The Honorable Marsha Blackburn

1. At the hearing, the claim was made that without fetal tissue, Zika virus research could not go forward. Only days later, the Washington Post1 cited a Cell Stem Cell research project in which induced pluripotent stem cells were engineered to study the characteristics of the virus's infectious potential in developing neural tissue. Since the breakthrough study produced vital information, why the insistence that fetal tissue is required to develop a vaccine or to study the infection's progress?

Answer: Respectfully, my own testimony did *not* state that "Zika virus research could not go forward. " I did, however, argue that it would be slowed without fetal tissue research. The Cell Stem Cell study cited in the Washington Post (Tang et al., Cell Stem Cell 2016) was indeed interesting in this context as it demonstrated enhanced infection of neural progenitors made from pluripotent stem cells. Thus, this paper perhaps provides an important clue to a cell type that Zika might infect. Interestingly, the Tang et al. paper uses HEK293, which are cells of fetal origin further underscoring the important need for fetal tissue in research. But, importantly, the Tang et al. Cell Stem Cell paper relies on the hypothesis that Zika directly infects the fetal brain as opposed to generating placental defects or other defects in the mother during pregnancy. This important evidence comes in part from a previous paper by *Mlakar et al., NEJM 2016 mar 10 vol 374 p951* that depended heavily upon fetal tissue for the conclusion that Zika virus infects the fetal brain directly. The genome structure of the Zika virus was also worked out in Mlakar et al, which is critical for future work. Without this analysis of donated abortion-derived fetal tissue provided in Mlakar et al., we would lack important evidence that the Zika virus actually infects the fetal brain, which was the foundation for the Cell Stem Cell paper.

Furthermore, the Tang et al. Cell Stem Cell paper is highly limited and only shows that one type of cell, a neural progenitor cell can be made with pluripotent stem cells and infected by Zika. But the Tang et al. paper provides no evidence that this progenitor is the actual cell type infected in the fetal brain. The fetal and adult brain are composed of many cell types including astrocytes, oligodendrocytes, and different types of progenitors in different regions. Neural progenitors themselves can be highly variable with regional identities that may or may not correspond to bona fide fetal brain cell types. The Tang et al. Cell Stem Cell paper does not examine any of these other cell types and so is highly limited. Most important, any cell type predicted by in vitro cell culture work to be the primary target of Zika will almost surely need to be verified in bona fide fetal brain tissue during the course of an infection before subjecting at-risk mothers to preventative or disease modifying therapies.

A more recent paper was just published in Cell Stem Cell by Nowakowski et al. (2016) that made extensive use of fetal brain tissue to determine in greater detail the cell type infected by Zika virus in the fetal brain and went further to determine the potential molecules that the virus uses to attack fetal brain cells. The paper by Nowakowski et al. goes well beyond the Tang et al. paper reported in the Washington Post and highlights how important fetal tissue is to identify the correct brain cells infected by the virus and the mechanism used so that potential therapies or vaccines may be developed.

2. First of all, you mention fetal cells related to spinal cord injuries – Why don't you tell us about any peer reviewed journal studies about the cures of spinal cord injuries from adult stems cells?

Answer: I am not sure which studies you are referring to, but I'll note that I am aware of a few sporadic studies of different cell types for spinal cord injury that claim results of varying quality. First, I told you about fetal tissue since that was the subject of the hearing. Second, some of the spinal cord injury studies claiming "cures" from injections of adult cells are poorly controlled and designed so that it is not possible to determine whether any beneficial effects were from the cells or from the accompanying intensive physical therapy or from occasional sporadic improvements. Third, patients in some of these reports suffered adverse events including benign tumors, payment of large amount of money without benefit, and worsening of function. One patient in particular treated with adult olfactory cells developed a spinal tumor at the site of transplant (Diouhy et al. J. Neurosurg Spine 2014). The point is that many types of cells need to be tested, including adult cells, embryonic cells, and fetal neural stem cells to find out what is the best therapy. It makes no sense to only try one approach and so the scientific community is pursuing many avenues in parallel to find relief for the many spinal cord injury victims that need help. Many such trials using different types of cells including fetal cells are listed in clinicaltrials.gov.

You told us that fetal cells are essential to make astrocytes but you did not mention that adult neural stem cells can make astrocytes and perform what the fetal cells are doing. Aren't the fetal cells in your testimony just "nurse" cells to spew growth factors out to support iPS cells.

Answer: Astrocytes are beginning to be recognized as cells that provide more than just growth factors. We use astrocytes in our experiments in a number of ways, sometimes just for the factors they produce. Not all astrocytes produced in vitro are identical to each other or to natural sources. We also don't yet know the nature of all of the factors produced by astrocytes. Continued work comparing fetal astrocytes to other sources of astrocytes will be useful. Moreover, astrocytes derived from fetal material grow more successfully in the lab environment than do adult-derived astrocytes.

Why did your testimony fail to mention that functional kidney "organoids" have already been grown using iPS cells and adult stem cells.

Answer: Thank you for raising the kidney organoid experiments, which are instructive. In the Nature paper reporting kidney organoids, stem cells were used to make structures that had some of the functional elements of kidneys although they were disorganized. Notably, the paper reporting on kidney organoids makes extensive use of *fetal tissue* as a comparison to evaluate how similar organoids were to *fetal kidneys*. This paper is a particularly good example of how important fetal tissue research is to ongoing research to develop organs from stem cells.

3. Prior to providing consent to donate fetal tissue, should a woman be advised that there is a possibility her child may be "born alive"?

Answer: This question is outside my field of expertise.

Is it not true that such information would allow a woman to provide a more informed consent prior to deciding whether to donate tissue, thereby making the consent more ethically sound?

Answer: This question is outside my field of expertise.

If you respond in the negative, please identify specific reasons why such information is ethically irrelevant.

4. In response to a question about "where do you guys get your fetal tissue," you testified that the "fetal neural stem cells that we obtain for our clinical trials come from our collaborating company called Neuralstem." You further testified that you "honestly don't know where they (Neuralstem) obtain their tissue." Since you are involved in transplantation research, do you know which DHHS regulations Title 45 Part 46 Regulations for an IRB were complied with? If so please provide these IRB approvals for the Panel.

Answer: The current work of the Sanford Stem Cell Clinical Center, which I direct collaborates with Neuralstem using an established cell line named NSI-566 and does not include procurement of new tissue. My understanding is that use of these cells is exempt from DHHS regulation Title 45 Part 46. DHHS regulations under Title 45 Part 46 apply only to federally funded human research. Nonetheless, our work with human subjects suffering from spinal injury is subject to federal regulatory oversight under Title 21 Part 50 and was approved by an IRB having satisfied all elements outlined in Title 21 Part 50. Moreover, this research is closely monitored by an IRB as required by Title 21 Part 50 . I have been advised that the IRB documents may be UCSD property and so should be requested from UCSD.

Do you currently obtain fetal tissue from sources other than Neuralstem? Have you obtained fetal tissue from other entities in the past?

Answer: My lab obtains fetal astrocytes from Lonza and immortalized fetal astrocytes from ABM. We also use the established cell line HEK 293, I have not personally obtained fetal tissue from other entities in the past.

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1 See https://www.washingtonpost.com/national/health-science/evidence-of-zikas-risk-to-pregnant-womencontinues-to-grow/2016/03/05/6c8e6152-e2aa-11e5-8c00-8aa03741dced_story.html.

The Honorable Joseph R. Pitts

Mr. Goldstein, you testified that the "form that says therapies for diseases such as Alzheimer's disease and all the rest have already been found, I agree, that is an inappropriate statement and it should not have been made on that form. I don't know who wrote it. That would not have made it past my IRB either." You were also asked where you get fetal tissue for your research. You responded that the fetal tissue neural cells come from Neuralstem and you don't know where they get the fetal remains from which to start the cell lines.

Following up on those statements:

1. How many research projects involving organs, tissue or cells from aborted babies have you participated in? Which of these were conducted in collaboration with Neuralstem?

Answer: My lab uses fetal astrocytes for our varied studies of Alzheimers Disease, therefore, approximately 6 projects use these cells. I also serve as director of a center (Sanford Stem Cell Clinical Center) that uses established fetal neural stem cell lines from a company called Neuralstem in clinical trials for spinal cord injury. I also chair an oversight committee that used fetal tissue in the past to develop a therapy for multiple sclerosis. Finally, I chair an executive committee of a multi-investigator project aimed at developing kidneys from stem cells. Several of the projects in this kidney collaboration use fetal material including the kidney organoid study that you cited during the hearing.

2. Did you obtain IRB approval for all of the projects involving human fetal tissue, organs, cells or cell lines that you have conducted or in which you have participated? What standards did your IRB set for ethically obtaining human fetal tissue in each?

Answer: We obtain IRB approvals as required in full accordance with federal regulations. My understanding of the rules is that if the research with established cell lines from fetal material does not involve interaction with living or identifiable human subjects (that is, laboratory work), that research is exempt from IRB approval. All of our work involving human tissue donors or patients participating in clinical trials is compliant with regulations under Title 45 Part 46 and is reviewed and approved by an appropriately convened IRB.

3. Please indicate the source of the fetal organs or cells for each project, the informed consent forms used in each (any patient-identifying information should be redacted), and the amount paid for each.

Answer: In our Alzheimer Disease studies, established fetal astrocyte lines and immortalized fetal astrocyte lines are grown and expanded substantially by a company, Lonza, using fetal brain as a starting material; these are effectively an established cell line and therefore exempt from DHHS or FDA regulations for protection of human subjects. Lonza normal human astrocytes are listed for $695 for one million cells. ABM immortalized astrocytes are listed for $1350 for one million cells.

My understanding is that informed consent documents are the property of the companies who grow the cells and are generally maintained by these companies to document the ethical procurement of the research material. For our work in 2012 published in Nature,

we obtained data from a colleague who had previously studied gene expression patterns in fetal brain. Since I was not the attending physician (I am not a physician), I do not have the relevant documents and have been advised that such documents may be UCSD property and so should be requested from UCSD.

In the collaborative spinal cord injury trials, fetal neural stem cells are obtained without cost in this collaborative trial. In the projects in which I participate in an oversight role, I do not have direct access to materials or documents.

4. With regard to your collaboration with Neuralstem or any similar intermediary, please obtain the information requested in question 3 from Neuralstem (or any other intermediary).

Answer: I do not have access to any of these documents. They belong to Neuralstem, to Lonza, and to ABM.

5. You indicated a form presented at the hearing that overstated cures would not make it past your IRB. That same statement appears on forms that Planned Parenthood uses (according to this). You co-authored this study regarding Alzheimer's. In the study, published in 2012, you thank "Planned Parenthood of the Pacific Southwest for fetal brain specimens." Redacting any patient identifying information, please provide a copy of the informed consent forms used for each specimen (fetal organ, tissue, cells or cell lines).

Answer: For our work in 2012 published in Nature, we obtained data from a colleague, another PI, who had previously studied gene expression patterns in fetal brain. Since I was not the attending physician (I am not a physician), and did not interact with the donors of material, I do not have the relevant documents and have been advised that such documents are UCSD property and so should be requested from UCSD. I would also like to point out that sharing of personal information (such as a signature or name on a research informed consent document) with individuals who are not otherwise authorized to access these documents (such as the FDA or the IRB), violates the confidentiality research participants expect (and is often made explicit in the consent form). For this reason, I would normally not be permitted to have access to consent forms signed by individuals from collaborating institutions or individuals (whether they are private companies or universities).

The Honorable Janice D. Schakowsky

During the March 2, 2016 Select Investigative Panel hearing, questions were raised concerning the significance of recent fetal tissue donation to advancing our understanding of human development, disease, and illness and to conducting research on potentially lifesaving treatments and cures.

As a distinguished practicing scientist for 40 years, you have a wealth of experience working with a range of cells and tissue as part of your efforts to understand and treat Alzheimer's disease, spinal cord injury, ALS (sometimes called Lou Gehrig's disease), and kidney disease. You are also likely familiar with the work of other researchers who use fetal tissue to understand and seek treatment for a range of other illnesses or diseases.

1. While some of your research may use established cell lines, is there still a need for ongoing fetal tissue donation? (If your answer is yes, please provide some representative examples that illustrate the ongoing need for fetal tissue donation.)

Answer: yes. There still is a need for new fetal tissue for studies of kidney development and development of other organs. While part of the work can be done with established cell lines, newer methods may generate better quality cell lines for therapeutic clinical trials. In addition, as investigators try to develop organs from stem cells in the lab, there will be continued need to compare the behavior of these organs to bona fide fetal tissue, especially for gene expression patterns in highly specialized cell types. Fetal tissue is the gold standard for these investigations and tissues otherwise destined for discard will be very valuable to the validity, quality, and reliability of studies trying to make organs in the lab from stem cells. It is likely that work that is developing organs from stem cells will continue to need comparative data from fetal and adult sources going forward. New ideas developed in the future about spinal cord injury treatment and other treatments, e.g., recent work about neonatal and fetal eye development, will likely continue to need new fetal tissue for comparative purposes or as actual sources.

I also want to point out that there are a number of clinical trials that are using cells derived from fetal tissue to treat patients for a variety of indications. For example:

- human fetal liver transplantation for the treatment of liver cirrhosis (Clinicaltrials.gov #NCT01013194)
- human fetal neuron transplantation for the treatment of huntington's disease (Clinicaltrials.gov #NCT00190450)
- human fetal neural progenitors for the treatment of parkinson's disease (Clinicaltrials.gov #NCT01860794)

Obviously, there are many physicians and scientists whose expert opinion is that treatment with fetal cells offers therapeutic options in certain contexts that do not exist otherwise.

2. Can anyone predict the types of cells or systems that will be necessary for answering particular research questions or developing new treatments or cures going forward?

Answer: Nobody can reliably predict the future. That is why we must as scientists pursue multiple paths in parallel to fight disease. Adult stem cells, fetal tissues, and embryonic stem cells all are potent weapons in the fight against disease and play a role

in investigations that should be pursued in parallel to find useful therapies as rapidly as possible for people suffering from disease. Some stem cells and tissues will be better for research and therapy than others. It is not one size fits all; we need a variety of cell types to fight disease just as we need more than one antibiotic to fight different types of infections. In addition, time matters to people suffering from fatal or disabling disease. Thus, we must proceed as rapidly, ethically, and efficiently as possible to help these people in need of medical and scientific help.