2015 Annual Meeting

Grow Your Mind

March 21-27
Hynes Convention Center | Boston, MA

USCAP
Pathology Excellence Through Education
Please Silence Your Cell Phones

Thank You
Cancer of Unknown Primary (CUP)

Overview, Morphological, Immunophenotypic and Molecular Profiling

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The Online CME/Evaluations/SAM claim process will only be available on the USCAP website until **October 2, 2015**.

*No claims can be processed after that date!*

After October 2, 2015 you will **NOT** be able to obtain any CME or SAMs credits for attending this meeting.
**Challenging Entities** Between Neuropathology and General Pathology

- **VARIED INCLUSION CRITERIA:**
  Cancer of **uncertain** primary or **suspected** primary, primary finally **identified** (during life or at autopsy) and primary never identified

- **VARIED PRESENTATIONS**

- Historically viewed as a distinct form of cancer!

**COLLECTION OF SCENARIOS → CANCERS** of unknown primary

- **How to validate assays when the primary location by definition can never be verified!!!!**

More powerful molecular genetic tools → **Increasing therapeutic opportunities**
Definition of Cancer of Unknown Primary

1. Generally no prior history of malignancy
2. Histology confirmed metastasis, but no detected origin
3. Detailed medical history, complete physical (H&N, rectal, pelvic, breast)
4. Lab tests (CBC, chemistry, UA, stool occult blood) negative
5. Histopathology review and immunohistochemistry not definitive
6. Imaging (CXR, CT of the thorax, abdomen and pelvis, MRI)
   Gender specific testing: mammography, transvaginal ultrasound, prostate ultrasound

In some cases:
   Radio-metabolic techniques; PET-scan (cervical carcinoma)
   (Guided by the above): endoscopies – cystoscopy, laryngoscopy, bronchoscopy, gastroscopy, colonoscopy
Epidemiology: Cancers of Unknown Primary

31,000 CUP in the US last year

Up to 5% of all patients diagnosed with cancers. MAY BE HIGHER – uncertain cases

Up to 15% of patients with brain mets have unknown primary (many turn out to arise from the lung)

Median age: 60 years old  Male slightly > Female

Median survival: 6-10 months

Not uncommon – not rare scenario!

Mayor Thomas Menino
Boston mayor from 1993-2014
Important Issues Relevant to Cancer of Unknown Primary

Aggressive phenotype (occult spread)

Rapidly invasive with early dissemination, provocative ideas

Unpredictable pattern of metastatic spread (go to places known primaries do not), e.g. occult pancreatic cancer will spread to lungs and bones

Lost or never had a clear identity
## Metastases of Known Primary to Brain

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>5-yr cumulative incidence(^1) (\text{Netherlands Registry})</th>
<th>Incidence proportion (MDCSS 1973-2001)(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>17.0%</td>
<td>19.9%</td>
</tr>
<tr>
<td>Renal</td>
<td>10.5%</td>
<td>6.5%</td>
</tr>
<tr>
<td>Melanoma</td>
<td>8.0%</td>
<td>6.9%</td>
</tr>
<tr>
<td>Breast</td>
<td>5.2%</td>
<td>5.1%</td>
</tr>
<tr>
<td>Colorectal</td>
<td>1.4%</td>
<td>1.8%</td>
</tr>
</tbody>
</table>

- Usually first spread to lung and then to brain (symptomatic)
- Except posterior fossa (often bypass lungs) – pelvic/renal and GI
- MCA territory in cortex-white matter junction (narrow, branching vessels); 15% PF

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Eight General Brain Metastasis Scenarios

1° vs 2°

Any scenario could be a CUP
15% of all brain mets are CUP

Solitary Brain Met: Breast, Colon, RCC

Multiple Brain Mets: Lung, Melanoma
1st step: CNS primary versus metastatic disease

GFAP or OLIG2

Cam5.2  MelanA  CD45/LCA  OCT-4

CK7/20
Lineage restricted TFs
TTF1
PAX2/8
CDX2
GATA3
p63

Gauri Varadhachary Clin Cancer Res 2013;19:4027-4033
Primary CNS tumor?

10% of glioblastoma are **multicentric**
10% of people w/ a prior diagnosis of peripheral malignancy actually have a GBM

- Typical features
- Invasive border

AE1/AE3
Cam5.2 (Ck8/18)
then CK7, CK20 and CK5/6

S-100
MelanA/MART1
HMB45
MITF

GFAP/OLIG2/SOX2

Epithelioid Glioblastoma

GFAP
OLIG2
SOX2
High-grade de-differentiated glioma (GFAP negative/Olig2 negative)

Sox2 – CNS progenitor cells
Dura or Intraventricular Masses

Meningioma

Atypical Regions

Anaplastic regions

Use aCGH to confirm meningioma identity

Not as helpful for GBM

Abedalthagafi, Bi et al., Cancer Genetics, in press
CUP – Generally Epithelial Tumors

Papillary Craniopharyngioma

Mutation specific antibodies
BRAF V600E (VE1)

Suprasellar region

Adamantinomatous Craniopharyngioma – Beta-catenin (CTNNB1) mutations

Brastianos et al., Nature Genetics 2014
Papillary Tumor of the Pineal Region

Posterior 3rd ventricular region
Signet ring cells, clear cells, pseudorosettes, true rosettes and tubules

Not Metastatic Papillary Carcinoma of Unknown Primary!
Not ependymoma and not choroid plexus tumors.

Like uveal melanoma frequently have GNAQ and GNA11 mutations

Primary melanocytic lesions of the CNS
- Melanocytoma
- Melanoma
- Melanosis
- Melanomatosis

S100+
MelanA/Mart1+
HMB45+

BRAF V600E

Like uveal melanoma frequently have GNAQ and GNA11 mutations

Neurocutaneous Melanosis

Murali et al., Acta Neuropath 2012
Küsters-Vandevelde HV et al., Acta Neuropath 2010
Paraganglioma

Chromogranin A

Germ cell tumors – Oct4/Nanog/Sox2
AFP, B-HCG, PLAP, c-KIT, CD30 (serum tests)

Lymphoma – CD45, CD3, CD20, etc...
Chordoma – Brachyury
Next: where is the primary site?

History
Radiology
Prior biopsy
Compare morphology to primary

Morphology clues and immunohistochemistry

Pathologists guide clinical thinking, work up and management

Favorable
(Subtype specific Rx)

Unfavorable
(NO-subtype specific Rx)

CUP: 20% 80%

EXCLUDE chemosensitive and potentially curable tumors
(i.e. lymphomas and germ cell tumors)
Good response cancers: breast, ovary, small cell lung (plus actionable lesions)
Poorly-differentiated Non-Small Cell Lung Carcinoma

Discriminate ACA from SCC (predicting response) → CK7/20, TTF1, napsin A vs. CK5/6 and p63
Metastatic Small Cell Lung Carcinoma

Combined neuroendocrine and primitive epithelium

1. Rule out CNS primary (PNET, poor-differentiated glioblastoma)
2. Lung vs. extrapulmonary (TTF1)

Good initial response to therapy
Rely heavily on lineage restricted markers such as TTF1 to assign primary location.

PAX8: Renal and Müllerian origin
Metastatic Clear Cell Renal Cell Carcinoma

CD10/CALLA and PAX2
Metastatic Sarcomatoid Renal Cell Carcinoma

PAX8
Metastatic Endometrioid Carcinoma

Faint, focal +

CK7+/CK20-/TTF1-/CDX2-/PAX8+
Important theme:
Discriminating Primary Brain Tumor
From Metastatic Tumor
Metastatic Breast Carcinoma

Diagnostic markers:
- CK7+
- CK20-
- TTF1 –
- **GATA3**+
- GCDFP-15
- Mammaglobin+

Prognostic/
Therapeutic markers:
- ER
- PR
- HER2

GATA3- can be positive in 80% of urothelial carcinoma – even sarcomatoid
Metastatic Alveolar Soft Part Sarcoma

Nests of large epithelioid cells, granular cytoplasm and crystalline cytoplasmic inclusions

3% of sarcomas have brain mets

Most common sarcomas: ASPS and Osteosarcoma

Chou YS, et al., J Surg Onc. 2011
Histology/Morphology of CUP

- **50%** well and moderately-differentiated adenocarcinoma
- **30%** poorly differentiated carcinoma (including adenocarcinoma)
- **15%** squamous cell carcinoma
- **5%** are undifferentiated malignant neoplasms (unspecified carcinomas, neuroendocrine, lymphomas, germ cell tumors, melanomas, sarcomas)

**In brain CUP, the % of poor-differentiated adenocarcinomas is higher**

Morphology (limited) + IHC
Gastrointestinal Lineage: CDX2
Brain Met CUP: IHC → Upper GI?

Maintain signature

- TTF1
- CK7
- CK20
- CDX2
Dx: Poorly differentiated malignant neoplasm

Do not maintain IHC signature of primary

Tissue preservation
Molecular Profiling of CUP

**GOAL:** Find predictive markers of response

- **Tissue of Origin (ToO) expression profiling (miRNA and mRNA)**
  - Discriminate >40 types of tumors
  - 85% diagnostic accuracy for mets with known primary
    - 65-75% diagnostic accuracy in ‘true’ CUP
  - Challenge has been how to **validate assays** when the primary cancer entity by definition can’t be verified
    - How to compare value against value of IHC
    - How to show impact on patient outcomes

- **Sequencing – Neoplasms of Unknown Target (NUT)**
Integrating IHC and ToO molecular profiling testing (poorly differentiated or undifferentiated) difficult-to-diagnose cancers.

H&E morphologic evaluation shows a poorly differentiated or undifferentiated cancer; clinical presentation not helpful; limited biopsy with difficult access to more tissue

- Keep tissue (2–3 cellular slides) aside for potential ToO profiling
- Proceed with a few (6-7) pertinent IHCs based on morphology

Poorly Differentiated Cancers

- No result with IHC: proceed to ToO MP instead of additional IHC

- No result with MP: consider next-generation sequencing
- Additional directed IHC to seek concordance with MP
FOR CNS CUP
most arise from the LUNG

Clinical and Histology Studies
- Le Chevalier et al., Cancer 1985
- Lagerwaard et al., Int J. Oncol Biol Physa, 1999
- Polyzoidis et al., Cancer Treatment Reviews, 2005
- Mavrakis et al., Neurology 2005

qRT-PCR miRNA ToO test
- Mueller et al., The Oncologist 2011

53 bona fide cases of brain mets with no primary

<table>
<thead>
<tr>
<th>Site of Origin</th>
<th># (of 53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>24</td>
</tr>
<tr>
<td>Head/Neck</td>
<td>10</td>
</tr>
<tr>
<td>Renal</td>
<td>4</td>
</tr>
<tr>
<td>Breast</td>
<td>4</td>
</tr>
<tr>
<td>Thyroid</td>
<td>3</td>
</tr>
<tr>
<td>Ovary</td>
<td>3</td>
</tr>
<tr>
<td>Colon</td>
<td>3</td>
</tr>
<tr>
<td>Melanoma</td>
<td>2</td>
</tr>
</tbody>
</table>

BAD NEWS and GOOD NEWS
Good news: A lot of information about drivers in lung cancer and some therapeutic successes!

Histology-based classification

- Adenocarcinoma: 62%
- Squamous Cell: 20%
- Small cell: 13%
- Carcinoid/ Large Cell/ Large Cell Neuroendocrine: 5%

Driver Mutations

- Adenocarcinoma:
  - Not defined
  - Double Mt
  - KRAS
  - EGFR
  - ALK
  - ROS1
  - MET
  - BRAF
  - NRAS

- Squamous Cell:
  - Not defined
  - FGFR1
  - KRAS
  - EGFR
  - DDR2
  - PIK3CA
  - BRAF

- Small Cell:
  - Not defined
  - PIK3CA
Only 10/200 (5%) were from brain mets.

- 96% had a genetic alteration (GA)
- Median of 4.2 GA’s per case
- 85% had 1 or more potentially targetable GA
- 79% of ACUP’s had a GA in an RTK
- Only 39% of Non-ACUPS’s had a GA in an RTK

### Table 2. Genomic Alterations in 200 Cases of Carcinoma of Unknown Primary Origin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>125 Adenocarcinoma (n = 125)</th>
<th>75 Not Adenocarcinoma (n = 75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total GAs, No.</td>
<td>494</td>
<td>347</td>
</tr>
<tr>
<td>GAs per sample, mean</td>
<td>4.0 (2.1)</td>
<td>4.6 (3.7)</td>
</tr>
<tr>
<td>Base substitutions, No. (%)</td>
<td>250 (51)</td>
<td>151 (44)</td>
</tr>
<tr>
<td>Indels, No. (%)</td>
<td>74 (15)</td>
<td>66 (19)</td>
</tr>
<tr>
<td>Amplifications, No. (%)</td>
<td>119 (24)</td>
<td>98 (28)</td>
</tr>
<tr>
<td>Homozygous deletions, No. (%)</td>
<td>39 (8)</td>
<td>27 (8)</td>
</tr>
<tr>
<td>Rearrangements/fusions, No. (%)</td>
<td>12 (2)</td>
<td>5 (1)</td>
</tr>
<tr>
<td>Samples with no GA detected, No.</td>
<td>0</td>
<td>8</td>
</tr>
</tbody>
</table>

Abbreviations: ACUP, adenocarcinoma of unknown primary site; GA, genomic alteration; indel, short insertion or deletion.

236 cancer genes; 19 genes for rearrangements

Jeff Ross et al., JAMA Oncology, 2015 Foundation Medicine
Frequency of Alterations in Key Genes

Complex genetic profiles → definitely NOT ONE DISEASE

Jeff Ross et al., JAMA Oncology, 2015
Foundation Medicine
## Actionable Genomic Alterations
Associated With Approved Targeted Therapies on the Market

<table>
<thead>
<tr>
<th>Genomic Alteration</th>
<th>ACUP</th>
<th>Non-ACUP</th>
<th>Total CUP</th>
<th>Associated Targeted Therapies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ellelalteration</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>Erlotinib, afatinib, gefitinib, lapatinib, cetuximab, panitumumab</td>
</tr>
<tr>
<td>ERBB2 amplification</td>
<td>4</td>
<td>2</td>
<td>6</td>
<td>Trastuzumab, lapatinib, pertuzumab, trastuzumab-DM1, afatinib</td>
</tr>
<tr>
<td>BRAF substitution</td>
<td>8</td>
<td>3</td>
<td>11</td>
<td>Vemurafenib, dabrafenib, regorafenib, sorafenib, trametinib</td>
</tr>
<tr>
<td>ALK substitution</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>Crizotinib, ceritinib</td>
</tr>
<tr>
<td>RET fus/substitution</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>Cabozantinib, ponatinib, sorafenib, sunitinib, vandetanib, regorafenib</td>
</tr>
</tbody>
</table>

~40 patients could be offered approved targeted therapies

Jeff Ross et al., JAMA Oncology, 2015
Foundation Medicine
Many could be directed to a clinical trial

| Alterations Associated With Active Clinical Trials for Novel Targeted Therapies |
|-----------------------------|------|---|------------------|
| MET amplification           | 2    | 3 | 5 Crizotinib, cabozantinib, agents in late-stage trials |
| ERBB2 substitution          | 8    | 1 | 9 Lapatinib, afatinib, pertuzumab, trastuzumab, trastuzumab-DM1, neratinib (late-stage trials) |
| KRAS substitution           | 31   | 9 | 40 Trametinib     |
| CDKN2A loss, substitution, truncation | 23   | 14 | 37 MDM2 inhibitors, CDK4/6 inhibitors |
| MCL1 amplification         | 13   | 6 | 29 CDK inhibitors, sorafenib, antitubulins |
| PIK3CA substitution, amplification | 10   | 7  | 17 Temsirolimus, everolimus, novel mTOR inhibitors (clinical trials), novel PI3K inhibitors (clinical trials), novel dual PI3K/mTOR inhibitors (clinical trials) |
| PTEN loss, substitution, truncation | 8    | 6 | 14 Temsirolimus, everolimus, novel mTOR inhibitors (clinical trials), novel PI3K inhibitors (clinical trials), novel dual PI3K/mTOR inhibitors (clinical trials) |
| STK11 truncation           | 7    | 6 | 13 Everolimus, temsirolimus |
| ATM substitution, truncation | 7    | 0 | 7 DNA-PKcs inhibitors, PARP inhibitors |
| NF1 loss, truncation       | 6    | 0 | 6 Temsirolimus, everolimus, novel mTOR inhibitors, trametinib |
| BRCA2 substitution, truncation | 6    | 5 | 11 PARP inhibitors |
| NOTCH1 substitution, truncation | 0    | 5 | 5 γ Secretase inhibitors |
| CCND2 amplification        | 0    | 4 | 4 CDK inhibitors |
| FGFR1 substitution, amplification, fusion | 4    | 0 | 4 Pazopanib, ponatinib |
| FGFR2 substitution, fusion | 4    | 0 | 4 Pazopanib, ponatinib |
| RICTOR amplification       | 5    | 7 | 12 Temsirolimus, everolimus, novel mTOR inhibitors |
| ROS1 fusion                | 1    | 0 | 1 Crizotinib, ceritinib |
Durable Response to Crizotinib in a MET-Amplified CUP Metastatic to Brain

1. 59yoF seizure (single brain met) in 5/2012
2. Mid-abdominal mass found on CT
3. Pathology: CK20-, p63-, S100-
4. 4 cycles of carboplatin and docetaxel
5. Sept 2012 Progression
6. Genomic profiling: mutant KRAS & P53
7. October 2012: Crizotinib 250mg bid PO
8. Stable disease out to 3 years

Approach is primary site agnostic
MET AMP and response seen in Lung ACA and SCC, Gastric and Esophageal CA, GBM

Palma et al., Case Rep Oncol 2014;7:503-508
Ross et al., JAMA Oncology, 2015
Do people benefit from being treated with a drug that specifically targets that variation than from being treated with drugs that do not?

**GENOMICALLY GUIDED CLINICAL TRIALS for patients with METASTATIC CANCER**

- **NCI-MPACT**: Molecular Profiling-Based Assignment of Cancer Therapy for Patients With Advanced Solid Tumors (phase 2), March 2013 to March 2017

  20 gene panel
  TMZ, Carboplatin, Everolimus (mTOR), Trametinib (MEK), ABT-888 (PARP), MK1775 (Wee1)

- **IMPACT 2**: Initiative for Molecular Profiling and Advanced Cancer Therapy Randomized Study Evaluating Molecular Profiling and Targeted Agents in Metastatic Cancer (phase 2) started recruiting to May 2019.

  MD Anderson – Foundation Medicine
Cancer mutations not distributed uniformly along genome.

Distribution is explained by chromatin accessibility which is associated with cell-type/site of origin.

Determine ToO based on the density and distribution of mutations along genome.

Future methodology?
Multiplexed Protein Analyses for theranostics

Small cell number (FNAs)
- Lineage/Tissue of Origin
- Pathway activation to guide treatment decisions

Ullal et al., Science Translational Medicine, 2014
Local lessons from genomic characterization of brain metastases

Whole exome sequencing of **100 trios of matched normal, primary and brain met**

Branched evolution and metastasis-specific mutations

Priscilla Brastianos (MGH), Scott Carter (Broad), Gad Getz (Broad), Bill Hahn (DFCI)
Brain metastases harbor clinically actionable mutations not detected in primary biopsies.
Brain metastases harbor clinically actionable mutations not detected in primary biopsies.

Priscilla Brastianos, Scott Carter, Gad Getz, Bill Hahn
Primary sites and mets in the brain share a common ancestor but evolve independently (branched evolution)

In >50% of cases - clinically actionable alterations in the brain met are absent in the primary (supporting the need to biopsy brain mets when possible)

Brain mets were similar to one another but different than mets outside the brain

Priscilla Brastianos, Scott Carter, Gad Getz, Bill Hahn
Summary

• CancerS of unknown primary (CUP) are a **challenging collection** of tumor types

• Many brain CUP arise from the **lung**

• Careful morphological examination is indispensable with IHC as the backbone

• Improved IHC, molecular testing and imaging enhance the ability to determine primary site

• Molecular genetic testing offers the hope of identifying **actionable mutations**

• Management of unknown primary and known primaries could become more similar, more personalized

• **Clinical trial testing** is essential to determine the best ways to use these technologies
Thank You!

Please go to the USCAP website to complete your Evaluation of the course and claim CME and/or SAMs Credits.
“And that is what happens when we resist change.”
Prevalence

Site of end stage failure of systemic therapies, BBB safe Haven

ROLE OF Neuropathologists

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<td>3. Ependymal tumours</td>
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<td>5. Other neuroepithelial tumours</td>
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<td>15. Metastatic tumours of the CNS</td>
<td>247</td>
</tr>
</tbody>
</table>

2007

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Metastatic Tumours of the CNS

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2015 Annual Meeting
What do we know? - and - What’s worth knowing?

Known knowns e.g. TTF1+ met from lung

Known unknowns e.g. IHC ? CUP

Unknown knowns e.g. genomic profiling (knowables)

CUP and/or NUT (Neoplasm of Unknown Target)