various CAs (first visit); 7% of 354 nonCA disease and <1% of 127 healthy persons reacted positively. Serum anti-T solid-phase immunoassay: 96% of 26 Tis CA and 85% of 229 CA patients (all stages) were positive. Of 152 benign-diseased and healthy persons 9.2% were positive (all $p<0.000$). T/Tn (HLA-free), chemically and physically fully defined, sterile, free of AIDS, Hepatitis and pyrogens is made from O RBC-derived type MN glycoprotein (quantity unlimited) by exhaustive enzymatic desialylation; this RBC T has ~10% covalently linked Tn (cf. Springer, G. F., Desai, P. R., *Molecular Immunol.* 22: 1303 – 1310, 1985). Since 1974, we use T/Tn Ag mixed with adjuvant for active specific therapy of breast CA patients. Each vaccine dose (1 ml) is injected intradermally in two 0.5 ml lots, ~7 cm apart at 6 – 12 week intervals, depending on induration at ~24 hrs (cytotox. T cell, monocyte, macrophage increase). A new body area is used each time; treatment is *ad infinitum;* no systemic toxicity was ever observed. Of 16 breast CA patients, pTNM Stages IV (5), IH (6) and H (5), all survived >5 yrs. Ten of these survived >10 to 18 yrs: three each are St III & IV. Three St IV patients are NED (no evidence of disease) at >14 yrs, one is stable with sacrum metastases (H0 performance, 5.0 yrs). One St IV (no mastectomy) and two St III patients died of CA within 10 yrs. The probability that our survival statistics are due to chance, using NCI "1990 standard treatment PDQ Data" as control is, for all three stages taken together — 5 yr survival: $p<1 \times 10^{-8}$ and 10 yr survival: $p<2 \times 10^{-6}$. (Aid: NCI grant 19083 & H. M. Bligh Cancer Fund).

S4.3
Glycosylation and the Metastatic Phenotype

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Neoplastic transformation in both murine and human cells is commonly accompanied by structural alterations in *N*-linked oligosaccharides, in particular, the expression of highly branched complex-type oligosaccharides, polylactosamine and Lewis antigens. Studies on tumor cell glycosylation mutants and drugs which inhibit oligosaccharide processing suggest that expression of branched complex-type *N*-linked oligosaccharides are required for efficient tumor cell metastasis. Specific cell surface oligosaccharides function as ligands for mammalian lectins and can enhance the retention of blood-born tumor cell in the microvasculature of host tissues. Expression of embryonic carbohydrate sequences (i.e., polylactosamine, extended-chain Lewis X and Y) following tumor progression or retinoic acid-induced differentiation of F9 teratocarcinoma cells is similarly dependent upon regulated expression of specific glycosyltransferases in the Golgi. These include the β1-6GlcNAc-branching of *N*- and *O*-linked core oligosaccharides; *N*-acetylglucosamine-transferase V (GlcNAc-TV), and core 2 GlcNAc-T, respectively. In several cell culture models of transformation and differentiation, the activity of GlcNAc-TV and core 2 GlcNAc-T are regulated and constitute an important rate limiting step in the biosynthetic pathway of polylactosamine and associated Lewis antigens.

Studies with somatic glycosylation mutants and with the processing inhibitor swainsonine suggest that multiple cellular phenotypes are affected, including tumor cell invasion and proliferation. These observations prompted our recent investigation to determine whether the loss of sialylated complex-type *N*-linked oligosaccharide in tumor cells affects the expression of genes which, in turn, could influence the malignant phenotypes. In both human and rodent tumor cells, we observed a selective increase in c-jun and tissue inhibitor of metalloproteases (TIMP) mRNA levels under conditions where *N*-linked processing was inhibited either by the alkaloid swainsonine or by stable glycosylation mutations. This suggests that *N*-linked oligosaccharide processing may be an integral element of the cellular phenotype controlling expression of select genes. Altered glycosylation of growth factor, lymphokines and/or their receptors on the cell surface are possible mediators of phenotypic charge; an avenue which is currently being investigated.

S4.4
Selection of Human Melanoma Variants Showing Concomitantly an Increase in their Metastatic Potential and a Defect in their Biosynthesis of Disialogangliosides GD3 and GD2

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The presence of gangliosides GM3, GM2, GD3 and GD2 on the outer surface of the plasma membrane of human malignant melanoma cells is well documented. The possibility of targeting these compounds by immunotherapy was investigated in several studies using either vaccines made with gangliosides or monoclonal antibodies (MAbs) directed to GD3 and GD2 gangliosides. Most of these trials were aimed to a single target ganglioside on melanoma cells. However, little is known about the exposure of gangliosides on tumor cells with regard to their capacity to form metastases. We recently developed an animal model to study the metatastic ability of human melanoma cells. Following subcutaneous injection of melanoma cells to immunosuppressed newborn rats, it was possible to select from the same parental cell line a series of variants showing, along with a 100% tumor take at the injection site, an increasing ability to give rise to lung metastases. The total glycosphingolipid content was not significantly different between the variants. The glycosphingolipid patterns of these cells were determined by TLC and showed that the increase in metastatic potential is concomitant with a decrease in the cellular content of disialogangliosides GD3 and GD2 which were absent in the most metastatic variants. These results were confirmed by flow cytometry using specific MAbs to GD2 and GD3 generated in our laboratory. Simultaneously, there was an accumulation of lactosylceramide in these cells, suggesting a defect in the activity of sialyltransferases. The use of specific MAbs to GD3 and GD2 to target human melanoma cells is therefore questionable.

S4.5
Membrane-Intercalated Heparan Sulphate Proteoglycan Phosphorylated at a Cytoplasmic Portion of the Core Protein