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Rapamycin increases survival in ALS mice lacking mature lymphocytes

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autophagy in ALS mice has not yet provided similar beneficial results.

Rapamycin is frequently used to pharmacologically increase autophagy by inhibiting the phosphorylation of the mammalian target of rapamycin (mTOR) [15]. In ALS mice, this drug has severely decreased survival [6] or did not affect survival [16]. Rapamycin is additionally used as a potent immunosuppressant as it inhibits the

the receptor was decreased by rapamycin (Figure 2D). Additional markers of autophagy, ATG5 and beclin-1, are also increased by rapamycin (Figure 2E & F).

To assess the effect of increased autophagy in ALS, we treated pre-symptomatic $SOD1^{G93A}$ mice with rapamycin. Rapamycin does not affect disease onset (Figure 3B), disease duration (Figure 3C) or survival of $SOD1^{G93A}$ mice compared to $SOD1^{G93A}$ mice fed vehicle diet (Figure 3D). However, a potential protective effect of increased autophagy by rapamycin in $SOD1^{G93A}$ mice may be masked by the detrimental immunosuppressive effect of rapamycin on lymphocytes in $SOD1^{G93A}$ mice. To circumvent this effect of rapamycin, we crossbred $RAG1^{-/-}$ mice, which are devoid of mature lymphocytes [22], with $SOD1^{G93A}$ mice to assess the effect of rapamycin in the absence of mature lymphocytes. Interestingly, when the immuno-

compounds that specifically target autophagy without immunosuppression is essential. As removing T-cells may be detrimental in ALS, $RAG1^{-/-}$ mice are useful to assess the role of autophagy in different disease models, such as in inclusion body myopathy [24], until specific autophagy-inducing compounds become available.

In summary, a protective effect of increasing autophagy is expected in ALS, but not yet been demonstrated pharmacologically *in i o*. We circumvented the negative effect of rapamycin on lymphocytes by removing these cells from $SOD1^{G93A}$ mice and found a moderate but significant effect on survival. This protective effect seems to be due to increased autophagy and indicates that this could become a therapeutic strategy to treat ALS.

Methods

Animal testing

Mice overexpressing $SOD1^{G93A}$ and recombination activating gene 1 knockout ($RAG1^{-/-}$) mice were purchased from Jackson Laboratories (Bar Harbor, USA) and maintained on a C57BL/6 background. Chow and water were provided *ad libit m* and mice were housed in the specific pathogen free facility of KU Leuven. A decrease of 10% in body weight compared to their average between day 90 and 105 is considered as disease onset. Mice no longer surviving were assessed as 0 g. End stage is defined as the age when mice could no longer right themselves from their back within 10 s and this is the measurement of survival. For Figure 3A-E both $RAG1^{+/-}$ $SOD1^{G93A}$ and $RAG1^{+/+}$ $SOD1^{G93A}$ mice were used, as their survival does not differ. All experiments were performed with the approval of the Animal Ethical Committee of KU Leuven (P020/2010).

Diet preparation

5. Shen X, Ying H, Qiu Y, Park JS, Shyam R, Chi ZL, Iwata T, Yue BY: