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Discussion

DR. TOM R. DEMEESTER (Los Angeles, California): Dr. Beattie's paper and Dr. Giuliano's paper that we heard earlier this morning provide us with some objective evidence for what we always imagined to be true, that is, there are unrecognized micrometastases in the node removed or left behind after a resection.

In the past, this has been difficult to measure and as a consequence, not much was done about it. Now, with the advent of the epithelial-specific clonal antibody, the assessment is possible and will cause us to rethink our surgical approach to malignant disease in the future.

The question, of course, is, are these stain specific? If you do detect cells, are they viable, can they grow, and does the finding affect survival?

It appears that Dr. Beattie has given us good evidence that the cells, when detected, have a dramatic effect on survival. In fact, he has shown that the observation may account for inaccuracies in staging, at least in early disease.

What would be important to know is whether the finding is a sign of systemic disease. Does he have information as to where the recurrences occurred? Were they systemic or local?

I would also be interested to know whether he has had an opportunity to study lymph nodes, as reported by Dr. Giuliano, and how many patients had histologically normal but histochemically positive lymph nodes with histochemically normal bone marrow.

That would be an interesting group of patients, because if indeed they do exist, it could be argued that our surgical resection for cancer should be more extensive rather than the current emphasis on limited resections. It may be that the survival difference observed between limited and extensive resection for node-negative patients may be explained by the removal of nodes histochemically positive with the more extensive surgery.

Now that he has developed this technique, it would be interesting to know how he might use this information in patient care. Would he encourage the searching for histochemical evidence of metastases preoperatively and then subjecting these patients to adjuvant therapy? Or would he suggest adjuvant therapy if the nodes were found in the surgical specimen? Does he have any evidence that micrometastases detected by histochemical techniques are more susceptible to chemotherapy? I think these types of studies will certainly challenge our thoughts concerning surgical oncology in the future.

DR. JOHN R. BENFIELD (Sacramento, California): Dr. Beattie's concept of extending the TNM nomenclature to TNM small "m" is heading in just the right direction, and I hope that he intended the small "m" to stand for micrometastases and not for marrow. I thought I might share with you some data that we presented just 2 months ago before The Society of Thoracic Surgeons, heading in somewhat the same direction in a different way (Johnson JR, et al. Successful xenotransplantation of human lung cancer correlates with the metastatic phenotype. Ann Thorac Surg 1995; 60:32–37).

We studied 81 patients who had lung cancer in various stages whose lung cancers were then propagated in xenotransplantation in nude mice. The mean follow-up was 22.5 (2–61) months. Twenty-one xenotransplants successfully took and seven metastasized in the nude mice. Neither the predominant cell type nor the incidence of lymph node metastases correlated with the results of xenotransplantation. Of 21 patients whose non—small-cell lung cancer (NSCLC) took in transplant, 13 (61.9%) developed metastases, and 9 (42.8%) died from their cancers. Among 57 patients whose NSCLC did not take, 14 (24.5%) developed metastases and 9 (15.7%) have died from their cancers. The higher incidence of metastases in association with xenotransplantation take was significant ($p = 0.0032$). We concluded that patients whose NSCLC take in transplant are at high risk for metastases, and we surmised that this method of propagating human lung cancers is a step toward facilitating precise cellular biologic definition of the metastatic propensity of such neoplasms.

I suggest that an extension of Dr. Beattie's approach to finding occult human lung cancers propagated in xenotransplantation might give us some insight as to the mechanisms that are involved and perhaps another tool to identify patients with systemic disease before the usual approach of staging.

We concluded that patients whose lung cancers take in our transplantation model are at high risk for metastases. We believe that this method of propagating human lung cancers will facilitate carrying out precise cellular biologic studies aimed at defining the metastatic potential of such neoplasms before metastases become apparent. The search for evidence of micrometastases, as described by Dr. Beattie, plus cellular biologic markers of metastatic propensity should eventually permit us to identify patients whose apparently early stage lung cancers should nonetheless receive adjuvant systemic treatment.

DR. DONALD MORTON (Los Angeles, California): Dr. Beattie asked me to discuss this paper, and I am happy to do so because I think we are seeing a recurrence of interest in a line of investigation that really began in the 1960s with the detection of circulating tumor cells. With the technology that was available at that time, primarily cytology, it was found that patients who could be otherwise cured of their cancers by surgery would on occasion have circulating tumor cells in their peripheral blood or in veins draining the neoplasm.

We are now looking again at this same problem with what I call ultrastaging, because it is very clear that many patients with solid tumors actually have circulating tumor cells in their blood and bone marrow. Most of the time these circulating tumor cells do not lead to metastases because the metastatic cascade that results in successful metastasis is a very rare event: only 1 in 10 to 10 million cells successfully implants at a distant site. This is because the establishment of a successful metastasis requires a series of events, including the presence of receptors on the tumor cell, which permits adherence of the cancer cell to the vascular endothelium of the particular organ. The tumor cell must then have the enzymatic capacity to lyse the intracellular cement between the capillary endothelium and invade the parenchyma of the organ. Finally, the particular organ must have the capacity to produce the necessary growth factors that allow the cancer cell to grow in the new organ site.

Our group has looked at circulating tumor cells with reverse transcriptase-polymerase chain reaction technology, which is much more sensitive than immunohistochemistry. In melanoma, we can detect as little as one tumor cell in 50 million peripheral blood lymphocytes. In breast and lung cancer, we can detect about 1 tumor cell in 10 million peripheral blood lymphocytes. We have focused on the blood as a more easily available specimen than bone marrow for repeated sampling and examination of circulating tumor cells.

It is interesting that not all of Dr. Beattie's patients who had tumor cells in their bone marrow recurred, at least thus far. I predict that a substantial portion, particularly stage I and II patients, will not recur. This means that detection of circulating tumor cells becomes a staging device and not necessarily a means of determining whether the patient will benefit from surgery. We know this because some patients who already have distant metastases to the lung or to the liver are cured of their cancers by surgical resection of the distant metastases, and others are not. Thus, a distant metastasis does not necessarily mean that the patient is incurable by surgery.

In closing, I would like to state that this is a very important paper that has far-reaching implications for lung cancer and other solid neoplasms. I want to ask Dr. Beattie if he has examined tumor cells in the bloodstream concomitantly with those in the bone marrow in these patients?

DR. RICHARD J. COTE (Closing Discussion): Thank you for allowing me the opportunity to discuss this paper and address the comments.

Concerning Dr. DeMeester's comments, the basis for recurrence in patients who have no clinical evidence of spread of tumor is the presence of undetected metastases. This concept forms the basis of the studies that my lab has been conducting to detect occult metastases in the bone marrow and lymph nodes of patients with early stage cancer.

The sensitivity and specificity of the detection system we have used is of paramount importance, and we have published several papers concerning this point. The assay is based on the ability to distinguish extrinsic (epithelial) populations of tumor cells from normal bone marrow cells using monoclonal antibodies that react with antigens expressed by epithelial carcinoma cells but not by normal hematopoietic elements. This assay also has an important morphologic basis because the extrinsic cells identified must be consistent with tumor cells. The biological significance of the extrinsic population of cells can be deduced by what eventually happens to the patients in whom such cells are detected.

We now have strong scientific evidence that the presence of occult tumor cells in the bone marrow (and lymph nodes) of patients with no clinical or pathologic evidence of regional or systemic spread of tumor has an important impact on tumor recurrence and patient survival. From this point of view such tumor cells are biologically significant. We are currently conducting studies to determine other aspects of the biology of these cells, including changes in gene expression, proliferative potential, and expression of proteins associated with invasion and metastasis.

Concerning the question about the evidence of systemic disease, we believe that the tumor cells we are detecting in the bone marrow are not necessarily cells at their final site of metastasis. Rather they are tumor cells in transit and probably more indicative of systemic tumor burden than a metastatic deposit in the bone marrow per se. Indeed, our studies in breast and lung cancer have demonstrated that the sites of recurrence in patients who have tumor cells detectable in their bone marrow are not restricted to the bone. We find metastases at all the typical sites one would expect for lung cancer (such as liver and brain) and for breast cancer (such as lung, liver, and bone).

Dr. DeMeester's question about the detection of occult metastases in lymph nodes is relevant to the work we have presented today. Several studies have conclusively shown that occult metastases in lymph nodes can be detected in patients with no histologic evidence of tumor in regional lymph nodes (node-negative lung cancer). The presence of occult lymph node micrometastases is clinically significant. We are currently conducting studies with Carmack Holmes, Alistair Cochran, and their colleagues at UCLA who have performed some of these studies to determine the significance of both occult nodal metastases and occult bone marrow metastases in patients with the earliest stages of lung cancer. We now have preliminary evidence in patients with node-negative breast cancer that those with occult nodal metastases are a distinct group from those with occult bone marrow metastases. Thus, the detection of occult spread of tumor to lymph node and bone marrow may provide complementary (and not simply redundant) information. This is quite exciting.

How might this information be used in patient care? For patients with stage I lung cancer, the detection of occult bone marrow (and lymph node) micrometastases identifies those most likely to recur, and therefore, could provide the rationale for

the administration of adjuvant chemotherapy in that group. With regard to patients with stage III disease, the identification of patients at lowest risk for recurrence—that is, those with negative bone marrows—may define the group in which aggressive surgical therapy might be warranted.

Dr. Benfield points out some extremely important studies that are complementary to our work. Cancer is a multistep process. We are looking at two apparently distinct events—spread of tumor by vascular or lymphatic routes. However, there are undoubtedly many factors responsible for the spread of cancer; Dr. Benfield's investigations will help to elucidate that.

Concerning Dr. Morton's comment, the immunochemical assay is indeed exquisitely sensitive, perhaps as sensitive as any other assay that is currently available, including those based on the polymerase chain reaction. Polymerase chain reaction assays do have some advantages, and these are something that we are currently looking into.

Concerning the examination of blood for micrometastases, we have found that the rate of detection in tumor cells in the blood of patients with early stage cancer is very low, compared with that in the bone marrow. However, the blood would be a convenient compartment to look for micrometastases, and is the subject of ongoing investigation.