Biomarker Driven, Targeted Cancer Therapeutics

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Cancer in the Molecular Era: Identifying the Drivers of Lung Cancer

**BEFORE:** One Disease

**TODAY:** Many different forms of lung cancer driven by different molecular defects – with more yet to be identified.

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ASCO’s Blueprint for Transforming Clinical and Translational Research
Smaller Trials, Bigger Chance for Success

**OLD MODEL:** Large numbers of patients, not selected by molecular characteristics; lower chance of demonstrating effectiveness, since many participants do not have the molecular defects being targeted.

**NEW MODEL:** Small patient populations, all with the relevant mutations or genetic defects; greater chance of desired results, since all participants have the potential to respond.

ASCO’s Blueprint for Transforming Clinical and Translational Research
# Improved Clinical Efficacy of Agents Targeting Genomic Alterations

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Genomic Alteration</th>
<th>Targeted Agent</th>
<th>Overall Response Rate</th>
<th>Reference</th>
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<tbody>
<tr>
<td></td>
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<td>PLX4032 (RG7204)</td>
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<tr>
<td>NSCLC</td>
<td>EML4-ALK</td>
<td>Crizotinib (PF-02341066)</td>
<td>57%</td>
<td>Kwak E et al. <em>NEJM.</em> 363, 1693-1703 (Oct 2010)</td>
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<tr>
<td>NSCLC</td>
<td>EGFR</td>
<td>Gefitinib</td>
<td>75%</td>
<td>Inoue A et al. <em>JCO.</em> 27, 1394-1400 (May 2011); Inoue A et al. <em>JCO.</em> 24, 3340-3346 (July 2006);</td>
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<tr>
<td>Basal Cell</td>
<td>PTCH1 (Hedgehog)</td>
<td>Vismodegib (GDC-0449)</td>
<td>55%</td>
<td>Von Hoff DD et al. <em>NEJM.</em> 361, 1164-1172 (Sept 2009)</td>
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</table>
IDEAS + TOOLS = PROGRESS

The human genome - 2001

- isomerases: 94; 0,5%
- receptors: 1076; 6,3%
- storage proteins: 15; 0,1%
- structural proteins: 280; 1,6%
- surfactants: 15; 0,1%
- cell junction proteins: 67; 0,4%
- chaperones: 130; 0,8%
- transcription factors: 2067; 12,0%
- phosphatases: 230; 1,3%
- membrane traffic proteins: 321; 1,9%
- transfer/carryer proteins: 248; 1,4%
- hydrolases: 454; 2,6%
- defense/immunity proteins: 107; 0,6%
- calcium-binding proteins: 63; 0,4%
- viral proteins: 7; 0,0%
- unclassified: 4061; 23,6%
- extracellular matrix proteins: 72; 0,4%
- proteases: 476; 2,8%
- cytoskeletal proteins: 441; 2,6%
- transporters: 1098; 6,4%
- transmembrane receptor regulatory/adaptor proteins: 84; 0,5%
- transferases: 1512; 8,8%
- oxidoreductases: 550; 3,2%
- lyases: 104; 0,6%
- cell adhesion molecules: 93; 0,5%
- ligases: 260; 1,5%
- nucleic acid binding: 1466; 8,5%
- signaling molecules: 951; 5,6%
- enzyme modulators: 857; 5,0%
Gene Expression (Transcription/Translation)

Gene

DNA

TRANSCRIPTION

RNA

TRANSLATION

Protein

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Transcription/Translation

DNA nucleotides: A C G T
RNA nucleotides: A C G U

Amino acid
Translation
Protein Sequence


NIH
Major Categories of Tumor Genomic Alterations

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

  - Activation of oncogenes-RAS in many cancers
  - Inactivation of TS genes-TP53 in many cancers

- **Copy number alterations**
  - Amplification
  - Activation of oncogenes-ErbB2 in breast cancer
  - Deletion
  - Inactivation of TS genes-Rb in retinoblastoma

- **Translocations**
  - +
  - Activation of many genes-Bcr-ABL in CML
  - ETS factors in prostate cancer

Point Mutations

- Single change in DNA sequence of gene
- Changes mRNA code (codon)
- Changes amino acid within a protein sequence
- Protein changed, resulting in altered function or loss of function
Gene Amplification

• Errors in normal DNA replication result in production of multiple copies of a gene in the region of the chromosome

• Multiple gene copies are transcribed and translated, leading to an overproduction of the protein
Chromosome Translocation

- One segment of chromosome arm breaks off and fuses to another chromosome
- Exchange of chromosome segments
Result of Chromosome Translocation

• Transcription and/or translation of the gene may change in the new chromosome location
  – Example: Movement of a gene could increase or decrease the amount of protein generated in cell

• Breaking and rejoining of parts of a gene can lead to inactivation, where no protein is produced

• Translocations are common in leukemia's and lymphomas less common in cancers of solid tissues
  – Example: Philadelphia chromosome - exchange of sections of chromosomes 9 and 22 leading to a shortened form of chromosome 22 seen in > 90% of patients with chronic myelogenous leukemia (CML).
Chromosome Deletion

- Deletion: A segment in the middle of a chromosome is discarded
- Inversion: Segment of DNA is released from a chromosome and then re-inserted in the opposite orientation
- Results in loss of gene or mutation affecting transcription/translation
  - Changes in amount or type of protein
  - Inactive protein
Gene Mutations that De-Regulate Cell Growth

• Oncogenes
  – Genes that express proteins that promote cell division and/or inhibit cell death
  – DNA mutations in oncogenes result in proteins that have gained functions or lost the ability to be controlled:
    • overexpression of an oncogene producing too much protein
    • too many copies of an oncogene sequence resulting in too much protein (gene amplification)
    • expression of an oncogene to produce and activate a protein when it normally should not be there or should not be active
    • expression of a mutated version of the gene, producing a mutated protein with altered functioning
Common Oncogenes

• HER-2: a growth factor receptor that promotes cell growth/division
  – Gene is amplified and overexpressed in ~ 30% of human breast cancers
• Ras: involved in signaling pathways that control transcription of genes that regulate cell division
  – Point mutation in gene affecting normal function is found in various cancers including pancreas, colon, lung, thyroid, etc.
• Myc: involved in transcription of genes that produce proteins required for control cell division
  – Chromosomal translocation within myc gene results in overexpression of protein in Burkitt's lymphoma, B-cell leukemia, and lung cancer
• Bcl-2: protein involved in cell signaling that prevents programmed cell death (apoptosis)
  – Chromosomal translocation leading to overexpression of Bcl-2 protein prevents apoptosis in mutated cells and promotes continued cell division
Gene Mutations that De-Regulate Cell Growth

• Tumor suppressor genes
  – Genes that express proteins that directly or indirectly prevent cell division or promote cell death
    • Key roles: regulation of transcription/translation of key proteins, function in DNA repair
  – DNA mutations in tumor suppressor genes result in loss of function of the protein it produces:
    • do not express a tumor suppressor gene so no protein is produced
    • do not express enough tumor suppressor gene so not enough protein is produced
    • cause a change in a tumor suppressor gene that de-activates the protein and prevents normal function
Common Tumor Suppressor Genes

• BRCA1 & 2: involved in DNA repair and regulation of gene expression
  – Mutations lead to unrepaired DNA damage that continue to divide and accumulate mutations in key genes, leading to cancer cell formation
  – Cancer cells with BRCA gene mutations typically display chromosomal breaks and severe aneuploidy
  – Inherited mutations increase susceptibility to breast & ovarian cancer

• p53: regulates transcription of genes that express proteins to control cell division and apoptosis
  – Senses DNA damage and stops the cell from dividing to allow DNA repair or target cell for death (apoptosis)
  – Mutated in ~50% of tumors from all cancer types

• Rb (retinoblastoma gene): indirectly involved in preventing transcription of genes that express proteins required for cell division
  – Mutations found in cancers of the eye in children (retinoblastoma) lung, breast, bladder and osteosarcomas
A Paradigm Shift: The Genomic View of Cancer

From Anatomy...
- Lung
- Breast
- Prostate
- Colon
- Brain

Genomic/Molecular Profiling

To Genetic Mutation
- KIT (Imatinib)
- EGFR (Erlotinib)
- HER2 (Trautuzamab)
- BRAF (PLX4032)
- PIK3CA (BEZ235)
The Path to Personalized Cancer Medicine

Obtain tumor biopsy material

Extract DNA/RNA from tumor to profile for somatic alterations

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<thead>
<tr>
<th>Targeted therapies</th>
<th>Gefitinib</th>
<th>Herceptin</th>
<th>PLX4032</th>
<th>PTK787</th>
<th>Imatinib</th>
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Mutated Genes
Accelerating Science - Concept to Clinic

Molecular characteristics

Preclinical models, cell lines and xenografts

Design clinical trial
Generation of xenopatients: specimen of liver metastasis cut in small pieces, pathologically and molecularly characterized, fragments implanted in 2 mice, passaged and expanded to two cohorts of 12 mice and randomized to placebo or cetuximab.

Xenopatient Trial

**HER2 overexpression/amplification**

B  Unselected (n = 2,340)  
2.7% (2.5–3.7%)  
2.7%  

KRAS wild type (n = 44)  
13.6% (P < 0.01)  
13.6%  

Quadraple negative (n = 11)  
36.4% (P < 0.001)  
36.4%  

Xenopatients derived from cetuximab resistant, quadraple wild-type, HER2-amplified tissue sample
Resistance to EGFR-Targeted Therapy

Activated pathway resistant to Anti-EGFR therapy

Adapted from Vlacich G & Coffey RJ. Cancer Cell 2011; 20: 423-425
Resistance to EGFR-Targeted Therapy

Activated pathway resistant to Anti-EGFR therapy

Adapted from Vlacich G & Coffey RJ. Cancer Cell 2011; 20: 423-425
Overcoming resistance to EGFR Targeted Therapy

Adapted from Vlacich G & Coffey RJ. Cancer Cell 2011; 20: 423-425
**Figure 1**

**NSABP FC-7 Overview**

- **Metastatic Colorectal Cancer with Primary Tumor for Wild-Type KRAS, NRAS, BRAF, PIK3CA**
  - Core biopsy to procure fresh tumor samples for correlative science studies
  - **Study entry/Dose level assignment**

**Treatment Regimen for All Patients**

- **Phase I and Phase II**
  - Cetuximab 400 mg/m² loading dose followed by weekly cetuximab 250 mg/m² until progression
  - (1 Cycle = 28 days)
  - + Neratinib* PO daily beginning on Day 1 of cetuximab and continuing until disease progression

* **Neratinib Dose Escalation Levels (Phase I)**

Dose-escalation will proceed on the basis of dose-limiting toxicity during Cycle 1.

- Dose level 1: 120 mg/day
- Dose level 2: 160 mg/day
- Dose level 3: 200 mg/day
- Dose level 4: 240 mg/day

Dose of neratinib in the Phase II part of the study will be at the recommended Phase II dose (RP2D). The cetuximab doses are the same in Phase I and Phase II.
NSABP Patient Registry and Biospecimen Profiling Repository

• MPR-1 protocol: Primary tissue collection and repository for patients w mCRC
  – Primary tumor sample profiled to identify druggable genetic alterations

• NSABP-Genome Assessment Guided Medicine (N-GAME)
  – A Clinical trials platform
    • Profile will be used to match an NSABP treatment trial
• NSABP committed to populate, maintain, and analyze through continuous patient recruitment.
• Testing will be performed by in NSABP pathology laboratory and by collaborating researchers
• Confirmation of eligibility will be performed at CLIA-certified lab before disclosure to treating physicians
Goal: 1000 – 2000 tumor specimens from living mCRC patients collected, stored, and analyzed as part of biospecimen repository.

Anticipated that 200-400 participants will be registered within first year.

First tissue sample entered 4/29/13

Number entered by 7/1/13 = 60
THE PRACTICAL VALUE OF SCIENCE.

“I have endeavored to state the higher and more abstract arguments by which the study of physical science may be shown to be indispensable to the complete training of the human mind, but I do not wish it to be supposed that because I may be devoted to more or less abstract and unpractical pursuits I am insensible to the weight which ought to be attached to that which has been said to be the English conception of Paradise—namely, ‘getting on.’ Now the value of a knowledge of physical science as a means of getting on, is indubitable. There are hardly any of our trades, except the merely huckstering ones, in which some knowledge of science may not be directly profitable to the pursuer of that occupation. An Industry attains higher stages of its development as its processes become more complicated and refined, and the sciences are dragged in, one by one, to take their share in the fray.”—Huxley.
EXTRA SLIDES
Properties of a Cancer Cell

- Changes in physical properties of cell
- Multiple DNA mutations
- Cell division no longer regulated by normal signaling mechanisms
- Unlimited number of cell divisions without normal cell aging
- Avoidance of cell death
- Overproduction of angiogenesis signals
- Tissue invasion/metastasis
Physical Properties of Cancer Cells

Cell Checkpoints/Regulation

- Entrance into M blocked if DNA replication is not completed
- Anaphase blocked if chromatids are not properly assembled on mitotic spindle
- DNA damage checkpoint: DNA replication halted if genome is damaged
- DNA damage checkpoint: entrance into S is blocked if genome is damaged

Cell Death

Apoposis

Cellular Senescence


Bandyopadhyay, D et al., Curr Protoc Cell Biol. 2005 Jul;Chapter 18:Unit 18.9. PMID: 18228464
Multiple Mutations $\rightarrow$ Tumorigenesis

NCI. (2005). Loss of Normal Growth Control from Understanding Cancer Series
Overcoming resistance to EGFR Targeted Therapy

Adapted from Vlacich G & Coffey RJ. Cancer Cell 2011; 20: 423-425
Process of Tumorigenesis

Summary of Cancer Development

Benign tumor cells grow only locally and cannot spread by invasion or metastasis.

Malignant cells invade neighboring tissues, enter blood vessels, and metastasize to different sites.

- Mutation inactivates suppressor gene
- Cells proliferate
- Mutations inactivate DNA repair genes
- Proto-oncogenes mutate to oncogenes
- More mutations, more genetic instability, metastatic disease
Conclusions

• In normal cells, all cell functions are tightly regulated by key proteins
• DNA found in our chromosomes contain genes that are transcribed into RNA and then translated into proteins
• When a cell divides (mitosis), DNA in the cell is replicated to create two identical copies of the cell
• Cancer cells develop when multiple mutations in DNA occur, leading to de-regulation of normal cell function and changes in cell characteristics and properties
• Cancer cells continue to divide in the absence of growth signals and ignore anti-growth signals, undergo infinite number of cell divisions, evade apoptosis, increase angiogenesis and promote tissue invasion and metastasis
Conclusions

• Various types of DNA mutations can occur that rely on functioning DNA repair mechanisms
• Mutations that are not repaired alter normal protein function and in turn alter cell function
• Oncogenes express proteins that promote cell division and inhibit cell death
• Tumor suppressor genes halt cell division in the presence of DNA damage to allow for DNA repair or targeting cells for apoptosis
• Multiple oncogenes and tumor suppressor genes are mutated in cancer cells, leading to uncontrolled cell division and prevention of cell death
Clinical Trial Design for Genomic Profiling of Tumor Biopsies