National Toxicology Program

Peer Review of Draft Report on Carcinogens (RoC) Monographs on Selected Viruses:
Human T-Cell Lymphotropic Virus Type 1, Epstein-Barr Virus,
Merkel Cell Polyomavirus, Kaposi Sarcoma Herpesvirus, and
Human Immunodeficiency Virus Type 1

December 17, 2015

National Institute of Environmental Health Sciences
Research Triangle Park, NC

Peer-Review Report

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Monograph on Selected Viruses**

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I.   Attendees*

Peer-Review Panel

Andrew F. Olshan (Chair), University of North Carolina Gillings School of Public Health

Blossom Damania, Lineberger Comprehensive Cancer Center, University of North Carolina-Chapel Hill

Paul F. Lambert, McArdle Laboratory for Cancer Research, University of Wisconsin

Margaret M. Madeleine, Fred Hutchinson Cancer Research Center

Edward L. Murphy, Jr., University of California, San Francisco

Charles S. Rabkin, National Cancer Institute

Rosemary Rochford, University of Colorado, Anschutz Medical Campus

National Toxicology Program Board of Scientific Counselors Liaison

Steven Markowitz, Queens College, City University of New York (by webcast)

National Institute of Environmental Health Sciences Staff

Abee Boyles	Ruth Lunn	Diane Spencer	Lori White
Gloria Jahnke	Robin Mackar	Nigel Walker	Mary Wolfe

NIEHS Contract Support Staff

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Stanley Atwood, Integrated Laboratory Systems, Inc. (ILS)

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II.   Welcome and Introductions

The National Toxicology Program (NTP) Peer-Review Panel (the "Panel") for the Draft Report on Carcinogens (RoC) Monographs on Selected Viruses convened on December 17, 2015, in Rodbell Auditorium, Rall Building, National Institute for Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. Dr. Andrew Olshan served as chair. Dr. Steven Markowitz attended by webcast as the NTP Board of Scientific Counselors (BSC) liaison. Representing NTP were Dr. Mary Wolfe, Deputy Division Director for Policy, Division of the NTP (DNTP); Dr. Nigel Walker, Deputy Division Director for Science, DNTP; Dr. Gloria Jahnke, RoC project leader; and Dr. Ruth Lunn, Director, Office of the RoC (ORoC). Dr. Lori White, Office of Liaison, Policy, and Review, served as the Designated Federal Official.

Dr. Olshan called the meeting to order at 8:30 AM, welcomed everyone to the meeting, and asked all attendees to introduce themselves. Dr. Wolfe welcomed and thanked the attendees. Dr. White read the conflict of interest policy statement and briefed the attendees on meeting logistics. Dr. Olshan briefed the Panel and the audience on the format for the peer review.

*The meeting was webcast. Individuals who viewed the webcast are not listed, except as noted.

III.   Process for Preparing Draft RoC Monographs

Dr. Ruth Lunn, presented background information on the RoC and the review of selected viruses (Human T-cell Lymphotropic Virus Type 1 [HTLV-1], Epstein-Barr Virus [EBV], Merkel Cell Polyomavirus [MCV], Kaposi Sarcoma-associated Herpesvirus [KSHV], and Human Immunodeficiency Virus Type 1 [HIV-1]**)**, including how they were selected for review, how the draft RoC monographs were prepared, and how NTP reached conclusions on their cancer hazards. The RoC is congressionally mandated under the Public Health Service Act, which directs the Secretary of Health and Human Services (HHS) to prepare a report that lists substances to which there is significant exposure in the United States and that are known or reasonably anticipated human carcinogens. Dr. Lunn noted that the listing process is a hazard evaluation, and not a risk assessment. The RoC is cumulative, including the profiles for newly listed substances and for all substances listed in previous reports.

The RoC is prepared for the HHS Secretary by the NTP according to a four-part formal process that consists of the following steps: (1) nomination and selection of candidate substances, (2) scientific evaluation of the candidate substances, (3) public release and peer review of the draft RoC monographs, and (4) submission of the substance profiles to the HHS Secretary for review and approval. The process incorporates public comment, scientific input, and peer review of the scientific information.

Dr. Lunn noted that for every candidate substance proposed for review, a concept document is written that explains the rationale and proposed approach for the review. Once a substance is formally selected for review, a draft RoC monograph is prepared, which consists of two parts: (1) a cancer hazard evaluation component that assesses the quality of the studies, reaches level-of-evidence conclusions, and proposes a preliminary listing recommendation and (2) the draft substance profile that summarizes the key studies on which the listing recommendation is based and provides information on exposure. If the substance is listed in the RoC, the profile becomes part of the RoC

Dr. Lunn outlined the steps of the process that had been completed for selection and evaluation of the five viruses under review. A private individual nominated the viruses as candidate substances. Following interagency review, the nominations were announced and public comment solicited in January 2012. A draft concept document was developed, released for public comment in March 2014, and presented to the BSC in April 2014. The rationale for conducting hazard evaluations of these five viruses included the large numbers of infected individuals in the United States and the large available database of cancer studies on these viruses, evaluating over two dozen specific cancer end points. She also noted the lack of vaccines for these viruses.

Dr. Lunn reviewed the protocol for preparing the RoC monographs, which included obtaining scientific input from technical advisors. The evaluation reviewed information from recent authoritative International Agency for Research on Cancer (IARC) reviews of the carcinogenicity of these viruses, supplemented by literature searches for key or more recently published information. Following interagency review, the draft monographs were released for public comment in November 2015.

Dr. Lunn reviewed the formats for the cancer hazard evaluation and substance profile components of the monographs. She then reviewed the considerations in reaching conclusions on (1) exposure of " a significant number of persons residing the United States" to the viruses and (2) the preliminary listing recommendations as *known to be a human carcinogen* or *reasonably anticipated to be a human carcinogen*, based on the RoC criteria for *sufficient* or *limited evidence of carcinogenicity* from studies in humans, *sufficient evidence of carcinogenicity* from studies in experimental animals (not relevant in evaluation of these viruses), and evaluation of mechanistic and other relevant data. In particular, she noted that data from the study of tissues or cells from exposed people could be used to meet the criterion of sufficient evidence of carcinogenicity from studies in humans, but that supporting mechanistic data are not required for listing of a substance in the RoC. For the five viruses, the evidence in humans comes from epidemiological, clinical, and/or molecular studies.

The charge to the Panel was as follows:

- To comment on each draft cancer evaluation component, specifically, whether it is technically correct and clearly stated, whether the NTP has objectively presented and assessed the scientific evidence, and whether the scientific evidence is adequate for applying the listing criteria.
- To comment on each draft substance profile, specifically, whether the scientific evidence supports the NTP's preliminary RoC listing status of each virus.

The Panel would be asked to vote on the following questions:

- Whether the scientific evidence supports the NTP's conclusion on the level of evidence for carcinogenicity from cancer studies in humans of the five viruses.
- Whether the scientific evidence supports NTP's preliminary listing decision of viruses in the RoC.

Dr. Lunn noted that the draft monograph would be revised based on NTP's review of the peer-review comments. The revised monograph, the peer-review report, and NTP's response to the peer-review review report would be provided to the BSC, after which the monograph would be finalized. Dr. Lunn acknowledged and thanked both NTP and contractor staff involved in preparing the draft monographs.

IV. Public Comments

No written public comments on the monographs were received, and no oral public comments were made at the meeting.

V. Overarching Issues in Evaluating the Carcinogenicity of Viruses

Dr. Gloria Jahnke, DNTP project leader on the monographs, briefly presented overarching issues in evaluating the carcinogenicity of the selected viruses. They included high prevalence of infection with low incidence of cancer for some viruses, the potential role of cofactors (such as immunosuppression, other infectious agents, or host genetics) on cancer outcomes, and the definition of some cancer end points by the presence of a virus. Factors to consider included (1) evaluation of human epidemiology studies based on the Hill considerations, (2) monoclonality of

the virus in malignant tissue (supporting causality and temporality), (3) expression of oncogenic protein by the virus, and (4) the percentage of tumors positive for the virus.

VI. Draft RoC Monograph on Human T-Cell Lymphotropic Virus Type 1

Dr. Jahnke presented an overview of the key information in the draft monograph on HTLV-1. HTLV is a single-stranded, enveloped RNA retrovirus with four subtypes, of which only HTLV-1 is known to be associated with neoplasia. HTLV-1 can be detected through measurement of anti-HTLV-1 antibodies, measurement of viral RNA or proviral DNA in peripheral mononuclear blood cells, or *in vitro* culture. Proviral load is used to monitor disease processes associated with HTLV-1 (including adult T-cell leukemia/lymphoma [ATLL] and inflammatory diseases). U.S. seroprevalence in blood donors has been estimated at 0.0051%. Prevalence in some parts of the United States is increased by immigration. Where HTLV-1 is endemic, notably Japan, prevalence can reach 15% in certain age and geographic subgroups, and it is estimated that 15 to 20 million people worldwide are infected. Transmission occurs via transfer of infected body fluids, including semen and blood, and is highest via breastfeeding. HTLV-1 infects mainly CD4 T lymphocytes, through cell-to-cell contact, and 2% to 4% of HTLV-1 carriers develop ATLL.

The viral gene *Tax* affects DNA repair and cell survival pathways, promotes cell proliferation through its effects on other genes and on host cell microRNAs, and triggers genetic instability. Although the viral protein Tax is immunogenic, clonal proliferation can occur over time (during a 40- to 60-year latency period). Tax has been shown to immortalize human T cells *in vitro* and *in vivo* in the absence of other viral factors. Cell transformation is maintained by another HTLV-1 protein HTLV-1 basic leucine zipper factor (HBZ).

Three cancer end points for HTLV-1 were evaluated: ATLL, liver cancer, and gastric cancer. The evidence for a fourth end point, cutaneous T-cell lymphoma, came primarily from case series studies, and no mechanistic evidence was available; the evidence was considered to be inconsistent and inadequate for assessment of this end point.

Human studies provide sufficient evidence that HTLV-1 causes ATLL. The original association between HTLV-1 and ATLL came from case reports and case series, primarily from Japan and South America. HTLV-1 carriers in eight cohorts developed ATLL, and four nested case-control studies found the risk of ATLL to be increased in carriers with higher viral or proviral loads. HTLV-1 is monoclonal in ATLL tumors. Over 90% of tumors are infected with HTLV-1, and the viral proteins Tax and HBZ are found in 40% and 100% of the tumors, respectively. HTLV-1 infection is now considered a diagnostic criterion for ATLL.

Human studies provide limited evidence that HTLV-1 causes liver cancer. Associations between HTLV-1 infection and liver cancer were found in six epidemiological studies, including those that excluded or controlled for a history of blood transfusion. All of the epidemiological studies were conducted in areas with very high prevalence of hepatitis C virus (HCV). One study found that HTLV-1 increased the risk of cancer in men infected with HCV, but it was not clear whether HTLV-1 was a cofactor or a confounder. Although the epidemiological evidence for a relationship between HTLV-1 and liver cancer was consistent, the influence of bias or confounding could not be ruled out, and no information was available from human tissue studies.

Human studies provide inadequate evidence that HTLV-1 causes gastric cancer. Epidemiological studies (three cohort studies and one case-control study) found decreased risks of gastric cancer in HTLV-1-infected individuals; however, the studies were limited by small numbers of infected cases. One of these studies found an inverse relationship between infection with HTLV-1 and *Helicobacter pylori*.

VI.A. Peer Review Comments, Actions, and Draft Substance Profile for HTLV-1

The Panel concurred with the statement that a significant number of persons living in the United States are exposed to HTLV-1.

Dr. Murphy stated that while the monograph provided a long description of viral entry and initial integration, more information could have been provided on the molecular biology, particularly on Tax and HBZ. He said that the estimate of 16,000 HTLV-1 infections in the United States was probably a significant underestimate, and that the paper by Gessain and Cassar (2012) cited in the monograph provided an estimate of 90,000 to 100,000. He noted that prevalence in blood donors significantly underestimates prevalence in the general population, and that prevalence in various high-risk groups should be included in the monograph.

Dr. Murphy said too much emphasis was placed on the sex differential of ATLL incidence in infected individuals (i.e., higher in males than females), given that this was seen only in Japan, as a result of higher HTLV-1 infection rates in women. He recommended using the more widely accepted incidence of 2% to 4% in infected persons of both sexes, and mentioning that males may have increased susceptibility in Japan. He noted that because of the long latency period, ATLL incidence calculations should be based on childhood infection rates from 40 years ago, which did not show as great a sex differential.

Dr. Margaret Madeleine said the section on human cancer studies was well written and complete.

Dr. Charles Rabkin commented that the associations of HTLV-1 with liver and gastric cancer were overemphasized in the monograph, given the limitations of the evidence for these end points and the convincing evidence for ATLL. In particular, featuring these end points in the tables seemed to inappropriately stress very tenuous evidence.

Dr. Paul Lambert suggested that the monograph discuss two recent papers reporting on studies that found ATLL in humanized mice infected with HTLV-1 (Villaudy *et al.* 2011, Tezuka *et al.* 2014). He considered these papers to provide probably the most compelling mechanistic evidence for the role of HTLV-1 in ATLL.

VI.A.1   Actions

Dr. Olshan suggested the Panel vote separately on the preliminary level of evidence conclusions for ATLL and liver cancer.

Dr. Murphy moved and Dr. Lambert seconded the motion that the Panel accept the preliminary level of evidence conclusion that there is sufficient evidence from studies in humans that HTLV-1 causes ATLL. The Panel voted unanimously (6 yes, 0 no, 0 abstentions) to accept the draft

NTP conclusion that the scientific information presented from human cancer studies on HTLV-1 supports the level of evidence conclusion of *sufficient evidence of carcinogenicity* for ATLL.

Dr. Rabkin was concerned about identifying HTLV-1 as a potential liver carcinogen because of the studies' limitations (weak associations and the inability to rule out confounding exposures). Dr. Murphy clarified that HTLV-1 has not been shown to have an immunosuppressive effect. He agreed with Dr. Rabkin that the problems with confounding of the liver cancer studies made them inadequate even as limited evidence of carcinogenicity. Dr. Rabkin moved and Dr. Lambert seconded the motion that the preliminary conclusion for liver cancer be revised to state that the data from human cancer studies are inadequate to evaluate the association between HTLV-1 and liver cancer. The Panel voted unanimously (6 yes, 0 no, 0 abstentions) to recommend the conclusion of *inadequate evidence* of carcinogenicity for liver cancer.

Dr. Murphy moved and Dr. Madeleine seconded the motion that the Panel accept the NTP's revised preliminary listing recommendation. The Panel agreed unanimously (6 yes, 0 no, 0 abstentions) with the NTP's preliminary policy decision to list HTLV-1 in the RoC as *known to be a human carcinogen* based on sufficient evidence from studies in humans. This conclusion is based on evidence from epidemiological and molecular studies, which shows that HTLV-1 causes adult T-cell leukemia/lymphoma and on supporting mechanistic data.

VI.A.2   Draft RoC Substance Profile for HTLV-1

Dr. Murphy suggested that the discussion of liver cancer in the profile be substantially downplayed, that transmission by blood transfusion and organ transplantation (rather than through a break in the skin) be highlighted, that the estimated number of infected people be increased, and that the reference to screening of organ donors be updated.

VII.   Draft RoC Monograph on Epstein-Barr Virus

Dr. Whitney Arroyave, Social and Scientific Systems, Inc., (SSS, support contractor to ORoC) presented an overview of the key information in the draft monograph on EBV. EBV, also known as human herpesvirus 4, is a double-stranded, enveloped DNA virus in the gammaherpes virus subfamily. It can be detected through measurement of anti-EBV antibodies in the serum or of viral RNA or DNA in peripheral white blood cells, plasma, serum, or tumor tissue. Transmission is primarily via saliva, and transmission by blood transfusion and organ donation has been reported. U.S. seroprevalence in 2009–10 was estimated at 50% in children aged 6 to 8 and 89% in young adults aged 18 to 19. The worldwide infection rate exceeds 90%, and the age at infection varies geographically. Infection by EBV is lifelong and asymptomatic in most individuals, but can cause infectious mononucleosis, as well as cancer.

Cancer end points evaluated for EBV included four types of lymphoma and three epithelial cancers. Four types of lymphoma were evaluated for association with EBV: Burkitt lymphoma (endemic and sporadic), Hodgkin lymphoma, nasal-type natural killer (NK)/T-cell lymphoma or leukemia, and immunosuppression-related non-Hodgkin lymphoma (NHL).

Activation of EBV transcription programs during latency mimics B-cell activation, resulting in B-cell proliferation and survival, and in some cases lymphoma. In Latency III, naïve B cells in the

tonsils are infected and activated. EBV produces Epstein-Barr nuclear antigens (EBNAs), latent membrane proteins (LMPs), and EBV-encoded small RNAs (EBERs), which protect the virus from immune destruction, promote genomic instability, increase reactive oxygen species, enable replicative immortality, and promote angiogenesis, inflammation, and dysregulation of cellular pathways. Latency III has been associated with immunosuppression-related NHL. Latency II takes place in the germinal center of the tonsils; it results in replicative immortality, angiogenesis, and inflammation and has been associated with Hodgkin lymphoma and NK/T-cell lymphoma. In Latency 0, memory B cells provide a lifelong reservoir for the latent virus. In Latency I, infected B cells exiting the germinal center resist apoptosis and avoid immune destruction, and genomic instability and reactive oxygen species are increased. Latency I has been associated with Burkitt lymphoma.

Human studies provide sufficient evidence that EBV causes endemic Burkitt lymphoma. Seven case-control studies and one cohort study found significant associations with EBV (based on the presence of anti-EBV antibodies or EBV DNA), with odds ratios (ORs) ranging from just under 3 to over 52. In all studies where dose-response relationships could be calculated (four case-control studies and the cohort study), dose-response relationships between ORs and viral titer were observed. EBV is monoclonal in endemic Burkitt lymphoma and found in 95% of tumors. The main viral protein expressed is EBNA-1, which protects defective B cells from apoptosis.

Human studies provide limited evidence that EBV causes sporadic Burkitt lymphoma. Four of five case-control studies (with 113 cases) found ORs greater than 2, but the ORs were moderate, and the association was statistically significant in only one study. EBV is found in 20% of tumors, although no data were available on clonality or EBV protein expression in sporadic Burkitt lymphoma.

Human studies provide sufficient evidence that EBV causes Hodgkin lymphoma. Of 19 case-control studies and one nested case-control that reported ORs based on the presence of anti-EBV antibodies, all but one found ORs greater than 1 (ranging from 1.1 to 67), and the association was statistically significant in 14 studies. Four case-control studies that based exposure on detection of EBV DNA support these findings. In addition, modest associations between infectious mononucleosis and Hodgkin lymphoma were seen in 10 of 11 case-control studies and all 7 cohort studies. EBV seropositivity differs among Hodgkin lymphoma subtypes. EBV has been detected in 20% to 50% of cases in North America and Europe, 65% in Asia, and 90% to 100% in Africa and South America. EBV is monoclonal in Hodgkin lymphoma, and the main viral proteins expressed are LMP-1 and -2A, found in 50% of cases.

Human studies provide sufficient evidence that EBV causes nasal-type NK/T-cell lymphoma/leukemia. Consistent evidence of an association was found in 16 case-series studies (with over 400 cases). In two case-comparison studies, EBV DNA was found in the plasma of cells positive for CD3 antigen (a marker for T cells) in case subjects but not in control subjects. EBV is monoclonal in nasal-type NK/T-cell lymphoma/leukemia. It expresses the viral proteins EBNA-1, LMP-1, and LMP-2A and has been found in the majority of tumors positive for CD56 (a surface marker for NK cells).

Human studies provide sufficient evidence that EBV causes immunosuppression-related NHL. The evidence primarily comes from molecular studies and the epidemiological evidence is

limited to two case-control studies, both of which found statistically significant increases in ORs. EBV is monoclonal in immunosuppression-related NHL. NHL is one of the diagnostic criteria for acquired immunodeficiency syndrome (AIDS). Among individuals who test positive for human immunodeficiency virus (HIV), EBV is found in 100% of central nervous system NHL tumors and 50% of systemic NHL tumors. EBV is also found in over 50% of post-transplant lymphoproliferative disease (PTLD) tumors, and treatment with EBV-specific cytotoxic T cells protects against PTLD and reduces viral load and tumor size. In immunosuppression-related NHL, the main viral proteins expressed are EBNAs, LMP-1, and LMP-2A.

Three types of epithelial cancer were evaluated for association with EBV: nasopharyngeal carcinoma, gastric cancer, and lymphoepithelial carcinoma of the salivary gland. EBV has been shown to transform lymphoblastoid cells in culture and to transform epithelial cells co-cultured with transformed lymphoblastoid cells. EBV Latency II proteins (EBNA-1, LMP-1, and LMP-2A) and EBERs are expressed in epithelial tumors.

Human studies provide sufficient evidence that EBV causes nasopharyngeal carcinoma. All 11 case-control studies found significant associations based on the presence of anti-EBV antibodies or EBV DNA, with relative risks (RRs) or ORs ranging from 21 to 820. Two cohort studies based on the presence of antibodies also showed significant associations (with RRs of 9 and 22). No associations were observed in two small nested case-control studies, which included a total of 14 cases and had short follow-up times. All six case-control studies based on the presence of EBV DNA in tumors found statistically significant associations, with very high RRs. EBV is monoclonal in nasopharyngeal tumors, and 98% of nonkeratinizing nasopharyngeal carcinomas contain EBV. The main viral proteins expressed are EBNA-1, LMP-1, and LMP-2A.

Human studies provide sufficient evidence that EBV causes gastric cancer. Although the epidemiological evidence is limited, there is strong mechanistic evidence. Significant associations were found in all three case-control studies, with high ORs, and two of three nested case-control studies, with modest ORs. EBV is found, in monoclonal form, in 8% to 11% of gastric tumors. The main viral proteins expressed are EBNA-1, LMP-1, and LMP-2A. In genomic studies of gastric cancer tumors, EBV-positive tumors were shown to have a unique molecular profile; compared with other gastric cancer subtypes, they showed more marked CpG methylation and downregulation of the CDKN2A tumor suppressor gene by methylation. *PI3K* oncogene mutations have been found in 80% of EBV-positive tumors. Additional signaling pathways altered in EBV-related gastric cancer include the JAK2 and NF-κB cell proliferation pathways.

Human studies provide limited evidence that EBV causes lymphoepithelial carcinoma of the salivary gland. In case-series studies, 208 of 209 cases were positive for EBV, and a case-case study found EBV DNA in lymphoepithelial carcinomas but not in other types of salivary-gland tumors. In one study, monoclonal EBV was found in 100% of lymphoepithelial carcinomas of the salivary gland. The main viral proteins expressed are EBNA-1, LMP-1, and LMP-2A. No additional supporting mechanistic data were available.

VII.A. Peer Review Comments, Actions, and Draft Substance Profile for EBV

The Panel concurred with the statement that a significant number of persons living in the United States are exposed to EBV.

Dr. Rabkin said the monograph should clarify that chronic active EBV disease is a very uncommon condition and that LMP-1 is not commonly expressed in gastric cancer. He said two studies cited on page 29 of the draft monograph (Lo *et al.* 2001 and Levine *et al.* 1995) needed to be more fully described. It should be mentioned that the association of elevated EBV antibody titer with EBV-associated gastric cancer in the Levine study was statistically significant.

Dr. Rosemary Rochford said the statement about "latent viral transcripts" should be corrected to read "latent viral proteins." She noted that primary EBV infection does not occur in the salivary glands and that additional routes of transmission have been observed, including genital secretions and breast milk. She said all of the latent EBV genes are expressed in PTLD. She wondered why HIV-associated Burkitt lymphoma, diffuse large B-cell lymphoma, and pediatric leiomyosarcoma were not included as cancer end points for EBV and questioned the inclusion of lymphoepithelial cancer of the salivary gland, given the weakness of the studies. Dr. Rabkin agreed. Dr. Jahnke clarified the origin of the decision to include the salivary gland cancer based on the IARC review.

Dr. Rochford suggested that the evidence for lymphoepithelial cancer of the salivary gland was inadequate, and Dr. Lambert agreed. Dr. Rabkin agreed with the description of the evidence for sporadic Burkitt lymphoma as limited. Dr. Rochford concurred, noting that although no mechanism has been proposed, EBV is consistently linked with a subset of sporadic Burkitt lymphoma cases.

Dr. Blossom Damania commented that the monograph focused mainly on the viral proteins and that more information should be added about the viral microRNAs, which can play a role in transformation. Dr. Rochford concurred. Dr. Lambert said the monograph should add discussion of studies of lymphoma in humanized mice infected with EBV (e.g., Ma *et al.* 2011).

VII.A.1   Actions

Dr. Rochford proposed revising the preliminary conclusion to state that the data from human cancer studies are inadequate to evaluate the association between EBV exposure and lymphoepithelial cancer of the salivary gland. The Panel concurred. NTP staff clarified that additional end points could not be included in the preliminary conclusion, because they had not been evaluated in the draft monograph. Dr. Rochford moved and Dr. Rabkin seconded the motion that the Panel accept the revised conclusion.

The Panel voted unanimously (6 yes, 0 no, 0 abstentions) to:
- Accept the draft NTP conclusion that the scientific information presented from human cancer studies on EBV supports the level of evidence conclusions of (1) *sufficient evidence of carcinogenicity* for Burkitt lymphoma (endemic), Hodgkin lymphoma, nasopharyngeal cancer, immunosuppression-related non-Hodgkin lymphoma, extranodal NK/T-cell lymphoma (nasal type), and gastric cancer and (2) *limited evidence of carcinogenicity* for Burkitt lymphoma (sporadic).

- Recommend the conclusion of *inadequate evidence of carcinogenicity* for lymphoepithelial cancer of the salivary gland.

The preliminary listing recommendation was revised by removal of reference to limited evidence for an association with lymphoepithelial cancer of the salivary gland. Dr. Rochford moved and Dr. Rabkin seconded the motion that the Panel accept the NTP's revised preliminary listing recommendation. The Panel agreed unanimously (6 yes, 0 no, 0 abstentions) with the NTP's preliminary policy decision to list EBV in the RoC as *known to be a human carcinogen* based on sufficient evidence from studies in humans. This conclusion is based on evidence from epidemiological, clinical, and molecular studies, which show that EBV causes endemic Burkitt lymphoma, Hodgkin lymphoma, immune-suppression-related non-Hodgkin lymphoma, extranodal natural killer-T-cell lymphoma (nasal type), nasopharyngeal carcinoma, and some forms of gastric cancer. There is also limited evidence for an association with Burkitt lymphoma (sporadic).

VII.A.2   Draft RoC Substance Profile for EBV

Dr. Rochford suggested that the discussion of lymphoepithelial cancer of the salivary gland be reduced or deleted.

VIII. Draft RoC Monograph on Merkel Cell Polyomavirus

Dr. Jahnke, presented an overview of the key information in the draft monograph on Merkel cell polyomavirus (MCV). MCV is a double-stranded, non-enveloped DNA virus that is part of the normal skin flora. It is also found in saliva and may be distributed systemically. It causes an asymptomatic lifelong infection in healthy individuals. Despite the virus's name, the cellular origin of Merkel cell carcinoma is uncertain. U.S. MCV seroprevalence rates have been reported at around 20% in children aged 1 to 5, rising to as high as 88% in adults. The mode of transmission is unknown, but the increase in prevalence with age suggests that MCV may be acquired from environmental sources or through close contact with family members. The virus has been found in up to 85% of environmental surface samples.

Three cancer end points for MCV were initially considered: Merkel cell carcinoma, lung cancer, and chronic lymphocytic leukemia. The evidence for lung cancer and chronic lymphocytic leukemia was based on case-series studies, without mechanistic data, and was considered inadequate for assessment.

In the development of Merkel cell carcinoma, at least two mutations are needed for cell transformation: integration of the virus into the host cell and truncation of MCV large T antigen by inactivation of helicase. The truncated antigen retains the retinoblastoma binding domain and is able to transform cells. Because the incidence of Merkel cell carcinoma is higher in people over the age of 60, and because the tumors occur on areas of the skin exposed to sunlight, it is thought that ultraviolet (UV) radiation exposure increases the mutations. MCV T antigens have been shown to transform cells both *in vivo* and *in vitro.* Both large and small T MCV antigens are expressed in MCV-infected tumors and are required for tumor growth and survival. Small T and truncated large T antigens derived from Merkel cell carcinomas showed oncogenic activity

in transgenic mice. The antigens cause cell-cycle progression, disrupt immune signaling, increase c-myc and cyclin E, and inhibit NF-κB mediated transcription.

Human studies provide sufficient evidence that MCV causes Merkel cell carcinoma. Associations between MCV and Merkel cell carcinoma were found in 21 case-series studies (which found MCV in 716 of 855 cancer cases); in all three case-control studies, with significant ORs ranging from moderate to high; and in one nested case-control study, which found significantly increased risk in females, but not in males. MCV is monoclonal in Merkel cell tumors (inserted at a specific site in the DNA), and is found in over 80% of tumors. The viral truncated large T antigen and the small T antigen have been found in 75% and 92% of Merkel cell tumors, respectively. Not all Merkel cell carcinomas have evidence of MCV infection, suggesting that another mechanism may be involved.

VIII.A. Peer Review Comments, Actions, and Draft Substance Profile for MCV

The Panel concurred with the statement that a significant number of persons living in the U.S. are exposed to MCV.

Dr. Lambert found the monograph to be a good overview and the review of both the *in vitro* and *in vivo* data to be very thorough; he had no changes to suggest. Dr. Madeleine suggested that Table 1-1 be revised to emphasize that the skin is the primary site of MCV detection, even if the viral load is lower than in the mouth, and that MCV is nearly undetectable at other body sites.

Dr. Madeleine said the human cancer section was well written. She commented that too much emphasis was placed on chronic lymphocytic leukemia. She noted that in the nested case-control study on Merkel cell carcinoma, the finding of no association with MCV in men could have been related to small sample size, and that no sex difference was seen in other studies.

Dr. Rabkin noted that there is continuing controversy about the fraction of Merkel cell carcinoma cases attributable to MCV, and that the existence of a MCV-negative subset is not universally accepted.

VIII.A.1 Actions

Dr. Rochford moved and Dr. Rabkin seconded the motion that the Panel accept the preliminary conclusion that there is sufficient evidence from studies in humans that MCV causes Merkel cell carcinoma. The Panel voted (5 yes, 1 no, 0 abstentions) that the scientific information presented from human cancer studies on MCV supports the NTP's preliminary level of evidence conclusion of *sufficient evidence of carcinogenicity* for Merkel cell carcinoma. Dr. Murphy voted no because he considered the evidence from human studies to be limited, rather than sufficient. He found the evidence from human studies to be weak and not comparable to the level of evidence for the other viruses, especially given that MCV is ubiquitous and Merkel cell cancer is rare.

Dr. Lambert moved and Dr. Rabkin seconded that motion that the Panel accept the NTP's preliminary listing recommendation that Merkel cell polyomavirus (MCV) is *known to be a human carcinogen* based on sufficient evidence from studies in humans. The Panel voted (5 yes, 1 no, 0 abstentions) that the NTP's preliminary policy decision to list MCV in the RoC as *known to be a human carcinogen* is based on sufficient evidence from studies in humans. This

conclusion is based on evidence from epidemiological, clinical, and molecular studies, which show that MCV causes Merkel cell carcinoma, and on supporting mechanistic data. Dr. Murphy dissented, citing the same reasons given above for his vote on the preliminary conclusion.

IX. Draft RoC Monograph on Kaposi Sarcoma Herpesvirus

Mr. Stanley Atwood, Integrated Laboratory Systems, Inc. (ILS, support contractor to ORoC), presented an overview of the key information in the draft monograph on Kaposi sarcoma herpesvirus (KSHV). KSHV (also known as human herpesvirus 8) is a linear double-stranded, enveloped DNA virus that infects many cell types, including endothelial and epithelial cells, B cells, and macrophages. Viral DNA, viral proteins, and anti-KSHV antibodies can be detected in tissues and blood; the most commonly used antibodies are latency-associated nuclear antigen (LANA) and lytically expressed capsid antigen K8.1.

Prevalence of KSHV varies widely, from 30% to 70% in high-prevalence endemic regions of sub-Saharan Africa, to 10% to 25% in lower-prevalence endemic regions around the Mediterranean and less than 10% in non-endemic areas, including the United States. However, some populations in non-endemic areas have seroprevalence rates equal to those of the endemic areas, including HIV-positive men and HIV-negative men who have sex with men (MSM). Transmission is not completely understood. KSHV is transmitted primarily via saliva, but also via blood, organ transplant, and sexual activity, especially among MSM regardless of HIV status.

KSHV latent infection is established when the virus enters the cell nucleus and releases the genome, which is maintained as a circular episome. CD19+ B lymphocytes serve as a long-term latency reservoir for the genome at a low copy number. Both latent and lytic viral genes also contribute to malignant transformation. Host-cell transformation and oncogenesis result from expression of viral genes for evasion of immune response, cell-cycle dysregulation, evasion of apoptosis, and promotion of angiogenesis and cell transformation. In a healthy host, KSHV rarely results in cancer, but in an immune-compromised host, lytic infection can escape immunosurveillance and contribute to pathogenesis via abortive lytic or paracrine mechanisms.

Three cancer end points for KSHV were evaluated: Kaposi sarcoma, primary effusion lymphoma (PEL), and multicentric Castleman disease (MCD). The data were insufficient to evaluate additional end points. Primary effusion lymphoma and multicentric Castleman disease are rare types of B-cell non-Hodgkin lymphoma.

Human studies provide sufficient evidence that KSHV causes Kaposi sarcoma. KSHV is a necessary etiologic agent for Kaposi sarcoma, based on epidemiological studies that demonstrate temporality and strength of association and on experimental and molecular evidence that confirms biological plausibility. Numerous case-control and cohort or nested case-control studies from several countries and including all four clinical subtypes – epidemic (HIV/AIDS related), iatrogenic (e.g., organ transplant recipients), classic (Mediterranean regions and Eastern European Jews) and endemic (sub-Saharan Africa) – have shown a strong association between Kaposi sarcoma and KSHV based on elevated ORs or RRs in both HIV-positive and HIV-negative populations and on dose-response relationships. Most tumors are oligoclonal, but monoclonality has been reported for a subset of advanced lesions. Almost all

Kaposi sarcoma tumors contain the virus at a low copy number and express the latent viral proteins and to a lesser degree lytic proteins.

Human studies provide sufficient evidence that KSHV causes primary effusion lymphoma (PEL), a rare B cell lymphoma. Positive associations between KSHV infection and PEL were found in case reports, case-series studies, and case-comparison studies. KSHV has been found in PEL tumors from both HIV-positive and HIV-negative patients. About 50% of PEL patients develop Kaposi sarcoma, and previous diagnosis of Kaposi sarcoma increases the risk of PEL. PEL tumors are monoclonal, and 100% of the tumors are infected with KSHV at a high copy number. KSHV is part of the diagnostic criteria for PEL. Expression of viral proteins is similar to that observed in Kaposi sarcoma and is required for survival of PEL cells in culture.

Human studies provide limited evidence that KSHV causes multicentric Castleman disease (MCD), which is another rare B cell lymphoma. Associations with KSHV were seen in all four case-comparison studies, with very high ORs. Tumors are typically polyclonal, but monoclonal B-cell expansions have been observed. KSHV is found in almost all tumors from HIV-positive patients and 40% to 50% of tumors from HIV-negative patients. Tumors express primarily lytic proteins, with high levels of viral interleukin 6. MCD frequently co-occurs with Kaposi sarcoma or PEL, and KSHV inhibitors have shown some therapeutic success with MCD.

Immunosuppression (e.g., from HIV infection or organ-transplants recipients) greatly increases the risk of all KSHV-associated cancers; increasing duration of HIV-1 infection and decreasing CD4 cell counts are associated with a more rapid course of development. Risk factors for which there is limited evidence include co-infection with other viruses (e.g., EBV and human papillomavirus [HPV]) and, for the classic subtype, either diabetes or corticosteroid use.

IX.A.   Peer Review Comments, Actions, and Draft Substance Profile for KSHV

The Panel concurred with the statement that a significant number of persons living in the United States are exposed to KSHV.

Dr. Damania stated that the section on properties was well written and adequate. She noted that KSHV is also transmitted via organ transplantation and suggested adding a citation (Barozzi *et al.* 2003). Because KSHV is almost 100% associated with the plasmablastic variant of MCD and never associated with the hyaline vascular form, she stated that the evidence that KSHV causes plasmablastic MCD in humans is sufficient, rather than limited. She suggested that the monograph include KSHV-associated inflammatory cytokine syndrome as a non-cancer disease associated with the virus.

Dr. Rabkin cautioned against overconfidence in the epidemiological knowledge about KSHV infection, as it is based on serologic tools with poor specificity, and the estimates of prevalence in the U.S. population are very uncertain; they could be either too high or too low. He noted that the difference in the incidence of Kaposi sarcoma between the endemic regions and the United States is much greater than the difference in KSHV seroprevalence. Drs. Rochford and Rabkin suggested that the reference on page 7 to insect bites as a possible risk factor for infection be clarified because the study cited did not suggest that KSHV is transmitted via insect bites.

Dr. Damania questioned the statement on page 18 that KSHV infection alone appears to be insufficient to cause Kaposi sarcoma. She would argue that KSHV alone is sufficient to cause Kaposi sarcoma, given that classic, pediatric, and iatrogenic Kaposi sarcoma occur in the absence of HIV co-infection, and that 95% to 99% of the tumors contain KSHV. On page 22, additional reports of PEL in HIV-negative patients should be cited (Dotti *et al.* 1999, Boulanger *et al.* 2008, Testa *et al.* 2010). In Table 3-6, percentage of Kaposi sarcoma tumors with KSHV should be changed to 95% (Moore and Chang 1998). On page 40, it should be clarified that KSHV is almost always associated the plasmablastic variant of MCD (Dupin *et al.* 2000), and Table 3-6 should state that KSHV is found in over 99% of tumors of the plasmablastic variant. Dr. Damania noted that KSHV has been associated with another type of HIV-associated non-Hodgkin lymphoma in addition to PEL and MCD.

Dr. Damania said it was important to note that cancer causation by viruses is not unusual in any way; cancer need not occur in all exposed individuals for an agent to be considered carcinogenic. For example, not all smokers get lung cancer, but there is a higher prevalence of lung cancer among smokers. She provided additional references to be cited in Section 4.2.1 on animal models for KSHV-associated cancer (An *et al.* 2006, Dittmer *et al.* 2015).

Dr. Lambert suggested that the mention of HPV as a possible cofactor is KSHV-associated neoplasia should be removed from Section 4.1.3, and Dr. Damania concurred. Dr. Damania confirmed that EBV is found in about 50% of PEL tumors, and Drs. Lambert and Rochford agreed that the last sentence of Section 3.5.1 should be revised accordingly. Dr. Rochford said the blanket statement on page 31 that KSHV is not oncogenic in a healthy host should be removed, as KSHV-associated cancer can occur in seemingly healthy individuals with no overt immune suppression.

IX.A.1   Actions

Dr. Damania proposed revising the preliminary conclusion to state that there is sufficient evidence from studies in humans that KSHV causes the plasmablastic variant of MCD. The Panel concurred. Dr. Rochford moved and Dr. Rabkin seconded the motion that the Panel accept the revised preliminary conclusion.

The Panel voted unanimously (6 yes, 0 no, 0 abstentions) to:
- Accept the draft NTP conclusion that the scientific information presented from human cancer studies on KSHV supports the level of evidence conclusion of *sufficient evidence of carcinogenicity* for Kaposi sarcoma and primary effusion lymphoma.
- Recommend the conclusion of *sufficient evidence of carcinogenicity* for multicentric Castleman disease (plasmablastic variant).

The preliminary listing recommendation was similarly revised. Dr. Rochford moved and Dr. Madeleine seconded the motion that the Panel accept the NTP's revised preliminary listing recommendation. The Panel agreed unanimously (6 yes, 0 no, 0 abstentions) with the NTP's preliminary policy decision to list KSHV in the RoC as *known to be a human carcinogen* based on sufficient evidence from studies in humans. This conclusion is based on evidence from epidemiological and molecular studies, which shows that KSHV causes Kaposi sarcoma,

primary effusion lymphoma, and multicentric Castleman disease (plasmablastic variant), and on supporting mechanistic data.

IX.A.2   Draft RoC Substance Profile for KSHV

Dr. Damania suggested qualifying the statement on page 46 that "decreasing CD4 cell counts are associated with an increasing risk of Kaposi sarcoma in both AIDS-associated and classic Kaposi sarcoma cases…." Although this was true a decade ago, HIV-infected individuals with higher CD4 counts are now developing Kaposi sarcoma as they age. Dr. Damania considered the statement about the role of HIV co-infection in Kaposi sarcoma too strong and suggested revising it to say that Kaposi sarcoma occurs in the context of immune suppression, including that associated with HIV co-infection. Dr. Rabkin added the caveat that the association between KSHV and Kaposi sarcoma is much more pronounced with HIV co-infection.

X.   Draft RoC Monograph on Human Immunodeficiency Virus Type 1

Dr. Pamela Schwingl, ILS, presented an overview of the key information in the draft monograph on human immunodeficiency virus type 1 (HIV-1). HIV-1 is a single-stranded, enveloped RNA retrovirus that infects a number of cell types. RNA viral load and infectiousness become very high soon after initial infection. The immune response produces CD8 killer T cells, which kill infected CD4 cells, resulting in decreased HIV-1 titer. HIV-1 remains latent, integrated into the host genome, at low titer, before symptoms occur. Latency is typically 10 to 12 years, but can range from 2 to 25 years. The virus can be detected in blood and sexual fluids through measurement of anti-HIV antibodies, HIV p24 antigens, or HIV RNA. Viral RNA can be detected as early as 10 days post-infection.

Transmission occurs during sexual activity, through sharing of infected needles, and vertically (*in utero,* during birth, and through breastfeeding). Transmission rates are highest in populations practicing unprotected sex and sharing needles, and the risk of transmission is increased by the presence of other sexually transmitted infections. An estimated 37 million people are infected worldwide, including over a million in the United States. Of the 50,000 new infections per year in the United States, 63% occur in MSM and 8% in injection drug users; 20% of new infections occur in women. Of those infected, 13% are unaware of their infection status and 65% are untreated; transmission by untreated individuals accounts for 90% of new infections. The overall infection rate is stable, but the rate is rising in young black and Hispanic gay men. Over 600,000 people with AIDS have died in the United States since 1981.

The main mechanism of HIV-1 carcinogenesis is immunosuppression as indicated by the following evidence: (1) the pattern of increased risk is similar in HIV-positive individuals and immunosuppressed transplant recipients; (2) cancer risk increases with decreased CD4 level or increased HIV RNA load; (3) cancer risks have changed since the introduction of highly active anti-retroviral treatment (HAART); (4) HIV infection results in increases in other infection-related cancers, suggesting diminished immune surveillance; and (5) of the cancers seen in HIV-positive individuals, 70% are infection-related, compared with 12% in HIV-negative populations.

Other potential mechanisms involve chronic inflammation and traditional risk factors such as smoking and alcohol consumption. The evidence for direct oncogenesis is unclear; there is

evidence that tumor cells do not harbor HIV-1 proviruses, nor does HIV-1 alone transform cells. However, HIV-1 RNA levels are associated with AIDS-defining malignancies, and HIV-1 proteins have been shown to interact with oncogenic viruses, disrupt cell-cycle regulation, inhibit tumor-suppressor genes and DNA repair, and promote chromosomal instability.

HAART, in use since 1996, combines different classes of medications based on viral load, viral strain, CD4 cell count, and symptoms. By controlling viral load, it prevents or delays the appearance of symptoms or progression to AIDS, prolonging survival. Although there is evidence that the HAART drugs zidovudine and zalcitabine are carcinogenic in animals and may be carcinogenic in humans, HAART does not appear to explain all the excess risk of cancer in HIV-positive individuals.

The NTP evaluation was based on the IARC review and literature, primarily searching for cohort studies and reviews, published since that evaluation (from 2009 through 2015). For many cancer sites, the new data were only evaluated for consistency with the 2012 IARC review; however, a more comprehensive review of the new studies was conducted for those cancer sites for which there were data gaps in the IARC evaluation. For the NTP evaluation, the cancer end points were categorized as follows: (1) AIDS-defining cancers: Kaposi sarcoma, non-Hodgkin lymphoma, and invasive cervical cancer; (2) infection-related, non-AIDS-defining cancers: Hodgkin lymphoma, invasive anal cancer, genital cancer (vaginal/vulvar and penile), and oral cancer; and (3) non-infection-related, non-AIDS-defining cancers: conjunctival cancer, non-melanoma skin cancer, lung cancer, and hepatocellular cancer.

Human studies provide sufficient evidence that HIV-1 causes the six types of cancer for which the evidence was sufficient in the 2012 IARC review: Kaposi sarcoma, non-Hodgkin lymphoma, invasive cervical cancer, Hodgkin lymphoma, invasive anal cancer, and conjunctival cancer. Each of these cancers has a viral cofactor except conjunctival cancer, for which UV radiation may be a cofactor. In the late HAART era, risks for the AIDS-defining cancers Kaposi sarcoma and non-Hodgkin lymphoma have significantly declined as post-treatment CD4 counts significantly increased, but have remained above those for the general population. For Hodgkin lymphoma, risk has significantly increased and is related to most recent CD4 count.

Human studies provide sufficient evidence that HIV-1 causes genital cancer (vaginal/vulvar and penile). Epidemiological studies and meta-analyses consistently showed significantly elevated RRs or standardized incidence ratios (SIRs). HPV is a cofactor for these cancers. Evidence for the effect of HAART on cancer risk was inconsistent. For vaginal/vulvar cancer, low CD4 count at AIDS onset was associated with increased risk. Risks were about 20-fold higher for *in situ* than for invasive cancers. Risk estimates were similar across injection drug users, MSM, and heterosexuals, suggesting that confounding by differences in risk factors was not likely.

Human studies provide limited evidence that HIV-1 causes oral cancer (oral-cavity and oropharyngeal cancers), based on RRs of 2 to 4 in at least 19 epidemiological studies and 2 meta-analyses. This grouping includes various types of oral cancers, both HPV-associated and non-HPV-associated, and the evidence for HIV-mediated immunosuppression in these cancers is inconsistent. The modest magnitude of the risks may be explained by sex differences in transmission dynamics of HPV, unmeasured variations in sexual behaviors across cohorts, or

confounding by tobacco or alcohol use; in one study, controlling for smoking reduced the SIR from significant (1.9) to nonsignificant (1.4).

Human studies provide sufficient evidence that HIV-1 causes non-melanoma skin cancer. Increased RRs were found in at least 19 epidemiological studies. RRs were consistently 1.5 to 6, with some as high as 20, and a meta-analysis found an mRR of 2.76. Increased risk was associated with high HIV-1 RNA level and low CD4 count. The most common subtype was basal-cell carcinoma. MCV may be a cofactor in non-melanoma skin cancer in HIV-positive individuals, but its overall contribution to risk is unknown.

Human studies provide sufficient evidence that HIV-1 causes lung cancer. Increased RRs of 1.5 to 6 were consistently found in over 48 cohort studies, and a meta-analysis found a significantly increased mRR. Associations with viral load and CD4 count were inconsistent, but risk has increased in the HAART era, with mSIRs of 2 to 3.5. There are no known viral cofactors. The most frequent subtype of lung cancer in HIV-positive individuals is adenocarcinoma, which often appears at an advanced stage at a younger age. Seven of eight studies of lung cancer that controlled for smoking or modeled the bias from smoking found statistically significant adjusted RRs, making it unlikely that smoking accounted for the excess risk in HIV-positive individuals.

Human studies provide limited evidence that HIV-1 causes hepatocellular cancer, based on a consistently increased RR of 2 to 16 in over 40 cohort studies and a significant mSIR of 5.6. The evidence for changes in risk in the HAART era is inconsistent. Interpretation of the data is complicated by co-infection with hepatitis B and C, as it is unclear whether they are confounders or cofactors. Few studies have measured the seroprevalence of viral cofactors, and the variability in risk in the HAART era may be due to differing prevalence of HCV among cohorts, longer survival, or differences in other liver cancer risk factors. Several studies reported no association between HIV and hepatocellular carcinoma among HCV-infected individuals or after adjustment for HCV infection, and one study found twice the risk of cancer among individuals infected only with HCV as among those co-infected with HIV-1.

The cancer end points evaluated in the draft monograph account for 90% of the excess cancer burden associated with HIV infection (based on 2010 data). The remaining 10% includes other cancers potentially related directly to HIV-1, to longer survival, or to behaviors or confounders common among HIV-positive individuals.

X.A. Peer Review Comments, Actions, and Draft Substance Profile for HIV-1

The Panel concurred with the statement that a significant number of persons living in the United States are exposed to HIV-1.

Dr. Madeleine suggested updating the reference to the NIH guidelines for HIV detection to reflect current methods. She noted that some studies were missing from Table 3-1. She suggested referring consistently to "vaginal/vulvar" cancer throughout the monograph, deleting the mention of hepatocellular carcinoma survival from Section 3.6.1, deleting the sentence beginning "NTP *in utero…*" from Section 3.11, citing the study by Robbins *et al.* (2015) in more places, and adding citations of de Martel *et al.* (2015) in Section 3.12. Dr. Murphy suggested mentioning that the incidence of ATLL in people co-infected with HIV-1 and HTLV-1 does not appear to be increased (Dhasmana and Taylor 2014).

Dr. Rabkin suggested revising the first paragraph on page 26 to read, "However, a role of hepatitis B and C viruses in NHL risk in the presence of HIV-1 infection has not been elucidated." He considered the evidence for the carcinogenicity of invasive cervical cancer to be limited, rather than sufficient, because associations with invasive cervical cancer are modest and heterogeneous in magnitude, confounded by HPV infection and access to care, and unrelated to CD4 count or HAART (in contrast to the definitive association of HIV-1 with *in situ* cervical lesions). Similarly, the associations with lung cancer are modest and heterogeneous in magnitude across studies, confounded by smoking behaviors, and unrelated to CD4 count or HAART; these factors decrease the credibility of the association as causal.

Dr. Rochford felt that the mechanistic section of the monograph was inconsistent. The argument for an immunosuppressive effect, which is fairly consistent across the studies, was not clear. The evidence for an indirect effect of HIV-1 should be spelled out for each cancer end point, and the section should discuss whether there is any evidence for HIV-1 being directly oncogenic. The section should focus more clearly on the mechanistic questions, rather than relying on the epidemiological studies.

Dr. Rochford commented that the mechanistic data for HIV and lung cancer are very limited. Few studies try to directly address an underlying cause for the increased risk. The one example cited to support a role for HIV-1 was based on a study of HIV infection of T cells, but there is no evidence that a similar mechanism could occur in epithelial cells. In further searching the literature, Dr. Rochford could not find any studies directly addressing the question of how HIV-1 mechanistically would increase the risk of lung cancer.

Dr. Rochford suggested adding more information on oral cancers and potential mechanisms. For example, the monograph did not mention whether oral cancers were HPV-associated and if so, which types were found. Also, it did not discuss whether the incidence of liver cancer in HIV-positive populations changed after introduction of HAART, which could provide evidence for the immunosuppression mechanism. Similarly, for non-melanoma skin cancer, she noted the monograph did not discuss whether the incidence of basal- or squamous-cell carcinoma changed following introduction of HAART.

Dr. Rochford commented that the statement (in the Synthesis section) that "direct oncogenic effects of HIV-1 may contribute to increased cancer risk" was not supported by the data in the section. It has been suggested that the HIV-1 protein Tat plays a role in Kaposi sarcoma and that the HIV-1 envelope has some capacity to stimulate B cells, but there is no direct oncogenic effect of HIV-1. Dr. Damania agreed. Dr. Rochford said that the HIV-1 monograph should also have discussed pediatric leiomyosarcoma.

Dr. Madeleine commented that while HIV clearly enhances the immunosuppression that allows other viruses to directly cause cancer, the evidence is stronger for some cancers (e.g., HPV-positive anal cancer) than for others (e.g., cervical cancer). Dr. Lunn confirmed that mechanistic evidence is not required for the conclusion that there is sufficient evidence of carcinogenicity in humans.

With respect to lung cancer, Dr. Lunn noted that a combined analysis of two cohort studies found the highest risk among HIV-1 patients with a history of pneumonia, suggesting a mechanism involving inflammation; however, Dr. Rabkin said that this has not been a consistent

finding. Dr. Lunn clarified that IARC had found HPV-16 to be associated with cancer of the oral cavity, tonsils, and pharynx, and that it has been proposed that HPV-32 might be more prevalent in HIV-positive individuals with oral cancer. Dr. Lambert noted that older transfection studies suggesting an effect of Tat on HPV early gene expression have no physiological relevance.

X.A.1   Actions

Dr. Rochford proposed classifying lung cancer as having limited evidence in humans, rather than sufficient evidence, for the reasons discussed above.

Dr. Rabkin proposed classifying invasive cervical cancer as having limited evidence in humans, for the reasons discussed above. He noted that associations of invasive cervical cancer with HIV-1 have been modest even in countries with poor screening for cervical cancer, and could be related to higher prevalence of HPV. He further noted that inclusion of invasive cervical cancer as an AIDS-defining clinical condition cannot be taken to mean that a causal link between HIV-1 infection and cervical cancer has been demonstrated. Dr. Madeleine considered the epidemiological evidence for invasive cervical cancer to be sufficient, based on the modest excess risks observed.

Dr. Olshan proposed voting separately on preliminary level of evidence conclusions for cancer end points with sufficient and limited evidence.

Dr. Rabkin moved and Dr. Lambert seconded the motion that the Panel accept the revised preliminary level of evidence conclusion that there is sufficient evidence from studies in humans that HIV-1 causes Kaposi sarcoma, non-Hodgkin lymphoma, Hodgkin lymphoma, invasive anal cancer, genital cancer (vaginal/vulvar and penile), conjunctival cancer, and non-melanoma skin cancer.

The Panel voted unanimously (6 yes, 0 no, 0 abstentions) to:

- Accept the draft NTP conclusion that the scientific information presented from human cancer studies on HIV-1 1 supports the level of evidence conclusion of *sufficient evidence of carcinogenicity* for Kaposi sarcoma, non-Hodgkin lymphoma, Hodgkin lymphoma, invasive anal cancer, genital cancers (vaginal/vulvar and penile), conjunctival cancer, and non-melanoma skin cancer.

In straw polls, the majority of the Panel agreed that the evidence for an association between HIV-1 and lung cancer in humans was inadequate. The preliminary level-of-evidence conclusion for end points with limited evidence was revised by the addition of invasive cervical cancer, but not lung cancer. Dr. Lambert moved and Dr. Rochford seconded the motion that the Panel accept the revised preliminary level-of-evidence conclusion that there is limited evidence from studies in humans that HIV-1 causes oral cancer, liver cancer, and invasive cervical cancer.

The Panel voted (5 yes, 1 no, 0 abstentions) to:

- Accept the draft NTP conclusion that the scientific evidence presented from human cancer studies on HIV-Type 1 supports the level of evidence conclusion of *limited evidence of carcinogenicity* for liver cancer and oral cancer.

- Recommend the conclusion of *limited evidence of carcinogenicity* for invasive cervical cancer.
- Recommend the conclusion of *inadequate evidence of carcinogenicity* for lung cancer.

Dr. Madeleine voted no on the conclusion because she considered there to be limited evidence from epidemiological studies that HIV-1 also causes lung cancer.

The preliminary listing recommendation was revised by removal of the reference to sufficient evidence for associations with invasive cervical cancer and lung cancer and by addition of a reference to limited evidence for an association with invasive cervical cancer. Dr. Lambert moved and Dr. Rochford seconded the motion that the Panel accept the NTP's revised preliminary listing recommendation.

The Panel concurred with the NTP's preliminary policy decision to list HIV-1 in the RoC as *known to be a human carcinogen* based on sufficient evidence from studies in humans. The Panel voted (5 yes, 1 no, 0 abstentions) that this conclusion is based on epidemiological studies showing that HIV-1 increases the risk of Kaposi sarcoma, non-Hodgkin lymphoma, Hodgkin lymphoma, invasive anal cancer, genital cancers (vaginal/vulvar and penile cancers), conjunctival eye cancer, and non-melanoma skin cancer, together with supporting evidence from mechanistic studies demonstrating the biological plausibility of its carcinogenicity in humans. Epidemiological studies also provide limited evidence for an association between HIV-1 infection and oral cancer, liver cancer, and invasive cervical cancer. Dr. Madeleine dissented, because she considered there to be limited evidence from epidemiological studies for an association between HIV-1 infection and lung cancer.

X.A.2 Draft RoC Substance Profile for HIV-1

Dr. Murphy said that the profile was nicely written and concurred with Dr. Madeleine's earlier suggestion that the profile should mention the more recent testing methods allowing earlier detection of HIV-1 infection, via HIV nucleic acid testing or BED serologic assays. Then suggested the profile highlight recent work indicating the importance of early treatment of all HIV-infected individuals.

XI. Closing Remarks on Draft RoC Monograph

Dr. Damania suggested that the NTP RoC evaluation could be used to support screening of organ donors for HTLV-1 and KSHV.

Drs. Wolfe and Olshan thanked the Panel for their thoughtful review and the staff for their work preparing for the meeting. The meeting was adjourned at 2:45 PM.

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{Signed}

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Dr. Andrew F. Olshan

Chair, Peer-Review Panel

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Date