End of Life In-patient Hospice and Rapid Autopsy to Study Tumor Heterogeneity in Lung Cancer

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I have no conflict of interest to disclose
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Outline of Talk

Tumor heterogeneity - Science and Controversies

A unique case report of unprecedented heterogeneity (the benefit of sequential biopsy protocols)

A review of a couple of published rapid autopsy series

Ethics guidelines for conducting such studies

Thoracic Malignancies Rapid Autopsy at the NIH Clinical Center.
validation that the mutant genes are expressed and have altered function. On top of this, the authors show widespread alterations in the total number of chromosomes in the tumor cells (aneuploidy) and detect many allelic imbalances at the chromosome level, in which one allele of a gene pair is lost. These imbalances can be due to chromosome loss or gene imprinting and may alter gene expression.

Another key finding is that different regions of the tumor have different mutations in the very same genes (so-called convergent evolution), including in SETD2, PTEN, and KDM5C, which under-

The news for personalized-medicine advocates is not all bad. The findings confirm that the genetic lesions that are found in the original tumor cells, the trunk of the evolutionary tree, are consistently expressed (e.g., the von Hippel-Lindau gene in renal-cell cancer). In addition, given that the tumor will do whatever is necessary to activate certain genes and inactivate others, the genes that are affected by convergent evolution may be suitable targets for functional inhibition or restoration. However, the simple view of directing therapy on the basis of genetic tumor markers is probably too simple.
Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing

Tumor biopsy samples analyzed from 4 consecutive patients with metastatic RCC

Whole exome sequencing performed on different regions of the specimens from patients 1 and 2—paired-end reads of 72 bp and 75 bp on Illumina Genome analyzer IIx and Hiseq platforms.

SNP array analysis on Illumina Omni2.5 (copy number)

mRNA expression profiling on Affymetrix Gene 1.0 arrays

Gerlinger et al., NEJM 2012
Biopsy and Treatment Timelines for the Four Patients.

Gerlinger et al., NEJM 2012
Samples for intratumor and intertumor heterogeneity - Patient 1

Gerlinger et al., NEJM 2012
Grey: mutation detected
Blue: NO mutation

Gerlinger et.al., NEJM 2012
Genetic Intratumor Heterogeneity and Phylogeny in Patient 2.
(119 somatic mutations detected)

Gerlinger et al., NEJM 2012
Mutation history and tumor’s past, present, and future

Darryl Shibata review, Science, 2012
Tumor evolution

LINEAR
- Founding clone
- Selection of more fitter clones

BRANCHED
- Multiple subclones
- Present simultaneously
  COMPLEX HETEROGENEOUS TUMOR
LEVEL 1: Trunk-driver mutations
Branches- neutral mutations

LEVEL 2: Trunk-driver mutations
Branches- neutral or additional driver mutations – convergent phenotypes.

(distinct mutations in SETD2 and PTEN in different regions of Renal cancer- converge on same pathway)

LEVEL 3: Level 1 and Level 2 events AND neutral mutations on trunk or branches that become drivers under selection pressure (T790M, ALK acquired resistance etc.)

Yap, Swanton et.al., Sci.Transl. Med. 4:1-4
A trunk branch model of intratumor heterogeneity
(\textit{Clonal architecture as a biomarker})

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“Palm-tree like” tumors- Ubiquitous genetic events \rightarrow heterogeneous genetic events
\textbf{Good prognosis!}

“Baobab tree-like” tumors – Heterogeneous genetic events \rightarrow ubiquitous events
\textbf{Bad prognosis!}
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Yap, Swanton et.al., Sci.Transl. Med. 4:1-4
that a meiosis I-specific factor from budding yeast, monoplin, generates kinetochores with more microtubule-binding elements and greater strength. These findings provide direct evidence that sister kinetochore fusion underlies the cosegregation of sister chromatids during meiosis I.

REFERENCES AND NOTES

9. Materials and methods are available as supplementary materials on Science Online.

LUNG CANCER EVOLUTION

Spatial and temporal diversity in genomic instability processes defines lung cancer evolution

Elza C. de Bruin,2,6 Nicholas McGranahan,2,3,4 Richard Mitter,2,6 Max Salm,2,6 David C. Wedge,2,6 Lucy Yates,4,5,† Mariam Jamal-Hanjani,1,† Seema Shafi,1 Nirupa Murugaesu,1 Andrew J. Rowan,2 Eva Grönroos,2 Madiha A. Muhammad,1 Stuart Horswell,2 Marco Gerlinger,2 Ignacio Varea,6 David Jones,4 John Marshall,4 Thierry Voet,6,7 Peter Van Loo,6,7 Doris M. Rassl,8 Robert C. Rintoul,8 Sam M. Janes,9 Slow-Ming Lee,1,10 Martin Forster,1,10 Tanya Ahmad,10 David Lawrence,10 Mary Falzon,10 Arigo Capitanio,10 Timothy T. Harkins,11 Clarence C. Lee,11 Warren Tom,11 Enoch Teefel,11 Sham-Ching Chen,11 Sharman Begum,2 Adam Rabinowitz,2 Benjamin Phillimore,2 Bradley Spencer-Dene,2 Gordon Stamp,2 Zoltan Szallas,12,13 Nik Matthews,2 Aengus Stewart,2 Peter Campbell,4 Charles Swanton1,2,†

WES and/or WGS on 25 spatially distinct regions

7 localized NSCLC tumor samples (surgical specimens)

1/3rd of all non-silent mutations were present in at least one region, but not other regions.

Branched evolution- key driver mutations present both before, and even after subclonal diversification.
Multiregion WES on 11 lung adenocarcinomas (48 tumor regions) (median depth- 277x)

20 out of 21 known cancer genes in all regions of individual tumors. 76% of all mutations were present in all regions.

3 patients with a larger fraction of subclonal population developed recurrent disease after surgery- intratumoral heterogeneity as a biomarker of poor prognosis.
Phylogenetic tree showing the clonal evolution of lung cancer

- Smoking-related genomic events occur quite early.
- Prolonged latency from the first driver events to clinical presentation.
- Even in presence of continued smoking, carcinogen related genomic events decreased over time.
- Increase in mutagenesis related to activation of a class of enzyme Apolipoprotein B mRNA editing-Enzyme-catalytic, polypeptide-like Cytidine deaminase (APOBEC)

Sequential biopsies to correlate proteo-genomic alterations with tumor heterogeneity and response to targeted treatment

(a Case study of an African American male never smoker)

<table>
<thead>
<tr>
<th>Event</th>
<th>Date</th>
<th>Notes</th>
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<tr>
<td>Dx</td>
<td>Nov 2007</td>
<td></td>
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<tr>
<td>Lung Bx</td>
<td>Oct 2008</td>
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<tr>
<td>LN Bx</td>
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<tr>
<td>Lung wedge</td>
<td>May 2011</td>
<td></td>
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<td></td>
<td>Dec 2011</td>
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<tr>
<td>LN Bx</td>
<td>Dec 2013</td>
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</tbody>
</table>

Whole genome sequencing

Ion torrent validation

Mass spectrometry: Proteome and phosphorylation
Less than 1% of SNVs and less than 2% of Indels are common between the two metastatic sites.

SNVs:
- Lung: 17,387
- Lymph node: 13,896
- Overlapping: 117

Indels:
- Lung: 678
- Lymph node: 404
- Overlapping: 7
Ch. 17 region with high copy number amplifications in lung tumor (red) and metastatic lymph node (blue) as accessed on WGS by CNV-Seq
ERBB2 L869R mutation is only present in the lymph node metastasis

Similar to EGFR L861R
Commonality in genomic alteration affecting a key hallmark - proliferation

**LUNG**

- **ERBB2**
  - **MAPK**
  - **AKT**
  - **mTOR**
- **KRAS**
- **PI3K**
- **CDKN2A**
- **CCND1**
- **CCNE1**
- **CDK12- G879V**
- **Inactivation** → **Unstable genome**

**Lymph Node**

- **ERBB2-L869R**
- **TP53- Del E339-F341**
- **KRAS**
- **PI3K**
- **MAPK**
- **AKT**
- **mTOR**
- **p21**
- **CCNE1**
- **No mutation in CDK12**
Rapid ("warm") autopsies to obtain tumor and normal tissues

- All possible areas of disease can be sampled
- Adjacent "normal" tissue can be collected
- Cell lines and xenografts can be generated
- Tissue can be sampled and stored to preserve quality
- RNA and Protein analyses can be performed
Rapid (“Warm”) Autopsy Study for Procurement of Metastatic Prostate Cancer


“Warm” autopsy or tissue procurement program at the University of Michigan.

The term “warm” derives from the short interval between time of death and acquisition of tissue samples during the autopsy. The primary goal of this program was to develop a tumor donor program that would allow men with metastatic hormone-refractory prostate cancer to agree to an immediate autopsy shortly after death. This program would serve as an

University of Michigan: Rapid Autopsy Study of Metastatic Prostate Cancer

- Median time to autopsy: 2.8 hours
- Delay beyond 2 hours was always because of transportation of the body from home or hospice to the hospital.
Tissue types involved in hormone refractory prostate cancer

Bony sites involved in hormone refractory prostate cancer

$n=14$ cases

A major goal of the Michigan rapid autopsy program:

Obtain high quality tumor tissue for prostate cancer research

- Bulky tumor metastases harvested and care to remove areas of necrosis.
- Good tumor histology
- Immunoreactivity for PSA
- Ability to develop xenografts
Warm autopsy program at the Univ. of Pittsburgh: Interstitial Lung Disease

• Lesson 1 - listen to the patient
• Lesson 2 - go to the people who have experience
• Lesson 3 - family members are often your best allies
• Lesson 4 - respect your patient’s last wishes
• Lesson 5 - allow space for patient leadership
Ethics guidelines for research with the recently dead

Rebecca D Pentz, Cynthia B Cohen, Mark Wicclair, Michael A DeVita, Anne Lederman Flamm, Stuart J Youngner, Ann B Hamric, Mary S McCabe, Jacqueline J Glover, Winona J Kittiko, Kathy Kinlaw, James Keller, Adrienne Asch, John J Kavanagh & Wadih Arap

Consensus Panel on Research with the Recently Dead (CPRRD)
CPRRD guidelines Nature Medicine Vol 11:1145-49

1. Receive scientific and ethical review and oversight

2. Involve the community of potential research subjects

3. Coordinate with organ procurement organizations

4. Not conflict with organ donation or required autopsy

5. Use procedures respectful of the dead

6. Be restricted to one procedure per day

7. Preferably be authorized by first person consent, though general advance directives and surrogate consent are acceptable

8. Protect confidentiality

9. Not impose costs on subject’ estates or next of kin and not involve payment

10. Clearly explain ultimate disposition of the body.
Metastatic castration-resistant prostate cancer reveals intrapatient similarity and interpatient heterogeneity of therapeutic kinase targets

Justin M. Drakea, Nicholas A. Grahamb,c, John K. Leed,e, Tanya Stoyanovaa, Claire M. Faltermeierf, Sudha Sudd, Björn Titzb,c, Jiaoti Huangg,h,i, Kenneth J. Pientaf,j, Thomas G. Graeberb,c,g,k,l, and Owen N. Wittesta,c,l,m,t

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Contributed by Owen N. Witte, October 23, 2013 (sent for review September 5, 2013)

16 metastatic CRPC samples from 13 different patients obtained at rapid autopsy (Michigan program)

Quantitative phosphoproteomics (mass spec, antibody arrays, Western blots) Evaluated active kinases
Anatomical location and histological characterization of metastatic CRPC samples used for phosphoproteomics.
Kinase activation patterns confirm intrapatient similarity across multiple, anatomically distinct metastases.

Drake J M et al. PNAS 2013;110:E4762-E4769
Heterogeneity of breast cancer metastasis

Primary tumor:
- ER: 80%+
- PR: 30%+
- % Methylation:
  - RASSF1A: 32
  - CYCLIN D2: 0.8
  - RARB: 7
  - APC1: 0.8

Liver Met:
- ER: Negative
- PR: Negative
- % Methylation:
  - RASSF1A: 26-50
  - CYCLIN D2: 2-10
  - RARB: 9-10
  - APC1: 10-20

Diaphragm Met:
- EGFR IHC: 1+
- MET IHC: 2+
- COX-2: Positive
- % Methylation:
  - RASSF1A: 95
  - CYCLIN D2: 11
  - APC1: 0
  - RARB: 12

Lymph node:
- EGFR IHC: 1+
- MET IHC: 2+

Primary tumor:
- EGFR IHC: 1+
- MET IHC: 1+
- COX-2: Negative
- % Methylation:
  - RASSF1A: 67
  - CYCLIN D2: 0
  - APC1: 6
  - RARB: 8

Liver Met:
- EGFR IHC: 1+
- MET IHC: 1+
- % Methylation:
  - RASSF1A: 0-72
  - CYCLIN D2: 7-12
  - APC1: 14-34
  - RARB: 12

Left Adrenal Met:
- EGFR IHC: 2+
- MET IHC: 1+
- COX-2: Negative
- % Methylation:
  - RASSF1A: 89
  - CYCLIN D2: 1
  - APC1: 0
  - RARB: 8

Bone Met:
- ER: Negative
- PR: Negative
- % Methylation:
  - RASSF1A: ~0
  - CYCLIN D2: 0
  - RARB: ~0
  - APC1: ~0

Omental Met:
- EGFR IHC: 1+
- MET IHC: 2+
- COX-2: Positive
- % Methylation:
  - RASSF1A: 69
  - CYCLIN D2: 6
  - APC1: 0
  - RARB: 7

Bone Met:
- EGFR IHC: 1+
- MET IHC: 1+
- COX-2: Negative
- % Methylation:
  - RASSF1A: 5
  - CYCLIN D2: 2
  - APC1: ~0
  - RARB: ~0
End-of-life in-patient hospice and rapid autopsy upon death (within three hours)
Collect multiple sites of disease and adjacent normal tissue
The patient, family and a comprehensive team

- Pain and palliative
- Social work
- Home hospice
- RAPID AUTOPSY
- Nurses
- Admission
- Physicians
- Pathologists
The promise of EGFR tyrosine kinase inhibitors (TKIs)

L858R
Erlotinib
6 weeks

And the inevitable problem.....

13 months
Influence of tumor heterogeneity on EGFR TKI resistance

Erlotinib

6 weeks

Resistance Mechanisms: A and "others"

Resistance Mechanism: A

What are Others??
End-of-life in-patient hospice and rapid autopsy protocol for thoracic malignancies (NSCLC, SCLC, TET, neuroendocrine, mesothelioma)

Hypothesis

Clonal evolution and selection of tumor cells can be assessed by examining genomic and proteomic alterations of tumor samples obtained from multiple sites of primary and metastatic sites
Primary objective

Procure tumor tissue from different sites shortly after death in order to study tumor heterogeneity - both intra tumor and between different metastatic sites Using integrated genomic and proteomic analysis.

Secondary objectives

-end of life inpatient hospice care
-compare genetic alterations of autopsied tissue with archival tissue
-compare genomic alterations in tumor tissue with those identified in isolated circulating tumor cells.
-generate cell lines and xenografts from isolated tumor tissue
study design

screening evaluation
- screening consent
- discussion of end-of-life directives
- discussion of DNR and limited treatment preferences
- history and physical examination
- social work screen
- pain and palliative care consult

Patient appropriate for inpatient hospice care at NIH

- consent to participate in study
- CT scan of neck, chest, abdomen
- identification of the patient’s next of kin
- designation of durable power of attorney
- completion of NIH advance directive for health care and medical research participation

Follow up visits q 2 weeks in OP12
Review of previously established advance directives

Study investigator estimates an expected survival of less than two weeks

Admission to 3 NW

Death of patient

Notification of next of kin
Obtain authorization for autopsy

Full Autopsy

Patient continues palliative care received at home or another institution

No

Yes
Proposed studies

Tissue obtained at rapid sterile autopsy (up to 10 sites each patient—preference to distant metastatic sites)

Formalin fixed
- Histology
- Generation of Tissue microarrays (TMAs)
- Immunohistochemistry

Flash frozen
- Whole exome sequencing (not more than 4 sites each patient)
- Transcriptome sequencing (RNA seq- up to 4 sites each patient)
- Comparative genomic hybridization (4 sites per patient)
- Proteomics studies (4-10 sites each patient)
  - proteome quantitation
  - Quantitation of phosphorylation (mass spectrometry, luminex assay, RPPA-reverse phase protein array)

Immediately dissociated (up to 3 sites each patient)
- Procedure for generation of cell lines from individual sites
Tissue collection from rapid autopsy

RA000 – Lung adenocarcinoma
**KRAS mutation**, MEK inhibitor $R_x$
Lung, Liver *(from home)*

RA002 – Mesothelioma
*(from ICU, Clinical Center)*

RA003 – Lung adenocarcinoma
**HER2 amplification**, Lapatinib $R_x$
Lung, Brain, Pleural fluid *(from home)*

RA004 – Lung adenocarcinoma
**KRAS mutation**, MEK inhibitor $R_x$
Lung, Lymph node, Liver, Kidney, Brain *(from 3NW, Clinical Center)*

RA005 – Lung adenocarcinoma
**EGFR mutation**, erlotinib $R_x$
Lung, Brain, Liver *(from 3NW, Clinical Center)*
Clinical Summary (RA005)

- 6/2009- 56yo male non-smoker with Stage IV lung adenocarcinoma s/p chemo referred to NIH
- 6/2009- started on sorafenib (brief holiday due to side effects)
- 2/2010- CT scan showed progression of disease; switched to erlotinib (presence of EGFR mutation)
- 3/2011- returned to NIH after care at WRNMMC and progression on erlotinib; started on pemetrexed and sirolimus
- 5/2011- returned to WRNMMC for care – again maintained on erlotinib or chemotherapy.
Clinical summary (RA005)

- 3/2013- Returned to NIH for Hsp90 trial
- 5/2013- stopped Hsp90i due to side effects
- 5/2013- Returned to WRNMMC and transferred to home hospice at some point, but maintained on erlotinib
- 6/12/2014- Transferred to NIH for rapid autopsy protocol.
- Wife signed protocol consent (no DPA- ethics involved)
- He expired on 6/13/2014 at 10:49 AM
- Autopsy initiated at 3.5 hours.
Lung – RA005 (R- 925g, L-1120g)
Lungs, all lobes (RA005)
RA005- Lung Adenocarcinoma- EGFR mutant

Cell line generated (RA005)
TMA construction to validate targets

Selection Of Tumor From Different Sites

Tissue Microarray Construction

Immunohistochemistry On Tissue Microarray

Proteo-genomics studies

Mass spectrometry
- Protein estimation
- Phosphoproteomics
  GUHA LAB

Simple western assays
- Target validation
- Pathway specific probes
  CPTR core facility

Genomics (NGS)
- Whole exome
- Transcriptome
  NCI Frederick core and Collaboration
  (Javed Khan, CCR)
Summary

Tumor heterogeneity – Science and Controversies
Branched tumor evolution – challenge for personalized medicine

A unique case report of unprecedented heterogeneity
(the benefit of sequential biopsy protocols)
Less than 1% similarity of sequential biopsies from metastatic sites

A review of a couple of published rapid autopsy series
Michigan, Hopkins, Pittsburgh programs. Striking intra-patient
similarity of metastatic sites and primary in prostate cancer

Ethics guidelines for conducting such studies
CPRRD guidelines

Thoracic Malignancies Rapid Autopsy at the NIH Clinical Center
5 rapid autopsies performed in less than a year
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Christopher Albanese, Georgetown Univ.

Intramural - NCI

David Schrump
Javed Khan
Stephen Hewitt
David Kleiner

Pain and Palliative Service

Social Work

3NW Nursing team

Admissions

3NW Nursing team