Biomarkers for Acute GVHD

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Disclosures

Financial Conflicts of Interest

- none

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- P01-AI33484, “Immunobiology of Tolerance Following Hematopoietic Cell Transplantation”
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Biomarkers in aGVHD

Goals and potential utility

- Improved diagnosis
  - lab based, objective
- Predict outcome
  - identify high risk patients
  - indication for pre-emptive or intensive therapy
- Monitor treatment response
  - guide dose adjustment and duration of IST
- Discovery
  - genes and pathways involved in pathogenesis of aGVHD
  - rationale for developing targeted therapy

Biomarkers in aGVHD

Tissue source

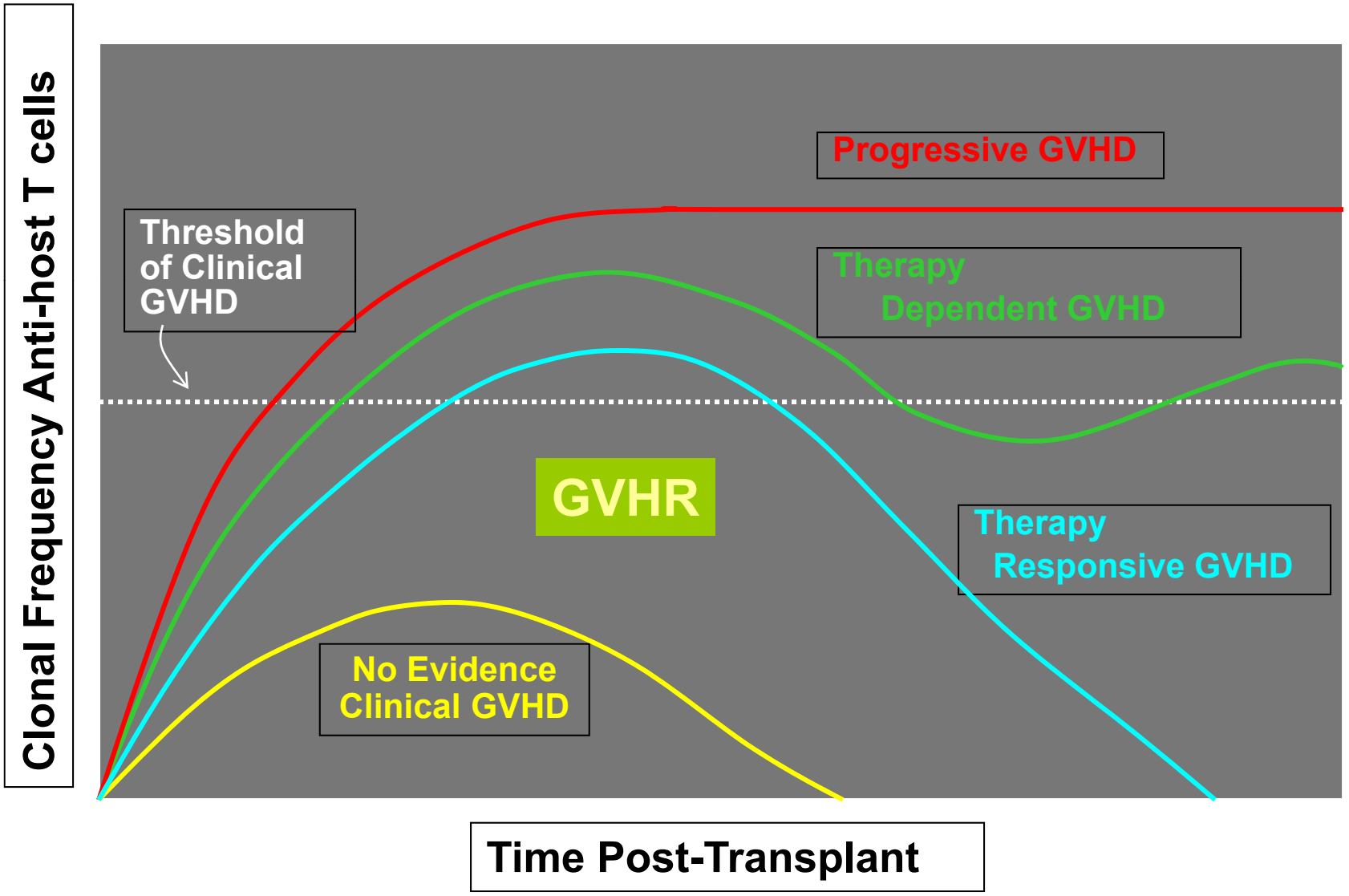
- Blood
  - plasma
  - WBC, PBMC, T cells
- Urine
- Tissue biopsy
  - skin
  - oral mucosa or gut
  - liver

Biomarker Approaches
(and methodologies)

- Immunophenotyping
  - enumeration of T cells, B cells, Treg
  - activation markers (expression of HLA-DR, CD25, FAS, etc)
- Proteomics
  - mass spec, discovery phase
  - antibody arrays
  - ELISA, bead-based multiplexing *(Luminex)*
- Genomics
  - analysis of gene expression ("transcription profile")

The Graft-vs-Host Reaction and GVHD

Severity = Strength [T cell response + inflammation] X Duration



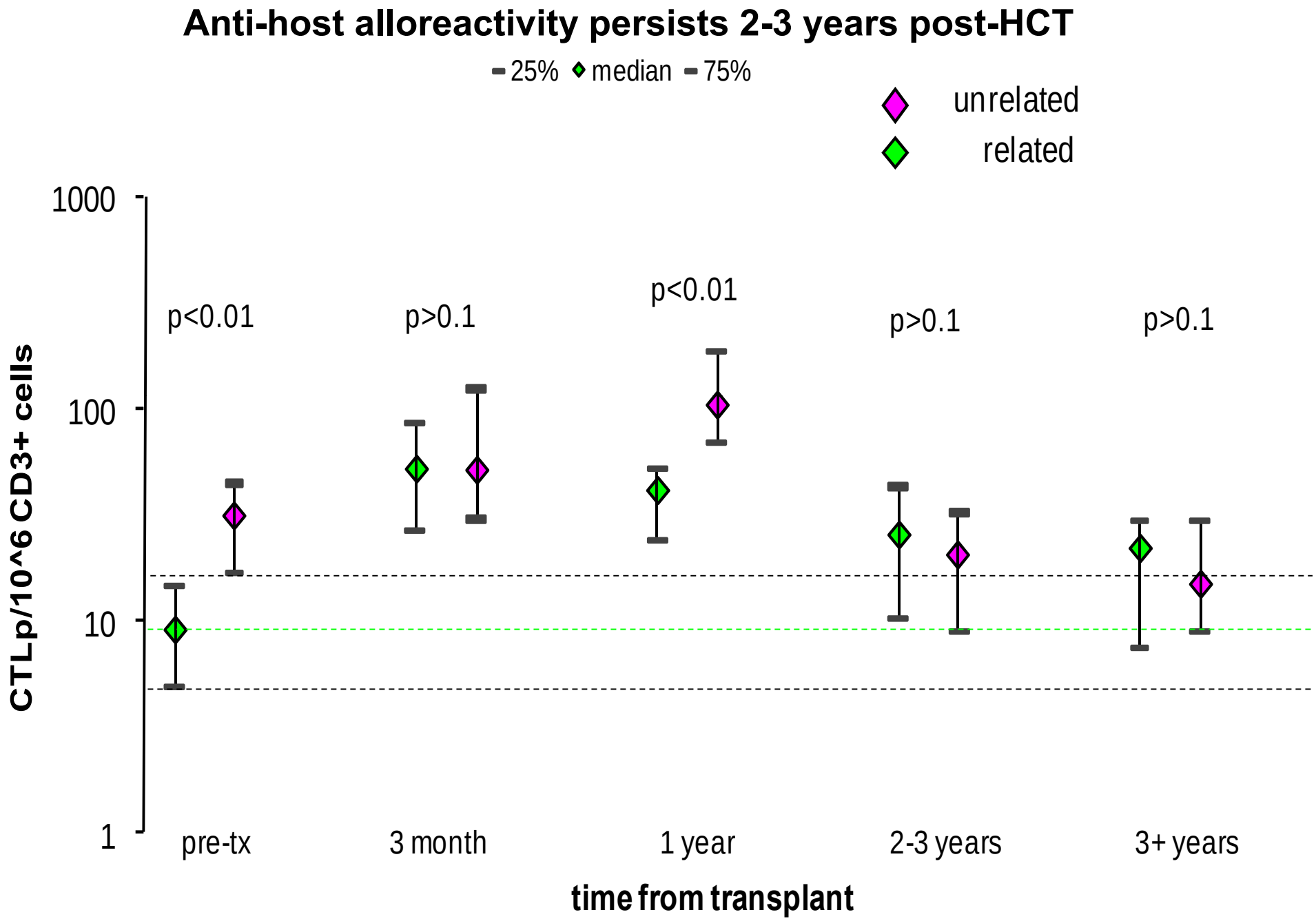
Alloreactivity and GVHD

Biology of the Graft-vs-Host Reaction

- initiated by donor T cells
- activation of both adaptive and innate immune systems > acute inflammation
- pre-HCT cytotoxic conditioning therapy > gut injury
  - translocation of bacteria
  - leakage of LPS > liver injury
- all the above,

  ⟹ amplification of inflammation > further tissue injury

Clinical GVHD is a complex multi-system syndrome

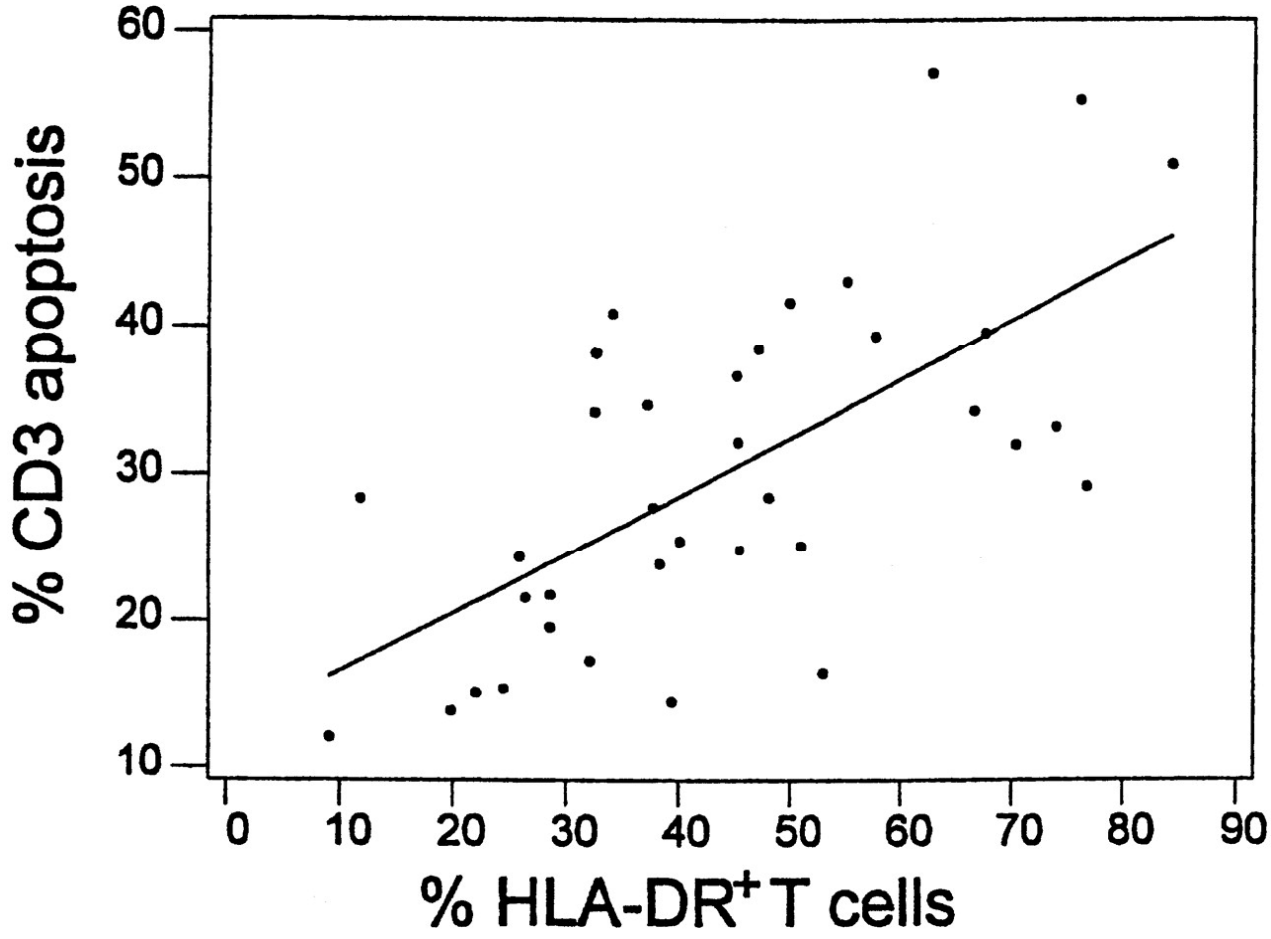

Anti-host alloreactivity persists 2-3 years post-HCT
25%
median
75%
unrelated
related
1000
100
10
1
CTLp/10^6 CD3+ cells
p<0.01
p>0.1
p<0.01
p>0.1
p>0.1
pre-tx
3 month
1 year
2-3 years
3+ years
time from transplant

Blood Lymphocytes as Biomarkers for acute GVHD

Activation and Apoptosis of Peripheral Blood Lymphocytes Early after Hematopoietic Cell Transplantation

Lin et al Blood 95:3832, 2000

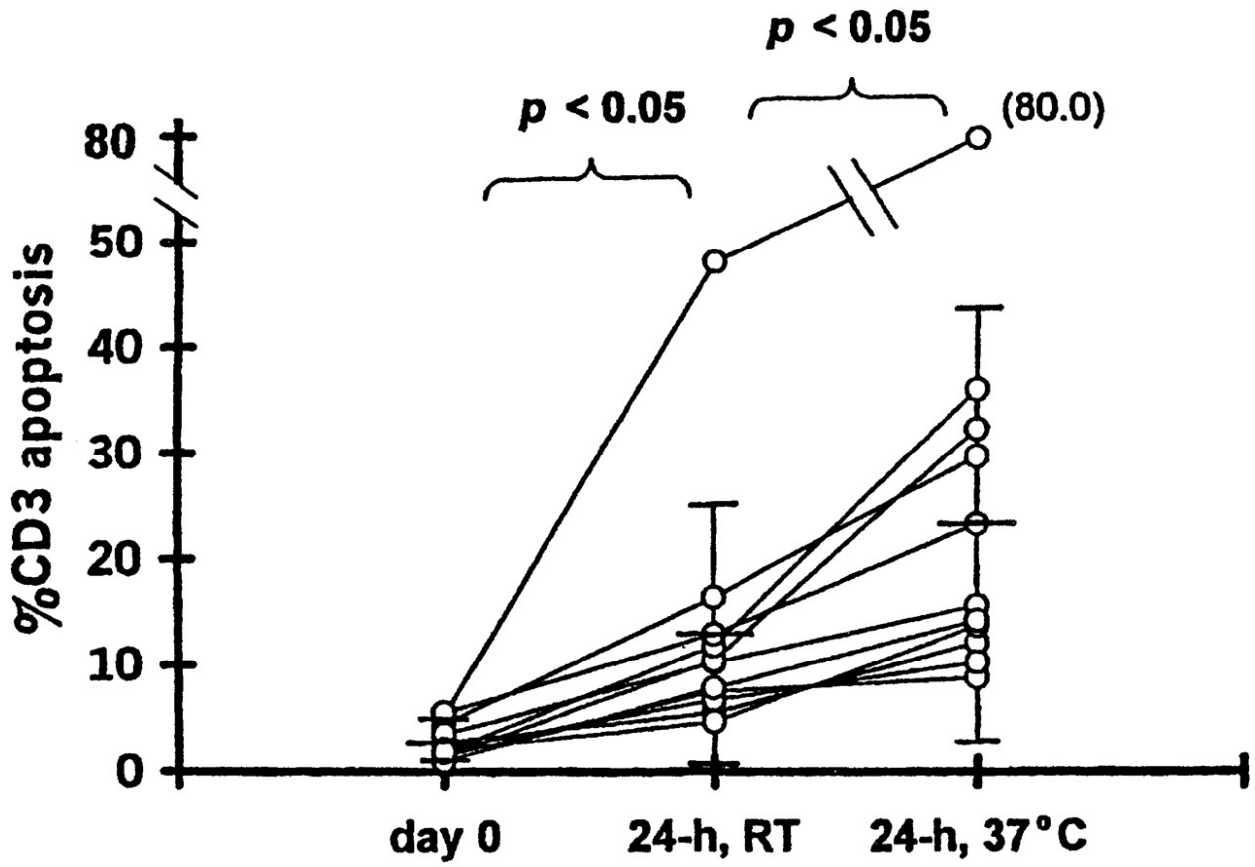
Correlation between T cell apoptosis after 24-hour culture and HLA-DR expression: 36 patients studied 19-23 days post-HCT



Lin, M.-T. et al. Blood 2000;95:3832-3839

Apoptosis of Peripheral Blood Lymphocytes Early after Hematopoietic Cell Transplantation

Patients



Lin, M.-T. et al. Blood 2000;95:3832-3839

Apoptosis of Peripheral Blood Lymphocytes Early after Hematopoietic Cell Transplantation

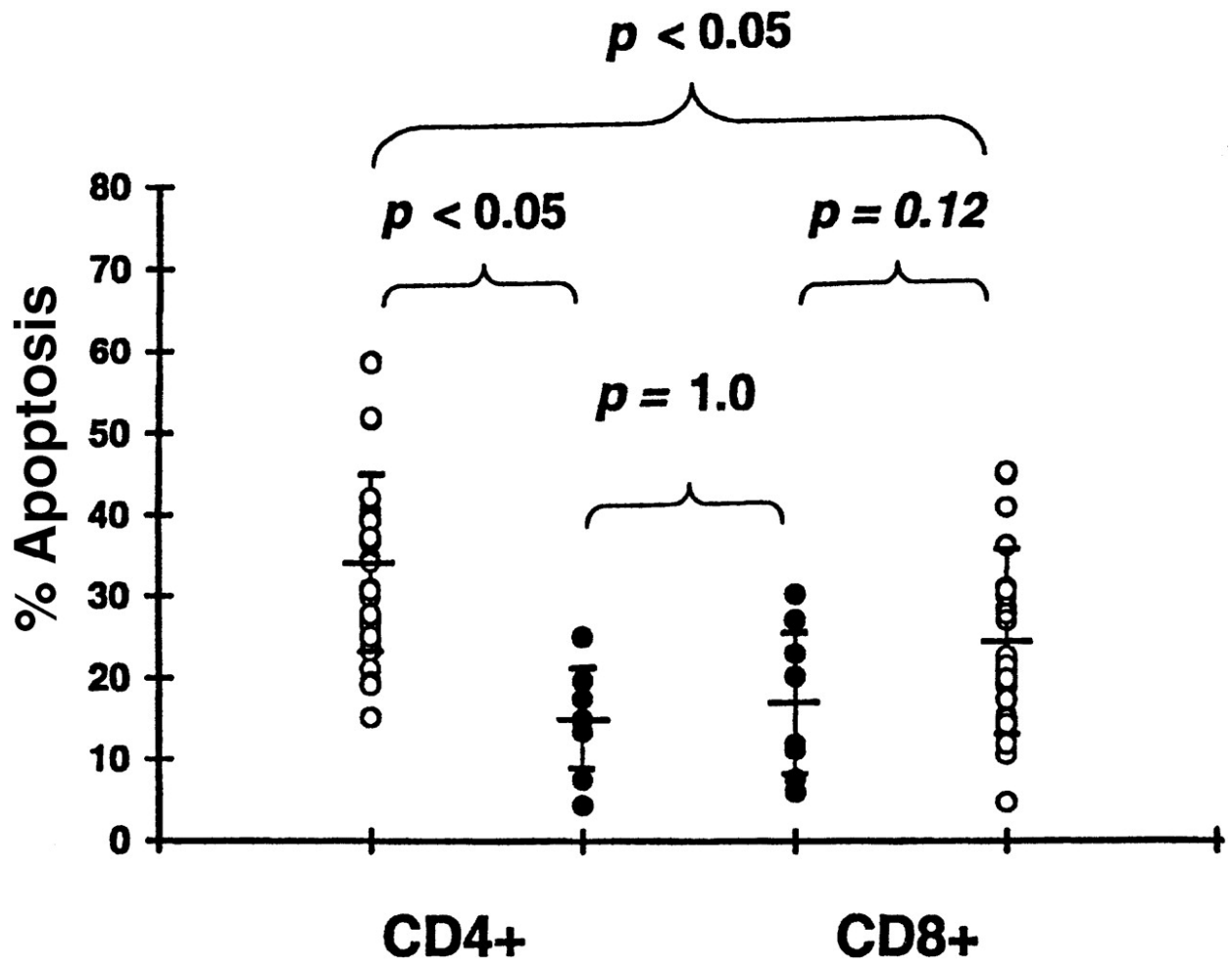
Lin et al Blood 95:3832, 2000

	% Apoptosis [1]		
	CD3+	CD4+	CD8+
Patients, 19-23 days post-HCT (n=51)	30.4±12.5 (5.1-60.0)	33.3±14.6 (4.3-68.6)	26.0±13.1 (5.9-59.7)
Normal controls (n=17)	4.0±1.5 (1.9-6.9)	4.2±1.6 (1.8-6.9)	3.8±2.0 (1.4-8.3)

[1] stained with 7ADD+ after 24-hour culture; apoptosis of CD56+CD3- NK cells, 2.2±1.2

Apoptosis of CD4+ T cells and grade 2-4 acute GVHD

(day 19-23 post-HCT, 24-hour *in vitro* culture)



Lin, M.-T. et al. Blood 2000;95:3832-3839

CD4+ T cell Apoptosis Correlates with grade 2-4 acute GVHD

Summary

- **increased apoptosis can be detected in freshly isolated blood**
- **amplified by short-term culture**
- **associated with T cell activation**
- **correlates with lymphopenia**
- **decreases after initiation of steroids**
- **recurrence may predict steroid-dependent disease**

Data pending replication by independent study

Blood Plasma as a Biomarker for acute GVHD

Plasma Proteins Associated with aGVHD

Summary of Published Reports

(does not includes negative findings)

Protein	aGVHD	TRM
IL2R	Miyamoto 1996; Grimm 1998; Foley 1998; Nakamura 2000; Visentainer 2003; Shaiegan 2006; Paczesny 2008	Paczesny 2008
IL-6	Imamura 1994; Malone 2007	
IL-8	Uguccioni 1993; Paczesny 2008	Schots 2003; Paczesny 2008
IL-10	Liem 1998	
IL-12	Nakamura 2000; Mohty 2005	
IL-15	Sakata 2001	
IL-18	Nakamura 2000; Fujimori 2000; Shaiegan 2006; Luft 2007	
CCL8	Hori 2008	
CXCL10	Piper 2007	
HGF	Okamoto 2001; Paczesny 2008	Paczesny 2008
IFNG	Imamura 1994; Nakamura 2000	
TNF	Holler 1990; Symington 1990; Imamura 1994	
TNFRI	Or 1996; Kitko 2008; Choi 2008; Paczesny 2008	Paczesny 2008
Syndecan-1	Seidel 2003	

Critique of Published Data

Questions and issues

- aGVHD risk and incidence rates vary between Centers
- sample collection and processing is not standardized
- little documentation of assay sensitivity, specificity and reproducibility
- mostly case-control study designs, but selection matching criteria may be variable and/or vague
- usually some degree of missing or excluded data
- studies rarely include 2 phase discovery & validation cohorts, or randomization

PLASMA CYTOKINE LEVELS BEFORE and AFTER THE ONSET OF ACUTE GVHD

George B. McDonald et al, Seattle

(unpublished data)

Experimental design
McDonald et al

Study I

- 146 patients receiving CY/TBI for hematological malignancy (MURD, 139); cyclosporine + methotrexate prophylaxis
- blood drawn weekly to day +56, processed rapidly
- plasma levels of 11 cytokines analyzed by ELISA

Plasma cytokine level changes over 15 day interval prior to onset of grade III/IV GVHD

Rising Levels (slope positive)	No change	Falling Levels (slope negative)
IL-1a +68±18% (p=.004) IL-6 +361±45% (p=.0003) sTNFRI +20±6% (p=.002)	IL-1-beta IL-2 IL-4 IL-10 TNFa IFNg IL-1RA	TGF-b1 -43±19% (p=.002)

Plasma cytokine level changes prior to onset of GVHD

GVHD grade	Δ IL-1α	Δ IL-6	Δ sTNFRI	Δ TGF-β1
0 – I (N = 16)	+22.8 ± 9.2% p=.03	-26.9 ± 64.7% p=.54	+27.2 ± 12.6% p=.06	+18.7±16.3% p=.27
II (N = 61)	+32.2 ±16.7% p=.08	+93.0 ±23.3% p=.003	+16.1 ± 3.5% p<.0001	-43.5±15.8% p=.0003
III – IV (N = 30)	+68.2 ±17.9% p=.004	+361.1 ± 45% p=.0003	+20.2 ± 5.6% p=.002	-43.4±18.5% p=.002

Median values for plasma cytokines prior to onset of acute GVHD

McDonald et al

Cytokine	Normal upper limit (NUL)	GVHD 0/I (N=16)	GVHD II (N=61)	GVHD III/IV (N=30)
IL-1α	225 pg/mL	39.8 (.18 x NUL)	43.5 (.19 x NUL)	75.5 (.34 x NUL)
IL-6	**0.7 pg/mL**	**15.4 (22 x NUL)**	**20.7 (30 x NUL)**	**34.9 (50 x NUL)**
TNFα	12 pg/mL	7.7 (.64 x NUL)	6.9 (.58 x NUL)	12.3 (1 x NUL)
TNFRI (p55)	925 pg/mL	1308 (1.4 x NUL)	1163 (1.3 x NUL)	1161 (1.3 x NUL)
TGFβ 1	9445 pg/mL	701 (.07 x NUL)	424 (.04 x NUL)	441 (.05 x NUL)

Conclusions – Study I [1]

McDonald et al, unpublished

1. Plasma levels of IL-6, IL-1a, and sTNFRI (p55) are increasing, and TGFb1 decreasing, prior to onset of clinical GVHD.
2. The slopes of plasma IL-6 levels before GVHD onset are steeper than those of IL-1a and sTNFRI.
3. Plasma IL-6 levels (but not IL-1a and sTNFRI) exceed the normal upper limit for this cytokine in healthy volunteers.
4. Plasma IL-6 level prior to onset of clinical GVHD correlates with severity of GVHD.

[1] Study I did not include IL-2Ra, IL-8 or HCF

Experimental design
McDonald et al

Study II

- 160 patients transplanted for hematological malignancy
  - myeloablative and reduced intensity
  - CSP+MTX, FK+MTX, and FK+MMF prophylaxis
- Blood routinely drawn weekly to day +56, and day 80
  - day 14 after starting IST for aGVHD Rx
- Plasma cytokine levels analyzed by Luminex
  - enlarge panel of analytes (IL-2Ra, IL-8, HCF and others)
- Randomly select cases & controls for discovery and replication cohorts
  - correlated with acute GVHD, treatment response, mortality

Status: analysis pending

“A Biomarker Panel for Acute GVHD”

Sophie Paczesny, James L.M. Ferrara, et al.

Blood 113:273-278, 2009

First GVHD biomarker study to include a separate and independent discovery and validation phase

"A biomarker panel for acute GVHD"
Paczesny et al, *Blood* 2009

Study plan

- 466 subjects receiving allo HSCT between 2001-2006 at the Univ of Michigan
- Excluded patients with VOD, IPS, septic shock (15%)
  - ~70% myeloablative, ~35% unrelated donor

"A biomarker panel for acute GVHD"
Paczesny et al, *Blood* 2009

Study plan

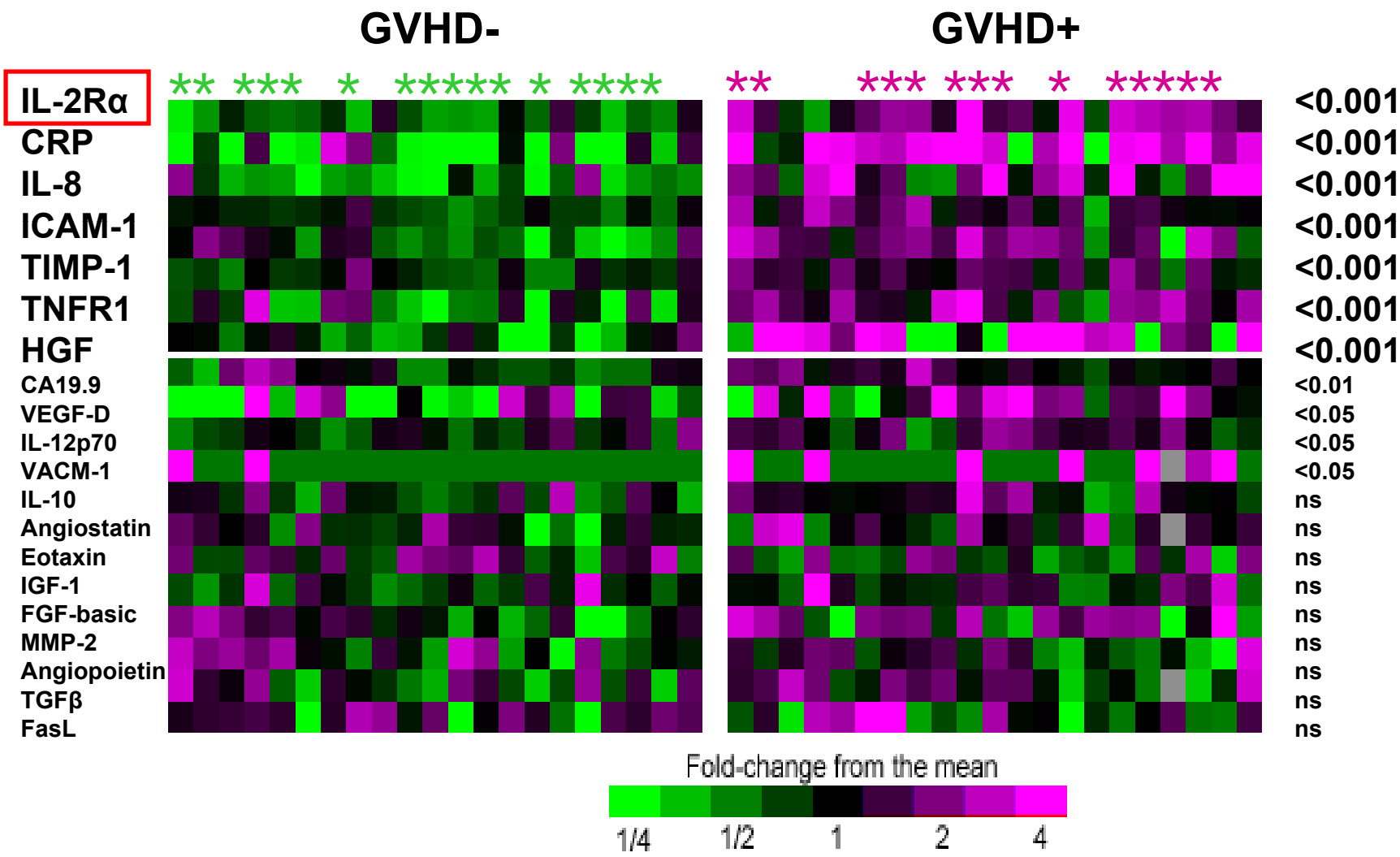
- Discovery phase, 42 patients selected for case-control study
  - 21 patients, grade 3-4 aGVHD
  - 21 patients, grade 0 aGVHD
- Replication phase, 424 patients randomly separated into:
  - training set, n=282; 166 GVHD (grade 0), 116 GVHD (grade 1-4)
  - validation set, n=142; 76 GVHD (grade 0), 66 GVHD (grade 1-4)

A biomarker panel for acute GVHD
Paczesny et al 2009

Results

- Discovery phase:
  - Antibody microarrays identified 35 of 120 plasma proteins significantly associated with severe grade 3-4 aGVHD
  - 23 of the 35 proteins selected for testing in a sequential ELIZA assay (to preserve sample)
  - 8 proteins gave p-value <.01 comparing GVHD+ and GVHD- patients

DISCOVERY PROTEOMICS
POTENTIAL GVHD BIOMARKERS



A biomarker panel for acute GVHD

Paczesny S et al, 2009

8 proteins selected from the Discovery phase antibody array + ELISA study for Validation

- IL-2Ra
- CRP
- IL-8
- ICAM-1
- TIMP-1
- TNFRI
- HGF
- CA19.9

"A biomarker panel for acute GVHD"
Paczesny et al, *Blood* 2009

Study plan

- Replication phase, 424 patients randomly separated into:
  - training set, n=282; 116 GVHD+, 166 GVHD-
  - validation set, n=142; 66 GVHD+, 76 GVHD-
- Sequential ELISA performed for 8 biomarkers
- Median values and individual AUCs determined for the training set

• GVHD+, grade 2-4; GVHD-, grade 0
• median onset, day 30

A biomarker panel for acute GVHD

Paczesny S, et al, 2009

Results – Training set:

- Linear regression determined that a linear combination of 4 proteins produced the best model to predict acute GVHD

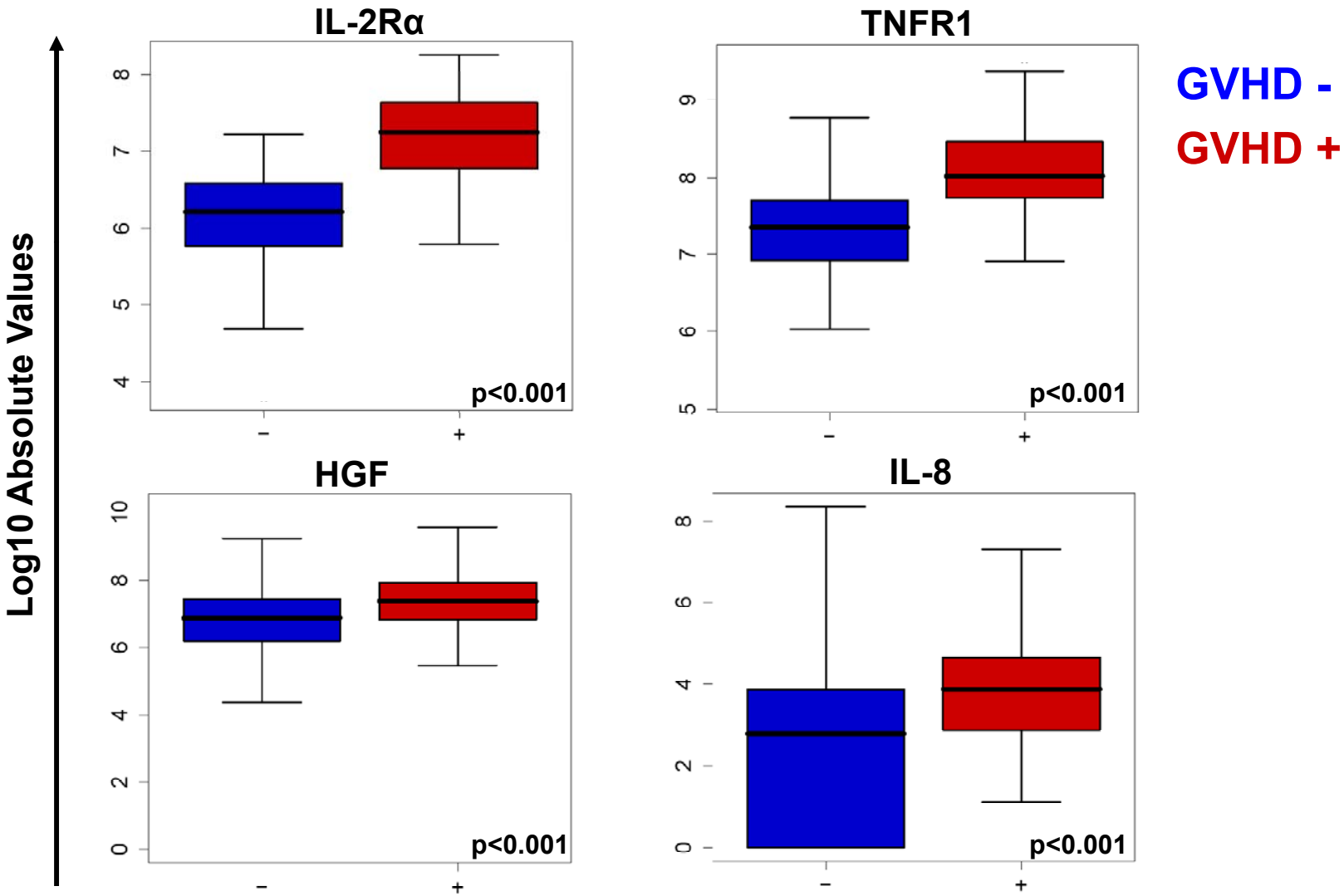
- IL-2Ra
- TNFR1
- HGF
- IL-8

Proteins failing conformation in the training set:

- CRP, ICAM-1, TIM-1, CA19.9

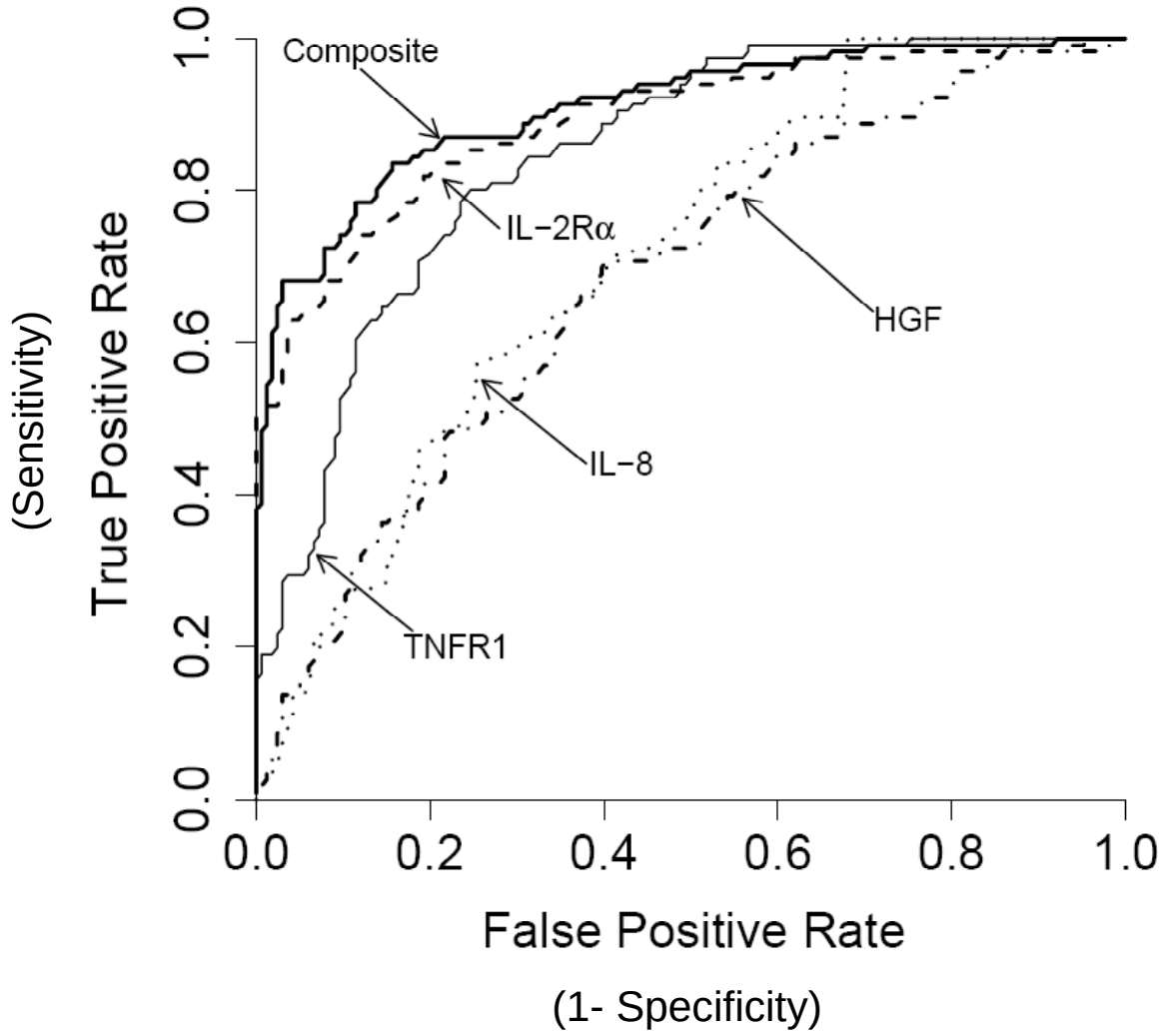
TOP FOUR PROTEINS IN THE TRAINING COHORT

Paczesny, S. et al. Blood 2009;113:273-278



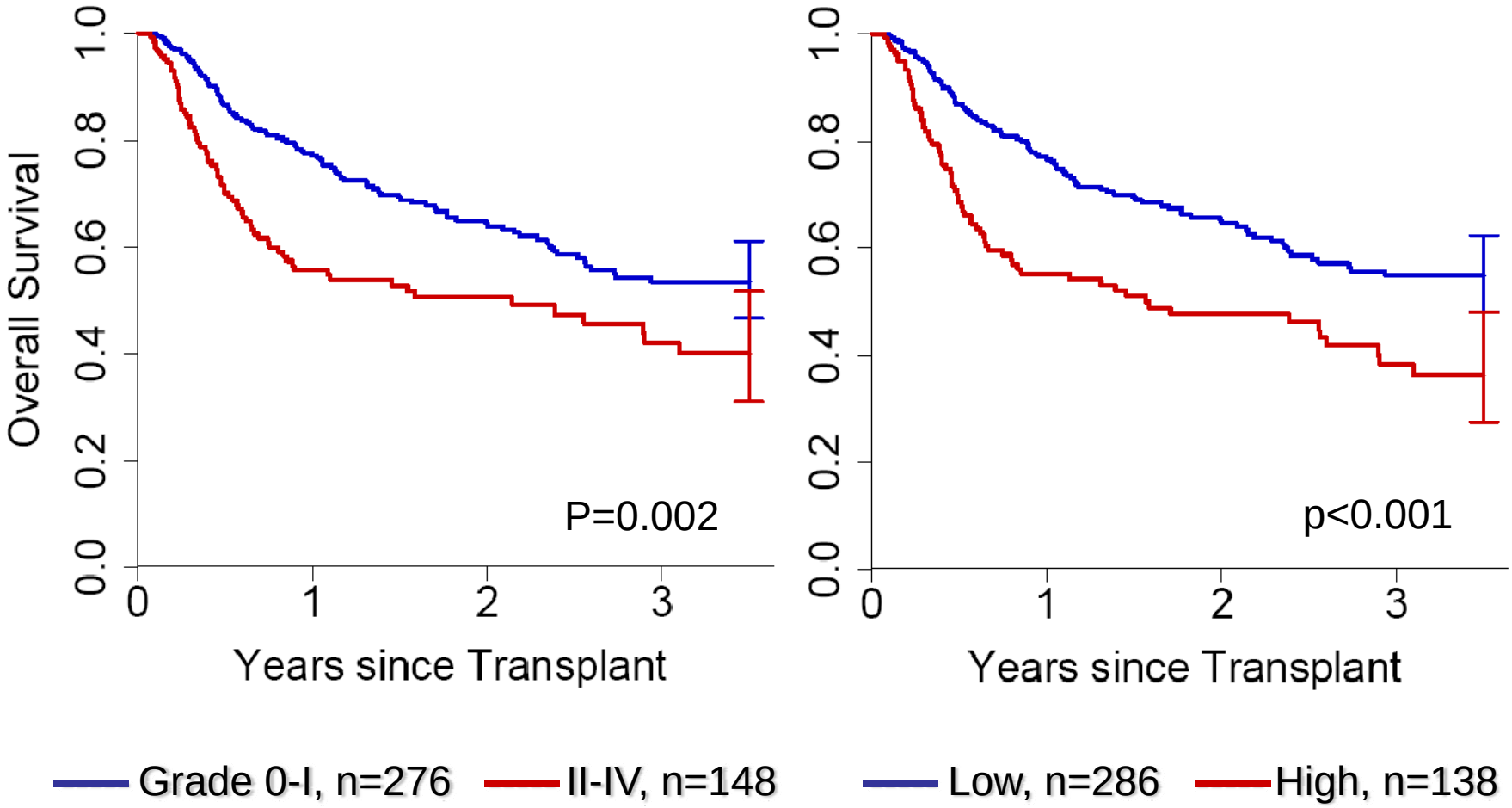
ROC Curve of the Training Cohort for Individual Proteins and Composite Panel

Paczesny, S. et al. Blood 2009;113:273-278



Survival is predicted independently by GVHD grade and Biomarker Panel

Paczesny, S. et al. Blood 2009;113:273-278



Are plasma biomarkers “ready”?

- Clinical trials
  - YES, candidate plasma proteins should be incorporated into prospective multi-center trials
    - adjunct or stand alone studies
    - avoid exclusions and missing data
    - evaluate specificity in patients with bacteremia, IPS, VOD, etc
    - determine time-dependent kinetics prior to onset
    - define early changes predictive aGVHD onset & severity
- Diagnostics, preemptive therapy
  - pending prospective validation and deeper analyses

Thank you!