VIRUS AS CANCER THERAPY

FROM LABORATORIES TO CLINICS

YUMAN FONG, MD

Sangiacomo Family Chair in Surgical Oncology
Chair and Professor, Department of Surgery
City of Hope

Disclosures

- Consultant for Boehringer Ingelheim, Eureka Therapeutics, and Imugene.
- Stock/Shareholder in Imugene.
- Royalties from Imugene, and Merck.

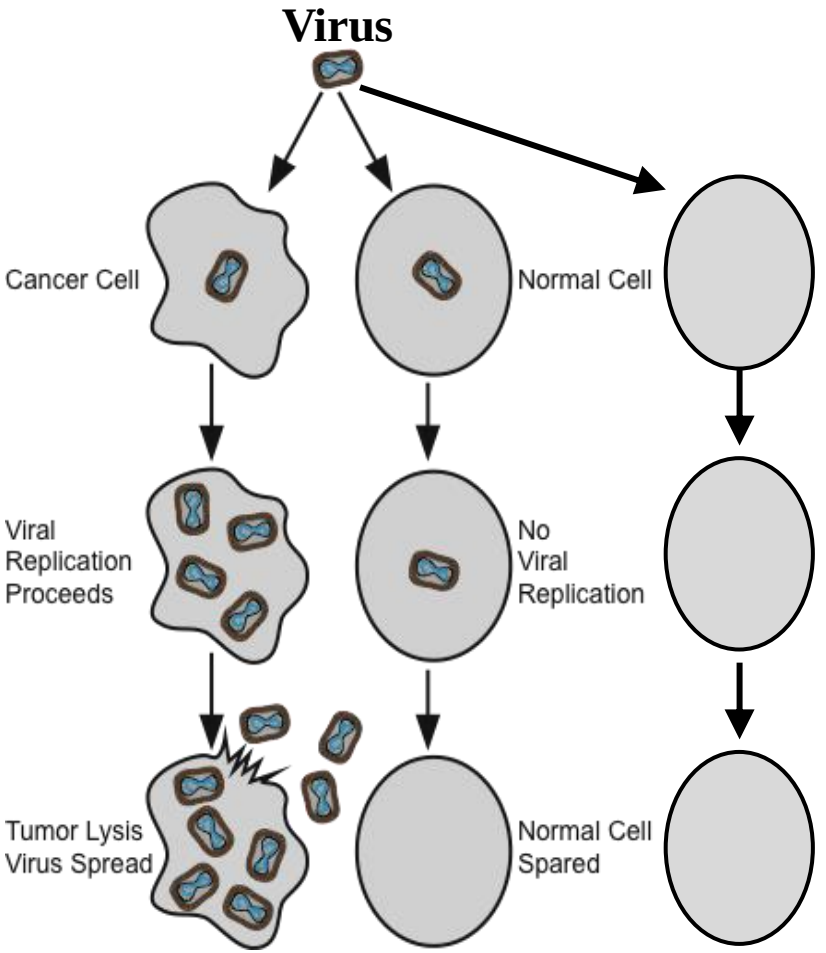
*The off-label or investigational use of **CF33, CF33-CD19** will be discussed.*

- 1859: Evolution, Charles Darwin
- 1865: Mendelian genetics, Gregor Mendel
- 1869: Isolation of DNA, Frederick Miescher
- 1879: Mitosis, Walter Fleming
- 1902: Chromosome theory of inheritance, Walter Sutton
- 1902: Concept of genetic diseases (alkaptonuria), Archibald Garrod
- 1909: Terms Gene, Genotype, Phenotype Used, Wilhelm Johannsen
- 1941: One gene, one enzyme hypothesis, George Beadle
- 1953: Structure of DNA, Watson and Crick
- 1961: First screen for genetic disease (phenylketonuria), Robert Guthrie
- 1961: mRNA, Sydney Brenner, Francois Jacob, Matthew Meselson
- 1972: First recombinant DNA
- 1973: First animal gene cloned
- 1975: DNA sequencing, Frederick Sanger
- 1983: First disease gene mapped (Huntington's disease)
- 2001: Sequencing human genome, Venter and Collins

- Since 1990: >1500 Gene therapy Trials

Oncolytic Viruses

- Goal: Genetically engineer viruses to specifically infect, replicate within, and kill cancers while sparing normal tissues
- Genetically Engineered Viruses
  - Adenovirus, Herpes simplex, Vaccinia, Newcastle Disease, Myxoma, Vesicular stomatitis, Measles, Reovirus



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Public and Media Fears

- A genetically re-engineered measles virus, originally created as a cure for cancer, turns into a lethal strain …. and mutates humans …. into predatory, nocturnal mutants

- Search of Zombie and Virus in the IMDB database yielded >50 movies

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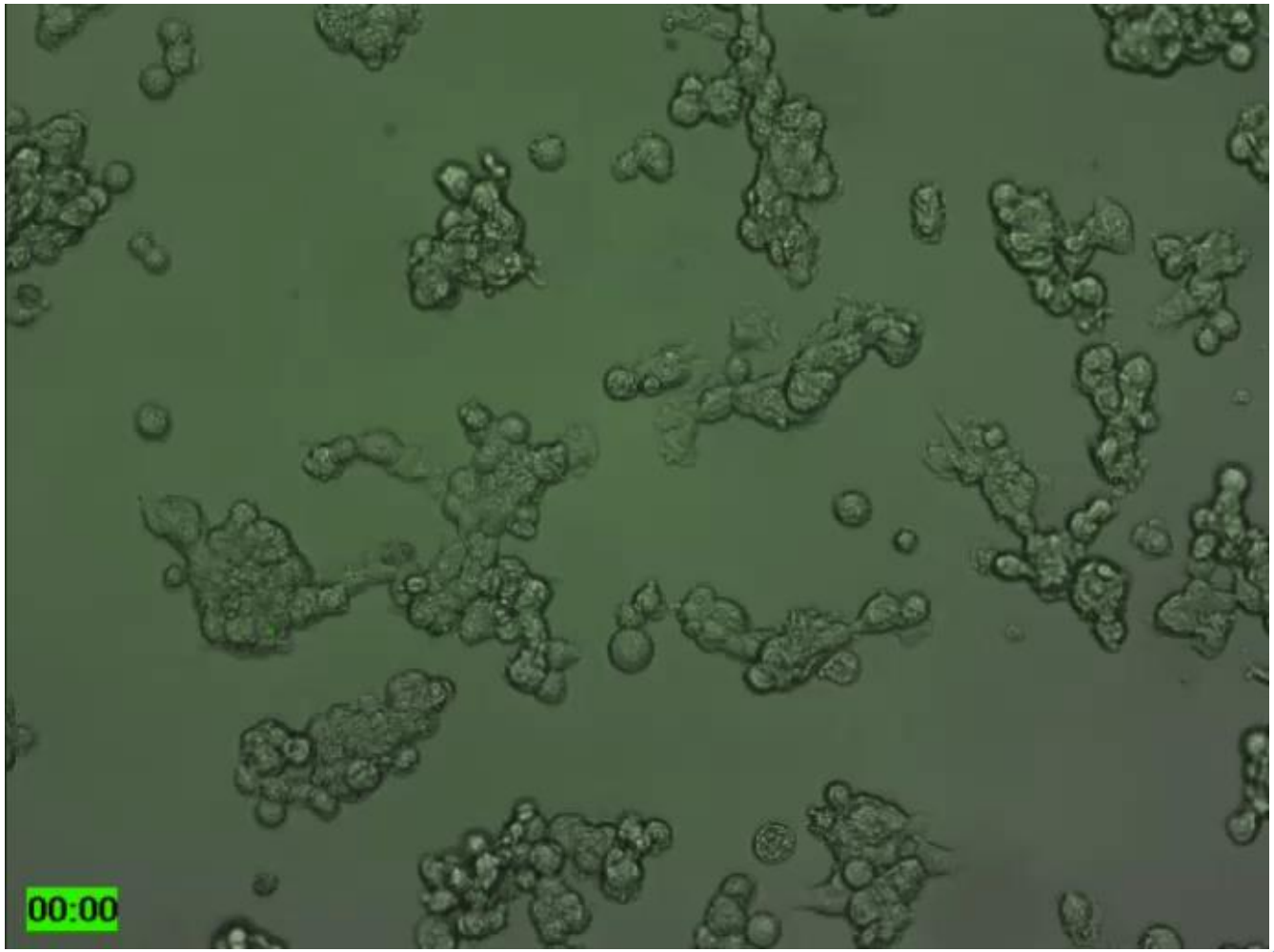
Infection and Killing of Resistant Cancers by Genetically Engineered Viruses



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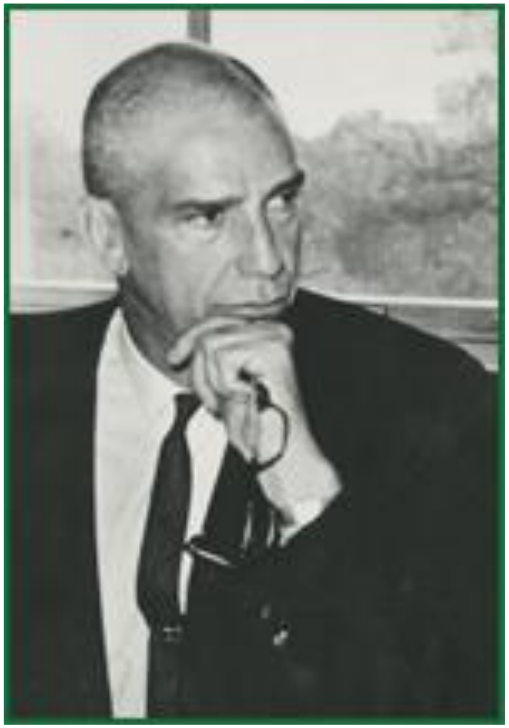
Natural Viruses as Treatment of Cancer



Chester M. Southam, MD
1919-2002
President, AACR 19

- West Nile Virus
- Measles Virus
- Ilheus Virus
- Russian Encephalitis Virus
- Newcastle Disease Virus
- Uganda S Virus
- Anopheles B Virus
- Bunyamwera Virus
- Theiler's G.D. VII Virus

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Natural Viruses for Treatment of Cancer

- Death of a child with cancer from the effects of measles treatment
  - Laski, B. JAMA 1973;225:1303
- Problems with natural viruses
  - Still too toxic for normal tissues
  - No treatments for toxiccities
- Challenge is to engineer viruses to be more toxic to tumor and less toxic to normal tissue

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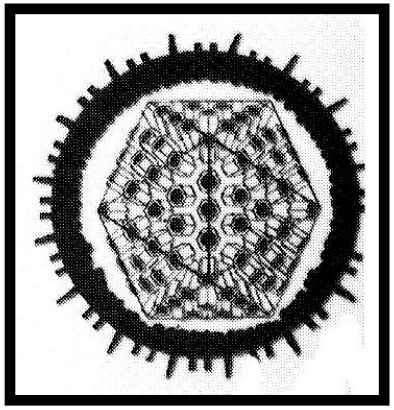
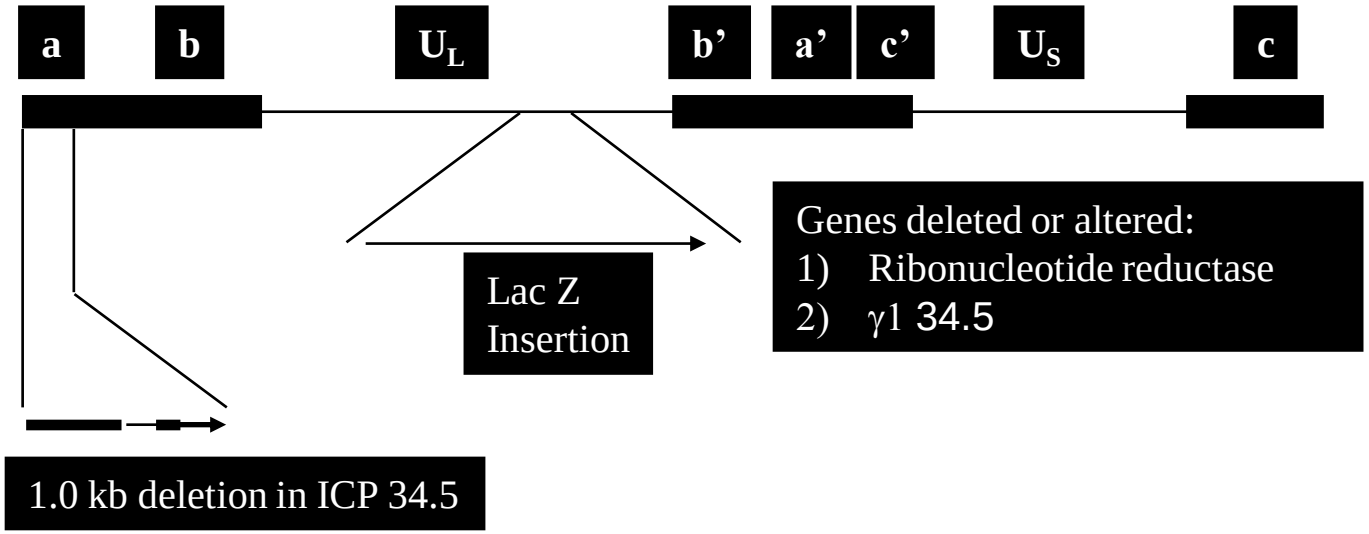
Herpes Simplex Virus



- DNA Virus
- 152 kbp
- Wild type virus is well tolerated
- Only 38 of 84 gene products are essential
- Acyclovir can treat severe infection



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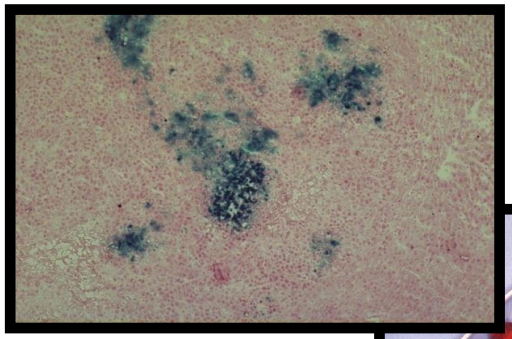
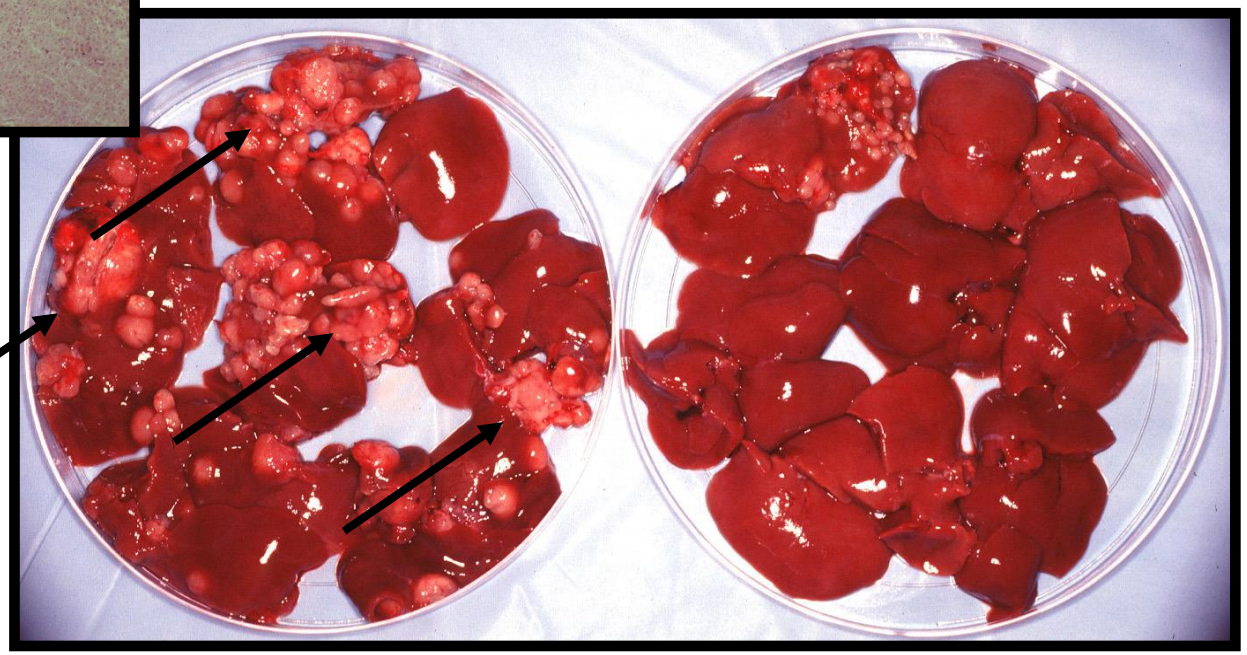
A Single Dose of Virus is Effective Treatment of Liver Metastases



Morris Hepatoma
Buffalo Rat Livers



Cancer

Controls **Treated**

Kooby and Fong., FASEB Journal, 1999

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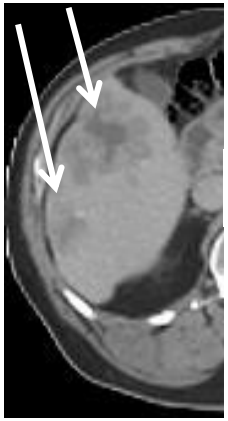
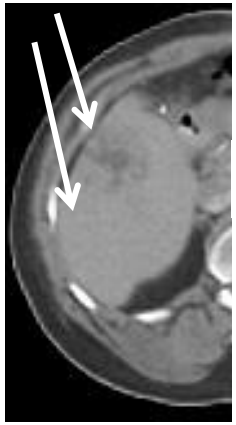
Single Dose of Virus Produced 50% Regression of Chemotherapy-resistant Cancer in Man (IND# 8447)

Before



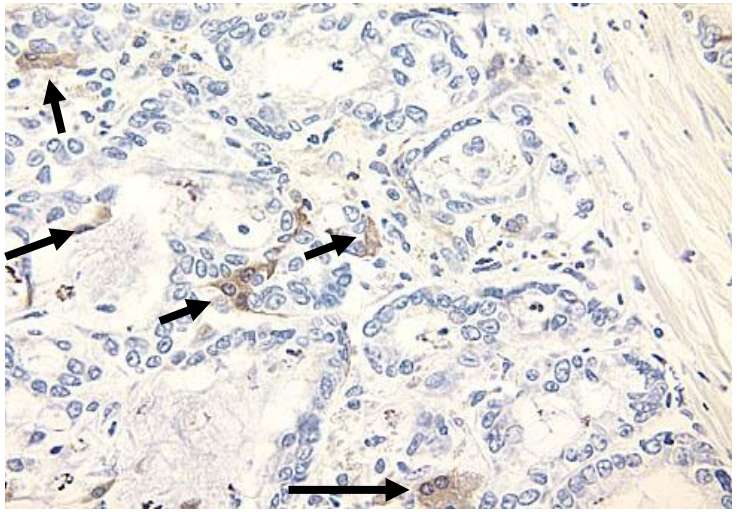
CEA = 825

1 Month After



CEA = 405

Virus detected in tumor biopsy



Fong et al., Molecular Therapy, 2008

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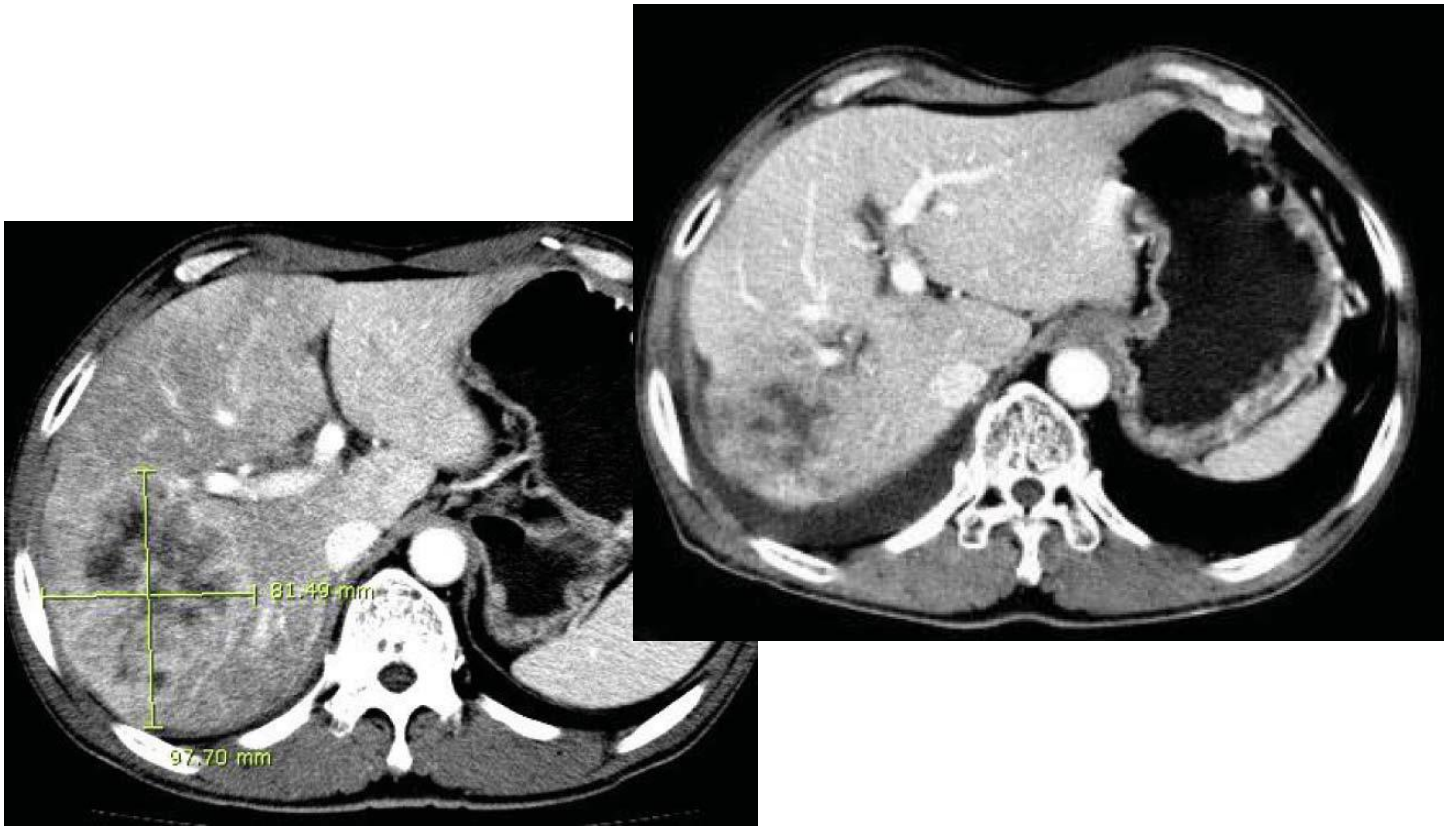
Repeated Administration for HCC



Park BH et al, Lancet Oncology, 2008

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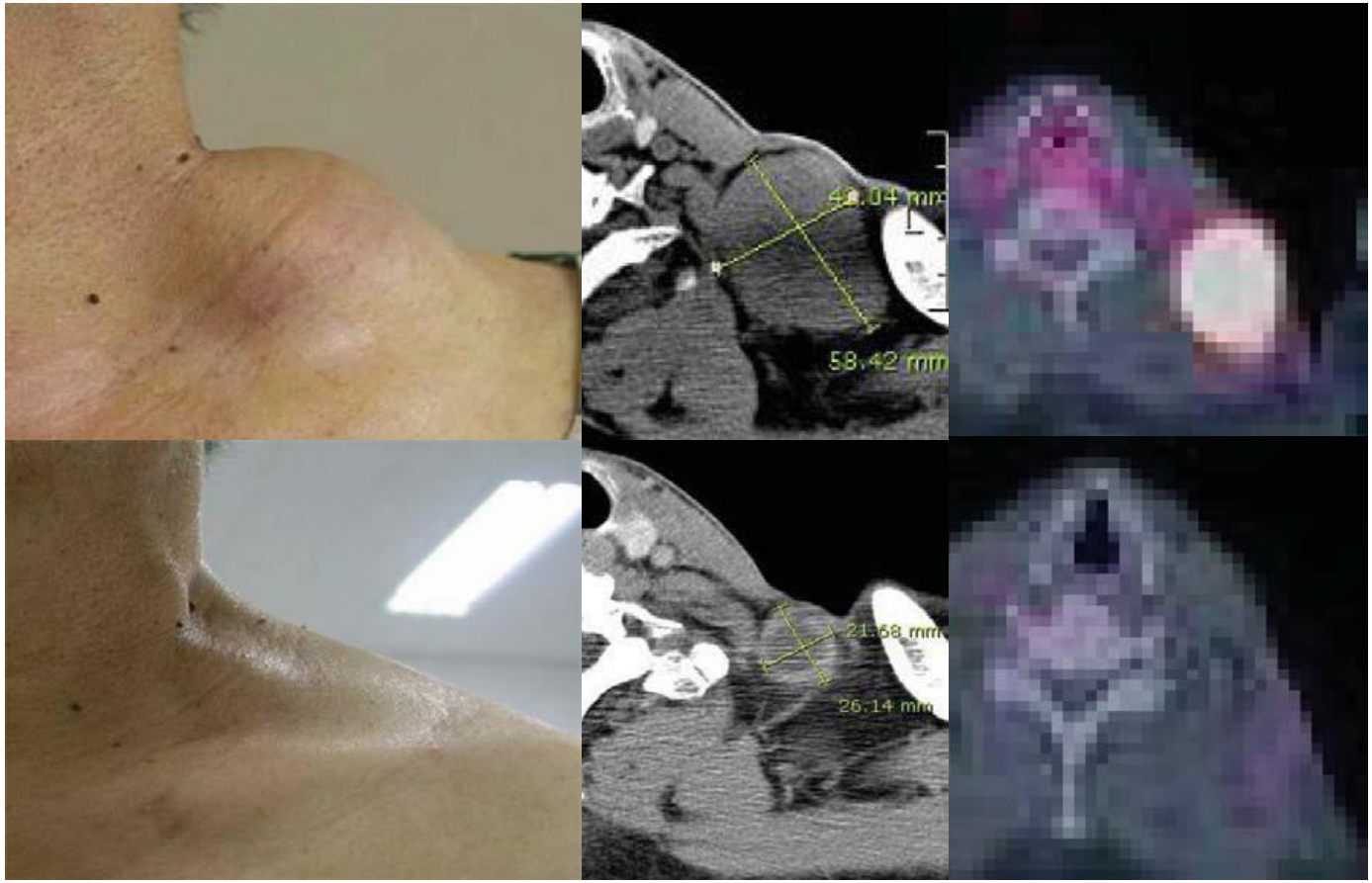
Repeated Administration for Melanoma Metastatic to Liver and Cervical LN



Park BH et al, Lancet Oncology, 2008

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Viruses Are Particular Effective for Targeting Chemotherapy-and Radiation-Resistant Tumors

- Chemo- and radio-resistant tumor cells are
  - Anti-apoptotic
  - Stem-cell like
  - Activated in DNA repair mechanisms
  - Hypoxic
- Tumor types
  - Pancreatic cancer, triple-negative breast cancer, HCC, poorly differentiated thyroid cancer, mesothelioma, cholangiocarcinomas

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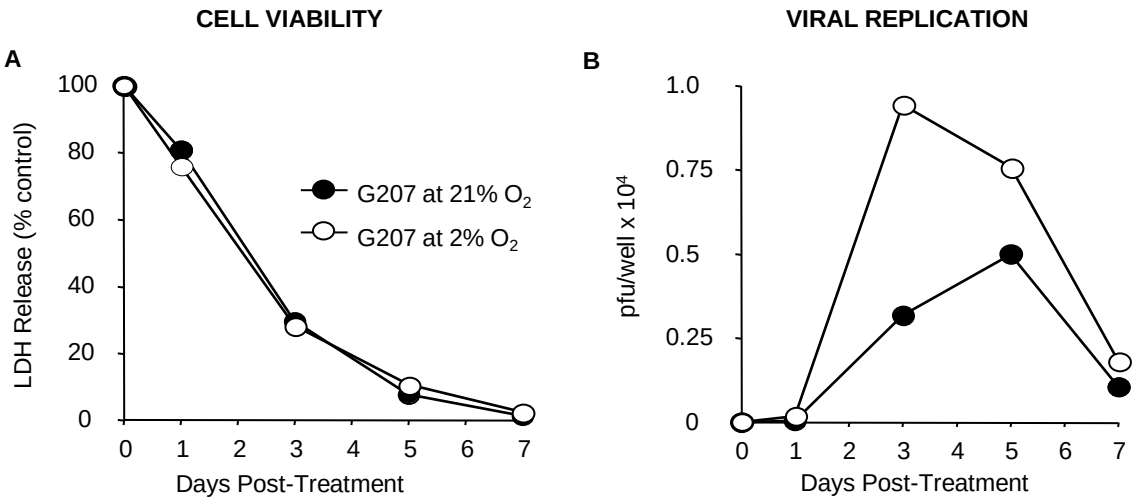
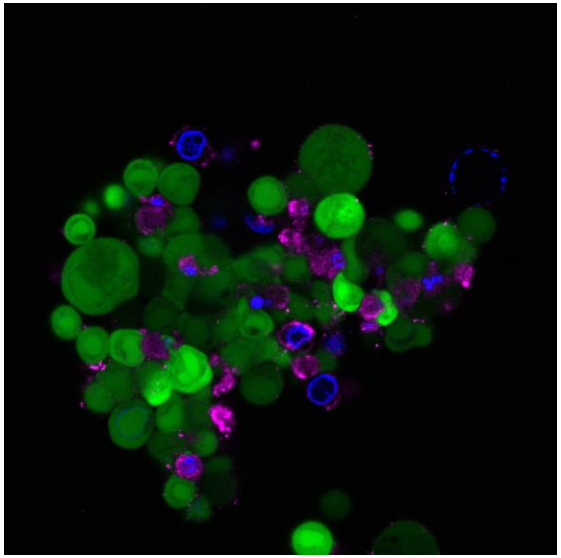
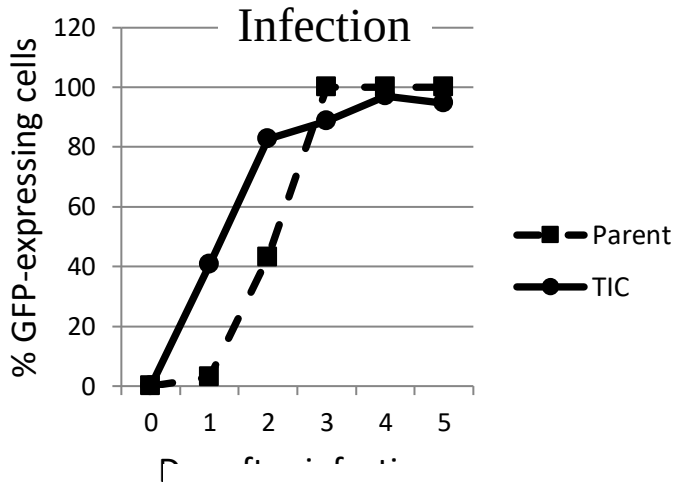
Viruses Can Kill Cancer and Replicate in Hypoxic Environment

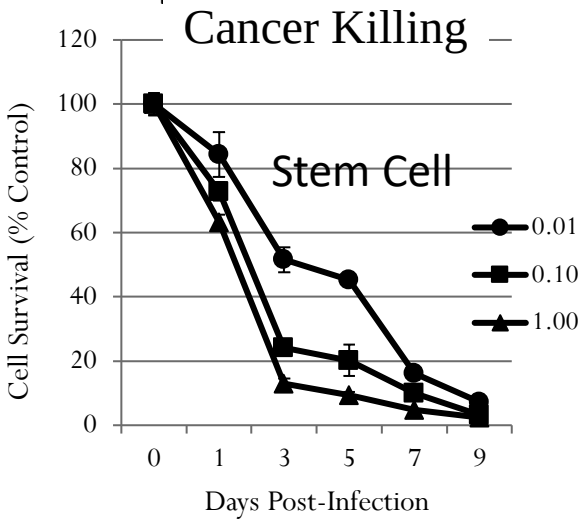


Infection, Viral Replication, and Killing of Cancer Stem Cells



Herpes Simplex NV1066 (F strain)
Human pancreatic Cancer
MiaPaca-2
CD133+CXCR4+





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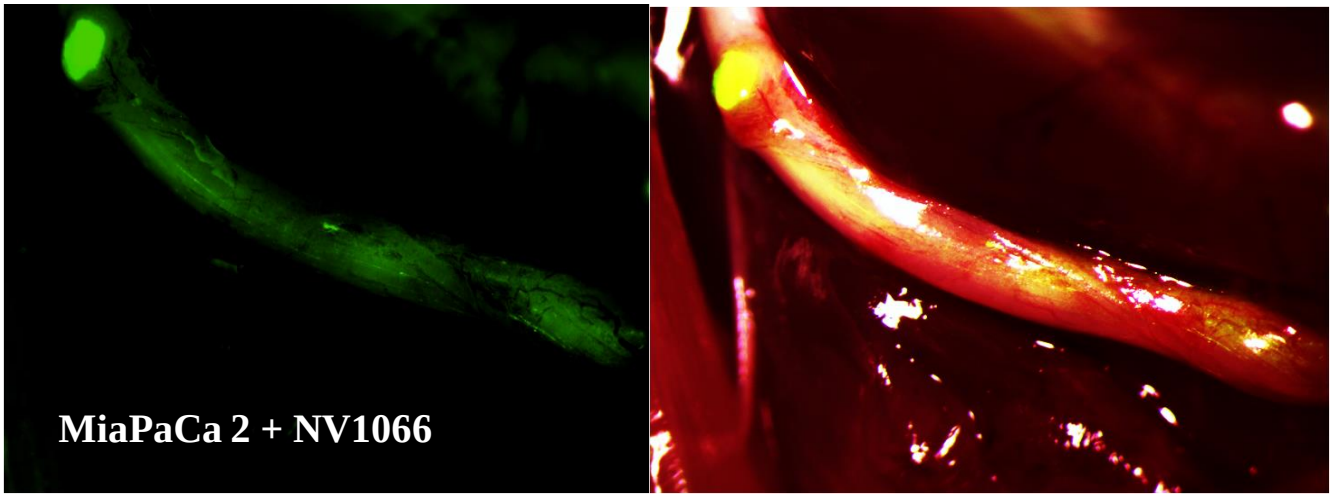
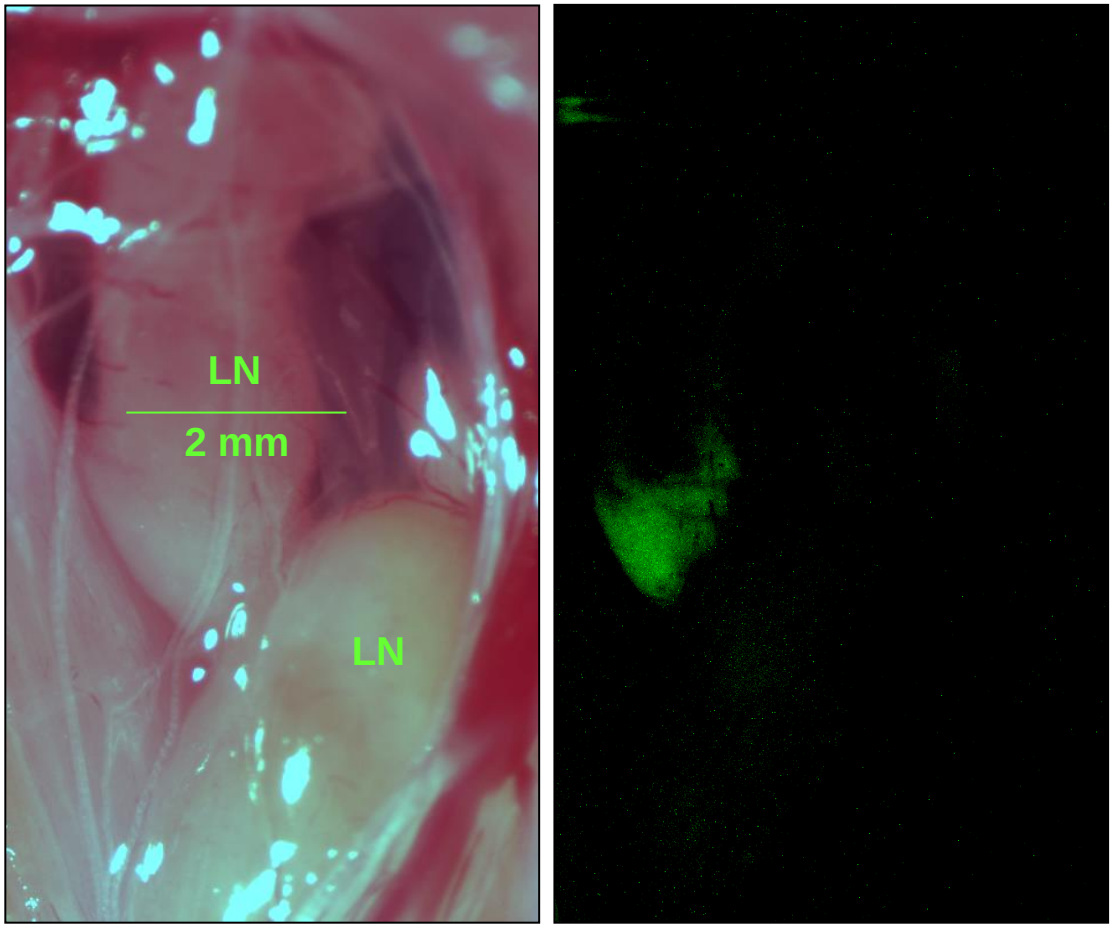
Virus Can Treat Peri-neural Invasion

- Cancers invade and travel within nerves
- Viruses can infect and kill tumor cells within nerves



©2015 GAC and COH; v.8.2.16

Virus Can Travel Along Lymph Vessels to Kill Cancer in Lymph Node



Viruses Can Deliver Other Payloads While Killing Cancer

- Immunologically modulatory genes
- Check-point inhibitors: anti-PD-1, PDL-1
- Immuno-stimulatory genes
- GM-CSF, IL-2, IL-12
- Anti-angiogenic genes
- Prodrug strategies
- Cytosine deaminase, thymidine kinase
- Cytotoxic genes
- Tumor necrosis factor
- Pre-differentiation genes: BMP-4

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T-Vec (Oncovec^{GM-CSF}) OPTiM Trial
Phase III : T-Vec intratumoral versus SQ GM-CSF

- Herpes simplex virus encoding hGM-CSF
- N=430
- Stage IIIB, IIIC, IV melanoma
- Response: T-vec: 33% vs sq GM-CSF: 2%
  - CR: 10.8% T-VEC, <1% GM-CSF
  - PR: 15.6% T-VEC, 5% GM-CSF
- Median OS: 23.3 months T-VEC, 18.9 months GM-CSF

- T-Vec was the first gene therapy approved in the US

Andtbacka et al.; J Clin Oncol. 2015;33:2780-2788.

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Imlygic (talimogene laherparepvec)

- ----------INDICATIONS AND USAGE----------
- IMLYGIC is a genetically modified oncolytic viral therapy indicated for the local treatment of unresectable cutaneous, subcutaneous, and nodal lesions in patients with melanoma recurrent after initial surgery.
- ----------DOSAGE AND ADMINISTRATION----------
- Administer IMLYGIC by injection into cutaneous, subcutaneous, and/or nodal lesions.
- Recommended starting dose is up to a maximum of 4 mL of IMLYGIC at a concentration of 10^6 (1 million) plaque-forming units (PFU) per mL. Subsequent doses should be administered up to 4 mL of IMLYGIC at a concentration of 10^8 (100 million) PFU per mL.

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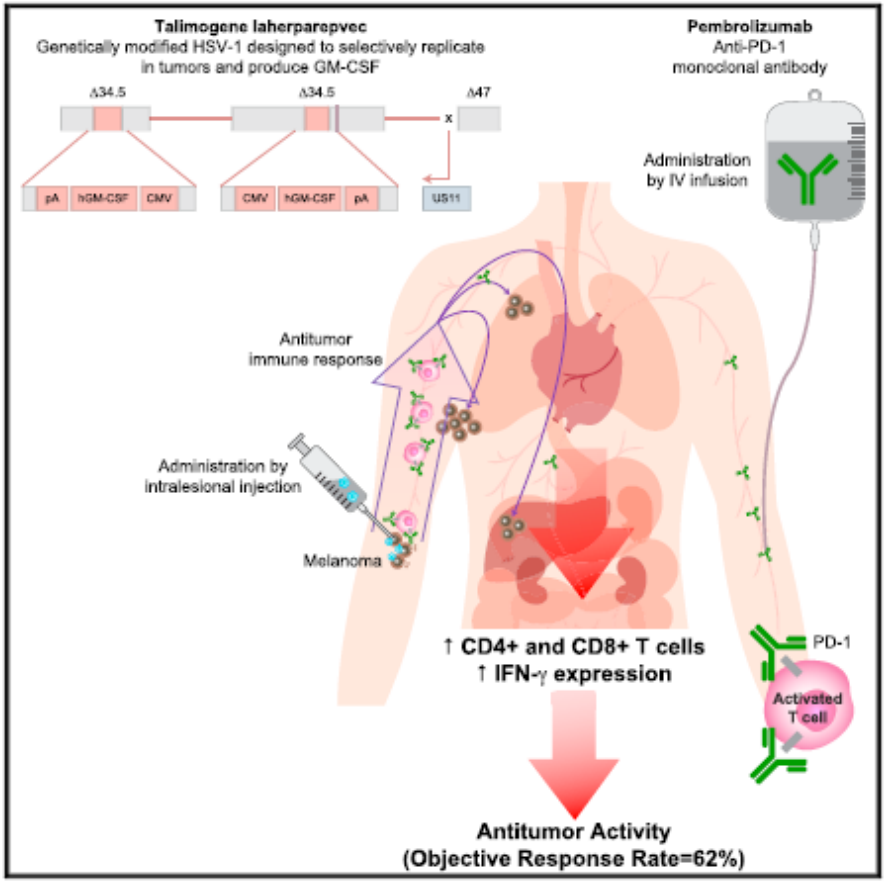
Oncolytic Virotherapy Promotes Intratumoral T Cell Infiltration and Improves Anti-PD-1 Immunotherapy

- Phase 1B Trial
- N=21
- Advanced melanoma (IIIB, V)
- Tvec + Pembrolizumab (200 mg q2W)
- 62% response; 14% stable



Ribas et al., Cell 170:1109, 2017

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Current Status of the OV Field
Oncolytic Virus Clinical Trials in Combination with CPI's

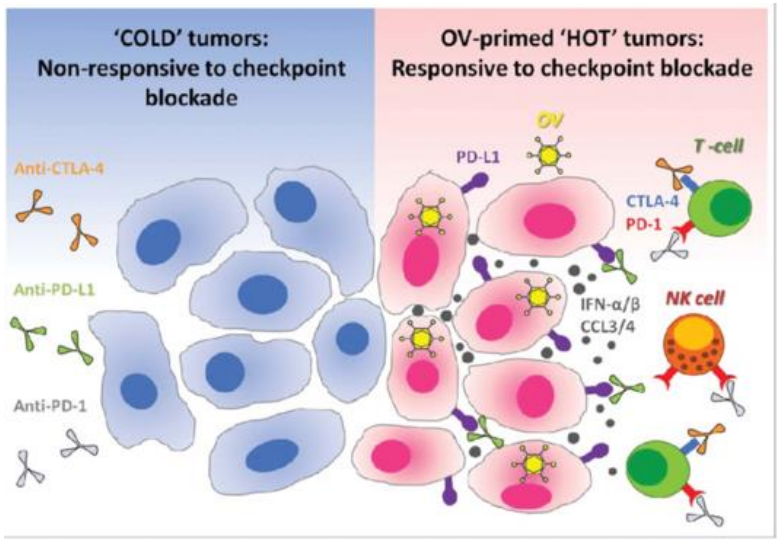

Table 3 | **Clinical trials of OV combined with ICBs**

OV	Checkpoint inhibitor	Indication	N	Response data	ClinicalTrials.gov identifier
T-VEC	Ipilimumab	Melanoma[154]	198	ORR 39% (T-VEC + ipi) versus 18% (ipi); $P = 0.002$	NCT01740297
T-VEC	Pembrolizumab	Stage IIIB–IV melanoma[155]	21	48% ORR	NCT02263508
T-VEC	Pembrolizumab	Stage III–IV melanoma	64[a]	N/A	NCT02965716
T-VEC	Pembrolizumab	HNSCC[87]	36	ORR 16.7% and disease control rate 38.9%	NCT02626000
T-VEC	Pembrolizumab	Sarcoma	26[a]	N/A	NCT03069378
T-VEC	Pembrolizumab	HCC, liver metastases	244[a]	N/A	NCT02509507
T-VEC	Nivolumab	Lymphoma and non-melanoma skin cancers	68[a]	N/A	NCT02978625
T-VEC	Atezolizumab	TNBC, CRC	36[a]	N/A	NCT03256344
HF10	Ipilimumab	Melanoma	28	N/A	NCT03153085
HF10	Ipilimumab	Melanoma[217]	46	BORR at 24 weeks 41%; median PFS 19 months; median OS 21.8 months	NCT02272855
HF10	Nivolumab	Stage IIIB, IIIC and IVM1a melanoma	20[a]	N/A	NCT03259425
Pexa-Vec	IT ipilimumab	Advanced-stage solid tumours	60[a]	N/A	NCT02977156
Pexa-Vec (IV)	Durvalumab/tremelimumab	CRC	35[a]	N/A	NCT03206073
Pexa-Vec (IV/IT)	REGN2810	RCC	89[a]	N/A	NCT03294083
Pexa-Vec	Nivolumab	First-line HCC	30[a]	N/A	NCT03071094
Enadenotucirev	Nivolumab	Metastatic or advanced-stage epithelial tumours (CRC, bladder, HNSCC, salivary gland cancer)	30[a]	N/A	NCT02636036
DNX-2401	Pembrolizumab	Glioblastoma, gliosarcoma	48[a]	N/A	NCT02798406
ADV/HSV-tk	Pembrolizumab	TNBC, NSCLC	57[a]	N/A	NCT03004183
CAVATAK	Ipilimumab	Uveal melanoma with liver metastases	10[a]	N/A	NCT03408587
CAVATAK	Pembrolizumab	Melanoma	50[a]	N/A	NCT02565992
CAVATAK	Pembrolizumab	NSCLC and bladder cancer	90	N/A	NCT02043665
MG1-MAGEA3 + Ad MAGEA3	Pembrolizumab	NSCLC	61	N/A	NCT02879760
Reolysin	Pembrolizumab	Pancreatic cancer adenocarcinoma	11	N/A	NCT02620423
VSV-IFNβ-NIS	Pembrolizumab	NSCLC and HCC	23[a]	N/A	NCT03647163
ONCOS-102	Pembrolizumab	Advanced or unresectable melanoma	12[a]	N/A	NCT03003676

Ad MAGEA3, adenovirus vaccine expressing melanoma-associated antigen A3; ADV/HSV-tk, adenovirus-mediated expression of herpes simplex virus by thymide kinase; BORR, best objective response rate; CRC, colorectal cancer; HCC, hepatocellular carcinoma; HNSCC, head and neck squamous cell carcinoma; ICB, immune checkpoint blocker; ipi, ipilimumab; IT, intratumoural; IV, intravenous; MG1-MAGEA3, Maraba virus expressing melanoma-associated antigen A3; N/A, not available; NSCLC, non-small cell lung cancer; ORR, objective response rate; OS, overall survival; OV, oncolytic virus; PFS, progression-free survival; RCC, renal cell carcinoma; TNBC, triple-negative breast cancer; T-VEC, talimogene laherparepvec; VSV-IFNβ-NIS, vesicular stomatitis virus encoding the interferon-β transgene and sodium–iodide symporter. [a]Estimated enrolment.

Kevin Harrington et al; 2019 Nature Review
Pic taken from: Article in OncoImmunology · February 2018

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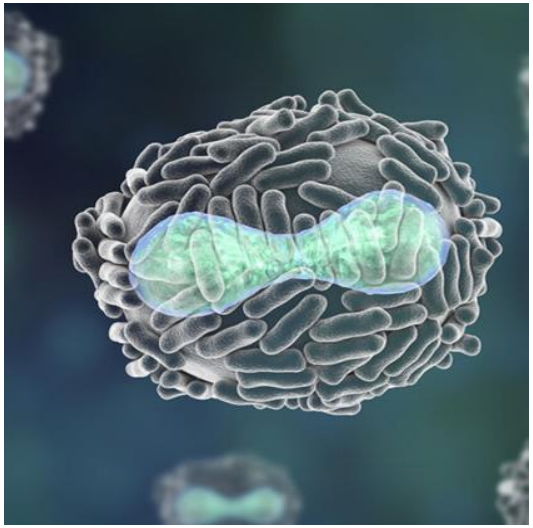
Lessons Learned

- Last generation OV too attenuated
  - Poor efficacy
  - High cost
- Most OV under clinical investigation are running out of IP
  - Field moved too slowly
- OV synergistic with other cancer therapies
  - ICI, radiotherapies, cell therapies
- Opportunity
  - Novel (IP), more potent OV, in combination studies with other immunotherapies

Company	Country	Viral Backbone	Lead Candidate	Development Status	
BioVex (Amgen)	USA	HSV-1	Talimogene laherparepvec (T-Vec)	Approved	
DNAtrix	USA	Adenovirus	DNX-2401	I	
Genelux Corporation	USA	Vaccinia	GL-ONC1 (GLV-1h68)	I/II	
Jennerex Biotherapeutics	USA	Vaccinia	Pexa-Vec (JX-594)	II	
Oncolys BioPharma, Inc.	Japan	Adenovirus	Telomelysin® (OBP-301)	I	
Oncolytics Biotech	Canada	Reovirus	REOLYSIN®	III	
Oncos Therapeutics Ltd.	Finland	Adenovirus	CGTG-102	I	
PsiOxus Therapeutics Ltd	UK	Adenovirus	ColoAd1	I/II	
Shanghai Sunway Biotech Co., Ltd	China	Adenovirus	Oncorine	Approved	Privately Held
VCN Biosciences SL	Spain	Adenovirus	VCN-01	Preclinical	Privately Held
Viralytics Limited	Australia	Coxsackie A21	CAVATAK™	II	Public
VIRTTU Biologics Ltd.	UK	HSV-1	SEPREHVIR®	I	Privately Held

WHY A VACCINIA VIRUS?



- Genus Orthopoxvirus in the family Poxviridae
- Large ds DNA virus, genetically very stable
- Most successful biologic therapy: eradicated smallpox
- 1st oncolytic virus demonstrating viral oncolysis (1922)
- Short, well characterized life cycle with rapid cell to cell spread
- Cytolytic for a broad range of tumor cell types
- Large insertion capacity (> 25 kb) for exogenous genes
- Amenable to large scale production
- Does not integrate into the host genome
- May be administered via intratumoral and intravenous routes

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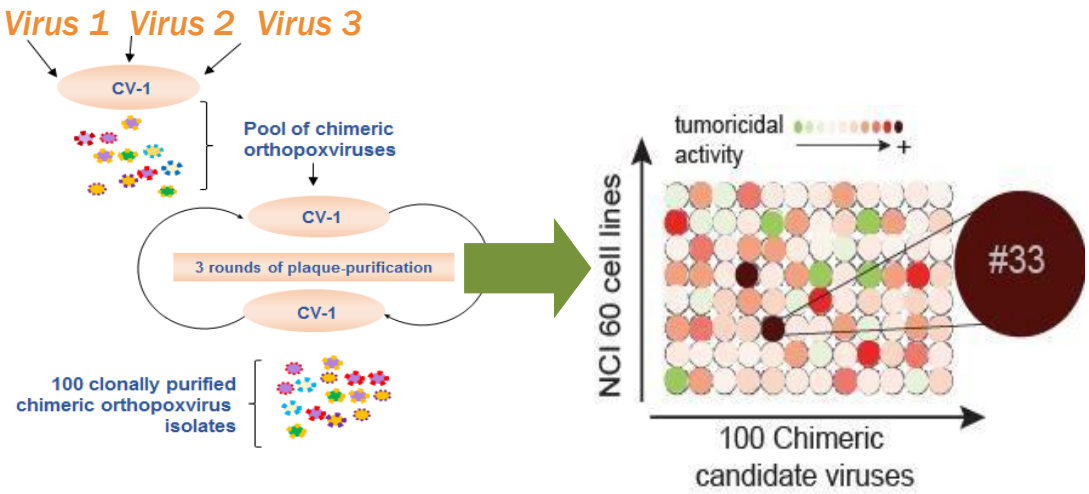
Generation And Evaluation Of Novel Chimeric Poxviruses



- 200 new backbones (new species)
- High through-put screening for cancer killing in the NCI-60 cell lines
- Arming with transgenes

STRATEGY

Engineer Novel Chimeric Viruses

High Through-put Screening for Efficacy Against NCI60

Safe in Animals

Arming with Additional Payloads

Hope Oncolytic Viruses (HOV)

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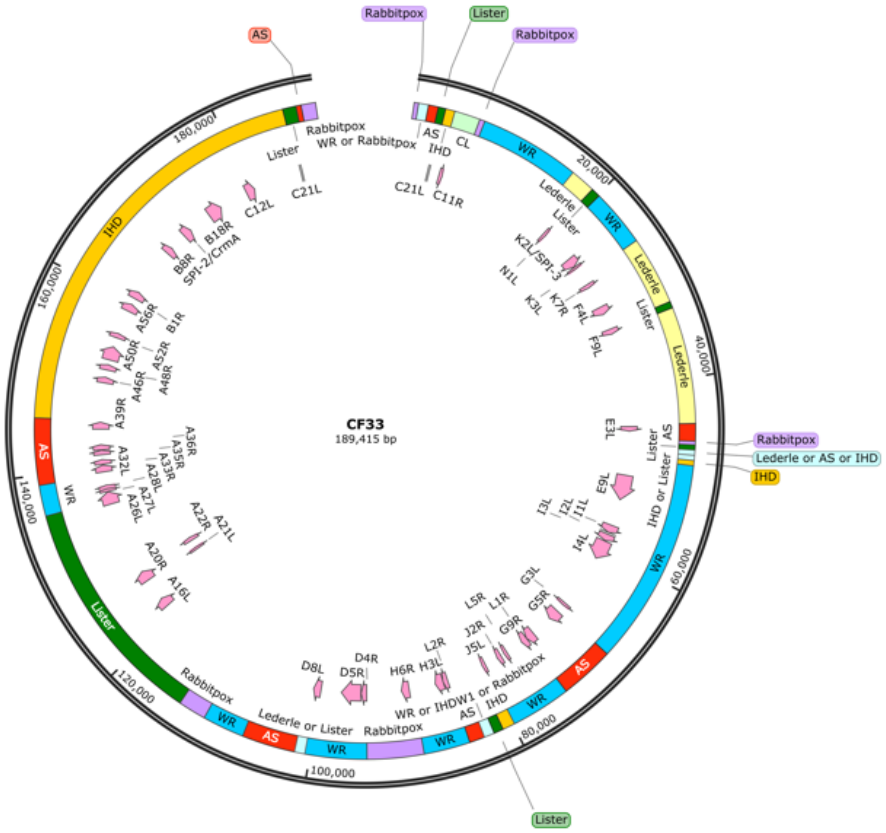
CF33 Genome & Derivatives



- Fully sequenced (no cowpox or raccoonpox)
- Genomic structure is unique and IP protection has been filed

  Remarkable that essential genes only appear once, including J2R (Thymidine Kinase, TK

FOUNDATION PATENT (2037)

PCT: US2017/046163

Title: Chimeric poxvirus compositions & use thereof

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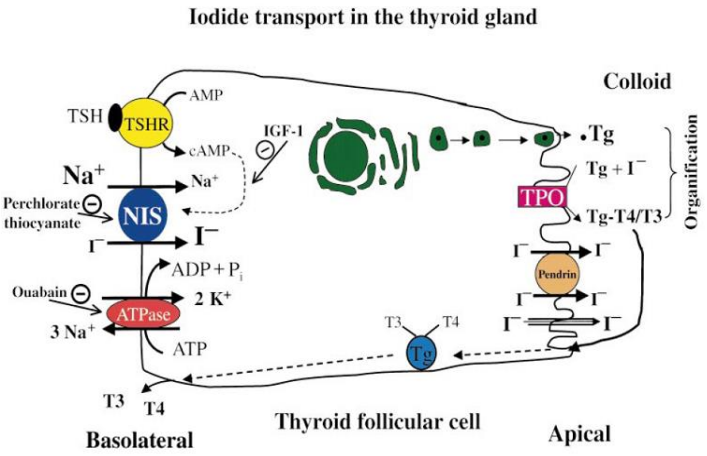
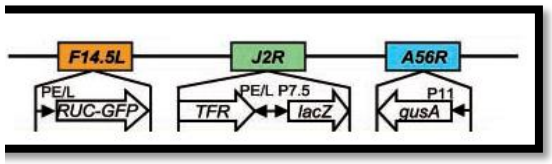
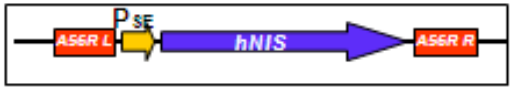
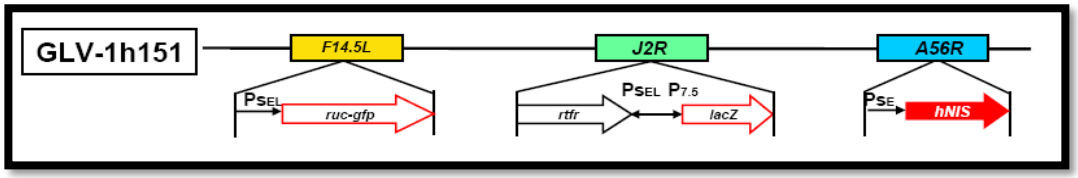
Vaccinia-hNIS Construct









Radioisotope	Half-Life	Therapeutic Emissions	Photon Energy (keV) and Abundance (%)
^{131}-Iodine	8 d	β^-, E_{av} = 0.134 MeV	364 (81)
^{99m}-Technetium	6 h	none	140 (89)
^{188}-Rhenium	16.9 h	β^-, E_{av} = 0.764 MeV	155 (15)
^{211}-Astatine	7.2 h	α, E = 5.87 MeV	79 (21)

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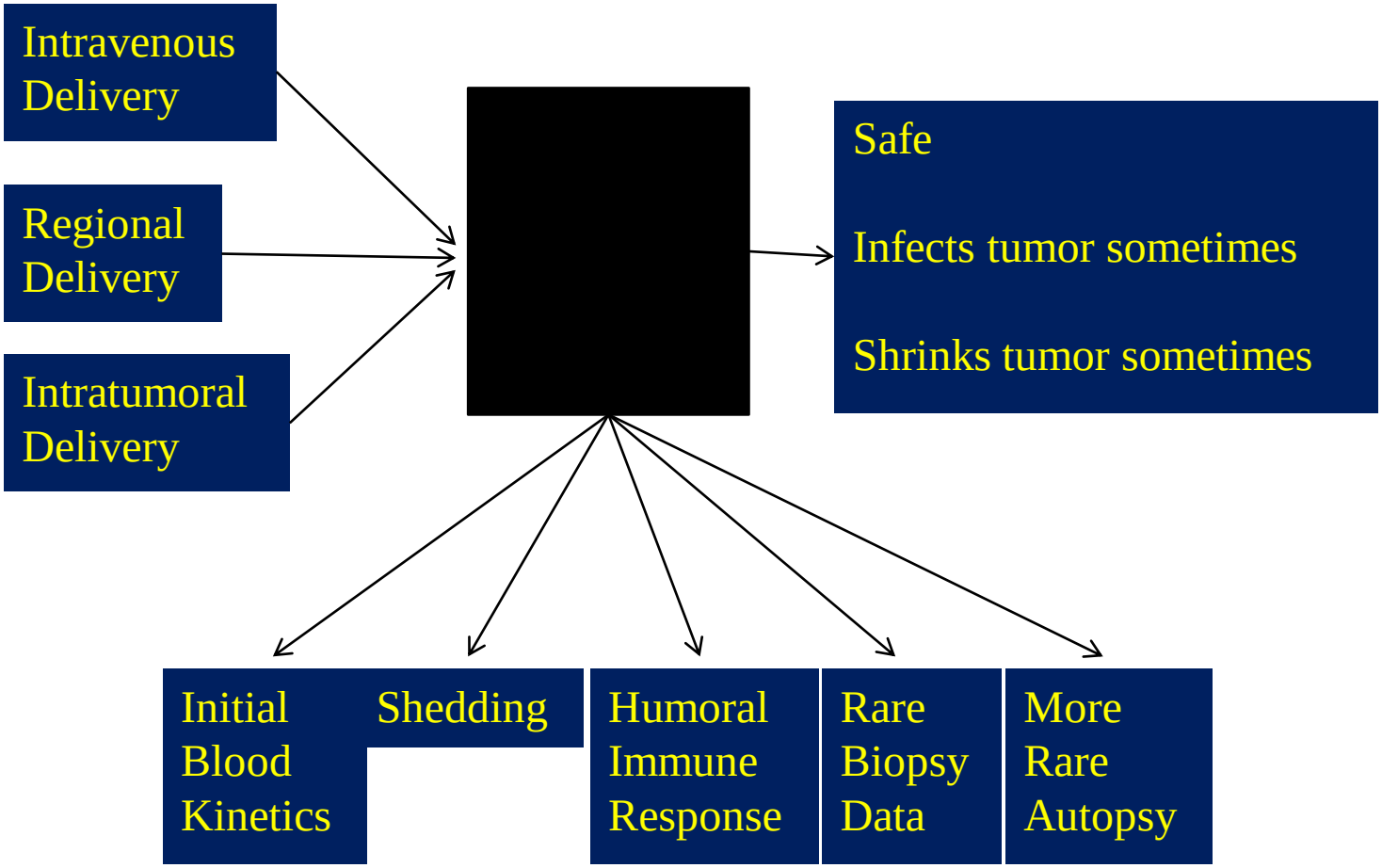
Trials in Oncolytic Viral Therapy



Challenge for Field of Gene/ Novel Therapy

- Tracking of viruses and vectors
- For proof of targeting
- Correlation with treatment response and toxicity
- What is the biodistribution of various virus?
- What is biodistribution according to route of delivery?
- Did immune response relate to biodistribution?
- Did virus replicate?
- Did virus persist in tumor?
- Did virus persist in normal organs?

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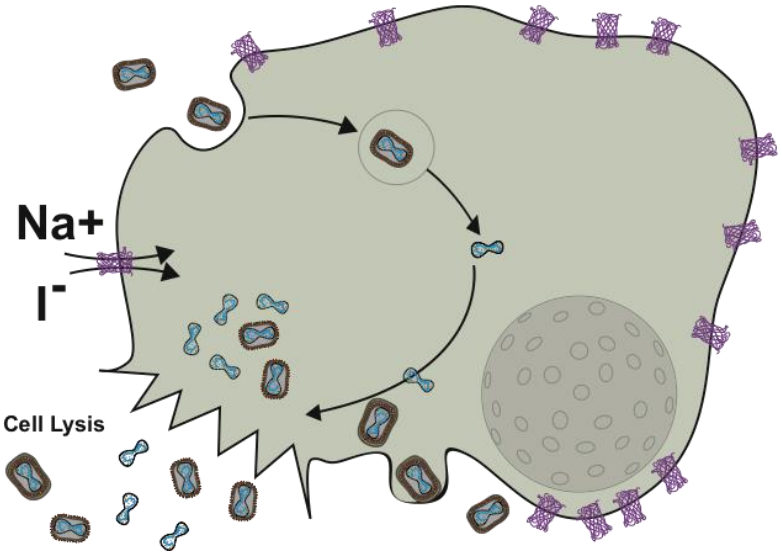
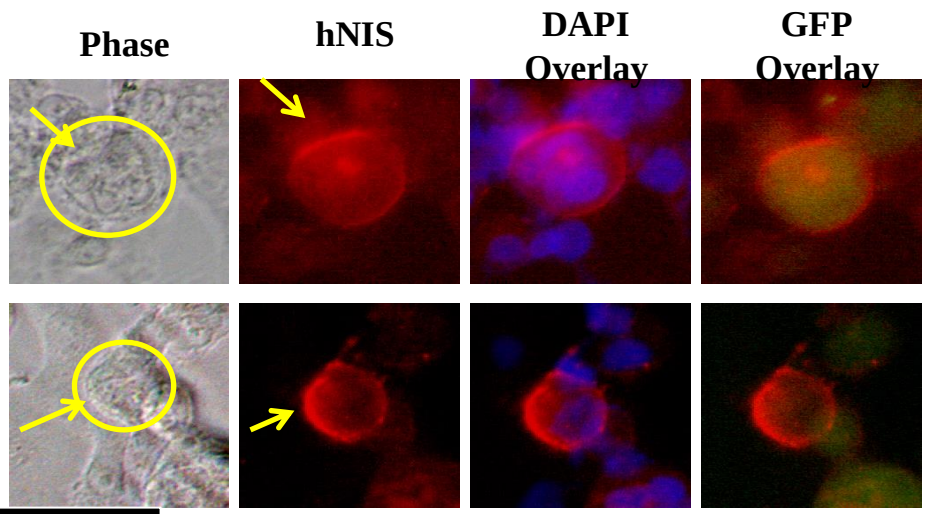
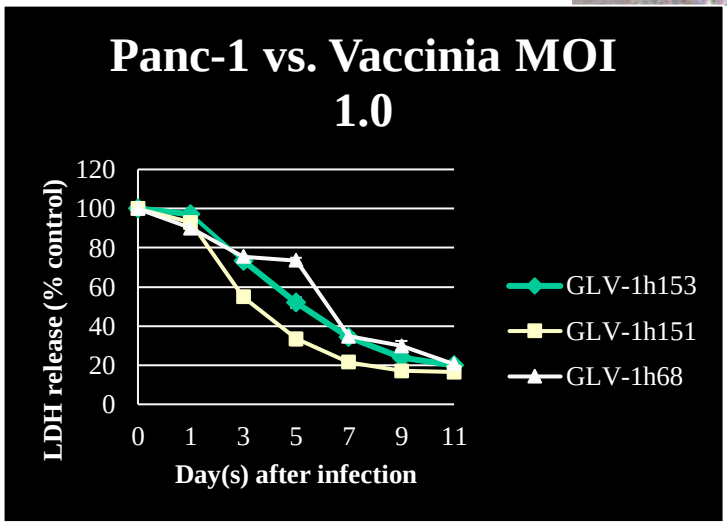
Oncolytic Virus Can Insert the Human Sodium Iodine Symporter onto Cancer Cells







Haddad et al.,
J Transl Med. 31;9:36, 2011

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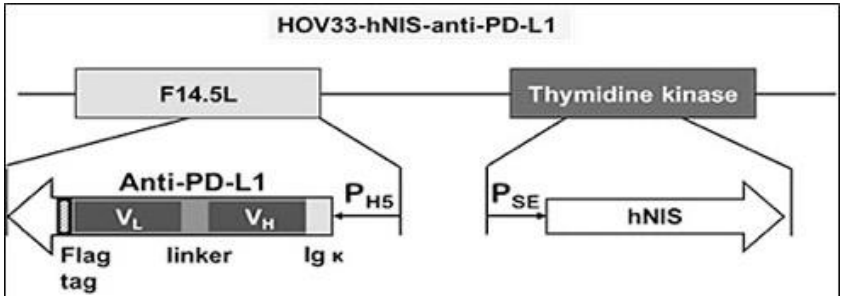
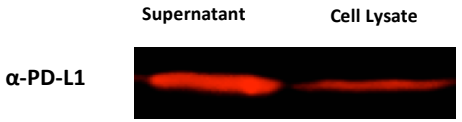
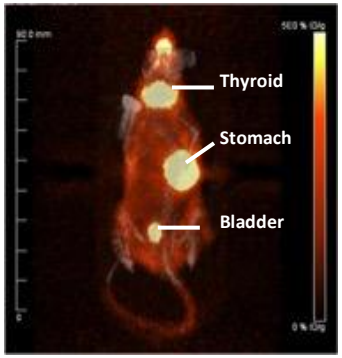
CHECKvacc: CF33-hNIS-αPD-L1



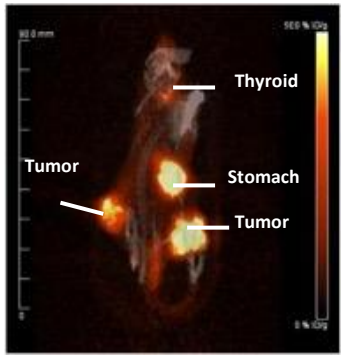


Cells infected with CHECKvacc produce High-levels of anti-PD-L1

Combines PET imaging, targeted radiotherapy, and local immune checkpoint blockade into one virus



PBS



CHECKvacc

- hNIS transgene inserted within J2R locus (Tk) to transport radioactive iodine, technetium, rhenium, and astatine for imaging or therapy
- Local secretion of immunotherapy
- Closest to tumor antigens
- Least chance of systemic autoimmune toxicity

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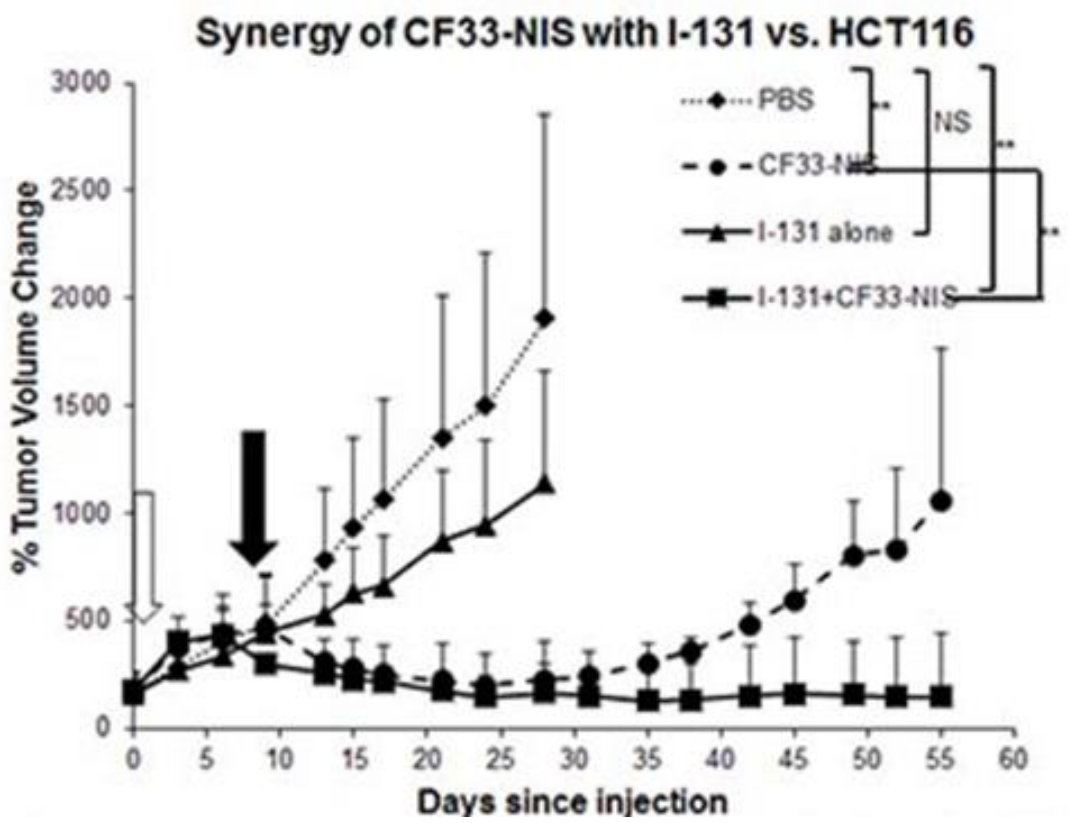
CF33-hNIS-αPD-L1 Synergizes with ^{131}I

- hNIS allows thyroid cancer to be highly treatable even when metastatic
- hNIS transgene allows transport of radioactive iodine for potential treatment



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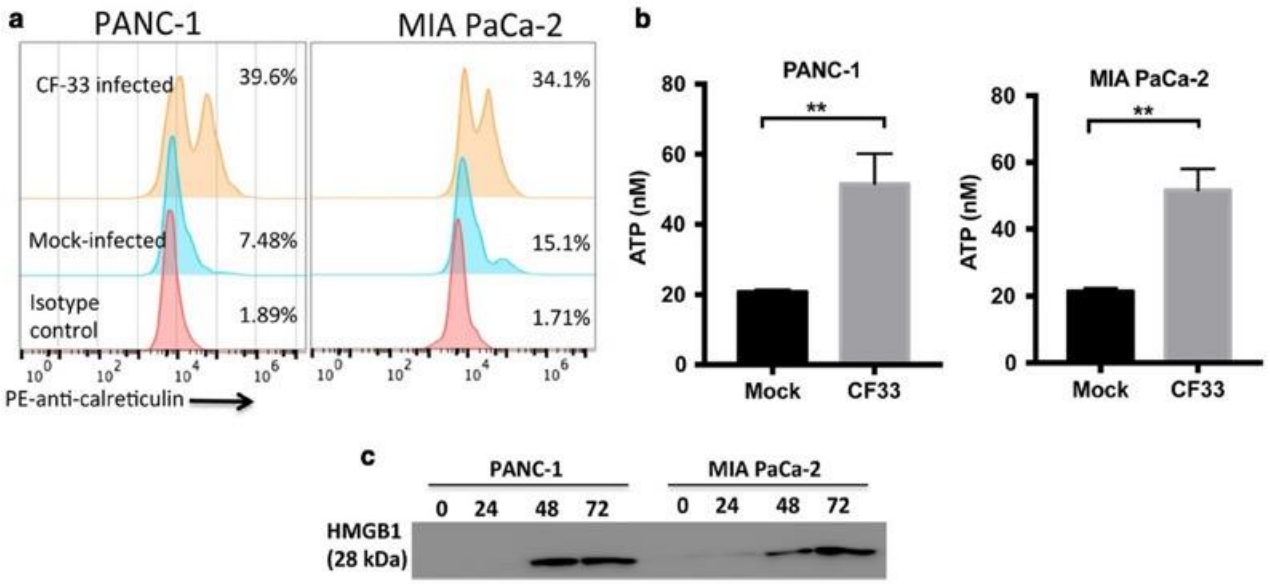
CF33 Induces Immunogenic Cell Death in Many Cancers



- PANC-1 and MIA PaCa-2 cells were mock-infected or infected with CF33 (MOI=5)
- Cell surface-exposed CRT was quantified by flow cytometry (a)
- Release of ATP was measured using ATP assays (b)
- The release of HMGB1 was analyzed by Western blot (c) **p < 0.01

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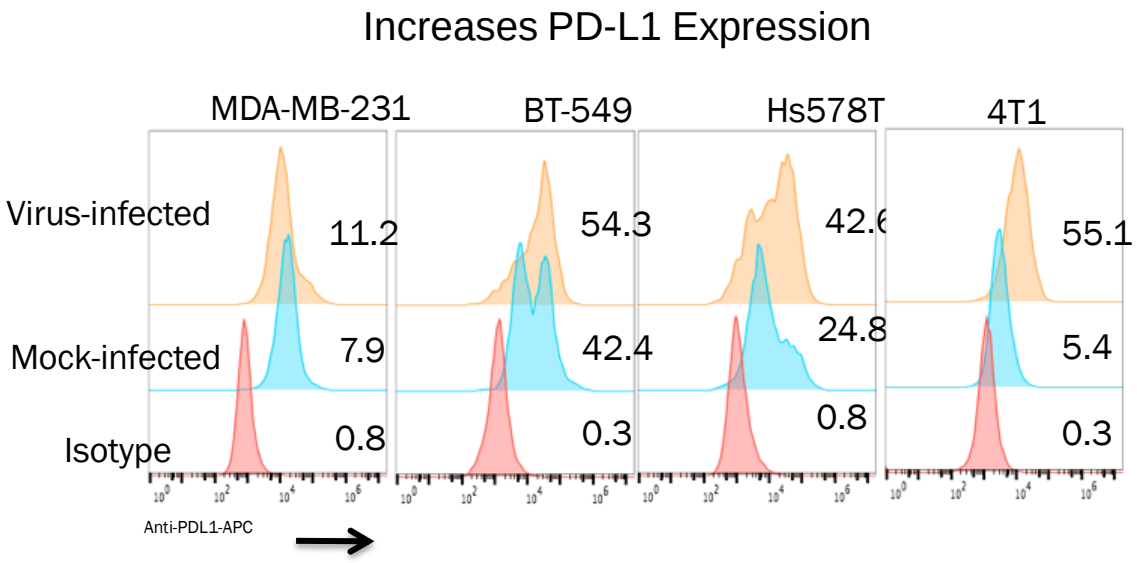
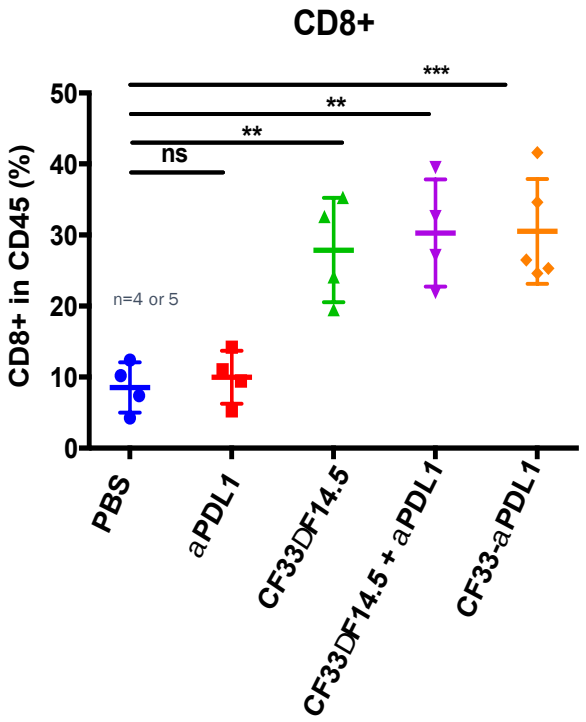
CF33 Upregulates PD-L1 Expression & Increases Infiltration by CD8+ T-cell





Chaurasiy et al., ASGCT 2019

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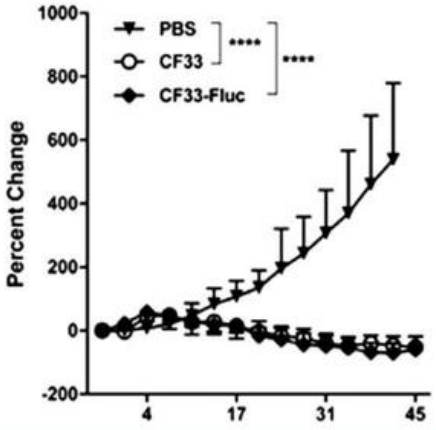
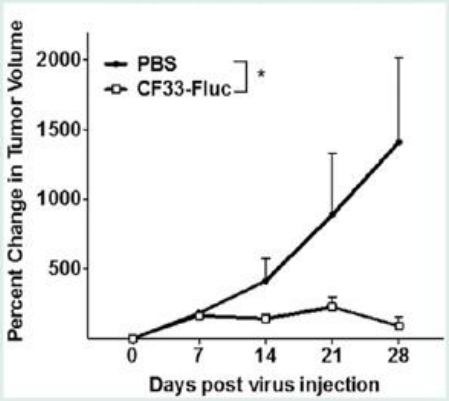
CF33 Kills Many Tumor Types at Low Doses

PANCREATIC



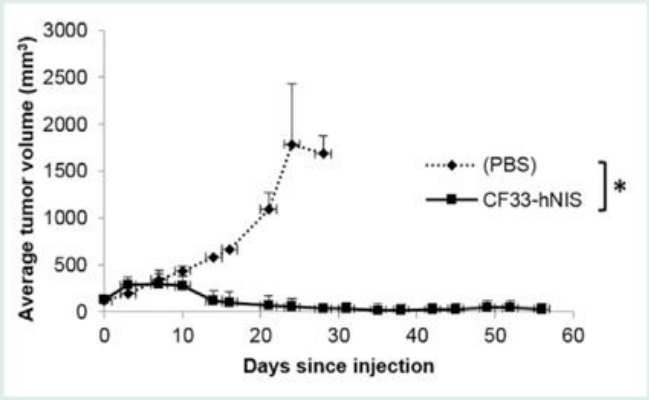
J Transl Med. 2018, 16, 110

COLORECTAL



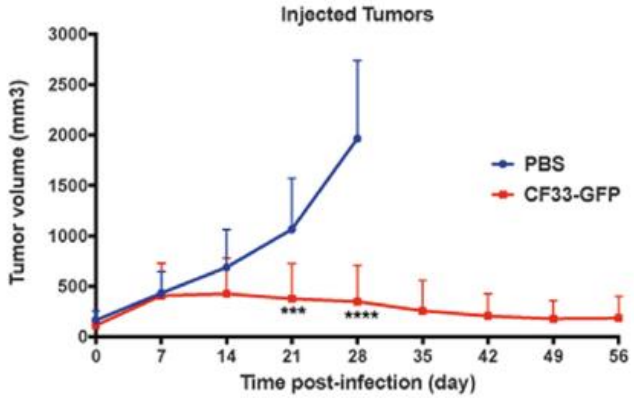
Mol Ther Oncolytics. 2018, 9, 13

COLON



Mol Ther Oncolytics. 2019, 13, 82

LUNG



Cancer Gene Ther. 2019

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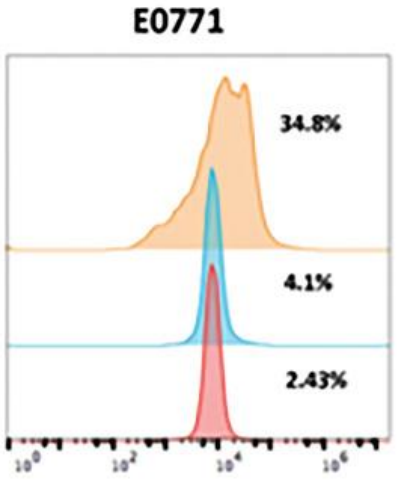
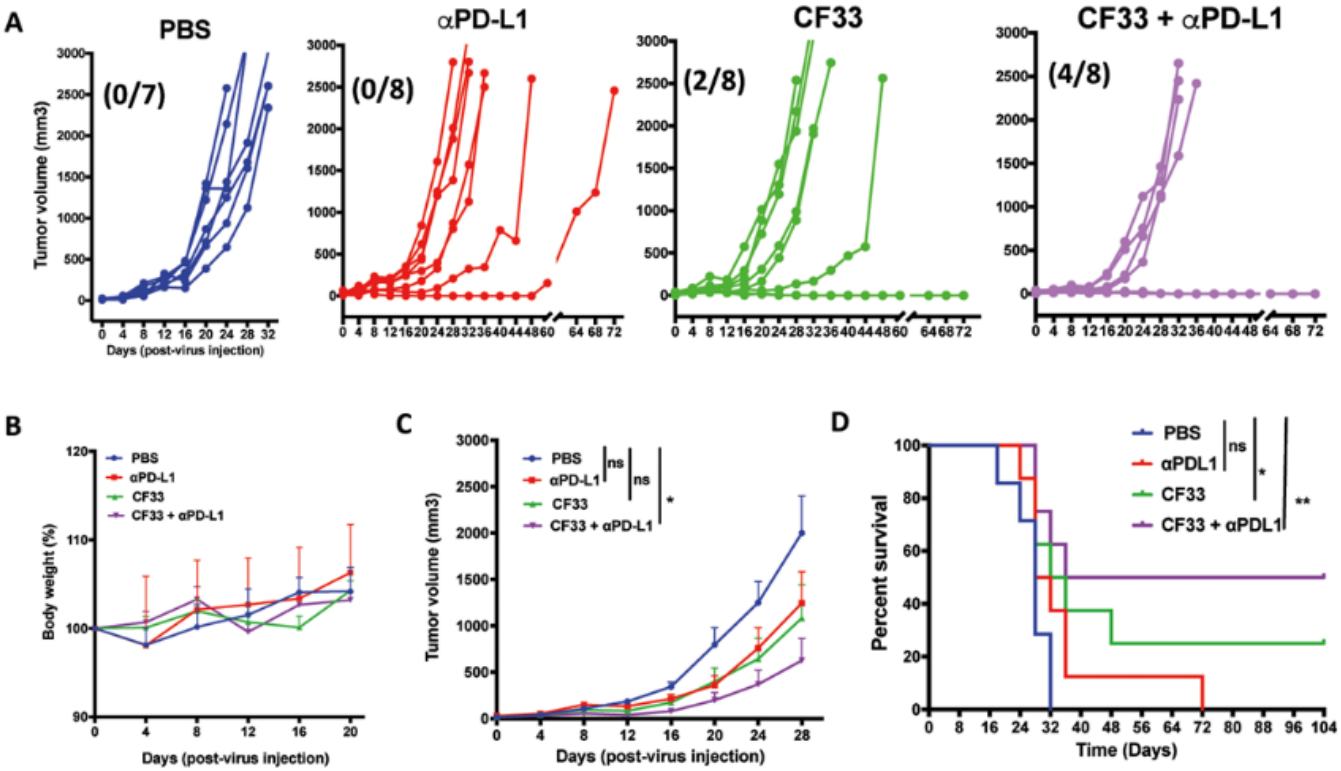
CF33-hNIS + Anti-PD-L1 Synergizes for Tumor Killing Breast Cancer

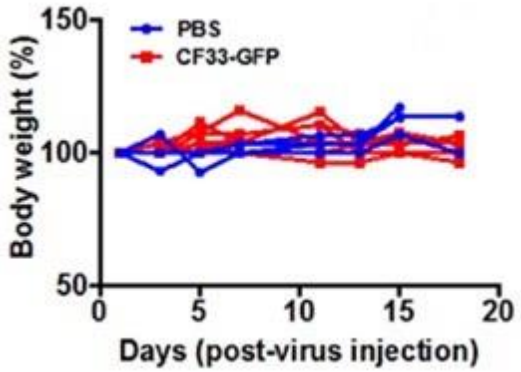
- E0771 TNBC in C57BL/6 mice
- 100 µg anti-PD-L1 (Bio X)

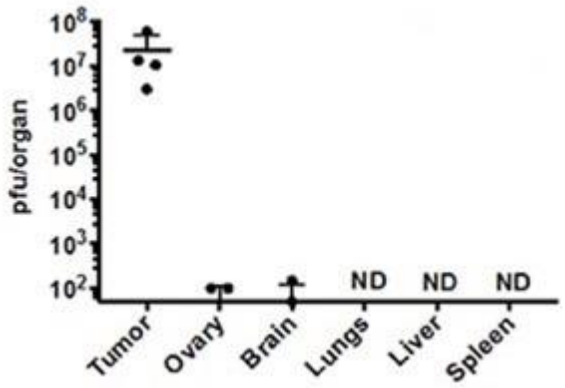




CF33 Can be Safely Delivered IT, IP, IV with Large Therapeutic Index

- In many tumor models, animals cured with a single injection of 1000 pfu
- NO TOXICITY UNTIL OVER 10^9
- Virus restricted to tumor





Virus	Mouse	# of Mice	Dose	Delivery	Toxicity
CF33-NIS	Nude	73	1e3-1e5	IT	No findings
CF33-miR	Nude	41	1e3-1e5	IT	No findings
CF33-Luc	Nude NSG	48 8	1e3-2e5 1e6	IT, IV & IP IT	No findings
CF33-GFP	Nude NSG	18 8	1e3-2e7 1e6	IT IT	No findings
CF33-hNIS-αPDL1	Nude Black/6 BALB/c	52 67 31	1e4 1e5-1e8 1e7	IT IT & IV (1e6) IT & IV	No findings
CF33-hNIS-Δ14.5	Nude Black/6 BALB/c	36 16 16	1e4 1e6 – 1e8 1e7-3e7	IT IT IT & IV (2e7)	No findings
CF33-CD19	NSG	288	1e6-1e8	IT	No findings

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FAQ

- What makes the virus specific for cancer?
  - Heparan sulfate is a cell surface glycosaminoglycan at high density on cancer cells. Many proteins on the vaccinia virus envelop (A27L, H3L) that binds onto Heparan sulfate
  - CF33 was selected by high through-put screening from a library of >200 new chimeric viruses for high infectivity for cancer and low animal toxicity
  - All of the clinical CF33-constructs have been engineered to lack a TK gene, restricting their replication to tumor cells
  - Even if it rarely infects a healthy cell, it can't replicate and will get degraded immediately without ever growing
- Will it work IV?
  - Yes, according to animal data, should work IT, IP, and IV
- Will it work in people vaccinated for vaccinia?
  - Yes, there is human data from prior vaccinia OV trials that humans with established B- and T-cell-based immunity can have tumors respond to vaccinia OV
  - In animal models, vaccinated animals respond to OV therapy
  - Might even work better because of immune effects of OV therapy
  - Vaccinated animals usually require an increase in dose. Starting treatment doses for CF33 are very low

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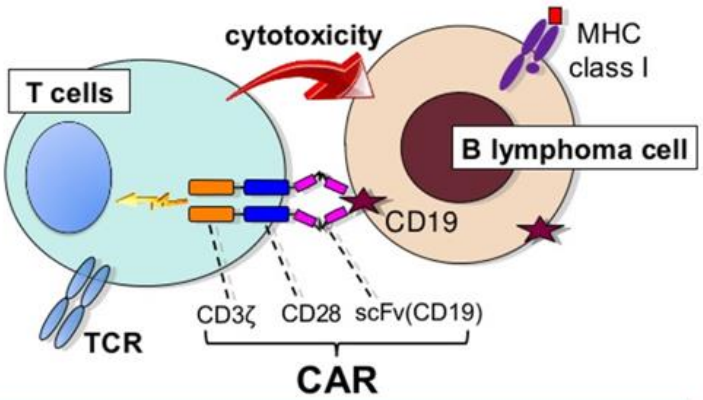
Chimeric Antigen Receptor T-cell (CAR T)

Cytotoxicity of CD19-specific CAR-expressing T Lymphocytes against B Cell Lymphoma



CD19-CAR T cells, which are engineered to express extracellular single-chain immunoglobulin variable fragments to CD19, linked to cytoplasmic T cell activation domains including CD3-ζ, showed remarkable therapeutic benefits toward CD19⁺ B cell malignancies.

- T Lymphocyte can kill cells it recognizes as cancers
- T-cells can be genetically engineered to recognize and kill cancer cells
- CAR-T directed at CD-19 (cell surface marker on B-cells) most successful so far
- CD-19 Car-T can cure refractory ALL
- August 2017: Novartis product approved for clinical use by FDA approval
- Targeting solid tumors has been more problematic
- Many cancer Ag expressed in normal tissues (e.g. Her-2, CEA)
- Many specific cancer proteins are intracellular (e.g. AFP)

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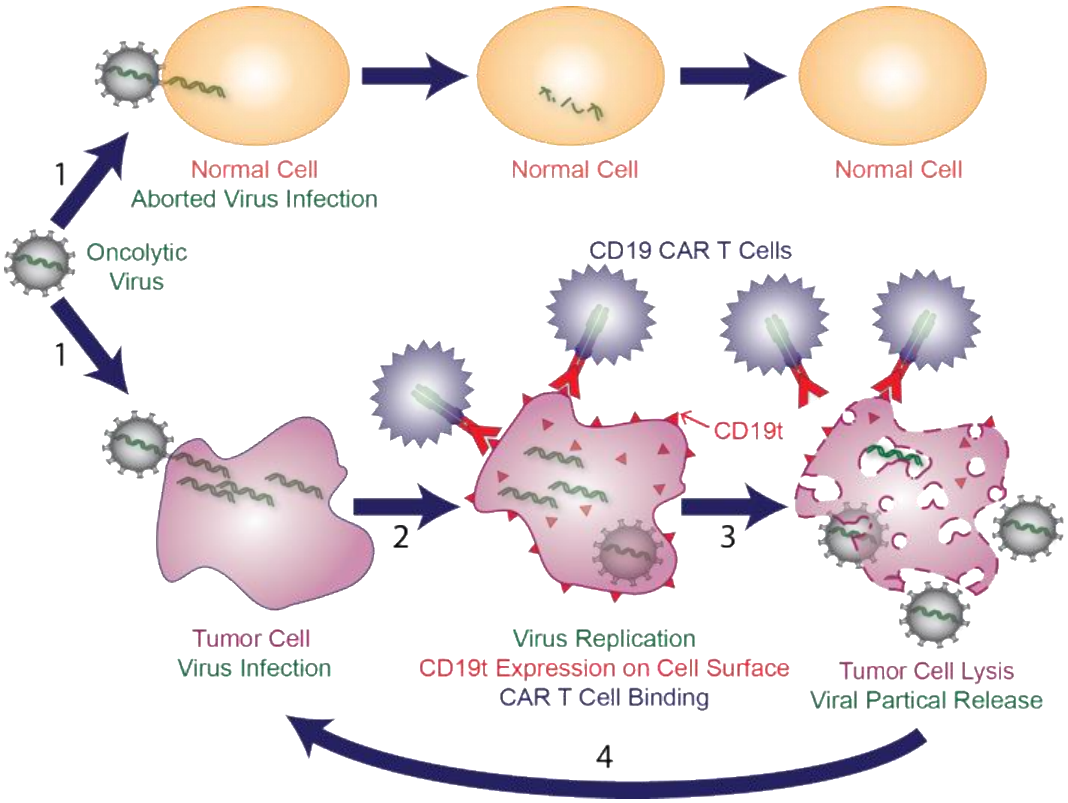
Oncolytic Viruses Deliver CAR Targets to "Targetless" Solid Tumors Delivering CD19 as Transgene



Strategy is to use an oncolytic virus that efficiently infects every known kind of cancer to deliver and express CD19 at tumor sites to become target for CD19-CAR T

Goals:

- Direct oncolytic effect
- Enhance local expression of death signals
- Attract T-cells and NK cells
- Produce targets for Car-T Therapy

Park et al. Science Translational Medicine 2020

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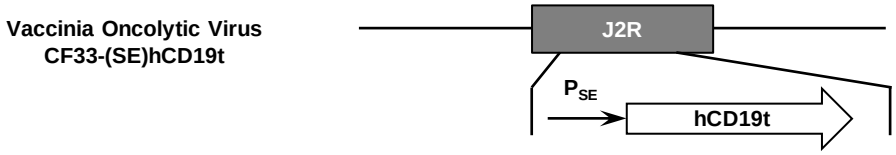
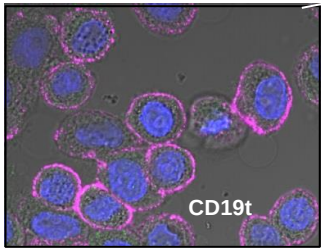
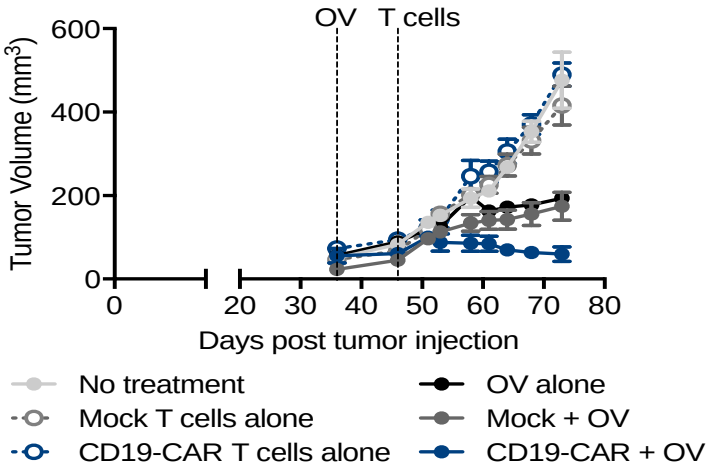
Oncolytic Viruses Delivering CAR Targets to Solid Tumors

- CD-19 CAR-T are now approved as clinical therapy
- Oncolytic virus designed to deliver gene coding CD-19 to bad solid tumors
- OV infection elicits CD-19 expression on solid tumors
- Infusion of CD-19 Car-T results in killing of infected tumor cells and neighboring cancer cells







Park et al., Science Translational Medicine, 2020

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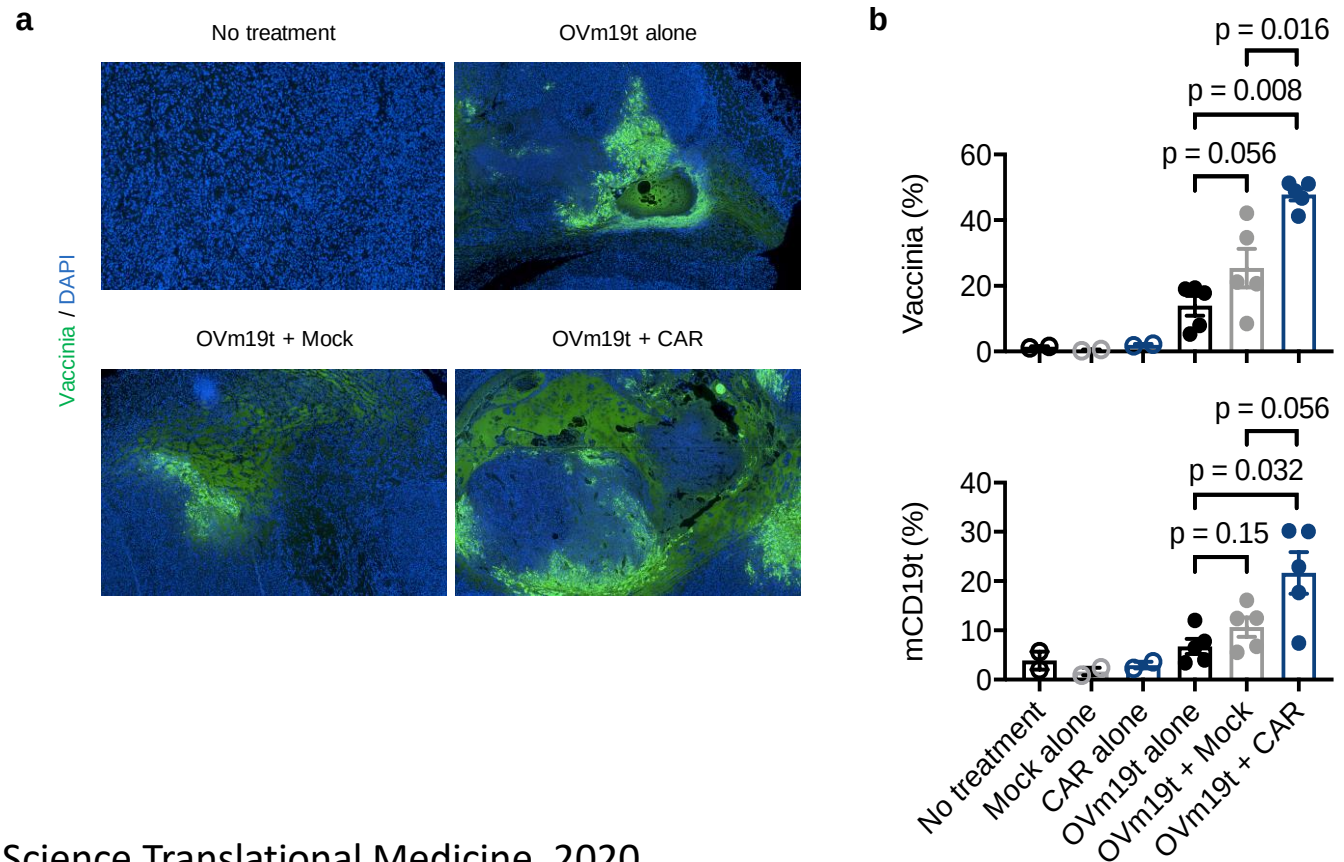
Biological Rationale:
CD19-CAR T cells enhance CF33-CD19t spread in tumors in vivo

Combination results in greater spread of CF33-mCD19t and higher cell-surface CD19t expression in tumors



Park et al., Science Translational Medicine, 2020

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CF33
Clinical Trials

- CHECKVacc (CF33-hNIS-aPDL1)
  - Triple negative breast cancer
  - Failed 1st line therapy
  - IT injection

- VAXinia (CF33-hNIS)
  - Many solid tumors
  - Failed 1st line therapy
  - IT or IV injection
  - Multicenter

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