National Toxicology Program Response to the Peer-Review Report

Peer Review of the Draft Report on Carcinogens Monographs on Selected Viruses

Public Meeting
December 17, 2015

National Institute of Environmental Health Sciences

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Table of Contents

Introduction

The NTP convened an *ad hoc* scientific panel ("Panel") to peer review five *Draft Report on Carcinogens (RoC) Monographs on Selected Viruses* at a public meeting held December 17, 2015, at the National Institute of Environmental Health Sciences, Research Triangle Park, NC (information on the meeting is available at http://ntp.niehs.nih.gov/go/38854). The five draft virus monographs include: Merkel cell polyomavirus, Epstein-Barr virus, Human T-cell lymphotropic virus type 1, Kaposi sarcoma-associated herpesvirus, and Human immunodeficiency virus type 1. Each draft RoC monograph consists of a cancer hazard evaluation component and a substance profile.

The Panel had a two-fold charge for each monograph:

1. To comment on the draft cancer hazard evaluation component, specifically, whether it was 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 RoC listing criteria.
2. To comment on the draft substance profile, specifically, whether the scientific justification presented in the substance profile supports the NTP's preliminary policy decision on the RoC listing status for each virus.

The Panel was asked to vote on the following for each monograph:

1. Whether the scientific evidence supports the NTP's conclusion on the level of evidence for carcinogenicity from human cancer studies on each virus.
2. Whether the scientific evidence supports the NTP's preliminary listing decision for each virus in the RoC.

The Panel's peer-review comments were captured in the *Peer Review of the Draft Report on Carcinogens Monograph on Selected Viruses* ("Peer-Review Report"). Per the process for preparation of the RoC, the NTP prepares a response to the Peer-Review Report and posts it on the RoC website (http://ntp.niehs.nih.gov/go/38854). For each virus, the NTP's response addresses the Panel's (1) recommendations concerning NTP's draft conclusions and (2) scientific and technical peer-review comments related to identifying scientific issues and improving the technical accuracy, clarity, and objectivity of the monographs. In addition, the NTP response also addresses the Panel's comments on the introduction to the monographs that discusses issues common to the evaluation of all five viruses.

The NTP carefully reviewed and considered the Peer-Review Report in revising the draft monographs. Revised draft RoC monographs[1] will be shared with the public and the NTP Board of Scientific Counselors (BSC) at their public meeting on June 15-16, 2016, and finalized following the meeting.

[1]Available at http://ntp.niehs.nih.gov/go/733995.

Selected Viruses Peer-Review Panel[2]

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[2] The selection of panel members and conduct of the peer review were performed in accordance with the Federal Advisory Committee Act and Federal policies and regulations. The panel members served as independent scientists and not as representatives of any organization, company, or governmental agency.

Overview and Introduction to the Monographs

The overview and introduction to the monographs describes the general methods for preparing the monograph as well as issues related to evaluating causality that are common to all five viruses.

Panel comments:

Two issues impacting text in the introduction and some sections of all monographs are:

1. Whether the presence of an oncogenic virus alone is sufficient for oncogenesis. For example, text in the draft monograph on KSHV stated that KSHV infection alone was insufficient for carcinogenesis. However, one Panel member considered KSHV alone sufficient to cause Kaposi sarcoma, in that classic, pediatric, and iatrogenic Kaposi sarcoma occur in the absence of HIV co-infection, and over 95% of tumors contain KSHV.

2. The Panel also noted that cancer causation by oncogenic viruses is not unusual; cancer does not need to occur in all exposed individuals for an agent to be carcinogenic. For example, a Panel member noted that smoking can cause lung cancer but not all smokers get lung cancer.

NTP response: As a result of these discussions, NTP has revised the introduction and sections of the monographs that discuss these issues.

Merkel Cell Polyomavirus

Panel's recommendation on NTP conclusions and NTP response

Exposure to Merkel cell polyomavirus (MCV)

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

Level of evidence from studies in humans[3]

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*. One panel member judged the level evidence from human studies to be limited, rather than sufficient, when compared to some of the other viruses reviewed; Merkel cell carcinoma is very rare but the Merkel cell virus is ubiquitous.

NTP's preliminary listing decision for MCV in the RoC

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 epidemiological, clinical, and molecular studies in humans and supporting mechanistic data. One panel member judged the level evidence from human studies to be limited for Merkel cell carcinoma, rather than sufficient, when compared to some of the other viruses reviewed, as Merkel cell carcinoma is very rare but the Merkel cell virus is ubiquitous.

NTP Response*:*

NTP concurs with the conclusion to list MCV as *known to be a human carcinogen* based on sufficient evidence from studies in humans for Merkel cell polyomavirus. Three case-control studies (Carter *et al.* 2009, Paulson *et al.* 2010, Viscidi *et al.* 2011) and one nested case-control study (Faust *et al.* 2014) found statistically significant associations between MCV infection (as measured by antibodies to MCV antigens or pseudovirions) and Merkel cell carcinoma, with odds ratios (ORs) ranging from 4.4 to 63.2. This information provided sufficient evidence from human cancer studies. Further, measurement of the total MCV burden does not reflect the tumor-causing form of the virus, which results from: (1) monoclonal integration into the host cell DNA and (2) mutational truncation of the LT antigen. Both events are needed for host cell survival and dysregulation of the host cell cycle (Moore and Chang 2014).

Scientific and Technical Peer-Review Comments

The Panel provided scientific and technical comments that addressed issues related to the cancer hazard evaluation and for improving the clarity and completeness of the *Draft RoC Monograph on Merkel Cell Polyomavirus*. The comments and NTP responses to those comments are discussed below; they are organized by the type of evidence stream (e.g., properties and human exposure, human cancer studies, mechanistic studies).

Comments and NTP's response on properties and human exposure

Panel Comments:

[3] Studies in humans also include molecular studies of tissues from exposed humans.

- Revise Table 1-1 to emphasize that the skin is the primary site of MCV detection and that MCV is nearly undetectable at other body sites.

NTP Response: Table 1-1 was deleted as some of the data may have been misleading. Instead, tissue sites with moderate to high levels of detection were reported in the text. The viral load values in Table 1-1 are based on an assumption of 10 virions per cell which is over 10-fold greater than what has been reported in publications that measured viral load. Further, skin was first cleaned before a swab was taken, which may have lead to lower viral loads than in other tissues. In addition, viral load was reported at low levels in prostate cancer; however, two other publications, as well as the Panel, disagreed with this finding.

Comments and NTP's response on the human cancer hazard evaluation

Panel Comments:

- Decrease the emphasis on chronic lymphocytic leukemia in the text.
- Note in the text that in the nested case-control study on Merkel cell carcinoma, a finding of an association in females but not in males could be due to small sample size.

NTP Response: Chronic lymphocytic leukemia discussion was in a single paragraph and the amount of emphasis was balanced; there was no change in this discussion. With regard to the nested case-control study, a sentence was added to the monograph to alert the reader that sex differences may have occurred due to small sample size.

Comments and NTP's response on mechanistic and other relevant data

Panel Comments:

- Discuss that the continuing controversy about the existence of a MCV-negative subset of Merkel cell carcinomas and their existence is not universally accepted.

NTP Response: NTP concurs with this and has revised the monograph to include this discussion.

Epstein-Barr Virus

Panel's recommendation on NTP conclusions and NTP response

Exposure to Epstein-Barr Virus (EBV)

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

Level of evidence from studies in humans

The panel agreed unanimously (6 yes, 0 no, 0 abstentions) to support NTP's level of evidence conclusions of *sufficient evidence of carcinogenicity* of EBV for Burkitt lymphoma (endemic), Hodgkin lymphoma, nasopharyngeal cancer, immunosuppression-related non-Hodgkin lymphoma, extranodal NK-T-cell lymphoma (nasal type) and gastric cancer; and *limited evidence of carcinogenicity* for Burkitt lymphoma (sporadic). The conclusion of *inadequate evidence of carcinogenicity* was recommended for lymphoepithelial cancer of the salivary gland.

NTP's preliminary listing decision for Epstein-Barr Virus in the RoC

Panel voted (6 yes, 0 no, 0 abstentions) that the NTP's preliminary policy decision to list Epstein-Barr virus (EBV) in the RoC as *known to be a human carcinogen* is based on sufficient evidence from epidemiological, clinical, and molecular studies in humans and supporting mechanistic data.

NTP Response: NTP concurs with the conclusion to list EBV as known to be a human carcinogen. NTP also agrees with the Panel's recommended level of evidence conclusions for all tumor endpoints and has revised the draft monograph discussion on lymphoepithelial cancer of the salivary gland. The available evidence for EBV-associated lymphoepithelial cancer of the salivary gland is primarily limited to case series and a case-case study. The molecular evidence in humans does not support a higher level of evidence conclusion since monoclonality in tumors is based on only a few tumor samples. Further, the interpretation of other cancer studies where EBV is found in salivary gland epithelial cells is unclear because EBV's role in carcinogenesis has not been elucidated for this tissue.

Scientific and Technical Peer-Review Comments

The Panel provided scientific and technical comments that addressed issues related to the cancer hazard evaluation and for improving the clarity and completeness of the *Draft RoC Monograph on Epstein-Barr Virus*. The specific comments and NTP responses to those comments are discussed below; they are organized by the type of evidence stream (e.g., properties and human exposure, human cancer studies, mechanistic studies).

Comments and NTP's response on properties and human exposure

Panel Comments:

- Clarify that chronic active EBV infection is not a common condition and that EBV infection does not occur in salivary glands. Also, note that EBV has been detected in breast milk and in genital secretions, suggesting forms of transmission other than saliva.

NTP Response: NTP verified the above information and included these changes and associated references (Thomas *et al.* 2006, IARC 2012, Daud *et al.* 2015) in the monograph.

Comments and NTP's response on the human cancer hazard evaluation

Panel Comments:

- Describe more fully the Lo *et al.* 2001 and Levine *et al.* 1995 studies; include statistical significance of elevated EBV antibody titer with EBV-associated gastric cancer (Table 3-4).

NTP Response: This information was added to the monograph.

- Shorten discussion of lymphoepithelial cancer of the salivary gland in the cancer hazard evaluation component and remove the discussion in the substance profile.

NTP Response: The discussion of lymphoepithelial cancer of the salivary gland has been revised in the cancer hazard evaluation component of the monograph and removed from the profile as the level of evidence for this cancer endpoint is inadequate.

- Inquired why HIV-1-associated Burkitt lymphoma, pediatric leiomyosarcoma, and diffuse large B-cell lymphoma were not included as EBV-related cancers.

NTP Response: All three cancers had limited databases for RoC cancer evaluation. HIV-1-associated Burkitt lymphoma and diffuse large B-cell lymphoma are noted in the HIV-1 monograph and are now cross-referenced in the EBV monograph. Diffuse large B-cell lymphoma and pediatric leiomyosarcoma are not discussed as IARC did not cover these endpoints, NTP only located a few case reports, and no human tissue mechanistic information was available.

Comments and NTP's response on mechanistic and other relevant data

Panel Comments:

- Note that LMP-1 expression is infrequent in EBV-associated gastric cancer.
- Add information on EBV-infected humanized mice (Ma *et al.* 2011) and on microRNAs (Marquitz *et al.* 2012).

NTP Response: This information was added to the monograph.

Human T-Cell Lymphotropic Virus Type 1

Panel's recommendation on NTP conclusions and NTP response

Exposure to Human T-Cell Lymphotropic Virus Type 1 (HTLV-1)

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

Level of evidence from studies in humans and human tissues

Panel voted (6 yes, 0 no, 0 abstentions) that the scientific information presented from human cancer studies on HTLV-1 supports the NTP's preliminary level of evidence conclusion of *sufficient evidence of carcinogenicity* for adult T-cell leukemia/lymphoma. In addition, the Panel voted (6 yes, 0 no, 0 abstentions) to recommend the conclusion of *inadequate evidence of carcinogenicity* for liver cancer, instead of limited evidence. The Panel recommended the preliminary conclusion for liver cancer be revised to inadequate due to weak associations and the inability to rule out confounding exposures.

NTP's preliminary listing decision for HTLV-1 in the RoC

Panel voted (6 yes, 0 no, 0 abstentions) that 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 epidemiological, clinical, and molecular studies in humans and supporting mechanistic data

NTP Response: NTP concurs with the conclusion of *known to be a human carcinogen* based on sufficient evidence from studies in humans for HTLV-1 and adult T-cell leukemia/lymphoma. The NTP also accepts the Panel recommendation that the level of evidence for liver cancer is inadequate because of several limitations, such as small number of studies or exposed subjects and potential confounding from infection with hepatitis B or C viruses.

Scientific and Technical Peer-Review Comments

The Panel provided scientific and technical comments that addressed issues related to the cancer hazard evaluation and for improving the clarity and completeness of the *Draft RoC Monograph on HTLV-1*. The specific comments and NTP responses to those comments are discussed below; they are organized by the type of evidence stream (e.g., properties and human exposure, human cancer studies, mechanistic studies).

Comments and NTP's response on properties and human exposure

Panel Comments on properties:

- Shorten the discussion on viral entry and integration and expand the discussion on the molecular biology of TAX and HBZ.

NTP Response: Monograph was revised as requested.

Panel Comments on human exposure:

- Remove the estimate of infected people based on blood donor rate to the U.S. general population because this is an underestimate due to selection of blood donors. Instead report Gessain & Cassar (2012) estimated 90,000 to 100,000 HTLV-1 infected persons in the United States.

NTP Response: The above estimated range was added to monograph (both the cancer hazard evaluation component and the substance profile).

- Provide more information about transmission by blood transfusion, organ transplantation, and injection drug users, which should be emphasized in the substance profile (Murphy *et al.* 1989, Matutes 2007).

NTP Response: Monograph was revised as requested.

- Check whether FDA regulations specify that organs for transplantation are currently being screened for HTLV-1.

NTP Response: FDA regulations (21 CFR 1271) prescribe procedures, including donor screening and tissue testing, to ensure that tissues intended for human transplant or other human cells, tissues, and cellular and tissue-based products (HCT/Ps) are free of HTLV-1; however, some particular HCT/Ps, e.g., vascularized human organs for transplantation, are excluded. NTP has updated the regulations and discusses this information as well as emphasizing and expanding the discussion of transmission by blood transfusion and organ transplantation in both sections of the revised monograph.

Comments and NTP's response on the human cancer hazard evaluation

Panel Comments:

- Clarify that the gender differential in adult T-cell leukemia/lymphoma (ATLL), which has only been seen in Japan and not seen in Jamaica (Murphy *et al.* 1989), and note that the estimated risk of an HTLV-1 carrier developing ATLL is about one per thousand person years or 2% to 4% over a lifetime post-infection (Matutes 2007).

NTP Response: NTP concurs with these comments and has revised the monograph accordingly. The recommended text and references were added to monograph.

- Shorten the text on liver cancer and gastric cancer as they are overemphasized. In addition, note that blood transfusion is a potential confounder for liver cancer because patients with liver cancer are likely to receive blood transfusions and thereby become infected with HTLV-1 or hepatitis viruses.

NTP Response: The NTP concurs with these comments. Text on liver and gastric cancer was shortened in the monograph and removed from the substance profile, as the data were judged inadequate. Text was added that transfusions pose a risk of HTLV-1 or hepatitis B or C virus infection.

Comments and NTP's response on mechanistic and other relevant data

Panel Comments:

- 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).

NTP Response: These two studies papers are briefly discussed and references added to the revised monograph.

Kaposi Sarcoma-Associated Herpesvirus

Panel's recommendation on NTP conclusions and NTP response

Exposure to Kaposi Sarcoma-Associated Herpesvirus (KSHV).

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

Level of evidence from studies in humans and human tissues

Panel voted (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, and to recommend *sufficient evidence of carcinogenicity* for multicentric Castleman disease (plasmablastic variant).

NTP's preliminary listing decision for Kaposi Sarcoma-Associated Herpesvirus in the RoC

Panel voted (6 yes, 0 no, 0 abstentions) that the NTP's preliminary policy decision to list KSHV 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 KSHV causes Kaposi sarcoma, primary effusion lymphoma, and multicentric Castleman disease (plasmablastic variant) and on supporting mechanistic data.

NTP Response*:* NTP concurs with the conclusion of *known to be a human carcinogen* based on sufficient evidence from studies in humans and supporting mechanistic data. NTP also agrees with the level of evidence conclusions of *sufficient evidence of carcinogenicity* for Kaposi sarcoma and primary effusion lymphoma and has revised the level of evidence to sufficient for multicentric Castleman disease (plasmablastic variant). NTP draft conclusion of limited evidence of carcinogenicity for multicentric Castleman disease did not include stratification by variant forms. KSHV is almost always associated with the plasmablastic variant of multicentric Castleman disease and is classified by the World Health Organization as "HHV-8 associated multicentric CD." By pathologic classification, KSHV is associated with the plasmablastic variant of multicentric Castleman disease.

Scientific and Technical Peer-Review Comments

The Panel provided scientific and technical comments that addressed issues related to the cancer hazard evaluation and for improving the clarity and completeness of the *Draft RoC Monograph on Kaposi Sarcoma-Associated Herpesvirus*. The specific comments and NTP responses to those comments are discussed below; they are organized by the type of evidence stream (e.g., properties and human exposure, human cancer studies, mechanistic studies).

Comments and NTP's response on properties and human exposure

Panel Comments:

- Add that test methods for KSHV have poor specificity and estimates of prevalence are uncertain.
- Add that transmission can occur from transplant donor to recipient (Barozzi *et al.* 2003).

NTP Response: This information and suggested citation was added to the monograph.

Comments and NTP's response on the human cancer hazard evaluation and mechanistic and other relevant data

Panel Comments:

- Correct statements in the document that decreasing CD4 counts (as with HIV-1 infection) are associated with an increasing risk of Kaposi sarcoma. Although this was true a decade ago, HIV-1-infected individuals with CD4 counts are now developing Kaposi sarcoma as they age. In addition, KSHV-associated cancer can occur in seemingly healthy individuals with no overt immunosuppression.

NTP Response: Statements concerning CD4 counts and risk of Kaposi sarcoma have been revised in all relevant sections of the monograph.

- Clarify that presence of KSHV alone is sufficient for development of primary effusion lymphoma and is not dependent upon HIV-1 co-infection (Dotti *et al.* 1999, Boulanger *et al.* 2008, Testa *et al.* 2010).
- Clarify that KSHV is found in over 99% of tumors of multicentric Castleman disease (plasmablastic variant) (Dupin *et al.* 2000, Damania 2010).

NTP Response: Added text and Panel-suggested references for case reports of HIV-1-negative transplant patients and development of primary effusion lymphoma. Noted that KSHV is found in over 99% of plasmablastic variant form of multicentric Castleman disease and suggested references added.

Human Immunodeficiency Virus Type 1

Panel's recommendation on NTP conclusions and NTP response

Exposure to Human Immunodeficiency Virus Type 1 (HIV-1)

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

Level of evidence from studies in humans

Panel voted (6 yes, 0 no, 0 abstentions) that the scientific information presented from human cancer studies on Human Immunodeficiency Virus Type 1 (HIV-1) supports the NTP's preliminary 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.

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-1 supports the level of evidence conclusion of *limited evidence of carcinogenicity* for liver cancer and oral cancer, and recommended the conclusion of *limited evidence of carcinogenicity* for invasive cervical cancer and of *inadequate evidence of carcinogenicity* for lung cancer. One Panel member, the epidemiologist primary reviewer for HIV-1, voted 'no' as she considered the epidemiological evidence for invasive cervical cancer to be sufficient, based on the significant excess risks observed and also limited evidence from epidemiological studies for an association between HIV-1 infection and lung cancer.

The Panel's rationale for recommending invasive cervical cancer as limited evidence: The 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 human papillomavirus (HPV) and access to care, and unrelated to CD4 count or highly active antiretroviral therapy (HAART) (in contrast to the definitive association of HIV-1 with *in situ* cervical lesions).

The Panel's rationale for recommending lung cancer as inadequate evidence: The associations with lung cancer are modest and heterogeneous in magnitude across studies, and are confounded by smoking behaviors. In addition, the mechanism is not known and no clear patterns were observed with markers of immunosuppression (e.g., CD4 count or HAART). These factors decrease the credibility of the association as causal.

Preliminary listing decision for HIV-1 in the RoC

Panel voted (5 yes, 1 no, 0 abstentions) that the NTP's preliminary policy decision to list HIV-1 in the RoC as *known to be a human carcinogen* is based on sufficient evidence from epidemiological studies in humans and from supporting evidence from mechanistic studies demonstrating the biological plausibility of its carcinogenicity in humans. This conclusion was based on the panel's level of evidence conclusions for tumor endpoints listed above. One Panel member, the epidemiologist primary reviewer for HIV-1, voted 'no' as she did not agree with the Panel conclusions for invasive cervical cancer and for lung cancer.

NTP Response*:*

NTP concurs with the conclusion that HIV-1 is *known to be a human carcinogen* based on sufficient evidence from studies in humans and supporting mechanistic data. NTP agrees with the conclusions 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 and *limited evidence of carcinogenicity* for liver cancer and oral cancer. NTP does not agree with the listing recommendations by the Panel for invasive cervical cancer and for lung cancer for the reasons noted below:

NTP believes that the evidence for HIV-1 association with invasive cervical cancer is sufficient based on consistent evidence of statistically significant increased risk, ranging from 2- to 25-fold in almost all cohort studies (greater than 17 studies). Although a clear association with indicators of immunosuppression (such as CD4 cells or with HAART treatment) was not observed, this is also true of other cancers linked to HIV-1 infection and thus is not a requirement for sufficient evidence. Chaturvedi *et al.* (2009) restricted their analysis to the HAART era and found, unlike in the pre-HAART era (Frisch *et al.* 2000, Mbulaiteye *et al.* 2003), that low CD4+ counts at AIDS diagnoses were associated with a non-significant elevated risk of cervical cancer. They hypothesized that high cervical cancer mortality in the pre-HAART era may have masked the association of immunosuppression with cervical cancer, and that prolonged survival with incomplete immunocompetence among those with very low CD4 counts at diagnosis provided time for cancer to develop. In addition, cervical cancer is an AIDS-defining malignancy.

NTP believes that there is limited evidence for lung cancer from studies in humans because almost all cohort studies that controlled for smoking or modeled smoking bias found at least 2-fold increased risks of lung cancer incidence or mortality, most of which were statistically significant. However, it is possible that residual confounding may still be present as smoking rates are much higher among HIV-1 infected individuals. Further, mechanistic data are not required for a listing in the RoC.

Scientific and Technical Peer-Review Comments

The Panel provided scientific and technical comments that addressed issues related to the cancer hazard evaluation and for improving the clarity and completeness of the *Draft RoC Monograph on HIV-1*. The comments and NTP responses to those comments are discussed below; they are organized by the type of evidence stream (e.g., properties and human exposure, human cancer studies, mechanistic studies).

Comments and NTP's response on properties and human exposure

Panel Comments:

- Add website addresses to current information on testing and clinical guidelines for HIV-1.
- Highlight recent work indicating importance of early treatment, of more recent testing methods allowing earlier detection of HIV-1 infection, and include the NIAMD web address that provides updated information on HIV-1 vaccine research.

NTP Response: This information was added to the text and bibliographies for the cancer hazard evaluation component and substance profile sections of the monograph.

Comments and NTP's response on the human cancer hazard evaluation

Panel Comments:

- Expand Surveillance, Epidemiology, and End Results (SEER) comment to: Provide more information on the incidence of server cancers including, changes in incidences of Kaposi sarcoma, anal cancer, and oropharyngeal cancer over time and incidence data for genital cancers.

NTP Response: The following information was added to the relevant sections of the monograph.

Kaposi sarcoma: the post-HAART incidence rate for 100,000 individuals (SEER DATA 2009 to 2013) was 0.5 in the United States and 4 for San Francisco (high-risk geographical area); the pre-HAART rate in San Francisco reached a peak of 34 in the early 1990s.

Invasive anal cancer: Over the past 10 years, anal cancer incidence rates have been increasing on average 2.2% per year, and the risk in men is approaching the risk in women (1.5 per 100,000 in men and 2.0 per 100,000 women per year based on 2008 to 2012 cases).

Genital cancers: U.S. (SEER 2008 to 2012) incidence (per 100,000 women or men) is 2.4 for vulvar cancer, 0.71 for vaginal cancer, and 0.8 for penile cancer.

Oropharyngeal cancer: Population-level incidence of human papillomavirus-positive oropharyngeal cancers has increased by 225% while the incidence for human papillomavirus-negative cancers declined by 50%.

Conjunctival cancer: Incidence rate of squamous-cell carcinoma of the conjunctiva, which is a rare cancer, varies geographically from 0.02 to 3.5 per 100,000 depending on the latitude of the population studied.

Panel Comments:

- Clarify that the risk of anal cancer is increasing in the post HAART era (the draft monograph said it was unclear) and discuss the findings reported by Chaturvedi *et al.* (2009).

NTP Response: The monograph was revised to provide a more comprehensive review of the studies evaluating the incidence of anal cancer pre- and post-HAART. While the database is somewhat inconsistent, the overall the body of literature suggests an increase in anal cancer incidence post-HAART. The monograph also discusses the Chaturvedi *et al.* 2009 study that found that low CD4 counts at diagnosis during the HAART era were associated with significantly increased anal cancer incidence. Such observed increases in anal cancer incidence may be due to the fact that mortality among individuals with a low CD4 T-cell count during the pre-HAART era may have masked an association between immuno-suppression and the risk of human papillomavirus-related invasive anal cancer. The

increased survival during the HAART era may provide adequate time for progression of premalignant lesions to invasive cancers.

Comments and NTP's response on mechanistic and other relevant data

Panel Comments:

- Mechanistic section of monograph is inconsistent and argument for an immunosuppressive effect was not clear; spell out evidence for indirect effect for each cancer endpoint, and discuss if there is evidence for HIV-1 being directly oncogenic. Focus more on mechanistic questions rather than epidemiology.
- Expand discussion on oral cancers and mechanism.

NTP Response: No information on direct oncogenic effect of HIV-1 found. More mechanistic information and discussion was added for some cancer sites, such as for NHL and liver cancer. For example, more information on HIV-1-induced viremia and NHL, co-infection with HPV and oral or anogenital cancer, or with the hepatitis viruses (B or C) and liver cancer were provided. In addition, a discussion on immunosuppression was included for all tumor endpoints. Information was also added to the oral and anogenital discussion on HIV-1 proteins facilitating HPV infection and cell-cycle disruption.

- Discuss EBV-positive pediatric leiomyosarcoma and HIV-1. EBV seems to be associated with smooth muscle tumors in HIV-1-positive but not negative patients.

NTP Response: The database on this topic is very limited for an evaluation in that only a few case reports were located.

References

Barozzi P, Luppi M, Facchetti F, Mecucci C, Alu M, Sarid R, Rasini V, Ravazzini L, Rossi E, Festa S, Crescenzi B, Wolf DG, Schulz TF, Torelli G. 2003. Post-transplant Kaposi sarcoma originates from the seeding of donor-derived progenitors. *Nat Med* 9(5): 554-561.

Boulanger E, Afonso PV, Yahiaoui Y, Adle-Biassette H, Gabarre J, Agbalika F. 2008. Human herpesvirus-8 (HHV-8)-associated primary effusion lymphoma in two renal transplant recipients receiving rapamycin. *Am J Transplant* 8(3): 707-710.

Carter JJ, Paulson KG, Wipf GC, Miranda D, Madeleine MM, Johnson LG, Lemos BD, Lee S, Warcola AH, Iyer JG, Nghiem P, Galloway DA. 2009. Association of Merkel cell polyomavirus-specific antibodies with Merkel cell carcinoma. *J Natl Cancer Inst* 101(21): 1510-1522.

Chaturvedi AK, Madeleine MM, Biggar RJ, Engels EA. 2009. Risk of human papillomavirus-associated cancers among persons with AIDS. *J Natl Cancer Inst* 101(16): 1120-1130.

Damania B. 2010. Targeting the PI3K/Akt/mTOR Pathway in AIDS-Associated Malignancies. In *Molecular Basis for Therapy of AIDS-Defining Cancers*. Dittmer DP, Krown SE, eds.: Springer Science+Business Media, LLC. 153-166.

Daud, II, Coleman CB, Smith NA, Ogolla S, Simbiri K, Bukusi EA, Ng'ang'a ZW, Sumba PO, Vulule J, Ploutz-Snyder R, Dent AE, Rochford R. 2015. Breast milk as a potential source of Epstein-Barr virus transmission among infants living in a malaria-endemic region of Kenya. *J Infect Dis* 212(11): 1735-1742.

Dotti G, Fiocchi R, Motta T, Facchinetti B, Chiodini B, Borleri GM, Gavazzeni G, Barbui T, Rambaldi A. 1999. Primary effusion lymphoma after heart transplantation: a new entity associated with human herpesvirus-8. *Leukemia* 13(5): 664-670.

Dupin N, Diss TL, Kellam P, Tulliez M, Du MQ, Sicard D, Weiss RA, Isaacson PG, Boshoff C. 2000. HHV-8 is associated with a plasmablastic variant of Castleman disease that is linked to HHV-8-positive plasmablastic lymphoma. *Blood* 95(4): 1406-1412.

Faust H, Andersson K, Ekstrom J, Hortlund M, Robsahm TE, Dillner J. 2014. Prospective study of Merkel cell polyomavirus and risk of Merkel cell carcinoma. *Int J Cancer* 134(4): 844-848.

Frisch M, Biggar RJ, Goedert JJ. 2000. Human papillomavirus-associated cancers in patients with human immunodeficiency virus infection and acquired immunodeficiency syndrome. *J Natl Cancer Inst* 92(18): 1500-1510.

Gessain A, Cassar O. 2012. Epidemiological aspects and world distribution of HTLV-1 infection. *Frontiers in Microbiology* 3(NOV).

IARC. 2012. Epstein-Barr virus. In *Biological Agents*. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, vol. 100B. Lyon, France: International Agency for Research on Cancer. pp. 49-92.

Levine PH, Stemmermann G, Lennette ET, Hildesheim A, Shibata D, Nomura A. 1995. Elevated antibody titers to Epstein-Barr virus prior to the diagnosis of Epstein-Barr-virus-associated gastric adenocarcinoma. *Int J Cancer* 60(5): 642-644.

Lo YM, Chan WY, Ng EK, Chan LY, Lai PB, Tam JS, Chung SC. 2001. Circulating Epstein-Barr virus DNA in the serum of patients with gastric carcinoma. *Clin Cancer Res* 7(7): 1856-1859.

Ma SD, Hegde S, Young KH, Sullivan R, Rajesh D, Zhou Y, Jankowska-Gan E, Burlingham WJ, Sun X, Gulley ML, Tang W, Gumperz JE, Kenney SC. 2011. A new model of Epstein-Barr virus infection reveals an important role for early lytic viral protein expression in the development of lymphomas. *J Virol* 85(1): 165-177.

Marquitz AR, Mathur A, Shair KH, Raab-Traub N. 2012. Infection of Epstein-Barr virus in a gastric carcinoma cell line induces anchorage independence and global changes in gene expression. *Proc Natl Acad Sci U S A* 109(24): 9593-9598.

Matutes E. 2007. Adult T-cell leukaemia/lymphoma. *J Clin Pathol* 60(12): 1373-1377.

Mbulaiteye SM, Biggar RJ, Goedert JJ, Engels EA. 2003. Immune deficiency and risk for malignancy among persons with AIDS. *J Acquir Immune Defic Syndr* 32(5): 527-533.

Moore PS, Chang Y. 2014. The conundrum of causality in tumor virology: the cases of KSHV and MCV. *Semin Cancer Biol* 26: 4-12.

Murphy EL, Hanchard B, Figueroa JP, Gibbs WN, Lofters WS, Campbell M, Goedert JJ, Blattner WA. 1989. Modelling the risk of adult T-cell leukemia/lymphoma in persons infected with human T-lymphotropic virus type I. *Int J Cancer* 43(2): 250-253.

Paulson KG, Carter JJ, Johnson LG, Cahill KW, Iyer JG, Schrama D, Becker JC, Madeleine MM, Nghiem P, Galloway DA. 2010. Antibodies to merkel cell polyomavirus T antigen

oncoproteins reflect tumor burden in merkel cell carcinoma patients. *Cancer Res* 70(21): 8388-8397.

Testa A, Baiocchini A, Comandini UV, Falasca L, Nardacci R, Maritti M, Loiacono L, Bibbolino C, Rizzi EB, Cristofaro M, Ettorre GM, Vennarecci G, Antonucci G, Del Nonno F. 2010. Fatal sclerosing peritonitis associated with primary effusion lymphoma after liver transplantation: a case report. *Transplant Proc* 42(9): 3849-3853.

Tezuka K, Xun RZ, Tei MM, Ueno T, Tanaka M, Takenouchi N, Fujisawa J. 2014. An animal model of adult T-cell leukemia: humanized mice with HTLV-1-specific immunity. *Blood* 123(3): 346-355.

Thomas R, Macsween KF, McAulay K, Clutterbuck D, Anderson R, Reid S, Higgins CD, Swerdlow AJ, Harrison N, Williams H, Crawford DH. 2006. Evidence of shared Epstein-Barr viral isolates between sexual partners, and low level EBV in genital secretions. *J Med Virol* 78(9): 1204-1209.

Villaudy J, Wencker M, Gadot N, Gillet NA, Scoazec JY, Gazzolo L, Manz MG, Bangham CR, Dodon MD. 2011. HTLV-1 propels thymic human T cell development in "human immune system" Rag2(-)/(-) gamma c(-)/(-) mice. *PLoS Pathog* 7(9): e1002231.

Viscidi RP, Rollison DE, Sondak VK, Silver B, Messina JL, Giuliano AR, Fulp W, Ajidahun A, Rivanera D. 2011. Age-specific seroprevalence of Merkel cell polyomavirus, BK virus, and JC virus. *Clin Vaccine Immunol* 18(10): 1737-1743.