Phenotypic Diversity in the Evolution of Breast Cancer Metastasis

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Cancer is a Genomic Disease

Stratton Nature 2009
Cancer is a Genomic Disease

The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups

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The landscape of cancer genes and mutational processes in breast cancer.


The clonal and mutational evolution spectrum of primary triple-negative breast cancers

Sohrab P. Shah1,2,*, Andrew Roth1,2,*, Rodrigo Goya1,2,*, Aruha Ologe1,2,*, Gavin Ha1,5, Yongjun Zhao1,5, Giulia Turashvili1,6,*, Jaruel Dine1,6,*, Kane Tie1,6,*, Golzhereva Haffari1,5,*, Ali Bashashiti1,5,*, Leah M. Prentice1,5,*, Jawawder Khata1,5,*, Angela Burleigh1,5,*, Damian Yap1,5,*, Virginia Bernard1,6,*, Andrew McPherson1,5,*, Karrey Shumansky1,5,*, Anamarla Cresh1,5,*, Ryan Giulian1,5,*, Adeena Heravi-Moussavi1,5,*, Jamie Rosen1,5,*, Daniel Lass1,5,*, Isaac Billett1,5,*, Richard Varhey1,5,*, Angela Tam1,5,*, Norine Shahe1,5,*, Thomas Zeng1,5,*, Kevin Ma1,5,*, Simon K. Chan1,5,*, Malachi Griffith1,5,*, Annie Moradian1,5,*, S. W. Grace Cheng1,5,*, Gregg B. Martin1,5,*, Peter Watson1,5,*, Karen Gilm1,5,*, Reuben Chiu1,5,*, Suet-Fung Chinn1,5,*, Christina Curtis1,5,*, Oscar M. Rueda1,5,*, Paul D. Hamnan1,5,*, Sanabasavaraj Damra1,5,*, John Mackey1,5,*, Kelly Ho1,5,*, Timothy Harkine1,5,*, Yaelie Tadjikov1,5,*, Mahnaz Agajani1,5,*, Philippe Gavard1,5,*, Thea Thr1,5,*, Joseph F. Costello1,5,*, Imirradh M. Meyer1,5,*, Connie J. Eaves1,5,*, Wyeth W. Wasserman1,5,*, Steven Jones1,5,*, David Hunnam1,5,*, Matthew Hirst1,5,*, Carlos Caldas1,5,*, Marco A. Moreno1,5,*, & Samuel Aparicio1,5

Sequence analysis of mutations and translocations across breast cancer subtypes

Shantara Banerji1,2,*, Kristian Chubinskis1,2,*, Claudia Rangel-Escareno3,*, Kristin K. Brown4,*, Scott L. Carter1, Abbie M. Frederick1, Michael S. Lawrence1,*, Andrey Y. Ylivahkas4,*, Carrie Sougnez4,*, Lihua Zou5, Maria L. Cortez6, Juan C. Fernandez-Lopez6, Shuyong Peng6,*, Kristin G. Arnold6,*, Daniel Rudolf7,*, Veronica Bautista-Pita6,*, Fujilko Duke1,*, Joshua Franches6,*, Joseph Goodson8,*, Antonio Maffuz-Azizi1,*, Robert C. Ondrusek1,*, Melissa Perkins1,*, Ham H. Pho2,*, Valeria Quintanar-Jarado8,*, Alex H. Ramirez1,*, Rosa Rebollar-Vega1,*, Sergio Rodriguez-Cuevas1,*, Sandra L. Romero-Cordoba1,*, Steven E. Schumacher1,*, Nicolas Strankson1,*, Kristin M. Thompson1,*, Laura Urbe-Jigueras1,*, Jose Ravelo1,*, Ramon Benavides3,*, Mercedes Polaya1,*, Dennis C. Sgroi1,*, Andrea L. Richardson1,2,*, Gerardo Jimenez-Sanchez1,*, Eric S. Lander1,*, Steven G. Kustra1,*, Francesca B. de Leiva2,*, Dave A. Garraway1,*, Todd R. Golub1,4,*, Jorge Melendez-Zagalin3,*, Alex Tokar1,*, Guad Getz1,*, Alfredo Hidalgo-Miranda1,2, & Matthew Meyerson1,2,8,9

Whole-genome analysis informs breast cancer response to aromatase inhibition

Matthew J. Ellis1,2,*, Li Ding1,2,*, Dong Shon Lee3,4,*, Jingjin Liu4,*, Vera J. Suman5,*, John W. Wall6,*, Brian A. Van Tine5,*, Jeremy Hoog4,*, Rene J. Goldberg4,*, Theodore C. Goldstein4,*, Sum Ng4,*, Li Liu6,*, Robert Crowder6,*, Jacqueline Spolder6,*, Karla Balmum6,*, Jason Weber5,4,*, Ken Chen5,*, Daniel C. Roboldt4,*, Corazzia Kandora4,*, William S. Scherling6,*, Joshua E. McMichael4,*, Christopher A. Miller5,*, Charles La6,*, Christopher C. Harris5,*, Michael D. MacLellan4,*, Michael C. Wendl5,*, Katherine Delchamps4,*, Craig Alfred3,*, Laura Elman6,*, Gary Undseth1,*, Julie Margenthaler1,*, G. V. Baber4,*, F. Kelly Marcom4,*, J. M. Gnant4,*, Marilyn Leitch4,*, Kelly Hagi6,*, John Oben4,*, Yu Tan4,*, Christopher A. Mahery4,*, Luisela L. Fabian5,*, Robert S. Fulton1,*, Michelle Harrison4,*, Ben Chromick7,*, Felipe De4,*, Ryan Demeter6,*, Tamir L. Vicker1,*, Adnan Elhammami4,*, Heleen Pienzca-Worms8,9,10,11, Sandra McDonald2,*, Mark Watterson4,9,10,11,9,10,11,9,10,11,*, David J. Doyte1,*, David Ora1,*, Li-Wet Chung1,*, Ken Morse1,*, Timothy J. Ley1,*, David Pienzca-Worms8,9,10,11,*, Joshua M. Stuart1,*, Richard R. Wilson7,8,12,13,3,*, & Elaine R. Marth1,2
• ~3000 breast tumours
• mRNA expression, DNA copy number, exome seq, miRNA expression, DNA methylation, protein profiling….

......what else is there??
Tumour Evolution & Intratumour Heterogeneity

Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution
Soheir P. Shah1,2,4, Ryan D. Morin1,4, Jaswinder Khattra1, Leah Prentice1, Trevor Pugh1, Angela Burleigh1, Allen Delaney1, Karen Gelmon1, Ryan Gullany1, Janine Senz2, Christian Steidl1,2, Robert A. Holt2, Steven Jones1, Mark Sun1, Gillian Leung1, Richard Moore1, Tesa Severson1, Greg A. Taylor1, Andrew E. Teschendorff1, Kane Tse1, Giulina Turashvili1, Richard Varhol1, René L. Warren1, Peter Watson1, Yongjun Zhao1, Carlos Cadlais1, David Huntsman1,2, Martin Hirst1, Marco A. Marra1 & Samuel Aparicio1,2,5

The patterns and dynamics of genomic instability in metastatic pancreatic cancer
Peter J. Campbell1,2, Shinichi Yachida1,4, Laura J. Mudie1, Philip J. Stephens1, Erin D. Pleasance1, Lucy A. Stebbings1, Laura A. Morsberger1, Callie Lattime1, Stuart McLaren1, Meng-Lay Lin1, David J. McHride1, Ignacio Varela1, Serena A. Nik-Zainal1, Catherin Leno1, Mingming Jia1, Andrew Menzies1, Adam P. Butler1, Jon W. Teague1, Constance A. Griffith1, John Burton1, Harold Swerdlow2, Michael A. Quail1, Michael R. Stratton1,4, Christine Iacobuzio-Donahue1 & P. Andrew Futreal1

Distant metastasis occurs late during the genetic evolution of pancreatic cancer
Shinichi Yachida1,2, Sihn Jones1,2, Ivana Bovic2, Tibor Antal1,6, Rebecca Leary1, Baojun Fu1, Mihoko Kamiyama1, Ralph H. Hiruban1,3, James R. Eshleman1, Martin A. Nowak1,2, Victor E. Velculescu1, Kenneth W. Kinzler1, Bert Vogelstein1 & Christine A. Iacobuzio-Donahue1,2,5,6

Genome remodelling in a basal-like breast cancer metastasis and xenograft
Li Ding1,2, Matthew J. Ellis2,4, Shuqiang Li1, David E. Larson1, Ken Chen2, John W. Wallis1,2, Christopher C. Harris1, Michael D. McLellan1, Robert S. Fulton1,2, Lucinda L. Fulton1,2, Rachel M. Abbott1,2, Jeremy Hoog1, David J. Dooling1,2, Daniel C. Koboldt1, Heather Schmidt1, Joelle Kalicki1, Qunyun Zhang1,2, Lei Chen1, Ling Lin1, Michael C. Wendl1,2, Joshua F. McMichael1, Vincent J. Magrini1,2, Lisa Cook1, Sean D. McGrath1, Tammie L. Vickers1, Elizabeth Appelbaum1, Katherine DeSchryver1, Sherri Davies1, Therese Guinot1, Li Lin1, Robert Crowder1, Yu Tao1, Jacqueline E. Snider1, Scott M. Smith1, Adam F. Dukes1, Gabriel E. Sanderson1, Craig S. Pohl1, Kim D. Deleaunay1, Catrina C. Fronick1, Kimberly A. Pape1, Jerry S. Reed1, Jody S. Robinson1, Jennifer S. Hodgues1, William Schierding1, Nathaniel D. Dees1, Dong Shen1, Devin P. Locke1, Madeline E. Wiechert1, James M. Eldred1, Josh B. Peck1, Benjamin J. Oberfell1, Justin T. Lolofie1, Feiyi Du1, Amy E. Hawkins1, Michelle D. O’Laughlin1, Kelly E. Bernard1, Mark Cunningham1, Glendora Elliott1, Mark D. Mason1, Dominic M. Thompson Jr1, Jennifer L. Ivanovich1, Paul J. Goodfellow1, Charles M. Perou1, George M. Weinstock1,2, Rebecca Aft1, Mark Watson1, Timothy J. Ley1,3,4, Richard K. Wilson1,2,3,5 & Elaine R. Mardis1,2,5

The Life History of 21 Breast Cancers
Serena Nik-Zainal1,2, Peter Van Loon1,2,3,18, David C. Wedge1,2,4,19, Ludmil B. Alexandrov1, Christopher D. Greenman1,4,5, King Wai Lau1, Keirin Raine1, David Jones1, John Marshall1, Manasa Ramakrishna1, Adam Shlien1, Susanna L. Cooke1, Jonathan Horton1, Andrew Menzies1, Lucy A. Stebbings1, Catherine Lero1, Mingming Jia1, Richard Rance1, Laura J. Mudie1, Stephen J. Gamble1, Philip J. Stephens1, Stuart McLaren1, Patrick S. Tarpe1, Elli Papaemmanouil1, Helen R. Davies1, Ignacio Varela1, David J. McBride1, Graham R. Bignell1, Kenric Leung1, Adam P. Butler1, Jon W. Teague1, Sancha Martin1, Goran Jönsson1, Odetta Marian1, Sandrine Boyault1, Penelope Miron1, Aquila Fatima1, Anita Langerad1, Samuel A.J.R. Aparicio1,2,15, Andrew Tutt1, Aineita M. Siewertsv1, Åke Borg1, Gilles Thomas2, Anne Vincent Salomon1, Andrew L. Richardson1,15, Anne-Lise Berensen-Dal,1,10,18 P. Andrew Futreal1, Michael R. Stratton1, Peter J. Campbell1,2,3,15 & Breast Cancer Working Group of the International Cancer Genome Consortium

Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing

Tumour evolution inferred by single-cell sequencing
Nicholas Navin1,2, Jude Kendall1, Jennifer Troye1, Peter Andrews1, Linda Rodgers1, Jeanne McIndoo1, Kerry Cook1, Ayna Stupansky1, Dan Levy1, Elane Espostio1, Lakohtut Muttunwamy1, Ales Krasnov1, W. Richard McComb1, James Hicks1 & Michael Wigler1
The Evolution of Cancer Genomes

MRCA – Most Recent Common Ancestor

Molecular Evolution of Breast Cancer

Molecular Evolution of Breast Cancer

- HUT
- CCL
- ADH
- Low grade DCIS
- Tubular
- IDC
- ILC
- Mixed Ductal-Lobular
- ALH
- LCIS
- PLCIS
- High grade DCIS
Intratumour Morphological Heterogeneity

E-cadherin

Da Silva Am J Surg Path 2008
Intratumour Morphological Heterogeneity

DCIS

ILC

LCIS

E-cadherin

β-catenin

1p-, 3p-, 4q-, 5q+, 8q+, 10p+, 11p-

DCIS

normal

11q+, 11q-, 16p+

1q+, 16q-

6q-, 8p-

Ecad-

LCIS

17q+ 22+

ILC

DCIS?

22+

IDC
Intratumour Heterogeneity in Metastatic Progression

2002
- WLE
- XRT
- Hormonal Therapy

IDC NST, grade 2

ER+, PR+, HER2-

2007
- Ceased Hormonal Therapy

Mastectomy
IDC NST, grade 2

ER-, PR-, HER2-

2009
- Subtotal gastrectomy
- Metastasis in stomach wall

2010

ER-, PR-, HER2-

Intratumour Heterogeneity in Metastatic Progression

E-cadherin

E-cadherin

E-cadherin

E-cadherin

P120 catenin
Mechanisms Underlying Ductal to Lobular Transition

- Mixed ductal-lobular carcinomas – 3-5% of all breast cancers
- Areas of ductal and lobular differentiation
- Either or both component can be capable of disseminating
- Genomic analysis - components clonally related rather than ‘collision’ tumours
- ‘Model system’ to study mechanism of transition
  - related to dysfunction of E-cadherin
  - aberrant localisation (15/17 (88%) cases) rather than complete loss
  - Not driven by epithelial to mesenchymal transition (EMT)
  - Genomic/epigenetic?

‘Transition zone’
Breast Cancer Autopsy Series

- 197 cases from Royal Brisbane Hospital (1957-2007)
- 945 metastases
- Clinical data – ages; treatment; dates of diagnosis, surgery, post mortem; PM details
- Morphological, Immunophenotyping & Genomic analyses

- 60% of cases with gynaecological metastasis were <50 yrs
- Association between metastases to bone and brain
- Association between metastasis to liver following surgery
Phenotypic Changes with Metastatic Progression

- 55 cases of primary tumour and multiple matched metastases
- IHC for ER, PR, HER2, Ki67, p53, EGFR, c-kit

<table>
<thead>
<tr>
<th>Metastatic site (N = number)</th>
<th>Estrogen receptor (ER)</th>
<th>Progesterone receptor (PgR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary vs metastatic status</td>
<td>Significance†</td>
</tr>
<tr>
<td>Lung or pleura (47)</td>
<td>13 pos to neg, 0 neg to pos, 24 neg to neg, 10 pos to pos</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bone (38)</td>
<td>11 pos to neg, 2 neg to pos, 15 neg to neg, 10 pos to pos</td>
<td>0.022</td>
</tr>
<tr>
<td>Liver (32)</td>
<td>10 pos to neg, 0 neg to pos, 18 neg to neg, 4 pos to pos</td>
<td>0.002</td>
</tr>
<tr>
<td>Non-axillary nodes (24)</td>
<td>8 pos to neg, 0 neg to pos, 14 neg to neg, 2 pos to pos</td>
<td>0.008</td>
</tr>
</tbody>
</table>
Brain Metastasis

HER3 and downstream pathways are involved in colonization of brain metastases from breast cancer.
Age – young women
• Primary breast tumour occurred at median age of 52yrs
• Gynae metastases occurred at median age of 57yrs
• Time to diagnosis – median 5yrs (range 0-17yrs)

Histological type – enriched for ILC
• IDC 54% (28/52)
• ILC 43% (23/52)

Immunophenotype – ER+, HER2-
• 35/37 (95%) primary tumours ER+
• 75% primary BC and 92% matched gynae met  ER+ (Bigorie 2010 Cancer)
• All HER2 negative
• Metastases: 87% ER+, 0% HER2+
Metastasis to Gynaecological Organs

**Metastasis often widespread and extensive**

**Distribution:**
- 21/58 (36%) had metastases only to gynae
- 37/58 (64%) had other metastases
  - peritoneum/omentum/ascitic fluid (68%),
  - abdomen/gastrointestinal tract (43%)
  - bone (26%)
  - lung (12%),
  - liver (9%)
  - skin (7%)
  - brain (2%)

GM49: ILC age 36, ER+/PR+/HER2-, gynae mets age 44
Genomics of Metastasis Progression

Autopsy Case 11 (2007): IDC, grade 3; ER-, PR-, HER2+
Clonal Evolution and Intratumour Heterogeneity in Metastatic Progression

Autopsy Case 7 (1989): mixed Ductal-lobular, grade 3; ER-, PR-, HER2+

Chromosome 2

Chromosome 10

Chromosome 17
Intratumour Heterogeneity - identifying the lethal clone?

Chromosome 2

Breast

Lung

Chromosome 10

Breast

Lung

Breast

Lung

Breast

Breast

Breast

Lymph Node

Lymph Node PM

Liver

Adrenal

Lung
Identifying Driver Genes of the ‘Lethal Clone’

Autopsy Case 33 (Primary tumour 1966; PM 1969): IDC, grade 3; ER-, PR-, HER2-
Summary

- Morphological and molecular features of breast cancer quite well characterised
- Huge molecular data resources now available from consortia such as ICGC, TCGA, METABRIC
- Intratumour heterogeneity
  - Limitations of single biopsy/single time point analysis
  - Evident throughout tumour development & progression to metastasis
  - Remains a considerable problem for patient management
    - Which is the lethal clone? Which clone to treat?
- We are investigating intratumour heterogeneity to unravel mechanisms of disease progression
  - Pre-invasive disease
  - Mixed ductal-lobular carcinomas
  - Metastatic progression
- Autopsy studies
  - End stage, but reveal important clinical and biological insight into the natural history of dissemination
- Contemporary longitudinal series
  - Support findings from autopsy series and effects of treatment selection on lethal clone.
Acknowledgements

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www.uqccr.uq.edu.au

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Queensland Centre for Gynaecological Cancer
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Helen Spiers (Ramaciotti), Dan Belluccio (Agilent)
## Clinical/pathology characteristics of gynaecological metastases

<table>
<thead>
<tr>
<th>All mets to gynaecology</th>
<th>n=53</th>
<th>Breast primary</th>
<th>Gynae metastasis</th>
<th>Other metastatic sites</th>
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</thead>
<tbody>
<tr>
<td><strong>Average age diagnosis</strong></td>
<td></td>
<td>52</td>
<td>57</td>
<td>57</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td></td>
<td>31-82</td>
<td>30-83</td>
<td>37-85</td>
</tr>
<tr>
<td><strong>Type</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>ILC</td>
<td>23 (43%)</td>
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<tr>
<td>IDC</td>
<td>28 (54%)</td>
<td></td>
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<tr>
<td><strong>Other (micropapillary/medullary)</strong></td>
<td>2 (3%)</td>
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<tr>
<td><strong>Grade</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5 (18%)</td>
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</tr>
<tr>
<td>2</td>
<td>10 (37%)</td>
<td></td>
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<tr>
<td>3</td>
<td>12 (44%)</td>
<td></td>
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<tr>
<td><strong>Size (mm) average n=38</strong></td>
<td></td>
<td>29</td>
<td>5-100</td>
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<tr>
<td><strong>Range</strong></td>
<td></td>
<td>5-100</td>
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<td></td>
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<tr>
<td><strong>LN n=43</strong></td>
<td></td>
<td>30/43 positive (69%)</td>
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<tr>
<td><strong>ER</strong></td>
<td></td>
<td>34/39 (87%)</td>
<td>23/24 (85%)</td>
<td>0/0 (90%)</td>
</tr>
<tr>
<td><strong>HER2+</strong></td>
<td></td>
<td>1/23 (4%)</td>
<td>4/18 (22%)</td>
<td>0/2 (0%)</td>
</tr>
<tr>
<td><strong>Time to diagnosis</strong></td>
<td></td>
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<td>0-17 years, Median 5 years</td>
</tr>
</tbody>
</table>

- Peritoneum/omentum/ascitic fluid: 28/41 (68%)
- Abdomen/GI: 18/41 (43%)
- Bone/bone marrow: 11/41 (26%)
- Lung: 5/41 (12%)
- Pelvis/iliac LN: 5/41 (12%)
- Liver: 4/41 (9%)
- Skin: 3/41 (7%)
- Kidney: 1/41 (2%)
- Gallbladder: 1/41 (2%)
- Bladder: 1/41 (2%)
- Thyroid: 1/41 (2%)
- Cerebrospinal fluid: 1/41 (2%)
- Brachial plexus: 1/41 (2%)
- Brain: 1/41 (2%)
ER and HER2 results

87 sites: 66 ER+ (75%)
49 gynaec sites on TMA: 43 ER+ (87%)

Her2 – negative 87/87 (100%)
Linear model of tumour evolution

- initial genetic and epigenetic alterations dominantly drive tumour phenotype
Non-linear development

- Clonal diversity arises as a consequence of genomic instability
- The dominant clone may change depending on the selection conditions
  - Micro-environmental factors
  - treatment
- more aggressive and harder to treat?
**Evolution of malignancy.** *(Top)* The late metastasis model places selection of genetic and epigenetic alterations mostly inside the primary tumor. If so, late-disseminating cells are genetically similar to the primary tumor, which can be used as a surrogate marker to choose a drug against disseminated tumor cells. *(Bottom)* By contrast, early-disseminated tumor cells accumulate such alterations at distant sites and diverge genetically from the primary tumors. Consequently, they may respond differently to drugs that are administered systemically.
Immunophenotype

% of cases

- ER+
- PR+
- HER2+
- Triple negative
- Basal-like
- p53+
- Ki-67 (>10%)

Primaries

Metastases
Ductal Carcinoma *In situ* and the Emergence of Diversity during Breast Cancer Evolution

D. Craig Allred,¹ Yun Wu,² Sufeng Mao,² Iris D. Nagtegaal,³ Sangjun Lee,¹ Charles M. Perou,⁴ Syed K. Mohsin,⁵ Peter O’Connell,⁶ Anna Tsimelzon,² and Dan Medina³

54% of DCIS of uniform grade

- 1 (29.2%)
- 2 (22.5%)
- 3 (2.5%)

46% showed diversity in grade

- 30% grade 1 + 2
- 6.6% grade 2 + 3
- 9.2% grade 1 - 3

Diversity in grade correlated with mutation of p53

A subset of ADH progresses to well-differentiated (low-grade) DCIS that progresses in a non-obligatory manner to more poorly differentiated (higher-grade) DCIS by stochastically acquiring additional genetic and epigenetic alterations that influence cell morphology and function.

**Modified Wellings Jensen Model:**

- TDLU → HELU → ADH → DCIS → IBC
- Growth → As Adhesion & Polarity → Invasion → IBC
- Diversity → IBC
Columnar Cell Lesions (CCL)

• spectrum of pre-invasive lesions of breast tdlu
• high frequency in biopsy due to microcalcification
• precursor to low grade DCIS and invasive carcinoma?
• co-existence with low grade DCIS & invasive carcinoma
• morphological & IHC overlaps
• molecular data
• clinical importance
Case 3

CCC + atypia
(class 6)

DCIS
(class 8)

CCH + cytological and architectural Atypia (class 5)
Right mastectomy for wide spread DCIS

20 samples analysed: normal tdlu – CCL – DCIS

Case 1
Case 3

wide local excision

CCL spectrum within TDLU

15 samples analysed: normal acini – CCL – ALH – DCIS
Case 3

‘normal’

CCC (class 1)

CCH (class 2)
Right mastectomy for invasive carcinoma

24 samples analysed: CCL – HUT – ALH – DCIS – IDC

Case 2
Distribution of metastases by patient age

<table>
<thead>
<tr>
<th>Main Site of Metastases</th>
<th>49 or less</th>
<th>Age at death</th>
<th>65 or older</th>
<th>Chi-squared</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>50-64 years</td>
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<tr>
<td><strong>Brain</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>No</td>
<td>40 (26.1%)</td>
<td>70 (45.8%)</td>
<td>43 (28.1%)</td>
<td>4.58, df=2</td>
<td>0.10</td>
</tr>
<tr>
<td>Yes</td>
<td>18 (40.9%)</td>
<td>19 (43.2%)</td>
<td>7 (15.9%)</td>
<td></td>
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</tr>
<tr>
<td><strong>Bone</strong></td>
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</tr>
<tr>
<td>No</td>
<td>10 (19.6%)</td>
<td>28 (54.9%)</td>
<td>13 (25.5%)</td>
<td>3.70, df=2</td>
<td>0.16</td>
</tr>
<tr>
<td>Yes</td>
<td>48 (32.9%)</td>
<td>61 (41.8%)</td>
<td>37 (25.3%)</td>
<td></td>
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</tr>
<tr>
<td><strong>Lung</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>10 (22.7%)</td>
<td>21 (47.7%)</td>
<td>13 (29.5%)</td>
<td>1.34, df=2</td>
<td>0.51</td>
</tr>
<tr>
<td>Yes</td>
<td>48 (31.4%)</td>
<td>68 (44.4%)</td>
<td>37 (24.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>11 (19.6%)</td>
<td>22 (39.3%)</td>
<td>23 (41.1%)</td>
<td>10.74, df=2</td>
<td>0.005</td>
</tr>
<tr>
<td>Yes</td>
<td>47 (33.3%)</td>
<td>67 (47.5%)</td>
<td>27 (19.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gynae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>37 (22.8%)</td>
<td>78 (48.1%)</td>
<td>47 (29.0%)</td>
<td>20.02, df=2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Yes</td>
<td>21 (60.0%)</td>
<td>11 (31.4%)</td>
<td>3 (8.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Number of main metastatic sites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-2</td>
<td>17 (19.5%)</td>
<td>39 (44.8%)</td>
<td>31 (35.6%)</td>
<td>11.64, df=2</td>
<td>0.003</td>
</tr>
<tr>
<td>3-5</td>
<td>41 (37.3%)</td>
<td>50 (45.5%)</td>
<td>19 (17.3%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Supp Fig 7 - Case 33

A Chromosome 5

B Chromosome 6

C Chromosome 8

D Chromosome 17

E Chromosome 20

F Chromosome X

Breast

Node non-axillary

Liver

Breast

Node non-axillary

Liver

Breast

Node non-axillary

Liver
Supp Fig 5 – case 13
Introduce what will be covering from molecular pathology point of view

Intra-tumour heterogeneity
- Path
- IHC phenotype
- Genomics

Maybe just speak about this on previous slide as a contents page?

Pre-invasive disease – how frequently heterogeneous? See Abdel fatah paper
- % of DCIS heterogeneous according to grade
- MDLs 3-5% of tumours share D and L features
- Heterogeneity within types – lobular carcinomas
- Heterogeneity during metastatic progression

HOW TO INTRODUCE THIS? HOW DOES FLOW WORK

2 parts to my talk: part 1 – morphological heterogeneity during progression from preinv-mets; part 2 use of a large collection of primary and mets samples from autopsy series and contemporary surgical series to examine Biology