Author’s response to reviews

Title: Immune targeting of autocrine IGF2 hampers rhabdomyosarcoma growth and metastasis

Authors:

Carla De Giovanni (carla.degiovanni@unibo.it)
Patrizia Nanni (patrizia.nanni@unibo.it)
Lorena Landuzzi (lorena.landuzzi@ior.it)
Marianna Ianzano (marianna.ianzano@unibo.it)
Giordano Nicoletti (giordano.nicoletti@ior.it)
Stefania Croci (stefania.croci@ausl.re.it)
Arianna Palladini (arianna.palladini@unibo.it)
Pier-Luigi Lollini (pierluigi.lollini@unibo.it)

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Point-by-point response letter

Zong Sheng Guo, PhD (Reviewer 1)

1. Figure 1a. A control siRNA is missing. Investigators may use a scrambled siRNA or a siRNA for a control gene as a control in such experiments.

Answer: control bar refers to cells cultured in the presence of control siRNA not homologous to any mouse mRNA, as described in the M&M section, page 5, line 51. Legend to Fig. 1 has been modified to include this information.
2. Figure 3b. Anti-hIGF2 levels were pretty high in 2 mice vaccinated with p-BLAST. Could you speculate on the reason?

Answer: Two mice vaccinated with control p-BLAST vector displayed an over-threshold reactivity against hIGF2, but they were devoid of any reactivity against mIGF2 (page 9, lines 14-17).

Dedong Cao, M.D. (Reviewer 2)

1. What is responsible for the overexpression of IGF2 in rhabdomyosarcoma. Is there any reference?

Answer: Loss of heterozygosity or loss of imprinting at the 11p15.5 locus (including the IGF2 gene) have been suggested as two mechanisms of the overexpression of the IGF2 gene (Zhan et al, 1994; Martins et al. 2011). A sentence has been added on this point (page 3, lines 7-9).

2. The authors mentioned "Immune targeting of IGFs has been reported in a few non-rhabdomyosarcoma experimental models, both in preventive and in therapeutic approaches" in lines 34-39. Please provide detailed key outcomes of these studies.

Answer: the sentence on non-rhabdomyosarcoma models targeted with anti-IGFs antibodies now includes details on the models and approaches (page 3, lines 39-49).

3. In the methods and materials section, "Two groups of vaccinated and control mice (n=5-6)" was presented. Please provide accurate number of mice for each group.

Answer: Number of mice for each group has been specified (page 4, line 44).

4. In the "Monoclonal antibodies against IGFs" section, it is mentioned "BALB-p53Neu male mice at a pre-neoplastic stage (5-6 weeks of age) were randomized to three experimental groups". Is there any imaging evidence supporting this stage, such as CT. Please also provide method of randomization.

Answer: The predictable time of onset of rhabdomyosarcomas in this transgenic model, from histological as well as molecular point-of view, was reported in previous works (Nanni et al.
5. Is CT or MRI applied to measure tumor diameters or detect metastasis number?

Answer: predictable and superficial site of onset renders the evaluation of tumor size possible with a caliper (see page 5, lines 14-19). Autotopically, tumor masses were isolated and weighted, and confirmed the measures. Lung metastases were evaluated as number of foci at the dissection microscope and as total weight of isolated lungs, with superimposable results.

6. It is better to present pathological tissue section of rhabdomyosarcoma and salivary gland carcinomas, and typical colony images in addition to the charts in results. It is essential to provide evidence demonstrating transgene model. In "Autocrine IGF2 circuit in the BALB-p53Neu murine model of rhabdomyosarcoma" result section, was the expression of IGF2 measured after treatment of inhibitor and siRNAs? Was there any changes before and after treatment?

Answer: For pathological tissue sections of rhabdomyosarcoma and salivary gland carcinoma see previous work (Nanni et al. 2003). Concerning treatments with inhibitor and siRNAs, they were performed in miniaturized agar cultures due to the availability of reagents, which renders post-treatment recovery very difficult.

7. In "Prevention of rhabdomyosarcoma by passive administration of anti-IGFs antibodies" result section, there is lack of enough data supporting "neutralizing the autocrine IGF loop".

Answer: the sentence has been modified (“targeting the autocrine IGF loop” instead of “neutralizing…”) (page 8, line 2, and abstract, conclusion).

8. The numbers of mice used in the survival assay is small. It is better to test the immune effects on more mice.

Answer: Data from a second experiment consistent with that previously reported were pooled to increase the number of mice tested. Now p-BLAST group is composed of 12 mice, whereas p-IGF2 group is composed of 8 mice (Figure 3d).