Challenges of personalized medicine: from clinical trials to practice

Fortunato Ciardiello

Oncologia Medica, Dipartimento Medico-Chirurgico di Internistica Clinica e Sperimentale “F. Magrassi e A. Lanzara”, Seconda Università degli Studi di Napoli
Issues for the development of molecular targeted therapies in cancer

- Identify a relevant molecular target for cancer development and/or progression.
- Develop anti-targeted agents which could be used as drugs.
- Identify patients whose cancers depend on the molecular target for growth and/or progression.
- Define one or more biomarkers for patient selection before treatment.
- Define optimal strategies for the use of the molecular targeted drug in combination and/or in sequence with conventional treatments (radiotherapy, surgery, chemotherapy).
- Manage novel side effects and toxicities.
- Identify and possibly overcome mechanisms of acquired resistance to molecular targeted therapies.
The efficacy of targeted therapy depends on **TUMOR HETEROGENEITY**
Target-based agents + predictive biomarkers: PERSONALIZED MEDICINE
Predictive biomarkers and personalized medicine

• Biomarkers that are associated with response to drugs (positive selection)
  – EGFR mutations and ALK rearrangements in NSCLC
  – BRAF mutations in melanoma
  – ERBB2 gene amplification in breast/gastric cancer

• Biomarkers that are associated with resistance to drugs (negative selection)
  – RAS mutations and resistance to EGFR monoclonal antibodies in CRC
Challenges in biomarker testing in NSCLC

- The number of potential biomarkers is increasing
- Oncogenic pathways are activated through different, peculiar genomic alterations in NSCLC
- The molecular landscape may affect tumor response to targeted agents even in tumors with driver mutations
- The molecular profile of NSCLC changes following treatment with target based agents
- Need of methods for molecular profiling of NSCLC
- Assessment of somatic mutations in blood samples
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Molecular subsets of lung carcinoma

NSCLC as one disease

Histology-Based Subtyping

Others 11%
Squamous 34%
Adenocarcina 55%

Adenocarcinoma

Squamous Cell Cancer

EGFRvIII
PI3KCA
EGFR
DDR2
FGFR1 Amp
Unknown

Li JCO 2013
# Biomarkers in NSCLC

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR mutations</td>
<td>Gefitinib, erlotinib</td>
</tr>
<tr>
<td>ALK rearrangements</td>
<td>Crizotinib</td>
</tr>
<tr>
<td>ROS-1 rearrangements</td>
<td>Crizotinib</td>
</tr>
<tr>
<td>RET rearrangements</td>
<td>Cabozantinib</td>
</tr>
<tr>
<td>MET amplification</td>
<td>Crizotinib</td>
</tr>
<tr>
<td>DDR2 mutations</td>
<td>Dasatinib</td>
</tr>
<tr>
<td>NRAS mutations</td>
<td>Selumetinib/Trametinib (preclinical)</td>
</tr>
<tr>
<td>ErbB-2 mutations</td>
<td>Afatinib/Lapatinib/Trastuzumab</td>
</tr>
<tr>
<td>KRAS mutations</td>
<td>Selumetinib</td>
</tr>
<tr>
<td>FGFR1 amplification</td>
<td>Dovitinib</td>
</tr>
<tr>
<td>BRAF V600E</td>
<td>Vemurafenib</td>
</tr>
<tr>
<td>BRAF Y472C</td>
<td>Dasatinib</td>
</tr>
</tbody>
</table>

*Approved*
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EGFR mutations and NSCLC

Lynch NEJM 2004; Paez Science 2004; Pao PNAS 2004; Sequist JCO 2007
Genetic alterations in Lung Adenocarcinomas

**Therapeutic Implications**

- **EGFR mutant**: tyrosine kinase inhibitors
- **ALK fusion**: ALK inhibitors
- **ROS1 fusion**: crizotinib
- **RET fusion**: cabozantinib, vandetanib
- **NTRK1**: AZ64, PLX7486 (in clinical development)

*Pao & Hutchinson, Nat Medicine 2012*
Fusion transcripts in lung adenocarcinomas

<table>
<thead>
<tr>
<th>3’ Gene</th>
<th>5’ Partners</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK</td>
<td>EML4, HIP1, KIF5B, KLC1, TPR</td>
</tr>
<tr>
<td>ROS1</td>
<td>CD74, EXR, GOPC, LRIG3, SDC4, SLC34A2, TPM3</td>
</tr>
<tr>
<td>RET</td>
<td>CCDC6, CUX1, KIF5B</td>
</tr>
<tr>
<td>NTRK1</td>
<td>CEL, NFASC, IRF2BP2, TFG, QSTM1, SSBP2, DYNC2H1, CD74, MPRIP</td>
</tr>
</tbody>
</table>
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Frequency of resistance mechanisms to TKIs in NSCLC

- MAPK1 amplification
- AXL kinase activation
- BRAF mutations

Takezawa Cancer Discov 2012

Doebele Clin Cancer Res 2012
Resistant cells are selected by Tyrosine Kinase Inhibitor (TKI) treatment.
The efficacy of targeted therapy is affected by TUMOR HETEROGENEITY.
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The major classes of genomic alterations that give rise to cancer

**Sequencing, Real Time PCR etc.**

**Point mutations**
- AGT Arg
- CGT Cys
- GGT Gly
- TGT Ser
- GAT Asp
- GCT Ala
- GTT

**Copy number alterations**
- Amplification
- Deletion

**Translocations**
- Activation of many genes
  - BCR-ABL in CML
  - EML4-ALK
  - ROS-1
  - RET
  - NTRK1

**Activation of oncogenes**
- EGFR
- ErbB-2
- MET
- ERBB2 in breast cancer
- RB1 in retinoblastoma

**Activation of oncogenes-RAS genes in many cancers**
- KRAS
- NRAS
- PIK3CA
- AKT1
- MAP2K1

**Checklist of alterations**
- FISH
- Immunohistochemistry

Modified from McConaill JCO 2010
Clinical cancer genomics

**High-throughput genotyping platforms (multiplexed screens):** detection of selected somatic mutations. Allows identification of rare mutations (such as BRAF\(^{V600E}\) in lung cancer) but are limited to the known variants that have been chosen for analysis. Favor oncogenes over tumor suppressor genes, for which only frequently mutated sites are evaluated. Not suited for the analysis of gene copy number variations and does not allow the identification of gene rearrangements. Difficulties in detecting small insertions or deletions. Decreased sensitivity in tumor samples with high stromal admixture.

**Targeted massively parallel sequencing:** detection of somatic mutations with increased sensitivity. Permits increased sequencing coverage of predefined regions of interest, such as coding exons of known oncogenes and tumor suppressor genes, in addition to pharmacogenomic polymorphisms. Also suited for detection of gene copy number variations and predefined gene rearrangements. Identification of small insertions/deletions remains difficult with current algorithms.

**Whole-exome sequencing:** detection of point mutations, small insertions/deletions, pharmacogenomic polymorphisms, and gene copy number variations with increased sensitivity. Not suited for discovery of gene rearrangements.

**Whole-genome sequencing:** discovery of novel gene rearrangements, complex insertions/deletions, and microbial infections as well as identification of copy number alterations. Accurate point mutation detection remains a limitation, requiring significantly more coverage.
## Next Generation Sequencing-based Cancer Panels

<table>
<thead>
<tr>
<th>Panel</th>
<th>Source</th>
<th>N. genes</th>
<th>DNA (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TruSight Tumor Sequencing Panel</td>
<td>Illumina</td>
<td>26</td>
<td>30-300</td>
</tr>
<tr>
<td>TruSeq Amplicon Cancer Panel</td>
<td>Illumina</td>
<td>48</td>
<td>250</td>
</tr>
<tr>
<td>Ion AmpliSeq™ Cancer Hotspot Panel v2</td>
<td>Life Technologies</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>Ion AmpliSeq™ Colon and Lung Cancer Research Panel v2</td>
<td>Life Technologies</td>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td>GeneRead DNAseq Tumor Actionable Mutations Panel</td>
<td>Qiagen</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>GeneRead DNAseq Clinically Relevant Tumor Panel</td>
<td>Qiagen</td>
<td>24</td>
<td>10</td>
</tr>
</tbody>
</table>

Slide courtesy of Nicola Normanno
Ion AmpliSeq™ Colon & Lung Cancer Panel
Developed and verified 8 labs experienced in colon & lung cancer screening

Prof. Ian Cree
Warwick Medical School
United Kingdom

Prof. Orla Sheils
Trinity College Dublin,
Ireland

Dr. Marjolijn Ligtenberg & Dr. Bastiaan Tops
Radboud University
Nijmegen Medical Centre
The Netherlands

Dr. Cristoph Noppen &
Dr. Henriette Kurth
VIOLLIER AG Basle,
Switzerland

Prof. Aldo Scarpa
ARC-NET University of
Verona Italy

Dr. Nicola Normanno
Centro Ricerche Oncologiche Mercogliano,
Italy

Dr. Ludovic Lacroix
Institut Gustave Roussy
Paris, France

Prof. Pierre Laurent Puig
Université Paris Descartes,
France
Ion AmpliSeq™ Colon and Lung Cancer Panel

Panel design and relevance

- **Genes included:** KRAS, EGFR, BRAF, PIK3CA, AKT1, ERBB2, PTEN, NRAS, STK11, MEK1, ALK, DDR2, CTNNB1, MET, TP53, SMAD4, FBXW7, FGFR3, NOTCH1, ERBB4, FGFR1, FGFR2

  - **New genes DDR2 and MEK1**
  - KRAS exon4 to include codons 117 to 146
  - EGFR exon12 to include codon 492
  - BRAF exon11 to include codons 466 and 469

- **Comparison with Ion AmpliSeq™ Cancer Hotspot Panel v2 (CHP2)**
  - 11 of 90 amplicons are new
    - DDR2, ALK, EGFR, MEK1
Molecular typing of lung adenocarcinoma on cytological samples using a multigene next generation sequencing panel

<table>
<thead>
<tr>
<th>Gene</th>
<th>Number of Mutations</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>EGFR</td>
<td>6/36 (16%)</td>
<td></td>
</tr>
<tr>
<td>KRAS</td>
<td>10/36 (28%)</td>
<td></td>
</tr>
<tr>
<td>TP53</td>
<td>7/36 (18%)</td>
<td></td>
</tr>
<tr>
<td>PIK3CA</td>
<td>3/36 (8%)</td>
<td></td>
</tr>
<tr>
<td>BRAF</td>
<td>2/36 (5%)</td>
<td></td>
</tr>
<tr>
<td>STK11</td>
<td>1/36 (3%)</td>
<td></td>
</tr>
</tbody>
</table>

36/38 (95%) adequate libraries

24/36 (67%) at least one
9/36 (25%) multiple
Colon and Lung Panel - a single workflow solution

DNA analysis

Colon and Lung AmpliSeq™ panelv2
DNA mutations

Tumor Sample

Ion PGM™ System
semiconductor sequencing

RNA lung fusion research panel
ALK, ROS, RET, NTKR1 fusions & expression

Ion Reporter™ Software
automated analysis & reporting

Sample to report in less than 36 hours
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Different sources of tumor DNA

DNA from tumor harvested by biopsy or surgery

Circulating DNA or tumor cells harvested by blood drawing

Fleischacker & Schmidt Nat Med 2008
Detection of EGFR mutations in plasma

Different methods have been used to detect EGFR mutations in the plasma/serum of NSCLC patients

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Therascreen</td>
<td>43.1%</td>
<td>Goto et al. J Thorac Oncol 2011</td>
</tr>
<tr>
<td>PNA clamp</td>
<td>59.1%</td>
<td>Rosell et al. N Engl J Med 2009</td>
</tr>
</tbody>
</table>

*ratio between positive cases on plasma and positive cases on tissue
Whole exome sequencing of plasma DNA

Murtaza Nature 2013
The future of biomarker testing in NSCLC
Challenges in genomics-driven oncology

• Alterations of “actionable” oncogenic pathways are not always predictive of response to targeted agents.
• Different molecular alterations of the same oncogene may not be equivalent.
• Intra-tumor heterogeneity may affect response to targeted agents.
• The molecular profile of solid tumors may significantly change following treatment with target based agents.
• Genomic testing programs should be strongly linked to matched clinical trials.
Genomics-Driven Oncology

Surgeon
Endoscopist
Radiologist

Fresh biopsy

Medical Oncologist
Pathologist, Molecular Biologist, Geneticist

Patient encounter
Omic profiling
Data interpretation
Management decision
Clinical response?
Drug resistance?
Salvage or new therapy?

• Aggressive/metastatic tumors
• Distinctive characteristics
• Enterprise-wide

“Cutting-edge” and emerging technologies

• Integrative heuristic algorithms
• Focused experimental validation?

• Evaluation committee
• Framework for decision making
• Hypothesis-driven phase I trials
• Mechanism-based clinical studies

• Registry studies
• Pharmaco-dynamic analyses

• Molecular mechanisms/correlates
• Integration with preclinical studies

Inform novel therapeutic trials or therapeutic combinations

Garraway JCO 2013