

Publishable executive summary



CHEMORES – first year report

CHEMORES is an integrated project involving clinicians and scientists at 17 universities, organizations for cancer research and research-oriented biotechnology companies in eight European countries. The purpose of the project is to improve cancer treatment by obtaining increased knowledge on mechanisms of chemotherapy resistance.

Cancer represents one of the most serious health problems in Europe. 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.

The main goals of the CHEMORES projects are to:

- **Provide a robust set of clinically and functionally validated markers for clinical response to chemotherapy in melanoma and lung cancer.** These may then be further developed into useful predictive tools to help identify patients with high probability to benefit from a certain therapy in general oncological practice.
- **Provide novel information on validated molecular mechanisms and pathways of chemotherapy resistance.** This should increase the possibilities to develop new strategies to overcome resistance by targeting the key molecules involved in such pathways.
- **Provide initial preclinical development of potential new modulators of chemotherapy resistance based on validated molecules and mechanisms of resistance.**
- **Provide new information on individual markers of toxicity caused by chemotherapy.** This will be developed into tools that can be used to predict toxicity in individual patients. Such tools may be used to provide information of use for individualized dosing of chemotherapy, minimizing the risk of suffering severe toxicity while optimizing treatment efficacy.
- **Provide validation of novel preclinical animal tumour model systems.** These can then be used in continued studies of chemosensitivity and resistance.

The focus of CHEMORES during the first year has included setting up and standardising methods, as well as experimental models. Another crucial part has been the tissue collections with related issues such as clinical record form development. Several publications have

already been generated with important discoveries. However, since most of the obtained results are yet to be published, they are presented here in more general terms.

A major goal of the project is to provide paired tissue samples procured both pre- and post-chemotherapy, for discovery of novel genes and pathways contributing to chemotherapy resistance, therapeutic escape and efficacy. Protocols are now in the final stages of approval by ethical and regulatory authorities in Manchester and Paris to activate prospective collection of samples in February 2008. A large number of retrospectively collected tumour samples have been identified for genomic analysis to commence during 2008. Information from both prospective and retrospective collections will be combined to generate novel data on molecular mechanisms of chemoresistance in lung cancer. Prospective collection of samples for toxicity studies is underway. Also, clinical trials being conducted in Sweden and in the UK are being identified for potential to contribute with samples to the toxicity studies.

All necessary validations for genomic investigations on clinical samples have been accomplished. Standard operating procedures have been made available and disseminated to all CHEMORES participants. The procedures include histological control of surgical specimens and biopsies, extraction of DNA, total RNA and microRNA from fresh-frozen and archived tissue, and preparation of cryostat sections for protein extraction. All technologies have been validated such as gene expression profiling and microRNA profiling, DNA-methylation studies and gene sequencing. Work is ongoing to allow use of formalin-fixed paraffin embedded tissues.

Several cutting edge methods have been developed. Optimisation of proteomics and down stream data analysis on chemotherapy sensitive and resistant cell line phenotypes by use of mass spectrometry (LC-MS/MS) proteomics and multivariate data analysis have been successful. Method development has also included peptide isoelectric focusing (IEF), pre-fractionation in combination with quantitative isotopic labeling (iTRAQ) proteomics, testing and development of plasma proteomics methods, membrane proteomics as well as multivariate data analysis tools for MS-data. For the first time, proteome effects caused by genotoxic stress in cancer cells have been identified using global proteomics and further data analysis is ongoing.

Experimental models for studying cancer stem cells in lung cancer and melanoma have been established and include melanospheres derived from lymph node tumour specimens or melanoma cell lines, subpopulations of cells expressing stem cell-associated markers e.g. CD133/ESA from primary lung tumours and established xenografts of human tumours in immunocompromised mice. Also, multiple lung cancer sublines resistant to cisplatin have been developed and are being evaluated for expression of the stem cell related markers CD133, ABCG2 and other ABC transporters. The variants display a stable drug resistant phenotype when grown in the absence of cisplatin for several months and are expected to be powerful tools to define the relationship between stem cell markers and drug resistance features.

The first gene products that are important for chemotherapy resistance have been identified, such as apoptotic protease-activating factor 1 and the ion channel VDAC1, and likewise strategies for overcoming chemotherapy resistance, in particular check point kinase 1 inhibitors and novel receptor tyrosine kinase inhibitors. Global gene analysis has identified differential expression of several genes and their products contributing to sensitization of non-

small cell lung cancer (NSCLC) cells to ionizing radiation. Validations have included quantitative real-time PCR and silencing or overexpression approaches.

One cause of impaired chemotherapeutic drug uptake is the acidic internal environment of tumours, where vacuolar ATPases contribute to the low pH. Inhibitors of vacuolar ATPases, i.e. proton pump inhibitors (PPIs), have been found to improve drug uptake by increasing the tumour pH, and also to induce tumour cell death and increase the cellular content of acidic vesicles. Lysosomes are known to be involved in certain forms of cell death, which have been found to be p53-independent. Currently, two inducers of lysosomal apoptosis have been identified and their primary target is being evaluated to examine their potential as anticancer drugs. Of particular interest is whether drug resistant cells that contain a large lysosome/acidic vesicle compartment are sensitive to these novel drugs and whether PPI-induced increases in the content of intracellular vesicles lead to sensitization to cathepsin-dependent drugs.

Biochemical assays are being developed, improved and implemented for the analyses of DNA damage and repair activities in preclinical and clinical trial samples, in relation to chemotherapy response. The effects of histone deacetylase inhibitors and insulin-like growth factor 1 receptor modulation on both DNA repair and drug sensitivity, and on melanosomes on drug sensitivity are also being investigated *in vitro*. Preclinical testing of DNA repair inhibitors as tumour-sensitizing agents has been initiated and potential lead compounds identified.

With the scope of targeted therapy of human cancers that are deficient in DNA repair, studies on inhibiting poly(ADP-ribose) polymerase (PARP) have been performed. Treatment with the novel PARP inhibitor AZD2281 (KuDOS/AstraZeneca, currently in phase I clinical trials) on tumour-bearing mice resulted in inhibited tumour growth, with no toxicity and a strongly increased survival. Although long-term treatment did result in drug resistance due to upregulation of *Abcb1a/b* genes, encoding P-glycoprotein efflux pumps, this could be reversed by co-administering tariquidar, a P-glycoprotein inhibitor. However, tumours that repeatedly responded to cisplatin could not be eradicated. Future experiments will focus on the therapy-surviving tumour-initiating cells. *BRCA1* expression is involved in NSCLC and may be associated with differential sensitivities to drugs used to treat NSCLC. Studies of responses in *BRCA1*-deficient tumours to anti-cancer drugs were performed in a mouse model for mammary tumours deficient in homology-directed DNA repair (*K14Cre, Brca1^{F/F}, p53^{F/F}*) with successfully mimicked acquired drug resistance to the maximal tolerable dose of doxorubicin, docetaxel and topotecan. In these *Brca1^{-/-}, p53^{-/-}* adenocarcinomas, the relevant resistance mechanisms have been identified.

One of the CHEMORES goals concerns validated markers for clinical response to chemotherapy in melanoma and lung cancers. Autoimmune antibodies (Abs) in sera of melanoma patients on adjuvant interferon (IFN) treatment have previously been reported to be important for prognostics and possibly also in prediction of outcome. Therefore, investigations on autoimmune Abs against cardiolipin, thyroglobulin and nuclear factors were studied in the sera of melanoma patients in 2 randomized controlled trials. Analyses in both trials clearly showed that the presence and or emergence of autoimmune Abs were not a (strong) prognostic factor and not a predictive factor for outcome in relation to IFN treatment. The assays used were independently validated and the results confirmed. One further trial from the Nordic Melanoma Group will be evaluated.

An important aim is that today's standardized chemotherapy should be replaced by personalized chemotherapy by which drugs are selected and doses adjusted to obtain optimal therapeutic effect without severe toxicity. It is well-known that differences in the individual genetic constitution can be important for toxicity differences between individuals, families and ethnic groups. Clinical record forms have been elaborated to allow a careful registration of chemotherapy-induced toxicity in non-small cell and small cell lung cancer patients receiving platinum-based combination chemotherapy. Genes of potential importance for drug effects have been identified and non-synonymous single nucleotide polymorphisms (SNPs) in these genes have been identified for analysis and correlation to toxicity in the patients.

With regard to dissemination to the public and the scientific community, a public website has been set up to provide information about the project to interested parties, including the EC, user groups, other international research groups and the general public. In addition, a web-based knowledge management system has been set up 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.

Contractors involved:

Academic organisations:

Cancer Research UK/Oxford, United Kingdom
Centro Nacional de Investigaciones Oncológicas, Spain
Erasmus University, Netherlands
European Organization for Research and Treatment of Cancer, Belgium
Institut Gustave Roussy, France
Institut National de la Santé et de la Recherche Médicale, France
Istituto Nazionale Tumori, Italy
Istituto Superiore di Sanità, Italy
Karolinska Institutet, Sweden
Linköping University, Sweden
Netherlands Cancer Institute, Netherlands
Royal Institute of Technology, Sweden
Technische Universität München, Germany
University Hospitals Leuven, Belgium
University of Manchester, United Kingdom

Companies:

Eurogentec, Belgium
Magnetic Biosolutions AB, Sweden

Public web-site: <http://www.chemores.org>

For more information: chemores@ki.se

Project coordinator: Johan Hansson, Karolinska Institutet, johan.hansson@ki.se

Project manager: Christina von Gertten/ Carolina Johansson, Karolinska Institutet, christina.von.gertten@ki.se / carolina.johansson@ki.se

Project administrator: Jenny Karte, Karolinska Institutet, jenny.karte@ki.se