## *Reply to Comment on* "Stability of human rapamycin-expanded CD4$^+$CD25$^+$ T-regulatory cells" Haematologica 2011;96(9):1357-65.

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We have read the letter by He and Lv[1] and we think the issues raised by the authors are not pertinent to our published work. Specifically, He and Lv raise three issues.

1) They claim that we cannot conclude from our study that rapamycin has an intrinsic role in the stability of Treg cells. We never asserted or even suggested that, based on our data, rapamycin has a direct effect on the stability of Treg cells. Our experiments show that a mixed population of Treg and non-Treg cells cultured for ten days in the presence of rapamycin is stable both *in vitro* and *in vivo*. We have never hypothesized a direct effect of rapamycin on Treg cells. Indeed, it was our group who first showed that rapamycin selectively inhibits the proliferation of effector T cells while sparing that of Treg cells[2] and not Strauss *et al.*,[3,4] as wrongly stated by the authors in their comment. Thus, it was us who had demonstrated that rapamycin does not have a direct role on the Treg cells themselves, and these findings were further confirmed in our recent paper in Haematologica.

2) He and Lv assert that we cannot claim that rapamycin-expanded Treg cells are stable *in vivo* since we only showed the frequency of FOXP3$^+$ Treg cells after *in vivo* transfer and not before. The phenotype of the cells before *in vivo* transfer was shown in detail in Figure 2 of our paper.

3) He and Lv wrote that "it was unfortunate that the authors did not obtain data in detecting the number of non-rapamycin expanded FOXP3$^+$ Treg cells recovered from the injected mice."[1] This information was reported in the "Design and Methods" section of our paper on page 1359, under the subtitle *Non-obese diabetic/severe combined immunodeficiency mouse model*.

In conclusion, we are convinced that our data support the notion that rapamycin fixes the phenotype of rapamycin-expanded Treg cells both *in vitro* and *in vivo*.

## References

1. He H, and Lv Y. Comment on "Stability of human rapamycin-expanded CD4+CD25+ T regulatory cells" Haematologica 2011;96(9):1357-1365. Haematologica 2012;97(5):e16.
2. Battaglia M, Stabilini A, Migliavacca B, Horejs-Hoeck J, Kaupper T, Roncarolo MG. Rapamycin promotes expansion of functional CD4+CD25+FOXP3+ regulatory T cells of both healthy subjects and type 1 diabetic patients. J Immunol. 2006;177(12):8338-47.
3. Strauss L, Czystowska M, Szajnik M, Mandapathil M, Whiteside TL. Differential responses of human regulatory T cells (Treg) and effector T cells to rapamycin. PLoS One. 2009;4(6):e5994.
4. Strauss L, Whiteside TL, Knights A, Bergmann C, Knuth A, Zippelius A. Selective survival of naturally occurring human CD4+CD25+Foxp3+ regulatory T cells cultured with rapamycin. J Immunol. 2007;178(1):320-9.