The Luminex Platform of the Infections and Cancer Biology Group

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International Agency for Research on Cancer

Lyon, France
% of cancer cases attributable to infections

**High-resource countries**
7.7% of all cancers

- HIV/HHV-8: 0.3%
- EBV: 0.3%
- HPV: 2.2%
- HBV & HCV: 1%
- H. Pylori: 3.9%

**Low-resource countries**
27% of all cancers

- HPV: 7.7%
- H. pylori: 7%
- HPV: 8.2%
- HBV & HCV: 8.2%
- Other: 0.2%
- HIV/HHV-8: 1.6%
- EBV: 1.6%
- Other: 0.2%
Events associated with the infection of several oncogenic viruses

- Deregulation of cell cycle, apoptosis, differentiation, senescence, cell polarity, signalling pathways
- Deregulation of immune response-related pathways
- Clearance
- Environmental and/or genetic factors
- Persistent infection
- Chromosomal instability

Infection → Oncoproteins → Progeny production
Development of novel diagnostic assays

- Rationale

1) Generation of diagnostic cheap tools with high sensitivity, specificity and throughput for broad spectrum of infectious agents to be used in epidemiological studies

2) Possible use of different types of human specimens, including archival material for retrospective studies
<table>
<thead>
<tr>
<th>Category</th>
<th>Types</th>
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<tbody>
<tr>
<td>Mucosal high-risk HPV types (n=21)</td>
<td>6, 11, 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68a, 68b, 70, 73 and 82. (+ Chlamydia T.)</td>
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<tr>
<td>Cutaneous beta HPV types (n=46)</td>
<td>5, 8, 9, 12, 14, 15, 17, 19, 20, 21, 22, 23, 24, 25, 36, 37, 38, 47, 49, 75, 76, 80, 92, 93, 96, 98, 99, 100, 104, 105, 107, 110, 111, 113, 115, 118, 120, 122, 124, 143, 145, 150, 151, 152, 159, 174</td>
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<td>HPV alpha, mu (n=5)</td>
<td>1, 2, 3, 27, 57</td>
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<td>Polyomaviruses (n=12)</td>
<td>BKV, WUV, KIV, MCV, JCV, HPyV6, HpyV7, HpyV9, HpyV10, TSV and SV40, HpyV12</td>
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<td>Herpes Viruses (n=8)</td>
<td>HSV1, HSV2, VZ, EBV (1 and 2), CMV, HH6, HH7, HH8,</td>
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<td>Other infectious agents (n=6)</td>
<td>Helicobacter Pylori, HBV, HIV, Chlamydia T., Schistosoma (3 different types), Bocavirus</td>
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</table>
Generation of Luminex platform for bacteria (n=100)

Bacteria that colonize the oral cavity, gut and genital tract: i.e Bacteroidaceae, Bifidobacteriaceae, Campylobacteraceae, Chlamydiaceae, Clostridiaceae, Desulfo bacteriaceae, Enterobacteriaceae, Enterococcaceae, Eubacteriaceae, Fusobacteriaceae, Helicobacteraceae, Klebsiella pneumonia, Peptostreptococcaceae, Porphyromonadaceae, Propionibacteriaceae, Ruminococcaceae, Streptococcaceae
Some examples of worldwide collaborative studies

- Senegal
- Algeria
- Chile
- India
- Mongolia
- Morocco
- Brazil
- Colombia
- Kenya
- Spain
- USA
- China
- Germany
- France
- Italy
- Qatar
- South Africa
- Iran
- Ethiopia
- Czech
- Russia
- Sweden
- Belgium
- Germany
- Greece

HNC

Ano-genital cancers

Other cancers: i.e. breast, lung, esophagus
Specimens successfully used so far

(i) Formalin fixed tissues in paraffin blocks
(ii) Frozen tissues
(iii) Exfoliated cervical cells in PBS or fixative (e.g. ThinPrep)
(iv) Urine
(v) Oral brushes, gargles and saliva
(vi) Breast ductal lavages/milk/colostrum
(vii) Cerebrospinal fluids
(viii) Eyebrow hairs
(ix) Skin cells collected using multiple swabbing techniques
(x) Buffy coat
(xi) Stool
(xii) Circulating Tumour DNA

Three small examples
Human papillomaviruses

(i) Mucosal low-risk HPV types, e.g. 6 and 11
(ii) Mucosal high-risk HPV types, e.g. 16 and 18
(iii) Benign cutaneous HPV types, e.g. 2, 3 and 10
Performance of the Luminex assay in detecting HPV DNA in urine and cervical samples

Concordance in HPV DNA detection in cervical smear and urine

<table>
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<tr>
<th>HPV Type</th>
<th>Accordance (%)</th>
<th>Kappa (95% IC)</th>
<th>McNemar* P value</th>
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<tr>
<td>HPV 18</td>
<td>96,2%</td>
<td>0,546 (0,486-0,605)</td>
<td>0,3711</td>
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<tr>
<td>HPV 16</td>
<td>93,6%</td>
<td>0,736 (0,697-0,775)</td>
<td>0,4927</td>
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<td>HPV 11</td>
<td>99,4%</td>
<td>0,767 (0,734-0,799)</td>
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<td>HPV 6</td>
<td>98,3%</td>
<td>0,815 (0,787-0,843)</td>
<td>0,0196</td>
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In collaboration with Alba Lucia Combita and colleagues, Bogota, Colombia
HPV flora in oral cavity

- 28% of the samples were positives for High risk mucosal types (HPV16 represented 20%)
- 6% were positives for Low risk mucosal HPV types
- 78% were positives for Cutaneous HPV types from the genus Beta (HPV23, 38, 15 …)
- 13% of the samples contains HPV from the genus Gamma
- Overall, 85% were HPV positives

Oral cavity of healthy individual is colonized by a HPV flora
Circulating tumour DNA

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<th>Number cases tested</th>
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In collaboration with Paul Brennan, IARC
Our interest in child cohorts

Infections

Persistent infection

Environmental and/or genetic factors

Cancer
Infections and environmental factors

**E6 and E7 from beta HPV38 cooperate with ultraviolet light in the development of actinic keratosis-like lesions and squamous cell carcinoma in mice.**


**The mycotoxin aflatoxin B1 stimulates Epstein-Barr virus-induced B-cell transformation in in vitro and in vivo experimental models.**

Acknowledgements (Main collaborators)

IARC - ICB group
Rosita Accardl-Gheit, Sebastien Chevalier, Tarik Gheit, Sandrine McKay-Chopin, Lucia Minoni, Laura Pacini, Cecilia Sirand

IARC - Director
Christopher P Wild

IARC - ICE group
Silvia Franceschi and all group members

IARC - GCS group
Catherine Voegele, Florence le Calvez, Geoffroy Durand, James McKay

ENS, Lyon
Henry Gruffat, Evelyne Manet

DKFZ, Heidelberg
Daniele Viarisio, Uli Kloz, Lutz Gissmann, Michael Pawlita, Martin Müller

Inserm Unit 851, Lyon
Uzma Hasan
E7 multiplex PCR

biotinylated primers

- HPV16
- HPV18
- HPV31
- HPV45
- HPV51
- HPV52

etc...

+ Clamydia Trachomatis

human beta globin

Reaction in a single tube

200bp amplimers
Luminex-based assay

- Multiplex PCR

- Biotinylated viral DNA PCR product/probe complex

- Streptavidin-R-phycoerythrin

One hundred of luminex beads with different fluorescent dye are commercially available.

Flow cytometer
High specificity of the Luminex-based assay

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International Agency for Research on Cancer
World Health Organization
Transfer of the Luminex-based assays to other Institutes

(i) the Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, India

(ii) Centro de Investigação Translacional em Oncologia – ICESP
Cerqueira César – São Paulo, Brazil