Characterization of Complete Response to IL-2 Using Gene Expression Analysis and Tissue Array Validation in Metastatic RCCa

Allan J. Pantuck, M.D.
UCLA Department of Urology
Society of Biologic Therapy
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Goals

• Identification of prognostic factors to select patients most likely to respond to IL-2 to maximize therapeutic efficacy and minimize toxicity

• To better understand mechanisms and pathways important for cytokine immune response

• To identify possible new therapeutic targets and design new treatment strategies
### Predicting IL-2 Response:

**Ancient History**

### Phenotypic Analysis of PBL in Responders and Non Responders

<table>
<thead>
<tr>
<th>Pt. #</th>
<th>Type of Response</th>
<th>Age</th>
<th>CD56^+CD3^-</th>
<th>CD56^-CD3^+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>57</td>
<td>CR (IFN-α/pTIL)</td>
<td>66</td>
<td>51</td>
<td>1</td>
</tr>
<tr>
<td>65</td>
<td>CR (IFN-γ/pTIL)</td>
<td>73</td>
<td>46</td>
<td>8</td>
</tr>
<tr>
<td>87</td>
<td>CR (TNF-α/pTIL)</td>
<td>42</td>
<td>73</td>
<td>28</td>
</tr>
<tr>
<td>63</td>
<td>CR (-/CD8+)</td>
<td>61</td>
<td>28</td>
<td>9</td>
</tr>
<tr>
<td>82</td>
<td>CR (-/CD8+)</td>
<td>71</td>
<td>41</td>
<td>18</td>
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<tr>
<td>78</td>
<td>PR (TNF-α/pTIL)</td>
<td>58</td>
<td>15</td>
<td>12</td>
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<tr>
<td>80</td>
<td>PR (-/CD8+)</td>
<td>45</td>
<td>28</td>
<td>24</td>
</tr>
<tr>
<td>90</td>
<td>PR (IFN-α/pTIL)</td>
<td>53</td>
<td>65</td>
<td>15</td>
</tr>
</tbody>
</table>

| Mean  | 43*  | 27** | 2.60*** |
| Mean ± S.D. | 20±9 | 12±9 | 2.24±0.19 |

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<th>Age</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>64</td>
<td>NR (IFN-α/pTIL)</td>
<td>79</td>
<td>3</td>
<td>29</td>
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<tr>
<td>67</td>
<td>NR (TNF-α/pTIL)</td>
<td>47</td>
<td>7</td>
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<tr>
<td>86</td>
<td>NR (TNF-α/pTIL)</td>
<td>70</td>
<td>14</td>
<td>18</td>
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<td>89</td>
<td>NR (IFN-γ/pTIL)</td>
<td>61</td>
<td>16</td>
<td>13</td>
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<tr>
<td>96</td>
<td>NR (IL-2/pTIL)</td>
<td>55</td>
<td>52</td>
<td>20</td>
</tr>
<tr>
<td>74</td>
<td>NR (-/CD8+)</td>
<td>61</td>
<td>33</td>
<td>24</td>
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<td>75</td>
<td>NR (-/CD8+)</td>
<td>57</td>
<td>14</td>
<td>26</td>
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<tr>
<td>81</td>
<td>NR (-/CD8+)</td>
<td>55</td>
<td>6</td>
<td>10</td>
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<tr>
<td>88</td>
<td>NR (-/CD8+)</td>
<td>70</td>
<td>13</td>
<td>9</td>
</tr>
</tbody>
</table>

| Mean  | 18 | 18 | 54 | 51 | 0.44 | 0.42 |
| Mean ± S.D. | 16±8 | 15±15 | 0.54 | 0.36 |

Normal value (n=3) 11±3 64±16 0.18±0.2

*p < 0.01 (R vs. NR) and p < 0.005 (A vs. B), ** P < 0.005 (R vs. NR) and p < 0.005 (A vs. B)
*** P < 0.05 (R vs. NR) and P < 0.05 (A vs. B), R = responder; NR = non-responder;
PR = partial responder

Belldegrun et al
JoI, 1996
## Predicting IL-2 Response

**The Middle Ages**

**Clinical Algorithms**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Hazard Ratio</th>
<th>p value</th>
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<tbody>
<tr>
<td>Constitutional Sx’s</td>
<td>1.9</td>
<td>0.005</td>
</tr>
<tr>
<td>N Stage</td>
<td>1.4</td>
<td>0.002</td>
</tr>
<tr>
<td>Metastasis location</td>
<td>2.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sarcomatoid Hist</td>
<td>2.3</td>
<td>0.003</td>
</tr>
<tr>
<td>TSH level</td>
<td>1.4</td>
<td>0.038</td>
</tr>
</tbody>
</table>

Leibovich et al.  
Cancer, 2002
Predicting IL-2 Response
The Modern Era

Paraffin Embedded Tissues
- Laser Capture/DNA Extraction
  - Array Based CGH
  - VHL Sequencing
- Clinical F/U Database
- IHC/FISH of RCC Tissue Array
- cDNA Microarray
- SELDI-TOF Proteomics
- RNA, Protein Extraction
- Fresh Frozen Tissues

Identify Genetic Markers
Validation
Identify Molecular Markers
Kidney Cancer Database

Kidney Tissue Data

AccessionNo | MedicalRecordNo | Ki67NuclearMax | Ki67Score |
---|---|---|---|
S98 | 2291892 | 3 | 30 |
S95 | 2056792 | 3 | 10 |
S90 | 1749272 | 2 | 5 |
S96 | 2080425 | 2 | 10 |
S97 | 1324859 | 3 | 32.5 |
S89 | 845760 | 1 | 0 |
S92 | 218122 | 3 | 30 |
S87 | 365277 | 3 | 0 |
S95 | 2068685 | 3 | 30 |
S94 | 2005848 | 3 | 20 |

1989-2002
- 1,498 Patients
- 1,349 Nx’s
- 622 study pts.
- 377 IL-2

263 Variables
TISSUE ARRAY CONSTRUCTION

396 Kidney Tumors
4 Cores per Tumor
1584 spots total
Affymetrix U133A Gene Chip Array

22,215 genes
500,000 oligonucleotide features
cDNA Microarray Data Analysis

CEL files were imported into dChip to compute the model based expression index for each gene.

To reveal the global pattern of the arrays, we used unsupervised learning analysis, (hierarchical clustering and multi-dimensional scaling plots).

To filter out significant genes, we used the standard two sample t-test in pair-wise comparisons involving different treatment groups. The selection criterion are as follows:

1. the fold change > 1.2;
2. |difference| > 100; and
3. p-value < 0.05
### Molecular Analysis of IL-2 Response

**Top 566 genes: PD vs CR**

<table>
<thead>
<tr>
<th>Probe Set</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>guanine nucleotide binding protein (G protein), alpha 11 (Gq class)</td>
<td>solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator)</td>
</tr>
<tr>
<td>tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein</td>
<td>KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2</td>
</tr>
<tr>
<td>phosphoglycerate kinase 1</td>
<td>RAN, member RAS oncogene family</td>
</tr>
<tr>
<td>heat shock 60kD protein 1 (chaperonin)</td>
<td>H2A histone family, member Z</td>
</tr>
<tr>
<td>high-mobility group (nonhistone chromosomal) protein 14</td>
<td>ADP-ribosylation factor 4</td>
</tr>
<tr>
<td>heterogeneous nuclear ribonucleoprotein F</td>
<td>bone marrow stromal cell antigen 2</td>
</tr>
<tr>
<td>myristoylated alanine-rich protein kinase C substrate</td>
<td>Lysosomal-associated multispanning membrane protein-5</td>
</tr>
<tr>
<td>Lysosomol-associated multispanning membrane protein-5</td>
<td>chaperonin containing TCP1, subunit 2 (beta)</td>
</tr>
<tr>
<td>ubiquinol-cytochrome c reductase hinge protein</td>
<td>v-myc myelocytomatosis viral oncogene homolog (avian)</td>
</tr>
<tr>
<td>phospholipid scramblase 1</td>
<td>glutathione peroxidase 2 (gastrointestinal)</td>
</tr>
<tr>
<td>splicing factor, arginine/serine-rich 3</td>
<td>actinin, alpha 2</td>
</tr>
<tr>
<td>N-myc (and STAT) interactor</td>
<td>low density lipoprotein receptor-related protein 6</td>
</tr>
<tr>
<td>DnaJ (Hsp40) homolog, subfamily B, member 6</td>
<td>catechol-O-methyltransferase</td>
</tr>
</tbody>
</table>
Top 73 Genes PD vs. CR
Hierarchical Clustering Dendrogram
VHL, Hypoxia, and RCC Tumorigenesis

**HYPOXIA**

- **TARGET GENE INDUCTION**
  - GLUT-1
    - Glucose transport
  - VEGF
    - Angiogenesis
  - IGF
    - Growth/survival
  - CAIX
    - Metabolism/pH regulation
  - CXCR4
    - Metastasis
      - Proliferation
      - Survival

**HIF1-α**

- HIF DEGRADATION

**VHL**

- HYPOXIA

**NORMOXIA**

Differentially Expressed Genes of Interest

**CR:**
- Integrin Associated Proteins (CD47, Fibronectin)
- Tumor Suppressors (PTEN)
- Heat Shock Proteins 60, 70, 90
- MCH Class II
- Pro-Apoptotic (Caspase I)
- MAP kinase
- Thyroid Autoantigen
- Carbonic Anhydrase IX

**PD:**
- Chemokines (CXCR4)
- Regulators of G protein Signaling
- Trefoil Factors
- Actin Associated Proteins (actinin)
- IGF binding proteins
Differentially Expressed Genes of Interest

**CR:**
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- Chemokines (CXCR4)
- Regulators of G protein Signaling
- Trefoil Factors
- Actin Associated Proteins (actinin)
- IGF binding proteins
CXCR-4 Pathways and RCC

SDF

CXCR-4

EGFR, HER-2/neu, IGFR

Cytoskeletal Actin Reorganization

Alterations in Morphology, Motility, Invasiveness

LY 294002

PI3K

PTEN

Raf

Bay 43-9006

Ras

FTI

Pten

mTOR

MEK

MAPK

2ME YC-1

Rapamycin
CCI 779
RAD001

TSC

AKT

HIF-1α

HIF 1α

ERK

17-AAG
17-DMAG

HIF 1β

VEGF  ➔ angiogenesis
CAIX  ➔ pH regulation
CXCR-4  ➔ metastasis
EGFR  ➔ tumor growth
TGFα/IGF  ➔ tumor growth
GLUT-1  ➔ glucose control

Normoxia

Proteosome

HIF-1α

VHL

Cul2

RB

E2

OH

PRO

Hsp90

Hypoxia
Increased CXCR4: A Marker of IL-2 Non-Responsiveness?

- Receptor for CXCL12/SDF-1
- Regulation of: metastasis proliferation survival
Cytokeratin + CXCR4+ circulating cells in mRCC pts. treated with high dose IL-2

- Pan-cytokeratin+ Cells
- Pan-cytokeratin+ CXCR4+ Cells

Cells/ml (x10^6)
Pancytokeratin+CXCR4+ cells/ml (x10^6)
Angiogenesis

Net neovascularization is determined by the balance of angiogenic and angiostatic factors within the local microenvironment.

- **Homeostasis**
  - Angiostatic
  - Angiogenic

- **Tumor growth**
  - Angiostatic
  - Angiogenic

- **Wound repair**
  - Angiostatic
  - Angiogenic
  - Chronic inflammation
## Angiogenic Factors and Immunotherapy Response

<table>
<thead>
<tr>
<th>CR</th>
<th>CXCL9</th>
<th>CXCL12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CXCL10</td>
<td>VEGF</td>
</tr>
<tr>
<td></td>
<td>CXCL11</td>
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</table>

<table>
<thead>
<tr>
<th>PD</th>
<th>--------</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>CXCL1</td>
<td>CXCR4</td>
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<tr>
<td></td>
<td>CXCL2</td>
<td>VEGF B</td>
</tr>
<tr>
<td></td>
<td>CXCL3</td>
<td>VEGF</td>
</tr>
</tbody>
</table>
Carbonic Anhydrase IX

Clinically, high levels (＞85%) associated with improved immunotherapy response (UCLA, CWG)

CAIX Gene Expression

CR: 1669       PD: 941       PD+ Lymph Nodes: 611
Tissue Array Validation:
Analysis of Genes and IL-2 Response

Carbonic Anhydrases IX and XII
EpCAM
Gelsolin
p53
Ki67
PTEN
Vimentin
CAIX by Ordinal IMT Response

P = 0.02
## Tissue Array Analysis of Genes and IL-2 Response

### Univariate Predictors

- **CAIX > 85%**  \( p = 0.027 \)
- **EpCAM > 5%**  \( p = 0.037 \)
- **P53**  \( p = 0.623 \)
- **PTEN**  \( p = 0.012 \)
- **Vimentin**  \( p = 0.301 \)
- **Gelsolin**  \( p = 0.794 \)

### Multivariate Predictors

| Estimate | Std. Error | t value |   Pr(>|t|) |
|----------|------------|---------|------------|
| (Intercept) | 0.0544731 | 0.2146256 | 0.254 | 0.8002 |
| CA9MemPos.mn | 0.0037908 | 0.0015443 | 2.455 | 0.0158 |
| EPDctPos.md | -0.0021168 | 0.0020954 | -1.010 | 0.3149 |
| p53Pos.md | -0.0009112 | 0.0049747 | -0.183 | 0.8550 |
| pTENMax.md | 0.1316415 | 0.0680478 | 1.935 | 0.0519 |
| VimMax.max | -0.0963226 | 0.0626230 | -1.538 | 0.1272 |
| GeMax.mn | 0.0045761 | 0.0742013 | 0.062 | 0.9509 |
### Tissue Array Analysis of Genes, Clinical Variables, and IL-2 Response

#### Multivariable Analysis

|                          | Estimate | Std. Error | t value | Pr(>|t|)  |
|--------------------------|----------|------------|---------|-----------|
| CA9MemPos.mn             | 0.005649 | 0.001695   | 3.333   | 0.00146 **|
| EpDctPos.md              | -0.003337| 0.002053   | -1.625  | 0.10931   |
| p53Pos.md                | -0.002777| 0.004600   | -0.604  | 0.54833   |
| pTENMax.md               | 0.140579 | 0.074246   | 1.893   | 0.06305   |
| VimMax.max               | -0.119671| 0.066276   | -1.806  | 0.07591   |
| GeMax.mn                 | 0.034811 | 0.083754   | 0.416   | 0.67914   |
| Male                     | 0.301309 | 0.115154   | 2.617   | 0.01118 * |
| ECOG                     | -0.244610| 0.099794   | -2.451  | 0.01712 * |
| nstage                   | -0.061457| 0.067538   | -0.910  | 0.36642   |
Receiver Operating Curve: Clinical + Molecular Prediction Model

AUC=0.629
CAIX Expression and Survival in mRCC Treated by IL-2

![Graph showing survival probability over time for CA9 Low and CA9 High groups. The graph indicates a statistically significant difference with a p-value of 2.4e-05.]

\[ p = 2.4 \times 10^{-5} \]
Gender and Survival in mRCC Treated by IL-2

p = 0.00159
CAIX, PTEN, Gender and Survival in mRCC Treated by IL-2

\[ p = 0.000245 \]
Conclusions

• Archived tumor tissue combined with a strong clinical data base: a powerful combination

• Proof of principle: gene expression analysis can identify relevant gene differences that translate into meaningful variables in an independent tissue array validation

• CAIX, PTEN, CXCR4, and other genes may play important roles in dictating IL-2 treatment response in RCC
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