Towards selective non toxic therapies against cervical cancer
Objectives

- Precision medicine biobanking study with integrative molecular analysis for future targeted therapeutic strategies
- Preclinical studies to identify new drugs and drug combination for precision medicine strategies
- Early phase clinical trials targeting the virus with therapeutic vaccines.

Achievements

- First prospective biobanking study in Europe with patient outcome data and early phase vaccine trials
- Patients’ stratifications into subgroups for future innovative treatment
- Preclinical pharmaco profiling studies for treatments’ combinations
- DNA vaccination early phase clinical trial finalized in The Netherlands
Whole exom and targeted DNA Sequencing

DNA sequencing was performed at Seqomics (Hungary). The molecular data generated by the consortium were integrated at Curie in the seamless information system KDI (Knowledge and Data Integration) developed in-house. Using bioinformatics pipelines, somatic mutations were identified from whole exome sequencing data. Unsupervised machine learning technique taking into account prior biological knowledge from gene interaction network (available from the Atlas of Cancer Signalling Network, [https://acsn.curie.fr](https://acsn.curie.fr)) was used to stratify the patients into subgroups. We identified 5 stable clusters (Figure 3). Two of these clusters (clusters 1 and 4) are notable for the high number of genetic mutations involving the mitochondrial energy production and RNA repair pathways.
Protein analysis

Reverse Phase Protein Array (RPPA) technology measures protein expression and activation in cervical cancer before and after standard treatment. We expect that, combined with DNA sequencing techniques, protein expression as assessed by RPPA will allow to identify predictive biomarkers and to refine patient stratification. (Figure 4).

Blood markers

Optimization of a non-invasive mutation detection method from cell free/circulating tumour (cf/ct) DNA (CURIE) for human papillomavirus insertion sites into the patients’ genomic DNA as well as the detection of cancer specific mutations was initiated from serum at Erasmus. To improve sensitivity (by 100 X) samples were analyzed by Digital PCR which allows for absolute mutation sample quantification.

Few patients revealed PI3KCA mutation changes in ctDNA during therapy. This needs to be validated and to be correlated with patients’ outcome in the next months (Figure 5).
Centralized review of imaging

Centralized review imaging will be performed at IOV and will allow the re-evaluation of treatment responses in all clinical centres as well as the comparison of the imaging techniques across the different countries (Figure 7).

Publications are planned with main objectives to report on correlations between Diffusion Weighted imaging parameters and 1° histological grade, 2° a prediction of the spread direction if the local cervical cancer is not controlled by standard therapy.

Radiotherapy

Radiotherapy (RT) remains a major tool in the treatment of cervical cancer. Image guided brachytherapy (IGBT) may offer 3 years local control rates of 92% [tumours > 5cm], and up to 98% [tumors 2-5cm], while also positively impacting overall survival. Training in contouring for external beam RT and IGBT was offered to RAIDs centres, via an innovative online contouring workshop, allowed the detection of discrepancies between trainee and expert (Figure 8). This on-site training was made possible by Gustave Roussy.
Main conclusion

Clinical accrual was ended on September 30th, 2016 with 419 patients included out of the 500 patients initially planned for the BioRAIDs study. Accrual rates have dramatically improved over the last 12 months. We have included more than 200 patients in the last 12 months. We have decided to stop inclusions in September 2016 rather than in April 2017 in order to insure good quality monitoring and curation of clinical data to meet the objectives of the BioRAIDs clinical protocol which is to correlate patients’ molecular data to clinical response. A clinical follow up at 18 months will be performed and clinical data will be correlated to integrated molecular pathways.

In total, approximately half of the inclusions are secured by French centres. IOV in Serbia is our single highest recruiting centre with 101 patients.

2/ Preclinical studies and biomarkers

Combination of immunotherapy (using a novel therapeutic vaccine for HPV: STxB-E7) and radiotherapy was tested using a HPV-associated head and neck cancer model in mice. Complete tumour remission following the combination therapy was visualized using in vivo imaging techniques (Figure 9).

A key component for tumour homing such as CCR2 expression on Tregs, might become a new biomarker of low-dose cyclophosphamide efficacy. Figure 9 shows a decreased proportion of CCR2+ Treg in the blood of patients with cancer (oral squamous cell carcinoma: OSCC) compared to healthy individuals. The frequency of CCR2+ Treg is higher within the tumours compared to normal gingival tissues. In a mouse model of MCA fibrosarcoma, a low-dose cyclophosphamide treatment resulted in a selective depletion of CCR2+Treg from the tumour draining lymph nodes.

Figure 9 – Efficacy of the combined Radiotherapy + Vaccination. Tumour growth is monitored by bioluminescence. Scatter plots show the proportion of CCR2+ regulatory T cells in the blood and the tumour of OSCC patients and the Bars graph show that CCR2+ regulatory T cells are specifically targeted by cyclophosphamide (CP). These results suggest that CCR2 expression could serve as a biomarker of tumour evolution and chemotherapy efficacy.
3/ DNA vaccines

Two clinical trials at NKI involving 2 DNA vaccine formats (Intradermal DNA tattoo):

1. fusion of HPV16 E7 shuffled and Tetanus Toxin Fragment C. (Figure 10);
2. minimalized helper sequences containing ER localization and retention and CD4 help. Both HPV16 E6 and E7 shuffled to improve immunogenicity (Figure 11).

Phase 1 / clinical trial with DNA vaccine Format 1:
- 12 VIN patients have been vaccinated.
- Well tolerated.
- Minimal vaccine induced T cell responses induced -> room for improvement.

Phase 2 / clinical trial with DNA vaccine Format 2
Max. 12 VIN patients will be vaccinated:
- Both E6 and E7 targeted vaccines.
- Validated superiority of vaccine in animals models in the clinic (Figure 12).
- Start early 2017.

4/ Perspectives

The BioRAIDS study identified challenges associated with the practical aspects of systematic biobanking which can lead to delays in clinical trial initiation. To address these challenges, there needs to be increased cooperation and standardization in terms of regulatory rules and practices across the EU.

BioRAIDS, an analytical study, sets the stage for future clinical trials of specifically targeted drugs or drug cocktails. Ongoing work on cell lines that compares mutational and proteomics data with pharmacological profiling should help identify common targeted drugs and drug cocktails to benefit patients.

The next step is to build on results from the present prospective study enabling to identify a series molecular markers of poor outcome to conventional treatment and to specifically target these using innovative therapies.

The RAIDs consortium’s future action plan is:
- to identify clusters that are associated with poor prognosis
- to implement early stage innovative adjuvant treatments in a future precision medicine clinical trial RAIDs 2 CURE.
RAIDs was supported by the European commission through the Seventh Framework programme for R&D and was launched on October 1st 2012 for 54 months. It gathered 16 partners from 7 European countries and has received 6 M€ of EC funding.

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If you have any questions concerning cervical cancer you can ask them here. Clinicians participating in RAIDs project will answer you.

[www.raids-fp7.eu/a-question.html](http://www.raids-fp7.eu/a-question.html)