Age related alterations of anti-tumour specific cytolytic CD8$^+$ T cells

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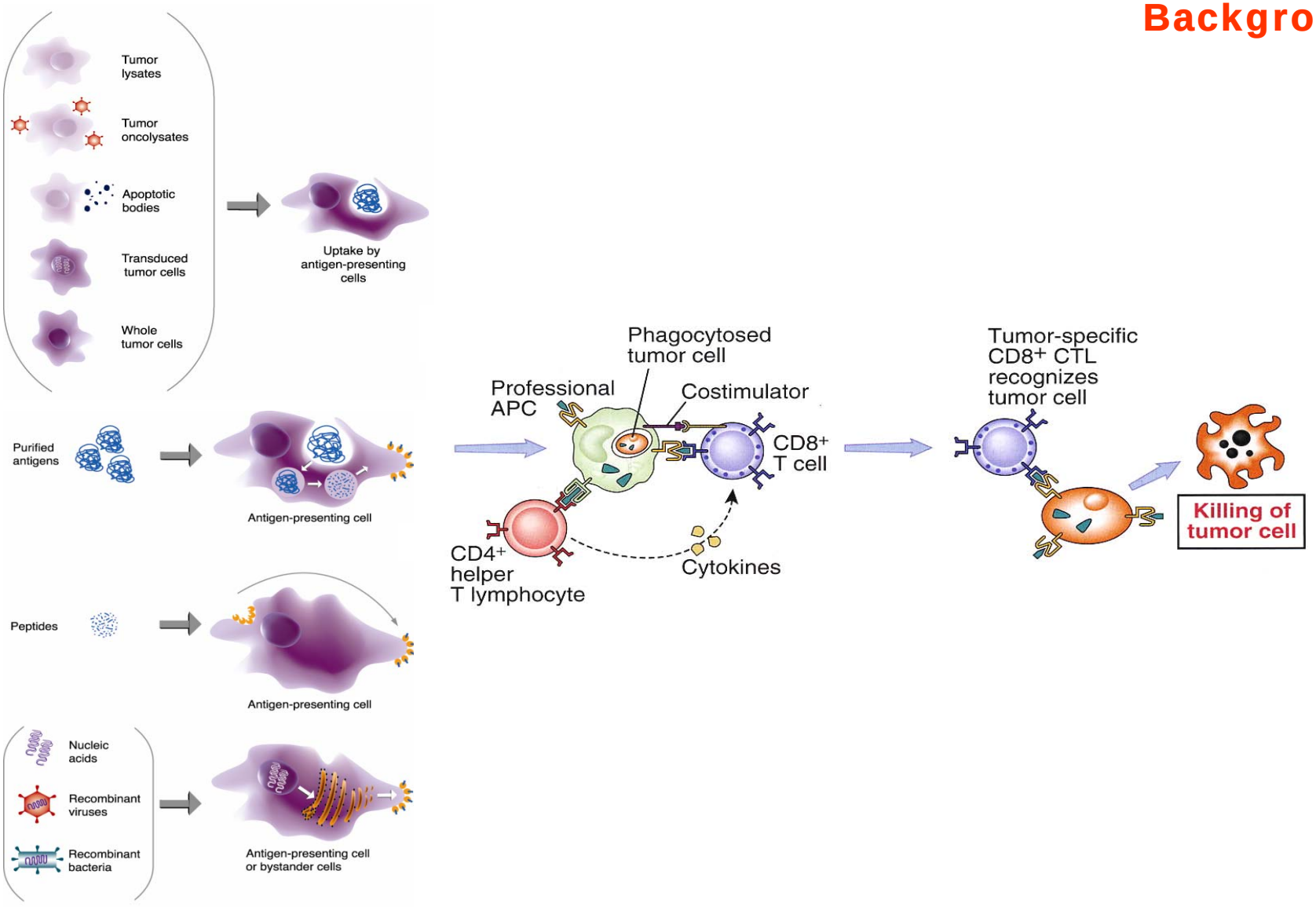
The basics of anti-cancer vaccination

Background



Immunotherapy over the last 2 decades

Responses	Clinical	Immunological
Group A	+	+
Group B	–	–
Group C	+	–
Group D	–	+

All trials

(irrespective of immunogens, Ag, adjuvants, sex, age, etc.)

<5% clinical response rate

Anti-tumour specific pCTL

Aim

Clarify the presence and role of naturally (pre-existing) occurring anti-tumor specific pCTL

Quantitation and function

in cancer patients and healthy subjects

Anti-tumour specific pCTL

Materials

Patients / Normals

15 with NSCLC

10 with SCLC

32 cancer-free individuals (19 >50y, 13 <40y)

Peptides

Antigen	Peptide	HLA-multimer
hTERT	ILAKFLHWL	A2.PE
	RLFFYRKSV	A2.PE
	VYAETKHFL	A24.PE
MAGE-A1	EADPTGHSY	A1/B35.PE
MAGE-A3	EVDPIGHLY	A1/B35.PE
	FLWGPRALV	A2.PE
	TFPDLESEF	A24.PE
BMLF1	GLCTLVAML	A2.APC
EBNA3C	RYSIFFDYM	A24.APC
PB1	VSDGGPNLY	A1.APC

Anti-tumour specific pCTL

Methods

- **Antigen expression**
- **Frequency**

  (multiple peptide MLPC – HLA multimer flow cytometry
  – Poisson analysis for rare events)
- **Isolation**
- **Functional characterization**
  - TcR Vβ analysis
  - Cytokine secretion – ^{51}Cr lysis assay
  - Phenotype

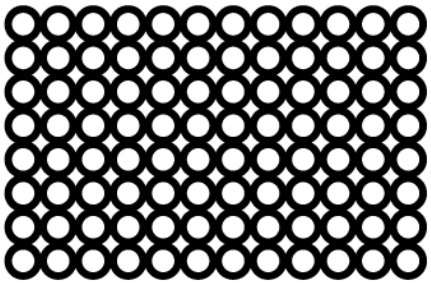
Anti-tumour specific pCTL

Multiple MLPC
HLA multimer flow cytometry
(Poisson analysis for rare events)

Day 0

PBMC ($2x10^5$ cells/well) **+peptides**

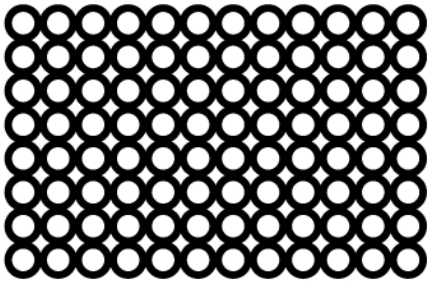
+IL-2, IL-4, IL-7



Day 7

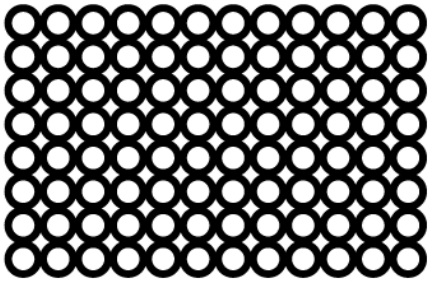
Restimulation with peptides

+IL-2, IL-4, IL-7



Day 14

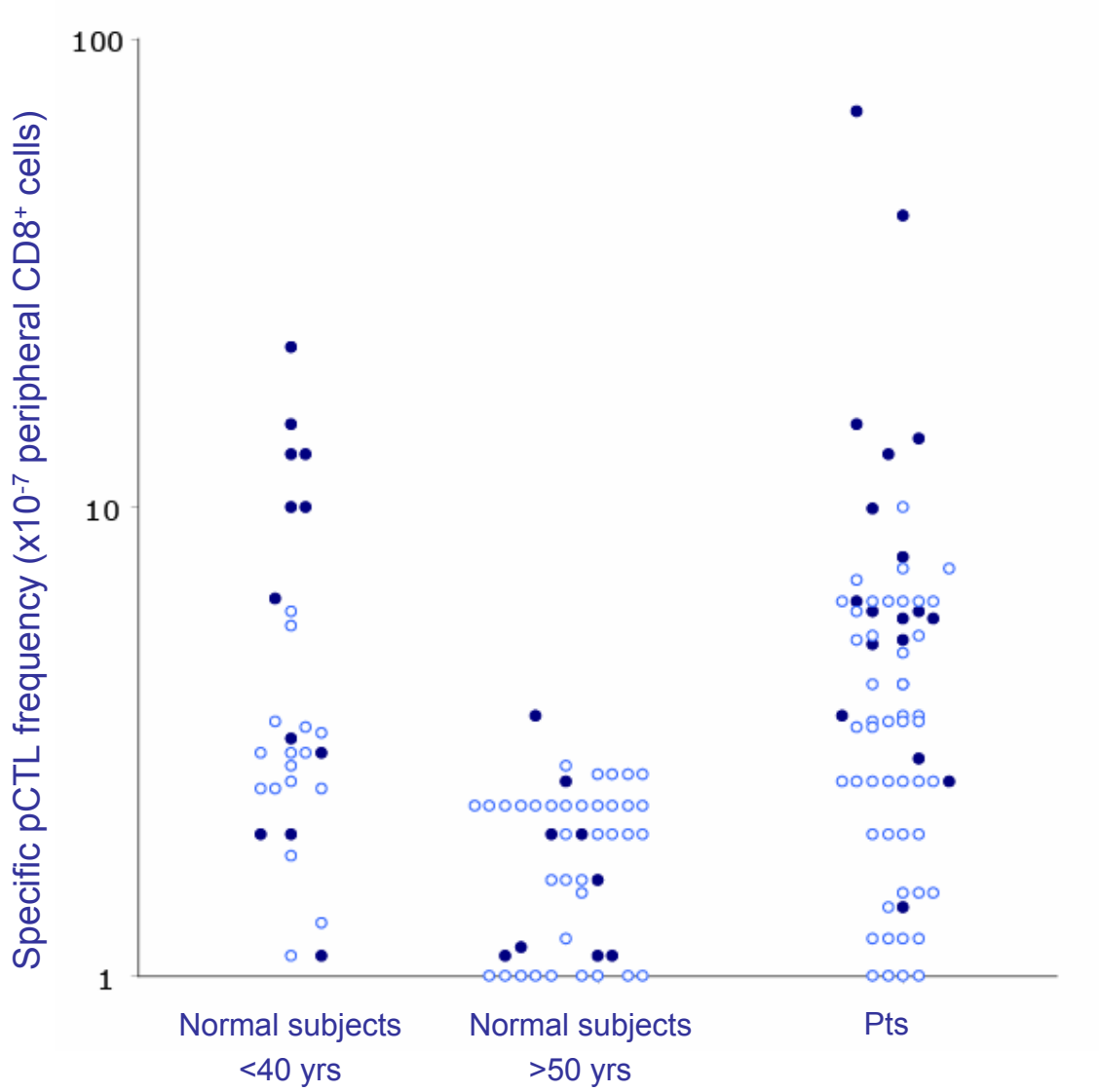
Staining with tetramers



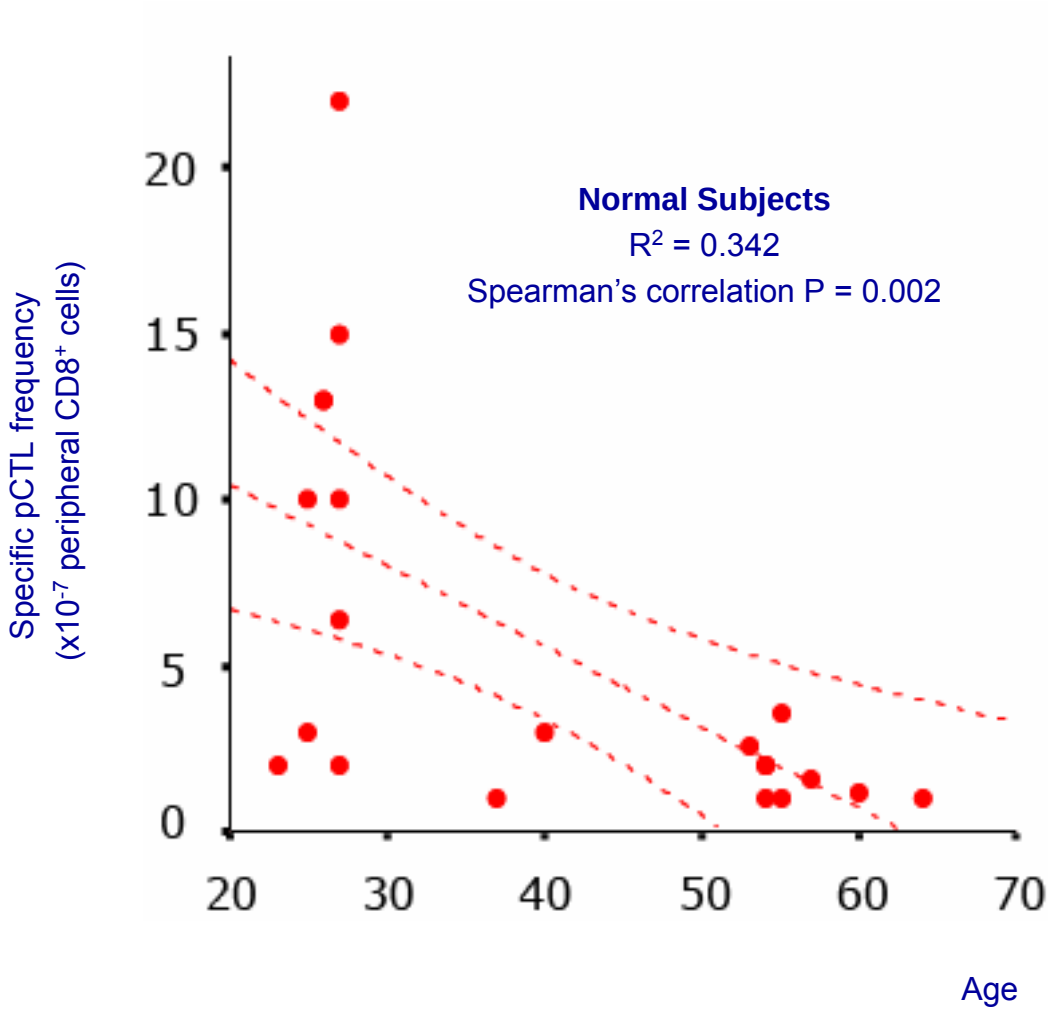
Cloning

Anti-tumour specific pCTL

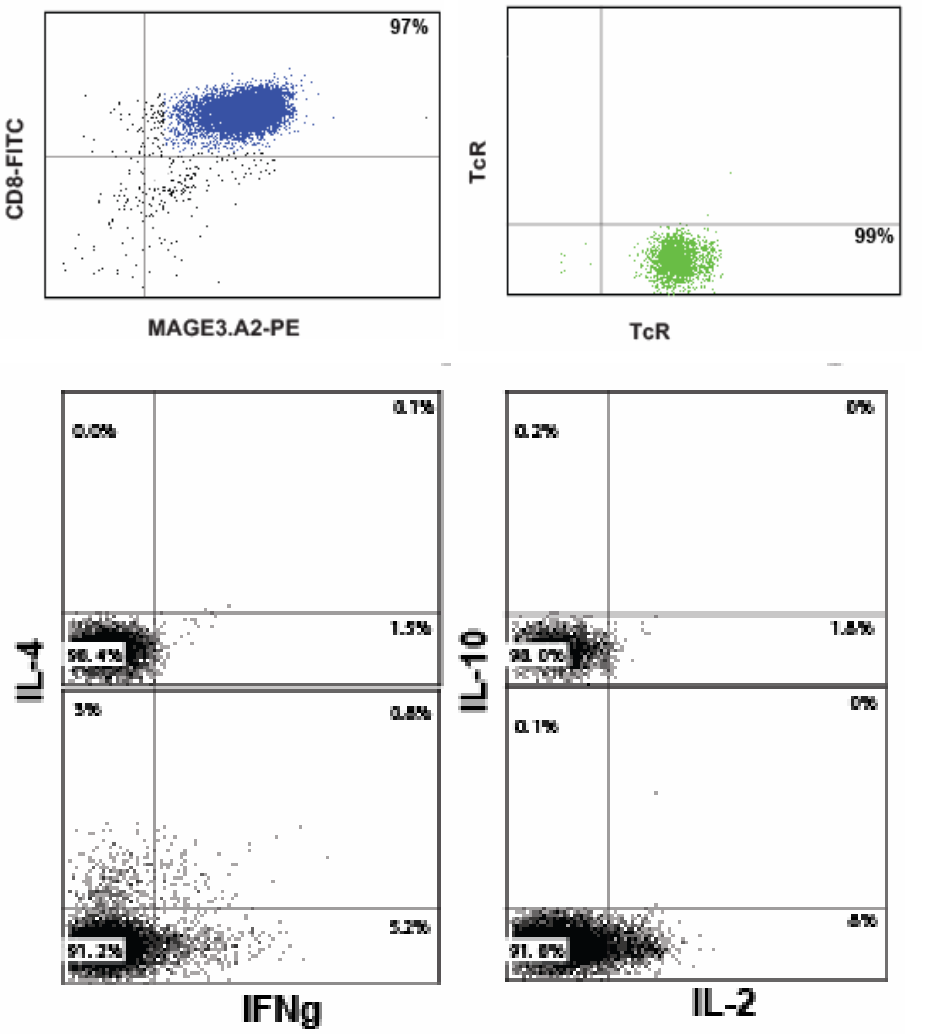
Frequency

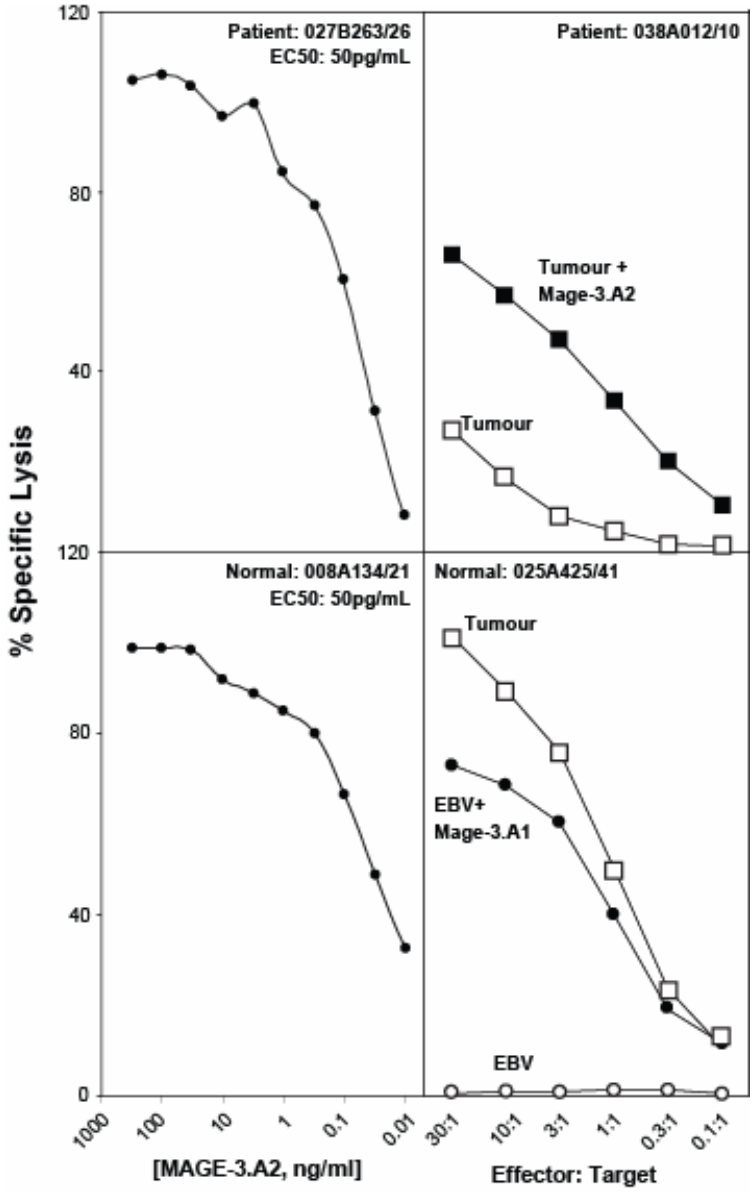


Anti-tumour specific pCTL

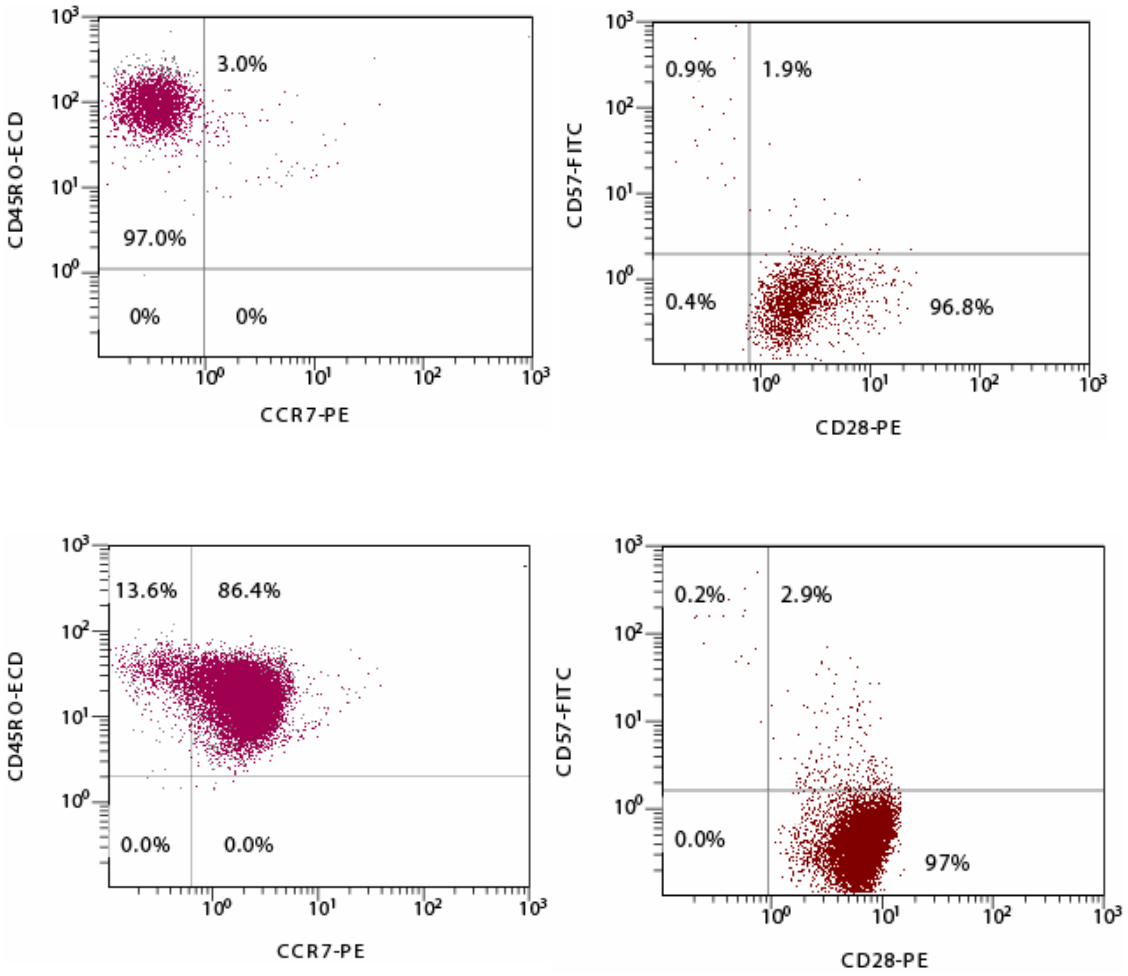


Anti-tumour specific pCTL

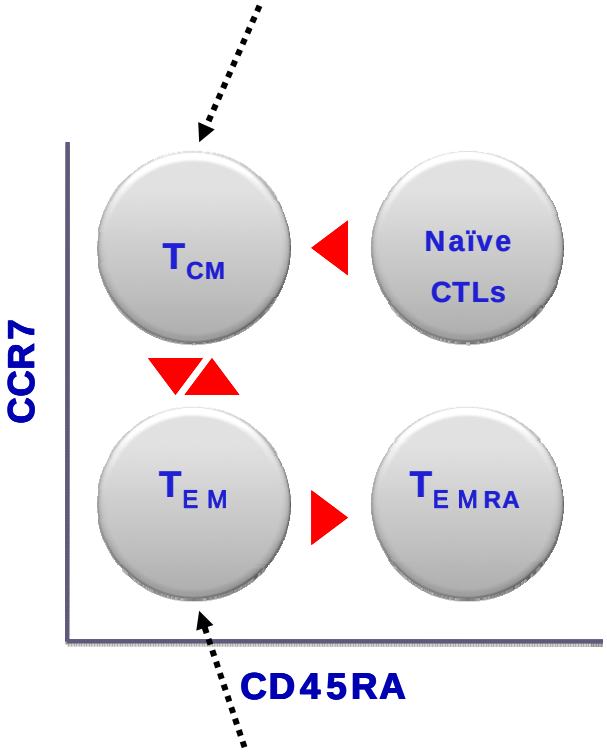




Anti-tumour specific pCTL



Clones from cancer-free individuals



Clones from cancer patients

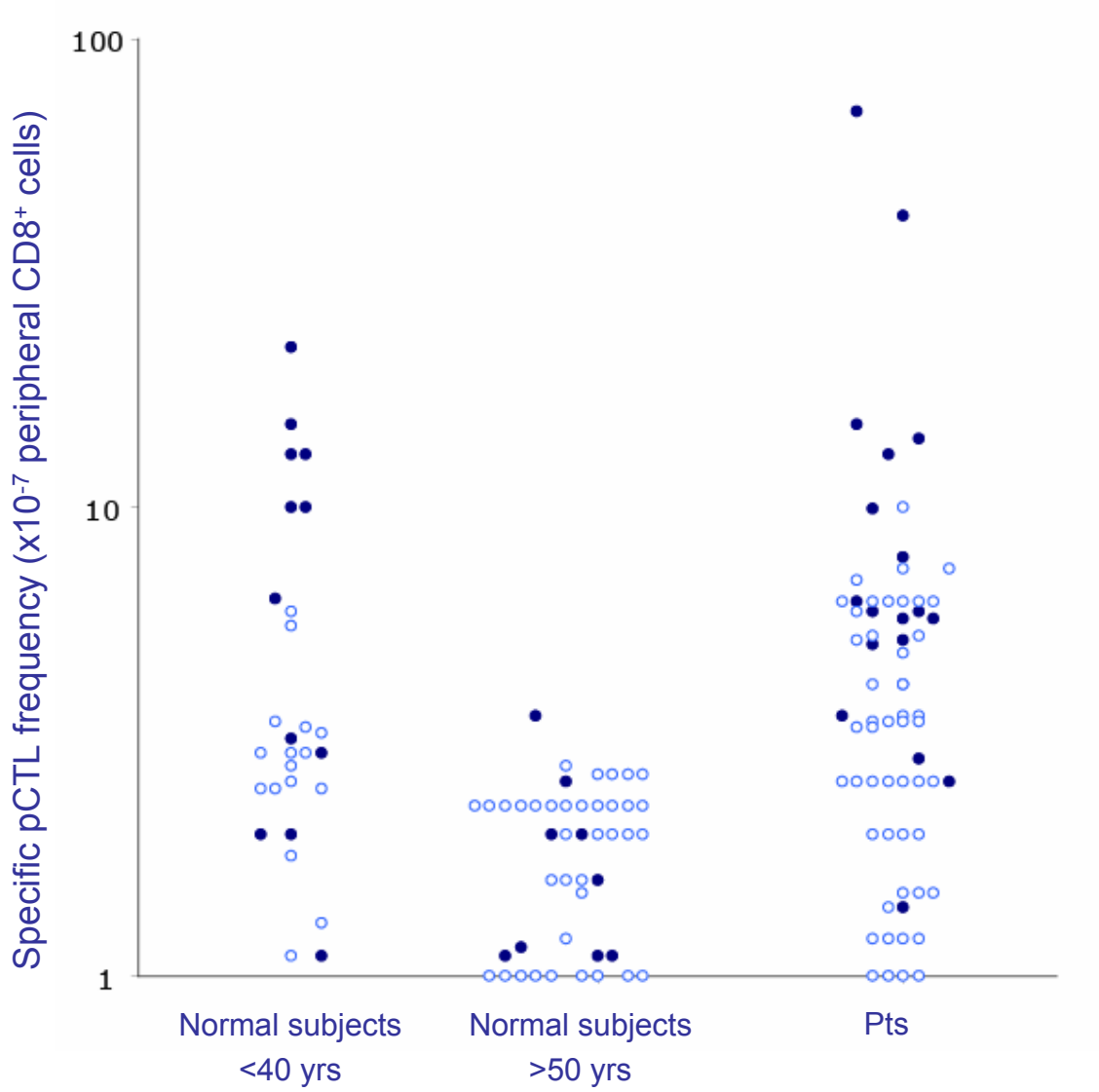
Anti-tumour specific pCTL

Age related decline in frequency of anti-tumour specific pCTLs

How can immunotherapeutic protocols benefit the aged cancer patient?

Anti-tumour specific pCTL

Frequency



Thank you for your attention

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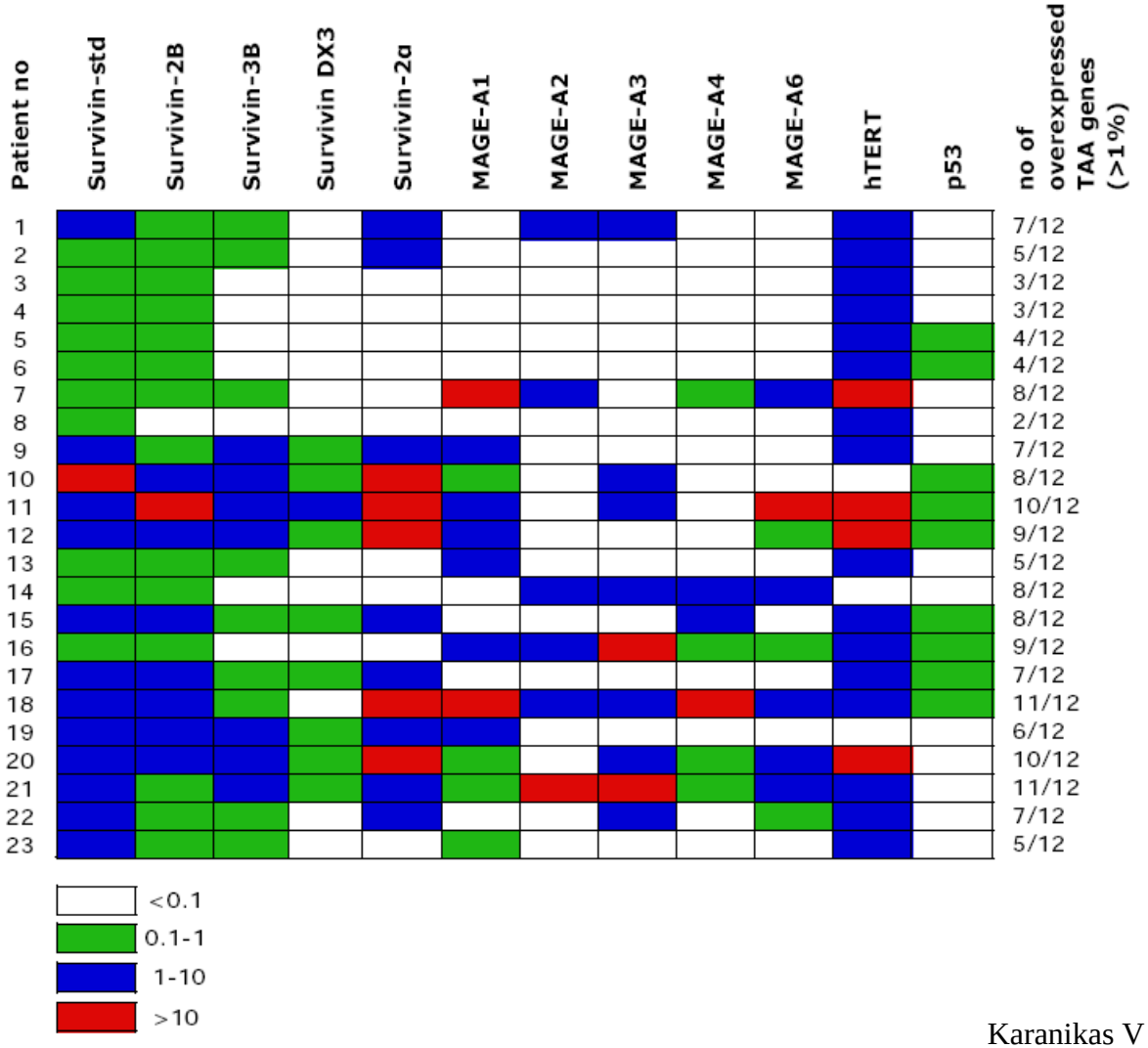
Anti-tumour specific pCTL

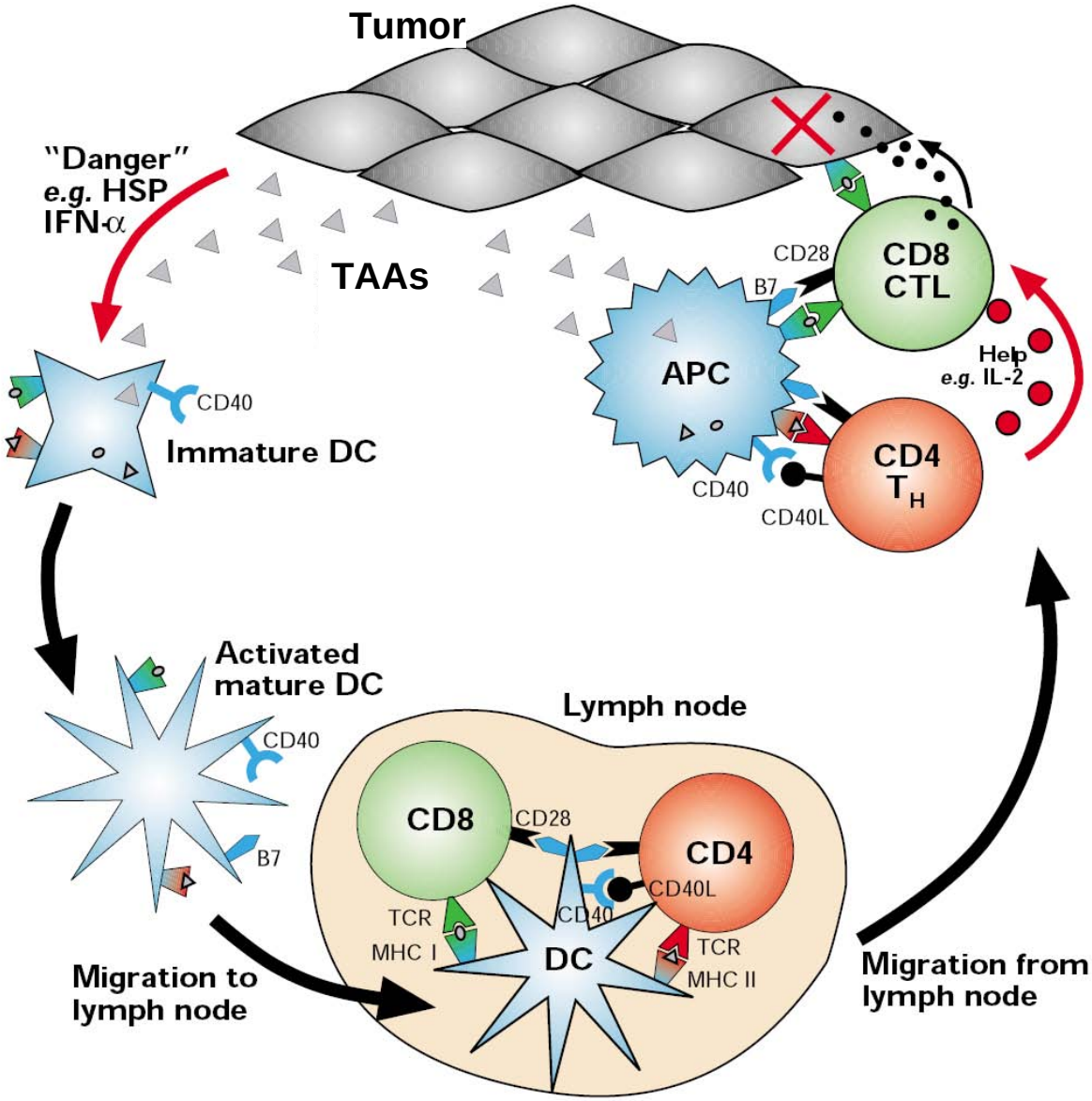
Antigen expression

RT-PCR, relative real time RT-PCR



Karanikas V et al. *Cancer Biol Ther* 2008, 7: 345

Requirements for effective immunotherapy



Background

A **sufficient number** of avid tumor reactive lymphocytes, must have appropriate **effector mechanisms** to destroy cancer cells