**ABSTRACT** (maximum 1000 words):

Peritoneal mesothelioma (PM) is a rare tumour, accounting for 10% to 20% of the 2200 cases of malignant mesothelioma registered each year in the United States. The prognosis for patients with PM is poor, with a median overall survival of 12.5 months in the best series. Diffuse malignant peritoneal mesothelioma (DMPM) is a subset of PM that account for 10% of all forms of mesotheliomas.

There is substantial interest in this disease on the part of the medical community and the general public, because millions of people have been exposed to asbestos fibers. The predicted total economic burden of malignant mesothelioma related to compensation for asbestos exposure in the next 40 years is up to $200 billion for the United States and $80 billion for Europe. The rising worldwide incidence of malignant mesothelioma is not expected to peak for another 10 to 20 years.

A variety of treatments options have been proposed, alone or in combination, but most of them have failed to demonstrate a significant impact in palliation or disease free/overall survival (Sugarbaker EJSO 2006). The chemotherapy resistance of the tumour is well known. Thus, it seems wise to try and combine systemic therapies, though still under development, with radical surgery and locoregional therapies. The recent advent of a combined approach of Cytoreductive surgery and Intraperitoneal hyperthermic perfusion (CRS+IPHP) has dramatically changed the natural evolution of the disease representing a effective salvage therapy for this clinical entity.

The most important prognostic factors are gender, completeness of cytoreduction, histological subtype, nuclear grade. On the other hand, in our previous experience we studied the prognostic value of clinico-pathologic and biological variables in patients affected by DMPM. We observed that completeness of cytoreduction, performance status, and mitotic count seem to be the best determinants of outcome. Another finding was that EGFR, MMP-2, and MMP-9 overexpressed and
p16 expression was absent/reduced in DMPM which might be involved in tumor pathogenesis and kinetics.

Starting from own experiences and in order to validate our preliminary results, a homogeneous group of patients with DMPM, defined on the basis of prognostic factors, will underwent a multidisciplinary treatment schedule which comprises CRS, IPHP and systemic chemotherapy.

The biology of DMPM is largely unknown and the cellular and molecular bases responsible for the proliferative potential and the relative resistance to current therapies of DMPM cells have not been elucidated yet. One of the hallmarks of cancer cells is their limitless replicative potential. In a high percentage of human tumors (>85%) the attainment of immortality is due to the re-activation of telomerase, an RNA-dependent DNA polymerase that stabilizes telomeres and allows cells to avoid the senescence checkpoint (Blackburn, Nature 350; 2001), and may therefore contribute to tumorigenesis and neoplastic progression (Hahn et al., Nature 400; 1999). Some tumors, however, do not have telomerase activity and maintain their telomeres by one or more mechanisms referred to as alternative lengthening of telomeres (ALT) (Bryan et al., Nat Med 3; 1997). Preliminary data, we obtained in a series of 28 PM specimens from patients who underwent CRS+IPHP at our Institute, show the presence of telomerase activity in 67.9% of cases and ALT mechanisms in the remaining 32.1% of specimens (unpublished observations). Such findings indicate the existence of multiple telomere maintenance mechanisms in DMPM and suggest the strict requirement for telomere maintenance during the development of this malignancy. As a consequence telomere-related proteins can be viewed as potential therapeutic targets in this malignancy.

Another hallmark characterizing the tumorigenic phenotype is represented by the disruption of cellular pathways that regulate cell death, and an acquired capability of tumor cells is the apoptosis escape. Preliminary immunohistochemical data we obtained in 32 PM specimens show overexpression of survivin and other IAP proteins in a high percentage of tumors, ranging from 69% to 100%, and in an elevated fraction of tumor cells within individual specimens. Accordingly, a low apoptotic index was consistently observed in these tumors (unpublished observations). Such finding would suggest IAP proteins as targets for the development of new therapeutic interventions in PM.

The primary end point is to evaluate the impact of CRS+IPHP and systemic chemotherapy on survival of patients affected by DMPM. Secondary end points are: ii) evaluation of preoperative CT scan accuracy in predicting disease operability; iii) to verify the relevance of the expression of tyrosine kinase receptor EGFR on clinical outcome; iv) to validate new therapeutic targets for DMPM taking advantage of the use of efficient and specific molecular inhibitors such as small
interfering RNAs (siRNAs) and locked nucleic acids (LNAs) directed against 1) human telomerase reverse transcriptase (hTERT) and telomere-related proteins and anti-apoptotic proteins such as survivin and other members of the IAP family (Apollon/BRUCE and XIAP).

DESCRIPTION OF THE COMPLETE PROJECT

Name of the applicant Institution: Istituto Nazionale per lo Studio e la cura dei Tumori di Milano

Title of the project: Innovative management of patients with Diffuse Malignant Peritoneal Mesothelioma: Clinical-Diagnostic Pathway and New Therapeutic Targets

Name of the project leader: Deraco Marcello

Qualification of the project leader: Responsible for the Peritoneal Surface Malignancies program of the Department of surgery of Istituto Nazionale per lo Studio e la cura dei Tumori di Milano

Publications of the project leader (10 most significant references)

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<th>Title</th>
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<td>Nonaka D, Kusamura S, Baratti D, Casali P, Rosai J, Younan R and Deraco M</td>
<td>Diffuse Malignant Mesothelioma of the Peritoneum: a clinicopathologic study of 35 cases treated locoregionally at a single institution</td>
<td>Cancer</td>
<td>2005</td>
<td>104(10)</td>
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Peritoneal mesothelioma (PM) is a rare tumour, accounting for 10% to 20% of the 2200 cases of malignant mesothelioma registered each year in the United States (Connelly, J Natl Cancer Inst 7: 1987; Antman, Lancet 11: 1985).

The prognosis for patients with PM is poor, with a median overall survival of 12.5 months in the best series (Weissmann, Proc Am Soc Clin Oncol 1988). Diffuse malignant peritoneal mesothelioma (DMPM) is a subset of PM that account for 10% of all forms of mesotheliomas (Cutler, Data of Bethesda NCI 1975). DMPM is histologically subclassified into the following types: epithelial, sarcomatoid, biphasic (mixed), and undifferentiated (poorly differentiated) (Battifora, Atlas of tumor pathology, 15: 1995; Weiss, Histological typing of soft tissue tumours, Second edition, 1994).

There is substantial interest in this disease on the part of the medical community and the general public, because millions of people have been exposed to asbestos fibers, and many articles about the dangers of asbestos have appeared in the press. In addition to its substantial personal and health care costs, malignant mesothelioma is associated with compensation costs that are a considerable problem for industry and government. The predicted total economic burden of malignant mesothelioma related to compensation for asbestos exposure in the next 40 years is up to $200 billion for the United States and $80 billion for Europe. The rising worldwide incidence of malignant mesothelioma is not expected to peak for another 10 to 20 years. It is possible that the disease has already reached its peak incidence in the United States, whereas the anticipated peaks in Europe and Australia are not predicted to occur for another 10 to 15 years. There is substantial concern that the increased use of asbestos in developing countries may result in an increase in the number of cases of malignant mesothelioma for many decades (Robinson, N ENGLJ Med 353, 2005).

A variety of treatments options have been proposed, alone or in combination, but most of them have failed to demonstrate a significant impact in palliation or disease free/overall survival (Sugarbaker EJSO 2006). The chemotherapy resistance of the tumour is well known. Thus, it seems wise to try and combine systemic therapies, though still under development, with radical surgery and locoregional therapies. The recent advent of a combined approach of Cytoreductive surgery and Intraperitoneal hyperthermic perfusion (CRS+IPHP) has dramatically changed the natural evolution of the disease representing a effective salvage therapy for this clinical entity. (Sugarbaker EJSO 2006).
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Starting from own experiences and in order to validate our preliminary results, a homogeneous group of patients with DMPM, defined on the basis of prognostic factors, will underwent a multidisciplinary treatment schedule which comprises CRS+IPHP and systemic chemotherapy.

The biology of DMPM is largely unknown and the cellular and molecular basis responsible for the proliferative potential and the relative resistance to current therapies of DMPM cells have not been elucidated yet. One of the hallmarks of cancer cells is their limitless replicative potential. In a high percentage of human tumors (>85%) the attainment of immortality is due to the re-activation of telomerase, an RNA-dependent DNA polymerase that stabilizes telomeres and allows cells to avoid the senescence checkpoint (Blackburn, Nature 350; 2001), and may therefore contribute to tumorigenesis and neoplastic progression (Hahn et al., Nature 400; 1999). Some tumors, however, do not have telomerase activity and maintain their telomeres by one or more mechanisms referred to as alternative lengthening of telomeres (ALT) (Bryan et al., Nat Med 3; 1997). Telomere dynamics in ALT cells are consistent with a recombination-based mechanism, and characteristics of ALT cells include unusually long and heterogeneous telomeres and subnuclear structures termed ALT-associated promyelocytic leukemia (PML) bodies (APBs) that contain telomeric DNA, telomere-specific binding proteins and proteins involved in DNA recombination and replication (Dunham et al., Nat Genet 26; 2000). Based on the limited information available thus far, it appears that ALT is more frequently present in tumors of mesenchymal origin than in those of epithelial origin, possibly because of a tighter repression of telomerase in normal mesenchymal than in epithelial cells (Henson et al., Oncogene 21; 2002). While it is well known that telomerase is largely expressed in pleural mesotheliomas (Kumaki et al., Am J Surg Pathol 26; 2002), no information is available in the literature concerning the presence of telomere maintenance mechanisms in DMPM. Preliminary data, we obtained in a series of 28 PM specimens from patients who underwent cytoreductive surgery at our Institute, show the presence of telomerase activity in 67.9% of cases and ALT mechanisms in the remaining 32.1% of specimens (unpublished observations). Such findings indicate the existence of multiple telomere maintenance mechanisms in DMPM and suggest the strict requirement for telomere maintenance during the development of this malignancy. As a
consequence telomere-related proteins can be viewed as potential therapeutic targets in this malignancy.

Another hallmark characterizing the tumorigenic phenotype is represented by the disruption of cellular pathways that regulate cell death, and an acquired capability of tumor cells is the apoptosis escape. Since apoptotic cell death is the major mode by which chemical and physical anticancer agents kill tumor cells, it is likely that dysregulations of the apoptotic pathways plays a role in sustaining DMPM cell chemoresistance as already demonstrated for malignant pleural mesothelioma cells. In fact, previous investigations have shown overexpression of anti-apoptotic proteins belonging to the Bcl-2 and inhibitors of apoptosis protein (IAP) families in pleural mesothelioma cell lines and surgical specimens. Moreover, through the use of antisense-mediated inhibition approaches, these studies also demonstrated a cytoprotective role of such proteins towards spontaneous and anticancer drug-induced apoptosis (Gordon et al., Carcinogenesis 23; 2002, Xia et al., Mol Cancer Ther 1; 2002). The identification of points in the apoptotic pathways at which dysregulation occurs in DMPM could open new opportunities for the design of novel therapeutic strategies targeting the molecular determinants of treatment resistance of this malignancy. Preliminary immunohistochemical data we obtained in 32 PM specimens show overexpression of survivin and other IAP proteins in a high percentage of tumors, ranging from 69% to 100%, and in an elevated fraction of tumor cells within individual specimens. Accordingly, a low apoptotic index was consistently observed in these tumors (unpublished observations). Such finding would suggest IAP proteins as targets for the development of new therapeutic interventions in PM.

Objectives (maximum 1000 words):

The primary end point is to evaluate the impact of CRS+IPHP and systemic chemotherapy on survival of patients affected by DMPM. Secondary end points are: ii) evaluation of preoperative CT scan accuracy in predicting disease operability; iii) to verify the relevance of the expression of tyrosine kinase receptor EGFR on clinical outcome of patients; iv) to validate new therapeutic targets for DMPM taking advantage of the use of efficient and specific molecular inhibitors such as small interfering RNAs (siRNAs) and locked nucleic acids (LNAs) directed against 1) human telomerase reverse transcriptase (hTERT) and telomere-related proteins and anti-apoptotic proteins such as survivin and other members of the IAP family (Apollon/BRUCE and XIAP).
Workpackages and Personnel (person/month)

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**Workpackages (WPs) 1: clinical diagnostic aspects**
*(Please, for each WP indicate the scientific responsible and for each WP describe objectives, protocols, methods and expected results)*.

**Scientific responsible for the WP:**
Marcello Deraco

**Objectives of the WP** (maximum 1000 words):

The primary objective of the present WP is to assess the overall and progression free survivals of patients affected by DMPM submitted to CRS+IPHP and systemic chemotherapy.

The second endpoint is to prospectively validate the pre-operative CT scan criteria for cyto-reducible DMPM disease defined by Yan and Sugarbake (Yan Cancer 2005).

**Protocols and methods available for the WP** (maximum 1000 words):

**Eligibility criteria for the treatment with CRS+IPHP**

- histologically diagnosed primary or recurrent DMPM-Epithelioid sub-type;
- age \( \leq 75 \) years;
- performance status ECOG \( \leq 2 \);
- good cardiac, renal, hepatic and bone marrow functions;
- informed consent.

**Exclusion criteria**

- age \( > 75 \) years;
- concomitant distant metastases, non resectable liver metastases, or other neoplasms;
- poor performance status (PS > 2);
- presence of important chronic diseases, organ insufficiency;
- refusal of the proposed treatment.
**Treatment plan**

The histologic diagnosis of DMPM will be confirmed by the following panel of immunostains; calretinin and WT as positive mesothelial markers, and polyclonal CEA and Ber-EP4 as negative markers. A preoperative staging will be done using routine methods (physical examination, abdominopelvic CT scan, chest x-ray).

Patients will be submitted to CRS+IPHP according to NCI Milan protocol followed by systemic chemotherapy (see *Protocols and methods available for the project*).

**Follow-up**

Physical examination comprising general physical examination associated with measurement of serum Ca 125 will be performed every 3 months during the first 2 years and every 6 months until the 5th year; 2) abdominopelvic CT scan and ultrasonography, and chest radiography will be performed every 6 months up to the 5th year.

**Statistical Analysis for the sample size calculation and assessment of survival results**

The primary end point of the study is overall survival. It is planned to enrol 35 patients over 3 years. Such a sample size yields an 80% power to detect, at the end of the accrual period, a doubling in median survival from an anticipated 12 months (baseline) to 24 months, with a one-sided test at the 5% significance level. For assessment of overall survival the unfavorable event will be death of any cause while for assessment of progression free survival the unfavorable event will be progression of disease as defined in the next section. The survival curve distribution will be calculated by the Kaplan-Meier method. The Log-rank test will be used to assess the significance of survival distributions.

**Validation of preoperative CT scan criteria of cytoreducible disease.**

The studies on prognostic factors conducted in our Institute demonstrated that factors associated with poor prognosis are completeness of CRS, nuclear grade and mitotic count and poor performance status. Here we propose to perform the combined approach in a subset of homogeneous patients with the exclusion of suboptimally cytoreduced patients and those with sarcomatoid component. Patients with these characteristics are deemed unanimously as of poor unchangeable outcome. Those who already received chemotherapy before the treatment will also be excluded.
As mentioned above, one of the most significant prognostic predictors for survival is the adequacy of cytoreduction. Patients who received an adequate cytoreduction, which resulted in residual peritoneal tumors \( \leq 2.5 \text{ cm} \), has improved survival compared with patients who receive a suboptimal cytoreduction, which results in residual peritoneal tumors \( > 2.5 \text{ cm} \). (Sugarbaker Surg Oncol Clin North Am 2003) However, the size of the residual peritoneal tumors is determined after the completion of surgery. This information is not available in the preoperative period in the patient selection process.

Thus Yan et al. assessed the radiologic findings which could be associated with a favourable outcome in PM patients treated with CRS+IPHP. They concluded that 2 CT findings are particularly associated with adequate cytoreduction: > 5-cm tumor mass in the epigastric region and loss of normal architecture of the small bowel and its mesentery. In a composite analysis of these 2 radiologic features, none of the patients with a > 5-cm tumor mass in the epigastric region and loss of normal architecture of the small bowel and its mesentery had an adequate cytoreduction. Patients who lacked these two preoperative CT scan findings had a 94% probability of an adequate cytoreduction (Yan Cancer 103; 2005).

For all patients, a preoperative CT scan will be performed with a minimum 1-cm continuous slice thickness through the entire abdomen and pelvis. In addition, the radiologic studies will be performed after the administration of oral and intravenous contrast media. All CT scans will be performed after an angiodynamic bolus at a rate of 1-2 mL per second, for a total volume of 150-180 mL of iothalamate meglumine 60% (Conray 60, Mallinckrodt, St. Louis, MO) or iohexol 240 (Omnipaque, Sanofi Winthrop, New York, NY). A 30-60-second delay occurred between the initiation of contrast administration and the start of CT scanning. Precontrast bowel preparation included 900-1200 mL of oral barium. Oral contrast ingestion started \( \geq 12 \) hours before the CT scan (2.1% barium sulfate suspension, E-Z-EM, Inc., Westbury, NY). Rectal barium contrast will be administered immediately before the CT scan. For clinical use, the CT scans will be read by a staff radiologist. For the current clinical research study, all CT scans will be reread by a single physician to score abdominal and pelvic tumor deposits using a standardized scoring system. The physician will be aware that all patients in the study had clinical evidence of peritoneal mesothelioma and that some anatomic sites present greater difficulty for adequate cytoreduction, but will be masked to the operative findings.

CT scan assessment of peritoneal disease will be evaluated using a peritoneal cancer index (PCI). First, the tumor size will be evaluated in 13 abdominopelvic regions: the umbilical region, right upper quadrant, epigastrium, left upper quadrant, left flank, left lower quadrant, pelvis, right lower
quadrant, right flank, upper jejunum, lower jejunum, upper ileum, and the lower ileum. For the PCI
data accumulation, it will be assumed that the upper and lower jejunum are in the left upper and left
lower quadrants, respectively, and that the upper and lower ileum are in the right upper and right
lower quadrants, respectively. Then, the tumor size will be assessed specifically at 16
abdominopelvic anatomic sites: the abdominal wall, greater omentum, right hemidiaphragm, left
hemidiaphragm, liver, spleen, stomach/duodenum, lesser omentum, subpyloric space, pancreas,
small bowel and its mesentery, large bowel and its mesentery, right paracolic gutter, left paracolic
gutter, pouch of Douglas, and the retroperitoneum.

In the 13 abdominopelvic regions and 16 abdominopelvic anatomic sites, CT scan assessment of
tumor size will be categorized into 4 groups: 0, no detectable disease; 1, minimal disease (tumor
thickness < 0.5 cm); 2, moderate disease (tumor thickness ≥0.5 cm and ≤5 cm); and 3, macroscopic
disease (tumor thickness > 5 cm).

**Expected results of the WP 1** (maximum 1000 words):

Results from this study should allow us:

a) to assess the a more accurate data concerning survival of DMPM patients treated uniformly with
CRS+IPHP and the same chemotherapy regimen. Usually data about survival of DMPM patients
usually result from studies on small sample, treated differently by different protocols.

b) to define accuracy of the preoperative CT criteria for completeness of cytoreduction defined by
Yan et al., in a prospective basis. This is of particular importance to help assistance in the
preoperative selection of patients eligible for the treatment with CRS+IPHP. The eventual decision
to operate a case that will be proven to be suboptimally cytoreducible at the moment and after the
surgery implicates not only in a substantial waste of personal and financial resources but also, the
submission of the patient to a unnecessary surgical risk without the guarantee of prognostic gain..
Workpackages (WPs) 2:
(Please, for each WP indicate the scientific responsible and for each WP describe objectives, protocols, methods and expected results).

Scientific responsible for the WP:
_Nadia Zaffaroni

Objectives of the WP (maximum 1000 words):
In this project we propose to validate new therapeutic targets for DMPM taking advantage of the use of efficient and specific molecular inhibitors such as small interfering RNAs (siRNAs) and locked nucleic acids (LNAs) directed against

1) human telomerase reverse transcriptase (hTERT) and telomere-related proteins, including the telomeric poly(ADP-ribose) polymerase tankyrase 1 and 2 and the human protection of telomere 1 (hPOT1) protein, which have been suggested to act as positive regulators of telomere elongation;

2) anti-apoptotic proteins such as survivin and other members of the IAP family (Apollon/BRUCE and XIAP).

Protocols and methods available for the WP (maximum 1000 words):
In this project we will take advantage of the availability of new molecular inhibitors such as siRNAs and LNAs to inhibit specific cyprotective factors and of the large experience acquired in our laboratory in the design and use of these molecules and in the development of strategies for their intracellular delivery.

Twenty-one nucleotide-long double-strand RNAs (siRNAs) are the effector molecule of the RNAi pathway, a natural process based on a sequence-specific, post-transcriptional gene silencing mechanism. Once entered a cells, siRNAs complementary to a target RNA are assembled to form an RNA/protein complex, the RNA-induced silencing complex (RISC), that guides the nucleolytic reaction leading to degradation of the mRNA target (Elbashir et al., Methods 26; 2002). LNAs are antisense oligonucleotides containing ribonucleotides with a methylene bridge that connects the 2’-oxygen of the ribose with the 4’-carbon. Such a chemical modification confers an enhanced stability against nucleolytic degradation and a high target affinity. Therefore, full LNAs display an attractive set of properties, resulting in a potent biological activity, and represent the most promising candidates for modulating the expression of cancer related genes (Kurreck., Eur J Biochem 270; 2003).

Sets of siRNAs and/or LNAs targeting hTERT, hPOT1, survivin and Apollon/BRUCE are already available for the study. Home-developed DMPM established cell lines, able to
generate tumors in athymic nude mice, are also available. Chemically synthesized siRNAs and LNAs will be delivered into cells through a cationic lipid-mediated transfer (e.g. Lipofectamine-2000 and/or Oligofectamine). Moreover, to further increase the efficiency of the cytosolic transport of the endocytosed molecules, the photochemical internalization (PCI) technique will be applied (Berg et al., Cancer Res.: 59; 1999; Folini et al., Cancer Res. 63; 2003). In addition, to obtain a more selective delivery of siRNAs, the therapeutic sequences will be inserted in plasmids bearing the promoters of genes that are specifically expressed in malignant cells. (Bilsland et al., Oncogene. 22; 2003, Li & Altieri,. Biochem J. 344; 1999).

The proposing group also developed specific competence in the isolation and in vitro propagation of stem/progenitor cells obtained from established cell lines and surgical specimens of other tumor types (i.e., breast cancer) (Ponti et al., Cancer Res 65; 2005).

**Expected results of the WP** (maximum 1000 words):
- Results from this study should allow us:
  - to gain information on genes/molecular pathways involved in i) telomere maintenance and immortalization, and ii) refractoriness to undergo apoptosis of mesothelioma cells;
  - to understand whether telomerase, telomere-related proteins and proteins belonging to the IAP family can be considered new therapeutic targets for this malignancy.
  - In addition, our experimental findings could provide a rational basis for the design of combined therapies, including inhibitors of specific cytoprotective factors, to enhance the responsiveness of DMPM to anticancer agents already available in the clinical setting.

**Workpackages (WPs) 3:**

(Please, for each WP indicate the scientific responsible and for each WP describe objectives, protocols, methods and expected results).

**Scientific responsible for the WP:**

Antonello Cabras

**Objectives of the WP** (maximum 1000 words):

In our previous experience studies on biological markers on DMPM patients treated uniformly by CRS+IPHP demonstrated that EGFR, MMP-2, and MMP-9 are overexpressed and p16 expression was absent/reduced in DMPM which might be involved in tumor pathogenesis and kinetics (Nonaka CANCER 104; Deraco Annals of Surgical Oncology, 13; 2006).
Based on these data we decided to continue studying the biological markers focusing on EGFR, EGFR-P, along with P53 and P185. The expressions of these markers will assessed again by standard immunohistochemistry technique.

**Protocols and methods available for the WP** (maximum 1000 words):

A representative paraffin block for each case of DMPM will be selected for immunohistochemical studies using the avidin-biotin-complex immunoperoxidase technique. Both EGFR and EGFR-P immuno-stained cells will be quantitatively evaluated and scored as follows: **score 0** if ≤ 1%; **score 1** if >1% and ≤ 20%; **score 2** if >20% and ≤ 50%; **score 3** if >50 and ≤ 80%; **score 4** if > 80% of neoplastic cells immunopositive, respectively. Staining intensity will be also recorded, using a categorical classification: **score 1** (weak); **score 2** (moderate); **score 3** (strong) immunostaining. Where the neoplastic population shows a patchy, not uniform staining intensity, the value is referred to the prevalent immunostained intensity.

**Expected results of the WP** (maximum 1000 words):

The confirmation of positive expression of these markers in particular of EGFR, EGFR-P could shed more light in the understanding of the tumor kinetics and progression. This finding could represent another valuable potential therapeutic targets for patients affected by DMPM.
Protocols and methods available for the project (maximum 1000 words):

**Cytoreductive surgery - peritonectomy**

At laparotomy we provide a full description of tumour size, location, level of invasion through the gastrointestinal wall, status of the liver, peritoneal surfaces and lymph nodes, and adherence/invasion of adjacent structures, as estimated by the surgeon, and reflected in the operative report. The Peritoneal Cancer Index (PCI) will be determined intraoperatively. Perioperative histology of suspicious lesions may be requested at the discretion of the surgeon.

If possible all tumour localisation at the peritoneal level will be removed by peritonectomy procedures. A minimal residual disease could remain with nodules not greater than 2.5 mm. Eventual residual disease will be reflected in the operative report. Neoplastic tissue biopsies are necessary for biological studies.

Cytoreductive surgery means a complete removal of all visible tumours into the peritoneal cavity. It could require peritonectomy procedures (Sugarbaker, Surg Oncol Clin North Am; 2003) eventually associated with multiple intestinal and/or organ resection.

Each procedure that composes the peritonectomy technique has a definite resection that requires an orderly sequence of surgical manoeuvres to create an optimum cytoreduction. One or more of following steps can be performed depending on the extension of primary surgical staging or disease extension at the time of CRS, in order to achieve optimal residual status: 1) greater omentectomy, right parietal peritonectomy ± right colon resection; 2) pelvic peritonectomy ± sigmoid colon resection ± hystero-bilateral salpingo-oophorectomy; 3) lesser omentectomy and dissection of the duodenal-hepatic ligament ± antrectomy ± cholecystectomy; 4) right upper quadrant peritonectomy with Glissonian’s capsule; 5) left upper quadrant peritonectomy ± splenectomy; 6) other intestinal resection and/or abdominal mass resection. Completeness of Cytoreduction will be assessed by the CC score (Sugarbaker).

**Intra Peritoneal Hyperthermic Perfusion**

The IPHP will be performed by the Closed abdomen technique: before closing the abdominal wall after cytoreductive surgery, four silicone catheters are placed in the abdominal cavity through the abdominal wall. Two in-flow catheters are placed respectively in the right subphrenic cavity and at the deep pelvic level. Two out-flow catheters are placed in the left subphrenic cavity and superficial pelvic site. A continuous peritoneal monitoring of temperatures during IPHP will be obtained by thermocouples placed in the abdominal cavity and subperitoneal site. After closure of the abdominal
skin the catheters are connected with the extracorporeal circuit. The preheated polysaline perfusate containing 43mg/l of CDDP and 15,2mg/l of Dx is infused into the peritoneal cavity using a heart-lung pump at a mean flow of 600 ml/min, for 60 min from the true hyperthermic phase (42.5°C).

Morbidity and toxicity of CRS+IPHP

Assessment of post-operative morbidity and toxicity will be done with the CTCAE v.3.

Systemic Chemotherapy

A systemic chemotherapy will be offered in the following possible situations: i) following an aggressive locoregional therapy (CRS+IPHP) in patients with high risk disease; ii) in those cases deemed as unresectable by open laparotomy. DMPM is an usually chemoresistant disease. Moreover, clinical studies and also case series analyses are substantially lacking in DMPM, hampering the assessment of clear activity of systemic chemotherapy in the disease. Thus, anecdotal reports of chemoresponsiveness should not be overlooked, and the possibility that DMPM is more responsive than its pleural counterpart should not be disregarded at the moment. Indeed, pleural mesothelioma is now viewed with somewhat more hopes. In fact, a combination regimen like Gemcitabine + Cisplatin has been associated in some series to response rates in the 40% range (Nowak Br J Cancer 87; 2002). A large randomised trial (Vogelzang J Clin Oncol. 15; 2003) reported a similar response rate for the combination of Cisplatin with the new antifolate compound MTA (Pemetrexed), proving significantly superior to Cisplatin alone also in terms of overall survival.

Since systemic chemotherapy should still be regarded as experimental in DMPM, the choice will be either the best regimen available at the moment for pleural its counterpart (e.g., Cisplatin/Carboplatin + Gemcitabine/MTA).

Feasibility of the project

The National Cancer Institute (NCI) of Milan meets all the requirements in terms of human, technical and laboratorial facilities to conduct this phase II clinical study on patients affected by peritoneal mesothelioma (PM) treated by CRS+IPHP.

Moreover, the referral characteristic of the institution guarantees the recruitment of patients according to the sample size calculation, in spite of the rarity of this disease. Since August of 1995 67 patients with PM has been treated with CRS and IPHP in the NCI of Milan. Furthermore, the growing recognition of his institute as a specialized center for PM is responsible for the increasing referral of patients affected by this disease to our institute in the last 3 years.
The PROJECT LEADER (Dr. Marcello Deraco) has a broad theoretical and practical background with locoregional treatments and has been working at the NCI of Milan since 1989. He initially acquired expertise in CRS+IPHP working as an associate researcher, at the Department of Surgical Oncology (Director Lasser MD), Unit of Digestive Surgery (Responsible Elias MD) of the Institute Gustave Roussy Villejuif in France, during 1991-1993. Then, he attended the Department of Surgery at the Washington Hospital Centre (Director Sugarbaker MD), in 1996, as a visiting MD. The PROJECT LEADER has already performed 350 surgical procedures of CRS+IPHP (the largest experience in Italy). Furthermore, he is Secretary of the Italian Society of Locoregional Treatment and the Co-ordinator in the same society of the “Study Group of Neoplastic Peritoneal Diseases”. The PROJECT LEADER participated as Researcher with an effort of 75% to 100% in three previous AIRC financed studies regarding the subject of the present proposed study. The first study was proposed in 1995 and allowed the establishment of feasibility of CRS+IPHP at the NCI of Milan. The second study was proposed in 1998 and permitted us to approach rare diseases as Pseudomyxoma Peritonei and PM. The third study evaluated the prognostic significance of biological markers in DMPM.

Given the multidisciplinary profile of present study, a co-operation with other specialities is fundamental for its feasibility. Accordingly, the project will involve the Unit of Experimental Oncology and pathology department.

Expected results of the project (maximum 1000 words):

Based on the results of clinical and biological studies conducted previously by our group we propose to refine the clinical pathway to approach patients affected by DMPM treating them homogenously with CRS+IPHP and systemic chemotherapy in a subset of patients with a favourable prognostic profile (exclusion of suboptimally cytoreduced cases, sarcomatoid sub-histotype). The diagnostic aspect of the DMPM patients management will also be approached by the validation of preoperative CT scan criteria for operability. This is could help the preoperative selection of patients for the treatment with CRS+IPHP. The eventual decision to operate a case that will be proven to be suboptimally cytoreducible at the moment and after the surgery implicates not only in a substantial waste of personal and financial resources but also, the submission of the patient to a unnecessary surgical risk without the guarantee of prognostic gain.
In the other hand we will exploit the biological insights obtained from the studies concerning the telomerase activity and apoptosis in DMPM in order to validate potential target therapies.

**Please remember:** for each WP indicate the scientific responsible and describe objectives, protocols, methods and expected results.

**Deliverables to be transferred to the National Health Service** (maximum 500 words):

If the WP-1 confirms the efficacy of the combined treatment CRS+IPHP+systemic chemotherapy for DMPM patients the results could support the employment of this therapeutic strategy in the routine clinical practice with the previous allowance of the Italian National Service. This decision should be taken despite the fact that the present study is uncontrolled. The conduction of a phase III randomized trial should be considered not feasible in an acceptable timeframe due to the extremely low incidence of the DMPM.

The results of the WP 2 instead will not have a immediate impact on the routine clinical because the study is experimental. If the results of the WP-3 should result positive concerning the expression of tyrosine kinase EGF receptors, this could support the development of phase I clinical studies testing biological therapies targeted against these receptors.

**Requested funds and co-fundings**

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**Duration of the project (in month, max 24):** 24

**Correlated ongoing projects**

The responsible for the present project is conducting another study entitled ‘Analysis of survival and biological markers in peritoneal mesothelioma managed with an innovative treatment’. This study was funded by AIRC for the year 2005.
It is a phase II clinical study aiming to determine whether patients affected by peritoneal mesothelioma (PM) may benefit from a multidisciplinary approach including extensive cytoreductive surgery (CRS)+intra peritoneal hyperthermic perfusion (IPHP)+/-systemic chemotherapy. The primary end point of the study is overall survival (OS). Secondary end points are: i) progression free survival; ii) analysis of morbimortality; iii) to characterise the clinical specimens obtained from PM for the expression of potential prognostic factors (anti-apoptotic and pro-apoptotic proteins, telomerase, GP170, MRP, BCRP, topoisomerase I and II and DNA repair enzymes, EGFR, p16, MMP-2 and MMP-9).

It was planned to enrol 35 patients over 3 years. Since the first presentation of the AIRC protocol in December 2004 (for the request of financial support year 2005) to December 2005 we accrued 12 patients. We are waiting the completion of the second year accrual to calculate the preliminary data on survival.

The results of the research line concerning the expression of epidermal growth factor receptor (EGFR), p16, matrix metalloprotease-2 (MMP-2), and MMP-9 were published on Cancer. Subsequently we performed a further evaluation of the prognostic value of pathological variables in a series of Diffuse malignant peritoneal cases followed-up by a longer period. The results were published on Annals of surgical oncology.

The other research line of the AIRC project was on apoptosis-related proteins and telomerase. We examined the expression of anti-apoptotic belonging to the Bcl-2 family (Bcl-2 and Bcl-XL) and IAP family (survivin, c-IAP1, c-IAP2 and X-IAP), and pro-apoptotic (SMAC/Diablo) proteins by immunohistochemistry in peritoneal diffuse malignant mesothelioma specimens obtained from 32 patients. Overexpression of survivin and other IAP proteins was observed in a high percentage of tumors and in an elevated fraction of tumor cells. Bcl-2 and Bcl-XL were also expressed in a high fraction of cases, with high intensity immunostaining, whereas SMAC/Diablo immunostaining was detectable in only 34% of tumors. In accordance with these finding, a low apoptotic index (median percentage of M30-positive cells, 0.45%; range, 0.01-5.8%) was consistently observed.

Telomerase catalytic activity, as detected by the telomeric repeat amplification protocol (TRAP) assay, was determined in 44 peritoneal diffuse malignant mesothelioma specimens, A positive TRAP signal was present in 29 out of 44 (66%) cases. The expression of the components of telomerase core-enzyme, hTR and hTERT, was also assessed by RT-PCR. Results obtained in these samples showed that hTR was expressed in all specimens, independently of telomerase activity. Since it has been demonstrated that alternative splicing of the hTERT is involved in the regulation of telomerase activity, we analyzed the expression of the different hTERT transcripts by RT-PCR using a specific primer set for the reverse transcriptase domain of the hTERT transcript, which allows the detection of four amplification products: the hTERT full-length transcript, and three splicing variants (α−, β−αβ−). Results of RT-PCR experiments showed that in mesothelioma specimens expressing telomerase activity, the FL transcript was always present, alone or with different combinations of splicing variants. Finally, we evaluated the expression of telomere-associated proteins, including TRF1, TRF2 and hPOT1. Results showed that such proteins were expressed in all mesothelioma specimens, and no significant difference in the level of TRF1, TRF2 and hPOT1 transcripts was observed between telomerase-negative and telomerase-positive samples.

The protocol financed by AIRC approached the clinical aspect of the PM patients treated by a combined treatment modality (cytoreductive surgery and intraperitoneal hyperthermic perfusion) focusing the results in terms of survival and prognostic factors. These effort allowed us to devise a better understanding of the PM tumor kinetics and the present project will attempt to develop new
systemic therapeutic alternatives for PM patients exploiting the results derived from the AIRC project as well as the refinement of the clinical pathway to manage patients affected by DMPM.

Milan 20.06.2006

Project Leader:

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