MELANOMA--A MODEL FOR BIOLOGICAL THERAPY

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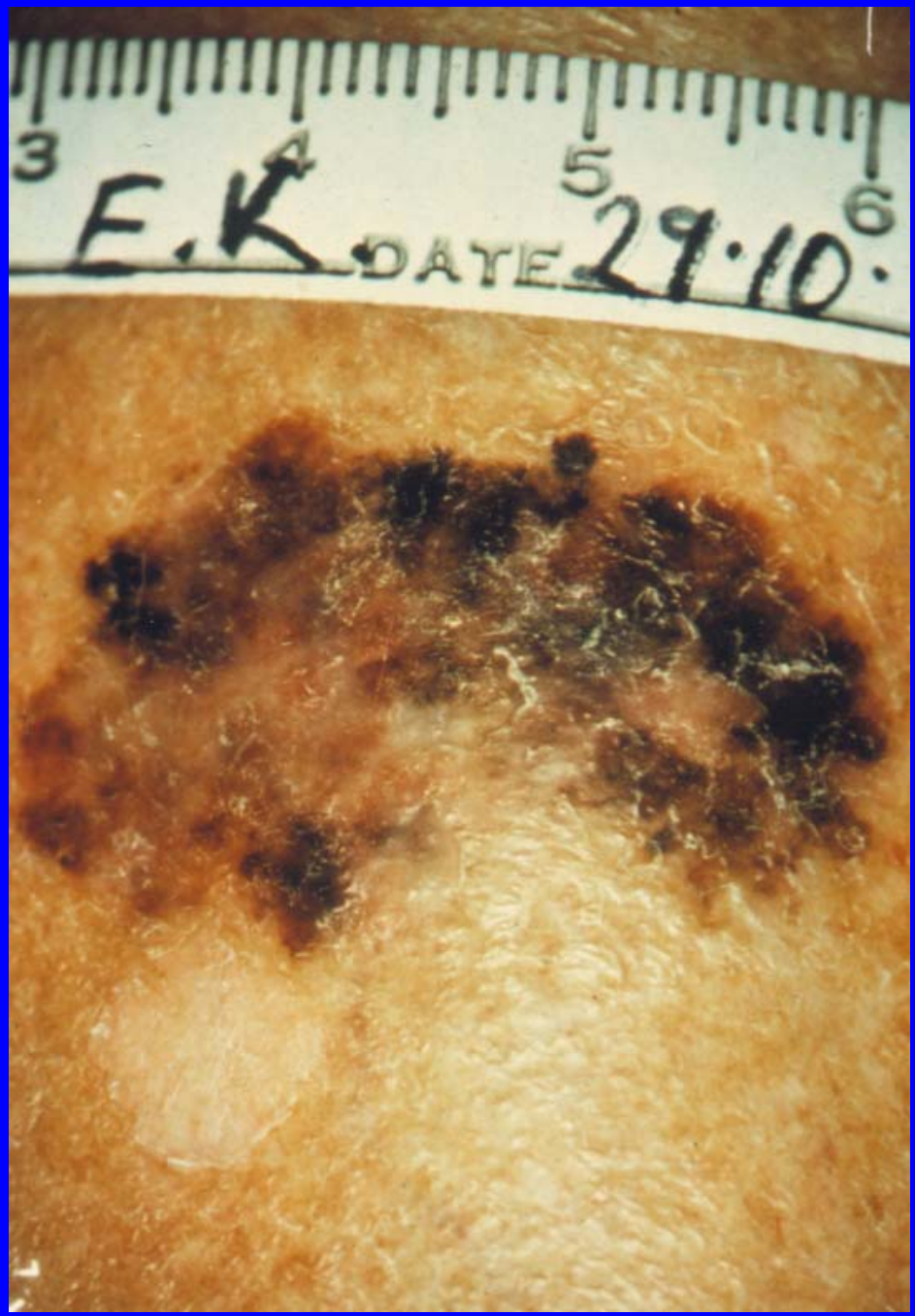
THE IMMUNE SYSTEM AND MELANOMA

- **Clinical evidence of Regression of melanoma**
- **Histopathological evidence of Lymphocytic infiltration Common**
- **Immune responses demonstrated in – Vitro**
- **Occasional spontaneous regression**
- **Tumor dormancy**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4
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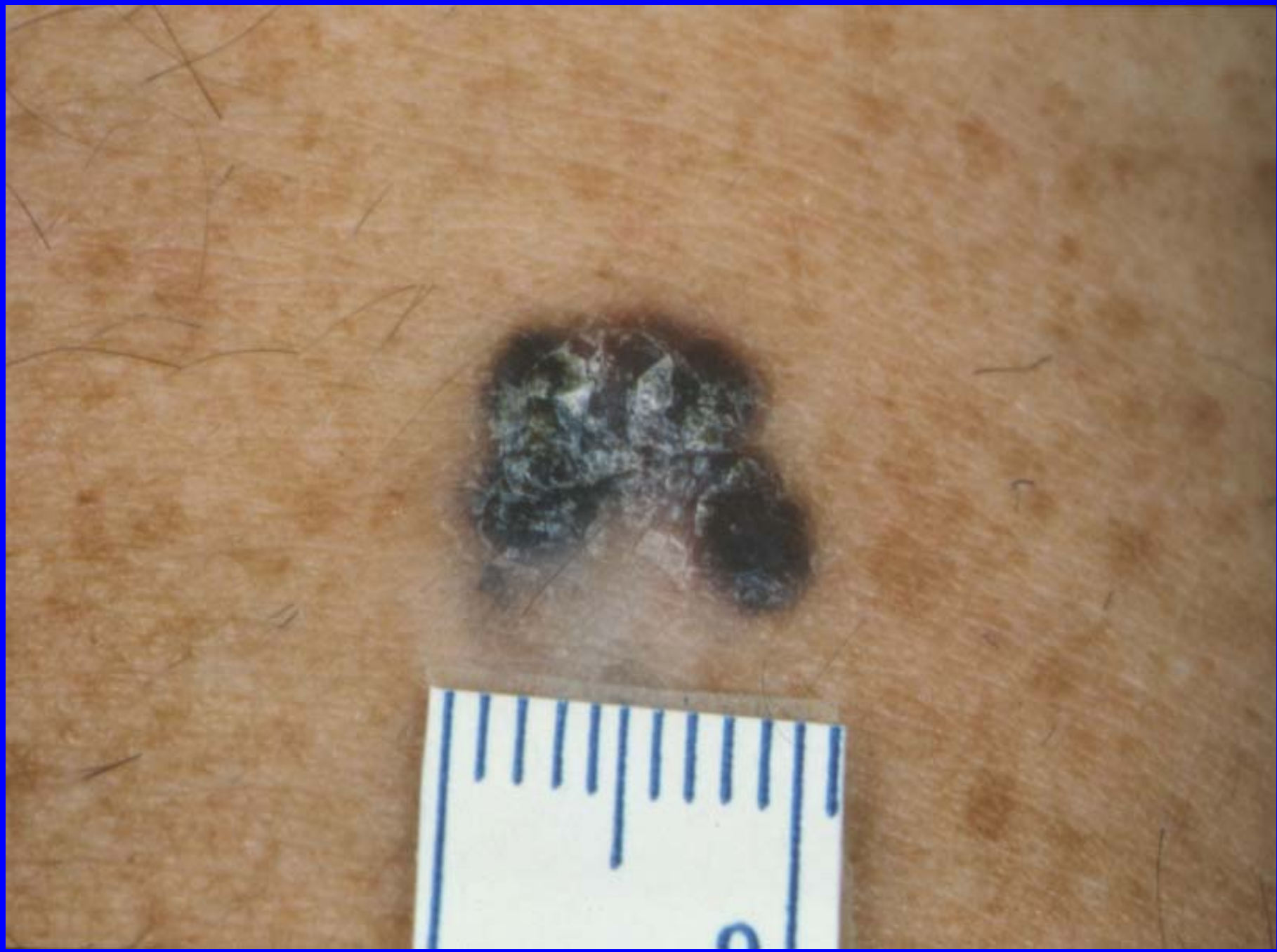
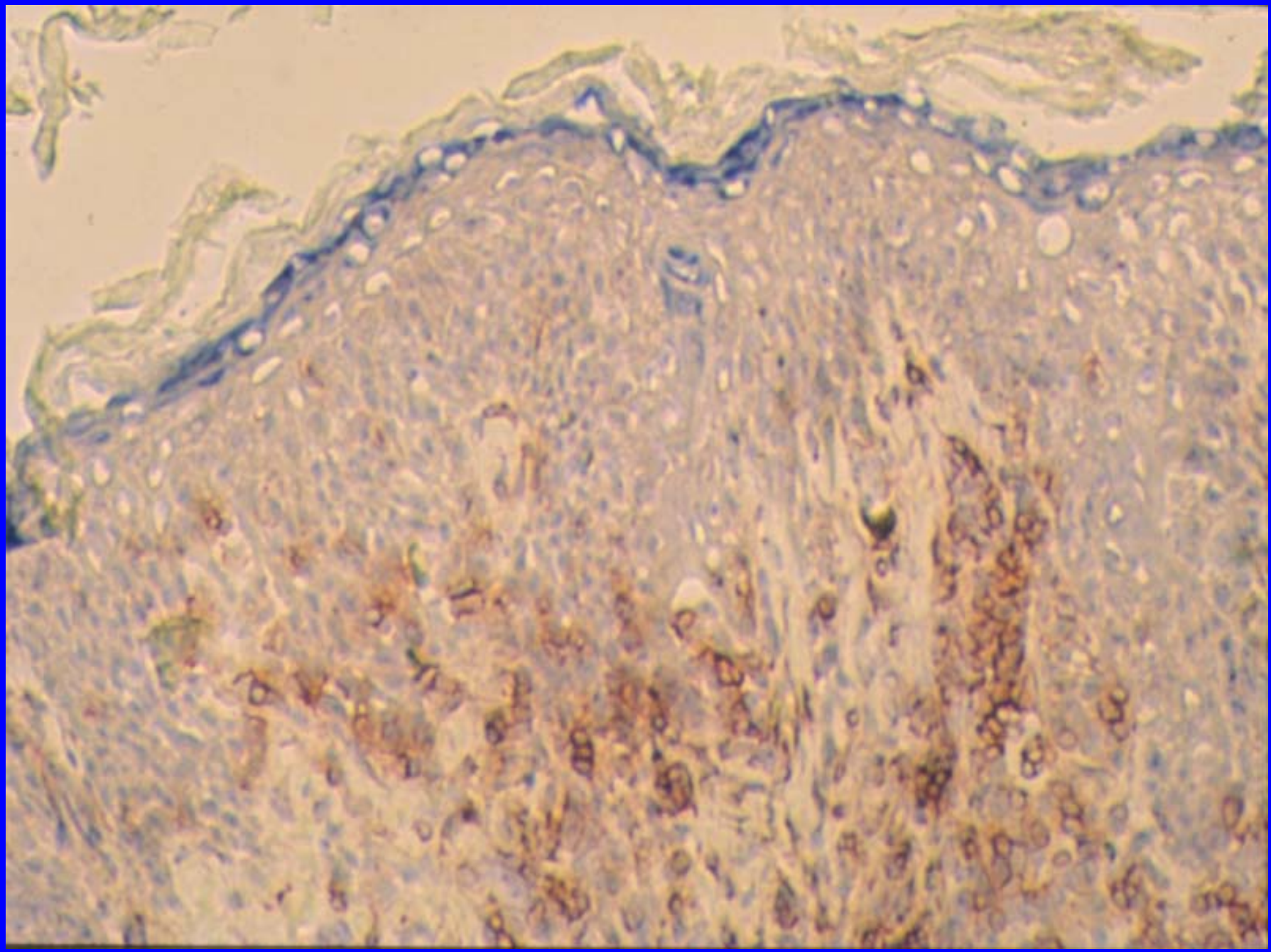
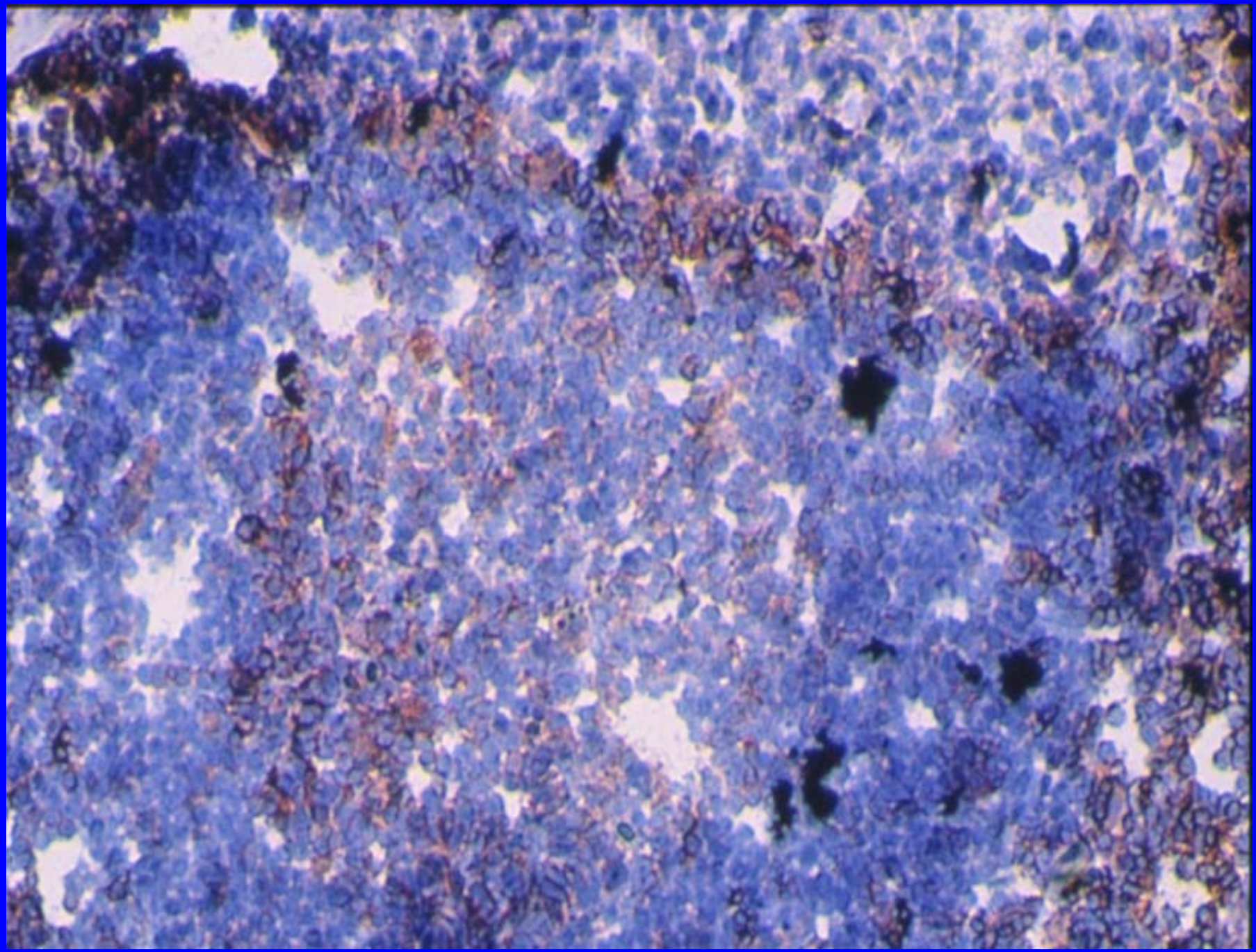
E.K
DATE 29.10.







MANY WELL DEFINED MELANOMA ANTIGENS

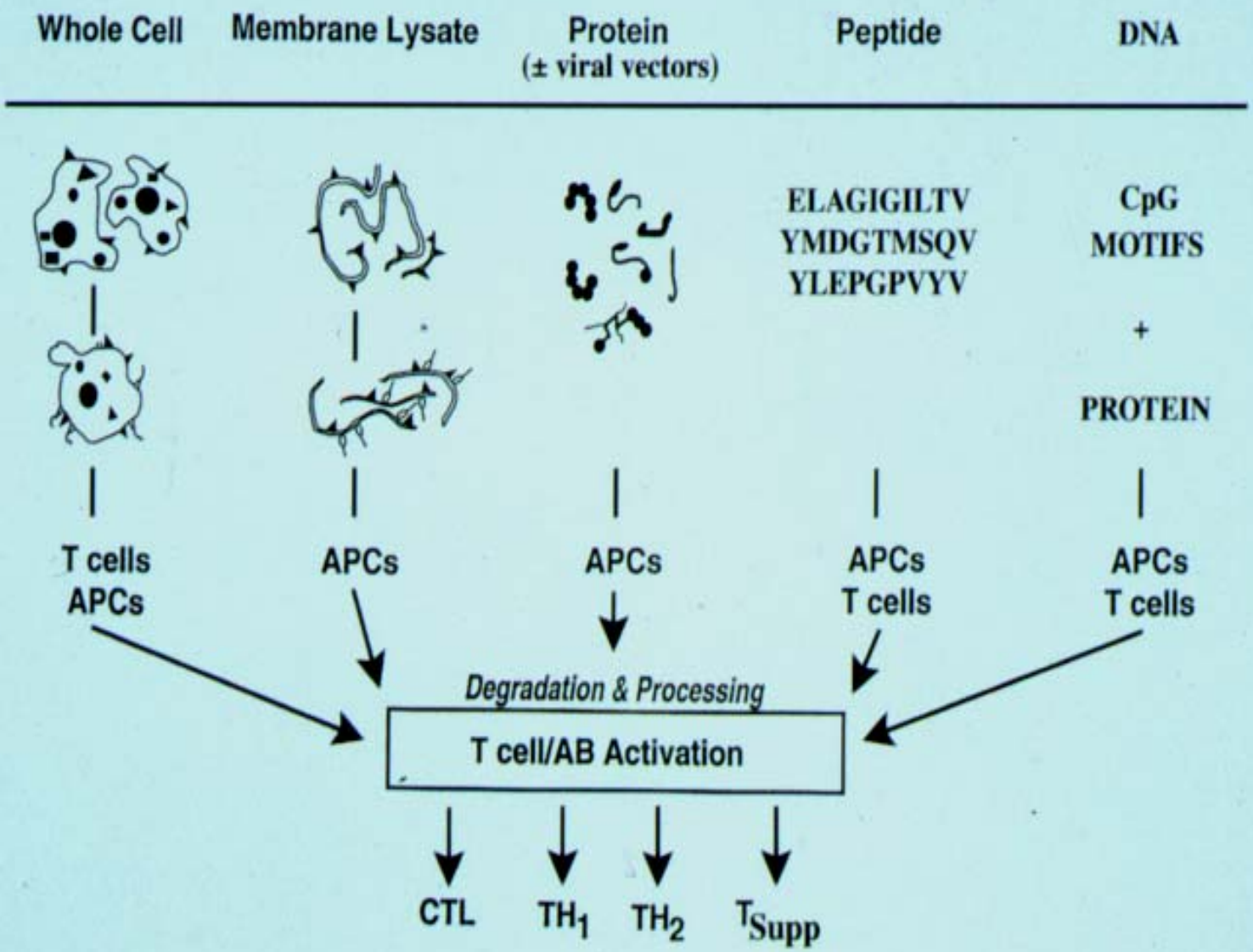
- **“Tumor Specific” Cancer Testis antigens**
- **Eg MAGE-3, NY-ESO-1**
- **Differentiation antigens MART-1, gp100**
- **Individual mutated antigens eg CDK4**
- **Overexpressed Antigens p15,Prame, CD63**

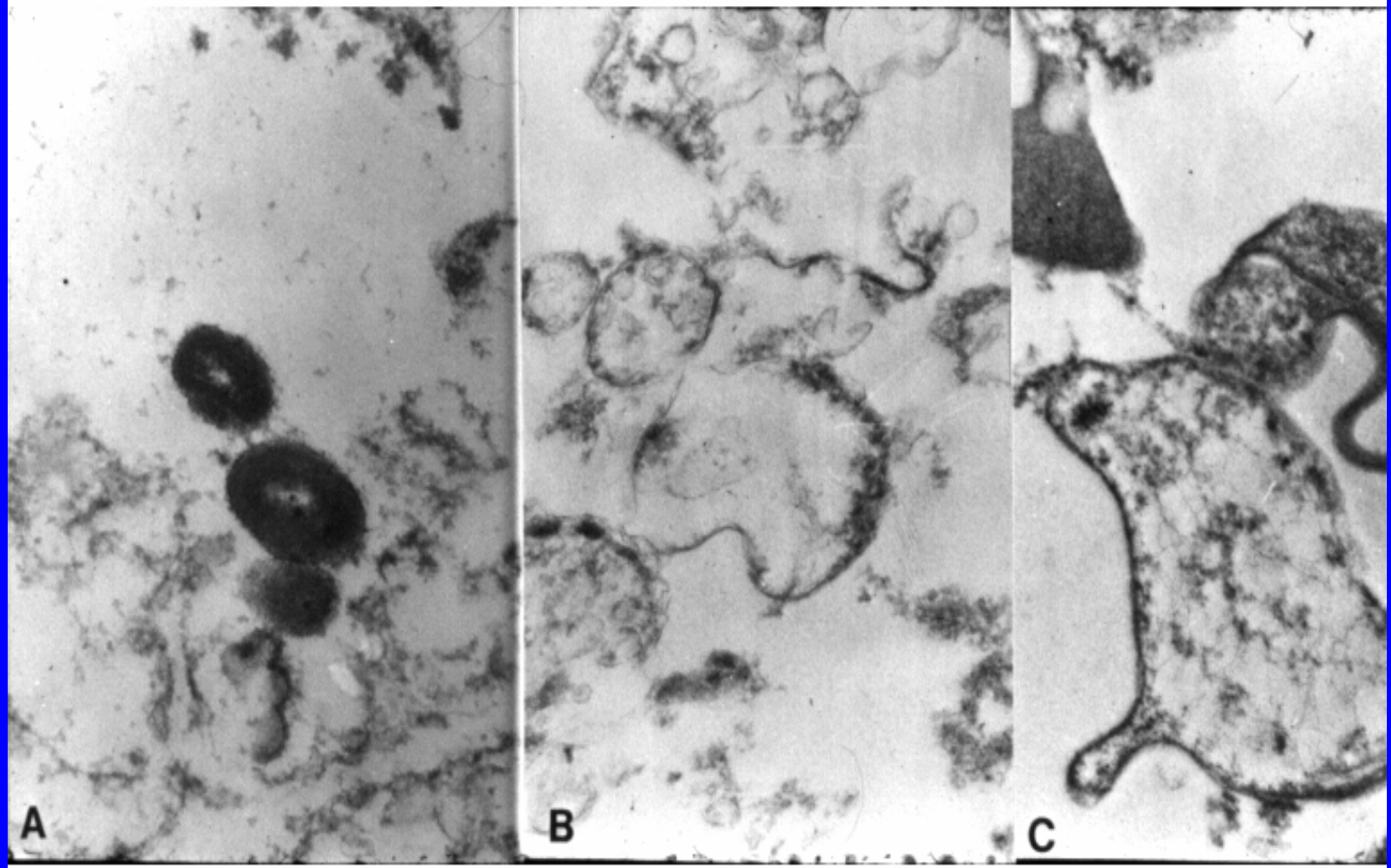
HYPOTHESIS-IMMUNE RESPONSE TOO WEAK --VACCINES WILL INCREASE STRENGTH OF IMMUNE RESPONSE

MANY VACCINES PRODUCED

- **A NUMBER OF TRIALS CONDUCTED**

CANCER VACCINES



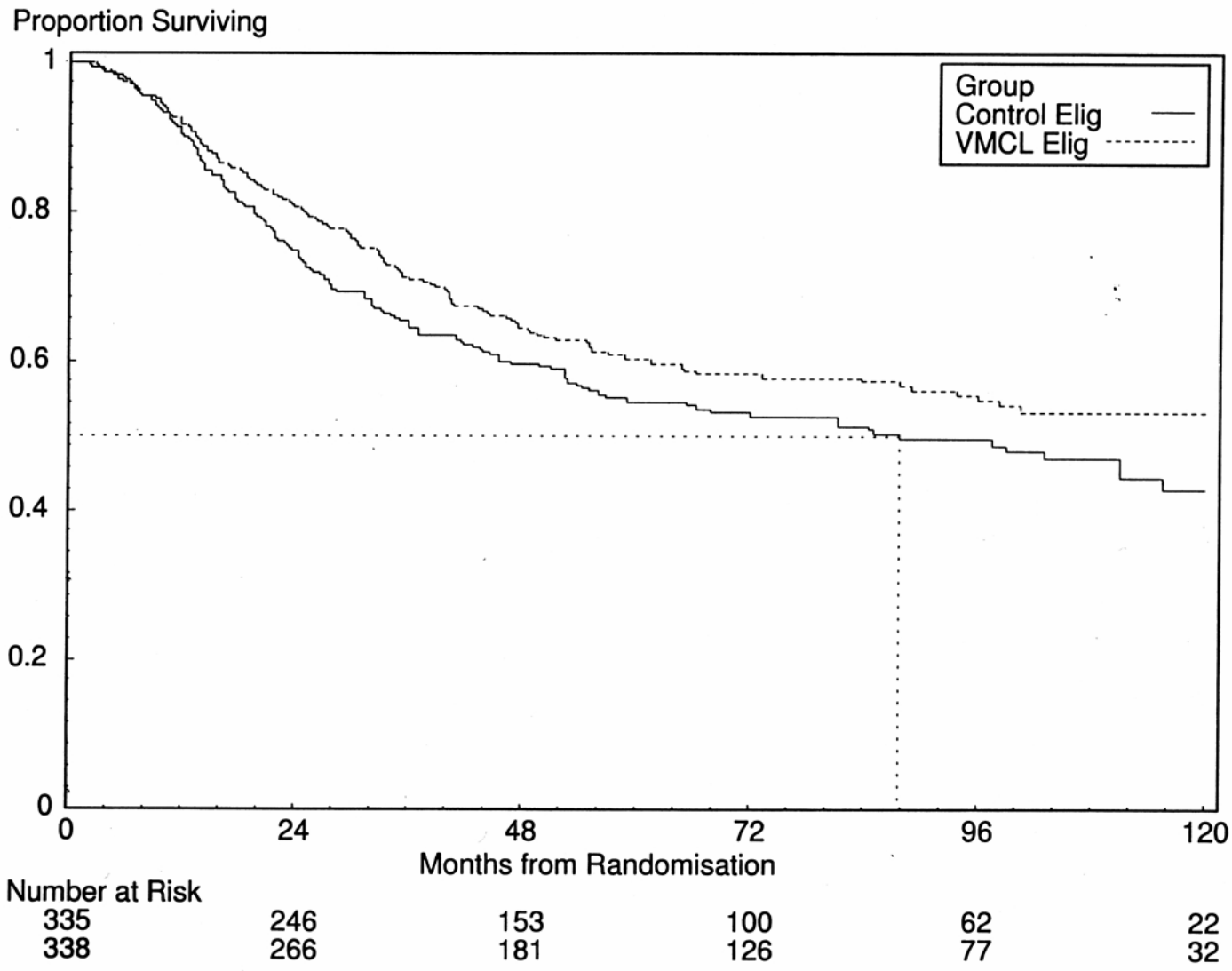

A
B
C

Summary Results of VMCL Trial
July 31st 2000

Treatment	Total	Alive	AWD	Dead
VMCL	353	207	21	146
Control	347	183	13	164

Overall Survival

Eligible patients



Summary of VMCL Trial

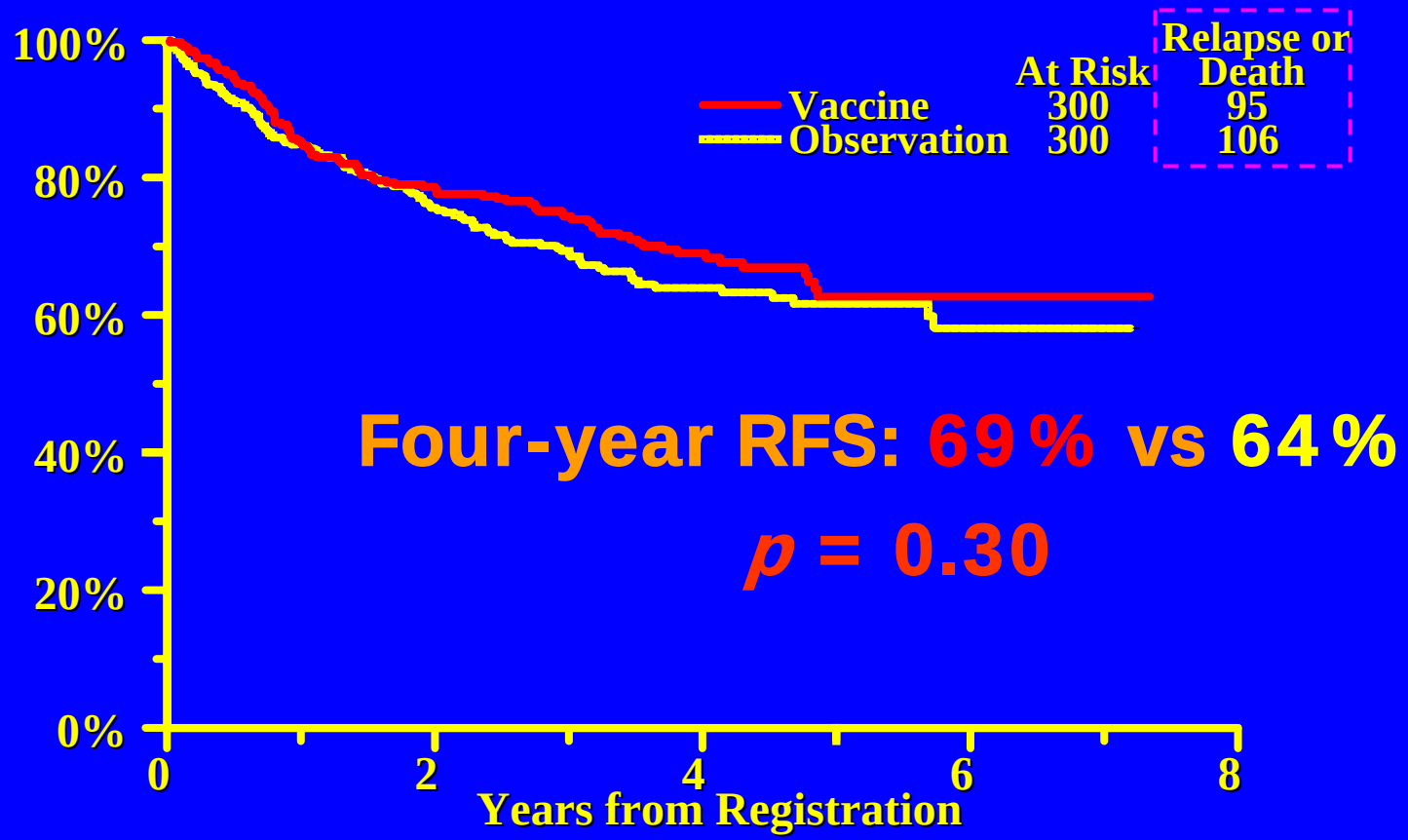
- 700 patients, median follow up 8 years

- Trend in favour of VMCL.
- HR for OS=.81 CI=.64-1.02 P=.068
- HR for RFS=.86 CI=.7-1.07 P=.17

- Median survivals 100 months overall
  88 months for Controls
  151 months forVMCL

SWOG-9035

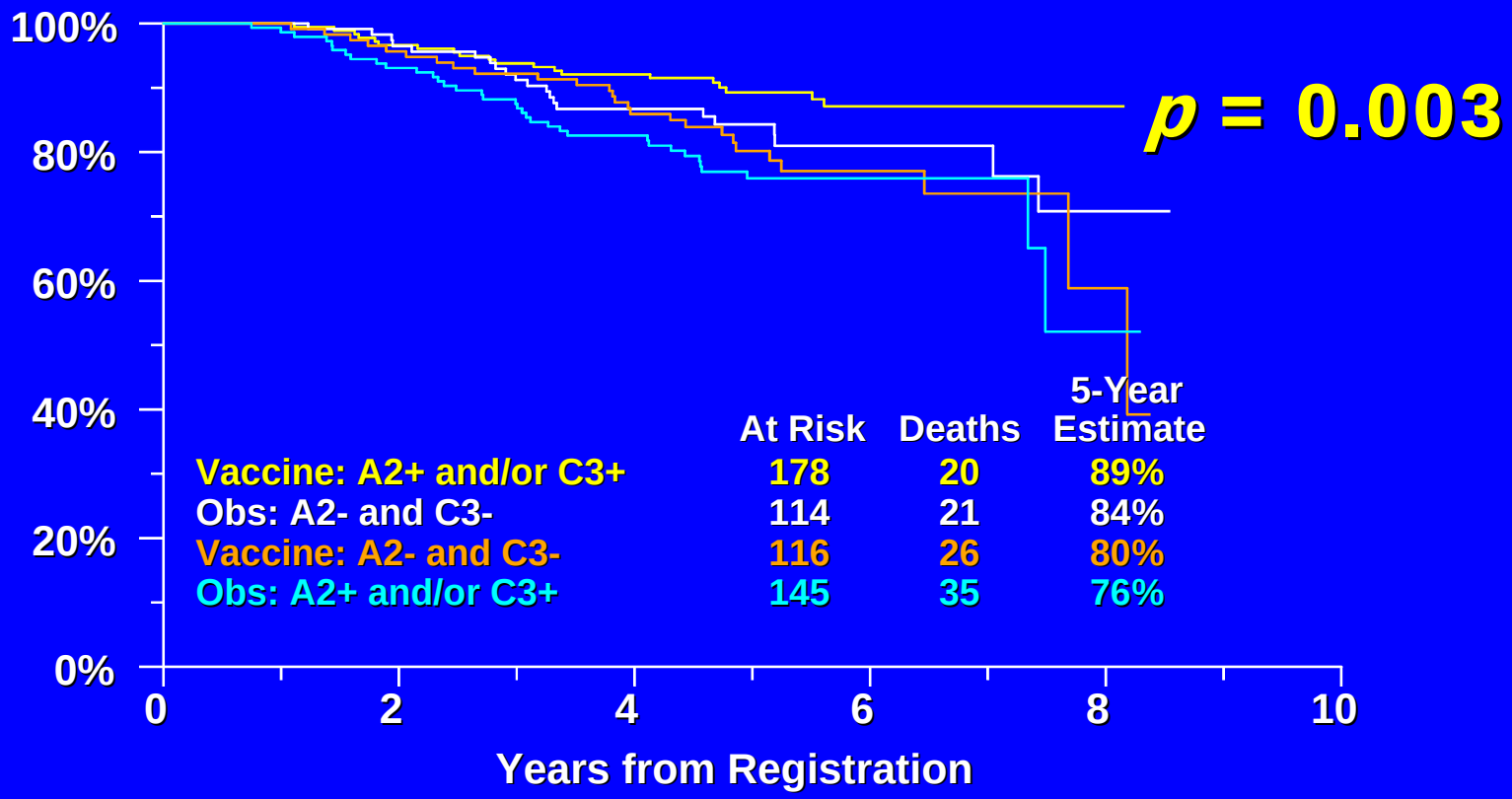
Relapse-free survival



SWOG-9035

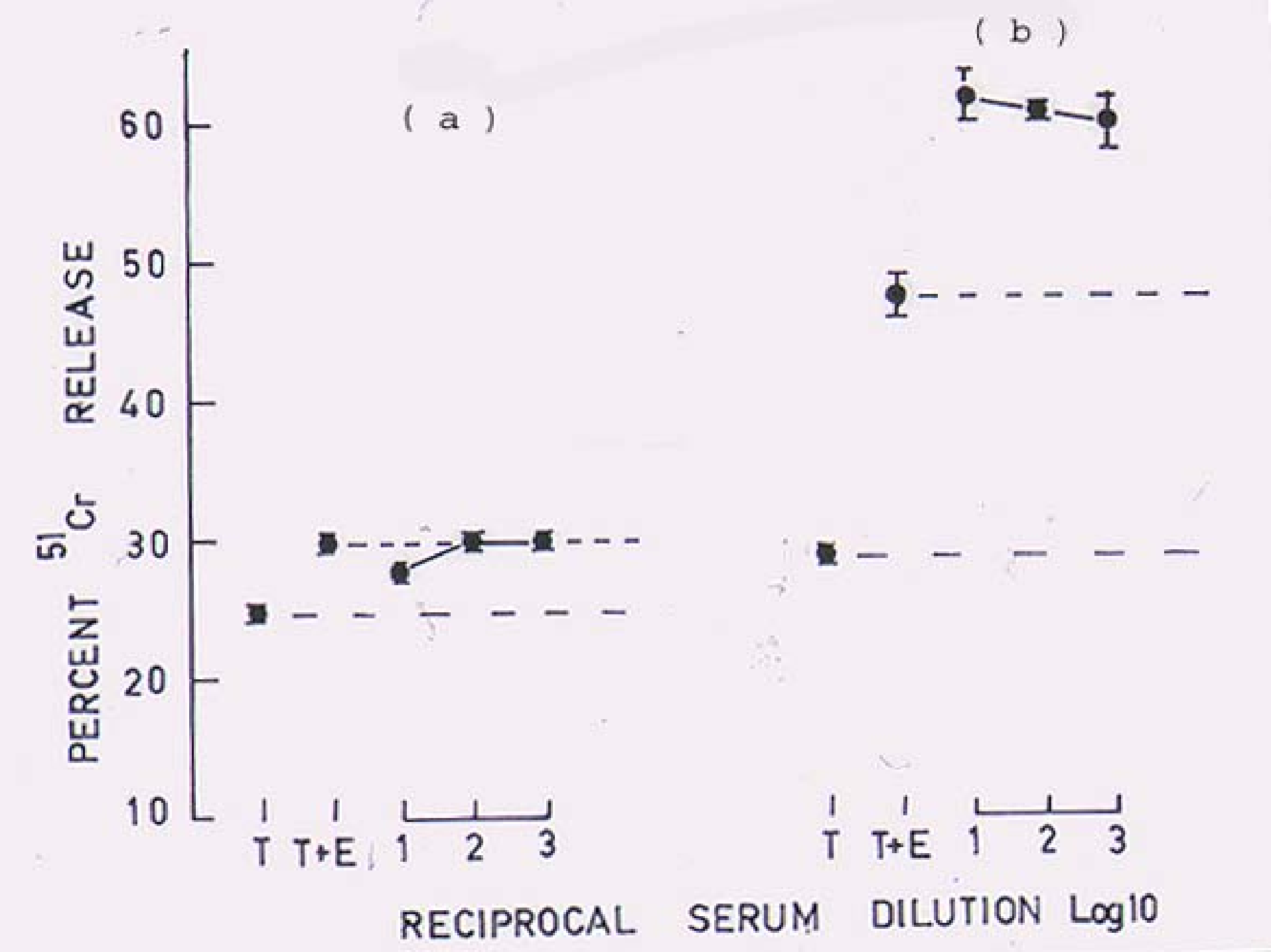
Overall survival

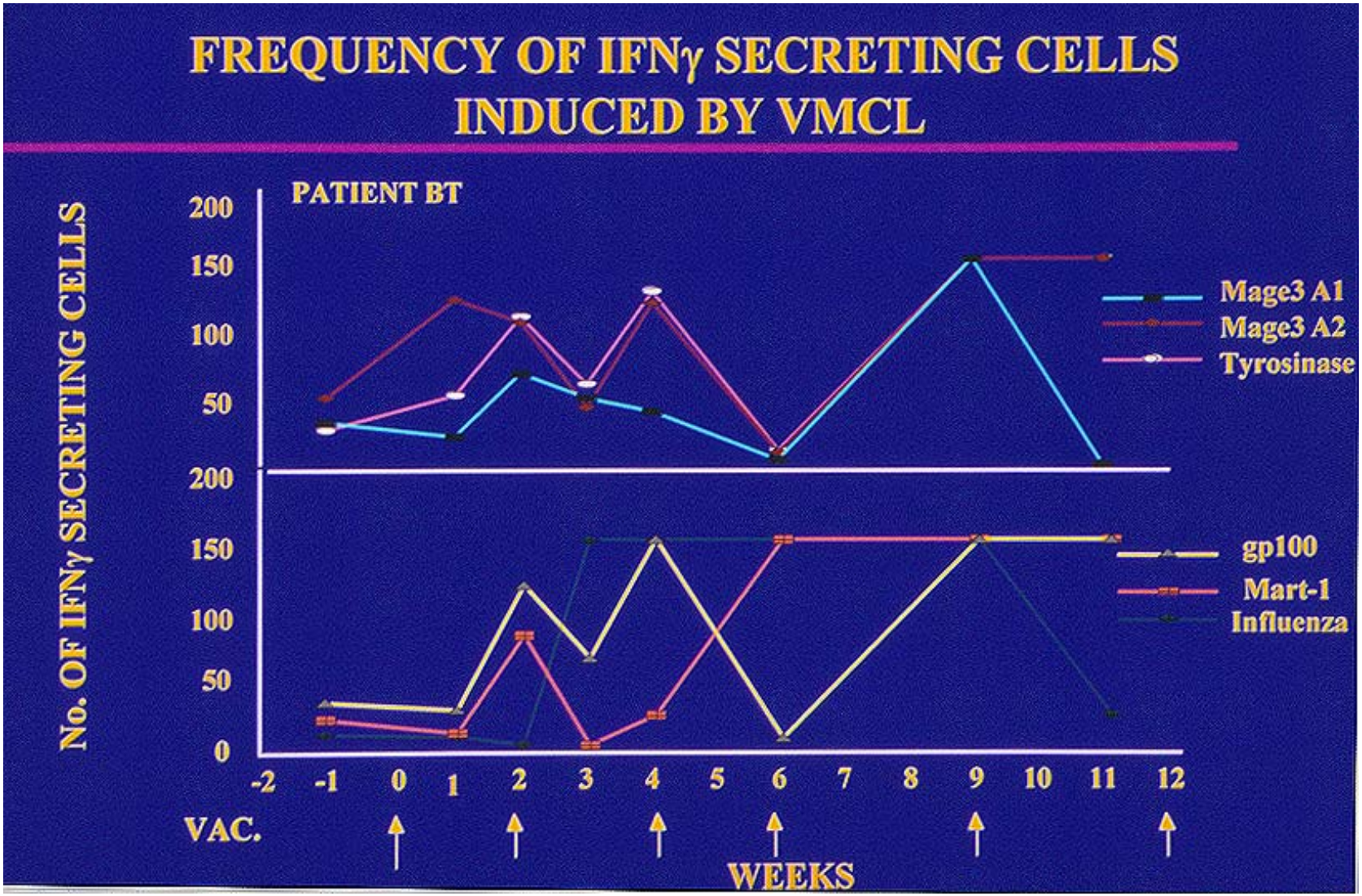
By Treatment and HLA-A2/C3 Status



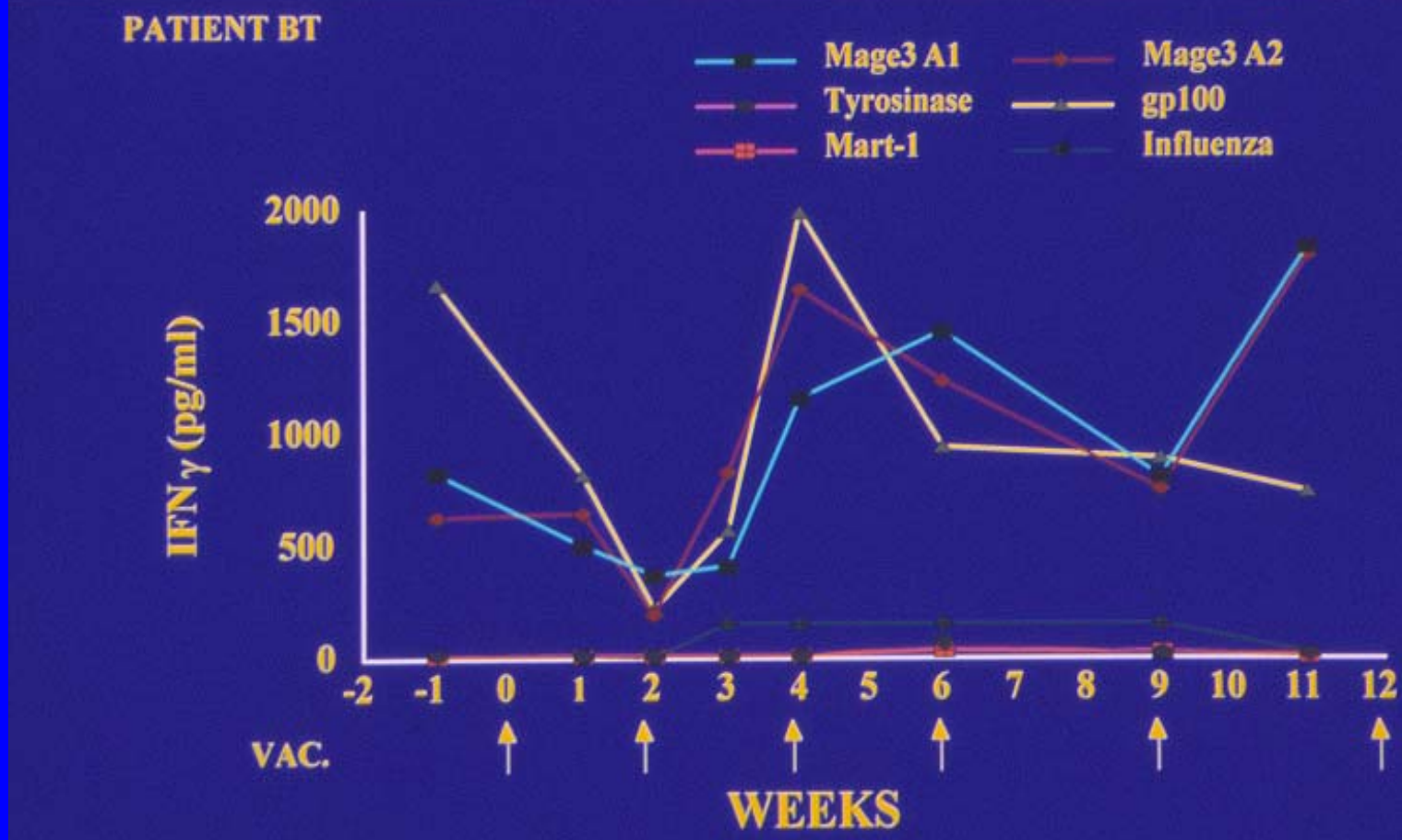
Sosman et al, JCO 20:2067-75 2002

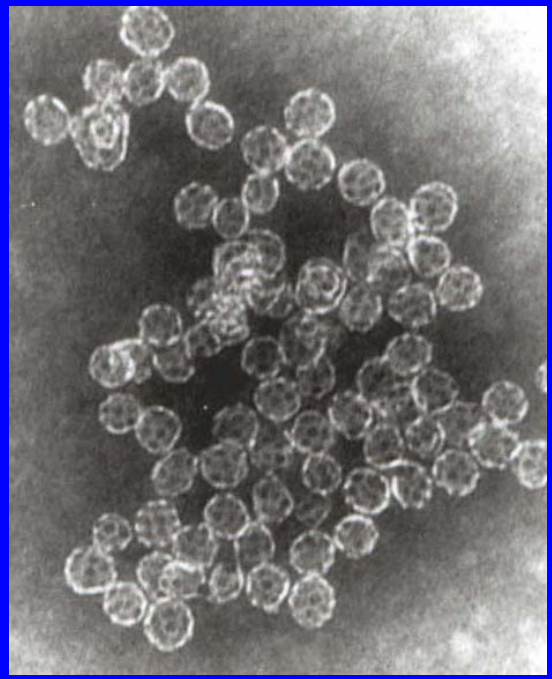
VACCINES INCREASE IMMUNE RESPONSES


(a)
(b)
60
50
40
30
20
10
PERCENT ^{51}Cr RELEASE
T
T+E
1
2
3
T
T+E
1
2
3
RECIPROCAL SERUM DILUTION Log10


FREQUENCY OF IFNγ SECRETING CELLS INDUCED BY VMCL
PATIENT BT
No. OF IFNγ SECRETING CELLS
200
150
100
50
200
150
100
50
0
Mage3 A1
Mage3 A2
Tyrosinase
gp100
Mart-1
Influenza
-2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12
VAC.
WEEKS

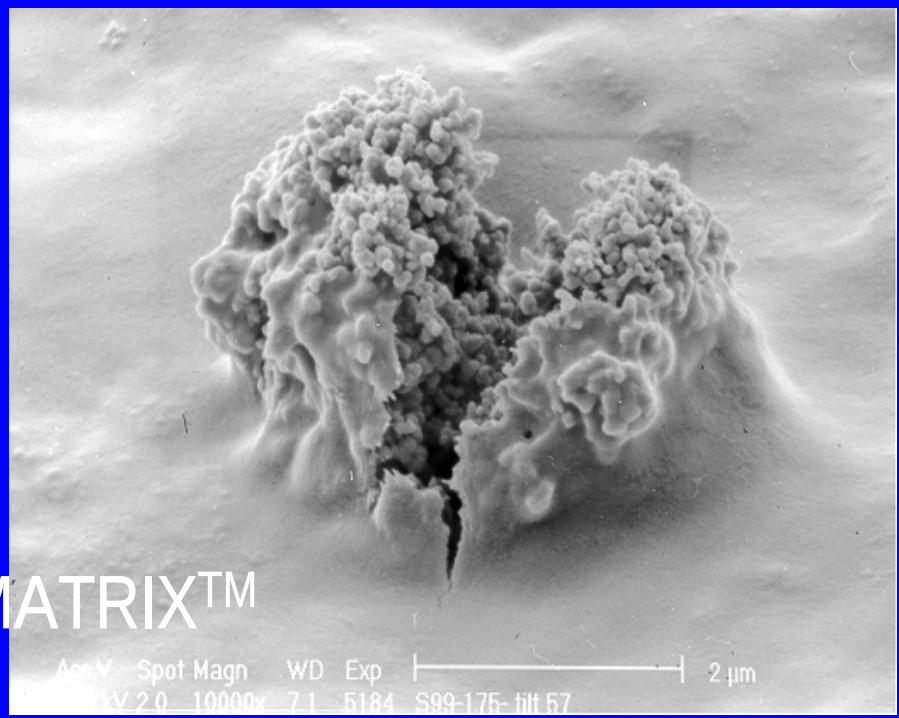
INDUCTION OF IFNγ PRODUCTION BY VMCL





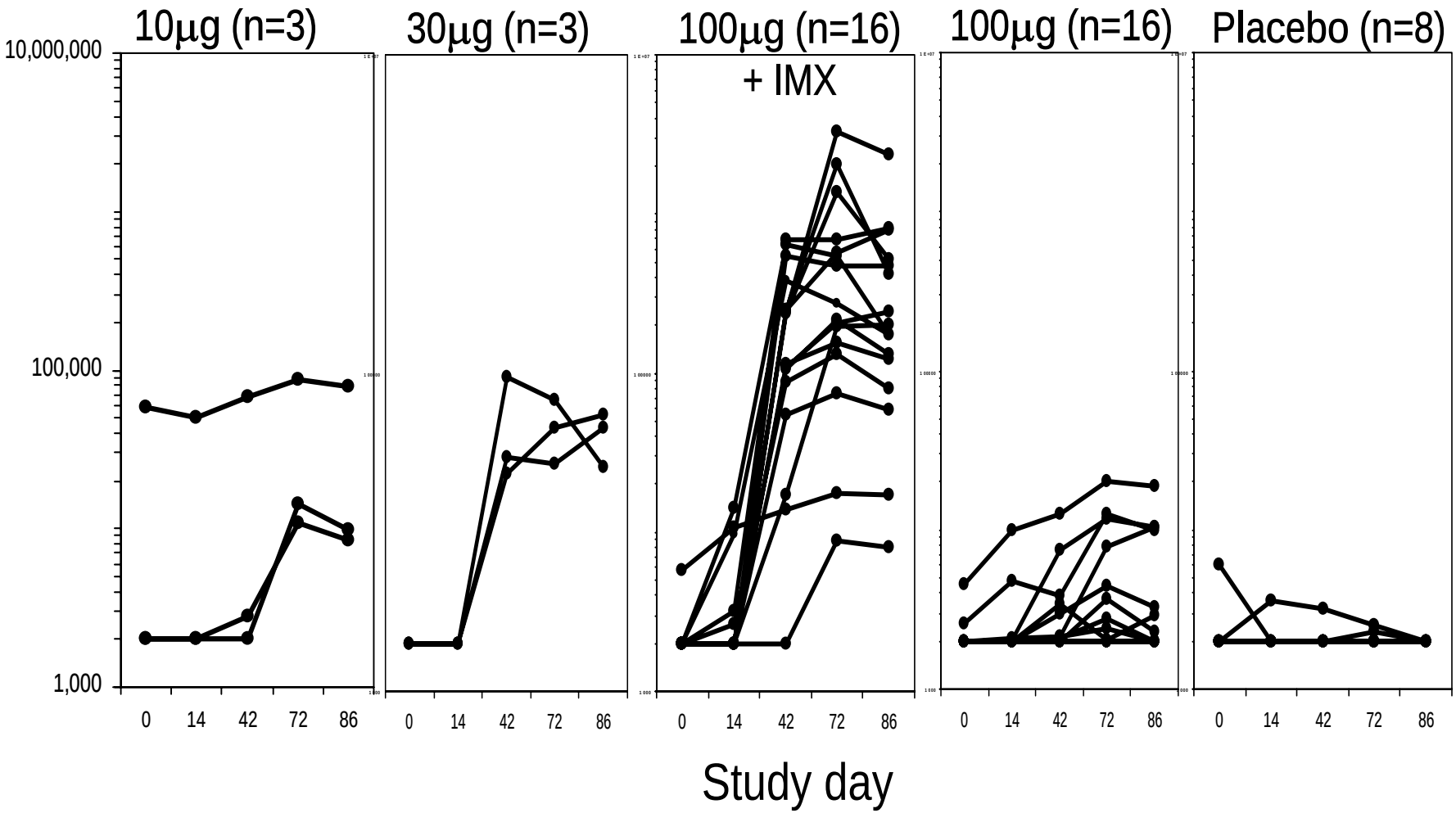
ISCOMATRIX™ adjuvant

- Non-living
- Cellular and humoral responses
- Components
  - Saponin
  - Phospholipid
  - Cholesterol

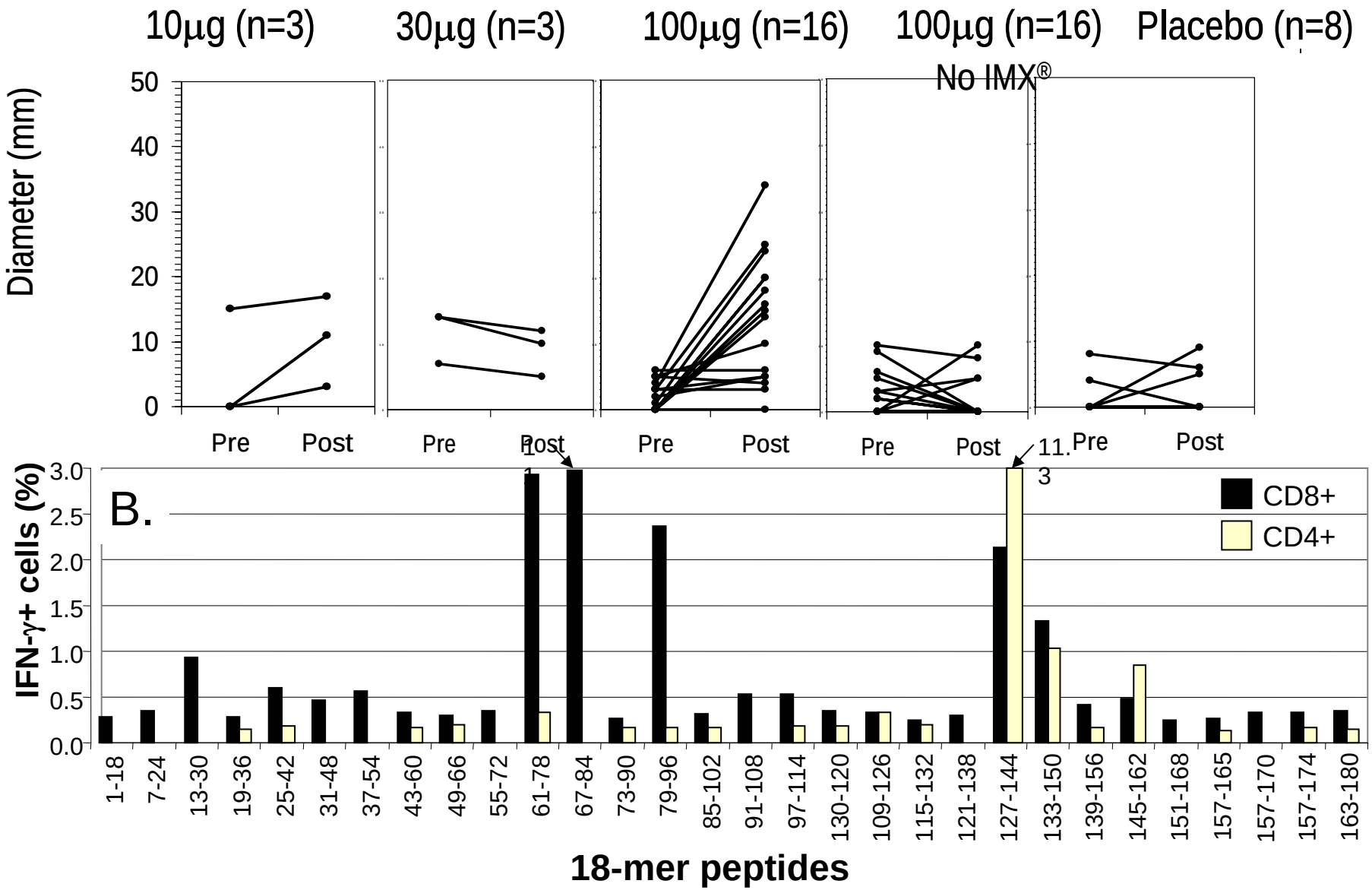


NY-ESO-1 ISCOMATRIX™

Antibody titre by cohort



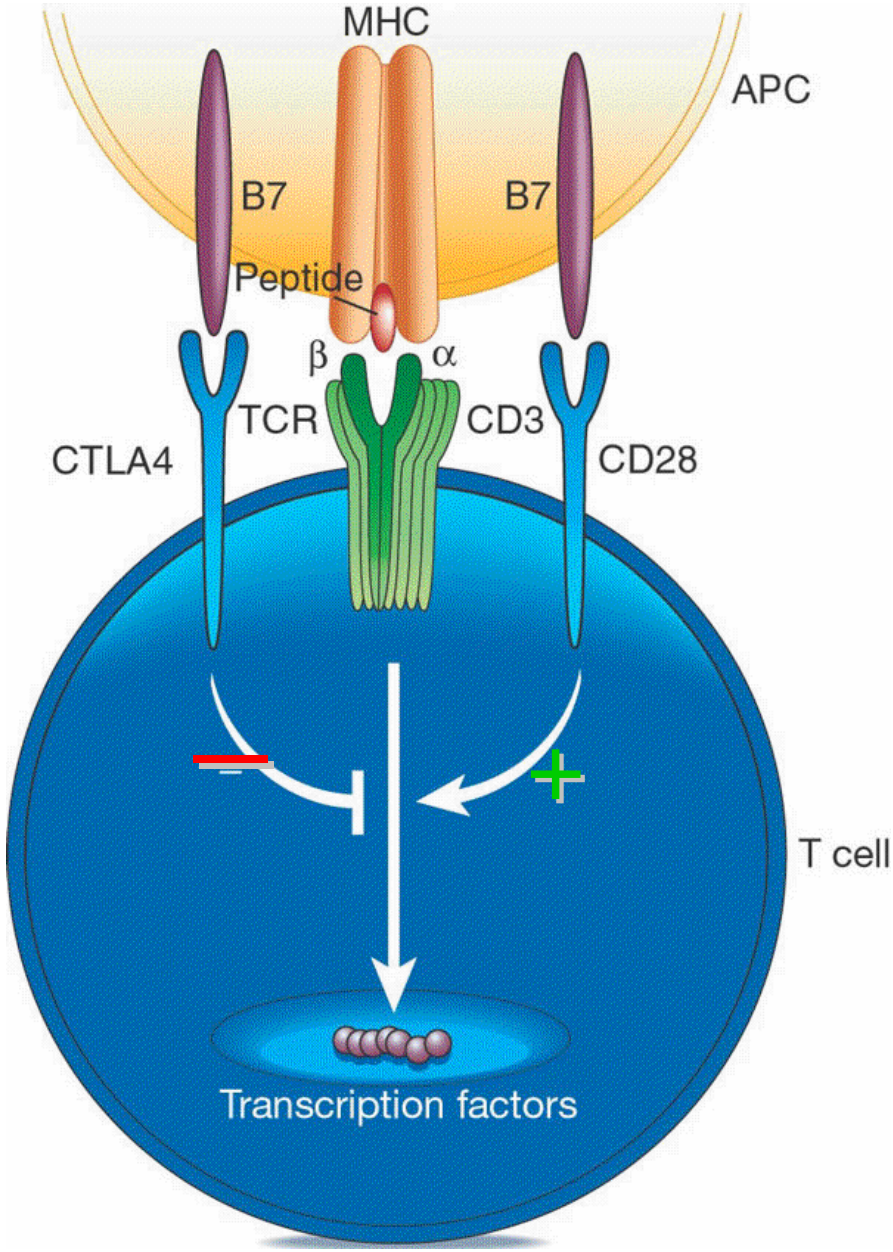
DTH Response (induration)



HYPOTHESIS----IMMUNE RESPONSES ARE INHIBITED BY REGULATORY T CELLS

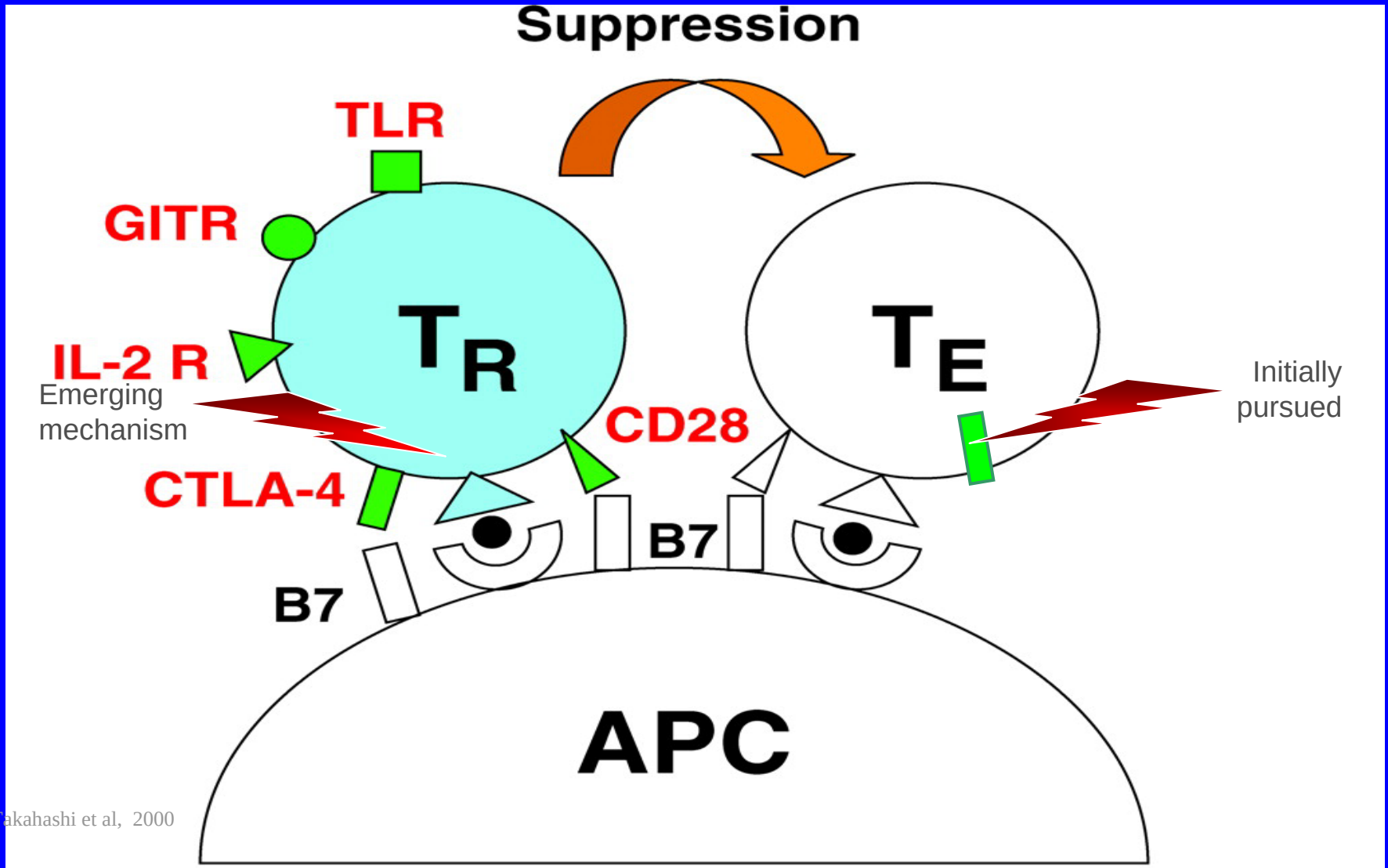
“TAKING OFF THE BRAKE” APROACH

CLT4 mediates a negative regulatory signal

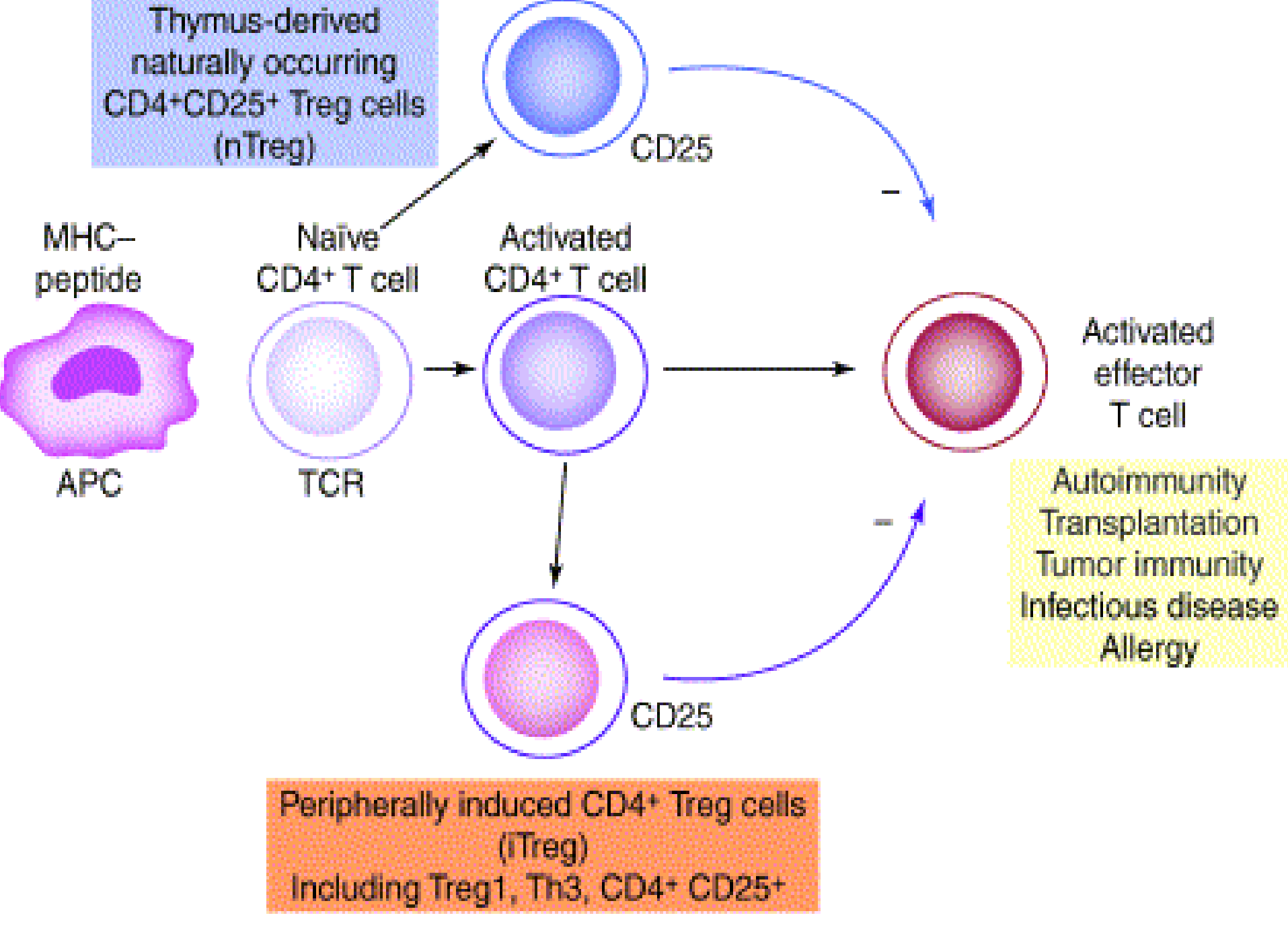


Appelbaum FR, 2001

Emerging Mechanism Extends the Impact of CTLA4 Blockade

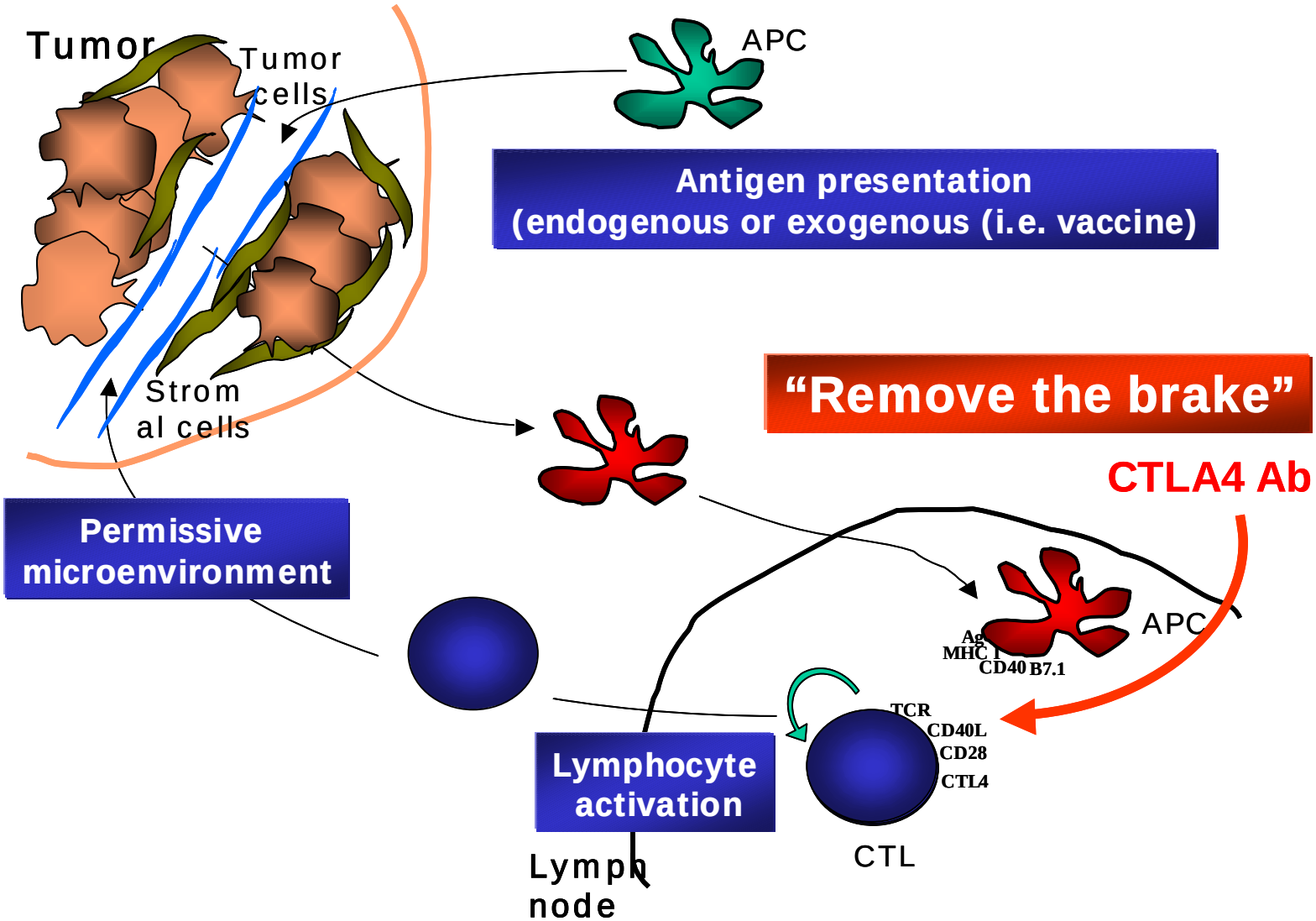


Takahashi et al, 2000


Thymus-derived naturally occurring CD4+CD25+ Treg cells (nTreg)
CD25
MHC–peptide
Naive CD4+ T cell
Activated CD4+ T cell
Activated effector T cell
APC
TCR
Autoimmunity
Transplantation
Tumor immunity
Infectious disease
Allergy
CD25
Peripherally induced CD4+ Treg cells (iTreg)
Including Treg1, Th3, CD4+ CD25+

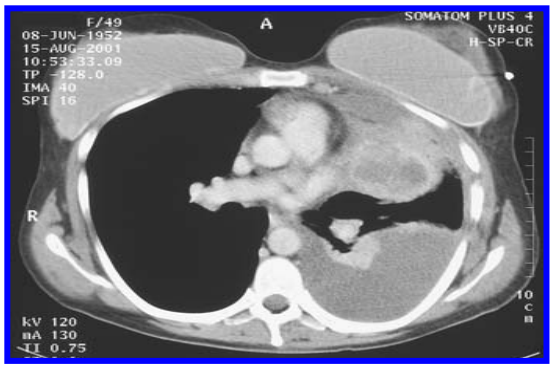
CTLA4 Program

Mechanism of Action Drives Strategic Plans

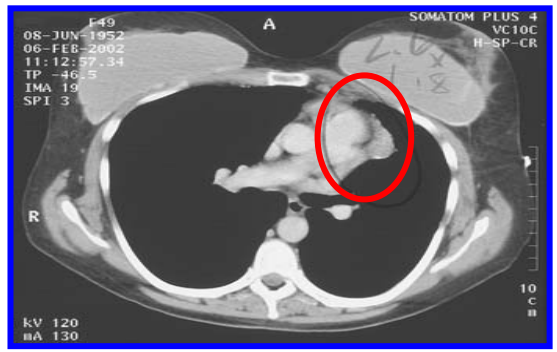


CTLA-4 (CD152)

- **Delivers a negative signal to T cells.**
- **May be constitutively expressed on Regulatory T cells**
- **Blockade in animal models results in tumor rejection**
- **Knockout mice have extensive Lymphoproliferation**
- **Polymorphisms of CTLA-4 associated with autoimmune disease in humans**

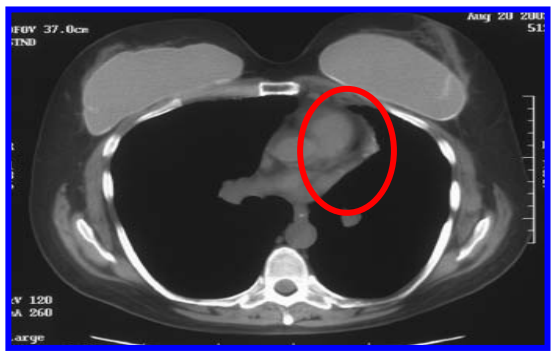


Pre-MDX (15AUG01)



Post-MDX dose
(6FEB02)

<u>Biopsy</u> confirmed persisting tumor



Post-CP-675,206 dose
(20AUG02; subsequently confirmed)

UCLA patient 113 treated with 3 mg/kg CP-675,206.
Previously received dendritic cell vaccine (3Q01) and MDX-010.

PHASE 1 STUDY WITH CP-675206

Dose mg/kg	Patient number	Clinical response	Duration mths
3	8	1CR,1 SD	24
10	11	1PR, 3SD	15
15	6	2CR,1PR, 1SD	14,13,14
	25	3CR,2PR, 5SD	

MEDAREX - 010

Anti CTLA-4 Antibodies As Adjuvant

	Patients	Vaccine	Response
Phan et al **Peptides 3wkly** **Anti CTL-4 3mg/kg**	14	gp100 peptides	2CR, 1PR (21%) (autoimmunity, g.i. skin, liver)
Weber, J **3 Peptides 4wkly** **MDX-010 up to 3mg/kg**	19	3 peptides	transient g.i. skin autoimmunity

Patient	Age/sex	Disease sites	Prior therapy	received*	(mos.)	Toxicity (grade III/IV)
1	52 M	Lung	I, S	2	PR (15+)	Enterocolitis; dermatitis
2	40 F	Supraclavicular lymph node	C, I, S	1	NR	Dermatitis; vitiligo†
3	39 M	Lung, mediastinum, subcutaneous	S	6	NR (mixed)	
4	55 F	Skin, subcutaneous	I, S	1	NR	Pulmonary infiltrates†
5	67 M	Liver, retroperitoneum, subcutaneous	C, I, R, S	4	NR	ANA+†
6	59 M	Lung, subcutaneous	I, S	4	NR	Vitiligo†
7	48 M	Lung, brain, adrenal, subcutaneous	I, S	2	NR	
8	48 M	Lung, liver, adrenal, mesentery, subcutaneous	C, I, S	2	NR	
9	53 M	Mediastinum, mesentery, skin	I, R, S	2	NR	Colitis
10	62 M	Lung, hilum	C, I, S	2	NR (mixed)	
11	54 M	Lung, brain, subcutaneous	C, S	5	CR (12+)	Hypophysitis
12	43 M	Subdiaphragm, muscle, subcutaneous	I, S	3	NR	Hepatitis; ANA+†
13	49 F	Lung, subcutaneous	C, I, S	4	CR (11+)	Dermatitis
14	63 M	Lung, pelvic lymph node	S	4	NR	

HYPOTHESIS----IMMUNE RESPONSES ARE OKAY. THE PROBLEM IS RESISTANCE OF MELANOMA CELLS TO KILLING BY THE IMMUNE SYSTEM

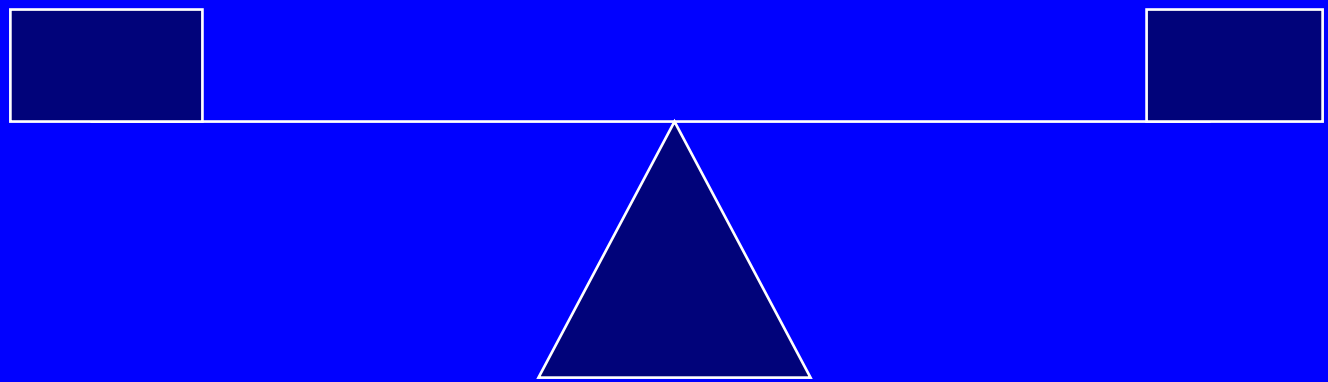
TUMOUR PROGRESSION

TUMOUR FACTORS

Cell Cycle regulation
Resistance to Apoptosis

HOST FACTORS

Immune System
Angiogenesis



CELL KILLING MECHANISMS USED BY LYMPHOCYTES DEPEND ON INDUCTION OF APOPTOSIS

1. Granzyme – Perforin Mediated Killing

CD8 CTL (CD4 CTL)

NK Cells and ADCC

2. TRAIL (FasL, TNF-α) Mediated Killing

CD4 T Cells

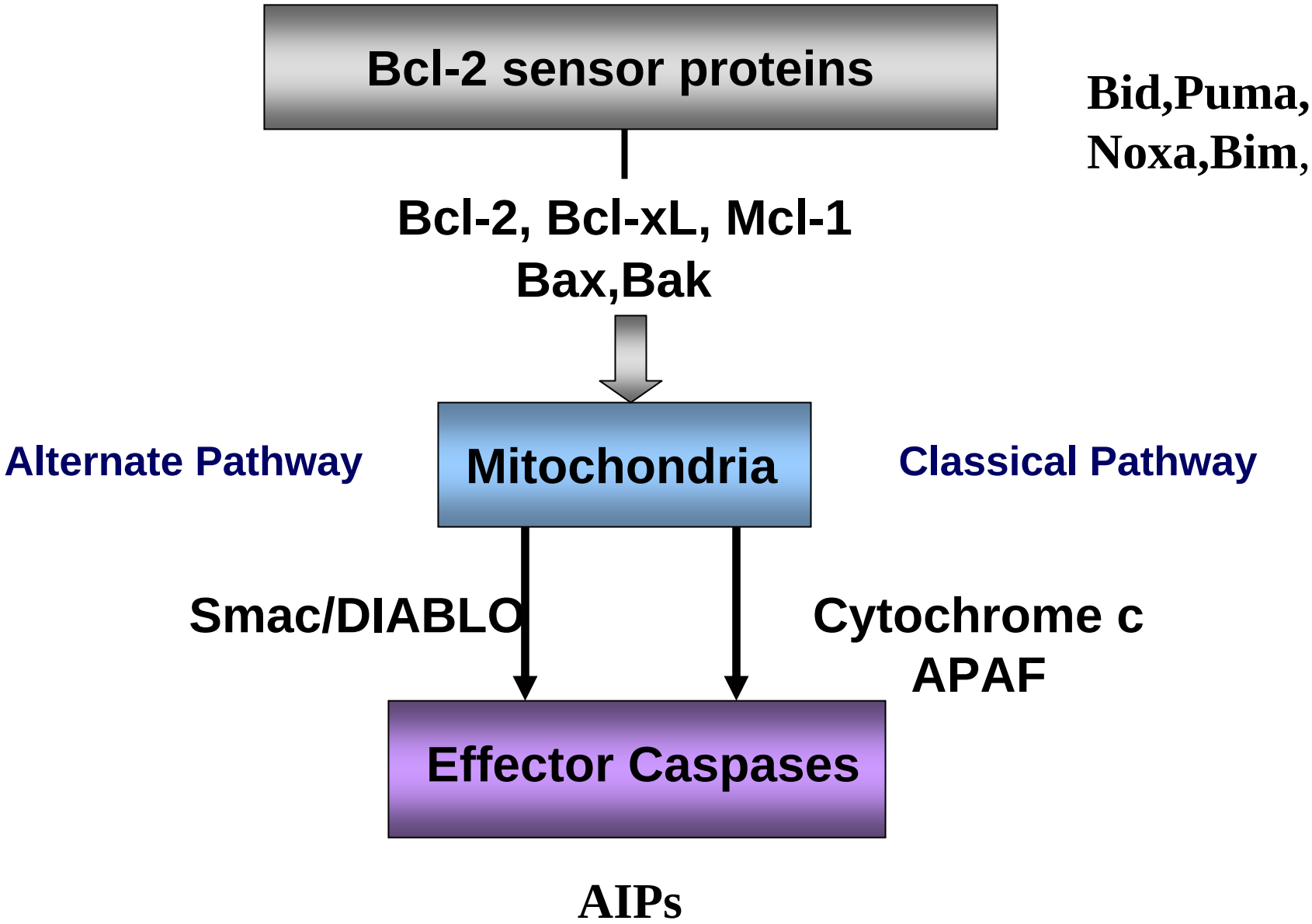
Monocytes, Dendritic Cells

Cytotoxic Activity of CD4 T-cells Against Autologous and Allogeneic Melanoma Cells

Cell Lines	TRAIL Induced Apoptosis	Specific Cytotoxicity			
		A4C2	C5C4	C5C5	2C4
Me 4405	61	35	58	17	7
Mel-CV	54	47	62	26	45
Mel-RM	76	92	83	32	64
Mel-FH	24	80	36	54	80
Me 1007	0	3	3	0	1
Mel-JS	0	3	3	0	2
K562	46	3	1	0	0

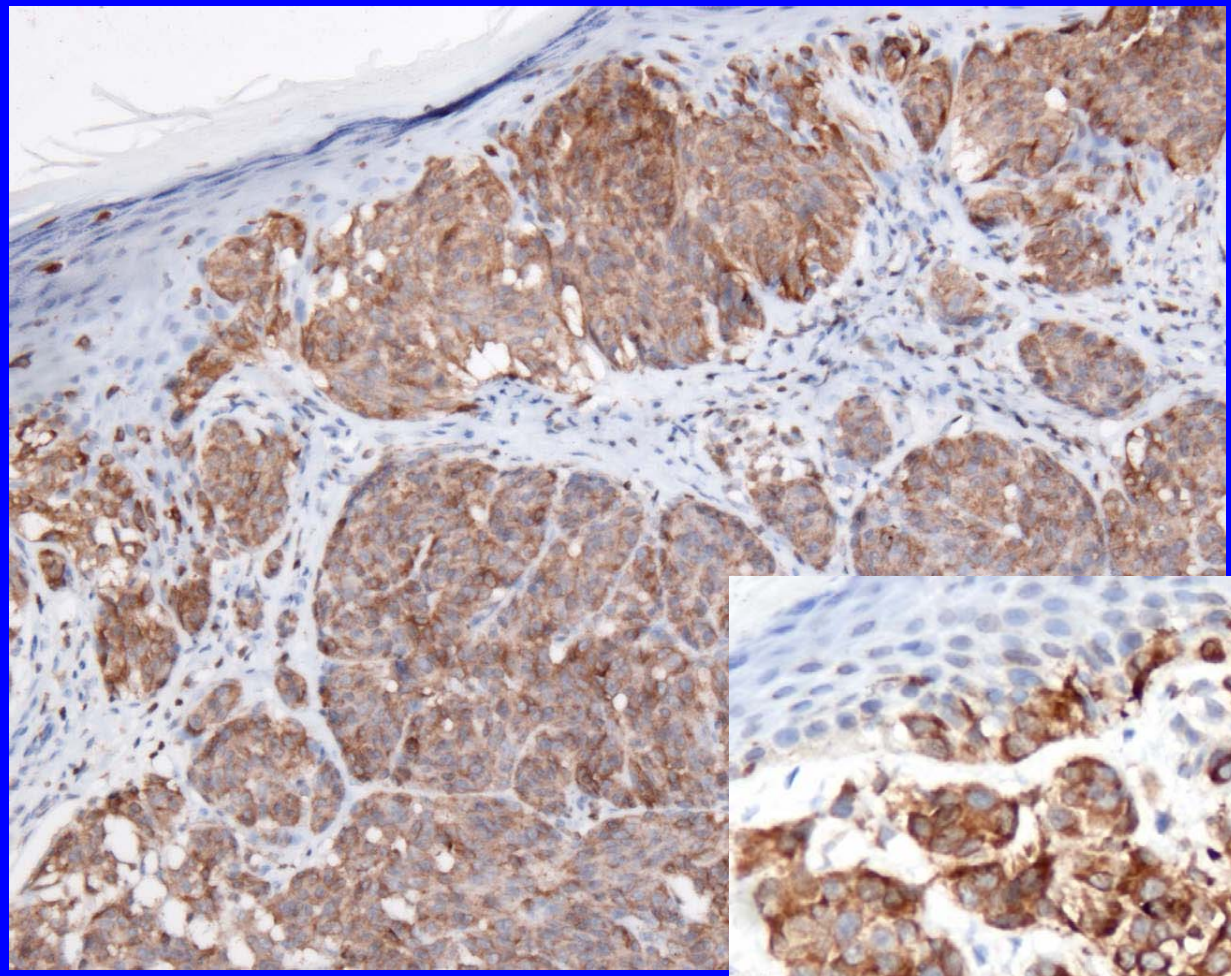
HYPOTHESIS--- OVERCOMING RESISTANCE TO APOPTOSIS WILL INCREASE SENSITIVITY TO IMMUNE RESPONSES

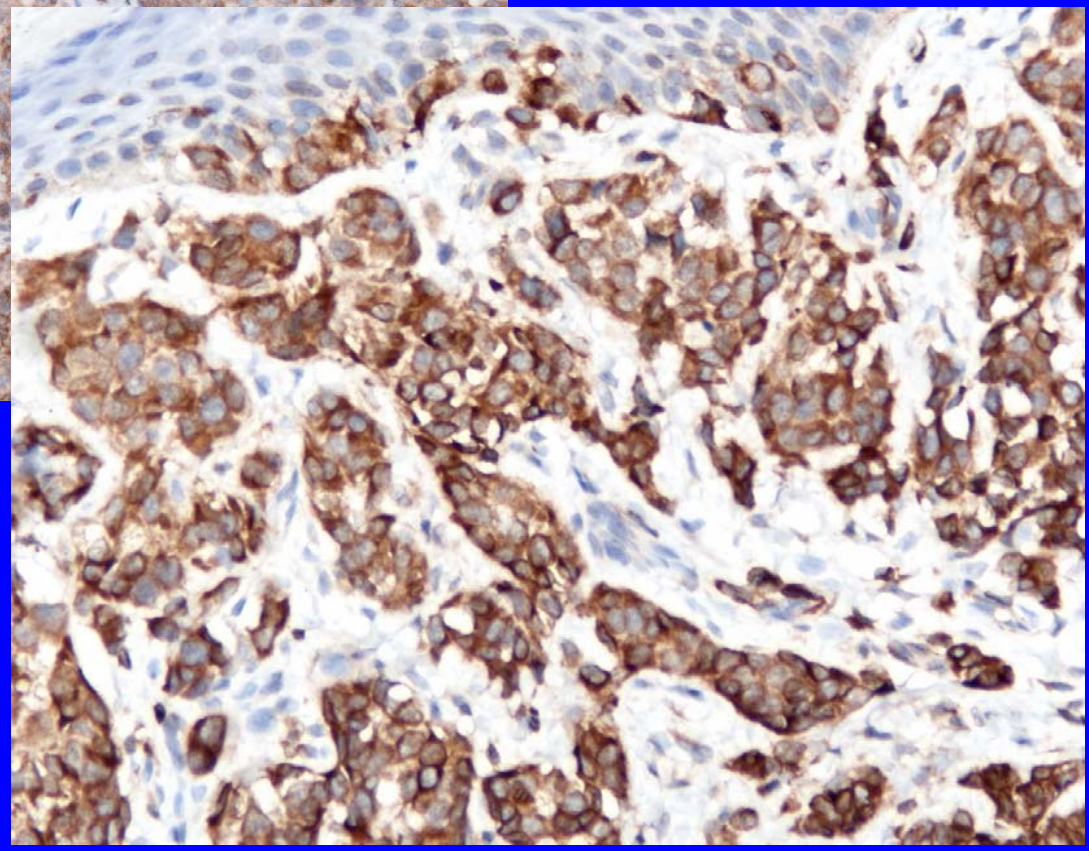
NEW CONCEPTS IN APOPTOSIS



MITOCHONDRIAL PATHWAYS TO APOPTOSIS ARE REGULATED BY BCL-2 FAMILY PROTEINS

- Pro-apoptotic BH3 only damage sensor proteins (Bid, Bim,Bmf,Noxa,Puma)
- Pro-apoptotic multidomain proteins: BAX, BAK
- Anti-apoptotic proteins: BCL-2, BCL-Xl, MCL-1, A1



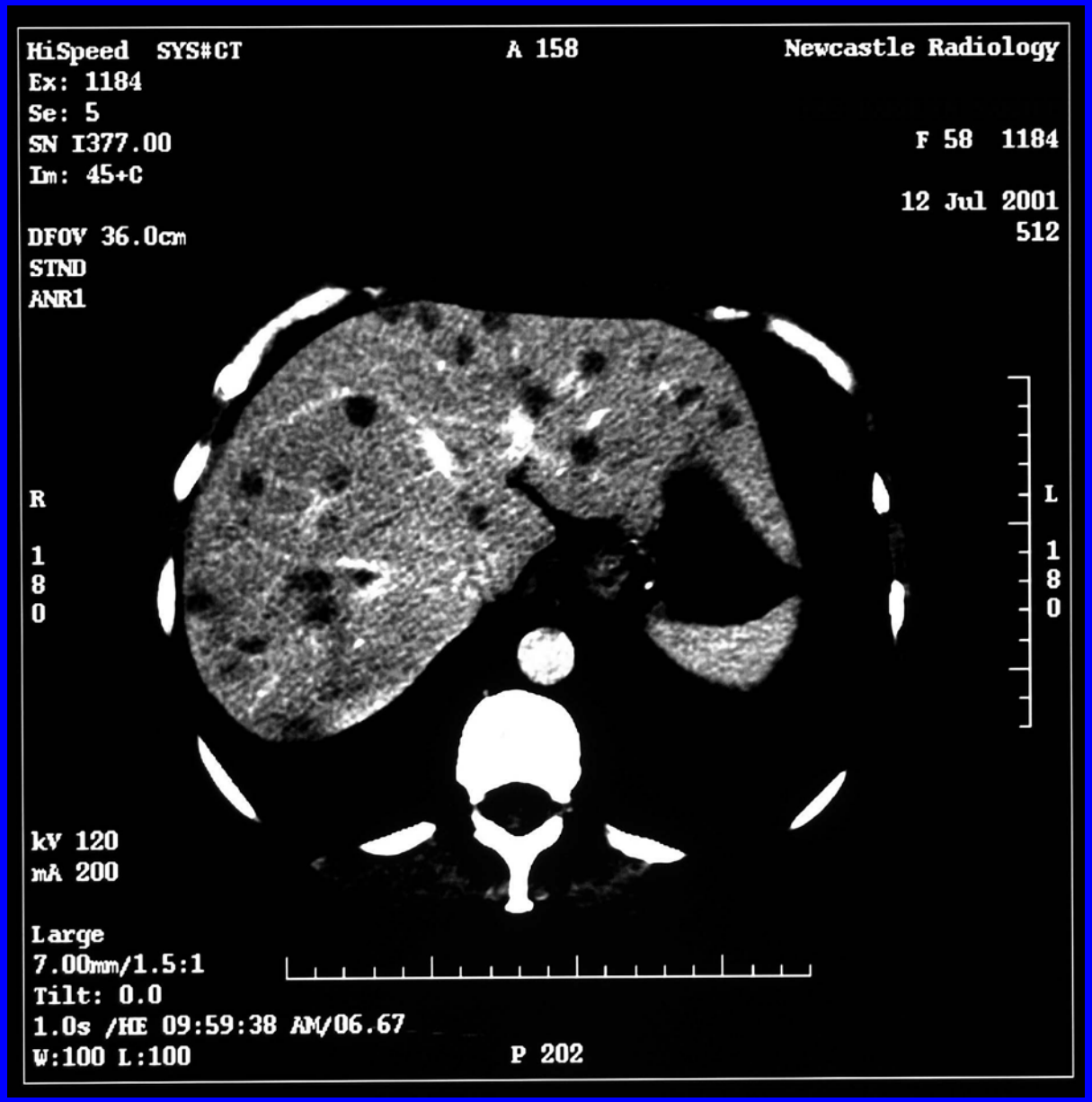


BCL-2 EXPRESSION IN 2 THIN PRIMARIES

CHANGING BCL-2 PROTEINS

- **INCREASE PRO-APOPTOTIC PROTEINS BY TREATMENT WITH MULTIPLE AGENTS**

- **DECREASE ANTIAPOPTOTIC PROTEINS eg BCL-2 ANTISENSE**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A 158
Newcastle Radiology
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Se: 5
SN I377.00
Im: 45+C
F 58 1184
12 Jul 2001
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DFOV 36.0cm
STND
ANR1
R
1
8
0
L
1
8
0
kV 120
mA 200
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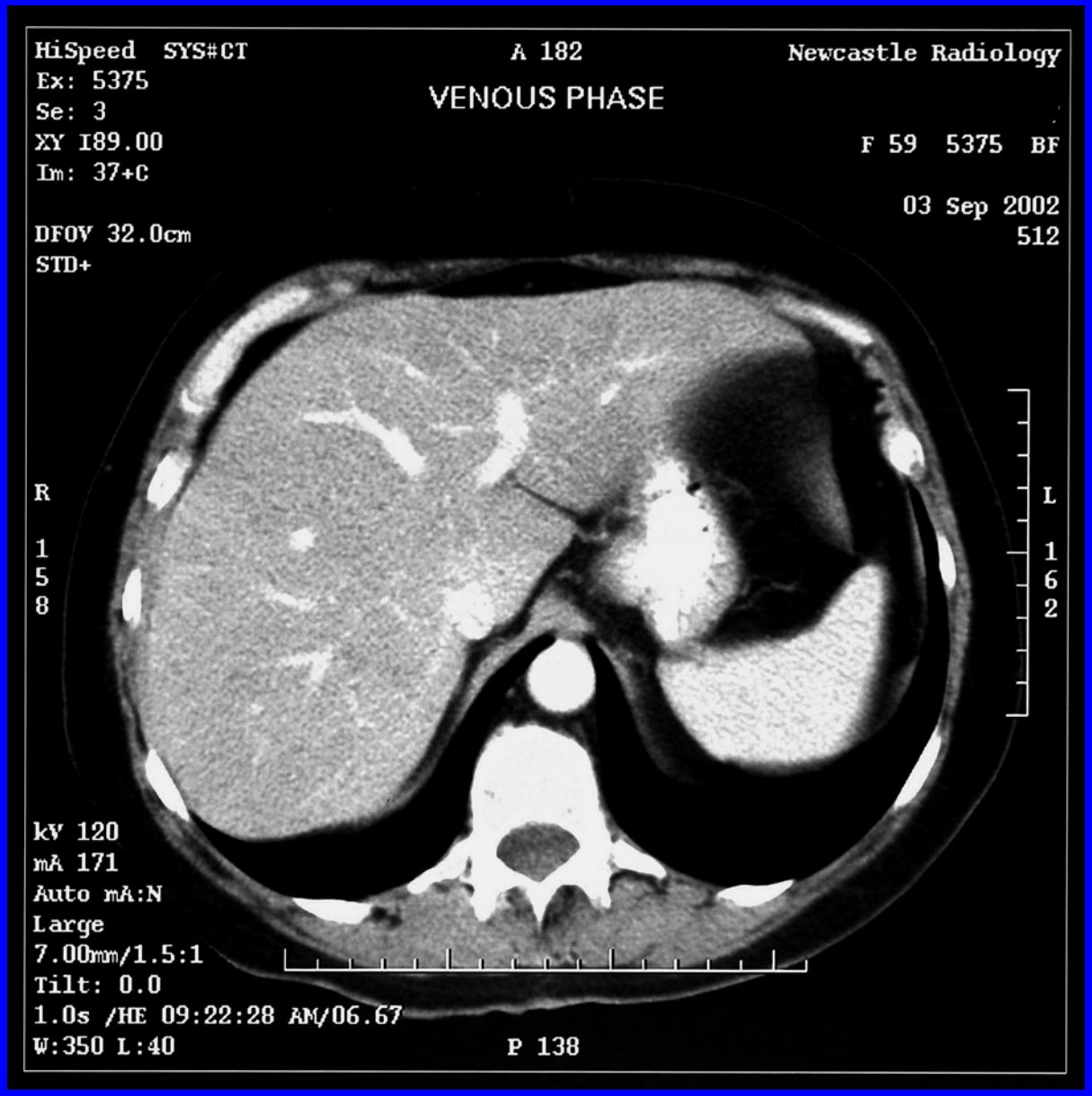
P 202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VENOUS PHASE
Newcastle Radiology
F 59 5375 BF
03 Sep 2002
512
R
158
L
162
kV 120
mA 171
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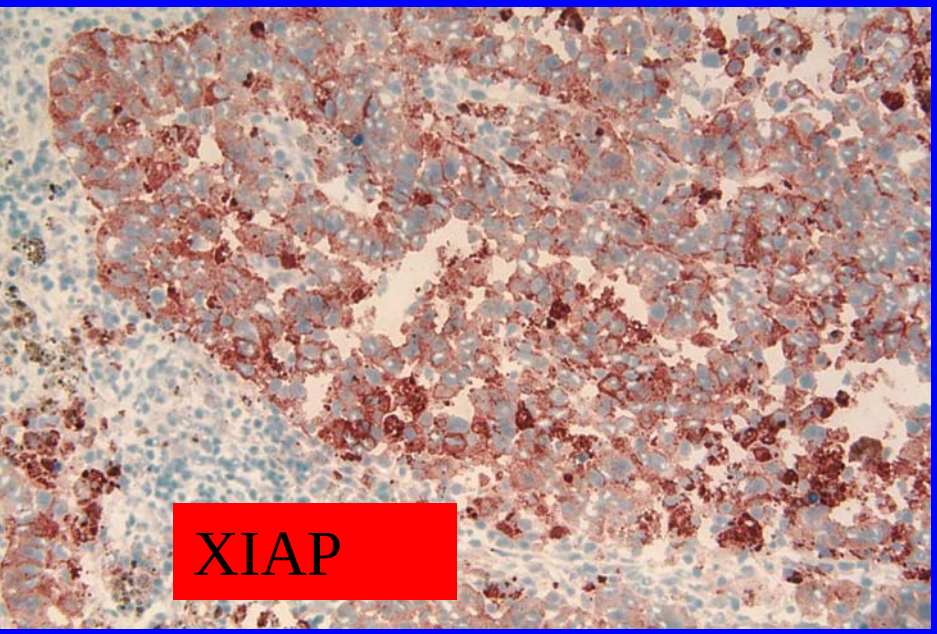
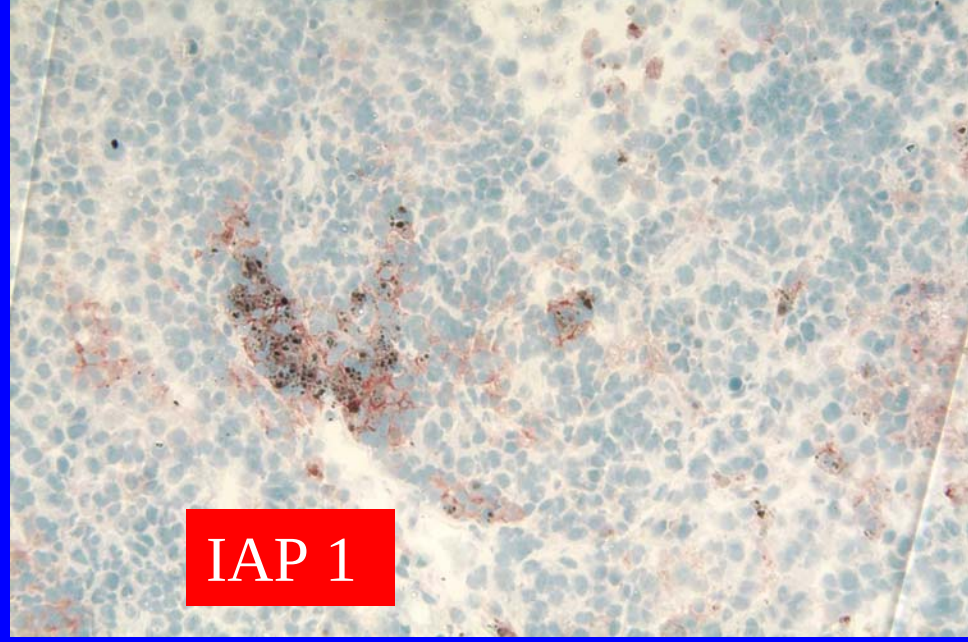
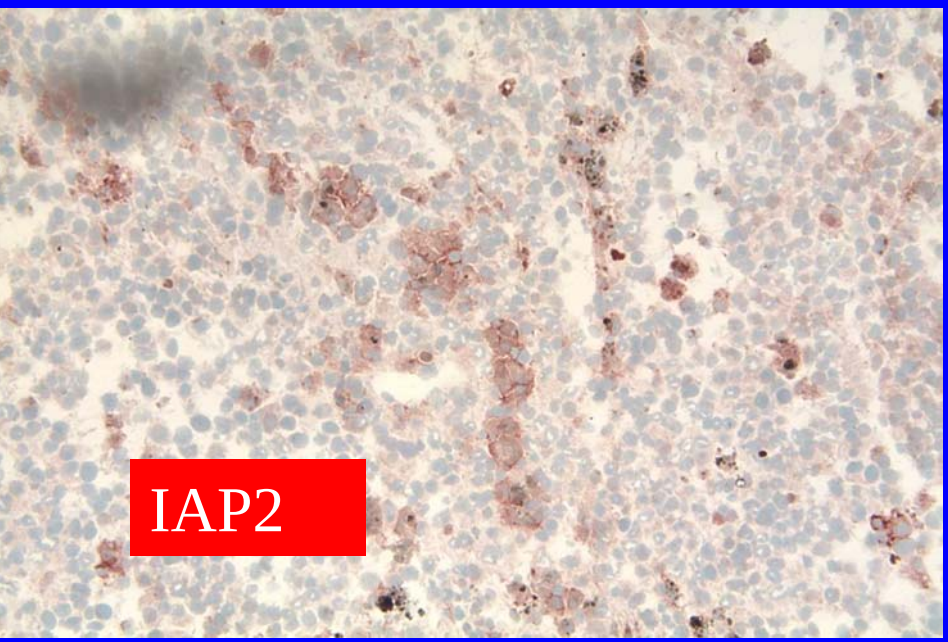






IAP PROTEINS IN A LN METASTASIS

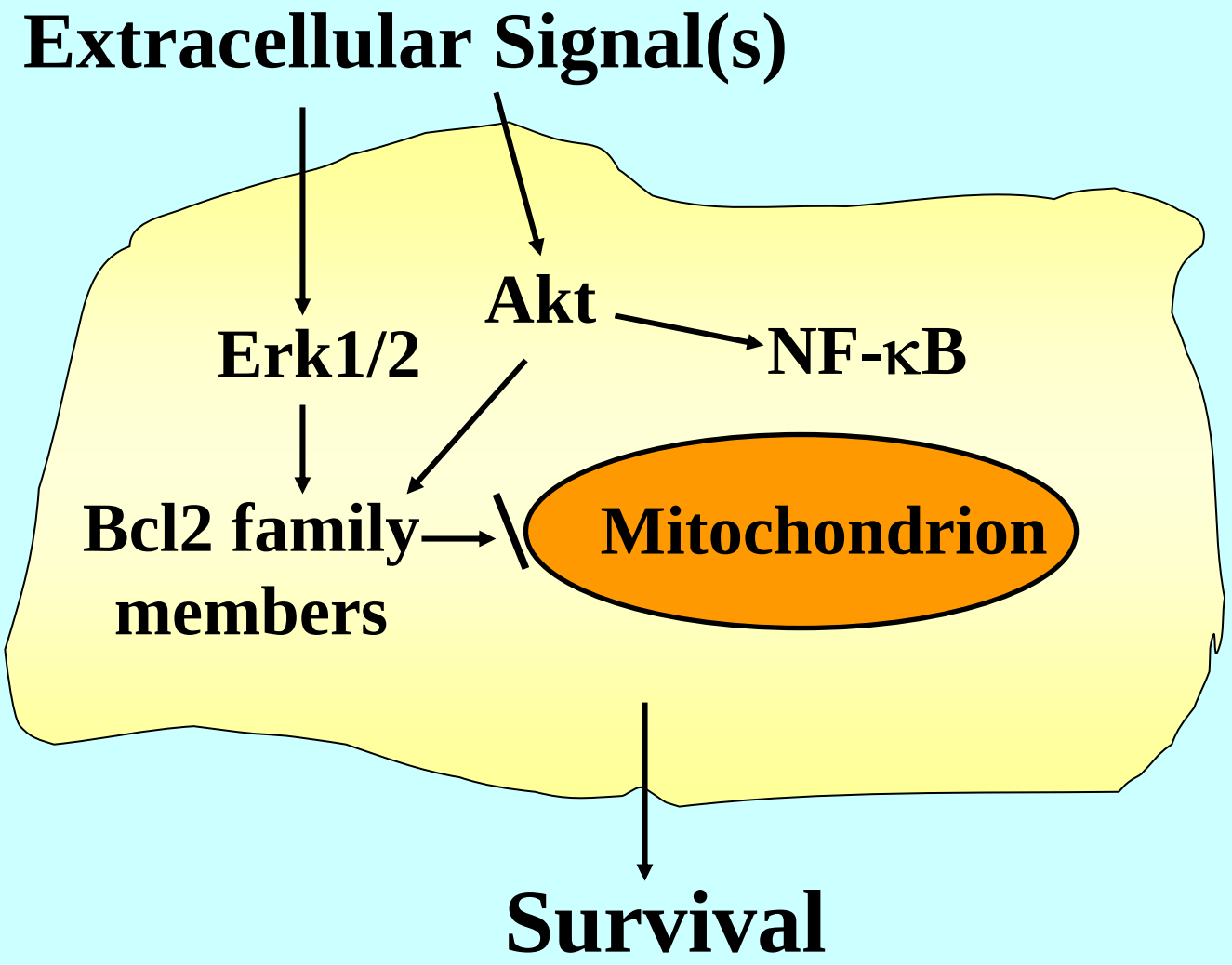
IAP PROTEINS-? TARGETS FOR SMALL MW INHIBITORS THAT MIMIC SMAC/DIABLO

- **Smac/DIABLO binds to all BIR domains in IAPs but particularly to BIR 3 whereas OMI binds to BIR 2 eg in XIAP**

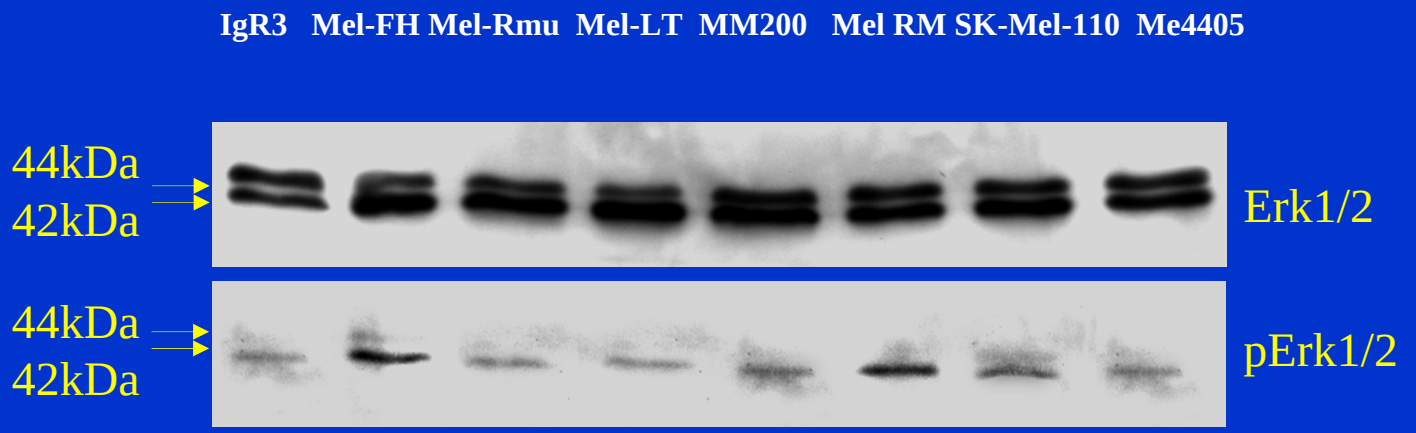
- **AVPI sequence in Smac is important for binding to BIR 3. Changing AVPA in OMI to AVPI allows it to bind equally to BIR 3&2 (Verhagen)**

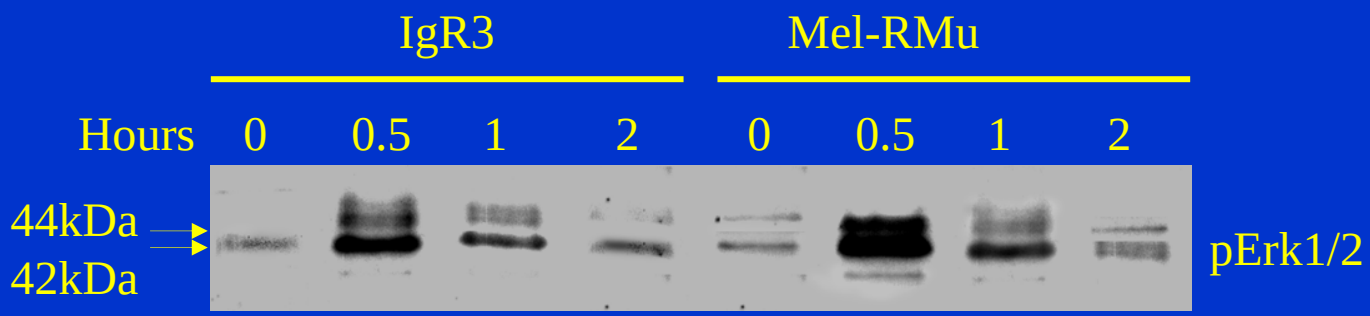
WHAT REGULATES THE REGULATORS?

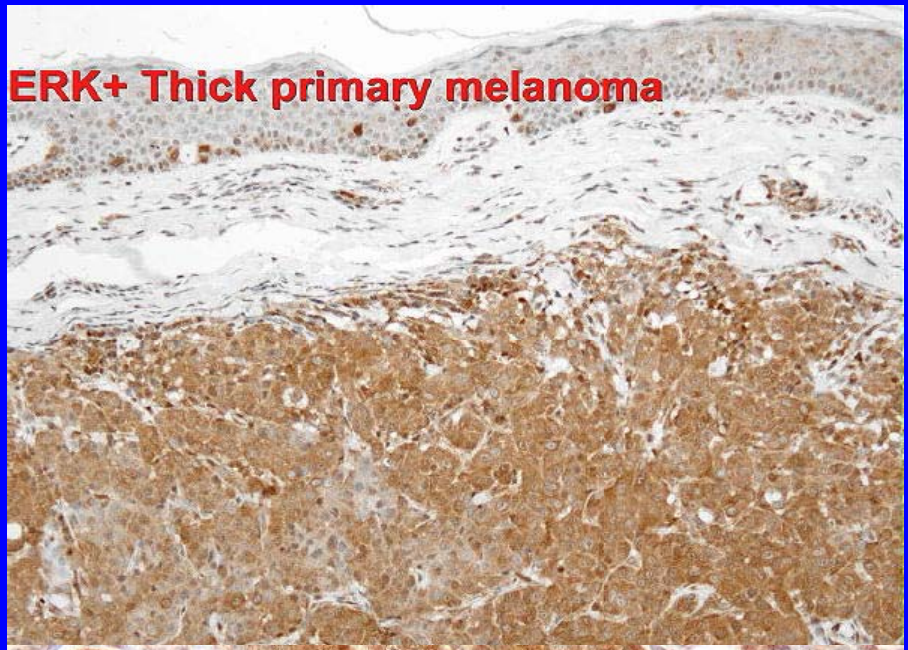
Erk1/2 and Akt Signal Pathways Protect Cells from Apoptosis

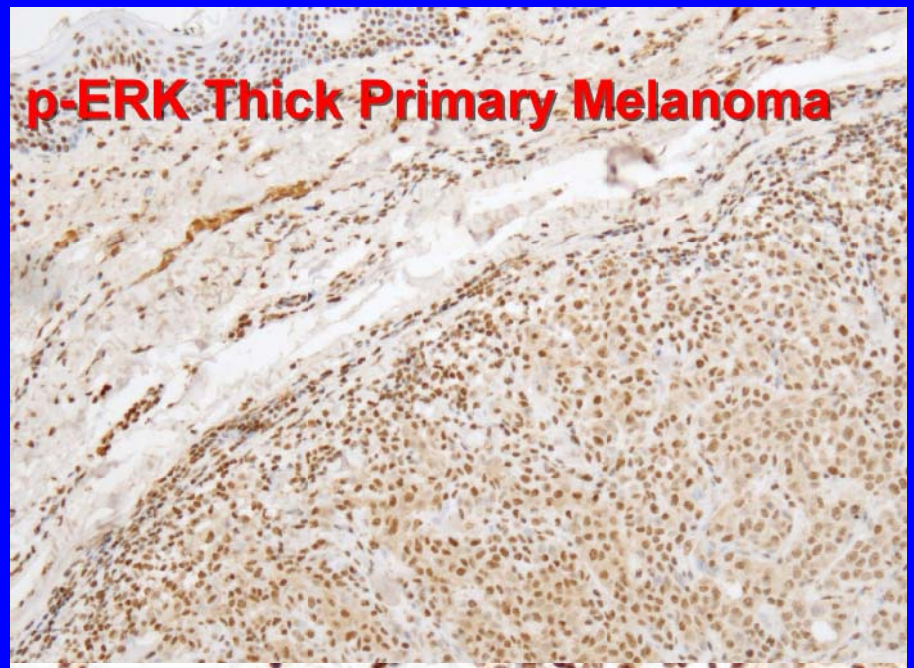


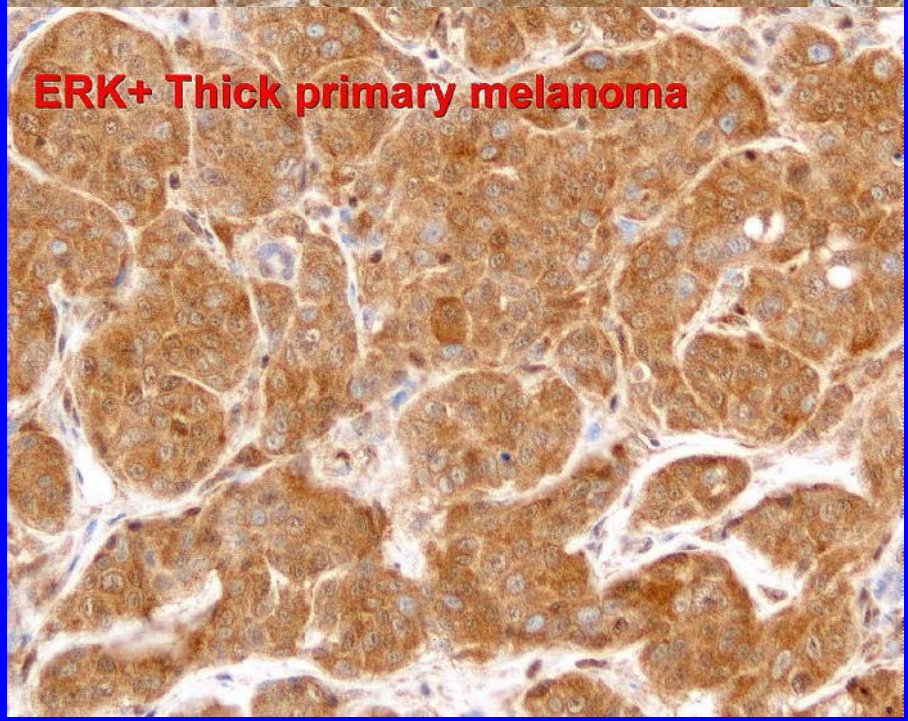
TRAIL Induces Rapid Erk1/2 Activation in Melanoma cells

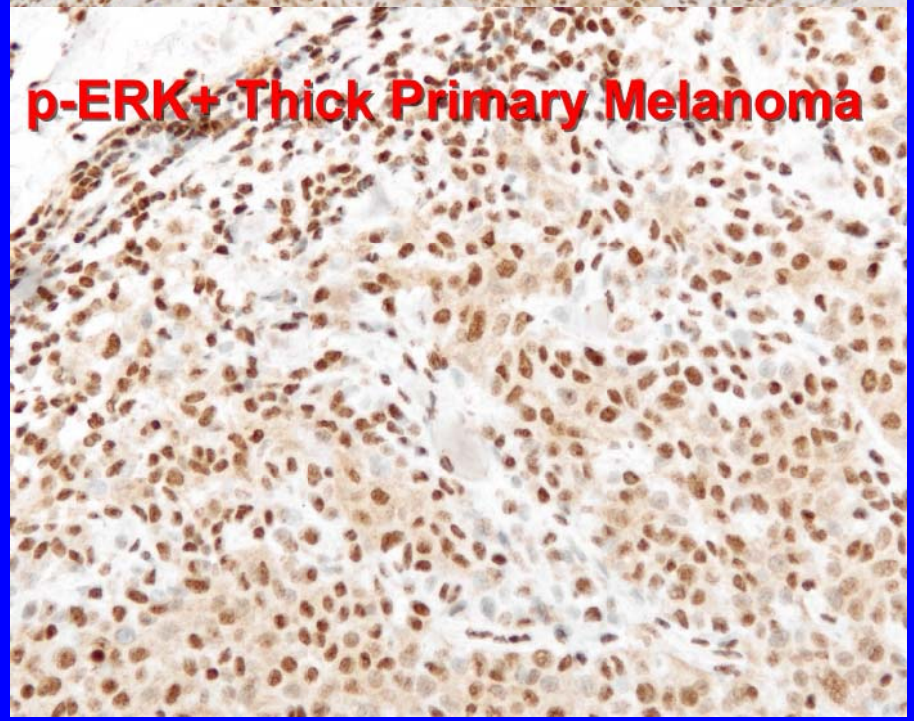




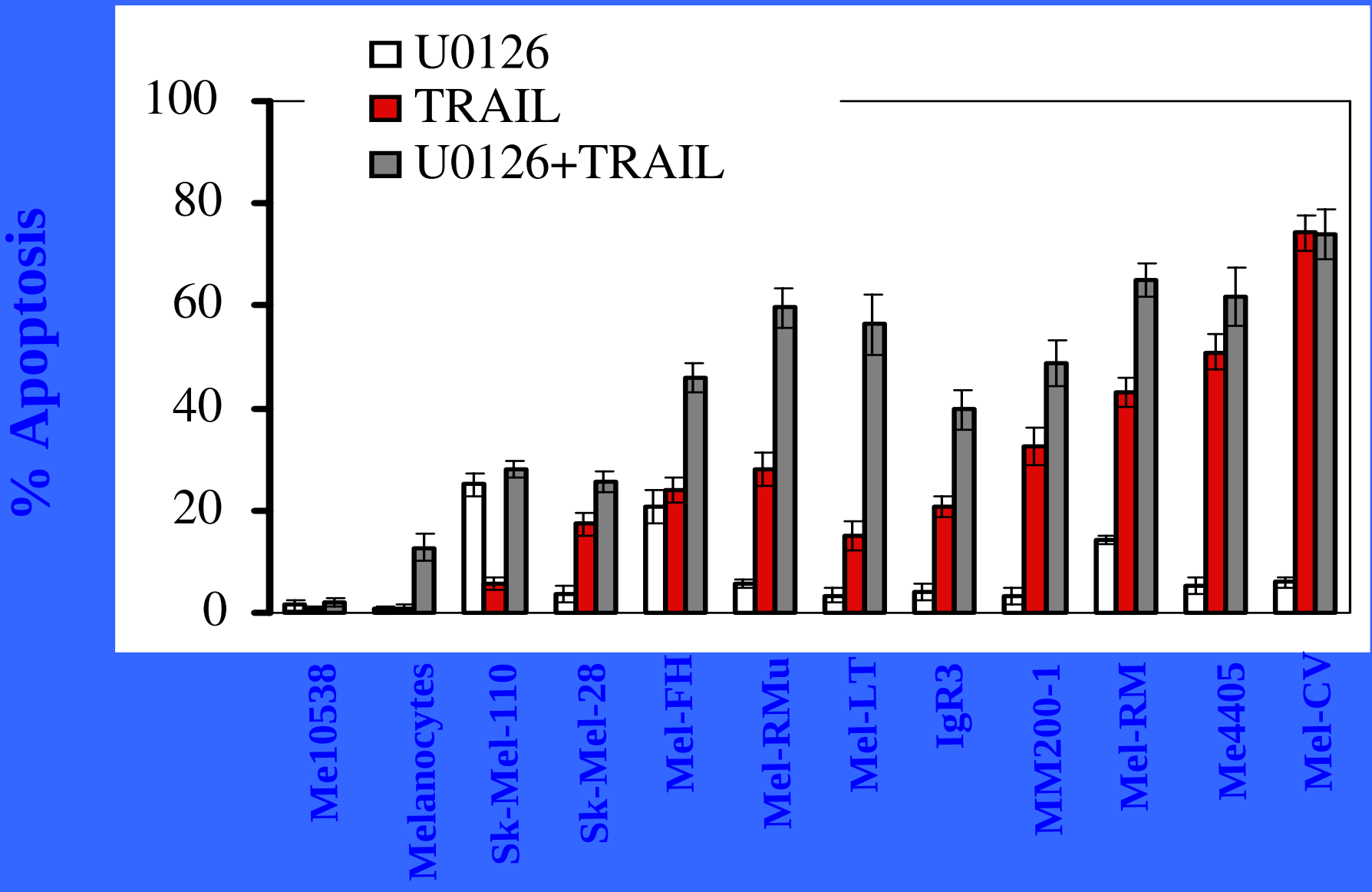

ERK+ Thick primary melanoma


p-ERK Thick Primary Melanoma

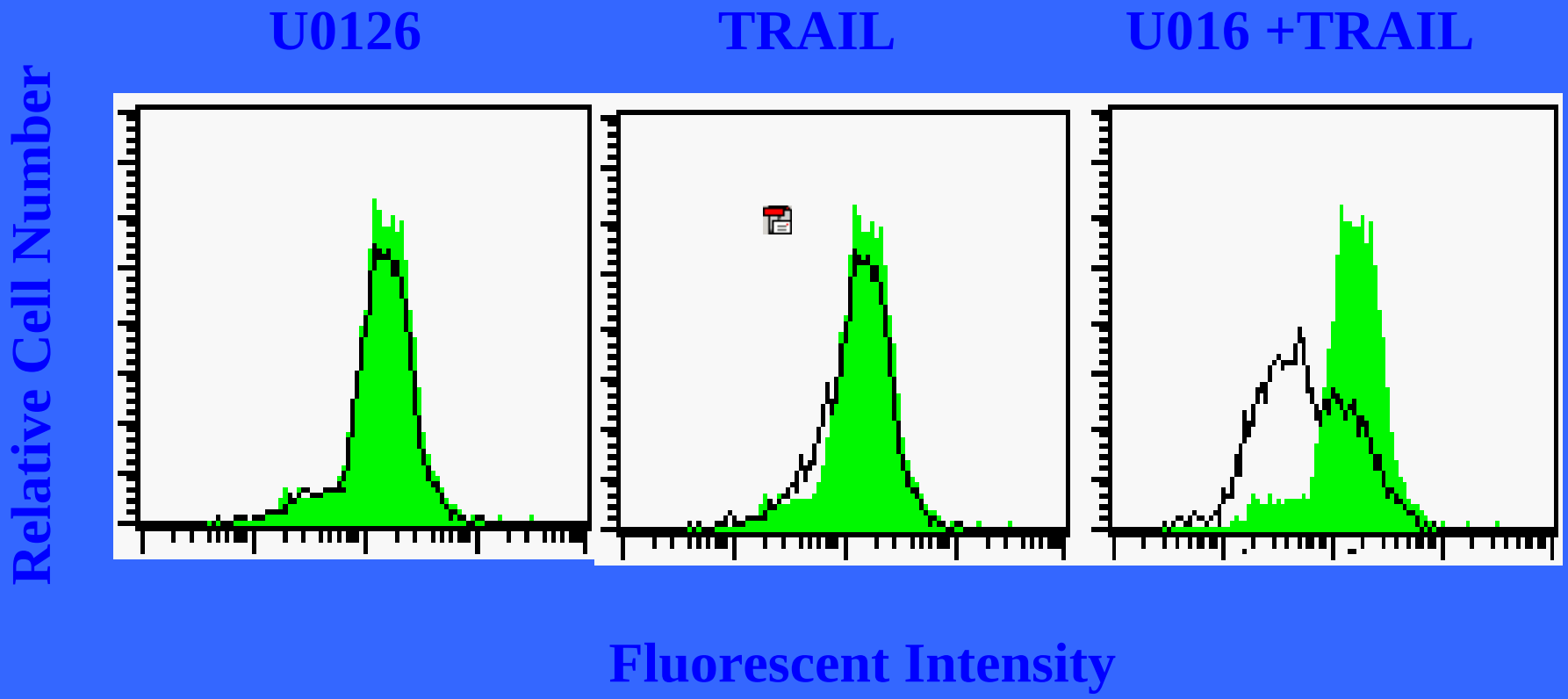

ERK+ Thick primary melanoma


p-ERK+ Thick Primary Melanoma

U0126 Sensitises Melanoma to TRAIL-Induced Apoptosis



TRAIL Induces a Marked Increase in Reduction of the Mitochondrial Membrane Potential in the Presence of U0126



MAP KINASE /ERK1/2 PATHWAY IN MELANOMA

- MAY BE ACTIVATED BY EXTERNAL STIMULI LIKE TRAIL OR BY ACTIVATING MUTATIONS IN BRAF(Davies et al)
- RAF KINASE INHIBITOR BAY-43-9006 SHOWING PROMISE IN TRIALS WITH CHEMOTHERAPY

BAY-43-9006 PLUS CHEMOTHERAPY IN MELANOMA

- 31 previously treated patients with metastatic disease
- Followed from 6-21 months

- 12PR,15 SD,1 PD. 3 early deaths

- Randomized trial now planned by ECOG

AGENTS WHICH SENSITIZE MELANOMA TO APOPTOSIS

- **↓ Bcl-2 proteins-eg Bcl-2 antisense**
- **↓ IAP proteins –eg PS 341, Act D , Smac/DIABLO mimics.**
- **MAP kinase inhibitors eg CI 1040, BAY –43 9006 BRAF inhibitor.**
- **Histone Deacetylase Inhibitors**

SUMMARY OF VACCINE STUDIES

1) **Melanoma vaccines generally non-toxic but as yet of unproven benefit.**

2) **Many new vaccine strategies are being developed.**

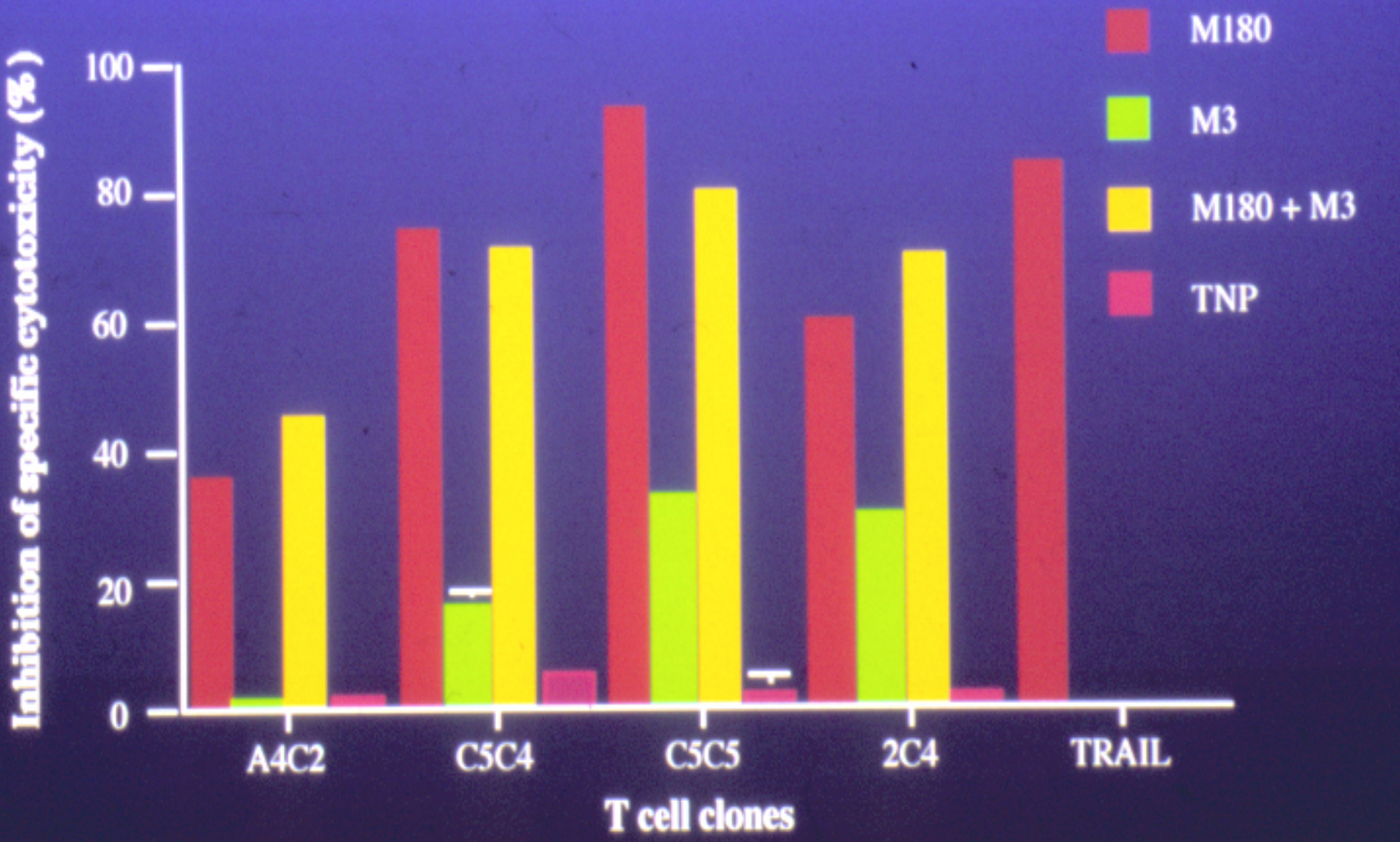
3) **Vaccine strategies may need to include agents that sensitize melanoma cells to apoptosis and reduce immunosuppressive factors.**

4) **All patients may not be suitable for immunotherapy.**

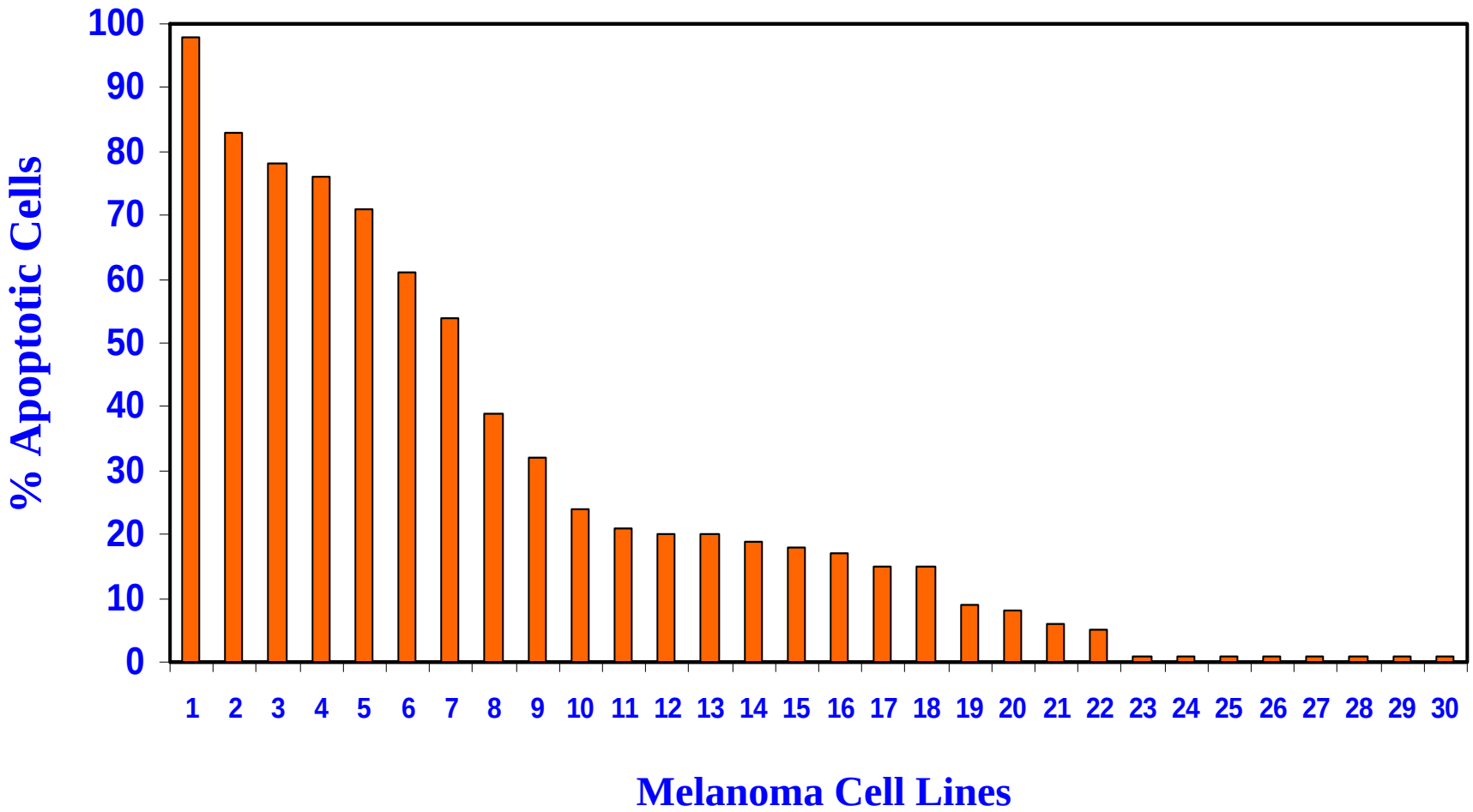
CONCLUDING HYPOTHESIS

- TO GET A CURE THE IMMUNE SYSTEM MAY BE NEEDED TO ERADICATE THE LAST CANCER CELLS

Cytotoxic mechanisms involved in CD4 T cell mediated killing of Jurkat T cells



TRAIL Induces Apoptosis in the Majority of Melanoma Cell Lines



SWOG-9035

Relapse-free survival

By Treatment and HLA-A2/C3 Status