What the clinician needs to know about molecular biomarkers in ovarian cancer.

Time to implement in routine care?

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I have been a speaker for Roche – honorarium paid to my clinic.

Diagnostics and Prediction

1. Your favorite biomarker will fail
2. Choice of treatment (differential diagnosis)
3. BRCAness
4. MSI-H
5. Prognostic risk groups

For today I will focus on epithelial ovarian cancer, and I will also make use of recent review articles.

Biomarker Studies – A Problem!

Biomarkers are highly relevant (everyone wants them) BUT few reach the clinic.

Estimated that <1% of cancer biomarkers make it to the clinic

There is a strong pressure to find and identify biomarkers, but it is considerably more difficult to actually validate them.

”Failure patterns formed a hierarchical logical structure, or outline, of an emerging, deeply complex, and arguably fascinating science of biomarker failure.”

Kern SE. Why your new cancer biomarker may never work: recurrent patterns and remarkable diversity in biomarker failures. Cancer Res. 2012;72(23):6097–101. pmid:23172309

Reasons for failure

- lack of clinical significance (UTILITY)
- hidden structure in the source data
- a technically inadequate assay
- inappropriate statistical methods
- unmanageable domination of the data by normal variation
- Implausibility
- deficiencies in the studied population or in the investigator system
- disproof or abandonment for cause by others

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REMARK

2005/6: Recognition of deep problems with biomarker studies. Publication of a checklist for reporting of biomarker studies.

Originally intended for prognostic tumor markers in blood, tissue and other body fluids.

However, the checklist is generally applicable.

2012: Publication of a detailed article with extensive explanation for each variable.

2018: Publication of an abbreviated article examining each variable.

Journals that require REMARK generally publish more studies that follow REMARK (about 60% of studies).

Sauerbrei W, Taube SE, McShane LM, Cavenagh MM, Altman DG. Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK): An Abridged Explanation and Elaboration. J Natl Cancer Inst. 2018 Aug 1;110(8):803-811.

INTRODUCTION

1 State the marker examined, the study objectives, and any pre-specified hypotheses.

MATERIALS AND METHODS

Patients

2 Describe the characteristics (e.g., disease stage or co-morbidities) of the study patients, including their source and inclusion and exclusion criteria.

3 Describe treatments received and how chosen (e.g., randomized or rule-based).

Specimen characteristics

4 Describe type of biological material used (including control samples) and methods of preservation and storage.

Assay methods

5 Specify the assay method used and provide (or reference) a detailed protocol, including specific reagents or kits used, quality control procedures, reproducibility assessments, quantitation methods, and scoring and reporting protocols. Specify whether and how assays were performed blinded to the study endpoint.

Study design

6 State the method of case selection, including whether prospective or retrospective and whether stratification or matching (e.g., by stage of disease or age) was used. Specify the time period from which cases were taken, the end of the follow-up period, and the median follow-up time.

7 Precisely define all clinical endpoints examined.

8 List all candidate variables initially examined or considered for inclusion in models.

9 Give rationale for sample size; if the study was designed to detect a specified effect size, give the target power and effect size.

Statistical analysis methods

10 Specify all statistical methods, including details of any variable selection procedures and other model-building issues, how model assumptions were verified, and how missing data were handled.

11 Clarify how marker values were handled in the analyses; if relevant, describe methods used for cutpoint determination.

RESULTS

Data

12 Describe the flow of patients through the study, including the number of patients included in each stage of the analysis (a diagram may be helpful) and reasons for dropout. Specifically, both overall and for each subgroup extensively examined report the numbers of patients and the number of events.

13 Report distributions of basic demographic characteristics (at least age and sex), standard (disease-specific) prognostic variables, and tumor marker, including numbers of missing values.

Analysis and presentation

14 Show the relation of the marker to standard prognostic variables.

15 Present univariable analyses showing the relation between the marker and outcome, with the estimated effect (e.g., hazard ratio and survival probability). Preferably provide similar analyses for all other variables being analyzed. For the effect of a tumor marker on a time-to-event outcome, a Kaplan-Meier plot is recommended.

16 For key multivariable analyses, report estimated effects (e.g., hazard ratio) with confidence intervals for the marker and, at least for the final model, all other variables in the model.

17 Among reported results, provide estimated effects with confidence intervals from an analysis in which the marker and standard prognostic variables are included, regardless of their statistical significance.

18 If done, report results of further investigations, such as checking assumptions, sensitivity analyses, and internal validation.

DISCUSSION

19 Interpret the results in the context of the pre-specified hypotheses and other relevant studies; include a discussion of limitations of the study.

20 Discuss implications for future research and clinical value.

Biomarkers: Observational, empiric research

Shares the problems of observational studies.

Not an interventional clinical trial (lacks randomization).

They cannot prove causal relationships.

Most studies are ”discovery” – key decisions cannot be planned

- scope/variety of data, QC, data analysis, ”lockdown” rules
- outcomes – erroneous and underreporting
- validation and significance are not the same as UTILITY

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Large p, small n

Using high-dimensional data to search for markers

p, the quantity of parameters in the data

n, the number of samples analyzed

Large p: microarray, mass spec, DNA sequencing

Small n: relatively few patients (millions of genes, hundreds of patients).

Most studies reporting markers from ”large p, small n” are either never externally validated by independent researchers or they fail validation.

Ex: 35 studies with new molecular classification. Cited 758 subsequent studies. Only 1/758 was an independent validation.

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What the clinician needs to know #1

1. It’s OK to be excited … just also be skeptical.
2. Keep a copy of REMARK on your desk.
3. Look for independent, external validation.
4. Beware ”large p, small n”

Diagnostics and molecular diagnostics

Correct tumor type and grade – biomarkers

Beyond histology pathologists use IHC to characterize difficult cases.

PAX8, P53, WT-1, ER, PR, NapsinA

These IHC markers are related to molecular pathways, and are valuable surrogates to sequencing.

Histology = Phenotype (what do the cells can actually do)

IHC / molecular testing = Genotype

Thus histology (i.e. phenotype) has an important roll in tumor diagnosis

Genotype vs. phenotype

The relationship between a gene and a phenotype is typically not simple.

Consider height. Eye color. These phenotypes are the product of networks of genes and environmental cues.

If I sequence your DNA I will have trouble determining your height.

If I look at you then I can just measure it.

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GENOTYPE

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PHENOTYPE

These same issues are present in cancer.

Molecular diagnostics

Is there a clinical role for routine genomics of gynecologic cancers?

Currently, diagnostics does not (primarily) depend on the demonstration of gene mutations.

Therapeutic choices, however, can depend on particular molecular defects (BRCA1,2 mutation status, MSI-H), and this process will continue as new therapies become available.

Research data

↓

Clinical trial data

↓

Adoption by NT rådet and Nationell vårdprogram

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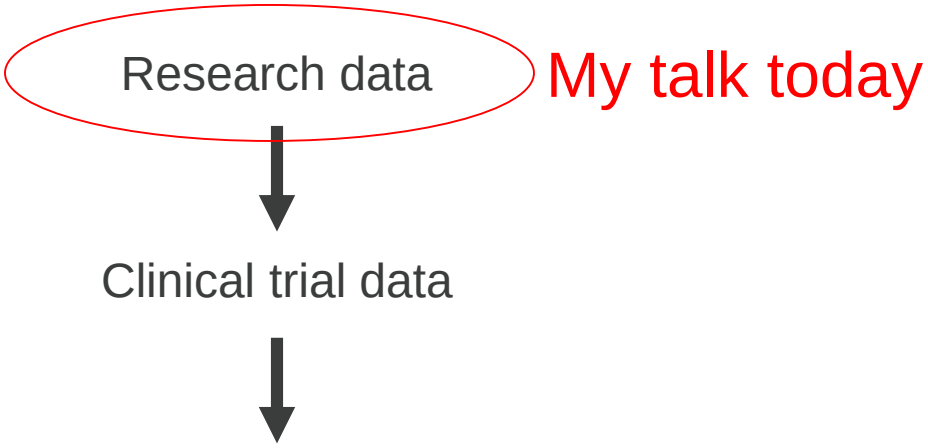

Local vs. external tests

The specific test determines if it can be set up by individual labs or if it is centralized.

Ex: Oncoscan Dx – The approval for this test in the US is based on it being performed at only one lab. All materials must be sent there to receive the ”Oncoscan score”.

Ex: EGFR mutation testing – The detection of this mutation can be done in any accredited lab, and can make use of various available kits.

… or folded into a larger, panel sequencing system.

The correct paradigm depends entirely on the details of the specific test.
There is not correct ”general answer”.

BRCAness

Tumors that share molecular features of tumors from BRCA1/2 syndromic patients may respond similarly to therapies.

BRCA1/2 dysfunction may lead to cancer by many mechanisms. Genomic integrity (HRR; homologous recombination repair) has received the most attention.

BRCA1/2 tumors show genomic instability and seem to be exclusively HGSOC. However, depending on the lab, FIGO3 endometrioid, undifferentiated, or even ”transitional cell” could also be BRCA1/2 related.

Proposed definition: BRCAness is a phenocopy of BRCA1 or BRCA2 mutation; it describes the situation in which an HRR defect exists in a tumour in the absence of a germline BRCA1 or BRCA2 mutation.

Lord CJ, Ashworth A. BRCAness revisited. Nat Rev Cancer. 2016 Feb;16(2):110-20.

BRCAness 1: Defects in individual HRR genes

Somatic mutations in genes related to HRR are present in a wide variety of tumors.

TCGA of HGSOC (489 cases) showed 50% of cases had a mutation in at least one HRR modulating gene

A subsequent study showed similar results.

Mutations in these genes can predict platinum sensitivity, but it depends on the genes.

Ex: A 12-gene panel was predictive.

Ex: BRCA1 promoter methylation is not (for example).

BRCAness 2: Transcriptional signatures of BRCAness

Multiple attempts with various levels of success in retrospective and observational cohorts.

BRCAness 3: Genomic signatures

Whole genome sequencing can reveal underlying patterns of mutations.

BRCA1/2 tumors show paticular mutational signatures
error-prone double strand break repair.

There are also particular DNA structural rearrangements.

Increased mutational burden.

BRCAness 4: Functional biomarkers

IHC for RAD51 localization.

Ex vivo DNA damage of fresh tumor biopsies or resections.

Either by radiation exposure or exposure to PARPi.

What the clinician needs to know #2

1. Pathology is largely based on phenotype (how the cells look and what they can do) with incorporation of selected biomarkers.
2. Introduction of molecular biomarkers to the clinic is happening and will continue to happen.
3. BRCAness is essentially a search for a phenocopy of BRCA1,2 carriers.
4. BRCAness will probably be an important predictive markers.
   - BUT which of the many methods will ”win” is not yet clear
   - Probably the one that shows benefit in a clinical trial!

Microsatellites

Microsatellites (also called Short Tandem Repeats) are regions of repetitive DNA in which certain DNA motifs (1-6 bp) are repeated, typically 5-10 times.

They occur at 1000's of locations within an organisms genome.

TATATATATATA – dinucleotide repeat

GTCGTCGTCGTC – trinucleotide repeat

They have higher mutation rates than typical DNA – they are noncoding

"Mutation" in microsatellites is slippage – they get longer or shorter

Mismatch repair deficiency and microsatellite instability

Mismatch repair deficiency – a distinct mechanism of carcinogenesis

Controlled by a number of specific genes: MLH1, MSH2, MSH6, PMS2

GENOTYPE

Leads to slippage of microsatelites during cell duplication.

The tumor thus shows high levels of microsatelite instability (MSI-H).

PHENOTYPE

These tumors are typically associated with a strong antitumoral immune response.

Colon cancer, endometrial cancer (and relationship to Lynch syndrome)

Mismatch repair deficiency in ovarian cancer

Approximately 7-22% of sporadic ovarian cancers are MSI-H.

Lynch related 2%

Approximately 2-29% of sporadic tumors show MMR protein loss.

Older studies before ”modern” subtyping. Small, heterogeneous cohorts.

Data indicating that MMR protein expression changed after treatment with cisplatinum. 73% changed from MSS to MSI-H. Perhaps associated with drug resistance?

Typically in non-serous subtypes – clear cell and endometrioid

Murphy MA, Wentzensen N. Int J Cancer. 2011 Oct 15;129(8):1914-22.
Dellas A, Puhl A, Schraml P, et al. Anticancer Res. 2004; 24: 361–369
King BL, Carcangiu ML, Carter D, et al. Br J Cancer. 1995; 72: 376–382.
Xiao X, Melton DW, Gourley C. Gynecol Oncol. 2014 Feb;132(2):506-12.

MSI-H and ovarian ca

Recently the FDA approved pembrolizumab for MSI-H tumors (independent of site).

Some labs in the US are performing MSI by IHC as an indication for pembrolizumab.

Clinical routine – close!

What the clinician needs to know #3

1. MSI-H is a relatively common mechanism present in ovarian ca
2. May be of clinical importance
3. Testing can be done in a variety of ways
   - IHC for MMR proteins
   - MSI testing
   - As part of a panel sequencing, thus including BRCA1,2 and MSI

Molecular subtypes of HGSOC

Gene expression analysis.

Australian Ovarian Cancer Study: C1, C2, C4, C5.

Confirmed by TCGA, renamed: immunoreactive, differentiated, proliferative, mesenchymal.

However, no differences in survival.

TCGA Network modified their groups to include purely prognostic gene signatures CLOVAR.

Thank you!