Rational molecular Assessments and Innovative Drug Selection

Project context and objectives

Cervical cancer (CC) is the second most common malignancy in women worldwide. While epidemiologists at the recent IGCS meeting in Melbourne (November 2014) judged that Human Papilloma Virus (HPV) was eradicable if women were screened worldwide, the reality is that regular screening for all can presently not be reinforced in highly endemic areas (such as India, Africa and certain areas in South America) where 4/5th of the population at risk may not be aware of screening practices. Although CC is a single diagnostic entity and infection of high-risk HPV is recognized as an important initiating event in tumourigenesis, CC exhibits differences in clinical behaviour. Stratification of CC into subclasses for progression and response to targeted treatment remains to be defined. At present, the dominant targets under scrutiny for innovative CC treatments are the following: EGFR/PI3K pathway, proliferation/DNA checkpoint and angiogenesis inhibition as well as anti-HPV vaccines. There have been recent publications (TCGA et al., 2017; Ojesina et al., 2014; Wright et al., 2013) on high resolution genetic investigations in CC. So far, there has been no prospective assessment on patient outcome based on whole genome/exome sequencing or protein profiling of their tumours together with quality control evaluation of patient treatment. Early prospective data is available from a small phase 2 clinical trial by Institut Curie, the coordinators’ center, suggesting that EGFR inhibition at the membrane is ineffective in the presence of a downstream PI3K pathway activation (De la Rochefordière et al., 2015).

- We are lacking prognostic and predictive biomarkers for CC treatment and there is a growing need for the development of biomarkers to follow up the course of the disease.
- More importantly, we need to learn which are the most important targets to address as well as for each target(s) the proportion of tumours which need corresponding innovative therapies.

RAIDs is a multidisciplinary co-operation between academic clinical centers, SMEs and translational research platforms. It combines Next Generation Sequencing (NGS) and Reverse Phase Protein array (RPRA) in a large patient population prior to standard therapy. It includes:

- a cognitive cohort study (BioRAIDs) intended to define tumour stratification for targeted therapies,
- as well as precision medicine trials using an HPV directed vaccine in combination with checkpoint inhibition.

In addition, high throughput screening techniques have been performed in CC cell lines, allowing to identify new drugs of relevance for advanced stage multi resistant CC. These molecules are to be validated in preclinical mouse models. Ongoing studies will assess in vitro efficacy of drug targeting according to molecular phenotypes.

The RAIDs consortium aims

- to provide a safer and more efficient therapy for the individual patient;
- to raise awareness in countries with lesser screening practices;
- to improve the quality of life for women with cancer via:
  - a) the acquisition of defined molecular data for better treatment decisions,
  - b) targeted pilot trials directed at specific alterations as well as targeted vaccine trials directed against the HPV
  - c) the continuous evaluation of standards of care by comparing standards and outcome in all the RAIDs centers
- to disseminate information on innovative practices in concertation with the help of other international structures, be they clinical trial orientated [EORTC (European Organisation for Research and Treatment of Cancer) and ENGOT (European Network for Gynecological
Work performed and main technical results achieved
RAIDs started October 1st, 2012 and ended March, 30th 2017 following the approval of an 18 months extension by the European Commission. The consortium is composed of 10 research centers (Institut Curie, INSERM, KEM, IOV, TEO HEALTH, IMSP IO, IGR, NKI, HCTL and ECRIN), 2 universities (Erasmus and EMAUG) and 4 companies (AmbTU, Seqomics, Quanticsoft and Ayming) in 7 European countries (France, Germany, the Netherlands, Serbia, Moldova, Romania and Hungary).

- **PROGRESS IN CLINICAL STUDIES**
  A. BioRAIDs STUDY (Ngo et al., 2015 and Samuels et al., 2016a) Patient accrual was closed end of September 2016 with the inclusion of 419 patients. Half of all patients are from French centers; IOV - Serbia accrued 101 patients. Clinical data curation is ongoing and will continue for the finalization of patients’ follow up till March 2018. The quality control for MRI imaging is almost completed at the radiological teaching centre in IOV in Serbia. Curation of the clinical data for the first 100 patients for whom exome sequencing was performed is finalized. Bioinformatics analyses are ongoing and correlation with clinical response identifies a set of genes and proteins associated with bad prognosis. These data will need to be validated in the whole patients’ cohort.
  B. DNA VACCINE TRIAL (Samuels et al., 2016b) The phase I DNA vaccine (HPV targeted therapy) trial sponsored by NKI in the Netherlands completed patient inclusions and immunological analysis finalized. The vaccine format is safe yet, only limited immune response is observed and no clinical response. A new phase I trial with a new more potent DNA vaccine is accepted by the competent authorities in The Netherlands and patients’ accrual in ongoing.

- **SCIENTIFIC WORK** achieved during the 48 months:
  - Sequencing on tumour samples is ongoing.
    - The first 100 exome sequencing pairs of tumour and constitutive DNA samples are finalized. Exome sequencing on 20 cervical cell lines has been completed and analyzed. Major somatic alterations and DNA copy number alteration from exome sequencing profiles confirm PI3K pathway mutations to be a dominant feature in CC (in at least 1/3 of patients).
    - Targeted sequencing on 600 differentially expressed genes based on the first clustering was finalized on 100 patients. Further sequencing and data analysis and integration will continue beyond the timelines of this project.
  - An efficient pipeline to isolate circulating (tumour) DNA from serum and plasma was developed at Erasmus Rotterdam together with methods for the detection of low-frequency mutations. The analyses of ct DNA from BioRAIDs patients is finalized and correlation to clinical response is ongoing.
  - RPPA analysis is finalized and first results were presented in a poster session at the AACR meeting 2017 (De Koning et al., 2017). The RPPA data identified three main clusters with different protein expression profiles representing the EMT, DNA damage and p38 MAPK/PI3K signalling pathways. Correlations with clinical data are ongoing. These 3 clusters compare with published data by a recent paper by TCGA in Nature 2017.
  - HPV integration sites and HPV viral gene detection in circulation are almost finalized on HPV16 and HPV18 positive patients. Statistical analysis for their impact on early detection of poorly responding/progressing tumours is ongoing.
  - Pharmacological profiling of cell lines has already detected a group of drugs that synergize with the “standard treatment” of advanced cervical cancers. The validation of these data is ongoing and the reformulation/use of old drugs is continued to be investigated according to molecular profiles.
- Preclinical mouse models for tumour micro-environmental studies have been developed and are published in journals with a high impact factor. Preclinical mouse data on combining vaccine and radiotherapy are published and report the efficacy of a novel dual targeting approach in a preclinically relevant animal model. A patent has been deposited.

**Dissemination to the public and scientific communities**

Dissemination actions have led to a high visibility of the RAIDs project on a European and on an international level and to the wider understanding of the need and the opportunity for targeted approaches in the field of cervical cancer.

We have made a very recent video interviewing patients in Serbia, while also showing doctors, scientists, lab workers, clinical trial developers asking their opinion on what RAIDs stands for. [https://vimeo.com/216824709](https://vimeo.com/216824709) (password raids).

Video clips explaining RAIDs have been produced ([http://www.raids-fp7.eu/press/videos.html](http://www.raids-fp7.eu/press/videos.html)).

A dropbox for patients (RAIDs drop box - [http://www.raids-fp7.eu/a-question.html](http://www.raids-fp7.eu/a-question.html)) allowing requests for information in the field of precision medicine has been inserted on the RAIDs website as well as in the cervical cancer factsheet of ESGO. The dropbox allows patients to ask questions in relation to standard treatment and to innovative protocols regarding precision medicine in their native language.

[**If you have any questions concerning cervical cancer you can ask them here**](https://www.raids-fp7.eu/a-question.html)

** Clinicians participating in RAIDs project will answer you.**

Answer, réponse, die antworten, odgovor, răspuns, antwoord

Interaction with patients and patient advocacy groups is ongoing. A patients’ working group was organized at Institut Curie around precision medicine issues: [http://curie.fr/actualites/échange-avec-patients-autour-projet-entreprise-2015-2020-007181](http://curie.fr/actualites/échange-avec-patients-autour-projet-entreprise-2015-2020-007181)

[https://www.facebook.com/InstitutCurie](https://www.facebook.com/InstitutCurie) le 25 avril

**Expected final results, potential impact and use**

RAIDs aims to define a set of stratification criteria based on molecular profiling. Its results will give insight into dominant genomic and protein signalling pathway alterations, enabling the identification of prognostic and predictive biomarkers for standard or targeted therapy in CC. Following the finalization of the molecular analyses and the correlation to clinical outcome, a clinical trial based on patient stratification identified in RAIDs will allow to provide patients with a personalized therapeutic strategy. The RAIDs2CURE trial is currently under preparation and negotiations with pharmaceutical companies are ongoing.

**RAIDs consortium**

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2. AmBTU, Amsterdam Biotherapeutics Unit, Netherlands
3. SeqOmics, Seqomics Biotechnologia Koriatolt Felelosseg Tarsasag, Hungary
4. KEOCYT, Keocyt SAS, France: **This partner has left the project**
5. INSERM, Institut National de la Santé et de la Recherche Médicale, France
6. AVL, Stichting Antoni Van Leeuwenhoek Ziekenhuis, Netherlands **This partner merged with partner 16**
7. Erasmus MC, Erasmus Universitair Medisch Centrum Rotterdam, Netherlands
References

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