Week 8, Immunology and Cancer

Ed Roy, Marie Roy, Sue Ingels, Mary Kuetemeyer

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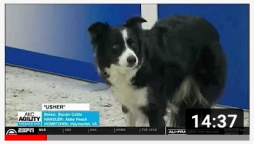

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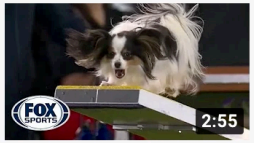

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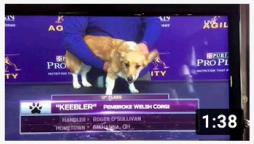

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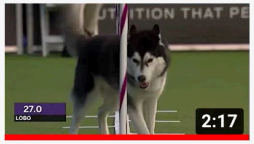

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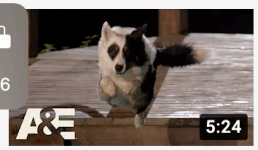

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A&E

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Border Collies | Breed Judging 2021

Westminster Kennel Club Dog...

FOCUS

- Develop immunotherapy that can eradicate brain tumors

Learning Objectives

- Does immune system play role in initiating and promoting cancer?

  Yes
- Does immune system play role in preventing cancer?

  Yes
- Can the immune system be directed to treat cancer?

  Yes

Chronic Inflammation promotes cancer

Chronic inflammatory conditions enhance a predisposition to cancer development.

Polymorphisms in genes that regulate immune balance influence cancer risk

Long-term usage of non-steroidal anti-inflammatory drugs and selective cyclooxygenase-2 inhibitors reduces cancer incidence.

.

Immune Cells are Part of Tumor Stroma that promotes Tumor growth

- Many immune cell types have two phenotypes, one pro-inflammatory and one anti-inflammatory
  - Mature dendritic cells vs tolerogenic DCs
  - M1 and M2 macrophages
  - Neutrophils and myeloid derived suppressor cells
  - Th1 CD4 cells vs Tregs

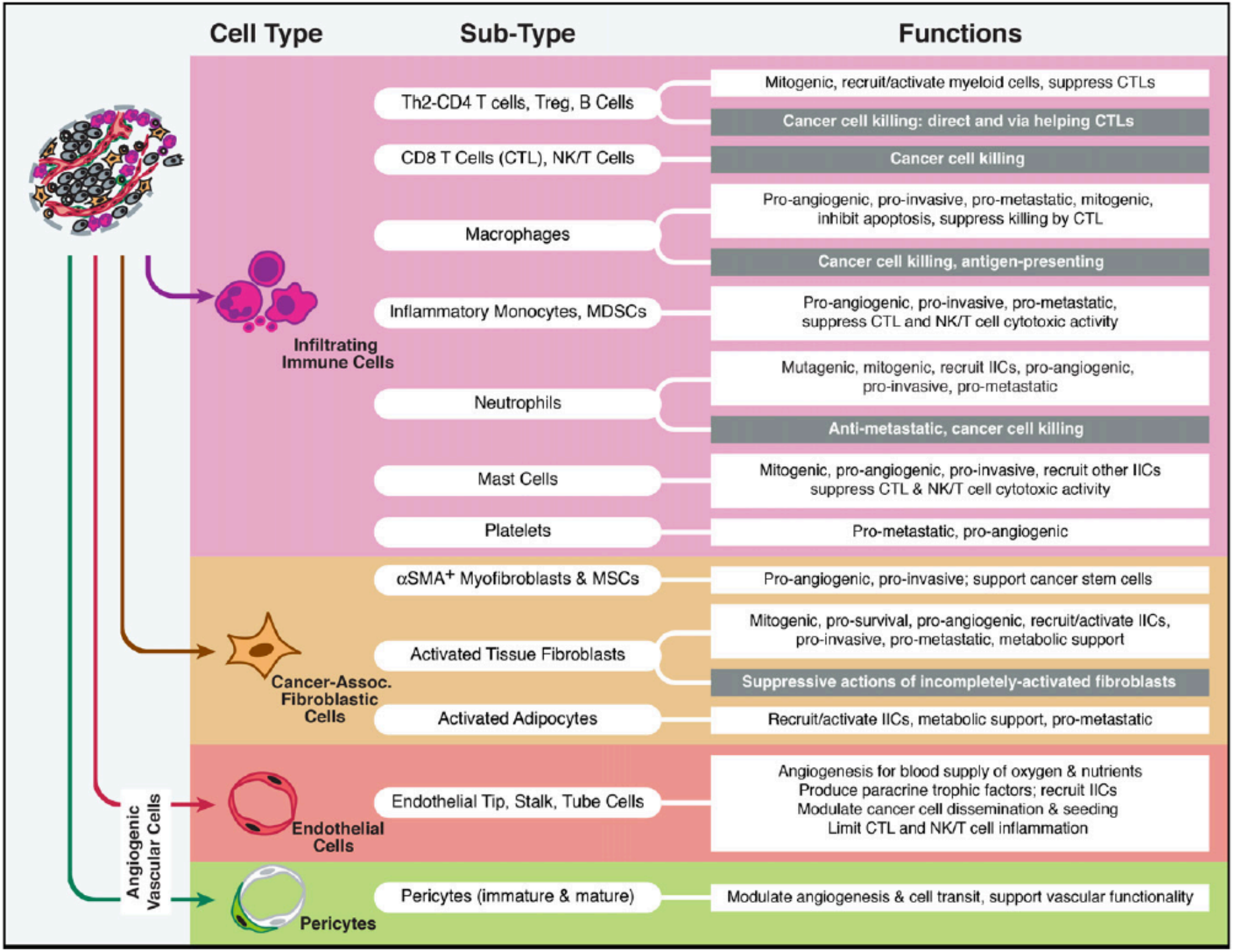

Cell Type
Sub-Type
Functions
Th2-CD4 T cells, Treg, B Cells
Mitogenic, recruit/activate myeloid cells, suppress CTLs
Cancer cell killing: direct and via helping CTLs
CD8 T Cells (CTL), NK/T Cells
Cancer cell killing
Macrophages
Pro-angiogenic, pro-invasive, pro-metastatic, mitogenic, inhibit apoptosis, suppress killing by CTL
Cancer cell killing, antigen-presenting
Inflammatory Monocytes, MDSCs
Pro-angiogenic, pro-invasive, pro-metastatic, suppress CTL and NK/T cell cytotoxic activity
Infiltrating Immune Cells
Neutrophils
Mutagenic, mitogenic, recruit IICs, pro-angiogenic, pro-invasive, pro-metastatic
Anti-metastatic, cancer cell killing
Mast Cells
Mitogenic, pro-angiogenic, pro-invasive, recruit other IICs suppress CTL & NK/T cell cytotoxic activity
Platelets
Pro-metastatic, pro-angiogenic
αSMA+ Myofibroblasts & MSCs
Pro-angiogenic, pro-invasive; support cancer stem cells
Activated Tissue Fibroblasts
Mitogenic, pro-survival, pro-angiogenic, recruit/activate IICs, pro-invasive, pro-metastatic, metabolic support
Suppressive actions of incompletely-activated fibroblasts
Cancer-Assoc. Fibroblastic Cells
Activated Adipocytes
Recruit/activate IICs, metabolic support, pro-metastatic
Endothelial Tip, Stalk, Tube Cells
Angiogenesis for blood supply of oxygen & nutrients
Produce paracrine trophic factors; recruit IICs
Modulate cancer cell dissemination & seeding
Limit CTL and NK/T cell inflammation
Endothelial Cells
Angiogenic Vascular Cells
Pericytes (immature & mature)
Modulate angiogenesis & cell transit, support vascular functionality
Pericytes

Figure 2. Multiple Stromal Cell Types and Subcell Types of the Tumor Microenvironment Can Variably Contribute to, or in Some Cases Oppose, Acquisition of the Seven Hallmark Functional Capabilities in Different Organ Sites, Tumor Types and Subtypes, and Stages of Progression

Major stromal cell subtypes are indicated, along with a synopsis of key functional contributions that such cell subtypes can make. The antagonistic functions of certain subcell types are highlighted in gray. The lists of subtypes and of their key functions are not comprehensive, but rather prominent examples. Not listed are molecular regulatory signals for, and effector agents of, the noted functions. Both lists will certainly be refined in coming years. Also not shown are the crucial cancer cells and cancer stem cells, with which these stromal cells dynamically interact to manifest cancer phenotypes (Hanahan and Weinberg, 2011). Th2, helper type 2; CD4 T cell, CD4-positive lymphocyte; Treg, regulatory T cell; CTL, cytotoxic T lymphocyte; NK/T, natural killer and natural killer T cell; MDSCs, myeloid-derived suppressor cells; αSMA, alpha smooth muscle actin; MSCs, mesenchymal stem cells.

Hanahan 2012

Immune cells prepare for metastasis

- Myeloid derived suppressor cells arrive before cancer cells

- Fusion of cancer cell and macrophages

- Is the immune system able to recognize cancer cells as dangerous and different?

Tumor Surveillance Hypothesis

- formally proposed by Thomas and Burnet in 1957.

- States that the immune system actively protects the host against altered self-cells including those that have undergone transformation.

Evidence	Conclusion
Histopathologic and clinical observations: lymphocytic infiltrates around some tumors and enlargement of draining lymph nodes correlate with better prognosis	Immune responses against tumors inhibit tumor growth
Experimental: transplants of a tumor are rejected by animals previously exposed to that tumor; immunity to tumor transplants can be transferred by lymphocytes from a tumor-bearing animal	Tumor rejection shows features of adaptive immunity (specificity, memory) and is mediated by lymphocytes
Clinical and experimental: Immunodeficient individuals have an increased incidence of some types of tumors	The immune system protects against the growth of tumors (the concept of "immune surveillance")

Evidence supporting the concept that the immune system reacts against tumors. Several lines of clinical and experimental evidence indicate that defense against tumors is mediated by reactions of the adaptive immune system.

T cells as prognostic factor

- Infiltrating effector T cells are the best prognostic factor after any type of therapy

Intratumoral T Cells, Recurrence, and Survival in Epithelial Ovarian Cancer

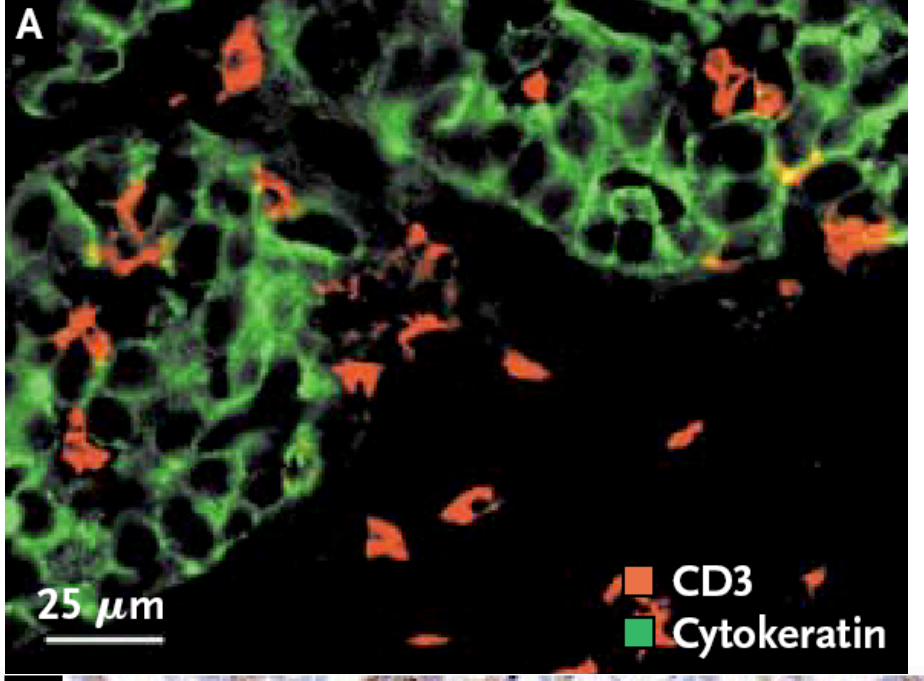
Lin Zhang, M.D., Jose R. Conejo-Garcia, M.D., Ph.D., Dionyssios Katsaros, M.D., Ph.D., Phyllis A. Gimotty, Ph.D., Marco Massobrio, Regnani, M.D., Antonis Makrigiannakis, M.D., Ph.D., Heidi Gray, M.D., Katia Schlienger, M.D., Ph.D., Michael N. Liebman, Ph.D., Ste M.D., and George Coukos, M.D., Ph.D.

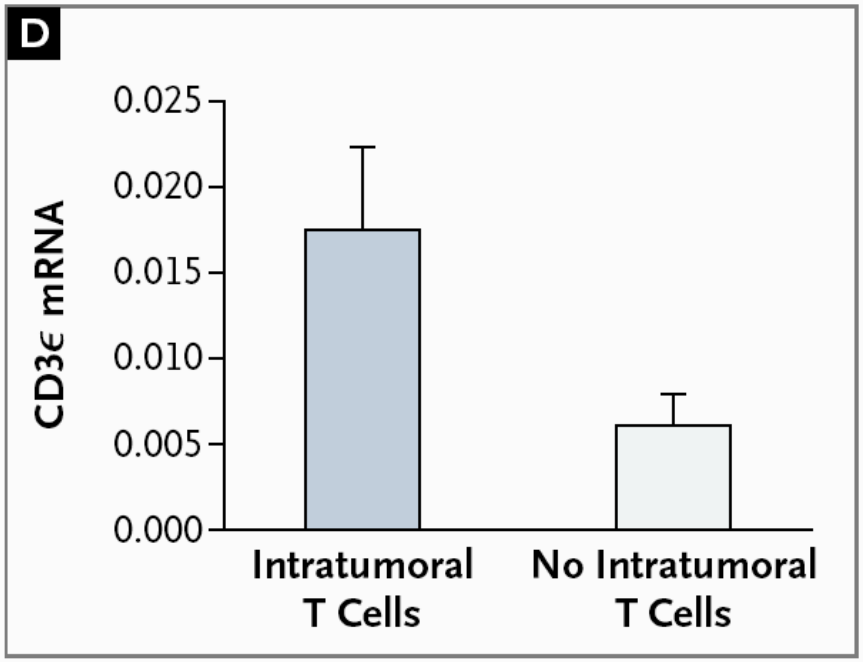
ABSTRACT

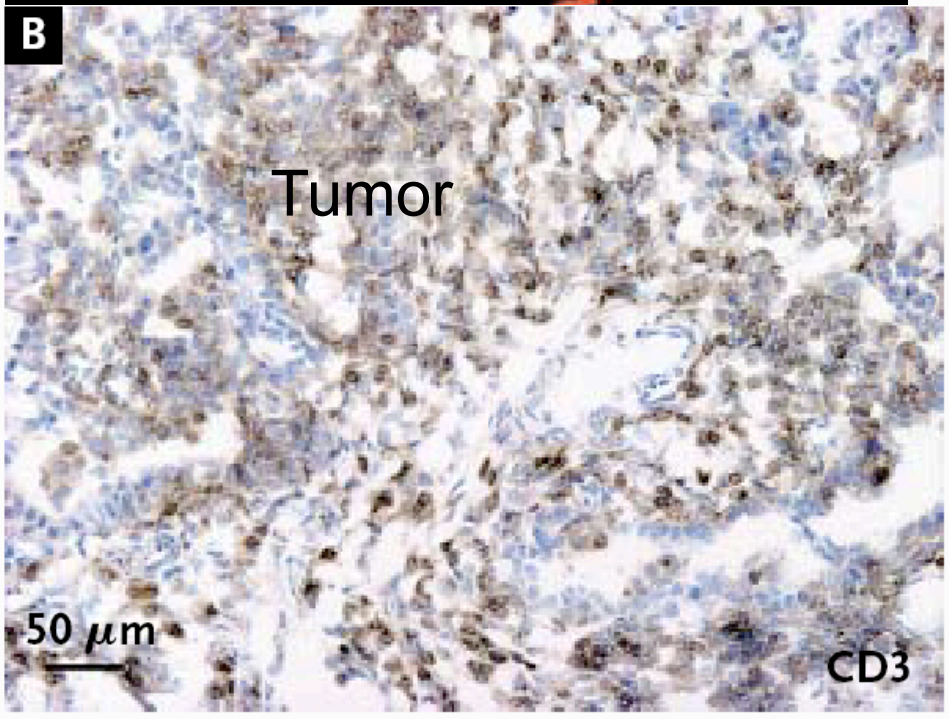
Background Although tumor-infiltrating T cells have been documented in ovarian carcinoma, a clear association with clinical outcome has not been established.

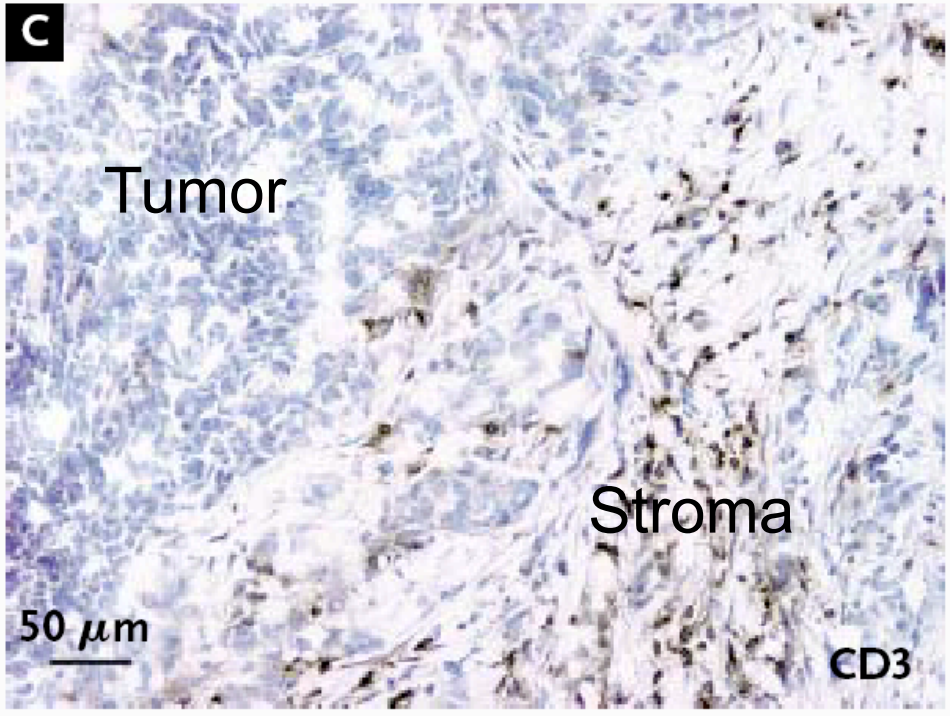
Methods We performed immunohistochemical analysis of 186 frozen specimens from advanced-stage ovarian carcinomas to assess the distribution of tumor-infiltrating T cells and conducted outcome analyses. Molecular analyses were performed in some tumors by real-time polymerase chain reaction.

Results CD3+ tumor-infiltrating T cells were detected within tumor-cell islets (intratumoral T cells) in 102 of the 186 tumors (54.8 percent); they were undetectable in 72 tumors (38.7 percent); the remaining 12 tumors (6.5 percent) could not be evaluated. There were significant differences in the distributions of progression-free survival and overall survival according to the presence or absence of intratumoral T cells (P<0.001 for both comparisons). The five-year overall survival rate was 38.0 percent among patients whose tumors contained T cells and 4.5 percent among patients whose tumors contained no T cells in islets. Significant differences in the distributions of progression-free survival and overall survival according to the presence or absence of intratumoral T cells (P<0.001 for both comparisons) were also seen among 74 patients with a complete clinical response after debulking and platinum-based chemotherapy: the five-year overall survival rate was 73.9 percent among patients whose tumors contained T cells and 11.9 percent among patients whose tumors contained no T cells in islets. The presence of intratumoral T cells independently correlated with delayed recurrence or delayed death in multivariate analysis and was associated with increased expression of interferon-γ, interleukin-2, and lymphocyte-attracting chemokines within the tumor. The absence of intratumoral T cells was associated with increased levels of vascular endothelial growth factor.


A
25 μm
CD3
Cytokeratin


D
CD3ε mRNA
0.025
0.020
0.015
0.010
0.005
0.000
Intratumoral T Cells
No Intratumoral T Cells


B
Tumor
50 μm
CD3


C
Tumor
Stroma
50 μm
CD3

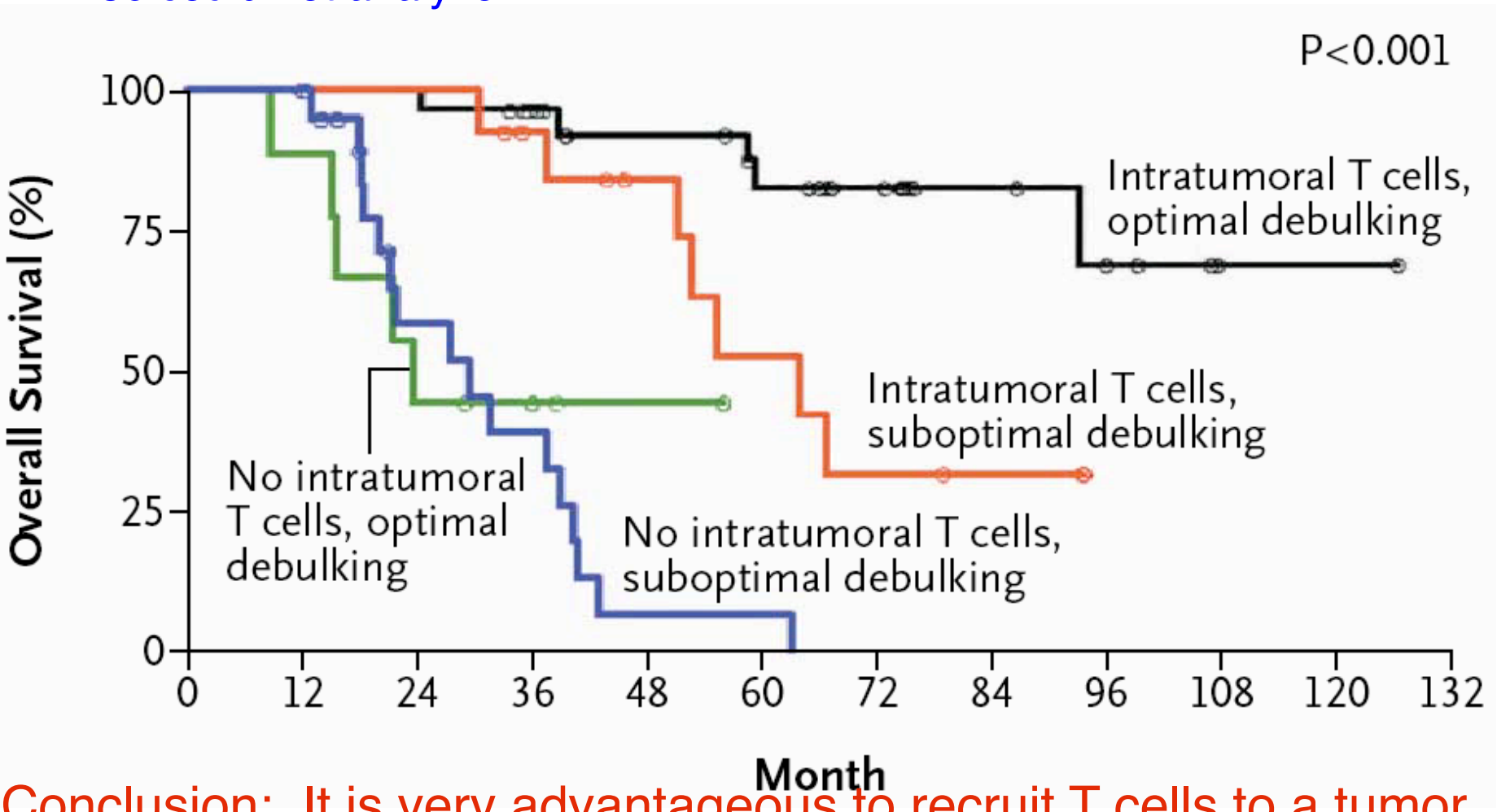
N Engl J Med 2003;348:203-13.

Immunohistochemistry of 186 frozen specimens, isolated prior to treatment

102/186 intratumor T cells

72/186 no intratumor T cells

12/186 could not analyze



Conclusion: It is very advantageous to recruit T cells to a tumor (and to also have a good surgeon).

Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome

Jérôme Galon,[1*†] Anne Costes,[1] Fatima Sanchez-Cabo,[2] Amos Kirilovsky,[1] Bernhard Mlecnik,[2] Christine Lagorce-Pagès,[3] Marie Tosolini,[1] Matthieu Camus,[1] Anne Berger,[4] Philippe Wind,[4] Franck Zinzindohoué,[5] Patrick Bruneval,[6] Paul-Henri Cugnenc,[5] Zlatko Trajanoski,[2] Wolf-Herman Fridman,[1,7] Franck Pagès[1,7†]

The role of the adaptive immune response in controlling the growth and recurrence of human tumors has been controversial. We characterized the tumor-infiltrating immune cells in large cohorts of human colorectal cancers by gene expression profiling and in situ immunohistochemical staining. Collectively, the immunological data (the type, density, and location of immune cells within the tumor samples) were found to be a better predictor of patient survival than the histopathological methods currently used to stage colorectal cancer. The results were validated in two additional patient populations. These data support the hypothesis that the adaptive immune response influences the behavior of human tumors. In situ analysis of tumor-infiltrating immune cells may therefore be a valuable prognostic tool in the treatment of colorectal cancer and possibly other malignancies.

Science '06

Tumor histopathology

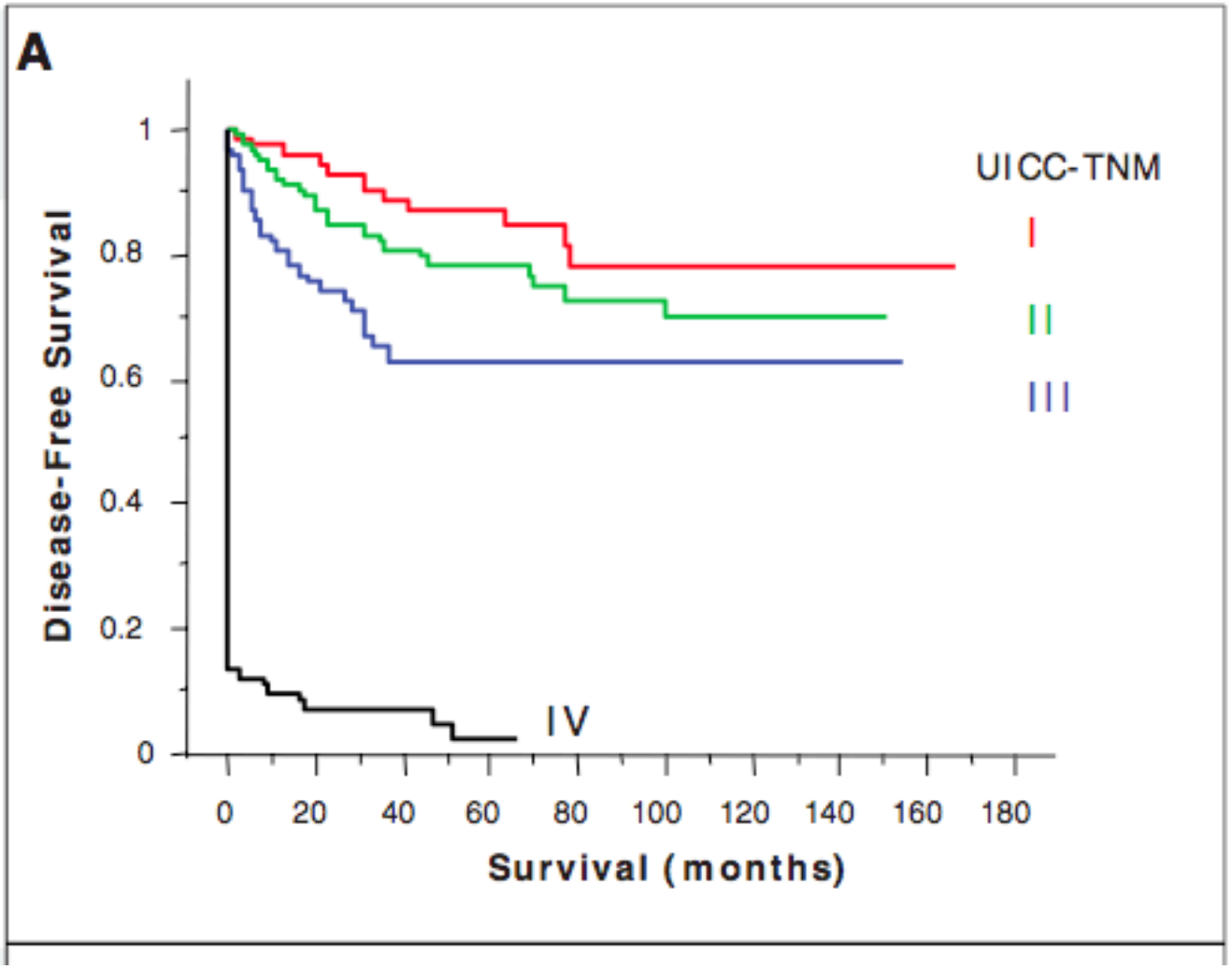
UICC-TNM Staging system

International Union against Cancer

Tumor

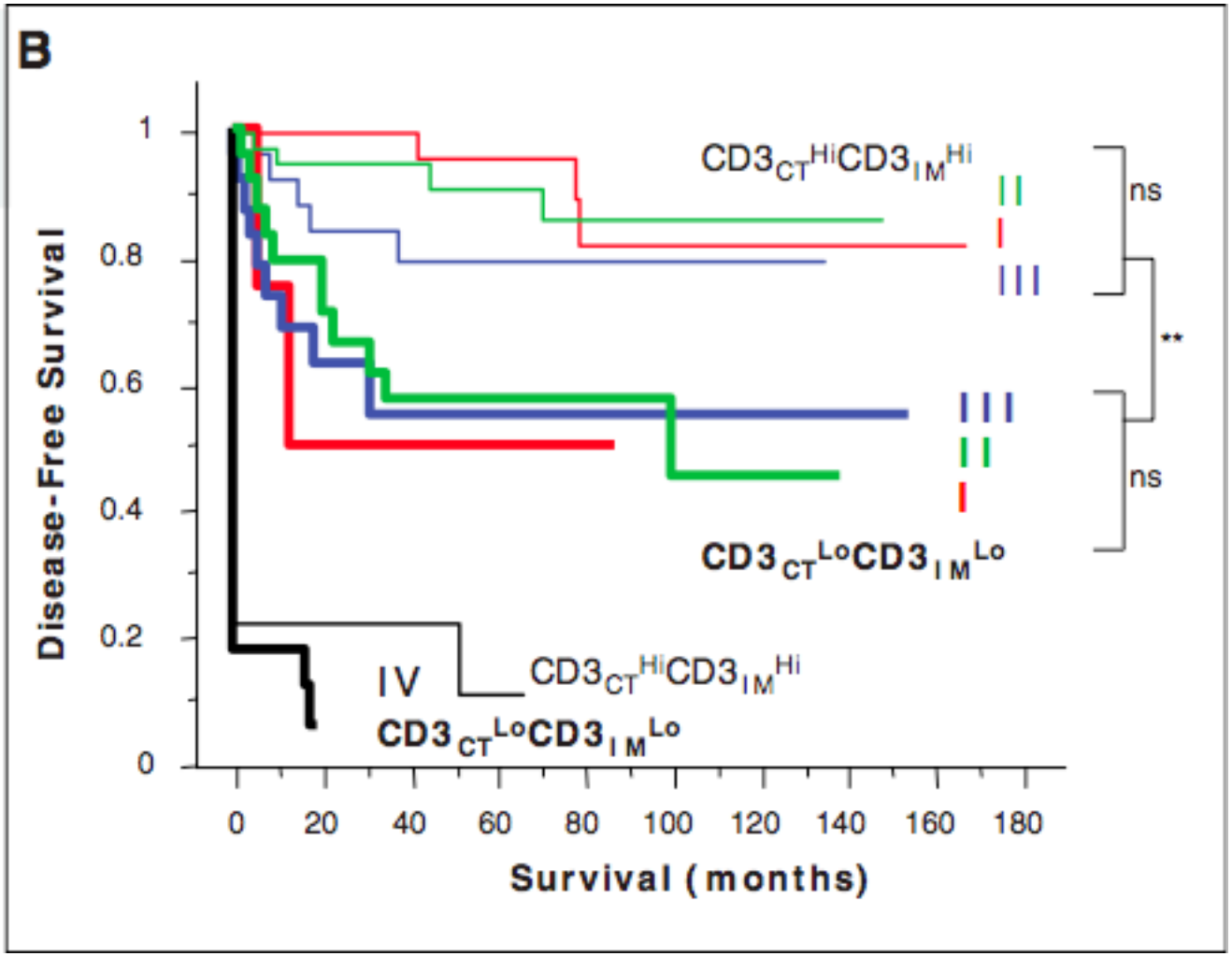
Nodes

Metastases



Tumor infiltrating immune cells

$CD3_{CT}CD3_{IM}$ evaluation



CT center of tumor, IM invasive margin

$CD3_{CT}CD3_{IM}$ evaluation

plus

$CD45RO_{CT}CD45RO_{IM}$ evaluation

Memory T cells

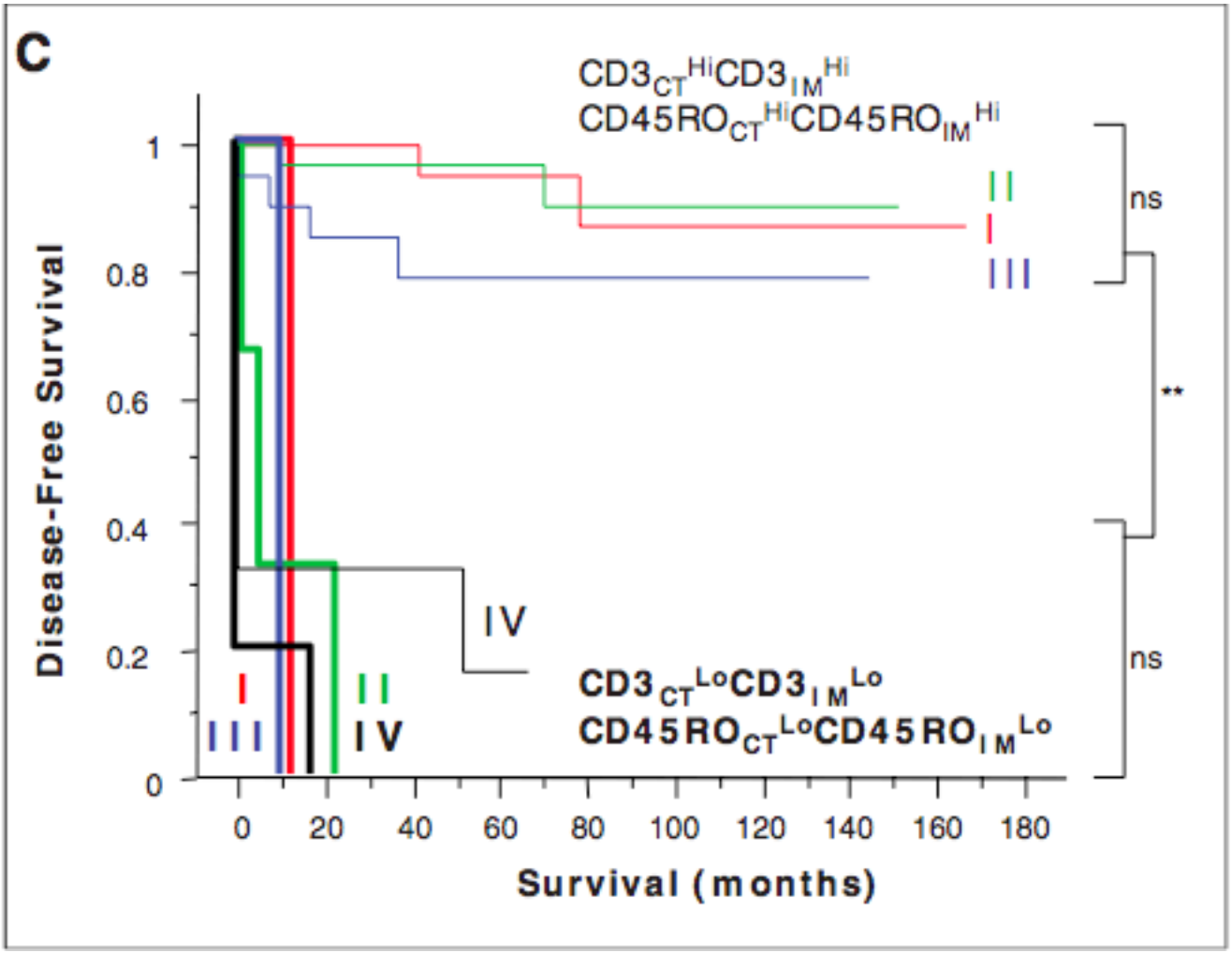
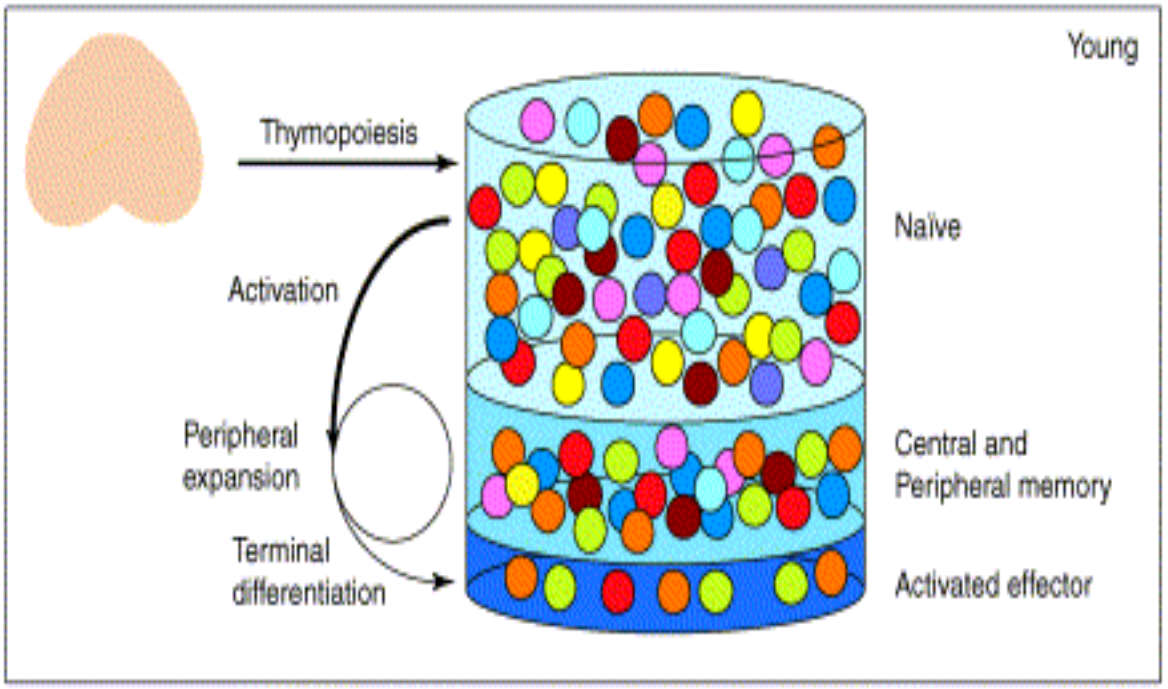
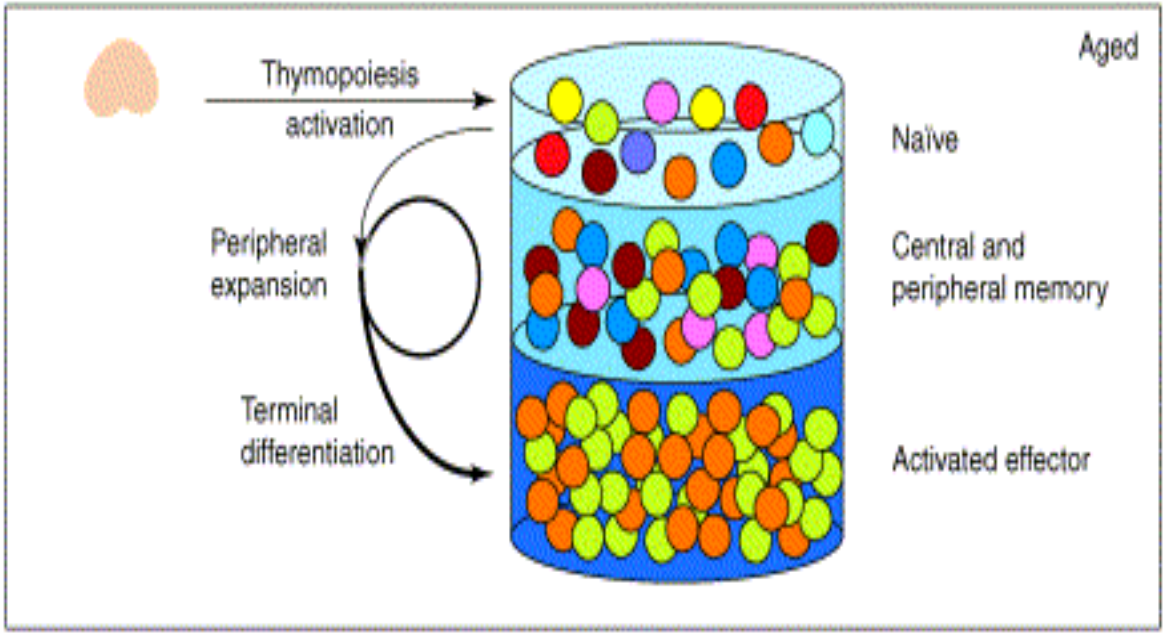


Fig. 3. (**A**) Kaplan-Meier curves illustrate the duration of disease-free survival according to the UICC-TNM stages [stage I, red line ($n = 75$ patients); stage II, green line ($n = 137$); stage III, blue line ($n = 99$), and stage IV, black line ($n = 95$)] in patients with CRCs. (**B**) Kaplan-Meier curves illustrate the duration of disease-free survival according to the UICC-TNM stages [as in (A)] and according to the density of $CD3^+$ cells in combined tumor regions ($CD3_{CT}{}^{Lo}CD3_{IM}{}^{Lo}$, thick lines, $n = 93$ patients; $CD3_{CT}{}^{Hi}CD3_{IM}{}^{Hi}$, thin lines, $n = 109$). The subgroup of patients that did not appear to have a coordinated in situ immune reaction in tumor regions (Hi/Lo or Lo/Hi for $CD3^+$ cell densities) presented similar Kaplan-Meier curves as the entire cohort (fig. S10).(**C**) Kaplan-Meier curves illustrate the duration of disease-free survival according to the UICC-TNM stages and to the density of $CD3^+$ and $CD45RO^+$ cells in combined tumor regions ($CD3_{CT}{}^{Lo}CD3_{IM}{}^{Lo}$ plus $CD45RO_{CT}{}^{Lo}CD45RO_{IM}{}^{Lo}$, thick lines, $n = 16$ patients; $CD3_{CT}{}^{Hi}CD3_{IM}{}^{Hi}$ plus $CD45RO_{CT}{}^{Hi}CD45RO_{IM}{}^{Hi}$, thin lines, $n = 88$). Cutoff values were 250, 640, 60, and 190 for $CD3_{CT}$, $CD3_{IM}$, $CD45RO_{CT}$, and $CD45RO_{IM}$, respectively. In (B) and (C), log-rank statistical test, ** $P < 10^{-4}$; ns, not significant.

- If tumor antigens exist, then tumors should develop more when immune surveillance decreases:
  - Aging
  - Immunodeficiencies
    - RAG mice
    - HIV/AIDS





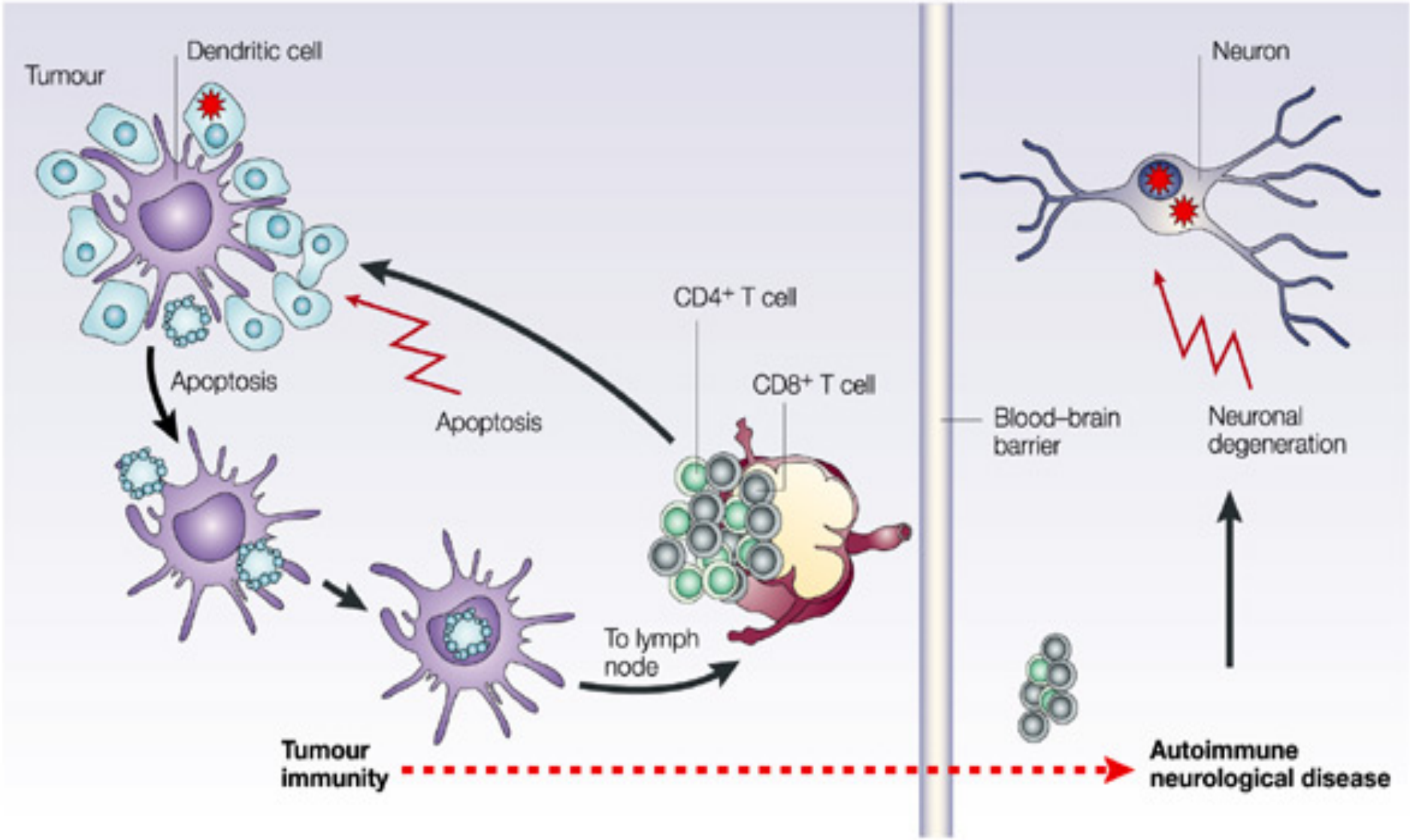
Current Opinion in Immunology

Young and aged thymocyte populations. The upper cylinder represents the peripheral T-cell compartment in the young, and the lower cylinder represents the smaller T-cell population found in the elderly. Naïve, memory, and activated effector cells are found at both ages, but the proportions change. The naïve cells comprise the largest proportion of T cells in the young, but are relatively few in the elderly. These naïve cells are the product of the thymus, which is large in the young but progressively diminishes with age. Naïve cells have the greatest TCR repertoire diversity. Upon activation, naïve cells move into the memory pool, where they may undergo peripheral expansion (circular arrow). The memory population is the source of most type 1 and type 2 cytokines. The enlarged memory component in the elderly may give rise to the increase in cytokines observed. Overall, the repertoire of memory cells in the elderly is less diverse than in the young due to a reduced input from the small naïve cell population. Upon repeated stimulation, memory cells give rise to terminally differentiated effectors. These activated effectors have the most severely limited repertoire within the three T-cell pools.

Table 1 | **AIDS-associated cancers**

Cancer type	Observed cases	Expected cases	Relative risk	Aetiologic or contributing factors
Kaposi's sarcoma				KSHV
Men	5583	57.3	97.5*	
Women	200	1.0	202.7*	
Non-Hodgkin's lymphoma				EBV and KSHV
Men	2434	65	37.4	
Women	342	6.3	54.6	
Cervical, invasive				HPV
Women	133	14.7	9.1	
Hodgkin's disease				EBV
Men	160	20	8	
Women	20	3.1	6.4	
Tongue				HPV and EBV
Men	17	9.3	1.8	
Women	5	0.7	7.1	
Rectal, rectosigmoidal and anal				HPV (anal carcinoma)
Men	75	22.7	3.3	
Women	9	3.0	3.0	
Liver (primary only)				HCV**, HBV, alcohol
Men	36	7.1	5.1	
Tracheal, bronchial and lung				Smoking***
Men	217	66.1	3.3	
Women	50	6.7	7.5	
Brain and CNS				EBV for CNS lymphoma
Men	42	13.4	3.1	
Women	7	2.0	3.4	
Skin, excluding KS				HPV**** and ultraviolet-light exposure
Men	133	6.4	20.8	
Women	8	1.1	7.5	

paraneoplastic neurological disease is evidence for silent, effective tumour immunity



Experimental Evidence that the immune system can eliminate tumors

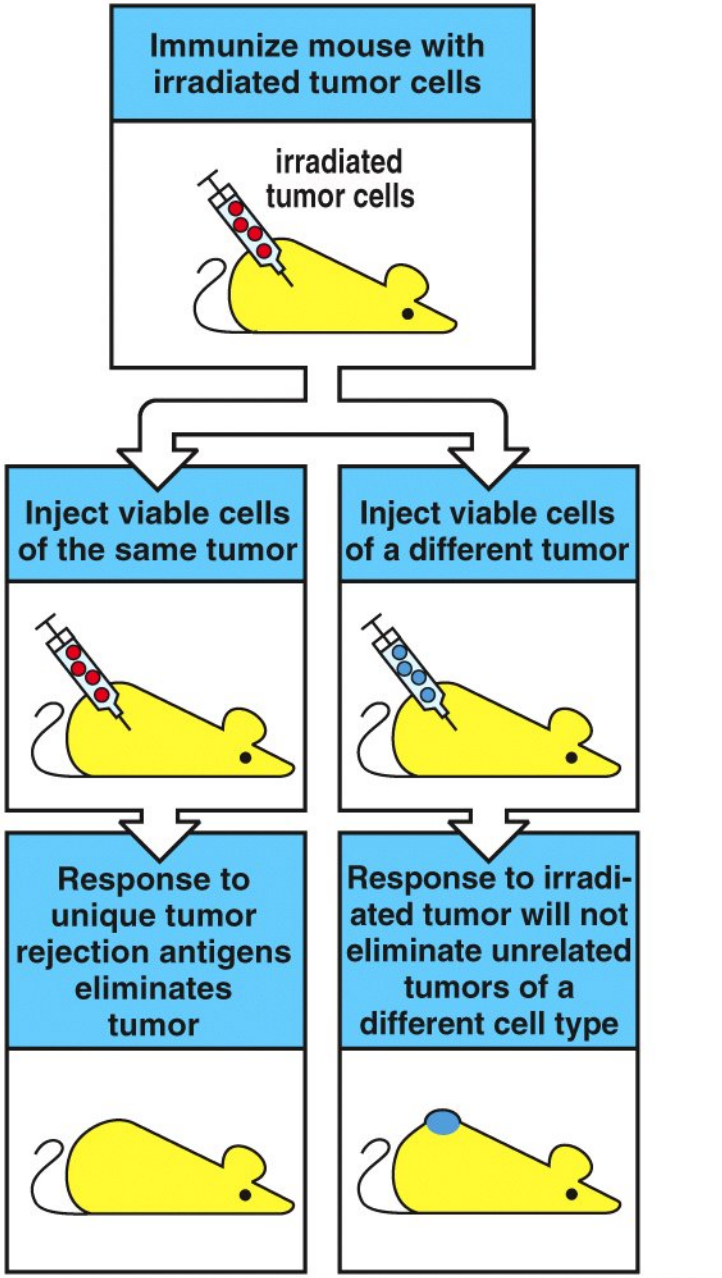
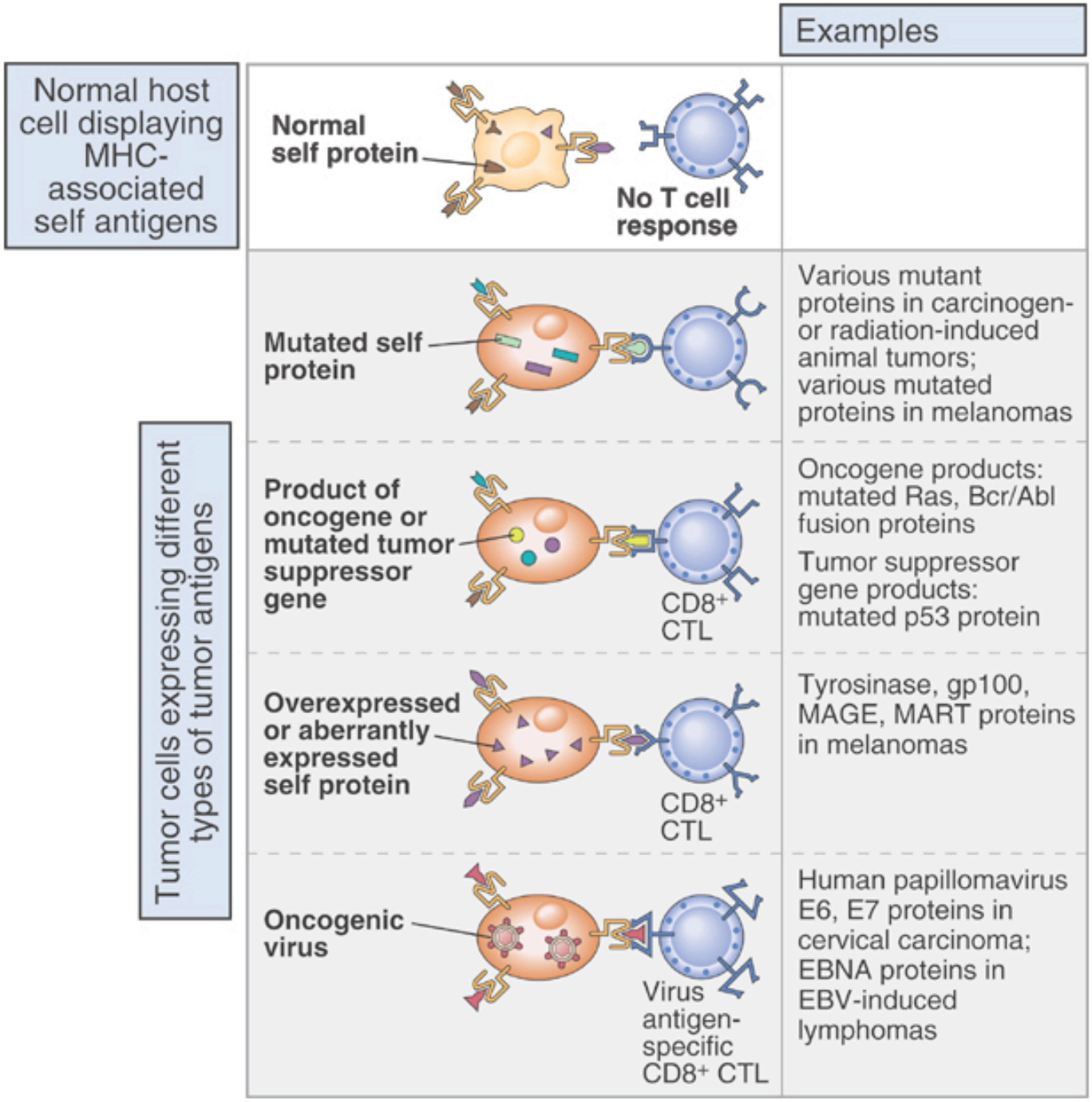


Figure 14-10 Immunobiology, 6/e. (© Garland Science 2005)

- What tumor antigens can be recognized by immune system?



- Why does immune response fail?

- Tolerance and immune evasion and suppression by tumor

Mechanisms by which tumors escape immune recognition

Low immunogenicity	Tumor treated as self antigen	Antigenic modulation	Tumor-induced immune suppression	Tumor-induced privileged site
No peptide:MHC ligand No adhesion molecules No co-stimulatory molecules	Tumor antigens taken up and presented by APCs in absence of co-stimulation tolerize T cells	Antibody against tumor cell-surface antigens can induce endocytosis and degradation of the antigen. Immune selection of antigen-loss variants	Factors (eg,TGF-β) secreted by tumor cells inhibit T cells directly	Factors secreted by tumor cells create a physical barrier to the immune system

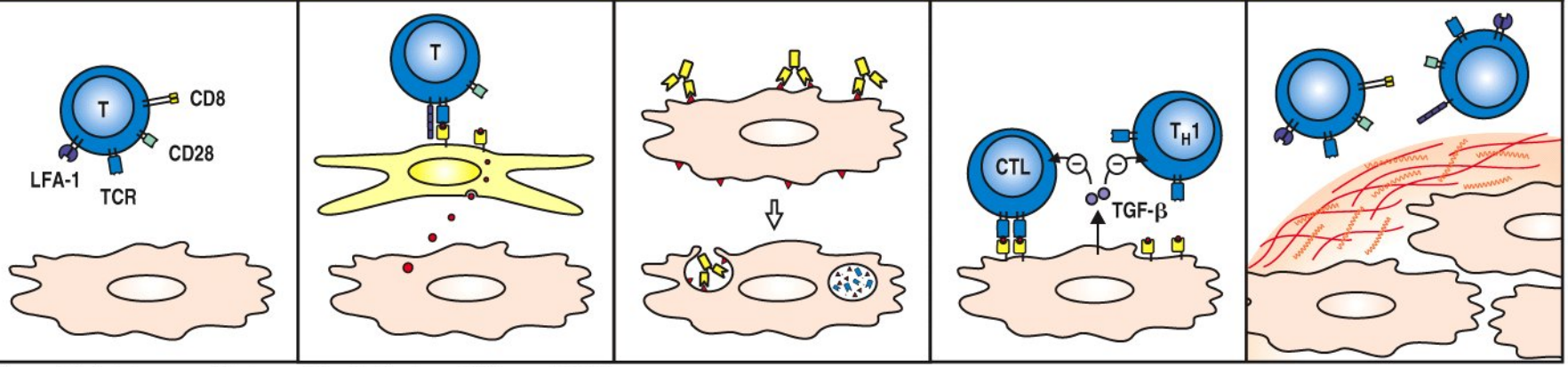
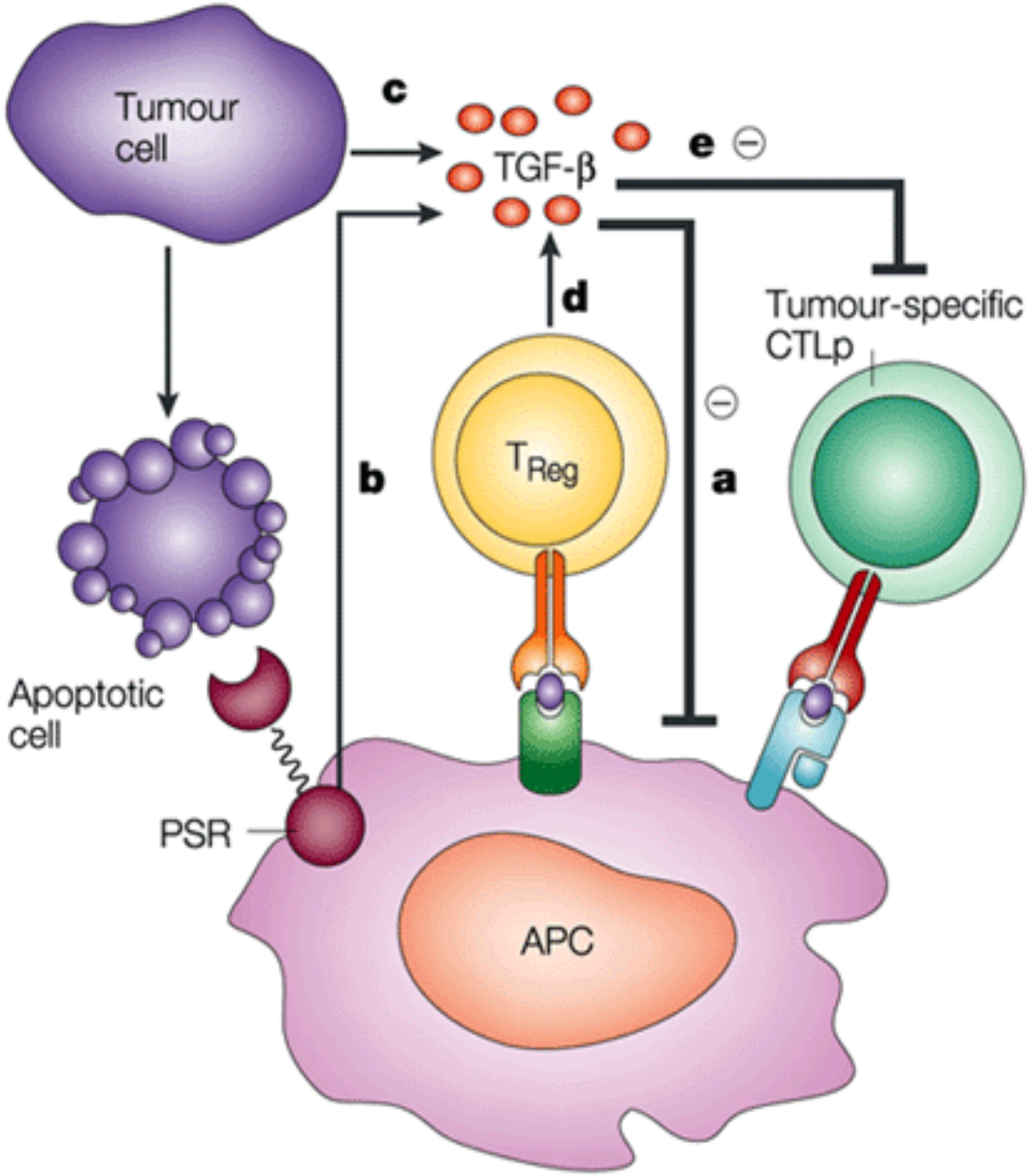


Figure 14-14 Immunobiology, 6/e. (© Garland Science 2005)

Active immunosuppression in tumor microenvironment

- Tumors secrete IL-10 and TGFb
- Regulatory T cells induced
- Tumors secrete IDO (catabolizes tryptophan)
- FasL, CTLA-4, PD-L1
- Local immunosuppression leads to systemic immunosuppression



Inhibition of antitumour response by TGF-β. **a** | Production of transforming growth factor- (TGF-) will inhibit maturation and antigen presentation by dendritic cells, thereby diminishing their T-cell-stimulating capacity. **b** | Apoptotic cell death of tumour cells can contribute to TGF-β production at the tumour site as well as inhibition of function of dendritic cells that process those cells. Once APCs bring the tumour antigen to the lymphoid organ to present it to T cells, activation of T cells can be inhibited by TGF-β produced by the tumour cells (**c**) or the immune cells in response to the tumour antigens (**d**). **e** | Even if some precursor cytotoxic T lymphocytes (pCTLs) are activated under such inhibitory conditions, TGF- would still inhibit their differentiation into effector CTLs. PSR, phosphatidylserine receptor; TReg, T-regulatory cell.

[*Cancer Research* 65, 5020-5026, June 15, 2005]

Priority Reports

Plasmacytoid Dendritic Cells Induce $CD8^+$ Regulatory T Cells In Human Ovarian Carcinoma

Shuang Wei[1], Ilona Kryczek[1,2], Linhua Zou[1], Ben Daniel[1], Pui Cheng[1], Peter Mottram[1], Tyler Curiel[1], Andrzej Lange[2] and Weiping Zou[1]

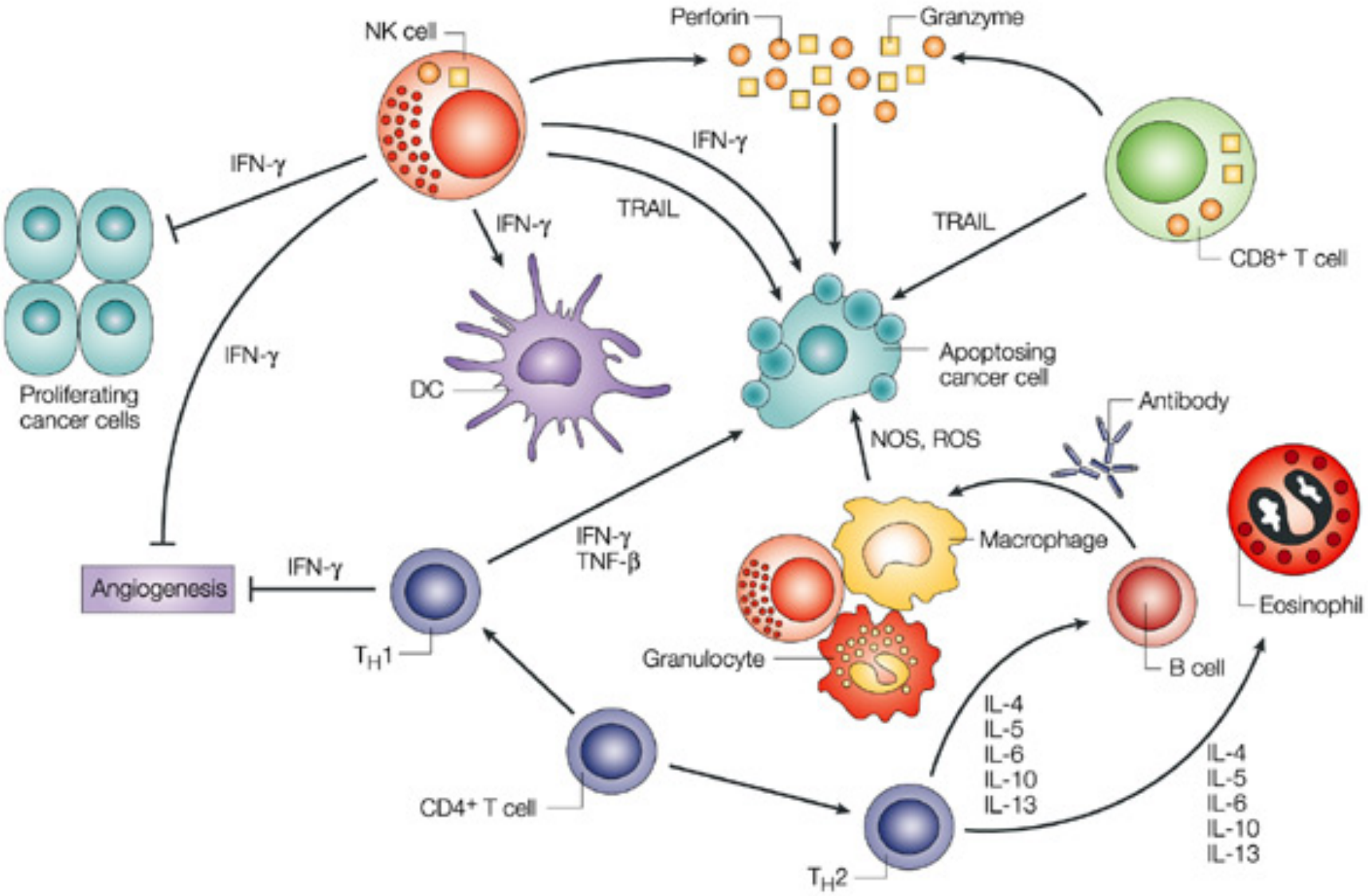
Abstract

▲ Top
▪ Abstract
▼ Introduction
▼ Materials and Methods
▼ Results
▼ Discussion
▼ References

To directly dissect the role of each immune component in human tumor immunopathogenesis, we have studied the interaction between dendritic cells and T cells in the tumor environment of patients with ovarian carcinoma. We previously reported that functional plasmacytoid dendritic cells, but not functionally mature myeloid dendritic cells, accumulated in tumor microenvironments. We now show that tumor ascites macrophage-derived dendritic cells induced tumor-associated antigen–specific $CD8^+$ T cells with effector functions. Strikingly, tumor ascites plasmacytoid dendritic cells induced interleukin-$10^+CCR7^+CD45RO^+CD8^+$ regulatory T cells. Four characteristics have been identified in tumor plasmacytoid dendritic cell–induced $CD8^+$ regulatory T cells: (*a*) induction of $CD8^+$ regulatory T cells is independent of $CD4^+CD25^+$ T cells; (*b*) $CD8^+$ regulatory T cells significantly suppress myeloid dendritic cell–mediated tumor-associated antigen–specific T cell effector functions through interleukin-10; (*c*) repetitive myeloid dendritic cell stimulation can recover $CD8^+$ regulatory T cell–mediated poor T cell proliferation, but not T cell effector function; (*d*) $CD8^+$ regulatory T cells express functional CCR7, and efficiently migrate with lymphoid homing chemokine MIP-3ß. Primary suppressive $CCR7^+CD45RO^+CD8^+$ T cells are found in the tumor environment of patients with ovarian cancers. Thus, tumor-associated plasmacytoid dendritic cells contribute to the tumor environmental immunosuppressive network. Collectively, tumors manipulate tumor microenvironmental dendritic cell subset distribution and function to subvert tumor immunity. The data are relevant to understanding tumor immunopathology as well as reevaluating tumor immunotherapeutic strategies.

- What are potential components of immune system that can be used to treat cancer?

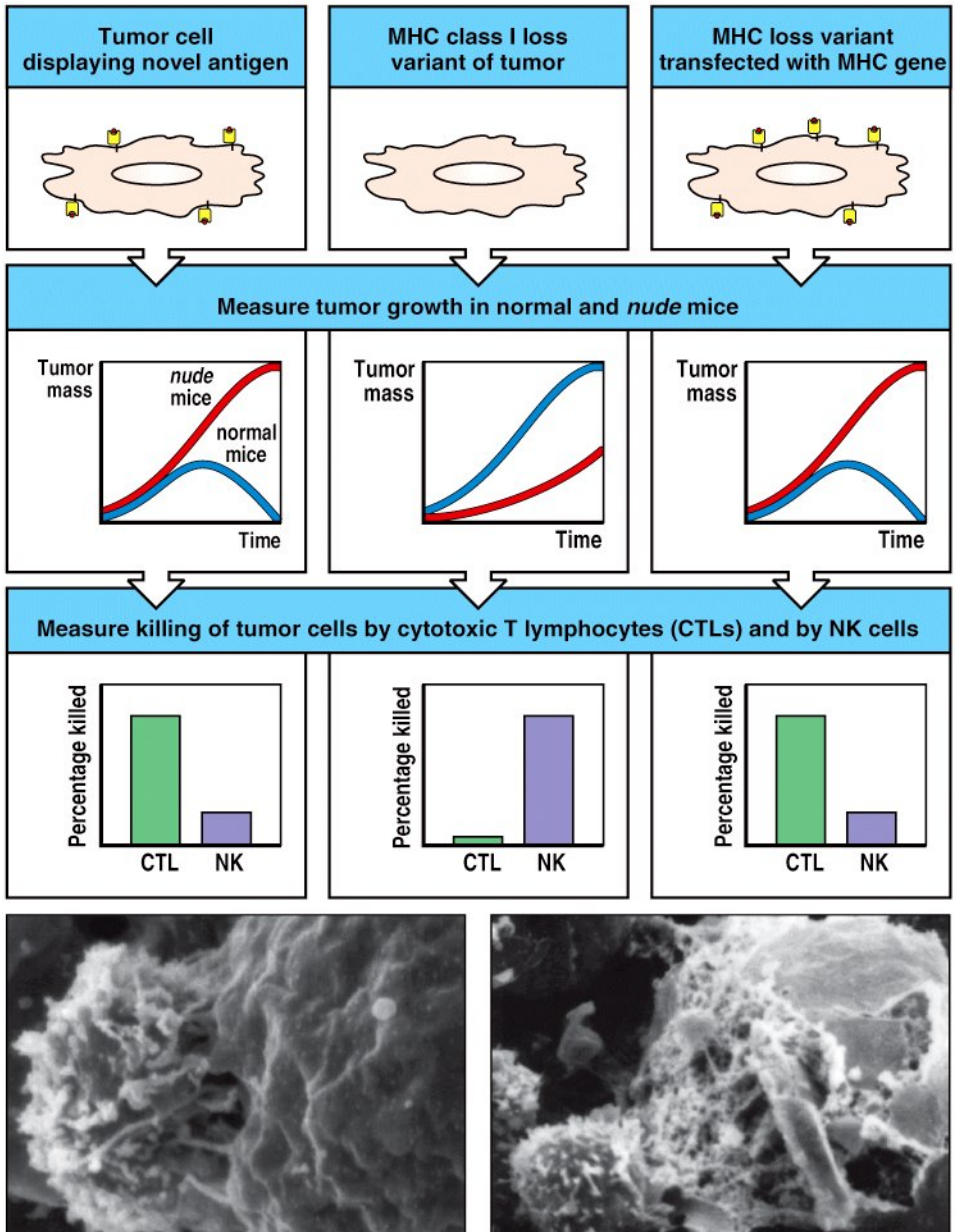
Immune defenses leading to tumor destruction.



Nature Reviews | Cancer

A coordinated cellular and humoral reaction mediates tumour destruction. Following stimulation, natural killer (NK) cells can lyse tumours through the perforin/granzyme pathway or apoptosis-inducing ligands such as tumour-necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL). NK cells secrete interferon- (IFN-), which inhibits tumour-cell proliferation, enhances tumour-cell apoptosis, improves tumour antigen presentation and inhibits angiogenesis. NKT cells also execute cytotoxicity and cytokine production. Cytotoxic CD8+ T cells lyse tumours through death ligands, such as TRAIL, and the perforin/granzyme pathway. CD4+ T cells can differentiate into T helper 1 (TH1) cells that secrete IFN- and TNF- or T helper 2 (TH2) cells that secrete interleukin (IL)-4, IL-5, IL-6, IL-10 and IL-13. The latter cytokines enhance eosinophil function and increase antibody production by B cells. Antibodies to cancer cell-surface molecules can inhibit oncogenic signalling and/or stimulate tumour destruction through engaging Fc receptors on macrophages, granulocytes and NK cells (not shown). Antibodies can further promote tumour antigen presentation by dendritic cells (DCs) through immune-complex formation. Macrophages can lyse tumours through the production of nitric oxide and reactive oxygen species. Tumour blood vessels can also be attacked by lymphocytes and granulocytes.

Complementary roles of CTLs and NK cells



How can the adaptive immune system be boosted to fight tumors?

- Passive immune treatments
  - Mabs
- Vaccinate against viral antigens (prophylactic)
- Vaccinate to initiate T cell or Ab response
- Enhance antigen presentation
- Adoptive Transfer and ex vivo modification of cells
- Reverse suppression

Monoclonal Antibodies

- Surface tumor antigens
- Normal effector mechanisms
- Can deliver toxins, radioactivity

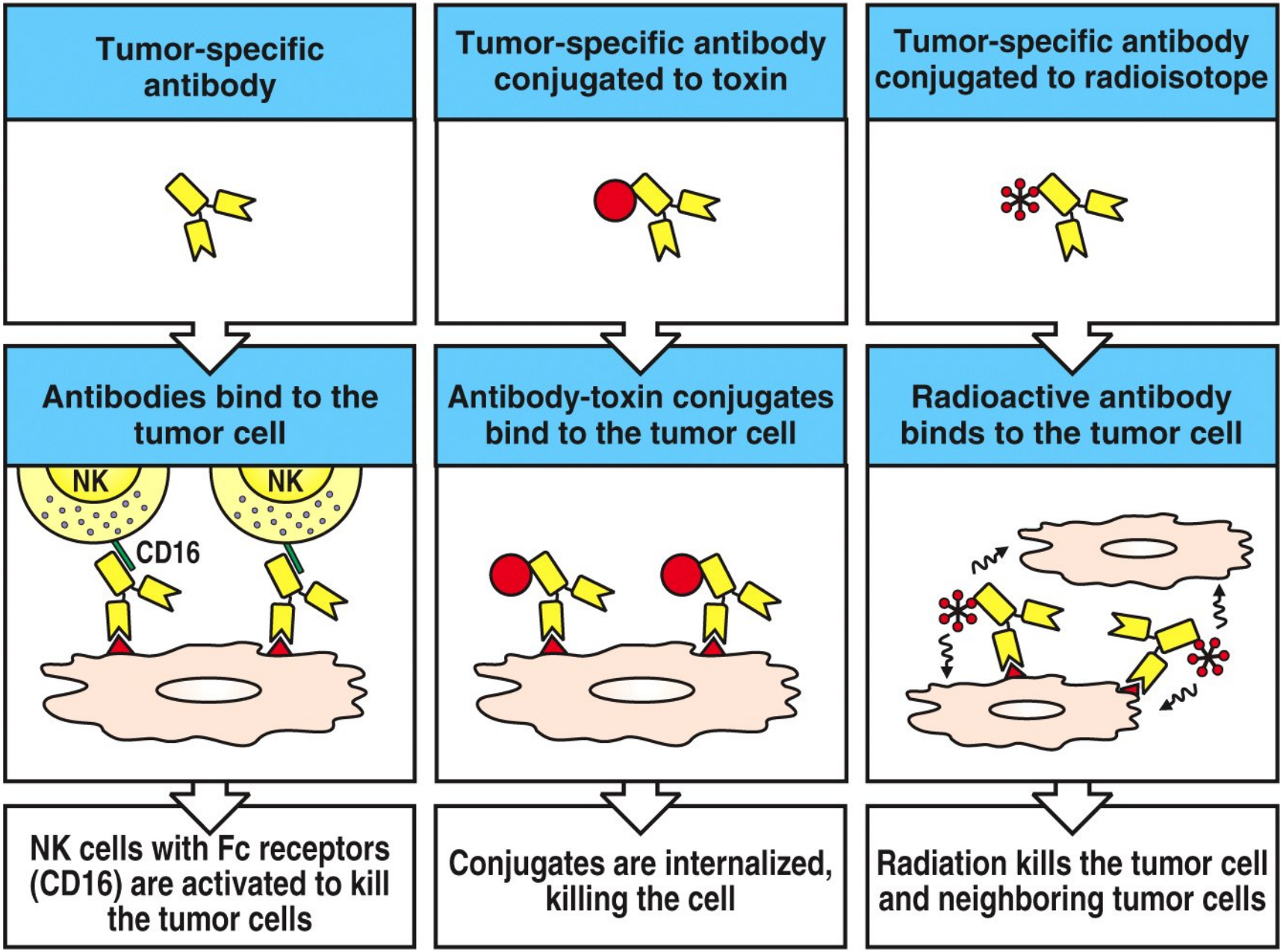


Figure 14-17 Immunobiology, 6/e. (© Garland Science 2005)

Monoclonal Antibodies

- Against tumor surface antigens - rituximab, CD20
- Against immune checkpoint inhibitors - anti-CTLA4, anti-PD1

How can the adaptive immune system be boosted to fight tumors?

- Passive immune treatments
  - Mabs
- **Vaccinate against viral antigens (prophylactic)**
- Vaccinate to initiate T cell or Ab response
- Enhance antigen presentation
- Adoptive Transfer and ex vivo modification of cells
- Reverse suppression

Prophylactic Vaccine for Cervical cancer

The second most common cancer in women worldwide
250,000 new cases each year, commonly in 30–50-year-old women
A consequence of infection with mucosal high-risk human papillomavirus (HPV)
Preceded by many years of persistent HPV infection
Premalignant lesions (cervical intraepithelial neoplasia, CIN) develop early after infection
Increased incidence after immunosuppression (HIV infection or immunosuppressive drugs)

Prevention and treatment
Largely prevented by cervical cytology screening programs (PAP smears)
Early stage (local) disease is curable by surgery and radiotherapy
Later stage disease kills by local invasion rather than distant metastatic disease

New treatment-vaccination against HPV.

Merck cervical vaccine works in trial

Drugmaker says Gardasil, to protect women against HPV, could reach the market next year.

April 7, 2005: 6:49 AM EDT

LONDON (Reuters) - An experimental vaccine from Merck & Co Inc has proved highly effective at stopping the virus associated with most cases of cervical cancer and genital warts, scientists said Thursday.

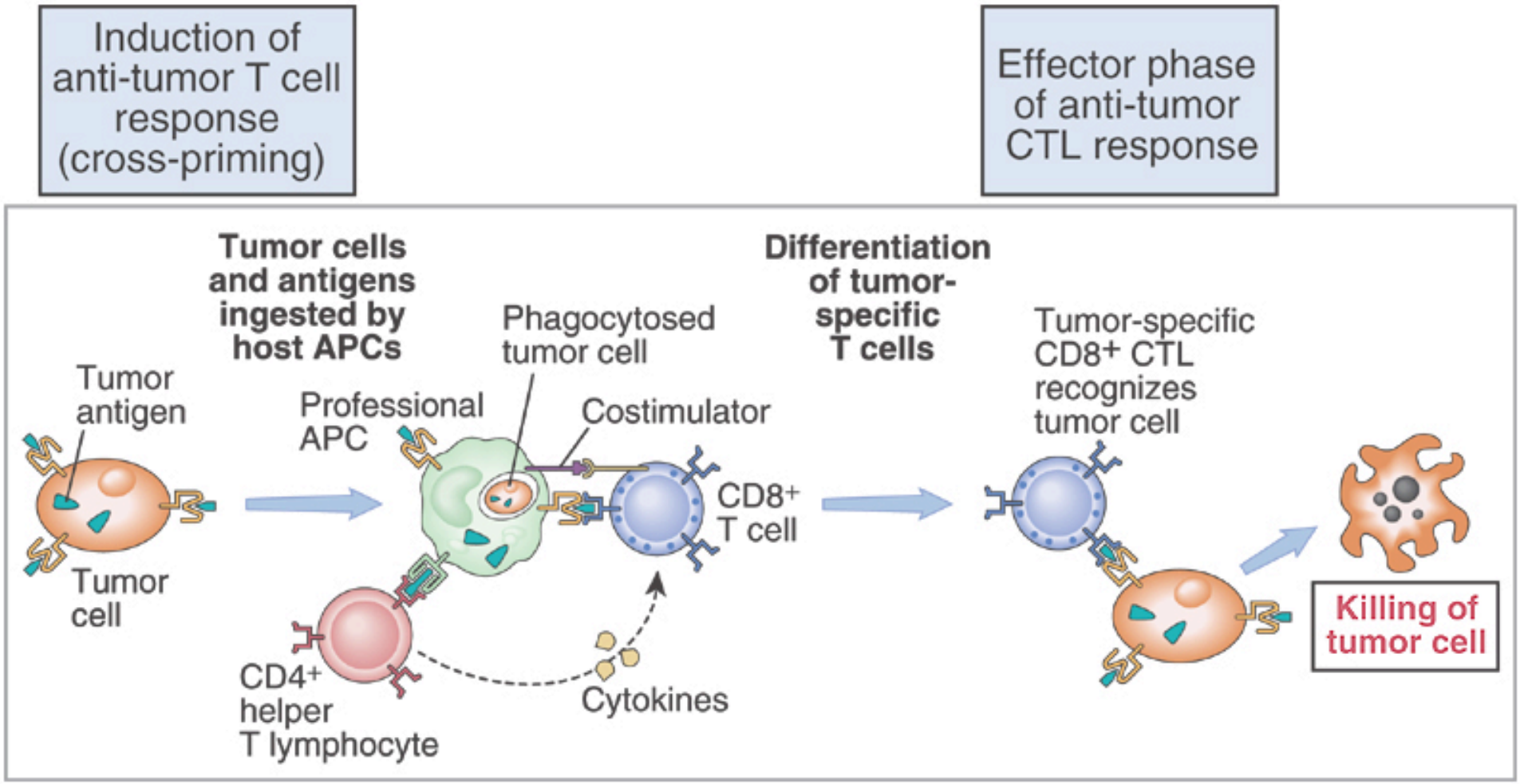
Gardasil, which could reach the market in 2006, is designed to protect women against four strains of the human papillomavirus (HPV).

It is competing against GlaxoSmithKline Plc's (Research) rival product Cervarix, which targets just the two strains responsible for cervical cancer -- a disease that kills a quarter of a million women each year.

Both treatments are now in final-stage clinical studies and are tipped by industry analysts to be multibillion-dollar sellers.

Gratis HPV-vaksine til unge jenter bør være en menneskerettighet i Norge!

Vaccine to elicit T cell response



Enhance APCs by increasing costimulation

- Make tumor an APC by transfecting with B7
- Attract APCs to tumor
- Expand APCs

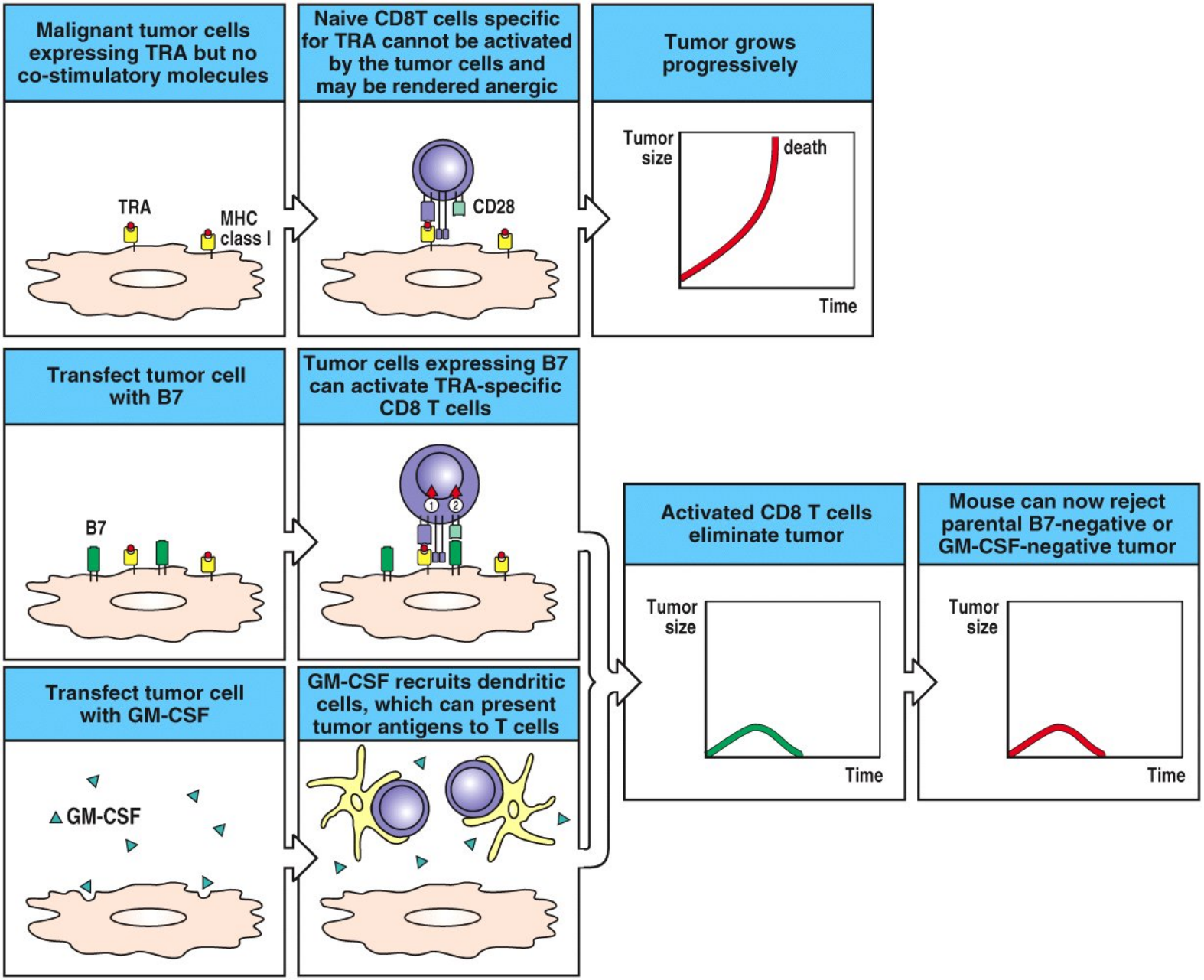
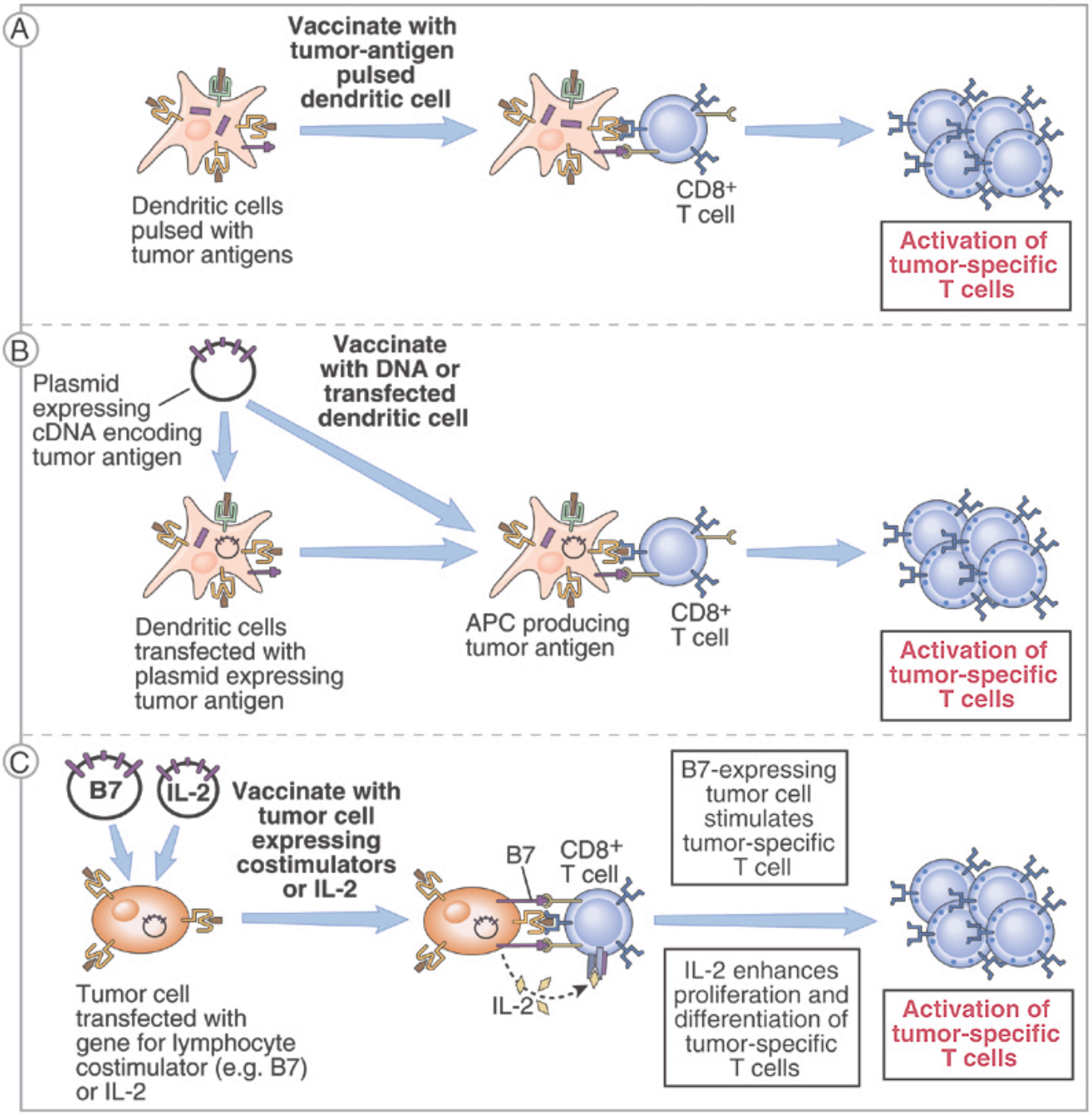


Figure 14-20 Immunobiology, 6/e. (© Garland Science 2005)

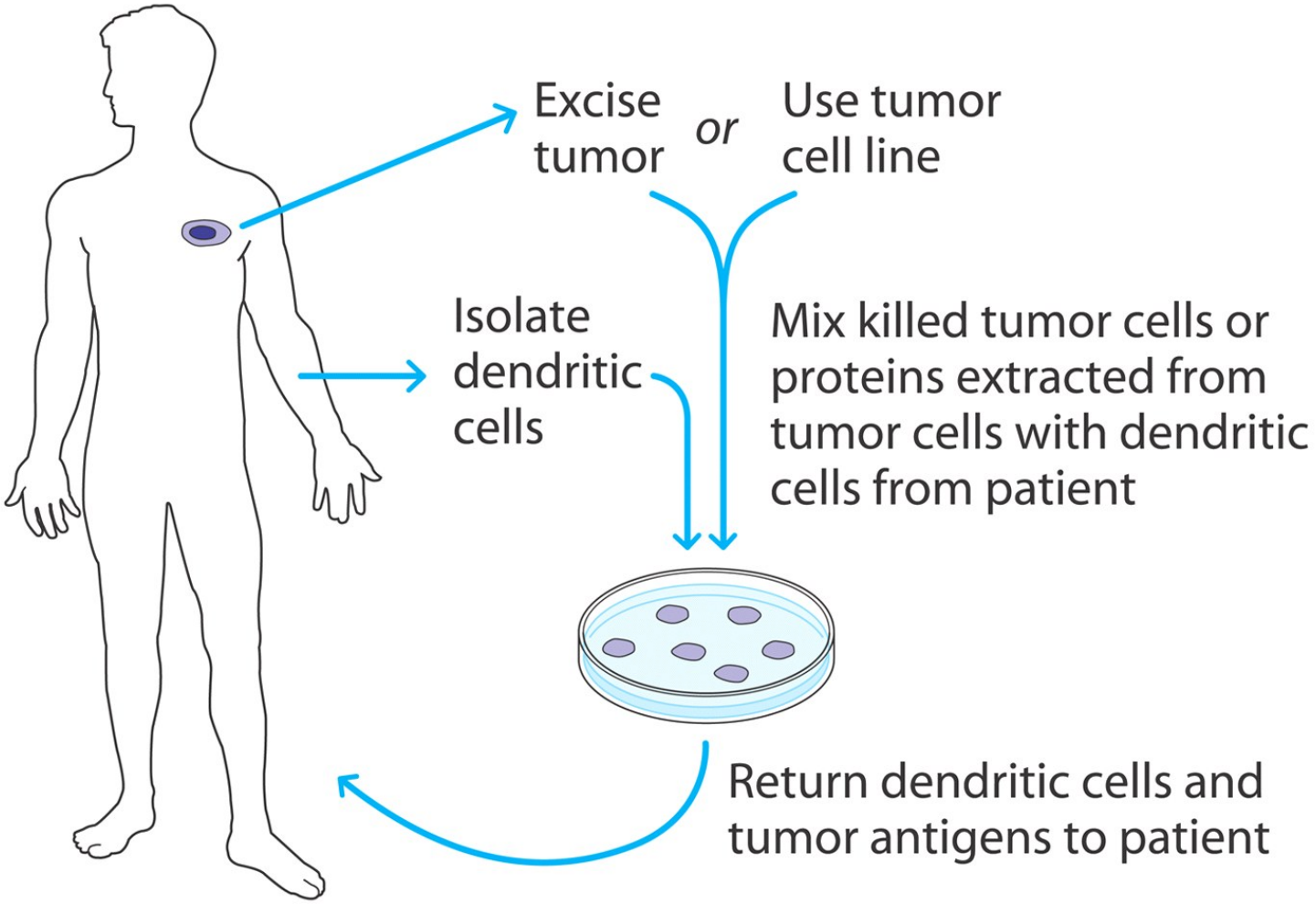


Other Tumor vaccination strategies

Adoptive Transfer: Ex vivo Expansion and Modification of cells

- Dendritic cells
- T cells

Ex vivo dendritic cell therapy for cancer.



F.D.A. Approves 'Vaccine' to Fight Prostate Cancer

By ANDREW POLLACK
Published: April 29, 2010

The Food and Drug Administration on Thursday approved the first treatment that uses a so-called cancer vaccine, a drug that trains the body's own immune system to fight the disease.

The drug, Provenge, developed by the Dendreon Corporation, was approved to treat advanced prostate cancer. In clinical trials it extended the lives of patients about four months compared with a placebo.

Getting the immune system to attack cancer has tantalized scientists for decades, because it promises to have fewer side effects than the harsh chemotherapy now used. But until now the approach has yielded little but disappointment.

"The big story here is that this is the first proof of principle and proof that immunotherapy works in general in cancer, which I think is a huge observation," said Dr. Philip Kantoff, chief of solid tumor oncology at the Dana-Farber Cancer Institute in Boston and the lead investigator in Dendreon's largest clinical trial for the drug. "

NY Times, 4/29/10

FDA approved dendritic cell vaccine for prostate cancer

- $93,000 for course of treatment
- “comparable to other treatments on life-extension per dollar”

Gene Therapy (2011), 1–10

www.nature.com/gt

ORIGINAL ARTICLE

Phase I/II trial of a dendritic cell vaccine transfected with DNA encoding melan A and gp100 for patients with metastatic melanoma

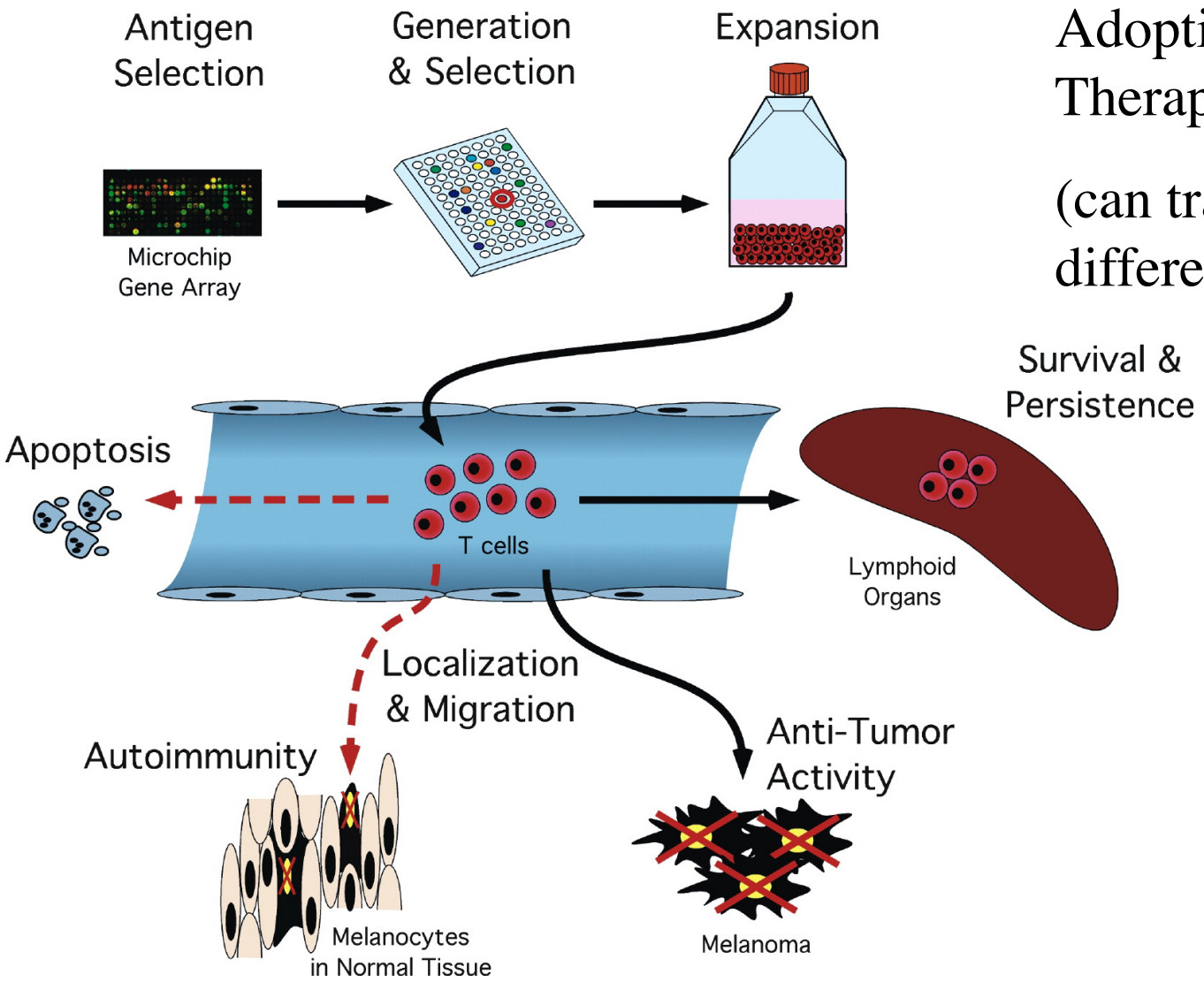
JC Steele[1], A Rao[1], JR Marsden[2], CJ Armstrong[1], S Berhane[1], LJ Billingham[1], N Graham[1], C Roberts[1,2], G Ryan[1], H Uppal[1], C Walker[2], LS Young[1] and NM Steven[1]

This trial tested a dendritic cell (DC) therapeutic cancer vaccine in which antigen is loaded using a novel non-viral transfection method enabling the uptake of plasmid DNA condensed with a cationic peptide. Proof of principle required the demonstration of diverse T lymphocyte responses following vaccination, including multiple reactivities restricted through both major histocompatibility complex (MHC) class I and II. Patients with advanced melanoma were offered four cycles of vaccination with autologous DC expressing melan A and gp100. Disease response was measured using Response Evaluation Criteria in Solid Tumours. Circulating MHC class I- and II-restricted responses were measured against peptide and whole antigen targets using interferon-γ ELIspot and enzyme-linked immunosorbent assay assays, respectively. Responses were analyzed across the trial population and presented descriptively for some individuals. Twenty-five patients received at least one cycle. Vaccination was well tolerated. Three patients had reduction in disease volume. Across the trial population, vaccination resulted in an expansion of effector responses to both antigens, to the human leukocyte antigen A2-restricted modified epitope, melan A ELAGIGILTV, and to a panel of MHC class I- and II-restricted epitopes. Vaccination with mature DC non-virally transfected with DNA encoding antigen had biological effect causing tumour regression and inducing diverse T lymphocyte responses.
Gene Therapy advance online publication, 10 February 2011; doi:10.1038/gt.2011.1

Keywords: dendritic cell vaccination; metastatic melanoma; whole antigen; immunotherapy; T-cell responses

Adoptive T cell Therapy

(can transduce with different TCR)



Critical checkpoints and pitfalls in adoptive T cell immunotherapy. Following selection of a target antigen, T cells are stimulated in vitro, and reactive cells isolated and expanded to large numbers. After infusion into patients, the T cells must persist, retain function, and localize to tumor sites to be effective. Failure to support in vivo survival can result in apoptosis of the transferred cells, which interferes with efficacy. Normal tissues may also express the targeted antigen, and strategies to avoid injury to such tissues must be considered.

1×10^{11} cells

Enhance APCs by Activation of TLRs

- CpG oligonucleotides for TLR-9
- Poly IC for TLR-3
- Link to tumor antigen

The Journal of Immunology, 2006, 176: 7335-7345.

Efficient Immunization and Cross-Priming by Vaccine Adjuvants Containing TLR3 or TLR9 Agonists Complexed to Cationic Liposomes[1]

Karen Zaks[3,*], *Michael Jordan*[3], *Amanda Guth*[*], *Karen Sellins*[*], *Ross Kedl*[‡], *Angelo Izzo*[*], *Catharine Bosio*[*] *and Steven Dow*[2,*]

[*] Department of Microbiology, Immunology, and Pathology and Department of Pediatrics, University of Cincinnati College of Medicine and Cincinnati Children's Hospital, Cincinnati, OH 45229; [‡] Integrated Department of Immunology, National Jewish Medical and Research Center and the University of Colorado Health Sciences Center, Denver, CO 80206; and Department of Clinical Sciences, Colorado State University, Ft. Collins, CO 80523

Enhance T cell Function with Cytokines

- IL-2, IL-12, IL-15
- Many ways to deliver cytokines

nature medicine

Interleukin-15 rescues tolerant CD8$^+$ T cells for use in adoptive immunotherapy of established tumors

Ryan M Teague[1,2], Blythe D Sather[1], Jilian A Sacks[1], Maria Z Huang[1], Michelle L Dossett[1,2], Junko Morimoto[1,2], Xiaoxio Tan[1,2], Susan E Sutton[3], Michael P Cooke[3], Claes Öhlén[1,2] & Philip D Greenberg[1,2]

Cancer Gene Therapy (2006) **13,** 969–974. doi:10.1038/sj.cgt.7700973; published online 9 June 2006

Regression of subcutaneous B16 melanoma tumors after intratumoral delivery of an IL-15-expressing plasmid followed by *in vivo* electroporation

K E Ugen[1,2], M A Kutzler[3], B Marrero[1], J Westover[1], D Coppola[4], D B Weiner[3] and R Heller[1,2]

IL15 Can Reverse the Unresponsiveness of Wilms' Tumor Antigen-Specific CTL in Patients with Prostate Cancer

Judy W. King,[1] Sharyn Thomas,[1] Fabrizio Corsi,[6] Liquan Gao,[1] Roberto Dina,[4] Roopinder Gillmore,[2] Katharine Pigott,[2] Amir Kaisary,[3] Hans J. Stauss,[1] and Jonathan Waxman[5]

Clin Canc Res 09

Abstract

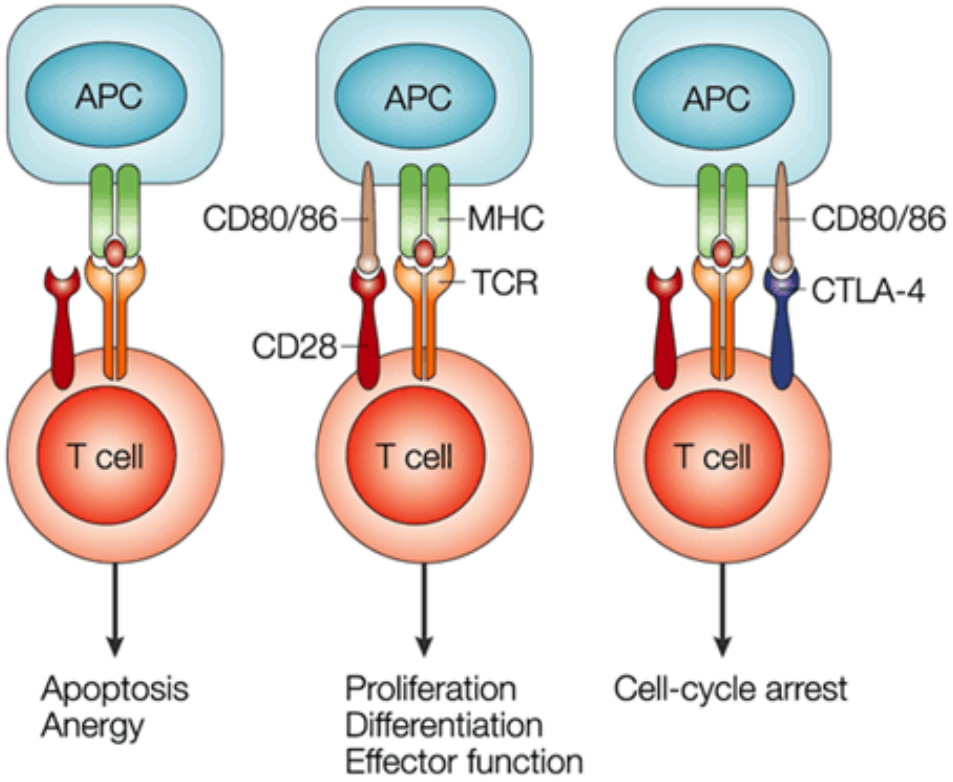
Purpose: The Wilms' tumor antigen 1 (*WT1*) is overexpressed in several leukemias and solid tumors, but there is currently limited information regarding its role in prostate cancer. This study aimed to investigate *WT1* expression in prostate cancer, and to determine the number and function of *WT1*-specific T cells in the peripheral blood of patients.

Experimental Design: Immunohistochemistry was used to assess *WT1* expression in cancer tissues. Human leukocyte antigen A2 (HLA-A2) tetramers served to detect *WT1*-specific T cells, and peptide-specific stimulation was used to assess T-cell function *in vitro*.

Results: Immunohistochemistry of tissue arrays comprising 36 cancer and 8 normal prostate samples revealed nuclear *WT1* staining in 39% of cancer samples, but not in normal prostate tissues. Tetramer analysis revealed a low frequency of *WT1*-specific T cells in 20 of 38 HLA-A2–positive patients. *In vitro* stimulation with *WT1* peptide plus interleukin 2 (IL2) and interleukin 7 (IL7) did not lead to an accumulation of *WT1*-specific T cells in any of the patient samples, although all patients were able to generate T-cell responses against Melan-A/MART1 control peptide. Stimulation with *WT1* peptide in the presence of interleukin 15 (IL15), a cytokine that was shown to reverse tolerance of murine tumor-specific T cells, was able to restore the expansion and IFNγ production of *WT1*-specific T cells in a subgroup of prostate cancer patients.

Conclusion: The observation that IL15 can restore the function of *WT1*-specific T cells that were

Activation sets in motion multiple mechanisms to end the response, e.g. CTLA-4 and PD-1 on T cells



Nature Reviews | Immunology

Anti-PD-1 Monoclonal Antibody Therapy

From Oncology, February 2014, Vol. 59, No. 2
From MD Anderson Cancer Center

A patient with melanoma is shown before (left), after one cycle, and after three cycles of treatment with the anti–PD-1 antibody MK-3475 (10 mg/kg every 3 weeks)

Oncolytic Viruses &Vaccination

- Tumor cells have reduced IFN responses, allowing viral infection by viruses that normally do not infect human or mouse cells (e.g., myxoma normally infects rabbits, not humans, but does infect tumor cells)
- Can be engineered to cause tumor cells to express immunoenhancing cytokines

[*Cancer Research* 65, 9982-9990, November 1, 2005]

Experimental Therapeutics, Molecular Targets, and Chemical Biology

Myxoma Virus Is a Novel Oncolytic Virus with Significant Antitumor Activity against Experimental Human Gliomas

Xueqing Lun[1,4], Wenqing Yang[1], Tommy Alain[1,4], Zhong-Qiao Shi[1], Huong Muzik[1], John W. Barrett[2], Grant McFadden[2], John Bell[3], Mark G. Hamilton[1], Donna L. Senger[1,4] and Peter A. Forsyth[1,4]

Reverse Suppression

- Eliminate Tregs (which suppress both T cells and NK cells)
  - Myeloablative chemo or radiation before adoptive T cell therapy
  - Anti-CD25
- Block TGF and/or IL-10

Mol Cancer Ther. 2006;5:1733-1743

Research Articles: Therapeutics

Blockade of transforming growth factor-ß signaling in tumor-reactive CD8$^+$ T cells activates the antitumor immune response cycle

Qiang Zhang[1], Ximing J. Yang[2,5], Shilajit D. Kundu[1], Michael Pins[2,5], Borko Javonovic[3,5], Robert Meyer[2], Seong-Jin Kim[7], Norman M. Greenberg[8], Timothy Kuzel[4,6], Richard Meagher[4], Yinglu Guo[9] and Chung Lee[1,5,6]

Departments of [1] Urology, [2] Pathology, [3] Preventive Medicine, [4] Medicine, and [5] Cell and Molecular Biology, Northwestern University Feinberg School of Medicine and [6] Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, Illinois; [7] Laboratory of Cell Regulation and Carcinogenesis, National Cancer Institute, Bethesda, Maryland; [8] Fred Hutchinson Cancer Research Center, Seattle, Washington; and [9] Institute of Urology, The First Hospital, Peking University, Beijing, China

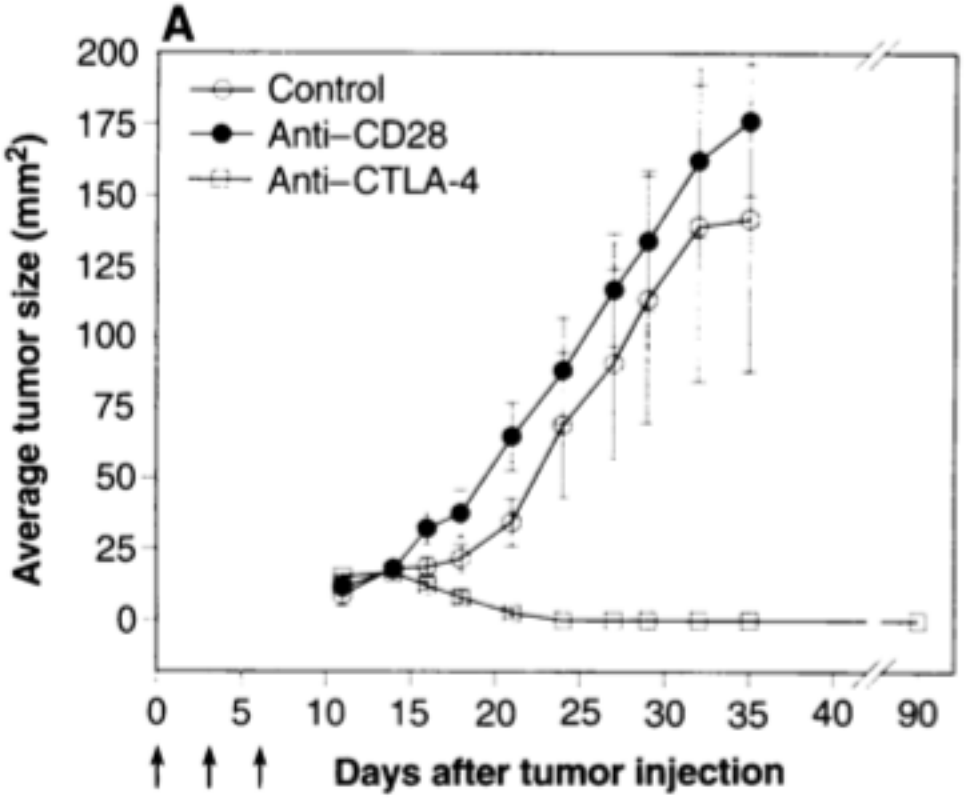
Check-point Blockade

- CTLA-4
- PD-1
- Monoclonal antibodies FDA approved

Enhancement of Antitumor Immunity by CTLA-4 Blockade

Dana R. Leach, Matthew F. Krummel, James P. Allison*

One reason for the poor immunogenicity of many tumors may be that they cannot provide signals for CD28-mediated costimulation necessary to fully activate T cells. It has recently become apparent that CTLA-4, a second counterreceptor for the B7 family of costimulatory molecules, is a negative regulator of T cell activation. Here, in vivo administration of antibodies to CTLA-4 resulted in the rejection of tumors, including preestablished tumors. Furthermore, this rejection resulted in immunity to a secondary exposure to tumor cells. These results suggest that blockade of the inhibitory effects of CTLA-4 can allow for, and potentiate, effective immune responses against tumor cells.


A
Control
Anti–CD28
Anti–CTLA-4
Average tumor size (mm²)
200
175
150
125
100
75
50
25
0
0
5
10
15
20
25
30
35
40
90
Days after tumor injection

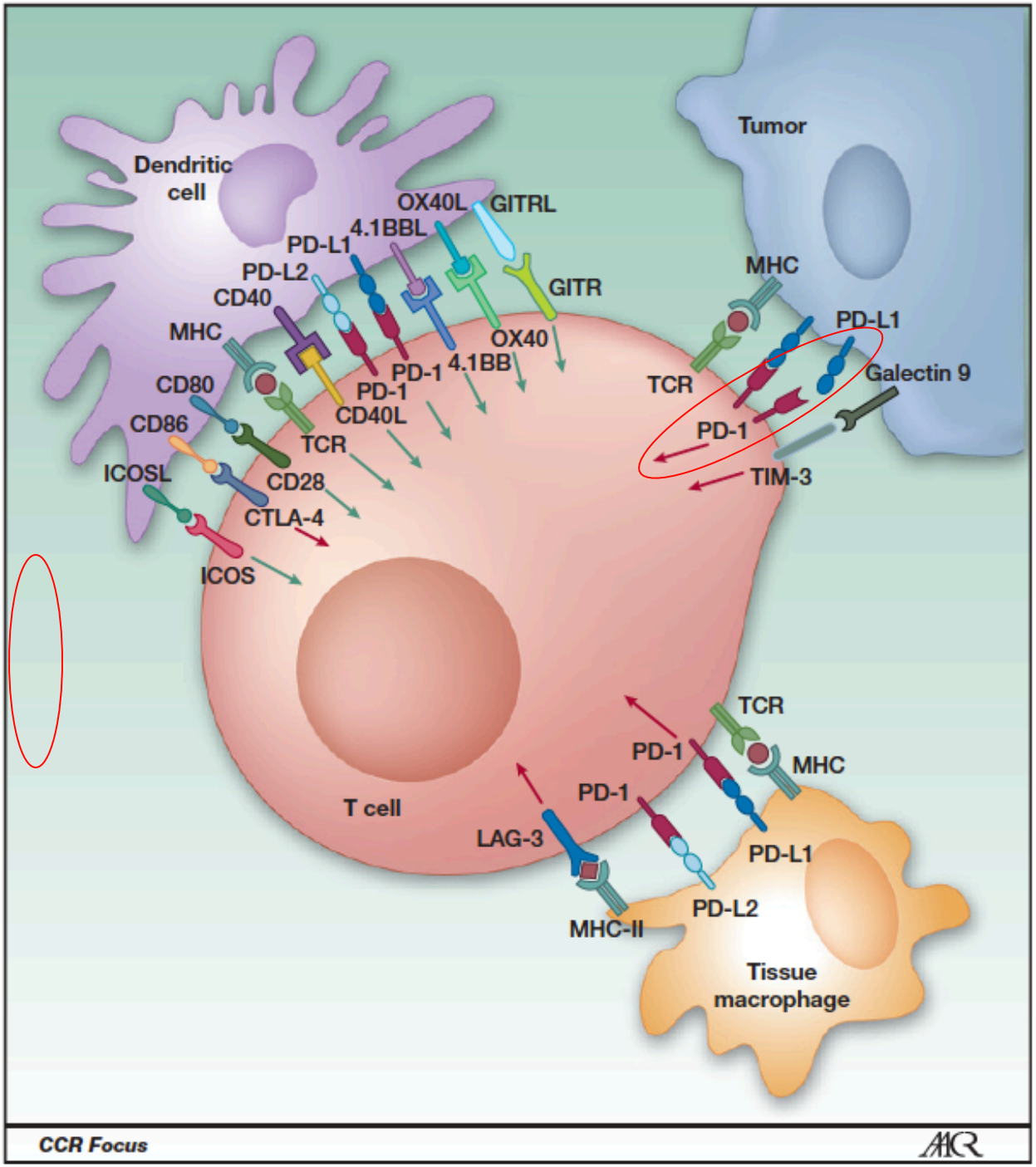


Figure 1. Costimulatory and coinhibitory ligand–receptor interactions between a T cell and a dendritic cell, a tumor cell, and a macrophage, respectively, in the tumor microenvironment. Figure adapted from Sharma (88) and Melero et al. (89).

Ott
2013

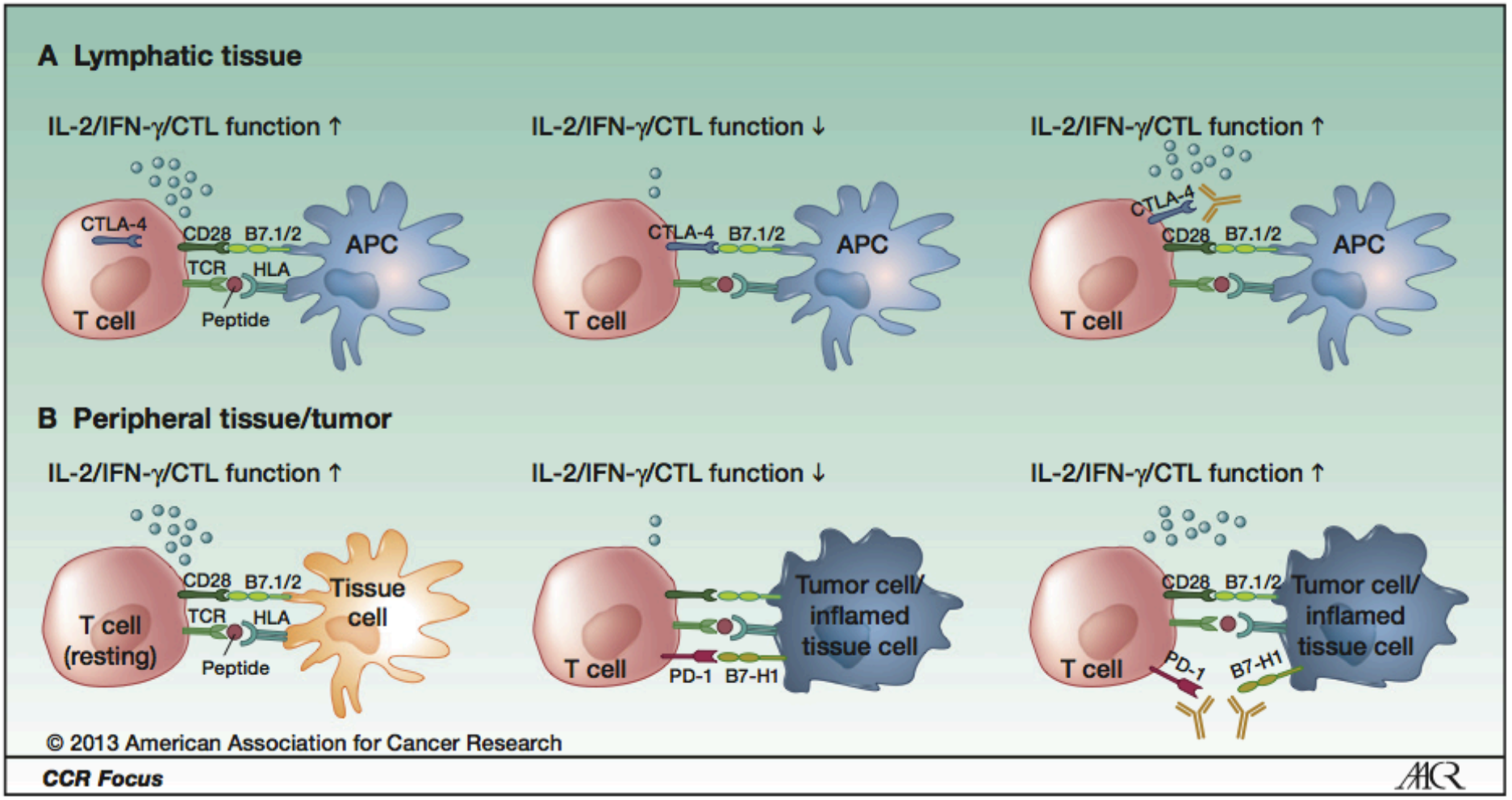


Figure 2. CTLA-4 and PD-1 modulate different aspects of the T-cell response: A, CTLA-4 is upregulated after antigen-specific activation of a naïve or memory T cell in lymphatic tissue, leading to decreased effector function (early activation phase). B, PD-1 is mainly expressed on antigen-experienced memory T cells in peripheral tissues cells. The immune modulation mediated by this pathway ensures protection of tissue from collateral damage during an inflammatory response. Tumor cells use this regulatory mechanism to evade a tumor-directed T-cell response by upregulating the PD-1 ligands B7-H1 and B7-DC.

Ott 2013

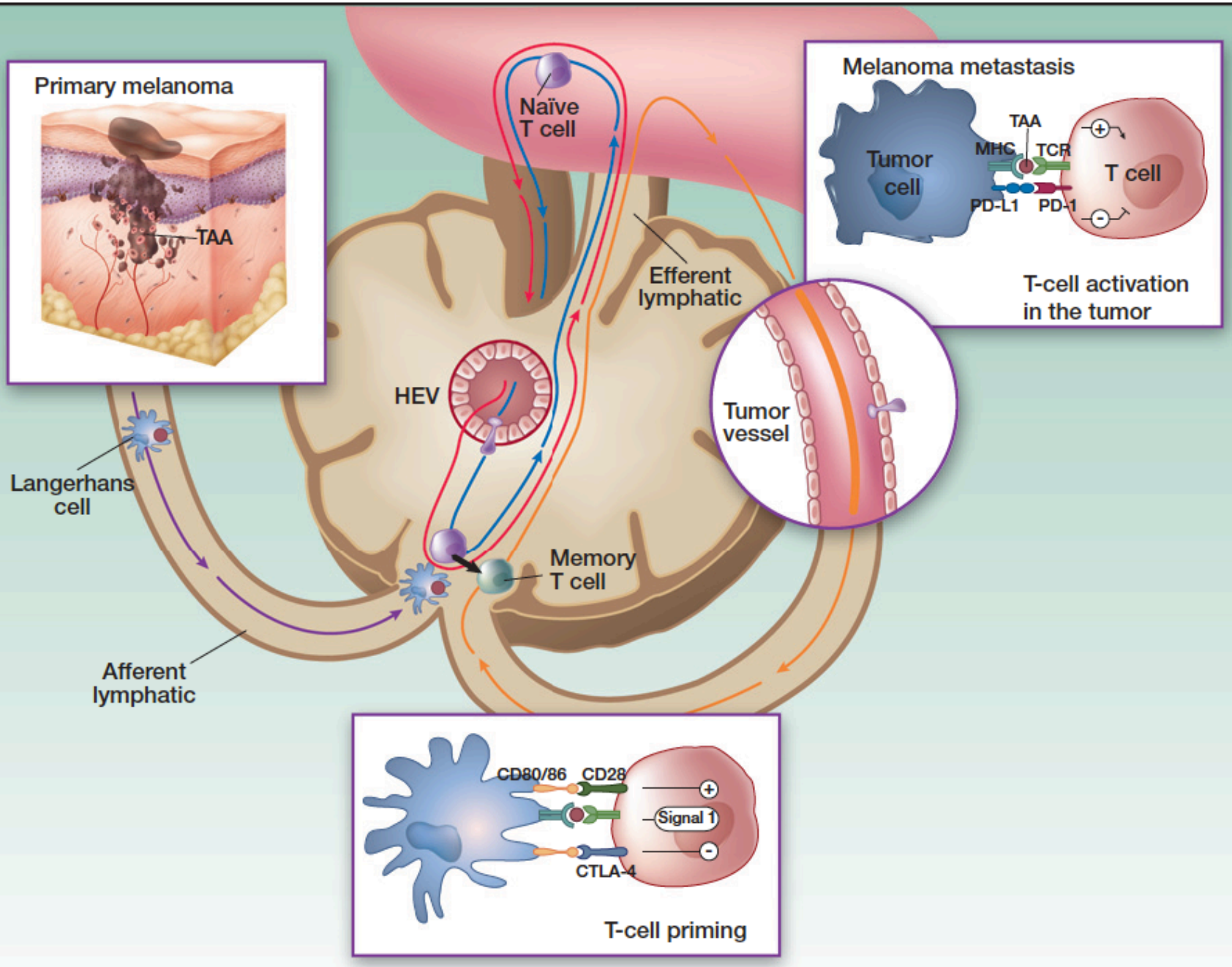


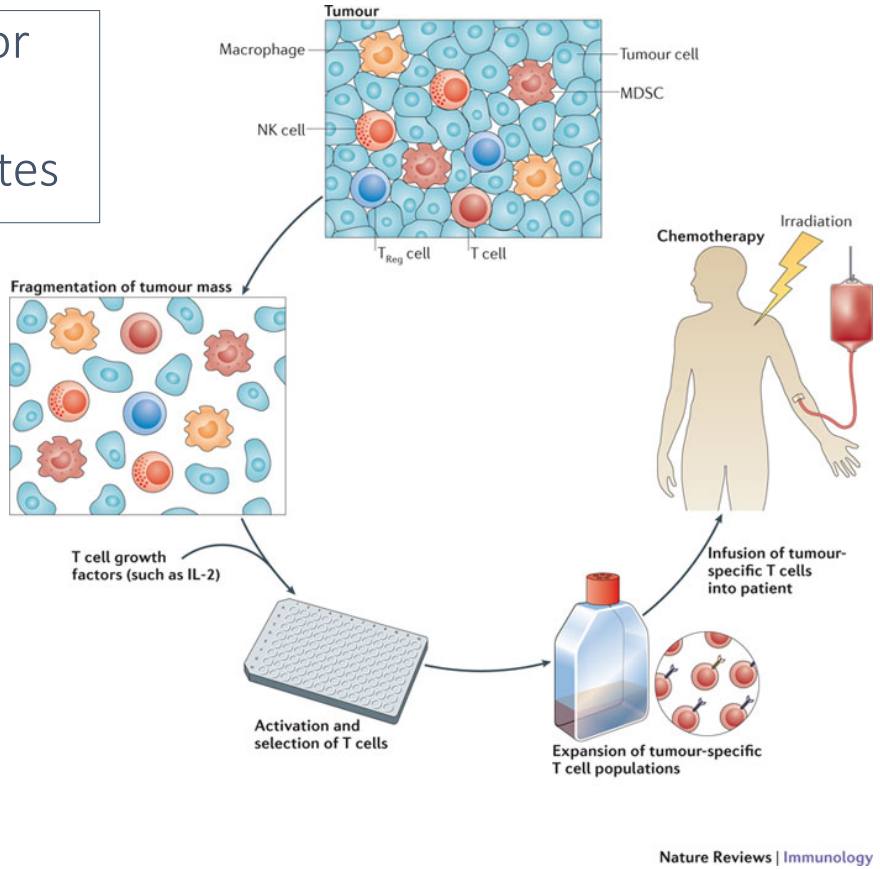
Figure 3. The figure shows a primary melanoma and tumor-associated antigens (TAA) that are taken up by an APC (a Langerhans cell; left). APCs then migrate via afferent lymphatics to the sentinel skin-draining lymph node, where they present TAA to naïve T cells. These naïve T cells continuously screen the lymphoid organs, extravasating through HEV until their corresponding peptide is presented to them in the context of the MHC. When a TAA is presented in the T-cell–dependent area of a lymph node to its specific naïve T cell (center), the latter requires signal 1 (antigen presentation to the TCR) and 2 (costimulation) for full activation. It will then proliferate and acquire "antigen memory" and a distinct and different set of adhesion molecules that will allow it to navigate outside of the blood vessels and the lymphoid organs, to the peripheral tissues and organs, like the melanoma metastases where it will be able to be reactivated upon re-presentation of the same TAA. However, in the lymph node, T-cell activation is interrupted when signal 3, mediated through interaction between CTLA-4 and CD80/86, takes over CD28 and CD80/86 interaction. This occurs 24 to 48 hours after the initiation of T-cell priming. When the memory T cell is recruited to the melanoma metastasis (right) and activated upon TAA re-presentation, the effector activation is decreased when PD1 is engaged with its ligand PDL-1 that can be expressed on the tumor cell constitutively or in the context of inflammation. The figure is adapted from the C. Robert and C. Verjat figure collection, Institute Gustave Roussy (Villejuif-Paris Sud, Paris, France).

Combination Therapies

- Lymphodepletion and Adoptive T cell therapy (Rosenberg 09)

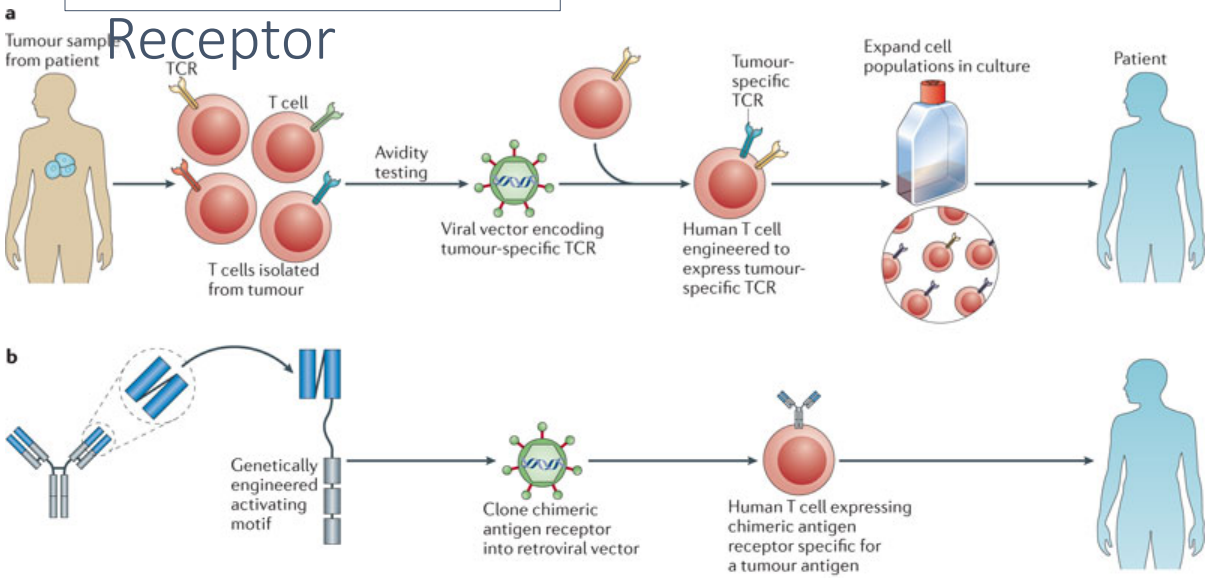
Adoptive T Cell Transfer (ACT)

TIL – Tumor Infiltrating Lymphocytes

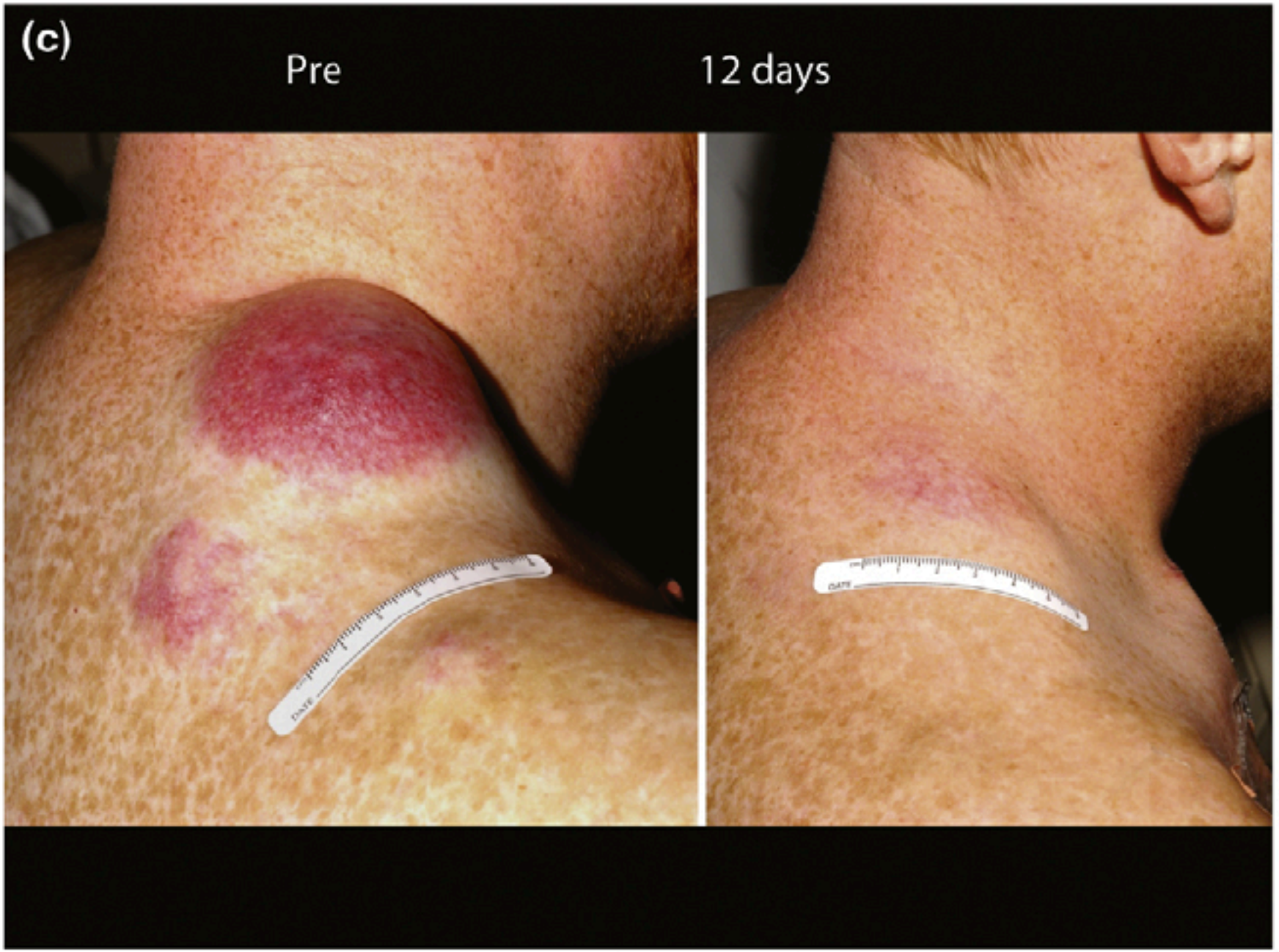


Adoptive T Cell Transfer (ACT)

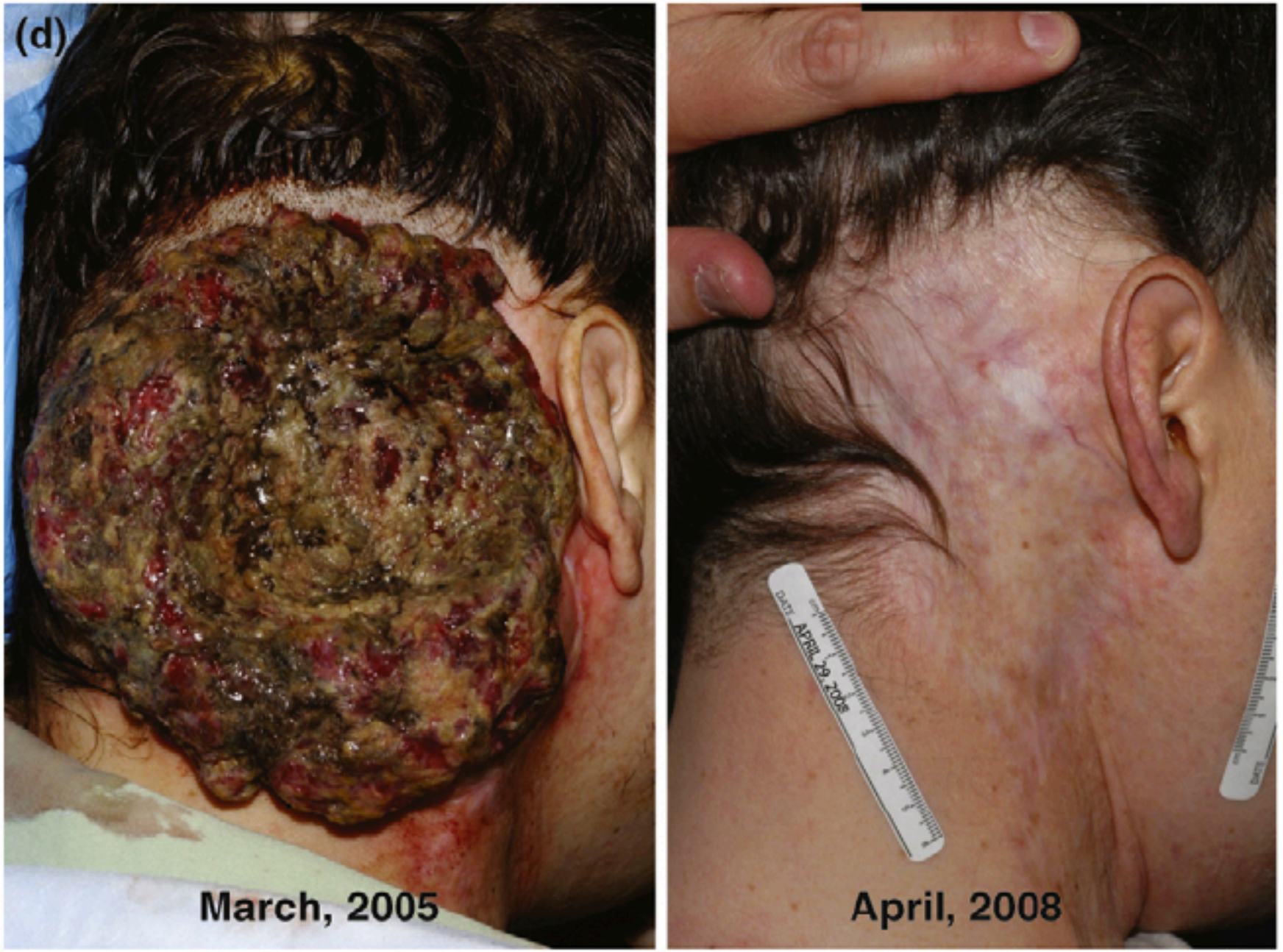
TCR– T Cell Receptor



CAR– Chimeric Antigen Receptor

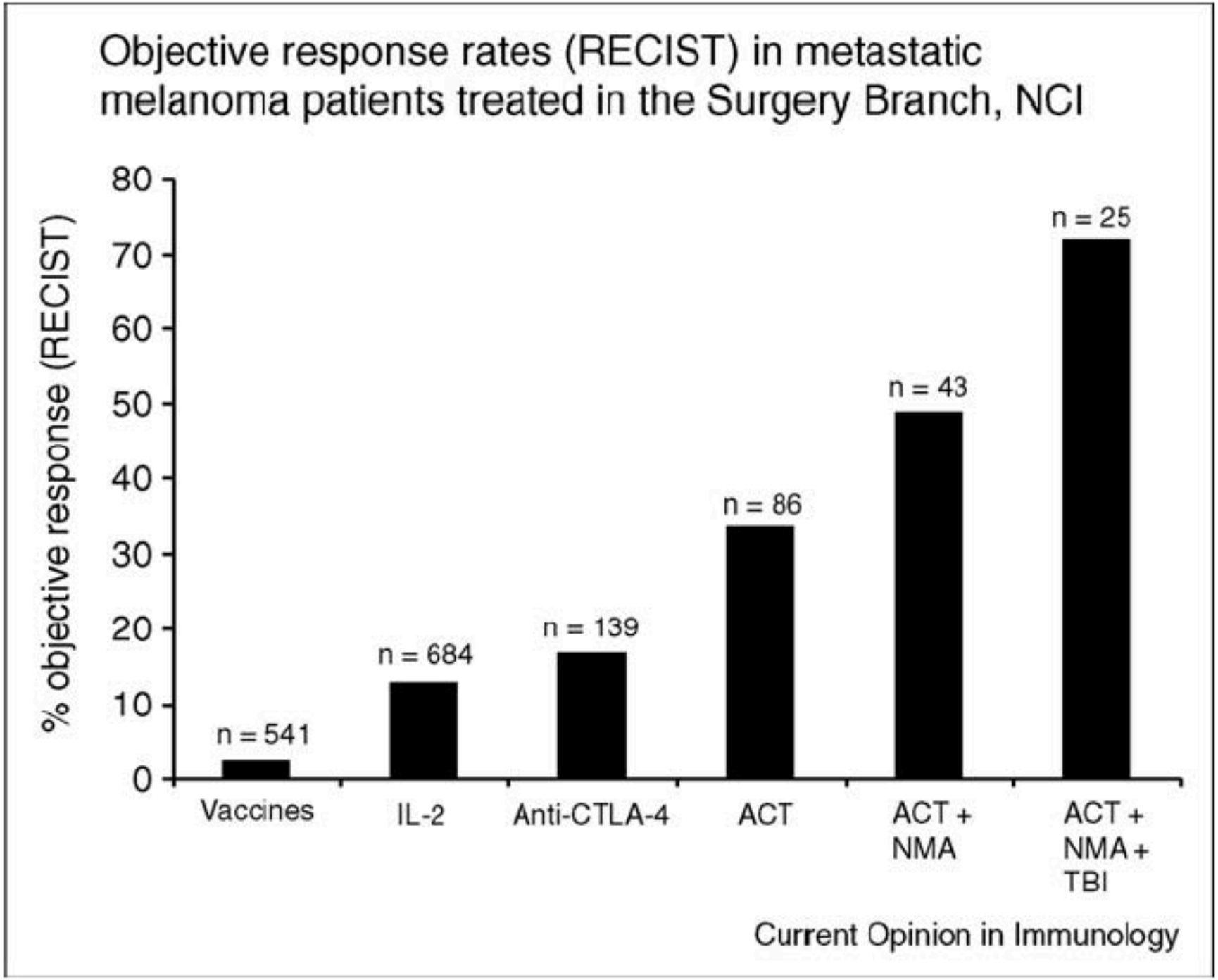


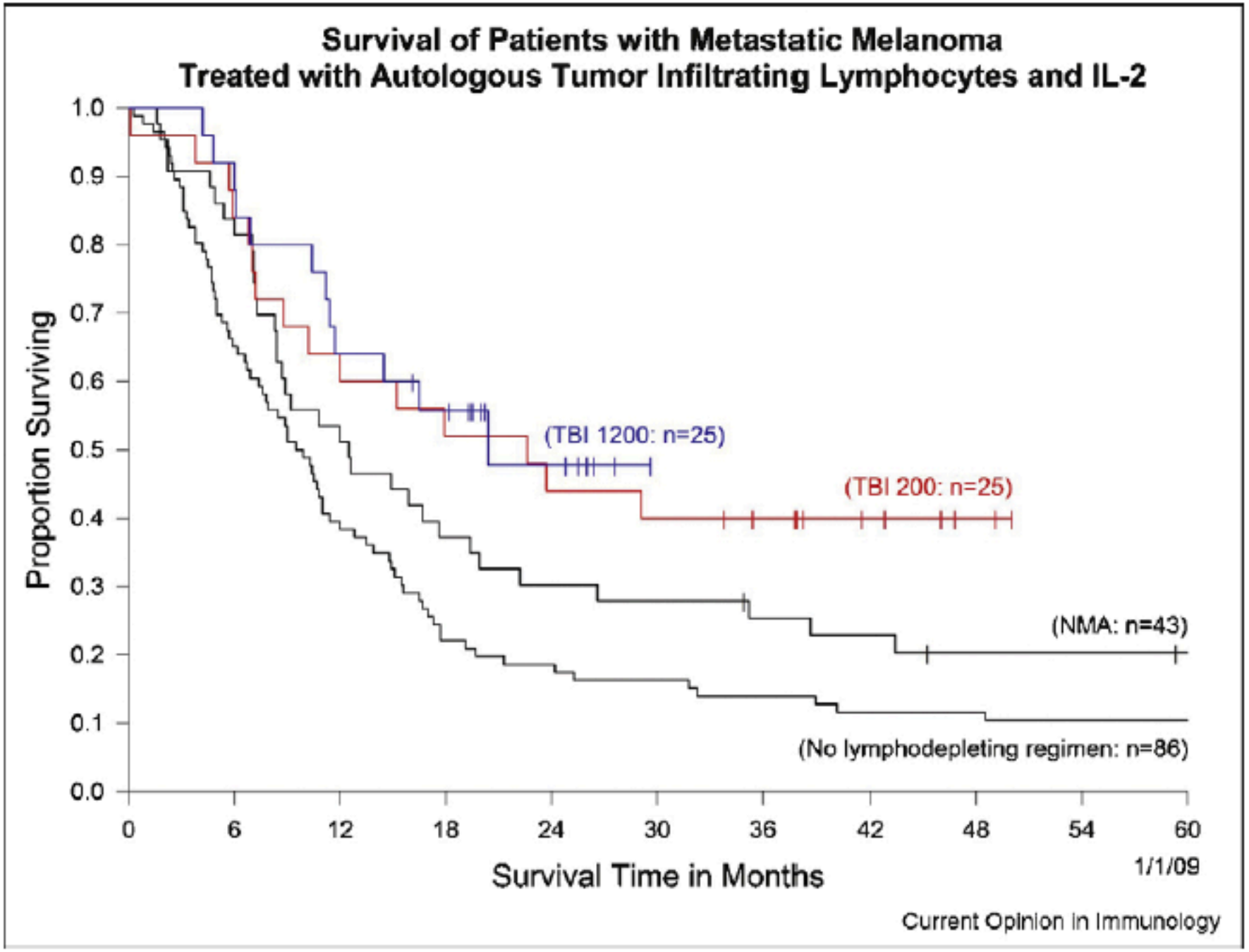
Rosenberg and Dudley 2009



Rosenberg and Dudley 2009

Current Opinion in Immunology

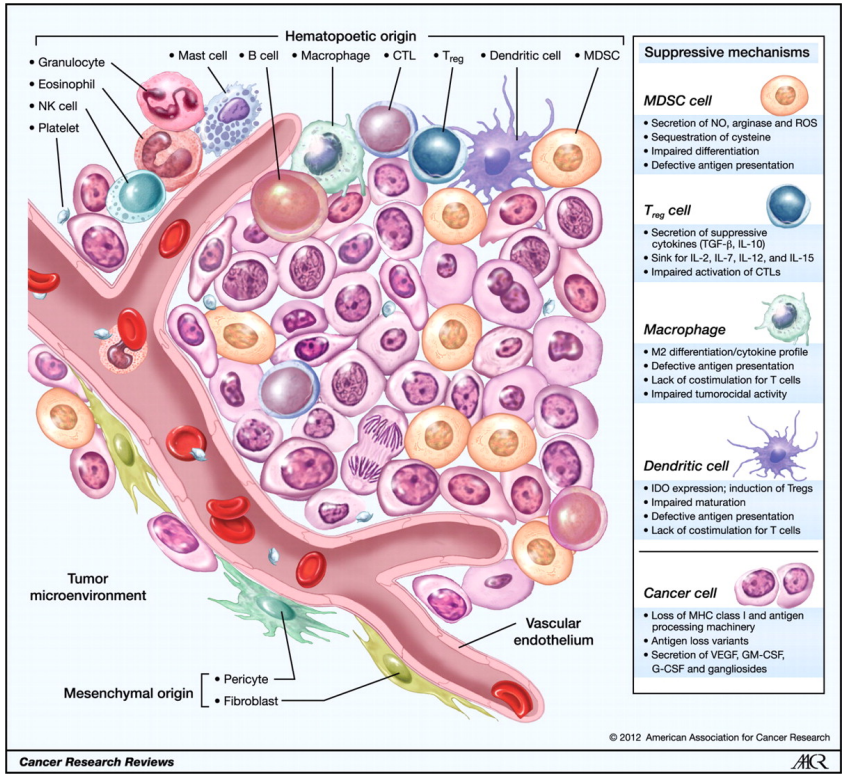

Objective response rates (RECIST) in metastatic melanoma patients treated in the Surgery Branch, NCI
% objective response (RECIST)
80
70
60
50
40
30
20
10
0
n = 541
n = 684
n = 139
n = 86
n = 43
n = 25
Vaccines
IL-2
Anti-CTLA-4
ACT
ACT + NMA
ACT + NMA + TBI
Current Opinion in Immunology


Survival of Patients with Metastatic Melanoma
Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2
Proportion Surviving
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
(TBI 1200: n=25)
(TBI 200: n=25)
(NMA: n=43)
(No lymphodepleting regimen: n=86)
0
6
12
18
24
30
36
42
48
54
60
Survival Time in Months
1/1/09
Current Opinion in Immunology

Why does immunotherapy fail?

- Most cancers do not have as many mutations as melanomas
- Cancers use natural suppressive mechanisms

Cellular infiltrates within the tumor microenvironment.

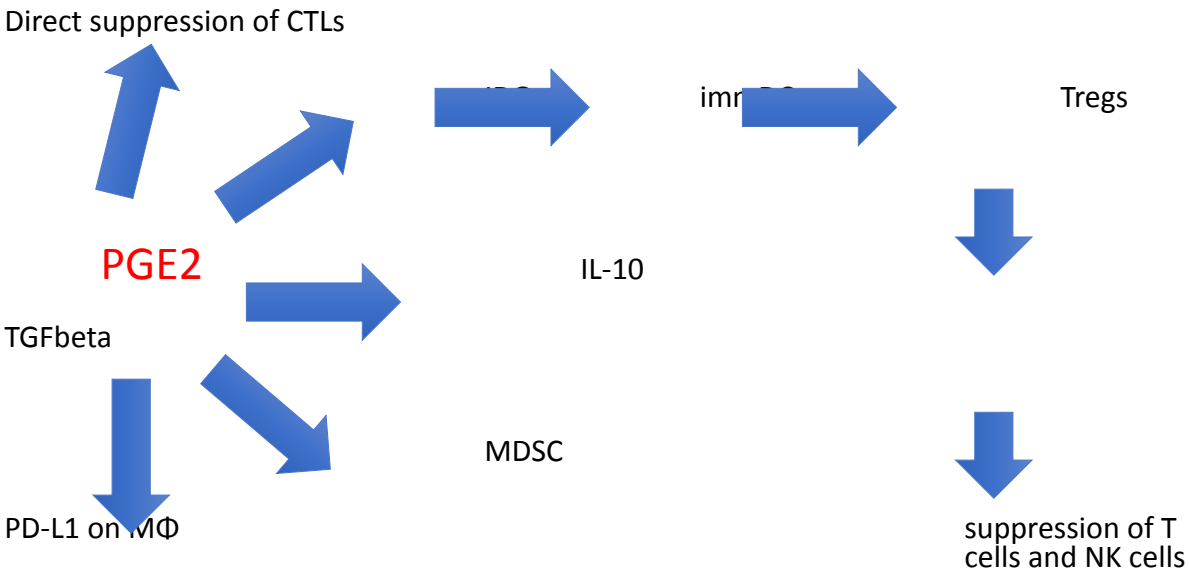


Kerkar S P , Restifo N P Cancer Res 2012;72:3125-3130

Immunosuppression Factors in Tumor Microenvironment

- TGFbeta
- IDO
- IL-10
- Arginase
- Treg
- MDSC
- Potassium
- M2 Macrophage
- Adenosine
- Prostaglandin E2 (PGE2)
- PD-1, CTLA-4, LAG-3, TIM-3
- Immunosuppressive DC

PGE2 may be pivotal

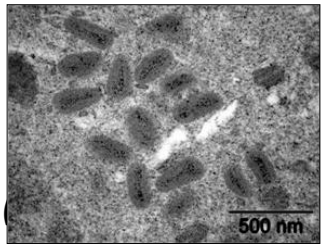


Our Focus

- Brain Tumors (gliomas and metastatic melanomas)
- T cells
  - Specific recognition directs cytolytic activity
  - Actively cross blood brain barrier
- Use treatments that increase both T cells and NK cells
- Reverse immunosuppression in tumor microenvironment with oncolytic viral delivery of IL-15 and blockade of prostaglandins with Celecoxib

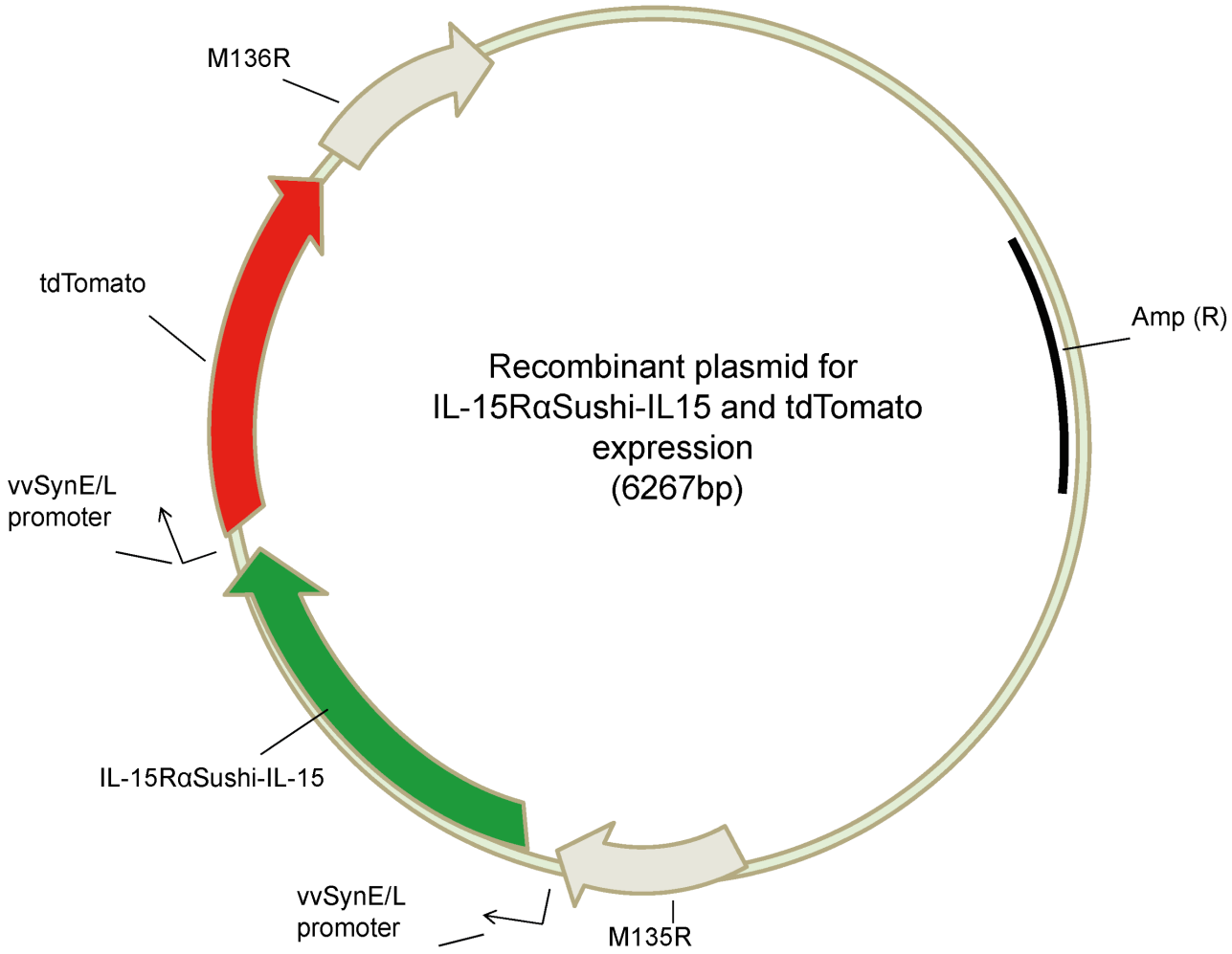
Myxoma virus

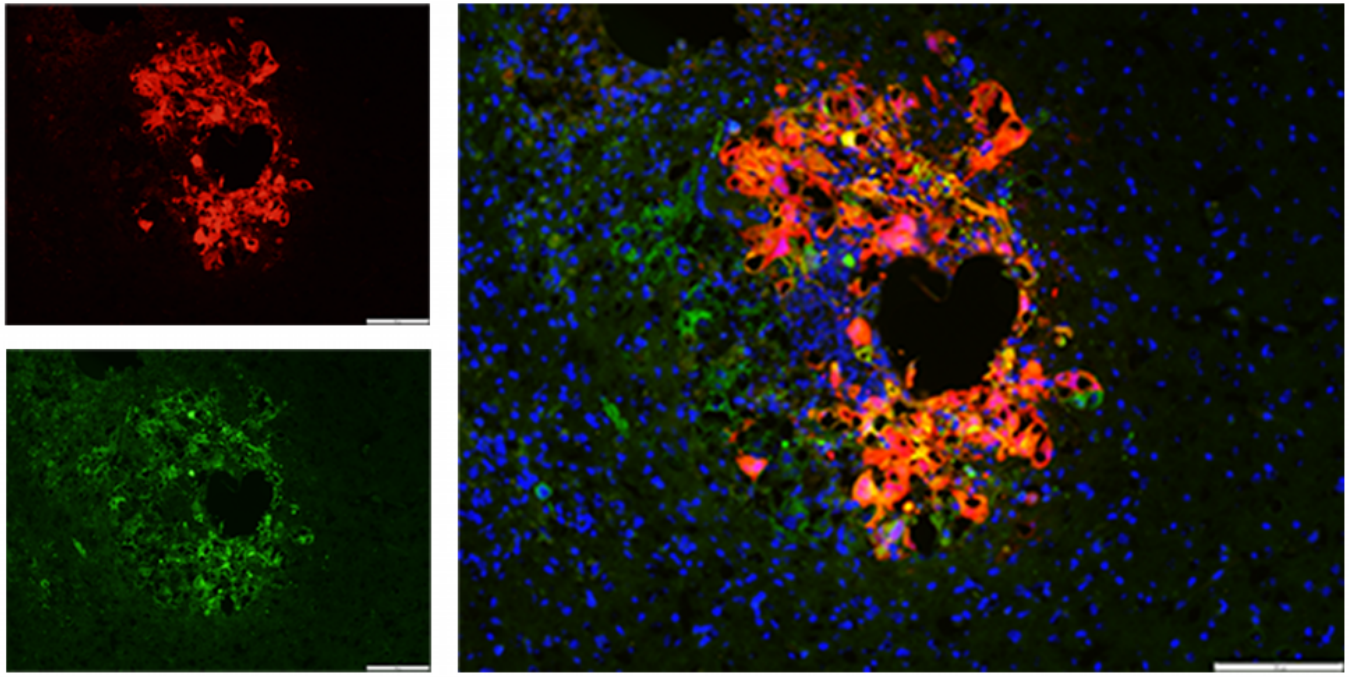
- Rabbit poxvirus - myxoma virus
  - non-pathogenic for all non-rabbit vertebrate species tested
  - selectively infects:
    - cancer cells in vitro *(Sypula 2004)*
    - human tumor xenografts in nude mice: glioma (medulloblastoma *(Lun 2007)*
    - syngeneic melanoma models *(Stanford 2008)*

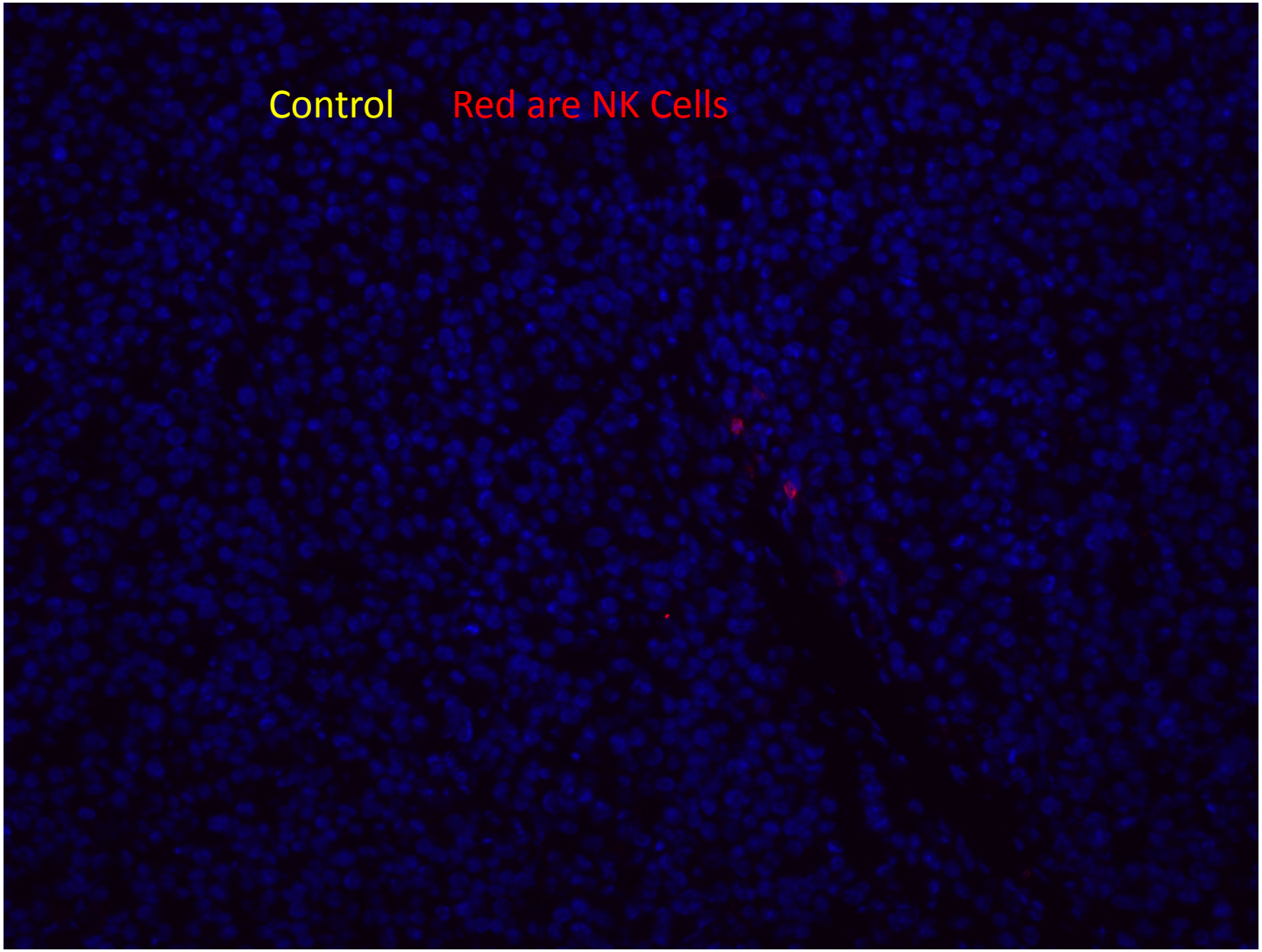


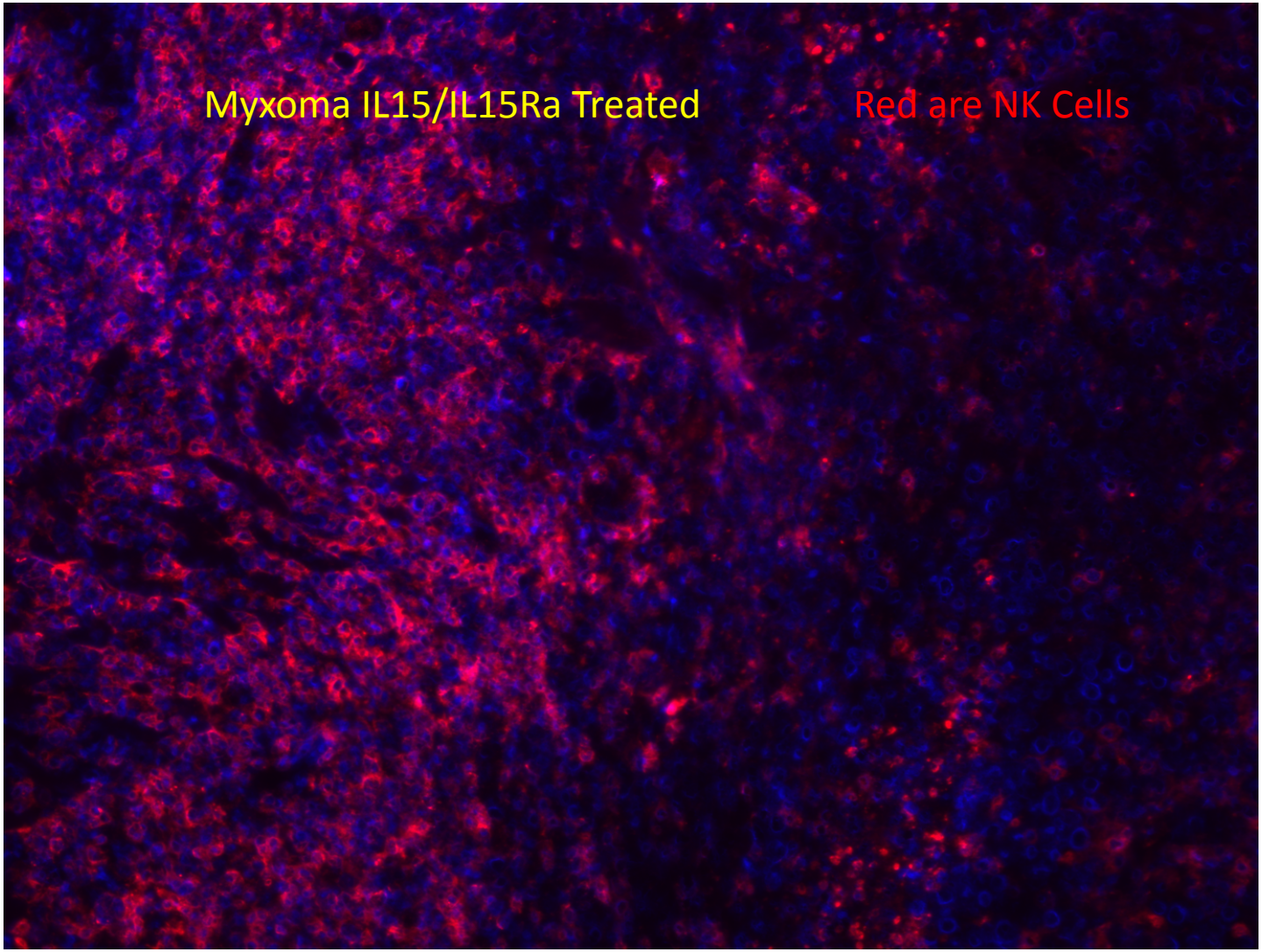
EM: Mature and immature virions in B16-SIY melanoma cells

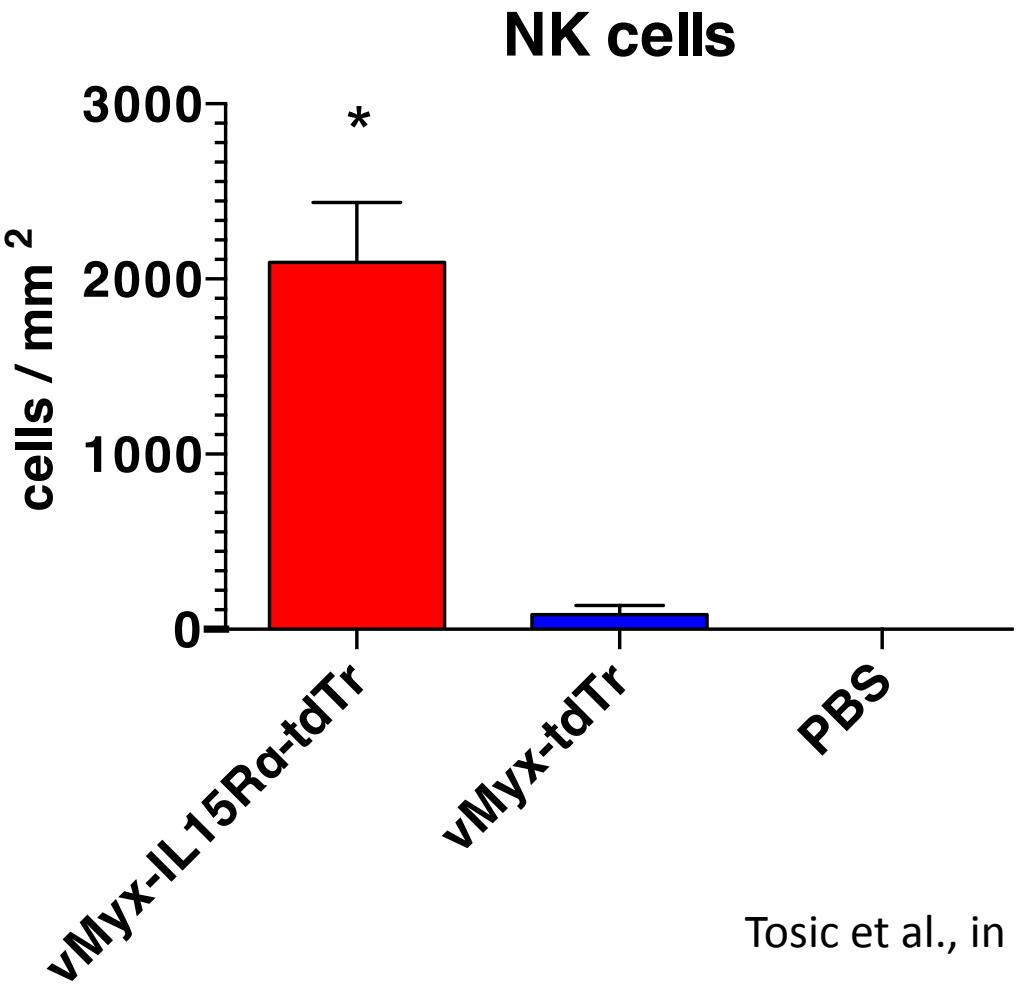
- <u>Advantages</u>:
  - No record of human infection, no prior immunity
  - Replicates in cytoplasm, no nuclear integration of viral DNA
  - Large dsDNA genome (161.8kb), can insert large genes
  - Tropism of myxoma to cancer cells is linked to hyperactivation of serine/threonine kinase Akt


M136R
tdTomato
Amp (R)
Recombinant plasmid for
IL-15RαSushi-IL15 and tdTomato
expression
(6267bp)
vvSynE/L
promoter
IL-15RαSushi-IL-15
vvSynE/L
promoter
M135R

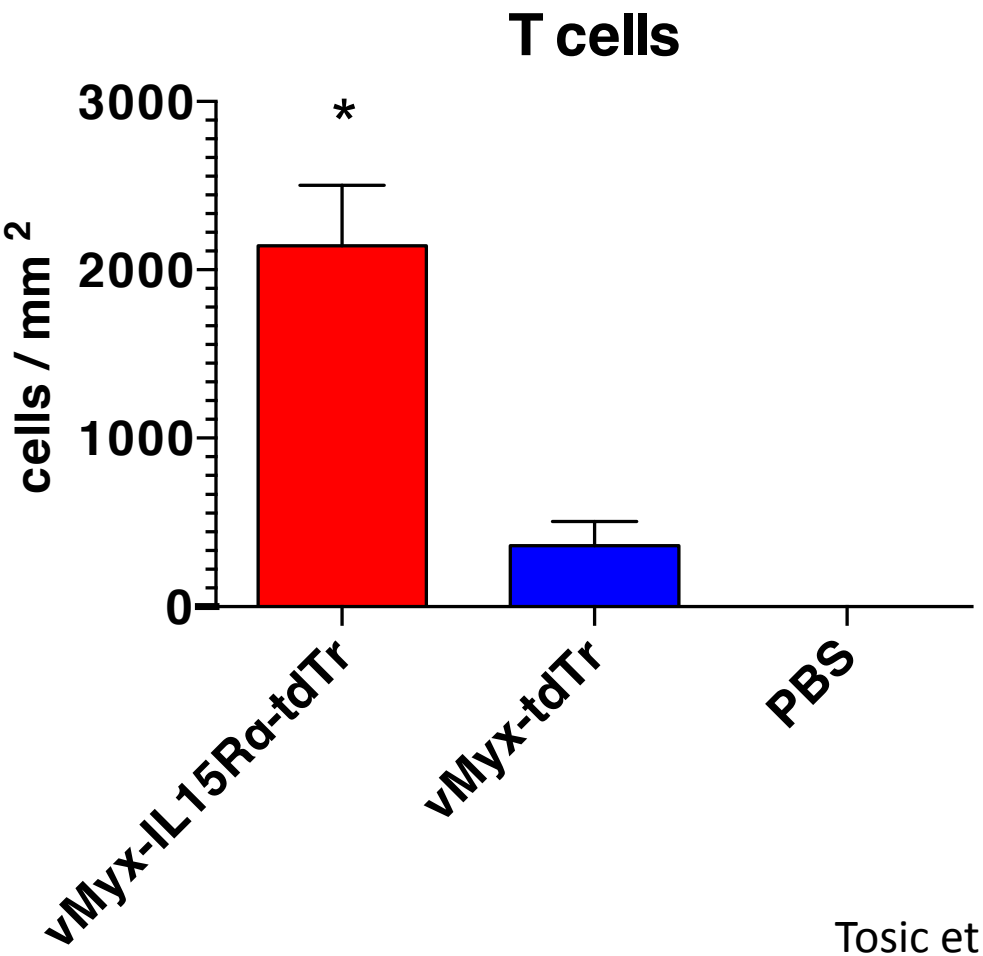



Control
Red are NK Cells

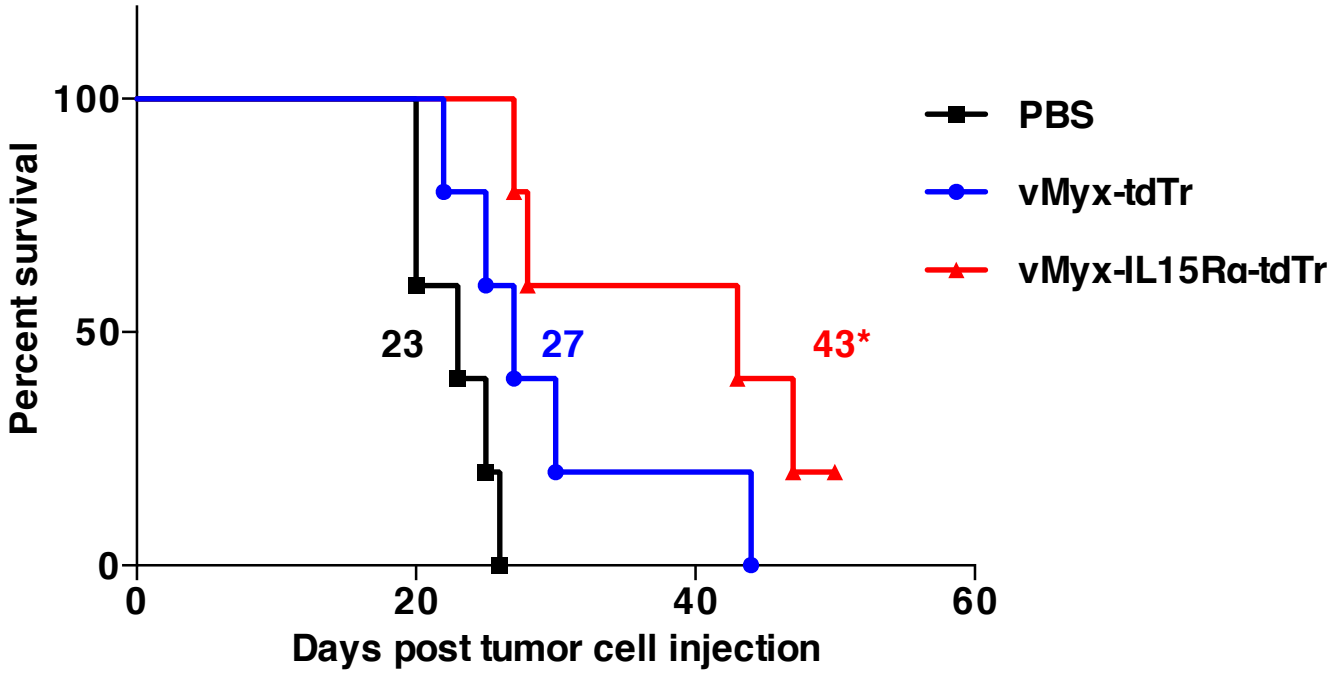

Myxoma IL15/IL15Ra Treated
Red are NK Cells



Tosic et al., in press



Tosic et al., in press

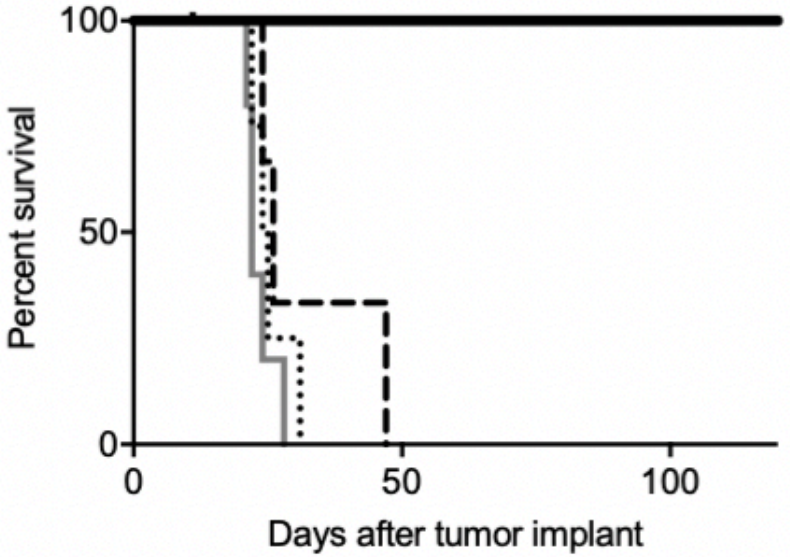


Tosic et al., in press

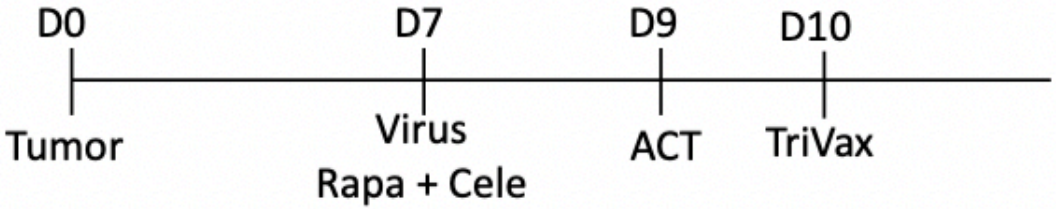
Roy Lab Combination Treatment for Brain Tumors (in mice)

1. Tumor-Specific T cells donated by vaccinated mice
2. Oncolytic Virus to Kill some tumor cells and provide locally expressed IL-15-IL15R$\propto$ to attract T cells and NK cells
3. Prostaglandin Synthesis Inhibitor (Celecoxib) to reduce Immunosuppression in Tumor Microenvironment

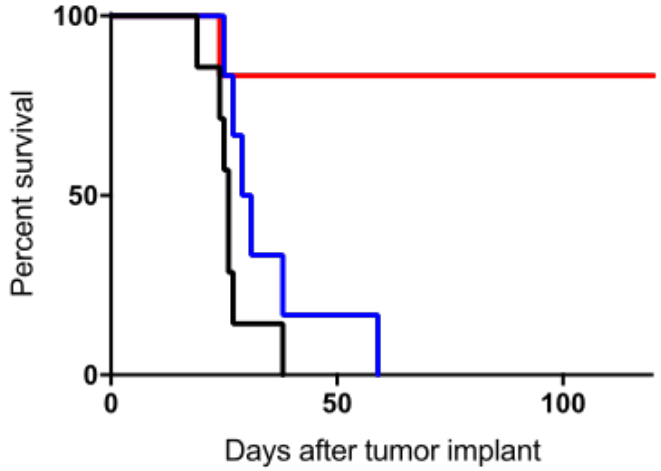
Survival experiment with combination treatment with vvDD-IL15Rα-YFP



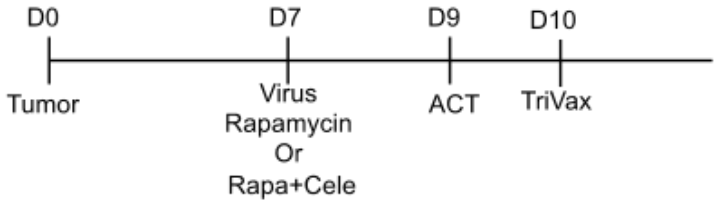
	Virus Treatment	Rapa+Cele	ACT (TriVax)	n
—	PBS	✓	—	5
– –	vvDD-IL15Rα-YFP	✓	—	3
····	vvDD-RFP	✓	✓	4
▬	vvDD-IL15Rα-YFP	✓	✓	5



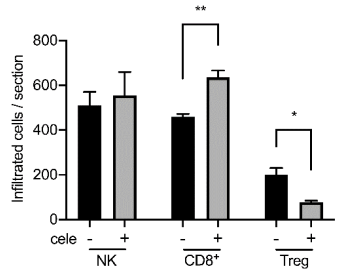
Myxoma Virus IL15/IL15Rα

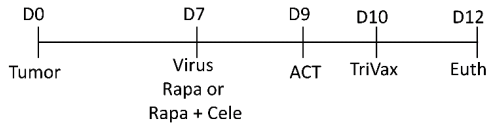


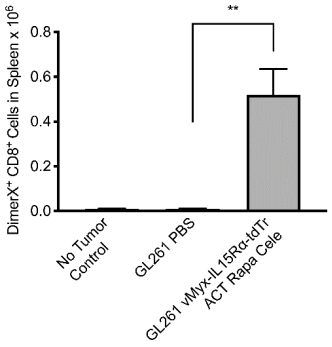
	Virus Treatment	Rapa	Cele	ACT (TriVax)	n
—	PBS	✓	✓	—	7
—	vMyx-IL15Rα-tdTr	✓	—	✓	6
—	vMyx-IL15Rα-tdTr	✓	✓	✓	6

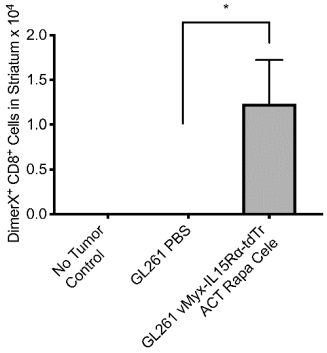


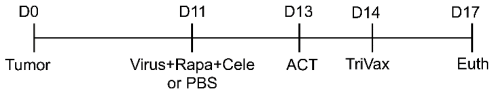
Potential mechanisms of the full combination treatment using vMyx-IL15Rα-tdTr









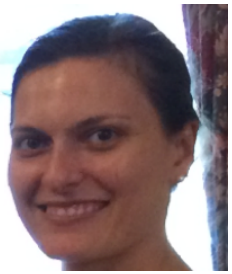


ILLINOIS

Conclusions

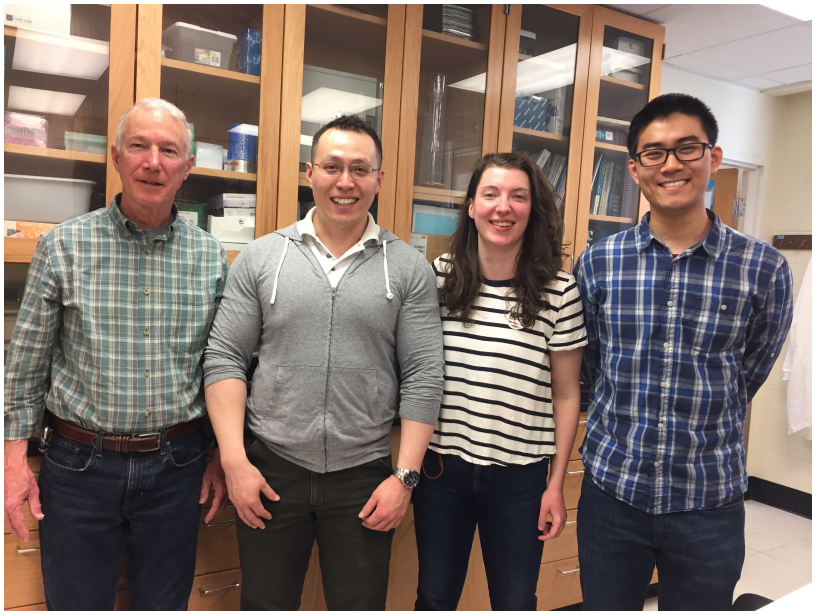
- Immunotherapies *can* eliminate established brain tumors
- Synergy of:
  - Adequate numbers of tumor-specific T cells
  - Local production of stimulatory cytokines such as IL-15
  - Blockade of immune suppression
- Someday something like these treatments will reach the clinic

Students who did the work



Diana Thomas Carolina Soto Vesna Tosic

Collaborators:
David Kranz
Joanna Shisler



Bingtao Tang Claire Schane David Yan

In the last year, **445,000** Americans died from COVID-19

Mass Graves in NYC

In the same time period, more than **600,000** Americans died from Cancer

The immune system is our best hope against both SARS-CoV-2 and Cancer

- The immune system is our best hope against both COVID-19 and cancer
- It is complicated but it works

- And here's a video of which booster shot to choose:
- https://www.youtube.com/watch?v=YhMlZczq4Ns

Thank you for participating


LMA
TO THY
OF T
THOSE
SEND