New Cancer Diagnostics: Opportunities and Challenges

Clinical Prof Sandra O’Toole
Head, Molecular Diagnostic Oncology
Royal Prince Alfred Hospital
Before and after 2 weeks vemurafenib treatment in melanoma in V600E mutant tumour
Before and After 5 days of gefitinib

Before

After

Images courtesy Prof Michael Boyer, RPAH
vemurafenib treatment in melanoma in V600R mutant tumour
Precision Medicine

- revolution in the diagnosis and treatment of patients with a range of malignancies
- patient selection is driven by molecular biomarkers
- pipeline of new targeted therapeutics
- Team approach needed – scientists, pathologists, oncologists
Outline

• Overview of current clinical mutation testing – lung cancer, melanoma
• Challenges of testing
• Need for multi-disciplinary collaboration and cross pollination of skills
• Future opportunities and challenges with NGS
‘Driver’ mutations

Oncogene ‘Addiction’

Cancer cells contain multiple genetic abnormalities but growth and survival can often be impaired by the inactivation of a single oncogene.

Protein tyrosine kinases frequently activated by mutation +/- gene amplification – important therapeutic targets.
## Pace of translation is accelerating

<table>
<thead>
<tr>
<th>Genetic change</th>
<th>Year target discovered</th>
<th>Time to translation years</th>
<th>Disease &amp; % eligible for Rx</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCR-ABL</td>
<td>1960</td>
<td>41</td>
<td>CML (100%)</td>
</tr>
<tr>
<td>EGFR</td>
<td>1978</td>
<td>36</td>
<td>Lung Ca (10%)</td>
</tr>
<tr>
<td>KIT</td>
<td>1996</td>
<td>16</td>
<td>GIST (85%)</td>
</tr>
<tr>
<td>BRAF</td>
<td>2002</td>
<td>8</td>
<td>Melanoma (40%)</td>
</tr>
<tr>
<td>ROS1</td>
<td>2007</td>
<td>5</td>
<td>Lung Ca (1%)</td>
</tr>
<tr>
<td>ALK</td>
<td>2007</td>
<td>3</td>
<td>Lung Ca (1%)</td>
</tr>
</tbody>
</table>

Adapted from Gerber & Minna Cancer Cell 2010
Personalised medicine
the success stories
Lung cancer – the challenges

- 7600 deaths from lung cancer in Australia in 2011
- leading cause of cancer death in men and women
- 70% of patients inoperable at diagnosis
- 15% 5 year survival
Lung cancer classification

- **small cell carcinoma**
  - approx 15-20% of lung Ca

- **non-small cell carcinoma** (NSCLC)
  - approx 80% of lung Ca

- **squamous cell carcinoma**
  - SCC
  - 44% of lung Ca in men, 25% in women

- **large cell carcinoma**
  - 9%

- **adenocarcinoma**
  - 28% of lung Ca in men, 42% in women
Targeted therapy in lung adenocarcinoma

- 10-15% patients with NSCLC have *EGFR* mutations
- *EGFR* TKI gefitinib & erlotinib
- oral agents, relatively low toxicity
- up to 70% response rates
- ALK/ROS inhibitor crizotinib
- pipeline of new agents

Gefitinib
Erlotinib
Crizotinib
Not all *EGFR* activating mutations are created equal!!
Targetable mutations in lung cancer

Pao and Hutchinson, Nat Med March 2012
Mutation Testing – challenges

• 70% of patients inoperable at diagnosis
• Small biopsies
• FFPE damaged DNA
• Rapid TAT, Cost constraints
• Accuracy and sensitivity
• desire for information on multiple genes, aberrations of which occur at very low incidence
Tissue is the issue!

- Specimens for KRAS testing
• Specimens for *EGFR* testing
Macrodissection

Dr Chiu Chin Ng
5% tumour cells

15% tumour cells

10% tumour cells

100% tumour cells

But not many of them!!!!!
Mutation testing - Which technique?

• PCR kit assays widely used
• Sanger sequencing
• single base extension assays eg SNaPshot and MassARRAY
• Next generation technologies
• No perfect technology!
Sequenom – sensitivity of PCR with specificity of mass spectrometry

used by MD Anderson & MSKCC

RPAH primary modality for lung cancer mutation testing
+ fragment analysis exon 19&20

A/Prof Bing Yu
Matrix Assisted Laser Desorption Ionization Time Of Flight Mass Spectrometry (MALDI-TOF MS)

Combines sensitivity of PCR with the accuracy of MS
L858R mutation

Wild type appearance

mutant peak

wild type peak
OncoCarta™ v1.0 Panel

- Use tumor samples from fresh, frozen, or formalin fixed, paraffin embedded tissues (FFPE) and/or cell lines.
- Analyze 238 mutations with as little as 500 ng DNA per sample.
- Detect and quantify mutation frequencies from >10%.

Covers 90-95% of currently “druggable” mutations

| ABL-1   | JAK-2   |
| AKT-1   | KIT     |
| AKT-2   | MET     |
| BRAF    | H-RAS   |
| CDK-4   | K-RAS   |
| EGFR    | N-RAS   |
| ERBB2   | PDGFα   |
| FGFR-1  | PIK3CA  |
| FGFR-3  | RET     |
| FLT-3   |         |
Sequenom massARRAY

- sensitive (10%)
- excellent performance EQMN, QAP
- works well FFPE, >1000 cases no fails, including samples >20 years old
- same assay for EGFR, BRAF, KRAS
- easy to read output
- information on multiple genes
Mutation profiling in early lung cancer

- 204 patients with operable adenocarcinoma, no adjuvant Rx
- Exon 18 mutations more common than prev reported
- >9% multiple mutations
- Resistance mutations detected de novo

FIGURE 1. (A) Distribution of number of mutations/variants present. (B) Frequency of different mutations/variants and ALK rearrangement.
Multigene profiling in lung cancer

- Mutations detected in 40% of single EGFR testing would have detected only 18%
- Information about resistance mechanisms
- Research and triage for clinical trials

N=234
Detecting structural rearrangements in lung cancer: FISH testing

Dr Christina Selinger

ALK FISH
ALK in lung cancer

2007 ALK inversion described

2008 phase I clinical trial

2010 NEJM 55% overall response rate and 72% PFS patients treated with crizotinib

ALK Fluorescent ISH (FISH)

Vysis ALK Break Apart Rearrangement Probe (Abbott Molecular)

Trial criteria for positive: split signals or extra red signals in >15% of tumour cells
Single Red ALK pattern - positive
Testing for \textit{ALK} rearrangement in lung adenocarcinoma: a multicenter comparison of immunohistochemistry and fluorescent \textit{in situ} hybridization

Christina I Selinger$^{1,13}$, Toni-Maree Rogers$^{2,13}$, Prudence A Russell$^{3}$, Sandra O’Toole$^{1,4,5}$, PoYee Yip$^{4,6}$, Gavin M Wright$^{7}$, Zoe Wainer$^{7}$, Lisa G Horvath$^{4,5,6}$, Michael Boyer$^{4,6}$, Brian McCaughan$^{8}$, Maija RJ Kohonen-Corish$^{5,9,10}$, Stephen Fox$^{2}$, Wendy A Cooper$^{1,10,14}$ and Benjamin Solomon$^{11,12,14}$

- \textit{ALK} rearrangement in cohort of 594 NSCLC cases from 4 centres in Sydney and Melbourne

- Is IHC effective to screen cases for ALK FISH testing (@ approx $300$)?
- *ALK* gene rearrangement in only 1% of Australian patient cohort
- Large scale FISH testing unlikely to be economically viable
ALK IHC sensitive and specific

- Must use lung specific protocols and strict controls

- We now use this to screen IHC for FISH

- Still require FISH confirmation before treatment

Table 4 ALK immunohistochemistry statistics

<table>
<thead>
<tr>
<th>Immunohistochemistry comparison</th>
<th>ALK1 antibody</th>
<th>5A4 antibody</th>
<th>D5F3 antibody</th>
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<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
<td>Specificity (%)</td>
<td>99</td>
<td>98</td>
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<tr>
<td>Positive predictive value (%)</td>
<td>54</td>
<td>39</td>
<td>54</td>
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<tr>
<td>Negative predictive value (%)</td>
<td>100</td>
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</table>
Personalised medicine
Melanoma
ORIGINAL ARTICLE

Improved Survival with Vemurafenib in Melanoma with BRAF V600E Mutation

Paul B. Chapman, M.D., Axel Hauschild, M.D., Caroline Robert, M.D., Ph.D., John B. Haanen, M.D., Paolo Ascierto, M.D., James Larkin, M.D., Reinhard Dummer, M.D., Claus Garbe, M.D., Alessandro Testori, M.D., Michele Maio, M.D., David Hogg, M.D., Paul Lorigan, M.D., Celeste Lebbe, M.D., Thomas Jouary, M.D., Dirk Schadendorf, M.D., Antoni Ribas, M.D., Steven J. O’Day, M.D., Jeffrey A. Sosman, M.D., John M. Kirkwood, M.D., Alexander M.M. Eggermont, M.D., Ph.D., Brigitte Dreno, M.D., Ph.D., Keith Nolop, M.D., Jiang Li, Ph.D., Betty Nelson, M.A., Jeannie Hou, M.D., Richard J. Lee, M.D., Keith T. Flaherty, M.D., and Grant A. McArthur, M.B., B.S., Ph.D. for the BRIM-3 Study Group

Dabrafenib in patients with melanoma, untreated brain metastases, and other solid tumours: a phase 1 dose-escalation trial


Figure 3: Change in tumour size in the 26 patients with Val600BRAF mutant melanoma with untreated brain metastases.
vemurafenib treatment in melanoma in V600R mutant tumour

22 Jan 2010

Feb 2012
# Clinically important mutations in melanoma

<table>
<thead>
<tr>
<th>Gene</th>
<th>Incidence</th>
<th>Treatment</th>
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<tbody>
<tr>
<td><strong>BRAF</strong></td>
<td>40% of cutaneous mel</td>
<td>BRAF inhibitors Vemurafenib dabrafenib</td>
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<tr>
<td><strong>NRAS</strong></td>
<td>13-25% of cutaneous mel</td>
<td>MEK inhibitors MET inhibitors PI3K inhibitors</td>
</tr>
<tr>
<td><strong>KIT</strong></td>
<td>15-20% mucosal mel and some mel arising in CSD</td>
<td>Imatinib Nilotinib sunitinib</td>
</tr>
</tbody>
</table>

Adapted from Lovly et al PLoS One 2012;7:e35309
Clinically important BRAF mutations

- **V600E** - 80% of BRAF mutations in melanoma, 81% RR vemurafenib
- **V600K** up to 20%, sensitive to vemurafenib
- **V600R** 5% mutant BRAF, recently reported to be sensitive to BRAFi*
- **V600M**, **V600G**, **V600D**, rare, predicted to be sensitive, but insufficient data currently
- **L597** and **K601** mutations possibly sensitive

*BRAF inhibitor activity in V600R metastatic melanoma.*

Patterns of mutations in melanoma

- No mutn 40%
- NRAS 21%
- All BRAF 33%
- V600 mutns 28%
- KIT 2.8%

Mean age NRAS 66.4 yrs
Mean age BRAF 60 yrs
BRAF mutations

BRAF V600E 59%

- BRAF V600K 18%
- BRAF Exon 11 10%
- exon 11
- L597
- K601
- V600*
- V600E
- V600K
- V600R
- V600M
- V600E2

Mean age V600E 56 years
Mean age V600K 63 years
Mean age non-V600 71.8 years
Multigene testing in melanoma

- >50% of patients had a mutation identified by the multigene panel (BRAF, NRAS, KIT), additional potentially actionable changes
- Compared to only 28% of cases harbouring mutations (BRAF V600) able to be detected by a single mutation specific assay
**KIT mutations in melanoma**

- Point mutations in the juxtamembrane domain and kinase domain.
- Imatinib sensitive mutations: L576P, K642E, V559A

Unexpected KIT L576P detected in cutaneous melanoma

http://www.biologicmodels.com/models-and-illustrations/
Benefits to multigene testing in melanoma

- Rapid identification of therapeutic targets
- Triage for clinical trials
- Time and cost effective
- Identification of potential resistance mechanisms
- May allow recognition of genotype/phenotype correlations and clinical activity of new drug combinations
Good histology is essential to accurate tumour mutation testing
M32
Stage IV melanoma
6/12 ago BRAF testing outside lab called negative
Retested at RPAH for NRAS/KIT/other potential targetable changes

Estimated 1-2% of tumour material on whole slide
Region selected for macrodissection
Melanoma estimated at 10% of cellular material
MD-12-1714

MD-12-1713

Estimated Mutant Proportion = 9%

BRAF V600E
Sanger sequencing

AMOY Diagnostics *BRAF* Scorpion-ARMS PCR assay

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
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<th>FAM_Ct</th>
<th>Hex_Ct</th>
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<tr>
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<td>Positive Ctrl</td>
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<td>&lt;28</td>
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<tr>
<td>8</td>
<td>MD-12-001714</td>
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<td>22.62</td>
<td>&lt;28</td>
</tr>
</tbody>
</table>

15.14 10 - 25
17.25 10 - 25
BRAF VE1 antibody
Sentinel lymph node biopsies
Mutation testing in small SLN mets

1st assay for BRAF V600

2nd assay for BRAF V600

Negative?
BRAF VE1 antibody

<table>
<thead>
<tr>
<th>Name</th>
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<th>Hex_Ct</th>
</tr>
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<tr>
<td>NTC</td>
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<tr>
<td>Positive-Ctrl</td>
<td>Positive Ctrl</td>
<td>10.73 &lt; 28</td>
<td>11.69</td>
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<tr>
<td>MD-12-563</td>
<td>Unknown</td>
<td>22.68 &lt; 28</td>
<td>20.82</td>
</tr>
</tbody>
</table>
Repeat test on new excision specimen
BRAF VE1 antibody

Only detects V600E mutant protein
BRAF VE1 antibody

- Detects BRAF V600E mutant protein
- 97% sensitive and 98% specific
- In 2/3 discordant cases, repeat analysis showed molecular assay was wrong i.e. false negative
- Antibody likely more sensitive than Sanger seq
- Will not detect V600K or other potentially sensitive BRAF V600 mutations
BRAF VE1 antibody

- Useful for rapid triage of patients for Rx
- Useful for specimens that may not be suitable for molecular testing
- Adjunct to molecular testing – confirmation, avoid molecular false negatives

Beware of melanin pigment interpreting VE1 IHC
Red chromogen can show non-specific staining
Personalised Medicine in 2014

- Only a handful of malignancies to date and only specific subsets of those
- New mutations/translocations are likely to occur at low incidence
- Challenge to identify those in a timely and cost-effective manner
The Future - Next generation sequencing

• huge promise for near future
• Can do pt mutns, indels, translocations, CNV if sufficient high quality fresh/frozen DNA and RNA

Ion Torrent (Life technologies)

• 10ng DNA
• Flexible sample number per run
• Short run time
• More manual prep
• quality DNA issues

MiSeq (Illumina)

• 250ng DNA
• Less flexible sample number per run (cost)
• need to batch
• Longer run time
• Less hands on sample prep
• Issue with FFPE quality
Challenges of NGS in practice

- Damaged DNA from routine pathology specimens – often small and low cellularity and high failure rates of FFPE samples
- Assays can be expensive but costs rapidly decreasing
- Analysing, interpreting and storing large amounts of data
- Generating meaningful reports to guide patient Rx in a reasonable time frame
- Validating and accrediting multiple mutations
Successful targeted therapy depends on ‘Driver’ mutations.

Oncogene ‘Addiction’

- BRAFi in BRAF V600 mutant melanoma
- Trastuzumab in HER2 amplified breast cancer
- Small molecule inhibitors of EGFR in EGFR mutant lung Ca
Genetic profile of most tumours

Passenger mutations

Multiple “drivers”
The landscape of cancer genes and mutational processes in breast cancer

73 different combinations of mutated cancer genes in 100 cancers
“a sobering perspective on the complexity and diversity of the disease is emerging.”

ER NOT IDENTIFIED AS A DRIVER MUTATION
Potential of NGS in novel biomarker discovery

- Phyllodes tumour of breast v rare (<1% of all mammary tumours), malignant PT exceedingly rare and difficult to treat if surgical Rx fails.
- Standard testing RPAH no mutations seen in a series of 29 PT including 3 malignant phyllodes tumour
- NGS comprehensive tumor panel Ion Torrent 3 malignant PTs - High confidence mutations in: TP53, RB1, PIK3C2B, NOTCH, PTCH1, ARID1A, MLL, GRM8, JAK2, CHEK1, DEK
- Potential insights into pathogenesis and possible new therapeutic targets

Masters of Surgery Project Dr Belinda Chan, supervised S O’Toole, A/Prof Bing Yu
Mutation testing in cancer

- Revolution in cancer medicine
- Need for multigene testing – NGS holds huge promise for the future, but significant practical issues to overcome before routine practice
- Hopefully will lead to better diagnosis and treatment → better outcomes
Thanks

Funding
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- Prof Richard Scolyer
- A/Prof Lisa Horvath
- Prof Michael Boyer
- Dr Tina Selinger
- Dr Chiu Chin Ng
- Dr Jerry Wei
- Dr Spiridoula Kraitsek
- Ms Cassandra Kavanagh

The Chris O'Brien Lifehouse at RPA
Sydney Catalyst
Australian Government
National Health and Medical Research Council
NGS

> single molecules of DNA for testing hybridize to the lawn of primers, new strand synthesised

Repeated cycles of synthesis, bridge formation and denaturation etc.

Results in multiple stands for “massively parallel sequencing”

Comprehensive, high accuracy, and sensitivity
Mutation testing in practice is not done well

- EQMN latest survey only 42 of 117 (46%) laboratories correctly genotyped all cases
- 20% of labs had an unacceptable error rate
- no single technology was clearly superior
- Australian QAP good concordance in EGFR, KRAS and BRAF mutation testing