Translational Research With Pemetrexed in Breast Cancer

Review Article [1] | November 02, 2004
By Axel R. Hanauske, MD, PhD [2]

Pemetrexed (Alimta) is a novel folate antimetabolite that primarily inhibits the enzymes thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyl transferase (GARFT), all of which are involved in pyrimidine and purine synthesis. In a phase II trial of patients with T3/4, N0–2 breast cancer, expression of thymidylate synthase (TS), dihydrofolate reductase (DHFR), glycinamide ribonucleotide formyltransferase (GARFT), p53, and c-erb-B2 (at the mRNA or protein level) was examined in tumor biopsy specimens before and 24 hours after the first dose of pemetrexed and after three cycles of single-agent treatment to establish correlations of biomarker levels and changes with clinical outcome and toxicity. Although final data are not available, initial indications are that clinical response may correlate with decreased or low TS expression. The results obtained from clinical data are supported by laboratory results in three cell lines (MDA-231, MCF-7, and ZR-75). These results suggest that in vitro transcript profiling to identify which genes are important predictors of successful cytotoxic chemotherapy, followed by a focused clinical trial to confirm the in vitro results, may be the best approach for translational research.

During the past decade, the integration of translational research into clinical trials has become an important aspect of cancer research. The goal of pharmacogenomic analysis is to identify individuals with specific genetic characteristics or molecular variables that correspond with clinical response or resistance. These analyses may also serve as a guide in developing targeted therapies that may improve tumor response and, ultimately, patient survival. The utility of translational research and the impact of patient selection for targeted therapies have been investigated in a variety of tumors. However, breast cancer has been the tumor probably most extensively studied to date, and several prognostic factors have been well established. The etiology of breast cancer is complex and it appears to involve numerous genetic, endocrine, and external factors. Most likely, a combination of events is required for the formation of cancer including overexpression of oncogenes that direct a cell to divide, inhibition of signals to stop replication or loss of maintenance of integrity of the genome by defunct tumor suppressor genes, and/or DNA replication and repair defects.[1] Available data in translational research may be confusing to oncologists who need to translate this data into therapeutic decisions. Translational clinical trials are under way to determine which genes are important predictors of cytotoxic chemotherapy response and resistance. Pemetrexed (Alimta) is a novel folate antimetabolite that inhibits thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyl transferase (GARFT), all of which are involved in pyrimidine and purine synthesis,[2,3] resulting in impeded synthesis of the nucleotide precursors of DNA and RNA.

Phase II

**Trial Design and Patient Characteristics**

A phase II neoadjuvant trial of pemetrexed in patients with breast cancer has been conducted. In this
study, biomarker expression was evaluated in patients with T4, N0-2, M0/1 breast cancer. Biopsies were obtained prior to the initial dose of pemetrexed, 24 hours after administration of pemetrexed, and after three cycles of therapy. Antifolates have been associated with severe sporadic toxicity, and evidence suggests that vitamin supplementation with folic acid and vitamin B₁₂ reduces plasma homocysteine levels, leading to a better safety profile with pemetrexed without adversely affecting efficacy.[4,5] As a result, baseline vitamin deficiency markers were drawn, and vitamin supplementation was given prior to administration of pemetrexed. The design of this study is shown in Figure 1. The median age of enrolled patients was 46 years of age (range: 31 to 72 years). The majority of patients (93%) received three cycles of therapy. The overall response rate was 31% (95% confidence interval [CI] = 20% to 44%). Fifteen patients (79%) exhibited the first evidence of a response after the first cycle.

**Figure 2: Distribution of Tissues**—DHFR = dihydrofolate reductase; FISH = fluorescence in situ hybridization; GARFT = glycinamide ribonucleotide formyl transferase; H & E = hematoxylin and eosin staining; PCR = polymerase chain reaction.

The emphasis of the biopsy tissue assessment was placed on messenger RNA (mRNA) expression of TS, DHFR, and GARFT with additional studies including quantitative polymerase chain reaction of p 53, and C-erb B2 as well as histology, immunohistochemistry, and/or fluorescence in situ hybridization (FISH) for TS, DHFR, GARFT, p53, and C-erb B2. For measurement of GARFT, a monoclonal antibody was specifically developed for this study. Additional material was retained and stored for later gene expression profiling. Frozen and paraffin-embedded specimens were evaluated to ensure that they contained sufficient tumor tissue for histologic analysis. Ninety-four percent of frozen specimens and 88% of paraffin-embedded specimens contained sufficient tumor tissue and were evaluable for histologic analysis. (The tissue analyses are illustrated in Figure 2.)
Preliminary results demonstrate that there was no real difference in HER2/neu classification between responders and nonresponders. Analyses of p53 mutations are still ongoing. However, preliminary data indicated that there were no p53 mutations in responders, and four mutations were observed in nonresponders. Using immunohistochemistry, no clear difference between TS responders and nonresponders was noted; however, the distribution of baseline TS as determined by polymerase chain reaction (PCR) revealed that there was a larger distribution in nonresponders with a trend toward higher values, and a more close clustering of low TS values in responding patients. When TS was determined and normalized to beta-actin, three groups emerged—very low TS expression, intermediate TS expression, and higher TS expression. Generally, up to 90% of the responding patients fell into the very low or lower intermediate TS expression as determined by mRNA and PCR analyses. When examining the data over time (ie, baseline, after the first dose of pemetrexed, following three cycles), questions regarding the ability of pemetrexed to induce resistance and differences in TS, and whether or not pemetrexed can modulate TS expression in patients following treatment were addressed. Preliminary analysis indicated that in responders (n = 17 with objective partial responses) and patients with stable disease (n = 31), pemetrexed does not induce TS expression. This also appears to be true among patients with stable disease; however, in resistant patients with progressive disease (n = 6), it appears that treatment with pemetrexed over time increased TS expression by a factor of two. Figure 3 illustrates differential TS expression in responders, patients with stable disease, and patients with disease progression. These preliminary data suggest that pemetrexed may not upregulate TS over time in those patients who benefit from the therapy. The role of DHFR, GARFT, and other molecular markers is currently under evaluation. The results of this type of translational research may prove to be one of the best indicators of a patient's potential response to cytotoxic chemotherapy; however, applying this technology in the clinical setting is an immense logistical challenge for all involved. Is it possible that modeling can provide the necessary information for making clinical treatment decisions? Three breast cancer cell lines—which included MDA 231, a very aggressive, estrogen receptor negative cell line; MCF7, a moderately aggressive cell line; and ZR-75 a less malignant cell line—were examined. With respect to TS, a consistent twofold increase in TS expression was noted, which corresponds to the clinical results observed. Discussion There are two approaches for conducting translational research, the first being clinical studies. For clinical studies tumors accessible for repetitive biopsies are required,
limiting the number of possible candidates to leukemia, certain kinds of breast cancer, head and neck cancer, and melanomas with cutaneous metastasis. These studies are difficult to perform. Accrual is slow, logistics are very complex, and these studies are very costly. The other opportunity for performing translational research is to conduct some preliminary work with patient-derived material. Using this approach, a large number of accessible tumors are available, because generally every cancer patient is operated on, thus increasing the opportunity of tissue accrual. However, verification in subsequent prospective clinical trials would be required, but in vitro investigations will allow investigators to decrease the number of potential prognostic candidate genes. This clinical approach could then be more focused than exploring numerous parameters, many of which will ultimately be eliminated. Both approaches for translational research require a centralized and validated laboratory. In conclusion, pemetrexed has demonstrated clinical activity in untreated breast cancers. Preliminary data indicate that the clinical response may correlate with decreased or low TS expression. Gene expression profiling using several hundred genes is in progress. The challenge, however, will be to sort out which genes are really important predictors of successful clinical treatment with pemetrexed. Analyses of biopsies during the treatment will provide information on the pemetrexed-induced modulation of genes at functional levels.

Disclosures: Dr. Hanauske has acted as a consultant for Eli Lilly.


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