

Translational Virotherapy in an Academic Setting



Stephen J Russell
Mayo Clinic

Attenuated measles as an oncolytic agent



Bluming and Ziegler (1971) Lancet ii, 105-106



SLAM only

SLAM + CD46

-targets CD46, ubiquitous receptor expressed at higher density on tumour vs. nontumour cells

-Efficiently and selectively infects and kills human tumour cells, spares normal cells

-Has potent antitumor activity against xenograft models of several human malignancies

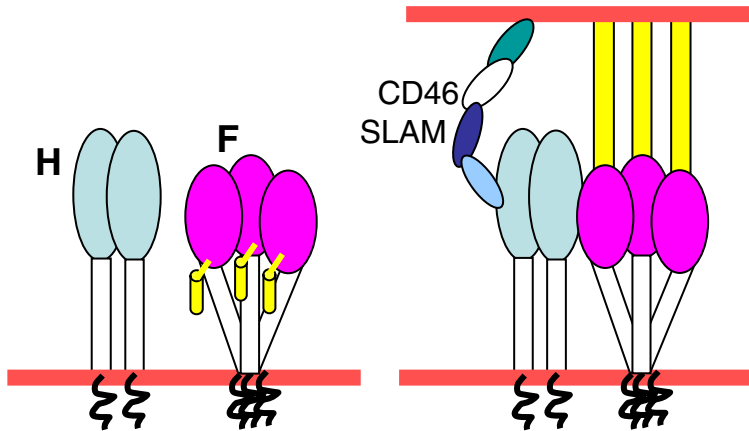
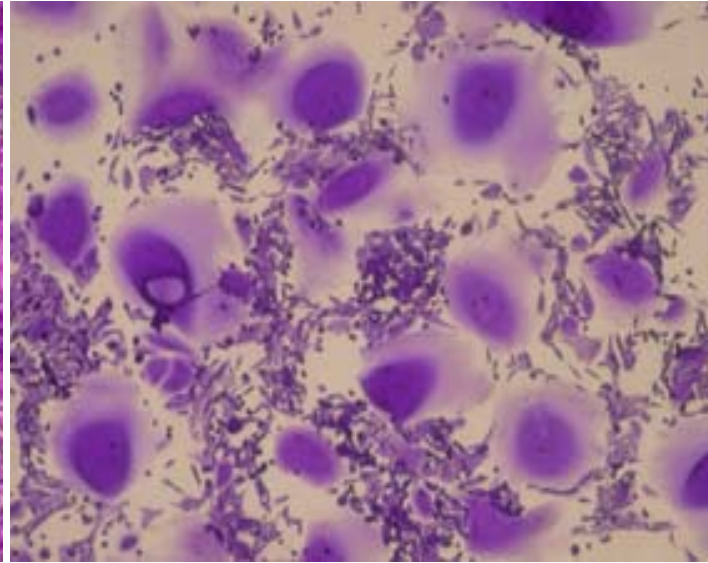
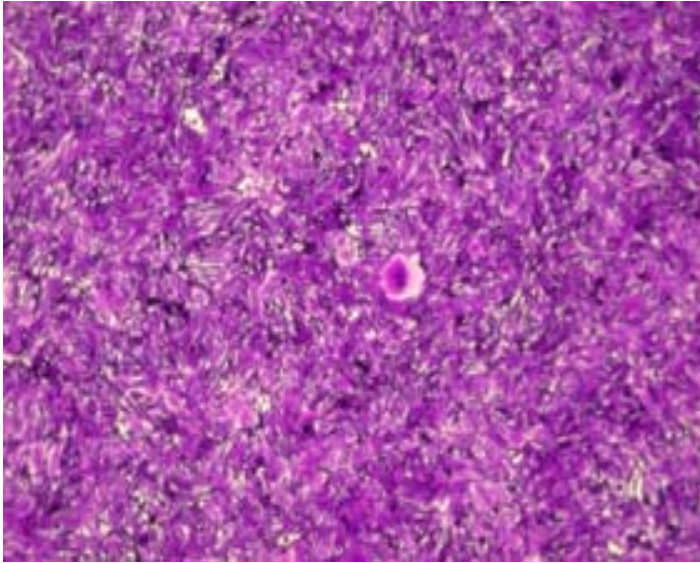
-Can be engineered to express additional genes; recombinants are extremely stable



Grote et al. Blood, 2001; 97: 3746-3754
 Peng et al. Blood, 2002; 98: 2002-2007
 Dingli et al. Blood, 2004; 103: 1641-1646
 Peng et al. Cancer Res, 2002; 62: 4656-4662
 Phuong et al. Cancer Res, 2003; 63: 2462-2469



Fusogenic proteins: measles F and H

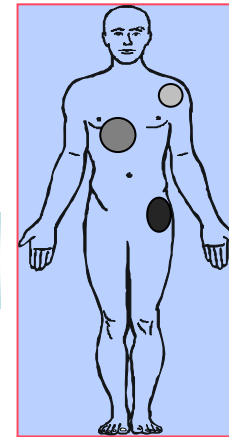


- H binds to CD46 or SLAM
- F triggers fusion

SLAM is expressed on activated immune cells.

CD46 is overexpressed on human cancer cells, a key determinant of oncolytic specificity

Measles engineering



C. Arming

Pwt

B. Imaging

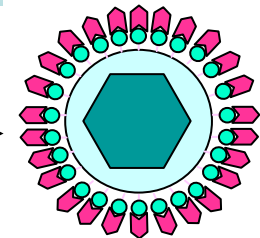
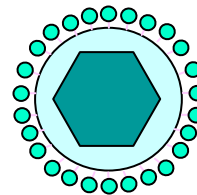
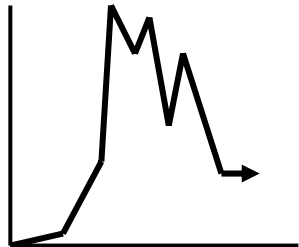
NIS



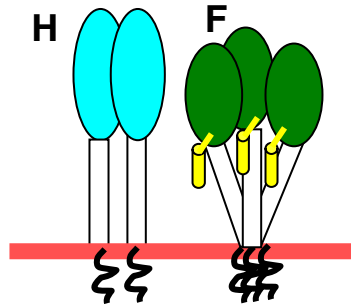
CEA

ligand

D. Targeting

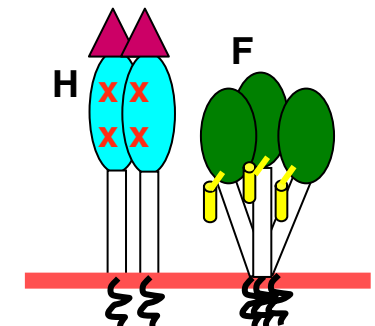


A. Monitoring



X ablate CD46 & SLAM binding

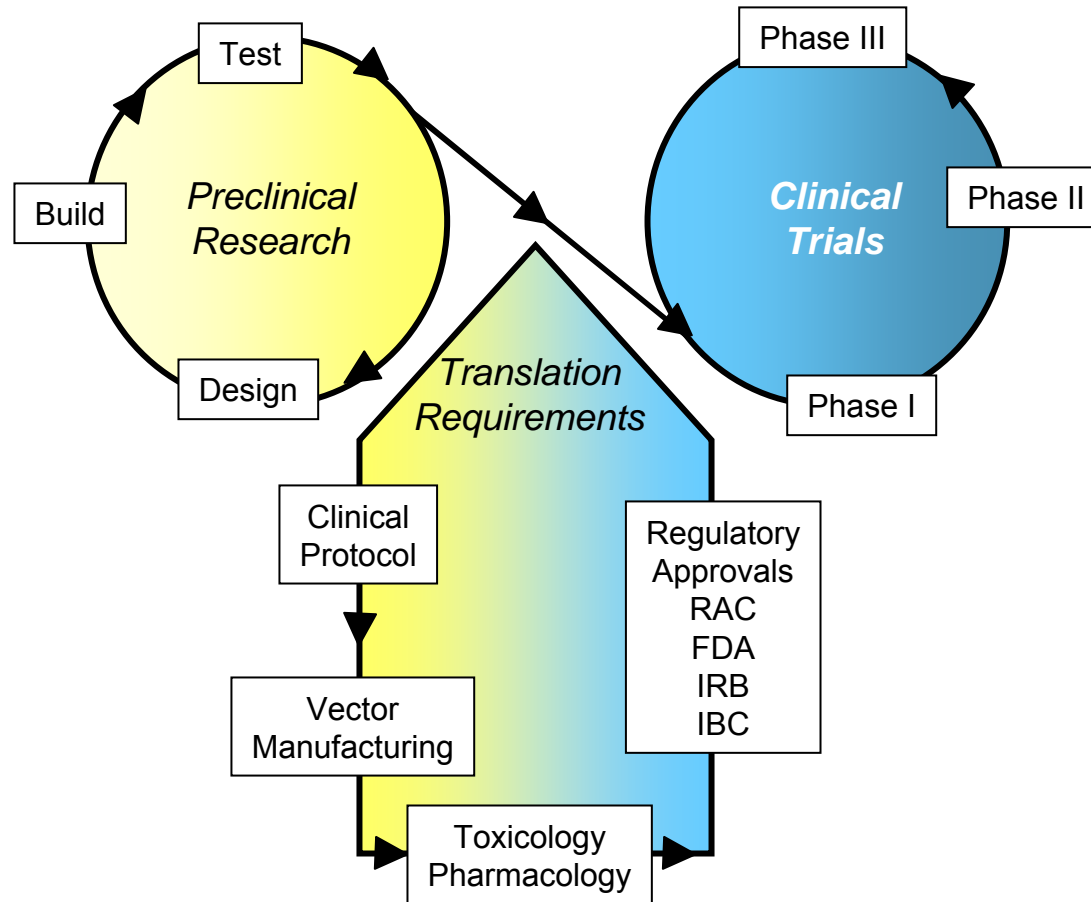
▲ display cell targeting ligands



CD46, SLAM able

CD46, SLAM blind

Translational Process



1. Which measles virus?
2. Which malignancies/routes of administration?

Virus monitoring:

- Was the virus delivered/expressed?
- How efficiently?
- Did the infection spread?
- Where?

Needed for evaluation of treatment failures

Serial biopsy is invasive/inconclusive

Pharmacokinetics:

The fate of the drug in the body

Direct analysis of multiple human tissues at multiple timepoints after administration of virotherapy is not feasible

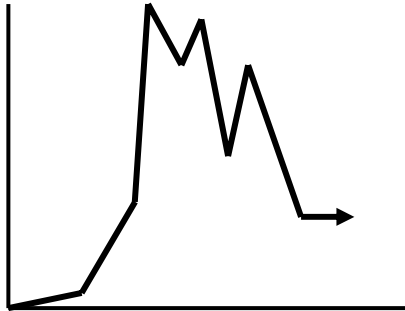
Even the most basic question of whether there was ANY infection of host cells remains unanswered for most patients treated with oncolytic viruses.

NONINVASIVE monitoring strategies are therefore required, especially for repetitive measurements of

1. Production of virus encoded proteins by infected cells
2. Distribution of infected cells expressing viral proteins

Which viruses to translate?

Soluble marker

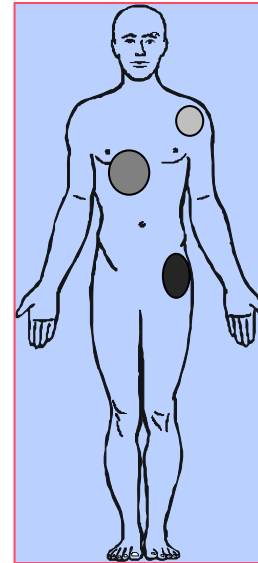


MV-CEA

CEA



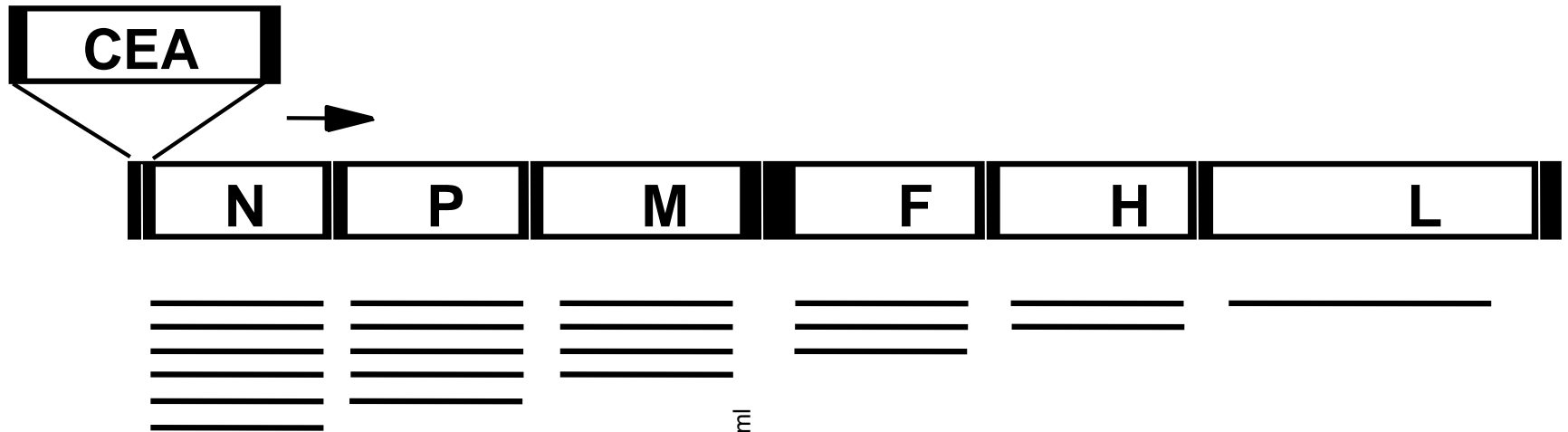
Imagable marker



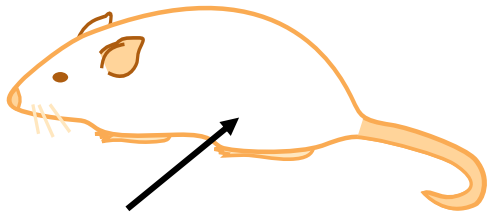
MV-NIS

NIS

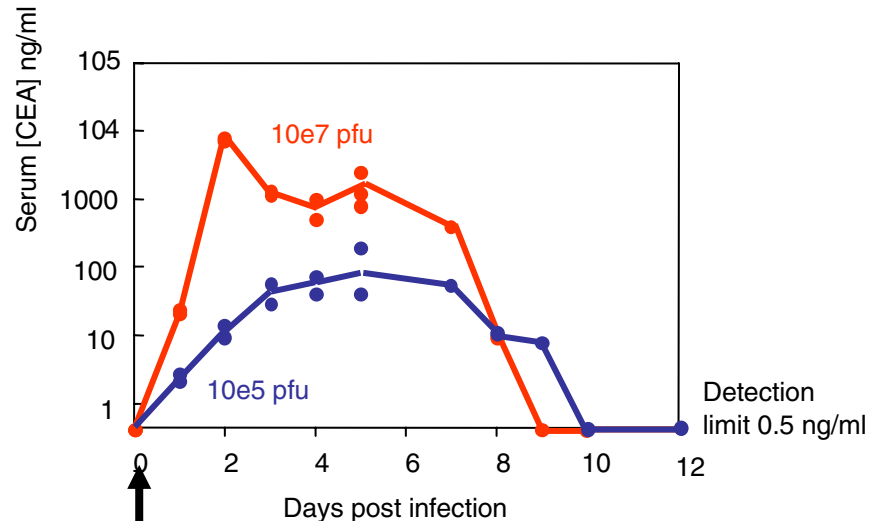
Expression monitoring - soluble marker peptide



IFNAR Ko CD46 Ge mice

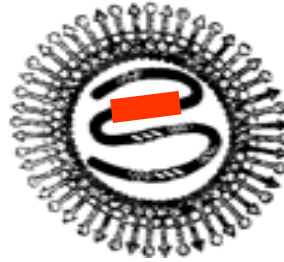


IP injection MV-CEA

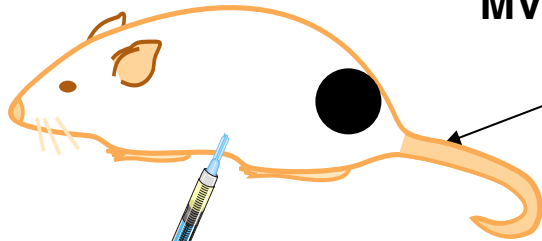
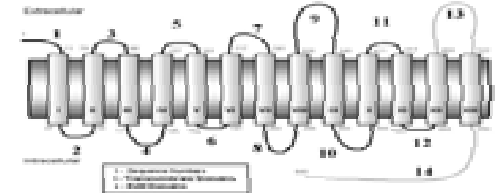


Imaging virus spread (MV-NIS)

NIS gene



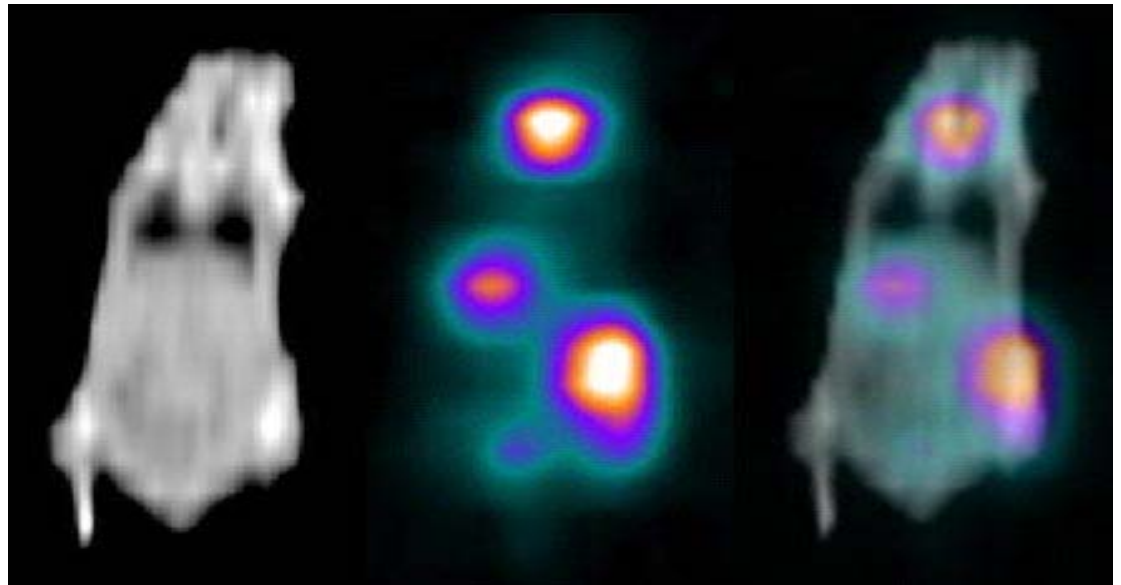
NIS protein



MV-NIS iv

Wait 7 days for virus to spread.
Give radioiodine

PET/CT imaging



Which disease targets?

Preformed antimeasles antibodies may block delivery via bloodstream

disease targets and delivery routes were therefore chosen to minimise this problem

Ovarian cancer	IP
Glioma	IT
Myeloma	IV

Animal models for efficacy studies.

1. Human xenograft models in nude or SCID mice (no antiviral immunity)
2. Patient-derived material (tumor and nontumor cells)

MV-CEA

Ca ovary (IP) Peng et al. Cancer Res, 2002; 62: 4656-4662

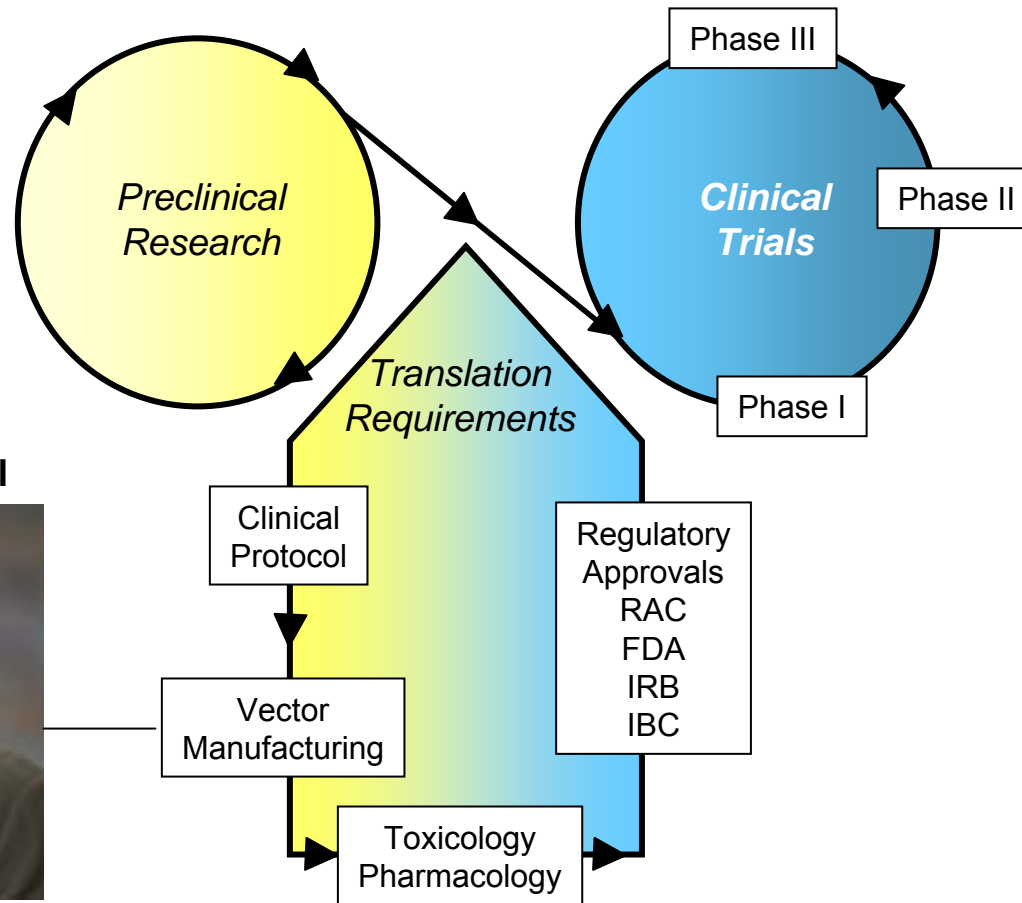
MV-CEA

Glioma (IT) Phuong et al. Cancer Res, 2003; 63: 2462-2469

MV_NIS

Myeloma (IV) Peng et al. Blood, 2002; 98: 2002-2007
Dingli et al. Blood, 2004; 103: 1641-1646

Manufacturing



Mark Federspiel

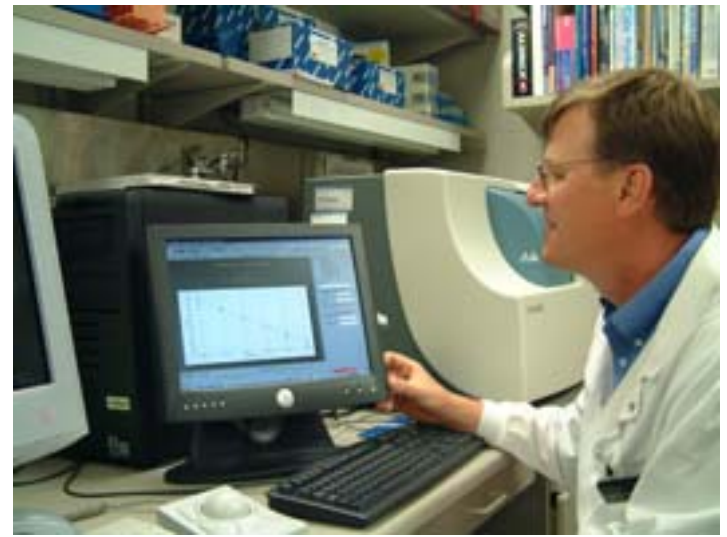




Large-Scale Production and Purification Capabilities



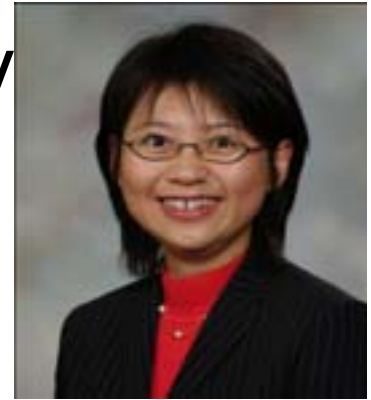
GMP Manufacturing Capabilities



Quality Control Assays

Toxicology/pharmacology

- Fate of the drug (virus) in the body
 - where does it go?
 - how is it eliminated?
- Effective dose
- Toxicity (in relevant species, protocol)



Choice of species for measles toxicology studies

No animal model fully recapitulates the human distribution of SLAM and CD46 measles receptors combined with intracellular permissiveness for measles replication

Information gained from animal toxicology studies is therefore of limited value for prediction of human toxicities and this is reflected in cautious clinical trial design.

Human experience with wt measles infection and with attenuated measles viruses of the Edm vaccine lineage is extensive and highly reassuring.

Toxicity of live measles vaccines

1. Vaccine dose is $>10^3$ TCID₅₀ by IM route
2. Billions of doses administered
3. Higher doses (up to 10^6) tested extensively in infants
4. Very impressive safety record
5. Main side effect is mild self-limited measles-like illness (fever, rash) but this is not seen in primates
6. Recommended even for HIV-positive children and immune-suppressed stem cell transplant patients

Ovarian Cancer Protocol

Oncolytic MV by IP route: Potential toxicities

1. Infusion reaction/peritonitis
 2. Measles-like illness (fever, rash, coryza)
 3. Transient immunosuppression (DTH)
 4. Measles pneumonitis, encephalitis
 5. Virus persistence (SSPE etc.)
- ? Relative roles of SLAM and CD46

Possible pharmacology/toxicology models

1. Monkeys

2. Rats

3. Pigs



4. Mice

CD46GeIFNARko mice

CD46 expression

Yes (human-like)

SLAM expression

No

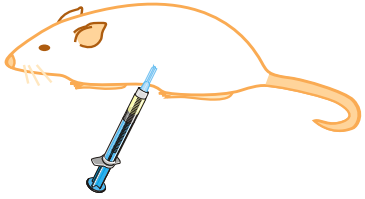
Cells permissive

Partial

- ***Not susceptible to wt measles***
- ***Can be infected by attenuated MV***
- ***Defective innate immune response***

MV-CEA toxicity studies

CD46 transgenic, IFN α Rko mice



→ Analyses

MV-CEA IP Injection

Group 1: 10^7 TCID₅₀

Group 2: 10^6 TCID₅₀

Group 3: 10^5 TCID₅₀

Group 4: 10^7 TCID₅₀ x 6

Group 5: Vehicle Control

Group 6: Uninjected

→ **General health**

→ **Weight**

→ **Blood**

Biochemistry

Hematology

Coagulation

CEA

→ **Histology**

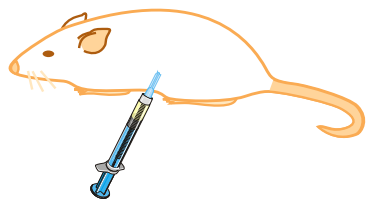
Acute

Subacute

Chronic

MV-CEA toxicity studies

CD46 transgenic, IFN α Rko mice



→ Analyses

MV-CEA IP Injection

Group 1: 10^7 TCID₅₀

Group 2: 10^6 TCID₅₀

Group 3: 10^5 TCID₅₀

Group 4: 10^7 TCID₅₀ x 6

Group 5: Vehicle Control

Group 6: Uninjected

No toxicities observed

Even at top dose (5×10^8 TCID₅₀/kg)

General health

Weight

Blood

Biochemistry

Hematology

Coagulation

CEA

Histology

Acute

Subacute

Chronic

Ovarian cancer clinical Protocol

1. Advanced ovarian cancer
2. IP administration of MV-CEA in 500 ml saline
3. Repeat administration
4. Dose escalation (10^3 to 10^9 TCID₅₀)
5. CEA monitoring to guide dose escalation

FDA approved starting dose of only 10^3 TCID₅₀
(~20 TCID₅₀/kg)

MV-CEA in Ovarian Ca: Response Assessment (RECIST)

21 evaluable patients

- Best objective response: stable disease (14/21)
- Median duration 88 days (range 54-277 days)
- Outcome was dose dependent: 9/9 patients with stable disease in dose levels 5-7, vs 5/12 in dose levels 1-4
- 5 CA-125 responses
- Median Overall Survival: 12.2 mo
- Historic experience in similar patient population: < 6 mo (Markman et al, 2006)

MV-CEA for glioma: Pilot MV-CEA neurotoxicity studies in transgenic mice

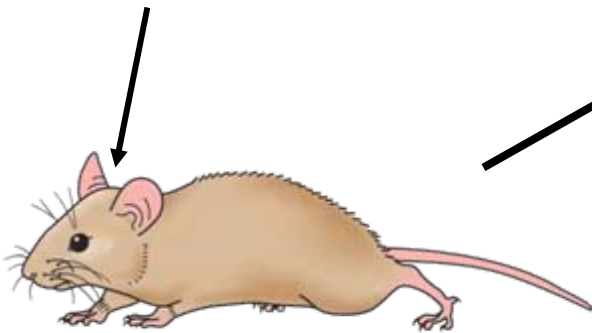
CD46 Ge IFNAR KO mice (5F+5M/group)

-Vehicle

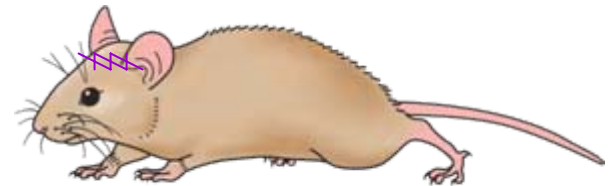
- 10^4 MV-CEA

- 10^5 MV-CEA

10 μ L intracerebral,
days 1 and 5



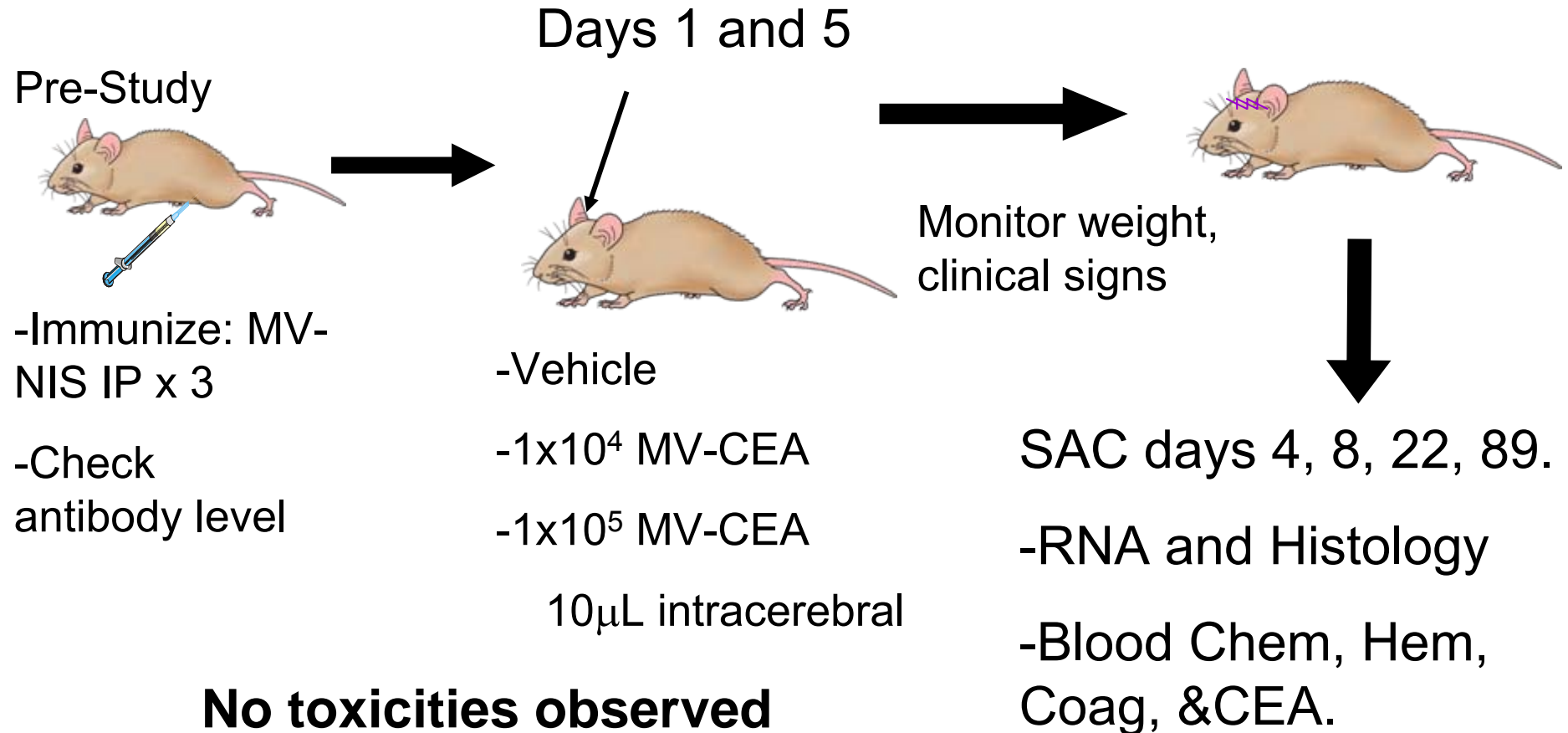
Monitor weight,
clinical signs



Weight loss
Focal neurological signs
Seizures
Death by day 8

Formal mouse neurotox study (measles-immunized transgenic mice)

CD46 Ge IFNAR KO mice (5F+5M/group)



Rhesus model for neurotoxicity studies?

1. Monkeys
2. Rats
3. Pigs
4. Mice



Rhesus monkeys

<i>CD46 expression</i>	Yes
<i>SLAM expression</i>	Yes
<i>Cells permissive</i>	Yes

But: *CD46 is expressed abundantly on rhesus rbc membranes
MV-Edm agglutinates rhesus rbc, (wt MV does not)
MV-Edm is unable to disseminate in rhesus*

Rhesus Neurotox Study

- 5 adult male rhesus
- Measles immune
- Non-terminal study

Group	MV-CEA (TCID ₅₀)	n
I	2×10^6	2
II	2×10^5	2
III	Vehicle	1

Species	Brain weight (kg)	Dose (TCID50)	Equivalent human dose (TCID50)
Human, adult	1.4		
Rhesus Macaque (<i>Macaca mulatta</i>)	0.090	2×10^6	3.1×10^7

Rhesus neurotox study outcome

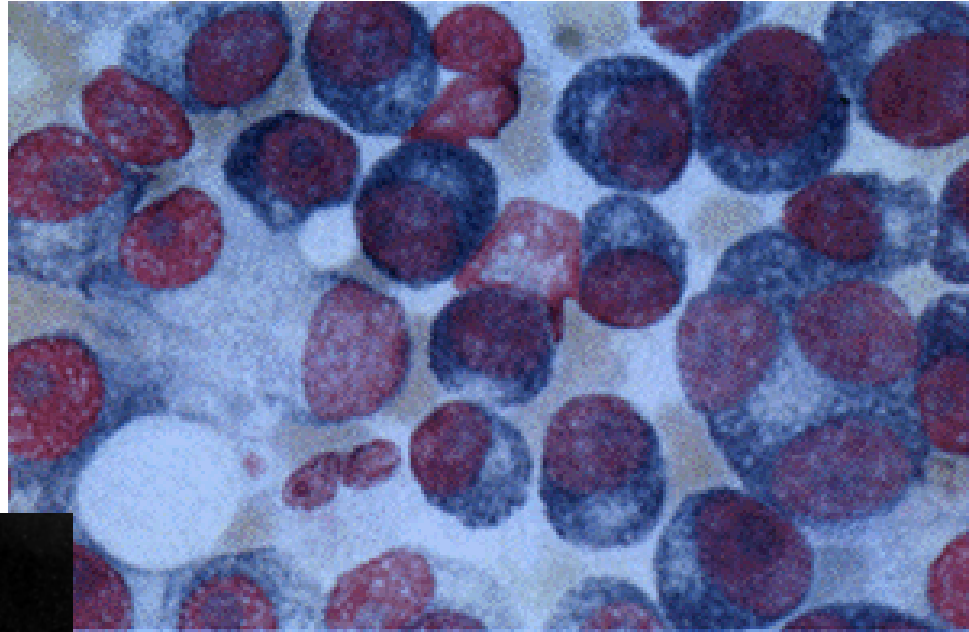
- No CEA detected
- Bloodwork normal
- No Neurological Abnormalities
- Normal MRIs at day 90, one year

MV-CEA Glioma clinical Protocol

1. Glioblastoma multiforme
2. IT administration of MV-CEA
3. Surgical removal of tumor (day 5)
4. Instillation of MV-CEA in tumor bed
5. Dose escalation (10^5 to 10^7 TCID₅₀)
6. CEA monitoring

Multiple Myeloma

Plasma cell malignancy
Disseminated from outset
Currently incurable
Survival ~ 5 yrs



High CD46 expression
High MV susceptibility
Low anti-MV Ab titers

Suitable for IV therapy

IV MV-NIS for myeloma: Species considerations

Old World or New World Monkeys?



*Rhesus monkey
(Old World)*



*Squirrel monkey
(New World)*

SLAM expression

Yes

Yes

Measles-like illness

Yes

Yes

CD46 on rbc

Yes

No

Squirrel monkey toxicology for MV-NIS

Saimiri sciureus
3M/group



*No toxicities observed
due to MV-NIS*



MV-N mRNA Levels from Cheek Swabs

Grp #	MV-NIS (TCID ₅₀)	Cyclophos(mg/kg)	Monkey #	Pre-Dose*	d1*	d2*	d8*	d15*	d29*	d91*
Grp I	0 (Ctrl)	0	775	<100	<100	<100	<100	<100	<100	
Grp I	0 (Ctrl)	0	793	<100	<100	<100	<100	<100	<100	
Grp I	0 (Ctrl)	0	803	<100	<100	<100	<100	<100	<100	<100
Grp 2	0 (Ctrl)	31	756	<100	<100	1230	<100	<100	<100	
Grp 2	0 (Ctrl)	31	770	<100	<100	<100	<100	<100	<100	
Grp 2	0 (Ctrl)	31	810	<100	<100	<100	<100	<100	<100	<100
Grp 3	10e ⁸	0	772	<100	1660	<100	1455	<100	<100	
Grp 3	10e ⁸	0	812	<100	<100	<100	<100	<100	<100	
Grp 3	10e ⁸	0	823	<100	66950	<100	896500	8830	<100	<100
Grp 4	10e ⁸	31	817	<100	2270	<100	988500	5880	<100	
Grp 4	10e ⁸	31	818	<100	53850	955	2465000	2379870	960	
Grp 4	10e ⁸	31	819	<100	150250	2520	516000	4590	690	<100

MV N gene analysis by RTqPCR, limit of detection ≤100 copies;

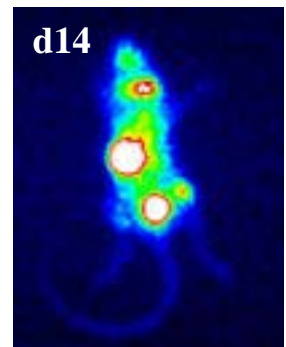
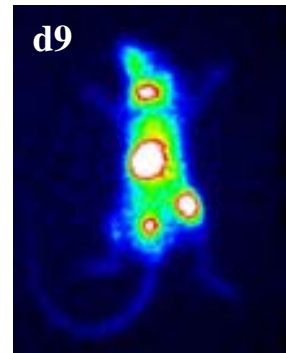
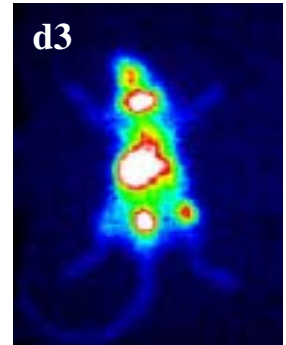
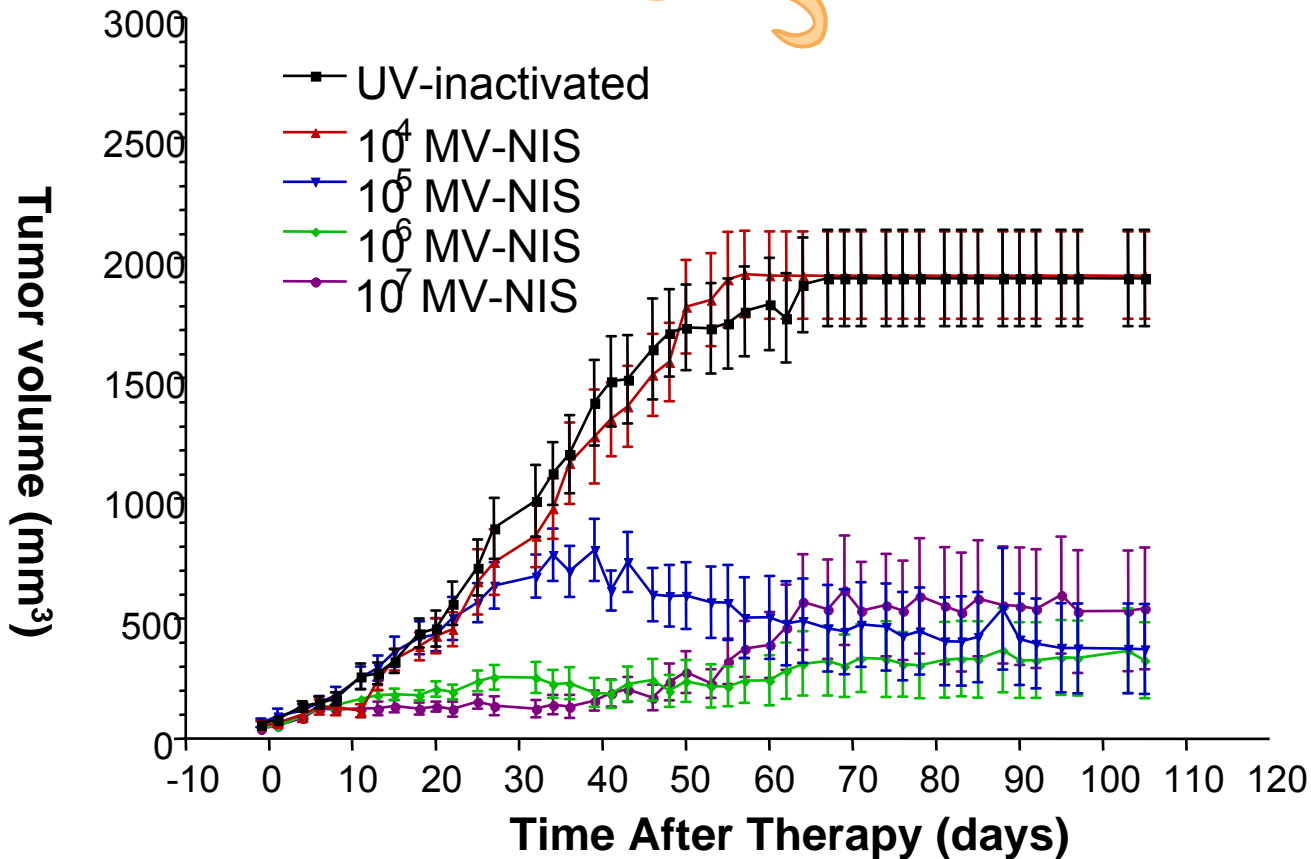
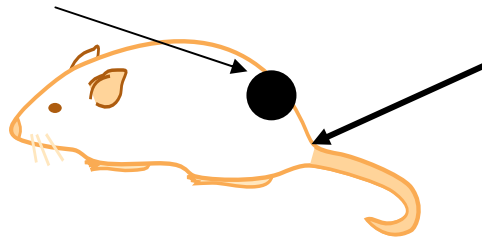
* Data represents Copies of MV N-gene in 1.0 µg RNA extracted from Buccal Cheek Scrapes

IV MV-NIS in myeloma: Dose-response studies

SCID mice

KAS6/1 xenografts, 0.5 cm

IV MV-NIS, one dose



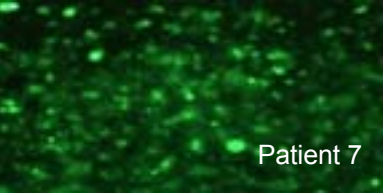
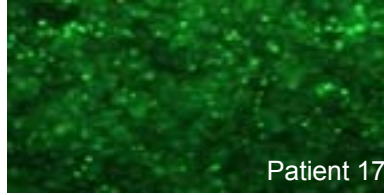
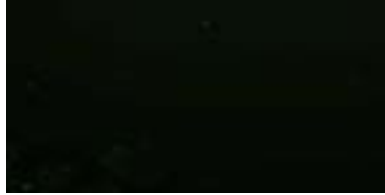
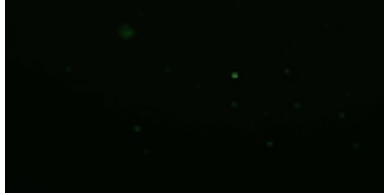
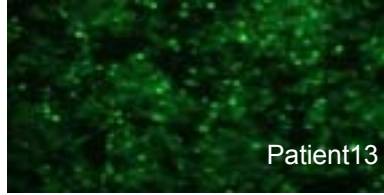
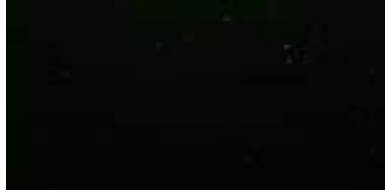
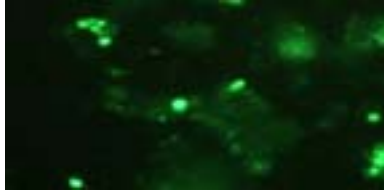
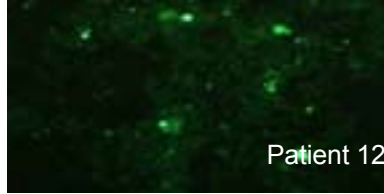
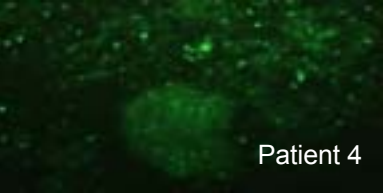
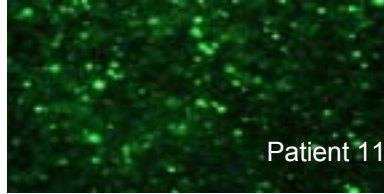
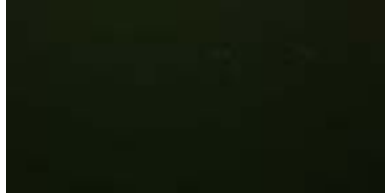
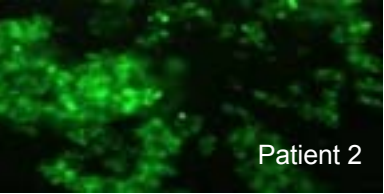
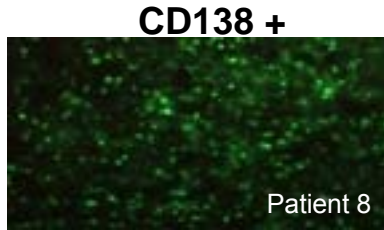
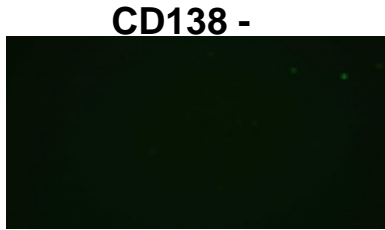
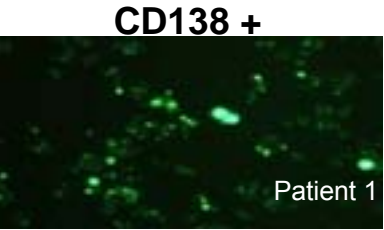
Minimum effective dose $5 \times 10^6/\text{kg}$

MV-NIS for Myeloma: Phase I Clinical Protocol

- Advanced multiple myeloma
- IV administration of MV-NIS in 100 ml saline
- Stage I: dose escalation 10^6 to 10^9 TCID₅₀
- Stage II: dose escalation with cyclophosphamide
- I-123 imaging to monitor virus spread

Manufacture and toxicity studies sponsored by
NCI RAID program (now NExT)

At 96 hours after infection, many MV-GFP+ cells were observed in CD138+ , but only few MV-GFP+ cells in CD138- cells

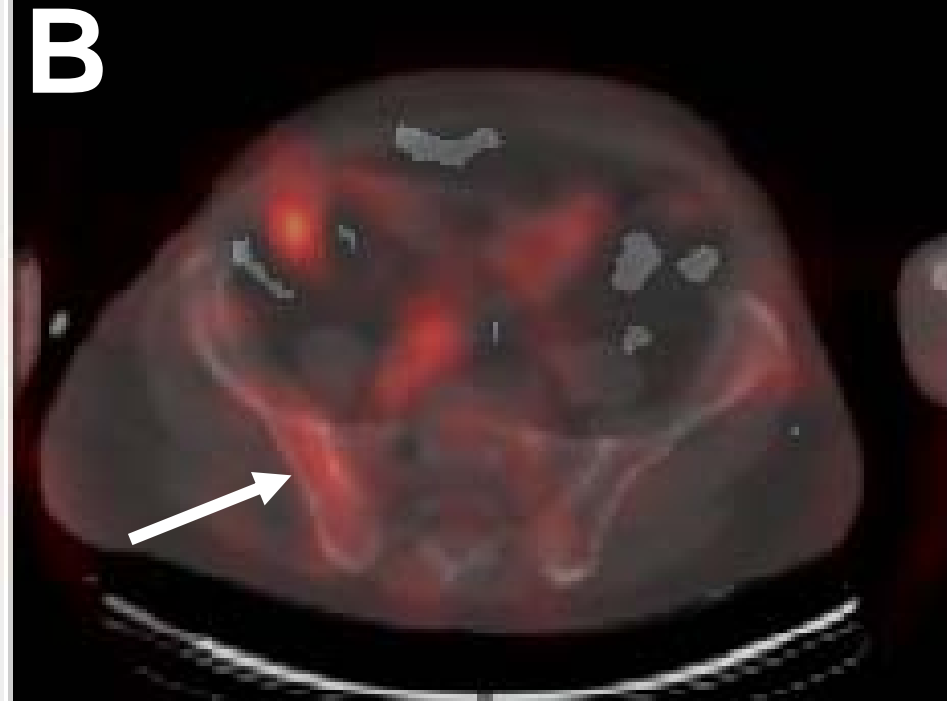
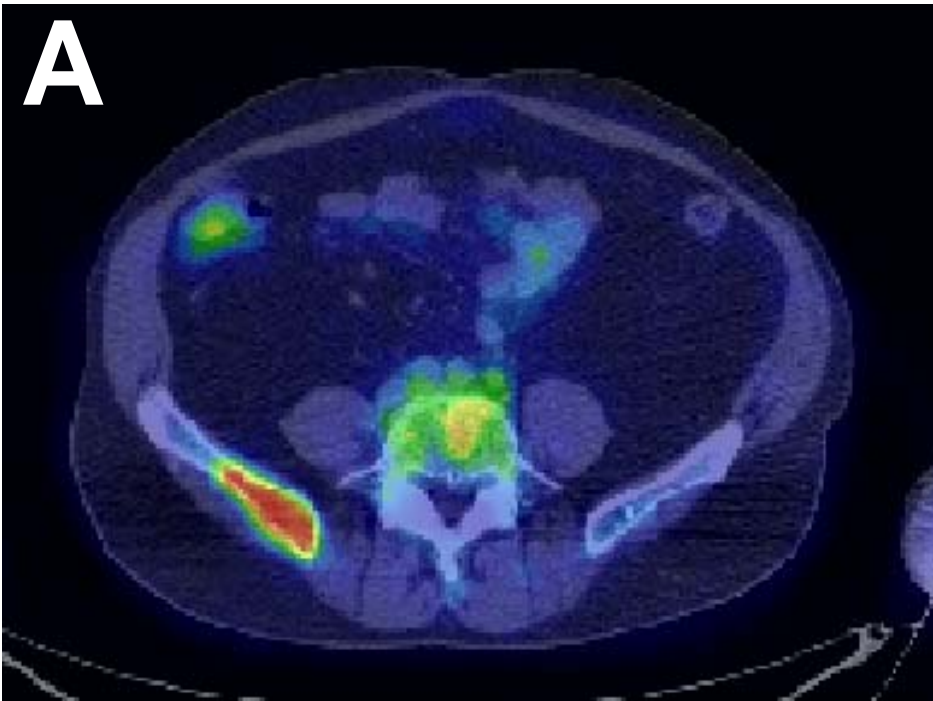


Patient 2 on day 8 after MV-NIS
 10^6 TCID₅₀ were administered by intravenous infusion

MV N mRNA was detected in the buccal swab
(16440 copies per μ g RNA)

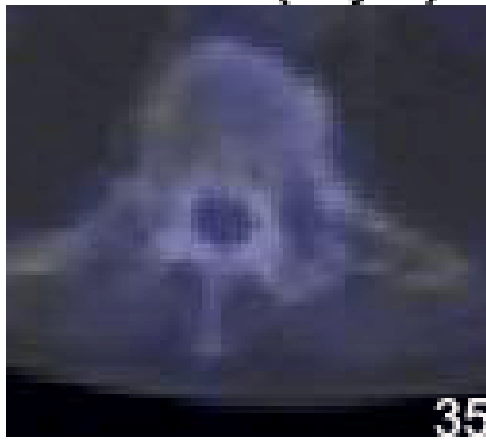
FDG PET/CT

I-123 SPECT/CT

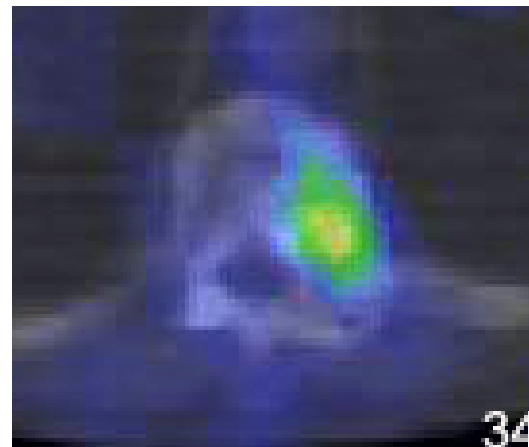
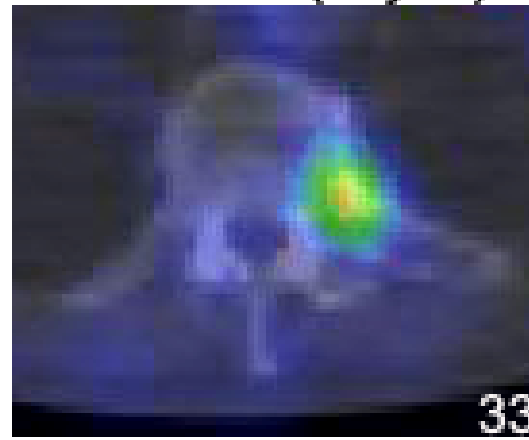


Patient 13 on day 8 (vs.day -1) after MV-NIS
 10^7 TCID₅₀ were administered by intravenous infusion. Preceded by cyclophosphamide

Baseline (day-1)

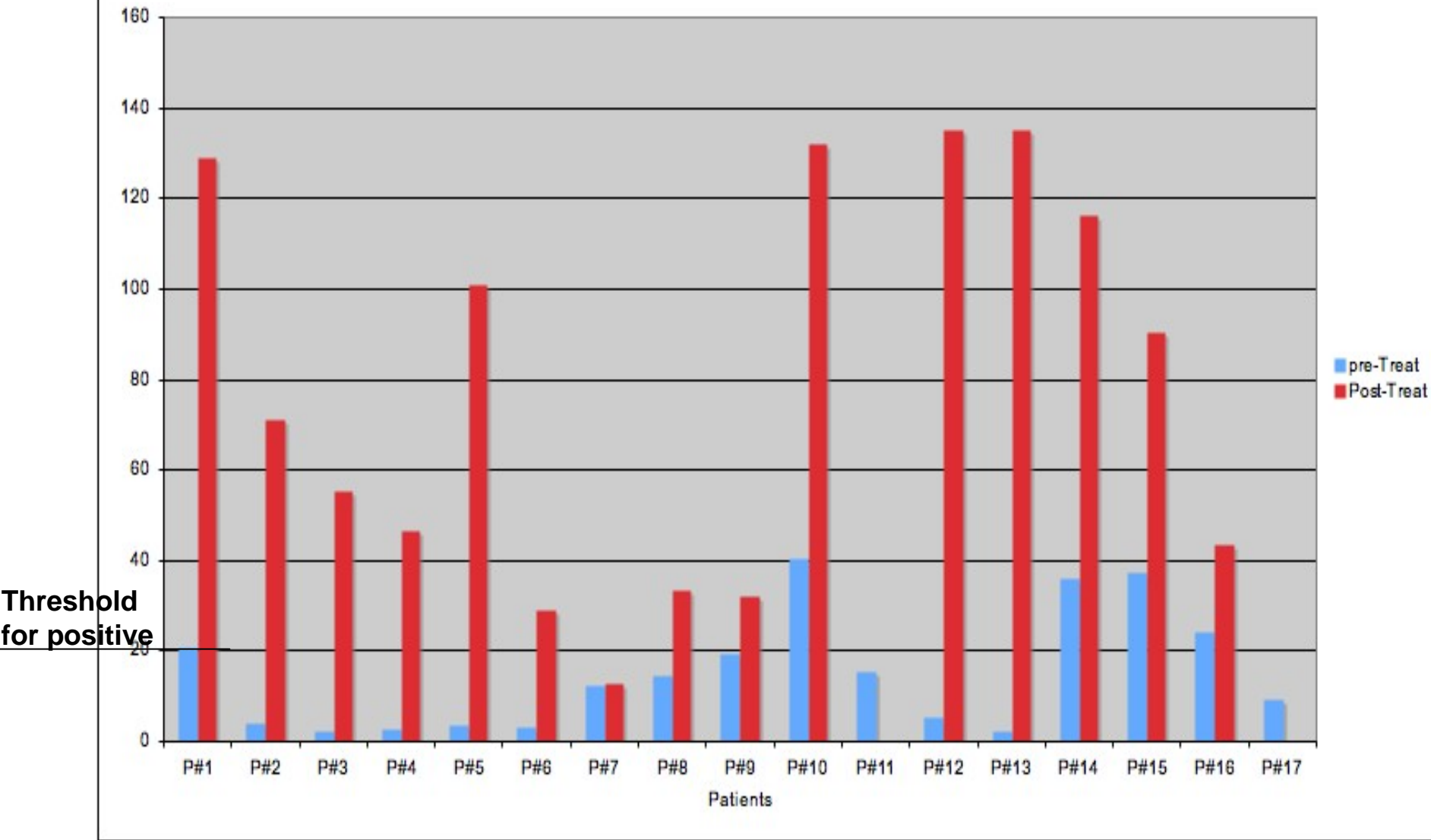


Post virus (day+8)



Humoral immune response to the injection of oncolytic MV-NIS (clinical data)

Measles IgG(with ELISA)



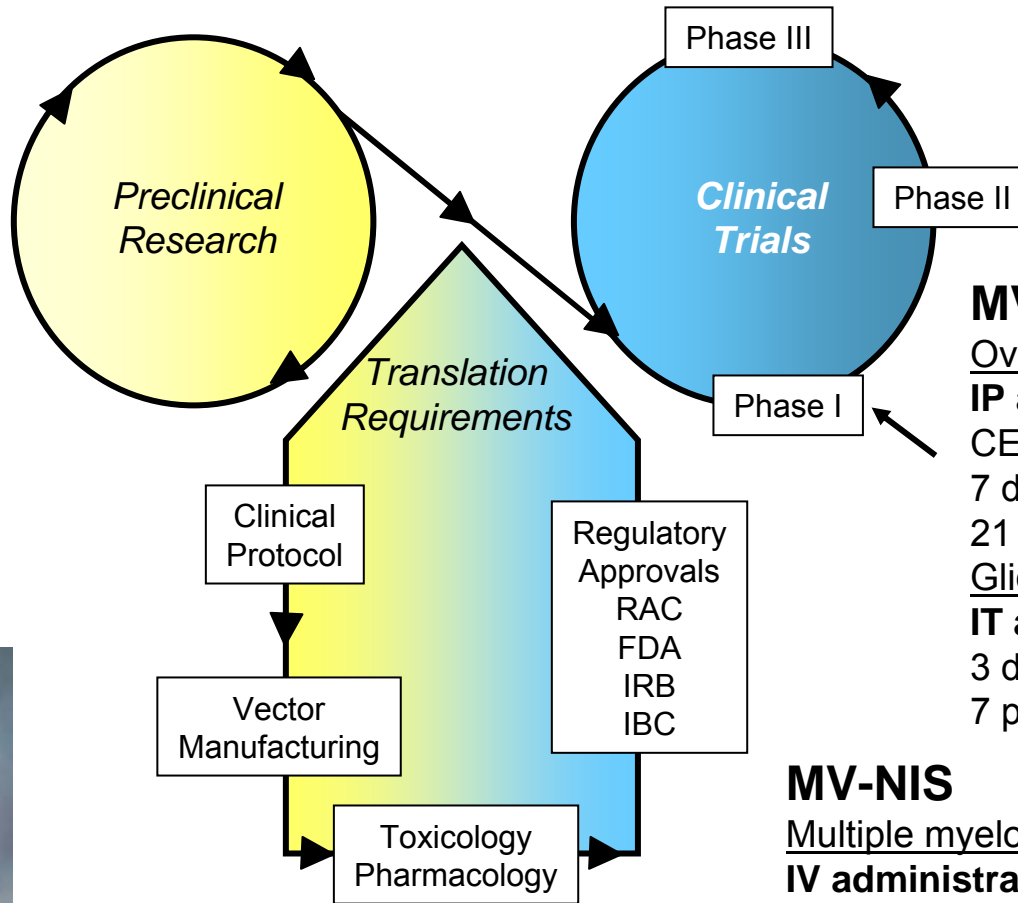
Oncolytic measles virus translation: open trials at Mayo



Evanthia Galanis



Angela Dispenzieri



MV-CEA

Ovarian cancer

IP administration

CEA monitoring

7 dose levels (10e3 to 10e9)

21 patients treated

Glioma

IT administration

3 dose levels (10e6 to 10e8)

7 patients treated

MV-NIS

Multiple myeloma

IV administration

CT-SPECT imaging

Four dose levels (10e6 to 10e9)

Cyclophosphamide addition

18 patients treated

Ovarian cancer

IP administration

5 patients treated

Future clinical studies (current translational activities)

Combining viruses with chemotherapy

CPA dose escalation with MV-NIS for myeloma

MV-NIS with chemotherapy for SCCHN

Using cell carriers to circumvent antibodies

MSCs infected with MV-NIS for ovarian cancer, IP

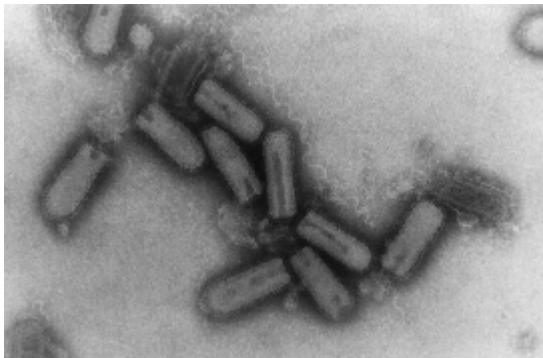
MM1 cells infected with MV-NIS for myeloma, IV

Using other viruses

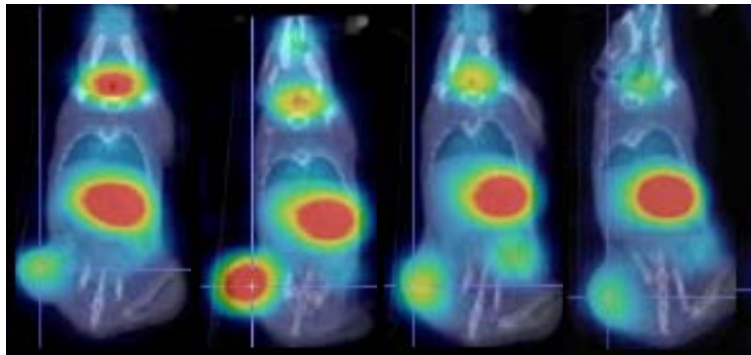
VSV-IFN β for HCC and SCCHN, IT

VSV-IFN β -NIS for myeloma, IV

VSV-IFN β -NIS: Serial imaging and potency in MM

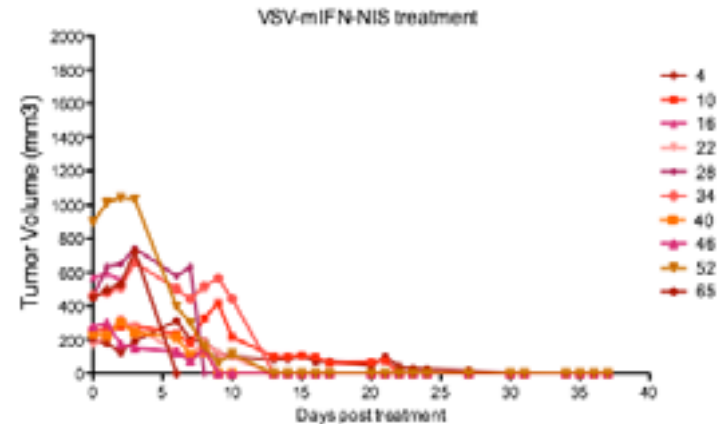
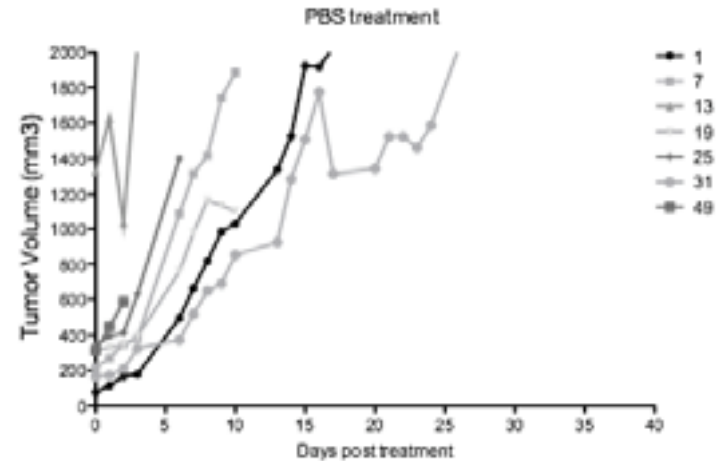


Days post treatment 1 2 3 4



SPECT CT imaging of intratumoral radio-isotope uptake in immune-competent C57Bl/KalwRij mice bearing subcutaneous syngeneic myeloma tumors treated with VSV-IFN-NIS

VSV as an anticancer agent (VSV-IFN-NIS, single intravenous dose) in myeloma



VSV-IFN-NIS treated tumors regress completely (immune-competent mice)

Oncolytic virotherapy with measles and VSV

Russell Lab

David Dingli
Betsy Hadac
Beth Kelly
Shruthi Naik
Hongtao Li
Stephanie Carlson
Linh Pham
Danielle Yarde
Patrycja Lech

Kah Whye Peng

Roberto Cattaneo

John Morris III

Richard Vile

Toxicology Core

Kah Whye Peng
Sue Greiner
Rae Myers
Cindy Reed
Pamela Ryno
Fareeda Hussain

Vector Core

Mark Federspiel
Linda Gregory
Guy Griesmann
Kirsten Langfield
Julie Sauer
Sharon Stephan
Henry Walker
Troy Wegman

Clinical Leaders:

Ovarian cancer

Evanthia Galanis

Glioma

Evanthia Galanis

Myeloma

Angela Dispenzieri

HCC

Mitesh Borad

Imaging

Mike O'Connor

