ANNEX 4: FINAL REPORT

MARIE CURIE ACTIONS

INDIVIDUAL FELLOWSHIPS

SEVENTH FRAMEWORK PROGRAMME

Project n°: 220209

Project Acronym: CISTAX

Project Full Name: Discovery of molecular markers for the inverse resistance relationship between platinum and taxane resistance in cancer

Marie Curie Actions

IEF-IOF-IIF- IIFR -Final Report

Period covered: from _1.10.2008______ **to** _30.9.2010_____

Period number: _____3_______

Start date of project: ____1.10.2008_________

Project beneficiary name: _Dr. Britta Stordal_____

Project beneficiary organisation name: _Dublin City University_____

Date of preparation: __27.9.2010__________

Date of submission (SESAM): 30.9.10

Duration: __1 year__________

Version: ____1________

1. FINAL PUBLISHABLE SUMMARY REPORT

This section normally should not exceed 2 pages.

This is a comprehensive summary overview of results, conclusions and the socio-economic impacts of the project. The publishable report shall be formatted to be printed as a stand alone paper document. This report should address a wide audience, including the general public.

Please ensure that it:

- *Is of suitable quality to enable direct publication by the REA.*
- *Is comprehensive, and describes the work carried out to achieve the project's objectives; the main results, conclusions and their potential impact and use and any socio-economic impact of the project. Please mention any target groups such as policy makers or civil society for whom the research could be relevant.*
- *Includes where appropriate, diagrams or photographs and the project logo, illustrating and promoting the work of the project.*
- *Provides the address of the project Website (if applicable) as well as relevant contact details.*

An EACR travel fellowship allowed me to spend a week at the laboratory of my collaborators Dr. Jean-Pierre Gillet and Prof. Michael Gottesman at the National Cancer Institute (NCI). My research investigates patterns of resistance and sensitivity in cancer cell lines to two chemotherapy drugs, cisplatin and paclitaxel. When cisplatin resistance is developed in the laboratory 68% of cells lines are not resistant to paclitaxel and some even become more sensitive to paclitaxel. The reverse is also true of paclitaxel-resistant cell lines. 66% showed no resistance to cisplatin or were more sensitive to cisplatin. This research suggests that two-thirds of cancer patients would benefit from receiving chemotherapy which alternates between cisplatin and paclitaxel, because developing resistance to one could make their cancer more sensitive to the other drug.

The challenge for me is how to identify which patients will respond to alternating therapy between cisplatin and paclitaxel. While two-thirds of cancer patients may respond well to this treatment strategy, the other third would respond poorly and need to be treated differently. Through collaboration with the Gottesman lab and several other groups I have put together a panel of drug-resistant cell lines which either have the inverse resistance phenotype between cisplatin and paclitaxel or are cross resistant to both drugs. I will be looking for "molecular markers": genes and proteins which correspond with the inverse resistance pattern. I will determine whether these molecular markers change in the same way in samples from ovarian cancer patients. Once validated in clinical samples, these molecular markers could be used as a clinical test to determine if an individual patient is likely to respond to cisplatin or paclitaxel therapy.

For the first part of the project we focused on a set of 6 samples which were a biological triplicate from one set of cell lines; parental IGROV-1 ovarian cancer cells and IGROV-1CDDP cisplatin-resistant cells. These cells are cross resistant to both cisplatin and paclitaxel and therefore model the smaller group of patients who are cross-resistant to both agents. We took a targeted approach to examining gene expression by performing profiling with a custom TLDA array designed by the Gottesman group. This assays the expression of 380 genes previously associated with drug resistance and response to chemotherapy. Gene families examined included; ABC transporters, DNA repair genes, apoptosis-associated genes, glutathione metabolism, copper metabolism and many others.

I extracted total RNA from the IGROV-1 cells in Dublin and sent the samples to the NCI. Dr. Jean-Pierre Gillet analysed my RNA samples for quality prior to my arrival and prepared the cDNA ready to go for the TLDA array. On my first day we set up the TLDA arrays, and the next day we had data to analyse. This is where it was extremely valuable to be in the lab in person to do each step of the analysis. This allowed me to understand how Jean-Pierre extracted the data from the TLDA array software and used BRB statistical software and Ingenuity Pathway software for analysis. This is something that could not have been achieved easily via email. Now, when I send over the rest of my RNA samples in the New Year and Jean-Pierre sends me the data I will be able to analyse the data myself.

The results obtained from my IGROV-1 and IGROV-1CDDP cells were very interesting. Almost 100 genes were differentially expressed between the cell lines and the reproducibility of the biological triplicates was very good. I will use this data as part of a characterisation study of this resistant cell line and as part of my data set analysing inverse resistance and cross resistance between cisplatin and paclitaxel. I presented this preliminary data at the Gottesman lab group meeting while I was there. Visiting the NCI in person cemented a collaboration which I hope will continue for many years to come.

Paclitaxel resistance in IGROV-1CDDP cells is mediated by over expression of ABCB1/P-glycoprotein (11.4 ± 0.4, $p<0.0001$) and is reversible with elacridar. P-glycoprotein is detectable at a low level by immunoblot in the parent IGROV-1 cells and has been up-regulated in response to cisplatin, even though cisplatin is not a substrate of P-glycoprotein. The mechanism of cisplatin resistance has not been fully elucidated, although it is not mediated by a decrease in cellular accumulation of drug as determined by atomic absorption. This is supported by no alterations in the mRNA expression of copper transporters ATP7A, ATP7B or CTR1 and no cross resistance to Cu^{2+}. Interestingly, we also found a significant decrease in mRNA expression of ABCC2/MRP2 (-3.3 ± 0.1 fold, $p<0.001$), which would be normally be associated with sensitivity to cisplatin rather than resistance. Other ABC transporters with significant changes in mRNA expression include ABCA4 (10.0 ± 1.4), ABCD3 (-3.2 ± 0.0) and ABCC4 (-4.3 ± 0.0). Other genes potentially involved in the resistance mechanisms include IGF1R, TGFB1, CCNE1 and HIF1A, which were all significantly decreased in IGROV-1CDDP cells. P-glycoprotein can be up-regulated in association with the development of cisplatin resistance and this can mediate cross resistance to many chemotherapeutic drugs, including paclitaxel.

2. USE AND DISSEMINATION OF FOREGROUND

Section A (public) – DISSEMINATION MEASURES

This section should describe the dissemination measures, including any scientific publications relating to foreground and specify any applications for patents etc in accordance with article II.11. Its content will be made available in the public domain thus demonstrating the added-value and positive impact of the project on the European Community.

- **Dissemination activities**

- **Publications (peer reviewed)**

The list of scientific publications (see articles II.11-12 of the grant agreement) starting with the most important ones, should specify:

- *publication name,*
- *date and page in order to be able to identify it (see proposed template).*

LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES								
NO.	**Title**	**Main author**	**Title of the periodical or the series**	**Number, date or frequency**	**Publisher**	**Place of publication**	**Year of publication**	**Relevant pages**
1	Biomarkers in ovarian cancer: early detection and chemoresistance	Stordal, B.	Contemporary Oncology	In Press			2010	
2	Taxane monotherapy for the treatment of platinum pre-treated ovarian cancer (Protocol).	Stordal, B.	Cochrane Database of Systematic Reviews	In Press			2010	

With regard to scientific publications published before or after the final report, such details/references and an abstract of the publication must be provided to the REA at the latest two months following publication. Furthermore, an electronic copy of the published version or the final manuscript accepted for publication shall also be provided to the REA at the same time for the purpose of publication by the REA if this does not infringe any rights of third parties.

Section B (confidential) - EXPLOITABLE FOREGROUND AND PLANS FOR EXPLOITATION

This section should specify the exploitable foreground and provide the plans for exploitation. It will be kept confidential and will be treated as such by the REA.

The applications for patents, trademarks, registered designs*, etc. shall be listed according to the template provided hereafter.*

The list should, specify at least one unique identifier e.g. European Patent application reference. If applicable, contributions to standards should be specified.

TABLE B1: LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.

Type of IP Rights: Patents, Trademarks, Registered designs, Utility models, etc.	Application reference(s) (e.g. EP123456)	Subject or title of application	Applicant(s) (as on the application)
Not Applicable			

Please complete the table hereafter:

TABLE B2: **OVERVIEW TABLE WITH EXPLOITABLE FOREGROUND**

Exploitable Foreground (description)	**Exploitable product(s) or measure(s)**	**Sector(s) of application**	**Timetable, commercial use**	**Patents or other IPR exploitation (licences)**	**Owner & Other *Beneficiary*(s) involved**
Not Applicable					

In addition to the table, please provide a text to explain the exploitable foreground
[One text box per row in table B2]

Open space (2 pages maximum) composed as following:

- *Its purpose*
- *How the foreground might be exploited, when and by whom*
- *IPR exploitable measures taken or intended*
- *Further research necessary, if any*
- *Potential/expected impact (quantify where possible)*

Not Applicable