IMUGENE

Developing Cancer Immunotherapies

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Executive Director, Clinical Science

Developing Cancer Immunotherapies

December 6, 2022

CF33 Oncolytic Virus
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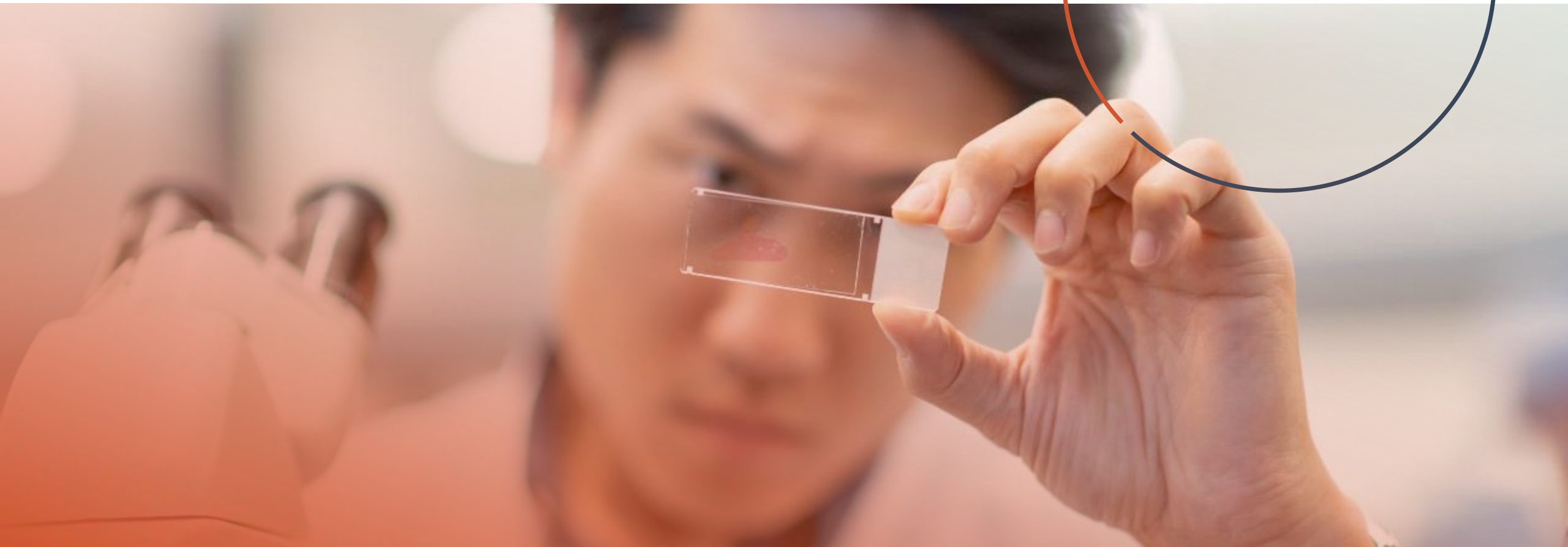
CF33 Oncolytic Virus



The Inventor & City Of Hope

Professor

Yuman Fong

the MIRACLE of SCIENCE with SOUL
City of Hope

The Sangiacomo Family Chair in Surgical Oncology and chair of The City of Hope Dept of Surgery is an *internationally recognized expert* in liver and pancreatic cancer. He has developed many new surgical techniques and instruments. He helped usher in robotic surgery for liver cancer. He has also led research efforts to use genetically modified viruses to destroy cancer cells.

Dr. Fong joined City of Hope in 2014 after more than three decades at Memorial Sloan-Kettering Cancer Center in New York City.

Dr. Fong has written and edited >1000 scholarly articles as well as 22 textbooks. He is the founding Editor-in-Chief of ***Molecular Therapy Oncolytics*** (Cell Press).

He is a fellow of the American Institute of Medical and Biologic Engineering, and the National Academy of Medicine.

Dr. Fong has had leadership roles in regulatory aspects of gene therapy, including serving as Chair or the Recombinant DNA Advisory Committee of the National Institutes of Health of the United States.

City of Hope, in Los Angeles, is *a leading research and treatment center* for cancer, diabetes and other life-threatening diseases. Founded in 1913, it is designated as a comprehensive cancer center, the highest recognition bestowed by the National Cancer Institute. City of Hope is also a founding member of the National Comprehensive Cancer Network, with research and treatment protocols that advance care throughout the US.
City of Hope has been ranked as one of the nation's "Best Hospitals" in cancer by *U.S. News & World Report* for over 10 years.
City of Hope has GMP facilities that produces clinical trials materials for many academic centers and is the alpha clinic trials site for CIRM

WHY A VACCINIA VIRUS?

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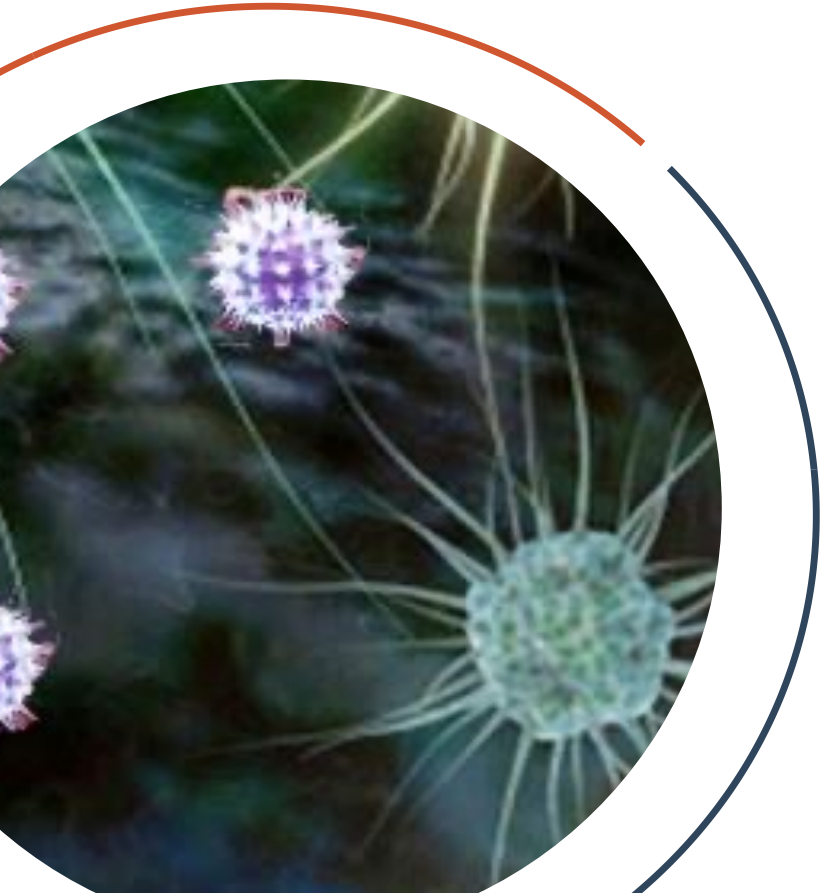
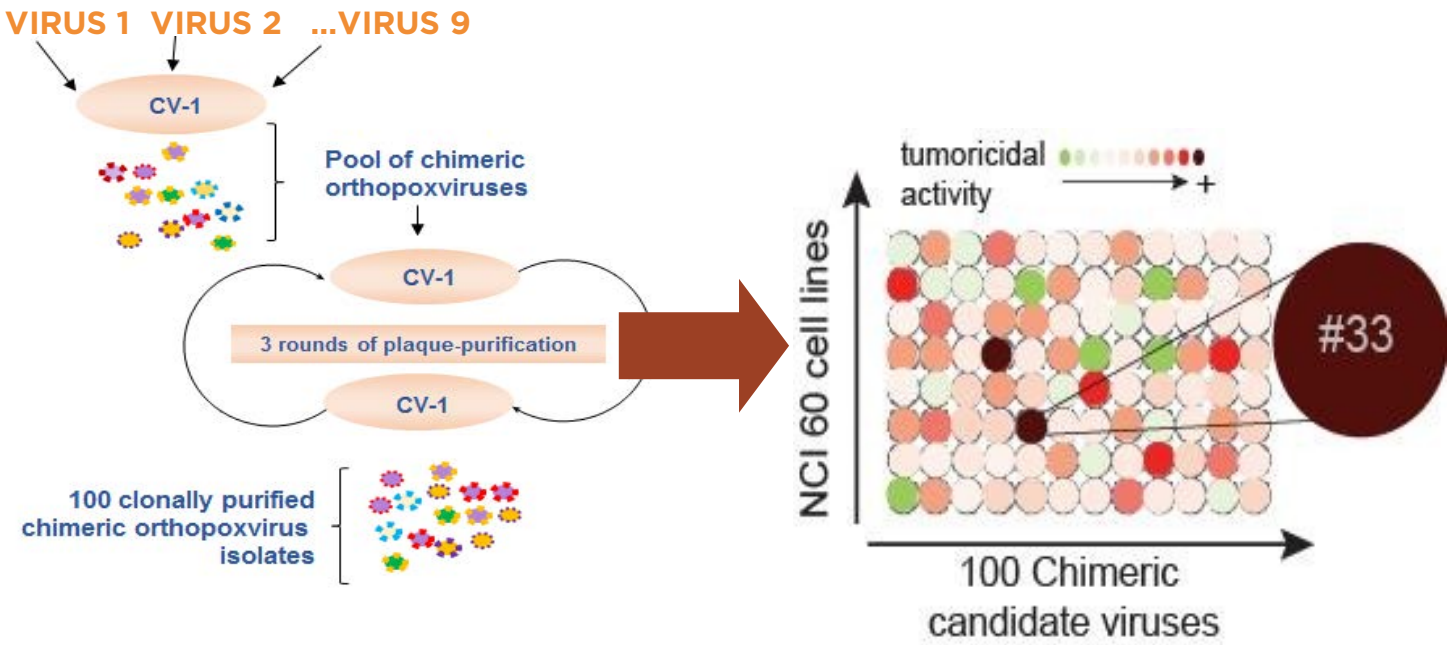


- Large DNA virus that is genetically very stable
- Most effective biologic therapy in history of man:

  vaccine that eradicated smallpox
- Highly cytolytic for a broad range of tumor cell types
- Amenable to large scale production
- Does not integrate into the host genome
- May be administered via intratumoral (IT) and intravenous (IV) routes
- Can carry large transgenes and large numbers of transgenes

GENERATION & 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

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STRATEGY

Engineer Novel Chimeric Viruses

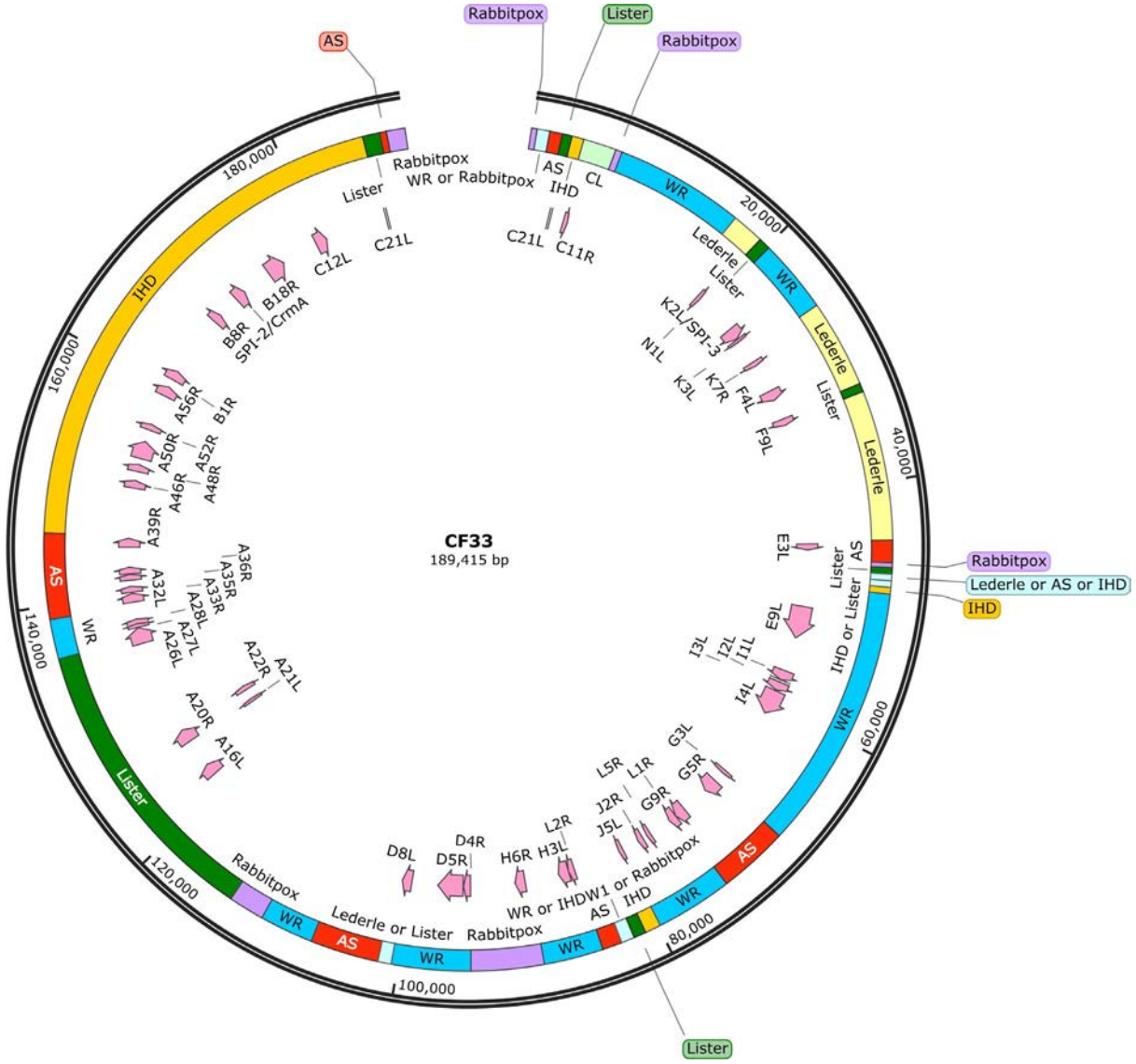
High Through-put Screening for Efficacy Against NCI60

Safe in Animals

Arming with Additional Payloads

Hope Oncolytic Viruses (HOV)

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

SAFELY DELIVERED IT, IP, IV WITH LARGE THERAPEUTIC INDEX

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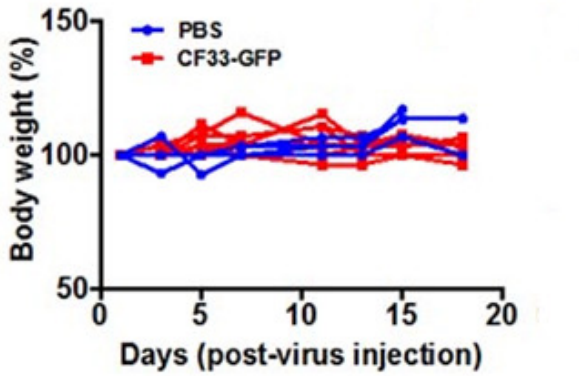
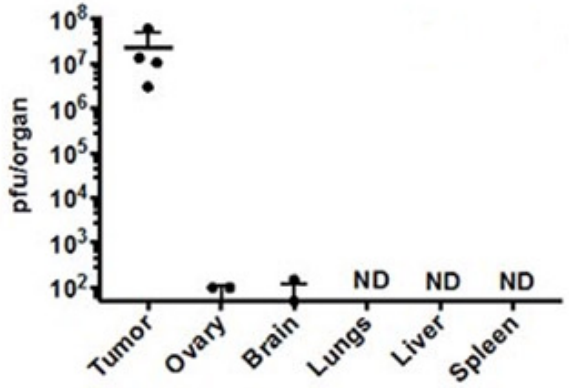
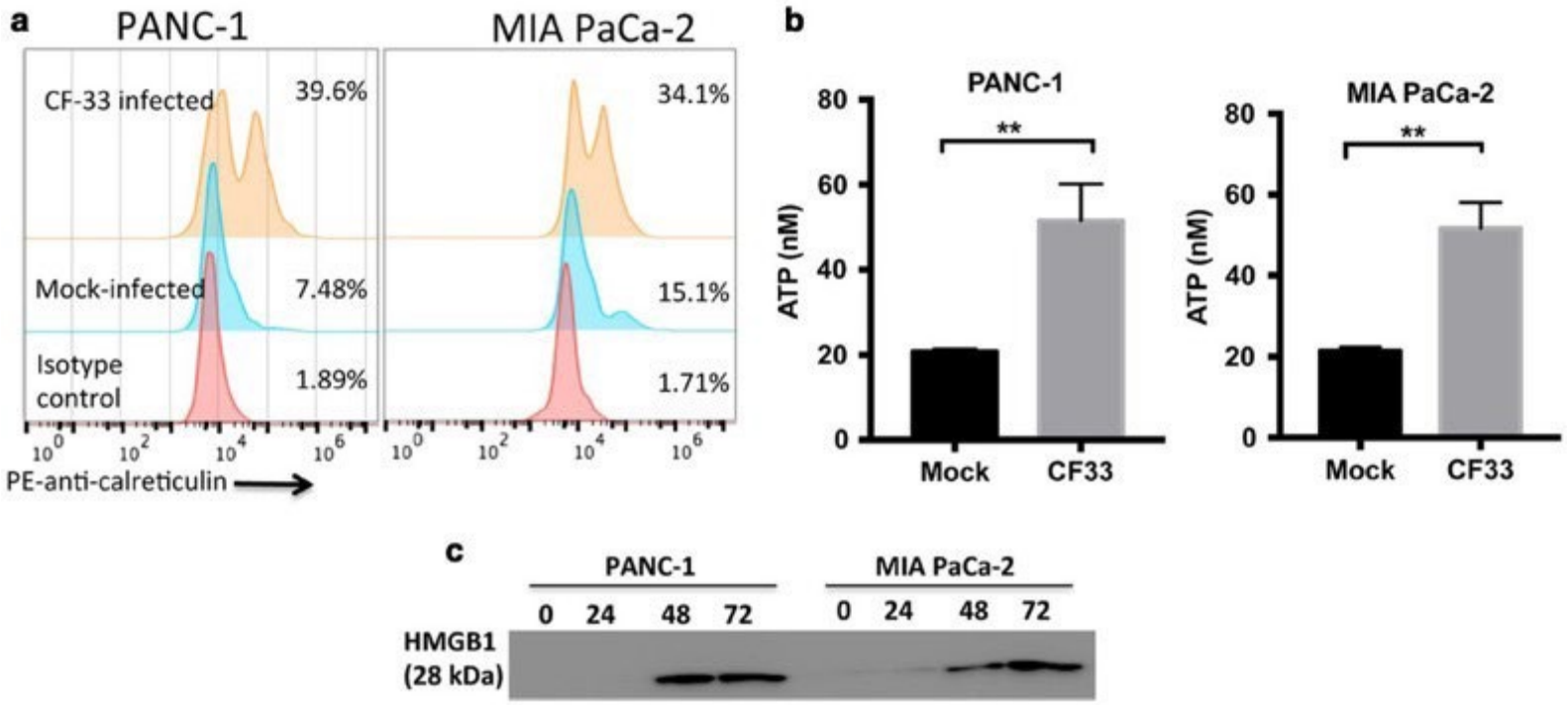




- In many tumor models, animals cured with a single injection of 1000 pfu
- NO TOXICITY UNTIL OVER 10^9
- Virus restricted to tumor

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

CF33 Induces Immunogenic Cell Death in Many Cancers

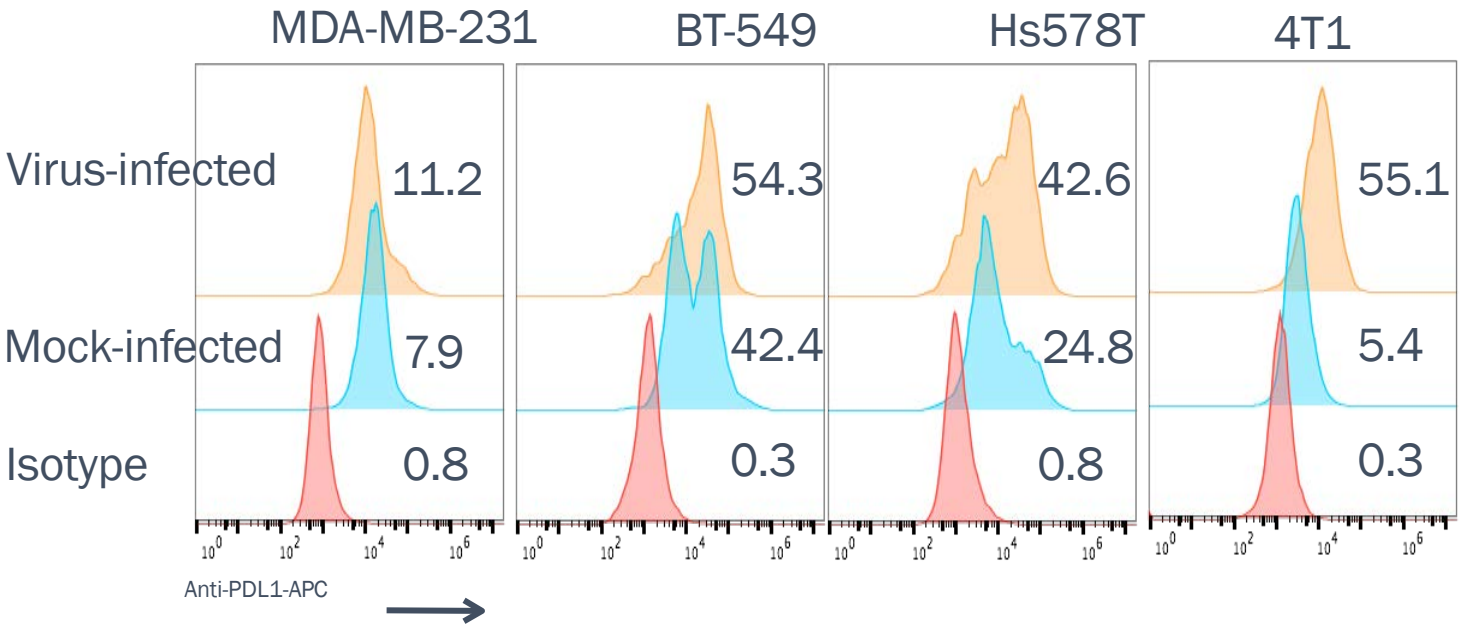


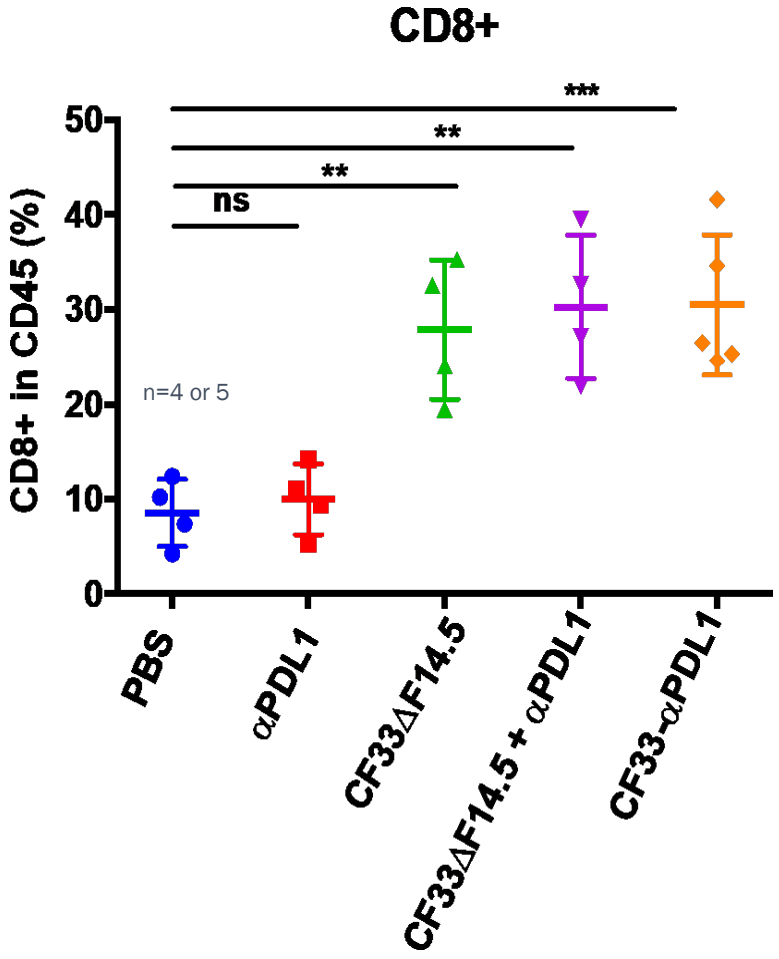
- Upregulation of calreticulin expression
- Release of ATP
- Increased expression of HMGB1

Source: O'Leary et al., J Transl Med 2018

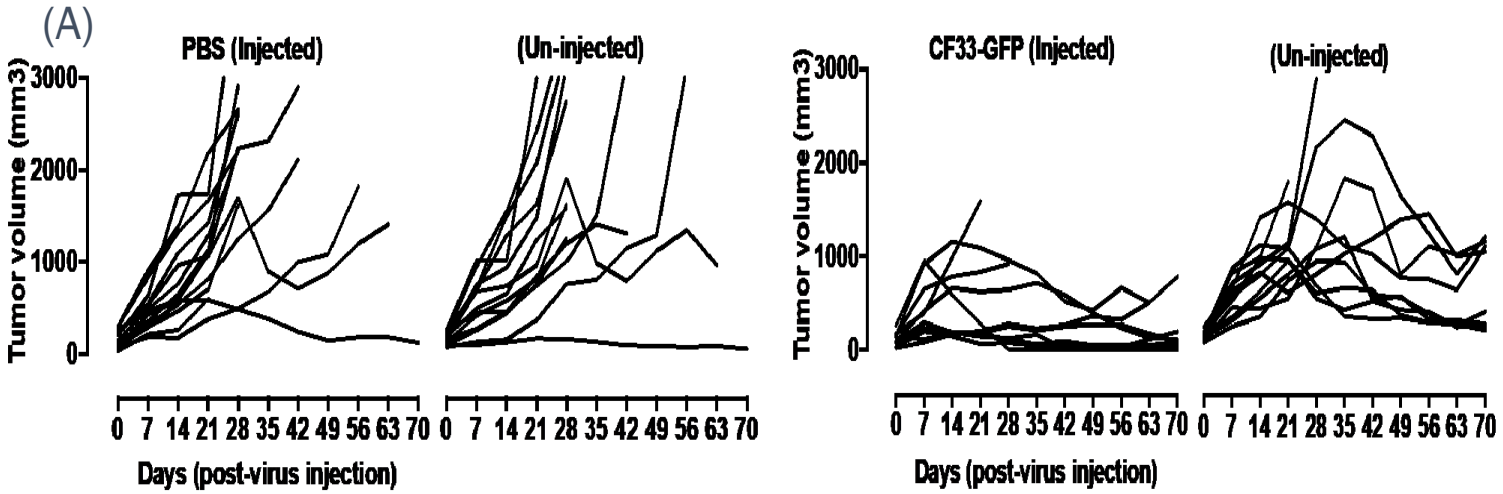
CF33 Upregulates PD-L1 Expression & Increases Infiltration by CD8+ T-cells

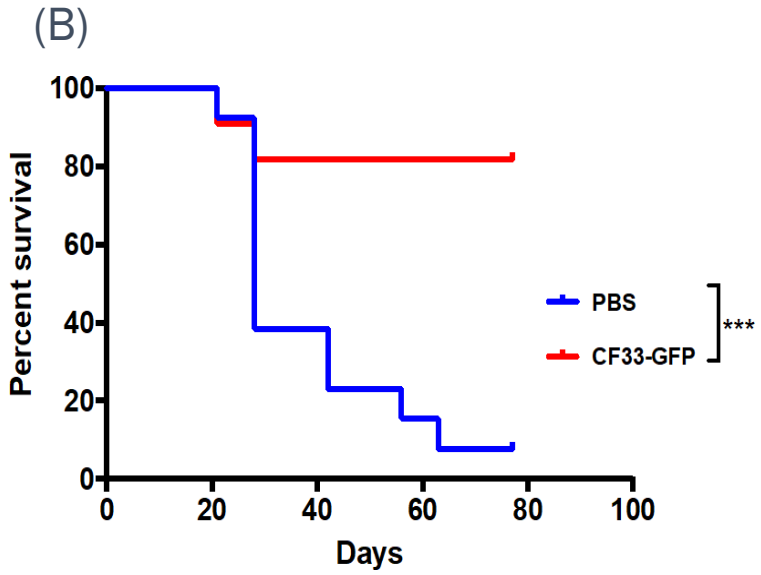
Increases PD-L1 Expression





CF33-GFP Killing in Lung Cancer Tumor Model

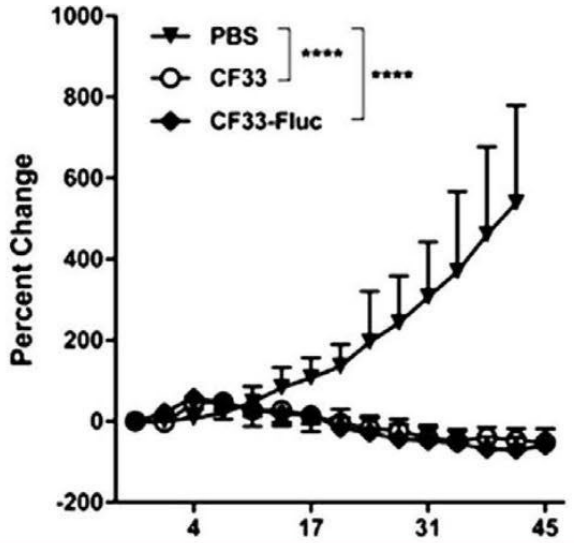




- IT injection model for animals with bilateral tumors
- Killing of cancer in injected and non—injected tumors
- Enhances survival and can cure with doses as low as 10^3 pfu
- Safe

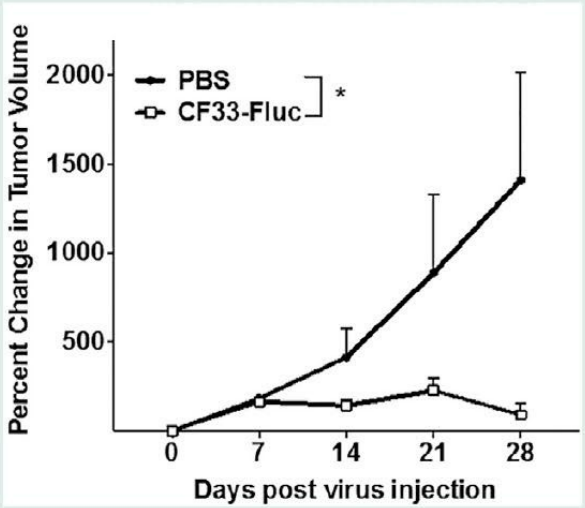
Compelling Killing of Many Tumour Types at Low Doses

PANCREATIC



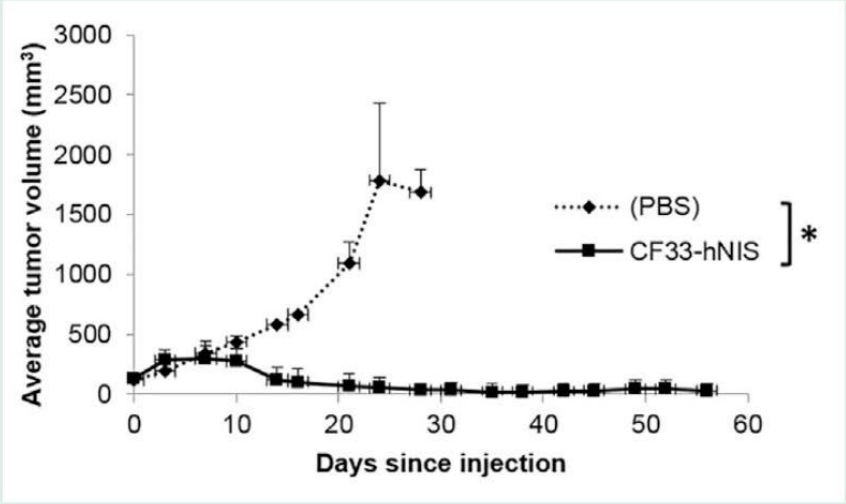
J Transl Med. 2018, 16, 110

COLORECTAL



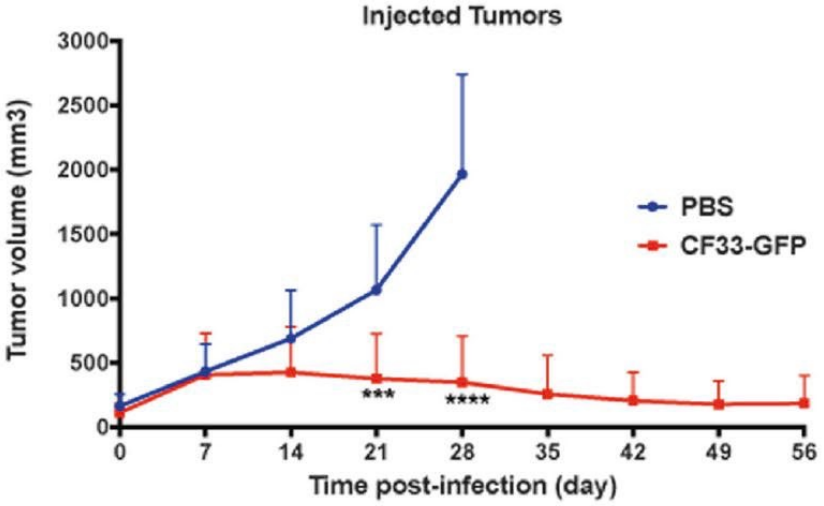
Mol Ther Oncolytics. 2018, 9, 13

COLON



Mol Ther Oncolytics. 2019, 13, 82

LUNG



Cancer Gene Ther. 2019

VAXINIA: CF33-hNIS “Parental Virus”

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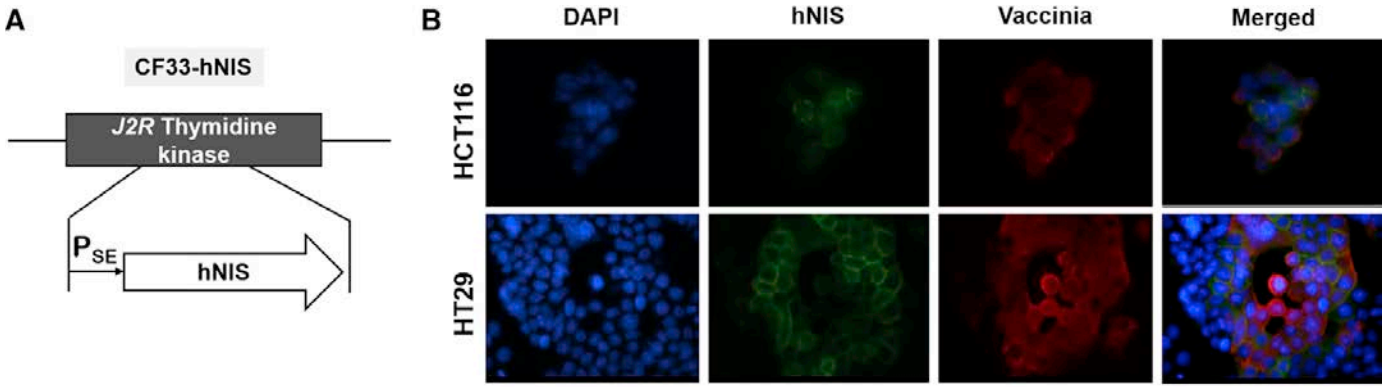
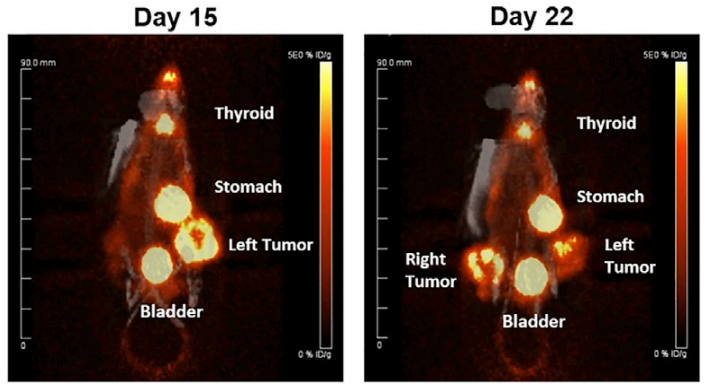
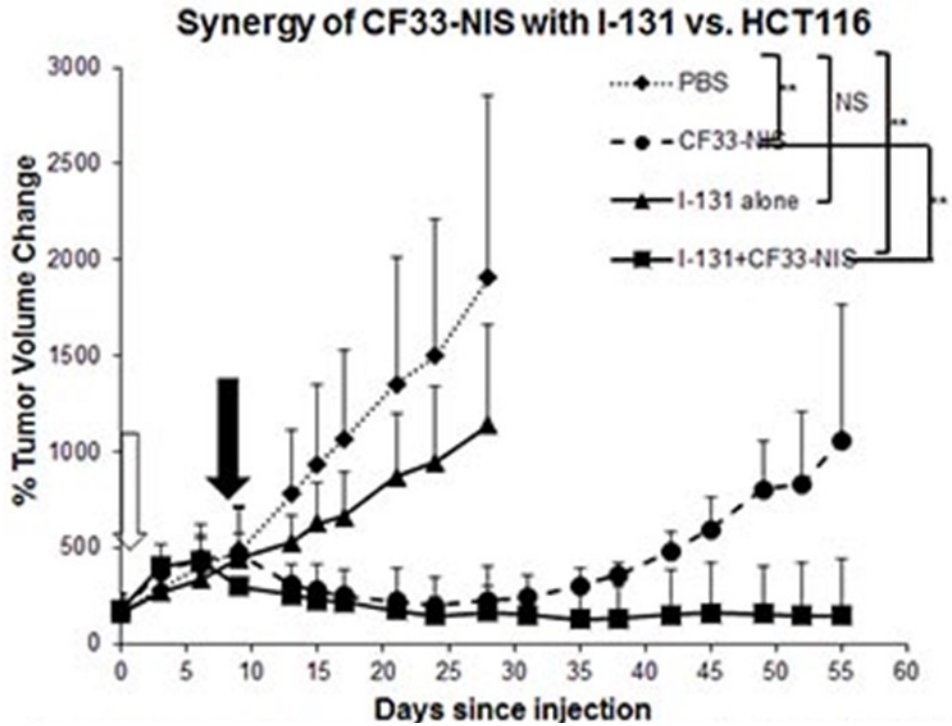


PET/CT I-124 imaging of CF33-hNIS



- hNIS transgene inserted within J2R locus (Tk) to transport radioactive iodine for imaging or therapy
- hNIS protein expressed on tumour cell surface (green)
- PET imaging shows virus in injected tumour at day 15 and virus infecting non-injected tumour by day 22
- CF33-hNIS infection is synergistic with I-131 radioisotope and induces sustained tumour growth abrogation in HCT116 colorectal cancer xenografts

Ref: Mol Ther Oncolytics, 2019, 13, 82



MAJOR ADVANTAGES OF CF33

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- Preclinical data has demonstrated that CF33 is more efficacious than all parental viruses and most viruses in clinical trials
- Can shrink multiple types of cancer at an extremely low dose (1000 pfu).

- Tumor type-agnostic: 'universal' approach to targeting solid tumors
- Turns immunologically 'cold' tumors to immunologically responsive 'warm' tumors
- CF33 shrinks not only injected tumors, but also non-injected distant tumors, indicating tumor tropism and abscopal effect

- Novel combination use of FDA-approved cellular immunotherapy (CD19-CAR T cells) along with OV that presents CAR target, CD19, on solid tumors
- CAR T cell-mediated cancer killing helps OV spread in tumors

KEY DIFFERENTIATION

1. CF33 OV Platform:
   - high potency in cancer killing
   - range of cancer cell types infectible
   - Big therapeutic window
2. CF33 can be made in high titres
3. Great stability profile
   - Genetic stability
   - Storage stability
   - Clinic stability after mixing
4. CF33 can be used in multiple doses without complete neutralization by host immune system

VAXINIA Phase 1 MAST Study
(Metastatic Advanced Solid Tumours)

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First Patient Enrolled May 2022, IT Cohort 1 Cleared Sept 2022

Dose Administration (Parallel Groups)

n=52-100

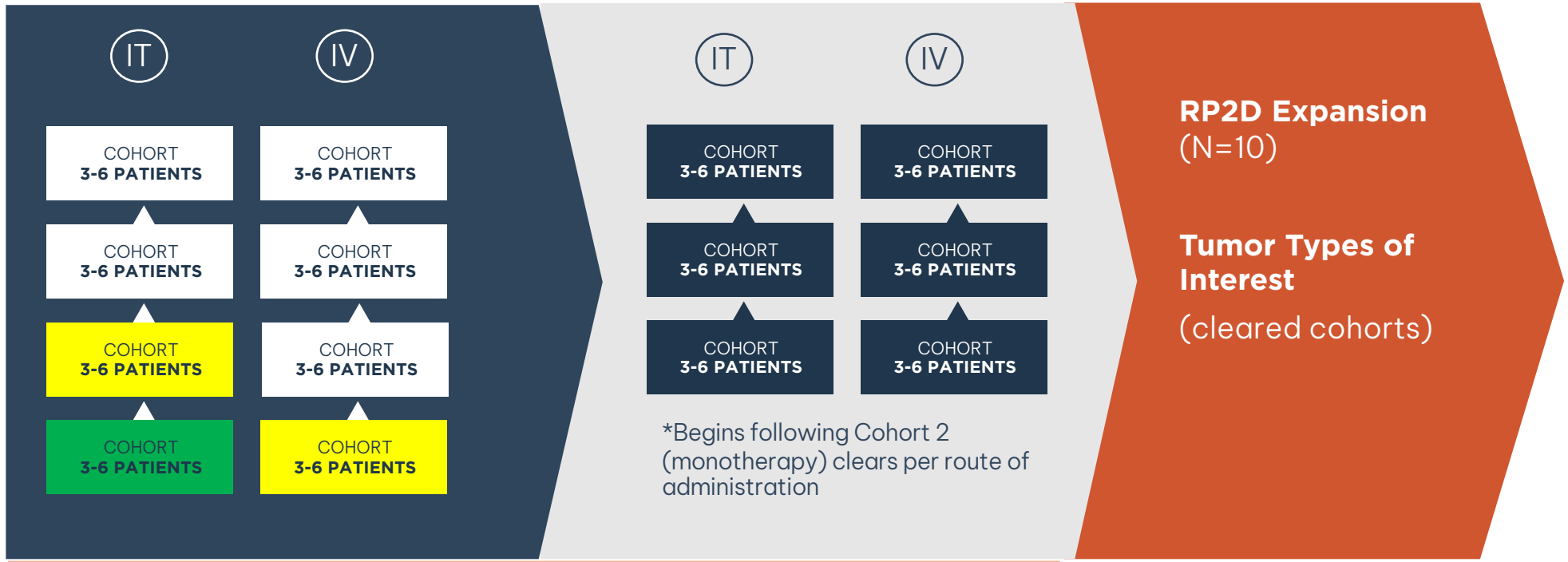
IT

IT Administration
Metastatic and Advanced Solid Tumours

IV

IV Administration
Metastatic and Advanced Solid Tumors

Site Location: USA, AUS

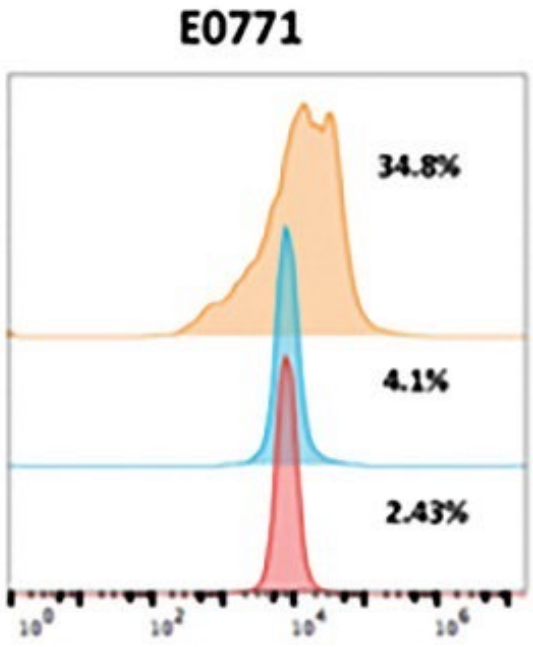


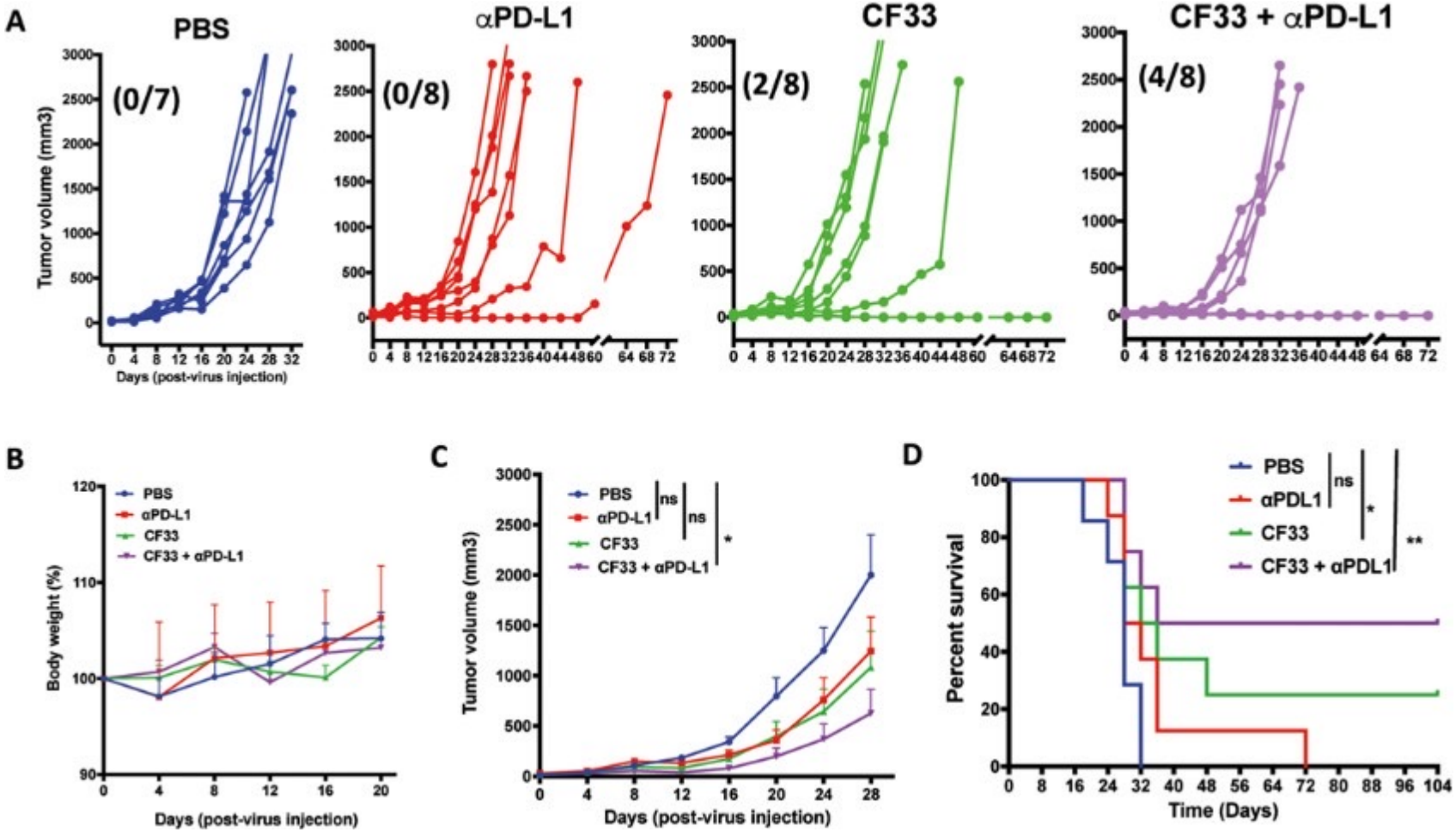
Identify: Recommended Phase 2 Dose (RP2D) – Monotherapy and Combination
Based on: Safety, Immunogenicity, Tumour Response

NCT05346484

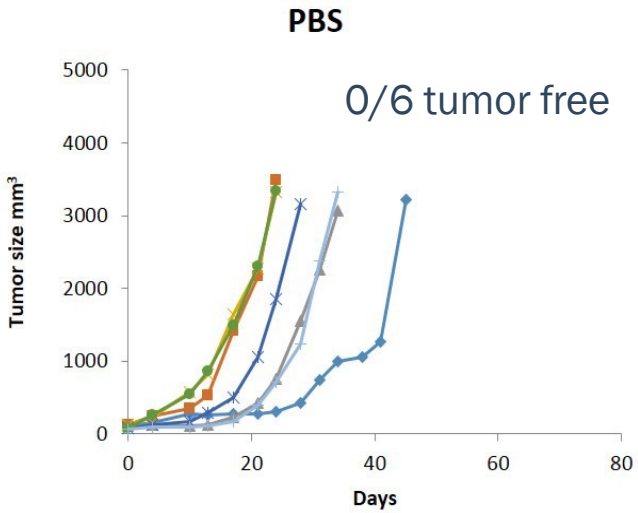
CF33-hNIS + Anti-PD-L1 Synergizes for Tumor Killing Breast Cancer and Other Cancers

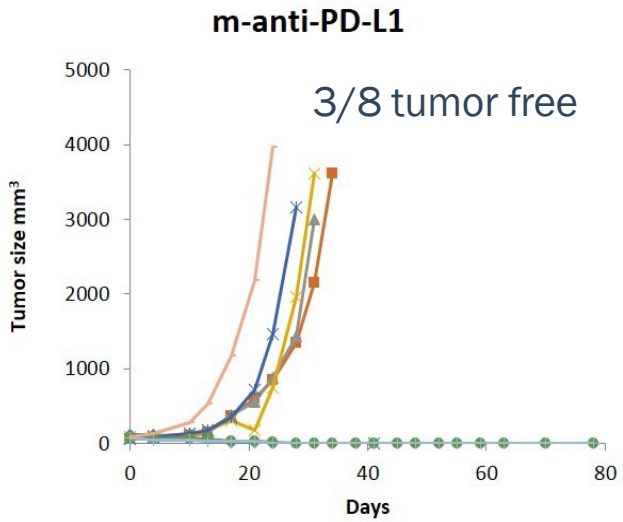
- E0771 TNBC in C57BL/6 mice
- 100 µg anti-PD-L1 (Bio X)

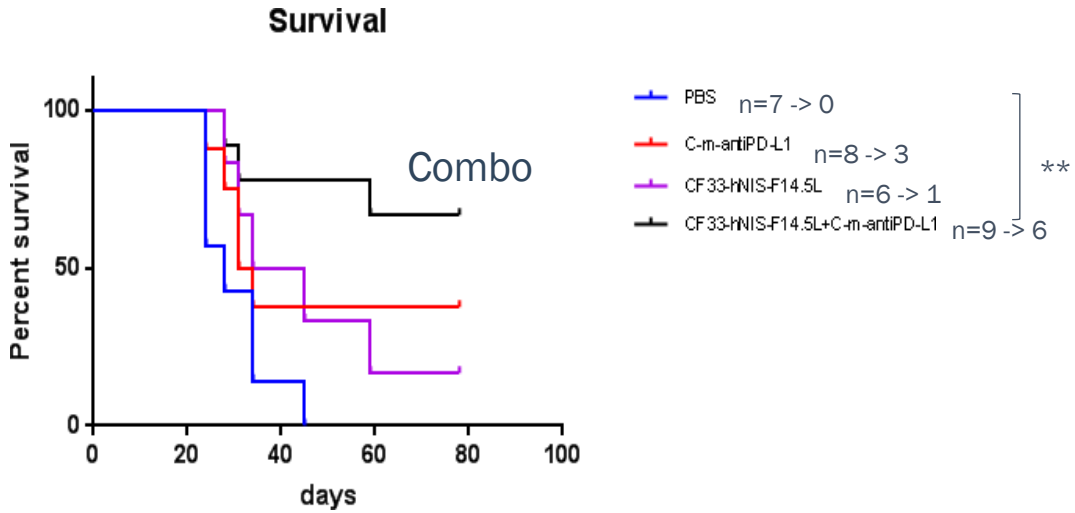


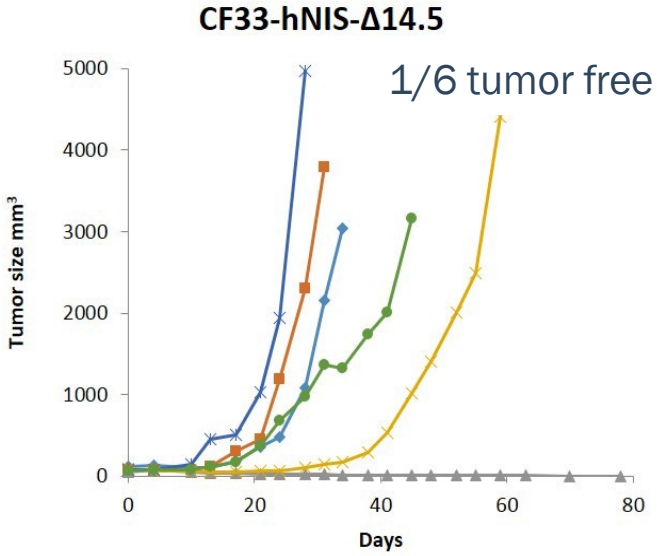


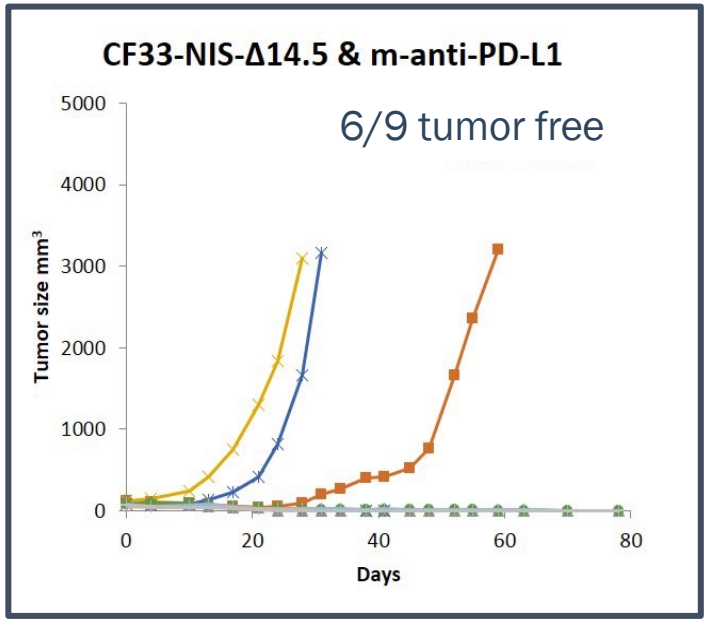
CF33-hNIS + Anti-PD-L1 Synergizes for Tumor Killing Colorectal Cancer











CHECKvacc PHASE 1 TNBC STUDY

CF33+hNIS+aPD-L1 ("Armed" Virus)

SAN ANTONIO BREAST CANCER SYMPOSIUM

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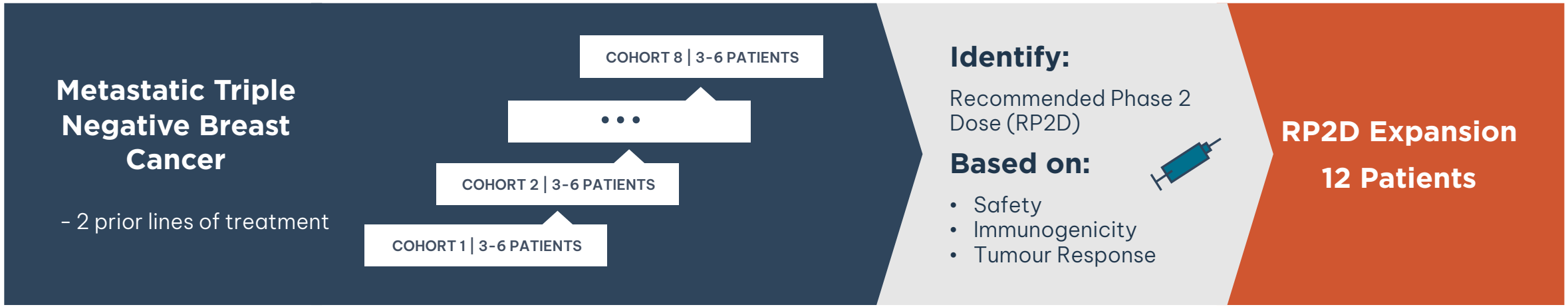
ACCEPTED TO SABC 2022



First Patient Enrolled October 2021

Disease of need

- 8-13 month survival for metastatic disease with few treatments

Potential target for immunotherapy

- Expresses PD1, PD-L1

Treatment responses to Atezolizumab (JAMA Oncology, 5:74, 2019)

- 1st line: 24%; 2nd line: 6%
- Approved by FDA 8 March 2019

Potential for registration in well-designed, randomised P2 study

Indication	TNBC
FDA IND	CHECKvacc: CF33-hNIS-aPDL1
N	33-78
Location	Single Center: COH
Admin Route	Intratumoral (IT)

NCT05081492

onCARlytics
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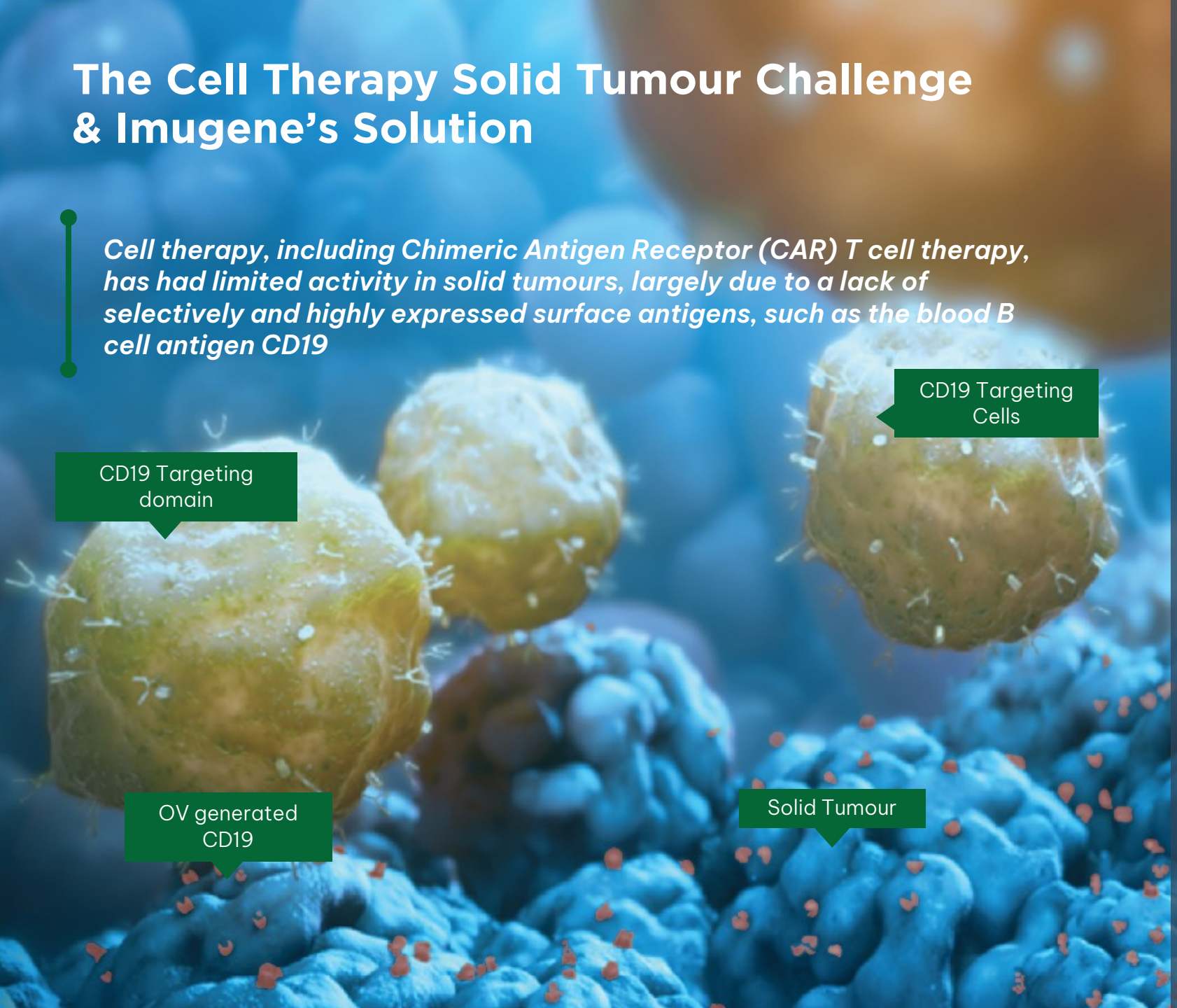
CF33-CD19

The Cell Therapy Solid Tumour Challenge & Imugene's Solution

Cell therapy, including Chimeric Antigen Receptor (CAR) T cell therapy, has had limited activity in solid tumours, largely due to a lack of selectively and highly expressed surface antigens, such as the blood B cell antigen CD19



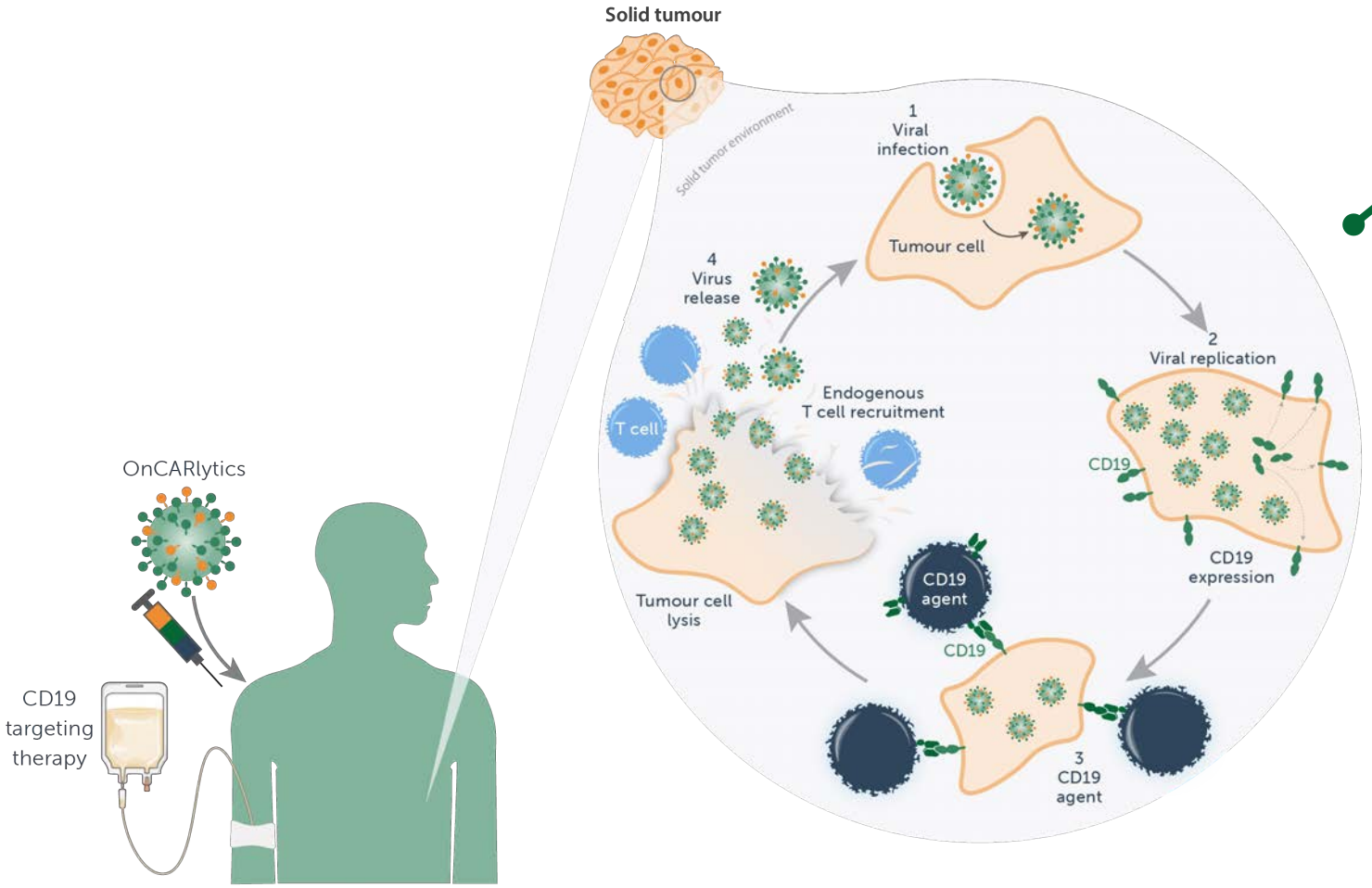
NEW CONCEPT

Utilise OV's as a delivery vector to deliver CD19 antigen to solid tumour cells

Engineer Imugene's CF33 to infect solid tumour cells and insert CD19 transgene to enable presentation of CD19 over the tumour cells during tumour cell infection, onCARlytics (CF33-CD19)

Combination use of CD19 targeting therapies, including autologous or allogeneic CD19 CAR Ts and bispecifics, with onCARlytics (CF33-CD19) presented CD19 targets on solid tumours

MECHANISM OF ACTION:
How does it work?



onCARIytics makes solid tumours "seen" by CD19 targeting therapies

1. OnCARIytics infects tumour cells
2. Virus replication and production of CF33-CD19 on the cell surface enabling CD19 cell targeting
3. Tumour cell lysis leads to viral particle release and the combination promotes endogenous immune cell recruitment to tumours
4. Released viral particles re-initiate virus infection of surrounding tumour cells.

onCARLYTICS DELIVERS CAR TARGETS TO "TARGETLESS" SOLID TUMOURS

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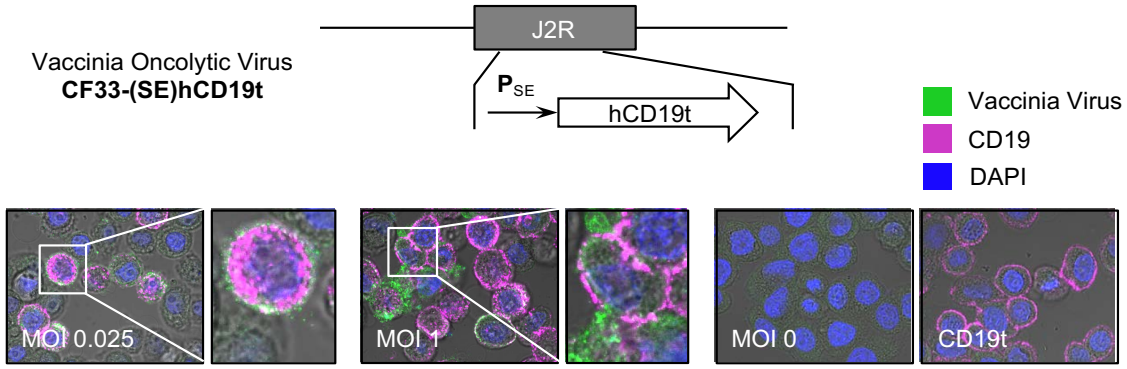
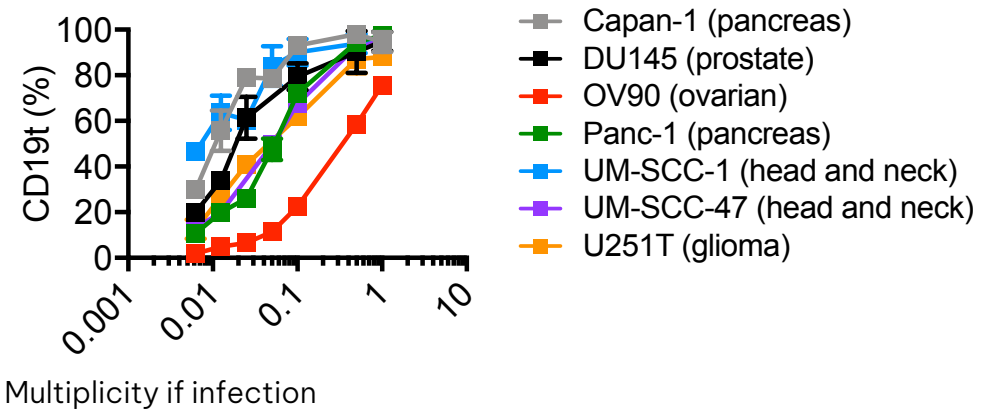
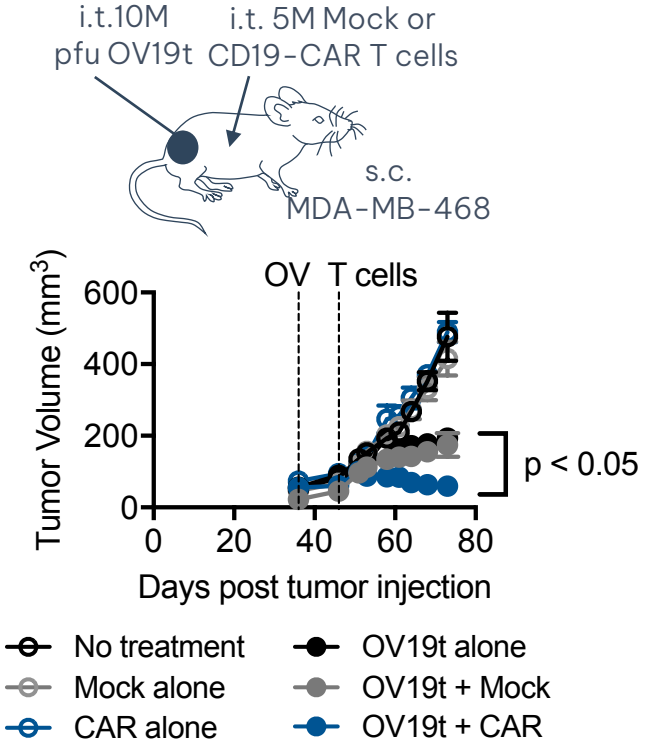


onCARlytics (CF33-CD19) infects a wide array of solid tumour cell lines, with dose-dependent CD19 cell surface expression



Combination of onCARlytics (CF33-CD19) and CD19-CAR T cells promotes tumour regression in xenograft model of TNBC



Park et al. *Science Translational Medicine* 2020

onCARLYTICS COMBINATION WITH CD19 TARGETING THERAPIES

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AUG 2021
Strategic Partnership with Celularity

NOV 2021
Strategic Partnership with Eureka

SEP 2022
Strategic Partnership with Arovella

sitc
Society for Immunotherapy of Cancer

3 x POSTERS PRESENTED AT SITC 2022

FDA APPROVED CD19 TARGETING THERAPIES

Approved and in-development autologous amd allogeneic CD19 CAR Ts and bispecifics can be partnered with Imugene's onCARlylics for treating solid tumours:

KYMRIAH® (tisagenlecleucel) Suspension for IV infusion
NOVARTIS

YESCARTA® (axicabtagene ciloleucel) Suspension for IV infusion
GILEAD

TECARTUS™ (brexucabtagene autoleucel) Suspension for IV infusion
Kite

Breyanzi® (lisocabtagene maraleucel)
Bristol-Myers Squibb

BLINCYTO® (blinatumomab) for injection 35 mcg single-dose vial
AMGEN

Figure 4

Activation of CYCART-19 by targeting of t cells expressing CD19t following onCARlyt infection

Imugene Limited, Sydney, Australia

Introduction

Autologous chimeric antigen receptor (CAR) T has shown impressive clinical responses again hematological malignancies and is being acti the treatment of solid tumors. However, sever precluded therapeutic responses in solid tumo tumor-restricted CAR targets and the immun microenvironment. We have recently reported combination immunotherapy using a novel chi based oncolytic virus (OV), called onCARlytic that is engineered to express a non-signaling, (CD19t) antigen for tumor-selective delivery, targeting of tumor cells by autologous CD19-(of the field's unanswered questions is whether allogeneic CAR T Cell are superior to cancer p T-Cells for product manufacturing to improve against solid tumors.

Here, we evaluated this combination strategy CAR T Cell products generated from peripher cells (PBMC) and placental T-Cells, respectiv CAR T Cell were manufactured from normal, h CYCART-19 (Celularity®, Inc.) Cells were deriv human placental T-Cells that are genetically r the CD19-CAR followed by CRISPR-Cas9- me the endogenous TCR and expanded to produc allogeneic "off the shelf" treatment.

CYCART-19 T-Cells induced potent cytolytic solid tumor cells infected with onCARlytics. In we observed comparable anti-tumor activity derived CD19-CAR T Cells and CYCART-19, si in cytokine secretion were detected. This warr that the placental-derived CAR T product ma potential in patients with maintained or impro combination approach demonstrated impress response in human tumor xenograft models. In have demonstrated that further development immunotherapy for the potential treatment of tumors is warranted.

Figure 1

Delivering truncated CD19t (CD19 cells using oncolytic virus (OV) as CD19-CAR T Cell.

onCARlytics selectively infect solid tumor cells ar CD19 (CD19t) as a target for CD19-CAR T Cell.

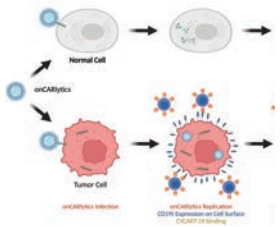


Figure 8

Anti-tumor activity of CYCART-19 in combination with onCARlytics in human xenograft triple negative breast cancer tumor model

Figure 2

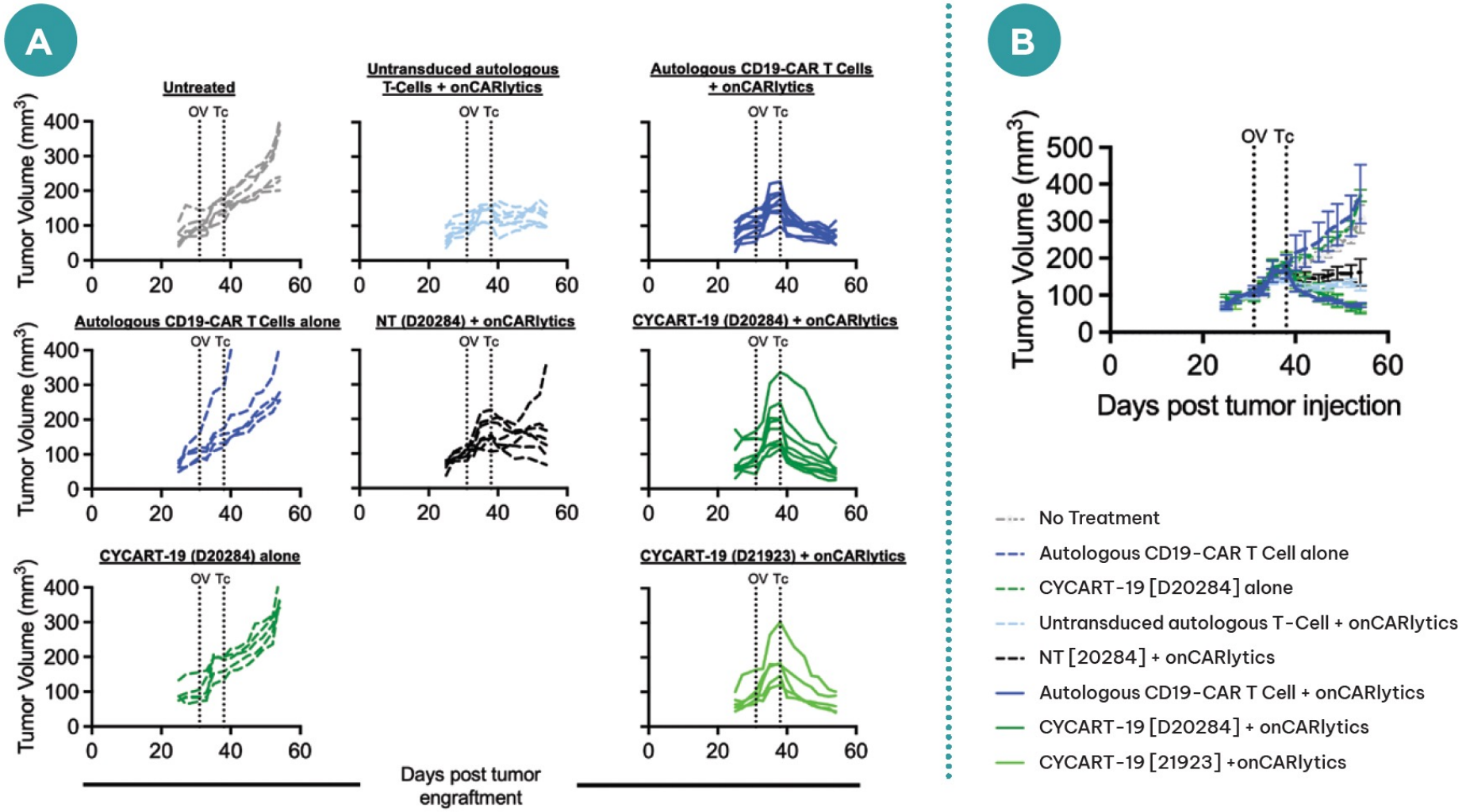


Figure 3

CD19 ARTEMIS® T-Cells effectively targets triple negative breast cancer cell line MDA-MB-468 following onCARlytics infection

Leslie M.O. Chong[1], Nimali P. Withana[2], [illegible]

[1]Department of Hematology and Hematopoietic Cell Transplantation, Beckma
[2]Department of Surgery, Division of Surgical Oncology, City of Hope National
[3]Eureka Therapeutics Inc., Emeryville, CA 94608
[4]Imugene Limited, Sydney, Australia

Introduction

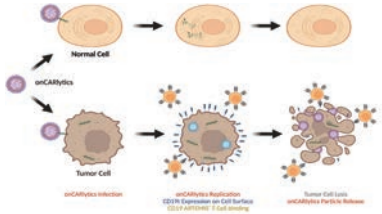
Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related deaths in the world with a 5-year survival rate at less than 12%. Currently, curative treatments include ablation, surgical resection, and liver transplantation. For majority of patients with advanced-stage disease, treatment with agents such as sorafenib, lenvatinib, and atezolizumab/bevacizumab and other investigational agents yield modest success rates and justify the need for further development of new therapies. T-Cell therapy against HCC targeting antigens such as alpha-fetoprotein (AFP) and glypican-3 (GPC-3) have shown some efficacy in clinical trials with conventional challenges against solid tumors including antigen heterogeneity, the immunosuppressive tumor microenvironment, and off-tumor on-target activity. Therefore, novel therapies are desperately needed to improve clinical outcomes for patients with HCC.

We have developed a novel chimeric vaccinia-based oncolytic virus, called onCARlytics (CF33-CD19t, Imugene Limited in collaboration with City of Hope®), that delivers a non-signaling, truncated CD19t (CD19t) antigen to tumors that allows for targeting of solid tumors by CD19 T-Cells. Once the CD19t is expressed on solid tumor cells, to enable cell killing, we have combined onCARlytics with CD19 ARTEMIS® T-Cell, a CD19-targeting adoptive engineered T-Cell powered by the ARTEMIS® antibody-T-Cell receptor (AbTCR) platform (Eureka Therapeutics®, Inc). ARTEMIS® AbTCR is distinct from CAR by recruiting the endogenous CD3 complex and utilizing the same activation and regulatory signaling pathways employed by natural TCRs, which enables both potent killing activity against CD19+ tumor cells and a superior safety profile. When administrated after onCARlytics, CD19 ARTEMIS® T-Cells were able to induce potent cytolytic activity against triple negative breast cancer and HCC tumor cells. OnCARlytics demonstrated expression of CD19t and robust in vivo anti-tumor efficacy against human HCC tumor xenografts. In summary, CD19 ARTEMIS® T-Cells combined with onCARlytics is a potentially effective immunotherapy strategy for the treatment of patients with HCC and can be applied to other solid tumors.

Figure 1

Delivering truncated CD19t (CD19t) to tumor cells using oncolytic virus (OV) as a target for CD19 ARTEMIS® T-Cells.

onCARlytics selectively infect solid tumor cells and deliver truncated CD19 (CD19t) as a target for CD19 ARTEMIS® T-Cells.



ARlytics) TARGETS HEPATOCELLULAR CARCINOMA (HCC)

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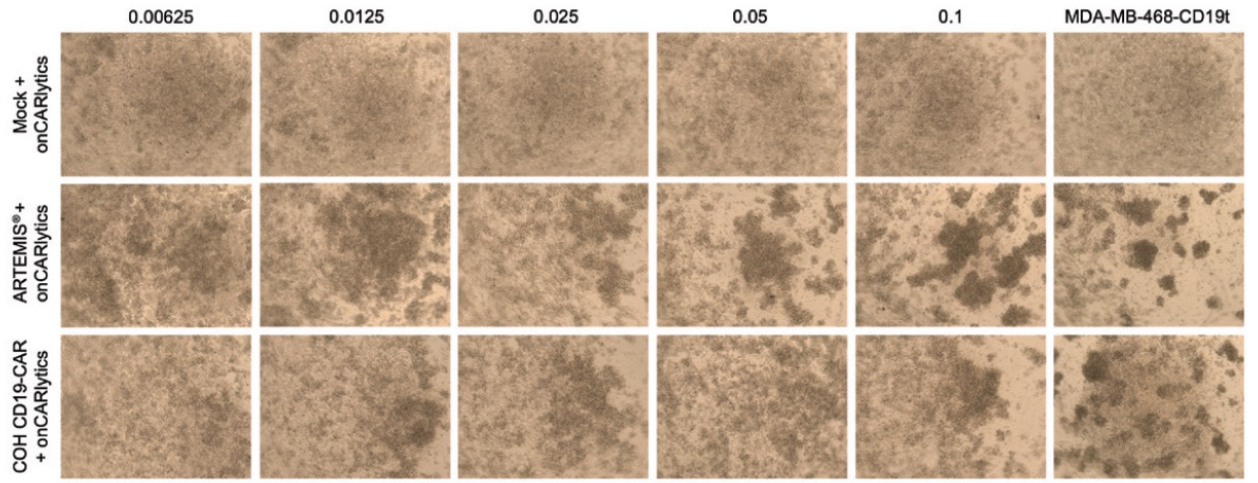
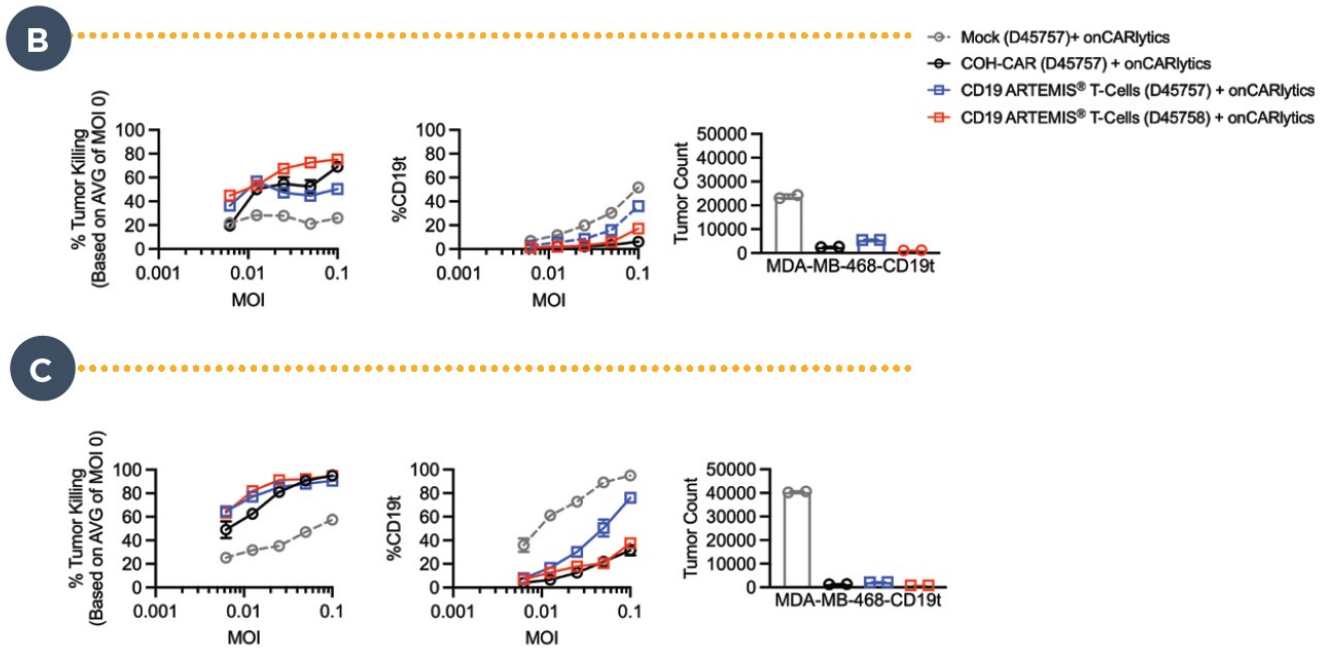




Summary

- onCARlytics can target triple negative breast cancer cell line MDA-MB-468 to express CD19t as a target for engineered T-Cells in an MOI-dependent manner.
- onCARlytics can target hepatocellular carcinoma cell lines HepG2 and Hep3B to express CD19t as a target for engineered T-Cells in an MOI-dependent manner.
- Eureka's CD19 ARTEMIS® T-Cells in combination with onCARlytics demonstrated greater in vitro efficacy against MDA-MB-468, HepG2, and Hep3B tumor cell lines compared to onCARlytics alone.
- There is an increasing trend in CD19 ARTEMIS® T-Cell activation in an onCARlytics MOI-dependent manner.
- CD19 ARTEMIS® T-Cells demonstrated higher trend of IL-2 production and lower IFNγ production compared to COH CD19-CAR T Cells when co-cultured with onCARlytics.
- CD19t expression was detected in tumors following onCARlytics infection in vivo.
- CD19 ARTEMIS® T-Cells and onCARlytics combination therapy efficacy will be tested in multiple in vivo models.

References

1. Park AK, et al. Effective combination immunotherapy using oncolytic viruses to deliver CAR targets to solid tumors. Sci Transl Med. 2020. **2.** McCart JA, Ward JM, Lee J, Hu Y, Alexander HR, Libutti SK, Moss B, Bartlett DL. Systemic cancer therapy with a tumor-selective vaccinia virus mutant lacking thymidine kinase and vaccinia growth factor genes. Cancer Res. 2001. **3.** Chaurasiya S, et al. A chimeric poxvirus with J2R (thymidine kinase) deletion shows safety and anti-tumor activity in lung cancer models. Cancer Gene Ther. 2020. **4.** O'Leary MP, Warner SG, Kim SI, Chaurasiya S, Lu J, Choi AH, Park AK, Woo Y, Fong Y, Chen NG. A Novel Oncolytic Chimeric Orthopoxvirus Encoding Luciferase Enables Real-Time View of Colorectal Cancer Cell Infection. Mol Ther Oncolytics. 2018. **5.** Yiyang Xu, et al. A novel antibody-TCR (AbTCR) platform combines Fab-based antigen recognition with gamma/delta-TCR signaling to facilitate T-Cell cytotoxicity with low cytokine release. Cell Discovery. 2018.

Figure 7

Blinatumomab dependent T-Cell infiltration following onCARlytics infection

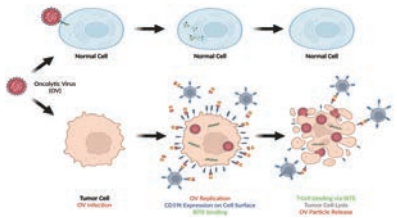
Introduction

Bispecific T-Cell engager (BiTE) monoclonal antibodies have emerged as a promising immunotherapy strategy for the treatment of hematological malignancies. Blinatumomab, an FDA approved BiTE carrying CD19 and CD3 scFv's has shown durable clinical responses for the treatment of B-Cell acute lymphoblastic leukemia (B-ALL) and non-Hodgkins lymphomas. Despite a wide array of research in hematological malignancies, BiTE therapies for the treatment of solid tumors have remained a significant challenge in demonstrating comparable efficacy. Solid tumors often lack amenable and targetable tumor antigens, and in many tumor types the tumor microenvironment (TME) is largely known to be immunologically "cold" and a barrier to immunotherapy responses.

Oncolytic viruses have recently gained traction in the field for the treatment of solid tumors because of their ability to target tumor-intrinsic properties and reshape the immunosuppressive TME. We have previously described the use of a chimeric oncolytic vaccinia virus (OV), CF33, for the treatment of a variety of tumor cell types, including triple-negative breast cancer, lung cancer, and liver cancer. Building on this, we generated an OV that expresses a non-signaling, truncated CD19 (CD19t) antigen called onCARlytics (CF33-CD19t), onto the surface of infected tumor cells prior to virus mediated tumor lysis, which redirected CD19-targeting chimeric antigen receptor (CAR) T Cell activity against solid tumors (Park et al. STM 2020). Using this OV, we have created a universal system that is agnostic to solid tumor type and can be provided with a targetable and well-characterized antigen. We now demonstrate that onCARlytics can redirect cytolytic functions of blinatumomab. We have demonstrated that tumors infected with onCARlytics in combination with blinatumomab show improved tumor cell killing, comparable to CD19-CAR T Cell. Using this approach, we show that a clinically-approved CD19-directed BiTE can be combined with onCARlytics to activate endogenous immune responses against solid tumors.

Figure 1

Delivering truncated CD19t (CD19t) to tumor cells using oncolytic virus (OV) as a target for bispecific T-Cell engagers (BiTEs)



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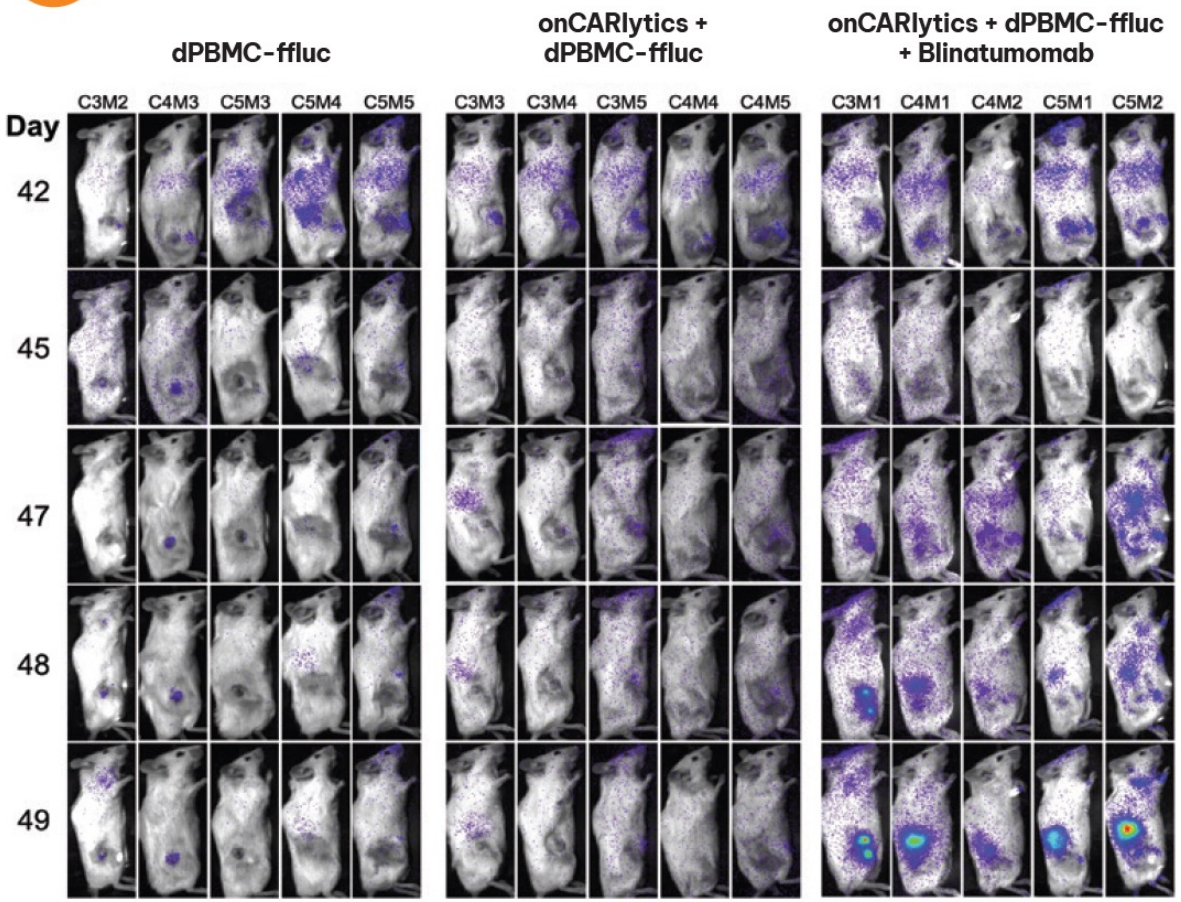

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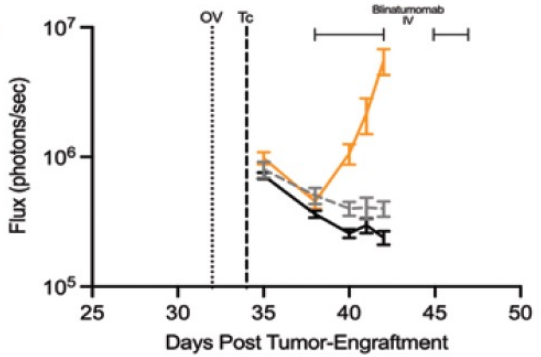

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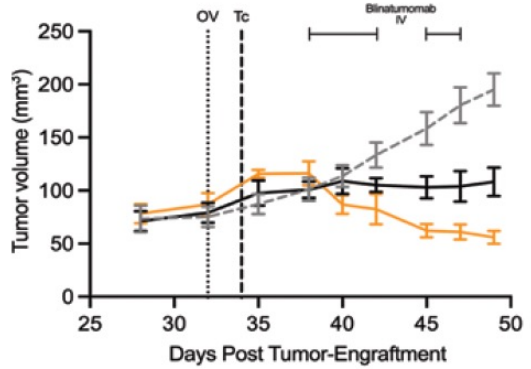

Summary

- **CF33 OV is an engineer novel chimeric orthopoxvirus platform that can be armed with differing "payloads".**
  - **Pre-clinical studies have shown that CF33 can infect and kill a range of cancer cell types.**
  - **VAXINA (CF33-hNIS) is being evaluated in a phase 1 study for patient with metastatic and advanced solid tumors.**
  - **CHECKvacc (CF33-PD-L1) is being evaluated in a phase 1 study in patients with metastatic triple negative breast cancer.**
- **onCARlytics (CF33-CD19) is an OV that infects and inserts CD19 into solid tumor cells "marking" these cells for killing by CD19 targeting agents.**
  - **Three independent studies showed that onCARlytics can target and mark solid tumors (TNBC, HCC). Combination with T-cell therapies (blinatumumab, CyCART-CD-19 T-cells, and CD-19-Redirected ARTEMIS T-cell therapy) confirmed the mark and kill strategy.**
- **Additional studies and currently being considered.**