HPV Program
Cervical cancer is a major public health problem

- 3rd most common cancer in women
- 275,000 deaths /year in the World
- Good prognosis: 5-years survival rate is ~ 70%, 92% when followed and treated

<table>
<thead>
<tr>
<th></th>
<th>World</th>
<th>U.S.</th>
<th>E.U</th>
<th>France</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence</td>
<td>530 000</td>
<td>75 000</td>
<td>31 000</td>
<td>3 000</td>
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<tr>
<td>Mortalité</td>
<td>275 000</td>
<td>33 000</td>
<td>13 000</td>
<td>1 000</td>
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Importance of Pap-smears in the cervical cancer screening

George Papanicolaou
Inventor of the "Pap smears" (1928)

This primary screening test has its limits:

- **Insufficient sensitivity** for the CIN2+ detection: ~53%
  → Repeatability of Pap-smears (every 2-3 years) increases the sensitivity to 70-80%
- Test operator-dependent
- **Ambiguous results are frequent** (ASCUS = 3-8%): only 5-10% of them are really associated with concurrent CIN2+

(Blavstein’s Pathology of the Female Genital Tract. 5th ed. New York, Springer-Verlag, 2002.)
HPV infection is the major cause of cervical cancer

- Nearly 100% of cervical cancer are linked to a **persistent infection by a high-risk HPV** (Human Papillomavirus)
- 70-80% of women will get an HPV infection at some point in life.
- In 90% of cases, the body “clears” HPV with its own immunity
- >75 different HPV genotypes
- **14 HPV strains are considered as “high risk” (HR) oncogenic isotypes**
- HPV types 16 and 18 are the most prevalent in about 70% of cervical cancer (in Caucasian population)
- **Integration of HR-HPV DNA is a critical step in the development of cervical cancer**
HR-HPV testing: a breakthrough in cervical cancer screening

Hallmarks of HPV testing are:

• **Higher sensitivity** than Pap smears

• **Very high NPV**: if HPV-, only 0.27% of CIN2+ risk after 5 years

• **Standardized** and automated tests: results are no operator-dependant

• But **lower specificity** than Pap smears

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**The cobas® HPV Test (Roche)** is the only clinically validated, FDA-approved assay that simultaneously provides pooled results on 14 high-risk genotypes and individual results on the highest-risk genotypes, HPV 16 and HPV 18.

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HPV testing and Pap-smears are complementary screening methods
Cervical cancer screening algorithm

- No consensus in cervical cancer screening guidelines among European countries and the U.S.
- HPV tests approved by the FDA are recommended for:
  - **Triage** of women (21 to 65 years) with atypical squamous cells of undetermined significance (ASCUS) pap-smears
  - Post-therapeutic control of CIN2/3 patients
  - HPV and cytology **cotesting** for women between 30 and 65 years
  - **HPV primary screening**, triage with cytology (only for Cobas test, Roche) for women between 30 and 65 years

### “Current” algorithm

- **Cytology**
  - Every 3 years
  - 94% Normal
  - 4% ASC-US/LSIL
  - 2% HSIL
  - HPV negative: 50% Routine screening
  - HPV positive: 50% Rescreen in 1 year
  - HPV testing: VPP: 50% Colposcopy

### “Future” algorithm*

- **HPV testing**
  - Every 5 years
  - 3% HPV 16/18 +
  - 7% other hrHPV+
  - 90% HPV -
  - 23% Normal
  - 77% ASC-US, LSIL, HSIL

* Already used in USA, to be generalized in a short term
HPV integration: Biomarker for the triage of HPV+ women

Women sexually active
HPV infection: HPV DNA detection
E6/E7 mRNA over expression

Women with HIGH risk to evaluate to cervical cancer

HPV test lack of specificity
• Useless colposcopies and follow-up
• Over-treatment
• Psychological impact
• Economic impact

Necessity of a more specific biomarker to distinguish patients with high risk to develop a cervical cancer
HPV integration: Biomarker for the triage of HPV+ women

Development of a Molecular Combing assay that detects directly HPV integration in the genomes of patients
Positioning of the Molecular combing HPV test

An optimal integrated screening and triage strategy

**A  “Current” algorithm**

- **Cytology**
  - Every 3 years
  - Normal: 94%
  - ASC-US/LSIL: 4%
  - HSIL: 2%

- **HPV testing**
  - HPV negative: 50%
  - HPV positive: 50%

- **Triage 2nd line**
  - Rescreen in 1 year

- **Routine screening**
  - Colposcopy
  - Follow-up in 12 months
  - Routine screening

**B  “Future” algorithm**

- **HPV testing**
  - Every 5 years
  - HPV 16/18 +: 3%
  - other hrHPV+: 7%
  - HPV -: 90%

- **Triage 1st line**
  - Normal: 77%
  - ASC-US, LSIL, HSIL: 23%

- **Cytology**
  - 50%
  - 94%

- **Follow-up in 12 months**
  - Colposcopy

- **Routine screening**

*Already used in USA, to be generalized in a short term
Objectives

**HPV**

**DIAGNOSTIC value**
- Improvement of the triage of women with ASC-US or LSIL
  - Reduction of useless colposcopies

**PROGNOSTIC value**
- Help to stratify the risk of progression to high-grade dysplasia/cancer
  - Better management and follow up of patients

**HPV test technical development**
**Clinical validation**
Added-value of Molecular Combing for the detection of HPV integration

- **Direct** observation of HPV genome integration
- **High-resolution**
  - Number of integration sites / patient’s genome
  - Single integrations/ tandem integrations (multiples)
  - Number of HPV genomes integrated / integration site

Hybridization of fluorescent probes specific for HPV-HR genomes

DNA extracted from pap-smears of HPV+ patients
Clinical validation

**Association between high-risk HPV genome integration detected by Molecular Combing and cervical lesions severity and/or evolution**

Set-up of 2 clinical studies

- IDAHO study: 8 Hospitals involved in France

- EXPL-HPV-002: 2 clinical sites involved in CZR, one active (Dr Dvorak)

  Prague Clinical Services (PCS) in charge of the study
PRAGUE CLINICAL SERVICES

Your partner in clinical development since 2004
EXPL-HPV-002 Objectives

Principal

The aim of this study is to validate the integration of the HPV-HR as an appropriate biomarker of the severity of precancerous lesions

 DIAGNOSTIC value of HPV integration

Secondary

To evaluate the pronostic performances of HPV integration as a biomarker of the clearance of HPV

To validate the integration of the HPV-HR as an appropriate biomarker of progression of cervical lesions towards cervical cancer

To evaluate the integration rate according to the HPV genotype

 PRONOSTIC value of HPV integration

Confidentiel
EXPL-HPV-002 Study design

- Exploratory Study
- GCP study
- Open-label
- Single arm
- Two parts:
  - cross-sectional part – one study visit
  - longitudinal part (follow-up) – up to 6 visits
Primary Endpoint

• Integration of HPV genomes studied by molecular combing (For each patient, several parameters will be assessed during the analysis by molecular combing)
• Lesion status
• Cytological classification
• Clinical outcome of cervical lesions (only for patients in follow up)
• Viral clearance (only for patients in follow up)
• Cure (only for patients in follow up)
EXPL-HPV-002 Site selection

- Site #6
  **Prof. MUDr. Pavel Ventruba, DrSc., MBA**
  Gynecological – obstetric clinic
  The University Hospital Brno

- Site #5
  **MUDr. Vladimír Dvořák** – Co-Ordinating Investigator
  Center for outpatients gynecological and primary care, Brno
EXPL-HPV-002 Laboratories

- **Genotyping: UHKT, Dr Tachezy**
  - Samples transported from the site to the lab on weekly basis
  - Samples evaluated approximately every two weeks
  - After evaluation, HR-HPV positive samples sent to GV labs

- **Histology: Aeskulab, Dr Trnkova**
  - Samples transported on daily basis
  - Results sent to the site on regular basis; to CRO per request
Enrolment goal: 400 subjects HR-HPV

• based on literature search concerning the primary goal:
  "association between the integration of high-risk HPV genomes and the severity of cervical lesions"

• based on distribution between HG cervical lesions and LG cervical lesions associated with HR-HPV on the main clinical site

• statistical considerations - difficult statistical calculation, limited data
• meta-analysis with the French HPV study data (IDAHO study)
EXPL-HPV-002 Recruitment details

- 303 patients recruited, 13 of them excluded by sponsor’s decision because of low DNA quantity
- 289 of them evaluated for HPV positivity
- 178 samples evaluated as HR-HPV positive
  - 106 High Grade
  - 65 Low grade
EXPL-HPV-002 Interim analysis

• **Analyzed samples:**
  • Patients enrolled by the end of 2016:
  • 138 HR-HPV positive patients of 203 enrolled patients
  • 12 samples excluded (low DNA quantity)
Preliminary encouraging results: DNA combing methodology has a power to improve the current diagnostic and treatment methods in cervical cancer

- More robust statistical data are needed
- Sufficient data will be obtained by the study continuation in both cross-sectional and longitudinal phases.