Προθυμοσίνη α: ένα πολυπεπτίδιο με διττό ρόλο & εναργή ανοσοδραστικότητα

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Τομέας Φυσιολογίας Ζώων & Ανθρώπου
Τμήμα Βιολογίας
Πανεπιστήμιο Αθηνών

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Cancer Immunotherapy

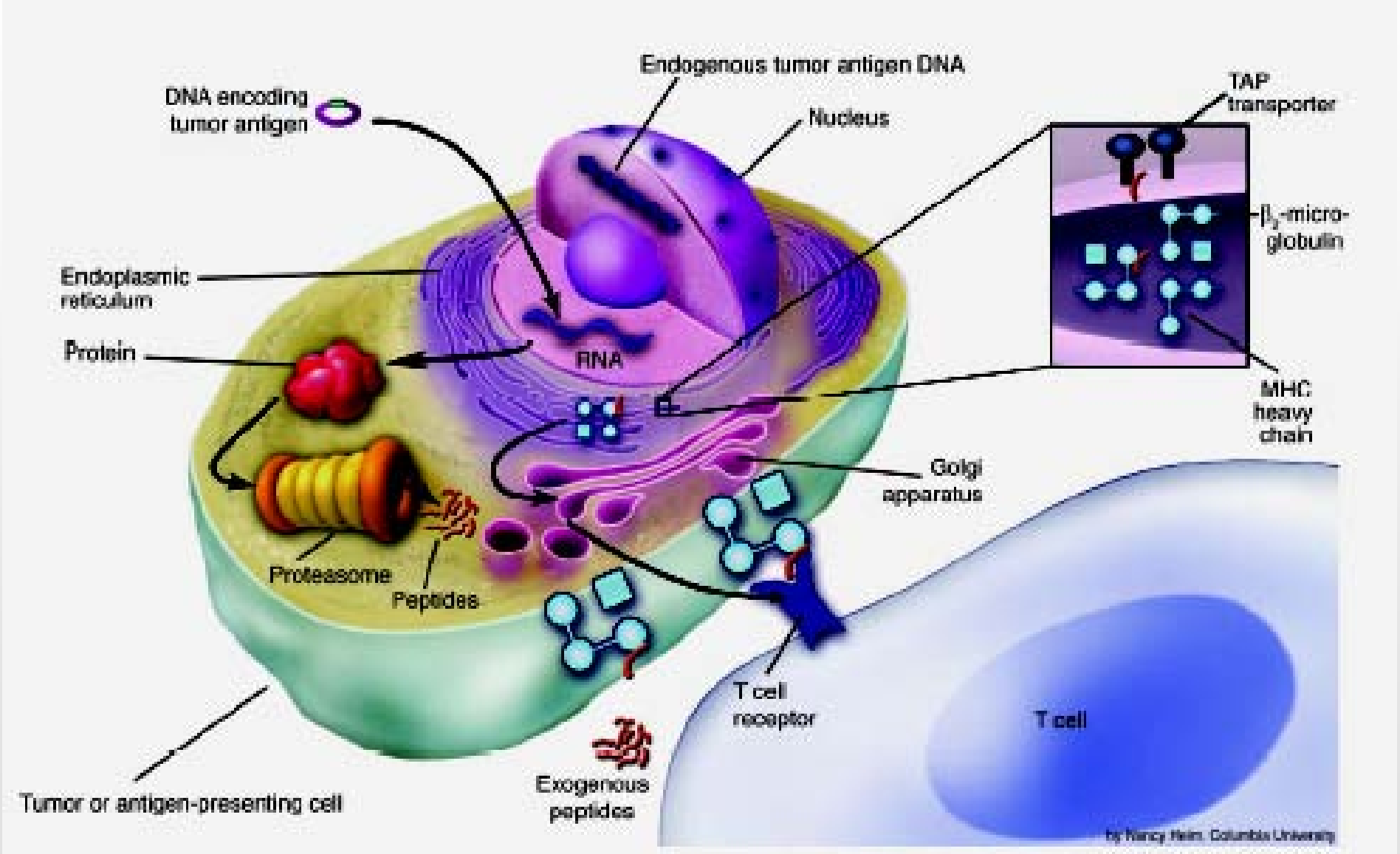
3 basic concepts

1. **Active immunotherapy** (vaccination)

2. **Adoptive or passive immunotherapy** (repeated infusions of ex vivo activated cells and/or cytokines or BRMs)

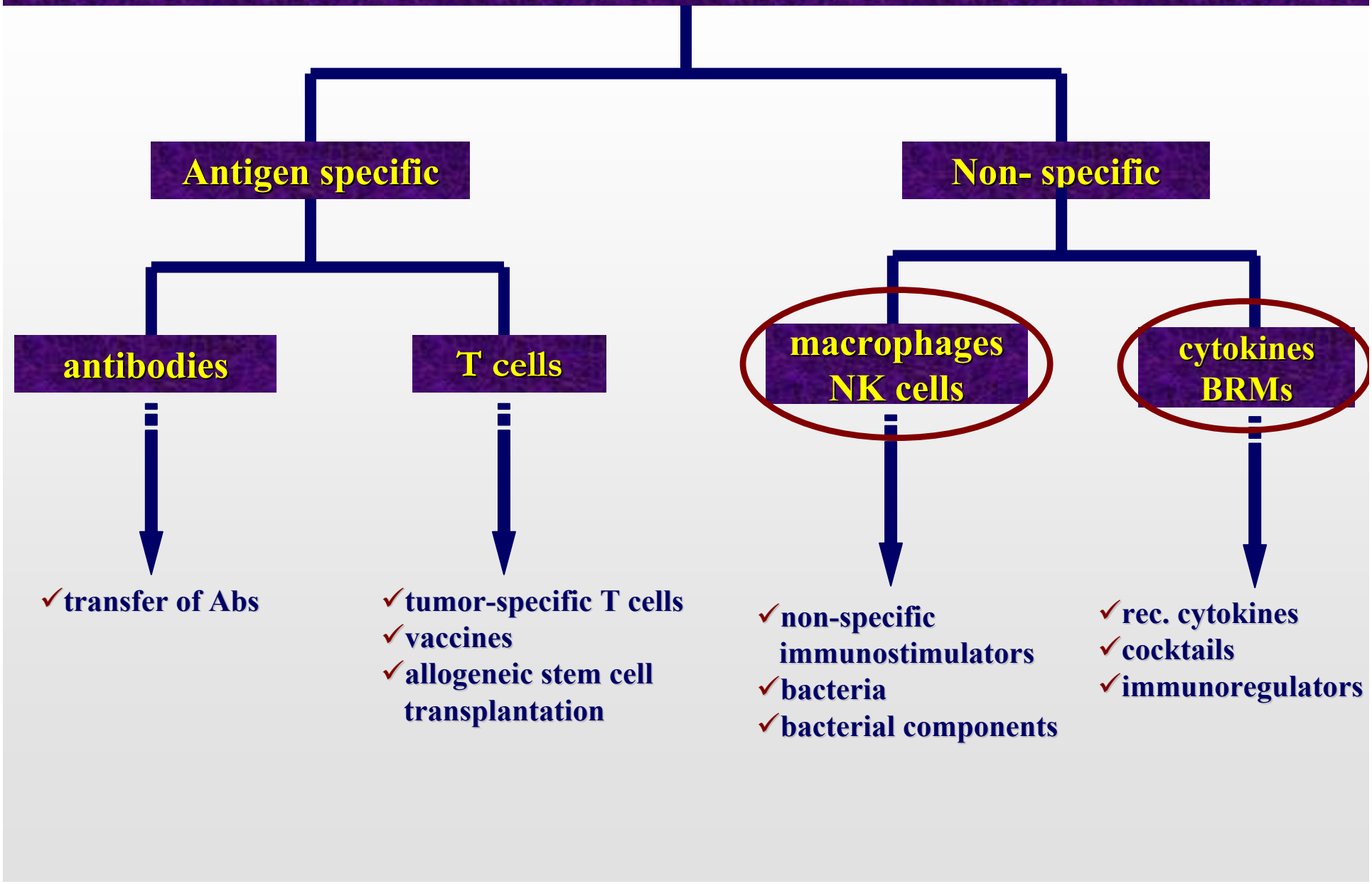
3. **Gene therapy** (arm the cell with impaired molecules or functions)

T cells can see inside a cancer cell



Berzofsky et al., 2004

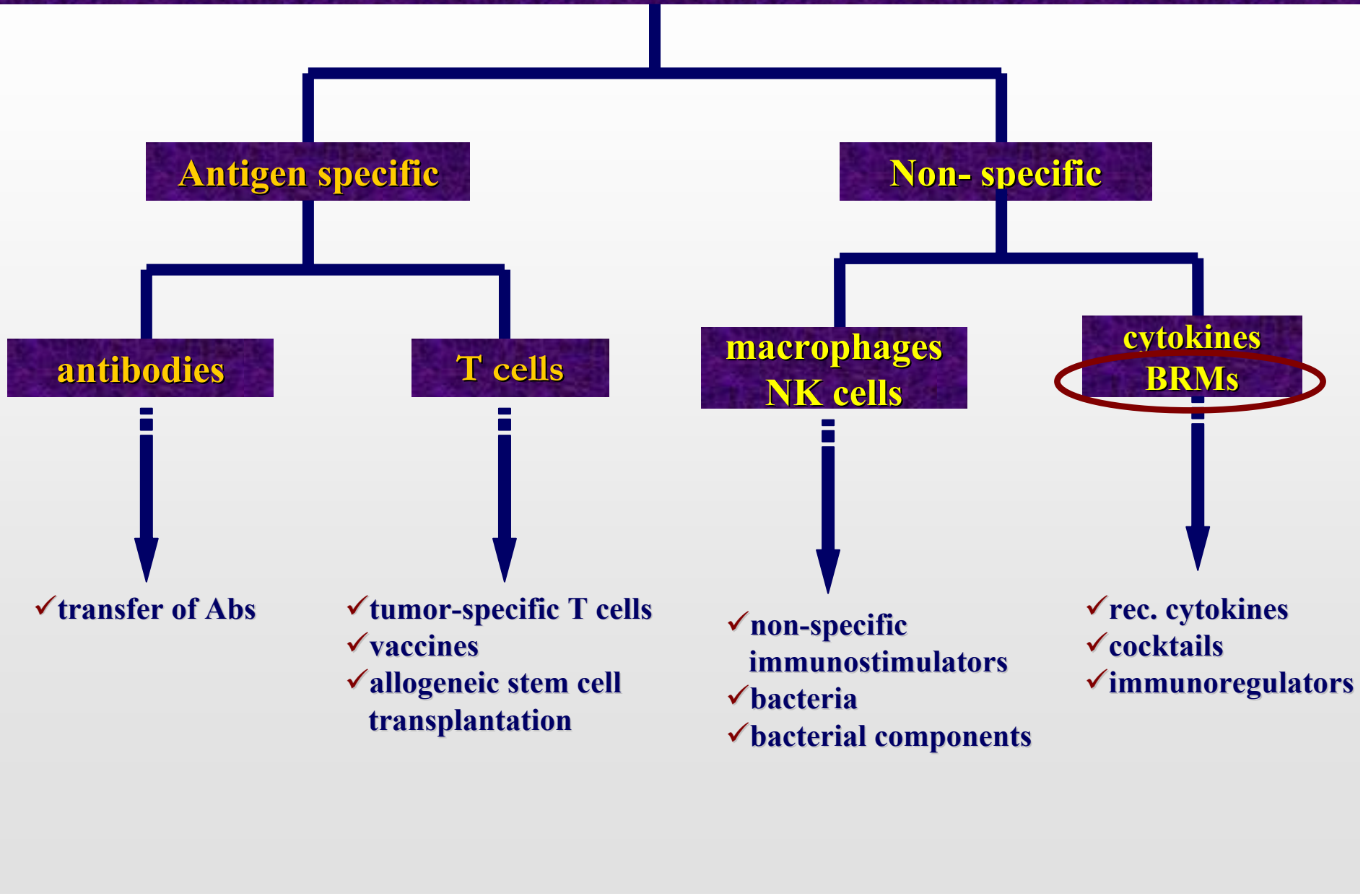
Mediators of Immune Defense



Immunostimulating agents / BRMs

- ***Interferon-α:*** *melanoma, renal carcinoma, hairy cell leukemia, CML*
- ***Interleukin-2:*** *melanoma, renal cell carcinoma, ALL, CLL, non-Hodkin's lymphoma*
- ***GM-CSF:*** *melanoma*
- ***TNF-α, IL-3, IL-4, IL-6, IL-12, IFN-β, IFN-γ***
- ***BCG, alum, CpG oligos***
- ***Thymic peptides:*** *thymic FV, thymosin α1, prothymosin α*

Mediators of Immune Defense



The α-thymosin story (I)

1966 : Thymosin (Goldstein et al.)

calf thymus; thermostable protein; 10 kDa

induces lymphocyte proliferation

1975 : Thymosin Fraction V (Hooper et al.)

calf thymus; 40 members; 1-15 kDa; **α-thymosins (pI <5)**

β-thymosins (pI 5-7)

γ-thymosins (pI >7)

1977 : Thymosin α1 (Goldstein et al.)

calf thymus; 3.1 kDa; pI 3.85; 28 amino acids

10-1000 times more active than TF V

1982 : Thymosin α11 & des-(25-28) thymosin α1 (Caldarella et al.)

calf thymus TF V; 35 & 24 amino acids

equally active to thymosin α1

The α-thymosin story (II)

	1	11	21
des-(25-28) thymosin α1	Ac-SDAAVDTSSE	ITTKDLKEKK	EVVE
Thymosin α1	Ac-SDAAVDTSSE	ITTKDLKEKK	EVVEEAEN
Thymosin α11	Ac-SDAAVDTSSE	ITTKDLKEKK	EVVEEAENGR DAPAN

Table 3. Effect of thymosin fraction 5 and thymic peptides on the growth of *C. albicans* in C3H/HeJ mice

Thymosin fraction 5		Thymosin α_1		Thymosin α_{11}	
Dose, ng per mouse	*C. albicans* cell count*	Dose, ng per mouse	*C. albicans* cell count*	Dose, ng per mouse	*C. albicans* cell count*
None	5,100				
2,560	8,500	80	5,870	80	4,200
5,120	440	160	190	160	510
10,240	320	320	280	320	320
20,480	1,600	640	1,410	640	1,260

Caldarella et al., 1982

The α-thymosin story (III)

1966 : Thymosin (Goldstein et al.)

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calf thymus TF V; 35 & 24 amino acids

equally active to thymosin α1

1984 : Prothymosin α (Haritos et al.)

rat thymus; 111 amino acids

more active than thymosin α1

Who isolated proTα

Haritos et al., 1984

Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 1008–1011, February 1984
Biochemistry

Prothymosin α: Isolation and properties of the major immunoreactive form of thymosin α_1 in rat thymus

(radioimmunoassay/thymic polypeptide/protection against opportunistic infections)

A. A. HARITOS*, GREGORY J. GOODALL†, AND B. L. HORECKER

Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110

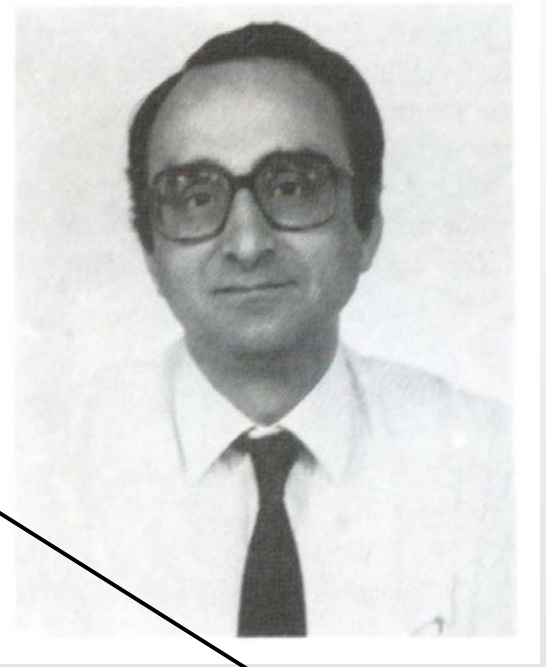
Contributed by B. L. Horecker, October 27, 1983

ABSTRACT A polypeptide containing ≈112 amino acid residues, with the thymosin α_1 sequence at its NH_2 terminus, has been isolated from rat thymus by using a radioimmunoassay with an antibody prepared against synthetic thymosin α_1. The new polypeptide, named "prothymosin α," was found to be the major substance crossreacting with thymosin α_1 antiserum in rat thymus extracts; peptides corresponding to thy-

terminus. We named this polypeptide prothymosin α because it appears to be the source of the thymosin α_1-related peptide fragments found in preparations of thymosin fraction 5.

MATERIALS AND METHODS

Rat thymuses from male Charles River CD rats, 5 weeks old



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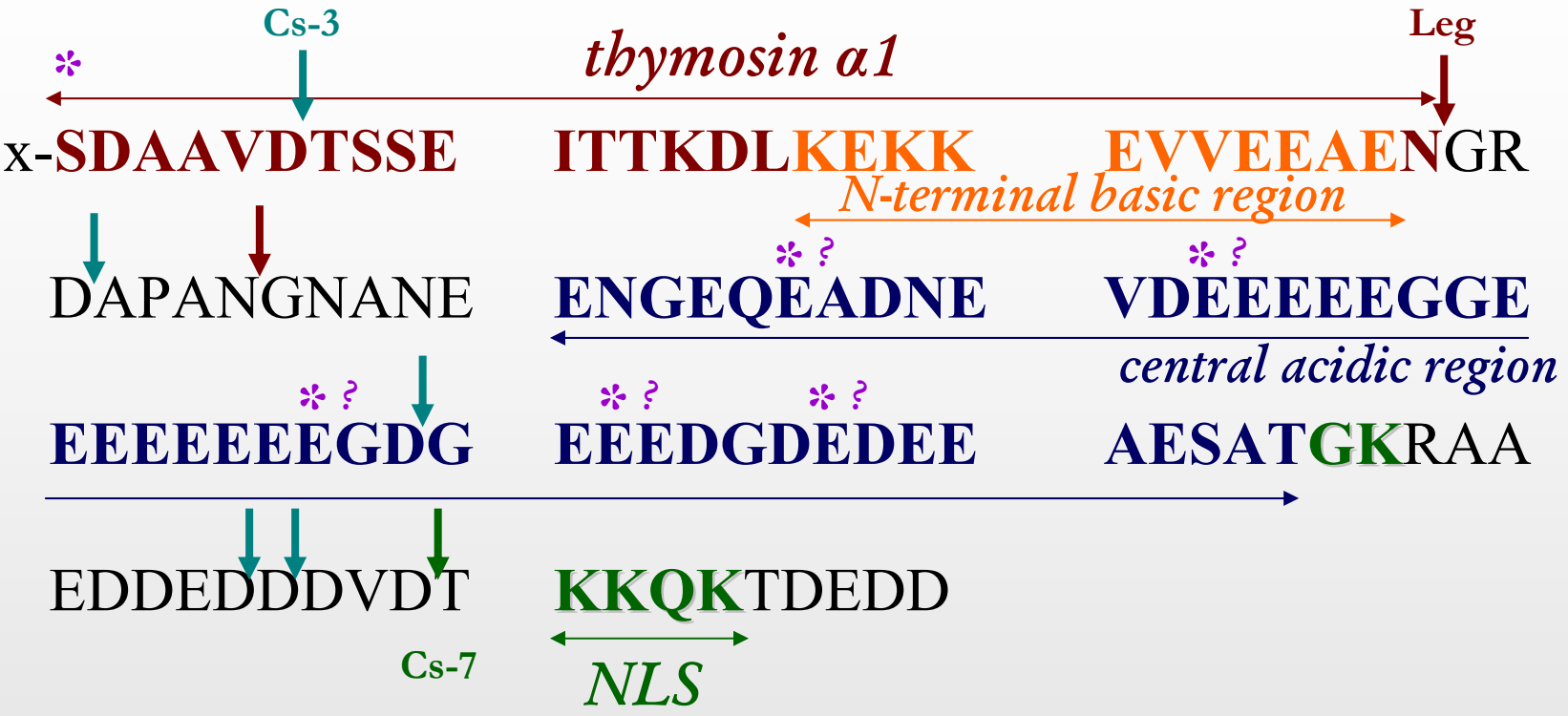
Structural properties of proTα



- *109 aa; 39 Glu + 18 Asp*
- *most acidic polypeptide*
- *no aromatic; no sulfur*
- *no absorption at 280 nm*

- *no signal peptide for secretion*
- *no secondary structure*
- *oligo or monomeric nature*
- *phosphorylated in vivo (aa 48-87)**

What does prothymosin α do?

INTRACELLULAR ROLE

Role of ProTα ?

EXTRACELLULAR ROLE
(IMMUNOLOGICAL)

Intracellular role of prothymosin α

- *essential for cell survival and cell proliferation*
  - cells deficient in ProTα cannot divide *(Sburlati et al., 1991)*
  - is highly upregulated in proliferating cells *(Tsitsiloni et al., 1993)*
  - increases during G1 *(Vareli et al., 1996)*
  - proTα antisense oligos inhibit cell division and siRNA induces apoptosis *(Jiang et al., 2003)*
  - is c-myc regulated throughout the cell cycle *(Orre et al., 2001)*
  - is actively transported in the nucleus, involved in chromatin remodelling via its central acidic region *(Karetsou et al., 2002)*
  - inhibits apoptosome formation *(Enkemann et al., 2000)*

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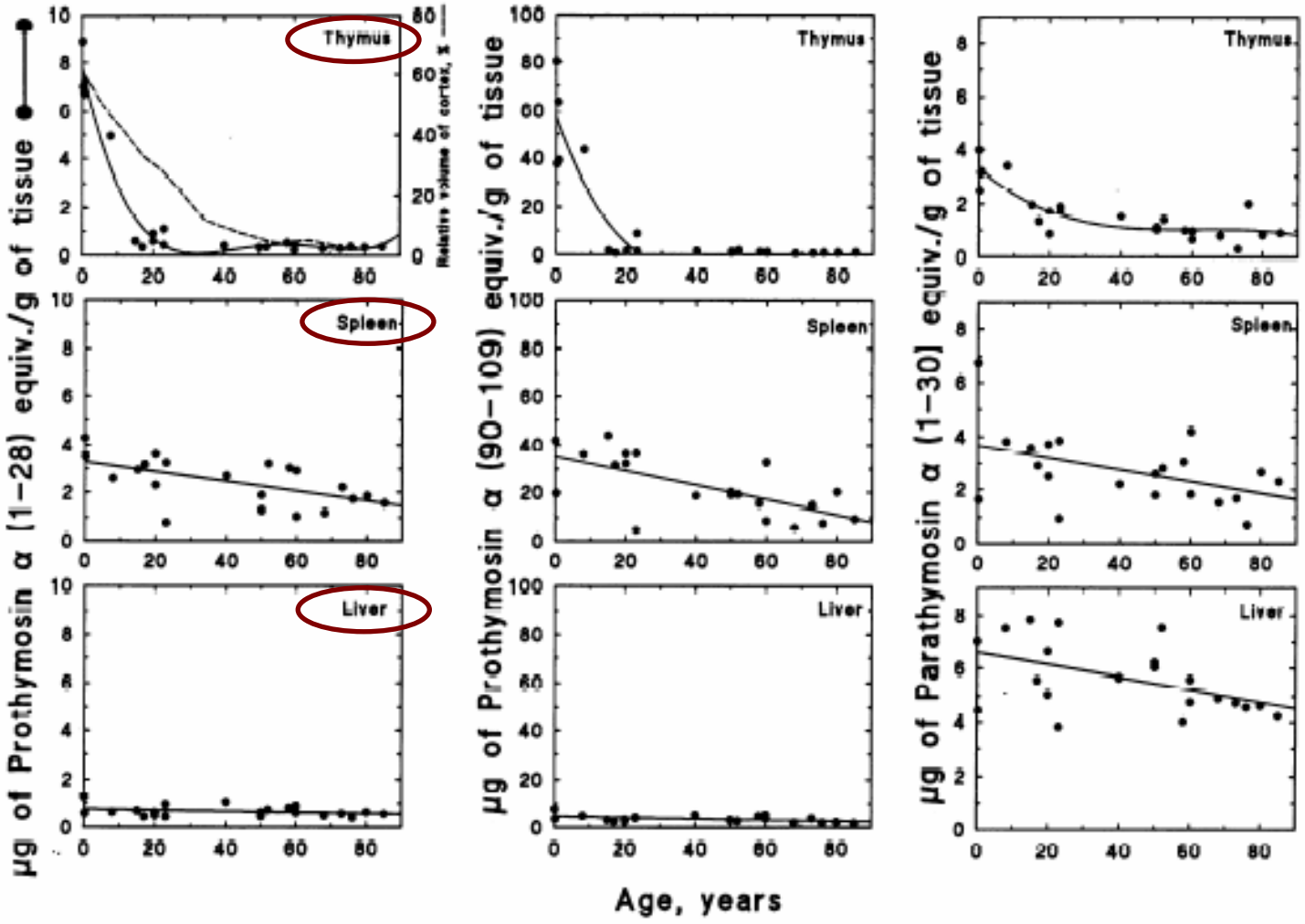
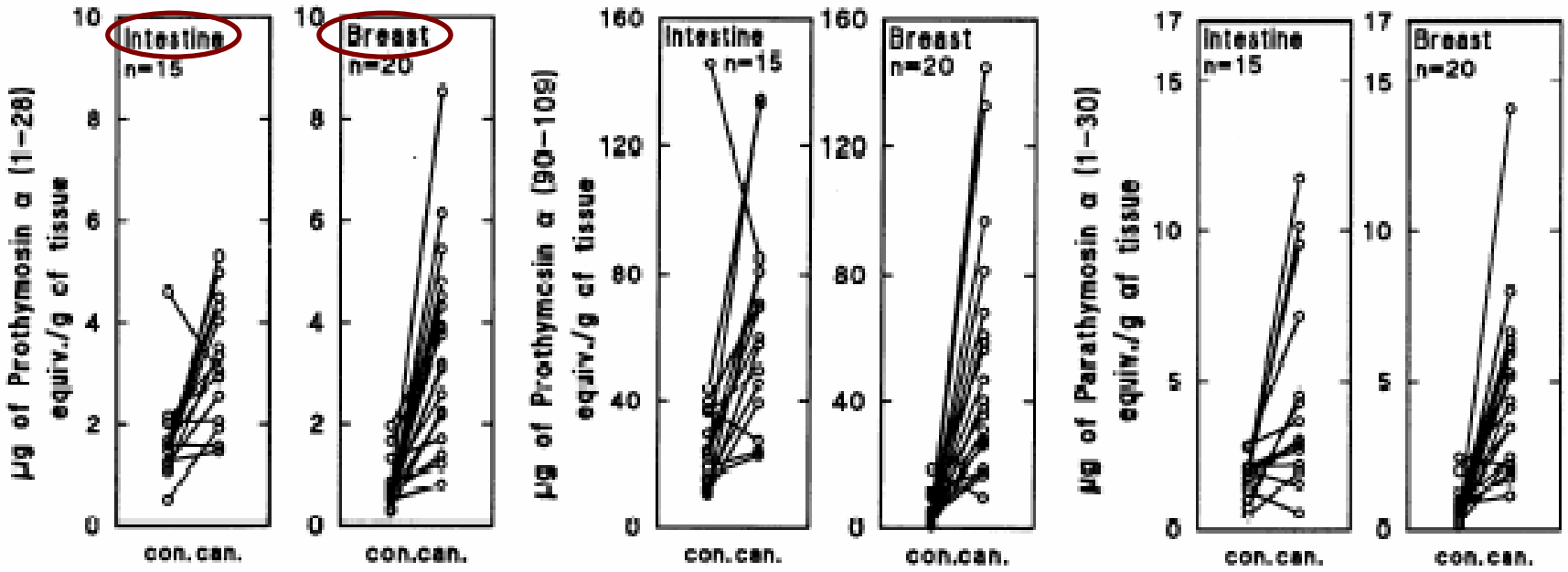
Levels of proTα in human tissues during ontogenesis



Tsitsiloni et al., 1993

Levels of proTα in human tissues during abnormal growth



Positive corelation of proTα levels with:

- *the grade of breast cancer*
- *ER status ?*
- *overall patients survival*

Tsitsiloni et al., 1993; Tsitsilonis et al., 1997

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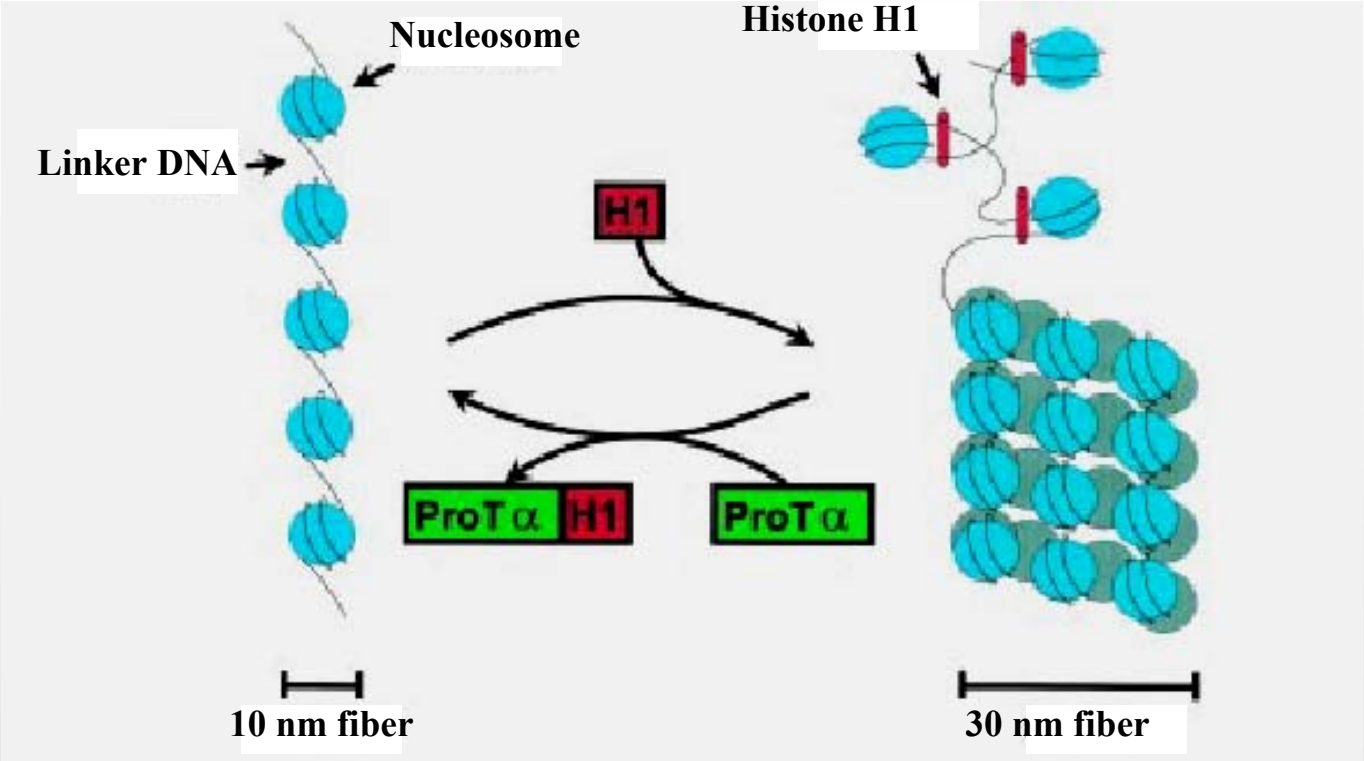
ProTα in chromatin decondensation



acidic region of ProTα + H1 = unfolding of the 30 nm chromatin fibres

Segade & Gomez-Marquez, 1999; Karetsou et al., 2002

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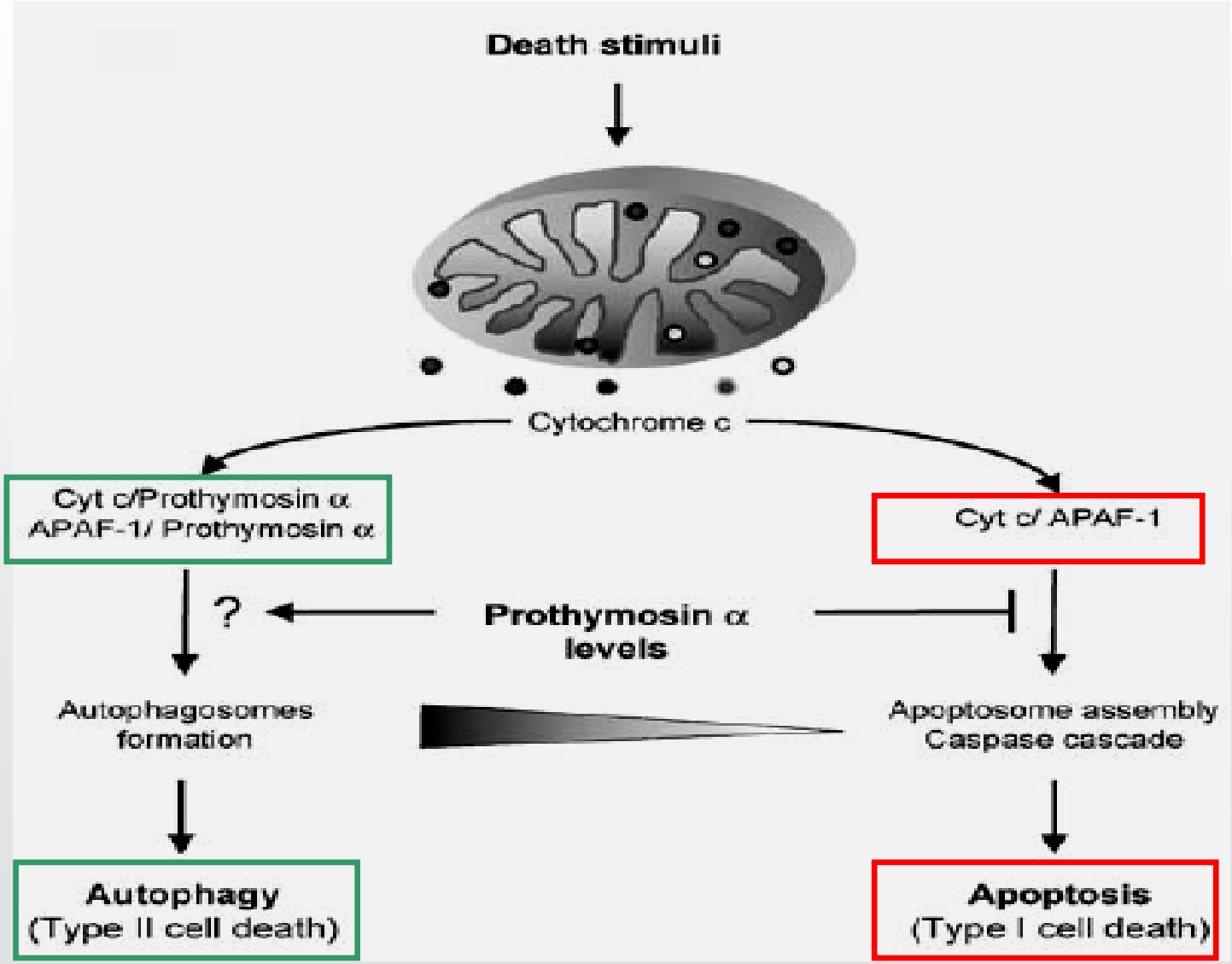
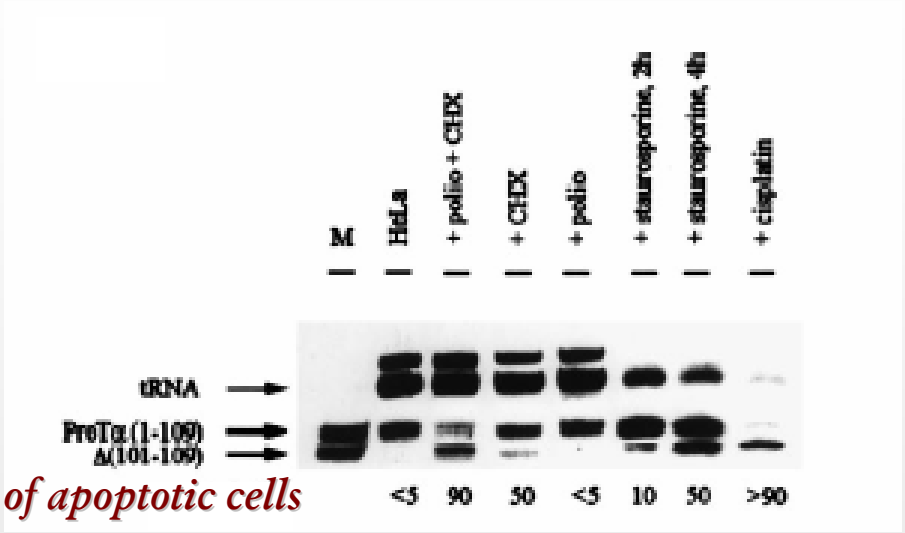
ProTα as a molecular "switch"



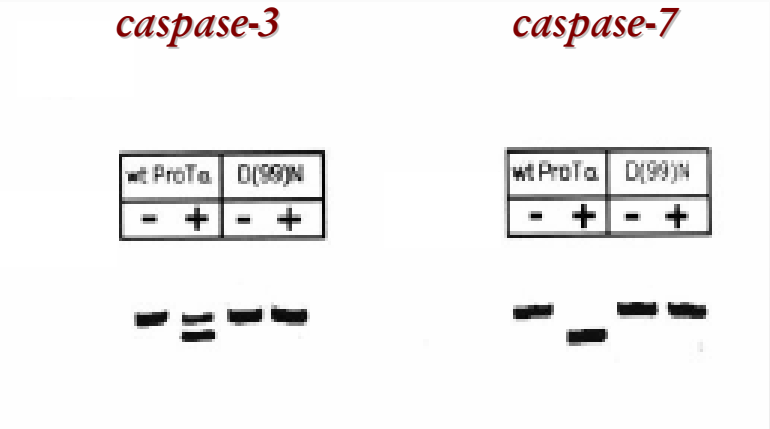
Piacentini et al., 2003

ProTα in apoptosis

HeLa cells



In vitro cleavage of proTα



x-SDAAVDTSSE ITTKDLKEKK EVVEEAENGR

DAPANGNANE ENGEQEADNE VDEEEEEGGE

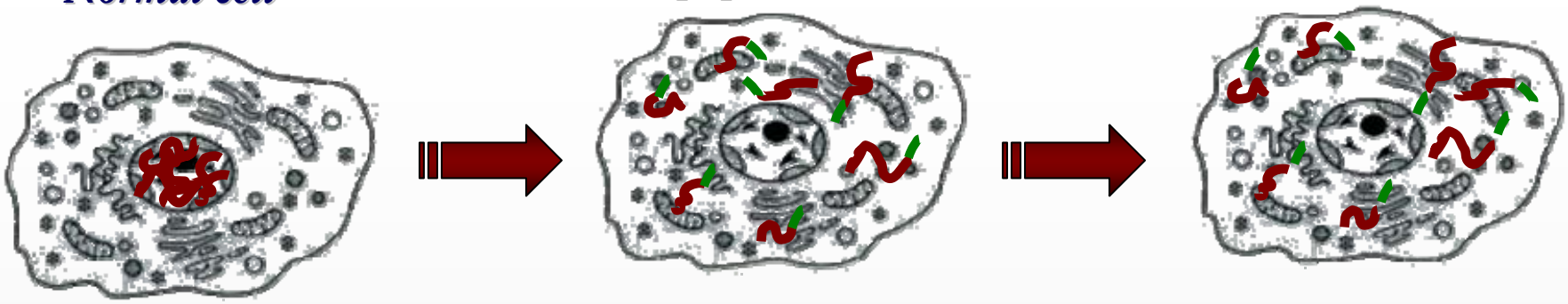
EEEEEEEGDG EEEDGDEDEE AESATGKRAA

EDDEDDDVDT KKQKTDEDD

Evstafieva et al., 2000

Normal cell

Apoptotic cell



Extracellular role of prothymosin α

immunoenhancing activity

- **induces T cell maturation and proliferation** *(Baxevanis et al., 1990)*
- **regulates IL-2 & PGE2 production, IL-2R expression, IFN-γ secretion** *(Garbin er al., 1997)*
- **upregulates MHC II gene expression and mRNA accumulation** *(Baxevanis et al., 1992)*
- **restores T, NK, LAK cell cytotoxicity** *(Eckert et al., 1997; Baxevanis et al., 1999; Voutsas et al., 2000)*
- **in vivo protects mice against opportunistic infections** *(Haritos 1987)*
- **in vivo antitumor activity in mice inoculated with leukemic cells** *(Baxevanis et al., 1995)*
- **stimulates chemotactic activity and function of PMN** *(Heidecke et al., 1997)*

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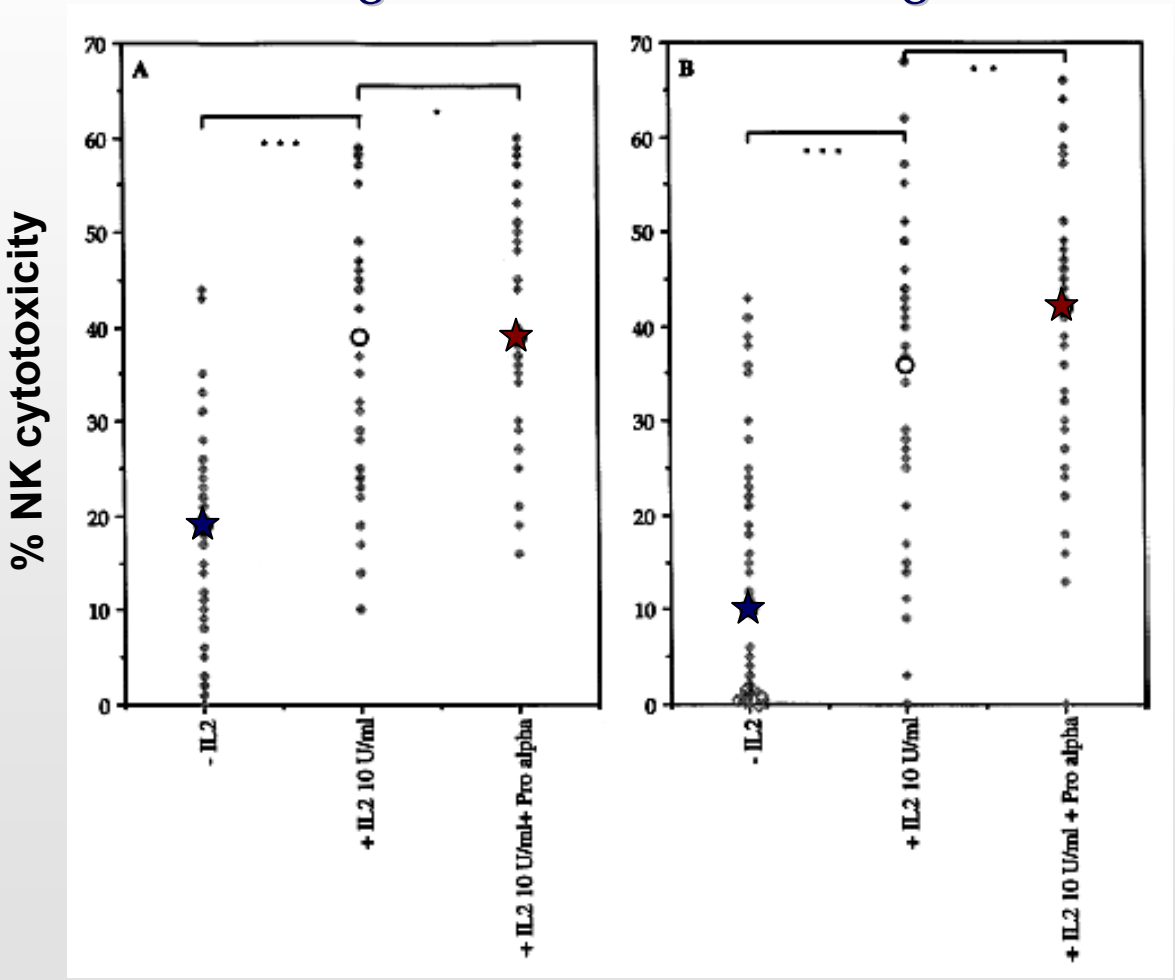
ProTα restores NK cell activity *in vitro*

Colorectal cancer patients

Dukes stages A/B *Dukes stages C/D*



Eckert et al., 1997

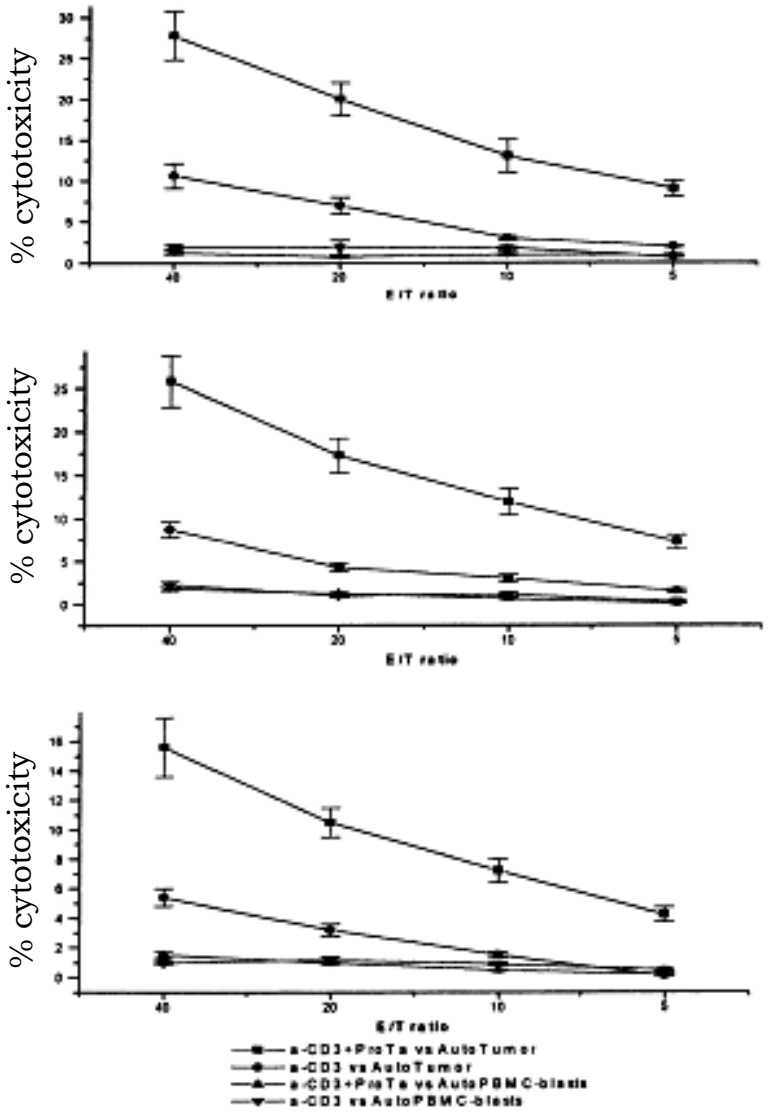
ProTα restores LAK cell activity *in vitro*

Assayed in synergy with anti-CD3

Donors	Cytotoxicity (%)					
	Raji cells			Daudi cells		
	–	+	Increase (%)	–	+	Increase (%)
Melanoma (n = 2)	22 ± 6	53 ± 12	141	10 ± 3	27 ± 6	170
Colorectal cancer (n = 6)	17 ± 5	52 ± 13	206	11 ± 2	31 ± 7	182
Lung cancer (n = 9)	12 ± 3	39 ± 7	225	9 ± 3	20 ± 6	122
Breast cancer (n = 12)	19 ± 5	35 ± 11	84	13 ± 5	29 ± 5	123
Ovarian cancer (n = 10)	18 ± 5	30 ± 10	67	7 ± 2	19 ± 6	171
Mean values for cancer (n = 39)	17 ± 3	42 ± 9	147	10 ± 2	25 ± 5	150
Healthy donors (n = 20)	42 ± 10	61 ± 9	45	25 ± 7	42 ± 10	68

Baxevanis et al., 1999

ProTα enhances cytotoxicity against autologous tumor targets *in vitro*



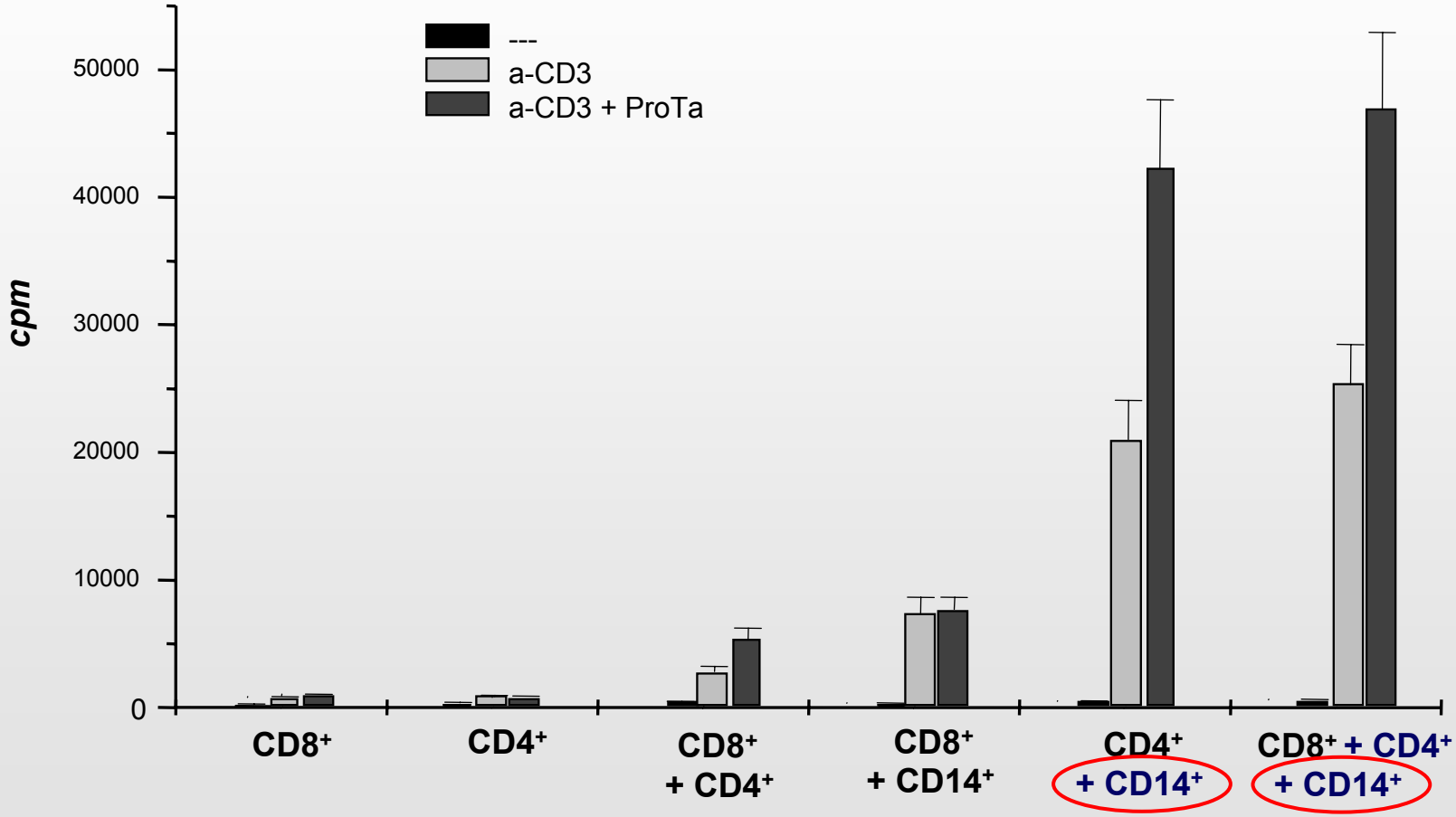
Lung cancer

Ovarian cancer

Breast cancer

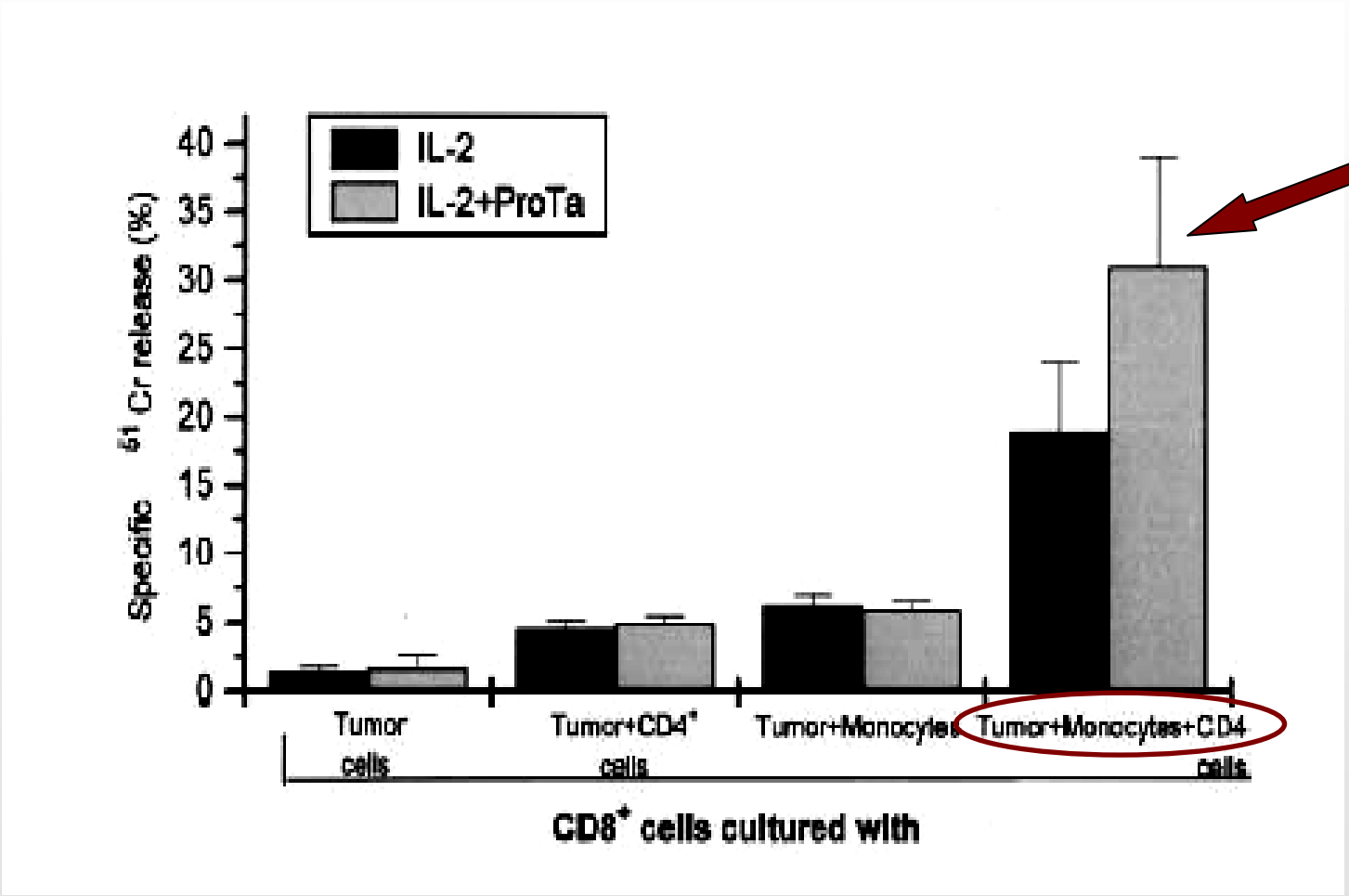
Baxevanis et al, 1999

Requirement of CD4+ T cells and monocytes for optimal induction of proliferation with prothymosin α



Baxevanis et al, 1999

ProTα-induced cytotoxicity is monocyte-dependent

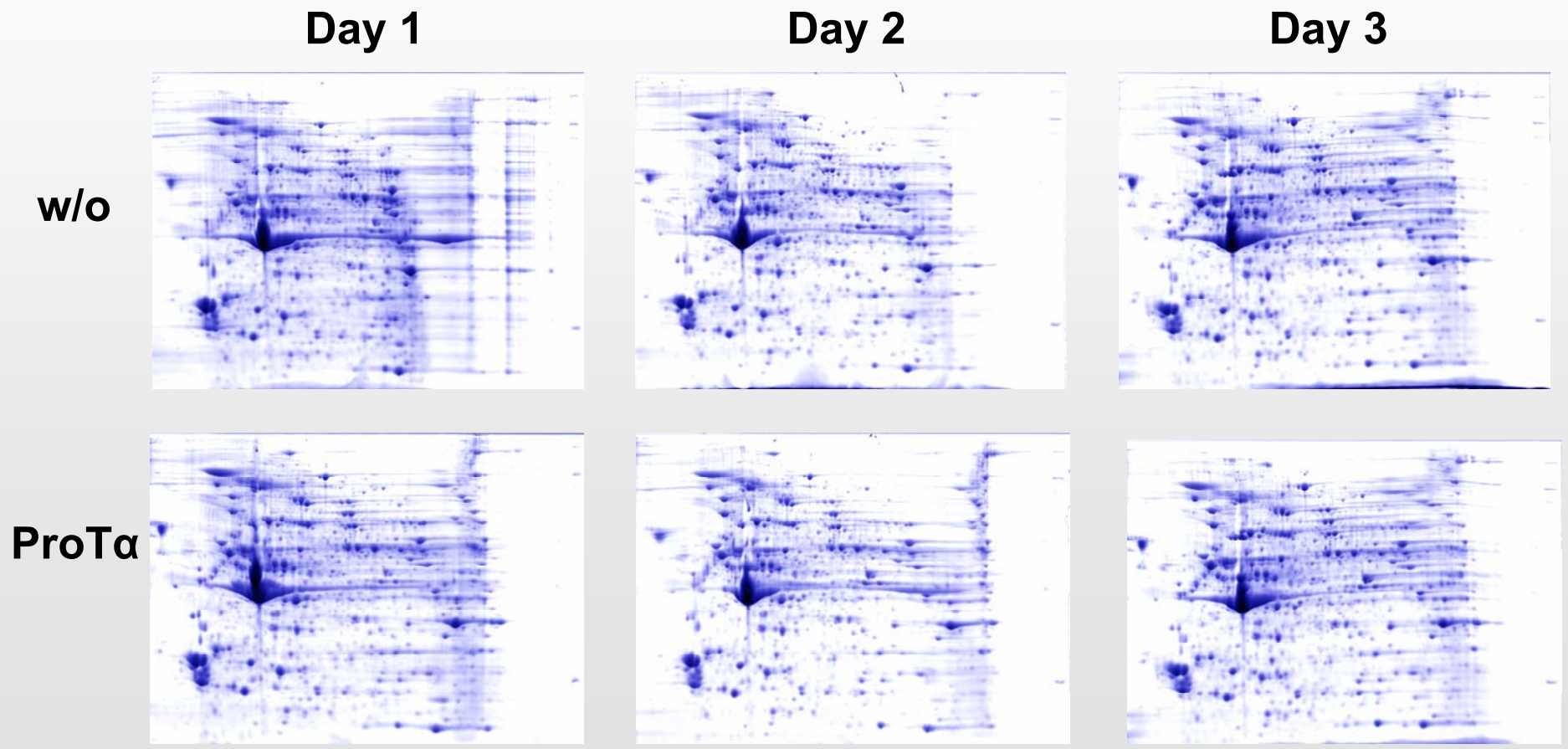


Voutsas et al., 2000

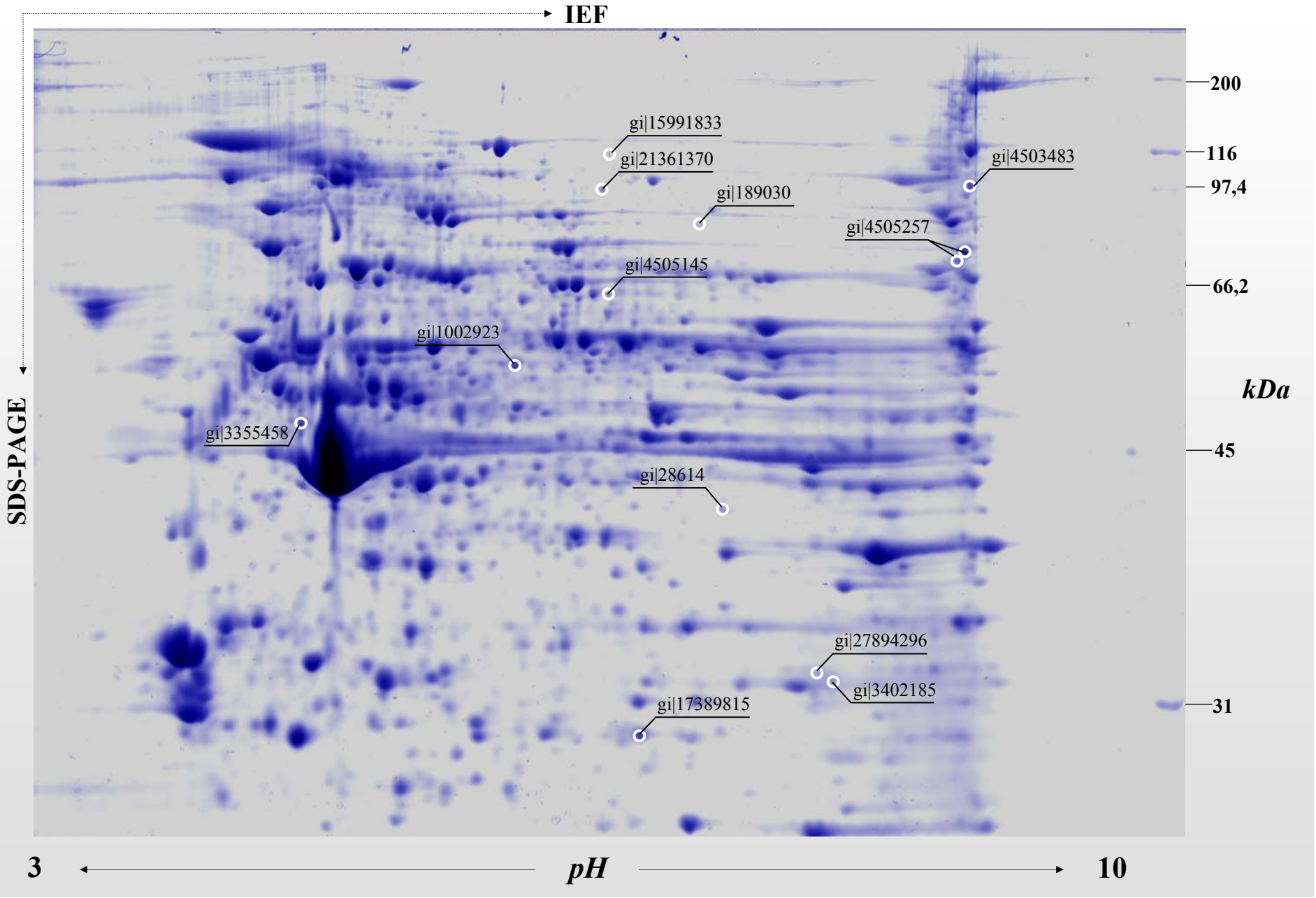
Summary on ProTα's mode of action

- is monocyte-dependent
- is CD4 T cell-dependent
- is IL-2-dependent
- exerted optimally after 3 days
- in principle on NK cells (cytotoxicity)

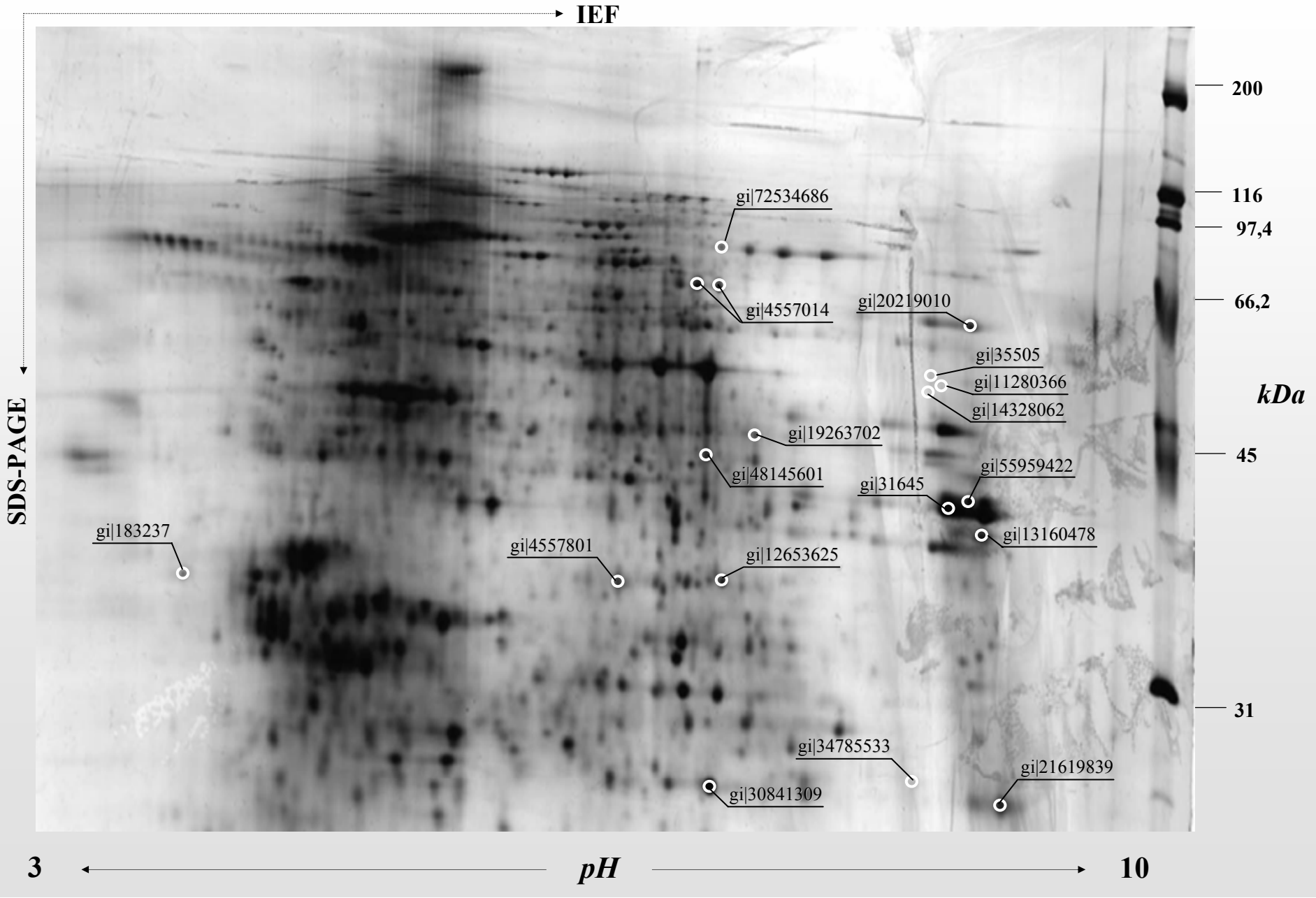
Proteomic analysis of proTα-stimulated PBMC extracts



Normal donor-derived PBMC extracts



Cancer patient-derived PBMC extracts



Healthy donor-derived PBMC

αριθμός κηλίδας	Όνομα πρωτεΐνης κατά NCBI	NCBI gi| number	Κάλυψη αλληλουχίας	Περιγραφή λειτουργίας
	ημέρα-1 αυξημένη έκφραση			
1	Vinculin	gi|24657579	10%	μόριο του κυτταροσκελετού
2	Vinculin	gi|24657579	14%	μόριο του κυτταροσκελετού
3	Heat-shock 90 kDa protein 1 (HSP90)	gi|40254816	21%	μόριο με ιδιότητες πρόσδεσης άλλων μορίων
4	alpha-(1,3)-fucosyltransferase	gi|520464	13%	μόριο ενζυμικής/καταλυτικής δραστικότητας
5	Tropomyosin 3	gi|88926	20%	μόριο του κυτταροσκελετού
6	Tropomyosin 3	gi|88926	30%	μόριο του κυτταροσκελετού
7	FLJ00035	gi|10440404	10%	άγνωστη λειτουργία
	ημέρα-2 αυξημένη έκφραση			
8	Eukaryotic translation elongation factor 2 (eEF2)	gi|4503483	8%	μόριο ρυθμιστής της μετάφρασης πρωτεϊνών
9	Moesin	gi|4505257	20%	μόριο του κυτταροσκελετού
10	Moesin	gi|4505257	15%	μόριο του κυτταροσκελετού
11	Triosephosphate isomerase 1 (TPI)	gi|17389815	33%	μόριο ενζυμικής/καταλυτικής δραστικότητας
12	Interleukin 1 family, member 7, (IL-1)	gi|27894296	19%	μόριο με χημειοτακτικές ιδιότητες
13	Protein tyrosine phosphatase sigma, partial CDS	gi|3355458	5%	μόριο ενζυμικής/καταλυτικής δραστικότητας
	μειωμένη έκφραση			
14	Hexokinase 1, isoform HKI-td	gi|15991833	14%	μόριο ενζυμικής/καταλυτικής δραστικότητας
15	Glycogen phosphorylase	gi|21361370	22%	μόριο ενζυμικής/καταλυτικής δραστικότητας
16	Myosin heavy chain-A, non-muscle	gi|189030	20%	μόριο του κυτταροσκελετού
17	Malic enzyme 2	gi|4505145	20%	μόριο ενζυμικής/καταλυτικής δραστικότητας
18	Galectin-3	gi|3402185	18%	μόριο με ιδιότητες πρόσδεσης άλλων μορίων
19	Aldolase A	gi|28614	27%	μόριο ενζυμικής/καταλυτικής δραστικότητας
20	Coronin-like protein	gi|1002923	9%	άγνωστη λειτουργία
	ημέρα-3 αυξημένη έκφραση			
21	LIM protein SLIMMER	gi|3859849	14%	μόριο με ιδιότητες πρόσδεσης άλλων μορίων
22	L-plastin polypeptide	gi|4504965	25%	μόριο με ιδιότητες πρόσδεσης άλλων μορίων
23	L-plastin polypeptide	gi|4504965	27%	μόριο με ιδιότητες πρόσδεσης άλλων μορίων
24	MX2	gi|2996644	11%	μόριο ενζυμικής/καταλυτικής δραστικότητας
	μειωμένη έκφραση			
25	SRC protein	gi|15079460	13%	μόριο ενζυμικής/καταλυτικής δραστικότητας
26	WD-repeat protein	gi|47606182	4%	άγνωστη λειτουργία
27	Macrophage migration inhibitory factor	gi|4505185	9%	μόριο με χημειοτακτικές ιδιότητες

Cancer patient-derived PBMC

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	ημέρα-1			
	αυξημένη έκφραση			
28	Catalase	gi|4557014	21%	μόριο με αντιοξειδωτική δράση
29	Catalase	gi|4557014	33%	μόριο με αντιοξειδωτική δράση
30	Glyceraldehyde-3-phosphate dehydrogenase	gi|31645	34%	μόριο ενζυμικής/καταλυτικής δραστικότητας
31	Purine nucleoside phosphorylase	gi|4557801	49%	μόριο ενζυμικής/καταλυτικής δραστικότητας
32	Actin-related protein 2/3 complex subunit 2	gi|12653625	37%	μόριο με ιδιότητες πρόσδεσης άλλων μορίων
33	Manganese-containing superoxide dismutase, chain B	gi|30841309	55%	μόριο με αντιοξειδωτική δράση
34	Lipocalin 2 (LPN2)	gi|21619839	32%	μόριο με ιδιότητες πρόσδεσης άλλων μορίων
35	Pyruvate kinase	gi|35505	26%	μόριο ενζυμικής/καταλυτικής δραστικότητας
36	Interleukin-1 receptor associated kinase 4 (IRAK4)	gi|20219010	9%	μόριο κυτταρικής σηματοδότησης
37	GTP-binding protein PTD004	gi|11280366	54%	μόριο ενζυμικής/καταλυτικής δραστικότητας
38	Isocitrate dehydrogenase 2, mitochondrial variant	gi|14328062	46%	μόριο ενζυμικής/καταλυτικής δραστικότητας
39	Cisplatin resistance related protein CPP9p	gi|19263702	7%	μόριο με ιδιότητες πρόσδεσης άλλων μορίων
40	N-acetylglucosaminyltransferase I	gi|183237	14%	μόριο ενζυμικής/καταλυτικής δραστικότητας
41	Ribophorin 1 (RPN1)	gi|48145601	12%	μόριο με ιδιότητες πρόσδεσης άλλων μορίων
42	Galactoside binding, soluble 8 galectin-8	gi|55959422	35%	μόριο με ιδιότητες πρόσδεσης άλλων μορίων
43	Inositol polyphosphate-5-phosphatase (INPP5)	gi|13160478	22%	μόριο ενζυμικής/καταλυτικής δραστικότητας
44	Huntingtin interacting protein C, isoform 1	gi|72534686	17%	μόριο με ιδιότητες πρόσδεσης άλλων μορίων
	μειωμένη έκφραση			
45	Profilin 1	gi|34785533	64%	μόριο με ιδιότητες πρόσδεσης άλλων μορίων
	ημέρα-2			
	αυξημένη έκφραση			
46	Hemoglobin subunit alpha	gi|57013850	47%	μόριο δέσμευσης θρεπτικών συστατικών
47	Hemoglobin subunit alpha	gi|57013850	55%	μόριο δέσμευσης θρεπτικών συστατικών
48	14-3-3 protein	gi|33872677	32%	μόριο κυτταρικής σηματοδότησης
49	Vimentin	gi|62414289	6%	μόριο του κυτταροσκελετού
	ημέρα-3			
	μειωμένη έκφραση			
50	Galectin-3	gi|4504983	27%	μόριο με ιδιότητες πρόσδεσης άλλων μορίων
51	Peroxiredoxin 1	gi|32455266	56%	μόριο με αντιοξειδωτική δράση
52	Transgelin 2	gi|55960375	55%	άγνωστη λειτουργία
53	Cofilin 1	gi|1177471	58%	μόριο με ιδιότητες πρόσδεσης άλλων μορίων

Open Access

Software

GoMiner: a resource for biological interpretation of genomic and proteomic data

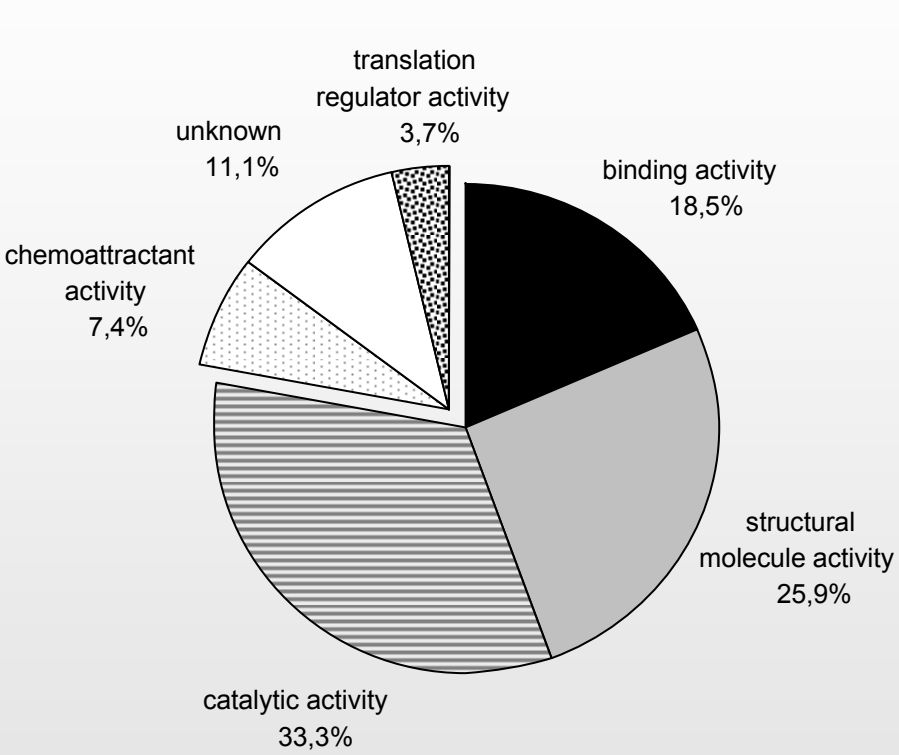
We have developed GoMiner, a program package that organizes lists of 'interesting' genes (for example, under- and overexpressed genes from a microarray experiment) for biological interpretation in the context of the Gene Ontology. GoMiner provides quantitative and statistical output files and two useful visualizations. The first is a tree-like structure analogous to that in the AmiGO browser and the second is a compact, dynamically interactive 'directed acyclic graph'. Genes displayed in GoMiner are linked to major public bioinformatics resources.

- **biological process**
- **cellular component**
- ***molecular function***

1. ***binding activity***
2. ***catalytic activity***
3. ***structural activity***
4. ***translation regulator activity***
5. ***chemoattractant activity***
6. ***nutrient reservoir activity***
7. ***antioxidant activity***
8. ***signal transducer activity***
9. ***enzyme regulator activity***
10. ***transporter activity***
11. ***triplet-codon-amino acid adaptor activity***
12. ***motor activity***
13. ***chaperone regulator activity***
14. ***chemorepellant activity***
15. ***energy transducer activity***
16. ***protein tagging activity***
17. ***obsolete molecular function***
18. ***unknown***

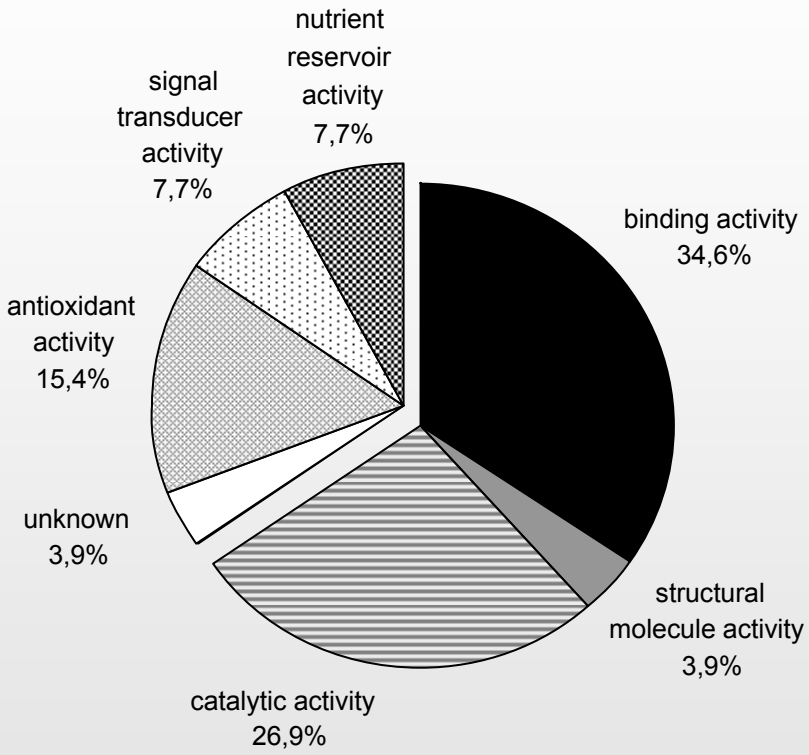
Protein organization according to GO

A. Normal donors



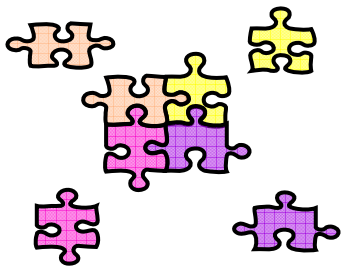
77,7 %

B. Cancer patients

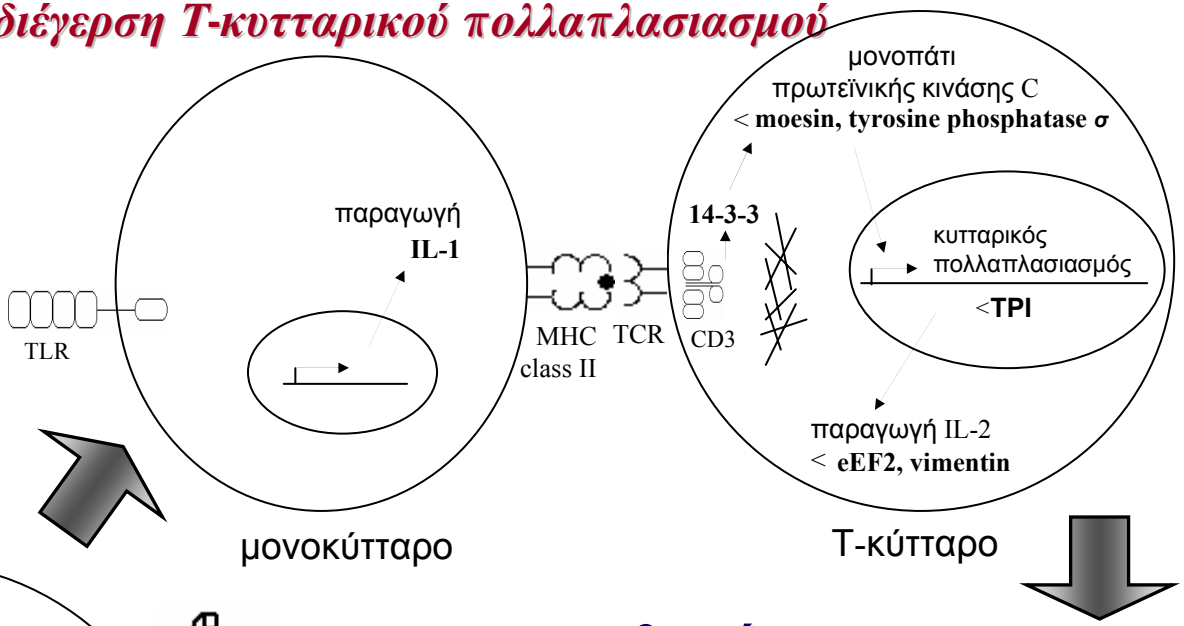


65,4 %

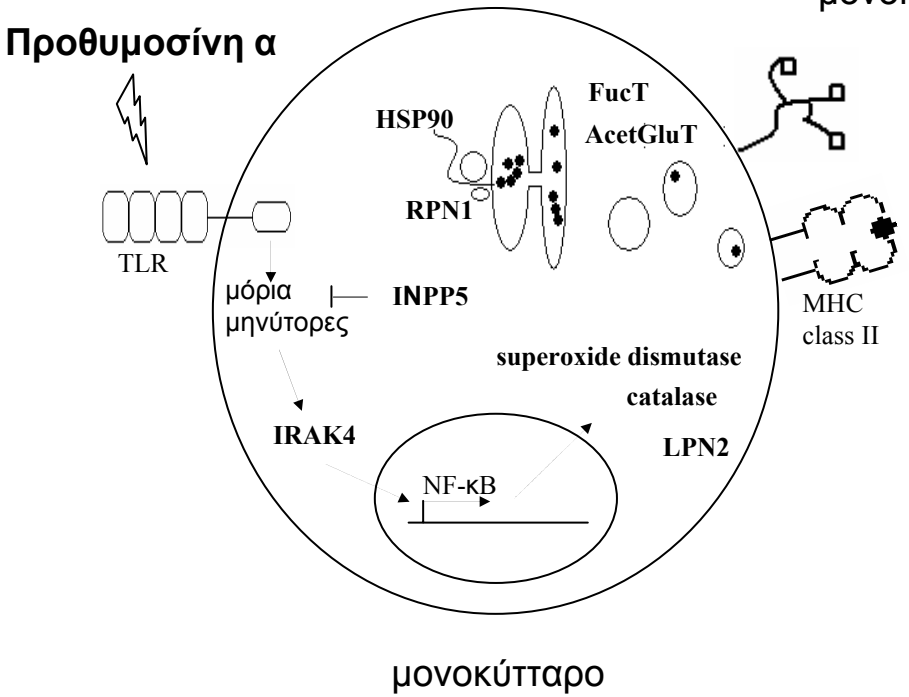
Skopeliti et al., 2007



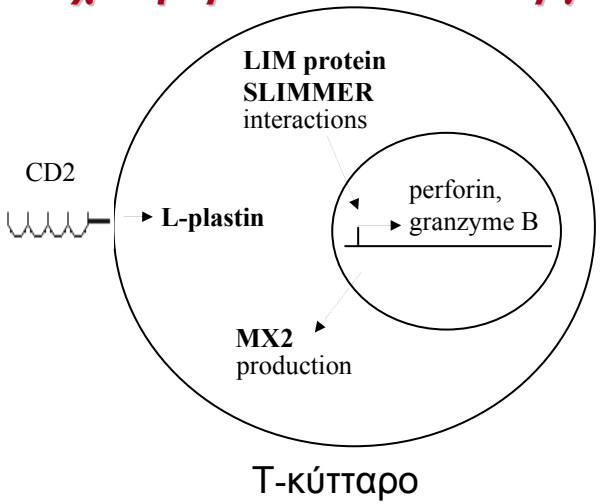
2η ημέρα:
σταθεροποίηση ανοσολογικής σύναψης
– διέγερση Τ-κυτταρικού πολλαπλασιασμού



1η ημέρα:
διέγερση μονοκυττάρων

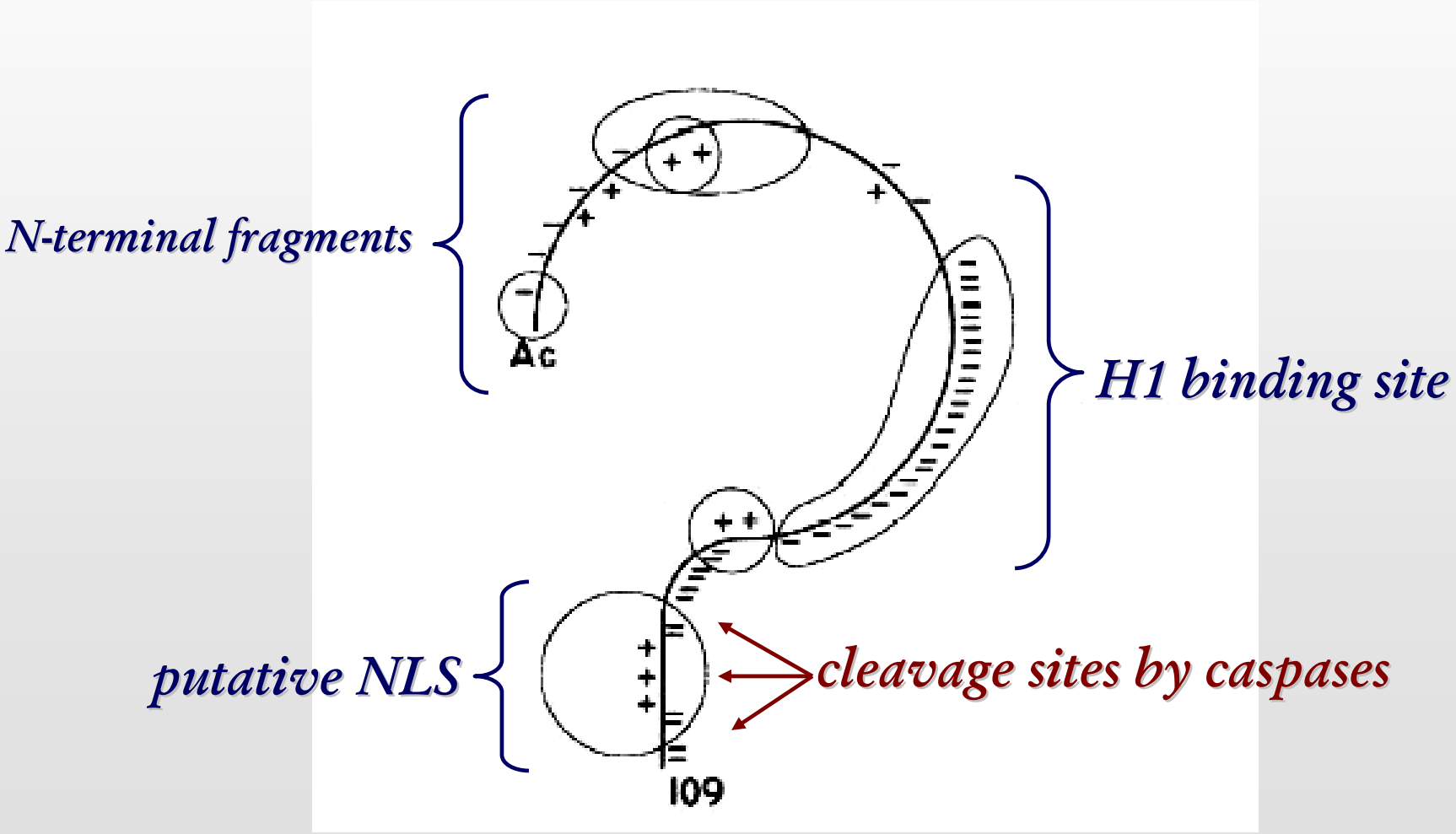


3η μέρα:
ενίσχυση δραστικών λειτουργιών

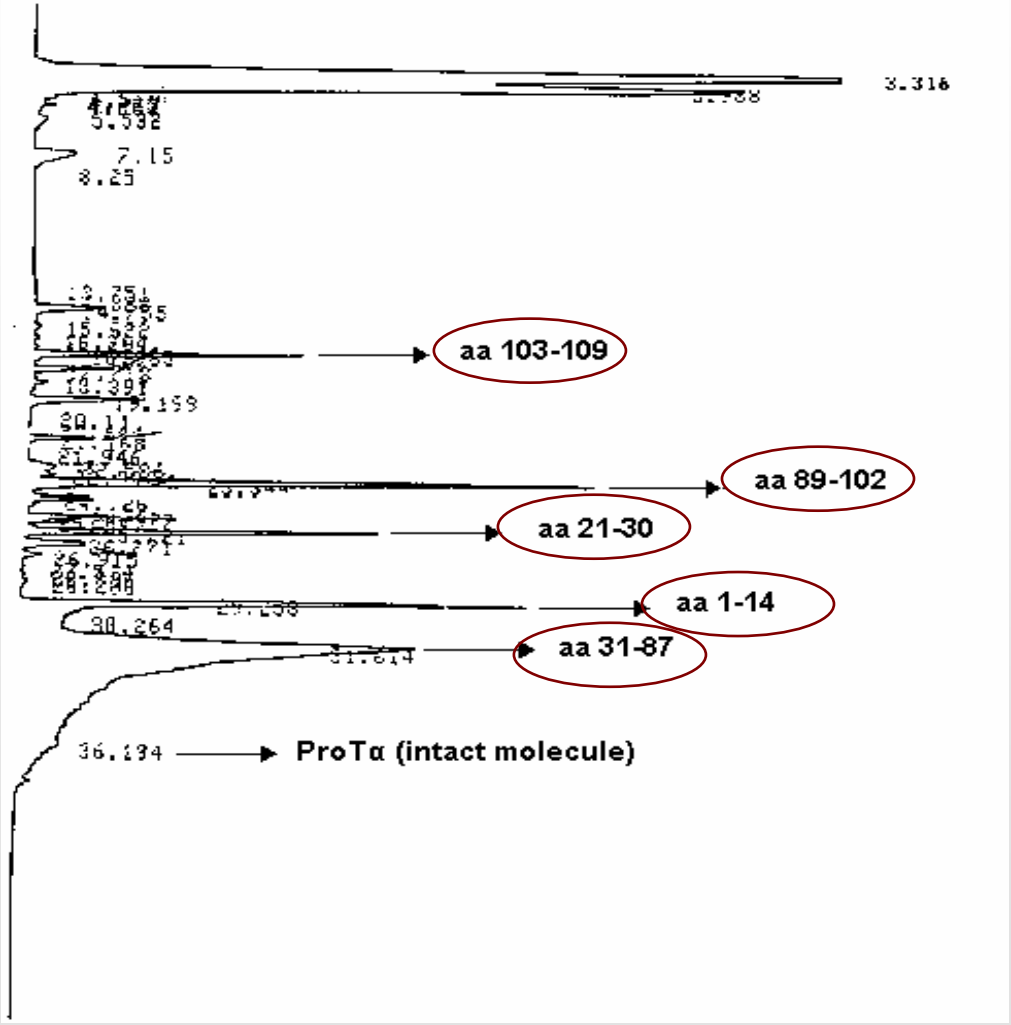


Skopeliti et al., 2007

Determining the immunologically active site of prothymosin α



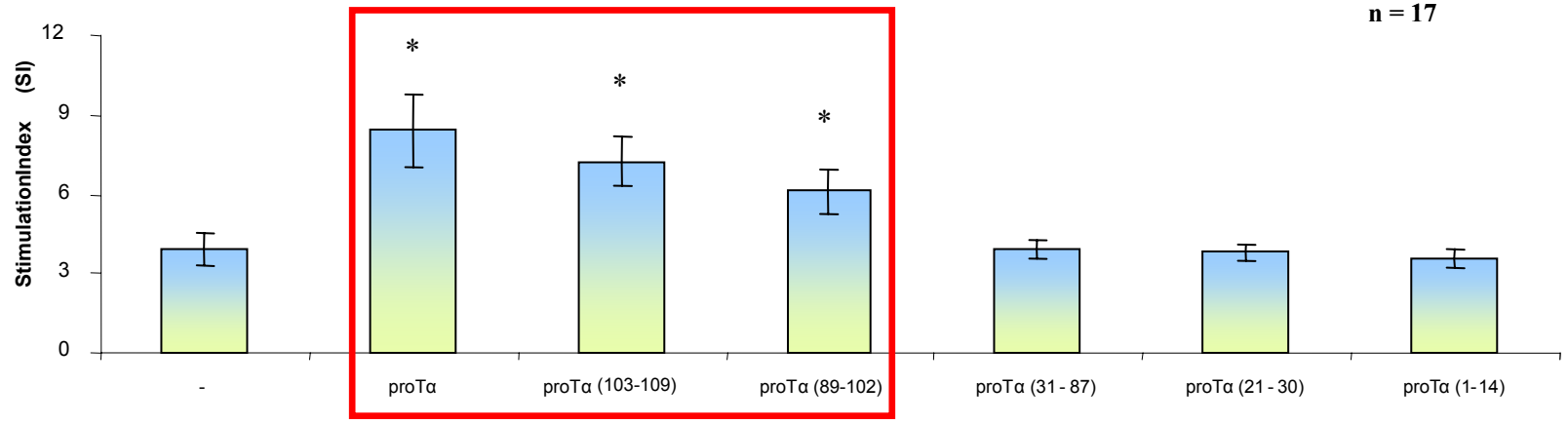
Tryptic digestion of prothymosin α



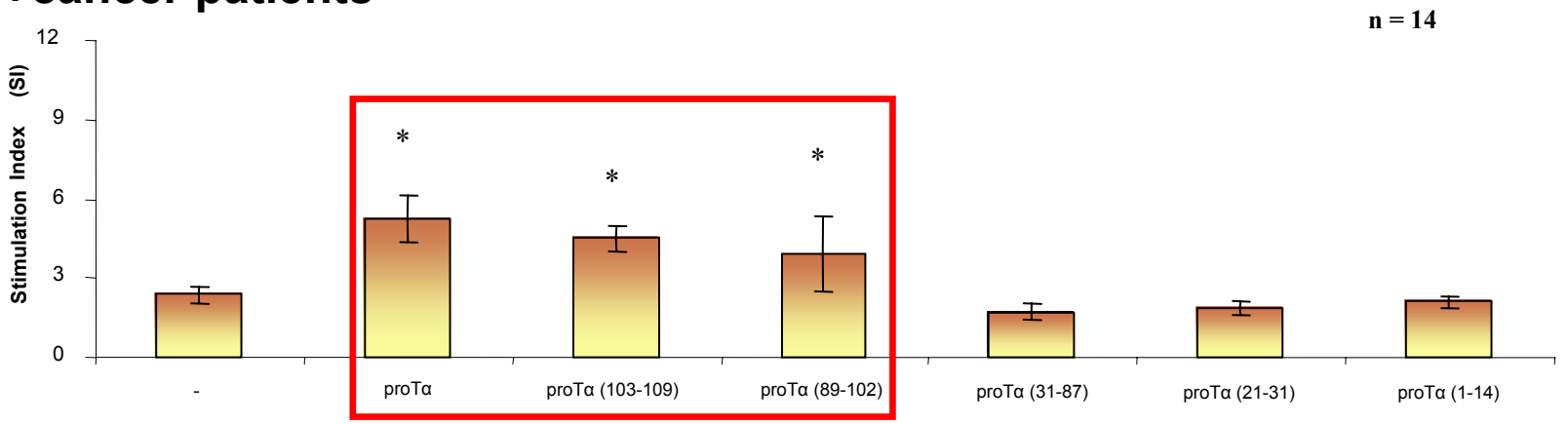
aa 1-14	SDAAVDTSSEITIK
aa 21-30	EVVEEAENGR
aa 31-87	DAPANGNANE ENGEQEADSE VDEEEEEGGE EEEEEEEGDG EEEDGDEDEE AESPTGK
aa 89-102	AAEDDEDDDVDTKK
aa 103-109	QKTDEDD

Prothymosin α fragments in AMLR

A .normal donors



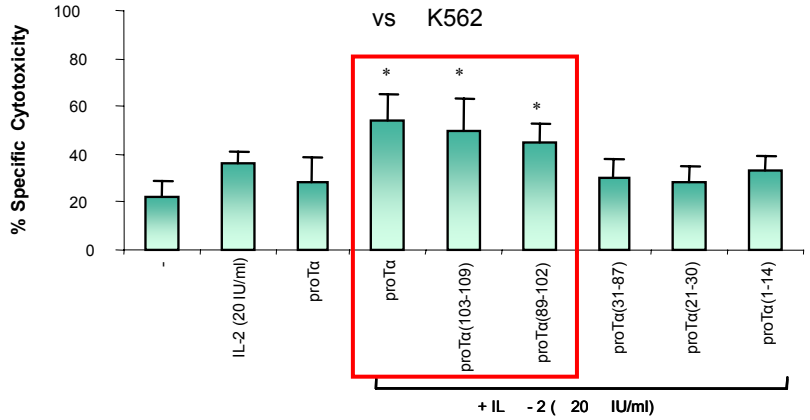
B . cancer patients

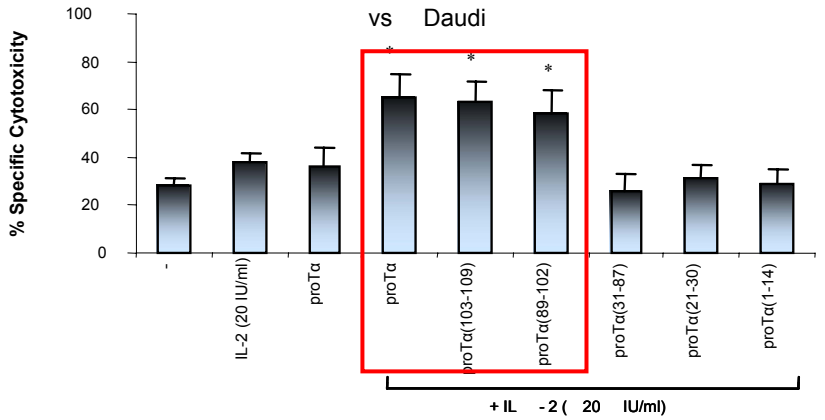


Skopeliti et al., 2006

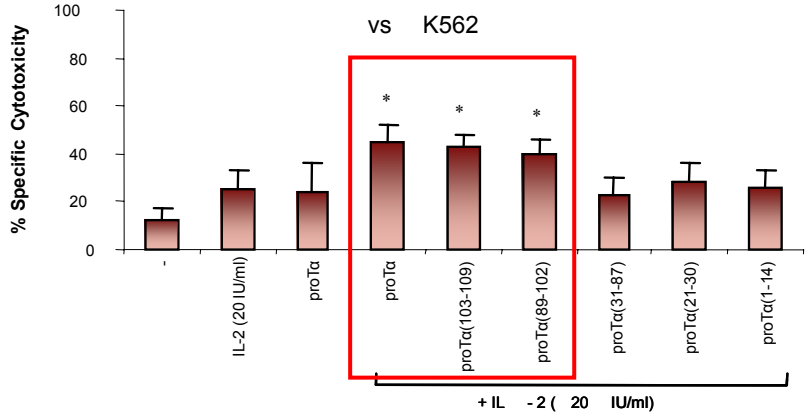
Prothymosin α fragments in cytotoxic assays

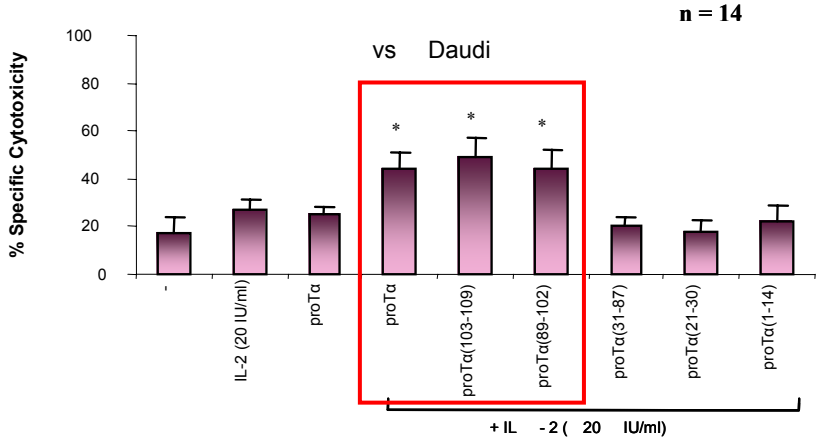
A . normal donors





B . cancer patients

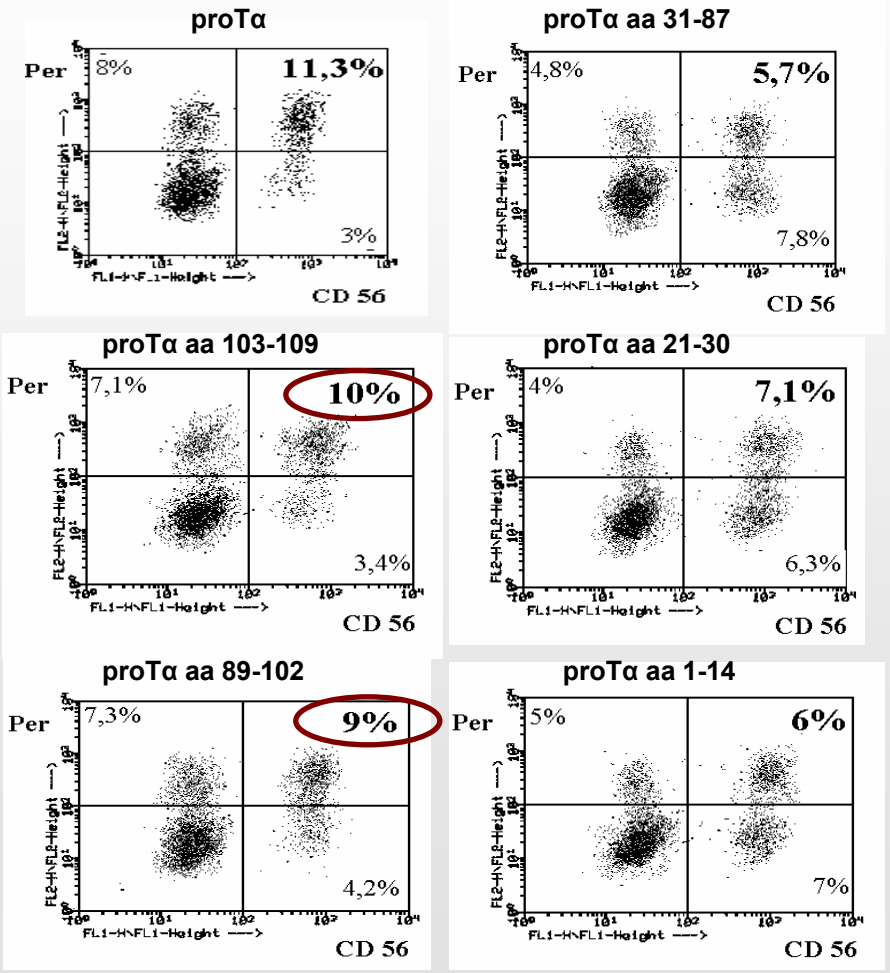




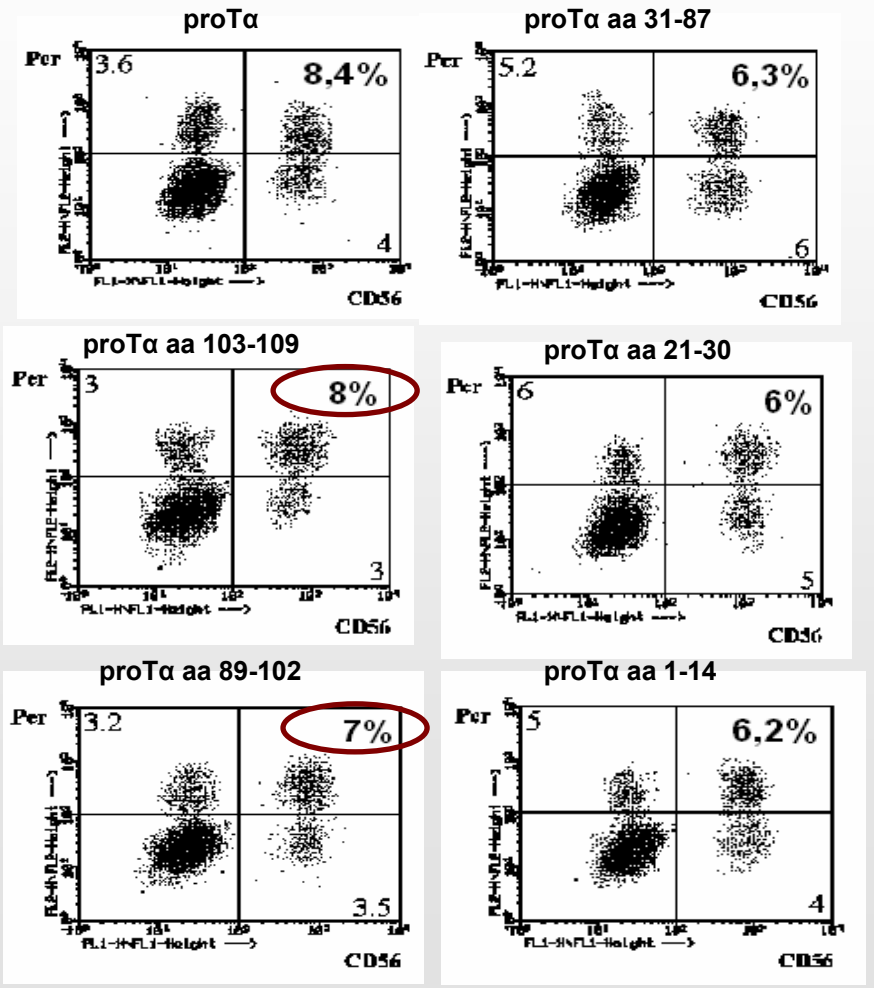
Skopeliti et al., 2006

C-terminal proTα fragments induce perforin production by NK cells

A. normal donor

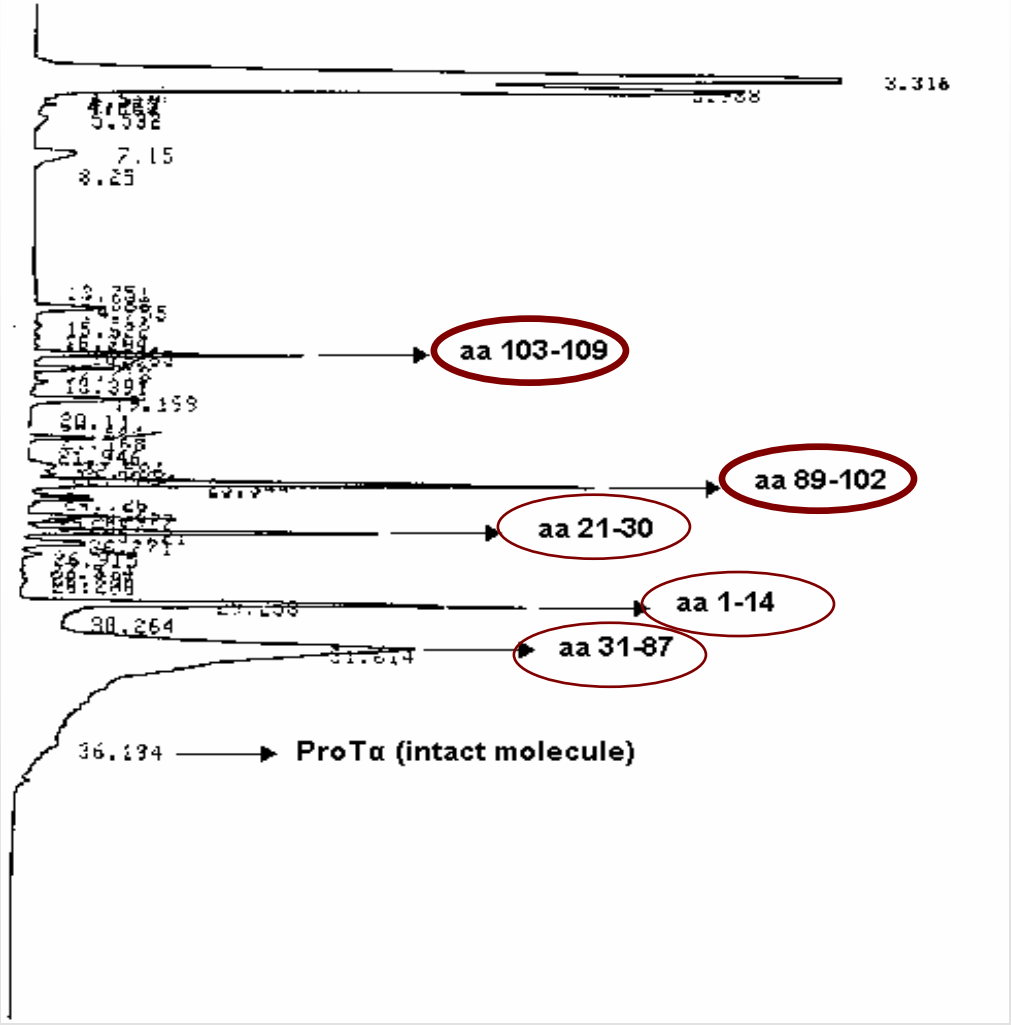


B. cancer patient



Skopeliti et al., 2006

Tryptic digestion of prothymosin α



aa 1-14	SDAAVDTSSEITIK
aa 21-30	EVVEEAENGR
aa 31-87	DAPANGNANE ENGEQEADSE VDEEEEEGGE EEEEEEEGDG EEEDGDEDEE AESPTGK
aa 89-102	AAEDDEDDDVDTKK
aa 103-109	QKTDEDD

Synthesis of C-terminal proTα fragments

94 *102* *109*

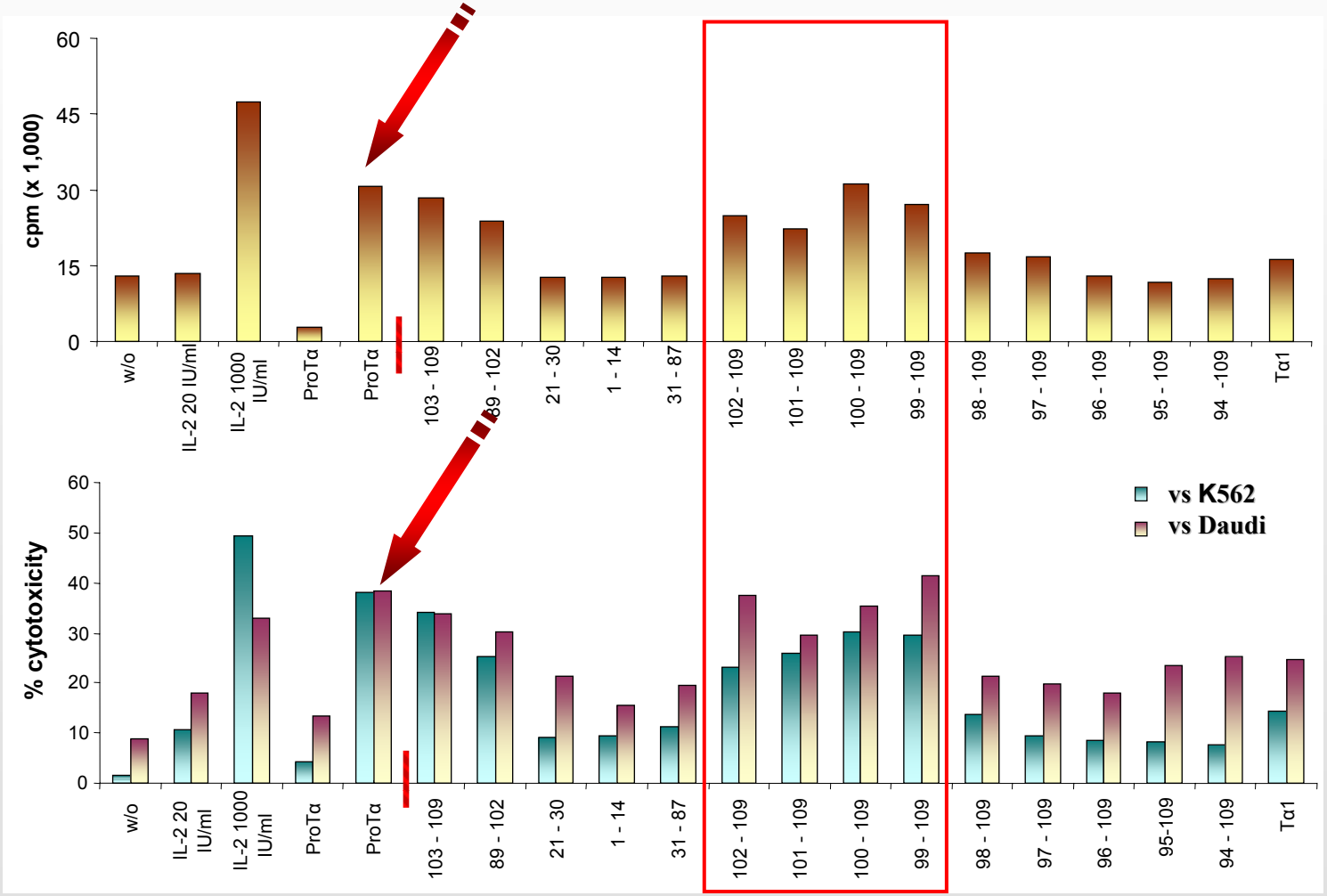
A A E D D E D D D V D T K K Q K T D E D D

K Q K T D E D D	*proTα 102-109*
K K Q K T D E D D	*proTα 101-109*
T K K Q K T D E D D	*proTα 100-109*
D T K K Q K T D E D D	*proTα 99-109*
V D T K K Q K T D E D D	*proTα 98-109*
D V D T K K Q K T D E D D	*proTα 97-109*
D D V D T K K Q K T D E D D	*proTα 96-109*
D D D V D T K K Q K T D E D D	*proTα 95-109*
E D D D V D T K K Q K T D E D D	*proTα 94-109*

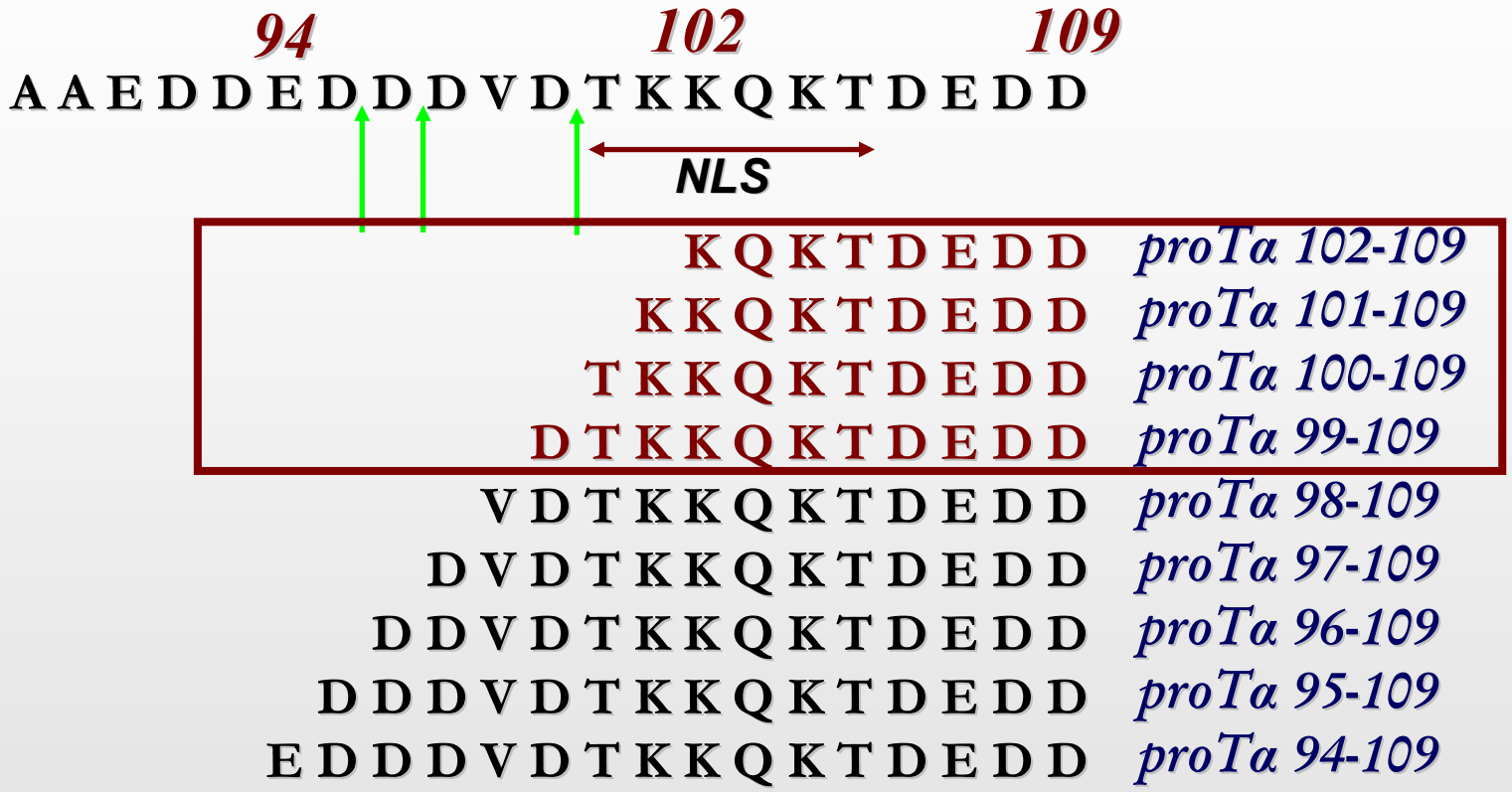
Immunological evaluation of C-terminal proTα fragments' effects

proliferation assay

standard Cr-release assay



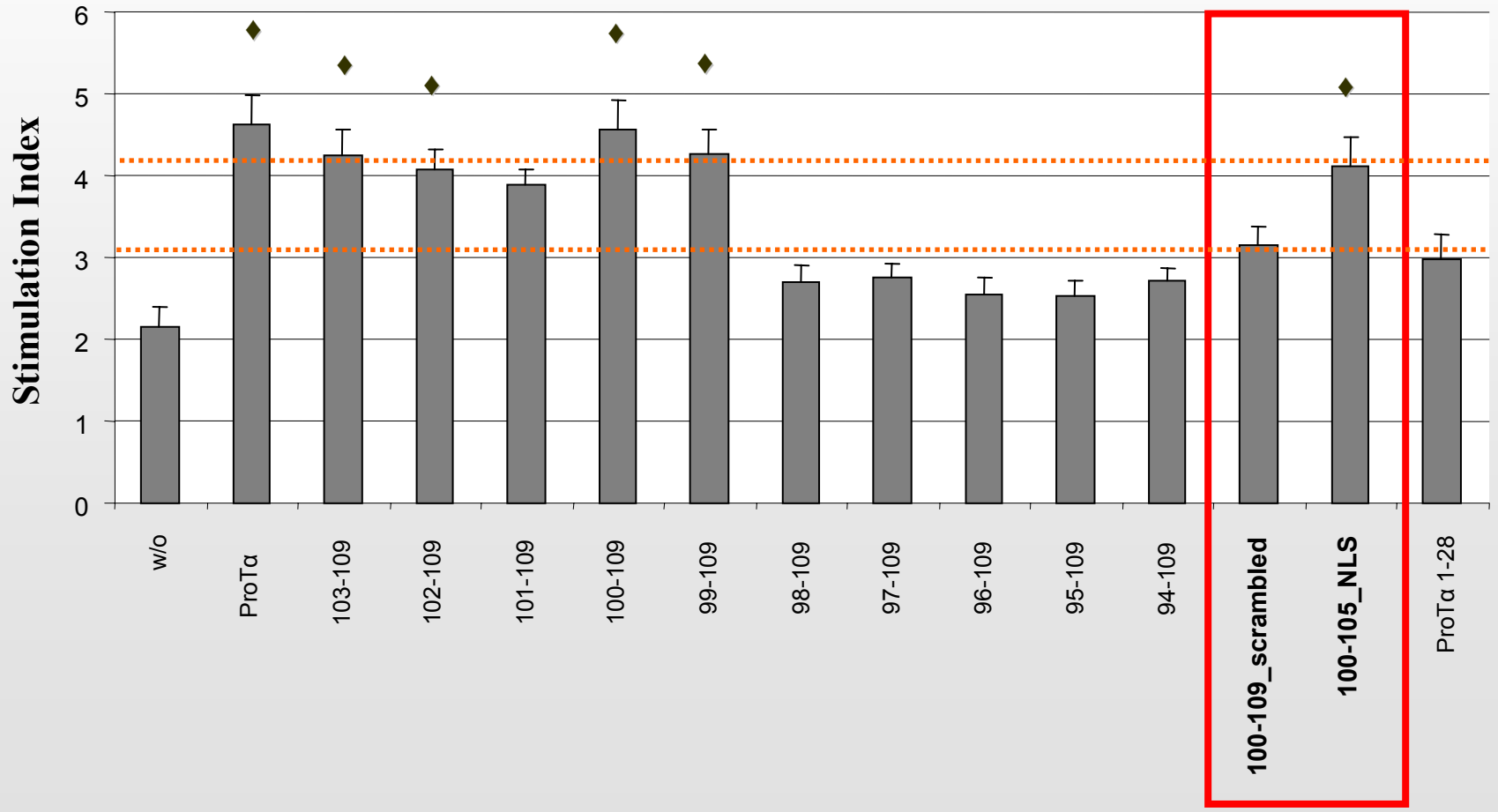
The peptide proTα (99/100-109) is equally active to intact proTα



proTα (100-109)_scrambled	K E T D K D K T D Q
proTα (100-105)_NLS	T K K Q K T
proTα (1-28)_Tα1	SDAAVDTSSEITTKDLKEKKEVVEEAEN

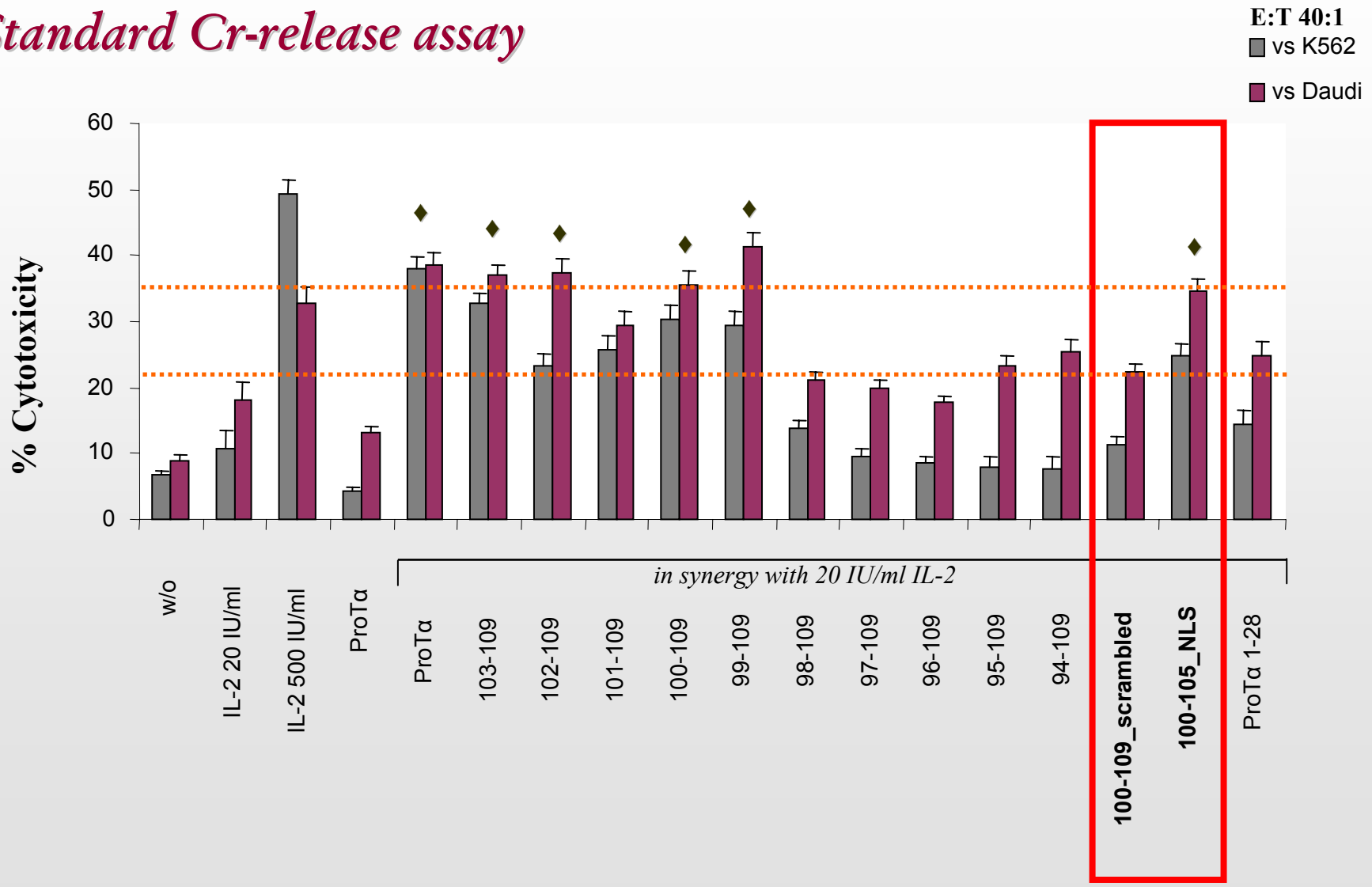
Sequence specificity of proTα (100-109)

Proliferation assay



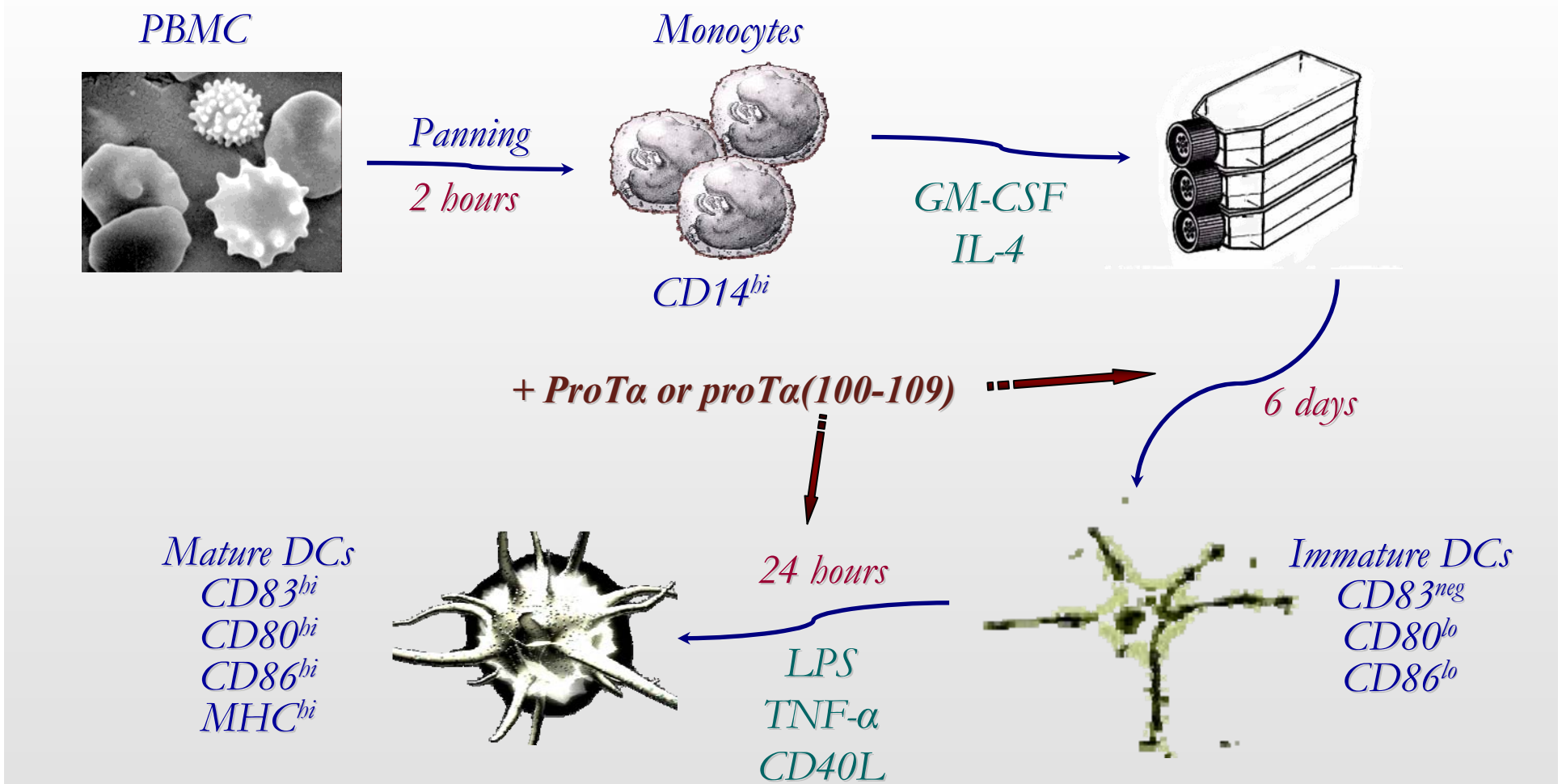
Sequence specificity of proTα (100-109)

Standard Cr-release assay

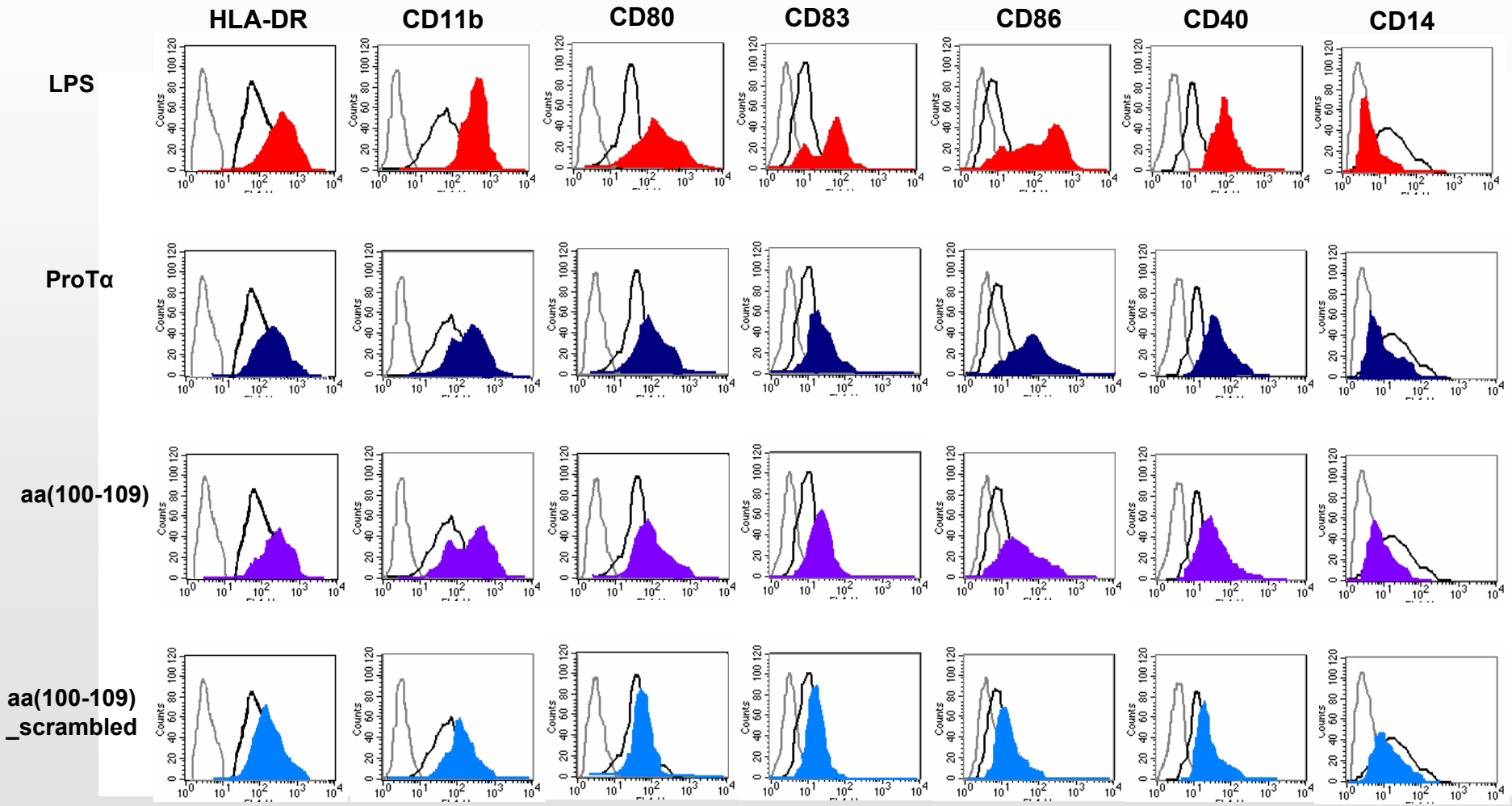


Skopeliti et al., 2008

Does proTα and/or proT α(100-109) activate innate immunity cells?



ProTα and proTα(100-109) on DC maturation



ProTα and proTα(100-109) on DC maturation

	HLA-DR	CD80	CD83	CD86	CD11b	CD40	CD14
isotype control	3,2	2,1	2,8	3,8	2,5	4,1	2,7
immature dendritic cells	156,4	36,1	12,3	7,8	73,7	18,2	22,3
LPS-matured dendritic cells	533,2	225,6	67,7	115,6	556,5	103,4	4,8
proTα-matured dendritic cells	339,4	159,9	27,1	75,9	229,8	41,3	7,4
proTα(100-109)-matured dendritic cells	369,9	146,8	22,1	28,3	354,3	35,2	7,9
proTα(100-109)_scrambled-matured dendritic cells	215,1	85,7	16,3	14,1	115,8	23,5	10,3

ProTα (100-109) activates neutrophils

Zymosan assay (phagocytosis)

(90 min)

Nitro Blue Tetrazolium (NBT) reduction assay (intracellular ROS production)

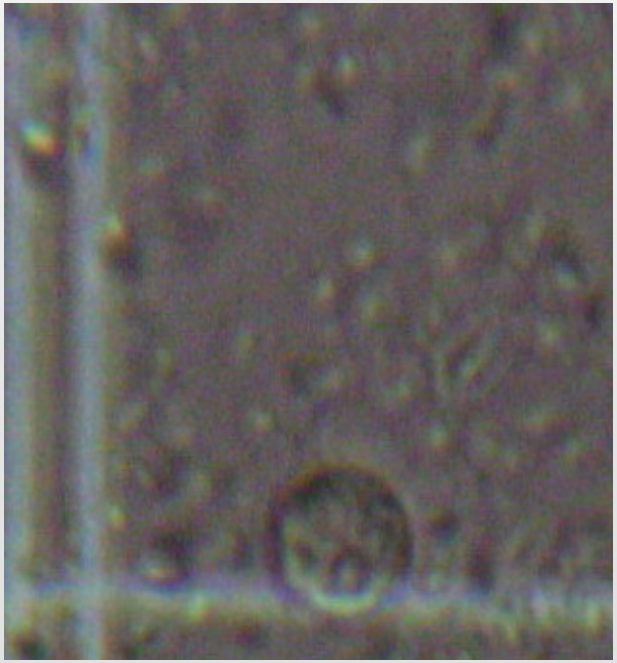
(20 min)

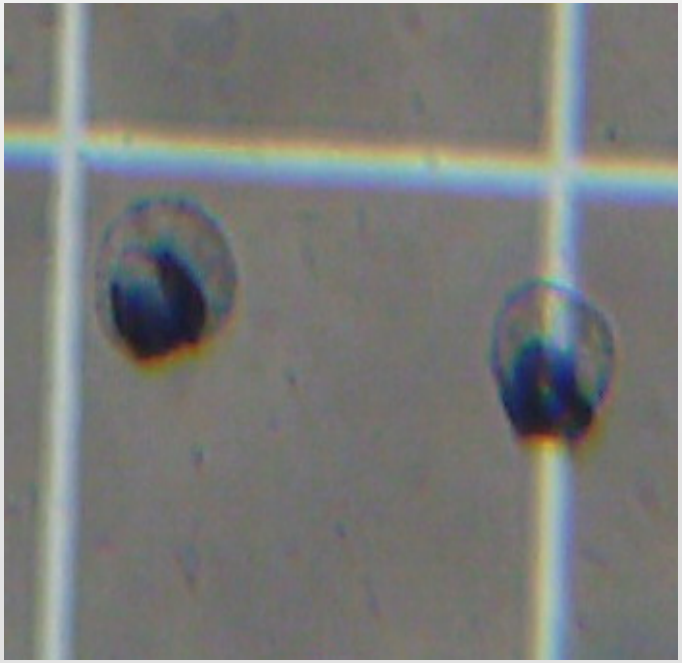
•*Photonic microscope*

Cytochrome C assay (extracellular ROS release)

(20 min)

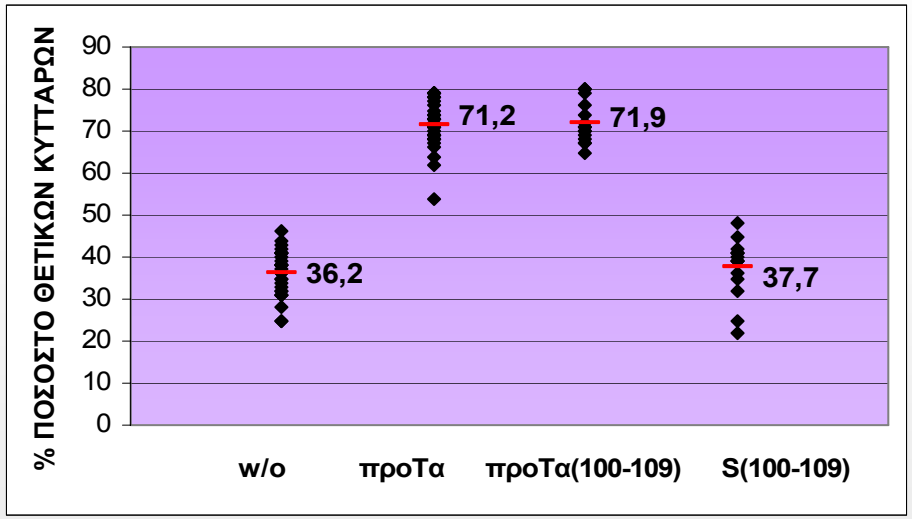
•*Photometer (535 & 550 nm)*



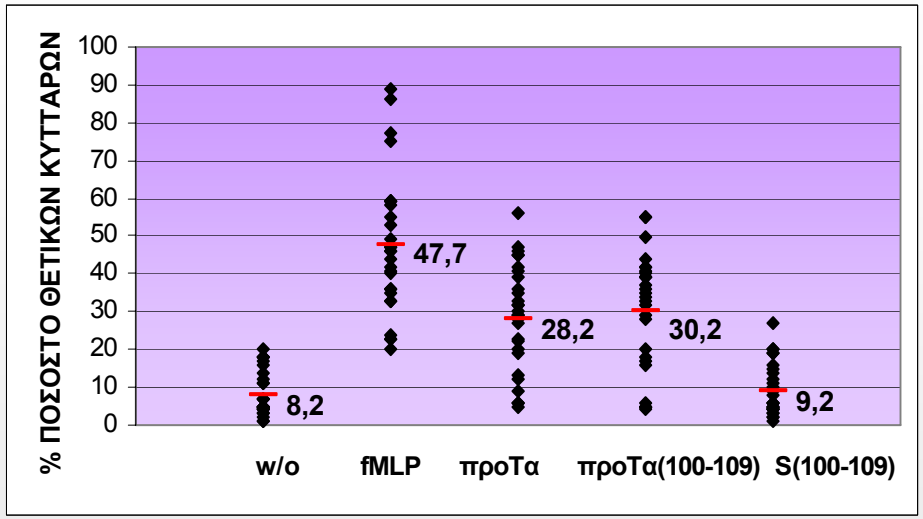


ProTα & proTα(100-109) ενεργοποιούν τα ουδετερόφιλα υγιών δοτών

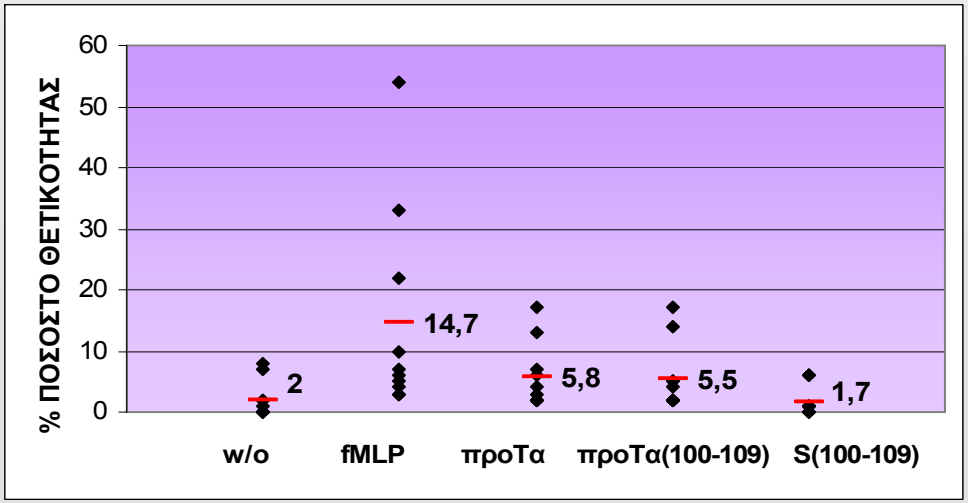
ΦΑΓΟΚΥΤΤΑΡΙΚΗ ΙΚΑΝΟΤΗΤΑ



ΕΝΔΟΚΥΤΤΑΡΙΚΗ ΠΑΡΑΓΩΓΗ O_2-

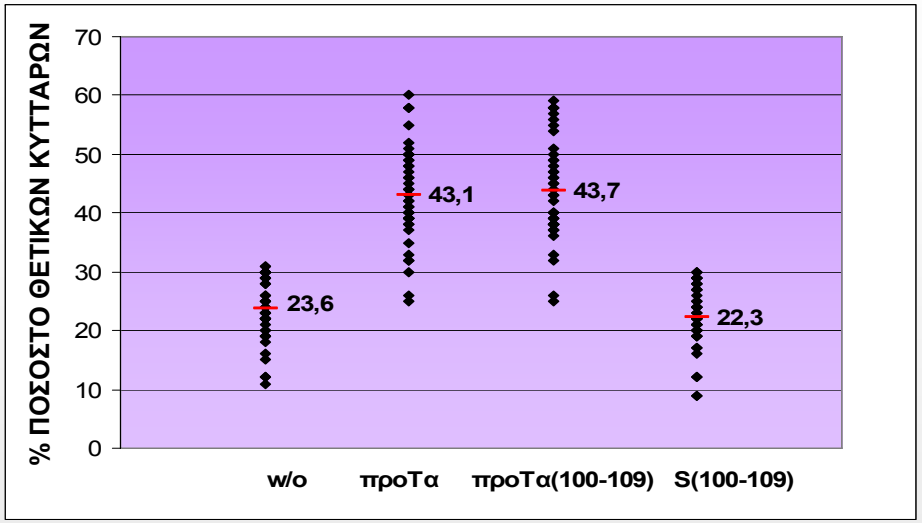


ΕΞΩΚΥΤΤΑΡΙΚΗ ΑΠΕΛΕΥΘΕΡΩΣΗ O_2-

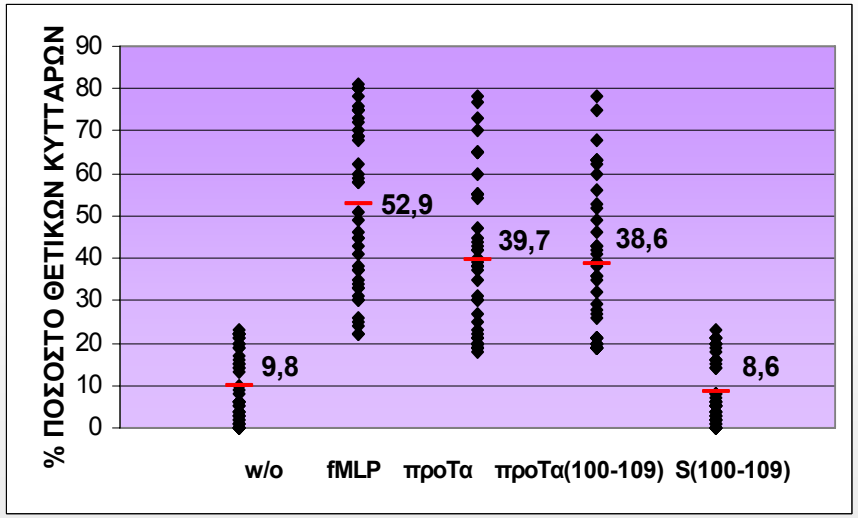


ProTα & proTα(100-109) ενεργοποιούν τα ουδετερόφιλα καρκινοπαθών

ΦΑΓΟΚΥΤΤΑΡΙΚΗ ΙΚΑΝΟΤΗΤΑ



ΕΝΔΟΚΥΤΤΑΡΙΚΗ ΠΑΡΑΓΩΓΗ O_2-



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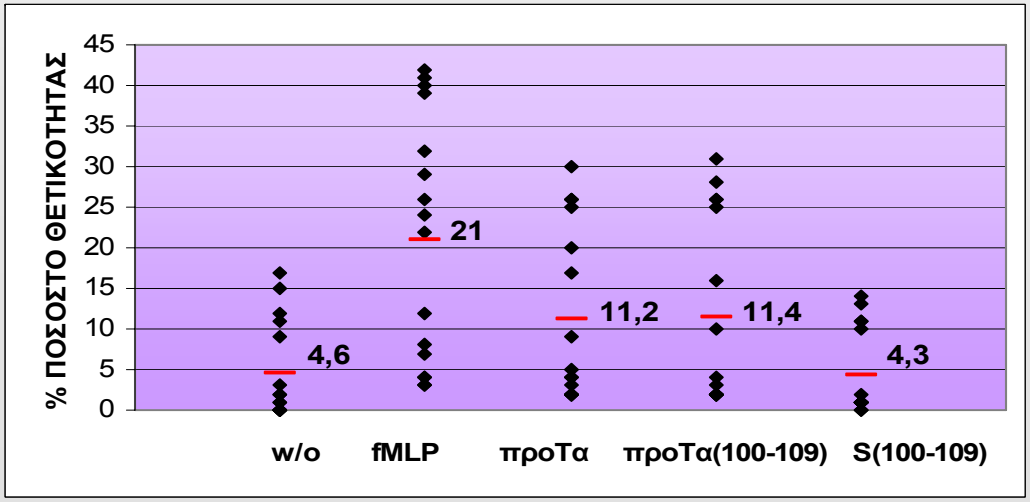
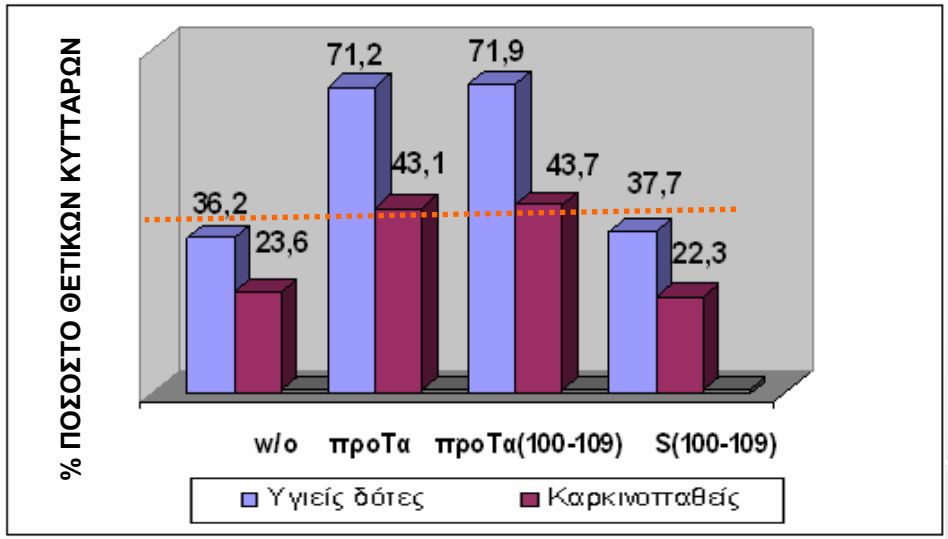
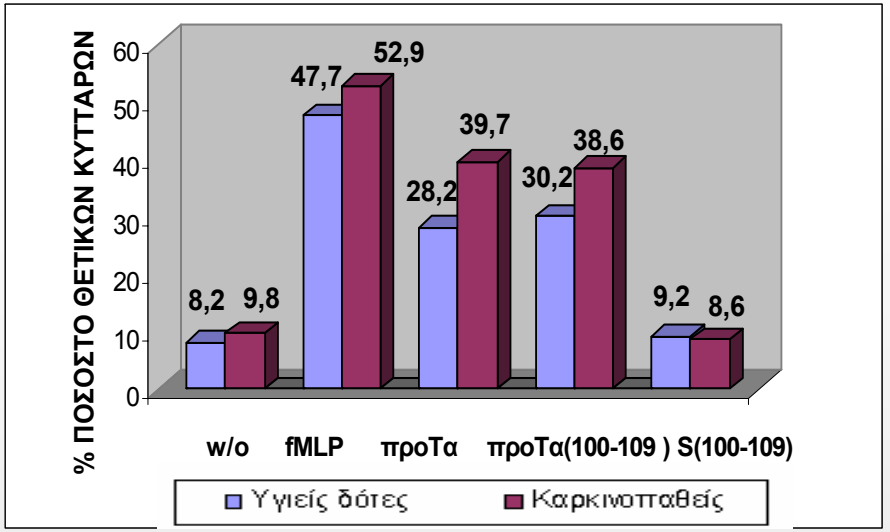


Συγκριτικά αποτελέσματα υγιών-καρκινοπαθών

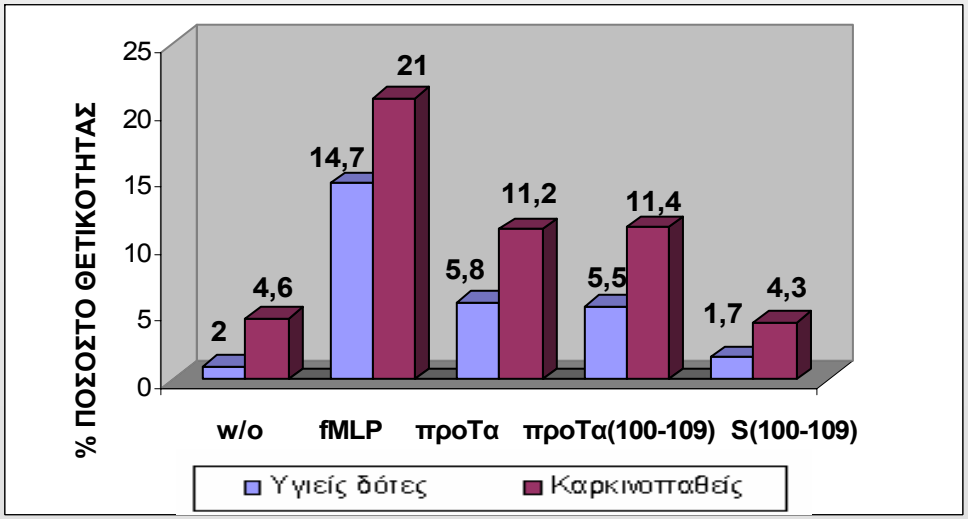
ΦΑΓΟΚΥΤΤΑΡΙΚΗ ΙΚΑΝΟΤΗΤΑ



ΕΝΔΟΚΥΤΤΑΡΙΚΗ ΠΑΡΑΓΩΓΗ O_2^-

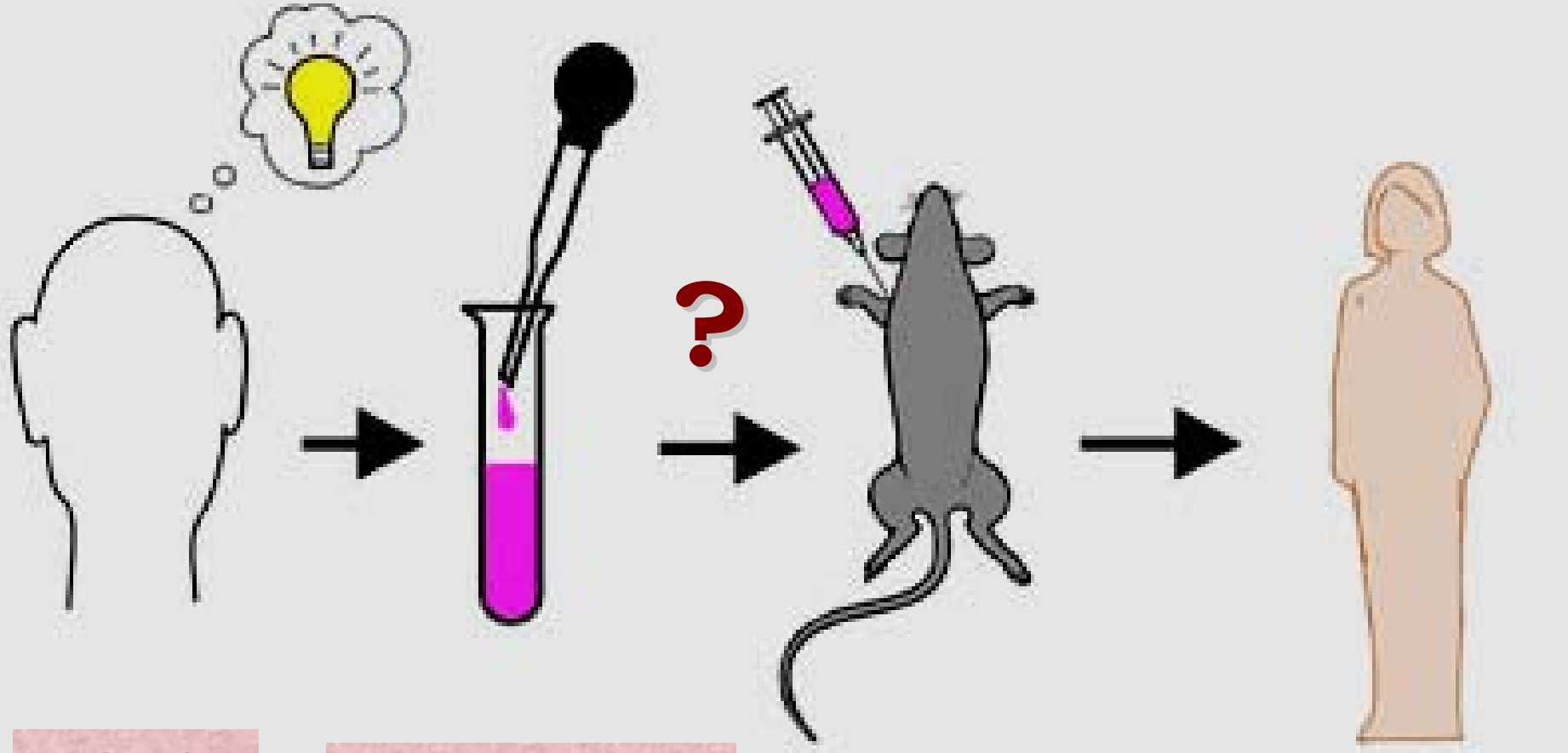


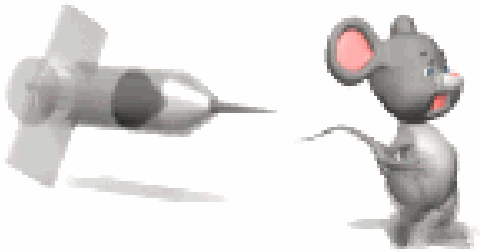
ΕΞΩΚΥΤΤΑΡΙΚΗ ΑΠΕΛΕΥΘΕΡΩΣΗ O_2^-



Samara et al., submitted

The design of cancer immunotherapeutic protocols





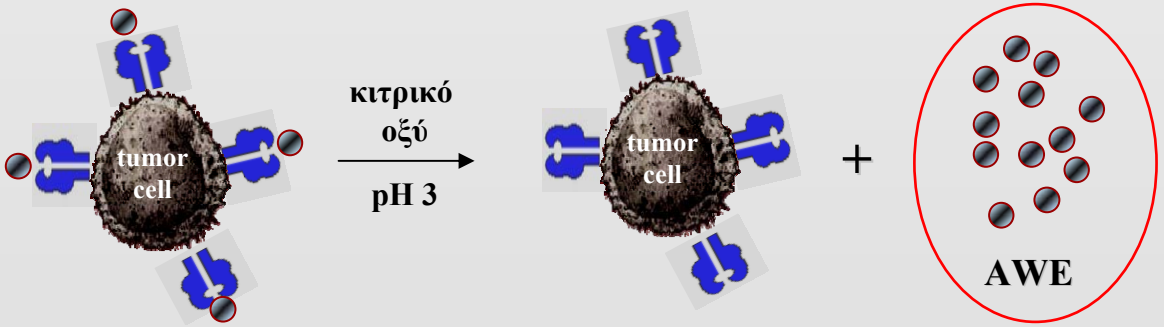
In vivo θεραπευτικό μοντέλο όγκου σε ποντίκια

- *ποντίκια C57Bl/6 ♂, 7 εβδομάδων*
- *5,000 κύτταρα της συγγενής σειράς B16 (κύτταρα μελανώματος)*

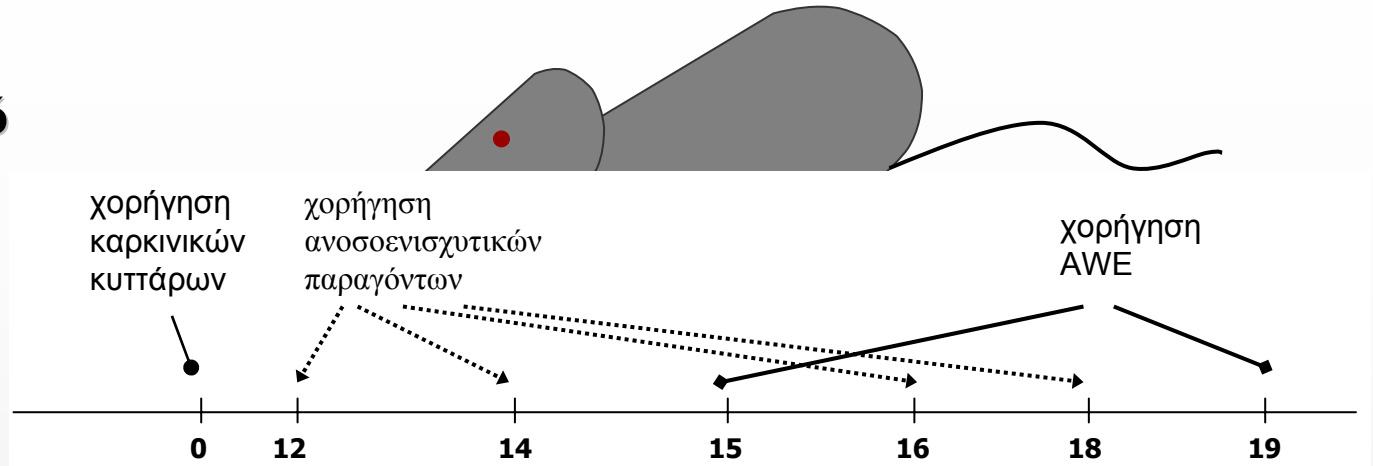
➢ *η ProTα δρα στα μονοκύτταρα, αυξάνοντας την αντιγονοπαρουσίαση και ωριμάζοντας προς DCs,*

μπορεί η ProTα και το ProTα αα100-109 να κατευθύνει την έκπτυξη ογκο-ειδικών Τ-κυτταρικών κλώνων άρα και την αναστολή ανάπτυξης του όγκου?

- **αντιγονοπαρουσιαστικό είδος καρκίνου**
  - ✓*μελανωματικά κύτταρα για την ανάπτυξη συμπαγών όγκων*
- **διάλυμα ογκο-ειδικών πεπτιδίων**
  - ✓*πεπτιδικό παρασκεύασμα AWE (Acid Wash Extract)*

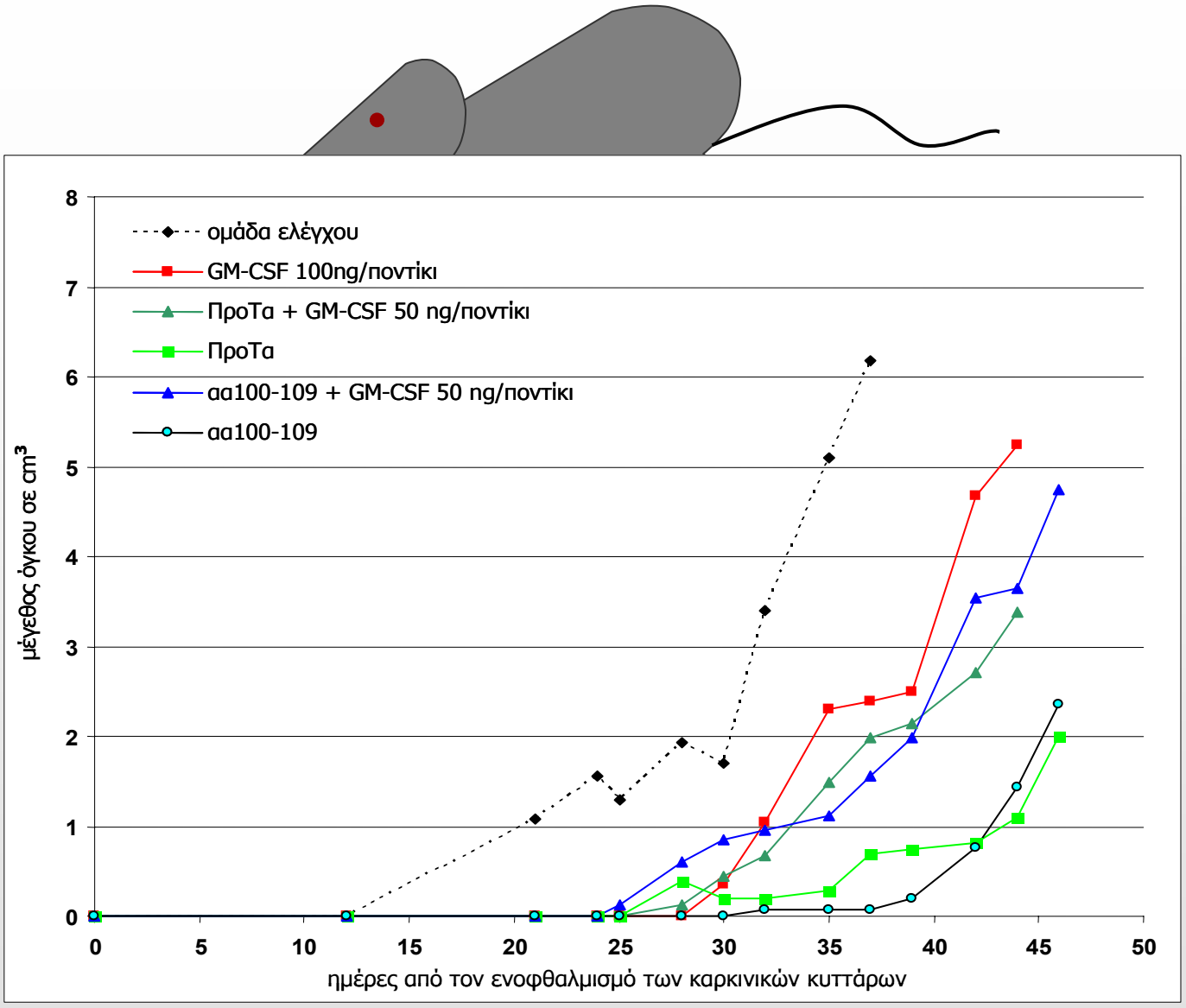


in vivo θεραπευτικό μοντέλο καρκίνου

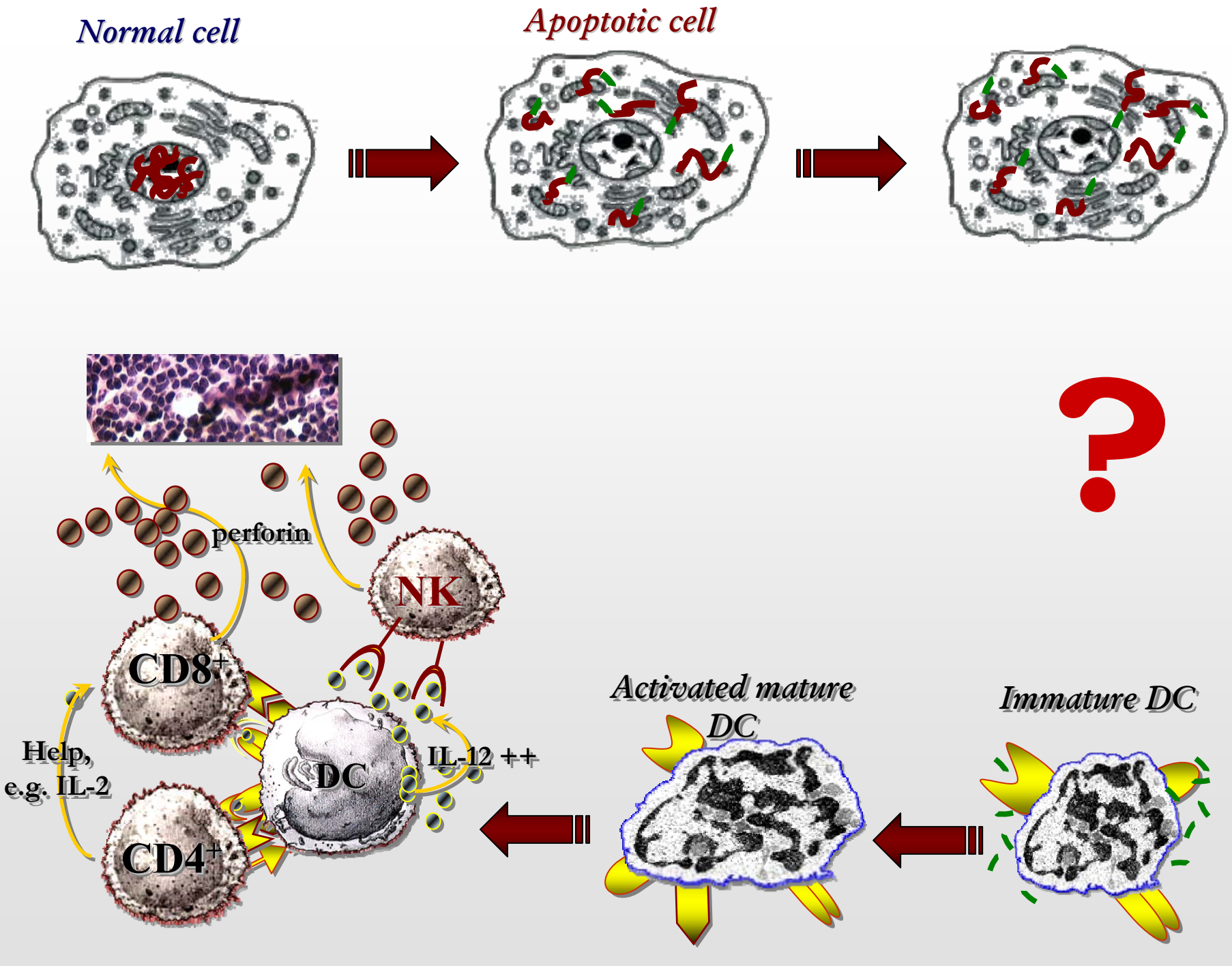


τρόπος χορήγησης	i.p.	i.p.	s.q. ουρά	i.p.	i.p.	s.q. ουρά
ημέρα	12	14	15	16	18	19
group A	PBS	PBS	PBS+IFA	PBS	PBS	PBS+IFA
group B	GM-CSF 100 ng/m	GM-CSF 100 ng/m	AWE+IFA	GM-CSF 100 ng/m	GM-CSF 100 ng/m	AWE+IFA
group C	GM-CSF 50 ng/m + ΠροΤα 350 ng/m	GM-CSF 50 ng/m + ΠροΤα 350 ng/m	AWE+IFA	GM-CSF 50 ng/m + ΠροΤα 350 ng/m	GM-CSF 50 ng/m + ΠροΤα 350 ng/m	AWE+IFA
group D	ΠροΤα 350 ng/m	ΠροΤα 350 ng/m	AWE+IFA	ΠροΤα 350 ng/m	ΠροΤα 350 ng/m	AWE+IFA
group E	GM-CSF 50 ng/m + αα100-109 100 ng/m	GM-CSF 50 ng/m + αα100-109 100 ng/m	AWE+IFA	GM-CSF 50 ng/m + αα100-109 100 ng/m	GM-CSF 50 ng/m + αα100-109 100 ng/m	AWE+IFA
group F	αα100-109 100 ng/m	αα100-109 100 ng/m	AWE+IFA	αα100-109 100 ng/m	αα100-109 100 ng/m	AWE+IFA

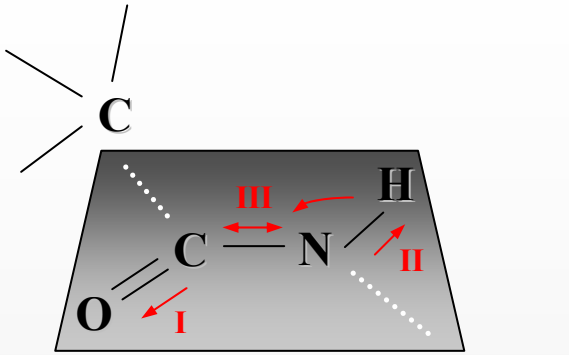
in vivo θεραπευτικό μοντέλο καρκίνου



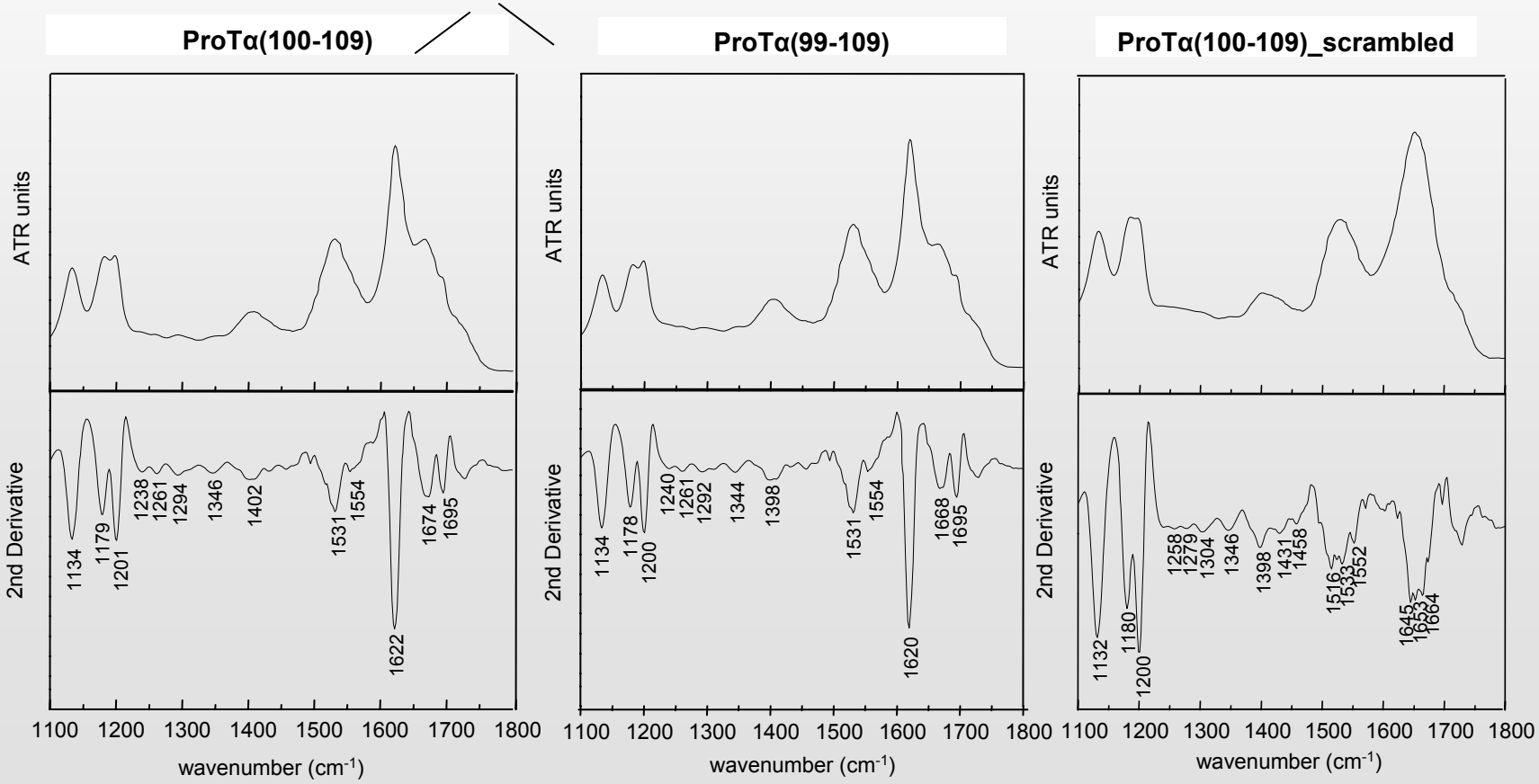
Μέγεθος όγκου (σε cm^3) = (α x β^2) / 2, όπου α= μεγάλη, β= μικρή διάμετρος του όγκου


Normal cell
Apoptotic cell
?
perforin
NK
CD8+
Help,
e.g. IL-2
DC
IL-12 ++
CD4+
Activated mature DC
Immature DC

ATR FT-IR



Η φασματοσκοπία ταλάντωσης υπερερύθρου βρίσκει εφαρμογή σε διαλύματα πρωτεϊνών βασιζόμενη στην απορρόφηση ενέργειας από χαρακτηριστικές ομάδες που προκύπτουν της δημιουργίας πεπτιδικού δεσμού.



ATR FT-IR

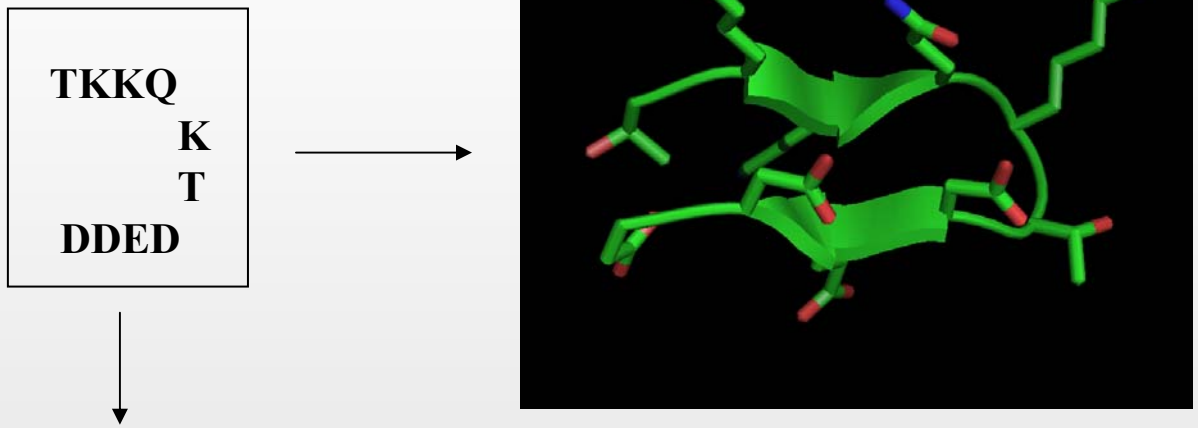
	Δευτεροταγείς δομές				Καρβοξυτελικά πεπτίδια της ΠροΤα		
ταινία	α-έλικα	β-πτυχωτή επιφάνεια	β-στροφή	τυχαία / ακανόνιστη	ΠροΤα (100-109)	ΠροΤα (99-109)	ΠροΤα (100-109)_scrambled
Αμιδική I	*1644-1649*[a] *ή* *1653-1660*[b]	**1691-1699**[a] or **1610-1640**[a,b]	**1662-1695**[d]	*1650-1654*[a] *ή* *1640-1650*[b]	**1695** **1674** **1622**	**1695** **1668** **1620**	*1664* *1653* *1645*
Αμιδική II	*1548-1553*[a] *ή* *1519-1521*[a]	1563[a] ή **1530-1535**[a]		*1546-1553*[a]	1554 **1531**	1554 **1531**	*1552* *1533* *1516*
Αμιδική III	*1280-1317*[c]	**1230-1245**[c]		*1245-1270*[c]	1346 1294 1261 **1238**	1344 1292 1261 **1240**	*1346* *1304* *1279* *1258*

Το πεπτίδιο ΠροΤα(100-109) παρουσιάζει δομή αντιπαράλληλων β-πτυχωτών επιφανειών

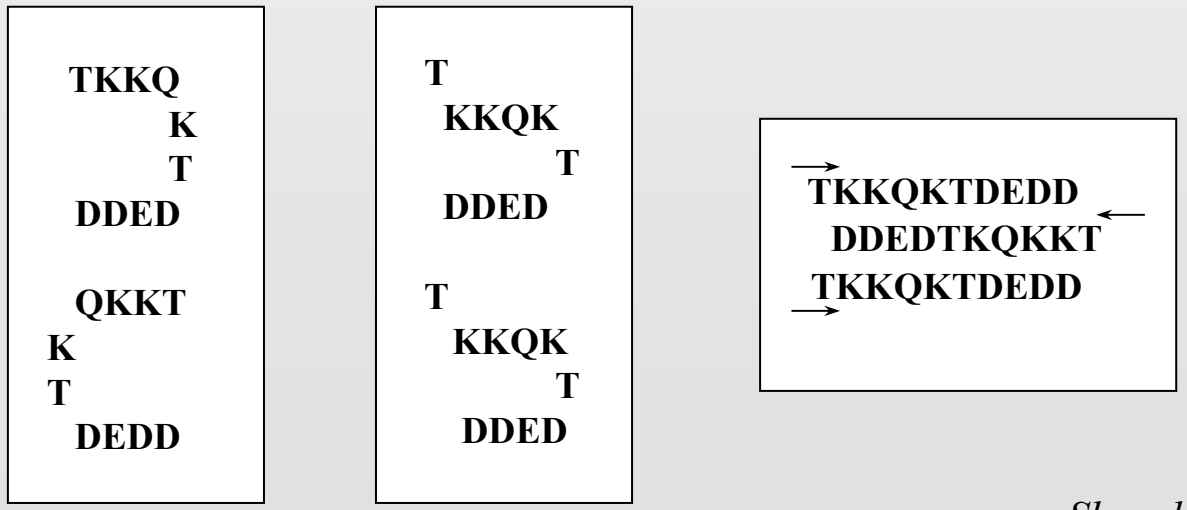
[a] *Venyaminov and Kalnin, 1990;* [b] *Griebenow et al., 1999;* [c] *Singh et al., 1990;* [d] *Orfanidou et al., 1995*

Modeling and ribbon diagram of proTα(100-109)

(a)

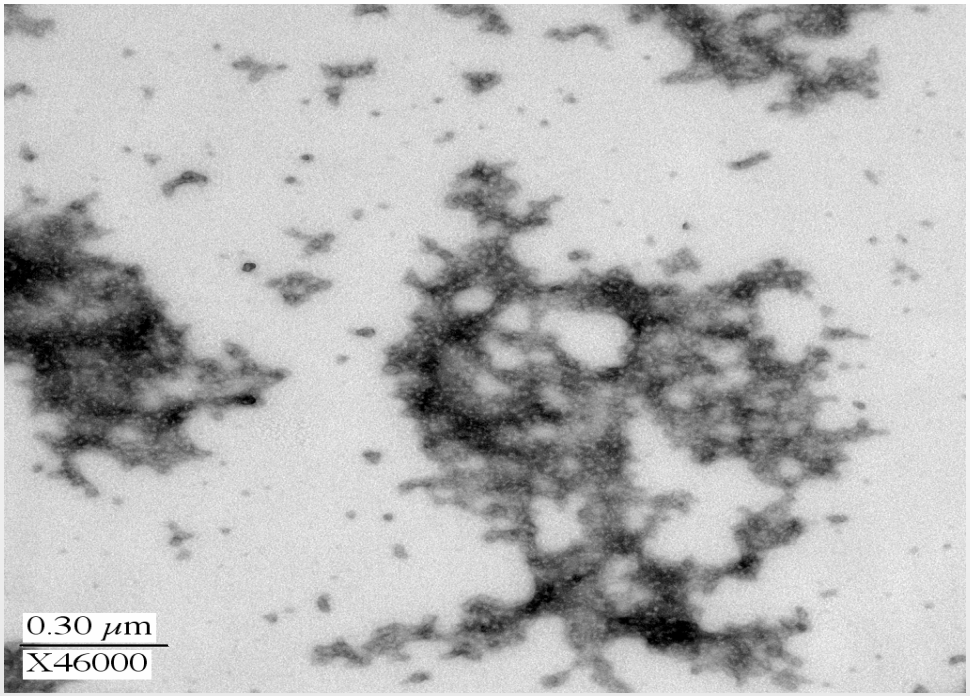


(b)

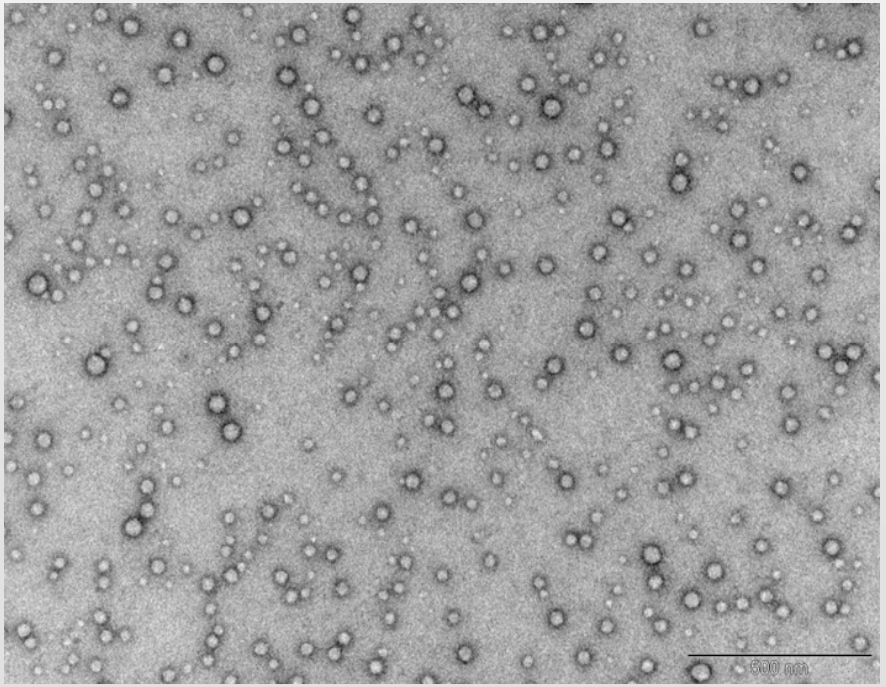


Skopeliti et al., 2008

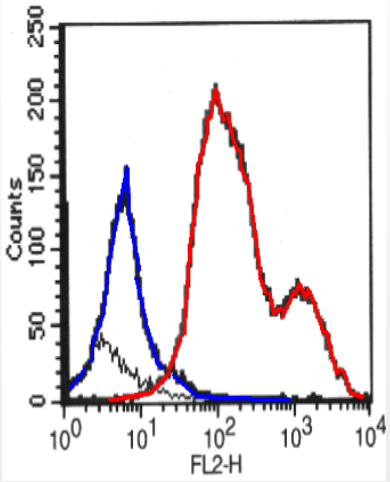
Transmission electron microscopy (negative stain)



- 5 mg/ml
- pH 5.5
- 1% uranyl acetate

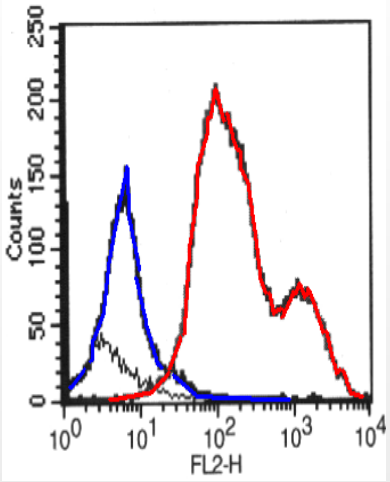


Extracellular localization of proTα(100-109) during apoptosis

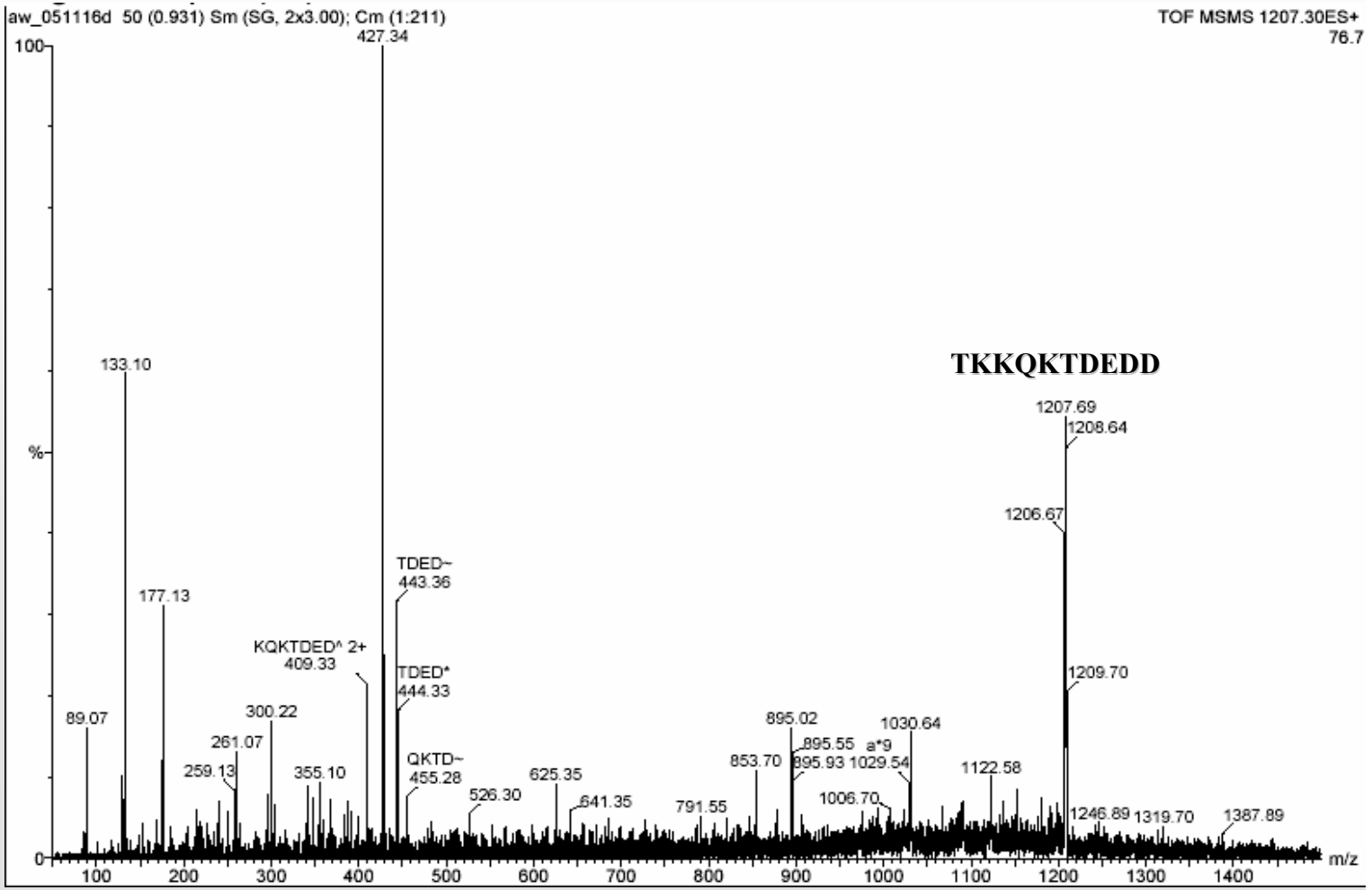


TNF-α and actinomycin D-induced apoptosis on HeLa cells

Extracellular localization of proTα(100-109) during apoptosis



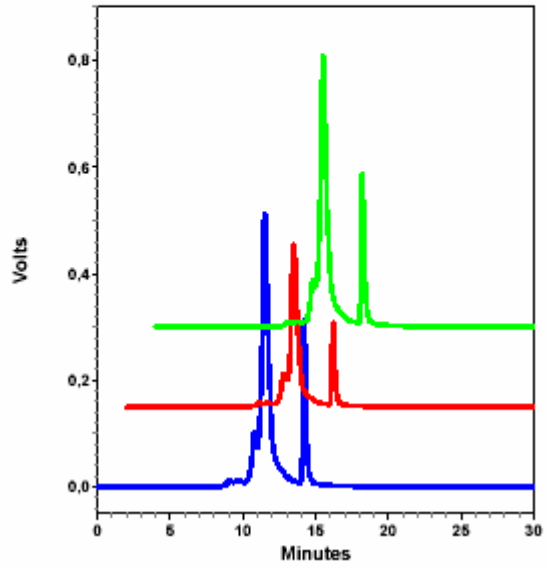
TNF-α and actinomycin D-induced apoptosis on HeLa cells



HPLC of apoptotic supernatants

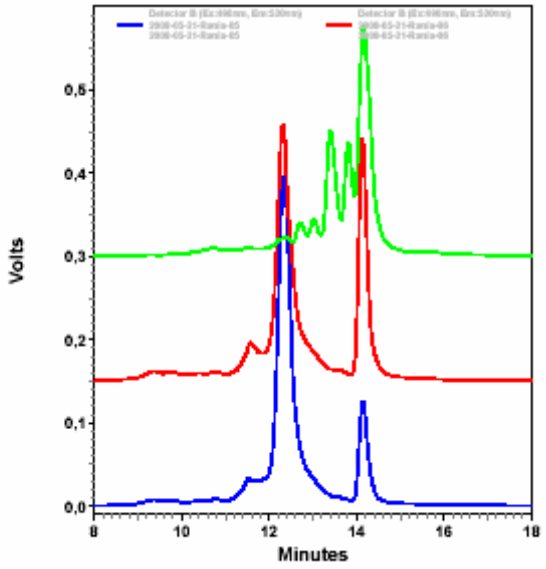
HPLC Run Report
Rania peptides #2 - #4

Sample ID:	2008-05-21-Rania-01
Run time:	21.05.2008 13:32:15
File name:	C:\CLASS-VP\DATA\Kalbacher\Andreas Dittmar\2008-05-21-Rania-01
Vial number:	0
Injection volume:	20 µl
column:	C 18; 125 X 2 mm; 5 µm; Wicom; 0.35 ml/min
Sample:	Rania peptides #2-#4



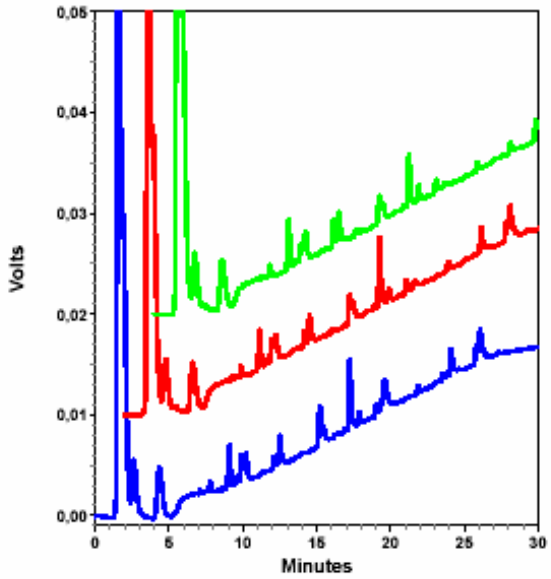
HPLC Run Report
Rania peptides #5 - #7

Sample ID:	2008-05-21-Rania-05
Run time:	21.05.2008 17:52:45
File name:	C:\CLASS-VP\DATA\Kalbacher\Andreas Dittmar\2008-05-21-Rania-05
Vial number:	4
Injection volume:	5 µl
column:	C 18; 125 X 2 mm; 5 µm; Wicom; 0.35 ml/min
Sample:	Rania peptides #5 - #7, Fluorescence Ex:492nm, Em: 520nm



HPLC Run Report
Rania peptides #8 - #10 (UV 214nm)

Sample ID:	2008-05-21-Rania-08
Run time:	21.05.2008 20:28:16
File name:	C:\CLASS-VP\DATA\Kalbacher\Andreas Dittmar\2008-05-21-Rania-08
Vial number:	7
Injection volume:	5 µl
column:	C 18; 125 X 2 mm; 5 µm; Wicom; 0.35 ml/min
Sample:	Rania peptides #8 - #10 , UV 214nm

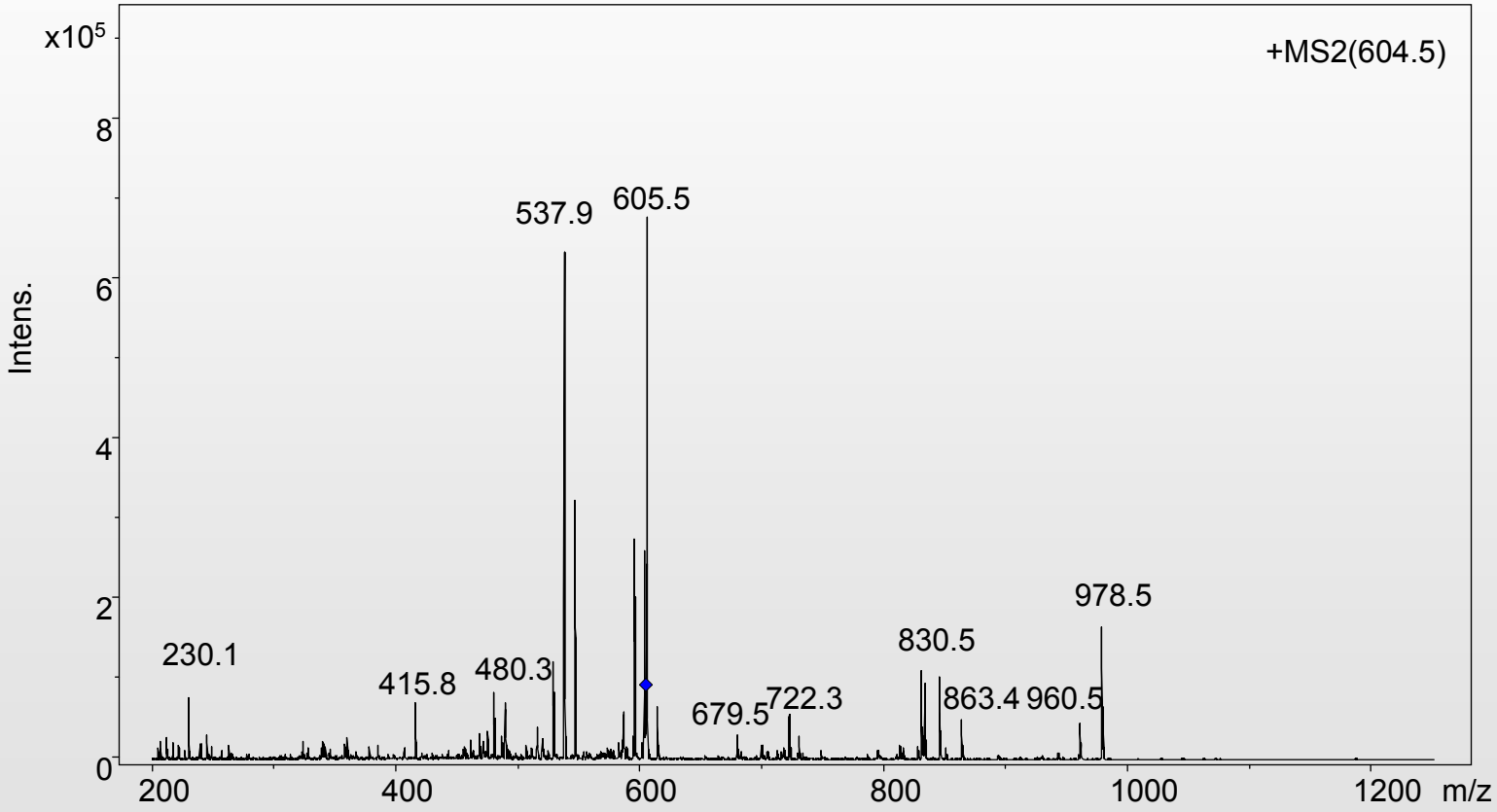


Significant fragmentation of proTα(100-109)

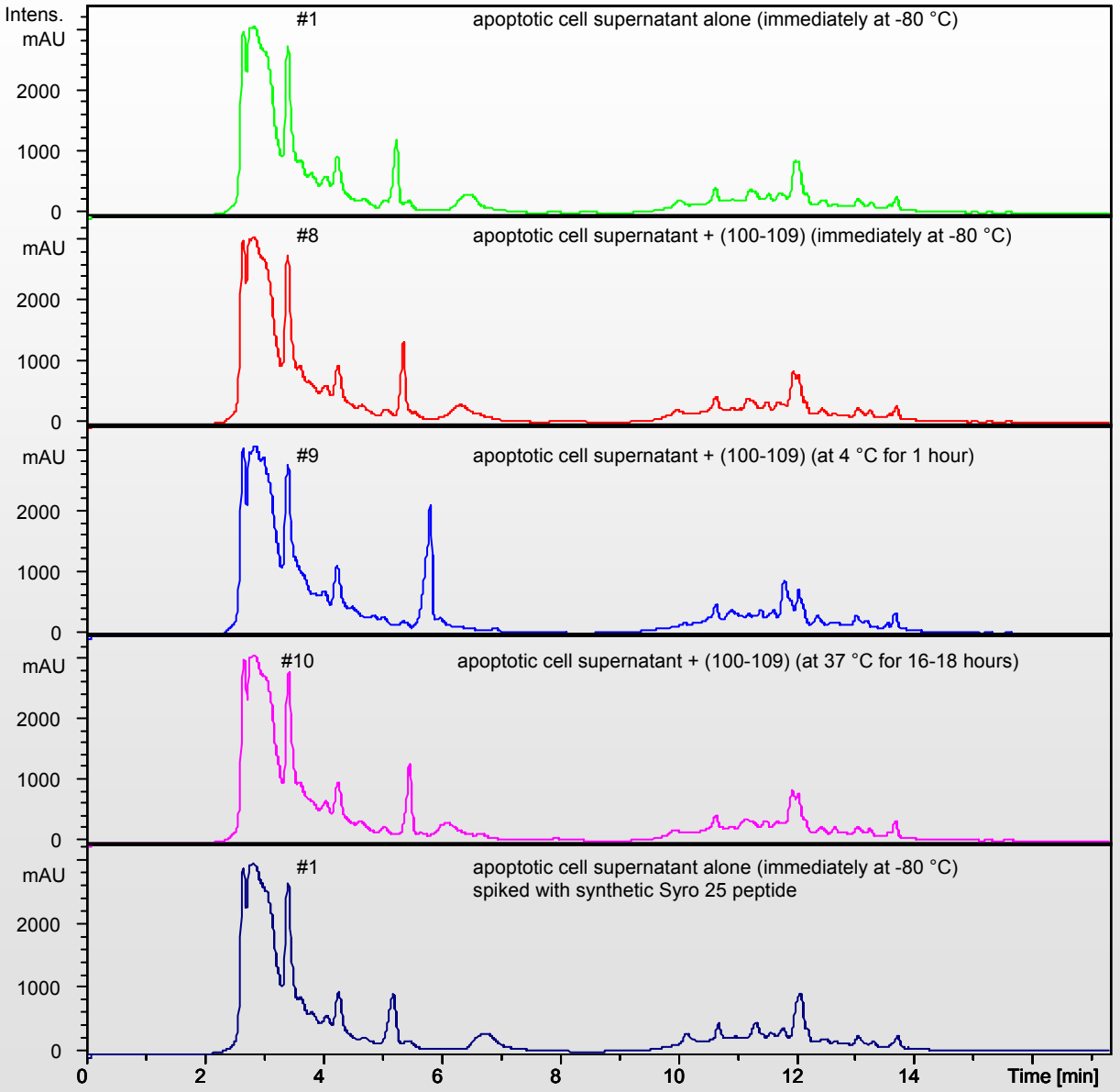
C-terminally labeled with FITC

MS/HPLC of apoptotic supernatants

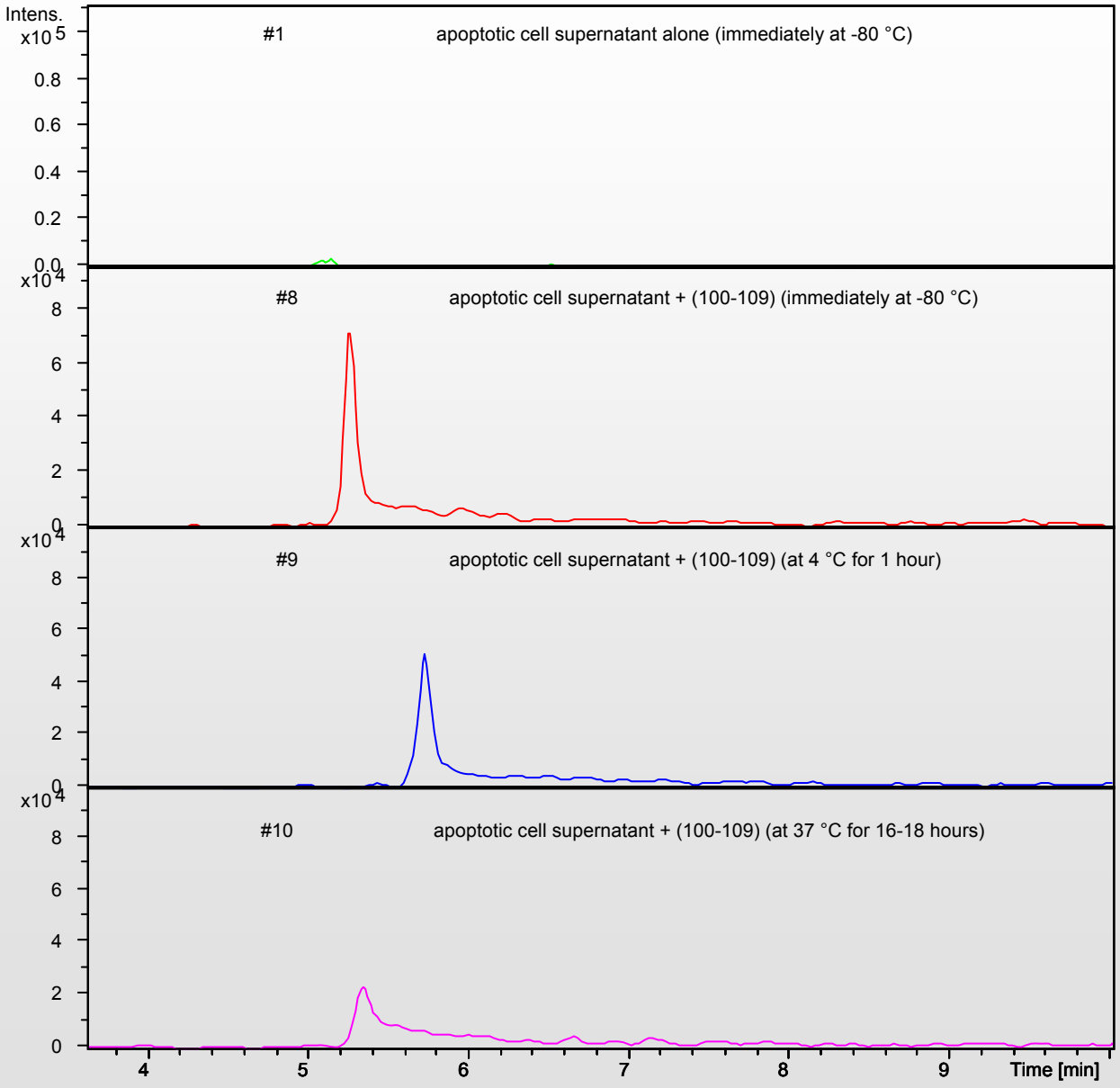
Syringe pump infusion Syro 25 (10 µg/ml) in 0.024% TFA, 40% ACN in water (v/v) at 4 µl/min flowrate
Positive ESI-MS/MS (m/z 604.5; $[M+2H]^{2+}$)
Quantifier: b_9^{2+} fragment ion at m/z 537.9

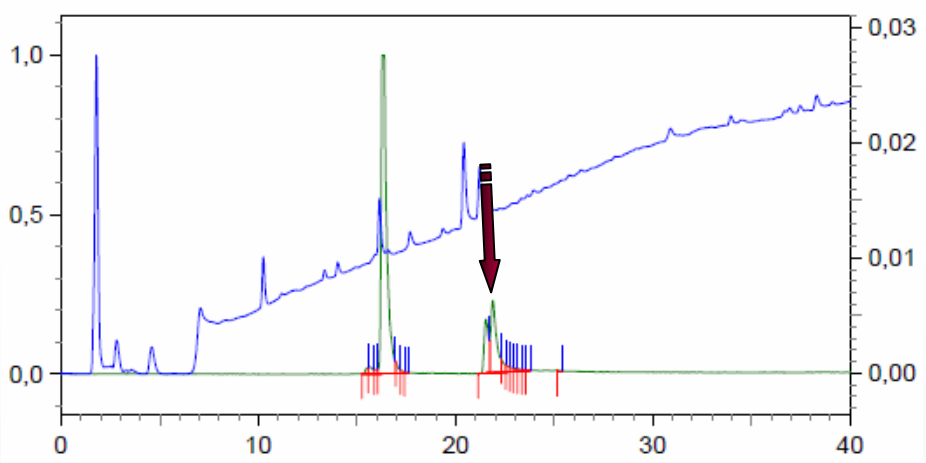


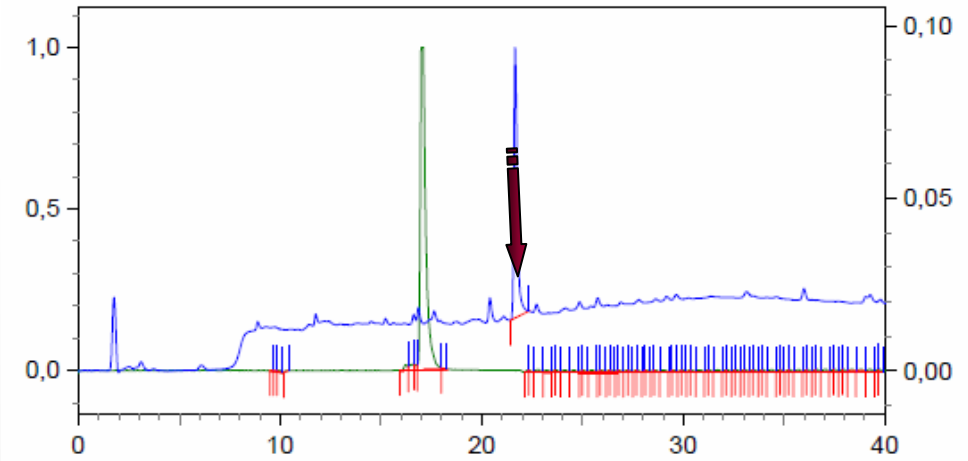
UV chromatograms of apoptotic supernatants (214 nm absorption)

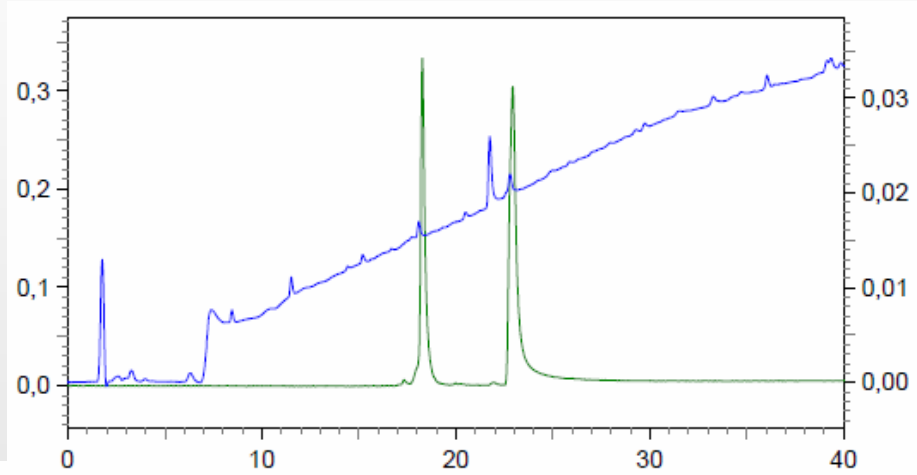


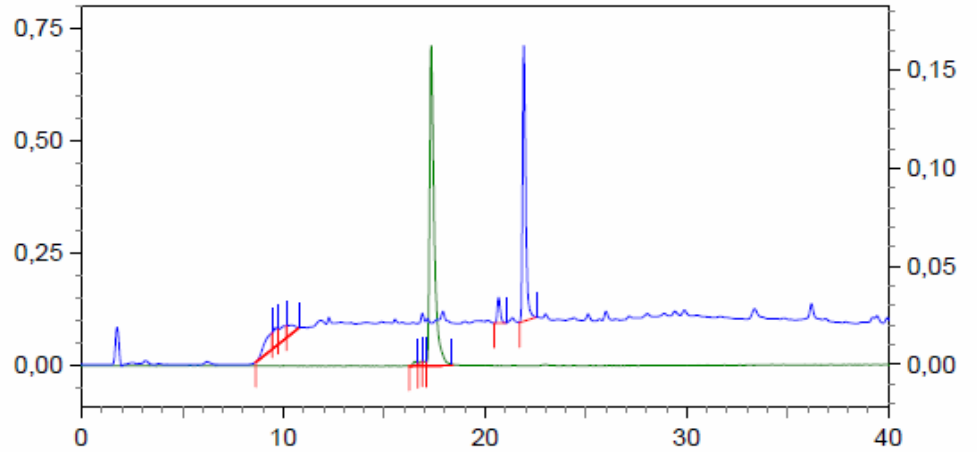
EIC (extracted ion chromatograms) + MS2 of apoptotic supernatants

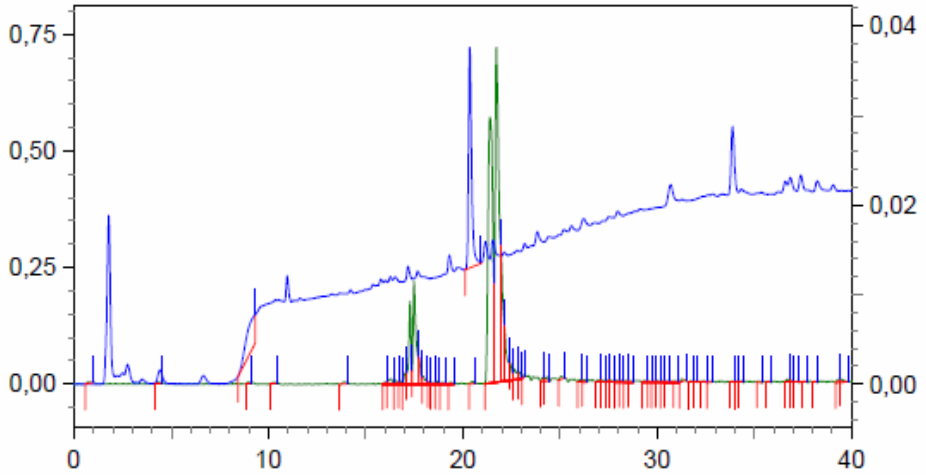


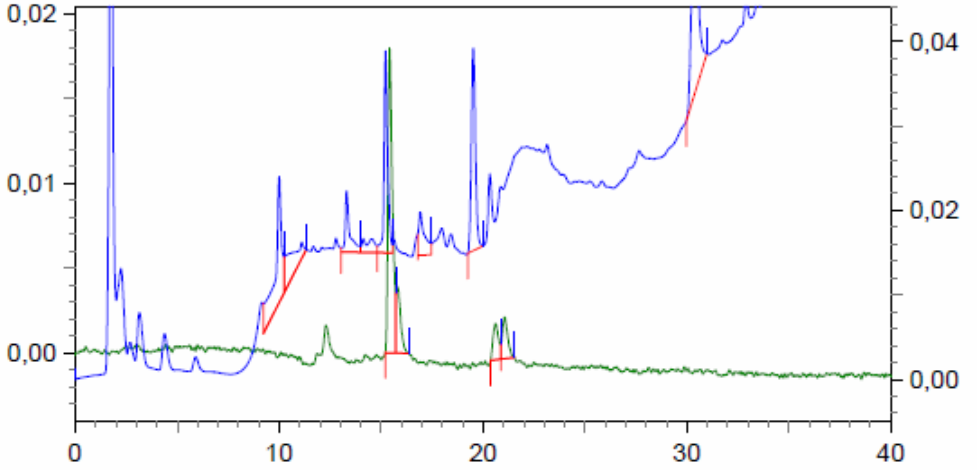

1,0
0,5
0,0
0,03
0,02
0,01
0,00
0
10
20
30
40

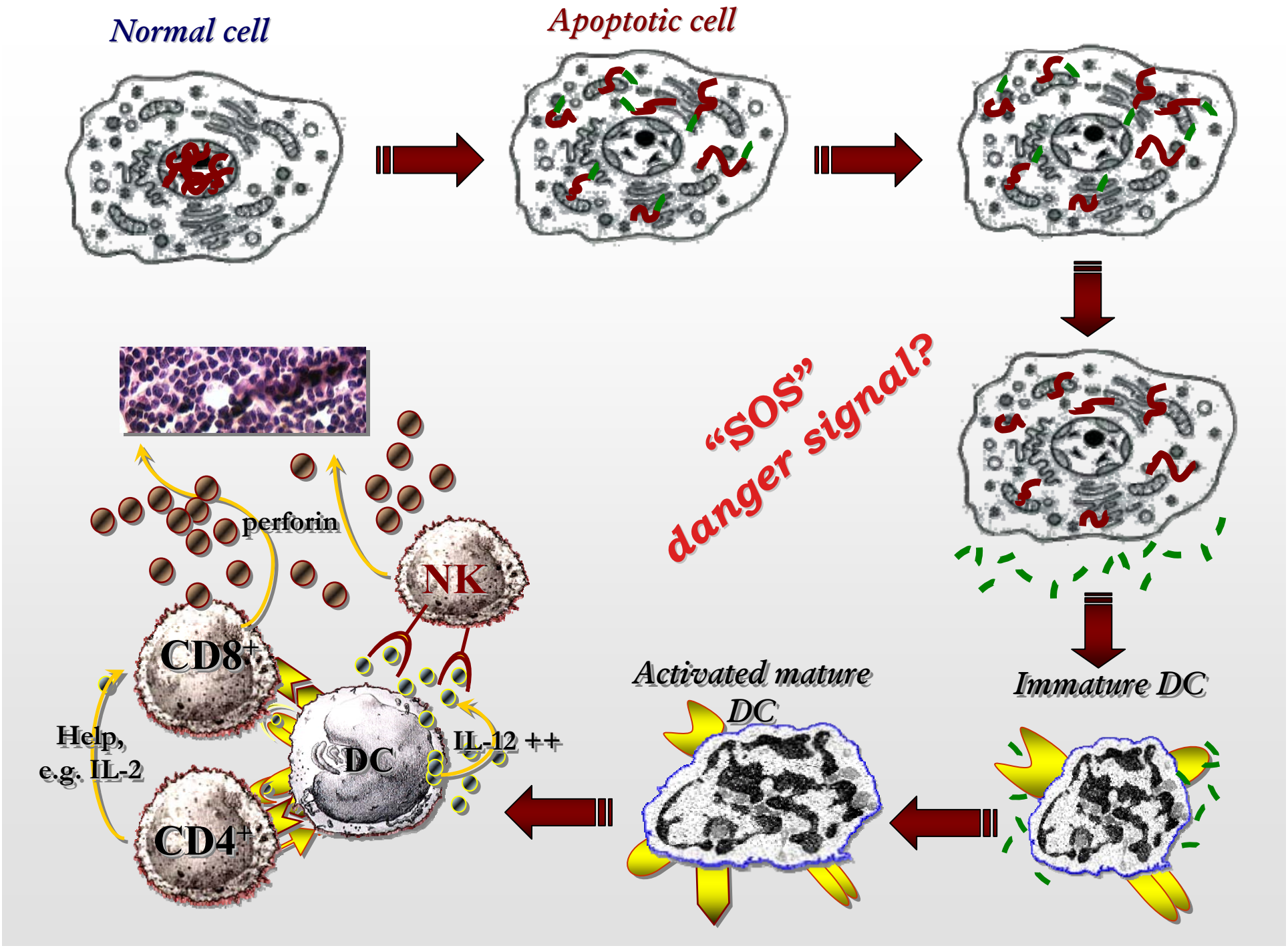

1,0
0,5
0,0
0,10
0,05
0,00
0
10
20
30
40


0,3
0,2
0,1
0,0
0,03
0,02
0,01
0,00
0
10
20
30
40

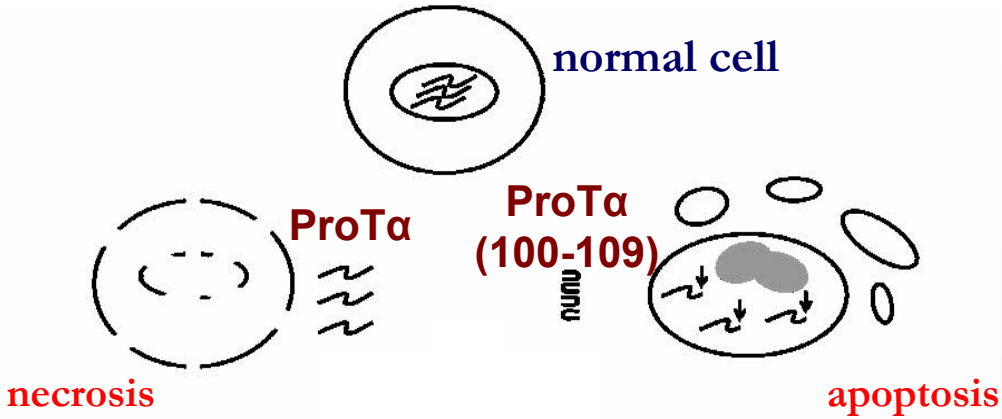

0,75
0,50
0,25
0,00
0,15
0,10
0,05
0,00
0
10
20
30
40


0,75
0,50
0,25
0,00
0,04
0,02
0,00
0
10
20
30
40

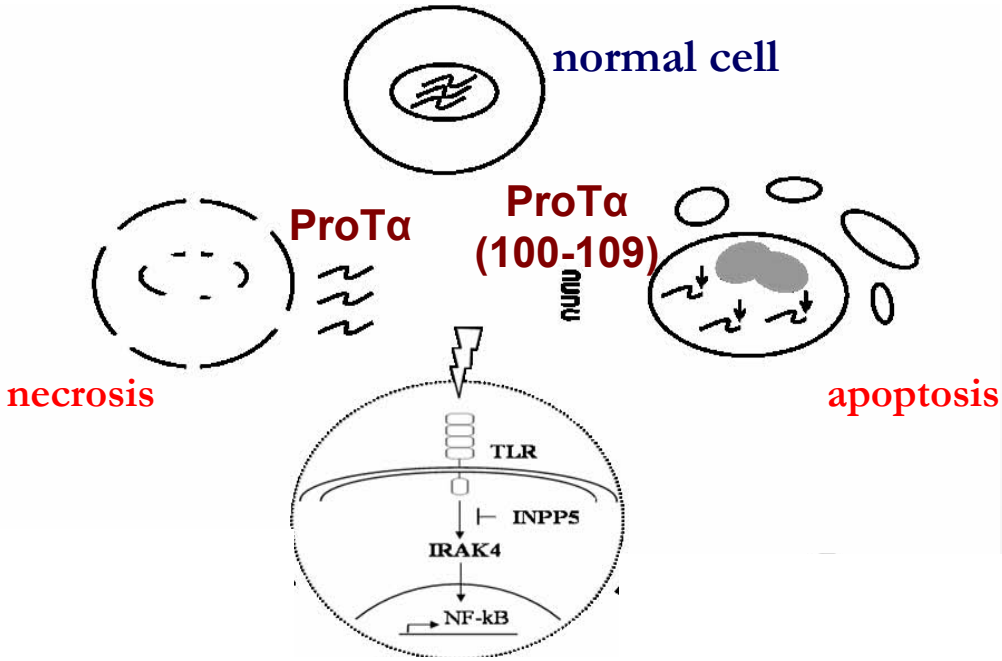

0,02
0,01
0,00
0,04
0,02
0,00
0
10
20
30
40


Normal cell
Apoptotic cell
"SOS" danger signal?
Immature DC
Activated mature DC
perforin
NK
CD8+
DC
CD4+
Help, e.g. IL-2
IL-12 ++

Intracellular role

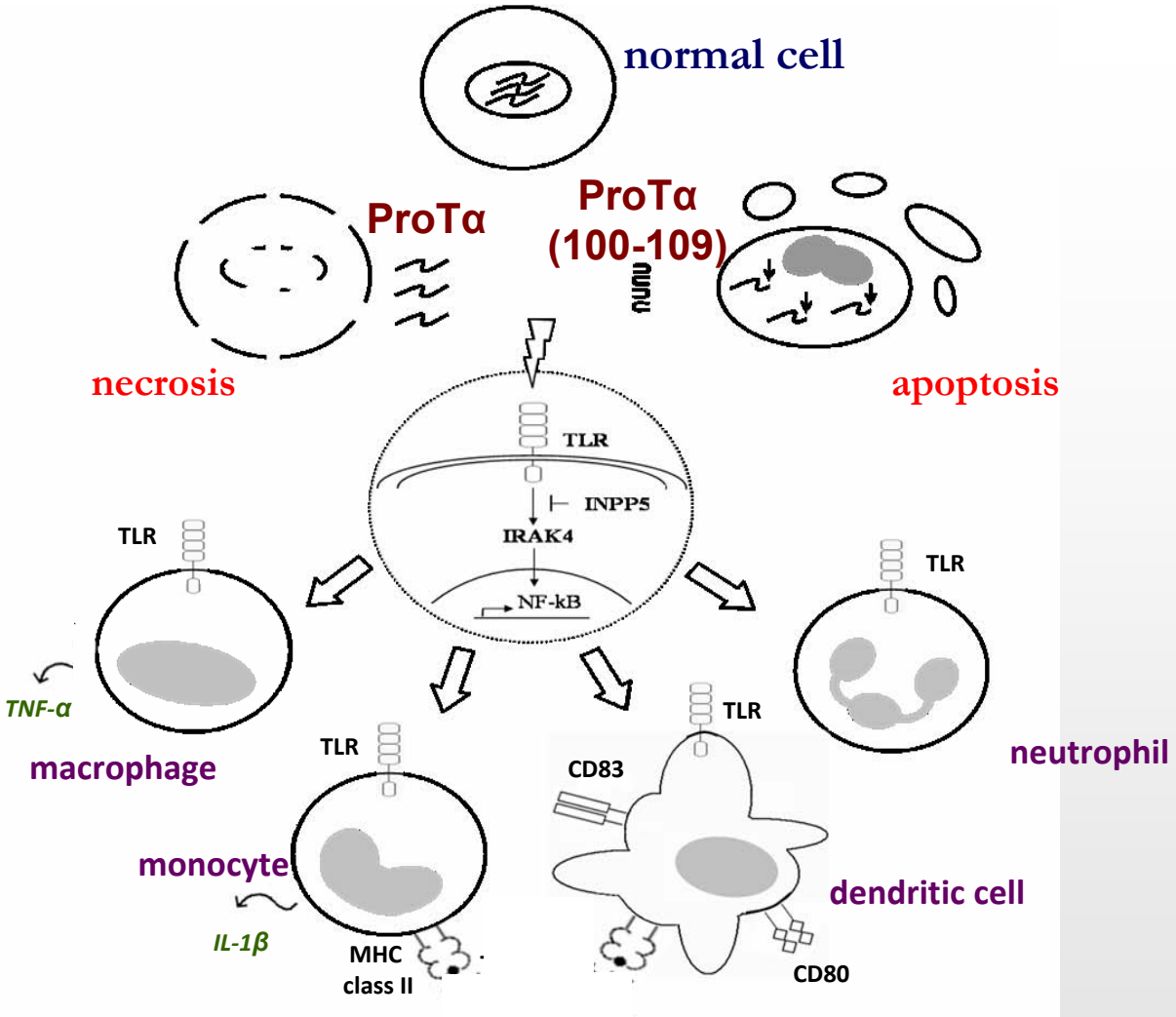


Intracellular role



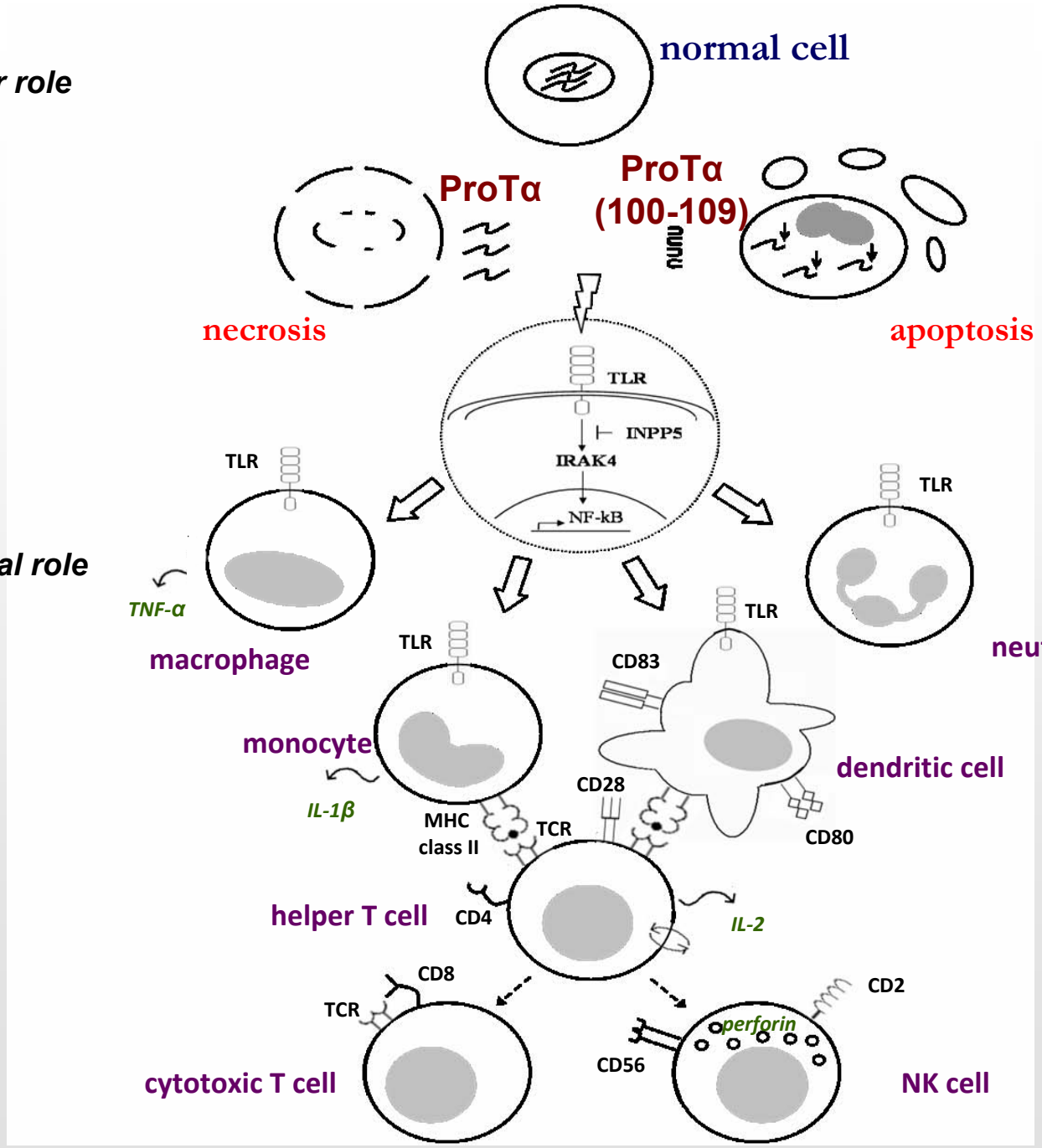
Intracellular role

Immunological role



Intracellular role

Immunological role



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