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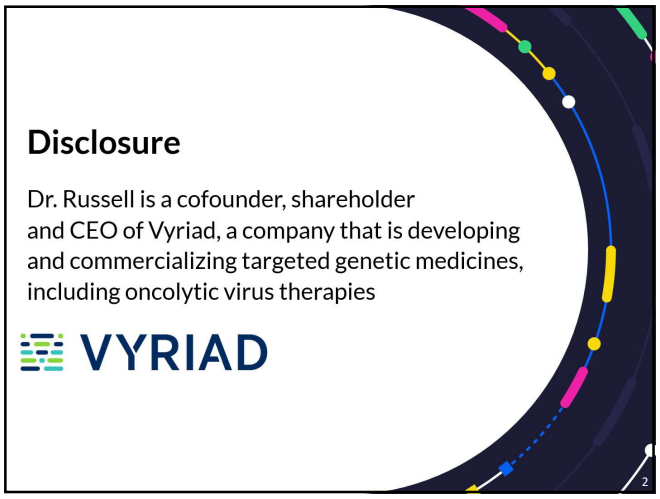
1



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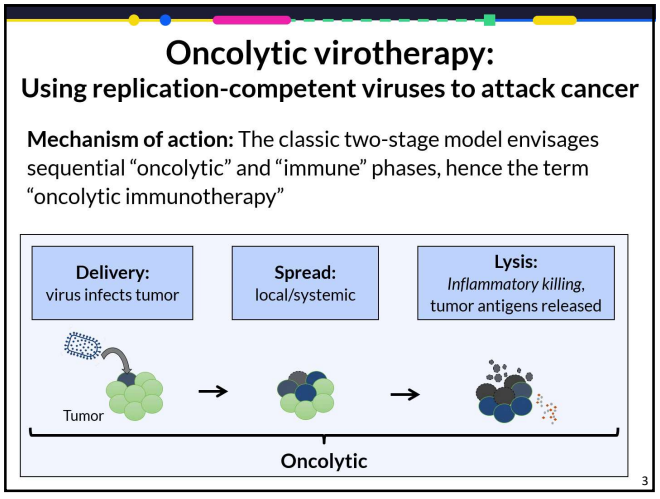


3

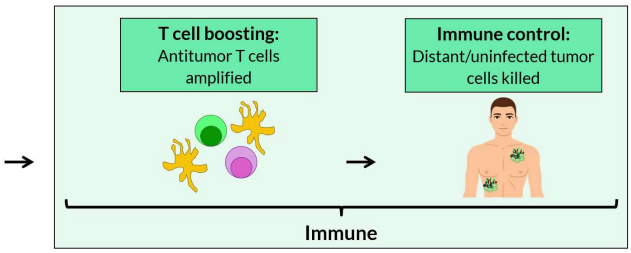
Oncolytic viruses: strategies, applications and challenges

Dr. Stephen J. Russell– CEO, Vyriad, USA

HSTalks

Oncolytic virotherapy:
Using replication-competent viruses to attack cancer

Mechanism of action: The classic two-stage model envisages sequential "oncolytic" and "immune" phases, hence the term "oncolytic immunotherapy"



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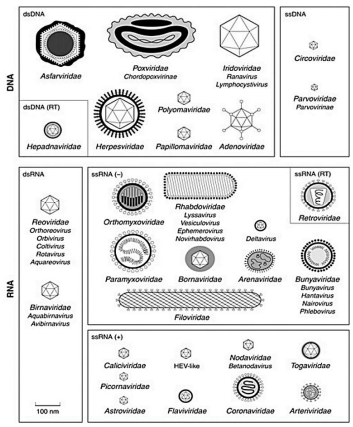
Any virus can theoretically be adapted or engineered to be an effective oncolytic agent

This represents a vast (largely) untapped bioresource…



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Key considerations in oncolytic virotherapy

Safety

- Natural virus biology/pathogenesis
- Stability of attenuating mutations
- Ability to control tropism (targeting and detargeting)

Efficacy

- Delivery to tumor and tumor-draining lymph nodes (TDLN)
- Replication and spread in tumor
- Immune activation and boosting of antitumor immunity

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Oncolytic viruses: strategies, applications and challenges

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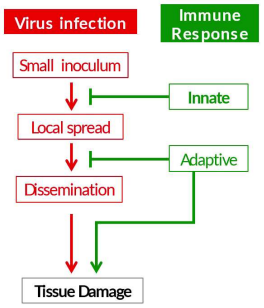
What can we learn from studies of natural virus pathogenesis?

1. Pathogenesis is an organized multistep process and plays out as a race between the spreading virus infection and the host immune response
2. Pathogenesis is shaped by virus tropism and the innate/adaptive host immune responses
3. Pathogenesis is heterogeneous between hosts and between viruses



Virgin S. in: Knipe D.M. & Howley P.M. *Fields Virology*. Wolters Kluwer Health. 2007; p.327

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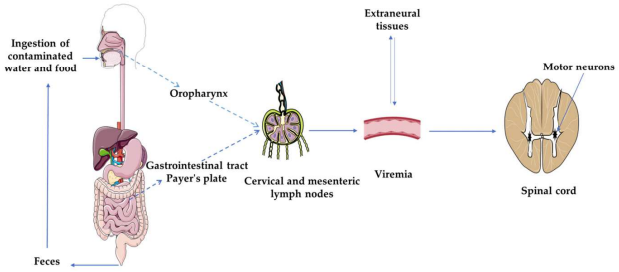
7

Poliovirus pathogenesis in humans and transgenic mice:

Implications for the design of OV therapies?



Blondel B. *et al. Curr Top Microbiol Immunol.* 2005; **289**:25-56

8

Poliovirus pathogenesis in humans and transgenic mice:

Implications for the design of OV therapies?

Poliovirus	OV therapies
Ingested inoculum amplifies stepwise in gut, then lymph nodes, then extraneural tissues, then spills into blood	IT injected virus amplifies stepwise in tumor, then draining lymph nodes, then spills into blood
Viremic threshold drives virus into motor neurons	Viremia level drives infection of metastases
95% infections asymptomatic. 1-2% lead to paralysis	Response heterogeneity expected, need to address
Off-target virus spread occurs in receptor positive mice with defective innate immunity (IFNAR KO)	IFNAR blockade might boost virus spread but targeting then key for safety
Anti-polio antibodies block pathogenesis	Anti-OV antibodies block efficacy

Blondel B. *et al. Curr Top Microbiol Immunol.* 2005; **289**:25-56

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Oncolytic virus examples:

- Measles virus
- Vesicular stomatitis virus
- Picornaviruses
  - Coxsackievirus A21 (infectious RNA)
- Herpesviruses
  - HSV (herpes simplex)
  - CMV (cytomegalovirus)

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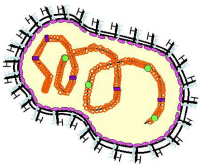
Measles pathogenesis



Nucleocapsid (N)
Phosphoprotein (P)
Polymerase (L)
Matrix (M)
F Fusion (F)
H Hemagglutinin (H)

Paramyxovirus, negative-strand RNA genome

9

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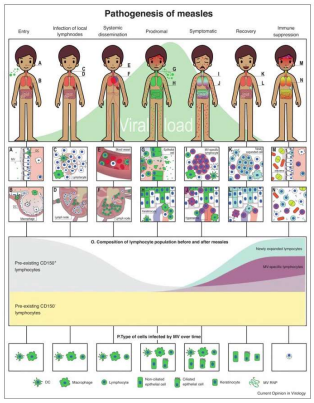
Measles pathogenesis



- Airborne (droplets) infection of tonsils leads to primary viremia (cell-associated)
- The virus amplifies in lymph nodes and spleen to drive secondary (cell-associated) viremia, rash, etc.
- Fever, coryza, conjunctivitis, rash, immune suppression, (pneumonitis, encephalitis)
- Mortality 0.1% in USA, 3% in Africa

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Origin of MV-NIS, a safe, trackable oncolytic measles virus

- David Edmonston was infected with measles 1954 (aged 11)
- A throat isolate was attenuated by serial passage (various cell substrates)
- Live Edm vaccines have since been successfully deployed to control measles
- Unlike wt measles, vaccine strains **use CD46 receptor** (abundant on cancer cells)

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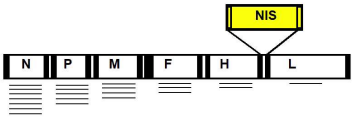
Origin of MV-NIS, a safe, trackable oncolytic measles virus



- **MV-NIS was derived from MV-Edm and is CD46-tropic, oncolytic**
- MV-NIS was administered intravenously to patients with cancer (single infusion)

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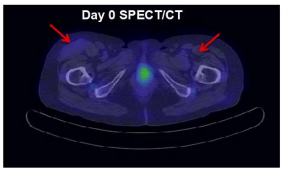
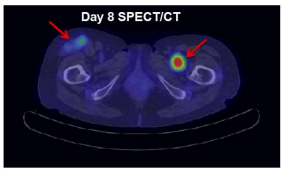
Origin of MV-NIS, a safe, trackable oncolytic measles virus

- Clinical responses were documented
- **Patient BJ:**
  - Heavily pretreated myeloma, multiple relapsing soft tissue plasmacytomas
  - NIS (reporter) imaging day 8 post-virus infusion shows MV-NIS-infected tumors





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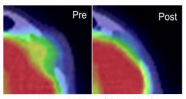
Stacy Erholtz: Durable complete remission of disseminated cancer after a single MV-NIS infusion

Medical history

- Relapsing disease (multiple myeloma) with multiple tumors and diffuse bone marrow involvement
- Refractory to all available therapies (including 2 stem cell transplants)

Treatment and outcome

- Single infusion of MV-NIS (1e11)
- 10-year (ongoing) durable CR at all disease sites



Resolution (PET/CT)

Russell S. *et al. Mayo Clin Proc.* 2014; **89**(7):926-33

16

Stacy Erholtz: Durable complete remission of disseminated cancer after a single MV-NIS infusion

Lessons learned

- Antitumor activity apparent only at top dose level (1e11)
- Only in patients with undetectable antimeasles antibodies

↓

But >95% of adults are measles immune

Russell S. *et al. Mayo Clin Proc.* 2014; **89**(7):926-33

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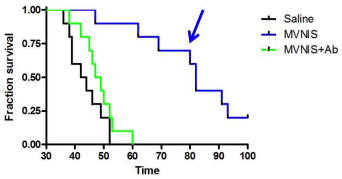
12

Anti-measles antibodies negate the efficacy of intravenous MV-NIS

Passive antimeasles serotherapy negates MV efficacy

Kaplan-Meier survival curve of SCID mice with systemic KAS 6/1 myeloma after a single infusion of MV-NIS



Days post KAS 6/1 implantation

Liu C. *et al. Mol Ther.* 2010; **18**(6):1155-64

18

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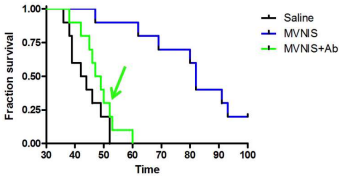

Days post KAS 6/1 implantation

Liu C. *et al. Mol Ther.* 2010; **18**(6):1155-64

19

Anti-measles antibodies negate the efficacy of intravenous MV-NIS

Hence strategies are in development to circumvent antiviral antibodies, e.g.:

95%
of people are measles seropositive

Liu C. *et al. Mol Ther.* 2010; **18**(6):1155-64

20

Anti-measles antibodies negate the efficacy of intravenous MV-NIS

Hence strategies are in development to circumvent antiviral antibodies, e.g.:

1. Use cell carriers to transport the virus
   - Ong H.T. *et al. Gene Ther.* 2007; **14**(4):324-33
   - Mader E.K. *et al. Clin Cancer Res.* 2009; **15**(23):7246-55
   - Ong H.T. *et al. J Heptol.* 2013; **59**(5):999-1006
2. Resurface measles with alternative F/H proteins (combine with targeting)
3. Use alternative (low seroprevalence) viruses e.g., vesicular stomatitis virus

Liu C. *et al. Mol Ther.* 2010; **18**(6):1155-64

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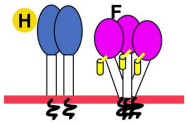
Targeting: Receptor-specific measles viruses displaying cell-targeting ligands



- Ablate natural specificities (CD46, SLAM, Nectin-4)
- Display cell targeting ligands (scFvs to CD38, EGFR, etc.)

Hadac E.M. *et al. Virology.* 2004; **329**(2):217-25; Nakamura T. *et al. Nat Biotechnol.* 2005; **23**(2):209-14

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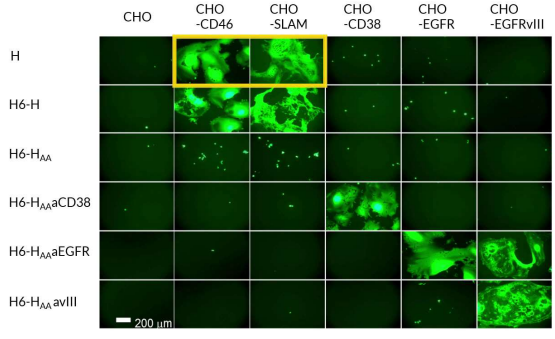

Hadac E.M. *et al. Virology.* 2004; **329**(2):217-25; Nakamura T. *et al. Nat Biotechnol.* 2005; **23**(2):209-14

24

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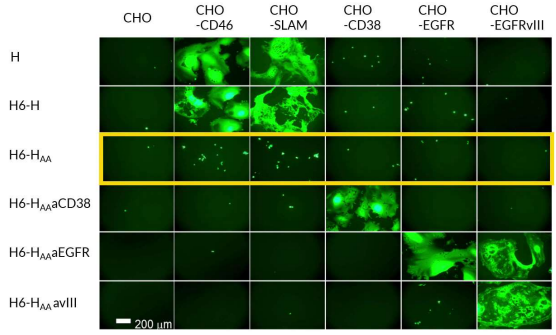
Targeting: Receptor-specific measles viruses displaying cell-targeting ligands



Hadac E.M. *et al. Virology.* 2004; **329**(2):217-25; Nakamura T. *et al. Nat Biotechnol.* 2005; **23**(2):209-14

25

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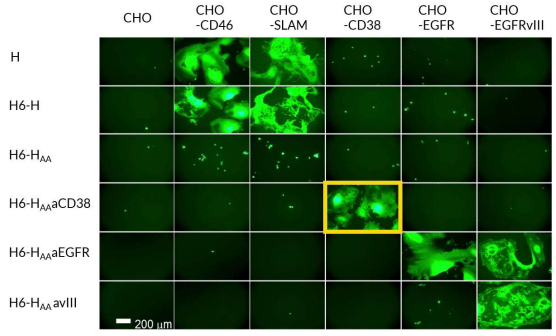
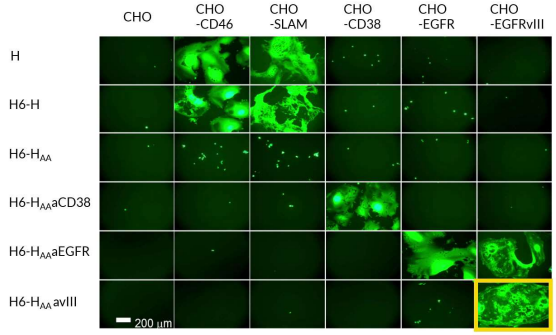


Hadac E.M. *et al. Virology.* 2004; **329**(2):217-25; Nakamura T. *et al. Nat Biotechnol.* 2005; **23**(2):209-14

26

Targeting: Receptor-specific measles viruses displaying cell-targeting ligands



Hadac E.M. *et al. Virology.* 2004; **329**(2):217-25; Nakamura T. *et al. Nat Biotechnol.* 2005; **23**(2):209-14

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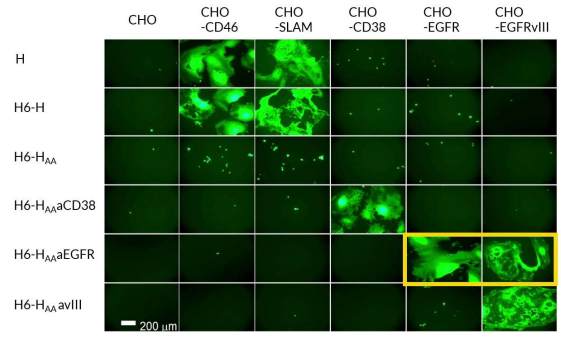
Targeting: Receptor-specific measles viruses displaying cell-targeting ligands



Hadac E.M. *et al. Virology.* 2004; **329**(2):217-25; Nakamura T. *et al. Nat Biotechnol.* 2005; **23**(2):209-14

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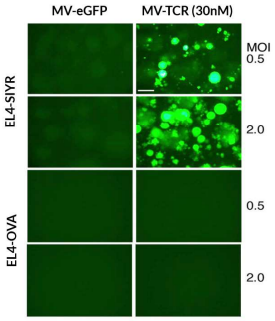
14

Targeting an MHC-peptide complex with a measles virus displaying a single chain T-cell receptor (scTCR)

Affinity matured scTCR-2C (binds SIYR peptide in context of mouse Kb) displayed on measles virus H protein, viruses rescued, encode GFP



Peng K-W. *et al. Gene Ther.* 2004; **11**(15):1234-9

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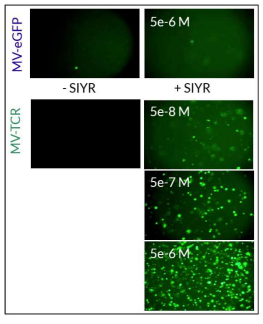
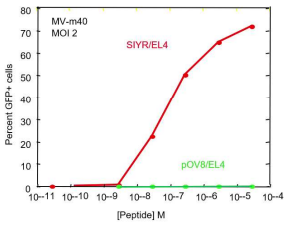
Targeting an MHC-peptide complex with a measles virus displaying a single chain T-cell receptor (scTCR)

Peptide feeding experiment (SIYRYYGL)





Infection occurs only when SIYR peptide is displayed by Kb, increases with increasing peptide concentration

Peng K-W. *et al. Gene Ther.* 2004; **11**(15):1234-9

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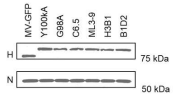
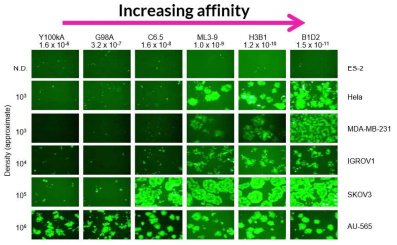
Discriminating receptor density on target cells using measles viruses

Measles viruses displaying Her2 antibodies of differing affinities for a single epitope

scFv	K_d (M)
Y100kA	$1.6X10^{-6}$
G98A	$3.2X10^{-7}$
C6.5	$1.6X10^{-8}$
ML3-9	$1.0X10^{-9}$
H3B1	$1.2X10^{-10}$
B1D2	$1.5X10^{-11}$



Immunoblots of measles virus



Fusion/killing of Her-2 positive targets

Lower receptor density requires higher antibody affinity

Hasegawa K. *et al. J Virol.* 2007; **81**(23):13149-57

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Stealthed, targeted, armed measles virus (MV-STAR)

MV-STAR circumvents antimeasles antibodies to enable systemic OV therapy

Developing the MV-STAR platform

- CDV surface glycoproteins were engineered using paramyxovirus F/H targeting technology
- Optimization of EGF receptor-targeted and CD38-targeted MV-STAR viruses armed with an IL12 transgene is underway

Nakamura T. *et al. Nat Biotechnol.* 2005; **23**(2):209-14; Bah E.S. *et al. Mol Cancer Ther.* 2020; **19**(10):205767; Munoz-Alia M.A. *et al. PLoS Pathog.* 2021; **17**(2):e1009283; Munoz-Alia M.A. *et al. Cell Rep Med.* 2021; **2**(4):100225

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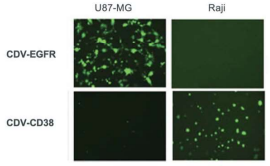

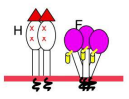

Canine Distemper F/H retargeting

Initiation of clinical testing is planned for 2024

Nakamura T. *et al. Nat Biotechnol.* 2005; **23**(2):209-14; Bah E.S. *et al. Mol Cancer Ther.* 2020; **19**(10):205767; Munoz-Alia M.A. *et al. PLoS Pathog.* 2021; **17**(2):e1009283; Munoz-Alia M.A. *et al. Cell Rep Med.* 2021; **2**(4):100225

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HSTalks

Oncolytic virus examples:

- Measles virus
- **Vesicular stomatitis virus**
- Picornaviruses
  - Coxsackievirus A21 (infectious RNA)
- Herpesviruses
  - HSV (herpes simplex)
  - CMV (cytomegalovirus)

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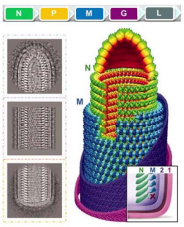
Vesicular stomatitis virus

- Bullet-shaped, enveloped virus, 5 genes, rapid replication, lytic (10,000 burst size)
- Naturally infects ungulates (cattle, horses) causing self-limited blistering illness
- Low human seroprevalence <5% population immune
  - Suitable for intravenous and intratumoral administration



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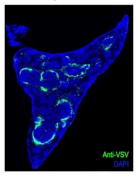
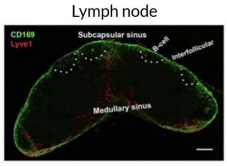
Vesicular stomatitis virus

- Recombinants with foreign transgenes are highly stable
- Targets tumor cells and sentinel macrophages in lymph nodes and spleen
  - Both cell types have suppressed innate immune responses

Spleen



Lymph node



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Vesicular stomatitis virus

VSV-IFNβ -NIS (Voyager-V1)

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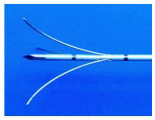
First-in-human clinical trial of VSV (VSV-IFNβ) as intratumoral therapy

VSV-IFNβ

(precursor of VSV-IFNβ-NIS, encodes human interferon beta)

Phase I trial of intratumoral injection of VSV-hIFNβ in patients with sorafenib refractory/intolerant HCC or tumors metastatic to liver



Multi-pronged Quadrafuse needle

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First-in-human clinical trial of VSV (VSV-IFNβ) as intratumoral therapy

- Preliminary evidence of anti-tumor efficacy was exhibited in patients with hepatocellular cancer including:
  - One partial response by RECIST v1.1 criteria
  - Three patients with stable disease, 2 of the 3 SD >4 months with AFP decrements

ID	Dose level	Tumor Type	Response
1	1	HCC	SD
2	1	HCC	PD
3	1	HCC	PR
4	2	HCC	PD
5	2	HCC	SD
6	2	HCC	PD
7	3A	HCC	PD
8	3A	HCC	SD
9	3A	Colorectal	PD
10	3A	Prostate	PD
13	3A	HCC	PD
14	3A	HCC	PD
11	4A	Cholangiocarcinoma	PD
12	4A	Colorectal	TLS

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First-in-human clinical trial of VSV (VSV-IFNβ) as intratumoral therapy

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  - One partial response by RECIST v1.1 criteria
  - Three patients with stable disease, 2 of the 3 SD >4 months with AFP decrements
  - One grade 5 toxicity (TLS)

ID	Dose level	Tumor Type	Response
1	1	HCC	SD
2	1	HCC	PD
3	1	HCC	PR
4	2	HCC	PD
5	2	HCC	SD
6	2	HCC	PD
7	3A	HCC	PD
8	3A	HCC	SD
9	3A	Colorectal	PD
10	3A	Prostate	PD
13	3A	HCC	PD
14	3A	HCC	PD
11	4A	Cholangiocarcinoma	PD
12	4A	Colorectal	TLS

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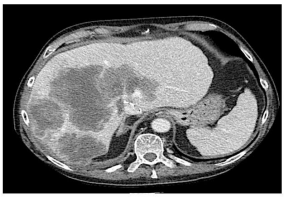
Case details: Tumor lysis after intratumoral VSV-IFNβ

- Age 67, metastatic colorectal cancer
- Previously treated with FLOX, irinotecan, bevacizumab, cetuximab, regorafenib, radioembolization
- High tumor burden
- Prior portal vein thrombosis



20

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Case details: Tumor lysis after intratumoral VSV-IFNβ

Day 1

- VSV-IFNβ injected into a single liver lesion

Days 5 to 10

- Biochemical features of tumor lysis, liver enzymes increasing, platelets dropping, CT scan and tumor biopsies showed extensive tumor necrosis

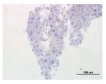
VSV had spread extensively, but only in the tumor deposits (injected and metastatic)

Day 13

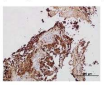
- Patient died from TLS/hepatorenal failure

VSV staining of tumor biopsies

Day 0 baseline



Day 8 post-therapy



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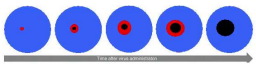
Postulated MOA for intratumoral VSV

1. VSV deposited in tumor, interacts with tumor cells via LDLR
   - Resistant tumors: Minimal infection/spread/killing
     - LDL competitively inhibits binding/entry
     - Antiviral state blocks intracellular replication

INTRATUMORAL DELIVERY



21

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Postulated MOA for intratumoral VSV

1. VSV deposited in tumor, interacts with tumor cells via LDLR
   - Permissive tumors (minority): Extensive spread/killing
     - Defective antiviral signaling allows intracellular replication
     - Direct contact-mediated intercellular transfer of progeny VSVs bypasses LDL barrier

INTRATUMORAL DELIVERY

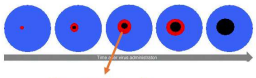
VSV + TAA traffic
to draining lymph node (TDLN)

44

Postulated MOA for intratumoral VSV

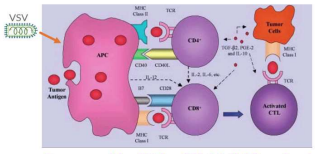
2. VSV + tumor cell vesicles travel in lymph vessels to TDLN
   - Capture by sentinel CD169+ macrophages in SCS//medulla
     - MFs enforce VSV replication (USP18)
     - Directly present viral/tumor antigens to B/T cells
     - Drive type 1 IFN response (via pDC)
     - Activate and transfer viral/tumor antigen to cDC

INTRATUMORAL DELIVERY



VSV + TAA traffic
to draining lymph node (TDLN)

Captured by
CD169+ SCS MFs



Synergy with αPD1, αCTLA4, IL12 payload

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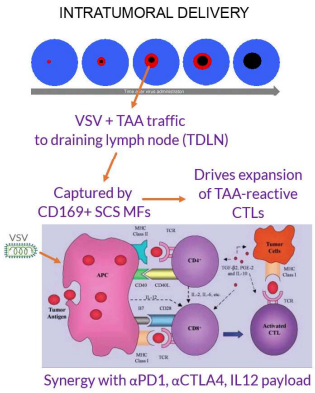
Postulated MOA for intratumoral VSV

2. VSV + tumor cell vesicles travel in lymph vessels to TDLN
   - CD169+ MFs drive activation/amplification of VSV- and TAA-reactive CTL (overrides insufficiency of tumor antigen)



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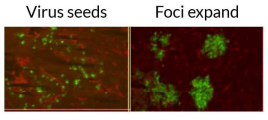
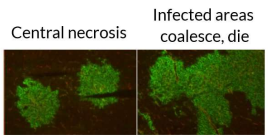
Intravenous VSV-IFNβ-NIS

5TGM1 myeloma model

Single IV dose of Voyager-V1





Naik S. *et al. Leukemia.* 2012; **26**(8):1870-8; Miller A. *et al. Mol Ther Oncolytics.* 2014; **1**:14005

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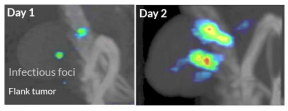
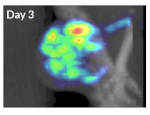
Intravenous VSV-IFNβ-NIS

5TGM1 myeloma model

Single IV dose of Voyager-V1

Daily NIS imaging of tumor and serial tumor biopsies (stained for VSV)





Naik S. *et al. Leukemia.* 2012; **26**(8):1870-8; Miller A. *et al. Mol Ther Oncolytics.* 2014; **1**:14005

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Intravenous VSV-IFNβ-NIS

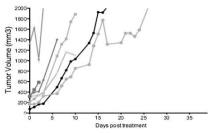
5TGM1 myeloma model

Single IV dose of Voyager-V1

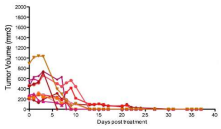
Daily NIS imaging of tumor and serial tumor biopsies (stained for VSV)

- Tumors shrink rapidly, infection resolves
- Long term immune control

Saline



Voyager-V1, single dose IV



Naik S. *et al. Leukemia.* 2012; **26**(8):1870-8; Miller A. *et al. Mol Ther Oncolytics.* 2014; **1**:14005

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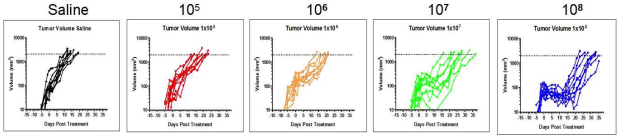
Intravenous VSV-IFNβ-NIS: Higher doses are more effective

5TGM1 myeloma, immunocompetent mice

Monitor tumor growth

Day 0: Single IV dose of Voyager-VI

Saline | 10^5 | 10^6 | 10^7 | 10^8



Naik S. *et al. Leukemia.* 2012; **26**(8):1870-8; Zhang L. *et al. Hum Gene Ther Clin Dev.* 2016; **27**(3):111-22

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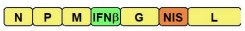
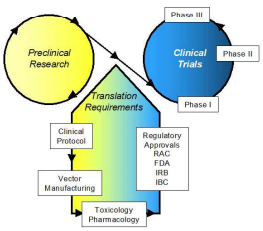
VSV-IFNβ-NIS canine lymphoma trial

Phase 1 trial in companion dogs with lymphoma

- Single intravenous infusion of VSV-IFNβ-NIS (10^{10} $TCID_{50}$ per 0.5 m^2)
- Well tolerated (8 dogs treated)





Naik S. *et al. Mol Cancer Ther.* 2018; **17**(1):316-26

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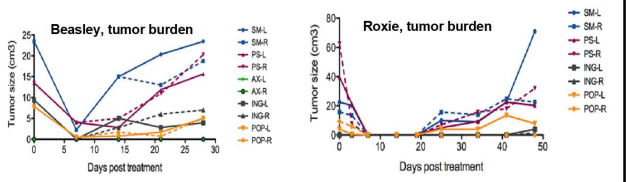
VSV-IFNβ-NIS canine lymphoma trial

Phase 1 trial in companion dogs with lymphoma

- Single intravenous infusion of VSV-IFNβ-NIS (10^{10} $TCID_{50}$ per 0.5 m^2)
- Well tolerated (8 dogs treated)
- Clinical responses observed (shrinkage of multiple lymphatic tumors) in two dogs with T cell lymphoma



Naik S. *et al. Mol Cancer Ther.* 2018; **17**(1):316-26

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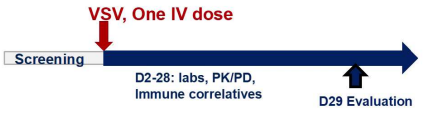
Human trial of intravenous VSV-IFNβ-NIS in hematologic malignancies

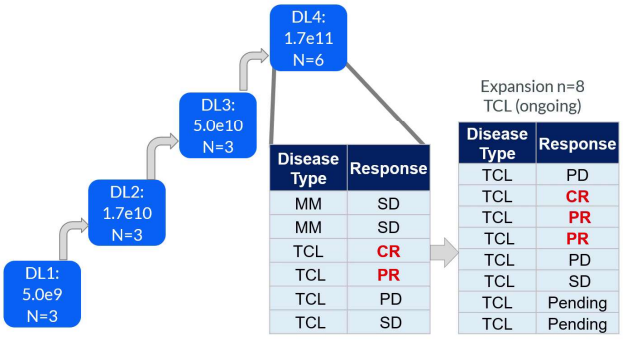
Phase 1 dose escalation (3+3) trial of single infusion of Voyager-V1 (IST) in patients with relapsed or refractory hematologic malignancies **(myeloma and T cell lymphoma)**



Cook J. *et al. Blood Adv.* 2022; **6**(11):3268-79

53

Human trial of intravenous VSV-IFNβ-NIS in hematologic malignancies



Disease Type	Response
MM	SD
MM	SD
TCL	CR
TCL	PR
TCL	PD
TCL	SD

Expansion n=8
TCL (ongoing)

Disease Type	Response
TCL	PD
TCL	CR
TCL	PR
TCL	PR
TCL	PD
TCL	SD
TCL	Pending
TCL	Pending

Cook J. *et al. Blood Adv.* 2022; **6**(11):3268-79

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Human trial of intravenous VSV-IFNβ-NIS in hematologic malignancies

Conclusion

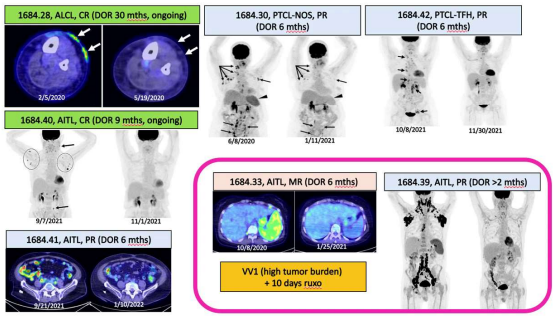
- Durable responses seen at top dose level (DL4) confirming efficacy dose threshold of ~1e11 $TCID_{50}$ as predicted by preclinical models
- T cell lymphoma displays a higher response level

Cook J. *et al. Blood Adv.* 2022; 6(11):3268-79

55

Voyager-V1 is active after a single infusion

Patients with treatment refractory peripheral T cell lymphoma (50% DRR)



26

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Voyager-V1 is active after a single infusion

A 20-patient T cell lymphoma expansion cohort has been added to the ongoing phase 1 trial, accrual to complete by Q4 2024

If data holds, we will proceed to a registration trial

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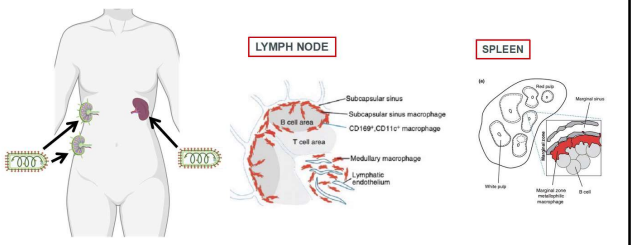
Postulated MOA for intravenous VSV-IFNβ-NIS in T cell lymphoma

- CD169 is expressed primarily on SCS and MS macrophages in lymph nodes and MZ macrophages in the spleen

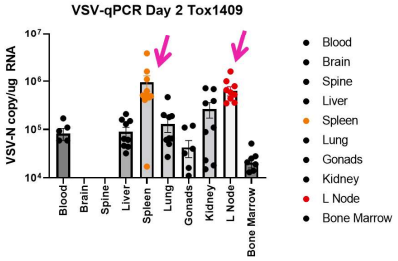


Martinez-Pomares L. & Gordon S. *Trends Immunol.* 2012; 33(2):66-70

58

Postulated MOA for intravenous VSV-IFNβ-NIS in T cell lymphoma

- CD169 is expressed primarily on SCS and MS macrophages in lymph nodes and MZ macrophages in the spleen



Martinez-Pomares L. & Gordon S. *Trends Immunol.* 2012; 33(2):66-70

59

Postulated MOA for intravenous VSV-IFNβ-NIS in T cell lymphoma

- CD169+ MΦs rapidly capture, endocytose VSV and are VSV permissive due to expression of USP18
- Because they reside in LNs and spleen, infected CD169+ MΦs may transmit virus to adjacent lymphoma cells

Martinez-Pomares L. & Gordon S. *Trends Immunol.* 2012; 33(2):66-70

60

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Combination therapy with VSV-mIFNβ-NIS + αPD1 + αCTLA4 antibodies

2206: 5TGM1 myeloma tumor S.C.

VSV-mIFNβ-NIS (1e8 $TCID_{50}$ IV, 1X)
ICB = αPD1 + αCTLA4 Ab
(IP, 2mg/mouse, 1X)

D-3	D0	D3
	Saline	
	ICB	
	VSV	
	VSV + ICB	
ICB	VSV	
	VSV	ICB

→ Monitor tumor volume

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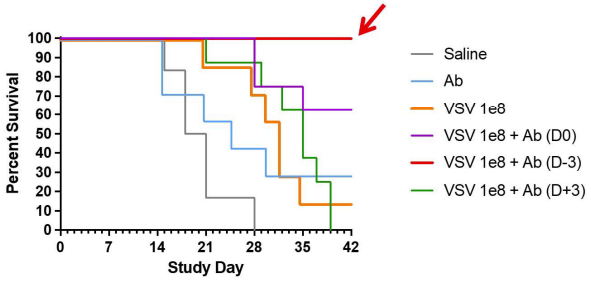
Combination therapy with VSV-mIFNβ-NIS + αPD1 + αCTLA4 antibodies



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VSV-IFNβ-NIS in human subjects: Experience to date

Manageable toxicities
- Short-lived CRS
- Lymphopenia
- AST elevation
- TLS/IFNβ toxicity (rare)

>160 patients have received VSV-IFNβ-NIS (29 IT, >130 IV)

Inflames tumors

Single agent antitumor activity
Lymphoma, others

Trackable
- IFNβ biomarker
- NIS imaging

Well tolerated with CPI
Anti PD-1

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HSTalks

Oncolytic virus examples:

- Measles virus
- Vesicular stomatitis virus
- Picornaviruses
  - Coxsackievirus A21 (infectious RNA)
- Herpesviruses
  - HSV (herpes simplex)
  - CMV (cytomegalovirus)

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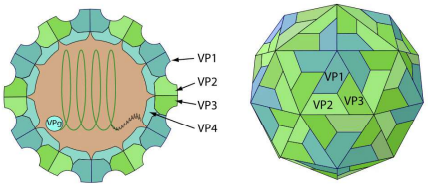
Infectious picornavirus RNA



Picornaviruses are promising oncolytic agents with small, simple +ve sense RNA genomes

The transfected cell will initiate a spreading viral infection

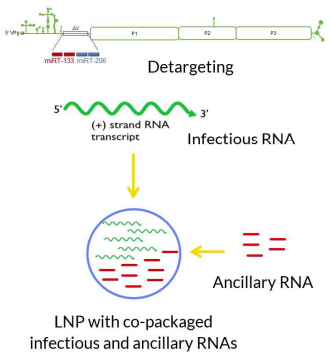
Hadac E.M. *et al. Mol Ther.* 2011; **19**(6):1041-7; Kelly E.J. *et al. Nat Med.* 2008; **14**(11):1278-83

65

Infectious picornavirus RNA

Synthetic approach

- Formulate detargeted picornavirus genomes as synthetic RNA of high specific infectivity (iRNA)
- Package iRNA into nonimmunogenic lipid nanoparticles (LNP) for repeat systemic stealth delivery
- Co-package ancillary RNAs encoding immune modulatory payloads for arming



Hadac E.M. *et al. Mol Ther.* 2011; **19**(6):1041-7; Kelly E.J. *et al. Nat Med.* 2008; **14**(11):1278-83

66

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Infectious picornavirus RNA

Detargeting

Hadac E.M. *et al. Mol Ther.* 2011; **19**(6):1041-7; Kelly E.J. *et al. Nat Med.* 2008; **14**(11):1278-83

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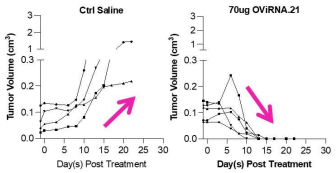
Coxsackievirus A21

LNP formulation bypasses serum neutralization, enables repeat dosing

Efficacy seen at very low dose of LNP encapsulated iRNA



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Coxsackievirus A21

iRNA/LNP platform technology

- Copackaging of iRNA with payload RNAs is simple
- Process developed for GMP-grade RNA synthesis
- IND planned appropriate activities underway

Elsedawy N.B. *et al. Mol Ther Oncolytics.* 2020; **17**:484-95; Ruiz A.J. *et al. J Virol.* 2016; **90**(8):4078-92

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Oncolytic virus examples:

Measles virus

Vesicular stomatitis virus

Picornaviruses

- Coxsackievirus A21 (infectious RNA)

Herpesviruses

- HSV (herpes simplex)
- CMV (cytomegalovirus)

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Status of the OV field Q1 2023

T-VEC (Imlygic)

- Intratumoral Herpes Simplex Virus (HSV1)
- The only FDA-approved drug
- JS strain – γ34.5 KO, ICP47 KO, GM-CSF transgene

Phase III melanoma trial started in 2009

- Unresectable stage IIIB, IIIC, IV disease
- Intratumoral T-VEC vs. subcutaneous GM-CSF
- Virus was administered every 2 weeks
- 430 patients randomised 2:1
- Durable (6 months) responses: **16% T-VEC, 2% control**
- Survival advantage (p=0.051)

Andtbacka R.H.I. *et al. J Clin Oncol.* 2015; 33(25):2780-8

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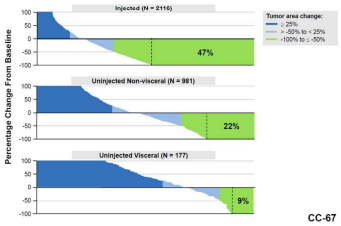
Status of the OV field Q1 2023

FDA approval October 2015, EU approval December 2015

- BUT, limited sales due to superior efficacy of checkpoint antibodies
- And inconvenience of intratumoral virus administration

Individual lesion responses
Amgen, CTGTAC/ODAC meeting
29th April 2015



Andtbacka R.H.I. *et al. J Clin Oncol.* 2015; 33(25):2780-8

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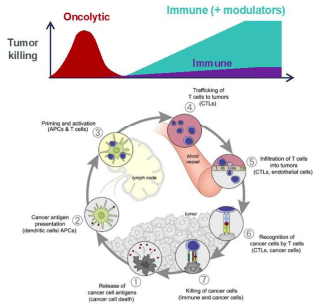
Boosting the clinical efficacy of T-VEC via combination therapy



The cancer immunity cycle

Chesney J. *et al. J Clin Oncol.* 2018; **38**(17):1658-67; Chesney J. *et al. J Clin Oncol.* 2023; **41**(3):528-40; Chen D.S. & Mellman I. *Immunity.* 2013; **39**(1):1-10

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Boosting the clinical efficacy of T-VEC via combination therapy

Ipilumumab (αCTLA4) +/- T-VEC, advanced melanoma

- Amgen 198 patient randomized phase 2 trial
- Response rates (PR plus CR)
  - 18% for Ipilumumab
  - 39% for Ipilumumab plus T-VEC

Pembrolizumab (αPD1) +/- T-VEC, advanced melanoma

- Amgen 692 patient randomized phase 3 trial
- No significant advantage for PFS or OS in combination versus pembrolizumab monotherapy arm (study closed due to futility)

Chesney J. *et al. J Clin Oncol.* 2018; **38**(17):1658-67; Chesney J. *et al. J Clin Oncol.* 2023; **41**(3):528-40; Chen D.S. & Mellman I. *Immunity.* 2013; **39**(1):1-10

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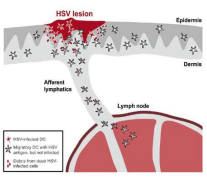
Combining herpesviruses (HSV+CMV) as an alternative approach for boosting OV efficacy

HSV-1

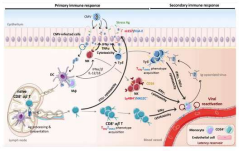
- Epithelial and neuronal tropic
- Kills most human cancer cells
- Releases tumor-associated antigens
- Induces systemic anti-tumor immune responses



Kills cancer cells, and releases tumor antigens

CMV

- Myeloid tropic
- Kills select human cancer cells (glioma, myeloid tumor cells)
- Locally recruits dendritic cells
- Suppresses NK response to HSV-1



Kills certain cancer cells, modulates tumor-associated myeloid cells, recruits antigen-presenting cells.

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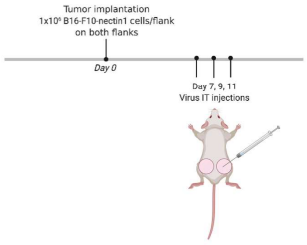
HSV1-IL12 + mCMV are synergistic in a bilateral B16-F10-nectin1 model



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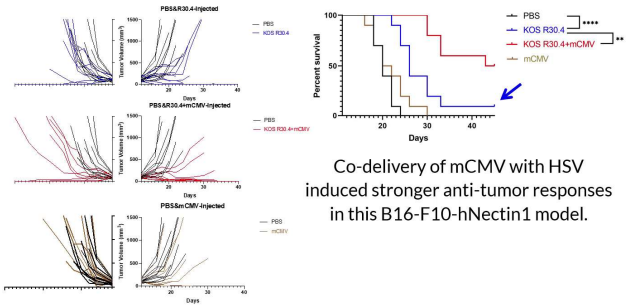
HSV1-IL12 + mCMV are synergistic in a bilateral B16-F10-nectin1 model



Co-delivery of mCMV with HSV induced stronger anti-tumor responses in this B16-F10-hNectin1 model.

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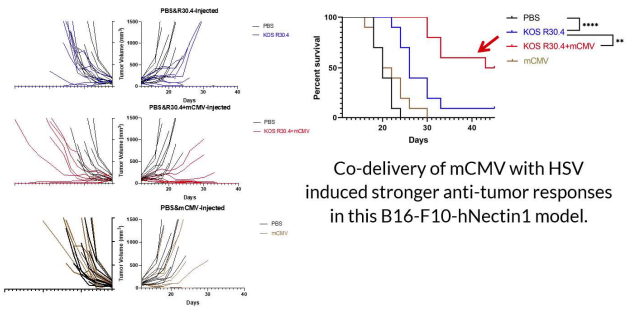
HSV1-IL12 + mCMV are synergistic in a bilateral B16-F10-nectin1 model



Co-delivery of mCMV with HSV induced stronger anti-tumor responses in this B16-F10-hNectin1 model.

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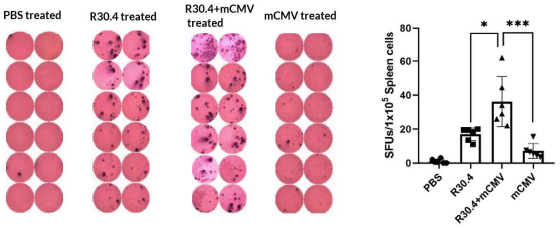
HSV1-IL12 + mCMV are synergistic in a bilateral B16-F10-nectin1 model

Elispot-IFN-gamma spleen cells anti tumor cells
(1x10^5 spleen cells + 1x10^5 tumor cells/well)



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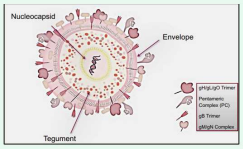
Taking intratumoral OVs to the next level

Advantages of CMV	Potential Applications
1. Large, stable dsDNA genome, higher capacity than HSV-1 to accommodate multiple foreign transgenes	I. Synergy with HSV
2. Slow, nonlytic replication cycle and distinct tropisms (myeloid, fibroblast, glial and endothelial cells)	II. *In vivo* engineering lentiviral vector production
3. Unique immune system interactions (T cell hyperinflation, NK suppression)	
4. High human seroprevalence but pre-existing immunity does not prevent re-infection	
5. Easily attenuated by deleting immune evasion genes, available antiviral drugs (ganciclovir)	



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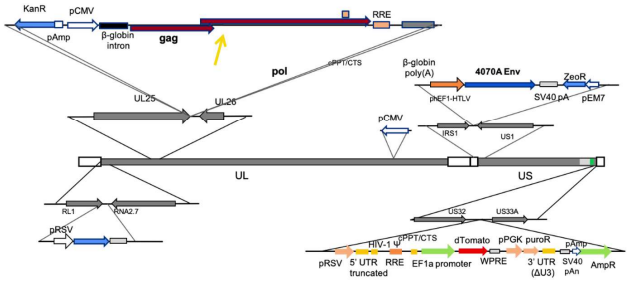
CMV to convert infected cells into lentiviral vector factories



Multiple CMV genes deleted, lentiviral helper and vector genomes inserted into the viral genome

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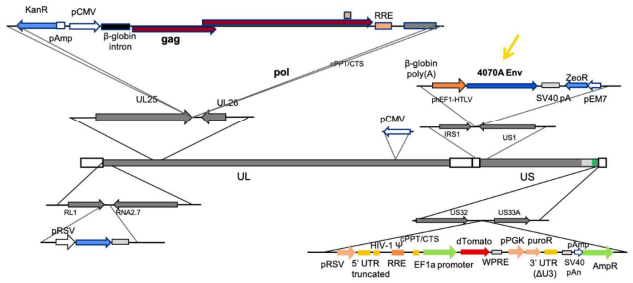
CMV to convert infected cells into lentiviral vector factories



Multiple CMV genes deleted, lentiviral helper and vector genomes inserted into the viral genome

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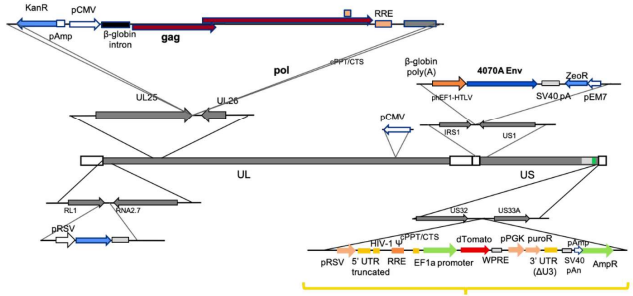
CMV to convert infected cells into lentiviral vector factories



Multiple CMV genes deleted, lentiviral helper and vector genomes inserted into the viral genome

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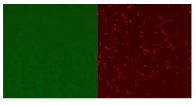
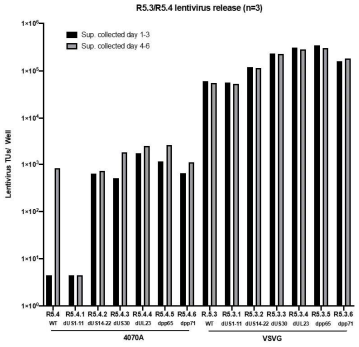
CMV to convert infected cells into lentiviral vector factories

Lentiviral transduction of 293T cells with filtered supernatant from CMV infected cells





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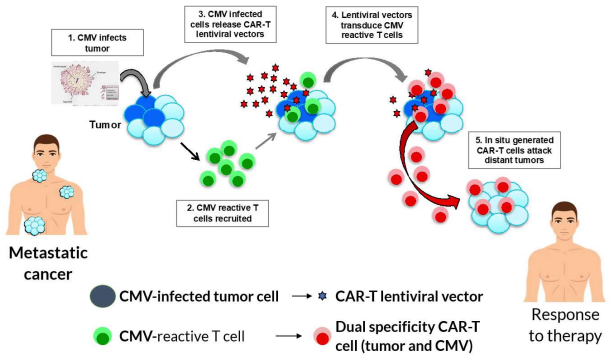
HSTalks

Envisioned strategy to use CMV as an oncolytic vector that also delivers lentiviral CAR-T therapy



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Conclusions

- Oncolytic viruses (OV) act by killing tumor cells *in situ* and reshaping the anticancer immune response
- There is still only one OV approved in the USA: Imlygic (T-VEC) for intratumoral therapy of late-stage relapsed melanoma
- Clinical trials have shown that a single intravenous infusion of a measles- or VSV-derived OV can lead to complete or partial remission of metastatic cancer
- Repeat intravenous OV dosing is associated with diminished efficacy due to rising levels of virus-neutralizing antibodies

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Conclusions

- Use of infectious picornavirus RNA in lipid nanoparticles may permit repeat systemic dosing
- Cytomegalovirus can synergize with intratumoral HSV1 and may facilitate complex *in vivo* tumor and myeloid cell engineering approaches

OVs are highly promising agents for use in combination with immune checkpoint blockade or adoptive immune cell therapies

Clinical progress is now accelerating due to recent technology breakthroughs impacting delivery, spread and immune system interactions

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(SJR moved from Mayo Clinic to Vyriad in December 2022)

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