TIDEGLUSIB, a drug candidate for the treatment of ALS

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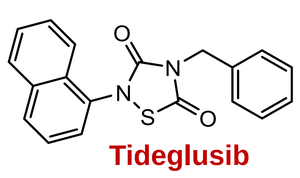
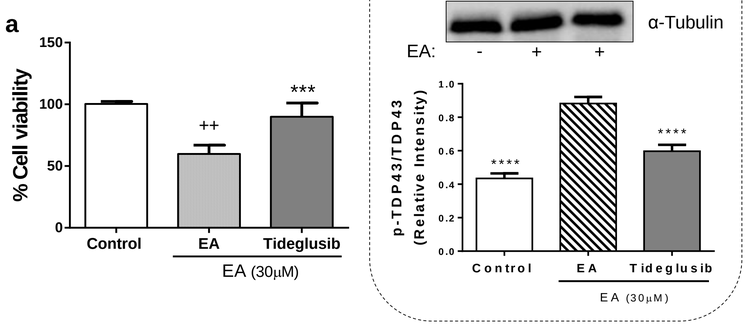
TDP-43 is the main pathological proteinopathy present in ALS, being the main therapeutic target of our research [1]. We have implemented a multidisciplinary and coordinated program called ALS_Madrid, with the main goal to discover small molecules able to recover the homeostasis of this nuclear protein. We used human-based cellular models (lymphoblasts from ALS patients) that are able to recapitulate TDP-43 pathology [2], to show differences (if any) between sporadic and familiar ALS samples in response to the different new treatments.

As different protein kinases are involved in TDP-43 pathology being responsible of some post-traslational modifications , some protein kinases inhibitors may have an important role in future ALS therapy. That is the case of tideglusib, a GSK-3 inhibitor currently in clinical trials for autism and myotonic dystrophy with a wide safety window. One of the main goals of ALS_Madrid is the repurposing of this drug for ALS based on different experimental facts that link GSK-3 with ALS in patients [3,4].

Here, we show how GSK-3 activity is increased in lymphoblasts from sporadic ALS patients, with a simultaneous increase in TDP-43 phosphorylation. Treatment with tideglusib decreased not only phospho-TDP-43 levels but also recovered its nuclear localization. Moreover, this effect is also observed *in vivo* after oral administration for 5 weeks to a tg-TDP-43 (A315T) mouse model. Further data of this latest experiment are under study.

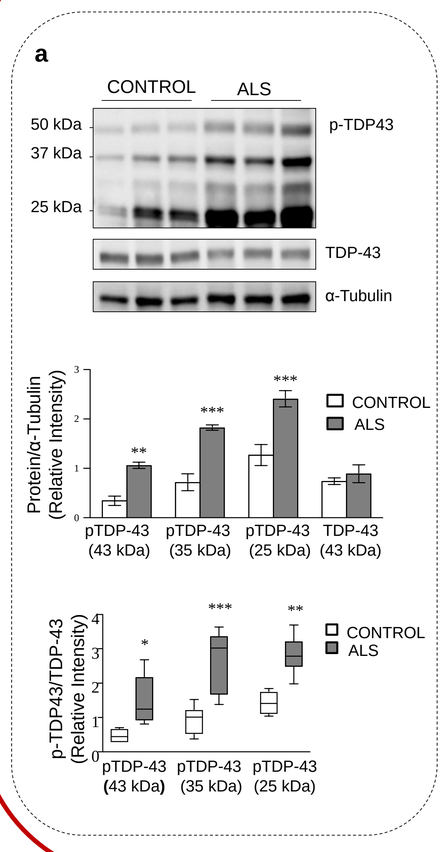
Tideglusib decrease TDP43 phosphorylation in a neuroblastoma ALS model

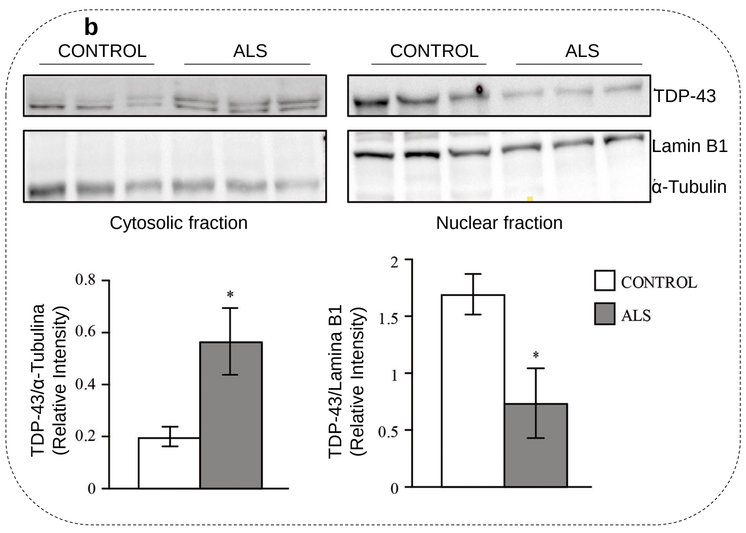




SH-SY5Y cells were treated with Tideglusib at 5 μM for 1 h and subsequently with EA (30 μM) for 24 h. a) Cell viability was determined by MTT methodology. b) p-TDP43 levels were analyzed by western blotting. Representative immunoblots are shown. Data represent the mean±SEM of 3 different experiments. (****p<0.001*, *****p<0.0001* significantly different from EA*)* (*++p<0.01 vs* Control*).*

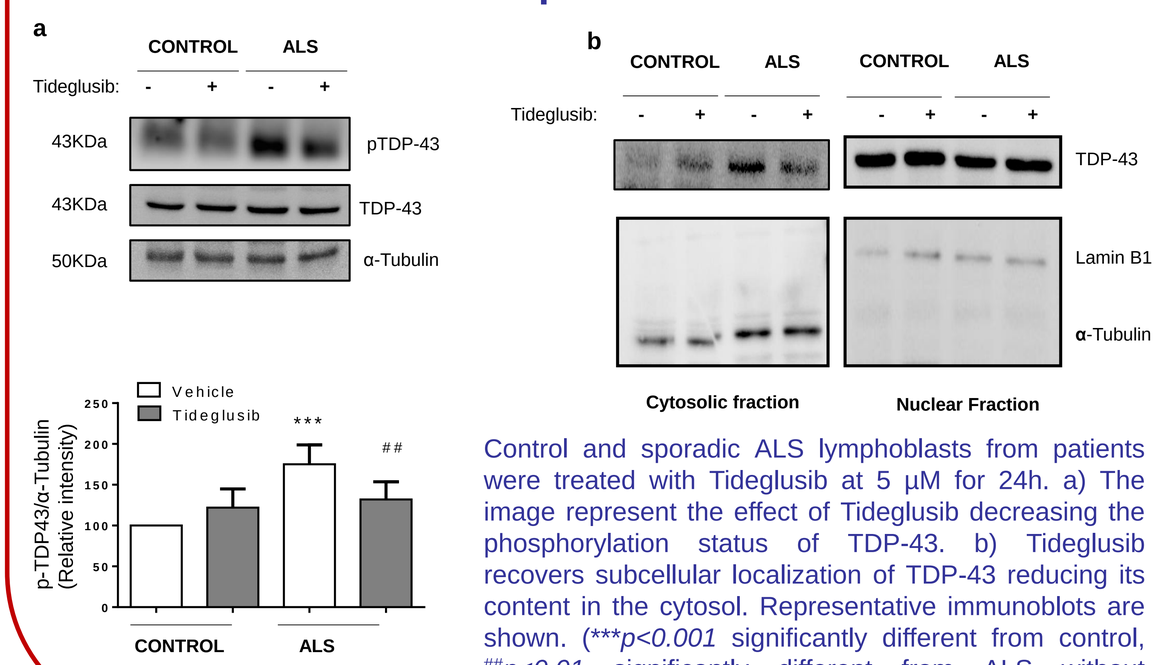
TDP43 characterization and GSK3β activity of lymphoblasts from sporadic ALS patients





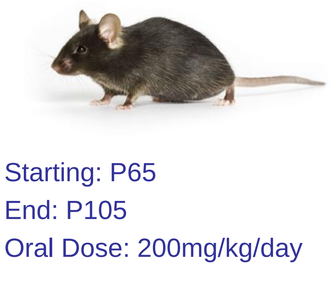
Cellular content and phosphorylation status of TDP43 in immortalized lymphoblast from control and sporadic ALS patients. Cells were seeded at a density of $1x10^6$ x mL and lysed after 24h. a) pTDP-43 (full length and truncated) and total TDP-43 protein levels were analyzed by western blotting. b) Subcellular localization of TDP-43 in control and ALS lymphoblast. Cytosolic and nuclear fragments were analyzed by western blotting. C) GSK-3β activity in lymphoblast was determined by analyzing the active (phosphorylated in Tyr216) and inactive (phosphorylated in Ser9) forms of GSK-3β. Data represent the mean±SEM of 3 different experiments. (**p<0.05*, ***p<0.01 ***p<0.001* significantly different from Control*).*

Tideglusib decreases pTDP-43 levels and recovers TDP-43 homeostasis in lymphoblasts from sporadic ALS patients

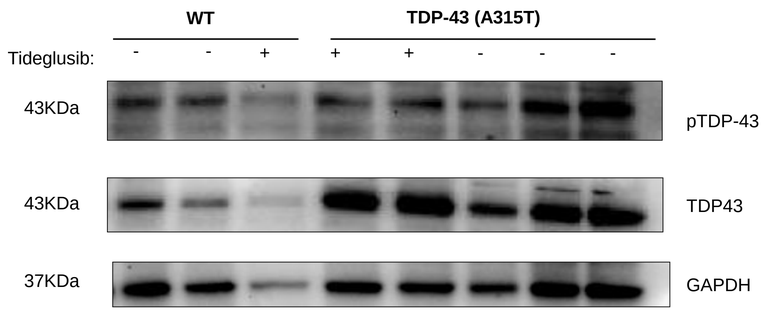


Control and sporadic ALS lymphoblasts from patients were treated with Tideglusib at 5 μM for 24h. a) The image represent the effect of Tideglusib decreasing the phosphorylation status of TDP-43. b) Tideglusib recovers subcellular localization of TDP-43 reducing its content in the cytosol. Representative immunoblots are shown. (****p<0.001* significantly different from control, *##p<0.01* significantly different from ALS without treatment*)*

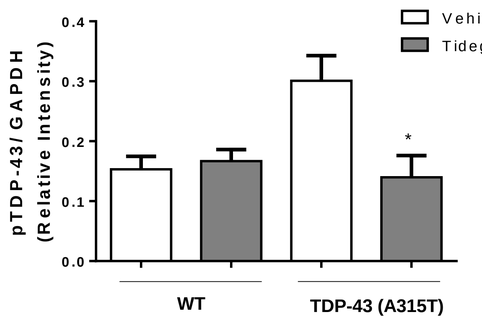
In vivo reduction of p-TDP43 by Tideglusib



Starting: P65
End: P105
Oral Dose: 200mg/kg/day



Spinal cord lysates from wild type and transgenic TDP-43(A315T) mice were used to determine the levels of p-TDP-43 and TDP-43 . Representative immunoblots are shown. Desintometric analyses represent the ratio of pTDP-43/GAPDH and are the mean ± SEM of 8 observations per group. (*p<0.05 $TDP43^{(A315T)}$tideglusib - $TDP43^{(A315T)}$vehicle)



CONCLUSION. Accumulation of TDP-43 in the cytoplasm of diseased neurons is the main pathological proteinopathy hallmark of both sporadic and familial ALS. Here we report how lymphoblasts from ALS patients recapitulate TDP-43 pathological processing in affected motor neurons such as increased phosphorylation, truncation and mislocalization of TDP-43. **Tideglusib**, a GSK-3β inhibitor, is able not only to reduce phosphorylation and normalized the nucleo-cytosol translocation of TDP-43 in lymphoblast of ALS patients, but also in spinal cord samples of TDP-43 (A315T) transgenic mice after chronic oral treatment. Based on these recent data we postulate the repurposing of **Tideglusib** as a new potential therapy for ALS ready to start clinical trials phase II.

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ACKNOWLEDGEMENTS

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