Targeted Therapy in Advanced Renal Cell Carcinoma

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## Rini Disclosures

<table>
<thead>
<tr>
<th>Nature of Relationship</th>
<th>Company</th>
</tr>
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<tbody>
<tr>
<td>Research funding</td>
<td>Pfizer, Genentech, Wyeth, Bayer/Onyx</td>
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<tr>
<td>Consultant</td>
<td>Pfizer, Genentech, Wyeth, Bayer/Onyx, Gerson-Lehrman group</td>
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</table>
Targeted Therapy in RCC

• Current clinical data with new agents
  – Monotherapy data with VEGF- and mTOR-targeted approaches

• Translational Efforts
  – Immunoregulatory properties of sunitinib
  – VHL status and correlation with clinical outcome
HIF-mediated degradation of HIF

**Normoxia and normal VHL function**
- HIFα
- OH
- VHL
- E3 ligase
- Ub
- Ub
- Ub
- Ub
- HIFα
- OH

**Protein Stabilization**
- HIFα
- OH
- mTOR
- Temsirolimus

**Transcriptional activation of HIF target genes**
- VEGF
- VEGFR
- Sunitinib
- Sorafenib
- PDGF
- PDGFR
- Sunitinib
- Sorafenib

**Hypoxia and/or VHL inactivation**
- HIFα
- OH
Bevacizumab (Avastin)
Bevacizumab: change in tumor burden in metastatic RCC patients

** 10% RR and median PFS benefit (2.5 vs. 4.8 months) vs. placebo in high-dose arm

Elaraj et al. *J Immunotx* 27(4), 2004
Bevacizumab ± IFN Phase III: Study Design

Eligibility Criteria
- Confirmed metastatic RCC with >50% clear cell histology
- Prior nephrectomy
- Karnofsky PS of ≥70%
- Measurable or non-measurable disease (by RECIST)
- No prior systemic treatment for metastatic RCC disease

Randomization 1:1
Stratified by: country Motzer score (n=649)

Bevacizumab 10mg/kg IV q2w + IFN-α2a 9MIU sc tiw (n=327)

IFN-α2a 9MIU sc tiw + placebo (n=322)

Adapted from Escudier B et al. Presented at ASCO Annual Meeting; June 1-5, 2007; Chicago, IL.
Bevacizumab ± IFN Phase III: Tumor Response

IFN + placebo (n=289)

*ORR 13%
CR 2% - PR 11%

Bevacizumab + IFN (n=306)

*ORR 31%
CR 1% - PR 30%

*P <0.0001

*Patients with measurable disease only; investigator assessed
Bevacizumab + Interferon (IFN) vs. IFN in untreated metastatic RCC

Median progression-free survival:
Bevacizumab + IFN = 10.2 months
Placebo + IFN = 5.4 months

Hazard Ratio = 0.63
p < 0.0001

• ORR: 31% vs. 13%

Adapted from Escudier B et al. Presented at ASCO Annual Meeting; June 1-5, 2007; Chicago, IL.
Sunitinib (Sutent)
Inhibitory Profile of Kinases for Sunitinib

Sunitinib: Phase II trials in RCC
Best Response by RECIST

<table>
<thead>
<tr>
<th></th>
<th>Trial 1</th>
<th>Trial 2</th>
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</thead>
<tbody>
<tr>
<td>Patients</td>
<td>63</td>
<td>106</td>
</tr>
<tr>
<td>Overall objective response</td>
<td>44%*</td>
<td>43%*</td>
</tr>
<tr>
<td></td>
<td>36%**</td>
<td>35%**</td>
</tr>
<tr>
<td>Median progression-free survival</td>
<td></td>
<td>8.2 months</td>
</tr>
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</table>

*investigator review; **independent review

Phase III Sunitinib vs. Interferon

N=750

Stratification Factors

- LDH ≤1.5 vs >1.5xULN
- ECOG PS 0 vs 1
- Presence vs Absence of Nephrectomy

Sunitinib (N=375)

IFN-α (N=375)

Motzer et al. NEJM, 2007
Sunitinib vs. Interferon in untreated metastatic RCC: PFS

- Median progression-free survival:
  - Sunitinib = 11.0 months
  - IFN-α = 5.1 months
  - Hazard Ratio = 0.538
  - p < 0.000001

- ORR: 39% vs. 8%
- No CR’s observed

Adapted from Motzer R et al. Presented at ASCO Annual Meeting; June 1-5, 2007; Chicago, IL.
Sorafenib (Nexavar)
**Sorafenib** phase III vs. placebo in cytokine-refractory RCC

**Eligibility criteria**
- Clear cell, unresectable and/or metastatic RCC
- Measurable disease
- Failed one prior systemic therapy in last 8 months
- ECOG PS 0 or 1
- No brain metastasis

**Randomization**
(1:1)
- n~884

**Stratification**
- MSKCC criteria
- Country

**Major endpoints**
- Survival (alpha=0.04)
- PFS (alpha=0.01)
Sorafenib vs. placebo in cytokine-refractory metastatic RCC: PFS

- Sorafenib (n=384) = 5.5 months
- Placebo (n=385) = 2.8 months

Hazard Ratio = 0.44
p < 0.000001

Final OS Analysis
16 Months Post-Crossover: Intent-to-Treat

Sorafenib (n=451) = 17.8 months
Placebo (n=452) = 15.2 months
HR (sorafenib/placebo) = 0.88
95% CI: 0.74–1.04
P=0.146*
Sorafenib vs IFN in untreated metastatic RCC: PFS

Median PFS (121 events/189 patients)
Sorafenib = 5.7 months
IFN = 5.6 months

Hazard Ratio = 0.883
p = 0.504 (log-rank test)

Adapted from Szczylik C et al. Presented at ASCO Annual Meeting; June 1-5, 2007; Chicago, IL.
Temsirolimus (Toricel)
Temsirolimus: Mechanism of Action

Growth Factors

extracellular membrane

PI-3 Kinase

PI-3K/AKT Activation

PTEN Loss

PTEN overexpression

PI-3 Kinase

Akt

PtEN

mTOR

S6K 4EBP1

Translation

Cyclin D1 overexpression

cMyc overexpression

HIF-1α, HIF-2α overexpression

Temsirolimus
Temsirilimus: Phase III trial in advanced RCC

Patients with previously untreated advanced RCC
Poor risk criteria (N = 626)

Minimum of 3 poor-risk features required:
1. LDH >1.5 X upper limit of normal
2. Hemoglobin <lower limit of normal
3. Corrected calcium >10 mg/dL
4. Time from diagnosis to first treatment <1 yr
5. Karnofsky performance status 60-70
6. Multiple organ site of metastasis

Interferon alfa SC up to 18 MU TIW as tolerated

Temsirilimus IV 25 mg weekly

Temsirilimus 15 mg IV weekly + Interferon alfa 6 MU SC TIW
Overall Survival by Treatment Arm

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IFN Arm 1</th>
<th>TEMSR Arm 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>207</td>
<td>209</td>
</tr>
<tr>
<td>Median Survival</td>
<td>7.3 months</td>
<td>10.9 months</td>
</tr>
<tr>
<td>Stratified Log-Rank P</td>
<td>0.008</td>
<td></td>
</tr>
</tbody>
</table>
Potential immunoregulatory properties of sunitinib

*Disclaimer: I am not a real Immunologist, nor do I play one on TV.
Potential Interaction between Immune Cells and Sunitinib

MSC

Myeloid Progenitor

CD11b

CD33

VEGF

RCC

Tumor

CD4

Treg

FoxP3+

CD25

Sunitinib

DC

NOS-2

Arginase 1

MSC (e.g.)

Myeloid Progenitor

RCC

Tumor

TGF-β

TDSF

(e.g.)

Th1

Th2

IFN-γ

IL-2

IL-4, IL-6

IL-5, IL-10

Bias

RCC

Tumor

EphA2

RCC

Tumor

Exepressed Antigen (e.g.)

Cell-Mediated Responses

Humoral Responses

Key

= Increased in RCC patients

= Inhibitory

= Stimulatory
Methods

- Peripheral blood obtained from cytokine-refractory, clear cell mRCC patients on day 1 (pre-treatment) and after 28 days of sunitinib 50 mg daily.

- T cell cytokine intracellular expression IL-4 (Th2) and IFN-γ (Th1) determined by stimulating PBMC with plate bound anti-CD3 and anti-CD28 antibodies for 72 hours.

- Percentage of CD25^{high} FoxP3^{+} cells within the CD3^{+}CD4^{+} cell population, and percentage of Treg that were FoxP3^{+} were evaluated using four color flow cytometry.
Sunitinib Reverses RCC Induced Th2 Bias in Peripheral Blood of Metastatic RCC Patients

### Anti-CD3/CD28 Antibody Stimulation of CD3(+) Cells (N=22)

<table>
<thead>
<tr>
<th></th>
<th>Day 1 (Median or Th2 Bias Ratio, Range)</th>
<th>Day 28 (Median or Th2 Bias Ratio, Range)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Th1 Response</td>
<td>2.1% (0.05-20.3)</td>
<td>9.6% (0.2-27.4)</td>
<td>.001</td>
</tr>
<tr>
<td>Th2 Response</td>
<td>26.1% (6.0-67.8)</td>
<td>19.3% (0.03-42.6)</td>
<td>.02</td>
</tr>
<tr>
<td>Th2 Bias</td>
<td>13.4 (1.2-234.0)</td>
<td>2.2 (0.01-61.3)</td>
<td>.002</td>
</tr>
</tbody>
</table>
mRCC Patients Show Increased Levels of T Regulatory Cells Compared to Age Matched Normal Donors

- **Range (CD25+ Hi)**: 1.0 – 2.8
- **Range (CD25+ Hi FoxP3+)**: 0.4 – 2.0

**p** = <0.001

**Normals (n=17)**

**mRCC (n=40)**

- **Range (CD25+ Hi)**: 1.5 – 9.2
- **Range (CD25+ Hi FoxP3+)**: 0.5 – 8.4
The Percentage of T-Regulatory Cells Decreases in the Peripheral Blood With Sunitinib Treatment in mRCC Patients

- **Range (CD25+ Hi)**: 1.5 – 9.2
  - **Range (CD25+ Hi FoxP3+)**: 0.5 – 8.4
- **Range (CD25+ Hi)**: 1.0 – 9.9
  - **Range (CD25+ Hi FoxP3+)**: 0.4 – 9.5
- **Range (CD25+ Hi)**: 0.9 – 5.6
  - **Range (CD25+ Hi FoxP3+)**: 0.3 – 4.2

- **p = 0.05**
- **p = 0.10**
- **p = 0.03**
- **p = 0.13**
Percent Suppression of CD4$^{+}$CD25$^{-}$ Cells by Tregs Decreases in Sunitinib Treated Patients

- C1D1 (n=4)
- C1D28 (n=4)

* Does not appear to be a direct effect of sunitinib
VHL status and clinical outcome to VEGF-targeted therapy
HYPOTHESIS

- Tumors with \textit{VHL} gene inactivation will exhibit a better clinical outcome after VEGF-targeted therapy.
MATERIAL AND METHODS

- 182 patients with metastatic RCC who received sunitinib, sorafenib, bevacizumab or axitinib (AG-013736) as initial anti-VEGF therapy on a clinical trial at Cleveland Clinic or UCSF between Feb. 2003 and Jan. 2006.

- 59 patients excluded:
  - Missing key data (n=3)
  - Pure non-clear cell histology (n=8)
  - Insufficient tissue for DNA extraction (n=12)
  - Unavailability of tissue at our institutions (n=36)

- 123 patients with available tissue/clinical data were included in the final analysis*.
  - sunitinib: n= 63 (51%), sorafenib: n= 28 (23%), bevacizumab: n=17 (14%), axitinib: n= 15 (12%)

* A subset of the data (n=45) previously reported: Rini BI, et al. BJU Int. 2006;98:756-62
Genomic DNA was extracted from frozen or paraffin-embedded tissue that contained >95% of tumor and manually dissected after pathology review.

One or more primer sets were used to amplify each of the exons (and exon/intron junctions) of the \( VHL \) gene.

PCR products were sequenced using Big Dye chemistry (Applied Biosystems) at the Core Sequencing Facility of each institution.

Sequences identified to harbor mutations were confirmed with a second round of PCR and sequencing reactions in the reverse direction.
## CHARACTERISTICS of VHL MUTATIONS

(49% mutated, 10% methylated)

<table>
<thead>
<tr>
<th>Location of VHL mutation</th>
<th>N (%) of 60 patients</th>
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</thead>
<tbody>
<tr>
<td>Exon 1</td>
<td>25 (42%)</td>
</tr>
<tr>
<td>Exon 2</td>
<td>19 (32%)</td>
</tr>
<tr>
<td>Exon 3</td>
<td>16 (27%)</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Type of mutation</th>
<th>N (%) of 60 patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frameshift</td>
<td>29 (48%)</td>
</tr>
<tr>
<td>Nonsense (Stop)</td>
<td>6 (10%)</td>
</tr>
<tr>
<td>Inframe deletion or insertion</td>
<td>7 (12%)</td>
</tr>
<tr>
<td>Splice</td>
<td>5 (8%)</td>
</tr>
<tr>
<td>Missense</td>
<td>13 (22%)</td>
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</table>
# RESPONSE AND VHL STATUS

<table>
<thead>
<tr>
<th>Factor</th>
<th>N*</th>
<th>ORR (%)</th>
<th>P Value</th>
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</thead>
<tbody>
<tr>
<td><strong>Response</strong></td>
<td>122</td>
<td>45/122 (37%)</td>
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<tr>
<td><strong>VHL Status</strong></td>
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<td></td>
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</tr>
<tr>
<td>Mutated</td>
<td>59</td>
<td>27 (46%)</td>
<td></td>
</tr>
<tr>
<td>Methylated</td>
<td>12</td>
<td>2 (15%)</td>
<td>41% ORR</td>
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<tr>
<td>Wild Type</td>
<td>51</td>
<td>16 (31%)</td>
<td>31% ORR</td>
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</table>

*One patient with inadequate follow-up
### RESPONSE AND VHL STATUS

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<th>ORR (%)</th>
<th>P-Value</th>
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<tbody>
<tr>
<td>Overall Response</td>
<td>122</td>
<td>45/122 (37%)</td>
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<tr>
<td>* One patient with inadequate follow-up was excluded</td>
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<tr>
<td>Frameshift</td>
<td>28</td>
<td>15 (54%)</td>
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<tr>
<td>Inframe (d/i)</td>
<td>7</td>
<td>4 (57%)</td>
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<td>4 (67%)</td>
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<td>Splice</td>
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<td>1 (20%)</td>
<td></td>
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<tr>
<td>Missense</td>
<td>13</td>
<td>3 (23%)</td>
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<td></td>
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<td>52% ORR</td>
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<tr>
<td></td>
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<td>vs.</td>
<td>p=0.04</td>
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### Objective Response in Relation to VHL Status by Specific Drug

<table>
<thead>
<tr>
<th>VHL Status</th>
<th>Sunitinib</th>
<th>Axitinib</th>
<th>Sorafenib</th>
<th>Bevacizumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutated</td>
<td>18/32 (56%)</td>
<td>3/9 (33%)</td>
<td>2/10 (20%)</td>
<td>4/9 (44%)</td>
</tr>
<tr>
<td>Methylated</td>
<td>2/6 (33%)</td>
<td>0/1 (0%)</td>
<td>0/2 (0%)</td>
<td>0/3 (0%)</td>
</tr>
<tr>
<td>Wild-type</td>
<td>13/25 (52%)</td>
<td>3/5 (60%)</td>
<td>0/16 (0%)</td>
<td>0/5 (0%)</td>
</tr>
</tbody>
</table>
Conclusions

• RCC is heavily reliant on the VEGF pathway

• VEGF pathway inhibition has produced robust clinical results in RCC and is now the standard of care

• Sunitinib may have favorable immunoregulatory properties
  • Immunotherapeutic combinations are being explored, e.g. + anti-CTLA-4 Ab, + vaccine

• The molecular geno/phenotype of response to VEGF-targeted agents in RCC requires further investigation