



EUROPEAN
COMMISSION

Community Research

AGENDA



Organised by

Diego Di Lorenzo

**Laboratory of Biotechnology
Civi Hospital of Brescia**

EXERA Project Coordinator

Genoa, September 6, 2008

Invited Partners

PARTNER 1 - Diego Di Lorenzo BRHSP/ Giovanna Mazzoleni UNIBS (Brescia, Italy)

PARTNER 2 – Adriana Maggi, UNIMI (Milan, Italy)

PARTNER 3 - Kalervo Väänänen, UTURKU (Turku, Finland)

PARTNER 4 – Paul Tomkins, BIOSERV (Athlone, Ireland)

PARTNER 5 - Richard Fry, CELLON (Bereldange, Luxembourg)

PARTNER 6 – Mikko Unkila, HORMOS (Turku, Finland)

PARTNER 7 - Laurent Gatto /Jean-Pol Detiffe, DV (Charleroi, Belgium)

PARTNER 8 - John Milne, BioUetikon (Dublin, Ireland)

PARTNER 9 - Aldo Tagliabue, ALTA (Siena, Italy)

Arrival in Genoa

Participants will stay at Star Hotel President, where the meeting will be held.

Star Hotel President

Corte Lambruschini, 4 - 16129 - Genova T: +39 010 5727 - F: +39 010 5531820
president.ge@starhotels.it

From Railway Station:

The hotel is situated close to Genova Brignole Railway Station, which is 200 m distant.

By car:

A12 Livorno/Genova (4 km), exit Genova Est, towards Centro-Stazione Brignole - From the A7 Milano/Genova and A10 Ventimiglia/Genova (8 km), exit Genova Ovest, towards Centro-Stazione Brignole. 200 mt. distance to the hotel.

From the Airport:

16 mins by taxi

Please, find the hotel and location on map at:

http://www.starhotels.com/hotel/president_genova/starhotels_president.php?idalb=4&idpag=52&lin=1

6th September

Opening

9.20		Diego Di Lorenzo , EXERA Coordinator The first 18 months
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Presentations

	Partner	Presenter
From 9,30 am	Partner's presentations	<p>Partner n° 1 Diego Di Lorenzo Giovanna Mazzoleni Nathalie Steimberg Claudia Montani (BRHSP/UNIBS)</p> <p>Partner n° 2 Adriana Maggi (UNIMI)</p> <p>Partner n° 3 Kalervo Väänänen (UTURKU)</p> <p>Partner n° 4 Paul Tomkins and Salwa Barkwan (BIOSERV)</p> <p>Partner n° 5 Richard Fry CELLON</p> <p>Partner n° 6 Mikko Unkila (HORMOS)</p> <p>Partner n°7 Laurent Gatto DNA Vision</p> <p>Partner n° 8 John Milne,</p> <p>Partner n° 9 Aldo Tagliabue ALTA</p>
At appropriate time		Lunch break
14.00		Round table General discussion on scientific, technical issues

MINUTE MEETING

Participants at the 5th EXERA meeting

Name	Institution	email
Diego Di Lorenzo	BRHSP	dilorenzodiego@yahoo.it
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Paul Tomkins	BIOSERV	ptomkins@ait.il
Salwa Barkwan	BIOSERV	sbarkwan@ait.il
Adriana Maggi	Università degli Studi di Milano	maggi.scientificsecretary@unimi.it ; adriana.maggi@unimi.it
Mikko Unkila	HORMOS	mikko.unkila@hormos-med.com
Lauraent Gatto	DNA Vision	l.gatto@dnavision.be
Aldo Tagliabue	ALTA	tagliabue@altaweb.eu
Paola Cesaroni	ALTA	cesaroni@altaweb.eu

The meeting starts at 9:00 a.m with presentation of participant's results.

- Nathalie Steimberg shows the successful cloning, amplification and characterization of cells from two tissues: hepatocytes and skin fibroblasts. Two different vectors were used: pRITA (conditional immortalization), Psv3TAneo (constitutive expression of the transgene).

Skin fibroblasts: Two most interesting clones were isolated from ERE-tk-Luc mice: FF4SV3 clone D6 and FF4RITAER clone 1. These clones have been cultured in 2D (Petri dish) and in 3D (RCSS). The characterization of immortalised phenotype is now concluded and the two clones are now ready to be sent to partner BIOU for cell banking.

Hepatocytes: Several immortalised clones were obtained but the characterization of their phenotype needs still to be completed. Hepatocytes clones were cultured either in monolayer and RCSS. 3D hepatocytes have been used to study the cell response to estradiol treatment compared to in vivo experiments. Hepatocytes clones have been sent to DNA Vision for expression profiles.

N. Steimberg concludes the presentation with some results on the development of 3D culture of bone made at the ELETTRA Synchrotron Light Laboratory.

- Claudia Montani refers that skin fibroblasts isolated from wild type and ERE-LUC mice and transfected with pSV3neo and pRITA have been cultured and several clones were isolated:
pSV3neo: P8 (pSV3Neo)
Pa5 (pSV3neo)

pSV3Neo+ERa)
PaE6 (pSV3Neo+ERa+ERE-tK-LUC)
Pb9 (pSV3Neo+ERb)
PbE14 (pSV3Neo+ERb+ERE-tK-LUC)

pRITA: In process: possible positive clones : R1, Rc2, Rα2

PSV3neo selected clones were treated with estradiol, bisphenolA (plasticizer) and Genistein and then analysed for Luciferase activity.

- ✓ 5 pSV3Neo clones are poorly inducibles by estradiol
- ✓ the best responsive clone to the estrogenic chemicals is **PbE14**

Further studies are in progress to evaluate if the immortalised fibroblasts are tumorigenic. The following samples were sent to DNA Vision for genome expression profiles: primary cells, P8 and

P8 } treated for 24h with estradiol 10nM
PbE14 }

The following samples were sent to BIOU for cell banking: P8 and PbE14

- Laurent Gatto describes the results obtained from samples of immortalised fibroblasts sent to DNAVISION by Diego Di Lorenzo. A total of 16 conditions in triplicates (48 samples), among which 2D and 3D samples (received at DNAVISION facility on September 2nd, 2008). The first check of RNA quality for 12 samples demonstrate the integrity and the good quality of samples except for two of them. The samples which passed the quality control were used for Affymetrix studies.

The whole genome expression profiles (DNA microarrays) of the immortalised cells in comparison to the pre-immortalization cell cultures for each tissue was analysed through the Affymetrix microarray platform. A list of genes associated to different pathways is now available. L. Gatto shows the expression of three genes as examples (Esr1, Esr2, Col1a2). The next step will be the selection of most interesting genes for further analysis.

DNA Vision just received 2D and 3D cultures (1 week culture) of immortalised skin fibroblasts and hepatocytes from Partner 1 for comparison of differentially regulated pathways.

- Kaisa Ivaska describes the transfection of mouse bone marrow cells (from femur and tibia) both from wild type and ERE-luc mice. She reports that 63 transfections have been performed in different conditions, for a total of 143 experiments: Electroporation (Bio-Rad), Amaxa nucleofection (for human MSCs, Lonza); Calcium phosphate co-precipitation; Transfection agents: FuGENE® HD (Roche), Lipofectamine™2000 (Invitrogen), jetPEI™ (PolyPlus Transfection), GenePORTER® (Genlantis). The construct used was linearized pRITA and modified pRITAdelta. Clones were formed in 17 out of 147 transfection experiments (~12%). Electroporation/nucleofection was the most successful method (75% of all clones).

The clones obtained are the following

Clones derived from wild type cells: 10 clones picked (by electroporation or Ca-phosphate method, transfected with pRITA), 8 clones survived, 4 of them characterized in more detail (WT1, WT4, WT5, WT6) express osteoblast/osteocyte-specific genes.

Clones derived from ERE-luc cells: 34 clones picked (by electroporation, nucleofection or GenePorter, transfected with pRITA or pRITAdelta), 23 clones survived. These clones are not yet characterized in detail.

K. Ivaska talks about experiments done in OSTER (Erα-) mice to evaluate Erα target genes in osteocytes. Preliminary results done by DNA VISION through Affimatrix microarray

show that 496 are differentially expressed, some are down regulated in the absence of ER α , others are up regulated in the absence of ER α . The most interesting genes will be selected for further studies.

- Paul Tomkins refers that a 3D immortalised Sertoli cell line (passage 85), which retains a significant number of differentiation markers was generated. Despite p53 & RB knockout, cells retain capacity to respond to & undergo apoptosis. Cells show relatively high levels of clastogenic induction, but does not imply abnormal DNA stability. Similar studies are in progress with immortalised granulosa cells.

The meeting then proceeds with a general discussion on the following issues:

- The project coordinator informs the participants the project is extended until the end of March 2010.
- An activity of dissemination was done with the organisation of a workshop entitled “New animal models and in vitro systems for the pharmaco-toxicological analysis of nuclear receptor-interacting compounds (NR-ICs)” held in Genoa (Italy) on 5th September 2008. This workshop occurred in conjunction with the 24th Conference European Comparative Endocrinologist and was open to its participants.
D. Di Lorenzo reminds to all partners that if their organizations are planning some dissemination activities, the announcements can be published in EXERA web site.
- Aldo Tagliabue reminds that three partners (HORMOS, DNAVISION and BIOU) have spent few money during the first 18 months, for these reason the exceeding amount not yet spent was asked back by project coordinator in order to allow the correct distribution of next EU payment to all partners.
- The next meeting will be organised by Paul Tomkins in Ireland. The most suitable date should be April 3rd, 2009.