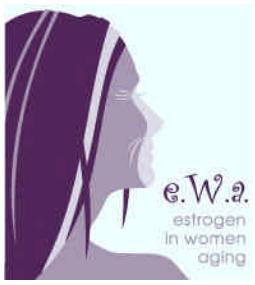

Project no. 518245

EWA

Estrogen and Women Ageing

Specific Targeted Research or Innovation Project
Life sciences, genomics and biotechnology for health -
Priority 1 -

Publishable final activity report

Period covered: from **01/07/07** to **31/12/08**
Date of preparation: **13/01/09**
Start date of project: **01/01/06**
Duration: **36 months**
Project coordinator name: Prof. Adriana Maggi
Project coordinator organisation name: UMIL

1. Project execution

1.1 Project objectives

The objective of the proposal was to capitalize on the European wealth of basic research in the field of sex steroid hormones action and estrogens in particular, to generate a research team devoted to the study of the physiology of woman aging and to the identification of suitable therapies to prevent disorders associated with woman aging and the post-menopausal period.

To this aim, in the research plan we proposed to: *i.)*generate cellular and animal models suitable to obtain a global view of the effects of estrogens and estrogenic compounds in young and aged female mammals; *ii.)* test the effects of long term treatment with estrogenic compounds in reproductive and non-reproductive organs; *iii.)* verify the relevance of estrogen anti-inflammatory activity in the manifestation of pathologies associated with woman aging (e.g. diabetes, neurodegeneration, development of skin ulcers).

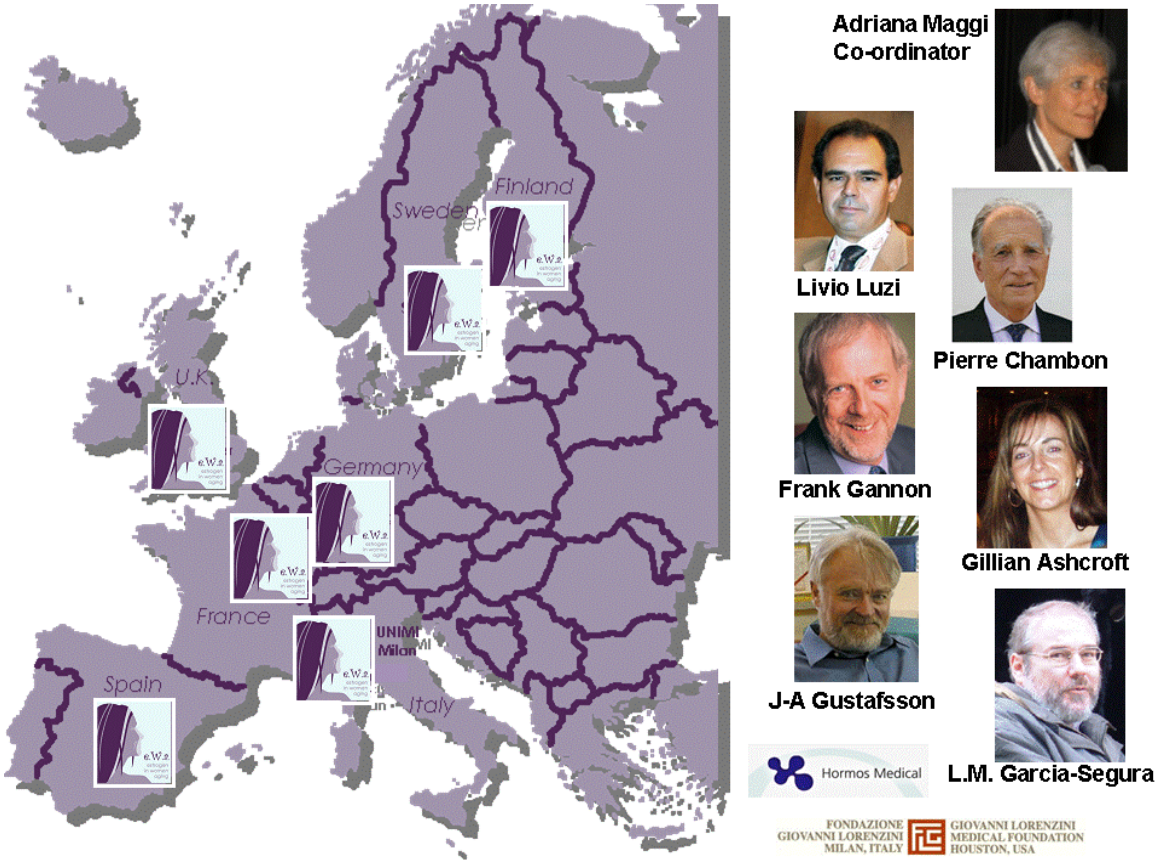

Fig. 1 : EWA consortium

1.2 Contractors involved

The Contractors involved in the EWA Consortium are widespread in Europe and include:
Italy: Adriana Maggi of the University of Milan,co-ordinator; Livio Luzi, Consortium for Research, prevention and Treatment of Metabolic and Endocrine diseases San Raffaele Hospital, Milan
France: Pierre Chambon, Centre Européen de Recherche en Biologie et Médecine – Groupement d'Intérêt Economique, Illkirch
Spain: Luis Miguel Garcia Segura, Consejo Superior de Investigationes Cientificas, Madrid
Germany: Frank Gannon, European molecular Biology Laboratory, Heidelberg
United Kingdom: Gillian Ashcroft University of Manchester,Manchester
Sweden: Jan-Åke Gustafsson, Karolinska Institutet, Stockholm
Finland: M. Unkila HORMOS Hormos Medical Ltd, Turku

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1.3 Work performed

What prompted us to propose the EWA research program was the awareness that in spite of the fact that estrogen and its receptors have inspired the research of a significant number of research groups worldwide we knew very little about their physiological functions, particularly with regard to non reproductive organs. We knew that reduced estrogen signaling or estrogen receptor malfunctioning was associated to a series of pathologies, but the exact mechanism at the basis of that phenomenon were unknown. Considering that:

1. with the increased life span women spend one third to their life with very low endogenous synthesis of estrogens,
2. this phenomenon correlates with an increased incidence of the skeletal, cardiovascular, immune and nervous diseases,
3. the efforts to prevent the disturbances associated to the post-menopause period with hormone replacement therapy has not given the expected research in terms of increased quality of life

we believed, and still do, that more research needed to be done in order to better understand the exact physiological role of estrogens in non-reproductive organs and design novel, more effective therapies to prevent osteoporosis, altered immune response and degeneration of the nervous and cardiovascular system typical of old age in women.

In our view, one of the reasons of the lack of progress in the field was the shortage of appropriate cellular and animal models enabling to rapidly evaluate the physiological consequences of altered ER activities in the whole organism and to identify the genes modulated by the hormone-receptor complex in specific non reproductive cell systems. Therefore we had originally proposed to generate novel model systems and to exploit them to further investigate the ER activity in brain, cardiovascular system, bone and skin.

We believe that our research very successful because:

1. we were able to generate a significant number of novel model systems (cells such as bone human bone and kidney cells, ER mutant mice and reporter systems where ER transcriptional activity can be evaluate in living animals). These innovative systems will immensely facilitate the detailed study of the physiological significance of the multimodal ways of activating ER activity (by direct binding of cognate ligands, by intracellular signaling regulated by membrane receptors such growth factors) and the diverse intracellular activities of the activated receptors (alteration of the rate of transcription of tissue specific genes by direct binding to ER responsive promoters or by protein- protein interaction with other transcription factors or molecules involved in intracellular signaling)
2. we created novel protocols for the study of ER activity in living animals using non invasive methodologies such as Bioluminescence-based molecular imaging
3. we started to apply the models and methodologies developed to:
    a. identify genes induced by estrogens in skin and bone and to define similarities and differences of ER activities in reproductive and non reproductive cells.
    b. generate a overall view of ER state of activity in the different phases of the cycle in whole organism
    c. evaluate the effect of specific pharmacological treatments testing novel estrogenic compounds and comparing their profile of activity in different organs
    d. evaluate estrogen effect in inflammatory disorders associated to neurodegeneration and skin repair systems
    e. set up novel parameters to translate the results of studies in animal models in therapies for aging women.

1.4 End results

Success in EWA research objectives

EWA programme has reached most of the ambitious goals set at the beginning of the study in particular in:

1. Aim #1 *generation of cellular and animal models suitable to obtain a global view of the effects of estrogens and estrogenic compounds in young and aged female mammals* we:

    a. With the work of P2 and P3 the Consortium generated unique cell model systems to study ER activity: of major interest the bone cell lines expressing the 46kd and 66kd ERalpha protein and the renal cells also expressing the ERs. These cell lines were unavailable before and are of relevance for the study of woman physiology.

    b. P1, P2, P3, P5, P7 and P8 performed micro-array in cells treated with estrogens and different estrogenic compounds. The data obtained are relevant for the identification of markers to be utilized for the study of the physiology of selected organs, the understanding of the molecular basis of pathologies associated with menopause and the analysis of the efficacy of treatments with natural and synthetic estrogens.

    c. P5 generated a tremendous array of novel ER mutant mice

    d. P1 and P5 generated unique reporter systems by crossing several ER mutants with the ERE-Luc reporter mouse, thus providing for the first time a wide array of mutants and reporters enabling a very detailed view of the repercussions of subtle deletions on ER molecules on the physiology of the entire organism.

2. *Aim #2*: test the effects of long term treatment with estrogenic compounds in reproductive and non-reproductive organs:

    a. we believe that the initial longitudinal studies carried out by P1 with the aid of reporter mice will set the ground for a novel methodology to be applied in preclinical testing of SERMs. Thus the technology of *in vivo imaging* set up for the longitudinal studies on the effect of treatments with SERM will be of fundamental interest for the evaluation of selected administration protocols aimed at finding safer modalities of treatment with estrogen. The future research will better define the technology and try to highline its validity over current methodologies.

    b. With the application of the reporter systems we were able to evaluate the relevance of liver in ER signaling and generate innovative hypothesis on the physiological significance of ER action in liver by showing that this organ may be

responsible for shutting down the estrous cycle in response to low calorie intake. We also dissected the molecular details of this phenomenon providing novel and very intriguing theories on the role or unliganded ER activation

3. Aim # 3 *verify the relevance of estrogen anti-inflammatory activity in the manifestation of pathologies associated with woman aging (e.g. diabetes, neurodegeneration, development of skin ulcers)*
    a. With the work carried out at the Karolinska Institutet where P7, showing a role for ERbeta in proliferation and apoptosis of human immune cells and proving that selected cells of the immune system release inflammatory agents (TNFalpha) when stimulated by ERbeta agonists. P7 has also shown that cells infiltrating tumors are enriched in ERbeta, the ERbeta cx variant.

    b. With the work carried out by P4 and P1 we have clearly shown the relevance of estrogen anti-inflammatory activity in different models of neuroinflammation including a murine model of Alzheimer. In all the models used we have shown that estrogens may limit neuroinflammation by acting both at the level of microglia and astrocytes, Most interestingly using specific KO mice we were able to show that in brain the anti-inflammatory activity of estrogens was mediated mainly through ERalpha; however when P4 studied the effects of domoic acid on astroglia proliferation observed that both receptors are implicated.

    c. With the work carried out in mouse skin by P2 we are demonstrating that the two ER isoforms are differentially involved in the process of wound healing and we are studying SERMs which would most be of use in the treatment of ulcers. Treatment with the ER alpha and ER beta selective agonists, 1,3,5-tris(4-hydroxyphenyl)-4-propyl-1H-pyrazole (PPT; 100 nM), and diarylpropionitrile (DPN; 1 nM) showed that in this model system also ERbeta is involved in the estrogen-dependent anti-inflammatori effect.

    d. With the work carried out in women by P6 we have generated the tools and technologies necessary to study the impact of HRT on energy homeostasis and insulin sensitivity. Studies in human epithelial cells showed an effect of estrogens on the expression of nephrin leading to the hypothesis that estrogens can directly reduce the pore-size of the glomerular filtration barrier.

Success of EWA impact on its industry or research sector

The results of the Women Health Initiative had a major impact on Industry research on women aging and in particular on hormone replacement therapies: most of them stopped research in the

field and blocked the development of the products under research. It is important to underline that the WHI study is widely recognized as biased by initial mistakes in the selection of the patients to be studied. Nevertheless the publicity given to the study produced a major effect on women: a study born in favour of women turned out to be most unfavourable because completely blocked the development of research on women. We have now to start again and to do that we need new tools and new good research: we hope that the Consortium provided some of that, but this is certainly not sufficient: we need to concentrate more of our research funds for gender physiology and aging. The work of some of the components will continue within the European Consortium CRESCENDO, we will also be very vocal with our Governments and within the European scientific community to induce more research in the field and to encourage the Pharmaceutical Companies to re-enter the field.

Some of the tools here generated, namely the reporter mice, might have a major impact on the drug discovery process in the Pharmaceutical Industry: to this aim, the University of Milan and P1 started a spin-off company of the University of Milan devoted to the generation of reporter mice for preclinical research.

Finally, the tools here generated may be of relevance also for the alimentary and environmental industry in particular with the research on Endocrine Disrupters.

EWA scientist will do their best to maintain the collaboration ongoing in the future years and are planning to maintain the logo we generated and publish their results in the EWA website: www.ewa.unimi.it.

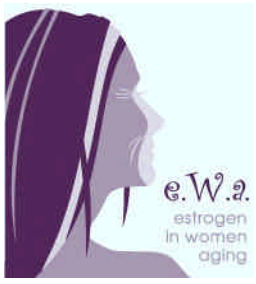

Fig. 2 EWA's logo

2. Dissemination and use

2.1 Intention for use and impact

The presence of Lorenzini Foundation was a key to EWA dissemination success which reached the highest visibility with a campaign in Italy which involved all communication media. This work

represented the seed for the generation of the European Society of Gender which is now operative.
We were also able to set up links with the Institute of Aging of NIH and we are currently planning a workshop to be held at lake Garda on October 2009 under the auspices of the DG research and NIH: the goal of the meeting is to initiate a collaboration among scientists leader of research on women aging in Europe and US.

We believe that the scientific publications and meeting presentations also had and will have a major impact in the field. The different groups were quite successful with the publication of several papers: however most of the collaborative publication are being finished at present time and will be published in the coming year.

2.2 Main elements of the published results

Most of the publications are at the moment in preparation and the elements of relevance have been presented in the previous sections. We are planning to write a review on all the achievements of the EWA Consortium and to submit it for publication in a peer reviewed journal: we hope that this will increase the visibility of the work done and will set the basis for novel research in the field. We are working very actively with national and international bodies to ensure an increase in the number of scientists devoted to research in the field of women aging.

2.3 Main elements of the plan for using and disseminating the knowledge

We believe that our programme generated research tools of unique value for the European research community and that will enable to set up novel and stronger research in the field of women aging. The final aim is the development of novel and safer therapies for the symptoms of menopause and, most importantly, for the prevention of disorders associated with menopause.