Author's response to reviews

Title: Phenotypic and functional analysis of lymphocytes infiltrating bone-associated tumors: use as a new therapeutic approach of osteosarcoma

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Dear Editor,

Please find enclosed the revised version of our manuscript which we propose for publication in BMC Cancer and our responses to the reviews (see below).

Thanking you in advance for your consideration

Best regards

Dr D. HEYMANN

Response to the reviewer 1 (Ian Frazer)
- According to the reviewer, we added in the legend of figures the tumor studied and the number of specimens analysed.
- The data represented in Figures 1 and 2 correspond to the median value of each CD analyzed and not to the mean +/- SD. Moreover, the small number of patient included in some subgroups (i.e. plasmocytoma, Ewing's sarcoma) did not allow to calculate the mean +/- SD. To present homogeneous data in the present paper, we decided to show the median value without SD.
- The data presented of Figure 5 are the mean +/- SD of three independent experiments. This point is added to the revised manuscript and the SD added in the Figure5.

Response to the reviewer 2 (Ruggero Ridolfi)
- The title of the revised manuscript is modified according the proposal of the reviewer.
- The references relating to studies on treatment with TIL are updated (see references 7, 9-11).
- According to the reviewer, the last sentence of the introduction is modified.
- Materials and Methods: the description of the patients included in the present work is completed (page 5, first paragraph)

"Twenty-seven bone-associated tumors [6 osteosarcomas, 2 Ewing's sarcomas, 2 chondrosarcomas, 7 giant cell tumors (GCTs), 2 plasmocytomas, 4 bone metastases (2 from kidney origin, 2 form unknown origin) and 4 other pathologies (1 chondromyxoid fibroma, 1 fibrous dysplasia, 1 chordoma, 1 undifferentiated sarcoma)] from 27 patients [12 women (38 + 17.8 years, range: 17-75) and 15 men (47.9 + 19.2 years, range: 16-75)] were included in the present study."
metastatic melanoma patients [35]. They demonstrated that in none of the patients, TAA-specific T cells were found both in tumor tissue and circulating blood at the same time. No significant changes in the frequency and specificity circulating TAA-specific T were found during the treatment period in all patients while inside melanoma tissue, TAA-specific TIL could be detected in 75% of tumor samples analyzed. These data suggested that a possible homing phenomenon of the tumor-specific T cell population to the tumor site could contribute to the effectiveness of antitumor immunity.

- The functional role of T-regulator lymphocytes CD4+CD25+ is now discussed in the revised manuscript (page 11, first paragraph and references 38-42):

"However, the role of the CD4+ T lymphocyte sub-populations on the CD8+ TIL activity remains to be clearly defined. The CD8+ T cell survival and activity against the most of the tumor is increased by CD4+ T helper cells (CD4+CD25-) and downregulated by CD4+ T regulator cells (CD4+CD25+) [38-41]. The CD4+CD25+ T cells maintain an microenvironment in the tumor sites that conceal the immunogenicity of tumor to permit progressive growth of antigenic tumors. In such cases, the suppression (in vivo or ex vivo from TIL extracted) of the T regulator cells represents a possible design of immunotherapeutic approach. In contrast to these data, the intestinal tumors which are strongly associated with an inflammatory microenvironment regress after injection of CD4+CD25+ T regulator cells [42]. The overall published papers raise the possibility of broader functions for regulatory lymphocytes in prevention and treatment of human cancers. Thus, CD4+ predominance in TIL population could not be prejudicial to the success of T lymphocyte-based immunotherapy but could be closely related to their CD25+ (low or high), CD25- status."

- According to the reviewer the first and the last sentence of the discussion are modified.