RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

June 10, 2015

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/biomedical-technology-assessment-recombinant-dna-advisory-committee/human-gene-transfer-protocols-registered-oba.]

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

June 10, 2015

The Recombinant DNA Advisory Committee (RAC) convened a special workshop entitled "Cytokine Release Syndrome after T Cell Immunotherapy Establishing Definitions, Developing Criteria, and Optimizing Management," at 8:30 a.m. on June 10, 2015, at the National Institutes of Health (NIH), Building 45, Conference Room E1/E2, Bethesda, Maryland. In accordance with Public Law 92-463, the meeting was open to the public from 8:30 a.m. until 4:37 p.m. on June 10, 2015. The following individuals were present, either in person or by teleconference, for all or part of this workshop.

Committee Members

Michael Atkins, Georgetown University School of Medicine
Paula Cannon, University of California, Los Angeles
Saswati Chatterjee, City of Hope National Medical Center
William Curry, Harvard Medical School
Kevin Donahue, University of Massachusetts Medical School
Rebecca Dresser, Washington University School of Law
Marie-Louise Hammarskjöld, University of Virginia School of Medicine
Patrick Hearing, Stony Brook University
Howard Kaufman, Robert Wood Johnson Medical School/Rutgers, The State University of New Jersey
Donald Kohn, (RAC Chair), University of California, Los Angeles
Joseph Pilewski, University of Pittsburgh
Lainie Ross, University of Chicago *(via teleconference)*
Michel Sadelain, Memorial Sloan Kettering Cancer Center
Richard Whitley, University of Alabama at Birmingham
Dawn Wooley, Wright State University
Laurie Zoloth, Northwestern University

NIH Office of Biotechnology Activities (OBA)

Lyric Jorgenson, Office of the Director (OD), National Institutes of Health (NIH)

Non-voting Agency Representatives

Denise Gavin, U.S. Food and Drug Administration (FDA)
Carrie Wolinetz, OD, NIH

NIH/OD/OBA Staff Members

Linda Gargiulo
Morad Hassani
Robert Jambou
Cheng Luan Li
Maureen Montgomery
Chris Nice
Marina O'Reilly

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

Gene Rosenthal
Aparna Singh

Attendees

There were 74 attendees.

Attachments

Attachment I contains a complete list of RAC members, nonvoting agency and liaison representatives, and ad hoc participants and speakers. Attachment II contains a list of public attendees. Attachment III contains a list of abbreviations and acronyms used in this document.

I. Welcome and Introductory Remarks

Dr. Kohn, the RAC Chair, called the meeting to order at 8:30 a.m. on June 10, 2015, and welcomed everyone to this specially convened workshop. Notice of this meeting under the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* was published in the *Federal Register* on May 13, 2015 (80 FR 27330).

Dr. Kohn noted that the current meeting builds upon two previous RAC workshops about T cell immunotherapy that were held in 2010 and 2013. As the field has matured and expanded, the community has seen many clinical successes. Of note are long-term remissions of various types of cancer, in particular, with chimeric antigen receptor (CAR) T cells engineered to target hematological malignancies. Clinical benefits have also been observed in protocols targeting a number of solid tumors. Consistent with and supportive of this growth, NIH's investment in clinical gene transfer protocols has nearly doubled in the past five years. In fiscal year 2011, the NIH budget included $14 million for gene therapy clinical protocols, while for fiscal year 2016, NIH funding alone is estimated to be $27 million. The potential of gene transfer technology is also attracting interest and funding from industry, spurring the establishment of numerous companies.

Despite the progress and promise of gene therapies, T cell immunotherapy is not without its challenges. Several technical issues are still in flux in terms of vector design, promoting T cell persistence, and finding and selecting targets. The safety of gene transfer research is also of concern. Multiple cases of severe adverse events (SAEs), manifesting as a collective set of symptoms referred to as cytokine release syndrome (CRS) constituted a central topic of discussion at the current meeting. CRS results from the release of cytokines by activated T cells, which can lead to systemic inflammatory reactions, manifesting as fever, hypotension, respiratory distress, and other serious conditions and events. Severe cases can lead to hypotension and organ damage. Clinical assessment and management of CRS and related symptomatology can be complicated. Most cases are well-managed, but strategies are evolving as more information is learned.

There currently is no standard definition of or grading criteria for CRS, resulting in inconsistencies in how the condition is diagnosed and treated, and making it difficult to compare outcomes across protocols and institutions. Several definitions for CRS have been proposed in the published literature, but as this field progresses toward the licensing of products by the FDA and commercialization of those products, efforts to harmonize those definitions into a single concept that can be broadly applied should be considered.

The goals for this workshop were to:

- Review incidence of severe CRS in T cell immunotherapy protocols,
- Develop a consensus definition and grading system for CRS, and
- Share best practices for managing CRS.

This workshop was designed to provide a forum for practitioners from different disciplines and settings to share their experiences in managing the occurrence of CRS in gene transfer clinical protocols. Discussions were facilitated through a series of sessions that focused on different types of CAR T cell immunotherapy protocols, which included targeted questions in support of the goals of the meeting. The first session was focused on CRS within the context of CAR T cell immunotherapy targeting human hematologic malignancies, for which there is more extensive experience, CAR T cell immunotherapy in solid tumors, and engineered T cell receptor immunotherapies targeting various malignancies. Subsequent sessions were focused on standardizing the definition and grading system for CRS and identifying strategies to optimize the management of CRS.

Following these introductory remarks, those in attendance, including RAC members, panelists, and other guests, introduced themselves by name, affiliation, and research interests.

II. Overview of T Cell Immunotherapy and Cytokine Release Syndrome (CRS)

Dr. Kohn presented background information on the frequency and distribution of T cell immunotherapy protocols registered with NIH/OBA over the past 20 years, an overview of CRS, and a summary of the initial characterization of CRS. For more than a decade, CARTCR (T cell Receptor) protocols comprised only three to four percent of all protocols in NIH's portfolio. A noticeable increase in the number and proportion of protocols was seen starting toward the end of the last decade. The most current data show that 25 percent (70/282) of all protocols submitted between 2011 and 2014 were T cell immunotherapy protocols. The number of TCR protocols has remained relatively constant over the past decade, while submission of CAR protocols has accelerated.

A review of the types of targeting moieties that were involved in the CAR protocols between 1994 and 2014 identified one protocol with a first-generation CAR with the gamma signaling domain, 25 utilizing first-generation CAR with CD3zeta as the signaling domain, 69 protocols utilizing second-generation CAR with CD3zeta and a secondary co-stimulatory domain (such as CD-28 or 4-1BB), and 11 protocols with third-generation CAR that have CD3zeta and two co-stimulatory domains. Three additional protocols compared two different generation CARs within the same study. In all, 108 CAR protocols have been submitted to the RAC. An additional 36 protocols using TCRs with chimeric alpha-beta chains have been submitted, bringing the total for all CAR/TCR protocols to 144. Among the 104 CAR-zeta protocols, 18 percent were first generation, 69 percent were second generation, and 13 percent were third generation.

The majority of the CAR/TCR protocols have been for oncology indications. Seven protocols used CAR-like molecules to target human immunodeficiency virus (HIV); many of the constructs in these studies had a CD4 extracellular domain, targeting gp160-expressing HIV-infected cells. Approximately 58 percent of the CAR-zeta oncology protocols targeted hematologic malignancies, and 42 percent targeted solid tumors. Further breakdown of these studies showed that hematologic malignancies are primarily the target for CAR, while melanomas and other malignant neoplasms are most prevalent for TCR-based approaches. CD19 accounts for nearly 50 percent of the antigen targets in protocols for hematologic malignancy. Small numbers of a variety of other antigens (e.g., CD20, CD30, CD22, CD123, BCMA, NK, GD2, ROR1, Kappa1) make up the other half of the targets in protocols for hematologic malignancy. When used in hematologic malignancies, TCRs have been primarily targeting cancer-testis antigens or Wilm's tumor antigens. The most commonly targeted antigens for solid tumor studies are the cancer testis and melanoma antigens (MART-1 and gp100).

In turning to the issue of the development of a draft definition for CRS, Dr. Kohn noted that the first reported grading scheme came from a group at Memorial Sloan Kettering. An initial working definition for severe CRS, secondary to CAR T cell immunotherapy, based on this grading scheme, included the following criteria:

- Fever lasting ≥ 72 hours,
- Two cytokines with at least a 75-fold increase in baseline levels or one cytokine with at least 250-fold increase in baseline levels, and

- At least one clinical sign of toxicity such as hypotension (requiring at least one intravenous vasoactive pressor) or hypoxia (PO2 <90 percent) or neurologic disorders (including mental status changes, obtundation, and seizures).

The Memorial Sloan Kettering group also found C-reactive protein (CRP) to be a good indicator of CRS. They reported high CRP levels (≥20 mg/dL) with 86 percent sensitivity and 100 percent specificity in the setting of participants who had received immunotherapy. Based on data from the Memorial Sloan Kettering team and investigators at other centers, a grading system for CRS was proposed, with Grade 1 CRS defined as fever and constitutional symptoms, and Grade 2 being defined as hypotension responding to fluids or one low-dose pressor, hypoxia, and organ toxicity. Per this grading system, Grade 3 CRS symptoms are more severe than for Grade 2 CRS; hypotension requires multiple pressors or high-dose pressors, hypoxia requires higher fraction of inspired oxygen (FiO_2), and participants have greater organ toxicity. Under this grading system, Grade 4 CRS requires either mechanical ventilation or involves severe organic toxicity.

A range of clinical signs and symptoms of CRS will need to be taken into consideration in drafting an initial definition of CRS. Constitutional symptoms are the most common features of CRS and include fever that may or may not be accompanied by rigors, malaise, fatigue, anorexia, myalgias, arthralgias, nausea, vomiting, and headache. Skin rash may also be present. Common cardiovascular and respiratory complications include tachypnea, hypoxemia, tachycardia, and hypotension. Participants may have increased cardiac output early in the course of CRS and decreased cardiac output as the condition progresses. There also may be coagulation abnormalities, including elevated D-dimers and hypofibrinogenemia, with or without bleeding. Some participants present with renal and/or hepatic complications such as transaminitis and hyperbilirubinemia, or with gastrointestinal symptoms including nausea, vomiting, and diarrhea. Neurologic complications, including symptoms that are variable but may include headache and a change in mental status presented as lethargy, somnolence, agitation, delirium, word-finding difficulty or frank aphasia, hallucinations, tremor, dysmetria, altered gait, or seizures. The nature and mechanism of these signs and symptoms are not fully understood and require further discussion and consideration. Consistent with the diversity of signs and symptoms of CRS, laboratory abnormalities and biomarkers of CRS are equally diverse. Neutropenia, thrombocytopenia, and anemia may occur but are difficult to attribute, particularly if conditioning chemotherapy has been given prior to the T cell infusion. Elevated levels of cytokines, CRP, ferritin, liver function tests, and creatinine are common.

The goal for the current meeting is to try to understand the extensive collection of signs, symptoms, and laboratory tests to develop a uniform definition of and a grading system for CRS. It was noted that as T cell immunotherapy transitions from the experience of the founding centers that pioneered these interventions towards commercialization, it will be important to develop definitions and identify the best practices that can become standard of care (SOC) and instituted broadly at hospitals and other research facilities that may not have the resources, for example, to obtain an Interleukin–6 (IL-6) level in a short period of time.

III. Session I: Protocol Updates and CRS Experiences

Presenter: Morad Hassani, M.D., Ph.D., OD/OBA, NIH

Relying on data from the NIH Genetic Modification Clinical Research Information System (GeMCRIS) over the 20-year span between 1994 and 2014, the NIH/OBA portfolio registered a total of 136 T cell immunotherapy protocols that targeted cancer (excluding HIV protocols and the single protocol using a CAR-gamma construct). Of these 136 protocols, 101 protocols (74 percent) involve CAR-zeta T cell products, while the remaining 35 protocols (26 percent) use TCR agents (CAR-alpha and -beta). Among the 101 tumor antigen-directed CAR-zeta T cell protocols, 59 protocols (58 percent) target hematologic cancers and 42 protocols (42 percent) target solid tumors.

The data used for the NIH/OBA analyses presented during the meeting were compiled primarily from GeMCRIS published studies, and the meeting summaries from two previous NIH symposia on T cell immunotherapy. Two key articles about CRS referenced during the meeting included one paper from Dr. Marco Davila and colleagues[2] from Memorial Sloan Kettering Cancer Center and a second by Dr. Daniel Lee and colleagues[3] from the NIH and multiple institutions across the country. Dr. Hassani noted that while prior studies have reported on CRS, these two publications included definitions and grading criteria for CRS, as well as information related to medical management of the condition. The NIH/OBA analyses were based on the definitions and grading system presented by Lee et al., as summarized below, because cytokine data, which was used as CRS criteria in the paper by Davila et al., was actually lacking in many of the serious adverse events reported to us on patients in gene transfer trials that resembled CRS. The CRS grading system considered by NIH/OBA is based on the criteria and grading defined by Lee et al.[3] and uses the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE v4.0). Per this system, mild, moderate, and severe CRS are graded and defined as follows:

- **Mild CRS:** Grade 1 CRS (common in this population and requiring only symptomatic treatment) and Grade 2 CRS events, including those with flu-like symptoms or constitutional symptoms, which are manageable with minimal intervention (e.g., hypotension responsive to intravenous [IV] fluids or one low-dose vasopressor or increased oxygen requirement responsive to less than 40 percent FiO_2). Note: These were excluded from NIH/OBA analyses.

- **Moderate CRS:** These CRS events have some features of Grade 2 CRS plus features of Grade 3 CRS after T cell immunotherapy.

- **Severe CRS:** Grade 3 or 4 CRS and/or neurologic toxicity after T cell immunotherapy.

Panelists were given the following guidance when considering the topics discussed during this session:

- How many protocols have your institution(s) conducted, and how many participants have been dosed?
- Which target diseases have been studied?
- Which tumor antigens have been targeted and by what type of CAR (second/third generation) and signaling domain (4-1BB, CD28) or T cell receptor?
- What has been your overall response rate by construct, target disease, and age group (adult vs. pediatric)?
- What definition and grading criteria were used to identify severe CRS (e.g., how similar or different from the referenced definitions[2,3])?
- What has the incidence of severe CRS been in your protocols? Has it differed by:
  - Construct?
  - Signaling domain?
  - Target disease?
  - Population (pediatric vs. adults)?

Panelists were also asked to consider the following questions during this session:

- What are the potential contributing factors for severe CRS? What role do the following have in severe CRS?
  - Participant-specific factors such as target disease, predisposing conditions, age, and host genetics
  - Tumor burden

[2] Davila, M., et al. (2014) Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. *Sci Transl Med* 6:224ra25. http://stm.sciencemag.org/content/6/224/224ra25.full.pdf

[3] Lee, D., et al. (2014) Current concepts in the diagnosis and management of cytokine release syndrome. *Blood* 124:188-195. http://www.bloodjournal.org/content/bloodjournal/124/2/188.full.pdf

  - Construct-specific factors such as signaling domains and tumor antigen targets
  - Other factors such as chemotherapy, lymphodepletion, and conditioning cytokines (e.g., IL-2)
- Are neurological symptoms and/or toxicities components of CRS or part of a distinct syndrome?
- What is the perceived need for a unified definition and grading system for CRS?

A. Chimeric Antigen Receptor (CAR) T Cell Immunotherapy Protocols Targeting Hematologic Malignancies

1. Presentation: CRS Events: OBA Data Analysis

Presenter: Morad Hassani, M.D., Ph.D., OD/OBA, NIH

NIH/OBA has 59 registered protocols targeting hematologic malignancies using CAR-zeta T cells. More than 400 participants have been dosed across all of these studies, with some participants receiving more than one infusion of the modified T cells, resulting in the number of infusions totaling more than 450.

For the analysis of CRS events, NIH/OBA had enrollment data from 36 of these protocols, and safety data from 35. Twenty-two of these 35 protocols with safety data reported SAEs, 75 adverse events (AEs) were considered related to investigational study product events; and 46 of the AEs were deemed to be CRS-related SAEs. Among these events, 36 met the criteria for severe CRS; six met the criteria for moderate CRS, and four involved neurologic symptoms only. Cytokine data were only provided for some of these events. IL–6 levels were reported for 18 of the 46 events; other cytokines were presented for nine events; and CRP levels were quantified and provided for seven events. In five events, it was only reported that CRP levels were elevated. In terms of CRS signs and symptoms, fever and hypotension were the most frequent events, as 37 cases reported fever and 36 cases reported hypotension. Twenty-one participants had some degree of respiratory distress, 15 had tachycardia, and 12 had acute renal failure with increasing creatinine levels. Thirty subjects had neurologic symptoms or toxicity, including six participants who had seizures. In terms of interventions, 20 participants had vasopressors, and ventilatory support was required for 25 participants for either respiratory distress and/or neurotoxicity. NOTE: a number of participants required ventilatory support due to neurologic toxicity (e.g., somnolence) and not necessarily due to respiratory distress. Corticosteroids were used in 23 participants, and this included few cases of adrenal insufficiency and DRESS syndrome (drug reaction [or rash] with eosinophilia and systemic symptoms), (1 or 2 cases). Anti-cytokine therapies, most predominantly IL-6 inhibitors, were used to manage severe CRS. Tocilizumab, an anti-IL-6 receptor monoclonal antibody, was administered in 28 cases. One event used siltuximab, another anti-IL-6 monoclonal antibody, and two cases used etanercept, an anti–tumor necrosis factor (TNF)–alpha antibody. Five participants required dialysis, and 41 of the 46 cases were treated in the intensive care unit (ICU), a good index of severe CRS. Three of the cases, which were not treated in the ICU setting, were graded as moderate CRS, suggesting that CRS symptoms requiring treatment in the ICU is a good indicator of severe CRS. Of note, a large number of the reported cases involving neurologic toxicity were from a single protocol, and the other reports of neurologic symptoms were from a small number of additional studies.

2. Panel Discussion

Moderator: Michel Sadelain, M.D., Ph.D., Memorial Sloan Kettering Cancer Center

Panelists: Noelle Frey, M.D., University of Pennsylvania
David Porter, M.D., University of Pennsylvania
Jae Park, M.D., Memorial Sloan Kettering Cancer Center
Renier Brentjens, M.D., Ph.D., Memorial Sloan Kettering Cancer Center
Helen Heslop, M.D., Baylor College of Medicine
Richard Champlin, M.D., MD Anderson Cancer Center
Brian Till, M.D., Fred Hutchinson Cancer Research Center
Michael Jensen, M.D., University of Washington
Daniel Lee, M.D., NIH, National Cancer Institute
Cameron Turtle, MBBS, Ph.D., Fred Hutchinson Cancer Research Center

David Maloney, M.D., Ph.D., Fred Hutchinson Cancer Research Center
L. Elizabeth Budde, M.D., City of Hope National Medical Center
Catherine Bollard, M.D., Children's National Health System

Dr. Sadelain emphasized that while the data collected and presented by NIH/OBA are in one place, challenges remain given that the data are incomplete, the denominator is not exactly known, and there is variability in the amount of data provided to NIH/OBA in each case. These issues make the analysis of the data difficult.

Drs. Frey and Porter reported that gene transfer protocols for hematologic malignancies conducted at the University of Pennsylvania (UPenn) primarily use a second-generation CAR with a 4-1BB (CD137-TNF family) co-stimulatory domain and CD3zeta signaling. Another feature of this construct (CTL019) is that it uses a lentivirus vector. A total of 174 participants with hematologic malignancies, including both pediatric and adult participants, have been treated thus far in these studies. Participants have been followed for two months to 59 months. The highest response to the CAR therapy is seen with acute lymphoblastic leukemia (ALL), followed by non-Hodgkin's lymphoma (NHL), and chronic lymphocytic leukemia (CLL). The complete remission rates for pediatric and adult ALL participants are 94 percent (45/48) and 80 percent (12/15), respectively. The overall response rate for NHL participants is 68 percent, with 58 percent having complete remission. Approximately 50 percent of CLL participants have had either a complete (24 percent) or partial (24 percent) remission following treatment. Preliminary results for a small cohort of myeloma participants indicate an overall response rate of 75 percent.

CRS is seen in nearly all responding participants in the UPenn protocols, but a higher incidence of CRS is seen in those with the highest response rates. More than 90 percent of ALL participants and slightly less than 50 percent of CLL participants have some CRS. Further analysis indicated that disease burden at baseline is associated with severity of CRS. For ALL participants, CRS symptoms usually occur one day to 14 days after cell infusion. Symptoms include high fever, rigors, myalgia/arthralgia, nausea/vomiting/anorexia, fatigue, headache, hypotension, encephalopathy, dyspnea, tachypnea, and hypoxia. Many of these are Grade 3 and 4 events, per the UPenn grading system for CAR-related CRS, which is similar to that published by Lee et al (2014). There have been three Grade 5 (death) events and three CRS-related deaths, all in adult ALL participants (i.e., the first three adults dosed). The investigators noted, however, that these three participants had very severe concurrent infectious illnesses throughout the entire treatment course. Neurologic toxicity varies and is not always connected to CRS. The highest rates of neurological symptoms are seen in participants with the highest response (i.e., ALL participants), but even in this group, less than 25 percent of participants had more severe symptoms (Grades 3 and 4). Measurement of cytokines show marked elevations in IL-6 and interferon–gamma and increased TNF–alpha. Dr. Porter pointed out that, under the UPenn CRS grading system, there is no quantification of oxygen requirements/hypoxia, based on the team's experience, and that this measure is not a good indicator of severity of illness. Grade 3 and Grade 4, respectively, distinguish between participants who are very sick but manageable and those who are critically ill and unstable and facing life-threatening complications. Participants with Grade 3 CRS require low-dose pressors, have oxygen requirements, and may require fluid resuscitation for hypotension. Participants with Grade 4 CRS, become hemodynamically unstable and require high-dose pressors or mechanical ventilation and very immediate and intensive care. Clinicians should always be aware of the high risk of concurrent infectious illness in these participants and consider interventions such as prophylactic antimicrobials. The investigators noted the challenge in trying to manage severe CRS concurrently with infectious illness.

The team at Memorial Sloan Kettering Cancer Center (MSKCC) is currently using a second-generation CD19-targeting CAR with a T cell–specific antigen that contains a CD3zeta signaling domain. A retrovirus is used to transduce the T cells. An 88 percent response rate (14/16 participants) to the CD19-specific CAR T cells was seen in one protocol of participants with relapsed or refractory B-cell ALL. About 23 percent of these participants had severe CRS based on a working definition that includes any requirement for vasopressors and/or mechanical ventilation for hypoxia. Another protocol for relapsed CLL has an overall response rate of 50 percent among the 19 participants treated to date. One participant experienced severe CRS. Similarly, one participant experienced symptoms of neurotoxicity. Another CLL protocol including adult participants uses CD19-targeted CAR T cells as a consolidation approach.

Participants in this study received pentostatin, cytoxan, and rituximab, the standard frontline regimen at MSKCC, following six cycles of chemotherapy. There has been an overall 50 percent response for the eight participants treated to date, with no severe CRS and no neurotoxicity. The MSKCC investigators agreed with the UPenn team that the risk of severe CRS differs between CLL and ALL participants and similarly found a correlation between CRS and disease burden at the time of dosing. These participants and their treating physicians face the additional challenge of concomitant infections because many participants enter a study in an immunocompromised state due to their underlying illness and/or treatments, including conditioning chemotherapy. Managing the constellation of symptoms and distinguishing between events related to CRS versus other causes is challenging.

Drs. Heslop and Bollard reported on Baylor's CD19 CAR studies, which have treated a total of 48 participants with hematologic malignancies, primarily lymphoma. These studies have tested first-, second-, and third-generation CAR T cells and multivirus-specific T cells after allogeneic transplant. For most participants, CRS has been moderate in severity. Milder cases have been seen in participants who received the multivirus-specific T cells after transplant. No central nervous system (CNS) toxicity was observed in these protocols. One participant with relapsed ALL who was recovering from CRS, died after developing a pulmonary embolism; this participant had other risk factors, including morbid obesity and diabetes that could have contributed to the participant's death. Studies with other targeted interventions (e.g., CD30 CARs) enrolled participants with high-grade lymphomas expressing the relevant antigens. The overall response rates for these protocols ranged between 30 and 40 percent. Some participants in these studies experienced milder CRS symptoms, such as fever, but none to date have had moderate or severe CRS, most likely due to differences in the intervention and disease burden. In participants given multivirus-specific T cells, the presence of viral antigen increased cell expansion but did not result in significant CRS.

Dr. Champlin's group at MD Anderson Cancer Center uses second-generation CD19 CAR T cells produced by the *Sleeping Beauty* (SB) system developed by Dr. Cooper at the same institution. The modified cells have been used primarily with hematopoietic transplants, following an autologous or allogeneic transplant at a time point of minimal disease, or in participants with low-bulk residual disease or in those who have relapsed. No Grade 3 or 4 CRS or CNS toxicities have been observed. Whether the mild CRS is related to responsiveness to the investigational product is not clear. The absence of a stronger cytokine response may be due to the characteristics of the participant population being treated or to the specific CAR construct. Dr. Champlin noted that *in vivo* expansion of the CAR T cells has been less than that reported by other centers, which might be another contributing factor. The investigators are redesigning the CAR construct in an effort to improve *in vivo* expansion in the future.

Dr. Till provided a brief summary of findings from two clinical protocols using a CD20-targeted CAR conducted at Fred Hutchinson Cancer Research Center (FHCRC). One protocol used a first-generation CAR, and the second had a third-generation CAR with CD28 and 4–1BB co-stimulatory domains. Unlike the other protocols, which use retroviral vectors or SB transposons, the second study used an electroporated DNA plasmid. Ten participants were dosed between the two protocols. Two participants had a partial remission. One participant had transient fever, hypoxemia, and orthostatic hypotension that resolved within 24 hours, which by some criteria does not meet the definition for CRS. No neurological toxicities were seen.

Dr. Jensen provided an update on his group's pediatric leukemia/ALL protocol for children and young adults with relapsed or refractory disease. The study uses a defined-composition product with an equal number of CD4 and CD8 T cells that have high-level CAR expression and a surface tag, EGFRt (truncated epidermal growth factor receptor) that also serves as a suicide gene. To date, 30 participants have enrolled; for most participants, refractory/relapsed disease occurred after allogeneic transplant. Most participants have only had Cytoxan as their conditioning regimen. Twenty-six participants have been dosed, and data are available on the first 22 participants. The complete remission rate for these 22 participants is 91 percent. There have been eight serious CRS cases and neurotoxicities but no toxicity-related deaths. Of the eight events, four involved CRS with neurologic toxicities, two involved severe CRS, and two involved only neurologic toxicities. Preliminary results indicate a poor correlation between these events and either disease burden or T cell dose. Some of the more severe toxicities have been

observed in participants with limited disease burden and at low doses. One participant had a cardiac toxicity that did not appear to be a cytokine-mediated response; rather, it looked like a self-limited hypocontractility phenomenon. A small number of participants at very high risk of severe CRS were dosed with tocilizumab, an anti-IL-6 receptor monoclonal antibody, and steroids at first fever. This approach appears to have mitigated progression to severe CRS, and these participants have had a high level of engraftment of the CAR T cells with B-cell aplasia and remission.

Dr. Lee reported on two National Cancer Institute (NCI) CAR T cell protocols in participants with hematologic malignancies. One is a CD19 CAR T cell study in pre-B ALL and B-cell lymphoma. The other is a CD22 CAR T cell study in pre-B ALL. The first study uses a second-generation CAR with a CD3zeta signaling domain and CD28 co-stimulatory domain. The initial preparative regimen included standard fludarabine/cyclophosphamide conditioning; the regimen was changed after the first 21 participants were dosed, based on disease burden at the time of eligibility screening in response to the number of Grade 3 and 4 CRS events in these initial participants. To date, 39 participants, including 28 children, have been enrolled and treated under the CD19 protocol; most of the participants have pre-B ALL. Twenty-three of the treated participants (64 percent) have had a complete remission, six have stable disease, and seven have progressive disease; an insufficient amount of time has elapsed to be able to assess response in the other three participants. Modified CTCAE v.4 grading was used for the first 21 participants in the CD19 protocol. The criteria were modified mostly for Grade 3 events to allow for clearer distinction between Grades 3 and 4. Three of the first 21 participants had Grade 4 CRS, which was treated with tocilizumab and corticosteroids. Three participants had Grade 3 CRS; two cases were driven by hypotension, and one participant was aphasic for about 8 hours. One of the three Grade 3 participants received tocilizumab, and all recovered. One of the participants treated for hypotension did not have any neurologic side effects but developed mild (Grade 1) hallucinations after he deffervesced and his pressors were discontinued. Dr. Lee noted that mild neurologic symptoms also developed post-tocilizumab in two other participants.

The investigators noticed that participants with more severe CRS tended to also have high disease burdens. To adjust for these factors, prep regimens were stratified by baseline disease burden, with the option to individualize therapy based on prior toxicities. Since making this change, there has been only one additional Grade 4 CRS (an elective intubation) and no Grade 3 CRS events among 18 newly enrolled subjects. The participant with the Grade 4 CRS received tocilizumab and corticosteroids and made a complete recovery. The CRS grading system used in the CD19 protocol was changed after dosing the first 21 participants to follow the definitions and system described in the paper by Lee et al. referenced earlier in the meeting. The CD22 protocol similarly follows this system.

The NCI's CD22 protocol uses as second-generation CAR with a 4-1BB co-stimulatory domain and CD3zeta signaling. The preparative regimen includes low-dose fludarabine/cyclophosphamide conditioning. Four participants have been treated to date with the lowest cell dose planned for the protocol (500,000 CAR cells/kg). All participants were CD-negative relapses following CD19 CAR at the time of enrollment. One of the treated participants had an MRD-negative complete remission, two had stable disease, and one had progressive disease.

Drs. Turtle and Maloney summarized CD19-specific CAR T cell immunotherapy protocols at Fred Hutchinson Cancer Research Center (FHCRC). Dr. Turtle focused on protocols of adult ALL using CD19-specific CAR T cells, while Dr. Maloney reviewed CD19 CAR T cell studies in participants with NHL and CLL. The protocols use a CAR with a 4-1BB co-stimulatory domain and lentivirus vector with a defined CD4/CD8 cell subset composition. Twenty-four ALL participants have been treated using a three-dose escalation regimen (2×10^5/kg, 2×10^6/kg, 2×10^7/kg) and a conditioning regimen. Thus far, 21 of the 23 participants (91 percent) evaluated have had a complete remission. Seven participants developed severe CRS, and one participant died 3 days after infusion with the highest dose of the CAR T cell product. Dr. Turtle noted that severe CRS criteria in this study was simply defined as "requiring ICU care". For most of these ALL participants, ICU care was for administration of pressors. Grade 3 or higher neurotoxicity was seen in 9 of the 24 participants. The risk factors for these SAEs are similar to those identified at other centers for CRS and neurotoxicity, including a high blast percentage, high CAR T cell dose, and lymphodepletion chemotherapy. Increased CAR T cell expansion and persistence was seen in

participants treated with a second dose level who received fludarabine as part of an intensified lymphodepletion chemotherapy regimen.

As seen in other centers, admission to the ICU for CRS is more likely in participants with high marrow blasts (and the related expansion of CD8 T cells). In this study, participants who had less than 20 percent bone marrow blasts (used as the cutoff for serious toxicity) did not require intensive care and had no cases of neurotoxicity. In contrast, among the 13 participants with greater than 20 percent blasts, seven required ICU case and nine had neurologic toxicity (delirium, focal deficit, seizures). CRS symptoms and neurotoxicity resolved with early intervention in all but one case. Dr. Turtle commented that the impact of treatment on outcome is difficult to discern, however. Whether the pre-dosing chemotherapy regimen is definitively associated with more toxicity is uncertain. Participants with elevations in interferon–gamma and IL-6 were more likely to need ICU care. CRP and ferritin in particular should be considered as possible makers of severe CRS risk.

Dr. Maloney summarized the data on another FHCRC study, which is a Phase 1/2 dose-finding protocol using the same CAR and doses described above in participants with NHL and CLL. Results in the first 12 participants showed relatively poor T cell expansion and proliferation with and without different lymphodepletion regimens. Based on these outcomes, the regimen was switched to cyclophosphamide followed by fludarabine for the second cohort of participants. The overall response rate for this group was 63 percent. Severe toxicity was seen in 3 of 23 participants, requiring ICU care; none of these participants were initially treated with cyclophosphamide or cyclophosphamide plus etoposide. Two deaths occurred within 3 weeks after infusion of the CAR T cells in participants with severe CRS; one had a CNS bleed, and the other died from a gastrointestinal bleed. Six of the 23 participants had some evidence of neurotoxicity, which was manifest primarily as delirium with or without focal deficits. There was one focal deficit, including a tremor, one focal deficit determined to be transient choreoathetosis, and a CNS bleed in the participant that died. Other than the CNS bleed, these events completely resolved with intervention (tocilizumab alone or with steroids). Results show a clear correlation between these SAEs and cell dose level. Participants with the most severe toxicity received the highest CAR T cell dose. As with ALL participants, the addition of fludarabine resulted in a marked increase in T cell expansion and persistence, and clinical activity. The increased toxicity in the fludarabine cohort treated at the highest CAR T cell doses caused the investigators to reduce the cell dose. There was no clear relationship with tumor burden. Results from five participants with CLL show complete remission in two participants, PRs in two participants with marrow/blood clearance and partial lymph node remission, and no response in one participant with significant splenomegaly and 100 percent marrow involvement. One participant with severe CRS required ICU care. Other events included two transient neurotoxicities characterized as delirium. No deaths have occurred in the CLL cohort. As reported in other protocols for hematologic malignancies, CAR T cell dose appears to correlate with toxicity.

City of Hope Medical Center currently has three active clinical protocols using a second-generation CD19 CAR with CD28 co-stimulatory domain and lentiviral transduction. In addition, Dr. Budde's group uses central memory T cells as the starting cell population. CRS grading follows the system criteria published in the Lee et al. article. A total of 11 participants across three protocols have been treated to date. No CRS was observed in two participants with relapsed and refractory acute and progressive leukemia, even when the dose was escalated to 200 million cells (flat dose) in the participant with progressive disease, who developed only a mild (Grade 1) fever. Similarly, no CRS or neurotoxicities were observed in the five participants treated at low and high doses under the protocol of relapsed and refractory lymphomas and in participants treated with the low dose (50 million) T cells in the protocol for diffuse large B-cell lymphoma or mantle cell lymphoma. However, one of three participants in the latter protocol had Grade 3 CRS symptoms (which resolved with a single dose of tocilizumab). This participant received 200 million cells. One participant recently treated at the 600 million cell dose level had a Grade 4 CRS that required ICU admission and intubation. Dr. Budde reported that the participant is improving clinically. No neurotoxicities have been observed in any of the three studies or at any dosage. Preliminary results appear to suggest a correlation between CRS and CAR T cell dose level. Two other protocols recently opened at City of Hope and will study CD123 CAR in participants with AML and a CAR T cell targeting the IL-13 receptor in participants with glioma.

Following the panelists' presentations, the discussion was opened for further comments and questions.

Dr. Sadelain commended each group for working on a CRS classification/grading schema for the clinical setting. Many of these systems are very similar and share multiple common features. However, because these systems have been adapted for use by individual centers, the definitions and criteria for CRS vary, making it difficult to compare data from one center to the other, one CAR protocol to another, and one target disease to another. Panelists and other participants were asked to comment on whether a unified grading system is needed and if so, what the process for developing such a system should look like and who the key players should be in this process (e.g., clinical researchers, academicians, sponsors, regulators).

Dr. Ribas supported the development of a clear definition of CRS but questioned the current nomenclature. The condition is referred to as "cytokine release syndrome" based on the assumption that cytokines released in response to an activating event (or events) are then involved in the development of toxicities that constitute this syndrome. There is little evidence, however, as to the specific cytokines that are inducing these adverse effects and little guidance in the published literature for establishing a unified definition for CRS.

Drs. Turtle and Park considered some definitions based on CTCAE to be appropriate for CAR T cell immunotherapy but agreed that there is an issue with terminology for CRS. Further clarification is needed as to whether the aim of a grading system for CRS is to distinguish acceptable vs. unacceptable toxicity and, in turn, to determine the level of toxicity that is considered unacceptable.

Dr. Sadelain noted that the term "cytokine storm" is used to describe a very dramatic and rapid increase in cytokines, typically following infusion of a CAR T cell product, and in reference to the subsequent development of serious CRS-related events. The focus the afternoon would be to define CRS, establish a CRS grading system, and identify the criteria for that guidance. The task for this session was whether to recommend standardizing the definition and grading system or to support proceeding with each site following its own policies and guidelines.

Dr. Champlin commented that a single scale, common definition, and qualitative rather than quantitative classification/categories of toxicities (i.e., mild, moderate, severe) should be considered. This approach is similar to what is done in the oncology field and would allow for direct comparison of symptomatology and treatments, constructs, and protocols and, in turn, designing and comparing future protocols with different targets and additional participant populations.

There was general agreement that having a uniform definition and grading system, established by consensus, would be helpful. The CTCAE grading system for CRS is appropriate for infusion-related reactions, but it does not take all clinical and biological responses into account. Better definitions, criteria, and grading for neurotoxicities are also needed. Dr. Lee explained that the reason for developing and publishing the novel CRS grading system and treatment algorithms was because the CTCAE criteria did not apply and were not relevant to the participants being seen across CAR T cell immunotherapy protocols. In anticipation of expanded use of immunotherapy, the ultimate goal is to provide guidance to centers and sites *without* prior T cell immunotherapy experience as to when to intervene with anti-cytokine therapies so that risks to participants are minimized and anti-tumor/anti-leukemia effects are not inhibited.

Dr. Jenson noted that T cell immunotherapy is a very different therapeutic modality than drug-based treatments in terms of the relationship of the dose to toxicities and the association of toxicities to clinical benefit. As a result, creating definitions to generate grading criteria and guidelines for CAR/TCR-related CRS may require a different approach or process. Underlying this process is what the definitions for CRS drive. For example, if the definitions drive toxicity ratings used in a dose-escalation protocol, they may not be beneficial or helpful in terms of deployment of CAR/TCR modalities outside of the initial clinical protocols. Because other features of these toxicities are linked to therapeutic efficacy and, for many participants, are self-limited, careful consideration of the kinetics or duration of any given toxicity as it relates to a severity score should be given in creating a unified definition and grading system.

Reversibility of these toxicities is another important feature to take into account in this process. Dr. Park and others noted the challenges in recording toxicities for all clinical protocol participants. Guidance is needed as to whether toxicities (e.g., fever, hypotension, respiratory problems) should be combined under one umbrella of CRS definition and grading system or whether they should be recorded separately in a different system. Clarification of this issue is needed for practical purposes in the clinical setting and to assure consistent and accurate recording of toxicities.

Dr. Yang commented that the severity of toxicity depends in large part on pre-emptive management of anticipated symptoms and side effects of CAR/TCR therapies. The degree of toxicity can be altered by anticipatory treatment, which is done routinely at the NIH. While different entities are observed, based on experience with more than 3,000 immunotherapy trial participants over 15 years, the team has identified commonalities across different immunotherapies. Dr. Yang noted that “universal” CRS includes multi-system organ failure in addition to specific target or geographic toxicities (e.g., neurotoxicity, focal symptoms) and on-target, off-tumor toxicities. He commented that each set of toxicities represents a different entity to some degree and that it is important to not conflate these symptoms, some of which are completely reversible and can be managed by treating prophylactically. All participants get CRS with IL-2, some participants also have multi-system organ failure, and a small number have neurotoxicity. In monitoring participants, the investigators focus on the symptoms and signs of multi-system organ failure, focal neurotoxicity, and on-target off-tumor toxicity because of the difficulty in managing these complications.

Dr. Kohn reminded the workshop attendees that other stakeholders in this arena are the regulators, the Data and Safety Monitoring Boards (DSMBs), and the Institutional Review Boards (IRBs) that review individual protocols with new constructs or new conditioning regimens that need to know that a particular product or regimen in a given protocol has undue toxicities.

Dr. Zoloth was struck by the marked variability among sites and inquired as to whether the basis for such differences is related to treatment, nomenclature, or other factors. She suggested that this issue should be sorted out before proceeding further. Dr. Powell noted that some variability is due to differences in doses and efficacy of the cell constructs and the participant populations and diseases being studied. For example, no CRS was seen in the initial CLL protocols at UPenn that included bulky disease, but dramatic CRS occurred in participants with ALL who were given 10-fold higher doses of the same product. Thus, the fact that a T cell immunotherapy is well tolerated does not necessarily indicate effectiveness. One part of this exercise should therefore be to focus on cases where the participants develop cytokine release syndrome with concomitant tumor response. Dr. Sadelain concurred with Dr. Powell and pointed out that there are objective differences in the incidence and severity of CRS due to different participants, types of CARs, T cell doses, and conditioning regimens. The question of semantics and definitions circles back to the starting point of having clear, uniform descriptions of symptoms to avoid ambiguity in documenting and reporting events. The choice of terms for these different entities is important to this endeavor.

The appropriate body to take on the task of developing the uniform definition and grading system for CRS and the mechanism to achieve this goal was also discussed. In addition to input from members of the RAC and panelists attending the meeting, feedback from study sponsors, industry representatives, and regulators should be considered. It was noted that these issues were scheduled to be discussed later in the meeting, including during the wrap-up.

The next issue for discussion focused on identifying the contributing factors that lead to more severe CRS and/or that affect the incidence and severity of CRS. The differential role of specific malignancies and disease burden needs to be taken into consideration in understanding the pathogenesis of CRS. For example, CRS was not seen in the initial CLL protocols that included bulky disease, but disease burden in the context of ALL appears to be critical risk factor for developing severe CRS. Dr. Powell pointed out that CRS was defined in part by the experience at various centers with participants with B cell ALL (B-ALL), which differs from other B cell malignancies in that it is more of a bone marrow-predominant disease. Why ALL may predispose to both better efficacy and more severe CRS are interesting questions. Whether the bone marrow-predominant feature of B-ALL has a role in how the CAR T cells function, where the T cells

traffic to, and whether or how those factors correspond to CRS remains to be defined. The protocols on CAR T cells in ALL probably provide the strongest data for investigating the mechanisms of CRS, given the frequency of this phenomenon and response rates in these participants. Identifying the source of the cytokines (T cells, ALL cells, other types of cells), the mechanism for activation of release of these cytokines, and the mechanism by which the cytokines are destroyed (e.g., by T cells?) all warrant further exploration.

For ALL, the correlation between disease burden and CRS appears to be due to T cell expansion in response to high tumor load/high levels of tumor antigen. Lymphodepletion may also play a role in CRS; in some studies, a change in the pre-conditioning regimen improved response rates and increased CRS, possibly reflecting better T cell expansion. Thus, even with a lower disease burden, if an environment with increased T cell expansion can be created, cytokine release may also increase, potentially obscuring the contribution of the tumor burden vs. T cell proliferation to the induction of CRS. Age also appears to influence the degree of toxicity, including neurotoxicities, for ALL, with adult participants having a higher frequency of and more severe CRS than pediatric participants. The greater difficulty for adults to get through CRS safely compared to younger participants is consistent with other ALL therapies. The basis for this age-related difference in ALL and the difference between diseases is not fully understood, however. The minimal to no CRS seen in CLL participants could be because CLL is a nodal disease; in contrast, in ALL, the bone marrow is the prime location for the disease as well as for T cell expansion. Dr. Powell suggested that the cytokines may also come from the tumor itself, as a result of destruction of the B cells by the activated T cells or from the activation of other immune cells by T cell-secreted cytokines. Evaluating patterns of serum levels of different cytokines following infusion of CAR T cells over the post-infusion period may be of some value in understanding whether this mechanism contributes to CRS.

A considerable amount of the experience in the ALL protocols involves blinatumomab, a T cell engager that is clearly associated with an increased toxicity profile (including neurotoxicities) that is similar to CRS. Other factors that warrant further investigation and analysis include roles of different chemotherapies, the timing of the T cell infusion, and T cell expansion and engagement with tumor antigen. Dr. Lee pointed out that a common feature of CRS is T cell expansion, which, in turn, appears to be the primary driver of the severity of CRS. Increased T cell expansion is seen with higher CAR T cell doses, more intensive lymphodepletion, use of IL-2, and more tumor targets. Under these conditions, T cells are more strongly activated, resulting in greater proliferation.

In addition to a central role of T cells in CRS, consideration should be given to cytokine receptor polymorphisms and host-specific factors that may dictate who gets more explosive CRS versus more subtle CRS, and may lead to variability in the capacity of T cells to undergo expansion. Participants who do not have strong expansion post-transplant may have less (or less severe) CRS than a participant with primary refractory leukemia who hasn't had a transplant or years of chemotherapy, who may have significant T cell proliferation and more significant CRS. These issues point to how clinicians dose these cells within the context of CAR/TCR clinical protocols and the "pharmacokinetics of living drugs". Dosing is based on the model for pharmaceutical agents, but T cells do not act in the same way as drugs. Instead of clearing from the body after infusion, the cells expand and, with the variability in T cell expansion among participants, the true "active dose" is not known, even when participants are given the same quantitative dose. Dr. Turtle raised the issue of the manufacturing process in affecting CRS in terms of the different CAR constructs, T cell subsets, and the composition of the engineered T cells. Experiments in xenogeneic models suggest that T cell products with a defined composition are more potent in tumor elimination and T cell proliferation and may affect the risk of CRS as well. While the impact of these factors is not binary, using a consistent product may present a better opportunity to tease out dose-response relationships.

Dr. Curry posed a two-part question regarding T cell dose. First, several centers report a relationship between "low" and "high" doses of T cells and CRS, but it isn't clear if the doses are the same across centers. Second, the basis for reduced risk of CRS with lower T cell doses needs further elaboration given the pharmacokinetics of the CAR/TCR constructs and the proliferation of T cells. As previously discussed, while there is a clear dose-response relationship in some cases, in other cases, no CRS develops even at a maximum dose. Several factors in addition to dose should be considered when

assessing these differences, including disease type and nature, disease burden, how the tumor is acting, timing of peak T cell expansion, and how long T cells persist. Differences between disease types may be due to the tumor expressing something that either inhibits T cells from proliferating (e.g., in large-cell lymphomas or CLL) or causes cells to explosively proliferate (e.g., in ALL). Tumor burden plays a role in terms of CRS, especially for ALL. In lymphoma, including follicular lymphomas with 60 or 70 percent marrow involvement, CRS is limited and not as severe as with ALL. Similarly, participants with 100 percent CLL in the bone marrow have had virtually no or only minimal CRS following T cell immunotherapy. Dr. Porter commented that while the target disease is important, he does not see a marked difference between CLL and ALL if T cell expansion occurs. He noted that although CRS is more common with treatment of ALL, CRS is as severe in CLL as in ALL with T cell proliferation. Further, the CLL participant population may be more informative because of the differential response within the population, which provides two subgroups for comparison.

Dose also matters in terms of providing the peak expansion and duration of CAR T cells needed to elicit an anti-tumor response. Identifying this dose can be challenging, however. At too low a dose, the cells may not expand or last long enough to provide long-term benefit, and if the dose is too high, the CRS response can overcome the anticipated treatment effect. Dr. Maloney noted, for example, that a participant with 90 percent marrow involvement becomes ill within hours after being given a high dose of CAR T cells. In contrast, when a fraction of that high dose is given, it can take 8 or 9 days before the participant becomes ill, at which point the cells have slowly expanded and killed most of the tumor cells without resulting in severe CRS.

Dr. Kohn asked whether there are any data to better understand the source of the cytokine storm; is it the T cells, the tumor, or both, and whether the data suggest a disease-specific response. Dr. Sadelain cited a recent study of a mouse model for ovarian cancer that showed variation in the severity of the CRS response following administration of human T cells (administered intraperitoneally). Results showed that the increased levels of cytokines were from the human T cells, but the elevation of mirroring cytokines, including IL-6, was not from the T cells. Dr. Porter and others noted that in most cases, increases in cytokines result from macrophage activation, also referred to as macrophage activation syndrome (MAS). Whether MAS is part of CRS or is a separate entity has not been determined, but in these cases, participants have both clinical and biochemical evidence of MAS, including significantly elevated ferritin levels and neurologic symptoms such as confusion. The source of the cytokines in MAS has not been identified, but immune activation likely contributes to some of the clinical features of this syndrome. Dr. Yang considered the initial triggers for cytokine release to be straightforward: The number of T cells that can quickly find their target antigen, how stimulatory that antigen is, and the array of functional cytokines that can be made.

Dr. Frey discussed risk factors for sepsis in participants in CAR/TCR protocols and suggested monitoring participants early in the post-infusion phase to identify those who may be more prone to hemodynamic collapse based on either their cytokine profile or disease burden at the time they receive the T cells. In addition, there is some evidence of endothelial cell polymorphisms that might predispose participants to different types of shock. Incorporating sepsis and shock evaluations into CAR/TCR protocols might provide better predictive measures given that these conditions are induced in hemodynamically stable participants with the study interventions. Dr. Atkins noted that similar evaluations done about 30 years ago for high-dose IL-2 in a model for sepsis and in the clinical setting were informative. Results of the clinical protocol showed that all of the participant deaths (six out of about 200) were related to bacterial infections. The investigators discovered that a neutrophil chemotactic defect developed with IL-2 therapy, which predisposed participants to catheter-related infections. These infections could be eliminated with prophylactic antibiotics, which greatly improved the safety of that therapy. In addition, because IL-2 therapy can be stopped, participants can be monitored and have the agent held if they start to become sick. Dr. Atkins suggested that data for participants with severe CRS who died should be reviewed to determine if any of the participants were neutropenic from their preparative regimens, and to consider if this factor needs to be taken into consideration in future participants. Mechanisms to block cytokines (other than IL-6) and/or shut off the T cell response, and the impact of such actions on the anti-tumor effects of the T cells, should also be explored.

Dr. Jensen stressed the importance of defining and quantitating CRS to have criteria and a grading system with prognostic and medical treatment value. The discussion at this point focused on whether toxicities may occur because of the failure to intervene preemptively, most likely due to concerns that use of agents such as corticosteroids will shut down the T cells. However, both pre-clinical and clinical data indicate that T cells are highly resistant to the effects of corticosteroid prophylaxis, possibly because of the nature of a CAR signal. Specifically, the corticosteroids might be shutting down signaling at the level of the responding tissues, rather than being focused predominantly on the T cells. Dr. Champlin questioned whether prophylactic anti-IL-6 treatment acts in a similar manner, by preventing or mitigating CRS without inhibiting the anti-tumor response of the T cell therapy. Without clarification of these mechanisms, many of the toxicities and factors contributing to a definition and grading system for CRS may be unfounded if an effective preemptive intervention strategy for these toxicities is available.

Dr. Yang was unimpressed with the reports of an anti-CRS response to tocilizumab, noting that his group at the NIH has not observed this effect. He relayed a recent case of a participant who developed severe CRS for which the investigators decided against using a cytokine-blocking antibody; the CRS resolved within about 24 hours and the participant then developed neurotoxicity. In this case, the participant was given CD19 CAR T cells for treatment of diffuse large B cell lymphoma. Dr. Yang noted that the lack of an anti-CRS effect by tocilizumab has been observed with other histologies as well. Dr. Lee pointed to the successful outcomes at many centers that used IL-6 receptor blockage and asked Dr. Yang whether the presumption is that participants would improve even without the antibody. Dr. Yang clarified that abrogation of symptoms is seen within minutes to hours of infusing a single dose of tocilizumab and that the CRS in some of those participants might have resolved spontaneously or with only aggressive supportive care.

The larger question is at what point and for what purpose is tocilizumab, or another antibody, given? Should it be soon after infusion of the T cells to prevent the onset of CRS, or later in the course of symptoms, to manage severity of CRS? IL-6 seems to be at least a principle driver in many cases, as demonstrated by the fact that inhibition of this cytokine can reverse CRS clinically. The source of IL-6 in this setting is not known, but experiments in the mouse model and *in vitro* experiments clearly show that it is not made by CAR T cells. In response to a question about the homogeneity (or lack thereof) and any patterns in the cytokine profiles in participants that meet the criteria for severe CRS, Dr. Turtle commented that the cytokine profiles appear to depend on the cell dose. With a low dose of T cells, it takes a long time for the cells to accumulate and toxicity to develop; during this time, there likely is downregulation of cytokine receptors. With a high dose of T cells, there is a much clearer correlation between toxicity and serum cytokines. In participants treated with high doses of T cells, thresholds about which severe CRS and severe neurotoxicity are more likely seen are with IL-6 and interferon–gamma. At the lower T cell doses when toxicity does not occur for several days, the relationship between CRS toxicity and cytokines is less clear. Similar results have been reported by Dr. Lee's group for a cohort of 21 participants; in addition, the relationship between TNF–alpha levels and CRS severity approached significance, but this analysis may not have been sufficiently powered to show a more definitive outcome. Dr. Powell noted that elevated cytokine levels are seen in participants in UPenn protocols, but no clear signal has emerged yet, perhaps in part because the sample size for this analysis is still relatively small.

Additional experience at UPenn shows that high levels of IL-6 correlate with severe CRS and that IL-2RA is associated with MAS. Interferon–gamma levels show no consistent pattern with degree of severity of CRS for CLL participants. However, there is a strong statistical difference between interferon–gamma levels and degree of severity of CRS for ALL participants, but no correlation with TNF–alpha or IL-2. Dr. Hassani reported that although complete cytokine data (including baseline levels before T cell infusion) were not available for every participant categorized as having CRS, NIH/OBA analyses showed that the highest cytokine levels after T cell infusion have been for IL-6, IL-8, IL-10, and interferon–gamma; TNF–alpha levels did not stand out per this analysis. In addition, in most cases, IL-6 levels increased after tocilizumab treatment, in support of literature suggesting that levels are elevated after anti-IL-6 therapy. This finding raises the additional question of what happens to T cell proliferation after IL-6 is inhibited and CRS is blocked. Dr. Turtle followed up this query by noting that ongoing proliferation is seen after giving both tocilizumab and dexamethasone to participants enrolled in protocols at Fred Hutchinson Cancer Research Center (FHCRC). It is not possible to discern whether proliferation would have been greater

without the use of these two agents, however. Dr. Frey noted that the same response (an increase in IL-6 levels after tocilizumab) has been observed in UPenn protocols, in part because the receptor for IL-6 is being targeted. The post-tocilizumab response makes some sense stoichiometrically given how the anti-IL-6 agent works. This response raises a further question, however, as to the location of the IL-6 receptors affected by tocilizumab. Dr. Sadelain pointed out that IL-6 receptor-positive cells are present in the CNS, which could explain why some participants develop neurotoxicity after being given tocilizumab. Dr. Lee described the team's experience with two participants who had CNS disease at the time of infusion. Cerebrospinal fluid (CSF) status was monitored closely for CAR T cells, leukemia cells, and a panel of ten cytokines. For both participants, there was a concomitant rise in the population of CAR T cells, IL-6, and interferon–gamma in the CSF as leukemia cells were cleared from the CSF. It is unclear if tocilizumab can cross the blood-brain barrier, but these data suggest that IL-6 is either processed or produced within the CSF space. Dr. Lee noted that no pharmacokinetic work on CRS has been done to date in the CSF, but his group is currently analyzing data from experiments in a non-human primate model to address this question.

Dr. Yang suggested conducting a small (e.g., 10 participants) randomized, blinded pilot protocol to explore these mechanisms further and to perhaps define thresholds for IL-6 and other relevant cytokines for anti-cytokine intervention. Other participants supported this or a similar effort. Dr. Hammarskjöld noted that the role of different CD19 CARs, co-stimulatory molecules, and other factors that may contribute to CRS should also be studied. Dr. Jensen asked whether correlation between severe symptoms and the relative lack of immunoregulatory cytokines such as IL-10 should be considered. He noted that his group has considered using *JAK* inhibitors in participants who were refractory to corticosteroids and tocilizumab but were concerned about results in animal models showing worsening of cytokine-related toxicities with this approach since this also leads to shutting down IL-10, which becomes a dominant mediating cytokine in response to cytokine storm.

Dr. Sadelain requested further elaboration on the relationship between the different signaling domains on the CARs and CRS, and on any observations on correlating CD4/CD8 T cell ratios or T cell subset composition and CRS. Dr. Hassani referred to studies showing differences in T cell persistence and/or proliferation and development of CRS based on the signaling domains used with the CAR. Such differences should be expected based on the nature of the constructs. Although it is difficult to analyze the full complement of data or infer strong conclusions at this time, it appears that the CAR T cells with 4-1BB signaling domain persist longer than the CAR T cells with CD28 signaling domain, at least in the pediatric data from UPenn. In contrast, adult data do not present a marked difference with the CD28 T cells infused at MSKCC. Based on his experience in studying CARs in mouse models, Dr. Sadelain cautioned against making general conclusions regarding whether an individual signaling domain (e.g., CD28, 4-1BB, OX40, ICOS [inducible co-stimulator]) is representative of that whole class or family. Further, the function of CARs is determined not only by the signaling domain, but also by the spatial structure in the high-affinity binding region and probably the position of the epitope. Dr. Park added that unless the same binding domains are being studied in the exact same population, comparisons will be difficult due to multiple confounding factors. Dr. Lee noted that although his group has not conducted any protocols to allow for a head-to-head comparison of different CAR signaling domains, results presented during this meeting suggest differences in cytokine response and CRS with CD28-containing CAR versus 4-1BB-containing CAR. He added that based on the team's experience, the degree of severe cytokine release reported for 4-1BB-containing CARs in other studies has not been observed in the pediatric ALL participants treated at the NIH. Dr. Park's group has found no differences in the CD4/CD8 T cell ratio for ALL and CLL participants. Future plans include analyzing the results to obtain additional details about the different T cell phenotypes.

The discussion continued with a focus on the relationship between conditioning regimens and CRS. Dr. Sadelain commented that if the same co-stimulatory domains are used, then modifying the conditioning regimen might also change the CRS. Dr. Turtle summarized results presented earlier in this session, which showed dramatically increased CD4 and CD8 CAR T cell proliferation and persistence in participants who received fludarabine and cyclophosphamide versus cyclophosphamide plus etoposide. In ALL, however, there has not been a difference in toxicity with the different conditioning regimens. In the small number of participants with non-Hodgkins lymphoma, all of whom were treated at the same CAR T

cell dose, severe CRS was seen much more frequently in the participants who received fludarabine. Whether this difference is related to CAR T cell expansion or another effect of fludarabine on the tumor environment is not known. Dr. Champlin pointed out that some toxicity is caused by the combination of the drugs used for the conditioning regimen, which are being given in principle to upregulate IL-15 and other endogenous cytokines to improve T cell expansion. The outcomes therefore reflect CRS in addition to the direct toxicity of the drugs, and the cytokines are released in response to the conditioning regimen.

Panelists addressed the questions posed by the RAC regarding the neurologic symptoms observed in participants given T cell immunotherapies, their relationship to CRS, and whether this neurotoxicity syndrome is distinct from CRS. The group agreed that clear definitions for this collection of symptoms are needed to reduce confusion as to whether neurotoxicities are associated with CRS. Dr. Maloney provided a brief history of experience with these symptoms. He noted that neurologic symptoms in the initial participants undergoing T cell immunotherapy were attributed to confusion associated with high fevers and the older age of the individuals, and were thought to be similar to IL-2-related neurologic adverse events. Upon closer examination, however, these participants were found to be aphasic with more focal neurologic deficits, suggesting the neurologic toxicity associated with CAR T cells is fundamentally different. Neither the investigators nor the participants could explain this loss of speech. Further, milder cases were often missed in the setting of severely ill, hospitalized participants. In addition to aphasia and ataxia, neurologic symptoms in some participants have included seizures, some of which have been life-threatening. Despite difficulties in diagnosing these symptoms, as with CRS, a common language for these complications should be established for uniformity in diagnosis and management. For example, do these events constitute encephalopathy, delirium, or confusion?

There was general agreement that the neurotoxicity syndrome in these participants is a separate entity from CRS. Its timing is often delayed, and the neurologic symptoms usually occur at the point when signs of CRS (e.g., hypotension, fevers) are resolving. In addition, neurotoxicity is typically seen only with severe CRS. Although most of the participants presenting with neurotoxicities have received tocilizumab for severe CRS, it is not clear if these symptoms are related to this agent.

Dr. Nath commented on the possible role of IL-6 and other cytokines in mediating any of these neurological symptoms. He noted that while there are a lot of data showing elevated cytokine levels in these participants, the literature does not support any neurological effects of elevated IL-6 levels on the brain, even at the highest levels measured. In a syndrome called neuromyelitis optica, participants develop optic neuritis and transverse myelitis in multiple segments of the spinal cord. These participants have very high CSF cytokine levels (e.g., in the pg/mL range) but show no signs of neurotoxicity; they are awake and alert. Some of these participants have been diagnosed with depression, but this diagnosis is more likely related to having neuromyelitis optica. There is evidence that treatment with interferon–alpha is associated with CNS toxicity. Use of TNF alone is not associated with neurotoxicity, and blocking TNF in neuroimmune disorders can have adverse effects. Limited experience in participants who receive anti-MAGE T cells or CD19 CAR T cells have identified very specific, discrete focal neurological symptom that may progress. Examination of participants who also have CRS or ‘cytokine storm’ revealed that this complication is not a generalized encephalopathy. Further analysis showed that the T cells were directly targeting the antigens in the brain, and the specific example of CD19 expression in certain parts of the brain was mentioned. Thus, the brain appears to be an on-target off-tumor site for CD19 CAR T cell treatments. Further, the data suggest that a high number of T cells are not needed in the brain to produce a standard cytotoxic immune response.

Dr. Yang underscored the importance of looking beyond the presumption that all side effects, including neurotoxicities, are due to cytokine storm to help identify any underlying causes of these deficits. His group has shown in studies involving immunotherapy treatment of participants with brain metastasis that T cells infiltrate the brain. Dr. Yang noted that while these participants develop other neurotoxicities, they also develop the specific focal defect mentioned by Dr. Nath, which appears to be a distinct entity. In this participant population, aphasia and ataxia are prominent and constitute a hypokinetic or kinetic mutism-like syndrome. The participants are awake and track with their eyes, but they do not follow commands or speak. All of the participants recovered fully, suggesting that the effect was not permanent. Dr. Yang commented that the neurotoxicity seen with CD19 CARs should be separate from all other

neurotoxicities. He pointed out that the collection of symptoms seen in this participant population is very different from the neurologic symptoms seen in approximately 300 participants given IL-2. The neurotoxicity seen with CD19 CARs is not the generalized cytokine syndrome seen with IL-2 or in the approximately 640 participants treated with T cells transfers that did not include CD19 targeting. The CD19-related toxicity falls between the frank on-target off-tumor toxicity seen with MAGE-2 and CEA and the generalized non-specific toxicity seen with IL-2. It does not appear that any critical neurons are destroyed with this therapy, suggesting that the targeted cells may be non-essential. The local cytokine reaction triggered in response to the T cells likely result in edema and other effects that temporarily impair those neurological regions but do not cause permanent damage. Dr. Nath pointed out that the CD19 antigen is present in the speech area and the Broca's area of the brain, thereby providing a basis for the participants' symptoms. However, he posited that direct T cell targeting of CD19 is probably responsible for impairing neurological function, not the cytokine response.

Dr. Lee noted that about 80 percent of participants his group's NIH protocols have had evidence of CAR T cells in the CSF through day 28 post-infusion. However, the presence or level of CAR T cells in the spinal fluid does not necessarily correlate with the on-target off-tumor neurological effects being discussed. Dr. Frey noted that several centers, including UPenn, have imaged participants to determine whether targeting causes local inflammation or edema in the brain. She has seen what she described as "very focused expressive aphasias" in participants in the UPenn protocols, but she does not recall seeing the same types of focal aphasia described by other sites. Dr. Nath noted that a neurologist at UPenn, Dr. Pruitt, has observed focal neurological deficits but no local inflammation on imaging. Dr. Lee described one participant with CNS-2 and a MERS-type syndrome, a mild encephalopathy with reversible splenial lesions, who had transient inflammation in the splenium of the corpus callosum that was associated with visual hallucinations. This participant did not have aphasia, but there was radiographic evidence of inflammation after infusion of CAR T cells. Dr. Yang's team has had one participant with MRI changes, but the imaging was not done at the peak of the participant's symptoms due to the difficulty in obtaining a quality MRI at that time. The investigators have not studied the CSF and MRIs of participants who do not have neurotoxicities over the post-infusion course, so there currently is no set of "normal" reference values for comparison.

Dr. Turtle's group takes a comprehensive approach to investigating neurotoxicity. Results from protocols at FHCRC show that the incidence of clinical neurological symptoms is much higher than what would be expected from lymphodepletion alone. MRI findings are inconclusive. Focal changes on MRI have been observed for some participants, while scans of some participants who have severe clinical neurotoxicities are completely normal. Speech abnormalities, ataxia, and mutism syndrome have been observed. In addition, several participants have developed cerebellar syndromes with MRI changes.

Dr. Park echoed the other participants' comments and supported the concept of a neurotoxicity syndrome that is separate from CRS, based on experience with participants on MSKCC protocols. Many of these participants have either no CRS or, at most, mild fever, but develop neurological symptoms. Cytokine patterns over the course of the post-infusion phase (e.g., for IL-6, IL-8, IL-10, interferon–gamma) did not necessarily coincide with the peak of CRS and typically subsided before neurotoxicities occurred. It is unclear whether anti-cytokine and steroid interventions improved the CRS, if the CRS resolved independent of these interventions, or if these agents worsened the participants' condition. In some cases, CRS began to ameliorate before tocilizumab was given, while in other cases, neurotoxicities subsequently developed days to weeks after these interventions. Analysis of daily IL-6 levels suggests some increase in this cytokine after tocilizumab, but to date no clear pattern has been identified. CSF analysis within the setting of concomitant acute neurological symptoms and thrombocytopenia is challenging. Among the participants for whom cytokine data have been collected, no clear correlation with neurologic toxicities has been observed. In addition, some participants who do not develop any neurotoxicity have very high serum and CSF IL-6 levels and T cells in the CSF.

Dr. Sadelain invited infectious disease experts to comment on the known CNS spectrum of encephalopathy, with or without seizures, and factors that may contribute to brain toxicity, including any role of cytokines. Dr. Nath noted that the literature on cytokine storms with influenza is limited, but there is evidence that the influenza virus may actually enter the CNS itself. A search of cytokine release

syndrome and the brain in PubMed yielded a single case report of childhood influenza from Japan, published in June 2015. This case involved a 2-year-old participant who died within 15 hours of diagnosis. The report includes pathology that shows swelling of the brain. Although the title of the article includes CRS, not a single cytokine was measured. The available literature therefore lacks evidence to demonstrate that cytokines are acting in the brain.

Dr. Hammarskjöld suggested that in addition to establishing common language for a distinct neurotoxicity syndrome, a standardized assessment should also be developed so that evaluations and diagnoses are based on objective measures and criteria. The input of neurologists in drafting a description of this syndrome and for this larger task is needed. For example, it was noted that some conditions, such as dysphasia and aphasia, have well-established batteries that clearly delineate the different types of events. Neurologists are well-versed in these batteries, and there is no controversy as to how they categorize these conditions and any subtypes. In contrast, the rates of dysphasia and aphasia differ when other medical specialists are assessing participants.

The final issue discussed during this session was whether this neurotoxicity syndrome falls under the umbrella of CRS or whether it is a separate entity, even if the two syndromes co-exist in the same participant. Investigators from several different sites stated that they have seen neurotoxicity separated from CRS. In some cases, the symptoms are mild (e.g., hallucinations) without any ongoing systemic CRS. The neurotoxicity syndrome in most cases is a delayed phenomenon that happens after CRS signs and symptoms have resolved. In some cases, neurotoxicity is not seen in participants who haven't had CRS. Whether different mechanisms of action are driving the different toxicities and syndromes warrants further investigation. However, severe CRS might be a potential predictor of a neurotoxicity syndrome. As Dr. Sadelain noted, "if CRS is the signature of strong T cell activation, and if this neurologic toxicity is an on-target off-tumor effect, then those with high tumor burden, severe CRS, and more activated T cells, would have a greater chance of this neurological syndrome". Dr. Yang expects that there is some statistical association between neurotoxicity, CRS, and tumor response. Multiple factors affect whether neurologic symptoms develop, including whether cells penetrate the CNS, and whether the cytokine profile for hypotension and fever is identical in all cases. T cells appear to be driving this neurotoxicity syndrome, but other factors likely contribute to this syndrome as well.

A key point going forward is whether to include neurotoxicity as part of the grading system for CRS and all of the downstream effects of systemic CRS, which will have implications for stopping rules and interventions. Dr. Frey commented that if the interventions for neurologic toxicity are going to be separate from the interventions for systemic toxicity of CRS, it makes sense to not include the neurotoxicity syndrome as part of the formal CRS grading system. However, guidance should be developed to assure that clinicians are aware that neurologic symptoms constitute a potential early treatment-related adverse effect of the CAR T cell immunotherapy.

There was general agreement that systemic CRS and neurotoxicity syndrome are distinct syndromes in participants treated with T cell immunotherapies, even if the two entities interact on some level. Understanding the underlying mechanisms and the etiologies of each of these phenomena should help distinguish them as separate entities and guide development of unified definitions and grading systems for both.

B. CAR T Cell Immunotherapy Trials Targeting Solid Tumors

1. Presentation: CRS Events: OBA Data Analysis

Presenter: Morad Hassani, M.D., Ph.D., OD/OBA, NIH

Approximately 200 subjects have been dosed across all 42 NIH/OBA protocols, targeting solid tumors using CAR-zeta T cells. For this analysis, OBA had enrollment data from 29 of these protocols and safety data from 14 of the studies that reported SAEs. Forty-three related SAEs were reported for these 14 studies combined. Only a small number of these events were assessed as CRS-like events. Two SAEs met the criteria for severe CRS, one met the criteria for moderate CRS, and one was considered a CRS-

like event (i.e., CRS symptoms in the setting of administration of a conditioning cytokine, such as IL-2). No neurologic events or seizures were reported. One of the two with severe CRS events was a case of hemophagocytic lymphohistiocytosis (HLH); the CRS-like event was due to an IL-2 overdose in a subject with neuroblastoma. Limited cytokine data were available for these events. CRS-related cytokine data included IL-6 levels presented for only one event and levels of other cytokines were mentioned for one case. No CRP data were provided. All four CRS/CRS-like cases had hypotension and hypoxia, three had fever, one had tachycardia, and one had acute renal failure. Interventions in these cases included vasopressors in one participant, ventilatory support by intubation in one case, hemodialysis in one case, corticosteroids in two cases, and rituximab (an anti-CD20 monoclonal antibody) in one case. Three of the four cases required ICU care.

2. Panel Discussion

Moderator: Howard Kaufman, M.D., Rutgers Cancer Institute of New Jersey

Panelists: Daniel Lee, M.D., NIH
Helen Heslop, M.D., Baylor College of Medicine
Michael Jensen, M.D., University of Washington
Nabil Ahmed, M.D., Baylor College of Medicine
Daniel Powell, Ph.D., University of Pennsylvania

Dr. Lee reported on the NCI Pediatric Oncology Branch's (POB's) single current protocol, a CAR T cell protocol for neuroblastoma and osteosarcoma. The study uses a third-generation CAR that incorporates a suicide switch, inducible caspase-9 (iC9), and is signaled with CD28, OX40, and CD3zeta. Participants undergo a preparatory conditioning regimen of cyclophosphamide as part of the study. Ten participants have been enrolled to date. One participant passed away before receiving the modified T cells. Of the other nine participants, six have stable disease and three have progressive disease at a dose of 1 million CAR T cells/kg. No Grade 3 or 4 adverse events have occurred, based on grading per modified CTCAE v.4.03 criteria in the paper cited above. A small number of Grade 1 CRS symptoms (fever) occurred in the first few days after dosing.

Dr. Heslop presented a summary and update of her group's protocols for neuroblastoma, targeting GD2 using both virus-specific and non–virus-specific T cells. The initial study used a first-generation CAR and was completed five years ago. The study enrolled 18 participants, 11 of whom had active disease. Three had complete responses, of which two have been durable; two other participants had some tumor necrosis but did not meet the criteria for an objective response. Mild CRS (low-grade fever) was seen in participants at the time of tumor necrosis. No other CRS events were reported. In a collaborative study with Dr. Meyer's group (Kansas) that used the first-generation GD2 CAR in virus-specific T cells post-transplant, one participant had a dramatic T cell expansion with a clinical response and no evidence of CRS during that response. The group's follow-up studies used the same third-generation vector as in the NCI's pediatric protocol described above. They have enrolled eight participants in a neuroblastoma study. To date, one participant had moderate CRS that did not require any intervention. A few other participants have had mild and/or transient symptoms. Although data are available for only a small number of participants, results across studies appear to suggest that responses with the third-generation CAR are better than with the first-generation construct.

Dr. Ahmed reported on CAR T cell solid tumor protocols conducted at Baylor, including studies led by Dr. Stephen Gottschalk, who was unable to attend the meeting. The CAR used in these studies has an exodomain based on the antibody, FRP5. The T cells have a CD28 signaling domain. A retrovirus is used to transduce the T cells. The original T cell platform was for osteosarcoma only; it was subsequently modified on a CMV-specific CTL platform to target sarcomas and glioblastoma multiforme (GBM). A total of 55 participants have been infused across all three studies. Doses range from 1 million cells/m^2 to 100 million cells/m^2. Lymphodepletion is not used in these protocols. One participant had a fever for two days, and some brain tumor participants reported having a headache after infusion. There have been no CNS toxicities attributable to any of the investigational products. Of the 19 participants treated in the first study, two had partial remissions, three had stable disease, and three are long-term survivors (more than two

years now). In another study, 17 participants with GBM, including six pediatric participants, were infused. One participant is in partial remission, and seven have stable disease for at least six weeks. Five of the seven subjects have been stable for ten weeks, and three are long-term survivors (two years to three years). The third protocol uses an EBV-DNR-bispecific CTL platform; the dominant negative TGF-beta receptor (DNR) is included to confer for TGF-beta resistance. This dose-escalation protocol enrolls any participant with a Stage 4 cancer. The infusions started at a very low dose and are now at a dose of 100 million cells/m^2. Eighteen participants have been infused to date. Three were discharged. Six participants with stable disease, several have been stable for ten weeks to 20 weeks, and one is a long-term survivor. Preliminary results from all three protocols indicate that participants do slightly better on the CMV and EBV platforms and with multiple infusions. Some participants have been given as many as six infusions. Cytokine profiles are not impressive. Elevated IL-8 levels are seen through six weeks after dosing, which could indicate either tumor or epithelial injury. Some participants have had quiescent response. Others have mild to moderate swelling in the brain within a few weeks after infusion, which appears to be a local reaction to the T cells, a sort of 'mini-cytokine' response. The Baylor group recently started a new protocol for sarcoma participants. This protocol includes preconditioning with fludarabine for five days, followed by infusion of the CAR T cells over five days. The first three subjects will be enrolled for safety assessment. Pending acceptable results, safety testing will proceed using cyclophosphamide in an additional six participants. Dr. Ahmed mentioned that they have recently infused the first participant with this and therefore it's too early to comment about safety.

Dr. Jensen described two solid tumor (neuroblastoma and glioblastoma) CAR protocols in development and one protocol that has been completed. The initial pilot study used a first-generation CAR that included a suicide gene. The design of that protocol followed an intra-participant dose escalation scheme in which doses increased every two weeks with dose escalating up to 10^9 T cells. No CRS was seen and the only major symptom reported was acute pain at sites of osseous metastases of the disease. The group reengineered the CAR into second- and third-generation constructs in a defined-composition CD4/CD8 T cell product and adjusted the extracellular space domain for infusion. The new protocol is now open at Seattle Children's Hospital and involves a head-to-head comparison of the second- versus third-generation products in glioblastoma participants. The three children enrolled thus far are currently at the first dose level with second generation CAR and one participant will be dosed soon with a third generation CAR. No clinical toxicities or detectable engraftment have been observed yet at this dose. Another glioblastoma protocol uses a human cytokine, IL-13, with a mutation in the sequence that has high affinity for IL-13 receptor a2. In this study, a first-generation CAR with this construct is injected directly into the brain, into a resection cavity reservoir. Some anti-tumor activity has been seen, along with some interesting neurotoxicities in the initially treated participants, including lethargy, aphasia, and some breakthrough seizures. These outcomes suggest that a locally delivered product may be making cytokines locally. This ongoing protocol is being conducted at City of Hope.

Dr. Powell described his group's experience with two solid tumor CAR protocols at UPenn. The first study targets mesothelin and is open to participants with pancreatic cancer and mesothelioma or ovarian cancer with confirmed mesothelin expression. This protocol study uses a second-generation CAR with a 4-1BB co-stimulatory domain. Expression is driven by a lentivirus. Two target doses are planned (10^7 cells/m^2,10^8 cells/m^2) with or without preconditioning with cyclophosphamide. Enrollment to cohort 1 (low dose, no conditioning) is complete (n = 6) and includes two participants with malignant mesothelioma, two with pancreatic cancer, and two with ovarian cancer. All participants were able to be treated, and there were no acute adverse events with infusions. Additional adverse events have been limited. The few Grade 3 and 4 AEs to date have been largely associated with leukocytosis, tachycardia, dyspnea, and hypotension. All six participants had stable disease at day 28 post-infusion; CAR T cells were shown to persist in peripheral blood at this time point. Most notably, there was no CRS in any of the dosed participants. The second protocol is studying a second-generation CAR that targets the EGF receptor (EFGR) variant III in glioblastoma. The construct has a 4-1BB co-stimulatory domain. Expression is driven by lentivirus and the Elongation Factor-1 alpha (EF-1-alpha) promoter. The study includes two participant cohorts, one with recurrent glioblastoma and one with newly diagnosed disease. Subjects receive a single infusion of CAR T cells. To date, six participants have enrolled on this protocol. Adverse events have been limited to neurologic symptoms, including a seizure, in a single participant. Despite evidence of persistence of CAR T cells in all dosed participants, no CRS has been observed. The lack of CRS is not

associated with the lack of T cells. No participant had a complete response in this study. The CAR T cell therapy appears to be safe. The CAR T cells persisted in the blood, were shown to traffic to the brain, and may have had some biological activity based upon evidence of antigen loss.

Following the panelists' presentations, the discussion was opened for further comments and questions.

Noting the difference in CRS events between solid tumors and hematologic malignancies, Dr. Kaufman asked whether these protocols include any other manipulation in terms of additional cytokine support (e.g., lymphodepletion chemotherapy). Dr. Heslop noted that her group is using fludarabine and Cytoxan for the GD2 studies. The neuroblastoma studies use a low dose of IL-2, administered subcutaneously after infusion of the CAR T cells. The Baylor protocols have not used cytokines, but two protocols now include lymphodepletion.

Dr. Lee questioned whether the low rate of CRS/CRS-like events in the protocols targeting solid tumors could have been due to ineffective or insufficient doses of the modified T cell products given to study subjects. Per this concern, he asked about the outcomes (i.e., efficacy of the intervention) for the 200 participants that have been dosed across all such protocols.

Additional information was requested regarding the levels of T cell expansion in these solid tumor protocols, whether these levels reach those seen with CD19 constructs targeting hematologic malignancies, and the method(s) used for such analyses. Dr. Lee noted that some expansion has been seen in some but not all participants in the NCI protocols. This may be due to the relatively low dose of cells administered. Testing is done via polymerase chain reaction (PCR). The expansion that has been seen is notably less than that of CD19 CAR T cells delivered at equivalent doses. While peak expansion has already occurred in most participants, there is some persistence even with tumor progression. The reason for this response isn't clear, but retrospective analysis of cytokines collected over the course of the study could be informative. Dr. Heslop noted a similar pattern for the GD2 studies, that is, expansion that persists for a short term (e.g., weeks) and that is less than what is seen for hematologic malignancy targets. Data from other studies of solid tumors show transient elevation in CAR T cell levels. In the osteosarcoma studies, the highest detectable levels were seen immediately after infusion; although cell levels drop off, many participants have maintained stable disease or remission for an extended period of time. Dr. Gavin noted that for both the EGFR- and mesothelin-targeted therapies, there is a short burst in T cell expansion, followed by a gradual decline in overall counts. The chain at the end of the mesothelin CAR might add an immune component to control persistence, which is being explored. Dr. Gavin noted that these cells can be measured in peripheral blood by flow cytometry and gene marking. Dr. Yang noted that in two additional solid tumor protocols in which the dose of CAR T cells was escalated to up to 10^{10} cells, there was no evidence of efficacy and all observed CRS events were directly attributed to the cytokine being given; all participants in these protocols underwent full lymphodepletion with fludarabine and cytoxan at high doses and received either high- or low-dose IL-2.

Dr. Kohn inquired about the possible relationship between tumor burden, clinical response and rate of CRS, and how results in solid tumors compare with outcomes for hematologic malignancies. In response, Dr. Ahmed noted that it might not be possible with solid tumors to reach an equivalent to the leukemia burden. The largest brain tumor to date among participants in the Baylor protocols was 8 centimeters, and sarcomas have been in the range of approximately 200 grams across all sites. Dr. Powell added that direct comparisons between solid tumors and hematologic malignancies may not be appropriate because the microenvironments of these tumors are very different and can impact efficacy of the T cell therapy. In one location or in one tumor microenvironment, these T cells can eradicate grams of tumor, while in another location or microenvironment, eradiation of 1 milligram of tumor may be difficult. Dr. Powell suggested that investigators start more extensive profiling of the different microenvironments of hematologic malignancies, for instance in the lymph nodes in CLL and the bone marrow in ALL. Whether similar profiles can be done in solid tumors is questionable.

The issue of tumor penetrance in eradicating bulky disease in solid tumors also needs to be taken into consideration. With a focus on solid tumors, investigators may need to target a larger number and more complex set of antigens. Dr. Yang noted that with mesothelin-targeted therapies, among the 13

participants dosed to date at 10^8 total T cells, the investigators have reached only about one one-hundredth of the desired dose. Similarly, even at very high doses of the vascular endothelial growth factor (VEGF)-targeted therapy, there was no evidence of efficacy or toxicity, which was unexpected. The investigators had anticipated this to be a very accessible antigen because it targets the vasculature. These results are consistent with other findings that suggest that the expression of this antigen in various solid tumors is not always clear. For example, intravenously delivered T cells are not effective in all participants with intra-cerebral metastases and even when these T cells appear to target or reach the CNS, the CNS tumors do not respond. Thus, there appears to be a differential effect between CNS and non-CNS targets that needs to be addressed. In these cases, although the target antigen is hard-wired in the CNS, how much is actually expressed on the cell surface under normal circumstances and the value of these antigens as targets for T cell immunotherapy are in question. Whether the CAR T cells are reaching solid tumors also remains unclear. In one analysis, a PCR amplicon from the HER2 CAR used to treat sarcomas was not detected in the peripheral blood or brain tumors of three participants in whom biopsies were obtained. Biopsies were not repeated given the risks to the participants. Other reports indicate that CAR T cells have been found at the tumor site in a small number of other cases. Dr. Yang suggested that target competition may be a real impediment to anti-tumor efficacy. Dr. Ahmed questioned the role of target competition on anti-tumor efficacy, however. He noted that the factors driving the T cells in leukemias and lymphomas are lacking in most of the solid tumor targets. Whether more aggressive lymphodepletion can push efficacy in solid tumors might also be a consideration.

C. Engineered T Cell Receptor (TCR) Immunotherapy Trials Targeting Human Cancers

1. Presentation: CRS Events: OBA Data Analysis

Presenter: Morad Hassani, M.D., Ph.D., OD/OBA, NIH

More than 250 subjects have been treated in the 35 NIH/OBA protocols of engineered TCRs targeting various malignancies, including 14 protocols targeting melanoma, 18 targeting other metastatic or advanced cancers, and three targeting hematologic malignancies. Fifteen of these studies reported a total of 58 research-related SAEs. For this analysis, NIH/OBA staff had data from 20 of the 35 protocols and safety data from 16 studies. We noted more CRS-like events than true CRS, involving other contributing factors. Five CRS-like SAEs were analyzed, although there are probably more of these in our database, and three events that met the criteria for severe CRS and two events that met the criteria for moderate CRS (i.e., 10 total events were analyzed); none of the events involved only neurologic symptoms. Three of the severe CRS events involved IL-2 toxicity– and I should also add that there were some product-related toxicities; these included a case of neurotoxicity with imaging abnormalities, a case of cardiotoxicity, and also a case of hepatobiliary toxicity that were reported in these protocols. In terms of signs and symptoms in the set of ten CRS-like events analyzed we noted fever in eight events, hypotension in all 10 events, hypoxia in eight events, and tachycardia in three events. Neurologic symptoms or toxicity were noted in five cases, including seizures, which were reported for two cases, and acute renal failure occurred in four cases. IL-6 levels were presented for one event, other cytokines were referenced for another event, and CRP levels and elevated CRP were presented for two events. In terms of interventions, vasopressors were used in five of the events, intubations were used in seven, corticosteroids were used in five, and tocilizumab was only used in one of the severe CRS cases. Interestingly, three interventions were used in one case, which included plasmaphoresis, to decrease IFN-gamma, Alemtuzumab (anti-CD52 antibody) to decrease T cell effects, and Ustekinumab (anti-IL-12 antibody) to decrease IL-12 levels. Hemodialysis was used in three cases. ICU care was provided for seven of the ten CRS-like events.

2. Panel Discussion

Moderator: Howard Kaufman, M.D., Rutgers Cancer Institute of New Jersey

Panelists: Antoni Ribas, M.D., Ph.D., University of California, Los Angeles
James Yang, M.D., NIH
Daniel Lee, M.D., NIH

Dr. Ribas focused on three clinical protocols conducted at UCLA, all using modified TCR constructs received from Dr. Rosenberg at the NCI Surgery Branch. In one study, 15 participants with melanoma were dosed with a MART-1 F5 TCR. Two participants on high doses of the TCR had adverse reactions that likely qualify as CRS. Both had hypotension as well as evidence of capillary leak and respiratory distress, with confounding factors such as neutropenic fever and potential infectious complications. Because MART-1 is expressed in skin, these participants had a full body rash that was followed in time by vitiligo. There was no planned plasma collection and analysis, but plasma and serum was collected from these participants during the acute event. There was some clinical response in the treated participants, but most tumor responses were not durable. Cytokine analysis showed that MCP-1 and IL-6 levels in the two affected participants were significantly elevated compared with baseline. There were not differences between the two participants with respect to T cell expansion and in vitro functionality of transduced cells when exposed to antigen. One participant had prolonged aplasia that might have been due to a TCR that was not recognizing the intended target. Two additional studies are using a TCR-targeting NY-ESO-1. One protocol has enrolled five participants, most of whom have synovial sarcomas. The participants are being given low-dose IL-2 twice a day for 14 days per a prior protocol. Several participants have eosinophilia, which is most likely due to IL-2 administration. One of the dosed participants has a potential breakthrough CRS. The third study uses TCR targeting NY-ESO and adoptive T cell transfer with conditioning and low-dose IL-2, given with ipilimumab, a CTLA-4 blocking antibody. Of the two participants enrolled to date, one had respiratory distress requiring transient intubation; this participant also had evidence of pneumonia on CT scan.

Dr. Yang summarized his group's work on eight protocols that have dosed approximately 130 participants. The antigen target differs across studies. Each protocol includes high-dose Cytoxan for conditioning in addition to high-dose IL-2, which is given until signs of CRS develop. Per this design, all participants have CRS or CRS-like events. Some evidence of efficacy (i.e., at least one objective responder) has been seen in each of the eight protocols. On-target, off-tumor neurotoxicity occurred in the protocol targeting MAGE with an HLA-A2 presenting epitope, suggesting that MAGE A-11 in the brain has a cross-reactive epitope presented by HLA-A2. Two of the four participants with neurologic symptoms in this study developed non-reversible neurotoxicity. Dr. Yang commented that these events probably do not contribute much to the interpretation or discussion of CRS or neurotoxicity events because all of these participants received both the conditioning chemotherapy and high dose IL-2.

Dr. Lee summarized the Pediatric Oncology Branch (POB) TCR-based protocol, which uses NY-ESO-specific T cells (at doses up to 10^9) in adult participants with synovial sarcoma. The preparation regimen for this study includes both fludarabine and cyclophosphamide. No IL-2 support is given post-infusion. To date, 11 participants (median age, 31 years) have been dosed, with an overall response rate of 55 percent. There has been one complete remission, five partial remissions, and five participants with stable disease. No Grade 4 CSR events (hypotension requiring pressors) have occurred, but there have been two Grade 3 CRS events involving hypotension. One SAE involving supraventricular tachycardia in a participant with pre-existing SVT is thought to have been associated with a tumor response.

Following the panelists' presentations, the discussion was opened for further comments and questions.

It was noted that at least some of the SAEs seen in these studies are more typical of IL-2 toxicity than TCR-associated CRS. Dr. Kaufman inquired whether a separate CRS grading system is needed for such participants.

Dr. Yang noted that there will be some CRS, and presumably efficacy, if adequate numbers of T cells are given. Preconditioning can substitute for cytokine support to some degree and will mitigate the frequent CRS symptoms seen with aggressive IL-2 dosing because participants won't have responses such as pulmonary edema or need to go on pressors for hypotension. Dosing can be titrated up or down depending on the degree of toxicity that can be managed in a particular setting. Dr. Lee pointed out, however, that as the dose escalates, there will likely be more on-target, off-tumor toxicities or other unknown or unanticipated adverse effects. Dr. Yang noted that the general, nonspecific cytokine-related toxicity seen in some studies is relatively straightforward and distinct from what is seen with CD19 and in

the MAGE A-2 protocols. Dr. Ribas commented that IL-2-related toxicities usually manifest several days after dosing, compared with CRS events, which typically occur much sooner (e.g., within one to two days). Thus, the timing of when symptoms first manifest could be a factor in distinguishing between CRS and more general events. A clear grading system and separate definition of the components or criteria for these nonspecific toxicities should be considered.

Dr. Atkins inquired about the gene-modified T cells that appeared to target a shared antigen in the MAGE protocol, resulting in serious on-target, off-tumor adverse events, and what difference in toxicity, if any, would be expected with other targeted antigens. Dr. Yang explained that, in any setting, administration of high-dose IL-2 and full conditioning, which is done in all eight of the group's protocols, would generate CRS. Whether anything other than the targeted TCR caused or contributed to the on-target, off-tumor toxicities have not been shown. The one participant with cholangiocarcinoma, who has nearly a 2-year ongoing response, has had no on-target, off-tumor toxicity that could be identified. After four doses of IL-2, however, the participant developed CRS, at which point the investigators stopped the IL-2, and the participant recovered completely. Closer assessment of other similar cases with known efficacy could be informative.

Dr. Sadelain asked about any differences in toxicities or response in studies that use two TCRs. Dr. Yang noted that responses were seen in both such protocols, albeit in a small number of participants (i.e., fifteen) and utilized a low-affinity receptor. The presumption is that a higher-affinity receptor would probably be more efficacious. Results in animals show both increased and reduced effectiveness. Thus, this remains an open question.

Dr. Atkins also asked about use of IL-12 or other agents to increase proliferation of armored, modified T cells and the impact of this type of regimen on toxicity. Dr. Yang's group has used IL-12 in some protocols, which changes the functional profile of the T cells in response to treatment. A high response rate was reported for a series of participants who were given T cells that were transduced to express IL-12 with a TCR-triggered promoter. The maximum number of T cells administered was 3×10^9, and participants did not receive systemic IL-2 because of the potential toxicity of giving the two cytokines together. CRS symptoms included fevers and some additional side effects. In the one participant who had profound toxicity, IL-12 expression and secretion was essentially uncontrolled; extremely high levels of IL-12 were released from a NY-ESO-1-transduced TCR that was also transduced to secrete IL-12. The systemic levels of IL-12 in this participant were in the 60,000 to 80,000 pg range; systemic interferon levels were approximately 200,000 pg. At these levels, survival is unlikely. One of the reasons this protocol was stopped was because the peak response rate of about 60 percent at the highest dose level did not seem to be durable. Use of these constructs may be considered for future protocols if a better way to control IL-12 release can be identified.

Dr. Turtle noted that the doses of T cells used are considerably higher in TCR-engineered studies (e.g., 10^{10} or 10^{11}) than in CAR T cell protocols. Given this difference, he inquired about the manufacturing process. Specifically whether repetitive *in vitro* stimulations with peptides are used to generate that many TCR-modified cells, or if the investigators follow the same process used in a lot of the CD19 CAR studies, that is, transducing cells without any *in vitro* stimulation. Dr. Yang explained that the only protocols in which very high T cell doses are not used are the CD19 CAR protocols, presumably because of the greater susceptibility of hematologic malignancies to much lower doses of T cells. He added that in every solid tumor protocol, except the IL-12 example described above, the objective has been to escalate the dose to between 10^{10} and 10^{11} total T cells. This number of cells is generated by anti-CD3 in feeders; cells are grown to about 50 million and then expanded by another 1,000-fold. Whether repetitive *in vitro* stimulation might be exhausting the cells to the point of affecting a difference in the response is being explored. Pre-clinical evidence exists to support that contention, but other groups have found a relationship between cell number/dose and efficacy.

Dr. Ribas's team uses a different process that involves growing the cells within a week without rapid expansion. The target is to have a total of 10^9 infused T cells. A percentage of those cells will be TCR-transduced, with the proportion ranging from 10-20 percent to up to 85 percent. Nine of the 15 participants in the MART-1 TCR protocol have had objective responses, but all of the responses have

been transient. The initial high rate of tumor shrinkage does not persist. The dose of NY-ESO-1 TCR cells is about one to two logs lower than what is used in the NCI Surgery Branch protocols; two of five participants in the UCLA protocol for synovial sarcoma have ongoing durable responses, including one participant who is close to one year post-treatment. In response to a question about whether co-stimulating TCR-transduced cells leads to an augmented cytokine production by those cells, Dr. Ribas commented that while co-stimulation will likely increase signaling, *in vitro* cultures show that co-stimulation results in release of huge amounts (i.e., at the hundreds of thousands picogram level) of interferon–gamma in a very antigen-specific manner in a very short period of time. Thus, co-stimulation is probably not needed.

Panelists were also asked to comment on use of corticosteroids, which seemed to be helpful in this group of participants. Dr. Yang noted that steroids are usually given later in the disease process, for example, after giving two pressors for two to three days if the participant is otherwise stable. The group will intervene sooner for participants who are not stable. There is little evidence, however, as to whether use of steroids will salvage participants at this stage. Thus, whether and when the use of steroids is effective remains an open question.

In brief, additional pre-clinical work is needed to better understand the mechanisms of CRS following TCR-modified immunotherapy interventions against various cancers. Use of mouse models or other appropriate models to test these mechanisms should be a high priority for translation into the clinical setting.

D. Public Comment

No comments were offered.

IV. Session II: Establishing a Uniform Definition and Grading System for CRS

A. Discussion Session

Moderator: Donald Kohn, M.D., University of California, Los Angeles

The following questions were posed for discussion:

- What should be the clinical criteria for a common definition of CRS?
- What grading systems currently exist? What should be the common grading criteria for CRS?
- How do CRS events differ in CAR immunotherapy protocols against solid tumors versus hematologic malignancies? Are CRS events in engineered TCR protocols different?
- What cytokines are most useful in identifying CRS?
- What are the optimum methods and timing for collection of cytokines for monitoring CRS?
- Are there other available or potential biomarkers for diagnosis and monitoring of severe CRS?
- Can cytokine or other biomarker data be expected to be available in time to inform therapeutic decisions?
- How does one best distinguish CRS from infection/sepsis?

An ultimate goal of this workshop was to establish a uniform definition and grading system for CRS. The ideal system should be simple and robust and use a scale that can be applied consistently across multiple sites but that still captures the nuances of this complex condition. Workshop participants were asked to consider whether the published CRS definition and grading system, as reviewed during the meeting, needs only minor revisions or whether new criteria and grading systems need to be developed.

There was general agreement about the definitions and criteria for Grade 1 and Grade 4 CRS. Grade 1 CRS is defined by constitutional symptoms (e.g., fever, nausea, malaise, myalgia) that are not life threatening and are relatively mild in nature. Grade 4 CRS is severe and considered life threatening;

symptoms are based on CTCAE criteria for organ toxicity or requirement for ventilator support. The challenge is how to clearly define and distinguish between moderate to severe CRS. Grade 3 CRS has been defined by symptoms that require and respond to aggressive intervention, such as hypotension, hypoxia, and persistent high-grade fever, while Grade 2 CRS involves symptoms that are moderate in severity and seriousness and that respond to limited or moderate intervention. Grade 3 organ toxicity is based on CTCAE Grade 3 toxicity definitions. Dr. Lee noted that the algorithm presented in the paper published in *Blood* (Lee et al., 2014) recommends administering anti-cytokine therapy for Grade 3 CRS in an effort to avoid progression to Grade 4; a similar approach is advised for severe Grade 2 CRS and Grade 2 CRS participants with persistent co-infections. Defining and managing neurologic symptoms was discussed separately during the meeting (see below).

Because certain early and aggressive treatments of CRS could limit the efficacy of the immunotherapy, some degree of CRS that would, in most cases, be reversible or managed with appropriate supportive care should be tolerated. Thus, such interventions should ideally be used to prevent severe and life-threatening symptoms while not interfering with the anti-tumor effects of the T cell therapy. Establishing a uniform grading of the severity of CRS could inform a treatment algorithm for management of CRS.

Reaching consensus on a uniform definition and its constituent components is needed on several levels, to address under- and over-reporting of CRS and CRS-related events, for participant safety, and for monitoring and management of adverse events. Any changes to the current definitions and system should be based on available data on toxicity, interventions, and outcomes for subjects with CRS or CRS-like symptoms following immunotherapy. Workshop participants recognized that while it would be ideal to have data from a much larger number of participants and more specific intervention- and treatment-related data, such as dose-limiting toxicities (DLTs), interim guidance can be developed using the existing data. Further modifications and refinements can be made as more participants are dosed and additional data becomes available. The goal was not to define how individual studies should be designed but rather to arrive at a common definition that can be used at multiple sites based on data pooled from different studies. Specifics regarding protocol design are left to the investigators.

Participants pointed out that many Grade 2 and Grade 3 symptoms and complications are similar, and that participants can have both Grade 2 and Grade 3 symptoms, making it difficult to establish a clear, objective definition and grading system. Dr. Lee explained that the intent of the published definitions and algorithms was that the grade of organ toxicity would be based on the CTCAE 4, with the presumption that participants could have a constellation of symptoms. For example, a participant could have renal failure with a rising creatinine that hasn't reached a Grade 3 yet but still require multiple vasopressors. In this case, based on the vasopressor requirement, the participant has fulfilled the criteria for Grade 3 CRS and should therefore receive anti-cytokine therapy. Participants recognized that each center has different policies and different styles of management of participants in the ICU. For example, some centers can give low-dose dopamine on a non-ICU floor, while at the NIH Clinical Center, vasopressors can be given only in the ICU. Given this requirement, NIH protocols include a rule where if a participant’s blood pressure drops by more than 20 percent below their baseline, despite two boluses of IV fluid, the subject is admitted to ICU because of the potential for decompensation. Given the range of strategies and policies across centers, clinical management should not dictate how to grade CRS.

One suggestion to address differences in center-specific policies and concerns about symptoms that overlap between grades was to consider developing a cumulative score that can be adjusted according to each participant’s baseline information and status instead of basing intervention thresholds and decisions on the highest NCI CTCAE grade. This approach would be similar to guidance based on symptoms and could be applied across clinical settings. In addition, it would be less dependent on clinical judgment as to the severity of CRS and suspected or known symptoms of CRS than the current guidance. Dr. Ahmed suggested following the strategy used to define and identify predictors of hemophagocytic lymphohistiocytosis (HLH), a life-threatening syndrome of excessive immune activation that manifests as severe sepsis, respiratory distress, and hypotension. It was not clear for a long time whether these participants were septic or if the constellation of symptoms constituted a different diagnosis. The condition eventually was defined using regression analysis based on the number of participants who responded to treatment for HLH and had these criteria. The symptoms and severity of these complications therefore

serve as a positive predictive value. An analogous assessment for CRS might be used by determining the number of participants at the end of the scale who have hypotension. Dr. Powell noted that such a scoring system would go beyond “reinventing” the CTCAE to identify and distinguish the degree of severity. Scores based on the severity of symptoms could be used to stratify the participant’s status and then serve as a predictive mechanism as to whether or which intervention is needed or would be helpful. For example, the guidance could specify that all Grade 4 participants should have aggressive intervention, while those with Grade 2 events should have limited interventions. UPenn investigators follow a modified version of this plan, under which few Grade 2 participants have been treated, while 25 percent of Grade 3 and more than 90 percent of Grade 4 participants received interventions. For the purposes of a unified CRS grading system, it might be preferable to avoid being overly quantitative in developing guidance and recommendations, allowing for introduction of a subjective element. Per this suggestion, instead of specifying the level of oxygen to start supportive care, the criterion would be hypoxia requiring oxygen. Dr. Champlin cautioned against going beyond what is known based on available data, given that the number of affected participants and interventions is small in several cases. Consistent with the suggestion to focus on qualitative rather than quantitative guidance, defining mild, moderate, and severe criteria should be considered.

Participants discussed whether there are any hallmark data or assays that could be incorporated into grading criteria and be used to assess participants following immunotherapy and when a participant begins to develop signs and symptoms of CRS. Most agreed that until more data are available, participant outcomes are better predicted based on clinical definitions (e.g., grading of symptoms) rather than biological definitions (e.g., cytokine levels). There is some relationship between cytokine levels (e.g., IL-6) and CRS but not in the absence of clinical symptoms. In addition, cytokine levels vary due to numerous factors. Furthermore, because the timing of an intervention can be critical in preventing or slowing the development of serious side effects, cytokines may not be clinically useful in most cases. Another issue is that elevated levels of cytokines are also detected in the presence of sepsis. Upon further review and discussion, meeting participants agreed that elevated IL-6 level is a good measure for post hoc confirmation but not for predicting participant outcome.

Elevated CRP levels may constitute a reasonably good predictor of CRS, especially when done in conjunction with clinical assessment. CRP is also a good surrogate for inflammation and elevated pro-inflammatory cytokines and can be determined relatively quickly. Dr. Lee noted, however, that because CRP is excreted from the liver in response to IL-6, there is about a 12- to 24-hour delay in when CRP levels increase. The timing of when CRP is measured is also important given the circadian patterns in the levels of this protein. Because CRP is also detected with sepsis, Dr. Turtle suggested using ferritin to distinguish between sepsis and CRS. Results from his studies show that ferritin levels in severe CRS are significantly higher than in sepsis.

Multiple groups are investigating correlative cytokine data to better define CRS with the hope that these data might be used to predict the severity of CRS and inform clinical decisions at some point in the future. Absolute cytokine values and “fold” increases in cytokines should not be included in a grading system, however, given the marked variability in cytokine levels among participants not only at baseline but also during the entire post-infusion period. While causative relationships may not ultimately be established, surrogate markers and correlative measures might be identified through ongoing data collection and analysis.

Because clinical decisions will be made based on the definition of CRS, it will be important to reach consensus, or at least have some level of agreement, about the threshold(s) to begin to treat the cytokine release or T cell activation syndrome. For example, should the intervention start when the participant needs one or two pressors or requires intubation? Regarding intubation, a further threshold to consider is the purpose of this intervention, that is, whether mechanical ventilation is being used for airway protection in the setting of neurologic symptoms (e.g., somnolence) or for respiratory failure.

While there was general agreement regarding the decision to always intervene at Grade 4 and either to not intervene or to proceed with limited interventions at Grade 2, questions remain regarding how to proceed with Grade 3 symptoms. Meeting participants also explored the proposal to set up a strategic

grading system that follows the natural history of toxicities to the point of intervention and that includes possible pre-emptive therapy at the onset of Grade 1 symptoms. Such a plan could be used to determine whether there is a cost for intervening too early and whether a pre-emptive intervention is unnecessary. Criteria would be needed to identify high-risk participants who would be treated at the first sign of CRS to blunt the evolution of severe CRS. Dr. Jensen noted that pre-emptive interventions (e.g., anti-IL-6 antibody and corticosteroids) have been used with a few high-risk participants in protocols at the University of Washington with excellent therapeutic effect (high-level engraftment and remission) without progression of CRS or the need for intensive care. Further studies of this approach are needed.

The timing of pre-emptive or prophylactic intervention is likely to impact subject outcomes. The effect of treating Grade 3 (or late Grade 2) participants with tocilizumab may be very different than that of prophylaxis at Grade 1 given that T cell expansion may correlate with both the degree of severity of CRS and the efficacy. Administering tocilizumab at Grade 1, when T cell expansion is minimal, might interfere with the full anti-tumor activity; whereas, anti-tumor efficacy might not be blunted if tocilizumab is given at Grade 3. Dr. Atkins asked whether a placebo-controlled pilot study would be useful to assess the effects of early tocilizumab intervention in Grade 3 CRS. One group that seems to have a predictable rate of CRS is ALL participants with a high percentage of blasts in the bone marrow. If a study could be designed with equipoise for each study group, this participant population might be eligible for a prophylaxis protocol that compares toxicity and proliferation with early tocilizumab versus placebo. Another approach in the cohort currently being followed by Dr. Lee's team is to give participants with a high tumor burden an intensive lymphodepleting regimen in an effort to lower the tumor burden, and, hypothetically, reduce the risk for severe CRS and the dose of tocilizumab needed to manage CRS. In chemotherapy refractory participants, however, more aggressive lymphodepletion could increase T cell expansion and increase toxicity.

While it isn't clear if tocilizumab changes the trajectory for T cell expansion, it can impact whether the subject needs intensive care or not. To that end, there has been a shift in some clinical settings to use tocilizumab earlier to prevent subjects from having to go to the ICU. This change in practice is analogous to treatment of graft-versus-host disease (GVHD), in which greater availability and use of effective drugs has led to a decrease in Grade 3 and Grade 4 disease. Regarding prospective studies, Dr. Atkins noted that a grading system is necessary to define populations to test the impact of interventions such as tocilizumab or corticosteroids on toxicity, T cell proliferation, levels of IL-6, CRP and ferritin. Dr. Kohn asked whether, given that more than 400 participants have been dosed, there existed comparable data that could be pooled and analyzed based on the intervention. Dr. Sadelain noted that an ideal definition would be both clinically and biologically based, available in real time, and allow for the grading of participants to define interventions. Until that can be developed, a clinical definition encompassing some cytokine data should help with reporting and analyzing data. Given the variability in cytokine levels, Dr. Kohn suggested, as a short term goal, the development of a method for standardized collection of data on cytokines, CRP, and ferritin as has been done by a consortium for primary immune deficiencies research.

Participants recognized that there will always be a "gray zone" when it comes to clinical decision making, but also acknowledged that having uniform thresholds should help standardize treatments between institutions as well as across disciplines. For example, leukemia and transplant doctors might be willing to wait before intervening while other disease specialists who are not familiar with the degree of toxicity seen with CRS might decide to intervene much earlier. In this sense, it might be useful to have clear thresholds to help guide the development of a uniform definition. To achieve this goal, investigators across sites and protocols should be collecting real-time data at the point of intervention (e.g., lung function, hemodynamic status, organ toxicities) as well as data on post-intervention outcomes. Both prospective and retrospective analyses should be done if the plan is to use this information to make clinical decisions going forward. Pooling data across sites should provide for more elaborate quantitation of toxicities.

Participants noted the importance of having a system or tool to predict how ill a participant may become, especially with a novel therapy, for development of safety and management plans within a regulatory context. Dr. George noted that from the FDA's perspective, a preliminary system that can be used to assess safety while data for a unified definition and more precise grading are being collected in a

standardized manner is a reasonable starting point. Whether the same system and definition encompass CRS in all settings—hematologic malignancies, solid tumors, CAR T cells, and TCRs—still needs to be determined. A better understanding of the underlying mechanisms and drivers of CRS in each scenario will help clarify this issue.

Dr. Hammarskjold returned to the question of the nomenclature for CRS and whether the name of the syndrome should be changed to something that reflects the more generalized adverse events, such as T cell immune-related adverse events or T cell activation syndrome. Although cytokines are the only factor identified in the current name for this syndrome, one of the reasons for considering a new definition is to make it easier to compare toxicities across protocols and to increase the safety of subjects enrolled in this type of clinical protocol. Several participants supported consideration of a name other than cytokine release syndrome. “T cell activation syndrome” was one of the preferred terms because it reflects what is actually happening as T cells proliferate and expand and encompasses the effects of cytokines and other factors as well.

Dr. Zoloth pointed out that there are two audiences to consider in determining how to describe or define a specific condition or complication and the participant’s status, investigators and participants along with their families. For investigators, the goal is education, which would entail providing the most specific information and guidance possible. For participants and families, the consent documents need to explain, in clear understandable “lay” language, the potential risks and side effects of the study treatment and how those risks will be monitored and managed. The level of uncertainty that has been discussed during this meeting may be difficult to convey in the consent documents and process, and it may also raise additional questions and concerns for participants and their family members.

There was general consensus that, while there is some overlap between CRS and neurologic symptoms, neurotoxicity seems to be a separate entity from CRS. In addition, criteria and terminology for the real-time defining event with neurotoxicity need to be clarified to ensure uniformity in the degree of toxicity. Delirium, confusion, and encephalopathy are currently all used to define neurotoxicity. CTCAE is often but not always used in these cases. Participants agreed that the field would be greatly enhanced by having a common language for how to define and assess neurotoxicity with and distinct from CRS.

Participants asked whether other organs, including the heart, need to be taken into consideration in developing guidance for grading and managing CRS in this subject population. Several participants identified the heart as a target organ in CRS. Dr. Atkins noted that about five percent of participants in his group’s studies with high dose IL-2 develop myocarditis and acute decreases in ejection fraction that resolve over time. Some participants continue to have arrhythmias after immunotherapy is completed. The mechanism for these effects isn’t clear. Dr. Yang added that participants who get frank myocarditis have elevations of cardiac enzymes, which can be easily identified through close (daily) monitoring. Some participants have profound decreases in cardiac contractility without enzymatic abnormalities. Testing cardiac output and enzymes could be part of a management plan. Most participants with treatment-related cardiac symptoms recover quickly, often within about a month. Whether cardiac abnormalities in these participants are a manifestation of elevated cytokines, monitoring for cardiac symptoms could be done via testing output and enzyme levels.

Coagulopathies are also important and present an opportunity for improved monitoring and management of hypoxia and hypotension. It was noted that nearly all severe CRS and even many Grade 2 CRS cases have some degree of coagulation abnormalities. Closer monitoring and increased use of colloids and fresh frozen plasma, where indicated, could help mitigate some of the other more serious toxicities seen in this participant population (e.g., pulmonary edema).

Participants noted the importance of not confusing the definition of CRS with a grading scale for severity. Identifying the threshold for when to intervene could help in establishing a uniform definition of CRS. A question was posed, however, as to how criteria for a CRS grading system can be developed without a clear definition for the condition. Consensus on the definition of CRS was not reached during the meeting. A decision regarding the naming of the grading system also needs to be made, specifically, whether to keep the numerical grades (i.e., Grades 1 to 5) or to use the descriptors mild, moderate, and severe.

Dr. Kohn suggested a shorter term goal of collecting data in a standardized manner in which each protocol captures the same information (e.g., levels of "X" number of cytokines, CRP, ferritin, etc.). He noted the example of a rare disease network consortium on primary immune deficiencies that is trying to scale lab results collected from various sites, investigators, and protocols over 20 to 30 years. Despite the challenges with this task, the group has progressed to the point of establishing a standardized definition and reference laboratory values. With time, planning, and coordination, a similar result is possible for CRS despite differences in participant populations, T cell constructs, and how and when cytokines and other factors are measured. A cataloguing of papers and presentations of CAR/TCR immunotherapy protocols and meta-analysis of data collected across protocols would be a significant undertaking but an important step in this process.

A plan for proceeding with draft definitions and grading systems for CRS and neurotoxicity was provided during the discussion wrap-up below. Dr. George clarified the FDA's role in this process, which she described as a predominantly scientific endeavor. The FDA will defer to the sponsors to come up with a consensus definition and will serve primarily in a supportive capacity to provide comments and suggestions on any consensus definitions and grading systems that are developed.

B. Public Comment

No comments were offered.

V. Session III: Optimizing the Management of CRS

A. Discussion Session

Moderator: Michael Atkins, M.D., Georgetown University School of Medicine

The following questions were posed for discussion:

- What are the potential contributing factors for severe CRS? What role do the following have in severe CRS? Are there common treatment strategies that could be elements of a CRS management algorithm?
  - What are the roles of corticosteroids and monoclonal antibodies (e.g., tocilizumab, siltuximab) in the management of severe CRS? Is there an optimum dose/frequency/timing of administration? Should there be concerns about anti-tumor effects?
  - What other strategies or agents are being successfully employed?
- What is the role of suicide genes, and has one been successfully used to abort CRS? For the different suicide genes, when is the expected onset of action compared to the pathogenic course of CRS?
- What are the contributing factors to neurologic toxicity, and how are these being managed?
- What data sharing mechanisms would facilitate optimization of CRS management?

Although participants were not able to come to consensus on a definition or a grading system for CRS in the prior session, the focus in this session was on reaching consensus regarding when to start therapy for CRS or CRS-like symptoms in participants given T cell immunotherapy. Absent a unified grading system, meeting participants were asked to consider if there are any factors such as the severity of certain side effects or a time point that could be used to inform this decision-making process.

The development of CRS is often, but not invariably, associated with proliferation of TCR- or CAR-modified T cells and anti-tumor activity. Several cytokines have been reported to be elevated in the serum following infusion of modified T cells, most commonly, interferon–gamma, TNF–alpha, and IL-6. Management of CRS has included supportive care, corticosteroids, and anti-cytokine agents such as tocilizumab, siltuximab, etanercept and alemtuzumab. As discussed below, the role of suicide genes in the management of CRS remains unknown. Regarding neurologic toxicity, per published reports and data

summarized during the meeting, several participants in CAR protocols across institutions have experienced obtundation, seizures, aphasia, and mental status changes, many of which have been reversible. Some of these may be related to CRS, but it is not clear whether they result from systemic cytokines crossing the blood–brain barrier and engaging cytokine receptors in the brain or from direct cytokine production in the CNS.

Dr. Jensen described the criteria used at Seattle Children's to determine when participants should be admitted to ICU. Transition to intensive care occurs when a participant needs to go on a pressor (e.g., after two bolus doses of fluids fail), shows an escalating need for higher doses of pressors (e.g., after low-dose pressors fail), or has another severe clinical complication. Tocilizumab is given first and is usually followed shortly thereafter by steroids. Others (Dr. Lee) noted that their facilities follow a similar paradigm for treating severe or worsening CRS: two pressors and intervention with tocilizumab and steroids. Dr. Park commented on how the approach at MSKCC has evolved as the clinical experience with immunotherapy has advanced. For example, the group has shifted in the past year or two to using tocilizumab as a first-line agent in response to early signs of severe CRS. Escalating up to the requirement of one pressor is usually the threshold to initiate the tocilizumab, and the usual trigger for intensive care is the need for any pressors. Dr. Park noted that the team does not require two pressors because the clinical difference between needing one vs. two pressors is minimal. If the participant does not improve in 12 hours in terms of hemodynamic instability or escalating pressor requirement with tocilizumab, then steroids are added to the treatment plan. In addition, for respiratory problems, the team tries to intervene after respiratory distress occurs and before intubation is required. These are the criteria and regimens included in current CAR/TCR protocols at MSKCC. The UPenn team uses a modified version of the algorithm published as a supplement to the group's article in *Science Translational Medicine* in all of their protocols.

Clinicians also have to take competing issues into account, including whether these issues involve treatment, participant population, or a participant's stated preferences or choices. Dr. Maloney noted that some participants, for example, do not want to be given corticosteroids under any circumstances because of the potential to impede the anti-tumor effect of immunotherapy. The kinetics of how the participant's status is changing also influences the type of front-line therapy that might be needed. If a participant is progressing rapidly from fever to rigors to needing intensive care and pressors over the course of a few hours, steroids will usually be given sooner rather than later. Decisions about use of steroids will be different for participants whose condition worsens over 24 to 48 hours. Approaches also differ for pediatric vs. adult participants. For example, for adult ALL, the UPenn team intervenes before participants need to go to the ICU. While the timing of this transition is difficult to predict, intervention for some participants occurs in accordance to the definition of Grade 2 CRS presented during the meeting. Thus, intervention for CRS will occur if the participant needs low-dose pressors, when supplemental oxygen is initiated, and with hypotension that is not responsive to fluid boluses. In contrast, UPenn clinicians treating pediatric participants tend to wait until Grade 3 or 4 CRS before intervening. Dr. Heslop concurred with the approach outlined by Dr. Jensen and with the UPenn strategy for adult vs. pediatric participants. Dr. Lee noted that at NIH, clinicians do not wait until severe toxicity (Grade 4) to intervene, but they also do not try to prevent ICU admissions. He added that the incidence of ICU admissions for CAR therapy is about the same as that for bone marrow transplant. NIH clinicians manage CRS strictly by the treatment algorithm published in the Lee et al. 2014, which was presented and discussed during this meeting; this algorithm is included in NIH protocols, with the goal of limiting Grade 4 cytokine release to less than ten percent.

The course of steroids may also need special consideration. Dr. Frey noted, for example, that septic management of participants includes stress-dose steroids (e.g., hydrocortisone), which differs from steroids given for aggressive management of CRS. Another variation in practice involves use of tocilizumab in conjunction with very low-intensity stress-dose steroids; the intention in this case is to administer corticosteroids for a limited time only, until the participant stabilizes. Participants whose CRS does not improve will be given tocilizumab first and the timeline for this practice varies, but if no improvement is seen within 24 to 48 hours after the initial administration of tocilizumab, a second challenge of the same agent is considered.

Dr. Atkins summarized the common features for current practice across institutions, as presented during this session. There appear to be two main strategies regarding transition to ICU care. Some sites treat before the participant gets to the ICU to try to prevent the most severe toxicities and admission to the ICU, which usually occurs if improvement is not seen with interventions such as low-dose pressors and bolus fluids. Other sites transition to ICU care earlier in the participant's course. A distinction between treatments of adult vs. pediatric participants was also made. The threshold for intervention is lower for adult participants, for whom the pace of deterioration might prompt quicker intervention. Sites also appear to differ as to use of tocilizumab alone vs. tocilizumab and steroids as the first treatment for CRS. Further discussion focused on whether any groups use steroids first and whether an agent other than tocilizumab would be used for intervention for non-neurologic symptoms. Participants (Dr. Ribas) noted results presented by Dr. Yang, who reported on the lack of benefit with tocilizumab. Dr. Park added that the response to tocilizumab is quick and participants who respond to this intervention typically improve within a few hours; fever is usually the first symptom to improve, and the need for pressor requirements also rapidly decreases. The Memorial-Sloan Kettering team has a time threshold for response to tocilizumab but has not studied use of steroids as a first-line intervention unless it is for treatment of a high-grade neurological event without hemodynamic and respiratory problems.

Dr. Nath commented that the decision to use steroids for neurotoxicities should be guided by whether the blood-brain barrier is compromised, which can be determined with gadolinium-enhanced MRI. Regarding antibodies to the IL-6 receptor and whether such agents can cross the blood-brain barrier, results of a small study recently published in *JAMA Neurology* (Ringelstein et al.) showed tocilizumab to be very effective in treating neurologic symptoms. Five of the eight participants in this study of neuromyelitis optica, an autoimmune disease of the central nervous system, responded very well to tocilizumab at doses of 6 to 8 mg/kg; the results suggested good efficacy of the drug to cross the blood-brain barrier and target IL-6 receptors.

Dr. Budde asked whether using a higher dose of tocilizumab reverses symptoms faster than lower doses (e.g., 4 mg/kg vs. 8 mg/kg). Dr. Turtle commented that both doses have been very effective in protocols conducted at FHCRC. It isn't clear if the higher dose abates symptoms more quickly than the lower dose, however. In addition, more data are needed to demonstrate whether giving an 8-mg/kg dose of tocilizumab first avoids the need for subsequent doses of 4 mg/kg. The UPenn team (Dr. Porter added) uses 4 mg/kg dose, which is the approved dose for adults and which works well for many participants. Interestingly, the recommended dose is 8 mg/kg in the pediatric population. However, since a number of participants with refractory CRS have not responded well to the 4-mg/kg dose, this may lead the UPenn group to start using the higher 8-mg/kg dose. Dr. Porter noted that while the higher dose has an acceptable safety profile, it does not appear to be more effective than the lower dose. He added that given high IL-6 levels, it is difficult stoichiometrically to block all circulating IL-6 but it might not be as difficult to block all IL-6 receptors at a higher dose of tocilizumab. Dr. Lee added at NIH, protocols in children through young adults use an 8-mg/kg dose of tocilizumab, which is consistent with the pediatric literature. Steroids and tocilizumab are given to rescue participants who present with rapid deterioration, which has happened in a small number of cases. Dr. Lee noted that because the NIH team can check cytokine levels in near real-time in a Clinical Laboratory Improvement Amendment (CLIA)-certified lab, results of this testing are used to guide decisions regarding which interventions to use and when to use them. For example, the NIH team looks for an initial spike in IL-6 to start tocilizumab treatment. Within a day or two, levels of cytokines that were elevated often drop dramatically.

Participants were asked about other strategies or agents that have been successfully employed to manage CRS. Plasmapheresis has been used in combination with other interventions in two or three cases TCR protocols. The UPenn team (Dr. Porter mentioned) has used the anti-IL-6 antibody siltuximab in two cases but remains concerned as to whether IL-6 is actually being blocked. As with participants who don't respond to tocilizumab or have other toxicities, the same IL-6 spike is seen within 24 hours after dosing with siltuximab. One participant appeared to respond to siltuximab, but additional information and experience are needed to draw any conclusions about the efficacy of this agent. Etanercept (an anti-TNF-alpha monoclonal antibody) has also been used in a few cases as mentioned earlier. To date, it appears that none of the groups have used *JAK* inhibitors to block cytokine signaling as kind of a rescue therapy in refractory participants. Dr. Maloney asked whether anyone has used plasmapheresis? Dr. Hassani

added that as he already mentioned there was one case, which was in a TCR protocol that used plasmapheresis as well as other interventions (i.e., Rituximab and alemtuzumab).

Dr. Park inquired about the earliest time point that can be considered for administration of these agents to avoid or at least minimize the risk of compromising the anti-tumor effect of immunotherapy. Specifically, what data, if any, show whether these treatments interfere with either T cell expansion or anti-tumor effects of T cell immunotherapy? Dr. Hassani noted that the NIH/OBA data analysis showed that steroids were used as early as day 2 after initial CRS symptoms were observed and as late as day 12, while tocilizumab was used as early as day 2 and as late as day 9. In some cases, steroids seemed to be used later in the course of CRS, and sometimes only for neurologic toxicities. In addition, some participants received only steroids, but a relatively large number of participants with severe CRS received both steroids and tocilizumab. In brief, in terms of responses, tocilizumab seems to have a very abrupt hemodynamic stabilizing force. The range of responses for the individual agents, and combinations of agents, was presented by the panelists and other participants. Dr. Maloney cited a few cases of explosive CRS in participants who maintained high levels of CAR T cells even with aggressive treatment for the CRS. In one case, CAR T cells were ablated after chemotherapy was given to treat the participant's progressing cancer.

Dr. Atkins asked whether an abrupt reduction in CAR T cell proliferation occurs with corticosteroids, similar to what is seen with cytokines following use of agents such as tocilizumab. Dr. Turtle noted that while an abrupt reduction in CAR T cells has not been seen in participants given 10 mg dexamethasone bid, T cell proliferation drops off with administration of 1 g methylprednisolone in severe cases. Dr. Ribas described one participant whose circulating MART-1 TCR cells 'disappeared' following administration of corticosteroids. He advised that steroids be used only when necessary, pointing out that it makes no sense to try to turn on the immune system for an anti-tumor effect only to turn it off. Per his perspective, the bar for use of steroids should be high within the parameters and priority of participant safety. However, Dr. Turtle noted that because steroids can affect TCR signaling and possibly CAR signaling, a low dose of steroids isn't necessarily bad and might even be beneficial if it dampens T cell signaling as it relates to toxicity.

Dr. Atkins commented that as clinical experience in this field has been gained, there has been less concern about breaking immune-related toxicities by giving steroids early because the majority of participants are responding to the T cell therapy they have received. While some off-target toxicities are stopped, results in many participants indicate that the T cells remain active even in the presence of high doses of steroids. Dr. Atkins noted that participants who have received CD19 CAR appear to have the best and most persistent anti-tumor outcomes, even after developing CRS. These outcomes suggest that, at least for this group of participants, CRS toxicities do not interfere with the efficacy of the T cell therapy. Participants agreed that further investigation of the impact of timing of CRS interventions on anti-tumor efficacy is warranted.

There was an extensive discussion on suicide genes and their potential role in preventing or mitigating CRS. Because T cells that have been genetically modified with artificial receptors may persist and expand in number, any adverse effect such as CRS or GVHD may be prolonged and even worsen as the cells expand. There has been considerable interest in including a suicide gene in constructs so that genetically modified cells can be destroyed as necessary by exposure to a specific signal. Although ablation of infused cells may not abrogate all adverse reactions if other immune effector mechanisms have been activated, suicide genes are being included in several constructs as a safety switch. These suicide genes may need to be activated at different stages of an adverse reaction, depending on the properties of each construct, such as speed and potency. The efficacy of suicide genes to prevent cytokine storm has been demonstrated in mice and non-human primates. Suicide genes are being studied but have not yet been validated in the clinical setting. Among the suicide genes that have been included in constructs infused in human protocols are the herpes simplex viral thymidine kinase (TK) gene, the inducible caspase 9 (iCas9) gene, and the truncated EGFR gene. The mechanism of action of these constructs and their potential roles in removing the engineered T cells after prolonged toxicities of T cell immunotherapy needs to be further investigated.

A growing number of CAR/TCR protocols submitted to the RAC involve modified T cells that have a suicide gene. Dr. Atkins noted, however, that many of the toxicities reported for these protocols involved participants who died of CRS without activation of the suicide gene despite being given CARs with suicide genes, therefore raising questions about the efficacy and safety of incorporating these genes into the modified T cell constructs. An additional concern within the context of CRS is whether an effective suicide gene also eliminates the T cell benefits, and whether it is better to use tocilizumab and/or steroids to curb the CRS without also destroying the therapeutic intervention. With this background, meeting participants explored the rationales for use of suicide genes and the conditions under which the suicide genes have been or might be activated.

Dr. Jensen responded that they have not yet included a suicide gene in their studies. In the pediatric leukemia/ALL protocol, for example, the team did not include a protocol-defined threshold to ablate the CAR T cells. They felt that ablation would not necessarily be desirable in the setting of CRS, but may be in the setting of B cell aplasia, thereby impacting the efficacy of the immunotherapy in participants who have long-term persistent B cell aplasia. The investigators are not convinced that an antibody-based self-inactivating suicide switch within the context of an acute severe CRS is the appropriate approach to use to stop a process that has already been initiated. Dr. Brentjens stated that whether suicide genes in all cases are useful for chronic toxicity only and are ineffective to treat an acute toxicity is not known at this point. In settings where a new antigen is being tested and there is potential for off-tumor toxicity, a suicide gene could provide an additional level of safety. And for a small subset of individuals where there is an indefinite persistence of cells, a suicide gene might be useful to terminate those T cells at some point. How effective the suicide gene is and where it fits into the algorithm for CRS treatment or management of chronic symptoms will be made clearer once it has been deployed in the clinical setting.

Dr. Powell provided the example of inserting a suicide gene (i.e., the truncated EGFR gene) in CARs for ovarian cancer that also includes an IL-12 gene. UPenn protocols using this construct demonstrated that activation of the EGFRt tag eliminates the CAR T cells. The algorithm in this case also includes Cetuximab to deactivate the marker. Subsequent removal of the suicide gene can be done after completion of Phase 1 dose escalation, although a new IND would probably be required because the construct has been changed. Dr. Heslop and her colleagues have used iCas9 in activated T cells after transplant in one participant who had CRS-like symptoms, including fever up to 106°F and high IL-6 levels; within about two hours after being given the dimerizer to activate the suicide gene, her temperature normalized, and her IL-6 levels returned to baseline. The Baylor team has not been able to routinely use iCas9 in their CAR T cell studies. Thus, for the cited case, the intervention was within the context of an activated T cell situation. The NIH team has used the same GD2 CAR construct as Dr. Heslop's group but without iCas9. Dr. Lee explained that because GD2 is expressed on peripheral nerves, the investigators were concerned about significant limiting neuropathies that could result from use of this construct. The intent with inserting a suicide gene would be to mitigate these neurologic symptoms; however, since symptoms have not occurred, the suicide vector does not appear to be needed.

Participants noted that not all suicide vectors are equivalent. For example, the EGFR tag has been seen more as a marker of transgene expression, rather than as a suicide gene. Cetuximab is included in NIH protocols as an added safeguard, specifically to avoid or manage unpredicted off-target effects of the modified T cells. To date, however, various suicide genes, deactivating agents, and other agents used to mitigate CRS have not been compared head-to-head. A suggestion was made to obtain data on the numbers of participants who do not respond to steroids and tocilizumab and to study the efficacy of other interventions before expanding the use of suicide genes. Until more is known about suicide genes, their use should be considered only as a last resort. There was general consensus among experts in this area that suicide genes should be used only in extreme cases, when a new target is being studied, and when a chronic effect or toxicity has already been managed. The iCas9 suicide approach has worked rapidly in a small number of cases and with T cells post-transplant. The efficacy of EGFRt in humans is not known, and in both murine and primate models, most of the modified cells are killed within 12 to 24 hours, which is slower than what has been seen for iCas9. An important message is that investigators and clinicians have a clear understanding that suicide vectors are unproven at this point in time.

Participants considered findings regarding neurologic toxicity, per published reports and data summarized during the meeting, and discussed strategies to treat the two different types of neurotoxicities observed in participants following T cell immunotherapy. Participants in CAR/TCR protocols across institutions have experienced confusion, delirium, and mental status changes as well as focal toxicities including seizures and aphasia. Some of these symptoms may be related to CRS, but it is not clear whether they result from systemic cytokines crossing the blood–brain barrier and engaging cytokine receptors in the brain or from direct cytokine production in the CNS.

Some sites (Dr. Brentjens added) have used an anti-seizure medication (Keppra) prophylaxis in addition to treating CRS, but this approach has not been consistently effective in ameliorating neurologic symptoms. In many cases, however, clinicians wait for neurotoxicities to resolve. Dr. Turtle explained that, depending on the circumstance, Keppra is given to minimize the risk of a life-threatening seizure regardless of EEG activity. Investigators at Memorial Sloan Kettering follow similar algorithms to treat CRS and neurotoxicity. Dr. Park noted that neurologic symptoms do not reverse as quickly as CRS symptoms such as fever and hemodynamic instability. The usual point for intervention is clinically observed seizure, which can be difficult to capture; while it often looks like the participant is having generalized convulsions, EEGs in some of these participants do not indicate epileptic activity. Steroids are given for this type of event, and intubation is used in the setting of severe encephalopathy, when airway protection is of concern. Dr. Park pointed out that these events are rare.

Dr. Kohn inquired about a case reviewed by the RAC Gene Transfer Safety Assessment Board (GTSAB) in which a participant was given intrathecal dexamethasone for a neurologic toxicity. Dr. Nath questioned the efficacy of steroids using this route of administration since such dosing would require delivery of the drug against the flow of the CSF.

Dr. Nath commented that the complications in these participants are not very well defined, even among those who have direct clinical experience with these populations. Discussions at the current meeting indicate confusion as to what constitutes neurotoxicity and neurologic symptoms of CRS, specifically with respect to whether participants have aphasias and if so, what type of aphasia, and whether participants have hyperactivity, hallucinations, delirium, or hypoactivity, which is an alteration in the level of consciousness. Just one example, noted by Dr. Park, involves participants who present with a clinically suspected seizure but have a negative EEG; these participants could have generalized myoclonus, which has been reported with CD19 therapy. Dr. Nath suggested assessing participants prospectively to define complications and develop a management plan based on correction of those complications. Retrospective analysis could miss subtle effects or potentially result in forgoing treatment of participants with severe symptoms or giving participants inappropriate/ineffective medications (e.g., anti-convulsants for generalized myoclonus). Dr. Park endorsed Dr. Nath's suggestion and noted that MSKCC protocols include a neurologist who examines participants before they receive CAR T cells. The pre-dosing neurologic evaluation does not require a baseline brain MRI unless medically indicated; in such cases, participants are scanned on a regular basis to monitor for any subsequent changes. In addition, participants with seizures or seizure-like activity have EEG monitoring for 24 to 48 hours so that this activity can be better characterized. The naming and classification of these "seizure-like" events and activities remain unresolved, however.

Dr. Turtle added that regardless of the character of the neurologic toxicity or the EEG findings if we see evidence of neurologic toxicity (e.g., participant with choreoathetosis) we put them on Keppra, as seizures are potentially life-threatening and treatment with Keppra is a low risk intervention.

Dr. Hammarskjöld suggested focusing efforts on determining whether any of the neurologic symptoms and toxicities seen in CAR/TCR protocols represent on-target, off-tumor effects given the potential risk of cross-reactivity with brain tissue. Regarding development of a unified algorithm for identifying and treating CRS-related neurotoxicities, Dr. Nath commented that this task is not possible because of confounding due to multiple underlying etiologies for neurologic symptoms seen in these participants. He revisited his suggestion to convene a panel for prospective review of events; to assure that a comprehensive review, the panel should include a neuro-oncologist, an epileptologist, a stroke specialist, a neuroimmunologist, and a neuroinfectious disease specialist.

The final topic for discussion focused on data sharing strategies to facilitate optimization of management of CRS. Dr. Ahmed noted that risk profile categorization would lead to different management plans and questioned whether participants need to be profiled when they get sick. Instead, algorithms could be developed through analysis of shared data of profiles of participant tumor load, dose of T cells, degree of lymphodepletion, and other relevant factors. This approach would require an extensive data set, which would be used to correlate data with participant outcomes and then reverse engineering these results into clinical practice. Dr. George suggested taking a step back before proceeding to data sharing to consider provisions for data collection. Initial steps in this process include identifying the data elements to capture for a shared database, delineating how to collect data in a uniform or systematic manner, and defining the format for these data. The data collection and submission process should be as “user-friendly” as possible. Specific types of information to collect include outcomes of participants who have CRS who have been treated with tocilizumab versus those who were treated with steroids or who were followed using a watch and wait approach. Results of cytokine panels and other laboratory tests (e.g., ferritin, CRP) in the pre- and post-treatment period would also be helpful in determining which markers are the best predictors of participant prognosis. Reporting the number of participants treated along with the number of positive outcomes and adverse events will put these events in context and will provide a much better picture of the frequency of more severe complications.

Most of the T cell immunotherapy clinical protocols conducted to date have been small, single-site protocols, which may have limited the opportunities for sharing information about SAE experiences and management of events that occur within the context of CRS. Optimizing the means of sharing study data would be beneficial for clinicians as well as participants across institutions and study sites. Workshop participants were interested in developing or expanding existing vehicles by which investigators are able to share study data, perhaps through some type of compendium of results. Information provided to a central site or database could include the number of partial and complete responses to the study intervention and treatments for CRS, with durability of responses broken down by adult versus pediatric populations.

A database such as GeMCRIS can be very useful in generating analyses for questions posed by study investigators and as discussed during this workshop. GeMCRIS serves as the repository for initial RAC submissions and subsequent annual progress reports on NIH/OBA protocols. There currently are gaps for some protocols, however, particularly in terms of updates and summaries after enrollment is initiated. GeMCRIS could serve as a central site for collection of broad sets of data from NIH/OBA studies, but increased awareness of the database and training on how to access and use the features of the system may be needed first to achieve this goal.

B. Public Comment

No public comments were offered.

VI. Discussion Wrap-Up

Dr. Kohn summarized the key issues discussed during each session of the meeting and the next steps proposed to address these issues.

<u>Session I</u>:

Regarding the need for a uniform definition and grading system for immunotherapy-induced CRS:

- There currently is no uniformity in defining or grading of immunotherapy-induced CRS. Descriptions of CRS vary considerably and include aphasia/dysphasia, delirium/encephalopathy, and CRS/cytokine storm/tumor lysis syndrome. In addition, there is no clear delineation between CRS and severe CRS. A standardized definition and grading system would greatly help

comparative studies and provide for uniform reporting, guidelines for interventions, and drug labeling).

- Academic centers and industry sponsors need to adopt uniform criteria in upcoming protocols to enhance the ability of investigators and regulators to evaluate safety and assess risk and benefit.
- As a next step, it would be ideal if all players (academia, industry, and regulators) could work together toward consensus definitions and a uniform grading proposal, rather than working individually or in separate groups. Industry sponsors should be part of these discussions. The purpose of this approach is to provide a noncompetitive space that fosters a collaborative effort. Consideration should be given as to whether GeMCRIS data can be used to test/model/validate proposed grading criteria.

The potential contributing factors for severe CRS include:

- Participant-specific factors, such as the target disease, predisposing conditions, age, and host genetics.
  - ALL was felt to be unique among hematologic malignancies. The extent of bone marrow disease (burden) is key in CRS risk.
  - Sepsis can mimic CRS, and infections are common predisposing factors to severe CRS and should be mitigated in this immunocompromised population.
  - Adults with ALL appear to be more prone to severe CRS than pediatric ALL participants following administration of CAR T cells. Although CRS prevalence data for adult ALL participants are not available at this time, younger participants seem to tolerate CAR T cell therapy better than older participants, who appear to have more severe consequences to T cell therapies. Additional studies are needed to better understand host predispositions to severe CRS.
- Tumor burden.
  - There is some correlation between tumor burden and prevalence of CRS events but not in all target diseases or for all CRS events.
  - Tumor burden is most predictive for ALL.
- Construct-specific factors, such as signaling domains and tumor antigen targets.
  - The role of signaling domains or other components of CAR constructs in severe CRS should be examined. Direct comparisons will likely prove difficult, however, because each group/study tends to use a particular construct.
- Other factors such as chemotherapy, lymphodepletion, and conditioning cytokines (e.g., IL-2).
  - The dose of T cells appears to be important, but low doses don't correlate well with CRS events.
  - Results from a small number of participants suggest that more intensive chemotherapy prior to T cell immunotherapy appears to reduce risk of CRS, mostly likely by decreasing tumor burden. Additional studies to better define this relationship are needed.
  - Fludarabine use in conditioning chemotherapy before T cell infusion correlated with T cell expansion and potentially with higher risk and severity of CRS.
  - High-dose IL-2 clearly induces some symptoms and signs of CRS. This effect is somewhat difficult to assess, however, because of confounding by the role of other T cell cytokines.

Regarding the question as to whether there is a neurological syndrome distinct from CRS:

- Neurotoxicity overlaps with severe (Grade 3/4) CRS, but further discussion is needed to determine whether neurotoxicity occurs with lower-grade CRS (Grade 1/2) or without CRS.
- Most participants felt neurologic toxicities observed during CRS events after T cell immunotherapy are distinct. Neurotoxicities occur later than CRS, do not have clear correlation with other symptoms and signs of CRS, and have specific CK profiles.
- Dr. Nath believes there is no correlation between IL-6 and neurologic toxicities associated with CRS events. In addition, he proposed that CD19 expression in parts of normal brain (including the language centers of the brain) may be responsible for some of the neurologic symptoms or toxicities seen uniquely in CAR T cell protocols, as CD19 CAR T cells may target these areas of

the brain. Whether there is a role of antibodies to CD19 or other brain antigens in these events isn't clear.

- The neurologic toxicity seen with IL-2 therapy was considered to be more metabolic in nature, suggesting the need for a different grading system for neurologic toxicities associated with adoptive T cell immunotherapy.
- If the field agrees that neurotoxicity should be considered separate from the canonical CRS definition, a uniform description of CAR- associated neurotoxicity and uniform grading criteria will be needed.
- It is recommended that a simpler and agreed-upon set of neurologic terminology to describe the neurotoxicity syndrome be developed to take AE and SAE reporting into consideration.
- A collaborative approach should be taken in designing preventive/treatment strategies (e.g., predictors, whether to use prophylactic anti-epileptics, how/when to intervene).

Session II:

- While there are common CRS features across T cell immunotherapy protocols, it isn't clear whether CRS events are the same for solid tumors versus hematologic malignancies. There is some suggestion that the observation of fewer CRS events in protocols targeting solid tumors may correlate with the decreased response rate of certain solid tumors to CAR T cell immunotherapy as compared to hematologic malignancies at the current time.
- Measurement of cytokines should be done as part of the goal of having clear criteria and a standard definition for CRS. Several cytokines might be useful for this purpose, but the optimal methods and time points for collecting samples for cytokine analysis need to be identified and tested. Commercial development of a CLIA-approvable cytokine measuring system that could be employed in multiple laboratories should be considered.
- Whether there are other potential biomarkers for diagnosis and monitoring of CRS needs to be studied. Clinical protocols collect extensive data, but the correlation potential for other measures, especially any routinely obtained in the hospital setting (e.g., CRP, ferritin), is not known. Analysis of available data would be challenging but could prove informative in defining other parameters that predict CRS severity and/or indicate when intervention is needed. As with cytokines, the optimal timing for collection of these data needs to be identified.
- Distinguishing CRS from infection/sepsis will be done through participant monitoring and microbiological culturing. However, as discussed, participants presenting with signs of infection will be treated up front. Thus, a differential diagnosis early in symptom presentation may not be critical.

Session III:

- Because sepsis can mimic CRS and infections can increase risk for severe CRS, antibiotic prophylaxis is routinely given as soon as fever begins. Further discussion is needed as to whether giving an anti-IL-6 agent (and/or corticosteroids) will decrease the effectiveness of fighting an infection or sepsis.
- Management of CRS requires situation-specific factors. However, some common treatment strategies, such as those identified by Dr. Brentjens' group (see Davila et al., 2014), could be elements of a CRS algorithm. Dr. Brentjens will make that information available for the group to review and comment on. Review of this information might, in turn, lead to a consensus algorithm for management of CRS.
- Corticosteroids and monoclonal antibodies against certain cytokines are used in the management of CRS, but optimal dose and timing and frequency of administration need to be more clearly defined. There seems to be some suggestion or trend in using these agents earlier during severe CRS. There currently are no definitive data to indicate whether these agents blunt the anti-tumor effects of T cell immunotherapy, but it appears that at least anti-IL-6 agents do not decrease anti-tumor efficacy.
- Limited information is available on use of other agents and strategies to treat CRS, such as plasmapheresis, because these approaches have been used in only a few cases.

- The general consensus regarding suicide genes is that, while there are reasons to use suicide genes, such as with a new antigen, they are not a primary or first-line approach for the management of CRS. There is limited clinical experience with the use of suicide genes, and the efficacy of one suicide gene versus another has not been established.
- CRS-related neurologic toxicity is relatively uncommon, making it difficult to identify predictive or contributing factors for such an event. Participants who have had other treatments for leukemia prior to T cell immunotherapy and perhaps those with history of CNS disease might be at increased risk of neurotoxicity, but there currently are no definitive data to support this suggestion.
- Data-sharing mechanisms to facilitate optimization include the GeMCRIS database, which is a very useful national repository of data. Investigators are encouraged to file annual reports in GeMCRIS and to include as much useful information as possible with these submissions. Having this information will provide the most accurate estimates of the prevalence of CRS events and outcomes given that the total number of participants enrolled across all studies (the denominator as brought up by Dr. Sadelain) is not clear at this point.

Next steps include preparation of revised definitions and grading systems and a report based on this workshop. Drs. Frey and Park offered to work with Dr. Lee on drafting a revised CRS definition and grading system based on the previously published criteria and definitions and the presentations and discussions from the current workshop. A separate definition and grading system for neurological toxicity will also be drafted. The proposed deadline for drafting this guidance is October 1, 2015. Dr. Park noted that this effort will focus first on a clinical grading system for CRS and then potentially on a system that also includes biological factors. Once in place, the draft criteria and definitions will be distributed via email as a starting point for further review and refinement. Conference calls may also be set up to discuss the draft materials following the initial review and receipt of comments.

A manuscript, or “white paper,” that would include the meeting proceedings will also be drafted. The draft manuscript would be provided for review and feedback prior to submission for publication, possibly in *Molecular Therapy*.

VII. Closing Remarks and Adjournment

Dr. Kohn thanked the RAC members, panelists, and other guests and the NIH staff and adjourned this June 2015 RAC workshop at 4:37 p.m. on June 10, 2015.

(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.)

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Lyric Jorgenson, 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: _____ _____

Donald B. Kohn, M.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. It does not include one individual whose name is illegible on the sign-in sheets.)

Tina Albertson, Juno Therapeutics
Khandan Boradoran, Dimension Therapeutics
Francois Lebel, Ziopharm, Inc.
Ke Liu, FDA
Viera Muzithras, Novartis
Maura O'Leary, FDA
Manisna Patel, Novartis
Raj Puri, FDA
Travis Quigley, bluebird bio, Inc.
Steven Rosenberg, NCI
Kapildeb Sen, Novartis
Robert Sokolic, FDA
Patricia Wood, Novartis

Attachment III:
Abbreviations and Acronyms

AE	adverse event
ALL	acute lymphoblastic leukemia
B-ALL	B cell acute lymphoblastic leukemia
bid	twice a day
CAR	chimeric antigen receptor
CAR T	chimeric antigen receptor T cell
CLIA	Clinical Laboratory Improvement Amendments
CLL	chronic lymphocytic leukemia
CNS	central nervous system
CRP	C-reactive protein
CRS	cytokine release syndrome
CSF	cerebrospinal fluid
CTCAE	NCI Common Terminology Criteria for Adverse Events
DLT	dose-limiting toxicity
DRESS	drug reaction (or rash) with eosinophilia and systemic symptoms
DNR	dominant negative TGF-beta receptor
EGFRT	truncated epithelial growth factor receptor gene
FDA	U.S. Food and Drug Administration
FHCRC	Fred Hutchinson Cancer Research Center
FiO_2	fraction of inspired oxygen
GeMCRIS	NIH Genetic Modification Clinical Research Information System
GTSAB	Gene Transfer Safety Assessment Board
GVHD	graft-versus-host disease
HIV	human immunodeficiency virus
HLH	hemophagocytic lymphohistiocytosis
iC9	inducible caspase-9
ICOS	inducible costimulator
ICU	intensive care unit
IL	interleukin
IND	investigational new drug
IV	intravenous
JAMA	Journal of the American Medical Association
MAGE	melanoma antigen gene
MART	melanoma antigen recognized by T cell
MAS	macrophage activation syndrome
MERS	mild encephalitis/encephalopathy with a reversible splenial lesion
MSKCC	Memorial Sloan Kettering Cancer Center
NCI	National Cancer Institute
NHL	non-Hodgkin's lymphoma
NIH	National Institutes of Health
OBA	Office of Biotechnology Activities, NIH
OD	Office of the Director, NIH
PCR	polymerase chain reaction
PO2	partial pressure of oxygen
POB	Pediatric Oncology Branch, NCI
RAC	Recombinant DNA Advisory Committee
SAE	serious adverse event
TCR	T cell receptor
Tk	thymidine kinase
TNF	tumor necrosis factor
UPenn	University of Pennsylvania
VEGF	vascular endothelial growth factor

Appendix A:
Public Comments

[No public testimony was provided at the June 2015 RAC workshop.]