

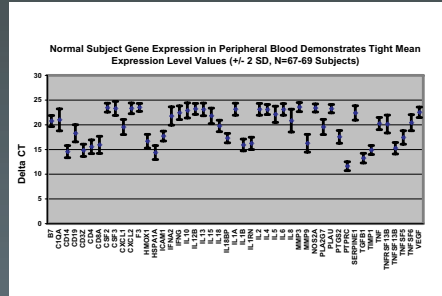
# HIGH-PRECISION GENE EXPRESSION ANALYSIS OF RHEUMATOID ARTHRITIS (RA) & OTHER INFLAMMATORY DISEASES

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## ABSTRACT

We applied high precision gene expression assays, disease/drug response databases & biomedical algorithms (Theratrax<sup>®</sup>) to examine mRNA levels in subjects with inflammatory diseases. Disease samples/data were obtained at clinical sites & normal subject samples at a blood bank with IRB review. Blood was drawn into PAXgene tubes (PreAnalytiX<sup>®</sup>) & cDNA analyzed by ABI Prism<sup>®</sup> 7700 (Applied Biosystems<sup>®</sup>). Disease groups showed elevated expression of numerous loci on a 72 gene panel compared to a normal group (n=69). Genes SERPING1 & ELA2 showed >4-fold increases between RA & normal groups & B-cell marker CD19 was suppressed in the RA study group. A number of genes were at similar levels in diseased & normal groups, such as IL8 & INF2A, acute inflammation & viral response markers. Use of a multi-gene algorithm showed marked differences between RA & normal subjects (3.6 SD units). Lupus flare and sepsis samples also show altered levels of inflammation gene mRNAs, revealing the utility of blood-based mRNAs as markers of inflammatory diseases.



Gene Loci	N	Mean	Median	Std Dev	%CV
VEGF	69	22.69	22.73	0.52	2.31
IFNG	69	22.66	22.64	0.59	2.60
IL18BP	69	17.43	17.39	0.47	2.69
ICAM1	69	17.93	17.95	0.49	2.71
B7	68	20.87	20.75	0.60	2.86
C1QA	67	21.29	21.23	0.94	4.41
HSPA1A	69	14.60	14.66	0.65	4.44
CD8A	69	16.19	16.17	0.77	4.73
IL8	69	20.80	20.91	1.10	5.28
MMP9	69	16.23	16.36	0.91	5.60

Table 1. Five Genes showing the Least and 5 Genes Showing the Most Variability Within the Source Normal Subject Reference Population (N=67 - 69)

Gene	RA mean dCt (n=19-24)	normal mean dCt (n=44-68)	Relative Expression (fold change from normal)	t test P value
SERPING1	16.7	18.9	4.72	<.0001*
ELA2	19.1	21.2	4.37	<.0001
ILTR1	18.8	20.7	3.93	<.0001
SERPINA1	11.9	13.8	3.77	<.0001
EGR1	19.1	20.9	3.57	<.0001
MMP9	14.6	16.4	3.45	<.0001
ADAM17	16.8	18.3	2.95	<.0001
TLR2	15.0	16.5	2.92	<.0001
IFIT6	14.6	16.1	2.84	<.0001
CYBB	13.0	14.5	2.78	<.0001
MPO	20.2	21.7	2.76	<.0001
HSPA1A	15.4	14.8	2.62	<.0001
SERPINE1	21.6	23.0	2.56	<.0001
TGFB1	12.2	13.6	2.52	<.0001
IL1RN	15.1	16.4	2.48	<.0001
PLA2G7	19.7	19.9	1.14	0.8591
INF2A	21.9	22.2	1.25	0.1285
IL6	21.0	21.0	1.01	0.9721
CD32	15.2	15.2	1.01	0.9495
CD19	19.3	18.7	0.64	0.009

Table 2. Disease Active RA Patient Population\* Expression Compared to the Source Normal Reference Population.

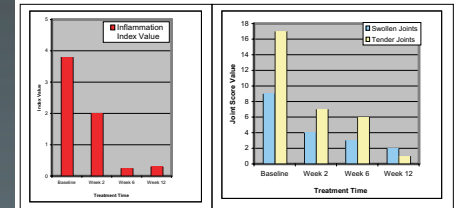
\* Active RA= 6 or more swollen joints, 9 or more tender joints, CRP > 2 mg/dL and may require introduction of more aggressive therapy.

Gene Loci	Lupus mean dCt (n=5)	Normals mean dCt (n=26-69)	Relative Expression (fold change from normals)	t test P value
Gene 1	17.46	20.89	10.76	<.0001
Gene 2	17.96	20.58	6.12	<.0001
Gene 3	13.49	16.00	5.70	<.0001
Gene 4	13.71	16.51	6.95	<.0001
Gene 5	17.55	17.73	1.13	0.4999
Gene 6	23.15	23.28	1.09	0.8177
Gene 7	19.16	17.40	0.29	<.0001
Gene 8	20.45	19.03	0.37	0.0009
Gene 9	20.47	19.17	0.41	0.001
Gene 10	20.73	19.53	0.52	0.004

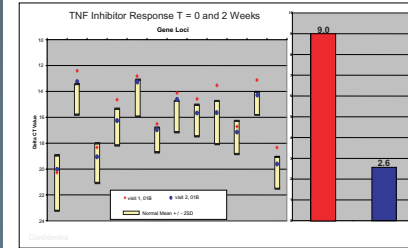
Table 3. Comparison of subjects experiencing lupus nephritis flares\* to normal subjects.

\* Active lupus nephritis flare was determined by a study subject's physician and defined as disease activity necessitating an increase in therapy (e.g., cyclophosphamide dosage increase)

Multi-gene Algorithm Correlates With Therapeutic Response in a RA Patient Initiating TNF Inhibitor Therapy



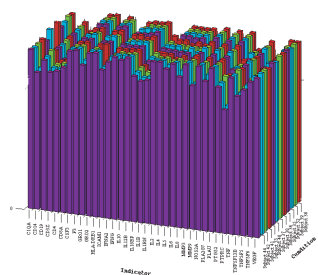
RA Subject Initiating TNF-Inhibitor Demonstrates Clear Gene Expression Responses at Individual Loci and in a Multi-gene Algorithm



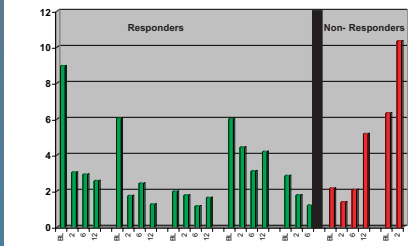
## Source TheraTrax Technology

- ❖ Precision Measurement of Gene Expression
- ❖ Normal Profiles
- ❖ Disease Versus Normal Profiles
  - Rheumatoid Arthritis
  - Lupus
- ❖ Tracking a Therapeutic Response

Gene Expression Pattern in Normal Individuals Is Remarkably Similar



Clinical Responders and Non-Responders Can Be Distinguished Using Gene Expression And Multi-gene Algorithms



\*Based upon physician's assessment

## SUMMARY AND CONCLUSIONS

- ❖ High-precision molecular analysis of whole blood gene expression shows great potential for the tracking of disease progression and therapeutic response.
- ❖ Populations of normal individuals exhibit consistent patterns of gene expression, making it possible to establish normal ranges.
- ❖ Gene expression in subjects with active RA and subjects experiencing lupus nephritis flare is significantly different across numerous gene loci compared to normal subjects.
- ❖ Multi-gene algorithms can be used to track responses to the introduction of new therapies in a clinical setting.
- ❖ Changes in gene expression in RA subjects, as measured by a multi-gene algorithm, correlate with clinical response.

## SOURCE PRECISION MEDICINE

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