Cytokines: Lessons From **Double Digit Cytokines**

IL-10, IL12, IL-15, IL18 and Counting [IL-35]

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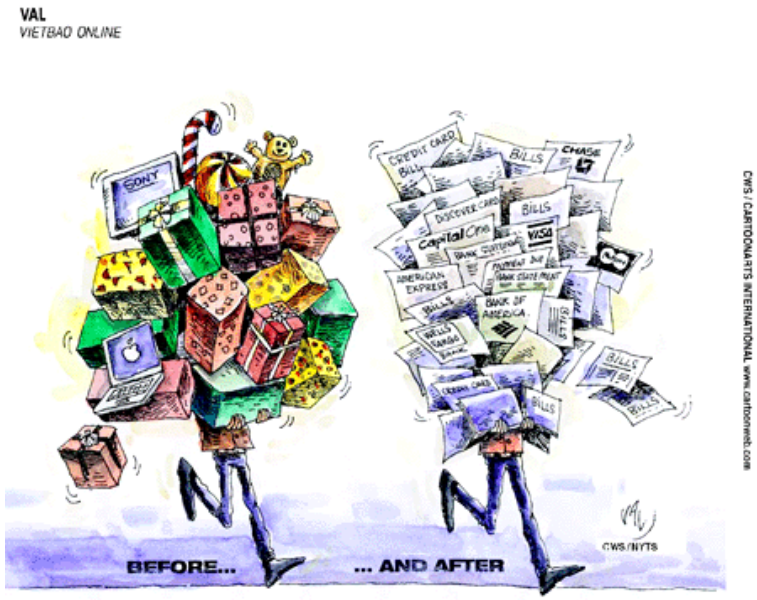

Cancer Vaccines 1990-2000

Study	# Patients	Objective Responses
Muc1+ BCG	63	0
Mel Peptide+Adj	28	0
Mel Peptide+IL-12	28	0
Mel Peptide+iDC	28	3
ANK+IL-2	6	0
TIL/IL-2/IL-4	4	1
IL-12 protein	40	2
IL-4 Gene Rx	18	3
IL-12 Gene Rx	44	4
Total	**259**	**13 [5%] 1 Long Term Survivor**

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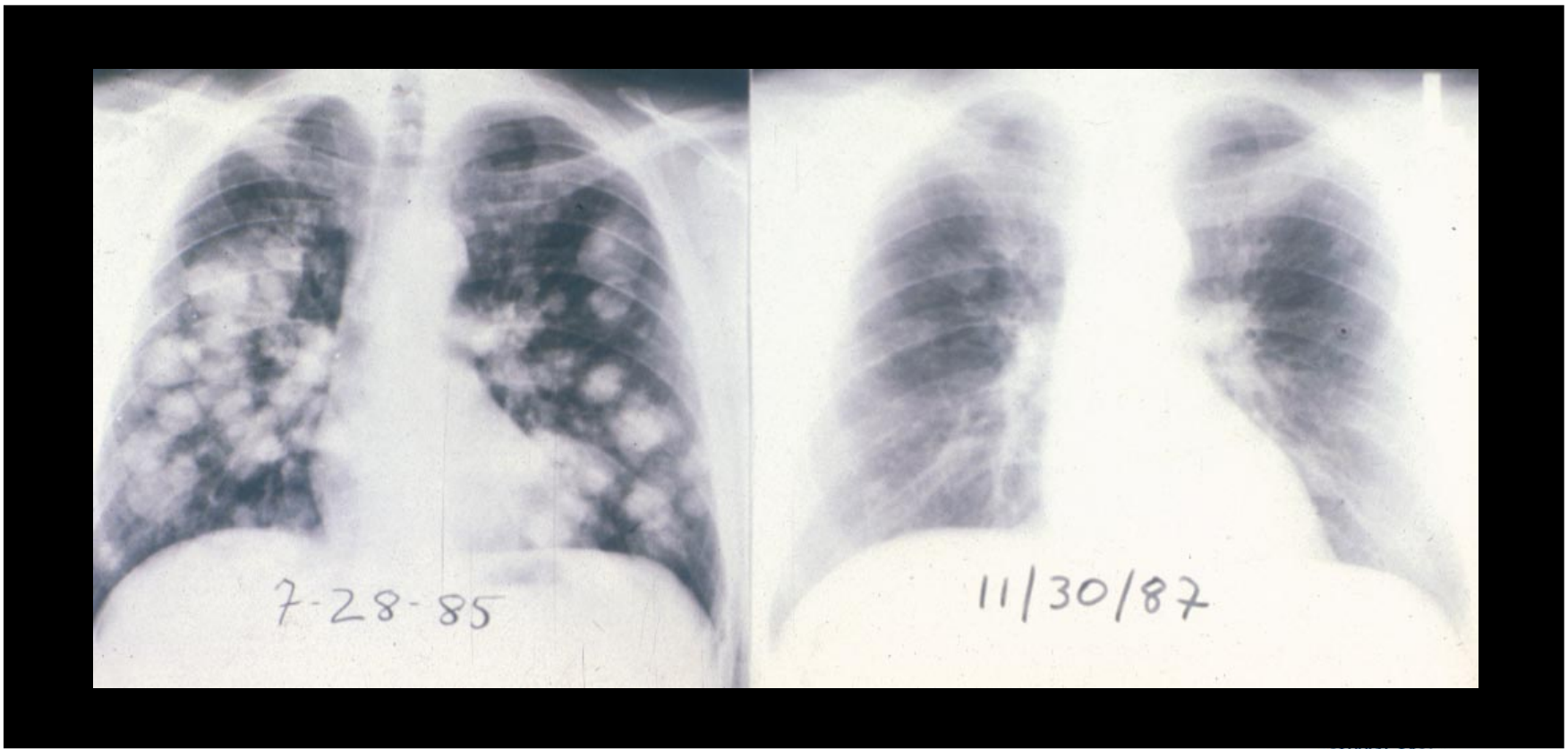
- During the early days of biologic therapy, novel purified recombinant cytokines were seen as suitable agents for exploration in clinical trials –"BRMs".
- Their major perceived role was to promote inflammation and to expand hematopoietic cells more generally and more specifically to enhance tumor mediated killing by NK cells and T-cells; similar to what we expected from ChemoRx and RØTx
- Many of these earnest early attempts at cytokine or cytokine gene therapies were based on an erroneous sense that there was defective recognition of tumors by immune effectors
- Most adult tumors arise in the setting of chronic inflammation, have an established T-cell response and simple cytokine therapies will unlikely be effective for most patients with cancer.

Cytokines: Lessons From Double Digit Cytokines

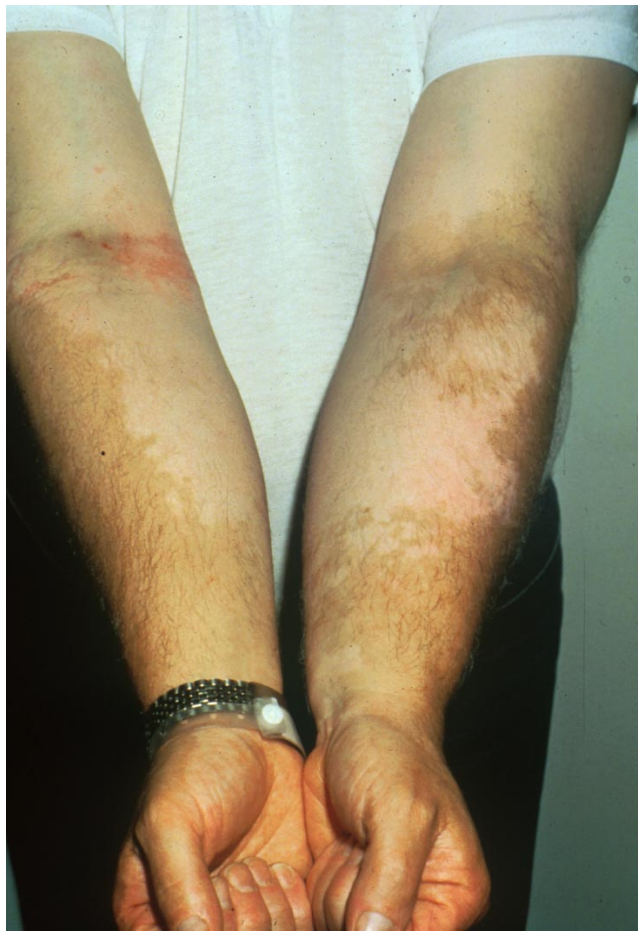
IL-10, IL12, IL-15, IL18 and Counting [IL-35]

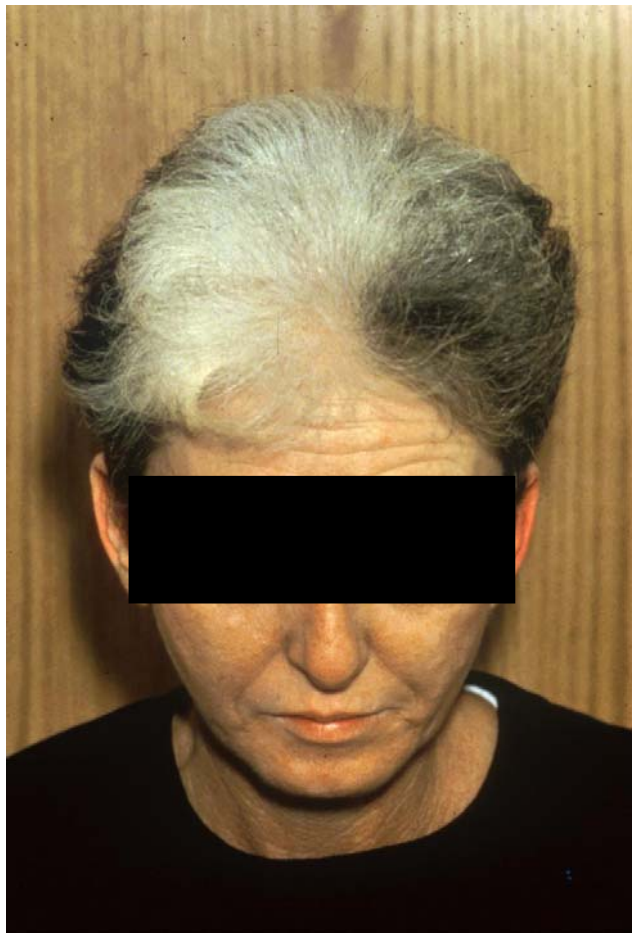
- Key Strategic Decisions Almost Always Made Based on Incomplete Information; Mouse Models Biased and Can be Misleading
- Impact of Regulatory Interactions – Fear of Adverse Outcomes and Decreased Tolerance of Risk for Patients with Limited Longevity and Options
- Financial Considerations: Projected Costs vs. Reality; Cost of Goods; Competition in the Marketplace; Corporate History
- Lessons Learned Not Used in Future Trials

Melanoma Patient Response Before and After High Dose IL-2



Vitiligo in Patients Receiving IL-2





A Crack in the Fabric of the Universe - IL-2

- Roche after substantial investment bowed to Cetus PEG-IL2
- Only one approved therapy for melanoma in Aug 1990, DTIC
- Cetus Application 8/90 - Sentiment that spontaneous regressions in the disease more common than currently believed
- 'Toxicities' including death had been realized by this time
- Risk/benefit ratio not thought to be suitable
- Safety pattern comparable to antibiotics was thought to be critical
- 100's of patients Rx with well tolerated regimen; 2% death
- ODAC did not recommend for approval

- TNF Gene Therapy approved by the RAC for clinical testing with no data in murine models

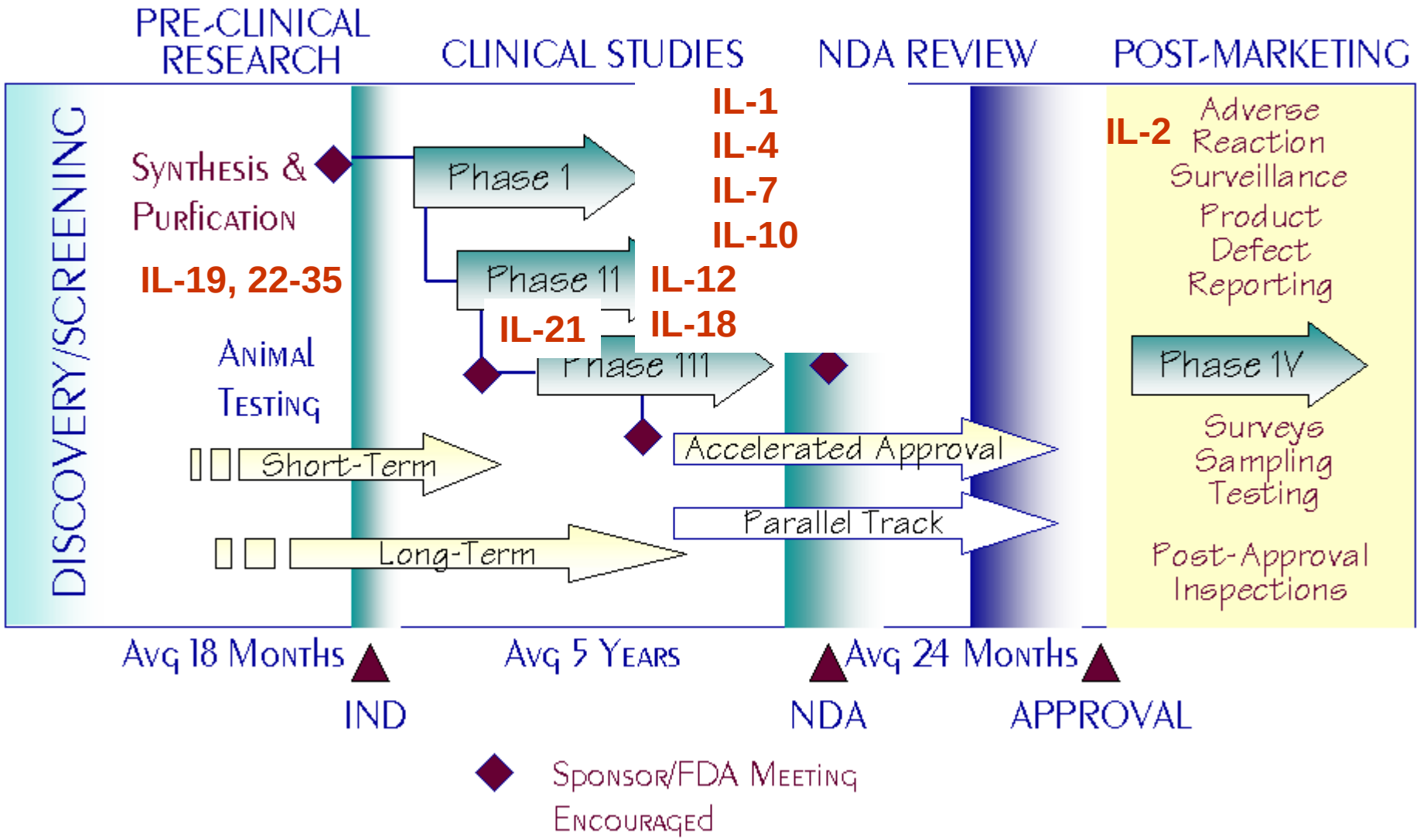
Other IL-2 Family [IL-9, IL-15, IL-21]

•**Interleukin 9.** Originally described as a TH2 cytokine, it has not been given to patients. Its inhibition is suggested by intriguing studies in allogeneic skin transplants in which it appears to be critical for maintenance of Tregs through a mast cell dependent process; Ab MedImmune.

•**Interleukin 15.** IL-15 has yet to enter clinical trials; Shares β and γ chains of IL-2; increases T-cells specific for tumor without impacting on Tregs and to be required for NK expansion; might be useful in the treatment of patients with human T cell lymphotropic virus I-associated myelopathy/tropical spastic paraparesis, rheumatoid arthritis, multiple sclerosis or refractory celiac disease, **mucosal protection**.

•**Interleukin 21.** Shares a common γ chain receptor with other members of the extended IL-2 family; has recently entered clinical trials in patients with renal cell carcinoma and melanoma and is associated with cytokine like effects. Anecdotal responses have been observed in patients with these diseases.

ROLE OF FDA IN NEW DRUG DEVELOPMENT



Interleukin 10 [IL-10, -19, -20, -22,-24]

- The IL10 family members are closely related to the interferons. Promotes NK and T cell cytotoxicity; retention
- IL-10 also exerts anti-inflammatory actions by counteracting many biological effects of interferon gamma (IFN-γ)
- IL-10 has never been tested in patients with cancer; has been given to patients with inflammatory bowel disease, with minimal improvement in patients treated
- Treated patients had significant increases in serum neopterin and PHA induced IFN-γ production
- The newer IL-10 family members, IL-19, IL-20, IL-22, and IL-24 have yet to be tested in the clinic or to have demonstrable antitumor activity.

Interleukin 10 [IL-10, -19, -20, -22, -24]

Berman RM, Suzuki T, Tahara H, Narula SK, Robbins PD, Lotze MT. Systemic administration of cIL-10 induces effective, specific and long-lived immune response against established tumors in mice. J.Immunology 157:231-238, 1996.

Virtually every gene therapy with IL-10 profound antitumor activity [vIL-10 promotes]

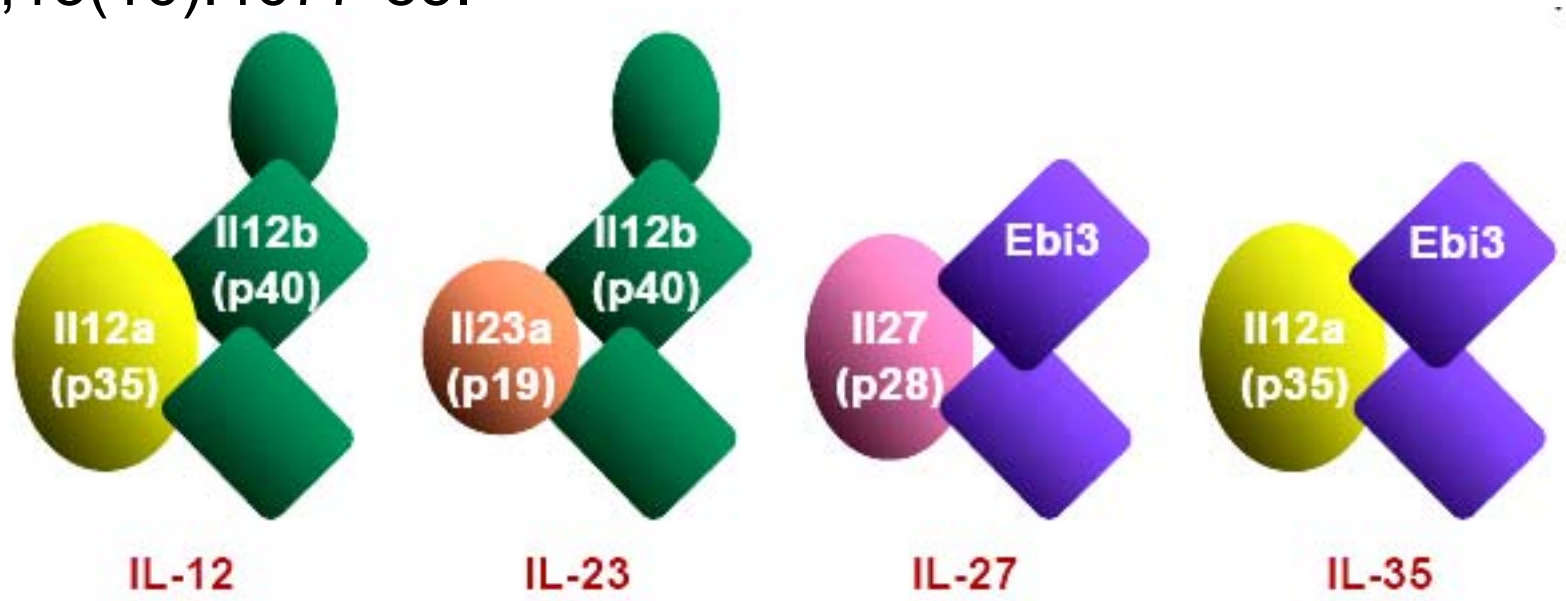
Being developed in a Small Drug Big Pharma

IL-2+IL-10=Interleukin 12

- First possible utility observed in murine gene therapy models
- Toxicity observed at much lower dose than expected [long half life of IL-12]
- Major collaboration [Genetics Institute and Roche] made impossible by financial considerations
- Corporate histories [Genetics Institute – Erythropoietin; Roche – Interleukin 2] complicated development
- Drugs developed by Oncology teams experienced with small molecule development; inexperienced with biologics
- Findings of early toxicity with two deaths likely unrelated to direct cytokine effects limited development
- Tachyphylaxis suggested alternative drug development strategies but these were not promoted.

Interleukin 12

Del Vecchio M, Bajetta E, Canova S, Lotze MT, Wesa A, Parmiani G, Anichini A. Interleukin-12: biological properties and clinical application. Clin Cancer Res. 2007 Aug 15;13(16):4677-85.



IL-10

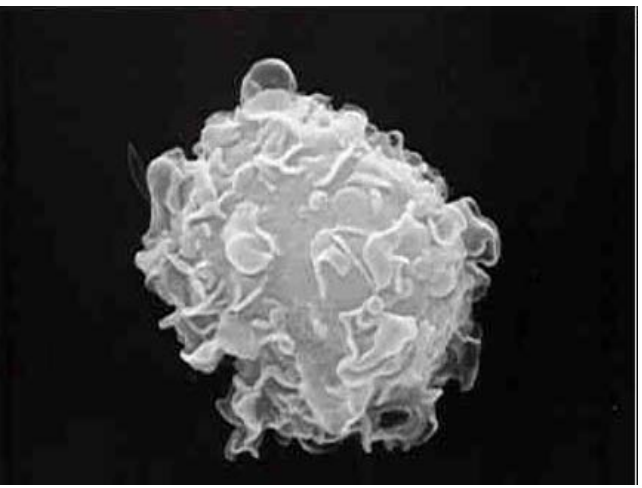
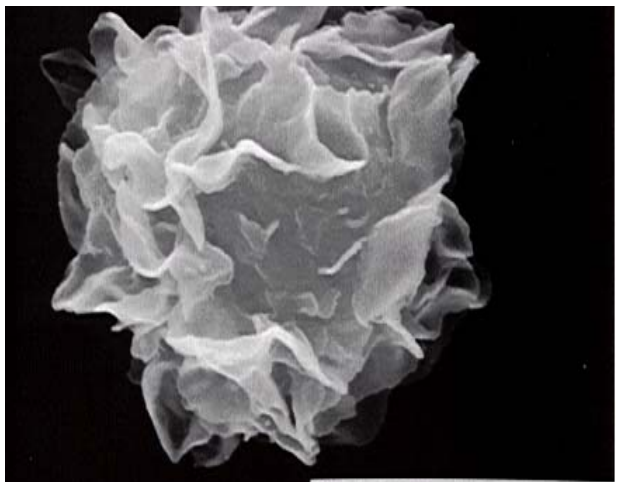
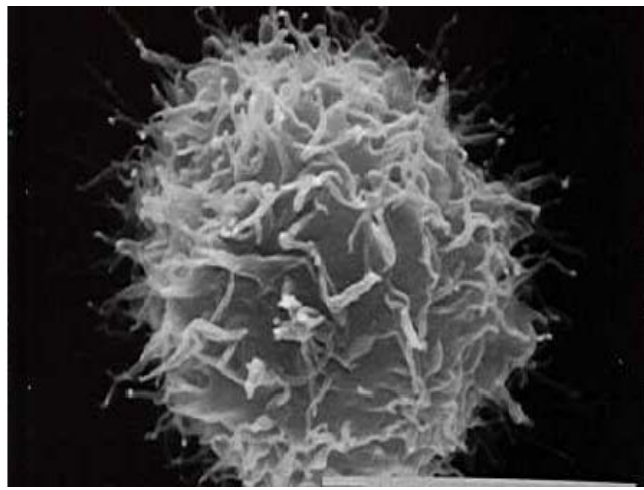
Interleukin 12

[a]Tumors	Route	Pt	O.R	[c]Immune modulation	[c]Angiogenesis-related effects
Different solid tumors*.	i.v.	40	5%	-Dose-dependent ↑ sIFN-γ;peak at 24-48 hrs -↓ $CD4^+/CD8^+$ and $CD16^+$ cells; nadir at 24 hrs -↑ of NK cell adhesion molecules (CD2, LFA-1).	N.D.
Melanoma *.	s.c.	10	0% 3 MRs	-↑ sIFN-γ within 24 hrs -↑IL-10 during the second cycle;	-↓ urine bFGF in 2/3 pts with MR
Renal cell carcinoma *	s.c.	51	2%	-↑ sIFN-γ with peak level at 24 hrs after the first maintenance dose	N.D.
CTCL	s.c. or i.t.	10	56%	-↑ $CD8^+$ and/or TIA-1^+ T cells in skin biopsy from regressing lesions.	N.D.
Melanoma, renal cell carcinoma	i.v.	28	3%	-Induction of IFN-γ, IL-15 and IL-18, maintained in pts. with tumor regression or prolonged disease stabilization	N.D.
Renal cell carcinoma ♦	s.c.	30	7%	-↑ sIFN-γ, IL-10 and neopterin, maintained in cycle 2.	N.D.
Abdominal tumors*	i.p.	29	7%	-↑ peritoneal $CD3^+$ and ↓$CD14^+$ cells	-↓ bFGF and VEGF in tumor;

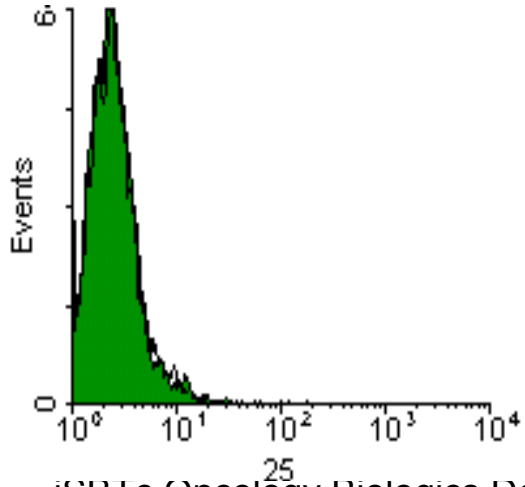
Interleukin 12

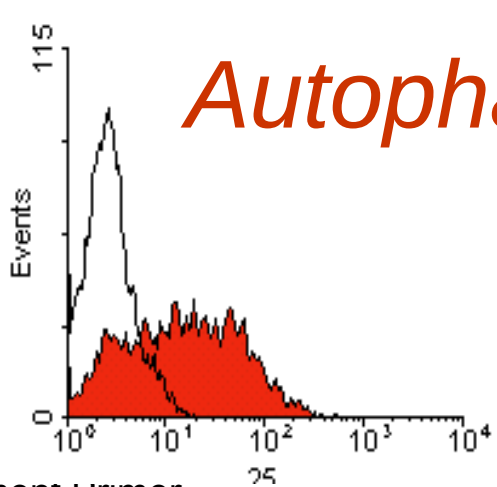
Bladder cancer*	Intra-vesical	15 pts	0% RR	No urine/serum IFN-γ induction	N.D.
Renal cell carcinoma *	s.c.	26	NA	-dose-dependent ↑ sIFN-γ, TNF-α, IL-10, IL-6 and IL-8 at first injection. -Lymphopenia; -Further ↑ IL-10 during treatment	N.D.
Cervical carcinoma ♦	i.v.	34	3%	-↑ lymphoproliferative responses to HPV 16 E4, E6 and E7 peptides.	N.D.
Head-neck carcinoma *	intratumoral	10	ND	-↑ $CD56^+$ NK cells in the primary tumor; -high IFN-γ mRNA expression at lymph node level	N.D.
AIDS-related Kaposi Sarcoma*	s.c.	34	50%	-↑ sIFN-γ after 1st dose, persisting after week 4	↑ sIP-10 after the 1st dose, persisting after week 4
Mycosis fungoides ♦	s.c.	23	43%	N.D.	N.D.

IL-2 Receptor-α (CD25) Expression Increases With DC Maturation

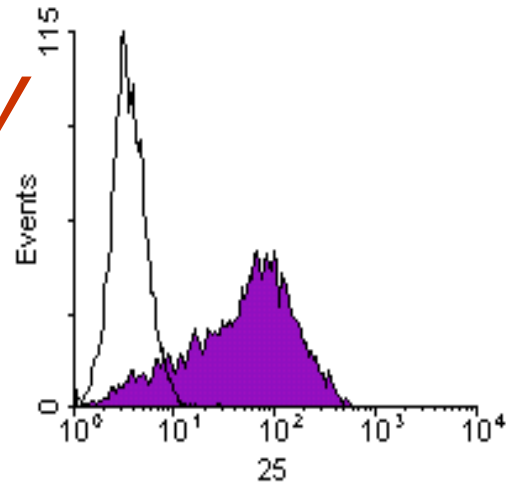




INTERLEUKIN 2 RECEPTOR ALPHA CHAIN



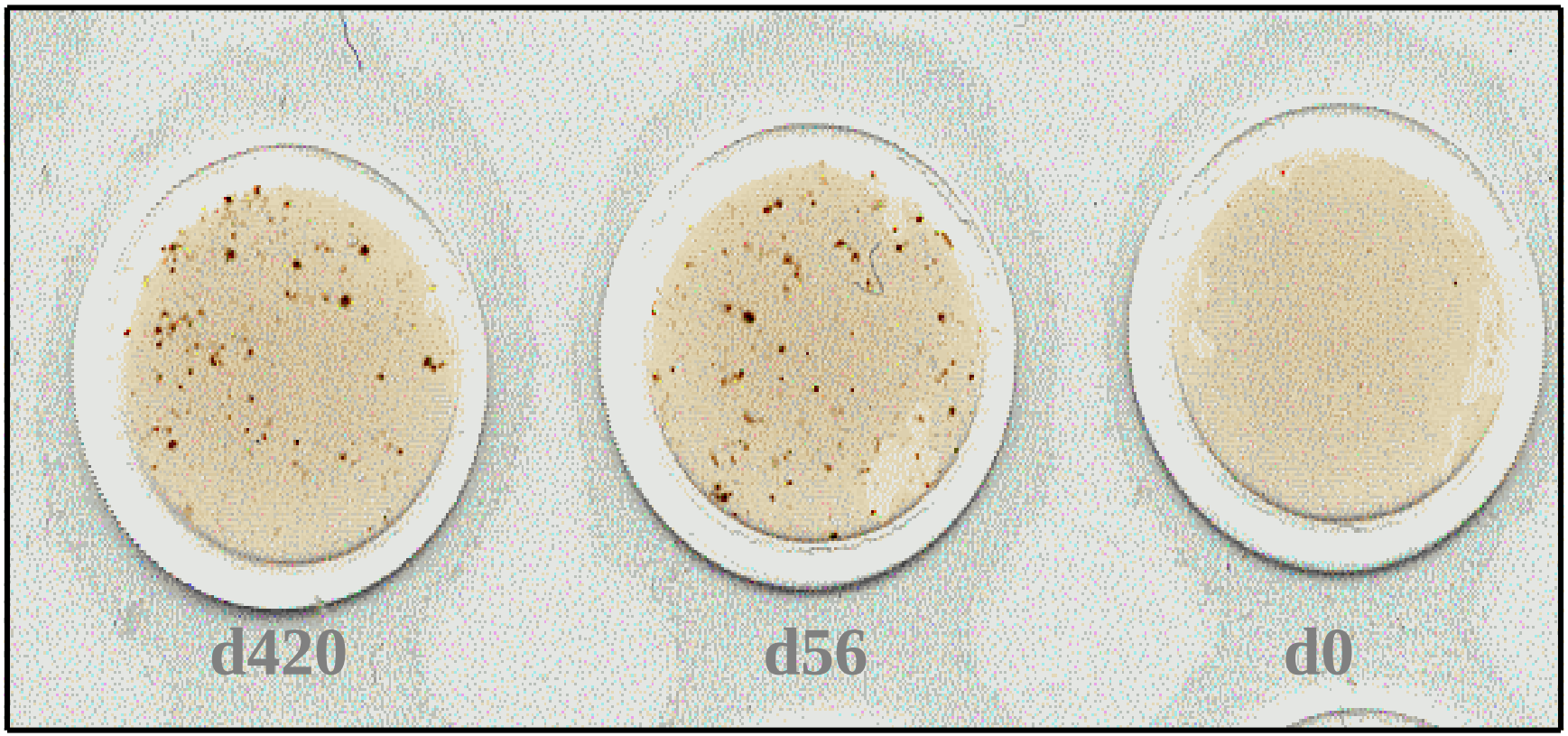


Autophagy

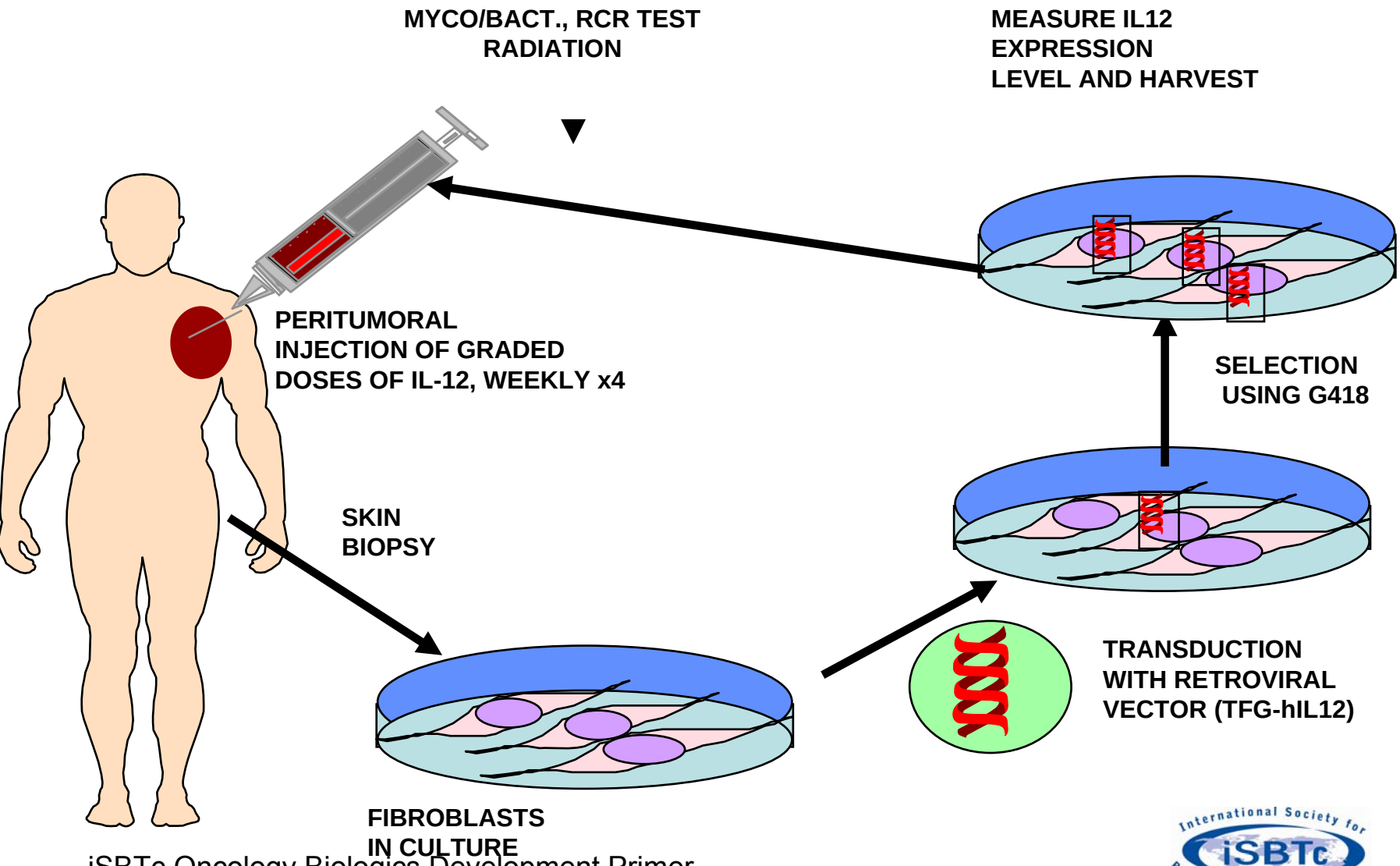


IMMATURE DC MATURE DC/MAC SUPE IL1,IL6,TNR,PGE2

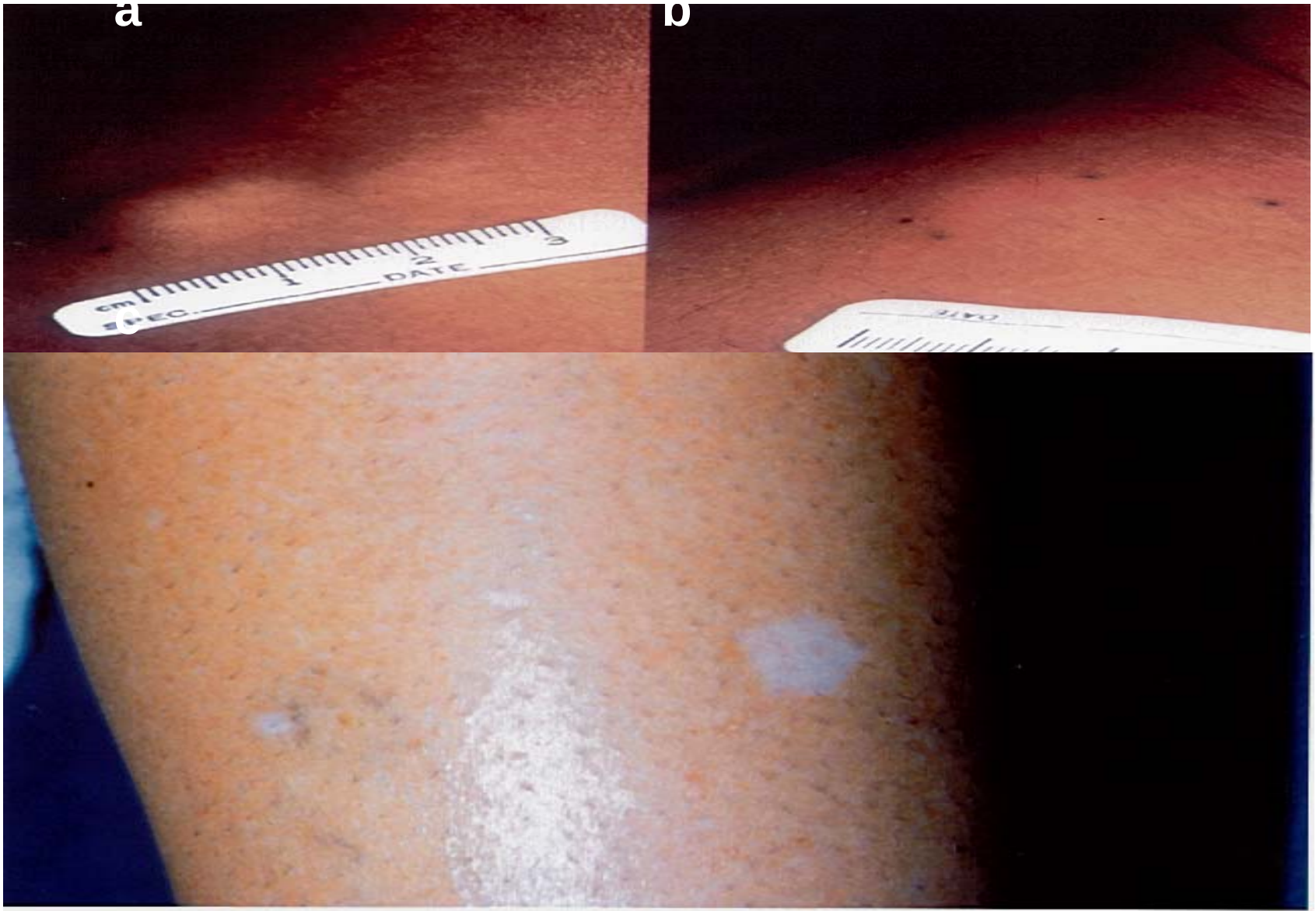
MM1 Anti-gp100 280-288 CD8+ PBMC-T Cell Response: IFN-g ELISPOT Analysis Pre-/Post- Vaccination



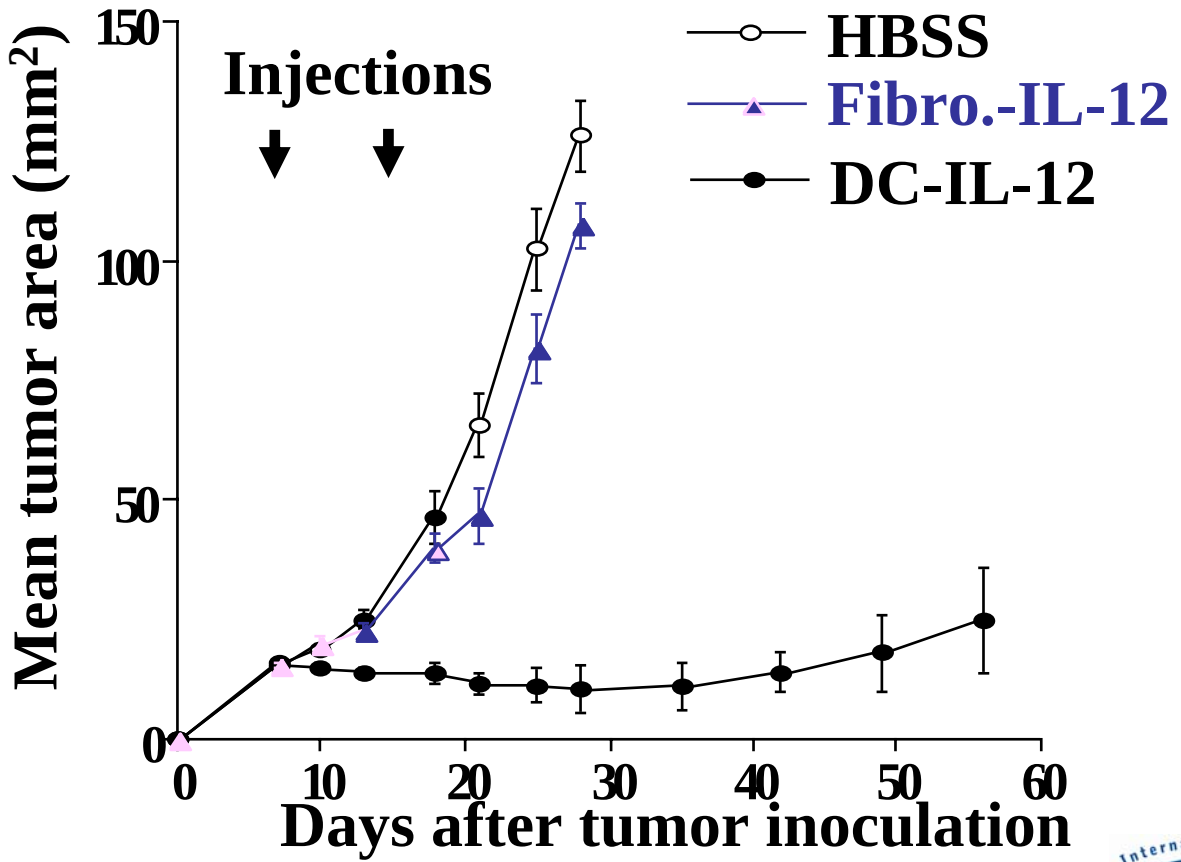
Interleukin 12 Gene Therapy



Responses to IL12 Gene Therapy in Patients with Melanoma/H&N Cancer



Repeated Administration of IL-12-DC Is Associated with Profound Antitumor Effects



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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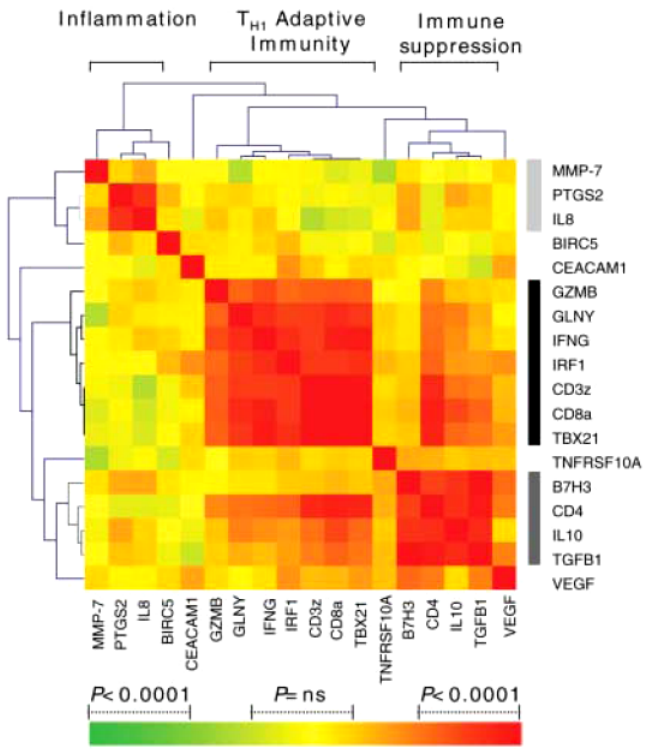


Tumor histopathology

UICC-TNM Staging system

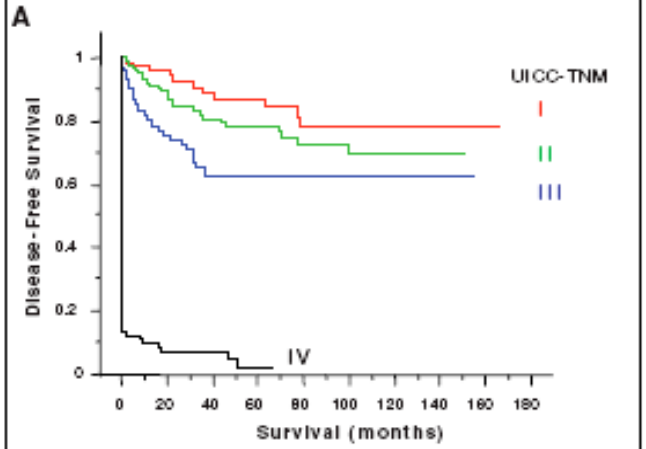
Tumor infiltrating immune cells

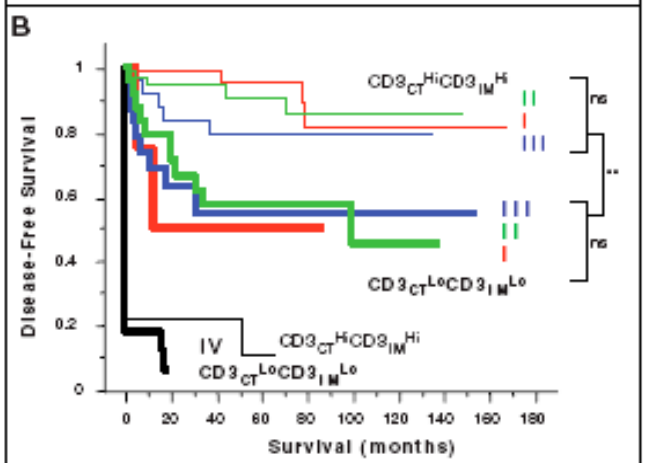
$CD3_{CT}CD3_{IM}$ evaluation

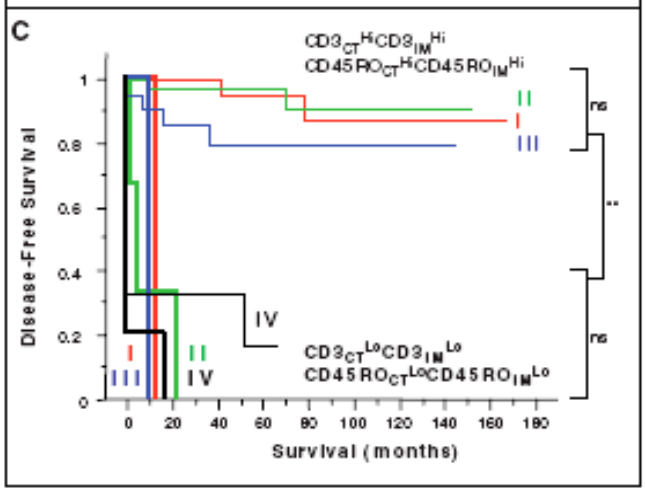
$CD3_{CT}CD3_{IM}$ evaluation

plus

$CD45RO_{CT}CD45RO_{IM}$ evaluation







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doi:10.1038/nature04808

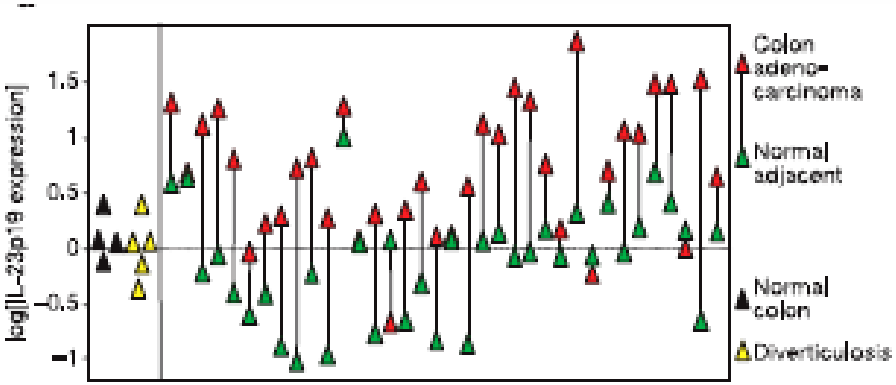
nature

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

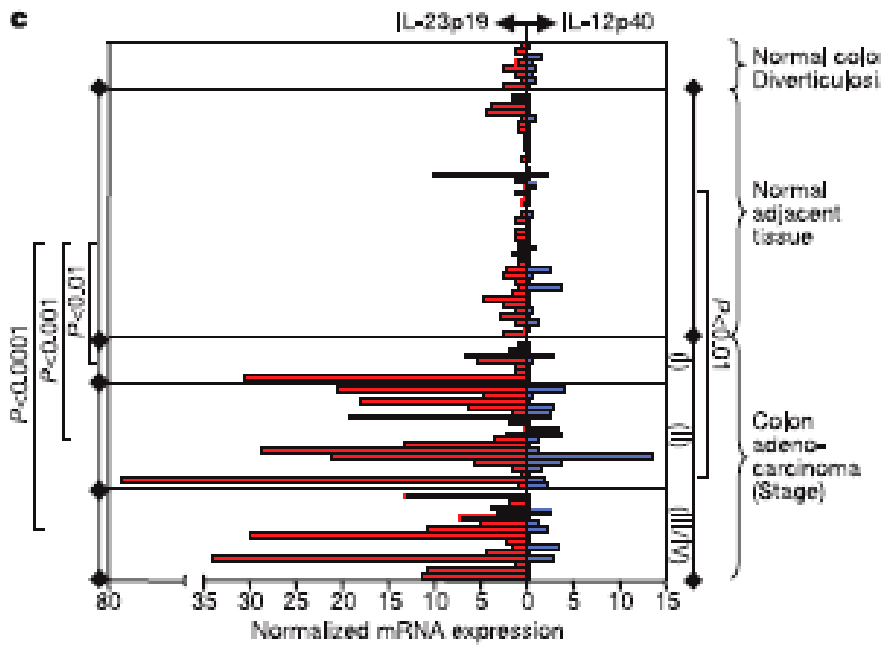
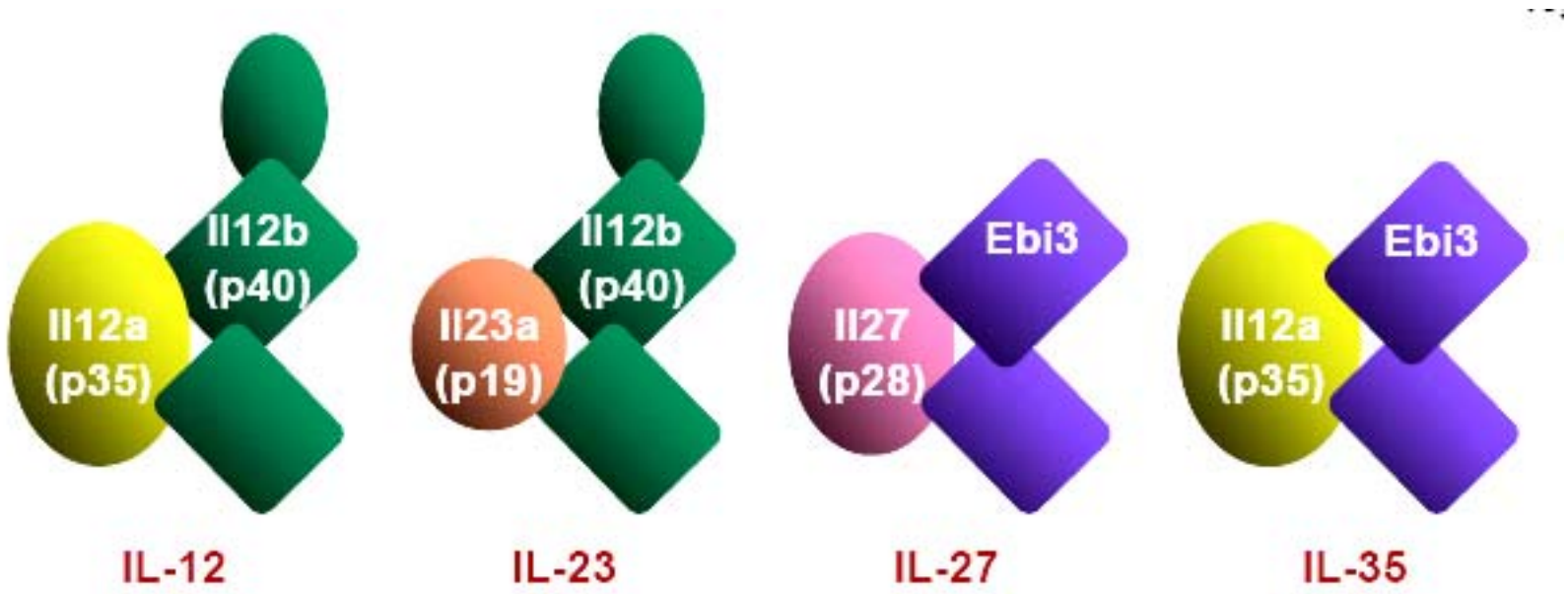


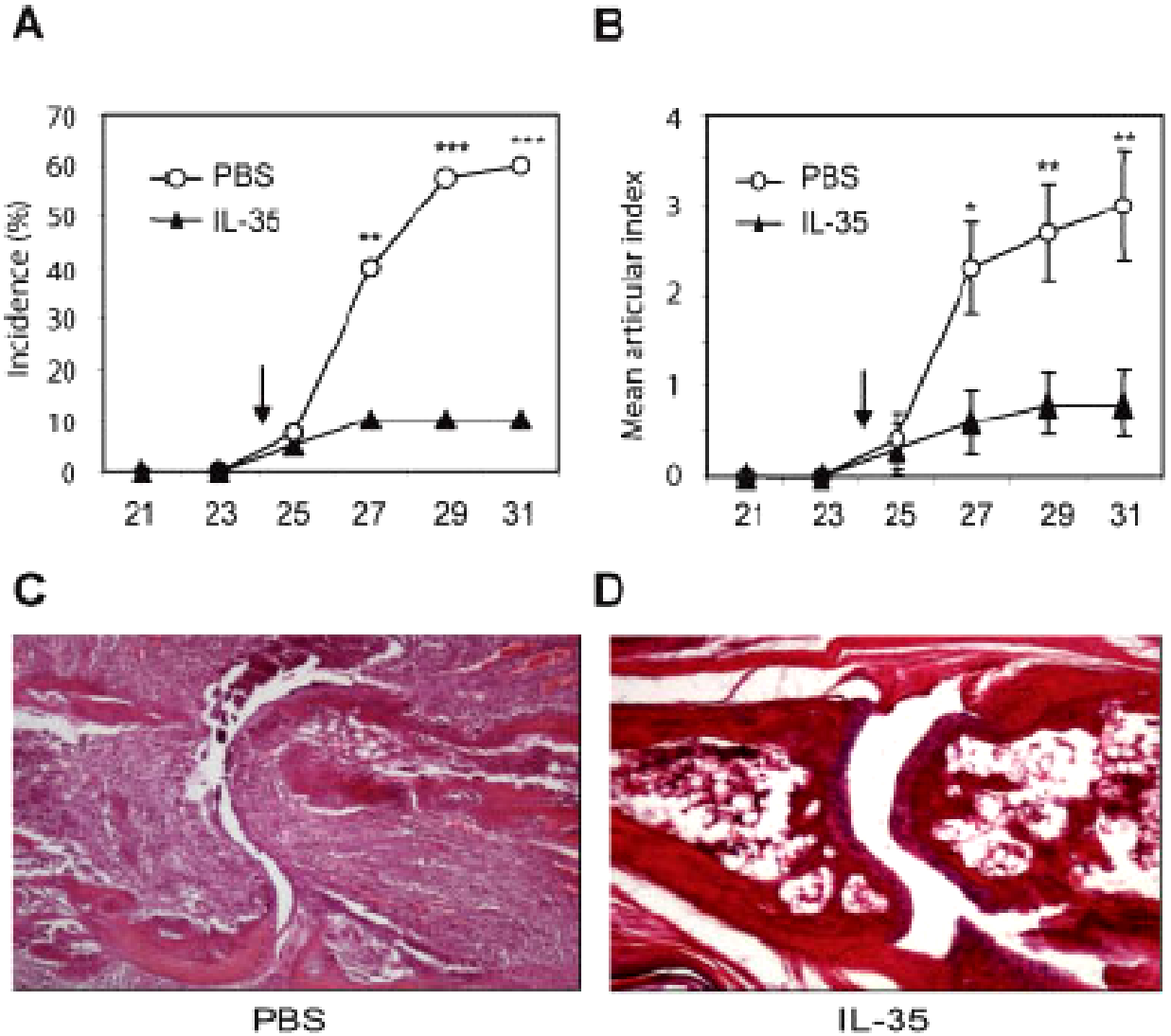
Figure 1 | Overexpression of IL-23 but not of IL-12 in human cancer.

Interleukin 35

Collison LW, Workman CJ, Kuo TT, Boyd K, Wang Y, Vignali KM, Cross R, Sehy D, Blumberg RS, Vignali DA. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. Nature. 2007 Nov 22;450(7169):566-9.



IL-35 suppressed disease development in CIA in DBA/1mice.

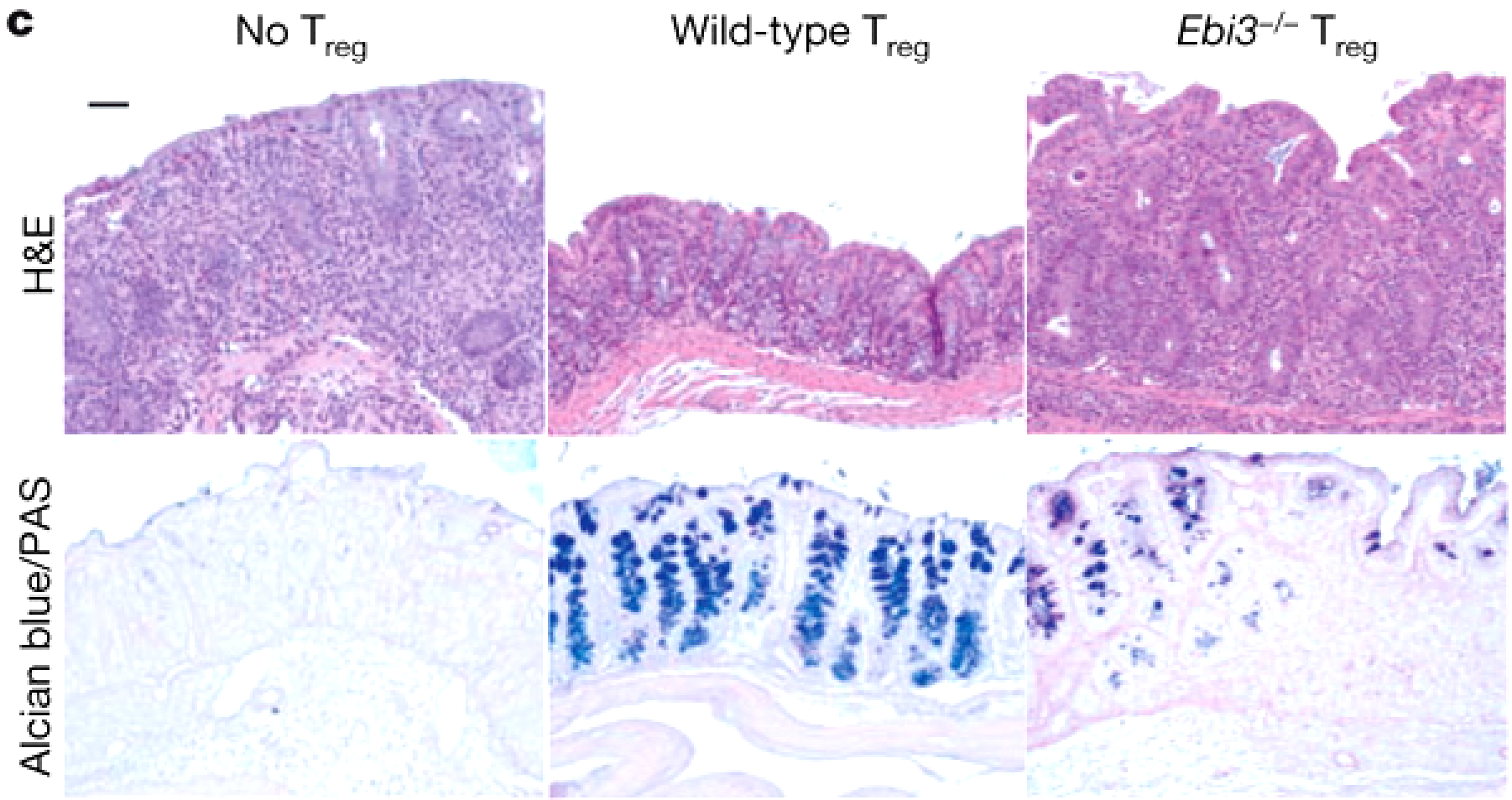


Wanda Niedbala, Xiao-qing Wei, Beilei Cai, Axel J Hueber, Bernard P. Leung, Iain B. McInnes and Foo Y. Liew.IL-35 is a novel cytokine with therapeutic effects against collagen-induced arthritis through the expansion of regulatory T cells and suppression of Th17 cells. Vol 37 (11) 2007, Correction Eur. J. Immunol. 2007. 37: 3293 3293

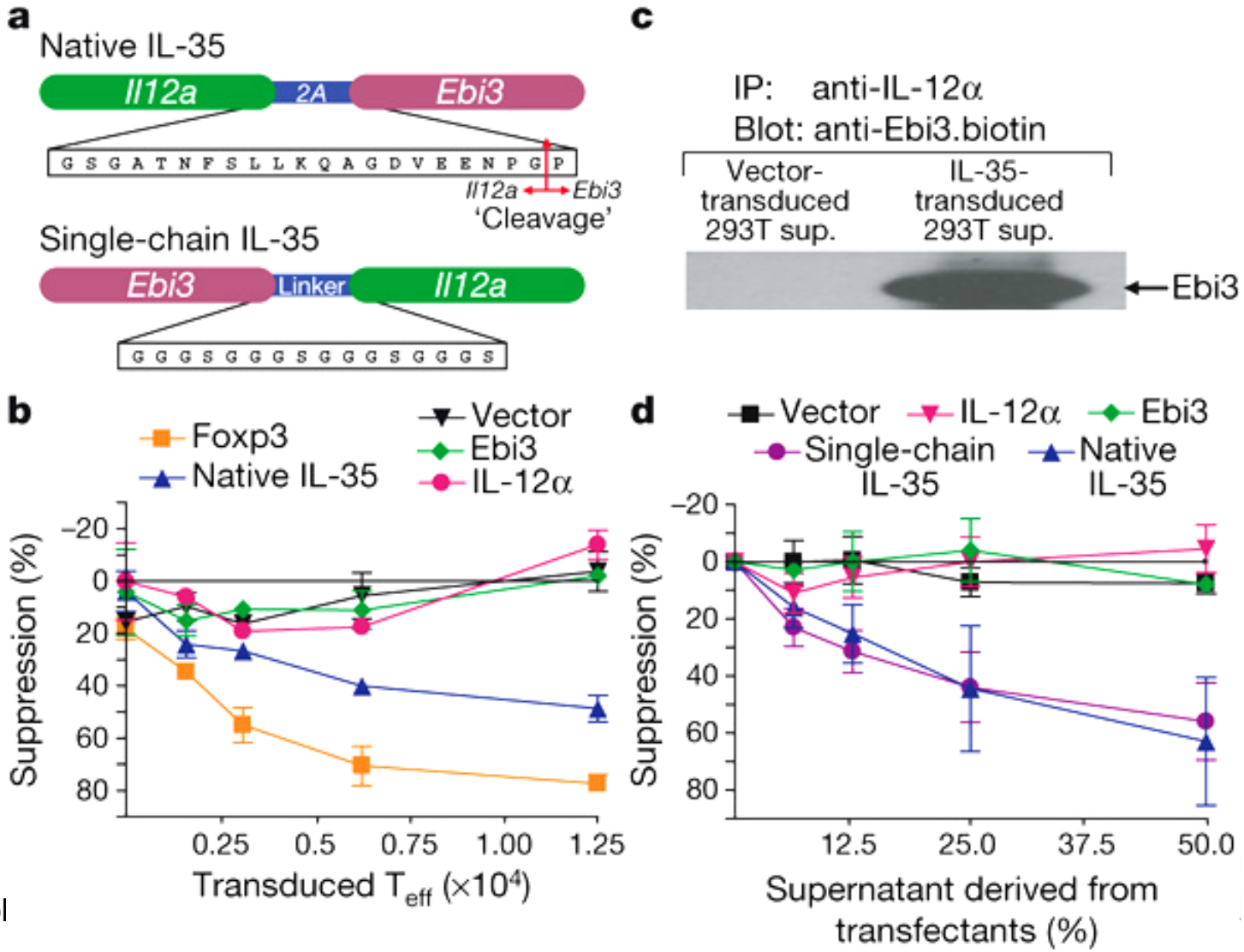
In our article (this issue), the two sentences “We have constructed a heterodimeric protein covalently linking EBI3 and p35, to form a novel cytokine which we now call IL-35” and “We propose to call the novel cytokine IL-35” imply that we were the first to propose this nomenclature; however, Dario Vignali first proposed the name IL-35 for the EBI3/p35 heterodimer at the 13th International Congress of Immunology, Rio de Janeiro, Brazil, as part of his presentation (Collison LW, Workman CJ, Kuo TK, Boyd K, Wang Y, Vignali K, Cross R, Sehy D, Blumberg RS and Vignali DAA.

The inhibitory cytokine IL-35 contributes to regulatory T cell function. Nature, in press). Dario Vignali also received confirmation of his proposed nomenclature from the International Union of Immunological Societies (IUIS) Subcommittee on Interleukin Nomenclature and by the HUGO Gene Nomenclature Committee on 25 June 2007. We would, however, like to point out that the construct of EBI3/p35-Fc and the biological functions described in Figures 1, 2, 5 and 6 of this manuscript were first published by FY Liew and Xq Wei in a patent application in 2005 (PCT/GB2005/001037, priority date 4/5/3).

Ebi3–/– and *Il12a–/–* Treg cells fail to cure IBD.



IL-35 suppresses Teff cell proliferation.



Interleukin 18 [IL-1F1-F10, IL-33]

•Hashimoto W, Osaki T, Okamura H, Robbins PD, Kurimoto M, Nagata S, Lotze MT, Tahara H. Differential antitumor effects of administration of recombinant interleukin 18 (rIL-18) or rIL-12 are mediated by Fas-Fas ligand and perforin-induced tumor apoptosis, respectively. Journal of Immunology 1999; 163:583-589.

•Osaki T, Hashimoto W, Gambotto A, Okamura H, Robbins PD, Kurimoto M, Lotze MT, Tahara H. Potent antitumor effects mediated by local expression of the mature form of the interferon-gamma inducing factor, interleukin-18 (IL-18). Gene Therapy 1999;6(5):808-815.

•Son YI, Dallal RM, Mailliard RB, Egawa S, Jonak ZL, Lotze MT. Interleukin-18 (IL-18) synergizes with IL-2 to enhance cytotoxicity, interferon-gamma production, and expansion of natural killer cells. Cancer Research 2001 61(3):884-8.

•Tanaka F, Hashimoto W, Okamura H, Robbins PD, Lotze MT, Tahara H. Rapid generation of potent and tumor-specific cytotoxic T lymphocytes by interleukin 18 using dendritic cells and natural killer cells. Cancer Research 2000 Sep 1;60(17):4838-44

Interleukin 18 [IL-1F1-F10, IL-33]

- My plan, rapid combination with IL2 and/or IL-12
- IL-18 and IL-18BP circulate in normal and cancer patients.
- Phase I/II trials of IL-18 have been carried out and recently reported.
- Patients given rhIL-18 ranging from 3 to 1,000 µg/kg had chills, fever, nausea, headache, and hypotension along with neutropenia, thrombocytopenia, anemia, hypoalbuminemia, hyponatremia, and elevations in liver transaminases but with limited, unconfirmed responses.
- Ongoing trials in combination with therapeutic monoclonal antibodies are ongoing (Z. Jonak, personal communication).

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)*

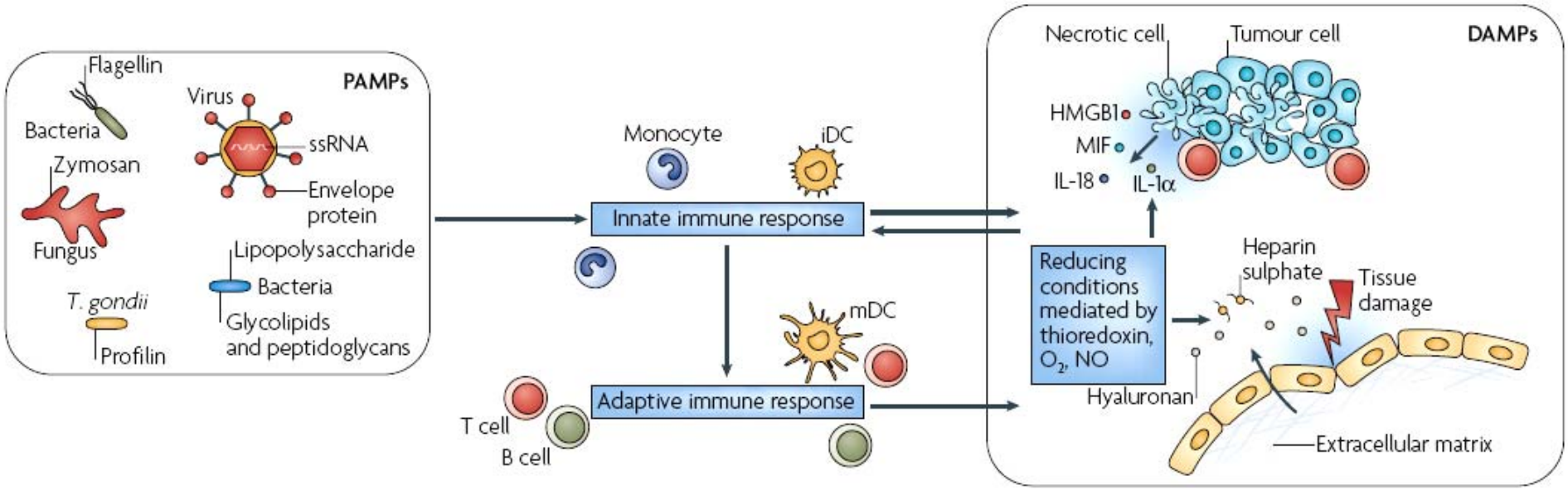
Biomarkers and Surrogates - DAMPS

- LDH
- S100b, S100p, HMGB1, HSPs
- DNA
- Uric acid, other purine metabolites

Damage-Associated Molecular Pattern Molecules (DAMPs)

Cell Constituents:	HMGB1 Heat shock proteins Uric Acid, ATP, Adenosine s100 proteins Hepatoma derived growth factor ?Cardiolipin Interleukin 1 Family DNA
Secreted molecules:	Fibrinogen domain A Surfactant protein A
Matrix elements:	Heparan sulfate Soluble hyluranan Fibronectin

PAMPS and DAMPS - Signal 0



Rubartelli A, Lotze MT. Inside, outside, upside down: Damage associated molecular pattern molecules and Redox. Trends in Immunology [2007].

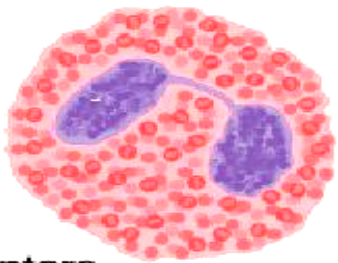
Eosinophils

Chemokine, complement and other chemotactic factor receptors

CD35	CCR1
CD88	CCR3
C3aR	CCR6
PAFR	CXCR1
LTB_4R	CXCR3
LTD_4R	CXCR4
fMLPR	CRTH2
Histamine (H4 receptor)	

Adhesion molecules

CD11a	CD44
CD11b	CD49d
CD11c	CD49f
CD15	CD62L
CD15s	CD162
CD18	CD174
CD29	
αd integrin	
β7 integrin	



Immunoglobulin receptors and other members of the immunoglobulin superfamily

CD4	CD58
CD16†	CD66
CD31*	CD89
CD32	CD100
CD33	CD101
CD47	HLA class I
CD48	HLA-DR†
CD50*†	$Fc_\varepsilon RI$**
CD54*†	

Enzymes

CD13
CD45
CD45RB
CD45RO
CD46
CD55
CD59
CD87
PAR-2

Apoptosis, signaling and others

CD9	CD97
CD17	CD98
CD24	CD99
CD28	CD137
CD37	CD139
CD39	CD148
CD43	CD149
CD52	CD151
CD53	CD161
CD63	CD165
CD65	Siglec-8
CD69†	Siglec-10
CD76	LIR1
CD81	LIR2
CD82	LIR3
CD86†	LIR7
CD92	
CD95	

Cytokines

CD25	CD124
CD116	CD125
CD117	CD131
CD119	IL-9R
CD120	IL-13R
CD123	TGFβR

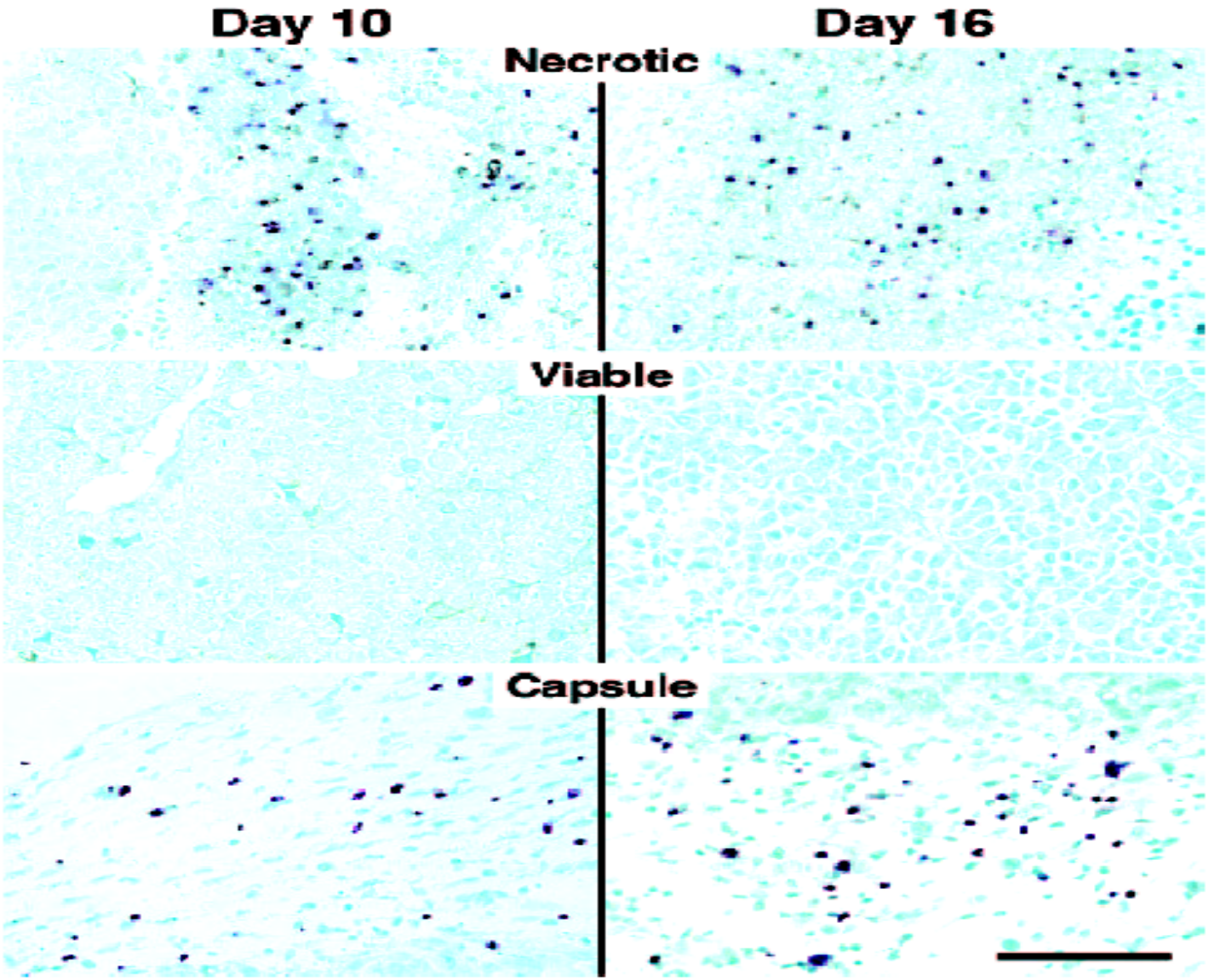
Wound Healing Eosinophils

- <1% circulating leukocytes
- Do not re-circulate
- IL-2, IL-4, GM-CSF, CTLA-4
- Associated with chronic inflammation including asthma, allergy, cancer, and transplant rejection
- Remove debris [opsonization]

MBP, EPO, EDN

Bochner et al., 2004, J.Allerg. Clin.Immunol.

Eos within necrotic tissue and capsule



Case Studies: Lessons and Issues

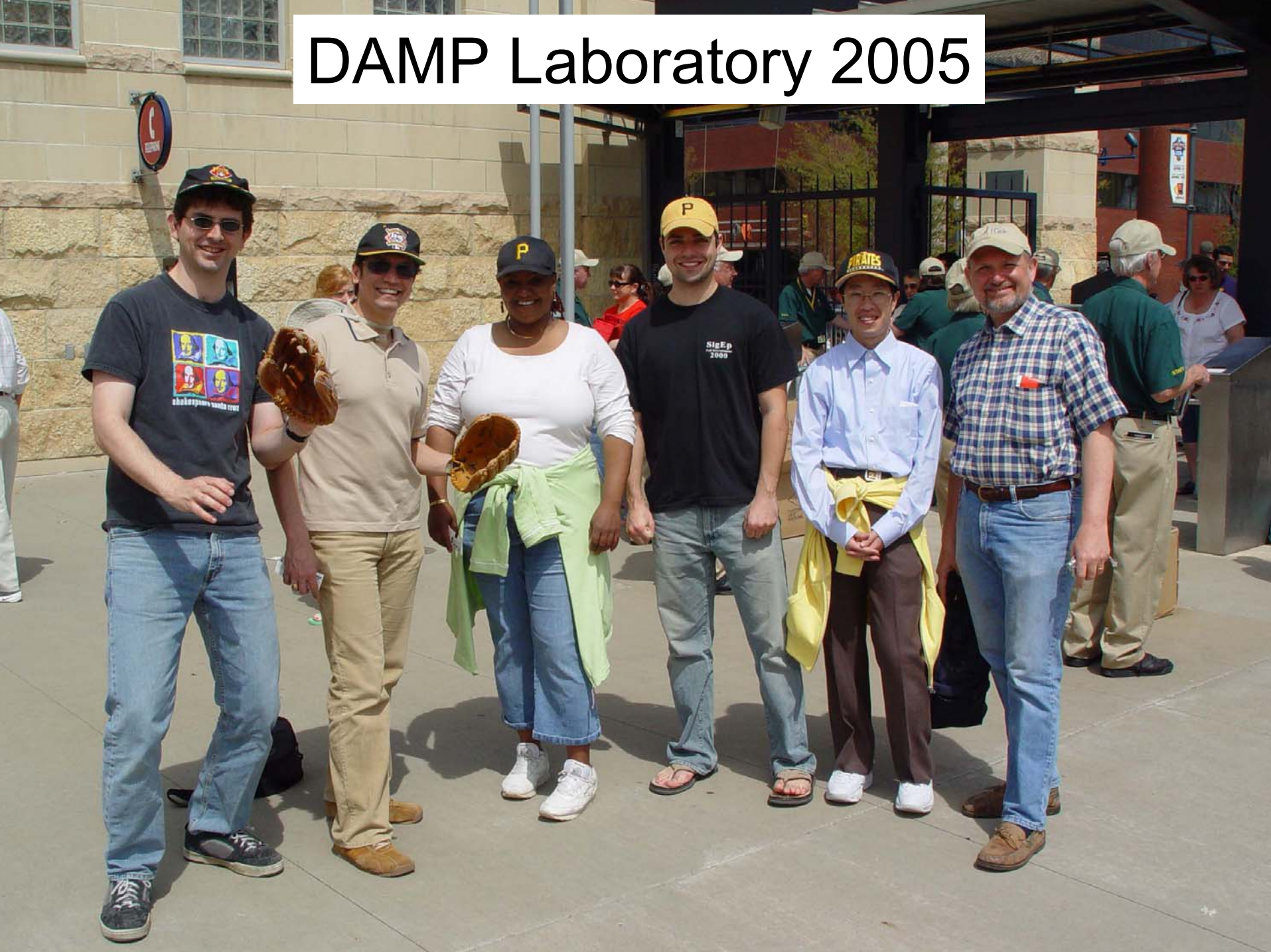
- What were the most important strategic decisions the team made that you would recommend to others facing similar issues? Follow the data and make decisions after MTD reached, not before

- Regulatory authorities
  - What feedback did you get from regulatory authorities that was helpful to the strategic development plan? Examine and reflect toxicity in animal models but don't be held to this completelyl
  - Did you get unanticipated feedback that led to changes in the plans? Yes. At all times.

- Funding of the project: projections vs. realities. Early success critical

- Lessons learned
  - What advice would you give to projects headed down a similar development path? Trust your biologic intuition and experimental evidence; early data dictates subsequent studies..
  - Mistakes or missteps that you will avoid in future projects? Define biologic endpoints that are credible and push to efficacy or unacceptable toxicity.

DAMP Laboratory 2005



DAMP Lab 2007

Why Women Live Longer Than Men