Reviewer's report

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

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Reviewer: Ruggero Ridolfi

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The study is an original piece of research work in the area of antitumor immunology. The authors aimed to verify the presence and functionality of tumor infiltrating lymphocytes (TIL) in bone cancer. There are numerous reports in the literature concerning the use of TIL in various tumor types, but no detailed data have ever been presented on the role of TIL in bone lesions. Phenotypic analysis of TIL in a series of human bone cancer samples was compared with that of TIL of rat osteosarcoma and a functional evaluation of cytotoxicity was performed with the 51Cr-released assay, which indicates potential TIL-specific antitumor activity. The following considerations can therefore be made:

1) The research is new and well-defined, although the authors are perhaps a little too hasty in their conclusions if we take into account the limited case series, which is also divided into different subgroups, and the fact that the efficacy of a future treatment remains an as yet unproven hypothesis.

2) The methods are well described and appropriate for the study, and also permit reproducibility.

3) The data reported are generally in line with those of the literature on TIL typizations of other pathologies, although for some subgroups (plasmocytoma, Ewing's sarcoma), the case series is too small to draw any conclusions.

4) The manuscript adheres to the most important standards for reporting and data deposition.

5) The Discussion and Conclusions are adequate, but I would suggest updating the references regarding new lines of research into T-regulator lymphocytes (CD4+ and CD25+), because the part about the CD4/CD8 ratio and its functionality could be strongly influenced by this type of regulatory element. Taking into account its pertinency with the specific subject matter of the manuscript, the authors could also comment on the relation between Tumor Associated Antigens (TAA) and TIL compared to that between TAA and PBL, an issue that was recently addressed by Haanen JB et al. (Cancer Immunol Immunother, 2005). Once again, I would suggest updating the references, this time relating to studies on treatment with TIL.

6) The abstract adequately summarizes the contents of the study, although the final sentence could perhaps be toned down in view of the limited case series, which only refers to osteosarcoma. With regard to the title of the paper, it would be advisable to place possible or theoretical in front of “use as a new therapeutic…” because the work does not provide a tangible evidence of a specific therapeutic approach, but simply hypothesizes such evidence.

7) The writing is acceptable.

Other suggestions regarding the text:

· Last sentence of the Introduction: in actual fact, the therapeutic potential of TIL was evaluated only for osteosarcoma, non for each pathology included in this study.

· Materials and Methods: in the description of patients, the sum of the tumor types does not correspond to 27 and the types of bone metastases are not specified (they are only reported in Table 1).

· Results: The fact that there is a consistent lytic activity against Daudi and K562 cell lines, in addition to that observed in osteosarcoma cell lines would seem to suggest a LAK-type Natural Killer activity which may not necessarily (or not only) be specific for the antigens of the tumor in question.
The penultimate and last sentences of this section are virtually identical and could be reduced to one sentence.

- Discussion: In the first and final sentences, the affirmation that TIL are easily isolated is not strictly true as no culture was obtained in 2 cases of condrosarcoma and in 2 of the 4 patients with bone metastases. The name of a cited author (Rivoltini L) is incorrectly (Rivoltani) written on two occasions. The authors might consider adding a comment on T-regulators in this section.

What next?: Accept after discretionary revisions

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

“I declare that I have no competing interests”