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THE PIG AS A LARGE PRECLINICAL MODEL FOR THERAPEUTIC HUMAN ANTI-CANCER VACCINE DEVELOPMENT

Gregers Jungersen

SSI Center for Vaccine Research

TOO MANY CLINICAL TRIALS FAIL

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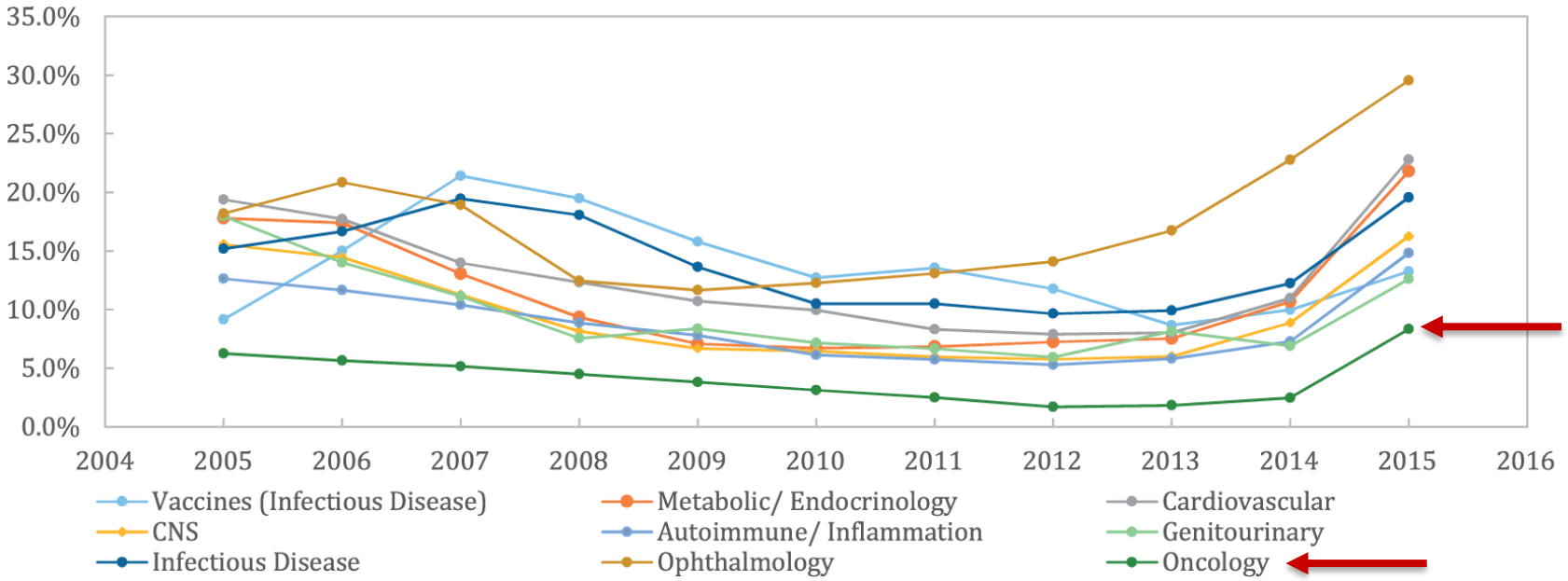
Probability of success for clinical trials:



Wong et al. 2019 Biostatistics, https://doi.org/10.1093/biostatistics/kxx069

PRECLINICAL MOUSE CANCER MODELS

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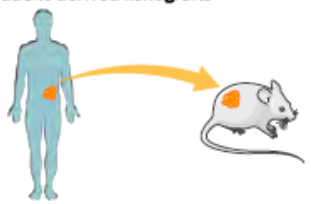
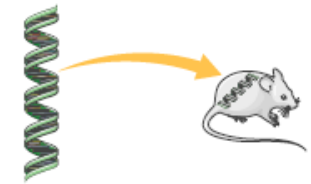
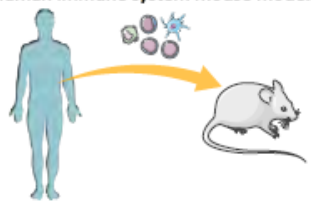
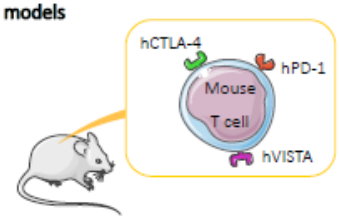
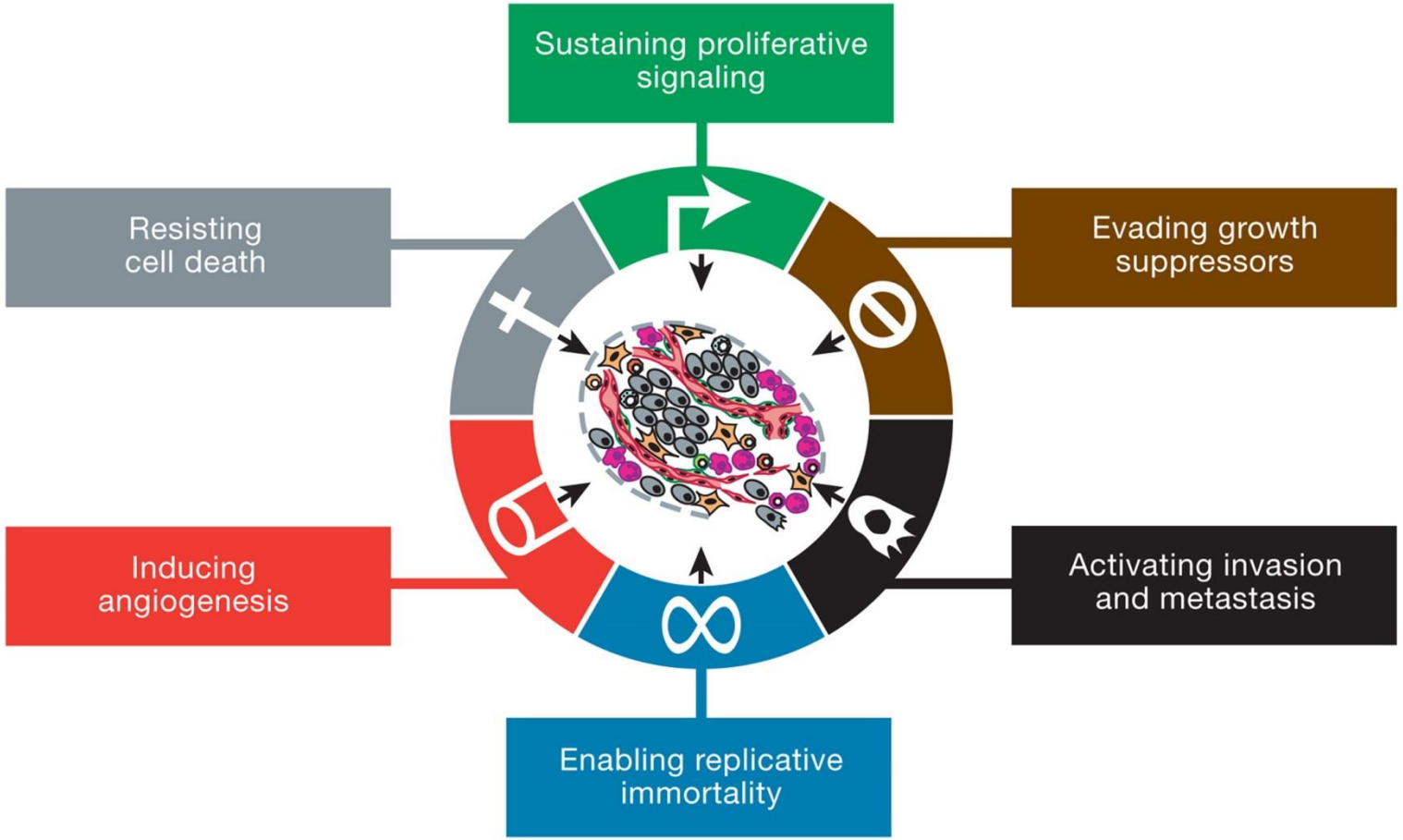
Cancer preclinical model	Advantages	Disadvantages
Cell line transplantation models 	• Simple and low cost • Rapid tumor growth • Highly reproducible phenotypes	• Mouse immune system • Insufficient number of simultaneous spontaneous tumors • Lack of intra- and inter-tumor heterogeneity
Patient-derived xenografts 	• Progressive tumor growth and amplification • Predictive therapeutic value • Maintenance of intra- and inter-tumor heterogeneity	• Immunodeficient model (i. e., no functional mouse immune system) • Physiological tumor microenvironment
Genetically engineered mouse models 	• Faithful recapitulation of human cancer development • Fully functional mouse immune system	• Mouse immune system • Time consuming and expensive • Unexpected and highly variable phenotypes
Human immune system mouse models 	• Studies on human immune cells' function in human tumor tissues	• Potential incompleteness and lack of physiological maturity of reconstituted human immune cells
Humanized immune checkpoint mouse models 	• Fully functional mouse immune system • Proper interaction between stroma, microenvironment, and immune cells	• Mouse immune system

Mouse models are/have been instrumental, but interpretation of immune responses is a problem

TRADITIONAL (2000) HALLMARKS OF CANCER



Hanahan & Weinberg, Cell, 2011

2011: THE INVOLVEMENT OF THE IMMUNE SYSTEM IN CANCER

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Hanahan & Weinberg, Cell, 2011; Couzin-Frankel et al., Science, 2013

THE CONCEPT OF CANCER IMMUNOEDITING

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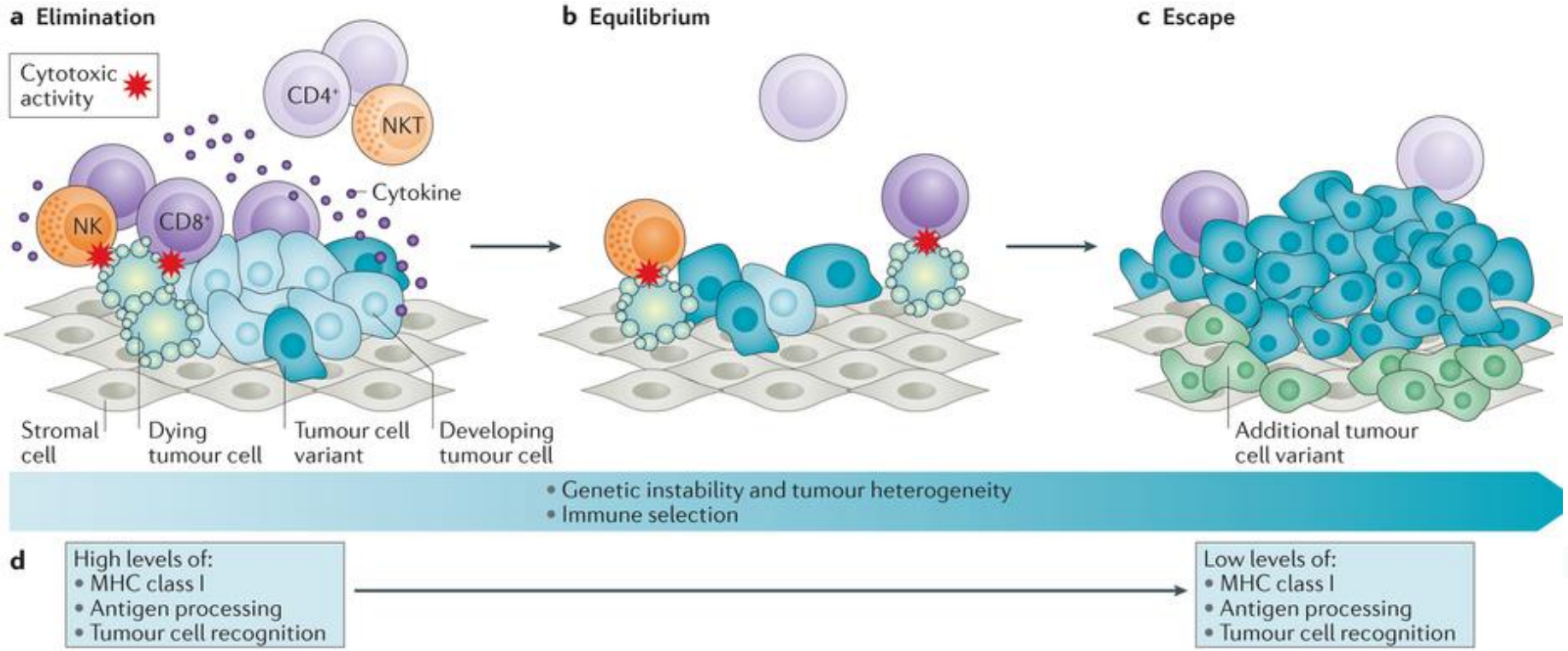

Hot tumor

Is this the normal?

Cold tumor

Uncontrolled tumor growth

Immunosuppressive tumor microenvironment

van der Burg et al, Nature Reviews Cancer, 2016

IMMUNE CHECKPOINT MOLECULES

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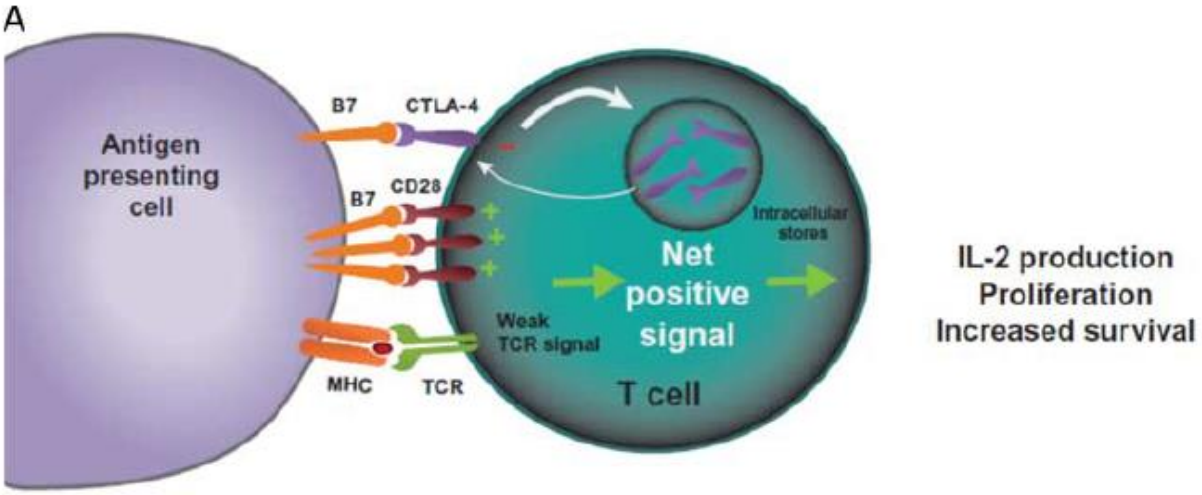
CTLA-4



Buchbinder and Desai, Am J Clin Oncol, 2016

IMMUNE CHECKPOINT MOLECULES

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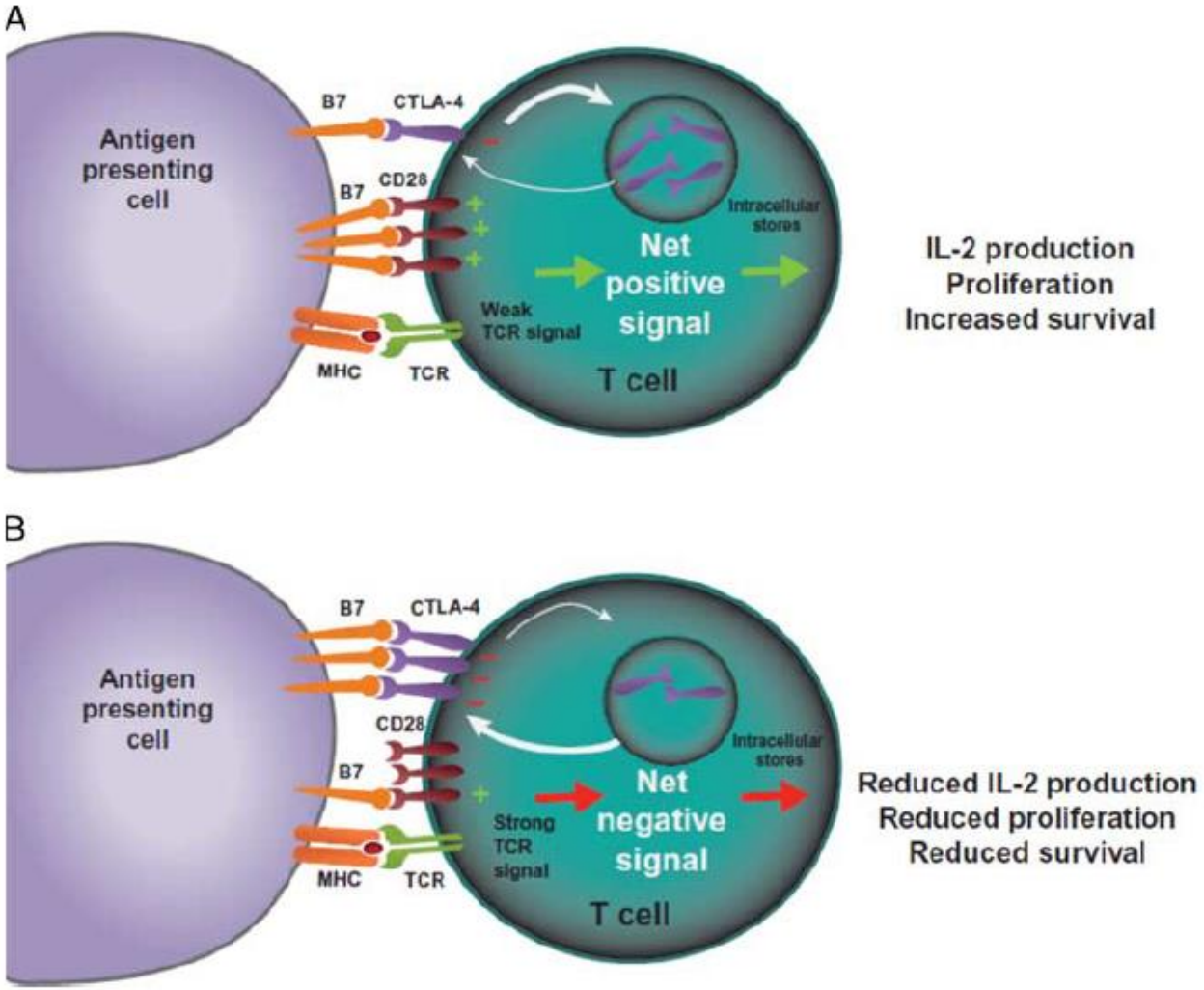
CTLA-4



Buchbinder and Desai, Am J Clin Oncol, 2016

IMMUNE CHECKPOINT MOLECULES

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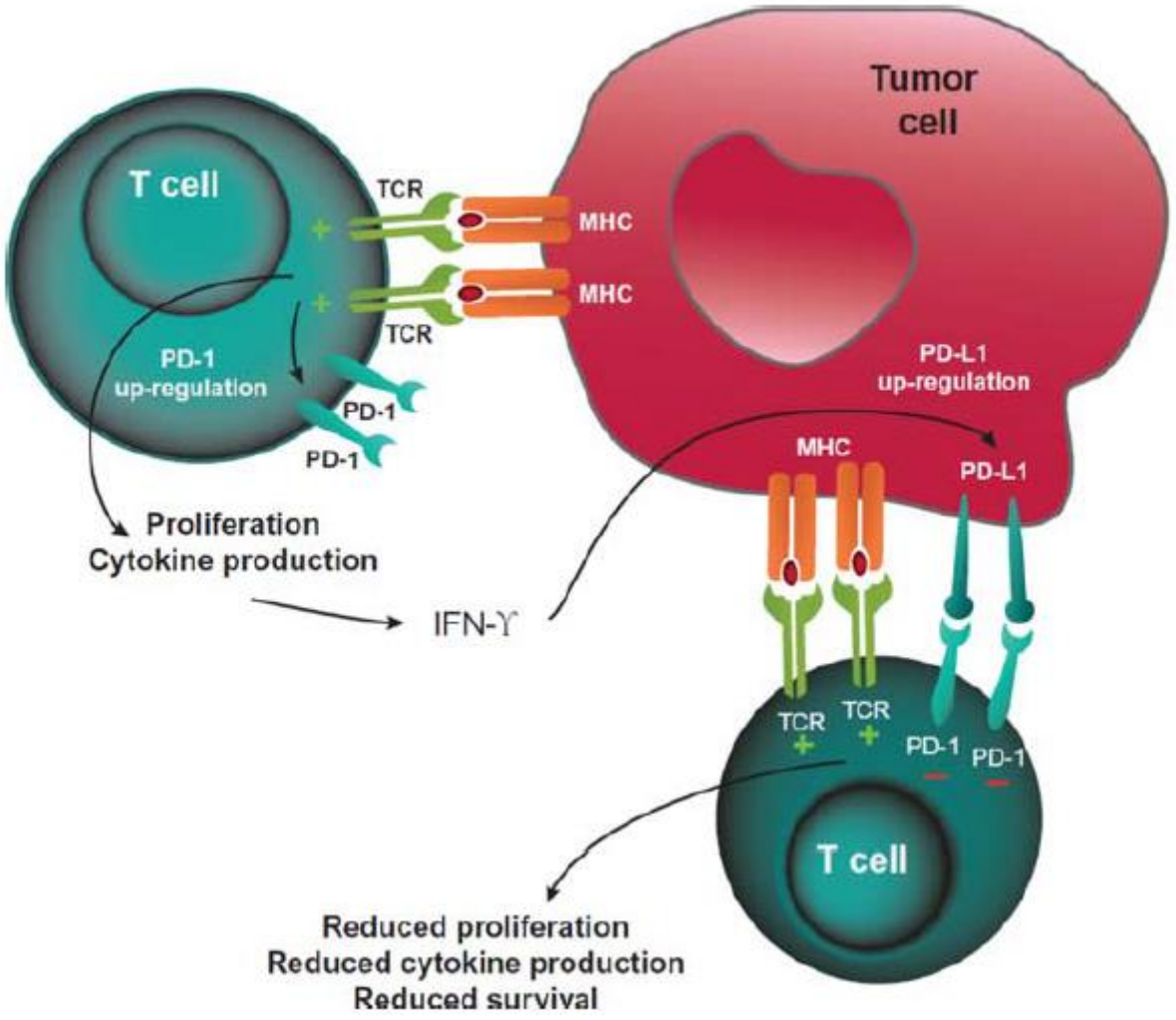
PD-1/PD-L1



Buchbinder and Desai, Am J Clin Oncol, 2016

IMMUNE CHECKPOINT MOLECULES

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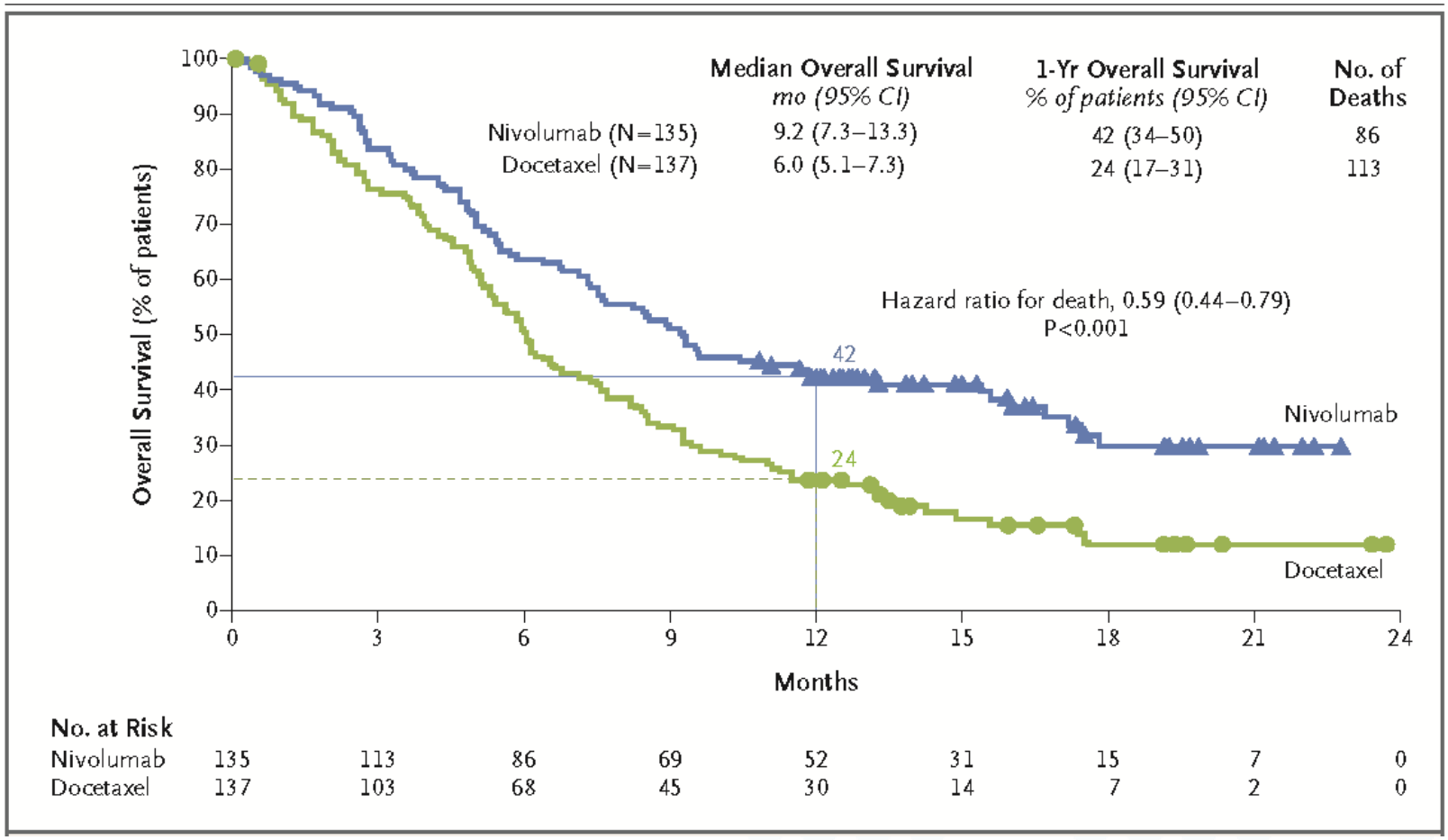
PD-1 blocking by Nivolumab in advanced lung cancer



Brahmer et al., NEJM, 2015

IMMUNE CHECKPOINT STUDIES IN PIGS?

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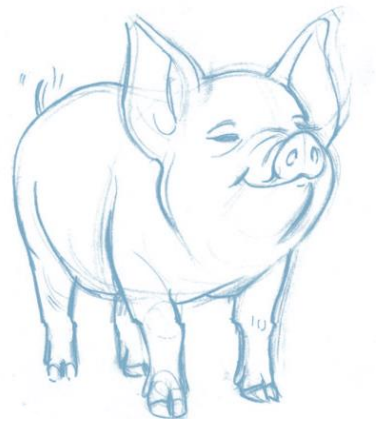
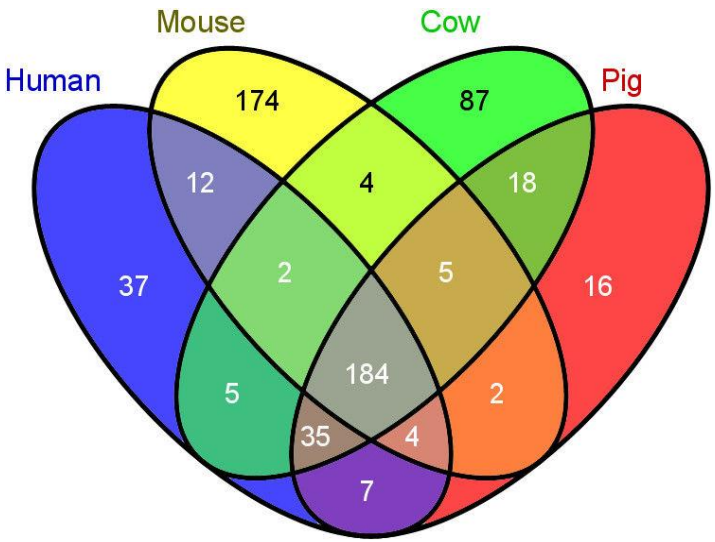
- Little if any cross reactivity for CTLA-4 and PD-1
- Humanized antibodies will elicit a neutralizing immune response in pigs
- Swine antibody isotype and Fc-receptor interactions are not known
- Porcinised check-point mAbs are needed
- Swine Antibody isotype research is needed

THE PIG AS A TRANSLATOR BETWEEN MICE AND HUMANS





Immunome

Table 1
Greater pig-human similarity revealed by gene family expansion analysis of pattern recognition receptors

Family Description	Human	Pig	Mouse
AIM2-like Receptor	4[a]	2	13
BPI Superfamily	12	14	16
CD1 Superfamily	5	4	2
CLEC Superfamily, Asialoglycoprotein and DC Receptor Subfamily	16	13	24
CLEC Superfamily, Collectin Subfamily	7	7	7
CLEC Superfamily, NK Cell Receptor Subfamily	24	23	57
CLEC Superfamily, Reg Subfamily	5	3	7
NLR Superfamily	22	20	43
RIG-I-Like Receptor Superfamily	5	5	5
Toll Like Receptor	10	10	12
TREM and TREM-like Receptor Superfamily	7	6	10

Key	
Expansion	Contraction
> 25%	> 25%
> 50%	> 50%
> 75%	> 75%

Dawson et al, BMC genomics, 2013; Dawson et al, Veterinary Microbiology, 2017

VALIDITY OF ANIMAL MODELS

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Denayer et al 2014
McGonigle & Ruggeri 2013

Face validity (appearance, clinic)

Similar clinical manifestation and symptoms of the human disease

Grafted tumors in mice looks like human tumors

Spontaneous tumors in pigs are very rare

Target/Construct validity (biology)

Similar biological role for the target of interest in the model compared to humans

Telomerase reactivation in human cancer

Telomerase reactivation in porcine cancer

Constitutive Telomerase expression in murine cells

Predictive validity (therapeutic effect)

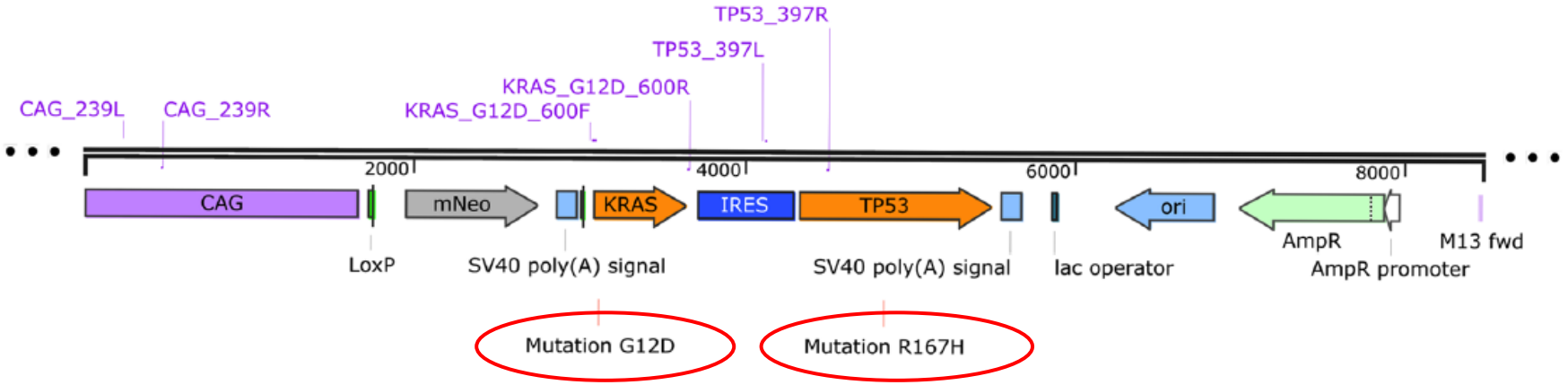
Similar effect of a drug/compound or treatment mimicked by the model

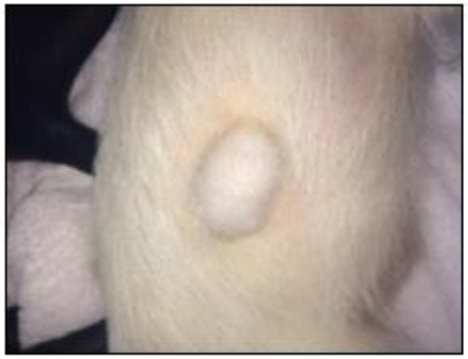
Dose in mice vs humans?

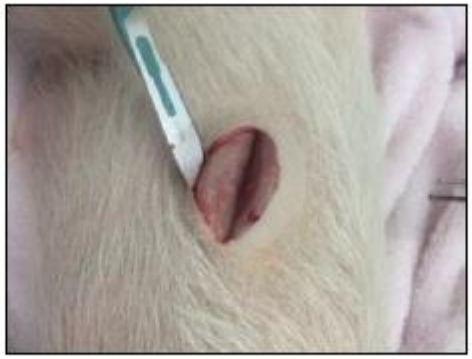
Immune deficient mice are per definition invalid

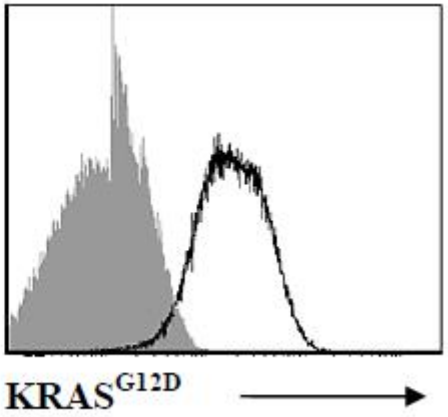
Immune redundancy in mice

THE ONCOPIG MODEL, UNIVERSITY OF ILLINOIS









Telomerase reactivation, tumor size, DNA methylation, and key transcriptional **features of human sarcomas**

Lawrence Schook et al, PLoS ONE, 2015

PERSISTENCE OF ONCOPIG TUMORS

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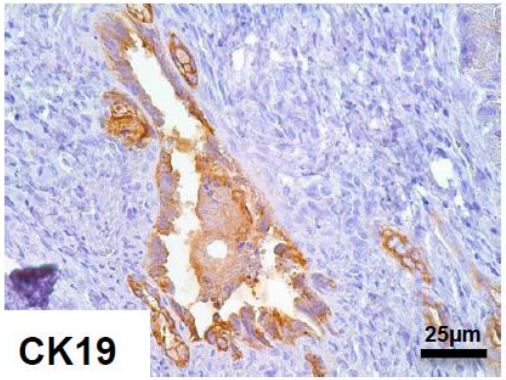
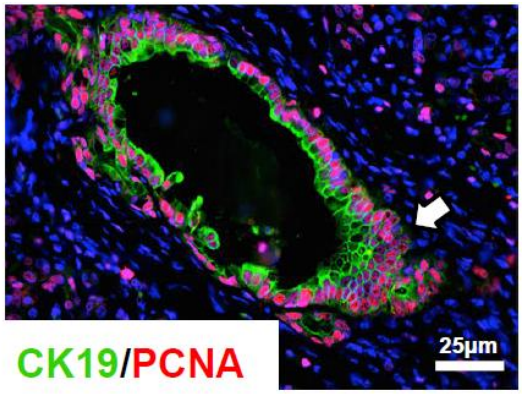
Pancreatic ductal adenocarcinoma: 1 year post AdCre





SCIENTIFIC REPORTS

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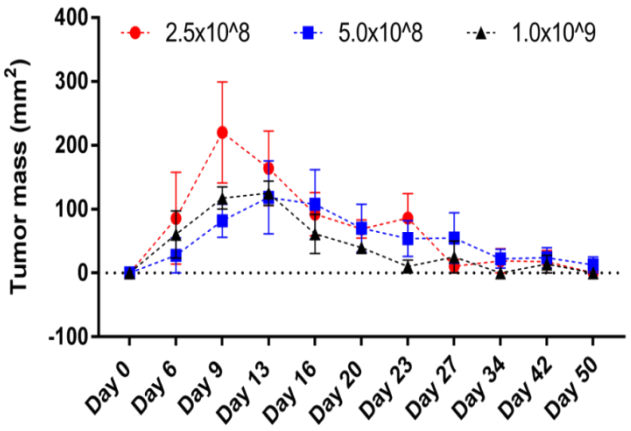
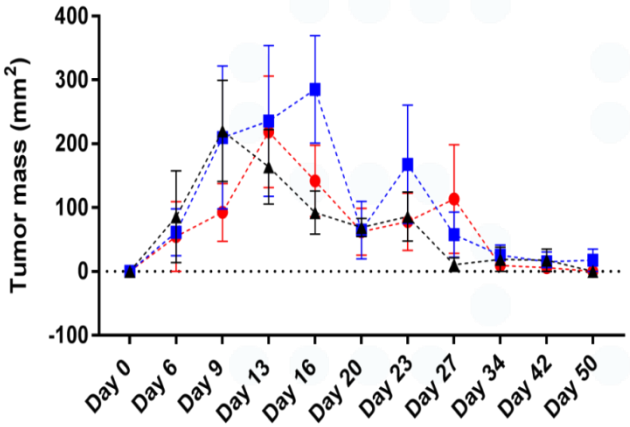
KRAS^{G12D} and TP53^{R167H} Cooperate to Induce Pancreatic Ductal Adenocarcinoma in *Sus scrofa* Pigs

Daniel R. Principe[1], Nana HaahrOvergaard[2,3], Alex J. Park[4], Andrew M. Diaz[4], Carolina Torres[4], Ronald McKinney[4], Matthew J. Dorman[4], Karla Castellanos[4], Regina Schwind[4], David W. Dawson[6], Ajay Rana[7], Ajay Maker[7], Hidayatullah G. Munshi[8], Lauretta A. Rund[3,5], Paul J. Grippo[4] & Lawrence B. Schook[3,5]

Subcutaneous sarcoma



Intramuscular sarcoma



Are Oncopig sarcoma tumors hot or cold?

T-CELL SUBSETS IN ONCOPIG TUMORS

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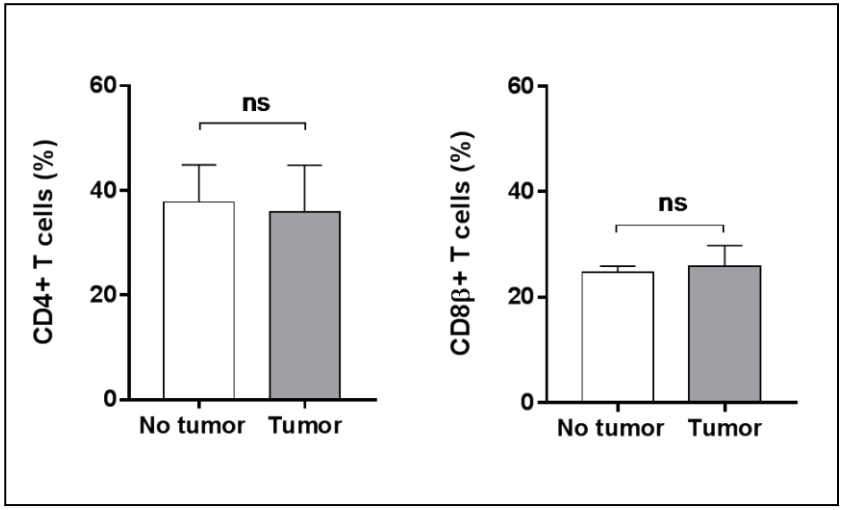
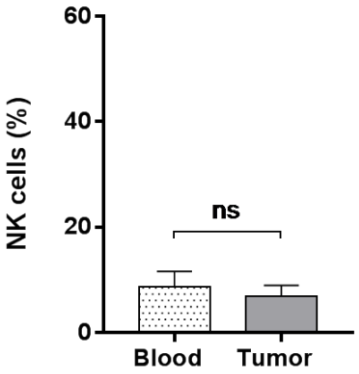
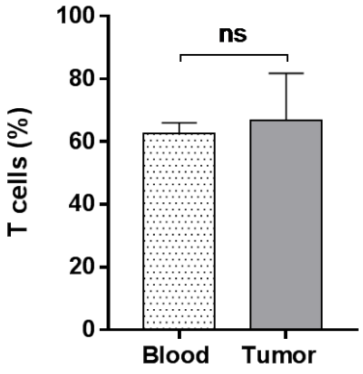
Peripheral blood



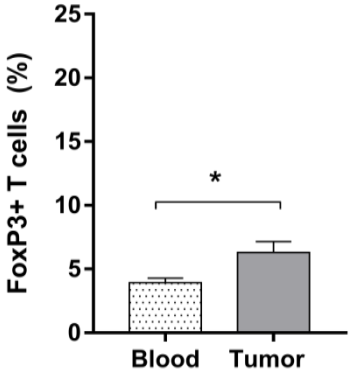
NK cells



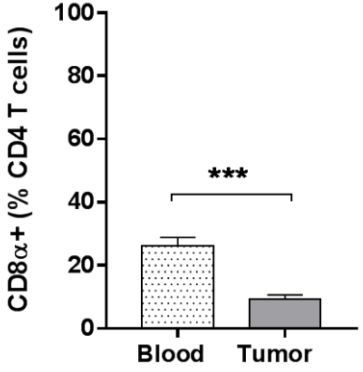
T cells



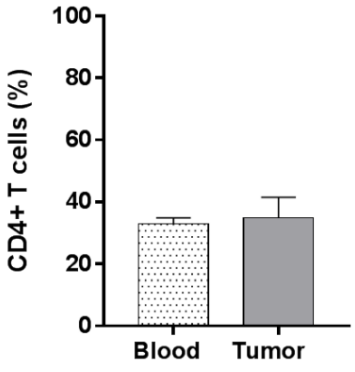
FoxP3⁺ T cells



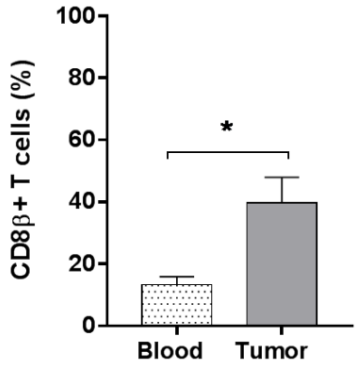
CD4⁺CD8α⁺ T cells



CD4⁺ T cells



CD8β⁺ T cells



Overgaard et al, Frontiers in Immunology 2018

PERFORIN+ AND GRANZYME B+ T CELLS

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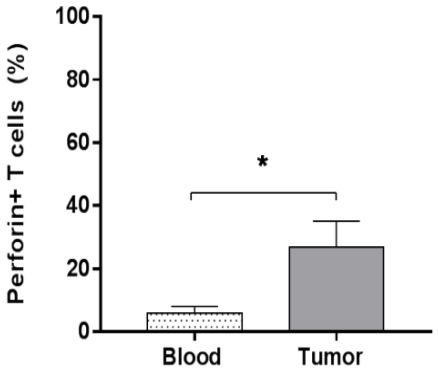
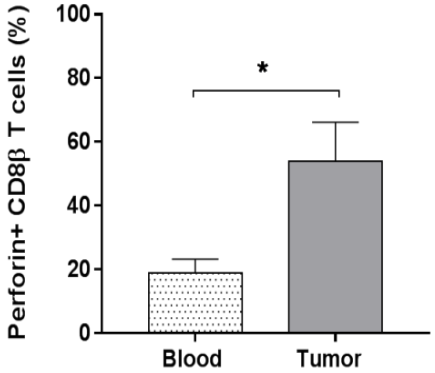
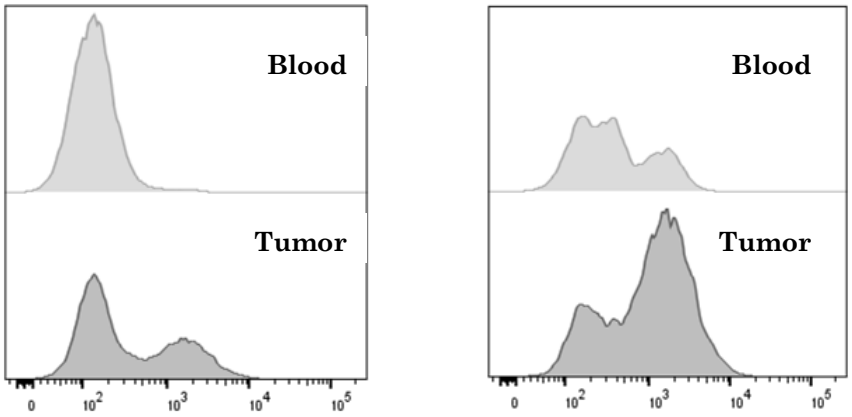
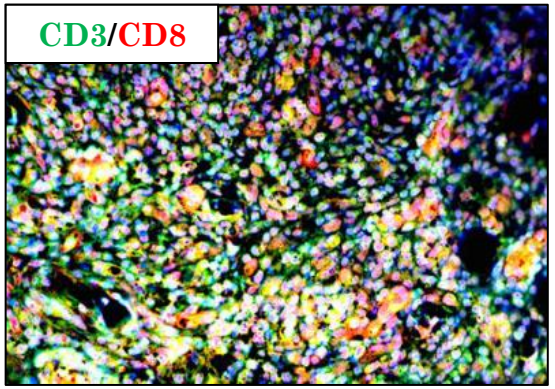
CD3+ T cells

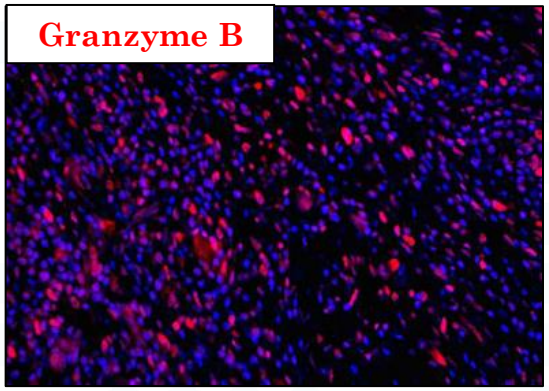


CD8β+ T cells



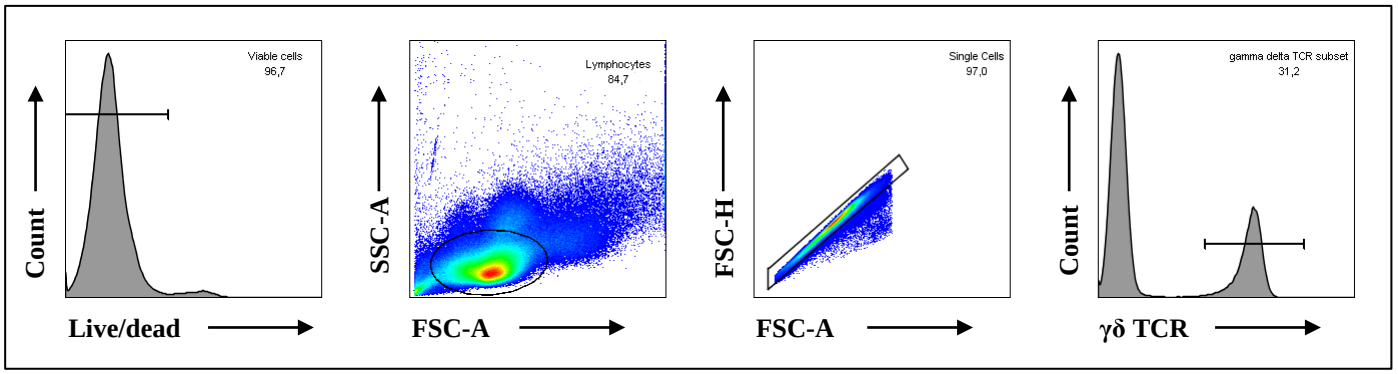




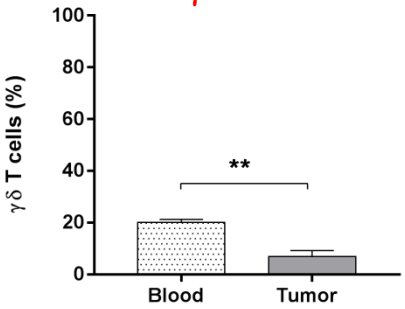


Overgaard et al, Frontiers in Immunology 2018

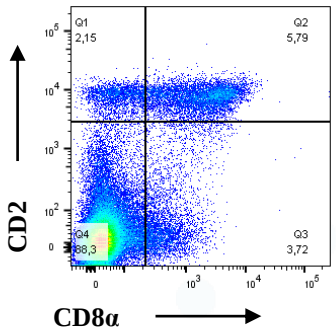
ACTIVATED γδ T CELLS ENRICHED IN ONCOPIG TUMORS



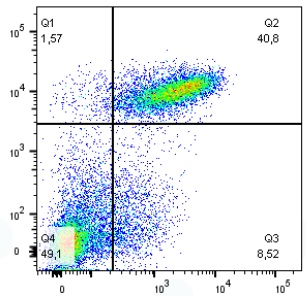
γδ T cells



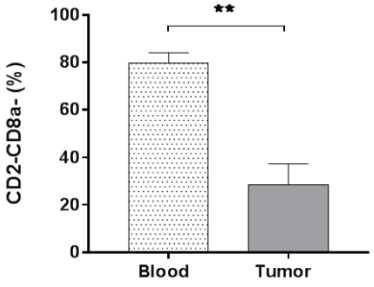
Blood

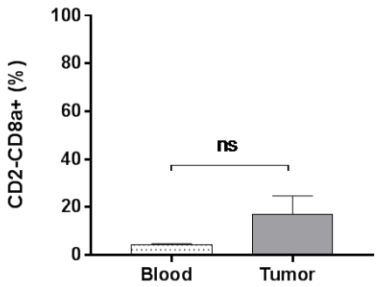


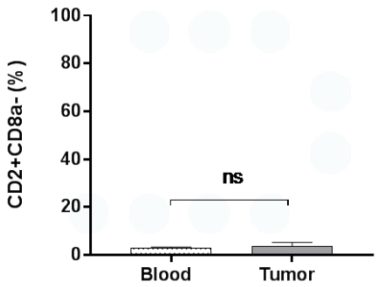
Tumor

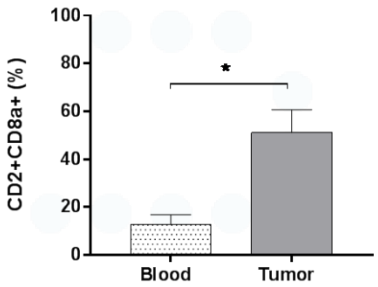


Activated γδ T cells









Overgaard et al, Frontiers in Immunology 2018

IN VITRO TUMOR CELL KILLING ASSAY

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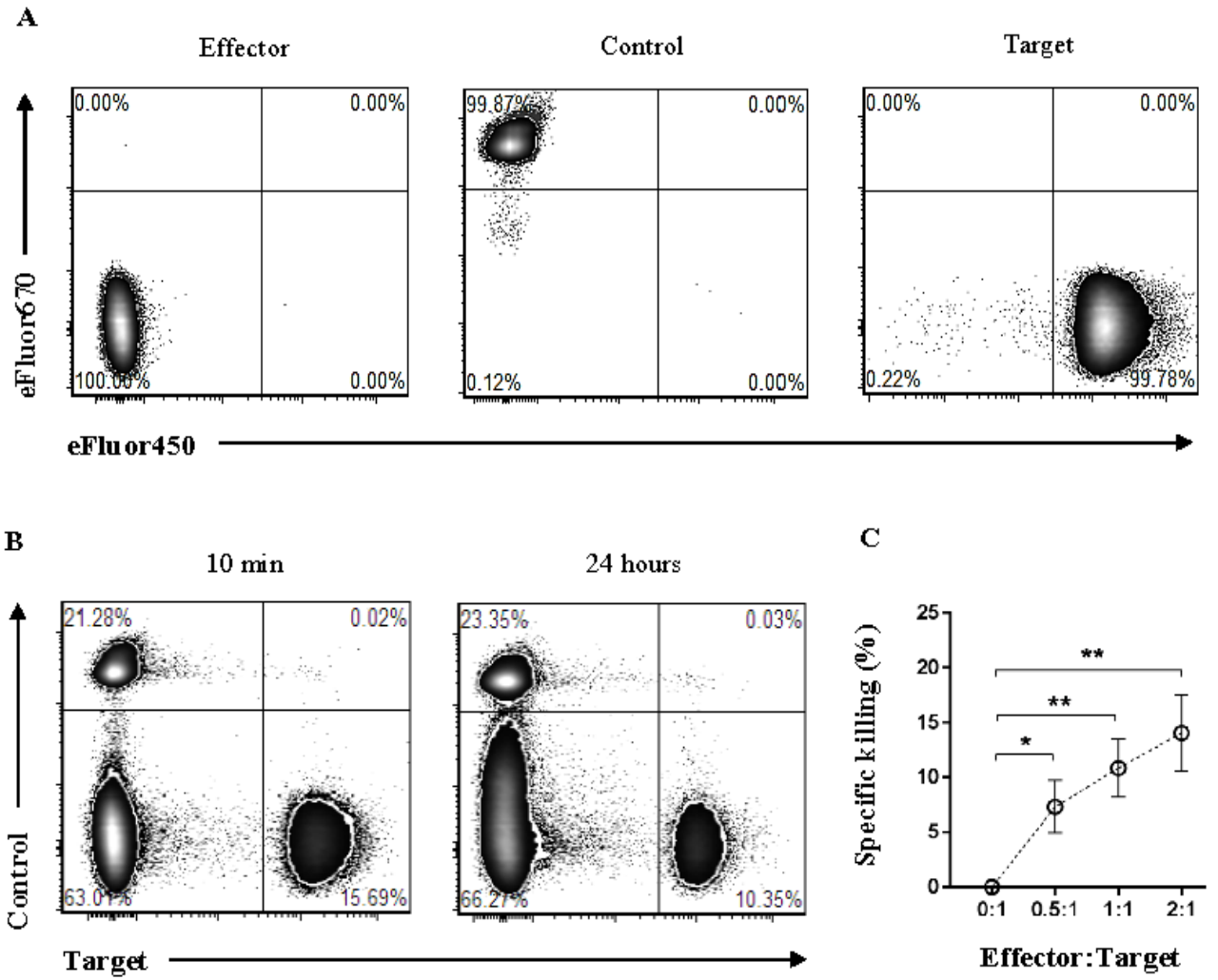
Tumor cells are specifically killed by autologous Oncopig immune cells



Overgaard et al, Frontiers in Immunology 2018

WHAT FACTORS MAY SUPPRESS THE ANTI-TUMOR IMMUNITY *IN VIVO*?

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Gene	Skeletal Muscle (FPKM)	Leiomyosarcoma (FPKM)	Log2 fold change	p-value	q-value	Significant
IDO1	0.488057	3.80091	2.96122	5.00E-05	0.000233877	yes
CTLA4	0.133311	1.01914	2.93448	5.00E-05	0.000233877	yes
PDL1	0.343398	1.08631	1.66148	0.00075	0.00276049	yes

Elevated expression is not a result of cellular transformation:

Gene	Primary Hepatocytes (FPKM)	HCC Cell Lines (FPKM)	Log2 fold change	P-value	Q-value	Significant
IDO1	1.15437	0.0406885	-4.82634	0.1494	0.23325	no
CTLA4	0	0	0	1	1	no
PDL1	1.15276	1.53313	0.411391	0.2771	0.370545	no

Possibly a good model to study therapies aimed at reactivating anti-tumor immunity *in vivo*

Overgaard et al, Frontiers in Immunology 2018

CONCLUSION

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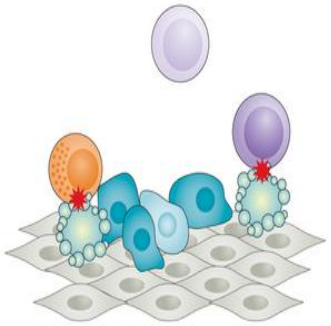
Oncopig tumors are generally hot with mixed population of activated immune cells and regulatory control mechanisms

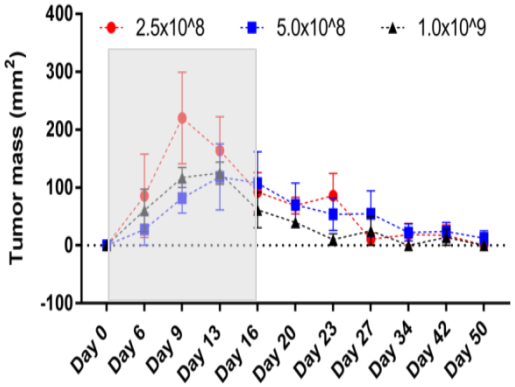
”Dynamic equilibrium”

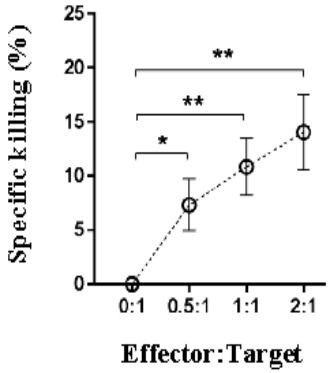


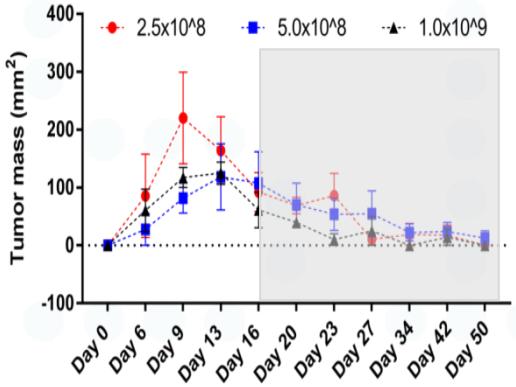
Tumor escape

Immune attack

Changes in *CTLA4, PD-L1, IDO1* expression?







CAN WE PUSH THE IMMUNE RESPONSE TOWARDS CYTOTOXIC CELL-MEDIATED OR ANTIBODY RESPONSE?

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Tetanus toxoid (model antigen)

1 µg | 10 µg | 100 µg

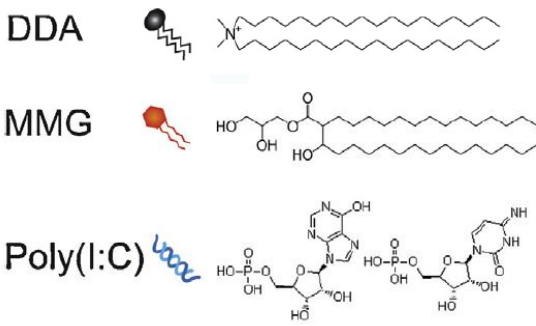
3x immunization

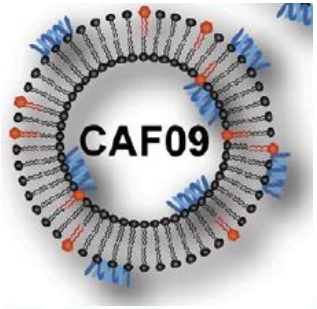
TLR3 stimulating adjuvant

DDA

MMG

Poly(I:C)





Korsholm et al, Vaccine, 2014

ADMINISTRATION ROUTE: I.P. DELIVERY TO MINIPIGS

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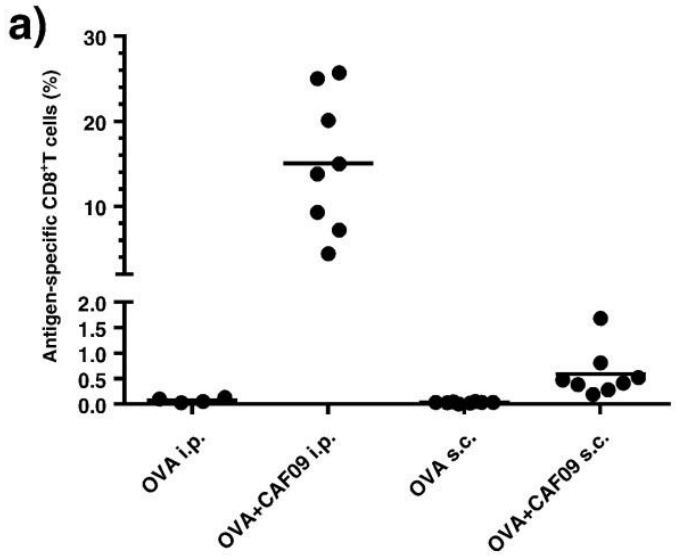
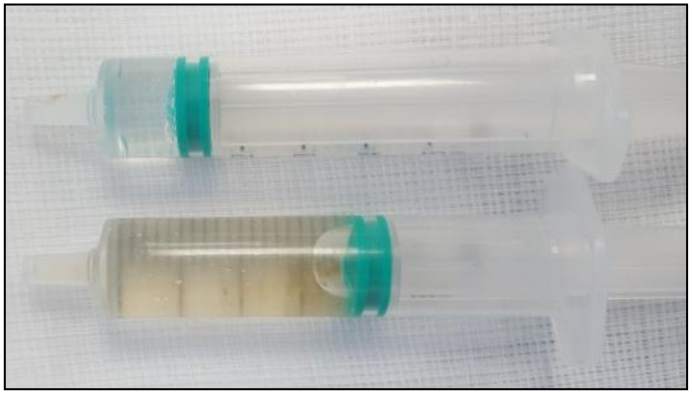
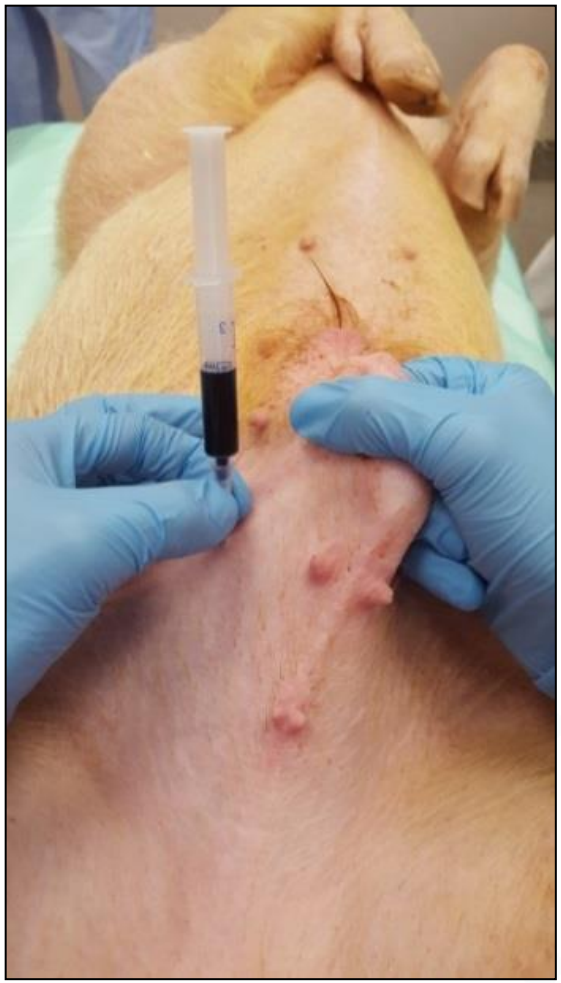
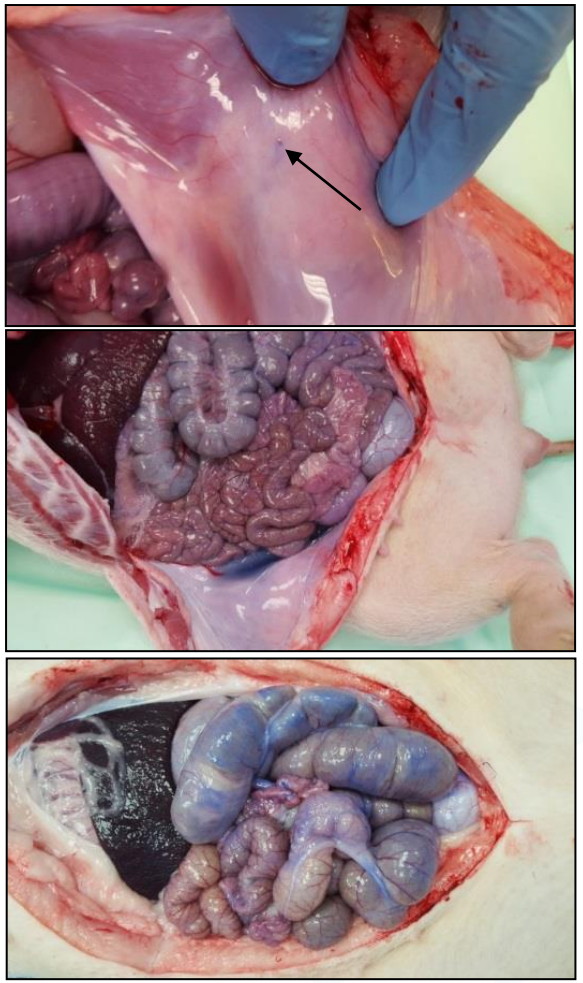


Schmidt et al, J Control Release, 2016







CAF09-FORMULATED LOW ANTIGEN DOSE FAVORS CELL-MEDIATED IMMUNE RESPONSE

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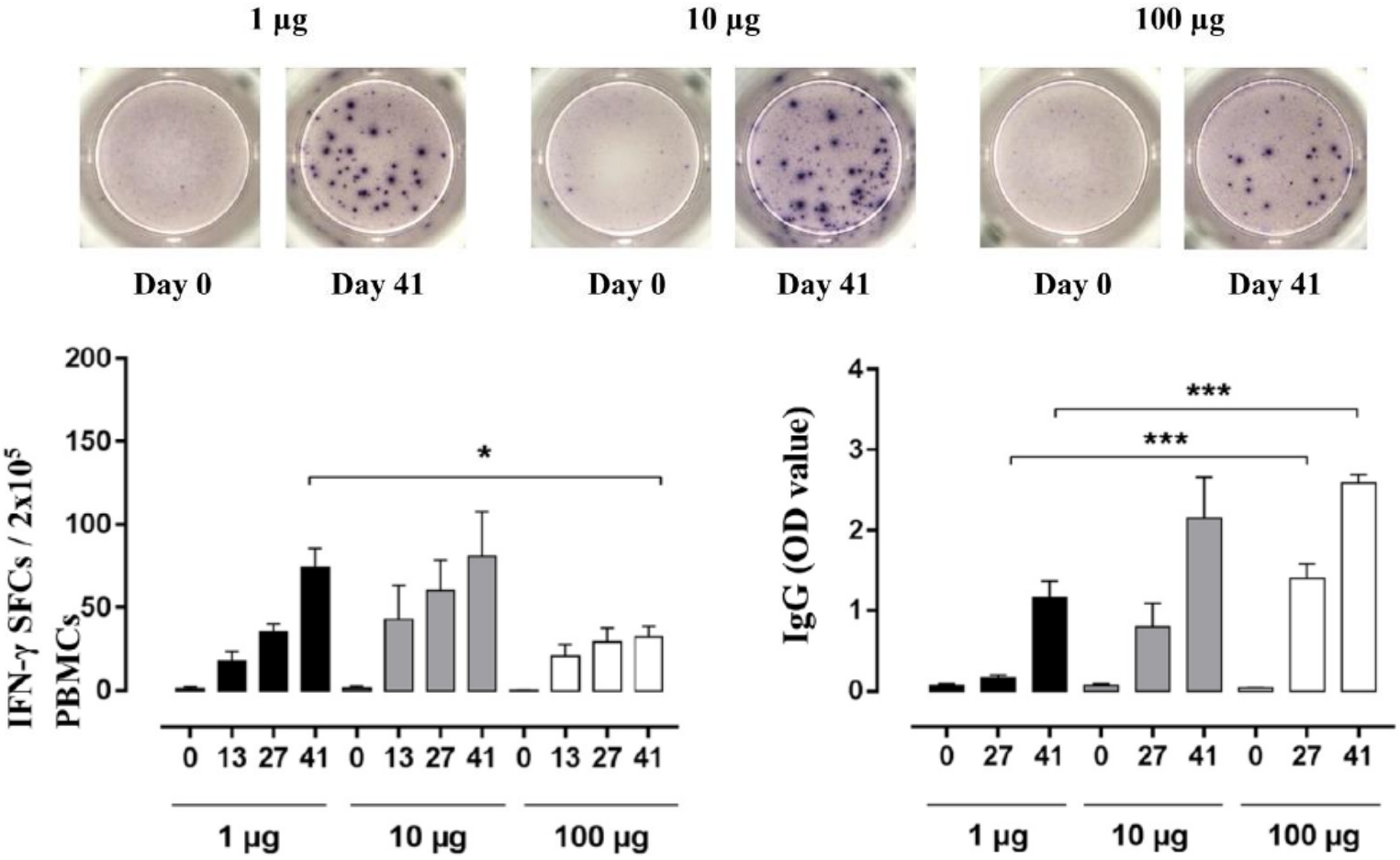

REPEATED I.P. IMMUNIZATION

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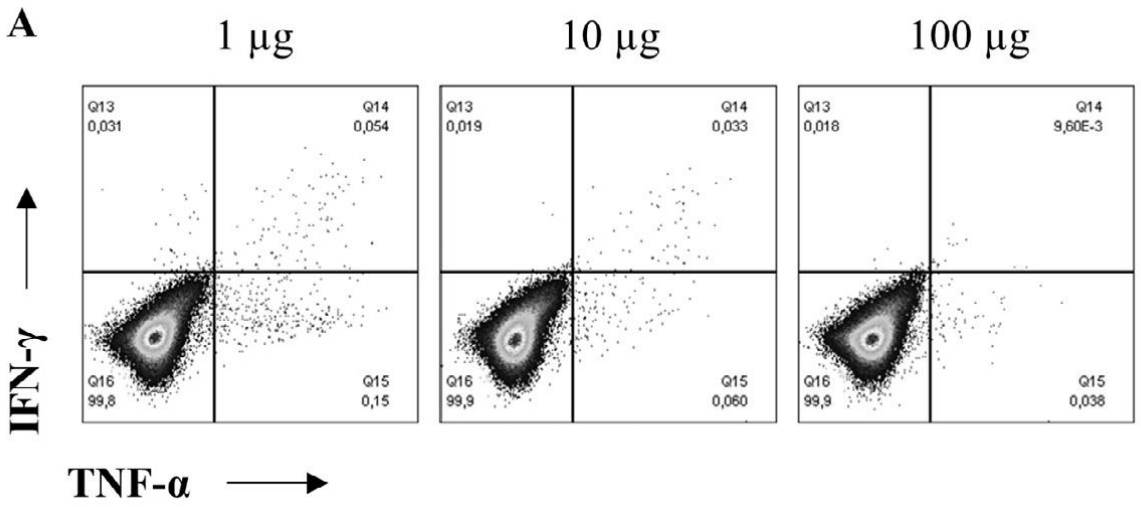
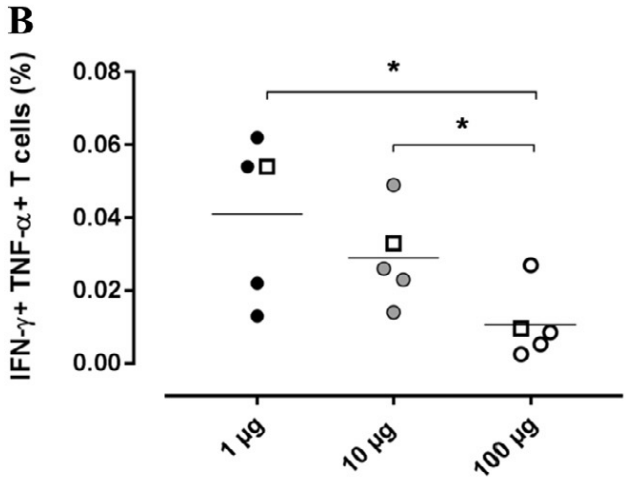
Low antigen dose induces a cytotoxic and polyfunctional T-cell response





N.H. Overgaard et al./Vaccine 35 (2017) 5629–5636

CAN WE BREAK THE PERIPHERAL TOLERANCE?

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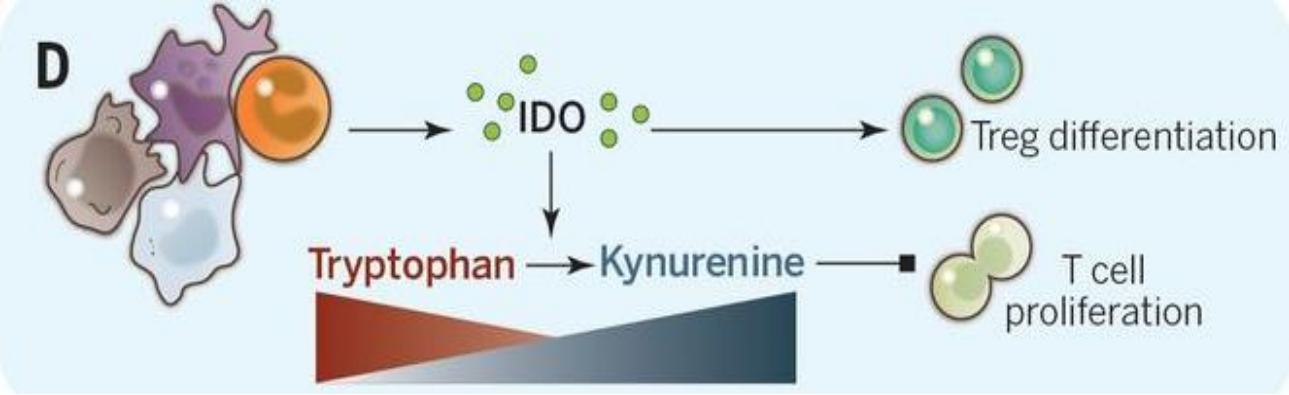
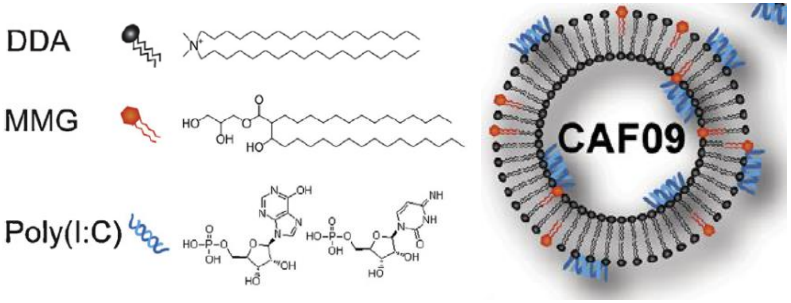
Indoleamine 2,3-dioxygenase (IDO)



Joyce & Fearon, Science, 2015



Korsholm et al, Vaccine, 2014

SLA-2*03:01

(NGS-based MHC class I allele typing)

Immunization administered i.p. every second week for a total of seven times

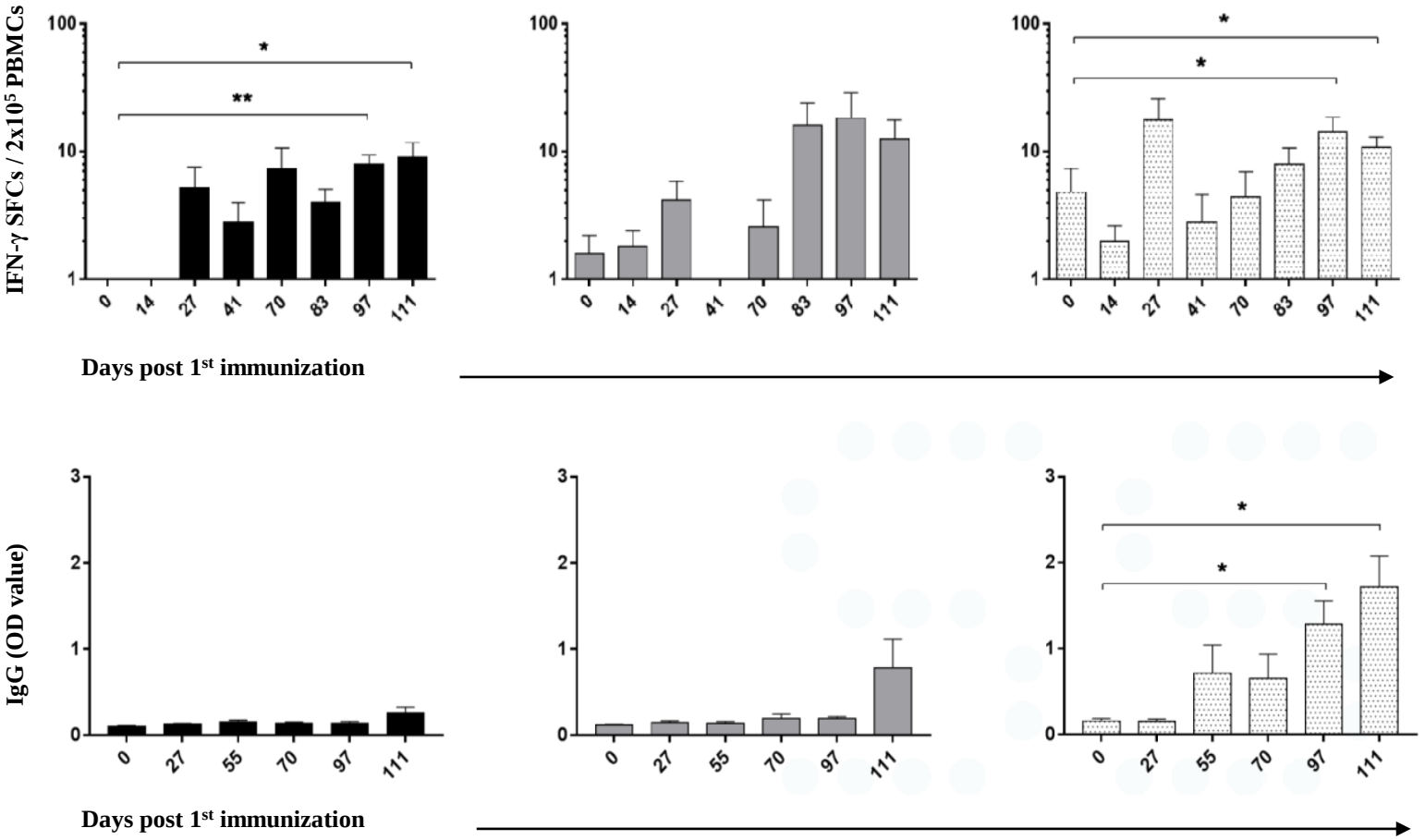
PEPTIDE DOSE DICTATES IMMUNE RESPONSE

STATENS SERUM INSTITUT

The CAF09-formulated peptide dose dictates the type of vaccine-induced immune response towards IDO

IDO2 peptide as example

1 µg | 10 µg | 100 µg

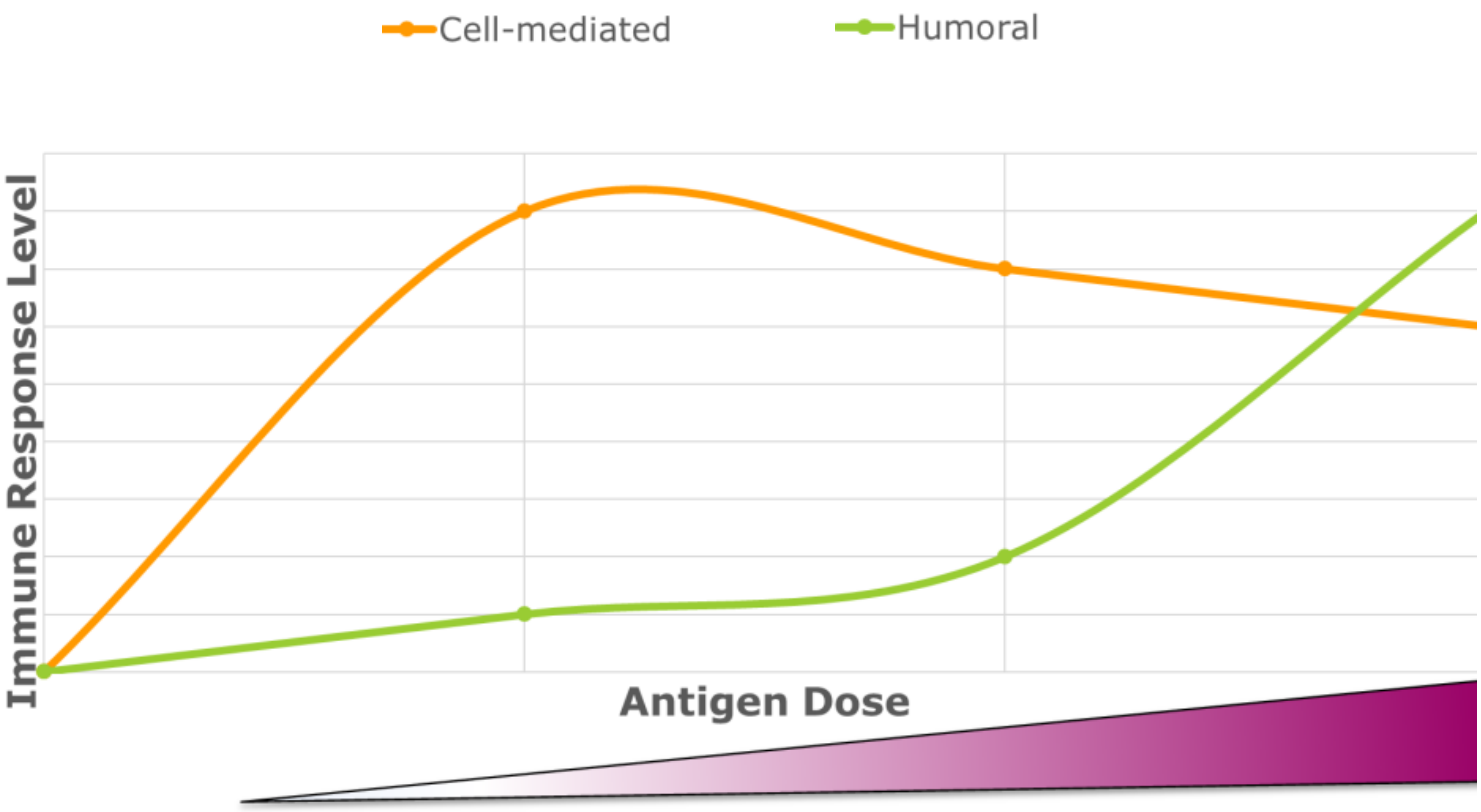


Overgaard et al, manuscript in preparation

ANTIGEN DOSE EFFECT

STATENS SERUM INSTITUT

Model of the antigen dose effect on the vaccine-induced immune response



CONCLUSION: No ”One Size Fits All”

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Mice:

Will continue to be the major animal model

Pigs: **Model of elimination and equilibrium phases?**

Spontaneous cancers are rare and regress

Genetically engineered models are emerging:

- Lymphoma
- Breast cancer
- Pancreatic duct adenocarcinoma
- Colorectal cancer
- Osteosarcoma, Soft-tissue sarcoma
- Hepatocellular carcinoma
- Basal cell carcinoma

Model of therapeutic vaccination?

Vaccination to break tolerance in outbred species

Dogs: **Model of escape phase and immunotherapeutics?**

Spontaneous cancers are common and persistent

- Six are NCI recognized tumor models

ILAR Journal, 2018, 1–16

doi: 10.1093/ilar/ily014
Review Article

Of Mice, Dogs, Pigs, and Men: Choosing the Appropriate Model for Immuno-Oncology Research

Nana H. Overgaard[1], Timothy M. Fan[2], Kyle M. Schachtschneider[3], Daniel R. Principe[4], Lawrence B. Schook[3,5], and Gregers Jungersen[6]

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