A Real-Time PCR Method to Assay the Molecular Expression of Multiple Tumor Antigens in Patient Biopsies

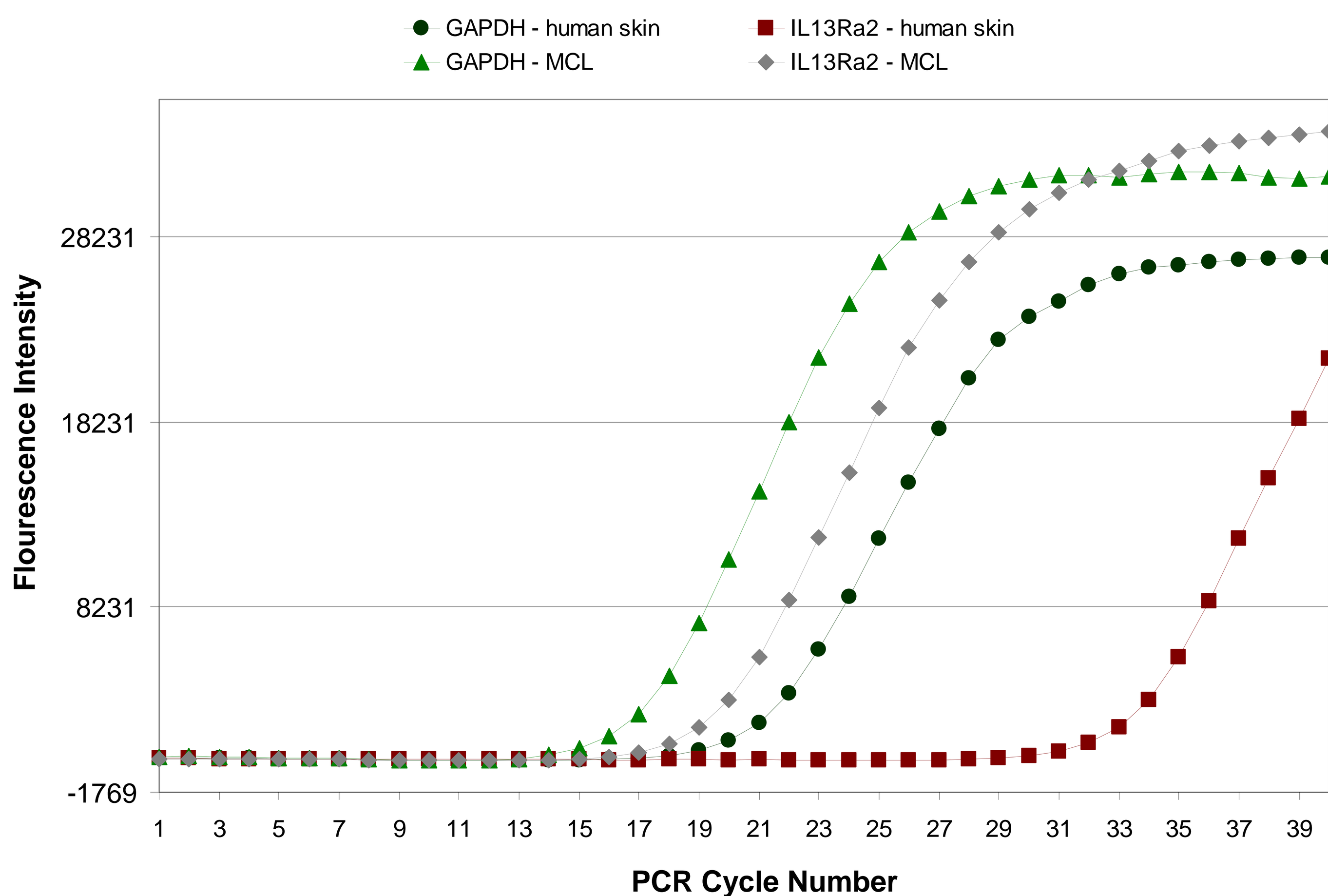
Brian Weinert[1], Francesca Milano[2], Sheila Krishnadath[2], Mai-Britt Zocca[1]

[1] *DanDrit Biotech A/S, Copenhagen, Denmark*
[2] *Department of Gastroenterology and Hepatology, Academic Medical Center, Amsterdam, Netherlands*

1. ABSTRACT

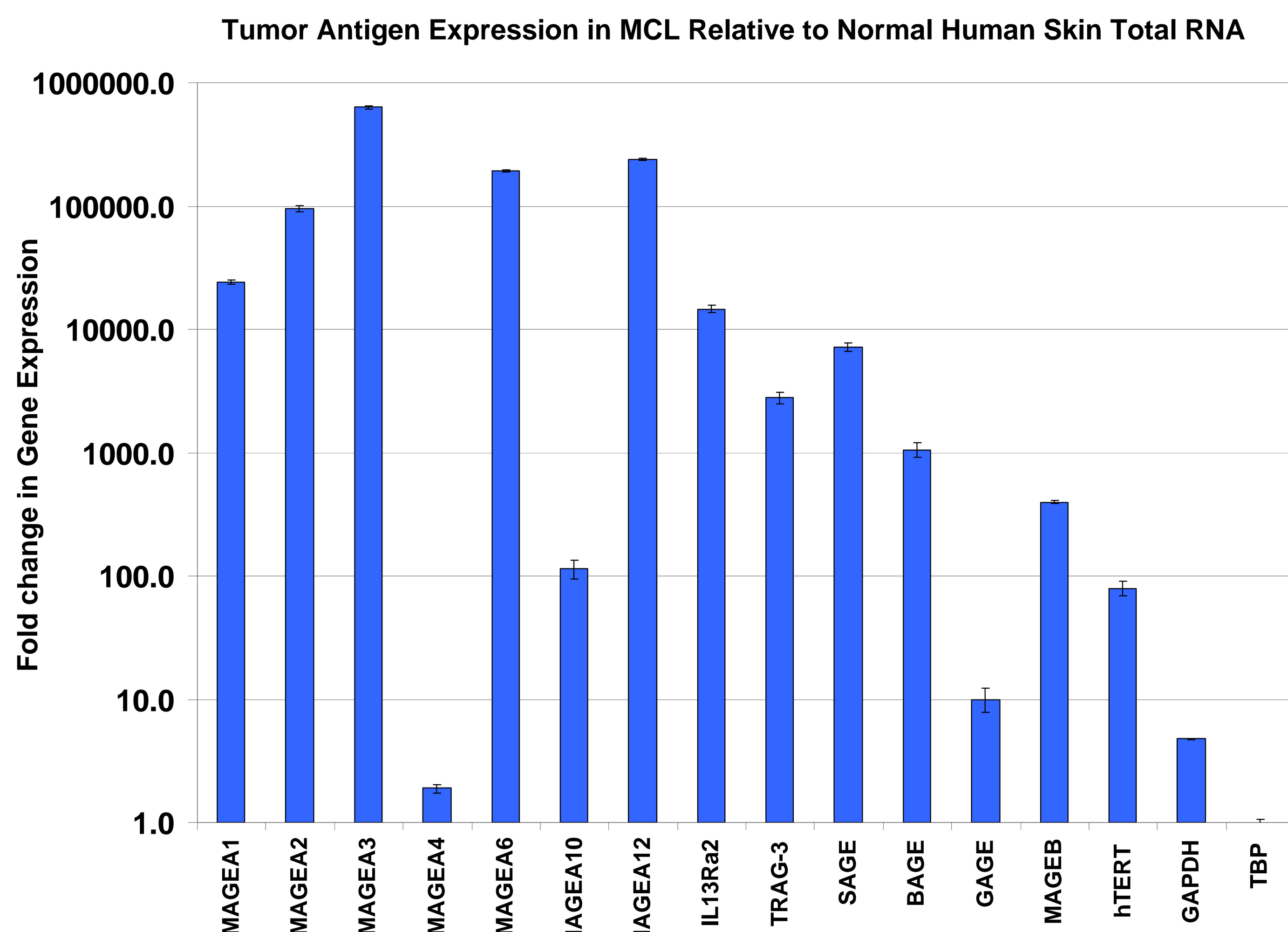
DanDrit Biotech A/S has developed a therapeutic cancer vaccine, MelCancerVac®, which is based on loading patient dendritc cells with a specifically selected allogeneic tumor cell lysate. In order to better define the tumor antigens expressed in DanDrit's tumor lysate we have developed real-time PCR assays for more than 40 different tumor antigen genes or gene families, including 19 genes with confirmed T cell peptide epitopes. In addition to testing for the presence of molecular expression in DanDrit's cell lysate we have begun screening patient tumor biopsies of both esophageal and colorectal cancers for the expression of tumor antigens. The real-time PCR assay we have developed is capable of assaying up to 37 different tumor antigen genes (+ three control genes) in a single run on a 96 well plate. However, we are currently screening for 29 genes in esophageal cancers and for 21 genes in colorectal cancers. Gene expression is determined as a ratio to GAPDH expression and is then compared to patient matched normal tissue in order to detect tumor-specific changes in gene expression. Preliminary results from esophageal tumor biopsies shows that ~50% of patients express tumor antigens and that cancer/testis tumor antigen expression is clustered. Our results indicate that this is a robust system to assay many tumor antigen genes simultaneously that is well suited to provide valuable information regarding the tumor antigen expression profiles of individual patients. In addition, this technique is both rapid and inexpensive. RNA isolation to real-time PCR analysis can be performed in as little as 48h at a cost of approximately 50-100€ per patient biopsy excluding labor. Although many studies have looked at the molecular expression of tumor antigens in cancers, few have looked at so many different genes in a single study.

2. RT-QPCR ANALYSIS OF THE IL13-RECEPTOR ALPHA2 GENE



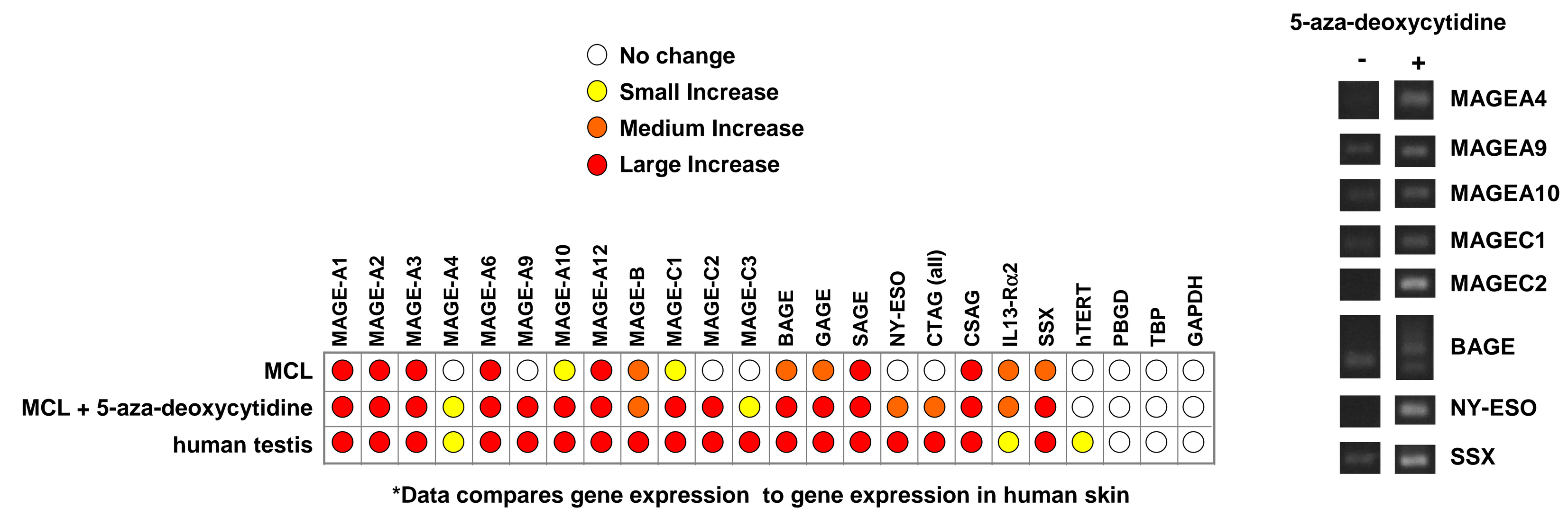
As described above, MelCancerVac® uses a specifically selected melanoma cell line as a source of tumor antigens for patient-derived dendritic cells. MCL = Melanoma Cell Line. Human skin total RNA is used as the normal tissue control. The difference in PCR cycle threshold between MCL and human skin is normalized to the difference seen for the control gene GAPDH. Here there is a large difference in amplification of the IL13-Receptor Alpha2 gene between melanoma cells and human skin. This difference corresponds to a ~1,500 fold difference in gene expression.

3. TUMOR ANTIGEN EXPRESSION IN MelCancerVac®



The above expression profile was generated by determining the expression level of each tumor antigen gene relative to a control gene, in this case TATA box-binding protein (TBP). The relative expression values for tumor antigens in MCL are then compared to the relative expression values in the control tissue, human skin, thereby generating the degree of gene expression seen in the chart above. Please note that for many of these genes there is no product detected in the control tissue (human skin). Therefore the fold change in gene expression is calculated by assuming that a product is detected in the last cycle of the PCR reaction. This results in an overestimation of the degree of gene activation, however it provides a method to compare the relative differences in gene expression.

4. TREATMENT WITH 5-AZA-DEOXYCYTIDINE INCREASES EXPRESSION OF CANCER/TESTIS ANTIGENS



*Data compares gene expression to gene expression in human skin

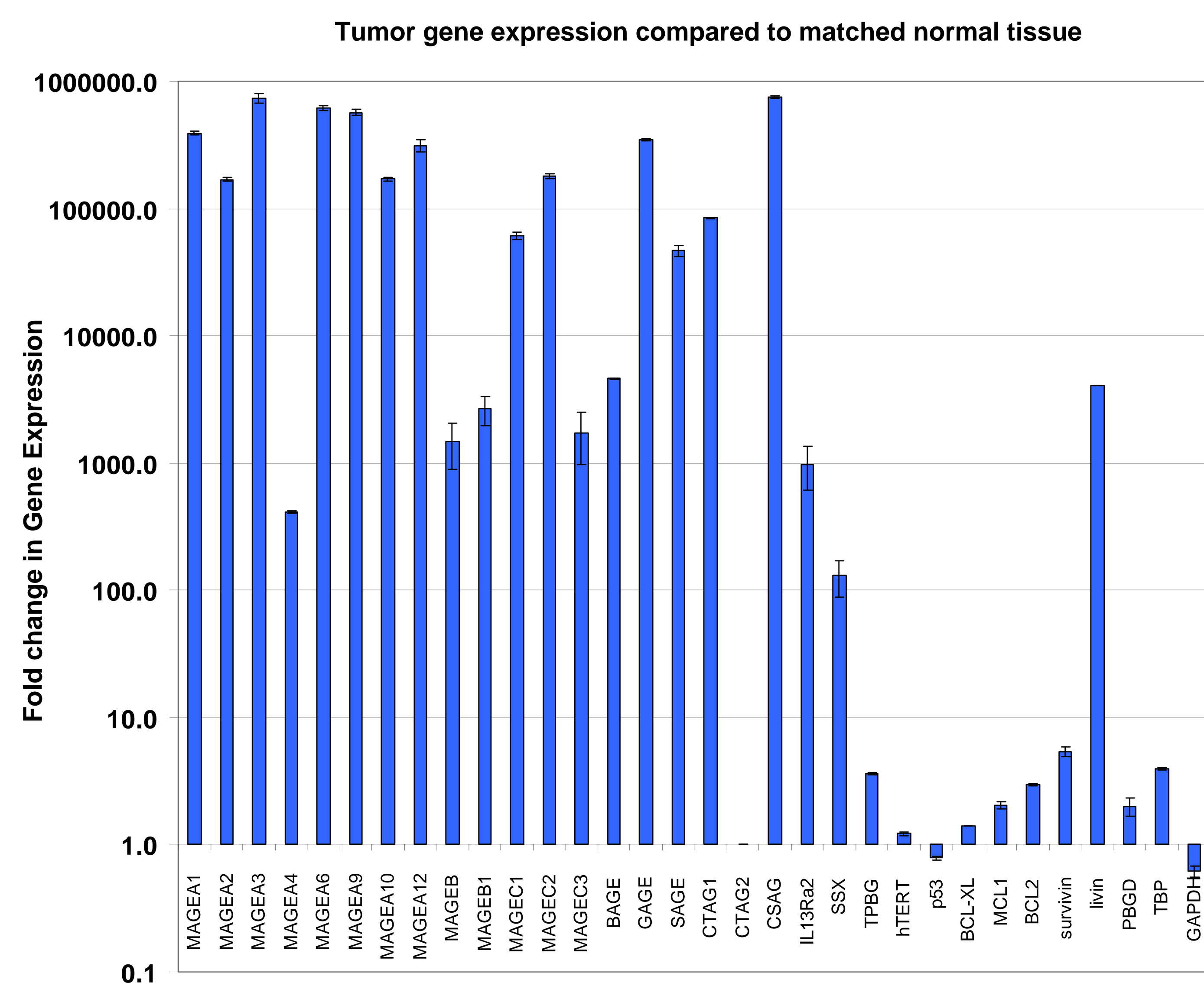
Cells were treated with 5-aza-deoxycytidine at 1uM for 72 hours. Changes in tumor antigen gene expression are shown relative to expression in a normal tissue, human skin. The expression profile of human testis shows that genes tested are cancer/testis antigens and further allows for comparison of the degree of expression in tumor cells versus the normal expression level in this tissue.

5. PROTOCOL FOR RECOVERY AND ANALYSIS OF ESOPHAGUS TUMOR BIOPSIES

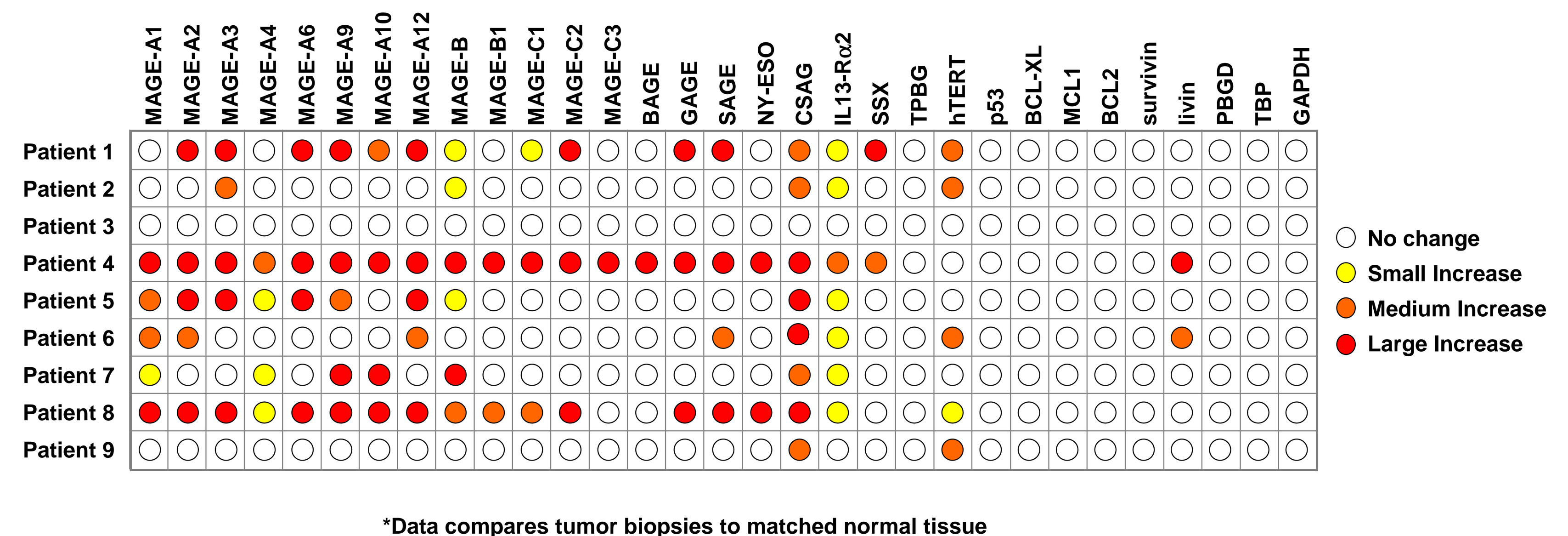
***Squamous cell carcinoma**

Recover patient biopsies of both normal and tumor tissue from patient esophagus using endoscopy → **Tissue is stabilized in *RNAlater* solution** → **Total RNA is isolated by QIAGEN RNeasy kit with DNAse treatment** → **mRNA is reverse transcribed to cDNA and analyzed by QPCR**

6. TUMOR ANTIGEN EXPRESSION IN PATIENT 4 TUMOR BIOPSY



7. TABLE OF TUMOR ANTIGENS IN ESOPHAGEAL SQUAMOUS CELL TUMOR BIOPSIES



*Data compares tumor biopsies to matched normal tissue

8. SUMMARY

The method described here facilitates the assay of many tumor antigen genes simultaneously. While many studies have examined tumor antigen expression in various tumor types, most of these studies only examined one or a few genes. Here we hope that by examining many genes we can reveal patterns in tumor antigen expression and gain valuable knowledge about the overlap between tumor antigen expression in our patient group and in our therapeutic cancer vaccine, MelCancerVac®. It is difficult to draw any conclusions from the esophagus tumor study with just 9 patients examined to date. However, it is clear that some patients express many cancer/testis antigens while others do not have detectable tumor antigen gene expression. This clustering of cancer/testis antigen expression has been observed elsewhere and is confirmed here.