

**European Society of Medical Oncology (ESMO)
Fellowships Program
ESMO Translational Research fellowship
Progress Report**

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Intrapleural Therapy after Surgery for Malignant Pleural Mesothelioma

Malignant pleural mesothelioma is an aggressive tumour with increasing incidence that is expected to peak in the next two decades. The management of these patients is still controversial, with currently the best survival data after multimodality treatment including induction chemotherapy, surgery and radiotherapy. Cisplatin in combination with an antifolate-compound is the recommended standard chemotherapy. While a response rate of 40% and survival benefit as compared to cisplatin alone has been reported with this combination, most patients ultimately will develop chemotherapy-resistant recurrent disease. Intrapleural therapy is an attractive treatment option for local tumour control with promising results in early clinical and experimental studies but further refinement is still necessary.

The Division of Thoracic Surgery and the Clinic and Policlinic for Oncology of Zurich hospital have become the major reference centre for the treatment of malignant pleural mesothelioma in Switzerland. Several research projects are being developed in collaboration between these two units. The applicant and her group developed a new animal model imitating mesothelioma recurrence so that she will be heavily involved in the *in vivo* experiments where the model will be used and surgical skills are necessary.

Project 1: The effect of DNA repair modulation on cisplatin-induced resistance to chemotherapy.

Cisplatin is a DNA-damaging agent and *in vitro* studies showed that cisplatin treatment significantly increased the formation of chemotherapy resistant cells. The repair of cisplatin induced DNA-adducts is dependent on several DNA damage repair pathways including translesion synthesis (TLS), which is the error-prone DNA replication of damaged bases.

Specific aim : To determine in vitro and in vivo the impact of TLS down-regulation on cell survival and chemotherapy resistance after cisplatin treatment

In vitro: These experiments were performed by the molecular oncology lab which has great experiences with siRNA and DNA repair. The original plan was to determine the effects of TLS down-regulation on cell survival after cisplatin treatment .

We failed to establish clones with reduced TLS expression in several cancer cell lines. At the same time, we were able to generate additional clones with reduced TLS expression from several normal cell lines. This observation resembled the synthetic lethality induced in BRCA-deficient breast cancer cells by PARP-inhibitors and we make the hypothesis that TLS inhibition induces synthetic lethality in cancer based on yet to be identified molecular differences between normal- and cancer cells. Because of these results we could not proceed with the in vivo part of the project where it was planned to use TLS-deficient syngeneic malignant mesothelioma cell line in the orthotopic malignant mesothelioma recurrence model in the immunocompetent rat ¹

Project 2: Intrapleural immunomodulatory therapy after surgery

Another approach for improved local tumour control is the intrapleural application of different immunomodulating substances. One particularly promising approach is to stimulate innate immunity. Toll-like receptor (TLR) belong to the family of pattern recognition receptors (PRR) and ligation of these receptors by conserved motifs of microorganisms (pathogen-associated molecules) results in activation of the innate immune response. TLR9 ligands bind unmethylated CpG clusters. Both bacterial DNA and synthetic unmethylated CpG oligonucleotides have been shown to enhance cellular and humoral immunity against cancers via TLR-9 (Krieg et al 2002). Furthermore experimentally CpG-ODNs were found to be potent enhancer of chemotherapy and radiotherapy {Mason et al 2006} and therefore might also qualify for multimodal treatment in mesothelioma

Mycobacterial preparations binding to TLR2 and TLR4 have been used in treatment of superficial bladder cancer, and since then Bacillus Calmette Guérin (BCG)-immunotherapy has a well-documented and successful clinical history {Morales et al. 1976}. SRL172, a suspension of heat-killed Mycobacterium vaccae, was evaluated in combination with chemotherapy in patients with advanced inoperable mesothelioma {O'Brien et al. 2000} {Mendes et al. 2002} with promising result, so that this treatment would be an attractive adjuvant treatment also in a curative intends for earlier stage MPM patients as part of multimodality concept.

Specific Aim : To evaluate the effect of intrapleural immunomodulatory therapy with ligands to the innate immune system (unmethylated CpG-ODN in solution or loaded to fibrin) on the extent of local tumour recurrence in an established rat model of malignant pleural mesothelioma (MPM).

Methods: Six days after subpleural inoculation of a syngeneic MPM cell line (IL-45), left-sided pneumonectomy and resection of the tumour nodule was performed. Animals were randomized into 4 treatment groups for intrapleural therapy: control (n=6), 500 µg CpG-ODN (Cytosine-phosphate-guanosine-oligodeoxynucleotide) (n=6), cisplatin-fibrin (n=6), cisplatin-fibrin + 500 µg CpG (n=6). 6 days later the volume of tumour recurrence was assessed, which was the primary endpoint. Secondary endpoints were quantification of the ratio host/tumour cells in the local recurrence and cytokine expression profile in the tumour tissue by real time quantitative PCR (qPCR). T lymphocyte subpopulations in the tumour recurrence

tissue were evaluated by immunohistochemistry. Treatment-related toxicity was monitored by measuring blood chemistry and complete blood count.

Results: The volume of tumour recurrence was significantly reduced from 610 mm³ in the control group to 11.7 mm³ in the cisplatin-fibrin group (p=0.004) and to 21.8 mm³ in the cisplatin-fibrin + CpG group (p=0.004).

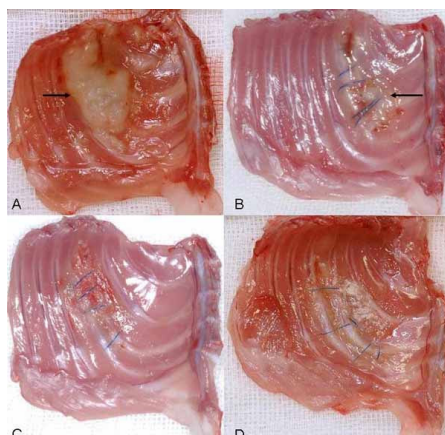


Fig. 1: Successful treatment of mesothelioma tumor with cisplatin-fibrin and cisplatin-fibrin + CpG: Photographs show ipsilateral chest wall at the time point of autopsy 6 days after intrapleural treatment; a) control group: arrow indicating tumour recurrence, b) CpG 500 µg group: arrow indicating tumour recurrence, c) cisplatin-fibrin group: tumour recurrence macroscopically not visible, d) cisplatin-fibrin + CpG 500 µg group: tumour recurrence macroscopically not visible.

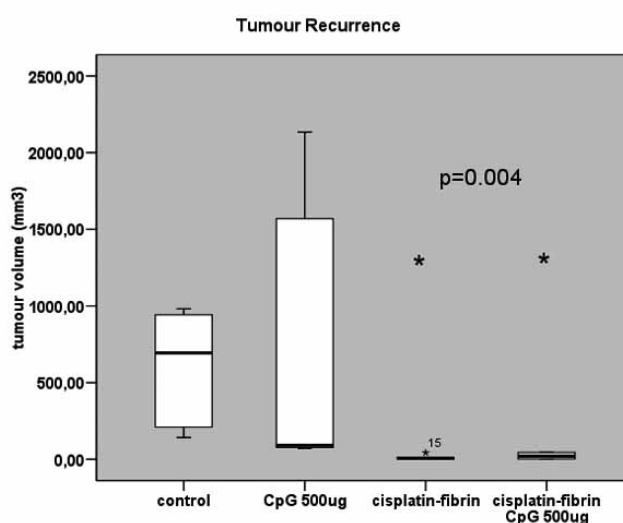


Fig. 2: Minimal tumour recurrence with cisplatin-fibrin or cisplatin-fibrin + CpG. In each group of rats n=6. Results presented as box-plot of volume of tumour recurrence.

Pro-inflammatory cytokines (IFN- γ , IL-6, IL-12) were increased after treatment with cisplatin-fibrin + CpG in comparison to cisplatin-fibrin alone but differences were not statistically significant.

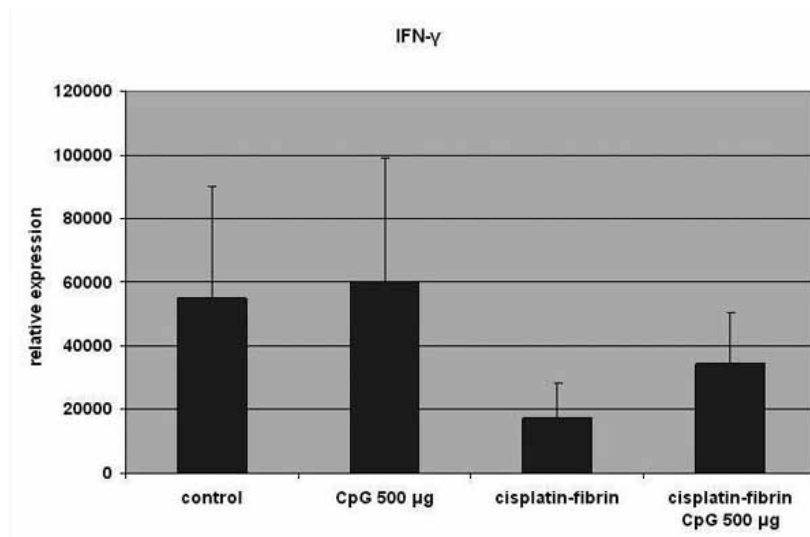


Fig. 3: The combination cisplatin-fibrin + CpG resulted in higher expression levels of IFN- γ in the tumour tissue as quantified by qPCR compared to cisplatin-fibrin alone but differences were not statistically different (Kruskal-Wallis test: $p=0.07$).

We found a higher ratio of host/tumour cells in the cisplatin-fibrin + CpG group (45/55%) compared to the cisplatin-fibrin group (27/73%).

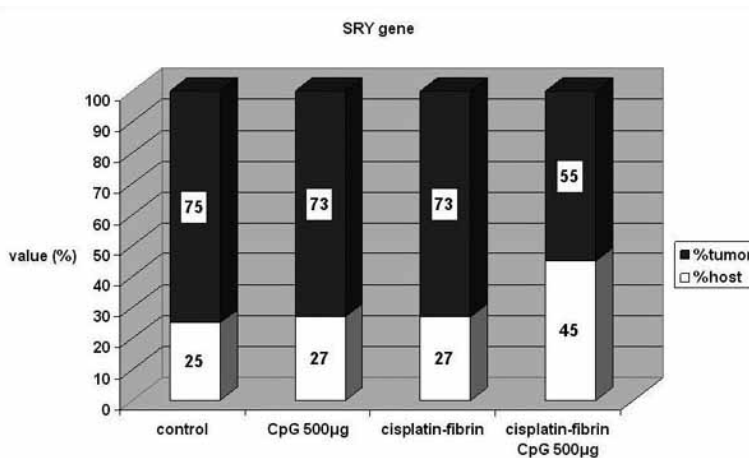


Fig. 4. Treatment with cisplatin-fibrin + CpG results in a higher content of host cells in the tumoral mass in comparison to the other groups. The content of host vs tumoral cells was determined by means of q-PCR analysis of SRY gene.

In comparison to the control group, animals treated with cisplatin-fibrin + CpG showed a higher number of CD8+ T-cells in the tumour tissue. No significant treatment-related toxicity was observed.

Conclusions: Adjuvant treatment with chemo- or immuno-chemotherapy leads to significant reduction of mesothelioma recurrence after surgery in this rat MPM model. Immuno-chemotherapy resulted in an increased recruitment of inflammatory cells to the site of tumorigenesis and elicited higher level of tumour growth inhibiting cytokines.

Project 3: Identification and characterisation of mesothelioma cancer stem cells

In order to open up new therapy options for mesothelioma patient we further want to define the characteristic of originating cancer stem cells. Cancer is increasingly being viewed as a stem cell disease, both in its propagation by a minority of cells with stem-cell-like properties and in its possible derivation from normal tissue stem cells. But stem cell activity is tightly controlled, raising the question of how normal regulation might be subverted in carcinogenesis. The long-known association between cancer and chronic tissue inflammation suggest that carcinogenesis proceeds by alteration of homeostatic mechanisms that govern tissue repair and stem cell self-renewal. This is potentially the case in mesothelioma which is associated to asbestos exposure leading to chronic pleura inflammation. Therefore a fraction of mesothelioma cells might be cancer stem cells with unlimited renewal potential as it has been proven for brain, colon and breast cancer. Characterisation of these stem cells might allow finding new therapy targets for treatment of mesothelioma patients.

Specific Aim : Establishment of malignant pleural mesothelioma stem cells and investigation of targeted therapy in vitro and in vivo

In vitro: This part has been performed with the collaborators of the oncology lab. Primary cultures of malignant pleural mesothelioma have been established in conditions maintaining self-renewal (stem cell) properties. Several primary mesothelioma cell cultures were established from surgical specimens. They expressed the Hedgehog pathway signaling molecules Smo, PTCH1 and Gli, although at variable amounts. Cyclopamine, an inhibitor of the Hedgehog signaling pathway, significantly inhibited cell growth in primary culture, while tomatidine, which was used as control, had no effect. This effect was accompanied by inhibition of Ptch1, Gli-1 and Snail1, which are genes downstream Hedgehog signaling. These results indicate that self renewal Hedgehog pathway is active in primary cultures from mesothelioma patients and blockade of this signaling regulates cell growth.

In vivo: A tumorigenicity xenograft model in NOD/SCID mice was set up by the applicant throughout injection of tumour cells under the renal capsule. Using this model we are currently testing the tumorigenicity of sorted tumor cells.