I am pleased and honoured to receive the Novartis award today. This award, as you know, recognizes the work I have done in developing the fluorescence-activated cell sorter (FACS) and monoclonal antibodies as complementary tools for immunology. However, as you must also know, it really recognizes both my work and the work of my collaborators, without whom I could not have succeeded in this effort.

There are many collaborators I could name. However, first and foremost among these is my wife, Lee Herzenberg, who joined my laboratory in a junior position when I first came to Stanford and now jointly runs the laboratory with me. You heard from Hugh McDevitt a moment ago that Lee played an important part in his work. Let me assure you, she has played an even more important role in my work and in my life. We have been married fifty-one years and have worked together almost all of that time. Perhaps this prize should also be given to her for enduring all these years or, more seriously, for all of the key contributions she has made to our work. In any event, we shall share equally in the benefits of the prize, particularly the support that it will give us for our joint laboratory.

I thank the jury for deciding to award this prize to me, and I thank Novartis for their decision to award these tri-yearly prizes to outstanding immunologists. While scientists don’t focus on winning prizes, it is certainly gratifying to receive one. I also commend the jury for making a perspicacious decision in recognizing that the FACS belongs neither to the clinical nor the basic science immunology community, but to the community as a whole. This decision, embodied in the choice of three, rather than the usual two, prize recipients, recognizes the broad uses to which biomedical science puts this instrument and its growing importance in translational projects that put work begun in the laboratory into the clinics.

In reviewing the work for which this prize is awarded, I think it appropriate to start by giving the young people here a sense of what life and work in immunology was like before the FACS (Fig. 1). The functional distinctions between T- and B-cell populations were just beginning to be known. However, they couldn’t readily be separated. They could be stained with fluorochrome conjugated conventional antisera and visualized by fluorescence microscopy; they could be obtained in various proportions depending on the organ source; and they could be enriched by gradient separation or similar nonspecific physical methods. However, evaluating the frequency of T and B cells in a given cell suspension required long hours of tedious counting with a fluorescence microscope, using methods introduced by Al Coons (Hugh McDevitt’s mentor at Harvard).

Al Coons, Max Cooper, Martin Raff and several other immunologists were masters of this technology. My eyes, unfortunately, weren’t that good. Nor...
were those of many other people. Therefore, not everyone agreed as to how many of what kinds of cells were obtained under various conditions. All in all, it became clear to me that we needed a more objective and reliable technology to empower the use of fluorescent antibody methods to count and sort cells in a way that would allow us to fully characterize the immune system. So, in the late 1960s, I set about trying to find a way to develop what ultimately became the fluorescence-activated cell sorter, or the FACS, as it is commonly known.

We were working in a world that was in turmoil. Remember, this was the time of the Vietnam War. There was a lot of resistance to the war at Stanford, where my laboratory was (and still is). I am proud to say that Lee and I took part in the protests (Fig. 2). We also brought the peace effort into our laboratory, much to the benefit of the FACS development effort. In particular, we established a working relationship with other scientists aimed at helping local engineers redirect their careers away from war-related work and into more humanly productive pursuits.

This political effort gave me the opportunity to recruit and work with some really talented engineers. It was definitely mutually beneficial. The engineers were very happy to be able to work on something that could be of use in the biomedical arena, and, as you can imagine, I was really happy to have the attention of seasoned engineers who would not normally have been so receptive to such a drastic mid-career switch.

Russ Hulett was one of the earliest engineers to join us; Bill Bonner came in slightly later. We began with a flow system that had been developed by Mack Fulwyler and Marvin Van Dilla at Los Alamos National Laboratory to sort particles according to size. We added fluorescence detection to the system. The first paper leading to FACS appeared in *Science* in 1969 (Fig. 3) and showed simply that we could sort viable fluorescent-labelled cells that were still functional after sorting. At this point, all we had was a very primitive machine. It really wasn’t the FACS, but it was a clear step in the right direction.

**The first FACS**

The FACS came along sometime later, when we added lasers to improve the illumination of the cells and thereby increase the fluorescence signals that were generated. We were fortunate in having laser experts at Stanford who worked with us on this part of the project.

Dick Sweet, who was working in a company called Varian, also joined us at this time. Dick had invented the ink-jet printer. He was pleased to find that Fulwyler and Van Dilla had incorporated his ink-jet droplet steering into their flow system, and we set to work improving it and much else in our early FACS (Fig. 4). Sweet stayed with us for many years, and is still doing FACS development work, now at BD Biosciences. People working with the new BD FACS Aria™ and BD FACSDiva™ encounter some of Dick’s recent work when they set up for sorting. The set-up procedure involves looking for the *Sweet spot*, which might appear to be just a
clever name but is actually named for Dick (or so I am told).

The first FACS was built in our lab by these and other engineers who were all dedicated to building an instrument that would really work for biological and medical studies. They were housed very close to our laboratory and knew all of the biologists in our research group. I spent a part of every day with the engineers, telling them what the instrument had to do if we were going to get any use out of it. Sometimes they thought I was hard to please, but they understood that I understood what we needed. If they told me this couldn’t be done, I came back with “Well, then there’s no point in building the instrument.” Ultimately, as the current FACS attests, these really resourceful engineers found ways to give me, and therefore the biologists, what was needed to do the kinds of cell analysis and sorting studies that we wanted to do.

By 1976, the FACS was a functional instrument. Our breadboard machine had been replaced with a commercial version built by Becton-Dickinson with our cooperation and already in operation in several laboratories throughout the world. *Scientific American* recognized the importance of this new technology by inviting us to submit an article, which Lee, Dick Sweet and I co-authored.

The diagram of the working components of the FACS instrument (Fig. 5), included in this article in the early 70s, still visualizes the essence of the technology. Anybody who has been working with a current FACS can recognize the components and how they operate. Basically, the FACS is still very much the same; it hasn’t changed in 30 years.

Some of the new instruments, such as the BD Biosciences Aria and other sorters, focus the lasers and detectors on the cells in a flow cell rather than interrogating the cells in the stream in air. And, of course, the newer instruments have more lasers and detectors, so that we can analyze and sort based on multiple measurements per cell. But still, when one comes down to it, our original concept of the fluorescence-activated cell sorter still drives today’s flow cytometry instruments.

Similarly, today’s FACS instruments, even the most modern, still sort cells by putting a tiny elec-
trical charge on a droplet carrying a desired cell and then passing it through an electric field (Fig. 6). Charged droplets move left or right, and fall into collection tubes down below. Uncharged droplets, which are empty or contain unwanted material, fall straight down into the waste collection vessel.

The droplets in the three sequential frames in this picture (from the *Scientific American* article) actually depict thousands of superimposed droplets, “frozen” by strobe illumination. This illustration is a composite of images in the *Scientific American* article. It is one of my original slides. I don’t have pictures of my children from back then, but I do have pictures of my droplets.

**Early FACS immunology studies**

As the FACS developed, it became progressively more capable of doing immunology studies. By current standards, these studies were fairly simple-minded, since we lacked the monoclonal reagents and the multicolour FACS capabilities that are the hallmark of modern immunology studies. Nevertheless, these studies provided key information that laid the groundwork for current understanding and practice, both at the basic science and clinical immunology levels (Box 1).

**Enter monoclonal antibodies**

About the time that the *Scientific American* article was published, I took a sabbatical and went to Cesar Milstein’s laboratory in Cambridge. Cesar’s paper describing the generation of antibody-producing hybridomas had already appeared in *Nature*. (This was in 1975; I arrived at his laboratory in the fall of 1976.) In my laboratory, Barbara Osborne and her husband Dick Goldsby were making T-cell hybridomas. So he and I were thinking along similar lines.

I didn’t go to Milstein’s laboratory intending to work on hybridomas. My plan, formulated before the hybridoma paper appeared, was to learn molecular biology and DNA sequencing, which was just emerging as a key methodology at the Cambridge lab. Nevertheless, with the hybridoma technology available in Milstein’s laboratory, it was natural to put part of my time into exploring its potential for alleviating the key problem facing FACS users at the

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**Fig. 5:** Diagram of the working components of the FACS instrument, circa 1976. Modern instruments work in much the same way.

**Fig. 6:** A FACS instrument passes charged and uncharged droplets through an electrical field to sort the droplets, including the cells within them, into collection tubes below.
time. In essence, FACS worked well, but FACS reagents were a disaster. They were made by absorbing antiserum to (hopefully) remove unwanted antibody contaminants, then purifying the antibodies, and fluorochrome-coupling them or detecting them with fluorochrome-coupled anti-Ig, prepared the same way. Everybody’s reagents were different, even in the same laboratory. Consensus was almost impossible to achieve.

What was needed, which monoclonal antibodies to cell-surface determinants could provide, was a single, disseminatable, readily purifiable source of such antibodies so that everyone interested in a particular determinant could work with the same reagent. When I realized that the hybridoma technology that Cesar had developed could be applied for this purpose, I began working in this direction, using a FACS machine at Oxford, since there weren’t any available in Cambridge.

Milstein didn’t think much of this FACS work, mainly because he didn’t think the FACS was very important. After all, it really was just a nascent technology at the time. (Later, it became quite important in Milstein’s laboratory.) But my student Vernon Oi joined me in Cambridge, where he and I were able to learn how to make hybridomas. Thus, we were able to bring the technology back to Stanford, where we immediately set about making monoclonal antibodies to a variety of mouse cell-surface antigens of interest to immunology (Fig. 7).

These hybridomas, which we freely disseminated, were the first to demonstrate what has now become a rule: that monoclonal antibodies and the FACS are complementary tools for immunology and for many other fields.

### Lymphocyte subsets: homologous human and mouse cell-surface antigens

Sometime after we and others had begun to make headway in obtaining monoclonal antibodies that identified previously known functional lymphocyte subsets in the mouse, Rob Evans, then at Sloan-Kettering Institute in New York, called and said that he had a series of monoclonal antibodies to human lymphocyte surface antigens and asked if we could help identify which of the corresponding mouse subset antigens these antibodies defined.

Jeff Ledbetter and others in our laboratory took on this task, whose outcome was by no means certain,
since there was no guarantee that surface molecules in mouse and man were homologous. However, to our surprise, FACS analyses rapidly identified candidate antigens (Fig. 8), whose homologies were then confirmed by gel analyses. This work, which laid the groundwork for identifying and studying human CD4 and CD8 T-cell subsets, provided yet another example of how monoclonal antibodies and the FACS work as complementary tools in immunology studies.

**Expanding FACS capabilities**

*Single cell deposition.* Although the FACS circa 1980 was a commercial instrument that was rapidly being acquired by many laboratories, we recognized that the potential of this technology had yet to be fully tapped. In particular, the scientists and engineers in our FACS development group recognized that some of the problems we were encountering in the laboratory could be solved by developing new FACS capabilities. The introduction of single cell sorting, which allowed us to clone hybridoma cells, is a good example. This new FACS method removed the uncertainties inherent in limiting dilution cloning (the method used at the time), where one could not be sure that a putative clone was generated from a single cell unless cells were plated such

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**Fig. 9:** FACS set-up for single-cell sorting (e.g., for cloning). Shown are a laser intersecting stream, deflection plates and a 96-well tray for collecting single droplets.
that a high proportion of the wells were empty.

David Parks, who has led the FACS development group for the past 25 years, did this work with Richard Stovel, who was one of the engineers we got from Lockheed thirty years ago. Importantly, it was because David is also a biologist and did “wet lab” studies that he was able to recognize the problem with limiting dilution cloning and develop the much more efficient single-cell deposition technology that is now a standard FACS tool (Fig. 9).

More fluorescence colours

The original FACS had one fluorescence colour, fluorescein, and we thought that was great. Soon, we were functioning with two colours (Fig. 10), which allowed us to clearly distinguish T and B cells. But there was more to be seen, and sorted. Next, David Parks brought up a two-laser machine, which opened the way to immunology studies as we know them today.

Randy Hardy and Kyoko Hayakawa did the first immunology studies with this dual-laser FACS. We consider it amusing that we decided to focus initially on characterizing splenic B cells, figuring that there were minimal subdivisions within this population. This work, we thought, would take a few months and give us some experience before we tackled T-cell subsets, which we knew were more complex. Little did we suspect that we would still be finding and characterizing B-cell subsets some twenty years later.

As soon as we conquered the basic technological problems in working with the nascent dual-laser FACS, we realized that the key limitation was the number of distinguishable fluorescent dyes that were available to be coupled to antibodies to detect surface markers. So, in addition to continuing to improve the FACS instrument itself, we started looking for new fluorochromes. Today, antibody–fluorochrome conjugates that can be combined to enable twelve-colour studies can be purchased. But there

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**Fig. 10**

**Dual immunofluorescence – new frontiers in cell analysis and sorting**

David R. Parks, Richard R. Hardy and Leonard A. Herzenberg

Investigations of the nature and functions of the immune system and its cell populations have been revolutionized by the technique of fluorescence-activated cell sorting (FACS) and flow cytometry. Over the last few years, however, it has become increasingly clear that making only a single immunofluorescence measurement on each cell is not adequate for many investigations of complex cell populations. This review deals with why adequate resolution of functional subsets and intracellular compartments require multicolor labeling.

Immunology Today

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**Fig. 11**: Seaweed, shown here under visible light (left) and ultraviolet light (right), produce phycobiliproteins.

**Fig. 12**: Jellyfish produce green fluorescent protein (GFP).
has been a long path to reaching this point.

Seaweed from the California coast (Fig. 11) provided the first breakthrough. It gave us phycobiliproteins like phycoerythrin and allophycocyanin. Randy Hardy did some of the first purifications and conjugations of these proteins.

Later, jellyfish (Fig. 12) gave us green fluorescent protein (GFP), which is key to many current studies. Scientists at Columbia University cloned the GFP gene and showed that it could be expressed by mammalian cells. Currently, GFP and its variants are used as transgenes that internally mark cells for FACS detection and as reporter genes that reveal promoter activity in vivo and in cell lines. By now, we (collectively) are up to seventeen usable colours. The laser illumination and the absorption and emission spectra for twelve dyes that are typically conjugated to proteins are shown in the diagram (Fig. 13). GFP is roughly equivalent to fluorescein and replaces it in staining combinations. Chemical dyes such as monochlorobimane, which we use to detect intracellular glutathione, replace other protein-conjugating dyes in the cocktails. The list keeps growing. As I indicated, seventeen independently distinguishable colours have now been fitted into a single cocktail.

Mario Roederer has made a major contribution to this continuous colour increment. Working in our laboratory, first as a postdoc and then as research associate, Mario spent nearly twelve years with us. He added many improvements to FACS technology and played a major role in the development of the FlowJo FACS data-analysis package now marketed by TreeStar, Inc. Somewhat tongue-in-cheek, he fashioned Roederer’s law, which states that the number of usable colours for FACS doubles every nine years, after Moore’s law (the number of transistors per integrated circuit double every two years) — and provided the data (Fig. 14) to back up his claim! Of course, Moore also said that the cost of the circuit goes down by two every two years. Would that the cost of fluorescent dyes do the same!

There has been some discussion in the community about how to refer to these multicolour analyses. We introduced the term multiparameter FACS to refer to the four-to-six–colour analyses done years ago.

![Hi-D FACS Diagram](image)

**Fig. 13:** The laser illumination and the absorption and emission spectra for 12 dyes that are commonly used in Hi-Dimensional FACS analysis.
However, as David Parks and Wayne Moore in our laboratory have taken pains to point out, parameter is incorrect if one considers how the term is used in other contexts. Therefore, we have recently been using the term Hi-Dimensional (or Hi-D) to refer to FACS analyses in which eight or more colours are used. (I use this term here.) Roederer has used the term polychromatic for this purpose. Perhaps simply multicolour, as Roederer uses in the slide he kindly loaned me, may suit best. In any event, the technology is here, and words of some sort are sure to follow.

Who needs so many colours?

Of course, there are many who question why anyone would need so many colours. People have often said to me that they “have enough trouble working with just two colours.” However, working with more colours enables resolution that is not possible with fewer colours. Even when the same set of stains is used, dividing them up into several staining cocktails with fewer reagents (colours) per cocktail loses key resolution: two 2-colour analyses, for example, can not resolve subsets that one 4-colour analysis can resolve, and so on. It is fine to measure calcium flux in combination with one other colour, perhaps to identify T cells in a stimulated PBMC sample. But just consider how much more information is available if one measures calcium flux in combination with reagents that detect naïve and memory T cells. That requires seven colours: indo-1 for calcium; CD3 for T cells; CD4 and CD8 for the major subsets; CD45 RA and CD62L to distinguish naïve and memory cells; and propidium iodide (PI) to gate out dead cells.

Measuring intracellular cytokine production, expression of activation antigens, receptor–ligand interactions, etc., all add colours, as do measures of intracellular kinase activity and production of intracellular proteins. Such studies may be done with intact cells or with cells that have been stained for surface markers and then fixed and permeabilized to enable antibodies and other reagents to reach internal markers (Box 2).

Studies in our laboratory and elsewhere have made great use of Hi-D FACS analysis and sorting to examine signal transduction and physiological markers such as calcium flux in lymphocyte subsets. However, we have also applied this methodology to ad-
vantage to examine the redox status of lymphocyte subsets, notably to measure glutathione levels in peripheral blood mononuclear samples of HIV-infected people, where we have shown that disease progression correlates with loss of CD4 T-cell glutathione (and others have shown that T-cell function improves when GSH is restored).

**Dead cells introduce important artefacts**

Hi-D FACS studies in which staining cocktails include eight or more separately distinguishable reagents include those examining signal transduction and those designed to characterize the physiologic status in subsets of T or B cells. In these assays, cells are stained with reagents that detect the surface markers that identify the subsets under study. In addition, they are either fixed and permeabilized to achieve access to internal targets, or are stained with chemical dyes that reveal particular aspects of cell physiology. It is essential to identify dead cells in both cases, since dead cells nonspecifically bind staining reagents. However, since fixation involves intentionally killing the cells during the staining procedure, different methods must be used to identify dead cells in each case.

Put simply, accurate analysis requires that dead cells be stained so that they can be identified and gated out of analyses. When intact cells are studied, it is sufficient to use PI staining just before FACS data collection. In assays that require fixation and permeabilization of cells to admit staining reagents, however, dead cells must be identified prior to fixation. Failure to identify these cells at this point will introduce false positives that can seriously compromise intracellular staining data. Under these conditions, ethidium monoazide (EMA) applied just prior to fixation is the method of choice.

To identify the dead cells, we basically substitute EMA for the usual PI dead-cell stain and stain with EMA before beginning the fixation and permeabilization procedure (Fig. 15). Like PI, EMA will intercalate into the DNA of any cell whose membrane is no longer intact. However, because intercalated EMA will covalently bind to DNA when exposed briefly to bright fluorescent light, it will permanently mark any dead cells that are present even after cells are fixed, permeabilized, stained for intracellular markers and washed several times.

I want to emphasize how important EMA is for intracellular cytokine measurements. Dead cells always stain as cytokine-positive. In our experience, measuring the frequency of cells producing cytokines like IL-4, which are not very abundant, can be seriously compromised by a failure to identify cells that were dead before the fixation and permeabiliza-

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**Box 2. Examples of Hi-D FACS studies to characterize the physiology and function of T- and B-cell subsets**

**Intracellular measurements in fixed and permeabilized cells**
- Ig isotype production
- Cytokine production
- Kinases and phosphatase protein levels
- Phosphoproteins (kinase/phosphatase activity)
- Caspases
- Redox enzymes (thioredoxin, thioredoxin reductase)

**Intracellular measurements in physiologically intact cells**
- Calcium flux
- Mitochondrial indices
- Redox status (glutathione levels)

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![EMA staining before fixation and permeabilization binds to dead cells and avoids false positives](image)

Fig. 15: Ethidium monoazide (EMA) staining.
tion step. Sometimes, the false positives due to the presence of dead cells can outnumber the true positives by twofold or more! EMA, which is available from Molecular Probes, provides a simple way of avoiding this problem.

The future of FACS

As all immunologists know, FACS is now big business. There are a bevy of instrument manufacturers that now offer some version of FACS, and many more purveyors of monoclonal antibodies that are (or can be) conjugated with fluorescent dyes to create what we have termed the complementary tools for FACS studies. We have made many key contributions to the development of this industry. Often, as I have indicated, we led the way to the inclusion of new capabilities in these instruments by initially developing and deploying these capabilities in our laboratory. Now, laboratories like that led by Mario Roederer have begun to provide similar leadership.

This is, perhaps, as it should be. As in the development of innovative medical therapies, academic laboratories provide fertile ground for the exploration of innovative aspects of instrumentation. Our experience indicates that demonstration that the innovations are useful leads (sometimes more slowly than we and other users wish) to dissemination by industry to the broader community. As a laboratory, we try to act as a gadfly, urging rapid commercial introduction of capabilities that we know will be of use to the biomedical community. In addition, we continue to work on the development of new capabilities.

So, we are still working on improving the FACS — as always, looking for ways to improve both the hardware and the FACS software (Box 3), which has become increasingly more important as flow cytometry technology has matured. Companies, especially BD Biosciences, have been (and are) quite helpful in this effort. TreeStar, Inc., which produces the FlowJo analysis package, has also been helpful, most recently by implementing the Logicle bi-exponential FACS data visualization method that Wayne Moore and David Parks have developed in our laboratory to eliminate data pile-up on contour-map axes. In addition, ScienceXperts, a nascent company, is about to release a software package that

Lee, Wayne Moore and others at Stanford have built to support the development of Hi-D FACS protocols. We have discussed much of this material in a 2002 article titled “The history and future of the fluorescence activated cell sorter and flow cytometry: A view from Stanford” that appeared in Clinical Chemistry [48 (10): 1819–1827].

Lee and I are pleased that our extended laboratory family also continues to work toward improving FACS capabilities (Box 4). As I have noted above, Mario Roederer and his group at the National Institutes of Health have also made significant contributions to this effort. Similarly, Gary Nolan, who did his PhD work in my laboratory, has recently been working with his group at Stanford to introduce innovative FACS technology, notably staining methodology and software to facilitate the detection of intracellular phosphoproteins. In addition, Diana Bianchi, who was a medical student in my laboratory, is working now at Tufts–New England Medical Center on the detection of rare cells in blood. She has shown that there is a connection between autoimmune disease and the persistence of fetal cells in the blood of women who have borne children. Collectively, the efforts by these young scientists, and by the industry that provides the instrumentation to

Box 3. Future improvements for FACS (some)

Hardware

- Increased number of fluorescence colours that can reliably be used, e.g., new types of illumination and detection to expand the usable spectrum
- Increased sensitivity and accuracy in data detection
- Improvements in drop charging and other sorting technologies to increase and stabilize high-speed sorting

Software

- Automated compensation and calibration tools
- Improved data display, e.g., Logicle axes
- Improved data analysis, e.g., reliable clustering
- Support for constructing protocols
- Reliable, long-term storage for FACS data and metadata (protocol and experiment descriptions)
Politics continues

We as scientists and physicians work very hard to make life better for people on this planet. Many years ago, one of the world’s great physician/scientists, Henry Kaplan, who helped me raise the funds to build the first FACS, did what was a very unusual thing for him. He stepped up to the microphone and spoke at an anti-war demonstration at Stanford and told the group assembled in the Medical School Courtyard that he had come to recognize that the war in Vietnam had to stop. It had, he said, already killed more people than he could ever hope to save in his whole career as a physician. “It has to end!” Coming from the man who had worked out the therapy for Hodgkin’s disease, this was indeed a strong statement.

Today, we are facing a new war in a new place, and the stakes are even greater. I spoke earlier of how Lee and I participated in the effort to end the Vietnam war and how we recruited engineers who did not want to do war-related work to help develop what we now know as the FACS. Jon Beckwith, a leading molecular biologist at Harvard, was also active in this anti-war movement. He recently wrote a book (Fig. 16) about his experiences protesting the Vietnam war and how he, like Lee and I, still continues to look for ways to improve the political landscape in which we are embedded.

I had heard of Jon Beckwith and his activities for many years, but I only met him recently. He sent me a copy of his book, which is entitled Making Genes, Making Waves: A Social Activist in Science. It is an exciting story that touched home for me in many places, one that is particularly relevant to my actions with respect to the Novartis prize. Jon received the Eli Lilly award in 1969 and decided to donate his prize money to a socially active organization. Reminded of this by his book, I was spurred to do something similar by giving a portion of the personal prize money that I receive today to truthout .org, a reader-supported Internet news site that provides one of the few places where one can get news and views that aren’t filtered by the corporate media (Fig. 17).

Box 4. Future applications for FACS (some)

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Fig. 16
Lee is on the board of *truthout*, and I strongly support its goals and activities. Interestingly, Steve Weissman and Marjorie Cohen, who were both leaders of the Stanford anti-war demonstrations in which Lee and I participated during the Vietnam era, have independently found their way to *truthout* as editors. So we have come full circle.

In any event, I am pleased to have the opportunity to share my personal prize money with the people at *truthout*, who are putting a great deal of time and effort into making today’s news freely available to me and to anyone else who has access to the Internet.

**A final note**

In the long and fruitful careers that Lee and I have had as scientists and professors, we have had the good fortune to have trained nearly one hundred younger colleagues who have now gone on to research careers in academia, medicine and industry. All of them deserve mention here, but merely presenting a list without discussion of their many contributions would serve little purpose. Therefore, I thank them all individually for the unique parts they have played in our lives. In addition, I thank them as a group for having helped to build the FACS and for doing the science that, in paper after paper, demonstrated the utility of the FACS and monoclonal antibodies and how these complementary tools could be applied to advance the understanding of the murine and human immune systems.

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