The Biology of CUP : Have we made any progress towards understanding the disease and in targeting strategic molecular pathways ?

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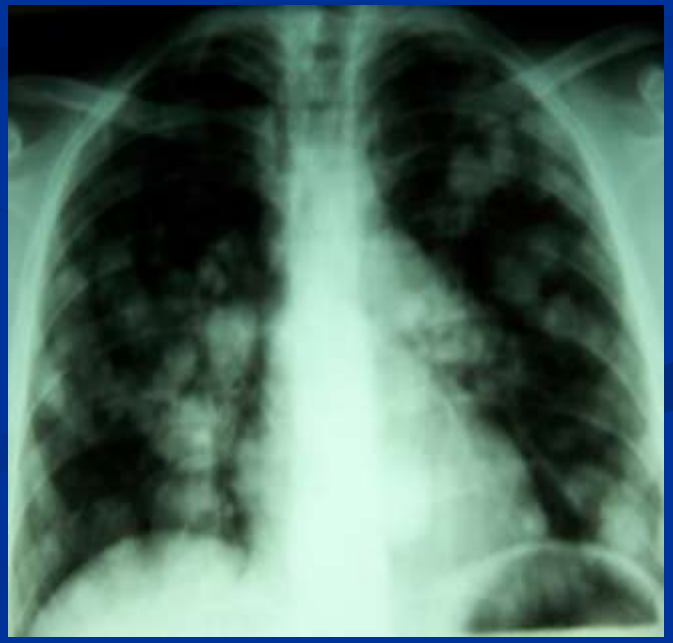
London, April 2012

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)

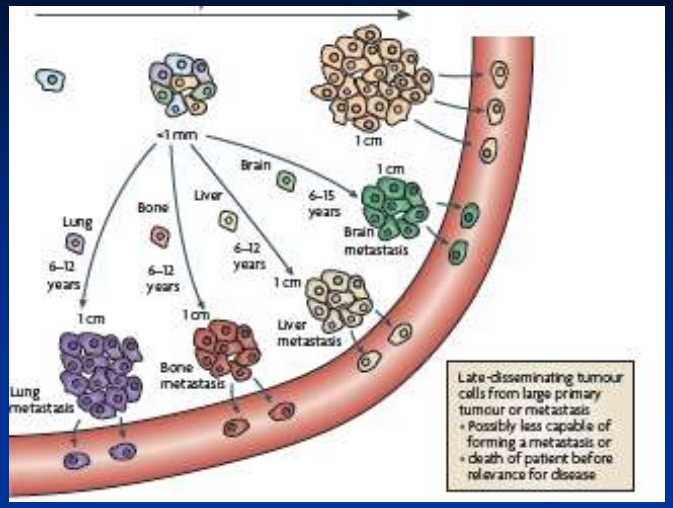
b u t

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

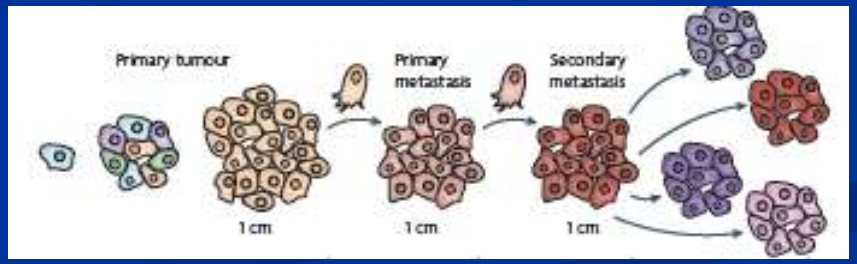
Hypothesis B

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

- Recent data from the Swedish Family Cancer Database suggest that the cause of death in CUP patients frequently matched the cancer diagnosed in a family member, suggesting that CUP had originated in that tissue.

- This implicates that the metastasis had probably undergone a phenotyping change complicating pathological tissue assignment.

- Interpretation : Some CUP cases are phenotypically modified primary cancers rather than cancers of unknown primaries.

Hemminki K, et al J CLin Oncol 29(4): 435-440, 2011

Hemminki K, et al 2012 (in press)

TRANSLATION RESEARCH ON CUP BIOLOGY

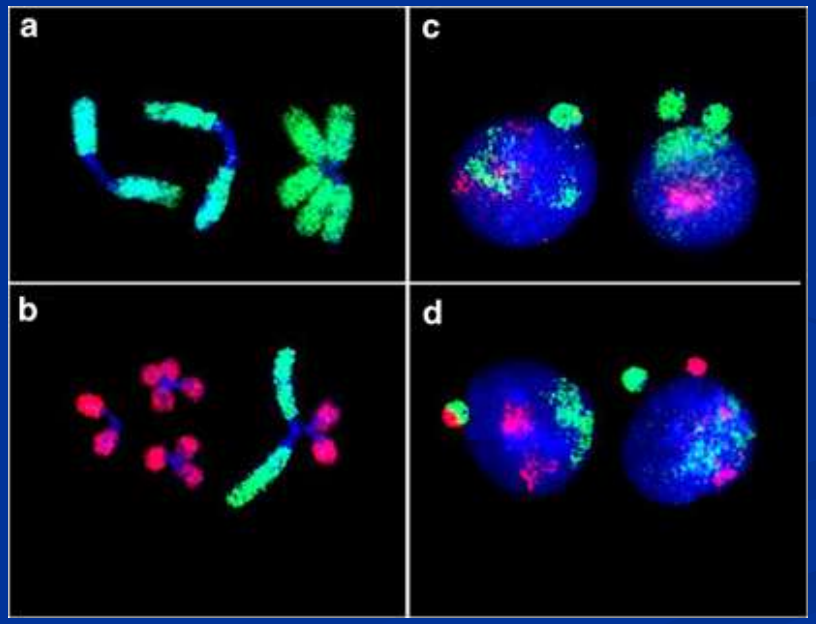
1. Chromosomal Instability
2. Oncogenes – Oncoproteins
3. Tumour and Metastasis Suppressor Genes
4. Angiogenesis
5. Metalloproteinases
6. Hypoxia
7. Epithelial Mesenchymal Transition and Stemness
8. Signaling Pathways
9. Molecular Diagnosis of the Primary

10. Targeting Treatment in CUP

PART I

Progress Towards Understanding the Disease

1. CHROMOSOMAL INSTABILITY



CHROMOSOMAL ABNORMALITIES

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

(Biochem Biophys Acta, 2011)

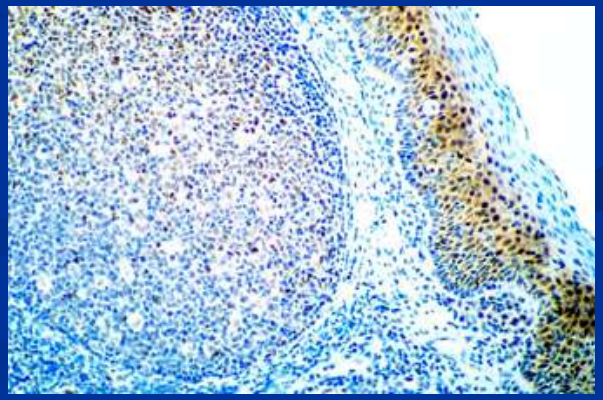
- **Aneuploidy in 70% of CUP adenocarcinoma**

(Eur J Cancer Clin Oncol , 2011)

Conclusions : **i) no correlation with metastatic spread or survival**

ii) overall data are similar to those of known primaries

2. ONCOGENES - ONCOPROTEINS



ONCOGENES – ONCOPROTEINS (I)

Oncoproteins	Method	Overexpression	Reference
HER-2	IHC	27%	Anticancer Res, 1995
HER-2	IHC	11%	J Clin Oncol, 2000
HER-2	IHC	4%	Proc ASCO, 2003
HER-2	IHC	24%	Proc ASCO, 2005
HER-2	IHC	4%	Br J Cancer, 2007
EGFR	IHC	61%	Proc ASCO, 2005
EGFR	IHC	12%	Clin Exp Metast, 2007
EGFR	IHC	35%	Br J Cancer, 2007

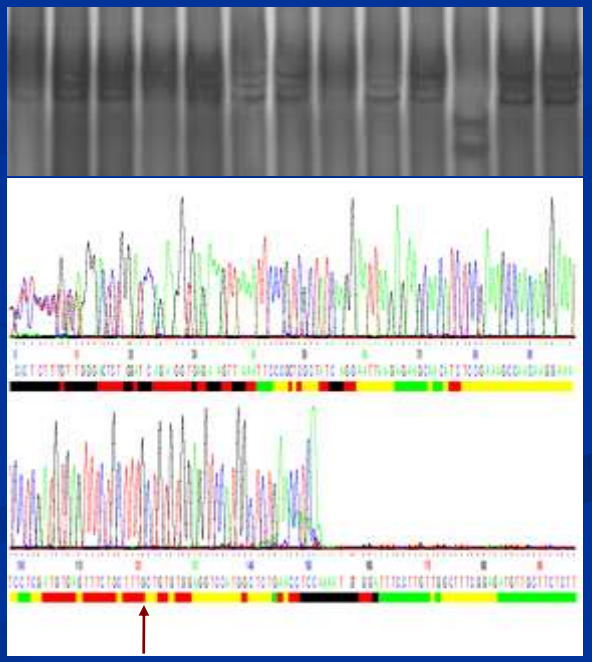
Screening EGFR exons 18, 19, 21

Dova et al, Clin Exp Metastasis. 2007; 24(2):79-86.

- **SYBR Green quantitative PCR:**
  Absence of amplification of exons 18, 19, 21 EGFR.

- **SSCP and sequencing:**
  Wild-type EGFR in 48/50 tumours.

- **No evidence for an activated EGFR axis in CUP**



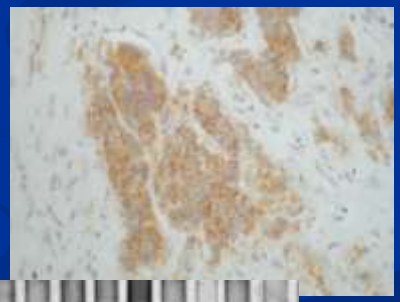
ONCOGENES – ONCOPROTEINS (II)

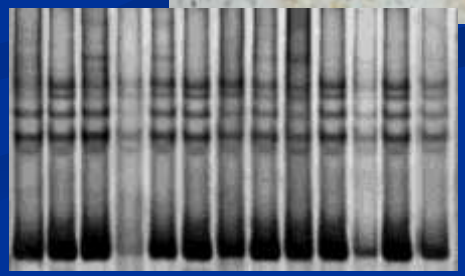
Oncoproteins	Method	Overexpression	Reference
cKit-PDGFR	IHC	13%	J Cancer Res Clin Oncol, 2008
cKit-PDGFR	IHC	4%	Proc ASCO, 2005
cKit-PDGFR	IHC	10%	Br J Cancer, 2007

C-KIT PDGFR activating mutations in CUP

J Cancer Res ClinOncol. 2008;134(6):697-704

- N=50 CUP
- **No exon 11 C-KIT mutations** were observed in SSCP mutational profiling.
- IHC CD117 overexpression in 13%.
- **No PDGFR exon 12** or **exon 18 mutations** were found.





ONCOGENES – ONCOPROTEINS (III)

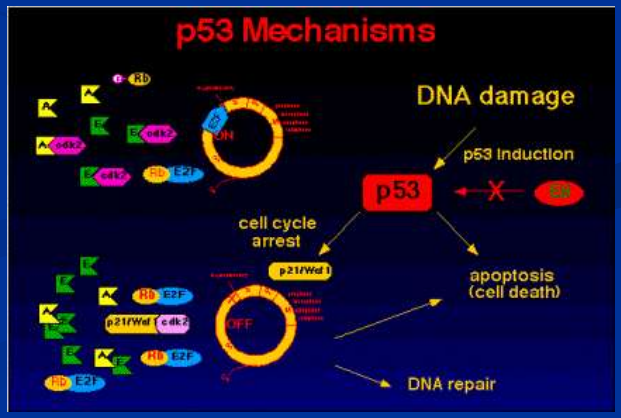
Oncoproteins	Method	Overexpression	Reference
BCL2	IHC	40%	Anticancer Res, 1998
cMYC	IHC	23%	Anticancer Res, 1995
Ras	IHC	23%	Anticancer Res, 1995

Implications :

- HER-2, EGFR, cKit-PDGFR, BCL2, cMYC, Ras oncoproteins although commonly expressed, seem to have no important role in the development of CUP
- No evidence of EGFR or cKit-PDGFR axes activation

Prognostic value : ● No significant association with patients prognosis

3. Tumour and Metastatic Suppressor Genes



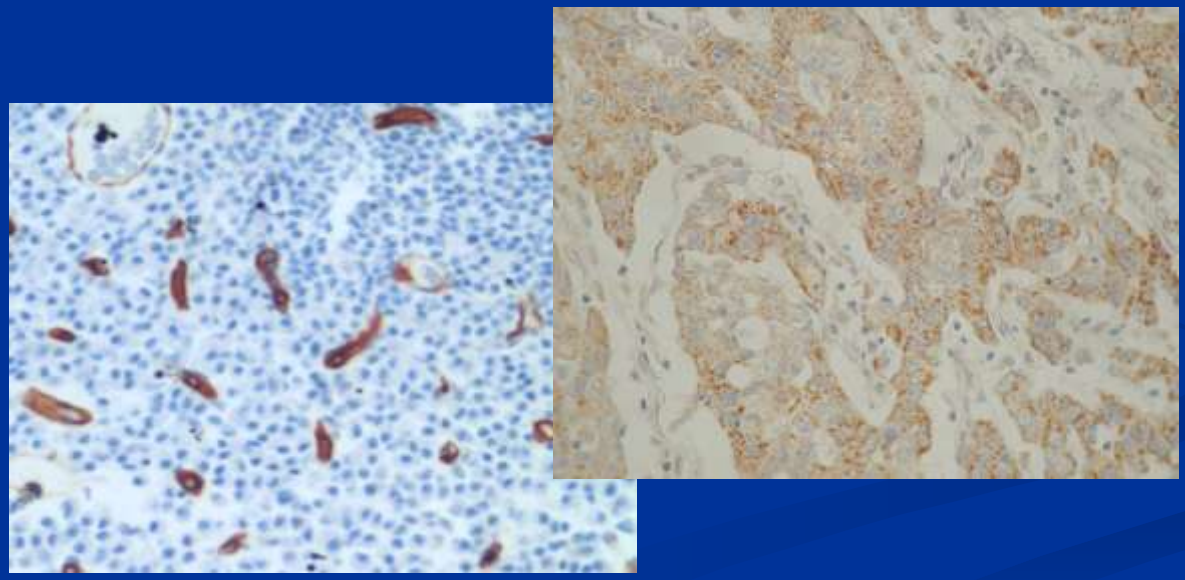
TUMOUR AND METASTATIC SUPPRESSOR GENES AND PROTEINS

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

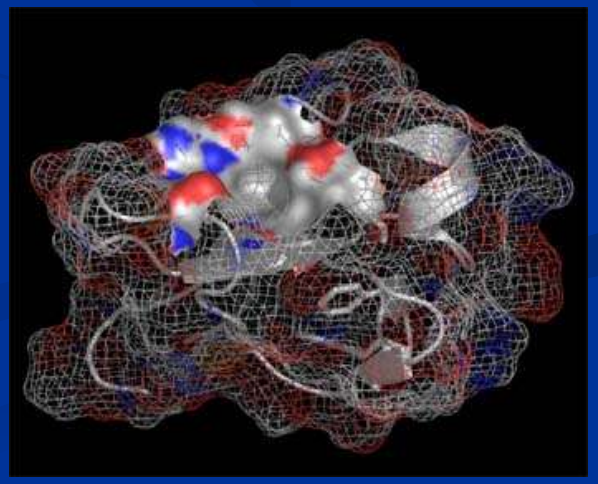
Proteins	Method	Overexpression	Reference
CD34 microvessel density	IHC		Int J Cancer, 1997
CD34 microvessel density	IHC	Median 56/mm^3	Anticancer Res, 2004
CD34 microvessel density	IHC	Median 59/mm^3	BMC Cancer, 2005
VEGF	IHC	83%	BMC Cancer, 2005
VEGF	IHC	26%	Anticancer Res, 2004
VEGF	IHC	29%	Proc ASCO,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

Implications : Angiogenesis is active in CUP, though 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



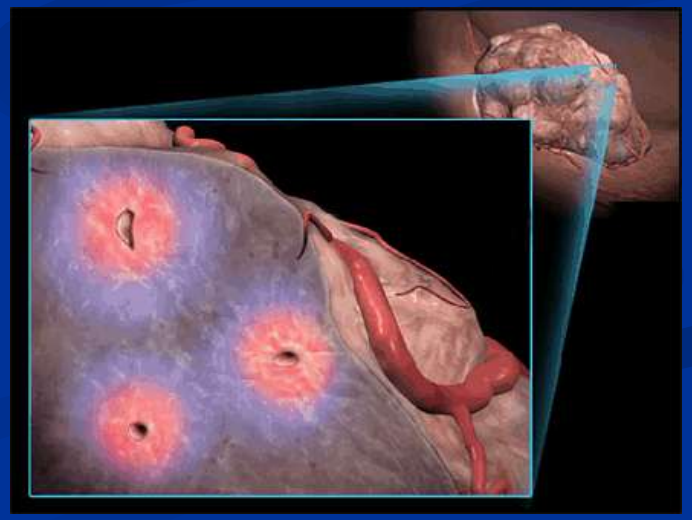
MATRIX METALLOPROTEINASES (Proteolysis-related molecules)

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)

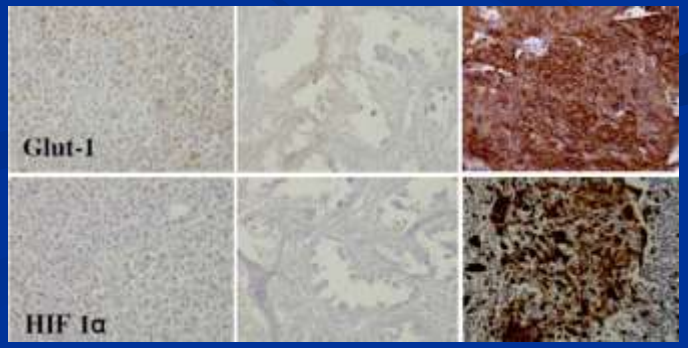
6. HYPOXIA



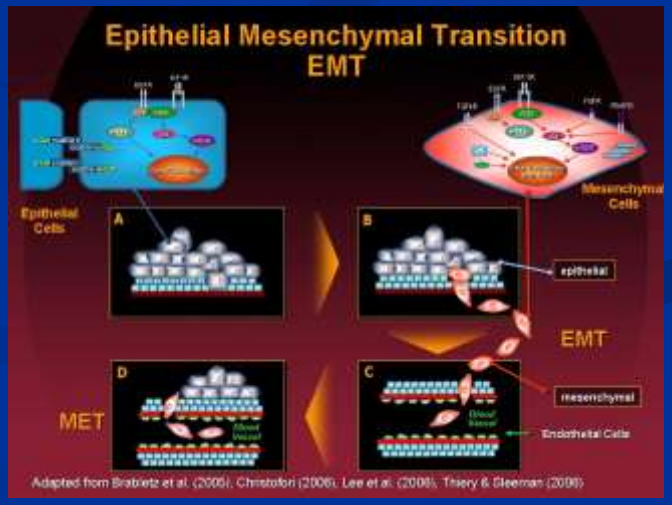
HYPOXIA

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

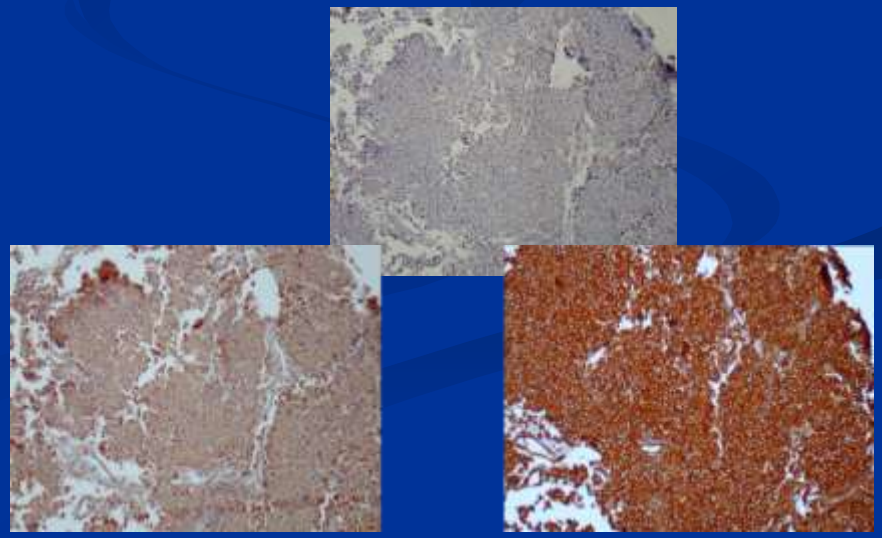


EPITHELIAL – MESENCHYMAL TRANSITION (EMT) AND STEMNESS

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%

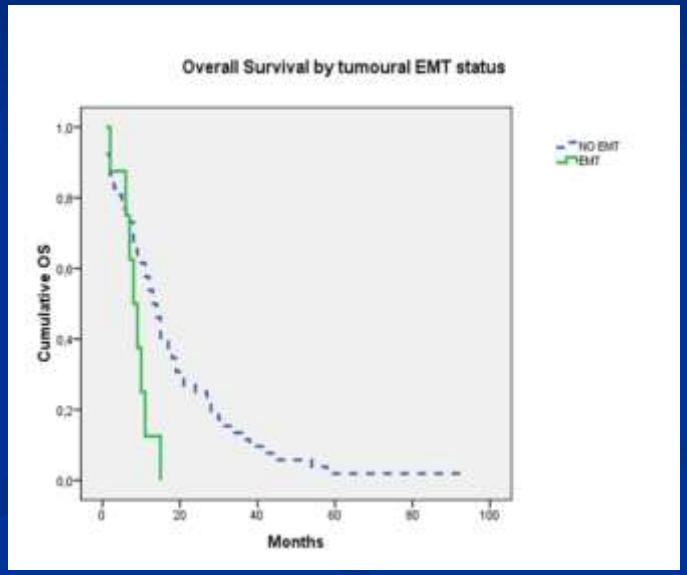
qRT-PCR : ongoing study



EPITHELIAL – MESENCHYMAL TRANSITION (EMT) AND STEMNESS

EMT phenotype was seen in :

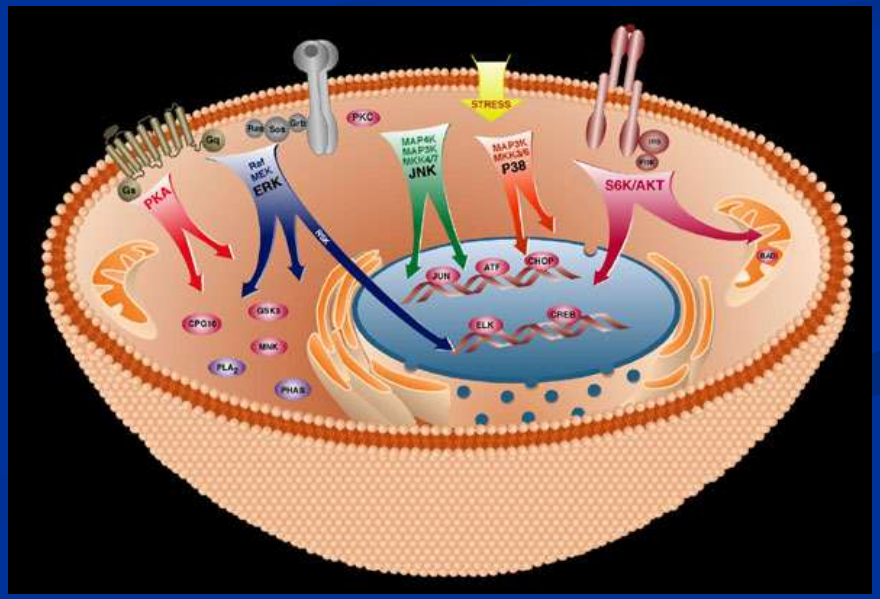
- **8.1 %** **of cases (by % stained cells)**
- **16.2 %** **of cases (by staining intensity)**



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



SIGNALING PATHWAYS IN CUP

cMET

pMAPK

Notch 1

Notch 2

Notch 3

Jagged 1

PTEN

pAKT

pRPS6

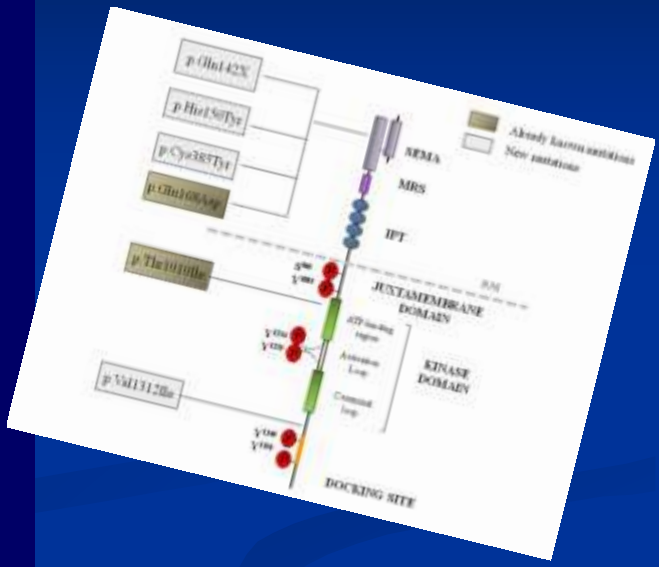
P21

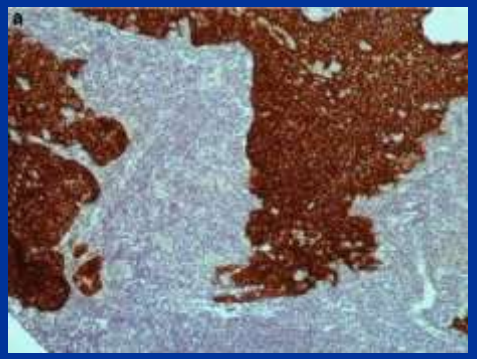
Cyclin D1

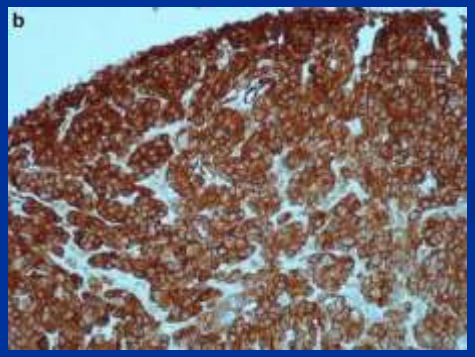
cMET and pMAPK Signaling Pathways

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 %







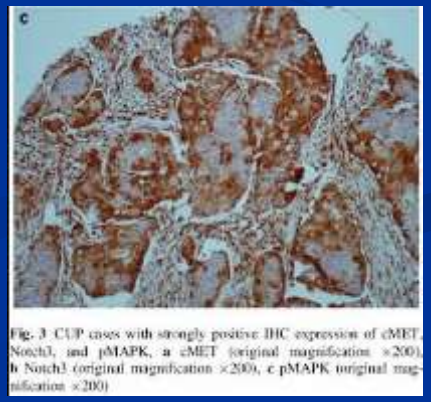
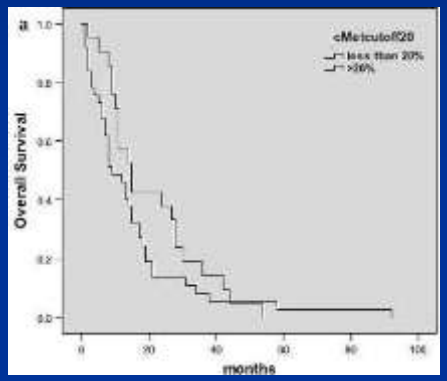


Fig. 3 CUP cases with strongly positive IHC expression of cMET, Notch3, and pMAPK. a cMET (original magnification ×200), b Notch3 (original magnification ×200), c pMAPK (original magnification ×200)

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 with **worse survival** (9 mos vs 17 mos – p=0.016)

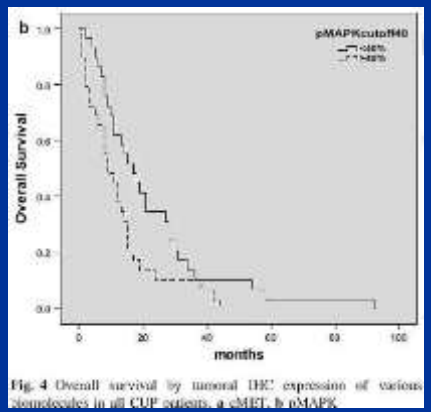


Fig. 4 Overall survival by tumoral IHC expression of various biomolecules in all CUP patients. a cMET, b pMAPK

* 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)

MET- Receptor Oncogene Mutations

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

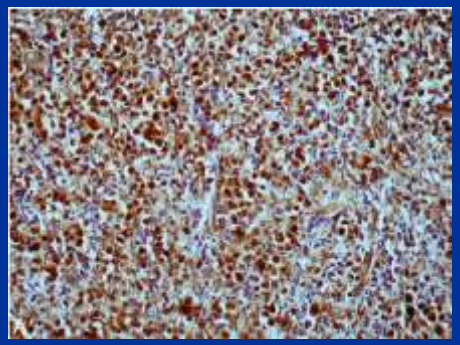
Implications and Prognostic value :

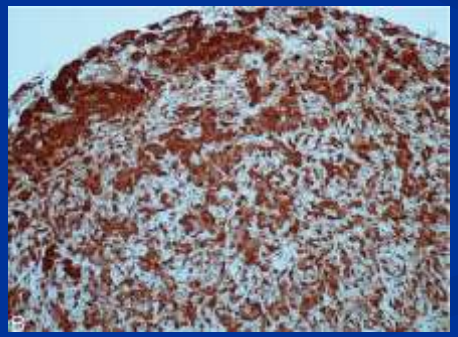
- Activating mutations clustering around kinase domain.
- Mutation rate 30%, as opposed to 4% in other solid tumors
- MET activating mutations are genetic markers associated with CUP

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 %





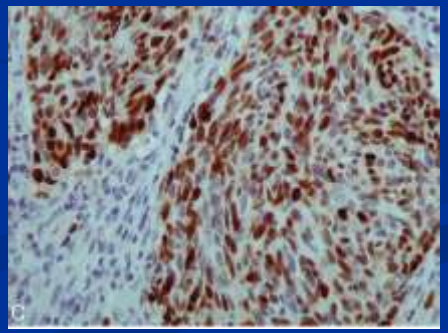
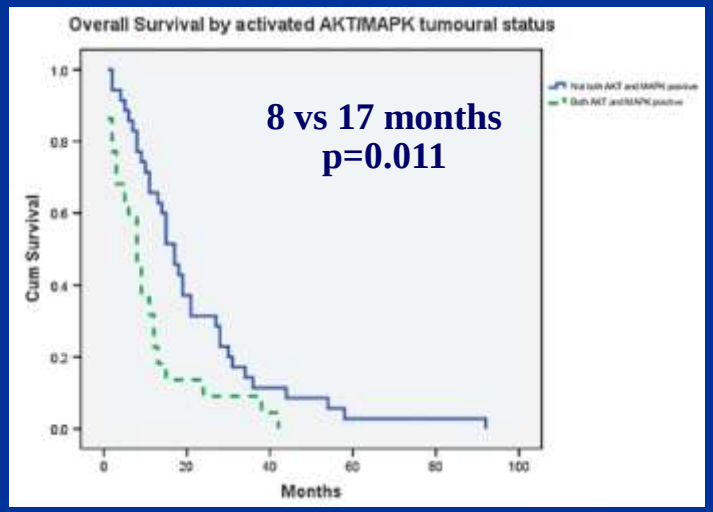


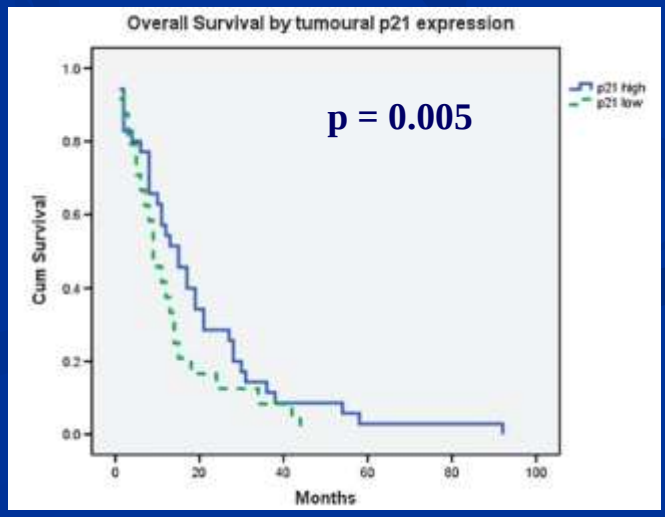
Figure 2. Immunohistochemistry carried out on tissue microarrays. (A) pAKT protein expression (×200); (B) pRPS6 expression (×100); (C) p21 expression in tumour nuclei (×400).

PTEN / AKT Signaling Pathway

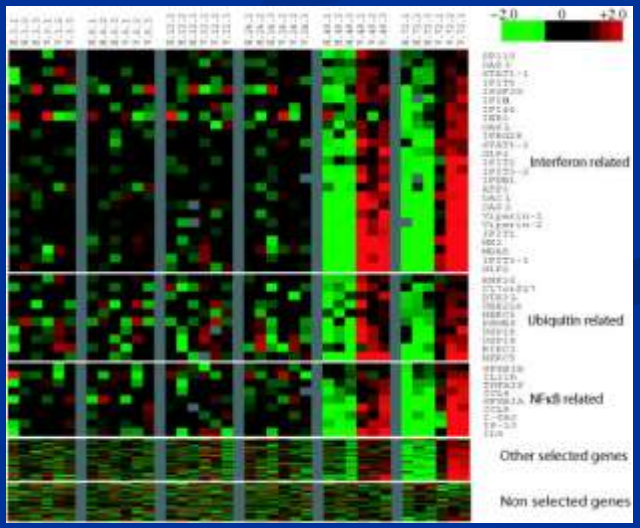
Prognostic values :

- 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





9. Molecular Diagnosis of the Primary



IDENTIFICATION OF PRIMARY SITE BY GENETIC PROFILING (MICROARRAYS) FROM ALL PUBLISHED CUP SERIES

Years of Publications : 2005- 2007

No of Samples : > 500 (cDNA)

Biological Assignment of Primaries (Accuracy) : 50 – 87 %

Primary Sites Identified :

Breast	15 %
Pancreas	12.5 %
Bowel	12 %
Lung	11.5 %
Genital system	9 %
Liver/bile duct	8 %
Kidney / adrenals	6 %
Bladder / ureter	5 %
Stomach	3 %
Other	18 %

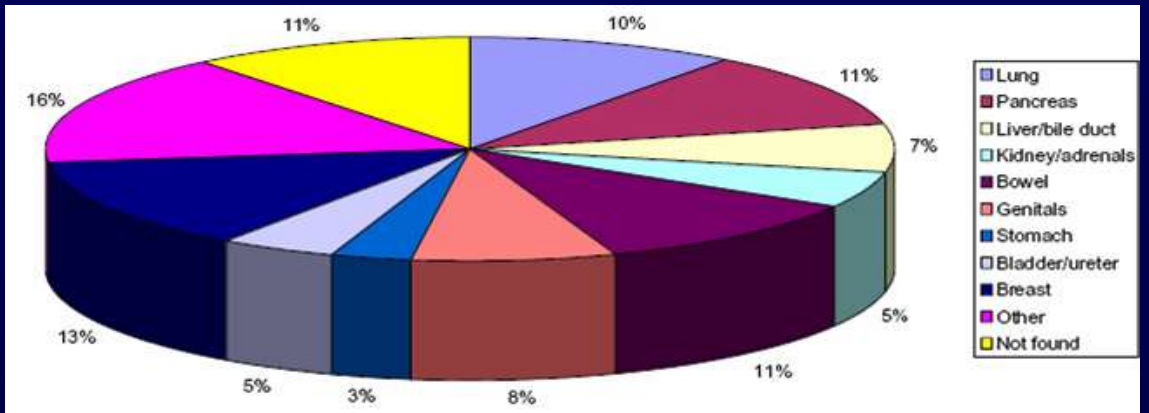
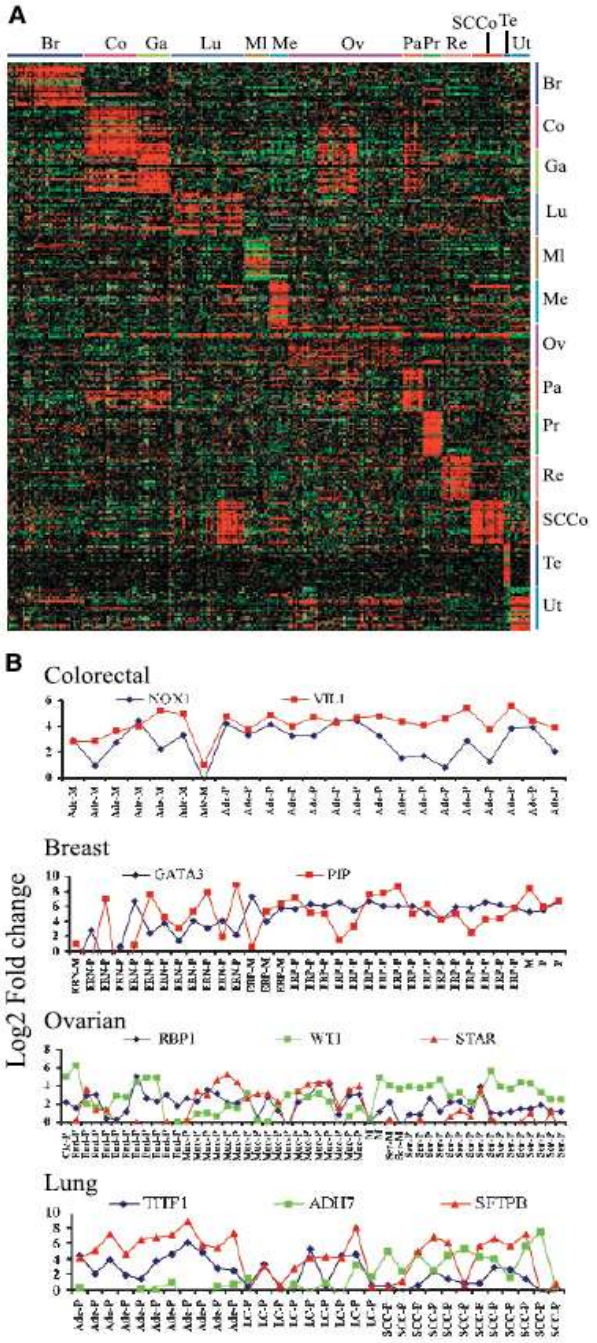


Fig. 2 – Relative proportion of molecularly-assigned primaries in published series.

Eur J Cancer 2026-36, 2007

DIAGNOSTIC MICROARRAY MOLECULAR PROFILING IN CANCER OF UNKNWON PRIMARY

Cancer Res 2005;65(100): 4031-4040



Gene Expression Profiling

Assays

Assay	Platform	Tissue	No. of Tumor types	Number of genes	Accuracy in known tumors (%)
Veridex	RT-PCR mRNA	FFPE	6 and "other"	10	76
Pathwork Diagnostics Tissue of Origin test	cDNA microarray	Frozen/ FFPE	15	1500	89
Rosetta Genomics MiReview met	RT-PCR miRNA	FFPE	22	48 miRNAs	86
bioTheranostics CancerType ID	RT-PCR mRNA	FFPE	39 (including subtypes)	92	86

CLINICAL AND THERAPEUTIC UTILITY OF GENE AND PROTEIN MICROARRAY TECHNOLOGIES

QUESTION 1

DOES MOLECULAR ASSAYS, INCREASE THE ACCURACY OF IDENTIFYING THE PRIMARY SITE?

ANSWER 1

YES : UP TO 90% ACCURACY

QUESTION 2

DOES THIS DIAGNOSTIC AID RESULTS IN IMPROVEMENT OF PATIENT OUTCOME ?

ANSWER 2 **?**

PART II

Targeting Strategic Molecular Pathways

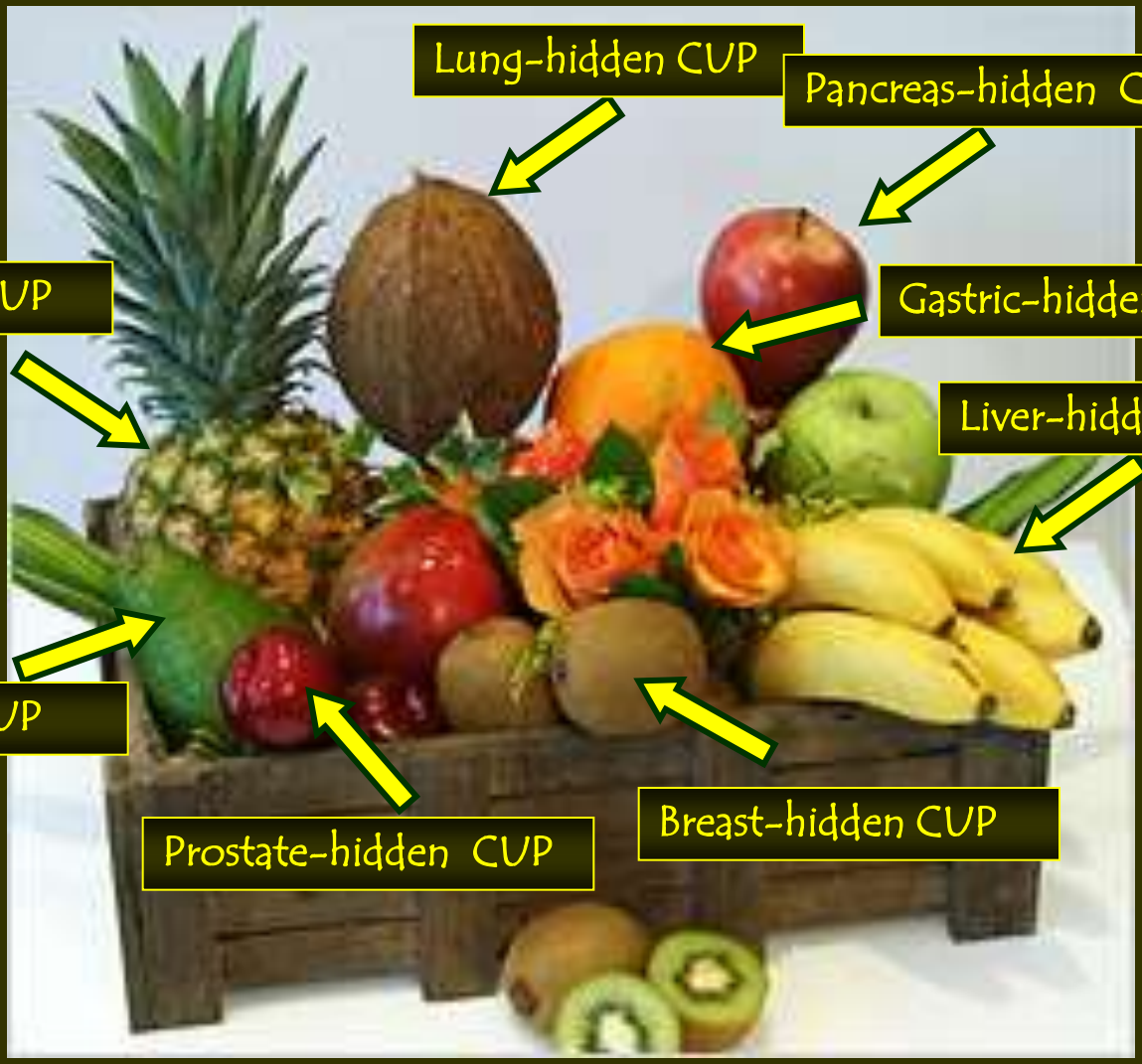
HOW DO WE TREAT CUP PATIENTS ?

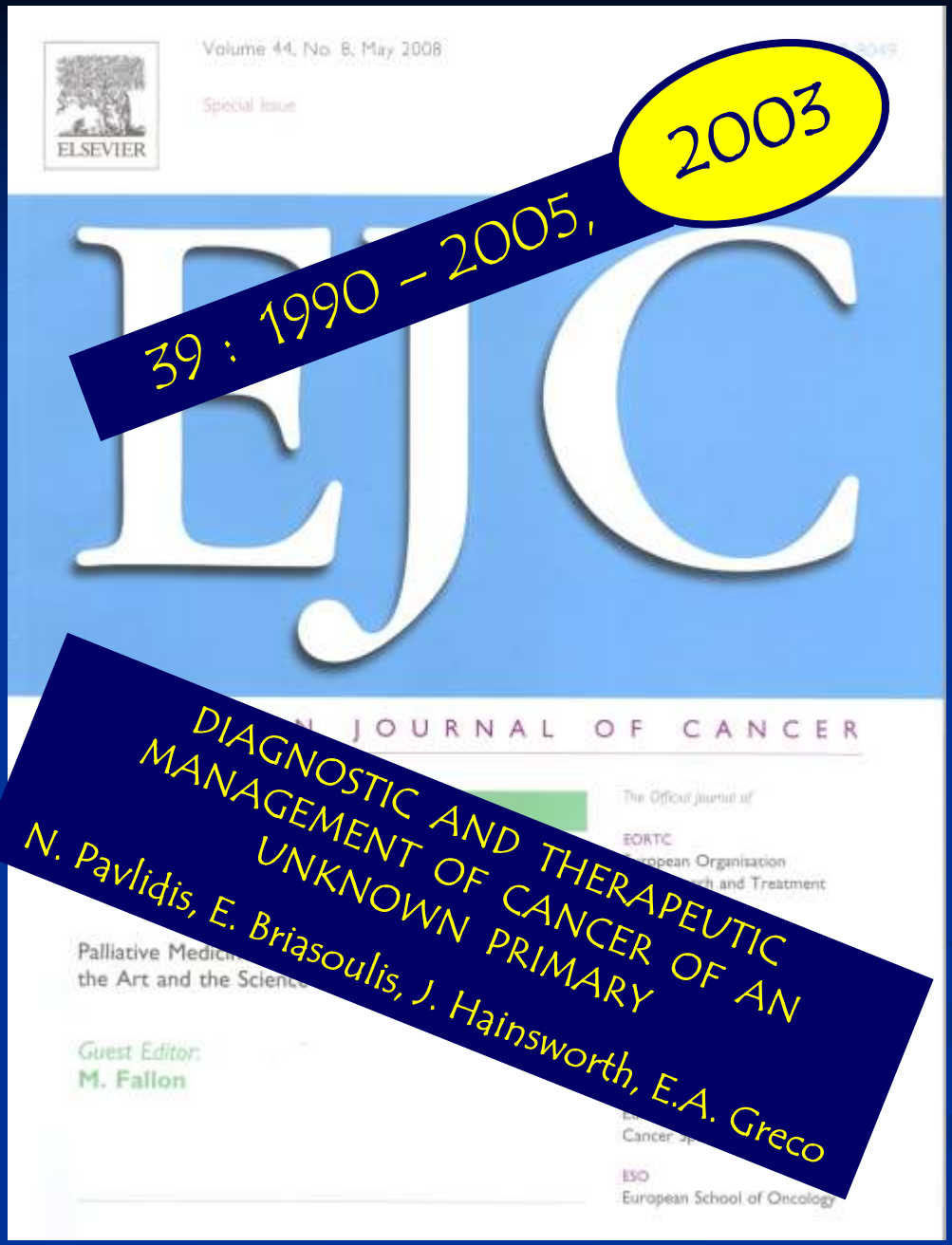
DO WE HAVE EFFECTIVE DRUGS FOR CANCER OF UNKNOWN PRIMARY

OR

WE JUST HAVE RESPONSIVE SUBSETS OF PATIENTS ?

WHAT IS CANCER OF AN UNKNOWN PRIMARY SITE ?




Volume 44, No. 8, May 2008
Special Issue
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DIAGNOSTIC AND THERAPEUTIC
MANAGEMENT OF CANCER OF AN
UNKNOWN PRIMARY
N. Pavlidis, E. Briasoulis, J. Hainsworth, E.A. Greco

CUP

FAVOURABLE OR GOOD PROGNOSIS SUBSETS — 20%

UNFAVOURABLE OR POOR PROGNOSIS SUBSETS — 80%

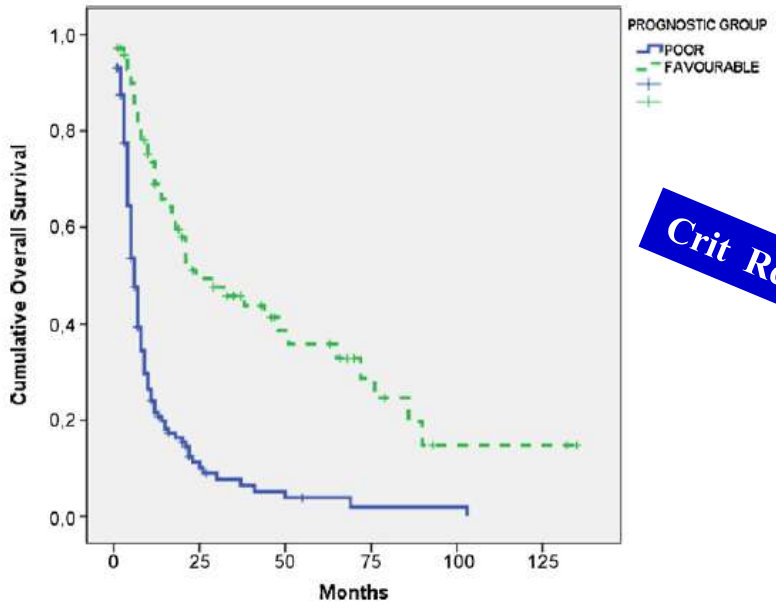


Fig. 1. Survival of favorable versus poor risk CUP patients treated at Ioannina University Hospital from 1995 to 2011.

Crit Rev Oncol Hematol (in press, 2012)

UNFAVOURABLE SUBSETS

80%

1. Adenocarcinoma metastatic to the liver or other organs
2. Non-papillary malignant ascites (adenocarcinoma)
3. Multiple cerebral metastases (adeno or squamous Ca)
4. Multiple lung/pleural metastases (adenocarcinoma)
5. Multiple metastatic bone disease (adenocarcinoma)
6. Squamous cell carcinoma of the abdominal cavity

Favourable Subsets

20%

1. Poorly differentiated carcinoma with midline distribution (extragonadal germ cell syndrome).
2. Women with papillary adenocarcinoma of peritoneal cavity.
3. Women with adenocarcinoma involving only axillary lymph nodes
4. Squamous cell carcinoma involving cervical lymph nodes
5. Poorly differentiated neuroendocrine carcinomas.
6. Men with blastic bone metastases and elevated PSA (adenocarcinoma).
7. Isolated inguinal adenopathy (squamous carcinoma
8. Patients with a single small, potentially resectable tumor.

HOW DO WE TREAT FAVOURABLE CUP SUBSETS ?

These patients are treated with locoregional treatment and/or systemic chemotherapy relevant to the hidden primary tumors

i.e.

- **isolated axillary adenoCa → like breast cancer stage II**
- **primary peritoneal parillary carcinoma → like ovarian cancer FIGO stage III**
- **squamous carcinoma of cervical nodes → like advanced head-neck cancer**

HOW DO WE TREAT UNFAVOURABLE CUP SUBSETS ?

With empirical chemotherapy :

i.e.

- **Cisplatin – based combinations**
- **Taxane - based combinations**

10. Do we Have any Edivence that Targeted Treatment is Drastic in Cup Patients ?

J Clin Oncol 2007 May 1;25(13):1747-52

Phase II Trial of Bevacizumab and Erlotinib in Carcinomas of Unknown Primary Site: The Minnie Pearl Cancer Research Network

John D. Hainsworth, David R. Spigel, Cindy Farley, Dana S. Thompson, Dianna L. Shipley, and F. Anthony Greco

No Patients : **47 (previously treated or poor-prognosis)**

Treatment : **Bevacizumab 10 mg/kg q 2wks**
Erlotinib 150 mg p.o. daily

Results : **10% PR**
61% SD
Survival : Median 7.4 mos
1-year 33%

Oncologist 2009, 14(12): 1189-97

Paclitaxel/Carboplatin plus Bevacizumab/Erlotinib in the First-Line Treatment of Patients with Carcinoma of Unknown Primary Site

JOHN D. HAINSWORTH,[a,b] DAVID R. SPIGEL,[a,b] DANA S. THOMPSON,[b] PATRICK B. MURPHY,[b] CASSIE M. LANE,[a] DAVID M. WATERHOUSE,[c] YUVAL NAOT,[d] F. ANTHONY GRECO[b]

No Patients : 60

Regimen : Carboplatin / paclitaxel / Bevacizumab / Erlotinib
As first-line and maintenance (Bev/Erlot)

Treatment : 49 pts completed 4 cycles
44 pts continued maintenance bevacizumab/erlotinib

Results : 53% major responses
41% stable disease
PFS - median : 8 mos
1-year : 38%
Survival – median: 12.6 mos
2-year : 27%

Clin Colorectal Cancer, 2011

A Retrospective Study of Treatment Outcome in Patients with Carcinoma of Unknown Primary Site and a Colorectal Cancer Molecular Profile

Haisworth JD, Schnabel CA, Erlander MG, Haines DW 3rd, Greco FA

32 CUP patients predicted by molecular profiling to have a colorectal site of origin had received colorectal cancer regimens

Overall response rate : 50%

Median survival : 27 months

Ongoing Clinical Trials on CUP

Trial	Phase	Regimens	Country
CUP-ONE	II	Epi / Cis / Capec ± Vandetanib	UK
UNUPRI 20	II	Standard chemotherapy based on molecular diagnosis of THE PRIMARY	US
	II (random)	Carbo / Paclit ± Belinostat	US
GEFCAPI 04	III	Cis / Gemc vs standard chemo based on molecular diagnosis of the primary	France
PACET-CUP	II (random)	Paclit / Carbo ± Cetuximab	Germany

FUTURE PESPECTIVES
IN
THERAPEUTIC TARGETING OF CUP

c-MET Driven Malignancies

(Mesenchymal-epithelial transition factor)

- **The HGF/c – MET pathway is implicated in the regulation of cancer cell growth, angiogenesis, invasion and metastasis.**
- **Activation of the c-MET signaling pathway can occur through activating mutations, overexpression, or autocrine, paracrine or endocrine loop regulation.**
- **c-MET has prognostic implications in patients with cancer**
- **C-MET is involved in resistance to VEGFR or EGFR inhibitors**

c-MET : An Exciting New Target for Anticancer Therapy

C-MET INHIBITORS UNDER CURRENT DEVELOPMENT

Agent	Company	Mechanism of Action	Phase
AMG 102	Amgen	Anti-HGF antibody	II
Tivantinib (ARQ 197)	ArQule; Daiichi Sankyo	Selective c-MET TKI	III
Cabozantinib (XL 184)	Exelixis; Bristol-Myers Squib	Nonselective c-MET, VEGFR2 and RET TKI	II
MetMAb	Genentech	Anti-c-MET antibody	II

Ther Adv Med Oncol 3(S1), S51-S60, 2011

COMBINATION STUDIES c-MET INHIBITOR PLUS OTHER PATHWAYS

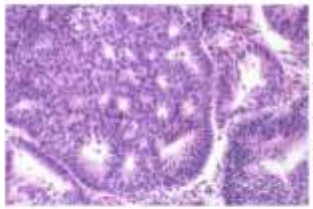
Combination	Phase
EGFR	
Tivantinib ± erlotinib	III
MetMAb ± erlotinib	II
Ficlatuzumab (AV-299) ± gefitinib	II
VEGF	
Rilotumumab (AMG 102) + bevacizumab or motesanib	Ib
Tivantinib + sorafenib	I
CHEMOTHERAPY	
Crizotinib + pemetrexed/docetaxel	III
Tivantinib + gemcitabine	I
Tivantinib + irinotecan and cetuximab	I/II

Ther Adv Med Oncol 3(S1), S51-S60, 2011

PHARMACOGENOMIC PROFILE IN PATIENTS WITH CARCINOMAS OF UNKNOWN PRIMARY (CUP)

ESMO Milan, Abstr 128P

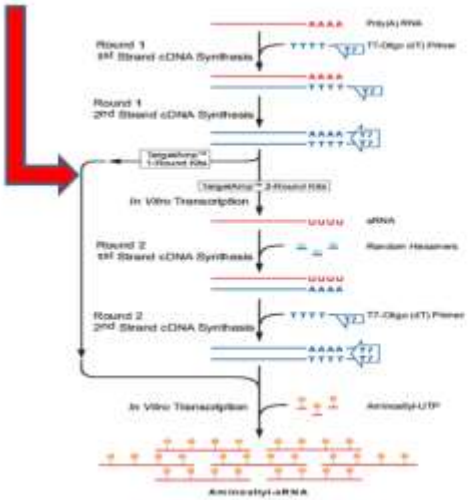
Patients and Methods



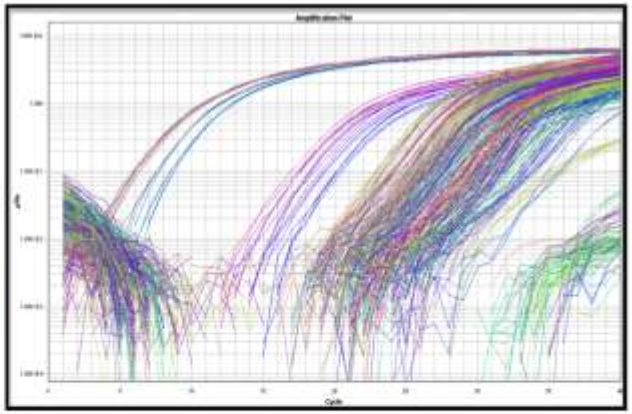
Tumor samples from 62 patients with Ca UP were collected. Sections of 5µm thickness were stained with H/E All samples reviewed by an independent pathologist

Malignant cells were procured using a piezoelectric microdissector

RNA extraction based on Trizol LS and DNase treatment



cDNA synthesis with 15ng of RNA using SuperScript III



Relative quantification using *b-actin* and *PGK1* as an endogenous control .In an ABI Prism 7900HT Sequence Detection System

Prognostic Significance of Gene Expression Profile in Patients with CUP

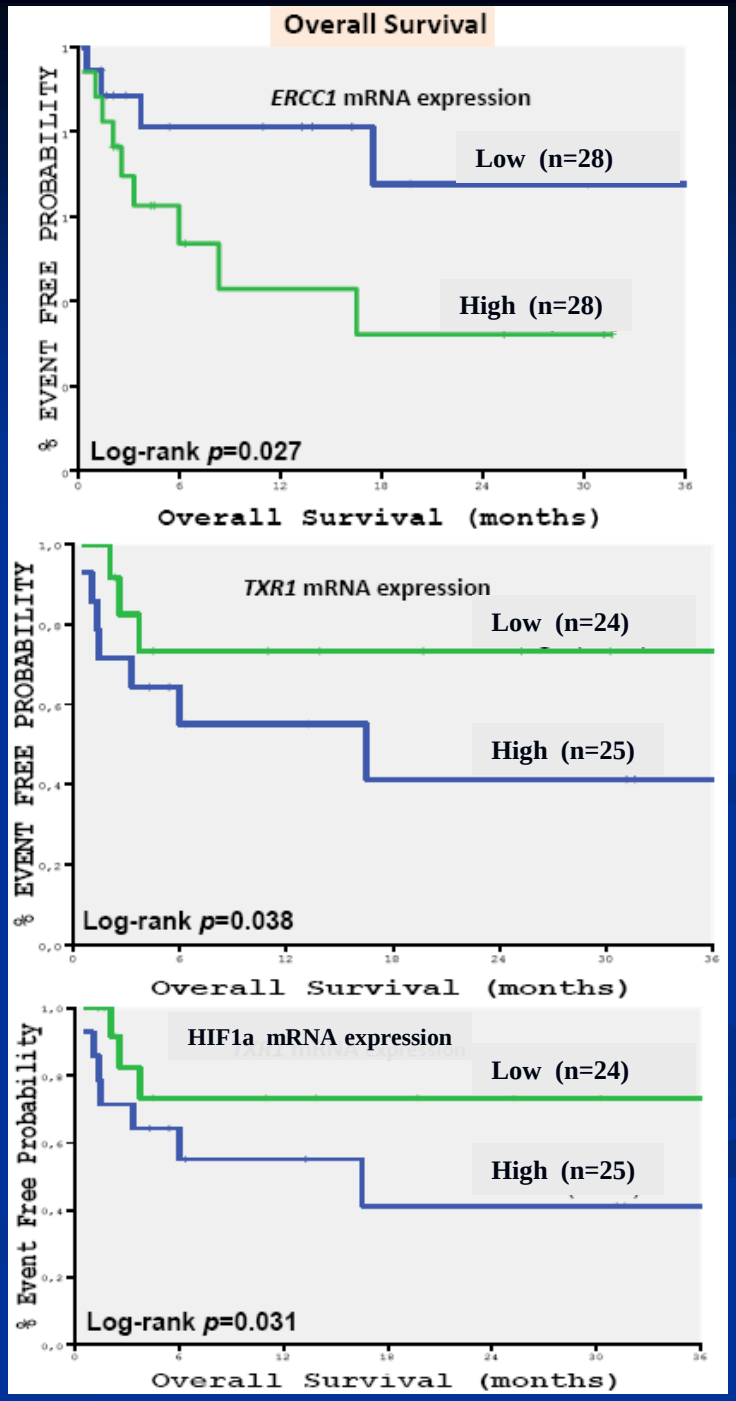
ESMO, Milan , Abstr 128P

In the present study we evaluated the **prognostic significance of gene expression** of specific genes correlated with DNA synthesis, DNA repair, apoptosis and angiogenesis in **62 patients** with CUP

Gene mRNA expression and patients' outcome

		Overall Survival (months)	
Genes	No patients	Median (95%CI)	p*
BRCA1 Low	27(49)	9.9 (6.4-13.6)	0.882
BRCA1 High	28 (51)	10.4 (6.9-14.2)	
ERCC1 Low	28 (50)	21.8 (11.6-28.5)	0.027
ERCC1 High	28 (50)	6.3 (1.6-10.8)	
RRM1 Low	24 (49)	11.6 (7.9 -1 5.3)	0.092
RRM1 High	25 (51)	8.9 (5.8-12.7)	
TOPO-I Low	24 (49)	11.1(8.9-18.6)	0.533
TOPO-I High	25 (51)	9.8 (7.1-15.2)	
TOPO-IIA Low	24 (49)	10.4 (8.0-15.5)	0.937
TOPO-IIA High	25 (51)	10.1 (8.1-14.9)	
TOPO-IIB Low	24 (49)	10.7 (8.2-17.2)	0.822
TOPO-IIB High	25 (51)	10.0 (7.4-14.8)	
TYMS Low	24 (49)	11.1 (9.0-16.3)	0.571
TYMS High	25 (51)	9.2 (7.6-14.3)	
HIF1α Low	24 (49)	6.9 (4.9-9.8)	0.031
HIF1α High	25 (51)	19.8 (10.3-21.4)	
TXR1 Low	24 (49)	18.3 (10.4-27.9)	0.038
TXR1 High	25 (51)	7.4 (4.4-12.7)	
TSP1 Low	24 (49)	8.2(5.2-11.6)	0.041
TSP1 High	25(51)	17.1 (11.1-39.2)	

CUP Pharmacogenomics



INTERPRETATION

- These data indicate that ERCC1, TXR1 and HIF 1a mRNA expression may be used as prognostic factors if these results will be independently validated.

- Further analysis is required for the predictive significance of these markers since the majority of them are also implicated in chemotherapeutic drugs metabolism or mode of action.

FUTURE RESEARCH SUGGESTIONS ON CUP

BETTER COLLABORATION

1. Establishment of **international electronic CUP registry** for data capture on presentation, management, outcome (may be **CUP Tissue Bank** as well ?)

2. Establishment of **CUP cell lines** and **CUP xenographs** from visceral CUP patients

3. Establishment of **International CUP Task Force** with meeting 1-2 times per years

4. Development of **international CUP trials**

SUGGESTED RESEARCH TOPICS:
Is there a CUP signature?

Genome - wide studies

1. **Compare via microarrays the expression of whole genome mRNAs or microRNAs between:**

   (i) **CUPs biologically classified according to a platfrom,** ***or***

   (ii) **Metastases from equivalent known primary tumours**

2. **Mutational profiling and FISH on commonly implicated oncogenes (MET, PTEN, P13K, HER 2, EGFR, KRAS, BRAF, AKT, TGFR, FGFR, ERK, MAPK)**

CONCLUSIONS

(I) BIOLOGY OF CUP [PART I]

- Although **HER-2, BCL 2, cMYC** and **Ras** are commonly expressed, they seem to have no important role in the development of CUP or in patients prognosis.
- The **EGFR** and **c-Kit - PDGFR** axes are not activated at their initiation and carry no mutations.
- **P53** is aberrant in 25-50% of cases but have no prognostic value.
- **Angiogenesis** is also active in CUP
- **Hypoxia-related proteins** are overexpressed in the nodal squamous head-neck subset and are associated with adverse prognosis.
- **EMT** is infrequently seen in a heterogeneous population of CUP tumours, however it carries significant adverse impact on patients outcome.
- The major **intracellular AKT** and **MAPK axes** are frequently activated in CUP and carry adverse prognostic significance.

(II) TARGETING TREATMENT IN CUP [PART II]

- **Bevacizumab** and **erlotinib** combinations have **moderate activity**
- Several subsets of CUP patients seem to benefit from **specific treatment** i.e. Colon - profile CUP
- **Randomized studies** are already ongoing to compare specific versus empirical treatment
- Studies on **novel agents** targeting signaling pathways are warranted
- **Pharmacogenomics** in CUP show promising results