Section of Infections (INF)

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The Section of Infections (INF) consists of two groups: the Infections and Cancer Biology Group (ICB) and the Infections and Cancer Epidemiology Group (ICE). The groups have similar goals in evaluating the role of infectious agents in human carcinogenesis using complementary strategies. ICB is mainly focused on the characterization of the biological properties of well-established and novel potential oncogenic viruses. In addition, ICB offers many laboratory assays that are widely used in epidemiological research. The work in ICE focuses on the elucidation of the spectrum of cancers associated with infections and the impact of prevention strategies.

In the 2014–2015 biennium, ICB has performed several functional studies on well-known and potential oncogenic viruses, such as Epstein–Barr virus (EBV) and members of the human papillomavirus (HPV) family. In particular, research by ICB highlighted the fact that oncogenic viruses share the ability to deregulate the same cellular pathways, although via different mechanisms. Thus, functional studies can be used as a tool to predict the role of novel viruses in human carcinogenesis.

Recent research efforts in ICE include the estimation of the global burden of cancer attributable to hepatitis B virus and hepatitis C virus infection, and that due to HIV after the introduction of antiretroviral treatment. Special efforts have been made to establish multiyear studies on the effectiveness of HPV vaccination and HPV-based screening in Bhutan and Rwanda, the first two low-income countries to successfully adopt HPV vaccination practices. Much energy has also gone into the improvement of statistical and other quantitative methods to estimate infection-associated cancers.

In addition, ICB and ICE have performed several collaborative studies that led to the characterization of the relationship between natural variations of mucosal high-risk HPV types, geographical distribution, and the severity of cervical disease. The two Groups have also intensively collaborated to better understand the natural history of HPV infection in the oral cavity and to further define the role of the viral infection in the etiology of cancer of the head and neck in Europe and Asia.

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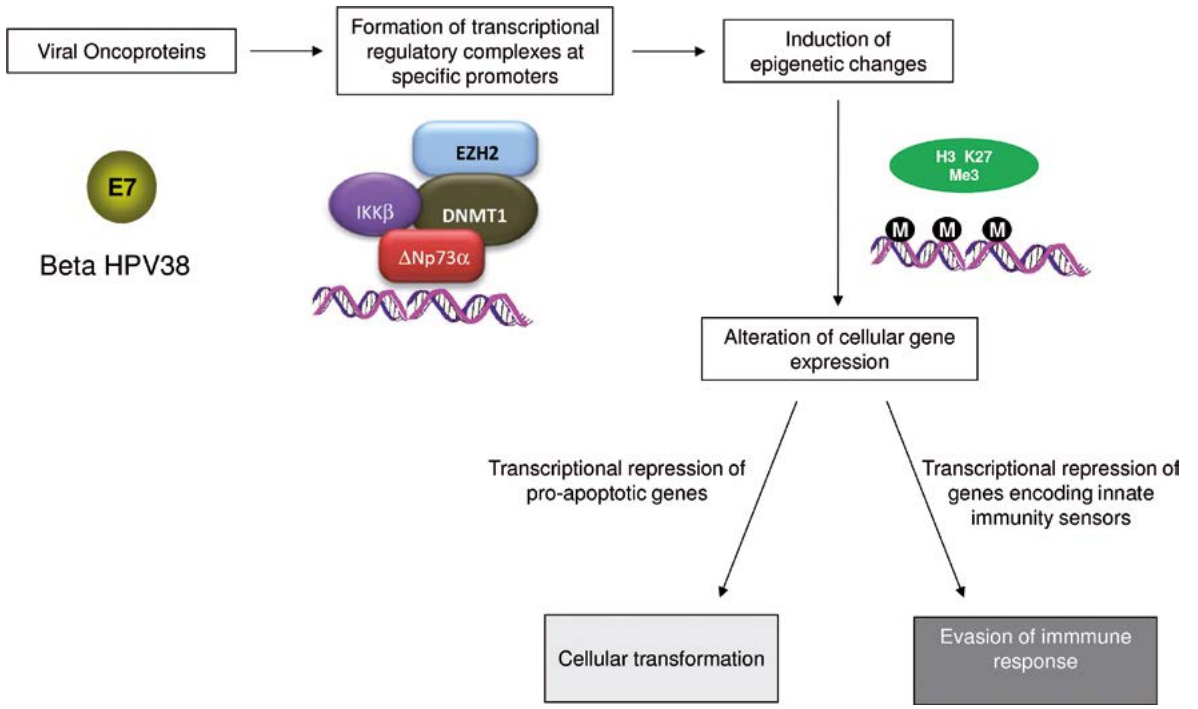
The main goal of the Infections and Cancer Biology Group (ICB) is to elucidate molecular mechanisms of both well-established and potential oncogenic viruses in deregulating pathways related to cellular proliferation and transformation as well as to the immune response. ICB's findings showed that several oncogenic viruses have the ability to induce epigenetic changes and deregulate cellular gene expression. As a consequence, these viruses promote cellular transformation and inactivate pathways involved in the innate immune response (Figure 1). In the 2014–2015 biennium, ICB has characterized new biological properties of some of these viruses (Bazot et al., 2014; Leitz et al., 2014; Shterzer et al., 2014; Siouda et al., 2014; Frecha et al., 2015; Pacini et al., 2015).

In addition to functional studies, ICB has performed several collaborative epidemiological studies using laboratory assays established by the Group, aiming to characterize the natural history of several viruses at different anatomical regions (Hampras et al., 2014; Donà et al., 2015; Franceschi et al., 2015; Hampras et al., 2015; Torres et al., 2015) and their contribution to cancer development (Anantharaman et al., 2014a; Bussu et al., 2014; Corbex et al., 2014; Gheit et al., 2014; Iannacone et al., 2014; Joshi et al., 2014; Toll et al., 2014).

Role of human papillomavirus infection and other co-factors in the etiology of head and neck cancer in Europe and India (HPV-AHEAD)

For four years (2010–2015), ICB has coordinated the HPV-AHEAD consortium, which included a multidisciplinary team in Europe and India, to evaluate the role of human papillomavirus (HPV) infection and other cofactors in the development of head and neck cancer (HNC) in Europe and India. The consortium collected and analysed plasma/serum samples (*n* = 4000) from many European centres and HNC tissues (*n* = 8000) from 42 centres in

Figure 1. Viral oncoproteins alter cellular gene expression, deregulating key pathways involved in cellular transformation and immune response. Many viral oncoproteins, such as LMP1 from Epstein–Barr virus (EBV) or E7 from several human papillomavirus (HPV) types, have the ability to induce the formation or alter the composition of transcriptional regulatory complexes, resulting in the deregulation of cellular gene expression. The figure shows, as an example, a mechanism characterized in ICB studies of E7 oncoprotein from beta HPV type 38 (reviewed in Tommasino, 2014). This virus belongs to genus beta of the HPV phylogenetic tree, which is suspected to be involved, together with ultraviolet (UV) radiation, in the development of non-melanoma skin cancer. Beta HPV 38 E7 oncoprotein has the ability to increase the level of a p53 antagonist, ΔNp73α, leading to the formation of a transcriptional regulatory complex containing ΔNp73α, IKKβ, and two epigenetic enzymes, DNA methyltransferase 1 (DNMT1) and enhancer of zeste homologue 2 (EZH2). This complex binds many promoters, inducing epigenetic changes (e.g. histone 3 K27 trimethylation and/or DNA methylation) and repressing the expression of genes encoding innate immunity sensors (e.g. Toll-like receptor 9) and pro-apoptotic genes (e.g. *Pig3*). © IARC.



16 European countries as well as from 6 centres in India. HNC tissues were analysed by several laboratory assays, including detection of viral DNA and RNA, to precisely determine the fraction of HNCs attributable to mucosal high-risk HPV types in many geographical regions in Europe and India. Analyses of the results are under way.

Epidermodysplasia verruciformis-associated genes *EVER1* and *EVER2* as potential novel DNA sensors of the innate immunity targeted by EBV

A recent study has shown that the products of two human genes, *EVER1* and *EVER2*, which appear to be associated with virus-induced carcinogenesis, may act as exogenous DNA sensors of the innate immunity (Frecha et al., 2015). Both genes were initially identified because they are mutated in patients with the rare genetic disorder epidermodysplasia verruciformis (EV). Patients with EV have an increased susceptibility to infection with cutaneous HPV types and development of squamous cell carcinoma. It has also been shown that specific single-nucleotide polymorphisms in *EVER1* and *EVER2* are associated with an increased risk of persistent infection with mucosal high-risk HPV types and consequent development of premalignant and malignant cervical lesions. ICB's recent findings showed that the expression of both genes was strongly upregulated immediately after infection with EBV or herpes simplex virus 1 in primary or immortalized human cells, suggesting that the activation of *EVER* expression could be part of the innate immune response to exogenous DNA. Importantly, *EVER1* and *EVER2* transcription were strongly repressed at a later stage of EBV infection. Finally, EBV infection was hampered in cells expressing ectopic levels of *EVER1* or *EVER2*. Together, these findings indicate a link between EVER proteins and oncogenic virus infections; the proteins most likely serve as DNA sensors as part of the innate immune response.

Natural history of beta HPV types and their role in human carcinogenesis

Due to the development by ICB of a Luminex-based diagnostic platform that enables the detection of more than 140 double-stranded DNA viruses, ICB has established a large number of collaborative epidemiological studies. One of the focuses of the Group's research is to evaluate the role of cutaneous HPV types in the development of non-melanoma skin cancer. The best candidates are the cutaneous HPV types that belong to the genus beta of the viral phylogenetic tree, which is subdivided into five species (beta 1–5). They were initially isolated in skin cancer of cancer-prone patients with the rare autosomal recessive genetic disorder EV (reviewed in Tommasino, 2014). Patients with EV are highly susceptible to infection with beta HPV types and ultraviolet (UV) radiation-induced skin cancers. It is now clear that beta HPV types are also abundantly present in the skin of healthy individuals. In a recent study, using anti-HPV antibodies and viral DNA as a marker of infection, ICB provided evidence that non-EV individuals with a history of skin cancer show a higher positivity for beta HPV infections compared with control subjects (Iannacone et al., 2014).

In agreement with other independent studies, ICB showed that the beta HPV types, in addition to the skin, can colonize different sites of the anogenital tract (Hampras et al., 2014; Donà et al., 2015; Torres et al., 2015), suggesting that their tropism is not strictly limited to the skin. Determination of the prevalence of beta HPV types in the anal canal of HIV-positive and HIV-negative men who have sex with men showed that HPV types belonging to the beta 1 and beta 3 species are increased in immunocompromised individuals. In contrast, the beta 2 species was equally distributed in the two groups (Torres et al., 2015). The fact that impairment of the host's immune surveillance affects beta HPV infections differently indicates that beta species have different biological properties.

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In 2014–2015, the Infections and Cancer Epidemiology Group (ICE) has contributed to progress in the understanding and prevention of cancer associated with infections by means of three main types of studies.

Implementation and monitoring of HPV vaccination and HPV-based screening in low-income countries

Bhutan and Rwanda, in which human papillomavirus (HPV) vaccination started in 2010 and 2011, respectively, are two model countries chosen by ICE to provide the first evaluations of the effectiveness and sustainability of an entirely HPV-based cervical cancer prevention strategy in low-income countries. Figure 1 shows the fairly high pre-vaccination prevalence of HPV in Bhutan (Baussano et al., 2014b; Tshomo et al., 2014).

Screening of HIV-positive women is a special challenge. In Kenya, ICE showed a relatively high efficacy of cryotherapy in treating cervical intraepithelial neoplasia grade 2 or 3 (CIN2/3) (77%; 95% CI, 66–86%) but very frequent persistence of HPV infection (78%) (de Vuyst et al., 2014). ICE also reported for the first time that in women co-infected with HIV and HPV, triage with a tri-marker methylation test was not inferior to cytology in predicting CIN2 or worse and was superior to visual inspection with acetic acid (VIA) (de Vuyst et al., 2015).

ICE also took advantage of the historical IARC cell repository (from 27 different countries) to study variants that may affect the transformation potential of high-risk HPV types. The distribution of HPV 33 variants, for instance, varies by region, and the A1 sublineage was strongly over-represented in cervical cancer cases compared with controls in Africa and Europe (Chen et al., 2014a, 2014b).

Spectrum, natural history, and prevention of infection-associated cancers other than cervical cancer

The contribution of HPV infection to head and neck cancer (HNC) is still ill defined and varies substantially by cancer site and world region. ICE carried out a meta-analysis of studies in which the prevalence of molecular and serological HPV markers was compared across different HNC and cancer-free controls (Combes and Franceschi, 2014). Data on markers of HPV-driven carcinogenesis, i.e. in situ hybridization or HPV E6/E7 mRNA, showed that HPV-attributable HNC is frequent in oropharyngeal cancer (OPC) (~50%) but rare in cancers of the oral cavity (~3%), larynx (~7%), and hypopharynx (~0%). ICE also showed that HPV prevalence differs by sex and country, possibly as a consequence of the vast international variation in smoking habits in men and women (Combes et al., 2014a). Nevertheless, HPV-positive OPC may systematically cause more OPC in men than in women, for reasons that are unclear but may include higher prevalence of HPV or lifestyle risk factors in men (Figure 2).

Figure 1. Age-specific prevalence of human papillomavirus (HPV) DNA and of cytological abnormalities among 2505 women in Bhutan in 2011–2012. Reprinted with permission from Tshomo et al. (2014). © 2014 Tshomo et al.; licensee BioMed Central Ltd.

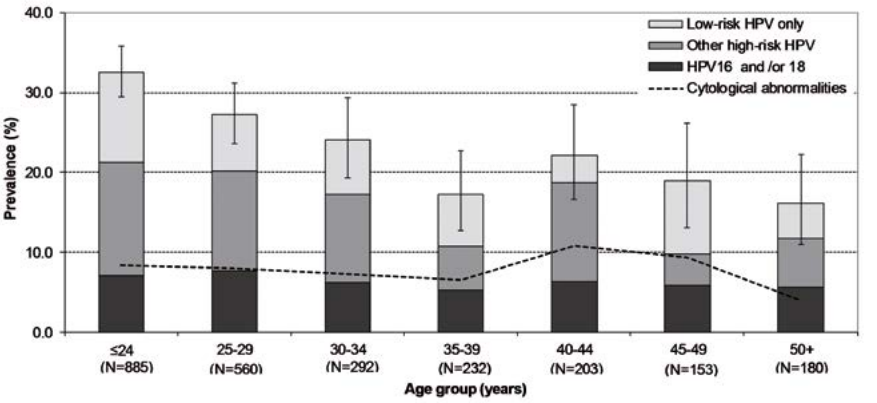
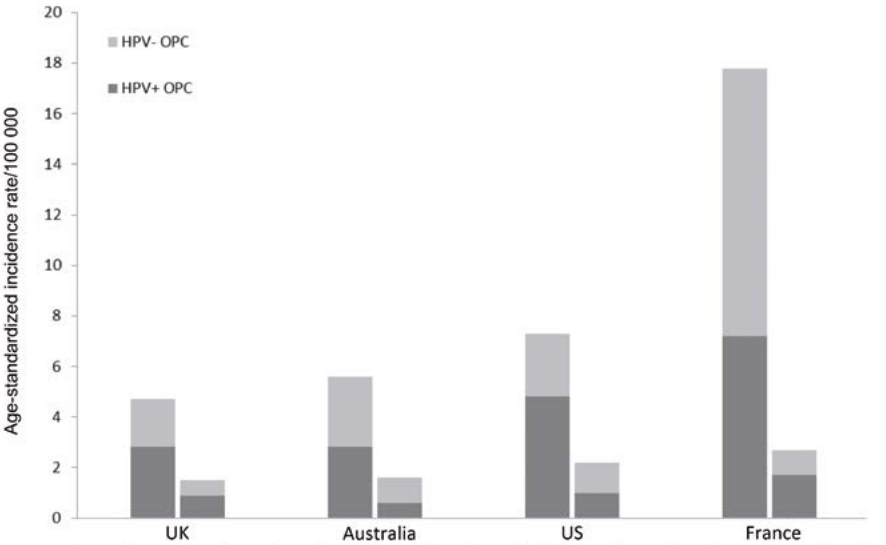


Figure 2. Age-standardized (world) incidence rates of oropharyngeal cancer (OPC) per 100 000 people stratified by country, sex, and estimates of human papillomavirus (HPV) status. Corresponding male (M) to female (F) ratios are also shown in the table. Reprinted from Combes et al. (2014a), by permission from the American Association for Cancer Research.



	UK			Australia			US			France		
	M	F	Ratio	M	F	Ratio	M	F	Ratio	M	F	Ratio
HPV+ OPC	2.8	0.9	3.3	2.8	0.6	4.4	4.8	1.0	4.9	7.2	1.7	4.3
HPV- OPC	1.9	0.6	2.9	2.8	1.0	2.9	2.5	1.2	2.0	10.6	1.0	10.2
Total OPC	4.7	1.5	3.1	5.6	1.6	3.5	7.3	2.2	3.3	17.8	2.7	6.6

Statistical and other quantitative methods to estimate infection-associated cancers

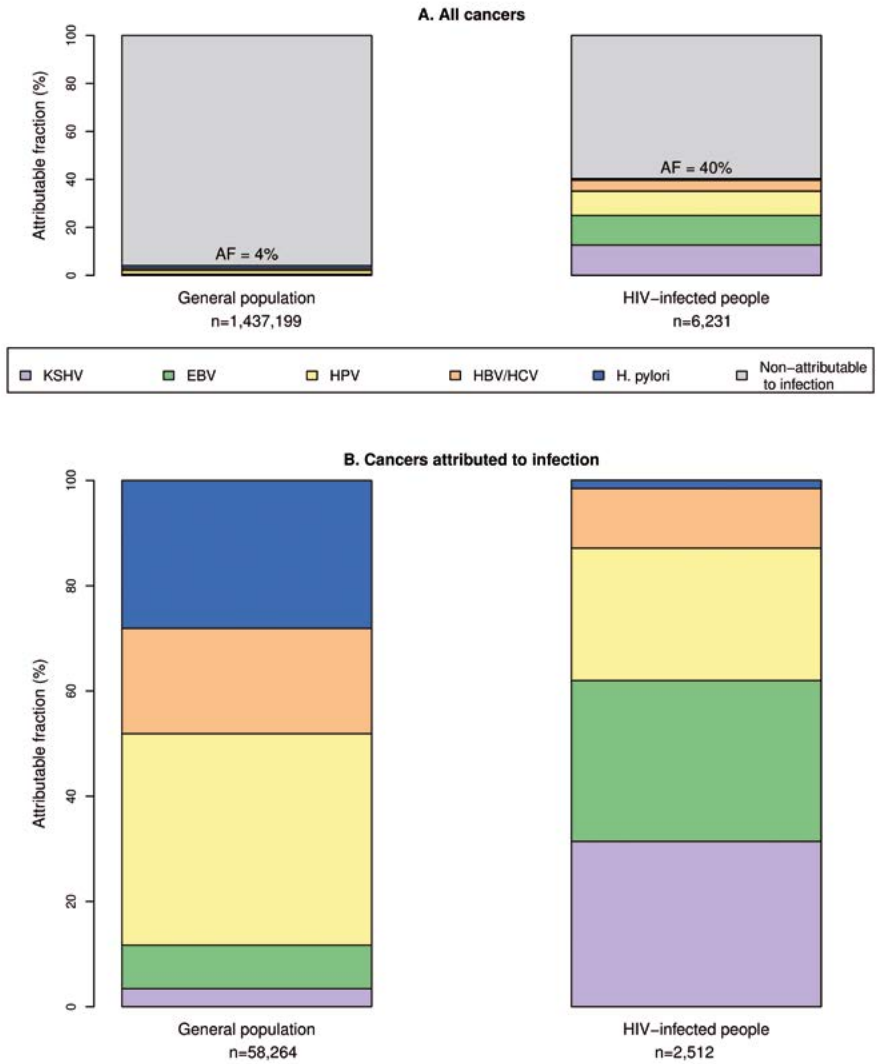
ICE regularly updates the fraction of cancer attributable to infections worldwide by region and individual carcinogenic infectious agent; see Plummer et al. (2015) for *Helicobacter pylori*. Recently, such a fraction was quantified in a particularly vulnerable population: HIV-positive people in the USA (Figure 3). The infection-attributable fraction was 40%, i.e. 10 times that seen in the general population (de Martel et al., 2015). The attributable fraction in HIV-positive people was also higher than that in the general population of any other world region, including sub-Saharan Africa, where 33% of cancers are attributable to infection.

ICE published several model-based reports on the natural history of HPV infections and on the performance and costs of different strategies of vaccination and screening, using high-quality data sets for IARC and European consortia (Franceschi and Baussano, 2014). ICE also reviewed the main principles of transmission dynamics, the basic structure of infection transmission models, and their use in combination with empirical data. The review also summarized models of carcinogenesis and their possible integration with models of the natural history of infections (Baussano et al., 2014a).

To disentangle the impacts of temporal changes in lifestyle, screening, and diagnostic practices on cancer trends, ICE produced ad hoc modifications of the age–period–cohort (APC) model in which non-identifiability was partly circumvented by making assumptions based on a consistent relationship between age and individual cancer incidence. For instance, it was shown that in the absence of cervical screening, incidence rates of cervical cancer for 2006–2010 in the Nordic countries would have been 3–5 times those observed (Vaccarella et al., 2014). Diagnostic changes (mainly the spread of neck ultrasonography and other imaging techniques) may account for more than 50% of differentiated thyroid carcinomas currently diagnosed in women younger than 80 years in many high-income countries, notably the Republic of Korea (> 80%), France, Italy, the USA, and Australia (Franceschi and Vaccarella, 2015; Vaccarella et al., 2015).

Finally, ICE staff participated in the development and dissemination of the R package for statistical computing.

Figure 3. Cancer attributable to infection in the general population and HIV-positive people in the USA in 2008. AF, attributable fraction; EBV, Epstein–Barr virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HPV, human papillomavirus; *H. pylori*, *Helicobacter pylori*; KSHV, Kaposi sarcoma-associated herpesvirus. Reprinted with permission from de Martel et al. (2015).



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