JOINT METING OF THE STERING BOARD AND TECHNICAL COMMITTEE MEETING

Acronym: CheTherDel

Project title: Chemo-hyperthermal Delivery - Combined chemo-hyperthermal control of hepatic tumors, based on microwave-activated subendothelial-targeted magnetic nano-assemblies

Vienna, November 2-4 , 2012

Revision Sheet

Release No.	Date	Revision Description
Rev. 1	5/11/2012	Steering Board and Technical Committee Joint Meeting
Rev. 2		
Rev. 3		

TABLE OF CONTENTS

1.0 Purpose

The purpose of this joint meeting of the Steering Board and Technical Committee is to present current research progresses and problems encountered by partners in achieving the goals of the CheTherDel project, to discuss future steps and possible changes in the initial protocol.

2.0 Outcome

Each member of the project consortium should understand better its role and perspectives while facing technical or scientifically limitations in developing individual parts of the project. Early results shall be discussed and a critical approach should bring in front problems and possible solutions.

VIENNA SB and TC MEETING AGENDA

Project Name:	**Chemo-hyperthermal Delivery - Combined chemo-hyperthermal control of hepatic tumors, based on microwave-activated subendothelial-targeted magnetic nano-assemblies**	Page	1/3

Purpose, Objectives and Elements of the Meeting:

The purpose of this joint meeting of the Steering Board and Technical Committee is to present current research progresses and problems encountered by partners in achieving the goals of the CheTherDel project, to discuss future steps and possible changes in the initial protocol.

.

As the agenda indicates, the meeting is divided into two parts. Both contain information of interest to all project partners. These sessions are devoted to presentations of early result by all attending partners, description of models and potential significance of early results. Data from all experiments will be discussed within all parts and potential problems will be outlined.

Attendees:	1. Dimofte Gabriel, Romania 2. Radu Iliescu, Romania 3. Vlad Porumb, Romania 4. Emanuele Papini, Italy 5. Alf Lamprecht, France 6. Mona Abdel-Mottaleb, France 7. Karlis Pajuste, Latvia 8. Arkadij Sobolev, Latvia 9. Brigita Vigante, Latvia 10. Aiva Plotniece, Latvia	**Date:** **Place:**	2-4 November 2012 Faberhaft Restaurant Vienna, Austria

Friday, November 2nd 2012, Vienna	
19.00	Partners arrival in Vienna and accommodation
19.30	**Welcoming all project partners**
20.00	**INFORMAL DINNER**

Satruday, November 3rd 2012, Iasi		
08.00-0.830	Registration of the participants, Seminar room, Faberhaft Restaurant, Vienna	
Part I – Early results in animal modeling and NP selection and vizualisation		
9.00-9.15	Welcome speech	Gabriel DIMOFTE, Project leader
9.15-10.00	G. DIMOFTE R. Iliescu V. Porumb	◆ Rat model for selective injection in hepatic artery ◆ Development a model for localized hyperthermia in a rat model ◆ Producing samples of heated and non heated rat liver for further evaluation by Italian group for best antigenic target selection and effects of short time heating ◆ Early data in evaluating intrahepatic distribution of NPs produced by the French group ◆ In vivo toxicity using provided NPs from the French group
10.00-10.15	Discussions	*Critical discussions regarding the models and techniques proposed by the partner
10.15-10.45	E. PAPINI	- toxicity models for ex vivo testing - early data of toxicity regarding the PLGA NPs produced in France - technique for future testing on heated samples of rat liver
10.45-11.00	Discussion	* Potential problems regarding the level of heating

		* Quantification of thermal effect and/or infLammatory response
11.00-11.45	A. LAMPRECHT M. Abdel Mottaleb	- nano-structure of the PLGA particles and the significance it caries regarding positioning of the magnetite or fluorescent tag - biodegradability of the PLGA NPs - solutions for better visualization of NPs using a more stable fluorochrome
11.45-12.30	Discussion	All partners should present their experience and problems regarding the topics as well as accumulation of data for early publications
12.30-13.00	**BREAK**	
13.00-14.00	**Part II – Round table discussion on the field of thermolabile lyposomes and drug delivery**	
13.00-13.30	K. Pajuste	- technical data regarding production of lyposomes and their PEG-ylation - pharmacotoxicity of compound used in the production of liposomes - liposome stability on time
13.30-13.45	A. Sobolev A. Plotniece B. Vigante	- significance of the carbon chain length for thermal stability of liposomes - problems in the evaluation of thermolability
13.45-14.00	G. Dimofte R. Iliescu V. Porumb	- thermolability requirements - concept of thermolable liposomes in the context of the project
14.00-15.00	Discussion	Solutions for the assessment of thermolability in dry or wet mass
15.00-16.00	**LUNCH**	
16.00-16.30	**Part IV – Concluding remarks**	
16.00-16.15	Clear steps for further developments	
16-15.16.30	Planning future meetings and common projects	
16.30	End of the meeting day - Discussion and Conclusion	

Detailed Meeting Minutes:

PART I: **Early results in animal modeling and NP selection and vizualisation**

G. Dimofte presented the results from the partner in Iasi regarding the early results in modeling a technique for intrahepatic injection with application on rats. Data were shown about the model being developed with significant benefits and potential drawbacks. The model is valid and it uses a Seldinger type technique associating a large laparotomy which allows for selective hepatic artery catheterization.

Discussions with E. Papini and R. Iliescu about potential problems arising from hepatic artery ligation. Vaculation of hepatocytes could partially be produced in non-heated lobes due to this technique.

G. Dimofte presented the prototype developed by I. Tudorancea and V. Porumb for local deployment of heat in the selected middle lobe. Data were presented regarding local effect and microscopic markers of heating

Discussions with E. Papini about future tests in Padua regarding effects of local hyperthermia.

G. Dimofte presented early results in heating liver lobes, demonstrating the technique. Samples were sent previously in Padua for antigenic recognition.

Discussion were carried by E. Papini, R. Iliescu and A. Lamprecht regarding the effect of heat on antigenic exposure and adequate choose of the antigen. Some alternatives for antigen staining were discussed regarding mostly the intravascular approach for antibody infusion in vitro.

G. Dimofte presented early results with NPs containing Nile red. The team was unsuccessful to visualize the fluorochorme and possible pitfalls were discussed. The decision was taken to exchange the fluorochrome with cumarin 6 for better stability. Data were also presented regarding magnetite containing NPs. The Iasi team was able to demonstrate large penetrability in both normal and heated liver and they have showed also a method to quantify the colorimetric effect using Pearls coloration.

Discussions were extensive regarding the cause of the failure and also the potential of adding cumarine 6 to the magnetite containing NPs. A decision was also take to send V. Porumb for a knowledge transfer in Besancon to work with Marta Abdel Mottaleb. R. Ilescu and V. Porumb made comments related to the visualization techniques employed and A. Lamprecht and Mona Abdel made significant suggestion regarding properties of the NPs and possibilities to recognize them. K. Pajuste and A. Sobolev informed the group that they can have access to TEM in order to visualize whole particles in tissue. A decision was taken to send M. Leon with tissue sample in Riga.

E. Papini presented protocols for ex vivo toxicity they are going to use as well as early data for toxicity studies already in progress. There are data suggesting that NPs produce in France have limited effect on macrophages.

Discussions were extensive with A. Lamprecht, R. Iliescu and G. Dimofte regarding the intended antigenic site we wish to target and possibility of developing a new model with liver metastatic disease. A. Lamprecht and M. Abdel informed the group about a model for induce colorectal cancer that might be used for further experiments. V. Porumb was given the task to learn and implement the model for further use.

A. Lamprecht presented the structure of the NPs and probable position of the substances absorbed in the NPs. Relevant data were presented regarding vizualisation and necessity to obtain high quality images using a scanning electron microscopy tool. The French team will do that on the NPs in order to better demonstrate the structure.

Discussions – G. Dimofte, R. Iliescu, E. Papini and A. Sobolev discussed numerous aspects about the particles and their destruction. A significant discussion was carried regarding the fate of the NP in tissue as well as the fate of magnetite crystals if freed in the interstitial tissue or cell. The panel was very convinced that the particle will maintain integrity for about a week, but the microcrystals will remain in tissue for much longer.

In the final discussion we decided that there are enough data, almost, for a publication regarding intrahepatic distribution of NPs containing PLGA.

Part II: **Round table discussion on the field of thermolabile lyposomes and drug delivery**

The team mostly involved with this task is the team from Riga. They took the leader position in this round table. They presented the structure of the liposomes they have synthesized for the research project with individual characteristic. There main problem was assessment of thermolability. A. Lamprecht and M. Abdel offered some practical solutions and a decision was teaken for samples to be sent in Besancon for testing. Samples of liposomes were also delivered for Iasi and Padua. K. Pajuste and other team colleagues presented significant data from their research program and also toxicity data on separated compounds. Cytotoxicity was performed in vitro and toxicity was performed in vivo with some degree of toxic effect.

Discussions were abundant regarding stability, chemo delivery and fate of liposomes. All participants demonstrated interest in using their own technology in augmenting the lyposomes and characterizing their thermolability which should deliver drugs at 42 degrees.

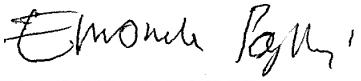
Dr. DIMOFTE moderates the final discussions and conclusions with the participants agreeing on the success of this meeting and the clear and straight ideas and steps for the immediate future of the project.

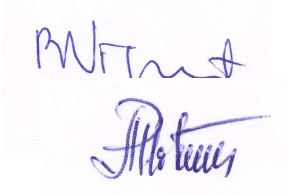
Signatures

Gabriel Dimofte 

Radu Iliescu 

Vlad Porumb 

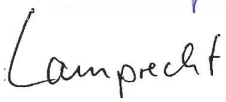
Emanuele Papini 

Brigita Vigante 

Aiva Plotniece

Arkadij Sobolev 

Karlis Pajuste 

Alf Lamprecht 

Mona Abdel-Mottaleb