

Commentary:

The Evolving Definition of Carcinogenic Human Papillomavirus

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“We have to live today by what truth we can get today and be ready tomorrow to call it falsehood.”—William James (1842-1910)

5 Abstract

It is now widely accept that approximately 15 human papillomavirus (HPV) genotypes cause virtually all cervical cancer worldwide. At a recent meeting of experts for the 100th International Agency for Research on Cancer monograph meeting on biological agents as causes of cancer, 12 HPV genotypes (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) were classified as
10 carcinogens and another (HPV68) was classified as a probable carcinogen (HPV68). Other HPV genotypes that are closely related to the carcinogenic and possibly carcinogenic were classified possibly carcinogenic. This category included HPV66, which was previously classified as a probable carcinogen in 2005. As a consequence, HPV66 was included as a target HPV genotype for detection by clinical HPV tests in development during this time period. The implications of
15 including HPV66 and other borderline carcinogenic HPV genotypes in clinical HPV tests are discussed.

Periodically the International Agency for Research on Cancer (IARC) convenes a meeting of cancer experts to review and update the evidence regarding potential carcinogens. In February of 2009, the meeting for the 100th IARC monograph convened on the carcinogenicity of biological agents, including human papillomavirus (HPV), now widely acknowledged as the obligate cause of cervical cancer and the important cause of other epithelial cancers, updating the evidence from the 90th monograph meeting in 2005 (1;2). One of the primary goals of this meeting was to examine the evidence for potential for each of the more than 100 HPV genotypes to cause cancer. The meeting report by Bouvard *et al.* (3) provides an overview of the meeting for all biological agents and Schiffman *et al.* (ref) provide the rationale for classify mucosotropic HPV as cancer-associated (carcinogenic) or not. Based on large case series, meta-analyses, and laboratory data of mechanistic studies, twelve HPV genotypes (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) were deemed carcinogenic and HPV68 was considered probably carcinogenic.

One of the important outcomes of the meeting was the downgrading of HPV66 from the category of probably carcinogenic, as judged in 2005, to possibly carcinogenic, which also includes HPV genotypes HPV26, HPV53, HPV67, HPV70, HPV73, and HPV82. As explained by Schiffman *et al.* (ref), these HPV genotypes had limited epidemiological evidence to cause cancer but are genetically related to other HPV genotypes that are carcinogenic. Unlike for HPV68, which is known to maintain a cancer cell line (ME 180), there are as yet only scarce laboratory data showing carcinogenic properties of few of the HPV genotypes classified as possibly carcinogenic (group 2B)(3). Unfortunately, laboratory assays that measure p53 degradation and immortalization of primary human keratinocytes (4) and E6 binding to PDZ domains on p53,

E6AP, Magi1 and hD1g and the degradation of hD1g and Magi1 (5) do not effectively distinguish between carcinogenic and non-carcinogenic HPV genotypes within the primarily carcinogenic HPV phylogenetic species alpha-5, -6, -7, -9, and -11.

5 Temporarily categorizing HPV66 as probably carcinogenic during the four-year interval meetings impacted the development of new clinical HPV assay. In 2005, there was only one FDA-approved HPV DNA test, hybrid capture 2 (hc2; Qiagen (formerly Digene), Gaithersburg, MD, USA), which targets the 13 certain and probably carcinogenic HPV genotypes as a pool of HPV genotypes. In 2009, one additional test, Cervista (Third Wave Technologies, Hologic, Inc.,
10 Madison, WI, USA), received FDA approval (<http://www.fda.gov/cdrh/mda/docs/P080015.htm>) and several other assays from other companies are being evaluated in clinical trials. Most have included HPV66 in their panel of targeted HPV genotypes.

What is the impact of including HPV66 in clinical tests? The lifecycle of these tests, from
15 development to validation, is 10 years or more. As a consequence, there will be a generation of HPV tests (even some that are not now in clinical trials but too far along in the formulation phase to alter) that include HPV66 and in theory will be slightly less specific than what might otherwise be achieved. Thus, more women will test positive for HPV when used in primary screening or as triage/reflex test for equivocal (atypical squamous cells of undetermined
20 significance [ASC-US]) cytology.

Worldwide, the prevalence of HPV66 estimated to be 0.4% (95%CI = 0.3-0.4%) although there are some regional variations in its prevalence (6). Therefore, an additional 1 in 250 all women

5 screened (400 in 100,000 women) will be called carcinogenic HPV positive because of the inclusion of HPV66[†]. Of those who are HPV66 positive, perhaps another $\leq 0.5\%$ will have a concurrent diagnosis of cervical intraepithelial grade 2 (CIN2), equivocal precancer (7;8), or CIN3, more certain precancer, both of which are typically treated by excision or ablative procedures. Thus, the inclusion of HPV66 will result in the identification of HPV66-related CIN2/3 in roughly 2 in 100,000 women screened with the current HPV tests. Whether these HPV66-related CIN2/3 cases are clinically important, i.e. have invasive potential, or not remains unclear.

10 Future meetings will no doubt try to resolve whether these borderline carcinogenic HPV genotypes (HPV26, HPV53, HPV66, HPV67, HPV70, HPV73, and HPV82) are cancer causing or not. Some HPV genotypes may shift up and down in classification with subsequent iterations due the inherent poor signal to noise of these borderline types. Since cancer events will be exceeding rare by those that are actually intrinsically carcinogenic, integrative epidemiologic and laboratory approaches will be needed for clarification. New investigative tools, such as micro-
15 dissection of the lesion and HPV genotyping or *in situ* methods if they are ever prove to be sufficiently sensitive, detection of over-expressed HPV genotype-specific E6/E7 transcripts or protein, or better *in vitro* models (9;10) may help to assign causal HPV genotypes.

20 In the end, perhaps resolving this issue of which of the borderline HPV genotypes are carcinogenic does not matter except for academic albeit important biological reasons: to understand the nature of HPV carcinogenicity and its relationship to viral genetics and

[†] For simplicity, the contribution of HPV66 to testing positive has ignored the unknown fraction of HPV66 positives co-infected with carcinogenic and probable carcinogenic HPV genotypes.

phylogenetics (11). We already know enough to decide which HPV genotypes to include in clinical HPV tests. As shown by others (12) using a receiver-operator curve (ROC)-like approach and CIN3 as the intermediate cancer endpoint, after including 8-10 most carcinogenic HPV genotypes there was only a slight increase in sensitivity and a larger cost in specificity with each additional HPV genotype included. However, CIN3 is subject to some diagnostic error (13;14), and some questionable or non-carcinogenic HPV genotypes can cause CIN3 that has little potential for invasion (14). Thus, one would expect even greater clarity by redoing this analysis with a cancer endpoint using the accumulated data presented at the 100th IARC monograph meeting. Experts then could come to a consensus about what is an acceptable tradeoff in sensitivity versus specificity vis-à-vis which HPV genotypes to include in clinical assays, accepting the notion that no test will achieve perfect sensitivity and that poor specificity has important implications, including potential unnecessary treatment, which can cause negative reproductive outcomes (15;16).

As a first approximation, a simple version of this ROC-like analysis approach based on previously reported data (6;17) is shown in the **Figure**. Notably, as previously noted (12), there is very little gain in sensitivity after the inclusion of the first ~10 HPV genotypes. Of note, the addition of the possible HPV genotypes decreases specificity by approximately 2%, or 2,000 additional HPV positives per 100,000 women, with virtually no benefit. Notably, most of these possible HPV genotypes (HPV53, HPV66, HPV67, HPV70, HPV73, and HPV82) are commonly detected by hc2 as the result of less than perfect targeting of the carcinogenic and possibly carcinogenic HPV genotypes (18). Despite the cross-reactivity to these weakly carcinogenic or non-carcinogenic HPV genotypes, hc2 has been proposed as the current benchmark for HPV test

performance (19;20). More fidelity for the important HPV genotypes will improve the performance of HPV tests by reducing the number of false positive results. A more formal ROC-like analysis of the data from the 100th IARC monograph (3) is needed to confirm these observations.

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The inclusion or exclusion of borderline carcinogenic HPV genotypes in a clinical assay for carcinogenic HPV becomes moot if robust strategies for management (risk stratification) of test positives become available. That is, there would be no significant penalty to include some of these borderline HPV genotypes if there was downstream, specific method of triaging HPV-
10 positive women to separate those in need of immediate colposcopy versus those who could be monitored by repeat screening in a year or two. The key is good stratification of risk for cervical precancer and cancer, and consistent, appropriate management of that risk (21). Some approaches, such as using cytology (22), HPV genotyping (23), and/or p16 immunostaining (24), look promising but need further validation. New, as of yet undiscovered, biomarkers may
15 provide still better solutions.

Although the inclusion of possibly carcinogenic HPV genotypes may hurt test specificity, it may indirectly increase the reassurance following a negative HPV test i.e. the negative predictive value of an HPV test for cervical precancer and cancer. Because non-carcinogenic and
20 carcinogenic HPV infections alike are sexually transmitted, identification of women with borderline carcinogenic or non-carcinogenic HPV identifies a subset of higher-risk women who are more likely to subsequently acquire a new carcinogenic HPV infection. That is, women who are HPV positive for any HPV type at any given time point represent a sub-group that engages in

more risky behavior, such as having more sexual partners, or have a partner(s) who is more likely to do so.

5 The future of cervical cancer screening in low-resource setting may include once-in-a-lifetime, low-cost and rapid HPV testing (25;26). The inclusion/exclusion of HPV genotypes, and therefore the tradeoff of sensitivity versus specificity, will depend greatly on the strategy for following-up and managing the screen positives and the resources to do so. Prevention programs that will require the use of the more traditional screen, colposcopic evaluation of screen positives, and treatment of women with precancer will need sufficient numbers of highly-trained
10 colposcopists to handle the clinical volume of screen positives, which could approach 10-15% of the population in many locations (27). The tradeoff of more false positives for greater reassurance may not be an acceptable if the local infrastructure cannot manage the screen positives.

15 Other prevention programs may adopt screen and treat approaches, in which all screen positives undergo some treatment. For example, HPV-positive women could then be evaluated by visual inspection with acetic acid to decide the best treatment option: chemotherapy or palliative care for women with obvious cancer, surgical lesion excision (by a physician at a regional medical center) for large precancerous lesions that are not amenable to cryotherapy, and cryotherapy for
20 all other HPV-positive women. Such an algorithm would only be applied to populations of older women, more than 10-15 years past the population median age of sexual debut, to minimize the over-treatment of mostly transient HPV infections in reproductive-age women. In general, because HPV-positive women will not undergo any further characterizations such as colposcopy

or a secondary test, there should be a greater emphasis on specificity i.e. inclusion of only the certain carcinogenic HPV genotypes. However, there is some evidence to suggest that cryotherapy provides a secondary benefit of reducing the likelihood of acquiring new HPV infections post-treatment (Dr. Thomas C. Wright, personal communication), which might
5 increase the acceptability of over-treatment of screen positives when applied once to a population.

In the end, it seems unlikely that companies that develop HPV tests will tailor their tests to individual country needs. Now is the time for the community of scientists, doctors, and public
10 health advocates to use the data presented at the 100th IARC monograph meeting to rationally decide the target HPV genotypes for the next generation of HPV tests for use in high-resource and low-resource settings. And provide guidance on the use of these tests for cervical cancer prevention programs. However, the adoption of any cervical cancer screening and prevention program will depend on its social and cultural acceptance as well as available resources and the
15 political willpower to implement it.

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Figure. The impact of adding individual carcinogenic, probable carcinogenic, and possible carcinogenic human papillomavirus (HPV) genotypes to a clinical HPV assay was estimated using a receiver-operator curve (ROC)-like approach of stepwise adding individual HPV genotypes according to their prevalence, from most prevalent (HPV16) to least (HPV53), in cervical cancer (6;17). Relative sensitivity (true positive fraction) was estimated based on those prevalences using the following assumptions: 1) HPV genotypes act independently such that cancers with multiple HPV genotypes detected were proportionally attributable to the HPV genotypes according to their overall prevalence; and 2) all cervical cancer is caused by HPV, and false negative results were independent of HPV genotype. Therefore, it was assumed that all cancers would have tested HPV positive with a perfectly sensitive test, and the relative contribution of each HPV genotype to cervical was a constant (although the absolute number increased). The relative false positive fraction, 1-Specificity, was estimated from the prevalence of individual HPV genotypes in the cytologic normal population, ignoring the overlap between HPV genotypes due to multi-HPV genotype infections (~25% of HPV positives).

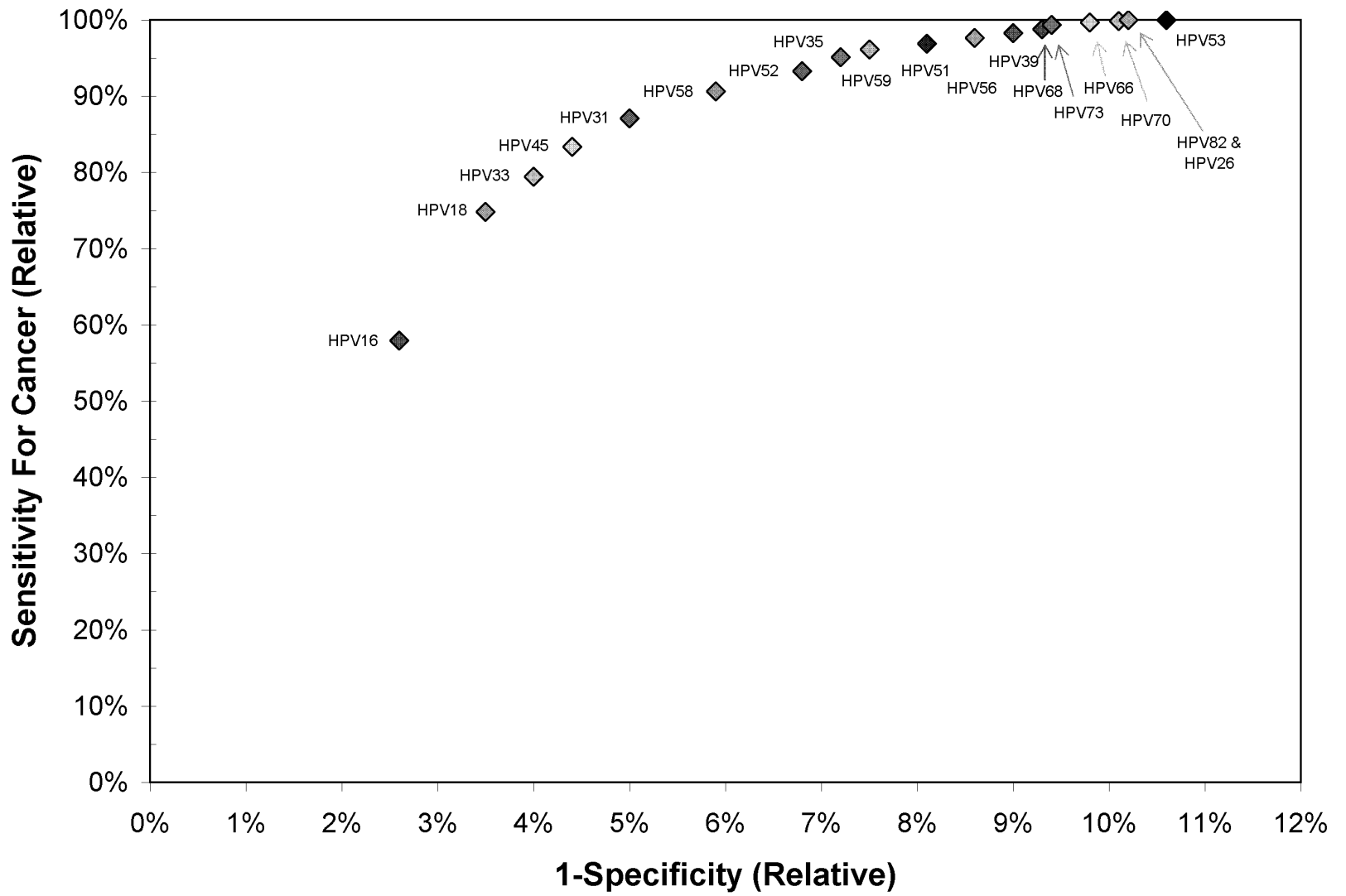


Figure 1

Additional files provided with this submission:

Additional file 1: iac .pdf, 79K

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