Effects of Antiagiogenic Therapy on the Immune System

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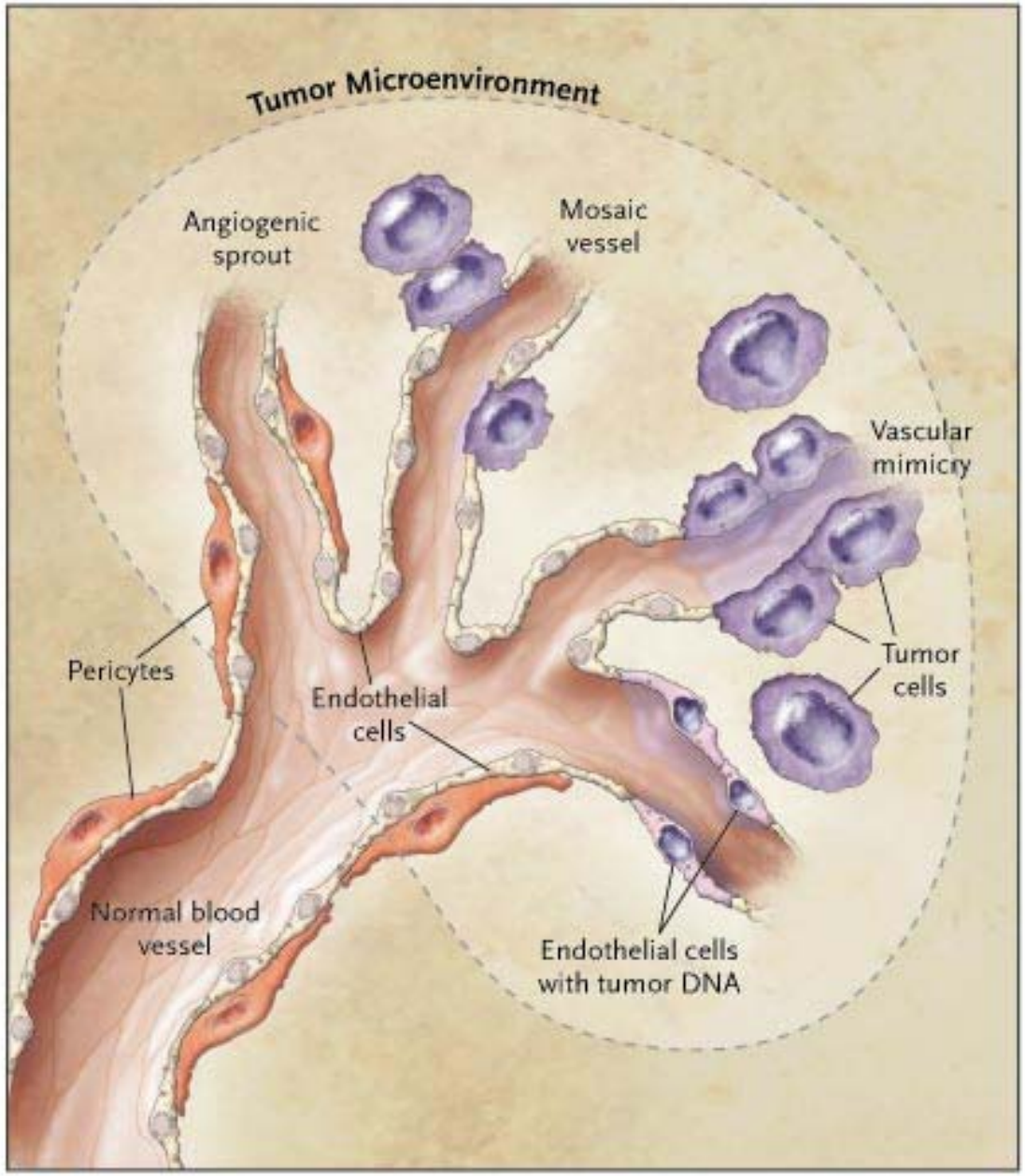
Clinical Observations

- 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

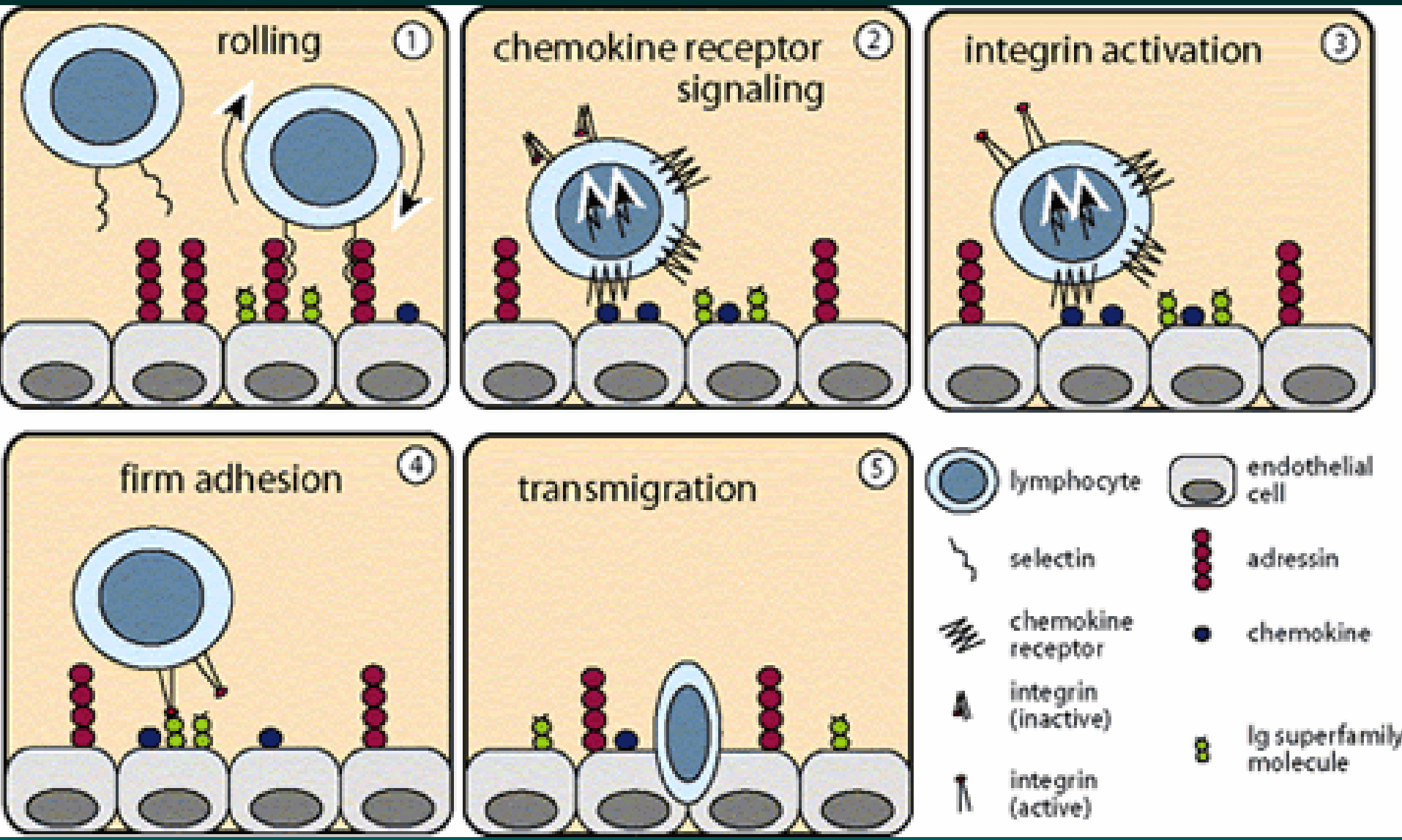
Cell Survival

- Supplies
  - Nutrients
  - Oxygen
    - Normal cell diffusion radius <200μm
    - $p53^+$ tumor cells diffusion radius <110μm
    - $p53^-$ tumor cells diffusion radius <150μm
- Waste
  - Toxic substances
  - CO_2
- Pathway
  - Vasculature

Cancer Producing Pro-Angiogenic Growth Factors

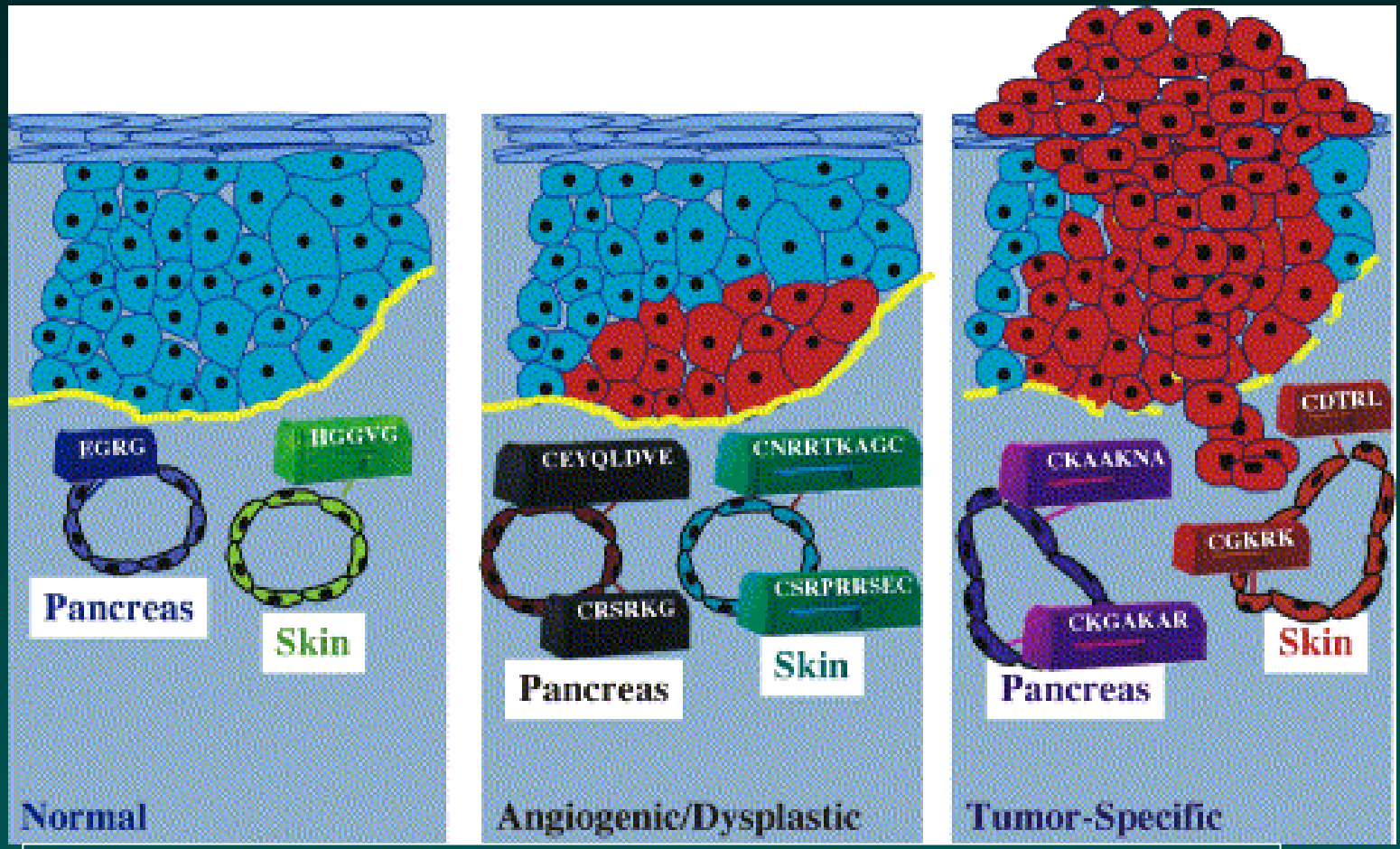
- Vascular Endothelial Growth Factor
- Basic Fibroblast Growth Factor
- Transforming Growth Factor β-1
- Placenta Growth Factor
- Platelet-derived endothelial cell growth factor
- Pleiotrophin

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

Motif	Protein	Location
EGRG:	Gelatinase – MMP-9	Pancreas
HGG:	Endothelial Growth Factor – Neuropilin	Skin
	Pro-PDGFR	Pancreatic Cancer
	Kallikrein-9	Squamous Cell/skin

Rafii et al 2003

Arap et al 2003

Leukocyte Trafficking

Memory T cell Vascular Attachment

Selectin type	Location
L-Selectin	Lymph Node
E-Selectin	Skin
P-Selectin	GI

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
Antiagiogenic 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 hematopoetic 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%)
  - Chromophobic
  - 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: HIFα & HIFβ subunits
- HIFβ constitutively expressed
- HIFα degraded in the presence of O_2
- Absence of O_2 prevents alteration in HIFα 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

Bevacizumab and Aldesleukin in Patients with Metastatic Renal Cell Carcinoma

A Cytokine Working Group Study
Update October 2006

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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
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- **Walter Urba, MD** Earle A. Chiles Research Institute
- **Theodore Logan, MD** Indiana University
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- **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

AGENT	TARGET
Anti-VEGF	VEGF
PTK787	TKI: VEGFr1, PDGFr
VEFG Trap	Cytokine Trap: VEGFr1, VEGFr2
STI-571 (Gleevec)	TKI: PDGFr, Bcr/Abl, c-Kit
Anti-EGFr (C225, ABX-EGF)	EGFR
ZD1839 (Iressa), OSI-774 (Tarceva)	TKI: EGFr
SU11248 (Sunitinib)	TKI: PDGFr, Flt 3, c-Kit, VEGFr2
ZD6474	TKI: VEGFr2, EGFr
BAY 43-9006 (Sorafenib)	RAF kinase, VEGF, PDGF
CCI 779 (Temsirolimus)	mTOR inhibitor

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 proinflamatory molecule in a tissue ishemia model and Rheumatoid Arthritis

- Bevacizumab is a recombinant, humanized monoclonal antibody that was selected for its affinity to VEGF.
  - Bevacizumab has been shown to in hibit 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

Anti-VEGF	10 mg/kg	3 mg/kg	Placebo
PFS at 4 months	64%	39%	20%
PFS at 8 Months	30%	14%	5%

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 ≥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)

		Cycle 1						Cycle 2
Drug	Days:							
	1	15	29	43	57	71	85	99…
Bev	X	X	X	X	X	X	X	X
IL-2		X	X					X…

Interim Safety Analysis at First Stage: Demographics

Bevacizumab and Aldesleukin in RCC Patients – A CWG Study

Patient Number	15
Median Patient Age	54 (range 40-73)
Gender	9 Male (60%), 6 Female (40%),
Karnosky Score	100% - 8 (53%) 90% - 4 (27%) 80% - 3 (20%)
MSKCC Criteria	14 (93%) Intermediate 1 (7%) Poor
Prior Treatment for RCC	15 Nephrectomy, 3 Radiation
Median Months from Dx to Regist.	16 (6-144)
Median Months from Dx to Mets	8 (2-20)
Diagnosis with Metastatic disease	7

Interim Safety Analysis at First Stage: Treatment

Bevacizumab and Aldesleukin in RCC Patients – A CWG Study

Cycle 1 of Treatment	Summary Statistics
# Pts Initiating Cycle 1	15 (100%)
# doses Bev	Median 7 (range 2-7)
2 doses	1 (7%)
3 doses	1 (7%)
4 doses	2 (13%)
6 doses	1 (7%)
7 doses	10 (67%)
# doses IL-2	
Cycle 1a	Median 11 (Range 6-14)
Cycle 1b	Median 6 (Range 0-14)
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):

Vascular Leak (3)	LFTs (3, 4)	Lymphopenia (3)
Non sustained V tach (3)	Electrolytes (3)	Neutropenia (3)
Afib (3)	↑Creat, Renal Failure (3,4)	Thrombcyotopenia (3,4)
Confusion (3)	Sepsis (3)	Dermatitis-allergic (3)
Dyspnea (3,)		

- 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

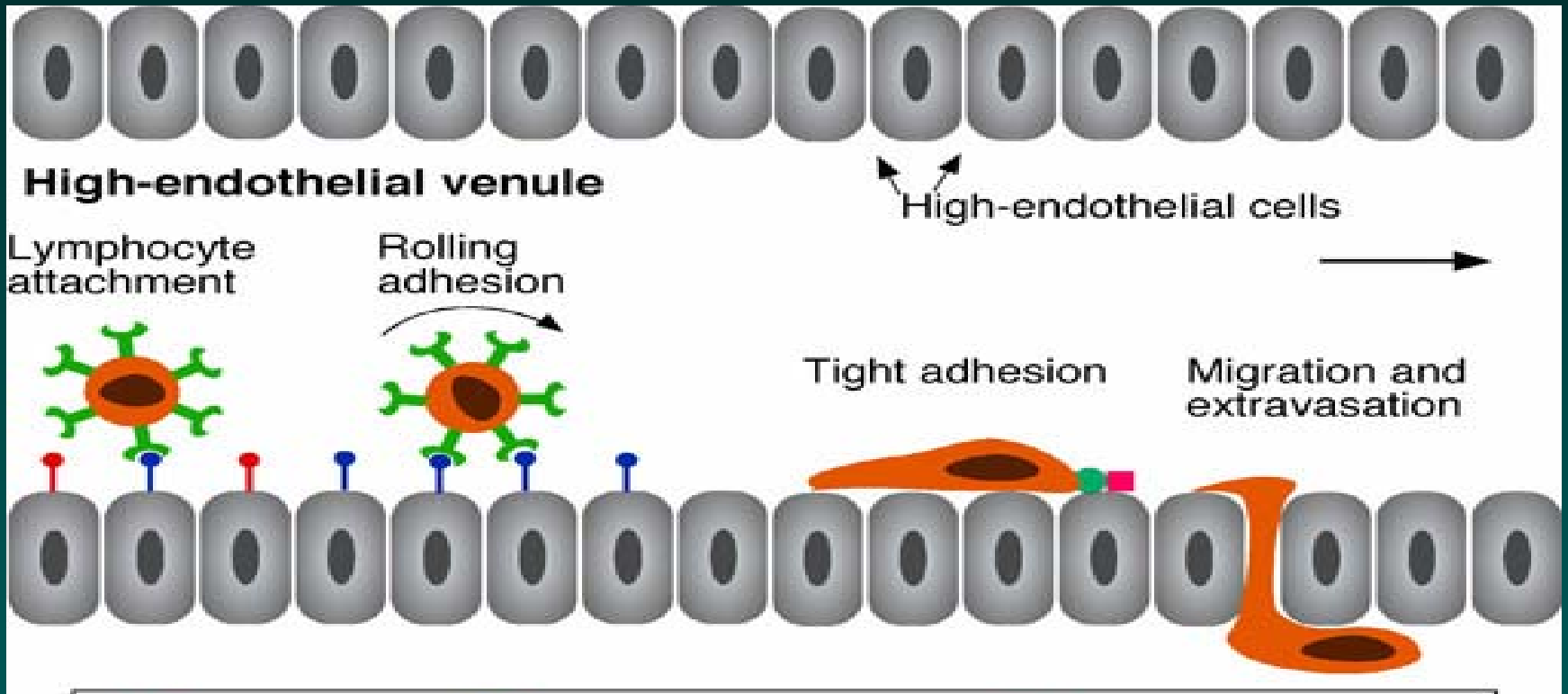

Figure 1. Lymphocyte-endothelial cell interactions in lymphocyte homing to secondary lymphoid organs

Neo-vascularization

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