Agena Bioscience: Role of DNA mass spectrometry in personalized medicine

Sept 2016
Abstract / key words

- Nucleic acid mass spectrometry (MS)
- Ionization
- Electrophoresis in vacuum
- Mass spectrum
- clinical laboratory
- Multiplex PCR
- Real time PCR
- MALDI TOF (MS)
- Sequencing & next generation sequencing
- diagnostic
- Prognosis
- clinical significance
- Personalized Medicine
- “Liquid biopsy”
- Pharmacogenomics
- cancer
- Solid tumors
- Single nucleotide polymorphism
- Mutation(s) & point mutations
- liquid biopsies
- Validated
- sensitivity
Abstract / key words

- minor allele detection
- Variants
- clinical tests
- in vitro diagnostic tests
- Human Papillomavirus (HPV)
- Mutation Profiling
- Flow Chart
- Non small cell lung cancer (NSCLC)
- Colorectal cancer (CRC)
- Breast cancer
- Cervix
- FFPE specimens
- RAS / RAF/ MAPK
- PIK3 / AKT
- EGFR T790M
- Ultra sensitive
- circulating tumour cells CTCs
- infiltrate into the blood stream, 1-10 cells per mL
- Pharmacogenetic
- Chemotherapy
- Fluorouracil or 5-FU
- human drug response
How it Works

Time of Flight Detection

Detector

Laser Desorption and Ionization

Matrix/Analyte

Acceleration and Detection

Mass spectrum

dAMP = 313.2 Da

dCMP = 289.2 Da

dGMP = 329.2 Da

dTMP = 304.2 Da
Cost-Effective, Targeted Genetic Analysis

Robust, Flexible, and High Throughput

- Real-Time PCR
  - Single / Selected Gene Testing
  - MassARRAY® System
    - MassARRA Y® System
  - Actionable Target Panels
  - Extensive Comprehensive Testing
- Multiplexing
- 10s – 100s
- 1,000s
- Single digits
Challenges in Oncology

- Early detection
- Classification of heterogeneous tumor mass
- Prognosis and outcomes assessment
- Therapy selection
- Non-invasive MRD and recurrence monitoring

All of the challenges rely on access to a sensitive accurate detection method
Medical Value

*Improve efficiency to patients and health care system*

- Prevent adverse events
- Improve disease management
- Improve quality of life
- More quickly target right treatment for patient

Benefits to Patient and Health Care System
- Earlier intervention
Applications for Ultra-Sensitive Detection

- Personalized Medicine
- “Liquid biopsy”
- Pharmacogenomics
Personalized Medicine / Oncology:
Targeted Therapies in Cancer

### Global Cancer Incidents

**The ten most common cancer deaths in males worldwide 2008 (with comparison to UK 2008)**

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>World (%)</th>
<th>UK (%)</th>
<th>UK Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>28</td>
<td>24</td>
<td>1st</td>
</tr>
<tr>
<td>Liver</td>
<td>11</td>
<td>2</td>
<td>12th</td>
</tr>
<tr>
<td>Stomach</td>
<td>11</td>
<td>4</td>
<td>7th</td>
</tr>
<tr>
<td>Colorectum</td>
<td>8</td>
<td>11</td>
<td>3rd</td>
</tr>
<tr>
<td>Oesophagus</td>
<td>7</td>
<td>6</td>
<td>4th</td>
</tr>
<tr>
<td>Prostate</td>
<td>6</td>
<td>12</td>
<td>2nd</td>
</tr>
<tr>
<td>Leukaemia</td>
<td>3</td>
<td>3</td>
<td>8th</td>
</tr>
<tr>
<td>Pancreas</td>
<td>3</td>
<td>5</td>
<td>5th</td>
</tr>
<tr>
<td>Bladder</td>
<td>3</td>
<td>4</td>
<td>6th</td>
</tr>
<tr>
<td>NHL</td>
<td>3</td>
<td>3</td>
<td>9th</td>
</tr>
<tr>
<td>All others</td>
<td>23</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

**The ten most common cancer deaths in females worldwide 2008 (with comparison to UK 2008)**

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>World (%)</th>
<th>UK (%)</th>
<th>UK Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>14</td>
<td>16</td>
<td>2nd</td>
</tr>
<tr>
<td>Lung</td>
<td>13</td>
<td>21</td>
<td>1st</td>
</tr>
<tr>
<td>Colorectum</td>
<td>9</td>
<td>10</td>
<td>3rd</td>
</tr>
<tr>
<td>Cervix</td>
<td>8</td>
<td>1</td>
<td>13th</td>
</tr>
<tr>
<td>Stomach</td>
<td>8</td>
<td>3</td>
<td>8th</td>
</tr>
<tr>
<td>Liver</td>
<td>7</td>
<td>2</td>
<td>14th</td>
</tr>
<tr>
<td>Ovary</td>
<td>4</td>
<td>6</td>
<td>4th</td>
</tr>
<tr>
<td>Oesophagus</td>
<td>4</td>
<td>3</td>
<td>9th</td>
</tr>
<tr>
<td>Pancreas</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Leukaemia</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>All others</td>
<td>27</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

*data source [http://info.cancerresearchuk.org/cancerstats](http://info.cancerresearchuk.org/cancerstats)*
Human Papillomavirus (HPV)
HPV Characteristics

- More than 120 different HPV types known
- DNA virus
- HPV16 and HPV18 are responsible for most HPV-caused cancers
- Cause several types of cancer (e.g. cervical cancer, anal cancer, oropharyngeal cancer)
- Cause appr. 5% of all cancers worldwide
- Don’t cause cancer
- Can cause skin warts
- Most infections occur without any symptoms
## HPV Risk Types according to WHO

### Classification according to IARC
(HPV type covered by Agena panel in bold)

<table>
<thead>
<tr>
<th>Carcinogenicity</th>
<th>HPV type according to IARC classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59</td>
</tr>
<tr>
<td>Group 2A</td>
<td>68</td>
</tr>
<tr>
<td>Group 2B</td>
<td>26, 53, 66, 67, 70, 73, 82, 30, 34, 69, 85, 97</td>
</tr>
<tr>
<td>Group 3</td>
<td>6, 11</td>
</tr>
<tr>
<td>„Low-risk“</td>
<td>40, 42, 43, 44, 54, 61, 62, 71, 72, 81, 83, 84, 89</td>
</tr>
</tbody>
</table>
Early detection

- Examine and treat women
- Do population screening
- Healthcare system to “weigh” on cost of screening vs. benefits of early detection
- Vision: Eradicate
- First countries to go for nationwide screening in EU
Lung Cancer (NSCLC)
Large scale NSCLC study on the MassARRAY® System

EGFR and KRAS mutational analysis in a large series of Italian non-small cell lung cancer patients: 2,387 cases from a single center

Authors: Riccardo Giannini, Cristiana Lupi, Elisa Sensi, Greta Alì, Agnese Proietti, Laura Boldrini, Adele Servadio, Mirella Giordano, Elisabetta Macerola, Rossella Bruno, Nicla Borrelli, Antonio Chella, Franca Melfi, Marco Lucchi, Alessandro Ribechini, Enrico Vasile, Federico Cappuzzo, Alfredo Mussi, Gabriella Fontanini

- Goals:
  - Determine incidence of EGFR & KRAS mutations in large cohort of Italian metastatic NSCLC patients
  - Compare two different routinely used testing methods for NSCLC mutation profiling
  - Investigate clinical utility of a multi-target methodology
Mutation Profiling Flow Chart

- MassARRAY enables:
  - Simultaneous testing of multiple targets
  - Reduces workflow complexity
  - Reduces TAT by 1 day

## Results from evaluating 2,387 NSCLC samples

<table>
<thead>
<tr>
<th></th>
<th>SSCP-Sanger EGFR</th>
<th>Pyrosequencing KRAS</th>
<th>Myriapod Lung Status Kit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tumor content</strong></td>
<td>&gt;20%</td>
<td>&gt;10%</td>
<td>&gt;10%</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>~10%</td>
<td>5% - 10%</td>
<td>2.5% - 10%</td>
</tr>
<tr>
<td><strong>Mean TAT</strong></td>
<td>4 days</td>
<td>4 days</td>
<td>3 days</td>
</tr>
<tr>
<td><strong>Sample throughput</strong></td>
<td>5</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td><strong>Additional Genes</strong></td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

- **Myriapod Lung Status CE-IVD Kit:**
  - Simultaneous testing of EGFR, KRAS, BRAF, NRAS, PIK3CA, ALK, ERBB2, DDR2, RET and MAP2K1 mutations
  - 40ng input DNA

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Key Advantages of the MassARRAY® System

- Significantly reduced the number of failed analyses due to low quantity or damaged DNA within cytological samples and small biopsies.
- Able to detect a wider range of mutations using a small amount of DNA.
- Allows for the rapid implementation and application of newly identified biomarkers.

...adopting the MALDI-TOF platform reduces the rate of missed samples.

The 3-day analytical TAT of the MALDI-TOF multi-target technique is appropriate for clinical management and reduces the overall treatment decision time.

Pan-Cancer Mutation Profiling
Identifying Potential Candidates for Target-Based Therapies

- 197 FFPE specimens from various tumor types were screened with OncoCarta v1.0 and two custom panels to identify potential candidates for targeted therapies and clinical trials – 25 oncogenes, 287 mutations
- Mutations were validated by sequencing with Junior 454 Roche sequencing
- 75 patients had actionable mutations and 26 had colon cancer with KRAS wild-type status
Co-occurring mutations

- 49.2% carried at least one mutation
- 32% had mutations in two genes
- RAS/RAF/MAPK (51.5%) and PIK3/AKT (36.1%) pathways were most frequently mutated
- CRC cases showed KRAS & PIK3CA co-occurrence while breast cancer samples showed PIK3CA & KIT co-mutations

The length of the arc corresponds to the frequency of mutations in the first gene, and the width of the ribbon corresponds to the percentage of patients who also had a mutation in the second gene.


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Conclusions

“MassARRAY technology is a rapid and effective method for identifying key cancer-driving mutations across a large number of samples, which allows for a more appropriate selection for personalized therapies.”

…this methodology makes it possible for a medium-sized laboratory to analyse multiple key hotspot mutations rapidly.

This procedure was a rapid, cost-effective method of identifying key cancer driving mutations across a large number of samples because it avoided complex bioinformatics analyses and assays were performed within two days.

This technology, in combination with the OncoCarta Panel v1.0, covers up to 95% of known druggable markers for an efficient mutation screening in clinical research trials and has an elevated grade of concordance with NGS technologies.

“Liquid Biopsy”
/ Ultrasensitive Detection
CTCs are circulating tumour cells that infiltrate into the bloodstream
- CTCs at 1-10 cells per mL

ctDNA is tumour DNA that has been shed into the bloodstream
- ctDNA can be present in 0.01% to >90% of the total cell free DNA (cfDNA)
- The amount of ctDNA is related to the tumour burden and varies highly between patients

Diaz and Bardelli, 2014 Journal of Clinical Oncology 32
Applications for Ultra-Sensitive Detection

- High-sensitivity detection may aide in personalizing therapy based on a patient’s mutational status
- Ideal for heterogeneous tumor samples and circulating plasma DNA
  - Heterogeneous tumor samples typically harbor low abundant mutations
  - Mutations may be informative for monitoring minimal residual disease
  - Clinical applications include early resistance monitoring (e.g. EGFR T790M- Gefitinib/Erlotinib)

» Non-invasive cancer diagnostics requires very low frequency mutation detection (<1%)
Pharmacogenetics
Why Pharmacogenetics?

Hereditary differences in human drug response
Fluorouracil or 5-FU (trademarked as Adrucil (IV), Carac (topical) etc.), is a drug that is a pyrimidine analog which is used in the treatment of cancer.
Thank You For Your Attention