THE BIOLOGY OF CUP

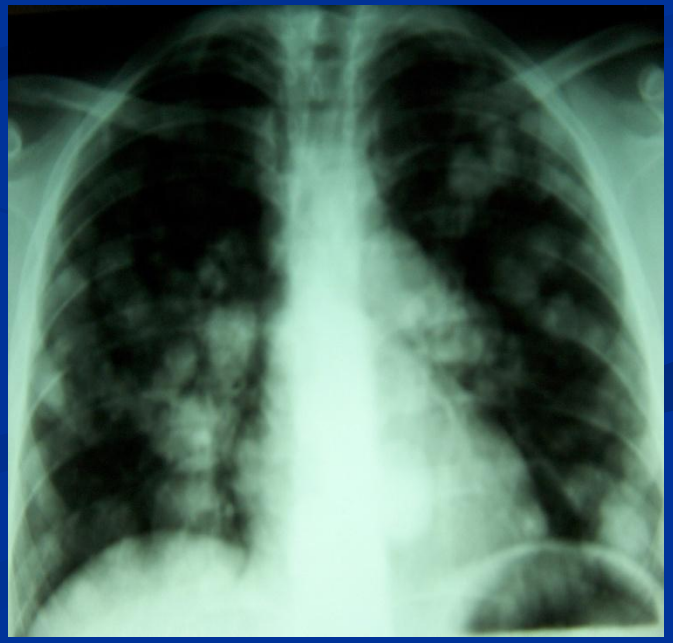
PROF. NICHOLAS PAVLIDIS, MD, PhD, FRCP (Edin)

London , September 2015

QUESTIONS TO BE ANSWERED

WHAT IS CUP ?

- **Metastases from a primary we simply cannot locate ?**
- **Tumors with not only a primary tissue-specific biology but also with a distinct biological signature, common for most CUPs ?**
- **Tumours that carry a peculiar and distinct biology compared to metastases from known primary tumours ?**



Hypothesis A

CUP does not undergo type 1 progression (from a premalignant lesion to malignant)

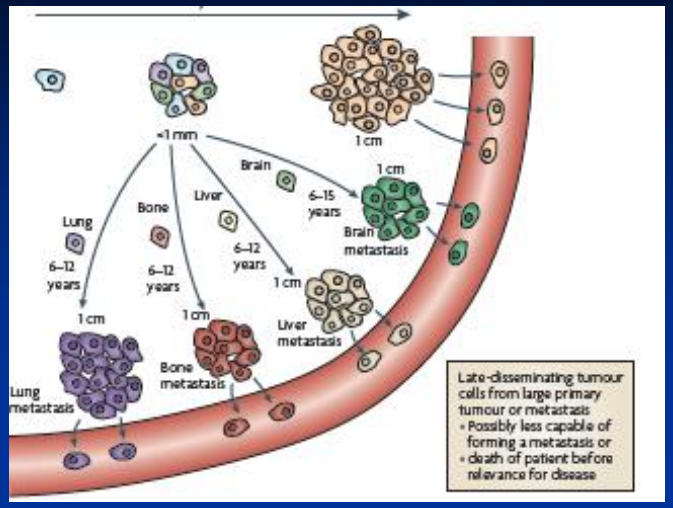
b u t

Follows a type 2 progression (malignant at the onset of the disease without forming a primary site)

Frost P et al, Cancer Bull 1989, 41, 139-141

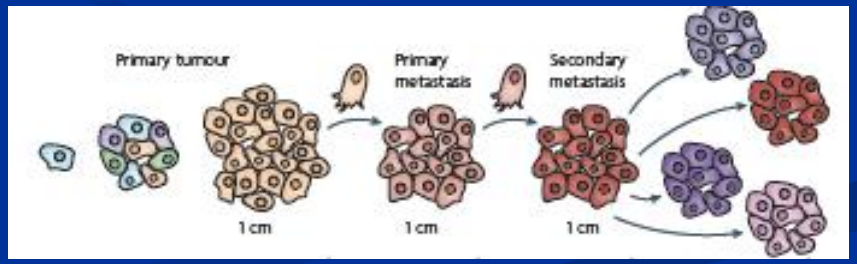
Hypothesis B

CUP follows the parallel progression model where metastases can arise early in the development of a malignancy …



In contrast to

the linear progression model where stepwise progression of accumulating genetic and epigenetic alterations accompanying cancer development

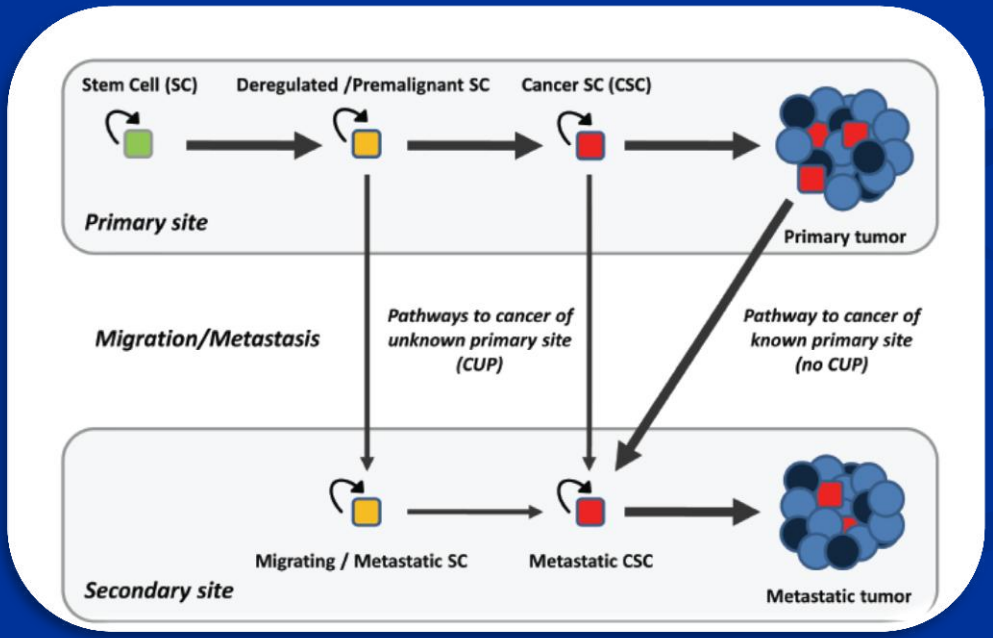


Klein C, Nature Reviews Cancer 9: 302-312, 2009

Hypothesis C

- **The migration ability of stem cells can explain the existence of CUP.**

- **Stem cells (deregulated, premalignant or cancerous) migrate away from their natural tissues and generate tumors in other locations.**

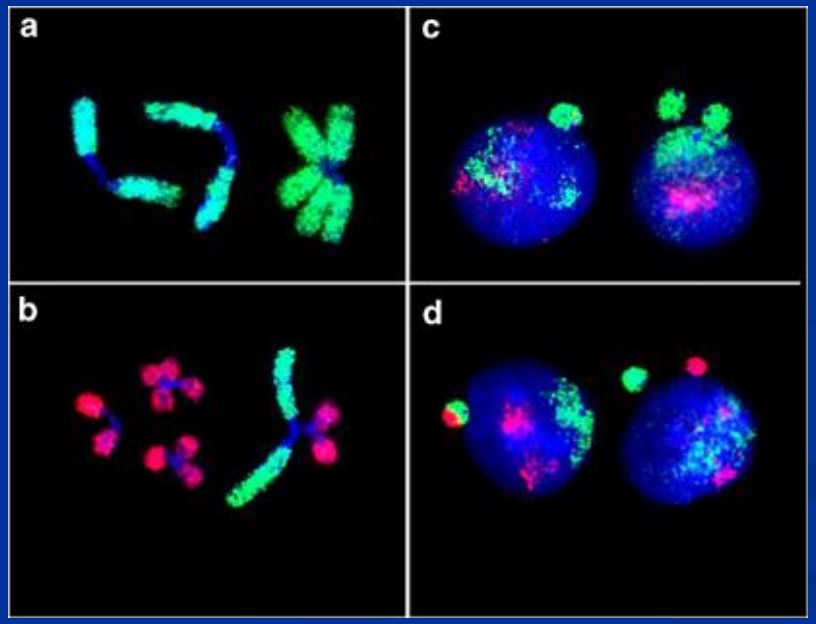


Oncoscience 2015: 2 (5) : 407-475

TRANSLATION RESEARCH ON CUP BIOLOGY

1. Chromosomal Instability
2. Oncogenes – Oncoproteins
3. Tumour and Metastasis Suppressor Genes
4. Angiogenesis
5. Metalloproteinases
6. Hypoxia
7. Epithelial Mesenchymal Transition and Stemness
8. Signaling Pathways

1. CHROMOSOMAL INSTABILITY



CHROMOSOMAL ABNORMALITIES

- **Aberrations of chromosomes 1, 6, 7 and 11**

(Biochem Biophys Acta, 2011)

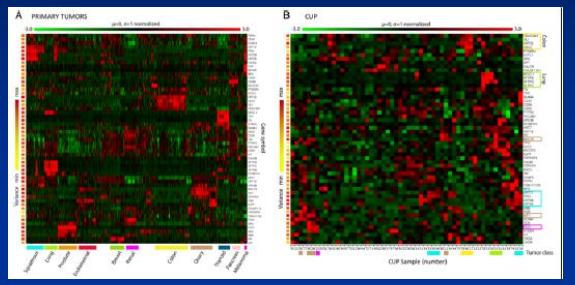
- **Aneuploidy in 70% of CUP adenocarcinoma**

(Eur J Cancer Clin Oncol , 2011)

BMC Cancer (2015) 15: 151

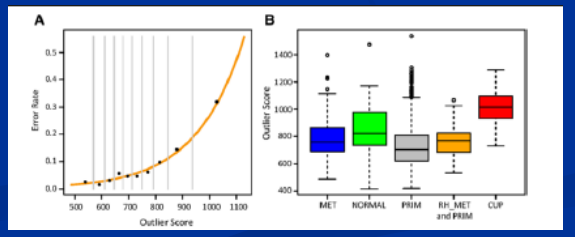
Cancers of unknown primary origin (CUP) are characterized by chromosomal instability (CIN) compared to metastasis of known origin

Jonas Vikesa et al



RESULTS

- **CUPs exhibit inconsistent expression of conventional cancer biomarkers**



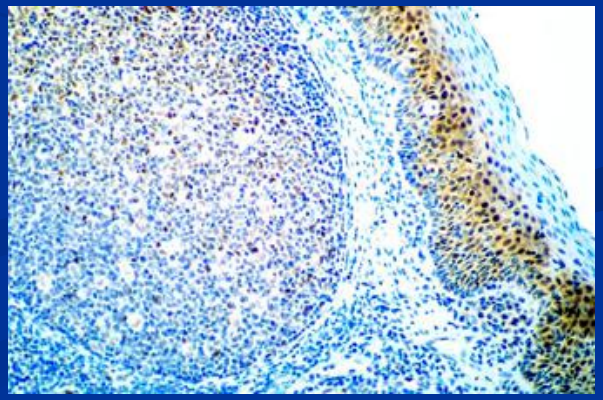
- **CUPs are more distantly related to their primary tumor class than corresponding metastases of known origin**

- **CUPs display increased expression of genes involved in DNA damage repair and experts mRNA signatures of chromosome instability**

CONCLUSIONS

- **CUPs are chromosome unstable compared to mets on known origin**
- **CIN may account for the uncommon clinical presentation, chemoresistance and poor outcome**

2. ONCOGENES - ONCOPROTEINS



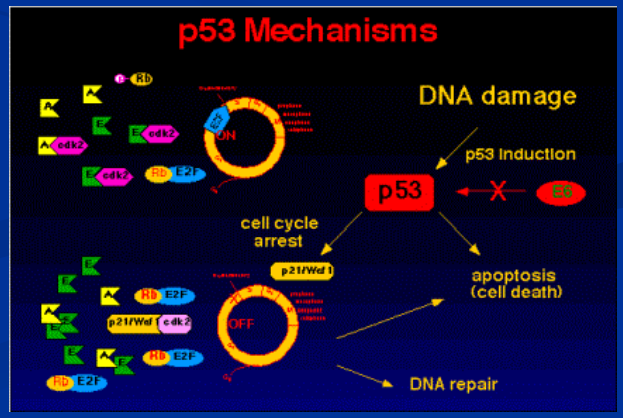
ONCOGENES – ONCOPROTEINS IN CUP

Oncoproteins	Method	Overexpression	Year / Publication
HER-2	IHC	4 – 27 %	1995 – 2007
BCL 2	IHC	40 %	1998
cMYC	IHC	23 %	1995
Ras	IHC	23 %	1995
EGFR	IHC + PCR	12 – 61 %	2005 - 2007
cKit- PDGFR	IHC + PCR (SSCP)	4 – 13 %	2005 - 2008

CONCLUSION *: No evidence of exons amplifications or axis activation or mutations.*

No association with patients' prognosis.

3. Tumour and Metastatic Suppressor Genes



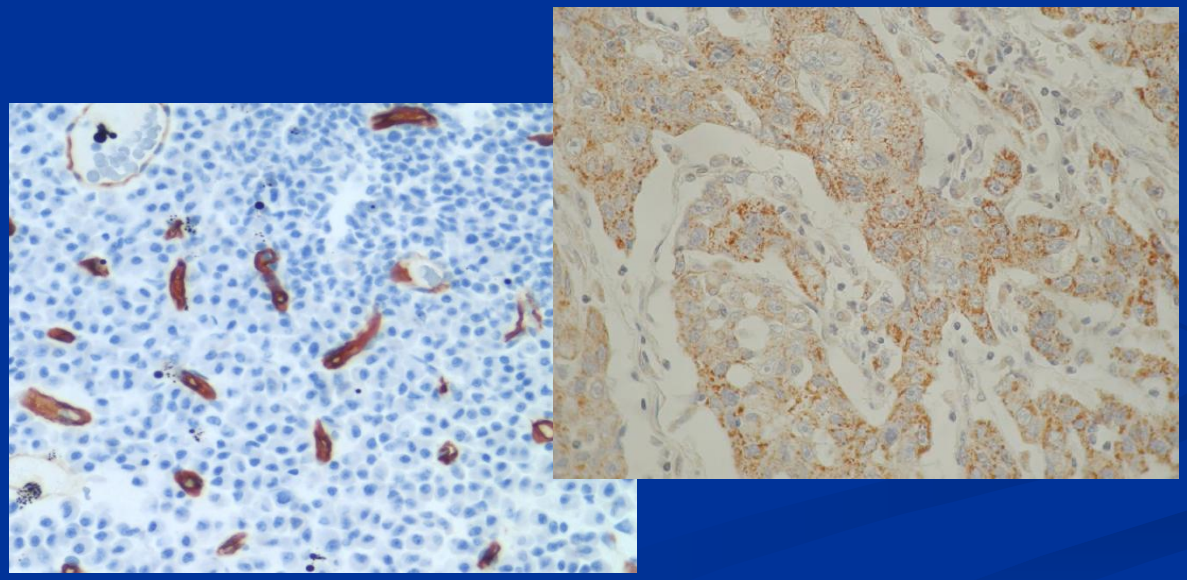
TUMOUR AND METASTATIC SUPPRESSOR GENES AND PROTEINS

Gene / Protein	Method	Overexpression/mutations	Reference
p53	IHC	53%	Anticancer Res, 1998
p53	IHC	48%	Anticancer Res, 2004
p53	PCR-SSCP	26% mutations in Exon 5-9 gene	Anticancer Res, 1993
KiSS-1	IHC	3%	Anticancer Res 2007
KiSS-1	PCR-SSCP	2% mutations in Exon 4a gene	Pathol Oncol Res, 2008

Implications :
- p53 is overexpressed and carries mutations.
- Kiss-1 is underexpressed with 2% mutations
- They role in CUP development is unknown

Prognostic value :
- p53 and KiSS-1 mutations are not correlated with patients' prognosis

4. ANGIOGENESIS



ANGIOGENESIS

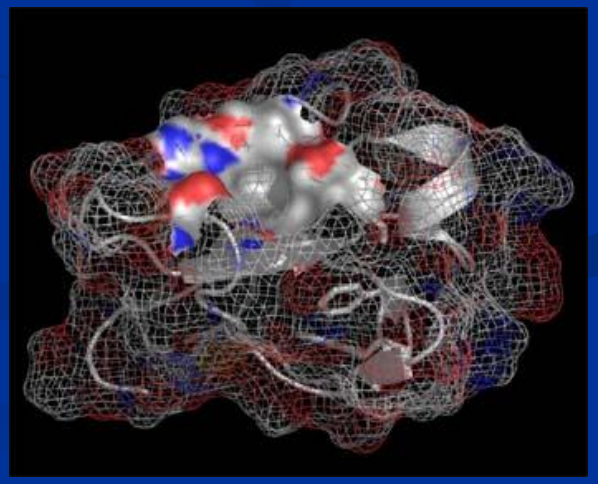
Proteins	Method	Overexpression	Reference
CD34 microvessel density	IHC		Int J Cancer, 1997
CD34 microvessel density	IHC	Median 56/mm^3	Anticancer Res, 2004
CD34 microvessel density	IHC	Median 59/mm^3	BMC Cancer, 2005
VEGF	IHC	83%	BMC Cancer, 2005
VEGF	IHC	26%	Anticancer Res, 2004
VEGF	IHC	29%	Proc ASCO, 2005
Stromal TSP-1	IHC	20%	BMC Cancer, 2005

Implications : Angiogenesis is active in CUP. However, this is a feature common in metastatic solid tumours in general.

Prognostic value : Microvessel density:

- Had positive correlation with VEGF
- Was higher in the unfavourable CUP group
- Was an adverse prognostic factor

5. METALLOPROTEINASES



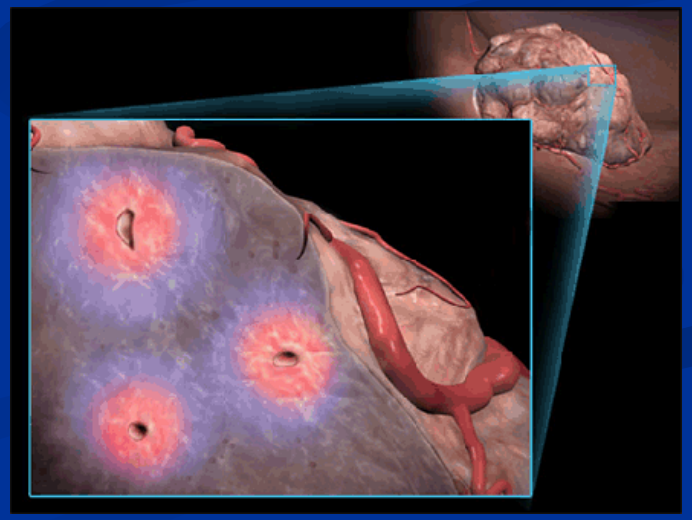
MATRIX METALLOPROTEINASES (Proteolysis-related molecules)

Proteins	Method	Overexpression	Reference
MMP-2	IHC	49%	Cancer 2005
MMP-9	IHC	36%	Cancer 2005
TIMP-1	IHC	44%	Cancer 2005

Prognostic value :

- TIMP-1 was significantly higher in unfavourable subsets
- It was associated with a shorter survival (7.5 vs 12 mos – p = 0.016)

6. HYPOXIA

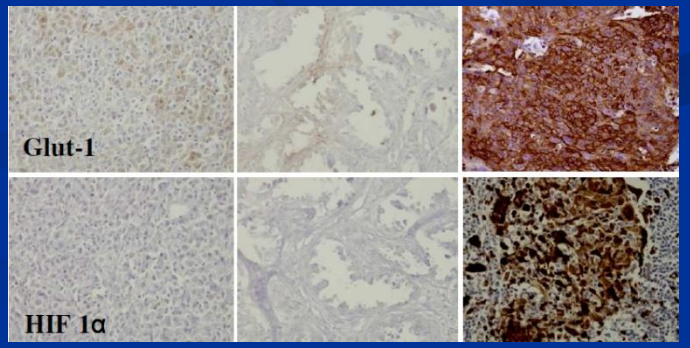


HYPOXIA

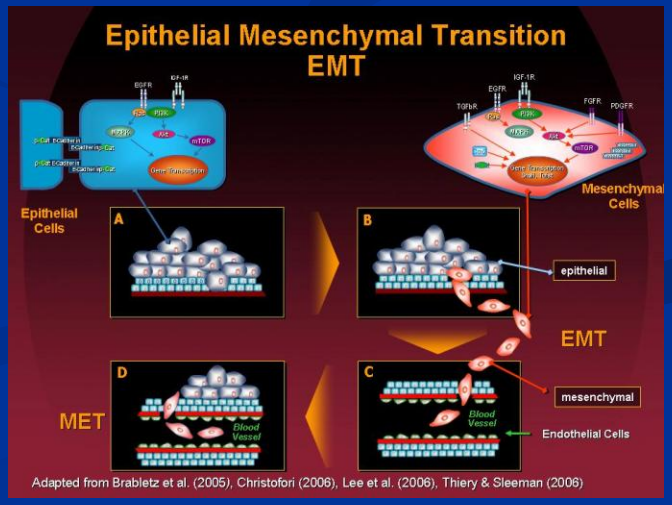
Proteins	Method	Overexpression	Reference
GLUT-1			
HIF 1a	IHC	25%	Tumor Biol, 2011
COX-2			

Prognostic value :

- Expression of hypoxia-related proteins was found in nodal squamous CUP of head and neck and was associated with poor prognosis



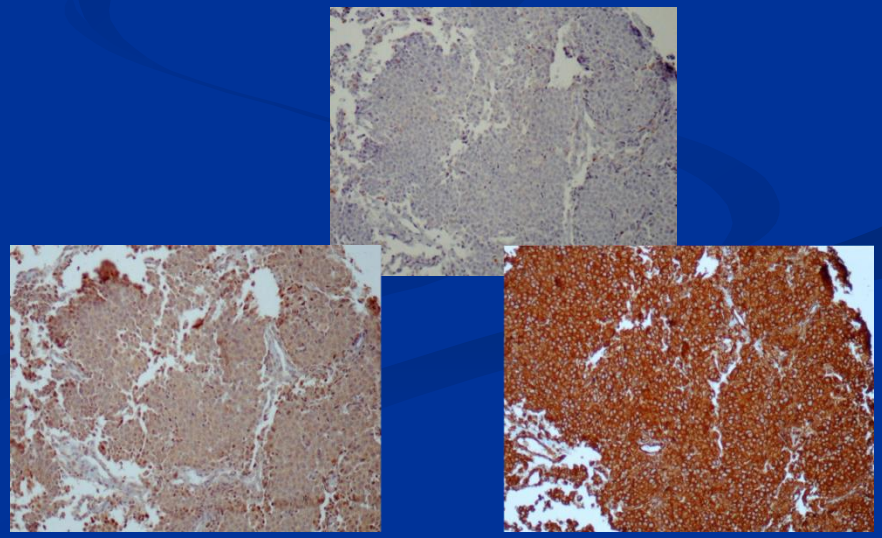
7. Epithelial Mesenchymal Transition and Stemness



EPITHELIAL – MESENCHYMAL TRANSITION (EMT) AND STEMNESS

Anticancer Res, 2012

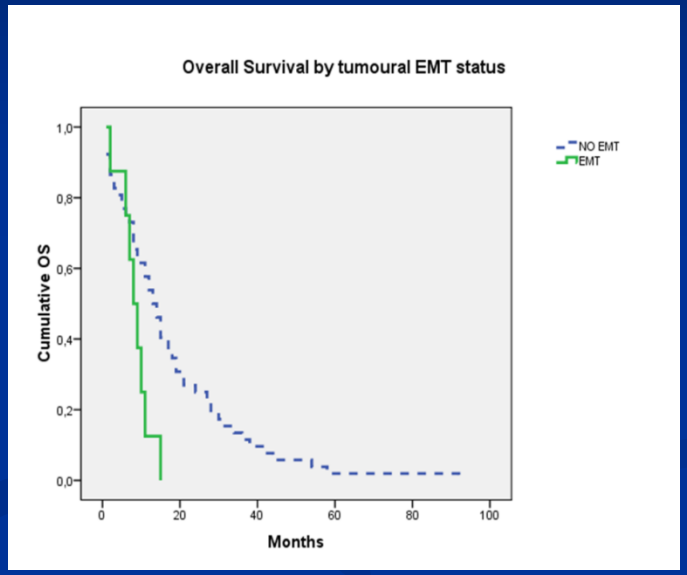
Biomolecule	Method	Cut-off (% + cells) Definition	Expression
E-Cadherin	IHC	≤ 60%	78.8 %
SNAIL	IHC	≥ 85%	61.9%
Vimentin	IHC	≥ 40%	23.2%
N-Cadherin	IHC	≥ 40%	13.8%
OCT4	IHC	-	0%



EPITHELIAL – MESENCHYMAL TRANSITION (EMT) AND STEMNESS

EMT phenotype was seen in :

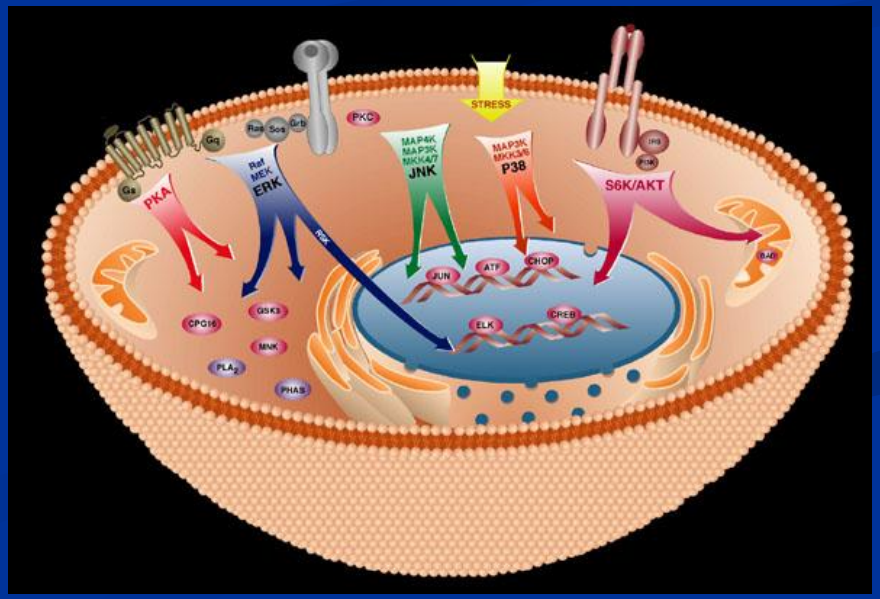
- **8.1 %** of cases (by % stained cells)
- **16.2 %** of cases (by staining intensity)



Implications and Prognostic values :

- EMT was **infrequently** seen in CUP
- EMT phenotype was strongly associated with **poor OS** (8 mos vs 13 mos p=0.023)
- EMT phenotype was correlated with **male gender**, **high grade** and **visceral disease** (p<0.05)

8. Signaling Pathways in Cup



MET- Receptor Oncogene Mutations

Oncogene	Method	Mutations	Reference
MET	PCR – SSCP	30 %	Hum Mutat, 2011

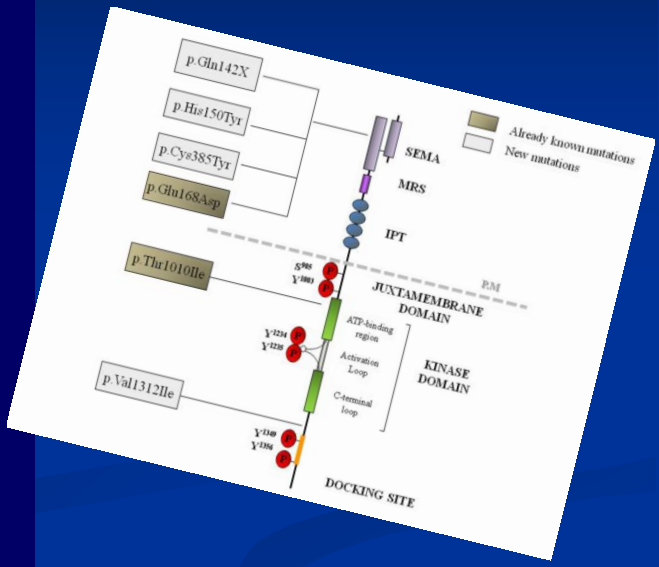
Implications and Prognostic value :

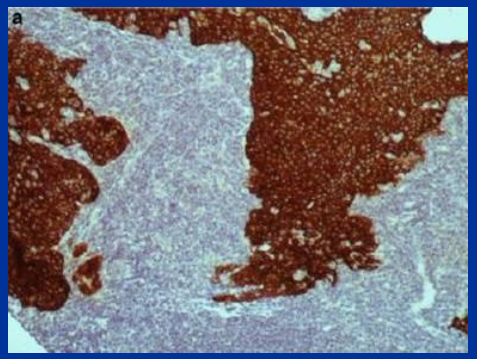
- Activating mutations clustering around kinase domain.
- Mutation rate 30%, as opposed to 4% in other solid tumors
- MET activating mutations are genetic markers associated with CUP
- c-MET: An exciting new target for therapy

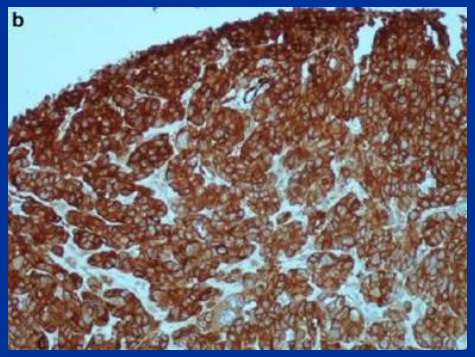
cMET and pMAPK Signaling Pathways

Clin Experim Metastases, 2012 (in press)

Biomolecule/ Oncogene	Method	Expression
cMET	IHC	42 %
pMAPK	IHC	54 %
Notch 2	IHC	56 %
Notch 3	IHC	73 %
Notch 1	IHC	2 %
Jagged 1	IHC	22 %







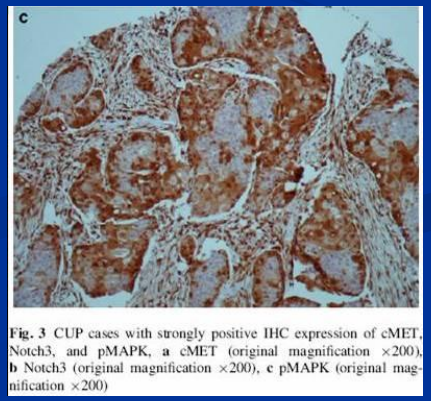
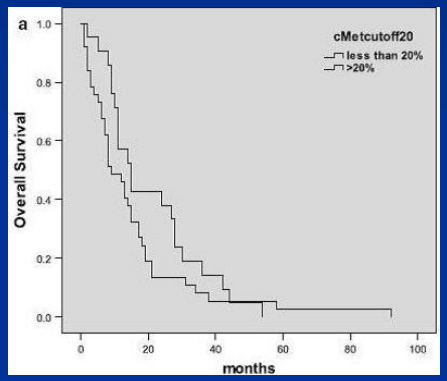


Fig. 3 CUP cases with strongly positive IHC expression of cMET, Notch3, and pMAPK, **a** cMET (original magnification ×200), **b** Notch3 (original magnification ×200), **c** pMAPK (original magnification ×200)

cMET and pMAPK Signaling Pathways

Prognostic value : * High cMET expression was associated with **better survival** (15mos vs 9mos , p=0.05) and **reduced risk of death** (p=0.025)



* High pMAPK expression was correlated to **worse survival** (9 mos vs 17 mos , p=0.016)

* Notch 3 overexpression was correlated to **worse survival in the midline nodal CUP subset** (12 mos vs 31 mos , p=0.05)

* Notch 1 overexpression was linked to **inferior PFS in the visceral group** (3 mos vs 7 mos, p=0.05)

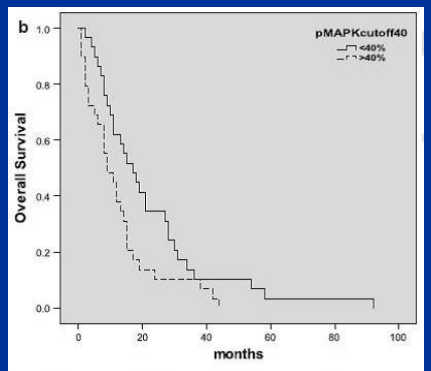


Fig. 4 Overall survival by tumoral IHC expression of various biomolecules in all CUP patients. **a** cMET, **b** pMAPK

Mutational profiling of the RAS, PI3K, MET and b-catenin pathways in cancer of unknown primary: a retrospective study of the Hellenic Cooperative Group

Pentheroudakis G et al

Clin Exp Metastasis, 2014; 31(7): 761-9

- **Tumor DNA from 87 CUP patients screened for B-CATENIN, MET, PIK3CA, KRAS, BRAF gene mutations**

	Incidence of mutated genes	Activating mutations
KRAS	12.6 %	10.2 %
BRAF	5.7 %	4.5 %
PIK3CA	9.0 %	6.6 %
MET	6.7 %	4.5 %
b-CATENIN	20.7 %	19.5 %

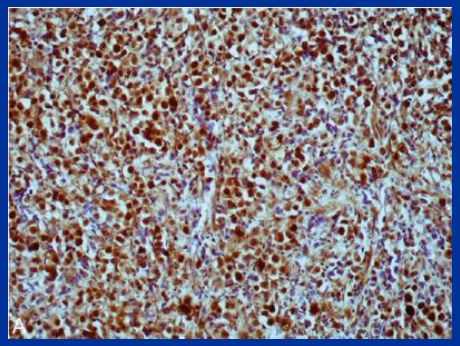
- **Prognostic significance :**
  - ✓ **MET activating mutations were prognostic for PFS (5 mos vs 9 mos, p=0.009) and OS (7 mos vs 20 mos, p=0.005)**
  - ✓ **The complex profile of either B-CATENIN or MET mutations had an adverse prognostic significance (OS 11 vs 21 mos, p = 0.015)**

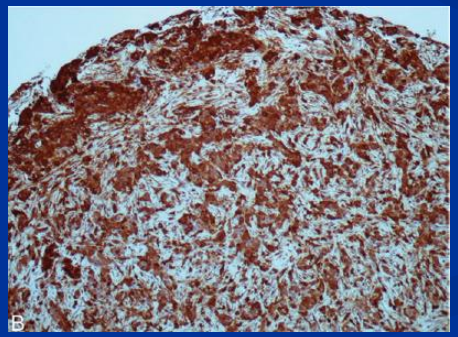
b – catenin gene mutations are frequent and along with MET mutations have an adverse prognostic effect in CUP patients

PTEN / AKT Signaling Pathway

Ann Oncol, 2012 (in press)

Biomolecule	Method	Expression
PTEN	IHC	50 %
pAKT	IHC	73 %
pRPS6	IHC	60 %
p21	IHC	61 %
Cyclin D_1	IHC	44 %





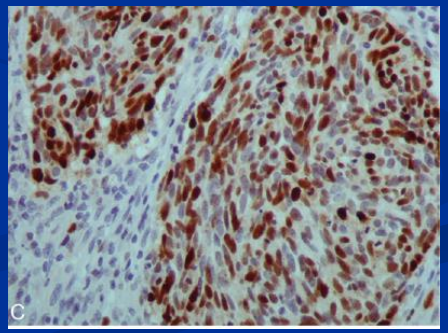
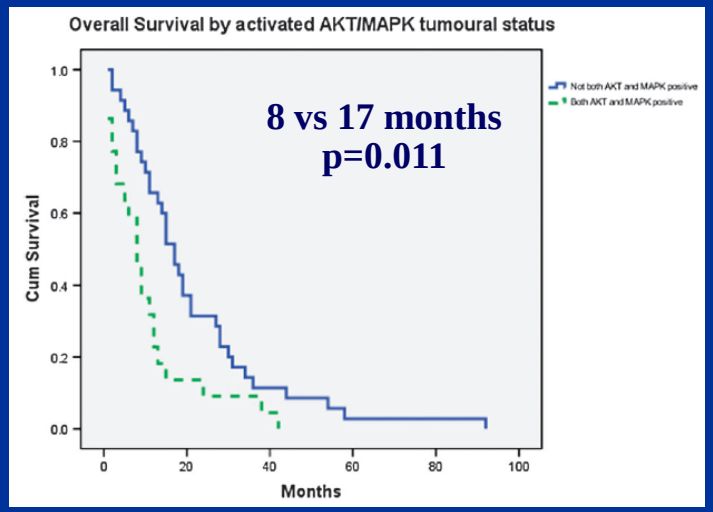


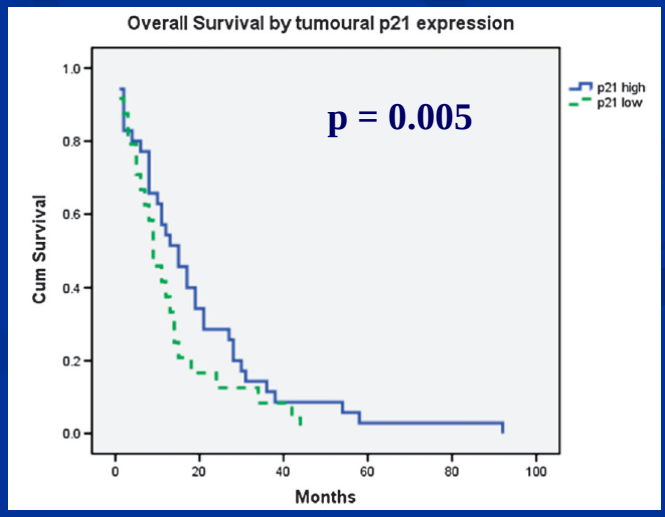
Figure 2. Immunohistochemistry carried out on tissue microarrays. (A) pAKT protein expression (×200); (B) pRPS6 expression (×100); (C) p21 expression in tumour nuclei (×400).

PTEN / AKT Signaling Pathway

Prognostic values :

- High p21 expression was associated with better survival , (p=0.005)
- High pAKT or pRPS6 expression predicted worse prognosis (p= 0.01 and p=0.008) in visceral CUP
- Concurrent pMAPK and pAKT expression had a marked adverse impact on survival, (8 mos vs 17 mos, p=0.011) in visceral CUP





Prognostic significance of WNT and HEDGEHOG pathway activation markers in Cancer of Unknown Primary.

Fotopoulos G, et al.

METHODS : Tissue microarrays and immunohistochemical expression of b-catenin, smoothened (SMO), TCF, LEF, GLI1 transcription factors.

RESULTS :

- **SMO expression** displayed a significant association with favourable outcome (OS 19 mos for (+) vs mos for (-), p=0.01).
- An **activated Wnt pathway** (expression of b-catenin, TCF, LEF) significantly associated with favourable PFS (9 mos vs 5 mos, p=0.037) and OS (19 mos vs 13 mos, p=0.04)
- No prognostic significance of the **Hedgehog pathway** activation was established
- A trend for association of **activated Wnt with response to chemotherapy** (67% vs 95%, p=0.07) in CUP adenocarcinomas

Eur J Clin Invest 2015

Conclusions

- No trace of a «CUP biologic signature», distinct from KPM
- CUP is characterized by chromosomal instability

Activated pathways in CUP:

- Angiogenesis with concurrent hypoxia
- AKT/S6RP axis with deficient apoptosis
- b-Catenin/Wnt axis
- MET axis
- EMT activity


SANTORINI
Thank you