CHEMORES is an integrated project involving clinicians and scientists in eight European countries. The purpose of the project is to improve cancer treatment by obtaining increased knowledge on mechanisms of chemotherapy resistance.

It is estimated that the two diseases that are studied in CHEMORES, lung cancer and melanoma, caused over 350,000 deaths in Europe in 2002. An important contributing factor in cancer mortality is the fact that the most common types of cancer do not respond well to systemic chemotherapy in the advanced stages. Increased understanding of the underlying processes will contribute to the development of predictors of both therapy response and toxicity, and in the end more efficient and personalised therapy.

During the second year, the project has progressed essentially as planned and there have been extensive interactions between the participants. The main challenge is the collection of clinical samples, which has been met by intensified efforts and identification of new sources of material. In this period, CHEMORES has generated interesting findings in both clinical and pre-clinical studies. The progress in the different workpackages (WPs) are summarised below.

WP1 – Clincal trials on melanoma patients
The main objectives of WP1 is to use clinical data and biobank material from large randomized trials in melanoma patients to study the molecular mechanisms for sensitivity and resistance to interferon (IFN) and DTIC/temozolomide (TMZ). Collection and test of sera for the presence of autoimmune antibodies has been performed in three IFN adjuvant trials. These analyses clearly show that the presence and/or emergence of autoimmune antibodies is not a (strong) prognostic factor and not a predictive factor for IFN-sensitivity and outcome in relation to IFN treatment.

Less biopsies than expected have been performed in a randomized trial in Stage IV melanoma patients comparing daily prolonged scheduling of TMZ *vs*. classic scheduling of DTIC. However, a plan has been put in place with all the CHEMORES melanoma centers, to collect also biopsy samples from other studies, performed in these centers, in patients treated with TMZ or DTIC to obtain sufficient samples to address the originally formulated questions and research program.

WP2 – Clinical trials on lung cancer patients
The overall aim of WP2 is to generate biological material (blood and tumour specimens) and clinical data for patients with non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) who are treated with chemotherapy. A major and challenging goal is to provide paired tissue samples procured before and after chemotherapy for discovery of novel genes and pathways contributing to chemotherapy resistance, therapeutic escape and efficacy. In 2008, 110 patients were recruited in Manchester and in Paris, archival frozen tumour and non-malignant lung tissue was obtained for 122 patients with complete clinical treatment and outcome data. These biological resources have been delivered to the genomics, proteomics and toxicity workpackages. Prospective sample collection will continue in Manchester, Paris and Oxford during 2009.

WP3 – Constitution of a Biological Resources Centre
Efforts during Period 2 have mainly focused on generating the first retrospective collection of lung cancer samples, as quickly as possible. Activities were delayed by the departure of the WP3 leader from the CHEMORES consortium. Deliverables are still expected to be completed according to schedule, although the exact number of patients to be included in the various collections will in some cases have to be redefined.

WP4 – Genome analysis
The main aim of WP4 is to identify molecular genetic mechanisms important for resistance or sensitivity to chemotherapy, using biological specimens from WP1-3. The objective for this year was to start analysing the retrospective lung cancer collection. This work includes identification of differentially expressed genes and microRNAs, characterization of promoter DNA methylation in

relation to therapy and clinical outcome, and screening of mutation status of a panel of genes commonly mutated in cancer.

A pilot study on 16 patients with NSCLC adenocarcinoma and treated with cisplatin and vinorelbin was accomplished. This study demonstrated proof of concept for the study design and methods used, and identified genes related to efficacy of the therapy. All microarrays, sequencing- and methylation-related technologies have been established and validated. The remaining experiments for completing the analyses of the retrospective tissue collection will be delivered on schedule.

WP5 – Proteome analysis

Method development for quantitative proteomics has resulted in the best membrane proteomics coverage reported so far in the literature, with the identification of 500 membrane proteins from a protein coverage of 3700 cellular proteins. Efforts on method improvement continue, with the purpose of gaining even more information rich proteomics data. The proteomic phenotypes of an SCLC cell line resistant to the cytotoxic drug Doxorubicin was analyzed and compared with its sensitive parental cell line. Among the many proteins identified, several have previously been characterized as important factors in chemotherapy induced cell death signalling. This strengthens the notion that the method is well suited for detection of signalling components in chemotherapy resistance. In additional studies, pathway analysis of proteomics data has lead to discovery of global changes in signalling status after DNA damage caused by radiation of different qualities. Studies of potential biomarkers from the S100-family have demonstrated a correlation with NSCLC stage I patient survival. We also showed that S100-proteins can affect degradation of anti-apoptotic and cell growth related proteins.

WP6 – Bioinformatics - Biostatistics

WP6 has designed, implemented and tested a web-based data-entry and data-base of clinical data for the prospective and retrospective lung cancer study. For the high-throughput molecular data, we have started developing the statistical analyses procedures on the pilot study that includes data on surgical cases with and without chemotherapy. The molecular data include comparative genomic hybridization (CGH) with Agilent 240K array, gene-expression data with Agilent 240K exon-array, and microRNA expression data.

WP7 – Animal tumor models

The objective of WP7 is to use established animal tumour models that reproduce the natural history of malignant melanoma, NSCLC and SCLC, to reveal molecular mechanisms responsible for chemotherapy resistance. All the compound strains for the NSCLC have been generated. We have implemented PET-CT as a non invasive technique to monitor lung tumor progression. This implementation will be essential for genetic validation studies of targets for therapeutic intervention.

In the *Adeno-Cre, $Rb^{F/F}$, $p53^{F/F}$* mouse model for SCLC we have treated spontaneous carcinomas of the lung with the maximum tolerated dose of cisplatin. Unfortunately, we only found a short increase in survival without substantial tumor shrinkage. After consultation with our clinical CHEMORES partners we decided that this is a substantial difference to SCLC in humans where tumors are initially very sensitive to platinum drugs and acquire resistance once tumors relapse from residual disease.

Regarding cisplatin resistance, we could show that irreversible defects of the *Brca1* or *Brca2* genes, which are essential for homology-directed DNA repair, impair the development of cisplatin resistance *in vivo*. In patients with BRCA1- or BRCA2-associated cancers genetic reversion of a frameshift mutation can restore BRCA function and result in acquired resistance. Our results suggest that other cisplatin resistance mechanisms than genetic reversion can not compensate the severe loss of BRCA function. Intriguingly, *Brca1* or *Brca2*-deficient tumors could hardly be eradicated, not even by combining cisplatin with a targeted PARP inhibitor. Future experiments will address which mechanisms cause the survival of remnant tumor cells.

WP8 – Lung cancer and melanoma stem cells

Since cancer initiating cells may be inherently resistant to the cytotoxic effect of chemotherapy, the identification of distinct phenotypic and functional markers associated with stem-like properties would

be instrumental for developing therapeutic targeting strategies in cancer patients. In lung cancer we found that a $CD133^+/ESA^+$ population is increased in primary NSCLC samples compared to normal lung tissue. $CD133^+$ cells isolated from tumors showed higher tumorigenic potential than the $CD133^-$ counterpart and were able to reproduce the original tumor heterogeneity in SCID mice. This phenotype was associated with an increased expression level of genes involved in stemness, adhesion and motility. Also, enrichment in several ABC transporters was noticed in $CD133^+$ cells compared to the $CD133^-$ fraction. Preclinical *in vitro* and *in vivo* models have been developed, including xenografts from primary lung tumors of patients. Cisplatin treatment resulted in enrichment of $CD133^+$ cancer initiating cells in both types of models.

In melanoma, cells obtained both from fresh tumor specimens and cell lines were cultured in Stem Cell Medium. Cells growing as melanospheres and displaying self-renewing capacity and multipotency were established. These cells had strong tumor initiating potential. Expression of stem cell and neural crest-associated markers was revealed. However, no direct correlation between any of these markers and the ability to form tumor in nude mice was evidenced. These data indicate that melanomas do contain cells endowed with cancer stem cell properties (self renewal, clonogenicity, multipotency and tumor initiating capacity), although further studies are needed to elucidate molecular and biological features of this subset of cells composing the tumor mass.

WP9 – Novel therapeutic strategies

A set of compounds with lysosomal mechanisms of action with regard to induction of apoptosis have been identified by screening. The most interesting compound has been proven to be an inhibitor of ubiquitin isopeptidases. This compound induces apoptosis of drug resistant cells and of cells overexpressing Bcl-2 and is currently being tested *in vivo.*

We have identified an interacting partner to cathepsin-D which is a good candidate for apoptosis induction. In addition, a chemical biology project aimed to identify compounds that induce AKT insensitive apoptosis has been completed. Most compounds identified proved to be mitotic inhibitors. Also, a novel topoisomerase inhibitor which is insensitive to MDR1 and MRP has been identified. This agent induces apoptosis of multicellular spheroids.

Proton pump inhibitors (PPIs) are tested with the aim of increasing tumor pH and to improve therapy results. *In vivo* experiments using SCID mice with human melanoma xenograft have shown that the minimal effective dose of PPIs is 2.5 mg/kg. An NMR-based *in vivo* imaging technique allowing *in vivo* measurements of tumor pH has been developed. The results clearly show that the PPI anti-tumor effects are consistent with a lowering of the pH gradient between the external and the internal microenvironments.

WP10 – Mechanisms involved in resistance to DNA damaging drugs

DNA repair assays, along with two DNA damage assays, have been established and an additional assay identified as being important is being developed. An alternative approach to the inhibition of alkylating agent-induced DNA repair has been initiated. Data on clinical samples, both peripheral blood mononuclear cells and tumour biopsies, have been generated. The DNA repair inhibitor lead compounds reported previously are disappointing in *in vitro* assays. Dual DNA repair inhibitors are in early assessment. A method has been developed for MGMT promoter methylation detection, also in paraffin embedded tumours. We have also identified histone deacetylase and insulin-like growth factor receptor inhibitors as therapeutic targets for modulating DNA damage response.

Candidate genes identified by gene expression profiling of melanoma tumours from responders and non-responders to TMZ/DTIC have been validated in two independent materials. As a follow up of these results, the role of melanosomes and their associated proteins in melanoma chemoresistance are studied in tumours and in cell lines with high and low number of melanosomes.

WP11 – Apoptosis regulation

WP11 deals with the identification of biomarkers that allow to predict chemotherapy resistance and susceptibility, as well as with the exploration of novel therapeutic targets for subverting chemotherapy

resistance in cancer. The overall strategy to achieve these long-term goals consists in a mixture of systems biology methods and hypothesis-driven approaches. For this, *in vitro* experiments are performed on cultured human cell lines exposed to chemotherapeutic agents. The consortium has been extremely successful in exploring chemotherapy-induced cell death (which is the therapeutic goal of anti-cancer therapy) and all milestones and deliverables that have been outlined in the project have been achieved, without any deviation from the plan. Several candidate biomarkers and targets have been identified.

WP12 - Studies of markers of individual chemotherapy-induced toxicity
The over all aim of WP12 is to clarify the role of constitutional genetic variability for the toxicity of chemotherapy in patients with NSCLC and SCLC. Work during Period 2 has focused on identifying genes of interest, establishment of analytical techniques and genotyping of the first patient materials received. Lists of genes have been prepared from literature searches on mechanisms and pathways of potential importance for toxicity of platinum analogues, gemcitabine and etoposide. In addition, bioinformatics searches have been performed in meta-analyses of expression microarray studies of the same drugs. Biogroups/biochemical pathways affected by these compounds have also been identified. SNPs in these genes will be screened in the first cohort of patients and correlated primarily to the myelosuppression in the first chemotherapy cycle. Genotyping has been initiated.

To validate the bioinformatics approach we have performed a similar meta-analysis on treatment of ovarian cancer with paclitaxel and carboplatin. 10 genes potentially important for myelotoxicity and neurotoxicity have been identified and 42 SNPs in these genes are being identified in a cohort of ovarian cancer patients with toxicity recorded prospectively.

PLAN FOR USING AND DISSEMINATING THE KNOWLEDGE
The knowledge produced within CHEMORES may be exploitable. Any innovations with such potential will be aided by the Technology Transfer Work Group which has put together a Technical Implementation Plan for the project.

With regard to dissemination to the public and the scientific community, the public website has been updated several times during the period, to provide information about the project to interested parties, including the EC, user groups, other international research groups and the general public. In addition, internal newsletters have been distributed and a web-based knowledge management system is in place to facilitate the daily transfer of information between the participants. The dissemination of knowledge includes publications in scientific journals, presentations at scientific meetings and workshops.

CHEMORES Contractors

Assistance Publique – Hôpitaux de Paris, France
Cancer Research UK/Oxford, United Kingdom
Centro Nacional de Investigaciones Oncológicas, Spain
Erasmus University, Netherlands
Eurogentec, Belgium
European Organization for Research and Treatment of Cancer, Belgium
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Royal Institute of Technology, Sweden
Technische Universität München, Germany
University Hospitals Leuven, Belgium
University of Manchester, United Kingdom

CHEMORES Project Managment

Info mail: chemores@ki.se **Public website:** http://www.chemores.org

Project Coordinator: Johan Hansson, johan.hansson@ki.se

Project Manager: Carolina Johansson, carolina.johansson@ki.se

Project Administrator: Jenny Karte, jenny.karte@ki.se