Final Report – Publishable Summary (01.01.2012 – 31.12.2015)

Grant Agreement number: 279113

Project acronym: OCTIPS

Project title: Ovarian Cancer Therapy – Innovative Models Prolong Survival

Funding Scheme: Collaborative Project (Small or medium-scale focused research project)

Date of latest version of Annex I against which the assessment will be made: 2012.06.06

Periodic report: 3rd

Period covered: from 01.01.2012 to 31.12.2015

Name, title and organization of the scientific representative of the project's coordinator:

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Declaration by the scientific representative of the project coordinator

I, as scientific representative of the coordinator of this project and in line with the obligations as stated in Article II.2.3 of the Grant Agreement declare that:

- The attached periodic report represents an accurate description of the work carried out in this project for this reporting period;

- The project (tick as appropriate):
  - o has fully achieved its objectives and technical goals for the period;
  - ⊗ has achieved most of its objectives and technical goals for the period with relatively minor deviations.
  - o has failed to achieve critical objectives and/or is not at all on schedule.

- The public website, if applicable
  - ⊗ is up to date
  - o is not up to date

- To my best knowledge, the financial statements which are being submitted as part of this report are in line with the actual work carried out and are consistent with the report of the resources used for the project (section 3.4) and if applicable with the certificate on financial statement.

- All beneficiaries, in particular non-profit public bodies, secondary and higher education establishments, research organisations and SMEs, have declared to have verified their legal status. Any changes have been reported under section 3.2.3 (Project Management) in accordance with Article II.3.f of the Grant Agreement.

Name of scientific representative of the Coordinator: Dan Cacsire Castillo-Tong

Date: 28.02.2016

Executive Summary

The FP7 EU Project aims at molecularly analyzing recurrent and resistant high grade serous ovarian cancer (HGSOC) by comparing them with their primary counterparts, creating model systems representing HGSOC, defining target molecules and developing innovative therapeutic strategies. The project started on 1.1.2012 and is closed on 31.12.2015. OCTIPS is structured into 7 workpackages, in which WP1 and WP7 are responsible for the management and dissemination activities, respectively.

Major management works are to monitor the project, to take decision on the basis of the OCTIPS progress and to coordinate the partners to produce scientific results. Having recognized the difficulties during the project, we developed different strategies to address the scientific objectives and to assure the quality of the works. Adjacent to the final OCTIPS meeting, we also successfully organized a training/networking session for young scientists.

WP2 has established two online databases, defined including criteria, coding system for samples and the standard protocols. In total, DNA, RNAs from 79 pairs of tumor tissues and germline DNA from 83 patients have been delivered. Tissue microarrays have been constructed from paired tumors of 123 patients. The clinical information of all patients has been delivered. Moreover, WP2 has also established 29 defined tumor cell lines from a total of 19 patients.

The main aim of the WP3 is to characterize relapsed ovarian cancer at genomic and transcriptional levels by comparing paired primary and relapsed ovarian cancer.

- Genomic analyses generate a platinum score of 13 copy number regions, including *MECOM, CCNE1* and *ERBB2* genes, which is correlated with platinum-free interval. Furthermore, chromosomal instability contributes to acquired resistance after a single line of platinum therapy.
- RNA sequencing data reveal that the patients can be sub-grouped according to the expression profiles of their primary and recurrent tumors and the immune reactive status around the tumors. By pairwise analysing the pathway difference between paired tumours, we found out that recurrent tumors resemble their primary counterparts in some sub-groups, while in other sub-groups, over-presentation of specific immune active pathways is the overwhelming difference.
- Two miRs (Hsa-miR-454-3p and hsa-miR-24-3p) were found to be upregulated in recurrent tumors.
- DNA-PKcs is amplified in HGSOC, correlates with poor survival, and is associated with shorter time to relapse. Furthermore, patients with an increase in nuclear pDNA-PK staining in relapse tumours tend to relapse quicker after initial diagnosis than patients without an increase in pDNA-PK expression.

Major aims of WP4 are to establish models, molecularly characterize them, and use them to test the therapeutic effects of the new treatment strategies.

- 29 novel cell lines and PDXs from 30 HGSOC patients have been established.
- Molecular characterization of the cell lines suggests that cellular response to carboplatin is an accumulative result of different genes with different mode of actions.
- Treatment of a DNA PK inhibitor NU7441 shows that PDX faithfully reproduce the sensitivity of the tumor of origin in the patient.
- We have calibrated a drug testing system *in ovo* for measuring the effects of particular chemotherapies on cancer cell metabolism. We confirmed that *in ovo* tumours represent a valid *in vivo* model for testing.

Major aim of WP5 was integrated analysis of molecular profiles characterizing drug resistant HGSOC for identifying molecular mechanisms and biomarkers aimed at overcoming resistance. The WP achieved the following major objectives:

- Molecular processes of relevance in HGSOC resistance to standard drugs could be identified
- Addressing the mTOR pathway via e.g. sirolimus could be postulated as promising in a combination with cisplatin/paclitaxel treatment
- The vascular endothelial growth factor (VEGFA) could be postulated as predictive marker for mTOR inhibition in HGSOC
- A set of drug combinations has been proposed targeting identified synthetic lethal protein pairs being involved in HGSOC resistance processes.

WP6 had the major objectives to demonstrate how validated molecular targets had been implemented into clinical applications. During the last period, three out of the four clinical trials have been closed and evaluated. The results could be presented in the context of the OCTIPS plan. One trial is under the evaluation.

Publishable summary

1. Project context and objective

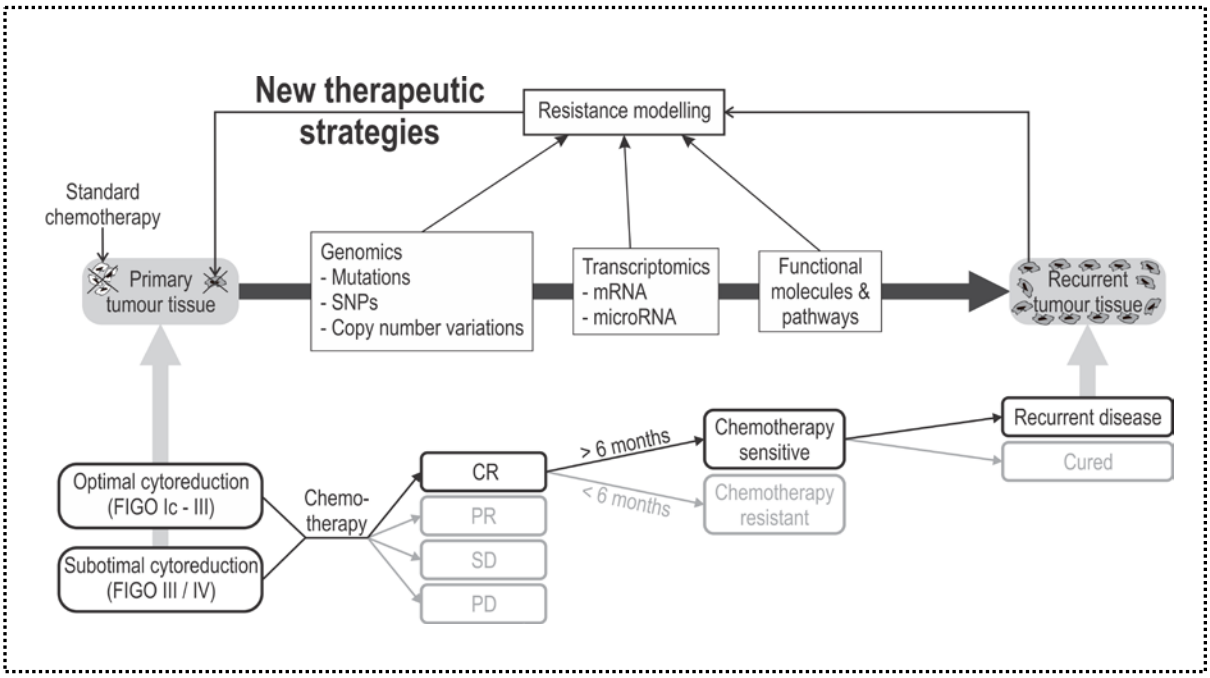


Figure 1. Concept of the project

Epithelial ovarian cancer (EOC) accounting for 4% of gynaecological cancer death in developed countries. Despite improved debulking surgery and the combination of platinum and paclitaxel-based chemotherapies, the 5-year survival is only about 30%. Even though about 75% of the patients have clinical complete remission after first-line treatment, most of them relapse and eventually die of cancer, indicating the need for further treatment improvements. We hypothesize that a small resistant cell population exists in the primary tumour and that this is responsible for ultimate relapse.

General objectives of the proposed project are:

- to compare and analyse molecular features of paired primary and relapse tumours from the same patient
- to define the molecular signatures specifically characterizing the cell population triggering relapse
- to define new therapeutic targets
- to define new therapeutic strategies specifically tackling this critical cell population in primary tumours
- to produce innovative model systems closely mimicking the relapsed ovarian cancer
- to validate the molecular features for relapse in innovative model systems
- to evaluate the molecular features and the resultant therapeutic approach in the clinical context

High grade serous ovarian cancer (HGSOC) accounts for more than 70% of EOC, and is the subtype most likely to present with advanced disease with higher risk for disease recurrence. In OCTIPS project, we focus only on HGSOC ovarian cancer.

The project will provide several levels of molecular characterization comparing paired primary tumours before first-line chemotherapy with those after relapse from the same patient. DNA, RNA, miRNA and proteins

predominating in relapsed tumours will be identified using high throughput technologies. Innovative models closely mimicking the relapse will be selected, including xenografts and cell lines that present platinum resistance, and have the newly defined molecular features. Through analysis of functional molecules and pathways in tumour tissues in the established model systems, and from computational modelling, new therapy targets for recurrent tumors will be defined. New therapeutic strategies that can be used in combination with platinum-based drug will be developed and validated in cell line models and xenograft models in avian eggs and mice.

Specific scientific and technological objectives:

- To collect and process paired tumour tissues at primary surgery and at recurrence from HGSOC patients
- To establish 12 paired cell lines from ascites or tumor tissues
- To molecularly profile paired ovarian tumour samples and to extract genomic and transcriptomic (mRNA and microRNA) features predominating in recurrent disease using next-generation sequencing (Illumina Hiseq2000), SNP array profiling and mRNA array
- To define known functional molecules and pathways specific for recurrent disease by immuno-histochemistry and RT-qPCR in tumour tissues
- To integrate "omics" data and to extract the general molecular features of relapsed tumours
- To select cellular and xenograft model systems mimicking platinum resistance
- To validate newly defined molecular features in model systems
- To define new therapeutic targets in resistant model systems and to design new therapeutic strategies against target molecules utilizing the concept of synthetic lethality
- To screen and to identify clinical drug combinations active against relapse producing HGSOC cells
- To validate therapeutic efficacy using different model systems
- To develop novel clinical trial designs based on OCTIPS project results

With the general objectives and specific scientific and technological objectives described above, we expect to successfully translate our research findings to the clinic, to expand the existing first-line therapeutic strategies for ovarian cancer, and finally to improve disease free and overall survival of the patients.

2. Impacts of the project

Major OCTIPS results can be clustered as follows:

New resources for scientific research:

- mRNAs and miRNA from 70 pairs of tumor tissues
- DNA from paired tumor tissues and matched germline DNA from 83 patients
- Tissue microarrays from paired tumors of 123 patients

  Paired tumors are from the same patient, all diagnosed with high grader serous ovarian cancer. All samples are accompanied with complete clinical information.

- 29 defined HGSOC cell lines from a total of 19 patients, all verified
- PDX from tumors of 30 HGSOC patients (4 of which with multiple sampling)

New knowledge of molecular biology of cancer, especially of ovarian cancer

- Genomic analyses generate a platinum score of 13 copy number regions, including *MECOM, CCNE1* and *ERBB2* genes, which is correlated with platinum-free interval. Furthermore, chromosomal instability contributes to acquired resistance after a single line of platinum therapy.
- RNA sequencing data reveal that the patients can be sub-grouped according to the expression profiles of their primary and recurrent tumors and the reactive status of host immune system around the tumors. By pairwise analyses of the pathway difference between paired tumours, we found out that recurrent tumors resemble

their primary counterparts in some sub-groups, while in other sub-groups, over-presentation of specific immune active pathways is the overwhelming difference.
- Two miRs (Hsa-miR-454-3p and hsa-miR-24-3p) were found to be upregulated in recurrent tumors.
- DNA-PKcs is amplified in HGSOC, correlates with poor survival, and is associated with shorter time to relapse. Furthermore, patients with an increase in nuclear pDNA-PK staining in relapse tumours tend to relapse quicker after initial diagnosis than patients without an increase in pDNA-PK expression.

Evaluated model system for studying molecular biology of cancer, especially chemoresistance of the tumors
- Newly established cell lines
- PDX
- in ovo models

New therapeutic targets and new therapeutic strategies
A number of targets have been proposed, for which the therapeutic strategies have been proposed and partly tested.

Clinical trials in the context with OCTIPS have been designed and proposed

Lead users of these results include ovarian cancer patients and their relatives, clinicians, hospitals, scientists, SMEs and biotechnology and pharmaceutical companies.
Indirect users include policy makers, health systems, society, biotechnological companies and pharmaceutical companies.

The project will have impacts on following aspects:

New knowledge and resources for scientific society

New knowledge on ovarian cancer, especially on relapsed ovarian cancer, on the functional pathways and molecules, have opened new aspects for the scientific society. Even though ovarian cancer is known as a very heterogeneous disease, molecular biologists use to believe that a certain phenotype could be explained with one certain mechanism for all cases. Most of the studies have been also constructed in such way to extract common genes, gene signatures or common pathways. The results of OCTIPS project on relapsed HGSOC indicated that in some sub-groups, tumors resemble their primary tumor counterpart, while in other cases, they presented different molecular profile. This demonstrated that HGSOC is indeed highly heterogeneous. Personalized analyses will be needed to achieve efficient therapy design. Not only the results, but also the pairwise analytical method provides a new way to perform molecular analyses.

The resources created in OCTIPS consortium are very valuable for not only basic scientific research, but also for drug development. For decades, undefined cell lines or “wrong” cell lines have been used for the investigation of HGSOC, which produced data that we cannot rely on. The cell lines and PDX created in our consortium present the same molecular profiles and are accompanied with the clinical information of the patients. They are already requested by the scientific society.

Paired DNA and RNA samples, in the same way, presented the first, largest and most precisely documented cohorts of HGSOC worldwide. They are very precious materials for further scientific works.

Societal impact

About 200,000 new cases of ovarian cancer are diagnosed in the world annually. The burden of this disease for the society is not only due to the morbidity and mortality but also due to the treatment itself, which has significant side effects, a low success rate and a very high burden on healthcare system. The OCTIPS consortium is in the front line of combating this threatening disease. The efforts on defining the new targets and new therapeutic designs against the targets will finally lead to efficient drugs that will kill the tumour cells and cure the patients.

This will have important social impact. It will free the patients from suffering both physical and psychological pain, thus improving their health and quality of life. Furthermore, it will also reduce the psychological and administrative burden for the family of the patients and the treatment costs for the society as well.

Economic impact

Our results could lead to the definition of biomarkers, such as mutations, specific copy number changes and SNPs, gene expression markers, miRNA markers, and protein markers, which predominate in relapsed disease but occur only in a small number in primary ovarian cancer. Detection of the biomarkers in primary ovarian cancer would make it possible to predict the outcome of the patients and thus help physicians to make new treatment strategies. Thus, methods, kits, reagents, or antibodies for the detection of relapsed cells can be developed by biotechnology companies. By definition of new therapeutic targets for relapsed ovarian cancer, a new dimension of drug development will be opened. In the case of P11, the work outlined in this project has the potential to expand the clinical rationale for their existing drug candidates, whilst addressing the unmet clinical need represented by relapse in HGSOC. Applying P11's established drug combination screening capabilities to the HGSOC models available through this collaboration, and subsequently mining the resulting data using P6's computation models will increase the clinical relevance of this approach, while also increasing the level of data interpretation beyond that achievable by each group individually. In addition to the potential for accelerated translation of these results to a clinical impact in HGSOC, it is expected that this interaction of P11 and P6 will lead to an improved approach for combination data analysis that can further accelerate the impact of combination screening in many other model systems, hence providing a competitive advantage for both SMEs. The exploitation of the project results will involve the activities of biotechnology and pharmaceutics companies, which will bring direct impact on the economic development of EU. For P9 and P11 this project holds the unique opportunity of further validating novel therapy approaches utilizing the precious sample and models material available. For P6 the OCTIPS data space provides the necessary basis for cross-Omics aimed at deriving a molecular relapse signature, which may subsequently enter patient stratification in clinical trials. P6 furthermore has the opportunity of implementing synthetic lethality screening with the potential of identifying novel targets and associated drugs.

Added value in carrying out the work at a European level

Ovarian cancer is very heterogeneous regarding its histotype, tumour stage and response to standard treatment. The proposed research works need a certain number of biological samples to ensure statistical power and reliability of the results. As ovarian cancer is a rare cancer, an approach at the European level is absolutely needed. The multi-platform research in specific scientific and technological areas also requires the cooperation at European level.

There are compelling reasons to organise such an effort at the European level. From the scientific point of view, our project has a conspicuous Community added value. It is inspired to the principle of a "laboratory without walls", in which no individual institution possesses the resources, the energy and the know-how to tackle the problem, but all participants share the resources and form a powerful synergy. This synergy is witnessed by the structuring of the experimental tasks, to which various components from different institutions are called to participate, and by the overall logical scheme of the project. The groups share the same general objectives but hold different expertise, experimental systems and philosophy of approach. The group composition is not casual but reflects an already established network of interactions (EORTC, http://www.eortc.be; TOC, http://www.toc-network.de; EUTROC, http://www.eutroc.org), which is witnessed in collaborative studies, as such OVCAD (http://www.ovcad.eu).

Through the OCTIPS project, therefore, the added benefit of formally coalescing many of these interactions into an organised framework. It is obvious that such a complex network of interactions cannot be achieved at the individual group level or even at the national level.

Building up further cooperation

The establishment of such collaboration on ovarian cancer research would not be possible in any Member State alone. Nevertheless, the scientific objectives pursued in OCTIPS fall with the research priorities of different Member Stats of the participants in the consortium, because cancer is one the major healthy concerns in all European countries.

Through the establishment of the external advisory board, the connection of the consortium and each participants to the research organisations abroad has also been set up, which will expand the established advantages in EU into an international level. By networking the "networks", we believe that the research on ovarian cancer will be extended into a new dimension, which will finally bring the benefit to patients.

Further impacts

As ovarian cancer is a rare cancer, an approach at the European level is absolutely needed. The multi-platform research in specific scientific and technological areas also requires the cooperation at European level.

OCTIPS includes eleven participants, each constructed with a research team and/or clinic. Through collaborative and interactive works, the network on ovarian cancer research in Europa will be certainly strengthened and expanded.

OCTIPS is also in line with the EU policies to drive sustainable growth and competitiveness through the stimulation of innovation, to help the SMEs realise their growth potential, to promote entrepreneurship and to create a healthier business environment for them, and finally to optimise national health systems.

In addition, OCTIPS focuses on identification and validation of drug targets, fully addresses the topic 2.4 Translational Research in Other Major Disease, 2.4.1 Cancer, and will contribute to the aims of FP7 of reducing cancer mortality and improving quality of life of the citizens. It also contributes towards the expected impact of HEALTH.2011.2.4.1-2: Translational research on cancers with poor prognosis.

3. Main dissemination activities and exploitations of the results

Dissemination

In the course of this project, valuable deliverables have been generated including biological materials (paired primary and recurrent tumor materials, RNA, DNA and TMA; newly established tumor cell lines; PDXs), databases, and processes. Our audience are basic scientists and physicians, the general public, especially the patients, pharmaceutical companies and the decision makers. The major results will be published in the scientific journals and presented in national and international scientific meetings.

The biological materials, processes, and knowledge generated during the course of the work will be made freely available to the project participants, whenever they are needed to achieve the project objectives, even if unpublished. For organisations outside OCTIPS, biological materials, processes, and knowledge will be made available for research purpose only, even when unpublished, when these are required and the confidentiality issues are resolved. The latter can also be done on a collaborative basis following agreements between the consortium, principal investigators and the legal departments of all related organisations. Published reagents will be made available to the scientific community, when the requesting institution is a non-profit organisation and the reagents are requested for research purposes. Individual participant might elect to distribute their reagents through common repositories. This policy will actually be encouraged within the consortium. Requests from profit-organisation (pharmaceuticals, biotechnology companies) will be handled by the coordinator under the advice of "Enterprise Europe Network" (EEN, http://www.enterpriseeuropenetwork.at). The decision will be taken on behalf of the OCTIPS consortium and within the framework of the policies established under "exploitation" (as described below).

The dialogue with the interested public will focused on ovarian cancer patients and their relatives. 5 clinical partners are involved in the project. They are in direct contact with patients and are able to give them more information on ovarian cancer disease, on the developments in science and technology, and on the treatment options. One of the OCTIPS participants P2, Charite has a distinguished success in performing the dissemination activities (http://www.stiftungeierstockkrebs.de/).

We also set up our project website and have managed to send out a numbers of press releases. Along with the increasing results of the OCTIPS, this activity to disseminate the project efforts and results to different levels of audience will certainly increase, even after the project will be closed.

Exploitation

There are a number of project results to be exploitable:

- The newly generated cell culture models are not only interested for the scientific society to perform research works on HGSOC, they are also useful tools for drug testing and drug development. We will provide the cell lines to the scientific society basically for free. The relevant projects will be evaluated and whenever possible, the collaboration will be pursued.

- In the similar way, the PDX will be exploited.

- OCTIPS also created huge data on paired HGSOC, including profiles of mutations, copy number variations, gene expression, and miRNA expression. We will provide our data for other research work whenever it is relevant.

- The results from most of the OCTIPS partners are under preparation for publication.

OCTIPS has established a tight network, in which many partners have already worked together and will certainly continue to collaborate, and pursue the final aims of the project. Should any potential be identified in the context with OCTIPS project, the coordinator will work together with The Office of Technology Transfer in The Medical University of Vienna (MUW) to determine the strategies for the exploitation.

4. The project public website

The project website (www.octips.eu) is publicly accessible and has a team area only for project participants. It aims at introducing OCTIPS to the public and disseminating new knowledge on ovarian cancer research to the scientific society and the clinical communities. Main resources for the webpage are from the project participants. The project is presented with its objectives, participants, and expected results. The website is linked to some established ovarian cancer research organisations. Support from European Commission is acknowledged.

Main S&T Results

1. Work progress and achievements

WP2. Recruitment of experimental materials and data repository

Summary of WP2

WP2 has successfully established two online databases, defined including criteria, coding system for sample documentation and the standard protocols as planned. In total, RNA and miRNA isolated from paired fresh frozen tissues at initial prognosis and relapse of 79 high grade serous ovarian cancer (HGSOC) patients were delivered to WP3. Similarly, paired DNA samples and matched germline DNA from 83 patients were delivered. Tissue microarrays including 123 pairs of FFPE samples were constructed and 29 cell lines from HGSOC patients have been established, verified and molecular characterized. The complete clinical data for a total of 151 patients, of whom the samples were involved in the final analyses, were collected, revised and delivered to all partners.

Thereby, we have created the biggest resource of paired HGSOC and the largest cohort of verified cell lines from HGSOC in the world.

The main objectives of WP2 are:
- To define the clinical materials needed for the OCTIPS project
- To collect, process, deposit and deliver all necessary clinical materials
- To build tissue microarrays
- To provide clinical data for all partners.

Deliverables of the WP2:
- D2.1. Both OCTIPS online databases released (M18)
- D2.2. DNAs, RNAs, microRNAs delivered (M18)
- D2.3. All necessary samples delivered to all partners (M36)
- D2.4. 12 pairs of cell lines established (M36)
- D2.5. OCTIPS TMA built (M36)
- D2.6. Complete clinical data delivered (M48)

Milestones of the WP2:
- M2.1. Inclusion and exclusion criteria, sample coding and standard protocol defined (M1)
- M2.2. Opening of the online databases (M2)
- M2.3. Delivery of retrospective materials (M4)
- M2.4. Delivery of all prospective collected samples (M20)
- M2.5. Release of complete clinical data (M46)

Publications

Kreuzinger C, Gamperl M, Wolf A, Heinze G, Geroldinger A, Lambrechts D, Boeckx B, Smeets D, Horvat R, Aust S, Hamilton G, Zeillinger R, Cacsire Castillo-Tong D. Molecular characterization of 7 new established cell lines from high grade serous ovarian cancer. Cancer Lett 362:218-28, 2015

Main results:

<u>Standard documentation and processing of clinical materials have been set up</u>

The inclusion criteria, the coding systems and sample documentation were determined at the project start.
Two dedicated data handling systems will be used in OCTIPS serving two distinct purposes: an online database for documentation of specimen and clinical data, and a data management system for organizing the experimental data resulted from these samples.

- Database for the documentation of clinical materials and clinical data

Alcedis MED trial OCTIPS (http://www.octips-project.com) is a web based electronic data capturing (EDC) and data management system which allows for an efficient data capturing of clinical data as well as sample recording and tracking (sending, receiving, isolation / extraction procedures). Furthermore, the software is aimed at supporting an efficient collaboration within the community as well as supporting project and process management. The system was adapted to OCTIPS project. Go Live of the demo system was set in September 2011. Data capturing of the clinical data began in February 2012. Further system expansions discussed on the OCTIPS Meeting in March 2012 are implemented.

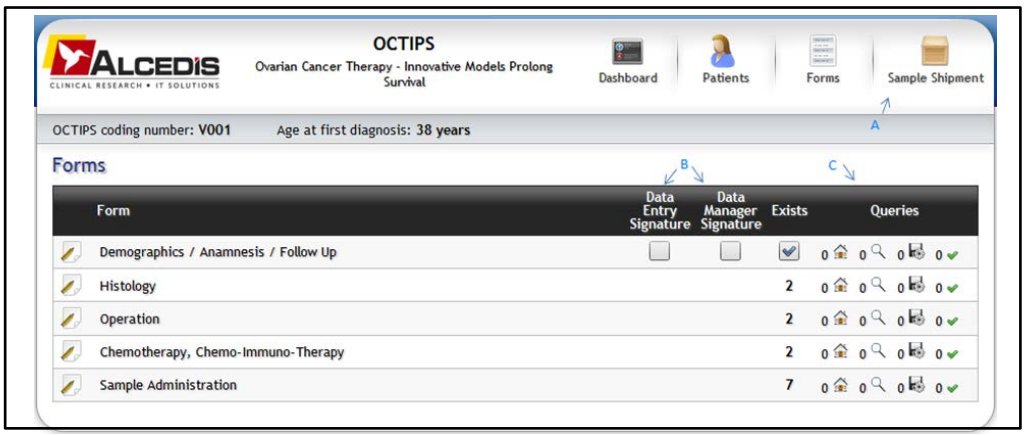


Figure 1: OCTIPS database showing the structure of the clinical documentation.

- Next to the clinical repository OCTIPS-BASE, a web-based data repository was established and used for OCTIPS-internal molecular data organization and data exchange.

<u>Clinical materials have been collected, processed and delivered to all partners</u>

- Fresh frozen tumor tissues for the isolation of DNA and RNA

As a result, we could deliver sections from 79 pairs of tumor tissues, from which RNA (including miRNA) was extracted and sent for the genomic and transcriptomic analyses. By further examining the gene expression data in WP3, 9 pairs of samples were excluded, resulting a final inclusion of 70 pairs of samples.

- Germline DNA

In order to perform the whole exome sequencing to obtain the information genomics of the ovarian cancer, germline DNA is need for the comparison. DNAs isolated from blood samples from a total of 83 patients have been sent to P3 for this purpose.

- Collection of FFPE samples and construction of an OCTIPS tissue microarray (TMA)

In total, we constructed a TMA with paired samples from 123 HGSOC patients.

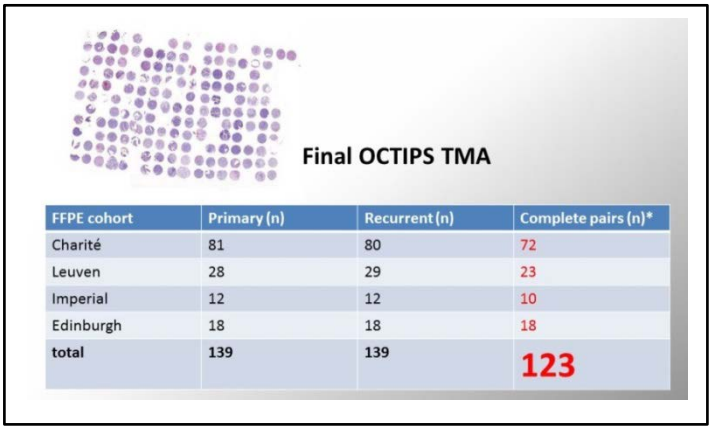


Figure 2. Overview of the OCTIPS TMA.

- All relevant clinical data have been collected and delivered

<u>29 cell lines from HGSOC have been established and verified</u>

In total we have established 29 cell lines from 19 of HGSOC patients. Sequencing of the TP53 confirmed that they all harbour the specific mutation as the tumors from the patients, where they derive from.

Figure 3. Cell lines and primary mesothelial cell culture established from high grade serous ovarian cancer. All tumor cell lines except one (8540) harbored specific *TP53* mutation.

WP3. Molecular characterization of relapsed ovarian cancer

Summary of WP3

Major results of the WP3 are the characterization of relapsed ovarian cancer at genomic and transcriptional levels by comparing paired primary and relapsed ovarian cancer:

- Genomic analyses generate a platinum score of 13 copy number regions, including *MECOM, CCNE1* and *ERBB2* genes, which is correlated with platinum-free interval. Furthermore, chromosomal instability contributes to acquired resistance after a single line of platinum therapy.
- RNA sequencing data reveal that the patients should sub-grouped according to the expression profiles of their primary and recurrent tumors and the immune reactive status around the tumors. By pairwise analyses of the pathway difference between paired tumours, we found out that recurrent tumors resemble their primary counterparts in some sub-groups, while in other sub-groups, over-presentation of specific immune active pathways is the overwhelming difference.
- Two miRs (Hsa-miR-454-3p and hsa-miR-24-3p) were found to be upregulated in recurrent tumors.
- DNA-PKcs is amplified in HGSOC, correlates with poor survival, and is associated with shorter time to relapse. Furthermore, patients with an increase in nuclear pDNA-PK staining in relapse tumours tend to relapse quicker after initial diagnosis than patients without an increase in pDNA-PK expression.

The main objectives of WP3 are:

- To define specific somatic mutations in relapsed ovarian cancer
- To define copy number alterations characterizing relapsed ovarian cancer
- To define gene expression signature predominating relapsed tumours
- To define microRNA signature specific for relapsed cancer
- To define functional molecules and pathways that are principal players in relapsed cancer

Deliverables of WP3

- D3.1. miRNA signature defined (M18)
- D3.2. Copy number alterations characterizing relapsed tumours determined (M18)
- D3.3. Gene expression profiles of 60 paired ovarian tumours obtained (M18)
- D3.4. qRT-PCR of STAT1, HDAC4, FOLR1-3, AKT 1-3, DNA-PKcs in 50 tumour tissue pairs performed (M18)
- D3.5. Somatic mutations specific for relapsed tumours validated (M36)
- D3.6. Gene signature predominating recurrent disease validated (M36)
- D3.7. miRNA signature validated in 100 samples (M36)
- D3.8. IHC results of TMA of STAT1, HDAC4, FOLR1-3, AKT 1-3, DNA-PKcs obtained (M36)
- D3.9. Mutation analysis of PI3K-MTOR-AKT-DNAPK associated pathway molecules completed (M36)

Milestones of WP3:

- M3.1. Accomplishments of molecular analyses at all levels (M18)
- M3.2. Definition of molecular features predominating relapsed ovarian cancer (M20)

Publications

Lambrechts S, Smeets D, Moisse M, Braicu EI, Vanderstichele A, Zhao H, Van Nieuwenhuysen E, Berns E, Sehouli J, Zeillinger R, Darb-Esfahani S, Cacsire Castillo-Tong D, Lambrechts D, Vergote I. Genetic heterogeneity after first-line chemotherapy in high-grade serous ovarian cancer. Eur J Cancer. 53:51-64, 2015.

Main results

A platinum score predictive of resistance to platinum therapy

A platinum score of 13 copy number regions, among other genes including *MECOM*, *CCNE1* and *ERBB2*, correlated with platinum-free interval (PFI) after first-line therapy, whereas an increase of this score in recurrent tumors predicted the change in PFI during subsequent therapy.

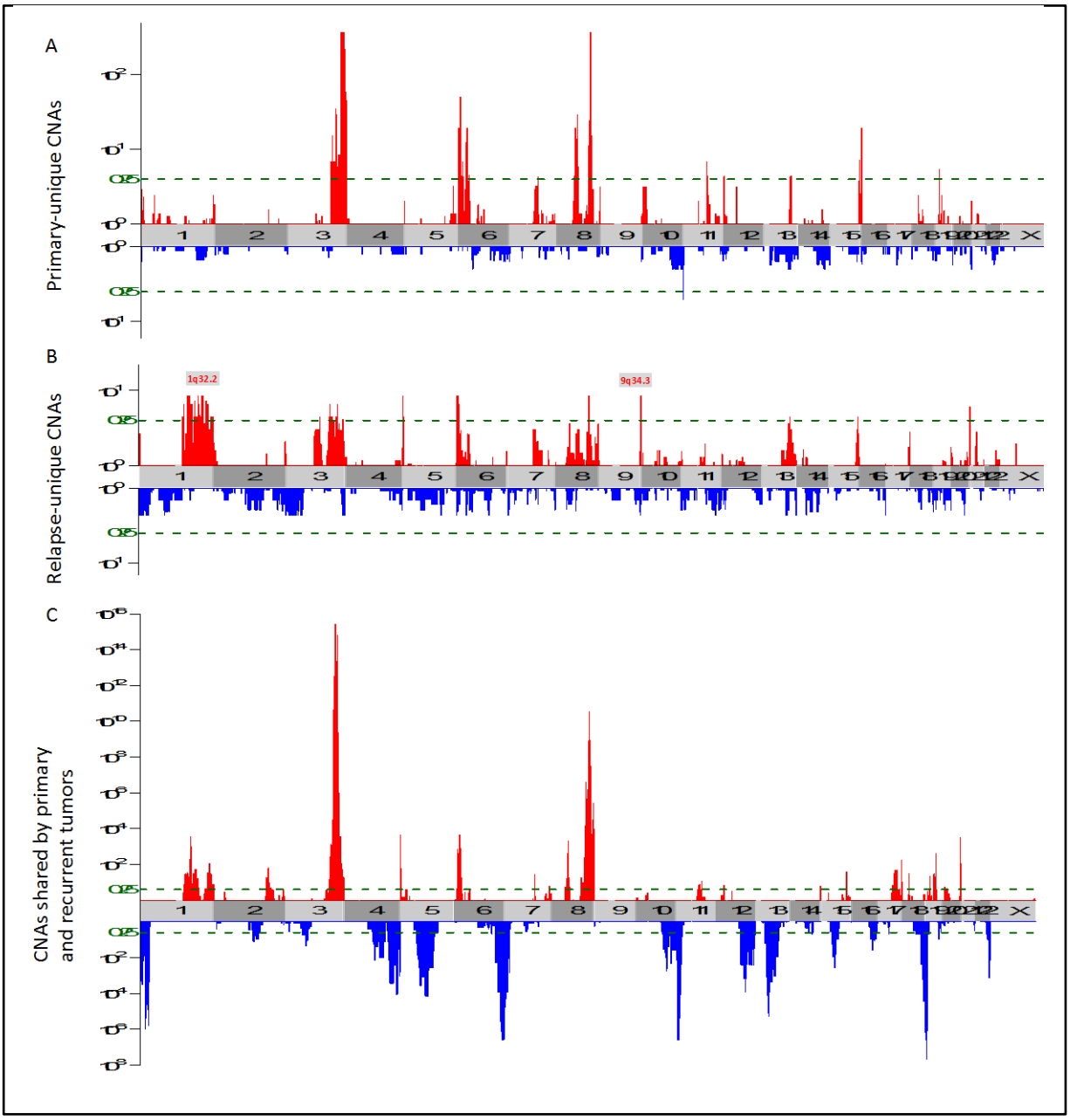


Figure 1. Recurrent CNAs in primary and recurrent tumors.

Gene expression profiling of paired primary and relapsed ovarian cancer revealed different sub-groups

- two types of the HGSOC have been identified

Unsupervised clustering revealed two clusters of tumors (Figure 2.).

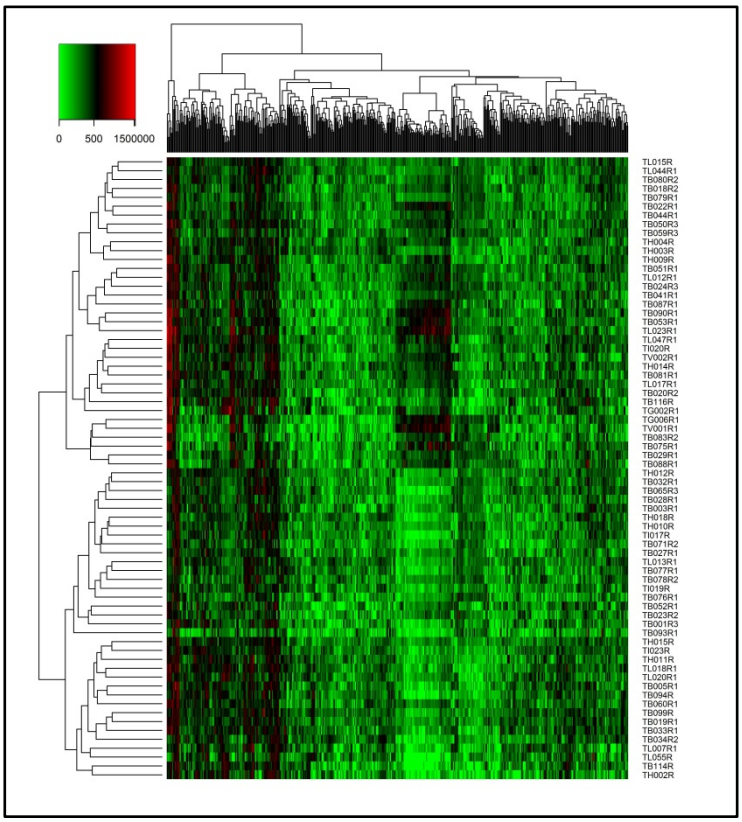


Figure 2. Unsupervised clustering of the relapsed samples using 500 genes with the highest mean values among the 1000 genes with the biggest variance across all samples.

241 genes out of the 283 genes with the highest difference among the two clusters code proteins specifically expressed by active immune cells (Figure 3).

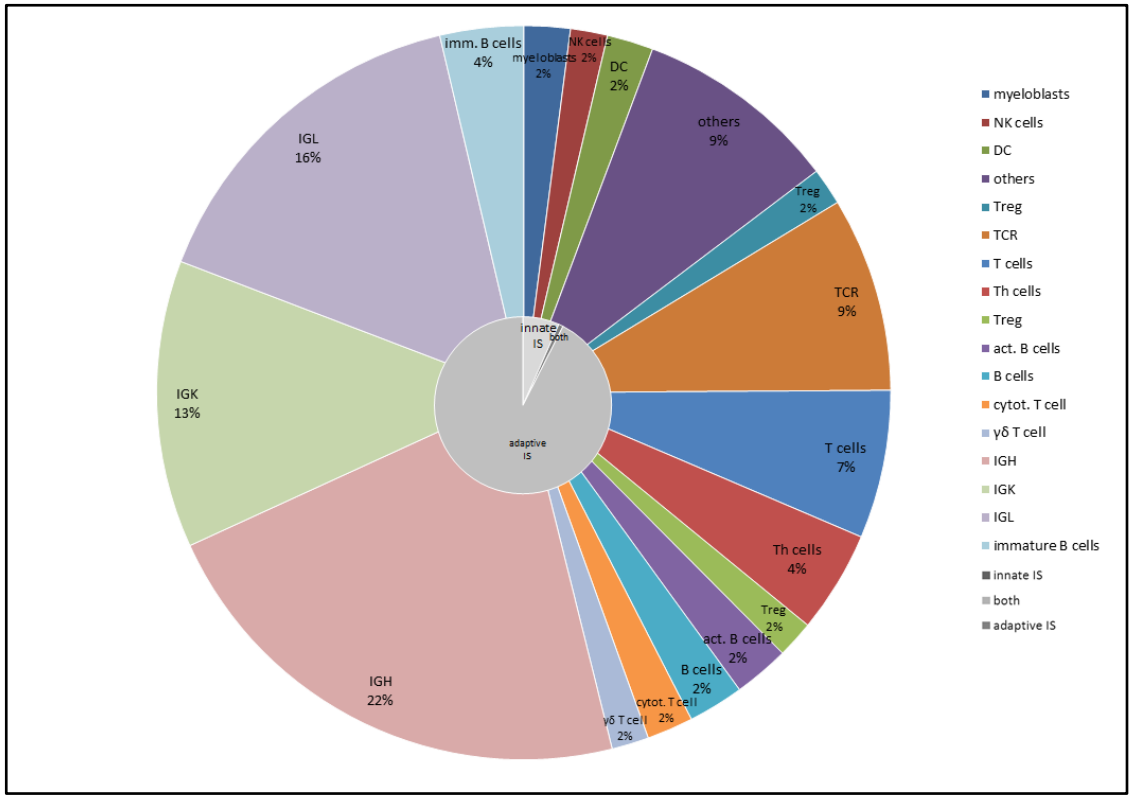


Figure 3. 241 differentially expressed genes.

- Paired primary and recurrent samples presented various immune reaction

Using these 241 immune related genes, we re-clustered the recurrent samples and the samples at diagnosis. 32 tumors are immune active and 38 are silent samples. In the primary samples, 28 tumors are clustered as immune active and 42 as immune silent (Figure 4).

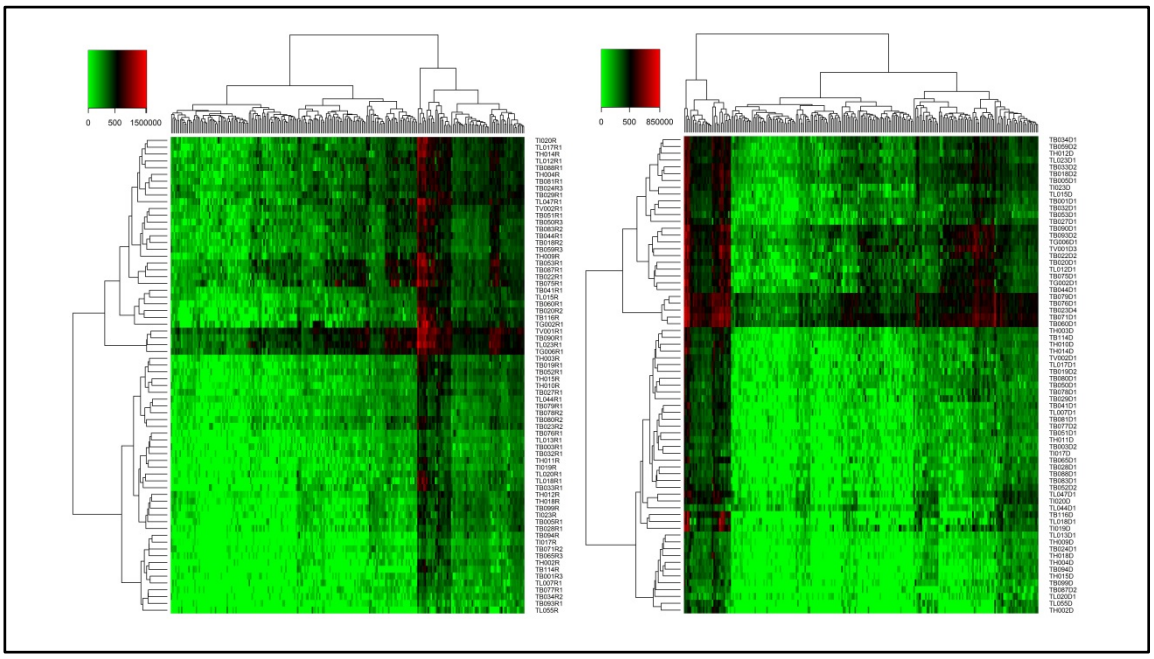


Figure 4. Clustering of recurrent (left) and primary (right) samples with the 241 immune cell associated genes.

We therefore define the patients with primary and recurrent tumors into 4 subgroups.

- primary immune active - recurrent immune active (active-active)
- primary immune active - recurrent immune silent (active-silent)
- primary immune silent - recurrent immune active (silent-active)
- primary immune silent - recurrent immune silent (silent-silent)

- Recurrent tumors show different nearness with their primary counterpart in different sub-groups

Pairwise analysis revealed that the recurrent samples resemble their primary counterparts in some sub-groups and in some other sub-groups not. In active-active and silent-silent sub-groups of patients, most of the tumors recurred with similar molecular type, showing no difference in pathway presentation. We assume that they would have similar response to the drug treatment. These account for about 50% of the patients in our cohort. In another 50% of the patients, recurrent and primary tumors differed significantly from each other in the way that one of the pairs was surrounded by a highly activated immune microenvironment and the other one not.

Besides these major differences, the other minor differences between recurrent and primary tumors were very heterogeneous. There were no or very few tumor pairs shared a common pattern, suggesting that personalized molecular analyses will be needed to define the treatment strategies.

<u>Study of miRNA and pathways involved in therapy resistance revealed two up-regulated miRNAs</u>

In the discovery phase we have performed miRNA expression analyses using TaqMan qRT-PCR fluidic cards of Applied Biosystems with which we were able to analyze 754 unique probes. Analysis of the fluidic cards on 44 paired samples revealed a total of 397 expressed probes. The data were normalized with the ΔCt method, with which the median of all expressed probes was used as the reference. Data were $\log_2$ normalized before further analyses.

147 miRNAs which had standard deviation equal or bigger than 2 were used for an unsupervised clustering cross all samples (figure 5). The most variable probes were selected by ranking their variability in expression across all samples.

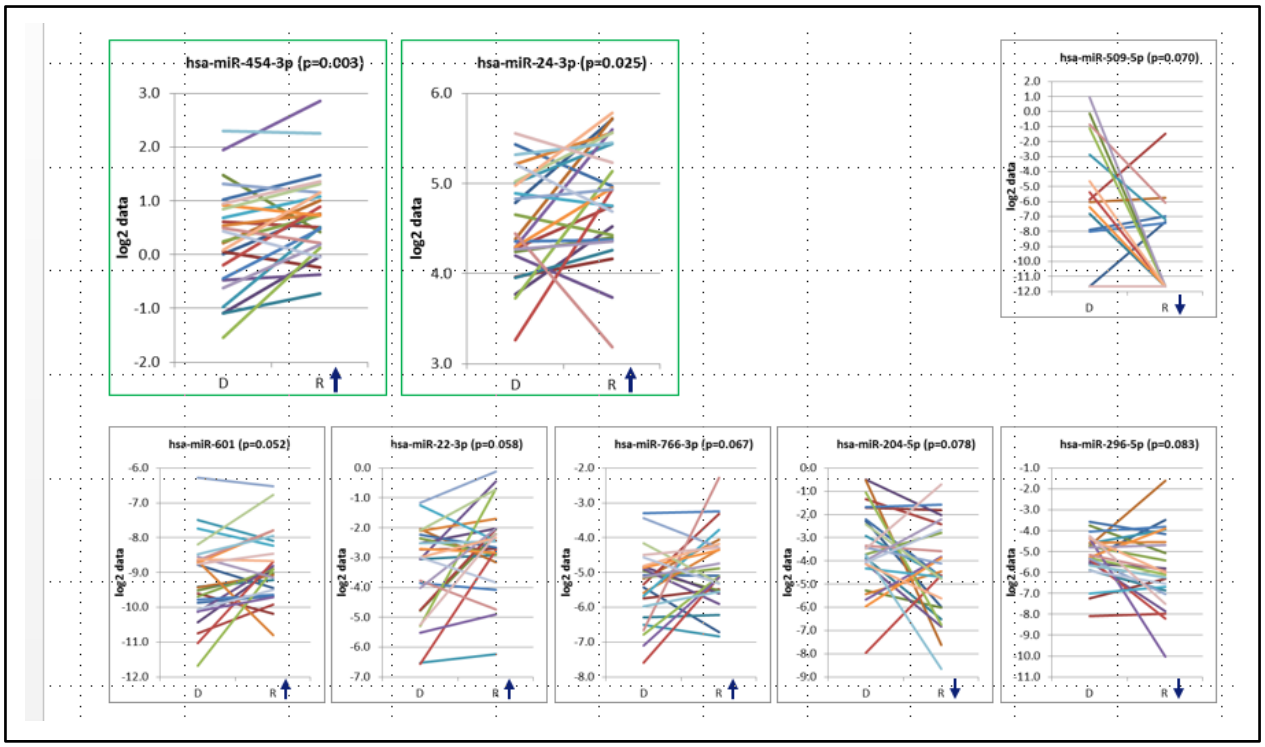


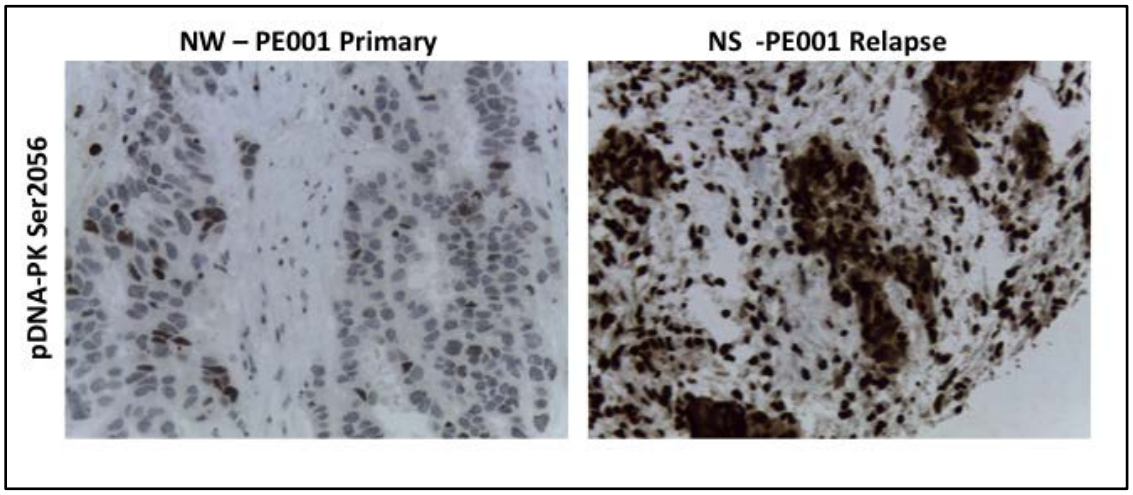
Figure 5. miRNA expression in primary and recurrent samples.

As a final results, Hsa-miR-454-3p and hsa-miR-24-3p are significantly upregulated in relapsed samples. The miRs may be tested in future studies in sera as diagnostic markers in ""liquid biopsies"" or extracelluar vesicles or exosomes.

DNA-PK as a therapeutic target in HGSOC

Using publically available datasets and DNA from the OCTIPS cohort of primary and relapse tumours, we have observed that DNA-PKcs is amplified in HGSOC, correlates with poor survival, and is associated with shorter time to relapse.

To determine the preclinical and clinical validation of the predictive and response biomarker status of DNA-PK, tissue microarrays of 120 pairs of primary and relapse tumours were stained for the presence of total DNA-PK and pDNA-PK Ser2056. We observed that phosphorylation of DNA-PK in relapse tumours may be linked to amplification of DNA-PK, given that patients with an increase in their DNA-PKcs copy number in their relapse tumour sample (relative to the copy number in their primary tumour sample) were more likely to show an increase in pDNA-PK staining in their relapse tumour sample (relative to the pDNA-PK staining in their primary tumour sample) (example of staining in Figure 6 upper panel). Patients with an increase in nuclear pDNA-PK protein in relapse tumours (relative to the level in primary tumours) tend to relapse quicker after initial diagnosis than patients without an increase in pDNA-PK expression (Figure 6 lower panel). Work is ongoing to determine the therapeutic potential of DNA-PK inhibition as a strategy in ovarian cancer.



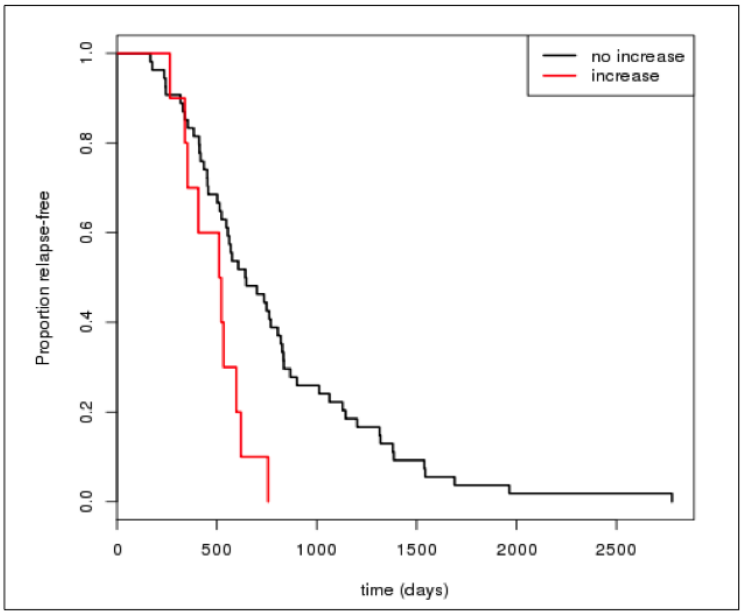


Figure 6 upper panel: Example images showing the differences in p-DNAPK Ser2056 staining intensity between primary and relapse tumour samples, PE001 denotes core 1 from Edinburgh TMA set. NW – Nuclear weak; NS – Nuclear Strong. Lower panel: Kaplan-Meier curves for progression-free survival of OCTIPS patients (n=64) stratified according to the change in nuclear pDNA-PK (as evaluated by IHC of TMA cores) between primary and relapse tumour samples (p=0.0084).

WP4. Innovative models to validate new therapeutic strategies

Summary

We successfully established and/or characterized following models
- 26 HGSOC cell lines from HGSOC
- PDX from 30 HGSOC patients, 4 of which with multiple tumor sampling
- 35 “old” commercially available cell lines
- *in ovo* system

The newly established models were shown to mimic their tumors and thus were optimal for studying the resistance of HGSOC. Some new therapeutic strategies were tested in these models.

Objectives of the workpackage are:
- To establish innovative models mimicking treatment failure for ovarian cancer – recurrent disease
- To design new therapeutic strategies to obviate resistance
- To validate the new strategies in well characterised model systems

Deliverables for WP4:
- D4.1. 10 pairs of sensitive and resistant tumourgrafts selected (M18)
- D4.2. 15 pairs of cell lines characterized (M36)
- D4.3. Molecular characterization of treatment resistance in 35 cell lines completed (M36)
- D4.4. Resistant tumourgraft models molecularly characterized (M36)
- D4.5. Pathways and principal molecules involved in relapsed defined (M36)
- D4.6. Active combinations of drugs for ovarian cell lines identified (M36)
- D4.7. New therapeutic strategies designed (M36)
- D4.8. *in ovo* HGSOC model using both cell lines and ascites validated and molecularly characterized (M36)
- D4.9. Therapeutic effects in *in ovo* models validated (M48)
- D4.10. New therapeutic strategies validated in tumourgrafts (M48)

Milestones:
- M4.1. Validation results of the molecular features for relapsed ovarian cancer from WP3 in all models (M24)
- M4.2. Determination of therapeutic targets (M27)
- M4.3. Design of new therapeutic strategies (M28)
- M4.4. Validation results of the new therapeutic strategies for all models (M48)

Publications

Kreuzinger C, Gamperl M, Wolf A, Heinze G, Geroldinger A, Lambrechts D, Boeckx B, Smeets D, Horvat R, Aust S, Hamilton G, Zeillinger R, Cacsire Castillo-Tong D. Molecular characterization of 7 new established cell lines from high grade serous ovarian cancer. Cancer Lett 362:218-28, 2015.

Colombo PE, du Manoir S, Orsett B, Bras-Gonçalves R, Lambros MB, MacKay A, Nguyen TT, Boissière F, Pourquier D, Bibeau F, Reis-Filho JS, Theillet C. Ovarian carcinoma patient derived xenografts reproduce their tumor of origin and preserve an oligoclonal structure. Oncotarget. 6:28327-40, 2015.

Beaufort CM, Helmijr JC, Piskorz AM, Hoogstraat M, Ruigrok-Ritstier K, Besselink N, Murtaza M, van IJcken WF, Heine AA, Smid M, Koudijs MJ, Brenton JD, Berns EM, Helleman J. Ovarian cancer cell line panel (OCCP): clinical importance of in vitro morphological subtypes. PLoS One 9:e103988, 2104.

Main results:

<u>Newly established cell line present different sensitivity to carboplatin which is related to the expression of some functional genes</u>

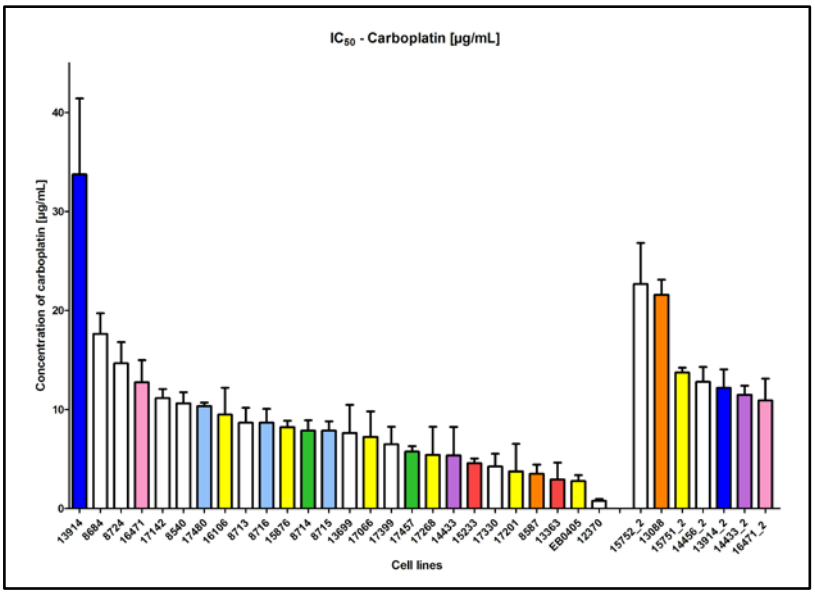


Figure 1. The group of cell lines at the right are primary mesothelial cell cultures and at the left epithelial tumor cell lines. The same color indicates the cell lines generated from the same patient, non-colored ones are single lines.

Gene expression of the *MLH1* and *GPX1*, both reported to be associated with carboplatin resistance of the cells, are highly expressed in the very resistant cell lines in comparison with the sensitive ones.

Tumourgraft representing relapsed HGSOC are produced and characterized

PDX from HGSOC of 30 patients have been established. Pairwise characterization (tumor of origin and PDX) at the molecular (transcriptome and CGH) and histological levels demonstrated that PDX perfectly reproduced the histology and morphology, as well as genetic characteristics of the tumors they originated from (Figure 2). These results thus confirmed that these PDX models are excellent representations of the disease in the patient.

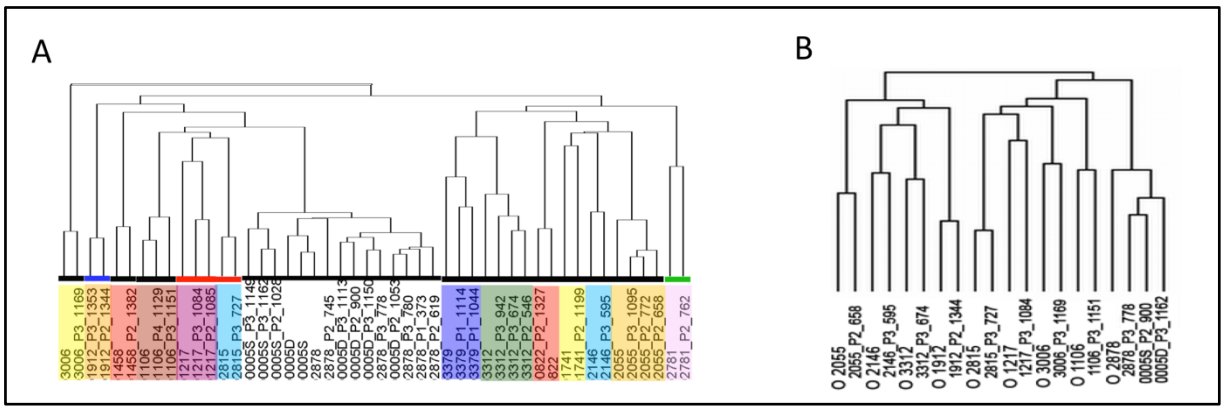


Figure 2. PDX reproduce genetic characteristics of their tumor of origin. A. CGH profiles of PDXs and tumors of origin cocluster in a hierarchical clustering analysis. B. coclusterization of PDXs and tumors of origin at transcriptomic level.

Preliminary results show that PDX faithfully reproduce the sensitivity to treatment of the tumor of origin in the patient (Figure 3.), suggesting they are optimal models to study the chemoresistance.

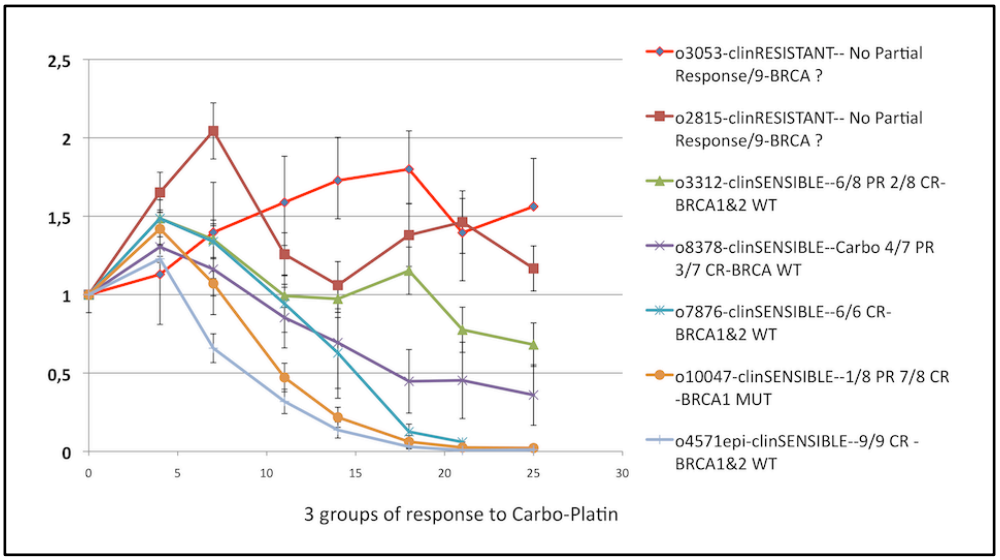


Figure 3. response to carboplatin of 7 EOC PDX models tested. Data indicate the existence of 3 groups of sensitivity

Screening of a drug library with paired cell lines revealed best combination

The following agents were the highest ranked of those confirmed to be additive or synergistic in combination treatment with cisplatin: belinostat (HDAC), vorinostat (HDAC), dasatinib (Src/Abl), decitabine (DNMT), AZD7762 (CHK), paclitaxel (Taxane), BKM120 (PI3K), YM155 (Survivin), MG132 (Proteosome), gemcitabine (antimetabolite), XAV-939 (Wnt). With the exception of paclitaxel and gemcitabine, which are already used therapeutically in combination with cisplatin, these agents are recommended for additional testing in OCTIPS models.

in ovo model is optimal for testing the new therapeutic strategies

- in ovo models showed similar histology as the tumors in patients

Cell lines established in OCTIPS consortium were engrafted into the egg in matrigel plugs, resulting in sizeable tumours containing viable and slowly proliferating cells. They are sensitive to carboplatin treatment (Figure 4).

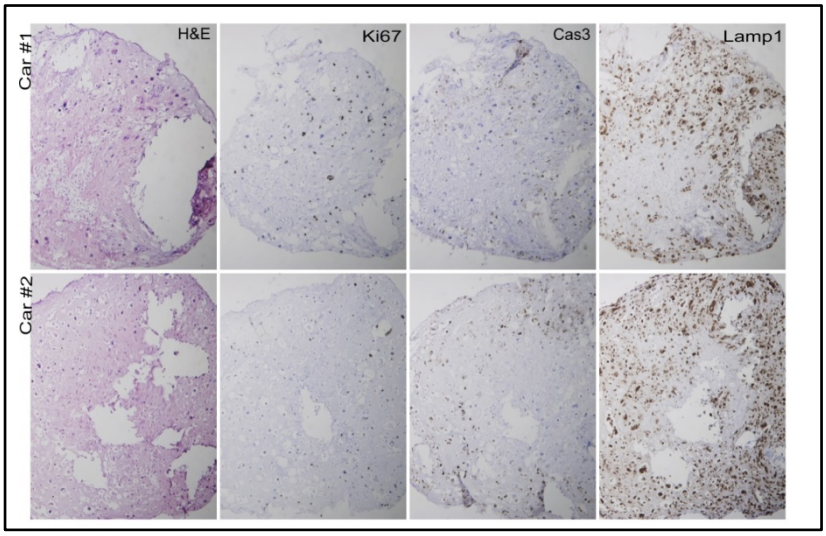


Figure 4. HGSOC cell line is sensitive to Carboplatin in the CAM tumour xenograft model. Immunohistochemistry for LAMP1, which stains profusely throughout the matrigel mass within the tumours, Ki67, which is greatly reduced in the 400ug Carboplatin group (Car) compared to the PBS group, and cleaved Caspase-3, which stains many cells in the Carboplatin group, and next to none in the PBS group.

WP5. Data integration and modelling

Summary

Significant results are:

- Molecular processes of relevance in HGSOC resistance to standard drug regimes (cisplatin, paclitaxel) could be identified based on generated network-based molecular models
- Addressing the mTOR pathway via e.g. sirolimus could be postulated as promising in a combination with cisplatin/paclitaxel treatment, specifically holding two synthetic lethal drug target interactions
- The vascular endothelial growth factor (VEGFA) could be postulated as predictive marker for mTOR inhibition in HGSOC, hence serving as marker candidate for patient stratification
- A publication entitled "Vascular endothelial growth factor A as predictive marker for mTOR inhibition in relapsing high-grade serous ovarian cancer" was submitted to a systems biology journal
- A publication covering the systematic integration of computationally inferred and experimentally determined synthetic lethal interactions on the background of cancer drug targets was drafted analysing synthetic lethal interactions in the context of ovarian cancer.

Publications:

Peter Andorfer, Alexander Heuwieser, Andreas Heinzel, Arno Lukas, Bernd Mayer, Paul Perco. Vascular endothelial growth factor A as predictive marker for mTOR inhibition in relapsing high-grade serous ovarian cancer: under review at BMC Syst Biol (acknowledging financial support from OCTIPS)

Andreas Heinzel et al. Synthetic lethality guiding selection of drug combinations in ovarian cancer, in preparation.

A central repository for managing heterogeneous molecular profiling data, the OCTIPS-BASE repository, has been established, serving as source for integrative analysis.

OCTIPS background data have been integrated

A manual search for HGSOC-relevant Omics profiles using keywords combined with one of the terms genomics, metabolomics, proteomics or transcriptomics, resulted in a set of Omics studies in the context of ovarian cancer. To further limit the results only studies whose content specifically fits HGSOC regarding relapse topic and study target were taken into account. The list of Omics studies formed the basis for extracting deregulated features in the context of ovarian cancer.

OCTIPS Cross-omics data integration and analysis revealed new therapeutic targets and strategies

- Integrative Omics in WP5 embeds the following components (Figure 1).

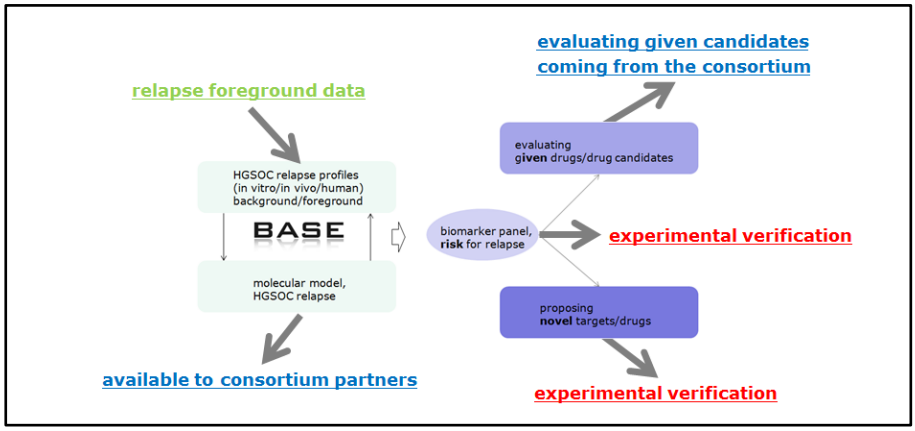


Figure 1: Central BASE holding background/consortium foreground data on relapse, being used for computing a molecular model representation of HGSOC relapse. This model is used for identification of biomarker candidates, further feeding evaluation of given therapy candidates/proposing novel candidates for experimental evaluation.

- Deriving a high-grade serous ovarian cancer resistance (HGSOCr) molecular model

We followed a cross-omics integration strategy for modeling molecular processes associated with HGSOC drug resistance. We concluded on a molecular model representation of drug resistant HGSOC, composed of 24 individual molecular processes embedding in total 409 protein coding genes:

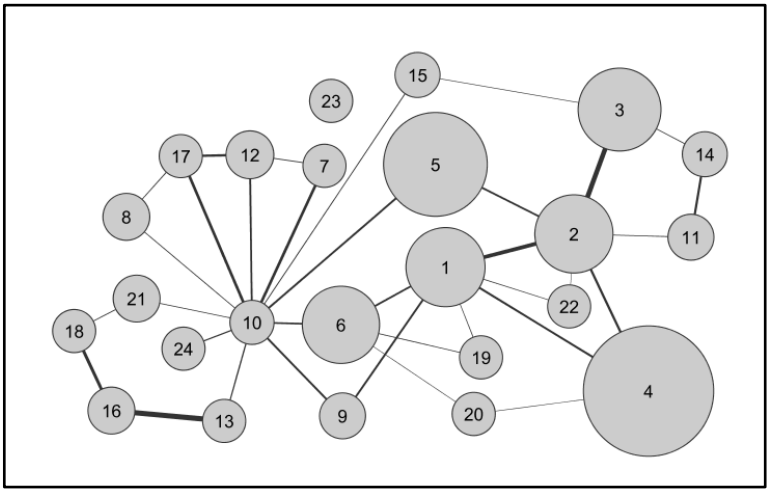


Figure 2. Schematic representation of the HGSOCr molecular model holding 409 proteins in 24 process units. Each node encodes a molecular process (node diameter scaling with number of protein coding genes embedded), edges resemble process dependencies.

The molecular model was used in order to identify molecular pathways of relevance in HGSOC resistance, evaluate interference with drug mechanism of actions, as well as to delineate marker candidates.

The following list of molecular pathways was identified being of high relevance in the context of HGSOC resistance:

Table 1: Relevant identified molecular pathways in the context of HGSOC resistance.

Pathway	# genes	estimate	p-value
TGF-beta signaling pathway	11	11.58	1.00E-05
mTOR signaling pathway	8	8.42	1.04E-04
ABC transporters	7	7.37	3.36E-04
Jak-STAT signaling pathway	9	9.47	0.00901
Neurotrophin signaling pathway	7	7.37	0.03265

Using a library of drug mechanism of action molecular models allowed us deriving hypotheses on drug interference for addressing resistance in HGSOC.
Significant interference with the drug mechanism of action of sirolimus, an mTOR inhibitor, was identified and VEGFA was identified as key molecule on the interference thus serving as predictive marker for mTOR inhibition.
Given analytics allowed us linking molecular mechanisms characterizing a drug resistant phenotype with an alternative drug mechanism of action, combined with a predictive biomarker candidate for patient molecular phenotyping.

HGSOC synthetic lethal hubs and drugs have been defined

The molecular model further served as basis for the identification of synthetic lethal interactions being addressable by drug combinations.

34 of the set of 102 drug targets in the molecular model were involved in 84 synthetic lethal interactions, addressed by 52 different compounds. We furthermore screened the indication fields for each drug in DrugBank to identify drugs currently being in clinical use in the field of oncology. Among the 52 compounds, 19 are currently approved as therapeutic option for at least one tumor type.

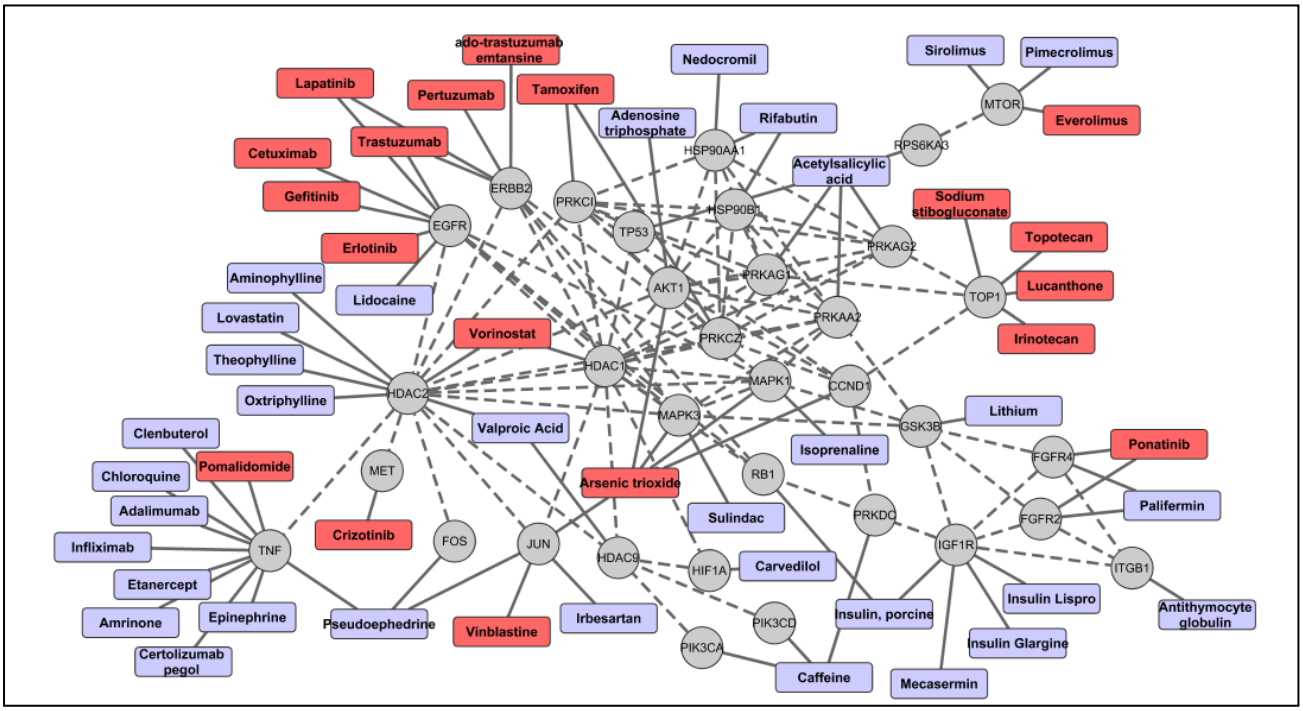


Figure 3: Graphical representation of synlet-target-drug interactions. Drugs already in clinical use in the field of oncology are highlighted in red. synlet edges are given by dotted lines, drug-target links are given by solid lines.
In summary, we (i) identified a set of molecular pathways of relevance in HGSOC resistance, (ii) proposed a drug interfering with such processes in combination with (iii) a predictive molecular marker. In addition, we (iv) proposed a number of drug combinations targeting synthetic lethal interactions linked to HGSOC resistance.

WP6: Clinical and therapy perspectives

Summary

During the OCTIPS project, three out of the four relevant clinical trials have been closed and evaluated, which results could be presented in the context of the OCTIPS plan. One trial is under the evaluation. A phase 1b study within the setting of platinum sensitive relapsed high grade serous ovarian cancer involving the clinical OCTIPs partners is almost complete, has established the dose of APR-246 to be used in combination with carboplatin and PLD, no new or surprising toxicities have been identified and encouraging levels of efficacy have been identified. These data have been presented at several international conferences (TAT 2014, AACR 2015, TAT 2015, ASCO 2015, ESGO 2015) and a manuscript is being prepared for submission to the Journal of Clinical Oncology. The phase II study is about to commence.

The main objectives of WP6 are:

- To demonstrate how validated molecular targets will be tested within the context of future OCTIPS
- clinical studies
- To supplement the data from WP3 and WP5 feeding into WP4 using biological materials collected from
- clinical trials, which are being performed by the participants and are near to completion
- To plan a phase II study relevant to OCTIPS results
- To plan future OCTIPS clinical trials using output from the whole project

Deliverables of the WP6:

- D6.1. Predictive AKTi biomarkers in conjunction with WP3 analysed (M36)
- D6.2. Efficacy readout of AKT inhibition utilising kits for PRAS40 and 4EBP1 in conjunction with WP3 analysed (M48)
- D6.3. Phase II study of sapacitabine in platinum-sensitive relapsed ovarian cancer closed (M48)
- D6.4. Define the molecular characteristics in the patient population of the EORTC 55041 and XL 184 trials (M48)
- D6.5. Correlate the molecular characteristics with response data (M48)
- D6.6. Effects of secondary BRCA1/2 mutations on the effect of primary therapy and of sapacitabine determined (M48)
- D6.7. Protocols for future phase I/II trials targeting the cell populations predominating at relapse completed (M48)

Milestones

- M6.1. Protocols for future clinical trials targeting the cell populations predominating at relapse (M48)

Publications

Cheraghchi-Bashi A, Parker CA, Curry E, Salazar JF, Gungor H, Saleem A, Cunnea P, Rama N, Salinas C, Mills GB, Morris SR, Kumar R, Gabra H, Stronach EA. A putative biomarker signature for clinically effective AKT inhibition: correlation of in vitro, in vivo and clinical data identifies the importance of modulation of the mTORC1 pathway. Oncotarget 6:41736-49, 2015

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chemotherapy in BRCA1/2 mutation carrier ovarian cancer in the setting of poly(ADP-ribose) polymerase inhibitor resistance: a multi-institutional study. Clin Cancer Res. 19:5485-93, 2013

Despierre E, Vergote I, Anderson R, Coens C, Katsaros D, Hirsch FR, Boeckx B, Varella-Garcia M, Ferrero A, Ray-Coquard I, Berns EM, Casado A, Lambrechts D, Jimeno A. Epidermal Growth Factor Receptor (EGFR) Pathway Biomarkers in the Randomized Phase III Trial of Erlotinib Versus Observation in Ovarian Cancer Patients with No Evidence of Disease Progression after First-Line Platinum-Based Chemotherapy. Target Oncol 10:583-96, 2015

Analysis in a clinical trial of PET guided dose findings of a GSK oral AKT inhibitor

The data shows that GSK2141795 inhibits AKT signalling in platinum-resistant ovarian cancer cells, that FDG-PET is an appropriate non-invasive pharmacodynamic marker and that patient response is predicted by pre-treatment measurement of an AKT pathway signature.

Patients enrolled in the Phase I clinical trial for GSK2141795 had biopsies taken prior to treatment and following 4 weeks (W4) of treatment with the drug. Lysates were prepared from the biopsies and profiled using RPPA as for the cell line and xenograft studies. The protein levels were evaluated in the paired biopsies for evidence of target inhibition by the AKT inhibitor and to examine the effect AKT inhibition has on signalling networks. Hierarchical clustering analysis indicate distinct changes in signalling behaviour following AKT inhibition in the patient group with clinical activity (CAS+), however in the CAS- group, very little effect is observed apart from changes in pAKT indicating drug activity but no downstream effects (Figure 1.). Total AKT and pAKT/total AKT ratio showed significant fold changes in expression levels in the post treatment biopsies compared to the baseline regardless of CA125 response, showing pharmacodynamic target engagement of GSK2141795.

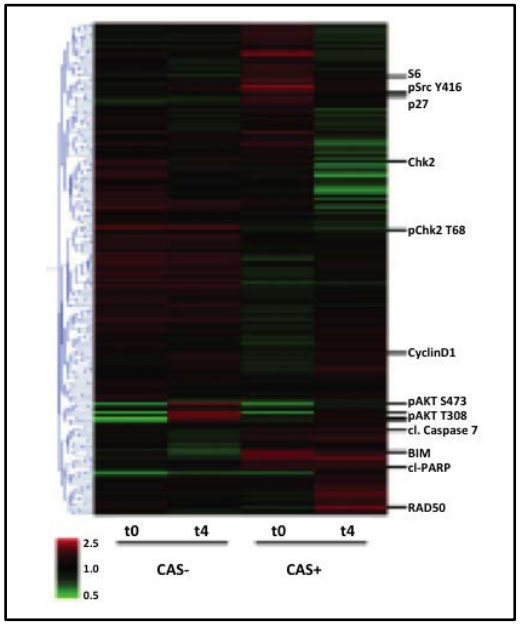

Figure 1. Genespring heatmap of protein expression detected in RPPA from pre-treatment and W4 on-treatment biopsies.

Genetic analyses in clinical materials collected from EORTC 55041

In the EORTC 55041 trial (NCT00263822), patients with ovarian cancer in remission after first-line chemotherapy were randomised to Erlotinib maintenance therapy versus placebo. Erlotinib inhibits epidermal growth factor (EGFR) which is overexpressed frequently in ovarian cancer. It is known that 32% of various solid tumour types had an EGFR amplification, 18% of these had focal amplifications and 12% carried high-level amplifications. Extracted DNA from paraffin embedded tissue of 318 patients included in the EORTC 55041 was available.

- EORTC 55041 trial (NCT00263822)

The EORTC-GCG 55041 trial is the first randomized phase III trial investigating the use of maintenance erlotinib in patients with ovarian, peritoneal or fallopian tube cancer. In this project, we investigated the status of EGFR and related pathways using immunohistochemistry, FISH, hotspot mutation analysis, and DNA sequencing to determine the frequency of these alterations in patients with ovarian cancer and to correlate these biomarker data with outcome and with the efficacy of erlotinib.
In contrast to mCRC and NSCLC, *EGFR* mutation did not predict responsiveness to erlotinib treatment, nor did gain-of-function mutations in the *EGFR* signaling cascades (*KRAS*, *BRAF*, *NRAS*, and *PIK3CA*). The limited frequency of mutated samples, however, prevents meaningful evaluation of clinical outcomes in relation to these mutations. However, when pooling all mutations together, patients with at least one mutation in either *KRAS*, *NRAS*, *BRAF*, *PIK3CA* or *EGFR* had a longer PFS (33.1 months) compared to those with wild-type tumors (12.3 months) (HR: 0.57; 95 % CI 0.33-0.99; *P* = 0.042).
Although the EGFR pathway appears to play an important role in ovarian cancer, particularly in tumor development, tumor cell survival, and metastasis, it is not yet clear how this pathway can be exploited to yield a therapeutic benefit.

Table 1: Correlation of biomarker results with overall survival (OS) and progression-free survival (PFS)

Biomarker	*n*	OS			PFS		
		Mos.	HR (95 % CI)	*P*	Mos.	HR (95 % CI)	*P*
KRAS							
Wild-type	309	NR			12.5		
Mutation	9	67.0	1.08 (0.43–2.69)	0.876	14.1	0.90 (0.42–1.91)	0.784
PIK3CA							
Wild-type	306	67.0			12.4		
Mutation	12	NR	0.53 (0.17–1.65)	0.262	NR	0.36 (0.15–0.86)	**0.017**
EGFR							
Wild-type	315	NR			12.9		
Mutation	3	37.9	1.80 (0.53–6.15)	0.345	4.01	2.09 (0.67–6.54)	0.195
KRAS, NRAS, BRAF, PIK3CA or EGFR							
Wild-type	294	NR			12.3		
Mutation	24	67.0	0.75 (0.38–1.49)	0.413	33.1	0.57 (0.33–0.99)	**0.042**

The limited frequency of mutated samples in this analysis prevents a meaningful evaluation of clinical outcomes in relation to individual mutations. As such, patients with at least one mutation in the EGFR signalling cascades were pooled. Patients with at least one mutation in KRAS, NRAS, BRAF, PIK3CA, or EGFR had longer PFS (33.1 versus 12.3 months, mean difference = 20.8 months, P = 0,042) compared to those with wild-type tumors (see figure 2.), but among the former, there was no significant benefit of erlotinib over observation (see figure 3). No significant difference in OS was seen when all mutations were pooled.

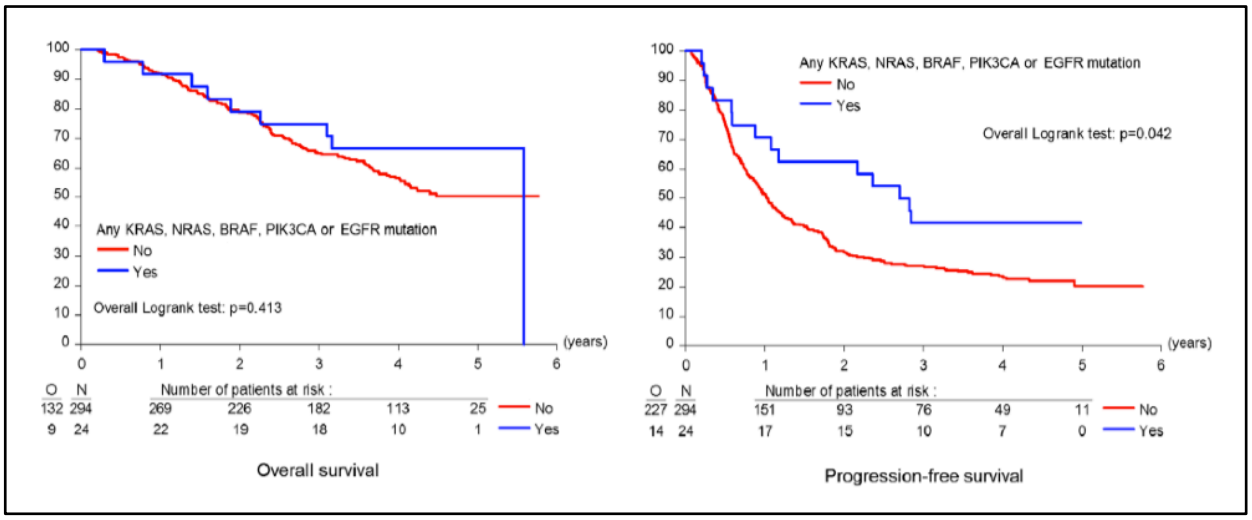

Figure 2: Kaplan-Meier curves for overall survival and progression-free survival, according to presence of mutations.

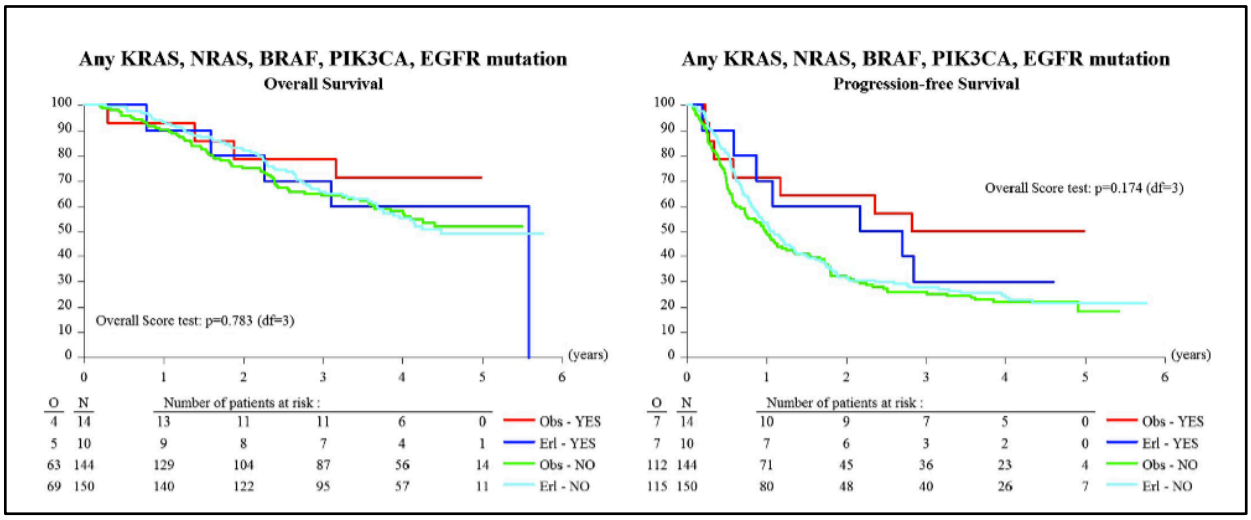

Figure 3: Kaplan-Meier curves for overall survival and progression-free survival, according to treatment allocation. Data were analysed according to presence of mutations.

Approaches in a sapacitabine clinical trial

Sapacitabine is an orally available 2'-deoxycytidine analogue which is converted to the active agent CNDAC in vivo. It has a low level of toxicity using a dosing schedule which has already been established in acute myeloid leukaemia (where the agent has entered phase III testing). CNDAC is incorporated into DNA producing breaks which cancer cells have varying abilities to repair. Preclinical data has shown that reducing homologous recombination repair capacity increases cancer cell sensitivity to sapacitabine up to 200-fold. In addition, using the PEO1/PEO4 matched ovarian cancer cell line pair described in WP4, it has been shown that the PEO1 cells (BRCA2 deficient) are over 10 times more sensitive to CNDAC than the PEO4 cells (BRCA2 functional). As such we plan to test sapacitabine in asymptomatically relapsing ovarian cancer patients (relapse defined by CA125/GCIG criteria). Samples of tumour will be obtained prior to and following therapy. These will be analysed for the presence of secondary mutations and the presence of these mutations correlated with clinical response.

The study investigating sequential treatment with sapacitabine and the CDK2 & 9 inhibitor seliciclib performed by P11 suggested that efficacy was evident in BRCA-deficient tumours of the breast, ovary and pancreas (figure 4). There were only two evaluable patients who had received prior PARP inhibitor therapy in this component of the

study and therefore a conclusion could not be drawn regarding efficacy in patients who had previously received PARP inhibitor treatment.

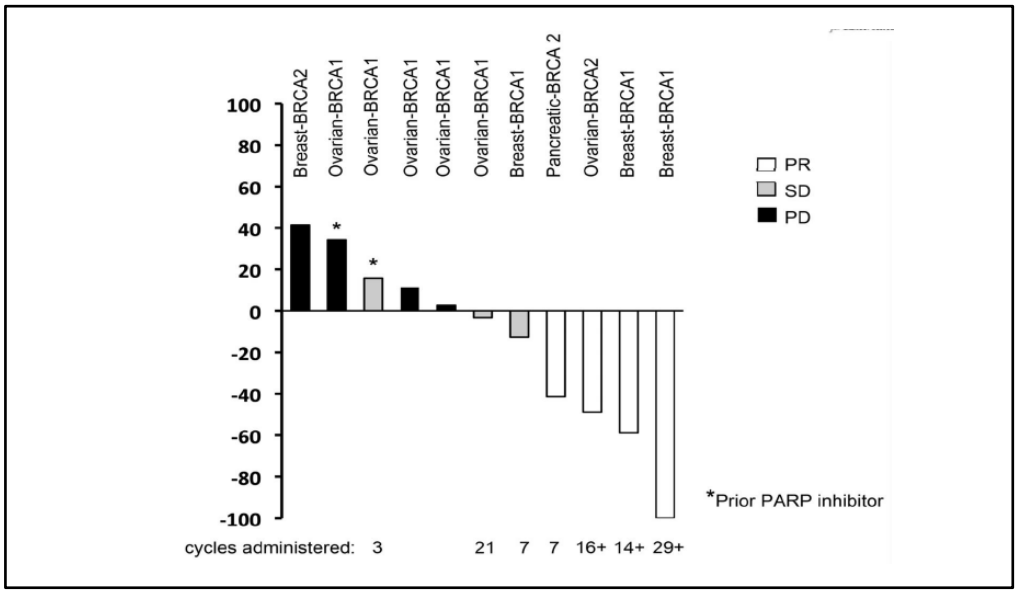

Figure 4. Change in size of tumour lesions in individual patients following sequential administration of sapacitabine and seliciclib. *denotes prior PARP inhibitor. (Shapiro et al, AACR 2013).

Due to the development of PARP inhibitors, such as olaparib which has recently been licensed in ovarian cancer, it was considered appropriate to further assess the impact of prior PARPi treatment on sapacitabine response in this patient population in this continuing study.

Planning of the future OCTIPS studies using the output from the whole project

Partners 3, 4 and 5 have collaborated to perform the PISARRO phase I/II study of the novel agent APR-246 in combination with carboplatin and pegylated liposomal doxorubicin (PLD) in the setting of platinum sensitive relapse of p53 mutant high grade serous ovarian cancer. APR-246 is an agent which stabilizes mutant p53 in the wild-type conformation, increasing the sensitivity of p53 mutant tumour cells to cytotoxic agents. This fits well within the remit of OCTIPS as the aim is to demonstrate the ability of the novel agent (APR-246) to kill otherwise chemoresistant cells within a patient population that is deemed platinum sensitive according to standard clinical criteria. This has to be demonstrated in the relapsed disease setting but if the trials are positive then there is an excellent case for testing the agent in the first line setting in order to increase the chance of clearing all disease and achieving cure.
The ongoing phase Ib/II study has enrolled patients with recurrent partially platinum sensitive (platinum free interval 6-12 months) and platinum sensitive (platinum free interval 12-24 months) HGSOC with positive p53 staining on immunohistochemistry. APR-246 was administered as a 6 hour intravenous infusion on four consecutive days every 4 weeks for 6 cycles. On day 4, APR-246 was given concomitantly with carboplatin AUC 5 and PLD 30 mg/m2. The Phase Ib component has a 3+3 dose escalation design with three planned dose levels (figure 5.) based on safety evaluation after cycle one for dose escalation.

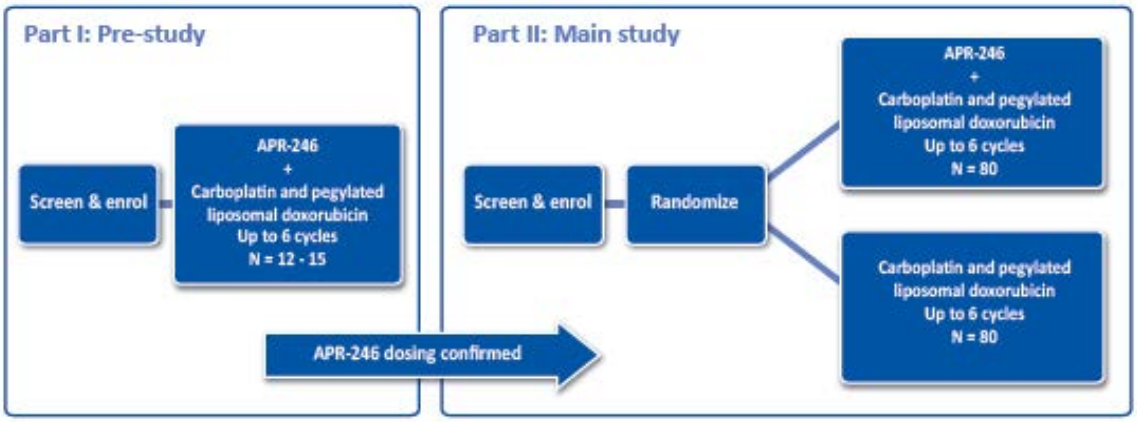

Figure 5. Design of the PISARRO phase Ib/II study.