MOLECULAR ONCOLOGY XXX (2014) 1–7

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/molonc

Review

Biomarker validation and testing

*Daniel F. Hayes**

University of Michigan Comprehensive Cancer Center, 6312 CCC, 1500 E. Medical Center Drive, Ann Arbor, MI 48109-5942, USA

ARTICLE INFO

Article history:
Received 9 October 2014
Accepted 9 October 2014
Available online ■

Keywords:
Tumor biomarkers
Validation
Level of evidence

ABSTRACT

A tumor biomarker is a molecular or process-based change that reflects the status of an underlying malignancy. A tumor biomarker may be identified and measured by one or more assays, or tests, for the biomarker. Increasingly, tumor biomarker tests are being used to drive patient management, either by identifying patients who do not require any, or any further, treatment, or by identifying patients whose tumors are so unlikely to respond to a given type of treatment that it will cause more harm than good. A tumor biomarker assay should only be used to guide management if it has analytical validity, meaning that it is accurate, reproducible, and reliable, and if it has been shown to have clinical utility. The latter implies that high levels of evidence are available that demonstrate that application of the tumor biomarker test for a given use context results in better outcomes, or similar outcomes with less cost, than if the assay were not applied. Use contexts include risk categorization, screening, differential diagnosis, prognosis, prediction of therapeutic activity or monitoring disease course. Very few tumor biomarker tests have passed these high bars for routine clinical application. However, if tumor biomarker tests are going to be used to drive patient care, than an understanding, and careful assessment, of these concepts are essential, since "A Bad Tumor Biomarker Test Is as Bad as a Bad Drug."

1. Introduction

The term "personalized medicine" has recently gained widespread acceptance among both the medical and lay communities. Fundamentally, "personalized medicine" implies getting the right therapy to the right patient at the right time, dose, and schedule. Of course since the beginning of medicine, physicians have tried to determine the correct diagnosis and match appropriate therapy to the patient at hand with the best evidence available (Schilsky, 2009). However, over the last five to ten years, the tools to aid clinicians in their quest to personalize medicine have become increasingly sophisticated, and perhaps no more so than in the field of oncology. The revolution in molecular biology over the last three decades has provided a much better understanding of the aberrant pathways that drive the malignant process. The pharmaceutical industry has exploited this better understanding of tumor biology to develop therapeutic agents that are targeted to these aberrant pathways. Finally, immunologic and molecular genetic technologies that were unthinkable as

* *Tel.*: +1 734 615 6725; fax: +1 734 647 9271.
E-mail address: hayesdf@umich.edu
http://dx.doi.org/10.1016/j.molonc.2014.10.004
1574-7891/

Please cite this article in press as: Hayes, D.F., Biomarker validation and testing, Molecular Oncology (2014), http://dx.doi.org/10.1016/j.molonc.2014.10.004

recently as a decade ago have permitted the generation of diagnostic approaches that illuminate the specific changes in cancer versus normal cells.

In spite of these advances, there seems to be more hype than reality. Very few molecular diagnostic tests have gained recommendation by major guidelines bodies, and only a few tumor biomarker tests have proven successful in the marketplace (Hayes et al., 2013). Further, some tumor biomarker assays are commercially available without documented evidence that they improve patient care, and yet are being ordered and used by many clinicians. What has led to this relative state of chaos? The remainder of this review will be dedicated to the theme that "A Bad Tumor Marker Test Is as Bad as a Bad Drug (Hayes et al., 2013)," detailing the current state of affairs and knowledge about what is needed to take a tumor biomarker test from a good idea to clinical reality.

2. What is a tumor biomarker test?

It is important to understand the distinction between a tumor biomarker and a test for it (Institute of Medicine, 2012). A tumor biomarker is an indication that a normal tissue is likely to or has become malignant, and/or it provides an indication of how a malignancy will behave, either naturally or in the context of therapy. A tumor biomarker might be a molecular change, such as in a nucleic acid, protein, or metabolite. It might also be a process change, such as an alteration in tissue appearance. Further, the presence of a benign process within malignant tissue might also be considered a tumor biomarker, such as neovascularization, that in itself is not malignant but may provide an indication of the expected biology of the cancer. Tumor biomarkers may be detected and/or monitored in tissue, blood, or relevant secretions, such as urine, stool, sputum, or breast nipple aspirates.

A tumor biomarker test is used to identify or measure the perturbations reflected by the tumor biomarker. There may be one or more assays or tests that provide some indication of the status of the tumor biomarker. These may measure the same thing, or they may measure very different perturbations in the biomarker. The erbB2 gene, which encodes for the HER2 protein, provides a good example of this issue. There are at least 3 commercially available assays for *in situ* hybridization to determine amplification of the gene, several assays, mostly based on immunohistochemistry, that quantify relative expression of the HER2 protein in cancer tissue, and others that quantify relative expression of the HER2 message (Wolff et al., 2013a,b). Recently, mutations in erbB2 that activate the protein without over-expression have been reported. Each of these may a give related indication of HER2 activity, but they are all very different and may or may not provide useful similar clinical information.

3. How is a tumor biomarker test used in the clinic?

To develop and validate a tumor biomarker test, several critical issues must be addressed. First, and foremost, one must establish the intended use or context (Table 1). These include risk categorization, screening, diagnosis, prognosis, prediction of therapeutic response, and monitoring (Henry and Hayes, 2006). A tumor biomarker test might be used to place an unaffected individual into one or more categories of risk, in which he/she might take preventive or screening strategies that would otherwise be unacceptable. Perhaps the best examples of this use is the presence or absence of a germline Y chromosome. Men do not generally undergo screening or prevention for breast cancer, while women do not need to be concerned about their risk of prostate cancer. A second use context is screening for the presence of a new cancer. Few if any tumor biomarker tests have been successfully developed for this role, although as an example, use of human papilloma virus assays have been incorporated into standard of care for screening for cervical cancers. Diagnosis, or more accurately differential diagnosis, is an important issue in pathology. Tumor biomarker tests, principally immunologically-based, are used on occasion to distinguish benign from malignant tissues, and more frequently to determine that an undifferentiated cancer is epithelial versus hematopoietic or mesenchymal.

Table 1 – Intended use contexts for tumor biomarker tests.

- **Risk categorization**
- **Screening for new cancer**
- **Differential diagnosis**
  - Cancer vs. benign
  - Epithelial vs. hematopoietic vs. mesenchymal
  - Organ of origin
- **Prognosis**
  - Early stage
  - Metastatic
- **Prediction of therapy activity**
  - Early stage
  - Metastatic
- **Monitoring disease status**
  - Early stage
  - Metastatic

The most commonly used tumor biomarker assays are used to predict the future behavior of an established cancer. The term "prognostic factor" refers to a tumor biomarker test that infers a high or low risk of a cancer-related event assuming the patient receives no more therapy than he/she has already received, if any. The most widely accepted prognostic factors in cancer are the size of the primary tumor, the presence or absence of regional lymph nodes or distant metastases. These have been codified into the now classic "TNM" staging system maintained by the Joint Commission on Cancer (AJCC, 2010).

In contrast, predictive factors, also designated response modifier elements, are used to estimate the relative likelihood that a cancer will respond to a class of, or even individual, therapeutic agents. Perhaps the oldest and most widely used example of a predictive tumor biomarker is the estrogen receptor (ER), which may be measured in many ways using different assays. Regardless, patients with ER negative breast cancers do not benefit from endocrine (anti-estrogen) therapy,

Please cite this article in press as: Hayes, D.F., Biomarker validation and testing, Molecular Oncology (2014), http://dx.doi.org/10.1016/j.molonc.2014.10.004