Workshop 5: Update on Diagnostic Techniques in Cancer Medicine

Canadian CTG: New Investigator Clinical Trials Course
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Workshop Objectives

At the end of this workshop, participants will be able to:
- List the characteristics of an effective diagnostic for the selection of a cancer treatment,
- Describe the challenges of developing effective diagnostics in cancer medicine,
- Develop strategies to collaborate with pathology/laboratory medicine to design clinical trials incorporating companion diagnostics.
Before and During the Workshop

• Before: Review this presentation, and included references. Prepare to discuss questions raised in this presentation.

• During: The workshop will include an interactive discussion of these case studies, with additional didactic materials and references.
Disclosure

• No relevant conflicts to declare.
Overview

• Diagnostics are Important – A Cautionary Tale from a Now-Established Biomarker

• Case Study 1: Diagnostic(s) for Cancer Immunotherapy
• Case Study 2: A Diagnostic for Ovarian Cancer Targeted Therapy
• Case Study 3: Multiple Mutation Analysis for Lung Cancer Targeted Therapy

• Diagnostic Techniques in Clinical Trials – Hints and Tips
“A Cautionary Tale” (Case Study 0)

The New England Journal of Medicine

USE OF CHEMOTHERAPY PLUS A MONOClonAL ANTIBODY AGAINST HER2 FOR METASTATIC BREAST CANCER THAT OVEREXPRESSES HER2

DENNIS J. SLAMON, M.D., PH.D., BRIAN LEYLAND-JONES, M.D., STEVEN SHAK, M.D., HANK FUCHS, M.D., VIRGINIA PATON, PHARM.D., ALEX BAJAMONDE, PH.D., THOMAS FLEMING, PH.D., WOLFGANG EIERMANN, M.D., JANET WOLTER, M.D., MARK PEGRAM, M.D., JOSE BASELGA, M.D., AND LARRY NORTON, M.D.*

Questions for Discussion:

a) What was the definition of “overexpresses HER2”?
b) What was the scientific basis of this definition?
c) How was the diagnostic for HER2 overexpression validated?
HER2: Human Epidermal Growth Factor Receptor 2

Importance in human breast cancer first shown in 1987

Slamon et al., Science 1987
(HER2 assessment by Southern blotting)
# HER2 Adjuvant Trials: What Diagnostics Were Used?

<table>
<thead>
<tr>
<th>Trial</th>
<th>IHC</th>
<th>ISH</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSABP B31 (n=2119)</td>
<td>Positive =3+ (≥10% strong)</td>
<td>FISH ratio ≥ 2 if IHC 2+</td>
</tr>
<tr>
<td>NCCTG 9831 (n=3505)</td>
<td>Positive =3+ (≥10% strong)</td>
<td>FISH ratio ≥ 2 if IHC 2+</td>
</tr>
<tr>
<td>HERA (n=5081)</td>
<td>Positive =3+ (unspecified)</td>
<td>FISH ratio ≥ 2 if IHC 2+</td>
</tr>
<tr>
<td>BCIRG 006 (n=3222)</td>
<td>Not used</td>
<td>FISH ratio ≥ 2</td>
</tr>
<tr>
<td>Finnish Trial (n=232 in HER+ arm)</td>
<td>All IHC 2+ or 3+ were confirmed by ISH</td>
<td>Single Probe CISH HER2 copies ≥ 6 in &gt;50% of cells*</td>
</tr>
</tbody>
</table>

* FinHer: If HER2 copies between 4 and 6, additional CEP17 probe used to assess for ratio ≥ 2.0
The Evolution of HER2 Diagnostics

MBC = Metastatic Breast Cancer

Reviewed in: Brufsky, Am J Clin Oncol 2010
“HER2 Positive”:

Before 2007; after 2013
• Positive (3+): >10% of cells with strong membranous staining
• Positive: HER2/CEP17 ratio > 2.0 (or HER2 > 6)

2007 to 2013
• Positive (3+): >30% of cells with strong membranous staining
• Positive: HER2/CEP17 ratio > 2.2 (or HER2 > 6)

Take Home Point:
Design of the original trials shaped eventual practice for both diagnostics and therapeutics. Because of how this drug was originally tested, we don’t know (and may never know) the best way to test for it.

Wolff et al., Arch Pathol Lab Med. 2007;131:18–43
Case Study 1: Diagnostic(s) for Cancer Immunotherapy

Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial

Lancet 2016;387:1540-50

• Questions for Discussion:
  a) What is the definition of “PD-L1-positive”?
  b) What is the scientific basis of this diagnostic approach?
  c) How might you design a trial to determine the patient population most likely to respond to drugs such as pembrolizumab?
PD-L1 and Immune Checkpoints

- PD-L1 contributes to a tumour immune microenvironment that inhibits T-cell function.
- Targeting PD-L1 enhances the host immune response.
- Does PD-L1 protein expression level make sense as a biomarker?

Gibney et al, Lancet Oncol 2016;16:e542
PD-L1: A Tale of 2 Trials

Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial

Roy S Herbst, Paul Boss, Dong-Wan Kim, Enrique Felip, José L Pérez-Gracia, Ji-Youn Han, Julien Molina, Joe-Hong Kim, Catherine Dubes Arici, Myung-Ju Ahn, Margarita Majim, Mary J Fidler, Gilberto de Castro Jr, Marco García, Gregory M Lubiniecki, Yue Shentu, Ellielin, Marisa Dalled-Filtsch, Edward B Golan

References: Reck et al, NEJM 2016:375
Herbst et al, Lancet 2016;387
PD-L1: A Tale of 2 Trials

- In untreated metastatic EGFR- and ALK-negative NSCLC, pembrolizumab vs. chemo.
- At least 50% PD-L1 IHC expression was an inclusion criterion. (Remind you of anything?)

This study led to the following FDA approval for pembrolizumab:

- patients with metastatic NSCLC whose tumors have high PD-L1 expression ([Tumor Proportion Score (TPS) ≥50%]) as determined by an FDA-approved test, with no EGFR or ALK genomic tumor aberrations, and no prior systemic chemotherapy treatment for metastatic NSCLC. (1.2)

Reference: Reck et al, NEJM 2016:375
PD-L1: A Tale of 2 Trials

• PD-L1 IHC score of 1% was used as an inclusion criterion, but stratified as part of the analysis.

• This was to decide maintenance therapy for previously-treated metastatic NSCLC.

• Led to the following FDA approval for pembrolizumab:

Reference: Herbst et al, Lancet 2016;387
For Diagnostics: The most informative strategy will require prospective tissue collection, along with retrospective multi-modality testing.
Case Study 2: A Diagnostic for Ovarian Cancer
Targeted Therapy

Part 1

• Question for Discussion:
  a) These studies led to FDA-approval for olaparib. What is the FDA-approved diagnostic?
  b) Can an independent laboratory develop a test for BRCA-mutation status?
Case Study 2: A Diagnostic for Ovarian Cancer
Targeted Therapy

Part 1

Patients and Methods
This multicenter phase II study enrolled individuals with a germline BRCA1/2 mutation and recurrent cancer. Eligibility included ovarian cancer resistant to prior platinum; breast cancer with ≥ three chemotherapy regimens for metastatic disease; pancreatic cancer with prior gemcitabine treatment; or prostate cancer with progression on hormonal and one systemic therapy. Olaparib was administered at 400 mg twice per day. The primary efficacy end point was tumor response rate.

The FDA Label was based on the subcohort of ovarian cancer patients. (Results on Next Slide.)

There are several limitations to this study. There was no central laboratory or central review of mutational status before enrollment. However, the vast majority of patients (265 [89%] of 298) carried truncating mutations, including 158 (53%) who carried one of the Ashkenazi Jewish founder mutations (and one patient carried two). Of the remaining patients, 17 carried missense mutations. Although it can be more difficult to establish the deleterious nature of missense mutations (and there is no universal standard for classification at this time), six missense mutations were BRCA1 C61G (clearly established pathogenic founder mutation). Thus, we feel that the chance of misclassification of mutation status is low.

A companion diagnostic was not included in this study, but evaluated separately in a retrospective “bridging study”.
Case Study 2: A Diagnostic for Ovarian Cancer Targeted Therapy

14 CLINICAL STUDIES

The efficacy of Lynparza was investigated in a single-arm study in patients with deleterious or suspected deleterious germline *BRCA*-mutated (*gBRCA*m) advanced cancers (Study 1). A total of 137 patients with measurable, *gBRCA*m-associated ovarian cancer treated with three or more prior lines of chemotherapy were enrolled. All patients received Lynparza at a dose of 400 mg twice daily as monotherapy until disease progression or intolerable toxicity. Objective response rate (ORR) and duration of response (DOR) were assessed by the investigator according to RECIST v1.1.

The median age of the patients was 58 years, the majority were Caucasian (94%) and 93% had an ECOG PS of 0 or 1. Deleterious or suspected deleterious, germline *BRCA* mutation status was verified retrospectively in 97% (59/61) of the patients for whom blood samples were available by the companion diagnostic BRACAnalysis CDx™, which is FDA approved for selection of patients for Lynparza treatment.

Efficacy results from Study 1 are summarized in Table 5.

**Table 5 Overall Response and Duration of Response in Patients with *gBRCA*-mutated Advanced Ovarian Cancer Who Received 3 or More Prior Lines of Chemotherapy in Study 1**

<table>
<thead>
<tr>
<th></th>
<th>N=137</th>
</tr>
</thead>
<tbody>
<tr>
<td>Objective Response Rate (95% CI)</td>
<td>34% (26, 42)</td>
</tr>
<tr>
<td>Complete Response</td>
<td>2%</td>
</tr>
<tr>
<td>Partial Response</td>
<td>32%</td>
</tr>
<tr>
<td>Median DOR in months (95% CI)</td>
<td>7.9 (5.6, 9.6)</td>
</tr>
</tbody>
</table>
Case 2: Summary of Part 1

- The “FDA-approved” companion diagnostic becomes a de facto “gold standard”, sometimes without full justification.
- A significant limitation arises when insufficient tissue is available for retrospective analysis.
- When “positive status” is an eligibility criterion, it is difficult to confirm what happens when you treat “test-negative” patients.
Case Study 2: A Diagnostic for Ovarian Cancer Targeted Therapy

Part 2

Olaparib combined with chemotherapy for recurrent platinum-sensitive ovarian cancer: a randomised phase 2 trial

Amit M Oza, David Cibula, Ana Oaknin Benzaquen, Christopher Poole, Ron H J Mathijssen, Gabe S Sonke, Nicoletta Colombo, Jiří Špaček, Peter Vuytske, Holger Hirte, Sven Mahner, Marie Plante, Barbara Schmalfeldt, Helen Mackay, Jacqui Rowbottom, Elizabeth S Lowe, Brian Dougherty, J Carl Barrett, Michael Friedlander

Lancet Oncology 2015;16:87-97
(see also Ledermann et al, Lancet Oncology 2016;17:1579-89)

• Questions for Discussion:
  a) What is the biologic rationale for olaparib in “recurrent platinum-sensitive” ovarian cancer?
  b) What is definition of “recurrent platinum-sensitive”?
  c) What is the diagnostic for “recurrent platinum-sensitive”? (Trick question.)
  d) What is the role for BRCA-mutation testing to select patients for this treatment strategy? (Not a trick question.)
Olaparib and BRCA

PARP Inhibition and Tumor-Selective Synthetic Lethality

Endogenous DNA damage (SSBs) → PARP inhibition → DNA DSBs accumulate with normal DNA replication.

Functional HR pathway (i.e., BRCA wt in normal cell) → HR-mediated DNA repair → Cell survival.

HR-deficient tumor cell (i.e., BRCA1/2/-) → Impaired HR-mediated DNA repair → Cell death.

DSB = double-strand break; HR = homologous recombination; SSB = single-strand break.

Olaparib and Platinum

**SSB**
(cellular metabolism, environmental exposures)

↓

PARP

*olaparib, etc*

**DSB**
(platinum agents, topoisomerase I inhibitors)

↓

BRCA

NHEJ

SSA

Mismatch repair

Base excision repair

Homologous recombination

Nucleotide excision repair
Case Study 2: A Diagnostic for Ovarian Cancer
Targeted Therapy

Part 2

• Questions for Discussion:
  a) What is the biologic rationale for olaparib in “recurrent platinum-sensitive” ovarian cancer?
  b) What is definition of “recurrent platinum-sensitive”?
  c) What is the diagnostic for “recurrent platinum-sensitive”? (Trick question.)
  d) What is the role for BRCA-mutation testing to select patients for this treatment strategy? (Not a trick question.)

There is none.

BRCA-testing is still needed for the on-label use of olaparib. This study supports expanding the use of olaparib to non-BRCA patients.
Case Study 3: Multiple Mutation Analysis for Lung Cancer Targeted Therapy


**Multi-institutional oncogenic driver mutation analysis in lung adenocarcinoma: The Lung Cancer Mutation Consortium experience**

Lynette M. Sholl, MD#1, Dara L. Aisner, MD PhD#2, Marileila Varella-Garcia, PhD#2,3, Lynne D. Berry, PhD#4, Dora Dias-Santagata, PhD5, Ignacio I. Wistuba, MD6, Heidi Chen, PhD4, Junya Fujimoto, MD PhD6, Kelly Kugler, BA3, Wilbur A. Franklin, MD2, A. John lafrate, MD PhD5, Marc Ladanyi, MD7, Mark G. Kris, MD7, Bruce E. Johnson, MD9, Paul A. Bunn, MD3,8, John D. Minna, MD10, David J. Kwiatkowski, MD PhD9, and on behalf of the LCMC Investigators

• Questions for Discussion:
  a) Why is tissue from a surgical resection specimen preferable for multiple mutation testing?
  b) What type(s) of error does inter-institutional validation reduce?
  c) What type(s) of error does proficiency testing reduce?
  d) Why is this type of study essential in developing novel diagnostics?
Case Study 3: Multiple Mutation Analysis for Lung Cancer Targeted Therapy

• Questions for Discussion:
  a) Why is tissue from a surgical resection specimen preferable for multiple mutation testing?

Results—1007 specimens had mutation analysis performed, and 733 specimens had all 10 genes analyzed. Mutation identification rates did not vary by analytic method. Biopsy and cytology specimens were inadequate for testing in 26% and 35% of cases compared to 5% of surgical specimens. Among the 1007 cases with mutation analysis performed, EGFR, KRAS, ALK, and
Analytical validity requires low systematic and low random error.

Two Types of Error

**systematic error**
- poor accuracy
- definite causes
- reproducible

**random error**
- poor precision
- nonspecific causes
- not reproducible

- “DRIFT”
Analytical validity requires low systematic and low random error.

<table>
<thead>
<tr>
<th>The ACCE framework</th>
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<tbody>
<tr>
<td><strong>A</strong>nalitical performance and validation</td>
</tr>
<tr>
<td><strong>C</strong>linical validation</td>
</tr>
<tr>
<td><strong>C</strong>linical utility</td>
</tr>
<tr>
<td><strong>E</strong>thical, legal &amp; social implications of the test</td>
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</tbody>
</table>

Case Study 3: Multiple Mutation Analysis for Lung Cancer Targeted Therapy

• Questions for Discussion:
  a) Why is this type of study essential in developing novel diagnostics?
Case Study 3: Multiple Mutation Analysis for Lung Cancer Targeted Therapy

• Questions for Discussion:
  
a) Why is this type of study essential in developing novel diagnostics?

What do we need to make circulating tumour DNA (ctDNA) a routine diagnostic test in lung cancer?

Reyes Bernabé a,b, Nicholas Hickson c, Andrew Wallace c, Fiona Helen Blackhall a,d,*

Table 2
Results for clinical outcomes of patients according to plasma EGFR mutation status in randomised trials of EGFR-TKIs versus chemotherapy and the IFUM phase IV study.

<table>
<thead>
<tr>
<th>Study [ref] (TKI)</th>
<th>Method (see Table 1 for abbreviations)</th>
<th>ctDNA test performance (tissue as reference)</th>
<th>Clinical outcome for TKI versus chemotherapy (CT)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Specificity (PPV)</td>
<td>Sensitivity (NPV)</td>
</tr>
<tr>
<td>IPASS [29] (G)</td>
<td>DxS ARMS</td>
<td>100%</td>
<td>43.1%</td>
</tr>
<tr>
<td>IFUM [41] (G)</td>
<td>Therascreen RGQ</td>
<td>99.8%</td>
<td>65.7%</td>
</tr>
<tr>
<td>EUR TAC [43] (E)</td>
<td>Taqman</td>
<td>100%</td>
<td>78%</td>
</tr>
<tr>
<td>FASTACT-2 [44] (E)</td>
<td>Cobas blood</td>
<td>96%</td>
<td>75%</td>
</tr>
<tr>
<td>(note CT ± E)</td>
<td></td>
<td>(94%)</td>
<td>(85%)</td>
</tr>
<tr>
<td>LUX-LUNG 3 [16] (A)</td>
<td>Therascreen 29</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LUX-LUNG 6 [16] (A)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ENSURE [12,34] (E)</td>
<td>Cobas v2</td>
<td>(96.8%)</td>
<td>(81.4%)</td>
</tr>
<tr>
<td>AURA3 [5,12] (O)</td>
<td>Cobas v2</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: TKI: tyrosine kinase inhibitor, PPV: positive predictive value, NPV: negative predictive value, G: gefitinib, E: erlotinib, A: afatinib, O: osimertinib, RR: response rate, PFS: progression free survival (median months) HR: Hazard Ratio, NA: not assessed (single arm study), NS: not stated, *significant interaction test for biomarker p value < 0.05, **E independent predictor in multivariate analysis, ***based on cycle 3 ctDNA EGFR mutation status.
Wrap-up

- Diagnostics is a relatively neglected area in clinical trials design.
- A good diagnostic must have analytical validity, clinical validity, and clinical utility.
- We are now beginning to learn from our past mistakes—the resulting biomarkers will be that much better for it!

Dan Hayes, 2013
References

• Case 0: Slamon et al, NEJM 2001;344:783-92
• Case 1: Reck et al, NEJM 2016:375
  Herbst et al, Lancet 2016;387:1540-50
• Case 2: Fong et al, NEJM 2009; 361:123-34
  Kaufman et al, JCO 2015;33:244
  Oza et al, Lancet Oncology 2015;16:87-97
  Ledermann et al, Lancet Oncology 2016;17:1579-89
• Case 3: Sholl et al, J Thorac Oncol 2015;10:768-777
  Bernabé et al, Eur J Cancer 2017;81:66-73