GENE DETECTION ASSAY FOR IMPROVING THE LIKELIHOOD OF AN EFFECTIVE RESPONSE TO A HER2 ANTIBODY CANCER THERAPY

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 11/690,304, filed Mar. 23, 2007 now U.S. Pat. No. 8,076,066, which is a continuation application of and claims priority to U.S. patent application Ser. No. 09/863,101 filed May 18, 2001 (abandoned) which claims priority under 35 U.S.C. §119(e) of provisional application 60/205,754, filed May 19, 2000, all of which are incorporated herein by reference in their entireties.

FIELD OF THE INVENTION

The present invention concerns the treatment of cancers characterized by the overexpression of a tumor antigen, such as an ErbB receptor, particularly HER2. More specifically, the invention concerns more effective treatment of human patients susceptible to or diagnosed with cancer, in which the tumor cells overexpress ErbB as determined by a gene amplification assay, with an ErbB antagonist, e.g., an anti-ErbB antibody. The invention further provides pharmaceutical packages for such treatment.

BACKGROUND OF THE INVENTION

Advancements in the understanding of genetics and developments in technology and epidemiology have allowed for the correlation of genetic abnormalities with certain malignancies as well as risk assessment of an individual for developing certain malignancies. However, most of the methodologies available for evaluation of tissue for the presence of genes associated with or predisposing an individual to a malignancy have well-known drawbacks. For example, methods that require disaggregation of the tissue, such as Southern, Northern, or Western blot analysis, are rendered less accurate by dilution of the malignant cells by the normal or otherwise non-malignant cells that are present in the same tissue. Furthermore, the resulting loss of tissue architecture precludes the ability to correlate malignant cells with the presence of genetic abnormalities in a context that allows morphological specificity. This issue is particularly problematic in tissue types known to be heterogeneous, such as in human breast carcinoma, where a significant percentage of the cells present in any area may be non-malignant.

The her2/neu gene encodes a protein product, often identified as p185HER2. The native p185HER2 protein is a membrane receptor-like molecule with homology to the epidermal growth factor receptor (EGFR). Amplification and overexpression of HER2 in human breast cancer has been correlated with shorter disease-free interval and shorter overall survival in some studies (van de Vijver et al. New Eng. J. Med. 317: 1239 (1988); Walker et al. Br. J. Cancer 60:426 (1989); Tandon et al. J. Clin. Invest. 7:1120 (1989); Wright et al. Cancer Res. 49:2087 (1989); McCann et al. Cancer Res 51:3296 (1991); Paterson et al. Cancer Res. 51:556 (1991); and Winstanley et al. Br. J. Cancer 63:447 (1991)) but not in others (Zhou et al. Oncogene 4:105 (1989); Heintz et al. Arch Path Lab Med 114:160 (1990); Kury et al. Eur. J. Cancer 26:946 (1990); Clark et al. Cancer Res. 51:944 (1991); and Ravdin et al. J. Clin. Oncol. 12:467-74 (1994)).

In an initial evaluation of 103 patients with breast cancer, those having more than three tumor cell positive axillary lymph nodes (node positive) were more likely to overexpress HER2 protein than patients with less than three positive nodes (Slamon et al. Science 235:177 (1987)). In a subsequent evaluation of 86 node-positive patients with breast cancer, there was a significant correlation among the extent of gene amplification, early relapse, and short survival. HER2 overexpression was determined using Southern and Northern blotting, which correlate with the HER2 oncoprotein expression evaluated by Western blotting and immunohistochemistry (IHC) (Slamon et al. Science 235:177 (1987); Slamon et al. Science 244:707 (1989)). The median period of survival was found to be approximately 5-fold shorter in patients with more than five copies of the her2 gene than in patients without gene amplification. This correlation was present even after correcting for nodal status and other prognostic factors in multivariate analyses. These studies were extended in 187 node-positive patients and indicated that gene amplification, increased amounts of mRNA (determined by Northern blotting), and increased protein expression (determined immunohistochemically) were also correlated with shortened survival time (Slamon et al. Science 244:707 (1989)); (see also U.S. Pat. No. 4,968,603). Nelson et al. have compared her2/neu gene amplification using FISH with immunohistochemically determined overexpression in breast cancer (Nelson et al. Modern Pathology 9 (1) 21A (1996)).

Immunohistochemical staining of tissue sections has been shown to be a reliable method of assessing alteration of proteins in a heterogeneous tissue. Immunohistochemistry (IHC) techniques utilize an antibody to probe and visualize cellular antigens in situ, generally by chromagenic or fluorescent methods. This technique excels because it avoids the unwanted effects of disaggregation and allows for evaluation of individual cells in the context of morphology. In addition, the target protein is not altered by the freezing process.

However, in the clinical trial assay (CTA), IHC of formaldehyde-fixed, paraffin embedded tissue samples only demonstrated 50%-80% sensitivity, relative to frozen IHC samples (Press, Cancer Research 54:2771 (1994)). Thus, IHC can lead to false negative results, excluding from treatment patients who might benefit from the treatment.

Fluorescence in situ hybridization (FISH) is a recently developed method for directly assessing the presence of genes in intact cells. FISH is an attractive means of evaluating paraffin-embedded tissue for the presence of malignancy because it provides for cell specificity, yet overcomes the cross-linking problems and other protein-altering effects caused by formalin fixation. FISH has historically been combined with classical staining methodologies in an attempt to correlate genetic abnormalities with cellular morphology (see, e.g., Anastasi et al., Blood 77:2456-2462 (1991); Anastasi et al., Blood 79:1796-1801 (1992); Anastasi et al., Blood 81:1580-1585 (1993); van Lom et al., Blood 82:884-888 (1992); Wolman et al., Diagnostic Molecular Pathology 1(3): 192-199 (1992); Zitzelberger, Journal of Pathology 172:325-335 (1994)).

To date, there has been no correlation of her2 gene amplification with anti-HER2 antibody treatment outcome, only with disease prognosis. The standard assay has been IHC on formalin fixed, paraffin embedded samples. These samples, when scored as 3+ or 2+, identify patients who are likely to benefit from treatment with an anti-HER2 antibody, like HERCEPTIN®. The 3+ and 2+ scores correlate with her2 gene amplification, e.g., as tested by FISH. However, there