# PROJECT FINAL REPORT

**Grant Agreement number:** 602783

**Project acronym:** CAM-PaC

**Project title:** “Integrative Analysis of Gene functions in Cellular and Animal Models of Pancreatic Cancer”

**Funding Scheme:** FP7 - HEALTH.2013.2.1.1-1

**Period covered:** from 01/11/2013 – 31/10/2018

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

Prof. Dr. Thomas Gress, Philipps Universitaet Marburg

**Tel:** +49 (0) 6421 58 66459

**Fax:** +49 (0) 6421 58 68922

**E-mail:** gress@med.uni-marburg.de

**Project website address:** www.cam-pac.eu

**Table of contents**

**1 An executive summary** ..... **3**

**2 A summary description of project context and objectives** ..... **4**

**3 A description of the main S&T results/foregrounds** ..... **6**

3.1 Summary of the main results/foreground of CAM-PaC ..... 6

3.2 Main results/foreground of the different work packages ..... 8

WP1: Novel genetically engineered mouse models of pancreatic cancer ..... 8

WP2: In vitro functional characterisation of pancreatic cancer target genes ..... 10

WP3: Definition of pancreatic cancer stem cell target genes ..... 11

WP4: Visualisation of tumour progression, therapy response and resistance phenotyping in GEMM ..... 12

WP5: High throughput culturing techniques for pancreatic cancer cells and CSCs ..... 14

WP6: Xenograft models for in vivo validation of candidate gene function ..... 16

WP7: Histopathologic and molecular characterisation of human primary tissues and model systems ..... 17

WP8: Large scale metabolic profiling of in vitro and in vivo models of pancreatic cancer ..... 20

WP9: Large scale transcriptomic profiling of in vitro and in vivo models of pancreatic cancer ..... 23

WP10: Data integration and pathway modelling ..... 23

WP11: Dissemination ..... 25

WP12: Project Management ..... 26

**4 The potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and exploitation of results** ..... **26**

4.1 Socio-economic impact and the wider societal implications of CAM-PaC ..... 26

4.2 The main dissemination activities of CAM-PaC ..... 28

4.3 Exploitation of results of CAM-PaC ..... 32

**5 The address of the project public website, if applicable as well as relevant contact details** ..... **33**

**Final publishable summary report**

## 1 An executive summary

Pancreatic ductal adenocarcinoma (PDAC) is the 4th to 5th most common cause of cancer related deaths in the Western world and is virtually resistant to any conventional therapeutic regimens. It is thus a health problem with a major socioeconomic impact for any society. Despite enormous advances in the identification of molecular changes associated with the disease, new treatment options have not emerged. Thus, 5-year survival rates remain unchanged at a dismal of 6%, the lowest for all solid tumours.The overall aim of CAM-PaC was to use a strongly SME-driven approach to contribute to solving the socioeconomic and health challenges of PDAC by an integrative and systematic functional analysis of pancreatic cancer candidate genes pre-selected and pre-characterised by members of the consortium in previous and ongoing high-throughput "omics" approaches. The scientific goals of the project were structured into 4 key objectives of research and implementation:

- **Key Objective 1: Creation of a portfolio of new and validated therapeutic targets associated with human disease by use of various cellular and animal models (WPs 2 & 3):**
  The program was designed to generate and use animal and cellular models to systematically analyze functions of genes and gene products in order to attain a better understanding of the disease, furnish a portfolio of new and validated therapeutic targets, compounds and therapeutic strategies for PDAC and serve as the basis for a translation into clinical applications.
  Detailed in vitro and in vivo analyses of a large number of candidate genes have been performed, resulting in several high-ranking publications. A pre-clinical trial with an inhibitor against candidate gene TTK (WP2) has been started and is ongoing; moreover, two clinical trials with agents targeting specific metabolic characteristics of cancer stem cells (WP3) have been initiated. In addition, small-molecule screening for new inhibitors of selected target genes has been performed or is ongoing.
- **Key Objective 2: Development of efficient, standardised and reliable tools, standardised operating procedures (SOPs ) and technologies for phenotyping (WPs 1-7 ):**
  A central goal of this project was the implementation of novel technologies for temporal and spatial control of transgene expression in GEMM (WP1) allowing for tissue-specific expression and control of target genes independently and in a reversible manner. A first novel system for transgene control through Erythromycin-responsive elements has been established in vivo; mice carrying this novel system have been generated and are currently being analyzed. Further, a standardised collection of patient-derived xenografts (WP6) as well as advanced methods for in vitro culture of single cell-derived clones and defined co-culture (WP5) have been established and used for coordinated phenotyping analysis. New technologies for non-invasive in vivo functional imaging (WP4), including advanced functional MRI techniques, have been developed in animal models and successfully transferred to clinical practice for human patients.
- **Key Objective 3: Large-scale histopathological, metabolic and molecular phenotyping in model organisms and in vitro model systems (WPs 4, 5, 6, 7, 8 & 9):**
  Comprehensive phenotyping is quintessential to take full advantage of the newly developed model systems as well as to validate their relevance for the human situation. To ensure standardisation, harmonisation and common ontology across different sites in the consortium, expert molecular pancreatic pathologists provided histopathological and molecular characterisation as well as SOPs for handling and exchange of materials from primary tumour xenografts and genetically engineered mouse models (WP7).
  Novel workflows for next-generation sequencing-based analysis as well as metabolic profiling from extremely small sample sizes have been developed within WP9 & WP8, respectively, and used to systematically analyse samples from the different model systems. Among others, histopathological and molecular evaluation of primary tumour tissues and their corresponding xenografts (WP7) has been combined with in vivo chemosensitivity data (WP6), metabolome profiles and functional data from organotypic cultures (WP8), 3D growth assays (WP5) and next-generation sequencing data (WP9) from the same cells.
- **Key Objective 4: Large scale data integration (WP10):**
  The CAM-PaC project has generated large amounts of diverse types of data on molecular and phenotypical traits of primary human tissues and in vitro and in vivo model systems of pancreatic cancer. Developing novel methods to utilise the knowledge that is generated on different levels and formulating scientific hypotheses from the comprehensive analysis of these data was the major aim of WP10. To this end, the necessary infrastructure as well as methods for integrating heterogeneous data from different sources and methodological approaches have been established and standardized tools made available to the consortium. Mathematical models of protein and pathway interactions have been established, and functional predictions derived from this model experimentally validated within WP2.

Overall, the work performed in CAM-PaC has resulted in ~90 publications in peer-reviewed journals as well as one patent already issued and several further patent applications in submission or preparation.

## 2 A summary description of project context and objectives

**Background:**

Pancreatic ductal adenocarcinoma (PDAC) is the 4th to 5th most common cause of cancer related deaths in the western world and is virtually resistant to any conventional therapeutic regimens. It is thus a health problem with a major socioeconomic impact for any society. Despite enormous advances in the identification of molecular changes associated with the disease, new treatment options have not emerged. Thus, 5-year survival rates remain unchanged at a dismal 6%, the lowest for all solid tumours.

At their core, cancers are genetic diseases arising from the accumulation of multiple molecular alterations in affected cells. For many human malignancies, large-scale genomic, transcriptomic and, to a somewhat lesser degree, proteomic analyses have been instrumental in establishing comprehensive catalogues of molecules that are altered in their structure and/or abundance in malignant tumours. Far less developed are concepts and methods to integrate data from different sources and to directly interrogate gene functions on a large scale in order to differentiate "driver" alterations, which directly contribute to malignant transformation and tumour progression, from "passenger" alterations, which have minimal or no influence on tumour biology. As a consequence, examples of successful translation of knowledge generated from "omics" approaches into novel clinical concepts and applications are few and scarce.

**Aim:**

The overall aim of CAM-PaC was thus to use a strongly SME-driven approach to contribute to solving the socioeconomic and health challenges of PDAC by an integrative and systematic functional analysis of pancreatic cancer candidate genes pre-selected and pre-characterised by members of the consortium in previous and ongoing HT-omics approaches. Therefore, the project was to develop novel cellular and animal models, as well as novel strategies to analyse and integrate large scale metabolic, transcriptomic and genetic data from these models, in order to systematically identify, characterise and validate novel targets for therapeutic intervention and bioinfomatic models for predictive diagnostics. In addition to the general tumour cell population, special consideration was to be given to sub-populations of tumour-initiating cells, a.k.a. tumour stem cells, which are of paramount importance for tumourigenicity, therapeutic resistance, metastasis and relapse, and are thus important to target in any novel treatment. In general, novel treatments based on single targets were considered to only be of limited impact to personalise PDAC treatment, since accumulating evidence indicates that PDAC is characterised by a marked genetic heterogeneity. Thus, integrative approaches for the discovery and validation of core signalling drivers and resistance mechanisms were deemed to better define individualised treatment strategies, which take into account the heterogeneity of PDAC subtypes.

**Objectives and Methods:**

- **Key Objective 1: Creation of a portfolio of new and validated therapeutic targets associated with human disease by use of various cellular and animal models (WP2 & WP3)**

  The programme was designed to generate and use various animal and cellular models to systematically discover and ascribe functions of genes and gene products identified in genome wide analyses.

  Partners in CAM-PaC had previously contributed to generating the largest collections of PDAC candidate genes in recent transpriptomic analyses, in parallelised cellular assays using reverse transfection technology, in genome wide synthetic lethality screens using siRNA and shRNA libraries as well as through PDAC genome sequencing approaches. Using deep RNA sequencing, CAM-PaC participants had identified additional candidate genes/pathways that are enriched in pancreatic CSC, including genes involved in "stemness", self-renewal, proliferation, invasiveness and metabolism. These candidate genes were to be subjected to systematic functional characterisation by use of cellular and animal models developed in the consortium to provide a portfolio of validated therapeutic targets as the basis for clinical translation. For the most promising candidate genes, CAM-PaC was to perform a limited number of HT-screens with compound libraries and characterise and validate the novel compounds identified therein.

- **Key Objective 2: Development of efficient, standardised and reliable tools, standardised operating procedures and technologies for phenotyping (WPs 1-7)**

  CAM-PaC was to generate in vitro and in vivo models for systematic functional characterisation of target genes that will also be of enormous value for the scientific community and pharmaceutical industry as models for cross-sectional and longitudinal studies of carcinogenesis in the pancreas, and to test or validate predictive diagnostic and therapeutic approaches.

  A central goal was to implement and validate novel technologies for temporal and spatial control of transgene expression in GEMM (WP1) allowing for tissue-specific expression and control of up to four target genes independently and in a fully reversible manner. Using, among others, established and newly developed cutting-edge technologies for in vivo functional

imaging (WP4), these systems were to be applied to the analysis of known oncogenes in pancreatic cancer, e.g. to assess questions of oncogene addiction, as well as to the analysis of novel target genes selected within the course of the project. WP6 was to employ patient-derived xenografts in pre-clinical therapeutic trials to address the exceptionally high inter- and intra- tumour heterogeneity in human PDAC and to identify novel determinants of therapy resistance in individual tumours. In addition, the relevance of expression of target genes identified in WPs 2 and 3 (see below) for tumourigenicity and therapy resistance of PDAC and cancer stem cell (CSC) lines in vivo was to be assessed.
Cell based in vitro model systems facilitate the characterisation of novel gene functions in a high throughput manner and allow to dissect cell type-specific molecular mechanisms in great detail. WP2 was to comprehensively analyse the functional roles of pre-selected target genes in PDAC cells as well as in pro-tumourigenic interactions between cancer cells and specialised stromal cells (pancreatic stellate cells, PSC). WP3 aimed at comprehensively characterising genes that mediate specific phenotypes in cancer stem cells (self-renewal, invasiveness, metabolism). Instrumental in these analyses were the development of novel cell-culture methodologies (WP5) based on different technological platforms, which provided an automated system of single- and multi-cell culture under non-adherent conditions in a highly controlled environment.

- **Key Objective 3: Large-scale histopathological, metabolic and molecular phenotyping in model organisms and in vitro model systems (WP4, WP7, WP8 &WP9)**
  Comprehensive phenotyping is quintessential to take full advantage of newly developed model systems as well as to validate their relevance for the human situation. To ensure standardisation, harmonisation and common ontology across different sites in the consortium, in WP7 expert molecular pancreatic pathologists were to provide histopathological and molecular characterisation as well as SOPs for handling and exchange of materials from primary tumour xenografts and genetically engineered mouse models. Among others, this regarded to be a basis for the validation of novel functional imaging technologies developed within WP4 for phenotypic characterisation of in vivo models. Novel workflows for next-generation sequencing-based analysis of extremely small sample sizes were to be developed within WP9 to facilitate large-scale transcriptomic profiling of in vitro and in vivo models. Finally, metabolic profiling performed within WP8 was expected to for the first time provide a comprehensive analysis of energy metabolism and metabolite-mediated paracrine signalling in primary pancreatic cancer and model systems

- **Key Objective 4: Large scale data integration (WP10)**
  The CAM-PaC project was expected to generate large amounts of diverse types of data on molecular and phenotypical traits of primary human tissues and in vitro and in vivo model systems of pancreatic cancer. Developing novel methods to utilise the knowledge that generated on different levels and formulating scientific hypotheses from the comprehensive analysis of these data was the major aim of WP10. This work package aimed at integrating, standardising and interpreting the molecular and phenotypical data gained within the consortium. Apart from storing, pre-processing and analysing the data, network models of the processes associated with progress and therapy resistance of pancreatic cancer were to be developed.
  Thereby, WP10 was expected to i) provide the platform for data integration and exchange to the project partners, ii) maintain the data in publically accessible web portals, iii) contribute to the identification of new and validated therapeutic targets in PDAC in collaboration with the partners, and iv) provide in-silico models for mechanisms of tumour progression and therapy resistance of PDAC.
  Targets and models were to be iteratively validated in the “wet-lab” CAM-PaC WPs using the cellular and animal models generated therein. Thereby, CAM-PaC was expected to contribute validated predictive models that may be characteristic for PDAC patients with different response patterns to therapy as well as new therapeutic targets for clinical translation.

- **Key Objective 5: Fostering the competitiveness of European SME in this market area (all WPs)**
  CAM-PaC was an SME-targeted consortium that included four research-intensive biotechnology SME and one SME for management and coordination. The technological expertise of the SME was to be combined with the expertise of clinical and academic partners with extensive experience in the management of pancreatic cancer patients, pancreatic cancer research, HT-analyses on multiple levels of the pancreatic cancer genome, data analysis and systems biology. We expected this integrative research programme to result in the development of novel models, tools and technologies with broad applicability to target gene-driven biomolecular research. The participating SMEs (EPO, MPS, CeGaT, PolyGene) and applied research institutes (IBA) were expected to directly profit from these innovations by development of new processes and applications and market-ready novel technologies and/or prototypes in order to broaden company portfolios. CAM-PaC would thus provide the SME with the possibility to develop and expand their portfolios of methods, applications and assays useful for academia and pharmaceutical industry in a clinically meaningful context where the patients’ need is

compelling. CAM-PaC included the end-users of the tools and technologies to be developed and thereby addressed one of the major needs of technology-focused SME`s. It was anticipated that CAM-PaC would improve the competitiveness of participating SME on the European and International market.

- **Dissemination:**
  Dissemination was to be achieved by:
  - reaching out to the scientific community, industry, patient organisations and other interested or potential stakeholders
  - disseminating results to the scientific community in the academic, healthcare and pharmaceutical sectors
  - fostering interaction and exchange with the scientific community and the public
  - identifying and valorising intellectual property rights generated in CAM-PaC
  - exploring possibilities to conduct clinical trials to overcome therapy resistance in pancreatic cancer

- **Impact:**
  The effort in CAM-PaC was expected to contribute to developing new technologies, new drugs, as well as new therapies in a clinically highly relevant area and thus contribute to economic goals in the health sector, while improving the quality of life of people in Europe and around the world.
  In particular, CAM-PaC was planned to:
  1. strengthen European SME and fostering private-public partnership through transfer of clinically relevant expertise and knowledge;
  2. provide novel experimental models reflecting the human situation for a rapid validation and characterisation of preselected drug targets;
  3. identify novel therapeutic strategies and targets to overcome drug resistance in PDAC patients;
  4. provide a portfolio of validated target genes and compounds derived from Omics-analyses.

# 3 A description of the main S&T results/foregrounds

## 3.1 Summary of the main results/foreground of CAM-PaC

Summary of the main results/foreground of the scientific work packages (WP1-10) in CAM-PaC:

WP1:
- New animal models, including mouse models that use regulated expression with the newly developed systems for regulated expression of candiadte or reporter genes such as EGFP or luciferase, Itgb6, Lrh-1, and RPIA, but also models with a conditional knockout of candidate gene Kdm5b, a Flp-controlled p53 mutant model, an Msi2-specific delete/indicator model, or knock-in models, were produced and delivered to the consortium partners.
- A novel functional paradigm based on erythromycin regulation, as well as a RU486-dependent gene regulation system were developed and implemented and led to an ICT patent application currently under evaluation.
- The RU486 system was applied to build an animal model for controlled modulation of the native, resident RPIA gene.

WP2:
- Novel Target genes were identified based on cell-based functional screens
- Sets of different target genes were selected for in-depth characterization. Detailed results have been obtained for the genes TTK, ADRBK1, PLAC8 and USP5 and the results published
- Based on functional analyses (UniMar) and RNASeq data (CeGaT), UULM developed mathematical models of signalling cascades involving the novel target genes; these were in turn experimentally validated by UniMar

WP3:
- Using in vivo functional taxonomy, three key regulators in pancreatic cancer stem cells identified – stemness signalling, metabolism and immune privilege
- Based on these data, actionable multimodal treatment strategies were developed
- New opportunities for pharmacological intervention were identified, and novel inhibitors are now undergoing further characterisation and optimization

WP4:

- GEMM with different genetic alterations that contribute to the development, progression and overall phenotype of PDAC were generated and characterised in translational multimodal imaging approaches
- Using T2w-MRI, we established protocols that allow precise detection of tumour onset and the growth of the progressing tumours.
- Diffusion-weighted (DW) MRI was intensively characterized for imaging of tumour cellularity in GEMM-based PDAC and delivered non-invasively very valuable information of the histological appearance and present intratumoural heterogeneity.
- GEMM were used to investigate matrix- assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS) as a tool to study drug delivery and spatial tissue distribution in PDAC.

WP5:

- A new cultivation platform was established, providing versatile 3D cultivation functionality in miniaturized format
- Methods to analyse the 3D cultures phenotypically within microscopical monitoring or with cell-based viability assays (luminometric) were established
- Protocols were established to study the several phases of carcinogenesis like initiation (clonal sphere formation starting with single cells), tumour promotion (proliferation analyses / growth kinetics), tumour progression (tumour - stroma-interactions) and chemo response (substance screening assay) without switching the cultivation platform.
- The newly established platforms were employed in experiments to generate functional data of a plethora of different cell lines with different genetic background and modifications.

WP6:

- PDAC tumour tissues, cell lines and CSCs were transplanted into immunodeficient mice to generate in vivo models for functional analyses
- A panel of cohorts derived from 14 patient-derived xenografts as well as of 6 circulating tumour stem cell derived models was systematically exposed to a clinically relevant panel of anti-tumoural drugs
- Exemplary data of tumour recurrence after stop of treatment as well as repetition experiments completed the characterization and provided meaningful information about tumourigenicity and treatment resistance of the models.
- “Clinical” data as well as biological sample material from these animals were distributed to project partners for molecular analyses and bioinformatic modeling

WP7:

- Six custom panels were created based on the information produced from exome and whole genome sequencing of PDAC primary samples within the International Cancer Genome Consortium
- PDAC xenografted samples (PDX) were produced from primary PDAC and both PDX and PDAC samples were sequenced with these panels (total 234 cases)
- A selection of these PDX were sent to partners involved in in-vivo testing of tumourigenicity and chemo resistance of PDAC and cancer-stem-cell CSC cell lines with altered expression of selected target genes
- Transgenic mouse models samples from consortium partners were evaluated and comprehensively morphologically characterized

WP8:

- To perform large-scale metabolic profiling of in vitro and in vivo models of pancreatic cancer, targeted LC-MS methods were developed to cover different classes of metabolites from major metabolic pathways consisting of polar lipids, oxylipins, central carbon metabolites, and amino acids and amines.
- Methods were developed to culture human tumour derived pancreatic organoids and stroma cells both in mono- and co-culture together with appropriate controls in the Organoplate platform.
- Medium of these cultures was analysed to determine the metabolic signatures of the interstitial fluid as well as to achieve a reference point for all different pancreatic cancer models
- For a better sensitivity for peak detection due to sample limitations, a miniaturized amine platform consisting of amino acids and biogenic amines was developed
- In vitro models were subjected to similar drug treatments as the in vivo models and subjected to metabolic profiling to compare and evaluate the drug effects

WP9:
- Differential transcriptomes of modulated cancer cells, clonal sphere cultures and sequenced xenograft samples or cells from treated mouse models were generated
- Methods for RNA isolation and sequencing library preparation for very limited sample material were developed.
- A standardised protocol for RNA panel sequencing was established
- Sequencing data which was generated during the project was interpreted to create a complete list of 41 genes suitable for use in an analytical pancreatic cancer RNA enrichment panel

WP10:
- New approaches for static modelling were developed and successfully applied them to the consortium's data
- A Boolean network model to hypothesise about the details of molecular dysregulation of cofilin-1 in pancreatic cancer was created
- To reduce the computational cost of modelling, we developed a new tool for modelling, visualization, and simulation of Boolean networks (ViSiBooL)
- We extended the basic ViSiBooL framework with advanced functionality for in-silico experiments to screen for potential drug targets. This "drug-simulator" was applied for our newly developed Boolean network model describing the dysregulation of cofilin-1 in pancreatic cancer to screen for possible druggable targets.

## 3.2 Main results/foreground of the different work packages

### WP1: Novel genetically engineered mouse models of pancreatic cancer

**Partners involved:** PolyGene, UniMar, UNIVR, CeGaT, Univ Leiden, QMUL, DKFZ
**Total WP progress** 100%

#### Background

The development of transgenic mouse models of pancreatic cancer has been instrumental in elucidating mechanisms of human pancreatic ductal adenocarcinoma (PDAC) progression as well as studying interactions of tumour cells with the tumour microenvironment. In these models, oncogenic mutated K-Ras alone or in combination with mutationally inactivated p53 is expressed through activation of the Cre recombinase under the control of the pancreas-specific promoter *Ptf1a* or *Pdx-1*. These mice show a progressive accumulation of precursor lesions and development of invasive tumours in the pancreas, which very closely mimic the histological and molecular features of PDAC. Subsequently, a plethora of models with additional genetic alterations and various phenotypes have been described which faithfully recapitulate a wide spectrum of histological phenotypes also encountered in human pancreatic tumours.

However, these models have a number of important **limitations**: 1) both promoters are **active at early embryonic stages**, so that the genetic alterations are already present in the developing pancreas. It has since been demonstrated that the dynamics of tumour formation are dramatically different when the mutations are activated in the adult pancreas. 2) Both promoters are active in embryonic pancreatic **precursor cells**, making it impossible to definitively identify the cell type of origin of cancer progression. 3) Due to the mechanism of transgene activation, activation of the transgenes **cannot be reversed**, making it impossible to address questions of **oncogene addiction** or the importance of target gene alteration in early vs. late tumour progression. 4) Due to the use of a single recombination system, genes cannot independently be targeted and are thus activated or inactivated simultaneously, in contrast to the sequential alteration in the human disease. Consequently, within **WP1** of this collaborative project, we set out to implement **novel and cutting-edge technologies for temporal and spatial control of transgene expression** in genetically engineered mice.

Partner PolyGene has previously developed the technological basis for controlling expression of transgenes using novel gene control systems on the basis of bacterial antibiotic resistance genes (Erythromycin/E-REX; Pristinamycin/Pip), antibacterial plant metabolites (Phloretin/PEACE), and hormones (Mifepristone/RuX). These paradigms have been functionally validated in tissue culture. Implementing these systems in transgenic mouse models in vivo for the first time allows for **tissue-specific expression and control of up to four target genes independently** and in a **fully reversible** manner, thus revolutionizing the study of gene functions in different temporo-spacial contexts and combinations in vivo. However, while these systems show a reliable functionality in a broad variety of cell culture applications, adaptations were necessary to implement them for the use in mice. In particular, the antibiotic systems were to be adapted to act in a reverse manner (ON-type, activated upon addition of drug), for better applicability in live setups.

**Overall objectives**

Sophisticated metabolic engineering and antibiotic-responsive expression concepts, such as the Tet-system, have enabled elucidation of gene–function relationships in many occasions. PolyGene adapted and developed alternative gene expression systems (E-Rex, Pip, PEACE, RuX). These systems become reversibly switched on/off depending on the availability of drugs such as erythromycin, pristinamycin, phloretin, or mifepristone within the cells. The various systems do not cross-react with each other and can therefore be used within the same organism, within the same cell, to switch on or off multiple genes at will, thus avoiding the limitations of recombinase-based systems. To further enhance their applicability, a main objective of WP1 was to alter the members of the Tet family (E-REX, Pip, PEACE) along the paradigm of rTet, changing the specificity of these molecules to bind to DNA upon drug addition, rather than drug removal (ON-type regulation).

The objectives of this WP were thus:

1. To develop, apply and evaluate reverse erythromycin (rE-Rex)/ pristinamycin (rPip) and phloretin expression system (rPEACE) for the reversible switch on/off of gene expression in vivo.
2. To use these novel systems of highly flexible temporo-spacial transgene control to generate GEMM allowing to re-assess and expand knowledge on the roles of known oncogenes and tumour suppressor genes in pancreatic cancer (Kras, p53, p16) in different phases of tumour initiation and progression.
3. To generate novel GEMM with targeted activation/inactivation of novel PDAC/CSC candidate genes identified in WPs 2 and 3

In addition to providing highly relevant information on the functional roles of oncogenes and tumour suppressor genes in various stages (development, maintenance, progression) of pancreatic cancer, the tasks of WP1 also included the development of efficient and reliable tools and technologies for in vivo phenotyping with broad applicability in many areas of biological or medical research. The intended goal was to generate a total number of 20 mouse models, to be made available to various groups and tasks of the consortium, using the mentioned regulation mechanisms, or independent of them.

**Results**

The efforts in WP1 yielded the initially intended number of animal models, amongst which the group of mouse lines that combine into the controlled regulation of K-RAS and p53, mouse models that use regulated expression with the freshly developed paradigms for test expression of EGFP or luciferase, suppression of Itgb6, Lrh-1, controlled modulation of RPIA, but also models along more traditional lines of design, such as a conditional knockout of Kdm5b, a Flp-controlled p53 mutant model, an Msi2-specific delete/indicator model, or knock-ins of plac8, Wnt5a, and Pglyrp1, or a Cre-regulator under Col11a1 expression as a knock-in model. Most of these models were delivered in time or ahead of time according to the consortium's plans, while the last two models defined late in the project, albeit mostly completed, will be shipped to the corresponding partners outside of the consortium's runtime.

The large goal of achieving ON-type regulation systems yielded a functional paradigm based on erythromycin regulation, while a second one (pristinamycin-based) failed for pharmacological reasons (the drug was ineffective in vivo), and a third paradigm, based on phloretin regulation, for which reverse clones could not be obtained, but proper validation criteria and testing systems established. A forth paradigm, RU486-based, glucocorticoid receptor-dependent regulation of genes was also developed and implemented. These efforts were largely successful and led to an ICT patent application currently under evaluation.

Thus, the initial goals of task 1 in WP1 were fully achieved. The two effective systems were applied to mouse models (Task 2), animal lines obtained and established – including the RAS2/p53 double-regulated model, as well as testing strains for establishing induction conditions – and the animals delivered to our partners. The combined mouse models can now be assayed. The tester strains unfortunately express weakly, and the tests were to this point inconclusive. However, the models were extensively tested in tissue culture setups and we believe that the oncogene mouse will express as expected. The models cannot be quickly brought to the point of using them in the final way, because of a complicated breeding scheme required for combining all genotypes into the final model. Thus, these final results are not yet available.

In a most promising approach, the RU486 system was applied to build an animal model for controlled modulation of the native, resident RPIA gene; a pilot project for another gene using the same technology was already successful. The ease of the model design – a CRISPR-based insertion of a small operator tag – allowed us to generate this model in a most rapid fashion. These mice are to be shipped as soon as possible, and assayed at partners' institutions. Detailed analyses will be performed in follow-up projects to the CAM-PaC project.

**Conclusion**

The complexity of the CAM-PaC tasks did not allow for a complete full list – rather an estimate – of the animal models required for the program's goals; nevertheless, all animal models intended and defined were built, and the number of initially planned additional animal models achieved.

The plan of reverting regulator molecules of the Tet family and implementing them into complex models that are in vivo applicable, was enormously ambitious. We are therefore extremely satisfied with the success of some of these large efforts - in the course of the project, it quickly turned out that both, synthesizing new functionalities, and converting them into functional entities, required substantially more resources than anticipated. Not too surprisingly, in retrospect, were some notable failures (e.g., with the Pristinamycin system), which however could be compensated by recruiting alternative techniques.
On the very positive side, however, is the generation of novel paradigms never planned or anticipated at the time of writing the program plan, most notably the possibility of modulating gene expression in resident genes by inserting an mphR(A) operator into an early intron. While it meanwhile became possible to affect gene expression by CRISPR-fusions, this technique still depends on harsh interference with the organism (e.g. adenovirus-based transfections), while here, we can apply a hormone several orders of magnitude away from side effects or even toxicity, by adding it to the drinking water. Ultimately, this can be a tool for true target validation for any target, in any field of biomedical research.

## WP2: In vitro functional characterisation of pancreatic cancer target genes

**Partners involved:** UniMar, IBA, EPO, UNIVR, CeGaT, UULM, MIMETAS, QMUL, DKFZ

**Total WP progress** 100%

**Background**

Our workgroup has previously generated and analysed expression profiles a total of ~2000 pancreatic cancer candidate genes to identify genes with expression patterns suggestive of important roles in tumour cell biology. For a selection of 80 of these candidate genes, first functional data were obtained in parallelised assays using reverse transfection technology. Additional candidates were identified in genome wide synthetic lethality screens using siRNA and shRNA libraries as well as through analysis of data from PDAC genome sequencing approaches among others generated by members (UniVR) in this consortium. Based on these data, sets of candidate genes were selected for validation and functional characterisation in vitro. Detailed results on cellular functions of these candidates were obtained during the runtime of the CAM-PaC project, and the most promising targets were further characterized in vivo in xenograft- and genetically engineered mouse models.

**Overall objectives**

The overall objectives of this WP were to:

1. comprehensively and systematically analyse the functional roles of selected target genes in pancreatic cancer cells in tumour progression and therapy resistance in vitro (tasks 1,2,3)
2. identify molecular mechanisms and signalling pathways involved in mediating these functional effects within epithelial cancer cells as well as between cancer cells and accessory stromal cells (tasks 2,3,4)
3. provide and phenotype stably transfected cell lines with inducible knockdown of selected target genes for further functional analyses in vitro and in vivo (task 5)

Thereby, WP2 aimed to i) contribute to creating a portfolio of new and validated therapeutic targets in PDAC, ii) provide in vitro model systems for large-scale metabolic and molecular phenotyping, iii) provide data and experimental validation for systems biology-based approaches to model mechanisms of tumour progression and therapy resistance of PDAC, which may help to guide personalised medicine, and iv) provide cellular models for clinically relevant situations such as therapy resistance that could serve as the basis for the SME`s in this consortium to develop and promote novel in vitro and in vivo assays of high scientific relevance and significant commercial potential.

**Results**

Results of a variety of cell-based functional screens were scored and analysed to identify potential novel target genes. These mainly included three sources of cell-based functional data: reverse transfection analyses as described in Buchholz et al., 2015 (PLoS One. 2015 Apr 7;10(4):e0122946), "dropout screens" using genome-wide barcoded shRNA libraries as well as analyses of apoptosis induction following transient transfections of a kinome-wide siRNA library (Sannino et al., Oncotarget. 7:73725-38).
All genes generating positive results in these screening approaches were compiled and further priorized for functional analyses. Two sets of candidate genes were generated: one set contains genes that are potentially druggable (generated by identifying candidate genes that belong to gene families that are generally suitable for targeting with small molecule compounds, e.g by

virtue of having known enzymatic functions), and a second set comprises genes that may be involved in tumour promotion via reciprocal stimulation within different cell-types in the tumour microenvironment (identified through known localization of the candidate genes in cell membrane or extracellular space and/or previous reports of functional roles of the candidates in stromal or inflammatory cells).

Based on first functional data in cell lines, a set of different target genes were selected for in-depth characterization. Detailed results have been obtained for the genes TTK, ADRBK1, PLAC8 and USP5 and the results published (Kaistha et al. (2016), Cancer Res. 76:96-107; Buchholz et al. (2015), PLoS One. 10: e0122946; Kaistha et al. (2014), Br J Cancer. 111:1780-7; Kaistha et al., Oncotarget. 8: 66215-66225). Research on FASTK and CFL1, including RNA-Seq data from CeGaT, is well advanced. The genes SMC2 and VCP have delivered promising results and will be further analyzed in the context of other funded projects.

Moreover, data from functional analyses for genes PLAC8, TTK, USP5, as well as RNASeq data from CeGaT and UULM (for PLAC8, FASTK and CFL1) has led to the identification of signalling cascades with high probability of being involved in the function of these genes in PDAC. A detailed model of the functional involvement of CFL1 in PDAC cell biology has been developed by UULM and has already in central parts been experimentally confirmed by UniMar, providing potential avenues for novel therapeutic intervention.

### Conclusion

Functional data from high-throughput screens obtained both before the start of the CAM-PaC project as well as during the first phase of the project provided a sound basis on which to select highly relevant candidate genes for further in-depth analysis. Further functional analyses in vitro and in vivo (for selected candidates) provided a plethora of novel insights into the basic biology of pancreatic cancer and potential vulnerabilities of pancreatic cancer cells to novel treatment options. Results have been published in several papers, and additional manuscripts are currently in preparation.

## WP3: Definition of pancreatic cancer stem cell target genes

**Partners involved:** QMUL, PolyGene, IBA; EPO, UNIVR, CeGaT, UULM, UNIV LEIDEN, DKFZ

**Total WP progress** 100%

### Background

Convincing evidence, from our lab and others, demonstrates that pancreatic cancer contains stem cell-like cells, which are uniquely capable of propagating the tumour much like normal stem cells fuel proliferation and differentiation in normal tissue. Genetic ablation of these cancer stem cells combined with effective debulking chemotherapy achieved long-term survival in advanced preclinical mouse models. These findings fundamentally change the way we should treat PDAC, but further clinical development of this concept will require a thorough understanding of the regulatory machinery of cancer stem cells in order to develop effective cancer stem cell-targeting treatments.

### Overall objectives

By understanding the epigenetic and metabolic biology of pancreatic cancer stem cells, we will be able to identify novel therapeutic targets and subsequently develop multimodal treatment approaches and improve the still miserable prognosis of patients with pancreatic cancer. Using deep RNA sequencing, we will identify specific candidate genes/pathways that are enriched in pancreatic cancer stem cells, including genes involved in "stemness", self-renewal, proliferation, invasiveness and metabolism. Thus, to assess the role and inevitable therapeutic value of these target genes, the following three objectives will be addressed in WP03:

Objective 1: To comprehensively analyse genes that mediate pancreatic CSC-specific phenotypes (self-renewal, invasiveness, metabolism, tumourigenicity) in vitro and in vivo (Tasks 1-3).

Objective 2: To dissect the role of candidate genes in pancreatic cancer, at the transcriptional and metabolomic level, by modulating target gene expression with RNA interference and subsequently modelling transcriptome and metabolomic alterations (Tasks 4).

Objective 3: To determine the role that target genes play in pancreatic cancer initiation and development by selectively controlling their expression in vivo using transgenic mice with hormone regulatable promoters (Task 5).

### Results

Using in vivo functional taxonomy we aimed to systematically dissect key regulators in pancreatic cancer stem cells. Three common schemes were identified – stemness signalling, metabolism and immune privilege – for all of which we have now provided strong preclinical data. For further clinical development, it was imperative to comprehensively study these schemes in the context of cancer stem cells and translate our findings into actionable multimodal treatment strategies. As such, we provided

novel information on how to most efficiently target PDAC including highly tumourigenic cancer stem cells. There translation into the clinic could lead to more durable treatment response and prevent disease relapse.

**Conclusion**

By the end of Year 5, WP3 provides thorough scientific evidence for the validity of our novel targets and thus justify their further clinical development, pending acquisition of sufficient funding. Eventually we envision novel phase I-II precision medicine trial, centred on targeting cancer stem cells and guided by analysing their circulating counterparts in the peripheral blood, and thus contribute to improving the still devastating prognosis of PDAC patients.

## WP4: Visualisation of tumour progression, therapy response and resistance phenotyping in GEMM

**Partners involved:** DKFZ, PolyGene, EPO, UNIVR, CeGaT, UULM, MIMETAS, QMUL, DKFZ

**Total WP progress** 100%

**Background**

Pancreatic Ductal Adenocarcinoma (PDAC) is still one of if not the deadliest cancer with fatal prognosis. Despite enormous academic and industrial efforts, the main reasons include ongoing late diagnosis in mostly an unresectable state, strong inherent/initial or acquired resistance to virtually any therapy. Reasons for this likely include the morphological and molecular tumour heterogeneity. Recent findings indicate the existence of at least two dominant PDAC subtypes, classical and quasi-mesenchymal that show morphological, molecular and therapy-related differences and may be approached with different targeting strategies. However, detection and diagnosis of these subtypes is difficult and at current is not part of the diagnostic workup due to the absence of robust diagnostic tools.

Genetically engineered mouse models with pancreas-specific activation of oncogenic KrasG12D and loss of tumour suppressor genes such as Tp53 recapitulate the morphological and molecular characteristics of human PDAC. In this project, we aimed to establish visualization tools and algorithms to gather information with regard to tumour heterogeneity, clinically relevant subtypes and earlier detection of therapy responses.

**Overall objectives**

In this work in the frames of CAM-PaC, we have used genetically modified mouse models (GEMM) as a model system closely resembling human PDAC. We developed imaging-based approaches for comprehensive non-invasive characterization of tumour properties with relevance for tumour progression, prognosis and therapy response markers. The overall aims of this working project were:

Objective 1: Investigate the sensitivity and robustness of individual parameters from multimodal imaging for detection and progression of established GEMMs of endogenous PDAC.

Objective 2: Establish non-invasive parameters for assessment of tumour heterogeneity and therapy response.

Objective 3: Apply multi-modal non-invasive imaging for characterization and monitoring of cancer biology with focus on assessing the role of key genes and signalling pathways in tumour maintenance and progression.

**Results**

To model the characteristic heterogeneity of pancreatic ductal adenocarcinoma (PDAC) and the influence of key genes and signaling pathways, we generated GEMM with different genetic alterations that contribute to the development, progression and overall phenotype of PDAC. We thus combined pancreas-specific activation of oncogenic KrasG12D with the additional inactivation of the tumour suppressor Tp53 (either heterozygous or homozygous loss of protein or activation of dominant-negative Tp53R17H) or the additional activation of the EGFR pathway, thus creating a wide spectrum of PDAC phenotypes with different morphologies and aggressiveness. In the most aggressive model, *Ptf1a*$^{+/Cre}$*;Kras*$^{+/LSL\text{-}G12D}$*;p53*$^{fl/fl}$ mice (termed CKP hereafter), PDAC develops in a short time span at 4-6 weeks and is characterized by moderately to poorly differentiated tumours (grading G2-G3) and, importantly, a very abundant stromal microenvironment (also called desmoplasia). As these mice are also highly resistant to chemotherapeutic approaches (Ardito et al., Cancer Cell 2012), this model recapitulates key features of human PDAC and is highly suitable for translational imaging approaches.

The various GEMM were subjected to multimodal imaging approaches. Specifically, we focused on multi-parametric Magnetic Resonance Imaging (MRI), which allows to obtain various tissue-specific and perfusion besides mere anatomical information. Since PDAC is often characterized by cystic lesions within or around the firm tumour nodules, we first used so-called T2 weighted (T2w) Magnetic Resonance Imaging (MRI) imaging sequences, which allow the identification of cystic/fluid lesions.

This is needed to perform segmentation of the tumour for correct tumour volume analysis. We thus developed a segmentation algorithm as described (Mazur et al, Nat Med 2015). Using T2w-MRI, we established protocols that allow precise detection of tumour onset and the growth of the progressing tumours. Thus, T2w-MRI can be considered a robust and well usable initial non-invasive imaging modality.

We further took advantage of additional MRI-based parameters that would allow us a more detailed visualization of tumour in terms of tumour burden (cellularity), the desmoplastic reaction and tumour vessel density and functionality, all parameters that are implicated as determinants of therapy response. Diffusion-weighted (DW) MRI was intensively characterized for imaging of tumour cellularity in GEMM-based PDAC and delivered non-invasively very valuable information of the histological appearance and present intratumoural heterogeneity. To validate the imaging findings, we established co-registration of imaging and histological sections, so that the imaging slices could be spatially assigned to the tissue appearance. We identified the DWI extracted Apparent Diffusion Coefficient (ADC) parameter to allow reliable distinction of preneoplastic and malignant lesions as well as tumours with abundant stroma from those with high cellularity. Lower ADC values indicate a more cellular phenotype and higher values point towards a ductal, more differentiated appearance. Thus, using this GEMM-based experimental platform, we optimized a DWI protocol for non-invasive morphological phenotyping of PDAC. Importantly, we used these imaging parameters for validation of our findings in human PDAC. Notably, we gathered the same information from MRI data sets of patients with PDAC. Moreover, we found the parameter ADC not only to correlate with the tumour cellularity but also provide prognostic information. Specifically, patients with resectable tumours and with higher cellularity both shown via histological assessment and lower ADC values, showed a significantly worse overall survival. This finding thus provides the basis to prospectively explore the value of ADC for future therapy stratification.

As stated above, tumour heterogeneity, both interindividual and intratumoural, is likely a central and heavily debated issue determining the clinical course and therapy resistance in PDAC. It is reflected through genetic, molecular, metabolic and histological heterogeneity among others, all of which strongly influence the course of disease and therapy response. We used multimodal imaging approaches not only for identification of PDAC but we also optimized protocols that allow better appreciation and characterization of intratumoural heterogeneity, e.g. tumour cellularity (as described above), intratumoural perfusion and distribution of drugs. We observed that in the GEMM used in this project, there is substantial inter- and intratumoural morphological heterogeneity (as determined via ADC), different metabolic activity as determined with 18F-FDG position emission tomography (PET), and tumour perfusion with dynamic contrast-enhanced (DCE) MRI.

The anatomical response of PDAC to treatment, e.g. changes in size, does not well reflect the degree of therapy response or resistance. This is likely due to the large amount of stroma in the tumours, thus tumours often do not with changes in size but composition to various treatments. So far, this limitation of anatomical imaging e.g. via computer tomography (CT) is an ongoing challenge when trying to determine therapy responses in PDAC. To assess the value of the aforementioned various imaging parameters and their use to identify better response markers, we submitted CKP mice to standard-of-care chemotherapeutic (gemcitabine) or targeted (MEK inhibitors targeting the RAS/MAPK signaling pathway) therapy regimens. We then followed the tumour response on several levels, including tumour volume, changes in tumour cellularity (DWI) and metabolic activity (FDG-PET). As observed in humans, treatment with gemcitabine induced only a slight tumour growth reduction and no changes could be observed on tumour cellularity. In contrast, the MEK inhibitor refametinib induced a very strong tumour size reduction with partial remission only 7-14 days upon therapy onset. This decrease in volume was preceded by a very early change in tumour cellularity, as we observed a uniform increase in ADC value in all treated animals. Post mortem analysis confirmed that the observed increase in ADC reflected changes in histology, namely massive apoptosis of tumour cells and reorganization of tumour stroma, especially an increase in stromal ground substance that filled-in the empty space left upon apoptosis (Trajkovic-Arsic et al, Sci Rep 2017). Thus, these data support the non-invasive parameter ADC as a potential early therapy response parameter that can easily be clinically applied and used to early identify the patients that favorable respond to therapy while excluding those from further harmful side effects that do not show an initial change in ADC. Indeed, when we validated the findings from the GEMM-based studies in human patients, we found first encouraging validation which led to the initiation of a clinical trial protocol to further evaluate ADC as an early response parameter.

The typical and prominent desmoplasia and the subsequent high intratumoural tissue pressure and low vascularization in PDAC are potentially also critical factors in drug delivery and intratumoural drug concentrations. We thus used GEMM to investigate the emerging technology of matrix- assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS) as a powerful tool to study drug delivery and spatial tissue distribution in PDAC. We examined the distribution of the approved targeted agent erlotinib, a small molecule inhibitor against EGFR, to investigate the drug tissue distribution and also that of erlotinib-derived drug metabolites, an area not well studied due to analytical limitations. We found that levels of erlotinib and metabolites were indeed significantly lower in PDAC compared to healthy pancreatic tissue. Moreover, we were able to localize the drug to

intratumoural complexes such as tumour glands, while we found decrease drug levels in stroma-rich areas without tumour complexes (Grüner et al, Mol Cancer Res 2016).

**Conclusion**

In conclusion, within the CAM-PaC framework we have developed a multiparametric imaging-based platform for identification, follow-up and therapy response evaluation in mouse models of PDAC. This platform is a highly useful preclinical tool for preclinical therapy trials. Moreover, many imaging parameters and algorithms have been validated in the human disease and can thus be translated in clinical scenarios. We believe that these approaches set milestones in preclinical imaging of this complex disease establishing strategies for imaging-based evaluation of relevant tumour phenotypes and early response evaluation. Our work can be used as a basis for further development and evaluation of novel treatment strategies in PDAC using these strategies to stratify or select for relevant subgroups in this disease and establish robust biomarkers for early therapeutic evaluation.

## WP5: High throughput culturing techniques for pancreatic cancer cells and CSCs

**Partners involved:** IBA, UniMar, MIMETAS, QMUL

**Total WP progress** 100%

**Background**

Pancreatic cancer is still one of the leading cause of cancer related deaths worldwide. This is addressed to limited therapeutic options associated with a high degree of resistance to chemotherapeutics and an aggressive tumour progression. The knowledge of candidate genes which drive chemo resistance and carcinogenesis, e.g. by promoting proliferation or tumour-stroma-interactions, might help to find new therapeutic strategies. The establishment of new, more effective drugs also might overcome the hurdles in the treatment of pancreatic cancer. The identification of these candidate genes and new substances is associated with huge effort, because a plethora of different genes and substances / molecules must be tested towards their role in tumour biology within suitable high-throughput *in vitro* platforms. Automation helps to stem this effort and realises large parallelized pre-clinical *in vitro* analyses. Unfortunately, the majority of these analyses are restricted to 2D cell cultivation. Results from these artificial cell models are hardly transferable to the *in vivo* situation and often leads to fails within *in vivo* studies. A better model for studying tumour biology *in vitro* is the spheroid model established by cultivating tumour cells under non-adhesive conditions in 3D. The main disadvantage of 3D cell models is the more complex handling. Associated to this, the implementation to high-throughput handling still is a hurdle.

**Overall objectives**

The goal of the present work package was to establish a new cultivation platform for high-throughput 3D cell cultivation and its implementation in cancer research. The new platform should be suitable for analyses regarding tumour initiation (clonal growth of cancer stem cells), tumour progression (cell proliferation, tumour -stroma-interactions) as well as substance screenings. The platform should be adaptable to the special needs of different cell types and therefor guarantee for different 3D cultivation methods (each with or without supplements).

The new platform should be sufficient for cultivation **requirements of pancreatic cancer cells and CSCs** including the **co-culture with stromal cell**s as a novel cell based model system (Task 1). Within the platform, functional analyses of **genetic changes** as well as **therapeutic treatments** in cellular model systems of pancreatic cancer should be possible (Task 2, 5).

Furthermore, the new platform should ensure an experimental **set up for laboratory experiments and downstream analytical procedures** based on automatized cell culturing technology (Task 2, 3, 4). Thereby WP5 will develop a novel *in vitro* cell-based model system for the efficient and standardised analysis of cellular phenotypes with broad applicability for cancer (stem) cell research.

**Results**

During the project, a new cultivation platform was established. The aim was to overcome the incompatibility of already developed platforms to the special requirements of pancreatic cancer (stem) cells and to provide a single device for versatile use in cancer research. The new cultivation platform is based on a commercially available low volume 384-well plate, thus in a miniaturized format and it comes up with an outstanding versatility [1]:

1. **Versatile in 3D cultivation method**
   As the optimal protocol for the formation of 3D culture is dependent on the cell type, the new cultivation platform enables the possibility to choose between two different 3D cultivation methods: either a liquid overlay protocol, where cells are seeded on top of an agarose coating or with a modified hanging droplet protocol. Here, the wells of the new cultivation

platform were coated with poly-Hema, cells were seeded in a well-overfilling volume and the platform was cultivated upside-down to guarantee for hanging droplets (Figure 1).

2. **Versatile in cultivation supplements**
Supplements for the formation of 3D cultures in liquid overlay as well as the modified hanging droplet protocol within the new cultivation platform are not needed, as cells interact with each other to build 3D structures. Unless this point, the addition of factors to the medium (e.g. extracellular matrix, growth factors, chemotherapy) is possible at every time point of cultivation.
3. **Versatile in the read out method**
As every scientific issue requires a unique read out, the new cultivation platform allows the experimenter to analyse the 3D cultures phenotypically within microscopical monitoring or with cell-based viability assays (luminometric). The main advantage is that no transfer of the cultures is needed.
4. **Versatile in analyses regarding tumour biology**
The study of carcinogenesis is accompanied by a huge effort, as different issues often require different devices (plates, dishes, chips). With the new cultivation platform, a plethora of different analyses can be performed within one single device. Protocols were established to study the several phases of carcinogenesis like initiation (clonal sphere formation starting with single cells), tumour promotion (proliferation analyses / growth kinetics), tumour progression (tumour -stroma-interactions) and chemo response (substance screening assay) without switching the cultivation platform.
5. **Versatile in sample preparation**
To guarantee for downstream analyses of the 3D cultures, the new cultivation platform offers the possibility to intervene at every time point of the cultivation and to prepare samples for different scientific issues – either metabolomic, genomic, transcriptomic or histological analyses.
6. **Versatile in handling**
Due to the standardized dimensions, the new cultivation platform is applicable for automated handling in standard liquid handling robots, microscopes and plate reading systems. Thus, it offers the possibility for standardized analyses in high-throughput manner. Despite automation, the new cultivation platform provides reproducible results with manual handling (Figure 2).

Within the project, the above mentioned experiments (section 4 and 5) were performed with a plethora of different cell lines with different genetic background and modification. The goal was, to identify genes, which can be connected to several phases in carcinogenesis and / or chemo resistance. In the following, the most promising results are summarized.

To get an insight in tumour initiation, an experimental set up for sphere formation from a single cell was established. Therefore, protocols for the generation of a complete single cell suspension and a single-cell-per-well seeding were optimized successfully. A clonal growth was observed in ≤ 3% of the seeded single cells, which is comparable to the amount of tumour-initiating cells in the well accepted cancer stem cell (CSC)-model of tumourigenesis.

In relation to tumour promotion through an enhanced cell proliferation, growth kinetics pointed out a differential potential for 3D growth in different cell lines. Most of the tested genes, like FastK and Plac-8, showed no distinct influence on tumour cell proliferation. In contrast to this, the gene for USP5 could be connected to the proliferative potential of the tumour cells, as its knock-down showed an impaired proliferation in all the three tested independent clonal cell populations [2].

Regarding to putative genes for chemo-response, the gene for FastK could be connected to chemoresistance to Gemcitabine in PaTu 8988t and Suit2-007 cells by extensive substance screening analyses. Furthermore, in Suit2-007, FastK also mediates resistance to Paclitaxel (Figure 3). An additive effect of combinatory therapies of standard therapeutics (5-fluorouracil, oxaliplatin, gemcitabine, paclitaxel and erlotinib) could not be detected, leading to the fact, that new substances are needed for the therapy of pancreatic cancer.

Concerning the tumour -stroma-interaction in pancreatic cancer, a stroma-induced sphere-formation of all tested pancreatic cancer cells (primary cells and cell lines) in the presence of human pancreatic stellate cells (HPSC) but not their conditioned media (secreted factors) was observed (Figure 4). This leads to the hypothesis of a strong and direct interaction between both cell types, which favours an increase in tumour -architecture. Experiments with supplements identified the necessity of an intact protein biosynthesis in HPSC to this interaction. Despite the mechanisms known from tumour -stroma-interactions in colorectal cancer [3], β1-Integrin played no role in the stroma-induced sphere-formation of pancreatic cancer cells, questioning the role of extracellular matrix in this interaction. Besides double-co-cultures, also triple-cultures were established within the new cultivation platform. Hence, complex cultures from tumour cells, HPSC and M2 polarized macrophages, comparable to the

histological results in patients, are possible to study *in vitro*. Only in combination with HPSC, M2 macrophages are able to induce sphere-formation in pancreatic cancer cells. Additionally, a distinct distribution of the several cell types within the spheroids was observed. This again leads to the idea of a strong and regulated interaction of the involved cell types. Furthermore, direct co-cultivation of tumour cells with HPSC as well as soluble factors of HPSC induce a gain in resistance towards gemcitabine in PaTu 8988t but not Suit2-007 cells. In contrast to HPSC, secreted factors of M2 macrophages have no effect on chemo-response, leaving the role of M2 macrophages unresolved.

**Conclusion**

With the present work, a new platform for 3D cell cultivation for its use in cancer research was established. For the first time, one device sums up all of the following main characteristics:

- Suitable for manual and automated handling
- applicable for different 3D cultivation methods
- useful for analyses of all stages of carcinogenesis (initiation, promotion, progression)

The analyses within this platform identified several putative candidate genes for tumour cell proliferation and chemo-resistance. Furthermore, an influence of tumour -stroma-interactions on chemo-resistance was detected.

The results gained in the new platform represent a valid basis for ongoing analyses, e.g. transcriptomic/metabolomic profiling or animal testing of interesting candidates. Furthermore, the implementation of the platform in personalized medicine might advance therapeutical strategies: As a logical implication to dose-response-relations of defined co-cultures and due to miniaturization, low-sized samples like biopsies can be analyzed to improve patient´s outcome.

**References**

1. Widder M, Lemke K, Kekeç B, Förster T, Grodrian A, Gastrock G. Eng. Life Sci. 2018 Feb;18(2):132-139. doi: 10.1002/elsc.201700008 “A modified 384-well-device for versatile use in 3D cancer cell (co-)cultivation and screening for investigations of tumour biology in vitro.
2. Kaistha BP, Krattenmacher A, Fredebohm J, Schmidt H, Behrens D, Widder M, Hackert T, Strobel O, Hoheisel JD, Gress TM, Buchholz M. Oncotarget. 2017 Aug 3;8(39):66215-66225. doi: 10.18632/oncotarget.19882 “The deubiquitinating enzyme USP5 promotes pancreatic cancer via modulating cell cycle regulators.”
3. Widder M, Lützkendorf J, Caysa H, Unverzagt S, Wickenhauser C, Benndorf RA, Schmoll HJ, Müller-Tidow C, Müller T, Müller LP. Int J Cancer. 2016 Feb 15;138(4):964-75. doi: 10.1002/ijc.29844 “Multipotent mesenchymal stromal cells promote tumour growth in distinct colorectal cancer cells by a β1-integrin-dependent mechanism.”

## WP6: Xenograft models for in vivo validation of candidate gene function

**Partners involved:** EPO, UniMar, IBA, UNIVR, CeGaT, MIMETAS, UNIV LEIDEN, QMUL

**Total WP progress** 100%

**Background**

The *in vivo* establishment and monitoring of xenograft models directly derived from patient tumour material simulates the most “clinic-close” situation to monitor tumour biology and the impact of therapeutics. In contrast to the human situation, primary xenografts allow an easy access to high quality pre- and even more importantly post treatment tissues (which is rarely available from clinical studies). In WP6, cell line-derived xenografts as well as large series of primary patient derived xenografts (“xenopatients”) were subjected to clinically relevant chemotherapeutic treatment regimens in a design similar to prospective and randomised clinical trials. Additionally, stably transfected PDAC and CSC cell lines were used to monitor the effects of modulation of target gene expression on tumourigenicity and tumour progression in a more complex microenvironment than provided by the *in vitro* culturing methods. The in vivo validation is an important step in the identification and characterisation of candidate genes as novel targets for therapeutic intervention and to reveal novel determinants of therapy resistance in individual tumours. Furthermore, due to inter- and intra- tumour molecular heterogeneity, defining key components of drug response pathways has proven challenging in cancer medicine. Since these aspects are closely mirrored by the "xenopatient" approach, analysis of the data and material provided by WP6 were instrumental in identifying novel mechanisms of drug resistance and combination strategies to enhance drug sensitivity and to identify bioinformatic models predictive of PDAC subgroups differing in their response to treatment regimens in collaboration with other project partners. This in turn is a prerequisite for the development of personalised treatment approaches adapted to the molecular phenotype of individual tumours.

**Overall objectives**

Preclinical research on new predictive biomarkers is often performed in a small number of cell lines that have been maintained in laboratories for several years. Even corresponding xenograft models derived from these established human cancer cell lines should be considered with care. Depending on the number of cell passages, cell lines and xenografts can behave very differently to the primary tumour, and, combined with other deficiencies in pre-clinical approaches, this can reduce the relevance of established cell lines or xenograft models for predicting the probability of success of biomarkers in clinical studies in some tumour localisations. To simulate the clinical situation (heterogeneity of tumours), a panel of patient-derived xenografts was supposed to be established and, in close cooperation with project partners, extensively characterised molecularly and histologically to ensure validity of the models.
In addition, the xenograft platform is employed to monitor the effects of modulation of expression of novel target genes on tumourigenicity and tumour progression in stably transfected PDAC and CSC cell lines in a complex microenvironment *in vivo*. Both types of xenograft models can then be used for treatment with chemotherapeutics relevant for the therapy of clinical pancreatic cancer. Phenotypic and molecular analysis of the xenograft material before and after treatment will be instrumental in identifying novel determinants of chemoresistance and chemosensitivity in PDAC.
In summary, the main objectives of WP6 are the establishment of a "xeno-factory" of comprehensively characterized primary PDAC-derived mouse xenograft models for preclinical therapeutic trials and to provide a central platform for *in vivo* testing of tumourigenicity and chemoresistance of PDAC and CSC cell lines with altered expression of selected target genes.

**Results**

Within the last five years of the project different PDAC tumour tissues, cell lines and CSCs from particular project partners were transplanted into immunodeficient mice. In doing so, a comprehensive preclinical platform with approved *in vivo* tumourigenicity as well as efficacy to standard of care was generated. Xenografts of both, primary and genetically modified pancreatic cancer cell lines as well as stem cells were examined in terms of tumour growth progression. Moreover, the panel of 14 patient-derived xenografts as well as of 6 circulating tumour stem cell derived models was exposed to a clinically relevant panel of anti-tumoural drugs consisted of Gemcitabine, Erlotinib, Abraxane, 5FU and Oxaliplatin (partly as combined therapy). Exemplary data of tumour recurrence after stop of treatment as well as repetition experiments completed the characterization and provided meaningful information about tumourigenicity and treatment resistance of the models. Additionally, different novel therapeutic approaches were submitted to the xenograft panel.
The gross of transplantated patient material showed progressive growth in immunodeficient mice with tumour doubling times between seven and twenty days. The response rate to Gemcitabine was 35% (thereof 10% partial remission) whereas the response rate to Abraxane was 80% (thereof 70% partial or complete remission). Trametinib monotherapy was only marginally efficacious, but Trametinib improved the therapeutic activity of Mocetinostat, JQ1 and Decitabine when given in combination. Furthermore, Minnelide was applied second line after 3 cycles of chemotherapy to all xenograft models and inhibited the tumour relapse up to 50% after stop of chemotherapy. The TTK inhibitor BAY1161909 prolongated tumour relapse in combination with Abraxane compared to Abraxane alone.

**Conclusion**

The CAM-PaC xenograft panel was successfully established from tumour material of 20 individual patients suffering from stage IIA/B (PDX) or stage IV (CDX) PDAC. This valuable *in vivo* platform identified responder and non-responder to standard of care and was applied to evaluate the efficacy of novel treatment strategies. Follow up analyses with material of the CAM-PaC xenofactory revealed histological and molecular consistency with primary tissues.
In summary, results from WP6 document the need of new sophisticated therapeutic approaches for pancreatic cancer patients as the standard of care is limited in efficacy and the curative potential of other drugs tested within this project was found to be marginal.

**WP7: Histopathologic and molecular characterisation of human primary tissues and model systems**

**Partners involved:** UNIVR, UniMar, PolyGene, EPO, QMUL, DKFZ

**Total WP progress** 100%

**Background**

The histopathological and molecular characterization of primary cancers remains a fundamental issue to properly describe cancer and address the issue of heterogeneity and genetic differences among the cancers in different individuals as well as within the same cancer in order to correctly stratify patient therapy. Analysis of data from Pancreas Ductal AdenoCarcinoma (PDAC) produced from genome sequencing approaches within the International Cancer Genome consortium by the University

of Verona [1] demonstrated the molecular variation of each cancer and highlighted the need for a molecular description of cancer tissue.
The same issue applies to murine models corresponding to both primary tumour xenografts (PDX) in immunodeficient mice as well as to genetically engineered mouse models (GEMM), in order to qualify their use in therapy validation. Novel transgenic mouse models and PDX are of enormous potential use in target validation for therapy but currently lacks adequate histo-pathological and molecular definition. As a result, PDX and GEMMs require both histo-pathological and molecular characterization to describe the generated cancer and to standardize models.
A major challenge in investigating PDAC mutations is the generally low cancer cell content of this cancer type implying that there is limited material available for genetic studies. Implanting the primary tumour tissue in immuno-deficient mice (xenografting) permits the increment of the cancer cellular component and the possibility of continual proliferation of tumour tissue for additional analysis [1].
However, there is a risk that differences may occur in the genomic makeup when the primary tumour tissue is transplanted in the murine host [2]. There is also the issue of heterogeneity of the primary tumour tissue implying that the implanted primary tissue may only partially represent the entire tumour. Furthermore, PDX suffer from a lack of human stromal components, which comprises the connective tissue and other elements of the microenvironment that surrounds the cancer cells. These are replaced by murine elements and the human immune system is missing.
Despite these issues, xenografts may be useful models for translational cancer research [3-6]. It has been suggested that successful xeno-engraftment may indicate poor prognosis and represent patient metastasis [2, 7]. Furthermore, there may be potential to use xenograft to determine patient personalized therapy based on xenograft response to specific drug combinations [8]. It is thus fundamental to investigate the molecular concordance between the primary cancer and that propagated in the murine model.

**Overall objectives**

The main objectives of this work package are:

- Histo-pathological and molecular characterization of primary cancers using classical methods and innovative next generation sequencing techniques
- Creation of a set of xenografted tumours from primary pancreatic cancers
- Histo-pathological and molecular characterization of cancer xenografts using classical methods and innovative next generation sequencing techniques
- Histo-pathological and molecular classification of neoplastic and pre-neoplastic lesions of novel transgenic murine models
- Creation of a central bio-bank of primary cancers and related xenografts and associated clinical and histo-pathological and molecular database for testing and validation.

Primary tumours will be molecularly characterized using a customized cancer panel using a personal genome sequencer querying a set of genes previously identified as altered through whole genome sequencing of pancreas ductal cancers carried out within the International Cancer Genome Consortium, of which the work package leader is a member. This information will be associated with standard morphological classifications to provide a complete analysis of the tumour tissue together with anonymised clinical and prognostic patient data. This novel approach to describing PDAC tumours will provide insight into the use of these tissues in other work packages.
The developed cancer panels will also provide the basis for the characterization of human-tumour derived xenografts to confirm the molecular correlation of the xenografts. This is an important step to confirm the homogeneity between the primary and xenograft used in testing and validation and to standardize and harmonize the xenograft models used in the consortium.
To date, transgenic mouse models have not been comprehensively characterized molecularly and so there is a need to analyse these models for use in the molecular identification and continuous quality control of the transgenic mouse models.

Therefore, an important result of this work package will be the creation of a portfolio of samples (bio-bank) completely characterized by expert pancreatic and molecular pathologists of:

1. Primary pancreatic cancers;
2. Human derived mouse xenografts;
3. Novel transgenic murine models.

This coordinated and harmonized bio-bank of samples for use in other work packages of the project for testing and validation, together with anonymised clinical, histo-pathological, molecular and prognostic patient data that will be required for predictive modelling in WP10. This will provide the technology oriented SME involved in WP1 & 6 with access to a sophisticated pancreatic pathology platform and expertise for the validation of PDAC animal models that is usually not available at SMEs.

**Results**

Six custom panels were created based on the information produced from exome and whole genome sequencing of PDAC primary samples within the International Cancer Genome Consortium [9].The PDAC / Peri-ampullary panel explores 20 cancer genes traditionally found mutated in cancer cells of pancreas and ampullary cancers which is found at the join of the pancreatic duct and the bile duct. The two DNA damage repair (DDR) panels explore mutations found in genes involved in repairing harmful breaks that occur on both strands of DNA, known as double-strand breaks and the other panels address the main pathways involved in Chromatin remodelling, TGFbeta signalling and WNT signalling.

PDAC xenografted samples (PDX) were produced from primary PDAC and both PDX and PDAC samples were sequenced with these panels. A total of 234 cases have been characterised. These have identified particular pathway specific mutations that permit not only a subgrouping of the pancreas cancer cases but also identify potential therapeutic directions for specific subgroups. Matched normal DNA samples were sequenced to identify whether mutations were germ-line (hereditary) or somatic (confined to the cancer cells).

We found that a large percentage (25%) of cases harboured mutations in the DNA damage repair panels. This is an interesting finding as tumours with DDR deficits have been associated with response to platinum therapies in patients and patient-derived xenograft models suggesting the possibility of a subgroup of PDACs defined by compromised DNA repair of double strand breaks [10] may identify patients benefiting from therapies targeting DDR pathways. PDAC patients with either, germ-line or somatic BRCA mutations or other mutations in DDR genes may benefit from platinum-based regimens and the newer class of drugs known as poly (ADP-ribose) polymerase (PARP) inhibitors [11]. Collectively, the traits that cancers share with those cancers harbouring BRCA1 or BRCA2 mutations are defined as 'BRCAness', we developed a BRCAness gene expression panel to investigate the expression of relevant genes involved in DDR. The two most differentially expressed genes in cancers with BRCAness alterations were CHEK1 and RAD51, which implies that aberrant expression of these genes might be used for therapeutic decisions.

We also investigated genes in other pathways that are known to have a potential prognostic and/or therapeutic implications. For genes in the TGFbeta signalling pathway, we found 33% of patients with pathogenic mutations, where a SMAD4 mutation has been suggested to segregate a responsive subgroup of patients with locally advanced pancreatic cancer to radio-frequency ablation [12]. For genes in the Chromatin Remodelling signalling pathway, we verified pathogenic mutations in 20% of patients and this also has an impact on prognosis [13].

In addition to investigating genetic mutations of cancer, we also analysed gene expression as this can also provide important information in defining subtypes that can infer differences in the molecular evolution of pancreatic cancer subtypes and identify opportunities for therapeutic developments [14]. We also developed specific innovative technology panels that were able to define further subtypes, in particular one related to immunogenic response. This and the other subtypes have potential prognostic and therapeutic impact.

By simultaneously evaluating PDAC and PDX samples from the same case set, we also demonstrated that there is high concordance between the primary and xenografted tissue with regard to genetic mutations. In our case set, this stood at 96%. This means that they are a useful model for genetic evaluation and validation of targets for therapy in in-vivo models of cancer.

A selection of these PDX were sent to partners involved in in-vivo testing of tumourigenicity and chemo resistance of PDAC and cancer-stem-cell CSC cell lines with altered expression of selected target genes. Once these tumours have been treated with therapeutic targets they were once again evaluated for morphological variations and sequenced with the panels to evaluate any molecular changes induced by the chemotherapy.

Transgenic mouse models samples were evaluated and comprehensively morphologically characterized, 4 KPC mouse models, 1 KC mouse model, 3 F+/KC models, 17 F-/-KPC models, and 17 F-/-KC models. These were also sequenced with the targeted sequencing panel PDAC-light redesigned for the genetic differences of the transgenic mouse and expanded to increase the coverage of all exons.

**Conclusion**

In this work package, we morphologically and molecularly characterised a set of primary cancers and related xenografts. This information led us to verify the existence of a "BRCAness" subgroup harbouring pathogenic mutations of BRCA1, BRCA2 genes together with other DDR genes. In addition to the verification of a significant subgroup with specific treatment potential including locally advanced cancer patients, we also verified subgroups prognostic impact. Furthermore, we verified additional subgroups

through gene expression profiling including an immunogenic subtype. In addition to this, we show that PDXs, primary tumour engrafted cancer models, despite tumour heterogeneity, demonstrate high concordance with the original primary PDAC. This might represent a valuable model that faithfully recapitulates the main genetic features of primary tumours and as such provide valuable material for extensive investigation of the cancer cell poor tumour type. Finally, the availability of molecularly characterized primary cancer and matched in vivo models paves the way for novel diagnostics and therapeutics based on molecular phenotype of individual tumours, as they have the potential of being used to predict drug responses through in-vivo testing as well as permitting the identification of effective therapeutic schemes.

**References**

1. Biankin AV, Waddell N, Kassahn KS et al. Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. Nature 2012; 491: 399-405.
2. Garrido-Laguna I, Uson M, Rajeshkumar NV et al. Tumor engraftment in nude mice and enrichment in stroma- related gene pathways predict poor survival and resistance to gemcitabine in patients with pancreatic cancer. Clin Cancer Res 2011; 17: 5793-5800.
3. Aparicio S, Hidalgo M, Kung AL. Examining the utility of patient-derived xenograft mouse models. Nat Rev Cancer 2015; 15: 311-316.
4. Hidalgo M, Amant F, Biankin AV et al. Patient-derived xenograft models: an emerging platform for translational cancer research. Cancer Discov 2014; 4: 998-1013.
5. Martinez-Garcia R, Juan D, Rausell A et al. Transcriptional dissection of pancreatic tumors engrafted in mice. Genome Med 2014; 6: 27.
6. Byrne AT, Alferez DG, Amant F et al. Interrogating open issues in cancer precision medicine with patient-derived xenografts. Nat Rev Cancer 2017; 17: 254-268.
7. Ding L, Ellis MJ, Li S et al. Genome remodelling in a basal-like breast cancer metastasis and xenograft. Nature 2010; 464: 999-1005.
8. Villarroel MC, Rajeshkumar NV, Garrido-Laguna I et al. Personalizing cancer treatment in the age of global genomic analyses: PALB2 gene mutations and the response to DNA damaging agents in pancreatic cancer. Mol Cancer Ther 2011; 10: 3-8.
9. Waddell N, Pajic M, Patch AM et al. Whole genomes redefine the mutational landscape of pancreatic cancer. Nature 2015; 518: 495-501.
10. Turner N, Tutt A, Ashworth A. Hallmarks of 'BRCAness' in sporadic cancers. Nat Rev Cancer 2004; 4: 814-819.
11. Schultz N, Lopez E, Saleh-Gohari N, Helleday T. Poly(ADP-ribose) polymerase (PARP-1) has a controlling role in homologous recombination. Nucleic Acids Res 2003; 31: 4959-4964.
12. Paiella S, Malleo G, Cataldo I et al. Radiofrequency ablation for locally advanced pancreatic cancer: SMAD4 analysis segregates a responsive subgroup of patients. Langenbecks Arch Surg 2018; 403: 213-220.
13. Sausen M, Phallen J, Adleff V et al. Clinical implications of genomic alterations in the tumour and circulation of pancreatic cancer patients. Nat Commun 2015; 6: 7686.
14. Bailey P, Chang DK, Nones K et al. Integrative genomic analysis of pancreatic cancer identifies subtypes with distinct histopathological characteristics. Nature in press.

## WP8: Large scale metabolic profiling of in vitro and in vivo models of pancreatic cancer

**Partners involved:** UNIV LEIDEN, UniMar, PolyGene, IBA, UULM, MIMETASQMUL, DKFZ

**Total WP progress** 100%

**Background**

Despite all the progress made in the identification of molecular alterations related to pancreatic cancer, effective treatments are still lacking to reduce the high mortality rate in this cancer. To move forward in pancreatic cancer research, CAM-PaC set out to develop concepts and methods to integrate data from transcriptomics and metabolomics studies to directly interrogate gene functions on a large scale in order to distinguish 'driver' mutations, which directly contribute to tumour progression. A novel element introduced by CAM-PaC is to develop new cellular and animal models as well as techniques to generate and integrate the aforementioned omics data from these models in order to systematically characterize and validate novel targets for therapeutic intervention. For this purpose, targeted metabolomics platforms that cover metabolites of major metabolic pathways including glycolysis, central metabolism, lipids, amino acids, and signaling lipids/ inflammatory mediators were developed in work package 8 (WP8) to analyse different pancreatic cancer cellular and animal models samples. In addition, the

metabolomics analysis of the different models will help to monitor tumour growth and regression and can hence contribute to our understanding of pathogenic mechanisms as well as improve monitoring of treatments.

**Overall objectives**

Within CAM-PaC, the overall goal of WP8 was to metabolically, profile patient derived pancreatic cancer cellular models from different cell types isolated from the tumour microenvironment including cancer cells and cancer stem cells and similarly generate metabolic profiles of patient derived animal models using novel mass spectrometry-based metabolomics approaches. For this, WP8 developed and miniaturized targeted metabolomics platforms and used them to define the metabolic phenotype of human pancreatic cancer, by identifying key metabolic pathways in *in vivo* and *in vitro* pancreatic cancer models and then validating the key metabolic pathways to identify new possible drug targets. To develop the cellular models, IBA and Mimetas focused on optimizing cell culture protocols by using respectively the hanging droplet and the Organoplate® technologies. Sources for samples of animal models were EPO (WP6) and the University of Verona (WP7).

**Results**

To perform this large-scale metabolic profiling of *in vitro* and *in vivo* models of pancreatic cancer, targeted LC-MS methods were developed to cover different classes of metabolites from major metabolic pathways consisting of polar lipids, oxylipins, central carbon metabolites, and amino acids and amines. To define the metabolic profiles of human cancer, novel patient derived xenografts, cell models and animal models were received from different work packages of the consortium. Samples were also regularly provided by Mimetas to establish protocols for metabolites extraction from the medium and from the cells (MS22).

Mimetas (WP8) worked together with the University of Verona (WP7) to evaluate a personalized medicine platform for Pancreatic Ductal Adeno-Carcinoma (PDAC). Organoids were derived from human PDAC patient samples retrieved from the ARC-Net biobank and seeded in the Organoplate® as single cells in matrigel®. Human tumour derived pancreatic organoids and stroma cells have been cultured both in mono- and co-culture together with appropriate controls in the Organoplate®. The medium of these cultures has been harvested in order to set up the analysis methods to determine the metabolic signatures of the interstitial fluid as well as to achieve a reference point for all different pancreatic cancer models (D8.2). The sampled media has been sent to Leiden University for metabolomics analysis to identify lipid mediator (oxylipins) which are secreted in tumour s. To achieve this goal, a 3D cell model system cultured using patient-derived organoids together with a stroma cell type, cancer associated fibroblasts (CAFs) were (co)cultured and treated with two inhibitors of kinases AKT and MAPK (MEK) that plays major roles in signaling pathways, also in cancer. Genetic alterations leading to the continuous activation of both kinases enhances cancer proliferation. CAFs are found in the tumour microenvironment and are known to contribute to the tumour proliferation, invasion and metastasis by secreting essential factors such as cytokines, chemokines and growth factor proteins. Interesting findings supporting the previously mentioned role of CAFs, from our metabolomics analysis, was that high levels of PGD2 and PGE2 prostaglandins were secreted by CAFs compared to cancer cells and decreased by the MEK inhibitor. PGE2 is known to play several roles in tumour progression, for instance, in tumour vascularization, however, the complex and interlinked pathways connecting PGE2 signaling with cancer is not yet fully understood. Our observations suggest that the secretion of the two prostaglandins by CAFs is induced by the activation of the MEK kinase. The methods optimized in this study was further employed to study medium samples to compare a variety of cell model interstitial fluids, cultured in the Organoplate® using different conditions.

For a better sensitivity for peak detection due to sample limitations, a miniaturized amine platform consisting of amino acids and biogenic amines was developed (D8.3). This platform was used to investigate if cancer stem cells have a characteristic metabolic profile distinguishing them from bulk pancreatic stem cells (D8.4). To answer this question, the cancer stem cells sorted with stemness surface marker CD133, were provided by WP3 and two cancer models, spheroids and organoids, were produced from 2 different patient derived primary cultures (12707&140114). The metabolic profiles of cancer stem cells showed that different cell donors have distinct amino acid profiles with increased levels of o-phosphoethanolamine compared to non-cancer stem cells. O-phosphoethanolamine belongs to the phospholipid (major component of the cell membrane) synthesis or is a breakdown product of phospholipids (HMDB). Higher levels of this amino acid in cancer stem cells suggests that phospholipid metabolism is highly active in these cells, that might be link to increased proliferation in this cell type compared to non-cancer stem cells.

In a different study, with the same goal of evaluating a personalized medicine platform for Pancreatic Ductal Adeno-Carcinoma (PDAC), Mimetas (WP8) in collaboration with the University of Verona (WP7) cultured human tumour derived pancreatic organoids and stroma cells both in mono- and co-culture together with appropriate controls in the Organoplate®. To optimize the co-culture, two different medium compositions were evaluated and compared to the complete expansion medium. To define

the phenotype of cancer cell and stromal cell interaction, the cell culture media representing the interstitial fluid of cancer cells and stroma cells of WP2 was analyzed using oxylipin platform to find extracellular/secreted metabolites. A 3D cell model system cultured using patient-derived organoids together with different stroma cell types including stellate and endothelial cells was generated and optimized (D8.5). Cell viability and proliferation were measured and metabolic profiling focusing on signaling lipids (oxylipins) was performed. The results showed that the co-cultures of patient derived organoids with stroma cells mimic the tumour composition and increased in oxylipins levels. Oxylipins are metabolites playing an important role in inflammation, but also propagate signal for signaling pathway activations and cell-cell interaction that in cancer will lead to a better outcome.

Further work developing similar cell models treated with anti-cancer drugs and the comparisons to xenograft tissue with the same treatment regimens is utilized to evaluate the clinical usage of these models for the evaluation of personalized medicine. If the models successfully mimic tumour susceptibility, it will be possible to screen different known and novel treatment options in a high throughput platform using material derived from an individual's biopsy to achieve the best possible outcome for the patient.

For this purpose, six anticancer agents or combinations were selected by the CAM-PaC consortium: gemcitabine, placlitaxel, abraxane and combinations gemcitabine/erlotinib, gemcitabine/abraxane, 5-Fluoruracil (5-FU)/oxaliplatin. Gemcitabine, oxaliplatin and 5-FU are anti-metabolites mainly targeting the nucleotide metabolism, erlotinib targets specifically the epidermal growth factor receptor (EGFR) and blocks downstream signaling transduction and abraxane or its protein-bound form, paclitaxel, targets the microtubules and prevent their depolymerisation. Four pancreatic cancer patient (12558, 12560, 12707, 140114) *in vitro* models were successfully developed from xenograft tumours and treated with drugs, by Mimetas (WP8). The cell models were treated with increasing drug concentrations, cell viability assays were performed to determine a drug concentration response curve for each (combination) therapy and patients cell models. Metabolomic screening was performed using intracellular extract of the cell populations with an effective concentration (EC) of 30% (EC30) and their cultured cell media. Mass spectrometry analysis focusing on two metabolic classes, amines and oxylipins, was performed. The metabolic profiles of the drug treated samples were compared to the non-treated cell models and the results show patient specific metabolic profiles for different drug treatments.

Similarly, metabolic profiling of pancreatic mice models from xenograft tumours upon similar drug treatments as the *in vitro* models were determined with the four mentioned metabolic classes (D8.6) to evaluate the drug effect on the metabolic profiles of patient derived xenografts. An expansion of patient 12556 and 12707 treated with different (combination) therapies provided by WP6 were analysed and the quantified levels of metabolites were compared to the non-treated control xenografts. Comparable to the cell model study, patient specific metabolic profiles were observed for different drug treatments. However, for the animal models, abraxane treatment appears to have had the most effect on the metabolic patterns when compared to the non-treated xenografts in both patients' animal models, although the levels of changes due to abraxane and co-treatment with gemicitabine was higher in patient 12707 than in patient 12556.
As part of validating key metabolic pathways in pancreatic cancer, intracellular and extracellular metabolic profile comparisons between *in vitro* and *in vivo* model systems were also performed using the data generated from patient 12707 models. For this comparison, the levels of oxylipins and amines metabolites found in both model systems shows that both models exhibit similar intracellular metabolic profiles but different extracellular profiles. The lack of correlation in the extracellular metabolites between both models might be explained by the fact that *in vivo* models do not have the same conditions as the *in vitro* models. In addition, a challenge is posed when sampling secreted oxylipins from the interstitial fluid of the *in vivo* tumour model. Therefore, during this metabolomic analysis, a blend of *in vivo* model tissue containing extracellular and intracellular oxylipins were analyzed while for the *in vitro* model, easily sampled culture media was used for the analysis of extracellular oxylipins. Thus, a method for sampling interstitial fluid from animal models was lacking although well developed cell models can serve as an alternative. Taken together, the results these studies performed during the past several years in CAM-PaC provided valuable insights into the metabolome of patient derived pancreatic cancer models.

**Conclusion**

The goal of WP8 was to generate large scale metabolic profiling of *in vitro* and *in vivo* models of pancreatic cancer using targeted mass spectrometry-based techniques. Employing the aforementioned technology, we were able to analyze a variety of cell and tissue samples and were able to produce data that can be used to better understand the disease. Most importantly, the metabolomics data will be integrated with the transcriptomic and genetic data from these models to help find and validate novel therapeutic targets. We were also be able to get valuable information on cancer cell interaction with stroma cells, and show that patient derived cell models could be used for metabolomics screening that could serve for personalized medicine purposes.

## WP9: Large scale transcriptomic profiling of in vitro and in vivo models of pancreatic cancer

**Partners involved:** CeGaT, UniMar, IBA, UNIVR, UULM, QMUL, DKFZ

**Total WP progress** 100%

### Background

Pancreatic cancer is one of the most lethal cancer types and new treatments are urgently needed. For development of novel therapeutic options, it is indispensable to have a detailed molecular understanding on the behaviour of pancreatic cancer cells. Very helpful to gain new insights into tumour biology of pancreatic cancer is RNA sequencing. With this analytical method e.g. differential gene expression, alternative splicing or previously unknown transcripts changes and much more can be detected, laying the basis for developing therapeutic strategies.

Work package leader CeGaT is a global provider of genetic diagnostics and mutation related disease analyses. The company is highly experienced in high-throughput sequencing and provided transcriptome sequencing service for CAM-PaC project partners. Different transcriptome analyses of animal and cellular models were performed in order to fulfil the tasks of WP9 in close cooperation with other project partners.

### Overall objectives

One objective was to do comprehensive profiling of global RNA transcription with concomitant mutation analysis of coding sequences using established state of the art next-generation-sequencing. In order to provide sequencing service also for samples with very limited sample material, the establishment of new low input protocols was another task.

Further, the generated sequencing data should be interpreted to generate a small set of genes suitable for use in RNA-Panel sequencing.

### Results

CeGaT generated differential transcriptomes of modulated cancer cells, clonal sphere cultures and sequenced xenograft samples or cells from treated mouse models generated by project partners. Reports on global gene expression profiles were provided to the cooperation partners for joined interpretation.

Methods for RNA isolation and sequencing library preparation were developed continuously. For very limited sample material CeGaT successfully established and validated a protocol for low input amount in the nanogram range (>100ng) and one for the analysis of a few hundred cells (200 pg).

The sequencing data which was generated during the project was interpreted to create a complete list of 41 genes suitable for use in an analytical pancreatic cancer RNA enrichment panel. Regarding this panel also a protocol for RNA panel sequencing was established.

### Conclusion

By providing transcriptome sequencing and molecular phenotyping of model organisms and in vitro model systems, CeGaT generated data for elucidating the function of genes and gene products in biological processes relevant for tumour progression and therapy resistance. Further efficient, standardised and reliable tools for molecular phenotyping in many research contexts were developed and established which expanded the company's portfolio and generated new market opportunities.

## WP10: Data integration and pathway modelling

**Partners involved:** UULM, UniMar, UNIVR, CeGaT, UNIV LEIDEN, QMUL, DKFZ

**Total WP progress** 100%

### Background

The CAM-PaC consortium generated a considerable amount of high-quality experimental data of very different kinds. DNA and RNA sequencing data, metabolomics data, and more were generated and analysed within the consortium. To provide easy access to this data for all consortium members a central data storage was required. The amount of data generated, e.g. from sequencing experiments, cannot be analysed adequately without standard bioinformatics operating procedures. These operating procedures cover pre-processing, alignment, and statistical analyses. They are supplemented with different methods from the area of machine learning such as clustering or classification algorithms. Therefore, we also developed new machine learning methods, which lead to interpretable static models by integrating data semantics.

In contrast to the static data analysis (e.g. classification/clustering) dynamic modelling of biological systems allows analysing a system's behaviour over time. Boolean networks are one of the simplest class of dynamic models based on two-valued logic. This model type can be used when only qualitative knowledge is available. Each component of a biological system is modelled

using a Boolean variable. Regulatory dependencies between the different components are described by Boolean functions. The state of a Boolean network is defined by the state of all its components at a particular point in time. Dynamic behaviour of the system can be observed by transitioning through consecutive states. This state transition is computed by applying Boolean functions. After several state transitions, a Boolean network eventually converges to a recurring number of states, which are called attractors. Attractors represent the long-term behaviour of the modelled system and can be linked to biological phenotypes. Hence, Boolean networks are used for predictions of biological phenotypes. Furthermore, Boolean network models are valuable tools to perform *in-silico* knock-out/over-expression experiments to generate hypothesis about the changing behaviour of the modelled system. Thus, the simulation gives valuable insights into the behaviour of complex systems and hypothesis about the system can be extracted.

**Overall objectives**

The first objective was to provide a central interface allowing for data distribution between the projects. Here, we created a repository to integrate all experimental data (D10.1). Additionally, several standard operating procedures (SOPs) were implemented enabling an exploratory analysis of this data (MS30). The server was extended with a distributed computation infrastructure (Völkel, Bioinformatics, 2015).
Another objective was the static and dynamic modelling of dysregulated pathways in pancreatic cancer to better understand the disease progression and therapy resistance (D10.3). To hypothesise about dysregulations, we applied the Boolean network approach.
In addition to the modelling process of specific pathways of interest, one objective was the development of new algorithms to analyse Boolean network models such as the “drug-simulator” (D10.4). The purpose of these methods is to hypothesise about the changing behaviour of the modelled system over time, which can help to identify meaningful conditions and potential targets. Therefore, it was used to predict drug-gable targets within our Boolean network model, which induce apoptosis (D10.5).

**Results**

We set up a web server with a reliable backup system that allows for the storage of the consortium’s data (MS29; D10.1). Due to the heterogeneous nature of the data, a noSQL database was used. This server has a freely accessible area as well as a secured, password-protected area. Additionally, the platform provides access to external databases like Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) and is able to perform basic data analysis (MS30; D10.2) along the consortium’s standard operating procedures for data (pre-)processing.
We developed new approaches for static modeling and successfully applied them to the consortium’s data (Kraus et al., Journal of Statistical Computation and Simulation, 2015; Schmid et al., Bioinformatics, 2016, Lausser et al., Archives of Data Science Series A, 2016; Lausser et al., Neural Processing Letters, 2017).
Based on the data and extensive literature research, we created a Boolean network model to hypothesise about the dysregulation of cofilin-1 in pancreatic cancer. Here, cofilin-1 is highly over-expressed what correlates with high invasiveness and poor prognosis for patients. In contrast, a cofilin-1 knock-out in pancreatic cancer cells was associated with cell growth inhibition, attenuation of the G1- to S-phase transition, but no induction of apoptosis. All these experiments generate many questions: 1) How is cofilin-1 over-expressed in pancreatic cancer? 2) How cofilin-1 influences the cell cycle? 3) Why is there no induction of apoptosis? With our Boolean network approach, we addressed all these questions and integrated the hypotheses of dysregulation into a single model. Simulations of this model reproduce the observations from the experiments. Additionally, the model could be supported by further laboratory validation experiments (unpublished).
To reduce the modelling effort, we developed a new tool for modelling, visualization, and simulation of Boolean networks. This Java-based framework, called ViSiBooL (Schwab et al., Bioinformatics, 2017), aims at a straightforward and easy-to-use modelling tool. ViSiBooL can predict local attractors from given initial states and the changing dynamics after knocking out and/or over-expressing components of interest can be observed. Additionally, ViSiBooL allows modelling of processes on different timescales using temporal predicates. The main purpose of the temporal logic is to support latency periods between gene expression and the observation of its product.
We extended the basic ViSiBooL framework with advanced functionality for *in-silico* experiments to screen for potential drug targets (Schwab & Kestler, Frontiers in Physiology, 2018). Using attractor search algorithms based on Boolean satisfiability and a parallelized thread-pool, ViSiBooL features multiple perturbation experiments (knock-out or over-expression of components) by its one to search for possible drug-gable targets. Here, the user can specify changes in the dynamics of the network, such as the removal of a specific phenotype. Based on a user-defined set of components of interest, the “drug-simulator” generates all perturbations and combinations of perturbations and returns sets of perturbations that lead to the desired change in the network’s dynamics. This method qualifies to widely screen over a large set of *in-silico* experiments, which is a time-consuming or even impossible process to do manually.
This “drug-simulator” was applied for our newly developed Boolean network model describing the dysregulation of cofilin-1 in pancreatic cancer to screen for possible druggable targets. The simulation identified a set of proteins, which are likely to induce

apoptosis in the context of our model. This set included the proteins CD44 molecule (CD44), signal transducer and activator of transcription 3 (Stat3) and twist family bHLH transcription factor 1 (Twist1). Further screens for combinations of targeted proteins revealed in many cases combinations with aurora kinase A (AURKA) or p21 activated kinase 1 (PAK1) as targets to induce apoptosis. Further evaluations with the R-package BoolNet (Müssel et al., Bioinformatics, 2010) revealed that a single knock-out of one of these proteins induces apoptosis for the majority of cases while the rest of cases show an attractor of an unstimulated cell (unpublished). Consequently, all these proteins are considered candidates for drug-targets.

**Conclusion**

The storage and analysis of high-throughput data is of immense importance. Furthermore, the data are accessible for all members of the consortium and will remain accessible after the funding period.
We were able to show that modeling via Boolean networks can reproduce the observations from pancreatic cancer and cofilin-1 knock-down cells. Additionally, our hypotheses about dysregulated pathways have further been supported by laboratory experiments.
New tools and algorithms for the analysis of Boolean networks were the main focus of this work package. With the Java-framework ViSiBooL we provide a light-weight tool to model and simulate Boolean networks in a visual representation. Using ViSiBooL does not require programming skills. The included drug-simulator enables to investigate the changing behavior of the modeled system after knocking-out or over-expressing components of interest. Additionally, a screening for potential targets in the system is implemented. Based on this tool, we identified targets to induce apoptosis in our in-silico cancer model. Three of the potential druggable targets are already in clinical trials (clinicaltrials.gov), two of which are tested for pancreatic cancer patients. The analysis of our model not only predicted three drug targets already in clinical trials which attests to the predictive capability of the model but also two new proteins to be evaluated.

**References**


1. Schwab, J., Burkovski, A., Siegle, L., Müssel, C. & Kestler, H.A., 2017. ViSiBooL-visualization and simulation of Boolean networks with temporal constraints. *Bioinformatics*, 33(4), pp.601–604.
2. Schwab, J.D. & Kestler, H.A., 2018. Automatic Screening for Perturbations in Boolean Networks. *Frontiers in Physiology*, 9, p.1275.
3. Müssel, C., Hopfensitz, M. & Kestler, H.A., 2010. BoolNet – an R package for generation, reconstruction and analysis of Boolean networks. *Bioinformatics*, 26(10), pp.1378-1380.
4. Völkel G, Lausser L, Schmid F, Kraus JM, Kestler HA. Sputnik: ad hoc distributed computation. **Bioinformatics** 31(8): 1298-301, 2015.
5. Kraus JM, Lausser L, Kestler HA. Exhaustive k-nearest neighbour subspace clustering. **J Stat Comput Simul** 85(1): 30-46, 2015.
6. Lausser L, Schmid F, Platzer M, Sillanpää M, Kestler HA. Semantic multi-classifier systems for the analysis of gene expression profiles. **Archives of Data Science Series A** 1(1), 157-176, 2016.
7. Schmid F, Schmid M, Müssel C, Sträng JE, Buske C, Bullinger L, Kraus JM, Kestler HA. GiANT: gene set uncertainty in enrichment analysis. **Bioinformatics** 32(12): 1891-1894, 2016.
8. Lausser L, Szekely R, Schirra LR, Kestler HA. The Influence of Multi-class Feature Selection on the Prediction of Diagnostic Phenotypes. **Neural Process Lett**, 2017.


**WP11: Dissemination**

**Partners involved:** concentris, UniMar, (all partners)

**Total WP progress** 100%

The overall aim of WP11 was to increase the visibility of CAM-PaC by reaching out to the scientific community, industry, patient organisations and other interested or potential stakeholders.

**Objectives:**

1. Make CAM-PaC known to the scientific community and the public
2. Disseminate the results to the scientific community in the academic, healthcare and pharmaceutical sectors and foster interaction and exchange with the scientific community and the public
3. Identify and valorise the intellectual property rights (IPR) generated in CAM-PaC
4. Explore possibilities to conduct clinical trials to overcome therapy resistance in pancreatic cancer

**Results:**

**Logo, website, corporate identity**
The CAM_PaC logo as well as the project website (www.cam-pac.eu) including a password-protected internal part and presentation slides have been created at the beginning of the project. The design elements appeared on all documents and helped to increase the visibility of the CAM-PaC project. The website has been updated on a regular basis and all publications as well as the project flyers can be found there.

**Dissemination for scientists**
~90 publications have been submitted to and accepted by peer-reviewed journals. 68 % of the publications are open access publications. ~150 oral presentations to a scientific event have been held by CAM-PaC partners. ~50 posters have been shown by CAM-PaC partners.

**Patents:**
Title of patent: Glucocorticoid-based gene regulation system
Application reference: WO2017134137
Owner: Polygene AG - URL

### WP12: Project Management

**Partners involved:** UniMar, concentris

**Total WP progress** 100%

Effective project management is a central element of successful research. This is because large research projects often entail a lot of administrative work, which needs to be dealt in an efficient and timely manner. In view of this, the purpose of WP12 was project management for the CAM-PaC project. This WP took care of all administrative and coordinating tasks.
To ensure compliance by beneficiaries with their obligations under the grant agreement, the project management office at concentris routinely supported the Coordinator in monitoring the partners' performance based upon the following:

- To make sure that tasks assigned to them were correctly and timely performed.
- Reports were submitted according to the guidelines and on time.
- Funds were used and claimed according to the rules.
- The partners fulfilled their obligations regarding dissemination, funding acknowledgements and intellectual property rights.
- Any changes to the work plan were communicated to the European Commission (EC) efficiently.
- Compliance to ethical regulations.

The Project management office acted as a helpdesk for all participants. It was the central node of communication on a day-to-day basis and communicated with the European Commission on behalf of the Coordinator regarding administrative and managerial issues (i.e. contract, amendments, reportings etc.).

# 4 The potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and exploitation of results

## 4.1 Socio-economic impact and the wider societal implications of CAM-PaC

Pancreatic ductal adenocarcinoma (PDAC) is the fourth most common cause of cancer-related deaths with the lowest survival rates of any major cancer type. Moreover, epidemiological data from the United States show that pancreas cancer incidence is related to the obesity epidemic, increased approximately 1.2% per year from 1999 to 2008 against the general trend of major cancers, and is projected to double by 2030. Conventional diagnostic and therapeutic approaches have so far failed to positively impact the disease. Since advanced PDAC is inherently resistant to most available therapies, most patients die four to six months after diagnosis. Furthermore, many patients suffer from rapidly declining performance and inanition. Thus, PDAC represents a major socioeconomic and humanitarian challenge to society. Innovative diagnostic and therapeutic approaches based on genes and gene networks involved in tumour progression and therapy resistance identified in high throughput genome analyses are urgently needed and will have a great impact on prolonging survival and reducing suffering of pancreatic cancer patients.
Though ample genome-wide data for this complex and heterogenic disease is available, progress towards the exploitation of these data to develop novel targeted approaches has been slow and examples for a successful clinical translation are scarce. This may in part be due to the lack of appropriate tools for a systematic in vitro and in vivo characterisation of potential PDAC

target genes. Given the long-standing history of futile efforts to identify novel therapeutic targets in pancreatic cancer cells, it is time to integrate efforts in academia and enterprises on an international basis to overcome this urgent and vital clinical dilemma. CAM-PaC represents a coordinated effort to structure and integrate the Europe-wide academic and SME-research into tumour progression and therapy resistance of pancreatic cancer, which will have a major impact on the development of future therapeutic strategies. The programme was designed to generate and use various animal and cellular models to systematically discover and ascribe functions of genes and gene products identified in genome wide analyses. This contributed to a better understanding of the disease, furnish a portfolio of new and validated therapeutic targets, compounds and therapeutic strategies for PDAC and served as the basis for a translation into clinical applications. Apart from the direct benefits for pancreatic cancer patients outlined above, CAM-PaC has and will impact a number of important areas:

- Establishing a structured and comprehensive research platform for a systematic functional characterisation and validation of pancreatic cancer target genes clearly requires pan-European collaboration and was unprecedented so far. Given the relatively low incidence of pancreatic cancer, European groups have by necessity in the past needed to collaborate in order to get access to adequate numbers of patients and clinical samples. As a consequence, pockets of specialist expertise have developed in individual sites which are only viable with continuing collaboration. Many of the groups involved in CAM-PaC have a long tradition of working together; these collaborations saw a much-needed further strengthening by the inclusion of other groups with essential technologies and abilities in CAM-PaC.
- The expected knowledge gain in this programme is considered to be of the utmost importance in many forms of translational tumour research. Therefore, the programme can provide leverage in academic research, increasing competitiveness of the European Research Area in a global context.
- A central purpose of the programme was to stimulate innovation by ensuring close and productive interactions between groups with expertise in a range of areas that we consider central for progress in cancer research and medicine. The participating groups individually have strong track records in various aspects of innovative, translational research which are shared among the whole consortium by cross-utilising numerous in vitro and in vivo platforms and resources such as:
  a. genetically engineered mouse models as preclinical in vivo platforms,
  b. large collections of clinical samples from pancreatic cancer patients,
  c. collections of xenografts from primary pancreatic tumours as in vivo platform
  d. various in vitro models for medium- to high-throughput analyses

- The combination of multiple levels of expertise from 10 leading groups and SME from 5 European countries has created a lasting network that will foster reverse-translation of clinical findings in the genetically engineered mouse models, the development of novel therapeutic strategies targeting genes or gene networks identified in HT-genome wide analyses and the rapid translation into clinical applications.
- CAM-PaC has generated in vitro and in vivo models for systematic functional characterisation of target genes that will be of enormous value for the scientific community and pharmaceutical industry as models for cross-sectional and longitudinal studies of carcinogenesis in the pancreas and other poor prognosis tumours, and to test or validate diagnostic and therapeutic approaches.
- One further benefit of the breadth of expertise among the partners brought together in this programme is the wide dissemination of radically new technologies among our respective scientific colleagues, widely distributed both geographically and by research fields. We will also be well placed to interact and mediate contacts with a considerable proportion of the European biotechnology industry, and with application areas, primarily in healthcare but also in other areas. The clinical partners in this collaborative project have key positions in national and international societies such as the cancer societies, clinical societies and national health care organisations, as well as in the editorial boards of the leading scientific journals in the field.
- The broad research programme of CAM-PaC was therefore expected to provide important added value at the European level, overcoming fragmentation, by creating a critical mass which would be difficult or impossible to achieve at the level of individual universities or countries. The groups of the consortium both from academic settings and from SME combined a broad expertise for a systematic characterisation of pancreatic cancer target genes that is unprecedented so far.
- In WP7, expert molecular pancreatic pathologists have provided histopathological and molecular characterisation as well as SOPs for handling and exchange of materials from primary tumour xenografts and engineered mouse models. Thereby, CAM-PaC has set the pace for a standardisation, harmonisation and common ontology of novel animal models of PDAC generated at different research sites.

- Exploitation of the technologies developed CAM-PaC in research, healthcare, and the pharmaceutical industry is central to the purposes of this research programme. The collaboration between academic and SME partners has stimulated the expedient transfer of IP generated in academia and can also promote the establishment of new SMEs.
- Novel compounds targeting genes or gene networks to be used alone or in combination with standard cytotoxic drugs such as gemcitabine have been identified and will be among the translational healthcare applications generated in CAM-PaC.
- WP10 was dedicated to standardised data gathering, warehousing, processing, statistical and bioinformatic analyses and modelling by a group experienced in systems biology and bioinformatics. Static and dynamic bioinformatic modelling of HT-data generated in vivo and in vitro models of gene networks and contributed to identify predictive subgroups of patients e.g. in order to predict those patients who are likely to respond to a certain treatment. CAM-PaC thus provided additional benefit for pancreatic cancer patients by generating models, data and compounds as a basis for the development of personalised treatment schedules. In the future, this may avoid unnecessary treatments and thus have an impact on important aspects such as safety and well-being of pancreatic cancer patients and the rationale use of health care resources.
- With its translational approach, CAM-PaC has provided the basis to develop new molecular targeted therapies. This will open new markets and will consequently contribute to the generation of new jobs.

## 4.2 The main dissemination activities of CAM-PaC

### 4.2.1 Publications

Within CAM-PaC, ~90 publications have been accepted by peer-reviewed journals. The 15 most important ones can be found below. An exhaustive list of CAM-PaC publications can be found in SESAM (final report).


1. **Intracellular autofluorescence: a biomarker for epithelial cancer stem cells.** Irene Miranda-Lorenzo, Jorge Dorado, Enza Lonardo, Sonia Alcala, Alicia G Serrano, Jenifer Clausell-Tormos, Michele Cioffi, Diego Megias, Sladjana Zagorac, Anamaria Balic, Manuel Hidalgo, Mert Erkan, Joerg Kleeff, Aldo Scarpa, Bruno Sainz, Christopher Heeschen. Nature Methods volume 11, pages 1161–1169 (2014). doi: 10.1038/nmeth.3112.

2. **Chloroquine Targets Pancreatic Cancer Stem Cells via Inhibition of CXCR4 and Hedgehog Signaling**. A. Balic, M. D. Sorensen, S. M. Trabulo, B. Sainz, M. Cioffi, C. R. Vieira, I. Miranda-Lorenzo, M. Hidalgo, J. Kleeff, M. Erkan, C. Heeschen. Molecular Cancer Therapeutics; Vol. 13/Issue 7, pages 1758-1771. American Association for Cancer Research Inc. (2014). doi: 10.1158/1535-7163.MCT-13-0948.

3. **ISG15 Is a Critical Microenvironmental Factor for Pancreatic Cancer Stem Cells**. B. Sainz, B. Martin, M. Tatar , C. Heeschen, S. Guerra. Cancer Research Vol. 74/Issue 24, pages 7309-7320; American Association for Cancer Research Inc. (2014). doi: 10.1158/0008-5472.CAN-14-1354.

4. **Next-generation metabolic imaging in pancreatic cancer**. Rickmer F Braren, Jens T Siveke. Gut 2016;65:367-369. doi: 10.1136/gutjnl-2015-310518.

5. **Combined inhibition of BET family proteins and histone deacetylases as a potential epigenetics-based therapy for pancreatic ductal adenocarcinoma**. Pawel K Mazur et al. Nat Med. Pages 1163-71 (2015). doi: 10.1038/nm.3952.

6. **Site-specific methylation of Notch1 controls the amplitude and duration of the Notch1 response**. K. Hein, G. Mittler, W. Cizelsky, M. Kuhl, F. Ferrante, R. Liefke, I. M. Berger, S. Jus , J. E. Strang, H. A. Kestler, F. Oswald, T. Borggrefe. Science Signaling; Vol. 8/Issue 369 (2015). doi: 10.1126/scisignal.2005892.

7. **MiR-93 Controls Adiposity via Inhibition of Sirt7 and Tbx**. Michele Cioffi, Mireia Vallespinos-Serrano, Sara M. Trabulo, Pablo Jose Fernandez-Marcos, Ashley N. Firment, Berta N. Vazquez, Catarina R. Vieira, Francesca Mulero, Juan A. Camara, Ultan P. Cronin, Manuel Perez, Joaquim Soriano, Beatriz G. Galvez, Alvaro Castells-Garcia, Verena Haage, Deepak Raj, Diego Megias, Stephan Hahn, Lourdes Serrano, Anne Moon, Alexandra Aicher, Christopher Heeschen. Cell reports. Vol. 12/Issue 10, pages 1594-1605. doi: 10.1016/j.celrep.2015.08.006.

8. **PLAC8 Localizes to the Inner Plasma Membrane of Pancreatic Cancer Cells and Regulates Cell Growth and Disease Progression through Critical Cell-Cycle Regulatory Pathways**. B. P. Kaistha, H. Lorenz, H. Schmidt, B. Sipos, M. Pawlak, B. Gierke, R. Kreider, B. Lankat-Buttgereit, M. Sauer, L. Fiedler, A. Krattenmacher, B. Geisel, J. M. Kraus, K. K.

Frese, S. Kelkenberg, N. A. Giese, H. A. Kestler, T. M. Gress, M. Buchholz. Cancer Research; Vol. 76/Issue 1, pages 96-107 (2016). doi: 10.1158/0008-5472.CAN-15-0216.

9. **RASSF1 tumor suppressor gene in pancreatic ductal adenocarcinoma: correlation of expression, chromosomal status and epigenetic changes**. Eliana Amato, Stefano Barbi, Matteo Fassan, Claudio Luchini, Caterina Vicentini, Matteo Brunelli, Giuseppe Malleo, Aldo Scarpa, Giorgio Malpeli. BMC Cancer; 16:11. doi: 10.1186/s12885-016-2048-0.

10. **Genomic analyses identify molecular subtypes of pancreatic cancer**. Peter Bailey, David K. Chang, Katia Nones, Amber L. Johns, Ann-Marie Patch, Marie-Claude Gingras, David K. Miller, Angelika N. Christ, Tim J. C. Bruxner, Michael C. Quinn, Craig Nourse, L. Charles Murtaugh, Ivon Harliwong, Senel Idrisoglu, Suzanne Manning, Ehsan Nourbakhsh, Shivangi Wani, Lynn Fink, Oliver Holmes, Venessa Chin, Matthew J. Anderson, Stephen Kazakoff, Conrad Leonard, Felicity Newell, Nick Waddell, Scott Wood, Qinying Xu, Peter J. Wilson, Nicole Cloonan et al. Nature; Vol. 531/Issue 7592, pages 47 – 52 (2016). doi: 10.1038/nature16965.

11. **Wnt signalling modulates transcribed-ultraconserved regions in hepatobiliary cancers**. Pietro Carotenuto, Matteo Fassan, Rosantony Pandolfo, Andrea Lampis, Caterina Vicentini, Luciano Cascione, Viola Paulus-Hock, Luke Boulter, Rachel Guest, Luca Quagliata, Jens Claus Hahne, Rachel Ridgway, Tam Jamieson, Dimitris Athineos, Angelo Veronese, Rosa Visone, Claudio Murgia, Giulia Ferrari, Vincenza Guzzardo, Thomas Ronald Jeffry Evans, Martin MacLeod, Gui Ji Feng, Trevor Dale, Massimo Negrini, Stuart J Forbes, Luigi Terracciano, Aldo Scarpa et al. Gut 2017;66:1268-1277. doi: 10.1136/gutjnl-2016-312278.

12. **Expression of DRD2 Is Increased in Human Pancreatic Ductal Adenocarcinoma and Inhibitors Slow Tumor Growth in Mice.** Pouria Jandaghi, Hamed S. Najafabadi, Andrea S. Bauer, Andreas I. Papadakis, Matteo Fassan, Anita Hall, Anie Monast, Magnus von Knebel Doeberitz, John P. Neoptolemos, Eithne Costello, William Greenhalf, Aldo Scarpa, Bence Sipos, Daniel Auld, Mark Lathrop, Morag Park, Markus W. Büchler, Oliver Strobel, Thilo Hackert, Nathalia A. Giese, George Zogopoulos, Veena Sangwan, Sidong Huang, Yasser Riazalhosseini, Jörg D. Hoheisel. Gastroenterology; Vol. 151/Issue 6, pages 1218-1231 (2016). doi: 10.1053/j.gastro.2016.08.040.

13. **ViSiBooL—visualization and simulation of Boolean networks with temporal constraints**. Julian Schwab, Andre Burkovski, Lea Siegle, Christoph Müssel, Hans A. Kestler. Bioinformatics, 33(4), pages 601–604, (2016). doi: 10.1093/bioinformatics/btw661.

14. **DNMT1 Inhibition Reprograms Pancreatic Cancer Stem Cells via Upregulation of the miR-17-92 Cluster**. S. Zagorac, S. Alcala, G. Fernandez Bayon, T. Bou Kheir, M. Schoenhals, A. Gonzalez-Neira, M. Fernandez Fraga, A. Aicher, C. Heeschen, B. Sainz. Cancer Research, Vol. 76/Issue 15, pages 4546 -4558 (2016). doi: 10.1158/0008-5472.CAN-15-3268.

15. **The VAR2CSA malaria protein efficiently retrieves circulating tumor cells in an EpCAM-independent manner**. Mette Ø. Agerbæk, Sara R. Bang-Christensen, Ming-Hsin Yang, Thomas M. Clausen, Marina A. Pereira, Shreya Sharma, Sisse B. Ditlev, Morten A. Nielsen, Swati Choudhary, Tobias Gustavsson, Poul H. Sorensen, Tim Meyer, David Propper, Jonathan Shamash, Thor G. Theander, Alexandra Aicher, Mads Daugaard, Christopher Heeschen, Ali Salanti. Nature Communications; Vol. 9/Issue 1 (2018). doi: 10.1038/s41467-018-05793-2.

### 4.2.2 Organisation of a CAM-PaC symposium

The CAM-PaC consortium organised an "EU FP7 CAM-PaC symposium" on 10th October 2018 in Verona, Italy. The symposium was chaired by the coordinators of the University of Marburg, Prof. Dr. Thomas Gress and Prof. Dr. Malte Buchholz. Five international experts have been invited as speakers and was attended by 76 persons:

- **Dr. Giulia Biffi** – Cold Spring Harbor Laboratory, NY, USA: **"Organoid and *in vivo* models of PDAC-CAF interactions"**
- **Dr. Paola Cappello** – University of Turin, Italy: **„Harnessing the tumour microenvironment to improve vaccine efficacy in pancreatic cancer"**
- **Dr. Marine Kraus** – Nestlé Institute of health Sciences SA, Lausanne, Switzerland: **„Humanized models for preclinical metabolic studies"**
- **Dr. Jennifer Morton** – CRUK Beatson Institute, Glasgow, UK: **„Modelling the genomic landscape of pancreatic cancer"**
- **Dr. David Chang** – Institute of Cancer Sciences, university of Glasgow, UK: **"The use of next generation preclinical models to inform biomarker-driven clinical trials in pancreatic cancer"**
- **Prof. Aldo Scarpa,** Università Degli Studi di Verona (Italy)*:* **Next generation histopathological diagnosis"**

### 4.2.3 Organisation of workshops for scientists & for civil society

1. UNIVERSITA DEGLI STUDI DI VERONA: Anatomia patologica e biologia molecolare nel carcinoma della mammella e del pancreas. 10/10/2014. Palermo. Audience: Scientific community (higher education, Research – Italy)
2. QUEEN MARY UNIVERSITY OF LONDON: Novel Precision Medicine Approaches for Pancreatic Stem Cells. 28/01/2016. Barts Cancer Institute, QMUL, London UK. Audience: Scientific community (higher education, Research) - Civil society, 30 pax – UK)
3. QUEEN MARY UNIVERSITY OF LONDON: Novel Precision Medicine Approaches for Pancreatic Stem Cells. 12/04/2016. Barts Cancer Institute, QMUL, London, UK. Audience: Scientific community (higher education, Research) - Civil society-Civil society, 30 pax – UK)
4. QUEEN MARY UNIVERSITY OF LONDON: Novel Precision Medicine Approaches for Pancreatic Stem Cells. 22/07/2016. Barts Cancer Institute, QMUL, London, UK. Audience: Scientific community (higher education, Research) - Civil society-Civil society, 30 pax – UK)

### 4.2.4 Flyers

1. concentris Research Management GmbH: CAM-PaC project flyer. 15/12/2014. Audience: Scientific community (higher education, Research) - Industry - Civil society - Policy makers – Medias, international
2. CEGAT GMBH: CAM-PaC Flyer. 11/12/2017. CeGaT Tübingen, Germany. Audience: Scientific community (higher education, Research)

### 4.2.5 Press releases

1. PHILIPPS UNIVERSITAET MARBURG: Dem Krebs auf der Spur, 18/11/2013. Uni Marburg, Medias, Germany
2. Concentris Research Management GmbH: Overcoming the standstill in pancreatic cancer research. 15/12/2016. CAM-PaC Website. Audience: Scientific community (higher education, Research) - Industry - Civil society - Policy makers – Medias, International

### 4.2.6 Website

concentris Research Management GmbH: www.cam-pac.eu. 28/02/2014. Audience: Scientific community (higher education, Research) - Industry - Civil society - Policy makers – Medias, International

### 4.2.7 Webinar

PHILIPPS UNIVERSITAET MARBURG: WEBINAR: The essential role of miRNA and mRNA on cancer progression and biomarker discovery. 15/11/2017. https://www.labroots.com/webinar/essential-role-mirna-mrna-cancer-progression-taqman. Audience: Scientific community (higher education, Research), International

### 4.2.8 Oral presentations

~150 oral presentations to a scientific event have been held by CAM-PaC partners. The exhaustive list of presentations can be found in SESAM (final report).

1. QUEEN MARY UNIVERSITY OF LONDON: Stem cells: Understanding the origins of pancreatic cancer. 02/02/2014 . Banff, Canada. Audience: Scientific community (higher education, Research), International.
2. UNIVERSITAET ULM: Linear Contrast Classifiers in High-Dimensional Spaces (ANNPR 2014). 06/10/2014. Montreal, Canada. Audience: Scientific community (higher education, Research), International.
3. KLINIKUM RECHTS DER ISAR DER TECHNISCHEN UNIVERSITAT MUNCHEN: Novel treatment strategies in RAS-driven pancreatic and lung cancer. 12/10/2015. Heidelberg, Germany. Audience: Scientific community (higher education, Research), International.
4. UNIVERSITA DEGLI STUDI DI VERONA: Digital sorting of 100% pure cancer cells: Resolving heterogeneity in FFPE samples and liquid biopsies. 19/04/2016. New Orleans, USA. Audience: Scientific community (higher education, Research), International.
5. QUEEN MARY UNIVERSITY OF LONDON: Intrinsic and extrinsic determinants of pancreatic cancer stem cells. 07/01/2016. The Function of Tumor Microenvironment in Cancer Progression, San Diego, USA. Audience: Scientific community (higher education, Research), International.
6. PHILIPPS UNIVERSITAET MARBURG: Funktionelle Bedeutung der Überexpression von Plac8 in neuroendokrinen Pankreastumoren. 13/09/2017. DGVS Dresden, Germany. Audience: Scientific community (higher education, Research), Germany.
7. UNIVERSITA DEGLI STUDI DI VERONA: Cancer heterogeneity and molecular diagnostics. 12/05/2017. Washington, USA. Audience: Scientific community (higher education, Research), International.
8. QUEEN MARY UNIVERSITY OF LONDON: Novel Precision Medicine Approaches for Pancreatic Cancer Stem Cells. 12/02/2017. Lucca, Italy. Audience: Scientific community (higher education, Research), International.
9. DEUTSCHES KREBSFORSCHUNGSZENTRUM: Epigenetic characterization in PDAC. 28/04/2017. Heidelberg, Germany. Audience: Scientific community (higher education, Research), Germany.
10. UNIVERSITEIT LEIDEN: Studying individual differences in cancer drug efficacy using cell lines derived from patients. 02/07/2015, San Francisco, US. Audience: Scientific community (higher education, Research), International.

### 4.2.9 Posters

~50 posters have been shown by CAM-PaC partners. The exhaustive list of posters can be found in SESAM (final report).

1. QUEEN MARY UNIVERSITY OF LONDON: Mitochondrial metabolism, the Achilles heel of pancreatic cancer stem cells. 18/05/2014. New Orleans, LA. Audience: Scientific community (higher education, Research), International.
2. MIMETAS BV: A novel high throughput personalized medicine platform using pancreatic ductal adenocarcinoma derived organoids in the OrganoPlate®. 13/10/2016. Heidelberg, Germany. Scientific community (higher education, Research), International.
3. DEUTSCHES KREBSFORSCHUNGSZENTRUM: Use of Diffusion-weighted magnetic resonance imaging for therapy response evaluation in pancreatic cancer. 21/02/2016. 32nd German Cancer Congress, Berlin, Germany. Audience: Scientific community (higher education, Research), International.
4. PHILIPPS UNIVERSITAET MARBURG: FUNCTIONAL RELEVANCE OF THE OVEREXPRESSION OF PLAC8 IN NEUROENDOCRINE PANCREATIC TUMORS. 28/10/2017. UEGW Barcelona, Spain. Audience: Scientific community (higher education, Research), International.
5. POLYGENE AG: Novel Glucocorticoid-based Gene Regulation System. 12/11/2016. EMBL Heidelberg, Germany. Audience: Scientific community (higher education, Research), International.

## 4.3 Exploitation of results of CAM-PaC

**Patents**

Application reference: WO2017134137
Title of patent: GLUCOCORTICOID-BASED GENE REGULATION SYSTEM
URL
Owner: Polygene AG

**Exploitable Foreground**

### 1. Novel target genes in pancreatic cancer

#### 1.1. Novel target genes/pathways in pancreatic cancer stem cells (CSCs):

Pancreatic cancer stem cells display a distinct metabolic phenotype based on their strong dependence on mitochondrial oxidative phospholyration (OXPHOS) and limited metabolic plasticity. While suppression of MYC upstream of PGC-1α represents the key determinant of this phenotype, partner QMUL also identified a subset of CSCs with reduced mitochondrial content, which rather showed resistance to mitochondrial targeting, but could be sensitized by inhibition of MYC, thus potentially offering a new avenue for anti-CSC treatment in pancreatic cancer by a combination of mitochondrial inhibition (e.g., by the well-known drug metformin) and MYC inhibition. Moreover, we demonstrated that PPARdelta is an upstream target of MYC in pancreatic cancer stem cells, and that metabolic reprogramming is driven by activation of PPARdelta and can be prevented by PPARdelta inhibitors.
At the same time, Peptidoglycan recognition protein 1 (Pglyrp1, Tag7), an anti-bacterial protein implicated in the innate immune responses, renders pancreatic cancer stem cells with yet unrecognized immune privilege. Genetic targeting experiments showed that Tag7 is protecting CSCs from lymphocyte cytotoxic effects rendering them more efficient in colonizing distant sites where cells are highly exposed to immune attacks as compared to established lesions where the stroma bears a strong immunosuppressive barrier. We now further demonstrated a striking phenotype for the conditional knockout of Tag7 in pancreatic cancer, and identified neutrophils recruitment as the crucial mechanism of action for Tag7 expression in murine cancer stem cells.
These data provide potential new opportunities for pharmacological intervention, and we have already identified four novel LHR1 inhibitors that are now undergoing further characterisation and optimization. In addition, we have initiated screening of small molecule libraries for novel inhibitors of Tag7 and PPARdelta as part of the consortium's central program for small molecule screening (performed by Dr. Jens v. Kries, Leibniz-Forschungsinstitut fuer Molekulare Pharmakologie, Berlin, Germany).

#### 1.2. Novel target genes/pathways in bulk pancreatic cancer cells:

In previous work, partner UniMar had generated and analysed expression profiles a total of ~2000 pancreatic cancer candidate genes to identify genes with expression patterns suggestive of important roles in tumour cell biology. For a selection of 80 of these candidate genes, first functional data were obtained in parallelized assays using reverse transfection technology. Additional candidates were identified in genome wide synthetic lethality screens using siRNA and shRNA libraries as well as through analysis of data from PDAC genome sequencing approaches among others generated by members (UniVR) in this consortium. Based on these data, two initial sets of candidate genes have been selected within the CAM-PaC project for validation and functional characterisation in vitro: one set contains genes that are potentially druggable and a second set comprises genes that may be involved in tumour promotion via reciprocal stimulation within different cell-types in the tumour microenvironment. A variety of cell-based functional assays were performed to analyse the influence of the expression of our target genes on growth (MTT assays, BrdU assays), apoptosis resistance (PARP cleavage, Caspase 3 cleavage) and chemoresistance (MTT and BrdU assays in the presence of chemotherapeutic agents Gemcitabine or 5-FU) of cultured pancreatic cell lines in vitro.
Data from functional analyses for the candidate genes PLAC8, TTK, and USP5, as well as RNASeq data from CeGaT and uulm (for PLAC8, FASTK and CFL1) has led to the identification signalling cascades with high probability of being involved in the function of these genes in PDAC. In the case of CFL1, a possible involvement in the MAL/SRF signalling pathway is under scrutiny, and the pathway interactions have been modelled by UULM. For Plac8 and FASTK, functional roles in vivo have been confirmed using novel genetically engineered mouse models produced within CAM-PaC. Moreover, cooperation has been established with industrial partner Bayer to test the efficacy of a new small molecule inhibitor against TTK (developed by Bayer) in xenograft and GEMM mouse models.
In addition, screening of small molecule libraries for novel inhibitors of Plac8 and FASTK as part of the consortium's central program for small molecule screening has been initiated (performed by Dr. Jens v. Kries, Leibniz-Forschungsinstitut fuer Molekulare Pharmakologie, Berlin, Germany).

**1.3. Novel target genes/pathways/technologies with special relevance in mouse models:**
The bromodomain and extraterminal (BET) family of proteins (BRD2, BRD3, BRD4, BRDT) are chromatin regulators ("readers"), which recognize acetylated lysines on histones. They are increasingly recognized as important key players in tumour development and progression. One major action is the transcriptional control of oncogenic drivers including MYC among many others in a context-dependent fashion. Partner DKFZ (formerly TUM-MED) has recently found the BET protein member BRD4 to be expressed in pancreatic ductal adenocarcinoma (PDAC), showing that inhibition of BET proteins by the small molecule JQ1 suppresses PDAC development and leads to cell death and inhibition of PDAC growth.
Regarding novel technologies for non-invasive characterization of tumour growth in genetically engineered mouse models, partner DKFZ identified Diffusion Weighted Magnetic Resonance based (DW-MRI) tumour imaging as a suitable method for differentiation of tumour cellularity and stromal content. The Apparent Diffusion Coefficient (ADC) resulting from DW-MR imaging procedure can be used as a direct measurement of tumour cellularity. Highly cellular PDACs in GEMMs show lower ADC values while low cellular and stromal rich tumours show higher ADC values. This finding was directly translational to patients. Furthermore, a subgroup of G2 differentiated tumours with lower cellularity and higher ADC value has been identified as group with better survival profile. All the findings are published by our group in 2016 in Clinical Cancer Research Journal (Heid et al, 2016).

**2. Development of novel transgene technology**

One of the goals of the CAM-PaC project is to generate new mouse models of "classical" pancreatic cancer-associated genes (WP1). Within these new mouse models, it is envisaged that the expression of multiple onco- and tumour suppressor genes, such as Kras, p53 and p16, can be regulated independently of each other in a reversible manner. This requires the development of conceptually novel model systems, including new so-calles "reverse expression systems" that are to be developed by partner PolyGene. In this context, PolyGene has developed a gene expression tool that can be combined with any gene of interest, in order to be regulated reversibly up (increased expression) or down (reduced expression) via addition of an artificial glucocorticoid hormone agonist, RU486 (mifepristone). A patent application covering this system has already been filed.
Additionally, PolyGene identified and validated a first reversed mutant of the erythromycin-dependent mphR(A) system which shows the intended activation of expression in the presence, but not the absence, of erythromycin. While this system has been shown to function in proof-of-principle studies, further work is ongoing in the form of characterization (e.g. examination of potential "leakiness" in different in vitro and in vivo systems) and refinement (targeted mutagenesis to increase range of expression induction upon erythromycin addition). First genetically engineered mouse models carrying this system are currently being analyzed by partner UniMAR. IP protection in the form of a patent application will be sought as early as feasible. Commercialization of the novel system will be seamless, since PolyGene is a commercial provider of transgenic mouse services and will add the novel systems to their commercial portfolio as soon as technical validation and IP protection is completed.

**3. Cooperation with pharmaceutical industry**

The CAM-PaC project interacts very closely with major players in the pharmaceutical industry, thus further accelerating translation of research results into pre-clinical and clinical application. This is most demonstrably visible in the application of proprietary pharmaceutical inhibitors in pre-clinical models of pancreatic cancer within cooperative projects between pharma companies and the CAM-PaC project. As an example, Bayer Healthcare (also represented in CAM-PaC's Scientific Advisory Board in the person of Dr. Anette Sommer) has provided the experimental MEK-inhibitor BAY 86-9766 as well as the TTK/MPS1-inhibitor BAY 1161909 for pre-clinical therapy trials in genetically engineered as well as patient-derived xenograft models of pancreatic cancer within CAM-PaC. Results of these studies will be exploited together with Bayer Healthcare. Further examples are the use of established therapeutic agents such as Metformin for novel combination therapies targeting pancreatic cancer (see above).

**4. Exploration of potential to conduct clinical trials**

While the CAM-PaC partners originally estimated that conducting clinical trials would likely be beyond the scope of the CAM-PaC running time, progress in this arena has been faster than estimated. Two clinical trials have been initiated that include clinical professionals from the CAM-PaC consortium and that are in part based on pre-clinical data acquired within the CAM-PaC project. Both, ongoing trials as well as the potential for additional trials have been described in detail in deliverable reports to the European Commission.

## 5 The address of the project public website, if applicable as well as relevant contact details

The public website of CAM-PaC has been continuously updated, and can be found under the following address:
www.cam-pac.eu.