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| 14. ABSTRACT<br>This project is funded in order to investigate immunoprofiles of serum anti-glycan antibodies recognizing Mesothelioma-derived aberrant glycans in human subjects and in animal models of Mesothelioma. This is accomplished using a one of a kind printed glycan array which has been developed by us at the New York University School of Medicine (NYU SoM) and has now been expanded by addition of 177 novel glycan probes, many of which are Mesothelioma-specific. It is expected that the results of these experiments will allow us to diagnose and prognosticate Mesothelioma earlier during its development. 14. ABSTRACT We have established the new glyco-laboratory with the dedicated print-room which now allows printing of large batches of glycochips of an enhanced quality, and with an increased efficiency. We are now in a process of printing large batches of glycochips for AGA immunoprofiling in both human and our model-Meso-rat sera. We have the protocols approved for the first long-term animal study for the immune responses to the exposure to asbestos in rats, and this experiment is now in its advanced stage. |                  |                          |                                      |                                                           |                                            |
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## INTRODUCTION

This project is funded in order to investigate immunoprofiles of serum anti-glycan antibodies recognizing Mesothelioma-derived aberrant glycans in human subjects and in animal models of Mesothelioma. This is accomplished using a one of a kind printed glycan array which has been developed by us at the New York University School of Medicine (NYU SoM) and has now been expanded by addition of 177 novel glycan probes, many of which are Mesothelioma-specific. It is expected that the results of these experiments will allow us to diagnose and prognosticate Mesothelioma earlier during its development. Results of our experiments using rat model of human Mesothelioma should also provide leads into the immuno-preventive and immuno-therapeutic approaches to treatments of the military personnel of high-risk for this malignancy due to their potential long-term exposure to carcinogenic form of asbestos during their service.

## BODY

The first arm of our study is carried out as scheduled: asbestos as carcinogen, silica dioxide as a non-carcinogen control for asbestos, and saline as a control for a process of injection have been administered as peritoneal injections and all animals have been bled and weighted monthly. We have collected two "baseline bleeding and weight" measurements prior to all injections. There have been nine monthly complete bleeding-weight sessions since the start of this long-term experiment. During each experimental session blood samples of individual animals have been kept on ice until blood draw was completed - usually within three hours - and then serum has been separated by centrifugation. Sera have then been stored in -80°C. Immunoprofiling of serum glycan-binding proteins will begin at the end of the experiment when all longitudinal serum samples are collected. To date - there have been no problems with the experimental animals, or with the planned schedule.

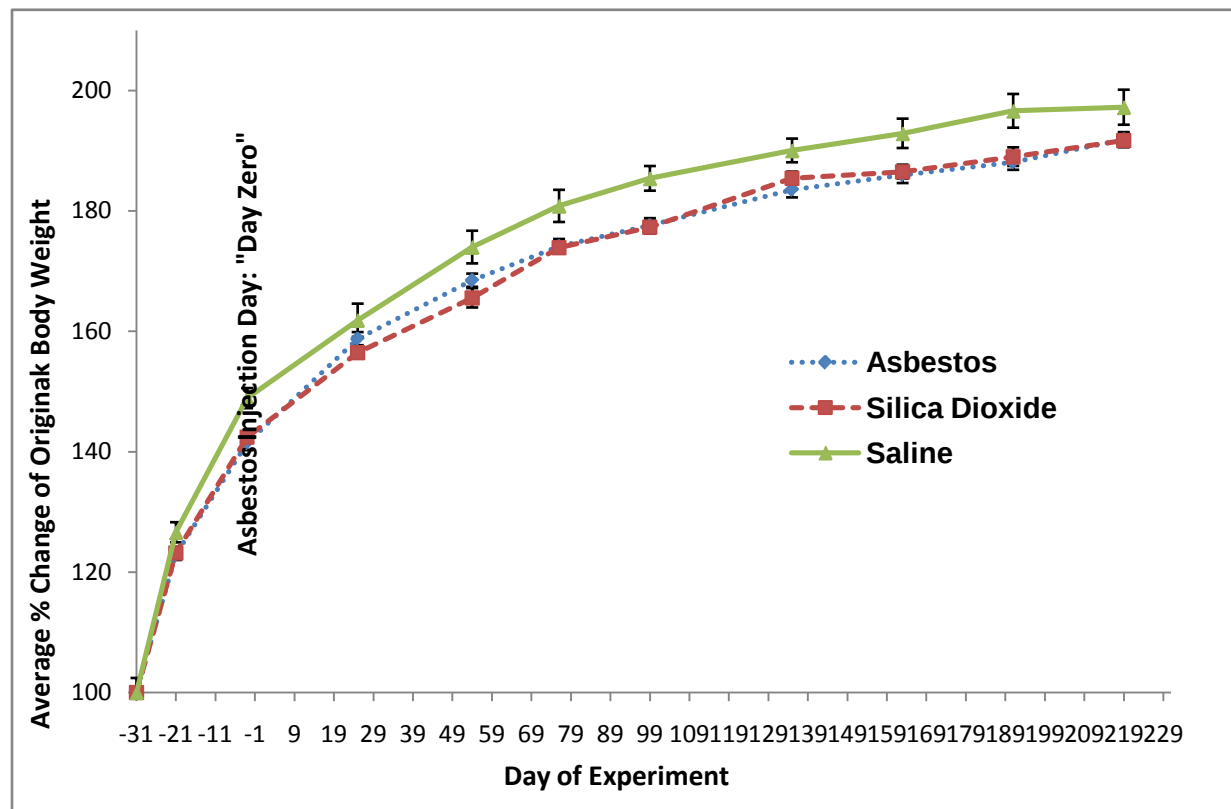
To-date plotted animal weight measurements are presented in **Figure 1**, and **Figures 2** and **3** illustrate examples of weight change patterns in selected rats injected with asbestos. There has been no specific weight change patterns observed in rats injected with silica dioxide and saline.

### Printed Glycan Array

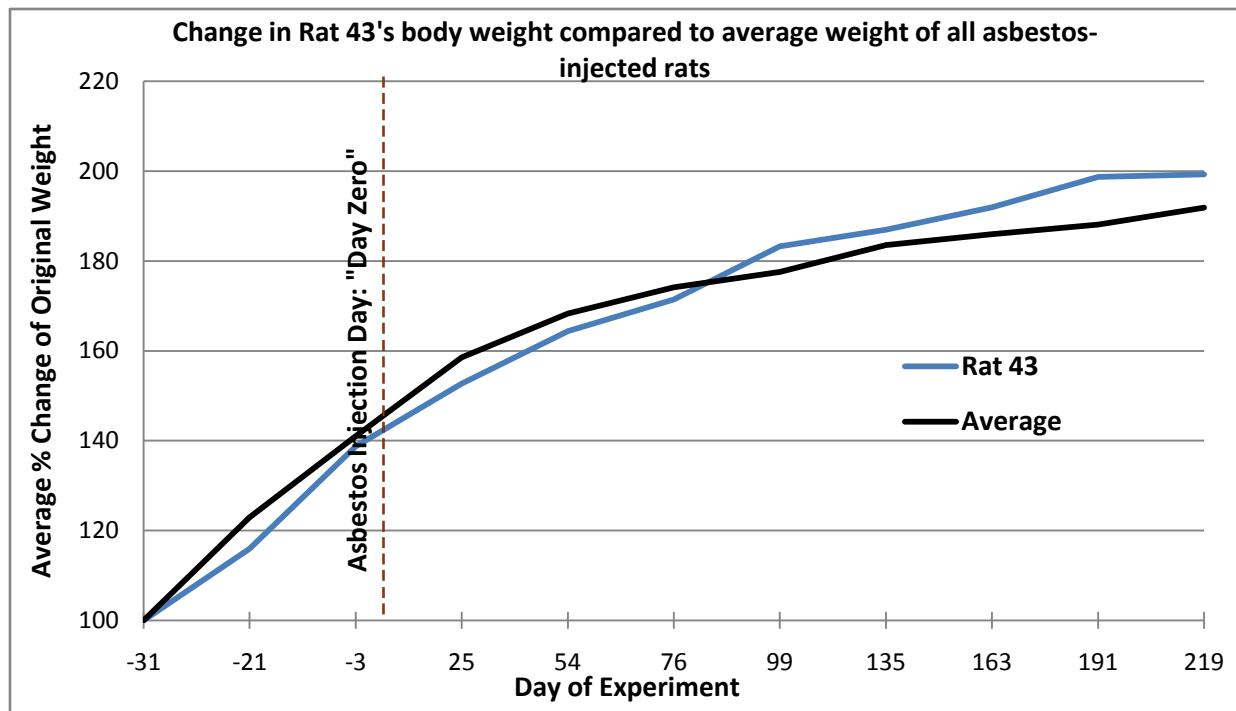
In preparation for the immunoprofiling of serum anti-glycan antibodies (AGA) in populations of asbestos-exposed individuals and patients with Malignant Pleural Mesothelioma, and in the animals in all arms of our study employing rat model of human Mesothelioma described in our original study plan, we have developed an expanded version of our glycochip, NYU PGA-400.

Our next generation of printed glycan array (PGA) includes now 377 synthetic glycans of pharmacological purity. Majority of novel glycan probes has been designed based on our experimental results obtained with the previously utilized PGA-200. Our current glycan library includes expanded categories of N-glycans such as fucosylated, sialylated and sulfated complex lactosamines, extended and modified blood group A, B, I and P glycans, extended and modified Lewis (Le)<sup>a</sup>, Le<sup>b</sup>, Le<sup>c</sup>, Le<sup>x</sup> and Le<sup>y</sup> glycans, as well as multiple tumor-associated glycolipid glycans, sialylated, sulfated and modified O-glycosylation core structures, synthesized on different amino- spacers that include peptide mimics and extended hydrophobic units. These glycans have been synthesized in the laboratory of Prof. Nicolai V. Bovin (Russian Academy of Sciences, Moscow, Russia). NYU PGA-400 glycochips are printed at 50 and 20  $\mu$ M concentrations, at eight replicates of each glycan at both concentrations. These glycochips are produced and quality-tested by a set of procedures that have been standardized and optimized for pre-clinical diagnostic research applications. Quality control steps and profiling of serum

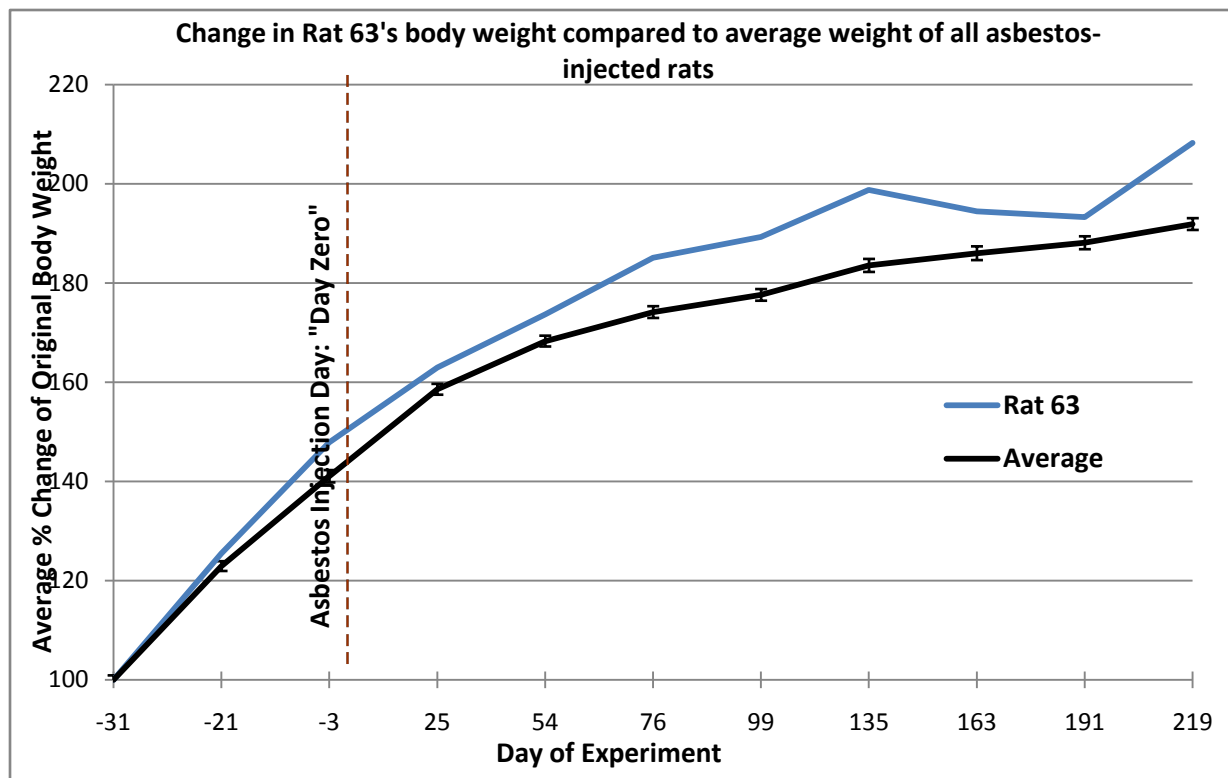
antibodies binding to printed glycans are performed as described in Huflejt et al. (2009) and Vuskovic at al. (2011). **Figure 4** shows AGA signals on the two sub-arrays with the complete library printed at 50  $\mu$ M. We are currently printing the large batches of glycochips, and immunoprofiling of serum AGA in the study populations will begin shortly.



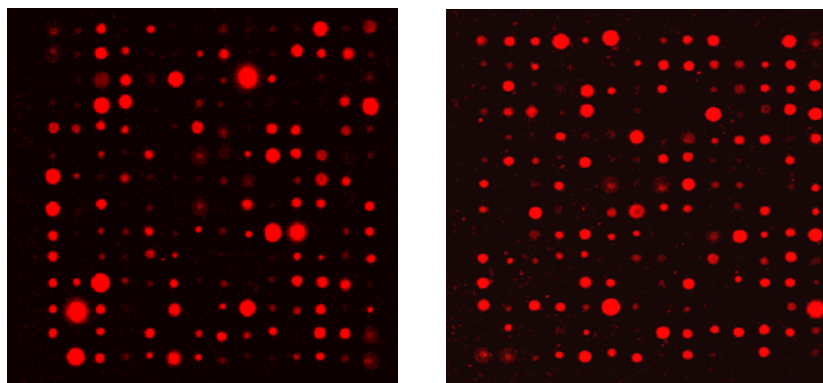
**Figure 1:** To-date weight measurements of all three groups of experimental animals.



**Figure 2:** A type 1 pattern of weight change shown on the example of Rat 43: a small but significant and steady increase in body weight since the third month following asbestos injection.



**Figure 3:** A type 2 pattern of weight change shown on the example of Rat 63: increasing body weight with fluctuations since the third month following asbestos injection.



**Figure 4: NYU PGA-400 glycochip** developed with human pooled serum, diluted 1:15 in a Carrier Buffer. Serum anti-glycan antibodies (AGA) are detected with biotinylated anti-human IgG+IgM+IgA and Streptavidin-Alexa<sup>555</sup>, then visualized and quantified with ImageJ-BioDiscovery software. Current library of 377 glycans is printed in two sub-arrays of 50 and 20  $\mu$ M, eight replicates each. The figure shows AGA signals on the two sub-arrays with the complete library printed at 50  $\mu$ M.

## KEY RESEARCH ACCOMPLISHMENTS

1. We have established the new glyco-laboratory with the dedicated print-room which now allows printing of large batches of glycochips of an enhanced quality.
2. We have developed the expanded glycan array platform, NYU-PGA-400 by adding 177 novel glycan probes, many of which are human Mesothelioma-specific. It is expected that this novel glycochip will allow us to diagnose and prognosticate Mesothelioma earlier during its development. We are now in a process of printing large batches of glycochips for AGA immunoprofiling in both human and our model-Meso-rat sera.
3. We have the protocols approved for the first long-term animal study for the immune responses to the exposure to asbestos in rats, and this experiment is now in its advanced stage.

## REPORTABLE OUTCOMES

None

## CONCLUSIONS

1. Our new glyco-lab with its dedicated, nearly particle-free print-room allows us now to print glycochips of much improved quality, and with the higher efficiency. We are therefore confident that we will be able to accomplish all originally proposed tasks despite initial delays due to the longer than expected time to the approval of animal protocols and previous sub-optimal conditions for glycochip printing.
2. Developed in a meanwhile, our new glycochip NYU-PGA-400 will no doubt provide far more asbestos exposure- and human Malignant Mesothelioma-relevant immuno-information as compared with our previously used PGA-200 – largely due to the addition of novel, Mesothelioma-specific glycan probes, the design of which has been based on our results obtained with the previous study cohorts and recently published in Vuskovic et al., 2011.

## REFERENCES

- Huflejt ME, Vuskovic MI, Vasiliu D, Xu H, Obukhova P, Shilova N, Tuzikov et al. [Anti-carbohydrate antibodies of normal sera: findings, surprises and challenges](#). *Mol Immunol*. 2009; 46: 3037-3049. PMID: 19608278.
- Vuskovic MI, Xu H, Bovin NV, Pass HI, Huflejt ME. [Processing and analysis of serum antibody binding signals from Printed Glycan Arrays for diagnostic and prognostic applications](#). *Int J Bioinf Res App*. 2011; 7: 402-426. PMID: 2211253.

## APPENDIX

Report from “Charles River Research Animal Diagnostic Services” confirming Pathogen-free status of II-45 rat Mesothelioma cell line.

**Sponsor: New York University Medical Center****Accession #: 2011-043499*****Diagnostic Summary Report***

Berg Institute MSB185  
 550 First Ave.  
 New York, NY 10016 USA

**Received:** 21 Sep 2011  
**Approved:** 27 Sep 2011, 13:41

**Attn:** Vanda Williams

**Bill Method:** PO# M000001030  
**Test Specimen:** frozen cells Rat

**Tel:** 212-263-2883

| Sample Set | Service (# Tested)         | Profile              | Assay | Tested | + | +/- | ? |
|------------|----------------------------|----------------------|-------|--------|---|-----|---|
| #1         | Infectious Disease PCR (1) | All Results Negative |       |        |   |     |   |

+ = Positive, +/- = Equivocal, ? = Indeterminate

**Service Approvals**

| Service                | Approved By*       | Date               |
|------------------------|--------------------|--------------------|
| Infectious Disease PCR | Alison L. Kelleher | 27 Sep 2011, 13:41 |

To assure the SPF status of your research animal colonies, it is essential that you understand the sources, pathobiology, diagnosis and control of pathogens and other adventitious infectious agents that may cause research interference. We have summarized this important information in infectious agent **Technical Sheets**, which you can view by visiting [http://www.criver.com/info/disease\\_sheets](http://www.criver.com/info/disease_sheets).

*\*This report has been electronically signed by laboratory personnel. The name of the individual who approved these results appears in the header of this service report. All services are performed in accordance with and subject to General Terms and Conditions of Sale found in the Charles River Laboratories-Research Models and Services catalogue and on the back of invoices.*

**Sponsor:** New York University Medical Center**Accession #:** 2011-043499**Product:** Not Indicated**Test Specimen:** frozen cells Rat**Received:** 21 Sep 2011***Molecular Diagnostics Infectious Disease PCR Results Report*****Department Review:** Approved by Alison L. Kelleher, 27 Sep 2011, 13:41\****Rat Essential Virus Panel***

| Sample #:<br>Code :         | <u>1</u><br>45 |
|-----------------------------|----------------|
| <b>REO 1 &amp; 3 PCR</b>    | -              |
| <b>LCMV PCR</b>             | -              |
| <b>LDV PCR</b>              | -              |
| <b>TMEV/GDVII PCR</b>       | -              |
| <b>Hantavirus Seoul PCR</b> | -              |
| <b>SEND PCR</b>             | -              |
| <b>RCMV PCR</b>             | -              |
| <b>RTV PCR</b>              | -              |
| <b>RPV PCR</b>              | -              |
| <b>IDIR PCR</b>             | -              |
| <b>RCV/SDAV PCR</b>         | -              |
| <b>Mycoplasma Genus PCR</b> | -              |
| <b>M. pulmonis PCR</b>      | -              |
| <b>DNA Spike</b>            | PASS           |
| <b>RNA Spike</b>            | PASS           |
| <b>NRC</b>                  | PASS           |

**Remarks:** - = Negative; I = Inhibition, +/- = Equivocal; + = Positive.

## Sample Suitability/Detection of PCR Inhibition:

Sample DNA or RNA is spiked with a low-copy number of a exogenous DNA or RNA template respectively. A spike template-specific PCR assay is used to test for the spike template for the purpose of determining the presence of PCR inhibitors. The RNA spike control is also used to evaluate the reverse-transcription of RNA. Amplification of spike template indicates that there is no detectable inhibition and the assay is valid.

## NRC:

The nucleic acid recovery control (NRC) is used to evaluate the recovery of DNA/RNA from the nucleic acid isolation process. The test article is spiked with a low-copy number of DNA/RNA template prior to nucleic acid isolation. A template-specific PCR assay is used to detect the DNA/RNA spike.