Latvian Biomedical
Research and Study Centre
research and education in biomedicine from genes to human

THERAPEUTIC MODULATION OF TUMOR MICROENVIRONMENT WITH RECOMBINANT VIRAL VECTORS

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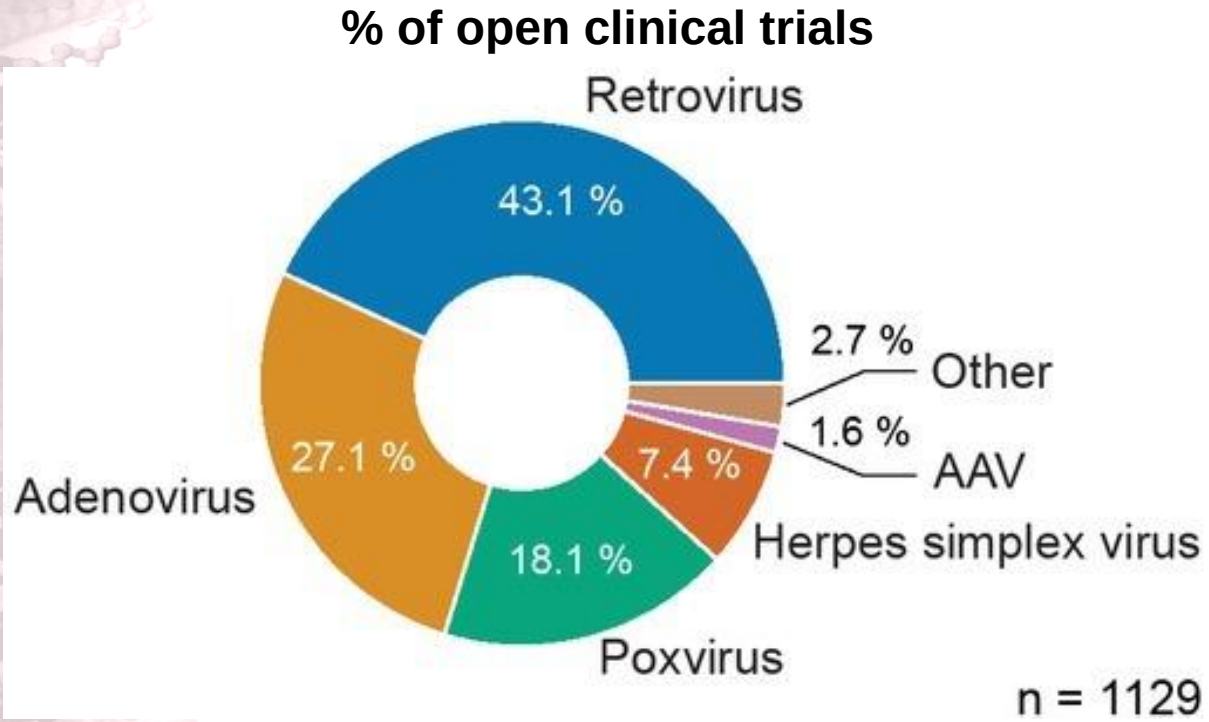
Application of viral vectors in clinical trials to treat cancer



Bezeljak, 2022
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8884858/

Pier Francesco Ferrucci, Laura Pala, Fabio Conforti, and Emilia Cocorocchio. Talimogene Laherparepvec (**T-VEC**): An Intralesional Cancer Immunotherapy for Advanced Melanoma. Cancers (Basel). 2021 Mar; 13(6): 1383. 2021 Mar 18. doi: 10.3390/cancers13061383

Zhang W-W, Li L, Li D, Liu J, Li X, Li W, et al. The first approved gene therapy product for cancer **Ad-p53** (**Gendicine**): 12 years in the clinic. Human Gene Therapy. 2018;29:160-179. DOI: 10.1089/hum.2017.218

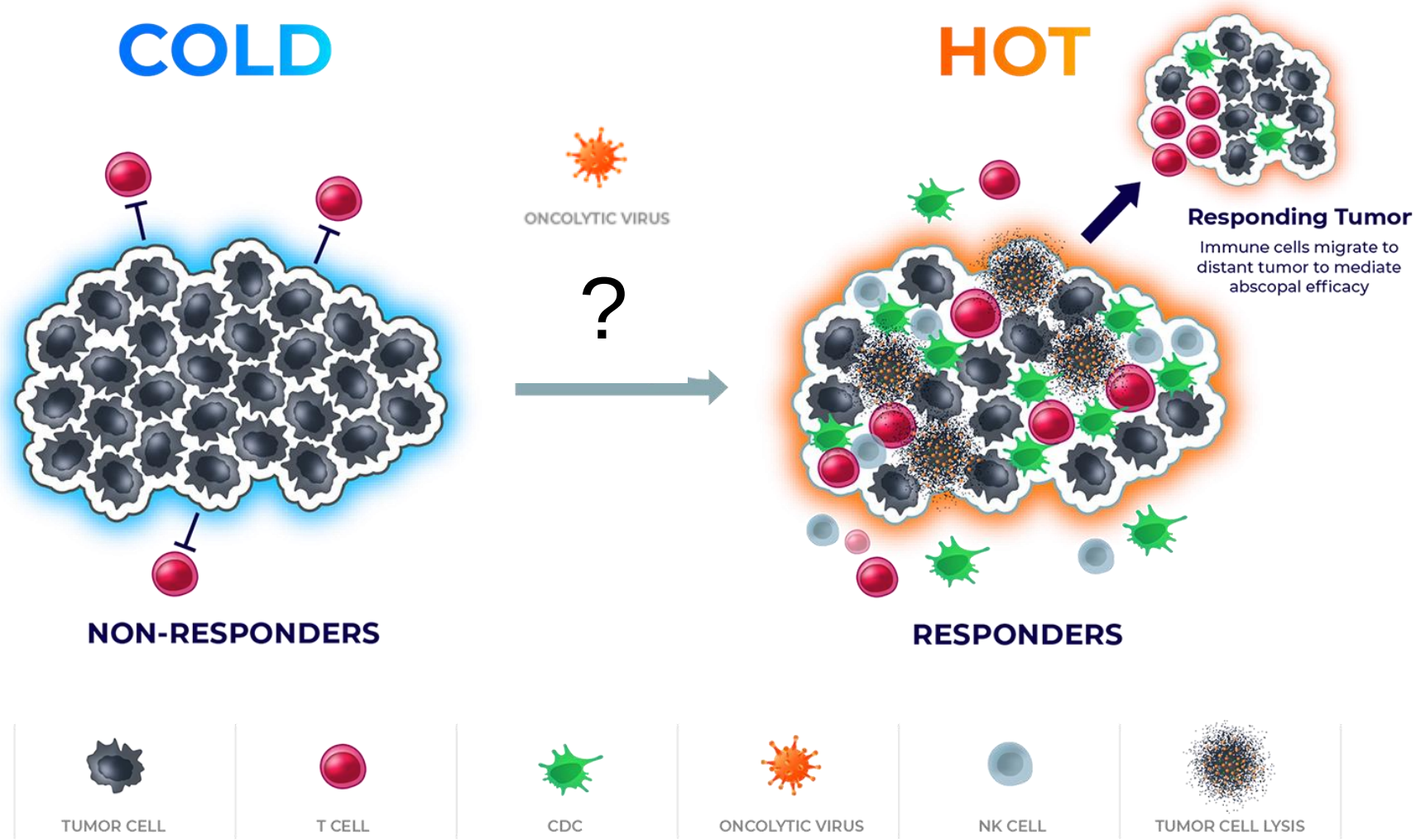
Liang M. **Oncorine**, the world first oncolytic virus medicine and its update in China. Current Cancer Drug Targets. 2018;18:171-176. DOI: 10.2174/1568009618666171129221503

Delytact

Jahan N, Ghouse SM, Martuza RL, Rabkin SD. In situ cancer vaccination and immunovirotherapy using oncolytic **HSV**. Viruses. 2021;13:1–27. doi: 10.3390/v13091740.

phase III clinical study
IFNα-2b rAd-IFNα/Syn3 (Ad5)+ polyamid surfactant (Syn3); patients with non-muscle-invasive bladder cancer (Boorjian, et al. Intravesical Nadofaragene Firadenovec Gene Therapy for BCG-Unresponsive Non-Muscle-Invasive Bladder Cancer: A Single-Arm, Open-Label, Repeat-Dose Clinical Trial. Lancet Oncol. 2021, 22, 107–117.)

GM-CSF T-VEC (∆ICP34.5 oHSV1) **anti-CTLA-4** (ipilimumab) clinical trials with metastatic stage IIIB/C–IVM1a melanoma (Ferrucci, et al, Talimogene Laherparepvec (T-VEC): An Intralesional Cancer Immunotherapy for Advanced Melanoma. Cancers 2021, 13, 1383.



https://www.oncorus.com/our-technology/

Table 1. Comparison of viral vectors.

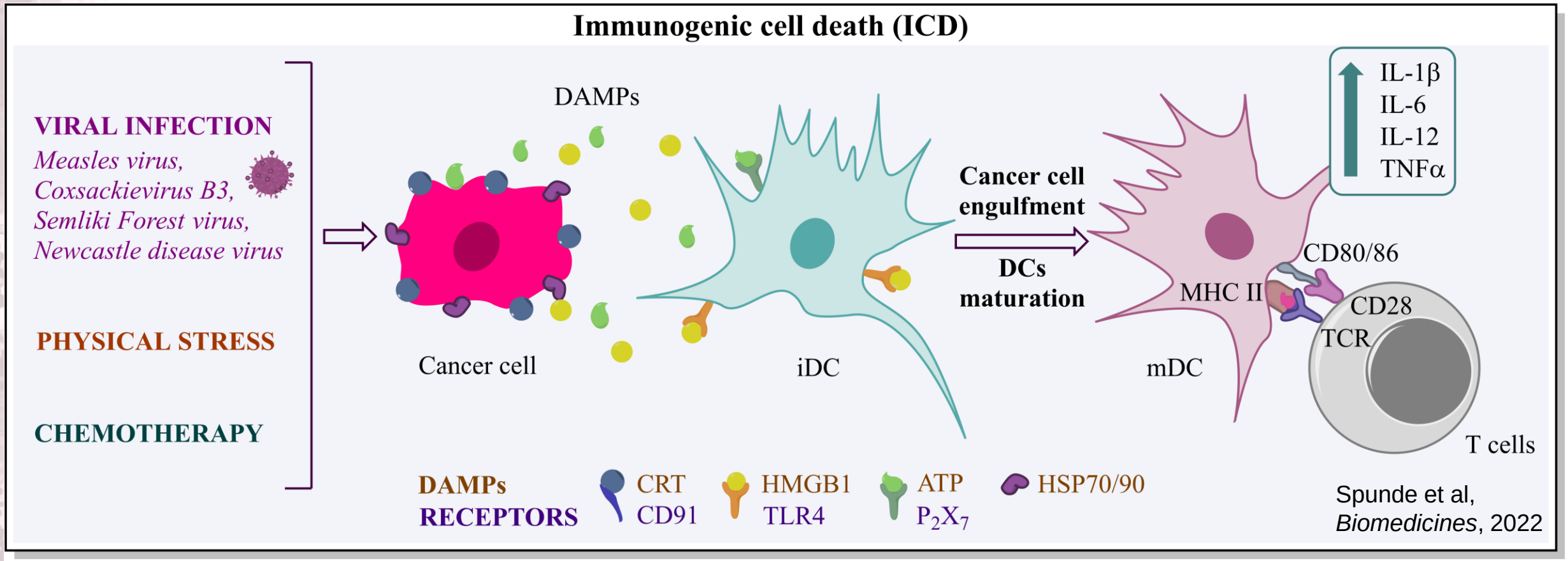
Virus Type	Insert Capacity	Cell Receptor/Tropism	Advantages	Limitations
		DNA vectors		
Adenoviruses	up to 7.5 kb up to 36 kb (fully deleted helper dependent Ads)	Coxsackie Adenovirus receptor (CAR); CD46	• high transduction efficiency • broad tissue tropism • availability of scalable production system • tumour-specific gene promoters	• pre-existing viral immunity • strong immune responses against vector proteins • biosafety concerns (random integration)
Poxviruses	up to 24 kb 7.5 kb (MVA)	binding to glycosaminoglycans following cell fusion; virus replication and spread are dependent on epidermal growth factor receptor (EGFR) signalling; preferential replication in cancer cells	• inherently tumour targeting • cytoplasmic replication • low prevalence of anti-vector immunity • large production of clinical grade preparations available • stable in blood following intravenous injection and highly efficient systemic delivery	• replication-deficient Poxvirus vectors encoding heterologous antigens have a lower ability to prime immune responses in humans than other viral vectors • adaptive immune response against the vector • large virus particles hampering their intratumoral spread
Herpesviruses	up to 40 kb (replication-deficient vector) up to 14 kb (HSV1)	Herpesvirus Entry Mediator and nectin 1 (HSV-1)	• selective replication in tumours • potent cytolytic capability • blood-brain barrier crossing • availability of scalable production system	• potential neurovirulence (HSV-related encephalitis) • genetically modified HSV vectors are not very efficient compared to oncolytic wild-type variants • pre-existing immune response • strong immune responses against vector proteins

Spunde et al, *Biomedicines*, 2022

https://www.mdpi.com/2227-9059/10/9/2142

Virus Type	Insert Capacity	Cell Receptor/Tropism	Advantages	Limitations
RNA vectors				
Rhabdoviruses	4–6 kb	multiple receptors were proposed (phospholipids and gangliosides, nicotinic acetylcholine receptor, neural cell adhesion molecule, and low-density lipoprotein gene family receptors (LDLR)	• selective and efficient replication in tumour cells including in metastases • high oncolytic properties • pseudotyping capabilities • ability to cross the blood-brain barrier (VSV)	• infection of tumour-associated DCs reduces their antigen presentation properties • potential neurovirulence (VSV) • insufficiently developed large-scale manufacturing technology
Alphaviruses	up to 5 kb	very low-density lipoprotein receptor (VLDL-R) and apolipoprotein E receptor 2 (ApoER2)	• low specific immune response against the vector • low pre-immunity • tumour tropism (SIN) • induction of immunogenic cell death • high level of transgene expression	• modest insert capacity • short time expression • potential neurovirulence (SFV) • insufficiently developed large-scale vector production system
Arenaviruses	up to 2 kb	preferentially infect monocytes, macrophages, and DCs through binding to α-dystroglycan (α-DG)	• non-lytic infection of dendritic cells • efficient $CD8^+$ T cell immunity • weak neutralizing antibody response against the vector • rare pre-existing anti-vector immunity • safe in human	• low insert capacity • limited direct oncolytic properties • insufficiently developed large-scale vector production system

Virus Type	Insert Capacity	Cell Receptor/Tropism	Advantages	Limitations
RNA vectors				
Enteroviruses	0.3–1.7 kb	immunoglobulin-like receptor, CD155; Nectin-like molecule 5 (Poliovirus), coxsackie-adenovirus receptor (CAR); RGD motif of integrins (Coxsackievirus), other co-receptors	• tumour tropism • neurotropism (Poliovirus) • low pathogenicity • oncolytic replication	• very low insert capacity • unstable genome • high levels of pre-existing immunity to polio vectors • insufficiently developed large-scale vector production system
Reoviruses	up to 1.5 kb within two RNA segments	the receptor is unknown, but is thought to include sialic acid and junctional adhesion molecules (JAMs)	• oncolytic properties • relatively non-pathogenic in adults • tumour tropism	• insufficiently developed recombinant vector platform • transient expression • anti-vector pre-immunity (neutralising Abs) • very low insert capacity
Paramyxovirus	up to 6 kb (Measles virus, MV) 4.5 kb (Newcastle disease virus, NDV)	different receptors: *MV*: signal lymphocyte-activation molecule (SLAM or CD150) CD46, Nectin-4 *NDV*: sialic acids on the tumour cell surface	• tumour tropism • pDC maturation • oncolytic properties • low seroprevalence (NDV) • safe for human	• pre-existing immunity (MV) • insufficiently developed recombinant vector platform



Ma et al. *Cell Death and Disease* (2020)11:48
https://doi.org/10.1038/s41419-020-2236-3

Cell Death & Disease

ARTICLE Open Access

Characterization of virus-mediated immunogenic cancer cell death and the consequences for oncolytic virus-based immunotherapy of cancer

Jing Ma[1], Mohanraj Ramachandran[1], Chuan Jin[1], Clara Quijano-Rubio[1,2], Miika Martikainen[1], Di Yu[1] and Magnus Essand[1]

Adenovirus initiates multiple cell death pathways including necroptosis, inflammasome activation and autophagy before the tumor cells die by Ad-mediated lysis.

VV mediated cell lysis is primarily accompanied by induction of necroptosis and autophagy

SFV4 induces rapid cell lysis accompanied by induction of immunogenic apoptosis

Necroptosis is not associated with SFV4 infection

Tumour Immune Microenvironment

"Cold" Immunosuppressive

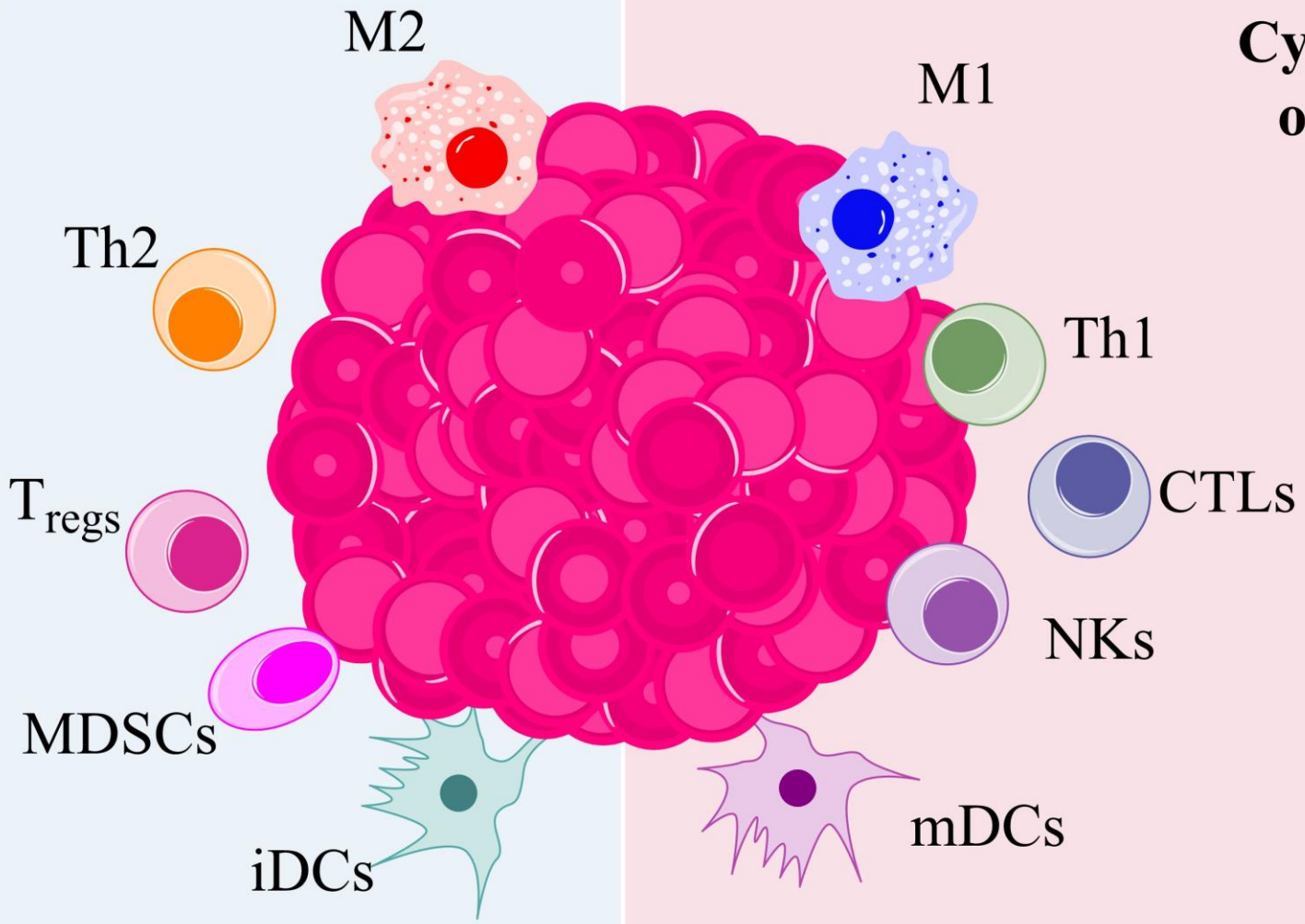
Cytokines and other factors

IL-4
IL-10
IDO
COX2
EGF
HGF
TGFβ
VEGF

"Hot" Immunostimulating

Cytokines and other factors

NO
IL-1β
IL-6
IL-12
TNFα
IFNγ



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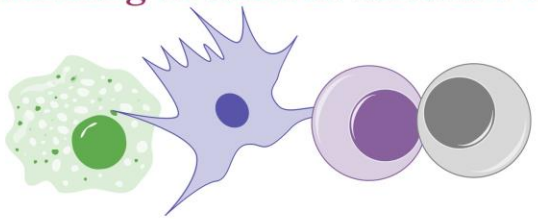
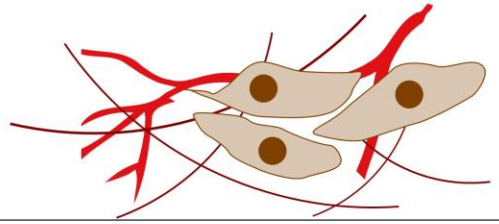
Therapeutic strategies

1 → Programming of tumour immune cells

2 → Programming of tumour stroma





1 Programming of tumour immune cells

	MDSCs	Macrophages	DCs	NKs	T cells
Transgene	IL-12 Anti-IL-10 (?)	IFNs TNFα CCL2 Anti-SIRPα	IFNs IL-12 MIP3α CD40L GM-CSF	IFNs IL-2 IL-12 IL-15 IL-18 IL-21	IFNs Anti-Treg Anti-PD-L1 IL-2 IL-12 IL-15 IL-18 CD80 CCL5
Effect	Reprogramming into DCs T_{reg} inhibition	M1/M2 ratio ↑ Phagocytosis Macrophage recruitment TAA presentation T cell stimulation	mDC/iDC ratio ↑ TAA presentation T cell priming	Tumour cell lysis	Th1 immune response CTL/T_{reg} ratio ↑ Tumour cell lysis T cell recruitment

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Programming of tumour stroma

2

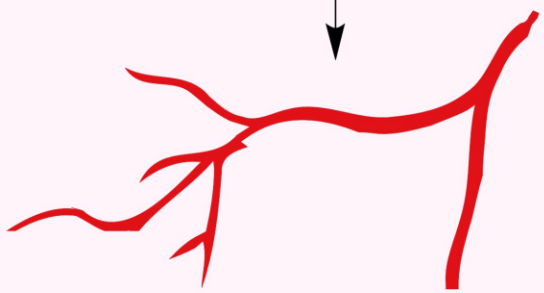
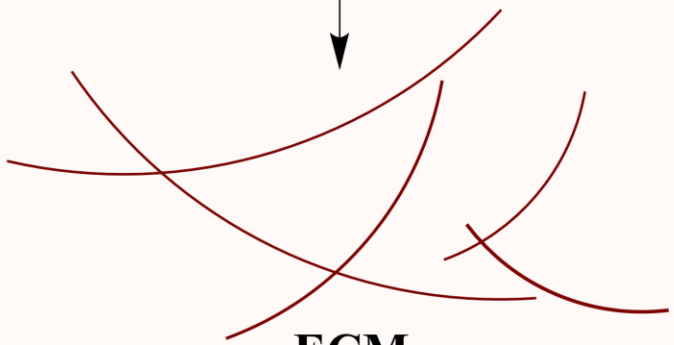
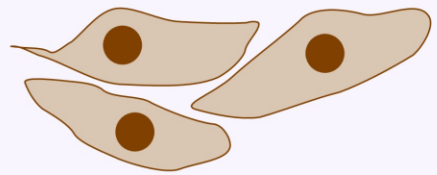
Transgene	Anti-TGFβ Anti-VEGF	Relaxin Hyaluronidase	Anti-TGFβ
			
	Vascular endothelial cells	ECM	CAFs
Effect	Angiogenesis ↓	Intratumoral virus spread, drug delivery and immune cell infiltration ↑	Invasion ↓

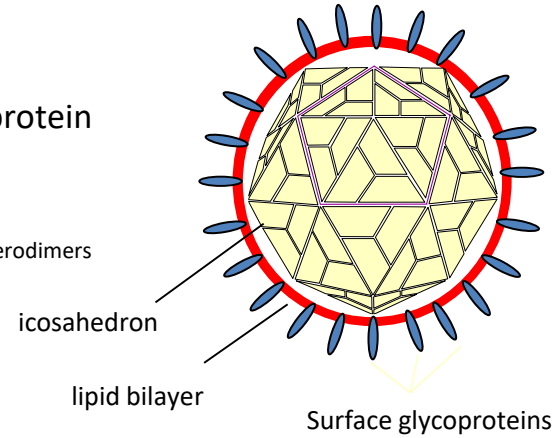
Table 4. Virus vectors encoding cytokines and other molecules for remodelling tumour multiple immune cell populations.

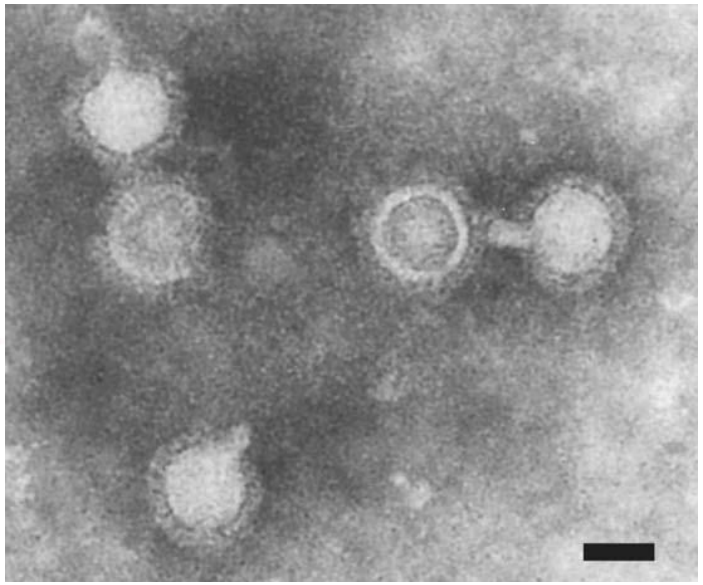
Transgene	Virus Vector (Virus Backbone)	Model	Effect
		Adenoviruses	
TNFα IL-2	Ad5-CMV-mTNFα Ad5-CMVmIL2 (Ad5)	murine B16.OVA melanoma; C57 BL/6JOlaHsd mice	complete tumour regression in all animals treated with anti-PD1 antibodies and corresponding viruses; Th1 immune response and increased intra-tumoral proportion of $CD8^+$ and $CD4^+$ T cells
GM-CSF CTLA-4 ab	SKL001 SKL002 Ad5	CMT-64 mouse small lung carcinoma, B16F10 murine melanoma, human A549 lung s.c. xenograft model	selective replication and anti-tumour activity after intravenous administration was shown in mouse B16F10 melanoma tumour and human tumour xenograft model; combination of the viruses potentiated anti-tumour activity
Anti TGFβshRNA GM-CSF (in one vector) plus MART1 (DNA/TAA)	AdGshT	murine B16BL6-CAR/E1B55 malignant melanoma; C57BL/6 mice	treatment by both DNA vaccine expressing TAA (MART1) and oncolytic adenovirus, encoding GM-CSF together with shRNA to TGF-β2 resulted in significant anti-tumour effects, however, complete regression of tumours was not achieved
IL-12p35, IL-12p40; GM-CSF and RLX (relaxin)	oAd/RLX oAd/IL12/GM-RLX	Syrian hamster s.c. and orthotopic pancreatic tumour models.	expression of IL-12, GM-CSF and RLX mediated by a single oncolytic Ad vector promoted remodelling of TME to potentiate antibodies-based therapies
IL-12 plus VEGF binding shRNA	RdB/IL12/shVEGF (Ad5)	murine B16-F10 melanoma; C57BL/6 mice	Efficient anti-tumour effect with massive tumour infiltration of differentiated $CD4^+$ T cells, $CD8^+$ T cells, NK cells, and DCs. Suppressed expression of VEGF, supporting the restoration of the anti-tumour immune response

		Herpesviruses	
CCL2 mIL-12	M010 M002 (ΔICP34.5 oHSV)	neuroblastoma Neuro-2a tumours s.c. syngeneic A/J mouse strain	combined treatment led to the most efficient tumour growth inhibition
IL-12 IL-15 PD1v GM-CSF IL7 plus CCL19	oHSV2-IL12, -PD1v, -IL15, -IL7-CCL19, -GM-CSF ΔICP34.5 ΔICP47 oHSV2 (HG52 strain)	breast cancer 4T1 and colon carcinoma CT26 murine tumour models; Balb/c mice	all vector variants used as a single treatment have had a similar anti-tumour activity; the most potent activity was demonstrated for all five virus vector combinations; the tumour re-challenge exhibited that cocktail therapy prevents secondary tumourogenesis
IL-12 GM-CSF (in one vector)	R-123 hHER2 retargeted ΔICP34.5 oHSV	human breast cancer SK-OV-3 cells, Lewis lung carcinoma murine cell line expressing hHER2 (HER2-LLC1) s.c. tumours; hHER2-transgenic C57BL/6 mice (B6.Cg-Pds5bTg(Wap ERBB2)229Wzw/J)	combined treatment with anti-PD1 led to significant inhibition of tumour growth with complete tumour resection in case, (mGM-CSF), mIL-12+mGM-CSF) expressing vector; systemic delivery of double-armed virus combined with anti-PD1 inhibited the development of tumour metastasis
		Poxviruses	
PD-1 fused with IgG1 Fc plus GM-CSF	VV-iPDL1/GM WR vvDD	murine s.c. tumours Luc B16-F10 melanoma; Murine breast cancer Py230 and MC38 colon adenocarcinoma; C57BL/6 mice	the highest tumour growth inhibition was observed in VV-iPDL1/GM treated animals, compared to single treatments; CD8 T cell depletion significantly abolished the systemic anti-tumour activity of VV-iPDL1/GM; increased DCs ($CD11c^+$) infiltration was observed in VV-iPDL1/GM treated mice

Alphaviruses

Enveloped virus
Icosahedron : 240 copies of 1 protein
Spherical : 65-70nm
Envelope : 80 trimer spikes
each spike = 3 x E1/E2 heterodimers

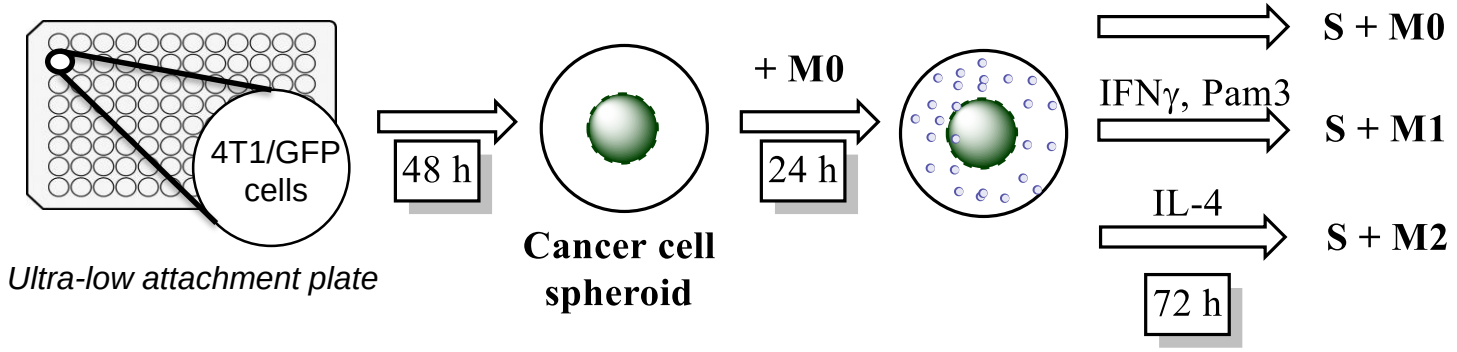


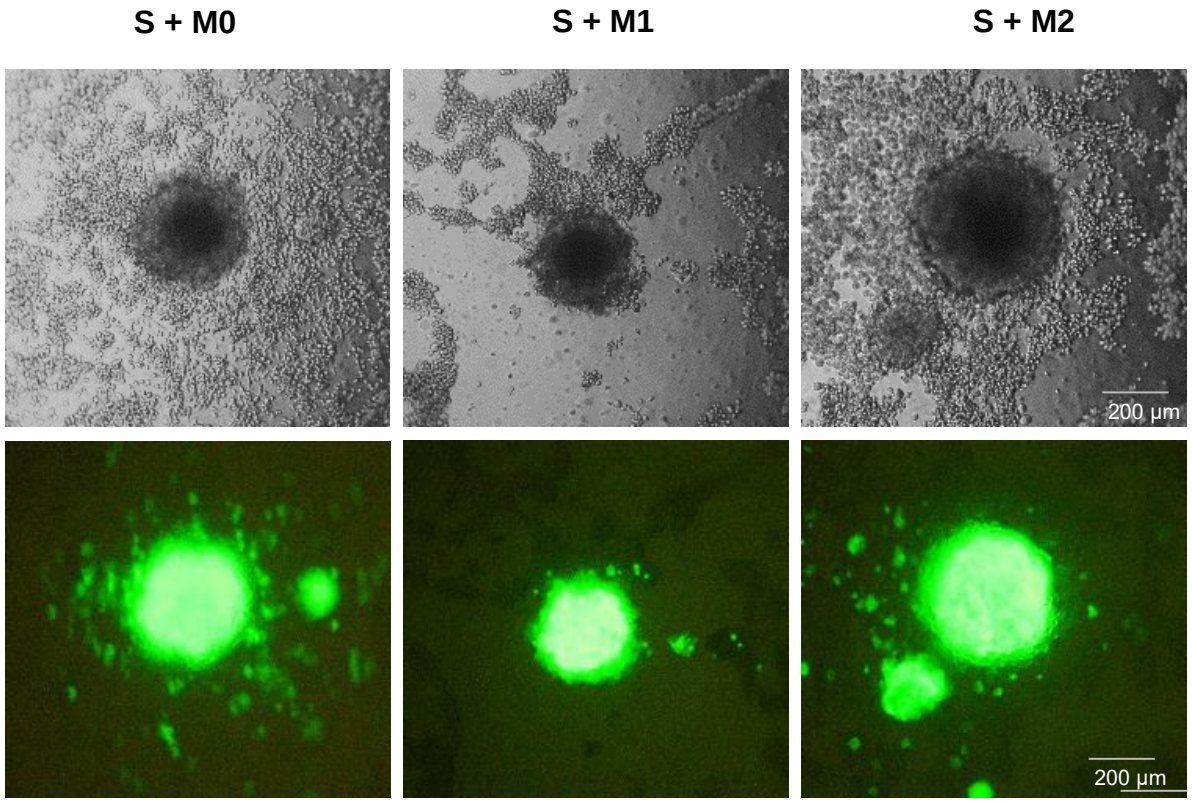


Electron microscopy of recombinant SFV particles. Negative staining, bar 50 nm. (Zajakina *et al*, 2010)

- RNA replication and high transgene expression
- No risk of integration
- Alphaviruses can target lymph nodes
- Dendritic cells infection
- Targeting cancer cells
- High virus titers
- Safe for human
- No vector preimmunity
- Transient expression
- Oncolytic properties
- **Induce immunogenic cell death**

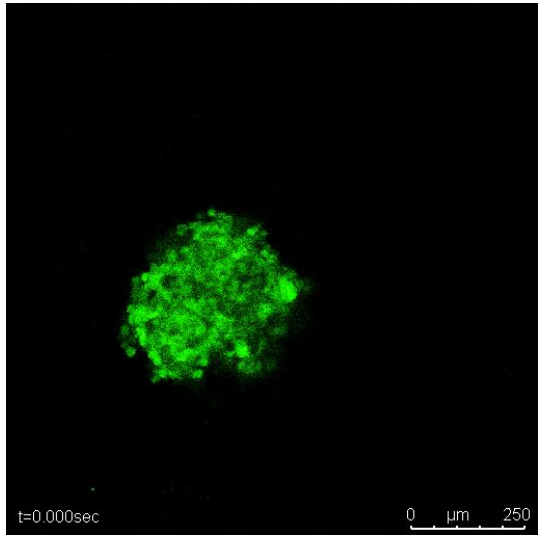
Co-culture of 4T1/GFP cell spheroids with BMDM



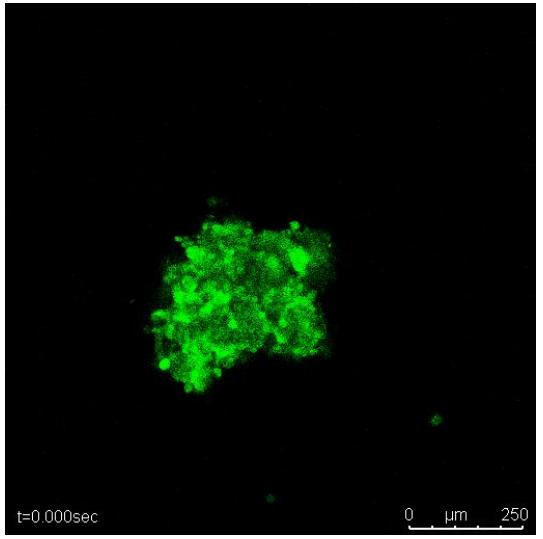


4T1/GFP (green), 48 h incubation with macrophages (unlabeled)

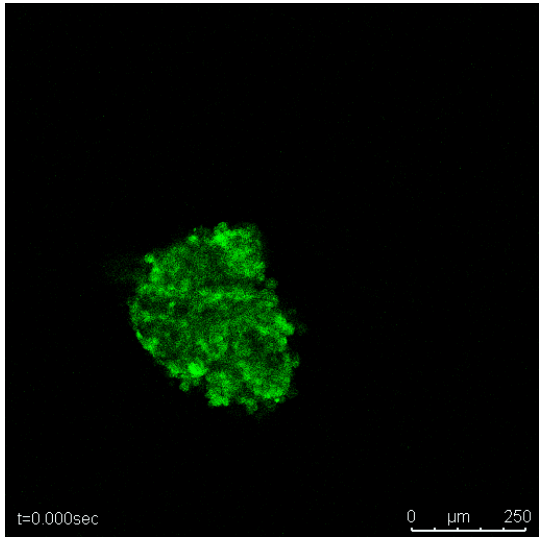
1_6 control (w/o M)



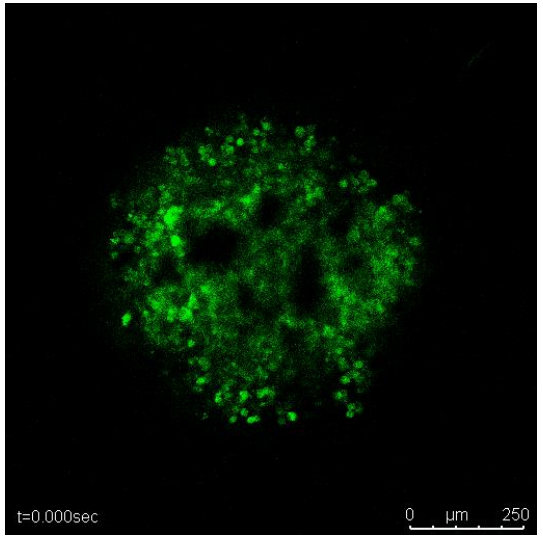
1_9 M0



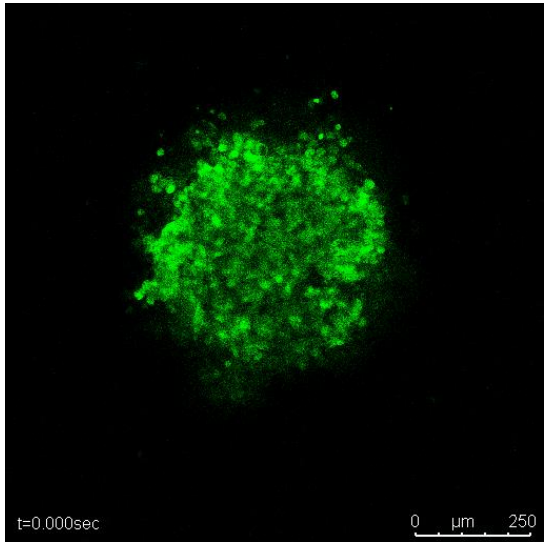
1_4 M1



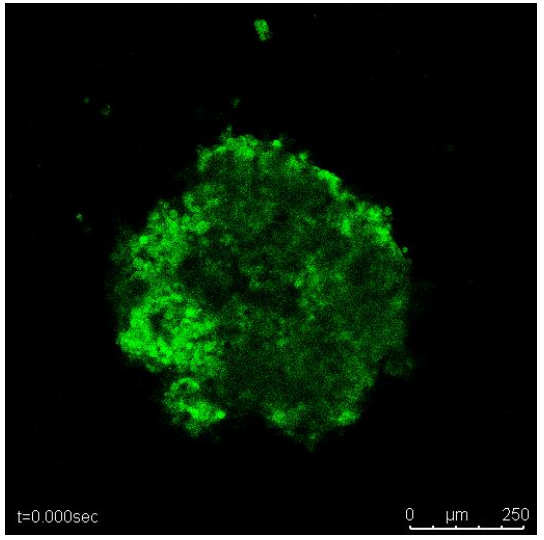
2_2 control



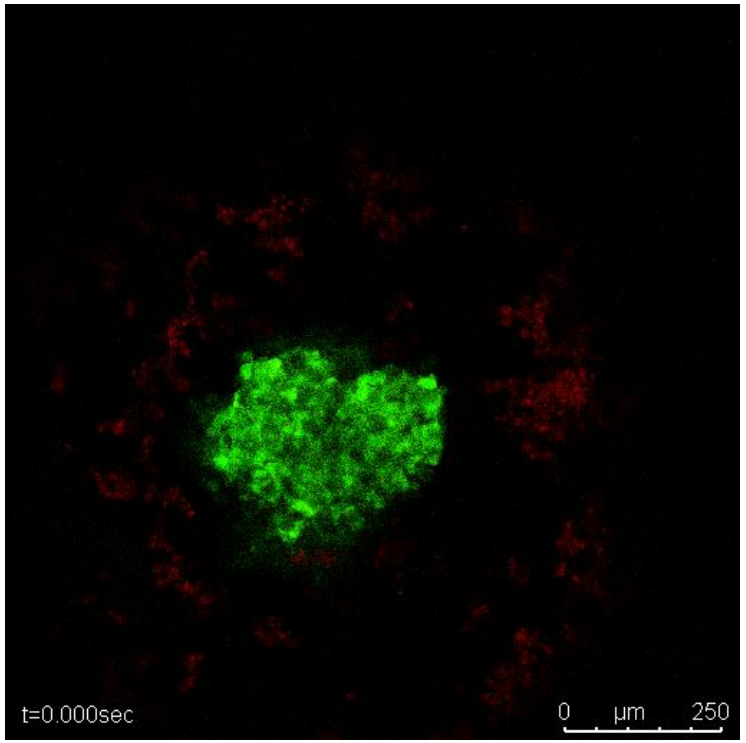
2_9 M0



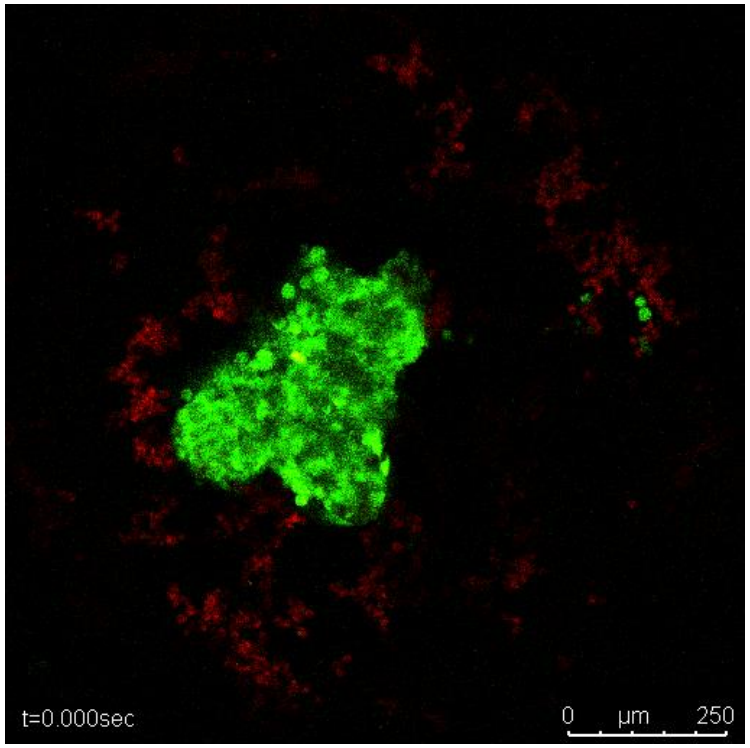
2_4 M1



4T1/GFP (green) + M1 (red)



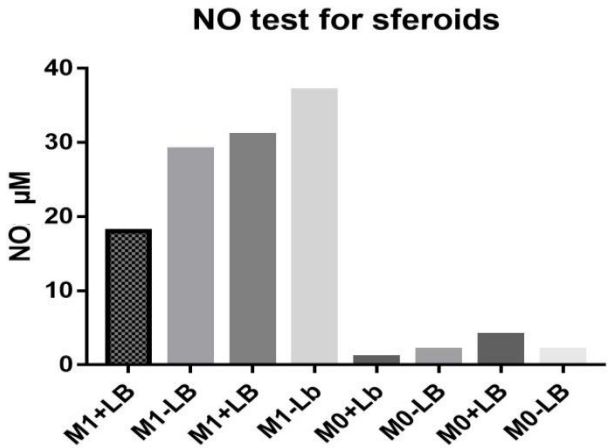
4T1/GFP (green) + M0 red



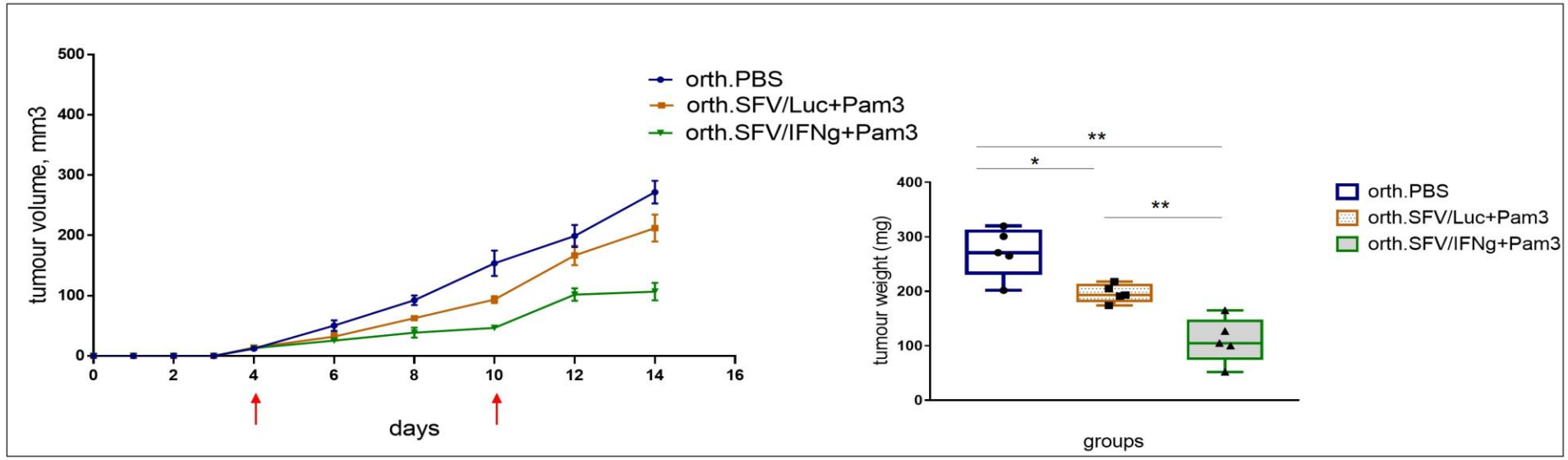
tumour spheroids (green) were incubated with macrophages (red) for 24 h

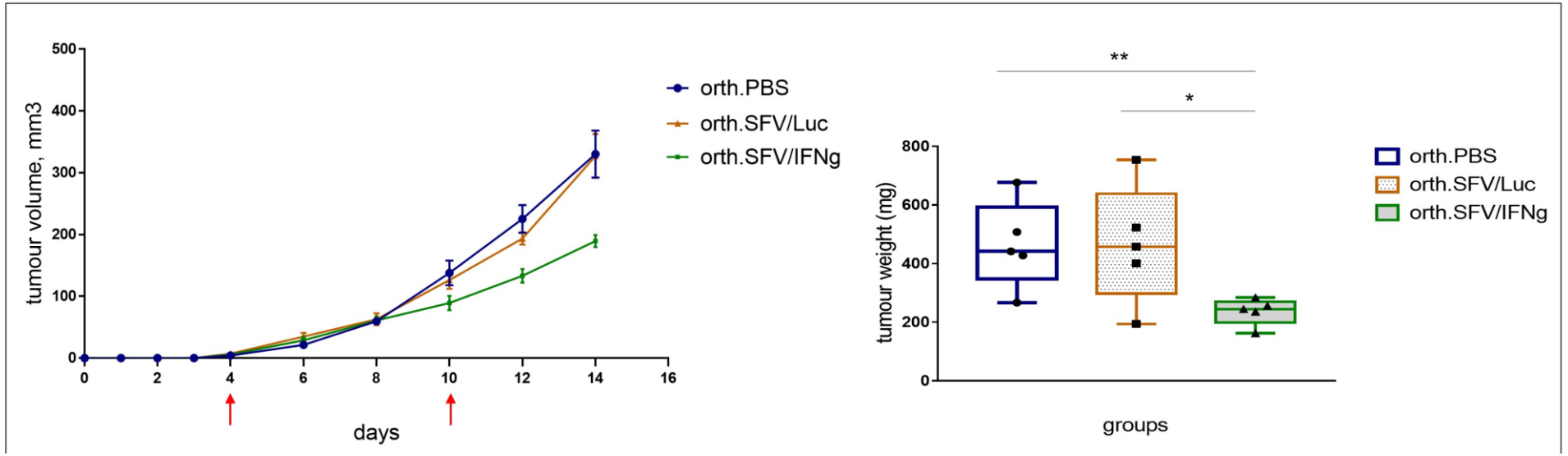
Observations:

- downregulation GFP intensity (M1)
- migration of GFP+ cells out of the spheroid (M0)
- NO test day 4



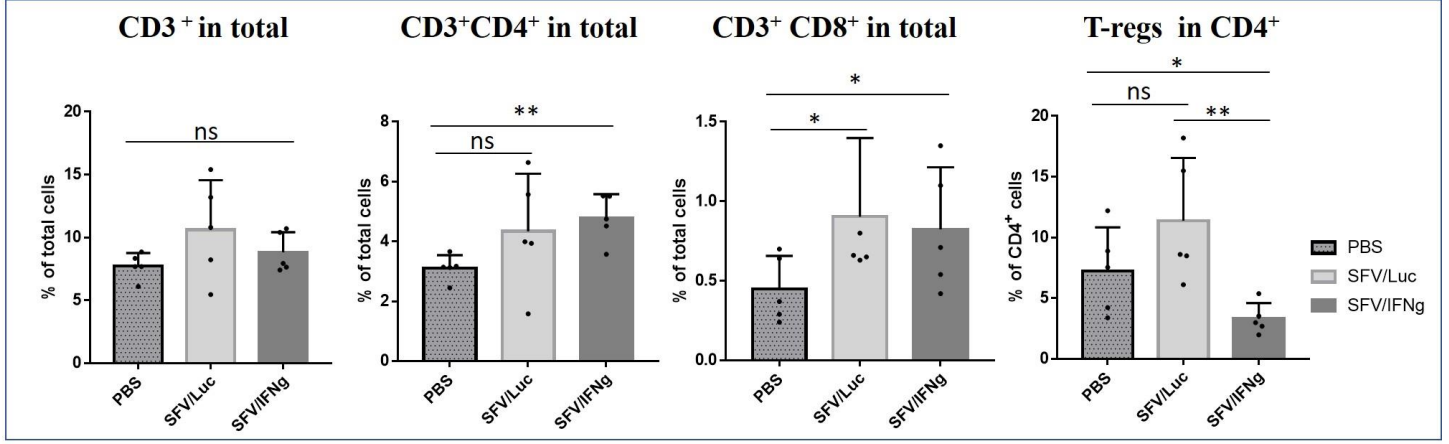
Inhibition of 4T1 tumour growth by i.t. injection of SFV/IFNg virus





Trofimova et al, *Vaccines* 2021

a.



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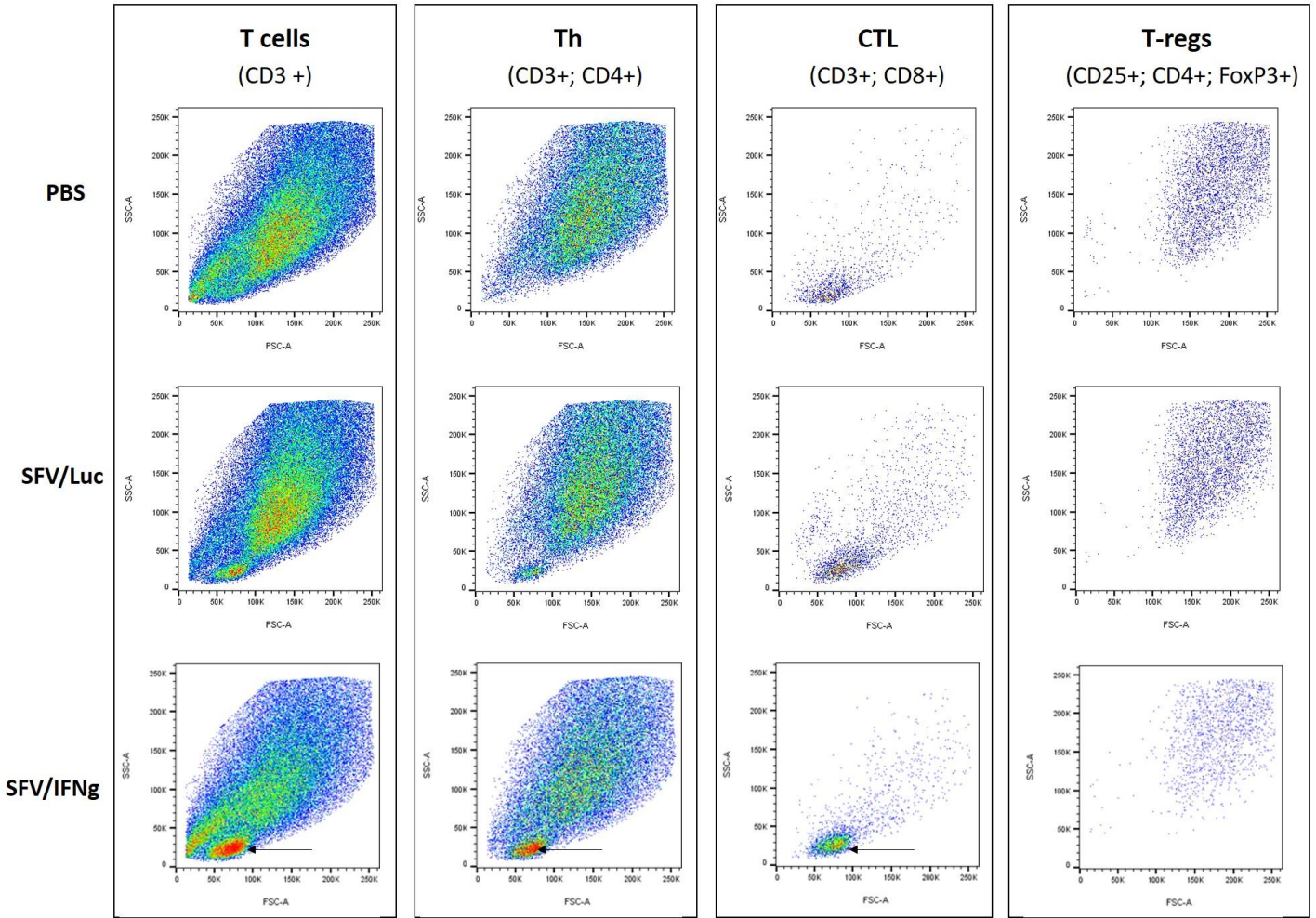
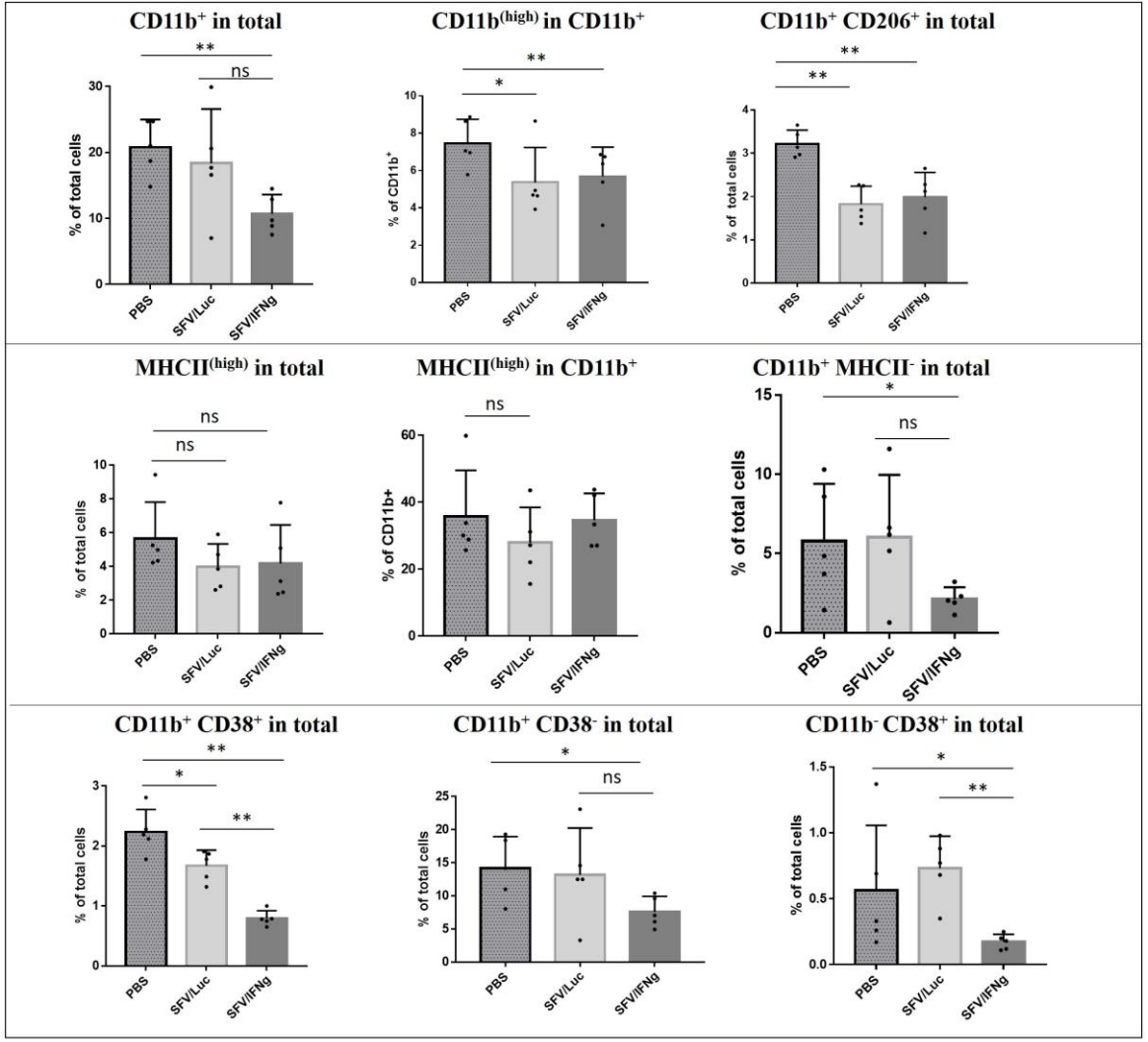
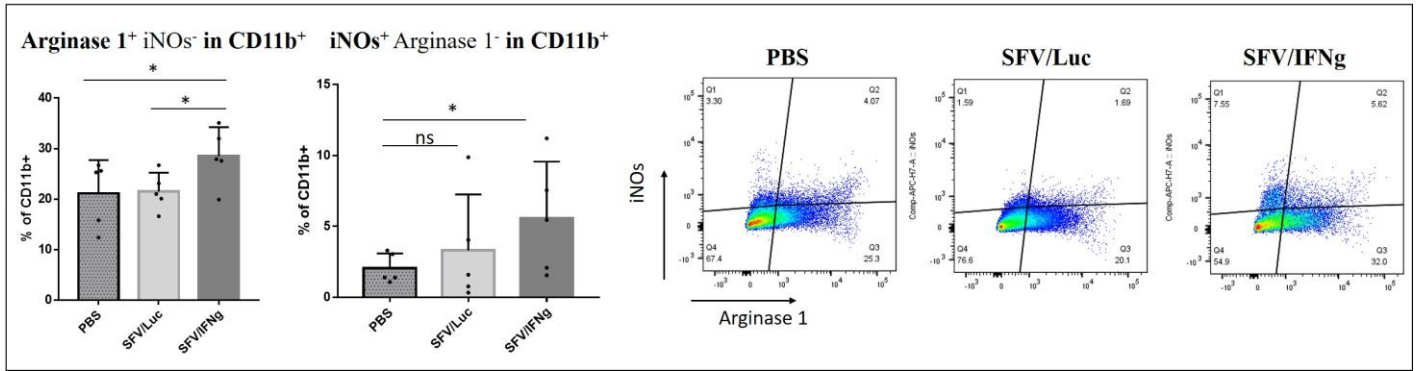
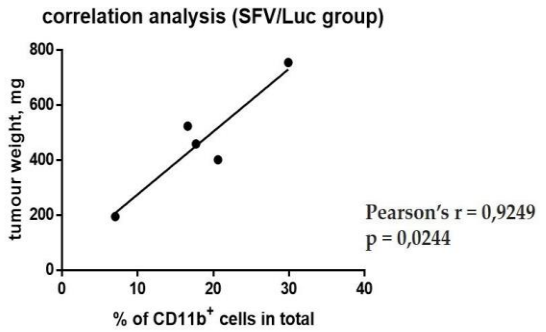


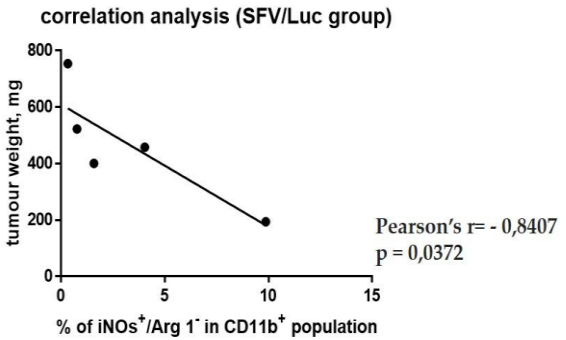
Flow cytometry analysis of T cells in the tumours treated with SFV/IFNg, SFV/Luc or PBS in orthotopic 4T1 mouse breast cancer model

Flow cytometry analysis of myeloid cells in the tumours treated with SFV/IFNg, SFV/Luc or PBS in an orthotopic 4T1 mouse breast cancer model

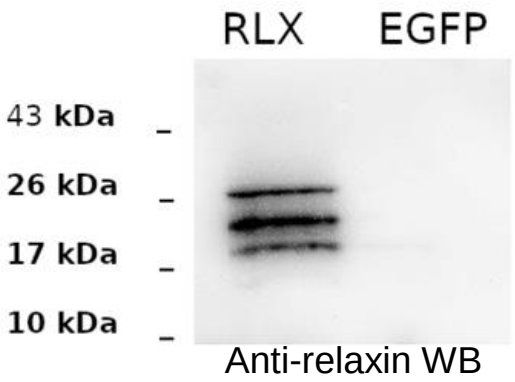




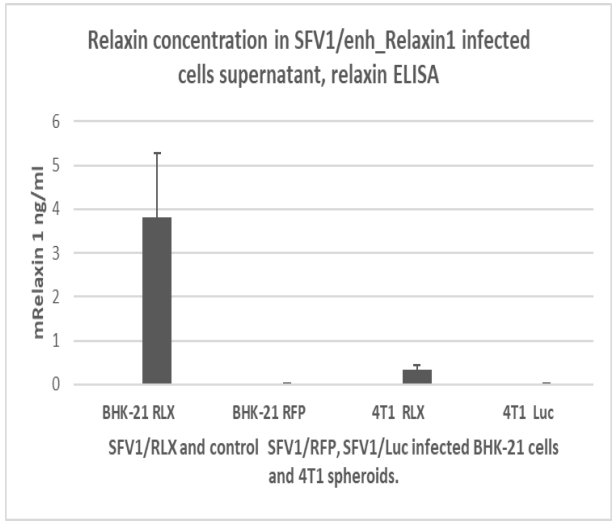


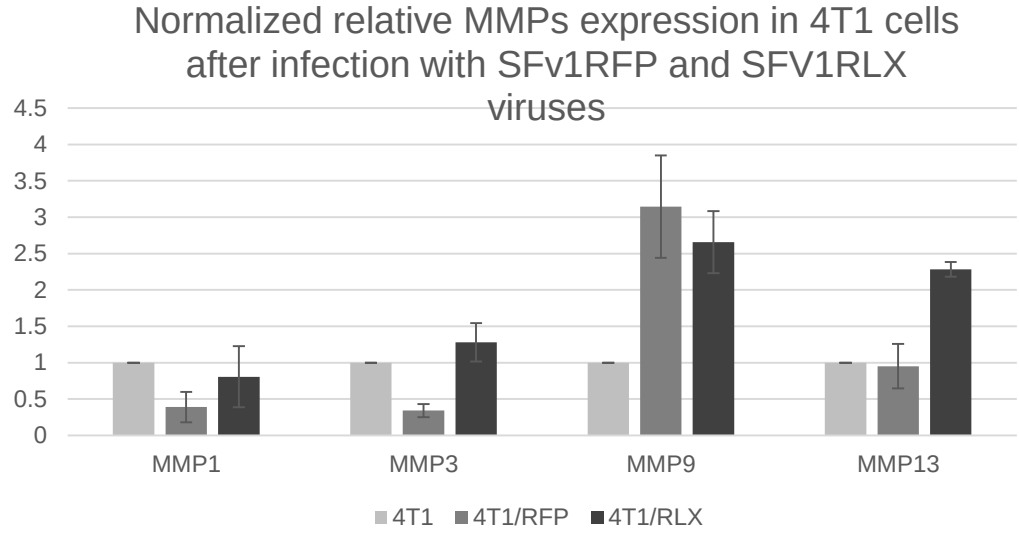


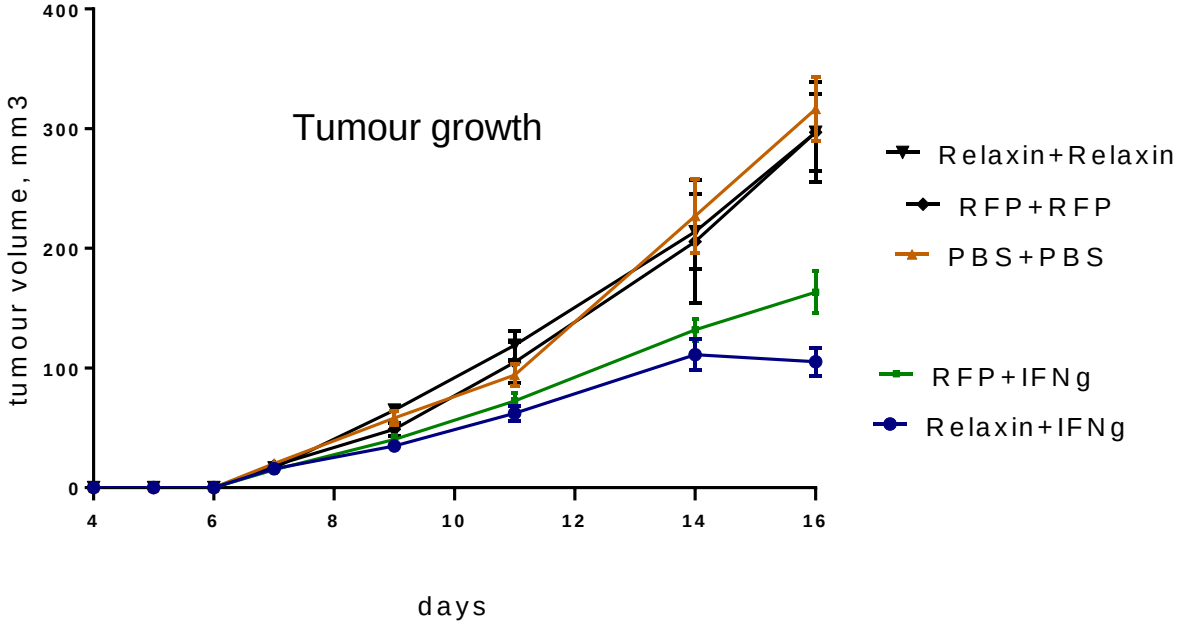
Inhibition of 4T1 tumour growth by i.t. injection of SFV/IFNg virus in combination with SFV/Relaxin

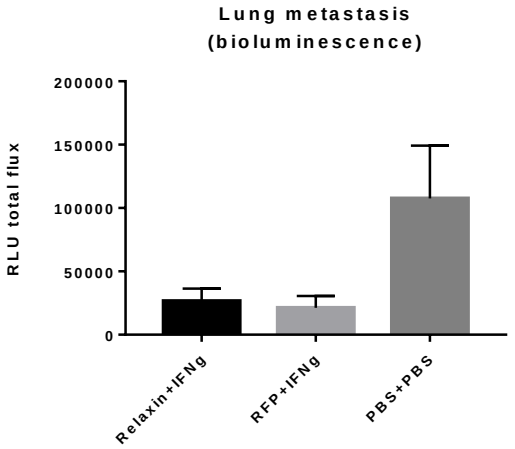


Anti-relaxin WB









SFV/IFNg vector

conclusions

- inhibition of tumor growth in an orthotopic 4T1 mouse breast cancer model
- significant increase in the populations of intratumoral Th cells and CTLs, and reduction of T-regs
- decreased intratumoral infiltration of myeloid cells expressing CD11b, CD206, or CD38
- enhanced inhibition of tumor growth in combination with Pam3CSK4 and Relaxin

SFV-based expression of IFNg benefits the antitumor immune response, representing a promising adjuvant to current immunotherapy and chemotherapy strategies.

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