>2× ULN; 3) serum aminotransferase levels were elevated and accompanied by possible signs or symptoms of hepatic injury; or 4) the event was assessed as medically serious by medical reviewers for any other reason.

In the 3.5-year period from June 15, 2007, to December 14, 2010, 10,927 patients were exposed to marketed ambrisentan in the United States with a total exposure representing 9,893 patient-years (Fig. 1). The mean time on ambrisentan was 330.5 days, with 62% of patients on ambrisentan for longer than 3 months and 50% longer than 6 months. The LEAP database included 314 (2.87%) post-marketing spontaneous reports of possible hepatic injury, of which 156 (1.43%) were medically confirmed. A total of 77 medically confirmed cases did not meet the criteria of clinically significant hepatic events (all of these cases reported small or unspecified elevations in liver function tests) with 79 (0.72%) remaining as clinically significant hepatic events. Six of the 79 clinically significant hepatic events had both elevated aspartate and alanine aminotransferase >3× ULN and serum total bilirubin >2× ULN and were considered as potential Hy's Law cases (Hy's Law is defined as >3× ULN for serum alanine or aspartate aminotransferase and serum total bilirubin >2× ULN), with no other reason to explain the combined increase and is considered a marker of possible drug-induced liver injury (3). In 5 of the 6 reports, probable alternative causes of the hepatic events were reported, with the sixth case having intermittent enzyme elevations for 2 years and a poorly documented medical history. Possible alternative causes or contributory factors for the hepatic events were also present in 55 of the 73 remaining medically confirmed clinically significant cases, and in 38 of these, ambrisentan was successfully restarted.

PAH is itself associated with congestive liver disease due to transient or progressive right ventricular dysfunction. PAH patients may also have abnormal serum aminotransferases due to concomitant medical conditions such as underlying diseases that cause secondary PAH. In placebo-treated patients in published clinical trials for ERA, background incidence of elevated aminotransferase levels of up to 6% has been reported in patients with PAH (4). In normal populations, 1% to 4% may also have an isolated or transient elevation of liver transaminases (5).

Differences in the structure of different ERAs may underlie differences in hepatic safety profiles. Bosentan and sitaxsentan are both sulfonamide-based, whereas ambrisentan is propanoic acid–based. As such, the compounds may have different effects on hepatocytes. In vitro analysis using sandwich-cultured human hepatocytes reveals that both bosentan and sitaxsentan reduced hepatic transport pumps. Ambrisentan, in contrast, showed no inhibition of influx or efflux pumps (6).

Based on the LEAP data, in March 2011, the FDA removed the requirement for mandatory monthly monitoring of LFT with ambrisentan therapy (the monitoring for pregnancy and the black box warning against the use of ambrisentan in pregnancy was maintained, in keeping with the known teratogenicity of ERA).

***Ori Ben-Yehuda, MD**
David Pizzuti, MD
Andrea Brown, MD
Marcus Littman, MBA
Hunter Gillies, MD
Noreen Henig, MD
Tobias Peschel, MD, PhD, MBA

*Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, California 94404
E-mail: Ori.Ben-Yehuda@gilead.com

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Please note: All authors are employees of Gilead Sciences, Inc., and own Gilead Sciences stock and/or stock options.

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Letters to the Editor

Human Papillomavirus and Cardiovascular Disease

We read with great interest the report by Hsu-Ko Kuo and Ken Fujise (1) titled "Human Papillomavirus and Cardiovascular Disease Among U.S. Women in the National Health and Nutrition Examination Survey, 2003 to 2006," recently published in the *Journal*.

The investigators report an association between the presence of human papillomavirus (HPV) deoxyribonucleic acid in the vagina and the risk for cardiovascular disease (CVD) in a population of women who participated in a national health and nutritional survey. They made the assumption that after HPV infection, the 2 major viral oncoproteins, E7 and E6, through their pRB and p53 degradation properties, may favor the development of atherosclerosis. The investigators argued that knock out for either p53 or pRB atherosclerosis development was accelerated in mice models. However, we would like to make some points that should be discussed in greater depth.

First, the overall HPV prevalence was high in the cohort. Kuo and Fujise (1) reported HPV deoxyribonucleic acid in 48% of women, 25% being infected by high-risk HPV, with a mean age of 35 years. Large epidemiological studies have reported a high HPV prevalence (up to 30% to 40%) in young women (age 20 to 24 years), while the prevalence decreases to 10% or less in women older than 35 years (2,3).

Second, women who were considered "nonresponders" were reportedly different from women included in the analysis. The investigators indicated that they were younger and thus more likely to harbor HPV deoxyribonucleic acid but probably less likely to present CVD than the "responders."

Third, of the 44 women with cervical cancer listed in the their Table 1 (1), 22 were HPV negative. This is not consistent with the fact that HPV is the etiologic agent for cervical cancer.

Fourth, in their Table 3, Kuo and Fujise (1) presented the odds ratios (ORs) for CVD comparing women with cancer-associated HPV types or other HPV types with those who are negative for HPV. In the first model, the OR was 2.87 for women with cancer-associated HPV types. The OR was 2.13 for women with other HPV types, namely, low-risk HPV types that are not able to induce p53 and pRB degradation. If the physiopathology hypothesis is correct, we would expect a nonsignificant OR. As for models 2, 3, and 4, we would also expect lower ORs for CVD in women with other HPV types.

Finally, the overall physiopathology hypothesis is very unlikely. Indeed, HPV fails to produce classic viremia. Rather the virus replicates locally until it is cleared by an efficient immune response. In case of viral persistence, the virus remains localized to the site of infection, and associated lesions can occur after an increased expression of E6 and E7. The virus does not disseminate throughout the body. It is therefore very unlikely that E6 and E7 have a systemic effect leading to atherosclerosis.

***Jean-Luc Prétet, PhD**
Mariette Mercier, PhD
Didier Riethmuller, MD, PhD
François Aubin, MD, PhD
Dominique Vuitton, MD, PhD
Christiane Mougin, MD, PhD

*Laboratoire de Biologie Cellulaire et Moléculaire
Bâtiment Diaclone
Hôpital J Minjoz
25000 Besançon
France
E-mail: jean_luc.pretet@univ-fcomte.fr

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Reply

We thank Dr. Prétet and colleagues for their insightful comments on our report (1). Brief, point-by-point responses provided here illuminate the need for further research on the link between human papillomavirus (HPV) infection and atherosclerosis.

First, on the basis of recent survey from the National Health and Nutrition Examination Survey 2003 to 2006, the weighted prevalence of genital HPV infection among 2,787 women was estimated to be 44.5% (2), which is close to the unweighted prevalence we reported (46.6%) (1). Using the research use–only Linear Array genotyping assay (Roche Diagnostics GmbH, Mannheim, Germany) has resulted in a higher HPV prevalence than previously reported (3).

Second, we do not know if the "nonresponders" (n = 543) were more likely to harbor HPV deoxyribonucleic acid, because they did not submit swab specimens or submitted inadequate swab specimens. The crude prevalence of cardiovascular disease (CVD) among responders and nonresponders was 2.6% and 2.2%, respectively (p = 0.612).

Third, ascertainment of cervical cancer was by self-report and may have suffered from recall bias and a certain degree of disagreement as to "true measures" of cervical cancer compared to "self-report." We reanalyzed the data for the HPV-CVD association without using self-reported cervical cancer as a covariate, and the results were the same.

Fourth, the association between other HPV types and CVD was significant in model 1 (odds ratio: 2.13; 95% confidence interval: 1.12 to 4.06) but not in models 2 to 5. Low socioeconomic status, associated with both HPV infection and CVD, is an important confounder for which we were not able to control (4).

Finally, although the mechanism by which HPV infection could promote atherosclerosis remains unknown, several possibilities exist. First, chronic local inflammation caused by HPV infection and resultant circulatory inflammatory mediators can facilitate atherosclerosis as seen in *Porphyromonas gingivalis* infection (5). Second, macrophages and monocytes (MΦ) recruited to HPV-infected tissue (6,7) can take up the virus (8), leading to the degradation of MΦ-p53 by E6 and E7. HPV-infected MΦ can then enter atherosclerotic plaques and facilitate atherosclerosis. The lack of functional p53 in MΦ facilitates atherosclerosis in mouse models of atherosclerosis (9,10). These hypotheses, although plausible, need to be experimentally tested.

***Hsu-Ko Kuo, MD, MPH**
Ken Fujise, MD

*University of Texas Medical Branch
Department of Internal Medicine
301 University Boulevard
Galveston, Texas 77555
E-mail: hskuo@utmb.edu

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