Cancer Biometrics: Results of the 2003 iSBTc Workshop

Theresa L. Whiteside, Ph.D., ABMLI

University of Pittsburgh Cancer Institute

The workshop objective

The objective was to consider state-of-the-art approaches to the identification of biomarkers and surrogate markers of tumor burden with the emphasis on assays in the blood, lymph nodes and within the tumor itself

Surrogate end-points

- Definition: end-points other than overall survival used to make conclusions or predictions about cancer progression/regression or responses to therapy

- Disease related:
  - Histologic markers: dysplasia, hyperplasia, CIS, tumor stages
  - Serum markers: CEA, PSA, CA125, etc
  - RR, TTP
- Mechanistic (biomarkers):
  - Immunologic
  - Genetic
  - Proteomics-based
  - Molecular
  - Functional

Areas of consideration

- Genomic analysis of cancer *
- RT-PCR for molecular markers of cancer
- Serum/plasma and tumor proteomics*
- Immune polymorphisms*
- High content screening by flow and imaging cytometry
- Immunohistochemistry and tissue microarrays
- Assessment of immune infiltrates and tumor necrosis

Genomics and proteomics in cancer

- Emphasis on high throughput screening/profiling followed by identification
- Recommendations *re* sample acquisition and banking:

  serial samples in order to get a dynamic view

  prospective collections linked to clinical trials

  standardized DNA, RNA amplification

  specimen processing/storage under GLP

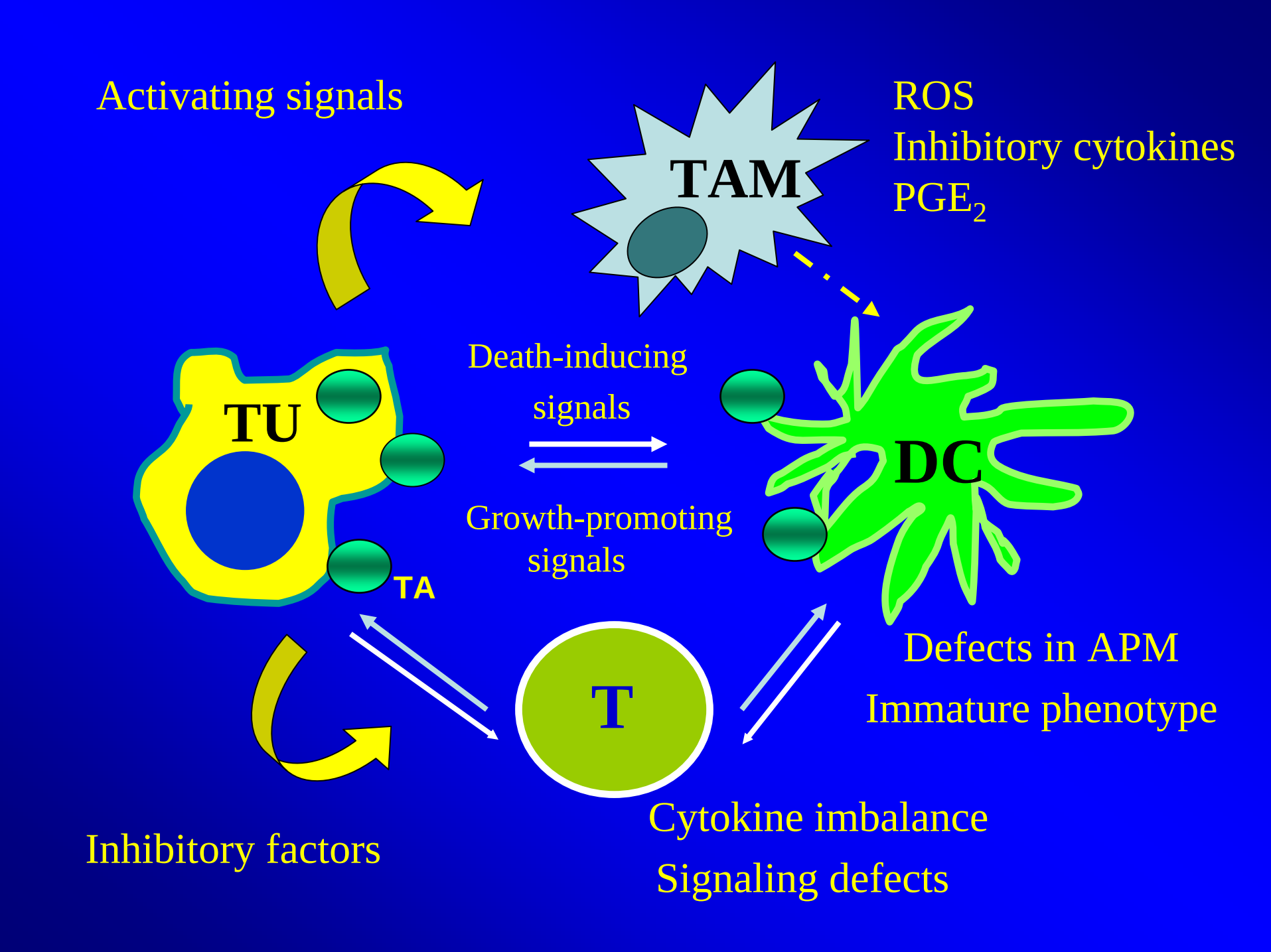
  serum or plasma for proteomics??

RT-PCR for detection of circulating tumor cells (CTC)

- Objective is to get “molecular footprint” of cancer in blood, LN, BM
- Need to have a marker gene for each tumor type
- Need RT-PCR for sensitivity (1-10 CTC/10^6 lymphocytes)
- Sample processing (whole blood vs. PBMC)
- Immunomagnetic bead enrichment in epithelial cells
- Emphasis on CTC validation vs. disease stages, recurrence, prognosis and survival to confirm clinical usefulness

High-content screening by flow or imaging cytometry to follow changes in immune cells

- Intimate and unique relationship of cancer and the host immune system
- How does tumor affect phenotype/ functions of immune cells?
- If tumor induces detectable alterations in phenotype/functions of immune cells, could we use these as biomarkers or surrogate endpoints?


Activating signals
TAM
ROS
Inhibitory cytokines
PGE_2
TU
TA
Death-inducing signals
Growth-promoting signals
DC
T
Defects in APM
Immature phenotype
Inhibitory factors
Cytokine imbalance
Signaling defects

What to measure, how and where?

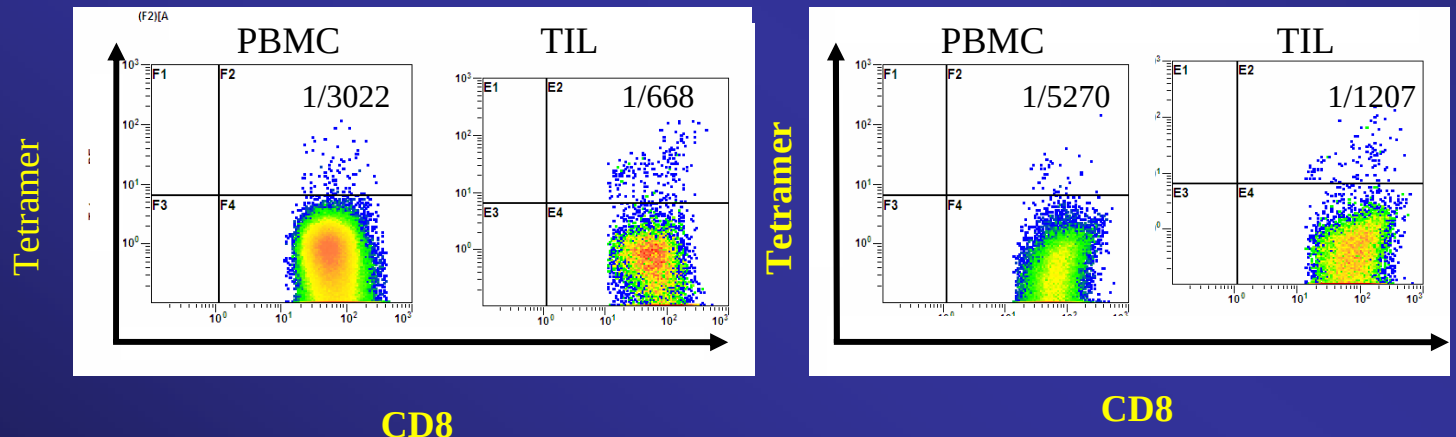
- Tumor site vs. blood vs. LN
- Selection of the immune cell type which is altered in marker expression, signaling, migration, cytokine production, etc in a tumor-bearing host
- Choice of methods (screening vs. confirmatory) that are robust but simple to use in correlative studies to determine clinical usefulness of the selected cancer biomarker

Frequencies of CD8+tetramer+ T cells in PBMC and TIL of patients with head and neck cancer

gated on CD3 and CD8

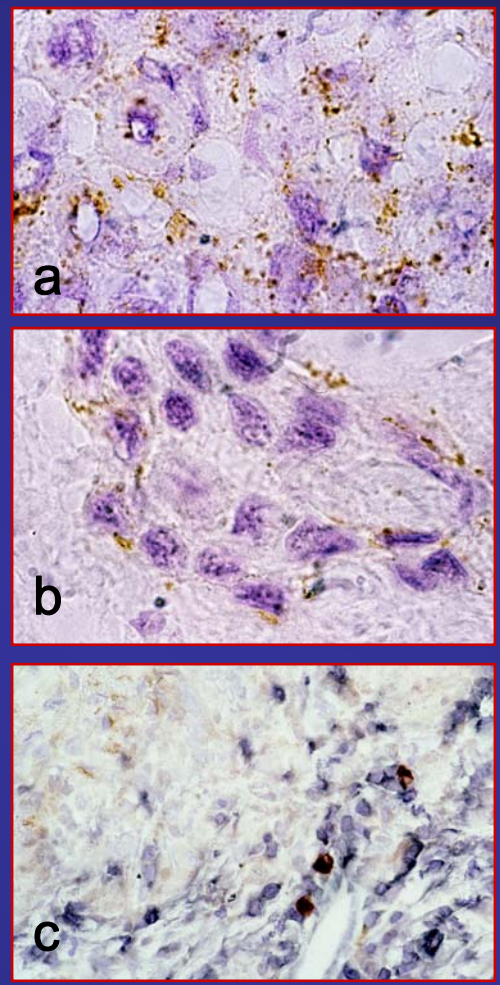
p53 tetramer$_{149\text{-}159}$ p53 tetramer$_{264\text{-}272}$

Patient # 1: Tetramer frequency



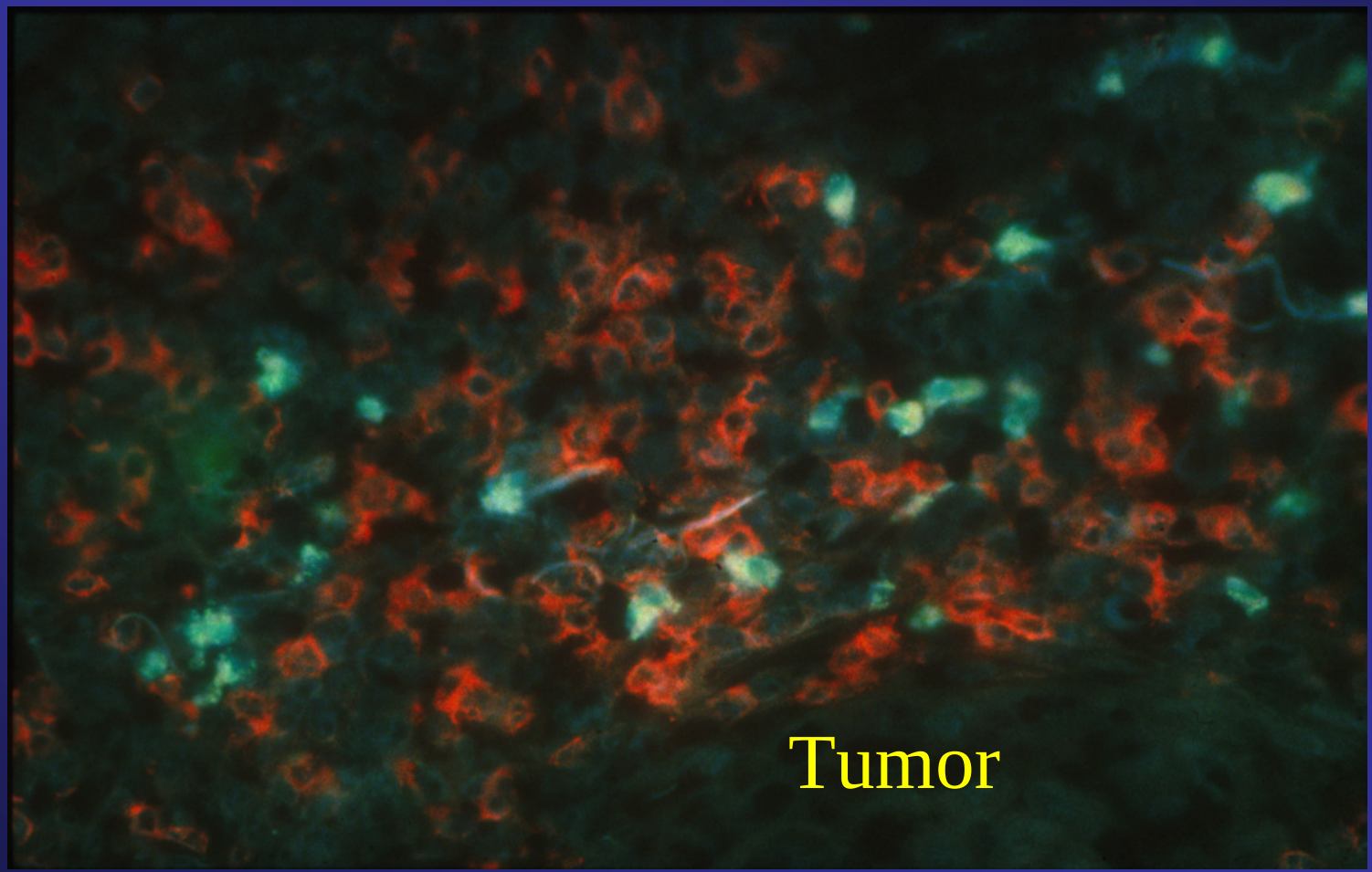
Fas-L expression on the tumor and TIL apoptosis

- Fas-L expression was seen on all tumors
  - high 17/28
  - Low 11/28
- High expression of Fas-L was associated with
  - Apoptosis in TIL
  - Reduced ζ expression in TIL



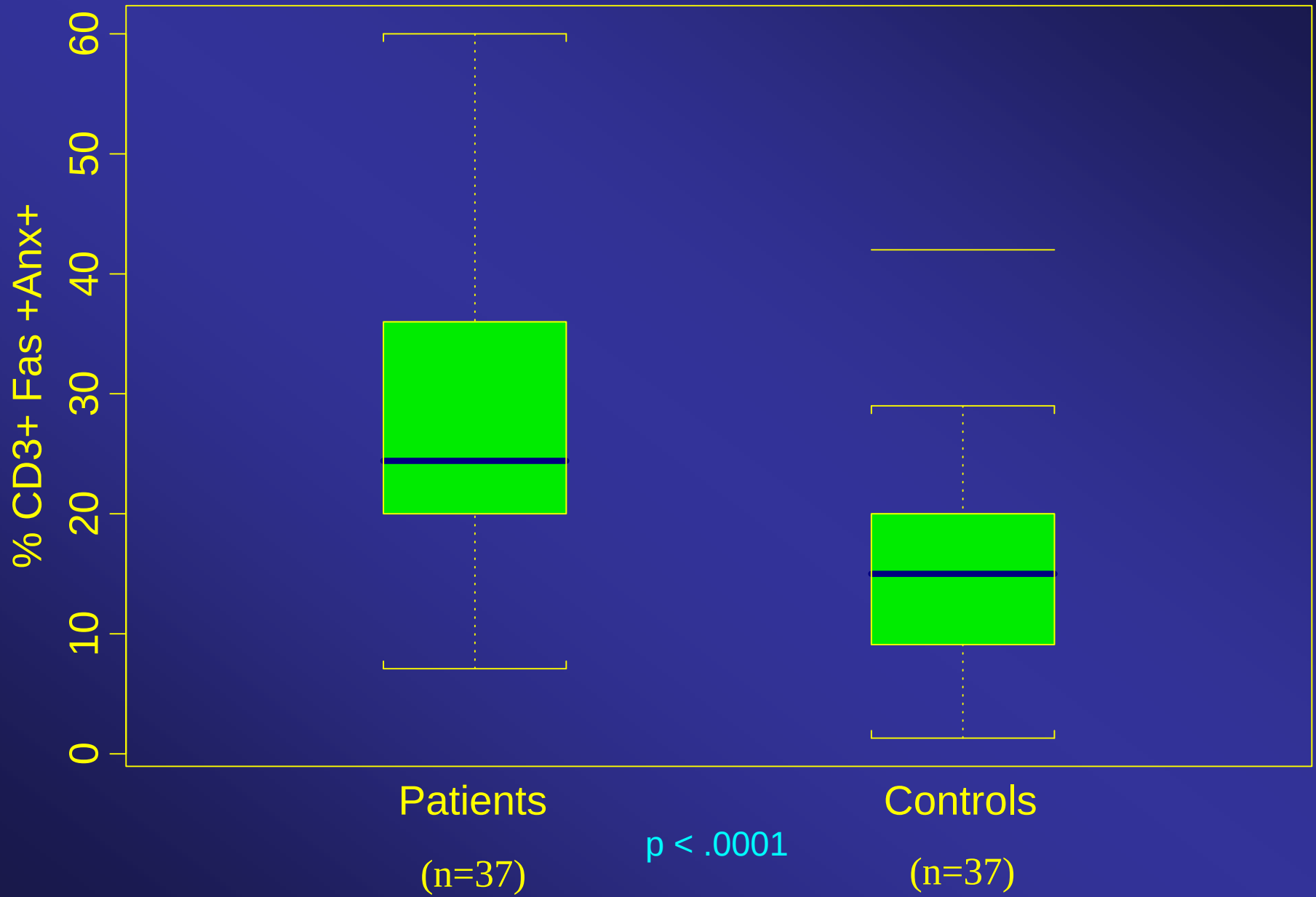
Reichert et al, Clin. Cancer Res.8: 3137,2002

Apoptotic CD8+ T cells in the nest of lymphocytes at the tumor site

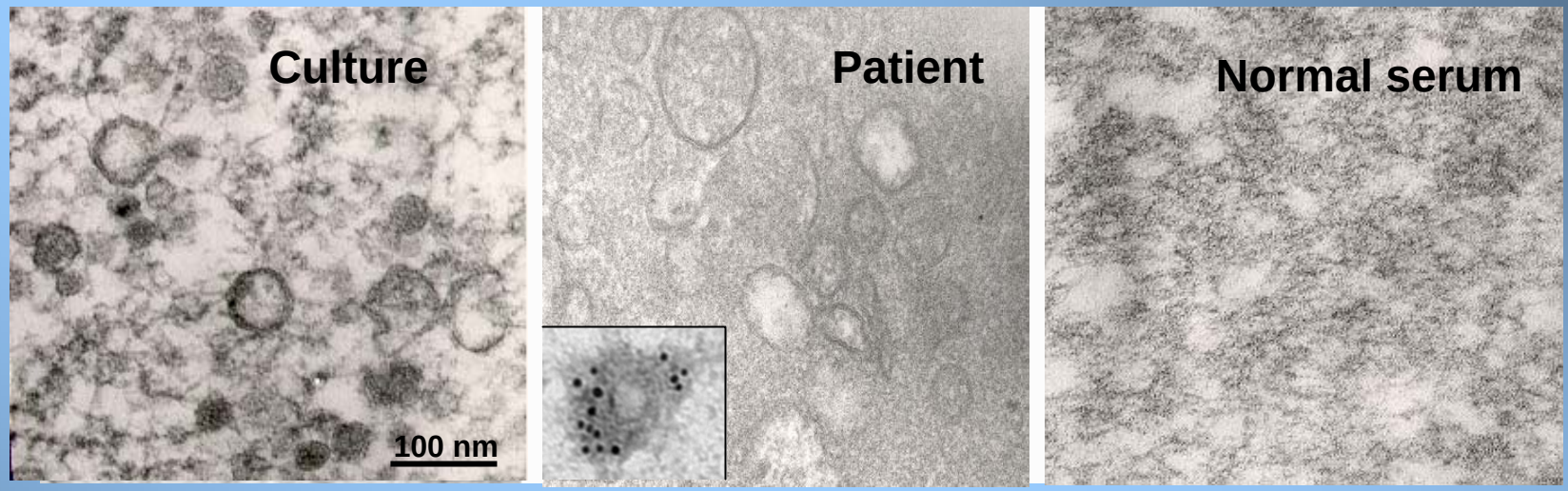


Red = alive CD8+ T cells Blue = dying CD8+ T cells

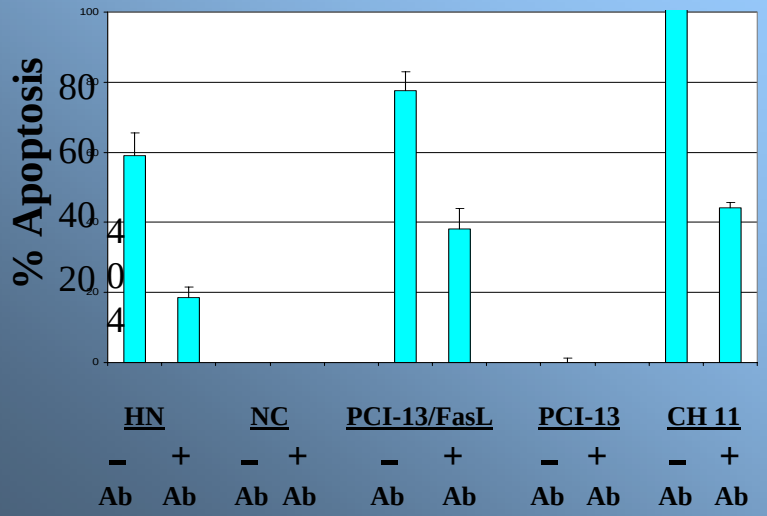
Circulating CD3+Fas+Annexin+ cells in patients with HNC and controls

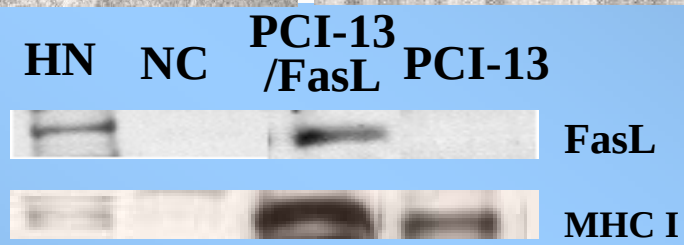


Isolation and characteristics of biologically-active MV in the sera of patients with HNC



Blocking with anti-Fas Ab





MV from patients' sera contain FasL (42 kDa) and HLA class I molecules and induce Jurkat cell apoptosis

Association of MV containing high, low or no FasL with disease in 27 SCCHN patients

MV/FasL vs T stage

	T1	T2	T3	T4
High FasL	4	0	0	10
Low FasL	1	5	2	5

$p = 0.0094$

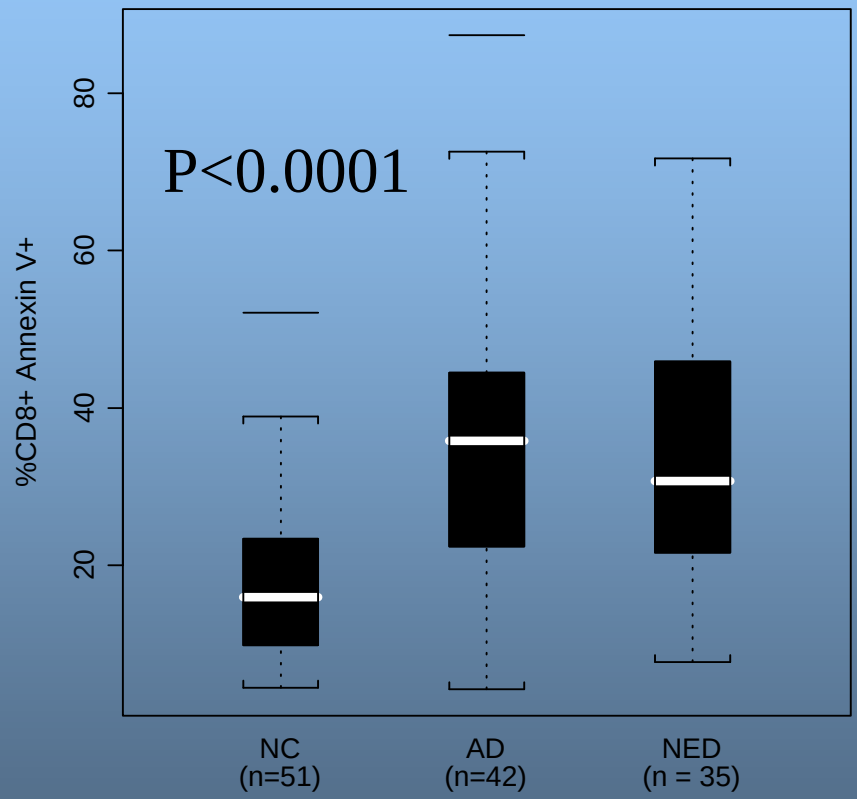
MV/FasL vs N

	N0	N1	N2	N3	**Total**
High FasL	4	3	6	1	**14**
Low FasL	8	1	4	0	**13**

$p < 0.12$

Sera of patients with stage IV disease and + nodes contain MV with the high level of FasL

Annexin V binding to circulating CD8+ T lymphocytes



NC : normal controls

AD : patients with active disease

NED : patients with no evidence of disease

Clinical significance of CD8+ T cell apoptosis in patients with cancer ?

- Discriminates patients with cancer from healthy controls
- Higher in patients with AD vs. those with NED, but not a significant discriminator
- Together with signaling defects (ζ chain) and CD95 expression on T cells, apoptosis correlated with the nodal involvement
- Potentially, could evolve into a marker of tumor aggressiveness or predictor of survival

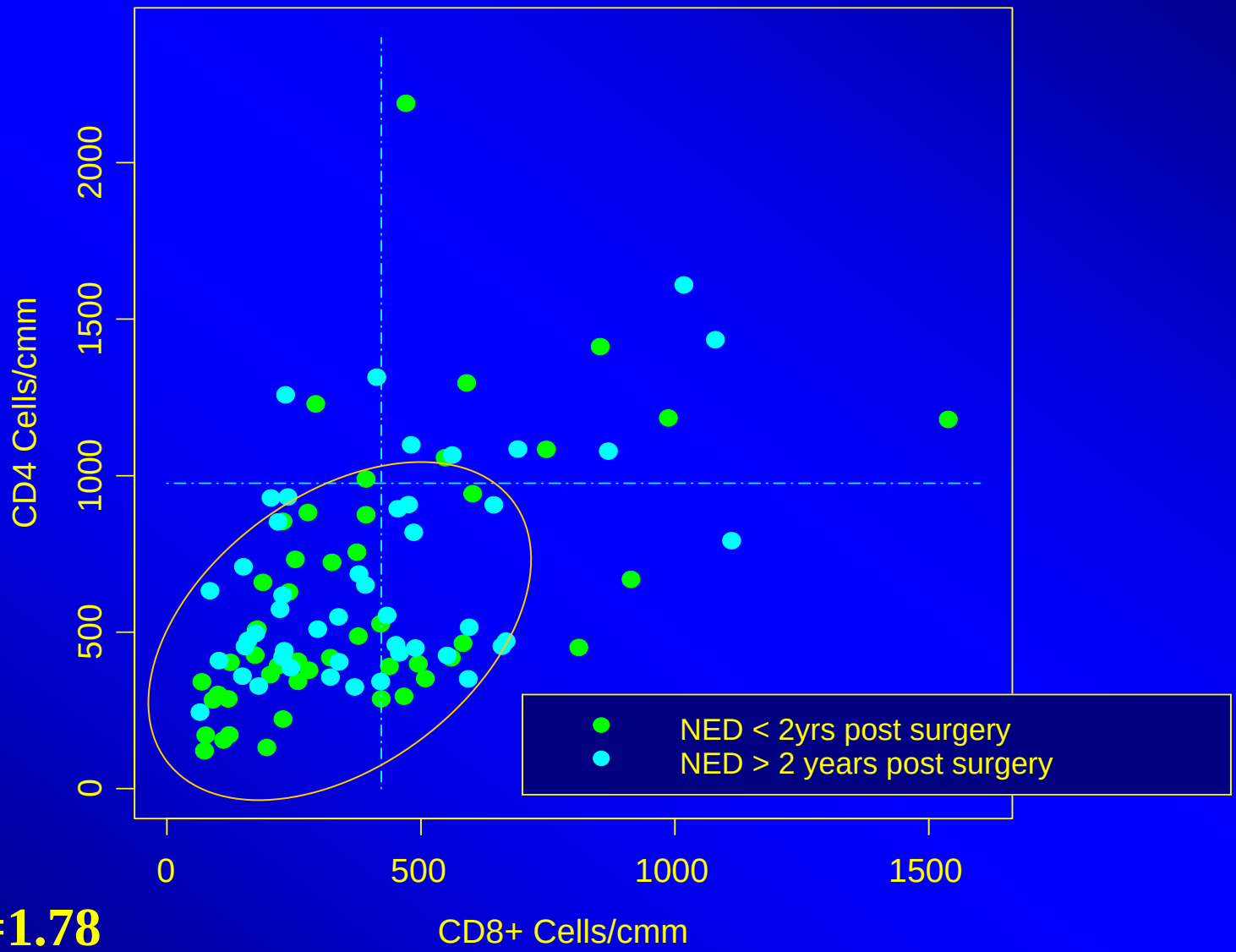
Characteristics that are often altered in circulating T cells

- T-cell absolute numbers
- T-cell subset changes (naïve, memory)
- Expansion of Tregs ($CD4+CD25^{high}$)
- Decreased ζ chain expression
- Increased apoptosis (CD95+, Annexin V+)
- Cytokine profiles
- Memory T-cell functions
- Tumor-specific T-cell responses

Absolute # vs. % (means +/- SD)

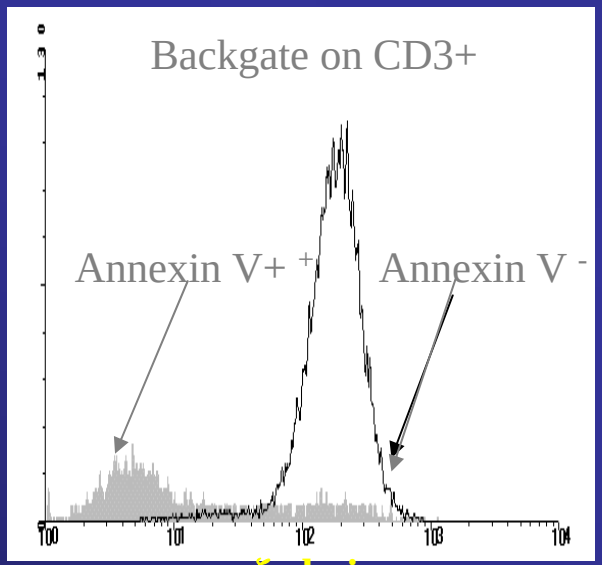
Absolute #		CD3+	CD4+	CD8+
N = 148	Patients	1081 +/- 601	670 +/- 412	392 +/- 269
N = 58	Normal Controls	1512 +/- 494	1005 +/- 360	476 +/- 208
	p value	< .0001	< .0001	.0012
Percentage				
N = 148	Patients	71 +/- 11	44 +/- 11	26 +/- 11
N = 58	Normal Controls	70 +/- 9	47 +/- 9	22 +/- 7
	p value	.6374	.1141	.0917

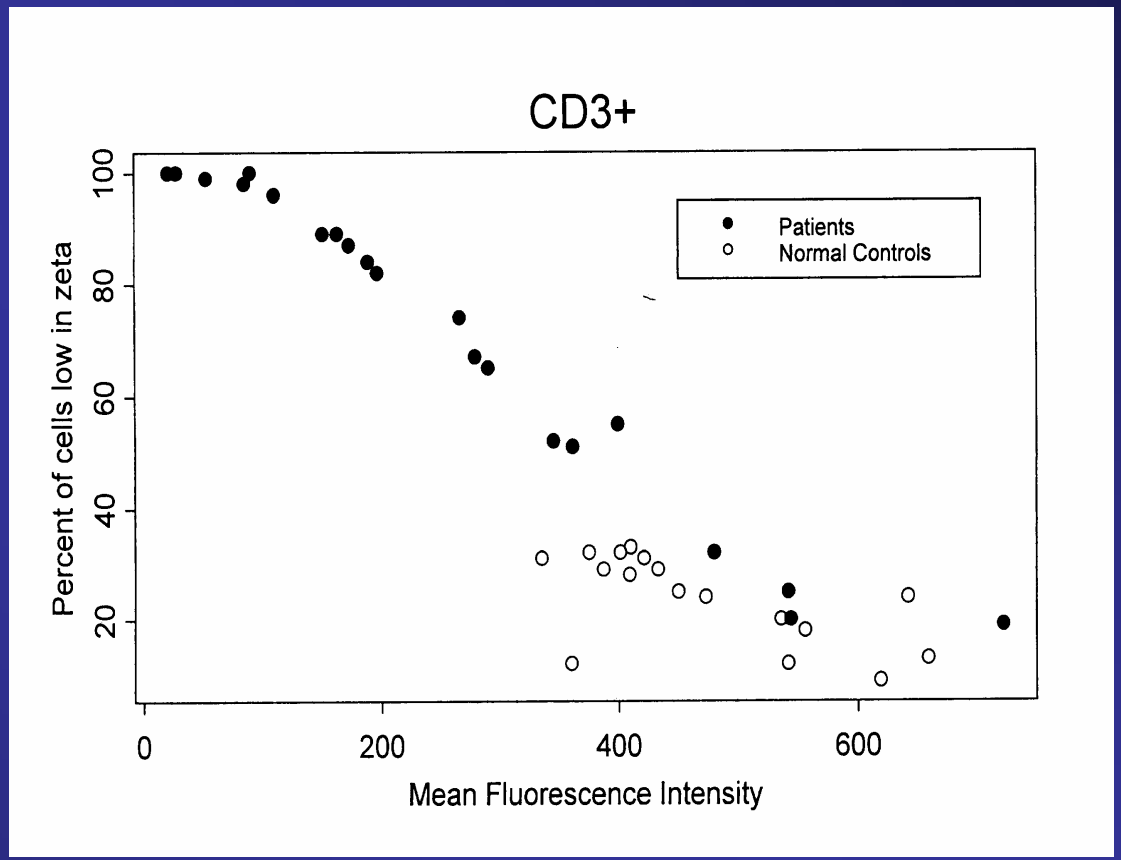
NED patients studied < 2 and >2 years after surgery

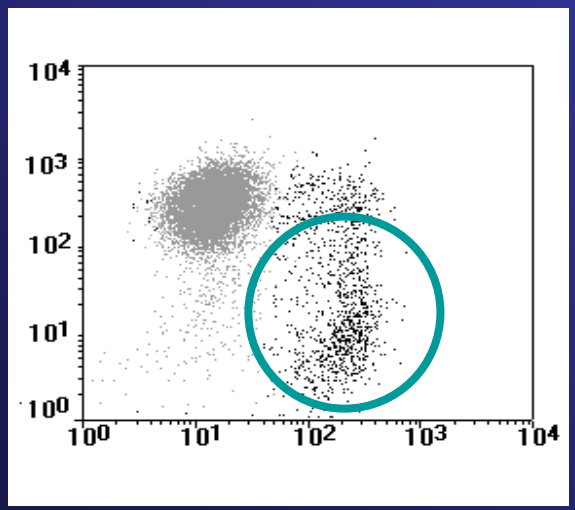


CD4/CD8=1.78

Decreased expression of ζ in Annexin+CD3+T cells in the peripheral circulation of patients with melanoma





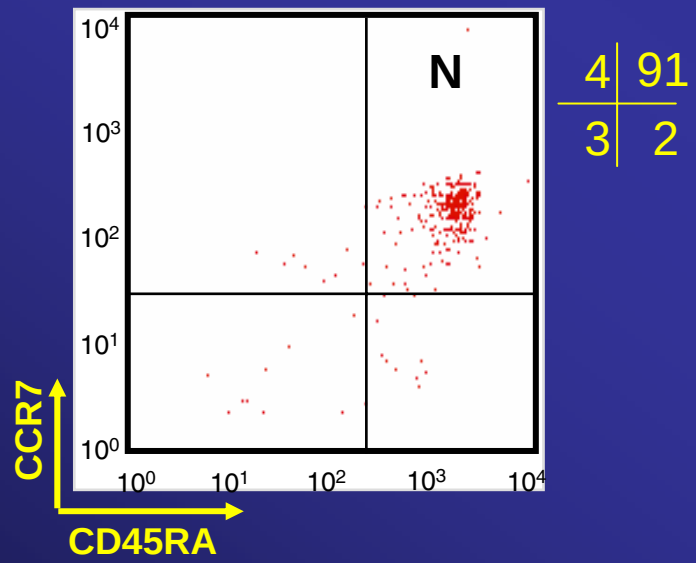


The % of cells positive for ζ vs. MFI for ζ in CD3+ T lymphocytes of the patients and normal controls

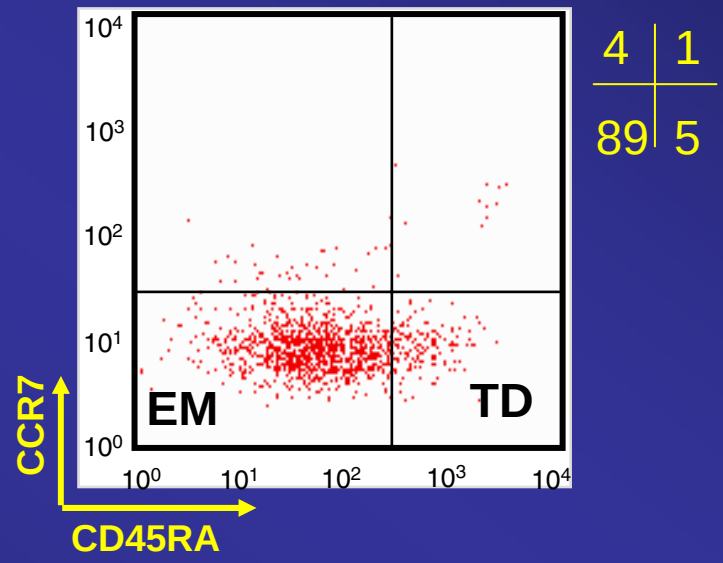
Characterization of Activation/Differentiation Status of Tetramer Stained Cells (MART 1)

Gated on tetramer MART 1^+ $CD8^+$ cells

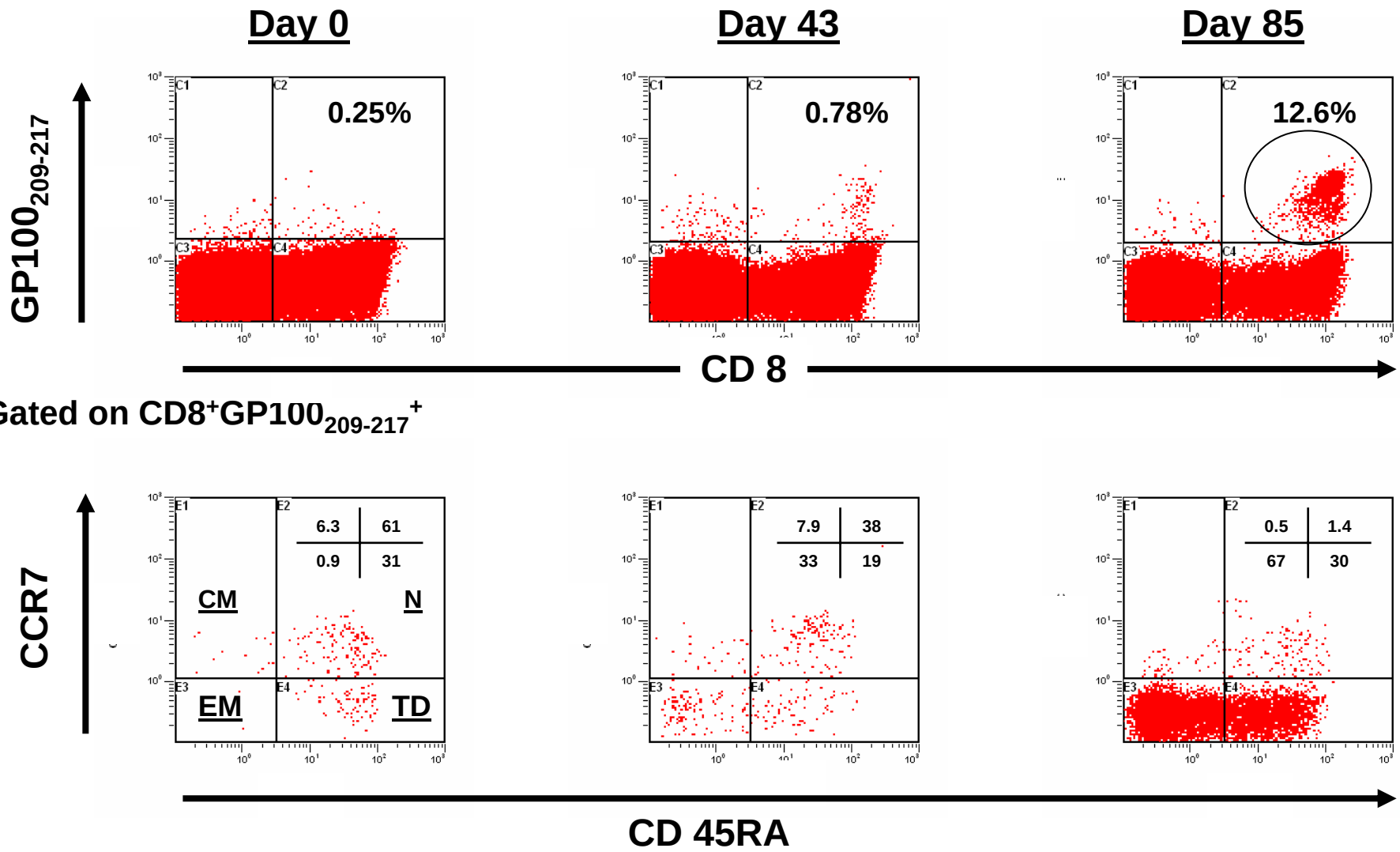
Healthy Donor



Melanoma Patient



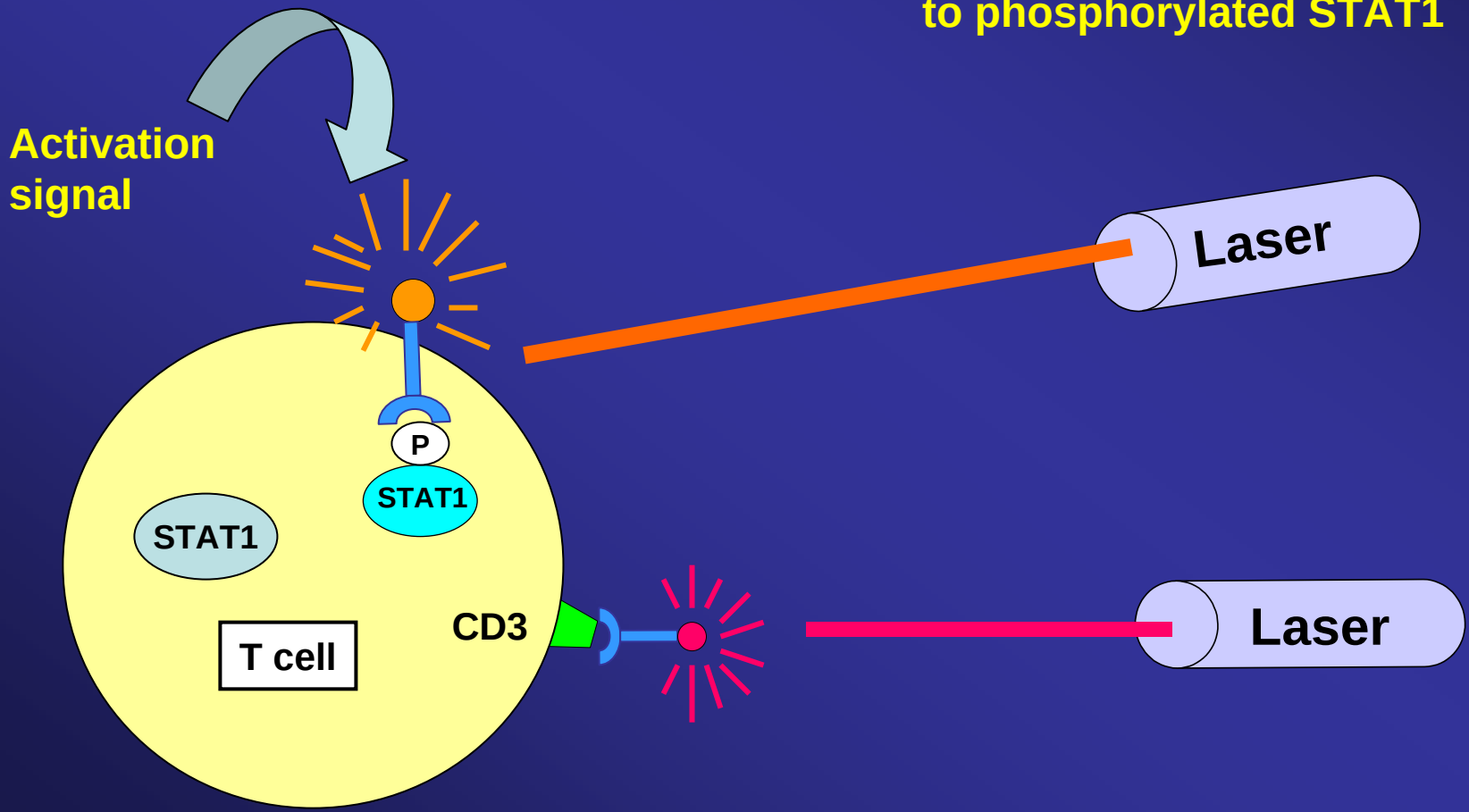
Expansion of $CD8^+GP100_{209-217}^+$- T cells and change of differentiation status in this subset seen in one melanoma patient treated with multi-epitope vaccine



Multi-color flow cytometry for phosphorylated STAT1 levels in activated immune cells

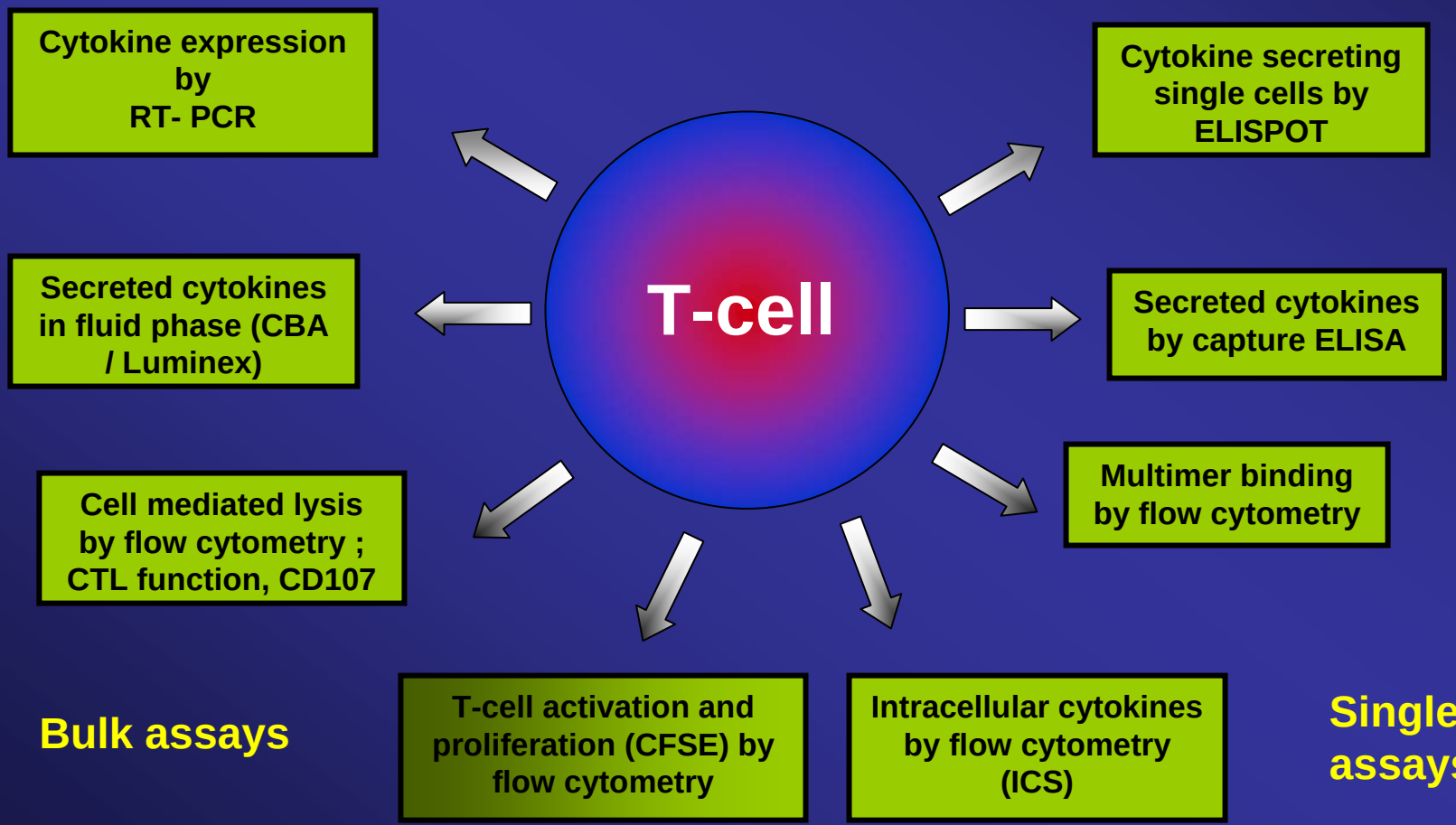
- Sensitive, quantitative, fast, uses few cells; measures early events

1. Surface staining with anti-CD3 Ab
2. Cell permeabilization
3. Intracytoplasmic staining with Ab to phosphorylated STAT1



Embarassing wealth of riches

- Emphasis on Ag-specific responses and multicolor high content screening

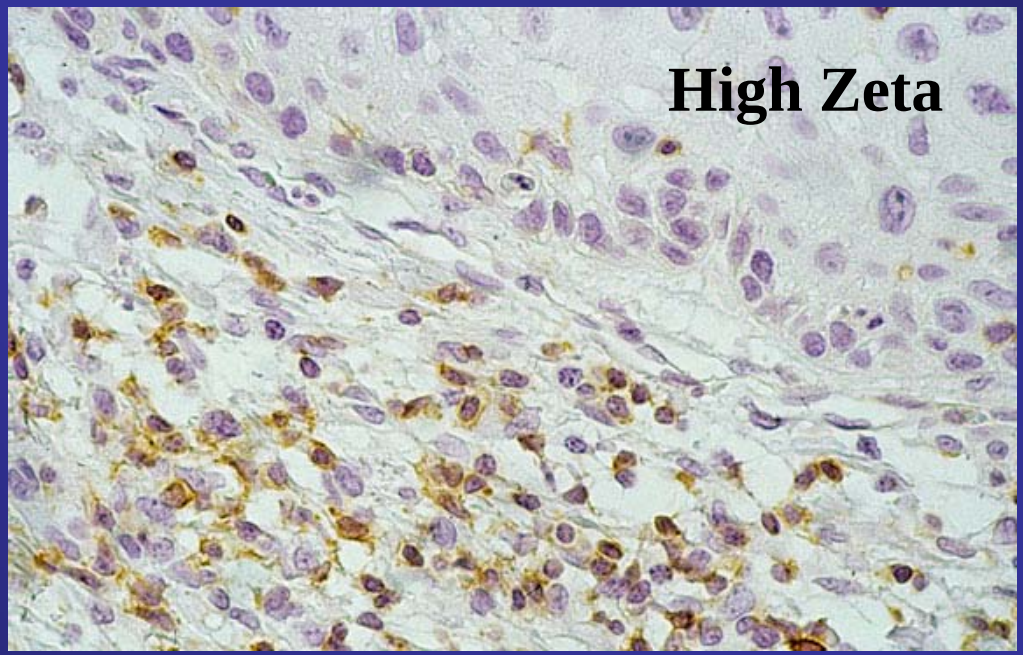


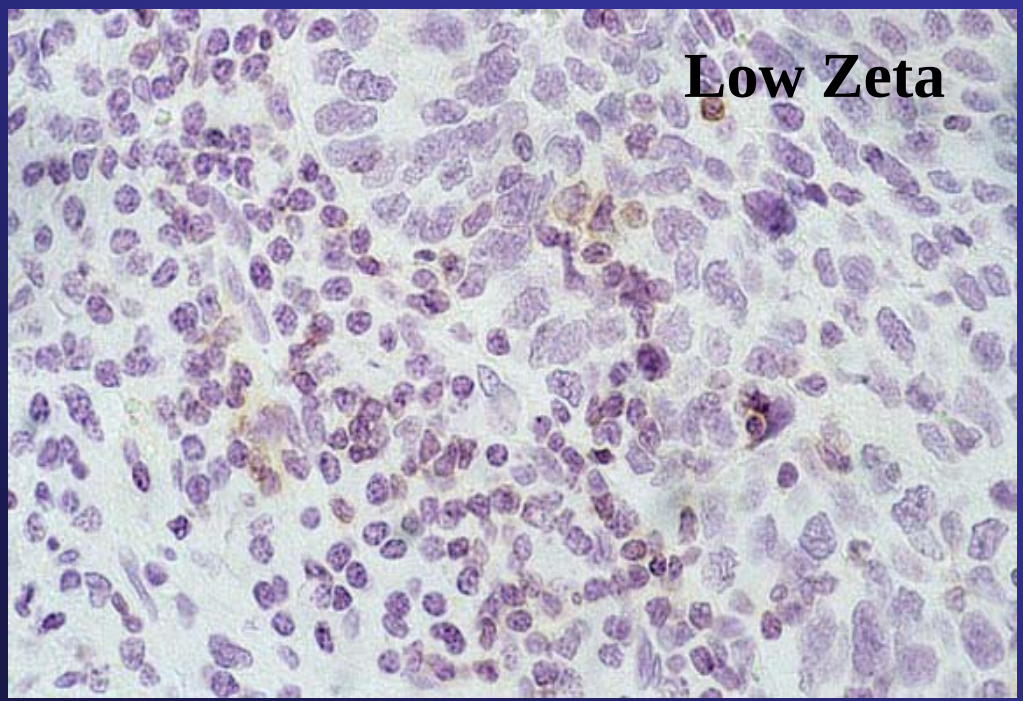
Immunohistochemistry/Tissue microarrays Immune infiltrates into tumor

- IHC/TMA useful clinically in estimating prognosis or responses to therapy
- In research, IHC/TMA is considered crucial for the identification and mapping of new biomarkers
- Tissue quality and epitope preservation
- Prospective collection in clinical trials
- Advancements strategies: multicolor labeling, confocal imaging, morphometry
- Need for standardization
- Data mining

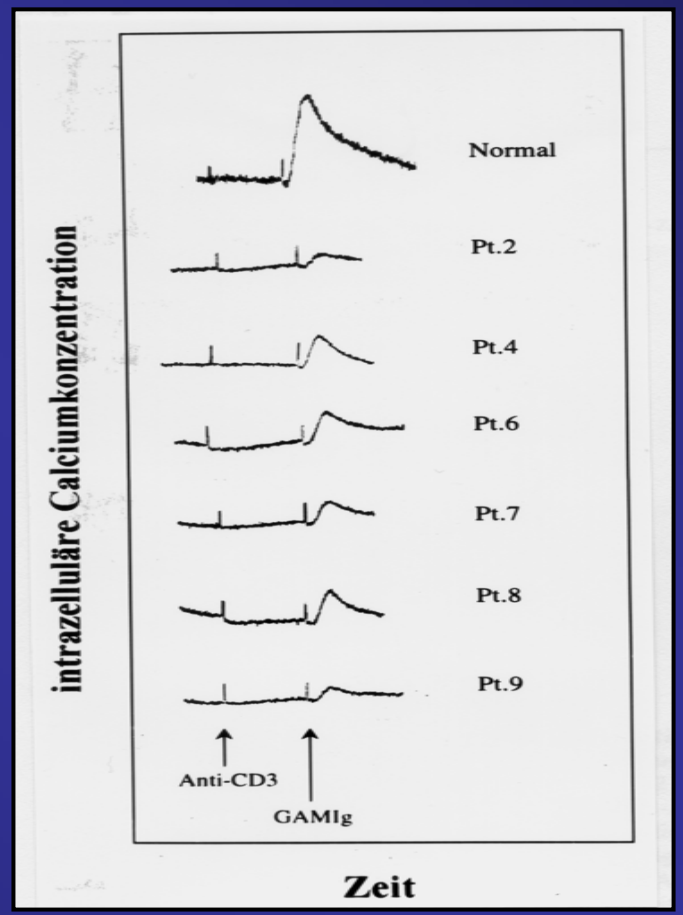
A retrospective study of tumor biopsies

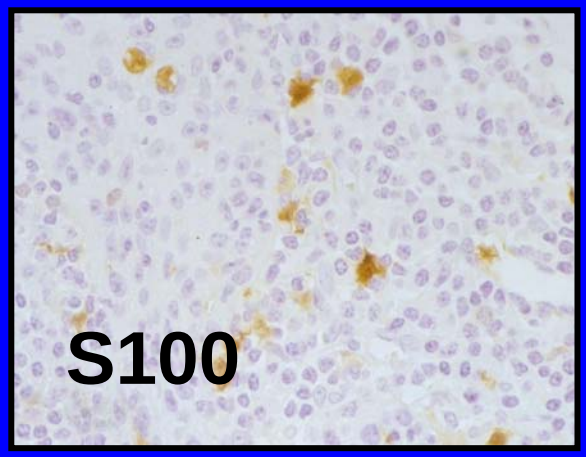
- 132 primary OSCC (Follow up > 5 Years)
- Immunohistochemistry for detection of DC and the ζ chain
  - Antibodies to S100, p55-protein, CD3 and CD247
  - Morphometrical analysis (cell number/HPF)
- Parameters evaluated:
  - Tumor size, TNM staging categories, grading, survival, recurrence
- Statistical analysis:
  - Proportional hazards regression
  - Multivariate survival analysis
  - Kaplan-Meier survival estimation

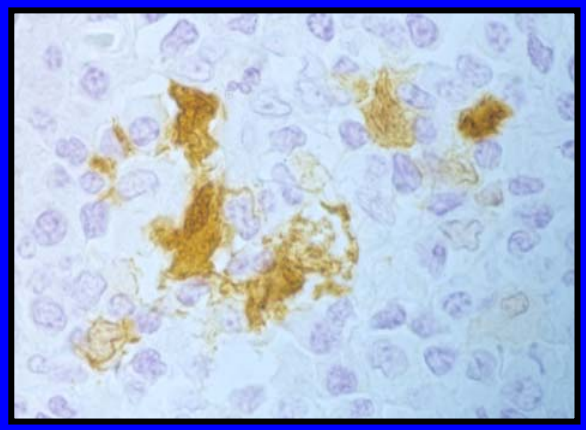


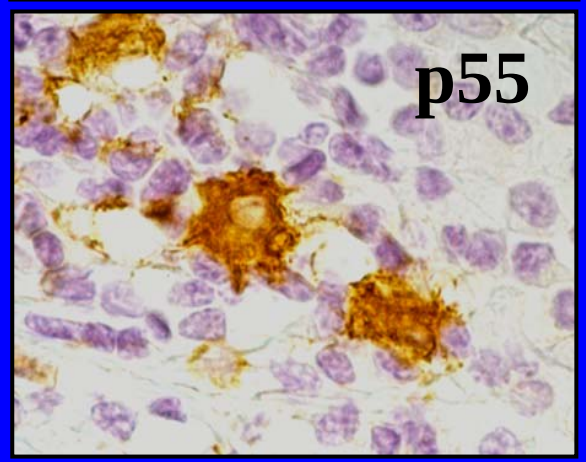


TIL in patients with HNC had variably but significantly decreased expression of TCR-associated ζ chain

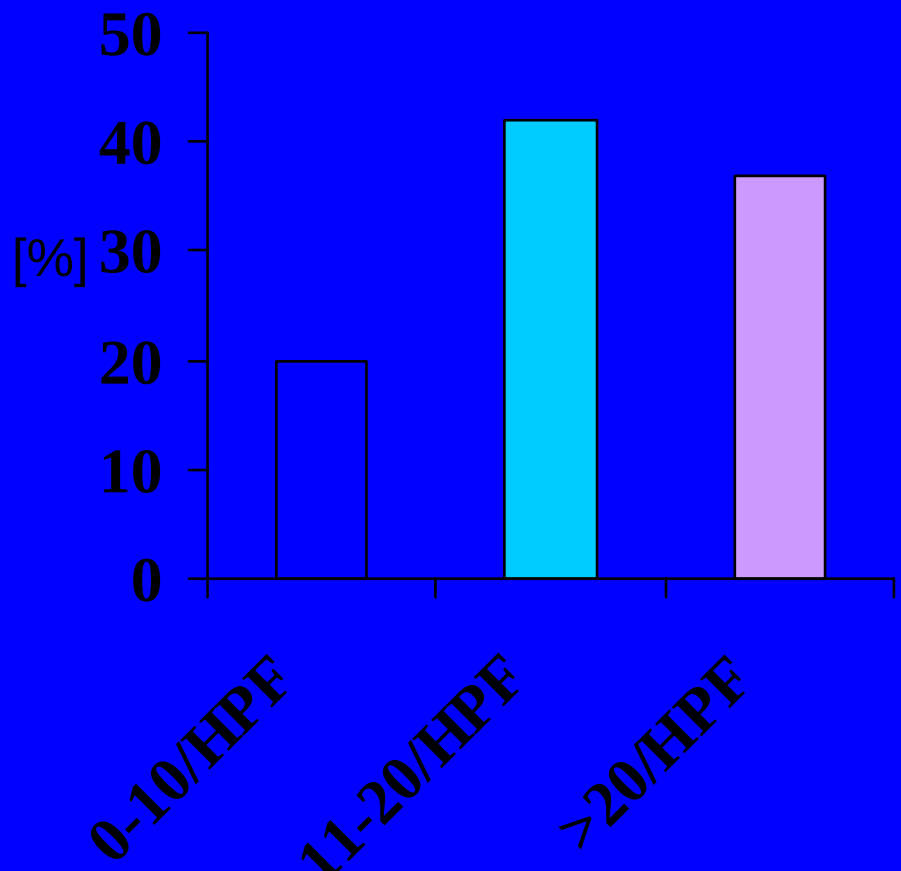






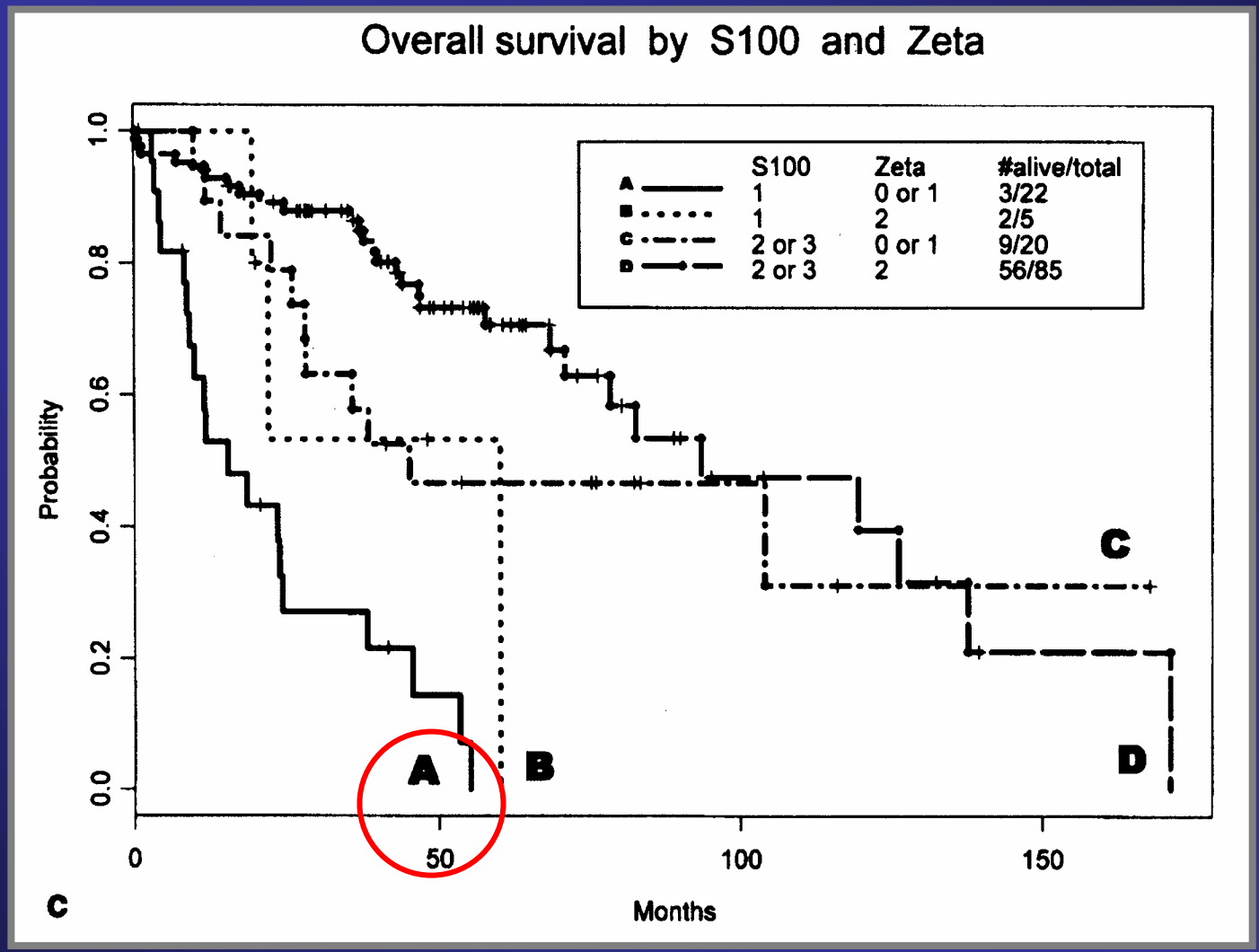


DC Counts in tissue



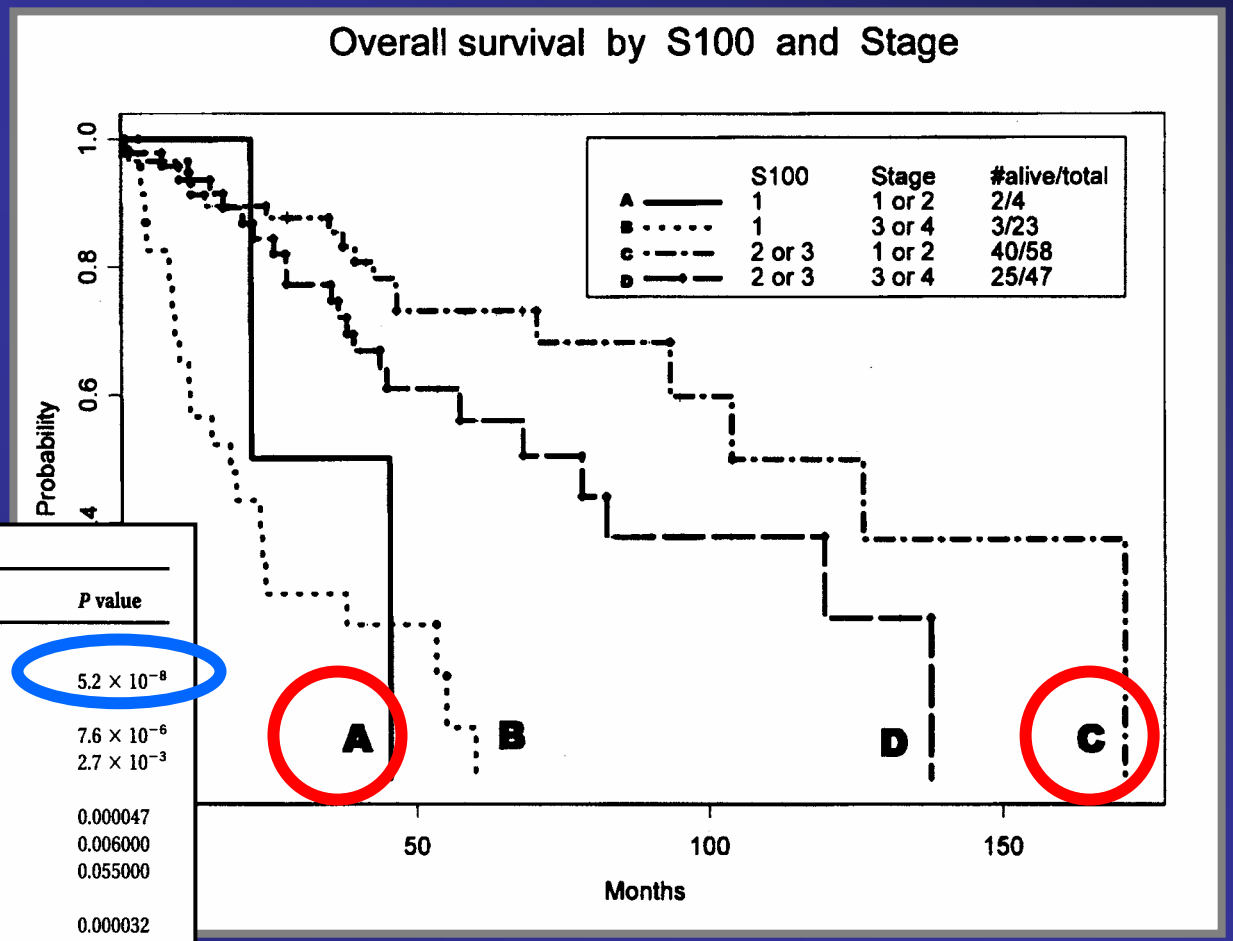
Multivariate Analysis

(Kaplan-Meier, S100 und zeta)



Reichert et al, Cancer 91: 2136-2147, 2001

Multivariate Analysis
(Kaplan-Meier, S100 and TU-Stage)



Stepwise Proportional Hazards Regression[a]

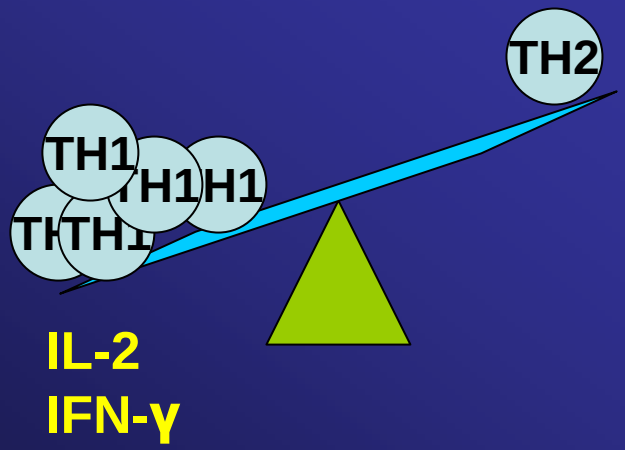
Model/predictor	Hazard ratio	*P* value
1		
S100	0.342	5.2×10^{-8}
2		
S100	0.40	7.6×10^{-6}
NS	1.54	2.7×10^{-3}
3		
S100	0.434	0.000047
NS	1.492	0.006000
T	1.240	0.055000
4		
S100	0.422	0.000032
NS	1.957	0.002800
T	1.497	0.015000
Stage	0.665	0.093000

[from: Reichert et al., *Cancer* 2001;91:2136-47]

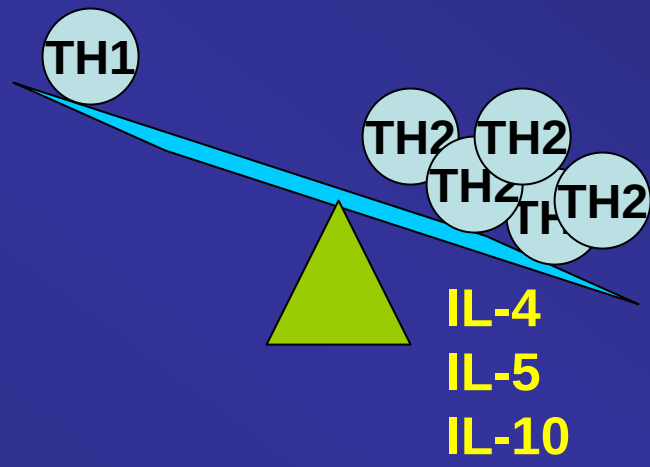
Cytokine balance in disease

- Therapeutic goal: shift the balance

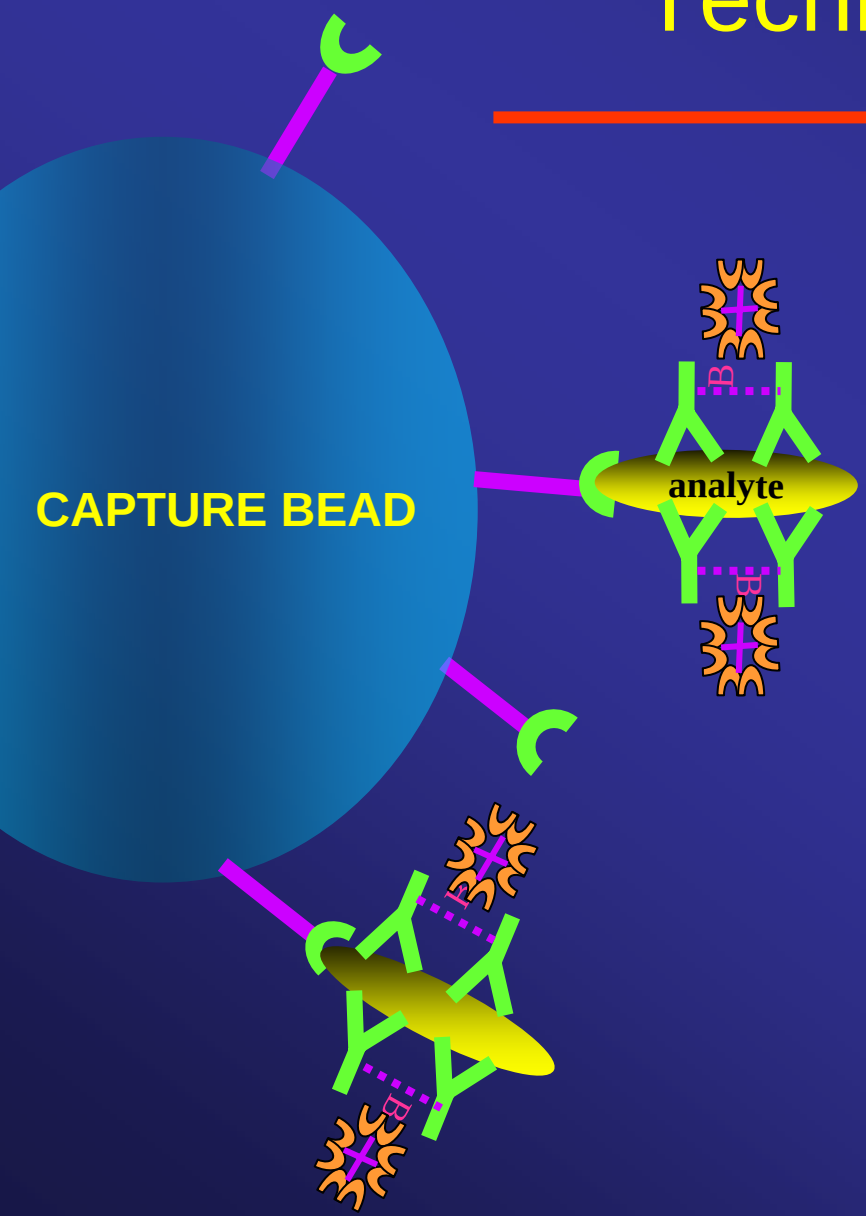
TH1-dominant diseases
Autoimmunity, GVHD



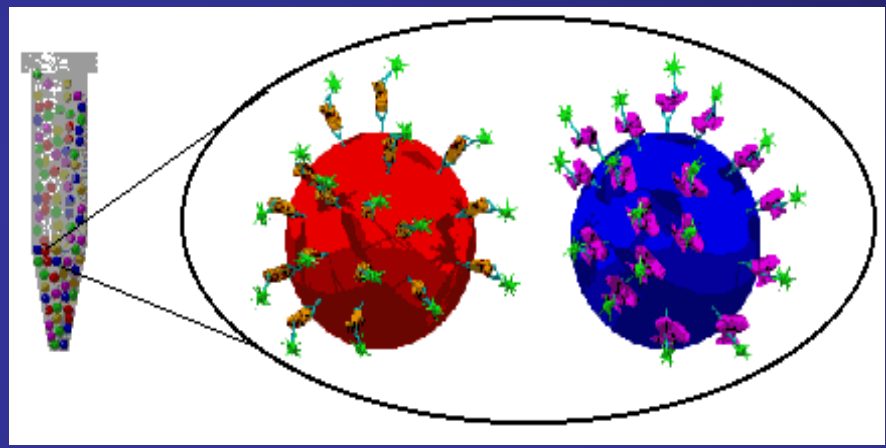
TH2-dominant diseases
Allergy, HIV, Cancer



Multi-Analyte Soluble Bead Array Technology



MICROSPHERE COLOR INDENTIFIES ANALYTE

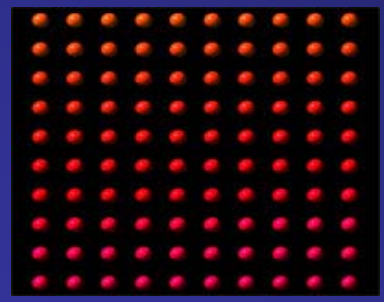


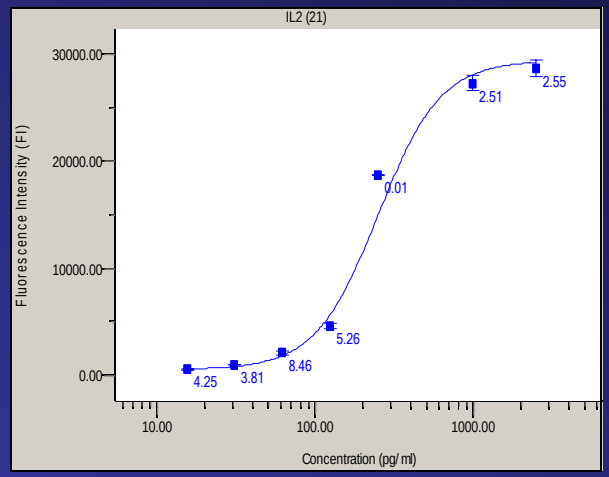
Analyte: body fluid supernatant

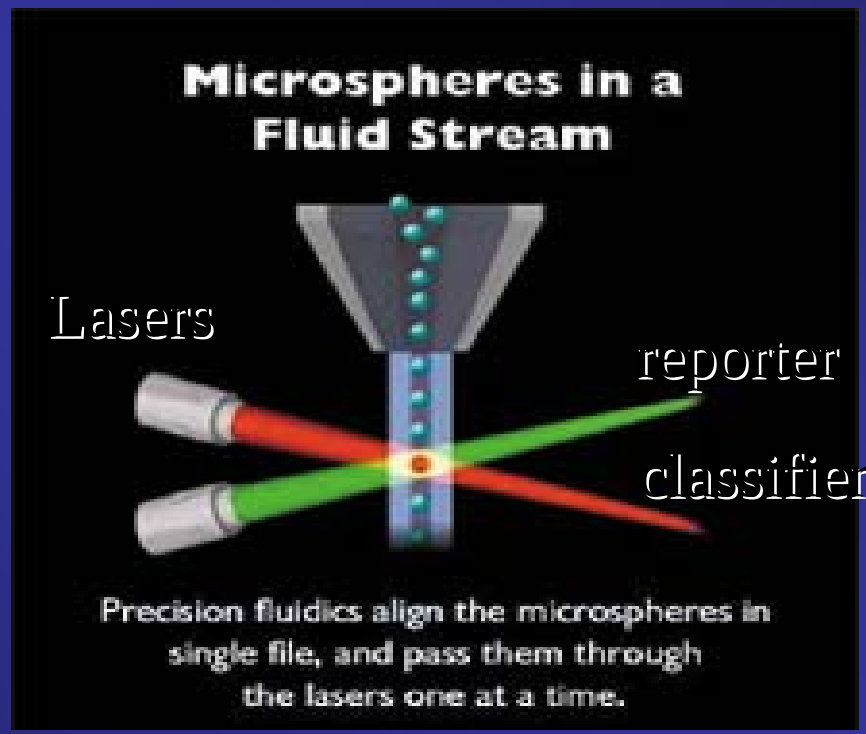
Volume: 50 uL

How The Bio-Plex protein array system works

- Up to 100 microspheres are in a bead set. Each is color-coded and conjugated with a MAb specific for a unique protein analyte
- A flow-based instrument with 2 lasers and associated optics measures biochemical reactions that occur on the surface of the colored microspheres
- A high-speed digital signal processor efficiently manages the fluorescent output.







Cytokines Chemokines & More

- Human
  - TNFα, IFNγ, TGF-β1, IL-1 Ra, IL-2 sR
  - IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6,IL-7,IL-8, IL-10, IL-11 ,IL-12, IL-13, IL-15, IL-18
  - G-CSF, M-CSF,GM-CSF, EGF, FGF-7, SCF, MIG, VEGF, HGF
  - FLT-3, MCP-1, MIP-1α, MIP-1β, Rantes, IP-10, LIF

Inflammation
G-CSF
IL-6
IL-8
TNF-α

Th-1/ Th-2	
IFN-γ	IL-4
IL-5	TNF-α
IL-2	IL-7
IL-12	GM-CSF
IL-13	IL-18

Autoimmune
IL-1b
IL-6
TNF-α
IL-12

Hematopoiesis
G-CSF
IFN-γ
IL-1β
IL-6
GM-CSF

Conclusions: high throughput assay platforms are here!

- *Technology is rapidly evolving*: 17-color flow, cytometric bead arrays, confocal immuno-microscopy, microfluidics, immunoassay-based microarrays, immuno-PCR. All aimed at a high throughput, small sample volumes, rapid detection
- *Profiling:* changes in several biomarkers
- *Potential future benefits*: identification of individual markers or profiles of immunologic markers which will serve as surrogate endpoints useful in predicting survival, clinical responses to therapy or in immunodiagnosis (e.g.,screening general populations)
- *Biomarker validation:* many promising biomarkers but few formally validated; we needmore cost-effective validation, based on solid mechanistic insights and clinical correlative studies

Advantages of a central laboratory operated as a GLP facility

- QA and QC in place assuring quality and reliability of monitoring
- State-of-the-art technologies
- Assay development, standardization and validation
- Decreased cost of immune monitoring which is essential for biotherapy protocols
- Result interpretation in conjunction with statisticians aware of immune-based analyses
- Banking of samples which are accompanied by clinical outcome data for future research

Acknowledgements

- Immunologic Monitoring and Cellular Products Laboratory (IMCPL)
- Many posdoctoral fellows
- My clinical colleagues:

Jonas T. Johnson, MD

Robert L. Ferris, MD, PhD

John M. Kirkwood, MD

Michael T. Lotze, MD

Development timelines and cost

Table 1: Typical vaccine development timeframes and costs

Phase	Years to Market	Probability of reaching market (%)	Cost at Stage ($m)
Research	11	10	400
Development (Preclinical)	8	20	350
Phase I	6	20-30	280
Phase II	5	30-50	200
Phase III	3	50-90	10
BLA Filed	1	90-95	5
Approval	0	99	0

Source: Jarvis (2002)

DATAMONITOR

Rational use of surrogate endpoints

- Mechanistic surrogate endpoint
- Disease-related surrogate endpoint
- Time-to-progression surrogate endpoint
- Overall survival endpoint