Protocol #0808-946: Phase I Trial of Intratumoral Injection of Vesicular Stomatitis Virus Expressing Interferon Beta in Patients with Hepatocellular Carcinoma

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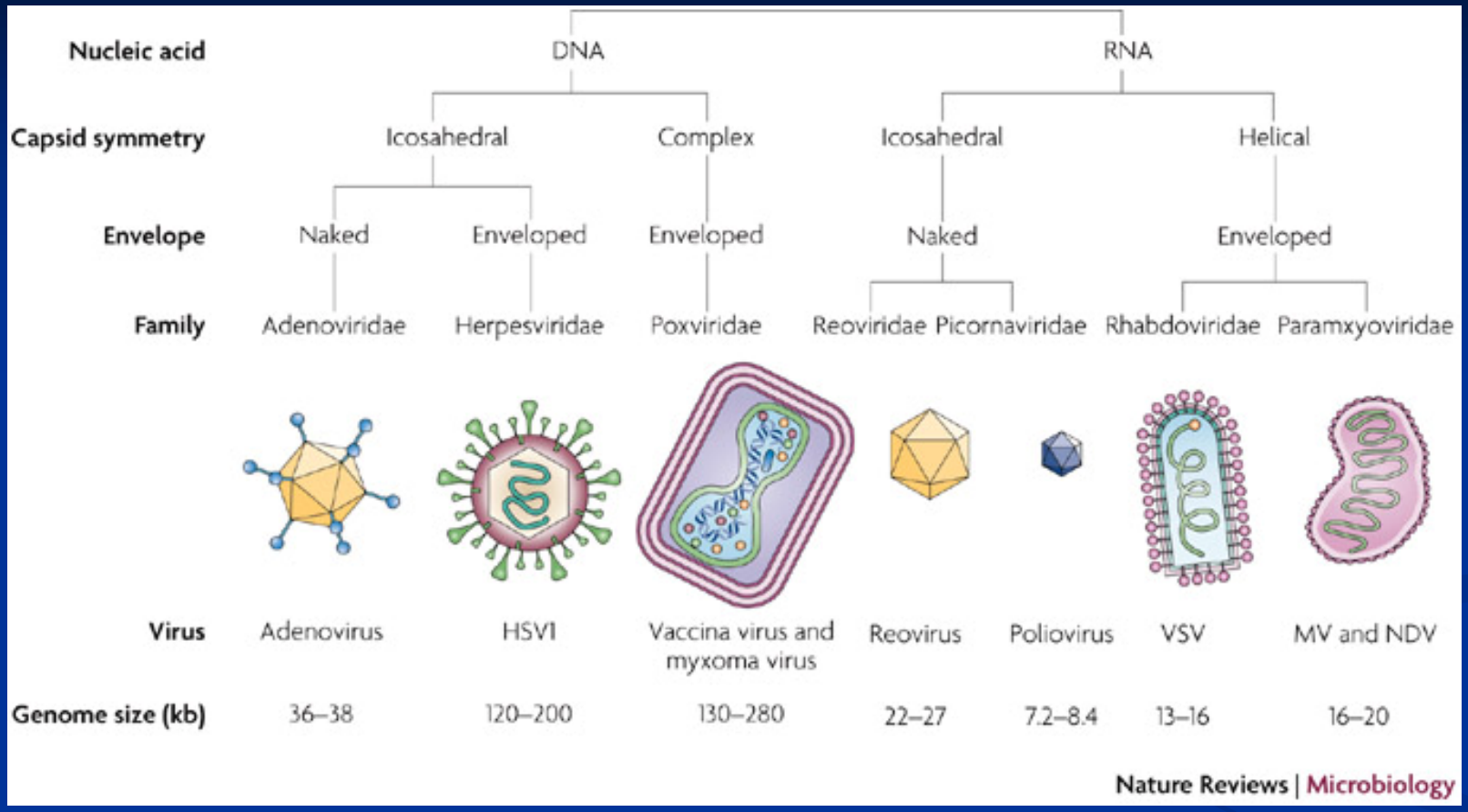
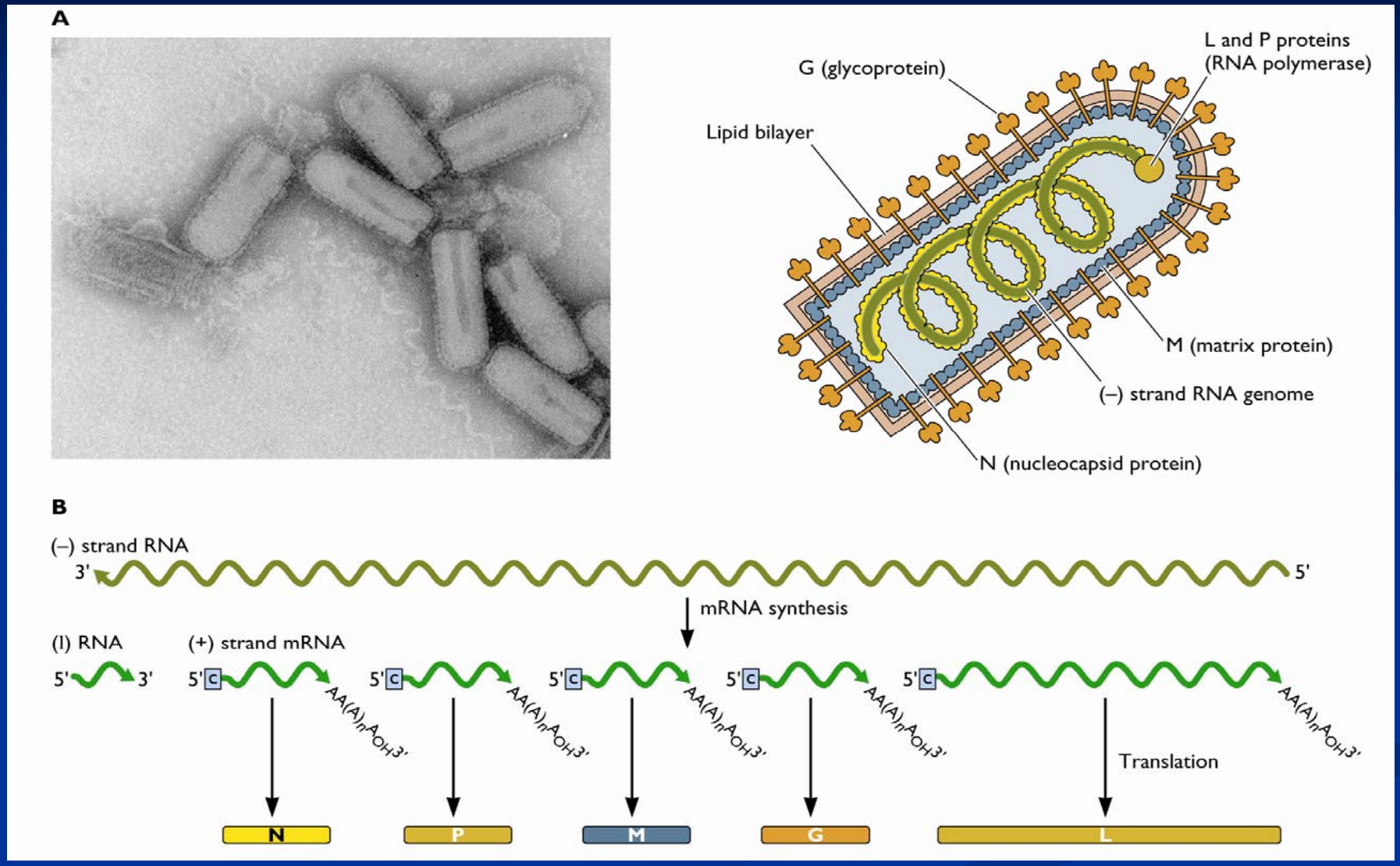
Aims:

1. To determine the safety and toxicity of intratumoral injection of vesicular stomatitis virus (VSV) expressing IFNβ into a hepatocellular carcinoma (HCC) tumor nodule in the liver of patients.
2. To determine the maximum tolerated dose of VSV expressing IFNβ in patients with HCC.
3. To observe any evidence of anti-tumor activity.



Oncolytic Viruses Being Developed For Clinical Trials

Vesicular Stomatitis Virus



*Images obtained from Principles of Virology, 2nd ed, by Flint *et al.*

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Vesicular Stomatitis Virus

- Two antigenically distinct species (serotypes*):
  - VSV-Indiana
  - VSV-New Jersey
- Causes nonfatal disease of significant economic importance in horses, cattle and swine
- Rare VSV infections in humans: flu-like illness; extensive sero-positivity without illness
- Classic example of a virus that inhibits production of IFNs

**based on G protein (serotype-specific antigen)*

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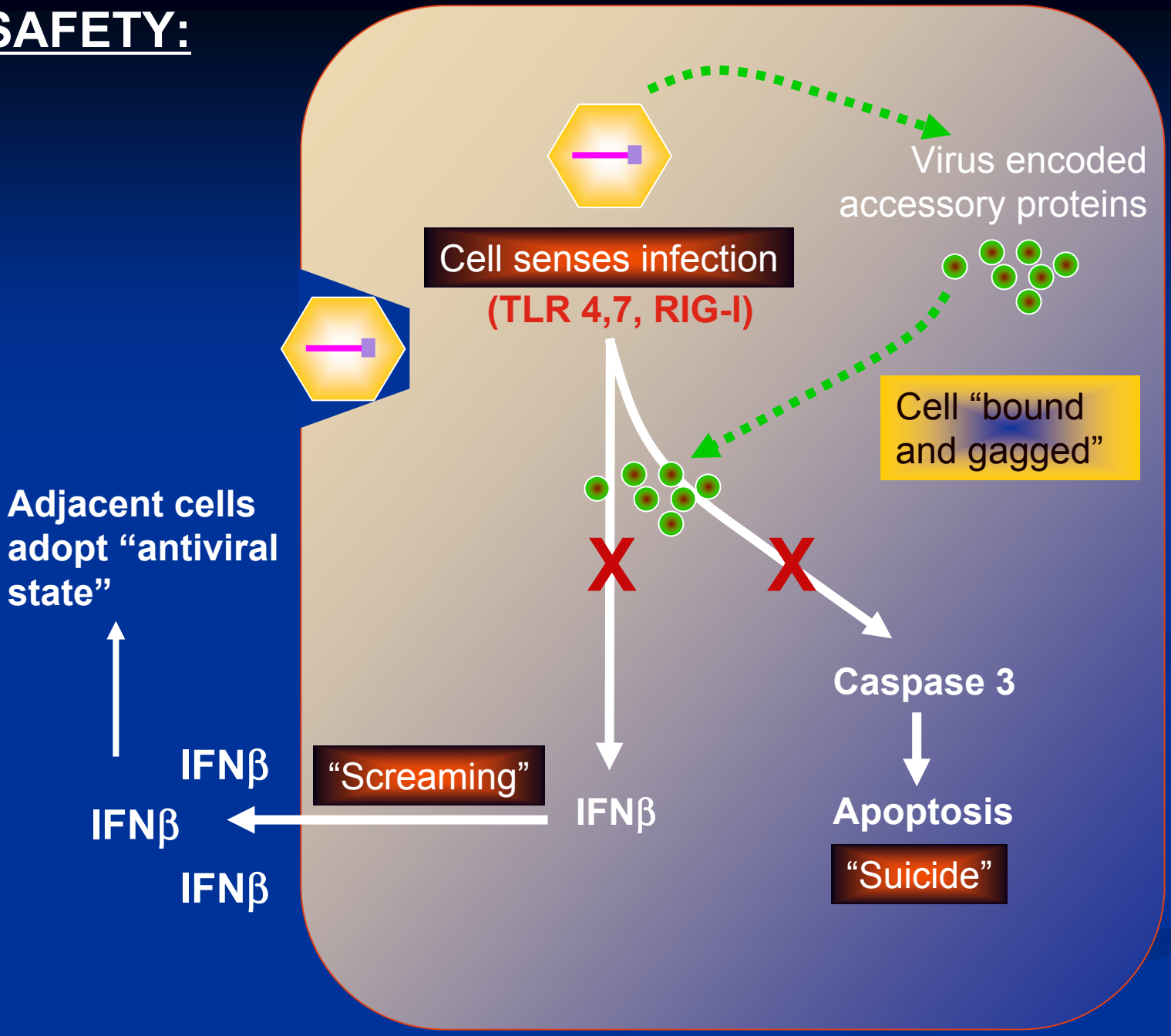
Vesicular Stomatitis Virus (VSV):

- Safety
- Efficacy

SAFETY:



Cellular Response To Infection

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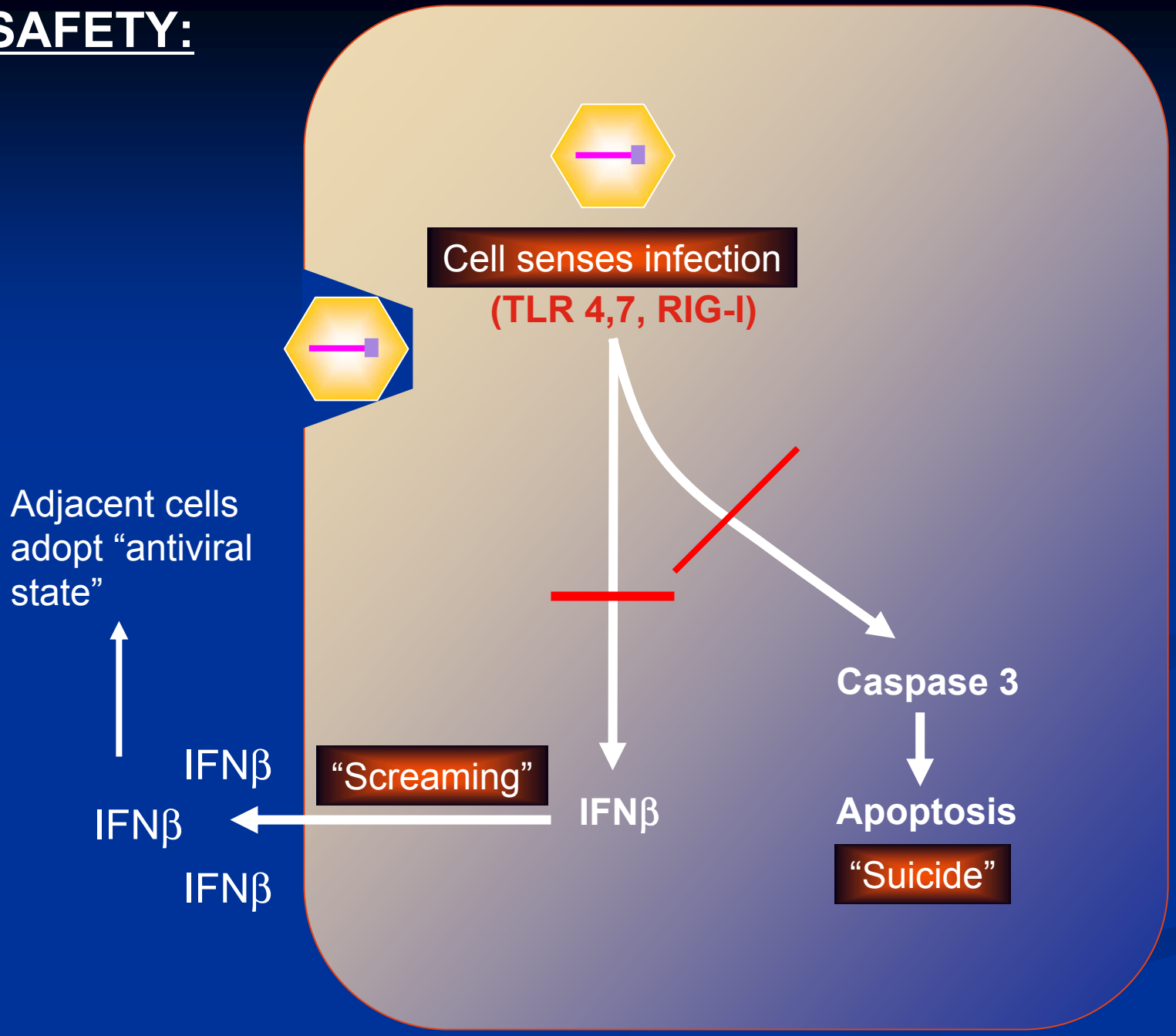
SAFETY:



Tumour Cells Might Scream But They Can't Hear…

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-Safety as an Oncolytic Agent: VSV is Extremely sensitive to the anti-viral effects of IFN

Mice harbouring a defective IFN system are exquisitely sensitive to normally innocuous exposure to VSV

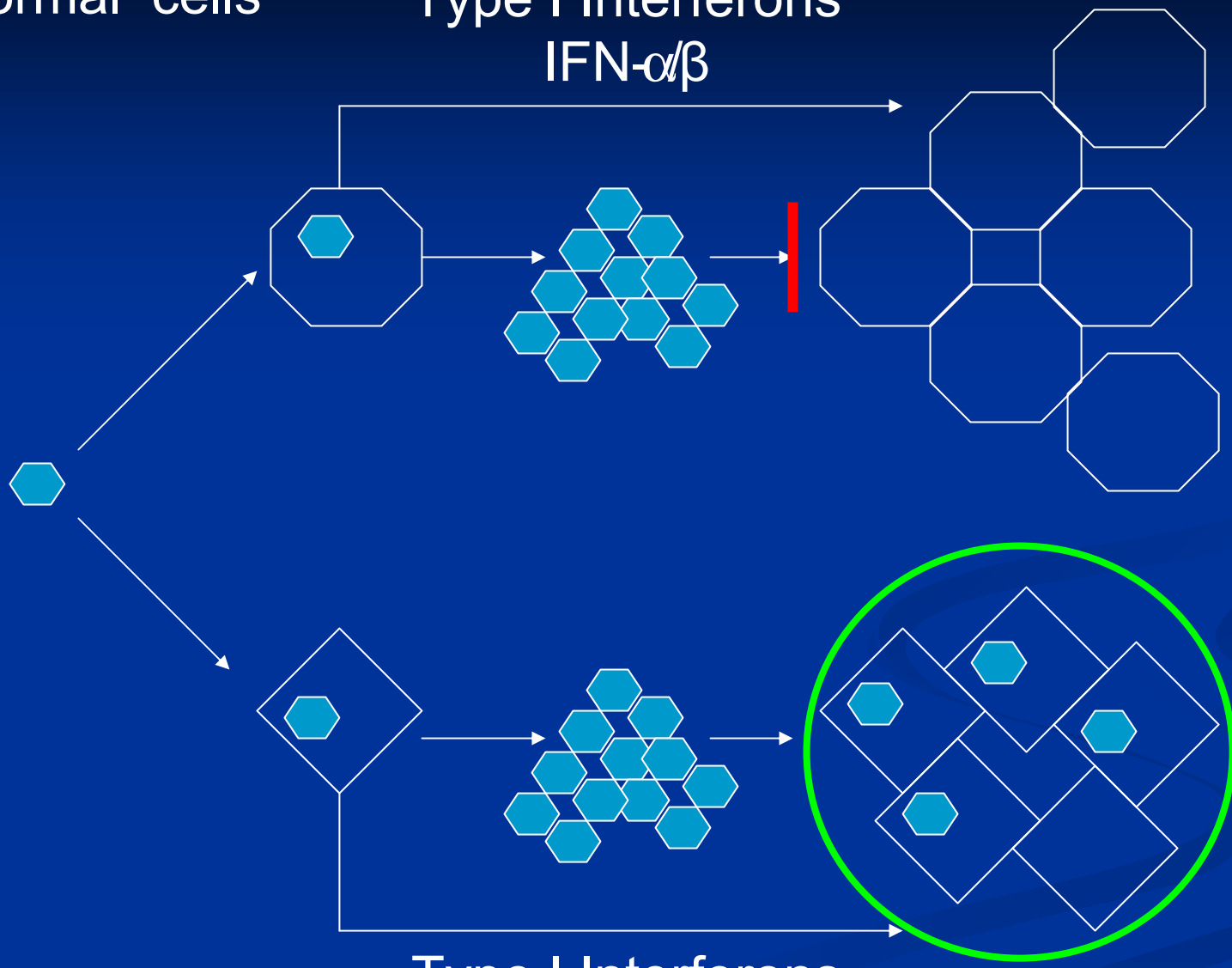
-Targeted disruption of the mouse stat1 gene results in compromised innate immunity to viral disease. Cell 1996;84:443-50.

-Targeted disruption of the stat1 gene in mice reveals unexpected physiologic specificity in the jak-stat signaling pathway. Cell 1996;84:431-42.

-Functional role of type i and type ii interferons in antiviral defense. Science 1994;264:1918-21.

1. Infection of Normal cells

Type I Interferons
IFN-α/β



2. Infection of tumour cells

Type I Interferons
IFN-α/β

Viral Spread Permitted

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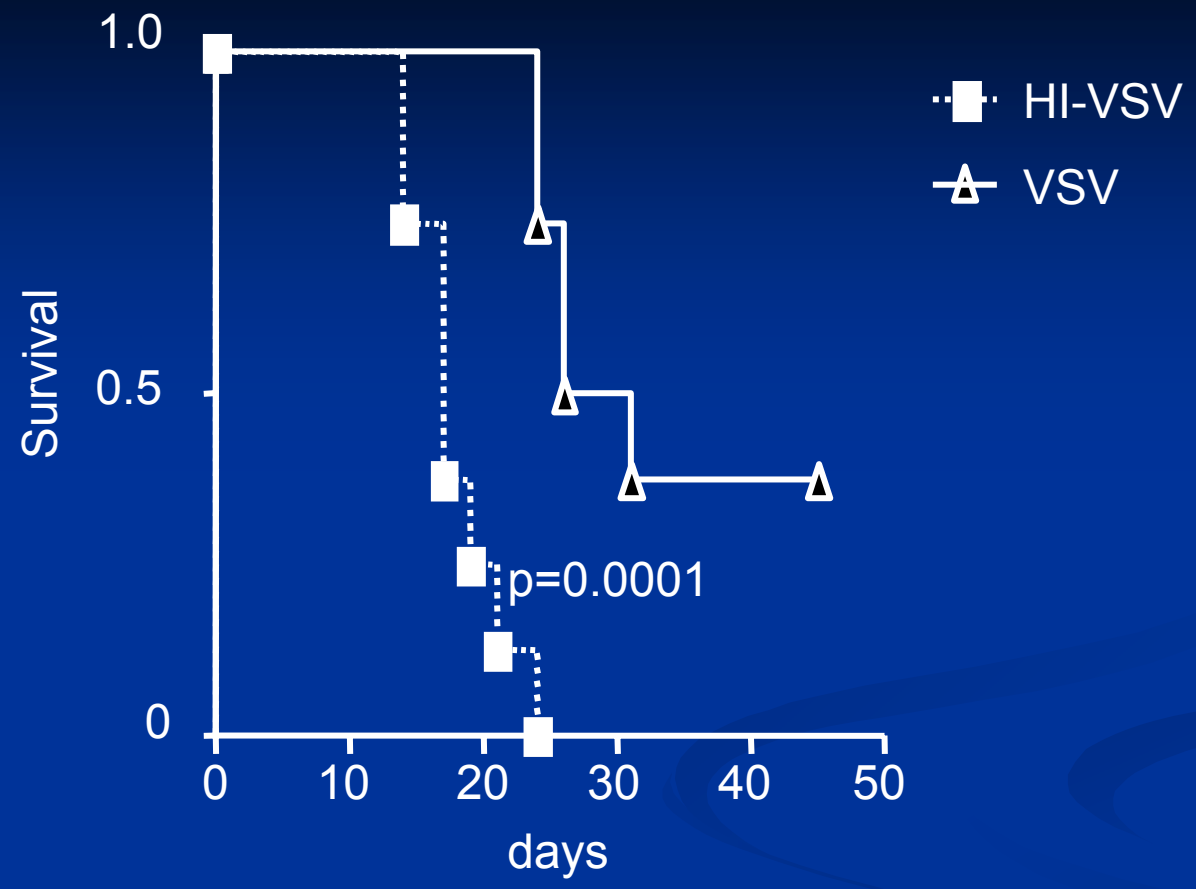
Are all cancer cells defective in their IFN-response pathways and, therefore, fully sensitive to VSV (Dr Roizman)?

Clearly NOT:

We will test patient samples for VSV replication +/- IFN; correlate with diagnostic assays on transcripts associated with innate immune response (Dr Barber).

Vesicular Stomatitis Virus (VSV):

- Efficacy as an Oncolytic Agent



EFFICACY: VSV induces tumour regressions *in vivo*
(Diaz et al. Cancer Res. 2007)

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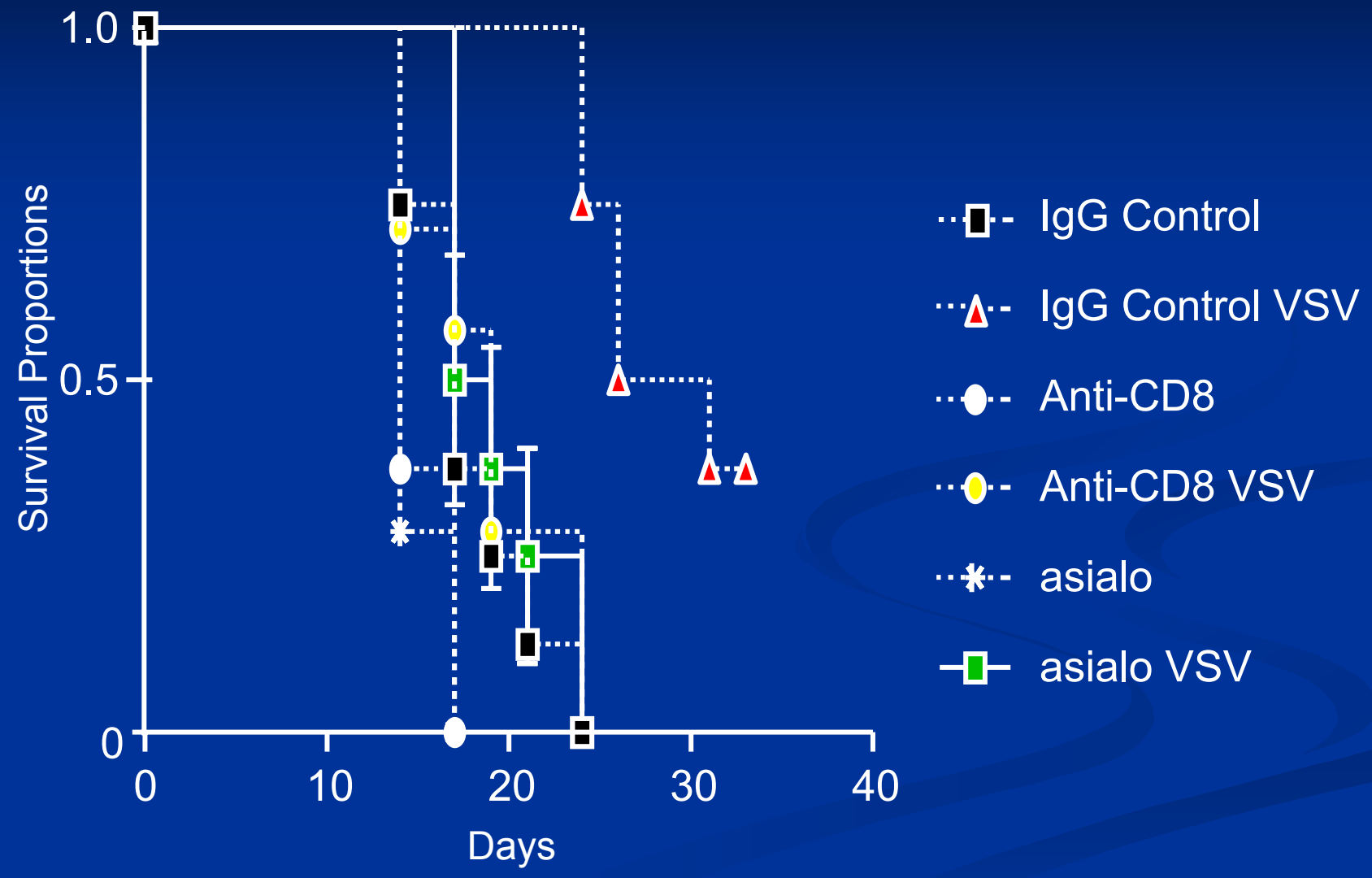
CD8 T cells and NK cells are critical for therapy in immunocompetent models:



(Diaz et al. Cancer Res. 2007)

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To improve further the safety of VSV as an oncolytic agent,we developed a genetically engineered VSV which also expresses IFN-β (Dr Glen Barber, University of Miami).

IFN-β has anti viral effects that protect normal cells from viral infection;

IFN-β also has considerable immune stimulatory effects that promote anti tumour immune clearance

A Phase I Clinical Trial of Single-Dose Intrapleural IFN-β Gene Transfer for Malignant Pleural Mesothelioma and Metastatic Pleural Effusions: High Rate of Antitumor Immune Responses

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Abstract

Purpose: This phase 1 dose escalation study evaluated the safety and feasibility of single-dose intrapleural IFN-β gene transfer using an adenoviral vector (Ad.IFN-β) in patients with malignant pleural mesothelioma (MPM) and metastatic pleural effusions (MPE).

Experimental Design: Ad.IFN-β was administered through an indwelling pleural catheter in doses ranging from 9×10^{11} to 3×10^{12} viral particles (vp) in two cohorts of patients with MPM (7 patients) and MPE (3 patients). Subjects were evaluated for (*a*) toxicity, (*b*) gene transfer, (*c*) humoral, cellular, and cytokine-mediated immune responses, and (*d*) tumor responses via 18-fluorodeoxyglucose-positron emission tomography scans and chest computed tomography scans.

Results: Intrapleural Ad.IFN-β was generally well tolerated with transient lymphopenia as the most common side effect. The maximally tolerated dose achieved was 9×10^{11} vp secondary to idiosyncratic dose-limiting toxicities (hypoxia and liver function abnormalities) in two patients treated at 3×10^{12} vp. The presence of the vector did not elicit a marked cellular infiltrate in the pleural space. Intrapleural levels of cytokines were highly variable at baseline and after response to gene transfer. Gene transfer was documented in 7 of the 10 patients by demonstration of IFN-β message or protein. Antitumor immune responses were elicited in 7 of the 10 patients and included the detection of cytotoxic T cells (1 patient), activation of circulating natural killer cells (2 patients), and humoral responses to known (Simian virus 40 large T antigen and mesothelin) and unknown tumor antigens (7 patients). Four of 10 patients showed meaningful clinical responses defined as disease stability and/or regression on 18-fluorodeoxyglucose-positron emission tomography and computed tomography scans at day 60 after vector infusion.

Conclusions: Intrapleural instillation of Ad.IFN-β is a potentially useful approach for the generation of antitumor immune responses in MPM and MPE patients and should be investigated further for overall clinical efficacy.

Malignant pleural mesothelioma (MPM) is a refractory neoplasm. Except for a few patients who benefit from aggressive multimodality approaches, the majority of patients die from the disease within 8 to 14 months of diagnosis (1, 2). Metastatic pleural effusions (MPE) also portend a poor prognosis and are typically treated solely with palliative measures.

Given this current lack of effective therapies, our group has focused on the development of adenoviral vectors for the treatment of intrapleural malignancies. We hypothesized that MPM and MPE would be particularly attractive targets for gene transfer studies given preclinical data showing evidence of effective adenoviral gene therapy in peritoneal and pleural models of tumor (3–5).

Our initial clinical trials used intrapleural delivery of adenoviral vectors expressing the suicide gene, herpes simplex thymidine kinase (Ad.HSV*tk*), into patients with MPM followed by 2 weeks of i.v. ganciclovir (6–8). Toxicity was minimal, no maximally tolerated dose was reached, and post-gene transfer

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Requests for reprints: Daniel H. Sterman, Interventional Pulmonology Program, Pulmonary, Allergy and Critical Care Division, University of Pennsylvania Medical Center, 833 West Gates Building, 3400 Spruce Street, Philadelphia, PA 19104-4283. Phone: 215-614-0984; Fax: 215-662-3226; E-mail: sterman@mail.med.upenn.edu.

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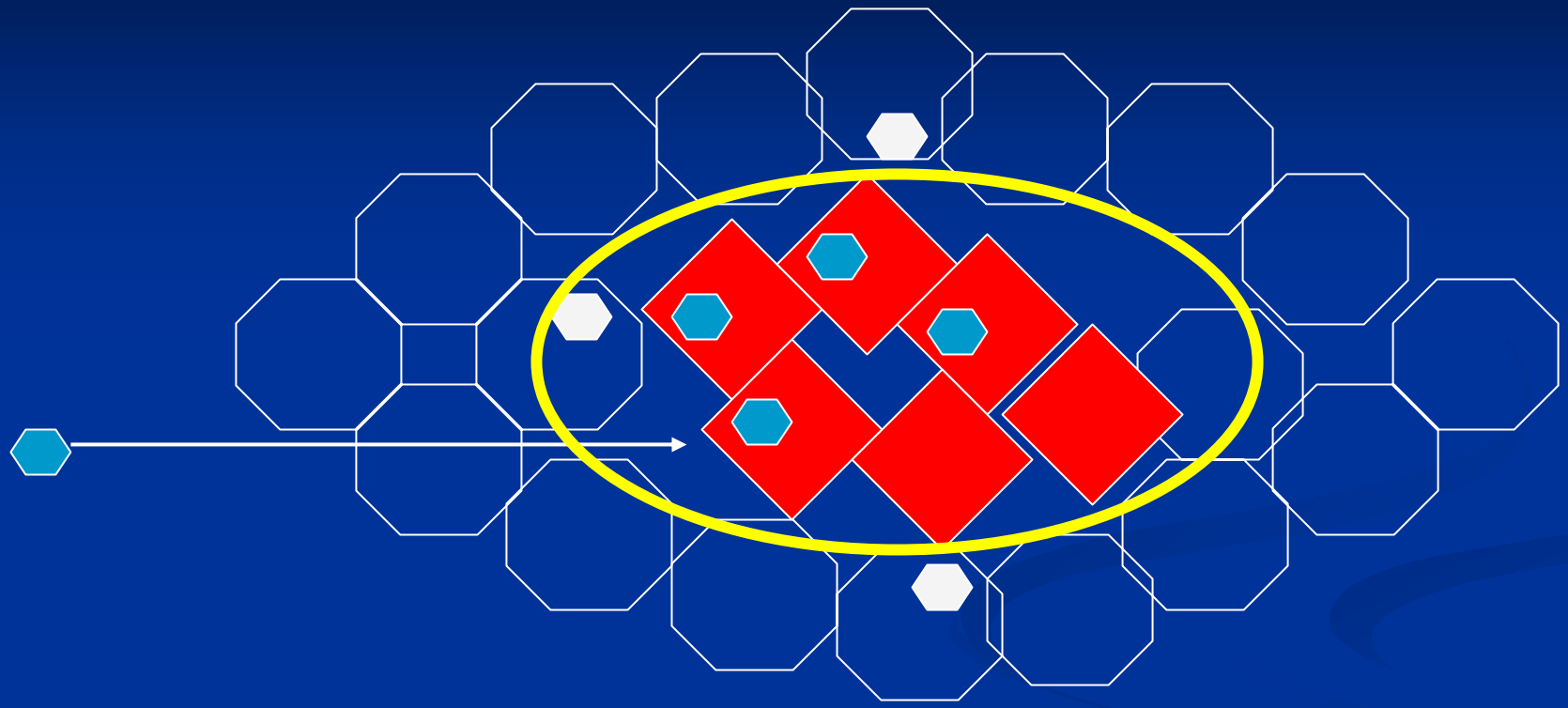
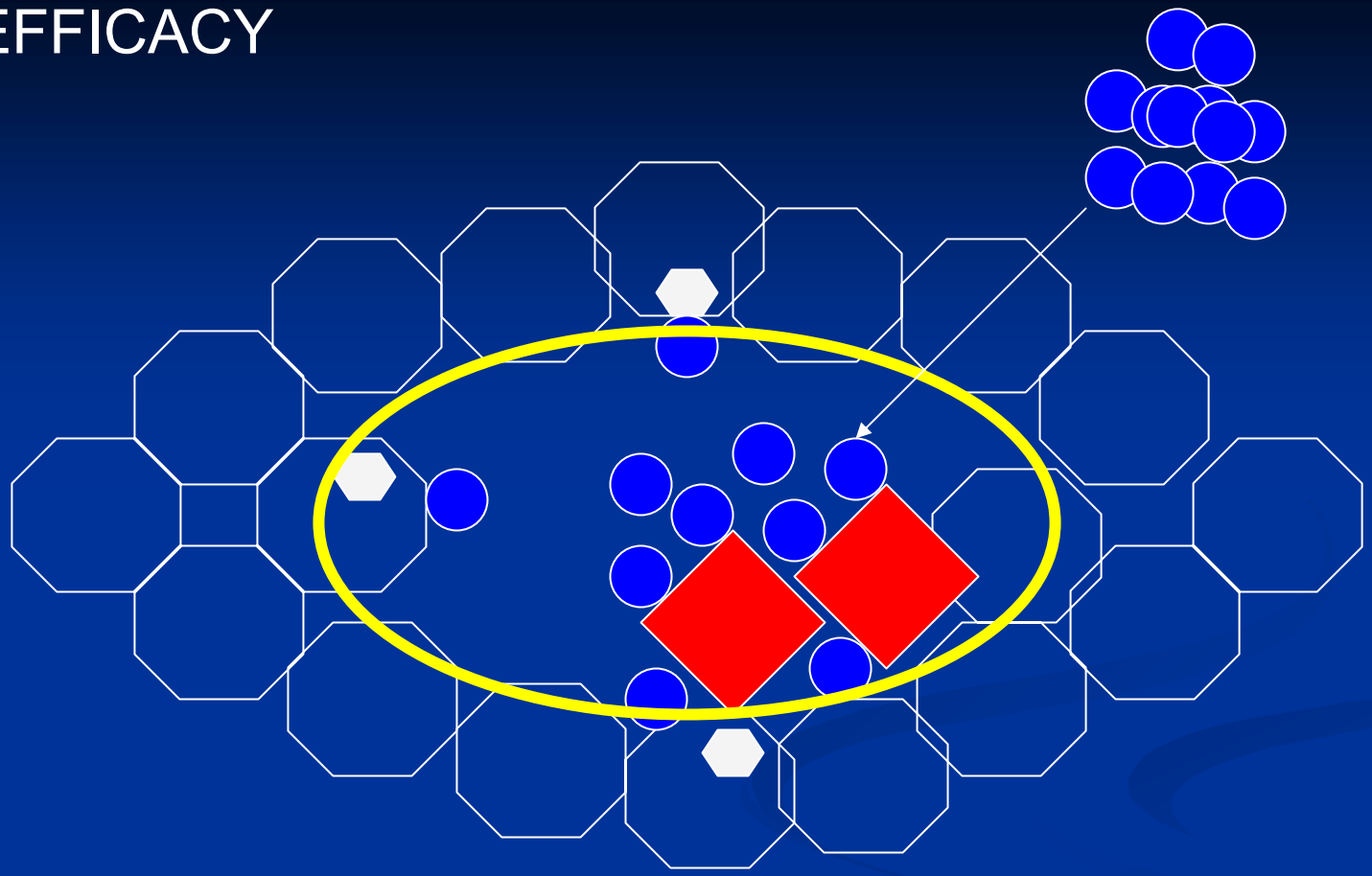
IFN-β - SAFETY



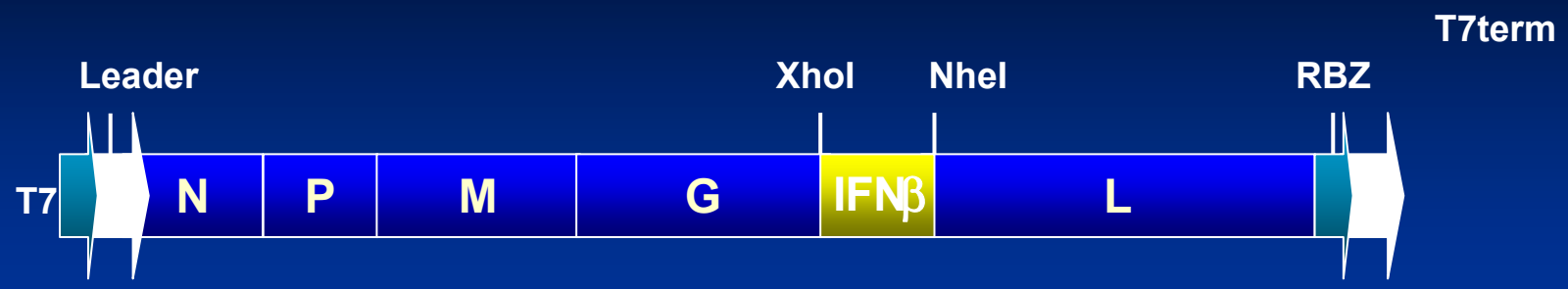
Molecular Basis of the Tumour Selectivity of VSV-IFN-β:
1) Direct Tumour Cell Oncolysis with a blanket of protective IFN-β

IFN-β - EFFICACY



Molecular Basis of the Tumour Selectivity of VSV:

1) Direct Tumour Cell Oncolysis
2) Immune Based Clearance



Full length infectious cDNA clone of VSV-IFNβ.
The IFN-β cDNA was inserted between the glycoprotein (G) and polymerase (L) protein.

Obuchi, M., Fernandez, M. & Barber, G.N. Development of recombinant vesicular stomatitis viruses that exploit defects in host defense to augment specific oncolytic activity. *J Virol* **77**, 8843-8856 (2003).

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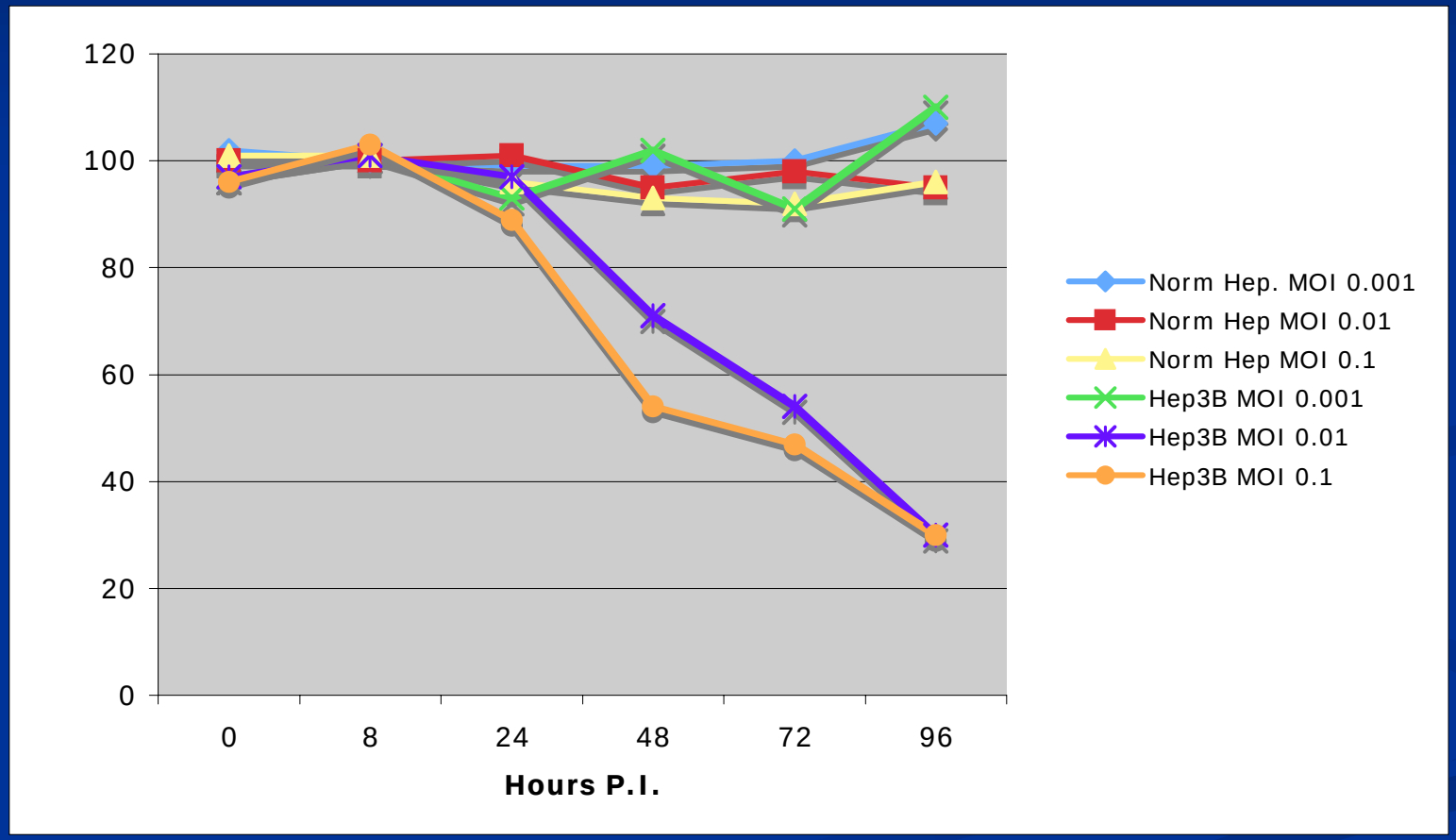
Our Experience with VSV:

1) Malignant Melanoma

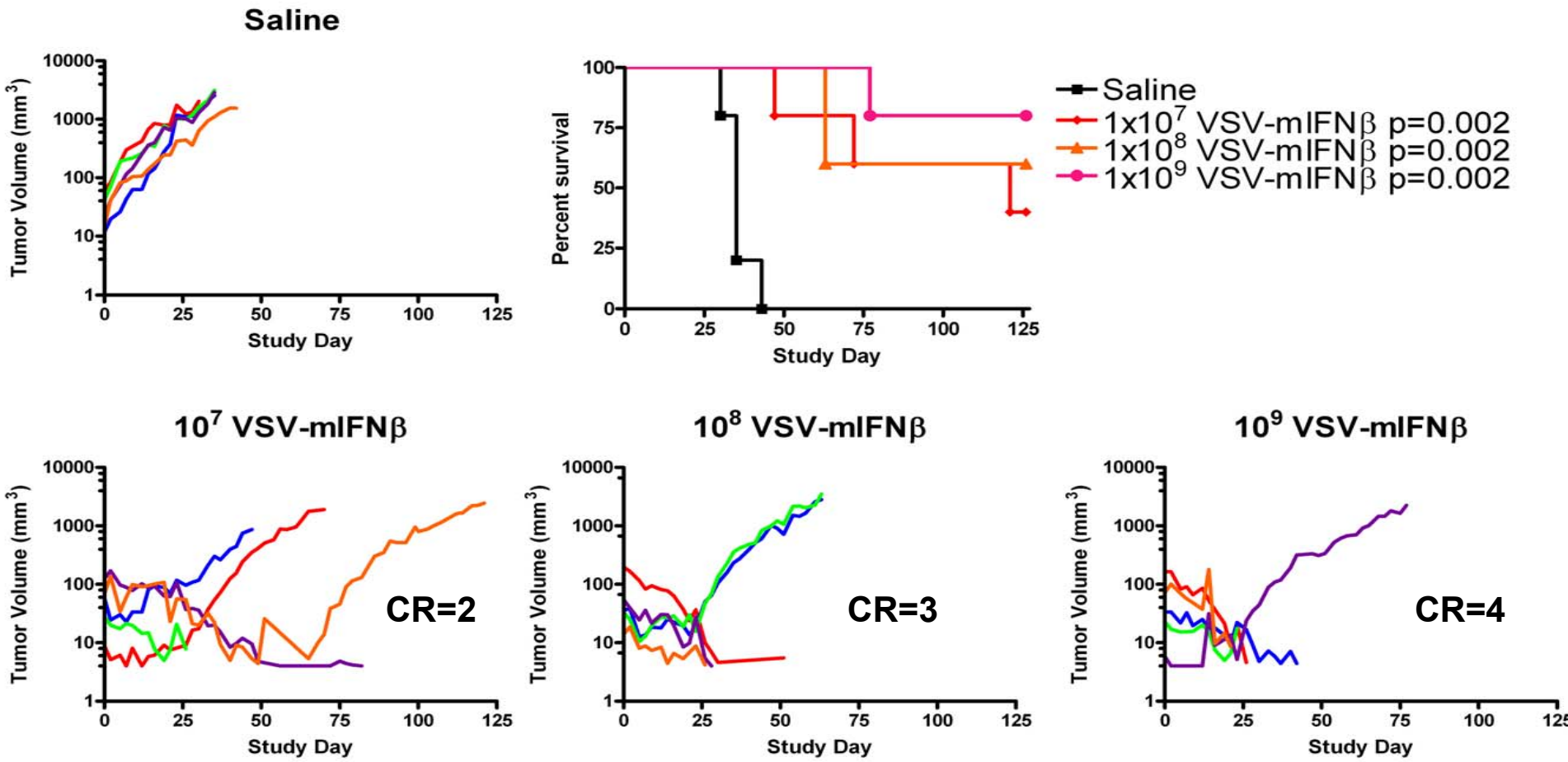
2) Hepatocellular Carcinoma

3) Mesothelioma

VSV-hIFN-β Selectively Kills Human Hepatoma Tumour Cells Over Normal Human Hepatocytes



VSV-mIFNβ has Anti-Tumor Activity Against Human Hep3B HCC Xenografts



Tumor volume and survival of nude mice bearing Hep3B subcutaneous tumors that were injected intra-tumorally with 10^7, 10^8, or 10^9 $TCID_{50}$ of VSV-mIFNβ. Single viral injection. CR = Number of animals with a complete response.

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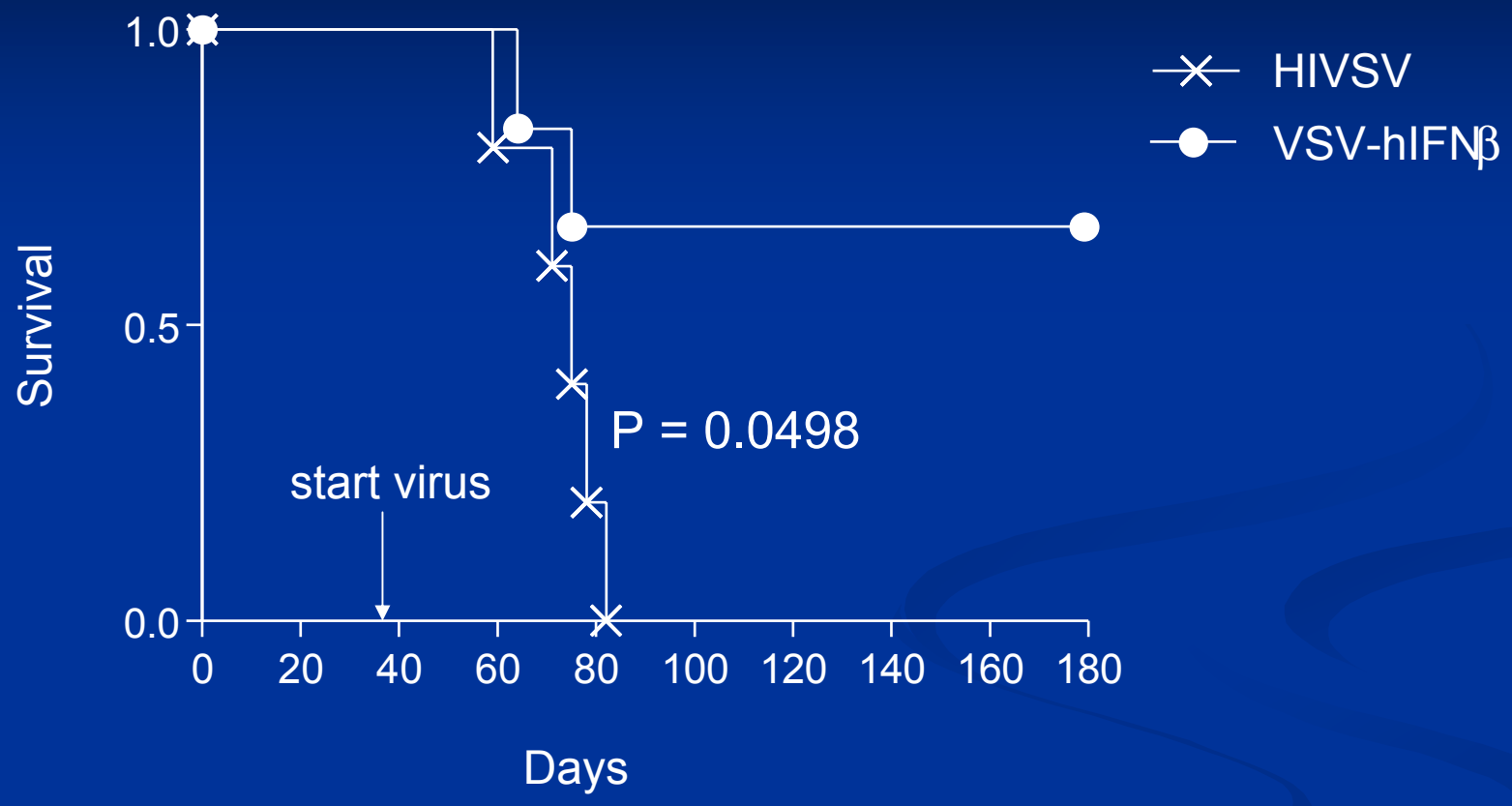
VSV-hIFNβ has Anti-Tumor Activity Against Established Human Hep3B HCC Xenografts



Nude mice bearing large established (50-80mm^3) Hep3B subcutaneous tumors were injected intratumorally with 5x10^8 pfu of VSV-hIFNβ for a total of 6 injections every other day for 2 weeks (5-6 mice/group). Survival of mice with time is shown. HI = heat inactivated control.

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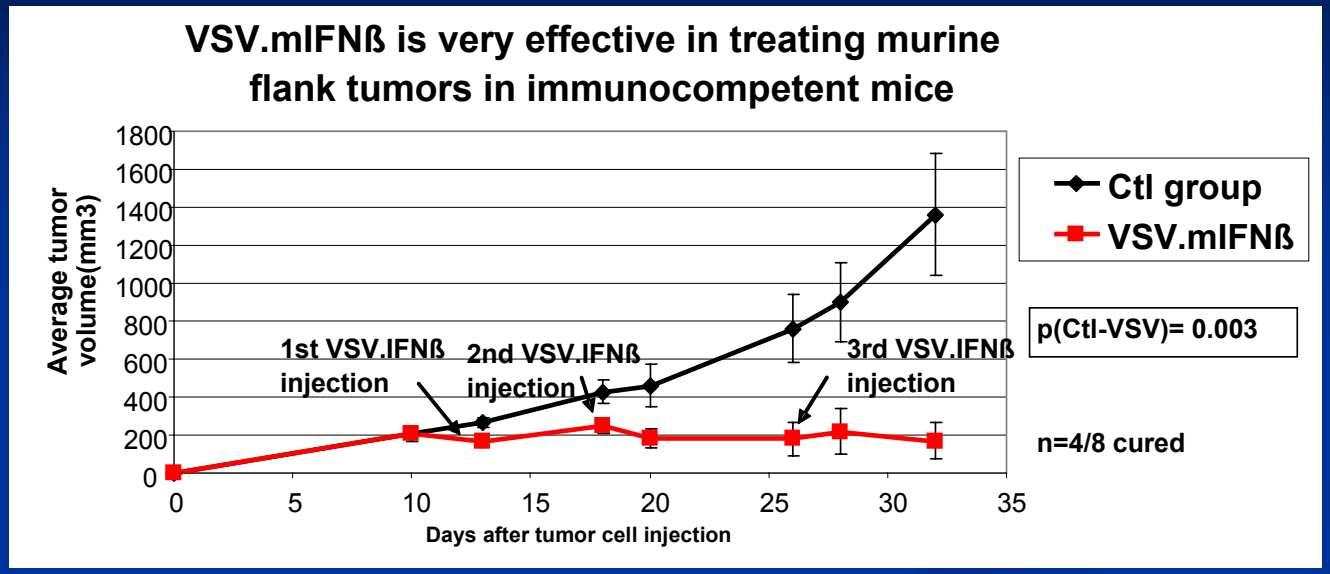
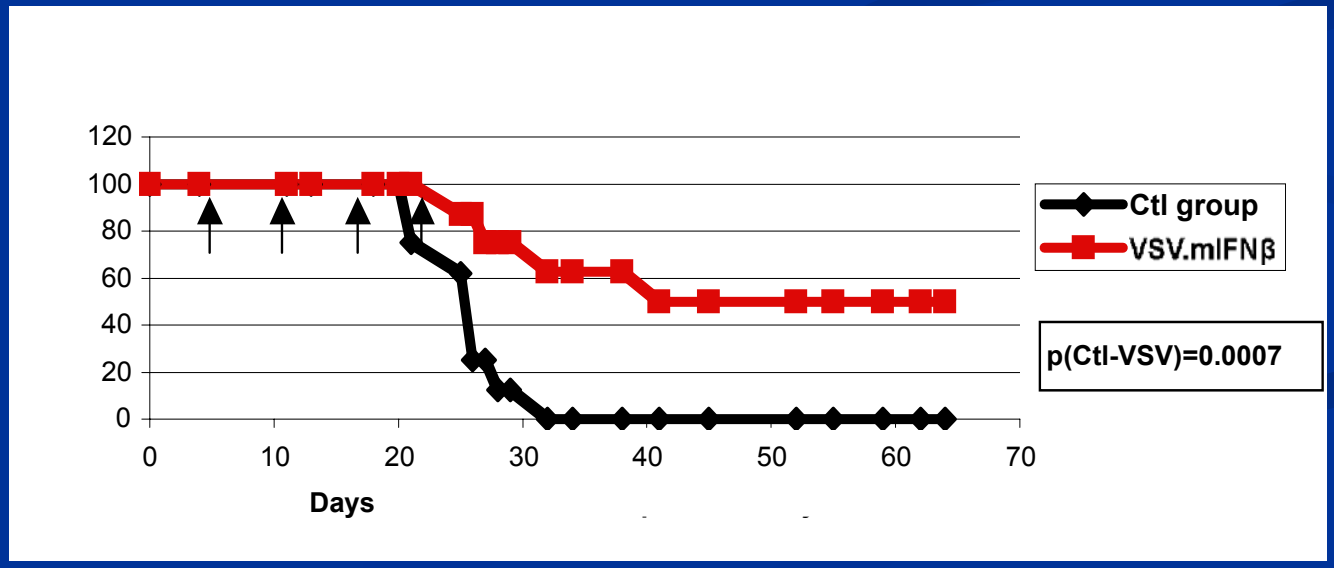
VSV-mIFNβ is effective in treating mice bearing tumors in a fully immunocompetent model





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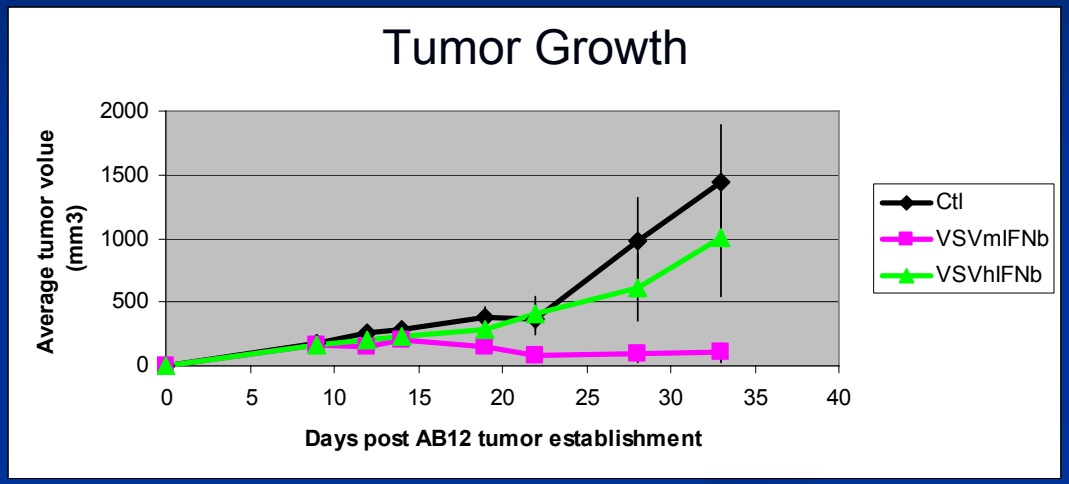
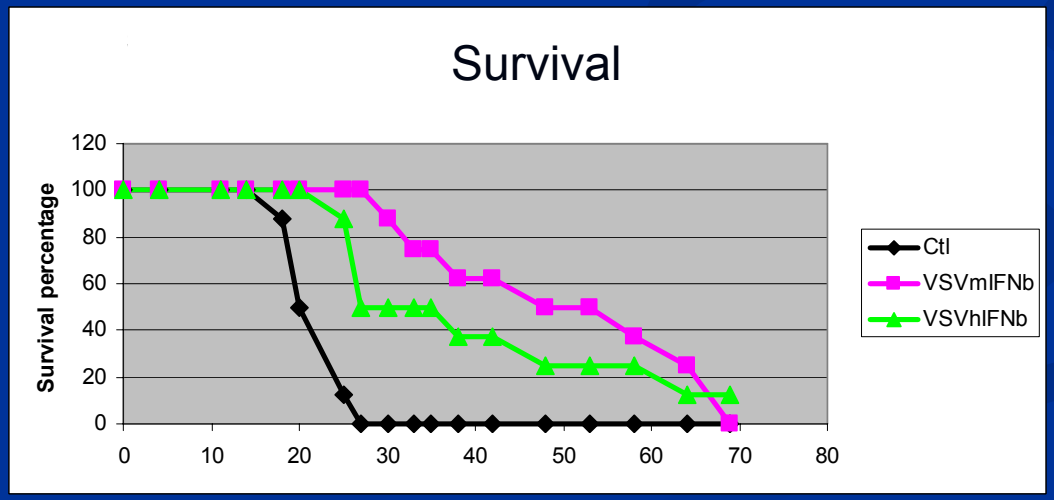
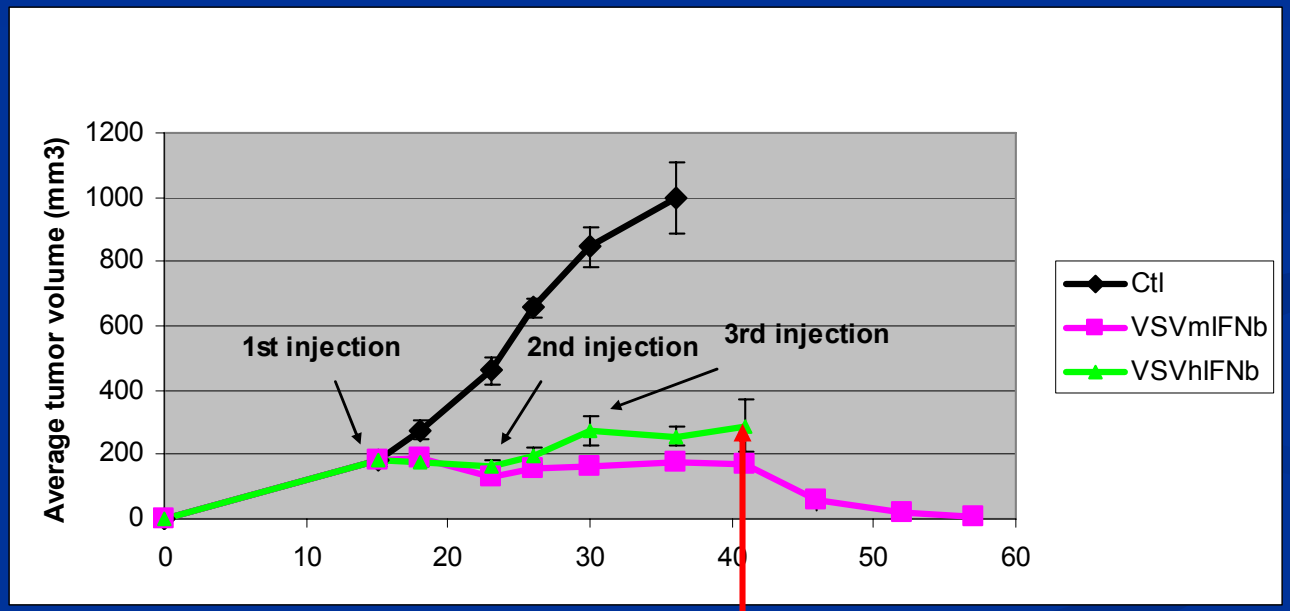
VSV-mIFNβ is more effective than VSV-hIFNβ inimmunocompetent mice - immune-mediated response augments therapy





Although both the human and mouse IFN expressing viruses appear to be similarly effective in nude mice, VSV-hIFNβ does not provide species-specific protection resulting in neurological toxicity:



TOXICITY

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Toxicity of VSV-IFNβ:

What are we concerned about?

Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Virology 360 (2007) 36–49
VIROLOGY
www.elsevier.com/locate/yviro

Neurovirulence properties of recombinant vesicular stomatitis virus vectors in non-human primates

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Available online 13 November 2006

Abstract

Although vesicular stomatitis virus (VSV) neurovirulence and pathogenicity in rodents have been well studied, little is known about VSV pathogenicity in non-human primates. To address this question, we measured VSV viremia, shedding, and neurovirulence in macaques. Following intranasal inoculation, macaques shed minimal recombinant VSV (rVSV) in nasal washes for 1 day post-inoculation; viremia was not detected. Following intranasal inoculation of macaques, wild type (wt) VSV, rVSV, and two rVSV-HIV vectors showed no evidence of spread to CNS tissues. However, macaques inoculated intrathalamically with wt VSV developed severe neurological disease. One of four macaques receiving rVSV developed clinical and histological signs similar to the wt group, while the remaining three macaques in this group and all of the macaques in the rVSV-HIV vector groups showed no clinical signs of disease and reduced severity of histopathology compared to the wt group. The implications of these findings for rVSV vaccine development are discussed.
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Keywords: Vesicular stomatitis virus; Pathogenicity; Neurovirulence; Viral vectors; Non-human primates; HIV vaccine; Intrathalamic

Neuroinvasiveness and Neurovirulence Protocol

- Macaques were inoculated either intranasally (IN) or intrathalmically (IT) with a total of 10^7 PFU of virus.
- Viruses tested: wtVSVin, rVSVin, rVSVin-GAG5 and rVSVin-CT1Gag5.
- Total of 4 Macques per group.
- Macaques were monitored for 21 days after challenge.

Neurovirulence properties of recombinant vesicular stomatitis virus vectors in non-human primates

-On intranasal inoculation, wt VSV showed no evidence of spread to CNS tissues;

-Inoculation intra-thalamically of wild type VSV induced severe neurological disease.

-Inoculation intra-thalamically of recombinant VSV induced no neurological disease.

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Inclusion of an IFN gene into VSV should protect normal brain cells from VSV:

JOURNAL OF VIROLOGY, Feb. 2007, p. 1479–1491 Vol. 81, No. 3
0022-538X/07/$08.00+0 doi:10.1128/JVI.01861-06
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Variable Deficiencies in the Interferon Response Enhance Susceptibility to Vesicular Stomatitis Virus Oncolytic Actions in Glioblastoma Cells but Not in Normal Human Glial Cells[▽]

Guido Wollmann,[1] Michael D. Robek,[2] and Anthony N. van den Pol[1]*

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Received 25 August 2006/Accepted 5 November 2006

'In contrast, IFN or poly(I:C) completely attenuated the infection of all primary control brain cells, whereas most glioblastoma cell lines treated with IFN or poly(I:C) showed little or no sign of protection and were killed by VSV. Together, our results demonstrate that activation of the interferon pathway protects normal human brain cells from VSV infection….'

Toxicology and Pharmacology Studies on an Indiana Strain Vesicular Stomatitis Virus Expressing the Human Interferon Beta Protein for a Phase I Trial in Patients with Recurrent Hepatocellular Carcinoma.

Viral Toxicology and Pharmacology Laboratory

Director: Dr Kah-Whye Peng

Choice of Toxicology/ Pharmacology Animal Model (In Discussion with the FDA):

- Susceptibility to VSV-hIFNβ virus infection
- Transgene is biologically active in chosen model
  - i.e. species specificity/cross-reactivity
- Representative of clinical situation
  - Tumor vs. non-tumor model

Preclinical Toxicology Studies Using VSV-IFN β In Buffalo Rats

NOAEL - No Observed Adverse Events Level.

MTD - Lowest level of virus at which an adverse event was observed.

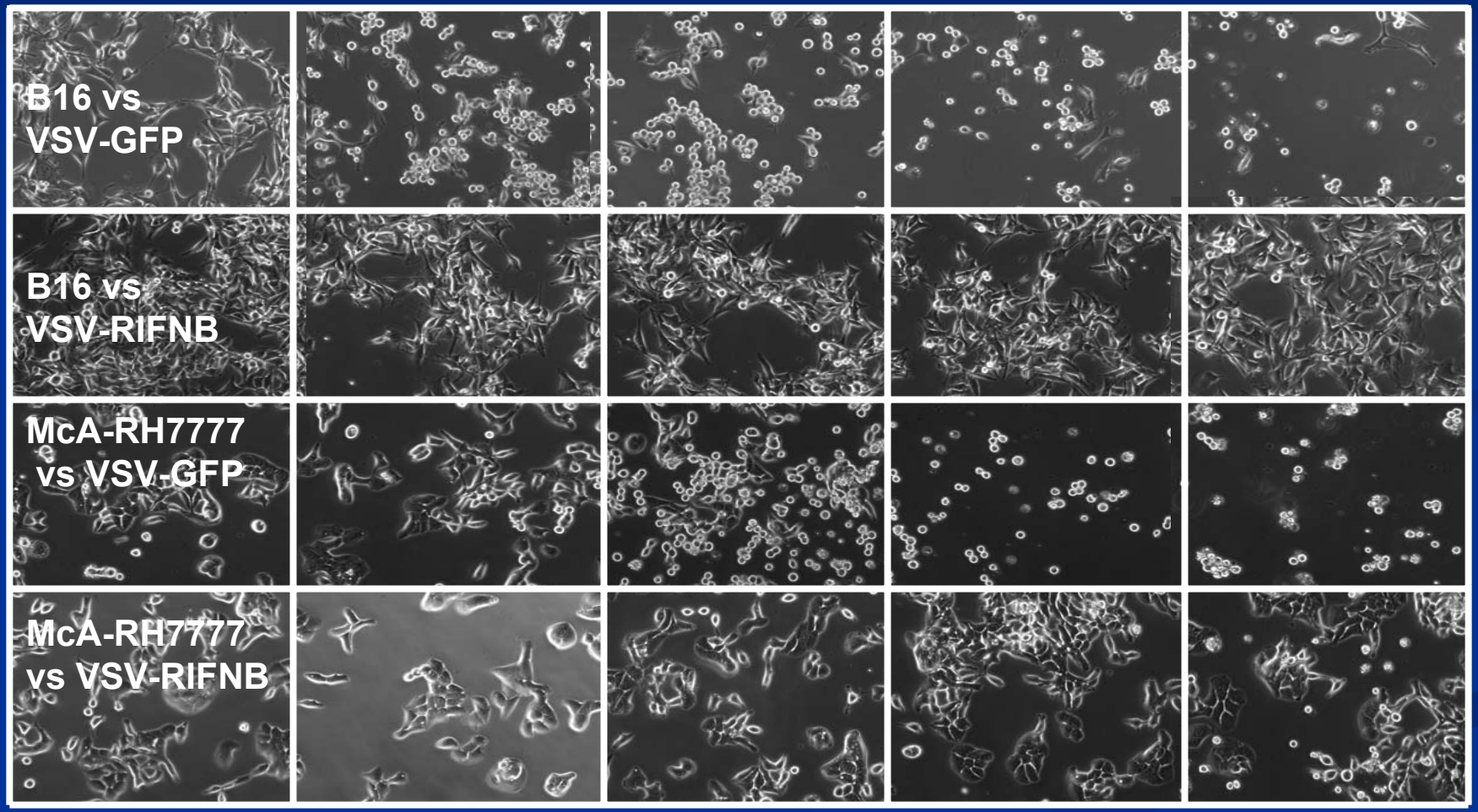
Toxicology Studies: Determining the MTD

Agent	Balb/c IH	Sprague-Dawley IH	Buffalo Rat IH	Buffalo Rat + Orthotopic HCC IT	Balb/c IV	Sprague-Dawley IV
VSV-mIFNb	$<3.00 \times 10^{9}$	$>6.61 \times 10^{8}$			1.00×10^{9}	$>1.00 \times 10^{10}$
VSV-hIFNb		$>7.59 \times 10^{9}$	1.00×10^{7}	$<7.59 \times 10^{7}$	1.00×10^{9}	$<1.00 \times 10^{8}$
VSV-rIFNb			$>1.00 \times 10^{10}$	$<1.00 \times 10^{9}$		

Table 1. Maximum Tolerated Dose (MTD) of VSV with different species of interferon beta transgenes in mouse and rat models, with local and systemic delivery. Reported toxicity was neuro-specific, with lethargy and partial to complete front and rear paralysis 3-8 days post virus. The presence of active virus cytotoxicity was confirmed with brain, spinal cord, tumor, and liver overlays on VSV-susceptible cells as well as by QRT-PCR analysis. Delivery routes defined as either IH (intrahepatic), IV (intravenous), or IT (intratumoral). MTD is displayed in $TCID_{50}$ units.

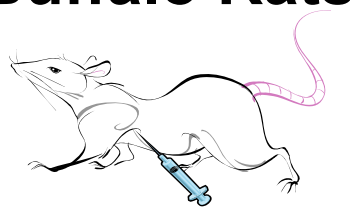
In Vitro Oncolytic Activity (24 hours)

MOI= Mock 0.001 0.01 0.1 1.0



Acute Rodent Toxicity, Biodistribution, & Efficacy Study in an Orthotopic Model

Buffalo Rats (6-7wks; 5F+5M/group)



Day -11: Surgical inoculation of $4x10^6$/20mL McA-RH7777 cells in left lateral lobe.

Day 0: Surgical intra-hepatic injection of 50mL agent into tumor nodule.

Monitor weight, clinical signs

Agent	Dose ($TCID_{50}$)
Saline	50mL
VSV-rIFNb	$1x10^8$
VSV-rIFNb	$1x10^9$
VSV-hIFNb	$1x10^7$

SAC days 2, 16.

-Histology

-Blood: Chemistry, Hematology, Coagulation, IFNb, IL-6.

-Anti-VSV & anti-IFNb Ab levels

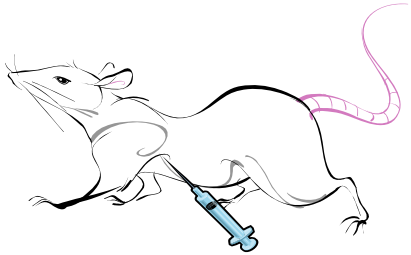
-Viral levels in organs and blood (QRT-PCR)

-Infectious titer of blood and tissues positive for VSV

Ebert et al Cancer Research (July 2003)

Chronic Rodent Toxicity, Biodistribution, & Efficacy Study

Buffalo Rats (6-7wks; 5F+5M/group)



Day 0: Surgical intra-hepatic injection of 50mL agent into left lateral lobe of the liver.

Monitor weight, clinical signs

Agent	Dose ($TCID_{50}$)
Saline	50mL
VSV-rIFNb	$1x10^8$
VSV-rIFNb	$1x10^9$
VSV-hIFNb	$1x10^7$

SAC days 44, 91.

-Histology

-Blood *(day 14 and terminal):* Chemistry, Hematology, Coagulation, IFNb, IL-6.

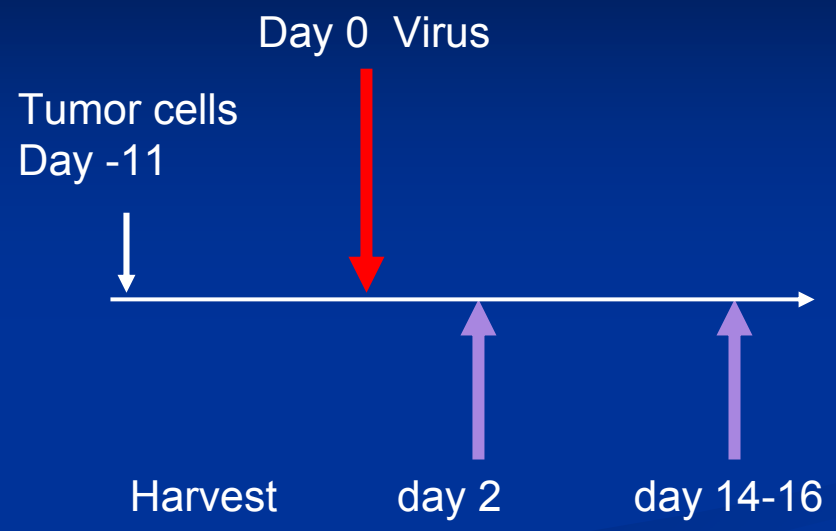
-Anti-VSV & anti-IFNb Ab levels

-Viral levels in organs and blood (QRT-PCR)

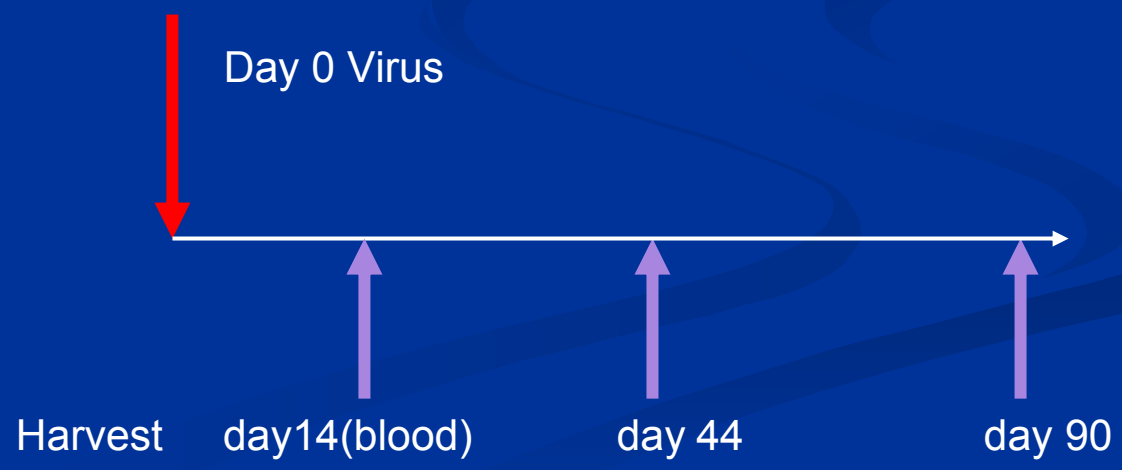
-Infectious titer of blood and tissues positive for VSV

Preclinical Toxicology Studies Using VSV-interferon β in Buffalo Rats

A. SHORT TERM



B. LONG TERM



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Preclinical Toxicology Studies Using VSV-IFN β In Buffalo Rats

	SHORT TERM	LONG TERM
VSVh-INFβ	$1x10^7$ $TCID_{50}$	$1x10^7$ $TCID_{50}$

NOAEL of VSV-hIFN-β in Rats,
with minimal/no protective effect of IFN-β, is
$1x10^7$ $TCID_{50}$

MTD is $7.59x10^7$ $TCID_{50}$

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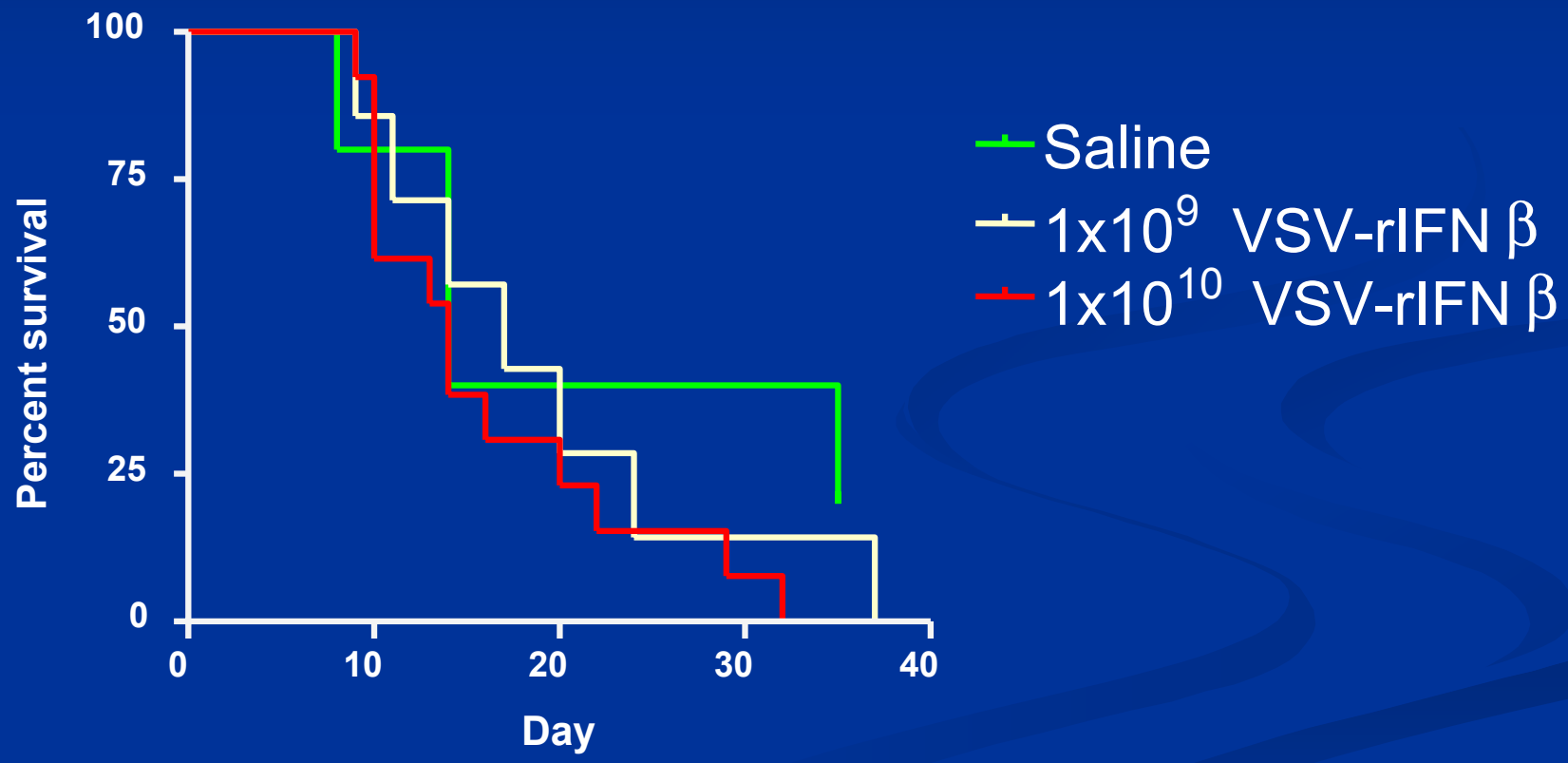
Preclinical Toxicology Studies Using VSV-IFNβ In Buffalo Rats

	SHORT TERM	LONG TERM
VSVh-IFNβ	$1x10^7$ $TCID_{50}$	$1x10^7$ $TCID_{50}$
VSVr-IFNβ	$1x10^{10}$ $TCID_{50}$	$1x10^{10}$ $TCID_{50}$ (NOT GLP)

NOAEL of VSV-rIFN-β in Rats,
with species-specific protective effect of IFN-β, is
$1x10^{10}$ $TCID_{50}$

MTD has not yet been reached

No Therapy was Observed in this Model (Dr Roizman)



MAYO CLINIC

Preclinical Toxicology Studies Using VSV-IFN βIn Buffalo Rats

Dose calculation: Rats to Humans - WITH Species Specific IFN-β Protection:

NOAEL of VSV-rIFNβ, <u>with species specific protection by IFN-β,</u> is $1x10^{10}$ $TCID_{50}$ in a 170 g rat

=

412 x 1 x 10^{10} $TCID_{50}$ for a 70kg human = 4.12 x 10^{12} $TCID_{50}$

Preclinical Toxicology Studies Using VSV-IFNβIn Buffalo Rats

Dose calculation: Rats to Humans - WITHOUT Species Specific IFNβ Protection

NOAEL of VSV-hIFNβ, <u>without species specific protection by IFNβ,</u> is 1×10^7 $TCID_{50}$ in a 170 g rat

=

412 x 1 x 10^7 $TCID_{50}$ for a 70kg human = 4.12 x 10^9 $TCID_{50}$

Preclinical Toxicology Studies Using VSV-IFN βIn Buffalo Rats

Dose calculation: Rats to Humans

-WITH Species Specific IFNβ Protection = 4.12×10^{12} $TCID_{50}$

- WITHOUT Species Specific IFNβ Protection = 4.12×10^{9} $TCID_{50}$

<u>Our Proposed Starting Dose = 1×10^{5} $TCID_{50}$</u>

Rhesus macaques:

4 males/8 yr

Direct intrahepatic administration by CT-guided injection

2@ 10^9 $TCID_{50}$ with VSVh-IFNβ out to 3 months

2@ 10^{10} $TCID_{50}$ VSVh-IFNβ out to 1 month

To Date: No observed neurotoxicity; blood parameters underway.

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VIROLOGY

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Neurovirulence properties of recombinant vesicular stomatitis virus vectors in non-human primates

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Abstract

Although vesicular stomatitis virus (VSV) neurovirulence and pathogenicity in rodents have been well studied, little is known about VSV pathogenicity in non-human primates. To address this question, we measured VSV viremia, shedding, and neurovirulence in macaques. Following intranasal inoculation, macaques shed minimal recombinant VSV (rVSV) in nasal washes for 1 day post-inoculation; viremia was not detected. Following intranasal inoculation of macaques, wild type (wt) VSV, rVSV, and two rVSV-HIV vectors showed no evidence of spread to CNS tissues. However, macaques inoculated intrathalamically with wt VSV developed severe neurological disease. One of four macaques receiving rVSV developed clinical and histological signs similar to the wt group, while the remaining three macaques in this group and all of the macaques in the rVSV-HIV vector groups showed no clinical signs of disease and reduced severity of histopathology compared to the wt group. The implications of these findings for rVSV vaccine development are discussed.
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Keywords: Vesicular stomatitis virus; Pathogenicity; Neurovirulence; Viral vectors; Non-human primates; HIV vaccine; Intrathalamic

Vector Manufacture

Viral Vector Production Laboratory (VVPL)

Director: Dr Mark Federspiel

Specific Concerns Raised by RAC Reviewers:

Dr Roizman

Dr Bartlett

Dr Kahn

Dr Roizman:

Fraction of HCC with defective IFN pathway:

-We do not know yet: - We will test patient samples for VSV replication +/- IFN; correlate with assays on transcripts associated with innate immune response (Dr Barber).

Effects of vigorous viral replication and high levels of IFNβ release:

-IFNβ should serve as a safety blanket; identified clinical toxicities: - We will test intra-tumoral, and systemic, IFNβ levels in animal models; we will treat with 100x levels for toxicity, tumor treatment; serum cytokines.

Dr Roizman:

Emergence of a mutant VSV-IFNβ defective in IFNβ:

-Intratumoral viral replication is limited (extinction by innate response); stable passage in the production procedure; We will sequence viral populations, and individual genomes, following *in vivo* passage through human HCC; and will mix VSV-IFNβ with wt VSV to gauge the proportion of viruses which need to retain IFNβ production.

What will be monitored in patients:

-Circulating virus; IFNβ, clinical signs; cytokine release syndrome.

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Specific Concerns Raised by RAC Reviewers:

Dr Bartlett: Will the chronic infection of normal hepatocytes with HCV, +/- prior therapy with IFN, abrogate their sensitivity to IFN and therefore make them susceptible to VSV infection/replication/cytotoxicity?

15-20% of normal hepatocytes are infected with HCV in a chronic carrier; neighboring normal hepatocytes should still respond (despite IFN treatment failure); we will infect normal hepatocytes with HCV and test VSV replication.

Specific Concerns Raised by RAC Reviewers:

Dr Kahn: Several issues related to the consent document.

We have altered the document in all of these respects.

Development of a Vesicular Stomatitis Virus Expressing IFN-β As an Oncolytic Agent For Direct Intratumoral Injection in Patients with Hepatocellular Carcinoma

Pre-clinical studies: Richard Vile, Mayo Clinic, Rochester;
Steven Albelda, U Penn.

Viral Toxicology and Pharmacology Laboratory, Mayo Clinic, Rochester:
Director Dr Peng

Viral Vector Production Laboratory, Mayo Clinic, Rochester,
Director: Dr Mark Federspiel

MITESH J. BORAD, M.D.

Clinical Protocol for Phase I Study of Intra-tumoral Injection of Vesicular Stomatitis Virus Secreting Interferon Beta (VSV-IFN-β) in Patients with Hepatocellular Cancer (HCC)

December 3rd, 2008

NIH RAC Meeting

BACKGROUND

- Advanced hepatocellular carcinoma (HCC) is an incurable disease
- Sorafenib was approved in 2007 for advanced HCC based on an improvement in survival (10.7 vs 7.9 months) [Llovet et al, ASCO 2007]
- Despite the approval of sorafenib, advanced HCC remains a largely incurable and fatal disease and novel therapeutics are desperately needed

BACKGROUND

Use of a targeted oncolytic poxvirus, JX-594, in patients with refractory primary or metastatic liver cancer: a phase I trial

Byeong-Ho Park*, Taeho Hwang*, Ta-Chiang Liu, Daniel Y Sze, Jae-Seok Kim†, Hyuk-Chan Kwon, Sung Yong Oh, Sang-Young Han, Jin-Han Yoon, Sook-Hee Hong, Anne Moon, Kelly Speth, Chohee Park, Young-Joo Ahn, Manijeh Daneshmand, Byung Geon Rhee, Herbert M Pinedo, John C Bell, David H Kirn

- GM-CSF secreting Pox Virus
- Trial in solid tumor patients (n=14) of which HCC (n=3) were treated
- One patient had a partial response by RECIST criteria
- Two patients had PET responses
- Three patients had AFP decreases (65-98%)

BACKGROUND

- Vesicular Stomatis Virus (VSV) is a negative strand RNA virus
- VSV appears to have a predilection for malignant liver cells compared to normal liver cells
- VSV-IFNβ has demonstrated efficacy in cell lines *in vitro* and animal models *in vivo*
- Anti-tumor effects are felt to be due to oncolysis and immune-mediated tumor destruction

OBJECTIVES

PRIMARY

- To Determine the Maximum Tolerated Dose (MTD) of Intra-tumoral Injection of VSV-IFNβ in Patients with HCC
- To Determine the Dose Limiting Toxicities (DLTs) and Other Safety Characteristics of VSV-IFNβ in Patients with HCC

OBJECTIVES

SECONDARY

- To Determine the Pharmacokinetic (PK) Profile of VSV-IFNβ in Patients with HCC by Measurement of VSV-IFNβ in Blood By Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)
- To Characterize the Pharmacodynamics (PD) of VSV-IFNβ By Way of Measuring Serum IFNβ and RT-PCR of VSV-IFNβ
- To Determine the Recommended Phase II Dose of VSV-IFNβ (RP2D)
- To Observe for Any Evidence of Anti-Tumor Activity

INCLUSION CRITERIA

- Age ≥ 18 years
- Histologically or cytologically confirmed HCC that is refractory or intolerant to sorafenib based therapy
- Following labs obtained ≤ 14 days prior to administration of study virus:
  - ANC ≥ 1000 /µL, Platelets ≥ 80,000 /µL, Hemoglobin ≥10 g/dL
  - AST/ALT ≤ 5 x ULN
  - Creatinine WLN for institution
  - Total Bilirubin ≤ 3 x ULN
  - aPTT ≤ 1.5 x ULN, INR ≤ 1.4 x ULN

INCLUSION CRITERIA

- Ability to provide informed consent
- Willingness to return to Mayo Clinic Arizona for follow up
- Life expectancy ≥ 12 weeks
- ECOG performance status (PS) 0-1
- Willingness to provide all biological specimens required
- Negative serum pregnancy test for women of childbearing potential done ≤ 3 days prior to administration of study virus
- Child Pugh Score of A or B

EXCLUSION CRITERIA

- Uncontrolled Infection
- Systemic anti-cancer therapy within 4 weeks of administration of study virus
- Known HIV infection
- Other concurrent chemotherapy, immunotherapy, radiotherapy or investigational therapy
- Pregnant or nursing women
- Patients for whom surgical resection or liver transplantation would be more appropriate
- Any condition, which in the opinion of the investigator, would render the patient unsuitable for study participation

SELECTION OF STARTING DOSE

Dose calculation: Rats to Humans

-WITH Species Specific IFNβ Protection = 4.12×10^{12} $TCID_{50}$

-WITHOUT Species Specific IFNβ Protection = 4.12×10^{9} $TCID_{50}$

<u>Our Proposed Starting Dose = 1×10^{5} $TCID_{50}$</u>

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DEFINITION OF DOSE LIMITING TOXICITY (DLT)

DLT will be defined as:

Grade 4 toxicity of any duration

Grade 3 toxicity lasting 5 days or longer

Context will be from time of administration of VSV-IFNβ to 4 weeks post-administration unless unrelated to study virus

Events other than those pre-defined may be considered DLTs if warranted to be such after discussion between study PIs

DOSE ESCALATION SCHEME

- "3+3" Modified Fibonacci Dose Escalation Scheme
- At any dose level (DL) 2 patients may be enrolled simultaneously and observed for 28 days for DLTs
- 3rd patient at that DL may be enrolled if no greater than 1 patient experiences a DLT
  - If 0 of 3 patients experience a DLT, then proceed to next DL
  - If 1 of 3 patients experience a DLT, then enroll up to 3 additional patients
  - If 2 of 3 patients experience a DLT, then stop dose escalation and go down one DL
- Toxicity Observation Period : 4 weeks after administration of study virus for purposes of DLT

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DOSE ESCALATION SCHEME

- Patients may be replaced if they fail to complete a 28 day observation period
- Investigator teleconferences will be held every 2 weeks to discuss safety findings
- Dose Escalations of 0.5 logs $TCID_{50}$/ml selected

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STATISTICS

- Dose escalation will be implemented at the MTD
- Up to a total of 12 patients will be treated at the MTD
- There will a power of 85% to detect a DLT that has a prevalence of 15% or higher
- Total number of patients will be contingent upon number of dose levels and occurrence of DLTs but will range from 12-48

DOSE ESCALATION SCHEME

DOSE LEVEL	$TCID_{50}$
DL-1	5×10^4
DL1	1×10^5
DL2	5×10^5
DL3	1×10^6
DL4	5×10^6
DL5	1×10^7
DL6	5×10^7
DL7	1×10^8

INVESTIGATIONAL CLINICAL PRODUCT

- GMP manufacturing plan is to produce 25-75 L of VSV-IFN-B infected cell supernatant
- This should yield about 500 ml of purified/concentrated product per 25 L Bioreactor with an estimated infectious titer of 1-2 x 10^{11} $TCID_{50}$/ml
- Product will be vialed in 1-10 ml aliquots
- Storage will be done at -60° Celcius
- Biosafety Level of product will be Biosafety Level 2 (BL2)
- Dilution will be done with normal saline at time of administration to achieve required dose based on dose level

INTRA-TUMORAL INJECTION OF VSV-IFN-B

- VSV-IFN-B will be administered in a single tumor using a 20-22 gauge needle using ultrasound guidance
- Viral dose corresponding to the dose level for the cohort will be thawed and diluted in normal saline to arrive at a final injectable volume of 15 ml
- 15 ml will be injected into 5 disparate sites into the tumor
- Tumor injections will be carried out under ultrasound guidance

RISKS ASSOCIATED WITH INJECTION PROCEDURE (SAFETY OF INJECTIONS)

- Needle length 15-20 cm and 20-22 gauge
- Risk of percutaneous liver biopsy which utilizes larger gauge needle (15-16) : Mortality of 0.031% (Smith et al, Radiology 1991)
- In Phase I study with JX-594 (Pox Virus) in HCC with similar patient population (platelets > 75,000 /µL, Child Pugh A/B), no injection related morbidities were observed (Park et al, Lancet Oncol 2008 and Kim et al, Molecular Therapy, 2006)
- VSV-IFN-B will be administered in a single tumor using a 20-22 gauge needle using ultrasound guidance

OBSERVATION AFTER INJECTION

- Observation will be for 6 hours after injection
- Vital signs (temperature, pulse, blood pressure and respiratory rate) will be recorded every 15 minutes for first 2 hours and then hourly for next 4 hours
- If there is any clinical deterioration, the patient will be hospitalized for continuous observation

RISK OF HUMAN TRANSMISSION

- VSV is an arthropod borne virus and the primary hosts are rodents, cattle, horses and swine
- Generally it causes non-fatal disease with clinical signs of vesicular lesions in the mucous membranes of the mouth and nose
- VSV rarely infects humans and as such most patients will be virus naive
- In the rare reported cases of human infection mild flu-like symptoms are the predominant manifestation
- One case of pediatric encephalitis has been reported
- In the event a patient develops vesicular symptoms, the patient will immediately be isolated and observed until no longer felt to be contagious

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	SCREENING	DAY 1 +++	DAY 2	DAY 3	DAY 8 +++	DAY 15 +++	DAY 22 +++	DAY 29 & ONWARDS ++	EOS
INCLUSION/EXCLUSION	√								
HISTORY/PHYSICAL	√	√	√	√	√	√	√	√	√
PERFORMANCE STATUS	√	√	√	√	√	√	√	√	√
CBC WITH DIFFERENTIAL	√	√	√	√	√	√	√	√	√
CMP	√	√	√	√	√	√	√	√	√
DIRECT BILIRUBIN	√	√	√	√	√	√	√	√	√
INDIRECT BILIRUBIN	√	√	√	√	√	√	√	√	√
INR/PT	√	√	√	√	√	√	√	√	√
PTT	√	√	√	√	√	√	√	√	√
ALPHA FETOPROTEIN	√	√						√	√
SERUM PREGNANCY	√								√
INFORMED CONSENT	√								
CHILD PUGH SCORE	√								
PK PCR-VSV BLOOD		√	√	√	√	√	√		√
ANTI-VSV ANTIBODIES		√	√	√	√	√	√		√
URINE-VSV PFA		√	√	√	√	√	√		√
SALIVA-VSV PFA		√	√	√	√	√	√		√
FECES-VSV PFA		√	√	√	√	√	√		√
SERUM INF-BETA		√	√	√	√	√	√		√
IMAGING		√	Every 8-12 weeks per investigator discretion						
INJECTION OF VSV-IFN-B		√							

PHARMACOKINETIC (PK) SCHEDULE

TIME POINTS
Baseline
15 minutes +/- 5 minutes
30 minutes +/- 5 minutes
60 minutes +/- 5 minutes
4 hours +/- 30 minutes
24 hours +/- 60 minutes
2 days +/- 3 hours
7 days +/- 3 hours
14 days +/- 3 hours
21 days +/- 3 hours

DISCONTINUATION FROM STUDY

- Patient experiences toxicity which in the opinion of the investigator would warrant discontinuation after requisite observation period
- Patient develops intercurrent illness which precludes further participation
- Non-compliance with study procedures
- Disease progression
- Withdrawal of consent
- In the opinon of the investigator, it would not be in the best interests of the patient to participate further in the study

DATA AND SAFETY MONITORING AND COLLECTION OF DATA

- Study PIs (Vile and Borad) will serve meet via teleconference every 2 weeks to discuss safety findings
- Adverse Events (AEs) will be reported using NCI CTC Version 3.0
- Serious Adverse Events (SAEs) will be communicated in a more expeditious fashion (within 24 hours of knowledge of event) between investigators and to other appropriate agencies

THANK YOU

Questions ?

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