

Oncolytic Virotherapy for Multiple Myeloma

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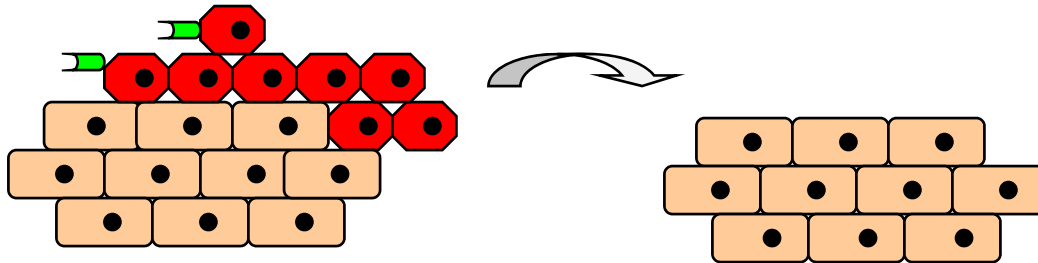
Rochester, Minnesota



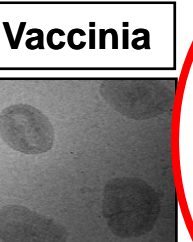
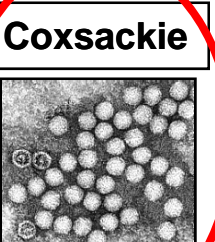
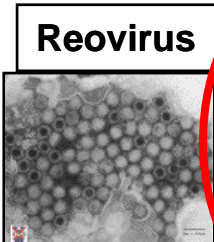
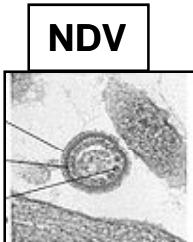
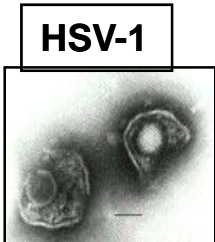
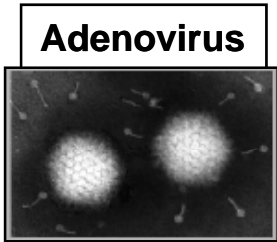
Jacksonville, Florida

Oncolytic viruses for cancer therapy

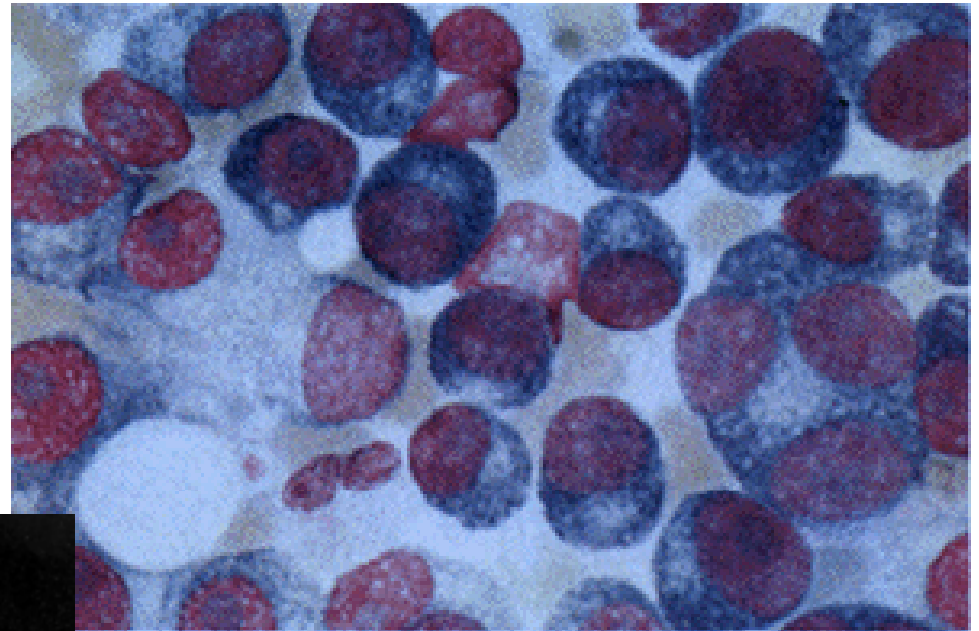
“Onco” = cancer “lytic” = killing



- Harness the destructive power of viruses
- Tumor specific
- Efficient killing of malignant cells
- Stimulate host’s anti-tumor activity
- Recombinant “armed” oncolytic viruses
- Can be combined with conventional therapies



Multiple Myeloma is an appealing target for intravenous virotherapy



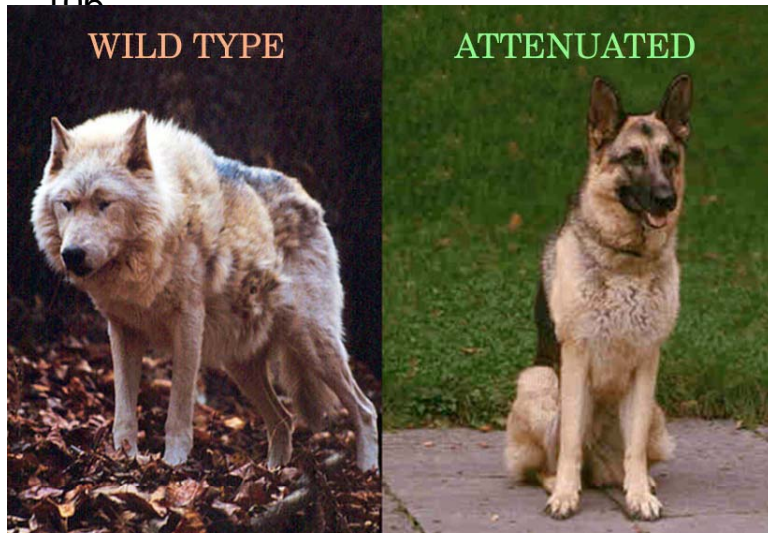
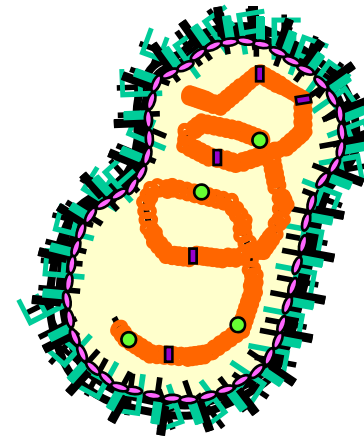
Disseminated from outset

Low antiviral Ab titers

Attenuated measles for myeloma



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



SLAM only

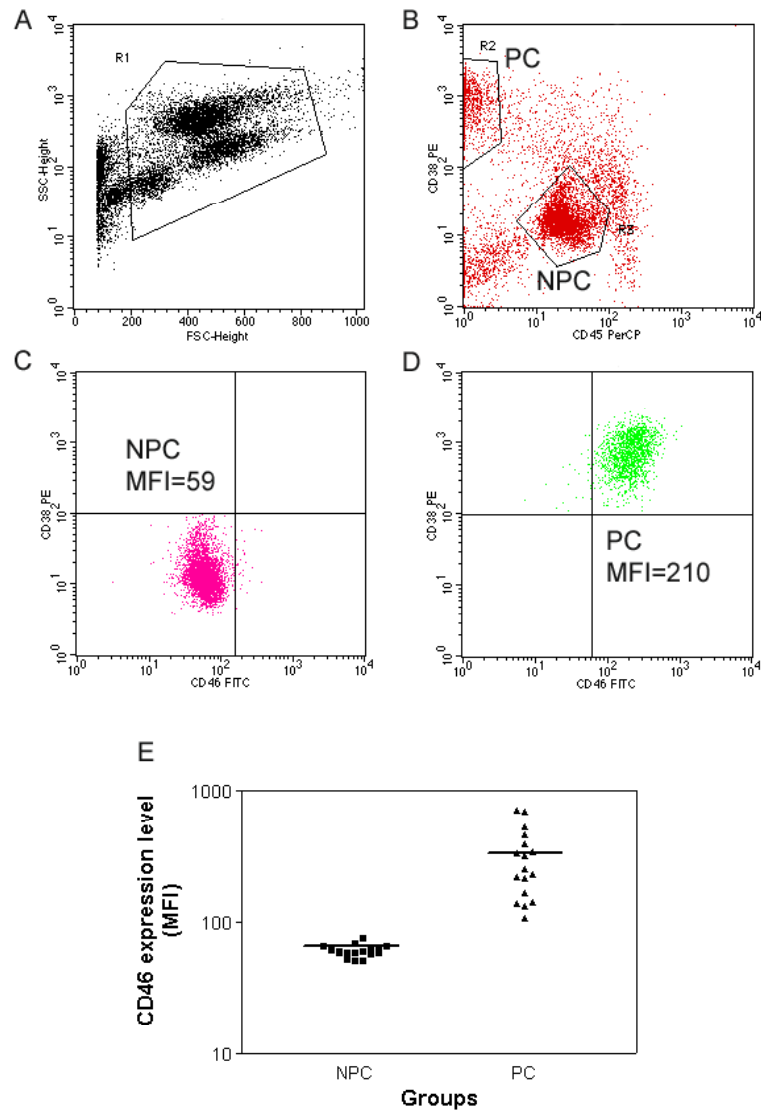
SLAM + CD46

- Efficiently infects and kills human myeloma cells (via CD46), spares normal cells
- Has potent antitumor activity against xenograft models of human multiple myeloma
 - Peng et al. Blood, 2002; 98: 2002-2007
 - Dingli et al. Blood, 2004; 103: 1641-1646
- Can be engineered to express additional genes; recombinants are extremely stable



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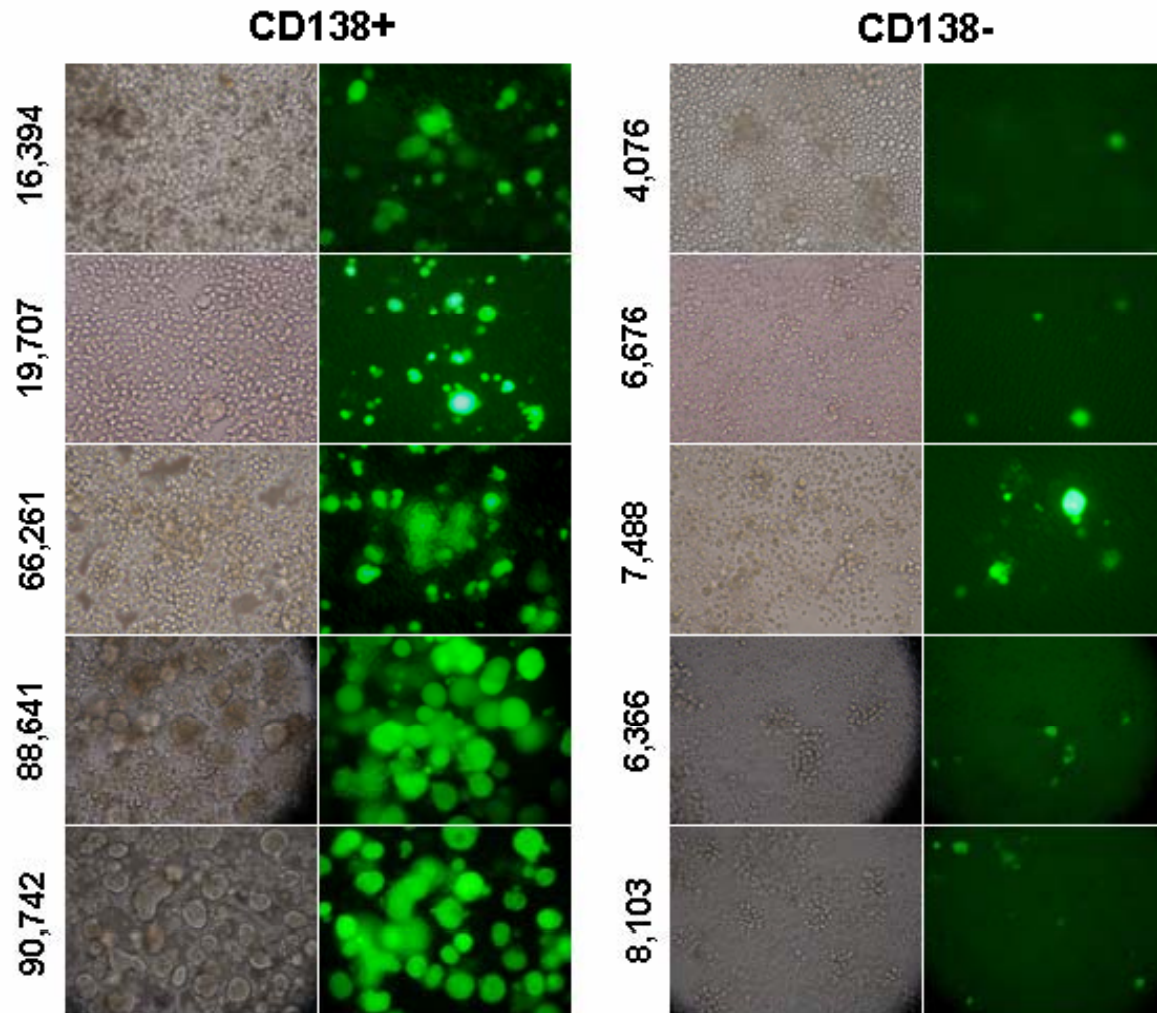
High CD46 expression in primary myeloma cells



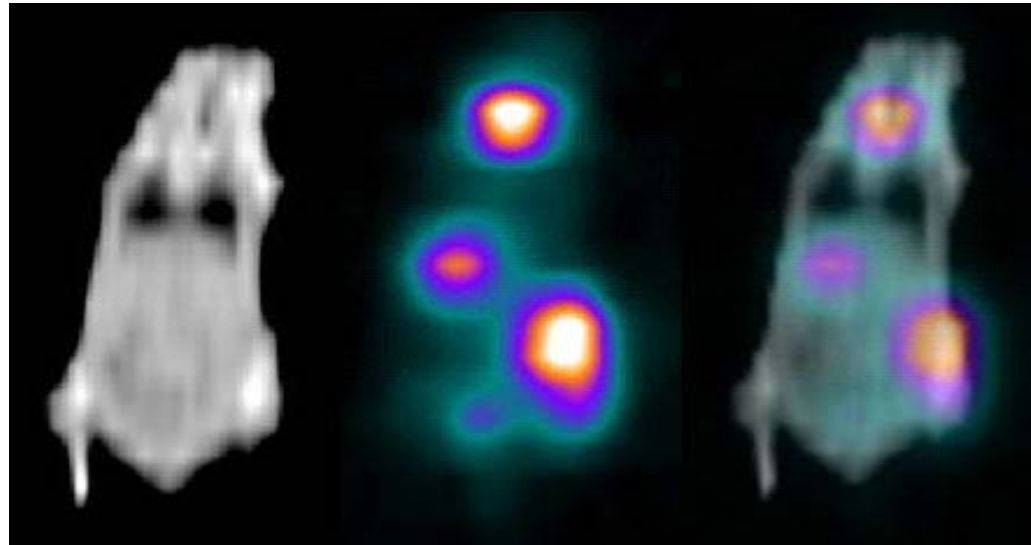
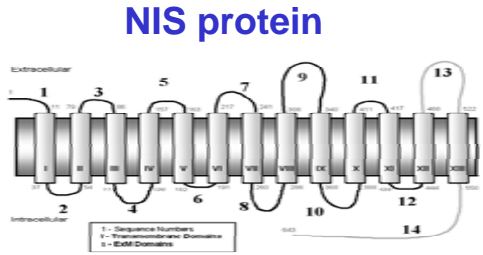
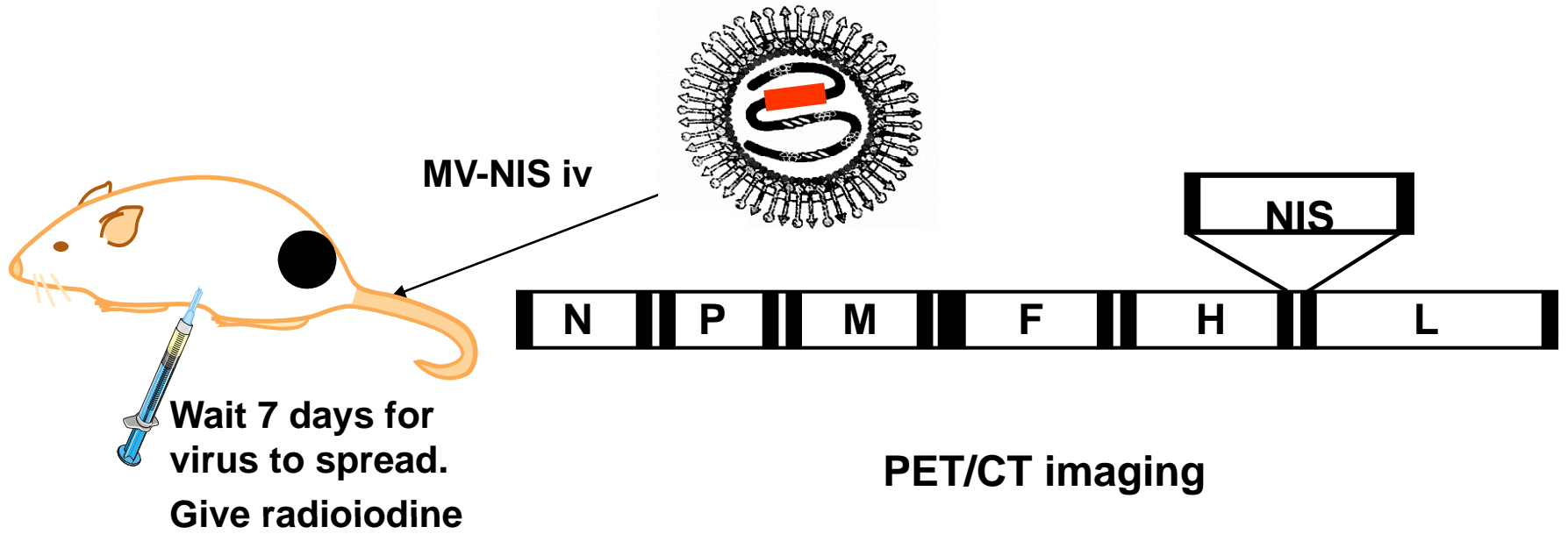
Unsorted bone marrow aspirated from patients with multiple myeloma

CD46 expression in primary myeloma cells correlates with measles susceptibility

B

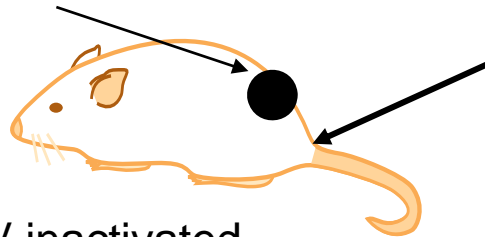


NIS gene insertion for imaging virus spread (MV-NIS)

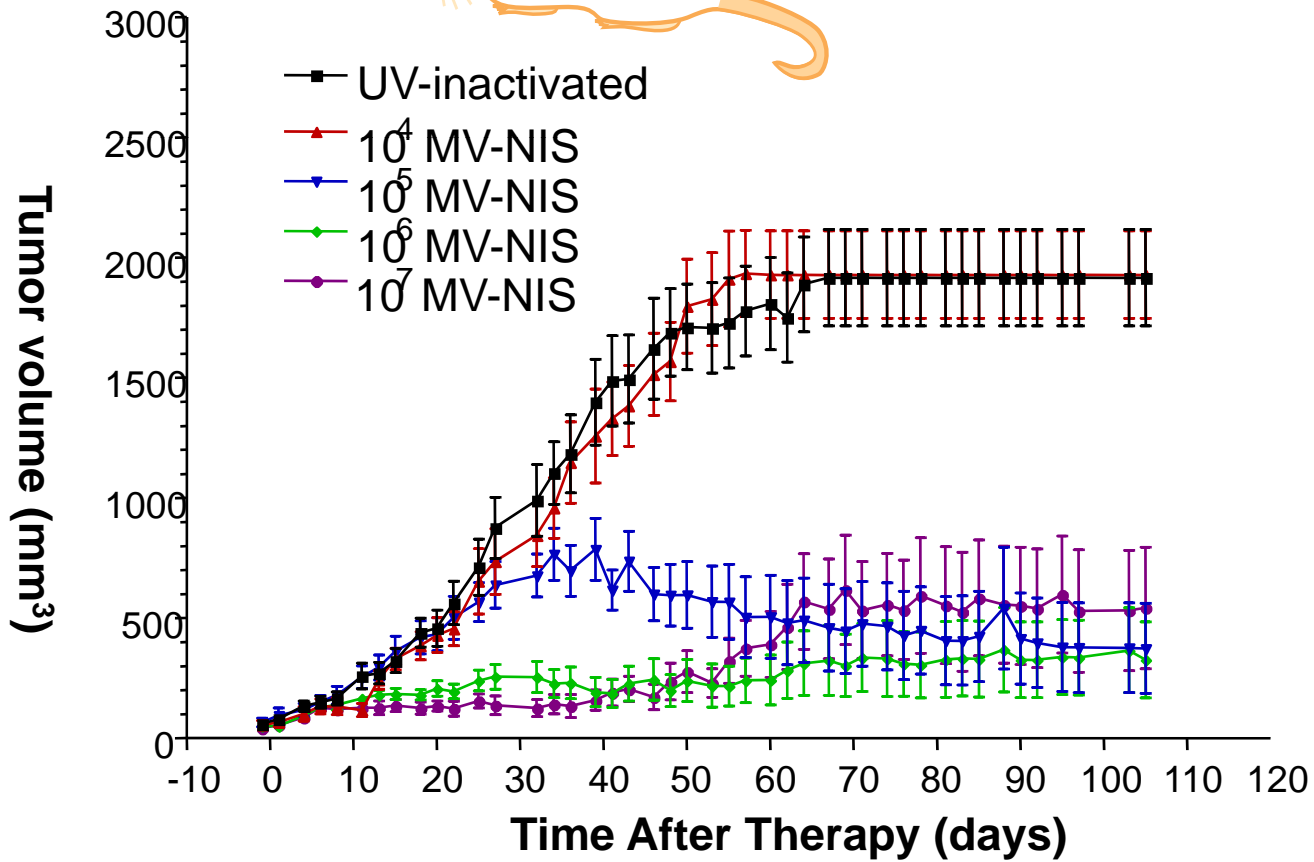


Intravenous MV-NIS: Dose-response studies

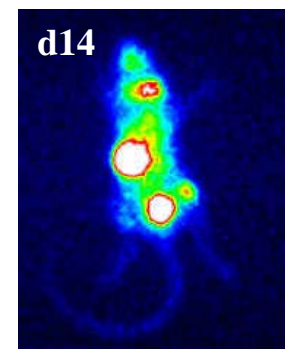
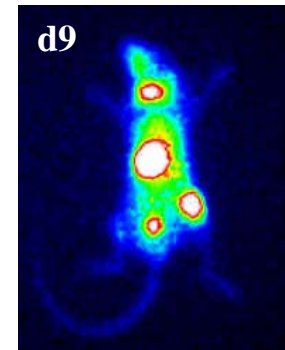
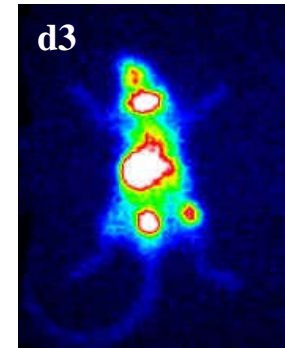
SCID mice
KAS6/1 xenografts, 0.5 cm



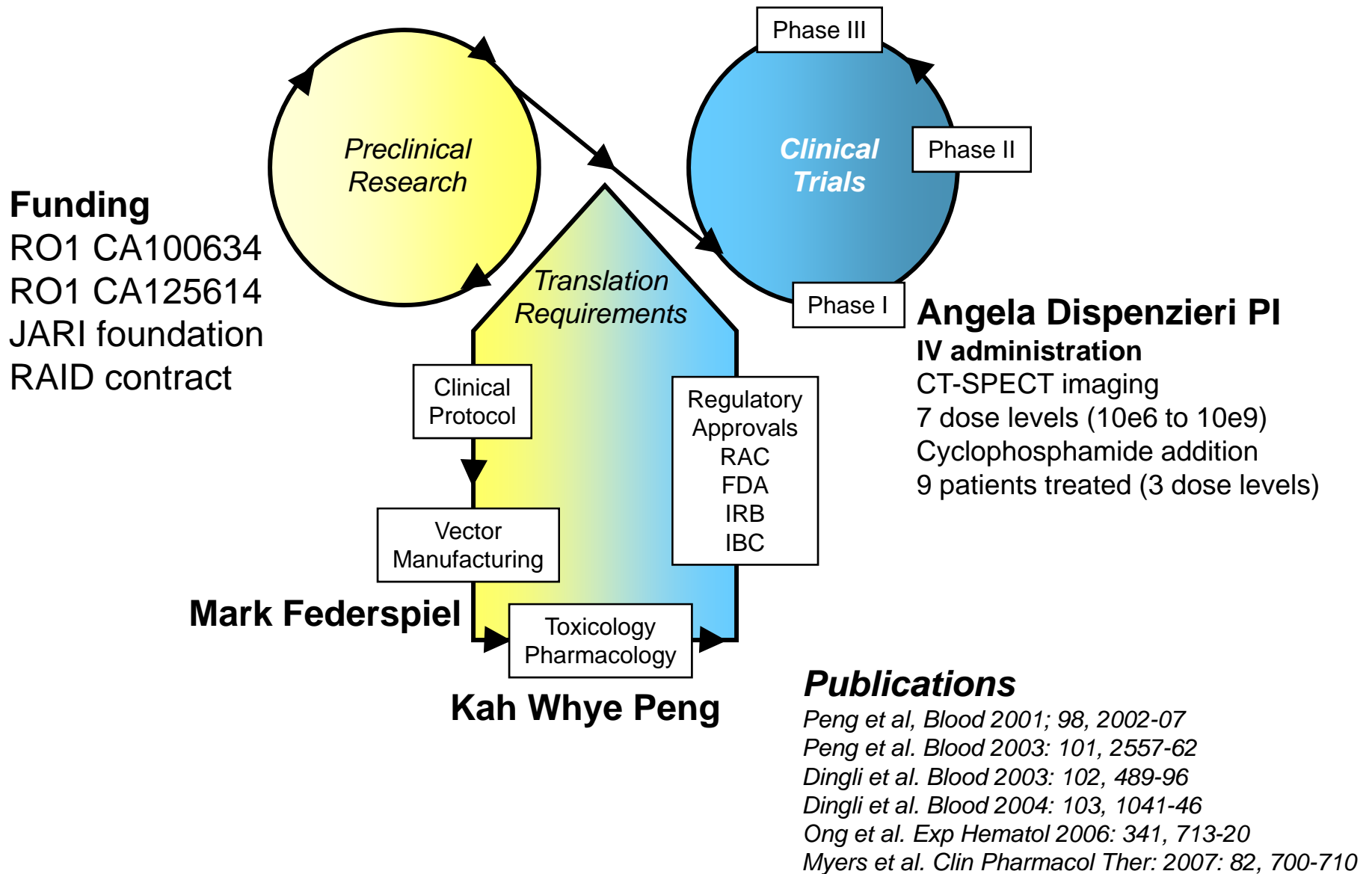
IV MV-NIS, one dose



Minimum effective dose 5 x 10⁶/kg

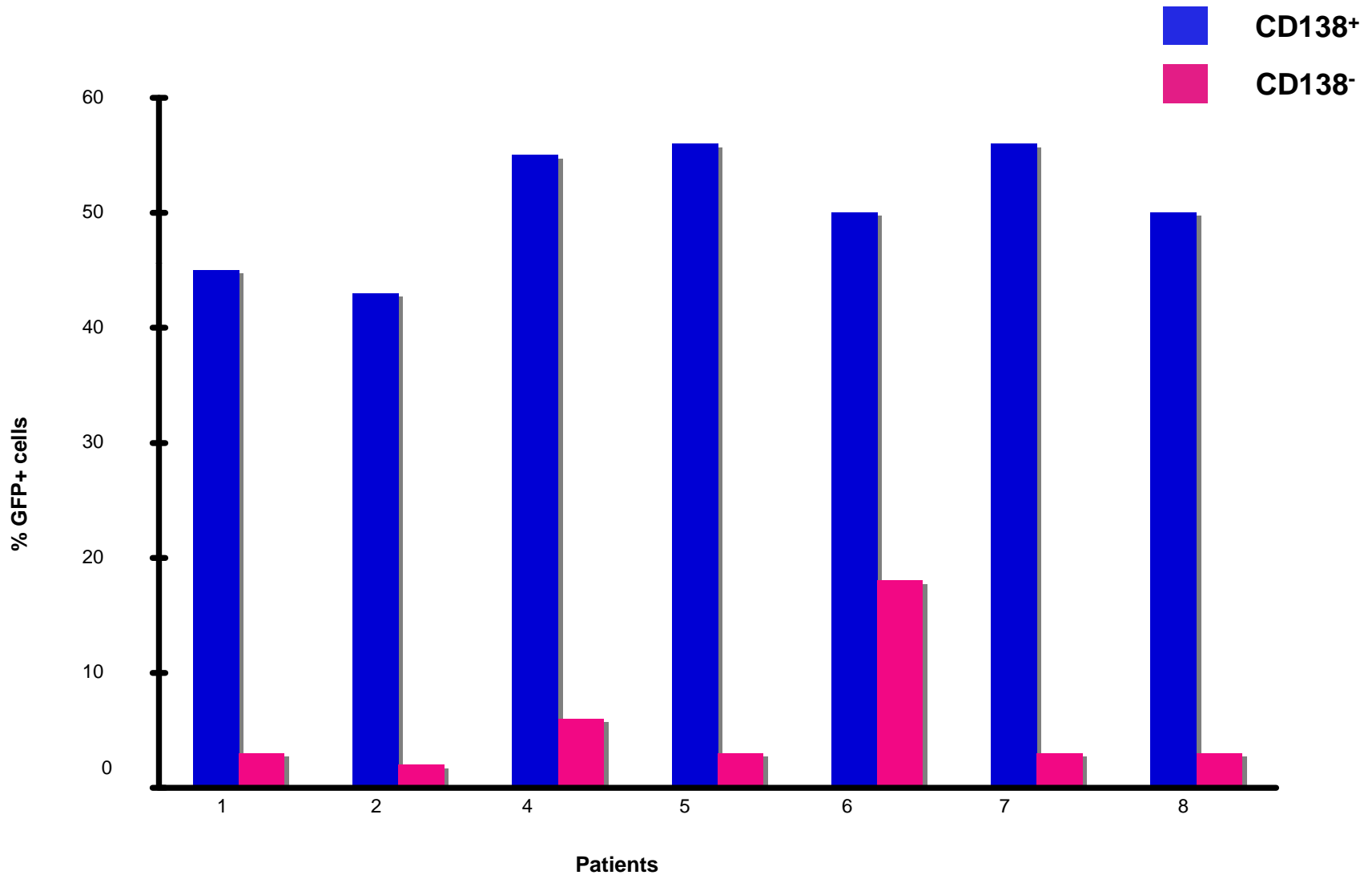


MV-NIS for myeloma: translation



MV-NIS trial data:

Malignant plasma cells are preferentially infected by MV-GFP



Anti-Measles antibody titers, CD46 receptor levels, QRT-PCR and ¹²³I uptake in nine treated MM patients

Patient #	1	2	3	4	5	6	7	8	9
Anti-MV Ab pre/ post- Rx	1:10 1:160	<1:10 1:20	< 1:10 1:10	<1:10 1:20	< 1:10 1:160	<1:10 1:10	< 1:10 <1:10	< 1:10 1:10	< 1:10 1:10
CD46 copies/cell CD138+/CD138-	79771/ 10322	140187/ 10145	28258/ 11228	75147 /9012	53048/ 12113	15136/ 5070	45332/ 8580	63391/ 7492	17356/ 6929
Q-RT-PCR gargle/blood/urine	- / - / -	+ ^a / - / -	+ ^b / - / -	- / - / -	+ ^c / - / -	- / - / -	- / - / + ^d	- / - / + ^e	- / - / -
¹²³ I uptake	-	+	-	-	-	-	-	-	-

^a 16440 and 1405 MV-N gene copies /mcg RNA on day 15 and 22, respectively

^b 1021 MV-N gene copies /mcg RNA on day 8, respectively

^c 3403 and 8250 MV-N gene copies /mcg RNA on day 8 and 15, respectively

^d 15800 and 702666 MV-N copies /mcg RNA on day 15 and 22, respectively

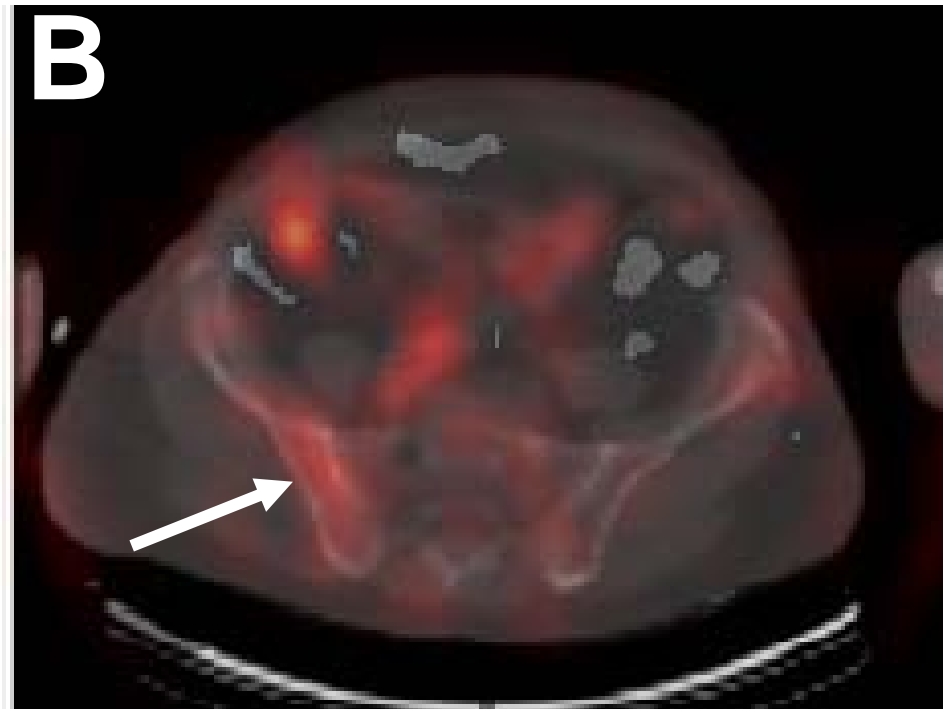
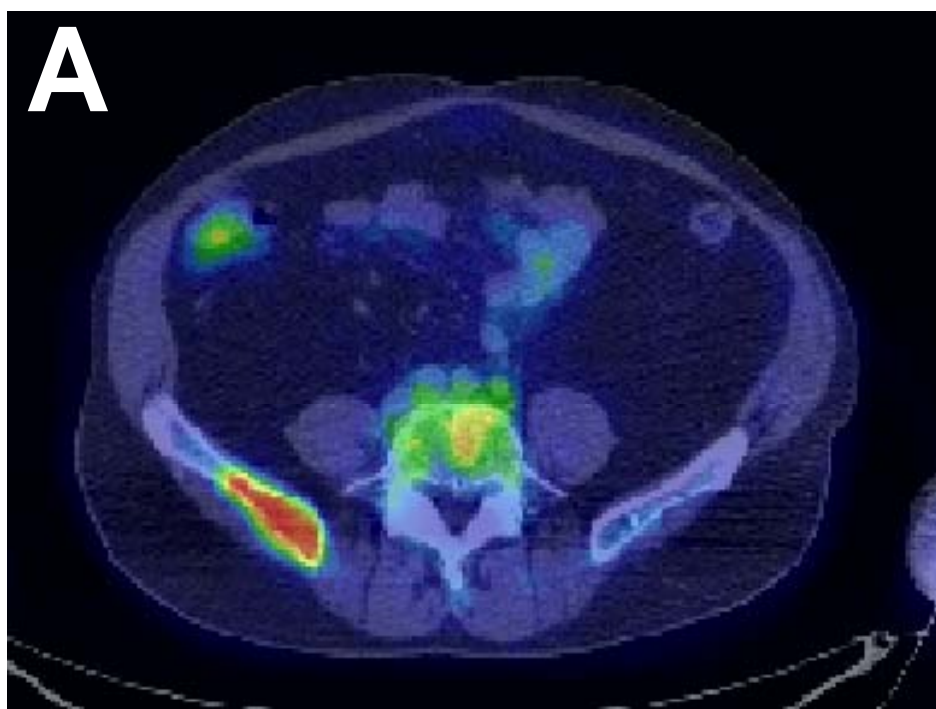
^e 2760 MV-N copies /mcg RNA on day 15, respectively

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)

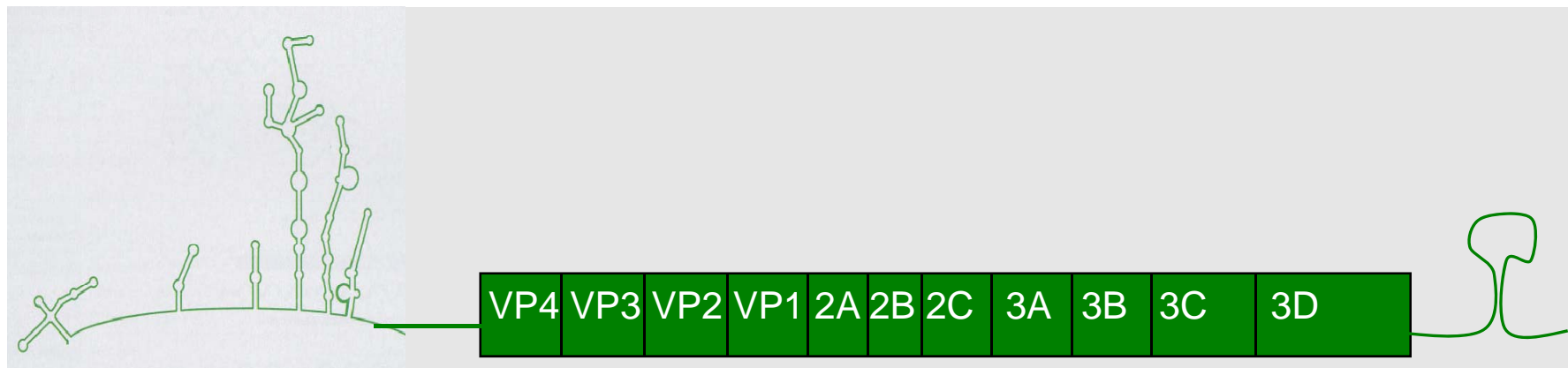
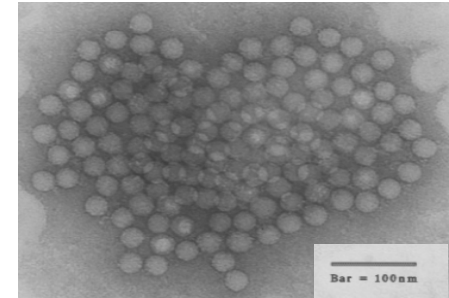
FDG PET/CT

I-123 SPECT/CT

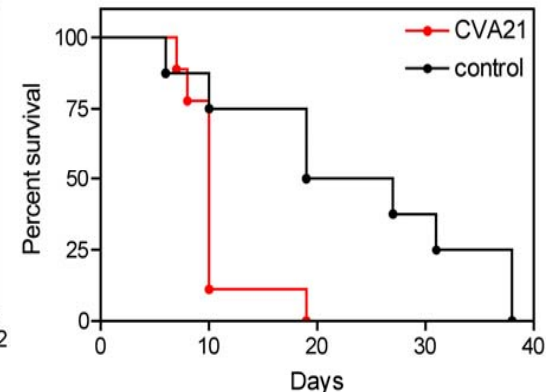
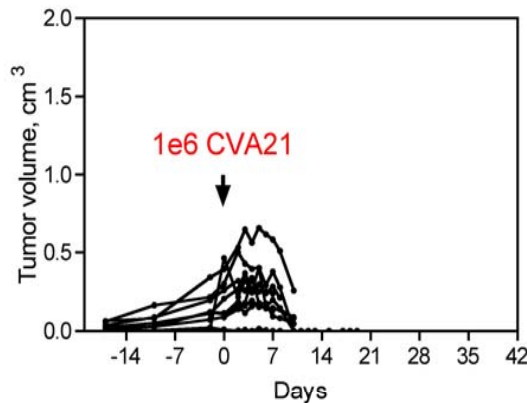
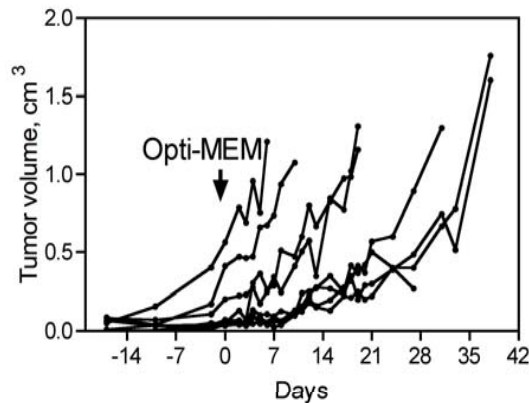


Coxsackievirus A21 (CVA21)

- (+) Sense RNA virus
- Enterovirus Closely related to Poliovirus
- ~7.5 kb genome
- Entry via ICAM-1 (CD54) and DAF (CD55)
- Shows potent oncolytic activity against melanoma xenografts, myeloma cell lines (DR Shafren 2004, 2007)
- Reported to cause hind limb paralysis and myositis in suckling mice, human (Yoeli et al 2002)



CVA21 – potent antimyeloma oncolytic, fatal toxicity



CPE of mouse tissue overlay on H1-HeLa cells

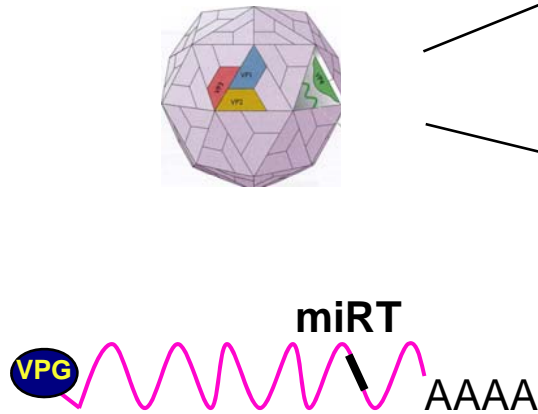
Mice	Tumor	Liver	Spleen	Brain	Skeletal Muscle
IV virus #1	+++	-	-	-	++
IV virus #2	+++	-	-	-	++
IV virus #3	+++	-	-	-	++
IT virus #1	+++	-	-	-	++
No virus #1	-	-	-	-	-

- **Coxsackievirus A21 (CVA21) injected into mice carrying SQ multiple myeloma tumors at 1×10^6 TCID50**
- **Complete tumor regression by 14 days**
- **Severe myositis developed 7-10 days post inoculation**
- **CVA21 recovered from tumor tissue and skeletal muscle only**

miRNA targeting concept

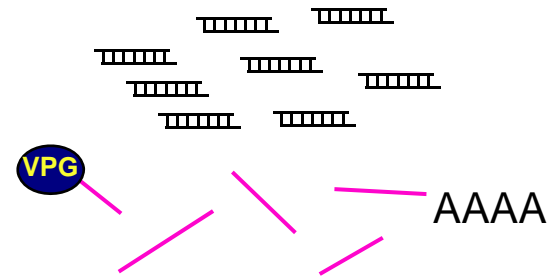
- MicroRNAs are small, 21-23 nt regulatory RNAs mediating translational repression & RNA degradation
- Incorporating microRNA target elements (miRTs) into the genomes of RNA viruses may impair their stability (only in cells expressing the cognate microRNA)
- Incorporation of tissue specific-miRTs into an oncolytic virus genome may provide a new modality for targeting tissue tropism

Recombinant CVA21 with muscle-specific miRT



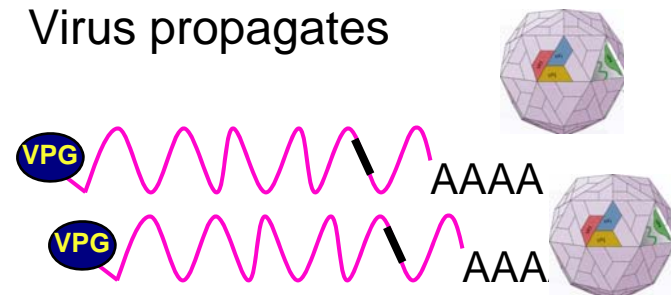
Muscle cell:

miRNA degrades viral genome



Tumor cell:

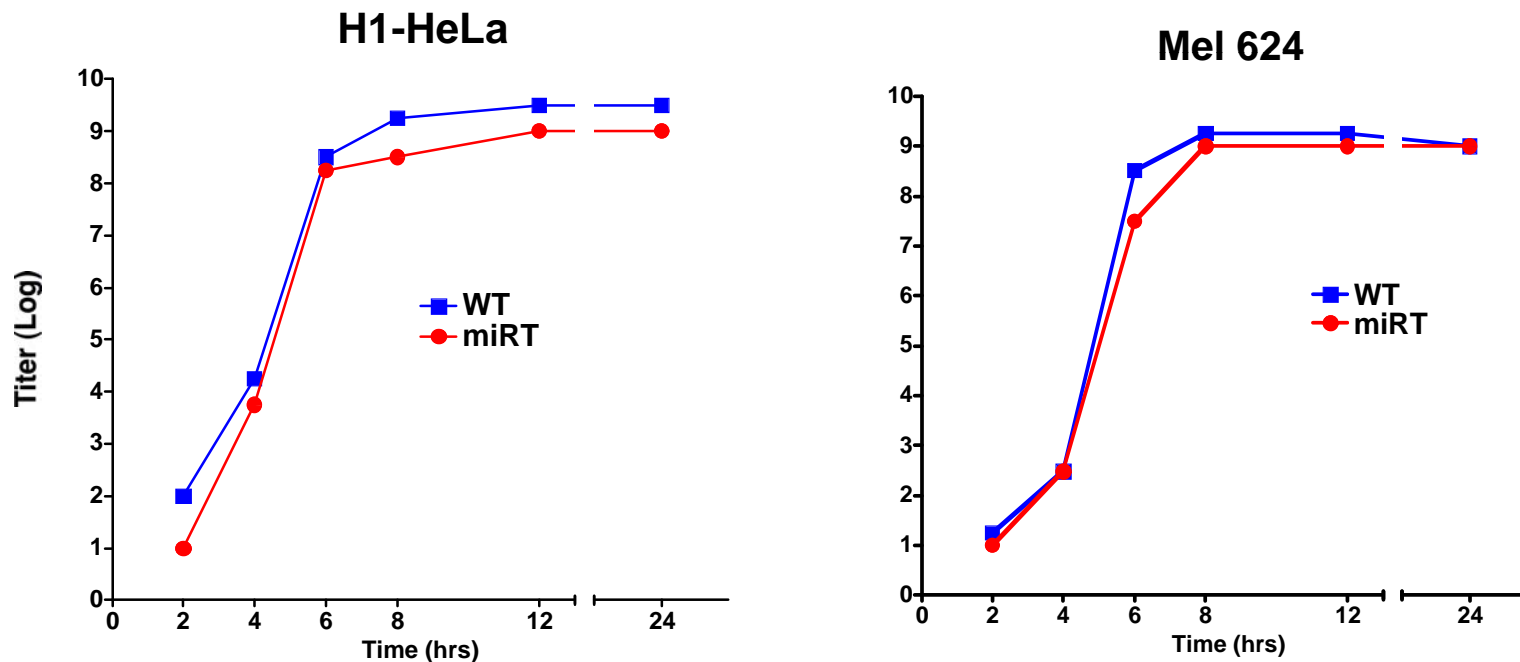
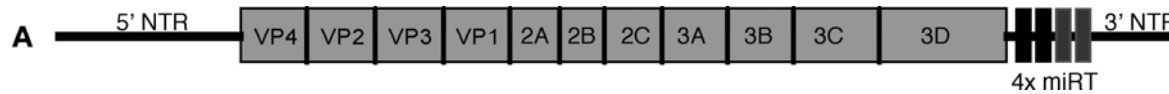
Virus propagates



Muscle-Specific miRNAs

- 3 highly conserved miRNAs (miR-1, miR-133, miR-206) highly enriched in skeletal muscle
- Screening, done using lentiviral vectors, identified a combination to two miR-133 plus two miR-206 target sequences as the best insert for muscle-specific shutdown of gene expression

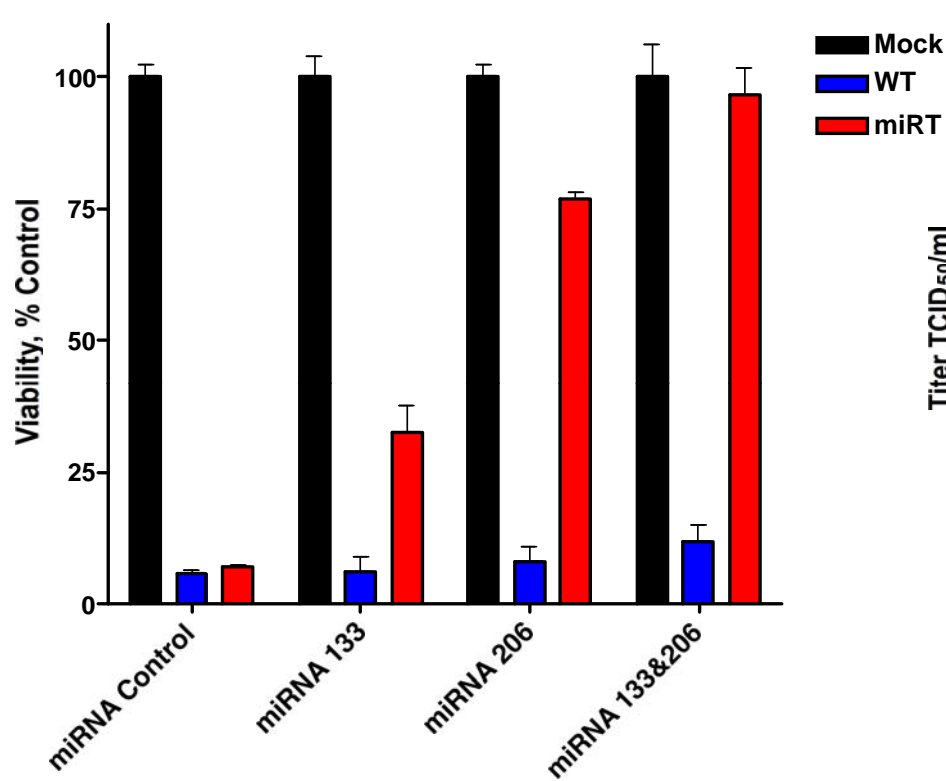
Isolation & Propagation of miRT-containing CVA21



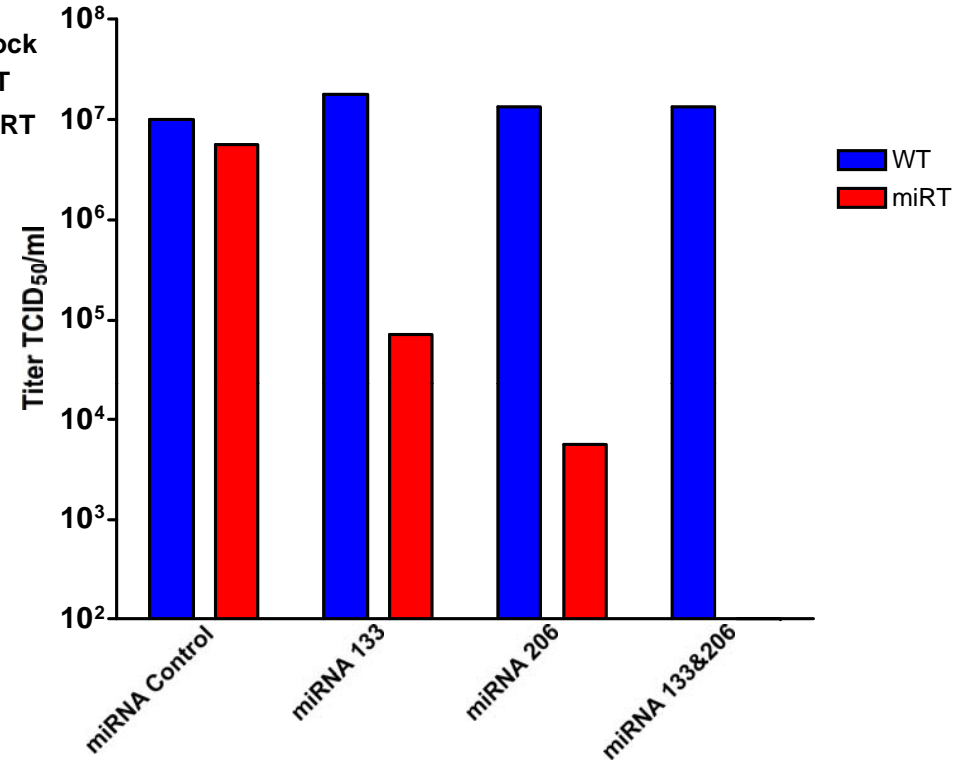
- **Growth kinetics of WT and miRT viruses similar in non-muscle cells**

Mechanistic Validation

Viability of infected cells



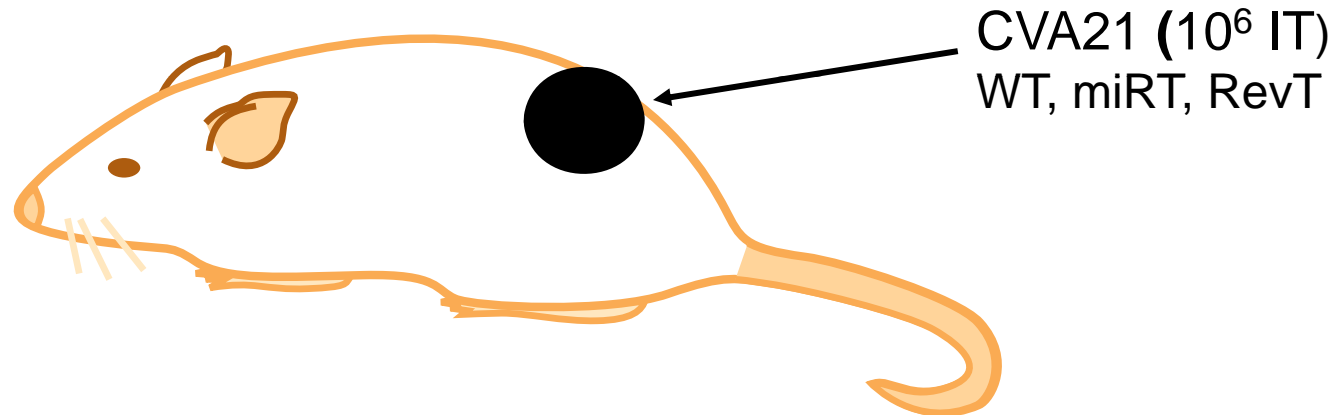
Virus yield from infected cells



- H1-HeLa cells transfected with control or muscle specific miRNA mimics
- miRNA 133 & 206 offer significant protection individually
- Combining miRNAs 133&206 completely blocks miRT virus toxicity and propagation on HeLa cells

In vivo studies (WT vs miRT vs RevT control)

KAS6/1 or Mel624 tumors
(SCID mice)

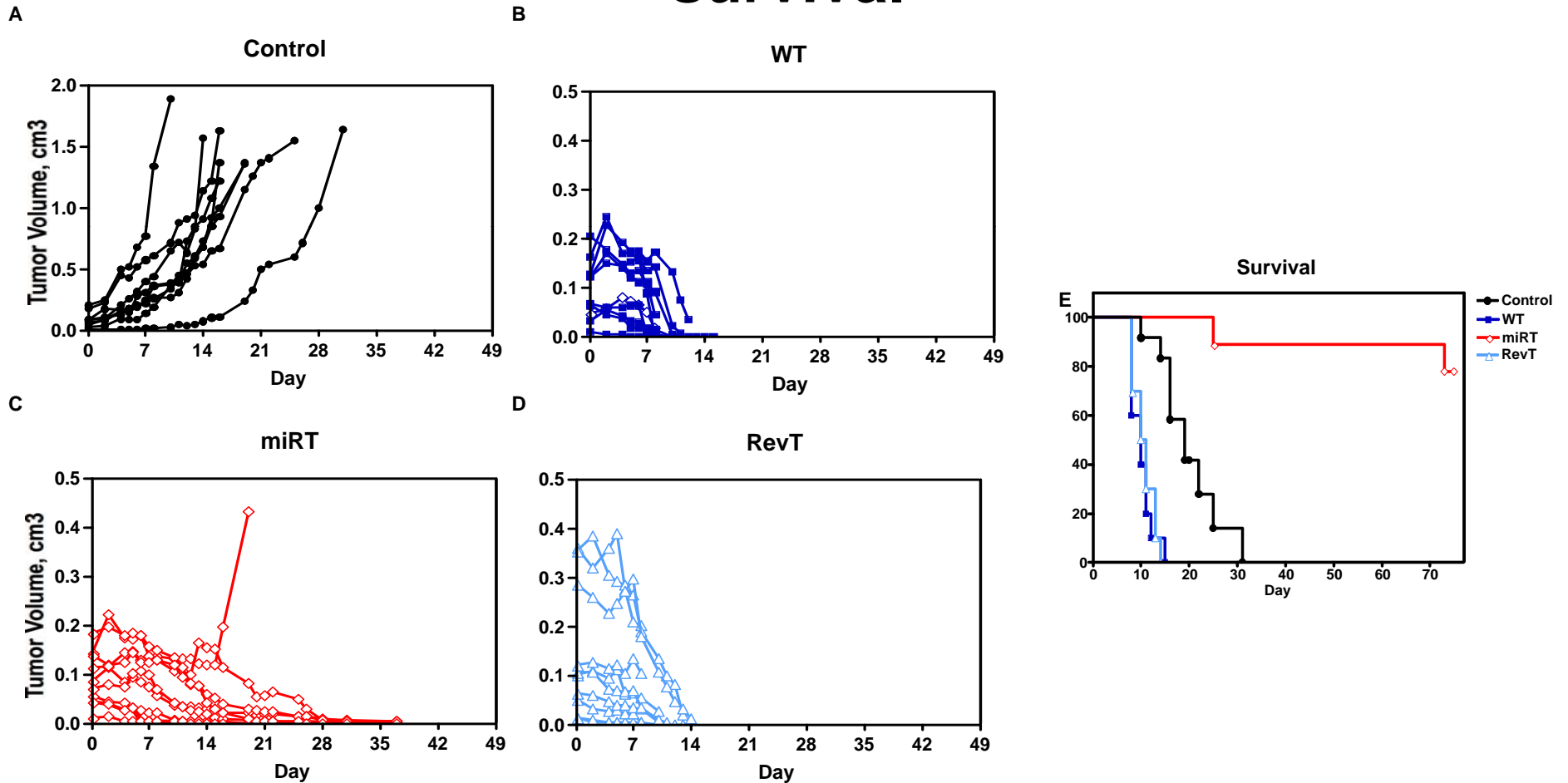


Monitor for tumor regression
and hind limb paralysis



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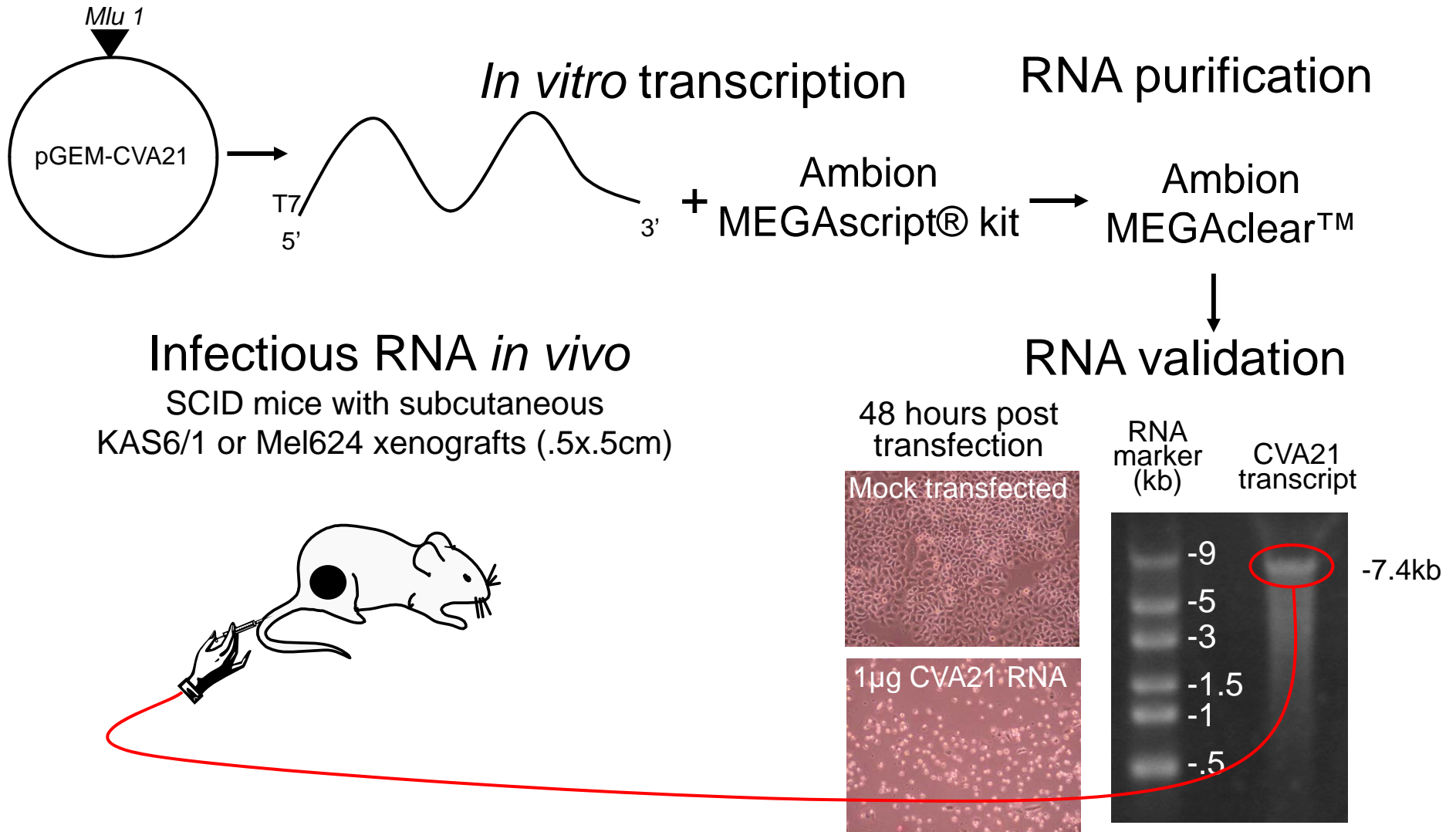
WT, miRT, and RevT viruses cause KAS 6/1 tumor regression but only miRT prolongs survival



microRNA targeting

- CVA21 host range can be restricted by incorporation of muscle specific microRNA target sequences (miRTs) into the genome
- This modification incapacitates the virus in cells expressing cognate miRNAs where the viral RNA is recognized and degraded
- The approach has subsequently been applied to poliovirus (neurotoxicity) and oncolytic adenoviruses (hepatotropism)
- But even targeted viruses remain immunogenic.....

CVA21 - infectious RNA production

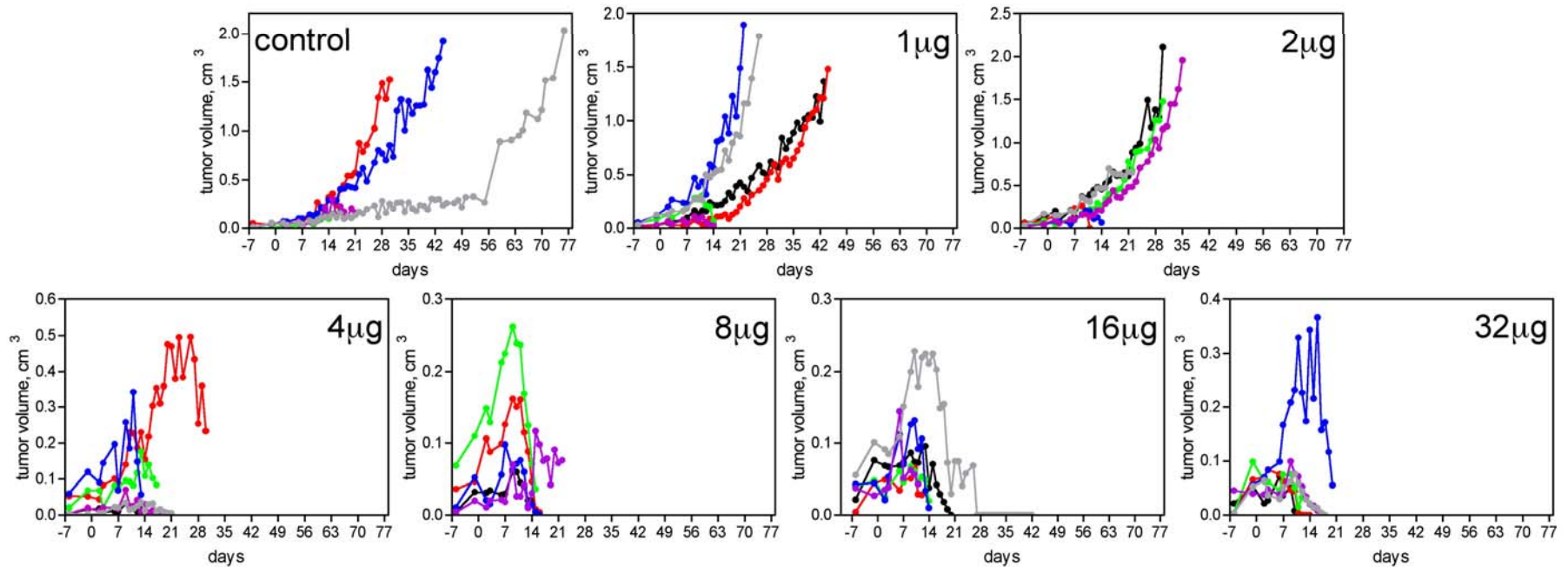




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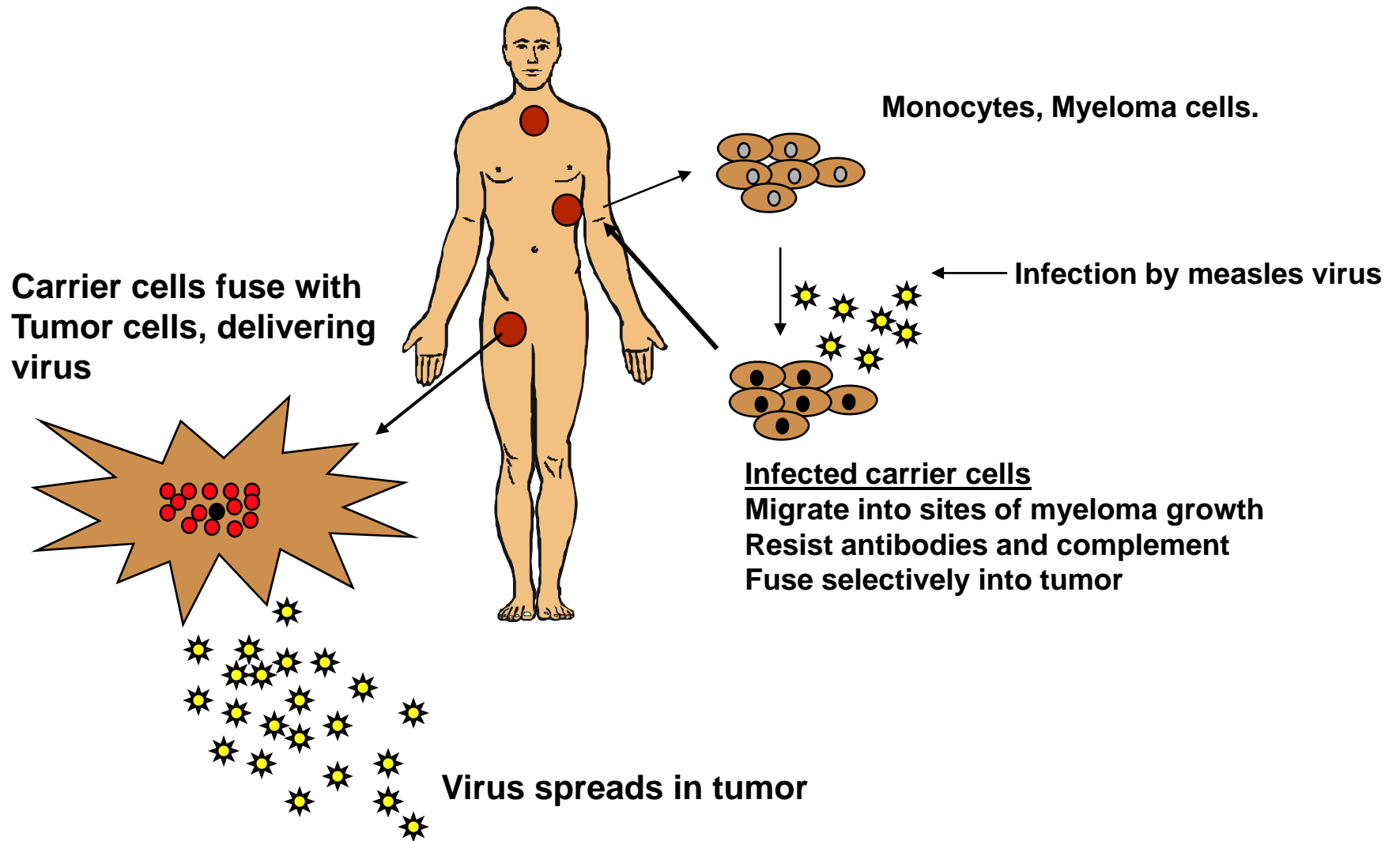
Dose dependence of infectious RNA oncolytic activity Multiple Myeloma tumor model

- KAS6/1 SQ xenografts in irradiated SCID mice
- Treated with one IT dose of CVA21 RNA (or Opti-MEM for control)

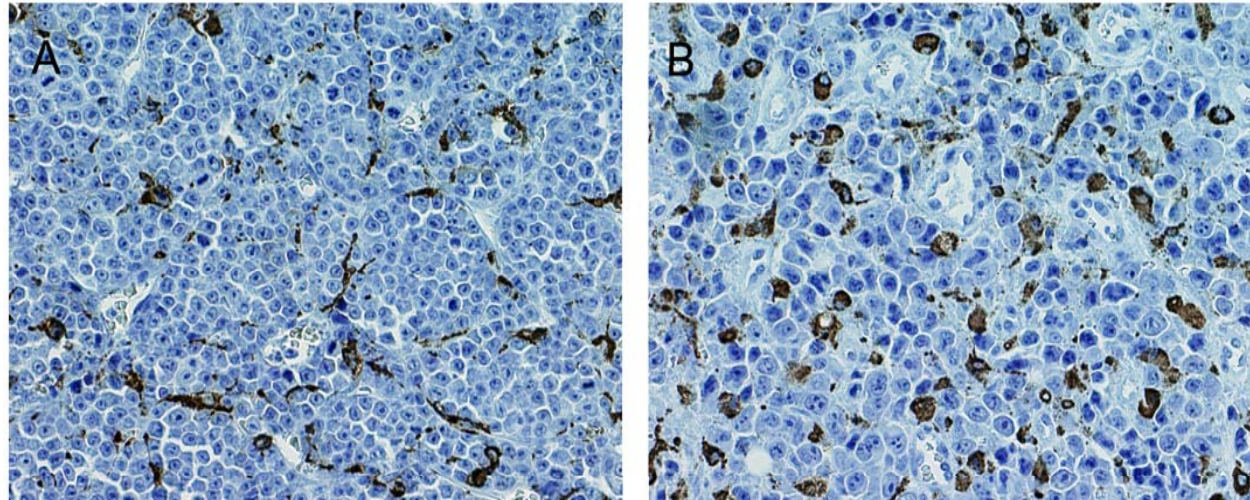


RNA dose	control	1µg	2µg	4µg	8µg	16µg	32µg
Mice with tumor reduction	0	33.3%	50%	100%	100%	100%	100%

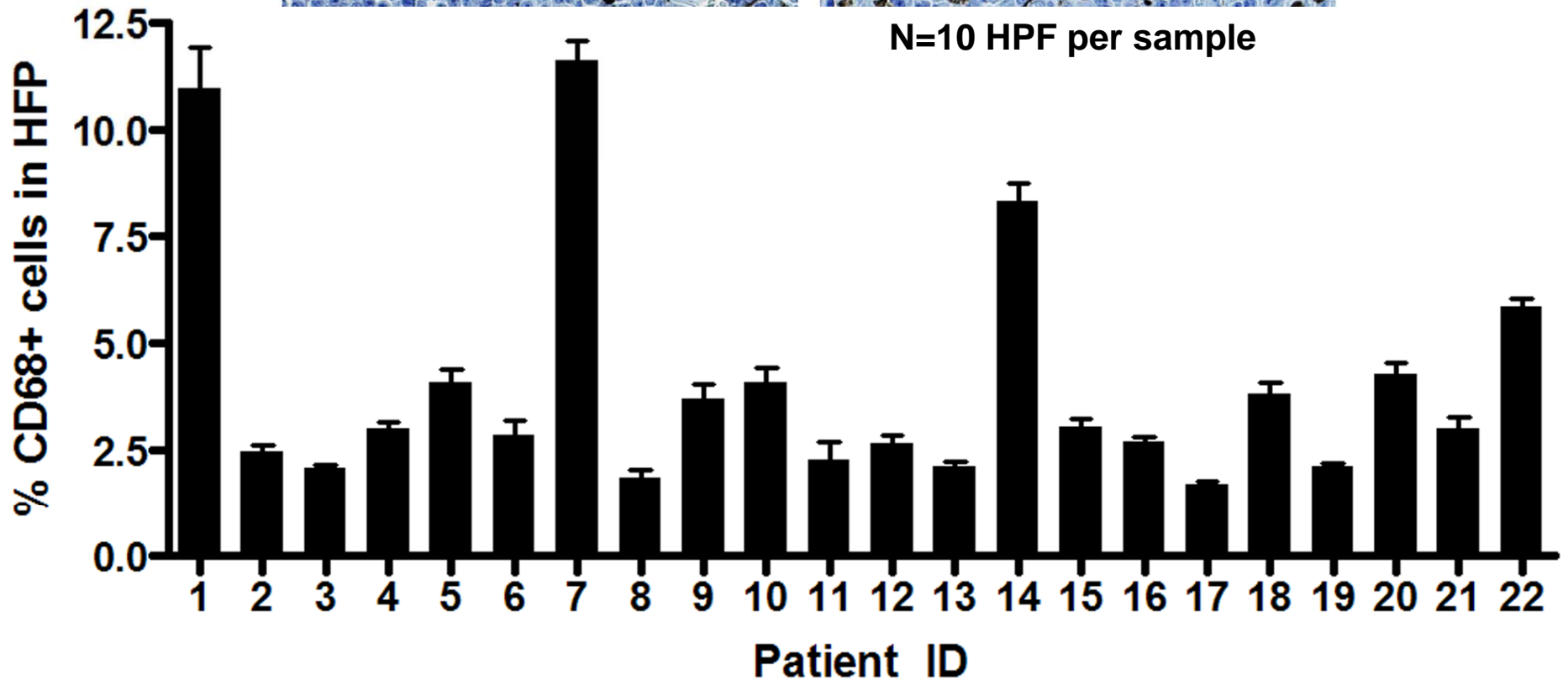
Circumventing anti-viral antibodies: Cellular vehicles for measles delivery



Plasmacytomas are infiltrated by CD68+ macrophages



N=10 HPF per sample



Conclusions

- Oncolytic virotherapy is a promising approach for myeloma
- Measles, CVA21, VSV, Vaccinia and Adenovirus have all shown promise in preclinical models
- Measles (MV-NIS) targets CD46 and is currently tested in a Phase I clinical trial
- microRNA target insertion is an effective new strategy to modify virus host range, abrogating toxicities
- Oncolytic viruses can be formulated as (nonimmunogenic) infectious nucleic acid or delivered in cellular carriers.



Oncolytic virotherapy for myeloma

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Sue Greiner

Rae Myers

Nathan Jenks

Andrew Greenslade

(Mary Harvey)

Vector Core

Mark Federspiel

Linda Gregory

Guy Griesmann

Kirsten Langfield

Julie Sauer

Sharon Stephan

Henry Walker

Troy Wegman

Clinical practice

Myeloma

Angela Dispenzieri

David Dingli

Martha Lacy

Morie Gertz

Susan Hayman

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