

Statement of:
Martin Wasserman, M.D, J.D.
Before the United States Senate
Committee on the Environment and Public Works
Subcommittee on Water and Wildlife
April 24, 2012

Chairman Cardin, Ranking Member Sessions, thank you for inviting me to speak today before the subcommittee in support of the Great Ape Protection and Cost Savings Act. My name is Dr. Martin Wasserman. I have lived in Maryland for 45 years. It is where I received both of my graduate degrees: in medicine from Johns Hopkins University, and in law from the University of Maryland. I have served as the chief health officer for Maryland's two largest jurisdictions as well as for Arlington County, Virginia. I have also served as State Health Secretary for both Maryland and Oregon and have been the Executive Director of MedChi, the Maryland State Medical Society, advocating for more than 25,000 physicians. I also served as Medical Director of Immunization Practices and Scientific Affairs in the Vaccine Division of GlaxoSmithKline Pharmaceuticals.

As a public health doctor and as a pediatrician, I have always placed patients first when balancing human needs against the needs of animal test subjects, but I have also considered my Hippocratic Oath, which constantly reminds me to "do no harm." And that is why I am here today to testify in support of the Great Ape Protection and Cost Savings Act.

This important piece of legislation is a bill of recognition, appreciation, and sensitivity. It recognizes the genetic, social, and behavioral similarities of chimpanzees and humans, who are 95 to 98 percent genetically similar. It also acknowledges that the expression of these genes is

dramatically different in chimpanzees and humans. It appreciates the past value of their contribution to medical research that has benefited humans but also recognizes the advances that have occurred in science since I was in medical school—advances that have rendered the use of chimpanzees unnecessary. It sensitively rewards these animals' service with lifetime care in a federal sanctuary. And it will save the government \$300 million over the next 10 years.

In addition to phasing out invasive experimentation on chimpanzees and releasing federally-owned chimpanzees to sanctuaries, this bill will codify the current National Institutes of Health's (NIH) voluntary breeding moratorium preventing any future violations like those that occurred between 2000-2011 when 137 chimpanzees were born to federally-owned chimpanzees at the New Iberia Research Center in Louisiana, and end the breeding of chimpanzees for the purpose of invasive research.

The timing of today's hearing is perfect. Just four months ago, the Institute of Medicine (IOM) released its report *Chimpanzees in Biomedical and Behavioral Research: Assessing the Necessity*, compiled at the request of Senators Harkin, Udall, and Bingaman. NIH has taken the IOM report seriously, and they are to be applauded for their efforts. But theirs is the response of current NIH leadership, and—as we all know—administrations change, leaders change, and policies change. Passage of this bill is essential to ensure that the unnecessary use of chimpanzees in invasive experimentation will not occur in the future.

In the Institute of Medicine report, the authors did not find a single area of human health research for which chimpanzees are necessary. Although there has been some discussion regarding hepatitis C, the authors of the report concluded the following: 1) "Chimpanzees are not necessary for hepatitis C antiviral drug discovery and development;" 2) "Chimpanzees are not necessary for the development and testing of a therapeutic hepatitis C vaccine;" and 3) it is "possible and ethical" to bring a hepatitis C preventive vaccine to human testing without using chimpanzees. In fact I'm proud that my former company, GlaxoSmithKline, publicly stated in

2008 that it would no longer use chimpanzees in their research. While I recognize that more than 130 million people worldwide live with chronic hepatitis C, and that this disease is a serious public health issue, it is indisputably clear that chimpanzee research is not a necessary tool in our battle against hepatitis C.

As a graduate of Johns Hopkins University I commend the schools of medicine and public health for their longitudinal studies of human hepatitis C patients. This long-term study of hepatitis C-infected intravenous drug users provides human-specific information regarding many aspects of hepatitis C acquisition, natural history, therapeutic responses, and vaccine opportunities. This, and similar studies, combined with the numerous human-based culture systems provides a more appropriate and reliable research methodology than redundant protocols using chimpanzees. The Modular IMMune In vitro Construct (MIMIC) System, for example, replicates the human immune system and is appropriate for every stage of drug and vaccine development. The MIMIC system, supported and funded by the U.S. Department of Defense specifically to develop biodefense vaccines, is an example of where science is now and could be used in the development of vaccines for human immunodeficiency virus (HIV), hepatitis C and other life-threatening diseases.

Let me also clear up a misunderstanding with regard to the Food and Drug Administration (FDA) and chimpanzee research. The FDA does not require the use of chimpanzees for either drug or vaccine testing. In 2011, the FDA approved two new therapeutics for hepatitis C—the first in 25 years— from Merck and Vertex Pharmaceuticals and there are two additional drugs from Pharmasset and Bristol-Myers Squibb in the pipeline. None of these four medications used chimpanzees for either development or testing. The IOM report described a variety of alternative research approaches to the continued use of chimpanzees, including cell-based testing and recombinant technologies, which are widely used for the development of monoclonal antibodies.

Significant advances have been made in the development of a malaria vaccine without the use of chimpanzees. A vaccine developed by GlaxoSmithKline—which as I stated earlier does not use chimpanzees—halved the risk for malaria infection in a final-stage trial of more than 15,000 African children. Ann-Marie Cruz, Ph.D., with the PATH Malaria Vaccine Initiative, told the IOM committee that chimpanzees were not essential to malaria vaccine research because humans can be used and represent a better model.

In the 1980s the U.S. expanded its breeding program because chimpanzees were believed to be critical for HIV research. Although, more than 85 HIV vaccines were developed and exhibited benefits in chimpanzees and other non-human primates, all failed in approximately 200 human trials. One vaccine that proved to be safe and effective in chimpanzees actually appeared to increase the chances of infection in humans. As someone who has worked in public health for 30 years devoted to finding solutions for patients infected with HIV and other diseases, I find it disheartening that millions of dollars were allocated toward HIV and other research using chimpanzees without significant benefits to humans when those dollars could have been better spent pursuing alternative methodologies.

At the outset, I mentioned the Hippocratic Oath to “do no harm.” Consider the following: The United States is the only nation in the world that is known to still use captive chimpanzees for large-scale invasive research. Chimpanzees respond to stress and trauma as we do. Published studies reveal that they suffer symptoms of post-traumatic stress disorder and that chimpanzees used in research become clinically depressed. Since there is little we will gain by continuing to use them in research there is no need to continue to keep them in laboratories.

Concerns have been expressed that the passage of this bill would forever preclude the use of chimpanzees in research, even in the case of a national emergency. To address this concern, the Institute of Medicine received testimony from experts in biodefense representing the National Institutes of Health and the Department of Homeland Security, who stated that chimpanzees

would make poor models for future emerging diseases. Nonetheless, I understand the bill's sponsors have agreed to include an amendment that would insert an "emergency clause" in case of a future dire public health crisis. From the public health perspective, I believe this new clause would address any concerns about the future need for chimpanzees in research.

In closing, I respectfully request that you pass the Great Ape Protection and Cost Savings Act in order to focus on new alternative research methodologies, end a cycle of wasteful and unnecessary research, and protect chimpanzees who have already given so much of their lives.

Thank you, and I look forward to your questions.

An Immunologic Model for Rapid Vaccine Assessment — A Clinical Trial in a Test Tube

Russell G. Higbee, Anthony M. Byers, Vipra Dhir, Donald Drake, Heather G. Fahlenkamp, Jyoti Gangur, Anatoly Kachurin, Olga Kachurina, Del Leistriz, Yifan Ma, Riyaz Mehta, Eric Mishkin, Janice Moser, Luis Mosquera, Mike Nguyen, Robert Parkhill, Santosh Pawar, Louis Poisson, Guzman Sanchez-Schmitz, Brian Schanen, Inderpal Singh, Haifeng Song, Tenekua Tapia, William Warren and Vaughan Wittman

VaxDesign Corporation, Orlando, FL, USA

Summary — While the duration and size of human clinical trials may be difficult to reduce, there are several parameters in pre-clinical vaccine development that may be possible to further optimise. By increasing the accuracy of the models used for pre-clinical vaccine testing, it should be possible to increase the probability that any particular vaccine candidate will be successful in human trials. In addition, an improved model will allow the collection of increasingly more-informative data in pre-clinical tests, thus aiding the rational design and formulation of candidates entered into clinical evaluation. An acceleration and increase in sophistication of pre-clinical vaccine development will thus require the advent of more physiologically-accurate models of the human immune system, coupled with substantial advances in the mechanistic understanding of vaccine efficacy, achieved by using this model. We believe the best viable option available is to use human cells and/or tissues in a functional *in vitro* model of human physiology. Not only will this more accurately model human diseases, it will also eliminate any ethical, moral and scientific issues involved with use of live humans and animals. An *in vitro* model, termed "MIMIC" (Modular Immune *In vitro* Construct), was designed and developed to reflect the human immune system in a well-based format. The MIMIC® System is a laboratory-based methodology that replicates the human immune system response. It is highly automated, and can be used to simulate a clinical trial for a diverse population, without putting human subjects at risk. The MIMIC System uses the circulating immune cells of individual donors to recapitulate each individual human immune response by maintaining the autonomy of the donor. Thus, an *in vitro* test system has been created that is functionally equivalent to the donor's own immune system and is designed to respond in a similar manner to the *in vivo* response.

Key words: *clinical trial, drug testing, functional assays, high-throughput, immune response, infectious disease, in vitro, vaccine.*

Address for correspondence: R. Higbee, VaxDesign Corporation, 12612 Challenger Parkway, Suite 365, Orlando, FL 32826-2784, USA.
E-mail: rhigbee@vaxdesign.com

MIMIC® System Technology Overview

The MIMIC System is based on the multidimensional interrogation of leucocytes. It can simulate a clinical trial, including the effects of immunotherapy on human population subgroups, where responses can be clustered into groups that capture genetic diversity and other important population characteristics, such as HLA haplotypes, age, autoimmune status, and gender. We hope that this dataset can guide the design of rapid and incisive adaptive clinical trials, as well as overcome limiting and misleading animal studies in predicting

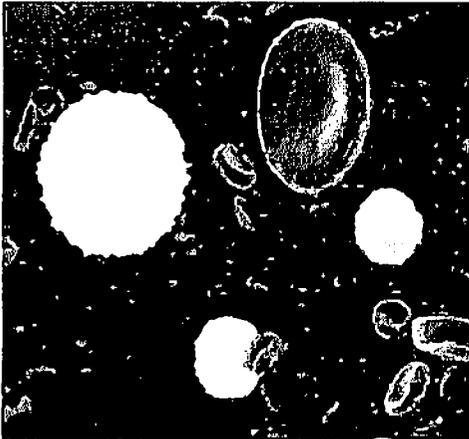
the immunogenic potential of non-homologous proteins and many vaccine candidates.

The MIMIC System is comprised of four different steps: 1) leucocyte collection and preservation, 2) the Peripheral Tissue Equivalent (PTE), 3) the Lymphoid Tissue Equivalent (LTE), and 4) functional assays for assessing the *in vitro* immune response (Figure 1).

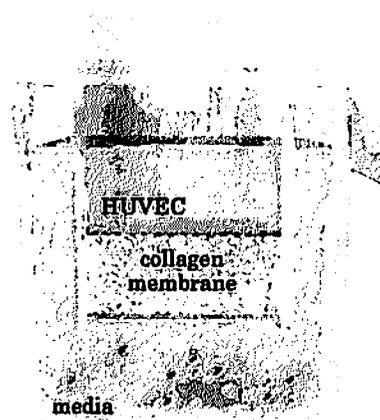
Step one begins with the collection of donor leucocytes by apheresis at a local blood bank. The processing of the leucocytes typically begins within an hour after collection, and the entire process to cryopreservation takes less than 4 hours. From a single apheresis donation, approximately 10 billion leuco-

Figure 1: The four modules of the MIMIC System

a) Blood cells



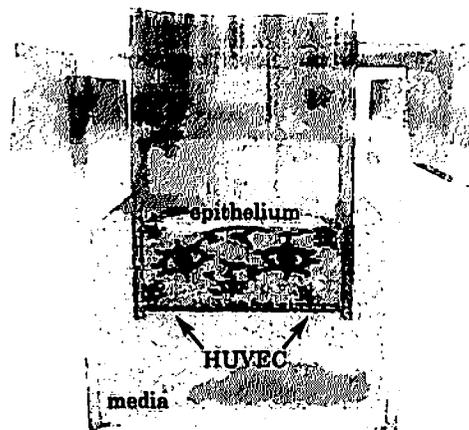
b) Innate immunity: PTE module



c) Adaptive immunity: LTE module



d) Effectiveness: functional assay or disease model



The four component modules of the MIMIC System: a) The collection of leucocytes from donors and their preservation; b) The second module, the Peripheral Tissue Equivalent (PTE) module, simulates innate immune responses. It comprises a monolayer of human umbilical vein endothelial cells (HUVEC) cultured above a 3-dimensional extra-cellular matrix upon which peripheral blood mononuclear cells (PBMCs) are applied; c) The third module, the Lymphoid Tissue Equivalent (LTE) module, simulates the adaptive immune response that would occur in the lymph node. Dendritic cells, follicular dendritic cells, T-cells and B-cells are applied in the correct sequential order to mimic the immune response expected in vivo; d) Functional assays, which indicate whether the immune response generated in the preceding modules is effective against the chosen stimulant or pathogen.

cytes are obtained and processed for cryostorage by standard methods, whereby the donor's cells may be used at a later date. This approach allows multiple experimental iterations, such as different compounds, doses or combinations, including enough of each to obtain statistically meaningful data. Because of the nature of the studies and the ability to cryopreserve cells, all the relevant controls, such as no treatment, drug alone and pathogen alone, can be run on the same "surrogate human" at the same

time. This is in striking contrast to what can be done with non-human primates (NHP) or in human clinical trials. An additional advantage over both NHP studies and human clinical trials is that the experiments can be repeated on the same "individual", as a portion of the primary cells can be frozen and stored for future use.

The second step is to simulate a peripheral tissue. For this, we developed innate immune responses in the Peripheral Tissue Equivalent (PTE) module. The

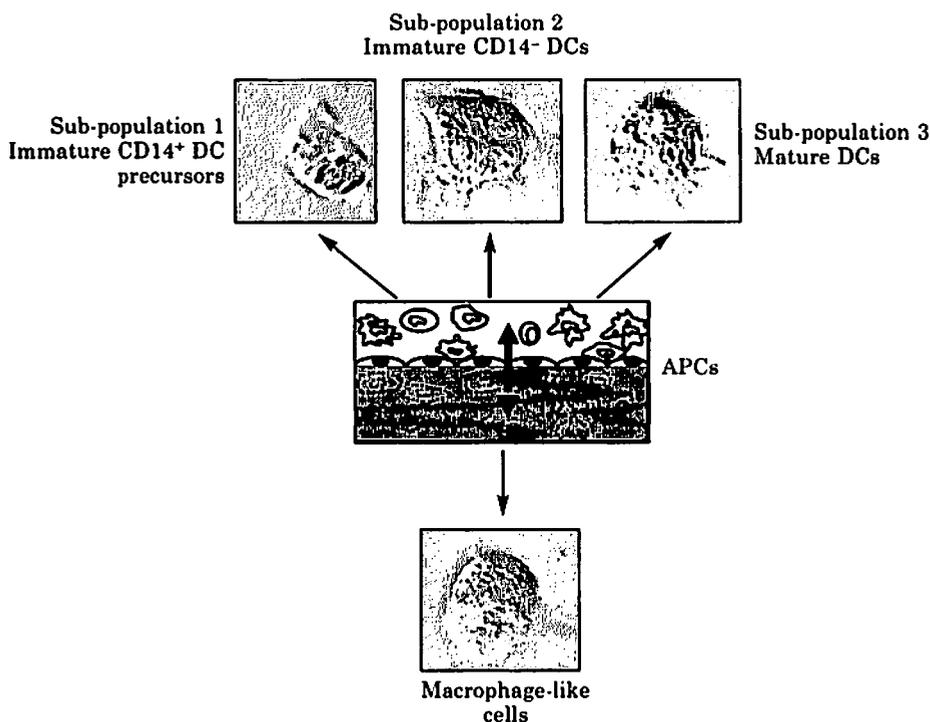
PTE module allows for a broad assessment of responses, from toxicity to proinflammatory immunoreactogenicity (testing vaccines, antigens, adjuvants, biologics, therapeutics and chemicals), to antigen processing, vascular leakage and leucocyte infiltration, maturation and extravasation. This module is a high-throughput, fully-automated, flexible and reproducible, 3-dimensional tissue-engineered construct, mimicking peripheral microvasculature and recapitulating peripheral circulatory and fast innate responses. The PTE also links to the adaptive arm of the immune system, allowing self-differentiation of extravasating monocytes into potent antigen-presenting migratory dendritic cells (DCs).

The third step is to simulate adaptive immune responses in the Lymphoid Tissue Equivalent (LTE) module. The LTE is essentially an artificial lymph node, where antigen-presenting cells from the immune system, the body's "sentinel" cells, start

working with the immune system's T-cells and B-cells. Specifically, the LTE is designed to reflect the spatio-temporal kinetics in a lymph node, e.g., DC-T-cell interactions, antigen-B-cell interactions, T-cell and B-cell interactions. Th1 or Th2 polarisation bias, antigen-specific antibody production or cytotoxic T-cells, can all be assessed from this *in vitro* module.

The fourth step is to assess the immunocytes and biomolecules from the previous modules in a functional assay, such as microneutralisation assays, haemagglutination inhibition, adherence inhibition, CTL responses, or disease modelling. Having all of these modules operating in a robotics platform provides a high-throughput, reproducible, platform where multiple drug/vaccine candidates can be tested on multiple donors at the same time, without subjecting the actual individual to a potentially dangerous substance.

Figure 2: The three populations of cells which arise in the Peripheral Tissue Equivalent (PTE) module



Three populations of cells arise following the application of peripheral blood mononuclear cells (PBMCs) to the PTE module. Sub-population 1 comprises immature CD14⁺ dendritic cell precursors while sub-population 2 comprises immature dendritic cells (CD14⁻), and sub-population 3 comprises mature dendritic cells. A fourth population is more macrophage-like and is retained in the collagen matrix. DC = dendritic cells, APCs = antigen presenting cells.

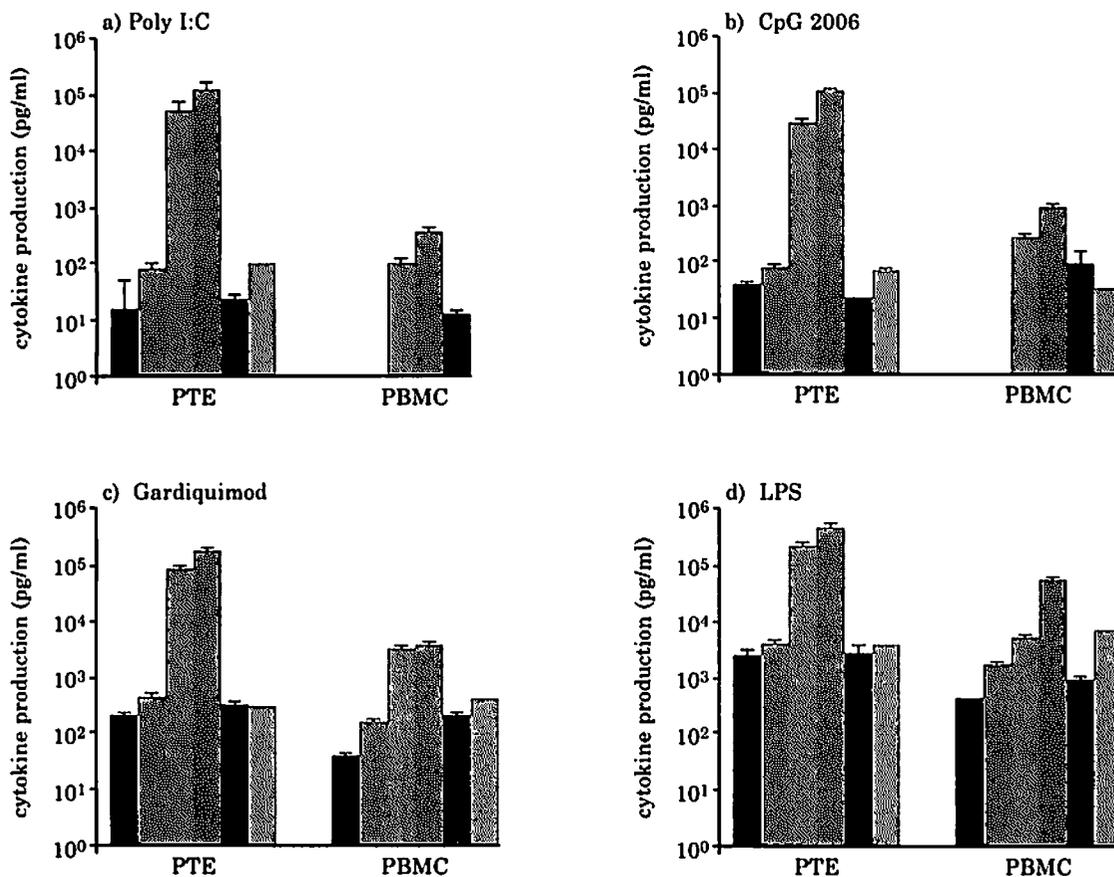
(Sanchez-Schmitz, G., Fahlenkamp, H.G., Ma, Y., Poisson, L., Warren, W.L., Mishkin, E. & Higbee, R. (2006). An autonomously driven *in vitro* human immune system for vaccine testing. Ninth International Congress on Dendritic Cells. Edinburgh, UK.)

The PTE Module

The PTE module has been well characterised, and is a unique method of generating autologous dendritic cells by using a 3-dimensional tissue engineered construct (1–3). Simply put, the PTE module is a high-throughput module that spontaneously and autonomously generates dendritic cells (DCs), the principal antigen processing cells (APCs) of the immune system. The PTE is comprised of a human endothelial monolayer grown to confluence over a 3-dimensional extracellular matrix (Figure 1), onto which purified peripheral

blood mononuclear cells (PBMCs) are placed. The immature monocyte population of cells extravasates across the endothelial monolayer, migrating into the matrix, where they spontaneously and autonomously differentiate into migratory DCs with different maturation states (Figure 2). The DCs then spontaneously reverse transmigrate across the endothelial layer — a process that reflects APCs crossing the lymphatics. Upon reverse transmigration across the endothelium, the APCs are then collected. At any point along their journey through the PTE, cultures can be stimulated with antigen/adjuvant/drug compounds of choice.

Figure 3: A comparison of the cytokine response to immunomodulators in the MIMIC System and the industry-standard PBMC assay



■ = IL-1α; ▨ = IL-1β; ▩ = IL-6; ▪ = IL-8; ■ = IL-10; ▨ = TNFα.

A comparison of the MIMIC System PTE module and the industry-standard PMBC assay was made using the same individual donor's blood cells. Immunomodulators, representing different classes, were applied to the PTE and the PMBC assay and the cytokine response was plotted on a log scale. PTE = Peripheral Tissue Equivalent; PMBC = Peripheral Blood Mononuclear Cell; IL = interleukin; poly I:C = polyinosinic : polycytidilic acid; CpG = unmethylated synthetic cytosine-phosphate-guanosine oligodeoxy nucleotides; LPS = lipopolysaccharide. Gardiquimod™ is an imidazoquinoline compound developed by InvivoGen.

The monocyte extravasation and the DC development kinetics match *in vivo* physiology. An important aspect of the PTE is that the migratory DCs remain largely immature in the absence of an external stimulus. We have found that these immature DCs can acquire and process antigen when properly stimulated by adjuvants, maturing into potent DCs capable of initiating antigen-specific immune responses in LTE co-cultures. These DCs have shown the capacity to induce antigen-specific lympho-proliferation, cell-mediated cytotoxicity and T-helper cytokine production.

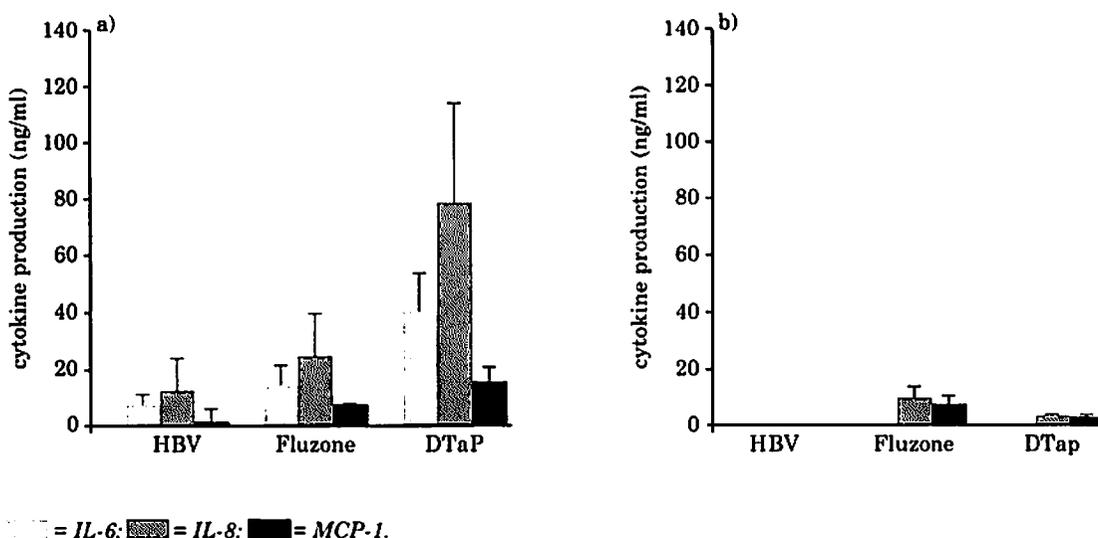
We have found that PTE-derived DCs are very similar to *in vivo* DCs. The PTE has shown that naturally extravasated monocytes constitutively and autonomously differentiate into either migratory DCs, or resident macrophages, in absence of stimulation, and this normally takes between 1–3 days; similar kinetics have been reported *in vivo* for both humans and animals (4–8). The transendothelial migration of blood monocytes promotes differentiation into potent antigen-presenting DCs in humans and animals (9–11), as observed in the PTE; extravasation of leucocytes is increased via endothelium activation in both the PTE and *in vivo* (12). Crossing the endothelium in the abluminal-to-luminal direction (reverse transmigration) in the PTE resembles the *in vivo* entrance of DCs into the lymphatics (13).

Similarly, *in vivo*, skin DCs will be one of the first cell types to engage a pathogen or foreign material, such as a vaccine or a topically applied chemical. The DCs produced by the PTE have been extensively characterised, and were found to be very similar in the expression of numerous surface markers to those of human dermal explants (14). Additionally, three subpopulations of DCs are also characteristic of PTE-generated DCs: immature DC precursors (CD14⁺), immature DCs (CD14⁻), and mature DCs (CD14⁻, HLA-DR⁺, CD86⁺, CD83⁺), along with a fourth population of cells that differentiate to a more macrophage-like phenotype and do not reverse transmigrate back across the endothelial monolayer (Figure 2).

The PTE has been found to largely recapitulate innate immune responses, when tested with vaccines, adjuvants, biologics, immunopotentiators, immunosuppressants and various pathogens. The PBMC assay is the accepted industry-wide standard for studying immune reactions (15). The MIMIC System has been found to produce a more physiologically relevant response than the PBMC assay (Figure 3) for various adjuvants and immunomodulators.

To evaluate the immunopotency of Toll-like receptor (TLR) agonists in the *in vitro* lymphatic PTE module, we measured TLR-induced cytokine production. Overall, TLR agonists induced higher levels of cytokines in the PTE module than in

Figure 4: The cytokine response to vaccines: A comparison of the MIMIC™ System PTE module and the industry-standard method



a) PTE-derived DCs; b) monocyte-derived DCs.

Dendritic cells (DCs) created in the MIMIC System PTE module and monocyte-derived dendritic cells were exposed to three vaccines and the production of three inflammatory cytokines (IL-6, IL-8 and MCP-1) was measured. PTE = peripheral tissue equivalent; HBV = hepatitis B virus vaccine; IL = interleukin; MCP = monocyte chemotactic protein; DTaP = diphtheria, tetanus toxoid and acellular pertussis vaccine; Fluzone[®] = a killed, trivalent influenza vaccine.

conventional PBMC cultures (Figure 3). For example, polyinosinic:polycytidylic acid (Poly I:C) and unmethylated synthetic cytosine-phosphate-guanosine oligodeoxy nucleotides (CpG 2006), both induced the proinflammatory cytokines IL-1 α/β in the PTE module, but neither of these cytokines were observed in PBMC cultures. Poly I:C also triggered the production of TNF α only in the PTE culture (Figures 3a and 3b). Moreover, Poly I:C and CpG 2006 treatments elicited approximately 100–1000 fold greater levels of IL-6 and IL-8 in the PTE module than in PBMC cultures. Although Gardiquimod and LPS dramatically induced IL-1 α/β , IL-6, IL-8, IL-10, and TNF α , both in the PTE module and in the PBMC cultures, the PTE module produced approximately 3–6 fold more IL-1 α/β and IL-10, and 10–50 fold more IL-6 and IL-8, than PBMC cultures (Figures 3c and 3d). Hence, the PTE module was found to be more sensitive than conventional PBMC cultures in response to TLR stimulation (Ma *et al.* *Assessing the immunopotency of Toll-like receptor agonists in an in vitro tissue engineered immunological model*. Manuscript in preparation).

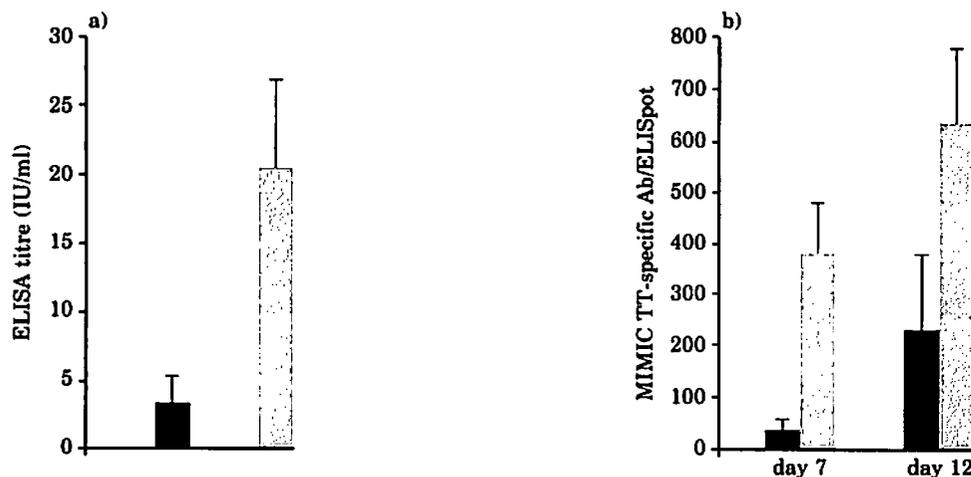
When stimulated by vaccines, the PTE module mimics the *in vivo* state by producing cytokines

known to be involved with inflammatory processes. Figure 4 shows the general innate reactivity of the commercially available vaccines, DTaP (Sanofi Pasteur, Inc.), Fluzone[®] (Sanofi Pasteur, Inc.) and Recombivax[®] (Merck & Co., Inc.), assessed by detection of a panel of proinflammatory cytokines in the PTE module. As can be seen, the reactivity is highest for DTaP probably as a result of a combination of bacterial components from diphtheria and acellular pertussis, and the presence of alum adjuvant. Fluzone shows moderate reactivity, that may be linked to a residue of egg albumin and the inherent stimulatory capacity of influenza virus proteins; and finally, the purified HBsAg sub-unit vaccine of Recombivax has less potency. Interestingly, several inoculations of the Recombivax vaccine are normally required *in vivo*, to elicit protective immunity.

The LTE Module

The Lymphoid Tissue Equivalent (LTE) module has been designed to largely recapitulate human adaptive immune responses in the lymphoid tissues of the body. Although there are many cellular

Figure 5: A comparison of the specific immune response to tetanus vaccine, *in vivo*, and *in vitro* in the MIMIC System



Thirteen volunteers were vaccinated with a commercial tetanus vaccine. Blood samples from each individual were taken before and after vaccination.

a) The levels of tetanus-specific antibodies in the individuals' sera were determined by enzyme-linked immunosorbent assay (ELISA), pre and post-vaccination.

b) Peripheral blood mononuclear cells, purified from both the pre and post-vaccination blood samples of the individual donors, were simultaneously evaluated in the MIMIC System. The number of tetanus-specific antibody secreting cells, after 7 and 12 days of *in vitro* culture, were determined by enzyme-linked immunosorbent spot (ELISpot) assay.

■ = pre vaccination; ▨ = post vaccination.

types within any given lymph node, the MIMIC system incorporates two-, three- and four-way interactions of the key immune cells (DCs, follicular dendritic cells, B-cells and T-cells). The application of these cells to the LTE is in sequential order, to mimic these immunologically-relevant responses, similar to what is known to occur *in vivo* (16). VaxDesign follows fundamental design observations, whereby the right cells (CD4⁺ T-cells, B-cells, DCs, and follicular DCs) are placed together at the right time and in the right order, in an automatable, scalable, reproducible system to get the appropriate response (Moser, J.M., Sassano, E.R., Leistriz, D.C., Eatrides, J.M., Gaucher, D., Filali-Mouhim, A., Phogat, S., Koff, W., Sékaly, R-P., Haddad, E.K. & Drake, D.R. [2009]. *Dendritic cell-based assay for the in vitro priming of naïve human CD4⁺ T cells*. Manuscript submitted).

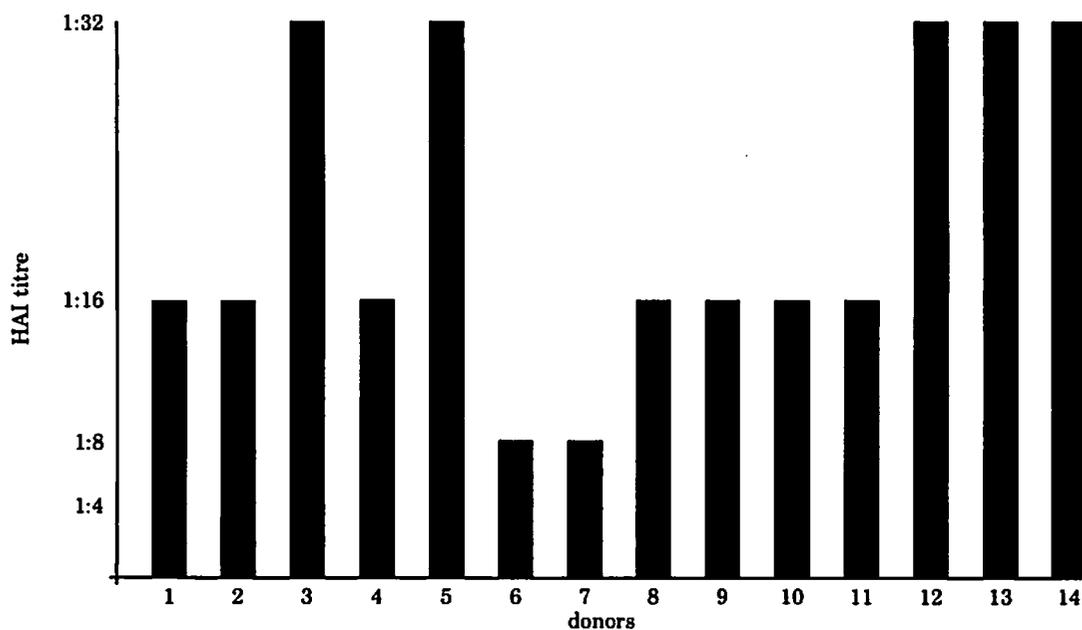
As one example to validate the potential of this approach, we monitored the *in vitro*-generated tetanus toxoid (TT)-specific antibody levels in a cohort of donors before and after receiving tetanus vaccination. Purified CD4 T-cell and B-

cell populations were combined with autologous tetanus vaccine-pulsed dendritic cells, to generate specific antibody. Enumeration of the TT-specific IgG antibody-secreting cells by enzyme-linked immunosorbent spot (ELISpot) assays displayed a significant increase in the magnitude of this population after vaccination. The relative magnitudes of the *in vitro*-generated TT-specific antibody response before and after vaccination, largely recapitulated the TT-specific IgG serum titre profiles measured in the same individuals, as shown in Figure 5 (17).

These findings provide evidence that the MIMIC System can be a rapid and representative *in vitro* method for measuring vaccine immunogenicity via induction of the memory B-cell response. In-house studies have clearly demonstrated that the use of purified lymphocyte populations and autologous DCs is more sensitive than bulk PBMC assays at generating both T-cell and B-cell immune responses (unpublished data).

Similar results have been obtained with other commercial vaccines, such as those for recall antigens (e.g. hepatitis B virus and influenza viruses),

Figure 6: Antibodies produced by the MIMIC System show effective neutralisation activity



Individual human donors' leucocytes were placed in the MIMIC System and stimulated with a commercial live-attenuated influenza vaccine. To test whether the antibodies produced by the B-cells in the MIMIC System are effective in neutralising the influenza virus they were tested, at serial dilution, in a standardised haemagglutination assay (HA). In this assay influenza virus particles cause agglutination of red blood cells (RBCs). Antibodies specific to influenza surface proteins will bind to the viral particles and will inhibit the haemagglutination. The greater the dilution of antibody-containing material, that still causes inhibition of agglutination, the greater the concentration of specific antibody. HAI = haemagglutination inhibition assay.

and primary response antigens, such as yellow fever virus.

Functional Assays

Functional assays are at the heart of the MIMIC System and determine whether the responses observed in previous modules of the MIMIC System are going to be effective against the original challenge material. For example, are the antibodies produced by B-cells effective at neutralising the original virus, as observed in the case for influenza (Figure 6), or do T-cell antigen-specific responses lead to an increase in cytokine production, cytolytic activity or overall proliferation? Cytotoxic T-cell assays examined CD107a and interferon-gamma (IFN- γ), both markers for cell killing.

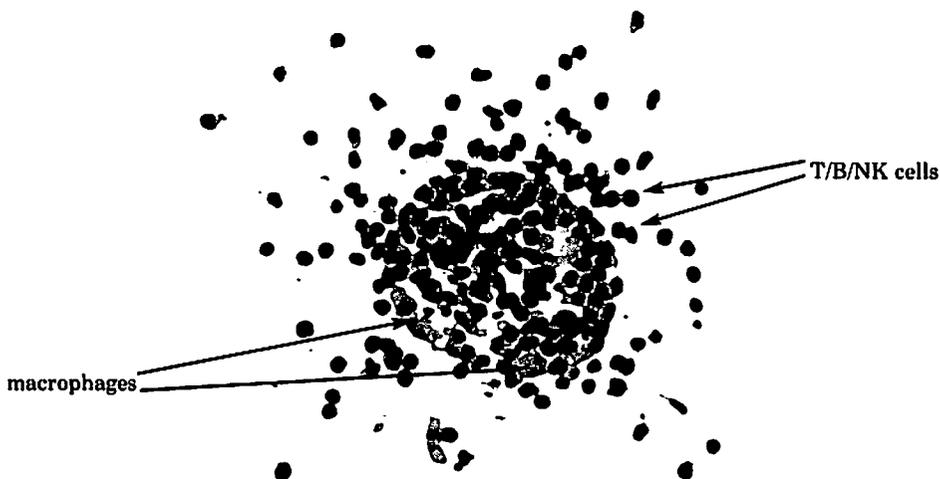
Since the MIMIC System can be re-stimulated *in vitro* many times, this is similar to the prime-boost scenarios used *in vivo*. This unique strategy allows for the possible application and testing of different primary and secondary stimulating antigen combinations to be delivered, all *in vitro*. CD8⁺ T-cell responses have been observed in the MIMIC System, for both recall antigens (live-attenuated influenza vaccine) and for naïve antigens (live-attenuated yellow fever vaccine).

Disease modelling can also be performed with the MIMIC System. Tuberculosis (TB) is classified as one of the most devastating granulomatous diseases world-wide (18). The MIMIC System has been able to successfully recapitulate granuloma formation *in vitro*, and to drive *Mycobacterium tuberculosis* to latency. Histological analysis of the PTE module seeded with *M. tuberculosis* and PBMCs revealed spontaneous granuloma formation. Haematoxylin and eosin staining of sectioned PTE modules seeded with *M. tuberculosis* and PBMCs, clearly showed initial stages of granuloma formation in culture (Figure 7). In this disease module, new antibiotics can be tested to determine whether they are effective on latent TB and could lead to new therapeutic regimens. (Pawar *et al.* *An in vitro model of human tuberculosis granuloma and Mycobacterium tuberculosis latency*. Manuscript in preparation).

Automation

The MIMIC system has been streamlined and automated from beginning to end, by using a unique, reliable and robotic system to construct and test each component of this *in vitro* cell-based technology. Automation allows for precise fluid handling, consistency between wells and tests,

Figure 7: A cross-section of a MIMIC System PTE module seeded with *Mycobacterium tuberculosis*



A MIMIC PTE module was seeded with *M. tuberculosis* and PBMCs. Examination of cross-sections, stained with haematoxylin and eosin, clearly showed the initial stages of granuloma formation. Macrophages, T-cells and B-cells, NK cells and fibroblasts are among the cells that aggregate to form the granuloma, with lymphocytes surrounding the infected macrophages. The MIMIC System has been able to recapitulate *in vivo* granuloma formation. This represents a novel disease model in which new antibiotics can be tested for efficacy against latent tuberculosis. PBMCs = peripheral blood mononuclear cells.

from one donor's cells to another, and provides a rapid platform to accomplish a very high-throughput system in the most cost-efficient manner currently available.

Conclusions

The MIMIC System is a high-throughput, automated, *in vitro* modular technology, which is capable of examining individual human donor immune cell responses to many different compounds, such as vaccines, adjuvants, proteins, chemicals and drugs. Innate immune responses primarily are observed in the PTE module, with the capacity to mimic multiple mucosal surface types, as well as different antigen delivery sites. LTE responses recapitulate *in vivo* adaptive immune response with the right cells, at the appropriate time, and under appropriate conditions, to permit the production of effective antibody production and/or T-cell responses to vaccines, biologics, biologicals, or pathogens. Functional assays test these antibodies or T-cells for performance against the stimulating antigen. Many of these involve cytokine production, increased titres *in vitro*, viral neutralisation, or cytotoxic T-cell assays. The MIMIC System allows testing for a variety of demographic groupings, such as for HLA typing, gender or age biases, and geographic regional differences.

Acknowledgement

This project was funded through the DARPA RVA Program (contract number NBCHC060058).

References

- Randolph, G.J., Beaulieu, S., Lebecque, S., Steinman, R.M. & Muller, W.A. (1998). Differentiation of monocytes into dendritic cells in a model of transendothelial trafficking. *Science, New York* **282**, 480–483.
- Randolph, G.J., Sanchez-Schmitz, G., Liebman, R.M. & Schäkel, K. (2002). The CD16⁺ (FcγRIII⁺) subset of human monocytes preferentially becomes migratory dendritic cells in a model tissue setting. *Journal of Experimental Medicine* **196**, 517–527.
- Shortman, K. & Liu, Y.-J. (2002). Mouse and human dendritic cell subtypes. *Nature Reviews. Immunology* **2**, 151–161.
- Zelivyanskaya, M.L., Nelson, J.A., Poluektova, L., Uberti, M., Mellon, M., Gendelman, H.E. & Boska, M.D. (2003). Tracking superparamagnetic iron oxide labeled monocytes in brain by high-field magnetic resonance imaging. *Journal of Neuroscience Research* **73**, 284–295.
- Pugh, C.W., MacPherson, G.G. & Steer, H.W. (1983). Characterization of nonlymphoid cells derived from rat peripheral lymph. *Journal of Experimental Medicine* **157**, 1758–1779.
- Smith, J.B., McIntosh, G.H. & Morris, B. (1970). The traffic of cells through tissues: a study of peripheral lymph in sheep. *Journal of Anatomy* **107**, 87–100.
- Fossum, S., Rolstad, B. & Ford, W.L. (1984). Thymus independence, kinetics and phagocytic ability of interdigitating cells. *Immunobiology* **168**, 403–413.
- Holt, P.G., Haining, S., Nelson, D.J. & Sedgwick, J.D. (1994). Origin and steady-state turnover of class II MHC-bearing dendritic cells in the epithelium of the conducting airways. *Journal of Immunology* **153**, 256–261.
- Larregina, A.T., Morelli, A.E., Spencer, L.A., Logar, A.J., Watkins, S.C., Thomson, A.W. & Falo, L.D. Jr. (2001). Dermal-resident CD14⁺ cells differentiate into Langerhans cells. *Nature Immunology* **2**, 1151–1158.
- Randolph, G.J., Inaba, K., Robbiani, D.F., Steinman, R.M. & Muller, W.A. (1999). Differentiation of phagocytic monocytes into lymph node dendritic cells *in vivo*. *Immunity* **11**, 753–761.
- Ginhoux, F., Tacke, F., Angeli, V., Bogunovic, M., Loubeau, M., Dai, X.M., Stanley, E.R., Randolph, G.J. & Merad, M. (2006). Langerhans cells arise from monocytes *in vivo*. *Nature Immunology* **7**, 265–273.
- Helintö, M., Renkonen, R., Tervo, T., Vesaluoma, M., Saaren-Seppälä, H., Haahtela, T. & Kirveskari, J. (2004). Direct *in vivo* monitoring of acute allergic reactions in human conjunctiva. *Journal of Immunology* **172**, 3235–3242.
- Robbiani, D.F., Finch, R.A., Jäger, D., Muller, W.A., Sartorelli, A.C. & Randolph, G.J. (2000). The leukotriene C₄ transporter MRP1 regulates CCL19 (MIP-3b, ELC)-dependent mobilization of dendritic cells to lymph nodes. *Cell* **103**, 757–768.
- Morelli, A.E., Rubin, J.P., Erdos, G., Tkacheva, O.A., Mathers, A.R., Zahorchak, A.F., Thomson, A.W., Falo, L.D., Jr & Larregina, A.T. (2005). CD4⁺ T cell responses elicited by different subsets of human skin migratory dendritic cells. *Journal of Immunology* **175**, 7905–7915.
- Assenmacher, M., Lohning, M. & Radbruch, A. (2001). Detection and isolation of cytokine secreting cells using the cytometric cytokine secretion assay. *Current Protocols in Immunology Unit* **6.27**, 1–10.
- Young, A.J. (1999). The physiology of lymphocyte migration through the single lymph node *in vivo*. *Seminars in Immunology* **11**, 73–83.
- Byers, A.M., Tapia, T.M., Sassano, E.R. & Wittman, V. (2009). *In vitro* antibody response to tetanus in the MIMIC™ system is a representative measure of vaccine immunogenicity. *Biologicals* **137**, 148–151.
- Toossi, Z. & Ellner, J.J. (2001). Pathogenesis of tuberculosis. In *Tuberculosis* (ed. L.N. Friedman), pp. 19–47. New York, NY, USA: CRC Press.

October 4, 2011

Bruce Altevogt, Ph.D.
Study Director
Institute of Medicine Committee on the Use of
Chimpanzees in Biomedical and Behavioral Research
500 Fifth Street, NW
Washington, DC 20001

Sent by e-mail (baltevogt@nas.edu)

Dear Dr. Altevogt:

I am writing to report recent developments in the field of therapeutics for hepatitis C, that will be of interest to the Committee. In recent months, two new hepatitis C drugs have been approved for market by the Food and Drug Administration, and two others have shown particular promise in clinical trials. All four drugs have reached these stages without testing in chimpanzees. These drugs are Merck's Victrelis (bocepravir), Vertex's Incivek (telaprevir), Pharmasset's uracil nucleotide analog PSI-7977, and Bristol-Myers Squibb's NS5A inhibitor BMS-790052.

Victrelis and Incivek were approved by the FDA in May 2011 for combined use with peginterferon alfa and ribavirin. A search of FDA and PubMed records reveals that the development and testing of bocepravir was completed without the use of chimpanzees. In response to our inquiry, Vertex Chief Scientific Officer and Executive Vice President Peter Mueller, Ph.D. stated that "chimpanzees were not used in the development of Telaprevir." Dr. Mueller's letter is attached.

Pharmasset's PSI-7977 and Bristol-Myers Squibb's BMS-790052 have completed successful phase II clinical trials demonstrating efficacy in the treatment of hepatitis C, and both drugs are advancing to later phase trials.

An inquiry to Pharmasset obtained a reply from Chief Scientific Officer Michael J. Otto, Ph.D., who stated: "In response to your e-mail and faxed letter, chimpanzees were not used in the development of PSI-7977. We do not use chimpanzees in our research or development and see no reason to change our approach." Dr. Otto's e-mail is attached.

A diligent literature search reveals that BMS-790052 has not been tested on chimpanzees. Inquiries to Bristol-Myers Squibb have not been answered.

Thus, both recently approved hepatitis C drugs and both new hepatitis C drugs showing particular promise in mid-stage clinical trials have been developed and tested without the use of chimpanzees. This is additional authoritative evidence that chimpanzees are not necessary to bring new effective hepatitis C drugs to the public.

We hope this information is useful for the Committee, and I would be pleased to answer questions or provide additional information at the Committee's request. Thank you for your ongoing careful consideration of the use of chimpanzees for biomedical and behavioral research.

Sincerely,

A handwritten signature in black ink, appearing to read "J. Pippin". The signature is fluid and cursive, with a large initial "J" and "P".

John J. Pippin, MD, FACC
Director of Medical Affairs
Phone and fax: (972) 407-9396
E-mail: jpippin@pcrm.org or jjpippin@sbcglobal.net