iSBTc 2004
Immune Monitoring Workshop
Functional Cytometry

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Functional Cytometry: Definition

Level 1 (not a functional experiment):

Assessment of the presence of molecules characteristic for functional properties (e.g. level of differentiation, migratory potential, cytotoxic molecules including granzymes and perforin).

Level 2

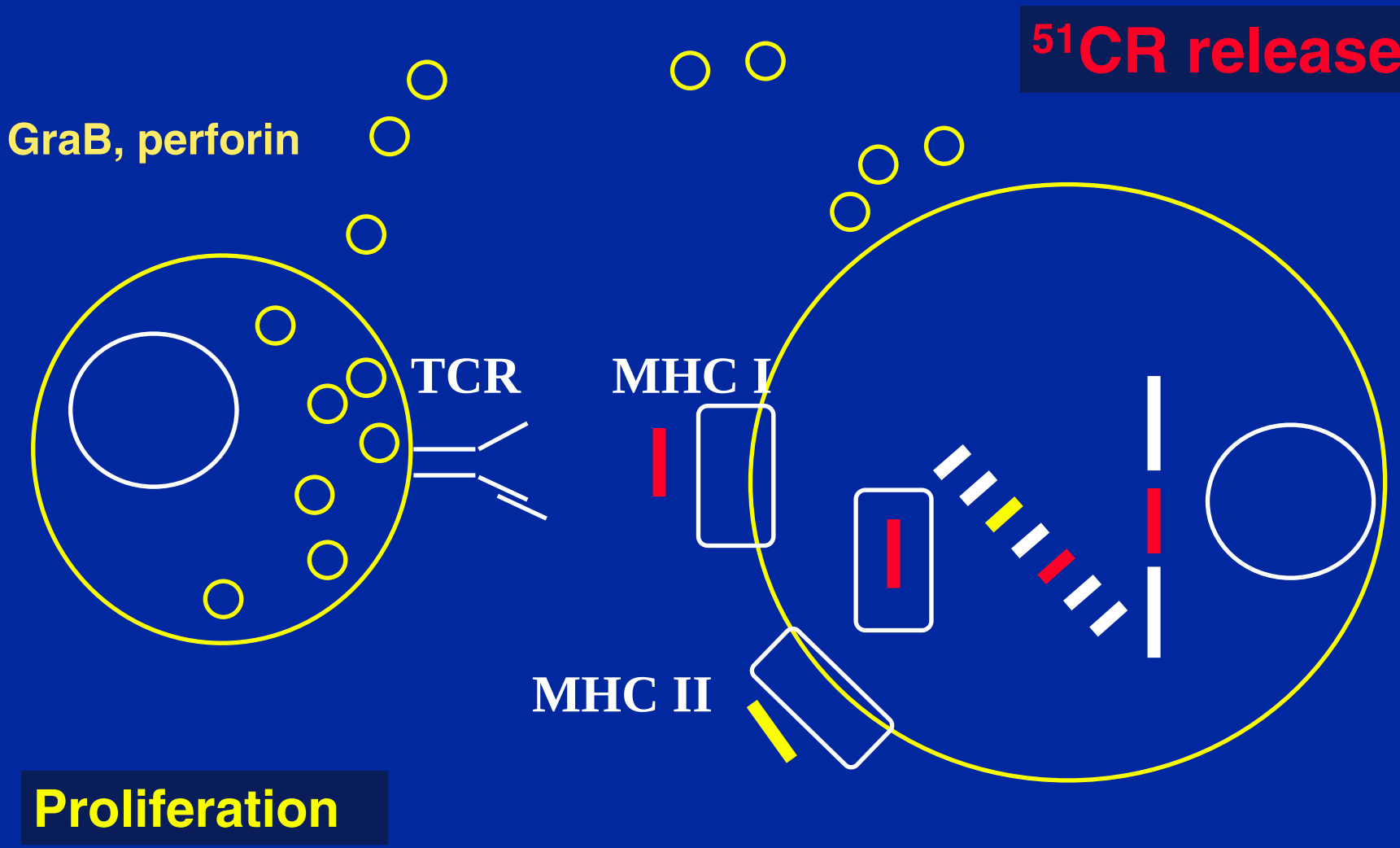
Assessment of functional consequences after exposure of cells to reagents that modify cellular processes (e.g. Ca flux, proliferation, activation, release of cytotoxic granules, apoptosis, signal transduction).

Level 3

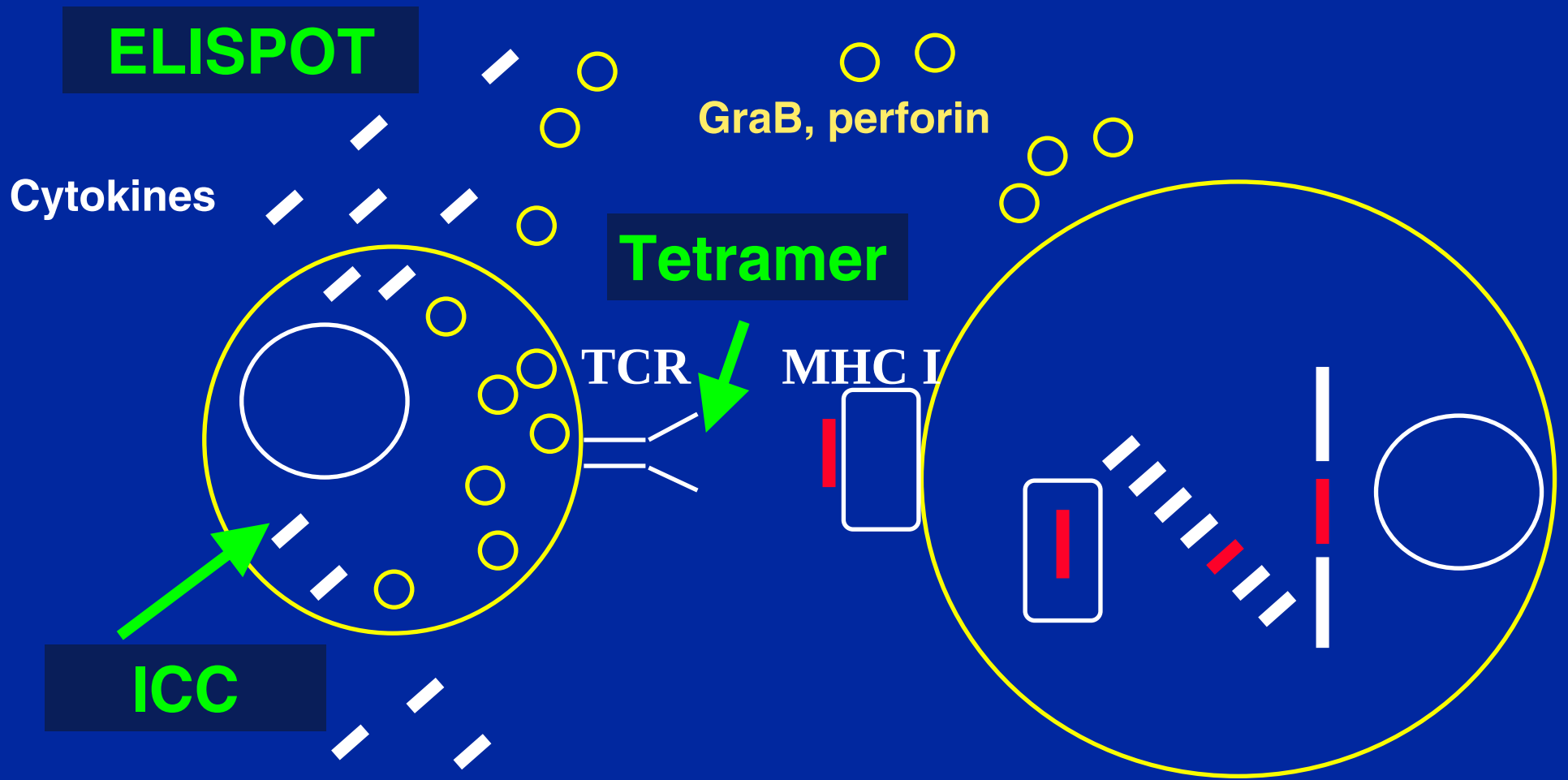
Cell-cell interactions, such as immune-tumor cell interactions, including cytotoxicity, can be assessed and classified as cell-cell engagement, target-cell apoptosis, and necrosis.

T cells

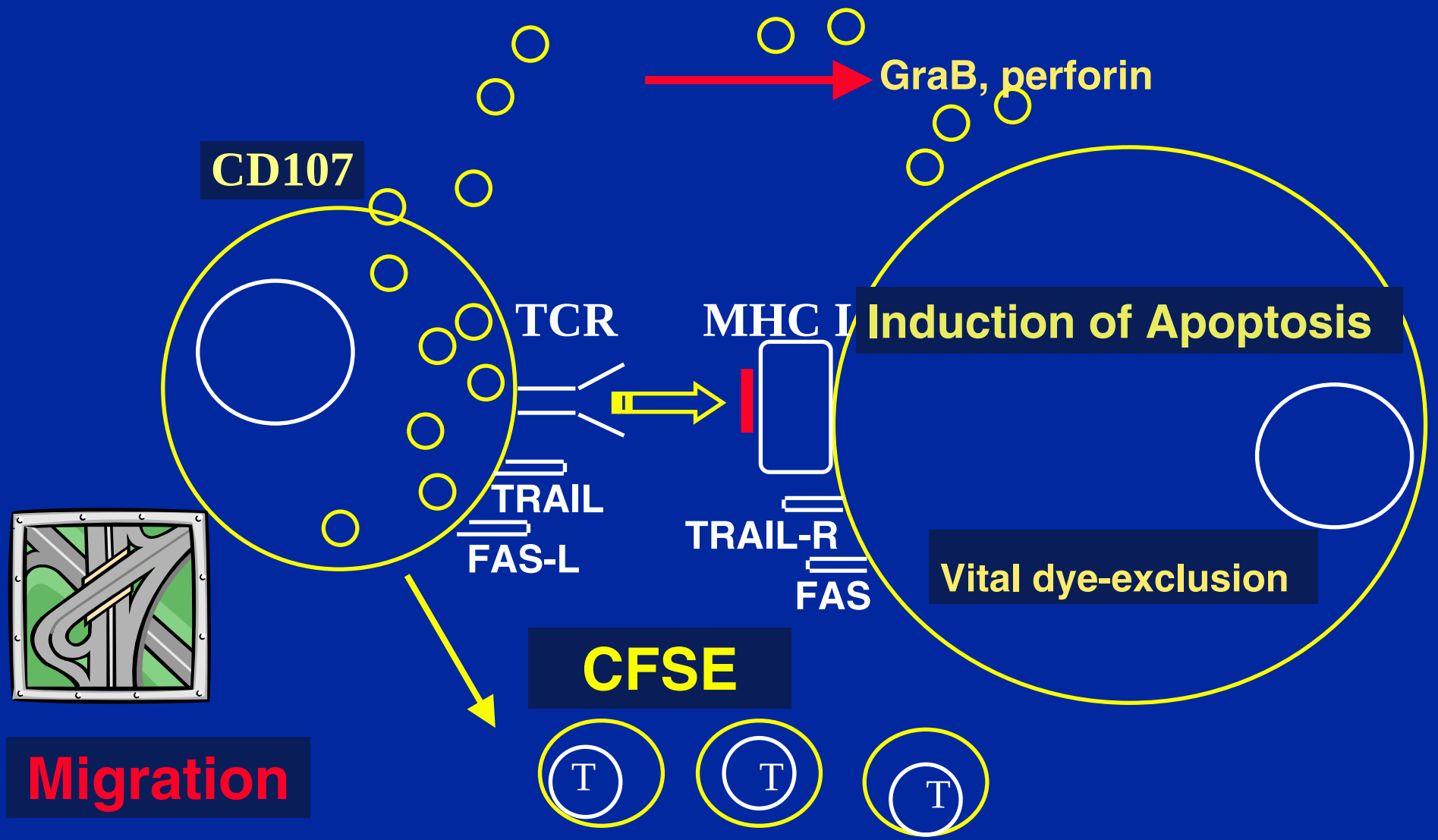
1st generation T cell assays: low sensitivity



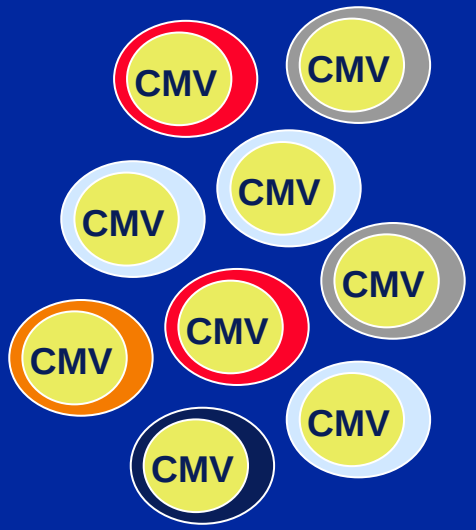
2nd generation T cell assays: „ex vivo“ quantification

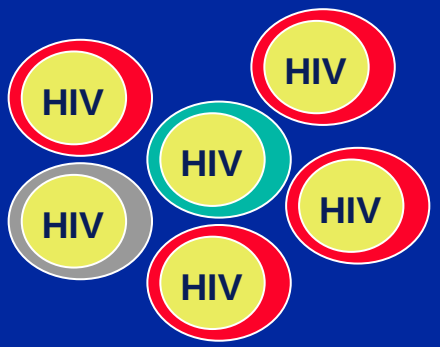


3rd generation T cell assays: specific T cell functions



Example: Antigen-specific T cell responses





Cytokine profile?

IFNγ vs. IL-2, TGFβ, IL-10, etc.

Memory/effector markers?

CD27, CD28, CD45RA, etc.

Functional avidity?

Response to limiting Ag conc.

Degranulation capacity?

CD107a+b

Presence of anergic cells?

Tetramer vs. cytokine

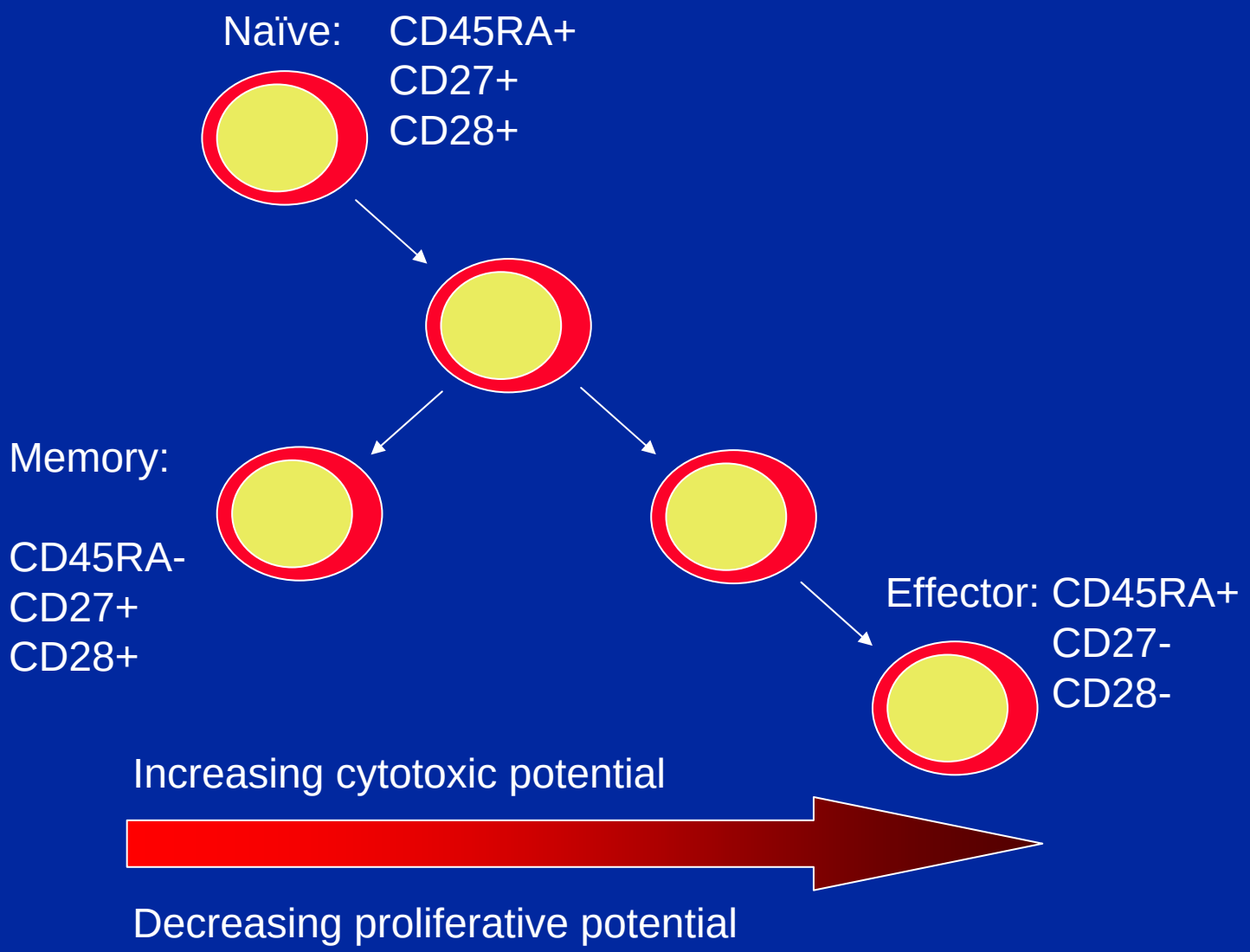
Functional characterization of vaccine-induced T cells by flow cytometry

- Differentiation subsets (effector/memory)
- Cytotoxic potential
- Proliferative capacitiy
- Type 1/type 2 T cells
- Migratory potential

Functional characterization of vaccine-induced T cells

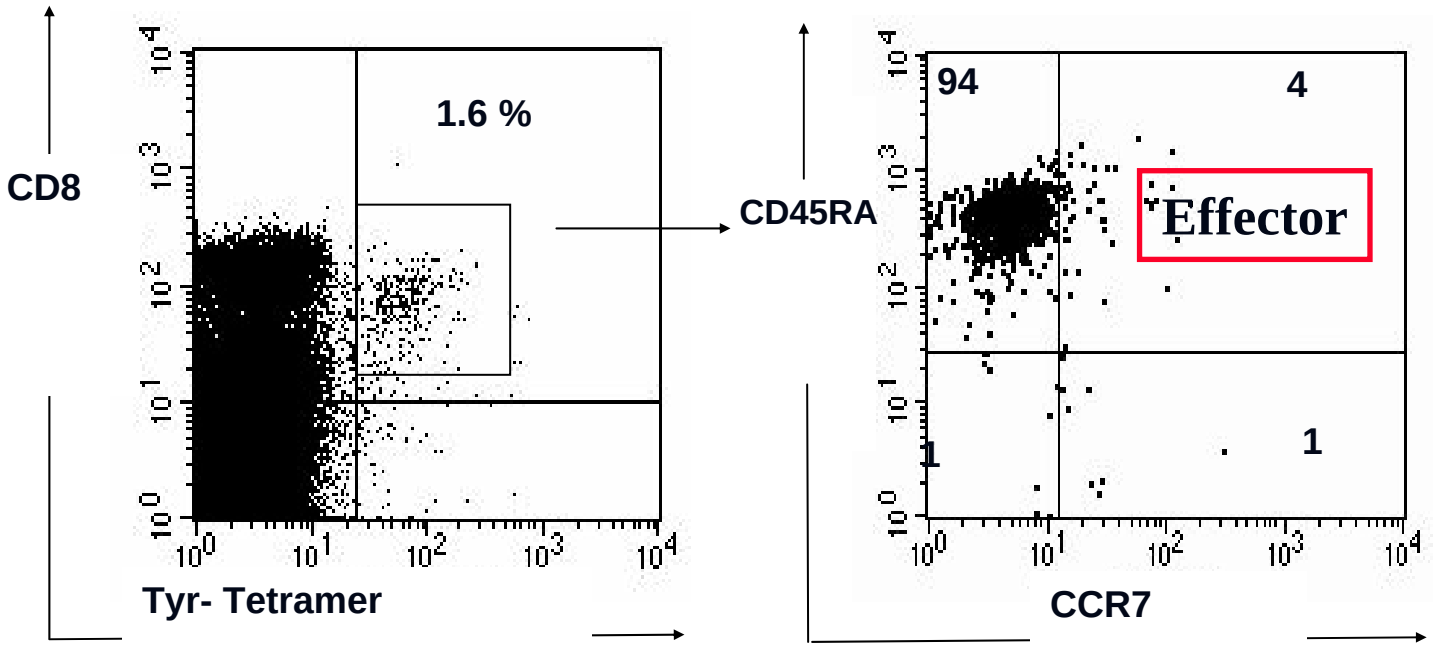
- T cell differentiation subsets

Simplified CD8+ T Cell Differentiation



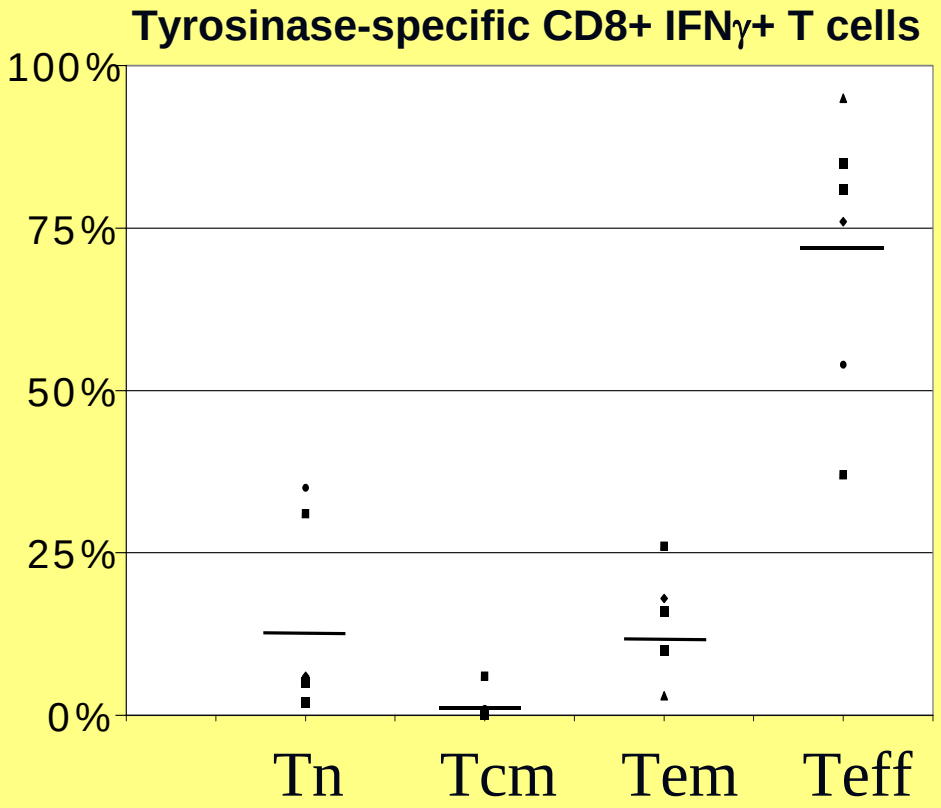
Differentiation subset of tyrosinase-specific T cells in peripheral blood following vaccination

C Pat 2: peripheral blood- tetramer

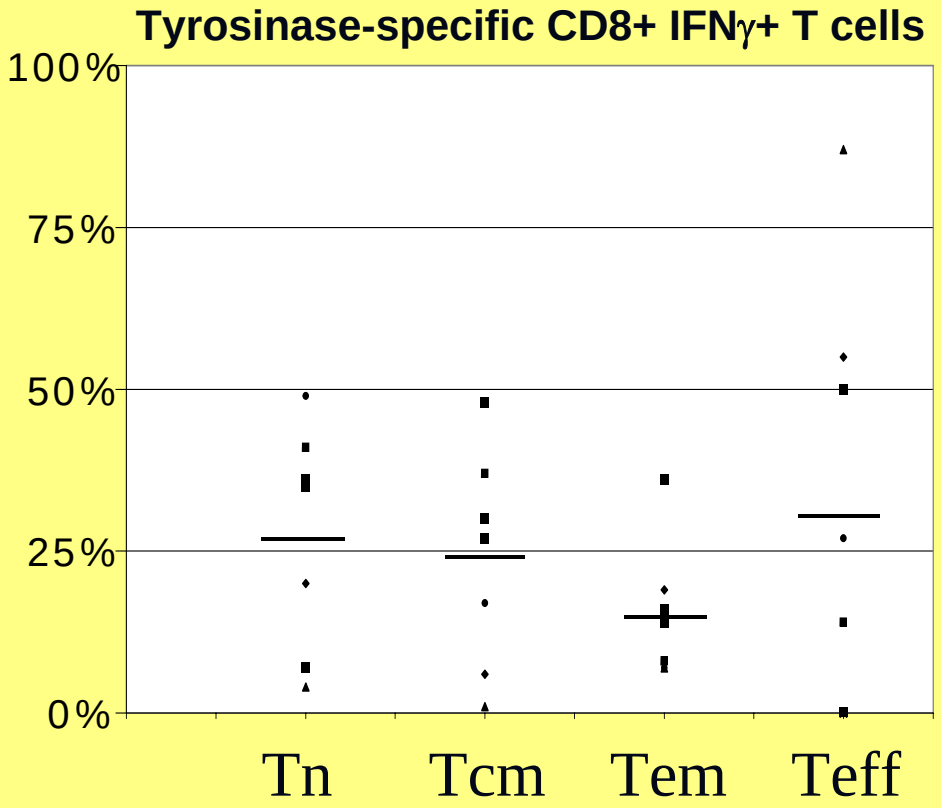


Vaccine-induced tyrosinase-specific central memory T cells reside in bone marrow

Peripheral blood



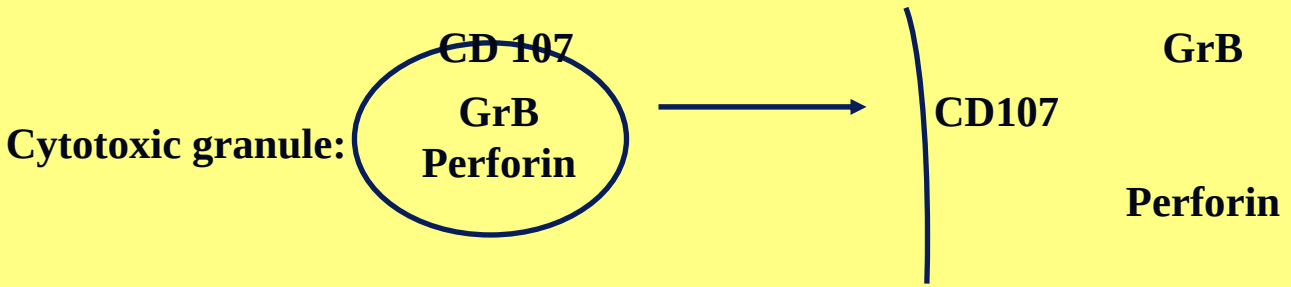
Bone marrow



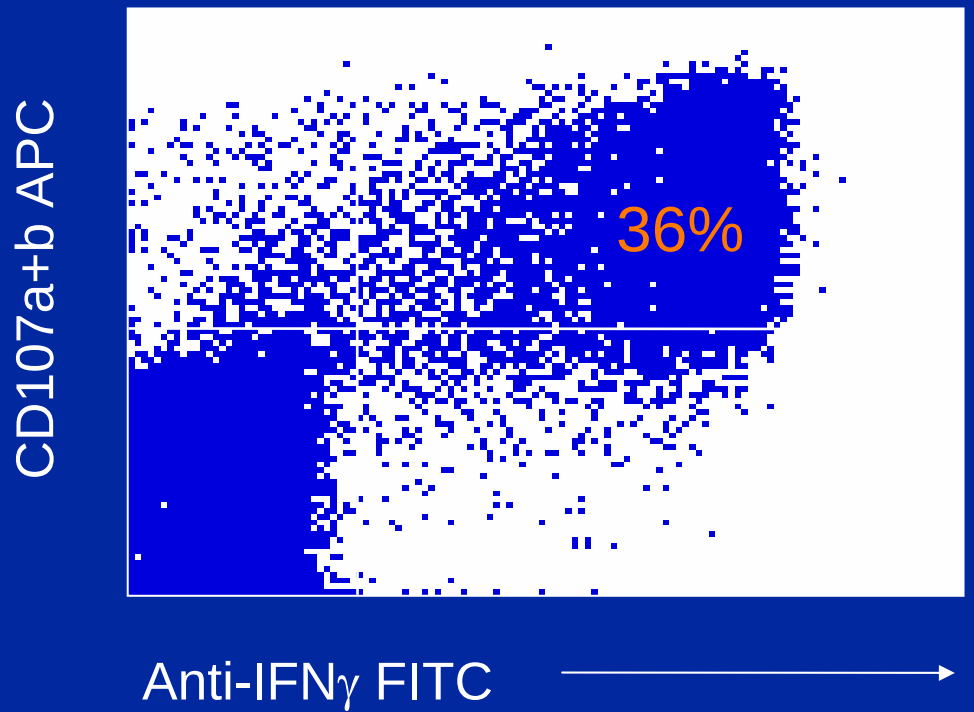
Functional characterization of vaccine-induced T cells

- Cytotoxic potential

- granzyme B/perforin
- CD107 mobilization (Betts MR, JIM, 2003)



CD107 : A new marker for CTL

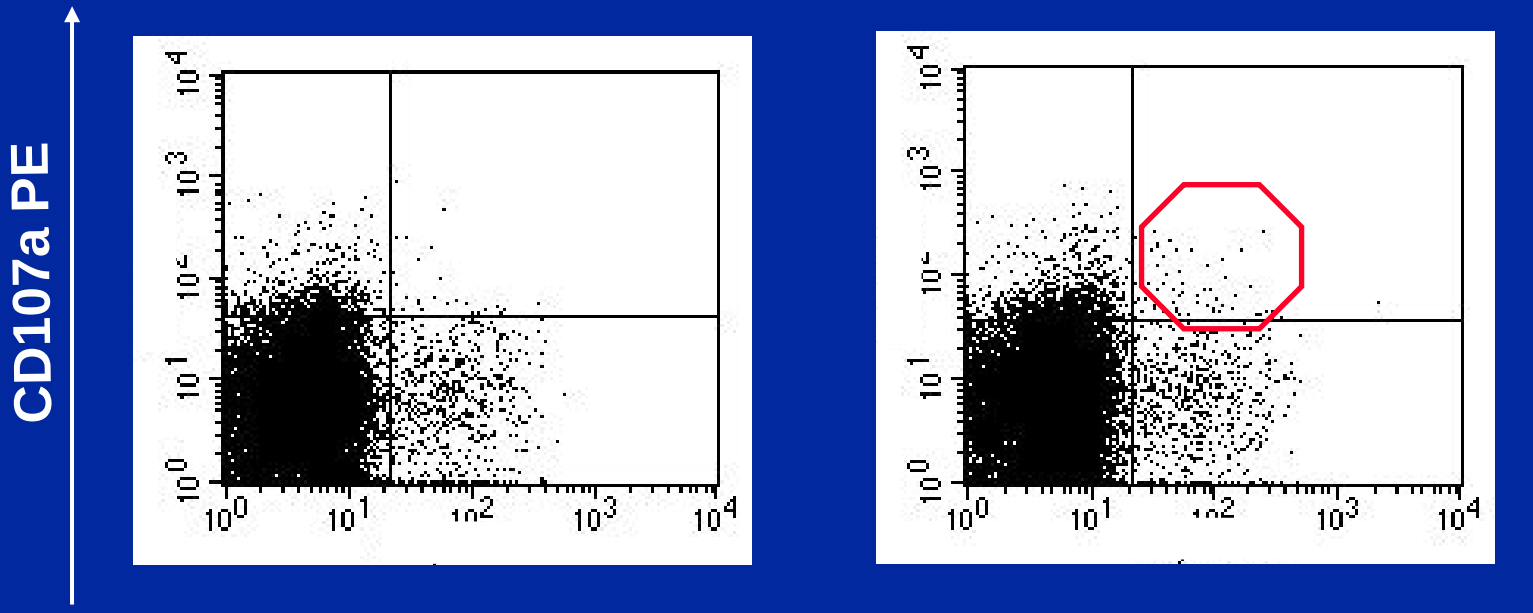


SEB-activated PBMC

A subpopulation of tyrosinase-specific T cells mobilizes the cytotoxic membrane protein CD107

+ irrelevant peptide

+ Tyrosinase.368-376 peptide



→ However, degranulation occurs in the absence of cytotoxicity!
(Wolint P, JEM, 2004)

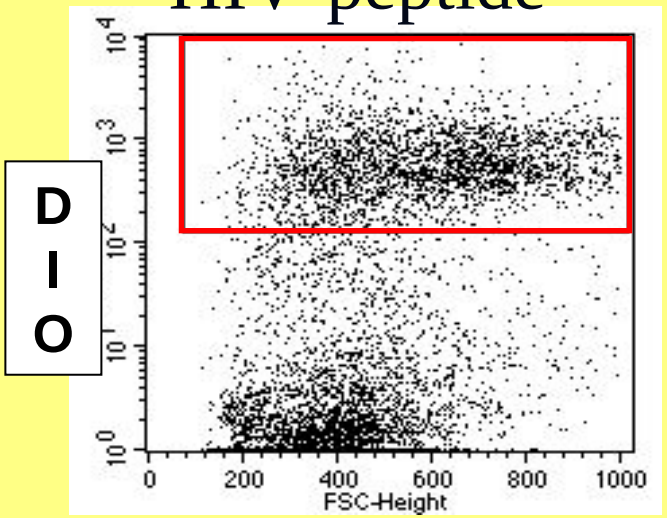
Functional characterization of vaccine-induced T cells

- **Cytotoxicity**

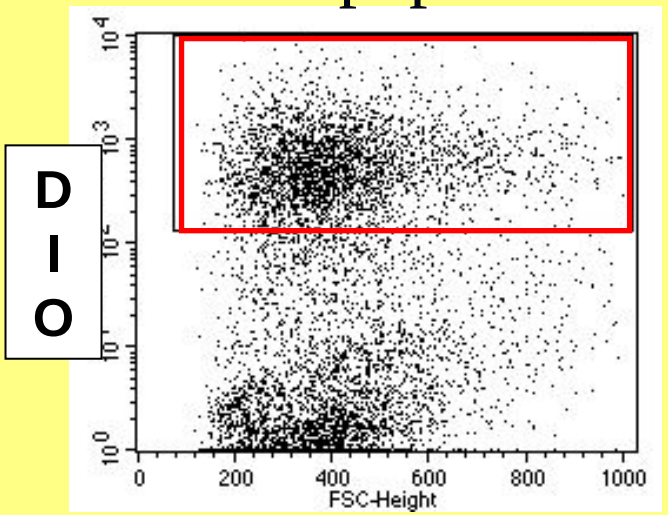
- **necrosis (propidium iodide)**
- **apoptosis (Annexin V, anti-caspase)**

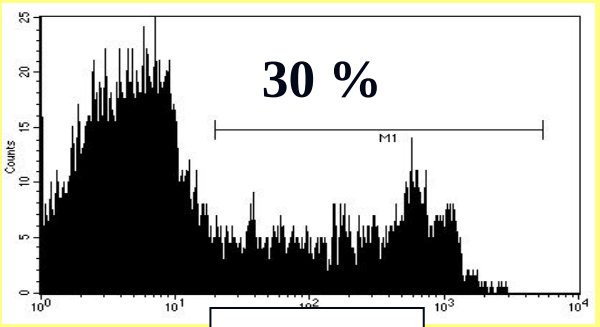
Propidium iodide assay with specific CTL

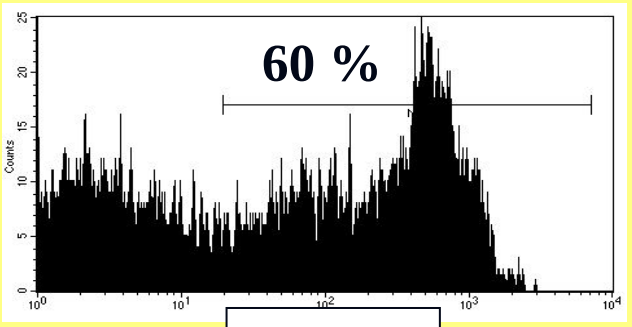
HIV-peptide



FLU-peptide







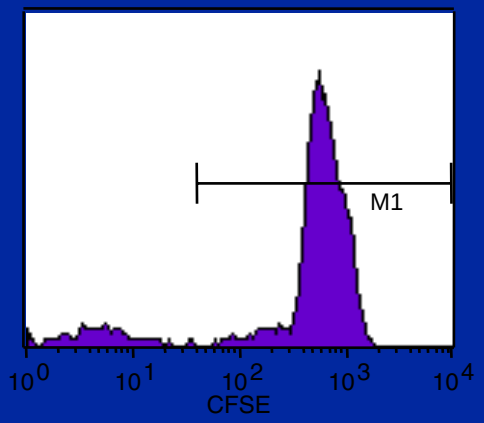
Flow Cytometric Techniques for Characterizing Proliferating T Cells

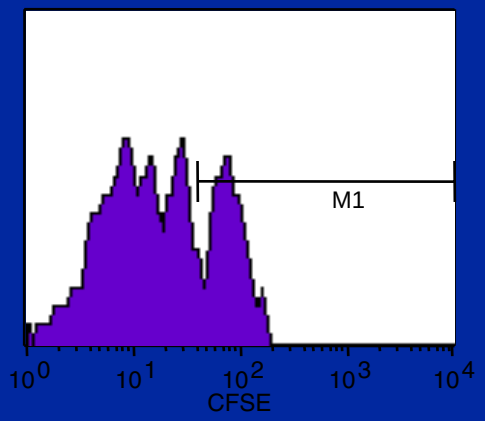
Ex-vivo	in-vitro
	CFSE
	BrdU
Ki-67	Ki-67

Multi-color Combinations

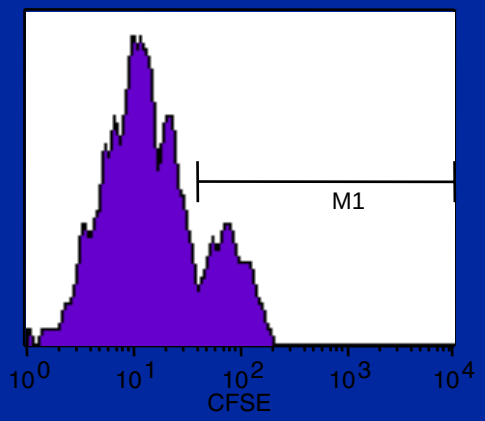
CFSE Example - anti-CD3 Stimulation

Day 0 ⟶ Day 4 - IL-4$^+$ cells



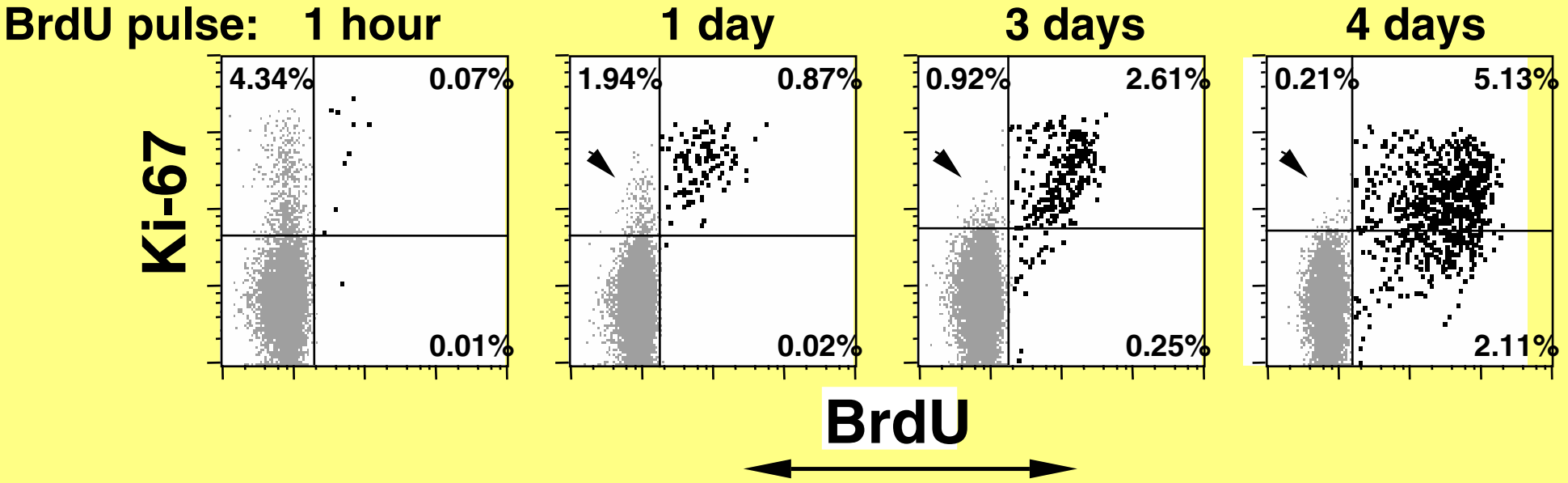


No CD81 costim.



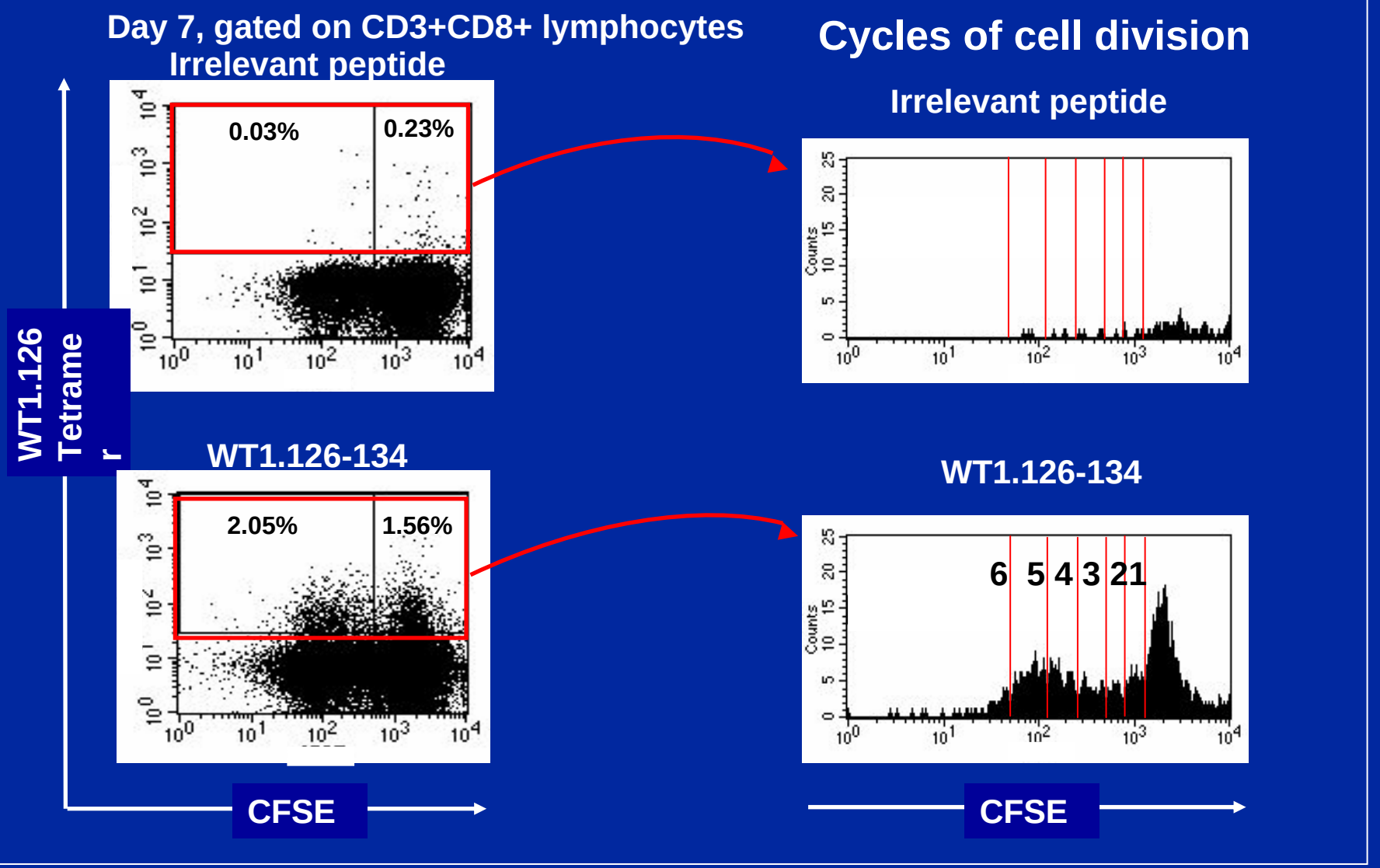
+ CD81 costim.

Ki-67 Expression: Correlation With BrdU Labeling Time



In-vivo administration of BrdU, monkey, SIV-gag restim. in vitro

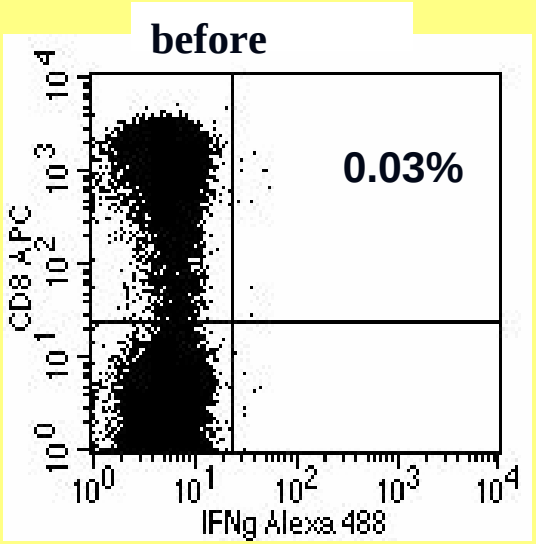
WT1.126-specific, vaccine-induced PB T cells proliferate in response to IL-2, IL-7 and WT1.126-134

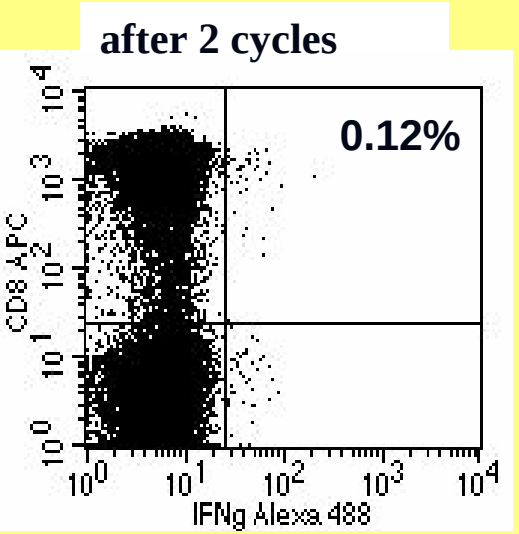


Functional characterization of vaccine-induced T cells

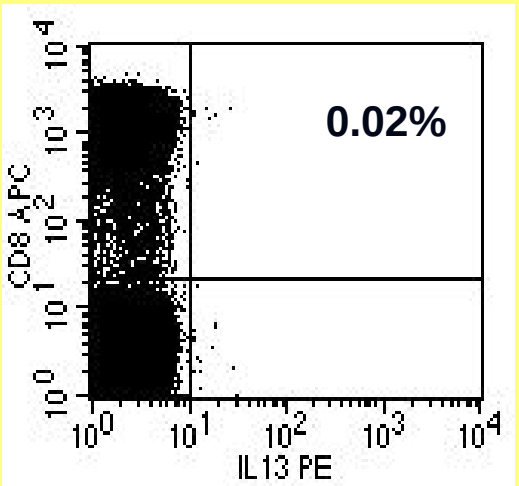
- Type 1/type 2 T cells

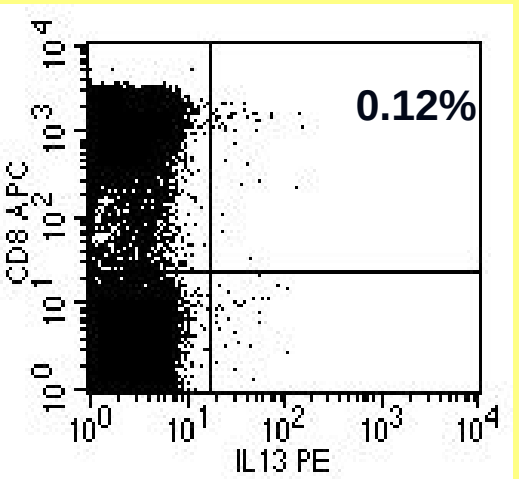
Type 1/type 2 T cell response to tyrosinase A2-peptide in 2 melanoma patients before and after treatment with IL-2





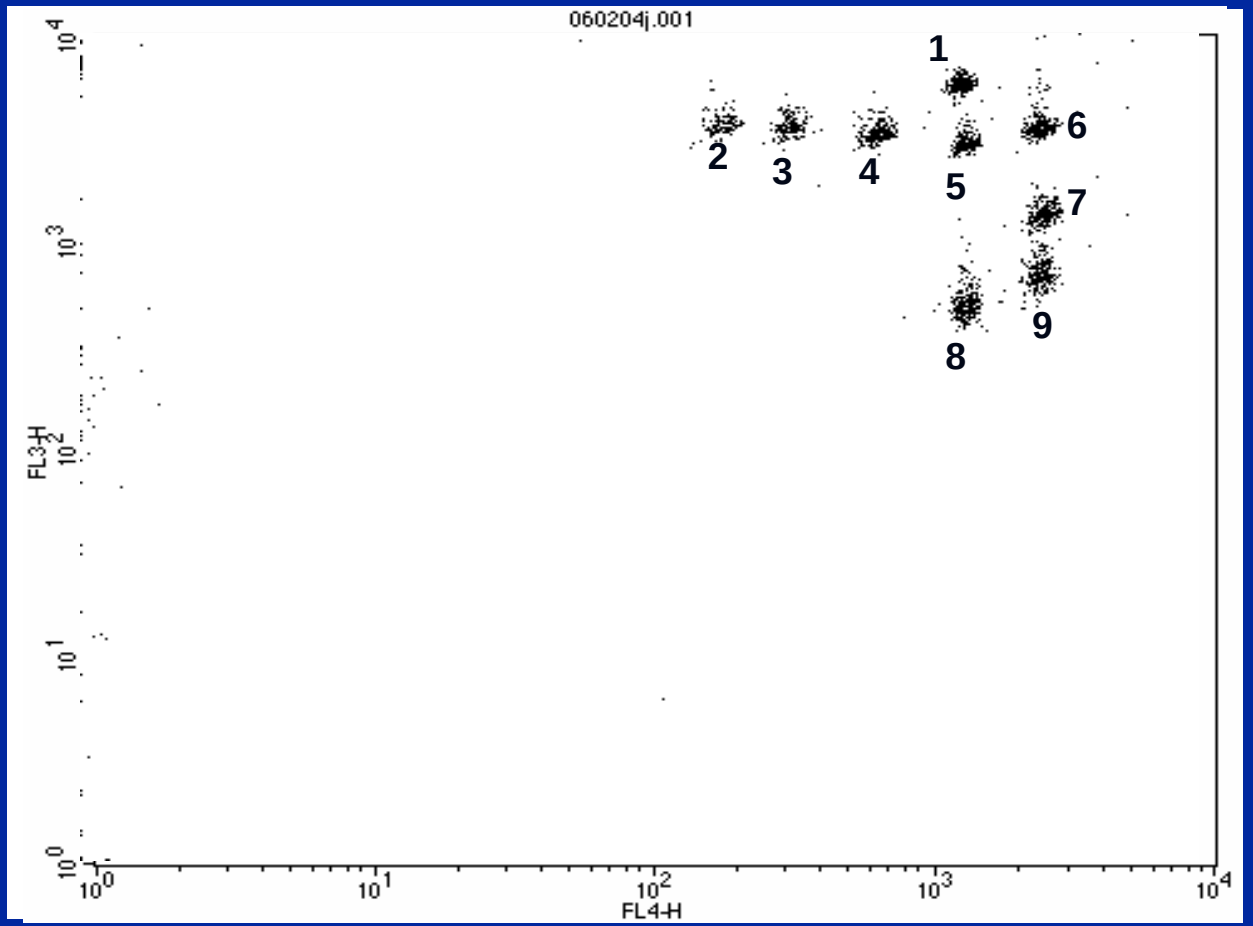
IFNγ - FITC





IL-13 - PE

9-Plex bead assay for detecting P-proteins in activated T cells



1. Itk (Y511)
2. ERK (T202/Y204)
3. JNK (T183/Y185)
4. P38 (T180/Y182)
5. PLCγ (Y783)
6. ZAP70 (Y319)
7. LAT (Y171)
8. c-Jun (S63)
9. RSK (S380)

Functional characterization of vaccine-induced T cells

Therapeutic vaccination:

- **ability to migrate into the tumor**

Adjuvant vaccination:

- **ability to migrate into many compartments**

Flow cytometry facilitates direct assessment of the functional characteristics of vaccine-induced T cells including:

- **production of type 1/type 2 cytokines**
- **Migratory potential**
- **Cytotoxic potential**
- **proliferative capacitiy**
- **differentiation into distinct T cell subsets**

Correlation with clinical efficacy?

Differences between various vaccines and adjuvants?

Tumor cells

Tumor cells

- Apoptosis induction
- Signal trunsduction pathways
- Antigen presentation machinery
- Migratory potential

Low Frequency Measurement and Validation of T Cell and Tumor Cell Characteristics in Vaccine Trials

Areas to cover in breakout session

Technical issues

- Sample Prep/Processing
- Analysis Methods
- Determining Assay Performance
- Management of data from multiple immune assays

Low Frequency Measurement and Validation of T Cell and Tumor Cell Characteristics in Vaccine Trials

Areas to cover in breakout session

Application

- Prioritize assays per question
- Judge assay development stage
- Compile examples of successful applications and reasons for failures