Effects of Antiangiogenic Therapy on the Immune System

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Clinical Observations

• Different levels of neo-vascularization occur between tumor types and between different tumors of the same type.

• Different levels of tumor infiltrating lymphocytes occur between tumor types and between different tumors of the same type.
Tumor Neo-angiogenesis

• Vessel Sprouting
• Vessel Splitting
• TC Vascular Mimicry
• CD11c Vascular Luekocytes
  • BM Derived
  • Resident Organ Derived
• CD137 expressed on tumor vessels

Fidler & Ellis 2004
Conego-Garcia 2004
Broll 2001
Cell Survival

• Supplies
  – Nutrients
  – Oxygen
    • Normal cell diffusion radius <200μm
    • p53+ tumor cells diffusion radius <110μm
    • p53- tumor cells diffusion radius <150μm

• Waste
  – Toxic substances
  – CO₂

• Pathway
  – Vasculature
Cancer Producing Pro-Angiogenic Growth Factors

- Vascular Endothelial Growth Factor
- Basic Fibroblast Growth Factor
- Transforming Growth Factor $\beta$-1
- Placenta Growth Factor
- Platelet-derived endothelial cell growth factor
- Pleiotrophin
Leukocyte Trafficking
Vasculature and Immune Cells

- Rolling of endogenous leukocytes is generally low in tumor vessels

- Stable adhesion (≥ 30 sec) is comparable between normal vessels and tumor vessels.
**Tissue Specificity**
Determined by Phage Display

**Vasculature Protein Expression**

<table>
<thead>
<tr>
<th>Motif</th>
<th>Protein</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGRG:</td>
<td>Gelatinase – MMP-9</td>
<td>Pancreas</td>
</tr>
<tr>
<td>HGG:</td>
<td>Endothelial Growth Factor – Neuropilin</td>
<td>Skin</td>
</tr>
<tr>
<td></td>
<td>Pro-PDGFR</td>
<td>Pancreatic Cancer</td>
</tr>
<tr>
<td></td>
<td>Kallikrein-9</td>
<td>Squamous Cell/skin</td>
</tr>
</tbody>
</table>

Rafii et al 2003
Arap et al 2003
### Leukocyte Trafficking

#### Memory T cell Vascular Attachment

<table>
<thead>
<tr>
<th>Selectin type</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Selectin</td>
<td>Lymph Node</td>
</tr>
<tr>
<td>E-Selectin</td>
<td>Skin</td>
</tr>
<tr>
<td>P-Selectin</td>
<td>GI</td>
</tr>
</tbody>
</table>
Vasculature and Immune Cells

• Activated immune cells adhered to blood vessels via cell specific receptors:
  – NK cells:
    • CD18 (B2 Integrin)
    • very large antigen-4 (VLA-4)
  – T cells:
    • CD62L (L selectin)
    • CCR7
    • CXRC3
    • CD103
    • CD4

• Blood vessel endothelial cell adhesion molecules are tissue specific:
  – ICAM-1 (intercellular adhesion molecule-1) on EC
  – VCAM-1 (vascular cell adhesion molecule-1) on EC
  – E-selectin on EC

• Regulation of adhesion molecules
  – upregulated
    • TNF-α
    • P90
    • VEGF
  – down-regulated
    • TGF-β
    • bFGF
Vascular Targets

• Normal vessels are well organized with even diameters.

• Tumor vessels are tortuous, with increased vessel diameter, length, density, and permeability.

• Anti-angiogenic therapies "normalize" the tumor vascular network and could ultimately reduce the vasculature to the point at which it provides inadequate support for tumor growth
Preclinical Observations
Antiangiogenic Therapy on the Immune System

- Adoptive Transfer of activated T-cells and radiation can induce High Endothelial Venules (HEV) and TIL
  Ganss 1998

- B7.1gene → Angiostatin gene induces ↓ tumor
  Sun 2001

- IL-2 / angiostatin fusion molecule induces ↓ tumor
  Dentelli 2004
Vascular Endothelial Growth Factor (VEGF)

- Vascular endothelial growth factor originally discovered in 1983 as the vascular permeability factor (VPF)

- Cloned in 1989

- **VEGF**
  - increases vascular permeability
  - promotes migration and proliferation of endothelial cells (ECs)
  - serves as an EC survival factor
  - upregulate leukocyte adhesion molecules on ECs
  - VEGF implicated in DC dysfunction
  - VEGF implicated in interfering with the development of T-cells from hematopoietic progenitor cells.
**VEGF**

- High levels of VEGF have been correlated with a poor prognosis for specific tumor histotypes.
  - Ligand-stimulated tyrosine kinases are induced in a tumor stage-dependent manner during cancer progression and are expressed in tumor vascular endothelial cells.

- Multiple VEGFR
  - Two high-affinity receptors: the tyrosine kinases VEGFR-1/flt-1 and VEGFR-2/flk-1,
  - Others (VEGFR-3) have been described as well
VEGF Pathways in RCC

• RCC
  – Clear cell (75%)
  – chromophilic (papillary) (15%)
  – Chromophoblic
  – Oncocytic
  – collecting duct

• Von Hippel-Lindau (VHL) gene
  – mapped to chromosome 3p25
  – VHL defect in Clear Cell
    • VHL inactivation >50-75% of sporadic cases
      – Somatic mutation
      – hypermethylation
VHL Pathways

- HIF is a heterodimer: HIF$\alpha$ & HIF$\beta$ subunits
- HIF$\beta$ constitutively expressed
- HIF$\alpha$ degraded in the presence of O$_2$
- Absence of O$_2$ prevents alteration in HIF$\alpha$ degradation domain, stops pVHL binding and leads to accumulation of HIF.
VHL Pathways

- Lacking functional pVHL results in the inability to suppress accumulation of Hypoxia-inducible (HIF) genes and proteins:
  - VEGF
  - PDGF
  - TGFα (a renal epithelial cell mitogen)
  - EGFr
  - Epo
  - CAIX
Support
Cytokine Working Group Study W 0454
(IND: BB-IND 12157)

- Chiron Inc, now Novartis Pharmaceuticals Corporation Oncology
- Genentech, Inc
Participating Members
Cytokine Working Group Study W 0454
(IND: BB-IND 12157)

- Michael Atkins, MD  Beth Israel Deaconess Medical Center
- David McDermott, MD  Beth Israel Deaconess Medical Center
- Todd S. Crocenzi, MD  Earle A. Chiles Research Institute
- Walter Urba, MD  Earle A. Chiles Research Institute
- Theodore Logan, MD  Indiana University
- Larry Flaherty, MD  Karmanos Cancer Center
- Ulka Vaishampayan, MD  Karmanos Cancer Center
- Joseph Clark, MD  Loyola University
- Janice Dutcher, MD  Our Lady of Mercy Cancer Center
- Robert Figlin, MD  University of California at Los Angeles
- John Kirkwood, MD  University of Pittsburgh
- Geoff Weiss, MD  University of Virginia
- Jeffrey Sosman, MD  Vanderbilt University
- Sabina Signoretti, MD  Pathology Core Director, Brigham and Women's Hospital
- Meredith Regan, ScD  Biostatistician, Dana-Farber Cancer Institute
- Nancy Crosby ARNP  Nurse Coordinator, DHMC
- Conrad Farnham  Protocol Coordinator, DHMC
- Cheryl Carlson, RN, BSN  Data Coordinator, DHMC
- Kim Margolin, MD  Consultant, City of Hope
Aldesleukin in Patients with RCC

- High-dose bolus IL-2 was approved by the FDA in 1992
- 600,000-720,000 IU/kg of recombinant human IL-2
  - every 8 hours x 14 doses.
  - A cycle of treatment consists of two 5-day treatment courses separated by 5-9 days of rest (maximum of 28 doses)
- Objective responses were seen in 37 of the 255 patients (RR 15%).
- There were 17 (7%) complete responses (CRs) and 20 (8%) partial responses (PRs).
- The median duration of response was 54 months for all responders,
- The median survival was 16 months for all 255 patients.
- Median overall survival was 20 months for PRs and has not yet been reached for CRs.
### VHL Targeted Pathways in RCC

<table>
<thead>
<tr>
<th>AGENT</th>
<th>TARGET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-VEGF</td>
<td>VEGF</td>
</tr>
<tr>
<td>PTK787</td>
<td>TKI: VEGFr1, PDGFr</td>
</tr>
<tr>
<td>VEGF Trap</td>
<td>Cytokine Trap: VEGFr1, VEGFr2</td>
</tr>
<tr>
<td>STI-571 (Gleevec)</td>
<td>TKI: PDGFr, Bcr/Abl, c-Kit</td>
</tr>
<tr>
<td>Anti-EGFr (C225, ABX-EGF)</td>
<td>EGFR</td>
</tr>
<tr>
<td>ZD1839 (Iressa), OSI-774 (Tarceva)</td>
<td>TKI: EGFr</td>
</tr>
<tr>
<td>SU11248 (Sunitinib)</td>
<td>TKI: PDGFr, Flt 3, c-Kit, VEGFr2</td>
</tr>
<tr>
<td>ZD6474</td>
<td>TKI: VEGFr2, EGFr</td>
</tr>
<tr>
<td>BAY 43-9006 (Sorafenib)</td>
<td>RAF kinase, VEGF, PDGF</td>
</tr>
<tr>
<td>CCI 779 (Temsirolimus)</td>
<td>mTOR inhibitor</td>
</tr>
</tbody>
</table>
Bevacizumab in Patients with RCC

- Vascular Endothelial Growth Factor (VEGF) is a pro-angiogenic factor, which has also been shown to have a negative influence on the immune system.
  
  VEGF is also implicated as a proinflammatory molecule in a tissue ischemia model and Rheumatoid Arthritis

- Bevacizumab is a recombinant, humanized monoclonal antibody that was selected for its affinity to VEGF.
  
  Bevacizumab has been shown to inhibit angiogenesis and tumor growth.
  
  Elevated levels of circulating VEGF have been shown to confer poor prognosis in numerous solid tumors.

- Toxicity observed: bleeding, thrombotic complications, hypertension, bowel perforation and proteinuria
## Bevacizumab in Patients with RCC

<table>
<thead>
<tr>
<th>Anti-VEGF</th>
<th>10 mg/kg</th>
<th>3 mg/kg</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFS at 4 months</td>
<td>64%</td>
<td>39%</td>
<td>20%</td>
</tr>
<tr>
<td>PFS at 8 Months</td>
<td>30%</td>
<td>14%</td>
<td>5%</td>
</tr>
</tbody>
</table>

4 PRs at 10mg/kg

Yang JC, 2003, 2004
Rationale for Bevacizumab and Aldesleukin

- Complementary immune regulatory effects of aldesleukin and bevacizumab (T cell activation, DC activation)
- Non-overlapping toxicities
- Potential prolongation of responses
- Ultimately, goal of improved survival compared to historical controls.
Rationale for Bevacizumab and Aldesleukin in RCC Patients – A CWG Study

- Negative influence of angiogenesis on anticancer immunity have shown that some angiogenic factors, such as VEGF, may induce immunosuppression.

- Some evidence of abnormally high blood levels of VEGF has been proven to be associated with resistance to IL-2 immunotherapy.

- Significant increase in the mean number of circulating mature DCs seen in IL-2 treated RCC patients.

  - Bonfanti A. 2000
Eligibility - Inclusion

Bevacizumab and Aldesleukin in RCC Patients – A CWG Study

• Histologically confirmed metastatic renal cell carcinoma with predominantly clear cell histology.

• Measurable or evaluable disease.

• KPS $\geq 80\%$

• Adequate end organ function

• No serious hemorrhage, bleeding diathesis, underlying coagulopathy, DVT, clinically significant peripheral vascular disease, or other thrombotic event.
Eligibility - Exclusion

Bevacizumab and Aldesleukin in RCC Patients – A CWG Study

- Patients who have received prior systemic therapy for metastatic RCC or have previously received bevacizumab or IL-2 are not eligible.

- Significant co-morbid illness such as uncontrolled diabetes or active infection that would preclude treatment on this regimen.

- Uncontrolled hypertension (BP >150/100 mmHg)

- Proteinuria dipstick > 3+ or > 2gm/24 hours

- Urine protein: creatinine ratio > 1.0 at screening
## Treatment Plan

Bevacizumab and Aldesleukin in RCC Patients – A CWG Study

One cycle = 84 days
HD bolus IL-2 IV Q8 hours (maximum 28 doses)
Bevacizumab IV (1-2 hours prior to IL-2) q 2 weeks x 12 weeks (6 doses)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Days:</th>
<th>Cycle 1</th>
<th></th>
<th>Cycle 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 15 29 43 57 71 85 99…</td>
<td>Bev X X X X X X X X</td>
<td></td>
</tr>
<tr>
<td>Bev</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>IL-2</td>
<td>X X</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Interim Safety Analysis at First Stage: Demographics

**Bevacizumab and Aldesleukin in RCC Patients – A CWG Study**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient Number</td>
<td>15</td>
</tr>
<tr>
<td>Median Patient Age</td>
<td>54 (range 40-73)</td>
</tr>
<tr>
<td>Gender</td>
<td>9 Male (60%), 6 Female (40%)</td>
</tr>
<tr>
<td>Karnosky Score</td>
<td></td>
</tr>
<tr>
<td>100% - 8 (53%)</td>
<td></td>
</tr>
<tr>
<td>90% - 4 (27%)</td>
<td></td>
</tr>
<tr>
<td>80% - 3 (20%)</td>
<td></td>
</tr>
<tr>
<td>MSKCC Criteria</td>
<td></td>
</tr>
<tr>
<td>14 (93%) Intermediate</td>
<td></td>
</tr>
<tr>
<td>1 (7%) Poor</td>
<td></td>
</tr>
<tr>
<td>Prior Treatment for RCC</td>
<td>15 Nephrectomy, 3 Radiation</td>
</tr>
<tr>
<td>Median Months from Dx to Regist.</td>
<td>16 (6-144)</td>
</tr>
<tr>
<td>Median Months from Dx to Mets</td>
<td>8 (2-20)</td>
</tr>
<tr>
<td>Diagnosis with Metastatic disease</td>
<td>7</td>
</tr>
</tbody>
</table>
## Interim Safety Analysis at First Stage: Treatment  
**Bevacizumab and Aldesleukin in RCC Patients – A CWG Study**

<table>
<thead>
<tr>
<th>Cycle 1 of Treatment</th>
<th>Summary Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong># Pts Initiating Cycle 1</strong></td>
<td>15 (100%)</td>
</tr>
<tr>
<td><strong># doses Bev</strong></td>
<td>Median 7 (range 2-7)</td>
</tr>
<tr>
<td>2 doses</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>3 doses</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>4 doses</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>6 doses</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>7 doses</td>
<td>10 (67%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong># doses IL-2</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cycle 1a</strong></td>
<td>Median 11 (Range 6-14)</td>
</tr>
<tr>
<td><strong>Cycle 1b</strong></td>
<td>Median 6 (Range 0-14)</td>
</tr>
</tbody>
</table>
| **Total**                         | Mean 17.8 +/- 5.3 95% CI 14.9-20.7)  
                                    | (Median 17 (Range 6-26)) |
|  **# not proceeding to cycle 2**  | 5 (33%)         |
Interim Safety Analysis at First Stage: SAEs Bevacizumab and Aldesleukin in RCC Patients – A CWG Study

- There is one treatment related death during cycle 2 of IL-2
- All but one patient remain alive as of August 3, 2006 (1 year after First Registered patient)
- Expected toxicity included (grade):

<table>
<thead>
<tr>
<th>Vascular Leak (3)</th>
<th>LFTs (3, 4)</th>
<th>Lymphopenia (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non sustained V tach (3)</td>
<td>Electrolytes (3)</td>
<td>Neutropenia (3)</td>
</tr>
<tr>
<td>Afib (3)</td>
<td>↑Creat, Renal Failure (3,4)</td>
<td>Thrombcytopenia (3,4)</td>
</tr>
<tr>
<td>Confusion (3)</td>
<td>Sepsis (3)</td>
<td>Dermatitis-allergic (3)</td>
</tr>
<tr>
<td>Dyspnea (3,)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Reported as Unexpected Toxicity (grade):

| Vascular Collapse & Death (5) | |
|-----------------------------||
Interim Safety Analysis at First Stage: Responses
Bevacizumab and Aldesleukin in RCC Patients – A CWG Study

• 11 evaluable patients thus far
  – 1 PR
  – 4 SD
Conclusions

• Combination high dose bolus IL-2 and bevacizumab may be given safely.

• New vascular directed agents are now making their way into clinical oncologic practice.

• Interaction between effector cells and vascular endothelium and the cytokines that effect their function make combination therapies attractive.

• New therapeutic combinations will need to be tested in clinical trials.
Leukocyte Trafficking

Figure 1. Lymphocyte-endothelial cell interactions in lymphocyte homing to secondary lymphoid organs.
Neo-vascularization

• Sprouting angiogenesis
  – Branching of new capillaries
• Non-sprouting angiogenesis
  – Enlargement & splitting of pre-existing vessels