Genomics and Proteomics in Immunotherapy Trials

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Francesco M Marincola
Immunogenetics Section, DTM/CC/NIH
Bethesda MD
Multidimensionality of tumor/host interactions in the context of T cell aimed immunization

1st dimension = TCR/HLA/epitope interaction
2nd dimension = Importance of co-stimulation
3rd dimension = Localization at tumor site
4th dimension = Evolving nature of immune response and genetic instability of cancer cells
5th dimension = Heterogeneity of the tumor micro-environment
T cell recognition of cancer cells

1) MAA
2) Proteasome
3) TAP
4) β2-microglobulin
5) HLA
6) Tolerizing agents: IL-10, TGF-β, EMAP-2, Antagonistic peptides, ETC.
7) FasL

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5th dimension = Heterogeneity of the tumor micro-environment
Most commonly expressed melanoma associated antigens

<table>
<thead>
<tr>
<th>RCC</th>
<th>Melanoma</th>
<th>EOC</th>
<th>Esophageal</th>
<th>CRC Primary</th>
<th>CRC Lymph Nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM</td>
<td>LN</td>
<td>CM (FNA)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The Systemic Response

Model: g209-2M peptide vaccine ± interleukin-2

Lee et al. J. Immunol. 1999, Kammula et al., 1999
Kammula et al. J Natl Canc Inst 2002
Monsurró et al. J Immunol 2000
Monsurró et al., J Immunol 2002

<table>
<thead>
<tr>
<th>Number of Immunizations</th>
<th>0i</th>
<th>8i</th>
<th>16i</th>
<th>24i</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8 FITC</td>
<td>0%</td>
<td>0.4%</td>
<td>0.3%</td>
<td>2.2%</td>
</tr>
</tbody>
</table>

No Clinical response
Few logical steps required for successful anti-cancer immunization

Immunogen → Local response

APC → LND → Loco-regional response

T cells → Circulation → Systemic response

Multidimensionality of tumor/host interactions in the context of T cell aimed immunization

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5th dimension = Heterogeneity of the tumor micro-environment
Functional heterogeneity of vaccine-induced CD8+ T cells

Pre-vaccination

Post-vaccination

Post-vaccination After IVS/209-2M

Monsurro’ et al. J Immunol 2002
Quiescent phenotype of tumor-specific CD8+ T cells following immunization

Perforin
Fas apoptotic inhibitor molecule 2
ENTPD1
DPP4
Granzyme A
Granzyme A
Nuclear factor of activated T cells 3
Cathepsin W
HLA-DM alpha
NK4

TNFRSF 1A modulator
LAK-4
IL F3
GPR 65
HLA-DRB4
Integrin α (VLA-4R)
TAL-1
NKG 5
Granzyme B
CD2
NKG7
TNFSF4 (ox40L)
Nuclear factor of activated T cells 3
Enolase 1
CD11a (LFA-1)

Monsurro’ et al. Blood 2004
Ex vivo cytotoxicity of immunization-induced CD8+ T cells

Monsurro' et al. Blood 2004
GFP-HLA fusion complex incorporation 

ex vivo by 

CD8+ immunization-induced T cells

Monsurro’ et al. Blood 2004
Preferential proliferation of Ag-specific T cell

EphB2 (ptk)
Bak=bcl2 family
Nitrogen activated PK3
Cyclophilin B
Annexin A8
Cyclophilin A
Nitrogen activated PK3
Mortality factor 4 like 2
ISGF3γ (IFN α/β responsive TF)
Nitrogen activated PK3
Eukariotic translation factor
Cyclin D3
NTF2
MPK1
Guanine nucleotide binding protein
Lck
Fibroblast growth factor 2
Tubulin α1
PTP type 6

*Monsurro’ et al. Blood 2004*
Global Approach: Biological Considerations

- **Immunogen**: Local response
- **LND**: Loco-regional response
- **Circulation**: Systemic response
- **Tumor site**: Peripheral response
- **Clinical Non-Response**
- **Clinical Response**

*Marincola et al. Trends in Immunol. 2003*
Studying the tumor micro-environment

Excisional Biopsies
- Good quantity of material to study
- Do not allow serial sampling of same lesion
- Do not allow prospective assessment of natural history of a given lesion

Fine Needle Aspirates
- Limited quantity of material to study
- They allow serial sampling of same lesion
- They allow prospective follow up of a given lesion

Wang and Marincola, Immunol Today 2000
Multidimensionality of tumor/host interactions in the context of T cell aimed immunization

1\textsuperscript{st} dimension = TCR/HLA/peptide interaction
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5\textsuperscript{th} dimension = Heterogeneity of the tumor micro-environment
**Kinetics of IFN-γ and gp-100 expression in melanoma metastases**

**Treatment:** gp100 based vaccine  
**Question:** Vaccine-elicited T cell may not localize at tumor site.  
**Results:**  
Vaccine-elicited T cells  
1) localize at tumor site  
2) interact with the tumor cells  
3) this is not sufficient for tumor rejection

<table>
<thead>
<tr>
<th>Site</th>
<th>IFN/CD8</th>
<th>Fold Inc</th>
<th>GP100/Actin</th>
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<tbody>
<tr>
<td>R axilla</td>
<td>568</td>
<td>1242</td>
<td></td>
</tr>
<tr>
<td>L thigh</td>
<td>7586</td>
<td>13.4</td>
<td>1310</td>
</tr>
<tr>
<td></td>
<td>11865</td>
<td>35.8</td>
<td>3955</td>
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<tr>
<td>L axilla</td>
<td>1187</td>
<td>6186</td>
<td></td>
</tr>
<tr>
<td>L thigh</td>
<td>7891</td>
<td>6.6</td>
<td>611</td>
</tr>
<tr>
<td></td>
<td>7788</td>
<td>13.5</td>
<td>894</td>
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<td>R thigh</td>
<td>2231</td>
<td>4452</td>
<td>235</td>
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<td></td>
<td>5532</td>
<td>5.5</td>
<td>18</td>
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<tr>
<td>L chest</td>
<td>1013</td>
<td>1</td>
<td></td>
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<td></td>
<td>1649</td>
<td>0.5</td>
<td>1</td>
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<td>L lat knee</td>
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<td></td>
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<tr>
<td></td>
<td>759</td>
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<td>R groin</td>
<td>692</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1474</td>
<td>2.1</td>
<td>12</td>
</tr>
</tbody>
</table>

**Kammula et al., J. Immunol., 1999**
Multidimensionality of tumor/host interactions in the context of T cell aimed immunization

Immunology

1\textsuperscript{st} dimension = TCR/HLA/peptide interaction
2\textsuperscript{nd} dimension = Importance of co-stimulation
3\textsuperscript{rd} dimension = Localization at tumor site
4\textsuperscript{th} dimension = Evolving nature of immune response and genetic instability of cancer cells
5\textsuperscript{th} dimension = Heterogeneity of the tumor micro-environment
Tumor variability and evolving with time

CR = Complete Response
NR = No Response

$p_2 = 0.003$

Proposed hypothesis of how antigen-specific therapy might affect target antigen expression

= Target antigen present
= Target antigen absent

Primary Effect

Inflammation

Effective

Not effective

Marincola et al., Trends in Immunology, 2003
Multidimensionality of tumor/host interactions in the context of T cell aimed immunization

1\textsuperscript{st} dimension = TCR/HLA/peptide interaction
2\textsuperscript{nd} dimension = Importance of co-stimulation
3\textsuperscript{rd} dimension = Localization at tumor site
4\textsuperscript{th} dimension = Evolving nature of immune response and genetic instability of cancer cells
5\textsuperscript{th} dimension = Heterogeneity of the tumor micro-environment
A global approach to Identify the algorithm responsible for tumor rejection in humans
Genes differentially expressed pre-treatment in immune responsive metastases

Wang et al. Nature Biotech 2000
Wang et al. Cancer Res. 2002
Genes differentially expressed between pre- vs post-treatment CR associated with responsiveness of:
- genital warts to Imiquimod
- carcinoid tumors to IFN-α
- CML to IFN-α

SDF-1 (CXCL12): T cell accumulation in RA recruitment of pDCs in lymph nodes and peripheral sites of inflammation.

Wang et al. Cancer Res. 2002
Genes associated with immune response during IL-2 therapy

Panelli et al Genome Biol 2002

CR LESSION POST IMMUNIZATION EXPRESSED GENE
SIMILAR EXPRESSION TO NK EXPOSED TO IL-2
UPREGULATED IN ACUTE REJECTION OF KIDNEY TRANSPLANT (PBMC AND RENAL BIOPSY TISSUE Sorwal, H. Immunol, 2001)

TIA-1=nucleolysin cytotoxic granule
NK4= natural killer cell protein 4
NKG5=granulysin
EBI3
TCR alpha
DAG kinase
HLA class II region expressed gene KE4
MHC class II DR beta
SERPINB1=Leukocyte elastase inhibitor
MIP-1 delta
FGF-13
STIM1=Stromal interaction molecule 1
VEGF
CD62 P selectin
GALECTIN 1
GALECTIN 1
N-Myc
DAP-1
53BP1=p53 binding protein
Postulated mechanism(s) of action of systemic IL-2 administration

**IL-2**

**PBMC**

Gene expression

- MCP-3
- MIP1-β
- MIP1-α
- MCP-1
- PARC
- MCP1
- IL-8
- GRO-1
- MIG

Philippe

Enhancement of innate effector mechanisms

- Chemo-attraction
- M1-immune stimulation
Unsupervised Hierarchical clustering of serum samples from RCC patients obtained pre, post 1 and post 4 doses of IL-2 (720,000IU/kg).

- = soluble factor minimally changing from pre to post 4 doses
- = soluble factor expression enhanced at 4 doses only
- = soluble factor expression enhanced post 1 dose
- = soluble factor expression enhanced at 1 and 4 doses

Panelli et al. J Translat Med, 2004
SAX2 SELDI ANALYSIS OF RCC serum

Rossi L. and Panelli MC manuscript in preparation
SELDI Immunoaffinity capture of SAA

Rossi L. and Panelli MC manuscript in preparation
Postulated algorithm of tumor immune responsiveness

Spontaneous release of immune modulators by tumor cells

Antigen-specific immunization
- IFN-γ
- MIP-1α
- IL-2
- TARC

Non-specific immune stimulation = IL-2

Immune stimulation/inflammation
- IL-4
- IL-13
- IL-8
- RANTES

Threshold for tumor regression

Spontaneous regression

Response to therapy

Spontaneous release of immune modulators by tumor cells

Tumor progression

Marincola et al., Trends in Immunol. 2003
Global Approach: Technical Considerations

Genetic Background
- DNA
  - SNP detection

Transcriptional Analysis
- RNA
  - cDNA arrays
  - Transcriptome array

Functional Product
- Protein
  - Proteomics

Functional Validation
Hypothesis of the study:

Whether two prototype populations can be segregated according to functional and genetic parameters?
Functional and genetic differences between Chinese and Caucasian subjects in response to IL-2 or LPS

- Chinese n=30 vs Caucasian n=30
- IL2 and LPS stimulation
- PBMC
- DNA
  - Cytokine polymorphism
- mRNA
  - Gene profiling
- SUPERNATANT
  - Proteomics
Identification of genes induced by IL2 or LPS in different ethnic groups
(Paired two sample t test, p<0.005, exp present >80%, 16,000 total gene used)

<table>
<thead>
<tr>
<th></th>
<th>IL2 vs No-STI</th>
<th>IL2 vs No-STI</th>
<th>LPS vs No-STI</th>
<th>LPS vs No-STI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Caucasian</td>
<td>Chinese</td>
<td>Caucasian</td>
<td>Chinese</td>
</tr>
<tr>
<td></td>
<td>N = 15</td>
<td>N = 12</td>
<td>N = 15</td>
<td>N = 12</td>
</tr>
<tr>
<td>IL-2-induced genes (paired p&lt;0.005)</td>
<td>808</td>
<td>209</td>
<td>989</td>
<td>344</td>
</tr>
<tr>
<td>Permutation</td>
<td>0.0017</td>
<td>0.06</td>
<td>0.0003</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Ethnic Distribution based on IL2 inducible genes

Genes inducible in **Caucasians**
808 genes

- IL-2-D3-Ch
- IL-2-D6-Ca
- IL-2-D5-Ch
- IL-2-D1-Ca
- IL-2-D19-Ca
- IL-2-D14-Ch
- IL-2-D11-Ca
- IL-2-D2-Ca
- IL-2-D9-Ca
- IL-2-D7-Ca
- IL-2-D13-Ch
- IL-2-D8-Ca
- IL-2-D26-Ca
- IL-2-D16-Ca
- IL-2-D24-Ca
- IL-2-D18-Ca
- IL-2-D25-Ca
- IL-2-D22-Ca
- IL-2-D20-Ca
- IL-2-D15-Ch
- IL-2-D10-Ch
- IL-2-D4-Ch
- IL-2-D12-Ch
- IL-2-D27-Ch
- IL-2-D23-Ch

**Fisher test Ca vs Ch cluster;** $p_2 < 0.001$

Genes inducible in **Chinese**
209 genes

- IL-2-D27-Ch
- IL-2-D23-Ch
- IL-2-D1-Ch
- IL-2-D12-Ch
- IL-2-D14-Ch
- IL-2-D24-Ca
- IL-2-D19-Ca
- IL-2-D21-Ch
- IL-2-D15-Ch
- IL-2-D4-Ch
- IL-2-D8-Ca
- IL-2-D7-Ca
- IL-2-D2-Ca
- IL-2-D13-Ch
- IL-2-D9-Ca
- IL-2-D18-Ca
- IL-2-D22-Ca
- IL-2-D26-Ca
- IL-2-D16-Ca
- IL-2-D24-Ca
- IL-2-D18-Ca
- IL-2-D25-Ca
- IL-2-D11-Ca
- IL-2-D5-Ch
- IL-2-D20-Ca
- IL-2-D2-Ch
- IL-2-D6-Ca
- IL-2-D10-Ch
- IL-2-D17-Ch

**Fisher test Ca vs Ch cluster;** $p_2 = 0.15$
Acknowledgments

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Polly Matzinger

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Harvey Klein
A new polymorphism-detection method for broad genome investigations in the context of clinical trials

Special Thanks
RCC
Melanoma
EOC
Esophageal
Colorectal Primary
Colorectal Lymph Nodes

Growth regulation
RCC predominance
Immunological Signature
Sub-cutaneous metastases
Blood Contamination in FNA
MDA-Specific cluster
L1CAM
TGFBR2
ENO2
ENO2
calumenin
MEF2C
PLD1
PTPRM
PTPRM
SYT6
CCL4
HBD1
HBD1
SLC25A4
IRAK1
GPR56
CPEB4
CPEB4
RASGDS
TRX2
TRX2
ADAMTS5
INGE34514
SLC1A2
MTF1
MTF1
SLC16A4
SDCCAG8
LAMA1
SNAI5
FUT11
SDC3
APOE
TNEM22

L1 cell adhesion molecule (hydrocephalus, stenosis of aqueduct)
insulin-like growth factor binding protein 2
ENO2-Enolase 2 (gamma, neuronal)
enolase 2 (gamma, neuronal)
calumenin
MEF2C box transcription enhancer factor 2, polypeptide C
procollagen-lysine, 2-oxoglutarate 5-dioxygenase (lysine
epoxide 5-dioxygenase)
R-PTP-MA-protein tyrosine phosphatase, receptor type, M
protein tyrosine phosphatase, receptor type, M
snare homolog 1 (Drosophila)
c-cbl-Casitas B-lineage lymphoma protein-syk-inhibiting a

cancer cell adhesion molecule

macrophage migration inhibitory factor (glycosylation-inhib

hepatitis B virus x interacting protein

hepatitis B virus x interacting protein

soluble carrier family 25 (mitochondrial carrier: adenosine

inhibitor of kappa light polypeptide gene enhancer in B-c

G protein-coupled receptor 56

cytoplasmic polyadenylation element binding protein 4

cytoplasmic polyadenylation element binding protein 4

Ras-related GTP binding D

T-box 2

T-box 2

a disintegrin-like and metalloprotease (reprolysin type)

SWAP1 protein

solute carrier family 7 (cationic amino acid transporter,

microphthalmia-associated transcription factor

microphthalmia-associated transcription factor

soluble carrier family 16 ( monocarboxylic acid transporter

serologically defined colon cancer antigen 8

laminin, alpha 1

SNAI5

fucosyltransferase 11 (alpha 1,3 fucosyltransferase)

syndecan 3 (syndecan)

apolipoprotein E

transmembrane protein 22
A new polymorphism-detection method for broad genome investigations in the context of clinical trials

A*0101/A*0101
a,a homozygosity

A*0201/A*0101
b,b homozygosity

A*0101,0201 / A*0101
a,b heterozygosity

Analysis of the effect of human polymorphism on immune responses:

Cytokine polymorphism chip

<table>
<thead>
<tr>
<th>Gene family Name</th>
<th>Gene number</th>
<th>Oligo number</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF</td>
<td>7</td>
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<tr>
<td>IFN</td>
<td>21</td>
<td>490</td>
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<tr>
<td>CSF</td>
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<td>TNF</td>
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<td>IL</td>
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<td>IRF</td>
<td>8</td>
<td>516</td>
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<td>STAT</td>
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<td>296</td>
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<td>JAK</td>
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<td>NF-kB</td>
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<td>1332</td>
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<tr>
<td>TLR</td>
<td>10</td>
<td>630</td>
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<tr>
<td>NK cell</td>
<td>25</td>
<td>544</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>255</strong></td>
<td><strong>10395</strong></td>
</tr>
</tbody>
</table>
Cytokine polymorphism detection chip
In Vitro Induction of Epitope Specific anti-Melanoma CTL

A) PBMC $3 \times 10^6$

- Peptide $\mu$M (AAGIGILTV)
- IL-2 30 IU/ml after 24 hrs

B) Weekly restimulations with irradiated (3,000 rads) autologous PBMC pulsed with peptide

Cytotoxicity Assay or Cytokine Release

Differential anti-MART-1 Reactivity in the Peripheral Blood of Melanoma Patients in Comparison to Healthy Donors

Rivoltini et al., J Immunol 1995

Marincola et al., J. Immunother 1996
Conclusions

• Comprehensive monitoring of cancer vaccines should be broadened from the study of systemic immune responses to include the evaluation:
  The immune responses at tumor site
  The genetic make up of each patient
  The genetic heterogeneity of individual patients’ cancers at the transcriptional and the protein level

• Tools are now available that could be applied in the context of immunotherapy trials at relatively low cost and effort if samples are appropriately collected
**MAA of the melanocytic lineage**
*Melanoma Differentiation Antigens*

(expressed by 70 to 80 % of melanoma lesions)

<table>
<thead>
<tr>
<th>Protein</th>
<th>Peptide Region</th>
<th>HLA-A*</th>
<th>Peptide Sequence</th>
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<td>MART-1</td>
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<td>Tyrosinase</td>
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<td>MLLAVLYLL</td>
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<td>YEIWRDIDF</td>
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<td>206-214</td>
<td>A2402</td>
<td>AFLPWHRLF</td>
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<tr>
<td></td>
<td>369-377</td>
<td></td>
<td>YMNGTMSQV</td>
</tr>
</tbody>
</table>
Specific activation of PBL *in vivo* by MAA derived epitopes as demonstrated by *in vitro* testing

(Cormier et al., Cancer J. Sci Am. 1997).
Response rate after peptide-based vaccinations against melanoma

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Responses</th>
<th>Patients</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>MART-1$_{27-35}$ in IFA</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>g209-2M in IFA</td>
<td>0</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>g209-2M in IFA + IL-2 (720,000 IU/Kg TID)</td>
<td>13</td>
<td>31</td>
<td>42</td>
</tr>
</tbody>
</table>

**TEST sample**
- 25 patients
- 37 melanoma metastases
- 63 FNA samples
  - CR
  - PR
  - SD
  - NR

**Reference sample:**
- Mixture of 6 normal donor PBMC
- 6,108 human cDNA array

**Control sample:**
- NHEM - Normal Human Epithelial Melanocyte
- FB - Fibroblast cell line from melanoma metastasis
- RCC - Renal carcinoma
- Ocu - Ocular melanoma

Wang et al. Cancer Res. 2002*
60% Epithelioid Melanoma
20% Pleyomorphic Transition Zone
20% Chondrosarcoma

Wang et al. Cancer Res. 2002
<table>
<thead>
<tr>
<th>Clone ID</th>
<th>Abbrev.</th>
<th>Title</th>
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<th>Relative expression</th>
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</table>
What is the role of interleukin-2

Panelli et al Genome Biol 2002

- Highest median across experiments: cell surface, adhesion, inflammatory proteins
- MHC class II DR alpha
- MHC class II DR beta
- Grancalcin Ca2+ binding protein in neutrophils and monocytes
- Calgranulin Ca2+ binding protein
- CD62L L-selectin
- CD45
- V-CAM-1
- CD64
- CD29 integrin=beta 1 fibronectin receptor (Fibronectin 1)
- Keratin 10
- IL-1 R
- IL-1 receptor antagonist
- IL-2 R beta chain
- TNF-a induced protein 3
- TGFβ receptor

- Interferon-γ IEF SSP5111upregulated protein (HSP70)
- MxA/interferon induced cellular resistance protein
- MxB interferon induced cellular resistance mediator protein (Interferon-a inducible protein IFI-6-16)
- Guanylate binding protein 1 interferon inducible
- IRF-1 interferon regulatory factor-1
- IFN induced 56KDa protein
- IFNγ receptor alpha chain
- Nmi=IL-2 and IFN-g inducible potentiator of STAT

- Human insulin like growth factor
- C-C chemokine receptor 1
- GRO-1 melanoma growth stimulatory activity chemokine
- MIG chemokine targeting T cells (MCP-1)
- MCP-3
- MIP-1beta
- MIP-1 alpha
- PARC=DC-CK1 chemokine targeting T cells not monocytes
- Monocyte neutrophils elastase inhibitor
- IL-8 chemokine
- Plasminigen activator urokinase
Primary Goal of this study: Is it possible to link functional to genetic information using high-throughput technology?

• Rationale:
  – Human immunology is a complex discipline encompassing **human polymorphism and epigenetic adaptation** to heterogeneous environmental stimuli.
  
  – **Transcriptional and/or post-translational analyses** (i.e. cDNA arrays) yield information about the cellular response to a given situation without segregating genetic predisposition from epigenetic adaptation.

  – **Genome wide screening** for genetic variation could lead to the identification of consistent patterns in a given population that could segregate the functional influence of genetic variability from that of epigenetic adaptation.

*Jin P and Wang E. Immune polymorphism: from HLA typing to immunogenetic profiling
Wang E and Falus A. Changing paradigm through a genome-based approach to clinical and basic immunology
http://www.translational-medicine.com*