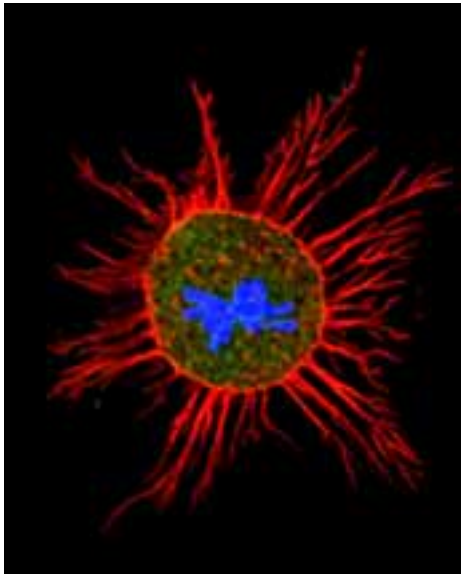


iSBTc Workshop on Cancer and Inflammation: Promise for Biological Therapy October 30, 2008

Michael T. Lotze, MD
Vice Chair Research
Department of Surgery
University of Pittsburgh Cancer Institute
Asst. Vice Chancellor, UPSHS

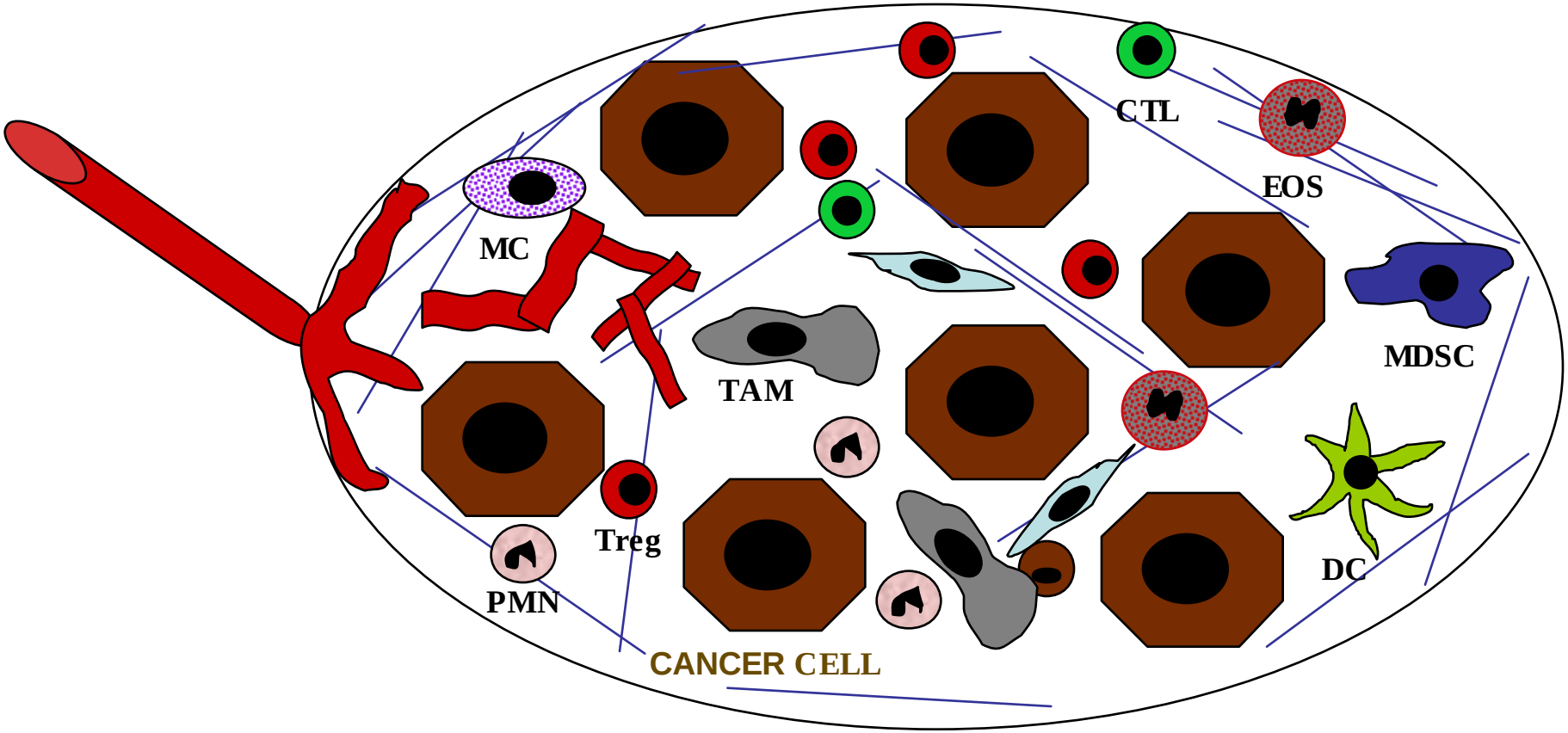
Chronic Inflammatory Conditions Associated with Cancer

Chronic inflammation	Associated cancer	Aetiological agent	Percent predisposed that progress to cancer
Bronchitis	Lung cancer	Tobacco smoke	11–24
Gastritis	Gastric cancer	*Helicobacter pylori*	1–3
Cervicitis	Cervical cancer	Human papillomavirus	<1
Warts	Non-melanoma skin cancer	Ultraviolet light, human papillomavirus	Varies with skin pigment and solar intensity
Asbestosis	Mesothelioma	Asbestos fibres	10–15
Inflammatory bowel disease	Colorectal cancer	Gut pathogens, altered gut permeability	1*
Pancreatitis	Pancreatic cancer	Tobacco, genetic factors	≤10%‡
Oesophagitis	Oesophageal cancer	Gastric acid, alcohol, tobacco	15
Sunburned skin	Melanoma, basal-cell carcinoma, squamous-cell carcinoma	Ultraviolet light	Varies with skin pigment and solar intensity, ≤9% of Caucasians
Hepatitis	Hepatocellular carcinoma	Hepatitis B virus, hepatitis C virus	10
Mononucleosis	Burkitt's lymphoma, Hodgkin's disease	Epstein–Barr virus	<1
Cholecystitis	Gall bladder cancer	Bacteria, gall bladder stones	1–2§
Cystitis	Bladder cancer	Gram-negative uropathogens, pelvic irradiation, carcinogens	<1

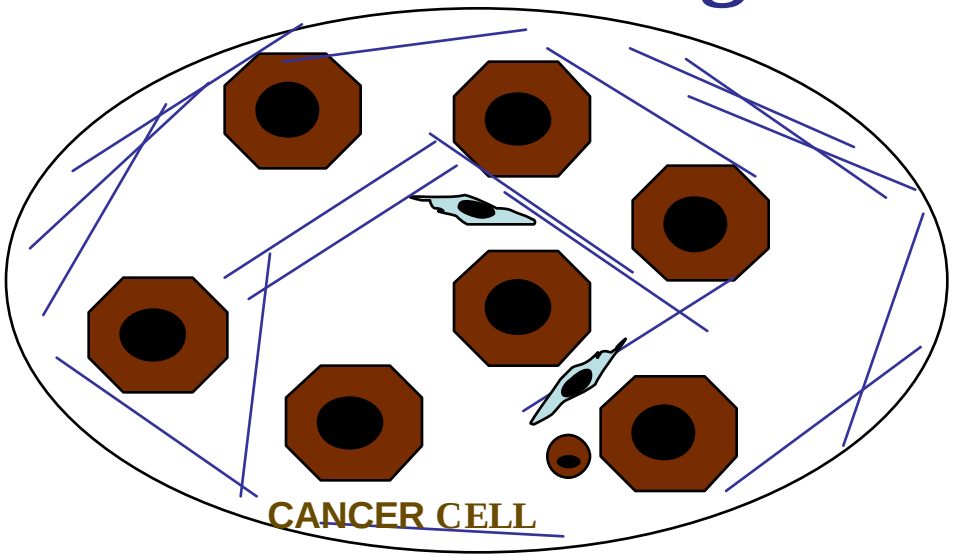
*Per year. ‡In susceptible populations. §At cholecystectomy.

Vakkila J, Lotze MT. Inflammation and necrosis promote tumour growth. *Nature Reviews Immunology* 4:641-647, 2004.

Inflammatory cells are present in all tumors



Current Focus Of Pathologic Evaluation

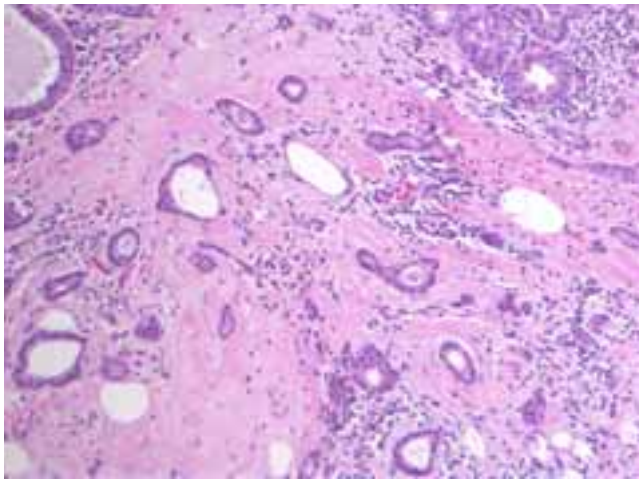


NEOPLASTIC CELLS:

Histological differentiation (architecture, nuclear grade, mitotic rate)

Biomarkers (e.g., hormone, growth factor receptors)

Gene expression profiling



STROMA:

"Desmoplastic stroma"

Presence of necrosis

Lympho/vascular invasion

Depth/extent of invasion

S. Demaria

Cancer Necrosis Correlates with Poor Prognosis

- Mesothelioma *(Edwards, 2003) p=0.008*
- Renal-clear cell carcinoma *(Cheville 2003; Tollefson 2007) p<.001*
- Colon carcinoma *(Hunter, 1983)*
- NSCLC *(Swinson, 2003) p=0.0016*
- Breast *(Gilchrist, 2003) p=0.0003; Kato, 2002) p=0.0068*
- Mucosal melanoma *(Prasod, 2002) p=0.007*
- Melanoma *(Balch, 2001)*
- Sarcoma *(Miyajima 2002; Gustafson 2003)*
- Transitional Urothelial Carcinoma (*Sang Eun Lee, 2007*)

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.

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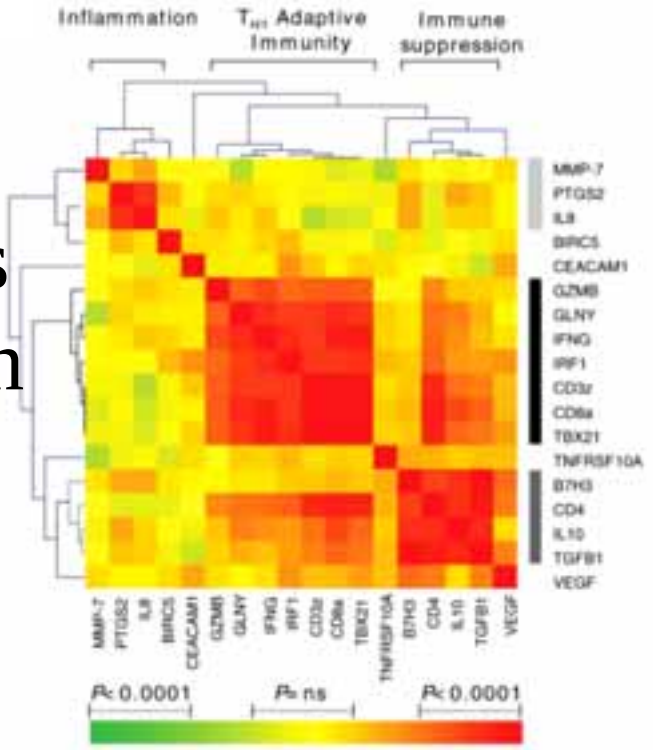


Coukos Ovarian Cancer

Tumor histopathology

UICC-TNM Staging system

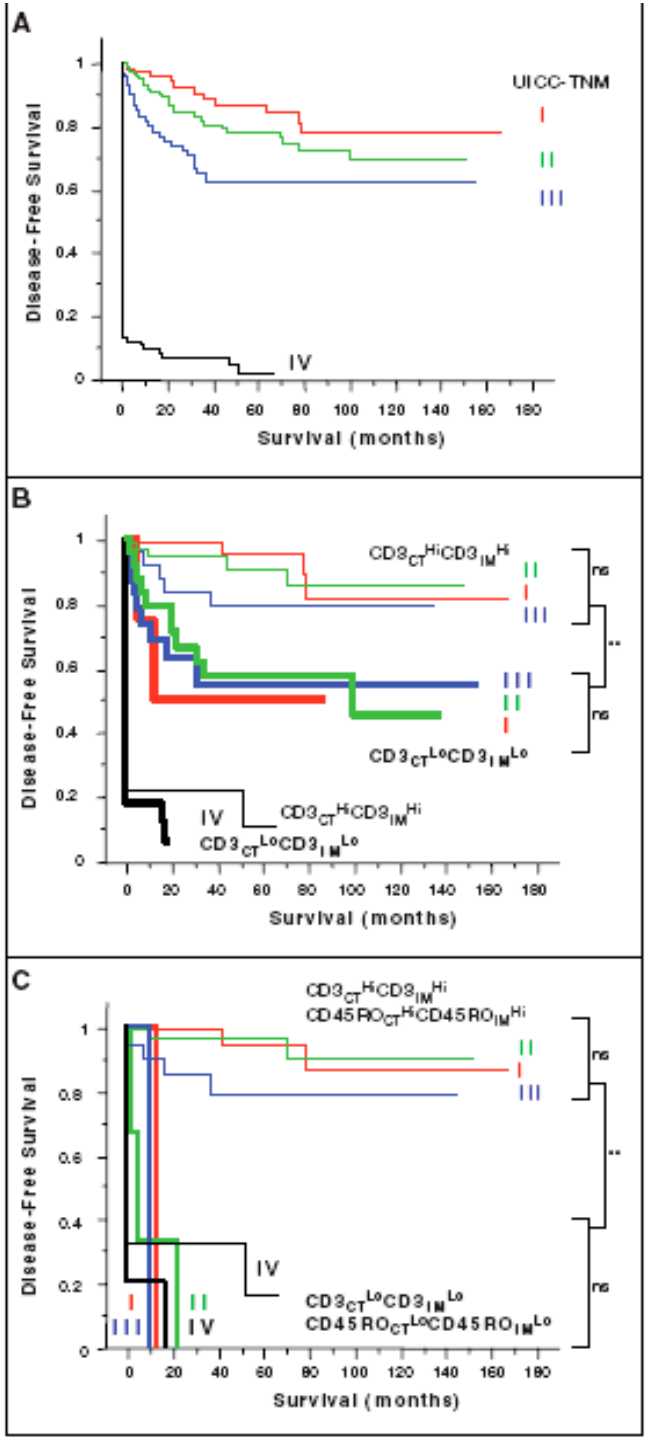
Tumor infiltrating immune cells

$CD3_{CT}CD3_{IM}$ evaluation

$CD3_{CT}CD3_{IM}$ evaluation

plus

$CD45RO_{CT}CD45RO_{IM}$ evaluation



State of the Science Sessions

- **Defining Inflammation**

  Michele Carbone, MD, PhD, *CRC of Hawaii*

  Sandra Demaria, MD, *New York University*

- **Genetic Polymorphisms and Factors which Modulate Inflammation and Cancer**

  Emad M. El-Omar, MB ChB, MD, *U. Aberdeen*

  Yen-Ching Karen Chen, ScD, SM, *National Taiwan University*

- **Animal Models of Cancer and Inflammation**

  Lisa M. Coussens, PhD, *UCSF*

  Michael Karin, PhD, *UCSD*

  *[*Eli Pikarsky, PhD *Hadassah]*

State of the Science Sessions

- **Causes and Molecular Targets in Cancer and Inflammation**

  Michael T. Lotze, MD, *UPCI*

  Giorgio Trinchieri, MD, *NCI*

- **Current Clinical Evidence for Targeting Inflammation to Prevent Cancer**

  Steven Dubinett, MD, *UCLA*

  Eva Szabo, MD, *NCI, NIH [*Jenny T. Mao]

- **Novel Therapeutics and Clinical Trial Development to Rx Cancer**

  George J. Weiner, MD, *University of Iowa*

  Arthur M. Krieg, MD, *Pfizer*

Defining Inflammation

1- Macrophages: how to differentiate TAM pro- and anti-tumor activities? Location within the tumor and/or markers?

2- MDSC: validate the use of IL-4Ra/? in human blood PBMC from cancer patients as a marker of immunosuppressive myeloid cells. How to evaluate the effects of pharmacological targeting of MDSC suppressive mechanisms in human tumors?

Defining Inflammation

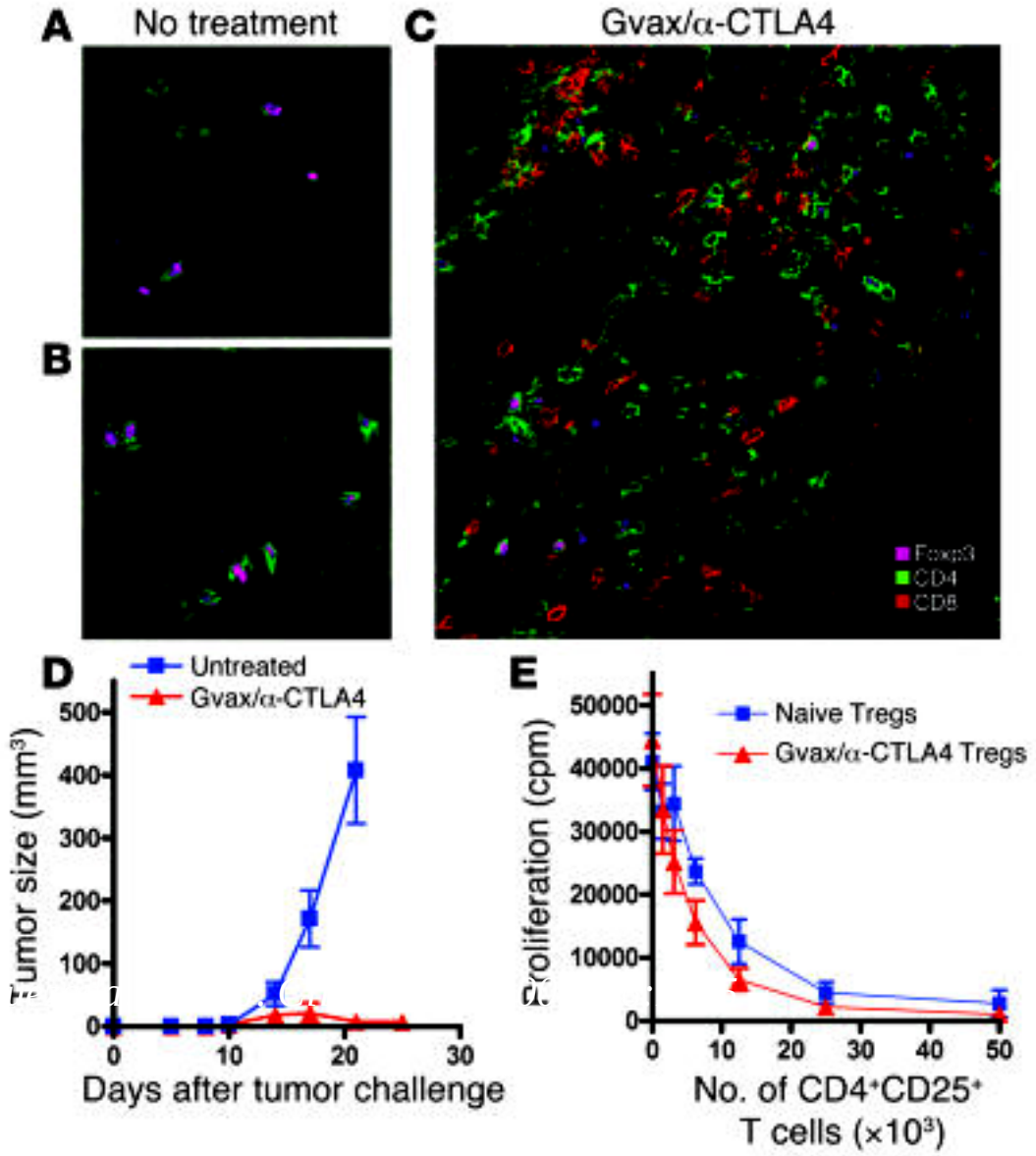
3- Mast cells: how to differentiate activities? Location?; degranulation (tryptase, heparin)?

4- Eosinophils: how to define their role in cancer? Markers of function? Location?

5- DCs: DC-LAMP and CD83; IL-13 and/or pSTAT6?

6- TILs: CD4/CD8, granzymeB, and FoxP3 to obtain a more comprehensive and reliable prognostic indicator? Ready for prime time in colorectal, ovarian cancers and HCC? hepatocellular? Others? Role of Th17?

The T-cell ratio: pre-clinical data



Mouse melanoma model:

intratumoral ratio of Teff/Treg determines tumor rejection

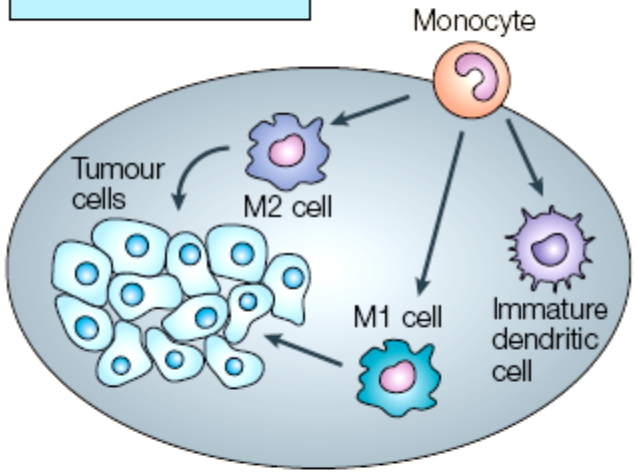
Defining Inflammation

7- Lymph Nodes: Should sentinel lymph nodes (SLN) be analyzed immunologically?

8- Methods for evaluation of prognostic/predictive parameters: Oncotype DX equivalent for “immunological signature” of a tumor? Do morphology and IHC provide additional/different information? Predictor of response to immunotherapy?

Tumor Infiltrating Macrophages-MDSC

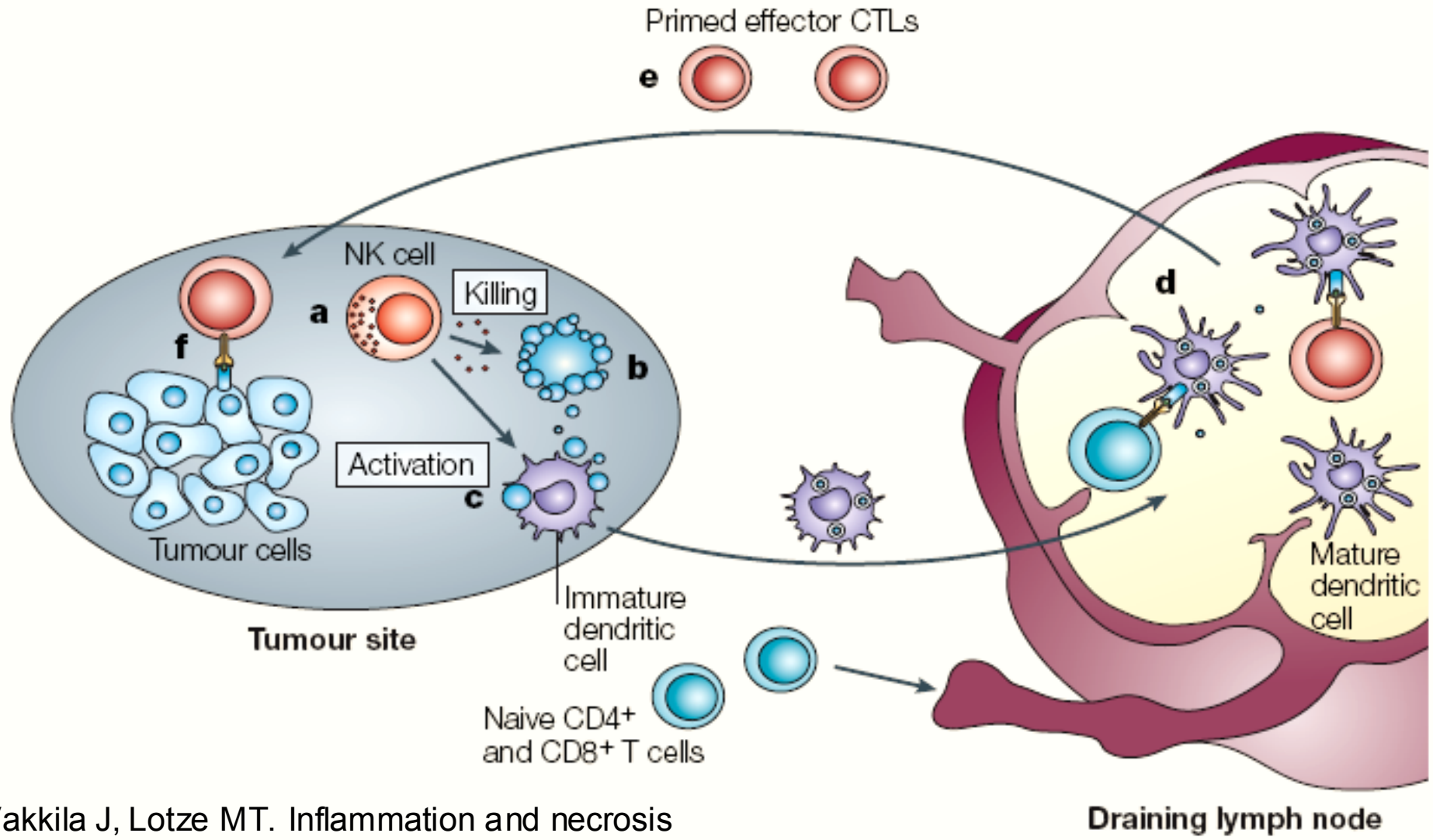
Factors produced by TAMs (M2 cells)
VEGF
bFGF
IL-6
IL-10
PGs
iNOS
arginase
IDO



Role of M1 cells and immature dendritic cells
Produce GM-CSF, IL-12, IL-18
IFN-γ and TNF
Kill tumour cells
Deliver 'message' to lymph nodes

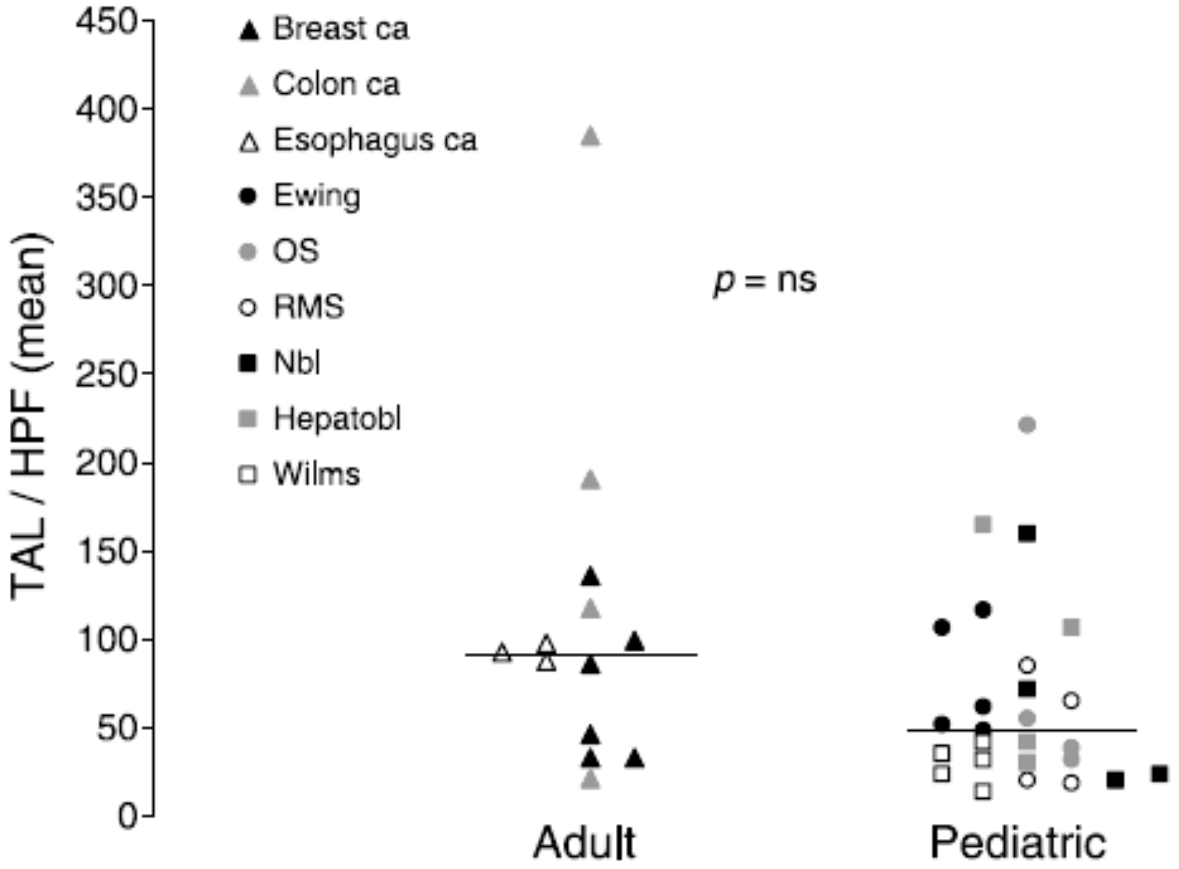
Vakkila J, Lotze MT. Inflammation and necrosis promote tumour growth. *Nature Reviews Immunology* 4:641-647, 2004.

The Crucial Role of T cells, NK Cells and DCs in the Tumor Microenvironment



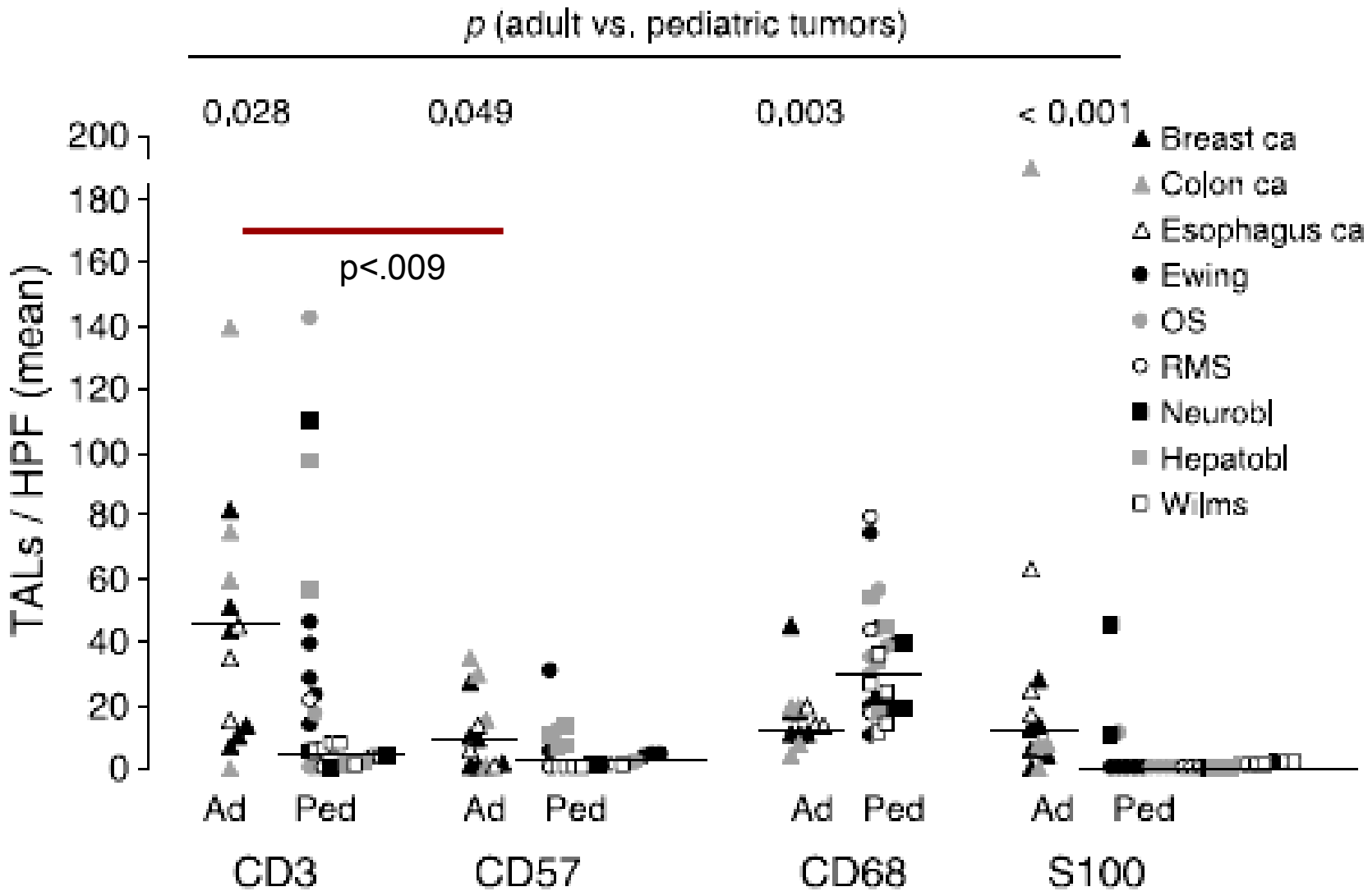
Vakkila J, Lotze MT. Inflammation and necrosis promote tumour growth. *Nature Reviews Immunology* 4:641-647, 2004.

No Substantial Difference in Number of Tumor Associate Leukocytes in Pediatric and Adult Cancers



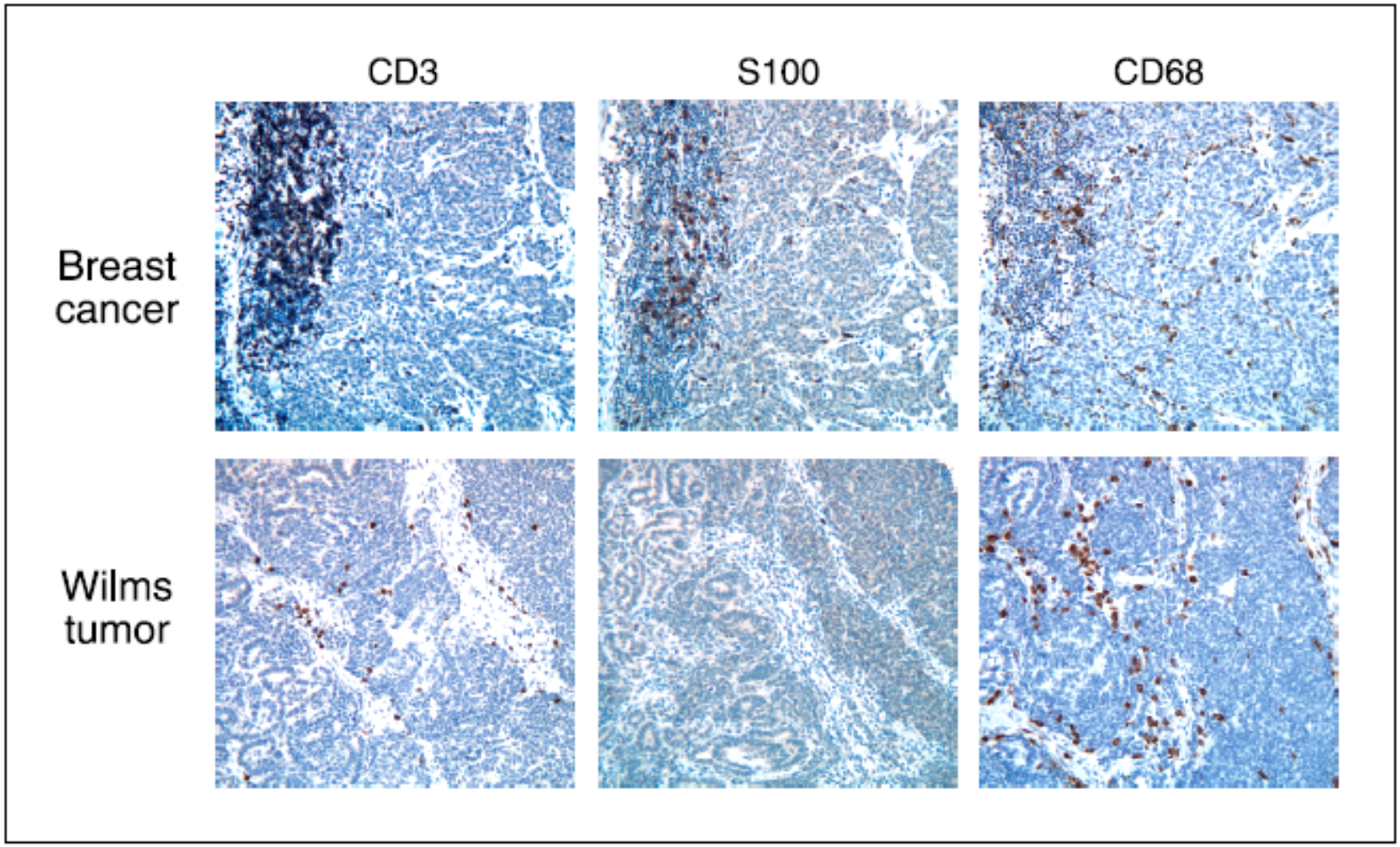
Vakkila J, Jaffe R, Michelow M, Lotze MT. Pediatric cancers are infiltrated predominantly by macrophages and have a paucity of dendritic cells. Clinical Cancer Research, 2006 Apr 1;12(7):2049-54.

Marked Decrease in Dendritic Cells in Pediatric Cancers



Vakkila J, Jaffe R, Michelow M, Lotze MT. Pediatric cancers are infiltrated predominantly by macrophages and have a paucity of dendritic cells. Clinical Cancer Research, 2006 Apr 1;12(7):2049-54.

Comparable Number of Macrophages and Decreased DCs in Pediatric Cancer



Vakkila J, Jaffe R, Michelow M, Lotze MT. Pediatric cancers are infiltrated predominantly by macrophages and have a paucity of dendritic cells. Clinical Cancer Research, 2006 Apr 1;12(7):2049-54.

Genetic Polymorphisms and Factors which Modulate Inflammation and Cancer

1. TLR4, P2X7, IDO2, TRAIL, Perforin polymorphisms?
2. Pathway Analysis
3. *IL-1* gene cluster polymorphisms; tumor necrosis factor-α (*TNF-A*) and *IL-10* polymorphisms
4. Include broader genotyping for known polymorphisms in clinical trials particularly of immunotherapeutic agents.

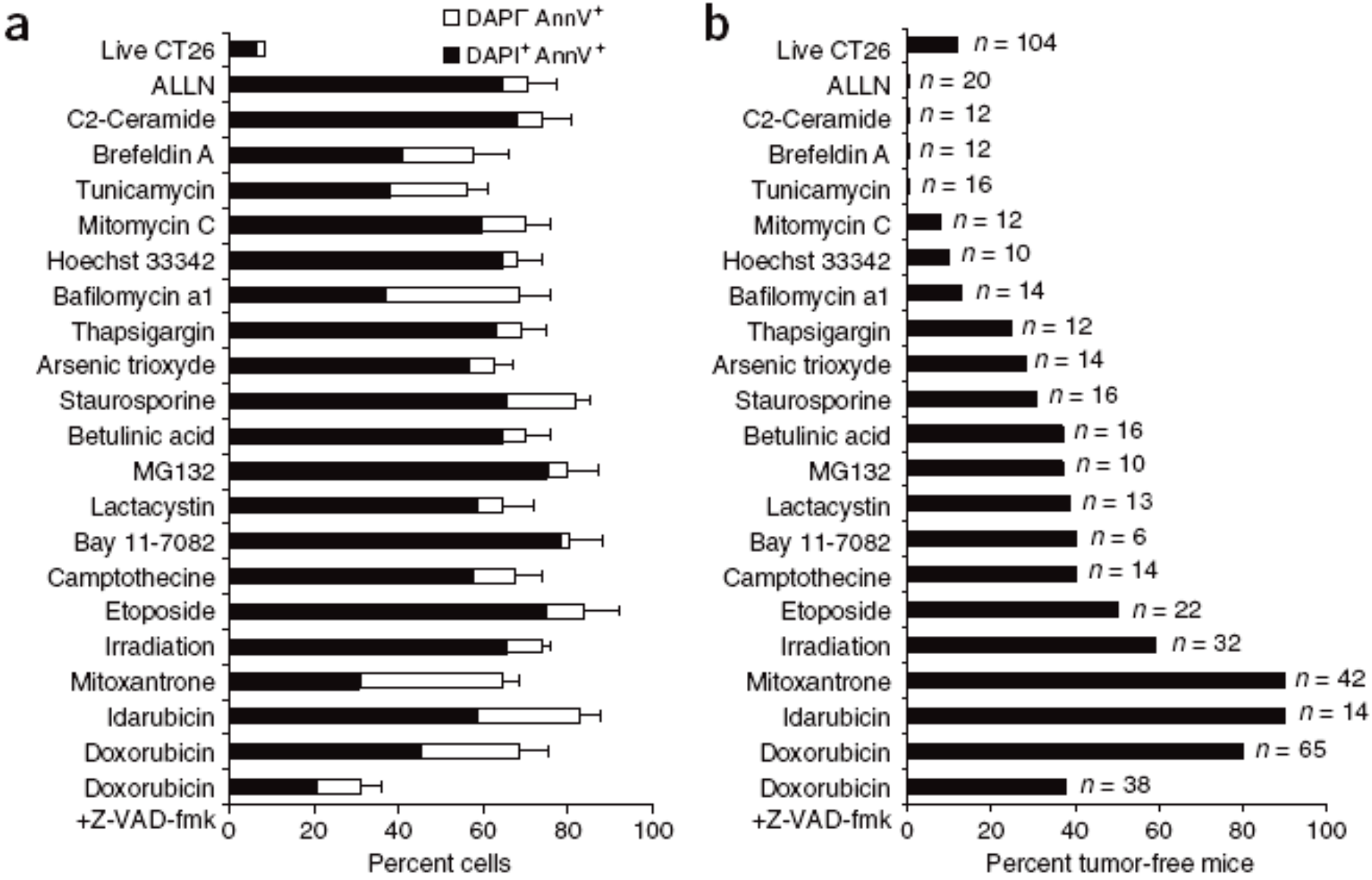
Animal Models

1. Can we identify cellular and molecular components that are common to all cancer-promoting inflammatory responses?
2. Innate immune cells directly and indirectly potentiate/limit cancer risk through the diversity of bioactive mediators they deliver to neoplastic tissues.
3. While the evidence for some mediators is strong (MMPs, cytokines, angiogenesis), for others there is less evidence (reactive oxygen and nitrogen species).

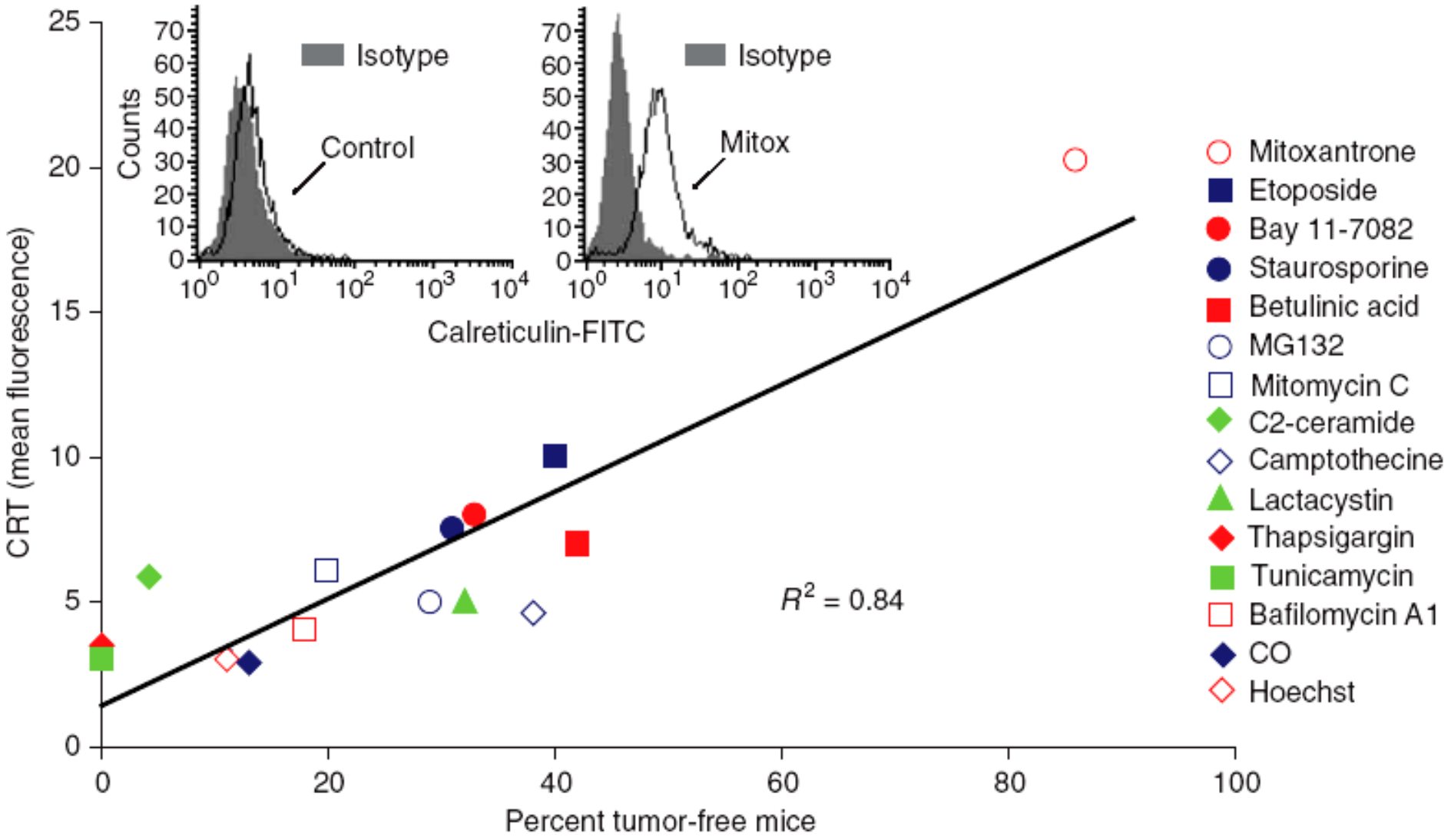
Animal Models

1. Define phenotypes and subtypes of hematopoietic cells (leukocytes, monocytes, mast cells, platelets).
2. Define the physiological roles of the immune cells and study the possible side effects resulting from neutralizing the pro-tumor properties of these cells utilizing immuno-depletion or pharmacologic inhibition strategies.
3. Better define the role of the various immune cells in the different stages of tumorigenesis.
4. Long-term usage of anti-inflammatory agents?
5. The adaptive immune system: etiology-, context- and organ-dependent roles [HCC, skin carcinogenesis examples]

Chemotherapy Induced Immunogenic Cell Death Does Not Correlate with Apoptosis/Necrosis



Calreticulin Exposure Correlates with Immunogenic Chemotherapy Induced Tumor Cell Death



Causes and Molecular Targets in Cancer and Inflammation

1. Role of Pathogen Associated Molecular Pattern Molecules – PAMPs [*H. pylori*, EBV, HCV, HBV, HPV, polyoma virus, HHV8]
2. Role of Damage Associated Molecular Pattern Molecules – DAMPs [HMGB1, S100p and others, purine metabolites – ATP, uric acid, HSPs]
3. Efforts to oxidize or neutralize factors

DAMPs -Chronic Tumor Lysis Syndrome

Cell Constituents:

HMGB1 – Cytochrome C

Heat shock proteins

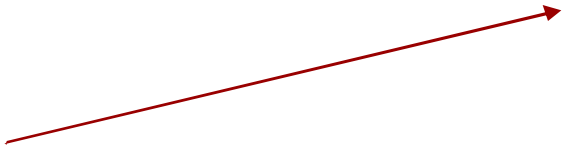
Uric Acid, ATP, Adenosine; CpG DNA

s100 proteins

Hepatoma derived growth factor

LDH

DNA



Acute Tumor Lysis Syndrome

Secreted molecules:

Fibrinogen domain A

Surfactant protein A

Matrix elements:

Heparan sulfate

Soluble hyluranan

Fibronectin

Prevention

- Chemoprevention refers to “the use of agents that can cause regression of existing preneoplastic lesions, prevent the progression of these lesions to cancer, prevent the development of new lesions” (Hong and Sporn, 1997).
- No recommended agents: interest in NSAIDs/COX2 inhibitors, steroids; ? others

Novel Strategies

A number of agents that would be expected to have a significant effect on inflammation within tumors are FDA approved (e.g. Bortezumib, Cytoxan and glucocorticoids) or under various stages of clinical development, yet we know little about the effect these treatments have on inflammation within tumors.

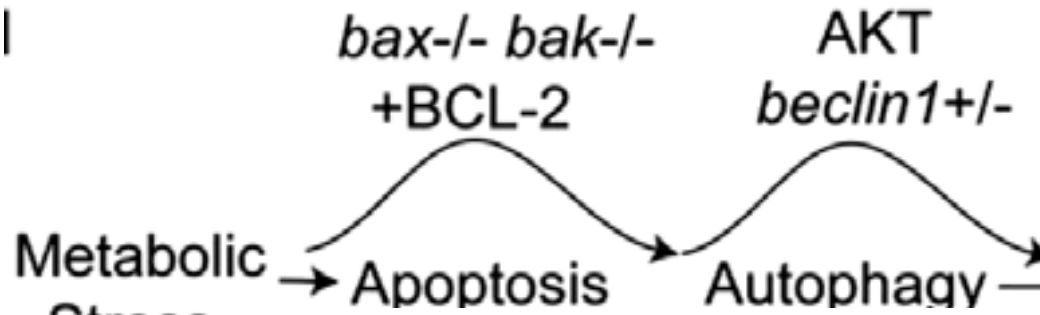
Start asking questions!

CANCER CELL 10, 51–64, JULY 2006

Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis

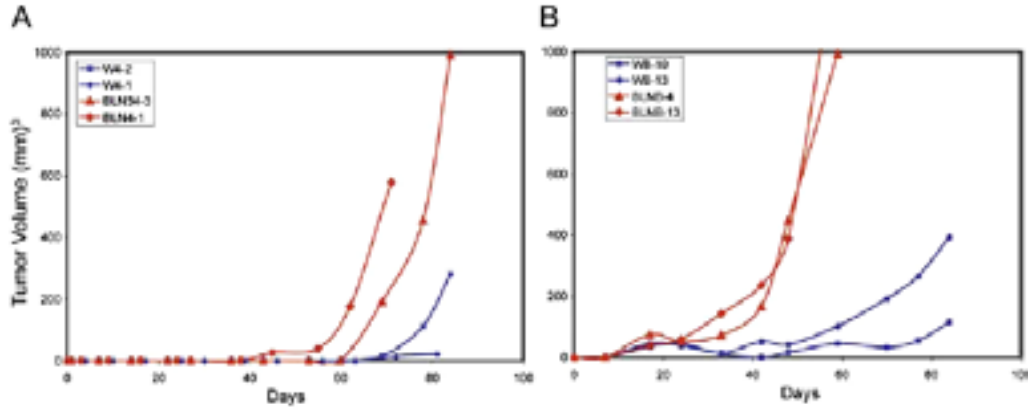
Kurt Degenhardt,[1,2,3,10] Robin Mathew,[1,9,10] Brian Beaudoin,[1,3,10] Kevin Bray,[2,3,4] Diana Anderson,[3] Guanghua Chen,[1,3,5] Chandreyee Mukherjee,[1,3,5] Yufang Shi,[6,9] Céline Gélinas,[1,8,9] Yongjun Fan,[1] Deirdre A. Nelson,[5] Shengkan Jin,[7,9] and Eileen White[1,2,3,4,9,*]

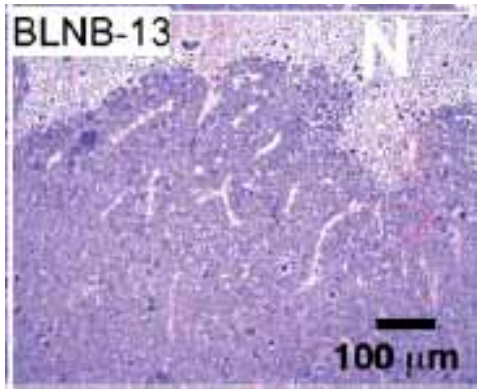
[1] Center for Advanced Biotechnology and Medicine, 679 Hoes Lane, Piscataway, New Jersey 08854



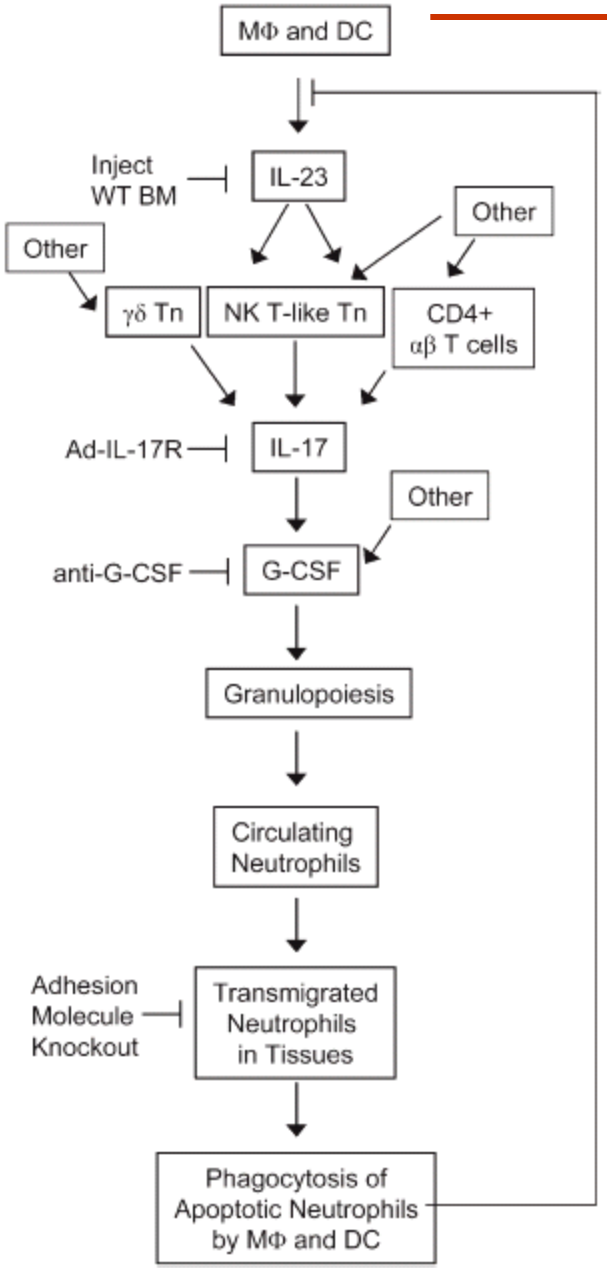
3:15 Cell Death

Mϕ Recruitment/Cancer





Life and Death



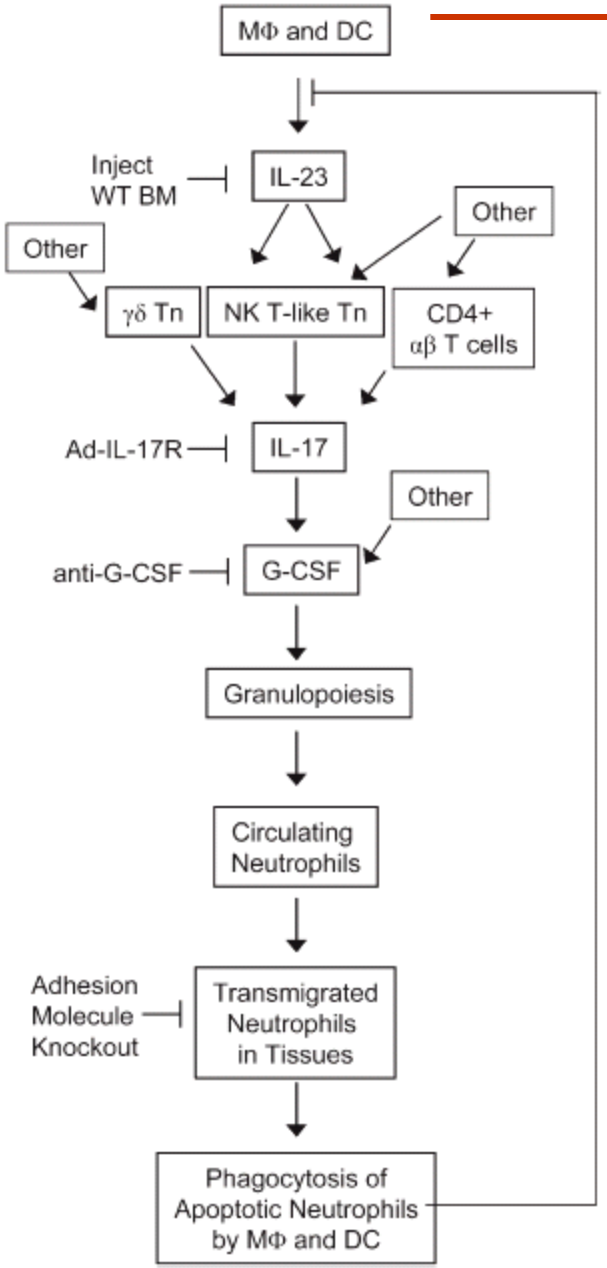
Immunity, Vol. 22, 285–294, March, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.immuni.2005.01.011

Phagocytosis of Apoptotic Neutrophils Regulates Granulopoiesis via IL-23 and IL-17

Matthew A. Stark,[1] Yuqing Huo,[3,4] Tracy L. Burcin,[3]
Margaret A. Morris,[2,3] Timothy S. Olson,[1,3]
and Klaus Ley[1,2,3,*]
[1]Department of Molecular Physiology and
Biological Physics
[2]Department of Biomedical Engineering
[3]Cardiovascular Research Center
University of Virginia
Charlottesville, Virginia 22908

1] Inflammation
2] DAMPs
3] Redox-Anti-DAMPs; hyaluronan
4] Eosinophils

Rube Goldberg/Heath Robinson Devices – Life and Death



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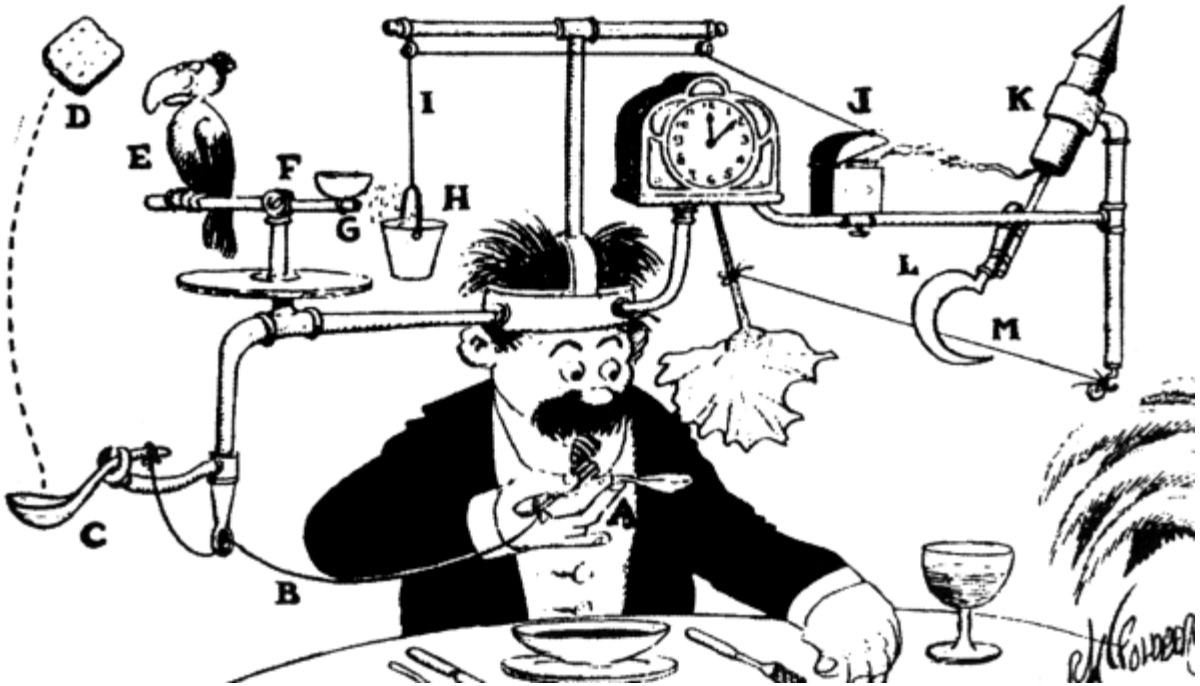
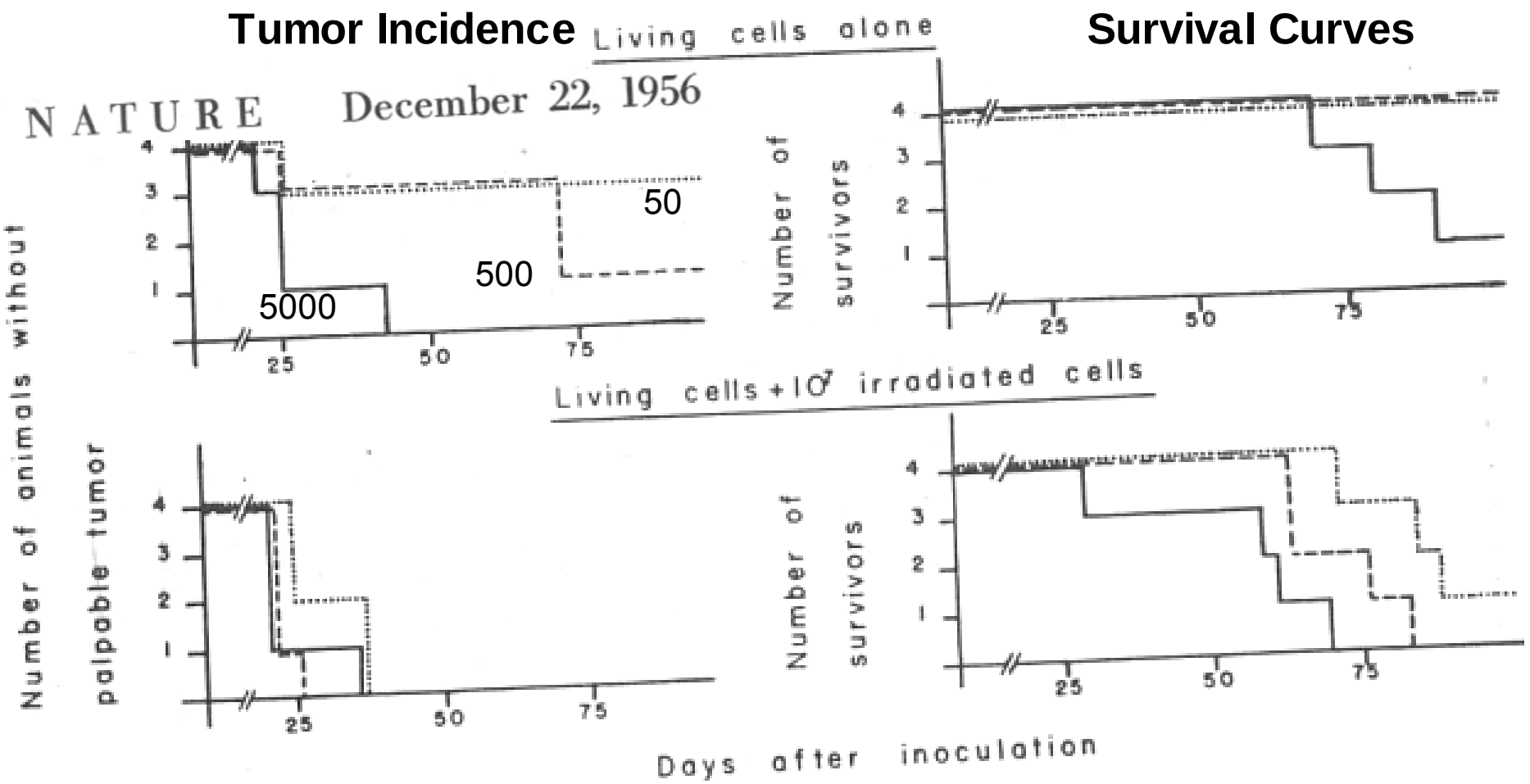


Effect of tumour cells killed by x-rays upon the growth of admixed viable cells. LÁSZLÓ [Laci] RÉVÉSZ (1926-2000) [Nature. 1956 Dec 22;178(4547):1391-2] Karolinska



Effect of cells lethally damaged by 15,000 R γ-irradiation on growth of viable cells + a spontaneous C3H mammary carcinoma. REVESZ L. Effect of lethally damaged tumor cells upon the development of admixed viable cells. J Natl Cancer Inst. 1958 Jun;20(6):1157-86.

The Four Step Cancer Model
Chronic Inflammation and Cellular Necrosis Progress to Cancer

Event	Chronic inflammation	Silencing of tumour-suppressor genes	Cell necrosis	Mutation of proto-oncogene(s)
Mediators and mechanisms of action	STAT3, prostaglandins, IL-1R1, IL-4, IL-10, TGF-β	Hypermethylation of CpG islands in promoter regions; enhanced by CI	CI-induced microthrombosis and micronecrosis results in release of HMGB1 and other necrotic factors	Autocrine growth or increased sensitivity to growth factors
Cells involved	T cells, macrophages, polymorphonuclear cells	Epithelial and stromal cells	Many normal and neoplastic cells distal and/or proximal to occluded arterioles and/or venules	Primarily epithelia, secondarily genetic and epigenetic changes in stromal cells
Consequences	Acute inflammatory reactions are downregulated; polarization to M2-type macrophages occurs; CI persists	Control of cell cycle or quality of DNA is lost; diminished apoptosis is promoted	Unscheduled cell death and reparative proliferation; increased probability of oncogenic mutations	Cancer

Vakkila J, Lotze MT. Inflammation and necrosis promote tumour growth. *Nature Reviews Immunology* 4:641-647, 2004.