect on action potential (APD_{60}, APD_{90}), RMP, UA, MRD, and triangulation, as measured in dog Purkinje fibers.

Pulmonary effects:

Study CVT3146.122-P: Pulmonary assessment of CVT-3146 in the anesthetized rat.

Sponsor study no.: CVT3146.122-P
Conducting laboratory: [redacted]

Date of initiation: 18 Oct 2005
GLP compliance: Yes (X) No () [COA was not GLP)
QA report: Yes
Drug, lot#, and % purity: CVT-3146, 0.08mg/mL, lot#803604, [redacted]

The purpose of this study was to evaluate the potential effects of CVT-1346 on pulmonary function in the rat.

Animal species/strain/sex per dose/weight/age: Rat/Sprague-Dawley/4males/dose/251 to 327 g/10 weeks old.

Methods:
The animals were assigned to 3 groups. After ~17-21 hours fasting period, animals received an intravenous bolus injection of the vehicle or CVT-3146 as follows:

Group # (4♂/group)	Treatment	Dose (µg/kg)	Volume (mL/kg)	Concentration (µg/mL)	HDM*
1	Vehicle	0	2.5	0	
2	CVT-3146	80	1.0	80	1.6 X
3	CVT-3146	200	2.5	80	4X

*HDM: human dose multiple based on a body surface area comparison for a clinical dose of 400 µg, equivalent to 296 µg/m^2 for a 50 kg adult.
The reviewer used a default adult weight of 50 because the clinical dose of 400 µg is not weight adjusted hence the need for a conservative weight of 50 kg adult to calculate the dose multiples.

Each rat was initially anesthetized using 1.5 g/kg of urethane. Catheters were placed in the esophagus to measure esophageal pressure and in the trachea to facilitate spontaneous

breathing while pulmonary function was assessed in a whole body plethysmograph. The animals were allowed to stabilize for a minimum of 5 min prior to measurement of the parameters. The following parameters were measured every minute for the first 5 min and every 5 min thereafter for 30 min: airway resistance, dynamic lung compliance, respiratory rate (breaths/min), tidal volume, and minute volume. All animals were euthanized without exsanguination at the end of study.

Statistical analysis:
Individual values of airway resistance, dynamic lung compliance, respiratory rate, tidal volume, and minute volume for the test article treated group were compared to the vehicle controls using ANOVA followed by Bonferroni Multiple Comparison Test (Systat). Differences with p values ≤ 0.05 were considered statistically significant.

Results:
Vehicle and a dose of 80 µg/kg test article had no significant effects on airway resistance, dynamic lung compliance, respiratory rate, tidal volume, and minute volume. A small and transient increase of 12% was observed in respiratory rate at 2 min post injection. At 200 µg/kg, immediately post-dose (1-5 min), respiratory rate was significantly increased by up to 28%; there was no significant effect on airway resistance, dynamic lung compliance, tidal volume, and minute volume.

Reviewer's comments:
The vehicle used was designated as placebo formulation. However, the formulation was not provided. At 200 µg/kg, the respiratory rate was significantly increased by up to 28%, immediately post-dose (1-5 min). The 12% increase in respiratory rate observed at 80 µg/kg was not statistically significant. Under the conditions of this study, the NOAEL for pulmonary functions was established in anesthetized rat at 80 µg/kg (~1.6 XHD).

Renal effects: Not conducted

Gastrointestinal effects: Not conducted

Abuse liability: Not applicable

Other: None

2.6.2.5 Pharmacodynamic drug interactions

Study CVT3146.130-P: Effects of caffeine on regadenoson-induced coronary vasodilation and changes in hemodynamics in conscious dogs (Study site:

Study objective:

Caffeine is a non selective adenosine receptor antagonist of all 4 subtypes of adenosine receptors. It was reported to antagonize the coronary vasodilatation caused by dipyramidole, therefore resulting in false negative in patients imaging. The present study was designed to evaluate the effects of caffeine on the coronary vasodilation and changes in hemodynamics caused by regadenoson in conscious dogs.

Study design:
Mongrel dogs (n=16) were instrumented: 9 dogs for measurement of blood pressure, coronary blood flow (n=9), and left ventricular pressure (LV dP/dt), and 7 dogs for pharmacokinetics.

Regadenoson was i.v. injected at 5 μg/kg 45 min before and 45 min after caffeine. CBF, MAP, HR, left ventricular systolic pressure (LVSP), and maximal rate of increase in LVP (LV dP/dt_{Max}) were recorded continuously. Peak CBF and the duration of time that CBF was increased to more than 2 fold of baseline were used as indices of drug-induced coronary vasodilation.

In the PK study, each dog received a total of 3 i.v. injection (1-3 min) of caffeine on different days, at 2, 4, or 10 mg/kg. MAP and HR were recorded continuously for 120 min and blood was collected at 2.5, 5, 15, 30, 60, 90, and 120 min. Plasma caffeine concentrations were determined to be relatively stable at 45 min post dosing.

Results:
Regadenoson (5 μg/kg) caused a significant increase in CBF, lasting ~ 10 min, with a peak of ~191 mL/min from a baseline of ~34 mL/min. Regadenoson caused a decrease in MAP (15%) and LVSP (9%), and an increase in HR (114%) and in LV dP/dt_{Max} (65%).

- Two mg/kg caffeine did not cause significant changes in MAP and HR, 4 mg/kg caffeine resulted in a significant increase in MAP (~12 mmHg at 2.5 and 5 min) and no change in HR; 10 mg/kg caused an increase in MAP (+5-9 mmHg at 2.5, 5, and 15 min), and a decrease in HR by 16-24 bpm at 30 to 120min post dose.
- The decrease in MAP was unaffected by 1 and 2 mg/kg caffeine but was no longer significant at 4 mg/kg caffeine (2±5% decrease from a baseline value of 112 mmHg). Ten mg/kg caffeine caused a 9 % increase in MAP from baseline value.
- Regadenoson-induced HR increase was further accentuated by 1 mg/kg caffeine (peak at 124%), but attenuated by higher doses in a dose-dependent manner (109, 79, and 74% from baseline at 2, 4, and 10 mg/kg respectively).
- Regadenoson-induced decrease in LVSP was not markedly affected by 1 and 2 mg/kg caffeine. The decrease was not significant in presence of 4 mg/kg (1±5 decrease from control). At 10 mg/kg caffeine, there was an 11% increase in LVSP.
- The effects of caffeine on regadenoson induced LV dP/dt_{Max} were inconsistent. At 1 mg/kg caffeine, the increase in LV dP/dt_{Max} was slightly greater and in the presence of 2 and 4 mg/kg caffeine, the increase was slightly attenuated. No remarkable effect was observed at 10 mg/kg caffeine.

Caffeine administration at 1, 2, 4, or 10 mg/kg resulted in plasma concentrations of 5, 10, 18, and 52 μM, respectively, at 45 min post dose. These injections did not cause significant changes in regadenoson-induced CBF peak increases (2, -0.7, -16, and -13 % respectively), but significantly lowered the duration of the 2-fold regadenoson-induced baseline CBF by 17, 48, 62, and 82% from control, respectively. The regadenoson-increased CBF remained at ≥2 fold baseline levels for ≥ 3 min in the presence of 1, 2, and 4 mg/kg caffeine.

Regadenoson peak plasma concentrations were not reduced by caffeine, and plasma caffeine remained at relatively steady levels from the time of pre-injection of regadenoson to 30 min following the second injection of regadenoson.

The second bolus injection of regadenoson (90 min apart) resulted in an identical coronary vasodilation.

Conclusions:
Doses of 1 to 10 mg/kg caffeine:

1) Did not alter baseline CBF and hemodynamics at 45 min, when caffeine plasma concentration was as high as 52 μM.
2) Did not significantly reduce the regadenoson-induced peak increases in CBF.
3) Caused a dose-dependent decrease in the duration of the regadenoson-induced coronary vasodilation; however, CBF still remained at 2-fold of baseline levels for ≥ 3 min in the presence of caffeine at 1, 2 or 4 mg/kg, and 4) blunted the regadenoson-induced sinus tachycardia and hypotension

Reviewer's comments:
This study indicates that caffeine may potentially affect the duration of increased CBF during MPI, hence the efficacy of imaging during MPI. This was an expected result in view of the affinity of caffeine for A_{2A} Ado receptors. Caffeine half-life is 5 hours in humans and 6 hours in dogs and according to the sponsor, a dose of 1, 2, and 4 mg/kg caffeine is equivalent to consumption of 1 to 2 cups of coffee. However, extrapolation to humans requires more accurate human PK evaluation of caffeine following oral consumption in order to extrapolate the results of dogs to humans.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Following a single i.v. dose administration of regadenoson, elimination $T_{1/2}$ was 18-24 min in rats, 16-32 min in dogs, and 37-60 min in rabbits. In rats, 90% of the drug was eliminated in 24 hours (55% in feces, 37% in urine). In dogs, 90% of the drug was eliminated in 48 hours. Plasma protein binding was 10-17% in rats and dogs. Following 7 and 28 day repeated dose i.v. administration, the toxicokinetics in rats and dogs were similar with peak at 2 min post dose, plasma levels proportional to administered dose and comparable between males and females. No accumulation of regadenoson was observed in rats or dogs following daily doses of up to 200 µg/kg/day for up to 28 days.

In a biodistribution study conducted in albino and pigmented rats, the highest concentrations of regadenoson were observed at 30 min post-dose in urinary bladder, bile duct, kidneys, and intestines and in addition in dorsal nerve roots in pigmented rats. In albino rats, significant concentrations were found in the skin up to 72 hr and in the lungs up to 120 h post dose, whereas in pigmented rats, radioactivity remained quantifiable up to 120 h post dose in the lungs, uveal tract and pigmented skin. The calculated half $life_{(24\text{-}120\ h)}$ of total radioactivity in the pigmented eye was approximately 7 days but was not provided for albino eye. Absorbance spectrum analysis of regadenoson identified 3 absorbance maxima below the UV-visible range; however, some absorbance was noted above 290 nm (290-320nm), which may potentially cause adverse photoeffects. However, regadenoson is intended for single administration, and no further evaluation is needed.

The metabolism of regadenoson was evaluated in plasma, urine, and bile in rats and dogs in vivo. No metabolites were detected in dogs, whereas in rats, three minor metabolites were found in urine (12% activity), which each represented 2% of total activity.

2.6.4.2 Methods of Analysis

[See under individual study reviews]

2.6.4.3 Absorption

Lexiscan is intended for intravenous administration; absorption is not applicable. However, the sponsor submitted the two following studies under "Absorption" section. They will be reviewed under this section to facilitate their location in the submission.

The pharmacokinetics of regadenoson were assessed in rats after single intravenous doses of 2, 20 or 200 µg/kg and in rabbits after single intravenous doses of 100, 300, and 500 µg/kg. In addition, the toxicokinetics of CVT-3146 were studied as part of the 7-day and 28-day repeated IV dose toxicology studies in rats and dogs (described in the Toxicology section and reviewed by Dr. Proakis).

Pharmacokinetics of CVT-3146 in Beagle Dogs after a Single IV Dose (Reviewed by Dr. Anthony Proakis)

The pharmacokinetics of CVT-3146 were determined in male and female Beagle dogs following a single intravenous dose of 2, 20 or 200 ug/kg. [*This was the first dose given on day 1 of a 7-day repeated dose study. Additional pharmacokinetic results from subsequent dosing and blood sampling is described under the respective repeated-dose toxicity study.*] Groups of 5 male and 5 female dogs were given single bolus IV doses via a cephalic vein and venous blood (via a jugular vein) was collected at various times (up to 180 min after dosing) for measurement of plasma concentration of CVT-3146.

The pharmacokinetics of CVT-3146 was characterized by a rapid clearance, moderate volume of distribution and a short terminal half-life (Table 5). The results from males and females were combined since no differences between male and female dogs were observed.

Table 5. Pharmacokinetics of CVT-3146 in Dogs after A Single IV Dose

Parameter	Dose, ug/kg IV		
	2	20	200
$AUC_{(0-\infty)}$, ng.hr/ml	ND	7.56	91.1
CL_p, ml/min/kg	ND	46.3	37.8
Vd, L/kg	ND	1.02	2.02
Distribution $t_{1/2}$, min	ND	5.35	6.54
Elimination $t_{1/2}$, min	ND	16.1	37.2

Values are the mean from combined 5 male and 5 female dogs
ND= No determined due to insufficient data (below level of measurement)

Study CVT3146.026-R: Pharmacokinetics of CVT-1346 in female rabbits after a single intravenous dose of CVT-3146 at 100, 300, or 500 µg/kg (CV Therapeutics, Feb 2000)

Study design

Pharmacokinetics of CVT-3146 was determined following a bolus intravenous dose at 100, 300 or 500 µg/kg to female New Zealand White rabbits. CVT-3146 drug product (lot #803604) was formulated in vehicle containing 15% propylene glycol in 0.1M phosphate buffer, containing 0.1% disodium edentate dehydrate, pH 7.0. Blood samples (1mL) were collected by venipuncture at pre-dose, 2, 5, 15, 30 min, and 1, 2, 4, 8, and 24 hours post-dose. Concentrations of CVT-3146 in plasma were determined by an LC/MS/MS assay.

Results

Following i.v. administration, plasma concentration of CVT-3146 decreased in an apparent biphasic manner consisting of a relatively rapid initial distribution phase and a slower elimination phase. AUC increased proportionally to the dose levels, whereas there was a less than proportional increase of C_0 attributed by the sponsor to the longer injection time at the highest doses. Values of CL_p, Vd_β and elimination $t_{1/2}$ of CVT-3146 in female rabbits did not change significantly between the dose levels studied, and are presented in the following table.

Dose (µg/kg)	100	300	500
Dose volume (mL/kg)	1.25	3.75	6.25
C_0 (ng/mL)	1550 ± 318	4270 ± 308	5780 ± 1740
C_{2min} (ng/mL)	927 ± 1 31	2670 ± 189	4040 ± 1105
AUC(0-∞) (ng.hr/mL)	199 ± 27.3	592 ± 71.8	1290 ± 602
CL_p (mL/min/kg)	8.51 ± 1.30	8.55 ± 1.03	7.46 ± 2.97
VD_β (L/kg)	0.456 ± 0.073	0.551 ± 0.129	0.552 ± 0.076
Elimination $t_{1/2}$ (min)	37.2 ± 2.54	45.5 ± 8.15	60.9 ± 36.1

Mean ± SD (n=4);
C_0: plasma concentration extrapolated to time zero.

Reviewer's comments:
Plasma concentrations at T_0 and T_{2min} appear to be higher than theoretically possible maximum concentrations at all doses. They are also much higher than those found in rats and dogs at similar dose levels. It is of note that the PK study in rabbits was conducted using the propylene glycol formulation, whereas the methylboronic acid formulation was used in the rats and dogs PK studies.

Study CVT3146.032-P: Pharmacokinetics of CVT-1346 in male rats after a single intravenous dose of CVT-3146 at 2, 20, or 200 µg/kg (CV Therapeutics, 02/2000)

Study design:
Pharmacokinetics of CVT-3146 was determined following a bolus intravenous dose at 2, 20, or 200 µg/kg to male rats (5/dose). CVT-3146 solution (lot #MHR-227-35D) was prepared in 20 mM carbonate buffer, pH 9.3, containing 0.1% methylboronic acid. Blood samples (0.5mL) were collected from carotid artery at 2, 5, 10, 15, 20, 30, 60, and 120 min post-dose. Concentrations of CVT-3146 in plasma were determined by an LC/MS/MS assay.

Results:
Following IV administration of 20 and 200 µg/kg, plasma concentrations decreased exponentially in a biphasic manner. AUC values increased proportionally to the dose. CVT-3146 dosing was characterized by rapid clearance, moderate volume of distribution and short terminal life.

Non-compartmental PK parameters are summarized in the following table

Dose (µg/kg)	2	20	200
Number of animals	5	5	4
C_{2min} (ng/mL)[b]	1.85 ± 0.41[a]	17.9 ± 5.12	147 ± 76.8
AUC(0-∞) (ng.hr/mL)	Not determined due to insufficient data	4.55 ± 1.63	38.0 ± 8.92
CL_p (mL/min/kg)		82.0 ± 32.0	91.8 ± 23.8
VD_β (L/kg)		1.87 ± 0.33	3.38 ± 1.81
Distribution $t_{1/2}$ (min)		6.42 ± 1.48	6.12 ± 1.27
Elimination $t_{1/2}$ (min)		17.7 ± 7.28	24.5 ± 6.70

[a]Mean±SD; [b]Concentration at 2 min

Based on the results of this study, the pharmacokinetics of CVT-3146 in rats appeared to be linear between the intravenous doses of 2, 20, and 200 µg/kg.

2.6.4.4 Distribution

Study CVT3146.001-N: Binding of CVT-3146 to rat and dog plasma in vitro (by CV Therapeutics, July 2003)

Study design
Binding of CVT-3146 to male Sprague-Dawley rat and male Beagle dog plasma was determined in vitro using an ultrafiltration method. [^{14}C]CVT-3146 at concentrations of 5, 25, 100 and 250 ng/mL was incubated with plasma pooled from 48 rats or with plasma pooled from 4 dogs, at 37°C for 30 minutes. After incubation, the unbound fraction of CVT-3146 in plasma was separated using an ultrafiltration technique and the radioactivity in plasma and filtrate was determined by liquid scintillation counting.

Results & Conclusions:
Mean values of CVT-3146 binding to rat and dog plasma were 9.59-16.9% and 12.2-15.4%, respectively, and were independent of concentrations between 5 and 250 ng/mL, which, according to the sponsor, represent the approximate range of the plasma concentrations in most toxicology studies. The extent of CVT-3146 binding in these two animal species is slightly lower than that observed in separate study in human plasma (22.2-24.9%).

Study CVT314.016-R: The disposition and tissue distribution of total radioactivity in the rat following intravenous administration of [^{14}C]-CVT3146

Conducting laboratory: [redacted]
Study date: January 30, 2003- May 13, 2003
Quality assurance statement: Yes
GLP: UK OECD principles
Formulation: [^{14}C]-CVT-3146 in 8% propylene glycol, 0.1M phosphate buffer, pH7.0

This study evaluated the excretion, distribution, and plasma kinetics of total radioactivity following intravenous administration of [^{14}C]-CVT-3146 to Sprague-Dawley and Lister Hooded rats at 250 µg/kg in 2.5 mL/kg (~5 times HD/50 kg adult)

1. **Excretion evaluation (n=5 male albino rats)**

Urine was collected at 0-6, 6-24 then at 24 hr intervals to 120 hrs post dose. Feces were collected at 24 h intervals to 120 h post dose. At the time of each feces collection, cages were washed and the wash retained for analysis of total radioactivity. Expired air was collected at 24 h intervals to 48 h post dose. At 120 h post dose the rats were sacrificed and blood sample (10 ml) was collected. The level of total radioactivity was determined in each organ and tissue collected.

Results:
Following i.v. administration of CVT-3146, 88.9% of the administered radioactivity was excreted in the first 24 h post dose. The main route was via the feces, with ~54.5% recovered over 120 hr collection period. Urinary excretion accounted for ~36.9% suggesting that biliary elimination plays a major role in the excretion. An additional 3.5% was recovered in the cage. Including cage wash and carcass, ~95.7% of the radioactivity was recovered in the 5 day collection period.

2. **Tissue distribution evaluation (n= 12 male albino rats)**

The rats were sacrificed at 30 min, 4, 24, and 72 hr post dose. Total radioactivity was measured in organs and body fluids.

Results:
Highest concentrations of total radioactivity were seen at the first sampling time (30 min post dose), and were found in tissues or organs associated with elimination of the compound; urinary bladder, bile duct, kidneys and intestines.

Concentrations of total radioactivity in the liver, bone marrow, skin, muscle, Harderian gland, pancreas, thyroid, salivary gland and heart contained means of 110, 90, 89, 82, 66, 66, 53 and 48 ng equiv/g, respectively, at 30 min post dose. All other tissues contained concentrations of total radioactivity lower than that seen in plasma at this time (45 ng equiv/g).

Significant concentrations were found in the skin up to 72 hr and in the lungs up to 120 h post dose.

3. **Plasma and whole blood kinetics were evaluated in 21 male albino rats.**

The rats were sacrificed (3/time point) at 2, 5, 10, 15 and 30 min and 2 h post dose, and blood (5-10 ml) was obtained by cardiac puncture.

Results:
Concentrations of total radioactivity in plasma decreased quickly from 261 ng equiv/mL at 2 min post dose, the first sampling time, to 4 ng equiv/mL at 2 h post dose, indicating a rapid elimination of total radioactivity associated with [^{14}C]-CVT-3146. Concentrations of total radioactivity in blood were approximately half those observed in plasma at each time, suggesting that radioactivity was predominately associated with the plasma fraction.

4. **Tissue distribution (n= 6 male pigmented rats)**

One rat /time point was sacrificed at 30 min, 4, 8, 24, 72 and 120 h post dose. Prior to sacrifice, whole blood (0.5 ml) was collected from the tail vein for radioactivity determination. Radioactivity was determined in the right eye and in all major organs and tissues.

Results:

Clinical signs observed in all pigmented animals but not seen in albino rats included: being subdued, walking with a rolling gait, irregular or slow and deep respiration and cold extremities. No signs were observed 2 hours post-dose.

At 30 min post dose, the highest concentrations of total radioactivity were noted in the kidney cortex, kidney (whole) and kidney medulla. Lower but significant concentrations were also noted in the dorsal nerve roots, small intestine wall, liver, aorta wall, Harderian gland, bladder, pancreas, uveal tract, skin (pigmented and albino), lungs, thymus, lymph node, pineal body and skeletal muscle. High concentrations were also noted in the pigmented skin, lungs, adrenal medulla, adrenal cortex, adrenal (whole), Harderian gland and albino skin at 8 hr post dose. Radioactivity remained quantifiable up to 120 h post dose in the lungs, uveal tract and pigmented skin.

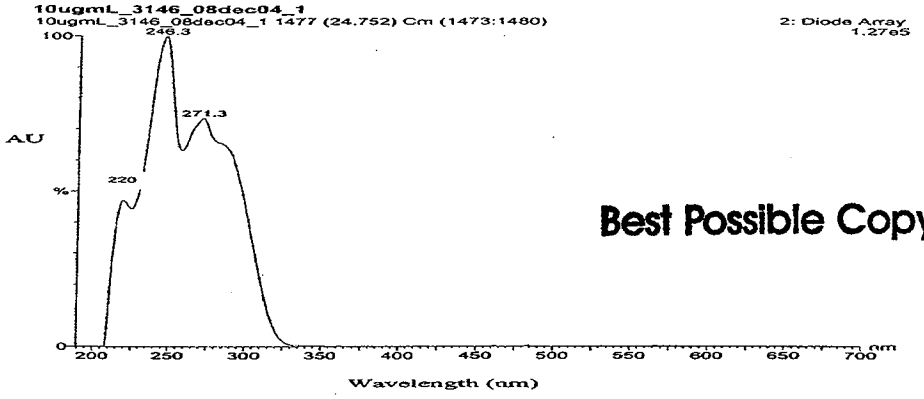
Comparison of the concentrations of total radioactivity in the albino eye and pigmented eye, as well as the albino skin and pigmented skin show that there is evidence for association of the radioactivity from [^{14}C]-CVT-3146 with melanin. The calculated half life$_{(24\text{-}120\ h)}$ of total radioactivity in the pigmented eye is approximately 7 days, but was not provided for the albino eye.

Reviewer's comments:
In pigmented rats relative to albino rats, regadenoson concentration (radioactivity) remained quantifiable up to 120 h post dose in the lungs, uveal tract and pigmented skin, with the calculated half life$_{(24\text{-}120\ h)}$ of total radioactivity in the pigmented eye approximately 7 days. Absorbance spectrum analysis of regadenoson (see below study CVT3146.046-N) identified 3 absorbance maxima below the UV-visible range; however, the absorbance noted above 290 nm (290-320nm) may potentially cause adverse photoeffects. Because the administration of regadenoson is intended as a single dose administration, no further phototoxicity evaluation is needed.

Study CVT3146.046-N: Absorbance spectrum of CVT-3146 (Conducted by CV Therapeutics, Inc., January 24, 2006, CVT-3146 lot 0 02011095051)

The ultraviolet (UV) and visible (VIS) absorbance spectrum of CVT-3146 between the wavelength of 190–700 nm was obtained by HPLC interfaced with a photodiode array (PDA) detector and a mass spectrometer. The identity of CVT-3146 was confirmed based on mass spectrometric analysis. CVT-3146 exhibited three absorbance maxima (λ_{max}) in the UV region at 220, 246.3, and 271.3 nm, with no absorbance maxima in the UV visible range of 290 – 700 nm. Some absorbance above 290 nm (290-320 nm) was observed due to the descending slope of the absorbance maximum at 271.3 nm as shown in the following figure:

Figure 3 Absorbance Spectrum of CVT-3146 (Retention Time = 24.72 min) Scanned at Wavelengths of 190-700 nm



2.6.4.5 Metabolism

Study CVT3146.009-MET: Metabolic profiles of CVT-3146 following intravenous administration of a single 250 µg/kg dose of [^{14}C]CVT-3146 to intact and bile duct-cannulated rats

Conducting laboratory: [redacted] (In life and total radioactivity measurement) and CV Therapeutics (Metabolite characterization)
Study date: January 2003- September 2003

The in vivo metabolism of CVT-3146 was investigated in rats. Plasma, urine, and bile samples collected following intravenous administration of 250 µg/kg dose of [^{14}C]CVT-3146 to intact and bile duct-cannulated (BDC) Sprague- Dawley rats were subjected to metabolite characterization using radiochromatographic and tandem mass spectrometric analyses following HPLC separation (LC/MS/MS).

Results
Radioactivity recovered in rat feces and urine was 54.5% and 36.9%, respectively. The radioactivity recovered in feces was due to biliary excretion, as suggested by studies with the BDC rat.

HPLC-radiochromatographic analyses of the plasma, urine and bile samples show that CVT-3146 was the major radioactive component, respectively accounting for greater than 93%, 87% and 95%, of the total radioactivity. Based on radiochromatographic and mass spectral analyses, no substantial levels of metabolites were detected in the plasma and bile. In urine, three minor metabolites, one unknown, a de-ribosylated derivative (CVT-8451) and a putative glucose conjugate of CVT-3146, were identified. According to the sponsor, the exact structural designation of the glucose conjugate remains to be elucidated. These two minor metabolites combined for less than 12% of the total radioactivity in urine and each represented 2% or less of the dose administered to rats. In

humans receiving CVT-3146, an average of 57% of the administered dose was excreted unchanged in urine.

Study CVT3146.011-MET: Metabolic profiles of CVT-3146 following intravenous administration of a single 200 µg/kg dose of [^{14}C]CVT-3146 to intact and bile duct-cannulated dogs

Conducting laboratory: [redacted], (In life and total radioactivity measurement) and CV Therapeutics (Metabolite characterization)
Study date: March 2004- October 2005

The in vivo metabolism of CVT-3146 was investigated in dogs. Plasma, urine, and bile samples collected following intravenous administration of 200 µg/kg dose of [^{14}C]CVT-3146 to intact and bile duct-cannulated (BDC) beagle dogs were subjected to extensive metabolite characterization using radiochromatographic and tandem mass spectrometric analyses following HPLC separation (LC/MS/MS).

Results:
Radioactivity recovered in dog feces and urine for 5 days was respectively 53.8% and 36.5%. Approximately 90% of the administered radioactivity was excreted in the first 48 hr after dosing. BDC dogs, receiving similar dose of [^{14}C]CVT-3146, excreted 43.6%, 30.6% and 14.1% in urine, bile, and feces, respectively. Most of the biliary excretion was completed within 8 hrs post-dose.

HPLC-radiochromatographic analyses of the plasma extracts, pooled urine, and bile samples showed that CVT-3146 was the major radioactive component accounting for greater than 85%, 95% and 96% of radioactivity respectively. Based on this study, no substantial levels of metabolites were detected in the plasma, urine and bile indicating that CVT-3146 was metabolically stable in dogs in vivo. It is of note that in humans receiving CVT-3146, on average 57% of the administered dose was excreted unchanged in urine. According to the sponsor, these results are also consistent with the in vitro observation that CVT-3146 was metabolically stable following incubation with dog liver microsomes.

2.6.4.6 Excretion

Study CVT3146-017-r: The biliary elimination of total radioactivity in the rat following intravenous administration of [^{14}C]-CVT-3146

Following i.v. administration of [^{14}C]-CVT-3146 to rats (n=5) at 250 µg/kg, 97.4% of the administered radioactivity was excreted in the first 24 hr post dose (urine: ~57.2%, biliary: ~31.2%, feces: ~8.3%). In the bile, the majority of the radioactivity was recovered in the first 2 hr post dose. The ratio urine excretion/feces excretion was

inverted in Study CVT314.016-R with the main route being feces. However the % excretion ranges were similar between urine and feces excretion in the two studies.

Study CVT3146.024-R: Disposition of [^{14}C]-CVT-3146 in male Beagle dogs after intravenous administration (GLP)

Following i.v. administration of [^{14}C]–CVT-3146 IV to the dogs (n=4) at 0.2 mg/kg mean total recoveries through 192 hr post-dose were 36.53% in urine, 53.77% in feces, and 4.11% in cage debris samples. Approximately 90% of the dose was recovered within 48 hr. Plasma concentrations were consistently greater than corresponding blood concentrations, with mean blood:plasma concentration ratios ranging from ~0.4 to 0.6.

Study CVT3146.025-R: Disposition of [^{14}C]-CVT-3146 in male bile duct-cannulated Beagle dogs after intravenous administration (GLP)

Following i.v. administration of [^{14}C]-CVT-3146 to 3 male bile duct-cannulated (BDC) beagle dogs at 0.2 mg/kg recovery of the administered radioactive dose through 168 hr (96 hr for bile) were 30.55% in bile, 43.64% in urine, 14.09% in feces, and 2.16% in cage debris samples. Nearly all biliary radioactivity was recovered within 4 hr of dosing. More than 80% of the activity was recovered within 48 hr, and elimination was nearly complete by 72 hr after dosing.

2.6.4.7 Pharmacokinetic drug interactions

Not conducted

2.6.4.8 Other Pharmacokinetic Studies

None

2.6.4.9 Discussion and Conclusions

2.6.4.10 Tables and figures to include comparative TK summary

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Comparison of single dose IV pharmacokinetics of regadenoson in rats, dogs, and rabbits to humans (As provided by the sponsor)

Species	Rat	Dog	Rabbit
Study	**CVT3146.032-P**	**CVT3146.015-T**	**CVT3146.026-R**
Dose (μg/kg)	200	200	500
$C_{2\ min}$ (ng/mL)	147	411	4040
$AUC_{(0-\infty)}$ (ng.h/mL)	38	91	1290
CL_p (mL/min/kg)	92	38	7
Vd_β (L/kg)	3	2	0.6
Distribution $t_{1/2}$ (min)	6	7	Not determined
Elimination $t_{1/2}$ (min)	25	37	61
Comparison to Human Data[a] (400 μg)			
C_{max}		14–24 ng/mL	
$AUC_{0-\infty}$		12–28 ng·h/mL	

[a] From Clinical Studies CVT 5112 and CVT 5121 non-compartmental PK analysis (see Sections 2.7.2.2.2 and 2.7.2.2.3). Values presented are the ranges of the means of individual groups of subjects.

C_{max} = maximum plasma concentration measured between 1–5 min postdose

$C_{2\ min}$ = plasma concentration measured 2 min postdose

AUC = area under plasma concentration

CL_p = plasma clearance

Vd_β = volume of distribution

$t_{1/2}$ = half-life

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

Single dose toxicity

In studies conducted in rats and dogs using a methylboronic acid formulation, administration of single i.v. doses of up to 1500 μg/kg to rats (30 XHD) and up to 2400 μg/kg to dogs (160 XHD) elicited no mortality and no clinical signs of toxicity. Single i.v. doses of 20 to 2400 μg/kg administered to dogs caused dose-dependent decreases in MABP, non-dose-dependent increases in HR and changes in T-wave polarity.

Since most of the preclinical studies were conducted using a methylboronic acid (MBA) formulation, a single dose bridging toxicity study was conducted in rats to evaluate the safety of the propylene glycol (PG) clinical formulation intended for clinical use. Four groups of rats received i.v. administration of vehicle, 0.08, 0.2, or 0.8 mg/kg PG regadenoson (1.6, 4, and 16 XHD). As a comparator, an additional group was given 0.2 mg/kg regadenoson in MBA formulation. Red discolored urine was observed with vehicle and drug treated animals in the PG formulation groups. Most of the clinical, hematology, and clinical chemistry changes occurred on day 2 in the group treated with the MBA formulation. One female in the PG vehicle group and one female in the 0.8

mg/kg PG group had similar, chronic, focal liver lesions consisting of mineralization and fibrosis. However, in the 0.8 mg/kg group female, these changes were associated with coagulative necrosis. Increased incidence of minimal cardiomyopathy characterized by scattered foci of lymphocytes and macrophages associated with few or no necrotic myocytes was observed on day 2 in males at doses of 0.08, 0.2 and 0.8 mg/kg (1/5, 2/5, and 5/5 rats) and in females (2/5) at 0.8 mg/kg. Cardiomyopathy was also seen in males (3/5) administered 0.2 mg/kg of MBA regadenoson. No cardiomyopathy was noted on day 15 in any of the groups: one control female and one low dose male in the PG group had minimal myocardial vacuolation, and one female treated with 0.2 mg/kg MBA regadenoson had a focal myocardial chronic inflammation. The cardiomyopathy was determined to be regadenoson related.

Repeated dose toxicity
Studies in rats and dogs of 7 and 28 days duration at doses of 2, 20 or 200 µg/kg/day (0.04, 0.4, and 4 XHD, and 0.13, 1.3, and 13 XHD for rats and dogs respectively) were conducted to evaluate the toxicity from repeated administration of regadenoson in MBA formulation.

In rats, no adverse effects on survival and body weight were seen at these dose levels for up to 28 days. Decreased MCH (35%) was seen in treated females, and higher than control serum levels (up to 3 fold) of creatine kinase and lactate dehydrogenase as well as higher bilirubin, potassium and phosphorus were seen in high dose female rats after 7 days, but not after 28 days of treatment. Gross and microscopic findings were limited to minimal inflammation at the site of intravenous injection and lymphoid hyperplasia of mandibular or mesenteric lymph nodes in the high dose groups. In view of the observed clinical chemistry changes, NOAEL in rats was considered to be 20 µg/kg/day (0.4 XHD).

In dogs, no adverse effect on survival or body weight was seen after 7 or 28 days of treatment. ECG recording showed changes in T-wave morphology (T-wave inversion) and slight elevation and arch of the T-segment in high dose males and females at one hour post dosing in the 28 day and (to a lesser extent) 7 day studies. Gross and microscopic lesions were limited to hemorrhage and inflammation at the site of injection in both treated and control animals and did not appear to be related to treatment. The NOAEL in dogs was considered to be 20 µg/kg/day (1.3 XHD).

General toxicology:

2.6.6.2 Single-dose toxicity

Study 124-001: Single dose (MTD) study of CVT-1346 administered intravenously to Sprague-Dawley rats. (Reviewed by Dr. Anthony Proakis)

Study Facility: [redacted]. Study No.: 124-001 Study Dates: 10/07/99 – 10/13/99

GLP Compliance: Compliance with GLP regulations attested. QA Report: Yes

Animals: Female Sprague-Dawley rats (approx. 8 weeks old; 172-231 gm)

Drug Administration: CVT-3146 (Lot # 315-67-2) was dissolved in 10% PEG 400 aqueous vehicle and administered IV via a lateral tail vein.

Dose Levels: 0, 300, 600, 900, 1200 and 1500 ug/kg (3/sex/dose group)

Observations/Measurements: Animals were observed twice daily for mortality and clinical signs of toxicity. Body weights were recorded predose and at study termination approximately 24 hours after dosing. No necropsies were performed.

Results

Mortality and Clinical Signs
No animals died during the study and no treatment-related adverse effects were observed.

Body Weight
Body weights among CVT-3146 treated animals were comparable to those of vehicle control.

The single-dose no-adverse effect-level in rats was 1500 ug/kg, the highest dose tested.

Study 124-002: Single dose escalating (MTD) study of CVT-1346 administered intravenously to beagle dogs (Reviewed by Dr. Anthony Proakis)

Study Facility: [redacted] Study No.: 124-001 Study Dates: 10/07/99 – 10/30/99

GLP Compliance: Compliance with GLP regulations attested. QA Report: Yes

Animals: Two male Beagle dogs (14.88 & 14.92 kg)

Drug Administration: CVT-3146 (Lot # 315-67-2) was dissolved in 10% PEG 400 aqueous vehicle and administered IV via a cephalic vein.

Dose Levels: 20, 50, 150, 300, 600, 1200, 1800 and 2400 ug/kg (the same 2 dogs were used for each dose up to 600 ug/kg, 1 dog was tested with the 1200, 1800 and 2400 ug/kg doses; dose administration occurred on study days 1, 2, 3, 4, 7, 15, 16, and 18).

Observations/Measurements: Animals were observed twice daily after each dose for mortality and clinical signs of toxicity. Body weights were recorded predose and at study termination approximately 24 hours after dosing. ECGs were recorded predose and

continuously for 15 min after each dose. Blood pressure measurements were recorded via tail cuff method prior to each dose and once every 5-min for 15 min after each dose. No necropsies were performed.

Results

Mortality and Clinical Signs
No animals died during the study and there were no clinical signs of toxicity observed for 15-min post dose.

Body Weight
Body weights were not affected during the experimental period.

ECG, HR and Blood Pressure
In all treated dogs, there was a change in T-wave polarity usually involving a change from a positive T-wave in Lead II to a negative T-wave immediately post dose that stayed negative for the entire 15-min postdose period. The T-wave inversion appeared more prominent with the higher doses of CVT-3146.

CVT-3146 caused dose-dependent decreases (23% at 20 ug/kg to 59% at 2400 ug/kg) in mean arterial blood pressure accompanied by non-dose-dependent increases in heart rate.

The maximum tolerated single intravenous bolus dose of CVT-3146 in dogs was 2400 ug/kg.

Study 124-009: Single dose escalating (MTD) study of CVT-1346 administered intravenously to Beagle dogs

Four female beagle dogs were given single intravenous bolus doses of 0.03, 0.1, 0.3, 1.0, 3.0, 10, and 20 µg/kg of CVT-3146 and vehicle (0.1% methyl boronic acid in 20 mM sodium bicarbonate). The same dogs were used for each dose level; dose administration occurred on 8 consecutive days.

Body weights (recorded on Days 1, 15, and 18) and clinical signs (recorded prior to each dose and continuously for 15' post- dose) were unaffected by treatment.

Blood pressure measurements were determined via tail cuff method, pre-dose, immediately post-dose, and at 5, 10, and 15' post-dose, and ECGs were recorded pre-dose, and continuously for 15' post-dose.

There was a change in T-wave polarity that occurred after 3 µg/kg, which was longer lasting and more prominent at the higher doses. A positive T-wave in Lead II changed to a negative T-wave immediately post dose and stayed negative for the entire 15-min post-dose period. There was also a prominent elevation and arch of the ST segment in most dogs after doses $\geq$3 µg/kg.

MABP response to dosing with drug did not differ significantly from MABP response to dosing with vehicle (-15 mmHg after 20 µg/kg of CVT-3146 vs. –13 mmHg after vehicle). The heart rate was statistically higher after dosing with 20 µg/kg of drug compared with heart rate obtained after dosing with vehicle (+3 bpm post dose with vehicle vs. +66 bpm post dose with CVT-3146).

The RR interval was statistically decreased after dosing with 20 µg/kg of CVT-3146 compared to the RR interval after dosing with the vehicle. There were no statistical changes in the PR, QRS, or QT intervals after dosing with CVT-3146. There was a statistical difference in the QTc interval over time. Post-dose QTc was longer than pre-dose values. The post-dose values exceeded the pre-dose values by an average of 6 ms. Some atrial premature depolarizations were observed; the sponsor claims they are a common finding in dogs.

The single IV dose no-adverse-effect-level, based ECG changes, in dogs was 1 µg/kg.

Study CVT-3146.056-T: Single dose intravenous bridging toxicity study of the clinical formulation of CVT-3146 in Sprague-Dawley rats

Study Objective
This study was designed as a bridging study to provide safety information for the clinical formulation containing propylene glycol (PG) since the previous 7 and 28 day toxicity studies that included microscopic evaluation were conducted with the methylboronic acid formulation (MBA). The rat was selected for this bridging study because of the absence of any species differences in the previous toxicity studies. The highest dose level of the MBA formulation of test article used in the 28 day repeated dose study is also being used in this study for comparison.

Key study findings: Increased incidence of minimal cardiomyopathy was observed on day 2 in males treated with the PG formulation regadenoson at doses of 0.08, 0.2 and 0.8 mg/kg (1/5, 2/5, and 5/5 rats respectively) and in females (2/5) at 0.8 mg/kg. These doses are respectively 1.6, 4, and 16 times the proposed clinical dose, based on a body surface area comparison and assuming a 50 kg adult. Cardiomyopathy was also seen in males (3/5) administered 0.2 mg/kg of regadenoson in the MBA formulation (4 times the human dose). No cardiomyopathy was noted on day 15: one control female and one low dose male in the PG group had minimal myocardial vacuolation, and one female treated with 0.2 mg/kg MBA regadenoson had a focal myocardial chronic inflammation. The cardiomyopathy was determined to be regadenoson related.

Study no.:	CVT-3146.056-T
Volume #, and page #:	N/A (e-CTD)
Conducting laboratory and location:	[redacted]
Date of study initiation:	08-October-2007

GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: Test article 1 (PG formulation): CVT-3146 Injection, 902624 (Bulk drug product), 902438 (bulk packaged product), [redacted]
Test article 2 (MBA formulation): CVT-3146, 971-049, [redacted]

Methods

Doses: 0, 0.08, 0.2, and 0.8 mg/kg (Test article 1 PG formulation); 0, 0.2 mg/kg (Test article 2: MBA formulation)

The animals were administered the test articles according to the following table

Group # (10M+10F/group)[a]		*Dose level (mg/kg)*	*Dose volume (mL/kg)*	*Human Dose Multiple*[d]
1	Vehicle 1[b]	0	10.0	-
2	Test article 1	0.08	1.0	1.6 XHD
3	Test article 1	0.20	2.5	4 XHD
4	Test article 1	0.80	10.0	16 XHD
5	Vehicle 2[c]	0	1.0	-
6	Test article 2	0.20	1.0	4 XHD

[a]Five animals/sex/group were necropsied on Study Day 2. The remaining animals were necropsied on Study day 15
[b]Vehicle 1 = 15% w/w propylene glycol, 0.1% w/w edetate disodium dihydrate, 10.9 mg/mL dibasic sodium phosphate dihydrate, and 5.4 mg/mL monobasic sodium phosphate monohydrate
[c]Vehicle 2 = 0.1% (w/v) methyl boronic acid in 50 mM $NaHCO_3$, adjusted to pH 9 with 2 N NaOH solution, with 0.55% (w/v) sodium chloride to achieve isotonicity
[d]Human dose multiple based on a surface body area comparison for a 50 kg human adult administered a single IV dose of 400 µg regadenoson. Please note that the sponsor had used 60 kg for human adults, compared to a more conservative use of 50 kg routinely used by the Division

Species/strain: Out-bred albino rats/ [redacted]CD®(SD)
Number/sex/group or time point (main study): 10/sex/group
Route, formulation, volume, and infusion rate: Intravenous/ test article injection 1 (0.08 mg/mL) and test article 2 (0.2 mg/mL)/ 10, 1, 2.5 and 10 mL/kg for test article 1, and 1mL/kg for test article 2/ bolus injection into the lateral tail vein over two minutes
Satellite groups used for toxicokinetics: None
Age: 9 weeks old
Weight: 258-319 g (males); 171-216 g (females)
Sampling times: Blood samples for hematology and coagulation parameters and clinical chemistry were obtained from animals on necropsy days 2 and 15.

Observations and times:

Mortality/Moribundity: Twice daily (a.m. and p.m.)
Clinical signs: Recorded pretest, on study day 1 at 15 min, 30 min, 1, 2, 4, and 8 hr post-dose, and once daily thereafter
Body weights: Recorded pretest and on study days 1 (predose), 2 (for necropsied animals), 8, 14 (prior to fasting), and 15 (prior to necropsy).

Food consumption:	Measured for animals necropsied on day 15 for study days 1 to 8 and study days 8 to 14.
Ophthalmoscopy:	Not performed
EKG:	Not performed
Hematology:	Blood samples for hematology and coagulation parameters were collected on necropsy days 2 and 15.
Clinical chemistry:	Blood samples for clinical chemistry were collected on necropsy days 2 and 15 (food withheld overnight prior to collection)
Urinalysis:	Semi quantitative analysis was conducted on animals submitted to necropsy on study days 2 and 15. The animals were placed in metabolism cages for overnight urine collection. Feed was withheld during the collection period.
Gross pathology:	On study days 2 and 5, five animals/sex/group were euthanized and submitted for a complete necropsy examination.
Organ weights:	Prior to fixation, the following organs were weighed: adrenal gland, brain, heart, kidney, liver, lung, ovary, spleen, testis, and thymus. Paired organs were weighed together.
Histopathology:	Organs and tissues were examined in situ, dissected and fixed in 10% neutral buffered formalin (except for the testes which were fixed in Modified Davidson's fixative and the eyes with optic nerve which were fixed in Davidson's fixative. Histopathology was performed on all tissues from all animals and all gross lesions. Slides were stained with hematoxylin and eosin.

Adequate Battery: yes (x), no ( )
Peer review: yes ( ), no (x)

Statistical analysis was performed on body weights, feed consumption, hematology, coagulation, clinical chemistry parameters, and organ weights.

To determine the appropriate statistical test, each data set was subjected to a statistical decision tree. A minimum of three animals per sex per group per interval was required for statistical analysis. Groups 2 through 4 of the same sex were compared to Group 1 at common time-points. Group 6 of the same sex were compared to Group 5 at common time-points. In addition, Group 6 of the same sex was compared to Group 3 at common time-points.

Results

Mortality:
All animals survived to scheduled termination.

Clinical signs:
A reddish discolored urine was noted within 2 hours postdose in the group administered the commercial vehicle alone (3 males and 8 females), and in 1 female each administered 0.2 mg/kg and 0.8 mg/kg regadenoson in the commercial formulation. Material around the nose was observed in the treated animals at higher incidence than in the two vehicle control groups.

Body weights:
No drug-related remarkable effects.

Food consumption:
Food consumption was significantly reduced for males treated with 0.2 mg/kg of MBA Test Article 2 compared to the respective control group on study days 1 to 8.

Hematology and clinical chemistry:
Statistically significant changes observed on day 2 are presented in the following table

Regadenoson	0.2 mg/kg in MBA	0.2 mg/kg in PG	Control MBA
Males Hematology			
Hemoglobin (g/dL)	15.27	14.14	14.08
Hematocrit (%)	50.10	46.76	47.04
Reticulocytes (%)	6.10		4.92
Basophil absolute number ($10^3/\mu L$)	0.077	0.034	
Prothrombin time (sec)	16.40		17.40
Females Hematology			
Platelets counts ($10^3/\mu L$)	1459.6	1253	
Males clinical chemistry			
Glucose (mg/dL)	108	92.8	
BUN (mg/dL)	10.2	11.8	
Alkaline phosphatase (U/L)	258.6	195.0	
Females clinical chemistry			
A/G	1.18	1.34	
Globulin	2.98	2.78	

These changes were not evident by Study Day 15. Since most of these values were not different from control, these changes were not considered test article-related. All other statistical differences in clinical chemistry parameters were considered incidental and unrelated to the test articles.

Urinalysis:
No remarkable test article related effects

Gross pathology:
No gross lesions at either necropsy that were clearly related to test article administration

Organ weights:
No test-article related significant findings

Histopathology:
Increased incidence of minimal cardiomyopathy was observed on day 2 in males at doses of 0.08, 0.2 and 0.8 mg/kg (1/5, 2/5, and 5/5 respectively) and in females (2/5) at 0.8 mg/kg in the propylene glycol regadenoson groups. Cardiomyopathy was also seen in males (3/5) administered 0.2 mg/kg of regadenoson in the methylboronic acid formulation. No cardiomyopathy was noted on day 15: one control female and one low dose male in the PG group had minimal myocardial vacuolation, and one female treated with 0.2 mg/kg MBA regadenoson had a focal myocardial chronic inflammation. Cardiomyopathy consisted of scattered foci of inflammatory cells (primarily lymphocytes and macrophages) that were associated with few or no necrotic myocytes and found primarily in the interventricular septum and the left ventricle near the heart apex. Foci were often adjacent to the endocardium in these areas. More rarely, inflammatory foci were seen in the right ventricular wall or closer to the heart base in the septum or left ventricle. The severity of the lesion was judged to be minimal for all affected animals. This effect was determined to be regadenoson related.

The incidence of cardiomyopathy on day 2 is summarized in the following table:

	PG formulation (mg/kg)				MBA formulation (mg/kg)	
	0	0.08 (1.6XHD*)	0.2 (4 XHD)	0.8 (16XHD)	0	0.2 (4 XHD)
Males (5/group)	0	1	2	5	0	3
Females (5/group)	1	0	0	2	0	0

*XHD: human dose multiple based on body surface area

Other histological findings included the following:

One high dose rat had minimal lymphocytic infiltration in the brain.

Injection site lesions (edema or acute hemorrhage) were seen sporadically in control and treated groups on day 2, and were reversible on day 15 except in a few treated animals. Minimal chronic inflammation of the epididymis was observed on day 2 in 1 control and 2 animals in each of the treated groups in the PG group, but was almost completely reversible on day 15. In lungs, a somewhat dose-dependent minimal chronic inflammation (1 low dose, 2 mid dose, and 2 high dose rats) was observed on day 2 but not on day 15

In the kidney, tubular cysts were found in low incidence in almost all groups including controls, and on both days 2 and 15; one MBA vehicle female had marked formation of tubular cysts in both kidneys (the same that had moderate numbers of hepatic biliary cysts). Kidney chronic inflammation and tubular mineralization were somewhat more prominent in the PG groups. Focal fibrosis was noted in one low dose rat and bilateral pyelonephritis in one MBA treated rat at 0.2 mg/kg.

One female in Group 5 (MBA vehicle) had marked formation of tubular cysts in both kidneys, as well as moderate numbers of hepatic biliary cysts. On day 2, one female in

the PG vehicle group and one female in the 0.8 mg/kg PG group had similar, chronic, focal liver lesions consisting of mineralization and fibrosis. In the 0.8 mg/kg group female, these changes were associated with coagulative necrosis. The sponsor considered these lesions to be chronic, occurring weeks to months before euthanasia, and therefore unrelated to administration of test article in these rats. However, the effect of vehicle may not be ruled out. On day 15, the PG groups rats showed a minimal liver chronic inflammation with higher incidence in treated rats (1/5, 2/5, 3/5, and 1/5 rats in control, low-, mid-, and high-dose groups, respectively) but a dose-dependent pattern was not clear; in addition, 2 high dose rats presented hepatocellular vacuolation.

Report's conclusion:
Although spontaneous cardiomyopathy is common in the Sprague-Dawley strain, the high dose response incidence, particularly in males, indicates that the lesion is drug-related. Histologically, the appearance of minimal cardiomyopathy in rats in this study is typical for the frequently reported spontaneous condition. The condition was less prevalent in females necropsied on day 2, with the greatest incidence (two of tem) in the high dose group.

The cardiomyopathy was not seen in the previous seven-day and 28 day studies in the same strain of rats at dosages up to 0.2 mg/kg CVT-3146 in the methyl boronic acid vehicle (Study Numbers 124-003 and 124-011, respectively).

Other than slight, transient changes in hematology parameters and feed consumption at 0.2 mg/kg of CVT-3146 in the methylboronic acid vehicle, there were no apparent differences in the toxicity profiles of the two formulations administered in this study.

Reviewer's comments:

Cardiomyopathy

It bears emphasizing that this study was the only toxicity study (including histopathology) that utilized the clinical formulation in preclinical development studies, To serve as an effective bridging study, the agency recommended that regadenoson in methylboronic acid be utilized as a comparator.

The major finding for this study is the occurrence of a dose related incidence of minimal cardiomyopathy on day 2 in males at doses of 0.08, 0.2 and 0.8 mg/kg (1/5, 2/5, and 5/5 rats, respectively) and in females (2/5) at 0.8 mg/kg in the propylene glycol regadenoson groups. Cardiomyopathy was also seen in males (3/5) administered 0.2 mg/kg of methyl boronic acid formulation (the highest dose evaluated). It seems reasonable to postulate that more male animals could have been affected had the sponsor tested a higher dose of the methylboronic acid formulation. For both formulations, no cardiomyopathy was noted in animals observed on day 15 following the dosing. The increased incidence of cardiomyopathy in males as compared to females indicates a possible sexual

predisposition since spontaneous cardiomyopathy occurs more commonly in males Sprague-Dawley rat. The dose related incidence of cardiomyopathy was not seen in the previous seven-day and 28 day studies in the same strain of rats at dosages up to 0.2 mg/kg regadenoson in the methylboronic acid vehicle (124-003 and 124-011), although interim sacrifice on day 2 was not performed as was the case in this study.

From a pathophysiological perspective, the sponsor ascribed the finding of minimal cardiomyopathy to a large and long-lasting increase in plasma norepinephrine concentration secondary to the vasodilating response to regadenoson, which in turn causes histological changes in the myocardium. To support their point, the sponsor cited study (CVT3146.129–P), in which i.v. administration of 10 µg/kg regadenoson to male rats caused a ~2-fold increase in plasma norepinephrine levels measured 1-5 min postdose, with levels returning to baseline by 1 hour postdose. The effect on female rats was not evaluated.

The sponsor ascribed the lack of histologic findings indicative of cardiomyopathy following repeated dosing for 7 or 28 days, to an adaptive conditioning to the catecholamine-induced changes, stating that even after a single dose, catecholamines are known to provide a cardioprotective effect relative to subsequent ischemic insults. This effect was referred to as cardiac preconditioning. Thus, the sponsor posits that repeat dosing will cause repeated increases in the release of endogenous catecholamines, providing a long-term cardiac preconditioning effect that protects the myocardium from subsequent catecholamine insult. No data with regadenoson are available to support this hypothesis.

Cardiomyopathy was observed on day 2 but not on day 15 following dosing, leading to the sponsor's claim that the necrosis of the heart resolved since it was absent in the recovery animals. However, there were no signs of lesions and/or regeneration in the heart of recovery animals.

It is of note that significant increases (up to 3 fold) were seen in creatine kinase and lactate dehydrogenase levels in the high dose female rats on day 8 in the 7 day repeat dose study but not after 28 days of treatment using the methylboronic acid formulation. While significant changes were noted in the levels of these enzymes, there were no histological correlates of cardiac injury in these studies. However, these parameters which may be associated with cardiac injury were not evaluated in the present study.

There was one occurrence of cardiomyopathy in males at 0.08 mg/kg (1.6 XHD based on body surface area comparison for a 50 kg adult) which could be attributable either to the drug or be considered spontaneous cardiomyopathy typical for this strain of rats. Ruling out the effect of the drug in this group, while reasonable, would be a subjective call. Therefore, the NOAEL may not be categorically determined to be 1.6 times the human dose and even if it does, a 1.6 dose multiple does not provide sufficient safety margin.

In their latest submission (N0012), the sponsor provided data showing that administration of regadenoson (6.7-800 µg/kg) causes prolonged decrease in MAP (up to 50%) and

increase in heart rate (up to 20%) in male rats. These changes were considered to be of greater magnitude than those observed in humans when normalized by body surface area or by body weight. The sponsor suggested using the body weight comparison to calculate human dose multiples stating that dose adjustment by body weight was more predictive of the human cardiovascular response in rats. Alternatively, the sponsor considered the use of C_{max} in rats and humans for comparison purpose. However, the challenge with this approach is that the sampling time is highly critical in defining the plasma peak concentration; hence such ratios could be variable. Moreover these approaches would imply that the mechanism of cardiomyopathy is known to be solely due to the drop in MAP. If one were to accept the sponsor's position that the body weight comparison is a more appropriate method of calculating human dose multiples for regadenoson, then the lowest dose at which equivocal finding of cardiomyopathy was obtained would give a safety margin of 10.

Human dose multiples for the lowest dose level tested (80 µg/kg) using different methods of calculation are shown in the following table

Parameter	**Human Dose Multiple**
C_0 (ng/mL)	6-11 XHD
$AUC_{0-\infty}$ (ng-hr/mL)	0.8-1.9 XHD
Body weight	10 XHD
Body surface area	1.6 XHD

In summary, regadenoson administration causes dose-dependent cardiomyopathy in rats. The safety margin would vary depending on the indices used for dose multiple comparisons as shown in the table above. Although more conservative, it is my considered opinion that a comparison based on the body surface area would be the most appropriate in this case since the risk of cardiomyopathy is a serious concern.

According to the sponsor, the reddish discolored urine noted for animals administered the commercial vehicle alone, and at 0.2 mg/kg and 0.8 mg/kg in the commercial formulation was possibly due to slight hemolysis from injection of a large dosing volume and/or possible changes in isotonicity. However, if that were the case, it should have been noted in the highest dose group animals at similar incidence since the animals received the same volume of vehicle than the control group.

One MBA vehicle female had moderate numbers of hepatic biliary cysts. On day 2, one female in the commercial vehicle group and one female in the 0.8 mg/kg propylene glycol group had chronic, focal liver lesions consisting of mineralization and fibrosis. In the 0.8 mg/kg group female, these changes were associated with coagulative necrosis. The sponsor considered these lesions to be chronic, occurring weeks to months before euthanasia, and therefore unrelated to administration of test article in these rats. However, the effect of vehicle may not be ruled out. On day 15, the PG rats exhibited minimal liver chronic inflammation with higher incidence in treated rats but no clear dose-dependent pattern; 2 high dose rats presented hepatocellular vacuolation.

2.6.6.3 Repeat-dose toxicity

Study 124-003: Seven-day repeated dose toxicity study of CVT-3146 administered via intravenous administration to Sprague-Dawley rats (Reviewed by Dr. Anthony Proakis)

Study Facility: [redacted]
Study No.: 124-003
Study Dates: 1/25/00-2/08/00

GLP Compliance: Compliance with GLP regulations attested. QA Report: Yes

Animals: Male and female Sprague-Dawley rats (males, 185-227 gm; females 152-185 gm). The animals were housed individually and maintained on [redacted] and filtered tap water, both provided ad libitum.

Drug Administration: CVT-3146 (Lot # MHR-227-34V) was dissolved in 0.1% methyl boronic acid in 20 mM sodium bicarbonate aqueous solution and administered IV via a lateral tail vein daily for 7 days.

Dose Levels: 0 (vehicle), 2, 20 and 200 ug/kg/day (8/sex/dose group)

Observations/Measurements: Animals were observed twice daily for mortality and clinical signs of toxicity. Body weights were recorded predose, on the day prior to necropsy and on the day of necropsy. An ophthalmologic exam was performed on each animal predose, prior to necropsy on Study day 8 (5/sex/group) and, for recovery animals, prior to necropsy on Study day 15 (3/sex/group). On study days 1 and 7, approximately 1ml of blood was collected (via orbital sinus) from 1 rat/sex/group at approximately 2, 15 and 60 min postdose for toxicokinetic measurements. Just prior to necropsy, blood samples were obtained by cardiac puncture for hematology and clinical chemistry analyses. Urine was collected prior to necropsy for urinalysis. Five rats/sex/group (study day 8) and 3 rats/sex/group (study day 15) were killed by carbon dioxide asphyxiation and examined for external and internal abnormalities. Major organs (brain, liver, kidneys, testes, ovaries, adrenal glands and spleen) were removed and weighed. Sections of major organs and tissues (listed in Histopathology Inventory attachment) of all animals were fixed onto slides and examined microscopically.

Results

Mortality and Clinical Signs
No animals died during the study and no clinical signs of toxicity were observed.

Body Weight
Body weights and body weight changes among treated animals were comparable to controls.

Ophthalmologic Exam
No treatment-related ophthalmologic effects were observed.

Hematology, Clinical Chemistry and Urinalysis
On study day 8, the mean corpuscular hemoglobin concentrations in the 20ug/kg/day and 200 ug/kg/day group females (34.9% and 34.8%, respectively) were significantly lower than control (35.7%). The relevance of this finding to treatment with CVT-3146 is unclear since there were no accompanying changes in other erythrocyte parameters.

No treatment-related effects on clinical chemistry parameters were noted in males on study day 8. Statistically significant increases (up to 3-fold) were seen in the levels of creatine kinase and lactate dehydrogenase in high-dose females compared to vehicle control at study day 8; other significant effects at the high dose level in females include increases in serum potassium, phosphate and bilirubin (Table 6). After a week of treatment-free recovery, serum phosphorus, potassium and bilirubin in females returned to baseline; the effects on creatine kinase and lactate dehydrogenase showed a modest, but incomplete recovery following the 7-day drug-free period.

No treatment-related effects on urinalysis parameters were observed.

Table 6. Mean Clinical Chemistry Values After IV Administration of CVT-3146

		IV Dose Group, ug/kg/day					
		Study Day 8				Study Day 15	
Parameter	Sex	0	2	20	200	0	200
Total Bilirubin, mg/dl	M	0.1	0.2	0.1	0.1	0.1	0.1
	F	0.1	0.2	0.1	0.3*	0.1	0.2
Creatine Kinase, U/L	M	590	385	394	352	234	236
	F	501	549	707	1485*	282	617*
Phosphorus, mg/dl	M	13.7	12.2	13.5	14.0	13.7	12.9
	F	12.0	13.2	13.1	14.5*	13.0	13.2
Potassium, mmol/L	M	7.3	7.0	7.5	7.7	8.5	7.1
	F	7.2	8.4	7.1	9.9*	9.1	8.4
Lactate Dehydrogenase, U/L	M	331	294	267	249	146	143
	F	366	331	427	963*	203	463*

* Significantly different from vehicle control ($p<0.05$).

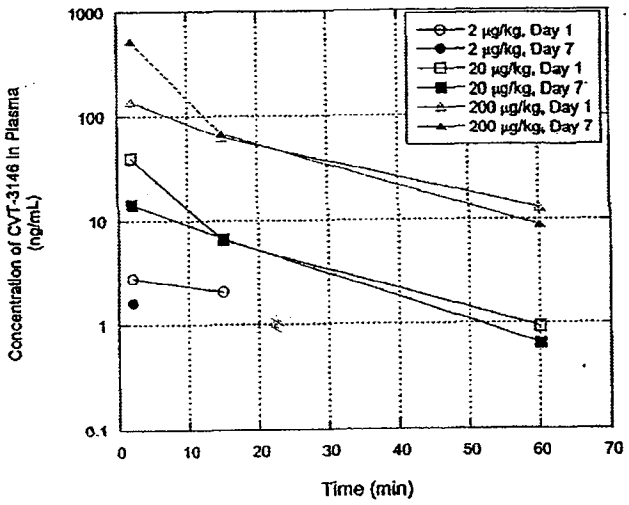
Organ Weights
Occasional differences in relative or absolute organ weights between treated and control were detected; however these were considered to be sporadic findings and unrelated to treeatment since there were no histological correlates for these effects in any of the organs examined.

Gross and Microscopic Pathology
No organ or tissue findings on gross examination were considered to be related to treatment. Minimal inflammation at the site of injection was the only apparent finding (2/8 HD males and 3/8 HD females) compared to control (0/8 males and 2/8 females) on microscopic examination. Other microscopic findings were considered incidental or in nature and unrelated to treatment with CVT-3146.

The IV no-observable-adverse-effect-level was considered to be at least 20 ug/kg/day.

Toxicokinetics

Fig. 4. Plasma Concentrations of CVT-3146 on Days 1 and 7 After IV Administration to Rats



Plasma concentrations of CVT-3146 following IV administration were comparable on Days 1 and 7 for each dose level (Fig. 4). Values of plasma AUC_{0-t} following IV administration of 20 and 200 ug/kg on day 1 were 5.82 and 45.7 ng.hr/ml, respectively and on Day 7 were 8.85 and 89.6 indicate that there was drug accumulation following 7-day repeated administration.

Study 124-004: Seven-day repeated dose toxicity study of CVT-3146 administered intravenously to Beagle dogs
(Reviewed by Dr. Anthony Proakis)

Study Facility: [redacted]
Study No.: 124-004
Study Dates: 2/03/00-2/18/00

GLP Compliance: Compliance with GLP regulations attested.
QA Report: Yes

Animals: Male and female Beagle dogs (males, 9.85-12.66 kg; females 8.18-10.34 kg) were housed 2/dog run and maintained on [redacted] and filtered tap water, both provided ad libitum.

Drug Administration: CVT-3146 (Lot # MHR-227-35D) was dissolved in 0.1% methyl boronic acid in 20 mM sodium bicarbonate aqueous solution and administered as an IV bolus via a cephalic tail vein daily for 7 days.

Dose Levels: 0 (vehicle), 2, 20 and 200 ug/kg/day (5/sex/dose group)

Observations/Measurements: Animals were observed twice daily for mortality and clinical signs of toxicity. Body weights were recorded predose, on the day prior to necropsy and on the day of necropsy. An ophthalmological exam was performed on each animal predose, prior to necropsy on Study day 8 (3/sex/group) and, for recovery animals, prior to necropsy on Study day 15 (2/sex/group). On study days 1 and 7, approximately 2ml of blood was collected (via jugular vein) from all CVT-3146 treated animals at approximately 2, 5, 15, 30, 60 and 180 min postdose for toxicokinetic measurements. Just prior to necropsy, blood samples were obtained via a jugular vein for hematology and clinical chemistry analyses. Urine was collected prior to necropsy for urinalysis. Three dogs/sex/group (study day 8) and 2 dogs/sex/group (study day 15) were killed by sodium pentobarbital and examined for external and internal abnormalities. The following organs were removed and weighed: brain, liver, kidneys, testes, ovaries, adrenal glands and thyroid. Sections of major organs and tissues (listed in Histopathology Inventory attachment) from all animals were fixed onto slides and examined microscopically.

Results

Mortality and Clinical Signs
No animals died during the study and no clinical signs of toxicity were observed.

Body Weight
Body weights and body weight changes among treated animals were comparable to controls.

Ophthalmologic Exam
No treatment-related ophthalmologic effects were observed.

ECG
There were some changes in T-wave morphology or polarity at doses =20 ug/kg; however, these effects on ECG were not dose related.

Hematology, Clinical Chemistry and Urinalysis
No consistent or distinct treatment-related effects on hematology parameters were observed. Sporadic findings included higher than control mean corpuscular hemoglobin concentration (+3%, +4% and +3%, respectively) in females from low, mid and high dose CVT-3146 treated groups, higher than control platelet counts (+40% and +48%, respectively) in mid and high dose females (end of treatment period) and lower than control hemoglobin and hematocrit (-9% and – 12%, respectively) in low dose males (end of the recovery period).

Clinical chemistry findings observed at the end of the treatment period (study day 8) included higher than control mean levels of serum calcium in low and high dose females

(23% higher in each group); these values were within normal limits and did not appear to be treatment-related.
Urinalysis parameters among treated groups were comparable to control.

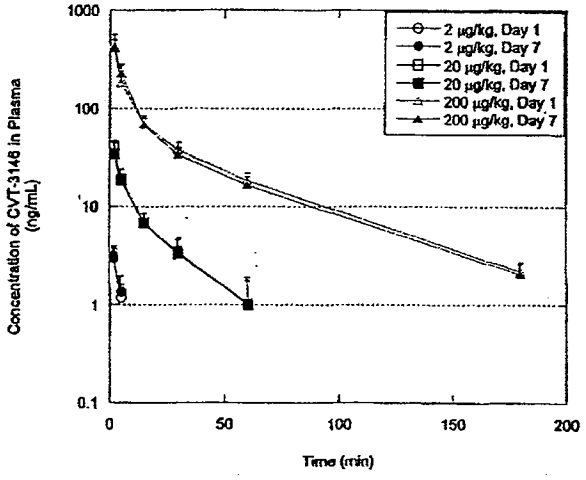
Organ Weights
Lower than control absolute thyroid weight for males given 2 and 200 ug/kg/day (24% and 33%, respectively) and lower than control relative thyroid weight for males treated with 2, 20 and 200 ug/kg/day (28%, 17%, 39%, respectively) was noted at the end of treatment. Higher than control relative and absolute kidney weight (29% and 25% higher, respectively) was noted for females given 20 ug/kg/day at the end of the treatment period; absolute and relative kidney weights in LD and HD animals were comparable to control.

Gross and Microscopic Pathology
No treatment-related gross lesions were observed. At the end of the treatment period, lesions of hemorrhage, subacute inflammation and thombosis were observed at several injection sites in vehicle control and CVT-3146 treated groups. These lesions were not evident in animals sacrificed at the end of the recovery period. No other treatment-related findings were noted.

The IV no-adverse-effect dose level for CVT-3146 in this study was considered to be at least 2 ug/kg/day.

Toxicokinetics
Plasma levels of CVT-3146 after intravenous administration of 20 and 200 ug/kg decreased rapidly in a biphasic manner (Fig. 6). Plasma levels of CVT-3146 after the 2 ug/kg dose decreased below the level of detection for most animals after 5 min; thus, pharmacokinetic parameters could not be determined at this dose level.

Fig. 6. Plasma Concentrations of CVT-3146 on Days 1 and 7 After IV Administration to Male and Female Dogs



Pharmacokinetic parameters are summarized in Table 9. The pharmacokinetics of intravenous CVT-3146 were approximately linear and were comparable between male

and female dogs. No accumulation of CVT-3146 was observed following daily doses up to 200 ug/kg.

Table 9. Pharmacokinetic Parameters Following IV Administration of CVT-3146 to Male and Female Dogs

Gender	Dosing Day	C_{2min} (ng/mL)	$AUC_{(0-\infty)}$ (ng.hr/mL)	CL_p (mL/min/kg)	Vd_β (L/kg)	Distribution $t_{1/2}$ (min)	Elimination $t_{1/2}$ (min)
2 µg/kg							
M[a]	1	2.85±0.745	Not determined due to insufficient data				
	7	3.43±0.908					
F	1	3.35±0.481					
	7	2.61±0.896					
M+F[a]	1	3.13±0.629					
	7	3.02±0.956					
20 µg/kg							
M[a]	1	44.4±7.37	8.01±1.33	42.6±7.92	1.03±0.110	5.47±0.308	16.9±2.21
	7	33.8±8.71	7.10±1.71	49.6±14.8	1.01±0.303	6.10±1.52	16.8±5.59
F	1	38.4±4.46	7.19±2.11	49.3±12.9	1.02±0.328	5.25±1.00	15.5±6.5
	7	36.1±10.6	7.21±3.02	52.9±20.4	1.02±0.153	5.87±0.74	15.2±6.1
M+F[a]	1	41.0±6.35	7.56±1.76	46.3±10.9	1.02±0.241	5.35±0.738	16.1±4.86
	7	35.1±9.79	7.16±2.38	51.4±17.1	1.07±0.164	5.98±1.08	15.9±5.58
200 µg/kg							
M	1	383±56.2	85.4±8.40	39.3±3.50	2.01±0.181	5.43±0.421	35.6±2.76
	7	394±73.6	84.8±11.9	39.9±5.27	2.17±0.391	5.04±0.40	37.7±3.44
F	1	439±223	96.9±24.1	36.2±9.37	2.02±0.480	7.65±5.23	38.7±2.05
	7	454±63.9	101±15.5	33.7±5.29	1.85±0.382	5.21±0.50	37.9±3.48
M+F	1	411±156	91.1±18.1	37.8±6.86	2.02±0.342	6.54±3.69	37.2±2.82
	7	424±72.1	92.8±15.5	36.8±5.94	2.01±0.402	5.13±0.44	37.8±3.26

[a]Data from male Dog #747 and #763 were not included in the calculation of the mean at the 2 and 20 µg/kg doses, respectively.

Study 124-011 28-day repeated dose toxicity study of CVT-3146 administered via intravenous injection to Sprague-Dawley rats
(Reviewed by Dr. Anthony Proakis)

Study Facility: [redacted]
Study No.: 124-011
Study Dates: 9/27/00-11/08/00

GLP Compliance: Compliance with GLP regulations attested.
QA Report: Yes

Animals: Male and female Sprague-Dawley rats (males, 201-257 gm; females 162-199 gm) were housed individually and maintained on [redacted] and filtered tap water, both provided ad libitum.

Drug Administration: CVT-3146 (Lot # MHR-227-43D) was dissolved in 0.1% methyl boronic acid in 20 mM sodium bicarbonate aqueous solution and administered IV via a lateral tail vein daily for 28 days.

Dose Levels: 0 (vehicle), 2, 20 and 200 ug/kg/day (15/sex/dose group)

Observations/Measurements: Animals were observed twice daily for mortality and clinical signs of toxicity. Body weights were recorded pre-dose, weekly during the dosing period and on the day prior to necropsy. Food consumption was measured weekly. An ophthalmological exam was performed on each animal pre-dose, prior to necropsy on study day 27 (5/sex/group) and, for recovery animals, prior to necropsy on study day 15 (5/sex/group). On study days 1 and 28, approximately 1ml of blood was collected (via orbital sinus) from 1 rat/sex/group (day 1) or 3/sex/group (day 28) at approximately 2, 15 and 60 min post-dose for toxicokinetic measurements. Just prior to necropsy, blood samples were obtained from all animals by cardiac puncture for hematology and clinical chemistry analyses. Urine was collected from all animals prior to necropsy for urinalysis. Ten rats/sex/group (study days 29 and 30) and 5 rats/sex/group (study day 43) were killed by carbon dioxide asphyxiation and examined for external and internal abnormalities. The following organs were removed and weighed: brain, liver, kidneys, testes, ovaries, adrenal glands and spleen. Sections of major organs and tissues (listed in Histopathology Inventory attachment) from all animals were fixed onto slides and examined microscopically.

Results

Mortality and Clinical Signs
No animals died during the study and no clinical signs of toxicity were observed.

Body Weight
The mean body weights for males treated with 200 ug/kg/day were significantly lower (6% to 8%) than control over study days 15 to 28. There were no consistent treatment-related effects on body weights of female rats (Table 7).

Food Consumption
Food consumption among CVT-3146 treated groups was comparable to control.

Table 7. Body Weights

BODY WEIGHTS - SUMMARY - FEMALE RATS

DOSE GROUP (MCG/KG/DAY)		1 0 (CONTROL)	2 2	3 20	4 200
RATS - TESTED		15	15	15	15
BODY WEIGHT (G)					
SD 1	MEANS.D.	181.9 ± 5.1	183.7 ± 7.2	183.1 ± 9.0	183.9 ± 8.9
SD 8	MEANS.D.	199.6 ± 7.4	203.6 ± 10.3	204.2 ± 12.2	199.8 ± 11.8
SD 15	MEANS.D.	221.6 ± 10.0	224.1 ± 11.3	223.1 ± 16.6	219.1 ± 16.1
SD 22	MEANS.D.	237.1 ± 14.2	240.7 ± 11.5	242.1 ± 21.5	233.9 ± 18.0
SD 29	MEANS.D.	247.7 ± 14.2	249.6 ± 14.3	252.7 ± 25.3	240.3 ± 20.8
SD 36	MEANS.D.	254.6 ± 17.6 [5]a	260.4 ± 13.1 [5]a	265.8 ± 30.8 [5]a	241.4 ± 18.7 [5]a
SD 42	MEANS.D.	257.4 ± 21.6 [5]a	267.2 ± 10.7 [5]a	282.6 ± 25.7 [5]a	251.0 ± 20.9 [5]a

BODY WEIGHTS - SUMMARY - MALE RATS

DOSE GROUP (MCG/KG/DAY)		1 0 (CONTROL)	2 2	3 20	4 200
RATS - TESTED		15	15	15	15
BODY WEIGHT (G)					
SD 1	MEANS.D.	231.3 ± 9.8	226.9 ± 10.2	227.5 ± 13.2	230.7 ± 11.6
SD 8	MEANS.D.	287.2 ± 14.5	281.5 ± 13.9	277.9 ± 17.2	273.9 ± 16.6
SD 15	MEANS.D.	326.6 ± 20.1	319.7 ± 16.4	312.7 ± 20.6	305.1 ± 20.8**
SD 22	MEANS.D.	356.9 ± 24.4	346.5 ± 21.6	342.3 ± 25.0	330.1 ± 22.5**
SD 28	MEANS.D.	372.5 ± 24.5	359.9 ± 24.3	356.3 ± 26.7	344.2 ± 25.8**
SD 36	MEANS.D.	402.6 ± 31.0 [5]a	387.4 ± 33.2 [5]a	381.2 ± 35.5 [5]a	353.4 ± 27.7 [5]a
SD 42	MEANS.D.	428.0 ± 29.3 [5]a	413.2 ± 33.7 [5]a	405.4 ± 36.4 [5]a	376.6 ± 26.0 [5]a

SD = STUDY DAY
[ ] = Number of values averaged.
a = Excludes values for animals that were a scheduled sacrifice.
** = Significantly different from Group 1 at p≤0.01.

Ophthalmologic Exam

Ophthalmologic exams revealed no treatment-related ocular changes.

Hematology, Clinical Chemistry and Urinalysis

Hematology parameters among CVT-3146 treated groups were comparable to control at the end of treatment (study day 29/30).

Clinical chemistry and urinalysis parameters among CVT-3146 treated groups were comparable to controls.

Organ Weights

Higher than control relative spleen weight was observed for low-dose and mid-dose males, at the end of the treatment period. At the end of the recovery period, higher than control relative brain weight was noted for high-dose males and lower than control relative kidney weight was noted for females in the low and high dose groups. These differences appear to be sporadic and unrelated to treatment.

Gross and Microscopic Pathology

Two gross lesions were observed (clear cysts on the left ovary of a control female and bilaterally enlarged uterus in a mid-dose female). These were considered incidental and/or unrelated to treatment. Thus, CVT-3146 treatment was not associated with any gross lesions at the end of dosing or recovery. All microscopic findings were incidental and spontaneous in nature and were not attributed to treatment.

The intravenous no-observable--adverse-effect-level was considered to be at least 20 ug/kg/day

Toxicokinetics

Plasma concentrations of CVT-3146 on Days 1 and 28 after IV administration were comparable for each dose level (Fig. 5). An approximate linear dose-proportional increase of AUC between 20 and 200 ug/kg was observed (Table 8). No evidence of drug accumulation or gender differences was observed.

Fig. 5. Plasma Concentrations of CVT-3146 on Days 1 and 28 After IV Administration to Rat

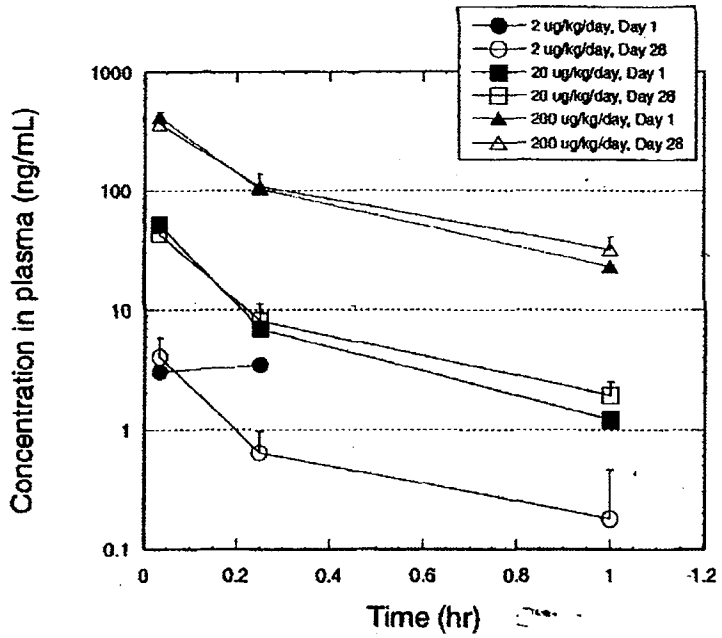


Table 8. Plasma AUC Values of CVT-3147 on Days 1 and 28 After IV Administration to Rats

IV Dose, ug/kg	Day 1 AUC_{0-t}, ng.hr/ml			Day 28 AUC_{0-t}, ng.hr/ml		
	Male (n=1)	Female (n=1)	M & F (n=2)	Male (n=3)	Female (n=3)	M & F (n=6)
2	ND	ND	ND	ND	ND	ND
20	[redacted]		9.39	10.1	8.71	9.41
200	[redacted]		104	110	103	106

ND= Not determined due to insufficient data (values below level of detection).

Study 124-012: 28-day repeated dose toxicity study of CVT-3146 administered intravenously to Beagle dogs
(Reviewed by Anthony Proakis)

Study Facility: [redacted]
Study No.: 124-012
Study Dates: 10/02/00-11/14/00

GLP Compliance: Compliance with GLP regulations attested.
QA Report: Yes

Animals: Male and female Beagle dogs (males, 9.04-12.05 kg; females 6.85-9.24 kg) were housed individually and maintained on [redacted] and filtered tap water, both provided ad libitum.

Drug Administration: CVT-3146 (Lot # MHR-227-43D) was dissolved in 0.1% methyl boronic acid in 20 mM sodium bicarbonate aqueous solution and administered as an IV bolus via a cephalic tail vein daily for 28 days.

Dose Levels: 0 (vehicle), 2, 20 and 200 ug/kg/day (5/sex/dose group)

Observations/Measurements: Animals were observed twice daily for mortality and clinical signs of toxicity. Body weights were recorded predose, weekly during the dosing period, on the day prior to necropsy and on the day of necropsy. Food consumption was measured weekly. An ophthalmological exam was performed on each animal predose and prior to necropsy on Study day 29 (3/sex/group) and, for recovery animals, prior to necropsy on Study day 43 (2/sex/group). On study days 1 and 25, approximately 2ml of blood was collected (via jugular vein) from all CVT-3146 treated animals at approximately 2, 5, 15, 30, 60 and 180 min postdose for toxicokinetic measurements. Blood samples were obtained from the jugular vein of all animals pretest, during week 2 and prior to each scheduled necropsy for hematology and clinical chemistry analyses. Urine was collected pretest, during week 2 and prior to necropsy for urinalysis. Three dogs/sex/group (study day 29) and 2 dogs/sex/group (study day 43) were killed by sodium pentobarbital and examined for external and internal abnormalities. The following organs were removed and weighed: brain, liver, kidneys, testes, ovaries, adrenal glands and thyroid. Sections of major organs and tissues (listed in Histopathology Inventory attachment) for all animals were fixed onto slides and examined microscopically.

Results

Mortality and Clinical Signs
No animals died during the study and no clinical signs of toxicity were observed.

Body Weight

Body weights and body weight changes among treated animals were comparable to controls during the dosing and recovery periods.

Food Consumption
Food consumption among CVT-3146 treated groups was comparable to control.

Ophthalmologic Exam
No treatment-related ophthalmologic effects were observed.

ECG
ECG recordings showed a trend towards increased heart rate, change in T-wave morphology (from positive to negative polarity) and slight elevation and arch of the ST segment in high dose males and females at one hour postdose.

Hematology, Clinical Chemistry and Urinalysis
Sporadic significant differences from control hematology parameters were observed and are summarized in Table 10. The differences detected were isolated, non-dose-dependent or did not coincide with CVT-3146 treatment.

Table 10. Mean Hematology Values in Dogs

Parameter	Sex	Study Period	CVT-3146 Dose Group, ug/kg/day			
			0 (vehicle)	2	20	200
Abnormal Lymphocytes, $10^3/mm^3$	M	Week 2	0.0	0.0	0.1*	0.0
	F		0.1	0.2	0.0	0.1
Platelets, $10^3/mm^3$	M	End of Dosing Period	281	347*	247	354*
	F		312	372	357	269
RBC, $10^7/mm^3$	M	End of Dosing Period	6.50	6.37	6.57	6.37
	F		7.33	6.43	6.38*	6.75
Mean Corpuscular Vol., μ^3	M	End of 14-Day Recovery	70.4	71.2	71.0	70.4
	F		68.4	69.8	71.6*	68.7

* Significantly different from vehicle control ($p<0.05$)

Sporadic significant differences from control clinical chemistry parameters were observed and are summarized in Table 11. The differences detected were isolated or within normal limits, non-dose-dependent or did not coincide with CVT-3146 treatment.

Table 11. Mean Clinical Chemistry Values in Dogs

Parameter	Sex	Study Period	CVT-3146 Dose Group, ug/kg/day			
			0 (vehicle)	2	20	200
AST, U/L	M	Week 2	47	42	47	36*
	F		48	55	47	47
Serum Albumin, g/dl	M	End of Dosing Period	3.2	3.2	3.4	3.6*
	F		3.6	3.4	3.5	3.6
Serum Calcium, mg/dl	M	End of Dosing Period	10.9	10.9	11.1	11.5*
	F		11.2	11.6	11.5	11.3
Serum Chloride, mmol/L	M	End of Dosing Period	112	113	114	116
	F		110	112	112	114*
Serum Glucose, mg/dl	M	End of 14-Day Recovery	110	104	111	104
	F		90	104*	104*	102*

* Significantly different from vehicle control ($p<0.05$)

Urinalysis parameters among CVT-3146 treated groups were comparable to control.

Organ Weights
Higher (24%) than control mean absolute adrenal weight was noted for high dose males and higher (21%) than control mean absolute liver weight was noted for mid dose males. Mean relative organ weights among CVT-3146 treated groups were comparable to control.

Gross and Microscopic Pathology
No treatment related gross lesions were observed. At the end of the treatment period, microscopic lesions of hemorrhage and inflammation were observed at several injection sites in vehicle control and CVT-3146 treated groups. These lesions were not evident in animals sacrificed at the end of the recovery period. No treatment-related findings were noted.

The IV no-adverse-effect dose level for CVT-3146 in this study was considered to be 20 ug/kg/day.

Toxicokinetics
Plasma levels of CVT-3146 after intravenous administration of 20 and 200 ug/kg decreased rapidly in a biphasic manner (Fig. 7). Plasma levels of CVT-3146 after the 2 ug/kg dose decreased below the level of detection for most animals after 5 min; thus, pharmacokinetic parameters could not be determined at this dose level.

Pharmacokinetic parameters are summarized in Table 12. The pharmacokinetics of intravenous CVT-3146 were approximately linear and were comparable between male and female dogs. No accumulation of CVT-3146 was observed following daily doses up to 200 ug/kg.

Fig. 7. Plasma Concentrations of CVT-3146 on Days 1 and 25 After IV Administration to Male and Female Dogs.

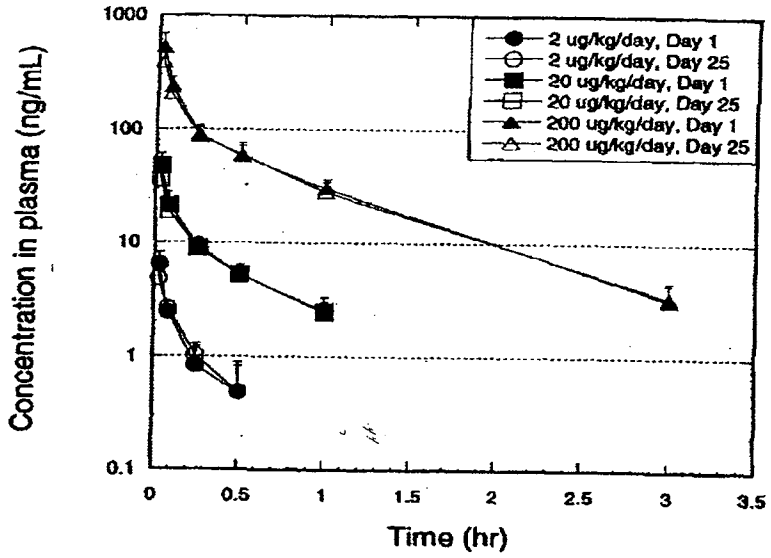


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Table 12. Pharmacokinetic Parameters Following IV Administration of CVT-3146 to Male and Female D

Gender	Dosing Day	C_{2min} (ng/mL)	$AUC_{(0-\infty)}$ (ng.hr/mL)	CL_p (mL/min/kg)	Vd_β (L/kg)	Distribution $t_{1/2}$ (min)	Elimination $t_{1/2}$ (min)
2 µg/kg/day							
M[a]	1	7.07±1.72	Not determined due to insufficient data				
	25	4.99±1.19					
F	1	5.03±0.725					
	25	4.54±0.479					
M+F[a, b]	1	6.16±0.169					
	25	4.79±0.924					
20 µg/kg/day							
M[a]	1	55.0±14.3	12.1±2.30	28.5±6.60	1.11±0.275	2.56±0.716	27.9±9.92
	25	42.1±10.7	11.3±2.26	30.6±6.35	1.41±0.568	3.35±1.16	33.4±15.0
F	1	40.3±8.39	10.3±3.46	35.1±10.5	1.39±0.253	3.29±1.06	29.7±12.6
	25	29.5±6.18	9.54±2.20	36.6±9.23	1.55±0.556	3.61±0.826	30.2±13.3
M+F[a, b]	1	47.6±13.5	11.2±2.92	31.8±8.9	1.25±0.289	2.92±0.936	28.8±10.7
	25	35.8±10.6	10.4±2.29	33.6±8.12	1.48±0.535	3.48±0.959	31.8±13.5
200 µg/kg/day							
M	1	485±182	126±20.1	27.0±3.97	1.29±0.228	3.55±1.17	33.3±4.86
	25	379±76.2	113±17.2	30.1±4.51	1.64±0.134	3.48±0.525	38.1±4.01
F	1	555±147	127±18.7	26.8±4.29	1.44±0.292	2.67±1.03	37.2±5.80
	25	392±115	118±32.6	30.0±8.68	1.61±0.594	3.29±0.543	36.5±4.86
M+F[b]	1	520±161	126±18.3	26.9±3.90	1.36±0.258	3.11±1.14	35.2±5.44
	25	385±92.0	116±24.7	30.0±6.52	1.62±0.406	3.39±0.514	37.3±4.28

[a]Data from male Dog #747 and #763 were not included in the calculation of the mean at the 2 and 20 µg/kg doses, respectively.
[b]Values of mean and standard deviation were determined from values of all animals excepted Dog #747 and #763 as noted in [a].

Histopathology Inventory for IND No. 62,862

Study No.	124-003	124-011	124-004	124-012		
Species	Rat	Rat	Dog	Dog		
Adrenals	X	X	X	X		
Aorta	X	X	X	X		
Axillary lymph node						
Brain	X	X	X	X		
Cecum	X	X	X	X		
Cervix	X	X	X	X		
Colon	X	X	X	X		
Duodenum	X	X	X	X		
Epididymis	X	X	X	X		
Esophagus	X	X	X	X		
Eye	X	X	X	X		
Fallopian Tubes						
Gall Bladder			X	X		
Gross Lesions	X	X	X	X		
Harderian Gland						
Head						
Heart	X	X	X	X		
Ileum	X	X	X	X		
Injection Site	X	X	X	X		
Jejunum	X	X	X	X		
Kidneys	X	X	X	X		
Lachrymal Gland						
Larynx						
Liver	X	X	X	X		
L nodes, cervical						
L nodes, mandibular	X	X	X	X		
L nodes, mediastinal	X	X	X	X		
Lungs	X	X	X	X		
Mandibular Gland	X	X	X	X		
Mammary Gland	X	X	X	X		
Nasal Turbinates						
Optic Nerves	X	X				
Ovaries	X	X	X	X		
Pancreas	X	X	X	X		
Parotid gland						
Parathyroid						
Pituitary Gland	X	X	X	X		
Prostate	X	X	X	X		
Rectum	X	X	X	X		
Salivary Gland	X	X	X	X		
Sciatic Nerve	X	X	X	X		
Seminal Vesicles	X	X	X	X		
Skeletal Muscle	X	X	X	X		
Skin	X	X	X	X		
Spinal Cord	X	X	X	X		
Spleen	X	X	X	X		
Sternum with bone marrow	X	X	X	X		
Stomach	X	X	X	X		
Testes	X	X	X	X		
Thymus	X	X	X	X		
Thyroid	X	X	X	X		
Tongue			X	X		
Tonsil						
Trachea	X	X	X	X		
Urinary Bladder	X	X	X	X		
Uterus	X	X	X	X		
Vagina	X	X	X	X		
Zymbal Gland						

2.6.6.4 Genetic toxicology

Genetic toxicology summary

Regadenoson was negative in the standard battery of genotoxicity tests. [redacted] impurities found in the drug substance were also evaluated: 1) [redacted] was positive in the bacterial reverse mutation assay; however, specification of NMT 10 ppm in the drug product are acceptable levels. 2) A drug formulation spiked with [redacted] each of the other [redacted] impurities [redacted] was negative in a bacterial mutation assay; the same formulation induced a statistical increase in the percentage of cells with numerical aberrations in a chromosomal aberration assay in CHO cells, in the non-activated 4-hour exposure group; however, because the percentage was within the historical solvent control, the results were considered negative.

Study 20608-1-422: Mutagenicity Test with CVT-3146 in the Salmonella-escherichia coli/Mammalian-Microsome Reverse Mutation Assay Preincubation Method
(This is a repeat study to confirm results of Study # 20608-0-422SC)

Key findings: Under the conditions of this study up to 5000 µg per plate CVT-3146 did not cause positive increases in the mean number of revertants per plate with any of the tester strains in either the presence or the absence of microsomal enzymes S9.

Study no.:	20608-1-422
Volume #, and page #:	N/A
Conducting laboratory and location:	[redacted]
Date of study initiation:	11/16/1999
GLP compliance:	Yes
QA statement:	yes ( ) no (x)
Drug, lot #, and % purity:	CVT-3146, 315-70-1, purity not provided

Methods
Mutation assay, pre-incubation

Strains/species/cell line: Salmonella typhimurium TA98, TA100, TA1535, TA1537, and Escherichia coli WP2*uvr*A

Metabolic activation: Liver microsomal enzymes (S9 homogenate) prepared from male Sprague-Dawley rats that had been injected (i.p.) with Aroclor™ 1254 (200mg/mL) at 500 mg/kg.

Doses used in definitive study: The pre-incubation assay was performed using tester strains TA98, TA100, TA1535, TA1537, and WP2uvrA in both the presence and absence of S9 mix. The doses tested in the assay were 7,690, 5,130, 1,540, 513, 154, and 51.3 µg of CVT-3146 per ml of pre-incubation reaction mixture in the presence and the absence of S9 mix. These doses are equivalent respectively to 5,000, 3,330, 1,000, 333, 100, and 33.3 µg per plate using the plate incorporation exposure.

Basis of dose selection: not provided

Negative controls: Vehicle controls were plated for all tester strains both in the presence and absence of S9 mix. The vehicle was plated using a 50 µl aliquot of vehicle equal to the maximum aliquot of test article dilution plated, along with a 100 µl aliquot of the appropriate tester strain and a 500 µl aliquot of S9 mix or phosphate buffer, when necessary, on selective agar.

Positive controls:

TABLE III. POSITIVE CONTROLS

Tester Strain	S9 Mix	Positive Control	Conc. per plate	Conc. per ml*
TA98	+	benzo(a)pyrene	2.5 µg	3.9 µg
TA98	-	2-nitrofluorene	1.0 µg	1.5 µg
TA100	+	2-aminoanthracene	2.5 µg	3.9 µg
TA100	-	sodium azide	2.0 µg	3.1 µg
TA1535	+	2-aminoanthracene	2.5 µg	3.9 µg
TA1535	-	sodium azide	2.0 µg	3.1 µg
TA1537	+	2-aminoanthracene	2.5 µg	3.9 µg
TA1537	-	ICR-191	2.0 µg	3.1 µg
WP2*uvr*A	+	2-aminoanthracene	25.0 µg	38.5 µg
WP2*uvr*A	-	4-nitroquinoline-N-oxide	0.4 µg	0.6 µg

* Expressed as concentration per ml of preincubation mixture (500 µl of S9 mix or buffer, 100 µl of tester strain, and 50 µl of control article dose).

Criteria for a positive response:
For a test to be considered positive, it had to produce at least a 2 fold increase (TA98, TA100, and WP2*uvr*A) or at least a 3 fold increase (TA1535, TA1537) in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control. This increase must be accompanied by a dose response to increasing concentrations of the test article.

Results

Study validity:
Criteria for assay validity were met.

Study outcome:
No positive increases in the mean number of revertants per plate were observed with any of the tester strains in either the presence or absence of S9 mix.

Report's conclusions:

The results of this study indicates that CV Therapeutics' test article, CVT-3146, did not cause positive increases in the mean number of revertants per plate with any of the tester strains in either the presence or absence of S9 mix.

Reviewer's comments:
Agree.

Study 6892-108: Salmonella-Escherichia coli/Mammalian- Microsome Reverse Mutation Assay

Key findings: Under the conditions of this study, [redacted] caused positive increases in the mean number of revertants per plate with tester strains TA1537 and WP2uvrA in both the presence and absence of S9 mix. No positive increases were observed with the other tester strains in either the presence or absence of microsomal enzymes.

Study no.:	6892-108
Volume #, and page #:	N/A
Conducting laboratory and location:	[redacted]
Date of study initiation:	01 October 2003
GLP compliance:	OECD principles of GLP
QA statement:	yes ( ) no (x)
Drug, lot #, and % purity:	[redacted], SAR-54-127, purity not provided

Methods
Plate incorporation assay

Strains/species/cell line: Salmonella typhimurium TA98, TA100, TA1535, TA1537, and Escherichia coli WP2*uvr*A

Metabolic activation: Liver microsomal enzymes (S9 homogenate) prepared from male Sprague-Dawley rats that had been injected (i.p.) with Aroclor™ 1254 (200mg/mL) at 500 mg/kg.

Doses used in definitive study: The assay was conducted using tester strains TA98, TA100, TA1535, TA1537, and WP2uvrA in both the presence and absence of S9 mix. Six doses of test article were tested in triplicate along with the appropriate vehicle and positive controls. The doses tested in the assay were 100, 333, 1000, 2000, 3330, and 5000 µg of [redacted] per plate.

Basis of dose selection: In a dose range finding study performed using strains TA100 and WP2*uvr*A in presence and absence of S9 mix, [redacted] was tested for cytotoxicity at concentrations ranging from 6.67 to 5000 µg per plate. Since no cytotoxicity was

observed in the dose range finding study, the highest dose level of [redacted] used in the mutagenicity assay was the same dose as that tested in the range finding study.

Negative controls: DMSO was used as vehicle.
Positive controls:

Table L Positive Controls

Tester Strain	S9 Mix	Positive Control	Dose (µg/plate)
TA98	+	benzo[a]pyrene	2.5
TA98	–	2-nitrofluorene	1.0
TA100	+	2-aminoanthracene	2.5
TA100	–	sodium azide	2.0
TA1535	+	2-aminoanthracene	2.5
TA1535	–	sodium azide	2.0
TA1537	+	2-aminoanthracene	2.5
TA1537	–	ICR-191	2.0
WP2*uvr*A	+	2-aminoanthracene	25.0
WP2*uvr*A	–	4-nitroquinoline-N-oxide	1.0

Incubation and sampling times:
All doses of the test article, the vehicle controls and the positive controls were plated in triplicate.

Criteria for a positive response:
For a test to be considered positive, it had to produce at least a 2 fold increase (TA98, TA100, and WP2*uvr*A) or at least a 3 fold increase (TA1535, TA1537) in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control. This increase must be accompanied by a dose response to increasing concentrations of the test article.

Results
Study validity:
Criteria for assay validity were met.

Study outcome:
The results of the dose range finding study were used to select the doses tested in the mutagenicity assay. The doses tested with all tester strains in the presence and absence of S9 mix were 100, 333, 1000, 2000, 3330 and 5000 µg per plate. The mutagenicity assay results were generated during Trial 25496-B1 and Trial 25496-B2 (Tables 3 & 4)

In the mutagenicity assay trial 25496-B1:

- Positive increases in the mean number of revertants per plate were observed with tester strain WP2*uvr*A in the presence (15.7-fold) and absence (8.6-fold) of S9 mix.
- No positive increases were observed with tester strains TA98, TA100, or TA1535 either in the presence or absence of S9 mix.
- The vehicle control value for tester strain TA1537 in the absence of S9 mix was not acceptable and vehicle control value for tester strain TA1537 in the presence

of S9 mix was higher than routinely observed in this laboratory. The data generated with tester strain TA1537 in Trial 25496-B1 were not used in the evaluation of the test article.

In the mutagenicity assay trial 25496-B2:

- The test article was retested with tester strain TA1537 in the presence and absence of S9 mix at 3.33, 10.0, 33.3, 100, 333, 1000, 3330 and 5000 µg per plate. Positive increases in the mean number of revertants per plate were observed with tester strain TA1537 in the presence (72.5-fold) and absence (40.8-fold) of S9 mix.

Reports' conclusions:
Under the conditions of this study, [redacted] (10-5000 µg per plate), caused positive increases in the mean number of revertants per plate with tester strains TA1537 and WP2uvrA in both the presence (72.5-fold) and absence (40.8-fold) of S9 mix. No positive increases were observed with any of the other tester strains in either the presence or absence of S9 mix.

Reviewer's comments:
Agree. This result was conveyed to the chemist reviewer. The specification for this impurity will maintain levels below 10 ppm, which is an acceptable level for genotoxic impurities.

Study CVT3146.046-T: Bacterial Reverse Mutation Assay with CVT-3146 Containing Impurities [redacted]

Key findings: Under the conditions of this study, CVT-3146 containing impurities [redacted] did not cause a positive response in the presence or absence of metabolic activation S9 mix, when tested at up to 5000 µg/plate. The level determined for each impurity [redacted] represented [redacted] of the individual impurity spiked in addition to the existing level already present in the drug substance.

Study no.: CVT3146.046-T
Volume #, and page #: N/A (eCTD)
Conducting laboratory and location: [redacted]
Date of study initiation: 09 October 2006
GLP compliance: Yes
QA reports: yes ( ) no (x)
Drug, lot #, and % purity: CVT-3146 containing impurities [redacted] 971-020, [redacted]

Drug	Lot#	% purity
CVT-3146 containing impurities [redacted]	971-020	[redacted]

CVT-3146 (Regadenoson)	17P03NJ00004	[redacted]
[redacted]	SAR-54-118	[redacted]
	SAR-55-123	[redacted]

The test article was provided by the Sponsor to the conducting laboratory as a stock solution of 150 mg/mL of CVT-3146 in DMSO spiked with impurities of [redacted] Subsequent test article dilutions were prepared by diluting in DMSO immediately before use.

Methods
Plate incorporation

Strains/species/cell line: The tester strains used were the Salmonella typhimurium TA98, TA100, TA1535 and TA1537, and Escherichia coli WP2*uvr*A.

Doses used in definitive study:
The dose levels tested were 15, 50, 150, 500, 1500 and 5000 µg per plate.

Basis of dose selection:
In an initial mutagenicity assay, the dose levels tested were 15, 50, 150, 500, 1500 and 5000 µg per plate. Precipitate was observed at 5000 µg per plate. No background lawn toxicity was observed but reductions in revertant counts were observed at 5000 µg per plate with a few test conditions. Based on the findings of the initial mutagenicity assay, the maximum dose plated in the confirmatory mutagenicity assay was 5000 µg per plate.

Negative controls: DMSO

Positive controls: Listed in the following table; all positive controls were diluted in DMSO except for sodium azide which was diluted in water.

Strain	S9	Positive Control	Concentration (µg/plate)
Salmonella Strains WP2 *uvr*A	Rat	2-aminoanthracene	1.0
			10
TA98	None	2-nitrofluorene	1.0
TA100, TA1535		sodium azide	1.0
TA1537		9-aminoacridine	75
WP2 *uvr*A		methyl methanesulfonate	1,000

Exogenous metabolic activation
S9 homogenate prepared from male Sprague-Dawley rats induced with an i.p. injection of Aroclor 1254, 500 mg/kg, five days prior to sacrifice, and used at 10% in S9 mix.

Incubation and sampling times:
S9 or sham mix (0.5 mL), 100 µL of tester strain and 50 µL of vehicle or test article dilution were added to 2.0 mL of molten selective top agar at 45±2°C. After vortexing,

the mixture was overlaid onto the surface of 25 mL of minimal bottom agar. When plating the positive controls, the test article aliquot was replaced by a 50 µL aliquot of appropriate positive control. After the overlay had solidified, the plates were inverted and incubated for approximately 48 to 72 hours at 37±2°C.

The bacterial background lawn was evaluated for evidence of toxicity by using a dissecting microscope. Precipitate was evaluated by visual examination. Except for positive controls, revertant colonies were counted either entirely by automated colony counter or entirely by hand.

Positive results criteria:

- For a test article to be considered positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article. A result was considered judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than 2 times (TA98, TA100 and WP2*uvr*A) or 3 times (strains TA1535 and TA1537) the mean vehicle control value.

- An equivocal response is a biologically relevant increase in a revertant count that partially meets the criteria for evaluation as positive. This could be a dose responsive increase that does not achieve the respective threshold cited above or a non-dose responsive increase that is equal to or greater that the respective threshold cited.

- A response will be evaluated negative, if it is neither positive nor equivocal.

Results

Study validity
The criteria for a valid study were met. The number of replicates and the counting method are acceptable

Study outcome:

Initial mutagenicity assay
The results of the initial mutagenicity assay were generated in Experiment B 1
Precipitate was observed at 5000 µg per plate. No background lawn toxicity was observed but reductions in revertant counts were observed at 5000 µg per plate. No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation.

Confirmatory mutagenicity Assay
The results of the confirmatory mutagenicity assay were generated in Experiments B3 and B4 (Contamination occurred in Experiment B2)

In Experiment B3: No positive mutagenic responses were observed with tester strains TA98, TA1535, TA1537 and WP2*uvr*A in either the presence or absence of S9 activation. Precipitate was observed at 5000 µg per plate. No appreciable toxicity was observed.
In Experiment B4: Strain TA100 was retested in this experiment B4 due to unacceptable positive control value (-S9) and unacceptable vehicle control value (+S9). No positive mutagenic responses were observed in either the presence or absence of S9 activation. Precipitate was observed at 5000 µg per plate. No appreciable toxicity was observed.

Report's conclusion:
All criteria for a valid study were met as described in the protocol. The results of the Bacterial Reverse Mutation Assay with CVT-3146 Containing Impurities [redacted] indicate that, under the conditions of this study, CVT-3146 containing impurities [redacted] did not cause a positive response in the presence or absence of Aroclor-induced rat liver S9.

Reviewer's comments:
Agree with the conclusion

This study was performed to evaluate the genotoxic potential of the impurities that may be present at [redacted] in the drug substance. The sponsor spiked the test article with [redacted] of each of the impurities.

The CVT-3146 solution (150 mg/mL, lot#971-020) spiked with approximately [redacted] was analyzed for % assay of CVT-3146 and % impurity levels of [redacted] CVT-3146 concentrations were found to be [redacted] of its nominal concentration (150 mg/mL). The levels of [redacted] respectively and those of [redacted] respectively. It should be noted that the CVT-3146 API lot 17PA03.NJ00004 already contained [redacted] Therefore, the levels of the two impurities determined [redacted] represented [redacted] of the individual impurity spiked in addition to the existing level already present in the drug substance.

Study CVT3146.047-T: In Vitro Mammalian Chromosome Aberration Test with CVT-3146 Containing Impurities [redacted]

Key findings: The percentage of cells with numerical aberrations in the test article-treated group was statistically increased (6.0%) above that of the solvent control at 1000 µg/mL in the non-activated 4-hour exposure group. Because, this percentage was within the historical solvent control range of 0.0% to 6.5%, it was not considered to be biologically significant by the sponsor. No changes were observed in the activated 4 hour exposure group. The results were negative when incubation for 20 hours with and without activation did not produce an effect.

Study no.: CVT3146.047-T
Volume #, and page #: N/A (eCTD)
Conducting laboratory and location: [redacted]

Date of study initiation: 10 October 2006
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: CVT-3146 containing impurities [redacted] 971-020, [redacted]

Drug	Lot#	% Purity
CVT-3146 containing impurities	971-020	[redacted]
CVT-3146 (Regadenoson)	17P03NJ00004	[redacted]
[redacted]	SAR-54-118	
	SAR-55-123	

The test article was provided by the Sponsor to the conducting laboratory as a stock solution of 150 mg/mL of CVT-3146 in DMSO spiked [redacted] with impurities of [redacted] Subsequent test article dilutions were prepared by diluting in DMSO immediately before use.

Methods
Chromosome aberration assay

Strains/species/cell line: Chinese hamster ovary cells (CHO-K_1)

Doses used in definitive study:
The dose levels tested in the chromosome aberration assay ranged from 15 to 1500 µg/mL (15, 50, 100, 250, 500, 750, 1000, 1250, 1500 µg/mL). The cells were treated for 4 and 20 hours in the non-activated test system and for 4 hours in the S9-activated test system. All cells were harvested 20 hours after treatment initiation. Doses selected for microscopic analysis were 100, 500, and 1000 µg/mL in the non-activated 4 hour and 20 hour exposure groups, and 100, 750, and 1500 µg/mL in the S-9 activated 4 hour exposure group. Selection of doses for microscopic analysis was based on mitotic index relative to the solvent control. The dose levels selected for microscopic analysis were 100, 500 and 1000 µg/mL. Higher dose levels of 1250 and 1500 µg/mL were not selected for microscopic analysis due to excessive mitotic inhibition relative to solvent control.

Basis of dose selection:
There was no evidence of precipitation or cell growth inhibition at any dose level under various exposure conditions. In the absence of both test article precipitation in the treatment medium and at least 50% toxicity (cell growth inhibition), selection of doses for microscopic analysis was based on mitotic index (the lowest dose with at least 50% reduction in mitotic index). Two additional lower dose levels were included in the evaluation.

Negative controls: The solvent for the test article was used as the solvent control at the same concentration as that found in the test article-treated groups.

Positive controls:
Mitomycin C (MMC) was dissolved and diluted in water and used as the positive control in the non-activated test system at final concentrations of 0.1 and 0.2 µg/mL.
Cyclophosphamide (CP) was dissolved and diluted in water and used as the positive control in the S9 activated study at final concentrations of 10 and 20 µg/mL.

Incubation and sampling times:
The cells were incubated with or without metabolic activation in a cell culture incubator at 37°C, 5% CO_2 according to the following table. Aroclor 1254-induced rat liver S9 used as the metabolic activation system was prepared from male Sprague-Dawley rats.

Summary of exposure conditions

Treatment condition	Treatment time	Recovery time	Test article concentration (µg/mL)
Non activated (-S9)	4 hr	16 hrs	15, 50, 100, 250, 500, 750, 1000, 1250, 1500
	20 hrs	0 hr	
Activated (+S9)	4 hr	16 hrs	

Results

The criteria for a valid study were met. The dose selection based upon mitotic index was acceptable.

- The percentage of cells with structural aberrations in the non-activated 4-hour exposure group was not increased above that of the solvent control at any dose level.
- The percentage of cells with numerical aberrations in the test article-treated group was statistically increased above that of the solvent control at 1000 µg/mL ($p \leq 0.01$, Fisher's Exact test). The Cochran-Armitage test was also positive for a dose response ($p \leq 0.05$). Since the percentage of cells with numerical aberrations in the treated group (6.0%) was within the historical solvent control range of 0.0% to 6.5%, it was not considered to be biologically significant by the sponsor.
- The percentage of cells with structural or numerical aberrations in the S9-activated 4 hour and the non-activated 20-hour treatment groups was not significantly increased above that of the solvent control at any dose level ($p > 0.05$, Fisher's Exact test).

Report's conclusion:
Based on the findings of this study, CVT-3146 containing impurities [redacted] was concluded to be negative for the induction of structural and numerical chromosome aberrations in CHO cells in both the non-activated and the S9-activated test systems.

Reviewer's comments:
The percentage of cells with numerical aberrations in the test article-treated group was statistically increased (6.0%) above that of the solvent control at 1000 µg/mL in the non-activated 4-hour exposure group. This percentage was within the historical solvent control range of 0.0% to 6.5%, and although the positive increase (6.0%) was seen towards the higher end of the historical control range (0%-6.5%) and there was a dose-response, the increase was not considered to be biologically significant by the sponsor.

Study CVTTOX04-004: Bacterial Reverse Mutation Assay

Key findings: Under the conditions of this study at concentrations of up to 5000 µg/plate, CVT-3146 did not cause positive increases in the mean number of revertants per plate with the tester strain TA1537, in either the presence or the absence of microsomal enzymes S9.

Study no.:	CVTTOX04-004
Volume #, and page #:	N/A (eCTD)
Conducting laboratory and location:	[redacted]
Date of study initiation:	03/09/2004
GLP compliance:	Yes (Exceptions: analyses to determine the uniformity or concentration of the test article mixtures and their stability were not performed by the testing facility or the sponsor)
QA statement:	yes (x) no ( )
Drug, lot #, and % purity:	CVT-3146 API, 0301CV301, [redacted]

Methods
Plate incorporation: Initial and confirmatory mutagenicity assays were conducted concurrently using independent bacterial cultures and independent test article dilutions at the sponsor's request.

Strains/species/cell line: Salmonella typhimurium TA1537

Metabolic activation: Liver microsomal enzymes (S9 homogenate) prepared from male Sprague-Dawley rats that had been injected (i.p.) with Aroclor™ 1254 at 500 mg/kg five days prior to sacrifice.

Vehicle solvent: DMSO

Basis of dose selection: In the initial mutagenicity assay, the maximum dose tested was 5000 µg per plate. The dose levels tested were 1.5, 5.0, 5, 50, 150, 500, 1500, and 5000 µg per plate. No positive mutagenic response was observed at. Precipitate was observed at 5000 µg per plate. No appreciable toxicity was observed.

In the confirmatory assay, no positive mutagenic response was observed. The dose levels tested were 1.5, 5.0, 5, 50, 150, 500, 1500, and 5000 µg per plate. Precipitate was observed at 5000 µg per plate. No appreciable toxicity was observed.

Positive controls:

Strain	*S9 activation*	*Positive control*	*Concentration (µg/plate)*
TA1537	Rat	2-aminoanthracene	1.0
TA1537	None	9-aminoacridine	75

Incubation and sampling times:

All dose levels of test article, vehicle control, and positive controls were plated in triplicate, with overnight culture of TA1537 on selective minimal agar in the presence or absence of S9-mix. A volume of 0.5 ml of S9 mix or sham mix, 100 µl of tester strain, and 50 µl of vehicle or test article dilution were added to 2.0 mL of molten selective top agar at 45 ± 2°C. After vortexing, the mixture was overlaid onto the surface of 25 mL of minimal bottom agar. After the overlay had solidified, the plates were inverted and incubated for ~48 to 72 hours at 37 ± 2°C. Revertant colonies (except for positive controls) were counted either entirely by automated colony counter or entirely by hand unless the plate exhibited toxicity.

Criteria for a positive response:
For the test article to be considered positive, it must cause a dose-related increase in the mean revertants per plate over a minimum of two increasing concentrations of test article such that the peak of the dose response is equal to or greater than 3 times the mean vehicle control value.

Results:

Study validity: Criteria for assay validity were met.

Study outcome: The results of both the initial mutagenicity assay and the confirmatory mutagenicity assay were similar: Precipitation was observed at the highest dose level tested, 5000 µg per plate. No appreciable toxicity was observed. No positive mutagenic response was observed with the tester strain in either the presence or the absence of S9 activation.

Conclusion:
Under the conditions of this study, CVT-3146 API Lot 0301CV301 at concentrations of up to 5000 µg per plate did not cause a positive response in either the presence or absence of S9 mix.

Reviewer's comments:

The batch analysis shows that the formulation contains impurities at the following %: [redacted], other inpurities were not detected. However, the specifications in the drug product are [redacted] for each of the impurities, which well exceeds the %age tested in this assay. Total impurities were present at [redacted] whereas the specifications are NMT [redacted] In addition, this assay was conducted with only one tester strain TA1537. Therefore, this study did not qualify the impurities, and the use of a single strain does not allow drawing conclusions on the mutagenic potential of the substance tested. The genotoxic potential was evaluated in other studies (see above)

2.6.6.6 Reproductive and developmental toxicology

Summary

Fertility and pre- and post-natal development were not evaluated. Embryofetal development was evaluated in rats and rabbits using a regadenoson formulation containing 8% propylene glycol (clinical formulation contains 15% propylene glycol).

When administered to rabbits during organogenesis, regadenoson caused maternal toxicity including tachypnea, soft, liquid or scant feces, and localized alopecia in all treated groups, and caused reduction in body weight and feed consumption at 0.3 and 0.5 mg/kg/day (12 and 20 X MRHD, respectively). At regadenoson doses equivalent to 12 and 20 times the MRHD, maternal toxicity occurred along with decreased number of live fetuses, reduced fetal body weight, and occurrence of fetal variations and malformations. At regadenoson doses equivalent to 20 times the MRHD, resorptions were increased and fetal body weights reduced. Fetal malformations included microphtalmia (1/116 at 20 X MHRD), interrelated vertebrae/rib alterations (2/145 and 2/116 each at 12 and 20 X MHRD), and misaligned caudal vertebrae (3/145 at 12 X MHRD). Fetal toxicity was only observed at maternally toxic doses. The no effect dose level for fetal toxicity is 0.1 mg/kg (4 X MRHD). A no effect dose level was not identified for maternal toxicity.

When regadenoson was administered to pregnant rats during the period of major organogenesis, 4/25 rats from the 1.0 mg/kg/day group (20 X MRHD) and 1/25 rats from the 0.8 mg/kg (16 X MRHD) group died immediately following the first dose of regadenoson. All dams had decreased motor activity and one was gasping post-dosing. At doses ≥ 0.5 mg/kg (10 X MRHD), maternal toxicity included decreased motor activity, increased limb extension, excess salivation, and reduction in body weight and feed consumption. At doses ≥ 0.5 mg/kg, fetal body weights were significantly reduced and significant ossification delays were observed in fore- and hindlimb phalanges and metatarsals. Skeletal malformations included delayed ossification of the skull (1/167), and hemivertebra present at a thoracic vertebra (1/167), observed at 16-20 X MHRD, and small arches of a lumbar and sacral vertebrae (1/174) observed at 2 X MRHD. The no effect dose level for maternal toxicity is 0.1 mg/kg/day (2 X MRHD).

Fertility and early embryonic development

Not conducted

Embryofetal development

Study 3003-005P: Intravenous dosage-range developmental toxicity study of CVT-3146 in rats.

Key study findings: Based on the results of this study, doses of 0, 0.1, 0.5, and 1.0 mg/kg/day (respectively 2, 10, and 20 times the human dose based on BSA) of CVT-3146 were recommended for the main developmental toxicity study in rats.

Study no.:	3003-005P
Volume #, and page #:	N/A (eCTD)
Conducting laboratory and location:	[redacted]
Date of study initiation:	Not provided (cohabitation November 2002)
GLP compliance:	Yes (With the exception that the test article analyses were performed according to cGMP regulations and the results of the end of study concentration analyses and the vehicle analyses were not reported to the study director)
QA reports:	yes (x) no ( )
Drug, lot #, and % purity:	CVT-3146/00918-030 & 0112CV302-6/ [redacted] (Two lot # were provided; COA was provided for lot # 0112CV302-6)

Study design:
Forty presumed pregnant Sprague-Dawley rats ([redacted]:CD®(SD)IGS BR VAF/Plus®) were assigned to 5 groups (8/group). The day of mating was considered as day 0 of presumed gestation (DG 0). CVT-3146 (0.1 mg/mL) or placebo was i.v. administered as bolus, on DGs 7 through 17 at doses of 0, 0.1, 0.3, 0.5 and 1.0 mg/kg/day in volumes of 10, 1, 3, 5 and 10 mL/kg, respectively. Placebo consisted of 8% propylene glycol, 0.1M phosphate buffer (pH7), 0.1% EDTA and 0.0005% butylated hydroxyanisole. Viability was checked twice daily, and clinical observations were recorded daily before, immediately after, and ~60 min post-dose, and daily thereafter. Body weights were recorded daily during dosing and post-dosing. Feed consumption was recorded on DGs 0, 7, 10, 12, 15, 18 and 21. All rats were C-sectioned on DG 21, examined for the number and distribution of corporea lutea, implantation sites and uterine contents. Uteri and ovaries of apparently nonpregnant rats were saved in neutral buffer 10% formalin. Gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Fetuses were weighed and examined for gross external alterations and sex.

Results:

All rats survived to scheduled sacrifice.

Adverse clinical signs included decreased motor activity, limb extension, tachypnea, tremors, excess salivation, ptosis, red perioral substance, urine-stained abdominal fur and swollen digit in the 1.0 mg/kg/day group. Excess salivation was also observed in one 0.5 mg/kg rat on D17. Most signs generally subsided at one hour post-dosing. Localized alopecia on the limbs, underside, back and neck occurred in the 0.3, 0.5 and 1.0 mg/kg/day groups and soft or liquid feces occurred in all treated groups.

Dose-dependent reduction was observed in body weights, body weight gains, and feed consumption values during the dosing period in the 0.3, 0.5 and 1.0 mg/kg/day groups compared to the control group. During the post-dosing period, the values rebounded in the 0.3 mg/kg/day group but continued to be reduced in the 0.5 and 1.0 mg/kg/day groups.

Caesarean-sectioning observations were based on 8, 8, 7, 8, and 8 pregnant rats in the five respective groups. Live fetal body weights were reduced in the 0.5 and 1.0 mg/kg/day groups. There were no dead fetuses and no dams had all conceptuses dead or resorbed. Examination of 110, 109, 96, 101, and 104 live fetuses in the respective groups revealed no fetal gross external alterations.

Conclusions: Based on these data, doses of 0, 0.1, 0.5, and 1.0 mg/kg/day (respectively 2, 10, and 20 times the human dose based on BSA) of CVT-3146 were recommended for the main developmental toxicity study in rats.

Study 3003-005: Intravenous developmental toxicity study of CVT-3146 in rats.

Study objective:
This study assessed the maternal and embryo/fetal effects of CVT-3146 when administered by IV injection to pregnant rats during the period of organogenesis.

Key study findings: Four/25 rats from the 1.0 mg/kg/day group (20 X MRHD) and 1/25 rats from the 0.8 mg/kg (16 X MRHD) group died immediately following the 1st dose of regadenoson. All dams had decreased motor activity and one was gasping post-dosing. At doses ≥ 0.5 mg/kg (10 X MRHD), maternal toxicity included decreased motor activity, increased limb extension, excess salivation, and reduction in body weight and feed consumption. At doses ≥ 0.5 mg/kg, fetal body weights were significantly reduced and significant ossification delays were observed in fore- and hindlimb phalanges and metatarsals. Skeletal malformations included delayed ossification of the skull (1/167), and hemivertebra present at a thoracic vertebra (1/167), observed at 16-20 X MHRD, and small arches of a lumbar and sacral vertebrae (1/174) observed at 2 X MRHD. The no effect dose level for maternal toxicity is 0.1 mg/kg/day (2 X MRHD)

Study no.: 3003-005
Volume #, and page #: N/A (eCTD)
Conducting laboratory and location: [redacted]

Date of study initiation: Not provided (Rats cohabitation Dec 2002)
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: CVT-3146/ 0112CV301-6/ [redacted]

Methods

One hundred and forty virgin female rats, weighing 209-260g (at assignment), age 62 days (at arrival), were placed into cohabitation for 5 days with 140 breeder male rats weighing 523-980 (at cohabitation), age 78 days (at arrival), one male per one female. Female rats with spermatozoa observed in a smear of the vaginal contents and/or a copulatory plug observed in situ were considered to be at DG 0.

Based on the results of the dose-range study [redacted] #3003-005P, doses of 0.1, 0.5, and 1.0 mg/kg/day were selected. Appropriate dosages were administered as a slow intravenous infusion (infusion rate not provided), once daily to rats on DGs 7 through 17, at approximately the same time each day. The animals were treated according to the following table:

Group	*Female rats #*	*Dosage[a] (mg/kg/day)*	*Concentration (mg/mL)*	*Dosage volume (mL/kg)*	*HDM*	*Assigned rat numbers*
I	25	0	0	8[b]	0	6601-6625
II	25	0.1	0.1	1	2X	6626-6650
III	25	0.5	0.1	5	10X	6651-6675
IV	25	0.8[b] (1.0)	0.1 (0.1)	8[b] (10)	16X (20)	1171[c], 6677-6680, 1172[d], 1173[e], 6683, 1174[f], 6685-6700

[a]The test article was considered 100% active for the purpose of dosage calculations.
[b]At the start of the study, the dosage volume for Groups I and IV was 10 mL/kg and the dosage level for Group IV was 1.0 mg/kg/day. Due to toxicity in Group IV, the dosage volume and dosage were reduced, at which time rats were at DGs 6 to 9. In Group I, four rats were administered two dosages and nine rats were administered one dosage at 10 mL/kg. In Group IV, three rats were administered two dosages and seven rats were administered one dosage at 10 mL/kg.
[c]Rat 6676 in Group IV was found dead after dosage on DG7 at 1.0 mg/kg/day and was replaced with rat 1171 on DG6.
[d]Rat 6681 in Group IV was found dead after dosage on DG7 at 1.0 mg/kg/day and was replaced with rat I 172 on DG6.
[e]Rat 6682 in Group IV was found dead after dosage on DG7 at 1.0 mg/kg/day and was replaced with rat 1173 on DG6.
[f]Rat 6684 in Group IV was found dead after dosage on DG7 at 1.0 mg/kg/day and was replaced with rat 1174 on DG6.

Species/strain: Rat/ [redacted] CD®(SD)IGS BR VAF/Plus®
Weight/age: 209-260g/62 days
Formulation: 0.1 mg/mL CVT-1346 formulated in 8% propylene glycol, 0.1M phosphate buffer (pH7), 0.1% EDTA and 0.0005% butylated hydroxyanisole
Satellite groups used for toxicokinetics: none

Parameters evaluated

Mortality/moribundity: All rats were observed at least twice daily for viability and weekly for general appearance during acclimation and on DG 0.

Clinical signs: Rats were examined for clinical observations, abortions, premature deliveries, and deaths before dosing, within 10 min, and 60±10 min after dosing. Observations were recorded once daily during the post-dosing period. The summary tables submitted incorporate the post-dosage clinical observations performed within 10 min and 60±10 min collectively.

Body weight and food consumption: Body weights were recorded weekly during the acclimation period, on DG 0, daily during the dosage and post-dosage periods. Feed consumption values were recorded on DGs 0, 7, 10, 12, 15, 18, and 21.

Gross necropsy:
(A table of random units was used to select one control group rat from which tissues examined at necropsy were retained, in order to provide control tissues for potential comparative histopathological evaluations of gross lesions)

Tissues with gross lesions were retained in neutral buffered 10% formalin for possible future evaluation. Unless specifically cited all other tissues were discarded.

All surviving rats were sacrificed by carbon dioxide asphyxiation on DG 21, Caesarean-sectioned and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Uteri of apparently non pregnant rats were examined to confirm the absence of implantation sites, and were retained with ovaries in neutral buffered 10% formalin. The number and distribution of corpora lutea were recorded. The uterus of each rat was excised and examined for pregnancy, number and distribution of implantation sites, live and dead fetuses and early and late resorptions. Placentae were examined for size, color and shape.

Each fetus was weighed and examined for sex and gross external alterations. Live fetuses were sacrificed by an intraperitoneal injection of sodium pentobarbital. Approximately one-half of the fetuses in each litter were examined for soft tissue alterations. These fetuses were fixed in Bouin's solution and the heads were subsequently examined. The carcasses were discarded. The remaining half of the fetuses in each litter were eviscerated, stained with Alizarin red S, and examined for skeletal alterations. The fetuses were initially fixed in alcohol and skeletal preparations retained in glycerin with thymol added as a preservative.

Rats that died were examined for the cause of death on the day the observation was made and examined for gross lesions. Pregnancy status and uterine contents were recorded. Conceptuses *in utero* were examined to the extent possible, using the same methods described for term fetuses.

Results:

Mortality (dams):
Four rats that were administered 1.0 mg/kg/day on DG 7 were found dead ~15 min post dose on the 1st dosing day. All four rats had decreased motor activity and one rat was gasping at the 10 min post-dosing observation. All tissues appeared normal at necropsy. Data for these four rats were maintained with the raw data and were not tabulated. This dosage level was subsequently reduced to 0.8 mg/kg/day and four rats were added to the group as replacements. Since several Group IV rats initially received 1.0 mg/kg/day, Group IV is referred to as 1.0/0.8 mg/kg/day dosage group.

One placebo rat was found dead on DG 9 one min after the 3rd dose. No change was noted in clinical signs and body weight gain and tissues appeared normal at necropsy for the slight degree of autolysis observed. The litter consisted of 18 embryos.

One rat of the 1.0/0.8 mg/kg/day dosage group was found dead on DG 12, 9 min after the 6th dose. This rat had been administered 1.0 mg/kg/day on DGs 7 and 8 and 0.8 mg/kg/day on DGs 9 through 12, and had decreased motor activity on DGs 7, 8, 10 and 11. Body weight loss occurred on DGs 8 and 9 and feed consumption on DGs 7 to 10 was the lowest in this group. All tissues appeared normal at necropsy for the slight degree of autolysis observed. The litter consisted of 15 embryos and one early resorption.

All other rats survived until scheduled sacrifice.

Clinical signs (dams):

There was no effect at 0.1mg/kg.

Within 10 min and 60±10 min collectively, decreased motor activity was observed in 23/25 rats in the 0.5 mg/kg/day group and in all 25 rats in the 1.0/0.8 mg/kg/day group. Significant increases in the incidence of limb extension and excess salivation were observed in the 1.0/0.8 mg/kg/day group. Excess salivation also occurred in 4 rats in the 0.5 mg/kg/day group. Most of the clinical signs occurred immediately after dosing and generally subsided at one hour after dosing.

Other clinical observations including soft or liquid feces, red perivaginal, perioral, and/or perinasal substance, scabs on tail, mass on lower midline or neck and scaly tail, were considered unrelated to CVT-3146 because the incidences were not dose-dependent or the observations occurred in only one to three rats. Localized alopecia of the limbs occurred in fewer rats in the treated groups compared to the placebo group.

No gross lesions were revealed by necropsy.

Body weight (dams):
Body weights and body weight gains were unaffected by the 0.1 mg/kg/day dosage of CVT-3146.

Body weight gains were significantly reduced on DGs 7 to 10 in the 1.0/0.8 mg/kg/day group and on DGs 15 to 18 in the 0.5 and 1.0/0.8 mg/kg/day groups. Body weight gains were significantly reduced for the entire dosage period (DGs 7-18) in the 0.5 and 1.0/0.8 mg/kg/day groups, and for the entire gestation period after the initiation of dosage (DGs 7 to 21) in the 1.0/0.8 mg/kg/day group. The average body weight on DG 18 was significantly reduced in the 1.0/0.8 mg/kg/day group as compared to the placebo control group value. Body weight gains during the post-dose period (DGs 18 to 21) were comparable among the four dosage groups.

Food consumption (dams):
Absolute and relative feed consumption values were reduced or significantly reduced on DGs 12 to 15, 15 to 18, 7 to 18 and 7 to 21 in the 0.5 and 1.0/0.8 mg/kg/day groups.

Absolute and relative feed consumption values were unaffected by the 0.1 mg/kg/day dosage of CVT-3146. Relative feed consumption values were significantly increased in the 0.1 mg/kg/day group on DGs 0 to 7 and 7 to 10. These significant increases reflected slightly increased body weight gains and/or absolute feed consumption values in this group during these intervals and were considered unrelated to the test article.

Toxicokinetics: Not performed

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

Pregnancy occurred in 25 (100%), 23 (92.0%), 22 (88.0%) and 23 (92.0%) rats in the four respective dosage groups. Because of the two reported deaths (control and high dose), C-section observations were based on 24, 23, 22 and 22 pregnant rats with one or more live fetuses in Groups I through IV, respectively.

Fetal body weights (total, male and female) were significantly reduced in the 0.5 and 1.0/0.8 mg/kg/day groups as compared to the control group. Fetal body weights were comparable for the 0.1 mg/kg/day group and the control group.

No other C-section or litter parameters were affected by dosages of CVT-3146 as high as 1.0/0.8 mg/kg/day. The litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, percent resorbed conceptuses, and percent live male fetuses were comparable among the four dosage groups and did not significantly differ. No dam had a litter consisting of only resorbed conceptuses, and there were no dead fetuses. All placentas appeared normal.

Table: C-section and litter parameters

PARAMETER	*Group I 0 mg/kg*	*Group II 0.1 mg/kg*	*Group III 0.5 mg/kg*	*Group IV 0.8/1.0 mg/kg*
Dams mated	25	25	25	25
Dams pregnant (#/%)	25/100	23/92	22/88	23/92
Found dead	1	0	0	1

Litters with one or more live fetuses	24	23	22	22
Dams with viable fetuses (#/%)	24/100	23/100	22/100	22/100
Dams with all conceptuses resorbed (#/%)	0/0	0/0	0/0	0/0
Dams with any resorptions N (%)	15 (62.5)	14 (60.9)	11 (50.0)	12 (54.5)
Mean Corpora Lutea	17.4	18.0	18.1	17.8
Mean Implantation	15.1	15.4	15.7	15.4
Total Live Fetuses	340	335	334	322
Mean Live Fetuses	14.2	14.6	15.2	14.6
Mean Total Resorptions	1.0	0.8	0.5	0.8
Mean Early Resorptions (N)	0.9 (21)	0.8 (18)	0.5 (12)	0.8 (18)
Mean Late Resorptions (N)	0.1 (2)	0.0 (1)	0.0 (0)	0.0 (0)
Dead Fetuses	0	0	0	0
Live male fetuses (N)	152	165	165	154
% Live male fetuses/Litter	42.7	50.5	49.8	48.0
Mean Live fetal body weight/Litter (g)	5.16	5.23	5.01*	4.80*
Mean male fetuses body weight (g)	5.45[a]	5.37	5.17*	4.90*
Mean female fetuses body weight (g)	5.03	5.07[b]	4.84*	4.71*
Mean % resorbed conceptuses/Litter	8.2	5.9	3.4	5.1

[a]Dam 6616 had no male fetuses
[b]Dam 6649 had no female fetuses
*Significantly different from the Group I value

Fetal alterations

Fetal evaluations were based on 340, 335, 334 and 322 live fetuses in 24, 23, 22 and 22 litters in the 0, 0.1, 0.5 and 1.0/0.8 mg/kg/day groups, respectively. Each of these fetuses was examined for gross external alterations. Of these respective fetuses, 164, 161, 161 and 155 fetuses were examined for soft tissue alterations, and 176, 174, 173 and 167 fetuses were examined for skeletal alterations and fetal ossification site averages.

The numbers of ossified fore and hindlimb phalanges and metatarsals were significantly reduced in the 1.0/0.8 mg/kg/day dosage group and the number of ossified hindlimb phalanges was significantly reduced in the 0.5 mg/kg/day group. The sponsor attributes these delays in ossification to the significantly reduced fetal body weights in these groups.

No gross external, soft tissue or skeletal fetal alterations (malformations or variations) were caused by dosages of CVT-3146 as high as 1.0/0.8 mg/kg/day. Incidental observations are described in the following table.

Offspring (malformations, variations, etc.):

	Variations & Malformations (Fetal incidence /litter incidence)	*Group I*	*Group II*	*Group III*	*Group IV*
	Litters with fetuses with alterations	5 (20.8%)	8 (34.8%)	7 (31.8%)	7 (31.8%)
	Fetuses with any alteration N	5 (1.5%)	9 (2.7%)	10 (3.0%)	7 (2.2%)
	% fetuses with any alteration/litter	5.5%	2.5%	3.4%	2.0%
Fetal gross external	Medially rotated hindlimbs	1	-	-	-

variations	Purple discolored skin at the neck	-	-	1	-
Fetal gross external malformations	Whole body edema, short hindlimbs, fused and short digits of fore and hindpaws,	1[a]	-	-	-
	Short tail	1	-	-	-
	Open eye lids, agnathia, small oral opening, absent tongue	-	-	1[b]	-
	Depressed eye bulge	-	-	1[c]	-
Fetal soft tissue variations	Enlarged right adrenal gland	1	-	-	-
	Absent innominate artery	-	1	1	-
Fetal soft tissue malformations	Absent tongue	-	-	1[b]	-
Fetal skeletal variations	Bifid centrum in a thoracic vertebra (fetuses/litters)	1/1	4/4	1/1	4/4[d]
	Cervical rib at the 7th cervical vertebra (fetuses/litters)	-	2/2	4/3[c]	1/1
	Wavy ribs	-	-	1	1[e]
	Fused manubrium	-	-	1	-
	Asymmetric sternal centrum	-	-	-	1
	Incompletely ossified 1st sternebra	-	2[f]	1[c]	-
	Incompletely ossified pubes	-	-	-	1[e]
Fetal skeletal malformations	Small left eye socket	-	-	1[c]	-
	Delayed ossification of the skull[g]	-	-	-	1[e]
	Right hemivertebra present as the 13th thoracic vertebra	-	-	-	1[d]
	Small arches of the 5th lumbar and 1st sacral vertebrae	-	1[f]	-	-
	Absent phalanges in all paws	1[a]	-	-	-

[a, b, c, d, e, f]Each letter represents a same fetus

[g]Delayed ossification of the skull includes incompletely ossified nasals, frontals, parietals, and tympanic rings, and not ossified interparietals and supraoccipitals.

Conclusions:

Clinical signs, body weights, and feed consumption were unaffected at 0.1 mg/kg/day in the pregnant rats. C-section parameters were not affected at 0.1 mg/kg. The NOAEL for both maternal toxicity was established at 0.1 mg/kg/day. Fetal skeletal malformations included delayed ossification of the skull (1/167), and hemivertebra present at a thoracic vertebra (1/167), observed at 16-20 X MHRD, and small arches of a lumbar and sacral vertebrae (1/174) observed at 2 X MRHD. The no effect dose level for maternal toxicity is 0.1 mg/kg/day (2 X MRHD).

Study 3003-004P: Intravenous Dosage-Range Developmental Toxicity Study of CVT-3146 in rabbits

Key study findings: CVT-3146 was i.v. administered to pregnant rabbits at doses of 0, 0.05, 0.1, 0.3 and 0.5 mg/kg/day from gestation day 7 through day 19 (2, 4, 12, and 20 times the human respectively). Based on the results of this study, doses of 0, 0.1, 0.3, and 0.5 mg/kg/day of CVT-3146 (respectively 4, 12, and 20 times the human dose) were recommended for the main developmental toxicity study in rabbits.

Study no.:	3003-004P
Volume #, and page #:	N/A (eCTD)
Conducting laboratory and location:	[redacted]
Date of study initiation:	Not provided (Around Nov 2002)
GLP compliance:	Yes
QA reports:	yes (x) no ( )
Drug, lot #, and % purity:	CVT-3146/0112CV302-6/ [redacted]

The vehicle consisted of 8% propylene glycol, 0.1M phosphate buffer (pH7), 0.1% EDTA, and 0.0005% butylated hydroxyanisole.

Study design:
Twenty-five naturally bred pregnant NZW rabbits were assigned to 5 groups (5/group). The day of mating was designated as day 0 of presumed gestation (DG 0). CVT-3146 (0.1 mg/mL) or placebo was i.v. administered as a slow infusion on DGs 7 through 19, at doses of 0, 0.05, 0.1, 0.3 and 0.5 mg/kg/day, in volumes of 5, 0.5, 1, 3, and 5 mL/kg respectively. Viability was evaluated twice daily; clinical observations were recorded daily before, immediately after, ~60 min post-dose, and once daily during the post-dosage period. Body weights and feed consumption values were recorded daily during the dosage and post-dosage periods. All rabbits were necropsied on DG 29 and examined for uterine contents. Fetuses were weighed and examined for gross external alterations and sex.

Study results:

All rabbits survived to scheduled sacrifice.

Tachypnea was observed in the 0.1 (2/5), 0.3 (5/5), and 0.5 mg (3/5) /kg/day groups immediately post-dose; rabbits were generally normal at one hour post-dosage. Dose independent observations included abnormal feces (scant, soft or liquid), abrasion on the neck, red substance in the cage pan, hyperpnea, and localized alopecia (head, limbs and back).

Body weights and feed consumption were generally comparable among the groups.

C-section observations were based on 5, 4, 5, 5 and 5 pregnant does in the five respective groups (0, 0.05, 0.1, 0.3, and 0.5 mg/kg/day). The number of late resorptions and percent resorbed conceptuses per litter were increased in the 0.3 mg/kg/day group. Total of 35, 25, 54, 40 and 41 live fetuses were examined for external gross alterations. There were no dead fetuses and no does with all conceptuses resorbed. There were no fetal gross external alterations observed.

Conclusion:

Based on the data of this study, doses of 0, 0.1, 0.3, and 0.5 mg/kg/day of CVT-3146 (respectively 4, 12, and 20 times the human dose) were recommended for the main developmental toxicity study in rabbits.

Study title 3003-004: Intravenous developmental toxicity study of CVT-3146 in rabbits.

Study objective:
This study assessed the maternal and embryo/fetal effects of CVT-3146 when administered by IV injection to pregnant Rabbit/New Zealand White [Hra©NZW)SPF] during the period of organogenesis.

Key study findings: Body weight and feed consumption were reduced in the 0.3 and 0.5 mg/kg/day group in pregnant females. Because of the clinical signs (tachypnea, soft or liquid or scant feces and localized alopecia) noted in all treated groups, NOAEL for maternal toxicity was not established in this study. Reproductive performance and fetal body weights were affected at 0.5 mg/kg. Fetal alterations were significantly increased in the 0.3 and 0.5 mg/kg groups. Fetal malformations included microphtalmia (1/116 at 20 X MHRD), interrelated vertebrae/rib alterations (2/145 and 2/116 each at 12 and 20 X MHRD), and misaligned caudal vertebrae (3/145 at 12 X MHRD). Fetal toxicity was only observed at maternally toxic doses. The fetal toxicity NOAEL was 0.1 mg/kg (4XHD).

Study no.:	3003-004
Volume #, and page #:	N/A (eCTD)
Conducting laboratory and location:	[redacted]
Date of study initiation:	17 December 2002 (date protocol signed)
GLP compliance:	Yes
QA reports:	yes (x) no ( )
Drug, lot #, and % purity:	CVT-3146/0112CV301-6 [redacted]

Methods

Species/strain: Rabbit/New Zealand White [Hra©NZW)SPF]
Weight/age: 2.7-4.7 kg (at assignment)/6months (at arrival)
Route, formulation, volume, and infusion rate: Intravenous, 0.1mg/mL formulation, 5, 1, 3, and 5 mL/kg in group I, II, III, and IV respectively/10mL/min slow injection.
Satellite groups used for toxicokinetics: none

Study design:
The rabbits were mated on four consecutive days and shipped to the testing facility. The day of mating was considered to be DG 0. Eighty timed mated rabbits were assigned to 4 dose level groups (20/group). CVT-3146 or placebo was i.v. administered as a slow injection on DGs 7 through 19, at doses of 0, 0.1, 0.3 and 0.5 mg/kg/day, in volumes of 5,

1, 3, and 5 mL/kg respectively. Doses were selected based on results of the dose-range study [redacted] #3003-004P in which all the rabbits survived for the duration of the study. The rabbits were administered at approximately the same time each day according to the following design:

Group	*Female rabbits #*	*Dosage[a] (mg/kg/day)*	*Concentration (mg/mL)*	*Dosage volume (mL/kg)*	*HDM[b]*
I	20	0	0	5	0
II	20	0.1	0.1	1	4X
III	20	0.3	0.1	3	12X
IV	20	0.5	0.1	5	20X

[a]The test article was considered 100% active for the purpose of dosage calculations.
[b]HDM: human dose multiple based on body surface area for a 50kg adult at a single dose of 400 µg (~300 µg/m^2)

Parameters and endpoints evaluated:

Mortality/moribundity: At least twice daily for viability and weekly for general appearance at least once during pre-dose period.

Clinical signs: Clinical observations, abortions, premature deliveries, and deaths before dosing, within 10 min and 60±10 min after dosing, then once daily during the post-dosage period.

Body weight: Recorded on DG 0, the day of arrival, and daily at dosage and thereafter.

Food consumption: Recorded daily after arrival.

Gross necropsy:

Tissues with gross lesions were retained in 10% formalin for possible future evaluation. (Except for the parovarian cysts common, spontaneous in rabbits according to sponsor)

All surviving rabbits were sacrificed by Beuthanasia®-D IV injection on DG29, C-sectioned, and gross necropsy was performed on the thoracic, abdominal, and pelvic viscera. Uteri and ovaries of apparently non pregnant rabbits were retained in 10% formalin. The number and distribution of corpora lutea were recorded. The uterus was examined for pregnancy, number, and distribution of implantation sites, live and dead fetuses and early and late resorptions. Placentas were examined for size, color and shape.

Fetuses were weighed and examined for gross external alterations. Live fetuses were sacrificed by i.p injection of Beuthanasia® and examined for sex. Cavitated organs were evaluated by dissection, and the brain was examined in situ. All fetuses were eviscerated, stained with alizarin red S and examined for skeletal alterations. The fetuses were initially fixed in alcohol. Skeletal preparations were retained in glycerin with thymol.

Rabbits sacrificed because of abortion were examined for the cause of abortion. Pregnancy status and uterine contents were recorded. Aborted fetuses or conceptuses in utero were examined to the extent possible, using methods described for term fetuses.

Data analysis:

One rabbit in the placebo group, one in the 0.3 mg/kg/day dosage group and two in the 0.5 mg/kg/day dosage groups had litters consisting of three or fewer conceptuses. The sponsor states that because such events can abnormally skew the distribution of the data, statistical analyses were made without the values for these rabbits and litters. Values for these rabbits and litters are presented in the individual report tables.

Results

Mortality/Abortions (does):
No test article related deaths occurred in the study. One rabbit in each of the control and 0.3 mg/kg/day groups aborted and were sacrificed. Neither of these abortions was considered related to CVT-3146 by the sponsor because the occurrences were not dose-dependent.

One control rabbit aborted and was sacrificed on DG20. Before abortion, this rabbit had soft or liquid feces on DG20, generally gained weight until DG19, and had reduced feed consumption on DGs 19 to 20. All tissues examined at necropsy appeared normal. The litter consisted of one fetus. This fetus was normal at gross external, soft tissue and skeletal examinations.

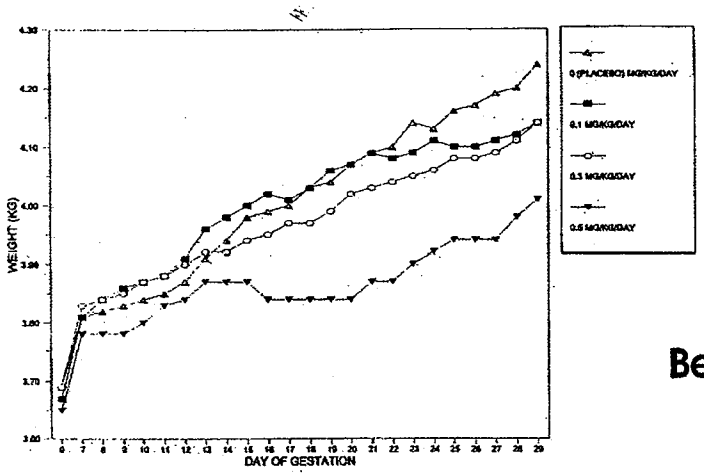
One 0.3 mg/kg/day rabbit aborted and was sacrificed on DG28. Prior to abortion, this rabbit had tachypnea on DGs 10, 14, 15 and 18, abnormal feces (scant or discolored) on DGs 14, 19 to 22, 26 and 27, localized alopecia on the underside on DGs 20 to 28 and a red substance in the cage pan on DG28. This rabbit generally lost weight throughout the study and feed consumption values were severely reduced after DG13. All tissues examined at necropsy appeared normal. The litter consisted of two late resorptions and nine fetuses. At gross external examination, one fetus had a missing tail that was presumed cannibalized and at skeletal examination, four fetuses had not ossified pubes; all other fetuses were normal at gross external, soft tissue and skeletal examinations.

Clinical signs (does):
Tachypnea occurred immediately after dosing and during the dosing period, in 17, 19 and 20 of the 20 rabbits in the 0.1, 0.3 and 0.5 mg/kg/day groups, respectively. Soft or liquid or scant feces and localized alopecia occurred in significantly increased numbers of rabbits in all treated groups. Discolored feces or no feces occurred in two or three rabbits in the 0.5 mg/kg/day dosage group, and one in the 0.3 mg/kg group. Other clinical observations observed in 3 rabbits were not dose-dependent and included ungroomed coat, red substance in cage pan or on the neck or nose, clear perinasal substance, scab on the neck or hindpaws, ulceration on the neck, lacrimation and redness of both eyes.

At necropsy, a raised clear area was observed on the kidney cortex of one rabbit in the 0.5 mg/kg/day group.

Body weight (does):
Body weights were unaffected at the 0.1 mg/kg/day dose level.
Significant body weight loss occurred on DGs 13 to 16 and significantly reduced body weight gain occurred on DGs 16 to 20 in the 0.5 mg/kg/day group. A non significant reduction in body weight gain occurred in the 0.3 mg/kg/day group on DGs 13 to 16. Thus, dose-dependent reductions in body weight gain occurred for the entire dosage period (DGs 7-20 in the 0.3 and 0.5 mg/kg/day groups, and body weight gain was significantly reduced in the 0.5 mg/kg/day group for the entire gestation period after the initiation of dosage.

Figure1: Maternal body weights



Food consumption (does):
Feed consumption values were unaffected at the 0.1 mg/kg/day dose level.
Absolute and relative feed consumption values were reduced or significantly reduced by 0.3 and 0.5 mg/kg/day on DGs 13 to 16, 16 to 20, for the entire dosage period (calculated as DGs 7 to 20) and for the entire gestation period after the initiation of dosage (DGs 7 to 29). Absolute and relative feed consumption values were generally comparable among the four dosage groups during the post dosage period (DGs 20 to 29).

Toxicokinetics: Not performed

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

Pregnancy occurred in 20 (100.0%), 19 (95.0%), 19 (95.0%) and 18 (90.0%) rabbits in the four respective dosage groups. One rabbit in each of the 0 and 0.3 mg/kg/day groups aborted and was sacrificed. One, one and two rabbits respectively in the 0, 0.3, and 0.5

mg/kg/day groups had litters consisting of three or fewer conceptuses. The sponsor states that such events can abnormally skew the distribution of the data, so statistical analyses were made without the values for these rabbits and litters. As a result, C-section observations were based on 18, 19, 17 and 16 pregnant rabbits with one or more live fetuses in the four respective dosage groups. Excluding litters consisting of three or fewer conceptuses in the statistical analysis is not an acceptable approach. However, in view that maternal toxicity occurred at dose levels in which these effects were observed, the conclusion of this study would not be affected.

Average litter sizes and numbers of live fetuses were reduced and total resorptions and early resorptions per litter, percent resorbed conceptuses per litter, and the percentage of does with any resorptions were increased in the 0.5 mg/kg/day dosage group. Fetal body weights were reduced in the 0.5 mg/kg/day dosage group. Although not statistically significant, these effects were ascribed by the sponsor to embryo/fetotoxicity. The litter averages for corpora lutea, implantations, late resorptions and percent live male fetuses were comparable among the four groups. No dam had a litter consisting of only resorbed conceptuses and all placentae appeared normal. C-Section parameters are summarized in the following table

Table1: C-Section parameters in rabbits

PARAMETER	*Group I 0 mg/kg*	*Group II 0.1 mg/kg*	*Group III 0.3 mg/kg*	*Group IV 0.5 mg/kg*
Does mated	20	20	20	20
Does pregnant (N/%)	20/100	19/95	19/95	18/90
Does aborted and sacrificed (N/%)	1/5	0/0	1/5.3	0/0
Rabbits pregnant and C-sectioned on DG 29	19	19	18	18
Included in analyses	18[a]	19	17[a]	16[a]
Litters with one or more live fetuses	19	19	18	18
Does with viable fetuses (N/%)	18/100	19/100	17/100	16/100
Does with all conceptuses dead or resorbed (N/%)	0/0	0/0	0/0	0/0
Does with any resorptions (N/%)	7/38.9	8/42.1	7/41.2	11/68.8
Mean Corpora Lutea	10.1	10.0	10.0	9.9
Mean Implantation	9.4	9.5	9.0	8.9
Mean litter size	8.8	8.7	8.4	7.0
Live Fetuses (N/mean)	158/8.8	165/8.7	143/8.4	112/7.0
Dead Fetuses (N/mean)	1/0	0/0	0/0	0/0
Mean Total Resorptions	0.6	0.8	0.6	1.9
Early Resorptions (N/mean)	5/0.3	4/0.2	3/0.2	23/1.4
Late Resorptions (N/mean)	5/0.3	11/0.6	7/0.4	8/0.5
Live male fetuses (N)	89	79	65	65
% Live male fetuses/Litter	57.4	48.7	45.1	54.6
Mean Live fetal body weight/Litter (g)	44.69	45.03	44.84	42.36
Mean fetal male body weight (g)	45.71	45.84	45.77	41.82 [15][b]
Mean fetal female body weight (g)	43.75	44.07	44.37	41.73
Mean % dead or resorbed conceptuses/Litter	6.2	7.7	5.8	21.2

[a]Excludes values for does that had litters consisting of three or fewer conceptuses
[b]Litter 1465 had no male fetuses, [ ]: number of values averaged

Fetal alterations

Fetal evaluations were based on 161, 165, 145 and 116 live, DG 29 Caesarean-delivered fetuses in 19, 19, 18 and 18 litters in the 0, 0.1, 0.3, and 0.5 mg/kg/day groups, respectively. Each of these fetuses was examined for gross external, soft tissue and skeletal alterations and fetal ossification site averages. One dead fetus in the control group was also examined and was normal at gross external, soft tissue and skeletal examinations. Fetal alterations are summarized in the following table.

Table 2: Offspring parameters (malformations, variations, etc.)

	Variations & Malformations (Fetal incidence /litter incidence)	*Group I*	*Group II*	*Group III*	*Group IV*
	Litters with fetuses with any alterations N (%)	**8 (42.1%)**	**10 (52.6%)**	**13 (72.2%)**	**9 (50.0%)**
	Fetuses with any alterations N (%)	**9 (5.6%)**	**16 (9.7%)**	**22** [**] **(15.2%)**	**14** [**] **(12.1%)**
	% Fetuses/litter with any alteration	**4.9**	**9.6**	**15.1**	**16.2**
Fetal gross external alterations	Accentuated fat pads,	-	-	1[a]	-
	Depressed left eye bulge	-	-	-	1[b]
Fetal soft tissue malformations	Left eye microphtalmia	-	-	-	1[b]
Fetal soft tissue variations	Absent intermediate lung lobe (N/Litter)	-	-	-	3/1[c]
	Absent gallbladder	-	-	1[d]	-
Fetal skeletal malformations	Small left eye socket associated with microphtalmia	-	-	-	1[b]
	Left hemivertebra present as the 4th cervical vertebra	-	-	1[e]	-
	Interrelated vertebral/rib alterations[z]	-	-	2	2[c]
	Misaligned 12th, 14th, 16th, and/or 17th caudal vertebra (N/Litter)	-	1/1[f]	3/3[g]	-
	Incompletely ossified 1st, fused 1st and 2nd & asymmetric 2nd sternal centra	-	1/1[f]	-	-
	Fused 3rd and 4th sternal centra	-	-	1[g]	-
Fetal skeletal	Irregular skull ossification[y]	4/4	7/5	10[**]/7[*]	0/0[*]
	Irregular ossification of nasals (total)	3/3	6/4	8[**]/5	0/0
	Intranasal/internasal ossification	-	-	3/2	-
	Irregular suture of nasals	-	-	1/1	-
	Displaced midline suture of nasals	3/3	6/4	5/3	0/0
	Irregularly shaped interparietal, misaligned manubrium and 1st sternal centrum and broad ribs	-	-	1[a]	-
	One or both hyoid alae angulated	1/1	3/3	3/2	4/4

variations	(N/litter)				
	Not ossified arch and unilateral ossification of the centrum of the 11th thoracic verteba	-	-	1[i]	-
	Thickened ribs	-	1	1	-
	Misaligned sternal centra (N/litter)	-	-	1/1	-
	Sternal centra fused	2/1	3/3	2/2	1/1
	Sternal centra incompletely osified	1/1	2/3	-	3/4
	Asymmetric sternal centra	-	2/2	-	-
	Pelvis not ossified pubes	-	1	-	-

**p≤0.01

a, b, c, d, e, f, g, h, i: Each letter represents a same fetus

[y] Fetus 1441-5 (0.3 mg/kg/day) had fused centra of the 5th and 6th thoracic vertebrae and fused left 5th and 6th ribs..
Fetus 1457-9 (0.3 mg/kg/day) had a left hemivertebra present as the 5th thoracic vertebra.
Fetus 1464-2 (0.5 mg/kg/day) had a right hemivertebra present as the 6th thoracic vertebra.
Fetus 1470-4 (0,5 mg/kg/day) had a split left 11th rib.

[z] Presence of small ossification sites within the sutures of the nasal or frontal bones and/or irregular shaping or fusion of the sutures or bones

Summary of fetal alterations:

Fetal malformations included eye microphtalmia associated with small socket and depressed bulge (1 fetus at 0.5 mg/kg), interrelated vertebrae/rib alterations (2 fetuses each at 0.3 and 0.5 mg/kg), misaligned or incompletely ossified or fused vertebrae (at 0.1 and 0.3 mg/kg). Fetal variations included absent intermediate lung lobe (3 fetuses from 1 litter at 0.5 mg/kg) absent gall bladder (1 fetus at 0.3 mg/kg); irregular ossifications of skull, nasals, ribs, and cervical and thoracic vertebrae were observed in higher incidence in all treated groups.

The significant increase in the fetal incidence of alterations in the 0.3 and 0.5 mg/kg/day groups reflected increases in specific fetal variations in these groups (irregular ossification of the nasal bones of the skull at 0.3 mg/kg/day; intermediate lung lobe absent at 0.5 mg/kg/day). Because the litter incidence of fetal alterations was not significantly increased in these groups, the sponsor does not consider these increases toxicologically important.

Reviewer's comments:
Because regadenoson caused maternal toxicity including tachypnea, soft, liquid or scant feces, and localized alopecia in all treated groups, the maternal toxicity NOAEL was not established. Reproductive performance and fetal body weights were affected at 0.5 mg/kg. Fetal alterations were significantly increased in the 0.3 and 0.5 mg/kg groups. Fetal malformations included microphtalmia (1/116 at 20 X MHRD), interrelated vertebrae/rib alterations (2/145 and 2/116 each at 12 and 20 X MHRD), and misaligned caudal vertebrae (3/145 at 12 X MHRD). Fetal toxicity was only observed at maternally toxic doses. Therefore the fetal toxicity NOAEL was 0.1 mg/kg (4XHD)

Prenatal and postnatal development

Not performed

2.6.6.7 Local tolerance

Local tolerance summary
Intravenous administration of Lexiscan to rabbits resulted in perivascular hemorrhage, vein vasculitis, inflammation, thrombosis and necrosis, with signs of reversibility except for the inflammation and thrombosis, which persisted through day 8 (last observation day). Perivascular administration of Lexiscan to rabbits resulted in hemorrhage, inflammation (acute or histiocytic), pustule formation and epidermal hyperplasia, which persisted through day 8 except for the hemorrhage which resolved. Subcutaneous administration of Lexiscan to rabbits resulted in hemorrhage, and acute inflammation, and on day 8 in fiber regeneration.

Study 124-021: Acute intravenous irritation study in rabbits with CVT-3146 in 15% propylene glycol with a saline flush

Study no.:	124-021
Volume #, and page #:	N/A (eCTED)
Conducting laboratory and location:	[redacted]
Date of study initiation:	February 19, 2003
GLP compliance:	Yes
QA reports:	yes (x) no ( )
Drug, lot #, and % purity:	CVT-3146, 0112CV301-6, [redacted]
Formulation/vehicle:	15% propylene glycol, 0.1M phosphate buffer (pH7), 0.1% EDTA

Study design
Six female New Zealand White rabbits each received 0.1 mL of a 100 µg/mL CVT-3146 solution in 15% propylene glycol by intravenous injection in the right ear (10 µg/animal). A comparable volume of vehicle was injected intravenously into the left ear of each rabbit. Each injection was followed by a 0.1 mL sterile saline flush. The Draize system was used to score the injection sites predose, at ~6 and 24 hrs post-dose, and on Day 8. Mortality/moribundity was assessed twice/day, and clinical observations recorded once/day. Body weights were recorded pretest, on Day 1 prior to dosing, and prior to necropsy, excluding the animals necropsied at 6 hour post dose. Feed consumption was measured approximately daily. Two rabbits were necropsied at ~6 and 24 hours post dose and on Day 8. The injection sites were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for histopathological evaluation.

Results
There was no mortality during the study. Clinical signs consisted of purple skin discoloration on the left ear (control) of 2 rabbits. No changes in body weight or feed

consumption occurred. Draize test revealed no erythema or edema. There was no finding at macroscopic examination.
Microscopic changes consisted of minimal to mild perivascular hemorrhage, inflammation, vein vasculitis, and coagulative vein necrosis. Findings are summarized in the following table

Table 1: Findings summary

	Treatment	*6 hours*	*24 hours*	*Day 8*
Perivascular hemorrhage	Vehicle (n=2)	2	2	0
	Test article (n=2)	2	0	0
Acute/subacute perivascular Inflammation	Vehicle (n=2)	2	0	1
	Test article (n=2)	1	0	2
Acute vein vasculitis	Vehicle (n=2)	0	0	0
	Test article (n=2)	2	0	0
Coagulative vein necrosis	Vehicle (n=2)	0	0	0
	Test article (n=2)	0	2	0

Report conclusion:
No mortality, drug-related overt clinical signs, changes in body weight, feed consumption, Draize scores, or gross changes occurred. The sponsor ascribed necrosis of the right ear vein injected with CVT-3146 noted microscopically at 24 hrs to leakage of the test formulation or injection into the perivascular tissue. Based on histological evaluations, the formulation was not considered to be irritating to the rabbit ear vein.

Reviewer's comments:
The formulation did not contain alcohol, [redacted] CVT3146 seemed to cause a slight increase in incidence and severity in the microscopic lesions. Necrosis was noted only in the two treated ears at 24 hours post-dose. The sponsor ascribed the effect to a leakage of the formulation into the perivascular tissue. Although it was noted on day 8, one may not assert that the effect is reversible since the leakage may have occurred only in those rabbits sacrificed at 24 hours. This issue was addressed in the local tolerance studies using perivascular and subcutaneous routes with the clinical formulation.

Study 124-022: Acute intravenous irritation study in rabbits with CVT-3146 in 15% propylene glycol

Study no.: 124-022
Volume #, and page #: N/A (eCTED)
Conducting laboratory and location: [redacted]
Date of study initiation: February 19, 2003
GLP compliance: Yes

QA reports: yes (x) no ( )
Drug, lot #, and % purity: CVT-3146, 0112CV301-6, [redacted]
Formulation/vehicle: 15% propylene glycol, 0.1M phosphate buffer (pH7), 0.1% EDTA

Study design:
Six female New Zealand White rabbits each received 0.1 mL of a 100 µg/mL CVT-3146 solution in 15% propylene glycol by intravenous injection in the right ear (10 µg/animal). A comparable volume of vehicle was injected intravenously into the left ear of each rabbit. The Draize system was used to score the injection sites predose, at ~6 and 24 hrs post-dose, and on Day 8. Mortality/moribundity was assessed twice/day, and clinical observations recorded once/day. Body weights were recorded pretest, on Day 1 prior to dosing, and prior to necropsy, excluding the animals necropsied at 6 hr post-dose. Feed consumption was measured approximately daily. Two rabbits were necropsied at ~6 and 24 hrs post-dose and on Study Day 8. The injection sites were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for histopathological evaluation.

Results
No mortality occurred during study. Clinical signs consisted of purple skin discoloration of the left ear (control) in 1 rabbit. No changes in body weight or feed consumption were observed. Draize test revealed no erythema or edema.

Microscopic changes consisting of minimal to mild hemorrhage, inflammation, and thrombosis were observed at the dose sites of both the right (treated) and left (control) ears. The incidence of findings is summarized in the following table

	Treatment	*6 hours*	*24 hours*	*Day 8*
Perivascular hemorrhage	Vehicle (n=2)	1	1	0
	Test article (n=2)	1	2	0
Acute/subacute perivascular Inflammation	Vehicle (n=2)	1	0	0
	Test article (n=2)	1	0	1
Vein (organized) thrombosis	Vehicle (n=2)	0	1	0
	Test article (n=2)	0	1	1

Table prepared by reviewer

The incidence of findings was comparable between control and treated ears at 6 and 24 hours. On day 8, minimal perivascular inflammation was observed in the treated ear of one rabbit and a non occlusive organized thrombus was present in the treated ear vein of the other rabbit.

Report conclusion:
The microscopic lesions at the injection sites were attributed to the trauma of injection. Based on this study, the administered formulations were not considered to be irritating to the rabbit ear vein.

Reviewer's comments:

The formulation did not contain alcohol [redacted]. CVT3146 seemed to cause a slight increase in incidence and severity in the microscopic lesions.

Study 124-023: Acute intravenous irritation study in rabbits with CVT-3146 in 20% propylene glycol

Study no.:	124-023
Volume #, and page #:	N/A (eCTD)
Conducting laboratory and location:	[redacted]
Date of study initiation:	February 19, 2003
GLP compliance:	Yes
QA reports:	yes (x) no ( )
Drug, lot #, and % purity:	CVT-3146, 0112CV301-6,
Formulation/vehicle:	20% propylene glycol, 0.1M phosphate buffer (pH7), 0.1% EDTA

Study design

Six female New Zealand White rabbits each received 0.1 mL of a 100 µg/mL CVT-3146 solution in 20% propylene glycol by intravenous injection in the right ear (10 µg/animal). A comparable volume of vehicle was injected intravenously into the left ear of each rabbit. The Draize system was used to score the injection sites predose, at ~6 and 24 hrs post-dose, and on Day 8. Mortality/moribundity was assessed twice/day, and clinical observations recorded once/day. Body weights were recorded pretest, on Day 1 prior to dosing, and prior to necropsy, excluding the animals necropsied at 6 hr post dose. Feed consumption was measured approximately daily. Two rabbits were necropsied at ~6 and 24 hours post dose and on Day 8. The injection sites were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for histopathological evaluation.

Results

There was no mortality during the study. Clinical signs consisted of purple skin discoloration of the left ear in control and treated ears (3 each). No changes in body weight or feed consumption were observed. Draize test revealed no erythema or edema. There was no macroscopic finding.

Microscopic changes consisted of minimal to mild hemorrhage, inflammation, and vasculitis. Findings are summarized in the following table

	Treatment	*6 hours*	*24 hours*	*Day 8*
Perivascular hemorrhage	Vehicle (n=2)	2	1	0
	Test article (n=2)	1	0	0
Acute perivascular	Vehicle (n=2)	1	0	0

Inflammation	Test article (n=2)	1	0	0
Acute vein vasculitis	Vehicle (n=2)	1	0	0
	Test article (n=2)	2	0	0

The incidence of findings was comparable between control and treated ears at 6 hours. At 24 hours, minimal perivascular hemorrhage was noted in the control ear of one rabbit. There were no microscopic findings on Day 8 in any of the rabbits.

Report conclusion:
Based on histological evaluations, the formulation was not considered to be irritating to the rabbit ear vein.

Reviewer's comments:
The formulation did not contain alcohol [redacted]

Purple left ear skin discoloration in control and treated ears appeared more frequent in this study, compared to the previous studies which used 15% propylene glycol (PG). Since the discoloration occurred at similar incidence in control and treated ears, the effect may be due to the higher PG % (20) used in the present study.
Overall, there was no remarkable difference in the findings between formulation containing 15% PG and 20% PG.

Study CVT3146.057-T: Local perivascular and subcutaneous tissue tolerance study of the clinical formulation of CVT-3146 in New Zealand white rabbits

Study no.:	CVT3146.057-T
Volume #, and page #:	N/A (eCTED)
Conducting laboratory and location:	[redacted]
Date of study initiation:	01-October-2007
GLP compliance:	Yes
QA reports:	yes (x) no ( )
Drug, lot #, and % purity:	CVT-3146, lot#902438 (Package) and lot#902624 (Bulk Manufacturing). [redacted]
Formulation/vehicle:	15% propylene glycol, 0.1M phosphate buffer (pH7), 0.1% EDTA

Study design:
Six female New Zealand White rabbits (4 month old/3.4-3.6 kg) each received 4 injections, two by perivascular administration (one placebo and one test article) and two by subcutaneous administration (one placebo and one test article). The perivascular injections were given adjacent to the marginal ear vessel. Placebo formulation was administered in the right ear, and test article formulation was administered in the left ear. The subcutaneous injections were given in the scapular region. Placebo formulation was

administered to the right of the midline, and test article formulation was administered to the left of the midline.

The experimental design was as follows:

Group number	Number of females	Placebo Animal's right side	Test article Animal's left side	Dose volume per site	Dose concentration (mg/mL, Placebo/Test article)
1	6	Placebo, Perivascular	CVT-3146, Perivascular	0.2 mL	0/0.08
		Placebo, Subcutaneous	CVT-3146, Subcutaneous	1 mL	0/0.08

Table provided by sponsor

Mortality/moribundity was assessed twice/day, and clinical observations recorded pretest and once/day. Body weights were recorded pretest and prior to necropsy.
On days 2 (24 hours post dose), 4 and 8, two animals per day were humanely euthanized via overdose with sodium pentobarbital and submitted for a necropsy examination limited to the four administration sites. Any gross lesions were recorded. The injection sites were examined in situ, dissected free, and were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for histopathology

Results

Mortality/moribundity:
There was no mortality during the study.

Clinical signs:
Black/blue discoloration of the skin was observed at the injections sites and the ears. This was mostly noted for the perivascular injection sites and was seen on Study Days 2 to 8. This observation was not considered test article-related, but was more likely related to trauma from the injection procedures since it was seen both at the sites treated with placebo and the sites treated with test article.

Gross necropsy:
On day 2, dark crust was observed in one control and two treated ears, and dark discoloration was noted in treated and placebo scapular region in one rabbit. On day 4, one treated ear had a dark focus and one placebo scapular region a dark discoloration. On day 8, findings were limited to a tan crust on the back skin in one rabbit. These gross observations were not considered to be related to the test article based upon the presence of similar observations at control article treated sites. The observations had a microscopic correlate of hemorrhage. Although most of the effects were reversible, their frequency and severity on days 2 and 4 following perivascular administration seemed higher in the treated ears than in placebo ears.

Histopatholgy:

Table 1: Histopathological findings summary

Perivascular administration						
Finding	**Day 2**		**Day 4**		**Day 8**	
	Placebo	Treated	Placebo	Treated	Placebo	Treated
Hemorrhage Minimal	0	0	2	1	0	1
Mild	1	2	0	1	0	0
Mild acute inflammation	1	2	0	0	0	0
Minimal histiocytic inflammation	0	0	2	2	1	2
Minimal stratum corneum pustule	0	0	0	0	1	1
Minimal epidermal hyperplasia	0	0	0	0	0	1

Subcutaneous administration						
Finding	**Day 2**		**Day 4**		**Day 8**	
	Placebo	Treated	Placebo	Treated	Placebo	Treated
Hemorrhage Minimal	0	1	0	0	0	0
Mild	1	0	1	0	0	0
Minimal acute inflammation	2	1	0	0	0	0
Minimal histiocytic inflammation	0	0	1	0	0	1
Minimal myofiber regeneration	0	0	0	0	0	1

Report conclusion:
The microscopic findings at the administration sites were considered to be related to manipulations (administration procedures). Hemorrhage, inflammation (acute or histiocytic), and pustule formation at test article-treated sites were not considered to be related to the test article based upon the presence of similar findings at control article treated sites. Additionally, the singular findings of epidermal hyperplasia and myofiber regeneration at test article treated sites were not considered to be related to the test article based upon their low incidence and their association with findings (pustule and/or histiocytic inflammation) that were also present in control article treated sites. Single perivascular and subcutaneous injections of the clinical formulation of CVT-3146 were well tolerated by New Zealand White rabbits with no signs of toxicity attributed to test article.

Reviewer's comments:
The frequency and severity of necropsy findings on days 2 and 4 following perivascular administration seemed higher in the treated ears than in placebo ears. However, most were reversible and on day 8, findings were limited to a tan crust on the back skin in one rabbit. Microscopic findings of hemorrhage, inflammation (acute or histiocytic), and pustule formation were seen for placebo and treated administration sites. However, epidermal hyperplasia (perivascular) and myofiber regeneration (subcutaneous) were observed in treated sites only on day 8. The sponsor ascribed these findings to manipulations, but because they were seen in the regadenoson treated regions, and because the number of animals is too low (2/group) it is not possible to rule out a drug effect.

[redacted]

2.6.6.8 Special toxicology studies

None

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:
The sponsor conducted the required safety pharmacology and toxicological evaluation for regadenoson. There were no unresolved toxicology issues in this NDA. With regard to the minimal cardiomyopathy observed in the single dose bridging toxicity study conducted in rats, a consult was requested from the Division of Cardio-Renal for assistance in interpreting the findings. Dr Albert F. Defelice's review of the data can be found in DFS (2/29/08).

Dr. Defelice considered the minimal focal cardiomyopathy (CM) to be irrelevant in a pharmaco-therapeutic context, whether veterinary or clinical. He further opined that the CM is expected to be without important sequelae based on chronic animal studies with other vasodilators, and his understanding that such lesions have not been observed at autopsy of patients treated with minoxidil - the prototype vasodilator for provoking such lesions (as well as coronary arteriopathy) in animals.

The cardiomyopathy findings have been incorporated into the label.

Unresolved toxicology issues (if any):
None

Recommendations:
Lexiscan is approvable from a pharmacology/toxicology perspective.

Suggested labeling:
The proposed labeling by the sponsor was edited as follows: the nonclinical pharmacology and toxicology edits are underlined or in ~~strikethrough~~.

[redacted]

2 Page(s) Withheld

____ Trade Secret / Confidential

✓ Draft Labeling

____ Deliberative Process

Withheld Track Number: Pharm/Tox- 5

[redacted]

Signatures (optional):

Reviewer Signature _____Siham Biade, Ph.D._____

Supervisor Signature_____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Siham Biade
3/24/2008 11:00:07 AM
PHARMACOLOGIST

Adebayo Laniyonu
3/24/2008 12:37:19 PM
PHARMACOLOGIST

Memorandum

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH
DIVISION OF CARDIOVASCULAR AND RENAL PRODUCTS

NDA 22-161

Date: February 29, 2008

From: A. DeFelice, PhD.
Supervisory Pharmacologist, DCaRP

Through: Norman Stockbridge, M.D., Ph.D.
Division Director
Division of Cardiovascular and Renal Products /CDER

To: Tiffany Brown
Regulatory Project Manager
DMIHP

Subject: Consult: NDA 22-161 (Regadenosan Injection, CV Therapeutics).

This memo responds to your consult requested of us on 02/21/08 for assistance in interpreting findings of focal cardiomyopathy (CM) in rats autopsied one day after a single 2-minute intravenous infusion of regadenoson. The latter is a selective A_{2A} adenosine receptor agonist for stress use prior to radionuclide myocardial perfusion imaging. I received and reviewed the following materials: 1) Summary of the Pharm/Tox review of the bridging study in rats; 2) Sponsor document submitted on February 7, 2008; 3) Summary document that the sponsor provided on Feb. 18,2008; and 4) the EDR link to NDA 22-161, amendment 0010, dated 12/10/2007 (which contains full references to the publications cited within this consult).
The conclusions and suggestions for follow-up expressed herein were also offered at an internal DMIHP meeting held February 25, 2008 at White Oak.

Reviewer comments :

The cardinal features of the minimal focal CM lesions elicited in the rat bridging study include their histopathology, sub- endocardial location, acute self-limiting trajectory, and association with levels of hypotension which reflexly promote plasma norepinephrine and tachcardic noradrenergic cardiac stimulation (especially since their agent also directly facilitates neuronal norepinephrine release). Such are fully consistent with the CM (and other CV pathology) provoked in the rat and other species by a variety of vasodilators at the excessive hypotensive and tachycardic dosages used in safety assays, and prevented by sympatholytic

intervention. The reviewer considers these to be irrelevant in a pharmaco-therapeutic context, whether veterinary or clinical. Even at the suprapharmacodynamic dosages needed to elicit them acutely in the rat,such lesions were self-limiting and not seen in 7 or 28 day studies (see below), typical of the CM provoked by other dilators at tachycardic dosages. Beyond that specific evidence, they are further expected to be without important sequelae based on chronic animal studies with other vasodilators, and the reviewer's understanding that such lesions have not been observed at autopsy of patients treated with minoxidil - the prototype vasodilator for provoking such lesions (as well as coronary arteriopathy) in animals.

Irrespective of the pathogenesis of the lesions, the reviewer has calculated safety margins for this pathology based not on mg/Kg or mg/M^2, but rather Cmax and AUC exposures. The 200ug/Kg threshold CM dosage , i.e., that which produces an excess CM incidence barely distinguishable from study or historical control, affords Cmax and AUC values which are about 18 and 4X, respectively, the corresponding parameters at the clinical dosage of 7 ug/Kg (standard 400 ug dose when given to a 60 Kg patient). Those projected safety factors assume the rat and human are equally sensitive to the hypotensive and tachycardic effect of regadenoson. In fact, data was provided showing that the human was appreciably less sensitive than the rat when comparing hemodynamic effects at equivalent AUC values. Accordingly, this reviewer believes these exposure-based safety margins are appreciably underestimated. How much cannot be projected as the overdose of regadenoson needed to lower blood pressure to the degree associated with excess CM in the rat (≥approx. 30%) is unknown. The clinical dose of 7ug/Kg lowers blood pressure by only a barely detectable 2-3 mmHg.

This reviewer is aware that excessive adrenergic stimulation can injure the myocardium in the context of pheochromocytoma, hyperthyroidism, myocardial infarction, and congestive heart failure. However, the extent and duration of the excess in such scenarios must be distinguished from the mild and transient excess expected in single use of regadenoson at a dose eliciting barely detectable blood pressure and heart rate changes.

Suggested future action:

The focal CM lesions appear indistinguishable from those produced in the rat by a variety of vasodilating hypotensive drugs (Greaves, 2000) and, as such, could also be considered to reflect an indirect ischemic (Balazs and Bloom, 1982) and noradrenergic intracellular calcium overload (Mann et al, 1992) pathogenesis rather than direct cytotoxicity. If so, pre-treatment of the rats with propranolol and phentolamine sufficient to block alpha and beta cardiac adrenoceptors should attenuate or prevent the CM. Such pretreatment prevents the focal CM in the rabbit provoked by a 90 minute infusion of norepinephrine, and prevents norepinephrine-provoked calcium overload and necrosis of adult cat cardiomyocytes in vitro. In extrapolating risk to humans, an indirect noradrenergic mechanism of the focal CM in the rat is only more re-assuring than direct toxicity in the sense that lesions so provoked are self-limiting. Based on behavior of a variety of vasodilators studied pre-clinically at up to and through suprapharmacodynamic dosages over the last four decades, acute CM occurs only at critical levels of hypotension and tachycardia, and in chronic studies, are without important sequelae, even in species most vulnerable to the lesions – namely, the dog.

Synopsis of bridging study:

The study of concern is a single intravenous infusion toxicity study in rats where regadenosan was administered at 0 or 0.2 mg/Kg via the [redacted] methyl boronic acid formulation or at 0, 0.08, 0.2, or 0.8 mg/Kg via a propylene glycol [redacted] formulation (N=10/sex/dose). Rats in this vehicle -bridging study were sacrificed 2 or 15 days post administration (N=5/sex/dose/time point). The highest dosage administered in the PG and the MBA formulations affords approx 16 and 4X the AUC observed in humans at the clinical dose of approx. 7 ug/Kg. The toxicity profile of the two formulations was comparable, and featured a dose-related, but minimal severity, excess focal CM seen at day 2, but not at day 15. The lesions occurred primarily in males at the following incidences for PG and MBA formulations, respectively: 0/5 (PG only), 1/5, 2/5, 5/5; 0/5(MBA only), 3/5. In females, the CM was observed only with the PG formulation, and only in the control and high-dose cohorts (1/5 and and 2/5, respectively) The CM was not seen in previous 7-day and 28-day intravenous studies (124-003; 124-011) of the MBA formulation in the same strain of rats at dosages up to 0.2 mg/Kg, i.e., that associated with CM in males in the subject bridging study.

The lesions were comprised of scattered foci of lymphocytes and macrophages, associated with few or no necrotic myocytes, and occurred primarily in the endocardial portions of the IV septum and LV apex, and, more rarely, in the RV wall. Although the incidence of lesions was dose-related, sponsor is silent as to whether, in addition to more animals being affected as dosage increased, there were more lesions in each affected rat. However, Sponsor asserts that all occurrences of CM were minimal, and histopathologically typical of that reported as commonly occurring spontaneously in the Sprague Dawley rat.

Sponsor ascribes the excess minimal CM to a noradrenergic effect on the heart secondary to direct stimulation of norepinephrine release from noradrenergic nerve terminals, but reviewer notes the profound vasodilating and hypotensive activity of their adenosine agonist. The hypotension is expected to reflexly enhance norepinephrine release at the heart, perhaps even over-riding direct adenosine receptor –mediated norepinephrine release. They cite study CVT3146.129-P in which 10 ug/Kg i.v. provoked approx. 200% increase in plasma norepinephrine titre in male rats, and return of such to baseline by about 1 hour post-dose.

Synopsis of Hemodynamic study:

Sponsor showed, in a separate study of conscious arterially cannulated rats, that over the dose range tested in the bridging study (80-800ug/Kg) ,mean arterial blood pressure was decreased to 50% of baseline for at least 90 minutes, an effect expected to markedly promote cardiac accelerator neuronal traffic and norepinephrine release, but not to be correspondingly tachycardic owing to adenosine well recognized ability to slow AV nodal conduction velocity and to increase the nodal refractoriness. Indeed, as noted above, even 10Ug/Kg doubled plasma norepinephrine level in the male rat.

Sponsor reports that the clinical dosage of approx 7 ug/Kg is virtually without appreciable effect on blood pressure or heart rate in human recipients.

Mechanism of absence of CM in repeated dose studies:

Sponsor ascribes the absence of CM in 7 and 28-day rat studies to a cardioprotective effect of such exposures to norepinephrine which protects cardiomyocytes from subsequent catecholamine insult, and submits an extensive literature on this phenomenon called cardiac pre-conditioning. While entirely plausible, no studies of protective effect of sympatholytics

(propranolol or phentolamine) on the emergence of the CM were performed as noted above.

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/s/

Albert Defelice
2/29/2008 02:35:12 PM
PHARMACOLOGIST

Norman Stockbridge
2/29/2008 05:39:17 PM
MEDICAL OFFICER