Reviewer's report

Title: Rapid cell culture and pre-clinical screening of a transforming growth factor-beta (TGF-beta) inhibitor for orthopaedics

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Reviewer: Ugo Ripamonti

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The study investigated the effect of the TGF-B receptor Alk-4/5/7 inhibitor SB431542 on osteogenic differentiation. Results showed pronounced different results in vitro vs. in vivo outcomes, i.e. inhibition and no inhibition of osteogenic differentiation, respectively.

Specific points:

1 ref 1 is not a proper reference to refer to BMPs as bone inductors; also BMPs do not "promote an osteogenic response"; BMPs induce the de novo differentiation of bone;

2 the use of the terminology osteogenic vs. non-osteogenic BMPs is unclear to this reviewer; in general, the terminology of the Introduction section is often unclear with several terms used to define equal if not similar outcomes, i.e. bone formation, promotion of bone formation (profoundly incorrect when in contexts with BMPs), osteogenic, osteogenic vs. non-osteogenic BMPs and the like; The above is very confusing also showing the lack of proper biological and literature knowledge, i.e. the statement "There is conflicting evidence on the effects of TGF-B signaling on bone formation". The authors when writing a manuscript on TGF-B in vitro and in vivo should at least know that the three mammalian TGF-B isoforms are endowed with the striking prerogative of inducing endochondral bone formation in heterotopic intramuscular sites.

3 Of concern to this reviewer is that BMP-2 "was solubilized in sterile water"; BMPs, as a rule, are non water soluble unless specific and laborious modifications are performed on the N-terminal proregions, usually and strictly confidentially by the biotech company in context.

4 pp 12 marrow cavity reaming is not done to produce fibrosis but to induce regenerative phenomena culminating in bone tissue regeneration.

5 the Discussion section discusses at length the use of the mammalian TGF-B isoforms reporting "subtle but positive effects" concluding however that the TGF-B isoforms may only increase the amount of fibrosis. Authors need to properly read the literature on the TGF-B isoforms.

6 Hypothesis of inhibition vs. enhancement of osteogenesis and/or chondrogenesis are related to results in vitro only;

To summarize, the ms proposes that in vitro TGF-B inhibition enhances differentiation of committed osteoprogenitor cells; the in vitro results do not
translate into increased bone formation in vivo.

The scientific background and flow of the ns is severely damaged by the lack of proper understanding of the literature on bone induction, by the lack of knowledge and quotation on the endochondral osteoinductivity by the three mammalian TGF-B isoforms;

The inhibitory action of the TGF-B protein on osteogenic differentiation is obviously an in vitro unrealistic results since TGF-B isoforms cooperatively work with hBMPs to induce significant amounts of newly formed bone both in rodents and non-human primates. The authors once again show ignorance of the published literature and need to read and meditate the published data on the synergistic induction of bone formation;

Finally, as a matter of scientific discussion only, the reviewer is simply surprised to say the least by the lack of substantial bone induction by 20ug hBMP-2 injected into the reamed medullary cavity; most probably the protein was not dissolved in water so the poor if any biological effect in

**Level of interest:** An article of insufficient interest to warrant publication in a scientific/medical journal

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I ahve no conflicts of interest when reviewing the submiited ms