

**HIV and Cancer Virology
Faculty Meeting
June 4, 2002
Gaithersburg Hilton**

Meeting Summary

Dr. James Goedert, the Faculty Head, welcomed the members of the faculty and guests to the faculty's third meeting. He noted the meeting's full agenda, which is to begin with business items approved by faculty steering committee members during a teleconference last week. Scientific presentations will follow.

Business Meeting

Mentoring: The Faculty steering committee has recognized that new mentoring initiatives would be helpful. One idea that has been would involve non-supervisory mentoring of postdocs not only on their scientific project interests but also on their broader career goals. Although not expected, postdocs could agree to contact between their mentors and their primary supervisors, particularly as this might both advance their careers and foster productive collaborations among NCI's Labs and Branches. Steering committee members also recommended surveying the DECG and CCR postdoc community about level of interest in such a program. If a new mentoring program moved forward following this survey, someone in NCI should be tapped to handle the administrative burden of pairing postdocs with mentors.

Dr. Goedert characterized the concept of non supervisory mentoring as potentially useful for postdoc recruitment--making NCI a more attractive place to come—and as potentially useful for “creating synapses” across NCI. A faculty survey last September indicated that mentoring had been a positive experience for faculty who had participated in the past and was not seen as a “major burden.”

Dr. Goedert asked for comments about the action item of going forward with a survey of the postdoc community to ascertain need/interest. He confirmed that the survey would be conducted by e-mail and that it would be useful for PIs to assist by forwarding the instrument to their postdocs. Guest Dr. Jonathan Wiest of CCR noted that his office would be happy to help with the mentoring program as well as continue his current responsibility of helping to match make between the postdoc needs of CCR labs and available postdocs (see *Networking* below). Dr. Goedert noted that DECG has already established a mentoring program across the DECG branches that works well.

Comments included the need for the program not just to be problem-oriented, such as when a postdoc might be experiencing difficulties with a primary supervisor. Another mentoring experienced faculty member noted that participation has led him to beneficial “science learning” contacts with other PIs.

Networking for future and current fellows (personnel recruitment and job placement assistance): Dr. Goedert characterized this concept as development of a list of former postdocs or “alumni” within faculty labs that could serve as a way for faculty to recruit postdocs and for postdocs to look for potential employment. A suggestion from the steering committee was to use the list and attendant information, such as curriculum vitae, that is assembled by each Lab and Branch in preparation for its quadrennial site visit. Names on the list would include those who have been in training in the past four-year cycle. Additional information could be where they “ended up.” Dr. Goedert noted that DECG put together such a list a year and a half ago and that “it was a very big job.” Therefore, he suggested something “on a more micro scale” that would involve a small committee to assemble a format and gather information.

Discussion included making sure the list was available (probably through the faculty web site) to different institutions around the country, given that NCI does receive the occasional letter from universities looking for junior faculty. Also suggested was posting the letters from the institutions like a bulletin board for postdocs to check. Again, Dr. Jonathan Wiest noted that his office is currently disseminating openings to postdoc fellows and invited faculty members to send applications to him that they are not interested in. At present in CCR, he said, “we have over 100 openings at any time. And, conversely, we have many postdocs looking for jobs.” Dr. Wiest noted that while his charge is to CCR and his fellows list serve only covers CCR (not DECG), he is available to discuss topics involving fellows, including new ideas. His e-mail address is wiestj@lmail.nih.gov.

In ensuing discussion about recruitment, it was suggested that labs could consider joint advertisements in the usual publications, such as *Science*, to reduce costs and potentially “to bring in a larger pool of applicants,” including those who could be invited to give seminars. Dr. Wiest mentioned that faculties can and “should” sponsor applicant visits to give seminars, noting that his office has also given thought to poster competitions/sessions on campus, thus saving travel budgets. Facilitation factors include the report that “joint advertisements” are deemed acceptable by administrators and that Dr. Janelle Cortner’s office is developing a database to assist PIs in requesting any necessary funding supplements. It was noted in general that Dr. Janelle Cortner and a computer network expert might be taking over both funding mechanisms and e-mail communication systems for the faculties.

Dr. Wiest also described a “Stargazer” database that he will make accessible on request that provides information about postdocs looking for work, including degrees, fields of interest, cvs, country of origin, e-mail addresses, and so on. He noted he adds names from such sources as *Science* and *Nature*. He added that most “will require visas.”

Faculty Membership: The steering committee suggested that individuals in the extramural division of NCI be invited to be participants in the faculty. Dr. Goedert mentioned Ellen Feigal and Jim Pluda as examples. It was proposed that others in NIH with virological interests be invited to give scientific presentations only—at least while the faculty is still forming its approach. It was proposed that the same be true of

scientists outside of NIH. Faculty members were urged to invite interested parties to sign up through the faculty's web site.

Next Meeting: It was noted that the proposed next faculty meeting date of Oct. 7, 2002 may conflict with a planned conference on the human papillomavirus virus and therefore is subject to change. Faculty members were encouraged to propose new meeting dates to Dr. Goedert. The meeting place is tentatively set again as the Gaithersburg Hilton.

Obtaining Visas (discussion with Dr. Philip Chen and Mr. Brian Daley):

Noting that visa problems are a "major" concern that affects every branch of the NIH intramural program, Dr. Goedert introduced Dr. Chen of the NIH Office of International Research and Mr. Brian Daley from the International Services Branch (ISB) and said they had come to try to answer faculty members' questions about visa obstacles to bringing in foreign scientists, particularly given the advent of new levels of scrutiny and lag times following the terrorists acts against New York City and the Pentagon of Sept. 11.

Background. Dr. Chen began by noting that there "has been more rigor and delay imposed on the system." President Bush specifically asked the White House Office of Science and Technology Policy to coordinate the granting of visas to scientific visitors or the exchange of scientists for vocational training to prevent the entry of terrorists and "other undesirables." Around the world, the U.S. State Department's consular offices are now looking at such visa requests with more rigor and "can turn them down if it is felt that the person in question might not return home in two years or if the area of study is suspect, like biological warfare, lasers, or some other area on a list that consular officers now use." State Department officials can ask for advisory opinions from headquarters, and such requests also all go to the FBI, the Commerce Department, the Treasury Department, and other government agencies now involved in visa restrictions.

In addition, Dr. Chen noted implementation of the new web-based SEVIS tracking system, which quickly alerts officials if someone has applied to several schools to obtain visas. Theoretically, in the past, someone might obtain I-20 forms from 10 schools, and 10 separate individuals could then use those forms to obtain student visas. "That can't happen now," Dr. Chen said. In addition, SEVIS has supposedly eliminated the problem of tracking visa holders who leave one institution for another. Since the spy plane incident (earlier this spring between the U.S. and China), a number of Chinese can't get J1 visas, and there are a few instances of Europeans also being denied, Dr. Chen reported. Apparently, consular officers are indicating that another type of visa that is available only to employees, not visitors, is not considered to be the same kind of problem.

Questions and answers

Q: Is there any chance to change policy? We're losing fellows.

A: It's not possible without an FTE position.

Q: A lot of universities bring postdoc fellows in. While we can't. So we're locked out of pool of qualified applicants even though we pay approximately at the same level. This is a severe problem for us.

A: H1B visas are common, but I also know that FTE positions are limited. If you have a good candidate, you can try to get the FTE position needed to get them an H1B visa. Or you could come to Dr. Gottesman for assistance.

Q: The problem is the definition of what a job is. We're the only ones who bother with J1 visas. ABL doesn't. Why can't we? The problem is the FTE. Why not call it something else? I may have someone with an H1 visa already to go, but I also have to have an FTE. We need to be as competitive as the rest of the world, including private industry.

A: NIH has been locked in position with an FTE requirement that is unrealistic and unfair. It should and needs to be changed. We try everyday to find individuals to do highly skilled jobs, and we're locked out from them. Universities simply do H1 then they don't keep the individuals forever. So we need within NIH to change the iron clad FTE rule. It is not necessary and serves no purpose.

A: It's not an NIH regulation.

Q: Then why do other institutions interpret the law differently?

A: They interpret it in the same way; they just have more FTEs.

Comment: As branch chief I see the struggle, and it is a serious one. We're putting up all these buildings, but those resources won't be used as well as they could unless you have good postdocs. The Bush Administration is eager to have things done on contract. Perhaps we can do that. We need a creative solution.

Q: Is there a nationwide limit? (On H1 visas.)

A: Government agencies are exempt.

Q: Can the J1 be transferred into an H1?

A: Yes, if they can get a waiver, and it is approved, but that's seldom. Provided you have an FTE and a research fellow. Now you don't have to be tenure track. That's a change.

Comment: That needs to be communicated.

Q: Are virology labs getting more scrutiny?

A: Yes, because they're probably on the consular officers' list.

Q: What about 4th and 5th year fellows? Are we having high rate of success of extensions through the State Department?

A: We had expected the State Department to give us J1 for 5th years. The department anticipated opening it up not only for us but for universities as well. But all that has been put on hold.

Observation (from Ms. Deborah Fountain, NCI Personnel): We have liaisons who know about all these new policies. All the key contacts know about them. There are individuals within each lab and branch who are familiar with them, such as office managers or ARC managers. I can be found in the global e-mail, so if you need good, accurate information, you can also ask me.

Comment: Reiterating the problem, I was shocked when Dr. Wiest made a presentation that showed over two-thirds of the postdoc fellows in NIH are on J1 visas. Clearly, that's a major portion of our work force. We're put at a selective disadvantage because we can't offer the H1 visa on a regular basis. It is a problem that has to be solved. We're supposed to be a premiere research institution.

Comment: We need to have a way to retain these folks. Perhaps an outside contractor could do this.

Observation: We have the 5-year/8-year rule. We can offer them an H1 if we have an FTE. By show of hands a majority in this room has lost excellent postdocs to universities who offered them an H1. Again, we are at a severe competitive disadvantage. We lose our first choices not because of money but because of this regulation.

Q: What about the Frederick contracts? Are they as restricted?

A: There used to be a cap on the H1's, but I'm not sure whether we are exempted.

Comment: Let's explore the contract mechanism. The question is whether there's an appropriate contract agency to do that. If we are exempt from the cap, the contract agency might not be, however.

Q: New topic: Are Italians supposed to pay U.S. taxes?

A (Brian Daley): ISB has a tax contractor responsible for looking for ISB into tax treaties between other countries and the U.S. She needs to research the matter. From what I know of the Italian treaty it is one of the few that doesn't U.S. tax-exempt fellows but does exempt employees. In other words, salary is different from stipend.

Conclusion (Dr. Chen): We heard your sentiments. We will do a little sniffing around and keep in touch. If you have ideas, let me know, and we'll look into them.

AM Scientific Sessions

Scientific sessions chair Marjorie Robert-Guroff [correct?] noted that the steering committee invited the sessions in a way meant to present faculty members with a broad representation of faculty research interests. All presentations were given in PowerPoint format.

Dr. Alan Rein gave the first presentation—"In vitro assembly of retroviral particles."

Dr. Rein explained that the problem he usually works on is how a retrovirus particle is assembled. A basic fact is that expression of the retroviral Gag protein in a mammalian cell is sufficient for efficient assembly and release of virus-like particles. HIV-1 Gag protein can easily be expressed in and purified from bacteria. And recombinant HIV-1 Gag assembles efficiently into virus-like particles that are only ~ 30 nm in diameter, which is significantly smaller than authentic virus particles (~100-120 nm). about 30 nm. Today, Dr. Rein explained, he would discuss the assembly of particles in a defined system in vitro, "what we've learned, and what good it is."

Dr. Rein noted that Dr. Stephen Campbell, formerly of Volker Vogt's lab, was able to isolate avian retroviral Gag protein and look for conditions under which it can assemble. What he found was that the conditions are high concentration of the protein, a little nucleic acid, and a little salt. Dr. Rein said the resulting particles are "good facsimiles of immature retroviral particle."

Dr. Campbell set about to do the same thing in Dr. Rein's lab with HIV-1 Gag protein, which can easily be expressed in and purified from bacteria, overnight—"a great virtue." Dr. Rein noted that this Gag is not full-length Gag. Another difference is that this protein is missing fatty acid modification at the terminus, which is involved in mammal cell

attachment at the membrane (but, Dr. Rein added, “We’re working in a solution system”).

The particles that result from Dr. Campbell’s method are small (about 30 nm, not 100 nm), and that is a “little problem,” Dr. Rein said. He also said he was surprised by the requirement of nucleic acid for assembly. He said he always thought of Gag protein as interacting with other Gag protein, and that the nucleic acid was simply cargo in the normal particle, “but Dr. Campbell set up conditions that showed that nucleic acid is necessary for assembly of the Gag proteins.”

Dr. Delphine Muriaux looked further into this question. MLV particles lacking viral RNA contain other, cellular RNAs in its place. She also asked, is RNA a structural element in MLV particles? She harvested immature MLV particles from mammalian cell culture fluid, stripped off their membranes, and treated the particles with RNase and then tested for disruption of the particles by centrifugation. The result showed that immature retrovirus particles could be disrupted by RNase after the membrane is removed with detergent.

Dr. Rein concluded that this is “quite strong evidence that RNA is an essential part of the structure of a retrovirus particle, as originally suggested by Dr. Campbell’s experiments.” Further, he stated that he suspects the RNA is the scaffolding for the protein.

Returning to the problem of small particle size, Dr. Rein noted that when mammalian cell lysate was added to the assembly, the size corrected. Dr. Rein noted that the active factor in the lysate is a small metabolite, IP5 (Inositol Pentaphosphate).

Dr. Rein noted that IP5 is highly charged and speculated that perhaps in mammalian cells, “it is a fatty acid derivative of this molecule.” I.e., perhaps the cofactor in mammalian cells is a phosphatidyl inositol phosphate; these are present in cellular membranes, where assembly takes place *in vivo*. Dr. Rein said his lab is now looking for evidence of IP5 function *in vivo* as well.

On another topic, Dr. Rein said his lab has screened chemical libraries for inhibitors, and although he would not discuss this at length today. In addition, he is testing VLPs as possible vaccines. As he mentioned earlier he “can make a lot of this stuff. ” Briefly, Dr. Rein mentioned giving mice leukemia virus, boosted, then challenged with Friend MLV. This virus preparation contains spleen focus-forming virus, which causes splenic hyperplasia in susceptible mice; the rate of spleen growth is a function of the rate of virus replication in the mouse. He noted that the rate at which the mice then died was related to how fast the virus was replicating in the mice. As a control, the lab used virus-like particles with HIV Gag. Dr. Rein noted he was not an immunologist and that this experiment had been run only once. The results are as follows: vaccination with MLV VLPs delays Friend MLV-induced disease; that is, the mice did contract Friend disease, but there was a 10-day delay. This vaccine work was done in collaboration with Dr. Sandra Ruscetti.

Questions and answers

Q: In a normal cell with HIV, are other cellular RNAs involved? Does the virus discriminate?

A: The virus discriminates very dramatically. What is the nature of the advantage of the viral RNA that wins the competition? That's a fascinating question.

Q: IP5. What is its mechanism?

A: Clearly interacts with internal domain of Gag. We're studying it. No real insights yet.

Q: Input of protein seems highly concentrated.

A: Yes.

Q: Tried with any other retroviruses?

A: Good question. Assembly *in vitro* of murine leukemia virus, avian leukosis virus, and equine infectious anemia virus (like HIV, a member of the lentivirus group of retroviruses) does not need IP5 or any other cofactor.

Q: Where does IP 5 bind?

A: We don't yet know where IP 5 binds yet; we're working on that.

Dr. Rob Gorelick gave the second presentation--“The function of the retroviral NC protein in infection processes.”

Dr. Gorelick noted that he began this work in Dr. Rein's lab. The protein he will discuss is part of Gag—NC—which contains zinc fingers. It is one of the most highly conserved elements found in retroviruses, it consists of invariantly spaced Cys and His residues, and it occurs once or twice depending on the retrovirus.

NC is a nucleic acid chaperone: it assists in melting and annealing reactions to create the most stable complementary base-pair nucleic acid regions.

In vitro studies on NC, it was determined that in reverse transcription (RT) processes:

- It functions in placement of the tRNA primer at the primer binding site (PBS)
- It assists in strand transfer reactions during reverse transcription
- It assists RT through regions of high secondary structures, preventing self-priming reactions that can deter viral replication

Also in integration processes, NC assists the viral IN with concerted integration reactions, obtaining enhancement of two-ended (coupled) joining of vDNA into model target DNAs in the presence of NC. In addition, according to results from the Bushman lab, integrated products are identical to those found in the authentic integration step during viral infections.

Dr. Gorelick explained that his lab is performing, *in vivo*, site-directed mutagenesis on the gene coding for retroviral NC, reconstructing proviral plasmids and transfecting into tissue culture cells, and harvesting viruses and comparing them to wild type to see what NC is doing in the virus life cycle.

The data to date is as follows: In examining virus properties for protein and nucleic acid composition as well as infectivity, Dr. Gorelick and his colleagues found, when they changed the 26th amino acid to the Zn²⁺-zinc fingers of MuLV (Moloney murine leukemia virus), a reduced level of genomic RNA is packaged and virions are replication defective. Dr. Gorelick reported that NC also seems to be involved in early infection processes. The lab has gone on to produce new mutants, and although they have wild type RNA packaging levels, they are defective in replication.

Dr. Gorelick explained that an obvious thing to check is reverse transcription in NC mutants. When the lab examined vDNA from infected cells, the finding was that mutant viruses had what appeared to be severely affected the reverse transcription process.

Dr. Gorelick presented the following summaries:

Summary I--

- WT MuLV NC with a CCHC Zn²⁺-finger assists in reverse transcription efficiency
- WT MuLV NC appears to be required for protection of the vDNA ends from degradation.

In extending their studies to HIV-1 viruses, Dr. Gorelick's team has found that an additional (NC_{H23}) mutant virus is replication defective and that it packages WT levels of genomes.

Summary II—

- The levels of reverse transcripts are reduced in the HIV-1 NC mutant
- The greatest defect in the vDNAs appears to be degradation of the ends, similar to results observed with MuLV NC mutants
- There is also a defect in removal of the "CA" dinucleotides from the ends of the vDNA which is performed by the viral IN protein

Summary III—

- NC and the CCHC Zn²⁺-fingers are required for: efficient reverse transcription; protecting the ends from degradation once the vDNA is synthesized; and setting the stage for the IN protein.

Now Dr. Gorelick and his colleagues are moving on to NC as a target for antiviral applications. In that regard, he made the following observations:

- One can inactivate viruses by treating with thiol reactive compounds (they react with NC's Cys thiols, and Zn²⁺ is ejected. In addition, exposed sulfhydryls oxidize to form inter- and intra-molecular disulfide linkages)
- Reagents do not affect surface molecules (gp120su or gp41tm) since they already contain disulfide linkages
- Inactivated viruses are being employed in vaccine studies using the SIV/monkey model for AIDS; and in immunological assays as stimulators for cell proliferation based analyses

Questions and answers

In response to questions, Dr. Gorelick indicated that NC is involved in the assembly process and once assembly has been achieved, to prevent nuclease degradation. In response to a question about differences between the first and second zinc fingers, he replied that it depends on the virus. For infectivity, you need both fingers. Mutation of the second reduces infection greatly, but the first finger may be more critical. He has not tried to remove the second zinc finger, but viruses with the first zinc finger removed are dead.

Dr. Bob Biggar gave the third presentation--“Studies of HIV infection in twins born to infected African women.”

Dr. Biggar noted that he collaborated in the study with the University of Malawi, Johns Hopkins School of Public Health, and the University of Ottawa. Dr. Biggar began by reporting HIV infection in American children based on CDC data through 1994, with the bulk coming from mothers who were infected. However, as only 25% of infants' mothers pass on the HIV infection, one question has become, why some and not others.

As published in 1991 (Goedert et al., *Lancet*), one study of this question looked at 66 pairs (37 HIV positive children) and found a higher rate of HIV infection in both the first born (A) and second born (B) twins when birthed vaginally, as opposed to via Caesarean section, with the A twin at “more risk than the B twin.” Dr. Biggar made the observation that some mothers have a higher viral load. The interest then became whether something that happened in vaginal births caused the A twin to protect the B twin.

When a second, expanded study involving 115 pairs (45 HIV positive children) was conducted, “we got a similar result again,” Dr. Biggar reported. So the team went to Malawi to investigate whether a certain routine of washing the birth canal with chlorohexidine would change the picture. Every woman in the study at every prenatal exam and over the course of labor (essentially every 4 hours) received the wash. And “it made no difference at all in terms of HIV infection of the infants,” Dr. Biggar reported, although it did make a difference in sepsis and overall mortality rates in the infants.

In 1994, the team returned to Queen Elizabeth Central Hospital in Blantyre, Malawi, where, at the time, HIV prevalence was 30% in pregnant women and 1:40 in twin deliveries (about twice the rate in the U.S.). The objectives were 1) to examine the role of birth order in transmission risk; 2) to examine genetic factors in fraternal vs. identical twins.

The team evaluated 315 twin pairs for in utero infection risk; 159 for perinatal infection risk; and 86 for postnatal infection risk (through breast milk).

Findings were that 39 of the 315 twin pairs tested positive, but the A and B discordant risk was “about equal,” and the concordant risk was “beyond chance.” Of the 159 twin pairs, 47 tested positive, but the A and B discordant risk difference was NS, and the concordant risk beyond chance. Finally, of the 86 twin pairs, with 11 testing positive, the direction of difference was greater levels of infection in the second born twin, “and that was not what was expected.”

Caesarian risk greatly lowers perinatal infection risk, most Caesarian deliveries being prior to labor in term infants, Dr. Biggar said. Presumably the transmission is across the placenta and lack of labor results in a lower risk of microtransfusions.

When the team looked at genetics, whether identical or fraternal, “almost all of the twin pairs had the same profile in viral load and remained virtually the same over 3 months of tracking.” In short, “it doesn’t seem to make a difference if twin pairs are identical or fraternal,” Dr. Biggar stated. Patterns were seen as “intrinsic to the virus rather than the host.”

Questions and answers

Q: Between America, Europe, and Africa, were the mothers with HIV sicker in Africa?

A: Hard to tell. Malawi is one of the 10 most poverty stricken countries in the world, and there are many causes of being thin and weak. But it was not obviously related to HIV infection.

Dr. Lauren Wood gave the fourth and last presentation before lunch—

“Antiretroviral and immune-based therapies in pediatric HIV infection.”

Dr. Wood began by outlining the reasons to pursue immune-based therapies:

- HIV specific cellular and lymphoproliferative immune responses are critical to control of viral replication and a major determinant of clinical outcome;
- Control or elimination of latent reservoirs may be facilitated by enhancement or manipulation of the immune system;
- Immune-based therapies may enhance immune function despite persistent detectable viremia, particularly in those patients with immunologic and virologic divergence.

In a prospective pilot study begun in 1995 of patients with immune suppression at entry, patients were observed for 8 weeks on a stable antiretroviral drug regimen (initially restricted to AZT + ddI) prior to the addition of IL-2. Then rIL-2 was administered subcutaneously BID for 5 days every 8 weeks. Amendments made in the study design over time allowed pts with minimal immune suppression to enroll, in addition to any combination of licensed antiretroviral drugs for the antiretroviral treatment regimen. Patients were enrolled sequentially into low dose then high dose cohorts.

Dr. Wood noted that dose modifications were made due to toxicity at the higher dose level. Low dose IL-2 was extremely well tolerated. Adverse events were tracked during the first 6 months/3 cycles of IL-2. A high incidence of fever, injection site reactions, nasal congestion, chills and malaise, all well documented and expected side effects of IL-2, were seen at both dose levels. These side effects were even more prominent at the higher dose. Only 3 patients went off study due to toxicity following receipt of IL-2 and in 2 of 3 the toxicity was due to antiretroviral drugs, *not* IL-2. Although the median duration on study for the cohort was 72 weeks (9 cycles of IL-2), the major reasons for discontinuation of the drug were patient choice (N=11) followed by study termination (N=9).

Dr. Wood noted that the HIV-1 RNA levels in 33 patients completing at least one full cycle of IL-2 did not statistically significantly change. Chronic changes in HIV-1 RNA levels in 24 patients completing at least 6 cycles of IL-2 included in week 24 showed no significant increase in HIV-1 RNA levels and at week 48, a statistical trend toward increased HIV-1 RNA levels, although the increase was deemed not clinically significant ($\leq 0.3 \log_{10}$, within the range of HIV-1 RNA level biologic variability).

In addition, there were chronic changes in CD4 counts: at week 24, there was a statistically significant trend toward increased absolute CD4 counts and at week 48 a statistically significant increase in absolute CD4 counts (median gain of 108 CD4 cells) associated with IL-2 administration, in a dose-dependent manner (trend). Dr. Wood reported that rIL-2 administration was also associated with increases in qualitative measures of immune function (DTH) as well.

Dr. Wood also discussed the recently terminated HIV-1 Immunogen vaccine study. Vaccine administration was “not associated with up regulation of viral replication.” Dr. Wood noted the following in the preliminary analysis of immunologic responses:

- There was a strong correlation between development of positive LSI responses to Immunogen and positive LSI to p24 antigen
- HAART therapy was associated with shorter time to development of a positive LSI response to Immunogen or p24 and maximal LSI response to Immunogen and p24
- HIV-1 RNA levels at baseline inversely correlated with maximal LSI responses (although baseline PCR had no association with development of a positive LSI response to Immunogen)
- Positive LSI response to recall antigens is associated with development of positive Immunogen LSI response, especially tetanus.

Dr. Wood also mentioned the terminated hydroxyurea study involving 7, heavily treatment-experienced patients with high viral loads. She noted that these viral loads declined significantly and rapidly associated with the administration of hydroxyurea in combination with ddI, d4T and efavirenz, but that viral rebound occurred in all patients. She also noted a median change for the better in absolute CD4 that “hasn’t been seen in adults.” Dr. Wood also mentioned interesting results in HIV 1 RNA levels versus changes in CD 8+/CD38+ (a marker of activation): the decline in HIV-1 RNA was

associated with a parallel decline in CD8+CD38+ cells. However when viral rebound occurred, the activated CD+ /CD38+ subpopulation continued to decline.

The bottom line at present, Dr. Wood said, is that

- Immune-based therapies may result in qualitative (functional) or quantitative improvements in the immune system
- Patients may exhibit immune system improvements despite incomplete suppression of viral replication
- Baseline viral load, CD4 count, CDC class, and type of antiretroviral therapy may not be predictive of immune responses to antiretroviral or immune-based therapies
- Antiretroviral therapy may improve immune dysfunction despite virologic failure or rebound. Dr. Wood posed the question of whether “this is due to the re-emergence of viral quasi-species with reduced replicative fitness”

Questions and answers

Q: In the children administered IL-2, how long did the CD4 increase last?

A: For those 11 patients who had remained on study and had been receiving IL-2 for years, we held the IL-2 and observed them for one year. Four of 11 patients needed to resume IL-2 for low CD4 counts as per protocol. Ultimately, 3 of the 4 required a change in ART therapy, suggesting that their diminished CD4 counts were due to lack of effective antiretroviral therapy (all had been on their current regimen for a substantial period of time).

Q: You noted that on the Immunogen study, patients who were receiving *no* antiretroviral therapy developed the most impressive CTL activity in response to vaccination, is that correct?

A: Yes.

PM Scientific Sessions

Dr. Robert Yarchoan opened the afternoon scientific sessions by introducing Dr. Zhi-Ming (Thomas) Zheng, an investigator in his branch (HIV and AIDS Malignancy, CCR).

Dr. Zheng’s presentation was on “Enhancement by 5’ capping of HPV 16 E6/E7 RNA splicing and destinations of the spliced and unspliced products in cells.”

Dr. Zheng opened his presentation by noting that his report mostly focuses on the human papilloma virus, which is associated with many cancers. His particular focus in this report will be on RNA splicing of HPV 16 E6/E7.

Summary of his presentation:

Expression of HPV 16 E6/E7 genes from promoter p97 produces a pre-mRNA which often undergoes extensive RNA splicing since an intron containing one 5' splice site (ss) at nt 226 and two alternative 3' ss, respectively at nt 409 and nt 526, exists in the E6

coding region. Dr. Zheng's lab is interested in understanding the mechanisms that promote recognition of the E6 intron leading to production of E6*I (226/409) and E6**II (226/526) mRNAs or that promote escaping of the intron recognition, resulting in a full-length E6 mRNA responsible for production of E6 protein.

In vitro splicing assay of HPV 16 E6/E7 pre-mRNAs conducted by using HeLa nuclear extracts demonstrated a role of the 5' cap structure in the E6/E7 pre-mRNA splicing. The E6/E7 pre-mRNA was spliced efficiently only when capped, and splicing reactions could be inhibited by adding m⁷GpppG cap analogues to the extract, suggesting that RNA 5' capping machinery promotes recognition of a cap-proximal nt 226 5' ss in the E6/E7 pre-mRNAs. However, complete removal of the cap-proximal E6 intron requires another enhancer element in a cap-distal exon. Further experiments were performed in 293 cells in transient transfection assay. HPV 16 E6/E7 coding region was cloned either upstream or downstream of an EGFP expression vector. RT-PCR and RNase protection analysis of the total cellular RNA isolated from 293 cells transfected with those two E6/E7 expression vectors showed a dramatic difference of their RNA splicing patterns. HPV 16 E6/E7 coding region cloned upstream of EGFP but immediately downstream of a CMV promoter resembled to its native position in the virus genome with the cap-proximal exon of 180 nts and its pre-mRNAs spliced very efficiently. When cloned downstream of EGFP, the cap-proximal E6/E7 nt 226 5' ss had a distance of approximately 910 nts from the cap and spliced poorly. The results imply a distance effect on capping-dependent splicing of the cap-proximal intron. Insertion of a non-specific sequence with a size of EGFP coding region between the CMV promoter and the E6/E7 coding region reduced the splicing to a background. Data indicate that spacing between cap structure and cap-proximal 5' ss is a limiting factor for the capping-dependent E6/E7 RNA splicing.

Question and answers

Q: What is the significance of this to the biology of the virus for HPV 16? Are there upstream promoters that would give rise to pre-mRNAs with different length E6 exon 1? Have you looked at E6 splicing in HPV 31 where multiple promoters have been mapped upstream of E6?

A: No, actually we haven't. HPV 16 E6 may be different from 18 and 31 E6 in oncogenicity. HPV16 has high oncogenic potential and its E6 RNA has multiple 3' splicing sites. In HPV 18, there is only one 3' splice site. We have no problem with detecting the full-length E6 RNA from HPV 18.

Q: Why do you get the difference in cell organization between these proteins? Which ones are the original loci for nuclear organization?

A: It looks like the high-level oncogenics like HPV 16 E6 and HPV 18 E6 interact with p53 and mainly locate at nucleus. Non-oncogenic HPV E6 do not have such function and primarily distribute in cytoplasm. Thus, different HPV E6 might be probably evolved in some ways at different location to perform their functions differently.

Comment: I'd like to give a word of caution on localization: when you study this like this, you see the steady state of a protein. But be wary of seeing it as primarily cytoplasmic or nuclear. That only reflects the steady state.

A: Good comment. We'd like to do studies to track the dynamic, but because of manpower, we haven't set them up yet.

Dr. Carl Baker gave the next presentation--“Development of novel therapies for cervical cancer and HPV infection using spliceosome mediated RNA trans-splicing (SMaRT)TM.”

Dr. Baker began by explaining that using the SMaRT strategy, one can reprogram 3', 5', or internal exons, and he showed a slide that displays how this works. SMaRT has broad applications in biotechnology, Dr. Baker noted. For example, in gene therapy it can be used to address genetic diseases such as cystic fibrosis and factor VIII, as well as cancers, such as cervical. Other applications noted include gene reprogramming, exon swapping, gene knockdown, target validation, and agricultural biotechnology.

Potential advantages of SMaRT for gene reprogramming include

- Targeting/specificity—SMaRT should occur only in cells that express the target pre-mRNA
- Regulation—PTMs not expressed in absence of trans-splicing

Dr. Baker said his team's specific goal is to use SMaRT to create a suicide gene therapy for cervical cancer by targeting expressions of a therapeutic molecule (e.g., a toxin) to cells expressing human papilloma virus pre-mRNAs.

Dr. Baker then proceeded to show demonstration slides and data regarding *trans*-splicing in 293 cells and specifically into endogenous HPV pre-mRNA targets in SiHa and CaSki cells.

In conducting co transfection experiments with the 293 cells, the team found that HPV-PTMs 1 and 5 efficiently and specifically *trans*-splice into the target splice site. HPV-PTM 8 and 9 also efficiently *trans*-splice but to two viral 5' splice sites. The control was CFTR-PTM 27, for its levels of *trans*-splicing to the HPV target are very low. Dr. Baker concluded that this shows that this kind of activity is site and target specific.

In assessing PTM *cis*-splicing, Dr. Baker noted that elimination of PTM *cis*-splicing improves *trans*-splicing efficiency for an endogenous target. He also noted that unspliced HPV PTMs are predominately retained in the nucleus. For PTM 8, the percentage was 83%; for PTM 11, 81%.

Dr. Baker noted that he had also looked at expression levels of the PTM in *trans*-splicing and found that *trans*-splicing efficiency is enhanced by a high PTM: target ratio. In short, one has to have “very high levels of PTM expression.”

In summary, Dr. Baker stated that

- HPV 16 pre-mRNAs were reprogrammed through targeted *trans*-splicing
- The PTM binding domain determines the target and splice site specificity
- *Trans*-splicing to an exogenous HPV 16 pre mRNA was highly efficient
- A high PTM: target ratio was required to achieve efficient *trans*-splicing
- About 80-85% of unspliced PTM was retained in the nucleus
- PTM *cis*-splicing reduces nuclear unspliced PTM levels and *trans*-splicing efficiency
- Significant *trans*-splicing levels were obtained in stable cell lines

In addressing efficiency and specificity for HPV-PTM 11, Dr. Baker analyzed cytoplasmic RNA from a co-transfection with 50-fold excess of PTM over target RNA. Efficiency of *trans*-splicing was 77%. Specificity was assessed by 5' RACE which showed 53% specific *trans*-splicing and 47% non-specific *trans*-splicing (three cellular targets showed up multiple times). Now, Dr. Baker added, "We have to find why the three cellular targets showed up multiple times."

So now the question has become what strategies would increase *trans*-splicing specificity. And Dr. Baker noted two

- Decrease PTM expression levels
- And design safety PTMs

Dr. Baker outlined future plans as

- Designing PTMS with increased specificity
- Substituting a toxin (eg DT-A) as the therapeutic molecule
- Using recombinant viruses for efficient delivery
- And going into animal models to see if this works

Questions and answers

Q: I would have thought a stable situation would be better. That you would get higher efficiency—because your SMaRT would be at a higher ratio. What's your explanation?

A: We have guesses at this point. One is that when you co transfect two plasmids, they probably go to the same place in the nucleus, and they get co-transcribed in close proximity. And that's going to favor *trans*-splicing. With an endogenous gene, you've got the gene someplace in the nucleus—one place—and you're putting in an expression vector that may go to a totally different part of the nucleus and so that reduces your efficiency. We're thinking of trying to do novel things, like trying to tether the PTM onto the CTD (of pol II) to deliver it directly to the endogenous gene.

Q: If you add a splicing enhancer to the PTM, do you see increased efficiency?

A: It should increase *trans*-splicing efficiency, and you might do that if you wanted to use a weaker 3' splice site. But we're already at 80% on co-transfection. It might help with endogenous targets, depending on whether you localize the RNA into the right place in the nucleus; it might not. Splicing enhancers have been shown in systems to enhance *trans*-splicing. I'm thinking of Adrian Krainer's work (PNAS 96:10655-60, 1999).

Comment: For a toxin application, specificity is even more of an issue.

A: Yes, that's right, for repair, you just need to get some functional protein. One can creatively engineer PTMs so that the toxin can be expressed only if it *trans*-splices onto the right molecule. There are combinatorial approaches.

Q: The design you have now runs the danger of expressing the toxin molecule in the absence of specific trans-splicing.

A: If you put no ATG in the PTM exon, then trans-splicing has to be in frame into an ORF with an ATG upstream; each of these things decreases the expression of the PTM when you don't want it expressed. There are lots of things we can do in a combinatorial approach.

Q: How do you envision delivering to a patient? Local injection? Also, on specificity, could a strategy be to put in thiamine kynase as a prodrug activating enzyme?

A: We're still evaluating toxins and delivery mechanisms. As a gene therapy, it has the same issues as all gene therapies.

Q: How about systemic delivery? Or local?

A: A local would avoid potential toxicity effects elsewhere. You could paint the surface of the cervix, for example.

Q: What is the ratio of *trans*-spliced to endogenous?

A: Of the pre *trans*-spliced? We're in 100 fold for the CMV to 10 fold for the SV40 promoter. We're currently testing the SV40 constructs.

Q: Do you see any therapeutic effect in cervical cancer cells?

A: We have a lot of toxicity in the SiHa and CaSki cells, and we can't really tell whether it is due to the transfection agent or a direct consequence of expression of chimeric E6 proteins. There is some evidence that E6 can act to block the degradation of p53. By making the E6/lac Z fusion protein, you potentially build up a lot of protein product. That's one of the strategies we could use.

Dr. Mark Schiffman gave the next to last presentation of the afternoon—“Natural history of oncogenic HPV types: persistence equals neoplastic progression.”

Dr. Schiffman began by explaining that from the epidemiologist's perspective, HPV 16 is a powerful carcinogen, and that the lessons from his research into this matter that he will report on were drawn from three NCI cohorts: from the Portland, Oregon, Kaiser Permanente cohort (20,000 plus women receiving Pap smears followed for 10 years); from Guanacaste, Costa Rica (9,000 plus women in a high-risk general population followed for 7 years); and from the ASCUS-LSIL triage study (5,000 plus women with mildly abnormal Pap smears followed for 2 years).

Dr. Schiffman suggested that one must reconsider the conventional histopathologic concept of the CIN continuum. Beginning in the 1970s, it was thought that a diagnosis of CIN 1 or mild dysplasia, equivalent to lack of differentiation up to about a third of the epithelium, was a process at high risk of progression to CIN 2, then CIN 3, then invasion. Today, although this is still the current histological grading, “we now know that this isn't the underlying biology.” For in the 1980s, “we came to understand that low grade lesions like CIN 1 were really flat warts, caused by human papillomavirus (HPV), very common infections”. High grade lesions were much less common and were the true cancer precursors. Therefore, we now think of the three major steps in cervical

carcinogenesis as HPV infection, progression to precancer, and invasion. However, HPV infections typically clear rather than persist and progress.

Today, Dr. Schiffman continued, “we know that HPV 16 appears to be uniquely carcinogenic, and if it persists, places the patient at high risk of pre cancer and cancer. “

Nearly the entire spectrum of cervical neoplasia, from mildly abnormal Pap smears to precancer to cancer, is caused by HPV infection. In terms of cervical cancer deaths, HPV 16 alone kills 100,000 out of a total of 200,000 cervical cancer deaths per year. Together with HPV 18, 31, 45, and approximately 10 other less important oncogenic types, HPV 16 “causes virtually every case of cervical cancer worldwide.” Other HPV-related cancers include fractions of vulvar, vaginal, penile, anal, and oropharyngeal neoplasia.

Because HPV causes cancer at transformation zones between different types of tissues (such as exists between the vaginal squamous epithelium and the glandular epithelium of the endocervical canal), Dr. Schiffman and his colleagues are investigating why this is so, with a focus for this talk on their recent data on HPV persistence, clearance and progression to precancer.

First, Dr. Schiffman observed about clearance that almost all infections go away, even oncogenic infections. When there is persistence of oncogenic types , “it seems associated with precancer (high-grade CIN).” Dr. Schiffman noted that this was important for vaccine development, because a vaccine that prevents persistence and promotes clearance could work very well.

In studying persistence and progression in women in Guanacaste, Dr. Schiffman examined the origins of precancer in a population that had not had prior effective screening and treatment. At present, HPV testing of all specimens and histopathology reviews of possible incident high-grade endpoints are underway.

The study was very large: 9,175 women underwent gynecologic screening at baseline, with greater than 90% participation. PCR performed by Robert Burk’s laboratory at Albert Einstein permitted categorization of these women into sub cohorts infected with specific HPV types. For example, 205 subjects were infected with HPV 16, but with only low-grade or no cytological abnormalities (women with prevalent precancers and cancers were censored).

These women were followed by repeat examinations for new cervical abnormalities. For safety, any evidence of precancer (CIN2+) was treated and censored. For this analysis, a complete repeat viral testing of the latest available specimens from years 5-7 or time of censoring for CIN 2+ was possible.

By year 7, most women who had HPV 16 at enrollment no longer had HPV 16, but most with persistent HPV 16 had precancer or cancer. “If you have persistent HPV 16 for 5-7 years, it is really worrisome, “ Dr. Schiffman concluded. In all, Dr. Schiffman’s team looked at nearly 40 types of HPV known to infect the cervix. HPV 16 is the highest risk

and also the most common. Dr. Schiffman said HPV 16's persistence—higher than any other type examined in the study--might explain why it is more prevalent. Persistence is not always associated with precancer, Dr. Schiffman said. In short, "There are a lot of HPV types that just persist for a long time and nothing happens, but persistence of oncogenic types denotes high risk of progression to precancer."

In summary, Dr. Schiffman said:

Persistent oncogenic HPV infection is highly associated with progression to precancer.

HPV 16 persists especially well, which might be important to its oncogenicity.

Other oncogenic types also lead to high risk of progression if persistent, while low-risk types do not cause progression even if persistent.

For clinical purposes, we might define persistence as repeat detection at 1-2 years.

Co-factors include smoking and multiparity, but viral load is a complicated issue (highest viral loads do not increase risk of CIN 3 and cancer).

Addressing the question of viral latency, Dr. Schiffman noted in conclusion:

Natural history studies through 10 years of follow-up suggest that latency is not a big factor, but these studies are still too short.

Latency is a theoretical concern, but if cancers arise from reactivated virus, this will affect screening and vaccination plans.

Evidence to date suggests very lowest viral loads (our inadequate surrogate for latency) are not strong risk factors for progression within 10 years.

Questions and answers

Q: Does persistence relate to the ability of the virus to integrate

A: We don't know this from formal study.

Q: How does one distinguish persistence from repeat infections?

A: Ideally, by type variants. For example, HPV 16 has several major variants.

Q: How about detection of co-infection? How does that affect the analysis?

A: In our studies, approximately thirty percent of infections are multiple infections. We have a good system for detection. Some women have 12 types at one time. If you have HPV 16 and 18 together, or 16 alone, they act the same over time. In other words, types do not affect each other very much from what we can see in the data so far.

Q: If you have 16 and clear it, do you have immunity?

A: We think so, but finding the exact marker for it has been difficult. Prevalence tends to go down over age despite continued exposure.

Q: So you think integration is a late event?

A: Yes.

Q: When you showed the percentage of CIN 2+, by HPV types, given persistence, and HPV 59 was higher than 16, it made me wonder, is 59 worse?

A: Not at all. HPV 16 is definitely the worst. That finding regarding HPV 59 was based on only a handful of persistent infections. And, if HPV 59 persisted, the lesions observed

were entirely precancers. The cancers related to the major oncogenic types, particularly HPV 16.

Dr. Doug Lowy gave the last presentation of the day—“Development of virus like particle vaccines for the prevention of human papillomavirus infection.”

Dr. Lowy said his talk would focus on

- The causal link between HPV infection and cervical cancer
- Development and clinical trials of the first generation prophylactic HPV vaccines
- Second generation HPV vaccines to improve effectiveness and/or implementation

Dr. Lowy noted that

- More than 600,000 cases of cancer per year are attributable to HPV infection
- This represents one-third of all cancers attributable to infectious agents
- HPV infection is etiologically involved in several types of cancer

Dr. Lowy went on to make the following points:

- In the most common cancers in women, there are enormous differences between more and less developed countries that are attributable to screening programs in the more developed countries
- Vaccines would most likely have a greater impact, therefore, in less developed countries
- E6 and E7 cellular changes are associated with high-grade dysplasia and many years of infection

Addressing whether vaccines to help combat these challenges should be preventive or therapeutic, Dr. Lowy made the following points:

- Approved vaccines against other infectious diseases are preventive (based on neutralizing antibodies), not therapeutic (based on cell-mediated immune responses)
- A combined therapeutic and preventative vaccine would be even better than a purely preventative vaccine

In addition, the preferred attributes of an HPV vaccine to prevent cervical cancer are:

- It must be safe
- It should confer long-term protection
- It should be suitable for widespread use in developing countries

Dr. Lowy then went on to introduce the key structural proteins encoded by papillomaviruses:

- L1, the major, structural protein, and the most abundant. Each viral particle has 360 copies.
- L2, the minor structural protein. Each part has 12 copies.

Dr. Lowy explained that L1 can self assemble to form virus-like particles (VLPs). He noted

- That L1 in authentic viral particles contains immunodominant neutralization epitopes
- L1 in VLPs contain these epitopes.
- Co expression of L1 and L2 together forms L1 /L2 VLPs.

Dr. Lowy said his team has done a few studies in animals. These include of oral papillomas in cows (BPV-4) and of cutaneous papilloma in rabbits. Findings include prevention by systemic immunization with VLPs. Specifically, both L1 VLPs and L1/L2 VLPs are effective (intact, non-denatured VLPs are required); the effect is prophylactic, not therapeutic; the vaccine is efficient with or without adjuvant; there was passive transference with immuneIg G (neutralizing antibodies); and the vaccine was type specific, conferring no cross protection.

Human trials conducted with L1 VLPs have also been effective, with the potential for reduction in cervical cancer from the addition to human vaccines of multiple HPV types, Dr. Lowy said. Findings in human trials to date with HPV 16 L1 VLPs include excellent response without adjuvant and only minor side effects (two times greater than saline controls). In summary, Dr. Lowy said, “we have not seen anyone who failed to respond to the vaccine.” There are quantitative differences in response, he noted.

In terms of making a more universal vaccine, Dr. Lowy noted that, a highly effective vaccine with HPV 16 alone would confers protection against about 58% of cases. If HPV-18 is added to HPV-16, the protection percentage goes up to 71.7%, and it could be as high as 95.7% if HPV genotypes 45, 31, X, 33, 52, 58, 35, 59, and 56 are all added.

When Merck researchers and Dr. Lowy’s team looked into the potential for the HPV 16 vaccine to confer long-term protection, they found some evidence of this. Merck re-examined Phase II vaccinees who were HPV DNA negative at the time of enrollment for acquisition of cervical HPV 16 DNA and found 9 out of 129 controls had acquired, but none of the 66 vaccinees.

Other findings include that those who were immunized and on oral contraceptives seemed to have the similar levels of antibodies over time but not at ovulation, when their titers of antibodies became “very low.” At present, it is not clear whether women are more at risk at this particular part of their cycle.

Pathways for exploring increasing HPV vaccine effectiveness include

- Development of mucosal vaccines as non-infectious VLPs in a live viral or bacterial vector
- Looking into vaccines that would confer both prophylactic and therapeutic benefits, including those using pseudovirions, genetic immunity, and/or chimeric VLPs
- Development of vaccines that provide protection against more virus types, for example stimulating cross-neutralizing antibodies to L2

Dr. Lowy then proceeded to explore chimeric VLPs, specifically those composed of an L2-E7 fusion protein that co-assembles with L1. He noted that chimeric VLPs retain neutralization epitopes of L1 and also induce cell immunity to E7. In vaccination studies with mice, Dr. Lowy's team has found that L1/L2-E7 chimeric VLPs protect mice against the challenge of tumor cells expressing HPV 16 E7. The mice were immunized with VLPs without adjuvant two weeks before the challenge.

HPV 16 L1/L2-E7-E2 chimeric VLP for human vaccine trials, including the two nonstructural viral proteins (E7 and E2), "should increase the likelihood of generating protective cell-mediated immunity," Dr. Lowy said. Subjects for the trials could include women with high-grade cervical dysplasia, with the goal of finding out whether chimeric VLP vaccination could induce regression in established lesions.

Last, Dr. Lowy briefly explored how L2 induces cross-neutralizing antibodies. He noted that the immunogen: L1/L2 VLPs "had high levels of neutralization titer for HPV 6, 16, and 18 when the immunogen was L2 protein."

In summary, Dr. Lowy said that L2 contains epitopes that induce broadly cross-neutralizing antibodies, but these epitopes are not exposed on intact VLPs; L2 type specific and cross-neutralizing titers are much lower than L1 type specific neutralizing titers; and "the future challenge" will be to develop vaccines that induce high titers of cross-neutralizing antibodies, perhaps by displaying L2 epitopes on the VLP surface.

Questions and answers

Q: Why not immunize young men?

A: There's no reason. It's a question of how you can demonstrate that the vaccine is or is not going to be effective. By far more data are available on women. The better the vaccine is, the less necessary it is to immunize men. It's the kind of public health question that will be addressed once it is clear the vaccine is effective.

Q: Why are VLPs produced with bacteria?

A: They form aggregates in bacteria, which makes it easier to make large amounts. Bob Garcia at the University of Colorado makes capsid smears and does that from reconstituted bacterial production.

Q: Do the VLPs package DNA?

A: In L1 VLPs, the amount of DNA is associated with purified particles is vanishingly small.

Q: Have you looked at other response, such as cellular responses?

A: Alan Hildesheim has been looking at that to some degree, and there were lymphoproliferative responses.

Q: In regards to L2 immunogenicity: if L2 is completely buried in the VLP, you couldn't get neutralization. Maybe L1 is so dominant L2 would not be exposed.

A: That's an excellent point.

Q: It could make a difference in your design.

A: I agree.

Q: What was the rationale for including 6 and 11?

A: Marketing—women are just as interested in protecting against genital warts as against cervical cancer. Young women, anyway.

Q: Do women on oral contraceptives have a higher rate of HPV 16?

A: That's a difficult issue. It is in the realm of a lab finding that requires follow up. No data suggest that women on oral contraceptives are protected against getting HPV 16 infection. If anything the opposite seems to be true.

Q: How do the vaccines work in animal models?

A: In the same animal experiments, they induce neutralizing antibodies, and they have been shown to be protective. Primarily in the rabbit system, but perhaps in dog.

Conclusion of the Faculty

Dr. Goedert announced that he would be happy to entertain via e-mail new ideas for the faculty, including about practical matters. He also asked members to check their contact information for accuracy to ensure good intra faculty communication.

