Clinical Applications of Molecular testing in Oncology and Hematology

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Molecular Diagnosis in Hematologic Malignancies.

Diagnostic Molecular Pathology

- Used as standard of care for
- Risk identification
- Diagnosis
- Prognosis
- Prediction of response to therapy
- Monitoring therapeutic responses

Morphology and IHC

- ALL with blasts in the peripheral blood (a) and marrow (b).
- IHC documents the blasts are positive for TdT (c) and PAX-5 (d).

Flow and Cyto

- Clonal population of B-cells expressing CD19 and CD5 and kappa restriction

Histology/Morphology
- What the cells look like

Immunohistochemistry (IHC)
- Staining the cells to identify specific markers

Flow cytometry
- Looks at individual cells based on staining for specific markers

Cyto genetics
- Chromosome analysis

FISH
- Targeting specific chromosomes

Molecular studies
- Identifying abnormal gene products
**DIAGNOSTIC ACCURACY**

**Translocations w/o gene fusion**

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Translocation</th>
<th>Activated Gene</th>
<th>Mechanism of Activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-ALL</td>
<td>t(1;14)(q32;q32)</td>
<td>TAL1</td>
<td>Relocation to TCR locus</td>
</tr>
<tr>
<td>B-ALL</td>
<td>t(8;14)(q24;q11)</td>
<td>MYC</td>
<td>Relocation to TCR locus</td>
</tr>
<tr>
<td>B-cell ALL</td>
<td>t(8;14)(q24;q11)</td>
<td>MYC</td>
<td>Relocation to TCR locus</td>
</tr>
<tr>
<td>Large Cell Lymphoma</td>
<td>t(3;14)(q27;q32)</td>
<td>BCL6</td>
<td>Relocation to IgH locus</td>
</tr>
<tr>
<td>Mantle Cell Lymphoma</td>
<td>t(11;14)(q13;q32)</td>
<td>Cyclin D1</td>
<td>Relocation to IgH locus</td>
</tr>
<tr>
<td>Follicular B-cell lymphoma</td>
<td>t(14;18)(q32;q21)</td>
<td>BCL2</td>
<td>Relocation to IgH locus</td>
</tr>
</tbody>
</table>

**FISH**

Red signal: ABL gene on a normal chromosome 9
Green signal: BCR on a normal chromosome 22
Yellow (combined): BCR/ABL fusion on the Philadelphia chromosome t(9;22)

**Polymerase Chain Reaction**

- Method to rapidly and highly specifically amplify DNA fragments
- Advantages
  - Common, fairly inexpensive
  - Rapid, sensitive and specific
- Disadvantages
  - Requires knowledge of the specific nucleotide sequence
  - Sensitivity may result in false-positive results

**Other Techniques**

- Gene Expression Profiling
  - Microarray technology to identify a molecular signature of a tumor
- Proteomics
  - Microarray technology to identify protein expression profiles of tissue/cell type

**Purpose of Molecular Tests**

- Diagnostic accuracy
- Prognostic markers to predict outcomes
- Monitor for minimal residual disease
Translocations w/fusion product: hematologic tumors

<table>
<thead>
<tr>
<th>TUMOR</th>
<th>Translocation</th>
<th>Gene fusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic myelogenous leukemia</td>
<td>t(9;22)</td>
<td>BCR-ABL(p210)</td>
</tr>
<tr>
<td>Acute promyelocytic leukemia</td>
<td>t(15;17); t(11;17)(q23;q11); t(11;17)(q23;q11); t(17;17)(p13;q21)</td>
<td>PML-RAR; PLZF-RAR; HMRA-RAR; ST15a-RAR</td>
</tr>
<tr>
<td>AML</td>
<td>t(8;21)(q22;q22)</td>
<td>AML1-ETO</td>
</tr>
<tr>
<td>AML with MLL (equivalent and post-Rx)</td>
<td>t(11q23)</td>
<td>MLL (25 partners)</td>
</tr>
<tr>
<td>Angiomyxoid liposarcoma (pediatric)</td>
<td>t(12;22)(q13;q12)</td>
<td>TPM-ALK</td>
</tr>
<tr>
<td>Ewing's sarcoma</td>
<td>t(11;22); t(21;22); t(7;22); t(12;22)</td>
<td>EWS/FLI1; EWS/ERG; EWS/ETV1; EWS/ETV4</td>
</tr>
</tbody>
</table>

Translocations w/chimeric products: solid tumors

<table>
<thead>
<tr>
<th>TUMOR</th>
<th>Translocation</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ewing's sarcoma</td>
<td>t(11;22)(q22;q11)</td>
<td>EWS/FLI1; EWS/ERG; EWS/ETV1; EWS/ETV4</td>
</tr>
<tr>
<td>Synovial sarcoma</td>
<td>t(X;18)</td>
<td>SYT/SSX1</td>
</tr>
<tr>
<td>Clear cell sarcoma</td>
<td>t(12;22)</td>
<td>EWS.ATF-1</td>
</tr>
<tr>
<td>Myxoid/round cell liposarcoma</td>
<td>t(12;22)</td>
<td>CHOP/FUS</td>
</tr>
<tr>
<td>Extraskeletal myxoid chondrosarcoma</td>
<td>t(9;22)</td>
<td>EWS/TEC</td>
</tr>
</tbody>
</table>

PROGNOSTIC MARKERS TO PREDICT OUTCOMES

AML Model based on molecular mutations

CEBP-α
- On chromosome 19q
- Normal function: Transcription factor for maturation of granulocytes
- Mutated in 15 – 20% of patients with AML
- Improved outcomes for patients with this mutation, independent of other mutations
**FLT3**
- Chromosome 13q
- Normal function: tyrosine kinase that is important for proliferation and differentiation of hematopoietic progenitor cells
- Mutated in 30 – 40% of AML patients
  - ITD, D835 point mutation, overexpression without mutation
- Uncontrolled proliferation leads to inferior overall and disease-free survival

**NPM1**
- On chromosome 5q
- Normal function: controls genomic stability
- Mutation in 50 – 60% AML
  - Either insertion or deletion
  - Increased in women
- Sole mutation present, improved outcomes
  - Outweighed by other negative mutations like FLT3

**MLL**
- On chromosome 11q
- Normal function: encodes enzyme that regulates homeostasis
- Mutation in 7 – 8% of AML patients as a partial tandem duplication
- Decreases overall survival

**IDH1 and IDH2**
- IDH1 on Chromosome 2q
- IDH2 on Chromosome 15q
- Normal function: critical to the Krebs cycle
- Mutations in 15 – 30% AML patients
- Results in increased expansion of HSCs and impaired differentiation

**BCL-2**
- On chromosome 18q
- Normal function: inhibit apoptosis and modulates cell cycle progression
- In Burkitt's lymphoma, moves upstream of IgH t(14;18)
- Overexpression leads to prolonged cell survival

**BCL-6**
- On chromosome 3q
- Normal function: represses transcription
- Often overexpressed in DLCL
- Mutation leads to increased proliferation
**TP53**

- On chromosome 17p
- Tumor suppressor that prevents uncontrolled cell growth
- Mutation of 17p found in many cancers
  - CLL, DLCL, solid cancers

**MONITOR FOR RESIDUAL DISEASE AFTER TREATMENT**

**WAS TREATMENT SUCCESSFUL?**

Routinely checked before and after treatment.

- AML: CEBP-α, FLT3-D835 point mutation, FLT3-ITD mutation, IDH1, IDH2, NPM1, MLL
- ALL: BCR/ABL, TEL-AML/AML1
- MDS: ASXL1, JAK2, ETV6, EZH2, P53, RUNX1
- Lymphoma: BCL-1 (CCND1), BCL-2, BCL-6, IgH, TCR

**WHERE DOES THIS ALL FIT IN?**

**CASES FROM MY PRACTICE**

**CASE 1**

- 52 yr old male. H/O hyperlipidemia, HTN, bipolar disorder, back injury
- Presented in Sep 2018, with 3 wk h/o dizziness, fatigue, abdominal pain.
- Exam: T 98.4, BP 123/65, Pulse 73, BMI 22, Spleen tip palpable.

<table>
<thead>
<tr>
<th>Basename</th>
<th>Value Date/Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC COUNT</td>
<td>189.7 09/26/2018</td>
</tr>
<tr>
<td>HGB</td>
<td>11.1 09/26/2018</td>
</tr>
<tr>
<td>HEMATOCTRT</td>
<td>36.0 09/26/2018</td>
</tr>
<tr>
<td>PLATELET COUNT</td>
<td>179 09/26/2018</td>
</tr>
<tr>
<td>PLATELETS,BLD,QL, MAN CT</td>
<td>CONFRIRMD 09/26/2018</td>
</tr>
</tbody>
</table>
CASE 1 WORK UP; Bone marrow biopsy report

• The peripheral blood smear is not available for the evaluation.

The biopsy shows fragments of crushed bone with little bone marrow elements. The bone marrow demonstrated about 80% cellularity with marked myeloid proliferation. The reticulin stain is not contributory due to scant amount of marrow elements.

The clot sections demonstrate hypercellularity bone marrow (about 100%) with marked myeloid proliferation.

The aspirate is satisfactory for the evaluation. The myeloid blasts are less than 1%. There is marked myeloid hyperplasia (M:E ratio about 39:1) and left shift. The megakaryocytes appear decreased number on the aspirate smears. Dwarf megakaryocytes are observed. The iron is present and no ring sideroblasts are identified.

FLOW CYTOMETRY

• A population of myeloid blasts accounts for approximately 0.7% of the acquired events. The blasts show equivocal partial expression of CD7, which, if confirmed, is an aberrant finding. Basophils account for approximately 2% of events. Correlation with the bone marrow and cytogenetic findings is suggested.

CYTOGENETICS and FISH

• Method: GTG FISH
• Number of cells/colonies: 20
• Band Level: 350
• Karyotype: 45-46,XY,t(9;22)(q34;q11.2)[cp20]
• FISH: Positive for Philadelphia chromosome translocation in 89% (89/100) of cells

CASE 1. Treatment

BCR/Abl

• Fusion protein that results in increased activity of a tyrosine kinase
• Present in CML, ALL (30–35% adult B-cell), and some AML
• Can be followed quantitatively with a Major Molecular Response (MMR) determined as ≤ 0.1% BCR-ABL (ratio of BCR-ABL/BCR)
Case 1. Treatment Course.

**6 mos Bone marrow and Cytogenetics**
- The bone marrow aspirate is relatively normocellular for age and shows trilineage hematopoiesis with a relative myeloid hyperplasia.
- Blasts are not increased. The patient's history of chronic myeloid leukemia is noted; correlation with cytogenetic and molecular studies is recommended for more sensitive markers of residual disease.

**Quant PCR**
- **BCR/ABL GENE TRANSLOCATION, PCR, QUANTITATIVE**
  - **Result IS%(p210)**: Positive
  - **Breakpoint Reference Range**
    - **e13a2/e14a2**
    - **LOD = 0.002 IS%**
    - **LOQ = 0.002 IS%**
    - **MMR = 0.10 IS%**

**CASE 2. Benign Hematology**

**Platelet Function testing.**
- Platelets play a key role in both hemostasis and thrombosis.
- Accurate measurement of platelet function is critical for identifying patients with platelet dysfunction or hyperfunction, but it also is becoming increasingly important for the monitoring of modern antiplatelet therapy.
- A major problem concerning the testing of platelet function is the difficulty in simulating hemostasis in vitro. Platelets are also sensitive to manipulation, and are prone to artifactual in vitro activation.
- The ability to test platelet function in the routine laboratory improved with the introduction of platelet aggregometry.

**Thromboelastography (TEG) and ROTEM**
- Thromboelastography (TEG) tests both platelet function and coagulation by assaying several parameters of clot formation dynamically in whole blood.
Case 2:
Platelet hyperreactivity noted.

• Added anti-platelet agent.

• Successful amputation with no further clotting

Molecular Diagnosis in Oncology- The age of Precision Medicine. Our goal for the Single patient.

Connecting specific medical treatments to individual characteristics; The "Right patient with the Right Intervention"

- Pathology
- Genomic
- Transcriptomic
- Proteomic
- Metabolomic
- Pharmacogenomic
- Microbiomics

Clinical Applications of Comprehensive Genomic Profiling in Oncology

CASE #

• 37 year old medical professional never smoker active, presented to ER 11/12 with worsening dyspnea of 3 weeks duration and decreased exercise capacity and back pain
• Initial labs unremarkable
• Exam: Absent BS right lung field, distended neck veins
• Admitted to hospital

CASE
CASE

Course in hospital
- Tube thoracotomy
- Pericardiocentesis
- Pericardial window
- Cytology: Adenocarcinoma. IHC consistent with Pulmonary origin.
- MRI Brain: Normal
- Bone scan: Extensive Mets involving spine, ribs, and pelvis.

DIAGNOSIS: STAGE IV NSCLC**

Lung Cancer Standard treatment for stage IV in 2011

The Evolution of Genomic Science.
1950-2016

Central Dogma

DNA is transcribed to RNA is translated to PROTEIN

During replication, DNA is copied to DNA

History of Genomic Medicine:
- 1953: Discovery of DNA structure by Watson and Crick
- 1973: First sequence of 26 bp published
- 1977: Sanger sequencing method published
- 1980: Nobel prize to Mullis and Khorana
- 1982: Genome sequencing started
- 1985: Development of PCR
- 1987: 1st automated sequencer (ABI 370)
- 1988: SANGER sequencer released
- 2000: Human Genome sequenced (COST approx. $3 billion)
- 2006: 1st SAGE (Serial Analysis of Gene Expression) system
- 2008: 1st single molecule sequencer
- 2012: Nanopore Technologies: Ultra long single molecule reads
Sanger sequencing

The Sanger sequencing method is a widely used technique for determining the nucleotide sequence of DNA by enzyme-catalyzed chain termination. In this method, a DNA template is used with a DNA polymerase enzyme that stops elongating the DNA strand once a specific nucleotide (such as dideoxyinosine triphosphate) is incorporated into the growing DNA chain. The resulting DNA fragments are then separated by electrophoresis and analyzed to determine the sequence.

Current strategies for sequencing

Presented By Richard Haspel at 2018 ASCO Annual Meeting

- Roche/454 FLX: 2004
- Illumina Genome Analyzer: 2006
- Applied Biosystems SOLiD™ System: 2007
- Helicos Heliscope™: recently available
- Pacific Biosciences SMRT: launched 2010
- Ion Torrent: Thermo Fisher; USED by STRATA (KPNC Collaboration)

Basic NGS Workflow

- Sample Prep: Isolation of material, PCR amplification, End repair, size selection
- Sequencing: Library QC, Cluster generation, Instrument operation
- Data Analysis: QC and pipeline analysis, Data interpretation

Platforms

- Helicos Heliscope™: recently available
- Pacific Biosciences SMRT: launched 2010
- Ion Torrent: Thermo Fisher; USED by STRATA (KPNC Collaboration)

Commercialization:

We bring the world of genetics to you.

- Next-day or 2-day shipping
- No monthly subscription needed
- Includes access to CLIA and laboratory testing services
- 1-year warranty

Order now $99
CASE

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DIAGNOSIS: STAGE IV NCSLC**

Lung Cancer: treatment of advanced stage 2011.

The Prevalence of EGFR Mutations
NSCLC Tumors

- Approximately 90% are exon 19 deletions or exon 21 (L858R) substitution
- Other EGFR mutations
- No EGFR mutations

- 10% - 15% EGFR Mutation - Positive NSCLC Tumors
- Exon 19 deletion or exon 21 (L858R) substitution

EURTAC: Tarceva (Erlotinib) Tablets Doubled Median PFS 64

For important safety information, please see slides 41 - 53 and full Prescribing Information for TARCEVA.

Investigator-Assessed PFS in EURTAC Intent-To-Treat Population

- Median PFS (95% CI), Months
- 66% Reduction in the Risk of Cancer Progression or Death

Erlotinib (n=86)
- 10.4 (8.7 - 12.9)
- HR=0.34 (95% CI, 0.23 - 0.49)
- Log-rank P<0.001

Chemotherapy (n=88)
- 5.2 (4.6 - 6.0)

ORR in the EURTAC Intent-to-Treat Population

- Objective Response Rate was 65% (95% CI, 54.1% - 75.1%) for patients treated with Tarceva (erlotinib) tablets and 16% (95% CI, 9.0% - 25.3%) for patients treated with chemotherapy

EURTAC: Objective Response Rate

- 65% Reduction in the Risk of Cancer Progression or Death

For important safety information, please see slides 41 - 53 and full Prescribing Information for TARCEVA.

- 5.2
- 10.4

Lung Cancer: Poster Child for Precision Medicine
Molecular Profiling Has Changed the Classification of Lung Cancer

Lung Adenocarcinoma

- EGFR
- ALK
- ROS1
- RET
- BRAF
- NF1
- NEK2
- MAPK7
- MEK1
- NEU1
- NEU2
- MET
- PI3K
- PIK3CA
- PTEN
- NRAS
- NRAS
List of Current Genes & select Intronic Alterations with actionable mutations or Investigational agents

CLINICAL RESEARCH KPNC ONCOLOGY

- STRATA MATCHED trials: 5 awaiting activation; PARP Inhibitor, RET Mutation, ERBB2 overexpression, FGF1 amplification, Her-2 Exon 20.

- NCI MATCH: ALK, EGFR, CDK4/6 Amplification, C-Kit, FGFR, GNAQ/GNA11, Her-2, MET, NTRK, PTEN (I/Del) Mutation, PIK3CA, ROS1, Smoothened (SMO) or patched 1 (PTCH1) mutations, TSC1 or TSC2 mutations.

Common Techniques for oncologic mutation testing: 'HOT SPOT'.

1. Sanger Sequencing
2. Gene Dosing: Measure presence or absence of a particular region of gene; Multiplex Ligation Probe Analysis, Quatitative RT-PCR
3. FISH: Gene rearrangement such as ALK

Approx cost to detect each alteration: $500-$2000.

ALL THESE TESTS DETECT JUST ONE SPECIFIC ALTERATION!

OR CAN DO THE SAME WITH GENOMIC SEQUENCING OR RNAseq (Reverse Transcribe)….

CASE #

37 year old medical professional never smoker active, presented to ER 11/11 with worsening dyspnea of 3 weeks duration and decreased exercise capacity and back pain.

Initial Labs unremarkable

Exam: Absent BS right lung field, distended neck veins

Admitted to hospital

Course in hospital

- Tuberculosis
- Percutaneous pneumothorax
- Percutaneous window
- Cytology: Adenocarcinoma
- MRI Brain: Normal
- Bone scan: Extensive Mets involving spine, ribs and pelvis.

TESTS: EGFR MUTATION, POSITIVE FOR EGFR MUTATION AT EXON 20;
NEGATIVE FOR ALK REARRANGEMENT BY FISH

Started on Chemotherapy for Stage IV NSCLC.
LIMITATIONS OF SINGLE/LIMITED ALTERATION ANALYSIS

- Not comprehensive:
- Not a practical approach to assess several genetic alterations.
- COSTLY.
- Not consistent with precision medicine/patient centered care.
- Older technology/methods, incapable of detecting all alterations.

THE CASE FOR COMPREHENSIVE GENOMIC PROFILING IN CANCER THERAPY

1. RAPID IDENTIFICATION OF ACTIONABLE MUTATION, "DRUG-ABLE" TARGET
2. IDENTIFICATION OF PREVIOUSLY UNKNOWN CANCER GENES, DISCOVERY OF ADDITIONAL PATHWAYS FOR DRUG DEVELOPMENT

Genomic Profiling of lung adenocarcinoma patients reveals therapeutic targets when standard testing is negative

- 31 percent of patients harbored clinically relevant genomic alterations that were not previously discovered by the prior clinical testing.
- 39% of patients had alterations within NCCN guidelines.
- 27% of patients had alterations for which clinical trials of targeted therapies could be considered
CGP/NGS has higher Sensitivity.

- Ability to detect alterations that were not previously detected.

CASE #

Course in hospital

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TESTED FOR EGFR MUTATION: POSITIVE FOR EGFR MUTATION AT EXON 20
NEGATIVE FOR ALK REARRANGEMENT BY FISH

Started on Chemotherapy for Stage IV NSCLC.

CASE #

Biopsy sent for Next Gen Sequencing (Foundation One)
Mutually Exclusive Mutations - questionable.

NGS in CLINICAL ONCOLOGY

- Comprehensive Genomic Profiling/Next Gen Sequencing of Cancers is rapidly gaining acceptance and represents a pivotal step towards precision medicine.
- Next Gen sequencing is comparable in price to routine imaging technologies
- It is expected to be as routine as special stains in the pathology lab
- As important as the X-RAY or Microscope in the diagnosis and management of cancers.
- Will lead to new targets for therapeutic exploitation
- Will require a new framework for the management of cancers in terms of regulatory issues, quality control and the conduct of efficient clinical trials.

Molecular Oncology Case Conference
Launched Feb 2019.

GOALS and OBJECTIVES

Conclusions

- The role of Laboratory medicine in management of cancers and blood disorders is invaluable and indispensable.
- Will have an expanding role in the era of precision medicine
- Close collaboration between physicians and laboratory scientists and technologists is critical in providing the best care for our members.
- Thank you.
- Questions?