June 7th, 2017
Torino

Cancer vaccines in the renaissance era of immunotherapy

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Breakthrough of the Year 2013

http://news.sciencemag.org/breakthrough-of-the-year-2013

2016: The Year of Cancer Immunotherapy

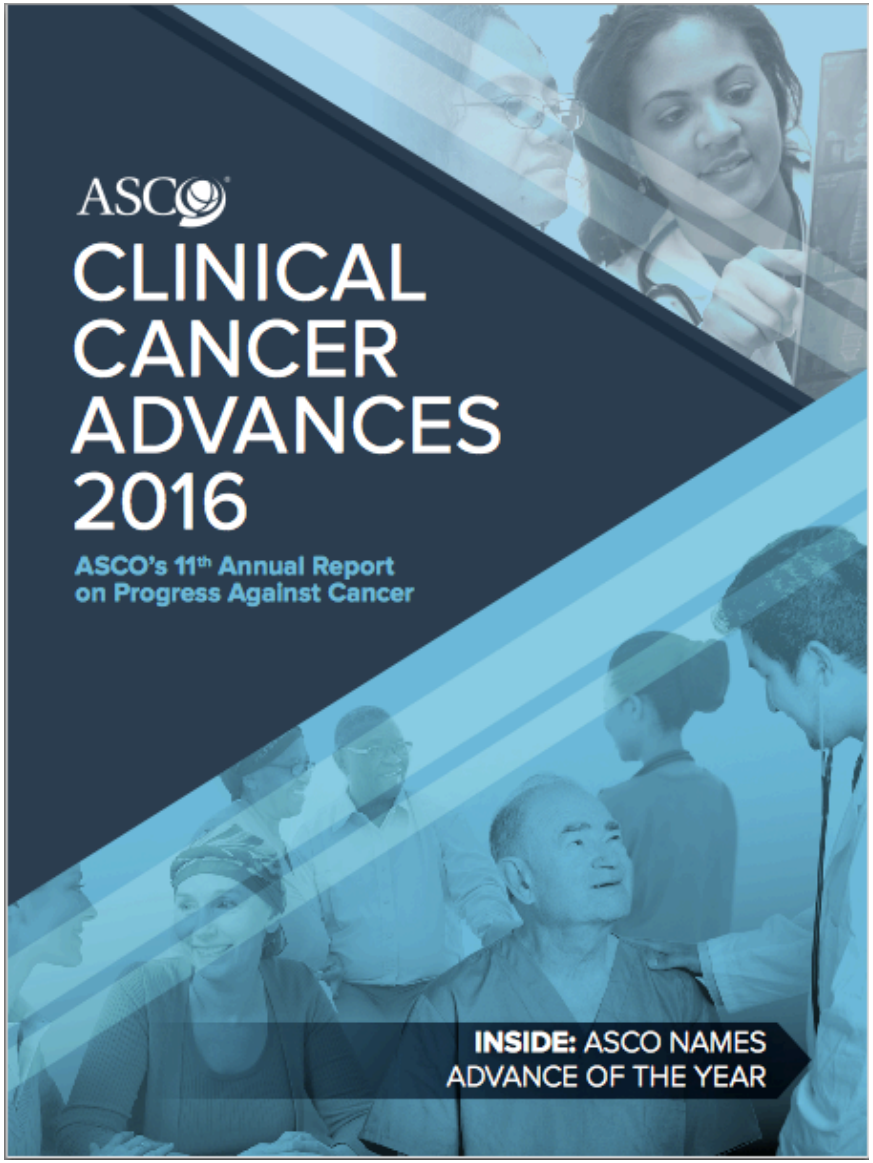
This has been another year of exciting progress in cancer immunotherapy, providing even more help and hope for patients, although it was not without its challenges.

After last year's breakthroughs, 2016 cemented immunotherapy's role in the future of cancer treatment, and it was recognized as the "Advance of the Year" by the American Society of Clinical Oncology.

http://www.cancerresearch.org/news-publications/our-blog/december-2016/2016-the-year-of-cancer-immunotherapy#sthash.2DaBandD.dpuf

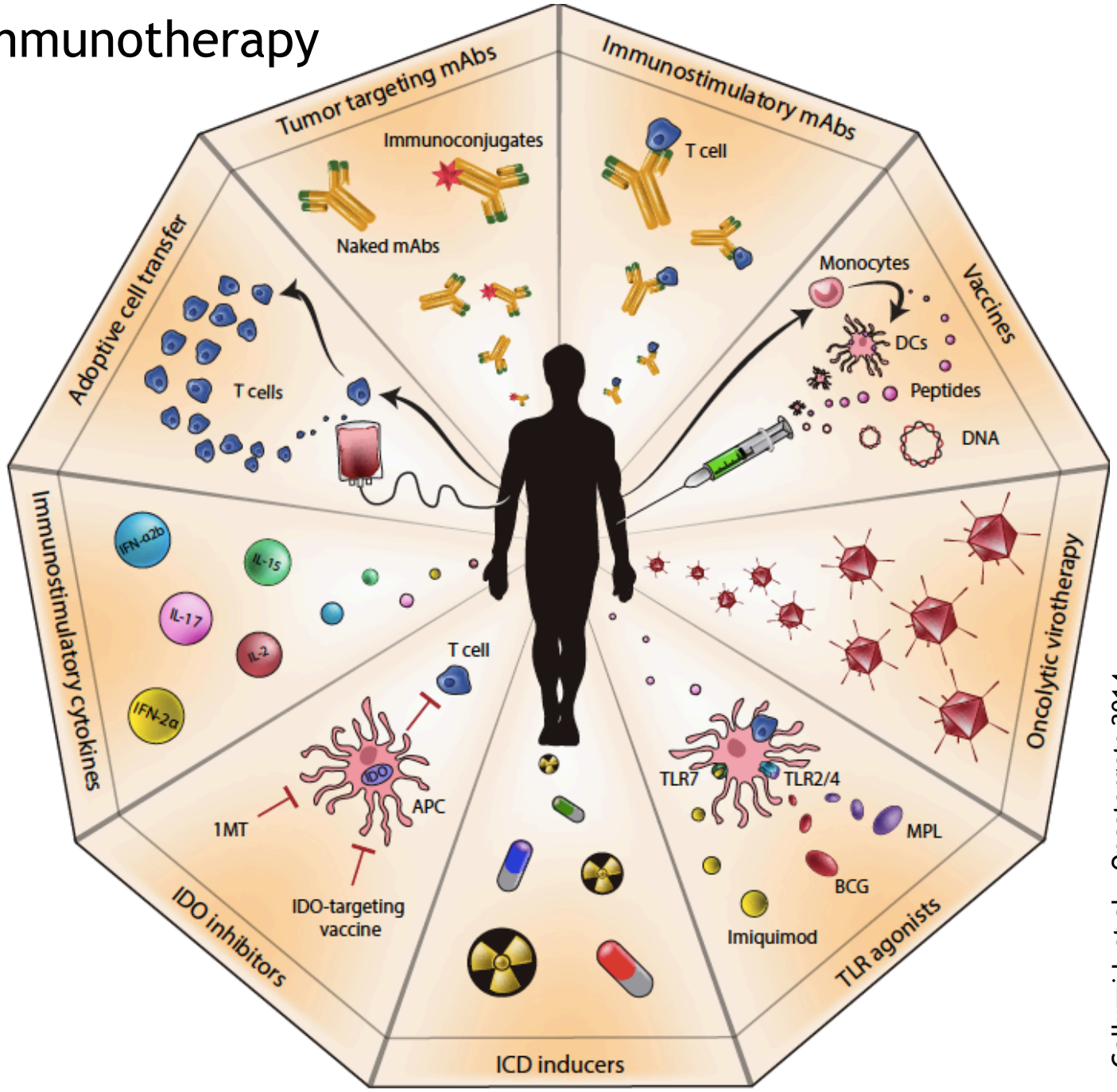
In its annual report, the American Society of Clinical Oncology (ASCO)—the world's leading professional organization representing physicians who care for people with cancer—has named cancer immunotherapy the "Advance of the Year".

"Although all research achievements highlighted in this report are remarkable, one area clearly stands out from the rest: cancer immunotherapy" the editors at ASCO note.



http://www.cancerresearch.org/news-publications/our-blog/february-2016/asco-names-cancer-immunotherapy-advance-of-the-year#sthash.raRg866V.dpuf

Anticancer immunotherapy



Galluzzi L et al., Oncotargets 2014

Anticancer immunotherapy

passive/adoptive

- Passive administration of tumor-targeting monoclonal antibodies (mAB)
- Adoptive therapy: isolation of killer cells from patients, ex vivo expansion and reinfusion in patients

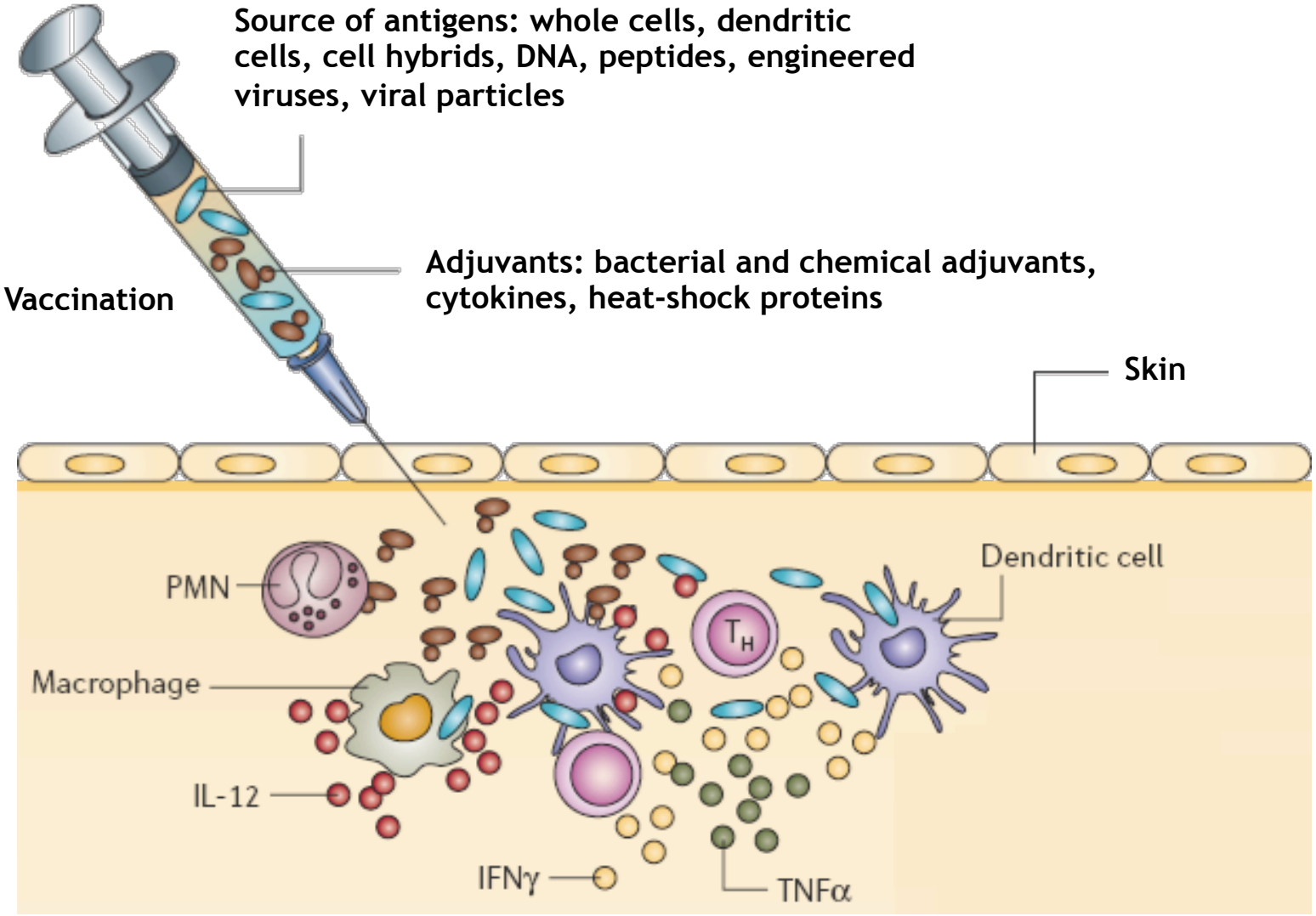
active

- Unspecific active immunotherapy (immunostimulatory drugs, cytokines, mAB; oncolytic viruses; PRR antagonists)
- Specific active immunotherapy: cancer vaccines

Cancer Vaccines

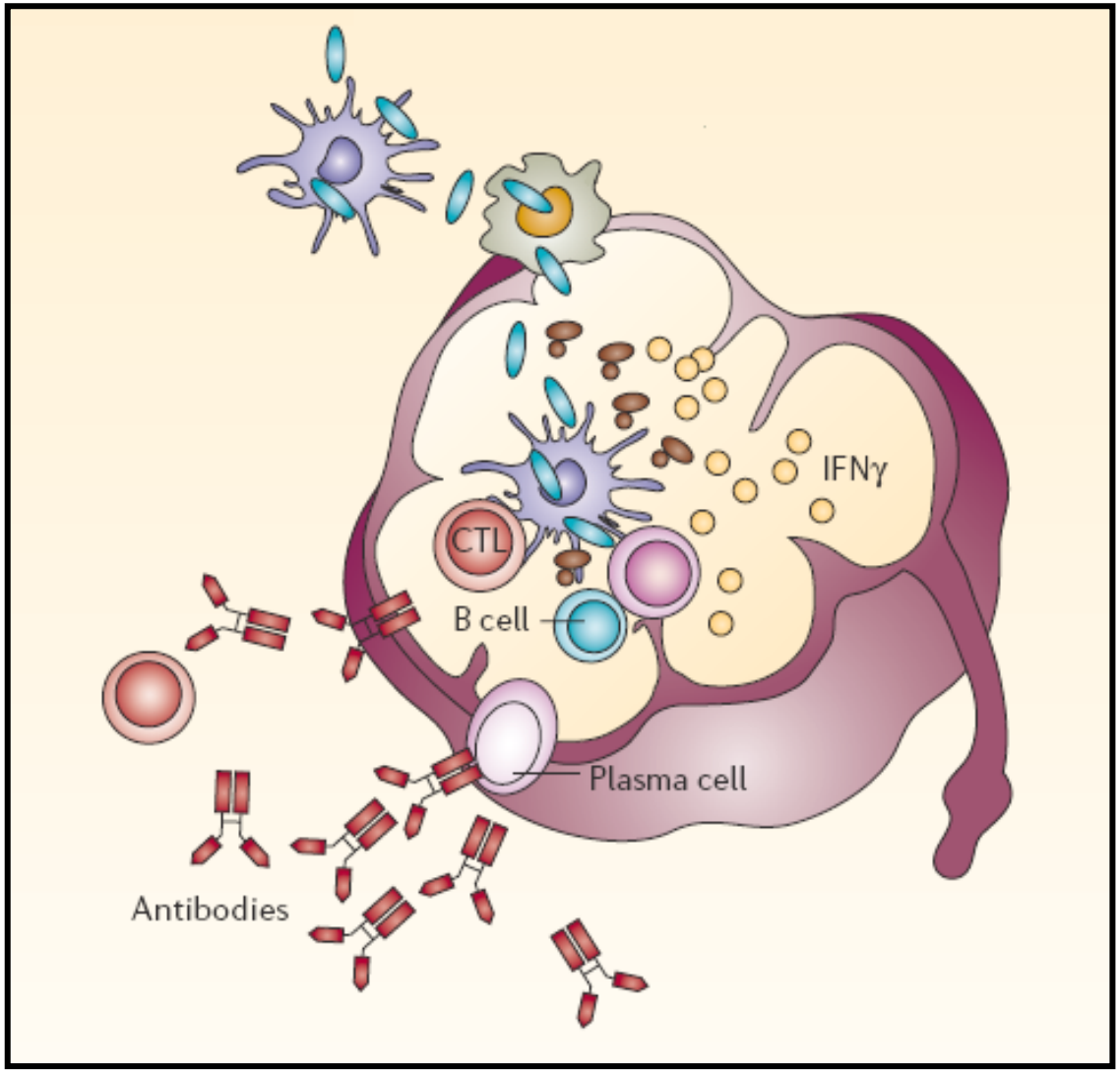
Cancer vaccine represent a non-toxic treatment modality potentially capable of inducing strong anti-tumor immune responses by esablishment of immune memory leading to tumor regression, or stabilisation of a tumor and also help in prevention of tumor recurrence and metastatization after a complete treatment.

The present knowledge and esperience of cancer vaccines are still in infancy, but cancer vaccines promise to be a good strategy for the future.



Lollini, Cavallo, et al, Nat Rev Cancer 2006 6:204-16

VACCINE-ACTIVATED IMMUNE REACTIONS



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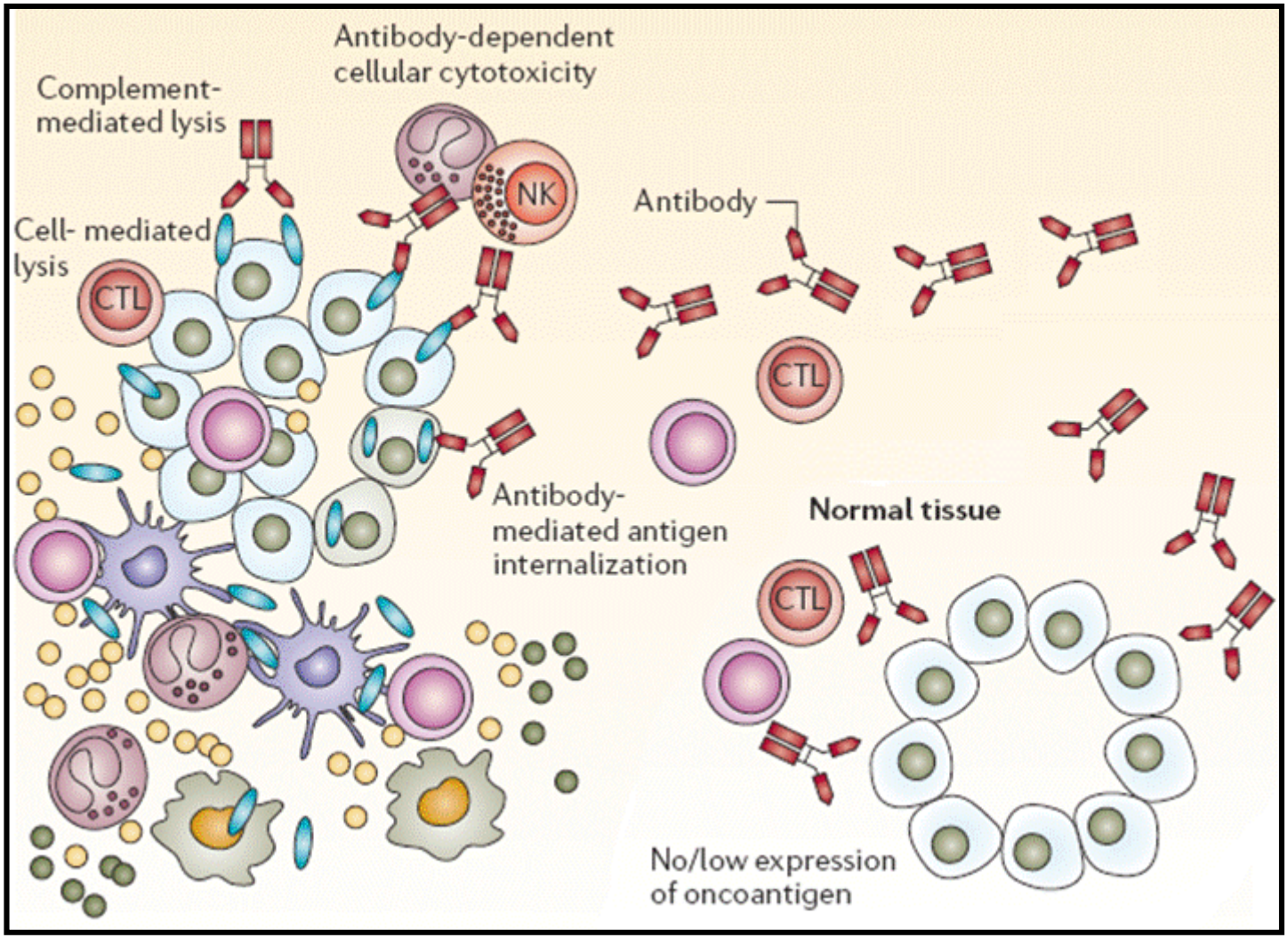


Lollini, Cavallo, et al, Nat Rev Cancer 2006 6:204-16

Tumor cells display:

Tumor specific antigens

unique to tumor cells
result of point mutations or gene rearrangements
viral antigens

Tumor associated antigens

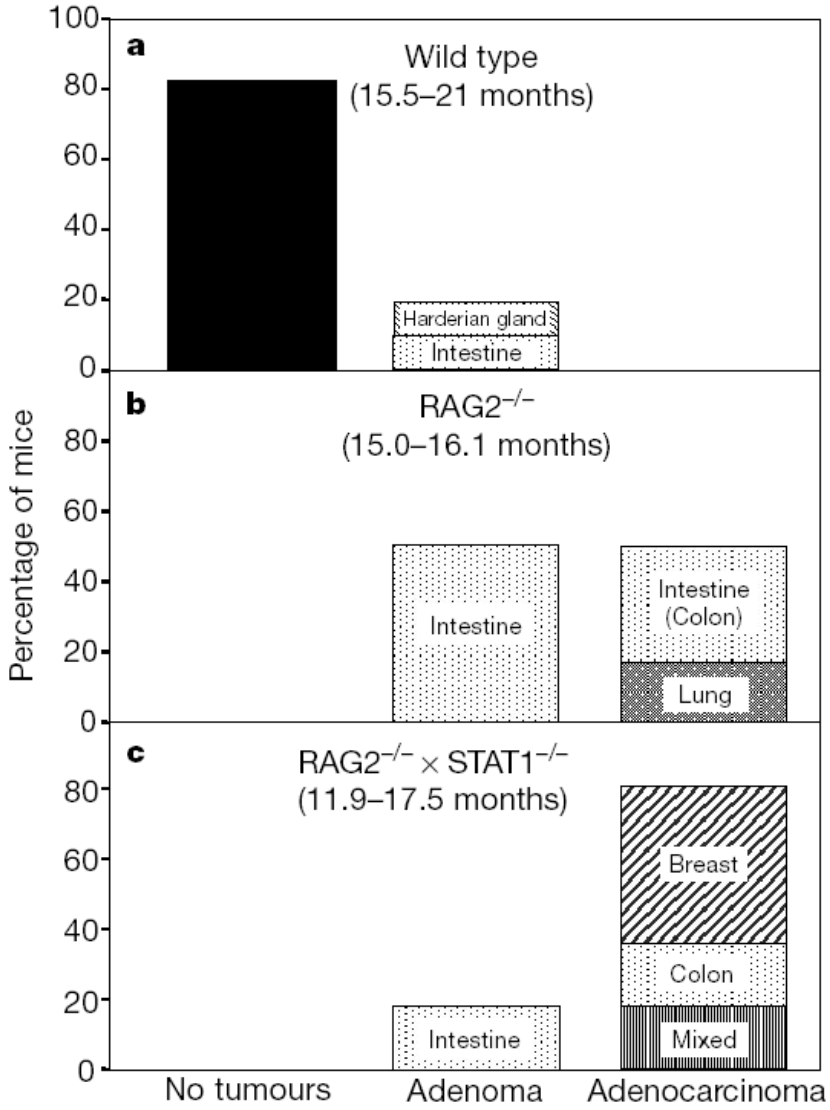
not unique to, but overexpressed in tumor cells
developmental antigens, which become derepressed
differentiation antigens; are tissue specific
abnormal post-translational modifications
…

Is tumor recognition a function of the immune system?

Immunodeficient mouse models

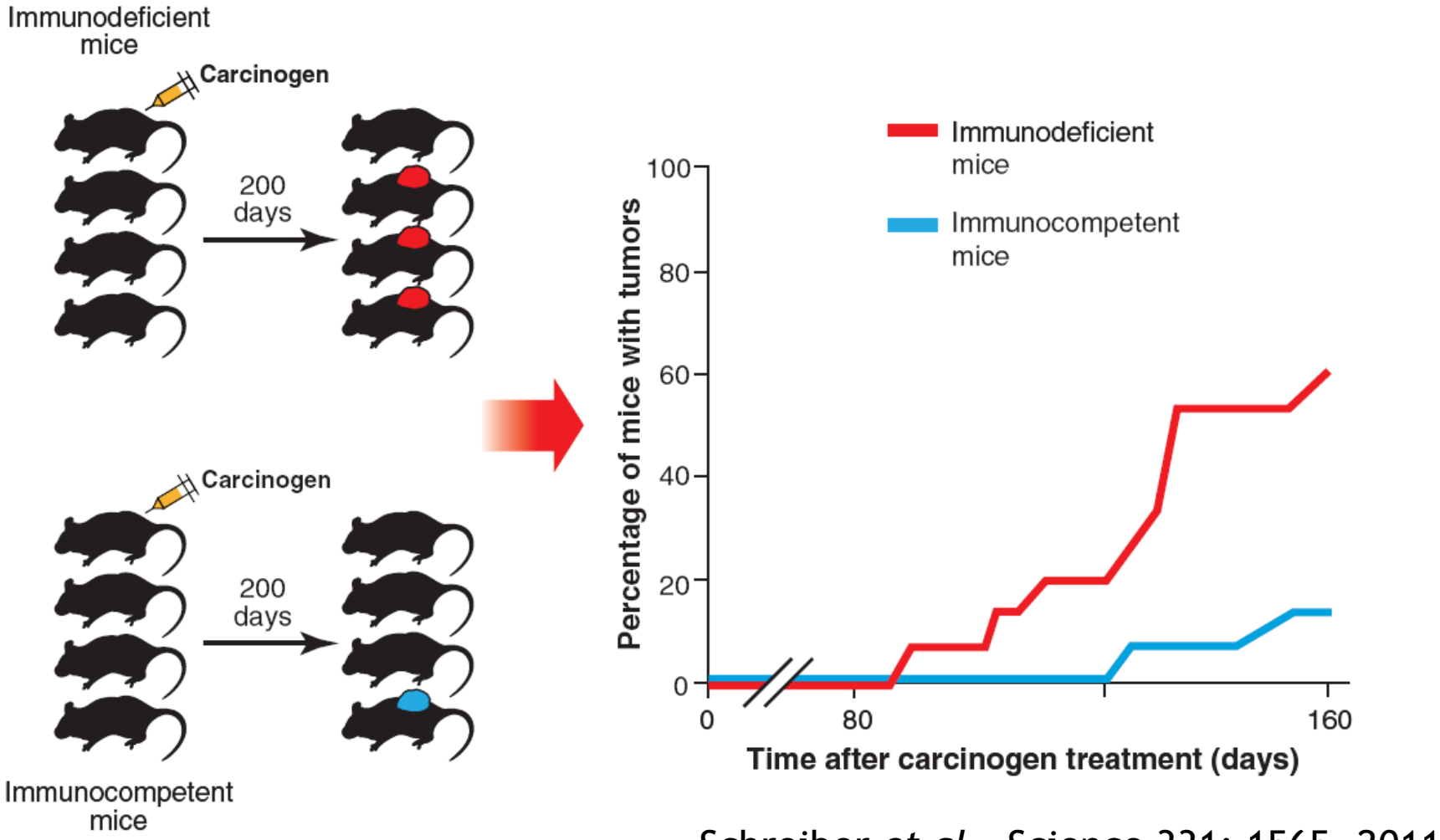
Spontaneous:
- partial immune deficit, partial recovery in older animals

Genetically modified:
- “designed” deficit, permanent, lifelong



Shankaran et al., Nature 410: 1107, 2001

The immune status of mice is critical for their susceptibility to chemical induced carcinogenesis



Schreiber *et al.*, Science 331: 1565, 2011

Immunodeficiencies and tumors:

- ✓ A *complete* and *long-lasting* immune depression significantly enhances the incidence of spontaneous, virally-induced and carcinogen-induced tumors
- ✓ A *partial* or *transient* immune depression is associated with increased incidence of some tumor types, e.g. virally-induced tumors, leukemias and lymphomas, sarcomas

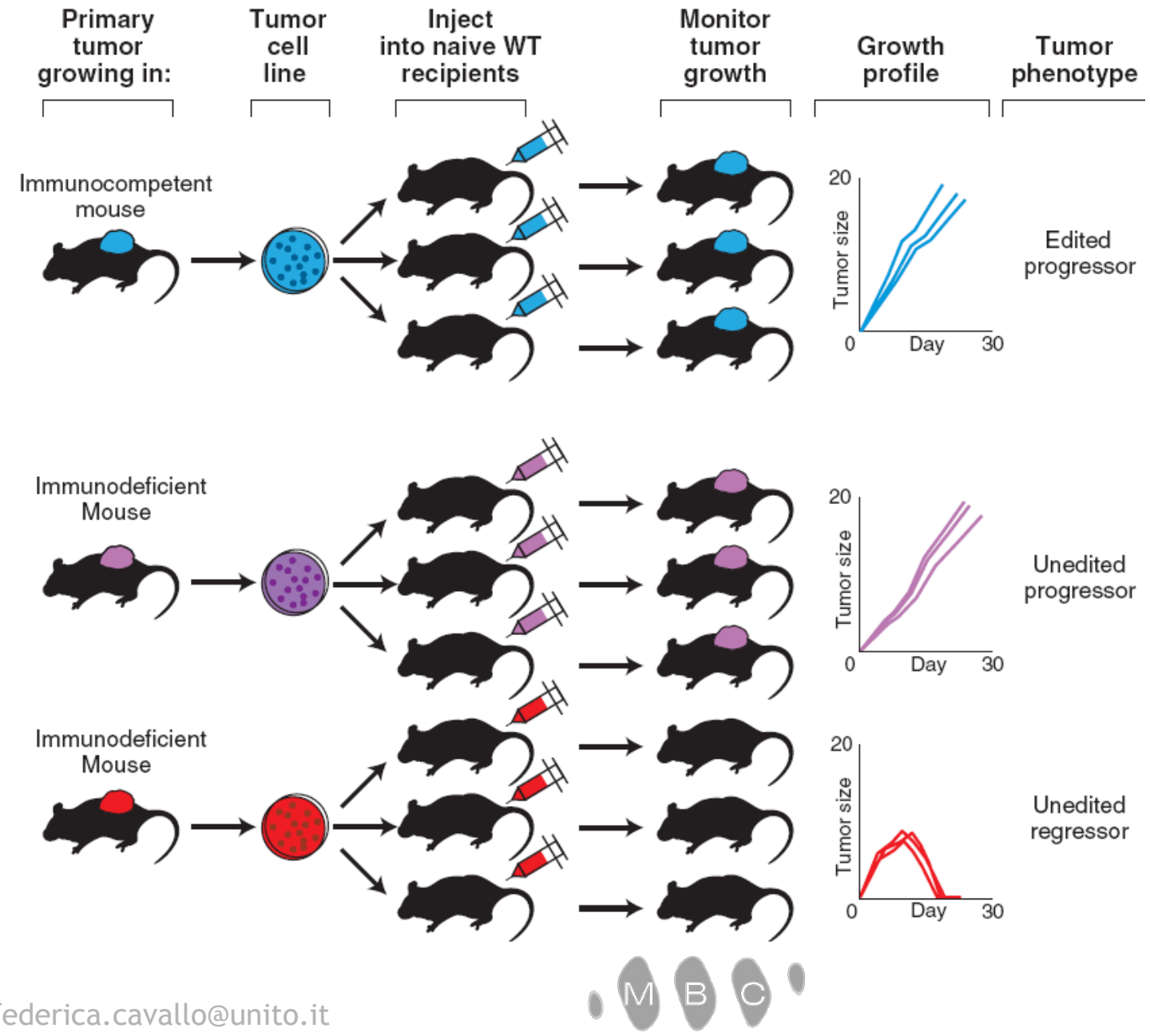
The concept of ***immunosurveillance:***

- ✓ Immunity is a critical mechanism for inhibiting cancer development and progression

- ✓ Tumors are antigenic and are spontaneously recognized by the immune system

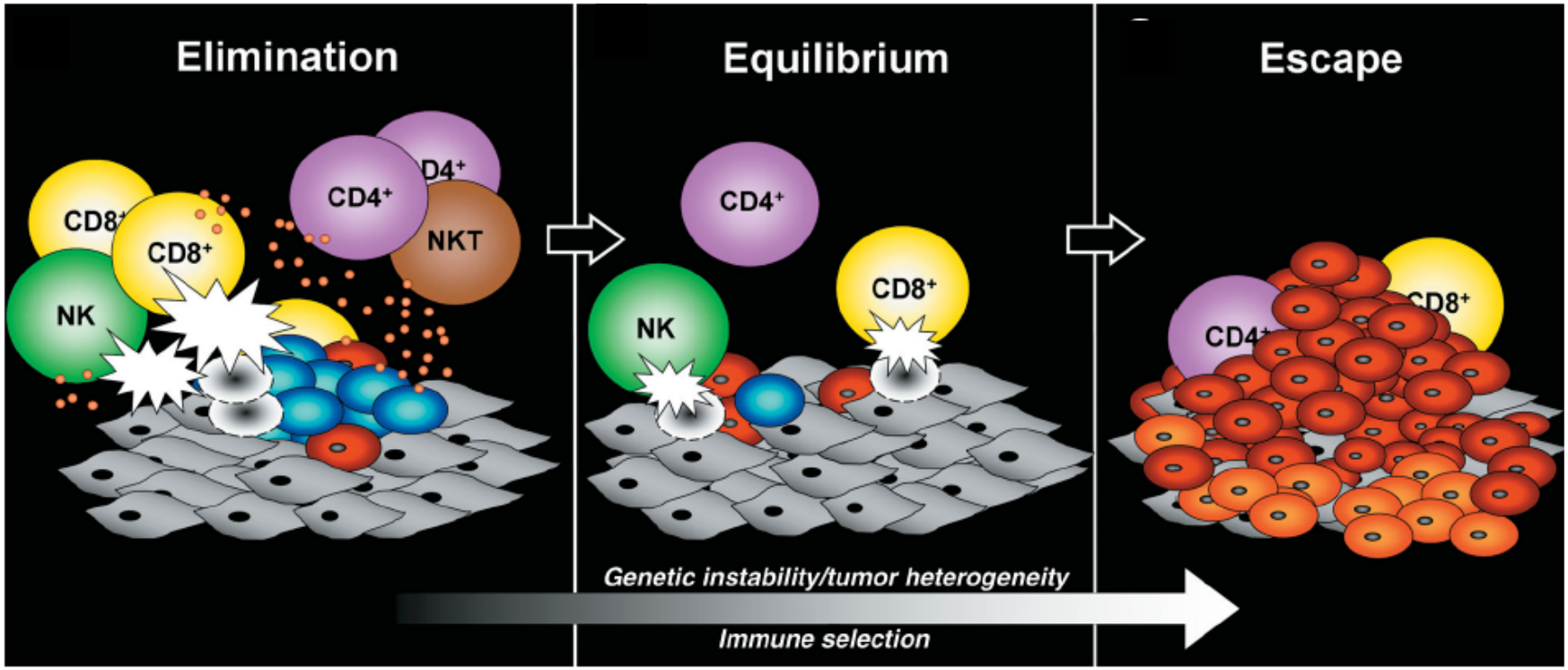
- ✓ What is the consequence of spontaneous immune reactions to tumors?

The immune system shapes tumor immunogenicity



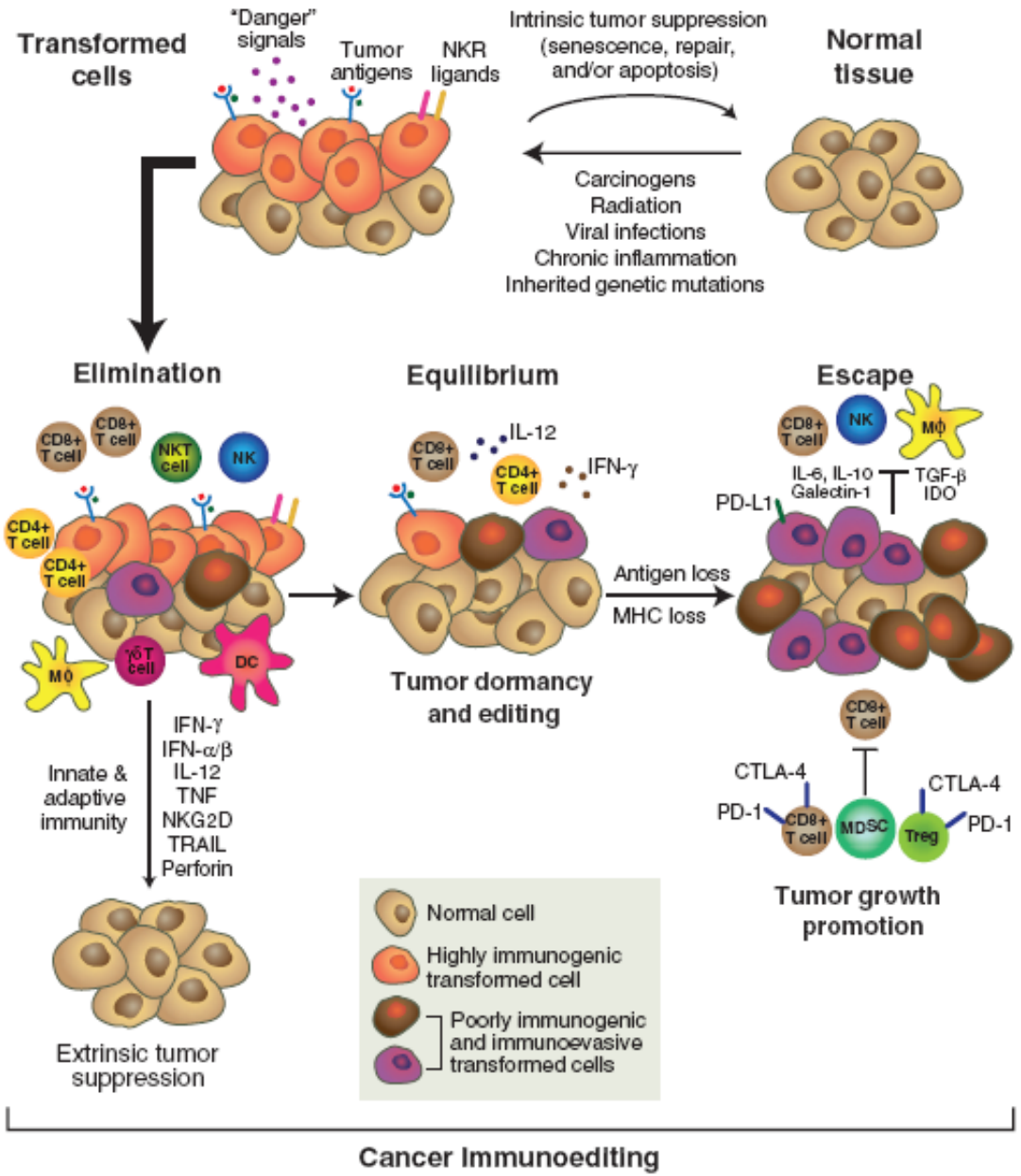
Shankaran et al., Nature 410: 1107, 2001
Koebel et al., Nature 450: 903, 2007
Schreiber et al., Science 331: 1565, 2011

The three Es of cancer immunoediting



Dunn et al., Nat Rev Immunol 2002 3:991-998

The cancer immunoediting concept



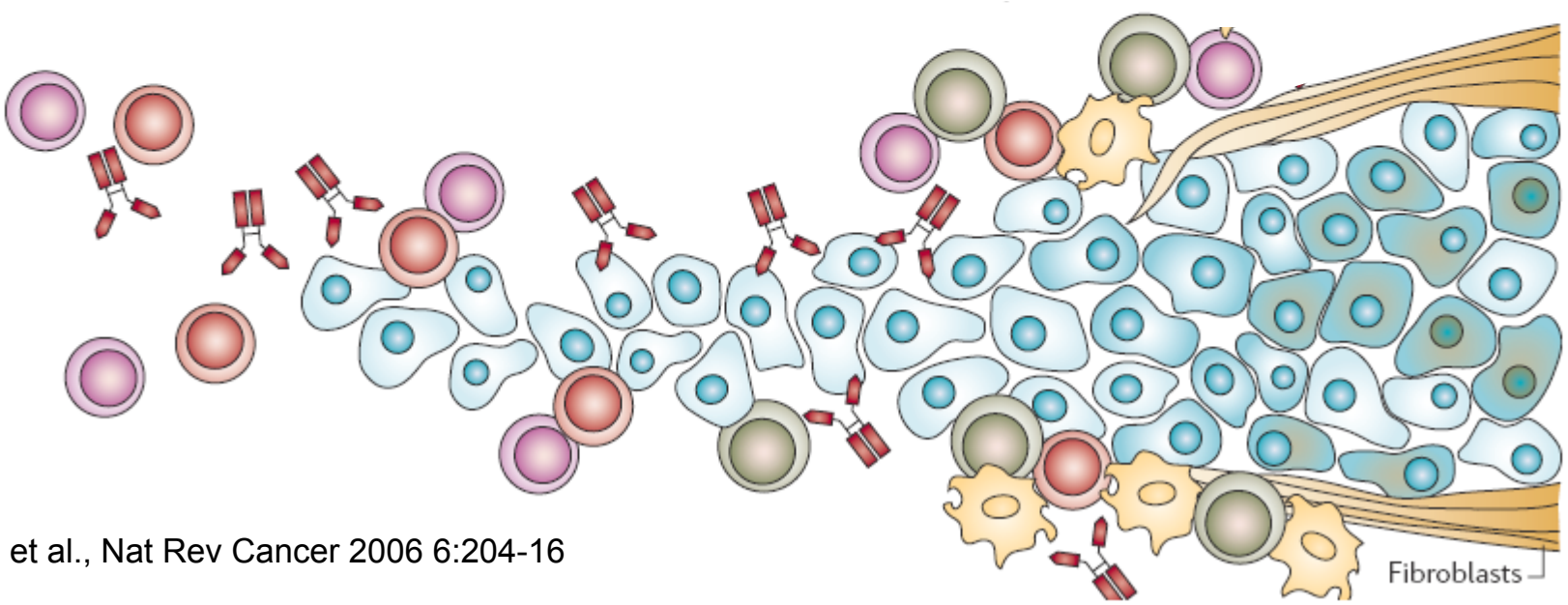
Schreiber *et al.*, Science 331: 1565, 2011

The immune hallmarks of cancer

Ability to

- ✓ thrive in a chronically inflamed microenvironment
- ✓ evade immune recognition
- ✓ suppress immune reactivity

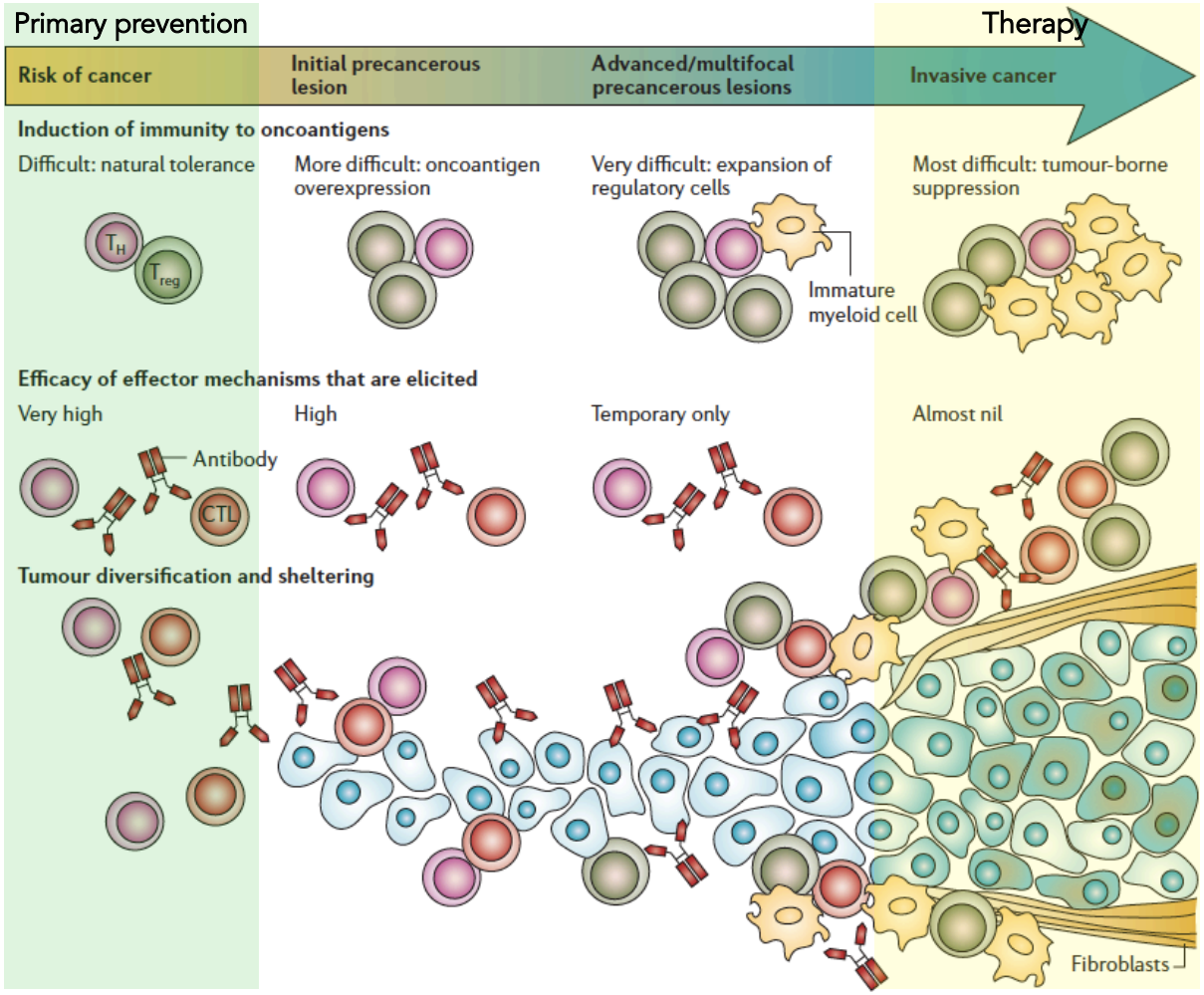
Cavallo et al, Cancer Immunol Immunother 2011; 60:319-26



Lollini et al., Nat Rev Cancer 2006 6:204-16

- ✓ Tumors are antigenic and are spontaneously recognized by the immune system
- ✓ This spontaneous immune reaction to tumors shapes tumor antigenicity
- ✓ A clinically evident tumor has acquired the ability to escape tumor recognition and to suppress the immune response
- ✓ These considerations must be taken into account to develop effective immunotherapies

Immunoprevention vs Immunotherapy



Adapted from Lollini et al, Nat Rev Cancer 2006 6:204-16

Primary immunoprevention: a futuristic option for non-infection associated tumors

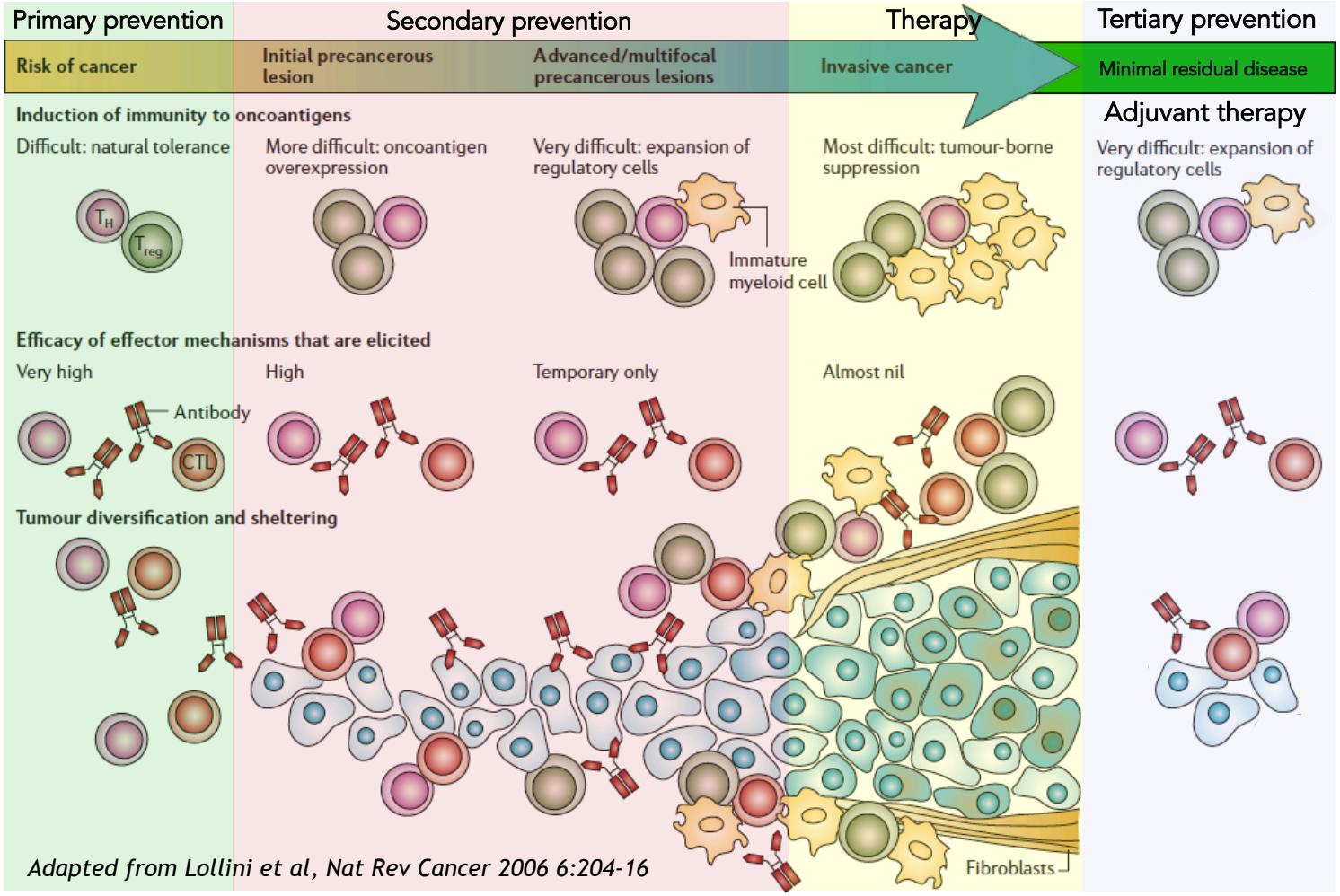
Vaccines against carcinogenic viruses, such as those against HBV and HPV, are currently used for primary prevention.

Application of primary immunoprevention for non-infection-associated tumors is still confined to pre-clinical experiments and futuristic projects.

The Artemis Project, supported by the US National Breast Cancer Coalition, is based on the identification of breast cancer-specific neoantigens expressed in the early phases of carcinogenesis and aims to stop people dying of breast cancer by 2020 (http://www.breastcancerdeadline2020.org)

"It may very well bring about immortality, but it will take forever to test it."

Immunoprevention vs Immunotherapy



Standard definitions of prevention

Type of prevention	Institute of Medicine of the National Academies, USA [2]	IARC, World Health Organization [3]
Primary	Primary prevention refers to health promotion, which fosters wellness in general and thus reduces the likelihood of disease, disability, and premature death in a nonspecific manner, as well as specific protection against the inception of disease.	Primary prevention is prevention of disease by reducing exposure of individuals to risk factors or by increasing their resistance to them.
Secondary	Secondary prevention refers to the detection and management of presymptomatic disease, and the prevention of its progression to symptomatic disease.	Secondary prevention (applied during the preclinical phase) is the early detection and treatment of disease. Screening activities are an important component of secondary prevention.
Tertiary	Tertiary prevention refers to the treatment of symptomatic disease in an effort to prevent its progression to disability or premature death. The overlap with treatment is self-evident, and perhaps suggests that preventive medicine has grandiose territorial ambitions. Be that as it may, there is a legitimate focus on prevention even after disease develops, such as the prevention of early cancer from metastasizing […]	Tertiary prevention (appropriate in the clinical phase) is the use of treatment and rehabilitation programmes to improve the outcome of illness among affected individuals.

Lollini PL et al., Vaccines 2015

Cancer immunoprevention

Cancer prevention	Aim	Target	Non-immunological examples	Immunological examples
Primary	Removal or avoidance of cancer risk factors	Healthy individuals	Healthy diet; Ban on carcinogens in the workplace; Quitting smoking; Tamoxifen in healthy women; Prophylactic mastectomy in hereditary breast cancer	Anti- HBV and HPV vaccines
Secondary	Early diagnosis and therapy	Pre-symptomatic cancer bearers	Pap test; Mammography; Colonoscopy	Anti- Her2 and MUC1 vaccines against preneoplastic or early neoplastic lesions
Tertiary	Prevention of relapse and metastasis	Survivors with occult neoplastic lesions	Prophylactic radiotherapy; Adjuvant chemotherapy	Adjuvant monoclonal antibodies; Adjuvant therapeutic vaccines; Intravesical instillations of Bacillus Calmette-Guerin

Lollini PL et al., Vaccines 2015

The rational repositioning of vaccines in the renaissance era of cancer immunotherapy

- Repositioning of cancer vaccines in the pre-malignant or in the adjuvant settings
- Combining cancer vaccines with blocking of endogenous immune-inhibiting mechanisms
- Utilization of enhanced vaccination platforms
- Testing cancer vaccines in reliable pre-clinical models

Lollini et al, Vaccines 2015
Bot et al., Expert Rev Vaccines. 2013;12:1219-34

Secondary immunoprevention: a future option whose efficacy is being tested now

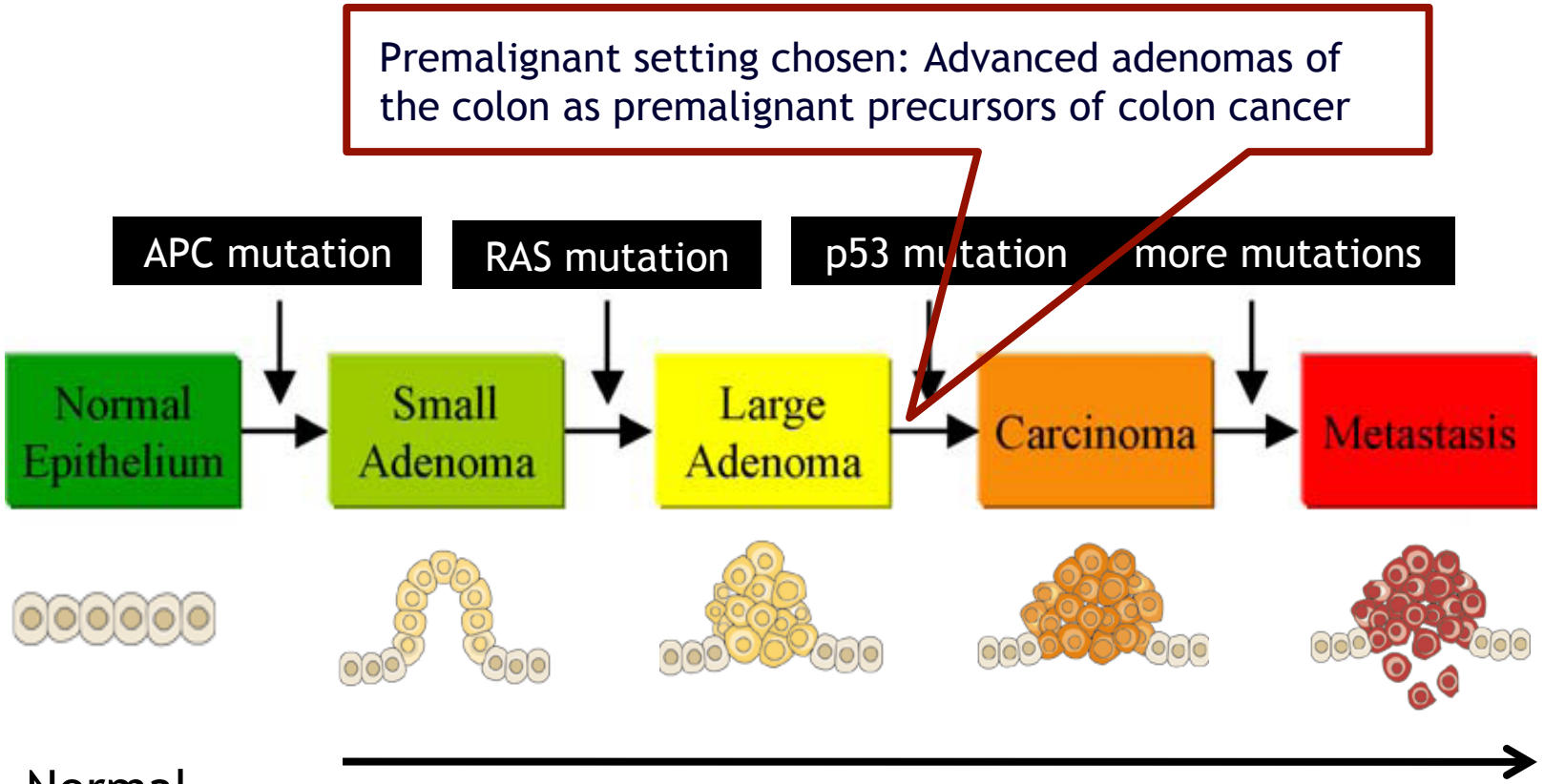
Testing a cancer vaccine based on a self/tumor associated antigen is possible in the premalignant setting.

High level immune responses can be achieved and memory responses can be evaluated.

Toxicity can be properly evaluated without the confounding effects of other previous or current treatments.

Many premalignant settings allow testing of vaccine efficacy in a relatively short time and in relatively small randomized trials.

MUC1 expression during colon cancer development



It plays a crucial role in progression of the disease

Adapted from Wilman Silk, Finn et al., Mol. Immunol. 2009

Randomized, placebo-controlled Phase II efficacy trial of the MUC1 prophylactic vaccine

- Patients: Diagnosis of advanced adenoma (removed)
- Vaccine: 100 mg MUC1 100mer peptide admixed with 500 mg of poly-ICLC (TLR3 agonist, Hiltonol®), 300 ml volume administered ID/SQ at W0, W2, W8, W52
- Primary endpoint: anti-MUC1 immune response
- Secondary endpoint: adenoma recurrence at Year 3
- Colon Cancer Prevention Network, NCI Division for Cancer Prevention
- Start date May 22, 2014, 13 medical centers involved
- 3-5 years follow-up
- Preliminary results suggest 52% of patients respond

Tertiary immunoprevention: the present option

The vast majority of cancer vaccines that have recently emerged from successful preclinical testing have been translated into clinical trials of tertiary cancer prevention (adjuvant therapy).

The odyssey of the first FDA approved therapeutic cancer vaccine, sipuleucel-T (Provenge), is quite an interesting example.

Provenge®

FDA approved for the treatment of asymptomatic or minimally symptomatic metastatic castration-refractory prostate cancer patients.

Withdrawal of the marketing authorisation in the European Union.

What's in a name?

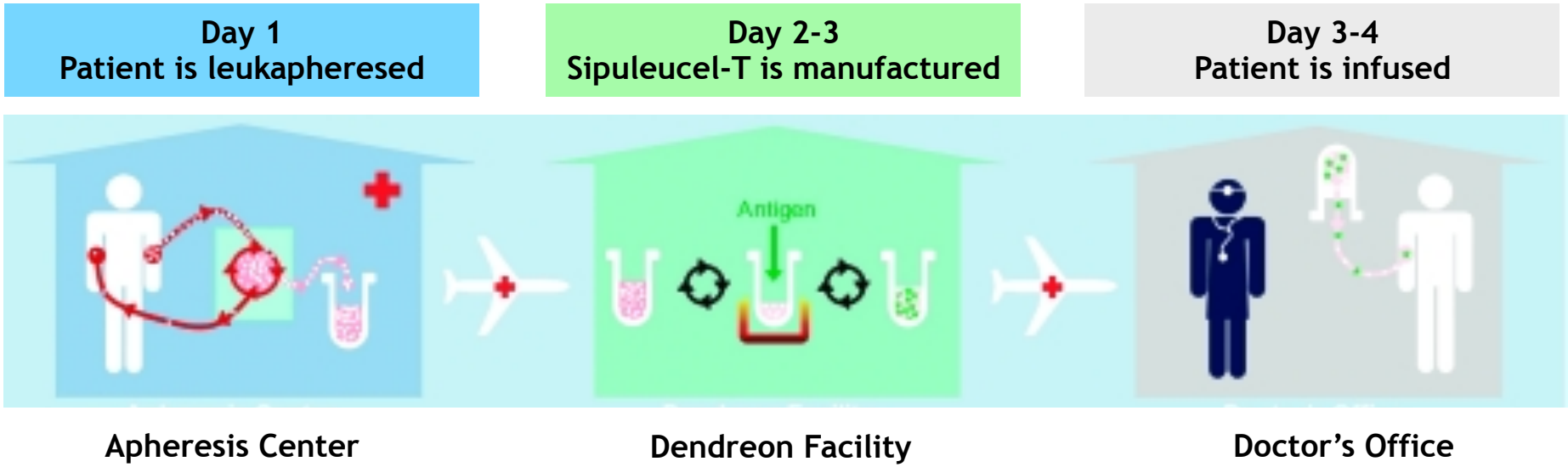
Sipuleucel-T (Provenge®)
approved by US FDA in 2010 for the treatment of asymptomatic or minimally symptomatic metastatic castration-refractory prostate cancer patients

Stem	What it means
si-	unique name
-pu-	pulsed with a cancer protein
-leu-	white blood cells
-cel	cellular therapy
-T	autologous

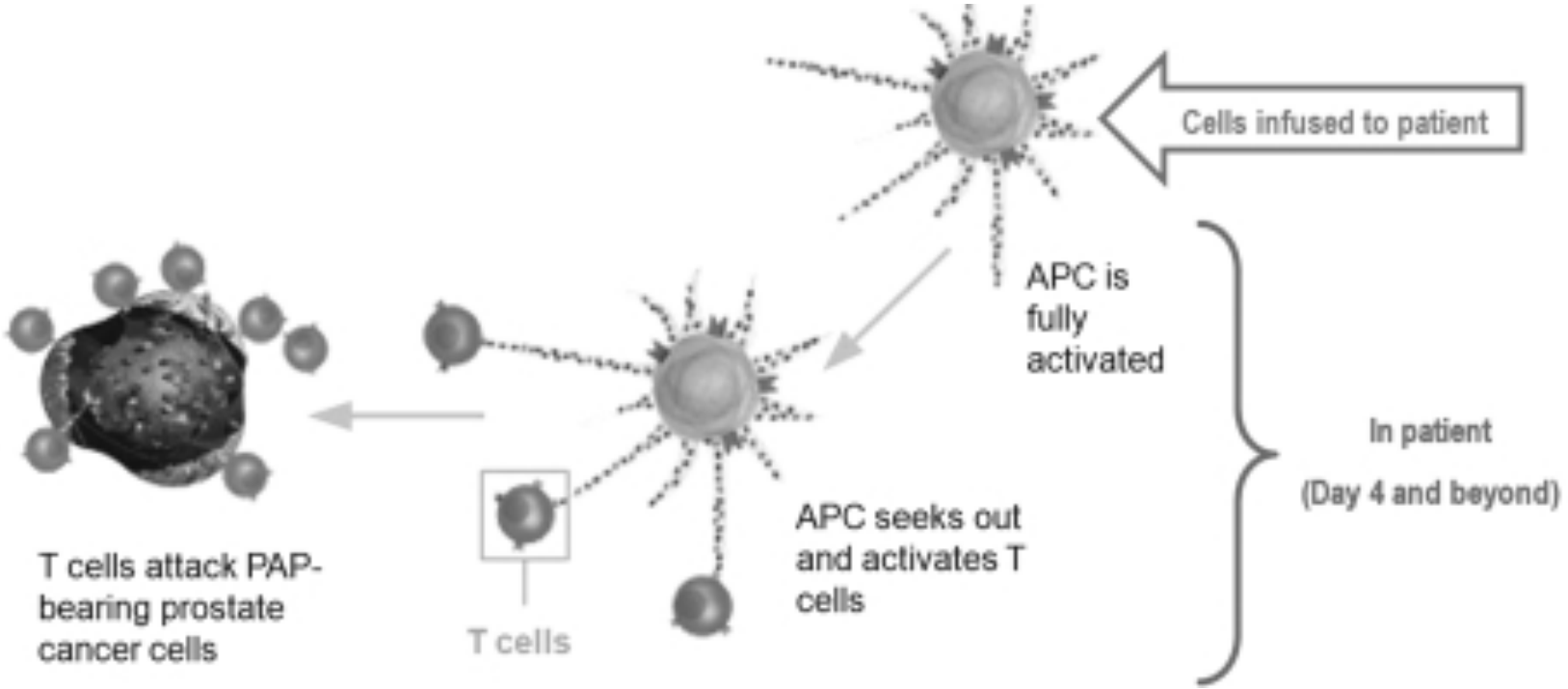
http://cancerresearch.org

Provenge®

Autologous peripheral blood mononuclear cells pulsed with a proprietary fusion protein (PA2024), which combines the antigen prostatic acid phosphatase (PAcP) with granulocyte macrophage colony stimulating factor (GM-CSF)



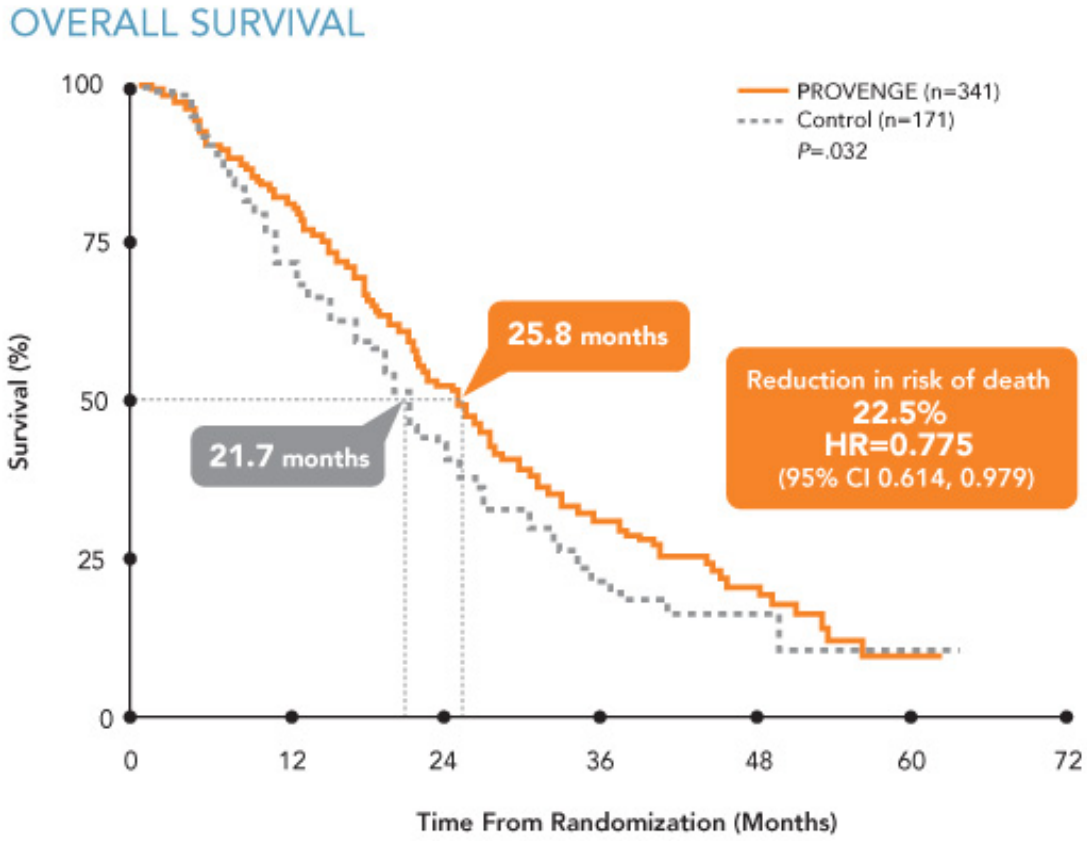
Adapted from Huber et al., J Natl Cancer Inst, 104: 273-279; 2012



Modified from Huber et al., J Natl Cancer Inst, 104: 273-279; 2012

The first problem with Provenge® is the *cost* (>$70,000), too expensive to justify its use by National Health Service.

Secondly, the *therapeutic benefit* in term of survival, about four months *versus* placebo, is modest.



Provenge®: modest therapeutic benefit
A possible explanation in the target antigen?

PAcP is a prostate epithelium-specific ***differentiation antigen***. In normal differentiated prostate epithelia, PAcP protein can be detected intracellularly and secreted in seminal fluid.

Serum PAcP level is low in healthy individuals, while it is elevated in individuals with metastatic prostate cancer and correlates with the stage of the disease.

Dual role in cancer: ***oncosuppressor*** (dephosphorylates p-Tyr of ErbB-2) and ***marker*** (overexpressed and released by prostate cancer cells). Its reduced intracellular amount correlates with increased tumor cell proliferation…

The rational repositioning of vaccines in the renaissance era of cancer immunotherapy

- Repositioning of cancer vaccines in the pre-malignant or in the adjuvant settings
- Combining cancer vaccines with blocking of endogenous immune-inhibiting mechanisms
- Utilization of enhanced vaccination platforms
- Testing cancer vaccines in reliable pre-clinical models
- Redirecting cancer vaccines to *more relevant targets*

Lollini et al, Vaccines 2015
Bot et al., Expert Rev Vaccines. 2013;12:1219-34

ONCOANTIGENS are tumour associated antigens (TAA) that

- have an essential role in tumor growth or progression and a restricted distribution in normal tissues

  unique TAA (neoantigens)
  derived from protein-altering "driver" mutations

  conserved TAA
  non-mutated proteins involved in the oncogenic process

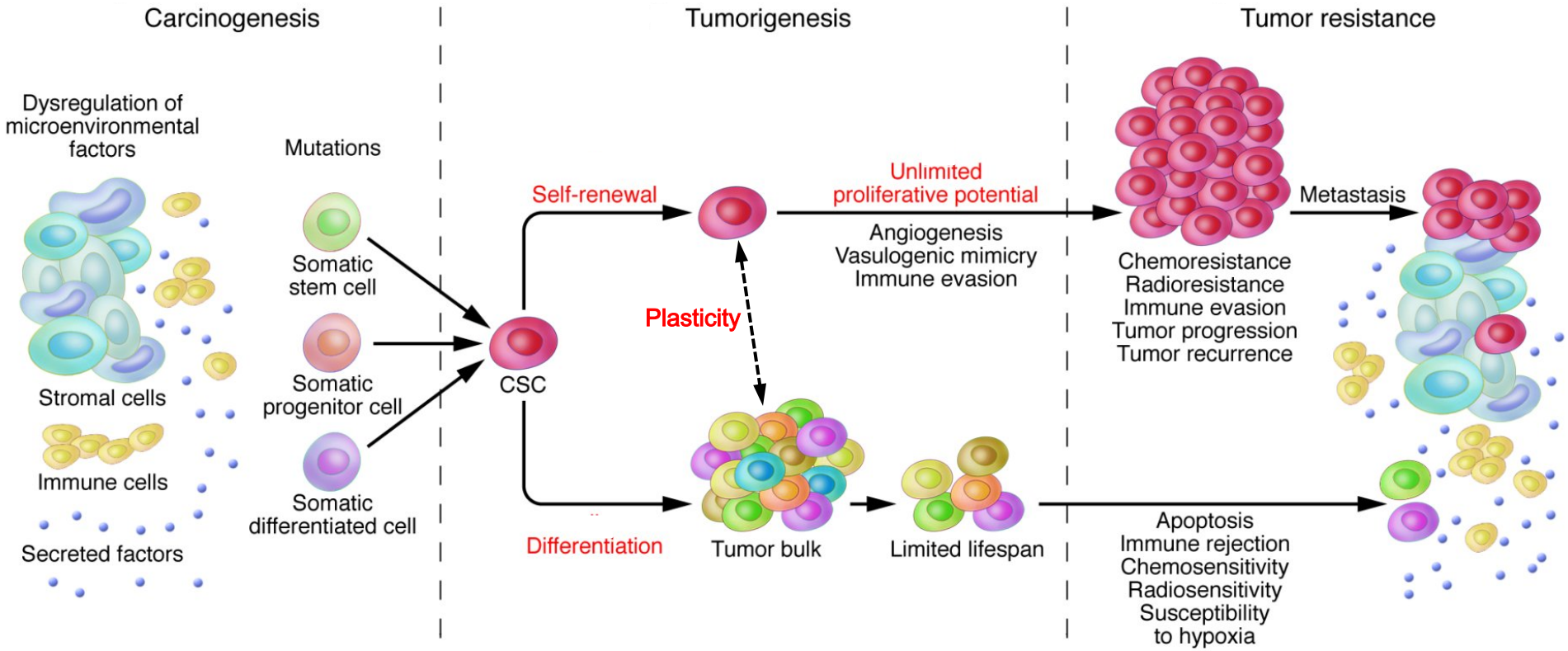
- cannot be easily down-modulated or negatively immuno-edited under the pressure of a specific immune attack

- when expressed on the cell membrane can be the target of both cell and antibody mediated immune responses

- if (over)expressed by cancer stem cells are ideal targets for anti-tumor vaccination

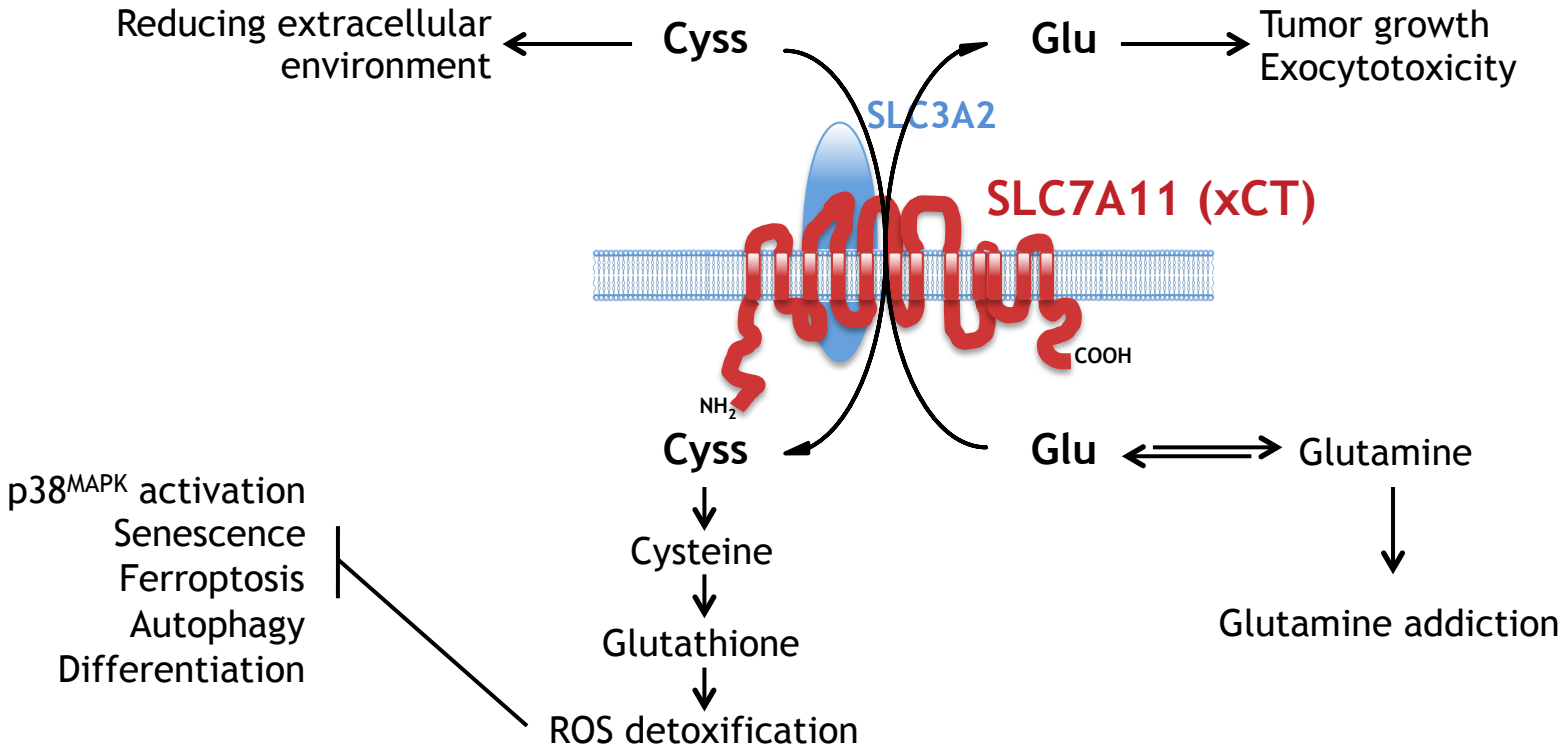
Lollini et al, Nat Rev Cancer 2006
Cavallo et al, Nat Rev Cancer 2007
Cavallo et al, Cancer Immunol Immunother 2011

Why targeting cancer stem cells (CSC)

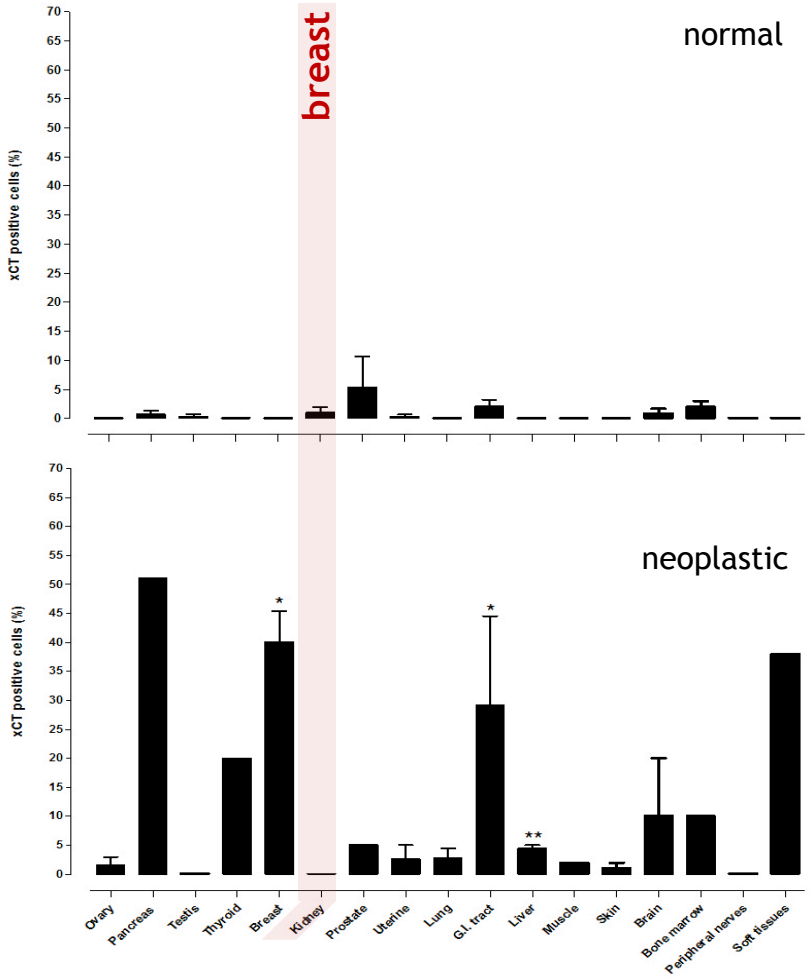


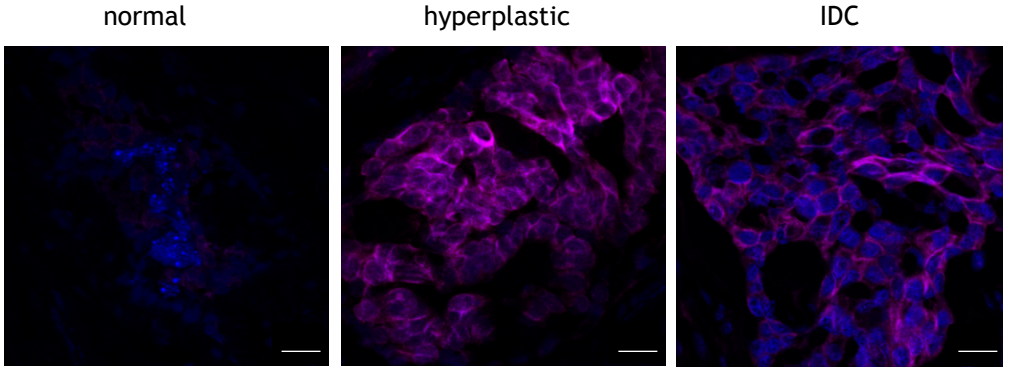
Adapted from Frank et al, J Clin Invest. 2010;120:41-50

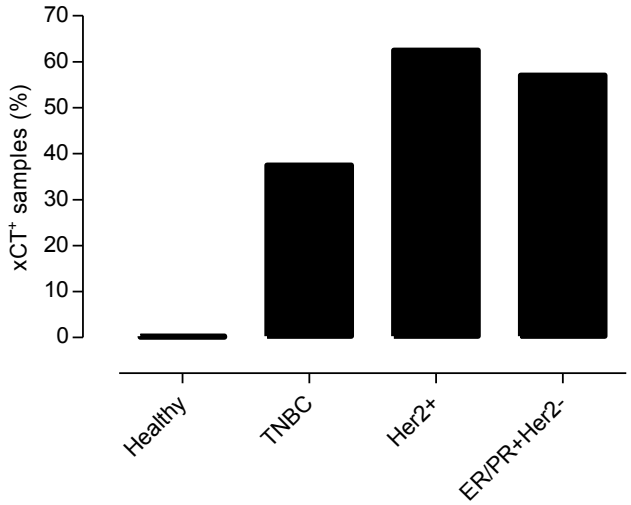
The cystine/glutamate antiporter system x_c^-



xCT expression in normal and neoplastic human tissues

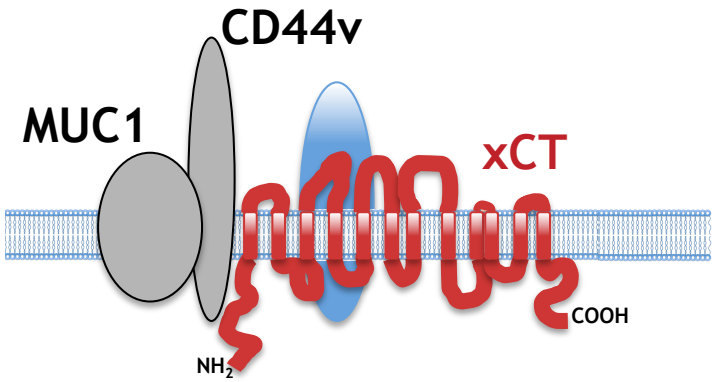


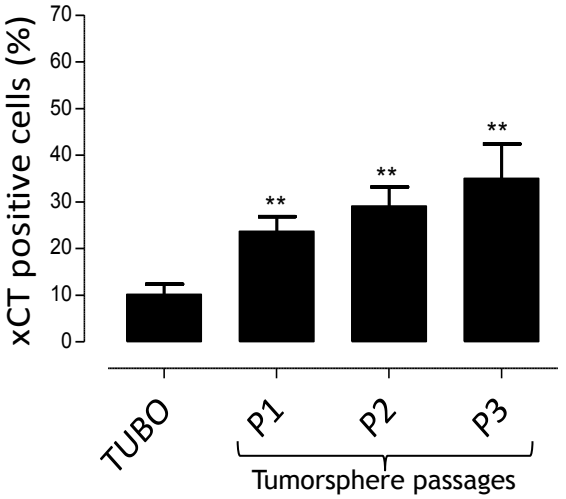


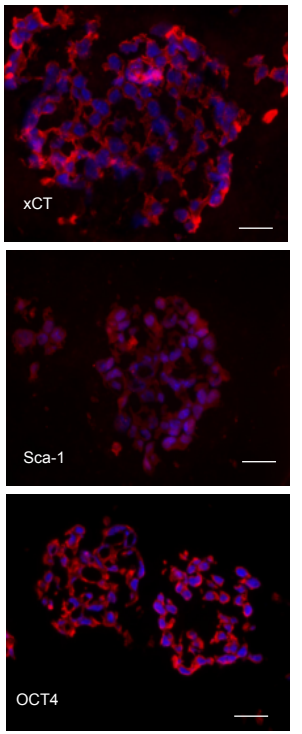


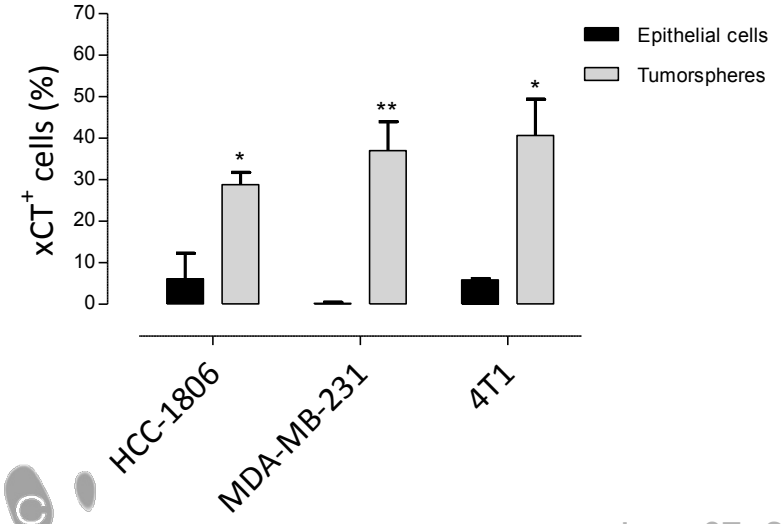
Lanzardo S et al. Cancer Res 2016; 76:62-72

xCT is overexpressed in CSC







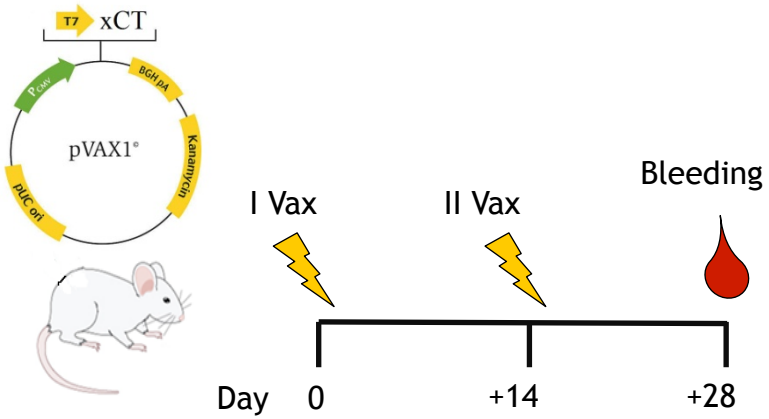


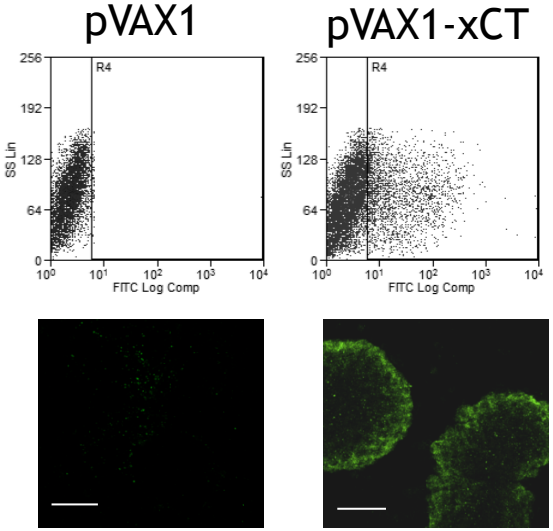
Ishimoto T. et al, Cancer Cell. 2011;19:387-400

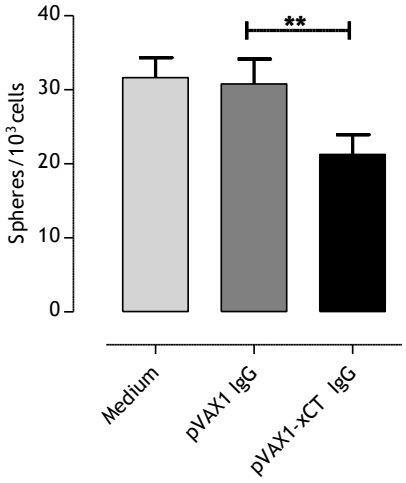
Hasegawa M. et al, Oncotarget. 2016;7:11756-69

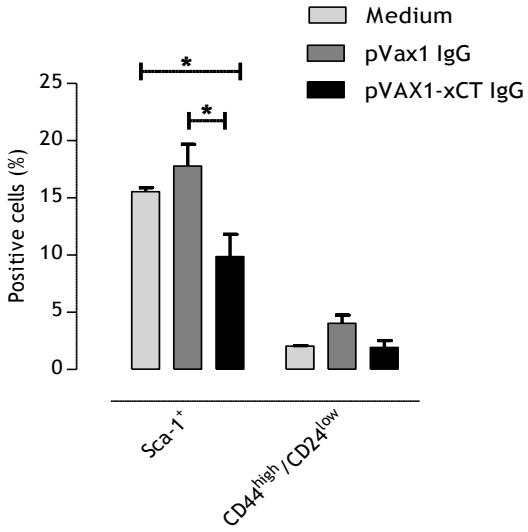
Lanzardo S et al. Cancer Res 2016; 76:62-72

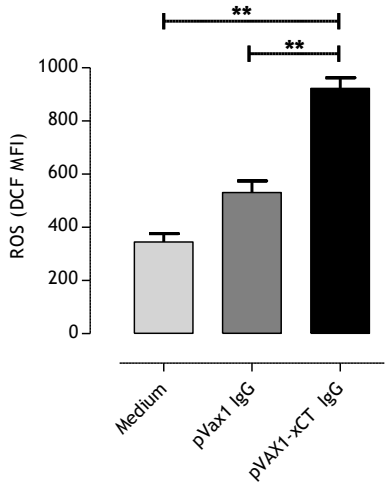
Vaccination against xCT induces antibodies that target CSC and affect their self-renewal and ROS flux





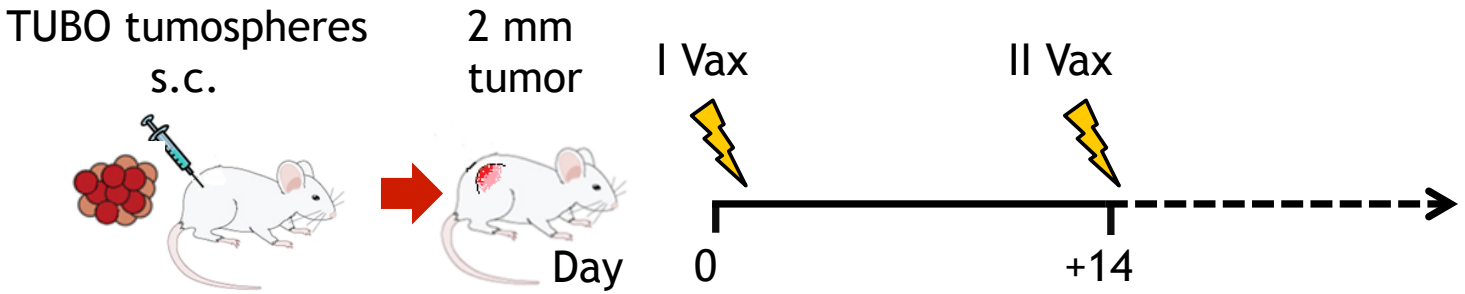


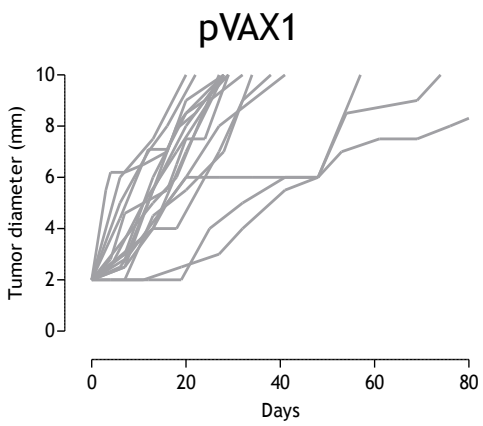


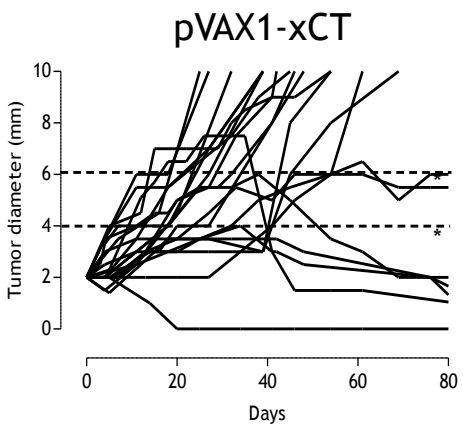


Lanzardo et al., Cancer Res 2016

Vaccination against xCT slows down tumor growth in vivo







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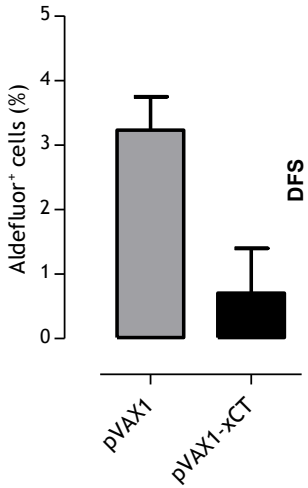
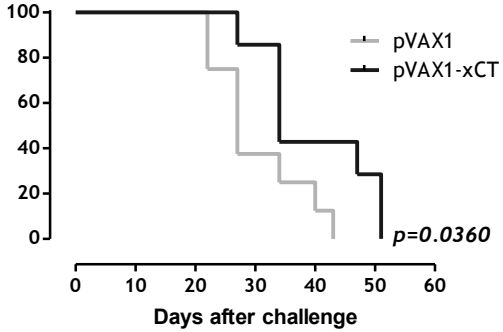
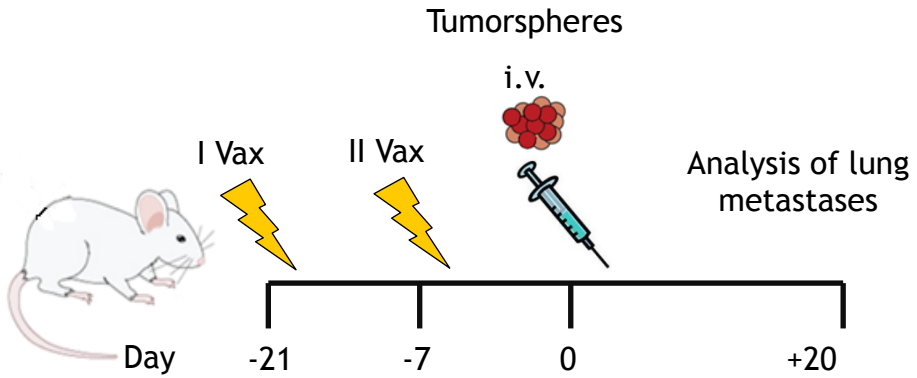
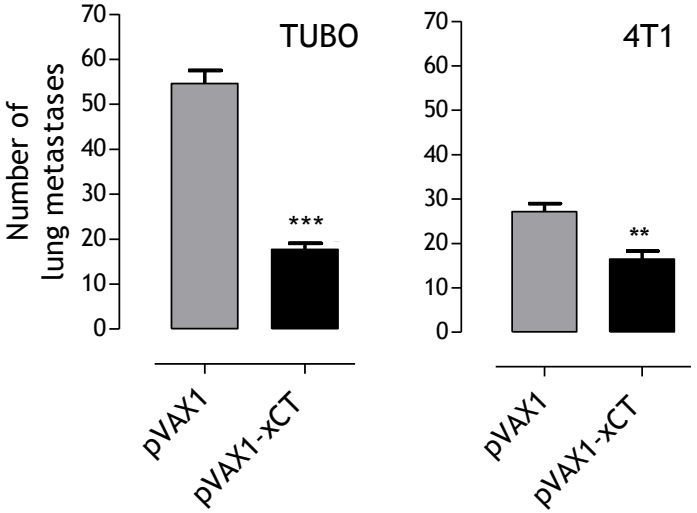


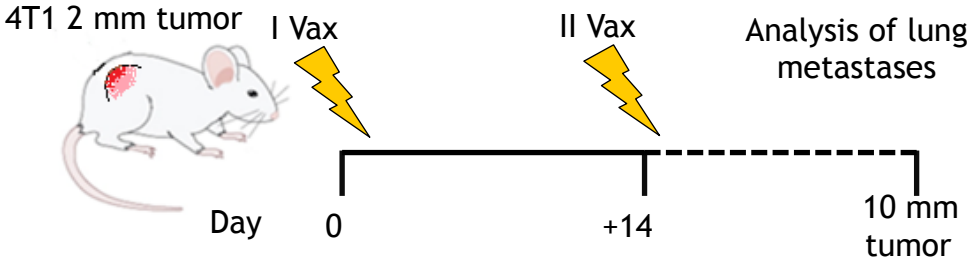


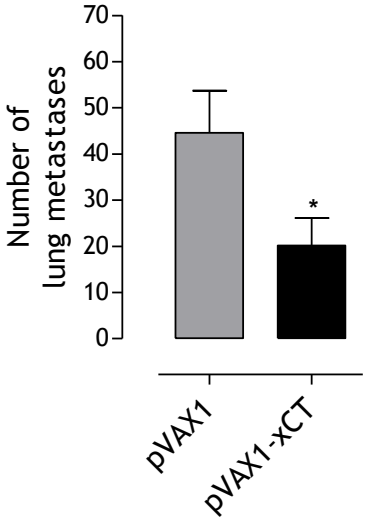
Lanzardo et al., Cancer Res 2016

Vaccination against xCT reduces lung metastasis formation



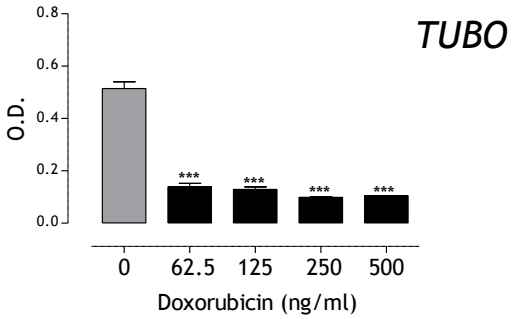


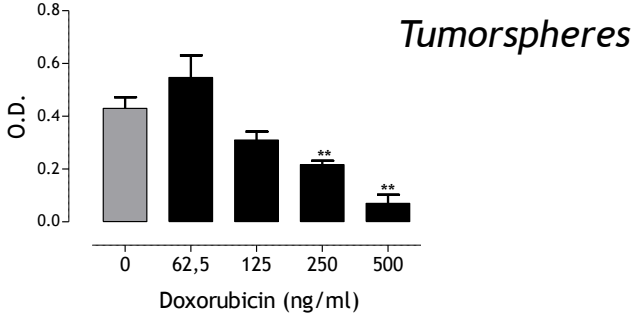


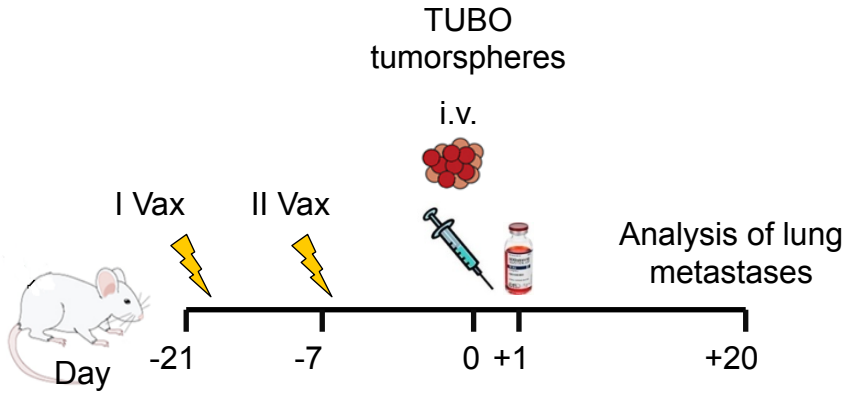


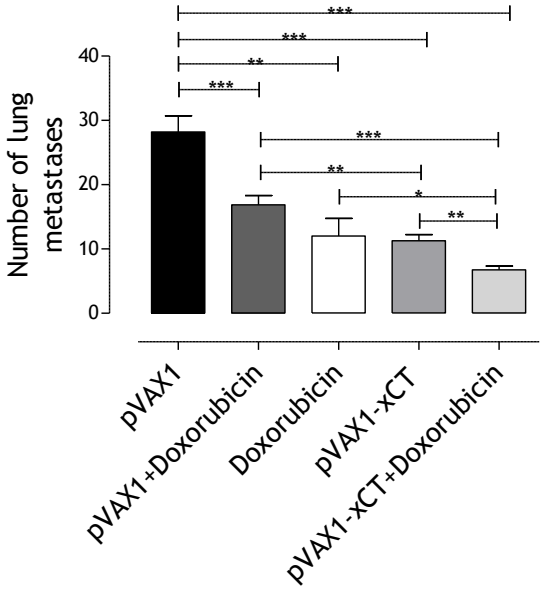
Lanzardo et al., Cancer Res 2016

Vaccination against xCT increases CSC chemo-sensitivity



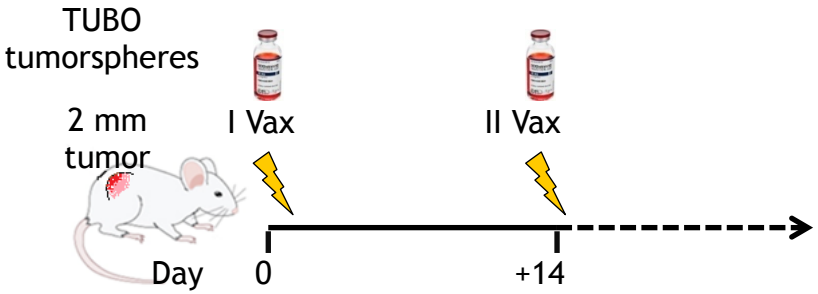




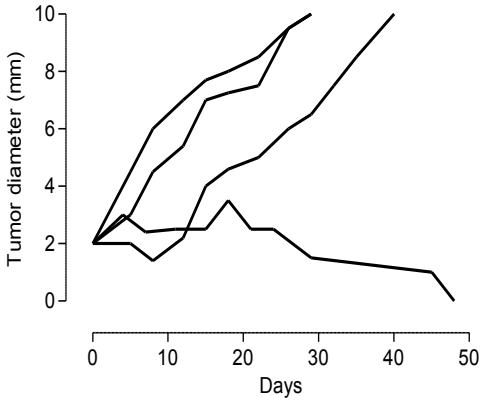


Lanzardo et al., Cancer Res 2016

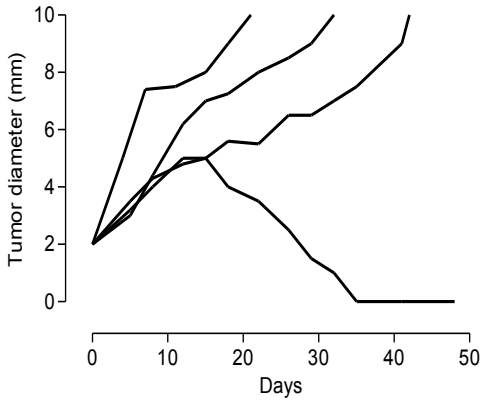
Vaccination against xCT increases CSC chemo-sensitivity



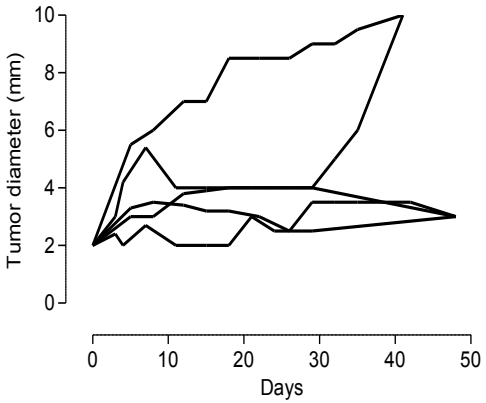
Doxorubicin



Doxorubicin + pVAX1



Doxorubicin + pVAX1-xCT



Lanzardo et al., Cancer Res 2016

Conclusions

- xCT is expressed in tumors and over-expressed in CSC
- xCT is required for CSC function (oncoantigen)
- xCT therapies should not lead to cancer escape mutants via antigen loss mechanisms
- xCT is an immunotherapeutic target for breast CSC
- Vaccination against xCT:
  - ✓ induces antibodies that impair CSC function
  - ✓ has limited (if any) effects on normal tissues
  - ✓ slows down tumor growth and impairs lung metastases formation in both $Her2^+$ and TNBC models
  - ✓ increases CSC chemosensitivity (combination therapies)
- xCT represents a novel target (aminoacid transporter) for a new class of anticancer vaccines directed against CSC functional molecules

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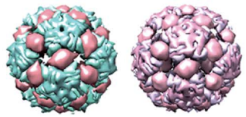
MS2 and QB VLP

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ASSOCIAZIONE ITALIANA PER LA RICERCA SUL CANCRO
AIRC

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Did you know?

1 in 3 people

1 in 4 dogs

1 in 5 cats

will develop cancer in their lifetimes

Naturally occurring cancer in companion animals

An undervalued resource at the center of growing attention because of the translational implications it may hold for human disease

CENTER FOR CANCER RESEARCH — Comparative Oncology Program — National Cancer Institute

https://ccrod.cancer.gov/confluence/display/CCRCOPWeb/Home

http://www.eurolupa.eu

Pets & People
Shared cancers, shared hopes

Similar in

- risk to develop tumors (association with age and behaviour)
- tumor onset, progression, histotypes
- antigen expression patterns
- interaction with the immune system
- response to therapy

Both may benefit from the testing of new therapies

The treatment of canine/feline patients today could be of great help for their owners tomorrow

Mirroring the human reality: the importance of the canine avatar

Syngeneic microenvironment shaped by the natural evolution of the tumor

Complex interactions between tumor and immune system

Recurrences and metastases

Naturally develop tumors growing over long time

Tumor genetics and molecular targets

Anatomical, histological, physiological similarities

Intratumoral and inter-individual heterogeneity

Similar genetic, nutritional, environmental risk factors

Scaling-up doses

Riccardo et al., Cancer Immunol Immunother, 2015

The rationale for evaluating (immuno)therapeutics in dogs before carrying out in-human studies

- high translational value
- complement other in vivo cancer models
- societal acceptance
- pet owners allow their dogs to participate in clinical trials
- reduced regulatory guidelines
- compressed life span allows rapid development and completion of clinical trials
- the treatment of canine patients today could be of great help for their owners tomorrow

Adjuvant immunotherapy of dog oral malignant melanoma with electroporated human chondroitin sulfate proteoglycan-4 (CSPG4) DNA

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Chondroitin Sulfate Proteoglycan 4 (CSPG4)

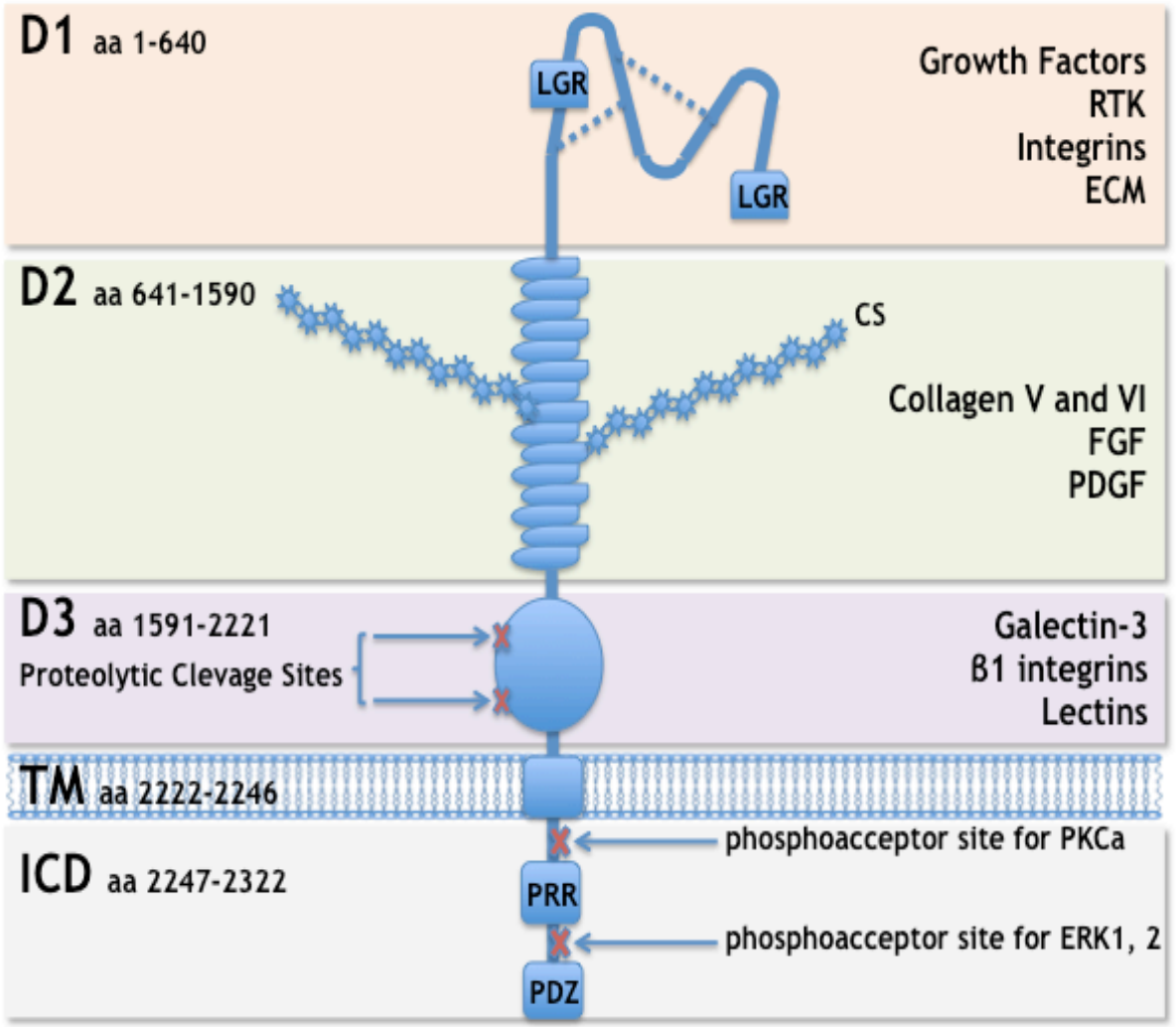
Key role in:

- proliferation and survival
- EMT
- cytoskeletal reorganization
- chemo resistance
- invasion and migration

High density expression on melanomas and various other tumor types

Overexpressed in cancer stem cells

Human vs canine aa sequence: 82% homology, 88% similarity



CSPG4 expression in canine MM

Tissue samples from 65 canine oral (55) and subungueal (10) MMs collected between 2000 and 2010 at the Diagnostic Laboratory of the Department of Animal Pathology of the University of Turin were examined

Immunohistochemical staining revealed 37 positive MMs

Histotypes	Melanotic (positive/total)		Amelanotic (positive/total)		All tumours (positive/ total)
	Oral	Digital	Oral	Digital	
Epithelioid	12/15	3/3	6/8	0/0	21/26 (80.8%)
Fusiform	2 / 6	1/2	3/6	0/1	6/15 (40.0%)
Mixed	5 /17	2/4	3/3	0/0	10/24 (41.7%)
Proportion of CSPG4-stained tumours	19/38 (50%)	6/9 (66.7%)	12/17 (70.5%)	0/1 (0)	37/65 (56.9%)

Lorda Mayayo et al., Vet J. 2011

Prognosis of canine oral MM

- *Surgery, radiotherapy and chemotherapy*, alone or in combination, control the tumor locally in up to 75% of animals; however the 1-year survival rate does not exceed 30% because of metastasis

- The *ONCEPT (Merial) vaccine* is labelled to extend median survival times in dogs with stage II or III OMM that have achieved local disease control via surgery and/or radiation therapy (*Grosenbaugh et al., 2011*). Its efficacy has been recently questioned (*Ottnod et al., 2013*)

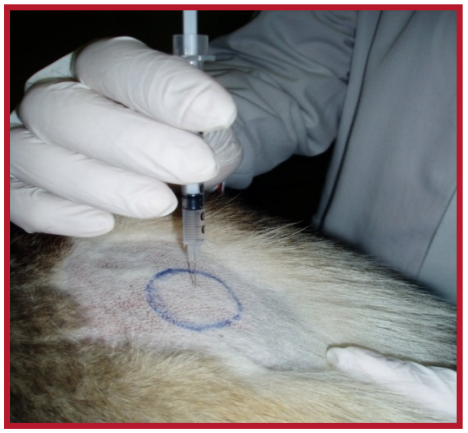
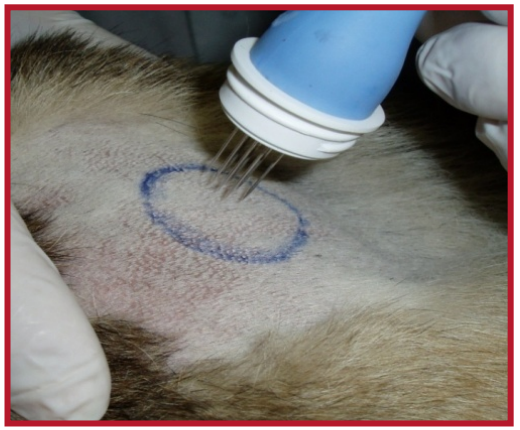
- Need to explore *new immunotherapeutic options* (new targets, different formulations, …)

Pilot study of DNA vaccination against CSPG4 in dogs with CSPG4^{+} spontaneous oral malignant melanoma

1. Histological diagnosis of oral malignant melanoma
2. Biopsy-evaluation of CSPG4 expression by IHC
3. Complete work-up for clinical staging
4. Estimated life expectancy of more than 4 months (absence of serious concurrent diseases)
5. Possibility of *"en bloc"* tumor and regional LN(s) resection (evaluation of excision margins and LNs)
6. Absence of distant metastases beyond the first draining LN
7. Written consent from the dog owner

Riccardo et al, Clinical Cancer Res 2014
Piras et al, Vet Comp Oncol. 2016

Xenogeneic DNA electrovaccination against CSPG4 in dogs

500 μg of a plasmid coding for ***human CSPG4*** injected i.m. into the semimembranosus semitendinosus muscle region of anesthetized dogs, followed by electroporation (Cliniporator™ IGEA, Carpi, Italy).

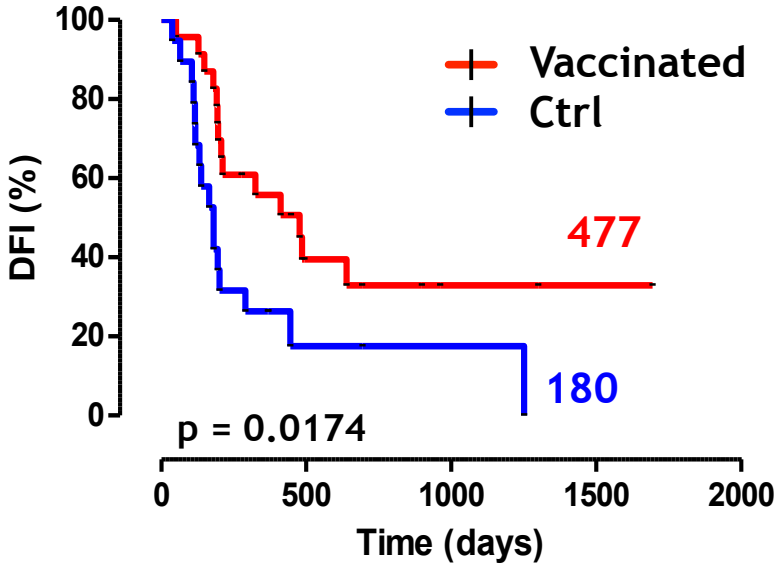
Vaccination started at the 3rd-4th post-operative week and was repeated after 2 weeks and then monthly; dogs surviving over 2 years were then vaccinated every 6 months.

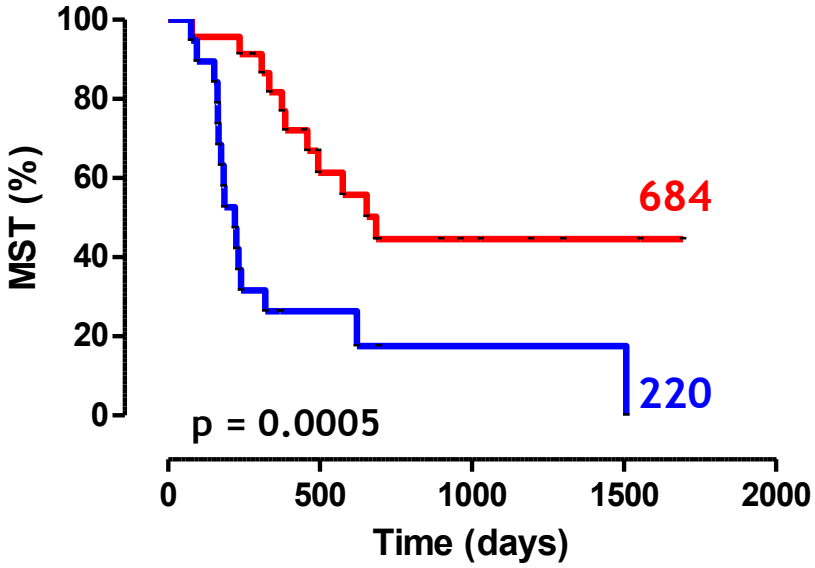
Riccardo et al, Clinical Cancer Res 2014
Piras et al, Vet Comp Oncol. 2016

DNA vaccination targeting CSPG4 is associated with increased disease free and overall survival

Population	Stage II	Stage III	Local Invasion	Excision Margins	
				Complete	Incomplete
Overall (n=42)	15 (35.71)[a]	27 (64.29)[a]	16 (38.09)[a]	34 (80.95)[a]	8 (19.05)[a]
Vax (n=23)	9 (39.13)	14 (60.87)	9 (39.13)	19 (82.61)	4 (17.39)
Ctrl (n=19)	6 (31.58)	13 (68.42)	7 (36.84)	15 (78.95)	4 (21.05)

[a] % in brackets





Piras et al, Vet Comp Oncol. 2016

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