Active Immunization Approaches

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Disclosures:

Advisory: Merck, Amgen, Castle Biosciences

Stocks: Pfizer, Celgene

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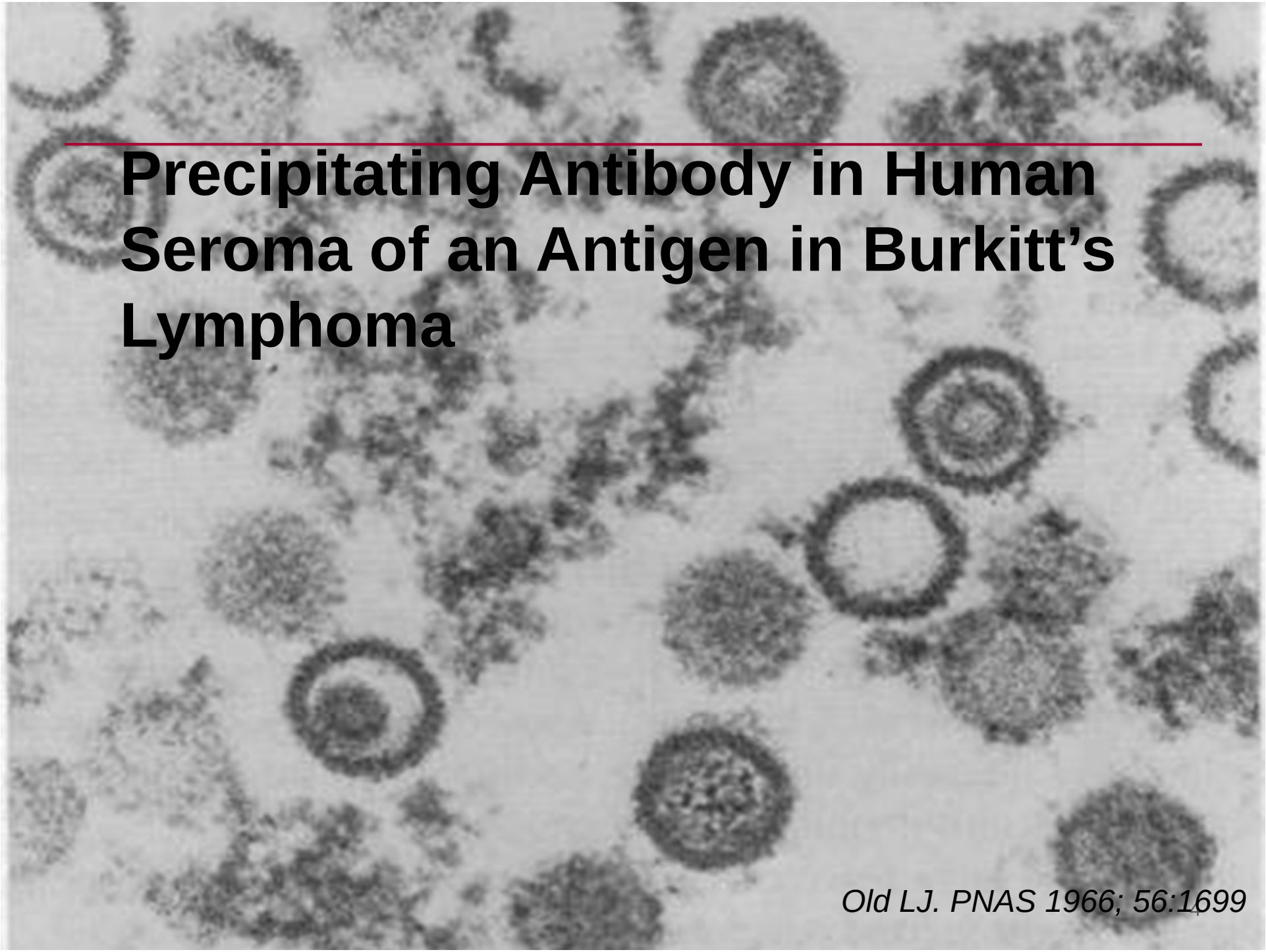
Potential Benefits of Coley's Toxins

End Results of 484 Cases of Malignant Disease with Histologic Confirmation in Which Coley's Toxins Were Used

Type of tumor	Total no. cases	Inoperable		Operable	
		Total	Five year survivals	Total	Five year survivals
Carcinoma	69	45	15	24	21
Malignant melanoma	24	19	4	5	3
Bone sarcoma	205	98	37	107	51
Soft parts sarcoma	123	91	53	32	25
Lymphosarcoma	49	45	24	4	4
Hodgkin's Disease	14	14	1	0	0
Total	484	312	134	172	105

Precipitating Antibody in Human Seroma of an Antigen in Burkitt's Lymphoma



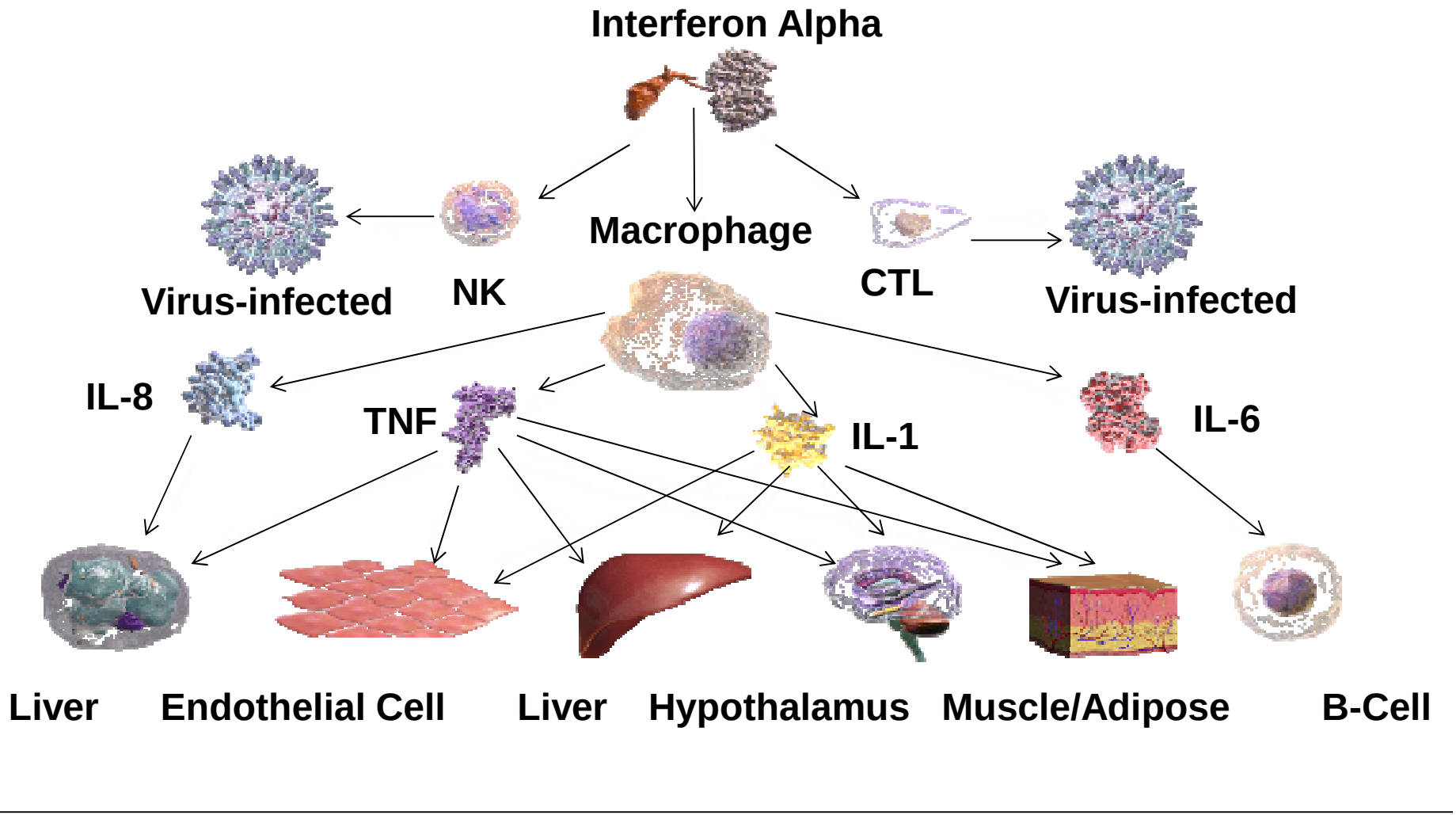
Old LJ. PNAS 1966; 56:1699

Potential Methods of Immunotherapy

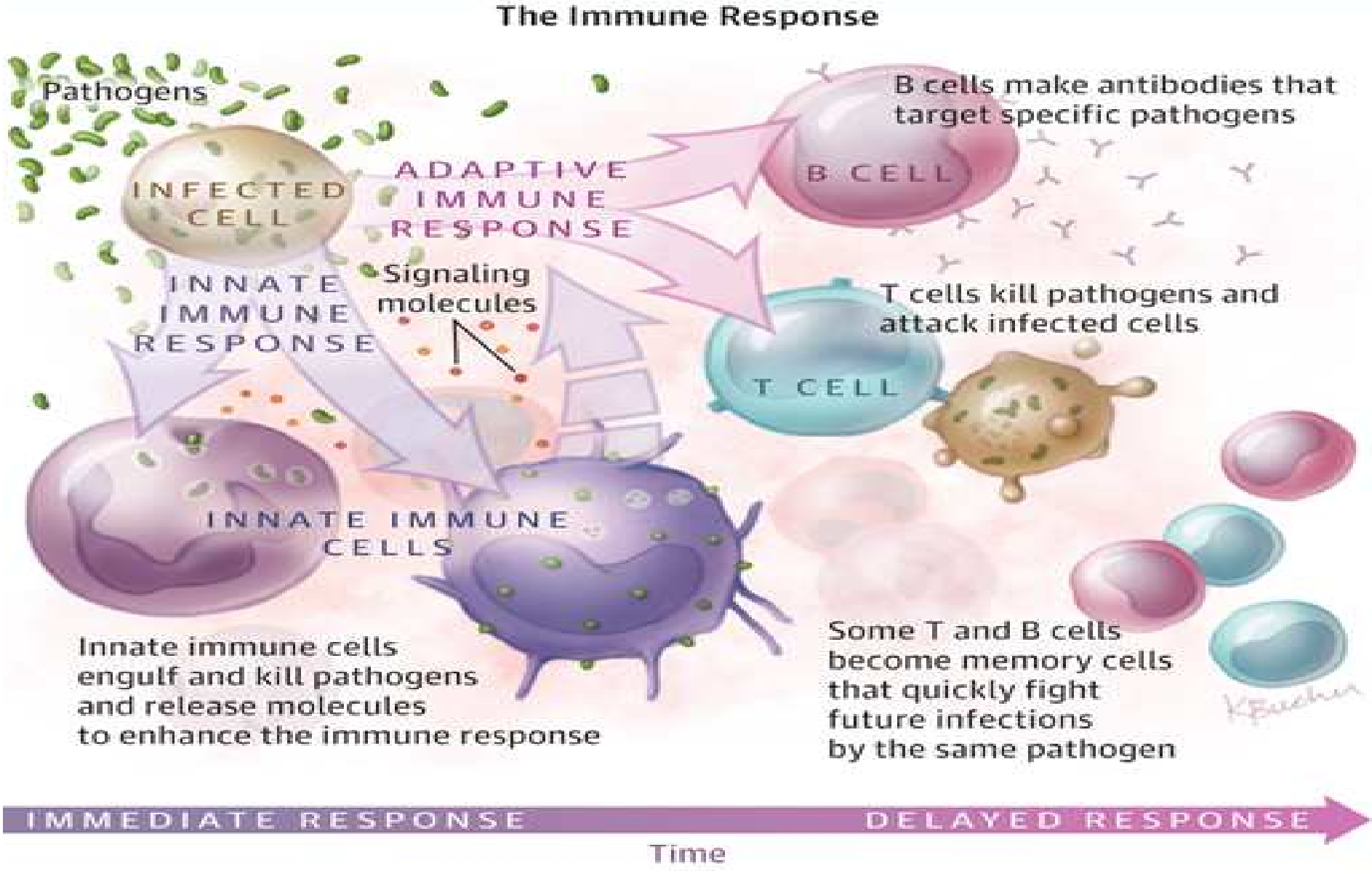
TABLE I.—*Potential Methods of Immunotherapy*

	Specific	Non-specific
Passive	Xenogeneic or allogeneic anti-tumour antisera	Non-specific serum factors Properdin, etc.
Adoptive	Xenogeneic or allogeneic sensitized lymphoid cells or extracts	Normal lymphoid cells—allogeneic or xenogeneic. Anti-tumour effect of GVH disease
Active	Tumour cells, extracts or chemically-modified tumour antigens. Foetal antigens	Non-specific stimulants of the immune response BCG, *C. parvum*, etc.

The Interferon Cascade



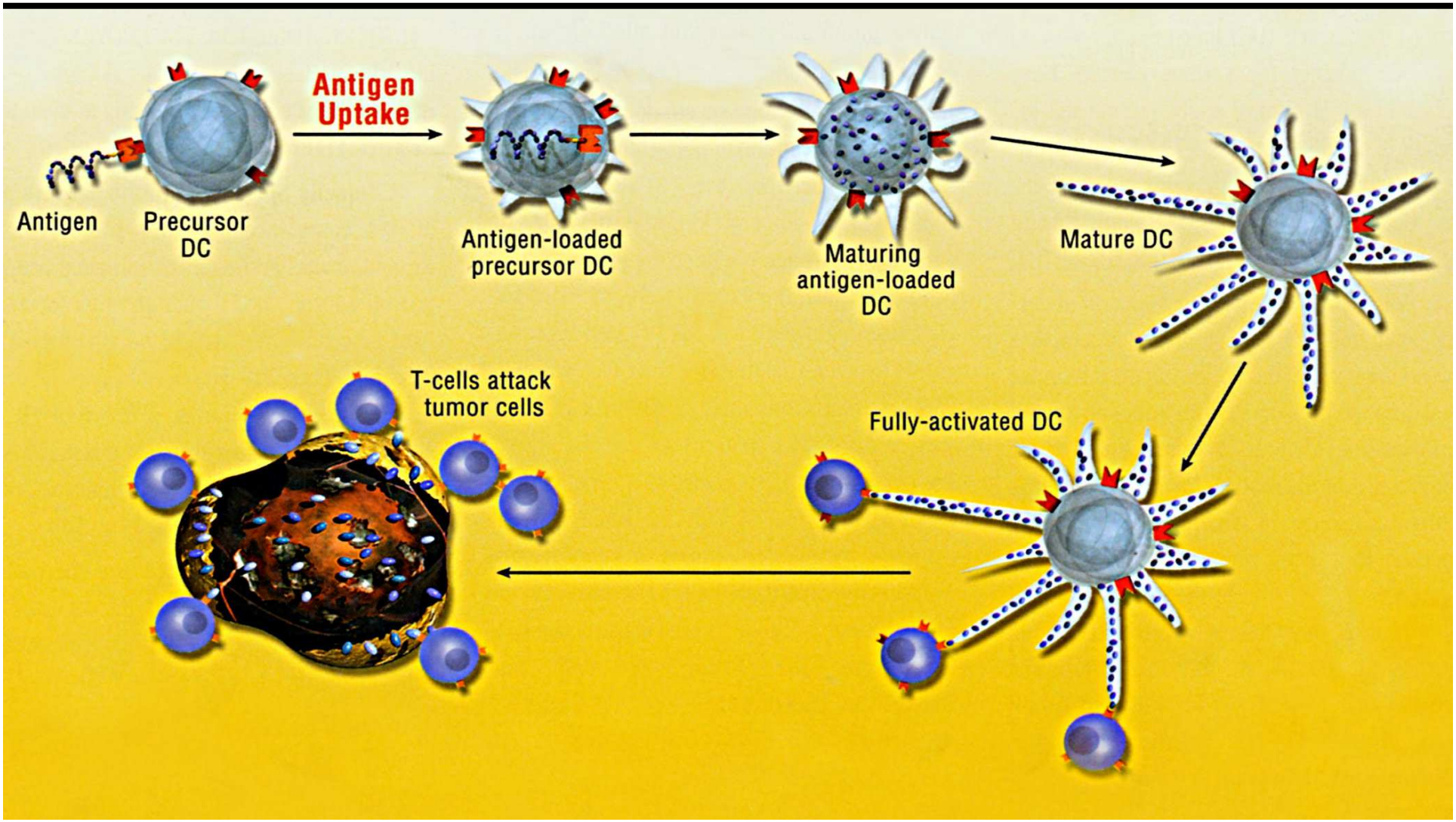
The Immune System



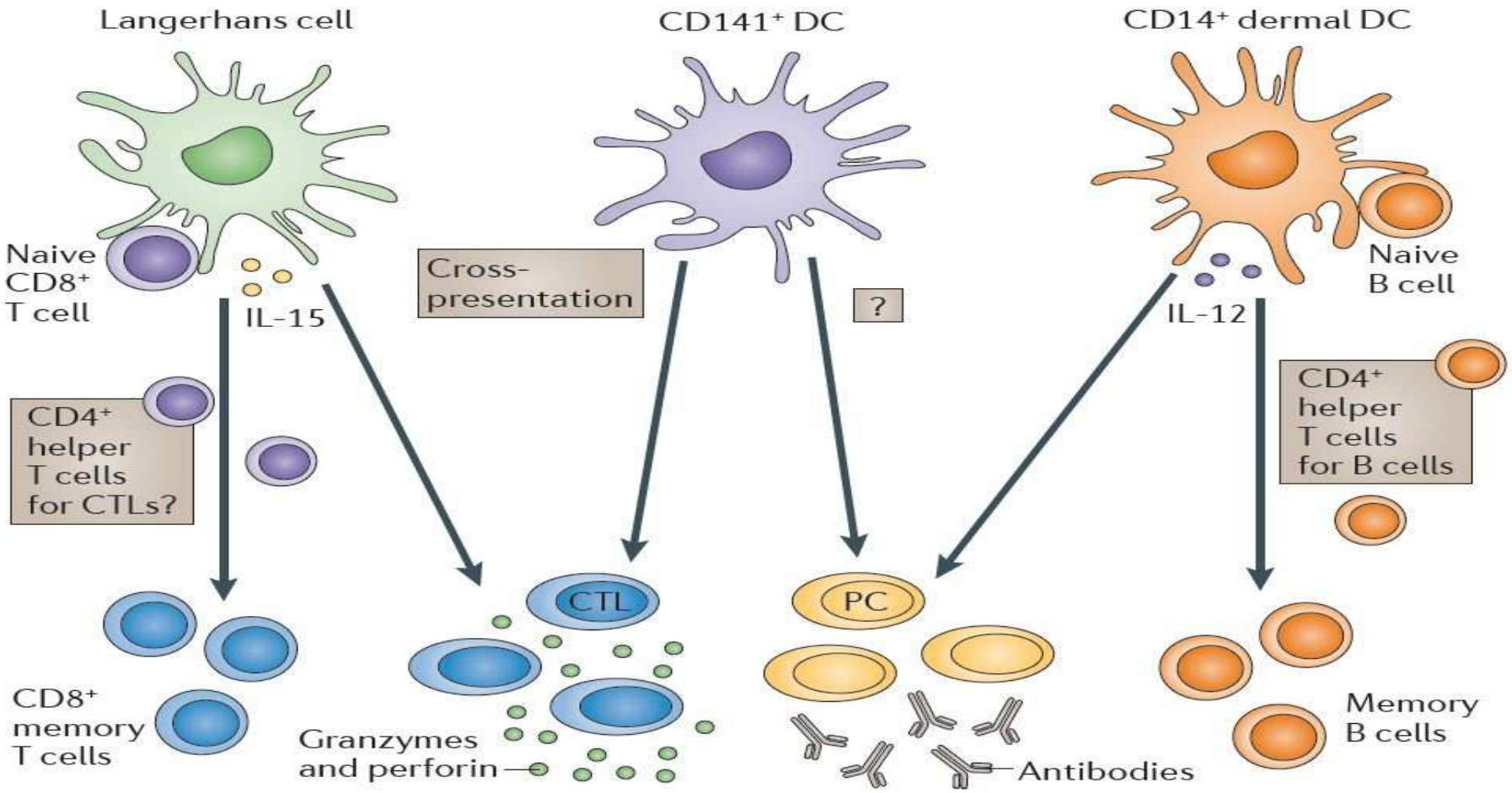
Immunity Cycle

www.melanomaantigens.com

Activation of the Immune Response by Dendritic Cells

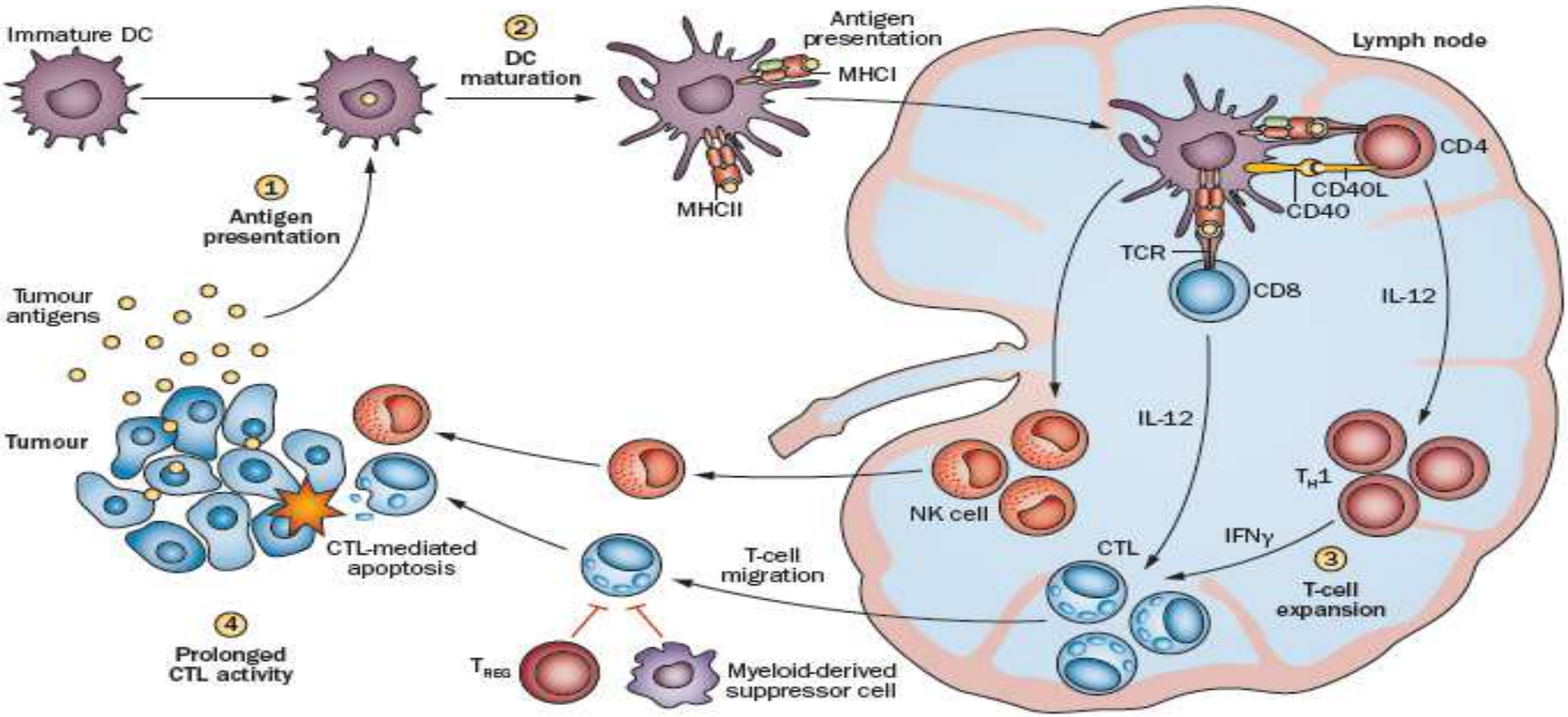


Subset of Dendritic Cells (DCs)

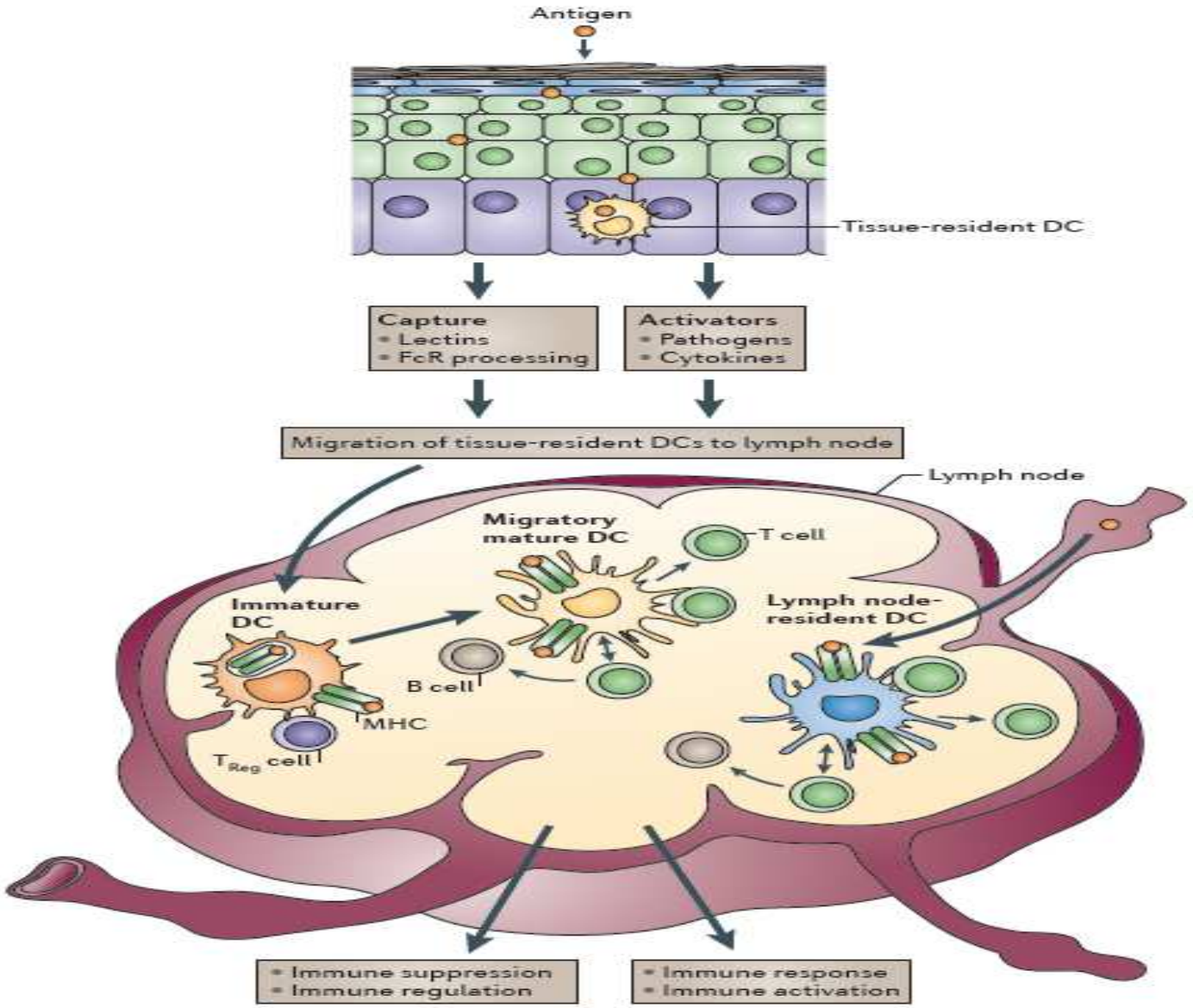


Nature Reviews Cancer 12, 265-277 (April 2012) | doi:10.1038/nrc3258

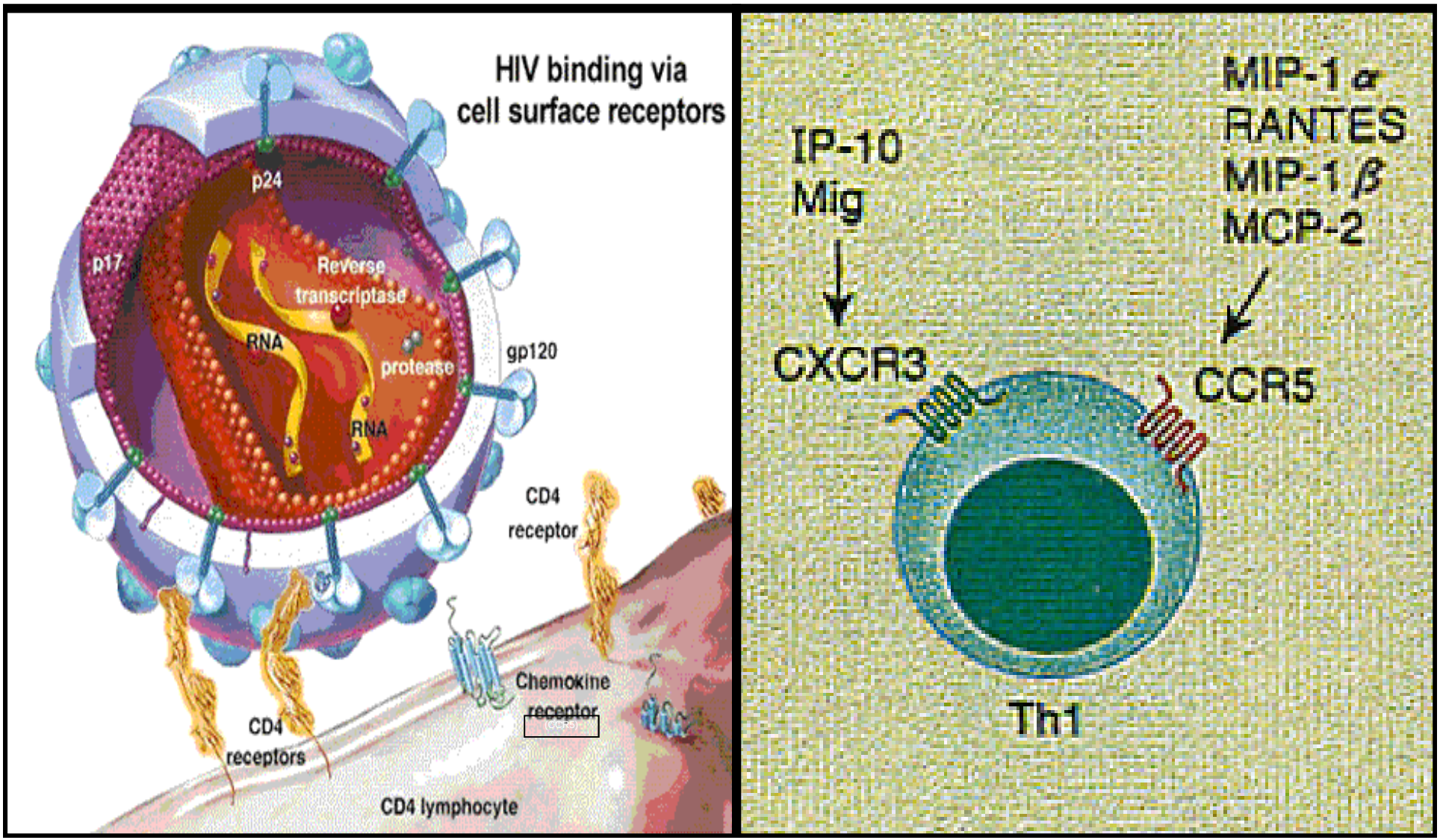
Stimulating an Immune System Response Against Tumor Antigen



Launching the Immune Response



Functional Aspects of Chemokine Receptor 5 (CCR5)



Immune Cell Types in Melanoma

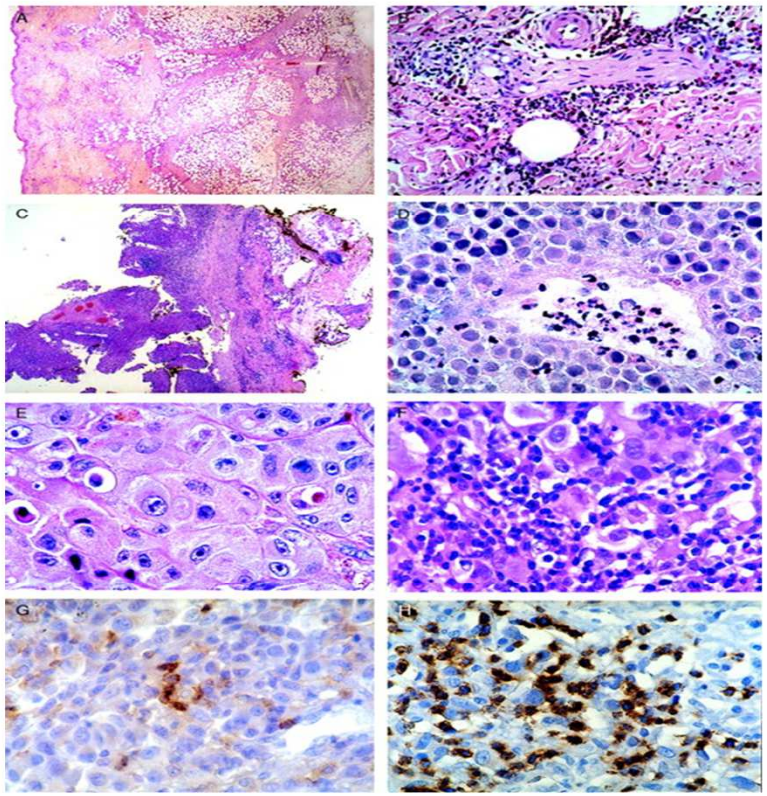
Table 1 Prevalence of immune cell types in sentinel vs. non-sentinel nodes and in positive vs. negative SLNs

Study (reference)	Tumor type	Number of patients (SLNs)	Number of + / - SLNs	Dendritic cells		Mature DCs		Plasmacytoid DCs		T cells		Regulatory T cells	
				SLN vs. NSLN	+ vs. - SLN	SLN vs. NSLN	+ vs. - SLN	SLN vs. NSLN	+ vs. - SLN	SLN vs. NSLN	+ vs. - SLN	SLN vs. NSLN	+ vs. - SLN
Compared to NSLNs of the same patient cohort													
Cochran et al. 2001 [3]	melanoma	11 (21)	10 / 11	lower	NT	NT	NT	NT	NT	NT	NT	NT	NT
Botella-Estrada et al. 2005 [4]	melanoma	10 (17)	1 / 16	higher	NT	no diff.	NT	NT	NT	NT	NT	NT	NT
Gerlini et al. 2007 [20]	melanoma	27 (39)	8 / 31	NT	NT	NT	NT	NT	higher	NT	NT	NT	NT
Speeckaert et al. 2011 [14]	melanoma	116 (116)	26 / 90	NT	NT	NT	NT	NT	NT	NT	NT	NT	higher
Ma et al. 2012 [15]	melanoma	84 (84)	31 / 53	NT	lower	NT	higher	NT	NT	NT	NT	NT	higher
Huang et al. 2000 [2][2]	breast cc.	21 (21)	not spec.	lower	NT	lower	NT	NT	NT	lower	NT	NT	NT
Kohrt et al. 2005 [7][2]	breast cc.	29 (29)	29 / 0	lower	NT	NT	NT	NT	NT	lower ($CD4^+$)	NT	NT	NT
Bembenek et al. 2008 [6]	breast cc.	79 (114)	51 / 28[3]	NT	NT	higher	NT	NT	NT	NT	NT	NT	NT
Ishigami et al. 2008 [11]	gastric cc.	27 (27)	8 / 19	no diff.	no diff.	NT	NT	NT	NT	no diff.	no diff.	NT	NT
Lee et al. 2011 [13]	gastric cc.	64 (64)	45 / 19[3]	NT	NT	NT	no diff.	NT	NT	NT	no diff.	NT	higher
Sakakura et al. 2005 [5]	oral cc.	12 (41)	0 / 41	higher	NT	no diff.	NT	NT	NT	NT	NT	NT	NT
Compared to non-tumor control nodes													
Mansfield et al. 2011 [10]	melanoma	20 (20)	8 / 12	NT	NT	lower	no diff.	no diff.	no diff.	lower ($CD8^+$)	no diff.	no diff.	no diff.
Poindexter et al. 2004 [12]	breast cc.	50 (50)	25 / 25	no diff.	no diff.	no diff.	lower	NT	NT	NT	NT	NT	NT
Mansfield et al. 2009, 2011 [8,9]	breast cc.	47 (47)	36 / 11	no diff.	no diff.	higher	lower	no diff.	no diff.	higher ($CD8^+$)	no diff.	no diff.	higher

Columns SLN vs. NSLN: comparison of negative SLNs to negative NSLNs or controls (except in studies [2] and [7]); columns + vs. - SLN: comparison of positive to negative SLNs.
[1]SLNs and NSLNs of unspecified status were compared; [2]positive SLNs were compared to mixed (+ and -) NSLNs; also compared to non-tumor controls, with similar results
[3]number of patients with + / - SLN status; NT: not tested; no diff.; no difference; not spec.: not specified.

Injection Site of Irradiated GM-CSF Secreting Melanoma Cells

(A) Injection site of irradiated GM-CSF secreting melanoma cells.



Robert Soiffer et al. PNAS 1998;95:13141-13146

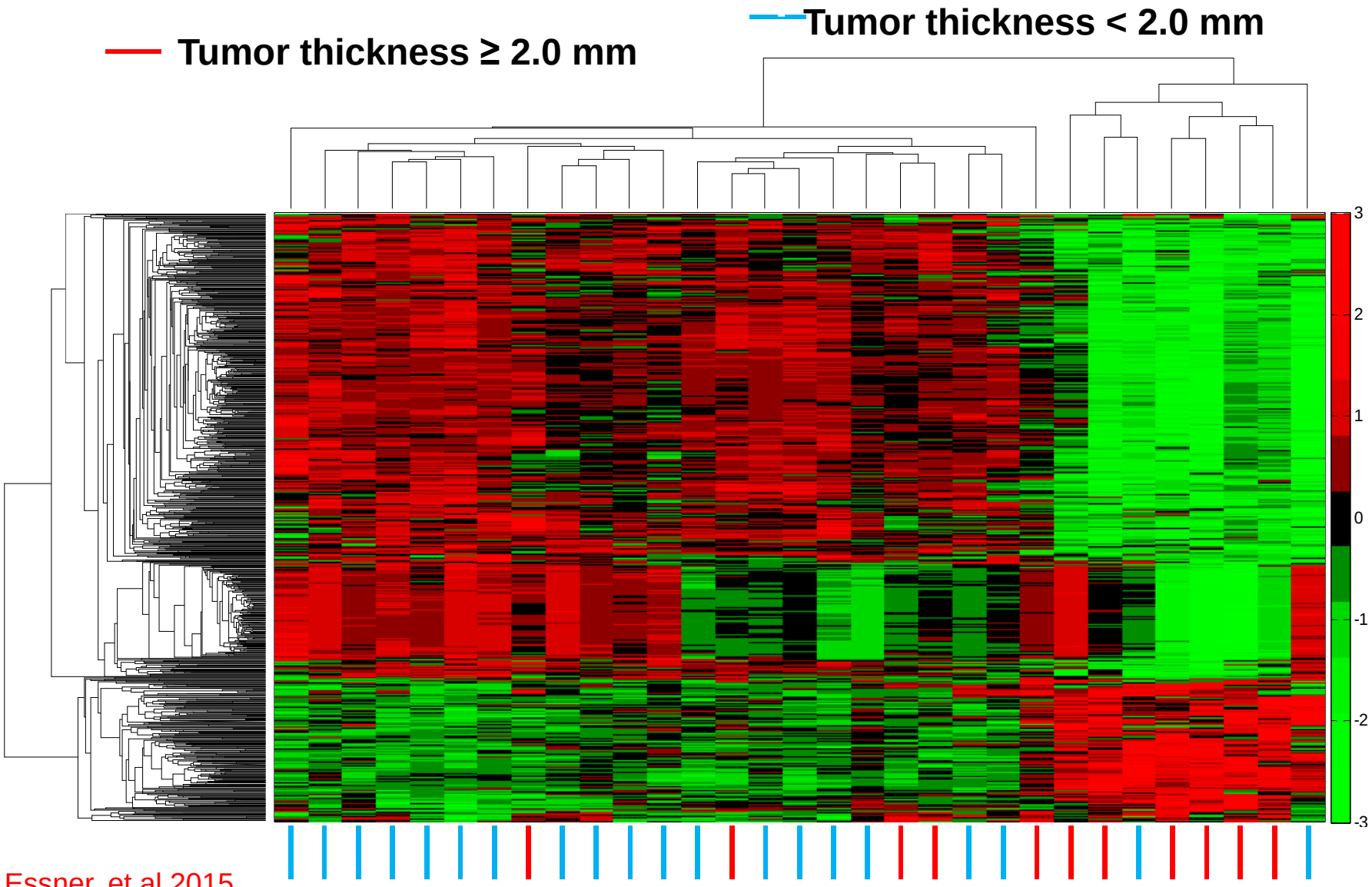
Antigens and Adjuvants

- Antigens are recognized by T cells and therefore act as targets for immunotherapy
- Adjuvants have proven useful as they direct cellular immune responses to these antigens
- Multiple adjuvants and/or antigens are generally used together in active immunotherapies in order to strengthen the immune response to the cancer

Antigen Approaches

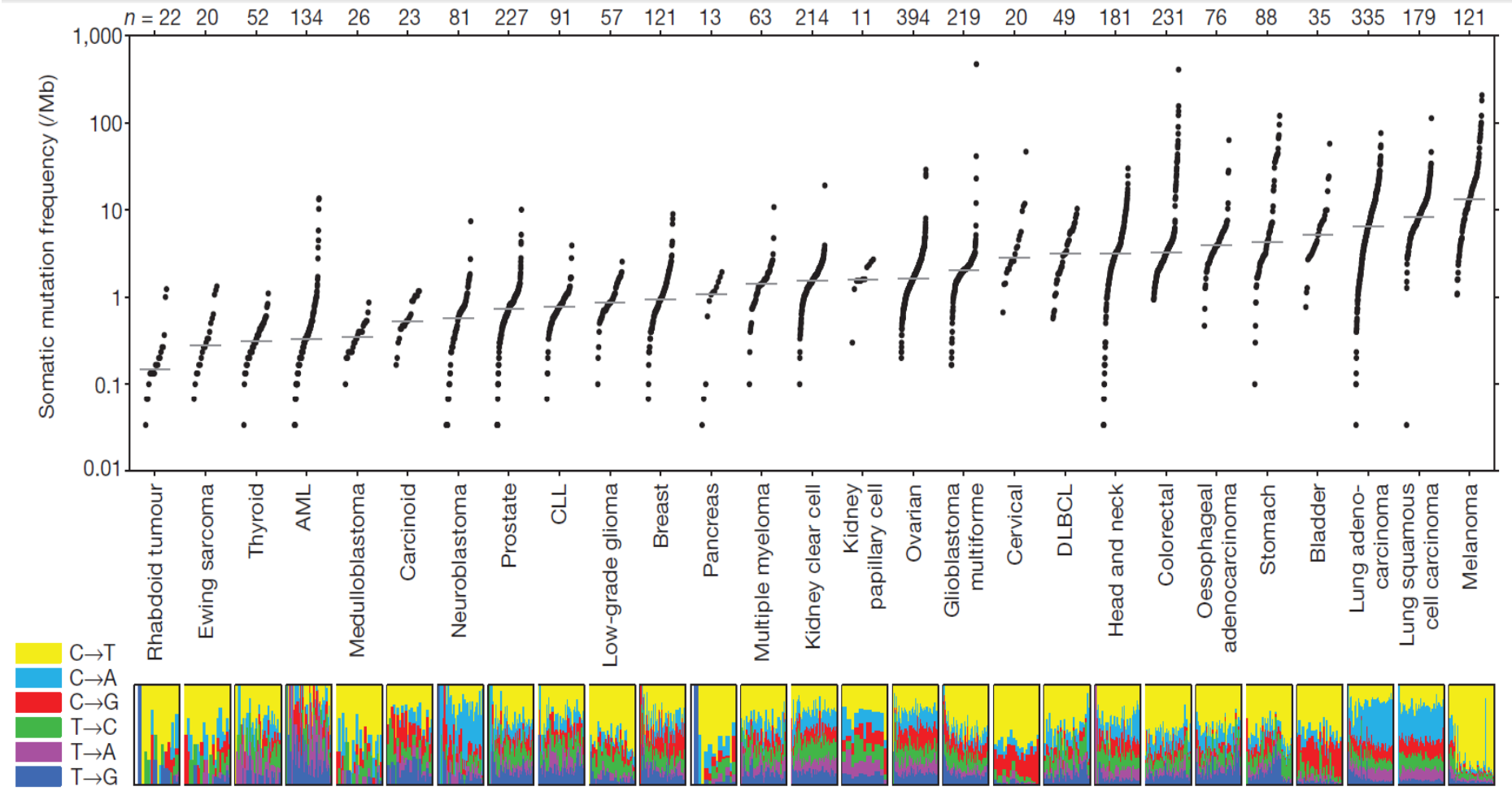
- Whole Cell
- Irradiated Cell Lysates
- Peptide
- Protein

Dendrogram of 981 differentially expressed genes



Essner, et al 2015

Somatic mutation frequencies from 3,083 tumor and normal tissue pairs



Lawrence et al. Nature 2013; 499

Antigens Presented by Cancer Cells

Antigen	Specific Types
Shared Antigens	
Cancer-testis antigens:	BAGE, GAGE, MAGE, NY-ESO-1
Differentiation antigens:	CEA, gp100, Melan-A, PSA, tyrosinase
Overexpressed antigens:	HER2, hTERT, p53, survivin
Unique Antigens	
Oncogene-associated antigens:	β-catenin-m, HSP70-2/m, KRAS
Shared antigens with unique mutations	
Glycans:	GM2, MUC1

Melero, I. et al. Nat. Rev. Clin. Oncol. 11, 509–524 (2014)

Tumor Expression Profile of Cancer-Germline Genes

Genes	Metastatic melanoma	Lung carcinoma	Colorectal carcinoma	Breast carcinoma	Prostate carcinoma	Refs
MAGEA1	46	46	0	19	18	69
MAGEA3	74	47	17	13	18	69
MAGEA4	25	51	11	6	0	69
MAGEA12	62	30	11	13	5	69
MAGEC2	43	11	0	15	1 of 10‡	46
BAGE1	31	10	0	12	0	69
GAGE1	41	38	0	10	15	69
XAGE1B	43	2 of 3‡	4 of 12‡			180
CTAG2	33	41	0	23	27	69
CTAG1	35	27	0	23	27	69
SSX2	50	0	26	19	25	181,182

BAGE1, B melanoma antigen 1; *CTAG*, cancer/testis antigen (*CTAG2* is also known as *LAGE1*; *CTAG1* is also known as *NYESO1*); *GAGE1*, G antigen 1; *MAGEA*, melanoma antigen family A; *SSX2*, synovial sarcoma X breakpoint 2; *XAGE1B*, X antigen family member 1B. *Percentage of tumours that express the gene.
‡The numbers of tested tumours are low, and the real numbers are shown.

Coulie. Nat Rev Ca 2014; 14: 135

Adjuvants for Cancer Vaccines

Adjuvant	Specific Types
Cytokines:	GM-CSF, IL-12
Microbes:	BCG, CpG, Detox, Lipid A
Mineral Salts:	Alum
Oil Emulsions or surfactants:	ASO_2, MF59, Montanide™, QS21
Particulates:	ASO_4
Viral Vectors:	Adenovirus, vaccinia, fowlpox, herpes

Melero, I. et al. Nat. Rev. Clin. Oncol. 11, 509–524 (2014)


NOVEMBER 25, 1985
$3.50
FORTUNE
SPECIAL REPORT
GORBACHEV VS.
THE SOVIET ECONOMY
CANCER
BREAKTHROUGH
NTERLEUKIN-2,
nyl, serine-125
For single use
sterile Water for
NANT INTERLEUKIN-2,
des-alanyl, serine-125
Lyophilized Preparation
2-8°C — For single use
with sterile Water for
Cetus Corp.'s
tumor-zapping
Interleukin-2

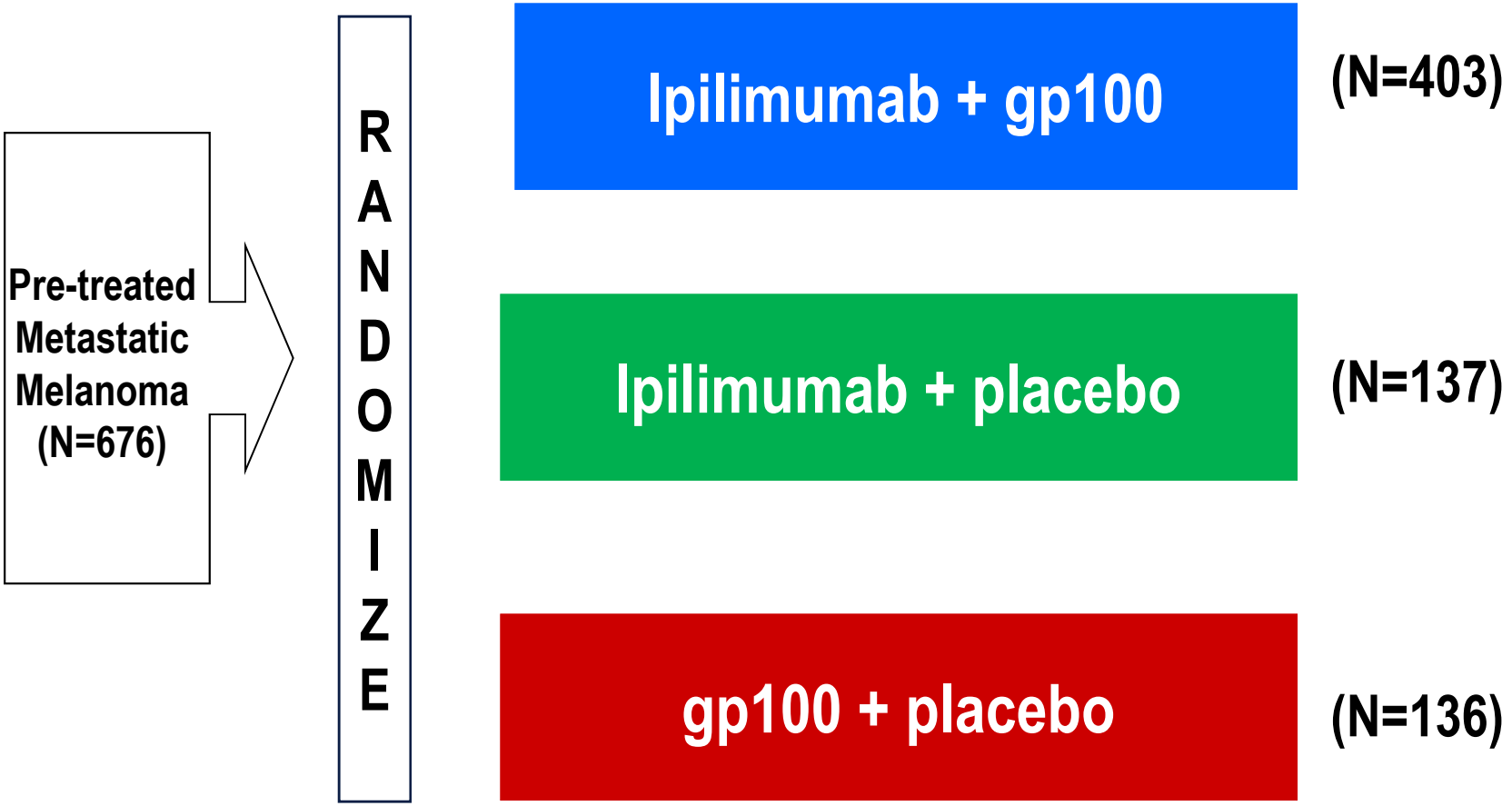
Immunotherapeutic Agents in Melanoma: IL-2

- Approved by the US FDA metastatic melanoma 600,000 IU/kg every 8 hours up to 14 doses x 2 cycles;
- Registration trial: single-agent IL-2 therapy
  - 270 pts; 8 trials; 22 institutions

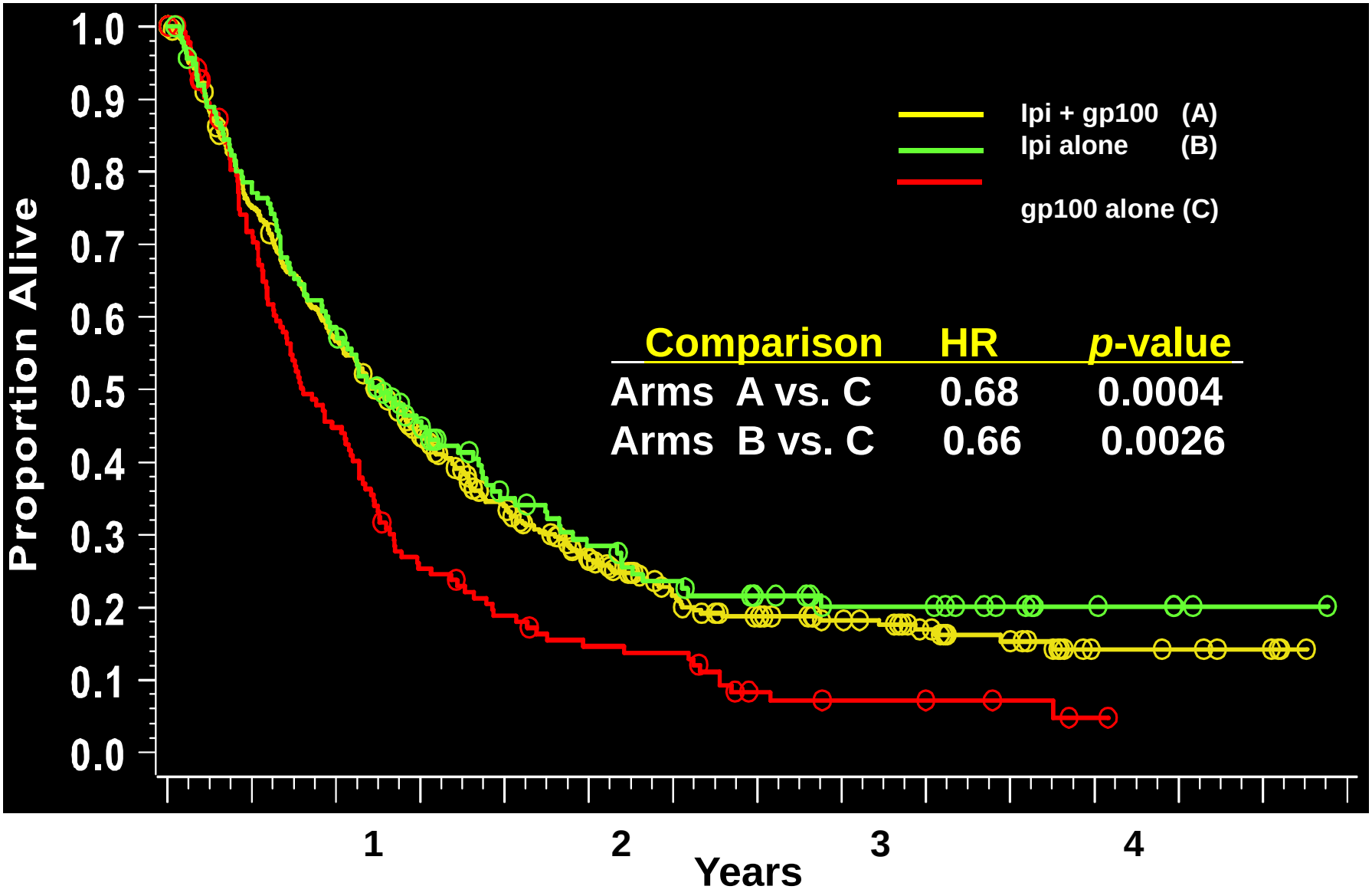
Response	RR (%)	DOR (mos)	Range of Response (mos)
ORR	16	9	1.5-106.2
CR	**6**	**>40**	**2.5-106.2**
PR	10	6	1.5-91.5

Atkins. J Clin Oncol. 1999;17:2105;
Agarwala. Expert Rev Anticancer Ther. 2009;9:587.

MDX010-20: Study Design for Ipilimumab



Kaplan-Meier Analysis of Survival



Ipilimumab Improves Best Objective Response Rate

	Ipi + gp100	Ipi + pbo	gp100 + pbo
BORR, %	5.7	10.9	1.5
P-value: B/C	0.0012		
DCR‡, %	20.1	28.5	11.0
P-value: B/C	0.0002		

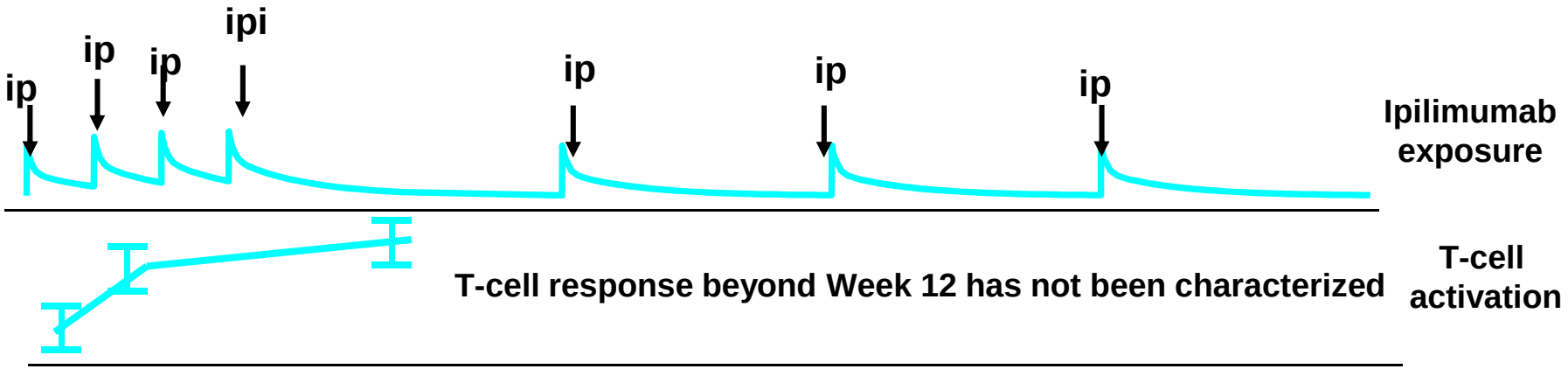
Clinical Activity of BMS-936558 in Melanoma Patients

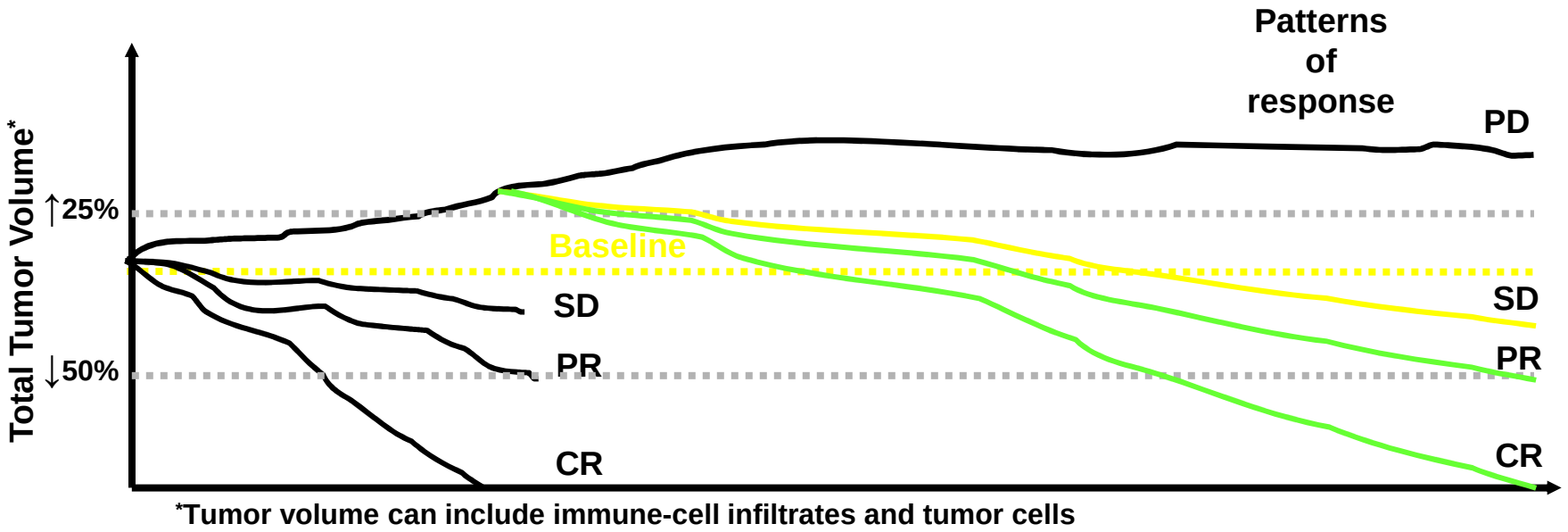
Pop	Dose (mg/kg)	Pts n	ORR n (%)	Duration of Response (mo)	SD ≥24 wk n (%)	PFSR at 24 wk (%)
MEL	0.1	14	4 (29)	5.6 to 7.5+	1 (7)	40
	0.3	16	3 (19)	1.9+ to 3.8+	1 (6)	31
	1	27	8 (30)	5.3+ to 24.9+	3 (11)	45
	3	17	7 (41)	9.2+ to 22.4+	1 (6)	55
	10	20	4 (20)	17.0 to 24.6+	0	30

- ORR was assessed using modified RECIST v1.0.
- 3 melanoma patients had a persistent reduction in baseline target lesions in the presence of new lesions but were not classified as responders for the ORR calculation

Hodi FS et al. 2012 ASCO Annual Meeting. Abstract 8507.

Immunotherapy: Evolution of Anticancer Effect





Immune-cell activation and proliferation begins early

Measurable clinical effect occurs at variable time points

Kinetics of Response

- **HD IL-2**
  - Rapid
  - CR or Bust
  - Acute Toxicity then Resolution

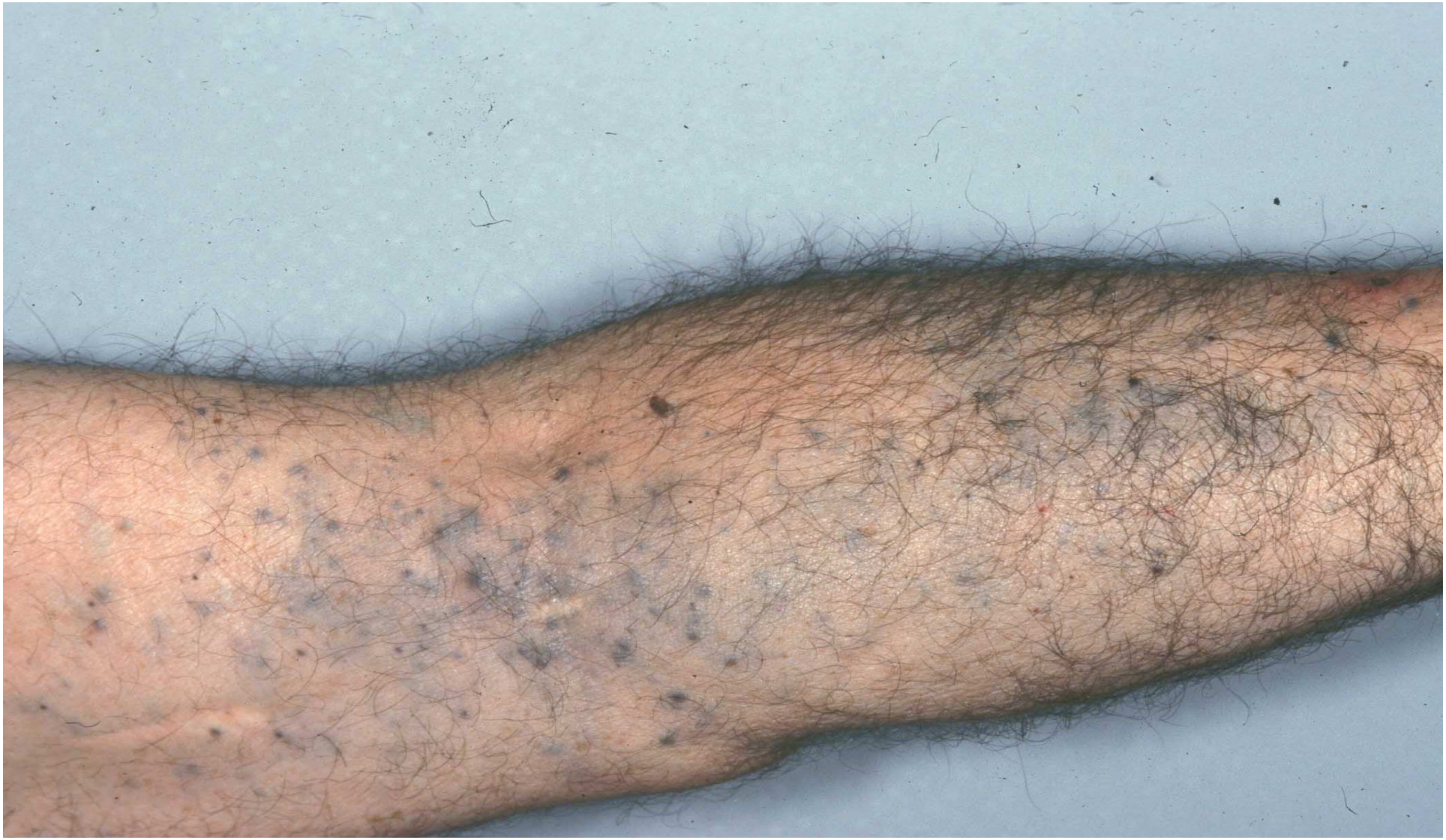
- **Ipilimumab**
  - Variable, Peak 3-6 months
  - CR rare, PR/SD/MR/MP Durable
  - Progression followed by Response
  - Toxicity Sub-acute then Resolution

Immune Checkpoint Inhibitors

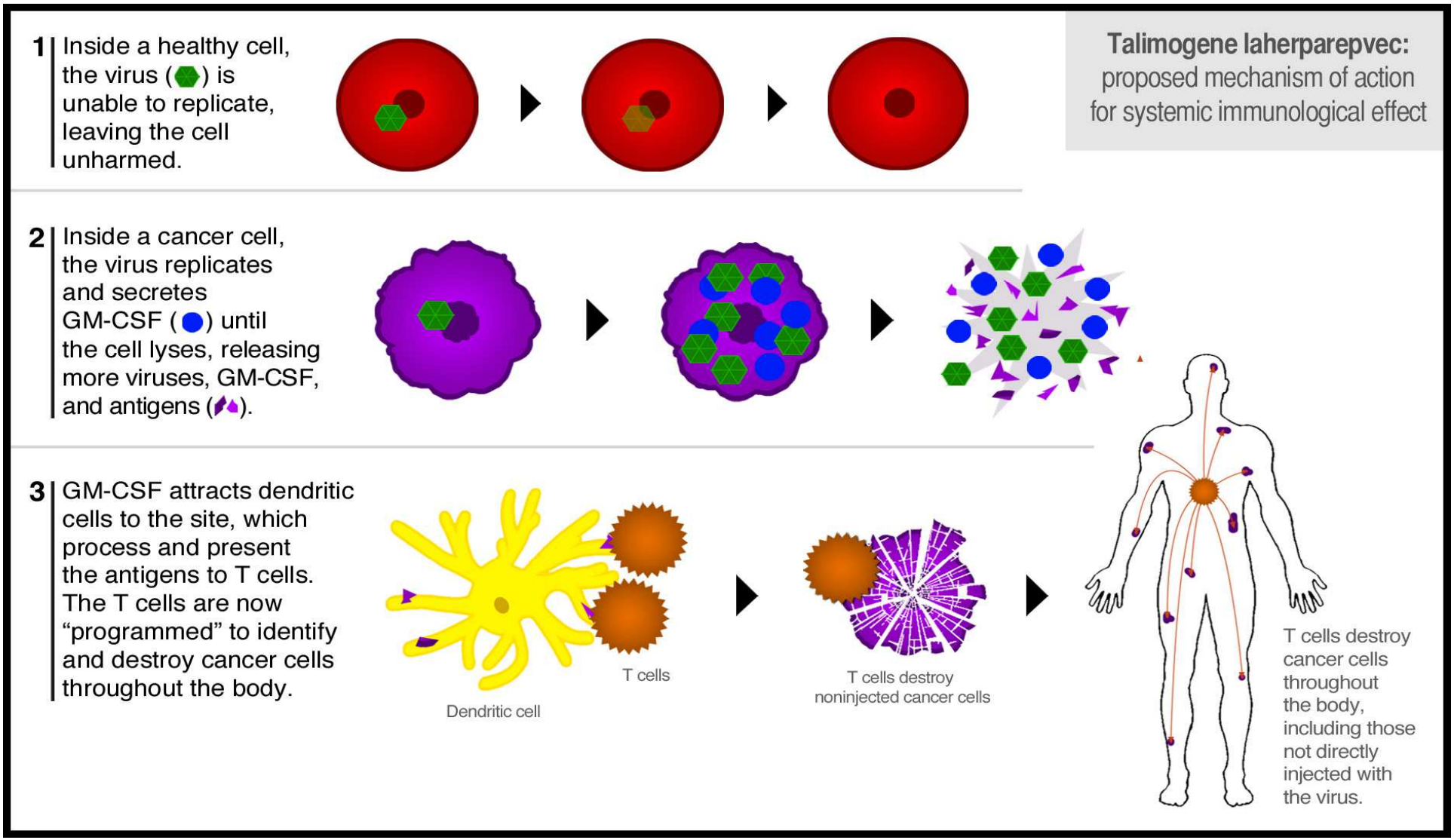
Inhibitor	Dose	N	6mPFS	RR	CR
Ipilimumab	3mg/kg	278	26	11.9	1.4
Pembrolizumab	10mg/kg (for 2 weeks)	279	47	33.7	6.1
	10mg/kg (for 3 weeks)	227	46	32.9	5

Robert. New England Journal of Medicine. 2015

Dermal Metastases—A Function of Diminished Primary Margins?—How do we treat?

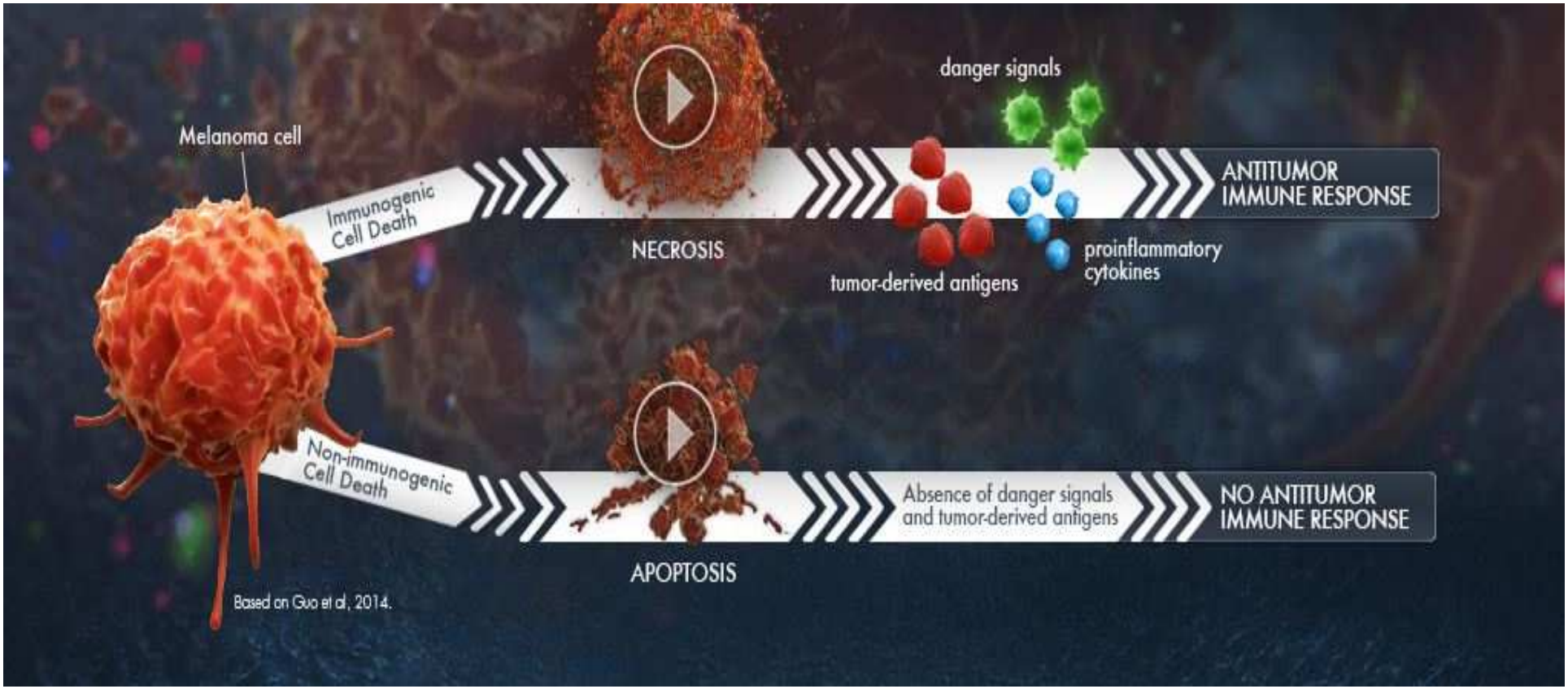


Oncolytic Virus Immunotherapy—Talimogene laherparepvec (T-Vec, Amgen)



Antigen Release: The Catalyst in Guiding the Immune System to Melanoma

- Tumor cell necrosis (a form of immunologic cell death) releases tumor-derived antigens (TDA)—the catalyst in guiding the immune system to melanoma



www.melanomaantigens.com

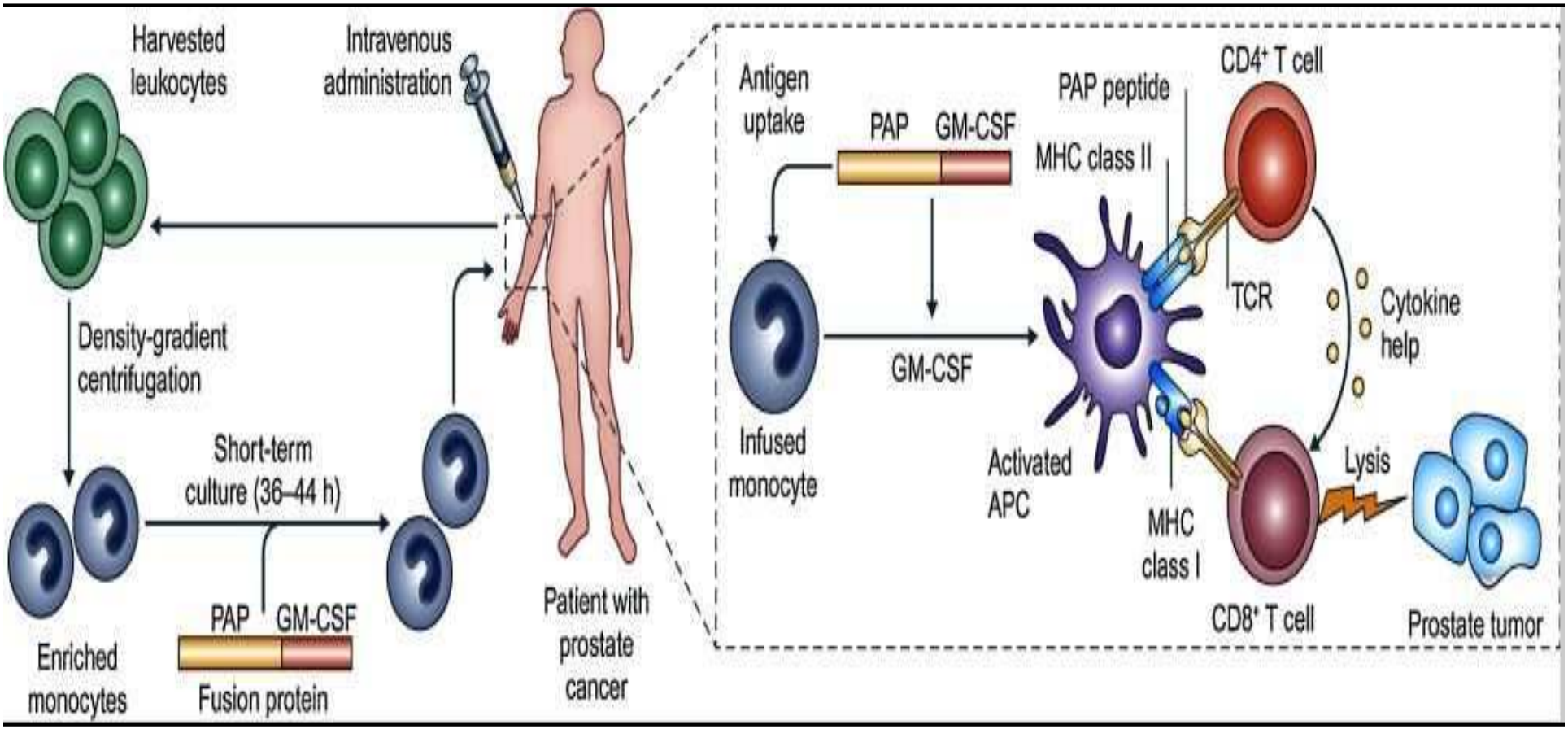
T-Vec Improves Durable Response Rate in Patients w/ Advanced Melanoma

- T-Vec is a herpes simplex virus type 1—derived oncolytic immunotherapy
- Its functions are carried out through replicating w/in tumor cells and producing GM-CSF to augment whole-body immune responses
- T-Vec is the first oncolytic immonotherapy to show a therapeutic advantage in combating melanoma in a phase III clinical trial.

	DRR	ORR	Median OS
T-VEC	16.3%	26.4%	23.3 months
GM-CSF	2.1%	5.7%	18.9 months

DOI: 10.1200/JCO.2014.58.3377

Sipuleucel-T (Provenge, Dendreon Corp) Immunotherapy and Proposed Mode of Action



Cancer Immunol Immunother. 2015; 64(6): 655–663. Published online 2015 May 30. doi: [10.1007/s00262-015-1707-3](10.1007/s00262-015-1707-3)

Evading Immunosurvellance

- As melanoma metastasizes, it develops genetic mutations, which result in continual presentation of evolving tumor-derived antigens (TDA)→Loss of recognition ability by the host's immune system→T-Cell activation ceases to occur

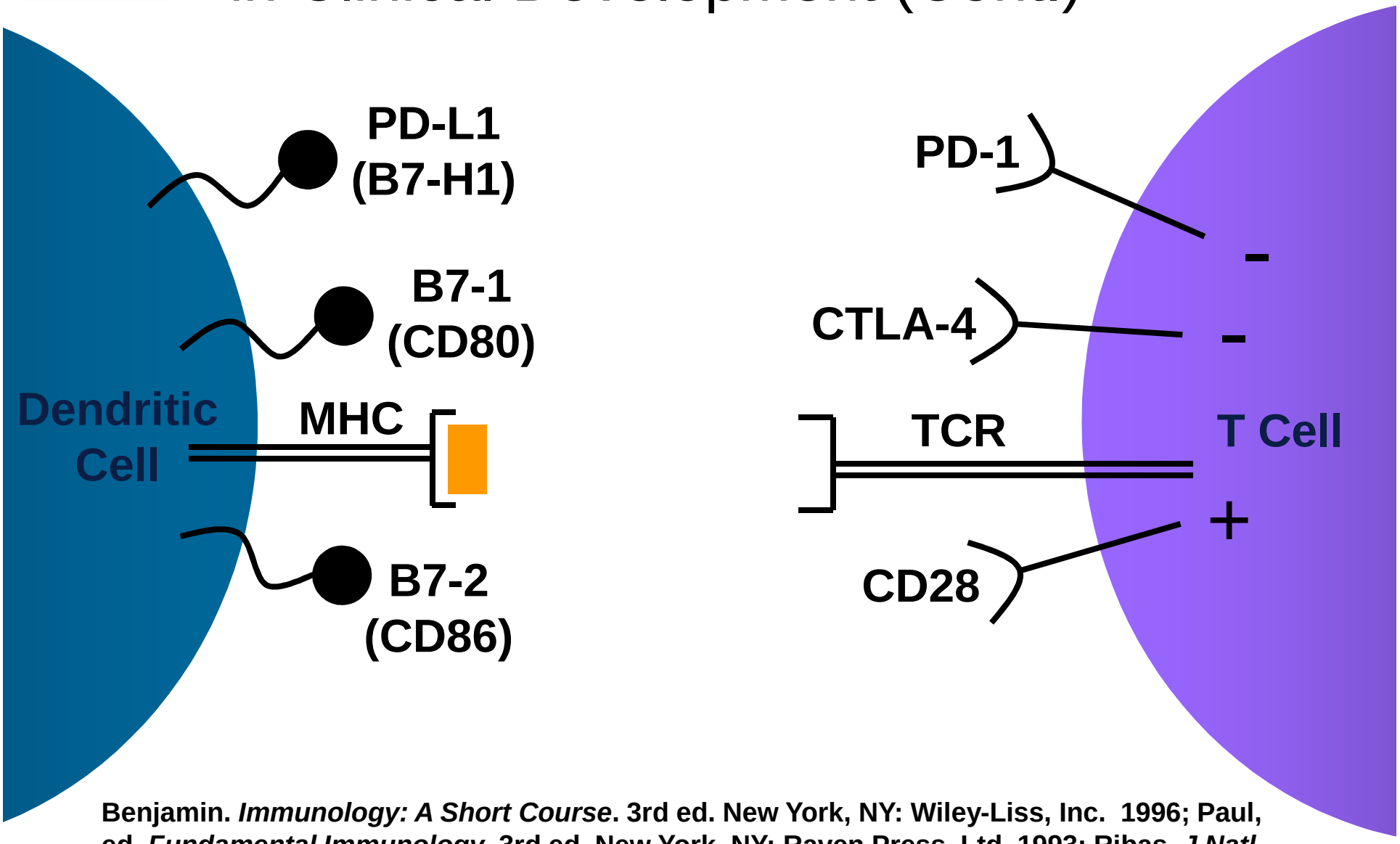
- We have revealed a molecular profile of melanoma that supports the presence of a tumor microenvironment that is immunosuppressive
  - →inducing the loss of function of antigen presenting cells (APCs/Dendritic cells)
  - →inducing a loss of T-Cell function (activation, differentiation, and migration into tumor cells)

- These mechanisms among others prevent melanoma cells from undergoing immunologic cell death

Organ Sites—Immune Related Toxicities

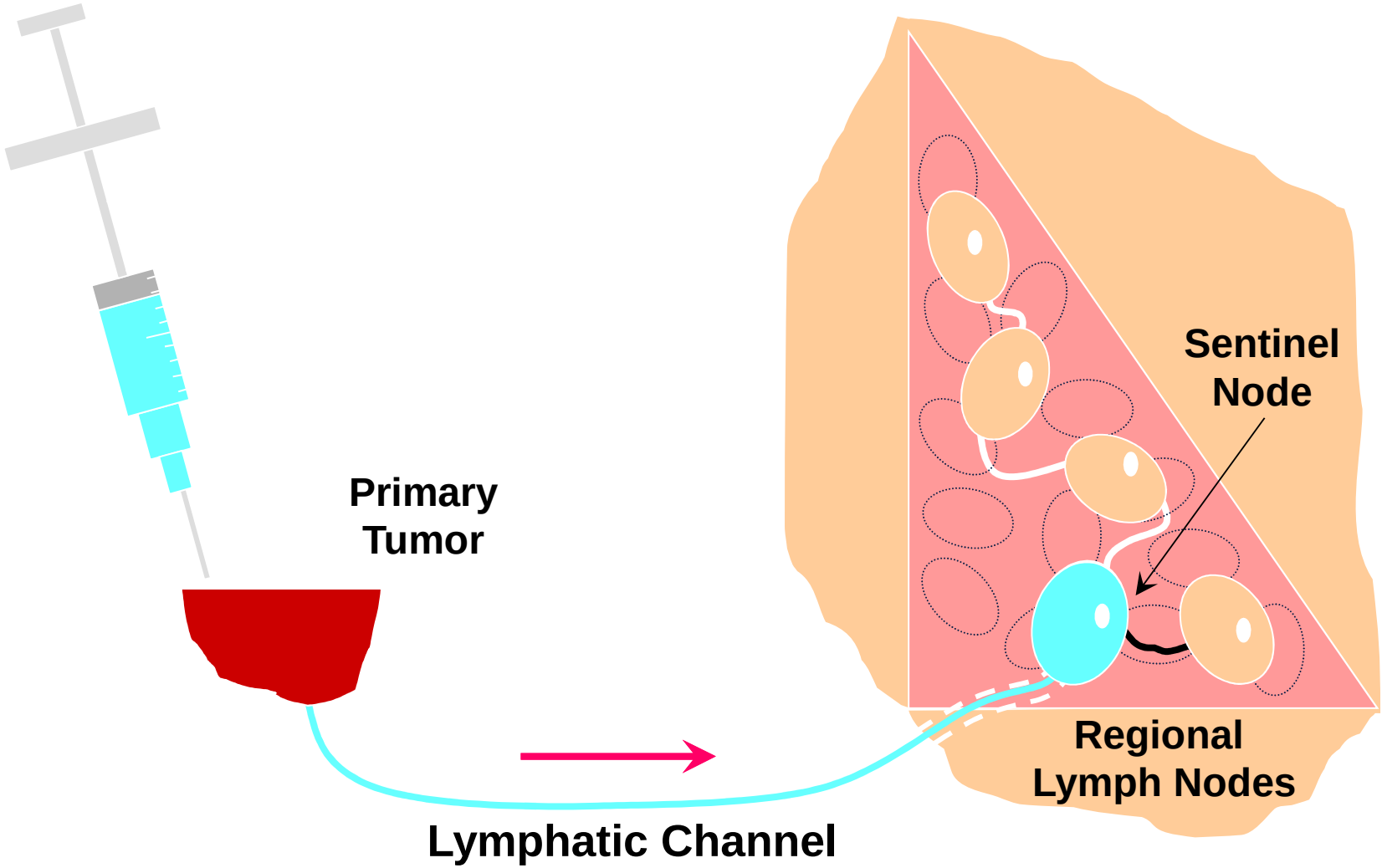
- Skin
- Gastrointestinal Tract
- Pituitary
- Liver
- Lung

Dendritic Cell/T-Cell–Activating Therapies in Clinical Development (Cont.)



Benjamin. *Immunology: A Short Course*. 3rd ed. New York, NY: Wiley-Liss, Inc. 1996; Paul, ed. *Fundamental Immunology*. 3rd ed. New York, NY: Raven Press, Ltd. 1993; Ribas. *J Natl Compr Canc Netw*. 2006;4:687.

Sentinel Node Technique



Cytokine Profiling of Sentinel Lymph Nodes by qRT-PCR

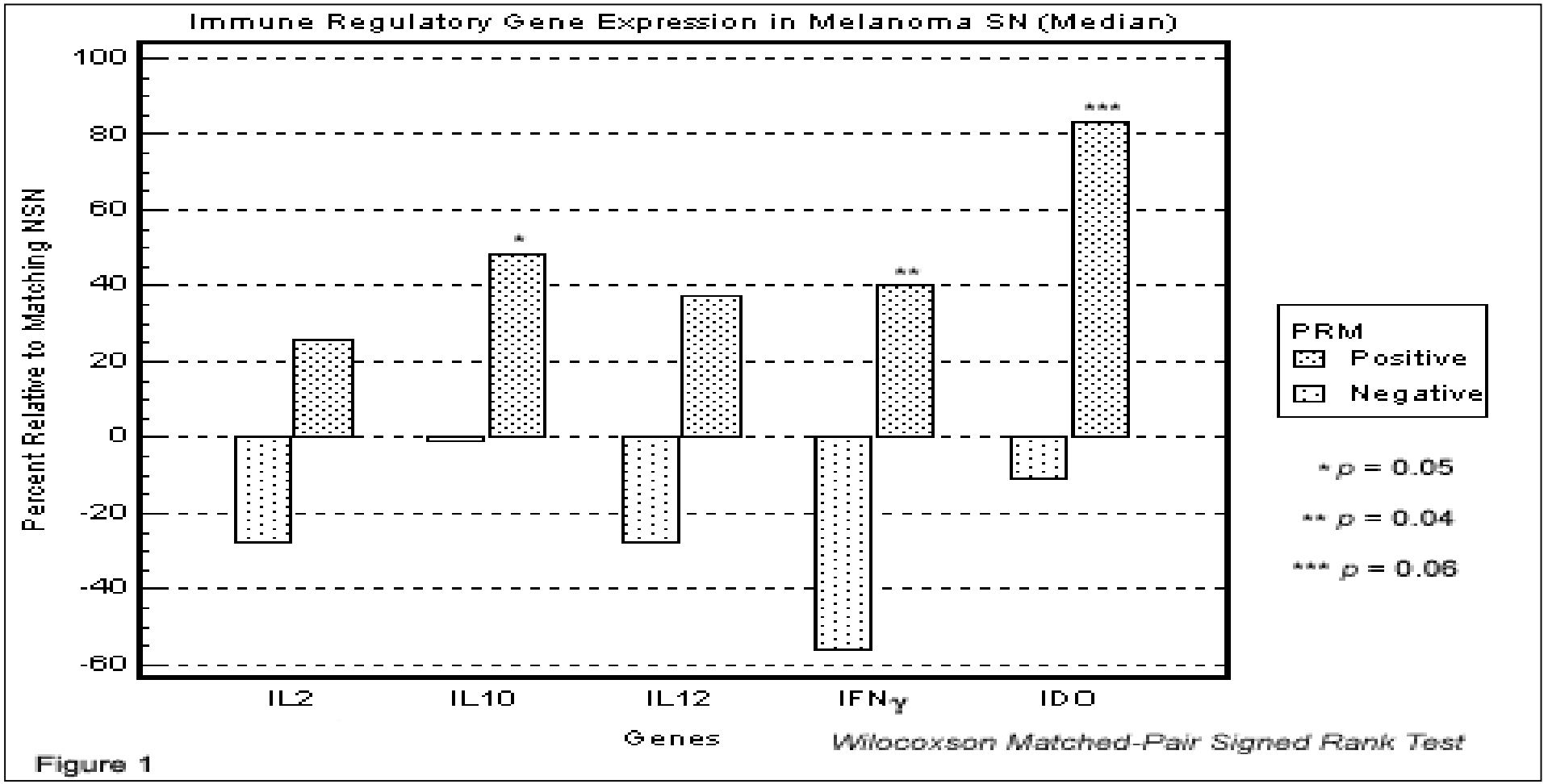
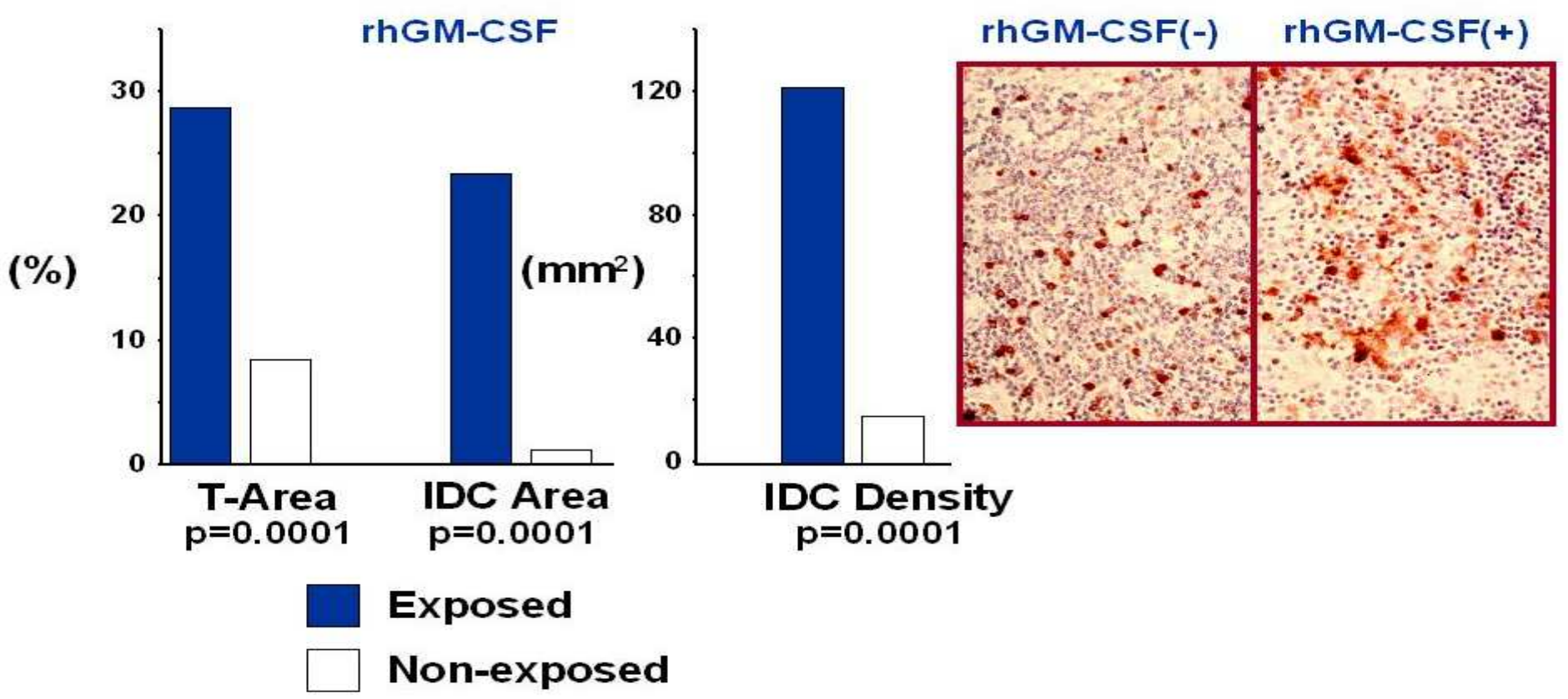


Figure 1

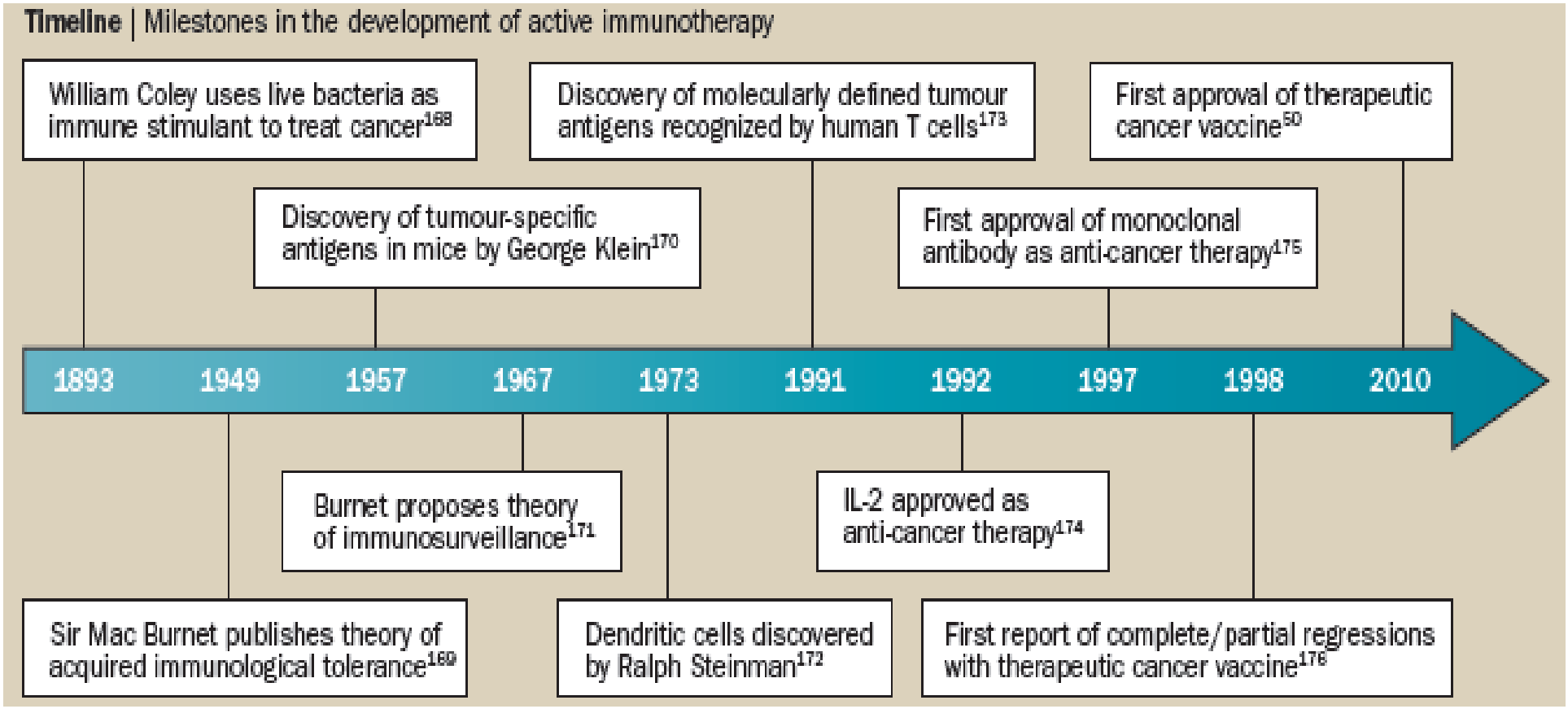
Lee JH. Clin Ca Res 2005;11: 107

Alterations in Sentinel Node Morphology With intradermal Administered GM-CSF



Lee JH. Clin Ca Res 2005;11: 107

Historical Context of Immunotherapy



Clinical Scenarios

- **High Tumor Burden BRAF Mutated (Rapid Growth, Symptomatic)**
  - Vemurafinib
  - What do you do at maximal response?
  - How do you incorporate Ipilimubab?
  - How does CNS disease factor in?

- **Low Tumor Burden BRAF WT**
  - Ipilimumad, then anti-PD1
  - Surgery
  - IL-2
  - Adoptive T-Cell Therapy
  - How does CNS disease factor in?

Lessons and Take Home Messages

- Cancer immunotherapy attempts to harness the strength and specificity of the immune system to combat tumor cells

- With a further understanding of the molecular and cellular bases of T-cell-mediated anti-tumor responses, we can employ the methods of **Active** immunotherapy to treat progressive cancer

- Focusing on antigens with limited expression (i.e. tumor associated antigens, TAA) should potentiate the evolution of increasingly potent therapies with enhanced response rates

- Prospective studies should aim to refine active immunotherapy, by studying and altering the tumor's microenvironment in order to perfect tumor-specific immune responses