Section C: Project Description

1. **Project description** - This is the **most important** component of your application. It should be prepared by yourself and have the approval of your home and host supervisors. The description *must not exceed* 2 pages. It must address in *sufficient* scientific, medical and/or technical detail all of the following points:

Purpose:

We have recently demonstrated that acquired antiprogestin resistance in mammary carcinomas is a reversible phenomenon (Wargon et al, submitted). In addition, we have demonstrated that antiprogestin resistance correlates with a diminished expression of PR A (Wargon et al, submitted), suggesting that PR A may be regulated by epigenetic mechanisms. The purpose of this study is to investigate whether the promoters or PR A or ERα are methylated in mammary carcinomas with acquired antiprogestin resistance.

a. Background

Ductal hormone-dependent (HD) mammary adenocarcinomas were induced in BALB/c mice by the continuous administration of medroxyprogesterone acetate (MPA). These tumors express estrogen (ER) and progesterone receptors (PR) and are maintained through serial syngeneic passages in MPAtreated mice. By transplantation into untreated animals, hormone-independent (HI) tumors that retained ER and PR expression were generated. Most of these HI tumors regress after antiprogestin or estrogen treatment and some of them show *de novo* antiprogestin resistance. To study the mechanisms involved in hormone resistance using C4-HI tumors we developed an acquired antiprogestin resistant variant (C4-HIR) by selective pressure under RU486 treatment. This tumor expressed lower levels of PR isoform A (PR A) and ERα than C4-HI ($p<0,05$). However treatment with 17-beta estradiol (E2), restored PR A expression. C4-HIR also showed to be resistant to ZK 230215, E2 or TAM. Interestingly, treatment with E2 or tamoxifen, restored RU 486 responsiveness. Antiprogestin resistance could also be reverted by successive transplantation in untreated animals generating the C4-HIRev variant which reacquired sensitivity to RU 486.-. The resistant tumor, C4-HIR, and the reverted tumor, C4-HIRev, proved to be more metastatic than the parental C4-HI. In summary, we demonstrated that antiprogestin resistance is a reversible phenomenon and that PR A expression is associated with antiprogestin responsiveness. The acquired metastatic phenotype on the other hand, followed a unidirectional pathway. The plasticity of of PR A expression suggest that epigenetic mechanisms may be silencing PR A expression.

b. Methods & Technologies:

DNA will be isolated from C4-HI, C4-HIR and C4-HIRev and will be treated with bisulphite to change all those cytosines which are not methylated to uraciles. This is currently used to differentiate the DNA with methylated CpG islands. Finally, DNA samples will be checked by Methylation Specific PCR (MSP) using the appropriate primers and controls. Specific primers will be designed for this aim. Finally, selected bands will be sequenced to confirm their identity.

c. What transferable skills do you hope to gain and how will you apply these after the Fellowship:

I will acquire expertise in the use of Methylation Specific PCR technique (MSP) and in primer design. I will also learn how to analyze this information. The experience acquired during this fellowship will be very useful to complete my thesis work. In addition these techniques might be also used by other members of our Laboratory who are studying the involvement of key proteins in breast cancer. I will be able to develop this technology once back in our laboratory since we have all the equipment necessary to carry out PCRs.

d. Relevance of the project to the cancer problem in your country:

Breast cancer is one of the most common cancers in women in Argentina. Two-thirds of breast cancers, when excised at the time of original diagnosis in women, contain estrogen (ER) or progesterone receptors (PR). This knowledge has led to the development of endocrine therapies that reduce 17-β-estradiol (E2) activity either by blocking its biosynthesis using aromatase inhibitors (AI) or competing with E2 for the ER with agents such as the selective ER modulator (SERM) tamoxifen (TAM) or the selective ER disrupter fulvestrant. In the clinical practice, resistance to one SERM is followed with the use of another SERM, or alternatively with an AI. Despite these recent therapeutic developments, resistance to all forms of endocrine therapy still limits the use of ERα inhibition in breast cancer treatment. The study of the mechanisms by which tumors may revert the acquired resistance might help to design a more rational use of the endocrine therapy.

e. Relevance and potential benefit to activities in the home institute:

Many key suppressor genes are regulated by methylation of their promoters. Thus, the expertise that I might acquire at Dr Russo's Laboratory may surely benefit other groups working in cancer research at this Institute. The MPA breast cancer model developed by Lanari and Molinolo is also used by two other groups in this Institute.

f. Reason(s) for choice of host institute:

Dr Russo´s Laboratory is the reference Laboratory in breast cancer research with 35 years experience in the subject. He has authored 330 peer review publications and trained more than 50 PhDs and MDs in breast cancer research. His studies in both rodents and humans have led him to conclude that the carcinogenic potential of a given chemical is in great part modulated by the biological conditions of the target organ, which determines its susceptibility to neoplastic transformation. This seminal work, carried out with the collaboration of a talented research team, led him to develop new concepts in the understanding of the susceptibility of the mammary gland to carcinogenesis. Concepts such as the role of differentiation and rate of cell proliferation of the mammary gland at the time of exposure to a given chemical carcinogen on its binding, DNA repair and tumor incidence have influenced the way in which the study of mammary cancer is focused by researchers worldwide. His studies have progressed form the understanding of the pathogenesis of mammary cancer in rodents to the validation of this model for the study of the human disease. Research performed during the last 16 years has led him to demonstrate that the undifferentiated structures of the human breast, designated lobules type 1, are the site of origin of the most common type of cancer, ductal carcinoma. More importantly, he has shown that the pattern of development and differentiation of the breast differs between nulliparous and parous women. Lobules type 1 are the most frequent structures present in the breast of nulliparous women, exhibiting a high rate of cell proliferation. In vitro lobules type 1 bind carcinogens to the DNA and their susceptibility to be transformed in culture by chemical carcinogens is greater than that of the differentiated lobules type 3 found in the breast of parous women. From these seminal studies he has further developed an in vitro system for testing the transforming abilities of different genotoxic agents. This is an active area of research in his group, with the goal of identifying which are the genomic and epigenetic changes responsible for the expression of immortalization and transformation phenotypes. The understanding of the mechanisms of initiation of cancer has led his group to develop strategies for its inhibition, capitalizing in the utilization of physiological mechanisms, such as pregnancy. This research has opened unsuspected avenues, such as the utilization of a single placental hormone, human chorionic gonadotropin (hCG), for protecting the mammary gland of virgin rats from chemically induced mammary cancer. The protective effect of hCG, like that of pregnancy, is mediated by the induction of full differentiation of the gland, which eliminates the undifferentiated terminal end buds that are the targets of the carcinogen. The relevance of these studies lies in their parallelism with epidemiological data that reveal that parous women have a four-fold lower breast cancer incidence than nulliparous women, providing therefore physiological bases for the prevention of cancer in humans. The discovery that hCG mimics pregnancy, and that the protection of the mammary gland from the initiation of the neoplastic process conferred is equal or more efficient than that induced by the gestational process, and that is also inhibits the progression of tumors, indicates that this model represents an ideal tool for breast cancer prevention and therapy. Finally his group has identified the genomic and epigenetic signature that determines the prevention of breast cancer in women.
For all the above reasons I believe that it will be a privilege for me to have the opportunity not only to learn the experimental work necessary to continue with my work but also to discuss all my project and data with investigators which are in the forefront frontier of breast cancer research.

g. Facilities available upon return to continue the work, apply and disseminate the newly acquired skills:

Our Laboratory has the facilities to perform Methylation Specific PCR technology. Back in the Laboratory of Hormonal Carcinogenesis I will be able to extend these results to other samples. In addition I will be able to investigate if other co-activators or repressors are regulated by this epigenetic mechanism. I will also be able to train other fellows from other Laboratories interested in this technology.

h. References to recent publications in the project field:

1- Progesterone receptor expression in medroxyprogesterone acetate-induced murine mammary carcinomas and response to endocrine treatment. Helguero LA; Viegas M; Asaithamby A; Shyamala G; Lanari C; Molinolo AA. Breast Cancer Res Treat, 2003.

2- Preclinical modelling of endocrine response and resistance: focus on aromatase inhibitors. Macedo LF; Sabnis G; Brodie A. Cancer, 2007.

3- Optimizing the Antihormonal Treatment and Prevention of Breast Cancer. Roshani R Patel, Catherine G.N: Sharma and V Craig Jordan. Breast Cancer 2007

4- Primary prevention of breast cancer by hormone-induced differentiation. Russo IH; Russo J, Cancer Res 2007.

i. Justification of project duration:

As I am already used to PCR technology, we believe that in one month I will be able to learn to design primers, to test different primers in the different tumor extracts. To excise bands for sequencing and to analyze the data.