Molecular Correlates with IL-2 Response in Renal Cancer

Phillip Febbo, MD

DFCI/Harvard Renal SPORE

Duke University
Duke Institute for Genome Sciences and Policy
IL-2 Therapy for RCC-2004

High dose IL-2 remains the preferred therapy for...

- appropriately selected patients
- with access to such treatment

Efforts to improve selection criteria are warranted
Additional Opportunities for Patient Selection: IL-2

- Histologic Factors
  (Upton et al Proc ASCO 2003)

- Molecular studies (CAIX Staining)

- Expression Profiling
Additional Opportunities for Patient Selection: IL-2

♦ Histologic Factors
  (Upton et al Proc ASCO 2003)

♦ Molecular studies (CAIX Staining)

♦ Expression Profiling
### Pathologic Correlates of Response to IL-2

Non-Clear cell histology associated with poor response

<table>
<thead>
<tr>
<th>Clear Cell Pathology</th>
<th>Risk Group</th>
<th>Primary N=146 RR (%)</th>
<th>Mets N=66 RR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alveolar &gt; 50% No papillary No granular</td>
<td>Good</td>
<td>39%</td>
<td>25%</td>
</tr>
<tr>
<td>Alveolar &lt; 50% Granular &lt; 50% No papillary</td>
<td>Intermediate</td>
<td>19%</td>
<td>9%</td>
</tr>
<tr>
<td>Others</td>
<td>Poor</td>
<td>4%</td>
<td>0%</td>
</tr>
</tbody>
</table>

*Upton et al Proc ASCO 2003*
Pathologic Correlates of Response to IL-2

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>Med Surv (yrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>2.9</td>
</tr>
<tr>
<td>Intermediate</td>
<td>1.4</td>
</tr>
<tr>
<td>Poor</td>
<td>0.9</td>
</tr>
</tbody>
</table>

P <0.001

Upton et al Proc ASCO 2003
Additional Opportunities for Patient Selection: IL-2

♦ Histologic Factors
  (Upton et al Proc ASCO 2003)

♦ Molecular studies (CAIX Staining)

♦ Expression Profiling
Methods

-Collected tissue blocks from patients enrolled in Cytokine Working Group (CWG) IL-2 trials (Upton, ASCO 2003)

-Enriched collection for responding patients- “nested case-control study”

-Selected representative tissue samples from each block

-Stained for CAIX expression using MN-75 Ab from Eric Stanbridge

-Correlated staining results with IL-2 response, survival, IL-2 dose, and pathologic risk group
CAIX Expression and IL-2 Response

Clinical Response

CAIX (% positive cells)

High CAIX (%)
Response 21/27 78%
No Resp  20/39 51%

P=0.04
OR = 3.3
CAIX Expression and IL-2 Response

Proportion Alive vs. Time from Initiation of IL2 (years)

- CAIX > 85% (n=41)
- CAIX ≤ 85% (n=25)

P=0.03
### Proposed New Model

<table>
<thead>
<tr>
<th>Refined Pathology Risk Group</th>
<th>Non-Responder (n=39)</th>
<th>Responder (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good risk path or intermediate path with high CAIX</td>
<td>Good</td>
<td>18 (46%)</td>
</tr>
<tr>
<td>Poor risk path or intermediate path with low CAIX</td>
<td>Poor</td>
<td>21 (54%)</td>
</tr>
</tbody>
</table>
IL-2 Related Survival and Refined Pathology Risk Group

Proportion Alive vs. Time from Initiation of IL2 (years)

- Good
- Poor

P<0.01
Additional Opportunities for Patient Selection: IL-2

- **Histologic Factors**
  (Upton et al Proc ASCO 2003)

- **Molecular studies (CAIX Staining)**

- **Expression Profiling**
Prediction of Response to IL-2

Expression Analysis

RCC Sample Flow

1) Obtained frozen samples from Renal SPORE Path Core
2) Cut frozen sections, reviewed with pathologist
3) Included samples with RCC without significant necrosis
4) Isolated RNA
5) Created Target
6) Applied to microarrays U133A
7) Performed Q/A to omit poor scans
Prediction of Response to IL-2

Unsupervised Analysis

No dominating gene expression pattern for response to IL-2
Prediction of Response to IL-2

NAME
chemokine (C-X-C motif), receptor 4 (fusin)
chemokine receptor CXCR4
leukocyte surface protein (CD31)
STAT6
integrin-linked kinase (ILK)
ras homolog gene family, member B
glutamyl aminopeptidase (aminopeptidase A) (ENPEP)
fenestrated-endothelial linked structure protein (FELS)
vascular endothelial growth factor
enolase like 1 (ENO1L1)
cysteine-rich protein 2 (hCRP2)
aquaporin 1 (channel-forming integral protein, 28kD)
alanyl (membrane) aminopeptidase (aminopeptidase N, aminopeptidase)
prominin (mouse)-like 1
carbonic anhydrase IX (CA9)
TSC501
N-acetyltransferase Camello 2 (CML2)
bcl-1
CD59 antigen p18-20 (antigen identified by monoclonal antibodies 16.3)
CD59 antigen p18-20 (antigen identified by monoclonal antibodies 16.3)
syndecan 1 (SDC1)
CD59 antigen p18-20
EST
GABA noradrenaline transporter
AKT and mTOR inhibition (Prostate)

Glut 1 Staining and RCC

Pathology Data: (N=66)

- Non-Clear Cell: 8
- Clear Cell: 58
  - Alveolar: 56
  - Granular: 33
  - Papillary: 4

Pathology Risk Group (Upton):
- good: 24 (36%)
- intermediate: 31 (47%)
- poor: 11 (17%)
Glut 1 Staining and RCC

Most RCC positive for GLUT 1
83.1% (51/61) with staining $> 1+$
Heterogeneous staining observed within tumors
Mean percentage of positive cells 30% (+/-27.6)

? Correlation with CAIX protein expression
? Correlation with IL2 outcome data
High CAIX expression appears to correlate with high Glut-1 expression.
Relationship between Glut-1 and IL-2 therapy

1. Glut-1 is NOT associated with response to IL-2 therapy

2. Glut-1 is associated with better survival following IL-2 therapy
Expression Analysis

Supervised Analysis

10 patients received HDIL-2

♦ Patient Characteristics-
  • 8 male/ 2 female
  • MSKCI criteria: 2 good, 6 intermediate, 2 poor
  • Response: 5 PR / 5 PD

♦ Specimens
  • All clear cell, 1 with papillary features
  • 8 with high CAIX protein expression
Prediction of Response to IL-2

Supervised Analysis

Non-Responders

Responders

variable charge, Y chromosome
superoxide dismutase 2, mitochondrial
hypothetical protein FLJ10815
hepcidin antimicrobial peptide
golgin-67
EST
interleukin enhancer binding factor 1
Superoxide dismutase 2, mitochondrial
Cytochrome P450, subfamily IIC (mephenytoin 4-hydroxylase), polypeptide 9
hexose-6-phosphate dehydrogenase (glucose 1-dehydrogenase)
neural proliferation, differentiation and control, 1
tissue inhibitor of metalloproteinase 3 (Sorsby fundus dystrophy, pseudoinflammatory)
acetylserotonin O-methyltransferase-like
MCM7 minichromosome maintenance deficient 7 (S. cerevisiae)
hypothetical protein FLJ22690
tissue inhibitor of metalloproteinase 3 (Sorsby fundus dystrophy, pseudoinflammatory)
metastasis-associated 1-like 1
KIAA0323 protein
EST
phosphatidic acid phosphatase type 2A

MnSOD
IL1RA

TIMP3
Prediction of Response to IL-2

Supervised Analysis

- 206 genes with expression > 2 fold higher in responders
  - TIMP3 (inhibits MM3- tumor less aggressive)
  - CD 9 (associated with immune responsiveness)
- 197 genes > 2 fold higher in non-responders
  - MnSOD
  - IL-1 RA
  - Both induced by inflammatory cytokines
  - MnSOD increases resistance to TNF mediated apoptosis
- CAIX expression
  - increased 1.8 fold in tumors from responding patients
  - Clustered with expression of HIF1 target genes
Prediction of Response to IL-2

Gene Set Enrichment Analysis (GSEA)

1) Choose Dataset

IL-2 Responsive v. Non-Responsive

2) Set Class Distinction

3) Determine position of genes in Gene Sets (Gene Ontology Sets)

4) Determine “running score” of gene set

5) Compare with permuted data

p < 0.01

Mootha et al (2003), Subramanian et al (Submitted)
**Prediction of Response to IL-2**

-3 -2 -1 0 1 2 3

- Non-Responsive
- Responsive

- fertilization (sensu Animalia)
- meiotic recombination
- plasma glycoprotein
- Chromatin Remodeling
- induction of apoptosis by extracellular signals
- cell adhesion receptor activity
- aldehyde dehydrogenase activity
- regulation of CDK activity
- actin binding activity
- cell-matrix adhesion
- "DNA-directed RNA polymerase II, core complex"
- pregnancy
- blood group antigen
- coreceptor activity
- actin cytoskeleton
- RNA catabolism
- Transcription, DNA dependent
- cell adhesion
- digestion
- pathogenesis
- actin cytoskeleton reorganization
- heterogeneous nuclear ribonucleoprotein
- structural constituent of cytoskeleton
- intracellular
- response to wounding
- potassium ion transport
- regulation of transcription from Pol II promoter
- vision
Prediction of Response to IL-2

Responsive

Chromatin Remodeling
induction of apoptosis by extracellular signals
cell adhesion receptor activity
aldehyde dehydrogenase activity
Conclusions

- Pathological and Molecular features of RCC can help anticipate an individual’s response to IL-2 therapy.
- There may be value in combining Pathological risk categories and CAIX staining in a response model.
- CAIX RNA expression correlates with other HIF targets at RNA and Protein level.
- Glut-1 expression does not correlate with response to IL-2: more to CAIX correlated response than HIF.
- Preliminary supervised analyses suggest there are additional expression correlates with IL-2 response.
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