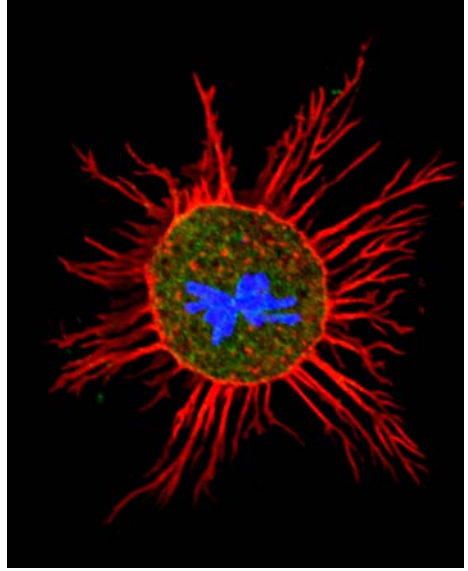
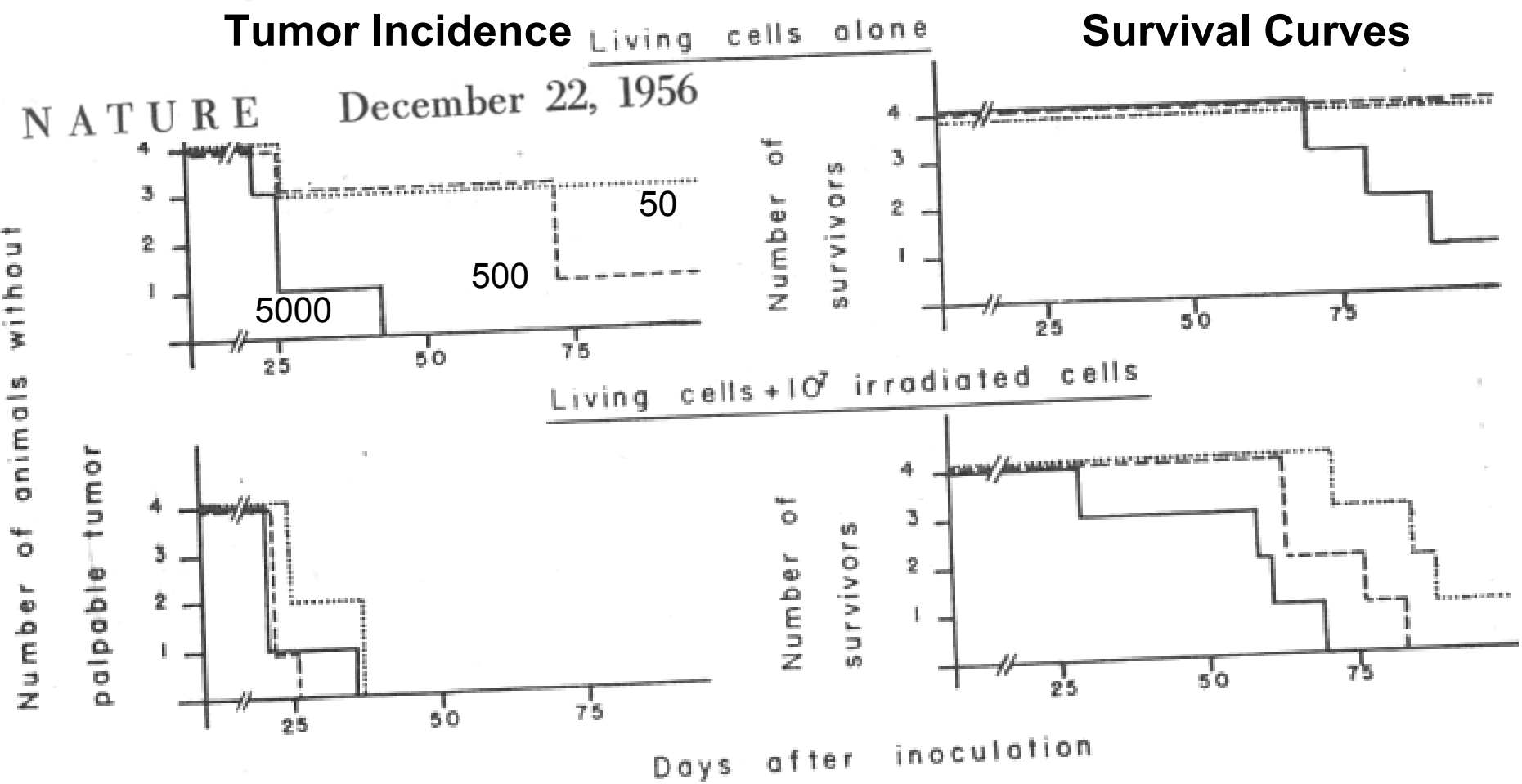


Cancer Is A Disorder of Cell Death:
Biologic Impact of Targeted Therapies

Michael T. Lotze, MD
Vice Chair and Chief, Translational Research
Departments of Surgery and Bioengineering
University of Pittsburgh Cancer Institute

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.

Stimulation Exerted by Dead Cells

- Specific stimulation by homologous cell products
- A ‘feeder effect’ in which the dead cells release essential nutrients
- Stimulation through provoking an inflammatory response and/or vascularization from the side of the host

TLRs, NLRs, and RLRs

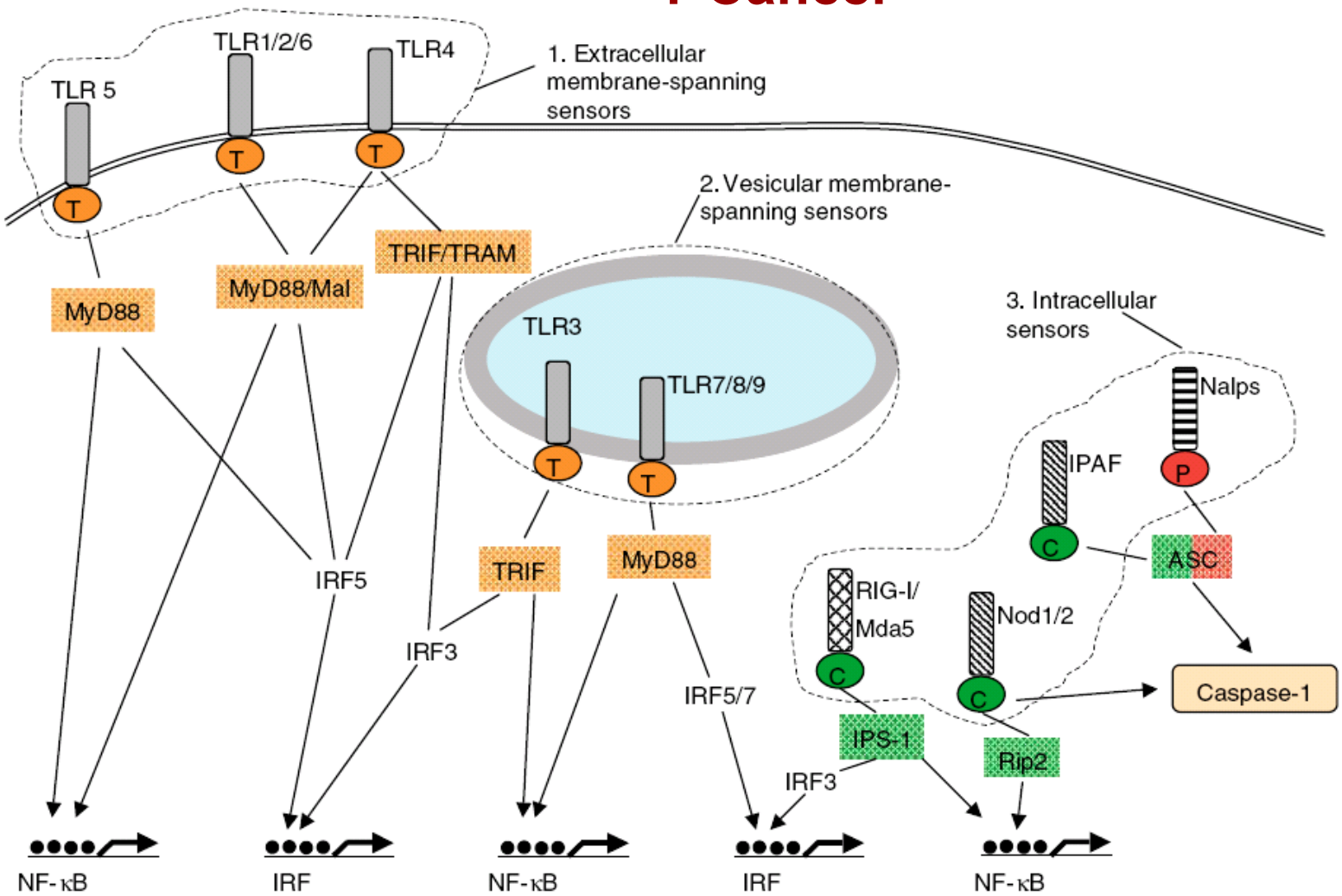
Cell Death and Differentiation (2006), 1–18

www.nature.com/cdd

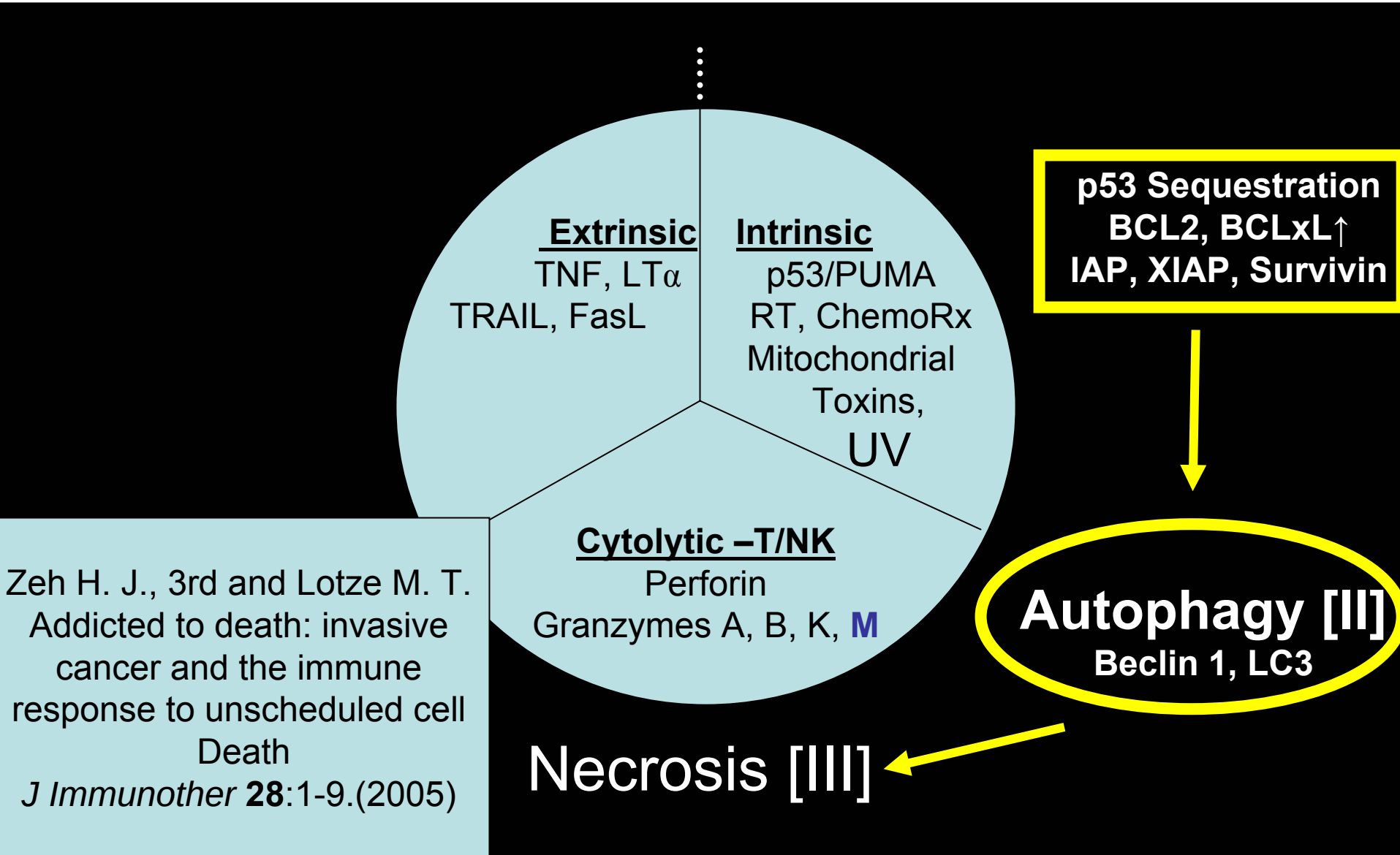
Review

TIR, CARD and PYRIN: three domains for an antimicrobial triad

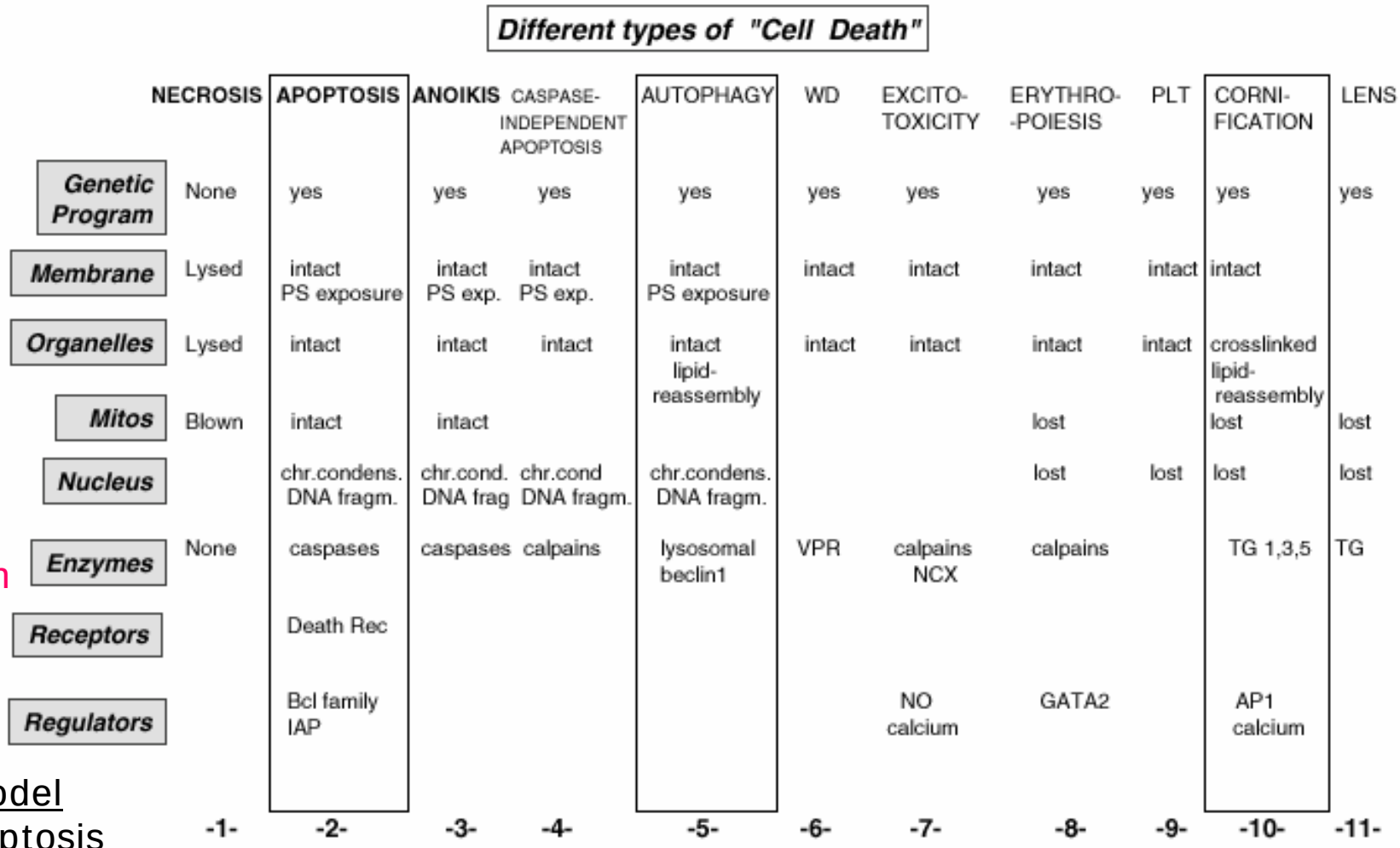
? Cancer



Death Used to be Simpler
Apoptosis [I], Autophagy [II] and Necrosis [III]



Zeh H. J., 3rd and Lotze M. T. Addicted to death: invasive cancer and the immune response to unscheduled cell Death
J Immunother **28**:1-9.(2005)



Current Model

11 forms of cell death

Classical Model

Type1: Apoptosis

Type2: Autophagy

Type3: Necrosis

G. Mollino, P. Nicotera et al., *Cell Death and Differentiation*, 12 (2005)

Oncogene (2006) 25, 4798–4811

www.nature.com/onc

REVIEW

Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy

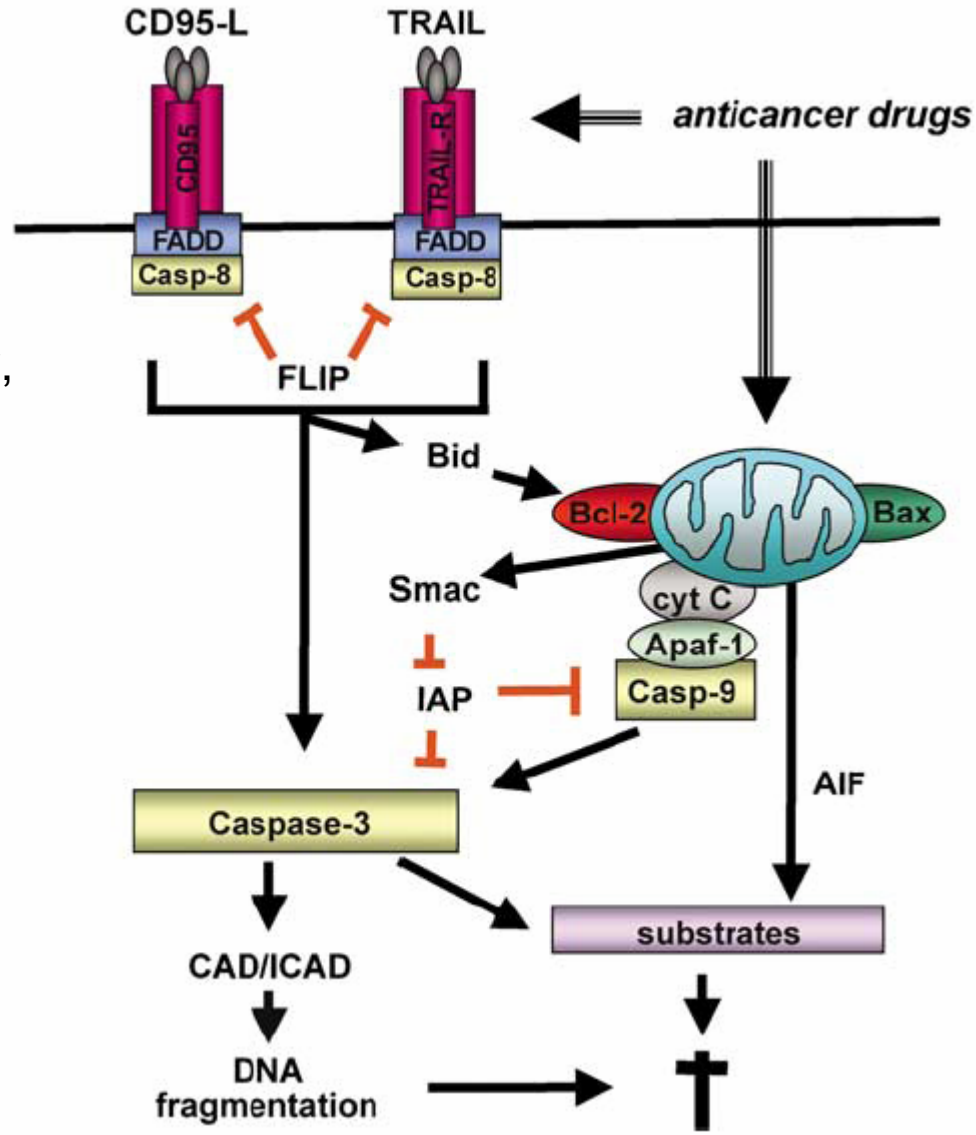
S Fulda and K-M Debatin

University Children's Hospital, Ulm, Germany

- Current cancer therapies, for example, chemotherapy, γ-irradiation, immunotherapy, or suicide gene therapy, primarily exert their antitumor effect by triggering apoptosis in cancer cells

- So far, no clear pattern has emerged between the level of apoptosis or proteins that regulate apoptosis and treatment response in most solid tumors

- Strategies Targeting the Intrinsic Pathway
  - Bcl-2 Family Proteins
  - Smac/Diablo Agonists
  - Irradiation



Process of Autophagy

- environmental stressors lead to isolation of double membrane-bound structures thought to be derived from the “phagophore”

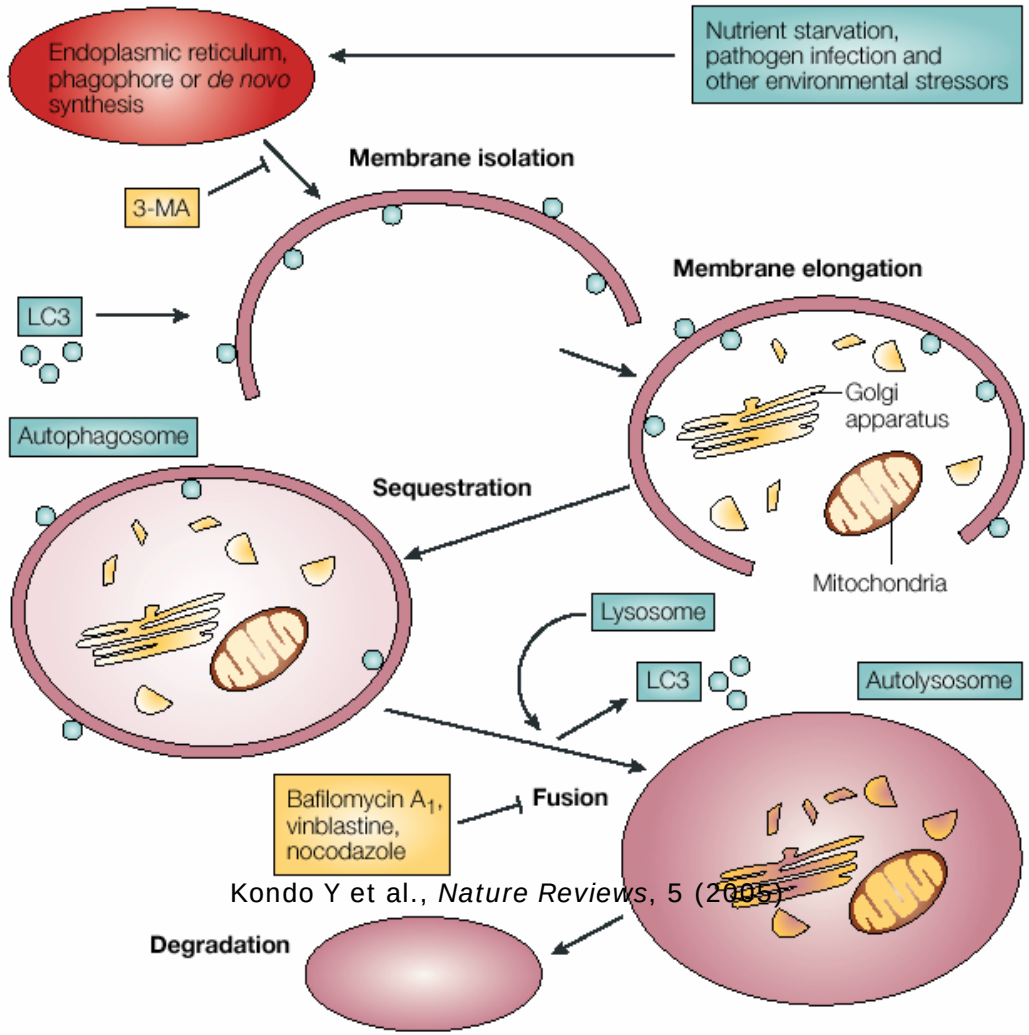
- membrane structures elongate and mature and MAP-LC3 is recruited to the membrane

- elongated double membranes form autophagosomes and sequester cytosolic proteins and organelles

- sequesteration requires ATP and microtubules

- process can be inhibited by blocking ATP production or microtuble assembly

- the autophagosome fuses with the lysosome which proceeds with degredation



Kondo Y et al., *Nature Reviews*, 5 (2005)

• Overexpression of beclin-1 (the yeast homolog of Atg6) induces autophagy in tumor cells and inhibits their tumorogenicity (acts as tumor supressor). Beclin-1 is regulated by BCL-2

• Other tumor supressor genes linked to autophagy include PTEN and tensin homologs

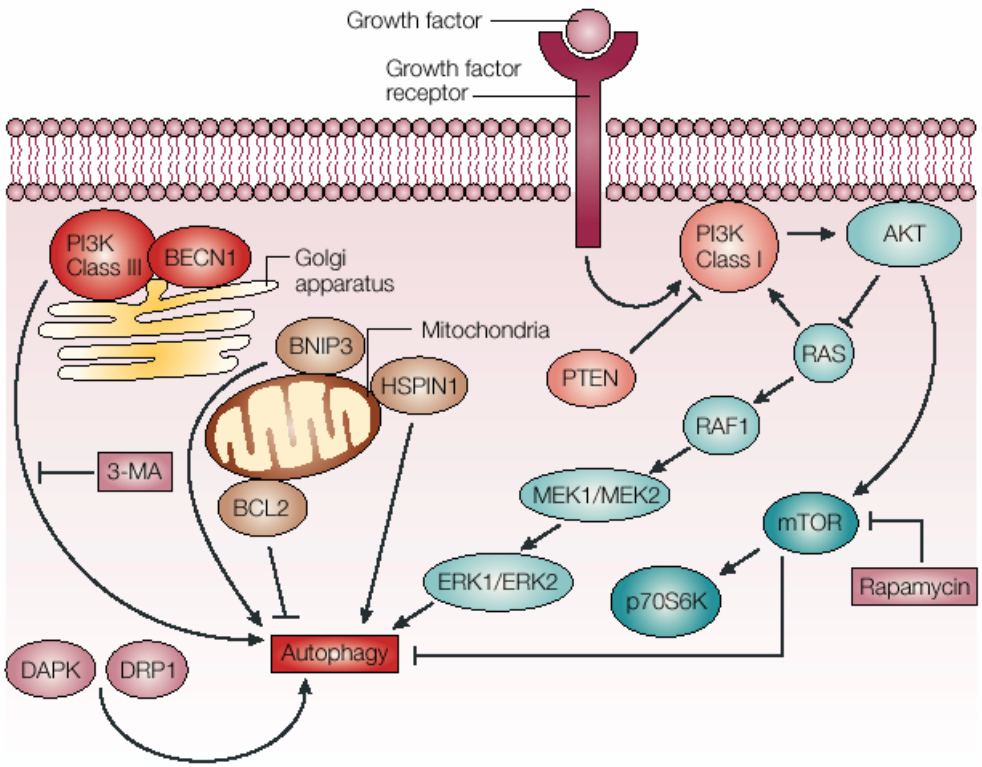
• Tamoxifen induces autophagic cell death of breast cancer cells through the oestrogen receptor and occurs through downregulation of AKT

Therapies that induce autophagy in cancer cells Kondo Y et al., *Nature Reviews*, 5 (2005)

Treatment	Proposed target	Cancer type
Tamoxifen	Oestrogen receptor	Breast cancer
Temozolomide	DNA	Malignant glioma
γ-Irradiation	DNA	Breast cancer, prostate cancer, colon cancer, malignant glioma
Sodium butyrate and SAHA	HDAC	Cervical cancer that overexpresses $BCL\text{-}X_L$
Hyperthermia	Unknown	Malignant glioma
Arsenic trioxide	Multiple targets (for example, mitochondria)	Malignant glioma
Resveratrol	Multiple targets (for example, oestrogen receptor and mitochondria)	Ovarian cancer
Soybean B-group triterpenoid saponins	Unknown	Colon cancer
Rapamycin	mTOR	Malignant glioma

HDAC, histone deacetylase; mTOR, mammalian target of rapamycin; SAHA, suberoylanilide hydroxamic acid.

Autophagy and Cancer



Inhibitors of autophagy

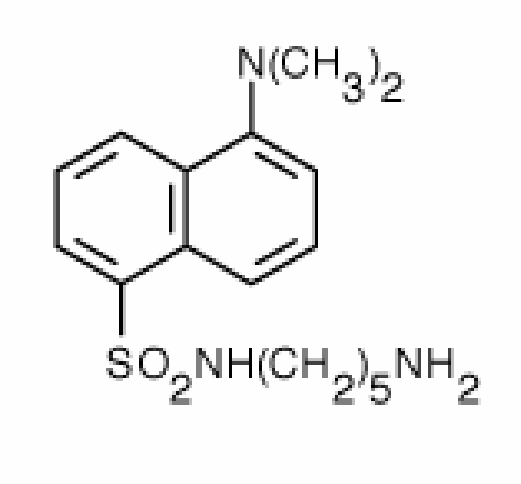
Compound	Modification of cellular component	Cancer type
3-MA	PI3K inhibitor. Inhibits the formation of pre-autophagosomal structure	Breast cancer, prostate cancer, colon cancer, malignant glioma and cervical cancer
Bafilomycin A_1	H^+-ATPase inhibitor. Blocks the fusion of the autophagosome and lysosome	Breast cancer, prostate cancer, colon cancer, malignant glioma, cervical cancer
HCQ	A lysosomotropic agent. Blocks the fusion of the autophagosome and lysosome	Cervical cancer
Monensin	Proton exchange for potassium or sodium. Blocks the fusion of the autophagosome and lysosome	Cervical cancer
siRNA against ATG5, BECN1, ATG10, ATG12	Blocks translation of these proteins	Cervical cancer

3-MA, 3-methyladenine; HCQ, hydroxychloroquine; PI3K, phosphatidylinositol 3-phosphate kinase; siRNA, small interfering RNA.

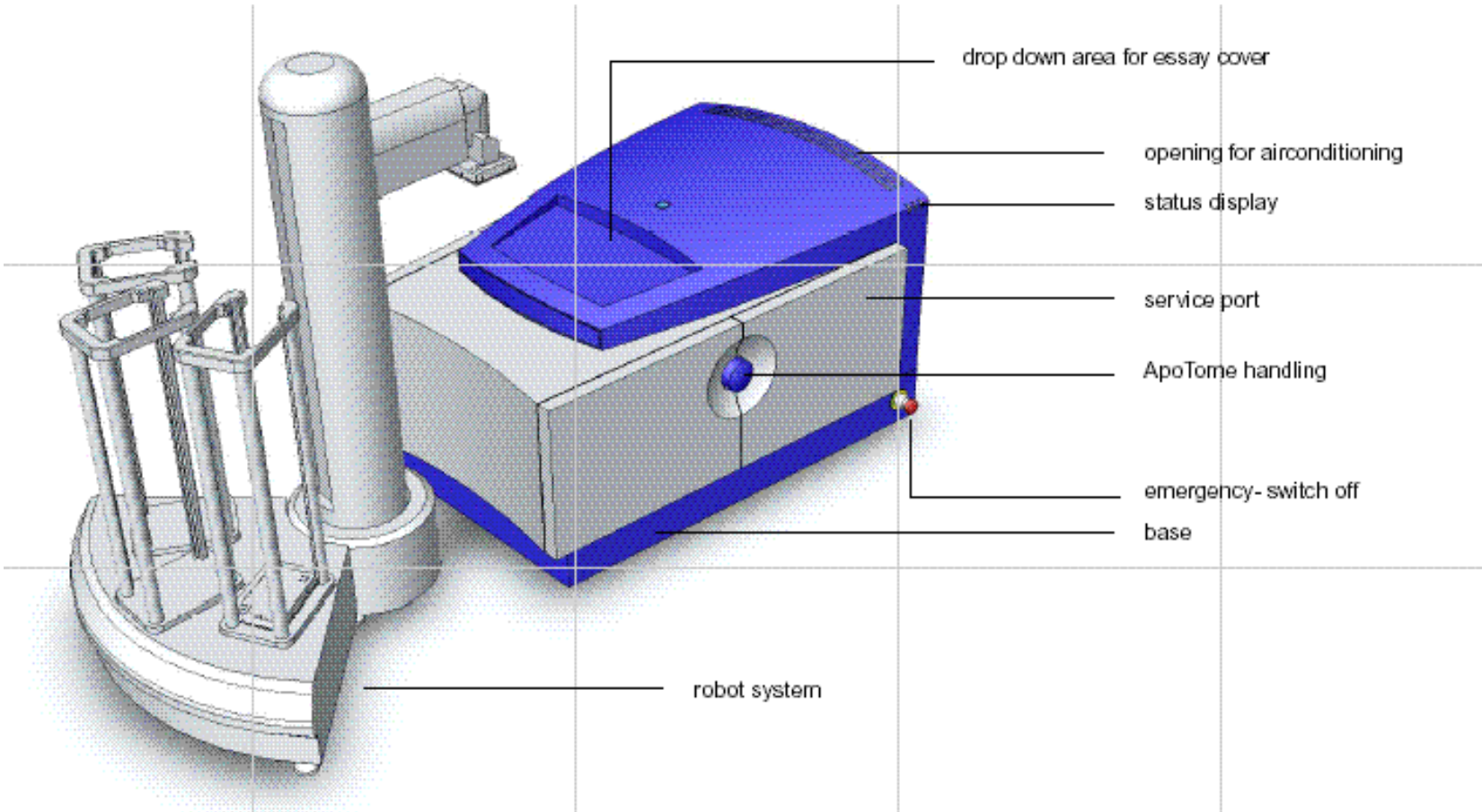
Kondo Y et al., *Nature Reviews*, 5 (2005)

• MAP-LC3
(Microtubule Associated Protein Light Chain 3)
Homolog of the yeast Apg8 protein which is essential for formation of autophagosomes. Present as two isoforms (I&II). LC3-II Localizes to the limiting membranes of autophagosomes after processing.

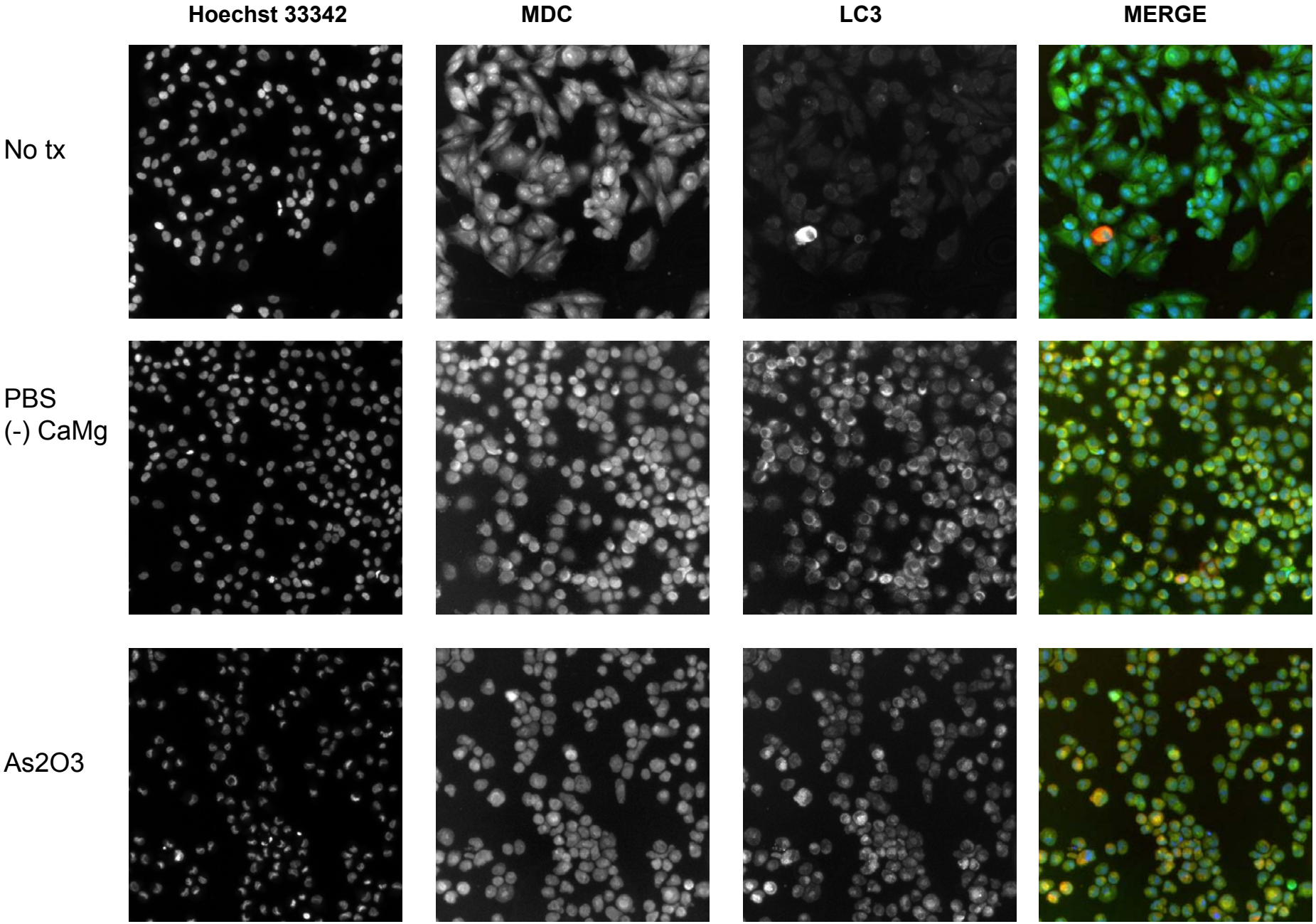
• Monodansylcadaverine (MDC)
lysomotrophic compound that has been shown to accumulate in autophagosomes (Biederbeck et al., 1995)



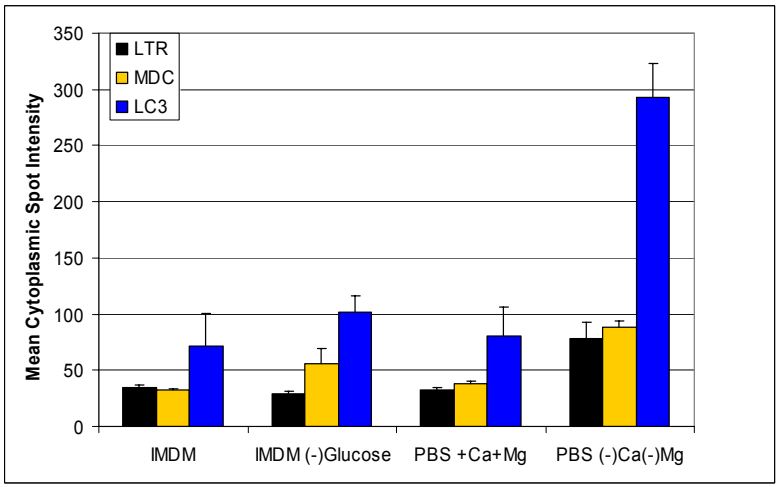
The ArrayScan VTI and High Content Screening (HCS)



Autophagy in Melanoma



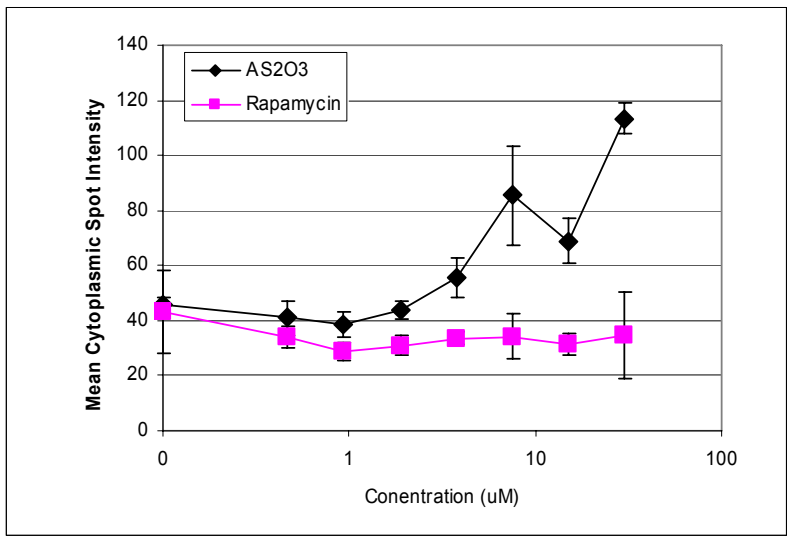
WM9 undergoes autophagy in response to starvation and arsenic trioxide as measured by an increase in cytoplasmic spot intensity



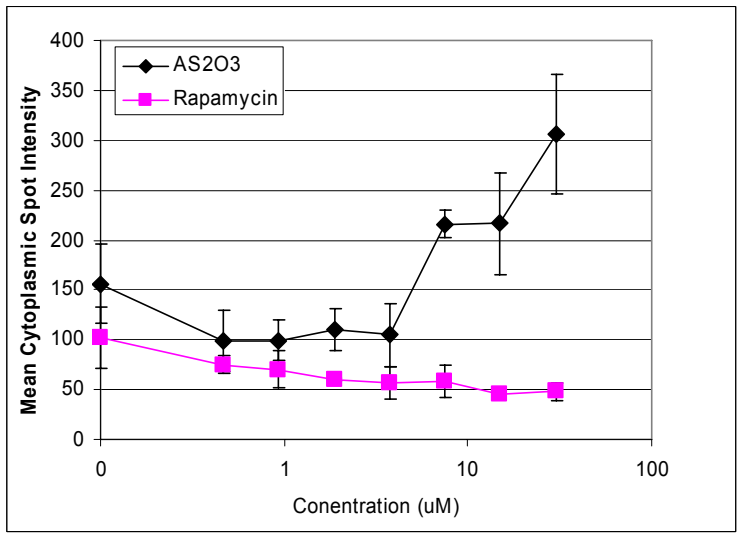
Cells appear to be protected from autophagy in the presence of Ca^{++} and Mg^{++} cations

LTR = Lysotracker Red
MDC = Monodansylcadaverine
LC3 = MAP LC3

MDC



LC3



Classes of Molecules That Initiate The Innate Immune Response – Signal 0

Pathogen-associated Molecular Patterns (PAMPs):

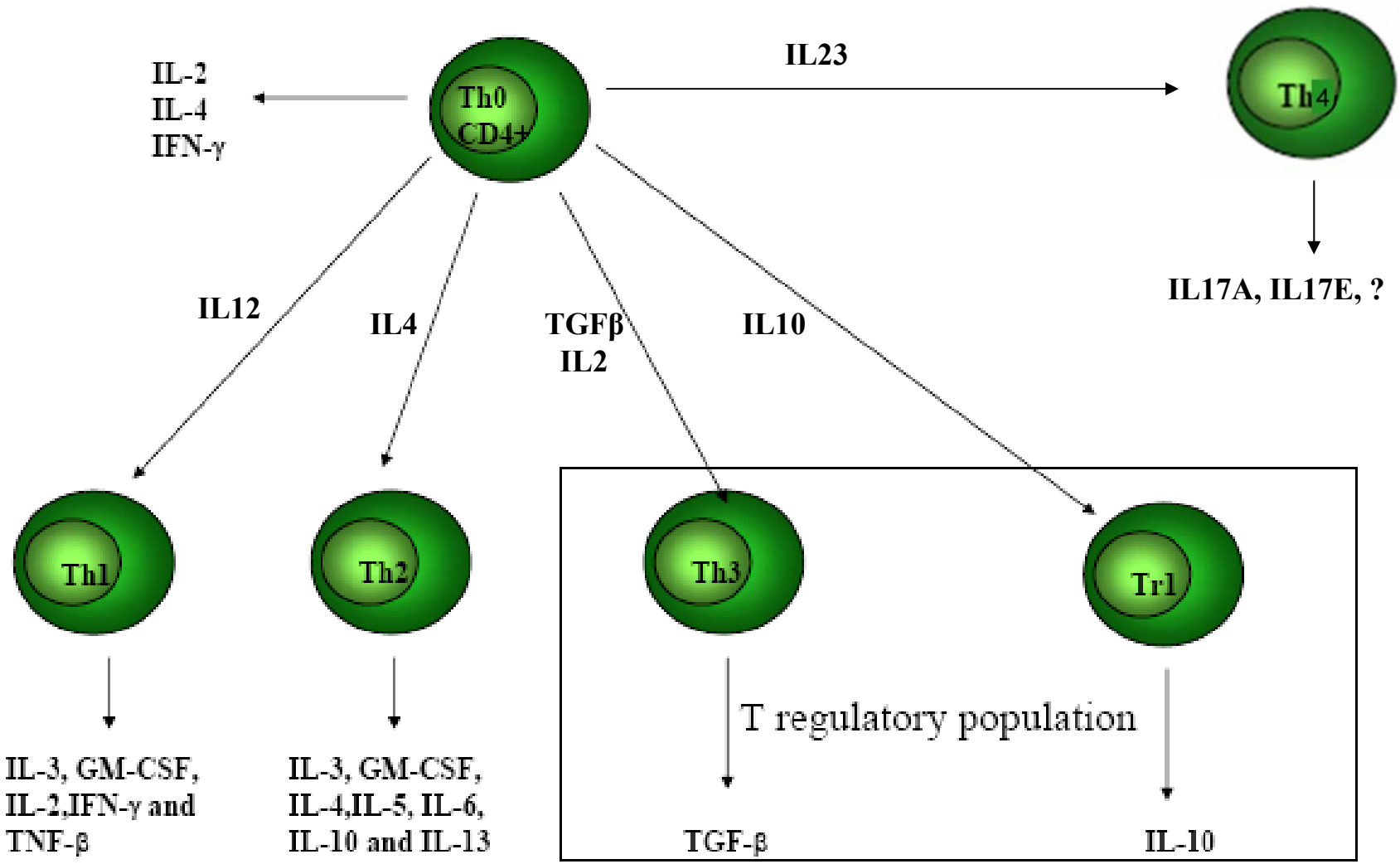
Molecules expressed or released by invading microorganisms that are structurally unique to the pathogen. Ruslan Medzhitov, 2000

Damage-associated Molecular Patterns (DAMPs):

Molecules expressed or released that are normally unavailable to the immune system but are released and recognized by immune cells following tissue injury.

Walter L Land, 2003

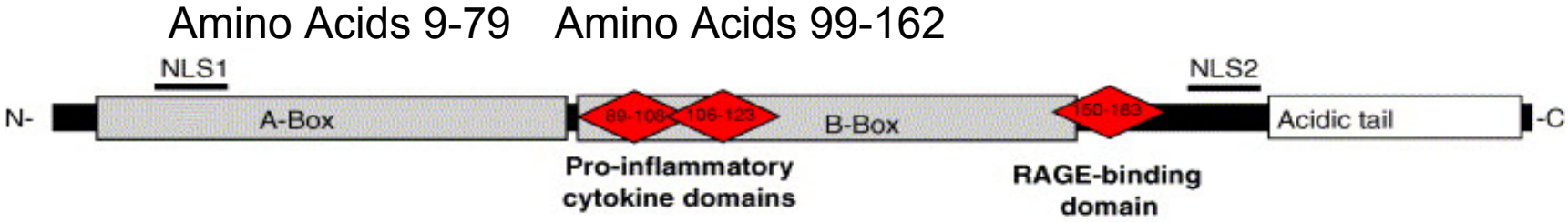
Classes of Molecules That Initiate The Adaptive Immune Response – Signal 3



Classes of Molecules That Promote Continued Adaptive Response – Signal 0 – Environmental Stress

T Helper	Signal 0 Signal 5	Inducer	Transcription Factor	Products
Th1	HMGB1, LPS, ?	IL12	T-bet	IFNγ
Th2	???	IL4	GATA3	IL4, IL5
Th3	???	IL10, TGFβ	FoxP3	IL10, TGFβ
Th4 [Th17]	↓?? Neut apoptosis	IL23	RORγ	IL17, IL6

HMGB1



Antagonist to B-Box activity

TNF stimulation

Stimulates transcription, binds chromatin , shields DNA-cisplatin from exonucleases

- The High Mobility Group Box-1 Protein, first identified in 1973.

Intracellular HMGB1

Co-transcriptional Factor

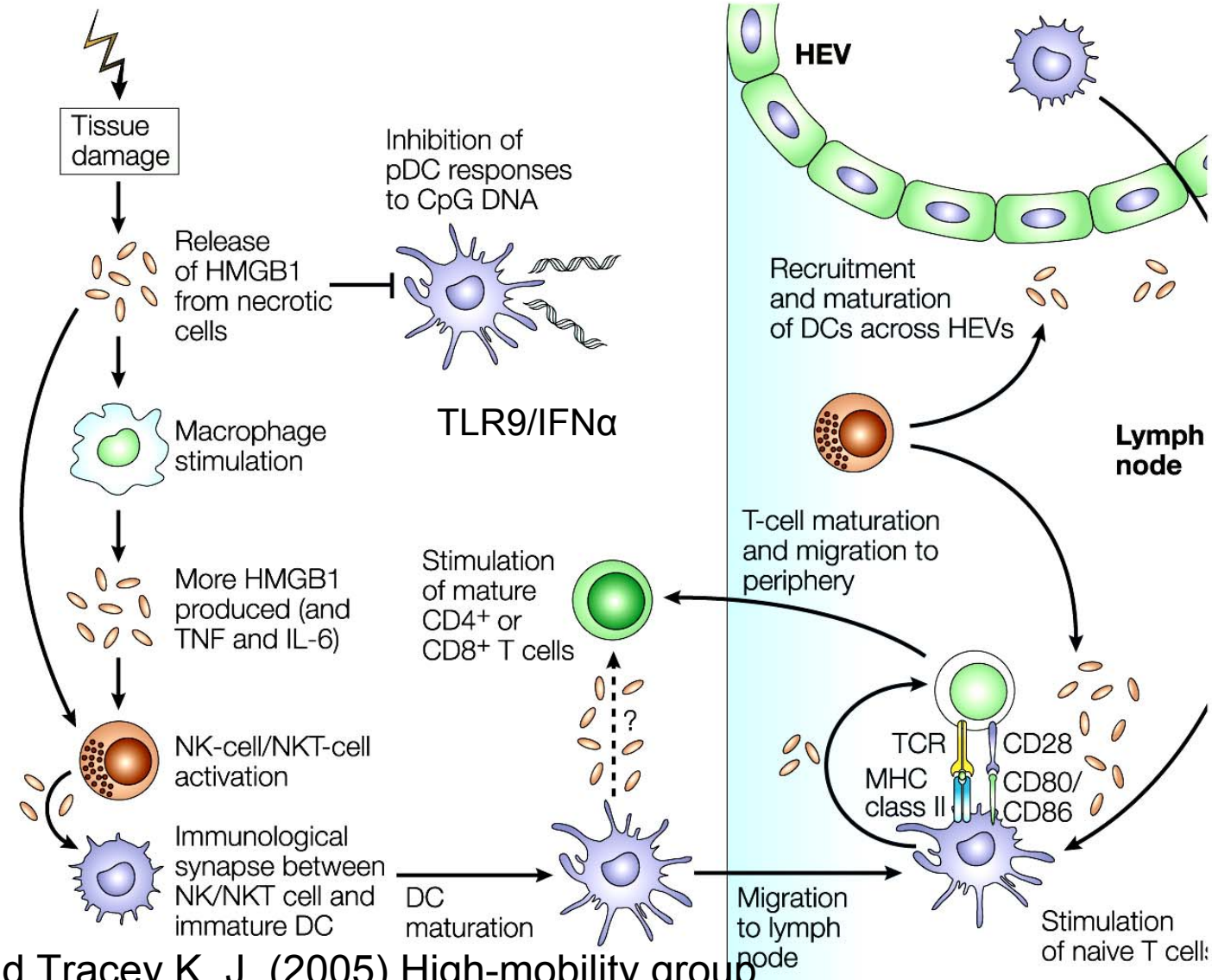
Structural DNA binding protein
Stabilizes Nucleosomes
Transcriptional regulation

Extracellular HMGB1

Inflammatory Cytokine

Neutrophil Chemotaxis
Macrophage activation
Dendritic cells maturation
Vascular Leakage
Acute Lung Injury
Hepatic Injury
Multiple Organ Failure

Immunobiology of HMGB1



Lotze M. T. and Tracey K. J. (2005) High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. *Nat Rev Immunol* **5**, 331-42.

doi:10.1038/nature04808

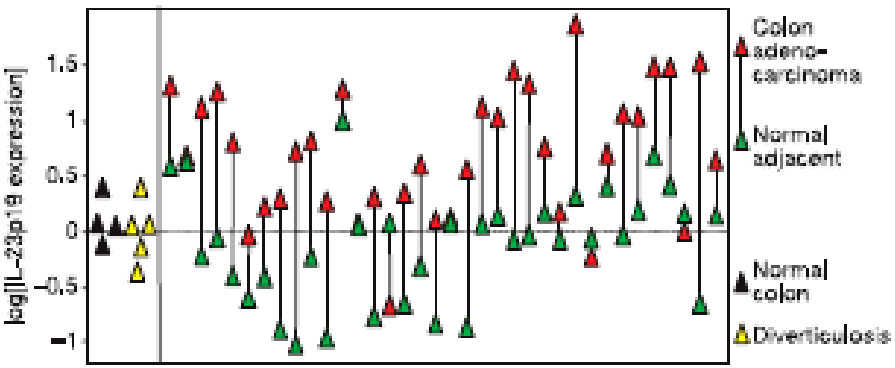
nature

May 2006

LETTERS

IL-23 promotes tumour incidence and growth

John L. Langowski[1]*, Xueqing Zhang[1]*, Lingling Wu[1], Jeanine D. Mattson[1], Taiying Chen[1], Kathy Smith[1], Beth Basham[1], Terrill McClanahan[1], Robert A. Kastelein[1] & Martin Oft[1]



b

Cancer type	Number of paired (tumour and normal) samples	Fold increase in expression: Average	Number >5×	Number >10×	P
Colon	36	15,33	23	17	0,0001
Ovarian	32	9,45	12	4	0,0001
Head and neck	44	3,41	11	4	0,01
Lung	114	3,03	20	8	0,0001
Breast	78	2,86	16	6	0,0001
Stomach	64	2,13	9	3	0,001
Melanoma	89	1,47	5	0	0,0001

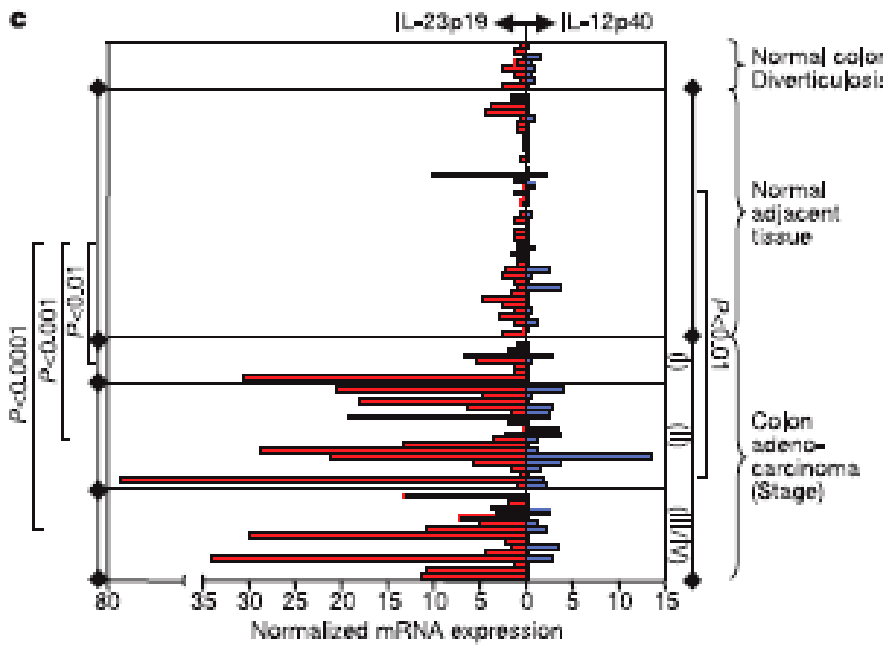


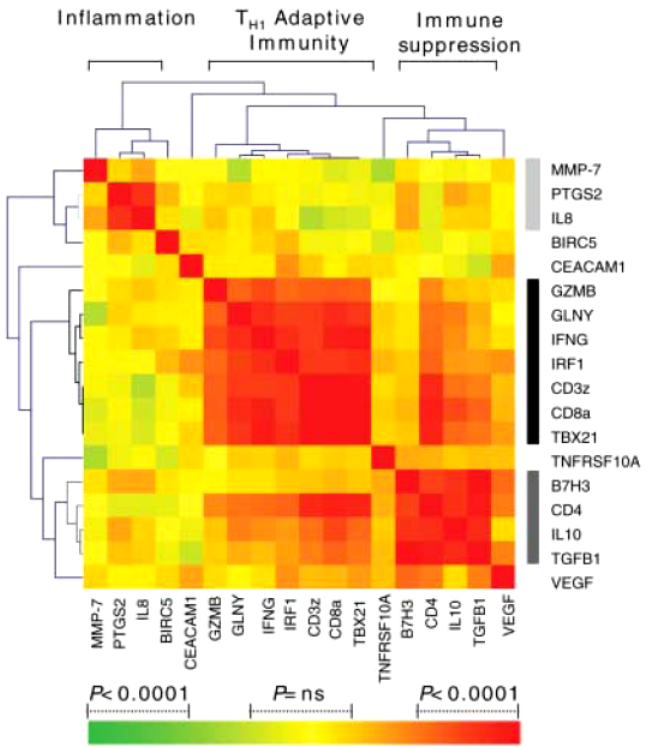
Figure 1 | Overexpression of IL-23 but not of IL-12 in human cancer.

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.

29 SEPTEMBER 2006 VOL 313 SCIENCE www.sciencem



Tumor histopathology

UICC-TNM Staging system

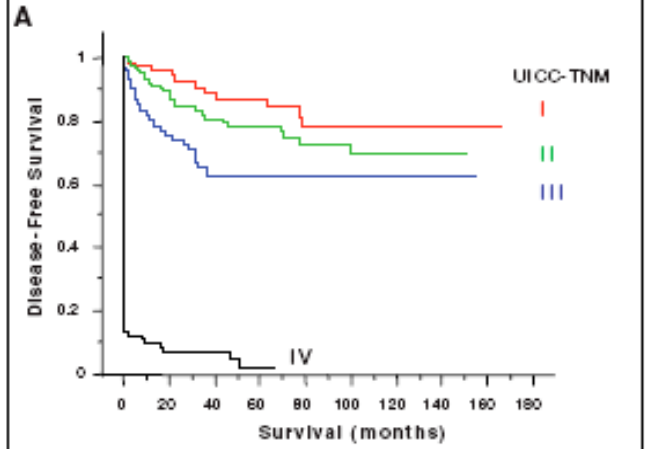
Tumor infiltrating immune cells

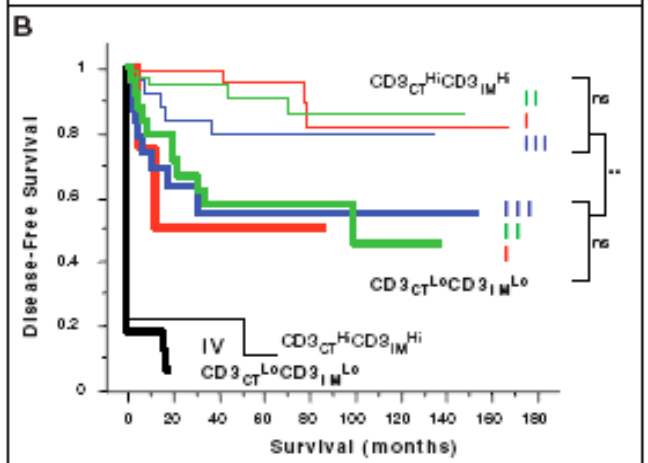
$CD3_{CT}CD3_{IM}$ evaluation

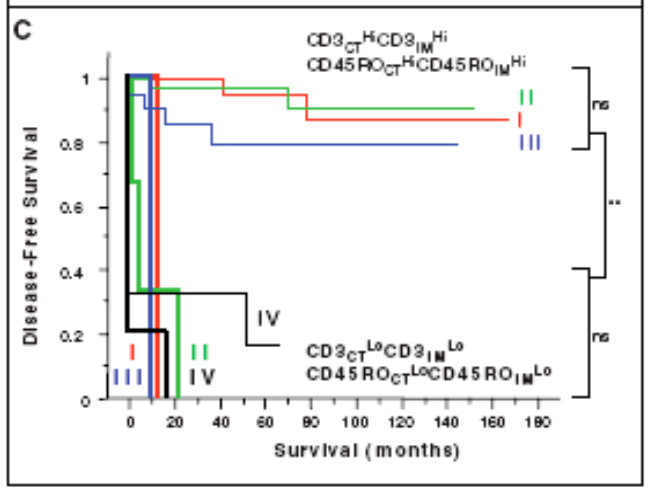
$CD3_{CT}CD3_{IM}$ evaluation

plus

$CD45RO_{CT}CD45RO_{IM}$ evaluation







Classes of Molecules That Promote Innate Response – Signal 0 – Environmental Stress

Sentinel	Stress	Antigen	Target	Signal 0 Signal 5
NK	Genomic Metabolic	-	MICA, MICB	HMGB1, LPS, ?
NKT	Cell Membrane	Glycolipid	CD1d	???
CD4	Cell membrane	13-20 Peptide	Class II	???
CD8	ER Stress	8-10 Peptide	Class I	↓?? Neut apoptosis

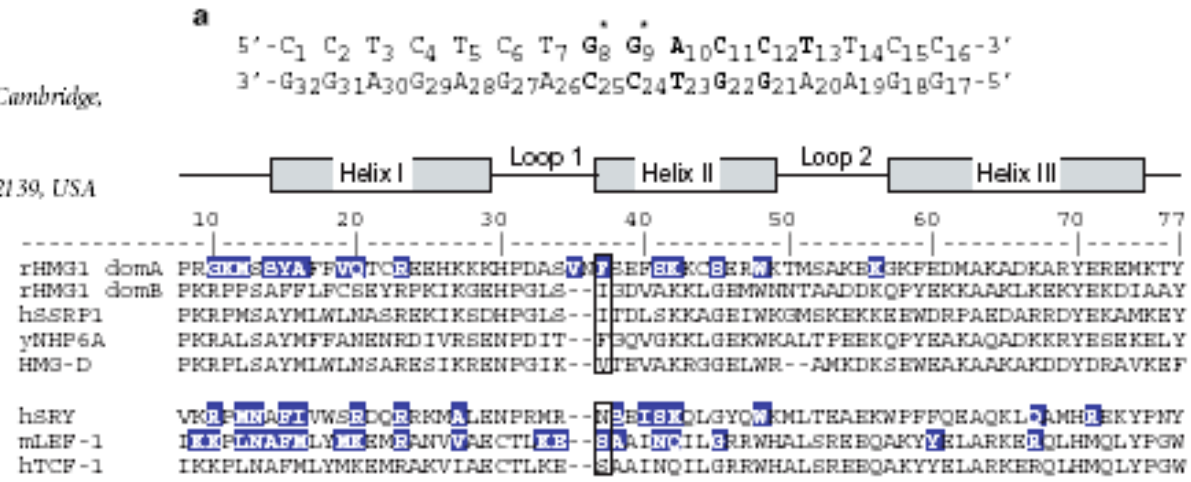
Basis for recognition of cisplatin-modified DNA by high-mobility-group proteins

Uta-Maria Ohndorf*, Mark A. Rould†‡, Qing He*, Carl O. Pabo† & Stephen J. Lippard*

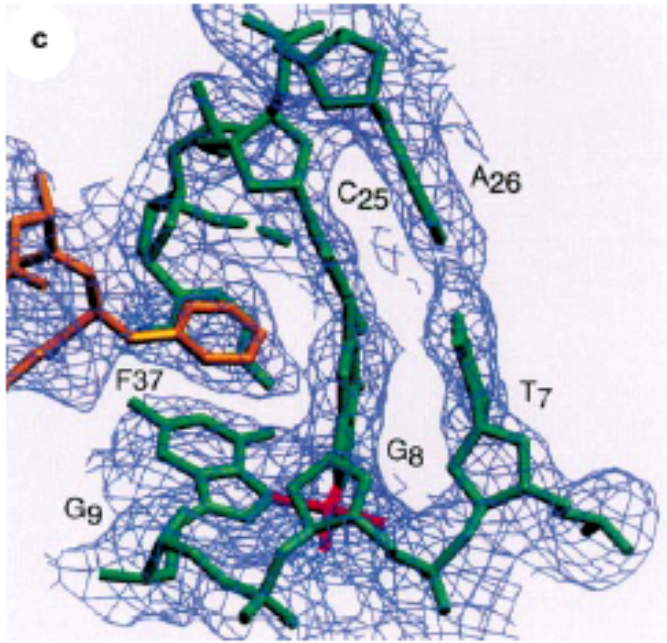
* *Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA*

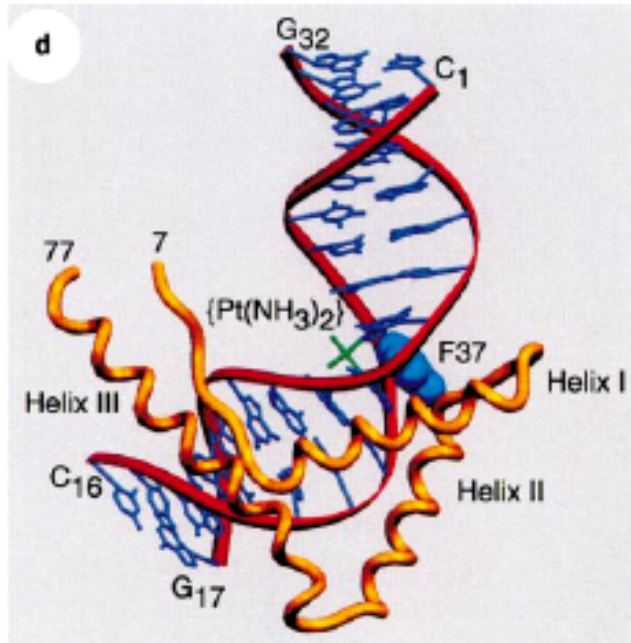
† *Department of Biology and Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA*

Nature. 1999 June 17 399:708-12.

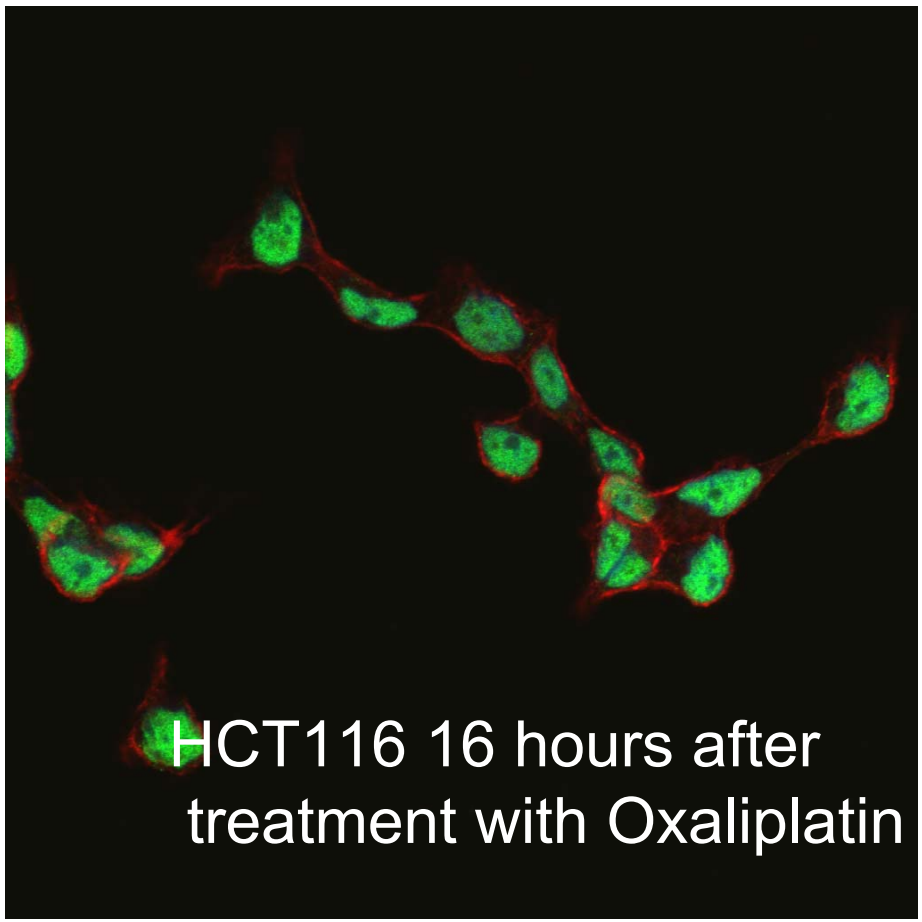


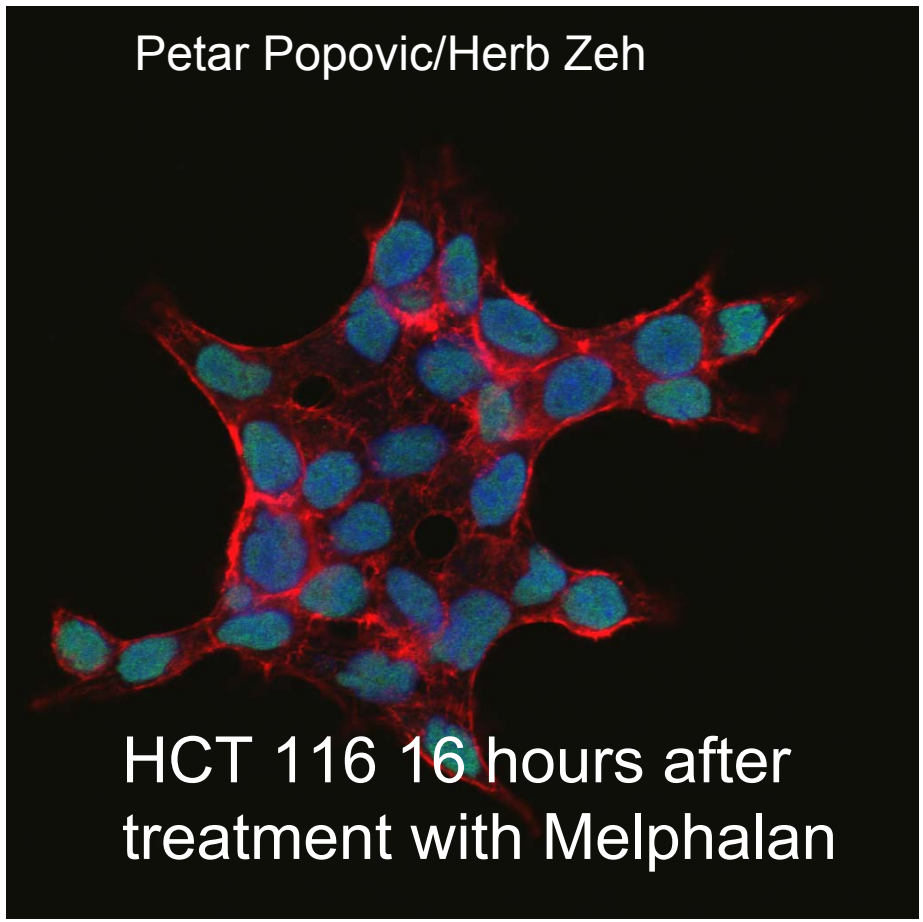
d(GpG) and d(ApG) adducts



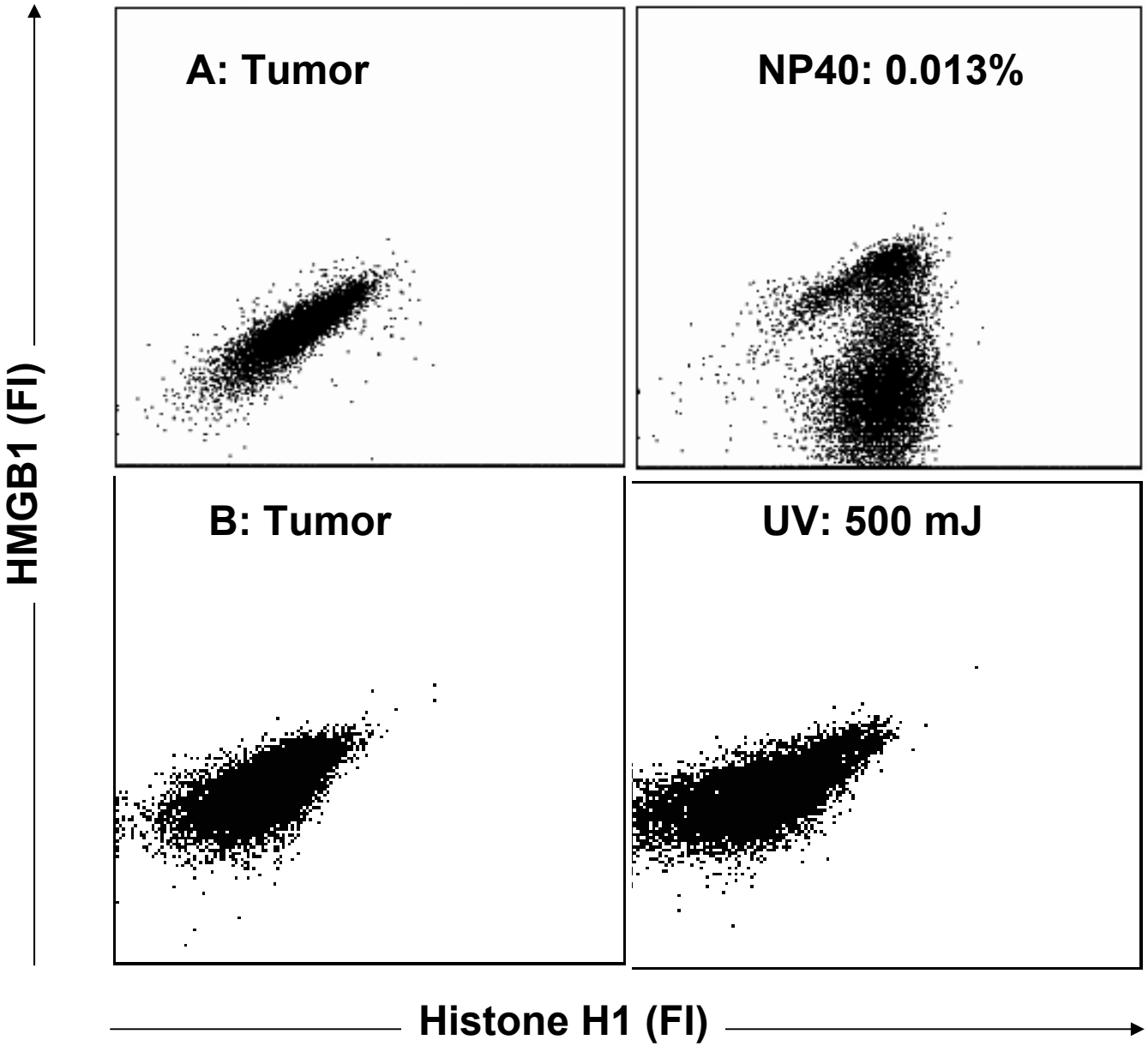


Release of HMGB1 from Melphalan But not Oxaliplatin Treated Cells

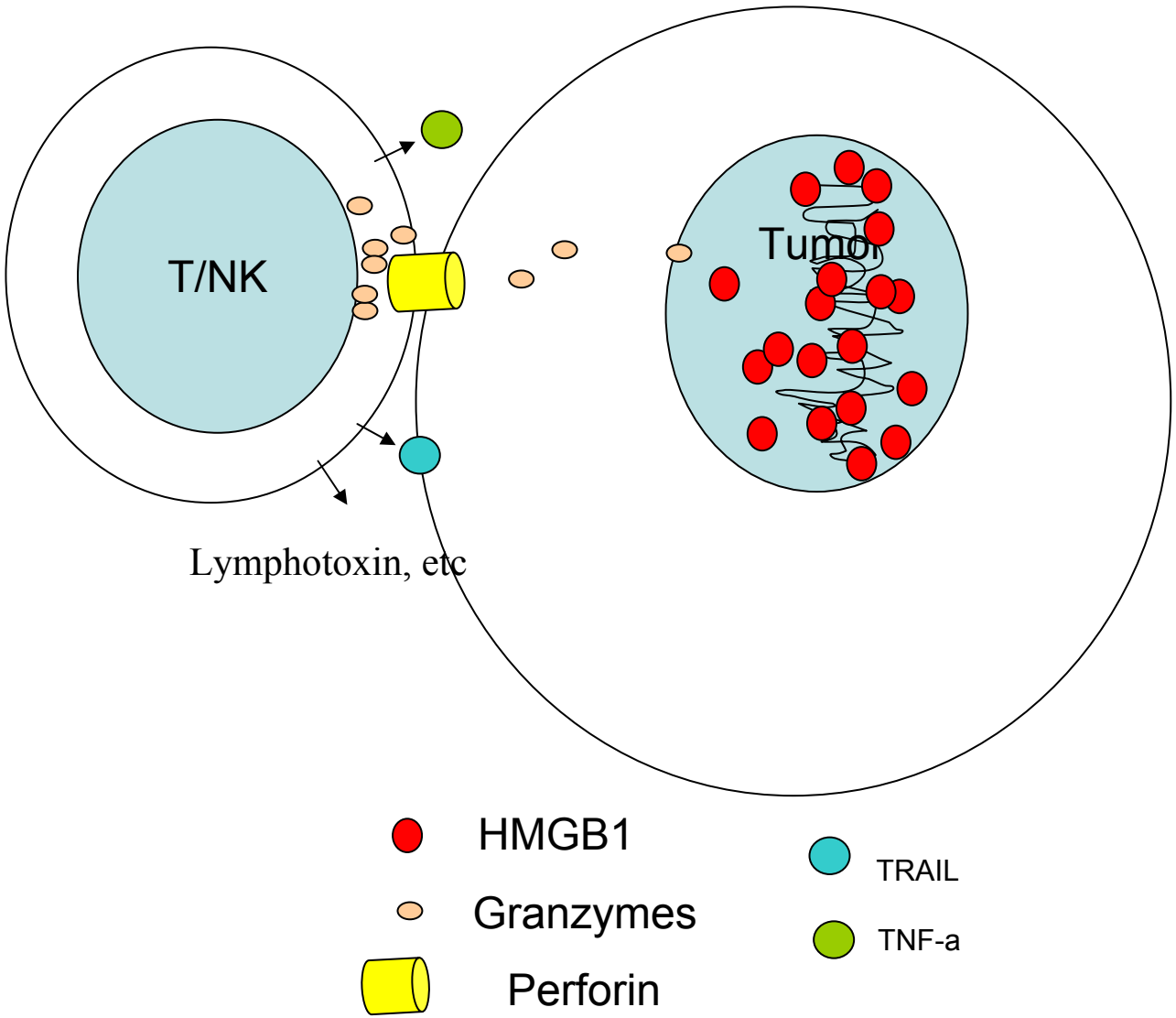




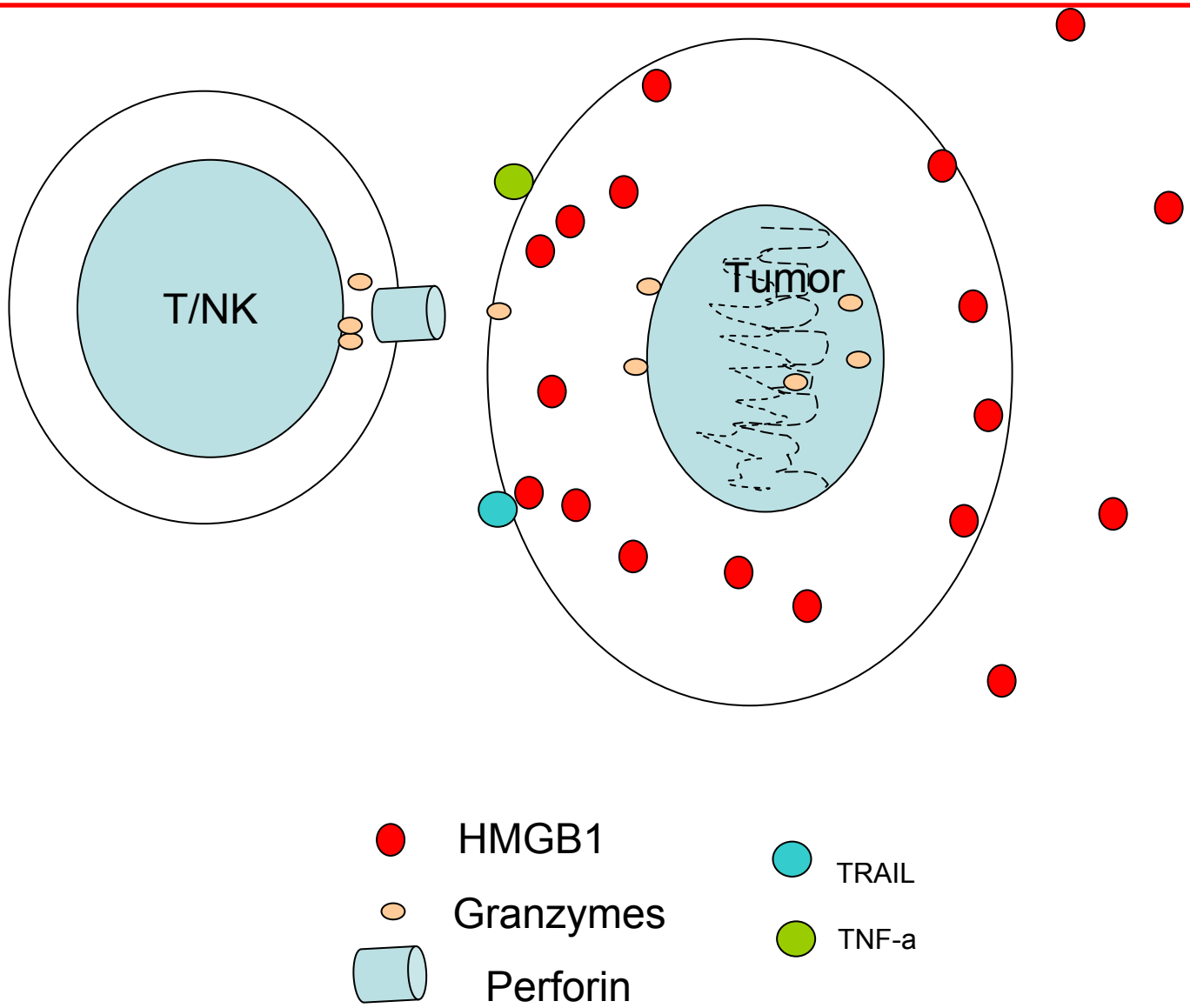
Flow Cytometry Intracellular HMGB1/Histone H1



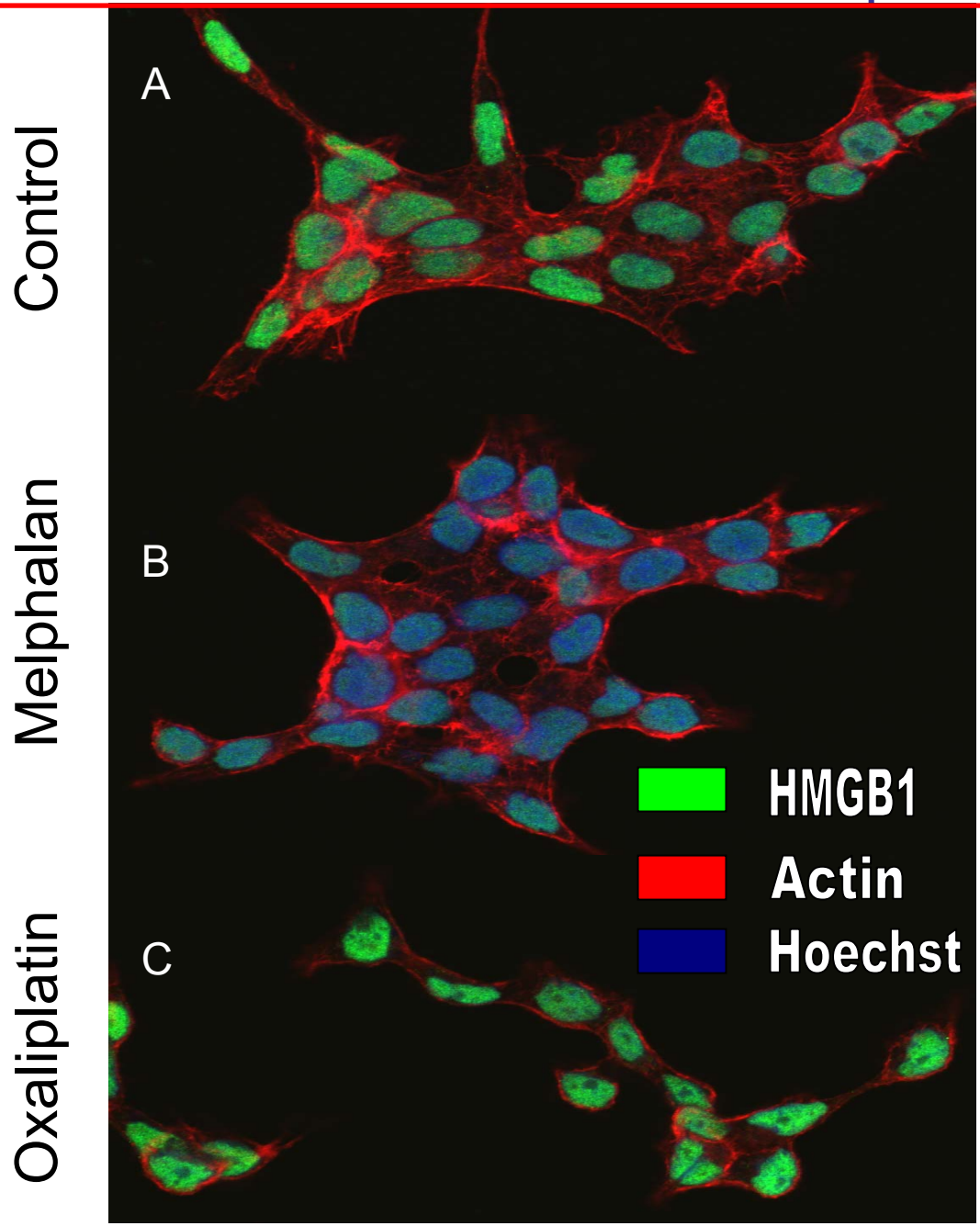
HMGB1 Release Following Immune Mediated Lysis?



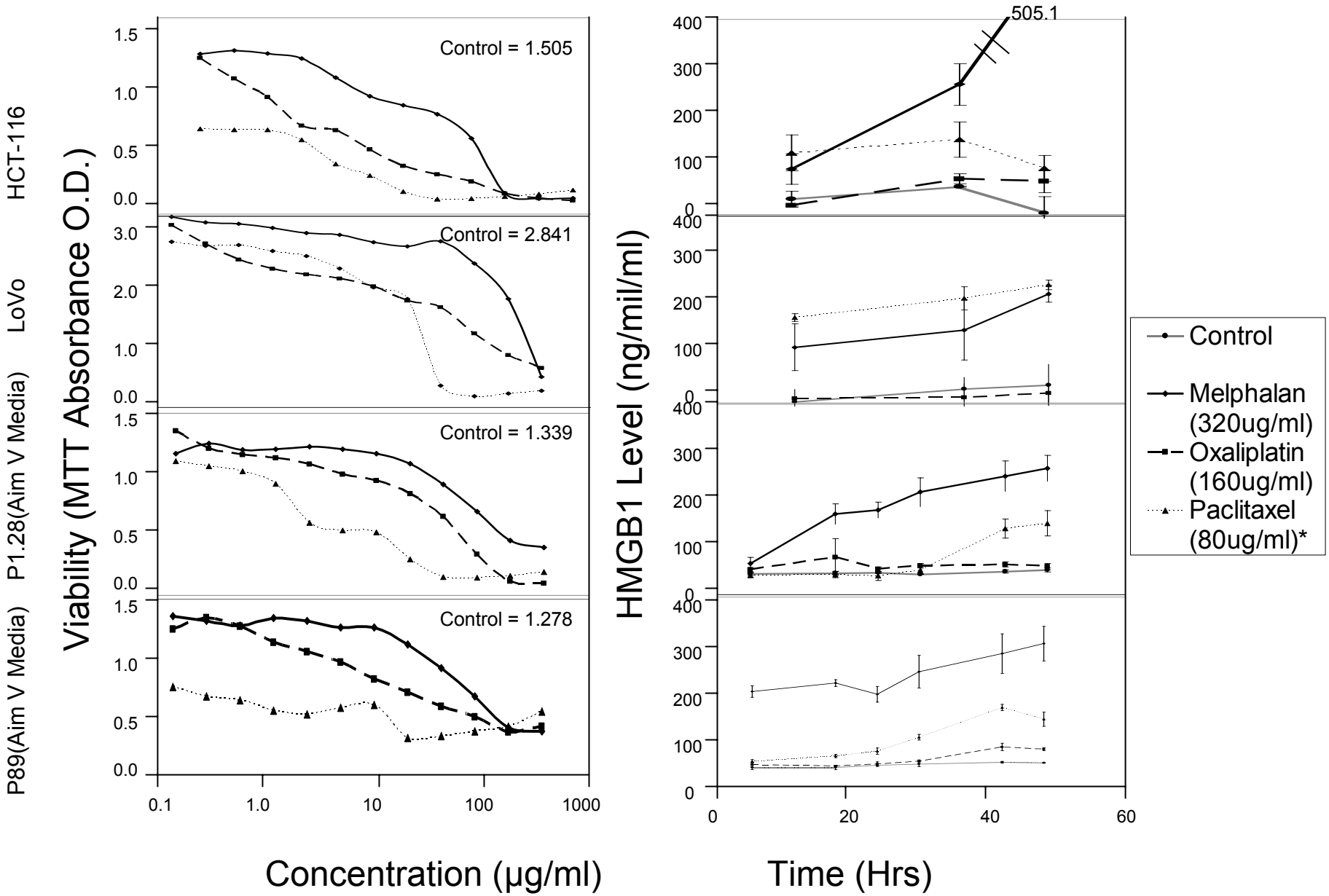
HMGB1 Release Following Immune Mediated Lysis?



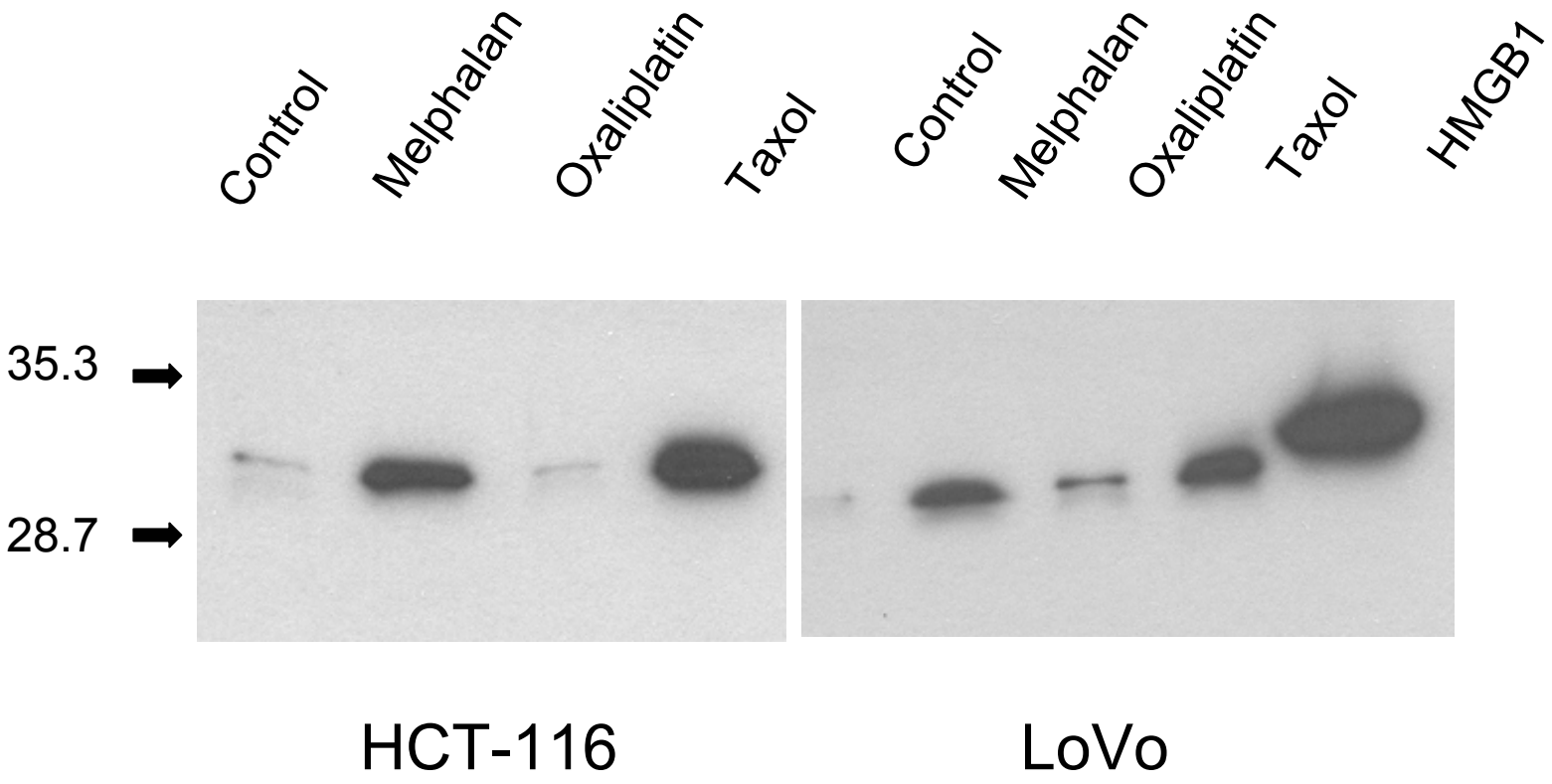
HCT-116 cells treated with chemotherapeutic agents



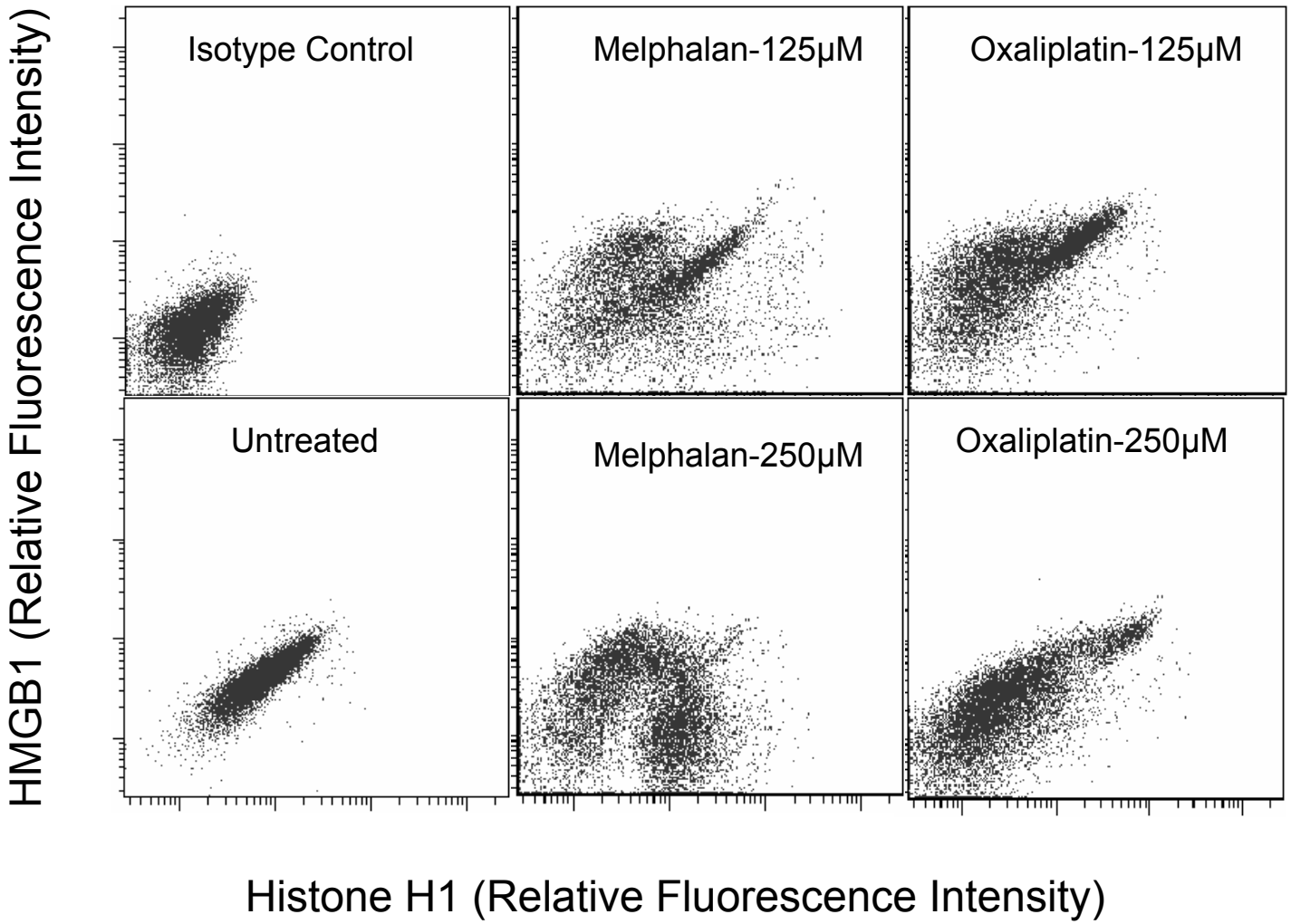
HMGB1 Release Assessed Within An ELISA



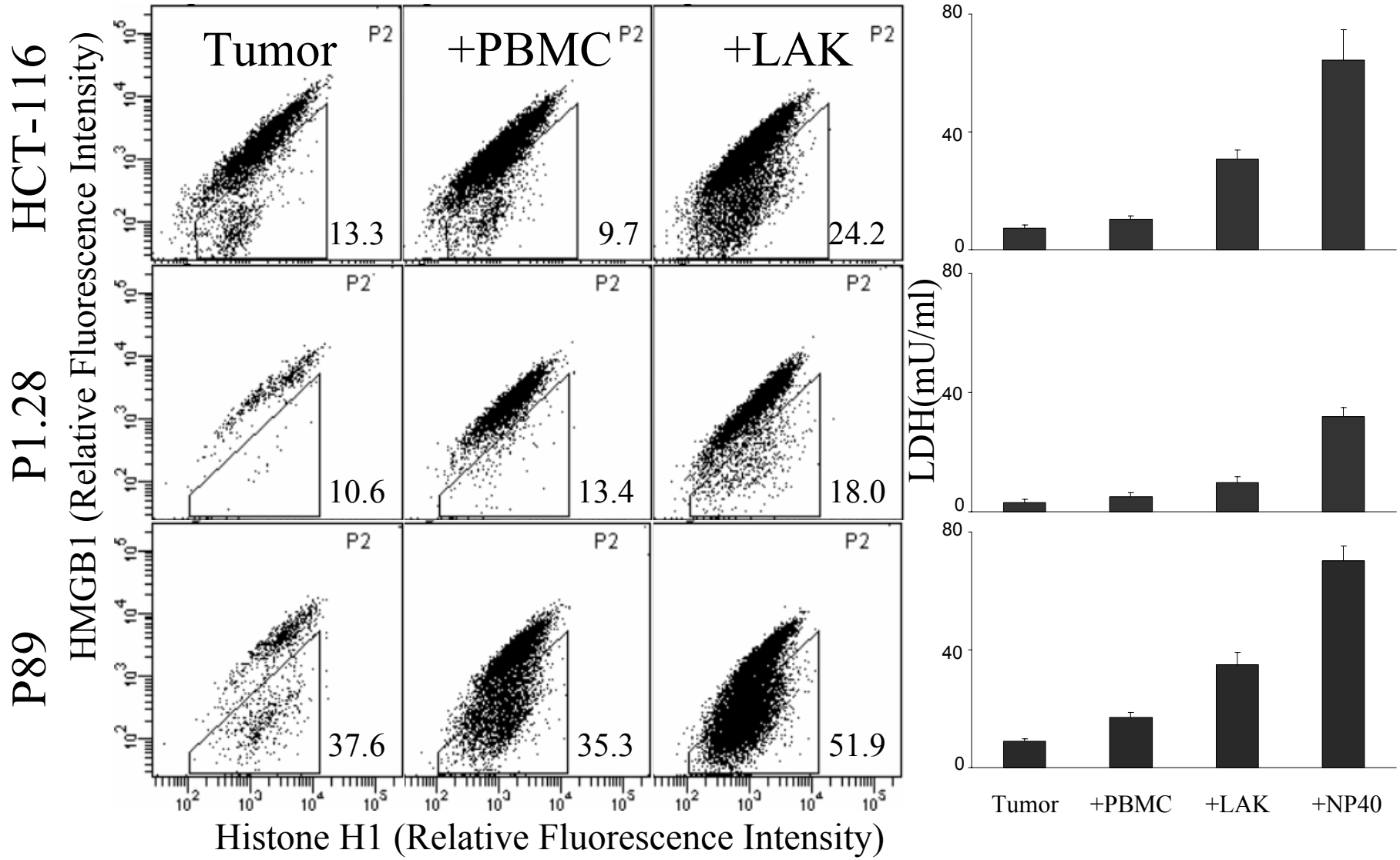
Western blot of supernatant derived from tumor cells incubated with chemotherapeutic agents [48 hours].



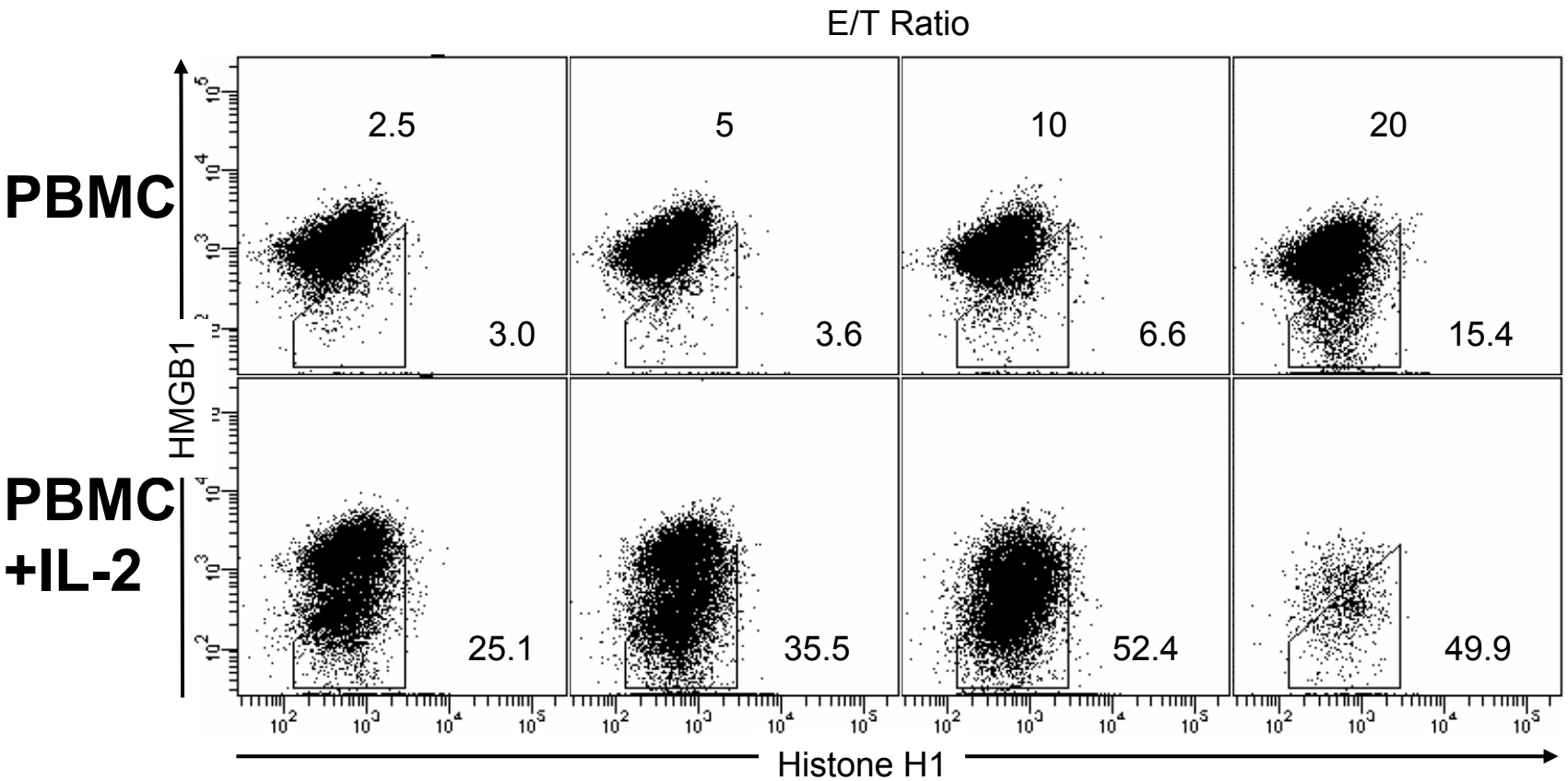
Intracellular nuclear staining following treatment with melphalan or oxaliplatin (WM2348 melanoma cell line)



Intracellular nuclear staining of HMGB1 diminishes following treatment with cells with LAK activity

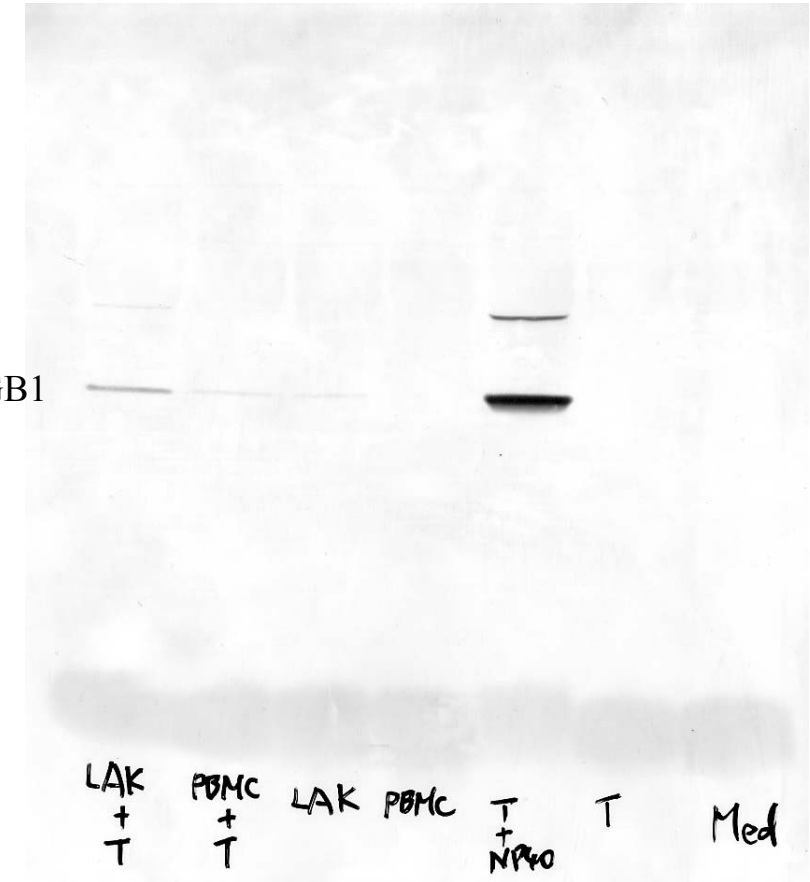


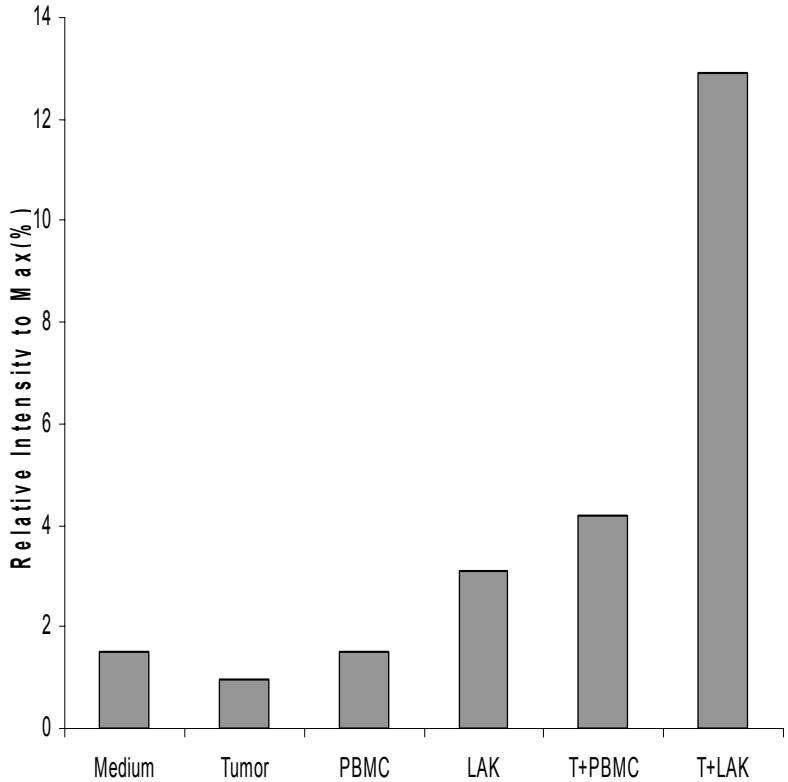
LAK Induces Intracellular HMGB1 Release



Immune Mediated Lysis of Tumor Cells Release HMGB1

HMGB1





Max(T+NP40)=100

Tumor: 451Lu, 4h, $5x10^6$/ml E/T=10/1

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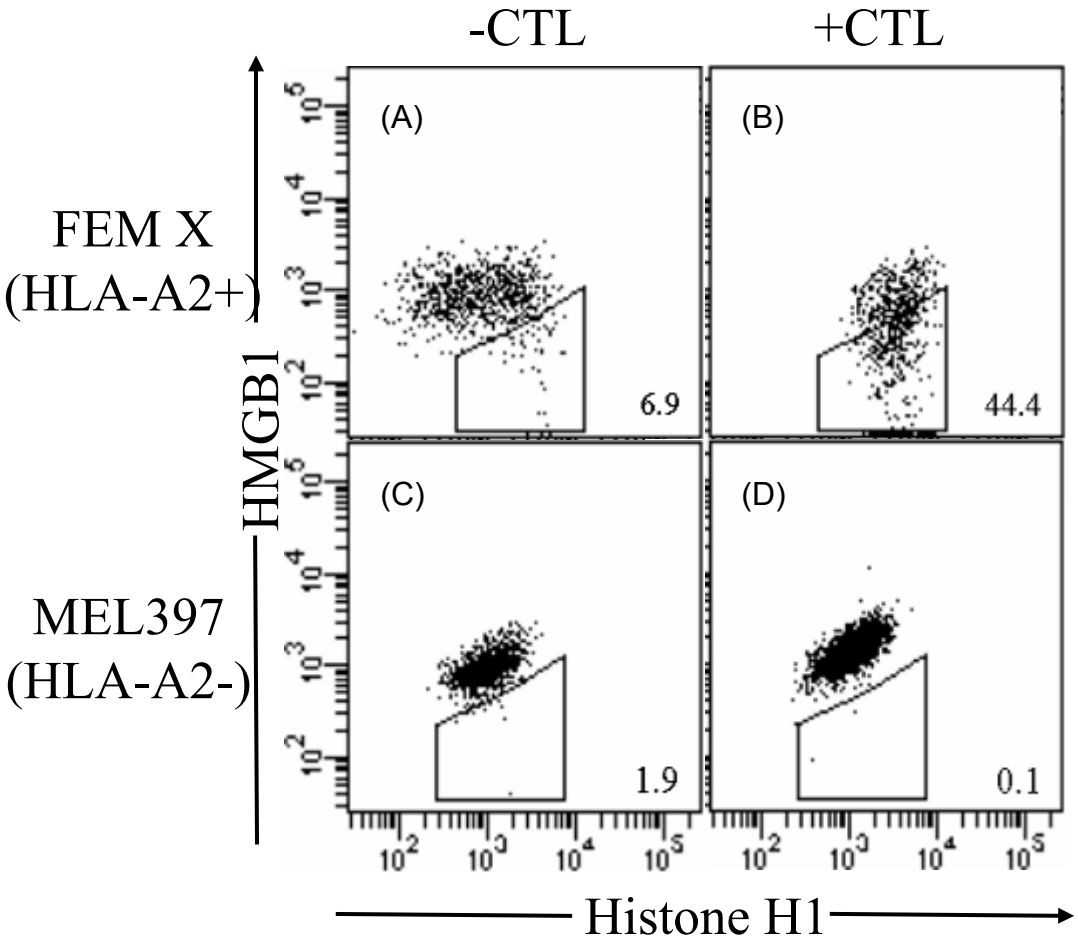

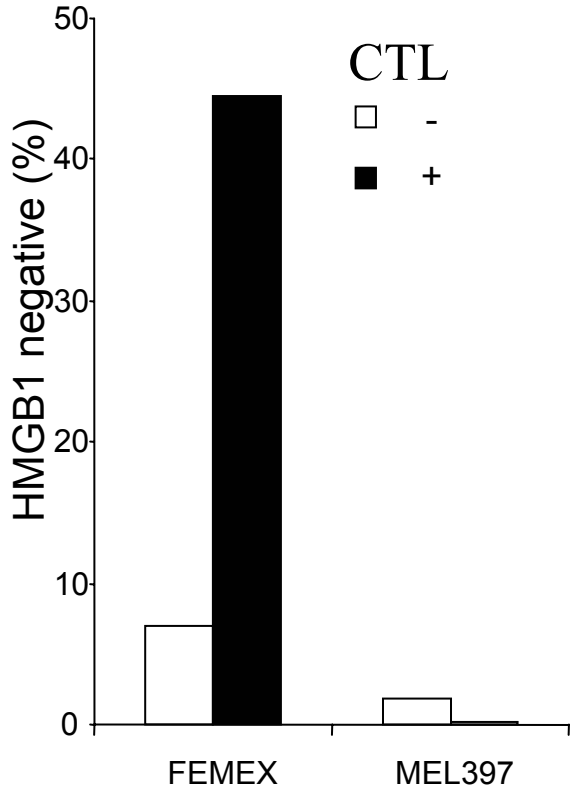

Apoptosis and Necrosis

At The Event Horizon

Conclusions 1: Necrosis and Apoptosis and HMGB1

- DAMPs and HMGB1 are links between inflammation and necrotic cell death
- Apoptotic cell death causes sequestration of HMGB1 in the nucleus [phosphorylation of Histone 2B] – preliminary Marco Bianchi
- Platination of dsDNA causes sequestration of HMGB1 in the nucleus [dGpG and dGpA]
- Treatment of cancer [and arthritis] with platinums may succeed because of their ability to sequester HMGB1

Conclusions 2

- HMGB1 is indeed a pleiotrophic endokine – an endogenous danger signal promoting DC maturation and found in the serum of acute and chronic inflammatory states
- HMGB1 synergizes with other cytokines in the mouse and man to promote acute immune reactivity [?Schlepping or Chaperoning]
- HMGB1 in chronic inflammation may promote PDC suppression, promoting healing
- Targeting HMGB1 with antibodies or soluble receptors may represent important strategies for treatment of microbial and inflammatory diseases

Contributors

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