Editorial on the original article entitled "Genetic validation of a therapeutic target in a mouse model of ALS" published in the *Science Translational Medicine* on August 6, 2014

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Abstract: Amyotrophic lateral sclerosis (ALS) still remains a deadly neurodegenerative disease, mainly characterized by the combined degeneration of both upper and lower motor neurons (MNs). The pathology perspective is changed after 2006 due to the demonstration of common inclusions in ALS and Frontotemporal Dementia (non-tauFTD). Genetics largely contributed to further define the common mechanisms of both diseases but the large numbers of sporadic cases still remain unsolved. Transgenic mice models demonstrated the non-cell autonomous nature of ALS, being surrounding cells as astrocytes, microglial cells, and olygodendrocytes crucial in determining MN degeneration. More recently, the use of embryonic stem cells (ESCs) and/or IPSCs contributed to provide *in vitro* models for the ALS pathology and biological assay of clinical relevance. The combined use of ESC and SOD1 transgenic model of ALS has been pioneering used. The prostanoid receptor DP1 has been elegantly demonstrated to mediate the glial toxicity to stem-cell derived MNs *in vitro*. This evidence has been translated *in vivo*: the genetic ablation of DP1 in the SOD1G93A mice extended life span, decreasing microglial activation and MN loss. This paper is quite compelling, at the cutting edge of the stem cell-transgenic translation, demonstrating that discoveries derived from stem cells can be corroborated *in vivo* and possibly translated to humans.

Keywords: Amyotrophic lateral sclerosis (ALS); stem cells; transgenic animals; prostanoid receptor DP1; microglia; motor neuron

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Amyotrophic lateral sclerosis (ALS) has been partially deciphered by the dramatic development of modern genetic technologies: more than 20 different genes have been demonstrated to play a role in the pathogenesis of both familial and sporadic ALS cases. As a result of these discoveries, different mechanisms of disease have been proposed, raising the question as to whether ALS is a proteinopathy, a ribonucleopathy, or both (1). ALS is also considered at the opposite end of the spectrum for a single disease with frontotemporal dementia (FTD), due to the fact that neurons in the prefrontal and temporal cortex are also affected to varying degree with frontal executive dysfunctions present in many patients and concomitant FTD in about 15% of ALS cases (2). As a consequence, FTD responsible genes must be considered in ALS patients with cognitive/behavioral changes. The most recent genetic discovery on *TUBA4A* (3) seems to point to the role of cytoskeletal proteins as responsible of the motor neuron (MN) loss after previous demonstration of *PFN1* (4), *DCTN1* (5), *PRPH* (6), and *NEFH* (7).

Although MN loss is the major characteristic of ALS, sustained activation of a neuroinflammatory response executed by a diverse range of glial cells is commonly found both in the spinal cords of ALS patients and of rodent models.

As these cells contribute to the progressive MN degeneration phenotype, the mechanism of neurodegeneration in ALS is established to be non-cell autonomous. In fact, studies of mutant SOD1 mice have demonstrated that astrocytes and microglial cells that surround MNs contribute to disease onset and progression (8). Glial cells become increasingly activated as the disease progresses in both animal models and ALS patients: a phenomenon called neuroinflammation. This reaction can have both deleterious and protective consequences (9). Recent evidence has greatly strengthened this concept. The fact that ALS astrocytes can induce MN death has been demonstrated both *in vivo* and *in vitro*. Of notice, this toxic effect was not only shown for astrocytes derived from the mutant SOD1 mouse but also for astrocytes from patients with SALS. Interestingly, wild-type SOD1 was found to elicit the toxic effect of astrocytes taken from patients with SALS, as knocking down SOD1 in these astrocytes abrogated toxicity (10). A dual (protective and toxic) effect of microglial cells in ALS is established but certainly not fully elucidated (11). Already at early disease stages, microglia recruits peripheral monocytes to the CNS. These monocytes are polarized to a macrophage phenotype in ALS mice and in patients with ALS, promoting neuronal loss (12). The role of T cells in ALS is only beginning to emerge. T cells infiltrate the spinal cord of patients with ALS and SOD1 mutant mice (13-15). $CD4^+$ T helper cells appear to modulate the inflammatory response beneficially, as deletion of these cells promotes neurotoxic action of microglia and astrocytes, responsible for a worsened disease outcome in mutant SOD1 mice (13,14). Regulatory T cells that infiltrate the spinal cord at the early symptomatic disease stages seem to have a beneficial influence by slowing disease progression, but their neuroprotective influence ultimately fails (13,16). Likewise, in patients with ALS, regulatory T cells influence disease progression rates: an early reduction in the expression of the regulatory T cell transcription factor FOXP3 was found to be predictive of rapid disease progression (17). In living ALS patients, the seminal paper of Turner *et al*. [2004] (18) corroborated the role of the neuroinflammatory mechanisms in the pathogenesis of ALS. The PET ligand 11C-PK11195 binds to the peripheral benzodiazepine receptor expressed by activated microglia and in ALS patients provided *in vivo* evidence of the widespread corticospinal tract and extra-motor microglial activation, notably lateralized within the hemisphere contralateral to the most affected body side in individuals with rare UMN forms of motor neuron disease (19).

To further clarify neuroinflammation in ALS, there are many examples of disease recapitulation using stem cell (SC) models obtained from patients, however few attempts have been made to determine whether mechanisms of disease learned from SC disease modeling can be validated *in vivo*.

The de Boer *et al*. paper (20) cogently supports the predictive power of SC systems: after preliminary demonstration of the neurotoxicity to MNs due to the treatment with prostaglandin D2 (PGD2) in a coculture system with astroglial cells similarly to that observed in coculture with primary glia from SOD1G93A mouse model, DP1 and DP2 PGD2 receptors have been investigated as responsible for mediating the resulting glial toxicity. To define the selective role of the DP1 receptors, ESC-derived human MNs have been tested with specific agonists and antagonists with the demonstration of the selective neurotoxicity on MNs mediated by glial toxicity. The negative effect on MNs of the SOD1G93A astrocytes was equivalently related to the DP1 receptor interaction. As a logical consequence, upon genetic removal of the *Ptgdr* gene (coding for DP1 receptor), the mouse glia of SOD1G93A revealed a dose-dependent reduction in toxicity to MNs. This evidence, gained using sophisticated technologies and different co-culture systems, finally indicates DP1 as an important modulator of both prostanoid signaling pathway transcription and *in vitro* glia toxicity using ESC-derived human MNs.

Being still unclear to what extent findings from SC models are predictive of outcomes *in vivo*, the genetic elimination of DP1 has been induced to test the life span in the SODG93A mouse model. Animals were demonstrated to live significantly longer. Unlike in the *in vitro* models, the complete elimination of the DP1 receptor did not provide additional MN protection or increase life span. In the further definition of the role of astrocytes *vs*. microglial cells on MN loss, a decrease in microglial activation has been demonstrated with a reduced inflammatory expression with decreased exhibition of distinct markers (*Ptgdr* or DP1, *Ptgs2* or Cox-2, *Ptgds* or L-PGDs) both *in vitro* and *in vivo*. The *SOD1*G93A/DP1 *in vivo* model provides combined evidence of both reduced microgliosis and decline in astrogliosis: this was a good argument for de Boer *et al*. to translate the question in the *in vitro* systems to test if DP1 was acting on microglial, astroglial, or both cell types to modulate toxicity to MNs. No MN toxicity was observed when purified astrocytes were tested in different experimental conditions: on the contrary, $CD11b^+$ microglia was isolated from nontransgenic and *SOD1*G93A glial cultures and subsequently tested on human ESC derived

MNs. The neurotoxicity due to the mutant microglial cells has been elegantly demonstrated. The changes of the DP1 genotypes both in the nontransgenic and *SOD1*G93A microglial cells accordingly influenced the MN survival and phenotype: these experiments conclusively showed that the effects of DP1 modulation on MN survival can be mediated through microglia.

The de Boer *et al*. (20) paper is impressive, representing a powerful translation from SC models to *in vivo* systems and vice versa. Beside the specific evidence for the microglial role in ALS due to DP1, the swing between the *in vitro* and the *in vivo* systems provides a proof of principle for an innovative strategic approach able to define complex mechanisms of disease. ALS as other neurodegenerative diseases has a non-cell autonomous mechanism of disease (21) and clarifying the role of the different cell phenotypes appears more and more critical. SCs can provide ideal diversified cell phenotypes to be tested in defined condition *in vitro*. Even more intriguing is the opportunity offered by the *in vitro* system to test molecules of clinical relevance and the translation to animal models may implement the results, offering further positive evidence for human clinical trials. Crossing the "valley of death" between basic science and clinical applications appears practicable because the SC systems can be derived from humans: the testing on ESC-derived human MNs has been applied in a large series of experiments by de Boer *et al*. (20).

As we recently pointed out (22), nearly 50 RCTs for disease-modifying treatments in ALS have been undertaken in the past half-century and riluzole is the only FDA and EMA approved drug, which emphasises the need for a crucial reassessment of methods used in ALS drug development. The recapitulation of disease mechanisms in SC models of disease and subsequent validation *in vivo* with animal models expressing the pathology may represent the way of the future: any possible option needs to be pursued to solve the ALS conundrum.

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