Abstract One of the most critical issues regarding management of early breast cancer is the proper detection and correct estimation of its malignant potential. In recent years, primarily through mammography, the frequency of ductal carcinoma *in situ* (DCIS) in malignant breast biopsies has increased several fold, from ~5% to over 20%. At present, DCIS classification is based exclusively on descriptive parameters and several attempts have been made to obtain uniform classification among pathologists. This goal has been elusive and further attempts both in North America and Europe have been made to better distinguish between DCIS lesions. However, even if this can be achieved to a satisfactory degree, the malignant potential of a DCIS lesion, either comedo or non-comedo, cannot be assessed without quantitative evaluation of these lesions. It may be possible to assess the malignant potential by estimating a variety of parameters, such as the presence of oncogenes or their products, presence of receptors, *etc.* To date, some of these have been shown either to be necessary or sufficient conditions for obtaining invasive cancer.

We have recently shown that the nuclear features of diagnostic cells, as well as nuclear features of the surrounding normal appearing cells, can be used for objective lesion classification and estimation of the malignant potential of DCIS lesions. A high resolution image cytometer (Cyto-Savant™, Xillix Technologies Corp.) has been developed. This device can be used for automated measurements of large numbers of cell nuclei stained stoichiometrically for DNA. Nuclear features such as size, shape, DNA amount, and foremost, the texture features describing the distribution of the DNA are extracted. The Cyto-Savant™ system has been trained to recognize the relevant cells in smears from fine needle aspirates or cytospins of cells extracted from tissue blocks using standard procedures. Several thousand cell nuclei per slide can be analyzed using only a few minutes of the pathologist's time. Combining qualitative assessment with quantitative data greatly improves the diagnosis of DCIS lesions and may provide prognostic and treatment monitoring information as well. The system will be discussed in detail and experimental data shown for several cases.

Bispecific Monoclonal Antibody Therapy (Anti HER-2/*neu* × Anti CD 64) for Human Breast Cancers That Overexpress HER-2/*neu*

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Abstract A Phase I clinical trial with a bispecific monoclonal antibody (BsAb) (anti HER-2/*neu* × anti CD 64) is currently being conducted in patients with Stage IV breast carcinoma or Stage III/IV ovarian carcinoma who are refractory to standard therapy and who overexpress the HER-2/*neu* antigen as determined by immunohistochemistry. The trial is a hybrid Phase Ia/Ib trial in which the principal endpoints are toxicity, determination of the maximum tolerated dose, biological efficacy, and BsAb pharmacokinetics. Clinical efficacy will be assessed employing standard cancer and leukemia group B (CALGB) criteria to categorize tumor responses. The BsAb, designated MDX-210, is a Fab′ × Fab′ construct which is designed to enhance tumor penetration owing to its relatively small molecular size (approximately 100 kD). CD 64 (FcγRI) is a high affinity Fc receptor for IgG and potent cytotoxic trigger molecule for monocytes, macrophages, IFN-γ-activated neutrophils, and G-CSF-activated neutrophils. The anti CD 64 employed in this study is uniquely constructed to bind to an epitope outside the normal

ligand binding site and thus should not be blocked *in vivo* by the relatively high levels of human IgG. Her-2/*neu* is overexpressed in human breast carcinomas with poor prognosis. *In vitro* studies with MDX-210 have shown effective killing of tumor cell lines that express the HER-2/*neu* antigen. Eight patients have been treated to date. The dosage levels tested to date are 0.35, 1.0, and 3.5 mg/m^2 infused intravenously at 6.0 mg/hour. Infusion of MDX-210 has been well-tolerated by all patients. The principal toxicities have been Grade I/II fevers and malaise that have fully resolved by 12 hours post infusion. Evidence of immunological activity has been observed even at the lowest dose tested. Plasma tumor necrosis factor alpha (TNFα) increased to as high as 500 picogram/ml in 5 of 6 patients tested. Peripheral blood monocytopenia, either preceding or concurrent with elevations of plasma TNFα, is consistent with binding of MDX-210 to both immune effector cells and target breast tumor cells. Significant dose-dependent *in vivo* binding of MDX-210 to CD 64 has been observed for more than 24 hours post infusion. It has been demonstrated in cell culture studies that MDX-210 triggers release of TNFα from immune effector cells in the presence, but not in the absence, of target tumor cells. The observation that MDX-210 is immunologically active at non-toxic doses forms the basis for considering MDX-210 as a candidate chemotherapeutic drug for recurrent or secondary breast cancers.

Estradiol Metabolism: An Endocrine Biomarker for Chemoprevention of Human Mammary Carcinogenesis

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Abstract A relationship between altered metabolism of estradiol (E2) and personal or familial risk for breast cancer suggests that endocrine changes associated with ovarian function may influence initiation or promotion of carcinogenesis. Evidence for a direct effect of E2 on non-involved mammary tissue (target for carcinogenesis) is equivocal. Explant cultures of human mammary terminal duct lobular units (TDLU) from breast cancer patients are utilized to examine whether (i) E2 metabolism in TDLU is altered in response to the chemical carcinogen benzo(*a*)pyrene [B(*a*)P], and (ii) perturbed E2 metabolism is modulated by naturally occurring polyunsaturated fatty acids (PUFA) and indole-3-carbinol (I3C). Treatment of TDLU with 40 nM B(*a*)P resulted in a >95% decrease in C2/C16α-hydroxylation ratio of E2 relative to that detected in solvent controls. This metabolic alteration was due to a specific increase in E2 C16α-hydroxylation. Exposure of TDLU prior to and during B(*a*)P treatment with omega-6 PUFA **decreased** C2/C16α-hydroxylation ratio by 38% ($p < 0.001$). Treatment with omega-3 PUFA and I3C **increased** the ratio by 318% and 376% respectively ($p < 0.001$), due to a specific increase in E2 C2-hydroxylation. Thus, carcinogen-induced perturbation of E2 metabolism in TDLU and its modulation by dietary modulators of rodent mammary tumorigenesis provide evidence for this endocrine biomarker as a clinically relevant endpoint for chemoprevention of mammary carcinogenesis.

Supported by CA 29502, The Wanda Jablonski Fund.