BRASS Registry: Strengths, Weaknesses and Tradeoffs in Obtaining Unique Information for Better Clinical and Public Health Knowledge

Nancy A Shadick MD, MPH
Brigham & Women's Hospital

Presentation Goals:

- Purpose and design of BRASS Registry
- Strengths, weaknesses and tradeoffs
- Registry results and potential for future investigation

Study Rationale

- Therapy in RA is empirically based
- Time consuming-requiring several months to find the correct combination
- irreversible joint damage occurs

RA treatment Challenges

- Lack of clinical or lab characteristics that reliably predict disease severity or phenotype
- Non genetic factors predict outcome such as age of onset, SES, RF and poor functional status
- Symptom improvement does not always alter the course of disease (erosions, deformity)

Opportunity in the BRASS study?

Human genome sequence, use of high throughput genomic technologies identify:

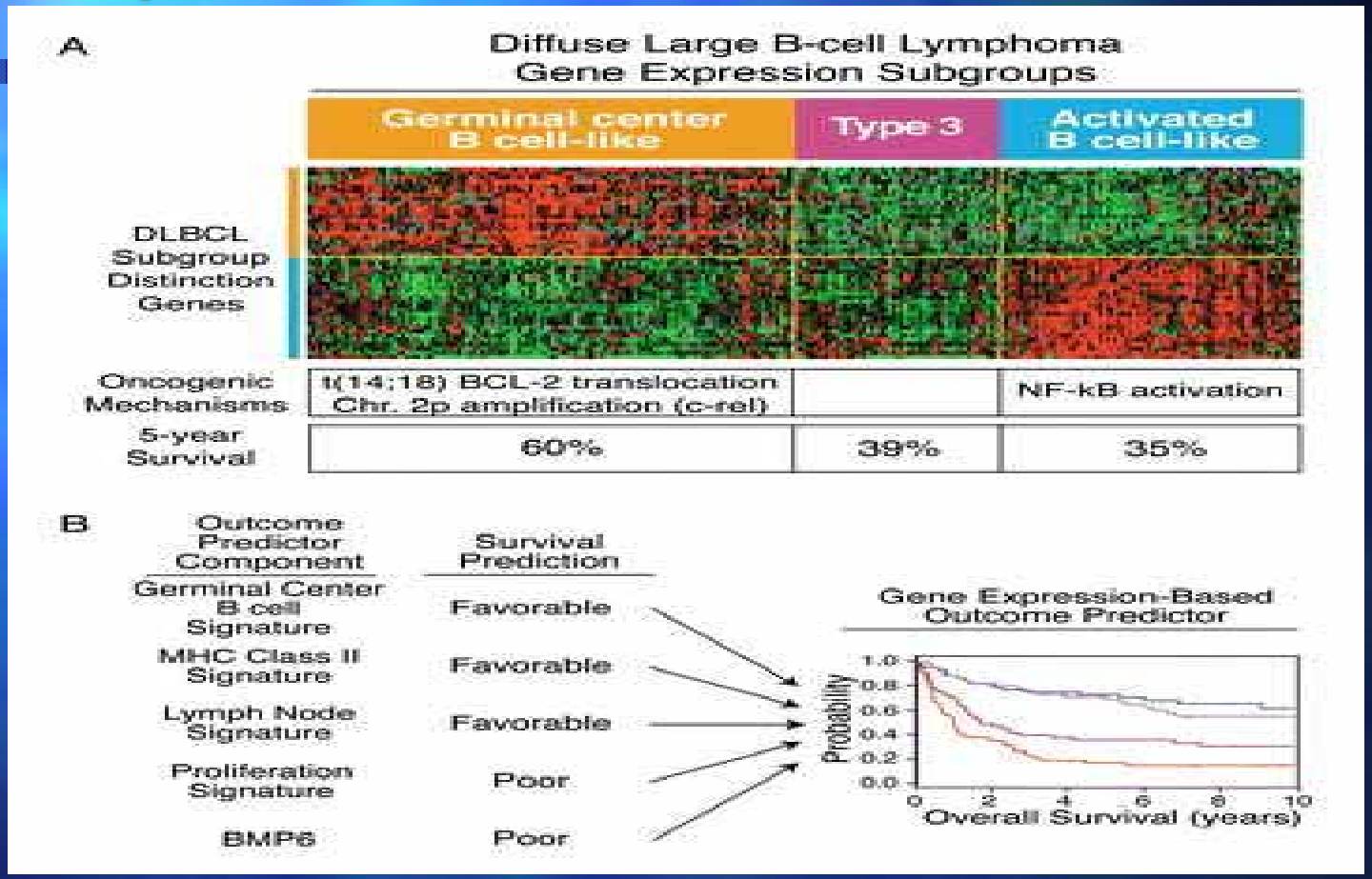
DNA, RNA and protein level

First time easily define molecular markers of disease susceptibility, progression and Rx response

BRASS goals

- Established in 2003 in initial collaboration with Millennium Pharmaceuticals
- To determine and validate biomarkers for disease activity and drug toxicity
- Monitor the natural history of disease and evaluate real world “effectiveness” of drug therapy
- Stimulate new research and knowledge in the field of RA and related inflammatory diseases.

Molecular Taxonomy: Lymphoma



Cancer Cell Vol. 2, No. 5, 11/02: 363 - 366

Structure of the RA registry

Registry	Structure	Data/specimens collected
B.R.A.S.S.	1000 RA patients per year for five years (30% new onset RA)	Physician data (yearly) Patient reported data (q6mon) Hand Radiographs DNA (once) RNA (yearly) Serum (yearly) Whole blood (fresh; yearly)
Prospective RA study	100 RA patients per year who are starting new therapy with either MTX or an anti-TNF (two year study)	Physician data (time zero, six and 12 weeks) Specimens (time zero, two, six, and 12 weeks): RNA, Serum, Whole blood (fresh)

Recruitment rate and followup

- First patient recruited March 2003
- 921 recruited to date.
- 98 dropouts, and 95 refusals
- Preliminary 6 month followup rate 90% after mailing

Data Collection

- Physician-based
  - RF, disease duration, RA Med/Surg History,extra-articular disease
  - Medications, Joint eval, Core set, VAS, Blood

Patient based

- Disease activity RADAI, Medications, employment, MDHAQ, medical history, SF-36, EuroQOL, FACIT, Resource Utilization.
- Hand Radiographs

Outcomes of Interest

- Mortality
- Erosive disease
- Decline in fn, QOL
- joint replacement
- extra-articular manifestations
- work disability
- CAM use
- CV, lung, osteoporosis
- Drug response- time to ACR 20,50
- osteoporosis, liver, malignancy, CHF, demyelinating disease
- DMARD patterns
- infection

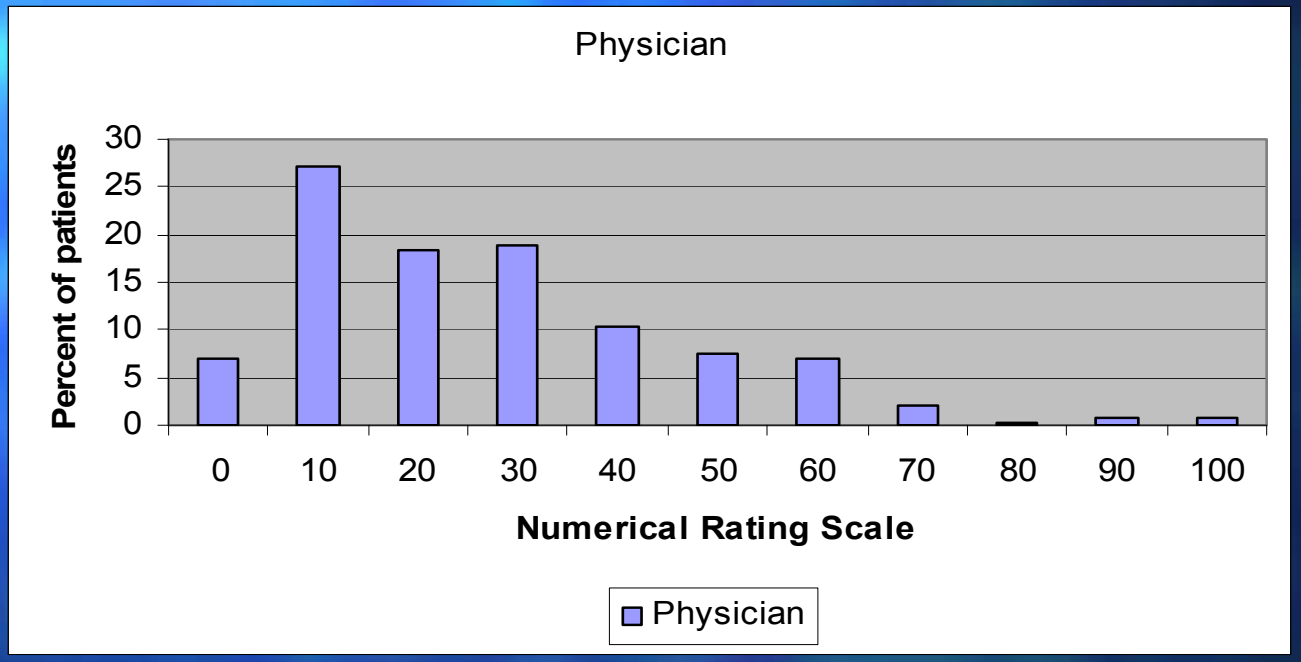
BRASS Cohort Characteristics

Number of Subjects	846 patients
Gender	82% female
Age	57.8±13.9 years
Duration of Disease	14.5±12.4 years
DAS28-CRP score	4.2±1.6
MD HAQ score	0.66±0.54
Use of DMARDS	736 patients (87%)
Methotrexate	406 patients (48%)
TNF-α inhibitors	311 patients (37%)

DAS Scores

- Low=34%
- Moderate=38%
- High=28%

Physician NRS



Strengths, Weaknesses, and Tradeoffs……

Weaknesses and Tradeoffs

- Disease only registry
- Clinic based- lose generalizability
- Expensive- sponsor

Registry Resources

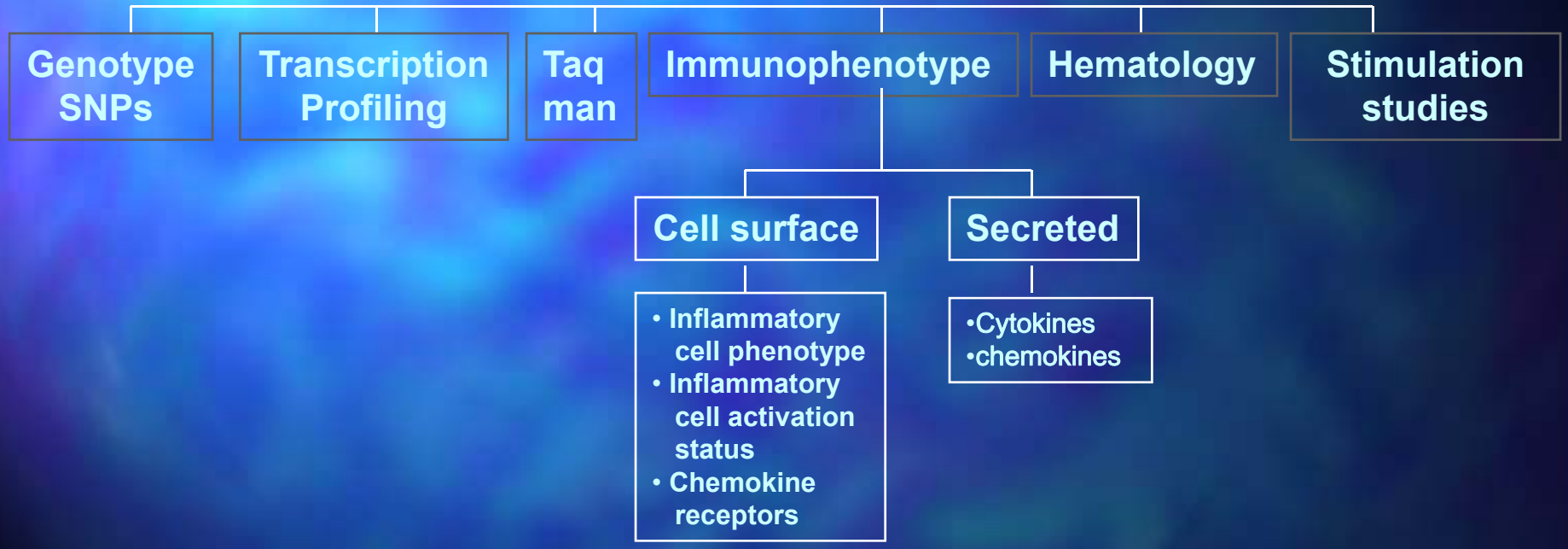
- Staff-
  - Sr project manager, Jr project manager
  - 4 RAs
  - Full time programmer
  - Statistician
  - Data entry personnel
  - Rheumatology fellow

What is the value of this effort?

- A well annotated sample bank at Millennium and Partners for future discovery
- Registries of this size and structure provide epidemiological data
- Ability to test biologic hypotheses related to disease related pathways and targeted interventions
- Potential to do biomarker discovery and validation activities for application towards
  - Early phase clinical trials (internal decision making around compound efficacy)
  - Late phase clinical trials (efficacy markers, pharmacogenomic markers)
  - Clinical assessment tools for practicing physicians
  - Opportunities for development of molecular diagnostics

Technology Platforms for biomarker discovery

Whole Blood (50ml)

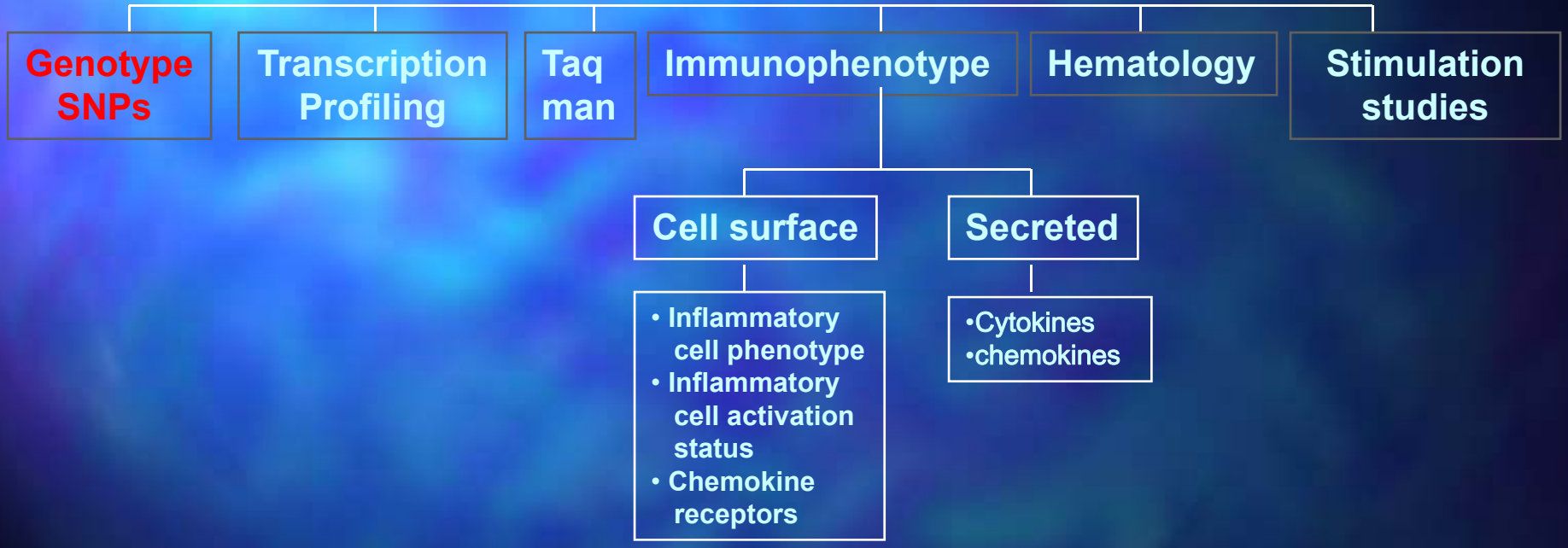


Biomarker Discovery

- Within individual components of the technology platform

RA Registry Studies

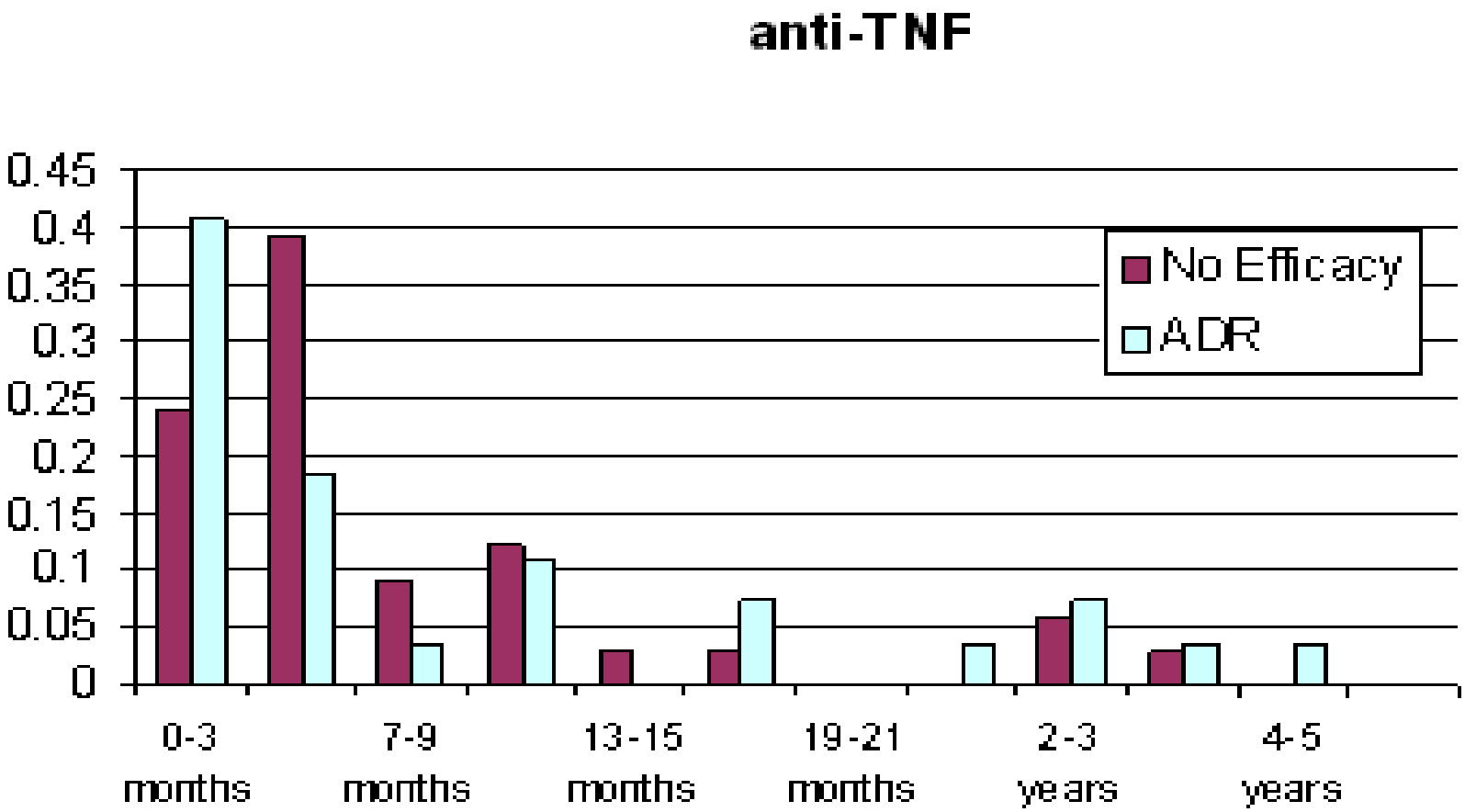
Whole Blood (50ml)



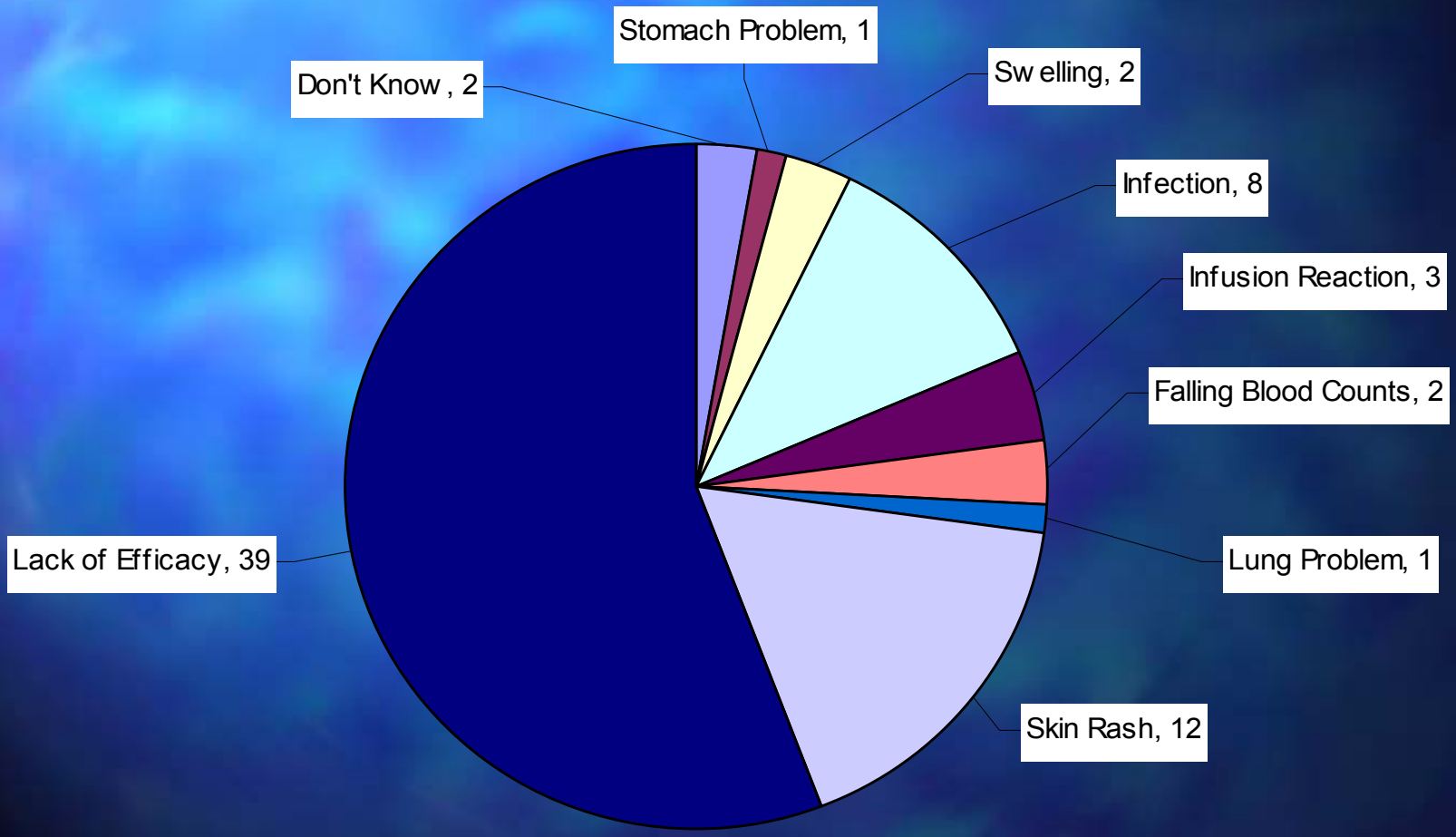
Objective

To identify genetic markers associated with efficacy and predisposition to adverse events during methotrexate (MTX) therapy or TNF-a blockade

Length of Exposure Prior to Discontinuation of anti-TNF Therapy



Reasons for Discontinuation of anti-TNF Therapy



Methods

- 31 genetic loci selected (including HLA-DRB1), all implicated in either risk for or severity of RA in at least 2 published studies
- Series of genetic markers, both VNTRs and SNPs, selected to characterize these genes in a recently-recruited RA registry
- Analyses made using contingency tables and multivariate logistic regression techniques

Table 2: Summary of Results

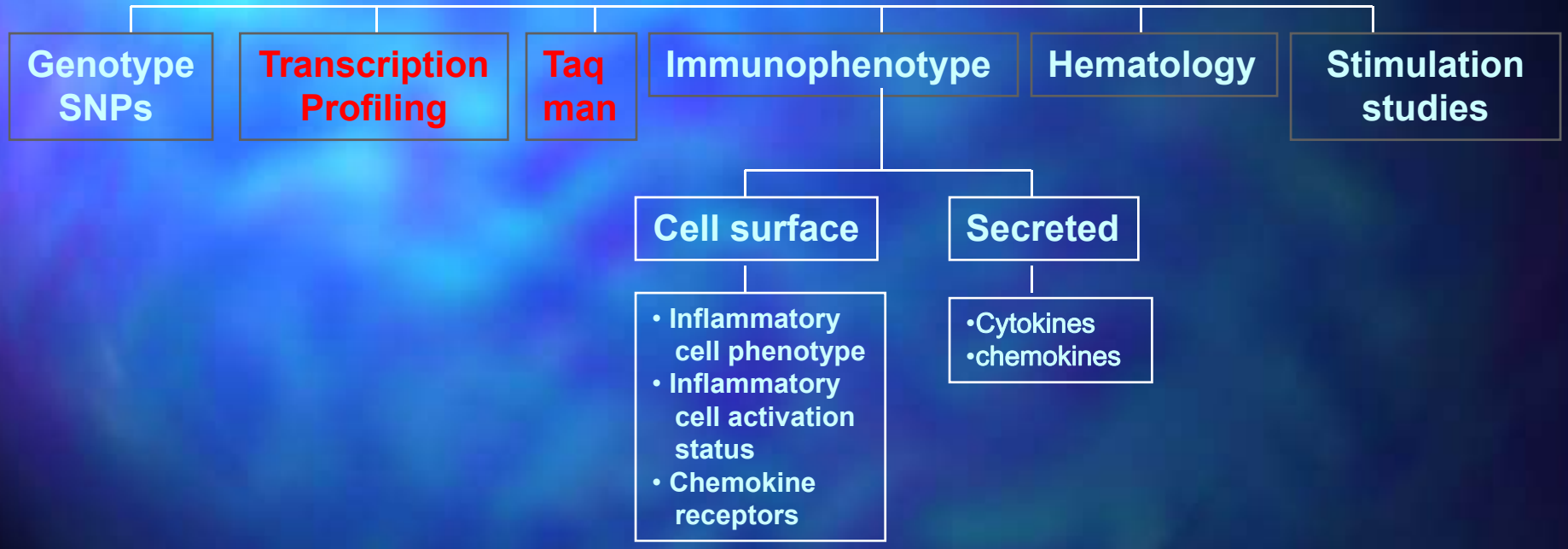
Phenotype Cohort	Drug Regimen	Locus	P-value
Lack of efficacy	MTX	CTLA4	0.0334
		IL1B	0.0079
		TNF	0.0217
		RUNX1	0.0034
		SLC11A1	0.0084
	TNF	FcGR2A	0.0176
		IL1RN	0.0086
		IL4R	0.0456
Adverse Events	MTX	IL1B	0.0140
	TNF	HLA-DRB1	0.0373
		IFNG	0.0495
		IL3	0.0405
		SLC19A1	0.0432
Severe Adverse Events	MTX	HLA-DRB1	0.0331
		CCR5	0.0077
	TNF	IL3	0.0072
		TNF	0.0148
		IL4R	0.0228
		PADI4	0.0192
		SLC19A1	0.0326
		SLC22A4	0.0496

Conclusion

- Results indicate a significant genetic component to the efficacy and toxicological profiles of two common RA therapies
- The non-overlapping sets of efficacy-associated genes suggest the potential for therapy-specific markers
- Our results also imply a central role for cytokines and their receptors in RA pharmacogenetics.

RA Registry Studies

Whole Blood (50ml)

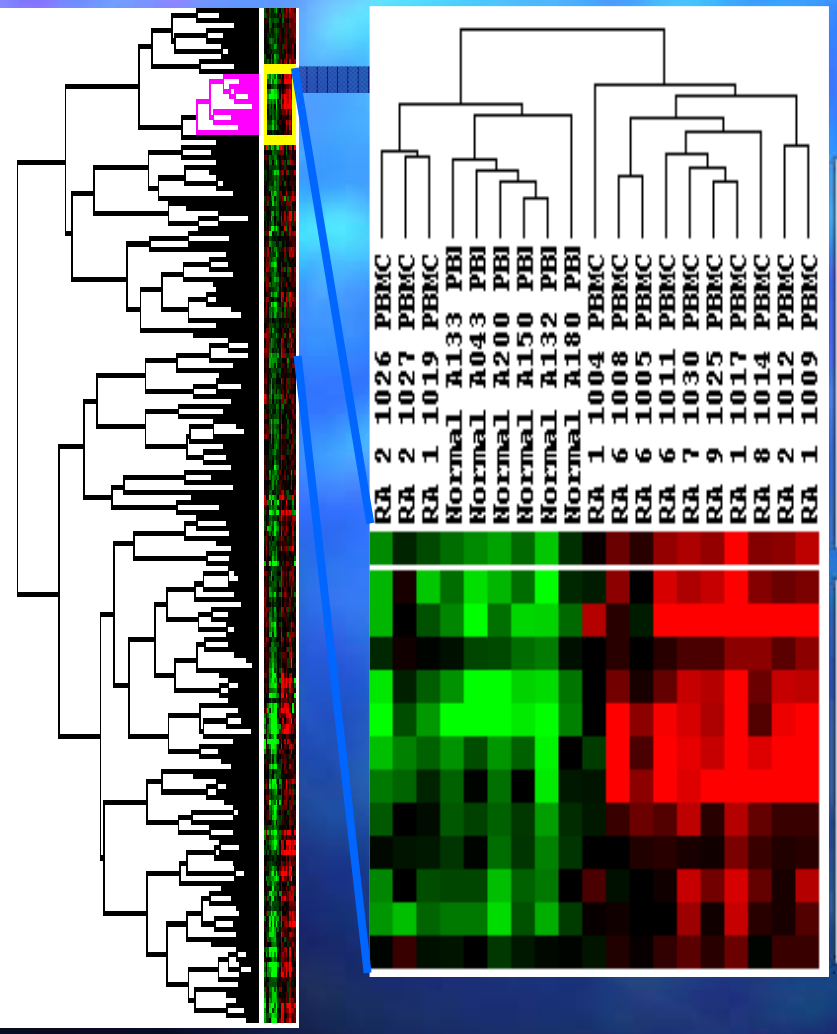


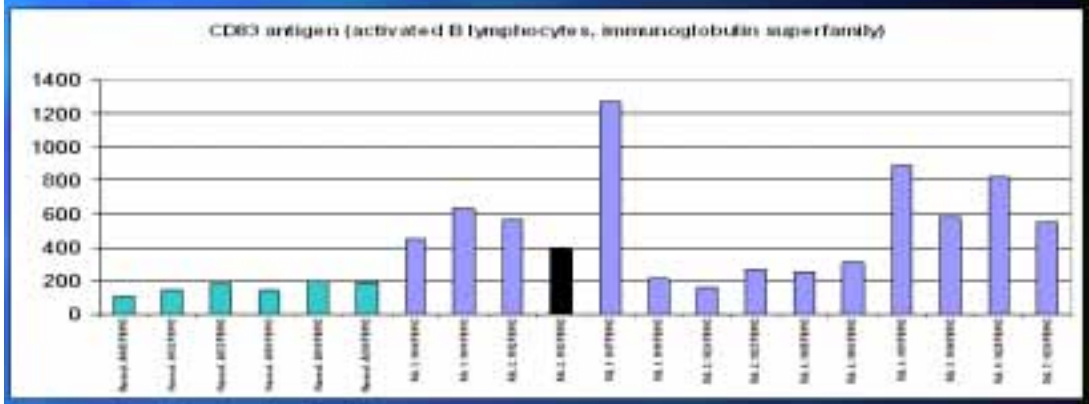
RNA analysis: Pilot study

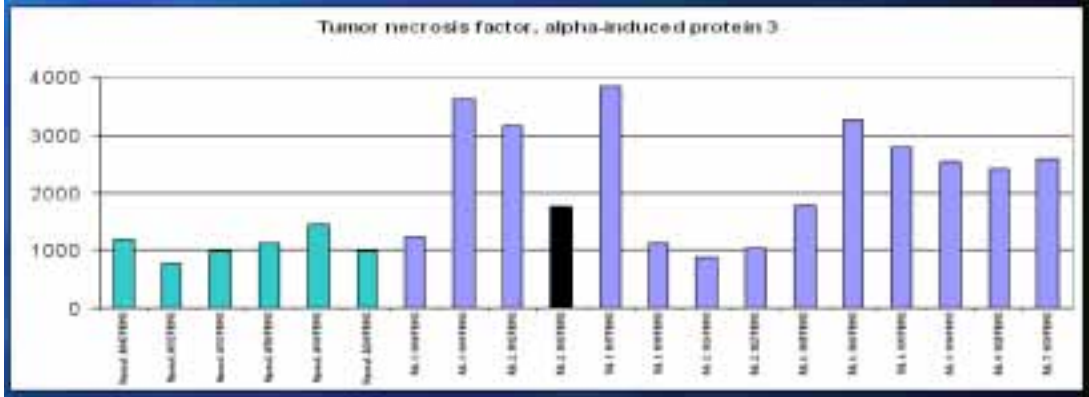
Results:

- Approximately 900 genes were significantly different between RA and NHVs, using PBMC transcriptional profiling
- Approximately 200 genes were significantly different between RA and NHVs, using Pax tube (whole blood) transcriptional profiling—reduction of detection likely secondary to rbc and PMN RNAs

Clustering Diagram Top 200 SNR Genes - PBMCs

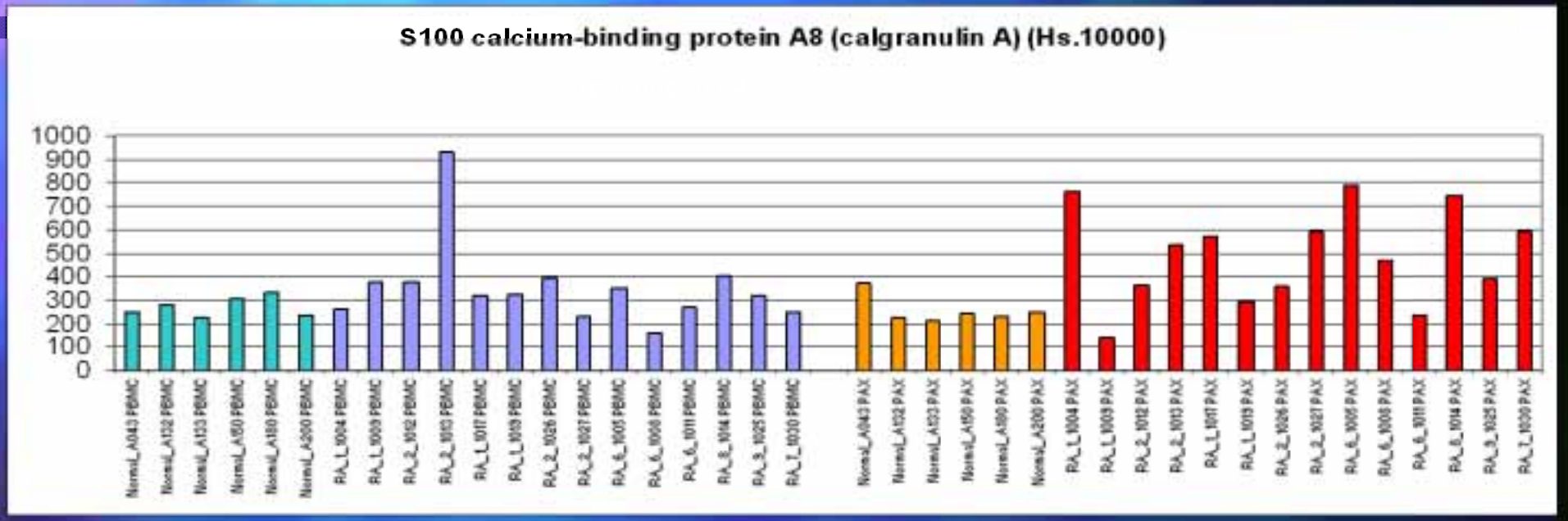






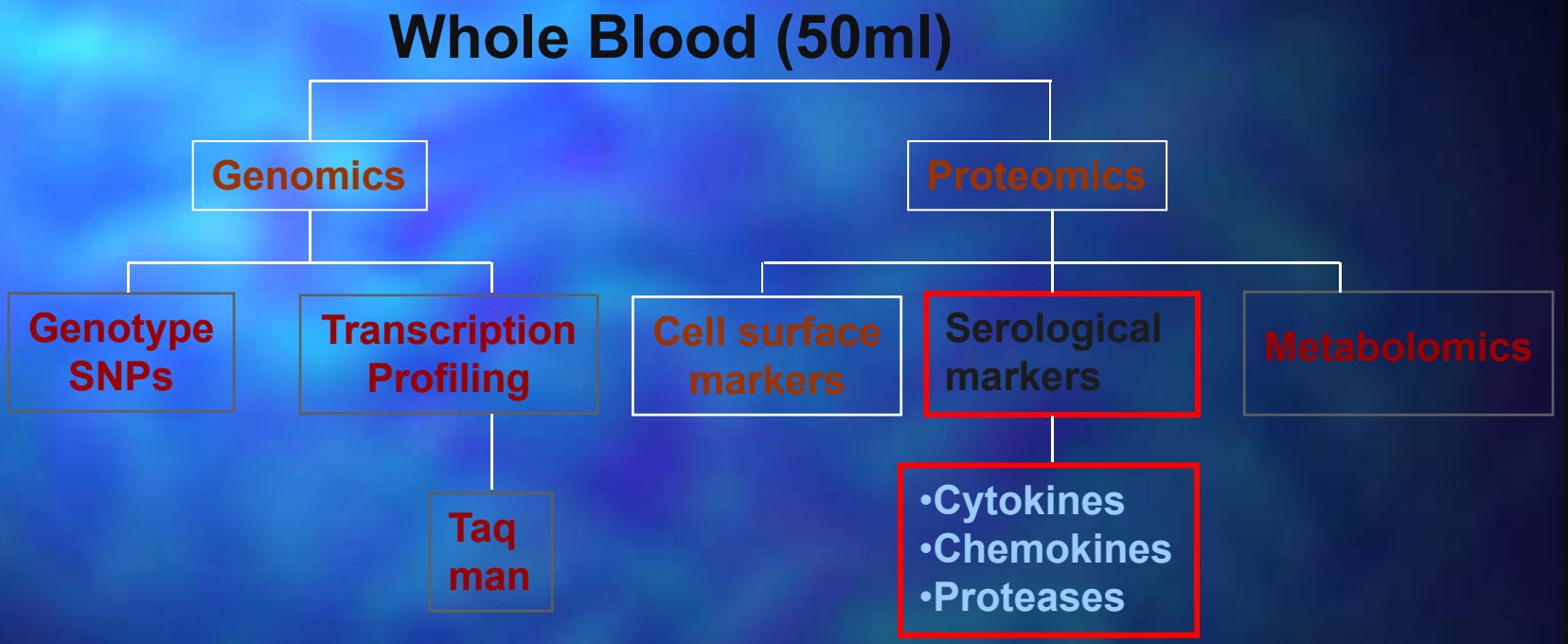
Top Genes for RA vs. Normal (PAX)

High SNR and POOF scores



Calgranulin A, Calgranulin C and the S-100 calcium binding proteins were identified by proteomic analysis as marker candidates for non-erosive RA

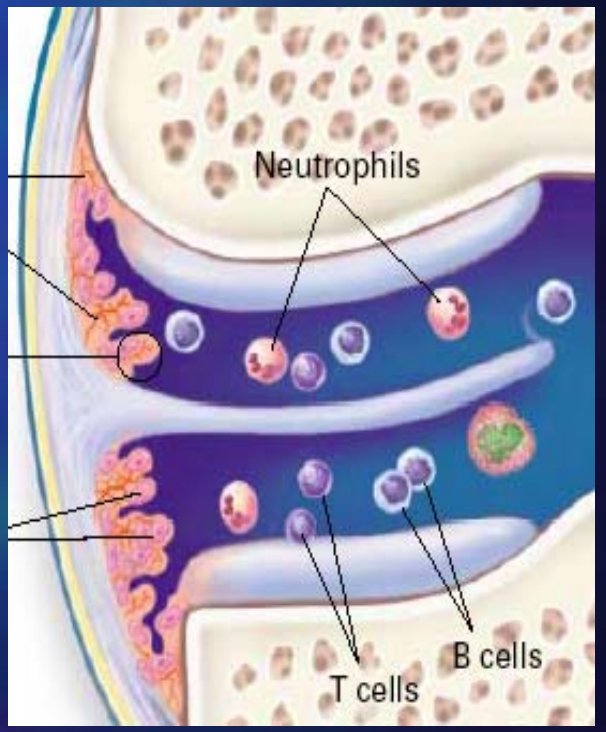
RA Registry Studies



Immunophenotyping
The Panel of Tests

- Cell phenotype markers
  - Monocytes, T cells, B cells, Grans, NK cells, APCs
- Monocyte activation markers
- T cell activation markers
  - CD4 effector/memory
  - CD8 Naïve/Memory
  - NKT cells
- B cell activation markers
- Chemokine receptor survey
- PD assay confirmation

NEJM, 2001, 344:907



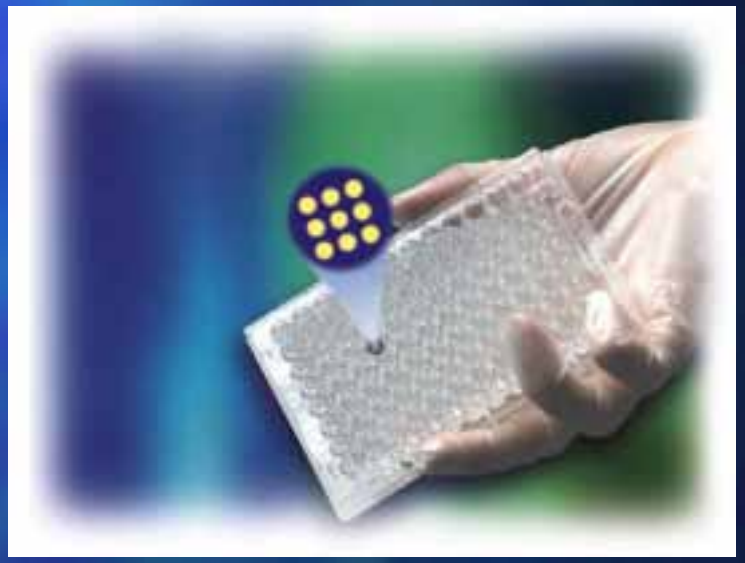
Proteomics

- Necessary due to lack of correlation between gene expression at the mRNA level with the amount of expressed protein.
- Protein-protein interaction, post-translational modification
- Can develop an ELISA test.

Biomarkers of Disease Activity

Panel I	Panel II
TNF-α	MadCAM
IFN-γ	I-CAM-1
IL-1β	vCAM
IL-4	E-selectin
IL-6	L-selectin
IL-7	RANTES
IL-8	VEGF
IL-10	MMP-1
IL-12 p70	MMP-2
IL-12 p40	MMP-3
IL-18	MMP-8
MCP-1	MMP-9
MIP-1α	TIMP-1
MIP-3α	TIMP-2
MMP-10	TNFR55
MMP-13	TNFR75

SearchLight™ Proteome Array (PerBio)



Association between DAS-28 and Protein Expression

Protein	P-value[a]	Odds Ratio	95% CI
MMP3	<0.0001	3.06	2.10-4.45
TNFR I	0.0005	3.61	2.05-6.37
IL6	0.0015	1.67	1.30-2.15
MMP1	0.0025	2.28	1.47-3.52
TNFR II[b]	0.0155	2.56	1.44-4.56
IL10	0.0185	1.47	1.17-1.85
IL4	0.0445	1.25	1.09-1.44

[a]Adjusted using permutation test (2000 permutations)

[b]Patients on Enbrel excluded

Proteins Most Associated with CRP

Protein	P-value	Odds Ratio	95% CI
IL6	<0.0001	2.25	1.63-3.11
MMP3	<0.0001	2.91	1.88-4.50
TNFR I	<0.0001	5.82	2.81-12.04
MMP1	<0.0001	3.61	2.11-6.18
IL4	0.0005	1.43	1.20-1.69
Rantes	0.0050	2.16	1.43-3.28
IL10	0.0205	1.54	1.19-2.01
IL18	0.0245	1.84	1.26-2.67
TNFR II[b]	0.0415	2.70	1.40-5.21

[a]Adjusted using permutation test (2000 permutations)

[b]Patients on Enbrel excluded

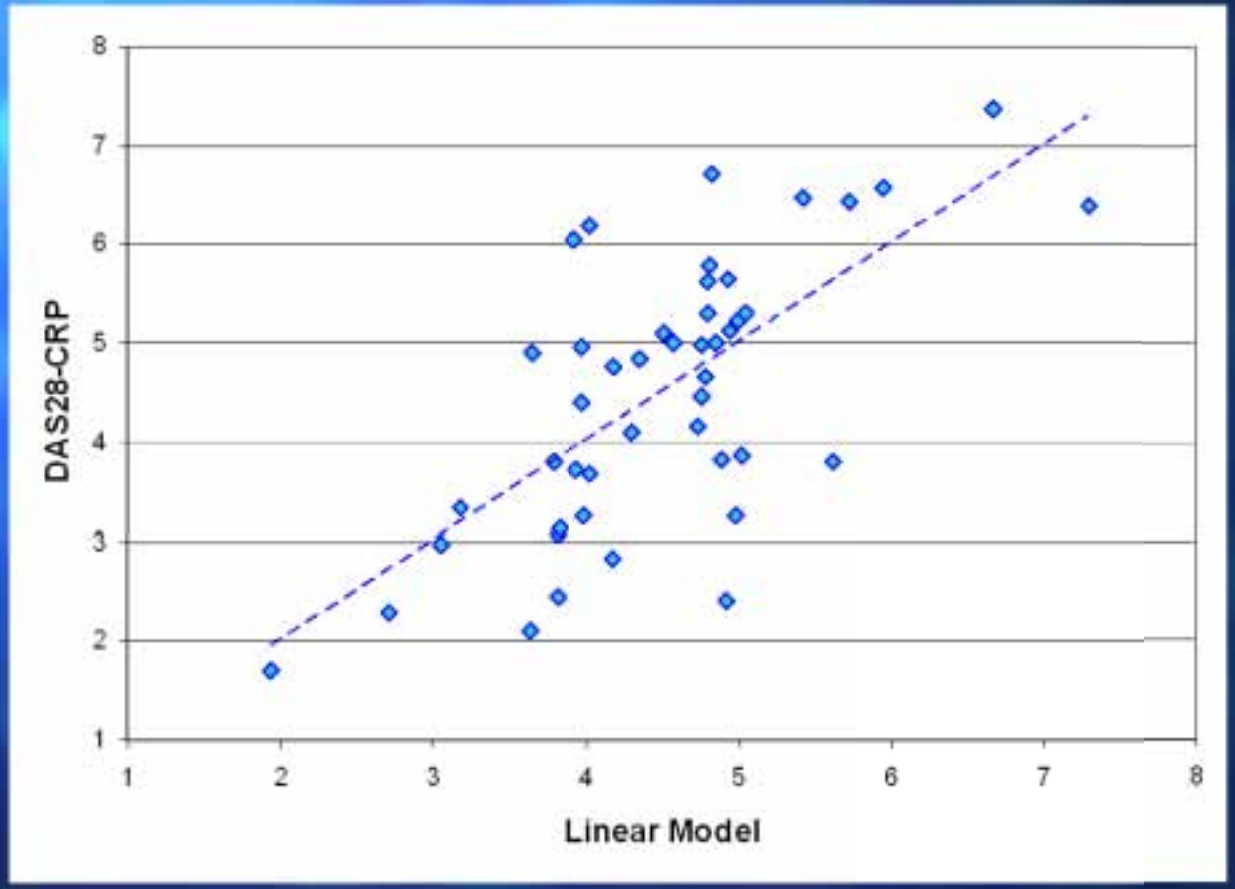
Conclusions

1. We have demonstrated that the expression levels of MMP3, TNFRI, IL6, MMP1, TNFRII, IL10 and IL4 are significantly associated with disease activity as judged by DAS 28 scores.
2. IL6, MMP3, TNFR I, MMP1, IL4, RANTES, IL10, IL18 and TNFR II were the proteins significantly associated with CRP.
3. Further steps in biomarker evaluation study will require validation with an independent set of samples.

Multidimensional marker sets predicting DAS score?

- Stepwise regression using:
- Clinical, genetic, proteomic and expression profiling data:
  - TNFRSF11, HLA-DRB1 genotypes,
  - serum MMP2 and MMP3 levels,
  - HLA-DQB1 mRNA abundance
  - rheumatoid nodule status

Multivariate linear model of DAS28-CRP score for BRASS subjects. Model incorporates TNFRSF11, HLA-DRB1 genotypes, serum MMP2 and MMP3 levels, HLA-DQB1 mRNA abundance, and rheumatoid nodule status. Multiple $R^2 = 0.421$, $P<0.0005$.



Future in genomics most exciting…..

Genome-wide association studies

- What is a genome wide scan?
  - Study whereby a dense set of SNPs across the genome is genotyped to:
    - survey the most common genetic variation for a role in disease or to:
    - identify the heritable quantitative traits that are risk factors for disease.

Why do a genome wide scan in BRASS?

- Find genes that influence RA
- Better understand the disease pathogenesis
- Rich clinical data for subphenotypes

Technology

- Illumina and Affymetrix chips (100K) and soon (500K) to comprehensively test a large fraction of common genetic variation (SNPs)across the genome

Genome-wide association studies

From vision…

How to test the role of *common variants* in complex disease such as RA

…**to reality**:

Practical with whole genome marker sets

Affymetrix 100K (116,204 SNPs)

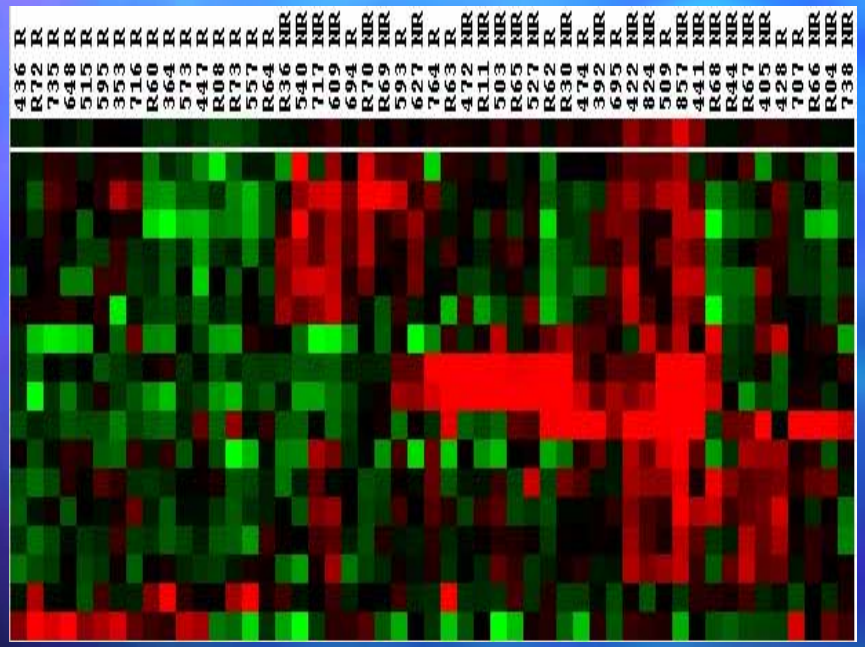
Hap Map Project

- International consortium to understand genetic variation in 269 samples from 4 geographic populations
- Set of closely linked markers on a chromosome tend to be inherited as a group
- Seeking susceptibility genes

First time…..Trifecta

- Large patient collections-registries
- Technology-products to efficiently test common genetic variation for its influence on disease (Affymetrix and Illumina chips)
- Understanding of genetic variation-millions of SNPs in public databases, Hap Map project

The Challenge of Translational Medicine/Personalized Medicine



Acknowledgements

Millennium Pharmaceuticals

Inflammation

Nancy Paz
Karen Anderson
Lisa Schopf
Miyoung Chun
Michael Hepperle
Hua Yang
Aileen Healy
Yajun Xu
Bruce Jaffee
Tim Ocain

Molecular Medicine

Michael Pickard
Andrew Damokosh
Joanne Meyer
Geoff Ginsburg
Julie Lekstrom-Himes
Alex Parker
Ronenn Roubenoff

BWH

Michael Weinblatt
Michael Brenner
Dan Solomon
Nancy Shadick
Nancy Maher
Robert Plenge
David Lee
Heather Matthews
Jessica Bilics
Ryan Lee
Lori Chibnik
Colin Maher
Jenny Heller
Roberta Glass

Pharmacogenomics

- **"Doctors will treat diseases like cancer and diabetes before the symptoms even begin, using medications that boost or counteract the effect of individual proteins… and they will know right from the start how to select the best medicine to suit each patient."**
- TIME 1/15/2001