

Recombinant DNA Advisory Committee

Protocol Reviews

#0808-934 and #0808-936

December 3, 2008

Presentation Outline

John Warner PhD, CSO, Juvaris

- JVRS-100 Preclinical Overview

Thomas Monath MD, CMO, Juvaris

- Prior Human Experience

John McHutchison MD, Principal Investigator, Duke Univ.

- Hepatitis C Clinical Protocol (#0808-934)

David Claxton MD, Principal Investigator, Penn State Univ.

- Acute Leukemia Preclinical Data and Clinical Protocol (#0808-936)

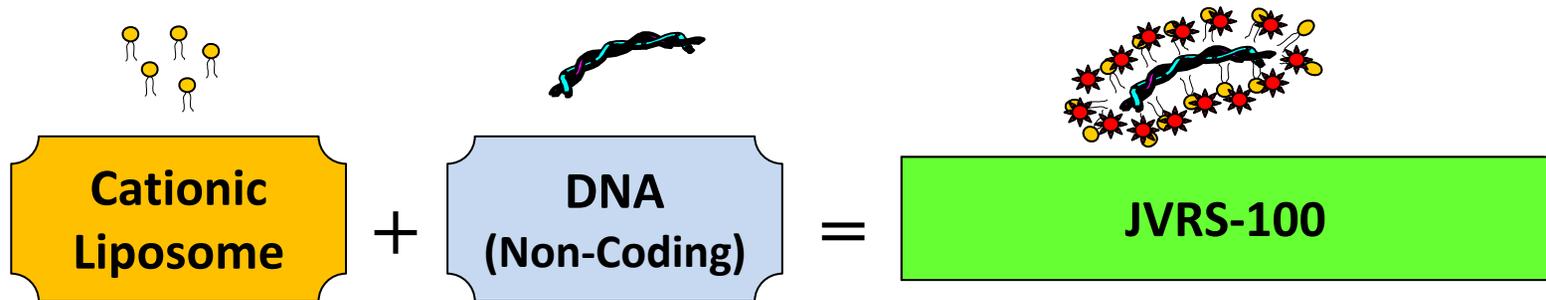
What is JVRS-100?

- Cationic liposome-non-coding plasmid DNA complex (CLDC)
- JVRS-100 is being developed as a stand-alone immunotherapy for infectious diseases and cancer and as a vaccine adjuvant when combined with antigen(s)
 - JVRS-100 activates innate immunity (TLR-9 and non-TLR-9)
 - JVRS-100 is not a gene therapy, as it contains no gene insert
 - JVRS-100 is not used for DNA vaccination, as it contains no transgene encoding a foreign protein
 - JVRS-100 is similar to VLTS-587 (same lipid & plasmid backbone, but no gene insert) providing prior preclinical & clinical information

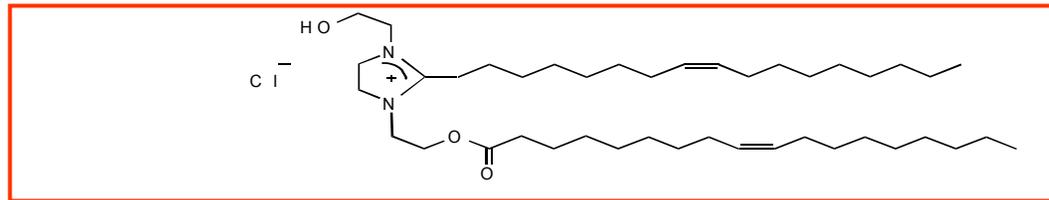
Regulatory Status

- JVRS-100 is the subject of three open, active INDs approved for different indications:
 - BB-IND#13745 JVRS-100 treatment of chronic HCV
 - FDA/CBER Office of Cellular, Tissue, and Gene Therapy
 - BB-IND#13766 JVRS-100 treatment of acute leukemia
 - FDA/CBER Office of Cellular, Tissue, and Gene Therapy
 - BB-IND #13695 JVRS-100 adjuvanted influenza vaccine
 - Phase 1 clinical trial (double-blind phase completed)
 - FDA/CBER Office of Vaccines Research & Review
- A similar product (VLTS-587) with an IL-2 transgene was previously tested by Valentis in a small number of patients with metastatic cancer
 - BB-IND #9456 (inactive)

JVRS-100 Product Description

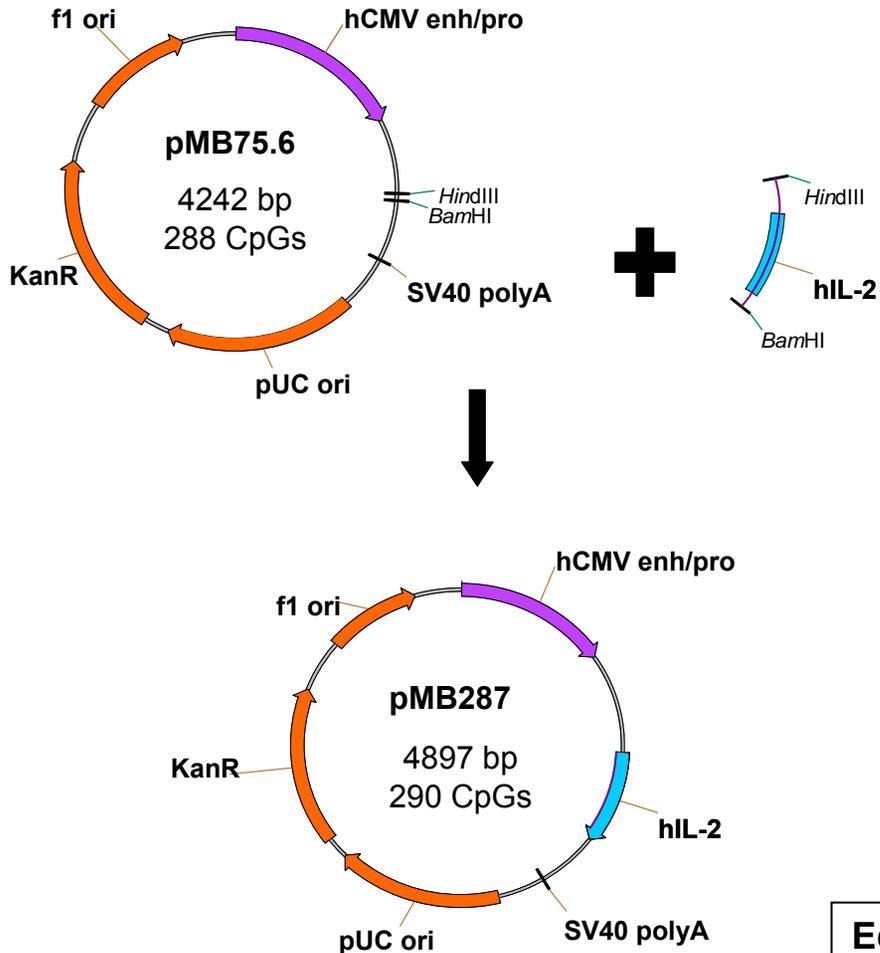


- **Product:** Cationic Liposome-DNA (non-coding) Complexes – (JVRS-100) lyophilized
- **Liposome:** Synthetic Cationic Liposome (DOTIM + Cholesterol > extrusion to 120nm)
 - DOTIM lipid: Octadecenoyloxy{ethyl-2-heptadecenyl-3-hydroxyethyl} imidazolinium chloride
 $C_{42}H_{79}N_2O_3Cl$ MW: 695.6



- Cholesterol: Synthecol
- **DNA Plasmid:** pMB75.6, 4242 bp; non-coding (no gene insert) double-stranded DNA
 - Contains 288 CpG motifs and other non-CpG immunostimulatory elements
- **Excipients:** Lactose monohydrate and Tris-HCl
- **Applications:**
 - Immunotherapeutic Setting: JVRS-100
 - Vaccine Adjuvant Setting: JVRS-100 + Antigen(s)

Comparison of pMB75.6 & pMB287(IL-2)



<u>CpG Motifs/Dose</u>			
JVRS-100	HCV/AML (min)	HCV (max)	AML (max)
Dose	0.5 µg/Kg	3 µg/Kg	5.4 µg/kg
CpG Motifs	2.2 x10 ¹⁵	1.3 x10 ¹⁶	2.3 x10 ¹⁶
VLTS-587(IL-2)	IV-IL2 (min)	IV-IL2 (max)	
Dose	2 µg/Kg	6 µg/Kg	
CpG Motifs	7.6 x10 ¹⁵	2.3 x10 ¹⁶	

Equivalent dose: pMB75.6 has ~13.7% more CpG motifs

Rabbit / Non-Human Primate Safety Studies: Summary

Parameter	Treatment	Species	# Studies (‘n’ animals each)	High Dose
Delivery Rate	VLTS-587(hIL-2) (IV)	Rabbits	2;(22),(22)	300 µg/kg
Dose Escalation	VLTS-587(hIL-2) (IV)	Rabbits	2;(22), (26)	600 µg/kg
Formulation	VLTS-587(hIL-2) (IV)	Rabbits	1; (19)	300 µg/kg
Pilot Primate	VLTS-587(hIL-2) (IV)	Cynomolgous Monkey	1; (4)	250 µg/kg
High/Low Dosing (GLP Tox)	VLTS-587(hIL-2) VLTS-587(empty) (IV)	Rabbits	1; (24)	600 µg (~171 µg/kg)
Multiple Dose Primate (GLP Tox)	VLTS-587(hIL-2) VLTS-587(empty) (IV)	Cynomolgous Monkey	1; (68)	200 µg/kg
Vaccine (GLP Tox)	JVRS-100- Fluzone (IM)	Rabbits	1; (80)	225 µg (~64 g/kg)

JVRS-100 proposed human clinical dose levels are 0.5 to 5.4 µg/kg

Rabbit GLP Toxicology Summary

- **Rabbit Toxicology (Gene Therapy Application – Valentis)**

Product: VLTS-587 (hIL-2 gene) or VLTS-587 (empty vector)

- IV infusion; 40 µg and 600 µg VLTS-587; 600 µg VLTS-587 (empty vector)

Treatments = day 0 or day 0 & 8; sacrifice two days after last dose (3 animals/grp)

- **Results:**

- Treatment and control groups comparable for Draize scores, histopathology, clinical chemistry and hematology

- Single High-dose VLTS-587(hIL-2) group:

Mild platelet reduction ($165 \pm 57 \times 10^3$ vs $332 \pm 33 \times 10^3$) in 2/3 animals
(not observed in 2X high-dose group)

Cholesterol elevation (1/3 animals)

Rabbit GLP Toxicology Summary (cont.)

- **Rabbit toxicology (Vaccine Adjuvant Application – JVRS-100)**
 - **Product:** JVRS-100 ± Fluzone[®] vaccine
 - IM administration (3X); 225ug JVRS-100 (64 µg/kg) ± Fluzone[®] (45 µg)
- **Results:**
 - No test article-related mortality
 - No remarkable test article-related clinical, clinical chemistry, coagulation or hematological findings, effects on body weight or food consumption
 - Body temperature transient elevation, slight erythema in all groups
 - No effect on ANA assessment
 - No adverse effects of systemic toxicity noted

GLP Non-Human Primate Safety Study Design: Multiple Dose (8X) Administration

IV Treatment	No. of M/F	Dose Level
		$\mu\text{g}/\text{kg}/\text{wk}$ (for 8 wks)
Vehicle	6/6	0
VLTS-587- Empty Plasmid (pMB75.6)	7/7	200
VLTS-587(hIL-2) Low Dose	7/7	20
VLTS-587(hIL-2) Intermediate Dose	7/7	80
VLTS-587(hIL-2) High Dose	7/7	200

Preclinical Non-Human Primate Toxicology Summary

Toxicology Outcome Profile VLTS-587(hIL-2) and VLTS-587(empty vector)

- No mortalities
- No biologically significant clinical, pathologic or histologic evidence of toxicity
- ECG and ophthalmologic evaluations negative
- Anti-nuclear antibody (ANA) not demonstrable
- Complement, fibrin split products and coagulation parameters normal
- Platelet count normal
- No evidence for disseminated intravascular coagulation (DIC)
- Dose-related, elevation in the neutrophil count, although below the upper normal reference value
- No significant increase in IL-6 levels
- No histopathological evidence of target organ toxicity

Preclinical HCV Studies

In Vitro Studies:

- T_H1 cytokine induction in Rhesus PBMC
- HCV replicon assay
- Stimulation of HCV-patient PBMC with JVRS-100 + HCV proteins

In Vivo Studies:

- Mouse cytokine induction
- HBV transgenic mouse model
[Morrey et al., Antiviral Res. (2008) 79:71]
- HCV chronically-infected chimpanzee model

JVRS-100 Preclinical HCV Efficacy Summary

In Vitro Studies:

- Increased T_H1 cytokine (IFN- α , IFN- γ) production in Rhesus PBMC compared to CpG ODN
- HCV Replicon Assay:
 - Decreased HCV RNA levels in IFN- α -sensitive cells (GSB1), but not IFN-resistant H801 cells
- HCV chronically-infected patient PBMC:
 - Increased ELISPOT T cell activation (IFN- γ) in the presence of JVRS-100 + core, NS3, NS4, or NS5 proteins

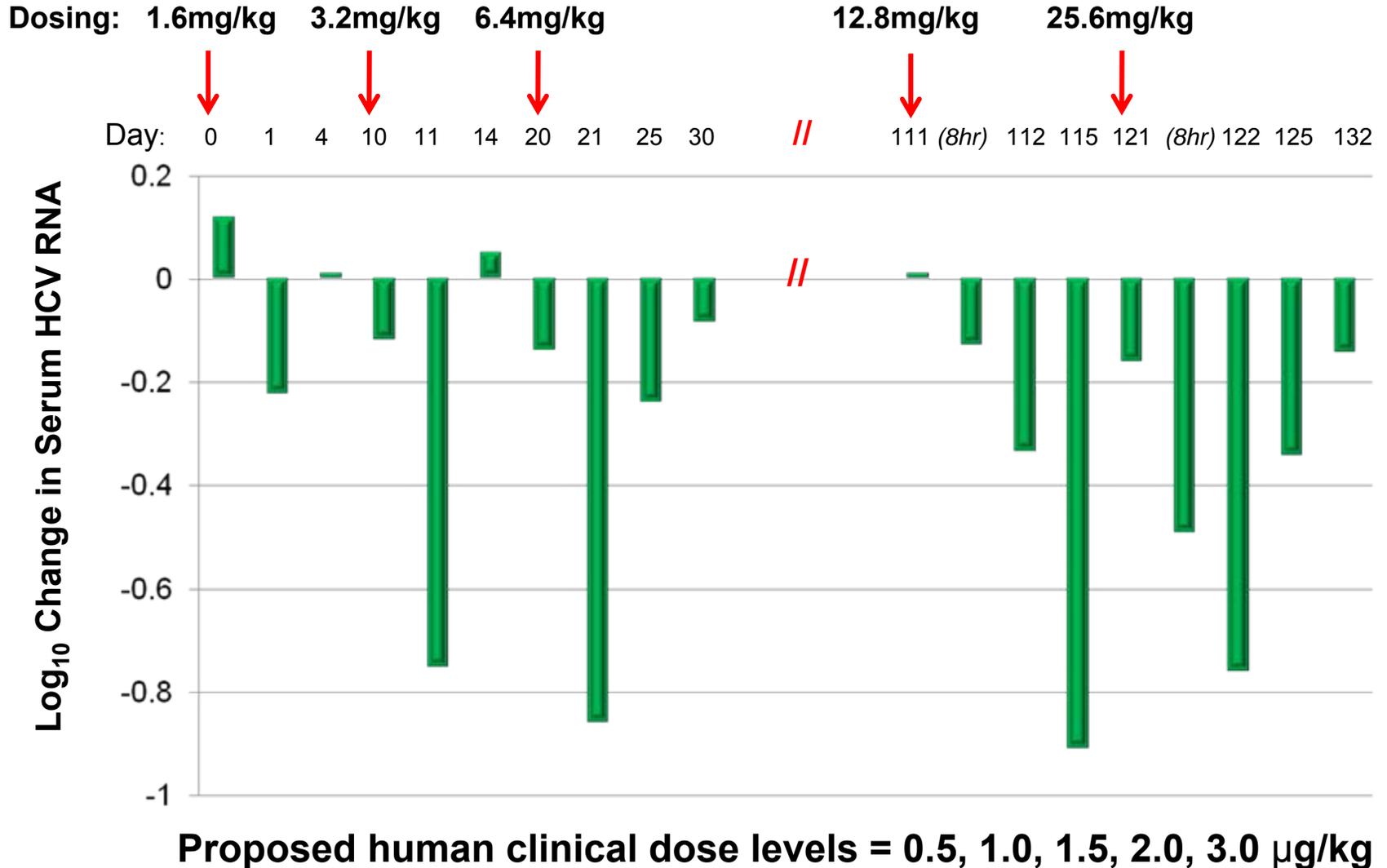
JVRS-100 Preclinical HCV Efficacy Summary

In Vivo Studies:

- HCV-Chronically-infected Chimpanzee Model (dose escalation)
 - JVRS-100 doses: 1.6, 3.2, 6.4, 12.8, 25.6 $\mu\text{g}/\text{kg}$
 - Intravenous administration
 - HCV RNA reduction, transient but associated with dosing
 - Limited cytokine induction (low doses); transient increased IFN- α , IL-6, IL-12 (high doses)
 - Limited interferon stimulating gene (ISG) activation at high doses
 - No significant clinical parameter (CBC, chemistries, etc.) changes outside of HCV disease condition

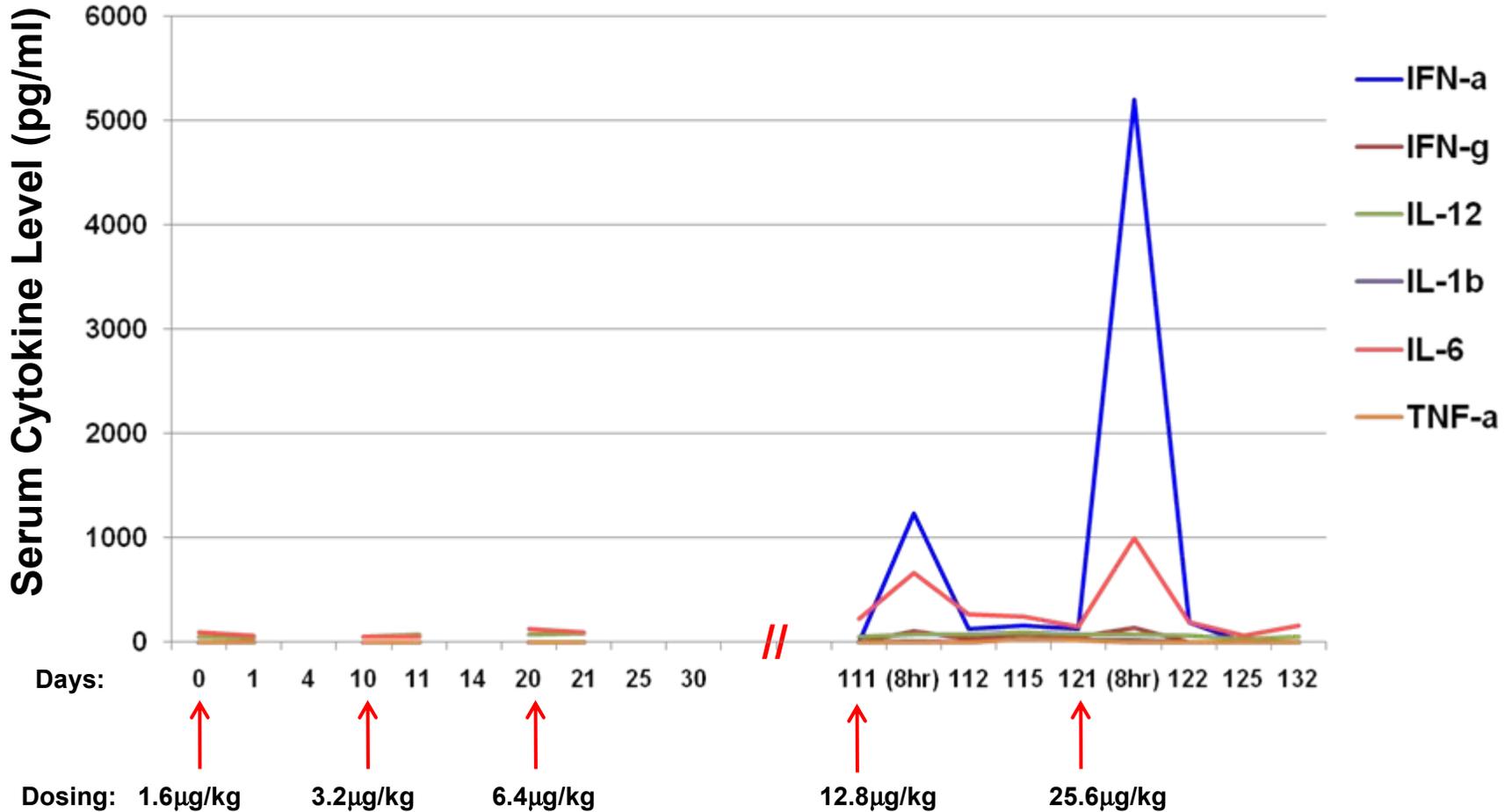
HCV Chronically-Infected Chimpanzee Pilot Study

JVRS-100 Induced Antiviral Activity in IFN Non-Responder



HCV Chronically-Infected Chimpanzee Pilot Study

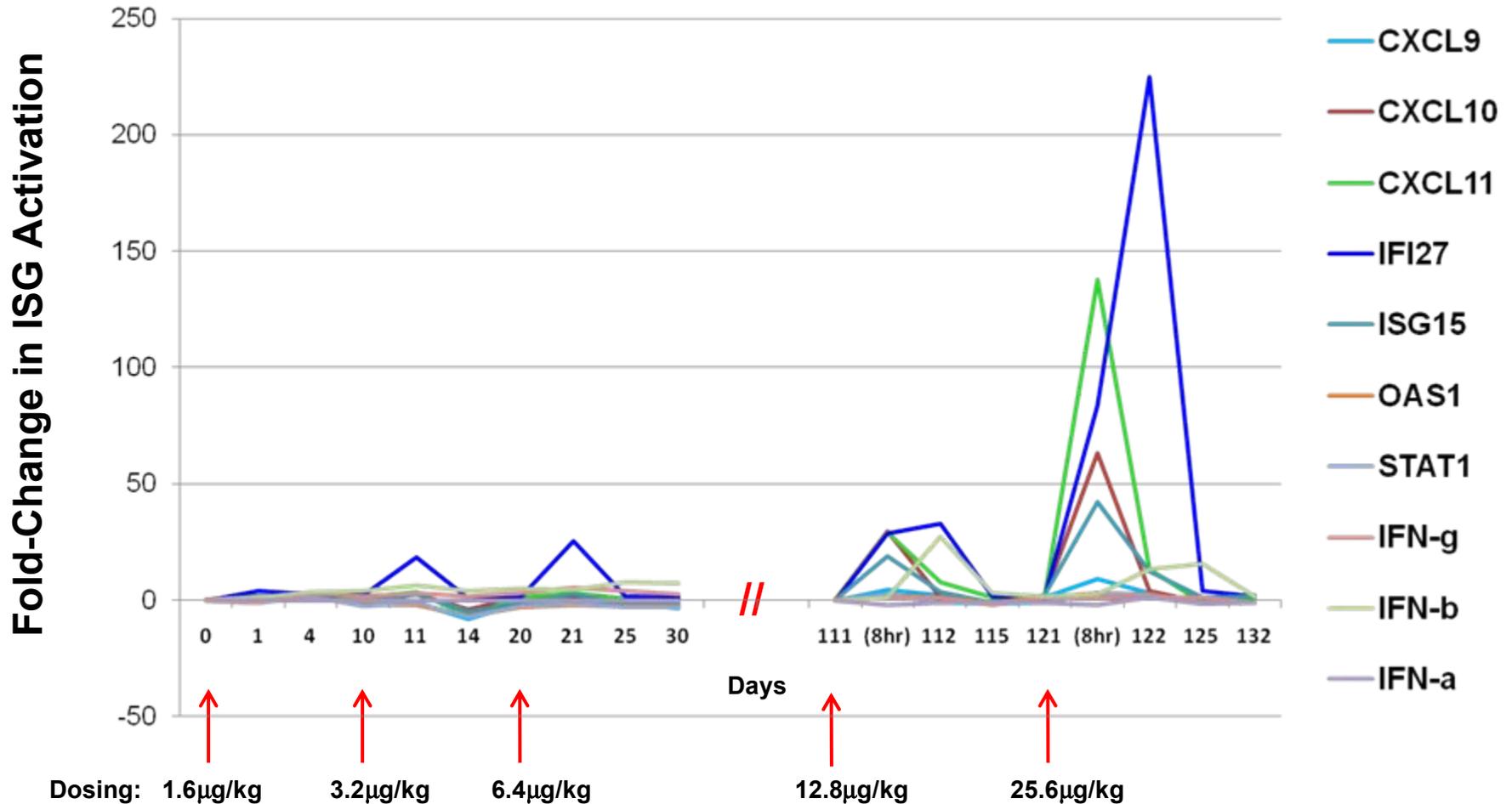
JVRS-100 Induced Serum Cytokine Responses



Increased IFN- α , IL-6, IL-12 at higher dose levels (12.8 and 25.6 $\mu\text{g}/\text{kg}$)

HCV Chronically-Infected Chimpanzee Pilot Study

JVRS-100 Induced Interferon Stimulated Genes (PBMC)



Increased expression - CXCL 9, CXCL 10, CXCL 11, IFI27, ISG15, IFN-β

JVRS-100 Treatment ↑

Prior Human Experience

Valentis and Juvaris Clinical Studies

**Tom Monath MD
Juvaris BioTherapeutics**

Valentis Clinical Study (IL2 Gene Therapy)

- Phase I, Multi-Center, Open-Label, Dose-Escalation Study of the Safety and Tolerability of Intravenously Administered VLTS-587 in Patients with Solid Tumors and the Presence of Metastases or Primary Cancer in the Lungs
- RAC # 0007-409; BB-IND 9456.
- VLTS-587: cationic liposome-plasmid DNA encoding IL-2.
- Administered twice IV at 1-week interval

Cohort (N=3)	Dose
1	2 µg/kg
2	6 µg/kg
3	20 µg/kg
4	60 µg/kg
5	80 µg/kg
6	100 µg/kg

Valentis Clinical Study Summary

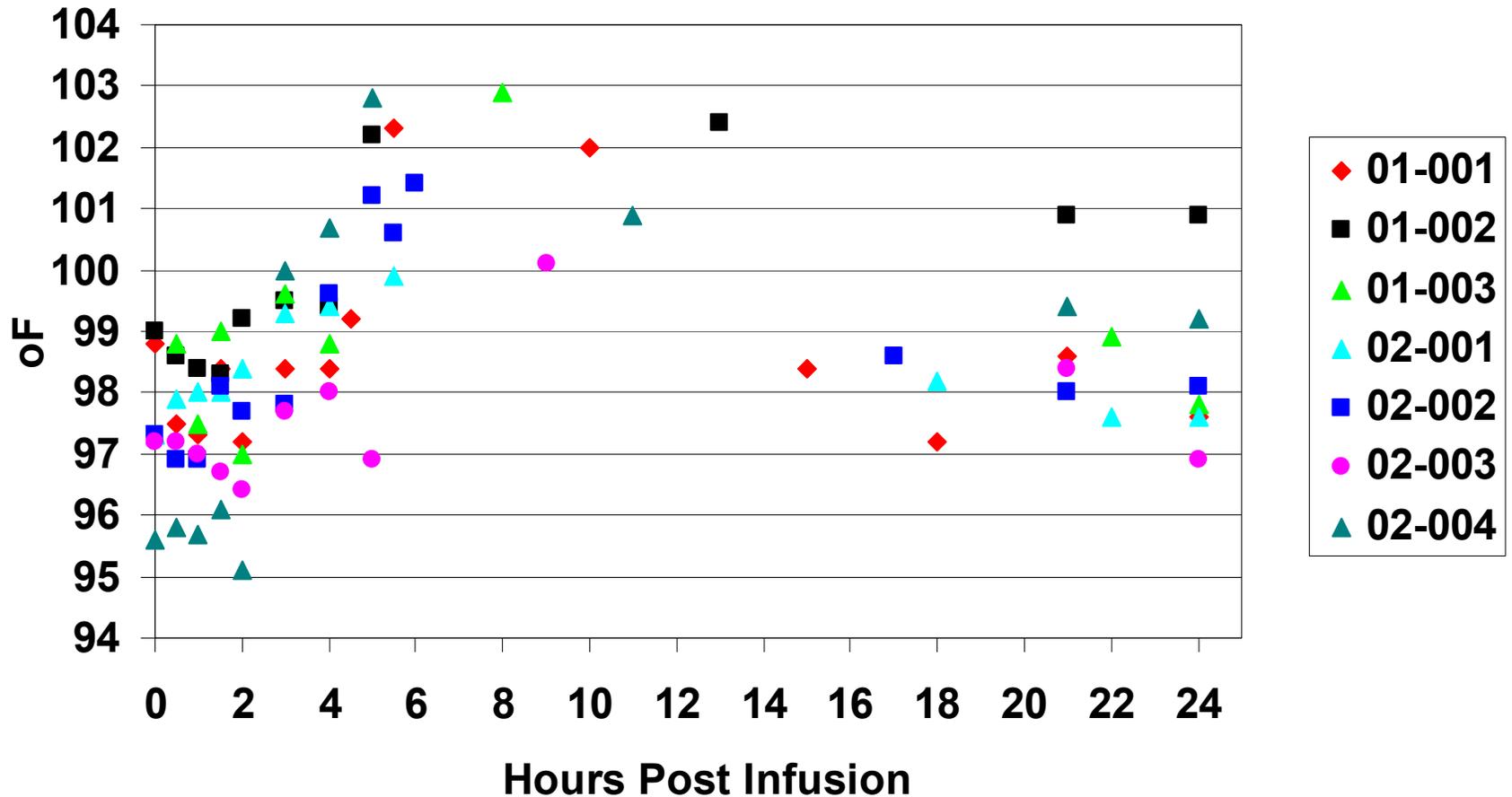
- 5 patients received 2 µg/kg Day 0, 7
- 1 patient received 2 µg/kg Day 0 (D/c'd progressive Ca)
- 1 patient received 2 x 6 µg/kg Day 0, 7
- 2 patients experienced SAEs (11 reported):
 - Unrelated to study medication
 - attributed to underlying malignancies.
- Early study termination:
 - Fever and other systemic side effects [Grade 1 or 2 = mild to moderate] at low doses, suggesting innate immune activation
 - Not possible to ascend study to high doses of VTLS-587 required for IL-2 gene expression

Incidence of Drug related Adverse Events

	Cohort 1 N = 6		Cohort 2 N = 1	
Body System/ Preferred Term	Grade 1	Grade 2	Grade 1	Grade 2
Cardiac disorders Tachycardia NOS			1	
Eye disorders Vision blurred	1			
Gastrointestinal disorders Aptyalism Diarrhea NOS Nausea Vomiting NOS		1 2 2 4		
General disorders and administration site conditions Chest pressure sensation Fatigue Pyrexia Rigors	1 3 2 1			1 1
Metabolism and nutrition disorders Anorexia		1		
Musculoskeletal and connective tissue disorders Muscle twitching Myalgia	1 2			
Nervous system disorders Headache NOS Incoherent	2 1	1		
Respiratory, thoracic and mediastinal disorders Wheezing	1			
Skin and subcutaneous disorders Sweating increased		1		1
Vascular disorders Flushing Hypertension NOS Hypotension NOS	1 1		1	

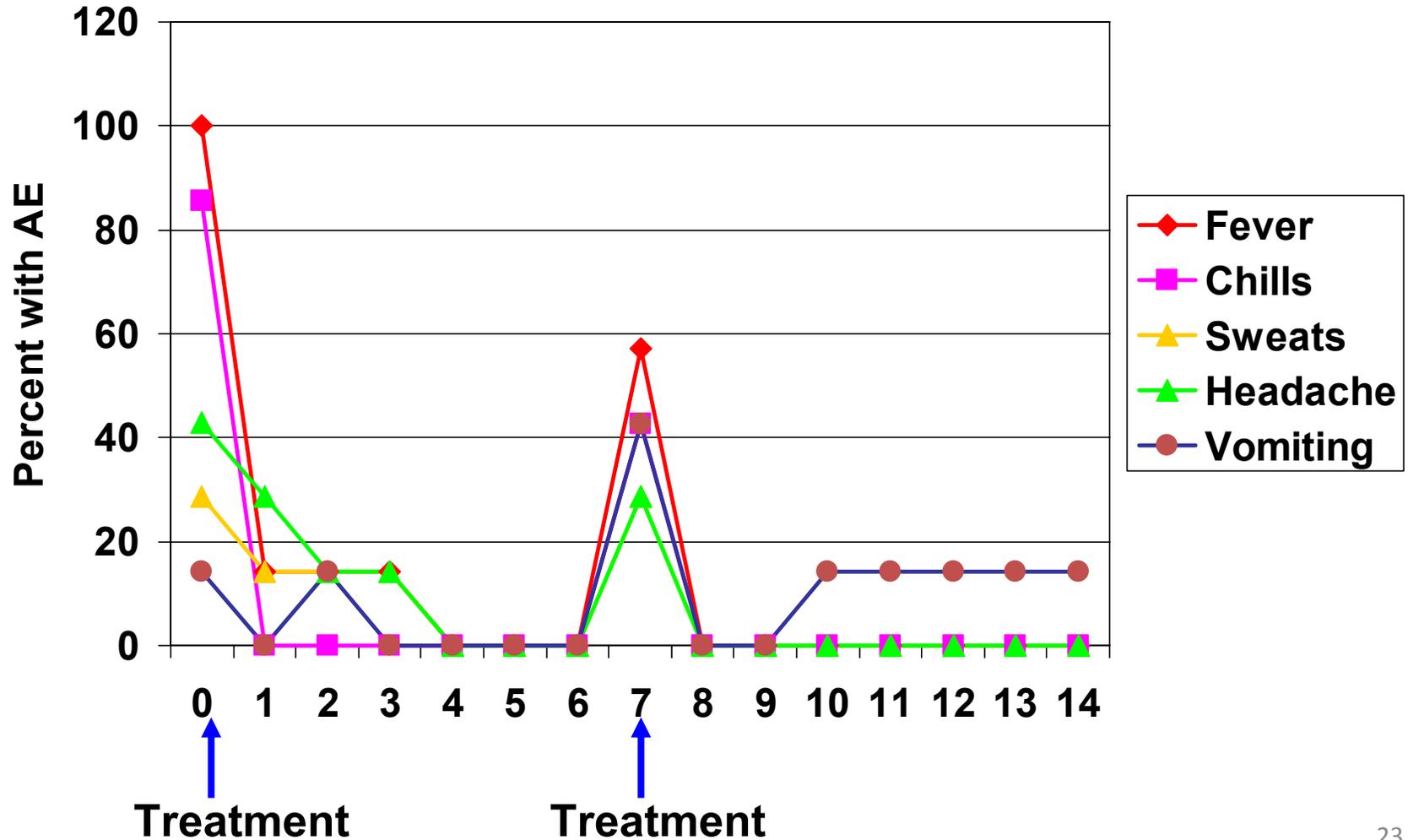
Valentis IL-2 Gene Therapy Febrile Responses

First Infusion



Adverse Events by Day

Valentis Study



Juvaris Adjuvanted Flu Study

- Randomized, Double Blind, Controlled Phase I Trial Fluzone[®] Trivalent Influenza Vaccine with Ascending Doses of JVRS-100 Adjuvant
- BB-IND 13695

Juvaris Adjuvanted Flu Study Design

- Pioneer Group of 8 subjects/dose cohort, Day 7 safety data reviewed by DSMB
- Dose ascension recommended by DSMB 7 days after review of safety data for last patient(s) in previous cohort

Group	N	Vaccine	JVRS-100 (µg)	Fluzone (µg)
1a	20	JVRS-100 + Fluzone	7.5	22.5
1b	6	Fluzone		22.5
1c	6	Fluzone		45
2a	20	JVRS-100 + Fluzone	25	22.5
2b	6	Fluzone		22.5
2c	6	Fluzone		45
3a	20	JVRS-100 + Fluzone	75	22.5
3b	6	Fluzone		22.5
3c	6	Fluzone		45
4a	20	JVRS-100 + Fluzone	225	22.5
4b	6	Fluzone		22.5
4c	6	Fluzone		45

Adverse Event by Cohort	JVRS-100 + Fluzone No. subjects (%)				Fluzone No. subjects (%)	
	7.5 µg N=20	25 µg N=19	75 µg N=20	225 µg N=20	22.5 µg N=25	45 µg N=24
Abdominal pain	0	0	0	2 (10)	2 (8)	1 (4)
Diarrhea	1 (5)	0	1 (5)	1 (5)	1 (4)	3 (13)
Nausea	1 (5)	0	1 (5)	4 (20)	1 (4)	1 (4)
Chills	2 (10)	1 (5)	2 (10)	4 (20)	2 (8)	0
Fatigue	5 (25)	1 (5)	8 (40)	13 (65)	5 (20)	5 (21)
Feeling hot	5 (25)	2 (11)	1 (5)	10 (50)	2 (8)	0
Injection site erythema	1 (5)	0	2 (10)	5 (25)	2 (8)	1 (4)
Injection site pain	10 (50)	13 (68)	18 (90)	18 (90)	9 (36)	14 (58)
Injection site pruritus	0	1 (5)	0	2 (10)	1 (4)	0
Injection site swelling	1 (5)	1 (5)	3 (15)	5 (25)	1 (4)	0
Malaise	4 (20)	2 (11)	1 (5)	10 (50)	2 (8)	4 (17)
Upper respiratory infection	0	1 (5)	0	0	4 (16)	1 (4)
Hemoglobin decrease	0	1 (5)	0	2 (10)	1 (4)	2 (8)
WBC increase	2 (10)	0	0	0	1 (4)	0
Arthralgia	1 (5)	1 (5)	2 (10)	4 (20)	4 (16)	1 (4)
Myalgia	5 (25)	1 (5)	4 (20)	7 (35)	5 (20)	3 (13)
Dizziness	0	2 (11)	0	0	0	0
Headache	7 (35)	4 (21)	5 (25)	9 (45)	3 (12)	6 (25)
Somnolence	3 (15)	1 (5)	3 (15)	8 (40)	3 (12)	3 (13)

Intensity of Adverse Events –All Moderate AEs

Adverse Event by Cohort	JVRS-100 + Fluzone No. subjects (%)				Fluzone No. subjects (%)	
	7.5 µg N=20	25 µg N=19	75 µg N=20	225 µg N=20	22.5 µg N=25	45 µg N=24
Diarrhea	1 (5)	0	0	0	0	0
Nausea	1 (5)	0	1 (5)	0	1 (4)	0
Vomiting	1 (5)	0	0	0	0	0
Chills	0	1 (5)	1 (5)	0	1 (4)	0
Fatigue	0	0	2(10)	1 (5)	1 (4)	2 (8)
Feeling hot	0	1 (5)	1 (5)	1 (5)	0	0
Injection site erythema	0	0	0	2 (10)*	0	0
Injection site pain	1 (5)	0	4 (20)	5 (25)	0	0
Malaise	2 (10)	1 (5)	1 (5)	0	2 (8)	0
Upper respiratory infection	0	0	0	0	1 (4)	0
Hemoglobin decrease	0	0	0	0	1 (4)	0
ALT/AST increased	0	0	0	0	0	1 (4)
Arthralgia	0	1 (5)	1 (5)	0	0	0
Myalgia	0	0	0	0	1 (4)	0
Headache	0	2 (11)	0	1 (5)	0	2 (8)
Somnolence	0	0	1 (5)	1 (5)	1 (4)	0

Adverse Events –Other

- A single grade 3 (severe) adverse event (JVRS-100 225 µg) group- injection site erythema (intensity based on diameter), resolved in <48 hr
- No elevated temperatures
- One thrombocytopenia, grade 1, Fluzone 45 µg group
- All adverse events resolved

Conclusion:

JVRS-100 appeared to be well tolerated, with mild injection site reactions occurring in highest dose groups (75 and 225 µg) and some systemic symptoms (fatigue, malaise, possibly nausea) at the highest dose (225 µg)

HCV Clinical Protocol

Hepatitis C Clinical Protocol (#0808-934)
BB-IND#13745

John McHutchison MD
Duke University

Juvaris HCV Phase 1 Study

- Phase I, Open-label Study of the Safety, Tolerability, and Therapeutic Activity of JVRS-100 Cationic Lipid-DNA Complex in Patients with Chronic Hepatitis C Infection Who Relapsed After Receiving Interferon-Ribavirin Treatment
- **RAC # 0808-934**
- **BB-IND 13745**

Juvaris HCV Phase 1 Study

- Study Design and Objectives
 - Patient population: Adults 18-64 years with HCV Genotype 1 pegIFN- α /ribavirin responder-relapsers
(N=18-36)
 - IV administration
 - 2 stages:
 - Dose Escalation Phase, single dose ascension to define MTD (N=18)
 - Repeat Dose Phase (q10 days, max 3 doses) at MTD (N=18)
 - Evaluate safety, tolerability, pharmacokinetics (pDNA)
 - Efficacy
 - HCV viral kinetics
 - T cell responses to structural, nonstructural HCV antigens
 - Serum cytokines

Juvaris HCV Phase 1 Study

Major Inclusion Criteria

- Inclusion
 - Age 18-64, good general health
 - HCV RNA >1000 copies/mL for >6 mo.
 - Prior treatment with pegIFN-Riba for 12 wk and documented relapse
 - Adequate bone marrow, renal, hepatic, coagulation function by specified minimum clinical lab values
 - Practicing birth control (males and females)

Juvaris HCV Phase 1 Study

Major Exclusion Criteria

- Exclusion
 - HBV, HIV infection
 - Cirrhosis, Child-Turcotte-Pugh score ≥ 6
 - History of cancer or autoimmune disease
 - Cardiac disease NYHA \geq class 2
 - Immunosuppressive medication
 - Positive ANA
 - EtOH abuse, significant psychiatric illness
 - Prior treatment with CpG oligodeoxynucleotides

Juvaris HCV Phase 1 Study

Stopping Rules

- Dose established by Valentis 2 – 6 $\mu\text{g}/\text{kg}$
 - Juvaris' starting dose is 4-fold lower at 0.5 $\mu\text{g}/\text{kg}$
 - Conservative dose ascension by 0.5 $\mu\text{g}/\text{kg}$ up to 3.0 $\mu\text{g}/\text{kg}$
- Stopping rules for individual patients:
 - + pregnancy test
 - development of medical condition qualifying for initial exclusion
- Stopping rules for the study:
 - SAE or Grade 4 AE at least possibly related to study drug; or
 - a severe (Grade 3) and unexpected AE at least possibly related to study drug; or
 - a Grade 3 change in bilirubin or a Grade 3 change in ALT and/or AST lasting 1 week or more.

Juvaris HCV Phase 1 Study Design

- Dose Escalation Phase
- Groups (N=3) receive ascending doses of JVRS-100 IV, single subject/day
- Subjects hospitalized for infusion and 24 hour observation
- Subjects within cohort dosed at 48 h intervals (increased from 24h)
- Dose ascension at 10 day intervals after Safety Review Committee review

Day	0	2	4	14	16	18	28	30	32	42	44	46	56	58	60	70	72	74
Subject	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Dose (µg/kg)	0.5	0.5	0.5															
				1	1	1												
							1.5	1.5	1.5									
										2	2	2						
													2.5	2.5	2.5			
																	3	3

Dose Ascension and Adjustment

- Cohorts receive the next highest dose after the safety and tolerability of the lower dose given to the previous cohort has been assessed by the Safety Review Committee.
- The dose regimen for the next highest dose may be modified during the study by the SRC based on the tolerability profile of the study drug according to a pre-defined algorithm

Efficacy Outcome Measures

Dose Escalation Phase

Day	0	+0.5, +4, +8 hr	+24 h	+48 h	7	10	14	30
HCV RNA	X	X (+8 h only)	X	X	X		X	X
T cells	X							
Cytokines IFN- α , IFN- γ , Il-2, IL-12, IL-10, IP- 10	X	X	X	X	X			
pDNA level	X	X	X	Sample collected	X		Sample collected	X

Juvaris HCV Phase 1 Study Design

- **Repeat Dose Phase**

- Subjects (N=18) receive MTD of JVRS-100 IV
- Subjects may have participated in Dose Escalation Phase
- Out-patient study
- Diary and temperature monitoring, visit +48 h
- Three sequential treatments at 10 day intervals
- Safety Review Committee monitors adverse events
- Algorithm for dose adjustment in the event of grade 3 expected AEs
- Follow up visits at Day 30, Day 60, 12 wk, 6 and 12 months

Efficacy Outcome Measures Repeat Dose Phase

Day	0	2	10	12	20	22	30	60	12 wk*	6 mo*	12 mo.*
JVRS-100 Rx	X		X		X						
HCV RNA	X	X	X	X	X	X	X	X	X	X	X
T cells	X						X				
Cytokines IFN- α , IFN- γ , IL-2, IL-12, IL-10, IP-10	X	X	X	X	X	X	X				
pDNA level	X	X	X	X	X	X	X	X	X	X	

* After final infusion

JVRS-100 for the Treatment of Relapsed or Refractory Acute Leukemia

David Claxton MD

Sponsored by:

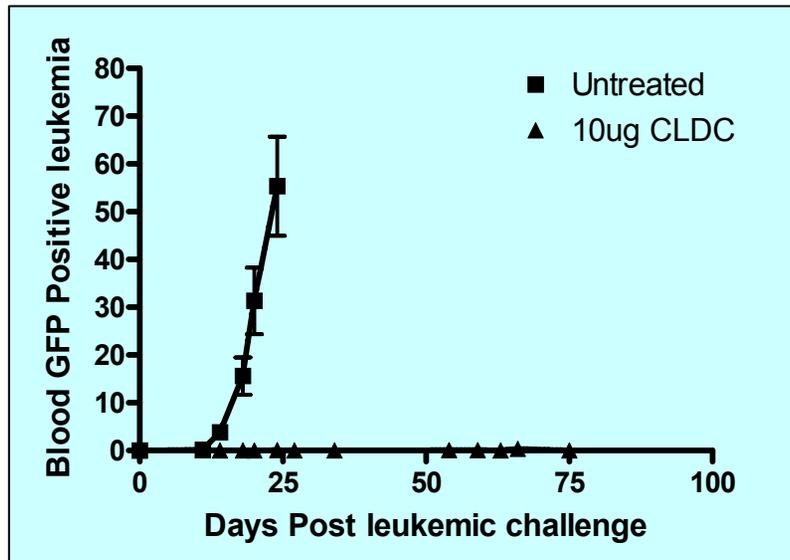
**Penn State Milton S. Hershey Medical
Center**

Preclinical Leukemia Studies

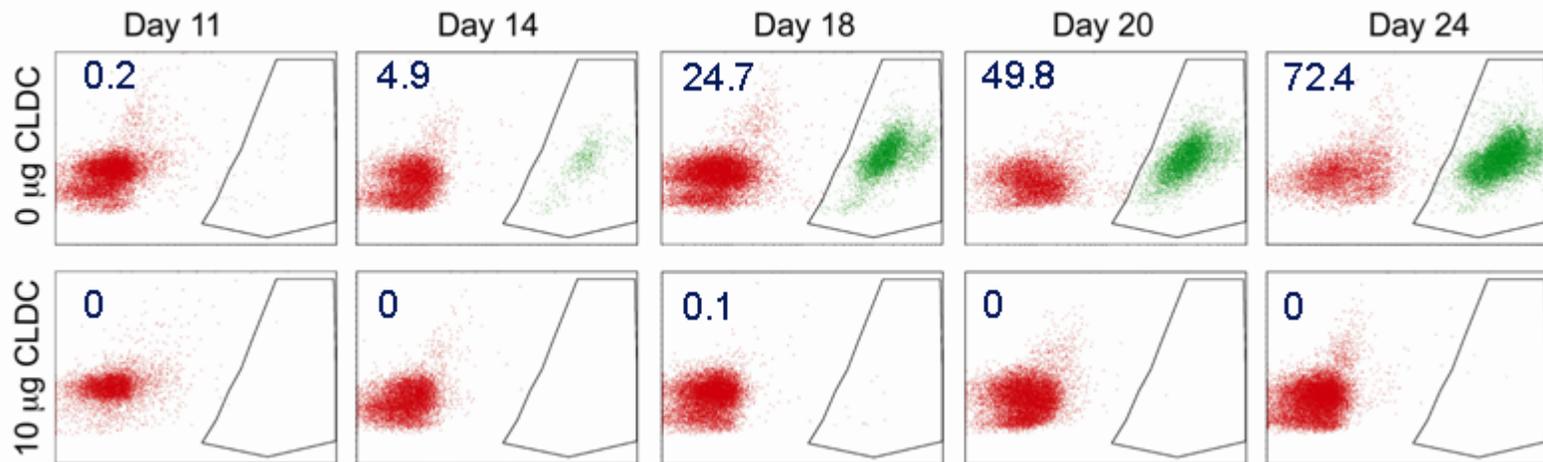
- Toxicity profile acceptable in multiple animal models
- Activation of T-cells, B Cells, NK cells, macrophages
- Active in MCA-205, B16 Melanoma and CT26 murine models (solid tumors).
- Tested in 32D-bcr-abl and Wehi-3B leukemias:
 - SC and IV routes both showed activity – prolonged survival and some animals cured – both models.
 - Anti-leukemic activity conferred by adoptively transferred lymphocytes and inhibited by anti-CD8.

Preclinical Studies - 1

JVRS-100 Treatment after Leukemic Challenge

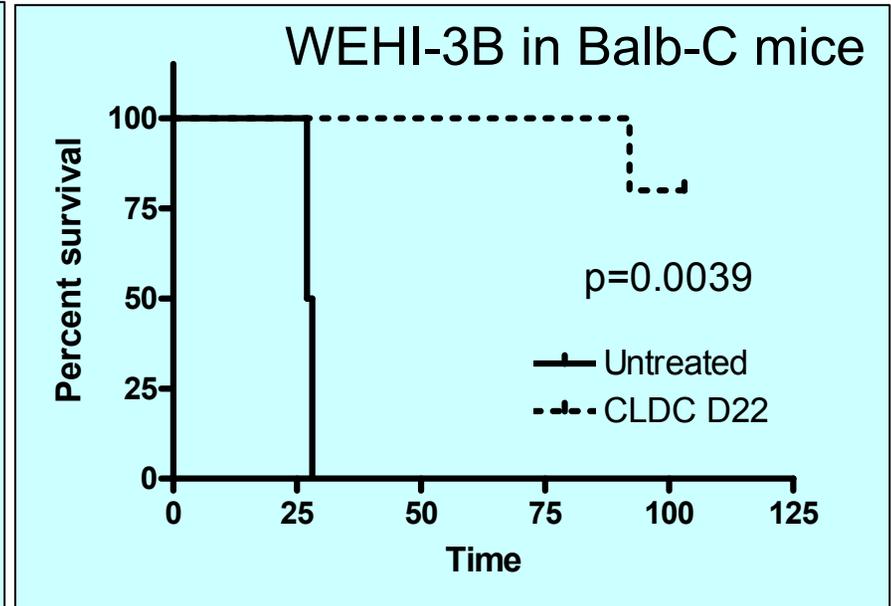
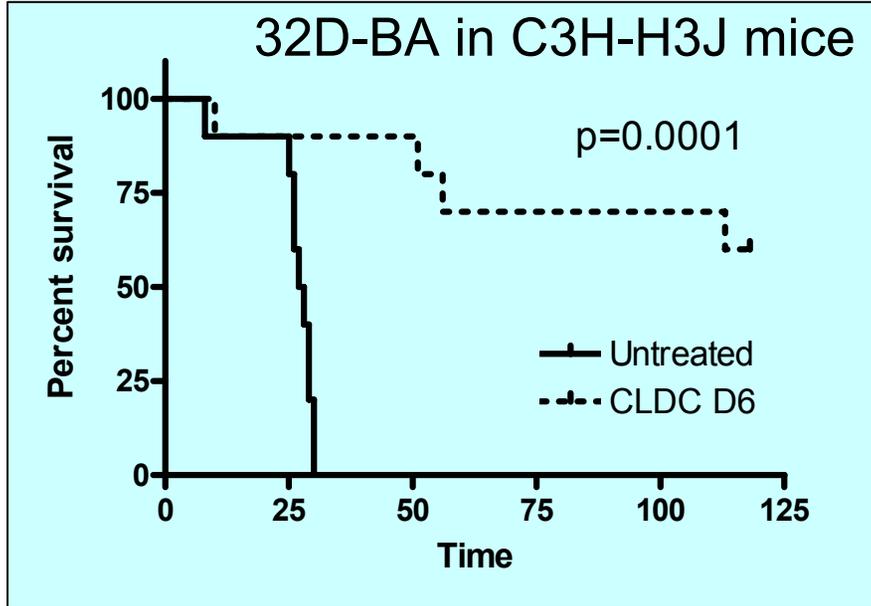


JVRS-100 (CLDC) effectively controls growth of GFP-labeled Leukemic Cells in C3H-HEJ Mice



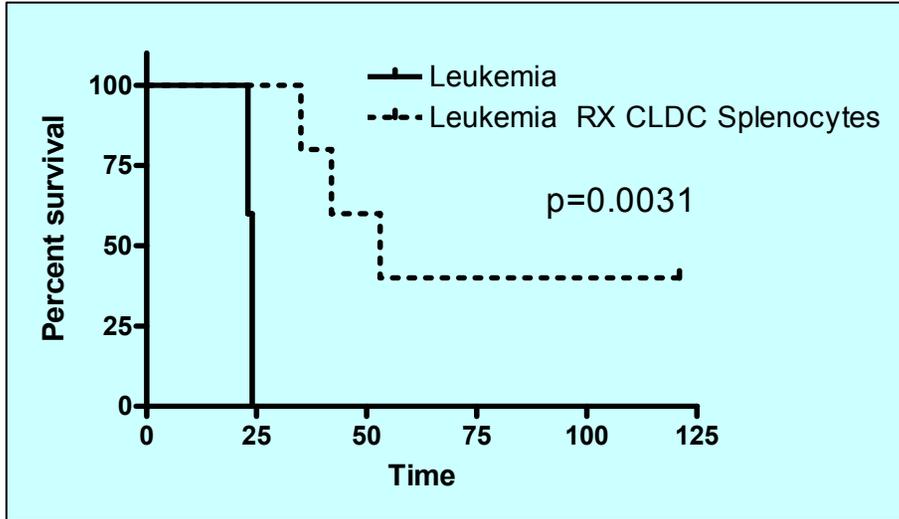
Preclinical JVRS-100 Studies – 2

Survival of Mice after Leukemic Challenge

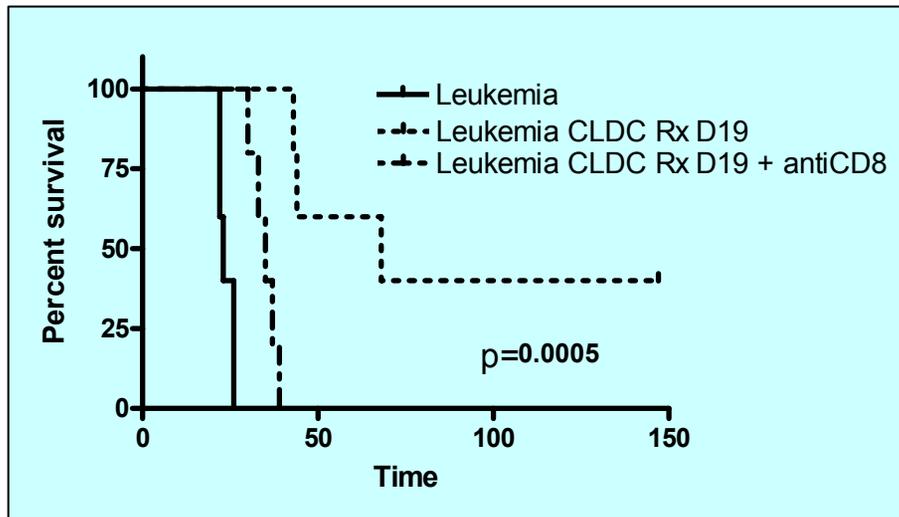


In studies using two mouse models significant cure fractions were observed even in animals treated with JVRS-100 well after establishment of leukemia.

JVRS-100 Anti-leukemic Effects are Apparently Mediated by CD8⁺ Cells



- Transfer of Splenocytes from animals treated with JVRS-100 to secondary animals prevented leukemic death.



- Treatment of animals with anti-CD8 abrogated the antileukemic effect of JVRS-100.

Penn State Milton S Hershey Medical Center Acute Leukemia Phase 1 Study

**A Phase 1 Trial of the Immunostimulant
JVRS-100 for the Treatment of Patients with
Relapsed or Refractory Leukemia**

RAC # 0808-936

BB-IND 13766

Acute Leukemia Phase 1 Study

Study Design and Objectives

- Objectives
 - Safety, MTD and RP2D
 - Efficacy: % blasts, durability of response and survival
- Patient population: ≥ 18 years w/ relapsed or refractory leukemia
 - 23 patients
 - JVRS-100 IV q7 days, 3 infusions per cycle, up to 6 cycles
- Stage 1: 1 patients at each dose level until \geq Grade 2 non-hematological toxicity *or* until Dose level 5 tolerated then:
- Stage 2: Dose escalation in cohorts of 3-6
- Final cohort at RP2D to be expanded to 12 patients

Acute Leukemia Phase 1 Study

Major Inclusion Criteria:

- Signed informed consent of at least 18 years of age
- Histologically or cytologically documented relapsed or refractory acute leukemia (AML or ALL)
- Unlikely to benefit from standard therapy or refuse standard therapy
- ECOG Performance Status of 0-2
- Adequate renal and hepatic function
- No hematologic criteria for WBC, Hgb, or platelets
- Negative virology screen for HIV, HBV, and HCV
- Females of childbearing potential must have a negative serum pregnancy test
- Practicing birth control (males and females)

Acute Leukemia Phase 1 Study

Major Exclusion Criteria:

- Active CNS leukemia. Prior CNS leukemia allowed provided current CSF cytology is normal.
- Off chemotherapy, radiation therapy, or immunotherapy for 2 wk (certain exceptions noted)
- Persistent CS tox from prior anticancer therapy, \geq Grade 2
- Bone marrow or stem cell transplant
- Chronic administration of immunosuppressive agents within 14 days of first dose
- Pregnant or lactating
- History of prior malignancy other than leukemia within the past 5 years
- Systemic fungal, bacterial, viral, or other infection not controlled

Dose Establishment, Delays, Stopping Rules

- Dose established by Valentis: 2 – 6 $\mu\text{g}/\text{kg}$
 - Starting dose is 4-fold lower at 0.5 $\mu\text{g}/\text{kg}$
 - Dose ascension 2-fold increases up to 4.0 $\mu\text{g}/\text{kg}$
 - 35% increments until DLT is reached in $\geq 2/6$, max dose 5.4 $\mu\text{g}/\text{kg}$
- Dose adjustment or dose delay in patients:
 - Fever $\geq 100.4^\circ\text{F}$ (38°C), within the previous 48 hours
 - Any AE \geq Grade 2 after treatment with JVRS-100. Further dose withheld until the event resolves to \leq Grade 1.
- Stopping rules for the study:
 - Death of a subject believed related to therapy
 - 2 AEs of Grade 4 non-hem tox believed related to therapy
 - 2 episodes of CS thrombocytopenia believed related to therapy

Phase 1 Study Design

- Subjects (N=23) receive JVRS-100 IV
- After 1st dose, patients hospitalized and 24 hour observation
- Subsequent doses are given in the out-patient area
- Drug administered on Days 1, 8, and 15 of 28-day cycle*
- Dose ascension after Cycle 1
- No more than 6 cycles to be administered
- Follow up visits: 24-hrs post dose, 28 days post last dose, and subsequently every 3 months until 12 months

* Chosen as a practical dosing schedule to be re-assessed based on clinical and correlative lab data

Stage 1: Dose Escalation Phase

- 1 patient enrolled at given dose level - escalation when patient completes 1 cycle with no \geq **Grade 2 Toxicity*** (*non-hematologic tox except nausea, vomiting or fever*)
- If one patient has **Grade 2 Toxicity**, shift accrual to stage 2 with a modified Fibonacci schema, with expansion of cohort at that dose level.

Dose Levels	Dose
Level 1	0.5 μ g/Kg D1, 8, 15
Level 2	1.0 μ g/Kg D1,8,15
Level 3	2.0 μ g/Kg D1,15
Level 4	2.0 μ g/Kg D1, 8, 15
Level 5	4.0 μ g/Kg D1, 15
Change to Stage 2 with starting dose of 4.0 μ g/Kg D1, 8, 15 if no toxicity by Level 5	

* ***This represents a change to be discussed, as consistent with Design 2 in: Simon et al Journal of the National Cancer Institute, Vol. 89, No. 15, August 6, 1997***

Stage 2: Recommended Phase 2 Dose

Stage 2 Dose-Escalation Schedule Cohorts of 3-6 patients (Modified Fibonacci)

Dose level	Dose
Level 1	To start when accrual to stage 1 is terminated
Additional levels	35% increments until DLT is reached in $\geq 2/6$ patients. Maximum dose that will be tested is 5.4 $\mu\text{g}/\text{kg}$
RP2D	Expand cohort to a total of 12 patients

Dose Limiting Toxicity Definition - 1

DLTs defined by toxicity observed during Cycle 1.

- Non-hematological toxicity: Any \geq Grade 3 toxicity other than fever that is drug-related (\geq possibly related).
- Fever: Grade 4 ($> 40.0^{\circ}\text{C}$ for >24 h), or $>39.0^{\circ}\text{C}$ for >72 h considered drug-related and uncontrolled despite optimal supportive therapy.

Dose Limiting Toxicity Definition - 2

- Non-hematological laboratory toxicity: Any Grade ≥ 3 toxicity considered study drug-related.
 - If baseline value elevated prior to treatment, an increase will not be considered a DLT unless there is an elevation by ≥ 2 grades and it is of clinical significance.
- Delay in starting Cycle 2 by ≥ 14 days due to study-drug related toxicity.

JVRS-100 for the Treatment of Relapsed or Refractory Acute Leukemia

- Enrolling patients with lethal diseases without reasonable alternative therapies.
- Conceived to bring an agent with *novel anti-leukemic properties* to the clinic.
- Designed to arrive at a recommended phase 2 dose expeditiously, potentially avoiding accrual of patients to ineffective doses.
- Accompanied by correlative laboratory studies expected to yield insights into the physiology of responses to this agent.

Back-up Slides

JVRS-100 Preclinical

Back-up Slides

JVRS-100 Manufacturing Overview

cGMP Manufacturing Process

Specifications

<u>Parameter</u>	<u>JVRS-100 Specifications (Current)</u>
Appearance (before reconstitution)	White to slightly yellow cake
Reconstitution Time	Report Results
Appearance (after reconstitution)	White translucent liquid
pH	6.3 – 7.3
Conductivity	0.2 – 2.0 mS/cm
Moisture Content	≤ 3 % (w/w)
Turbidity	< 0.5 OD @ 400 nm
Plasmid Identity	Conforms to plasmid reference standard
Plasmid Concentration	0.25 – 0.35 mg/ml
Plasmid Integrity	≥ 90 % Super-coiled + % Open-circular
Lipid Identity	Conforms to DOTIM and cholesterol reference standards
DOTIM Concentration	1.5- 3.5 mM
Cholesterol Concentration	1.5- 3.5 mM
Particle Size Distribution	Report Results
Particulate Matter In Injections	Meets USP
General Safety	Meets CFR
Endotoxin	<100 EU/mg DNA
Sterility	Sterile

Comparison of CMC Processes

Component/Process	Valentis	Juvaris BioTherapeutics
Plasmid DNA	pMB75.6	pMB75.6 plus human genes
Master Cell Bank Manufacture Analytical	Host cell line DH5α Charles Rivers Laboratories	Same host and process Same Enhanced/updated test
Plasmid DNA Purification Process Manufacture Analytical	Patented process Cangene Corp.	Same Progen Pharmaceutical Ltd. Enhanced/updated tests
Lipids Cholesterol DOTIM Analytical	SAFC Animal derived R&D production Synthetic R&D process Accepted on C of A, limited tests	SAFC Synthetic GMP process Synthetic GMP process Enhanced testing, qualified tests
Liposomes Manufacture Analytical	R&D process SAFC	GMP process designed for endo-free production, SAFC Enhanced/updated tests
DNA/Liposome Complex Manufacturer Analytical	Patented GMP Process Cangene Corp.	Same Althea Technologies, Inc. Enhanced/updated tests

Clinical Trial Formulations with pMB75.6

Sponsor/ Formulation	Plasmid	Plasmid Dose	Cationic Lipid	Neutral Lipid	Route
Juvaris/ JVRS-100-Flu (vaccine)	pMB75.6	Escalating to 225µg	DOTIM	Cholesterol	IM
Valentis/ C192 (cancer)	pMB75.6 plus IL-2 gene & pMB75.6 plus SEB	Escalating to 2,000µg	DOTIM	Cholesterol	IT
Valentis/ VLTS-587 (cancer)	pMB75.6 plus IL-2 gene	Escalating to 7 mg	DOTIM (previously called BODAI)	Cholesterol	IV
Valentis/ VLTS-589 <u>Multiple trials including Phase 2 (PVD)</u>	pMB75.6 plus Del-1 gene	Escalating to 84 mg	Does not contain Lipids	Does not contain Lipids	IM, Retrograde IV
Valentis/ VLTS-582 (Physician Sponsored- Esophagitis)	pMB75.6 plus MnSOD gene	30 mg	DOTIM	Cholesterol	Interaso-phageal

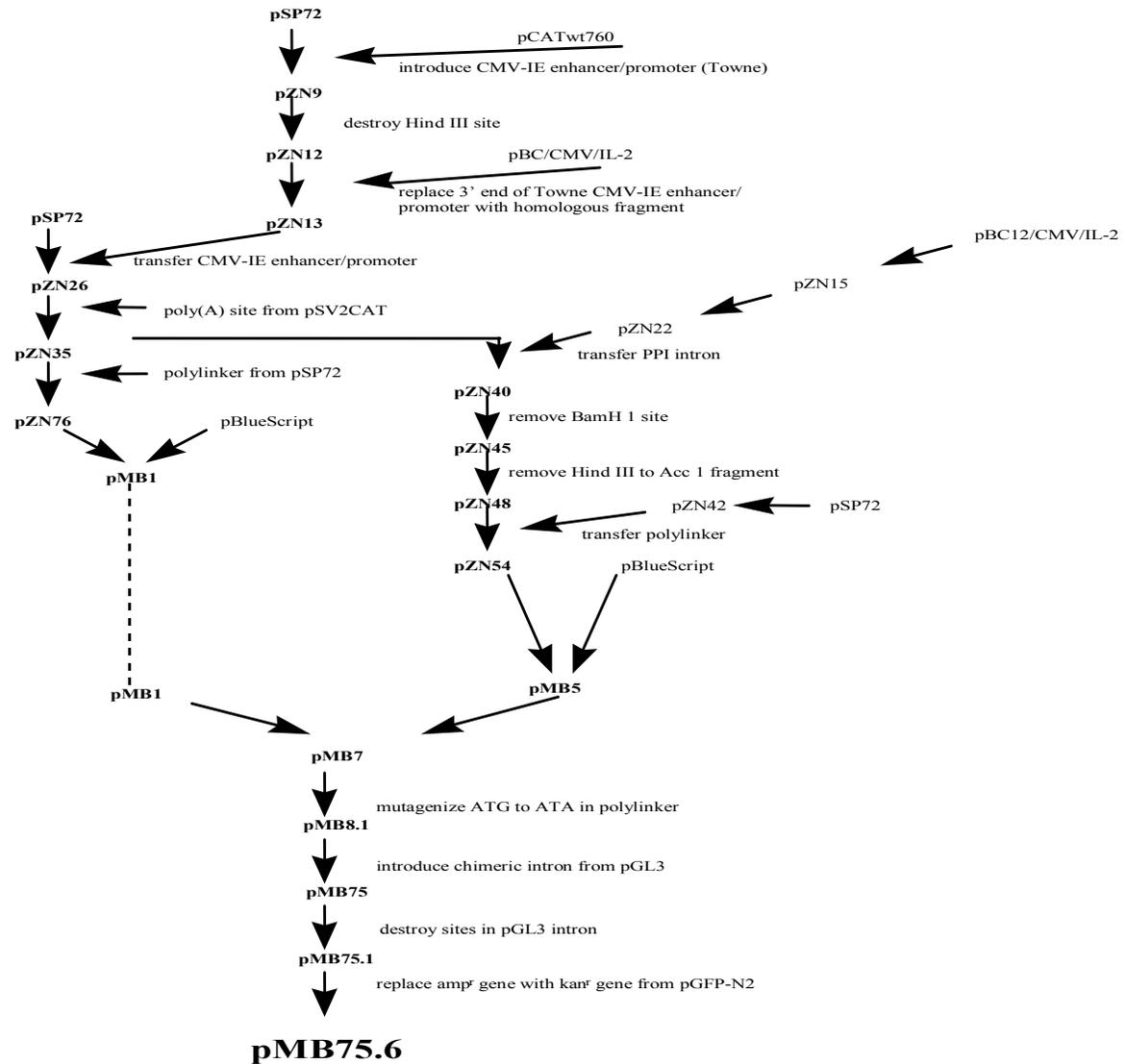
Production Host & MCB

- **Escherichia coli strain DH5 α**
 - F- ϕ 80*dlacZ*D Δ M15-D(*lacZYA-argF*)U169 *deoR recA1 endA1 hsdR17*(rk-,mk+) *phoA supE44 thi-1 gyrA96 relA1*
 - GMP Master Cell Bank for production host
 - Well characterized, stored offsite
 - Transformation and testing protocols in place
- **GMP Master Cell Bank: pMB75.6 / DH5 α**
 - Produced, tested, released
 - pMB75.6 plasmid sequenced

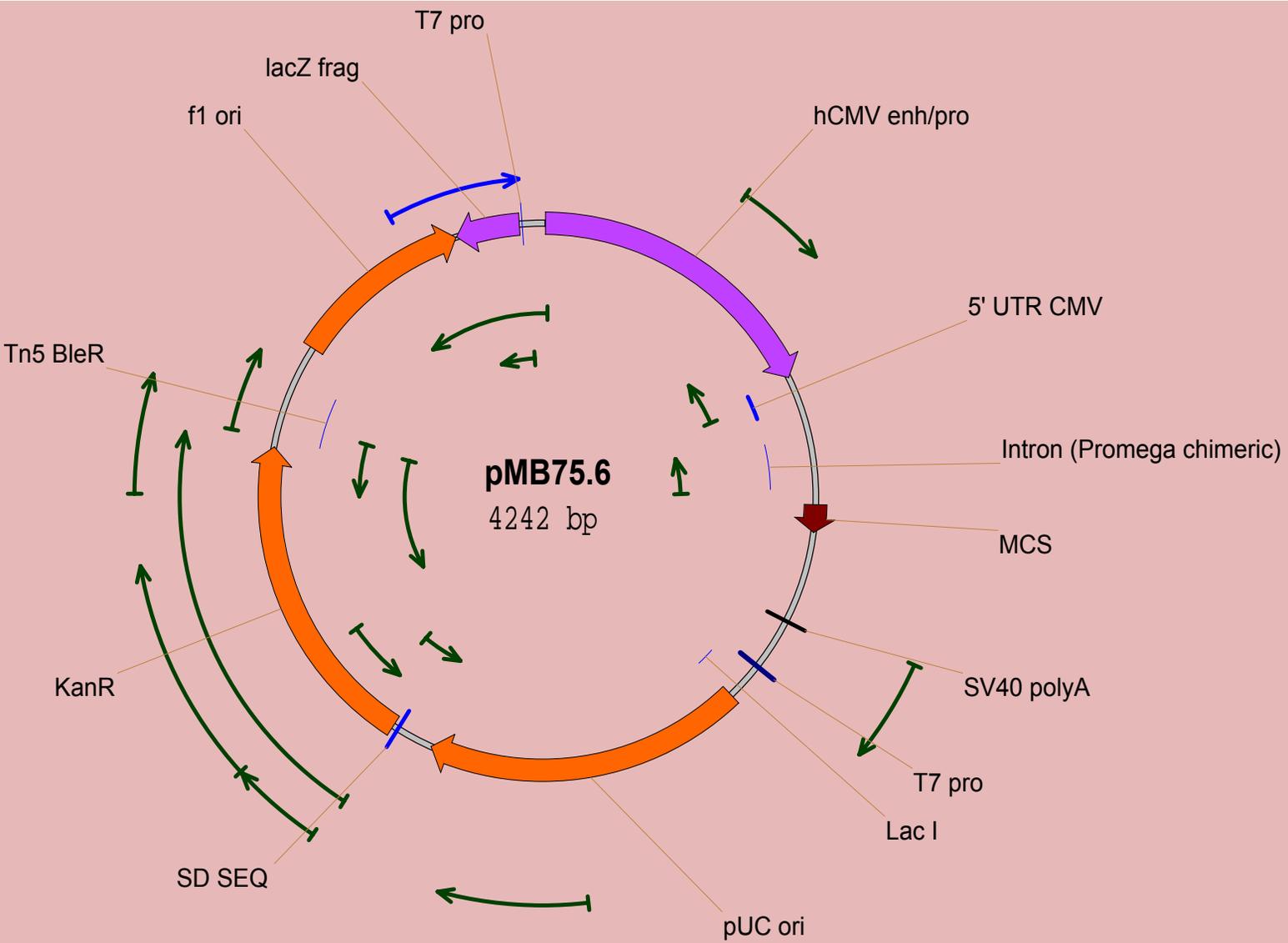
Lipid/Liposome Derivation

- Development of cationic lipids (Megabios/Valentis)
- Combinations of cationic lipid and helper lipids evaluated
 - Cationic lipids: DOTIM, EDMPC, DOTMA
 - Helper lipids: Chol, DOPE, DMPE, DOPC, DSPE, DLPE, DLPC
- Design considerations: cleavage point for natural breakdown (e.g., enoyl)
DOTIM and EDMPC
- Screening:
 - Liposome formation, DNA binding, membrane fusion capability, particle stability, DNA dissociation from complex, gene expression, safety
- Safety testing: rabbits, dogs, monkeys, humans
- DOTIM/Cholesterol combination selected for gene therapy application:
 - Stable particle formation
 - Enhanced gene expression in vivo
 - DNA binding to complex
 - Safety

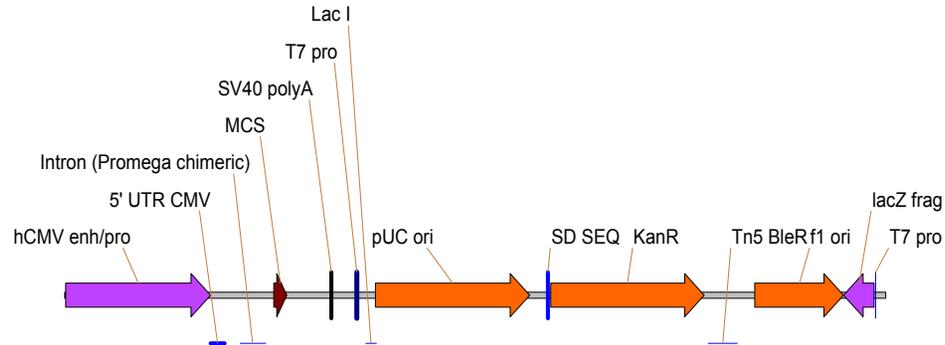
Construction of pMB75.6



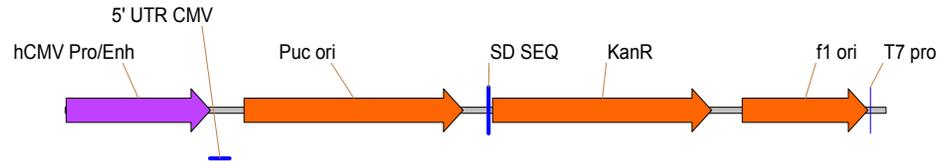
pMB75.6 Open Reading Frames



Deletions of pMB75.6



pMB75.6
4242 bp

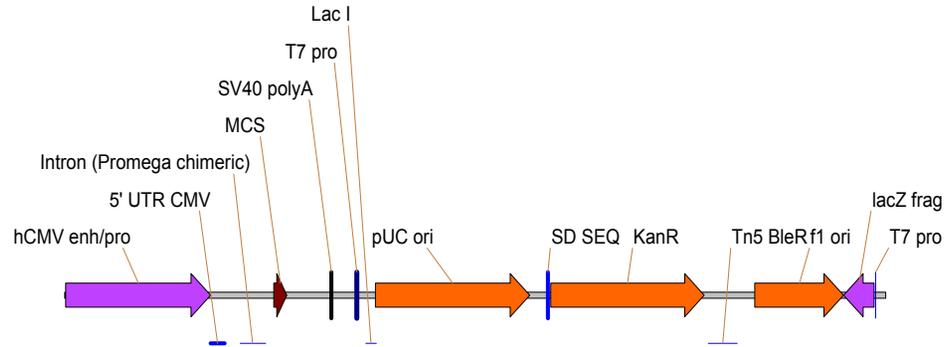


pJB1903
2971 bp

<i>Lac Z</i>	5' CMV remnants	Tn5 Ble ^R	T7 Pro #1	<i>Lac I</i>	MCS	Promega Intron	SV40 Poly A
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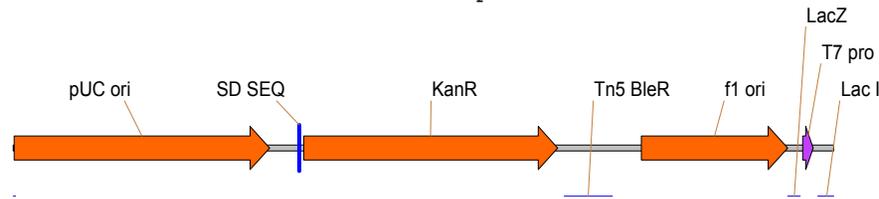
Low production yields

Deletions of pMB75.6



pMB75.6

4242 bp



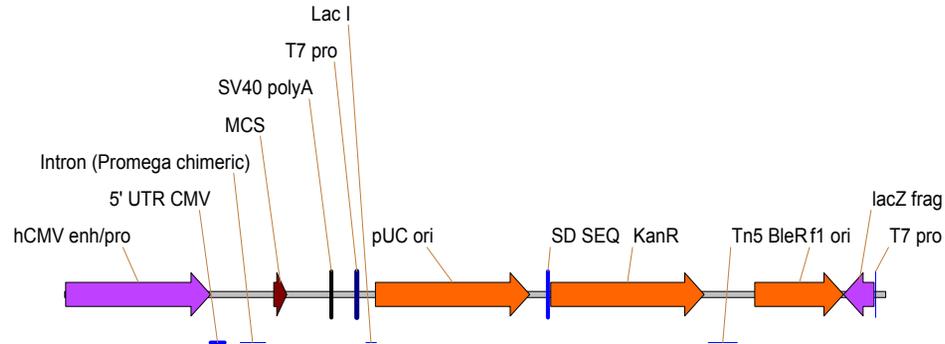
pJB1906

2565 bp

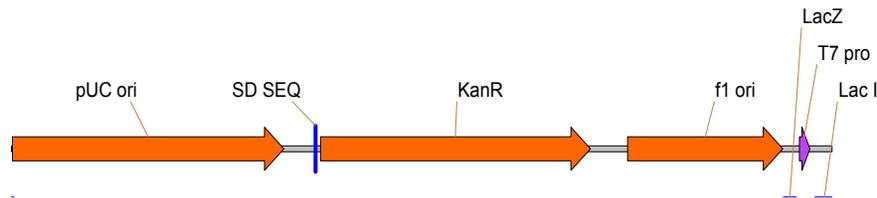
<i>Lac Z</i> (portion)	MCS	Promega Intron	SV40 Poly A	T7 Pro #2	hCMV pro/enh	5' UTR
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Low production yields

Deletions of pMB75.6



pMB75.6
4242 bp

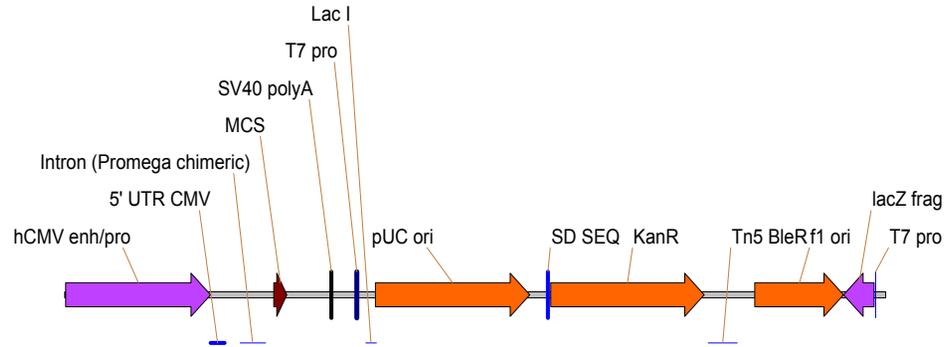


pJB1907
2412 bp

<i>Lac Z</i> (portion)	Tn5 Ble ^R	MCS	Promega Intron	SV40 Poly A	T7 Pro #2	hCMV pro/enh	5' UTR
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Low production yields

Deletions of pMB75.6



pMB75.6
4242 bp



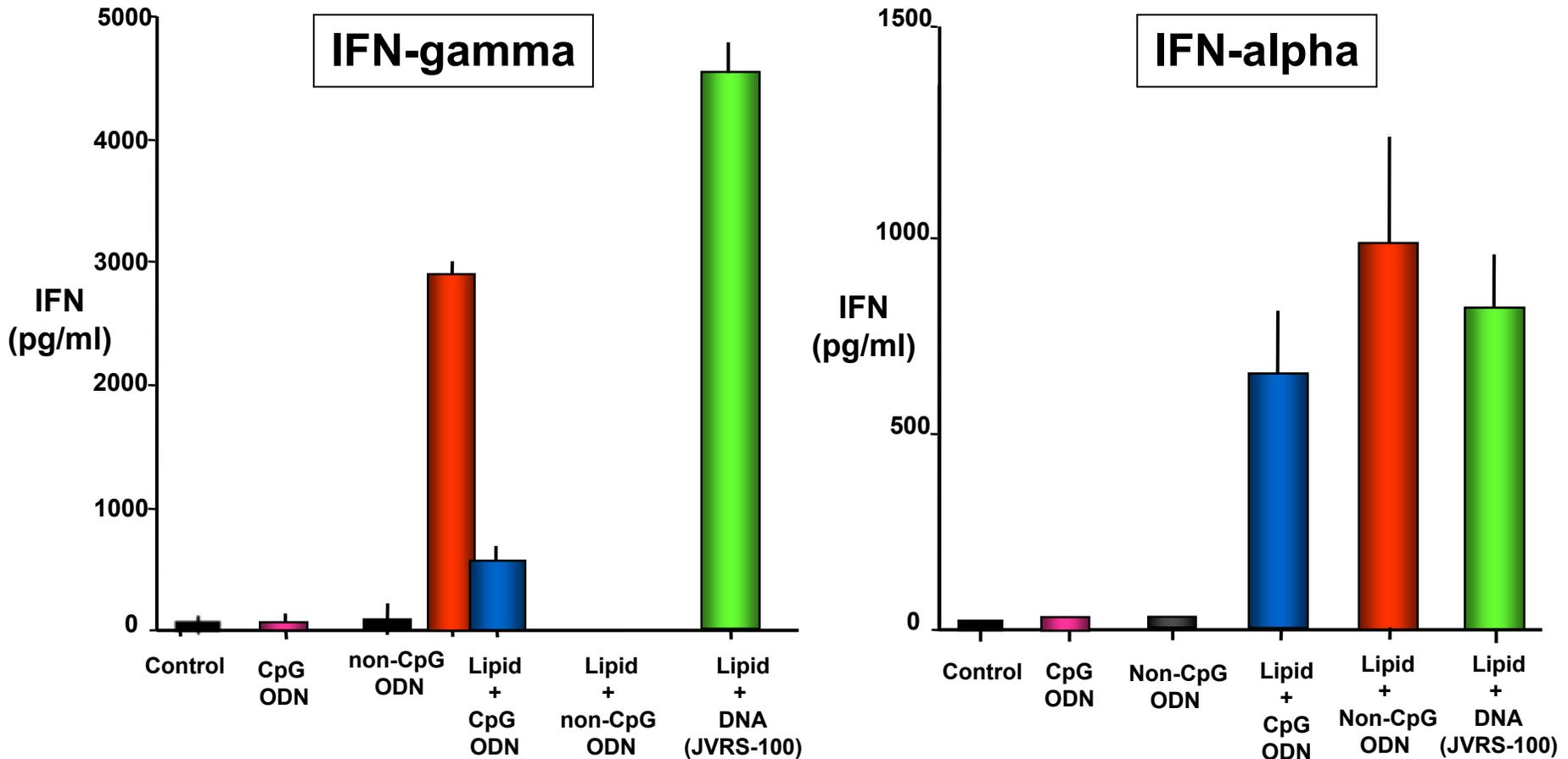
pJB1908
2300 bp

<i>Lac Z</i>	Tn5 Ble ^R	T7 Pro #1	<i>Lac I</i>	MCS	Promega Intron	SV40 Poly A	T7 Pro #2	hCMV pro/enh	5' UTR
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Low production yields

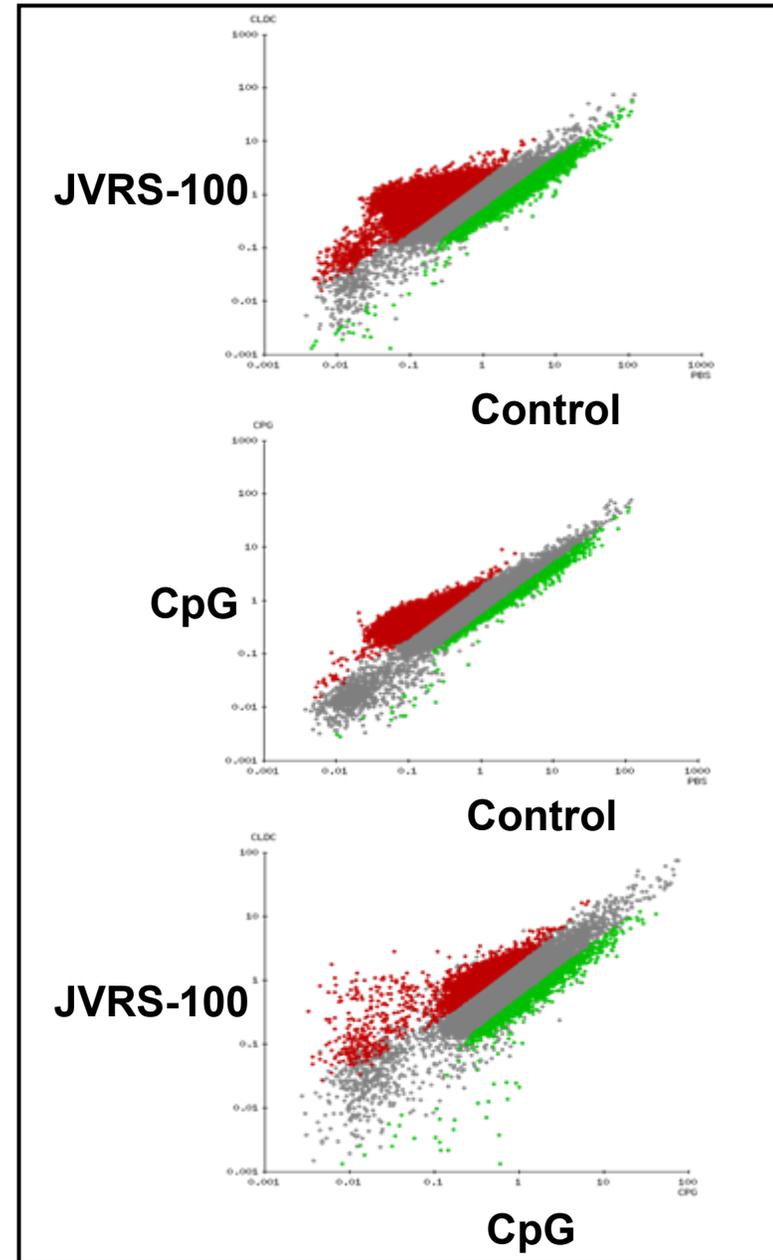
Liposome-Nucleic Acid Induction of Interferon

JVRS-100 Mechanism Involves Both CpG and Non-CpG Elements



Transcriptional Comparison of JVRS-100 and CpG ODN

- JVRS-100 vs. Control
 - 17,599 differences out of 36,177 (49%)
 - Immune system process (590 transcripts)
 - 211 up in JVRS-100 (36%)
 - 54 down in JVRS-100 (9.2%)
- CpG vs. Control
 - 12,328 differences out of 36,177 (34%)
 - Immune system process (590 transcripts)
 - 157 up in CpG (26%)
 - 19 down in CpG (3%)
- JVRS-100 vs. CpG
 - 5,727 differences out of 36,177 (16%)
 - Immune system process (590 transcripts)
 - 64 up in JVRS-100 (11%)
 - 33 down in JVRS-100 (5%)



Cytokine-Cytokine Receptor Interactions

Increased by JVRS-100

- Kinase insert domain protein receptor
- Activin A receptor, type 1
- IL-7 receptor
- CSF-1
- VEGF-C
- Thyroid peroxidase
- TNFr superfamily, member 11a
- Epidermal growth factor receptor
- Activin receptor IIB
- IL-18r accessory protein
- CXCL1
- CCL2
- TNFr superfamily, member 19
- CXCL4
- TNFr superfamily, member 25
- CD70
- Platelet-derived growth factor, D polypeptide (2x)
- IL-17, beta
- IL-12r, beta1
- IL-15
- IFN- α
- TNFr superfamily, member 19-like
- Growth hormone receptor
- TNFr superfamily, member 25
- IL-10r, alpha
- IFN-kappa
- IFN-g
- Bone morphogenic protein receptor, type II
- TNFr superfamily, member 4
- TNF ligand superfamily, member 18

Increased by CpG ODN

- IL-2r, gamma (CD132)
- CSF-3r
- Csf2ra (CD116)
- IL-6 signal transducer
- IL-1r, type 1
- TNF ligand superfamily, member 13b

Preclinical Mouse Biodistribution Summary

Group	Treatment	Plasmid Dose* (mg/Kg)	No. of Males/Females	Injection Days	Sacrifice Time Points			
					Day 1	Day 7	Day 8	Day 35
1	VLTS-587	0.25	10/10	Day 0	5 (m) 5 (f)	5 (m) 5 (f)	-	-
2	VLTS-587	0.25	10/10	Days 0, 7	-	-	5 (m) 5 (f)	5 (m) 5 (f)
3	5% dextrose	0	5/5	Day 0	-	5 (m) 5 (f)	-	-
4	5% dextrose	0	5/5	Days 0, 7	-	-	-	5 (m) 5 (f)

Study Design to Assess the biodistribution of VLTS-587 following IV administration in mice

* Dose per injection. Dose levels are approximate. All dosing was by fixed volume and was not adjusted for individual animal weight.

Biodistribution Study Summary

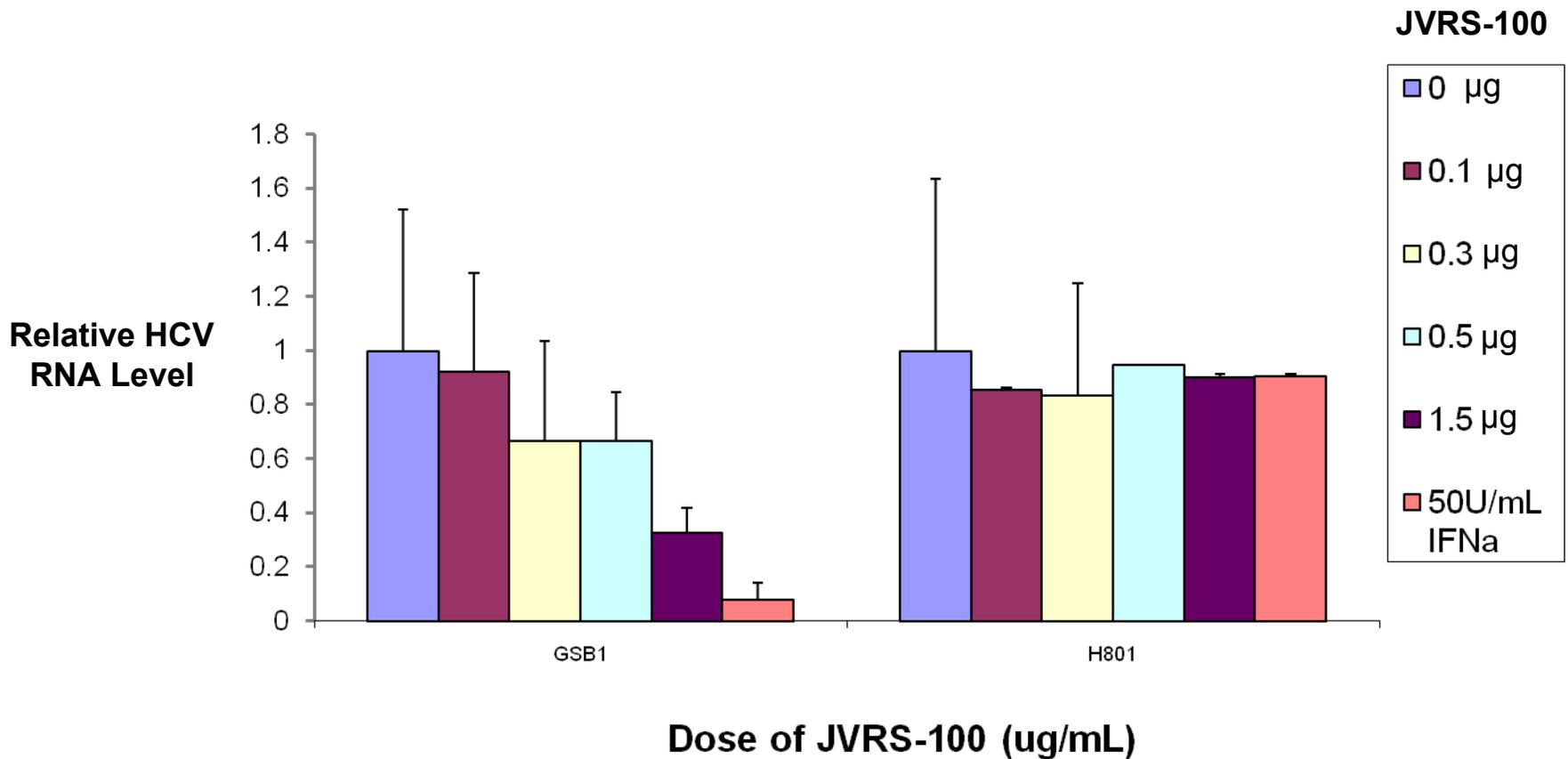
- **No apparent sex differences in plasmid biodistribution pattern**
- **No preferential accumulation of plasmid in any particular organ**
- **Organ distribution of DNA 24-hrs post single administration**
 - Day 1: Injection site (1.7×10^6) > Blood (9.8×10^5) > Lung (3.2×10^5) > Spleen (2.2×10^5) > Liver (5×10^3)
 - Day 7: Blood (2.8×10^4) > Spleen (7.1×10^3) > Lung (2.2×10^4) > Injection site (6/6)
- **Organ distribution of DNA 24-hrs post second administration (days 0, 7)**
 - Day 8: Injection site (3.3×10^6) > Blood (4.6×10^5) > Spleen (2.9×10^5) > Lung (4.1×10^4) > Liver (1×10^4)
 - Day 35: Injection site (5.6×10^5) > Blood (6.9×10^3) > Spleen (6/6) > Lung (6/6)
- **Plasmid distributed to all tissues 24 hrs. after single or two-dose IV injections**
- **Plasmid levels decrease substantially 7 days post-single injection**
- **Quantifiable DNA levels at injection site and blood 28 days following second dose**
- **Plasmid DNA below limit of quantitation in lung, spleen, liver, kidney, heart, brain, BM**
- **Plasmid DNA negative in the brain and gonads of most animals at day 35 (i.e., 28 days post-second injection)**

Preclinical Mouse Biodistribution Summary

Time Interval (Days)	Inj. Site	Lung	Spleen	Liver	Kidney	Heart	Brain	Bone Marrow	Gonads	Blood
Day 1	1.7 x 10 ⁶	3.2 x 10 ⁵	2.2 x 10 ⁵	5 x 10 ³	2.4 x 10 ³	4.7 x 10 ³	1.3 x 10 ³	NA	(6/6)	9.8 x 10 ⁵
Day 7	(6/6)	7.1 x 10 ³	2.2 x 10 ⁴	(6/6)	(6/6)	(6/6)	(5/6)	(5/6)	(5/6)	2.8 x 10 ⁴
Day 8 (day 0, 7 treatment)	3.3 x 10 ⁶	4.1 x 10 ⁴	2.9 x 10 ⁵	1.0 x 10 ⁴	4.0 x 10 ³	1.3 x 10 ⁴	1.7 x 10 ³	1.5 x 10 ³	1.3 x 10 ³	4.6 x 10 ⁵
Day 35 (day 0, 7 treatment)	5.6 x 10 ⁵	(6/6)	(6/6)	(5/6)	(6/6)	(6/6)	(2/6)	(4/6)	(2/6)	6.9 x 10 ³

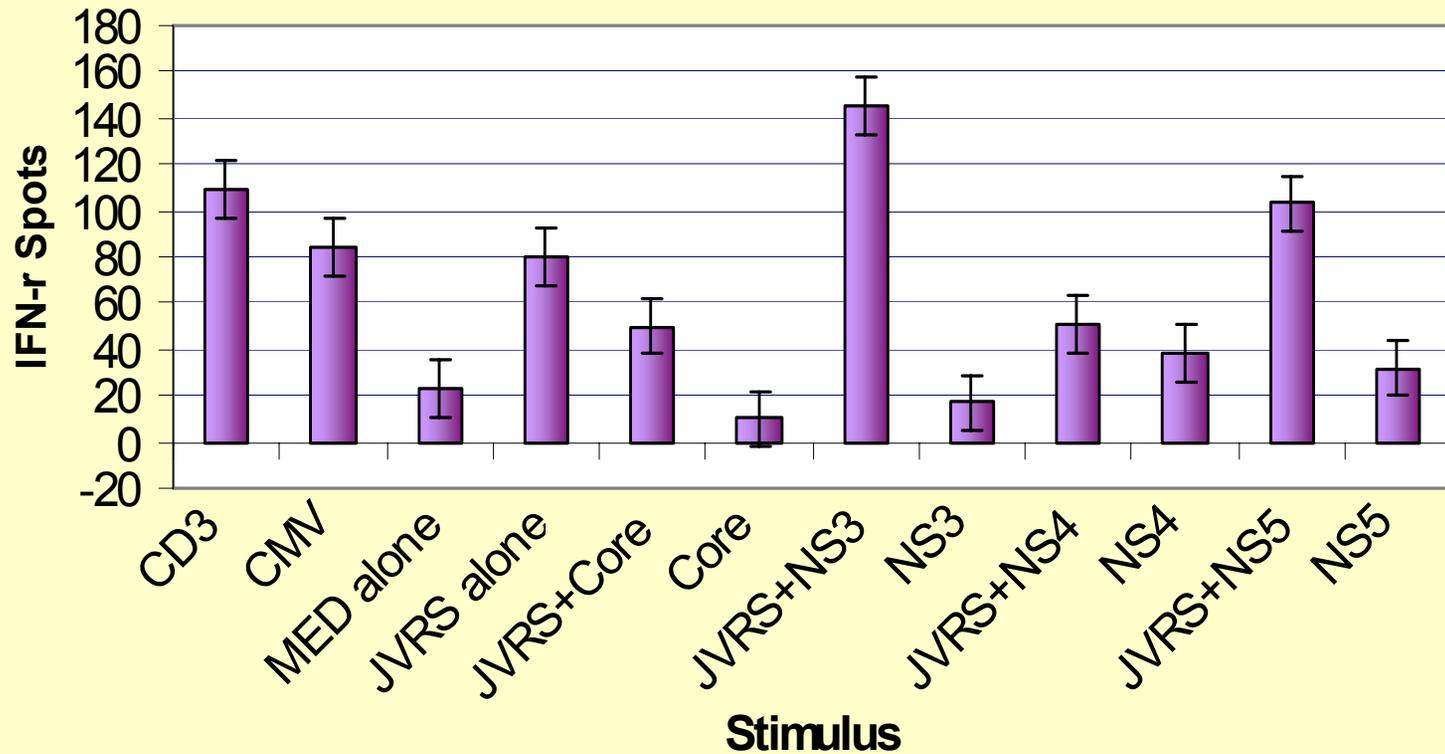
The data are presented as copies of plasmid per microgram of total DNA (- mean value combining both sexes n=6/group). Three animals /sex/group were analyzed for plasmid DNA; the remaining tissues were reserved for analysis in the event of sample loss during processing. In cases where none of the samples contained quantifiable levels of plasmid DNA, values in parenthesis represent number of tissues positive/total number tissues tested. NA = tissue not available.

JVRS-100 Effect on HCV RNA Replication in Subgenomic HCV Replicon Cell Line

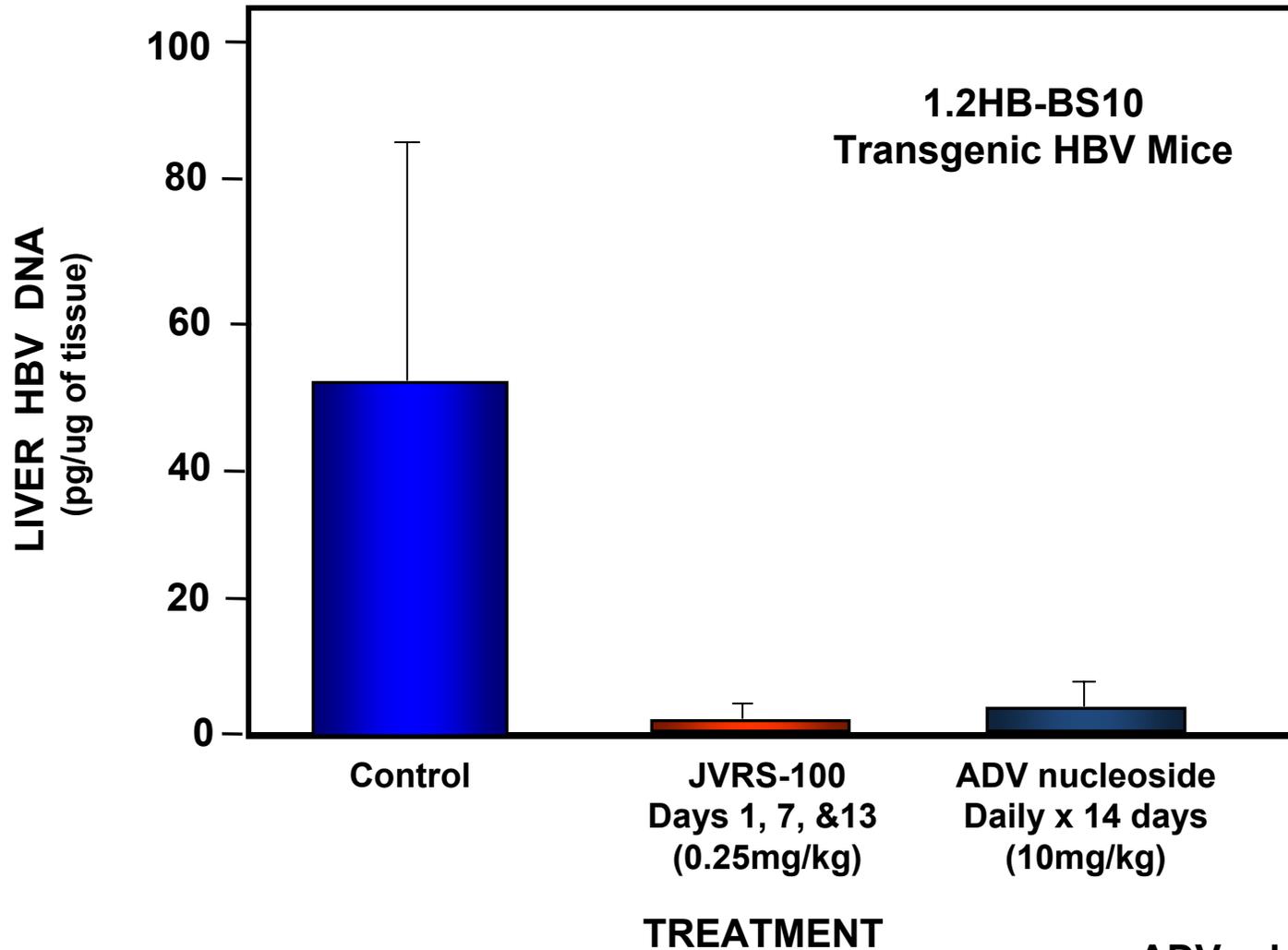


In Vitro Effect of JVRS-100 + HCV Antigens on HCV Patient T Cell Activation

Interferon-gamma Elispot Assay



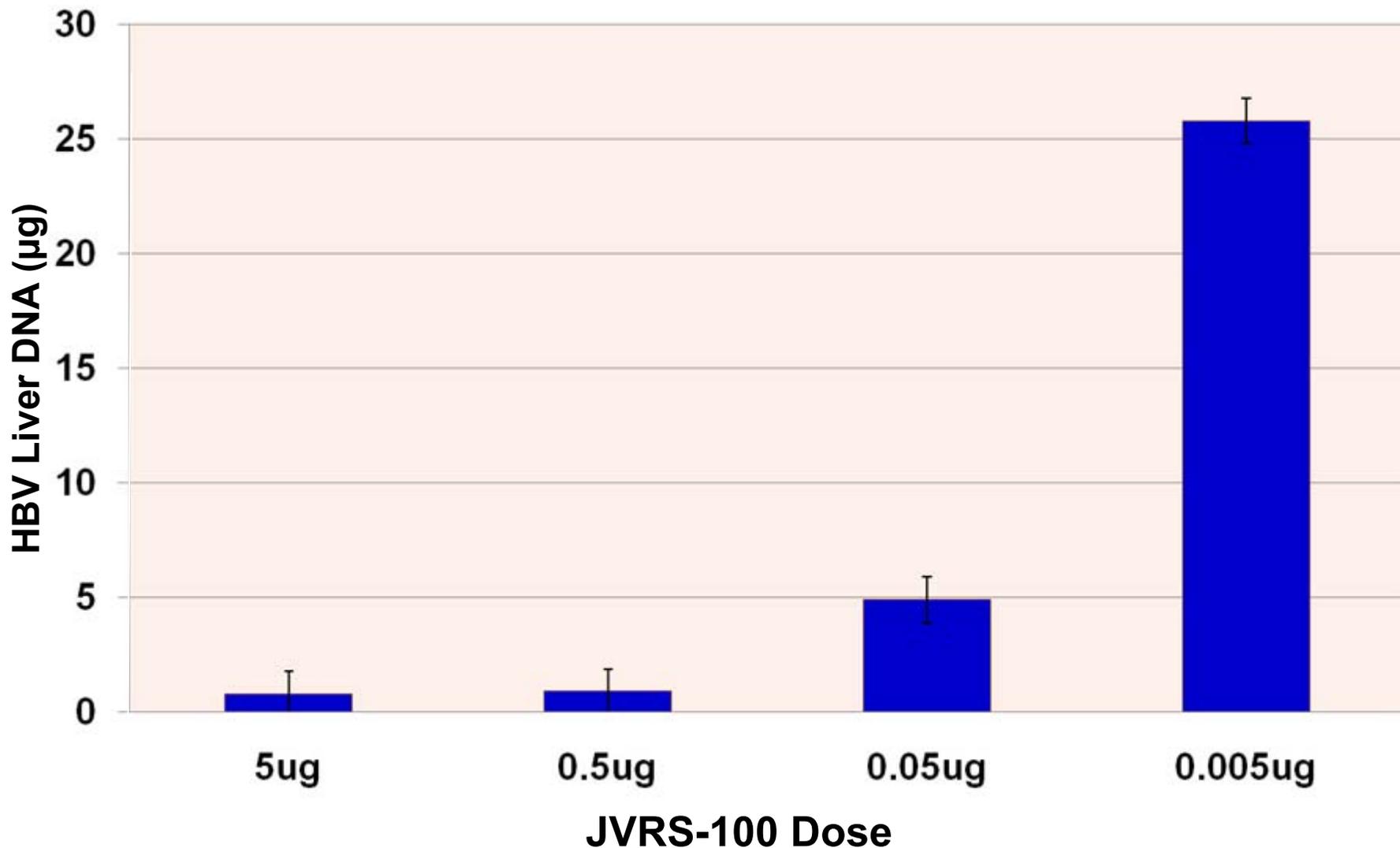
JVRS-100 Therapeutic Response in HBV Transgenic Mice



Institute of Antiviral Research,
Utah State Univ. (John Morrey)

ADV-adefovir dipivoxil
(nucleoside analog)

JVRS-100 Dose Titration in HBV Transgenic Mice



JVRS-100 Preclinical Efficacy Summary

Hepatitis Models

- **In Vivo Studies:**

- TH1 cytokine (IL-12, IFN- γ) induction in mice (>LPS, Poly I:C or CpG ODN)
- HBV Transgenic Mouse Model:
 - HBV DNA reduction comparable to Adefovir
 - Cytokines: Increased IL-1a, IL-12, MCP-1, RANTES
 - Nominal: IL-1b, IL-2, 3,4,5,6,9,10, TNF- α , MIP-1, GM-CSF
 - Normal ALT, normal liver histology
- HCV-Chronically-infected Chimpanzee Model (dose escalation)
 - HCV RNA reduction, transient but associated with dosing
 - Limited cytokine induction (low doses); increased IFN- α , IL-6, IL-12 (high dose)
 - Limited interferon stimulating gene (ISG) activation at high doses
 - No significant clinical parameter (CBC, chemistries, etc.) changes outside of HCV disease condition

Preclinical Efficacy Summary - HCV Model

- **HCV Chronically-infected Chimpanzee Model:**
 - JVRS-100 dose escalation (1.6, 3.2, 6.4, 12.8, 25.6 µg/kg); 10-day treatment interval
 - Intravenous administration at 200-400 µL/min infusion rate
- **Study Results:**
 - Reduced HCV RNA levels (0.9 log reduction)
 - No significant clinical or hematological changes outside of HCV chronic disease condition; no disease exacerbation
 - Clinical chemistries: unchanged from HCV disease condition (most within normal limits)
 - Hematology: unchanged from HCV disease condition (most within normal limits)
 - Cytokines: Limited and transient TH1 cytokine induction
 - increased IFN-α, IL-6, IL-12 at higher dose levels (12.8 and 25.6 µg/kg)
 - nominal IL-1β, TNF-α, IFN-γ
 - Interferon stimulated gene (ISG) activation:
 - increased expression - CXCL 9, CXCL 10, CXCL 11, IFI27, ISG15, IFN-β
 - nominal change - OAS1, STAT1, IFN-α, IFN-γ

Preclinical Non-Human Primate Toxicology Summary

- **Cynomolgus Monkeys (gene therapy application): (Valentis)**
 - Product: VLTS-587+hIL-2 gene; VLTS-587 (no gene insert) equivalent to JVRS-100
 - IV infusion (200 μ L/min) of VLTS-587 at 20, 80, & 200 μ g/kg with single weekly doses x8
 - IV infusion (200 μ L/min) of VLTS-587(no gene) at 200 μ g/kg with single weekly doses x8
 - Dose volume standardized at 6 mL; injection sites alternated weekly (left & right cephalic)
 - Study duration was 10 weeks (necropsy) with equal male/female groups

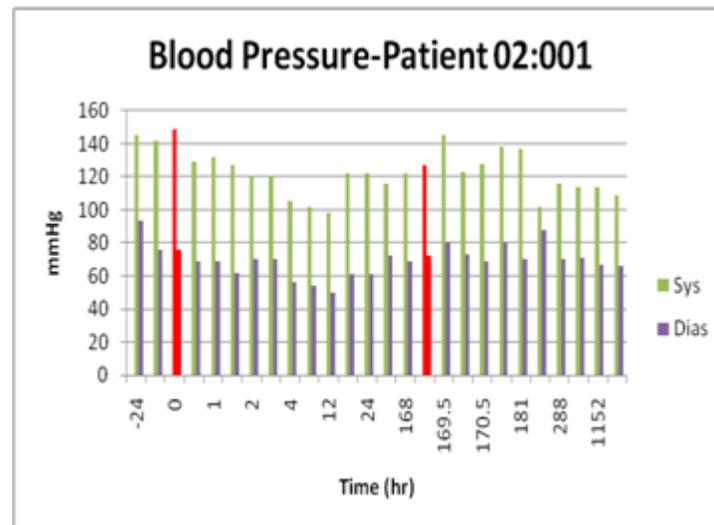
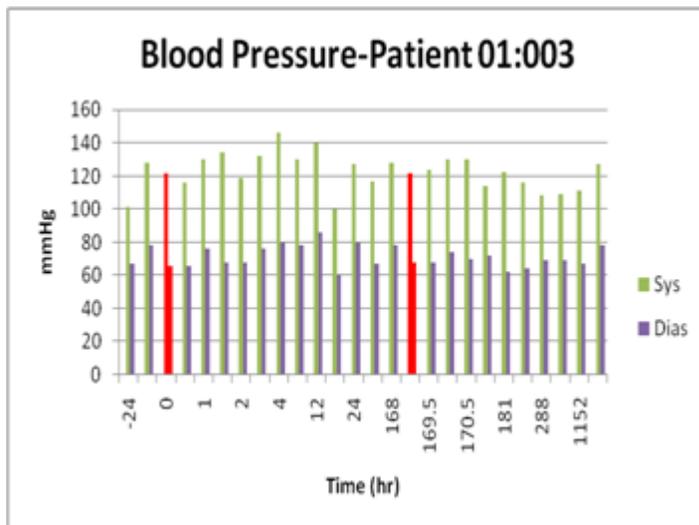
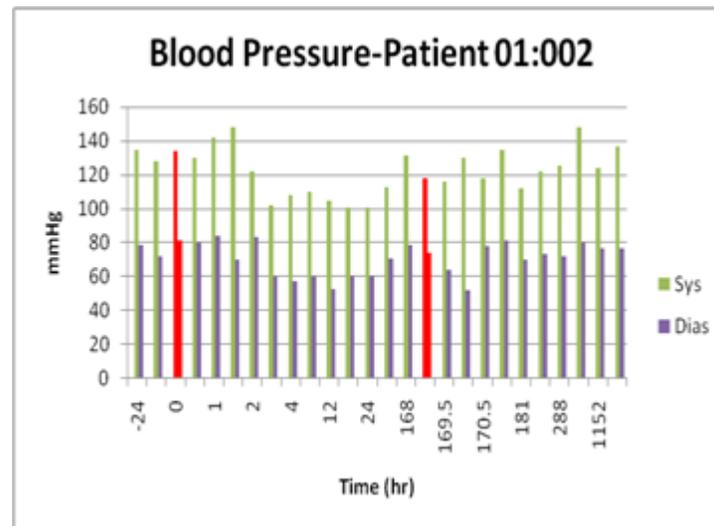
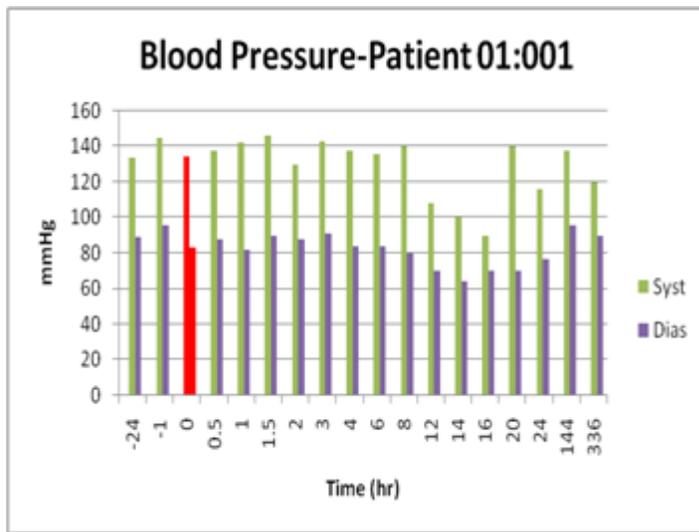
Valentis Clinical

Back-up Slides

Cancer Diagnosis and Prior Treatment

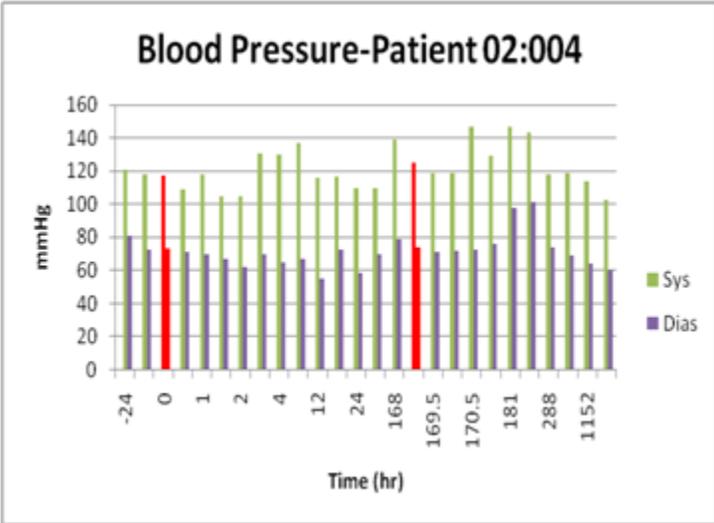
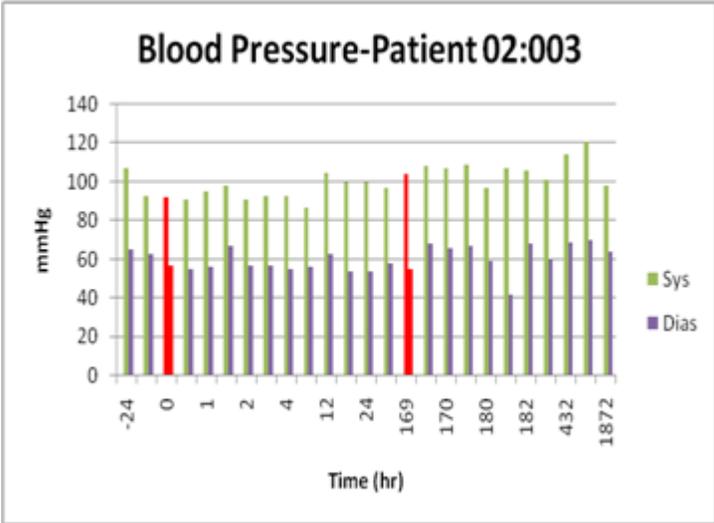
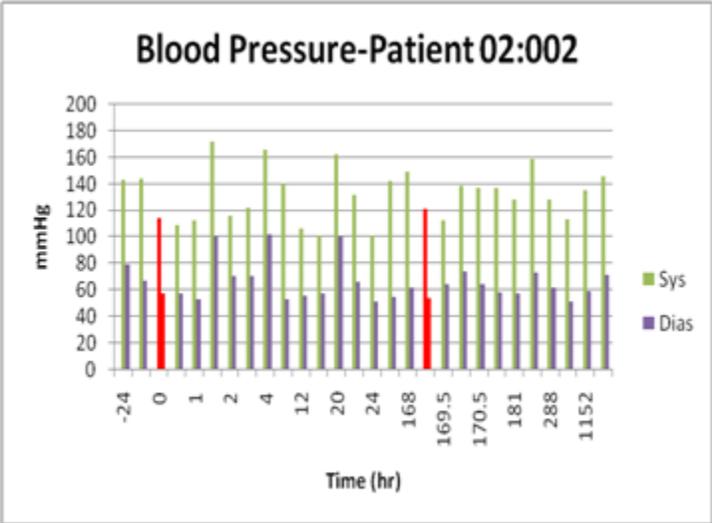
Patient No.	Cancer Diagnosis'	Prior Cancer Treatment		
		Chemotherapy	Surgical Therapy	Radiotherapy
01-001	Breast cancer	Yes	Yes	Yes
01-002	Colonic adenocarcinoma	Yes	Yes	Yes
01-003	Colonic adenocarcinoma	Yes	Yes	Yes
02-001	High grade fibrosarcoma	Yes	Yes	Yes
02-002	Ovarian carcinoma	Yes	Yes	No
02-003	Renal cell carcinoma	No	Yes	No
02-004	Renal clear cell carcinoma	No	Yes	No

Blood Pressure After VLTS 587 Dosing



Red Lines Indicates Dosing

Blood Pressure After VLTS 587 Dosing

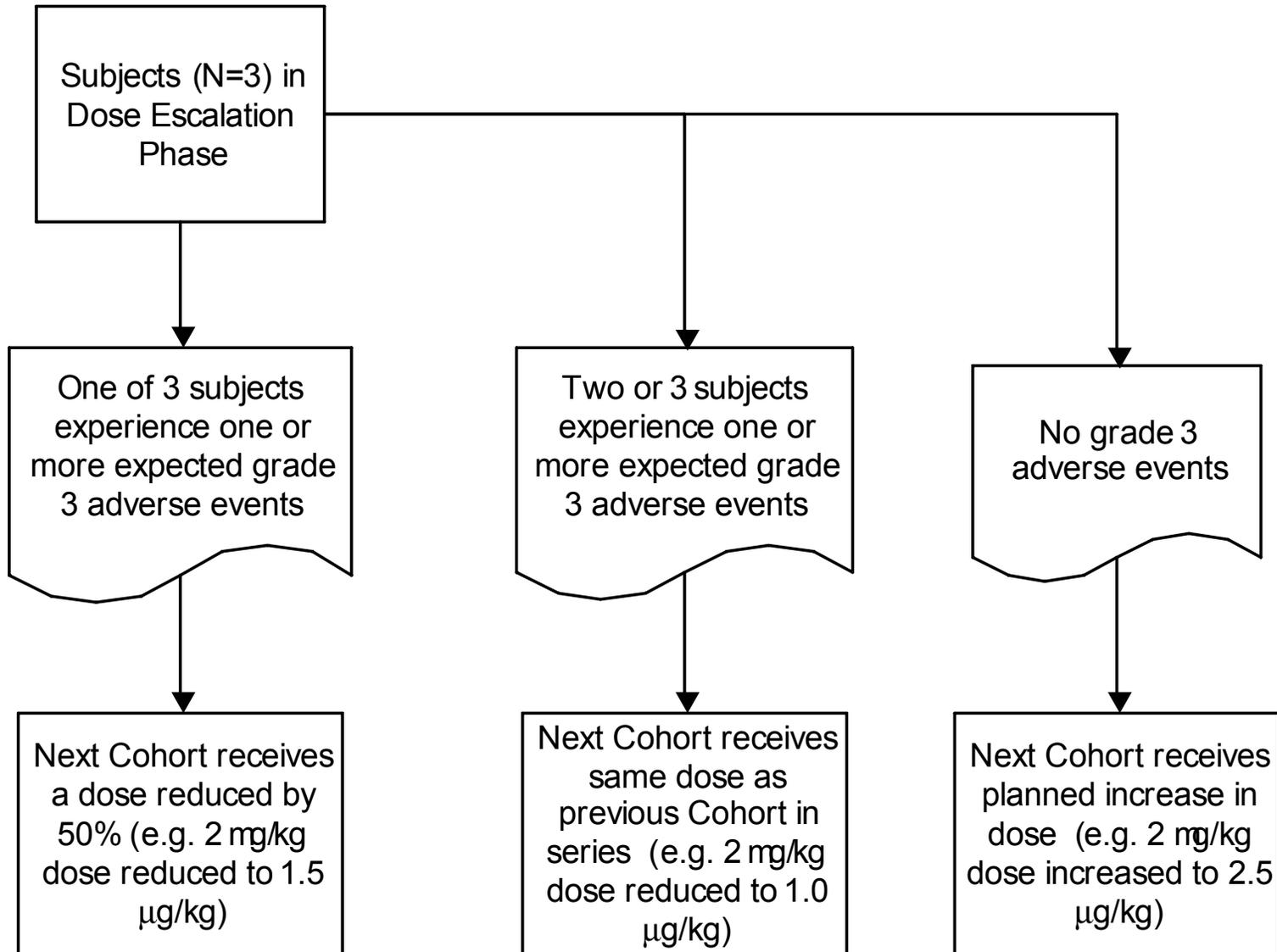


Red Lines
Indicates Dosing

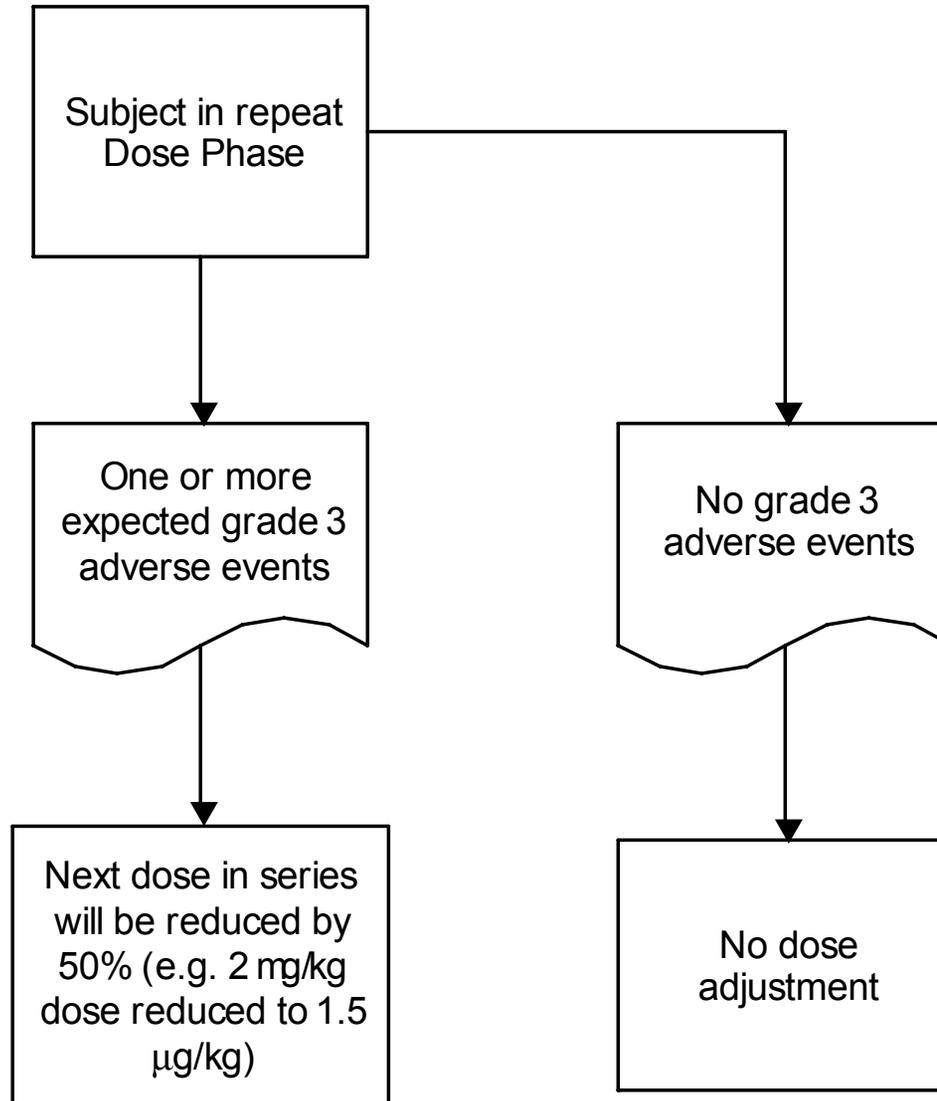
HCV Clinical

Back-up Slides

Dose Adjustment for Cohorts (Dose Escalation Phase)



Dose Adjustment for Individual Subject (Repeat Dose Phase)



AML Preclinical & Clinical

Back-up Slides

Stage 1: Dose Escalation Phase

- 1 patient enrolled at given dose level
- Dose ascension when patient completes 1 cycle with no DLTs
- If a DLT is seen in one patient, 5 additional subjects will be accrued at that dose level.
- If ≥ 2 patients w/ DLT, the dose level below evaluated, considered for RP2D.

Dose Levels	Dose
Level 1	0.5 $\mu\text{g}/\text{Kg}$ D1, 8, 15
Level 2	1.0 $\mu\text{g}/\text{Kg}$ D1,8,15
Level 3	2.0 $\mu\text{g}/\text{Kg}$ D1,15
Level 4	2.0 $\mu\text{g}/\text{Kg}$ D1, 8, 15
Level 5	4.0 $\mu\text{g}/\text{Kg}$ D1, 15
Change to Stage 2 with starting dose of 4.0 $\mu\text{g}/\text{Kg}$ D1, 8, 15 if no toxicity by Level 5	

Dose Limiting Toxicity

The definition of MTD will be based on the occurrence of DLTs during the first cycle of therapy with JVRS-100.

# of Patients with DLT at a Given Dose Level	Escalation Decision Rules for Stage 2 and Definition of RP2D
0 out of 3	Enter a min of 3 patients at the next dose level
≥ 2 out of 3	Stop Dose escalation. This dose level will be the MTD Add 3 more patients to previous dose level
1 out of 3	Add 3 more patients at this dose • If 0 of 3 have DLT, proceed to the next dose level. • If ≥ 1 patient have DLT, dose escalation is stopped, this is MTD. Add 3 more patients to previous dose level
≤ 1 of 6 at highest dose level below MTD	This is the RP2D. Once determined, the cohort will be expanded to a total of 12 patients.

Safety Monitoring

Data Safety Monitoring:

1. Data continuously monitored by PI and PSCI data management staff.
2. Penn State Cancer Institute Data Safety Monitoring Board to formally review data on patient by patient basis 6 monthly.
3. An independent pharmacovigilance group reviews SAE(s) and creates narratives for reporting
4. Juvaris internal personnel frequently monitors all data for protocol adherence, safety events, and regulatory compliance.

Efficacy Outcome Measures

Dose Escalation Phase

Day	0	1	Post-Dose	8	Post-Dose	15	Post-Dose	28
Clinical hem, chem, coag labs	X	X	X	X	X	X	X	X
Blood & bone marrow studies	X							X
Radiographic imaging	X							X
Immune monitoring: cytokines and flow cytometry at 2, 4, 24 hrs, functional studies at 24 hrs *w/in 24-hrs of dose		X*	X	X*	X	X*	X	X