

Subject: *Investigation summary of findings related to the Expression of Concern: Inhibition of TPO-induced MEK or mTOR activity induces opposite effects on the ploidy of human differentiating megakaryocytes (doi:10.1242/jcs.02784). Guerriero R, Parolini I, Testa U, Samoggia P, Petrucci E, Sargiacomo M, Chelucci C, Gabbianelli M, Peschle C. J Cell Sci. 2019 May 31;132(11). pii: jcs233684. doi: 10.1242/jcs.233684. PMID: 31152055*

The Bioethics Unit and the Research Ethics Committee (REC) of the Istituto Superiore di Sanità (ISS: Italian National Institute of Health), in charge of dealing with research integrity matters, carried-out an independent investigation following the *Expression of Concern*¹ published by the Journal.

The corresponding author, Dr. Raffaella Guerriero, provided relevant and pertinent documentation and information related to the expression of concern¹, including a chronology of events that is consistent with the one given by the executive editor, Dr. Sharon Ahmad.

In summary, the Guerriero et al. study² shows that the mTOR pathway has a prevalent role in the megakaryocitopoiesis by regulating proliferation, maturation, poliploidy and cell size².

As already notified, original data related to Figure 5, object of concern, are no longer available.

The authors assert that western blotting results of phosphorylated kinases shown in figure 5 were obtained from samples loaded within gel and equal loading was checked by probing again the membranes with antibodies recognising total proteins.

The panel of figure 5 includes six lanes with thrombopoietin (TPO) in combination or not with four kinase inhibitors and the paper does not discuss all results relative to the figure; some data were already known or additional, but not meaningful for the findings of the work.

Conclusions are based on several data-based findings and on a review of the literature in this topic at the time of preparation of the manuscript. Moreover two independent following papers describe and confirm part of the conclusions reported by the authors^{3,4}.

Raslova et al (2006)³ showed that rapamicin, a mTOR inhibitor, causes the inhibition of megakaryocytes poliploidization in cultures of human megakaryocytes, as observed by Guerriero et al².

Liu et al. (2011)⁴ have evidences that mTOR is responsible for differences during the development of human megakaryocytes. Neonatal megakaryocytes are hyper-proliferative with a lower ploidy during the maturation in comparison with the adult ones. These developmental differences are

associated with up-regulated thrombopoietin signalling through mammalian target of rapamycin (mTOR) and elevated levels of full-length GATA-1 and its target.

In conclusion, the ISS Bioethics Unit and the ISS REC reviewed and confirmed results and conclusions of the paper by Guerriero et al. (2006).

The Head of the ISS Research Coordination and Support Service was informed about the results of such investigation.

The President of the ISS, Prof. Silvio Brusaferrò, approved the results of the investigation and the present document.



(Carlo Petrini)
Director of the Bioethics Unit
Chair of the Research Ethics Committee

References

1. Expression of Concern: Inhibition of TPO-induced MEK or mTOR activity induces opposite effects on the ploidy of human differentiating megakaryocytes (doi:10.1242/jcs.02784). Guerriero R, Parolini I, Testa U, Samoggia P, Petrucci E, Sargiacomo M, Chelucci C, Gabbianelli M, Peschle C. *J Cell Sci.* 2019 May 31;132(11). pii: jcs233684. doi: 10.1242/jcs.233684. PMID: 31152055
2. Guerriero R, Parolini I, Testa U, Samoggia P, Petrucci E, Sargiacomo M, Chelucci C, Gabbianelli M, Peschle C. Inhibition of TPO-induced MEK or mTOR activity induces opposite effects on the ploidy of human differentiating megakaryocytes. *J Cell Sci.* 2006 Feb 15;119(Pt 4):744-52. Epub 2006 Jan 31. Erratum in: *J Cell Sci.* 2019 May 31;132(11): pii: jcs233676. doi: 10.1242/jcs.233676.
3. Raslova H1, Baccini V, Loussaief L, Comba B, Larghero J, Debili N, Vainchenker W. Raslova et al. Mammalian target of rapamycin (mTOR) regulates both proliferation of megakaryocyte progenitors and late stages of megakaryocyte differentiation. *Blood* 2006; 107: 2303-2310.
4. Liu ZJ1, Italiano J Jr, Ferrer-Marin F, Gutti R, Bailey M, Poterjoy B, Rimsza L, Sola-Visner M. Liu et al. Developmental differences in megakaryocytopoiesis are associated with up-regulated TPO signaling through mTOR and elevated GATA-1 levels in neonatal megakaryocytes *Blood.* 2011 Apr 14;117(15):4106-17. doi: 10.1182/blood-2010-07-293092. Epub 2011 Feb 8.



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