UICC
global cancer control

IJC
International Journal of Cancer

Author's reply to: multiple human papillomavirus genotype infections in cervical cancer progression in the study to understand cervical cancer early endpoints and determinants

Salvatore Vaccarella[1], Gary M. Clifford[1], Rebecca Howell-Jones[1], Peter J.F. Snijders[2] and Silvia Franceschi[1] and for the International Agency for Research on Cancer Multicentric Cervical Cancer Study Group

[1] Infections and Cancer Epidemiology Group, International Agency for Research on Cancer, Lyon, France
[2] Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands

Dear Editor,

The attribution of specific human papillomavirus (HPV) types to invasive cervical cancer (ICC) is important to predict the efficacy of current and future HPV vaccine programs and to decide which HPV types should be included in HPV-based screening tests. Although ICC is assumed to originate from a single transformed cell, its attribution to an individual HPV type is complicated by the increasingly frequent detection of multiple HPV types in cervical samples.[1]

A previous article by Wentzensen *et al.*[2] in this journal used different algorithms to assess the potential range of attribution of specific HPV types to various grades of cervical disease in the United States. This cross-sectional study included 1,670 women who were referred to colposcopy because of abnormal cytology or histology, among whom only 107 had ICC. The HPV detection method was based on the linear array assay, a very sensitive test capable of detecting 37 HPV types.

In this report, we have replicated the algorithms used by Wentzensen *et al.* in a much larger number of ICC cases (1,728 histologically confirmed ICC cases) derived from the IARC Multicentric Case–Control Study (IMCCS). The IMCCS was carried out between 1990 and 1999 and included nine study areas (Brazil, Mali, Morocco, Paraguay, Philippines, Thailand, Peru, India and Algeria). In the IMCCS, the overall presence of HPV DNA was determined using a general GP5+/6+ primer-mediated PCR. HPV positivity was assessed by hybridization of PCR products in an enzyme immunoassay using two HPV oligoprobe cocktails that, together, detect 33 HPV types: HPV6, 11, 16, 18, 26, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 57, 58, 59, 61, 66, 68, 70, 72 (equivalent to CP4137), 73 (MM9), 82 (equivalent to IS39 and MM4 subtypes), 83 (equivalent to MM7), CP6108 and CP8304. Subsequent HPV typing was performed by reverse-line blot hybridization of PCR products, as described earlier.[3,4] For India and Algeria, the oligoprobe cocktail was extended to include HPV types 55, 71 (equivalent to CP8061) and 84 (equivalent to MM8). The IMCCS also included two studies from Spain and Colombia which, however, employed different primers (MY09/11 consensus primer) and, therefore, were not included in the present analysis.

By analogy with Wentzensen *et al.*[2] and Insinga *et al.*,[5] attribution of multiple HPV types in our present report was based on the "proportional" and "hierarchical" algorithms. In the "proportional" approach, attribution of cervical disease to an HPV type was based on the frequency of that type as a single infection within the study. For example, in a multiple infection consisting of HPV16, 31 and 53 where frequencies in single infections are 50%, 15% and 20% for the respective types, the case would be split between the three types (50/85 for HPV16, 15/85 for HPV31 and 20/85 for HPV53). Conversely, in the "hierarchical" approach, instead of a partial attribution, a case with multiple HPV types was completely attributed to the type, among those present in the coinfection, most frequently found in single infections. In the above example, the case would be, therefore, fully attributed to HPV16. Due to the small number of single infections (only 69 out of 107 ICC, of which 44 with HPV16), Wentzensen *et al.* had to base attribution algorithms for ICC on the overall frequency of HPV types, rather than on the frequency of types in single infections. To note, while the proportional algorithm can attribute cases to HPV types with low oncogenic potential, the hierarchical attribution favors the most frequent types in cancer, particularly HPV16.

Ninety-four percent of cervical cancer cases in the IMCCS were infected with at least one HPV type. Among HPV-positive women, two types were detected in 9.8%, and three or more types in 1.1% of women. Overall, the most common HPV types were, in decreasing order, HPV16, 18, 45, 33, 31 and 52. The attribution to HPV16 ranged from a minimum of 50.1% among single infections to 54.4% using the proportional attribution, to 55.2% using the hierarchical attribution (Table 1). The corresponding attribution estimates for HPV18 were 14.8%, 17.2% and 17.0%, respectively. The combined percentage for the two oncogenic types HPV16 and 18, which are included in the current vaccines, ranged from 64.9% (single infections) to 72.1% (hierarchical attribution) while the combined percentage for the seven most important oncogenic types (16, 18, 45, 33, 31, 52 and 58) varied from 77.7% (single infections) to 86.9% (hierarchical attribution; Table 1).

The fraction of multiple infections among HPV-positive cases in our study was substantially lower (11% *vs.* 34%)

Table 1. Attribution of HPV types to 1,728 cervical cancer cases, IARC Multicentric Case–Control Study, 1990–1999

	Single infection		Proportional attribution[1]		Hierarchical attribution[1]		Any	
Type	*N*	%	*N*	%	*N*	%	*N*	%
16	866	50.1	940.3	54.4	953	55.2	953	55.2
18	255	14.8	297.4	17.2	293	17.0	342	19.8
45	86	5.0	100.2	5.8	96	5.6	123	7.1
33	23	1.3	32.8	1.9	33	1.9	56	3.2
31	40	2.3	45.7	2.6	48	2.8	54	3.1
52	40	2.3	42.5	2.5	41	2.4	49	2.8
58	32	1.9	36.5	2.1	38	2.2	44	2.5
35	18	1.0	23.6	1.4	18	1.0	38	2.2
59	19	1.1	21.7	1.3	23	1.3	28	1.6
51	19	1.1	20.8	1.2	21	1.2	26	1.5
39	10	0.6	12.1	0.7	12	0.7	20	1.2
56	12	0.7	14.3	0.8	14	0.8	18	1.0
66	6	0.3	8.2	0.5	8	0.5	14	0.8
73	8	0.5	8.5	0.5	8	0.5	12	0.7
6	1	0.1	2.2	0.1	2	0.1	8	0.5
26	3	0.2	3.0	0.2	3	0.2	5	0.3
68	2	0.1	3.3	0.2	3	0.2	5	0.3
40	0	0.0	0.0	0.0	0	0.0	4	0.2
53	1	0.1	1.6	0.1	1	0.1	4	0.2
11	1	0.1	1.0	0.1	1	0.1	3	0.2
16/18	1,121	64.9	1237.7	71.6	1246	72.1		
16/18/45/33/31/52/58	1,342	77.7	1495.4	86.5	1502	86.9		

[1]See text for the description of the two methods.

than that of Wentzensen *et al.* In fact, the HPV detection method we used, GP5+/6+ PCR, is known to be less sensitive for multiple HPV infections than linear array,[6] the method used by Wentzensen *et al.* Most likely, the GP5+/6+ PCR assay (that uses two universal primers) suffers more from competition between different HPV types than the linear array (that uses multiple type-specific primers). Therefore, we observed smaller differences in the attributions. Nonetheless, the two studies showed fairly similar attribution estimates according to the proportional or hierarchical algorithms, although results were based on smaller numbers in Wentzensen *et al.* The range of attribution to HPV18, however, shifted toward higher values in the IMCCS compared to Wentzensen *et al.*

The ICC cases of the two studies differed by age (mean age = 46.8 years in Wentzensen *et al.*; 49.5 years in the IMCCS) and by study location, which was the United States in Wentzensen *et al.*, but a wide range of medium/low-resource countries in the IMCCS. Attributable fractions for specific and combined HPV types are, however, known to vary little across geographical areas,[1,7] with the possible exception of a slight over-representation of HPV58 in Eastern Asia.[1]

Whether the lower sensitivity of the GP5+/6+ PCR assay may favor the HPV type most likely to be the one associated with ICC is unclear. Without using complex functional assays to explore the mechanistic basis for assigning an etiological role to a given HPV type, the attribution of an ICC to an individual type when multiple types are detected is impossible, and different algorithms can thus be helpful. The present analysis showed that, despite the use of a PCR technique with a relatively lower sensitivity for the detection of multiple types and the consequently smaller fraction of multiple HPV infections, attribution estimates within the IMCCS were similar, although more robust, especially for less frequent HPV types, to those presented by Wentzensen *et al.*

Yours sincerely,
Salvatore Vaccarella
Gary M. Clifford
Rebecca Howell-Jones
Peter J.F. Snijders
Silvia Franceschi

References

1. Li N, Franceschi S, Howell-Jones R, Snijders PJ, Clifford GM. Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: variation by geographical region, histological type and year of publication. *Int J Cancer*, 2010 Apr 19. [Epub ahead of print].
2. Wentzensen N, Schiffman M, Dunn T, Zuna RE, Gold MA, Allen RA, Zhang R, Sherman ME, Wacholder S, Walker J, Wang SS. Multiple human papillomavirus genotype infections in cervical cancer progression in the study to understand cervical cancer early endpoints and determinants. *Int J Cancer* 2009;125:2151–8.
3. Jacobs MV, Roda Husman AM, van den Brule AJ, Snijders PJ, Meijer CJ, Walboomers JM. Group-specific differentiation between high- and low-risk human papillomavirus genotypes by general primer-mediated PCR and two cocktails of oligonucleotide probes. *J Clin Microbiol* 1995; 33:901–5.
4. van den Brule AJ, Pol R, Fransen-Daalmeijer N, Schouls LM, Meijer CJ, Snijders PJ. GP5+/6+ PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes. *J Clin Microbiol* 2002;40: 779–87.
5. Insinga RP, Liaw KL, Johnson LG, Madeleine MM. A systematic review of the prevalence and attribution of human papillomavirus types among cervical, vaginal, and vulvar precancers and cancers in the United States. *Cancer Epidemiol Biomarkers Prev* 2008;17:1611–22.
6. Woo YL, Damay I, Stanley M, Crawford R, Sterling J. The use of HPV Linear Array Assay for multiple HPV typing on archival frozen tissue and DNA specimens. *J Virol Methods* 2007;142:226–30.
7. Muñoz N, Bosch FX, Castellsagué X, Diaz M, de Sanjosé S, Hammouda D, Shah KV, Meijer CJ. Against which human papillomavirus types shall we vaccinate and screen? The international perspective. *Int J Cancer* 2004;111:278–85.

Key words: human papillomavirus, cervical cancer, multiple infections
DOI: 10.1002/ijc.25774
History Received 12 Aug 2010; Accepted 15 Oct 2010; Online 15 Nov 2010

Correspondence to: Dr Salvatore Vaccarella, International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon Cedex 08, France, Tel.: +33-4-72-73-8402, Fax: +33-4-72-73-8345, E-mail: vaccarella@iarc.fr

Antabuse (disulfiram) as an affordable and promising anticancer drug

Liz Zhe Lin[1] and Jianqing Lin[2]

[1] Upper Dublin High School, Fort Washington, PA 19034
[2] Department of Medical Oncology, Thomas Jefferson University, Philadelphia, PA 19107

Dear Editor,

Recently, Cvek proposed that special clinical trials be funded by government or charities for "old" drugs like antabuse (disulfiram).[1] We are raising both hands in support of his idea. Believe it or not, a novel mechanism of disulfiram on prostate cancer growth inhibition was recently published.[2] Noting this, we think that serious action is needed to move these findings forward.

In the mid of financial crisis, spending thousands of dollars to buy brand new drugs to extend only a few weeks of "precious" life is a hard decision to make. The fact is, a plethora of old drugs maybe better than the newcomers for a specific indication. They are available: not in the middle of the rain forest but right up in a local pharmacy some blocks away. These are the many known drugs that have not been studied thoroughly or have been brushed aside because of their generic status.

If clinical trials can be conducted in a scale to establish efficacy of a known drug, it can be repurposed for the treatment of, say, cancer, a disease of huge social and economic burden. There were many successful examples of this approach.[3,4] Disulfiram, a drug with multiple targets for cancer control, costs less than a dollar a day and is being evaluated in a phase II clinical trial for prostate cancer, thanks to the generosity of philanthropist David Koch (Clinicaltrials.gov Identifier: NCT01118741). Although this is an exciting development based on promising preclinical data, it is only the start. More of such trials need to be done. Although we do not expect one drug to save the American Health system, the possibilities make any testing worthwhile.

The question here on in lies in how the medical society moves to examine and redefine these old drugs for new answers. We completely agree with the notion that leading organizations and government should fund clinical research, especially clinical trials, to speed up the off-patent drug development process and eventually bring down painfully high costs.[1]

In addition, we should develop mechanisms to encourage profit-seeking manufacturers to be involved in these developmental processes. One option is a temporary revision of generic drug prices to compensate the manufacturer for the studies and ensure sufficient tax revenue for the government organization sponsoring such clinical trials. The benefits of raising generic drug prices by a few dollars far outweigh the cost to the individual consumer.

Yours sincerely,
Liz Zhe Lin
Jianqing Lin

References

1. Cvek B. Antabuse (disulfiram) as a pilot case of non-profit drug. *Int J Cancer* 2010;127:2486.
2. Lin J, Haffner MC, Zhang Y, Lee BH, Brennen WN, Britton J, Kachhap SK, Shim JS, Liu JO, Nelson WG, Yegnasubramanian S, Carducci MA. Disulfiram is a DNA demethylating agent and inhibits prostate cancer cell growth. *Prostate* 2010 Aug 31 [Epub ahead of print].
3. Aronson JK. Old drugs—new uses. *Br J Clin Pharmacol* 2007;64: 563–565.
4. Chong CR, Sullivan DJ, Jr. New uses for old drugs. *Nature* 2007;448: 645–646.

DOI: 10.1002/ijc.25780
History Received 27 Sep 2010; Accepted 19 Oct 2010; Online 17 Nov 2010
Correspondence to: Jianqing Lin, Department of Medical Oncology, 834 Chestnut Street, Thomas Jefferson University, Philadelphia, PA 19107. USA, Tel.: 215-503 6442, Fax: +215-503-7515, E-mail: Jianqing.lin@jefferson.edu

Antabuse repurposing: we need more knowledge and wide international support

Boris Cvek

Department of Cell Biology and Genetics, Palacky University, Olomouc, Czech Republic

Dear Editor,

I thank Lin & Lin for their warm response to my letter "Antabuse (disulfiram) as a pilot case of nonprofit drug." There are, in my view, two crucial points to get Antabuse as the first nonprofit drug against cancer into the clinic. One of them is mechanism of action. Antabuse in the human body is metabolized to diethyldithiocarbamate and its derivatives,[1] which are quite reactive compounds[2] targeting many cellular proteins.[3] However, the active compounds in Antabuse-mediated cancer suppression seem to be relatively nonreactive complexes of diethyldithiocarbamate with zinc or copper formed in the body, not Antabuse on its own.[4] That is why Antabuse is tested with copper supplementation in Utah clinical trial (ClinicalTrials.gov Identifier: NCT00742911). The complexes are potent proteasome inhibitors able to target the proteasome most probably in a quite unusual way when compared with first-in-class proteasome inhibitor Velcade (bortezomib).[5,6] We have to elucidate the mechanism of action of the complexes against cancer and translate the knowledge to the clinic: which malignancies and why could be sensitive to the complexes?

The second point is the funding of the nonprofit drug development. Dr. Branco Weiss, Swiss entrepreneur and founder of Society in Science at ETH in Zurich, truthfully described the situation as follows (http://www.society-in-science.org/directorate/index): "For decades I have faced the challenge of turning scientific discoveries into practical innovations. If there is one thing that I have learnt from this pursuit, it is that while the problems which occur during implementation and application may sometimes be technical, much more often they are of a social nature. Social resistance towards innovation can be observed at all levels of society – in small teams, in entire companies, and in the public at large. People who are reasonably well-off have little interest in change. This aspect is constantly underestimated." First, world nations invest huge money in development of new drugs that are too expensive for majority of mankind and whose prices threaten even their own healthcare systems.[7] What we need is change of mentality internationally and globally.

The first sign of the change is the initiative of GlobalCures (http://www.global-cures.org/), "a not-for-profit medical research organization that intends to develop scientifically-based, peer-reviewed clinical data that will support the use of certain commonly available compounds and other therapies in the treatment of deadly diseases." Governments, organizations such as Union for International Cancer Control or World Health Organization, charities and patients' right advocates should fund GlobalCures and promote its basic idea worldwide.

The best encouragement for profit-seeking manufacturers to be involved in the drug repurposing will be success of GlobalCures and clearly expressed public demand for the change. We can learn from the case of neglected diseases R&D: "There is a clear disjunct between the reality of neglected disease activity and current government thinking, which is focused on 'commercialising R&D to bring big companies back into the field.' This thinking is... now significantly out of kilter with the industry neglected-disease drug landscape. ...there is an urgent need to support the new model of neglected-disease drug development, in particular the public-private partnership approach, which is already generating new drugs, is highly cost-effective, appears to offer the highest health value, and is a crucial factor in continuing cost-effective industry involvement in neglected disease R&D."[8]

Yours sincerely,
Boris Cvek

References

1. Johansson B. A review of the pharmacokinetics and pharmacodynamics of disulfiram and its metabolites. *Acta Psychiatr Scand Suppl* 1992;369:15–26.
2. Thorn GD, Ludwig RA. The dithiocarbamates and related compounds. Amsterdam: Elsevier Publishing Company, 1962. 298 p.

3. Cvek B, Cvorak Z. Targeting of nuclear factor-κB and proteasome by dithiocarbamate complexes with metals. *Curr Pharm Des* 2007;13: 3155–3167.
4. Brar SS, Grigg C, Wilson KS, Holder WD, Dreau D, Austin C, Foster M, Ghio AJ, Whorton AR, Stowell GW, Whittall LB, Whittle RR, et al. Disulfiram inhibits activating transcription factor/cyclic AMP-responsive element binding protein and human melanoma growth in a metal-dependent manner in vitro, in mice and in a patient with metastatic disease. *Mol Cancer Ther* 2004;3:1049–1060.
5. Chen D, Cui QC, Yang H, Dou QP. Disulfiram, a clinically used anti-alcoholism drug and copper-binding agent, induces apoptotic cell death in breast cancer cultures and xenografts via inhibition of the proteasome activity. *Cancer Res* 2006;66:10425–10433.
6. Cvek B, Milacic V, Taraba J, Dou QP. Ni(II), Cu(II), and Zn(II) diethyldithiocarbamate complexes show various activities against the proteasome in breast cancer cells. *J Med Chem* 2008;51: 6256–6258.
7. Cressey D. Health economics: life in the balance. *Nature* 2009;461: 336–339.
8. Moran M. A breakthrough in R&D for neglected diseases: new ways to get the drugs we need. *PLoS Med* 2005;2:e302.

DOI: 10.1002/ijc.25779
History Received 6 Oct 2010; Accepted 19 Oct 2010; Online 17 Nov 2010
Correspondence to: Boris Cvek, Slechtitelu 11, Olomouc 78371, Czech Republic, Tel.: 420585 634 904; Fax: 420585 634 905, E-mail: cvekb@seznam.cz