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, 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 third year, the project has progressed essentially as planned and there have been extensive interactions between the participants. The collection of clinical samples, which has been the main challenge in the project, has largely been completed for the discovery studies and the project is gradually moving into the validation phase. In this period, CHEMORES has generated interesting findings in both clinical and pre-clinical studies and the progress in the different workpackages (WPs) are summarised below:

WP1 and WP3 – Clincal trials on melanoma patients
Work in WP3 overlaps with that of WP1 and WP2. In general work during Period 3 has been focused on finalizing the collection of clinical materials for the discovery phase, and switching the effort to obtaining samples for validation. For melanoma the analyses of multiple sequential serum samples, from melanoma patients in three large randomized trials, demonstrated that emergence of auto-immune antibodies is NOT a strong prognostic factor and is NOT a predictive factor for IFN sensitivity in melanoma. Additional studies showed thath high preoperative serum YKL-40 levels are associated with poor survival in untreated patients, but not in IFN treated patients. Increases in serum YKL-40 during treatment or follow-up is associated with poor prognosis. IL-1β and IL-6 serum levels were determined by using and comparing two different techniques. We could not confirm the predictive value of initial high IL-6 levels nor the importance of IL-1β levels and improved outcome in IFN-treated patients. This study illustrates the complexity of cytokine determination in sera and stresses the importance of validating assays.

WP2 and WP3– Clinical trials on lung cancer patients
The overall aim of WP2 is to generate biological material (blood and tumor 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 to the CHEMORES project and archival frozen tumor 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. In 2009 ~ 210 patients were recruited and a retrospective collection of frozen resected lung tumors was procured. Prospective sample collection will continue during 2010.

WP4 – Genome analysis
During year 3, WP4 has completed the molecular profiling of all samples in the retrospective lung cancer material (NSCLC), with a large panel of system biology approaches including gene expression analysis using a unique exon array with 244 000 probes, microRNA profiling, Comparative Genome Hybridization (CGH) and sequencing of selected relevant genes. The molecular data were delivered according to the planned schedule to WP6 for statistical analysis. The objectives of WP4 in the upcoming period is to complete this molecular data set with next generation sequencing (for all human exons, the exome) and epigenetic screening in a panel of representative patients from the cohort. In

addition, the ongoing gene expression profiling of melanoma biospecimens will be finalized and the initiated validation studies will continue.

WP5 – Proteome analysis
Method development and analysis of lung cancer proteomes have been performed with successful application of multidimensional fractionation on plasma and pleural effusion. This has been achieved using high abundant protein depletion, peptide isoelectric focusing and LC-MS/MS analysis. In addition to improved clinical proteomics, several novel technological methods have been developed and published. We have initiated tissue microarray analyses, applied for both discovery and validation purposes, and the first publication on protein markers has demonstrated a prognostic role of S100A6. Interestingly, results from *in vitro* studies points to S100A6 as a regulator of anticancer therapy response, which could serve as a potential target in cancer therapy. Work is ongoing with tissue proteomics efforts to complement the genomics data generated in WP4 using the retrospective lung cancer cohort, and the project is proceeding according the plan.

WP6 – Bioinformatics - Biostatistics
Collection of molecular data for the 123 retrospective samples from lung cancer patients was completed during 2009. The molecular data include comparative genomic hybridization (CGH) with Agilent 240K array, gene-expression data with Agilent 240K exon-array, and microRNA expression data. All standard analyses of the molecular data, based on analyses of the platforms separately, were performed. Key variables in the clinical data included the dates of relapse and whether or not the patient received chemotherapy. For each platform we developed and implemented statistical analyses for the purpose of identifying the molecular signature of response to chemotherapy, allowing testing whether chemotherapy worked better in certain subgroups defined by molecular data.

WP7 – Animal tumor models
We have unveiled a synthetic lethal interaction between *K-Ras* oncogenes and *Cdk4* in a mouse tumor model that closely recapitulates human NSCLC. Ablation of *Cdk4*, but not *Cdk2* or *Cdk6*, induces an immediate senescence response only in lung cells that express an endogenous K-Ras oncogene. No such response occurs in lungs expressing a single *Cdk4* allele or in other K-Ras expressing tissues. More importantly, targeting *Cdk4* alleles in advanced tumors detectable by computed tomography scanning also induces senescence and prevents tumor progression. These observations suggest that robust and selective pharmacological inhibition of Cdk4 may provide therapeutic benefit for NSCLC patients carrying K-*RAS* oncogenes. A melanoma mouse model carrying a conditional knock-in for the BrafV600E mutation is now also available in the project.

It was found that the responses of the *Adeno-Cre, Rb$^{F/F}$, p53$^{F/F}$* mouse model for SCLC to the maximum tolerable dose of cisplatin differ from what is observed in the clinic. In particular the development of multifocal lung tumors complicates the *in vivo* imaging and monitoring of tumor responses to therapy. We continue to optimize this model by testing orthotopic transplantation of tumor tissues into the lungs of mice. In parallel, the analyses of resistance to cisplatin, topotecan, docetaxel, doxorubicin and the poly(ADP-ribose) polymerase (PARP) inhibitor olaparib in the *K14Cre, Brca1$^{F/F}$, p53$^{F/F}$* and *K14Cre, Brca2$^{F/F}$, p53$^{F/F}$* models were continued.

WP8 – Lung cancer and melanoma stem cells
The identification of tumor-initiating cells and associated markers may be useful for optimization of therapeutic approaches and to provide predictive and prognostic information in lung cancer and melanoma patients. Concerning lung cancer, we have reported the presence of a highly tumorigenic CD133$^+$ subpopulation of cells displaying stem-like features and chemoresistance to conventional drugs in primary NSCLC. We found that a CD133$^+$ESA$^+$ population was increased in primary NSCLC compared to normal lung tissue and displayed higher tumorigenic potential in SCID mice and increased expression of genes involved in stemness, adhesion, motility and drug efflux than the CD133$^-$ counterpart. A tendency towards shorter progression free survival was observed in CD133$^+$ NSCLC patients treated with platinum-containing regimens. These results indicate that chemoresistant populations with highly tumorigenic and stem-like features are present in lung tumors. The molecular

features of these cells may provide the rationale for more specific therapeutic targeting and for defining predictive factors in clinical management of this lethal disease.

The presence, nature and frequency of cancer stem cells (CSCs) in human melanoma are still unclear. In our study, we provide evidence that supports the notion that human melanomas contain cells endowed with features of CSC. Taken together, our data provide further evidence for the heterogeneous nature of human melanomas, and show that melanospheres and their corresponding tumors, generated *in vivo* in immunocompromised mice, are useful models to investigate melanoma biology.

WP9 – Novel therapeutic strategies
WP9 aims to develop novel therapeutic strategies for drug resistant cells. A number of compounds that induce apoptosis of tumor cells have been identified by screening. The most interesting compound is an inhibitor of two cellular deubiquitinylating enzymes (DUBs). These compounds induce apoptosis of drug resistant cells and of cells overexpressing Bcl-2. A number of structural analogues have been synthesized and tested. Robust effects have been observed in a SCID mouse xenograft model.

The DUBs induce cathepsin-D-dependent apoptosis by unclear mechanisms. A cathepsin-D interacting protein has been identified which is known to be involved in apoptotic processes. Experiments are ongoing to understand the importance of this partner for induction of apoptosis by compounds identified in WP9. Several compounds that induce apoptosis of cells grown in multocellular spheroids have also been identified by screening.

Protonpump inhibitors (PPIs) are tested with the aim of increasing tumor pH and to improve therapy results. Our studies show that the PPI anti-tumor effect is consistent with a lowering of the pH gradient between the external and the internal microenvironments.

WP10 – Mechanisms involved in resistance to DNA damaging drugs
The transfer and expression of a gene, the product of which strongly inhibits the action of MGMT *in vitro*, has been achieved in human tumor cells *in vitro*. This approach is being used to explore novel DNA damage response pathways conferring chemoresistance, as is an *in vitro* assay for the possible nucleotide excision repair of O^6-alkylguanine in cell free extracts of melanoma cell lines.

The impact of MGMT promoter methylation as a predictive marker of chemotherapy response has been investigated. Extensive studies have been performed, both *in vitro* and using tumor biopsies, exploring the potential role of melanosomes and other organelles and their associated proteins in melanoma chemoresistance.

WP11 – Apoptosis regulation
WP11 deals with the identification of genes and processes that mediate the resistance of melanomas, non-small cell lung cancer cells or small cell lung cancer cells against chemotherapy, with the scope of delineating strategies to overcome chemotherapy resistance by enforcing the induction of programmed cell death. This workpackage is advancing well and all deliverables have been achieved.

WP12 - Studies of markers of individual chemotherapy-induced toxicity
Blood samples for preparation of DNA for SNP analyses have now been collected from more than 500 lung cancer patients treated in Paris, Manchester and Stockholm with platinum based chemotherapy. Most patients with non-small cell lung cancer have been treated with carboplatin in combination with either gemcitabine or vinorelbine. Data on treatment-related toxicity have been retrieved from clinical and laboratory records with focus on bone marrow toxicity (neutropenia and thrombocytopenia) and neurotoxicity. When analyzing data from the first chemotherapy cycle, there is a pronounced inter-individual variability in the reduction of blood cell counts one week after chemotherapy as compared to pretreatment values.

We have previously made a list of candidate genes of importance for toxicity based on knowledge of metabolism and transport proteins for drugs used in lung cancer as well as a bioinformatics approach. Published data from gene expression arrays of cells or tissues exposed for drugs used in lung cancer have been collected and up- and down-regulated genes have been identified. In a first attempt to validate this strategy, we have analysed 42 SNPs in 10 genes in 32 ovarian cancer patients treated with

carboplatin and paclitaxel (material from our biobank). In this limited material, variability was found in 11 SNPs. A correlation was found between SNPs in the ABCA1 gene and neurotoxicity.

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

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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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