Title
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Journal
Journal of the National Cancer Institute, 109(4)

ISSN
0027-8874

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Publication Date
2017-04-01

DOI
10.1093/jnci/djx007

Peer reviewed

JNCI J Natl Cancer Inst (2017) 109(4): djx007

doi: 10.1093/jnci/djx007
First published online March 14, 2017
Commentary

COMMENTARY

Current State of PCR-Based Epstein-Barr Virus DNA Testing for Nasopharyngeal Cancer

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Abstract

Clinical studies have shown plasma Epstein-Barr virus (EBV) DNA level to be an independent prognostic biomarker for nasopharyngeal carcinoma (NPC). However, the proportion of NPC patients whose tumors are associated with EBV vary with geographic location, and there are a variety of assays for plasma EBV. To develop the level of evidence needed to demonstrate the clinical utility of plasma EBV DNA detection for NPC patients and encourage widespread adoption of this biomarker test in clinical laboratories, validated harmonized assays are needed. In 2015, the National Cancer Institute (NCI) convened a Workshop on Harmonization of EBV Testing for Nasopharyngeal Cancer, where experts in head and neck oncology and laboratory medicine addressed the limitations of currently available polymerase chain reaction–based EBV DNA quantitation assays and discussed strategies for advancing the development of harmonized EBV DNA assays and their appropriate clinical use. This article presents the key recommendations to direct future efforts in assay harmonization and validation.

Epstein-Barr virus (EBV) is well known as the cause of infectious mononucleosis, but it is also linked to cancers such as Burkitt lymphoma, B- and T-cell lymphomas, Hodgkin lymphoma, gastric cancer, and nasopharyngeal carcinoma (NPC). The incidence of NPC varies dramatically by geographic region, ethnicity, and gender. NPC is a rare cancer in the United States and Europe, with an overall incidence of less than one per 100 000 person-years, but it is much more common (>20 per 100 000 person-years) in certain parts of China and Southeast Asia (1). There have been a number of studies showing elevated IgA antibody titers to EBV antigens, such as EBV viral capsid antigen (VCA) and EBV early antigen (EA), in NPC patients compared with healthy individuals, supporting the use of EBV IgA serology as a screening tool for NPC in high-risk endemic populations (2–4). Some studies have shown superior sensitivity of circulating EBV DNA levels over EBV IgA serology for the detection of NPC (5–7). However, not all NPC patients have detectable circulating EBV DNA. According to a review of 15 studies involving EBV DNA quantitation, the reported sensitivities ranged from 53% to 96% (8). One explanation could be the use of assays with different performance characteristics, which would depend on PCR assay design and procedures, extraction method, and cutoff values. For example, it has been shown that there is a statistically significant inverse correlation between polymerase chain reaction (PCR) amplicon size and the percentage of patients positive for circulating EBV DNA (9).

Received: September 26, 2016; **Revised:** December 14, 2016; **Accepted:** January 9, 2017

Early studies using a PCR approach targeting a repeat sequence within the EBV genome confirmed a statistically significant association between NPC pathology and an elevated level of EBV DNA in plasma (10,11). In 2006, Leung et al. demonstrated that the EBV DNA load in plasma as measured by a quantitative PCR assay correlated inversely with overall survival and could be used to refine estimated survival for early-stage disease at diagnosis (12). Specifically, when patients with stage I or II NPC were stratified using a pretreatment cutoff of 4000 copies EBV DNA/mL plasma and followed for eight years, patients whose EBV DNA load fell below the cutoff (<4000 copies EBV DNA/mL) had a five-year survival of 91% (95% CI = 85 to 97), while patients whose EBV DNA load was higher than the cutoff had a five-year survival of 64% (95% CI = 53 to 75). Cutoff levels of EBV DNA are different in many studies: Some use 0 copies/mL, some 100 copies/mL, and others 200–4000 copies/mL. Because the cutoff value greatly affects the prognostic value as well as the sensitivity and specificity of EBV DNA assays, it has to be taken into consideration when interpreting study results and conclusions reported by various investigators. The ability of the test to detect low levels consistently (lower level of detection [LLD]) is also relevant.

Investigators have also studied the use of post-treatment plasma EBV DNA level in NPC patients undergoing radiation or chemoradiation therapy as a biomarker of post-treatment risk of disease recurrence and found it to be a better prognostic marker than pretreatment EBV DNA level or stage for progression-free and overall survival in patients with locally advanced NPC (13–15). However, in many published studies, the timing of "after treatment" is not consistently defined. Some measure EBV DNA after initiation of but still during treatment (16) and some immediately after treatment completion (13–15). This lack of standardization in the timing of specimen collection could lead to variability in the reported post-treatment EBV DNA levels, and findings of correlation between DNA levels and clinical outcome may not be reproducible. For example, it is possible that soon after treatment, acute and variable tumor effect and clearance activities might affect EBV DNA release and levels in circulation. They may increase (especially early during treatment), decrease (as tumor is killed by treatment), or remain the same (if treatment has no effect on tumor).

Finally, a large-scale screening study involving patients from Hong Kong and Toronto suggested that EBV DNA in nasopharyngeal epithelial cells, obtained using a transoral brushing biopsy and quantitated by PCR, was highly sensitive and specific in detection of NPC (17). Another cross-sectional study in Hong Kong on post-irradiated patients also demonstrated the value of EBV DNA from brushed nasopharyngeal samples in the detection of local recurrences (18). Other studies evaluating EBV DNA load in direct nasopharyngeal brushing or swab specimens also revealed good diagnostic value in detecting primary NPC (19,20) as well as local recurrent disease (21). Further refinement of noninvasive nasopharyngeal sampling technique and DNA preservation methodology will likely increase adoption of the methods for routine clinical use. The utility of transoral and direct nasopharyngeal brushings as a screening tool for early-stage NPC and post-treatment surveillance warrants additional clinical study.

Issues With Quantification Of EBV DNA by PCR

At present, there is no gold standard assay for quantification of EBV DNA for clinical or analytical purposes, and prior to 2011 there was no internationally validated reference material to calibrate EBV DNA assays or to account for the many variables that can influence EBV DNA quantification in different laboratories. Currently, there is no US Food and Drug Administration (FDA)–approved assay for the detection and quantification of EBV DNA. Clinical and analytical laboratories have laboratory-developed tests/assays (LDTs) for quantifying EBV DNA or have relied on a combination of commercially available research use only (RUO) and/or analyte-specific reagents (ASRs), with DNA purified from EBV-infected cells as a source of quantitative standards (ie, calibrators). Labs may also use plasmids with the cloned EBV target. Calibrators comprised of EBV virions are also commercially available (eg, EDX, Zeptometrix, Acrometrix). More than 30 distinct LDTs for quantifying EBV DNA are reported in the clinical literature, and a number of factors could lead to variability in EBV DNA quantitation by different LDTs, including the biological source (ie, plasma, serum, or whole blood), extraction and purification method of EBV DNA, and PCR reagents and techniques (eg, amplicon length, target gene, and target sequence). A comparison of several characteristics of six EBV LDTs is presented in Table 1, which provides some insight into real-world variability in EBV DNA quantification methods among the laboratories.

Several studies have also reported detailed side-by-side comparisons of the performance of some of these assays. For example, in 2009, Preiksaitis et al. were tracking EBV DNA in plasma of organ transplant patients in order to identify risk of post-transplant lymphoproliferative disorder (PTLD). This group distributed a set of plasma samples to 28 laboratories for EBV DNA quantification by nucleic acid amplification testing and then compared the results (reported as genome copies/mL) across labs (22). They reported that the variation in reported results on individual samples ranged from 2.28 $\log_{10}$ to 4.14 $\log_{10}$, that more than 52.0% of all data points fell outside acceptable standards for variation (ie, variation was more than 0.5 $\log_{10}$ outside of the expected value), and that interlaboratory variability for replicate samples was markedly greater than intralaboratory variability. This study demonstrated that the existing procedures could not be reliably used to provide an accurate estimate of EBV viral load. Several other publications have also examined and compared the performance of other quantitative assays for EBV DNA (23–28).

In preparation for the NRG-HN001 clinical trial (NCT02135042), the NRG Oncology Group evaluated the concordance and performance of a PCR-based assay for EBV DNA (referred to as the "BamHI W" assay) in the four laboratories participating in the trial. The results revealed relatively poor interlaboratory concordance, which triggered an attempt to harmonize the assay for improved performance (29). Prior to the harmonization effort, the intraclass correlations (ICCs) for 40 tested samples when the three laboratories were compared with the fourth indexed laboratory were 0.62 (95% confidence interval [CI] = 0.39 to 0.78), 0.70 (95% CI = 0.50 to 0.83), and 0.59 (95% CI = 0.35 to 0.76) (29). After the harmonization effort, the ICC values for 10 archival frozen samples improved to 0.83 (95% CI = 0.50 to 0.95), 0.95 (95% CI = 0.83 to 0.99), and 0.96 (95% CI = 0.86 to 0.99). This study also demonstrated that the largest improvement in interlaboratory concordance came from using the same assay calibrator. Additional data on the assay's limit of detection, precision, accuracy, cross-reactivity, and endogenous and exogenous interfering substances were collected at the Clinical Virology Laboratory for Stanford Health Care and Stanford Children's Health. The Stanford laboratory acts as the US reference site for the NRG-HN001 trial, and laboratories at

Table 1. Laboratory-developed tests for quantification of EBV DNA

	Sites/laboratories					
Assay parameters	Stanford University Medical Center	University of Washington/ Harborview Medical Center	Viracor-IBT Laboratories	Primex Clinical Laboratories	Mayo Clinic	Brigham and Women's Hospital
Specimen type	EDTA plasma	Blood, plasma, serum, CSF, DBS, tissue	Plasma, serum, whole blood, bone marrow, CSF, lower respiratory sample, tissue, fecal	Nasopharyngeal swab	Whole blood (for quantitative PCR); whole blood, body fluid (eg, CSF, amniotic, ocular), respiratory fluid (eg, bronchial washing, BAL fluid, NP aspirate, sputum), bone marrow, fresh tissue (for qualitative PCR)	Whole blood
Specimen volume	1 mL	3 mL blood, 1 mL CSF, 1 full circle DBS	Varies	500 μL	200 μL required	50 μL
EBV DNA extraction method	Automated magnetic bead-based method QIAsymphony DSP Virus/Pathogen Midi kit Cellfree 1000 protocol	Automated magnetic bead-based method, with pre-extraction lysis step if DBS or tissue is the source material, depends on specimen type	Easy mag or QiaCube, optimized by specimen type	Automated Roche	Roche MagNA Pure LC 2.0	Automated total nucleic acid isolation
Extraction instrument (if automated)	Qiagen QIASymphony SP/AS	Roche MP96 or Magnapure, Promega Maxwell 16	Easy mag or QiaCube-optimized by specimen type	Roche Magnapure	Roche MagNA Pure LC 2.0	Roche MagNA Pure 2.0
Elution volume	90 μL	100 μL	varies by specimen type	100 μL	100 μL	100 μL
PCR reaction volume	25 μL	50 μL; 32.2 μL (for EBV IR1 [large internal repeat])	_*	25 μL	20 μL	20 μL
Eluate input volume	10 μL	20 μL; 15 μL (for EBV IR1)	_	10 μL, samples standardized for DNA content prior to PCR	5 μL	5 μL
PCR enzyme	Qiagen Artus EBV (for routine); Roche FastStart Taqman Probemaster (for BamHI W assay for NPC)	Applied Biosystems AmpliTaq DNA Polymerase; Bio-Rad Sso7d fusion polymerase (for EBV IR1)	_	Taq polymerase	Roche FastStart	Taq polymerase
PCR mastermix	Qiagen Artus EBV (for routine); Roche FastStart Taqman Probemaster (for BamHI W)	In-house PCR mix; Bio-Rad SsoAdvanced Probes Supermix with ROX (2x) (for EBV IR1)	_	Thermo Taqman Universal Mastermix includes *Uracil N-glycosylase* (*UNG*)	Roche analyte-specific reagents	Qiagen *artus* EBV LC PCR kit (product has been discontinued by manufacturer)

(continued)

Table 1. (continued)

Assay parameters	Sites/laboratories					
	Stanford University Medical Center	University of Washington/ Harborview Medical Center	Viracor-IBT Laboratories	Primex Clinical Laboratories	Mayo Clinic	Brigham and Women's Hospital
EBV gene target(s) for PCR amplification	EBNA-1 (for routine); BamHI W (multicopy)	EBER (single copy); EBV IR1 gene (multicopy)	EBNA-1 and EBER - dual target	EBNA-1; EBER1/ EBER2 (single copy)	Latent membrane protein (single copy)	97 bp region of EBNA-1
PCR chemistry	TaqMan	TaqMan	TaqMan	_	FRET	Hydrolysis
PCR instrument	Qiagen Rotor-Gene Q	ABI 7500; ABI StepOne (for EBV IR1)	ABI	QuantStudio 12K	LightCycler 2.0	LightCycler 2.0
Cycling conditions	95C (10 min), 45 cycles (95C [15 sec], 65C [30 sec]) (touch-down 10 cycles 1C) 72C [20 sec]) (for routine); 95C (10 min), 45 cycles (95C [15 sec], 56C [30 sec]) (for BamHI W)	50C (2 min), 95C (2 min), 45 cycles (95C [20 sec], 60C [1 min]); (for EBV IR1) 50C (2 min), 95C (20 sec), 45 cycles (95C (3 sec), 60C [30 sec])	_	50C (2 min), 95C (10 min), 40 cycles (95C (10 sec), 60C (1 min)	_	45 cycles (3 steps each [95C, 55C, 72C])
Calibrator composition	Primary standard WHO, secondary standard = plasmid, Namalwa	Acrometrix EBV Panel – used to calibrate plasmid standard curve, Raji	Primary standard WHO, secondary standard = plasmid	EBV virus particles in plasma (Acrometrix EBV plasma panel) 100 to 1 000 000 copies	Roche calibrators (200 000 000 copies/mL to 2000 copies/mL), Acrometrix	EBV DNA plasmid
Calibrator extracted/not extracted	Extracted (WHO standard), not extracted (plasmid)	Extracted	Extracted (WHO standard), but not with every run	Extracted	Not extracted	Not extracted
Harmonization to IU/mL units (yes/no)	Yes (calibrated to WHO standard but awaiting international sites to adopt IU/mL units before implementing)	Yes	Yes	No	Not currently (data acquired to convert to IU/mL using either WHO or Acrometrix standards)	No
Unit	Copies/mL and IU/mL	IU/mL	IU/mL	Copies/mL, Ct value	Copies/mL	_
Assay contains internal control (yes/no)	Yes (for routine), no (for BamHI W)	Yes	Yes	Yes	Yes	Yes (provided in Qiagen PCR kit)
Quantitative/ qualitative	Quantitative for routine tests; reported as qualitative for NRG-HN001 trial	Quantitative for plasma, CSF, DBS; qualitative for tissue	Quantitative for multiple specimen types; qualitative for fecal and tissue specimens	Quantitative	Quantitative for whole blood; qualitative for multiple specimen types	_

*No response provided in survey. CSF = cerebrospinal fluid; DBS = dried blood spots; EBNA-1 = Epstein-Barr nuclear antigen 1; EBV = Epstein-Barr virus; IU = international unit; NPC = nasopharyngeal carcinoma; PCR = polymerase chain reaction; WHO = World Health Organization.

Singapore General Hospital and Fudan University Shanghai Cancer Center have been credentialed for international testing.

Subsequently, the World Health Organization (WHO) International Standard for EBV was established in late 2011 and made available to clinical and analytical laboratories (30). For assessment of the EBV International Standard, study samples of EBV were sent to 28 laboratories representing 16 countries across Europe, North America, Asia, and Africa. Variability between the individual laboratory mean estimates for the samples was up to 2.5 log10 copies/mL, and when the estimates for the samples were expressed relative to the EBV International Standard (a lyophilized, cell-free, whole virus preparation of the EBV B95-8 strain), there was improvement in the agreement between laboratories (31).

In 2014, the Stanford University team determined that the WHO reference material for EBV DNA demonstrated commutability for two EBV DNA PCR assays: the BamHI W assay and the commercial artus EBV QIAsymphony Rotor-Gene Q (QS-RGQ) assay (which is "European Conformity" [CE] marked and intended for in vitro diagnostic use in Europe) (32). The availability of the WHO reference material for EBV DNA simplifies future attempts to establish a validated, harmonized assay for use in research and clinical settings (see discussion below) and allows for successful credentialing of additional international sites to the HN001 trial.

Requirements for Commutability, Traceability, and Harmonization

The integrity of diagnostic medicine and good laboratory practice will be seriously challenged if multiple analyses of a single patient sample yield markedly different values for the same analyte being measured (ie, measurand). The relevant criteria for performance of quantitative laboratory assays for a biological quantity, such as EBV DNA, can be found in the Clinical Laboratory Standards Institute (CLSI) guidance documents EP30-A (*Characterization and Qualification of Commutable Reference Materials for Laboratory Medicine*) (33), MM06-A2 (*Quantitative Molecular Methods for Infectious Diseases; Approved Guideline, Second Edition*), and ISO 17511 (*In Vitro Diagnostic Medical Devices, Measurement of Quantities in Biological Samples, Metrological Traceability of Values Assigned to Calibrators and Control Materials*) available at http://shop.clsi.org/method-evaluation-documents/ep30.html, http://shop.clsi.org/molecular-methods-documents/mm06.html, and http://shop.clsi.org/iso-documents/iso-17511_2.html, respectively.

In brief, accurate quantitation of a biological quantity can only be assured if a suitable reference material is used as a primary standard. A reference material is suitable and is said to be "commutable" when measurement results for the reference material and for a native clinical sample have the same relationship when two measurement procedures are compared, independent of the assay procedure used to quantify the specific measurand (34). Measurement procedures calibrated with commutable reference material will then produce clinical sample results that are equivalent among different measurement procedures and different LDTs (eg, there is no calibration bias or matrix bias).

Clinical laboratory assays are said to be "harmonized" when the results of all assays are independent of the specific assay procedure/protocol and where and when the assay is performed. If a commutable reference material is available and the unit of measurement for the measurand has been standardized, the assays can be harmonized by requiring that all procedures be directly or indirectly calibrated to the primary reference material using the standardized unit of measure. This assumes that all measurement procedures measure the same measurand with suitable analytical specificity and are free of interference from all other substances within the sample. For a set of assays for a specific measurand, traceability is the set of relationships that ensure that all measurements are calibrated to a common reference standard.

Recommendations for Developing a Harmonized Assay for EBV DNA Quantitation

The harmonization of laboratory procedures as presented above is highly relevant to developing quantitative assays for EBV DNA. Given the emergence of PTLD in the United States and Europe, the endemic nature of NPC in Southeast Asia and other parts of the world, and the ubiquitous nature of EBV and its association with many rare and a few common pathological syndromes, there is sufficient clinical relevance as well as urgency behind the need for a reliable quantitative assay for EBV DNA. Key stakeholders and thought leaders gathered to discuss the need for such an assay in a National Cancer Institute (NCI) Workshop on Harmonization of EBV Testing for Nasopharyngeal Cancer, held in November 2015. The workshop was convened by the NCI's Cancer Diagnosis Program, whose mission is to support research and education to improve the performance of diagnostic technologies and biomarker tests for cancer and move them into clinical practice, and was cosponsored by the NCI's Center for Global Health and the National Institutes of Health Office of Rare Diseases Research. The discussions among the head and neck oncologists, laboratory medical directors, and representatives from commercial reference laboratories providing EBV testing services have identified important points of consensus and some unresolved questions and concerns. Specific goals and recommendations are discussed in this section and summarized in Box 1.

One point of consensus is that several practical problems will need to be solved and more studies conducted before clinical practice guidelines can be formulated and the full potential of using plasma EBV DNA as a quantitative biomarker for NPC and other conditions can be realized. For instance, there is no clear consensus on the optimal timing, time interval, or frequency of testing, and these conditions will need to be established through clinical studies and guidelines development. Furthermore, the cutoff values established for therapeutic intervention vary with the local laboratory/center applying their own management algorithms specific to their LDTs, making it challenging to establish broadly applicable clinical guidelines at this time. Identification of the optimal cutoff value and refinement based on validation studies will need to be critical aspects of future studies using harmonized assays. The cutoff will also depend on the intended use of the assay (eg, screening, case finding, surveillance, etc.).

To begin to address these problems, a standardized assay should be developed and suitable reference materials (ie, secondary or tertiary reference materials that are traceable to the WHO International Standard for EBV DNA) must be identified. More importantly, members of the relevant clinical organizations and laboratories should make a collective effort to harmonize the assay procedure(s) for clinical use worldwide. This would require a multicenter study to evaluate the performance of one or more EBV DNA assay protocols, including the limit of

Box 1. Recommendations for developing harmonized assays for EBV DNA quantitation*

1) Identify and develop a set of standard conditions and reagents that will help produce comparable data on EBV DNA abundance independent of time and place, including
   A) sample type and preparation method (plasma, serum, or whole blood);
   B) standardized EBV DNA extraction and isolation method and materials (eg, sample spiking with control DNA molecule for DNA recovery efficiency and quality control, use of automated platforms); and
   C) aliquoting and short-term storage conditions.
2) Focus on developing a PCR assay that
   A) uses a standardized PCR protocol and reagents (kits);
   B) is suitable for analysis of plasma, nasopharyngeal cells, or tumor cells;
   C) uses primers and probes that target conserved sequence regions;
   D) targets two EBV sequences (amplicon size < 100 bp) in parallel, including a single-copy sequence and a multiple-copy sequence;
   E) reports in international units (IU) per mL (or for tissue testing, normalized to a human gene, such as beta globin); and
   F) is calibrated using secondary or tertiary reference materials that are traceable to the WHO reference standard for EBV DNA.
3) Examine whether and how much the EBV DNA quantitation can be improved by adapting next-generation sequencing-based analytical procedures or digital PCR.
4) Conduct a multicenter study to evaluate the performance of the EBV DNA assay protocol,
   A) using a panel of well-characterized clinical samples that includes positive and negative controls (eg, non-NPC H&N cancers, healthy EBV carriers), samples that cover a large range of concentrations, and several sample types;
   B) to define the analytical performance, including the limit of detection, linear range, precision, reproducibility (inter- and intra-assay variability), sensitivity, and specificity; and
   C) to establish cutoff values tailored to sample types and to the intended use (eg, prognostication of progression or recurrence, early detection, etc.)
5) Initiate clinical validation studies through multi-site clinical trials.

*EBV = Epstein-Barr virus; H&N = head and neck; NPC = nasopharyngeal carcinoma; PCR = polymerase chain reaction.

detection, limit of quantification (as applicable), linearity of the assay across the measuring range, precision, and reproducibility (inter- and intra-assay variability). This study should use a panel of well-characterized clinical samples that includes positive and negative samples, samples that cover a large concentration range (ie, the assay measuring range), and several sample types. Without appropriate harmonization, it will not be possible to make optimal use and/or to properly interpret the data collected with the existing assays because of the unaccounted effects of many variables that influence assay outcomes. In addition, the assay as well as the clinical guidelines for use of assay data may need to be optimized differently for different clinical indications or sample types.

Another point of consensus is that the quantitative accuracy of the EBV DNA assay needs to improve. Data from clinical and analytical laboratories show that the lower limit of detection of the EBV DNA assay is lower when the target sequence is represented by multiple copies in the EBV genome (eg, the BamHI W repeat sequence). However, because the target copy number varies in different viral clones or strains, it is challenging to compare absolute values when samples are collected from different patients. For example, the virus in one patient's tumor may harbor five copies of the PCR target sequence per viral genome, while the virus from another patient's tumor may harbor two or three copies per viral genome, such that the abundance of EBV DNA in the plasma of these two patients may not directly correlate with tumor burden. Moreover, amplification from the BamHI W repeat sequence could result in a variable number of the targets per EBV genome across different patients, which cannot easily be controlled and may lead to inaccurate quantification. Amplification from the BamHI W repeat sequence may impact the development of quantitative thresholds for clinical decision-making, though it will be important to determine whether approximately two- to threefold differences in repeat number is clinically significant in the context of overall assay imprecision and other sources of biological variation.

One approach to address this problem would be to test two different sequences in the EBV genome in parallel (ie, via two PCR assays with independent readouts), where one target is in a single copy gene, such as EBNA1 (BKRF1), p134 (BNRF1), or DNApol (BALF5), and the second target is in a region that is present as multiple copies per EBV genome. Evaluating both a multicopy and a single-copy target could potentially maximize sensitivity without compromising quantitative accuracy. While this approach could enable more accurate quantification of EBV copy number when sufficient levels are present, if the levels are so low that only the multicopy amplicon is detected, the inability to accurately characterize the EBV copy number present must be acknowledged, for example, by reporting that the EBV DNA is detectable but not quantifiable. The quantitative readout from the assay targeting a single-copy amplicon can be used for measuring tumor loads across different patients, whereas the assay targeting the multicopy gene can provide maximal sensitivity for detecting the tumor cells present in low amounts (eg, in the detection of minimal residual disease after treatment or screening of asymptomatic patients). In addition, strain variation exists in the EBV genome, with eight different strains completely sequenced as of 2014 (35), such that primers for PCR amplification should target highly conserved sequences in the genome to enable reliable quantification of EBV DNA across different EBV strains/isolates/regional variants. Other approaches to improve the sensitivity of the assay should also be pursued. For example, next-generation sequencing-based analytical procedures and novel technologies such as digital PCR may provide increased sensitivity, though evidence supporting the use of these methodologies remains to be gathered.

Conclusion

In summary, there is an urgent need to harmonize and validate a quantitative assay for EBV DNA. Development of clinical

practice guidelines and dissemination of knowledge about the utility of EBV DNA in the clinical setting will depend on the availability of a reference standard that is commutable and assays that have been harmonized on a large scale, which are integrated into clinical validation trials that will generate the level of evidence to support the recommendations. It is anticipated that such an assay could become an effective tool to support safe and appropriate decisions on how to treat and manage patients affected by EBV-related pathologies.

Despite consensus among stakeholders on this need, a precise path toward harmonization of a quantitative assay for EBV DNA has not yet been defined or agreed upon. However, the investigators participating in the November 2015 NCI workshop on this topic have outlined initial recommendations and suggestions for working toward this goal, as discussed in the previous section and in Box 1. The workshop participants were encouraged to work toward validating and harmonizing quantitative assays for EBV DNA. The workshop participants agreed on the immediate next steps of collaborating on selection of primers and amplicons for the EBV DNA assay and mounting a multicenter comparative study on a set of clinical samples.

Acknowledgment

This project was supported by grants U10CA180868 (NRG Oncology Operations) from the National Cancer Institute (NCI).

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