# Signaling pathways, mechanisms of resistance and treatment decisions in renal cell cancer

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*Director, Division of Medical Oncology*
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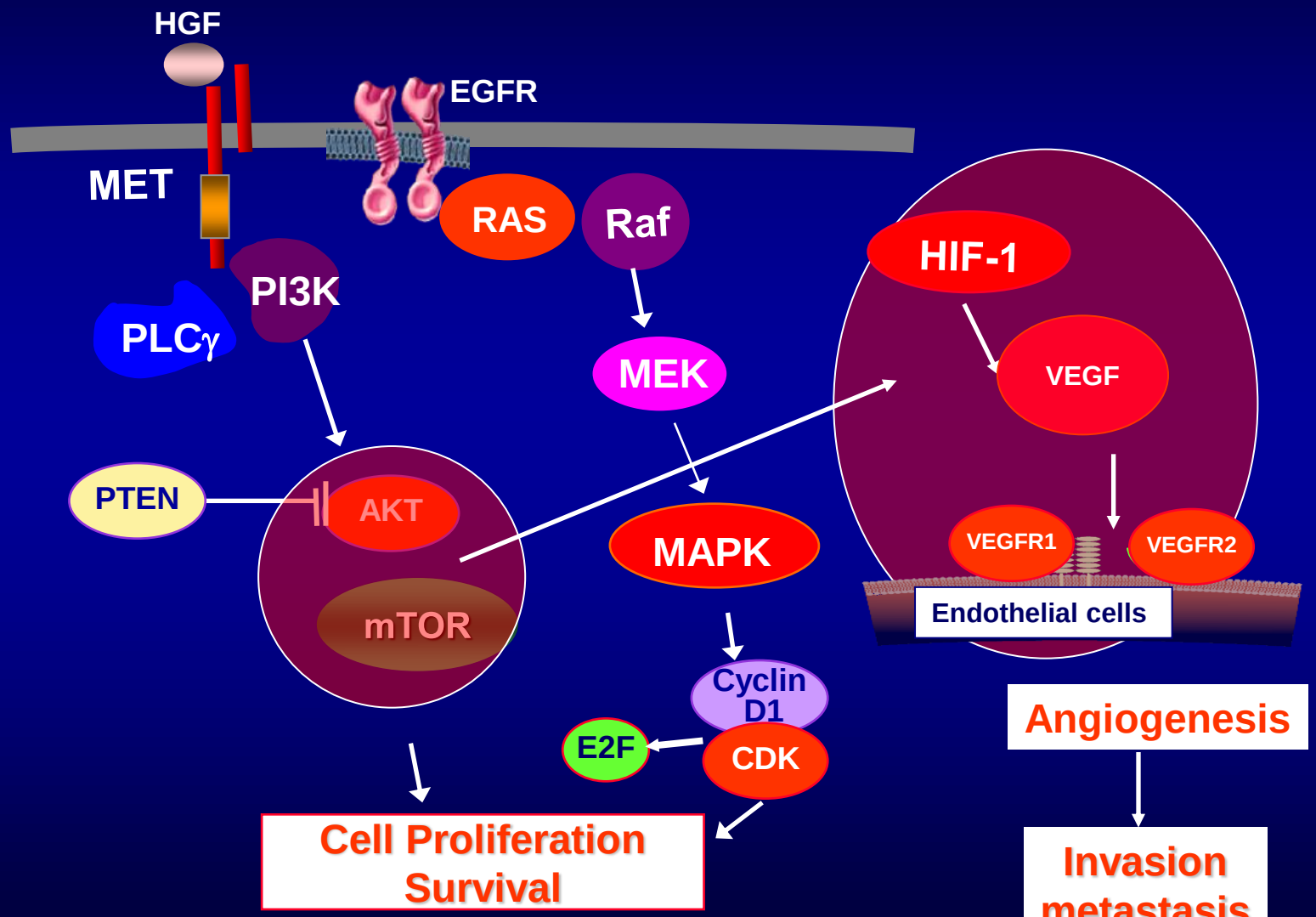
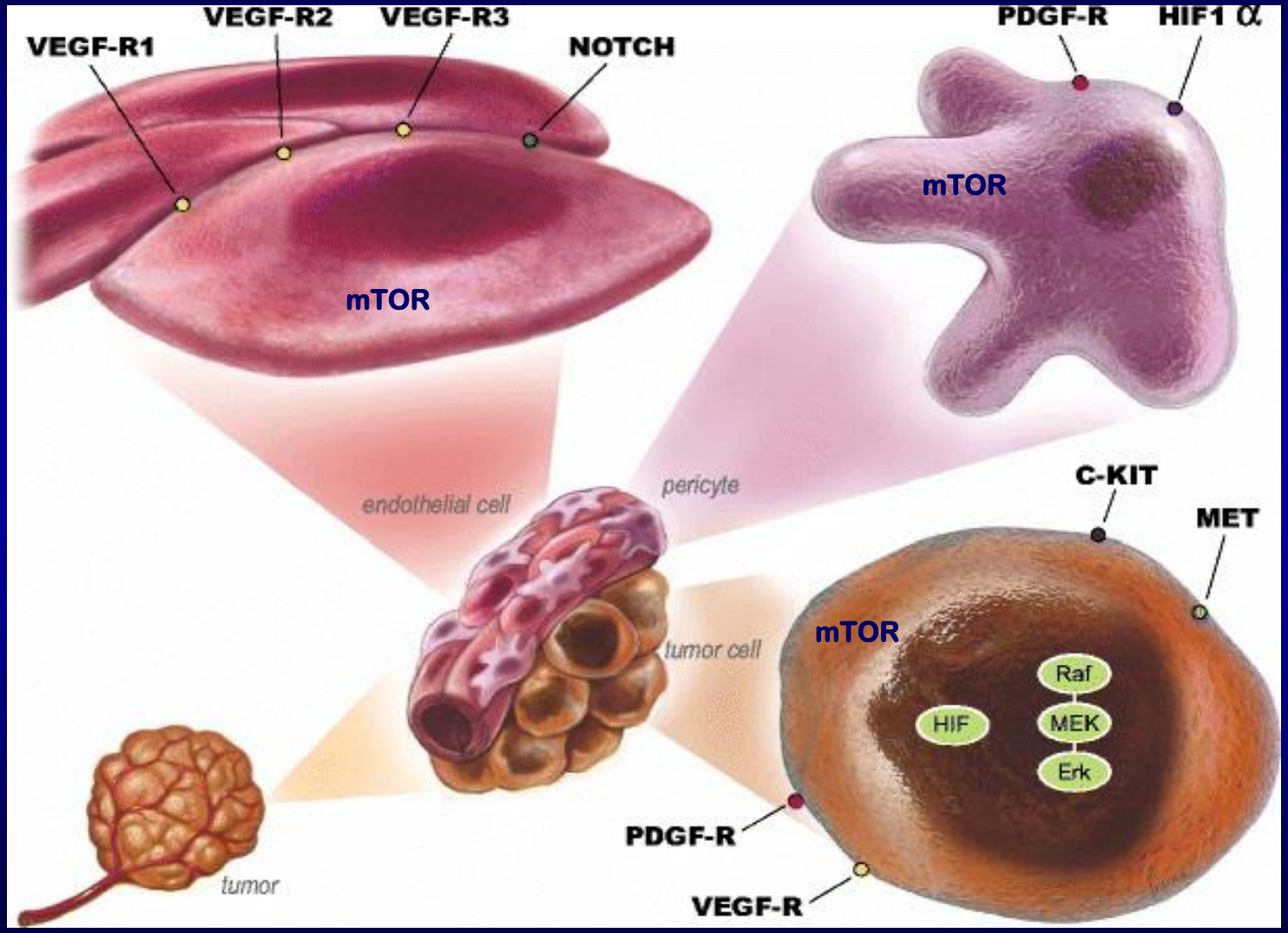
*Italy*

# Signalling pathways in Renal Cell Cancer




Tortora et al.,2010

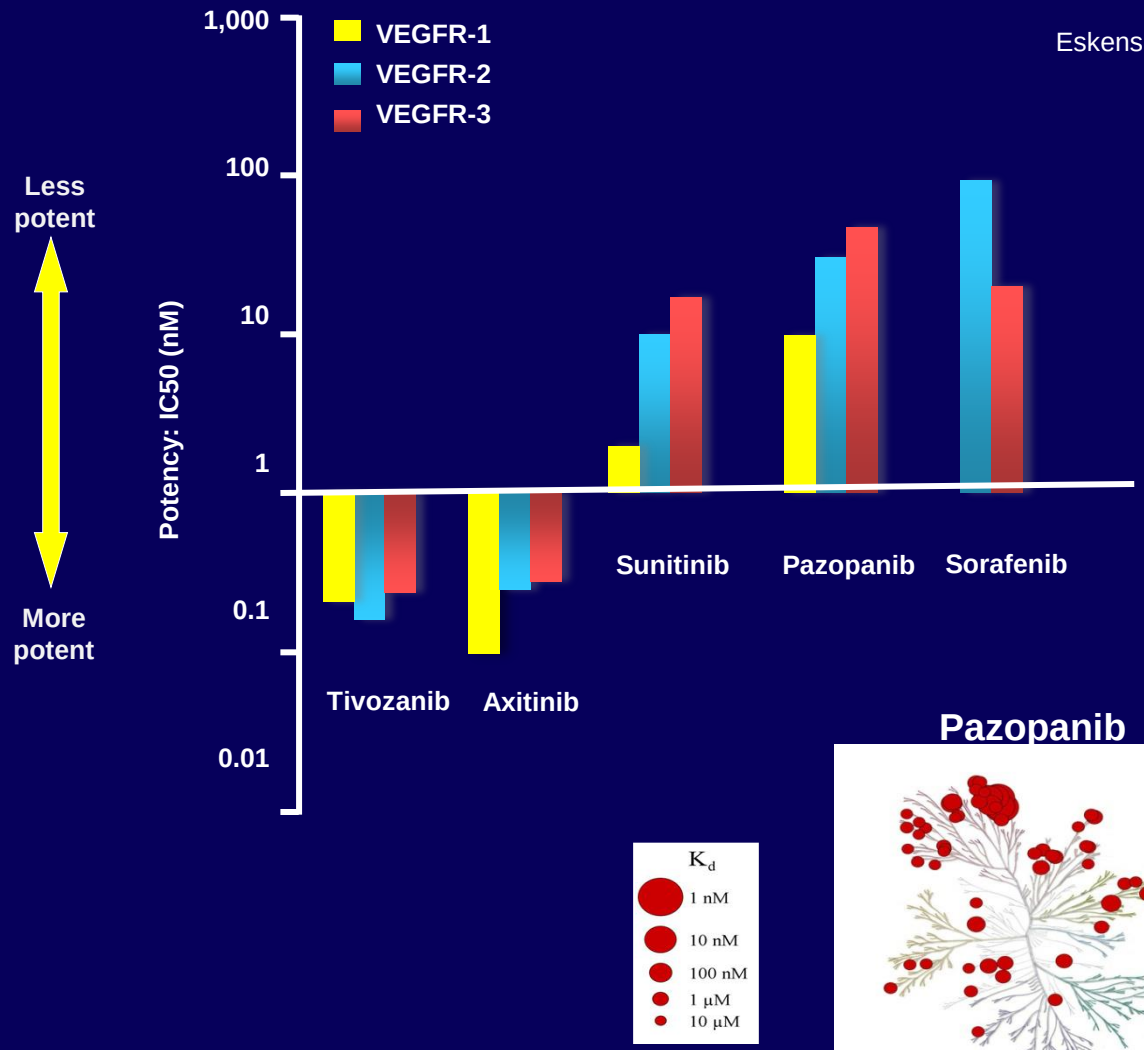
# Site of action of VEGF and VEGFR inhibitors on endothelial and cancer cells




Modified from the original Kelly R.J., Targeted Oncology 2009

# Anti-VEGFR TKIs have different potency and off-target activity

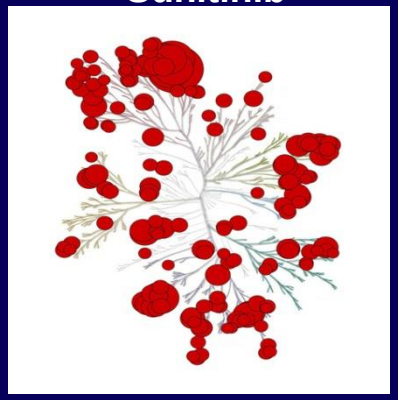
Figure modified using data from Chow LQM, Eckhardt SG. *J Clin Oncol*. 2007; Eskens FALM, et al. AACR 2008. Abstract LB-201; Hu-Lowe DD, *Clin Cancer Res* 2008

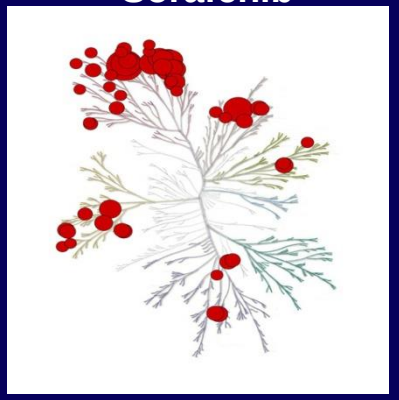








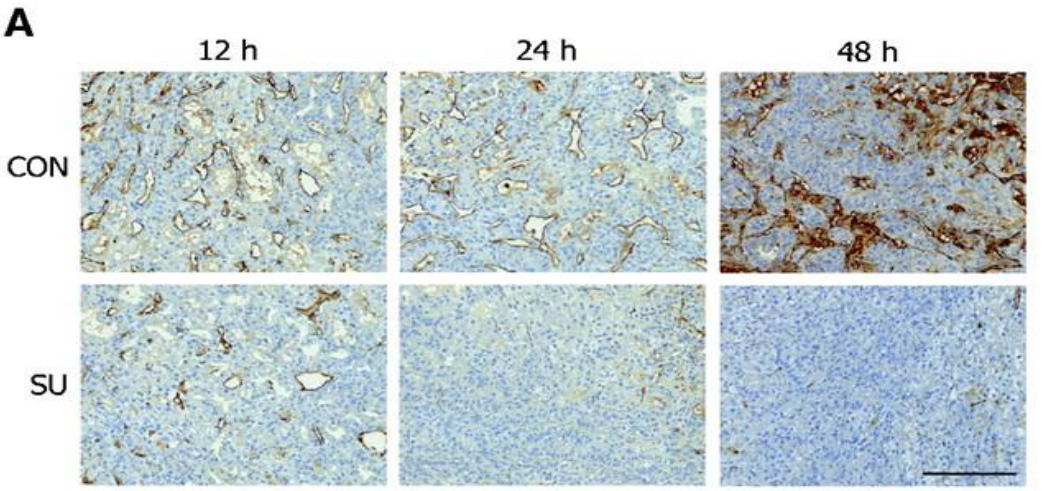



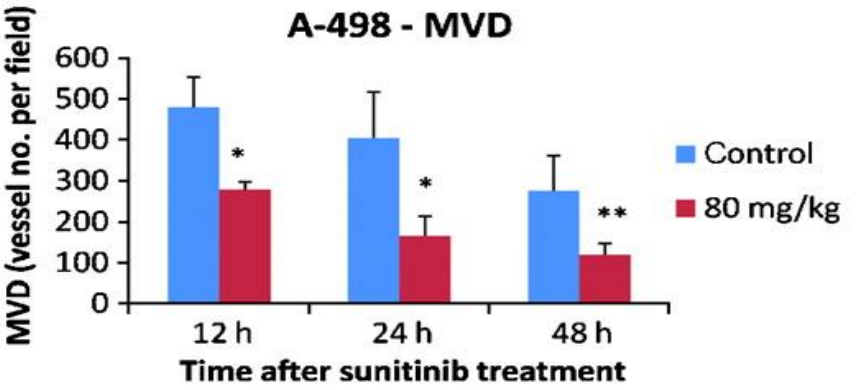

Adapted from Karaman et al. Nat Biotech. 2008;26:127

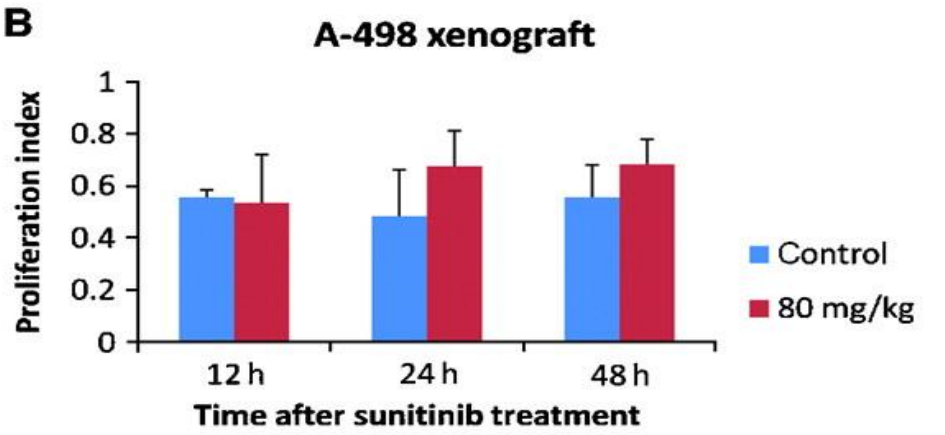
# Sunitinib primarily acts on tumor endothelium rather than via direct targeting of ccRCC cells in vivo.

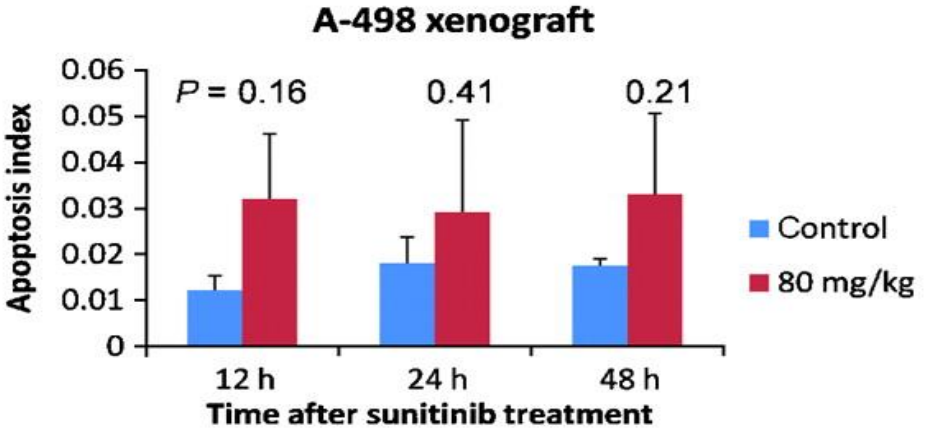
**sunitinib inhibits A-498 tumor angiogenesis as soon as 12 h after treatment**

**sunitinib shows minimal effect on tumor cell proliferation and no induction of apoptosis up to 72 h after treatment in vivo.**



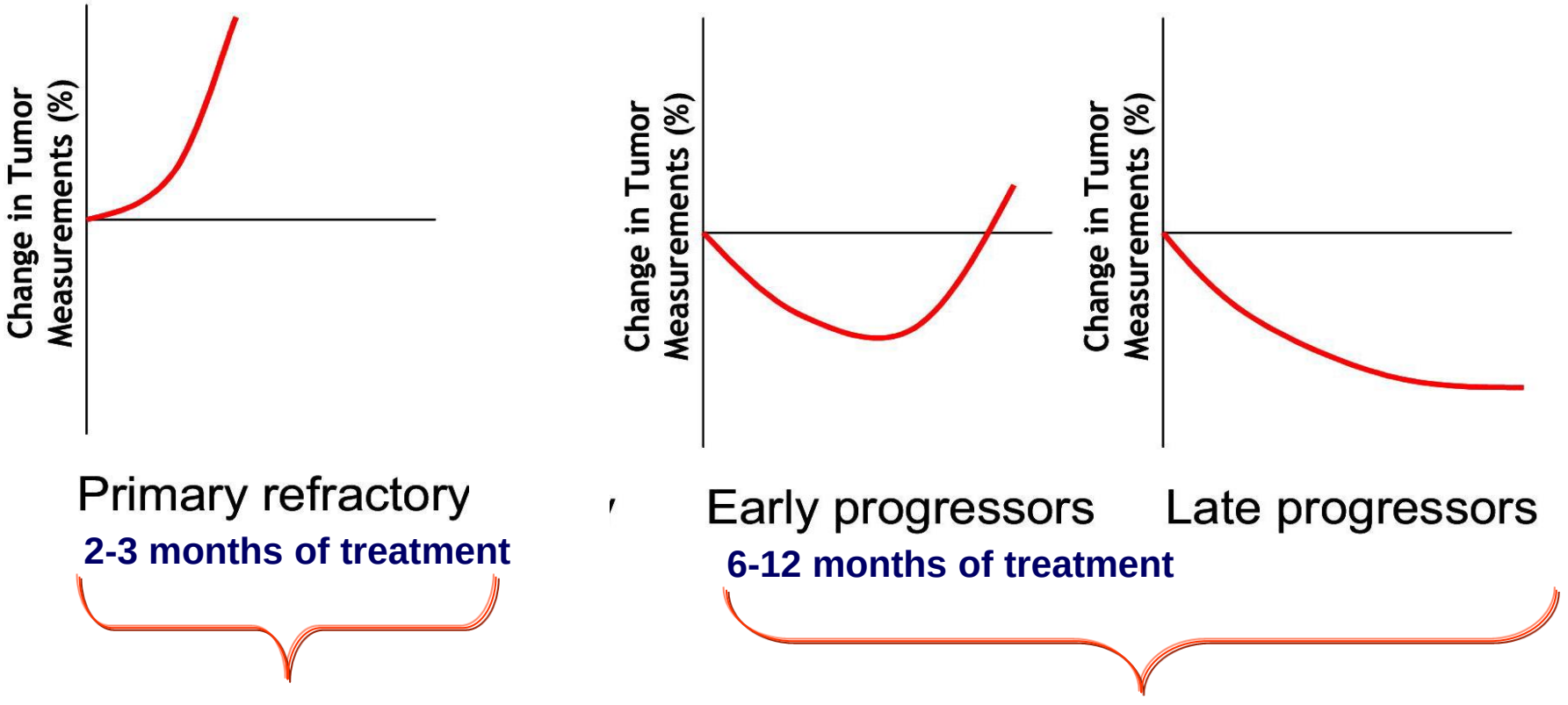




Huang D et al. Cancer Res 2010;70:1053-1062

# PRIMARY AND SECONDARY (EVASIVE) RESISTANCE

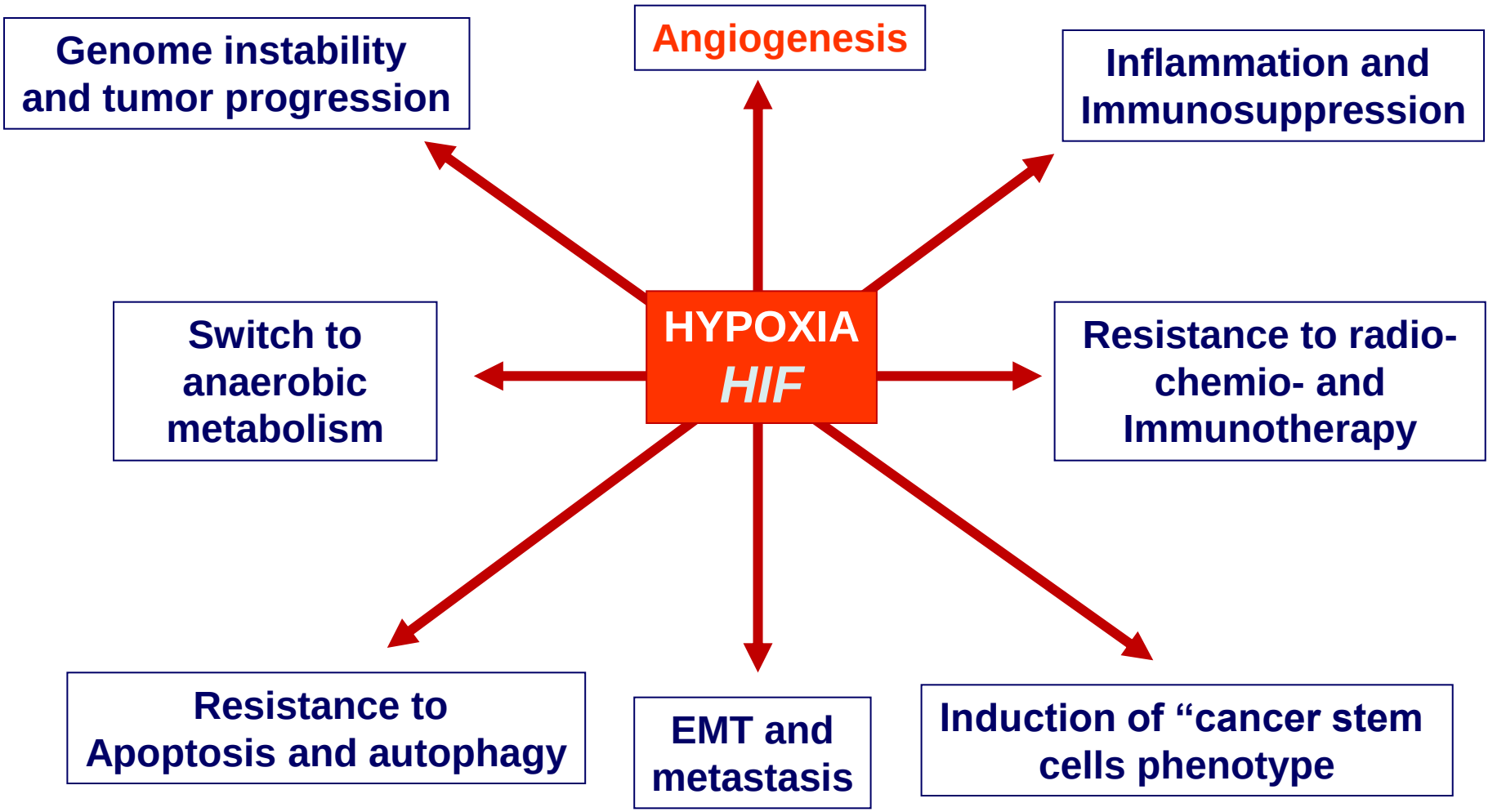



Modifiied from the original Rini B and Flaherty K, Urology Oncology 2008

# Key factors in the resistance to treatment in RCC

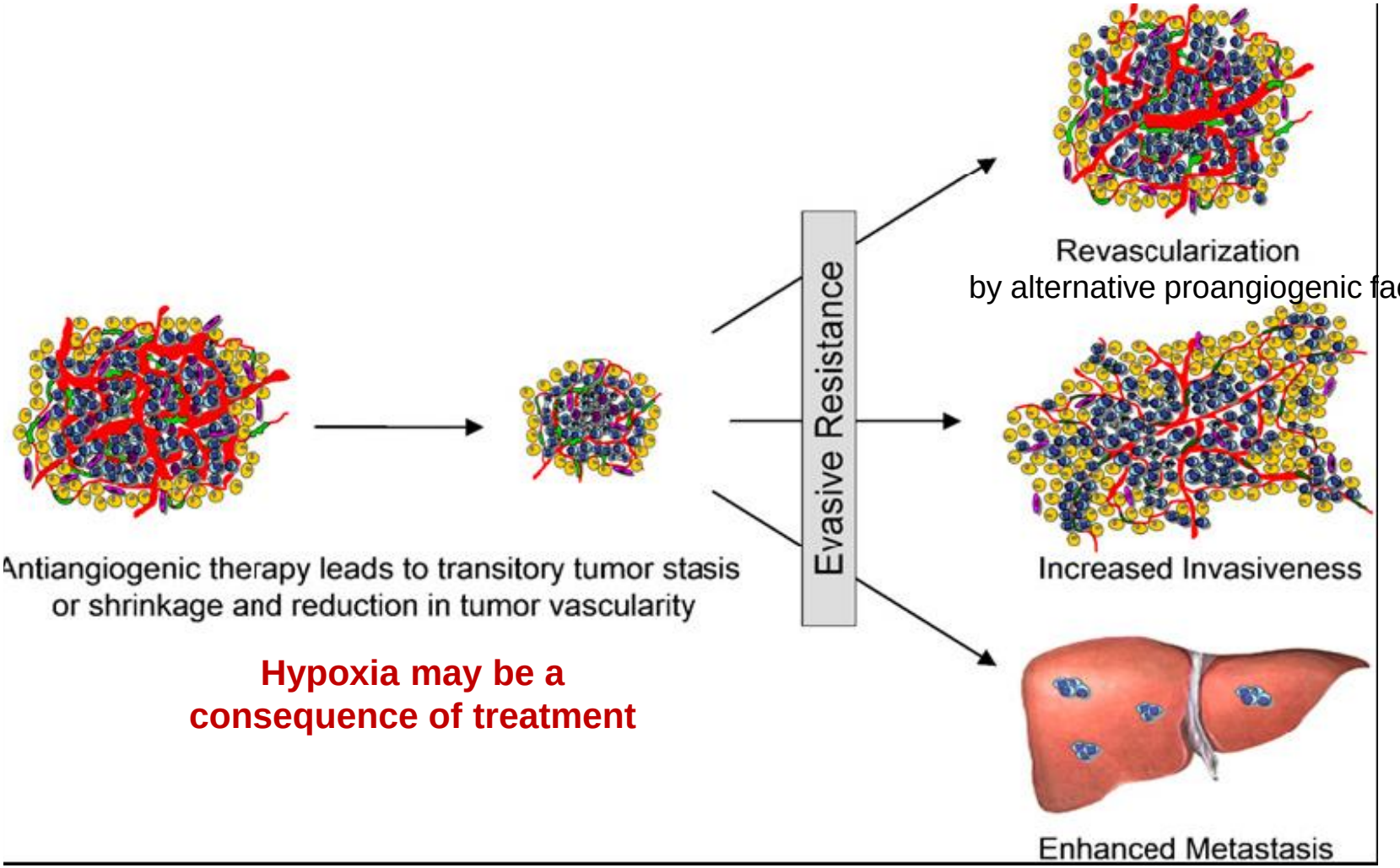
- **Hypoxia and angiogenesis**
- **Production of alternative pro-angiogenic factors (IL-8, FGF, HGF/MET).**
- **Activation of alternative signalling pathways (mTOR, PI3K/Akt etc.)**
- **Microenvironment**
- **Tumor heterogeneity**

# HYPOXIA AFFECTS MANY FUNCTIONS



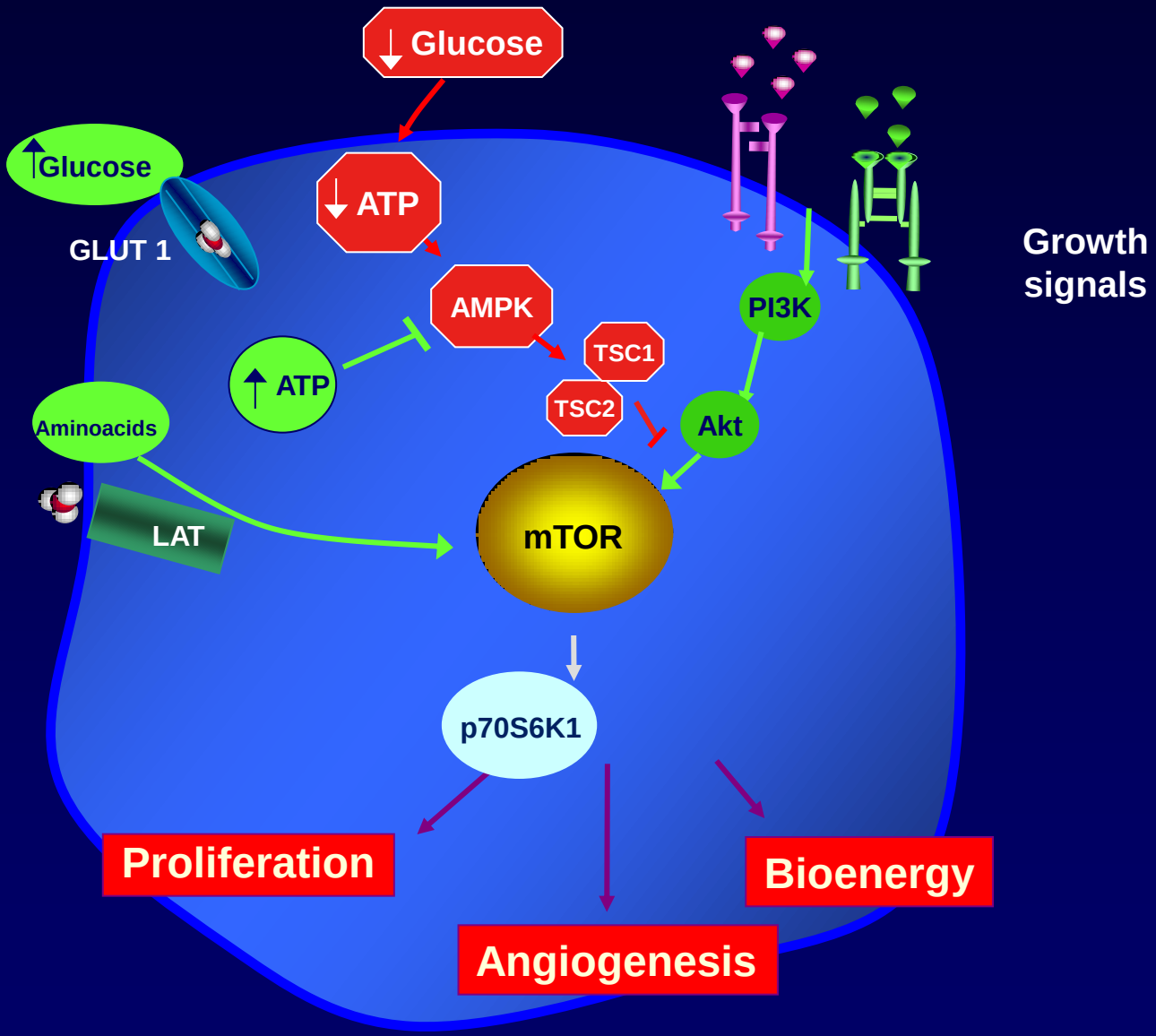

Jain R. AACR 2012

# Adaptive-Evasive Responses by Tumors to Antiangiogenic Therapies

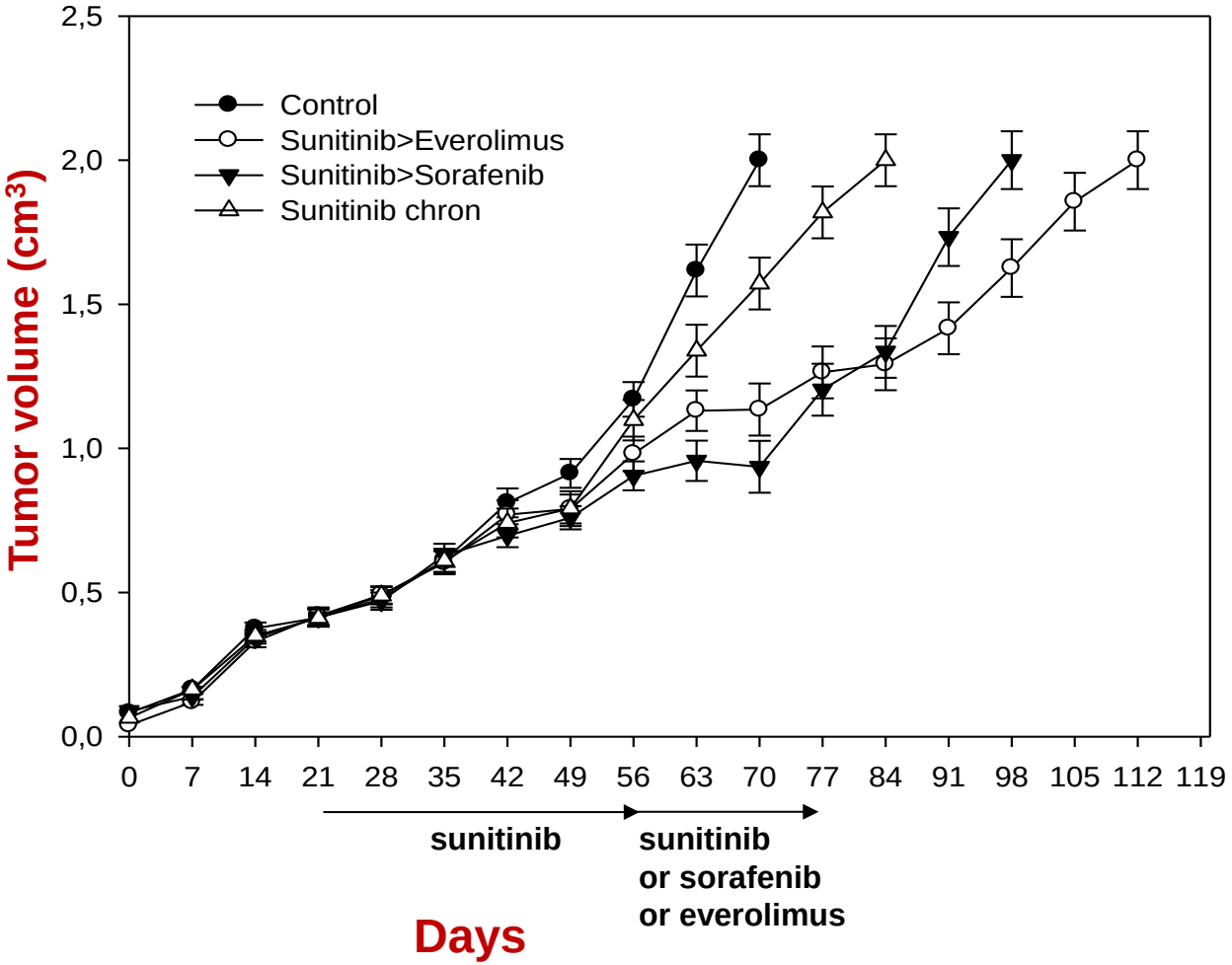


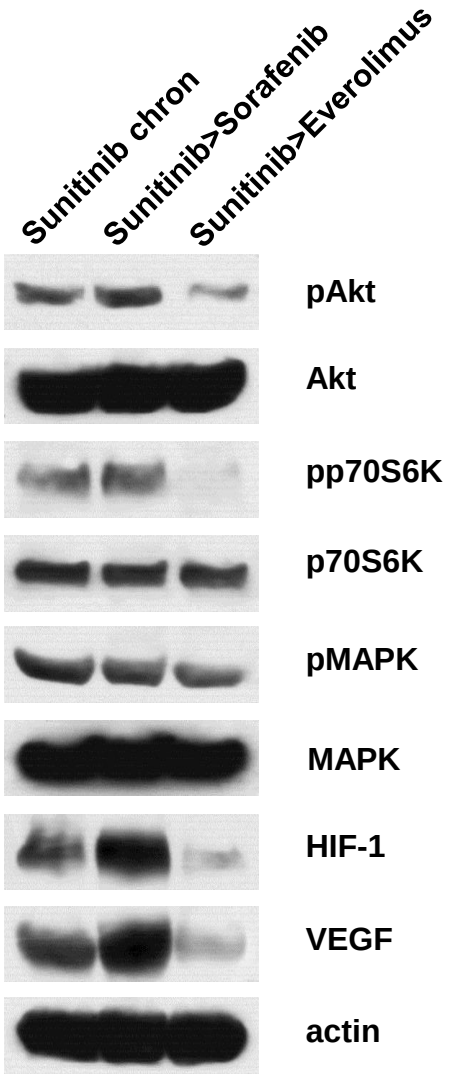

Paez-Ribes et al., Cancer Cell 15, 220-231, March 2009

# mTOR pathway is involved in angiogenesis, proliferation and bioenergetics



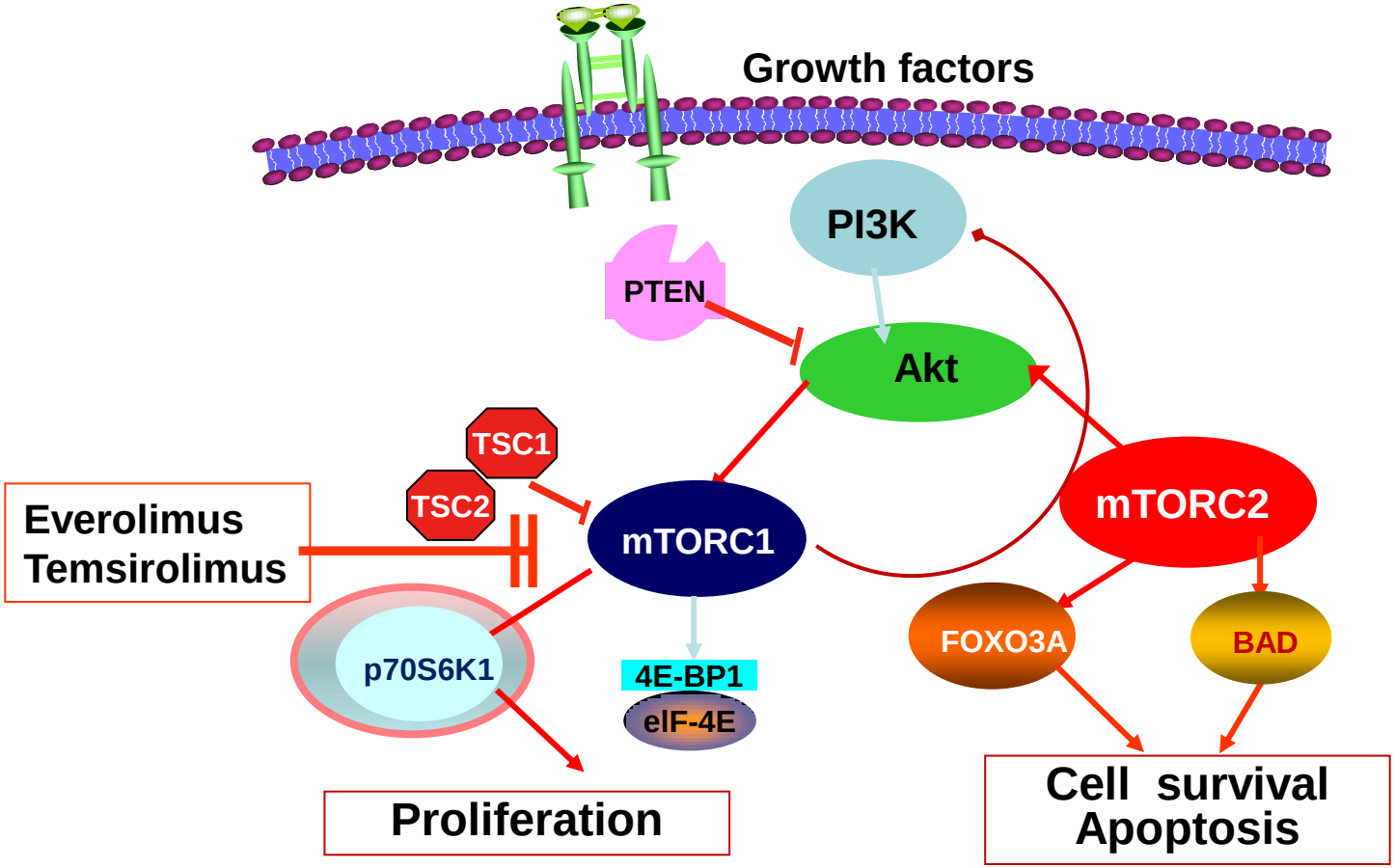
# Effects of drug sequences on growth of 786-O tumors in nude mice



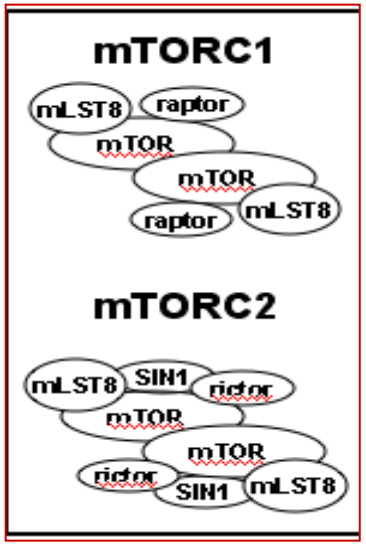

Western blot analysis on tumors lysates on day 77, **at the end of second treatment**.

# mTOR Signaling pathway and TORC




**Resistance**

**mTOR complexes**



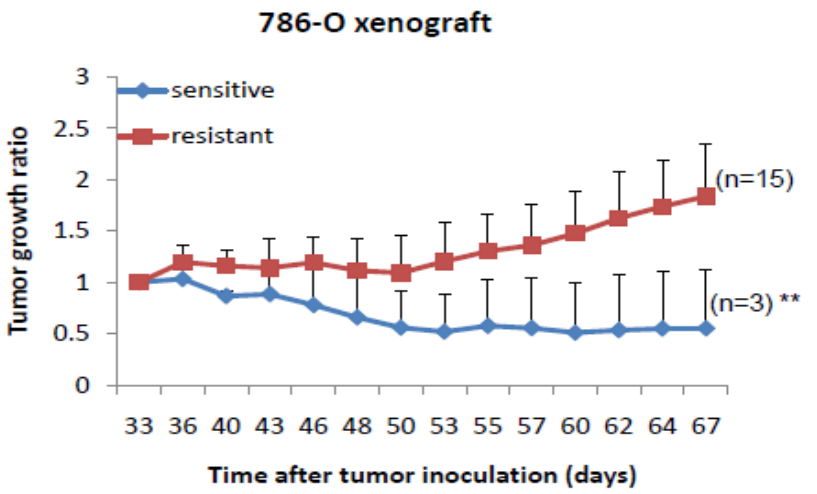

- Double inhibitors of PI3K and mTORC1 (BEZ235, etc.)
- Two inhibitors in combination
- Novel catalytic inhibitors of mTORC1 and mTORC2

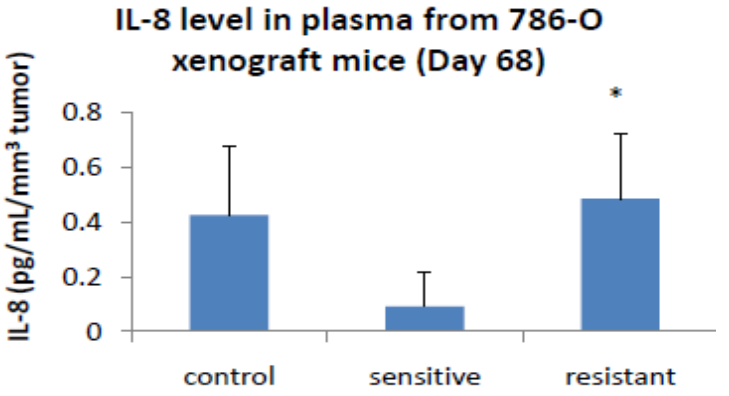
# Other proangiogenic factors relevant in RCC and resistance to treatment

- IL-8
- bFGF and FGF-Rs
- HGF/c-MET

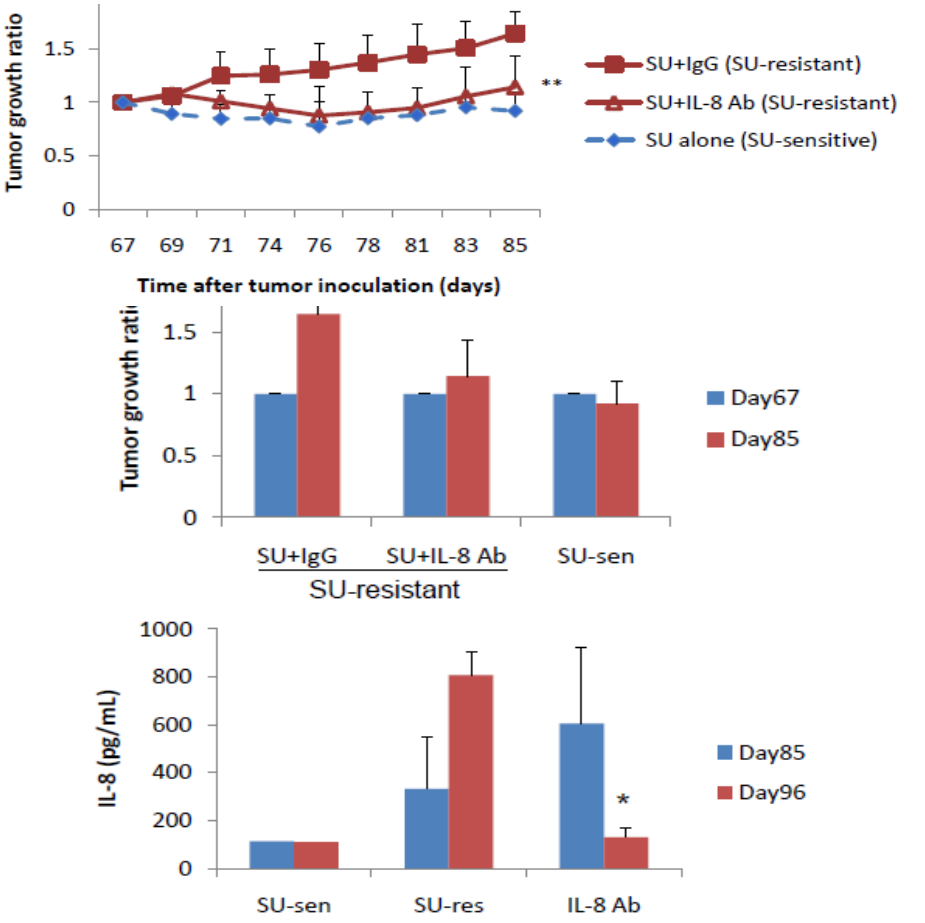
# IL-8 mediates resistance to sunitinib in RCC

## Phenotypic resistance of 786-O ccRCC xenografts is associated with elevated plasma levels of IL-8 in sunitinib-resistant mice treated with continuous dosing



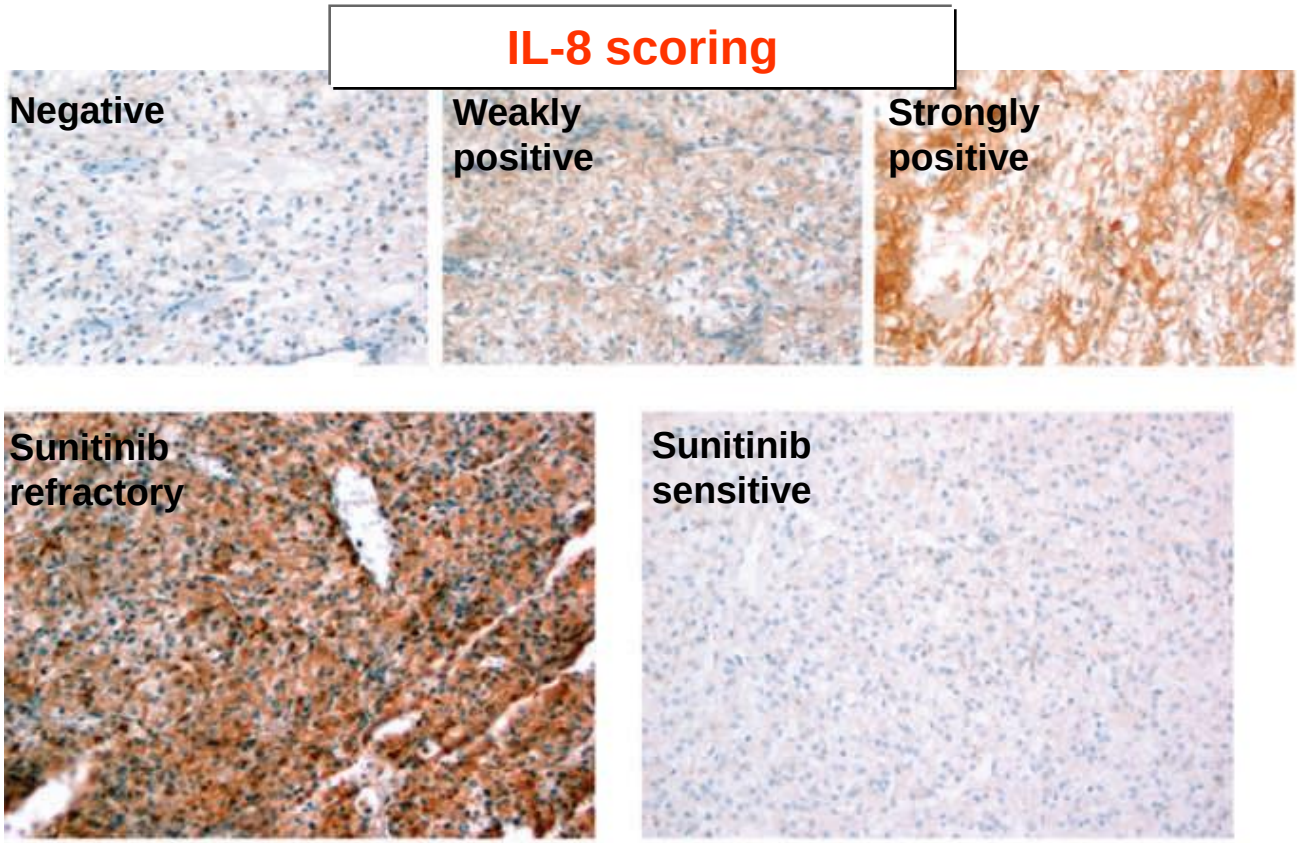




## Neutralization of IL-8 resensitized 786-O ccRCC xenografts to continous sunitinib




Huang D et al. Cancer Res 2010

# IL-8 expression is increased in human RCC tumors with intrinsic resistance to sunitinib

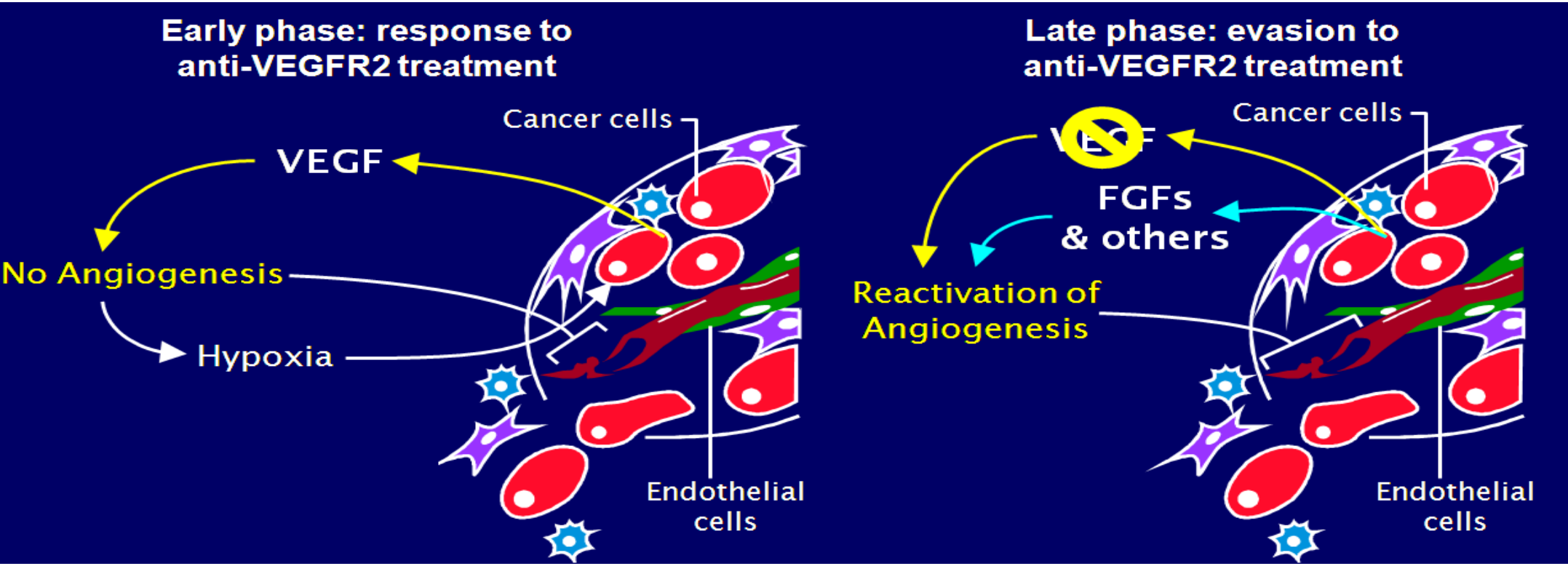



| | Progression on sutent | | *P* |
|---|---|---|---|
| | Yes (*n*=9) | No (*n*=11) | |
| IL8 composite score | 112.2±33.8 | 44.5±31.8 | 0.0002 |

Huang D et al. Cancer Res 2010

# FGF Mediates Escape From Antiangiogenic Therapy

**FGF2 is expressed by numerous tumor types and exerts its proangiogenic activity by interacting with TKRs, heparan-sulfate proteoglycans, and integrins expressed on the endothelial cell surface**



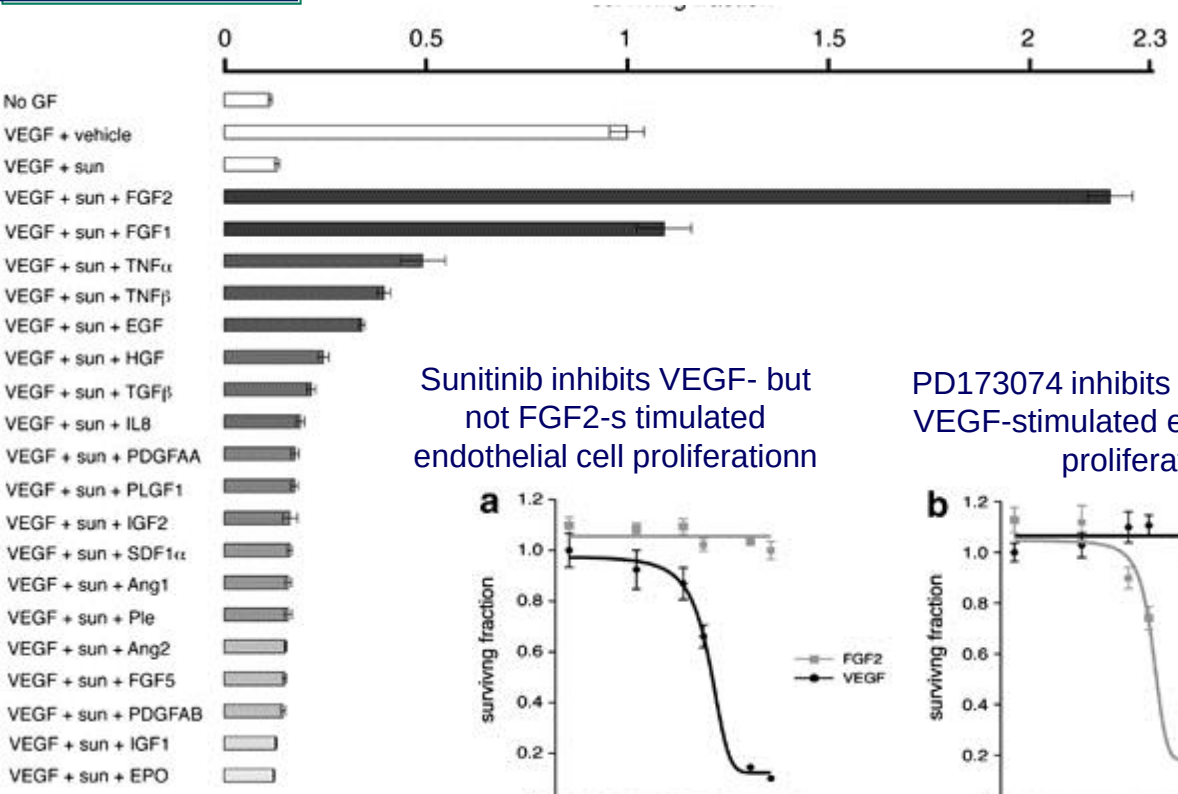

**Early phase: VEGFR2 blockade transiently stops tumor growth and decreases vascularity**

**Late phase: Reactivation of angiogenesis after anti-VEGFR2 therapy through FGF activation leads to tumor progression**

Casanovas O, et al. *Cancer Cell.* 2005;8(4):299-309.

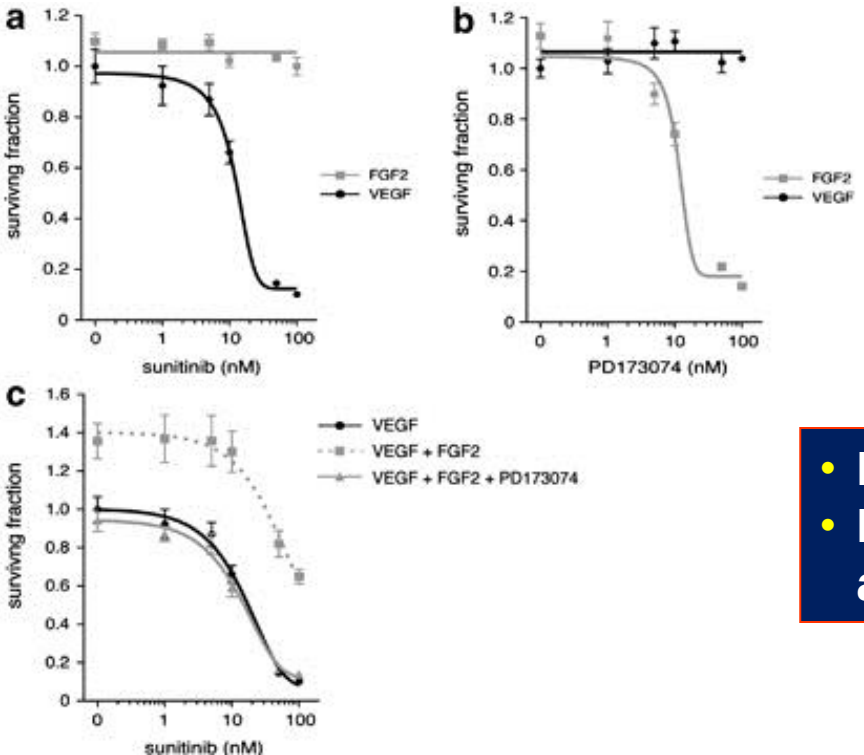
# FGF2 is a potent mediator of endothelial cell resistance to sunitinib

**HUVEC cells**

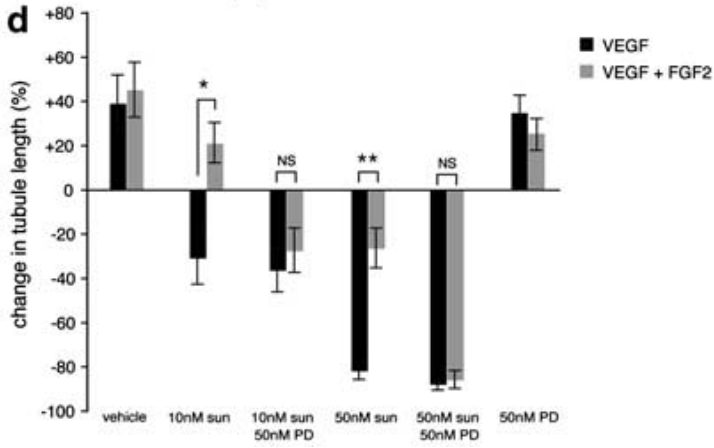



Sunitinib inhibits VEGF- but not FGF2-s timulated endothelial cell proliferationn

PD173074 inhibits FGF2- but not VEGF-stimulated endothelial cell proliferation




PD173074 prevents effect of FGF2 on sunitinib-induced retraction of tubules

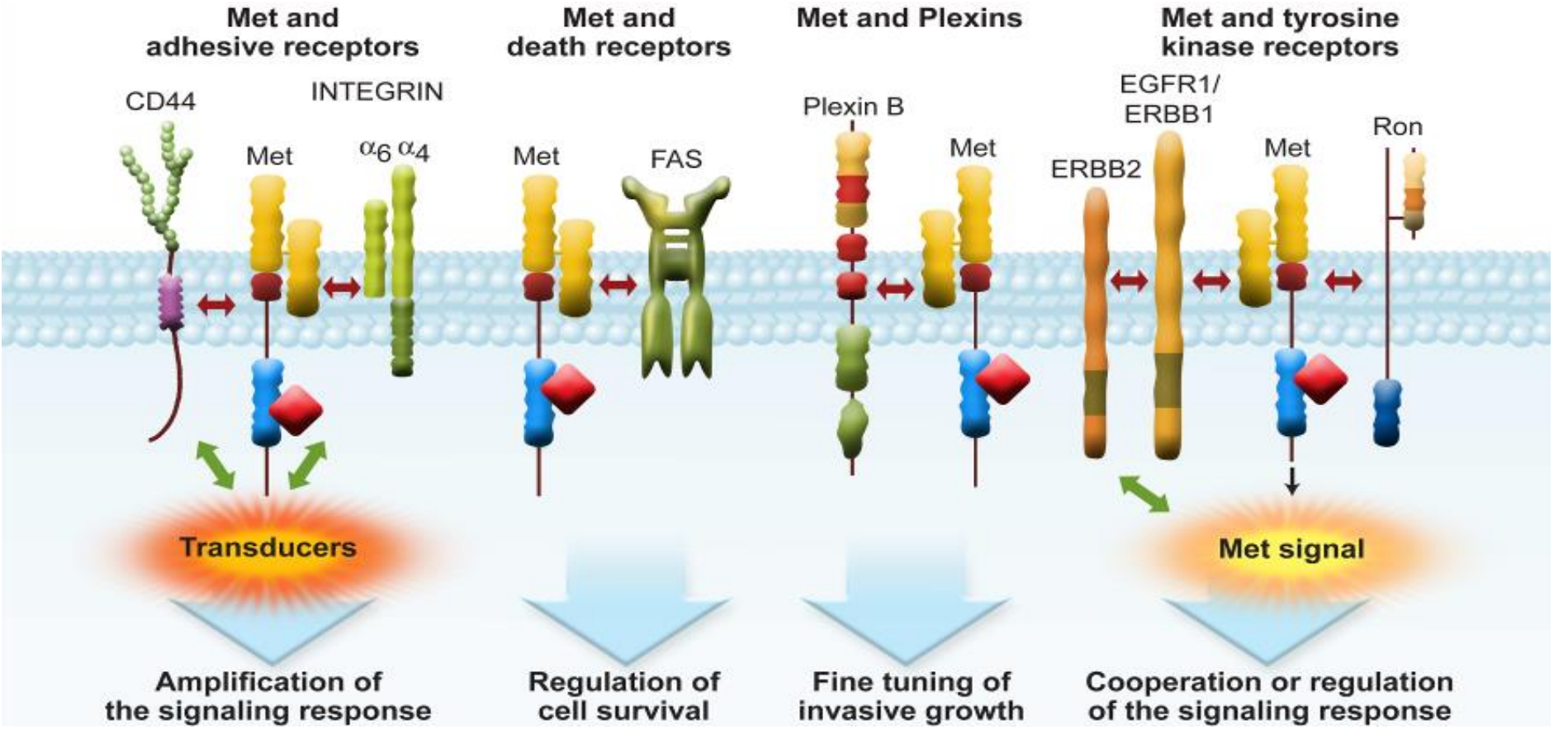



- **Inhibitors of FGF or FGFRs**
- **Multikinase inhibitors of FGRs and VEGFRs (Dovitinib, etc.)**

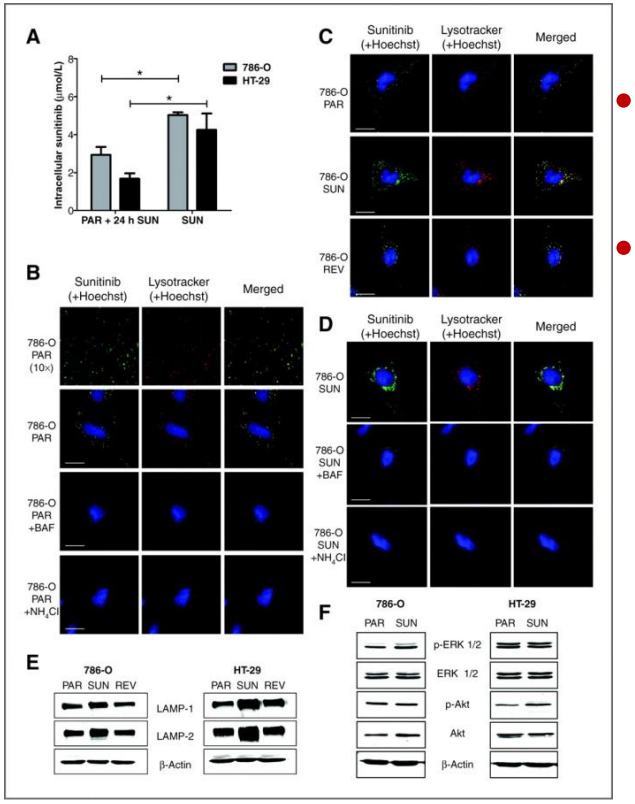
**JC Welti et al., Oncogene 2010**

# HGF and its receptor MET

- **One of the most frequently genetically altered RTKs in human cancers (activating mutations, amplifications)**
  - **Activating mutations**
    - **Hereditary papillary RCC: 100%, sporadic papillary RCC (13%)**
    - **HNSCC: 10%**
    - **NSCLC (8%) and SCLC (13%)**



# Resistance to Sunitinib and Lysosome sequestration

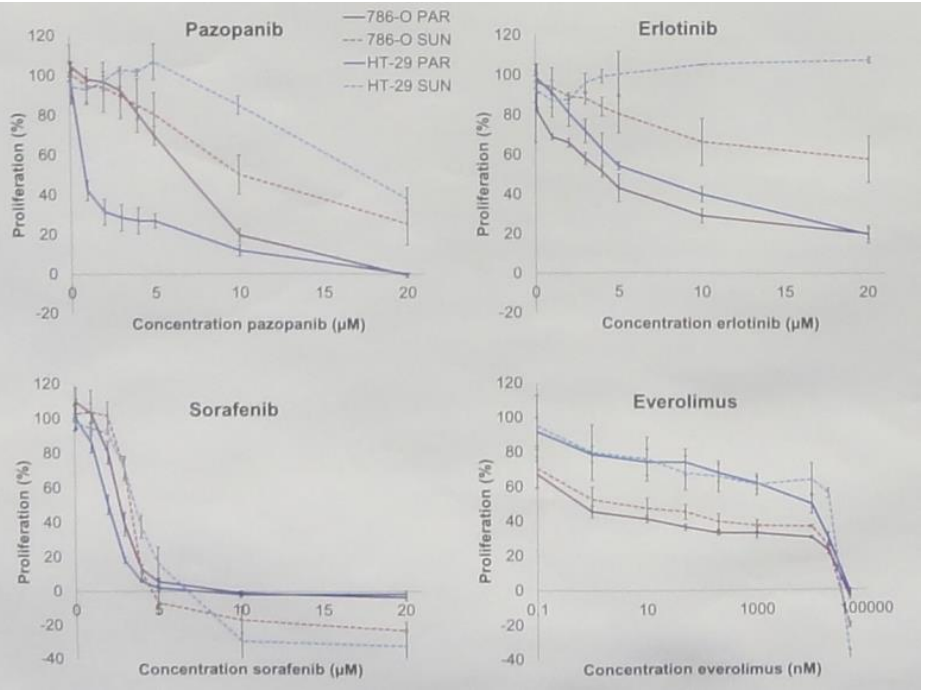



- **Resistance by an adaptive mechanism : increased intracellular lysosomal sunitinib sequestration.**
- **Upon entering acidic organelle such as a lysosome, sunitinib becomes protonated and cannot cross back membranes**

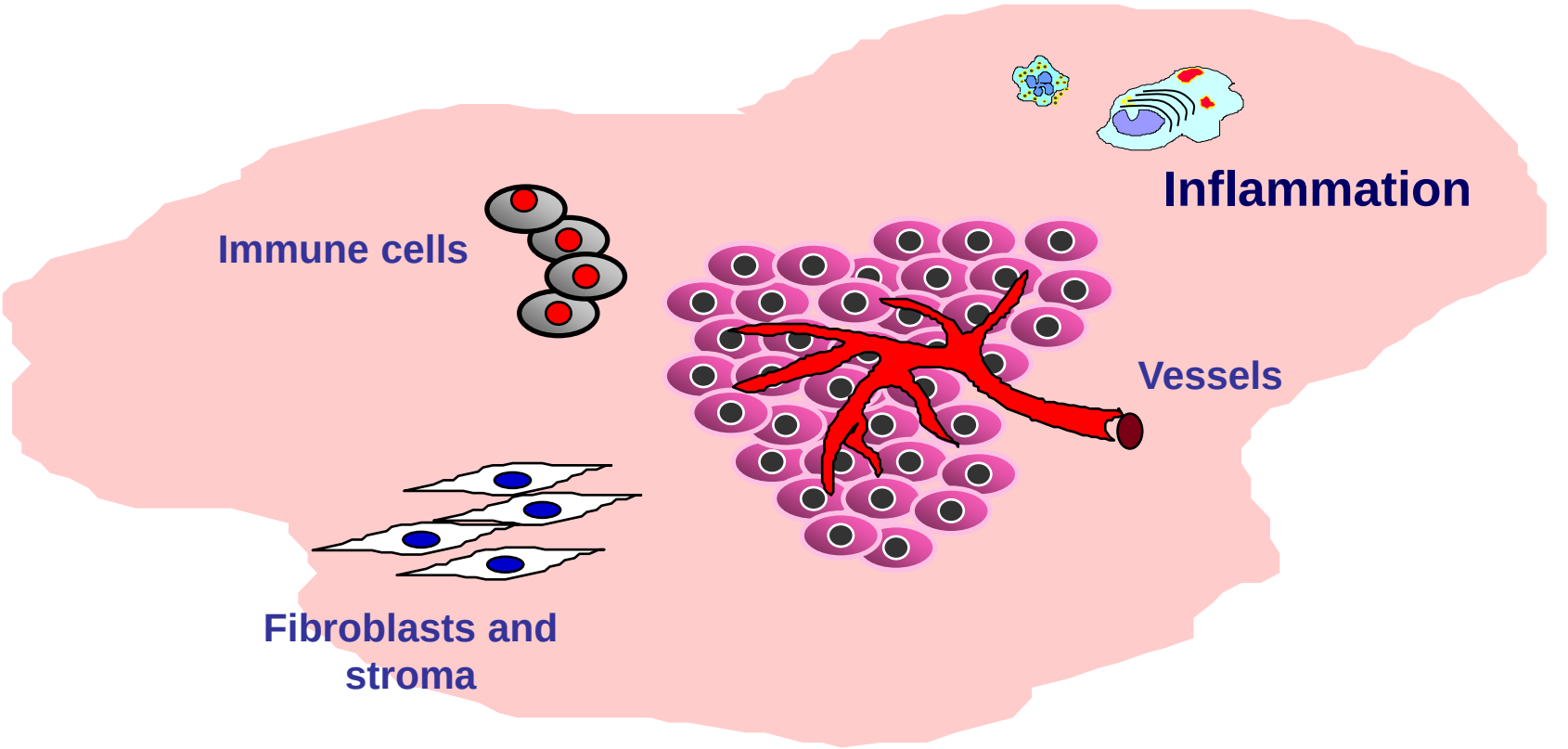
Gotink KJ. Clin Cancer Res; 17(23); 7337-46. 2011

**Sunitinib-resistant cells are cross-resistant to pazopanib and erlotinib but are sensitive to sorafenib and everolimus.**

Gotink KJ. Et al. AACR 2012

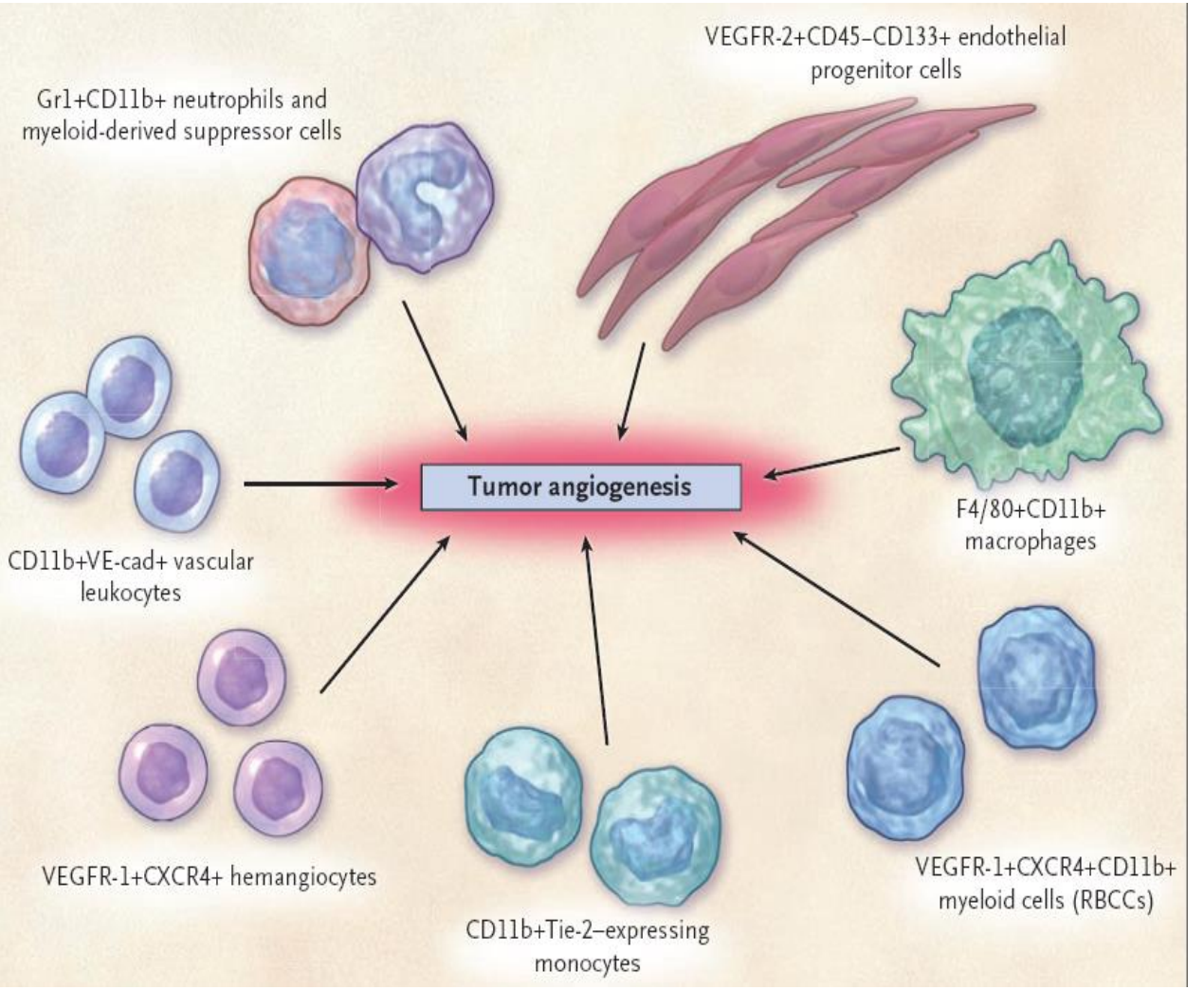


# Tumor, vessels, surrounding stroma, inflammatory and immune cells : The Tumor Microenvironment



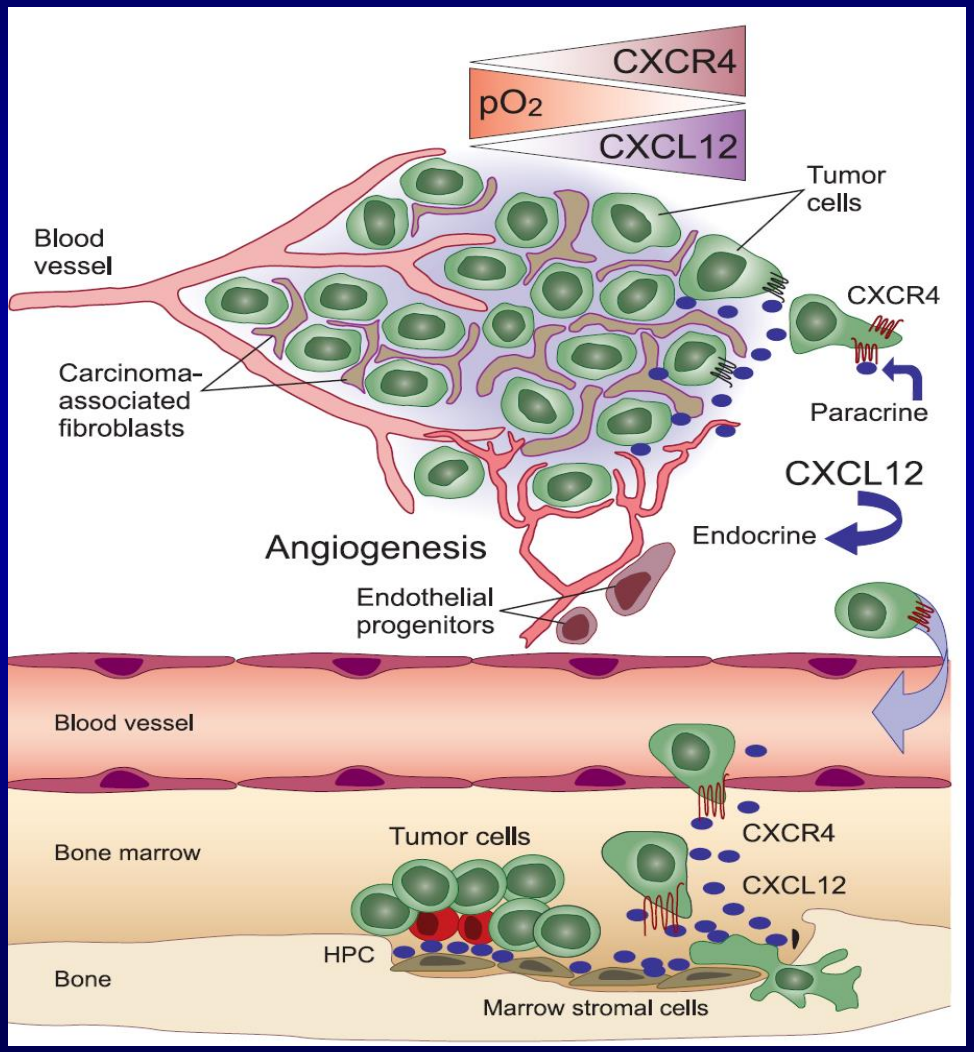

Tortora G., 2012

# Circulating Bone Marrow–Derived cell populations that stimulate or amplify tumor angiogenesis



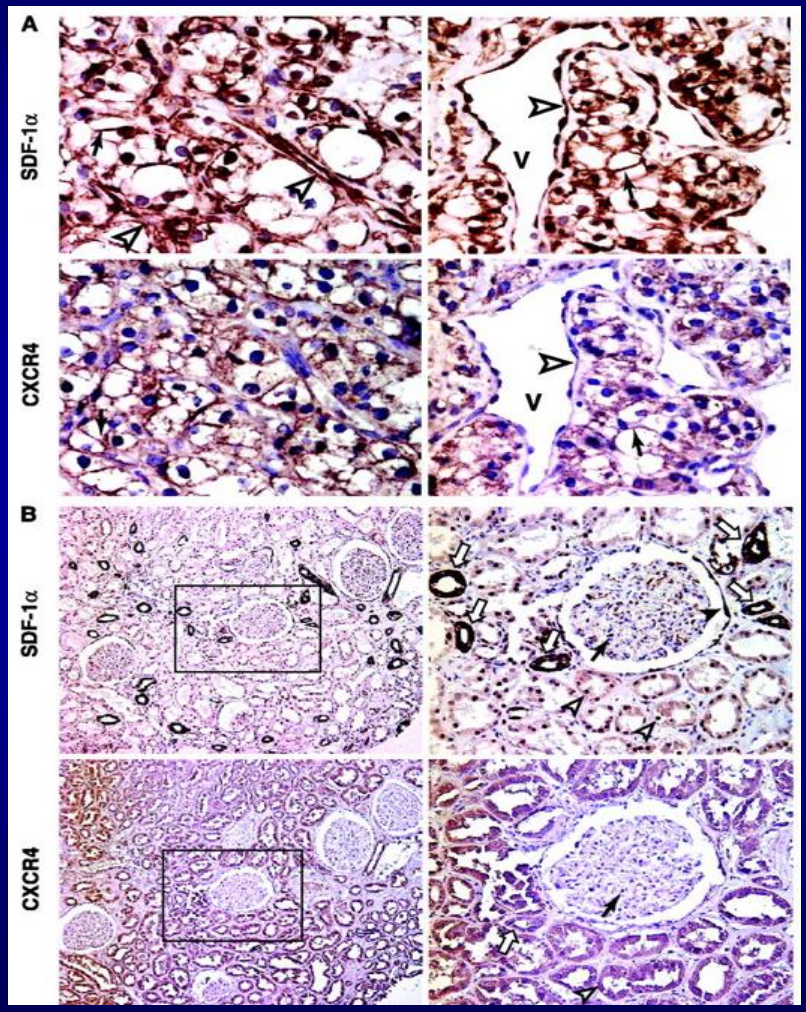

Kerbel R, *New England Journal Medicine*, 2008

# CXCL12/SDF-1 - CXCR4 Axis and expression in RCC and normal kidney




Burger e Kipps, Blood, **107**,1761 (2006)

**TUMOR**




**Controlateral normal kidney**

Tumor cells (*arrows*) and vascular cells (*arrowheads*).

Zagzag, D. et al. Cancer Res 2005 ;65:6178-6188

# Biomolecular markers under investigation

| Category | Markers |
|---|---|
| **Adhesion molecules** | cadherin-6, E-cadherin, MUC1-EMA, ICAM-1, VCAM-1, ELAM-1, KSA) |
| **Inducers of immune-suppression** | HLA class 1, IL-6, IL-8, IP-10, MIG, MIP1β, B7-H1, B7-H4, CD44 |
| **Growth factors receptors** | VEGFR-3, TGFβR-II |
| **Hypoxia-induced molecules** | CAIX, CAXII, CXCR-4, HIF-1α, VEGF, IGF-I |
| **Markers of proliferation** | Ki-67, PCNA, Ag-NORs |
| **Cell cycle regulatory proteins** | p53, bcl-2, PTEN, cyclin A, Akt, p27 |
| **Others** | VHL, mTOR, ribosomal protein S6, survivin, IMP3, caveolin-1, PCR, vimentin, fascin, seric amiloide A, NGAL, IGF-1 |

# Detection of prognostic and predictive biomarkers

- **Immunohistochemistry**
- **Circulating in plasma or serum**
- **Polymorphisms (SNPs)**

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 MARCH 8, 2012 VOL. 366 NO. 10

# Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing

Marco Gerlinger, M.D., Andrew J. Rowan, B.Sc., Stuart Horswell, M.Math., James Larkin, M.D., Ph.D., David Endesfelder, Dip.Math., Eva Gronroos, Ph.D., Pierre Martinez, Ph.D., Nicholas Matthews, B.Sc., Aengus Stewart, M.Sc., Patrick Tarpey, Ph.D., Ignacio Varela, Ph.D., Benjamin Phillimore, B.Sc., Sharmin Begum, M.Sc Neil Q. McDonald, Ph.D., Adam Butler, B.Sc., David Jones, M.Sc., Keiran Raine, M.Sc., Calli Latimer, B.Sc., Claudio R. Santos, Ph.D., Mahrokh Nohadani, H.N.C., Aron C. Eklund, Ph.D., Bradley Spencer-Dene, Ph.D., Graham Clark, B.Sc., Lisa Pickering, M.D., Ph.D., Gordon Stamp, M.D., Martin Gore, M.D., Ph.D., Zoltan Szallasi, M.D Julian Downward, Ph.D., P. Andrew Futreal, Ph.D., and Charles Swanton, M.D., Ph.D.

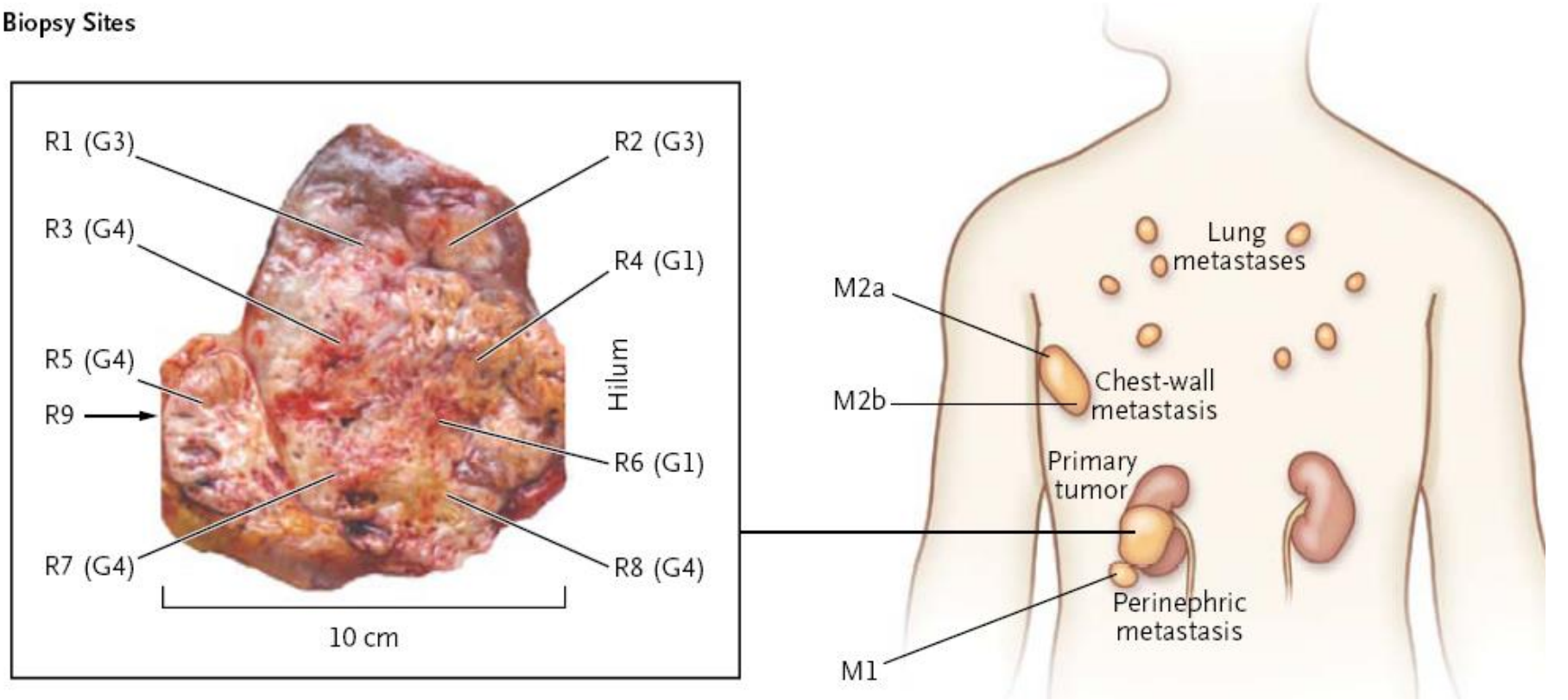
The NEW ENGLAND JOURNAL of MEDICINE

EDITORIALS

## Tumor Heterogeneity and Personalized Medicine

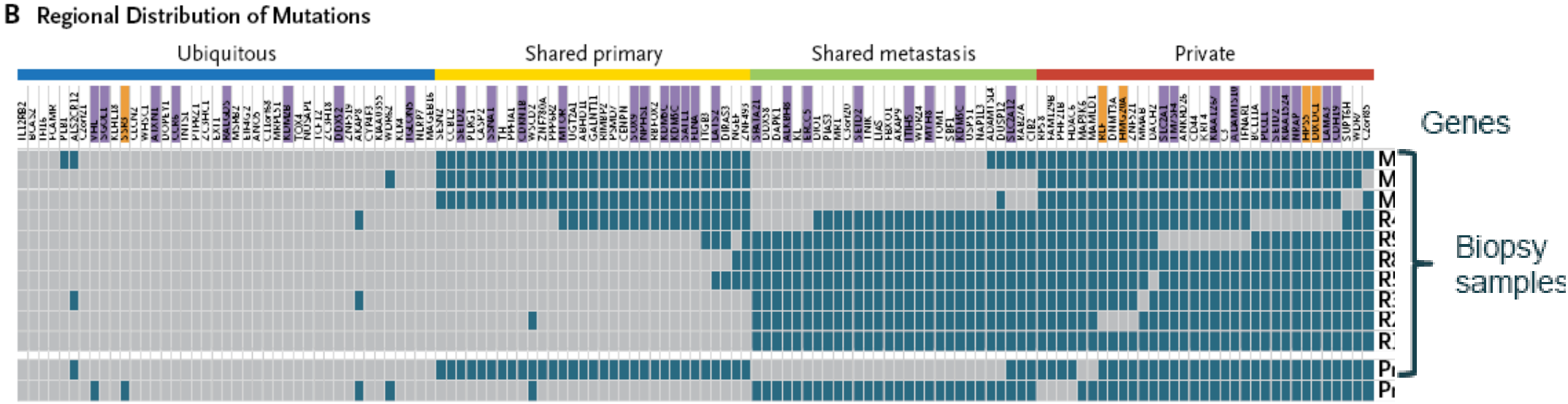
Dan L. Longo, M.D.

**Image-guided biopsies of large tumours might not be representative of the entire primary and may be misleading for a personalized therapeutic approach**




Gerlinger M et al. *N Engl J Med. 2012;366:883-892.*

# A Single biopsy sample cannot provide an accurate genetic picture of a tumor




Gray, presence of mutation.
Dark blue, absence of mutation.

- ***Only 55% of all somatic mutations* (70/128) from the primary tumor were *detected in a single biopsy sample***

- **When multiple samples from the same tumor were taken from different regions, *only 34% of mutations were detected in all samples***
  - ***Number falls to 31% if pre-treatment and metastases are included***

Gerlinger M et al. *N Engl J Med. 2012;366:883-892.*

## No Relationship Between *VHL* Gene Mutations and Treatment Outcome with Cytokines, Temsirolimus, Pazopanib

1. Kim JH et al. *Oncol Rep.* 2005;13:859-64.
2. Cho D et al. *Clin Genitourin Cancer.* 2007;5:379-85.
3. Hutson et al. J Clin Oncol 2008;26(15S):Abstract 5046 and oral presentation.

## No relationship between *VHL* gene mutation status and RR and PFS in patients treated with initial sunitinib, sorafenib, bevacizumab, or axitinib

Choueiri TK et al. *J Urol.* 2008;180:860-5.

## Carbonic Anhydrase IX (CAIX) is not a predictive marker for IL-2 response

1. Atkins M et al. *Clin Cancer Res.* 2005;15:3714-21;
2. McDermott DF et al. *J Clin Oncol.* 2010;28:4514.

## CAIX Is Neither Predictive Nor Prognostic for Sunitinib or Sorafenib Response

Choueiri TK. Prognostic and Predictive Factors in RCC, ASCO-GU 2011

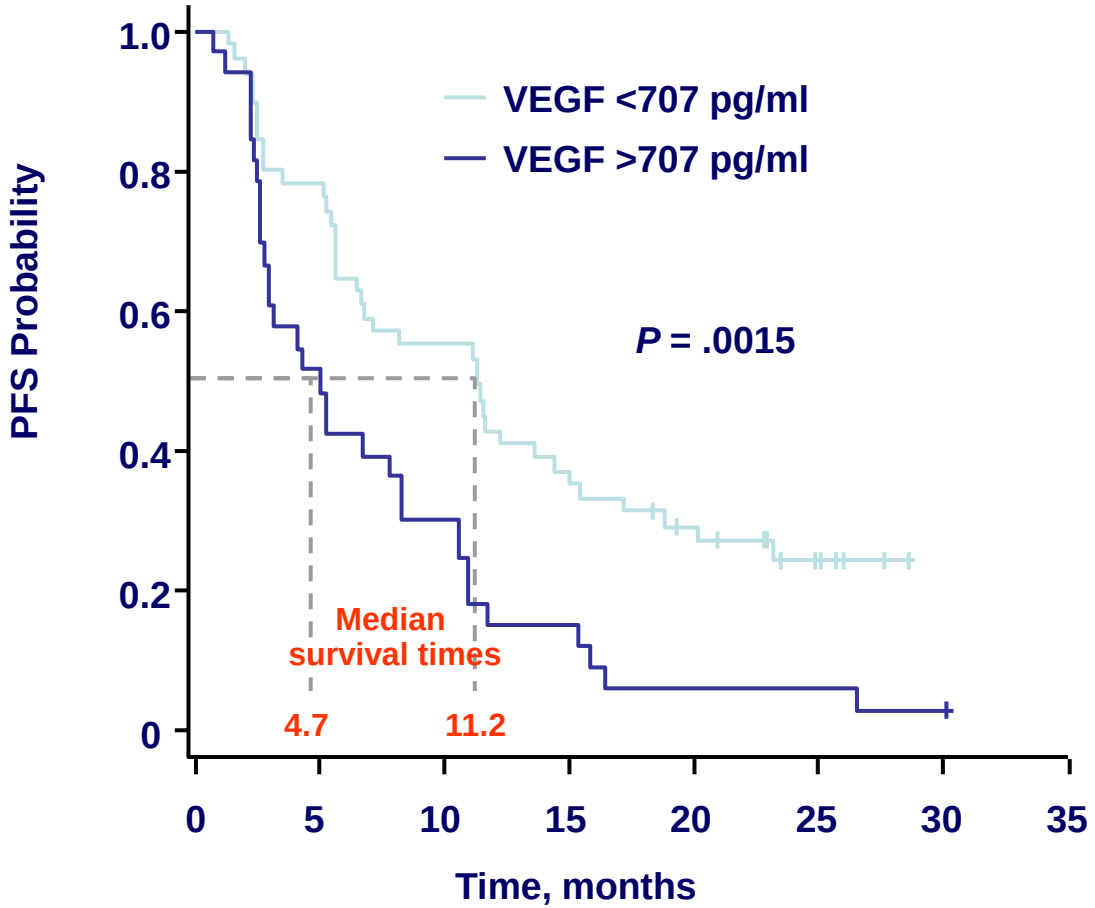
## CAIX Does Not Appear to Be Predictive for Response to Temsirolimus

Cho D et al. *Clin Genitourin Cancer.* 2007;5:379-85.

# VEGF as a Predictive Tool in Sunitinib-treated Patients

Sunitinib-treated patients with baseline VEGF <707 pg/ml had prolonged median PFS vs those with VEGF >707 pg/ml

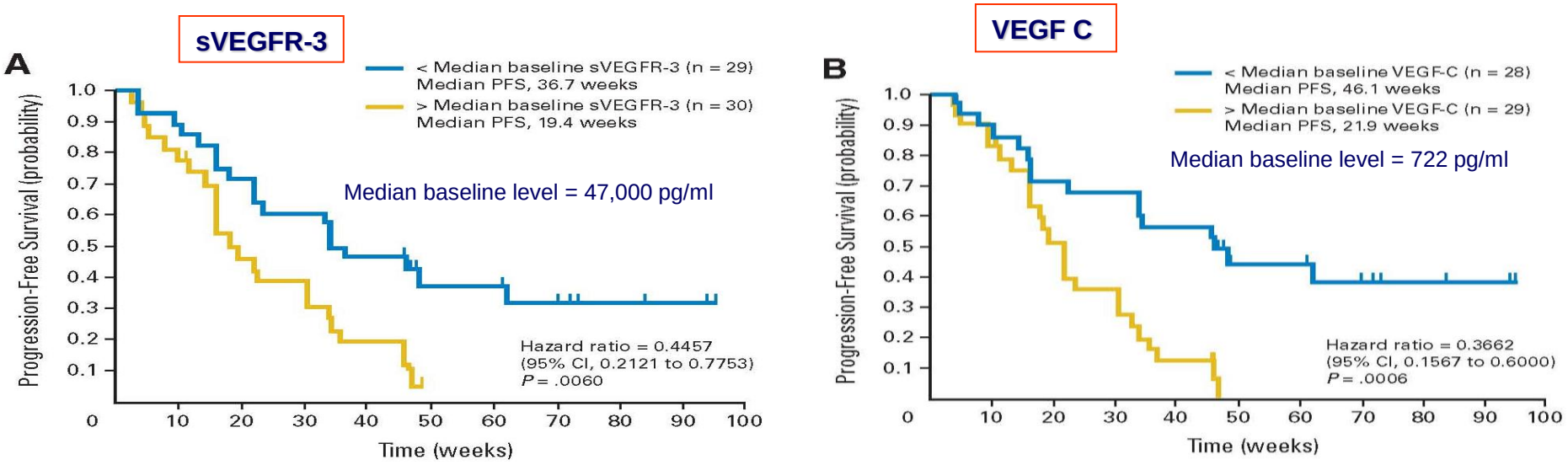
**Deprimo SE et al. *J Transl Med.*, 2007;5:32.**



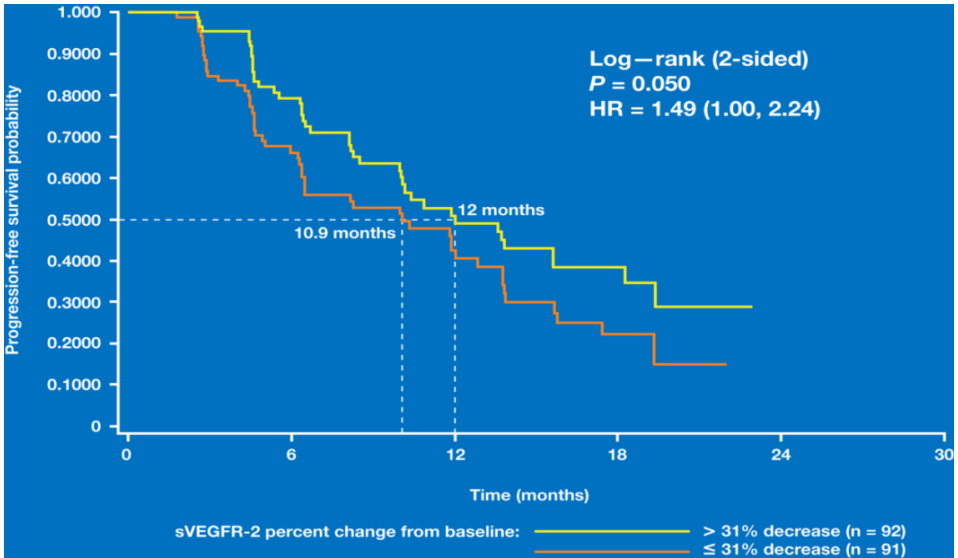

Rini BI et al. JCO 2008 Aug 1;26(22):3743-8

# Lower baseline levels of sVEGFR-3 and VEGF-C are associated with longer PFS to sunitinib in RCC patients refractory to bevacizumab

Rini BI et al. JCO 2008 Aug 1;26(22):3743-8



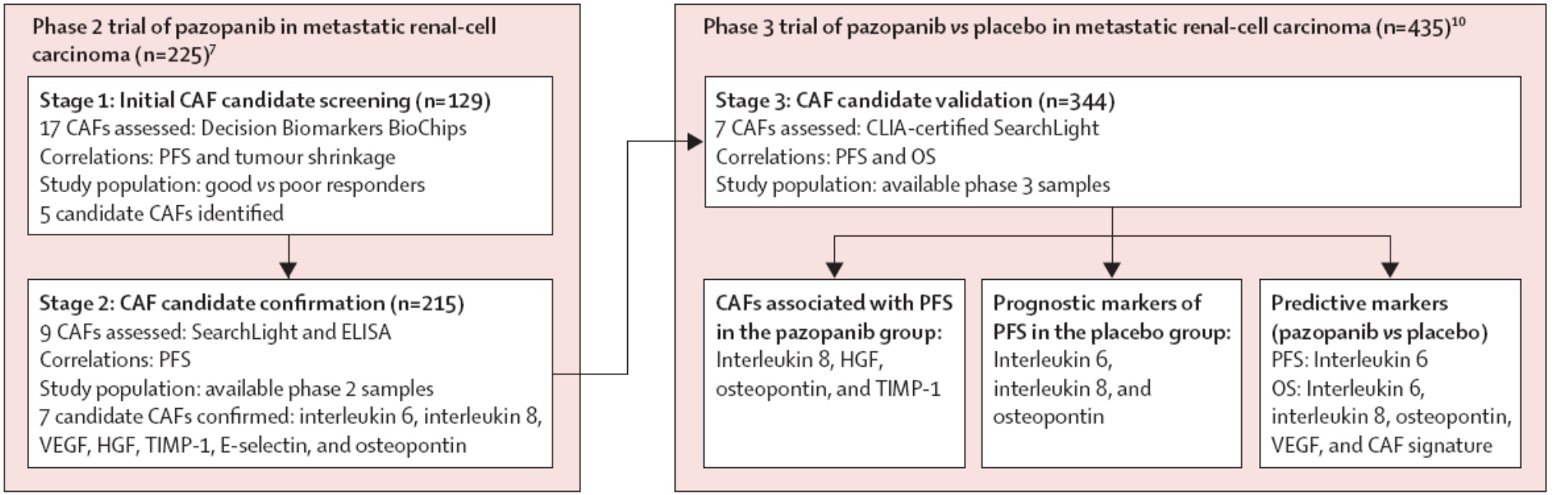

# Decrease of sVEGFR-2 correlates with PFS and tumor response to Pazopanib




Hutson *et al*. *J Clin Oncol* 2008;26(15S)

# Prognostic or predictive plasma cytokines and angiogenic factors for patients treated with pazopanib for metastatic renal-cell cancer: a retrospective analysis of phase 2 and phase 3 trials

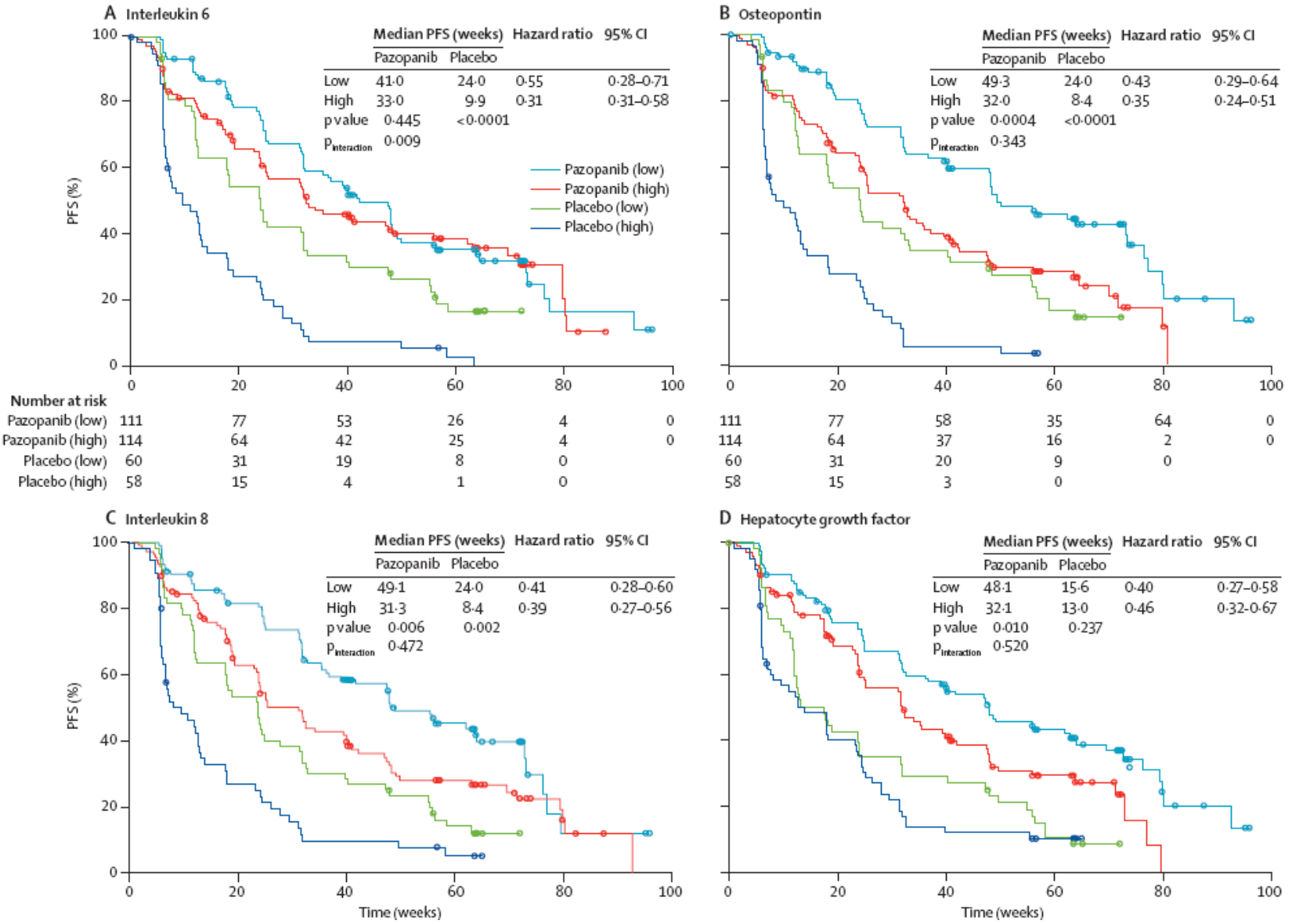
*Hai T Tran, Yuan Liu, Amado J Zurita, Ying Lin, Katherine L Baker-Neblett, Anne-Marie Martin, Robert A Figlin, Thomas E Hutson, Cora N Sternberg, Rafael G Amado, Lini N Pandite, John V Heymach*




**IL-6, IL-8, VEGF, HGF, Osteopontin, TIMP-1**

Tran et al. Lancet Oncol 2012; 13: 827–37

# Cytokine and angiogenic factors (CAFs) associated with PFS in the randomised phase III trial




Tran et al. Lancet Oncol 2012; 13: 827–37

# A cytokine and angiogenic factor (CAF) analysis in plasma for selection of sorafenib therapy in patients with metastatic renal cell carcinoma

**Baseline biomarkers with significant predictive effect on PFS**

| CAF | Baseline concentration[a] | Median PFS (months) | | Hazard ratio | *P* |
|---|---|---|---|---|---|
| | | SOR | SOR + IFN | | |
| Osteopontin | Low | 3.8 | 11.1 | 0.36 | 0.02 |
| Osteopontin | High | 7.7 | 3.9 | 1.94 | 0.09 |
| VEGF | Low | 7.7 | 11.1 | 0.33 | 0.02 |
| VEGF | High | 5.7 | 4.2 | 1.57 | 0.26 |

**Predictive value of CAF index based on the baseline signature of six biomarkers (sCAIX, Osteopontin, VEGF, TRAIL, Collagen V, and sVEGFR2)**

| CAF index (cut-off) | Signature positive (≥cut-off) HR (95% CI) SOR + IFN versus SOR | Signature negative (<cut-off) HR (95% CI) SOR + IFN versus SOR | *P* value for Interaction |
|---|---|---|---|
| 3 | 1.63 (0.82–3.24) | 0.16 (0.05–0.53) | 0.0005 |
| 4[a] | 2.25 (1.02–4.96) | 0.20 (0.08–0.55) | 0.0002 |
| 5 | 10.8 (1.17–99.6) | 0.48 (0.25–0.91) | <0.0001 |

Zurita A J et al. Ann Oncol 2012

# Genetic Variations in Angiogenesis-Related Factors

## SNPs as Predictive Factors in RCC

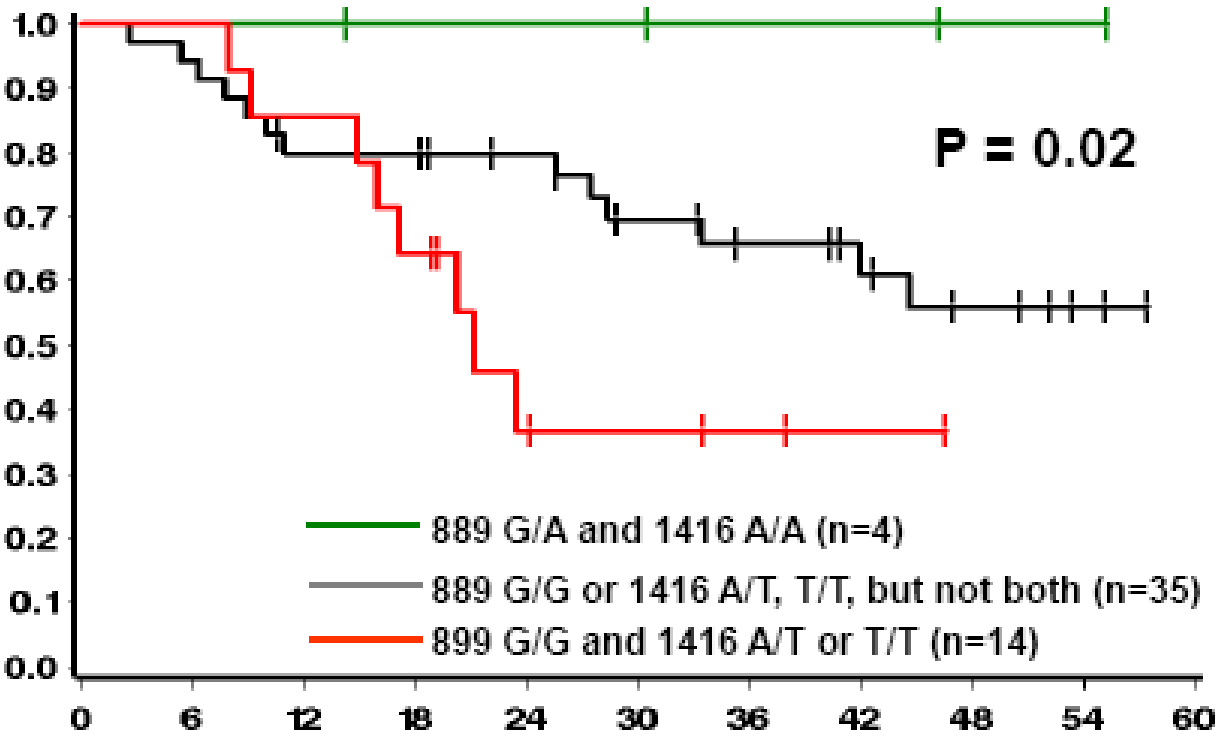
| Biomarker | Association With Outcomes |
|---|---|
| VEGF SNP 936<br><br>VEGFr2 SNP 889<br><br>VEGFr2 SNP 1416 | In patients receiving sunitinib[1]<br>• VEGFr2 SNP 936 associated with tumour shrinkage<br>• Presence of combined VEGFr2 SNP 889 and 1416 associated with improved OS, whereas presence of VEGF SNP 936 CC genotype in combination with VEGFr2 SNP 889 GG genotype associated with increased risk of death |
| IL8 2767<br><br>FGFR2 IVS2 + 906C>T<br><br>VEGFA-1154G>A<br><br>NR1IR<br><br>FLT4 | In patients receiving pazopanib<br>• OS longer in patients carrying<br>– Wild-type IL8 2767 AA genotype compared with wild-type IL8 2767 TT genotype[2,3]<br>– Wild-type FGFR2 IVS2 + 906C>T CC genotype compared with FGFR2 IVS2 + 906C>T TT variant genotype[2]<br>– Wild-type VEGFA-1154G>A GG genotype compared with VEGFA-1154G>A variant GG genotype[2]<br>• Variant genotypes of NR1IR (-25385C>T) and of FLT4 (1480A>G) polymorphisms associated with reduced OS[2] |
| ABCB1<br><br>ABCG2 | In patients receiving sunitinib[4]<br>• Presence of A-allele in drug transporter ABCG2 associated with a trend toward prolonged PFS and OS<br>• Presence of TCG in ABCB1 haplotype (3435C/T, 1236C/T, 2677G/T) significantly |

1. Kim JJ, Vaziri SA, Elson P, et al. J Clin Oncol. 2010; 28:15 (suppl):abstr 4629. 2. Xu C et al. J Clin Oncol. 2011;29:7(suppl):abstr 303.
3. Ball HA et al. J Clin Oncol. 2010;28(suppl 15):abstr 4520. 4. Eechoute K et al. J Clin Oncol. 2010;28(suppl 15):abstr 4521.

# VEGFR2 SNPs 889 and 1416 may predict Overall Survival in mRCC patients receiving sunitinib

VEGFR SNPs, 889 and 1416 were marginally associated with overall survival (p=0.13, 0.17)

The combined genotype remained statistically significant after adjusting for prognostic factors (p=0.03 for each factor)



# SNPs in IL8, FGFR2, VEGFR3, VEGFA, and NR1I2 Associated with OS in Pazopanib-Treated RCC Patients

| GENE | SNP (NCBI) | *P* Value | Allele Frequency Caucasians, %[1] | Allele Frequency Asians, %[1] | Allele Frequency Blacks, %[1] |
|---|---|---|---|---|---|
| *IL8* | rs1126647 | 0.003 | 39 | 32 | 6 |
| *IL8* | rs4073 | 0.01 | 40 | 35 | 83 |
| *FGFR2* | rs2981582 | 0.01 | 42 | 25 | 52 |
| *VEGFR3* | rs307826 | 0.04 | 7 | 0 | 0 |
| *VEGFA* | rs1570360 | 0.05 | 25 | 17 | 3 |
| *NR1I2* | rs3814055 | 0.03 | 41 | 27 | 27 |

- None of the SNPs associated with OS in patients who did not receive pazopanib (N = 37)

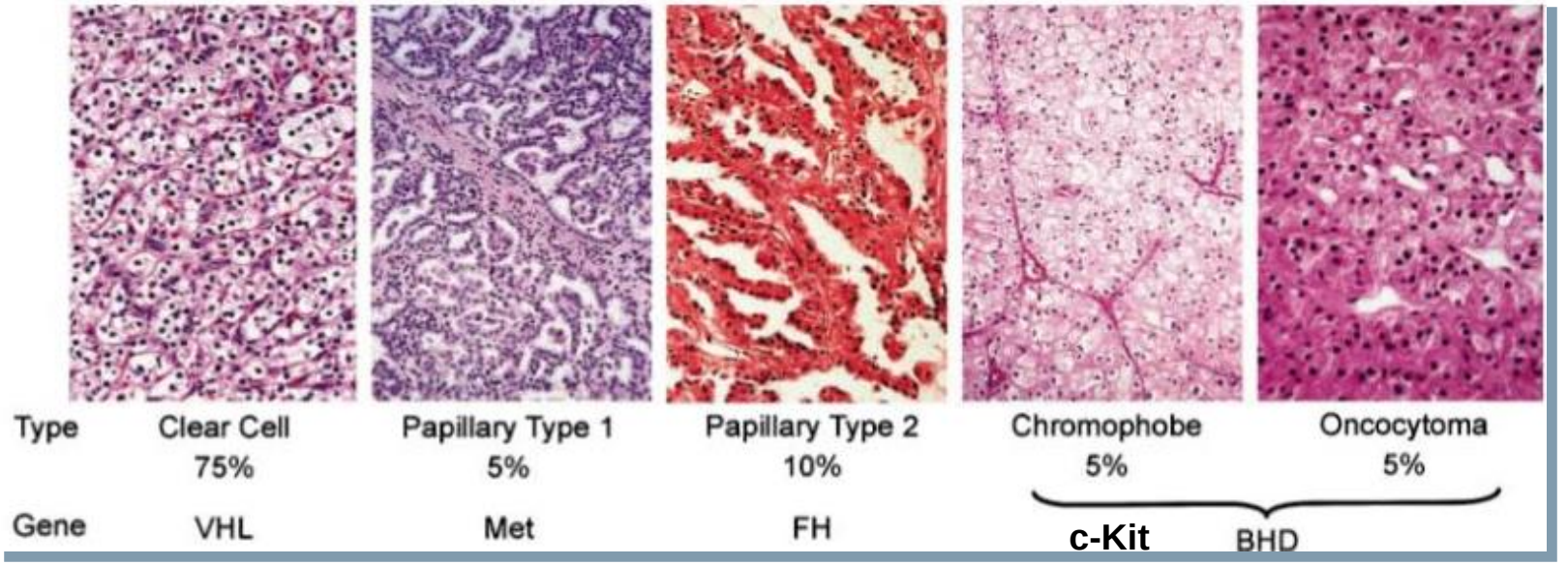
***NR1I2* encodes a key regulator for CYP3A4 expression affecting Pazopanib clarance**

Xu CF et al., J Clin Oncol. 2011 29(18):2557-64

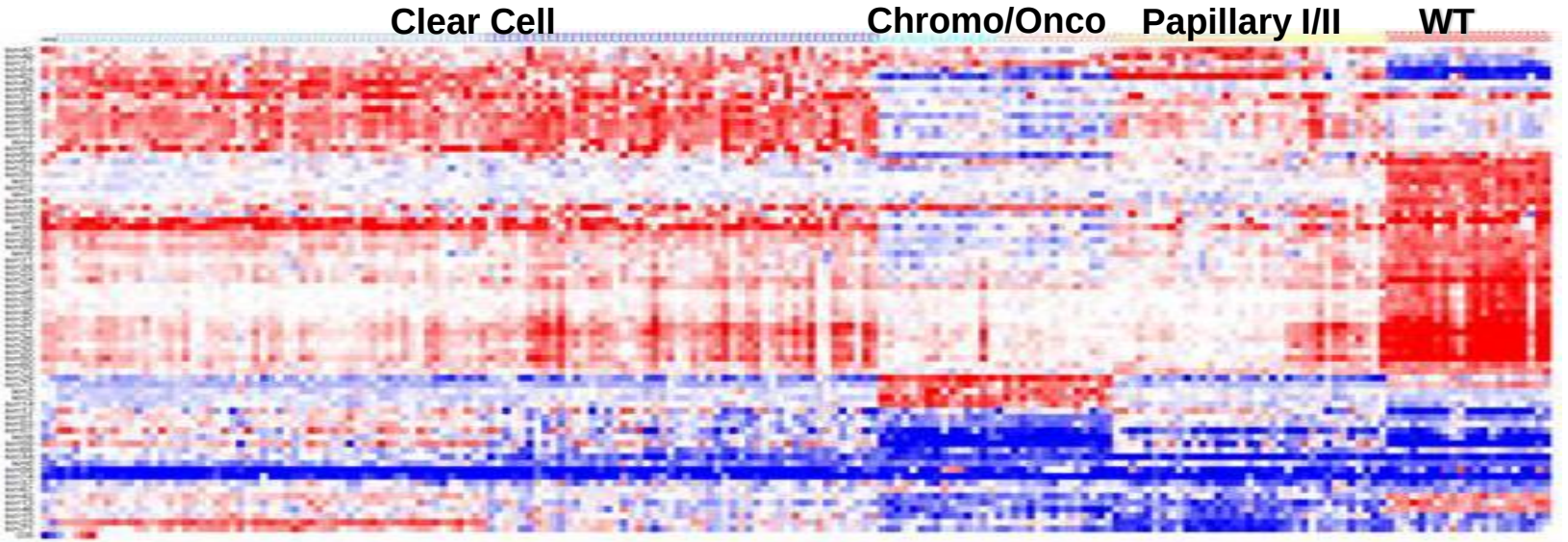
# Conclusions

- Several factors involved in signaling and resistance to treatment, that may also have a potential role as predictive biomarkers, have been identified in RCC.
  - Tumor, Plasma and Genetic markers
- Validation of these markers is ongoing, with several noteworthy recent results.
- Additional challenges remain:
  - Standardize sample collection and analysis
  - Consider always tumor heterogeneity
  - Make an effort to collect large amount of tumor samples and matched response data to have reliable results.

# Histotypes and kinome in RCC




Linehan, W. M. et al. Clin Cancer Res 2007;13:671s-679s




Kinome expression (74/518 kinases with >3-fold differential expression vs.normal kidney

(Teh, ASCO 2007 and Lancet Oncology 2010)