MESSAGE FROM THE PRESIDENT
It is a great privilege for me to have been elected President of IMIG for the period 1995-1997. IMIG has remarkably grown since its creation in Paris in September 1991. Credit should be given to my two predecessors as Presidents of IMIG, Marie-Claude Jaurand and Bruce Robinson, as well as to all the members of the Executive Committee. IMIG has truly become an international forum of outstanding scientists of different background and expertise, covering all the basic research and clinical aspects of mesothelioma.

Because of the wide range of scientific issues addressed by IMIG, its scope goes well beyond mesothelioma and extends to many key topics including, to name a few of them, epidemiology, molecular biology, carcinogenesis, pathology, oncology, experimental therapeutics. The summary of the last IMIG meeting in 1995 included in this newsletter by Marjan Versnel, who patiently collected all the data for it, is by itself proof of this remarkable variety of contributions made by many outstanding worldwide investigators.

Our task is now to prepare the next IMIG meeting which will take place in the United States in 1997. After many discussions with members of the Executive Committee, we have decided to hold the next meeting from May 13 to 15, 1997 in Philadelphia, where our colleagues at the University of Pennsylvania are conducting a trial of gene therapy of mesothelioma and have accepted to host the meeting. Also this appears to be an easily accessible location, and the dates do not compete with other major medical conventions.

Looking forward to an exciting meeting and hoping to see you all next year,

A. Philippe Chahinian, M.D.

Report of 3rd International Mesothelioma Interest Group meeting
Paris, France, September 1995

Biology of mesothelioma
M.A. Versnel, PhD
Dept. of Immunology, Erasmus University Rotterdam, The Netherlands
The conference was started by Balmain (Glasgow, United Kingdom), who reviewed the studies on carcinogenesis and the role of tumour suppressor genes in a mouse model for skin carcinomas. In a mouse strain extremely resistant to tumour induction, markers to resistance for papilloma development were identified and expression of genes involved in the initiation, promotion and progression of the tumour was analyzed.

Gerwin (Bethesda, USA) addressed the role of tumour suppressor genes in malignant mesothelioma. Several studies on p53 and Rb revealed that these genes are infrequently involved in malignant mesothelioma. The
MDM2 gene, encoding a gene product that stabilizes and inactivates p53, was found to be normally expressed at the mRNA and protein level. The p16INK4 gene, encodes a CDK4 inhibitor. Inactivation or deletion of p16INK4 will block Rb product mediated pathway of cell cycle control. In the majority of malignant mesothelioma cell lines p16 was found to be homozgyously deleted. Normal mesothelial cell lines and T-antigen immortalized human mesothelial cell lines were found to express p16INK4 mRNA and protein. Transfection of p16 into a malignant mesothelioma cell line significantly decreased the colony formation. In vivo p16INK4 was found to be lost in 70% of the malignant mesotheliomas, while in colon, lung and ovarian tumours no deletions were observed. These observations suggest that loss of function of p16INK4 can play a role in the pathogenesis of malignant mesothelioma.

Björkqvist (Helsinki, Finland) showed results of fluorescent Comparative Genomic Hybridization (CGH) applied to malignant mesothelioma specimen and cell lines. Using CGH loss and gain of DNA can be detected. However, no balanced chromosomal aberrations, small mutations or changes in peri-centrometic and heterochromatic regions can be detected. The advantage of CGH is that only DNA is required and compared to cytogenetic techniques and no culturing is needed. In malignant mesothelioma tumour samples the changes detected were gain of chromosome 1q, loss of 9p pand 14q.

Versnel (Rotterdam, the Netherlands) reviewed the studies on PDGF (platelet-derived growth factor) in malignant mesothelioma. Several groups have found elevated PDGF-BB expression in human and mouse malignant mesothelioma cell lines compared to normal mesothelial cells. Malignant mesothelioma cell lines were found to express PDGF-β-receptors, while normal mesothelial cells predominantly express PDGF α-receptors. In vivo malignant mesothelioma cells were found to express PDGF and the PDGF β-receptor, whereas the α-receptor was occasionally found. Studies on the regulation of PDGF chain and receptor expression revealed that differences of the PGDF A- and B-chain and the β-receptor are determined at the transcriptional level. Using various methods two regions (-9.9 kb and bp -64/-61) were identified as relevant for the elevated PDGF B-chain expression in malignant mesothelioma cell lines. Future studies will be directed towards identification of the transcription factor(s) for PDGF B-chain expression and interference in the possible PDGF driven autocrine loop.

Mossman (Burlington, USA) studied rat pleural mesothelial cells (RPM) exposed to asbestos and erionite. In these exposed cultures induction of c-fos and c-jun mRNA was observed. Analysis of the signalling pathways and transcription factors involved in the asbestos induced c-fos and c-jun expression revealed that the Mitogen-Activated Protein Kinase (MAPK) and NF-κB pathways are both activated. Upon asbestos exposure induction of PKCα was observed. Suramin, a polyanionic compound that inhibits the interaction between growth factors and their receptors, was found to downregulate this asbestos induced PKCα. Subsequently, it was demonstrated that asbestos exposure induces phosphorylation of the Epidermal Growth Factor-receptor. These studies indicate that asbestos might interact with the EGF receptor.

Jaurand (Créteil, France) studied the early changes in RPM upon asbestos exposure. Upon asbestos exposure oxygen derivatives, as suggested by experiments with anti-oxidants and by the observed poly (ADP) ribose polymerase activation, lead to DNA damage. DNA-damaged cells can be eliminated by apoptosis. In vitro asbestos was found to induce apoptosis in RPM as detected by DNA fragmentation analysis. However, in situ only 5% of the RPMC are apoptotic after asbestos exposure and no evident induction of p53 was detected by immunocytochemistry. These results indicate that asbestos exposed RPMC follow a DNA repair pathway.
Immunology and inflammation in mesothelioma
M.A. Versnel, PhD
Dept. of Immunology, Erasmus University Rotterdam, The Netherlands

McCluskey (Adelaide, Australia) gave an introductionary lecture on "Immune recognition of cellular antigens - implications for tumour immunity". In summary, tumour cells can evade T cell surveillance by (1) defective antigen presentation, (2) secretion of suppressive factors like TGF-β, and (3) secretion of regulatory cytokines e.g. switch from Th1 phenotype to a Th2 phenotype.

Broadus (San Francisco, USA) postulated that pleural inflammation precedes malignant transformation. Early events observed upon in vivo asbestos exposure are influx of neutrophils and macrophages, cytokine production by macrophages and mesothelial cells and mesothelial hyperplasia. The inflammatory potential of mesothelial cells is evident by their propensity to phagocytose and to produce prostaglandins, oxidants and cytokines. Malignant mesothelioma was thought to share many elements with the inflamed mesothelium and may be considered as unregulated inflammation.

Robinson (Perth, Australia) demonstrated that during tumour progression in a mouse model the cytokine production profile by tumour infiltrating lymphocytes deviated from Th1 to Th2. Th1 cytokines have a cytotoxic antitumour effect and Th2 cytokines are considered to be more tumour protective. A switch from Th1 to Th2 production may cause escape by immune recognition. Changing this immunodeviation towards a Th1 response by the potent Th1 stimulator IL-12 could result in tumour destruction.

Malignant mesothelioma cell lines were found to produce the immunosuppressive molecules TGF-β1 and TGF-β2. In malignant mesothelioma TGF-β was considered as an autocrine growth factor. Antisense TGF-β studies showed in vitro a decrease in anchorage - independent growth in soft agar and prolonged survival accompanied by increased expression of tumour-infiltrating T cell markers in vivo. Blockade of TGF-β seems to reduce tumour growth and inhibit immunosuppression. Immunotherapy by intraresional IFNα application in malignant mesothelioma patients resulted in partial responses. GM-CSF caused in one out of six treated patients local necrosis indicating that a specific anti-malignant mesothelioma response can be generated. Combination therapy with several cytokines like GM-CSF, IL-2 and IL-12 may be more effective.

Garlepp (Perth, Australia) presented data on the effect of introduction of the costimulatory molecule B7-1 in murine malignant mesothelioma cell lines. Malignant mesothelioma cell lines do not express B7-1. The introduction of B7-1 expression on malignant mesothelioma cells would enhance tumour antigen expression and result in an immune response. Murine malignant mesothelioma cell lines transfected with B7-1 had a delayed growth in vivo, but eventually all inoculated cell lines formed tumours. Introduction of expression of allogeneic MHC and B7-1 resulted in a strong decrease in tumour development. These data suggest that B7-1 expression enhanced the immunogenicity but tumour antigen in combination with costimulation are required for effective antitumour response. Introduction of IL-2 into tumour cells resulted depending on the cell line used in a decrease in growth rate or a decrease in tumour induction, indicating that activation of NK cells is effective. It was concluded that (1) growth of malignant mesothelioma can be slowed down or prevented, (2) tumour specific cytotoxic T lymphocytes can be generated and (3) malignant mesothelioma cells bear antigens recognizable for autologous T cells.

Mesothelioma pathology
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Kane (Rhode Island, USA) studied several biomarkers of response to asbestos exposure in a mouse model of malignant mesothelioma. Intrapleural asbestos injection resulted in an increase in mesothelial proliferation, while PMA rather caused an inflammatory response and little mesothelial cell proliferation. DNA damage was increased in mesothelium of asbestos treated cells as detected by
micronuclei formation. An increase in Gadd45, an intermediate in the p53 DNA repair pathway, was found in asbestos exposed mesothelioma. However, ionizing radiation was causing an earlier and a greater increase in Gadd45.

Van Marck (Antwerpen, Belgium) reviewed immunohistochemical markers available to establish malignant mesothelioma diagnosis. Analysis of glutathione-S-transferase (GST) expression revealed that 51% of malignant mesothelioma and all non-neoplastic mesothelium cases were positive for at least one GST subclass. Expression of at least one GST correlated positively with a slightly prolonged survival. Several groups showed that expression of the Wilm's tumour suppressor gene (WT1) was highly specific for malignant mesothelioma, while primary lung carcinomas and metastatic tumours to the lung or pleura were negative. Renal cell carcinoma was found the only non mesothelial tumour with WT1 positivity. Studies on p53 and MDM2 revealed that the wild type and mutated form of p53 could be detected in 10 out of 15 malignant mesothelioma cases and MDM2 in 6 out of 15 cases indicating that other molecules than MDM2 are involved in p53 inhibition.

Donna (Alessandria, Italy) presented results of the markers AMAD-1 and AMAD-2 for malignant mesothelioma. Both markers recognize cytoplasmic antigens and were found to be highly specific for malignant mesothelioma. AMAD-2 is a rabbit polyclonal antibody reactive with paraffin-embedded tissue. All malignant mesothelioma cases investigated, were positive for AMAD-2 in more than 50% of the cells. No reactivity was observed in the majority of other tumours only adenomatoid tumours of the epididymis, serous cystadenoma and synovial carcinomas were positive.

**Gene therapy for malignant mesothelioma**

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Peschanski (Créteil, France) gave an introduction on gene therapy in the nervous system. The various methods of gene therapy and their advantages, disadvantages and limitations were discussed.

Albelda (Philadelphia, USA) reviewed the experimental studies on gene therapy using the Thymidine Kinase (TK) "Suicide gene" in an adenoviral vehicle. TK is able to form a toxic product from the drug ganciclovir (GCV). An advantage of this system is the bystander effect probably from the toxic product on cells that are not transfected. Disadvantages of adenoviral vectors are transient expression and induction of an immune response. In SCID mice intraperitoneally injected with human MM cell line did not develop tumours and had a prolonged survival upon TK gene transfer in combination with GCV treatment. In an immunocompetent rat model with intrapleural injection of a syngeneic rat mesothelioma a marked reduction in tumour weight was observed but the survival was not prolonged. This was possibly caused by an immune response against the adenovirus and might be circumvented by immune suppression with cyclosporin. Indeed cyclosporin application resulted in prolonged transgene expression, better tumour killing and prolonged survival.

Kaiser (Philadelphia, USA) discussed the progress in the clinical application of the TK suicide gene therapy. The preclinical toxicity in non human primates did not reveal significant toxicity of GCV, viral shedding or gross lesions. A phase I trial was expected to start within 1 month using increasing dose levels and monitoring a number of parameters like TK expression by RT-PCR and immunohistochemistry, inflammatory responses, neutralizing antibody generation. Experimental studies in the SCID model revealed that a replication competent virus is more effective in inducing tumour reduction than a non replication competent virus.

Robinson (Perth, Australia) discussed the various possibilities for gene therapy using immune and antisense approaches. In murine malignant mesothelioma cell lines antisense TGF-β studies in vitro and in vivo upon direct injection in the tumour showed a decrease in tumour growth and a prolonged survival. Stable transfection of the B7-1 in the parental
line was found to reduce the tumor growth. A prolonged survival was obtained after IL-2 transfection and GM-CSF inhibited tumor growth. Combinations of these approaches should be tried.

Chemotherapy of malignant mesothelioma
A.P. Chahinian, MD
Mount Sinai School of Medicine, Division of Neoplastic Diseases, New York, USA
Current results of chemotherapy for malignant mesothelioma were summarized at the Third International Mesothelioma Conference. Most response rates to single agents rarely exceed 15% in large series. This is the case for the most commonly tested agents, for which cumulative response rates (and total number of patients treated) are: doxorubicin = 15% (112), carboplatin = 12% (97), cisplatin = 14% (74), epirubicin = 12% (69), mitoxantrone = 5% (64) (Chahinian AP, in Cancer Medicine, Holland and Frei, eds, 3rd Edition, Lea and Febiger, 1993, pp 1335-1355). There is no evidence of a dose-response relationship for doxorubicin, but pilot trials with high-dose cisplatin seem to reach 30% response rates in very small series.

Newly evaluated agents include edatrexate, with an interesting response rate of 25% in 20 patients, whereas preliminary results with trimetrexate (12% response in 51 patients), ifosfamide (8% response in 26 patients) and paclitaxel (Taxol, 13% response in 15 patients) are more disappointing.

A large number of combination chemotherapy trials have been reported. In general, there is no clear-cut evidence that results are superior to single agents, and very few trials are randomized. The Cancer and Leukemia Group B recently reported the result of a randomized Phase II trial which evaluated the combination of mitomycin + cisplatin (26% response rate with two complete responses in 35 patients) versus the combination of doxorubicin and cisplatin (14% response rate in 35 patients). There was no difference in overall survival between the two groups (J. Clin. Oncol. 11:1559, 1993).

Dr. Chahinian also presented at the meeting his latest results of experimental chemotherapy of malignant mesothelioma in nude mice xenografts, testing new agents such as suramin and paclitaxel. It appears at this time that suramin has little antitumor activity in this system, and paclitaxel and cisplatin, however, produced better antitumor activity and deserves a clinical trial.

The effects of paclitaxel in vitro and in vivo in murine and human cell lines were presented at the meeting by Drs. Davidson and Robinson (Perth, Australia), who failed to detect significant activity. Dr. Mattson and co-workers (Helsinki, Finland) reported on the use of high-dose methotrexate, combined with interferon alpha and gamma. Among 18 patients, they observed one complete and three partial responses after 3 cycles. The accrual continues as a Nordic Phase II trial. Dr. Shinjo et al. (Hyogo, Japan) studied the pharmacokinetics of cisplatin and irinotecan (CPT 11) in patients with pleural mesothelioma and concluded that effective levels of these drugs can be obtained in pleural fluid when using the intravenous route. Dr. Soulié et al. (Créteil, France) have treated a large number of patients with the combination of cisplatin and interferon alpha in weekly schedules, and observed a 30% response rate in a total of 50 evaluable patients.

Mesothelioma: clinical trial
C. Boutin, MD
Service de Pneumologie, Hôpital de la Conception, Marseille, France
A new TNM classification for mesothelioma replacing the one proposed by A.P. Chahinian was first used by V. Rush (New York, USA). In Stage I (T1 N0 M0) the pleura is adhesions-free and the patient generally presents pleurisy. In Stage II the tumor is circumferential with involvement of adjoining thoracic organs. In Stage III patients are still operable but radical pleuropneumonectomy must be performed. In Stage IV, as with other types of cancer, treatment is ineffective.

Thus early diagnosis is the major concern in the management of methestelioma patients. A.P. Chahinian proposed that measurement of hyaluronic acid may be used as a method of screening patients at risk for mesothelioma.
mesothelioma cell lines.

We solicit comments from the International Mesothelioma Interest Group on this project. Specifically we would like to know what research groups would be interested in using these o-nitrotoluene mesothelioma cell lines in their research studies.


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Internet page
At the last committee meeting in Paris it was decided to open an internet page for posting of information, reagents, advertisements for positions, new protocols etc. This page is accessible by the following directory:
http://www.eur.nl/FGG/IMMU/meso.html
For those who are interested to leave a message on this page please fill the form on that page or send it by fax to Marjan Versnel, Dept. of Immunology, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR ROTTERDAM, the Netherlands, +31 10 4367601 (FAX).

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American Thoracic Society
ALA/ATS International Conference
May 17-21, 1997
San Francisco, USA
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Lung Cancer (IASLC) Meeting
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Dublin, Ireland
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