RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

March 8–9, 2016

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

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[Note: The latest Human Gene Transfer Protocol List can be found on the Office of Biotechnology Activities website at http://osp.od.nih.gov/office-biotechnology-activities.]

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
Minutes of Meeting[1]

March 8–9, 2016

The Recombinant DNA Advisory Committee (RAC) convened for its 145th meeting at 9:00 a.m. on March 8, 2016, at the National Institutes of Health (NIH), Building 35, Conference Room 620/630, Bethesda, Maryland. Dr. Hans-Peter Kiem, RAC Chair, presided. In accordance with Public Law 92-463, the meeting was open to the public from 9:00 a.m. until 3:15 p.m. on March 8, 2016, and from 9:00 a.m. until 2:35 p.m. on March 9, 2016. The following individuals were present, either in person or by teleconference, for all or part of the March 2016 RAC meeting.

Committee Members

Michael Atkins, Georgetown University School of Medicine
Mildred Cho, Stanford University School of Medicine
William Curry, Harvard Medical School
David DiGiusto, Stanford University, Stanford Medicine (incoming)
Kevin Donahue, University of Massachusetts Medical School
Angelica Hardison, Augusta University *(via teleconference)*
Patrick Hearing, Stony Brook University School of Medicine
Hans-Peter Kiem (RAC Chair), University of Washington School of Medicine/Fred Hutchinson Cancer Research Center
Dean Lee, University of Texas MD Anderson Cancer Center (incoming)
Douglas McCarty, Ohio State University College of Medicine
Joseph Pilewski, University of Pittsburgh *(via teleconference)*
Lainie Ross, University of Chicago Medical Center
Richard Whitley, University of Alabama, Birmingham School of Medicine *(via teleconference)*
Dawn Wooley, Wright State University
Laurie Zoloth, Feinberg School of Medicine, Northwestern University

NIH Office of Biotechnology Activities (OBA)

Lyric Jorgenson, Office of the Director (OD), NIH

Nonvoting Agency Representatives

Kristina Borror, Office for Human Research Protections, U.S. Department of Health and Human Services
Denise Gavin, U.S. Food and Drug Administration (FDA)
Carrie Wolinetz, Office of Science Policy, NIH

NIH/OD/OBA Staff Members

Shayla Beckham
Linda Gargiulo
Morad Hassani
Robert Jambou
Chengyuan Li
Maureen Montgomery
Marina O'Reilly
Eugene Rosenthal

[1] The Recombinant DNA Advisory Committee is advisory to the National Institutes of Health, and its recommendations should not be considered final or accepted. The Office of Biotechnology Activities should be consulted for NIH policy on specific issues.

Aparna Singh
Jyoti Singh

Attendees

There were 41 attendees at this 1-day RAC meeting.

Attachments

Attachment I contains a list of RAC members, nonvoting agency and liaison representatives, and attendees present for the bioethics discussions. Attachment II contains a list of public attendees. Attachment III contains a list of abbreviations and acronyms used in this document.

I. Call to Order and Opening Remarks

Dr. Kiem, the RAC Chair, called the meeting to order at 9:00 a.m. on March 8, 2016. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)* was published in the *Federal Register* on February 23, 2016 (81 FR 8975). Issues addressed by the RAC at this meeting included a report from the Gene Transfer Safety Assessment Board (GTSAB, a subcommittee of the RAC), public review and discussion of four gene transfer protocols, public review and recommendations of biosafety issues involving a proposal to transfer chloramphenicol resistance to several *Rickettsia* species, and public review of an FDA pilot project to develop a clinical and chemical manufacturing and controls database to examine safety in trials that use chimeric antigen receptor (CAR) T cells.

RAC members introduced themselves by name, affiliation, and research interests.

Dr. Jorgenson reminded RAC members of the rules of conduct that apply to them as Special Government Employees, read into the record the conflict-of-interest statement, and suggested that related questions be addressed to the OBA Committee Management Officer.

II. Minutes of RAC Meeting, December 4, 2015

RAC Reviewers: Drs. Cannon and Zoloth

Dr. Cannon was not able to attend the current meeting but had the opportunity to review the minutes of the December 2015 RAC meeting. Dr. Kiem relayed Dr. Cannon's comments on the minutes, which Dr. Cannon found to be accurate. Dr. Zoloth agreed that the minutes accurately reflected the Committee's business. Both Dr. Cannon and Dr. Zoloth recommended approval of the minutes. No additional comments or changes to the minutes were suggested by other RAC members.

A. Committee Motion 1

Dr. Kiem asked the RAC to approve the minutes of the December 4, 2015, RAC meeting. The RAC voted unanimously by voice to do so.

III. Review and Discussion of Human Gene Transfer Protocol #1601-1495: A Phase I Dose Escalation Study of the Safety of AAV8-VRC07 (VRC-HIV AA V070-00-GT) Recombinant AAV Vector Expressing VRC07 HIV-1 Neutralizing Antibody in Antiretroviral-Treated, HIV-1–Infected Adults with Controlled Viremia

Presenters: Julie Ledgerwood, D.O., National Institute of Allergy and Infectious Diseases (NIAID), NIH

Jason Gall, Ph.D., NIAID, NIH
Barney Graham, M.D., Ph.D., NIAID, NIH

RAC Reviewers: Drs. Hearing, Kiem, and Ross

Dr. Whitley was recused from consideration of this protocol due to a conflict of interest.

A. Protocol Summary

The Vaccine Research Center (VRC), NIAID, NIH, plans to evaluate the potential clinical uses of the investigational AAV8-VRC07 gene transfer product that expresses an HIV-specific broadly neutralizing human monoclonal antibody (MAb), VRC07 (VRC-HIVAAV070-00-GT). The AAV8 vector has been extensively used in investigational gene transfer products for many years and has a well-established safety profile. VRC07 is a novel monoclonal antibody that has high neutralizing activity against HIV-1; it has never been used in humans before. VRC07 is a clonal relative of VRC01, a highly potent and broadly neutralizing HIV-1 human MAb targeted against the HIV-1 CD4 binding site that was discovered by VRC researchers. VRC01 was isolated from an individual who was infected with HIV-1 for more than 15 years and whose immune system controlled the virus without antiretroviral therapy (ART). The VRC01 MAb is currently in clinical trials for prevention and therapeutic indications.

The proposed study is a Phase I dose escalation trial of the safety and tolerability of AAV8-VR07 in HIV-1–infected adults. The study will examine the pharmacokinetics of VRC07 expression following intramuscular (IM) administration of AAV8-VRC07 in research participants on ARV. The hypotheses are that (1) AAV8-VRC07 will be safe for human administration and will not elicit hypersensitivity or anti-drug antibody to VRC07 and (2) IM delivery of AAV8-VRC07 will result in production of biologically active VRC07 antibody at a concentration in serum that is measurable and safe. The three planned doses are 5×10^{10} vector genomes (vg)/kg, 5×10^{11} vg/kg, and 2.5×10^{12} vg/kg. Up to 25 research participants will be enrolled in the protocol. Study participation will continue for a total of five years and will include a total of 34 clinic visits, including 26 clinic visits in the first year and 8 additional visits over the subsequent four years (one visit every six months). Cumulative safety data will be reviewed weekly by a Protocol Safety Review Team (PSRT) that includes an Independent Safety Monitor (ISM) while injections are being administered. The second subject in each dose group will be injected after the four weeks' safety assessment for the first subject. Decisions regarding dose escalation and subject enrollments will be based on safety data and on VRC07 concentration in blood achieved at four weeks after product administration.

B. Written Reviews by RAC Members

Ten RAC members voted for in-depth review and public discussion of this protocol. The trial was found to warrant public review because it involves first-in-human use of the AAV8-VRC07 product in HIV-infected subjects using a novel a gene transfer approach to generate a monoclonal antibody.

Three RAC members provided written reviews of this proposed Phase I trial. They found the protocol to be well written and thoughtful. The study team is highly experienced in this field.

Dr. Hearing had the following comments and questions:

- Appendix M refers to "UBC Enh" in the immunoglobulin G (IgG) expression promoter. This element was not in the AAV8-simVRC07 vector used in non-human primates (NHPs), and its function is not described.
- NHPs were infected IM with 1×10^{13} vg AAV8-simVRC07. How does this convert to viral genomes/kg body weight? At this dose without immunosuppression, the VRC07 target dose of 50 µg/ml was not achieved (VRC07 peaked between weeks 2 and 4 at 2.5–7.7 µg/ml). Is the highest dose proposed in the protocol higher than the highest dose in the NHP study?
- In the NHP study, VCR07 became undetectable by week nine. Was that outcome due to loss (clearance) of vector-infected cells?

- In the informed consent document (ICD), the terms “mucosal” and “neutralizing antibody” need to be clearly explained or replaced with simpler wording, since research participants may not know these terms. Similarly, research participants may be confused by the meaning of “integration” as a possible risk of the AAV8 vector. It might be preferable to use “insertion” instead or to define “integration” as “insertion of AAV8-VRC07 DNA into human DNA.”

Dr. Kiem had the following comments and questions regarding the protocol:

- The process for screening AAV8-seronegative patients needs to be delineated, and the percentage of patients in the target population who are AAV8 seropositive should be specified.
- While an advantage of IM delivery is potentially longer persistence of rAAV8 and VRC07, this can also be of potential concern. What is the plan to address any immune responses (at the tissue level) to AAV8 and any responses to the VRC07 antibody production? As described by the investigator in the protocol and consent document, these responses/reactions can be mild or serious allergic reactions. It is not clear, however, whether there any plans for immunosuppressive treatment in case of immune responses.
- What plans, if any, are in place to analyze tissues for adeno-associated virus (AAV) genome integration? If such analysis is anticipated, how will this be done?
- It would be helpful to have a schematic of the AAV8 vector in Appendix M.
- Discussion of recent reports on potential oncogenicity of AAV should be provided.

Dr. Ross’s comments focused on areas related to human subjects protections. The consent form clearly explains that this is a Phase I trial, that VRC07 has never been given to humans before, and that the study is not meant to be curative. Research participants are encouraged to stay on their ART during the study. The exclusion of minors is appropriate for the proposed research. The monitoring plan is satisfactory and includes the addition of an independent monitor if a research participant is an NIH employee.

Dr. Ross had the additional following comments and suggestions:

- Genetic tests results will not be in medical records except for human leukocyte antigen (HLA) testing. The reason why results of this testing will be put into the research participants’ medical charts is not clear, however, and needs to be provided and included in the consent form.
- Per the protocol, after two research participants are observed at one month post-dosing, the decision is made to continue to give the same dose to a total of five research participants or to increase the number of research participants at that dose based on pharmacokinetic (PK) data in mice and NHPs and stabilization of transgene concentration. Dr. Ross noted that the decision to modify the number of research participants based on whether there is any response is ethically appropriate. The protocol does not explicitly state, however, whether the response includes any serious adverse events (SAEs) that could be attributed to AAV8-VRC07.
- In some sections of Appendix M, the “response” to a specific question is cited as “Response: The list of studies and results is provided in Error! Reference source not found,” which needs to be corrected.

The ICD needs to explain that once delivered, the construct and the antibody production are likely to persist for a very long time, cannot be removed, and may not be reversible should there be side effects. As a result, subjects who “withdraw” from the study are only withdrawing from being followed. No additional doses of the vector product will be given, and so the experimental intervention is complete following the IM injection. The consent can then point out that the investigators will only know whether the product persists by collecting samples over time; research participants can be encouraged to continue with ongoing sampling, even if they refuse to participate in further research data collection.

C. RAC Discussion

During the meeting, the following additional questions, concerns, or issues were raised by RAC members:

- Dr. Kiem also asked about how the investigators plan to manage immune responses following delivery of the investigational product via IM injection, which is expected to provide for longer

persistence of the product than by other routes. In addition, he asked about the potential for local damage if is there is a stronger than anticipated immune response.

- Drs. Kiem and McCarty raised the issues of AAV8 genome integration and potential risk of cancer with exposure to AAV based on recent reports of wild-type AAV. Dr. McCarty pointed out that even if the vector is considered non-integrating, the possibility of oncogenic or proto-oncogene interactions cannot be ruled out. Random events could occur, especially with high numbers of modified cells. The product has two enhancers, including one very strong enhancer element (i.e., the chicken β-actin (CBA) promoter). Dr. McCarty also expressed concern about potential leaking of the agent out of the muscle during the injections and traveling to other tissues, in particular, the liver, which is a mitotically active site. Given these issues, the protocol should include long-term monitoring for potential neoplasms in the tissue being studied as well as off-target tissues where there is increased risk for integration.
- Dr. Lee inquired about the correlation between serum antibody concentration and viral control and the choice of using antibody levels in the rules for dose escalation. Since the goal is viral control and antibody levels are being used as a surrogate for predicting viral control, he asked how the investigators plan to proceed if viral control occurs at lower dose levels and whether dose escalation would continue in such a case.
- Drs. Zoloth and McCarty requested clarification regarding issues relating to pregnancy risks and subject follow-up. Risks to the developing fetus and nursing infant from possible exposure to AAV8-VRC07 are not known. Women who are pregnant or lactating cannot enroll in this protocol. Men and women of childbearing potential will be required to use an effective form of birth control for the first 52 weeks of the study. Subjects who become pregnant will continue to be followed through pregnancy outcome but will not have any additional samples collected for research purposes. Further clarification is needed regarding potential risks during pregnancy and lactation with respect to persistence of the product issue. Dr. McCarty noted that risks to research participants involve two products: the AAV delivery vector and the gene/gene product. In terms of pregnancy risk, the short-term persistence of the AAV gene delivery vector poses the greatest danger. Regarding persistence of the gene and gene product, there does not appear to be greater risk for a woman than a man in terms of germline transmission. Given these considerations, Dr. McCarty suggested separating out the different risks in the consent. Dr. Zoloth noted that congenital anomaly/birth defect is included in the definition of an SAE, as described in the protocol. Since this is a first-in-human study, subjects must have a clear understanding of the possible risks of participation. In addition, she suggested following participants who become pregnant for the full five years of the study, rather than only until pregnancy outcome.
- Drs. Ross and Zoloth questioned the rationale and plan to withdraw research participants who develop a serious illness that needs ongoing medical care (e.g., diabetes) instead of continuing to follow them.
- Drs. Ross and Zoloth noted the large number of visits (26) in the first year of this single-dose study, which will likely be an inconvenience and potentially a burden to research participants. The investigators need to make sure that research participants have a clear understanding of what the protocol entails and that these visits will require them to travel to NIH.
- Dr. Zoloth asked the investigators and the RAC to consider whether the maximum compensation for five years' participation in this trial, approximately $6,000 to $7,000, is an undue inducement or potentially coercive. There was some concern that research participants are being considered, albeit perhaps in a limited sense, as a sort of worker on this single-dose study in terms of the proposed compensation, which does not seem to be based on a clear, rational evaluation. If the rates are determined using a market-based analysis, it is not clear why the mid-level rate was chosen instead of the highest rate, for example. Dr. Zoloth suggested providing either no compensation for research participants' time and inconvenience or the equivalent of the current living wage ($15) as an alternative to the current rates. Dr. Ross noted that the research participants' time and inconvenience should be appreciated and acknowledged, especially given the requirements of the study, which is done in part with some level of payment. However, the inclusion of commuting time, which is not part of the protocol, as part of the compensation for study visits, is a newer concept.

- Dr. Lee commented that the RAC's discussion on compensation should focus on whether or not the planned payment is an undue incentive or coercive within the context of gene therapy and other similar clinical trials approved by the IRB; if the rates are comparable to those in other studies, which appears is the case, then local IRB rules should prevail.
- Dr. Zoloth requested further clarification as to how genetic information will be protected, since such information cannot be fully anonymized because it is tied to the individual and may impact not only the individual but family members as well. The consent should explain in simpler terms the purpose and limits of genetic testing and why results of this testing cannot be guaranteed to be kept entirely confidential.
- The investigators will give the subjects a quiz after going through the informed consent document to assess their understanding of the protocol and its risks. Additional information about this quiz and the related assessment process was requested.
- Dr. Zoloth expressed concern that although short-term medical care is provided, the protocol does not offer any long-term medical care, and research participants presumably will be required to cover costs for research-related injuries without receiving any compensation for such injuries. She objected to these provisions, which appear to be NIH policy, adding that it is the obligation of the clinicians and the institution to provide care to study participants.
- The investigators were asked to clarify which samples will be collected under this protocol, including any tissue samples.

D. Investigator Response

1. Written Responses to RAC Reviews

"UBC Enh" is the abbreviation for "human ubiquitin C gene enhancer," whose function is to increase expression of the gene at the transcription level. The monoclonal antibody expression cassettes are the same in the clinical AAV8-VRC07 vector and the AAV8-simVRC07 vector.

The dose of the vector product in the NHP study cited is approximately 2.5×10^{12} vg/kg. Animals infused with AAV8-simVRC07 generated strong anti-drug antibody responses. Those responses may have eliminated simVRC07 from plasma. It is also possible that anti-simVRC07 cytotoxic lymphocytes (CTLs) were elicited that would eliminate transduced cells, but the investigators were unable to detect them. In subsequent experiments using the same dose of AAV8-expressing natural NHP monoclonal antibodies against simian immunodeficiency virus (SIV) Env (ITS01 or ITS06.02), expression levels greater than 10 µg/mL were achieved in a majority of NHPs, and levels were maintained as long as six months, the maximum time studied. No anti-drug responses to the natural NHP antibodies were detected (*VRC unpublished data*).

Screening for pre-existing antibodies to AAV8 will be done by enzyme-linked immunosorbent assay (ELISA), as described in the protocol. The prevalence of pre-existing immune responses in the target population is unknown.

Allergic reactions (if they occur) will be treated by appropriate clinical methods. In cases of immune response development to AAV or VRC07, cells expressing these proteins will be eliminated by immune responses. There are no plans for immunosuppressive treatment for the study.

The vector that will be used in the proposed trial is considered non-integrating by the FDA; therefore, there are no plans for analysis of AAV genome integration. The schematic of the AAV8-VRC07 construct is shown in Appendix M (Figure 2 in response to question M-II-B-1a). Per the schematic, AAV8 inverted terminal repeat (ITR) packaging signals are included in the construct.

The investigators are not aware of any reports of carcinogenesis in human studies with AAV vectors. The AAV8 vector used in the team's studies, including the proposed trial, does not have any viral sequences aside from ITRs and therefore is designed to remain episomal. Furthermore, the FDA guidance "Gene Therapy Clinical Trials—Observing Subjects for Delayed Adverse Events" classifies recombinant AAV (rAAV) vectors as "non-integrating." The investigators summarized the following relevant reports in the

response to the reviewers regarding this issue. Copies of the FDA guidance and the papers cited were submitted with the response memo.

A recent study in a neonatal mouse model with intrahepatic delivery of AAV showed that 50 percent of AAV-treated mice developed hepatocellular carcinoma (HCC) compared to 10 percent of untreated mice in the control group. The study's primary limitation on risk assessment for human exposure to AAV vectors was the reliance upon a tumor- and disease-prone neonatal mouse model, wherein insertion sites resulting in HCC were in a specific gene in the *Rian* locus, which is not present in humans. Another recent report implicated wild-type AAV2 as a potential cause for human hepatocellular carcinoma tumors via insertional activation of proto-oncogenes. This is the first report of a potential pathogenic effect of wild-type AAV virus in humans. The authors identified a genome fragment including the 3' end of the *Cap* gene of the AAV2 as required for insertional activation. The investigators point out that this fragment is not present in the AAV8 vector used by the VRC team. In a study reported in 2013 in *Nature Medicine*, insertion site frequency was investigated following intramuscular delivery of an AAV1 vector expressing lipoprotein lipase (LPL) to research participants with LPL deficiency. The study showed that integration occurred randomly and that integration could occur at any breakpoint of the vector genome. The authors concluded that there was no preferential integration at transcription start sites or gene coding regions, that the frequency of integration was approximately three logs lower than for retroviral vectors, and that "AAV integration is potentially safe." Thus, AAV vectors may integrate, but there does not appear to be a dominant process for preferential insertion near proto-oncogenes, unlike for some retroviral vectors.

Overall, the investigators consider the AAV8 vector to be non-integrating. They conclude that recent reports do not provide significant additional insight into a risk assessment of AAV vector-induced oncogenicity in humans.

As stated in the protocol, the dose escalation rule refers to "no safety concerns" as a condition for a dose escalation. The PSRT will review safety weekly, and a special safety review PSRT meeting for the dose escalation will be convened in which all adverse events (AEs) and reactogenicity data will be discussed and a decision regarding the dose escalation will be made.

Per the NIH Clinical Center (CC) procedures, results for all tests ordered through the NIH CC medical laboratory, including HLA typing, are placed in the research participant's medical chart at NIH. The difference in record keeping between the genetic tests performed in research labs and the HLA test done through the NIH CC laboratory is outlined in the ICD.

The consent language regarding persistence of the investigational product and withdrawal from the protocol has been revised to read, "If you get a dose of AAV8-VRC07, the study product administration will be completed at this point, but production of VRC07 may continue in your body for some time. Therefore, you will be asked to keep follow-up visits so we can monitor your health and VRC07 amount in your blood. Collection of samples that are for research purposes only may be stopped."

The investigators agreed with the other recommendations regarding the ICD and will modify the proposed ICD accordingly.

The statement in Appendix M with the message "Error! Reference source not found" has been corrected and now refers to Table 11, which provides the results of preclinical animal studies.

2. Responses to RAC Discussion Questions

The team is developing an antibody anti-lipase type assay against the AAV8 capsid and will screen all research participants using that assay. This testing will ensure a homogenous population for assessment of immune responses to the vector. Dr. Ledgerwood noted that it is unlikely to find no immunity in this population but that by screening patients as planned, the baseline immune response will be similar among research participants in the study cohort. Between three and 20 percent of the target population is expected to be AAV8-positive; this number will be clarified with review of the screening data.

Any clinically apparent reactions will be treated by physicians on the study team, which includes allergists and infectious disease specialists, and/or through consult with specialists at the NIC CC. The protocol includes a management plan for adverse events that may occur over the course of the study. The investigators do not anticipate needing immunosuppressive treatment but will address events on a case-by-case basis. The available evidence suggests that the self-immune response will clear the modified cells and eliminate the vector that could provide antigen for any kind of immune reaction. Given the small number of muscle cells overall in that tissue, a significant local clinical effect is not expected with IM dosing, which may be thought of as being similar to gene-based vaccine administration.

Dr. Ledgerwood noted that the vector that will be used for this study is considered non-integrating and is specifically designed to not integrate into the genome. While this does not rule out a theoretical possibility that some integration could occur (as with any virus), it is not expected in this trial. Biodistribution of the vector genome was assessed in preclinical good laboratory practice (GLP) toxicology studies, but not in the NHP studies. The vector is widely distributed, with some distribution in the liver. Based on the design of this construct and the publicly available data, the investigators have found no link between the AAV8 vector for this trial and any reports of oncogenicity. In the case of intrahepatic delivery of AAV8 vector in mice that developed paracellular carcinoma, it was subsequently determined that the integration and the clinical carcinoma outcome required the presence of the *Rian* locus, which is not present in humans. In another case, the *Cap* gene of the AAV2 vector is required for the outcome that was seen in that study (i.e., insertional activation of proto-oncogenes). The AAV8 vector for the proposed trial does not have the *Cap* gene. Given these differences between vectors, integration and oncogenicity are not expected. As an added safeguard, however, the investigators plan to test for the presence of the vector by PCR but will not assess specific tissues. They will consider adding monitoring of the muscle tissue, as suggested, with post-injection imaging at specific intervals and other possible methods (e.g., palpation).

The investigators will address the different potential and unknown pregnancy risks of the vector and gene/gene product and for male versus female participants. Research participants who become pregnant after enrollment and dosing will not be withdrawn from the study and will continue to be followed for safety and pregnancy outcome and for the duration of the trial these provisions will be clarified in the protocol and consent. The investigators acknowledged that statements that pregnancy should be prevented for as long as the product is persistently expressing the gene may be overly cautious, given that there does not appear to be any plausible way for the vector to integrate into the genome or the germ line. They will consider modifying the consent language to say that the risks to the fetus are not clear or are not known at this time and that if new information about the persistence of the vector is discovered, the recommendations for pregnancy prevention may need to be reconsidered in the future.

Regarding the plan to withdraw subjects who develop a serious medical illness, Dr. Ledgerwood explained that some individuals might develop a condition that should be addressed in another protocol or under a different IND product. Further, the outcomes of the proposed trial could be confounded if research participants who require additional treatment because of a new illness remain in the protocol. In contrast, research participants who become pregnant would continue to be followed for data collection only, involving minimal risk procedures (e.g., blood sampling) and pregnancy outcome (e.g., for any endpoints involving fetal defects). Dr. Graham made the distinction between removing a research participant's data from the analysis and removing the research participant from follow-up or from the protocol. Pregnant research participants will be followed for the duration of the study for safety parameters; they may follow a slightly different schedule than non-pregnant research participants, however, and they may not be included in the overall analysis for antibody PK. The language regarding the reasons for removing research participants, and when data will or will not be used in the study analysis, needs to be revised to reflect the actual plans and provisions for this study, as discussed.

The PI noted that the number and frequency of visits in the proposed trial (i.e., about one visit every other week in the first year) is similar to that in other studies. She provided background to explain the rationale for the planned compensation for completion of the trial and all follow-up visits and tests over five years, which is estimated as between $6,050 to $7,175. Actual compensation will be based on the number and type of study visits completed. Dr. Ledgerwood explained that investigators at the CC spent a considerable amount of time discussing and reviewing parameters to determine compensation rates for

visits and procedures done under NIH protocols and specifically in trials conducted by NIAID and the Vaccine Research Group (VRG). Intensive deliberations started in 2005 and involved multiple consults with the NIH Bioethics group and IRBs; review of the literature; and polling of compensation provided to participants in other clinical trials, specifically not-for-benefit research, conducted at academic and private centers in the Mid-Atlantic area. Based on assessment of this information, rates were set in 2007. The per-visit rates for VRG trials are in the middle range of compensation in studies conducted at local institutions. The investigators recognize the huge time commitment required by study participants. Subject inconvenience and burden, including travel/commute time, the length and number of individual visits, and time needed to complete various aspects of a study outside formal study visits (e.g., recording symptoms between visits), were taken into account in setting these rates. The rates are not calculated on a per-hour or per-unit basis in large part because when polling was done to determine how to compensate study participants, it became apparent that factors related to time and inconvenience were missing from those metrics. Dr. Ledgerwood noted, for example, that compensation for a two hour outpatient visit that includes oral swabs and blood drawing is $175 under the rates used in VRG trials. She commented that paying for time and inconvenience is different than being paid to do work. Research participants cannot be paid for pain or risk, but the rates used in the group's trials allow for compensation for more complex, longer visits, as planned for the proposed study. The PI recognized the altruistic nature of volunteering for clinical research and the suggestion to not paying subjects at all, in balance with the considerable amount of time research participants are asked to commit to complete this trial and similar studies.

The investigators will modify the consent language for genetic testing and risks of such testing, as suggested, to clarify that genetic information will be protected to the extent possible but that this information cannot be fully anonymized because it is tied to the individual and may impact not only the individual but potentially family members as well.

The "quiz" that research participants take as part of the informed consent process is a working document that is included in the "Assessment of Understanding." Prior to enrollment, study candidates complete multiple screening visits that occur over one to two months to determine eligibility. At the initial screening visit, the research participant is introduced to the consent form for this study to begin to learn about what is entailed in this clinical trial. At that visit, the investigators also explain that the informed consent process is part of an ongoing discussion between the research participant and the study team, that the investigators are interested in feedback and questions from participants, and that it is important that research participants understand the study and what their participation involves. The investigators, in turn, need to know that research participants have a good understanding of the trial and its risks at the time the consent document is signed (at study entry). They spend an additional 1.5 hours reviewing the document with each research participant on the day of enrollment. And at the end of that session, research participants are given the assessment of understanding questionnaire. It is basic but includes the key points and concepts of the trial to reflect whether the individual is properly informed or not. A study nurse or physician will assess the number of questions that are correctly vs. incorrectly answered and what information the research participant does not appear to understand. The investigator then goes through the consent again with the research participant to review and address anything that is not clear. Upon retaking the questionnaire, most research participants are able to answer all questions correctly or miss only one question. Thus, it's not a pass/fail type of test but an ongoing assessment of understanding. The Assessment of Understanding quiz needs to be completed correctly, however, before the study consent is signed.

To be eligible for the proposed study, patients must be stable on their antiretroviral regimen and have a low viral load (50 copies/mL). Given these criteria and that most research participants will likely remain on an ARV medications while enrolled in the study, use of viral load as an outcome measure for viral control will not be informative. As a result, serum antibody levels (against VRC07) will be used as a surrogate for viral control. The investigators considered results of both PK and NHP studies in determining the antibody levels that will be used to inform dose escalation. PK studies in mice suggested that a threshold of 10 mcg/mL would be sufficient for safety. Results from NHP studies, however, indicated that a serum level of 50 mcg/mL has a biological effect and was chosen as the dose escalation threshold. At this point, it is not known if the 50-mcg/mL threshold will be reached, or if a higher threshold will be needed. Serum samples

will be assessed for VRC07 level, and a kinetic profile will then be built for each research participant over time. The study team will work with the PK consultant in real time to assess these data. Analyses will be done in real time as required for assessment of dose escalation. The presence of the vector will also be checked by PCR. Per consultation with experts in the field, samples should initially be assessed for the vector (by PCR) at a baseline and then compared within the first 1–2 days after dosing (injection). Samples will be collected weekly and stored in freezers for subsequent testing, working backwards if a sample is found to be positive.

Sample collection will be limited to blood and mucosal secretions to determine whether VRC07 can detected in serum and these secretions, where the product is presumed to be present. There are no plans to collect tissue samples.

E. Public Comment

Dr. Borror suggested that terms such as “vector,” “encoding,” and “secretions” should be simplified or more clearly defined in the ICD so that research participants have a better understanding of what these words mean.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The following observations and recommendations were made during the RAC’s in-depth review and public discussion:

- In the event that tumor development is reported by research participants during long-term follow-up, the protocol should include a plan for the analysis of AAV vector integration in the tumor tissue if available.
- The informed consent document should be modified to:
  - Define more clearly complex terms and concepts (e.g., mucosal, integration, neutralizing antibody).
  - Convey concerns to research participants of both sexes about the short and long-term risks to pregnancy, which may be associated with the product.
  - Clarify the language regarding the removal of participants from data analyses, since this level of detail is unnecessary for participants and they will continue to be followed throughout the course of the study.

G. Committee Motion 2

Dr. Kiem summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Kiem requested a vote, and the RAC approved these summarized recommendations by a vote of 12 in favor, 0 opposed, 0 abstentions, and 1 recusal (Dr. Whitley).

IV. Review and Discussion of Human Gene Transfer Protocol #1601-1494: Efficacy of Local Delivery of MicroRNAs in Promoting Osteogenesis and Modulating Local Inflammation: A Pilot Clinical Trial Using the Tooth Socket Model

Presenters:	Gustavo Avila-Ortiz, D.D.S., Ph.D., University of Iowa College of Dentistry and Dental Clinics (*via teleconference*) Brad Amendt, Ph.D., University of Iowa College of Dentistry and Dental Clinics Liu Hong, M.D., Ph.D., University of Iowa College of Dentistry and Dental Clinics
RAC Reviewers:	Drs. Cho, Donahue, and Whitley

Ad Hoc Reviewer: Ilias Alevizos, D.M.D., M.M.Sc., National Institute of Dental and Craniofacial Research, NIH

A. Protocol Summary

Regenerative medicine has been in continual development over the past two decades, with the primary objective of developing cost-effective, novel therapies associated with clinical success and lower morbidity. Advances in the understanding of cell biology and molecular signaling pathways have allowed for the translation of fundamental knowledge into clinical applications to promote regenerative outcomes. Particularly in the field of bone biology, identification and characterization of key molecules involved in bone growth and fracture healing has provided with critical information that can be used to develop safe and efficient tools for bone regeneration. For example, due to their strong osteogenic capability and promising effects, recombinant human bone morphogenetic proteins (rhBMPs), which are FDA-approved, represent one of the most extensively used biologic agents to induce bone formation in clinical settings in the United States. However, rhBMPs are extremely expensive. The dose needed to achieve a therapeutic local effect has also raised long-term, systemic safety concerns. Thus, safe alternative approaches to achieve predictable and adequate bone regeneration are warranted.

MicroRNAs (miRs) are small non-coding RNAs that function in post-transcriptional regulation. These small molecules can modulate gene expression via translational repression or target degradation. Evidence suggests that miRs are involved in bone development and osteogenic differentiation. miR therapeutics therefore have the potential to become novel and alternative interventions for the promotion of bone regeneration (e.g., after tooth extraction). miRs are abundantly expressed in humans, including in saliva. No adverse effects or toxicity has been reported in preclinical rodent studies following overexpression of miRs and miR inhibitors in the oral cavity using plasmid delivery vectors.

The proposed dose escalation pilot study will assess bone regeneration following tooth extraction. The purpose of this study is to evaluate the efficacy of locally delivered plasmid DNAs encoding miRs and/or miR inhibitors in the promotion of osteogenesis and the modulation host inflammatory response to minimize alveolar bone atrophy in a tooth socket model in humans. The miRs will be delivered as plasmids in a bovine collagen vector. Non-viral plasmid DNA will be used to deliver the plasmids of interest to avoid risks with viral plasmid DNA. The protocol will enroll 24 healthy adults requiring single-rooted tooth extractions and future tooth replacement therapy with an implant-supported fixed partial denture. Each subject will be randomly assigned to one of eight study groups, which include a control group (tooth extraction and bovine collagen sponge) and seven experimental groups, as follows:

- Tooth extraction and bovine collagen sponge containing 5, 10, or 20 µg of miR-200c plasmid
- Tooth extraction and bovine collagen sponge containing 10 µg miR-200a inhibitor
- Tooth extraction and bovine collagen sponge containing 5, 10, or 20 µg miR-200c plasmid and 10 µg miR-200a inhibitor

A core bone biopsy will be obtained at the time of implant placement, from the osteotomy site. This biopsy will be processed for histologic/histomorphometric analyses. Subjects will be informed that if primary stability cannot be achieved during implant placement, the standard of care is either to attempt placement of a larger (wider, longer, or both) implant or to abort implant placement at that time, graft the area, allow further healing for an additional period of time (at least 3 months), and re-enter the area for another implant installation attempt. Volunteers will be followed at regular intervals through 12 months after tooth extraction. A radiograph will be obtained at baseline (before tooth extraction) and at 14 weeks (prior to implant placement) to assess bone changes. At some visits, a fluid sample (e.g., wound fluid from the healing site or saliva) will be obtained. Blood samples will be collected to monitor systemic inflammation.

B. Written Reviews by RAC Members

Eleven RAC members voted for in-depth review and public discussion of this protocol. The trial was found to warrant public review, because the study involves the administration of plasmid DNAs encoding

microRNAs and/or microRNA inhibitors to promote osteogenesis and modulate host inflammatory response to minimize alveolar bone atrophy in a tooth socket model in healthy volunteers.

Three RAC members and one ad hoc reviewer provided written reviews of this proposed pilot study.

The reviewers found the proposed research to be interesting but identified several issues that needed clarification or additional consideration.

Dr. Donahue had several comments and questions regarding the general study design. The protocol cover letter includes a statement that the overexpression of miRs and miR inhibitors in humans and in saliva and the oral cavity pose “no threat to the research participant.” Dr. Donahue pointed out, however, that the presence of miRs as a general category is not necessarily an indicator of their safety. Because every miR has a variety of functional effects, overexpression, off-target expression, or repression of any miR could have significant consequences. Thus, there is a real risk of adverse events, and this needs to be carefully considered in the study design.

Throughout the application, the proposed therapy is referred to generically as “miR overexpression and inhibition,” and a dose range of 5–10 µg for the plasmid is given. The study design is a placebo-controlled, randomized single dose. Dr. Donahue noted that the proposed therapy, which presumably is miR-200c, needs to be clarified. Drs. Donahue and Whitley commented that the exact dose and the rationale for how that dose was chosen should be provided. Furthermore, given the current state of development of this approach, the investigators should consider whether a dose escalation study design might better inform future clinical development.

The preliminary data provided in the application reflect a compilation of basic studies tracking the signaling pathways affected by miR-200c and delivery of plasmid to the temporomandibular joint (TMJ) in rats where expression was observed. Dr. Donahue inquired whether there are any specific preclinical data to show that miR-200c overexpression will accomplish the aims of the clinical trial. In particular, the application mentions that the investigators have found other miRs that play a role in this process. Given this background information, are there data to indicate that miR-200c is necessary and is sufficient to achieve the goal of improved bone growth?

Dr. Donahue also asked whether there are any data to show that the proposed delivery tools, matrix, and gene dose are successful in the proposed environment of tooth socket and whether the investigators have assessed the proposed clinical product to see whether the collagen matrix affects plasmid availability or stability.

The clinical monitoring plan assumes little if any off-target delivery. Because the study uses plasmids, this is likely to be the case. However, the proposed miR has a variety of immune modulating effects, and the investigators need to be sure that off-target delivery does not occur. It would be prudent to monitor subjects more closely for immune-mediated side effects in case the clinical situation differs from the preclinical models. At a minimum, a more thorough and better-defined monitoring plan for local wound healing should be considered, and some level of monitoring for local and systemic immune suppression and/or autoimmunity reaction should be performed. This is important under any circumstance, but it is particularly important in the planned patient population of otherwise healthy individuals with a therapy for a non–life-threatening condition.

Dr. Donahue requested additional information regarding the current standard of care for tooth extraction/dental implantation and how the proposed trial differs from the standard. In addition, he asked (1) whether the proposed bone biopsy will affect study outcomes or add risk to a patient population where local bone availability is the problem and (2) whether the 16-week period of time between extraction and implantation mirrors usual clinical practice.

Dr. Donahue had the following additional comments and questions on information in Appendix M:

- The investigators state that the usual bone atrophy in the proposed model is predictable. However, in the introduction to the clinical protocol, they state that the response to current

therapy in this model is variable within a range of 60-100 percent. These statements need to be reconciled.
- Data supporting the conclusion that 80 percent of target cells will express the transgene (with the proposed delivery method, investigational product, and model) need to be provided.
- Details of the specific experience of the team's facility for gene transfer clinical studies and the biosafety controls that will be in place for this study are needed.

Dr. Donahue identified the following issues in the ICD:
- The page 2 description of the active treatment group should be rephrased to more clearly describe the therapy. People who know the difference between DNA and RNA will likely be confused by the label "plasmid DNA micro RNA," and people who do not understand that concept are unlikely to be helped by the current description.
- Issues of patient privacy with respect to the photographic and video data need to be addressed, including how long these data will be kept.
- Biosafety, adverse effects of on-target gene expression, and any effects of off-target gene expression risks need to be addressed more thoroughly in the risks section. Since this is a Phase I study, the consent should also explicitly state that the risk profile cannot be adequately described because these experiments have never been performed in humans before.
- The section on leaving the study should clearly explain that there is no reasonable way to extract or inactivate the DNA once transferred, so that leaving the study is only leaving the follow-up, not leaving the initial treatment. Risk of interrupted or decreased follow-up after administration of a biological therapy should also be presented in this section. Likewise, the section on an investigator deciding to end a subject's participation should clarify the difference between pre-DNA administration termination and post-DNA administration termination, which is really just reduced follow-up.

Drs. Donahue and Whitley found the information regarding the actual gene product and the plasmid promoter to be confusing. The appendices list two different plasmids: a cytomegalovirus (CMV)–driven construct that is generically labeled "siRNA," and a U6-driven construct that appears to be designed for a miR inhibitor that is not otherwise mentioned in the application. It is not clear which construct will be used for the clinical product in the proposed trial. Dr. Donahue also asked whether the investigators have considered (1) how the non-mammalian DNA sequences in the planned vector may affect gene expression and host responses to the gene transfer and (2) the potential effects from promoter selection on cellular toxicity and miR expression.

Dr. Whitley had the following additional general comments and questions:
- Why is the proposed approach better than the standard of care, particularly for volunteers at younger ages, where normal healing would be anticipated?
- The investigators state that they will study "efficacy" in the Purpose and Objectives section of the protocol but then later state that the study is not powered to determine efficacy. This should be clarified.
- Because this is a pilot study, really, all that will be learned is in respect to safety. The investigators should consider following volunteers for safety more rigorously, particularly considering off-target effects, which are not insignificant, since the age range of the volunteers is very broad. Older individuals will be at greater risk for adverse events as well as delayed healing.
- Consistent with issues related to safety, although miRs are present in saliva and the mouth, overexpression may not equate with safety, further supporting more rigorous assessment of potential risks.
- The investigators state that they will use biomaterials acceptable in dentistry, particularly the bovine collagen sponge. This does not appear to be the case for miR-200c, however. If this presumption is correct, what preclinical toxicity testing has been done to meet FDA standards? Furthermore, will miR-200c be the investigational material? In addition, if miR-200c will be supplemented by other miRs, what are they?
- What is known about the stability of the bovine collagen construct and the miR(s) of choice?

Dr. Whitley requested that the following points be addressed in the ICD:

- A statement regarding the advantage for participation in this trial should be added near the start of the document.
- The exact number of subjects the investigators plan to enroll should be specified instead of giving the approximate number of subjects.
- It is unlikely that a layman with a high school education will know what plasmid DNA and micro RNA. This must be clarified.
- Clarify that the intervention given under the experimental group cannot be reversed.

Dr. Cho noted that overall, the proposed research appears to pose low risks. She raised several concerns, however, regarding the study design and objectives, particularly within the context of subject safety. Measurements of systemic risks is lacking, and supporting evidence of safety and efficacy from preclinical studies is very thin. The study is set up as a very small (N = 12) randomized "pilot study" that is optimized to assess neither safety nor efficacy. For example, safety parameters such as systemic effects of plasmid gene expression will not be measured. A proposed level of clinical significance of primary outcomes is not proffered, and with such a small sample, it would be difficult to interpret the findings in terms of efficacy.

Dr. Cho had the following comments and questions on the protocol and Appendix M:

- The protocol states that recombinant bone morphogenic proteins are expensive and raise systemic safety concerns as a justification for conducting the proposed study. However, the proposal does not address how it will evaluate the systemic safety of the plasmid/microRNA approach.
- The alternative surgical approaches to enhance bone regeneration in the context of tooth replacement are described as "predictable", with success rates of 60-100 percent. However, a compelling rationale for why the proposed approach will be superior to these alternatives (either in healthy or high-risk populations) was not given in the protocol. The Appendix states that evidence regarding effectiveness of current ridge preservation techniques is "limited" and that effectiveness "is likely to be attenuated in subjects presenting systemic conditions that predispose an impaired healing response, such as uncontrolled diabetics and patients suffering from immune disorders." However, no evidence supporting this assertion was given. Furthermore, in the appendix, there is a strong assertion that "the main advantage of the proposed experimental therapy…is the robust 'dual' osteogenic and local anti-inflammatory effect that has been observed in previous experiments and that has not been previously reported for any biologic."
- The Appendix also states that the "sustained production of miR200c" as a "tangible advantage" over a single dose of, for example, BMP. However, the data presented on in vivo gene expression in rats is underwhelming. Expression of miR200c 5 days after injection into the temporomandibular joint (TMJ) and trigeminal (TG) nerve of rats, as measured by real-time PCR, was 0.89±0.24 in the TMJ and 5.5±4.85 in the TG, as compared to 0.56±0.66 TMJ and 0.89±0.17 TG in tissue injected with empty plasmid. No data on gene expression or other effects outside the local sites of injection were provided. The investigators state in the Appendix that the procedure "has no chance of causing death or injury" but supporting evidence of this claim is not provided.
- Given the implication of miR200 in promoting late stages of metastasis, it seems prudent to exclude individuals who have been diagnosed with cancer, rather than only those who are currently undergoing cancer treatment and those within 18 months of radio- or chemotherapy.
- The intervention group is said to receive "collagen sponge containing plasmids (5-10ug) encoding microRNA and/or microRNA inhibitor." This statement and the diagrams of gene constructs are unclear regarding which plasmids will actually be administered and to which participants. In addition, the subjects are said to be randomized to intervention or control (no plasmid), but it does not appear that the investigators will be blinded to allocation, either during the procedures or analysis, increasing the potential for bias introduced in the procedures and analysis. Because all subjects will receive a sponge in the tooth extraction site, such blinding seems feasible.
- It is not clear why no direct assessments of gene expression are proposed, either locally or systemically.

Dr. Cho questioned whether the plan to waive the fees for tooth extraction ($400) and the CT scan ($205) and to reduce the fee for implant placement (from $1,500 to $875) presents an undue incentive to potential participants. (Participants also will be paid $20 for each of the four follow-up visits.) Her main concern was that individuals might hide information that would disqualify them from participation. To avoid this potential problem, the inclusion/exclusion criteria should be independently verified to the extent possible, and the assessment of eligibility should be performed before the potential subjects are told about the financial incentives. Dr. Cho agreed with the other reviewers that the consent form needs to explain that dropping out of the study after receipt of the intervention will not guarantee that any effects of the plasmid will be terminated.

Dr. Alevizos commented that the possibility of safe local delivery of microRNAs through plasmids would be a major advancement in the field and could pave the way for other applications where direct tissue access is easily achievable. Like the other reviewers, however, he identified several issues that need to be clarified in order to minimize the potential risks of a human clinical trial and maximize the scientific knowledge obtained by the clinical trial. Dr. Alevizos had the following comments and questions for the study team:

- Have safety studies been established for the plasmid delivery of miRs in the bone in animal models? There is no mention of such studies except for injection to the TMJ, which involves a completely different delivery method than the one suggested by the investigators.
- The protocol cover letter states that the investigators “have proven that our method of delivery and expression of microRNAs and microRNA inhibitor has no adverse affects or toxicity in mice.” However, there is no evidence in the provided materials that collagen sponges were used to deliver plasmids in teeth sockets.
- The design of the trial is very confusing as there are sporadic mentions of the use of miRNA and/or its inhibitor in the six subjects that will receive the treatment. In addition, there is no detailed description of the use of the miR and the inhibitor in the protocol. The definition of the primary outcome measure is missing, and it is not clear how efficacy is defined in this study. Dr. Alevizos recommended that this intervention be studied first as a safety trial. Efficacy with only three subjects per group will not show any relevant clinical or statistical significance.

Dr. Alevizos posed the following additional questions and issues to the investigators:

- The definition of the primary outcome measure is missing. What defines efficacy in this study?
- Additional detail is needed on how adverse events will be reported.
- Will this study have a Data Safety Monitoring Board?
- The upper limit of age inclusion is too high and the range too wide for such a small sample size.
- One hour for the total first visit is inappropriate. Discussion of the informed consent might take as long.
- What is the plan for enrollment if subjects drop out or develop infection and are in need for antibiotics?
- How does delivery of the plasmid occur in the cells, and what is the half-life of the plasmid in the sponge? What is the transfection efficiency? There is a need to demonstrate uptake of plasmids in animal models (e.g., rats), before a trial takes place in humans.
- The investigators should consider excluding individuals taking biologics or disease modifying agents, those with a history of oral cancer treated within 5 years, smokers in general and not only “heavy smokers”, persons with history of osteosarcomas and chondrosarcomas and osseous and odontogenic tumors.
- How will the plasmid be manufactured (e.g., in good manufacturing practice (GMP) facilities, under FDA certification)? Details of the QC/QA procedures and how and whether the product will be stored need to be delineated.
- A monitoring plan needs to be added to the protocol.
- Details should be specified as to the frequency of enrolling and treating subjects and the number of days between enrolling new subjects to ensure safety of the product.

Dr. Alevizos had the additional following comments regarding the ICD:

- A brief lay description of what microRNAs are is needed
- What is the reading level of the consent?
- Inconsistencies regarding procedures in the protocol need to be addressed. For example, a brief description of what computed tomography (CT) entails and the risks associated with it need to be clearly stated in the consent.
- The contraception policy during the study should be well defined.

The reviewers noted that participants are unlikely to recognize or understand some of the technical terms in the consent (e.g., plasmid DNA, microRNA). Brief descriptions of these terms in lay language are needed.

C. RAC Discussion

During the meeting, the following additional questions, concerns, or issues were raised by RAC members:

- The reviewers found the presentation to be clear and their concerns and questions to be well addressed. They went through their comments and the investigators' responses to their queries and suggestions.
- Several RAC members agreed with the reviewers that additional preclinical studies in an appropriate animal model are warranted to assess adequate gene delivery and ensure the safety and efficacy of the planned delivery method and in the specific microenvironment of the tooth socket. Testing in other tissues and models (e.g., TMJ, calvarial bone defect model) does not replicate or necessarily predict what might happen in the tooth socket. The periodontium has a variety of resident cells not present in other tissue, and cells and bacteria exposed with the planned tooth extraction will likely result in interactions not seen in other settings. The data from the calvarial model are encouraging, but it is not clear why the proposed experiment, including tooth extraction and subsequent injection of the miR plasmid (with or without inhibitor) into the tooth socket, has not been done in an animal yet. A dose-response study in a larger animal (e.g., a dog model) should be conducted before testing is done in healthy volunteers. The investigators should design the animal study to set up the Phase I trial, using both the intervention and the bovine collagen sponge that is planned for the clinical protocol. As with all preclinical models, the results will not necessarily predict what is seen in humans, but they will be able to inform how to proceed and what factors in the specific microenvironment will afftect delivery. Aspects of the preclinical models that are applicable to humans should be more fully described to better convey translatability. It was noted that the FDA will likely require a rigorous assessment of the intervention and delivery method in at least two animal models and that safety and toxicity data in animals are needed to set up a Phase Ib protocol. That assessment will include preclinical toxicity data and pre-specified and pre-defined endpoints for the clinical trial.
- The premise of using naked plasmid should be pursued because it is easily accessible for a number of diseases, but data demonstrating the efficacy of this approach are needed. Dr. Alevizos noted, for example, that his lab has not been able to achieve a very high efficiency of transfection with plasmid in the oral cavity. As the investigators point out, use of a barrier membrane to seal the socket has been reported in some clinical trials to provide an additional benefit. However, the proposed research involves a different microenvironment for the extraction socket than in other studies, and use of a barrier membrane may not be as effective as anticipated in preventing significant alveolar ridge resorption after tooth extraction.
- The RAC expressed concern about the proposed study design. The protocol is currently written as a Phase II trial, but it is important to establish the safety, risks, and appropriate dose of this intervention in humans before evaluating efficacy. Similarly, animal studies that show safety and efficacy and inform dose levels for human testing should be done before initiating a clinical trial. Once preclinical data are available as recommended by the RAC, the protocol should be re-written as a Phase I pilot study to assess safety of the planned intervention before proceeding to a Phase Ib or II trial.
- If the appropriate supporting animal and other preclinical studies have been done and show that the gene product is expressed and the combination and delivery method are safe and effective,

the investigators might be able to proceed to a dose-escalation Phase Ib trial in the target population.

- Drs. Whitley and Cho noted further that given the complexity of the design, specifically, the number of cells, and the small number of subjects per cell, it will be difficult to obtain the desired information, particularly the dose. Drs. Alevizos and Cho suggested initially pursuing only the dose-escalation part of the study (e.g., the first four arms of the proposed trial), without the inhibitor, given the lack of safety data and data on the feasibility of delivering the microRNA plasmid into the dental bone socket. The investigators should consider consulting their institution's biostatisticians to see if there is an alternative approach that will yield usable data to the research questions without having to employ a complex clinical trial design. An adaptive design might provide answers to questions of safety as well as provide preliminary evidence of efficacy. In such a scenario, the design might initially look at the first one or two groups only to determine whether the expected effect is demonstrated; if so, the study could then proceed with an expanded cohort.
- Dr. Whitley noted that the upper age range for eligibility was lowered from 75 to 65, but he remained concerned about the potential impact of age on healing; specifically, differences in how well and how quickly the extraction and socket site will heal in younger versus older participants, and, in turn, how (or whether) these differences affect the final outcome. Dr. Zoloth expressed concern about testing this intervention in healthy adults and asked whether the study will be done in the anticipated or appropriate target populations. If the target is older rather than younger persons, for instance, some participants could be unnecessarily exposed to research risks.
- Dr. Donahue commented that the investigators have done a lot of work regarding off-target delivery, including effects with whole-body exposure, which addresses concerns about potential off-target events. Subjects will receive a standard modality of care for tooth extraction and dental implantation, including the 16-week healing period, so that any added risks are with the research procedures.
- Dr. Donahue was concerned that the initial steps in this research may be too focused on the ultimate goal. It is not clear how the trial would proceed if the "wrong answers" are generated early in the study, for example, if there is no delivery because salivary DNAse consumes all of the plasmid. Dr. Cho asked whether the 80 percent of cells that take up the plasmid do so in an in vitro setting only or if this result is also seen any in vivo models. The reviewers noted that the matrix appears to stabilize the DNA, which should mitigate DNA loss from degradation by the inflammatory cells that flood the socket or from exposure to saliva and other factors in the oral environment. Other aspects of the design need to be addressed, however.
- The investigators addressed questions and concerns about the proposed compensation and reduction in and waiving of some costs associated with the standard dental procedures that participants will undergo. However, they need to make sure that sufficient protections are in place to avoid undue inducement of potential subjects.
- The ICD should clarify that withdrawal from the study refers to withdrawal only from study follow-up, procedures, and monitoring for potential long-term adverse effects of the experimental intervention and that the product itself cannot be withdrawn.
- Dr. Zoloth questioned why participants who become pregnant will not be followed in the same manner as male subjects and subjects who develop other conditions while enrolled. Individuals who are pregnant or lactating are not eligible to participate due to the unknown risks of the experimental product and the risks associated with radiation (x-rays). However, it is not clear whether both male and female participants of reproductive potential are required to use effective birth control for the duration of the study. The consent says that subjects should "consider" using contraception during the active phase of the study (16 weeks). These provisions need to be clarified and stated explicitly in the consent form.
- It similarly is not clear why someone whose "condition becomes worse" would not continue to be followed. In addition, the kinds of adverse conditions that might result from a tooth extraction or the research intervention should be specified (e.g., severe infection). Since this study will include only healthy adults, the subjects are not expected to have serious underlying medical conditions or illnesses.

- The reason for including a request for an autopsy is not clear, particularly given that systemic effects and distribution of the experimental product are not anticipated. While the consent says the autopsy would be done to collect information about the safety and efficacy of gene transfer, this seemed inconsistent with the objectives of this research.
- Dr. Zoloth expressed concern that the cost of medical care to treat any injury or illness resulting from participation in this study will be the responsibility of the subjects. Given that this is a Phase I trial, the potential harm that could occur is not known. Contrary to what is stated in the consent document, medical and hospital insurance carriers will not cover any costs related to experimental research; such statements are therefore misleading. Dr. Zoloth objected to these provisions, which appear to be NIH policy, adding that it is the ethical obligation of the clinicians and the institution to provide and pay for care to study participants.
- The consent refers to “someone else” being able to end a subject’s participation in the study. This language is vague and needs to clarify if anyone other than the investigators could make a decision regarding withdrawal of an individual subject from the study.
- The investigators need to ensure that the study participants are fully aware of the potential risks of the study and adequate and appropriate safeguards are in place so that subject are protected to the extent possible.

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators agree with the reviewers that each microRNA can target many transcripts. They note, however, that tissue specificity is important, as not all genes are expressed in each tissue. Overexpression of miR-200c in mice models yielded only small changes in bone growth, with no other effects. Similarly, effects in mice exposed to miR-200c and miR-200a inhibitors were limited to defects and growth due to inhibited bone growth. In another rodent model, calvarial bone defects restored by miR-200c adsorbed collagen sponge and demonstrated no adverse effects. In this study, a type-I bovine collagen sponge loaded with naked plasmid miR-200c (the same approach proposed for the human socket model) was implanted into rat calvarial bone defects. There were no reports of death, inflammation, or infection among operated animals during the 4-week post-implant observation period. Micro-CT and histology-based evaluation showed bone formation in the treatment group without side effects. Transgenic mice that overexpress miR-200c, miR-200a, or microRNA inhibitors are born and mature normally with no side affects, including no liver toxicity. The localized delivery and specificity of the miRs and the inhibitor demonstrate the efficacy of the teams’ approach. In addition, in vitro toxicity tests show that plasmid miR-200c does not affect the metabolism of human bone marrow cells, gingival fibroblast, or periodontal ligament fibroblasts. The investigators clarified that they have not shown that delivery of microRNAs or inhibitors via a sponge in tooth sockets does not cause toxicity because they have not performed these specific experiments yet.

The proposed study will supplement overexpression of miR-200c with an inhibitor to miR-200a. The optimal dose of miR-200c in humans is not known yet. Evidence suggests, however, that the response is related to the dose responsive, and doses of 5, 10, and 20 µg of each plasmid will be tested in the pilot trial to determine the best dose. The investigators have revised the study to follow a dose escalation design, as suggested by the reviewers. The protocol now includes one control group and seven experimental groups in which subjects will receive 10 µg miR-200a inhibitor plasmid alone or 5, 10, or 20 µg miR-200c plasmid alone or in combination with 10 µg miR-200a inhibitor plasmid. All participants will undergo tooth extraction, and the control group will receive only the bovine collagen sponge. The investigators think a combined approach is the best strategy, because miR-200c overexpression by itself may not be as efficient in promoting bone growth and reducing inflammation.

Although there are no data for a tooth socket model, studies have been conducted in a calvarial bone defect model, TMJ disorders, and periodontitis rat models. Results of these studies show that cells can take up the plasmids and express the miRs and inhibitors. In the teams’ recent studies, the collagen matrix appears to offer increased stability for the plasmid. The collagen sponge binds the plasmid DNA

and sequesters it to the fibers; as the cells infiltrate the sponge, they then take up the plasmid and express the microRNA. While this is not controlled release, the plasmid is taken up over time as more cells enter the matrix, and it is progressively broken down to form the biologic scaffold that is essential for bone formation.

The stability and biocompatibility of the bovine collagen sponge is well established; it is a product widely used in clinical practice in both private and academic settings. The matrix is stable for 3 to 5 days, and it is completely resorbed and replaced after 10 to 14 days. The plasmid DNA binds to the collagen and is delivered to the cells as they invade the sponge. miR stability in the cell can range from hours to several days with a gradual decline in transcripts. The investigators have found that miR-200c is one of the most stable microRNAs, with a half-life of approximately 48 hours in the cell. The inhibitor is more stable, with a half-life of 5 days.

The plasmid described in Appendix I uses the CMV promoter to express miR-200c. This plasmid can express high levels of miR-200c, which is needed for an efficient response. The Pol III U6 promoter (Appendix II) is used to drive expression of the inhibitor; this promoter is less efficient and results in less expression of the inhibitor, which is the desired outcome, since overexpression of the inhibitor can overwhelm the miR processing system. These vectors were identified and modified in response to extra DNA sequences that could affect gene expression and gene transfer. No defects or problems with cell or animal death or adverse effects have been identified with these plasmids. In addition, no cellular toxicity effects with either miR-200c or inhibitor overexpression have been found.

The investigators follow GMP guidelines for production, testing, and storing of the study products. The plasmid DNA has been sequenced and confirmed to be specific for the microRNA or inhibitor. The plasmids are amplified in modified bacteria for replication in high copy numbers without degradation of the plasmid DNA. Plasmid DNA is isolated by standard laboratory methods and purified to high standards. The GMP includes controlled environmental conditions and clearly defined manufacturing processes that are rigorously controlled through protocol design and constant evaluation of practices. The group includes trained laboratory staff with more than 20 years of experience preparing plasmids. Records are maintained for quality and quantity, instrument measurements are documented and stored, and batch information on purity and concentration is kept up-to-date. All products are stored independently in a locked freezer at the University of Iowa under the PI's supervision. Plasmid DNAs encoding microRNAs and/or microRNA inhibitors have been tested in multiple in vitro and in vivo settings. They are not FDA approved at this point, but the investigators plan to apply for FDA approval as the study progresses.

Data show that naked plasmid DNA is taken up by cells via endocytosis. While this method is not as efficient as nanoparticle or other lipid delivery systems, an advantage is that the delivery of naked plasmids is not toxic or inflammatory. Delivery in a collagen sponge adsorbed with the plasmid DNA allows for continuous uptake by cells with migration into the sponge, and the DNA in the sponge is stable for weeks. Although the time profile of plasmid expression is not clear, the levels of microRNAs and inhibitors in cells remain high through 1 week after treatment. In vitro expression of microRNAs and inhibitors from naked plasmid is dependent on dose; in the team's in vitro study, about 15-and 80-fold higher miR-200c expression was detected in human bone marrow stem cells after treatment with 2 and 20 ug/mL, respectively. This system is highly efficient, without evidence of toxic effects.

Patients will be evaluated on a regular basis as per the study protocol. Measurements of bone growth and inflammation will be obtained. Blood samples will be analyzed by the University of Iowa Pathology Clinical Laboratory Team for infection and inflammatory responses. The investigators have developed a data safety and monitoring plan that will be reviewed by the university's Human Subjects Office, Clinical Research Office, and study team.

There currently is no universally accepted standard of care following tooth extraction. Some health providers do nothing but curettage and irrigation of the socket. Others also deliver a collagen sponge to stabilize the blood clot and subsequently aid postsurgical healing, as well as to prevent complications associated with food impaction. Recent evidence indicates that grafting of the socket with a bone replacement material, with or without the adjunctive use of an occlusive membrane, may be beneficial in

most clinical scenarios. Such grafting attenuates the typical alveolar bone atrophy observed after tooth loss by promoting osteogenesis via osteoconduction and, depending on the rate of resorption of the applied biomaterial, limits the volumetric reduction of the alveolar ridge after complete healing (i.e., within 14–20 weeks after tooth extraction). The control therapy in the proposed trial does not differ significantly from one of the standard modalities of care after tooth extraction (i.e., application of a collagen sponge). The planned bone biopsy will not affect bone availability, as the bone tissue will be harvested from the exact location where the dental implant will be placed for future prosthetic rehabilitation. In other words, this is bone that would be otherwise discarded. The 16-week healing period is standard of care in the typical clinical practice.

The investigators explained that alveolar bone atrophy is a well-known consequence of tooth extraction and loss and therefore is to be expected if no alveolar ridge preservation technique is applied. If tooth replacement therapy using dental implants is desired by the patient, ancillary bone augmentation procedures are often necessary for the reconstruction of atrophic alveolar ridges to facilitate dental implant placement and for adequate bone support to ensure long-term success of the prosthetic therapy. The 60–100 percent success rate reported by another group (Chiapasco et al., 2006) refers to these ancillary bone augmentation procedures. Recent evidence strongly suggests that grafting of the socket with a bone replacement material, with or without the adjunctive use of an occlusive membrane, may be beneficial in most clinical scenarios. However, as shown in a recent meta-analysis co-authored by some of the investigators in the proposed study, alveolar ridge preservation via socket grafting is not fully efficient in preventing alveolar bone loss after tooth extraction (Avila-Ortiz et al., 2014). This can be attributed to inter-individual local and systemic factors, some of which may be directly related to a robust local inflammatory response, which can lead to upregulation of osteoclastogenesis and increased bone loss. The experimental therapy proposed in this trial is expected to both promote osteogenesis and modulate the local inflammatory response that follows tissue trauma (as it normally occurs after tooth extraction).

The investigators clarified that references to 80 percent expression of the transgene in target cells mean that 80 percent of the cells that take up the plasmid DNA express the microRNA or inhibitors at high levels. Experiments demonstrating these findings used control reporters and controlled cell numbers to identify expression. The investigators do not believe that 80 percent of the cells are transfected with the naked plasmid. However, new cells that associate with the collagen matrix appear to take up the plasmid in high levels (i.e., an estimated 60 percent of cells). In the TMJ disorder experiments, the tissue also expressed the microRNAs efficiently.

Gene therapy studies per se are not conducted at the University of Iowa; most studies are conducted using viral vectors, not plasmid-based gene therapy. As described in more detail below, the study investigators will be working with the University of Iowa Pathology Clinical Laboratory Team to conduct specific tests on participants' samples. The trial follows a standard GMP plasmid quality control protocol to assure that the plasmids comport with stringent specifications.

In addressing the reviewers' overarching concerns regarding the study aims and subject safety, the investigators noted that the planned measures to assess safety were limited to clinical assessments and monitoring of the participants, which may be considered insufficient in a study of this nature. The investigators have modified the protocol to improve this aspect of the study, and they have redesigned the protocol to be a randomized, parallel-arm, double-blind clinical trial with four treatment groups, with safety as the primary focus. The outcomes and goal of this trial (to generate dense bone and repair the alveolar ridge defects in individuals with tooth loss or dental defects) and the clinical need for this type of study have been more clearly delineated. The patients will be evaluated on a regular basis per the study protocol, and measurements of bone growth and inflammation will be obtained. The adverse events in this study may include inflammation, necrosis of the tissue surrounding the tooth socket, overgrowth of tissue, and abnormal bone growth. The clinical coordinator and the lead clinician will monitor and document any adverse changes in all patients. Blood samples will be collected at multiple time points (1, 2, 3, 4, and 14 weeks post-extraction) and analyzed by the University of Iowa Pathology Clinical Laboratory Team for infection and inflammatory responses. There will be a minimum of 2 weeks between subjects within an experimental group to ensure that there is no evidence of adverse events at either the

clinical or biomolecular level before continuing with additional subjects. The investigators understand that the low sample size (six subjects per group as originally proposed, three subjects per group in the revised protocol) is not sufficient to extract major conclusions regarding the effectiveness of the proposed experimental therapy. The original sample size (12 subjects) was selected for feasibility.

The investigators believe that the study design is valid to obtain pilot data in terms of the efficacy of the proposed treatment in the promotion of osteogenesis and the modulation of the local inflammatory response in a tooth socket model. If the experimental therapy is proven safe and superior to the control, the data generated in this study will be used to guide the conduct of additional clinical trials involving larger populations on the basis of a proper power analysis.

The following additional modifications and clarifications have been made in response to the reviewers' comments. The age range for eligibility has been changed to 25 to 65 years, and the length of the first study visit, which includes review of the consent document, has been increased from 60 to 120 minutes. Subjects who drop out or otherwise meet an exclusion or withdrawal criterion will be replaced; the protocol will remain open to new enrollment until 24 subjects complete the study. The investigators noted that they do not intend to enroll any smokers. The clinical study coordinators are well trained and typically verify the eligibility criteria prior to discussing any financial incentives associated with participation in the study with potential subjects.

The investigators agreed with the recommendations regarding the ICD and have modified the proposed consent document accordingly. The investigators confirmed that they plan to collect photographic data and video recordings, with the intention of keeping these materials indefinitely, in conjunction with the rest of the study data, for possible publications and scientific presentations.

2. Responses to RAC Discussion Questions

The investigators will address the RAC's concerns, as discussed during the meeting and per the committee's recommendations. They requested further clarification regarding what the RAC would consider an appropriate in vivo model for the additional preclinical studies (e.g., dog, NHPs).

Dr. Hong noted that there is no established socket model to test the proposed intervention but that the calvarial defect model is an established model for bone regeneration. Studies using this model and the same bovine collagen sponge show that cells can take up the plasmids and express the miRs and inhibitors. Dr. Amendt added that in vivo mouse studies show overexpression of miRs (miR-200c) with no adverse effects. Craniofacial defects are seen with the knockout model, but these animals have no tumors, liver toxicity, or other abnormalities. The advantage of the proposed plan is that subjects will undergo the same type of tooth extraction routinely performed in dental clinics in the United States and will develop a typical socket that forms due to alveolar bone atrophy following such an extraction. If bacteria enter the tooth socket, it will mirror the real-life situations, with potential for infection. Delivery of plasmid DNAs encoding miR200c as planned is expected to modulate the proinflammatory cytokine response at the local level, thereby knocking down inflammation and enhancing bone formation in the socket. The investigators will consider the recommendation to conduct an in vivo animal study to assess the safety and efficacy of the miR using the same delivery method in the specific microenvironment of the tooth socket as planned for the proposed trial.

The investigators explained that one of the main reasons for testing this method is to preserve bone and maintain the height of the maxillary ridge following extraction. This, in turn, will be beneficial for any subsequent dental implant. Without any intervention, the area will heal, but no new bone will form. Further, the bone in the socket will decrease in size, regardless of the age of the patient. Testing to date shows no adverse effects beyond local infection and inflammation even with overexpression of the miRs. Although time to heal may differ somewhat, the research risks should not differ between older and younger volunteers.

The investigators follow team's GMP protocol to purify their DNA. The purification process uses sodium chloride. The purified plasmid is free of proteins and toxins, and the purity of the product is known. The

collagen sponge appears to degrade after about 10 days in the socket, at which point the plasmid absorbs to the sponge, acting as a sort of delivery system. Within another 3-4 days, the cells begin to take up the plasmid. By adding the inhibitor with the RNA polymerase (Pol) III U6 promoter, which is a stable molecule, expression can be controlled to more targeted levels. Dr. Amendt noted that it is not clear why naked DNA works as well as it does in this system, but results are consistent across different cell cultures and in mouse models.

The PI explained that the compensation and discounted rates for the proposed trial are comparable to other similar studies. Subjects typically receive $20-$25 per visit and are reimbursed for parking and gas, as required by the local IRB. Monetary compensation is provided because many subjects travel a relatively long distance to participate onsite at the University of Iowa.

E. Public Comment

No comments from the public were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The following observations and recommendations were made during the RAC's in-depth review and public discussion:

- More preclinical data from an appropriate animal model are warranted, specifically, to assess adequate gene delivery and to ensure safety and efficacy in the context of the specific delivery method in the specific microenvironment of the tooth socket.
- Given that this product is being administered to an otherwise healthy population, this protocol is recommended to be characterized as a Phase I study with a simplified experimental design. The investigators should consult a clinical trial specialist for the trial design.
- Consider reducing the number of arms in this study and employ an adaptive trial design, or consult with a biostatistician, to ensure that you gain relevant information about dose and safety.
- The investigators should assess whether miR200c expression driven by the CMV promoter would be safer and equally effective with a POL III promoter.
- The informed consent document should be modified to:
  - Ensure complex terms and concepts are understandable at a 5th-grade reading level.
  - Clearly articulate unknown risks of the intervention, including the possibility that the investigational agent may worsen typical outcomes.
  - Clarify that participants who develop comorbid conditions or complicating factors (such as pregnancy) should continue to be followed and not excluded from the study.
  - Explicitly state that while a research participant may withdraw from the trial, the administration of the gene transfer product is not reversible.

G. Committee Motion 3

Dr. Kiem summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Kiem requested a vote, and the RAC voted to approve these summarized recommendations with 13 in favor, 0 opposed, 0 abstentions, and 0 recusals.

V. Discussion Regarding the Deliberate Transfer of Chloramphenicol Resistance to *Rickettsia felis*, *R. rickettsii*, and *R. typhi*

Co-Chairs: Drs. Whitley and Wooley

A. Presentation by Dr. Wooley

Dr. Wooley summarized the key aspects of this proposal and request as discussed during the December 2015 RAC meeting. She then presented the charge to the RAC for the current meeting.

The request under consideration, as presented during the December RAC meeting by Olaf Schneewind, M.D., Ph.D., University of Chicago, is to introduce chloramphenicol resistance (Cm^R) into the following *Rickettsia* species:

- ***Rickettsia rickettsii*** (Risk Group 3), which causes Rocky Mountain spotted fever (RMSF), spreads to human from small mammals via ticks and is found in the United States.
- ***Rickettsia typhi*** (Risk Group 3), which causes murine typhus, is spread to humans from rodents, cats, and opossums via fleas and is found in tropical and subtropical areas, including parts of the United States.
- ***Rickettsia felis***, an emerging pathogen that causes spotted fever-like illness, is spread to humans from rodents, cats, and opossums via fleas and ticks, and is found in the United States. The risk group for *R. felis* is unclear.

The proposed research has two main parts. The first is to introduce the selectable marker chloramphenicol acetyltransferase (CAT) into *Rickettsia* species, using a transposon system that has been modified so that it is no longer mobilizable. Although transposons usually insert, excise, and move around in the genome, the system that would be used to introduce CAT into the *Rickettsia spp*. as proposed has been genetically engineered so that it cannot excise itself from the chromosome.

A primary goal of this aspect of the research is to generate a mutant library in *R. rickettsii* and/or *R. typhi* with CAT as the selectable antibiotic marker. Using this library, Dr. Schneewind plans to study the mutants obtained as a result of random transposon-mediated insertional mutagenesis in order to correlate genes with their functions and thus to learn more about *Rickettsia* biology. Chloramphenicol has been chosen over other selectable antibiotics given its reported significantly lower background rate of spontaneous resistance frequency in *Rickettsia* (i.e., typically greater than 10^{-10} to 10^{-11}), compared to other agents such as erythromycin and rifampin, which have background spontaneous resistance frequency rates of about 10^{-6} to 10^{-8}. This difference is particularly important because rickettsial species are obligate intracellular parasites, propagating inside mammalian cells and must be placed under continuous selection to maintain the desired resistance trait which allows one to efficiently select genotypically resistant organisma from the phenotypically false-positive organisms in the background population. Having to screen large numbers of suspected mutants to isolate the the truly genotypically resistant organisms, as would need to be done with markers for antibiotics other than chloramphenicol, would be very labor-intensive and costly.

The second part of the proposed research involves creating a shuttle vector. Generally, shuttle vectors can propagate in more than one species. In this case, the investigators plan to create a shuttle vector by fusing the pET/pUC plasmid from *Escherichia coli* with the CAT antibiotic marker and the pRF plasmid from *R. felis*, which is believed to have the ability to conjugate with other *Rickettsia spp*. Through conjugation, the cells can transfer genetic information can from one organism to another in horizontal fashion. This process raises some concern because if the chloramphenicol resistance carried by a plasmid that has conjugative ability, then the resistance could spread. As pointed out in Dr. Schneewind's proposal, the research therefore goes beyond using Cm^R as a selectable marker to include a novel genetic method whereby shuttle vectors are transferred via *R. felis*-mediated conjugation to other pathogenic *Rickettsia* rendering these resistant to chloramphenicol.

In some cases, the transfer of antibiotic resistance to certain organisms is considered a major action under the *NIH Guidelines*, and the request to carry out such research must be brought to the RAC in accordance with Section III-A-1-a of the *NIH Guidelines*, which states in part: "The deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally […] if such acquisition could compromise the ability to control disease agents in humans, veterinary medicine, or agriculture, will be reviewed by the RAC." In the case of experiments involving *Rickettsia*, the RAC approved a prior request in 2007 to deliberately introduce a gene encoding chloramphenicol resistance into *R. conorii* (the causitve agent of Meditterranean spotted fever). In another major action, also decided in 2007, the RAC disapproved a request to introduce chloramphenicol resistance into *R. typhi* (the causative agent of endemic or murine typhus).

In the previous 2007 RAC decision that allowed the transfer of chloramphenicol resistance into *R. conorii*, a number of requirements were incorporated into the experimental protocol:

- Only the laboratory making the request is permitted to carry out this experiment.
- The experiment must be conducted at BL-3 physical containment level with restricted access.
- Only well-trained personnel essential to the conduct of the experiment are permitted in the containment area. A standard training procedure should be in place for initial and ongoing training.
- The laboratory should be off-limits to anyone with a known allergy or sensitivity to doxycycline or any other tetracycline.
- A back-up power source must be in place to maintain the laboratory's security system, which is controlled by a computer.
- A unique identifier (a genetic "bar-code") should be engineered into the genome of the drug-resistant strain to facilitate the identification of the laboratory-created strains.
- The health surveillance program for laboratory personnel should involve storage of a baseline blood sample, training of all personnel, and development of a detailed standard operation procedure in the case of a laboratory exposure or infection.

Prior to consideration by the full RAC, Dr. Schneewind's request was reviewed by the RAC Biosafety Working Group (BSWG) during a conference call that was attended by members of the BSWG and several subject matter experts, Dr. Stephen Dumler (University of Maryland), Dr. Ted Hackstadt (NIAID/NIH), and Dr. Greg Dasch (Centers for Disease Control and Prevention (CDC)). A separate call to discuss the proposal was held among NIH staff and another subject expert, Professor Didier Raoult (Université d'Aix-Marseille), who was not able to participate in the BSWG teleconference. The RAC reviewed the issues and questions raised during the BSWG and NIH staff teleconferences during the December 2015 RAC meeting.

Dr. Wooley summarized the main BSWG discussion points, as listed under the following three categories:

- *Antibiotic resistance*
  - The therapeutic landscape for antibiotic treatment of Rickettsioses has not changed much since 2007.
  - There is no known naturally occurring Cm^R in *Rickettsia*.
  - Treatment failure data, which may indicate resistance, are crude and uncontrolled. There are many reasons for treatment failures other than antibiotic resistance, such as starting treatment too late in the course of infection, the immune status of the host, and other host factors.
- *Therapeutic Options for Rickettsioses*
  - The first line treatment is doxycycline, a member of the tetracycline family.
  - The second line treatment is chloramphenicol, which has more side effects than doxycycline and is available only as an IV formulation in the United States. In some countries, chloramphenicol is the first line treatment due to its availability and low cost. Additional information is needed on worldwide usage of chloramphenicol (i.e., from the World Health Organization (WHO)).
  - Other agents showing promise in clinical trials and anecdotal reports include ciprofloxacin, a member of the quinolone family, and azithromycin, a member of macrolide family.
- *Public Health Considerations*
  - A treatment option (chloramphenicol) that is known to be effective would potentially be taken away.
  - Chloramphenicol is the first line treatment in some countries and certain situations (e.g., allergy to doxycycline, pregnancy).
  - Mortality can be high in certain settings (e.g. immunocompromised hosts, meningitis).
  - Some researchers believe that public health risk is low because the disease does not spread directly from human-to-human; however, if resistance were introduced into the reservoir population, it could still spread via arthropod vectors (ticks/fleas).

The BSWG was asked to provide individual recommendations regarding the introduction of resistance into *R. rickettsii, R. typhi*, and R. felis; creation of the shuttle plasmid with conjugative ability; whether

work with arthropods should be allowed; and whether the prior RAC approval to allow the transfer of chloramphenicol resistance into *R. conorii* could serve as proof of principle for the similar proposed work in *R. rickettsii* and *R. felis*. None of the BSWG members were in favor of introducing Cm^R into *R. rickettsii* or to allow work in arthropods with any of the Cm^R species. A few BSWG members supported proceeding with some aspects of these experiments, but the majority of opinions were against these issues.

During its December 2015 meeting The RAC considered the issues raised by the BSWG. Regarding the availability of alternate therapeutic options for the treatment of Rickettsioses, the committee noted that few studies of alternative antibiotic treatment for *R. typhi* and *R. rickettsii* infections exist and that no animal model is available for *R. rickettsii* studies. Where comparisons were made between doxycycline, ciprofloxacin, and chloramphenicol for the treatment of murine typhus infection (by *R. typhi*), the study numbers were small (i.e., less than 30 subjects for any antibiotic treatment) and the results were not conclusive. The RAC noted that RMSF is not confined to the Americas but is widespread with outbreaks in Central and South America, and that murine typhus has a world-wide distribution with frequent outbreaks in low-income countries. The RAC also reviewed the WHO Lists of Essential Medicines for rickettsial diseases, which does not include parenteral doxycycline but does include both oral and parenteral chloramphenicol. Despite the known drawbacks related to chloramphenicol, treatment using parenteral chloramphenicol may be the sole option in many countries for severe rickettsial infections and other febrile illnesses that mimic Rickettsioses (e.g., typhoid). In addition, the RAC considered biosafety concerns regarding exposure to *Rickettsia* and the potential risks of introducing Cm^R into *Rickettsia spp*. Being obligate intra-cellular parasites, Rickettsial infections do not occur by human-to-human transmission; arthropod vectors (tick and/or flea) are the primary routes of transmission. Laboratory workers experimenting with tissue culture systems may become infected with *Rickettsia* as a result of a parenteral exposure (e.g. skin abrasion, cut or needle-stick). Such infections would be treated with doxycycline. Some evidence suggests that *R. typhi* may be transmitted by the aerosol route, indicating that use of Cm^R as a selectable marker for in vitro work with *R. typhi* is of concern.

One letter of comment was received in response to the *Federal Register* notice of December 19, 2015, regarding the proposed research. The letter was distributed to RAC members and reiterated many of the same concerns regarding the transfer of Cm^R into *Rickettsia* species raised by the RAC and the BSWG.

Dr. Wooley noted that Dr. Schneewind did not have any updates or additional information regarding the proposal or request since his presentation at the December 2015 RAC meeting. In addition, there is no published evidence of successful transformation of *R. conorii* using Cm^R as a selectable marker.

The charge to the RAC at the March 2016 meeting was to weigh the evidence regarding the use of chloramphenicol resistance as a selectable marker for work with three *Rickettsia* species, and to provide a recommendation to the NIH Director on whether this line of research—which, under the *NIH Guidelines*, is a major action—should go forward.

B. RAC Discussion

Dr. DiGiusto requested further clarification on the charge to the RAC and whether the committee was being asked to decide whether to change the previous ruling from 2007 to allow the proposed research. Drs. Whitley and Wooley commented that nothing has changed since the RAC's discussion at the December 2015 meeting and that no new information has emerged that would change the committee's decision in 2007, that is, to allow transfer chloramphenicol resistance into *R. conorii* but not into *R. typhi*. They both supported keeping the 2007 recommendations as previously approved.

Dr. Jorgenson pointed out that the current request includes two additional species, *R. rickettsii* and *R. felis*, as well as *R. typhi*. The committee is therefore considering the proposal to transfer Cm^R to three species and needs to decide whether to broaden the 2007 action to include the two additional that were not in the prior request. Dr. Jambou explained further that the decision to introduce chloramphenicol resistance or chloramphenicol as a selectable marker into *R. conorii* was discussed in 2007. The RAC's recommendation at that time was to allow that particular experiment to go forward because of the availability of alternate therapeutic options to treat infections with this organism. Specifically, certain

macrolides and fluoroquinolones were proven to be effective against *R. conorii* (the causative agent of Mediterranean spotted fever).

The current charge to the committee was to evaluate the risks of introducing chloramphenicol resistance into *R. Rickettsii*, the agent of RMSF; *R. typhi*, which causes endemic typhus; and *R. felis*, which is recognized as an emerging pathogen that is found in ticks, fleas, and a variety of other arthropods. *R. felis* has worldwide distribution and primarily infects cats; human infection is most likely due to close proximity to pets. Based on this assessment, the committee needed to vote on whether to allow the introduction of Cm^R into these three species of *Rickettsia*. The assessment included revisiting the 2007 decision regarding *R. typhi*, with no new resistance data for this organism presented since that time.

The committee weighed the risks and benefits of creating a new, resistant strain for which one of two treatment options would be obviated versus the public health risk of doing nothing to address the disease. As part of this discussion, Dr. DiGiusto considered whether there has been any change in the perception of the public health risk of rickettsial illness, given that the science has not recently changed. He noted CDC data showing a marked increase in the number of reported cases between 1920 and 2010. In contrast, fatality rates, at least in the United States, have not followed this trend. The criteria to justify a recommendation to proceed, given the potential public health risks, need further consideration (e.g., type of drugs available, type or resistance).

Although doxycycline is the first-line treatment for Rickettsial diseases in the United States, in some countries and in certain situations (e.g., pregnancy, in patients with severe hypersensitivity to tetracyclines), chloramphenicol is the only option to treat rickettsial diseases. In these cases, the diseases may not be as treatable as in the United States, especially if these organisms were to get into the environment. The WHO Lists of Essential Medicines for rickettsial diseases include both oral and parenteral chloramphenicol but not parenteral doxycycline. Dr. Whitley noted that macrolides and fluoroquinolones show promise as potential treatments for rickettsial diseases; however, there currently are no definitive data as to their efficacy for this indication.

The WHO has argued against going forward with the proposed experiments because chloramphenicol may be the only antibiotic in certain countries for treating Rickettsioses. By introducing Cm^R into these species, an important and effective treatment would no longer be available for many people around the world. Dr. Zoloth pointed to the serious ethical problem with the disproportionate burden that would be placed on people who cannot access or otherwise use doxycycline if chloramphenicol were eliminated as an option to treat rickettsial diseases.

Dr. DiGiusto also inquired about provisions in place to contain and prevent the escape of the mutant organisms from the lab. As discussed at the December 2015 meeting, Dr. Schneewind has not previously worked with *Rickettsia*, but he has an established track record with other bacterial species, including plague bacteria. The proposed experiments will be conducted and contained in the biosafety level 3 (BSL-3) facility located within Argonne National Laboratory outside Chicago. The facility is designed to meet or exceed requirements outlined by the CDC, NIH, and Occupational Safety and Health Administration specifications for biocontainment and is also certified by CDC as part of the Select Agent Program. All staff involved in this research will be required to undergo specialized training, and a comprehensive emergency response plan in case of occupational exposure is in place and was delineated at the RAC meeting. Even with these facilities and multiple safeguards in place, there is still a concern regarding risk to public health when an investigator or lab is new to working with these agents.

Dr. Wooley commented that the proposed experiments, specifically, the creation of the library and the study of the mutant strains, are not directly translatable to disease prevention or treatment. This research may or may not yield generalizable knowledge. Whether that is worth the risk of a chloramphenicol-resistant stain of *Rickettsia* getting into the environment is a key question. One suggestion made at the December 2015 meeting was to re-direct research efforts toward a better biological selection system instead of introducing chloramphenicol resistance into the rickettsial species as proposed.

Dr. DiGiusto suggested outlining a path forward to define the criteria under which this type of work could be done so that the public interest is served. Studies demonstrating clear alternatives to chloramphenicol, for example, could change the current landscape and facilitate this effort. Dr. Kiem noted that a working group might be best suited to take on this type of task.

C. Public Comment

No comments from the public were offered.

D. Synopsis of RAC Discussion and RAC Observations and Recommendations

The RAC serves as an advisory group to the NIH Director. As such, the committee's recommendations and deliberations will be transmitted to the NIH Director, who will make a final determination of whether the proposed experiments can proceed. The Director's decision will be presented to the RAC for confirmation.

Dr. Jorgenson clarified that the RAC needed to have a separate vote on whether chloramphenicol resistance can be introduced into each of the three species under consideration in the proposed request: *R. typhi*, *R. rickettsii*, and *R. felis*. The following motions and votes reflect the recommendations based on RAC's in-depth review and public discussion.

E. Committee Motion 4

Dr. Kiem requested a vote on the motion to deny the request to introduce chloramphenicol resistance into *R. typhi*. The RAC approved the motion unanimously by show of hands (11 in favor, 0 opposed, 0 abstentions and 1 recusal [Dr. Ross]).

F. Committee Motion 5

Dr. Kiem requested a vote on the motion to deny the request to introduce chloramphenicol resistance into *R. rickettsii*. The RAC approved the motion unanimously by show of hands (11 in favor, 0 opposed, 0 abstentions and 1 recusal [Dr. Ross]).

G. Committee Motion 6

Dr. Kiem requested a vote on the motion to deny the request to introduce chloramphenicol resistance into *R. felis*. The RAC approved the motion unanimously by show of hands (11 in favor, 0 opposed, 0 abstentions and 1 recusal [Dr. Ross]).

VI. Gene Transfer Safety Assessment Board Report

RAC Reviewers: Drs. Atkins, Curry, Donahue, Kaufman, Kiem, Lee, Pilewski, and Whitley

A. GTSAB Report

Dr. Kiem opened the session by presenting the charges to the GTSAB:

- Review in closed session, as appropriate, safety information from gene transfer trials for the purpose of assessing toxicity and safety data across gene transfer trials.
- Identify significant trends or significant single events.
- Report significant findings and aggregated trend data to the RAC and thereby disseminate it to the scientific and patient communities and to the general public.

The current GTSAB roster includes eight RAC members and two FDA representatives.

Dr. Kiem then presented the GTSAB report for the first quarter of 2016. Within the past 3 months, NIH/OSP received a total of 28 protocol submissions, 23 of which were not selected for public review at this RAC meeting. Of the five protocols selected for in-depth public review, one was deferred to the June 2016 RAC meeting. Of the 23 protocols not selected for public review, 18 were oncology protocols, 3 were monogenic disease protocols, 1 was an elimination graft-versus-host disease (GVHD) protocol, and 1 was an osteoarthritis protocol.

Among these 23 protocols, 6 used adenoviruses, 4 used AAVs, 3 used retroviruses, 3 used herpes simplex virus, 2 used vaccinia virus/fowlpox, 1 used plasmid, 1 used vaccinia virus, 1 used poliovirus, 1 used measles, and 1 used lentivirus.

For the first quarter of 2016, the GTSAB reviewed initial and follow-up reports on 21 SAEs from 17 protocols. (Information about these trials will be made available on the OSP website after this RAC meeting and in the future will be available in the NIH Genetic Modification Clinical Research Information System, also known as GeMCRIS.)

Dr. Kiem provided an update from the last GTSAB meeting regarding an event involving listeriosis in a Phase II trial (Protocol 1082) evaluating ADX11-001, an attenuated *Listeria monocytogenes (Lm)* vaccine strain for the treatment of persistent or recurring squamous or non-squamous cell carcinoma of the cervix. An SAE suggested a possible delayed listeriosis and bacteremia more than two years after the last dosing of a subject with the attenuated *Lm* vaccine strain. Protocol 1082 and other protocols using this *Lm* vaccine strain were placed on hold while the company investigated the case. The Principal Investigator (PI) and sponsor concluded that progressive disease was the cause of the death for this SAE. The delayed *Lm* infection was thought to have resulted from biofilm formation in the prosthesis and bone grafts that this particular individual received after surgery, following the initial dose and before additional doses of the attenuated *Lm* vaccine strain. The protocol is being revised to exclude participants with prosthetic devices and implants that are not easily removed and will implement antibiotic regiments to prevent persistent infections. Due to the specific circumstances of this event, the GTSAB members did not recommend that the protocol revisions be generalized to the other attenuated *Lm* strains at this point.

Another protocol using engineered T-cell receptor (TCR) immunotherapy against synovial sarcoma identified potential risk factors for prolonged pancytopenia, including age (>50 years), treatment with fludarabine (Flu), and extensive prior myelosuppressive therapy. The proposed protocol modifications include allowing the removal of preconditioning for high-risk subjects (e.g., older patients), enrollment of high-risk subjects on Flu-based regimens only after discussion with the medical monitor, and addition of pancytopenia to the development core safety information and informed consent form as an AE associated with the study product.

Most of the other SAEs are from CAR and TCR studies, as described below. Dr. Kiem noted that similar events, including signs and symptoms of cytokine release syndrome (CRS), were seen across different protocols:

- **CD19 CAR for B-cell malignancies** (Protocol 1213): Severe CRS with fever and hypotension complicated by hypoxia, neurotoxicity, severe cerebral edema, leading to brain herniation and death. *Protocol modification*: Cell dose de-escalated to dose level 1 for future subjects with acute lymphoblastic leukemia (ALL) regardless of bone marrow (BM) blast counts, and a CRS management algorithm was added (Lee et al., 2014).
- **HLA-A2 restricted NY-ESO-1 for sarcoma** (Protocol 1071): Severe CRS plus tremors, complicated by respiratory failure, strial fibrillation, renal insufficiency, sepsis, and prolonged pancytopenia leading to death.
- **MAGE-A3 TCR-gene engineered lymphocytes and interleukin-2 (IL-2)** (Protocol 1253): CRS-like reaction with hypotension, hypoxia, shock liver, renal failure, atrial fibrillation, pleural effusions, mental status changes complicated by arrhythmia-related hypotension, respiratory failure, and sepsis. The patient recovered without tocilizumab or corticosteroids.
- **Anti-BCMA CAR targeting multiple myeloma** (Protocol 1303): Two severe CRS cases—one with fever, hypoxia, hypotension, and muscle weakness; one with fever, hypotension, and

neurotoxicity. Both patients had elevated creatine phosphokinase (CPK) and were complicated by prolonged pancytopenia. There was not evidence of myeloma on last BM analyses in either case.

- **Phase II CD19 CAR trial against B-ALL** (Protocol 1351): Four SAEs were reported for this protocol, including three cases of severe CRS. One CRS case was thought to be related to rapid progressive leukemia leading to death about ten days after the CD19 CAR T cell infusion). Additional details regarding each CRS case are as follows:
    - One patient with severe CRS also developed fever, hypotension, and respiratory distress, followed by neurotoxicity. Labs showed pancytopenia, coagulopathy, and elevated transaminases, CRP, and ferritin levels. Both CRS and encephalopathy resolved.
    - In another patient, CRS was complicated by renal failure, respiratory failure, pancytopenia, coagulopathy, and eventually intracranial hemorrhage leading to death.
    - In the third case of severe CRS, the patient also had neurotoxicity and respiratory failure complicated by sepsis in the setting of adrenal insufficiency and multi-organ failure.

For the first quarter of 2016, OBA received notification that 13 new protocols opened, two of which were publicly reviewed:

- **Protocol 1401-1287:** Phase I Study of Cellular Immunotherapy Using T Cells Lentivirally Transduced to Express a CD123-Specific, Hinge-Optimized, CD28-Costimulatory Chimeric Antigen Receptor and a Truncated EGFR for Patients with Relapsed or Refractory CD123+ Acute Myeloid Leukemia
- **Protocol 1307-1240:** A Phase I, Open-Label Study to Assess the Safety, Feasibility, and Engraftment of Zinc Finger Nucleases CCR5 Modified Autologous CD34+ Hematopoietic Stem/Progenitor Cells (Sb-728mr-Hspc) with Escalating Doses of Busulfan in HIV-1 (R5)–Infected Subjects with Suboptimal CD4 Levels on Antiretroviral Therapy

Results of several OBA protocols have been published recently, as summarized below:

- **Allogeneic T Cells That Express an Anti-CD19 Chimeric Antigen Receptor Induce Remissions of B-Cell Malignancies That Progress After Allogeneic Hematopoietic Stem-Cell Transplantation Without Causing Graft-Versus-Host Disease:**
    - Eight of 20 treated subjects achieved remission. The response rate was highest for patients with ALL. None of the subjects developed GVHD after CAR T-cell infusion. Peak blood CAR T-cell levels were higher in subjects who had remission than those who did not. The presence of B cells before CAR T cell infusion was associated with higher post-infusion CAR T cell levels.
    - Conclusion: Allogeneic anti-CD19 CAR T cells can effectively treat B-cell malignancies that progress after alloHSCT (Brudno et al., *Journal of Clinical Oncology*, 2016)

- **Ibrutinib Enhances Chimeric Antigen Receptor T-cell Engraftment and Efficacy in Chronic Lymphocytic Leukemia (CLL):**
    - T cells in CLL have proliferative defects that reduce *ex vivo* expansion. Five or more cycles of ibrutinib therapy appear to reverse this defect and improved expansion and engraftment of CD19-directed CAR T cells. Ibrutinib does not impair CAR T-cell function in vitro and improves CAR T cell engraftment, tumor clearance, and survival in human xenograft models of resistant ALL and CLL when used concurrently.
    - Conclusions: Ibrutinib enhances CAR T-cell function, and clinical trials with combination therapy are warranted. Clinical trials with ibrutinib lead-in and subsequent continued treatment could enhance the efficacy and engraftment of adoptively-transferred T cells, including CAR T cells (Fraietta et al., *Blood*, prepublished online, January 26, 2016).

- **Convergence of Acquired Mutations and Alternative Splicing of CD19 Enables Resistance to CART-19 Immunotherapy:**
    - Relapses with epitope loss occur in 10-20 percent of pediatric responders. Hemizygous deletions of *CD19* and mutations in exon 2 of *CD19* were noted in the relapse cases; alternatively spliced *CD19* mRNA was also found. Exon 2 skipping bypasses exon 2

mutations in B-ALL cells and allows expression of the N-terminally truncated CD19 variant, which fails to trigger killing by CART-19 but partly rescues defects associated with CD19 loss.
  - An unexpected finding in this trial was a rare case of relapse due to a CD19-negative ALL that appeared to express CAR19 scFv. Investigation of this case revealed that contaminating B-ALL cells, which can persist at very low frequencies in the apheresis product, were transduced by the lentiviral vector and were the source of the relapsed leukemia. According to the investigators, this is a very rare event in the manufacturing process that occurred in only one case among more than 90 subjects infused with the same IND product.
  - Conclusion: This mechanism suggests a possibility of targeting alternative CD19 ectodomains, which could improve survival of patients with B-cell neoplasms (Sotillo et al., *Cancer Discov* 5(12):1282-95, 2015*).*

- **First Clinical Application of TALEN Engineered Universal CAR19 T Cells in B-ALL:**
  - A novel strategy, TALENs, was used to enable "off-the-shelf"' therapy with mismatched donor CAR19 T cells. Use of TALENs was able to overcome HLA barriers by preventing GVHD and rejection via disruption of the T-cell receptor and CD52 (the target for alemtuzumab).
  - A single pediatric subject with-high risk CD19+ ALL received this experimental therapy, which was the first-in-human application of TALEN engineered cells that provides early proof-of- concept evidence for a ready-made T cell strategy that will now be tested in early phase clinical trials (Presented at the ASH Meeting, Gene Therapy and Transfer, December 5, 2015, Orlando, Florida).

B. RAC Discussion

No comments from RAC members were offered.

C. Public Comment

No public comments were offered.

VII. Day 1 Adjournment

Dr. Kiem adjourned Day 1 of the March 2016 RAC meeting at 3:15 p.m. on March 8.

VIII. Day 2 Opening

Dr. Kiem opened Day 2 of the March 2016 RAC meeting at 9:00 a.m. on March 9.

IX. Review and Discussion of Human Gene Transfer Protocol #1601-1492: A Phase I Dose Escalation Study Evaluating Safety and Feasibility of BPX-701 in Patients with Relapsed or Refractory Myeloid Neoplasms

Presenters:	Rachel Cook, M.D., Oregon Health and Science University (OHSU) Richard Maziarz, M.D., OHSU Aaron Foster, Ph.D., Bellicum Pharmaceuticals, Inc.
Sponsor:	Bellicum Pharmaceuticals, Inc.
RAC Reviewers:	Dr. Atkins, Dr. Wooley, and Ms. Hardison

Dr. Kiem was recused from consideration of this protocol due to a conflict of interest; Dr. Atkins chaired this section of the March 2016 RAC meeting.

A. Protocol Summary

Patients with relapsed or refractory acute myeloid leukemia (AML) or other myelodysplastic syndromes (MDS) have a poor prognosis, characterized by low survival rates and few proven viable treatment options. Overall survival of primary refractory AML is reported to be 7 percent at 5 years for patients under age 60 and 4 percent at 2 years in patients over the age of 60. One retrospective analysis study of 594 patients with AML undergoing second salvage therapy with standard therapies, including stem cell transplant, reported a median survival of 1.5 months, with a 1-year survival of 8 percent. Currently, the universal goal for patients with refractory disease is to pursue allogeneic hematopoietic stem cell transplant (HSCT), if they are able to withstand the rigors of the procedure. These poor outcomes for this population demonstrate the need to identify interventions that can bring the patients' disease under control so that they can qualify for potentially curative transplant.

PRAME (preferentially expressed antigen in melanoma) is overexpressed in melanoma, AML, and other cancers and at a level much higher than that detected in normal tissues. PRAME is also expressed at high levels in the testes; at intermediate levels in the ovaries, endometrium, and adrenal glands; and at low levels in several other normal tissues. A mechanism that can target PRAME antigens expressed on cells of patients with myeloid neoplasms might be effective in controlling the disease. Bellicum Pharmaceuticals, in collaboration with Leiden University Medical Center, has developed a TCR-based investigational agent, BPX-701, which targets the PRAME antigen and includes a "safety switch" (CaspaCIDe) to remove the gene-modified T cells in the event of uncontrollable T-cell toxicity. The TCR expressed in the BPX-701 T-cell product recognizes and binds to a PRAME peptide bound to HLA-A2 on a cancer cell surface, resulting in apoptosis in the tumor cell.

The proposed trial is a Phase I/IB, open-label, non-randomized feasibility, safety, and dose-finding study of BPX-701 in adults with refractory AML or MDS. An *ex vivo* strategy is proposed in which a murine retrovirus is used to engineer a participant's own T cells with a TCR that can recognize PRAME. The engineered cells will be infused into the participant, where they will target the specific cancer cells. The design consists of five dose cohorts of BPX-701. Each cohort will enroll three to six participants, who will receive BPX-701 following a 3 + 3 dose escalation/de-escalation schema. The Phase I stage of the protocol will be designed to identify the maximum tolerated dose (MTD). During the Phase Ib expansion stage, participants will receive highest tolerated dose of BPX-701. Salvage/lymphodepleting chemotherapy (fludarabine plus cyclophosphamide) may be given up to 3 days prior to T-cell reinfusion if necessary and as tolerated to control rapidly growing disease. The study will enroll up to 36 participants (18 per stage). Participants will be monitored and managed for side effects after dosing. Uncontrolled toxicity will trigger the use of the dimerizer drug, rimiducid, which activates the "CaspaCIDe" suicide switch to eliminate the BPX-701 PRAME-reactive T cells. Long-term follow-up will continue after the active participation phases. An aim of this pilot is to generate hypothesis for further drug development.

B. Written Reviews by RAC Members

The trial was found to warrant public review because of the potential risk for on-target, off-tissue toxicity and the use of a suicide switch in the setting of TCR-based immunotherapy.

Three RAC members provided written reviews of this proposed Phase I/Ib protocol.

Both Dr. Atkins and Dr. Wooley expressed concerns regarding off-tumor/on-target effects. Dr. Wooley identified this as her overall concern and main question about the proposed study, calling particular attention to potential effects in the testes, where PRAME is expressed at high levels. She noted that off-tumor/on-target effects in the testis could lead to delayed puberty in young male participants and that germline effects must be considered, even though the probability is low, because the experimental agent targets an antigen found on testes.

Dr. Atkins found the study to be well-designed but had the following additional questions:

- Is there evidence of T-cell persistence in the absence of IL-2 in this patient population?
- What specifically will trigger a decision to administer rimiducid?
- Is there evidence of rimiducid efficacy in humans in the autologous gene-modified T-cell therapy setting?
- What is the effect of rimiducid on anti-tumor effects?
- What will happen if participants develop symptomatic disease deterioration after leukapheresis but before therapy can be administered? Will such patients be replaced? Will the protocol need to be modified to take such cases into account?
- Explain the thinking about allowing intervening “dealer’s choice” lymphodepleting chemotherapy after leukapheresis and whether this will introduce toxicity biases in the small subset of participants.

Dr. Wooley had the following additional specific questions and comments:

- The description of how the virus is prepared (in Appendix M) is confusing. A flowchart or diagram of this process would be helpful. The description of how the two different producer lines are used is unclear. The write-up switches back and forth between 293VEC, RD114, and GALV cell lines/cultures. Does the final, stably transfected cell clone contain the vector or the helper constructs? If it contains the vector, has it been completely sequenced? If it contains the helpers, how can the integrity of the vector sequence be ensured, considering that the process of transfection can be mutagenic?
- The replication competent retrovirus (RCR) testing (as described in Appendix M) described only a 3-day process. The investigators should consider whether performing a 21-day amplification process, similar to that described by Sastry et al. (*Molecular Therapy*, 8(5), 830-839, 2003) would be more sensitive.
- Regarding the integrity of the transduced DNA, additional detail is needed as to whether analyzing cells expanded from a single clone be representative of the entire transduced population.
- The investigators state in Appendix M that "The lack of IFN response with T cells treated with rimiducid is consistent with the dimerizing drug activation of the iCasp9 suicide switch triggering apoptosis in the iCasp9 containing T cells." Dr. Wooley asked whether there are any other assays to more directly test for apoptosis.
- Per the information provided, the suicide switch is not 100 percent, and about 7 percent of the transduced cells were still present in mice after flipping on the suicide switch. This is a concern since off- tumor effects could not be evaluated *in vivo*. Further detail regarding this issue is needed.
- Regarding DNA entering other cell types or germ line cells, Dr. Wooley asked about the type of washing procedure that is done after the T cells are infected with vector virus *ex vivo* to make sure there are no extracellular viral particles in the mixture, and whether the washes been tested for retroviral particles or nucleic acids.
- Is there a cutoff amount for the proportion of cells receiving the transgene (i.e., a minimum fraction successfully transduced to move forward)?
- The language regarding potential benefit of participation states that the “hoped for” benefits are persistence and expansion of the cells and controlling tumor. Because this is a Phase I study, the anticipated benefits should be safety of the agent so that future efficacy studies can be performed. This should be clarified across all documents.

Dr. Wooley offered the following comments on the ICD:

- The document states that a study drug (rimiducid) will be given by vein to eliminate the modified T cells. Since the T cells cannot be completely eliminated, this should be reworded to say, "…to reduce the number of modified T cells."
- Should a barrier method of birth control be recommended?
- If sterility is a potential risk, it should be mentioned in the consent document.

- The statements regarding potential benefit should be revised to read, "There will likely be no benefit to you from being in this study .However, by serving as a participant, you may help us learn how to benefit patients in the future."

Ms. Hardison's comments focused primarily on the ICD. The ICD contains a lot of information and needs some editing to describe the study procedures more clearly. Ms. Hardison offered the following comments to address this concern:

- In the section titled "During the Study," Ms. Hardison provided language to define and describe the purpose of the suicide gene (a "self-destruct switch") so that participants have a better understanding of this complex concept.
- In the section titled "Discussion of Tests and Procedures," the investigators need to clearly differentiate tests associated with regular cancer treatment versus tests and procedures performed solely for research purposes.
- In the section titled "When you are finished taking the cellular therapy," a statement regarding the administration of the study drug rimiducid should be added. Ms. Hardison noted that this information appears late in the ICD, but she recommended keeping the order in which tests and procedures are described as chronological as possible to add clarity to the process.
- In the section titled "How long will I be in the study?", the tests that will be performed using the blood samples collected need to be specified, and the document should explain what is meant by "checkups." Ms. Hardison suggested adding the following sentence in the paragraph describing follow-up for possible side effects: "We will also request to monitor your health condition long term by calling you on the phone to discuss your health." In addition, she added the underlined text to the following statement: "The investigator may withdraw you from this study <u>before you have completed all of the steps</u> if your cancer does not improve ..."
- In the section on risks associated with gene transfer, Ms. Hardison provided language to explain the process of and potential risks with integration, including possible cancer risk based on results of other preclinical and clinical gene transfer studies. This section should more clearly delineate between side effects and risks.

C. RAC Discussion

During the meeting, the following additional questions, concerns, or issues were raised by RAC members:

- While the patients who will enroll in this study are facing a grave situation, appropriate and adequate provisions need to be in place to protect their safety so that their quality of life is maintained to the extent possible, particularly for those in the terminal stages of disease.
- Dr. Wooley noted that she and the other reviewers had the same primary concern, that is, the potential for on-target, off-tumor effects, which is the main reason this protocol was selected for in-depth public discussion. The investigators presented a detailed response to this concern. They have looked at levels of expression in normal cells and determined that in most tissues, the expression level is low, below 1 percent. For some tissues, however, the level is either at or greater than the 100-copy threshold. Dr. Atkins asked how (or whether) this threshold relates to any *in vivo* toxicity models and whether this cut-off is relevant to the proposed research. In addition, he asked whether data can beyond the PRAME experience could be cited in support of use of this expression assay for assessing on-target, off-tumor effects, or if another assay to detect *in vitro* expression of an antigen might be more relevant for this study.
- Although there may not be a good animal or disease model for AML, it is not clear why toxicity testing in mice or another animal has not been done.
- Dr. Wooley requested further clarification regarding preparation of the BPX-701 retrovirus and subsequent manufacture of the BPX-701 T cells. In the schema provided, the process appears to include two rounds of transduction, but the rationale for the additional step is not clear. She noted that the process usually involves a cell clone that has the vector integrated. The clone is then sequenced to ensure the integrity of the integrated virus (vector), and the helpers are introduced to a single batch for production purposes. In the current case, it appears that virus particles that could reinfect the cells and potentially cause recombination are being produced. If this is the

case, integrity of the vector cannot be ensured. Whether superinfection could occur during the production process, with the viral particles produced re-infecting the cell during the timeframe for the entire process, needs to be clarified.

- Dr. Wooley also inquired about the status of testing for the investigational product and whether the tests performed on cells expanded from a single clone are representative of the final product or if additional or multiple clones need to be analyzed. Additional information was requested about the assays for apoptosis with regard to the suicide system.
- The consent document has been revised to reword the statements regarding anticipated benefit to say there will likely be no benefit from being in this study but that by serving as a participant, subjects may help investigators learn how to benefit patients in the future.
- Many participants will already be sterile because of prior treatments, and banking of sperm and eggs will be offered for those who are not infertile. As noted in the response to the reviewers, there is a small but real risk of severe adverse effects to the male reproductive system that includes the potential need to remove the testicles. This possible outcome and related psychological impact should be added to the consent as possible risks of the study intervention.
- Dr. Atkins noted that while most of the reviewers' concerns regarding off-tumor, on-target toxicities in the patients have been addressed, he did not see references to monitoring for possible effects to the retina. PRAME is expressed in the retina, but it is not clear if that expression is in the retinal cells or melanocytes. Even though the eye is supposed to be an immune-protected site, with some immune therapies, inflammation in the eye is seen (e.g., as uveitis). In addition, skin rash can be a problem with a lot of the treatments for melanoma, which presumably involve reaction against melanocytes in the skin. Given that PRAME is expressed in skin cells, particularly in melanocytes, it may be useful to pay attention to those sites.
- In other studies in which there is a risk of cytokine release syndrome (CRS), subjects without any significant heart or lung disease have sometimes succumbed to CRS. Since testing of BPX-701 is still in the early stages, the potential risks are not well known. Given these factors, the investigators should consider having more stringent eligibility criteria, especially related to cardiac, lung, and renal function and disease, as an added patient safeguard.
- IL-2 is administered in conjuction with many T-cell therapies to mitigate potential adverse effects from the cytokine response (e.g., CRS). The investigators do not think IL-2 is necessary for the proposed trial, even though the study participants will have a high tumor burden and expansion of T cells will occur. Dr. Atkins requested further clarification as to the rationale for not planning to administer IL-2 and whether the goal is to allow for T cell persistence or not. Although stem cell transplant might be an option, it was noted that the participants who will be eligible for the proposed trial will generally not be transplant candidates. Additional details of the plan to monitor participants for potential toxicities in these off-tumor on-target sites should be provided.
- Dr. Atkins requested additional information on the factors that would trigger a decision to administer rimiducid, which will shut off the study agent, and how easily such a determination can be made.
- The protocol states that any particpant who withdraws prior to day 180 post-BPX-701 infusion for any reasons will be replaced. Participants who deteriorate during the period of time between the leukapheresis and when the product is ready to be given also will be replaced. Whether the day 180 time point includes an additional administration of the study drug is not clear. In addition, the protocol should specify whether any data from participants who withdraw prematurely from the trial will be used in the study analyses.
- Dr. Atkins asked which lymphodepleting chemotherapy will be used in the proposed trial and what would happen if the chemotherapy is found to be working and the pre-treatment biopsy shows only a few or no blasts in the bone marrow.
- Dr. Zoloth raised concerns regarding the invasive nature of some of the procedures, such as bone marrow biopsies, and the large amount of blood drawn for leukapheresis, in these vulnerable patients, some of whom may have an expected survival of only 2 months. In addition, she noted that despite some potentially very serious risks associated with participation (e.g., blindness, loss of testicles), it appears that patients would have to sue for the cost of any research-related injuries.

- Dr. Zoloth requested clarification as to whether participants will be treated as inpatients or in an outpatient setting. The presentation indicated that participation would involve a hospital stay, but the consent document says that participants will be asked to visit the investigator's office after they receive the cellular therapy.
- Drs. Wooley and Zoloth noted the possible risk of sterility and inquired about banking of both sperm and eggs prior to receiving the study agent. This risk and any banking options available to participants should be clearly described in the consent form.
- Ms. Hardison was satisfied that all of the pertinent information is included in the ICD and that her recommendations to simplify, clarify, and organize some of the language to improve readability and comprehension were addressed.
- As proposed, the dose escalation scheme provides for reassessment at each level to determine whether to add more participants at that dose, go to the next higher dose, or go back to the next lower dose. Dr. Donahue suggested building in an iterative analysis into the study design should the on-target, off-tumor effects not reach the pre-set threshold or signal (e.g., a minor rash vs. a severe rash), which is likely at the lower doses, and to revise the dose de-escalation plan if one participant has a severe reaction to determine if that adverse effect is a dose-limiting toxicity (DLT).
- Dr. DiGiusto requested additional information regarding the algorithm for deciding when to treat CRS, specifically when to eliminate the modified T cells versus when to continue to manage symptoms. Dr. Lee noted that elderly patients in CAR T-cell studies tend to have more serious responses than younger patients, with respect to CRS and CAR T-cell related marrow suppression and pancytopenia-related aplastic anemia. Given these and other risks and concerns identified in the protocol and through the RAC review, the investigators should consider having clearly defined parameters for when to intervene for responses related to the stusdy agent. Leaving these decisions to individual clinicians will likely result in different strategies, based on their varied experiences. Dr. Lee also asked whether rimiducid will be available onsite and ready to use as needed, and if not, how long it would take to have the drug available for use.
- Dr. Lee requested additional information about the TCR and how it has been modified, specifically, whether this is a human TCR that was modified for greater affinity to the HLA-A2 PRAME complex. He also asked what has been done to assure that the affinity modification has not increased affinity to similar peptides from other proteins.
- Dr. Cho commented that the consent wording appears to suggest that an individual subject would participate in both "active" phases of the study, the dose-escalation and expansion stages. This needs to be reworded to better explain the study design in terms of the separate dose cohorts and when "expansion" (additional participants) might be needed. The consent should clarify that each participant will receive only one dose of the investigational product.
- The section of the consent that describes risks of rimiducid needs to be revised to state that the participants' condition could worsen because of immunosuppressive factors and their underlying disease. The example involving GVHD is confusing and should be clarified.

D. Investigator Response

1. Written Responses to RAC Reviews

The investigators provided a detailed response to questions and concerns regarding potential off-tumor/on-target effects of BPX-701. Preclinical *in vitro* and *in vivo* data indicate that that PRAME expression is much lower in normal cells than in tumor cells, by several orders of magnitude in most cases. Expression of PRAME in normal tissues has been investigated using reverse transcription polymerase chain reaction (RT-PCR) by a number of research groups. In one report (Ikeda, 1997), the level of PRAME messenger RNA (mRNA) was generally less than 1 percent of that in LB33 melanoma cells in a variety of normal tissues, including skin, muscle, heart, liver, colon, stomach, lung, breast, bladder, and prostate; brain, kidney, adrenals, and ovary contained less than 10 percent of the level in LB33 melanoma cells; and endometrium contained about 50 percent. Testis was found to contain over twice the level of PRAME mRNA compared to LB33 melanoma cells.

Results of another study (Amir et al., 2011) demonstrated that expression of PRAME (as determined by quantitative RT-PCR) in target cells correlated with recognition of the cells by the PRAME-specific T-cell clone HSS1, the source of the TCR used in BPX-701. This naturally occurring TCR recognizes a specific peptide (termed SLL) presented on the surface of the target cell by HLA-A2. Target cells with high or intermediate PRAME expression were effectively or intermediately recognized by the T cells, respectively, while targets that did not express PRAME or expressed PRAME at a very low level were not recognized. When the HSS1 clone was tested for recognition of various HLA-A2+ nonmalignant cell types, it showed no reactivity against numerous normal cells, including fibroblasts and keratinocytes with or without pre-treatment with interferon gamma, primary bronchus epithelial cells, hepatocytes, intrahepatic biliary epithelial cells, colon epithelial cells, or B cells.

Recently, PRAME copy number in human normal and tumor tissues was determined by colleagues in Leiden. Using an *in vitro* killing assay, they demonstrated that 100 copies of PRAME were required for BPX-701 cell-killing activity. Except for a limited number of tissues, including testis and epididymis, normal cells have PRAME copy numbers below the 100-copy threshold level.

The investigators acknowledge that although expression of PRAME in normal tissues is low compared to that in tumor tissues or germ cells, the chance of on-target/off-tumor toxicity cannot be completely ruled out, especially because of the lack of *in vivo* data due to the unavailability of appropriate animal models. Participants receiving BPX-701 TCR T-cell therapy will be closely monitored for evidence of toxicity; if toxicity cannot be controlled by standard means, rimiducid will be administered to activate the iCasp9 suicide system, which eliminates the BPX-701 T cells by triggering apoptosis.

The iCasp9 system developed by the study sponsor, Bellicum Pharmaceuticals, has been shown to eliminate BPX-701 T cells in preclinical *in vitro* studies as well as *in vivo* studies in immunodeficient NGS (next-generation sequencing) mice. The iCasp9 system has been incorporated into other Bellicum investigational drug products, including BPX-501 (gene-modified T cells), and has been approved for use in multiple clinical trials (NIH Protocols 1204-1158, 1407-1325, and 1504-1422).

Thus, although the investigators cannot say with certainty that the off-tumor toxicity will not be a problem, the overall risk in this population with high-risk AML and no treatment options is mitigated by the following factors:

- Low overall expression of PRAME in most normal tissues,
- Presence of suicide gene to eliminate T cells,
- Clinical tracking of signs and symptoms related to potentially affected tissues (kidney and testes) using blood tests and ultrasound, and
- High likelihood of sterility in males due to prior chemotherapy for underlying disease with prior sperm banking.

Based on the *in vitro* data above and experience in treating inflammation and CRS, the investigator will monitor participants in the study for signs of toxicity, especially in the organs most at risk for being recognized by the PRAME-specific TCR in BPX-701: testis, dendritic cells, and kidney. Testis is the normal tissue with the highest level of PRAME expression but is considered to be an immunological “sanctuary site” and is therefore not expected to be exposed to high levels of BPX-701.

As delineated in the protocol, participants will be monitored throughout the study for changes in hematology and chemistry, especially alterations in cellular and lymphocytic profiles and changes in chemistry that may indicate decreased renal or hepatic function. Participants will also have regular physical examinations, allowing for close monitoring of signs and symptoms of renal or pelvic problems, followed by noninvasive ultrasound evaluation as necessary. In addition, levels of C-reactive protein (CRP) and cytokines will be tracked as a measure of inflammation.

In participants with refractory AML who have undergone chemotherapy, the standard of care is to offer sperm or egg banking prior to initiating treatment, as the chemotherapy will likely result in sterility or abnormal sperm. It would be difficult to assess the damage to the sperm or eggs from PRAME targeting versus the effects of chemotherapy, but any sign of inflammation will be tracked, followed, and treated as

necessary. There is a small possibility that the testes would be affected by BPX-701. If a participant becomes symptomatic, he will be treated with standard-of-care; in extreme cases (e.g., severe inflammation of the testes), orchidectomy may be required. Male study participants will be given the opportunity to bank sperm samples prior to BPX-701 treatment. Infertility is not expected to be a frequent occurrence, and although it is a serious outcome, it is a less severe situation than the current refractory myeloid disease.

In the event that a participant's condition deteriorates after leukapheresis but before BPX-701 infusion occurs, the participant will be discontinued and replaced. The protocol has been revised to include the specific criteria for determining when a subject will be replaced.

The investigators clarified that the proposed study has only one clinical site in the United States and that the PI is willing to use the same lymphodepleting chemotherapy for all study patients, instead of allowing use of different lymphodepleting chemotherapy agents following leukapheresis. The protocol has been updated accordingly.

The investigators provided a detailed graphic and an accompanying description of the process for preparation of the BPX-701 retrovirus. The clone that produced the highest titer of BPX-701 retrovirus was expanded, banked, and used for retrovirus production after testing for sterility and mycoplasma. The final, stably-transduced cell clone contains the vector, which has been completely sequenced. No helper constructs are present.

RCR testing includes a 3-day culture prior to sampling the cell culture media for testing. This particular assay is conducted on the packaging cell line 293VEC RD114 before transduction by the BPX-701 retroviral vector to confirm the lack of RCR in this banked packaging line. The 3-day culture step is standard for an assay at this stage of virus production. RCR testing during later stages of virus production is performed using more standard sensitive culture assays. Testing of the vector production master cell bank, the supernatant from the cell bank, the retroviral supernatant product utilizes the 21-day amplification process. Testing of the final BPX-701 T cell product is conducted using a qualified PCR assay for RCR-specific sequences.

Assessment of the integrity of the transduced DNA in the BPX-701 T cells is ongoing. Genomic DNA isolated from the total population of BPX-701 cells manufactured using the clinical process will be analyzed by PCR amplification of multiple overlapping regions of the integrated provirus. The presence of the expected size PCR products will demonstrate sequence integrity and a lack of rearrangements. Flow cytometry for Vβ1 will be performed on the final BPX-701 T-cell product to determine the proportion of the cells that contain the transgene, a measure of transduction efficiency. The minimum target transduction efficiency is 20 percent. In the event that the minimum is not reached for a given manufactured product, the product will be evaluated by the clinician in order to determine whether to proceed with infusion.

The investigators confirmed that apoptosis will be assessed as part of the release characterization of each lot of BPX-701 manufactured. A flow cytometry-based assay to detect changes in the cell membrane by measuring binding to a protein (Annexin-V) is used to confirm the induction of apoptosis *in vitro* during development of BPX-701. This assay measures the ability of the BPX-701 cells to respond to the dimerizer drug, that is, how well cell apoptosis is initiated and cell death occurs with addition of the AP1903 dimerizing agent. The team has developed another assay that measures a key downstream target of caspase 9 (i.e., the 'effector' caspase 3), which, once activated, drives irreversible apoptosis; in this assay, measurement of caspase 3 activation is directly proportional to the cells undergoing apoptosis/death.

The following responses were provided to questions about T-cell persistence and the use of rimiducid to eliminate the BPX-701 T cells in the event of uncontrolled toxicity.

TCR gene-modified T cells proliferate in response to the tumor cells *in vivo*. No exogenous IL-2 is given to participants. The investigators have no direct evidence for T-cell persistence in this population. However, these participants will have a high tumor burden, so T-cell levels are expected to be high.

Rimiducid will be administered in the event of uncontrolled toxicity associated with off-tumor/on-target side effects in normal tissues or CRS. The decision to administer rimiducid will be made based on the risks and benefits of the toxicity being treated and the disease response of the participant. It is expected that participants will lose the anti-tumor effects once rimiducid has been administered.

Per the protocol, participants will be monitored throughout the study for changes in hematology and chemistry, especially alterations in cellular and lymphocytic profiles and changes that may indicate decreased renal or hepatic function. For male subjects, serial hormone testing (testosterone, luteinizing hormone [LH], follicle-stimulating hormone [FSH], and inhibin B) and clinical exams will be used to monitor potential testicular toxicity. As specified in the protocol, the investigator may initially treat toxicity due to CRS with tocilizumab and corticosteroids. If the toxicity does not resolve, rimiducid will be administered.

Five autologous gene-modified CAR T-cell protocols that incorporate the *iCasp9* suicide gene have been registered and submitted to the FDA. The investigators are not aware of use of rimiducid in any of these protocols to date. A Phase I trial (NIH Protocol #0704-849) of allogeneic T cells expressing the *iCasp9* suicide gene in patients undergoing T-cell–depleted haploidentical transplantation demonstrated the efficacy of rimiducid to eliminate the gene modified cells; this study has been completed, and results have been published. In this protocol, a single dose of rimiducid, given to four participants in whom GVHD developed, eliminated more than 90 percent of the iCasp9-modified T cells within 30 minutes after administration; the GVHD ended without recurrence (up to 1 year). No immediate or delayed adverse events associated with rimiducid were noted. Similar results were reported (Zhou, 2015) for a second study performed at the same clinical site (NIH Protocol #1102-1092).

The administration of rimiducid will activate the iCasp9 suicide system, thereby eliminating BPX-701 T cells and thus the potential for tumor control. The number of PRAME-specific T cells remaining is expected to be very small, but the precise number is uncertain. Whether there would be a sufficient number of BPX-701 T cells after rimiducid treatment to provide a clinically functional level of anti-tumor activity is unknown. The proposed trial includes analysis of tumor burden and regular collection of blood samples to assay for BPX-701 T cells, potentially addressing the question in the situation of rimiducid dimerization of the BPX-701 T cells.

The investigators point out that because off-tumor/on-target side effects cannot be evaluated in preclinical animal models, it is not possible to determine whether the small number of BPX-701 T cells likely to remain after rimiducid administration would express the TCR/iCasp9 construct and would be sufficient to cause toxicity. Therefore, participants will be closely monitored for signs of toxicity, especially in the target tissues, such as the testis, dendritic cells, and kidney following rimiducid treatment. An anti-T cell reagent such as ATG (antithymocyte globulin) could be used to remove all T cells. Persistence of BPX-701 T cells in peripheral blood, and any other cellular specimens available, will be monitored by qPCR and or flow cytometry assay throughout the study. In prior clinical studies, apoptosis of T cells containing the iCasp9 retroviral gene construct (after induction by the rimiducid dimerizer molecule) was correlated with cells that express high levels of the iCasp9 transgene. In these studies, the clinical symptoms of GVHD caused by the *iCasp9* gene-modified T cells completely resolved upon administration of rimiducid. The remaining cells expanded over time, but they continued to lack high expression and were not associated with any clinical symptoms.

The informed consent document has been revised to address the comments of the reviewers. The schedule for follow-up study visits is now clearly stated, and the reason for collection of blood samples is specified. The term “check-up” has been replaced with “physical exam.” Barrier methods of birth control for both men and women have been added to the document, and the risks of BPX-701 treatment to men are described. Male participants will be given the opportunity to bank their sperm prior to administration of the investigational product.

2. Responses to RAC Discussion Questions

Animal models do exist in which HLA-A2 malignancy in tumors can be eradicated, for example, in immune-deficient mice. However, while some animals are transgenic for HLA-A2, they generally do not express human PRAME in any tissues. The strains that are engineered with that HLA type, in turn, could not be further engineered to express the PRAME antigen. These animals therefore are not good models for testing toxicology in the tissues of interest for this research. This is the case for other gene therapies where chimeric molecules are used or transplanted into T cells. Some models have been developed for different types of antigens where there is clear cross-reactivity. However, getting the appropriate antigen expression for a human protein in the tissue of interest is very challenging from a gene expression perspective. Dr. Foster noted that the study team, in collaboration with their colleagues at Leiden University Medical Center, has tried to address this problem by looking at expression in normal tissue. This is done by taking the TCR transgenic product, BPX-701, as a probe against human tissue that is HLA positive; this assay, in turn, revealed different levels of expression, as reported. In addition, by using this approach, the investigators found that in tissues with fewer than 100 copies, there is very little recognition and toxicity. Based on these findings, the threshold cut-off for copy number was set at 100. Gene expression patterns in normal tissues are being used to develop a toxicology profile for the investigational product.

The investigators recognize that some toxicity occurs with different TCRs and that labs use different technologies to determine whether antigen suppression is sufficient to cause such toxicities. The OHSU study team uses a PCR-based approach, which probably is not as sensitive as assays that use mass spectroscopy. The team's experiments show a correlation between copy number of PRAME in the analyzed tissues and cytotoxicity of the BPX-701 product towards those cells. The investigators are not aware of other studies that have looked at a greater level of resolution to determine whether or not a peptide copy number or the amount of protein is related to particular toxicities.

Regarding production of the virus and modified T cells, Dr. Foster explained that in making producer cell lines for this virus, the investigators prefer to use the RD114 envelope, which has worked well in their transfection protocols. To do that, the retroviral backbone is introduced into the 293VEC RD114 producer cell lines, which can be done in a number of different ways. The team typically has pseudotyped a virus with a GALV vector and then transduced the RD-114 producer cell lines. This is the preferred approach because using an envelope that is similar to the one for the virus usually results in poor viral titers. The additional step in the production of BPX-701 retrovirus therefore increases efficiency and yield. The resulting clones of the producer cell lines (i.e., packing cells) have the retroviral vector and backbone and thus are able to produce virus. The viral particles, however, do not include those components and therefore are not replication competent. Both cell lines are GMP grade.

While the transient method is most commonly used for lentiviral production, it is increasingly being used for retroviral production for GMP trials. Packaging cell lines are used to create a producer cell line, and the viral supernatant is then tested for replication of the recombinant virus (e.g., using plaque assays) to make sure a replication-competent virus is not being generated. Dr. Foster noted that the current study team and other groups have found the integrity and functionality of the provirus in transduced cells to be intact, using deep sequencing. Major mutations and similar problems have not been identified. The process and assays used for the BPX-701 retrovirus and proposed trial are validated technologies that are used in the majority of gammaretroviral gene therapy studies for TCRs and CARs. A producer cell line produces a large amount of virus in bulk fashion that can then undergo quality assurance to confirm integrity and functionality of the viral particles.

The TCR is a natural TCR from a patient who had chronic myelogenous leukemia (CML). It is a high-affinity TCR that is HLA-A2 positive and was isolated through reactivity assays. The affinity for this TCR is about 1 nanomolar, which is in the range of what is seen for the safety profiles of most TCRs. Through the tissue expression screening, the investigators determined that there is no cross-reactivity between this TCR and other proteins. They have not yet run more complex *in vitro* toxicity assays using, for example, IPS cells. Dr. Foster noted that the PRAME peptide is not conserved in the A2 mouse, thereby eliminating that animal as a possible model for this research.

The investigators are analyzing clones for the IND filing. They have created multiple clones and then tested those clones for viral titer production. The different clones are banked, and one that performs well in the validation studies is then selected for further characterization through sequencing. Analysis for the integrity of the DNA in different clones has not yet been done.

Several different assays are available to test for apoptosis, both directly and indirectly. Interferon gamma response (reduction) in the tumor killing assay is done with PRAME-expressing cell lines. One flow cytometry-based assay detects changes in the cell membrane and is used to confirm the induction of apoptosis in vitro during development of BPX-701. Another intracellular assay measures a key downstream target of caspase 9, the 'effector' caspase 3, which, once activated, drives irreversible apoptosis; in this assay, which also uses flow cytometry, measurement of caspase 3 activation is directly proportional to the cells undergoing apoptosis/death. The caspase 3 assay is under development. In the initial clinical study of BPX-501, which uses iCasp9 for T-cell add back. The investigators have used flow cytometric analysis to assess apoptosis. The newer assay may be a better test in that it can directly measure a single molecule involved in the cascade for apoptosis, that is, caspase 3. Prior clinical use of rimiducid in transplantation trials for the mitigation of GVHD suggests a strong safety profile for the agent. Preclinical *in vivo* data demonstrate tumor killing and the functionality of the CaspaCIDe suicide switch in removing BPX-701 T cells that might give rise to off-tumor/on-target toxicities. Extensive monitoring is done for these effects, and other anti-T cell drugs could be employed if necessary.

Dr. Cook agreed that the inclusion of an ophthalmologic exam as part of a formalized assessment (e.g., as part of the standard physical exam) is a good idea and will add it to the protocol. The investigators will consult an OHSU expert in the field to provide guidance on the monitoring and management of ophthalmologic conditions and will consider adding this expertise to the protocol. The study already includes routine examination of the skin.

Dr. Cook explained that the aim of the proposed regimen is to have homeostatic T cell expansion to address the residual blasts that are present with long-term persistence but that are not well characterized. Ideally, there will be relatively extensive killing of tumor cells earlier in the process but persistence of some cancer cells also needs to be taken into consideration. IL-2 is not needed because there is no evidence of expanded T cell persistence with this investigational agent. Dr. Maziarz added that if needed, stem cell transplantation, which is standard of care, could be done to eradicate the transduced cells and for disease control. The PI noted that in a Phase I trial such as the proposed protocol, perfect disease control is not expected and that taking participants to transplant because of disease persistence is usually not done as part of the study. Patients will be monitored for up to 15 years, so they will be followed for an extended period of time if they are a long-term survivor.

The decision to use rimiducid is based on the risk:benefit assessment of the toxicity being treated and the disease status of the participant. Subjects with relatively mild symptoms whose leukemia is in remission would probably not derive benefit from rimiducid. In contrast, rimiducid would be given to a participant with grade 4 toxicities and serious complications (e.g., severe CRS). Dr. Cook noted that such decisions are not easy and are made on a case-by-case basis based on clinical judgment and experience in conjunction with results of monitoring for changes in hematology and chemistry, especially alterations in cellular and lymphocytic profiles and decreased renal or hepatic function. Per the protocol, toxicity due to CRS may initially be treated with tocilizumab and corticosteroids; if the toxicity does not resolve, rimiducid is administered. The investigators anticipate that participants will lose the anti-tumor effects of BPX-701 once rimiducid has been administered. Dr. Maziarz added that particpant safety comes first and noted, as Dr. Cook pointed out, that determining how to proceed needs to balance between dodging the disease itself and the toxicity of therapy. Tools that can decrease the risk of toxicity are available, and a process for a consensus management decision that includes the participant will be used. The consent document describes the potential toxicities that can occur and that a decision might need to be made at some point as to whether the study agent gets shut off or not. The PI confirmed that rimiducid will be readily available at the onsite pharmacy for use as needed.

Dr. Cook clarified that the day 180 post-BPX-701 infusion is a time point for reassessment only and does not include an additional administration of the modified cells. Subjects who are withdrawn by the investigators (in accordance with the protocol) or who voluntarily leave the study prior to this time point will be replaced. Subjects who die or go on to transplant will not be considered “withdrawn,” and their data will be used in the study analyses. Subjects who will be replaced or not counted will include those who did not receive the product because their disease is not controlled. Dr. Cook noted that some of these details need to be clarified.

All subjects needing lymphodepleting chemotherapy will receive the same regimen of fludarabine, cytarabine, and granulocyte colony-stimulation factor (GCSF) (FLAG), which is standard of care for this population. Salvage/ lymphodepleting chemotherapeutic agents may be given up to 3 days prior to T-cell infusion if necessary to control rapidly growing disease. If there are any contraindications (e.g., a previous reaction to cytarabine), the options would need to be reconsidered for that specific subject. If the pre-treatment bone marrow biopsy shows few or no blasts, the investigators would still proceed with the T-cell infusion given the poor prognosis of these patients unless another treatment option were available (i.e., transplant from a matched donor). Results of the biopsy are typically available within a day, so a decision regarding any alternative therapy could be made quickly. Patients with relapsed refractory myeloid malignancy will be identified for a pre-treatment regimen based on the kinetics of the subject’s leukemic cells. Part of the rationale for originally allowing different types of lymphodepleting chemotherapies was to take into account malignancies that have a much higher kinetic growth rate and need a more aggressive regimen than malignancies with lower rates. To avoid introduction of toxicity biases in the small numbers of subjects that would be in each subgroup, the investigators agreed to proceed with a single lymphodepleting chemotherapy regimen. Dr. Maziarz noted that the chosen regimen is effective against myeloid malignancies and will reduce any residual lymphocyte populations in the host that, in turn, will give the best chance for efficacy of the investigational product.

The investigators recognize the risks and discomfort associated with invasive procedures such as bone marrow biopsies. The PI explained, however, that bone marrow biopsy is the only way to confirm the patient’s disease status and that serial blood and bone marrow sampling is needed to determine tumor response to treatment and to monitor for any toxicity. These procedures are only done for subjects on active treatment and would not be done if a patient were receiving supportive care.

To date, no subjects in the team’s trials have had to cover any out-of-pocket expenses. The investigators will revise the consent language to reflect more patient-friendly provisions for coverage of medical expenses for research-related injuries and harm.

Both male and female participants will be offered sperm or egg banking prior to T-cell infusion. The ICD mentions sperm banking but needs to include information on egg storage. The investigators will add information about the potential risk of severe damage to the testicles and the circumstances surrounding the rare possibility that the testicles might need to be removed.

Whether participation occurs in the inpatient or outpatient setting will depend on the status of and potential risks to the subject. Dr. Cook noted that for most subjects, it will be a combination of both settings. For the proposed trial, the infusion will be done in the hospital; a subject who shows good progress after the infusion may be able to continue as an outpatient.

The investigators appreciated the feedback on the dose escalation and de-escalation plans and will incorporate the suggested changes to the protocol.

Tociluzimab and corticosteroids will be given initially to treat grade 3 or higher CRS. Dr. Maziarz noted that both disease bulk and time after dosing impact the likelihood of the development of CRS and the severity of the response. Patients with very high white blood cell counts can have evidence of CRS within 24 hours of infusion, while in those with more indolent disease, signs of CRS may not develop for 2 to 3 weeks.

The team is working on translating established algorithms for CRS management based on experience in other protocols. The investigators are working with collaborators in the ICU to develop criteria for implementation of therapy to shut down the CRS response before patients develop refractory hypertension or requiring intubation.

The investigators agreed that the exclusion criteria should be more restrictive to reduce risk of CRS and cardiac, lung, and kidney complications.

The ICD will be revised to better explain the study design and to clarify that each subject will receive only one dose of BPX-701. The section on risks of rimiducid will be revised to state that participants' condition may worsen.

E. Public Comment

Dr. Borror noted that the ICD refers to the study intervention as "cellular therapy," which implies or is likely to be interpreted by participants to mean a proven treatment. The investigators should refer instead to the name of the agent or call it an "experimental therapy" or "intervention."

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The following observations and recommendations were made during the RAC's in-depth review and public discussion:

- Because the final producer clones contain both the viral vector and helper constructs, monitoring of the integrity of the transduced DNA is needed to assess for possible rearrangements and mutations that may occur during superinfection in the production process. Also, the proposed plan to analyze the participants' cells for the integrity of the transduced DNA needs to be implemented.
- Use the caspase 3 assay on the T cells when the assay is developed, as it is likely to yield a readout of apoptosis that is more directly proportional to the administration of the suicide drug.
- Clarify the procedure for replacing participants withdrawing from the study. Ensure inclusion of all participants receiving product in data analyses.
- Develop a more flexible toxicity management plan including a more specific dose de-escalation scheme as a response to untoward effects.
- Consider adding ophthalmological expertise to the trial, and in particular examine for potential changes to the retina given the high level of PRAME expressed in this tissue.
- Develop a more formal process for determining when to administer rimiducid.
- Implement stricter exclusion criteria to ensure that any observed toxicities are not due to potential comorbidities, specifically heart and lung conditions.
- Revise the informed consent document to:
    - Explicitly state the range of potential negative outcomes, even those considered to be extremely rare (e.g., removal of testicles), including the possibility that administration of rimiducid may worsen their outcomes.
    - Offer fertility preservation options for both sexes (e.g., banking services) when appropriate.
    - Address cost coverage for negligence.
    - Clarify that although there are two stages to this trial, each individual will participate in only one stage and therefore will receive only one dose.
    - Ensure that "treatment" is not used to describe BPX-701, as it is still an experimental therapy at this point.

G. Committee Motion 7

Dr. Atkins summarized the RAC recommendations to be included in the letter to the investigators.
Dr. Atkins requested a vote, and the RAC approved these summarized recommendations by a vote of 11 in favor, 0 opposed, 0 abstentions, and 1 recusal (Dr. Kiem).

X. Review and Discussion of Human Gene Transfer Protocol #1601-1493: A Feasibility and Safety Study of PSCA-Specific Chimeric Antigen Receptor–Engineered T Cells (BPX-601) in Subjects with Non-Resectable Pancreatic Cancer

Presenter:	Carlos Becerra, M.D., University of Texas Southwestern Medical Center, Dallas
Sponsor:	Bellicum Pharmaceuticals, Inc.
RAC Reviewers:	Drs. Atkins, Kaufman, and Zoloth

Dr. Kiem was recused from consideration of this protocol due to a conflict of interest. As a result of Dr. Kiem's recusal, Dr. Atkins chaired this section of the March 2016 RAC meeting.

A. Protocol Summary

Pancreatic adenocarcinoma is the fourth most common cause of cancer-related death in the United States. It is estimated that 49,000 people will be diagnosed with pancreatic adenocarcinoma and approximately 41,000 people will die of the disease in 2015. The majority (85–90 percent) of cases of pancreatic cancer are advanced at the time of diagnosis, and by the time symptoms are present, pancreatic tumors are typically unresectable with no curative options.

A high percentage of pancreatic cancer cells overexpress the prostate stem cell antigen (PSCA), which has low basal expression in normal tissues. Recognizing the lack of curative treatment options for patients with advanced pancreatic cancer, Bellicum has developed an investigational agent, BPX-601, that uses PSCA as a target for gene therapy. BPX-601 is a first generation PSCA-specific CAR containing iMC, a molecular switch activated by rimiducid (AP1903) to enhance T-cell persistence. The iMC switch with rimiducid has been used clinically in Bellicum's dendritic cell vaccine, BPX-201. CAR T-cell immunotherapy in general has been limited by the lack of T cell persistence and thus the limited duration of the activity of the modified T cells. The dimerization of the iMC by rimiducid provides a controlled activation and proliferation signal, thereby improving the persistence of the CAR T cells and potentially their prolonged activity against solid tumors.

The proposed study is a Phase I single-center, open-label, non-randomized dose-finding trial. The aims of the protocol are to determine the feasibility and safety of the administration of the anti-PSCA CAR T cells (BPX-601) in subjects with non-resectable pancreatic adenocarcinoma, the safety of the activation of the CAR T cells by rimiducid, and the evaluation of the persistence of the CAR T cells over time after a single rimiducid infusion. The MTD design consists of five cohorts with three to six subjects per cohort who will be treated following a 3 + 3 dose escalation/de-escalation schema. The five planned doses of BPX-601 are 0.3×10^6 cells/kg, 0.625×10^6 cells/kg, 1.25×10^6 cells/kg, 2.5×10^6 cells/kg, and 5×10^6 cells/kg. Subjects will receive one dose of BPX-601 on Day 0 followed by one dose of 0.4 mg/kg of rimiducid on Day 14. The initial three subjects will be enrolled sequentially, with a period of observation (14 days) post-BPX-601 administration to assess safety at each dose level prior to administration of rimiducid. Subjects will be evaluated for dose-limiting toxicities (DLTs) after each of the BPX-601 and rimiducid infusions. The study will enroll up to 30 adults with histologically confirmed adenocarcinoma of the pancreas with non-resectable disease.

B. Written Reviews by RAC Members

Eleven RAC members voted for in-depth review and public discussion of this protocol. The protocol was found to warrant public review because it is the first trial to employ a novel stimulatory domain (iMC) and signaling strategy to improve the persistence of first-generation CAR T cells to prolong the activity of the engineered T cells.

Three RAC members provided written reviews of this proposed Phase I trial. Dr. Atkins read Dr. Kaufman's comments into the record during the meeting.

The reviewers considered the proposed study to be well-designed and appropriate for a patient population with a very poor prognosis and no effective treatment options.

Dr. Atkins noted that the planned intervention seems cautious, and the study has already been approved by the local Institutional Review Board (IRB) and Institutional Biosafety Committee. Nonetheless, it tests a novel approach for potentially maintaining the persistence and activity of CAR T cells and thus poses some theoretical risks. Furthermore, given the patient population and the delay in rimiducid treatment, the study may not yield useful information. Dr. Atkins posed the following questions regarding the protocol:

- What is the evidence that BPX-601 will persist for 14 days in patients with pancreatic cancer in the absence of IL-2 infusion?
- What will be done if insufficient CAR T cells are present at Day 14 when rimiducid is administered? Has consideration been given to an earlier administration?
- Why is above-grade-5 hematologic toxicity, rather than above-grade-4 toxicity, considered a DLT both for BPX-601 and after rimiducid? What grade reflects bone marrow failure?
- What if patients don't get lymphodepletion? What is the plan to address this, and will reducing the dose of T cells make a difference in these cases?
- What will happen if patients develop symptomatic disease deterioration after leukapheresis and lymphodepletion but before therapy can be administered? Will they be replaced? Will the protocol need to be modified?
- How long does the effect of rimiducid last? What if the modified cells persist after rimiducid administration, and toxicity develops? What is the plan to treat any toxicity that develops?
- Explain the thinking about allowing intervening "dealer's choice" lymphodepleting chemotherapy after leukapheresis and whether this will introduce toxicity biases in the small subset of patients.

Dr. Kaufman commented that proposal is based on intriguing preliminary data and builds on the company's experience with retroviral-transduced dendritic cells. He found the clinical protocol to be well written with an acceptable and well-thought-out clinical trial design. Dr. Kaufman had the following comments and questions:

- What was the rationale for waiting 14 days to give the rimiducid? This may be a long time period in patients with advanced pancreatic cancer.
- The investigators mention the potential for CRS, and studies of CD-19 CAR T cells have suggested the potential for neurotoxicity. These potential adverse events are not mentioned in the ICD. This information should be added to the document.
- It is not clear how T cells will be depleted. The investigators may want to consider defining the chemotherapy regimen prior to treatment and then try to standardize this regimen across subjects.
- Is there a plan for control of cytokine release or overactive T cells? Are the modified cells sensitive to corticosteroid administration?

Dr. Zoloth identified two types of ethical issues raised by the project. The first is related to the project design, in which very ill and frail patients are required to have two interventions, BPX-601 with CAR T cells and rimiducid, both of which carry significant risks. Given this design, patients must stay close to the site or leave and return in two weeks. The second ethical issue arises in the consent form. The consent has many strong parts, but the overall tone of the consent form, which has been carefully constructed by the team, is unduly optimistic.

The proposed study is a Phase I clinical trial. No benefit is to be promised, yet the consent form talks about "good and bad" effects. Dr. Zoloth suggested stating explicitly that participants "should not expect to get better in this kind of study" to avoid any misperception that the study could provide a "chance for a cure." In addition, the consent describes a 15-year follow-up period in a study that will enroll a group of extremely vulnerable patients and their attendant families. If that number is used, it gives the clear

impression that these subject can expect to live that long. The same impression is given by the section on pregnancy. This suggests a future, tied to the entrance into this clinical trial. Dr. Zoloth recommended not using the 15-year number for the follow-up phase and modifying the language regarding pregnancy to more realistically reflect the status of this patient population.

Dr. Zoloth suggested modifying some of the language in the form. For example, the word “doctor” should be replaced by “scientist” or “researcher” so that “treatment” is not emphasized as an aim of the trial. The fact that the cancer is overwhelmingly fatal also needs to be directly addressed. The value of the patients’ volunteering is their altruism for the sake of future patients. A clear description of how the process of de-escalation will work needs to be incorporated into the document. In addition, the consent should clarify how long the cells persist and why gene therapy trials are different than drug trials. The warning about central nervous system (CNS) risks needs to be clarified.

Dr. Zoloth was very concerned that a for-profit company using dying patients to test their drug is not paying for treatment-related injuries or costs of care. This protocol goes a further step and states in the consent that the cost of “charges for tests and examinations done as part of a research study may or may not be covered.” Dr. Zoloth commented that the authors of the consent document surely know that insurance companies are not going to pay for Phase I tests or examinations. She did not see any justification for requiring patients to cover these costs and stated that the ethical course seems clear: The company needs to cover the costs of the trial, not shift it onto patients. In addition, the company needs to bear the responsibility for harms or injuries caused by their interventions.

C. RAC Discussion

During the meeting, the following additional questions, concerns, or issues were raised by RAC members:

- The reviewers found the presentation to be clear and their concerns and questions to be well addressed. They went through their comments and the investigators’ responses to their queries and suggestions. Dr. Atkins reviewed Dr. Kaufman’s questions and the investigators’ responses.
- Drs. Atkins and Kaufman inquired about the rationale for timing of administration of rimiducid, to enhance T cell persistence and function. Dr. Cho asked whether 25 days is sufficient to assess T cell persistence in the setting ofusing the activator and the desired goal for treatment, that is, to have an anti-tumor effect and to control tumor growth. The investigators plan to wait 14 days before giving rimiducid, which appears to be a long period of time for patients with advanced pancreatic cancer. The reviewers asked about the evidence showing that the CAR T cells will persist for 14 days. Furthermore, they asked what will be done if not enough T cells persist through day 14 in order to take advantage of the added rimiducid (activator). There are no clinical data on the persistence of the modified CAR T cells, but preclinical models suggest that the BPX-601 cells will persist for at least 28 days in the absence of rimiducid. Data shown during the presentation suggest that BPX-601 cells persist in the presence of tumor antigen without rimiducid and that the cells have a negative effect on tumor growth when rimiducid is administered.
- The investigators plan to discuss with the FDA the option of modifying the protocol as it is currently written in order to be able to administer rimiducid earlier on day 7 after T cell infusion to a subgroup (cohort) of patients in the study if no safety issues are observed. Dr. Atkins noted that this plan is premature given the absence of clinical data to assess the toxicity of the cells and whether a sufficient number of cells are still present at certain time points after infusion of BPX-601.
- A DLT in the protocol was changed from grade 5 to grade 4 toxicity, but it is not clear how the investigators will be able to distinguish between grade 4 adverse events from the lymphodepletion vs. grade 4 hematologic toxicity from the BPX-601 cells or rimiducid. If this distinction cannot be made, the investigators may need to reconsider how the DLT will be defined in terms of the adverse effects of the modified T cells.
- The plan to control CRS and overreactive T cells involves administration of methylprednisolone and tocilizumab. Corticosteroids are not always effective in managing CRS in all patients and can be weaned over a relatively short period of time (several days).

- Neurotoxicities may also result from exposure to CAR T cells; some adverse neurologic effects of CD19 CAR T cells may be due to the expression of CD19 antigen in the brain. The consent document should be revised to explain the potential risk of neurotoxicities as a consequence of CAR T cell infusion and how neurologic symptoms will be managed.
- Dr. Zoloth agreed with the reviewers' comments regarding the frailty of this patient population and the question of whether there is direct benefit with participation. The prognosis for metastatic pancreatic cancer is very poor. Most patients survive less than 2 years, and there currently are no good options to treat the disease. The proposed trial involves giving two experimental products to this vulnerable population. Prior to receiving these products, patients undergo leukapheresis and also may need lymphodepleting chemotherapy after receiving standard care.
- Dr. Zoloth discussed two main ethical issues raised by this project. The first involves the 14-day lapse between administration of the two study agents. Investigators are asking very ill and frail patients not only to be given two different interventions, but to also wait 14 days between the administration of the two products. The way the trial is designed will therefore require participants to remain close to the study site. Provisions to accommodate patients during the study need to be clearly delineated, including whether the subjects will remain in the inpatient setting and whether lodging will be provided in between hospitalizations. Additional details of the monitoring plan during this time are also needed, especially given that some of the patients may be very sick.
- The second ethical issue involves the tone and language of the ICD; specifically, the document is unduly optimistic regarding the experimental intervention and the subjects' medical status. The language also conveys a sense that participants are serving as "guinea pigs" for the proposed testing. Dr. Zoloth appreciated that the study participants will be treated with compassion and care but pointed out that they are research subjects, not patients, and that the intervention could make them significantly worse and potentially contribute to their death. A statement that metastatic pancreatic cancer is not operable and is always fatal should be made near the start of the ICD. As a Phase I trial, this study offers no direct clinical benefit. The patients who volunteer for this protocol have a terminal illness and are altruistic in agreeing to participate in this research. This study adds risks beyond standard of care for metastatic pancreatic cancer, but the consent does not present a clear picture of the serious nature of some of those risks, including neurological adverse effects (e.g., nerve and brain damage) and potentially death. These risks need to be explained to participants and delineated in the ICD. The risks associated with leukapheresis also need to be clearly stated in the consent document. The consent document should state that there are no benefits to patients enrolled in this Phase I study but by volunteering, participants may help medical science and other patients in the future.
- Dr. Zoloth noted further that describing the 15-year follow-up in patients whose life expectancy is 2 years is inappropriate and gives a sense of false hope. The investigators should consider replacing this timeframe with wording such as "as long as you live with your cancer." Pregnancy and breastfeeding are exclusionary for this trial due to the unknown risks to the developing fetus and nursing infant. However, references to patients who are pregnant (and therefore not eligible to participate) or who might become pregnant after enrolling in this study can similarly give individuals false hope. Pregnancies in this population are the exception, and discussion of the potential for pregnancy should be tempered. Participants who are still capable of becoming pregnant or fathering a child are required to use effective forms of birth control or abstinence for 3 months after BPX-601 administration; pregnancy testing is done at screening and over the course of the study for women who are able to become pregnant.
- Dr. Zoloth was very concerned that a for-profit company using dying patients to test their drug is not paying for treatment-related injuries or costs of care. This protocol goes a further step and states that the cost of charges for tests and examinations done as part of a research study may or may not be covered. Dr. Zoloth reiterated her comment in the written review that the study team and sponsor are undoubtedly aware that insurance companies do not cover the cost of Phase I trials, as well as the examinations, or injuries that may be involved, and pointed out that many specifically exclude experimental treatment. She did not see any justification for requiring patients to cover these costs and stated that the sponsor should cover the costs of the trial. In addition, the company sponsor needs to bear the responsibility for harms caused by their interventions. Whatever final provisions are in place, participants need to know what will happen if they become injured or ill while taking part in this study, including any costs they will be

required to cover themselves, without reimbursement through the study or from their insurance carrier.

- Dr. Zoloth clarified some of the terminology used in the consent. She noted that the proposed trial is a test, not a research trial and explained that in a test, scientists try out an idea for a drug to see if it is safe to use on people. If it is shown to be safe in initial testing (e.g., Phase I study), then scientists will try to see if it is effective (e.g., Phase II study). The investigators need to be careful to not overstate the purpose of the test and to convey through the language in the consent the altruistic decision of the patients who are volunteering for this study to advance the science. The consent should make clear that the study drugs are not intended to extend the patient's life but that the agents are being tested for safety and for possible further testing in other patients in the future. Use of the term "effective" is confusing and is not consistent with the aim of this Phase I study. Dr. Zoloth also suggested that the terms scientist and investigators should be used instead of doctor, except when referring to the patient's doctor, who has nothing to do with this study. In addition, patients need to be reassured that if they choose to leave the study and no longer be followed that they will still receive care from their own doctor. The language regarding potential risks of genetic testing could be stronger and clarify that it is not possible to absolutely guarantee that any tests for DNA remain anonymous.
- Dr. Zoloth suggested language to more clearly explain T cells and their function; the specialized T cells that will be tested, BPX-601; and the drug rimiducid in the consent document. She also provided a description of the catheter used for leukapheresis and how blood will be collected that could be added to the ICD.
- Dr. Cho identified several additional issues that need to addressed or clarified in the consent document, including identifying the risks of lymphodepletion, listing death as a potential outcome of CRS, and describing what a dose-escalation design is and that the severity of side effects may increase as the dose increases. In addition, the consent should point out that the reported side effects of rimiducid were seen in healthy volunteers but that patients with pancreatic cancer may or may not have the same responses since the agent has not been tested yet in this population.
- The suggested revisions and clarifications for the consent are to assure that potential subjects, and those who agree to enroll in this trial, have a clear and realistic understanding of what the study participation entails, particularly the risks and responsibilities (including any medical costs) of participation, and the provisions in place to mitigate those risks.
- Dr. Ross noted that discomfort to the patient also needs to be taken into account in addition to more serious risks. Dying individuals may not want to go through the added pain of research procedures at the end of life, whether it involves an extra 10 blood draws after treatments have weakened their veins or undergoing an experimental intervention knowing that it is not yet being tested for efficacy and is likely to "fail". The investigators therefore need to think carefully about how information is presented.
- Effective treatments for numerous diseases including pancreatic cancer are needed, and clinical trials play a critical role in identifying such treatments. As Dr. Zoloth and other RAC members pointed out, however, most interventions do not proceed beyond the early stages of clinical research. Moving from a Phase I to a Phase II trial often does not occur. Scientists learn from failures as well as from successes, but in incremental steps. It is important to keep this perspective and the overall body of evidence and experience when reviewing protocols and consents.
- Dr. Atkins pointed out that the RAC has had extensive discussions about the issues raised by Dr. Zoloth. While there is not universal agreement on all of the points made regarding the current protocol and consent, the suggested language is relevant to this trial and should be considered by the investigators.
- Dr. DiGiusto agreed that subjects need to be as fully informed as possible and that the consent should convey that the investigators cannot promise any benefit in this trial. However, he also was concerned about presenting an inappropriately or intentionally pessimistic or optimistic message. He noted, for example, that the vast majority of individuals with pancreatic cancer will die from their disease, but the fatality rate for this patient population is not 100 percent. In another trial cited by Dr. Becerra, survival was extended in a small number of treated patients with metastatic pancreatic cancer; thus, saying in the consent document for the proposed study that

all individuals with this diagnosis will die within a few months or a year is not completely accurate. Several other RAC members noted the low rate of survival for patients with metastatic pancreatic cancer (<1 percent). While a small number will survive, the investigators need to be careful not to overstate the anticipated outcome and craft appropriate language (e.g., regarding pregnancy, duration of follow-up) within the context of the life expectancy.

- Dr. DiGiusto commented on some of the preclinical data used in support of the scientific rationale for the proposed trial. One study in mice showed no difference between treatment with the BPX-601 cells alone and treatment with the cells plus the activator (i.e., rimiducid/the CID panel). In the clinical setting, a second signal is needed (e.g., CD28 or 4-1BB cDNA), which the investigators have elected to not use with this intervention. Dr. DiGiusto noted that from a safety perspective, with testing of an investigational product, it makes sense to not use CD28 or 4-1BB. In a subsequent study, the number of T cells in the CAR construct increased in the presence of rimiducid with CD34 signaling, but there was no demonstrable increase in the rate or frequency of eliminating the tumor. The relevance of the CD34 in this setting is not clear, however. In contrast, Dr. DiGiusto noted the innovative approach of controlling the proliferation of the modified cells using dimerization induction of iCasp9, even though preclinical evidence did not suggest that dimerization would benefit this model. It might be preferable to use iCasp9 as a safety valve instead of a second signal, given that while it leads to T cell persistence, there does not appear to be preclinical evidence that it has a clinical effect (i.e., removal of tumor cells).
- Dr. Lee asked whether this is the first time the dimerizer will be used in a T-cell setting where it is activating immune response rather than serving a safety function in a clinical trial. He also requested specific data from the pharmacokinetic studies done in humans, including the half-life of the drug and how long the drug would continue to act if it were stopped in response to uncontrolled proliferation causing toxicity.
- Dr. DiGiusto asked if the investigators have any data on whether the sensitivity of corticosteroids is affected with co-stimulation versus no co-stimulation of the T cells, given that corticosteroids are given to treat CRS. Another words are corticosteroids sensitive for treating toxicities such as CRS in this trial, where no co-stimulatory domains are present in the CAR construct?
- RAC members encouraged the investigators to present the best preclinical data to the FDA and suggested including some information in the protocol about the pharmacokinetics of rimiducid.

D. Investigator Response

1. Written Responses to RAC Reviews

The PI provided the following responses to the multiple questions about the rationale for the timing of rimiducid. In pre-clinical experiments in mice bearing HPAC tumors, the study team demonstrated that the BPX-601 cells persist for at least 28 days in the absence of rimiducid. As shown in Appendix M, spleens were analyzed by flow cytometry 28 days after BPX-601 administration with and without rimiducid. BPX-601 cells accounted for 49 percent of the human T cells in the absence of rimiducid (control panel) and 87 percent with rimiducid (CID panel). An intermediate dose of 1.25×10^6 BPX-601 cells/kg without rimiducid slowed tumor growth for 14 days. Administration of rimiducid on Day 14 resulted in increased tumor control and a reduction in tumor size. This demonstrated that sufficient numbers of BPX- 601 cells were present at Day 14 even starting with an intermediate dose to mount an effective antitumor response upon activation by rimiducid. In addition, these data demonstrate that BPX-601 cells persist in the presence of tumor antigen without rimiducid and have an additional effect on tumor growth when rimiducid is administered. There have been reports of clinical toxicities in some patients receiving CAR T cells due to T-cell activation. CRS may be mild and flu-like with fevers and occurs within hours of T-cell infusion, or the response can be more severe and life threatening. Evidence of delayed-onset CRS, occurring between 7 and 50 days after infusion, has also been reported. To separate the potential safety outcomes of the first generation BPX-601 T cells alone from any safety events resulting from the activation and proliferation of the BPX-601 cells by rimiducid, the protocol provides for a period of observation (14 days) post-BPX-601 administration to determine the safety of the cell administration at each dose level prior to administration of rimiducid. The sponsor, Bellicum, will discuss with the FDA the option of modifying the protocol to treat the first cohort of patients with rimiducid at day 14 post–BPX-601 injection (as is currently

written in the protocol) and, if no safety issues are observed, to administer rimiducid on day 7 in the remainder of the patients.

In response to questions about the clinical trial design and patient safety following leukapheresis, the PI noted that protocol has been modified to clarify that patients will be treated with one of two standard chemotherapy regimens for first-line treatment of metastatic pancreas cancer include gemcitabine/ abraxane and fluorouracil (5-FU)/oxaliplatin/irinotecan. Patients will be treated up to 2 weeks prior to administration of BPX-601. The protocol has also been modified to state that patients will receive lymphodepletion therapy with cyclophosphamide (Cytoxan).

The protocol has been changed regarding the DLT for hematologic toxicity to grade 4 or higher. All patients will be lymphodepleted, which is not expected to cause bone marrow aplasia. Furthermore, PSCA is not expected to interact with the hematopoietic system.

BPX-601 cells are expected to persist approximately 1 month after administration of rimiducid. If toxicity develops, symptoms will be treated according to the protocol. In vivo data in tumor-free mice showed an order of magnitude drop in bioluminescence (BPX-601 cells were co-transduced with luciferase) at 30 days post-rimiducid administration. Bioluminescence dropped to baseline by day 10 in mice that did not receive rimiducid.

Per the protocol, for subjects with grade 3 or higher CRS, tocilizumab can be administered over a period of 1 hour at a dose of 4 mg/kg in adults and 8 mg/kg in children, with an option to repeat the dose if clinical improvement does not occur within 24 to 48 hours. Corticosteroids should be considered as second-line therapy for CRS. The initial dose includes methylprednisolone (2 mg/kg/day), which can generally be weaned over several days. For subjects with severe neurologic symptoms, dexamethasone (0.5 mg/kg; maximum, 10 mg/dose) can be considered due to more efficient penetration of the blood–brain barrier. The use of tocilizumab and/or corticosteroids to manage grade 3 or higher CRS has been suggested at previous NIH RAC meetings. Some centers have reported use of low-dose ATG to remove transduced T cells. This can be considered as the third-line therapy, as noted in the protocol. While not tested in vivo, the BPX-601 T cells behave as normal T cells. All other CAR T cell immunotherapies have been responsive to corticosteroids.

The informed consent document has been revised to address the comments of the reviewers.

2. Responses to RAC Discussion Questions

Dr. Becerra agreed with the reviewers that little is known about the toxicities associated with infusion of BPX-601 in humans. The issues of safety and toxicity will be assessed in the proposed Phase I clinical trial, which is designed to proceed in a step-wise fashion so that the potential toxicities of one intervention (BPX-601 alone) can be assessed before adding the second intervention (rimiducid) and evaluating any further toxicities of that product. The 14-day gap between administration of BPX-601 and rimiducid was chosen based on preclinical data in mice, which indicate that the BPX-601 cells persist for at least 28 days in the absence of rimiducid and that the effect of rimiducid on cell persistence lasts approximately a month. The protocol may need to be modified, however, if this timeframe does not result in enhanced T cell persistence as anticipated and/or unacceptable toxicities are seen. One option might be to administer rimiducid earlier than day 14 if the dose escalation is tolerable. Dr. Becerra pointed out that enrollment of patients will be staggered to allow sufficient time to assess safety following administration of the BPX-601 cells and infusion of rimiducid before proceeding to the next subject.

The study team has conducted preclinical experiments that followed T cell persistence through 100 days. For this particular trial, determining the duration of clinically significant T cell persistence takes potential toxicities from the different components of the study into consideration. In preclinical studies, however, the agents have been used somewhat differently (i.e., as a growth factor for amplifying cells in vivo) than in a clinical trial. Preliminary results in tumor models indicate that amplification continues to increase with repetitive dosing. Rimiducid is therefore functioning as a growth-promoting molecule in the context of

tumor. Without tumor, it provides a survival signal. When the agents are used together, there is a greatly amplified frequency in the absolute number of CAR T cells.

With additional data on the kinetics of these interventions in humans, the drugs might, for example, be administered multiple times to gradually increase the dose of CAR T cells to therapeutic levels. Studies of other CAR T cell products indicate that in vivo proliferation and activation of CAR T cells correlate with anti-tumor effects. Results of some solid tumor studies show that at larger doses, therapeutic levels of T cells decrease very rapidly over the course of 2 to 4 weeks, and that the durability of the responses are lost with the drop in T cells counts. Ultimately, the goal is to use this amplification strategy to maintain cells at therapeutic levels for as long as needed for the patient. If, at some point, an anti-tumor effect occurs and there is a curative effect, the drug might be withdrawn so that the number of cells drops off. The investigators hope to solve this problem with a switch that provides a survival effect on the BPX-601 CAR T cells. The optimal dose of the T cells and rimiducid, and the timing of the dosing for each agent, are not known at this point. Determining these parameters is an aim of preliminary studies that are planned and underway.

The investigators have tested corticosteroids with some immunosuppressive drugs, which showed a response against cytokine release, but not specifically with co-stimulation of the BPX-601 T cells. For example, one agent, rapamycin, causes T cells to produce significantly less IL-2 with activation. The investigators do not expect to see any marked difference in terms of sensitivity with this system but acknowledge that follow-up experiments with the actual agents that will be used are warranted.

The investigators probably will not be able to differentiate between hematologic toxicity related to the lymphodepletion versus the study product. The PI noted that from a clinical standpoint, the greatest concern is neutrophil count and risk of infection in this patient population regardless of the causative agent. The team will request feedback from those with more experience in treating patients with CAR T cells and lymphodepletion on criteria that will better define hematologic toxicity for the purposes of establishing the DLT for BPX-601.

The PI noted that patients who live more than 50 miles from where they will be treated will be able to stay free of charge (courtesy of the American Cancer Society) at the Hope Lodge, which offers a hotel-like setting near the study site. Accommodations at this facility should help reduce at least some of the burden and inconvenience of having to travel long distances, particularly when patients are sick. Patients will also have 24/7 access to a clinical cancer center as an added safeguard for management of their disease and symptoms by oncology specialists. These options and the importance of being able to closely monitor side effects and toxicities are discussed with patients and their families. Information about these options will be added to the consent document.

The investigators appreciated the concerns regarding the vulnerable status of this patient population and the suggestions for how to better inform participants about the risks of participating in this trial. The team has tried to balance testing a novel intervention in desperately ill patients and providing care to the participants without being overly optimistic about their illness or the aims of the trial. Although the primary objective at this stage of clinical research is to assess the safety and tolerability of an internetion, testing is done because of the potential for benefit in the future. Early-phase clinical trials build the foundation for subsequent studies, when applicable. Dr. Becerra will review the additional specific recommendations from Dr. Zoloth and follow up with revisions to the consent document to address her suggestions and comments.

Dr. Becerra acknowledged the poor survival rate for patients with metastatic pancreatic cancer but pointed out that patients treated with combination regimens (FOLFIRINOX [fluorouracil, folinic acid, irinotecan, and oxaliplatin] or gemcitabine plus nab-paclitaxel) have improved overall outcomes, including significantly higher survival rates at 1 and 2 years compared with patients given single-agent regimens. Of the more than 850 subjects in the IMPaCT trial, for example, patients given combination therapy (gemcitabine plus nab-paclitaxel) had significantly higher survival rates at 1 and 2 years compared with patients given gemcitabine only. A few patients are still alive after 5 years. In other clinical trials, survival rates are significantly higher in patients given FOLFIRINOX versus those given gemcitabine. Despite

these encouraging outcomes, the overall survival rates for patients with metastatic pancreatic cancer are approximately 20 percent at 1 year and only 2 percent at 5 years. The consent language could be modified to include these rates and results from other trials and say that the study intervention is “not intended to benefit” or “unlikely to benefit” participants.

Dr. Foster explained that a minimal epitope built into the CAR is based on CD34 and that that transgene expression will be determined by using flow cytometry to detect CD34+ cells. As to whether or not inducibilty is required for CAR function, results of experiments in other tumors and dose titration of the group’s CAR T cells show that dimerization is required at lower dose of T cells (e.g., ≤1 million cells). Dimerization therefore does function as a proliferative signal in these T cells, drives their expansion in vivo, and contributes to anti-tumor efficacy. The inducible co-stimulatory domain (iMC) provides a very strong signal of cell survival in the absence of antigen and gives an indication of engraftment. This coordinated approach of the CAR T cells plus dimerization of the iMC molecule generates cell proliferation. What still needs to be determined is the dose levels required for pancreatic cancer.

The question of whether iCasp9 and a different co-stimulatory molecule should be employed has been explored in in vitro and in vivo studies comparing iMC to CD28 and 4-1BB. In those experiments, iMC was found to be superior in terms of T cell proliferation and function; in addition, it appears to provide better control of T cell persistence in the absence of tumor, which is a concern for pancreatic cancer in scenarios where access to the tumor by T cell therapy might be limited. In such cases, because there may not be a signal from the CAR molecule, it might be important to extend the persistence of these cells through a controllable activation switch. The investigators have studied the signaling mechanism of iMC in the modified T cells using gene analysis and protein analysis of phosphoric pathways. iMC appears to turn on pro-survival genes; the signal is sufficient to keep the cells alive in both in vitro and in vivo settings, but it is not sufficient to cause them to divide by themselves. Additional experiments in mice showed dimer dependency in terms of tumor control at a lower dose of 1.25 million cells. The number of CAR T cells was more elevated in mice that received the drug (rimiducid) compared to those that did not receive the drug. In the absence of the tumor and iMC-directed dimerization, the T cells go away completely. Although these findings are from an animal model, they fit the hypothesis that these signals work together to drive proliferation. The same molecular switch (iMC followed by administration of rimiducid) has been tested in a Phase I clinical trial in more than 75 administrations of a genetically modified dendritic cell vaccine, BPX-201, against metastatic prostate cancer; no DLTs were observed, and the switch was deemed to be safe.

The pharmacokinetics of rimiducid have been assessed in healthy volunteers enrolled in a Phase I, placebo-controlled intravenous dosing study and in the iCasp9 clinical trials for GVHD. The effects of IV administration of rimiducid on T cells bearing the *iCasp9* gene are detected within 30 minutes, and serum levels of the dimerizer are nearly undetectable within 2 hours. The half-life of the drug is about 30 minutes. A somewhat different response to rimiducid might be expected when iMC rather than a suicide gene is activated in T cells. Additional experiments show that it acts as an activation signal in primary T cells and that this function decreases over time, based on factors such as cytokine response, activation markers, and proliferative changes. The investigators anticipate that once the modified T cells are activated, there will be a lag time in the activation of T cells bearing iMC.

E. Public Comment

No comments from the public were offered.

F. Synopsis of RAC Discussion and RAC Observations and Recommendations

The following observations and recommendations were made during the RAC’s in-depth review and public discussion:

- Describe in the protocol how you will determine the optimal timing for rimiducid administration following the administration of the study product (BPX-60, PSCA-specific CAR T cells).

- Describe in the protocol the potential hematologic dose-limiting toxicities of the study product (BPX-601, PSCA-specific CAR T cells) and how they will be differentiated from the toxicities of the preparative regimen.
- Revise the informed consent document to:
  - Clearly describe complex terms and concepts, including the description of investigational agents.
  - Emphasize that this is a Phase I study, which is not necessarily intended to benefit the participant, but rather to assess the safety of the product in a human clinical trial.
  - Explain that this is a dose-escalation study.
  - Clarify that although participants can withdraw from the study, they may not be able to avoid the effects and long-term consequences of the study product.
  - Maintain the consistency of inclusion/exclusion criteria (e.g., pregnancy should be listed as an exclusion criterion across documents).
  - Include more information on options for local accommodations and any cost coverage.
  - Provide more information regarding cost coverage during the study.
  - Explicitly state the variety of potential negative outcomes in this study, such as the risks of lymphodepleting chemotherapy, cytokine release syndrome (CRS), and even adverse events that are considered to be extremely rare (e.g., risk of death from CRS).

G. Committee Motion 8

Dr. Atkins summarized the RAC recommendations to be included in the letter to the investigators, expressing the comments and concerns of the RAC. Dr. Atkins requested a vote, and the RAC approved these summarized recommendations by a vote of 12 in favor, 0 opposed, 0 abstentions, and 1 recusal (Dr. Kiem).

XI. FDA Presentation: Pilot Project to Develop a Clinical and CMC (Chemical Manufacturing and Controls) Database to Examine Safety in Trials Using CAR (Chimeric Antigen Receptor) T Cells

Presenters: Maura O'Leary, M.D., FDA
Kimberly Schultz, Ph.D., FDA

A. Presentation by Dr. O'Leary and Dr. Schultz

Drs. O'Leary and Schultz highlighted key aspects of the FDA's plans to create two new databases to better assess clinical and manufacturing information for anti-CD19 CAR modified T-cells.
The Chemistry Manufacturing Control (CMC) database will use information previously submitted with individual Investigational New Drug (IND) protocols for CAR T-cell therapies and through additional sponsor inquiries. The Clinical Safety database will use the standard Clinical Data Interchange Standards Consortium–Study Data Tabulation Model (CDISC–SDTM) format to facilitate submission of clinical and safety information from CAR T-cell INDs. Through the databases, the FDA hopes to answer questions about CAR T-cell therapies, such as how dose, CAR T-cell levels, and cytokine levels might affect patient outcomes and adverse events.

The databases are needed because each adverse event generates complex manufacturing and clinical data, because it is important to understand the complex relationships between manufacturing and clinical factors (e.g., dose) that relate to safety, and because the existing system of data collection is cumbersome and does not provide for uniformity in how data are formatted, all of which make it difficult to analyze available information. Efficient data analysis, in turn, requires collection of manufacturing and clinical data in a standardized manner, systematic organization of manufacturing and clinical data in a central location, and scientific computing to perform the data analyses.

The first phase of this project is to build a pilot CAR T-cell database to help evaluate the safety of these products across INDs, as described by Dr. O'Leary. Dr. Schultz then reviewed the proposed CMC database.

The initial focus of this task is on CAR T cells directed at CD19, a protein that occurs on the surface of normal B cells and in B-cell malignancies, such as acute lymphoblastic leukemia and non-Hodgkin's lymphoma. Anti-CD19 CAR T-cell therapies have been widely reported in peer-reviewed journals as inducing remissions in refractory acute lymphoblastic leukemia in both children and adults. In one report in patients refractory diffused large B cell lymphoma and indolent non-Hodgkin's lymphoma, the anti-CD19 CAR T cells achieved an 80 percent overall response rate, with the duration of the responses lasting between 9 to more than 22 months. The media have featured reports of these successes, including the initial patient in the pediatric trial at the Children's Hospital of Philadelphia (CHOP), who is still in remission and recently visited the White House.

Despite these promising results, there are safety issues with use of CAR T-cell therapies, including deaths in rare cases. The main safety concern with this intervention is the complex clinical entity, cytokine release syndrome, which has an acute phase that can be and often is life-threatening. While experiencing CRS, patients often develop renal, pulmonary, cardiac, and neurological complications that can linger after the initial phase of CRS. Deaths have been reported both in the acute phase and weeks later. The neurologic toxicity occurs with or without CRS and appears to be reversible in most cases. Infections are also common in these patients. Their immunosuppressed state is exacerbated by the lymphodepletion chemotherapy, which is given prior to the cell infusion, and by the CAR T-cell treatment itself. These adverse effects further complicate the patients' clinical course as well as the course of CRS. The modified T cells can persist from months to years. Patients are followed for up to 15 years after infusion to assess the long-term risks and adverse events, particularly second malignancies. One of the aims of the proposed databases project is to better define risks with transduced CAR T cells.

The sample sizes of studies supporting individual INDs for anti-CD19 CAR T-cell therapies are too small to develop a complete picture of their safety characteristics and make risk assessments difficult; building these databases is expected to help overcome these obstacles and establish a better-defined safety profile for this product class. Anti-CD19 CAR T cells were chosen for the pilot based on the number of INDs available, as well as the preliminary evidence that the products have potential for substantial benefits and substantial risks. To date, the FDA has received 105 INDs for genetically engineered T-cell therapies, 36 of which are specifically for anti-CD19 CAR T-cells from more than 15 different sponsors. The agency has data on at least 275 patients who have been treated with anti-CD19 CAR T-cell therapies and who in total have received more than 500 individual doses of the CAR T-cell products. The dosing regimens include single doses, split doses, and sequential dosing, which explains the discrepancy between the number of subjects and the number of doses administered.

The objectives of the pilot project are to evaluate the feasibility of feasibility of creating databases to facilitate analysis of cross-IND safety issues for CAR T-cells, inform and build risk-prediction and risk-mitigation models, and use the knowledge from a central database to advise IND sponsors on safety issues. The agency understands that sponsors are often unwilling to share information about their products with one another given the sensitive nature of this information. Because there are no data-sharing limitations within FDA, safety analyses can be performed across INDs from multiple sponsors. Maintaining confidentiality is top priority in developing and using these databases and integrating the data provided by sponsors, including patient, manufacturing, and other product information.

Along with the potential to be curative, even for severely refractory CD19-positive malignant diseases, the anti-CD19 CAR T cells have complex in vivo activity and complex manufacturing processes that may relate to clinical safety issues. The manufacturing of anti-CD19 CAR T cells involves a multi-step process that starts with a patient-specific, heterogeneous apheresis product which, in turn, comes in contact with many biologically active reagents. Many companies developing anti-CD19 CAR T-cell therapies are transitioning from manufacturing processes developed in academia to commercial manufacturing processes. During this transition, many sponsors will be refining their manufacturing controls and more closely defining their product attributes such as potency. The FDA's CMC database is being designed to

capture the steps in this process and any changes that have occurred across the lifecycle of the INDs. As with the Clinical Safety database and pilot project, data collected for the CMC database and used for analyses conducted by the FDA will be done under the highest confidentiality standards; both patient data and proprietary product development information will be kept private. The totality of data will be used to identify safety trends across INDs and make data-driven regulatory decisions.

Data on both the manufacturing process and product attributes accumulate throughout the path to licensure, from development through the preclinical and clinical stages of testing. Each step varies by sponsor. Most sponsors of INDs for anti-CD19 CAR T-cell products are in the late stages of the manufacturing process and are working on refining potency tests and characterizing their process to show comparability. Given the status of most products, it is an opportune time to use the available information to fill gaps in the collective knowledge about these products and how they are produced. Factors for consideration include the selection of the cell population; whether the apheresis product is selected for before it is transduced, and if so, how; the process for activation of T cells; how long T cell expansion takes, and the impact on the final cellular profile. During the manufacturing process, T cells come in contact with many biologically active reagents that, in turn, may also affect the final product. The FDA is especially interested in understanding whether it is possible to predict safety outcomes by profiling the incoming apheresis characteristics. The FDA is also interested in identifying deviations and inconsistencies in the manufacturing process, as independent process parameters are among the most likely factors to affect the quality attributes of a product.

Once these factors are identified and their impact on the final product are characterized, many of the individuals steps in the manufacturing process could be better designed and controlled to generate as consistent a product as possible. Consistency is assessed through the identification and characterization of critical quality attributes, many of which are already included in the sponsor's Certificate of Analysis of the manufactured CART19 product. These attributes may include the percent of the product that is transduced to express the CAR and the types and levels of cytokines produced by the CART19 cells, which are often used by the sponsors as a potency test. An important safety measure such as the number of integrated gene copies per cell will also be considered. A main goal of this analysis will be to understand the level to which the cellular profile of the final product is consistent and how it relates to the actual profile of transduced cells, specifically how different T cell subsets and ratios of T cell subsets contribute to clinical safety and whether or not receptor density can provide any indication of clinical safety.

To achieve the objectives of objectives of analyzing the impact of the manufacturing process on the quality of the CART19 product and determining how critical product attributes contribute to the safety and efficacy of CART19 therapies, the FDA has categorized the manufacturing process into three main categories. The first, the IND-specific category, captures general information about both the IND and the vector design. The second category, the manufacturing process, is divided into six different modules to allow for tracking of changes in the manufacturing process throughout the IND life cycle (apheresis, selection and activation, gene delivery and expansion, harvest and formulation, clinical delivery, and clinical reagents). The third category, referred to as the patient lot, will encompass information specific to each batch of CART19 cells, including apheresis composition, the vector lot used to transduce the T cells, the cell product, the cell product fluorescence-activated cell sorting (FACS) profile, and any deviations. The patient lot information in particular will be critical to linking the CMC database to the Clinical Safety database.

Modules across categories have been linked to ensure data integrity and to assess how the factors in the subcategories relate to variance in the manufacturing process and how they contribute to the actual manufacturing process. For example, critical reagents and deviations affect every step in the manufacturing process, whereas other factors, such as vector design and vector lot, are linked only to certain aspects of the process, in this case, to the gene delivery and expansion steps.

Several preliminary hypotheses have been identified as the CMC database has been developed, including testing of the following:

- The effect of apheresis composition on T-cell population doubling rate.

- The correlation of potency measurement (e.g., IFN-g production) to final CART19 cell composition and CRS.
- The correlation of other product attributes to clinical safety, such as apheresis composition, CART19 dose, and final product composition.

Information for the databases will be gathered from published reports, INDs that sponsors have already submitted to the FDA, and additional information voluntarily provided by the sponsors. Data contributions from the sponsors will facilitate the accuracy of input and will be critical for the development of true cellular profiles of both the apheresis and the final product cell composition. Information such as details about the transduced cells and the overall cell population will be linked to a unique ID related to both the patient and the batch ID. Upon collection of these data, statistical analyses will be performed with FDA biostatisticians. Trends will then be reported to the CART community via various venues and outreach strategies, such as through regulatory reviews and presentations at conferences.

The pilot database is designed to be user-friendly for both FDA staff and sponsors. It relies on an existing database system, called HIVE (High performance Integrated Virtual Environment), and will use standardized data formats that sponsors are already familiar with. Three phases for development of the pilot database are planned, and include:

- **Phase 1:** Collection of data in a standardized manner using existing format.
- **Phase 2:** Store data in the FDA database (HIVE) using an integrated data format to enable fast cross-study/cross-IND data queries.
- **Phase 3:** Conduct cross-study/cross-IND analysis of data retrieved from HIVE.

The phases are not sequential and can overlap.

The HIVE database is optimized for the storage, retrieval, and analysis of large amounts of data, and is an ideal environment for developing the CAR T-cell database. It enables the agency to capture the complex structure and relationships found in clinical and manufacturing data. Data safety elements that will be collected for analysis include descriptive adverse event data and correlative information such as the dose of the transduced CAR T cells received, cytokine information, the disease being treated, and response data. Data standardization will be done with the assistance of CDISC, an organization that works on developing data standards for collecting and sharing clinical data. This process will employ SDTM, a CDISC standard that is widely used for submitting study data to the FDA. The domains defined in SDTM are being used as templates to develop a standardized data collection and storage format for CAR T-cell safety data. The advantage of using existing tools is that neither the sponsors nor the FDA will need to add new systems. The agency is flexible, however, and will accommodate other similar data formats as needed.

The pilot project teams include members with expertise in manufacturing, pharmacology, toxicology, and clinical practice and research. In addition, assistance is provided from a scientific computing team, the FDA Office of Biostatistics and Epidemiology, and the HIVE database team. The FDA already has some data for the pilot and has completed data formatting per the CDISC-SDTM domains and standards and has created a data dictionary. The next step will be to test the data archiving and retrieval process in HIVE. Once data collection is adequate, the computing team will run analytics for output and will perform validation.

Future analyses will focus on the following:

- Analysis of risks, including:
  - Relationships between cell dose, in-vivo CAR T-cell levels, and cytokine levels, and the characteristics (e.g., frequency, type, onset, severity, duration) of adverse events.
  - Whether there any interactions between demographic features and disease characteristics that influence the characteristics of adverse events.
  - Identifying any concomitant treatments that influence, either favorably or unfavorably, the characteristics of adverse events.
- Whether, and if so, how the construct of the CAR affects safety.

- Whether there interactions that affect toxicity related to disease type, tumor burden, CNS disease, CRS, expansion, persistence, and vectors.
- Whether there is a correlation between events (e.g., CRS and CNS events).

These analyses will provide historical safety information to allow for knowledge-based advice for the CAR T-cell products. For future analysis of SAEs as well as overall safety analysis for these products, the FDA will be able to expand beyond single-episode/single-IND evaluations to allow for more consistent review of safety concerns. For the sponsor, FDA can provide more reliable advice regarding product development. This pilot project may inform future efforts in the Office of Cellular, Tissue, and Gene Therapies and other Offices and Centers of the FDA to look at adverse events across INDs.

The FDA expects to have enough additional data from sponsors to perform some preliminary analyses in the next 6 to 9 months. All sponsors with CAR T-cell therapy INDs will be asked to collaborate on this project but will have the option to decline.

B. RAC Discussion

Dr. DiGiusto asked whether the analyses will be hypothesis driven or if the plan is to see what is generated by the data. If the latter, has a power calculation or predication been done to estimate how much data needs to be collect before doing the analyses? Dr. O'Leary explained that this is not a research project but rather a plan to collect, organize, and assess the safety and manufacturing data that have accumulated to date across INDs for CAR T-cell products. It is not a hypothesis-driven project at this point.

Dr. Kiem asked how the FDA plans on sharing data to individual investigators for feedback or potentially to other investigators if any significant information or important trends are identified in the databases. Dr. Cho asked whether the FDA is planning to share or present the analyses or aggregate data from the pilot. Dr. O'Leary noted that as the analysis progresses, it will inform the agency as to when to provide advice to sponsors on an individual basis (e.g., with respect to specific known complications). The planning has not yet reached the stage for broader reporting of results, however, but sharing of high-level data would be considered and would likely be presented in a setting such as a workshop. Given the prevalence of severe CRS with CAR T-cell products (e.g., 10-40 percent in INDs for leukemia), this database should be helpful, and the findings are expected to be shared with as wide an audience as possible.

Dr. Whitley pointed to the value of this project and suggested that the FDA consider expanding it to include oncolytic virus, which adds some variables to the process and analysis. He inquired about the process that will be used for validation (e.g., a double- or triple-entry system) and how subjective interpretation of the data (if applicable) will be avoided. Dr. George, the lead on the CAR T-Cell Project Team, explained that an initial set of test samples will be validated and, as more data are collected and added to the databases, further validation will be done. Once the analytics are created, a second batch will be tested to confirm the validation process and outcome. Given the sample size and the number of variables being considered, the agency anticipates being able to offer sponsors advice to mitigate risk rather than specific provisions for regulatory decision making. Dr. O'Leary noted that if this model is successful, it (or a similar approach) will be used for other products. Dr. Atkins commented that this approach could be particularly useful, for example, in identifying factors associated with CRS across protocols.

Dr. Atkins requested further information as to involvement of investigators in this project, specifically, whether researchers will be asked or required to participate or provide any other form of support. Dr. Schultz replied that investigators are being asked to voluntarily provide the requested information. The FDA has received positive feedback from several sponsors about their support for this project and analysis. By asking all investigators to participate, the agency is seeking to develop as consistent a process as possible (e.g., nomenclature, steps in the manufacturing process) and to provide as consistent advice as possible across sponsors. Dr. Zoloth suggested working with journals to require compliance with the FDA's request as a condition of publication. Dr. O'Leary commented that the

sponsors have been very cooperative in providing information in response to detailed questions for this project (e.g., how the products are produced and administered, clinical results to date, etc.) and that an additional “stick” isn’t needed.

Dr. Zoloth noted that the National Cancer Institute (NCI) presents what could be considered an overly optimistic view of CAR T-cells by referring to such interventions as “treatments” but with little reference to the rate of severe CRS in trials of CAR T-cell products. She suggested advising the NCI to revise this information to not overstate the potential benefit of these agents, which are being referred to as “a fifth pillar of cancer treatment,” until their efficacy has been more fully demonstrated and confirmed. Dr. O’Leary acknowledged this concern but noted that the results to date are encouraging, particularly for certain cancers. Further, it is beyond the role of the FDA to direct the NIH’s actions in this regard.

Dr. Lee suggested consulting the appropriate Advisory Board for guidance on which data should be collected and what analyses should be conducted, such as looking at correlative data to discern differences between central memory versus effective memory subsets based on, for example, which assays and markers of CRS are used and over what timeframe. Standardizing these parameters will in turn strengthen the analyses. Dr. Schultz noted that flow cytometry data, including the specific cell populations and categories being sorted, are being collected, as well as descriptive data such as definitions of central memory cells. This information will be used to identify correlates and correlations. Cytokine production is another main area of interested, and ways of standardizing that information in the databases are being explored since different labs use different assays for cytokine analysis. The team is working with statisticians to determine how to normalize data provided.

Dr. Lee noted that the acronym CART19 was initially used by CHOP but is now Novartis's term. Given this, he asked whether the FDA’s use of the term is limited to the Novartis products or if the term refers to all anti-CD19 CAR T-cell products across all INDs. Dr. O’Leary clarified that the databases and terminology refer to all anti-CD19 CAR T cells and agents.

Dr. Lee also inquired about the information being collected about individual components of the CARs and CAR T-cell domains clones. Dr. Schultz noted that this issue has been discussed at length with the T-Cell Working Group and that sponsors are being asked to provide details not only about the CD19CARs but also about the clones, transmembrane domains, and organization of the intracellular domains.

Dr. Jorgenson requested additional information regarding the timeline and next steps for the pilot project. Dr. O’Leary noted that for the clinical arm of the project, the FDA already has some data on SAEs and other safety parameters, such as cytokine levels, but more information is needed for the planned data sets and analyses. Dr. Schultz noted that information regarding the manufacturing process and related parameters that are already included in the INDs is being entered into the CMC database. Forms have been developed for distribution to sponsors to provide feedback and responses to the specific questions asked. The forms are in the final stages of review. Additional data collection is expected to begin within the next month or so, with the initial “seed” data sets in place within 6 to 9 months. The preliminary data sets are being reviewed to make sure they meet the needs of the project. The databases will include information on historic products as well as current and future agents. As noted during the presentation, this is expected to be an ongoing project as CAR T-cell products become more widely studied and used.

C. Public Comment

A follow-up question was posed regarding validation of the quality of the data received and whether the FDA is relying on sponsors only or if other sources (e.g., published data) are also being accessed given the differences in, for example, assays used across labs. Dr. Schultz noted that the FDA is relying primarily on sponsors to provide individual information. She recognized that statistical analysis done by a sponsor conducting the same assay on different products is going to be much more consistent than analyses between sponsors. However, biostatisticians involved in this project are focused on figuring out which comparisons, including any assay-specific comparison, can be made across sponsors and INDs. Dr. O’Leary noted further that there already are built-in audits for data and product manufacturing to

document the reliability of the information provided and that this project will not go past the standard operating procedures for the FDA.

Keith Wonnacott, Ph.D., Director of Regulatory Affairs at Novartis, asked how the FDA will protect confidential information if the agency plans to share data from the pilot. He also asked how the agency will balance potential negative vs. positive consequences of sharing results, for example, cases in which a specific parameter may lead to a negative outcome that is then universally applied. Dr. Schulz explained that the data would represent trends or ranges of information rather than individual data points that could be tied back to the companies that provided them, thus protecting individual sponsors' commercial interests. Details of the provisions to protect privacy and confidentiality will be more fully characterized as the project develops.

As both Drs. O'Leary and Schultz stressed during the presentation, the agency has made protecting the confidentiality of the information in the databases, including all patient data and proprietary information, a priority for this project. Dr. George added that this project does not involve looking at any product-specific outcomes, including efficacy outcomes. Rather, the goal is to collect and analyze aggregate data for a class of products to assess safety. Because of the many variables across the data set, the analyses may need to focus on very broad common themes or parameters rather than very specific characteristics of the product. It will be important to collect the data to the greatest granularity possible based on the available information. Given the volume of data anticipated, and the range of variables, the information and advice provided may be more general than specific. Further, as noted earlier, the entire process is being vetted.

Elizabeth Smith from Juno Therapeutics asked whether the FDA ever anticipates a time when providing information for the databases would be mandatory. In addition, she inquired about the frequency of data collection. Dr. O'Leary stated that submission of data for this purpose will always be voluntary. At this point, early in the pilot phase of the project, it is not known how often the agency will ask investigators to provide data. The goal is not to burden sponsors. Requesting and releasing information in batches makes sense now, but this approach may change depending on how the project progresses. The FDA will continue to dialogue with sponsors on the scope of the project. As these products proceed to licensure, it is going to be very important to have a safety profile for the entire class of agents to use for labeling and to inform clinicians, especially those who are not familiar with this type of product.

XII. Closing Remarks and Adjournment

Dr. Jorgenson provided an update on the status of changes to the RAC review process that were proposed in October, followed by the open comment period that closed in December. The comments have been reviewed, and the planned changes, which are based largely on the original proposal previously reviewed by the RAC, will be announced in a final rule, to be released in the near future. The new process for RAC protocol reviews will be implemented following the June meeting. When available, the final rule and guideline changes and the specific implementation date will be distributed to RAC members. Per the Institute of Medicine's (IOM's) proposal, an oversight body such as an IRB will make the recommendation for RAC review. Those recommendations will be based on criteria outlined in the IOM report, that is, that the proposed intervention is sufficiently novel or has a safety characteristic that should be evaluated by this committee. In some cases, the NIH Director could designate a proposal for RAC review if the therapy poses a significant safety, ethical, or scientific consideration.

The NIH will continue to monitor the gene therapy landscape field and will still receive all submissions from all investigators, including, for example, information on SAEs and novel applications of previously used vectors. As currently done, that information will be incorporated into the existing protocol registry database and will remain accessible to the RAC (via GeMCRIS). A summary of why a specific protocol is not recommended for in-depth RAC review will continue to be provided. Dr. Jorgenson noted that the number of RAC meetings will not necessarily change once the new process is in place. RAC members will continue to have standing meeting times to review protocols as needed and to address other issues such as review of FDA proposals and consideration of emerging issues in the field.

Dr. Jorgenson informed the committee that a new OBA Director has been named and that the new Director will serve as the RAC Executive Secretary starting with the June meeting. She thanked the members for their contribution to this field, noting that she has enjoyed working with the group.

Dr. Kiem thanked the RAC members and the OBA staff and adjourned the March 2016 RAC meeting at 2:35 p.m. on March 9, 2016.

[Note: Actions approved by the RAC are considered recommendations to the NIH Director; therefore, they are not considered final until approved by the NIH Director.]

_____
Jessica Tucker, Ph.D.
RAC Executive Secretary

I hereby acknowledge that, to the best of my knowledge, the foregoing Minutes and the following Attachments are accurate and complete.

This Minutes document will be considered formally by the RAC at a subsequent meeting; any corrections or notations will be incorporated into the Minutes after that meeting.

Date: _____

_____
Hans-Peter Kiem, M.D., Ph.D.
Chair, Recombinant DNA Advisory Committee

Attachment I:
Recombinant DNA Advisory Committee Roster

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Attachment II:
Public Attendees

(This list includes only individuals who are not identified elsewhere in this document.)

Nina Berkowitz, NIAID, NIH
Lizbet Boroughs, Association of American Universities (AAU)
Chris Boshoff, NINDS, NIH
Grace Chen, NIAID, NIH
Jasmine Daniel, AAU
Adam Dezure, NIAID, NIH
Martha French, Bellicum Pharmaceuticals
Annemarie Moseley, Bellicum Pharmaceuticals
Yonatan Negash, FDA
Manisha Patel, Novartis
Elizabeth Smith, Juno Therapeutics
Ramjay Vatsan, FDA
Keith Wonnacott, Novartis
Galina Yamshchikov, NIAID, NIH

Attachment III:
Abbreviations and Acronyms

AAV	adeno-associated virus
AE	adverse event
ALL	acute lymphoblastic leukemia
AML	acute myeloid leukemia
ART	antiretroviral therapy
ATG	antithymocite globulin
BM	bone marrow
BMP	bone morphogenetic protein
BSL	biosafety level
BSWG	Biosafety Working Group
CAR	chimeric antigen receptor
Cas9	CRISPR-associated protein 9
CASP3	caspase-3
CAT	chloramphenicol acetyltransferase
CBA	chicken β-actin
CC	Clinical Center
CCR5	chemokine receptor type 5
CDC	Centers for Disease Control and Prevention
CDISC–SDTM	Clinical Data Interchange Standards Consortium–Study Data Tabulation Model
CHOP	Children's Hospital of Philadelphia
cDNA	complementary DNA
CLL	chronic lymphocytic leukemia
CMC	chemistry and manufacturing controls
CML	chronic myelogenous leukemia
Cm^R	chloramphenicol resistance
CMV	cytomegalovirus
CNS	central nervous system
CPK	creatine phosphokinase
CRISPR	clustered regularly interspaced short palindromic repeats
CRP	C-reactive protein
CRS	cytokine release syndrome
CT	computed tomography
CTL	cytotoxic lymphocyte
DLT	dose-limiting toxicity
ELISA	enzyme-linked immunosorbent assay
FACS	fluorescence-activated cell sorting
FDA	U.S. Food and Drug Administration
5FU	5-fluorouracil
FLAG	fludarabine, cytarabine, and GCSF
Flu	fludarabine
FSH	follicle-stimulating hormone
GCSF	granulocyte colony-stimulation factor
GeMCRIS	Genetic Modification Clinical Research Information System
GLP	good laboratory practice
GMP	good manufacturing practice
GTSAB	Gene Transfer Safety Assessment Board
GVHD	graft-versus-host disease
HCC	hepatocellular carcinoma
HIVE	High-Performance Integrated Virtual Environment
HLA	human leukocyte antigen
HSCT	hematopoietic stem cell transplant
ICD	informed consent document
IGG	immunoglobulin G
IL-2	interleukin-2
IM	intramuscular

IND	Investigational New Drug
indel	insertion or deletion mutation
IOM	Institute of Medicine
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ITR	inverted terminal repeat
IV	intravenous
LH	luteinizing hormone
Lm	*Listeria monocytogenes*
LPL	lipoprotein lipase
MAb	monoclonal antibody
MDS	myelodysplastic syndromes
miR	microRNA
mRNA	messenger RNA
MTD	maximum tolerated dose
NGS	next-generation sequencing
NHP	non-human primate
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OBA	Office of Biotechnology Activities, NIH
OD	Office of the Director, NIH
OHSU	Oregon Health and Science University
PCR	polymerase chain reaction
PI	principal investigator
PK	pharmacokinetic
Pol	polymerase
PRAME	preferentially expressed antigen in melanoma
PSCA	prostate stem cell antigen
PSRT	Protocol Safety Review Team
QC/QA	quality control/quality assurance
qPCR	quantitative polymerase chain reaction
rAAV	recombinant adeno-associated virus
RAC	Recombinant DNA Advisory Committee
RCR	replication competent retrovirus
rhBMP	recombinant human bone morphogenetic protein
RMSF	Rocky Mountain spotted fever
RT-PCR	reverse transcription polymerase chain reaction
SAE	serious adverse event
SIV	simian immunodeficiency virus
SOP	standard operating procedure
TCR	T-cell receptor
TG	trigeminal
TMJ	temporomandibular joint
UBC Enh	human ubiquitin C gene enhancer
vg	vector genomes
VRC	Vaccine Research Center
VRG	Vaccine Research Group
WHO	World Health Organization