MEMORIA DEL PROYECTO CIENTÍFICO DE COLABORACIÓN CNIO-FUNDACIÓN INTHEOS

Background:

We propose to generate a collection of xenografts from human tissues or from biopsies of patients affected from TN Breast Cancer (TNBC). Experimental studies give evidences that xenografts would be considered an excellent tool to this aim (3-5). In fact, xenografted tumors seem to retain the main biological features of the human tumor of origin. Another plus is that a grafted tumor preserves the background of the original tumor (3, 6). Another advantage with respect to an *in vitro* study is that the use of xenografts permits to evaluate the potential cytotoxicity of the employed treatment.

Objective:

1) To generate 3 usable PDXs models, using primary tumors obtained from biopsies of 3 patients with advanced TNBC.

After patient surgery, primary tumor would be implanted subcutaneously into the interscapular fat pad of nude mice. The reason for creating models is the establishment of more effective treatments against the disease.

2) To study the efficacy of the combination of abraxane

Study design.

A) **Method:**

-TNBC derived xenograft models:

Under the auspices of a prospective IRB approved protocol, a collection of 3 usable TNBC xenograft models will be generated by implanting and propagating, in athymic nude mice, human samples of primary tumor from advanced TNBC patients undergoing surgery.

60 females 6-8 weeks old athymic nude mice per included patient will be used in this project. At the beginning, small pieces (aprox. 3x3x3 mm) from fresh surgical TNBC specimens of patients operated at the Grupo Hospital de Madrid, will be implanted subcutaneously, in the interscapular fat pad, in groups of 10 mice (2 cages of 5 mice) for each patient, with 1 small piece in each mouse. This will be the first passage, Px1 (see Table 1). When the tumors grow, they will be dissected, cut and grafted in a second group of mice. That group would be made of 25 mice divided in 5 cages. Passage 3 would be identical to second one (see Table 1).
In order to increase tumor mass per specimen for sample storage purposes, we plan to make three sequential passages per specimen.

a. **Primary Measures:** Tumor sizes will be measured twice weekly in two dimensions using a caliper, and the volume will be expressed in mm3 using the formula TV= width2 x length x 0.5. Mouse body weight will be measured weekly.
b. **Data Collection:** Data collection and analysis will be conducted under the PI supervision.
c. **Sample storage:** three types of samples will be obtained from each tumor passage: cryopreserved viable samples (LN2), Formalin Fixed Paraffin Embedded (FFPE) samples and snap frozen samples (-80 degrees).

Table 1:

Xenograft culture (cages/passage)		
Px1	**Px2**	**Px3**
2 (3 months)	**5** (2 months)	**5** (2 months)

-Efficacy studies:

Pretreatments - All animal-related work will be done on aseptic conditions. For general anesthesia, we will use the Viking Medical Vaporizer, which combines oxygen and isofluorane into an anesthetic gas. After recovery, mice will be monitored daily for deterioration until healing of incision, and euthanized by CO_2 inhalation in case of signs for lethargy, cachexia or weightloss.

Experimental treatment – Sixty mice will be inoculated per each original patient and will be randomized upon accomplishment of the tumor size of 200 mm^3 into the following 6 groups, with 10 mice in each group:

- **Group 1:** Control (vehicle)
- **Group 2:** Abraxane as single agent (50mg/kg, once/4 days x 3, i.v.).
- **Group 3:** Capecitabine.
- **Group 4:** Gemcitabine (100mg/kg, twice a week, i.p.).
- **Group 5:** Abraxane + capecitabine at the above mentioned doses.
- **Group 6:** Abraxane + gemcitabine at the above mentioned doses.

Animals will be checked daily for any symptom of toxicity or discomfort. On the 28th day or when the tumor reach 1,5cm^3 ([width2 x height]/2), whichever comes first, mice will be euthanized by inhalative CO_2.

Evaluations of antitumor activity – Tumors will be measured using calipers 3 times per week and tumor volumes will be calculated using the formula: [(width)2 x height]/2. The efficacy of a drug will be evaluated in terms of:

•Tumor growth inhibition: T/C= (mean tumor volume of drug-treated group/ mean tumor volume of control group) x 100
•Tumor growth delay: T-C= difference between the mean values of the time required by tumors of treated and control animals to double their volume, divided by the mean value of the time required by the tumors of the control mice to double their volume
•Tumor regression will be defined as partial regression (PR) if the tumor volume decreased to >50%, or a minor regression (MR) if the tumor volume decreased from 0 to 50% of that at the start of treatment.

Biological studies:

Tumor specimens will be collected from all groups at the end of the study. The status of the tumor stroma will be evaluated by means of Inmunohistochemistry and Masson's Trichrome staining in the collected specimens.
The following IHQ markers will be used in this evaluation:

-SPARC
-alpha-Smoth Muscle Actin (a-SMA)

Budget:

		Cost per patient (Primary		Total cost (3
1) Patient derived xenograft		Mice (around 60 for banking to passage	1,680.00 €	5,040.00 €
		Animal Facility (42,10 euro/ cage-5 mice-/month; an average of 3.7 cages/month for 7	1,090.39 €	3,271.17 €
		Histopathologic studies of	400.30 €	1,200.90 €
		Supplies (storage of	300.30 €	900.90 €
		Subtotal (1)	**3,470.99 €**	**10,412.97 €**
2) Efficacy studies		Mice (around 60 mice per model)	1,680.00 €	5,040.00 €
		Animal Facility (42,10 euro/ cage-5 mice-/month; 12 cages/month for three months)	1,515.60 €	4,546.80 €
		Subtotal (2)	**3,195.60 €**	**9,586.80 €**
		Total Direct Costs	**6,666.59 €**	**19,999.77 €**

Payment Schedule:

Deliverables	Date	Payment
Execution of Agreement and Upon Receipt of Invoice (100% of budget)	Nov 2014	**19,999.77 €**