

# Future therapeutical strategies dictated by pre-clinical evidence in ALS

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## ABSTRACT

*Classic concepts on amyotrophic lateral sclerosis led to define the disease as a selective degeneration of upper and lower motor neurons. At present such selectivity is questioned by novel findings. For instance, the occurrence of frontotemporal dementia is now increasingly recognized in the course of ALS. Again, areas outside the central nervous system are targeted in ALS. In keeping with motor areas other cell types surrounding motor neurons such as glia and interneurons are key in the pathogenesis of ALS. This multiple cell involvement may be due to a prion-like diffusion of specific misfolded proteins which are altered in ALS. This is the case of FUS and TDP-43 which harbor a prion domain prone to pathological misfolding. These misfolded proteins are metabolized by the autophagy, but in ALS there is evidence for a specific deficit of autophagy which impedes the clearance of these proteins. These concepts lead to re-analyze the potential therapeutics of ALS. In fact, mere cell substitution (stem cell) therapy appears insufficient to contrast all the alterations in the various pathways affected by ALS. Although preclinical data speed the application of stem cells in human clinical trials, several hurdles limit their translation into new therapies. Future treatments are expected to consider the need to target both motor neurons and neighboring cells which may contribute to the diffusion and persistence of the disease. On this basis the present manuscript describes which future strategies need to be pursued in order to design optimal therapeutic trial in ALS.*

## Key words

*Frontotemporal dementia • Autophagy • Protein clearing system • Prion-like proteins*

ALS has been considered for decades to be the prototypical pyramidal motor system neurodegenerative disease: degeneration of upper and lower motor neurons (MNs) with MN cytoplasmic inclusions immunoreactive for ubiquitin and degeneration of the corticospinal tract were considered to be diagnostic for ALS (Hirano et al., 1996). Recently, researchers have begun to recognize an important connection between frontotemporal dementia (FTD) and ALS: TDP-43 (ubiquitinated TAR DNA-binding pro-

tein) is a multifunctional DNA/RNA-binding factor implicated in the regulation of neuronal plasticity. The notion that pathological TDP-43 is involved both in ALS and FTD was proposed when it was discovered by Neumann et al. (2006) that this protein is the major constituent of pathological inclusions in FTD with U (FTD-U, now known as FTD-TDP) but also in FTD-MND, and ALS. Therefore, a common pathogenesis linked to TDP-43 abnormalities in these disorders has been suggested and further

confirmed. This scheme reflects the considerable overlap of clinicopathological features between neurodegenerative diseases: ALS, FTD-MND, and FTD-U may be situated at different points along a continuous and broad spectrum of a multisystemic degeneration (Armstrong et al., 2005; Strong et al., 2008; 2009). Recently, it has been demonstrated that elevated expression of TDP-43 in mouse fore-brain causes neuropathological patterns similar to FTD-U, mimicking its specific behavior phenotype (Tsai et al., 2010). Moreover, both TDP-43 and Cu/Zn superoxide dismutase-1 (SOD-1) proteins modulate sequestration of neurofilament mRNAs in the aggregates characteristic in ALS MN degeneration (Volkering et al., 2009). Mutations in a gene encoding another DNA/RNA-binding protein with striking structural and functional similarities to TDP-43 named FUS (fused in sarcoma) or TLS (translocation in liposarcoma) have been recently reported to trigger degeneration of MNs and be responsible also for FTD (Ticozzi et al., 2009). Although this gene was initially identified as a component of a fusion pro-oncogene resulting from a chromosomal translocation seen in liposarcomas, it belongs similarly to a subfamily of RNA-binding proteins, involved in MN (patho) physiological biology/metabolism. The emerging scenario of multiple regional involvements due to the TDP-43/FUS neuropathological inclusions in the central nervous system of ALS patients entails a significant impact on the therapeutic strategies applicable to ALS and, particularly, on the stem cell (SC) approach: RNA-binding proteins appear as key regulators of signaling networks responsible for neuronal development and homeostasis, as well as neural SC biology (Deschenes-Furry et al., 2006; Bolognani et al., 2008). Therefore, healthy transplanted cells cross talk with the surroundings may be compromised by the abnormal cellular RNA metabolism, able to trigger MN degeneration, thus impeding any therapeutic outcomes related to SC. Furthermore, FUS and TDP-43 harbor also a "prion domain" very similar to the specific one present in several yeast prion proteins prone to pathological misfolding transmissible within or between healthy cells or species (Fuentealba et al., 2010; Cushman et al., 2010; Liu-Yesucevitz et al., 2010; for a review see Udan and Baloh, 2011). This concept lead to a potential explanation of multiple cell types involvement in the

anterior horn of the spinal cord where motor neurons can be affected along with interneurons (Fornai et al. 2008a; Chang and Martin, 2009; Pasquali et al., 2009). On the other hand it is likely that the spreading of the disease along different brain regions reported above may be justified by a prion-like spreading of the disorders. In this case, no SC strategy could maintain positive therapeutic outcomes in the long term, without a supportive treatment able to prevent the spread of the disease (Cushman et al., 2010). Future treatments are expected to consider the need to target both MNs and neighboring cells and pathways which may contribute to the diffusion and persistence of the disease.

These novel concepts may help to explain why ALS and frontotemporal lobar degeneration (FTLD) are anatomo-clinical overlapping disorders which initiate in different location to progressively cover similar brain areas.

Additional data demonstrate that ALS affects brain regions beyond the pyramidal system and frontotemporal cortex. In fact, ALS can now be defined a multisystemic disorder with a critical involvement of the upper and lower MN which determine the disease progression and the dramatic course of the disease. Nonetheless, a variety of neuronal and non-neuronal cells are altered. Sensory neurons may be involved in ALS (Isaacs et al., 2007) as well as extrapyramidal neurons (Vogels et al., 2000) and the autonomic nervous system. In fact, recent literature demonstrates the occurrence of autonomic dysfunction in ALS, which leads to alterations of cardiovascular system and gut as an early step in ALS progression (for a review see Baltadzhieva et al., 2005).

The occurrence of multi-system pathology in what originally believed to be just ALS led to the concept of ALS-plus syndrome (see McCluskey et al., 2009). By definition, ALS-plus syndrome meets clinical criteria for ALS but also includes one or more of the following: dementia, geographic clustering, extrapyramidal signs, objective sensory loss, autonomic dysfunction, cerebellar degeneration, or ocular motility disturbance. If originally kept distinct from ALS, the concept of ALS-plus syndrome is now involving almost all ALS cases since one or more items listed above represent a constant ALS feature. This led Pradat and Bruneteau (2006) to write about classical and atypical features of ALS.

Occurrence of pathological alterations extending beyond the central nervous system was bound to the finding of systemic biochemical alterations which recently were indicated in ALS patients (Keizman et al., 2009; Miana-Mena et al., 2010). These recent data offer a new perspective to understand ALS. In fact, starting from mechanism-based pre-clinical studies, systemic alterations offer a key to define pathophysiology as well as peripheral markers of the disease (Meininger, this issue).

In fact, the spreading of cell dysfunctions beyond what was once considered a selective MN alteration led to dissect ubiquitous pathways which appear to be significantly involved in ALS pathogenesis.

Consequentially, mere cell substitution appears insufficient to contrast all the alterations in the interrelated complex cerebral pathways activated by ALS.

Among this pathway the autophagy is now taking a leading role since most forms of identified familial forms of ALS are characterized by autophagy failure (Fornai et al., 2008a,b; Madeo et al., 2009; Crippa et al., 2010). This applies, for instance, to the autophagy failure occurring in the SOD-1 mutation, dynein mutations; dynactin mutations; ESCRT mutation; alsin mutation (for a recent review see Pasquali et al., 2010). Interestingly, genetic or pharmacological treatments which induce autophagy produce protective effects on motor neurons (Hetz et al., 2009; Crippa et al., 2010).

For all these reasons, mere cell substitution appears insufficient to contrast all the alterations in the interrelated complex cerebral pathways activated by ALS. Preclinical data demonstrate in principle the feasibility of SC application to ALS animal models speeding the route towards human clinical trials, but several hurdles still limit a direct translation into new therapies, as discussed by Lindvall and Kokaia (2010). Retrieval of inconsistent behavioral improvements after SC grafting in animal models have not been paralleled by adequate comprehension of the underlying regulatory mechanisms exploitable for the development of standardized protocols applicable to ALS patients. Altogether, the preclinical data show the feasibility of SC therapy for ALS, but more definitive answers are needed on the biological cascades activated by transplantation, such as the regulation of grafted SC behavior in terms of survival, proliferation and migration, as well as novel functional synaptogenesis, before widespread

human trials can be contemplated (Garbuzova-Davis and Sanberg, 2009).

Multiple small pilot trials in ALS patients, using a variety of different SC types, have been published, but inconsistency on safety procedures, optimal cell dose/source, and delivery route reduce the interpretation of their potential efficiency (Cova and Silani, 2010). An impressive debate on expensive SC treatment offered worldwide has enlightened a complex reality where both meaningful interpretations and anecdotal reports are strictly linked. Nevertheless, since efficacious therapy is lacking, the severity of ALS might justify the potential risks of intervention in patients to demonstrate clinical feasibility of pioneering SC techniques (Gornall, 2010). A clinical trial aimed to evaluate the safety of human SC implant in ALS patients is currently ongoing in the United States, which follows the preclinical validations of the surgical procedures (Riley et al., 2009). The SCs used in the study, prepared from cultured neural SCs, have previously been shown to extend the life of rats with ALS (Xu et al., 2006) and reverse the paralysis in rats affected by ischemic spastic paraplegia (Cizkova et al., 2007). Direct neuronal differentiation of the grafted cells and release of growth factors to host MNs via graft-host connections have been suggested as the mechanisms responsible for the positive effects observed in both models.

The Phase 1 trial directed by Dr Glass at Emory University (Georgia, USA) will enroll up to 12 ALS patients who will receive 5-10 SC injections in the lumbar area of the spinal cord. The patients will be examined at regular intervals after surgery, with final review of the data to come about 24 months later. Depending on the success of this initial trial, a follow-up phase 2 trial or a modified Phase 1 trial is expected to implement the surgical and experimental procedures. This clinical trial is now recruiting ALS patients.

Intravenous, intrathecal, and more often intraparenchymal administrations of hematopoietic SCs derived from peripheral blood or bone marrow have been tested in a small series of patients. Even if safety and lack of early side effects have been claimed, the majority of these studies did not exhibit solid preclinical evidence as recommended before translation to clinical application (Silani et al., 2004; Badayan and Cudkovicz, 2008). Clinical efficacy appears

unproven and long-term safety needs to be demonstrated. A large number of ALS patients have been recently reported after intracerebral transplantation with olfactory ensheathing cells (Chen et al., 2007; Huang et al., 2008; 2009) with disabling side effects reported for a patient who received this therapy in Beijing, China (Chew et al., 2007). No sham operations have been documented, and the interpretation of the reported data is difficult because it is generated mostly outside the construct of a well-designed clinical study. Finally, novel surgical techniques for efficient SC delivery within the spinal cord have to be developed and tested to maximize safety and to support grafted cell integration in the host circuits, as recently suggested by several reports (Feron et al., 2005; Blanquer et al., 2010).

Guidelines for clinical trials using SCs need to be specifically designed for ALS patients after selection of the most appropriate end points to reach clinical significance: the historical data related to cell transplantation in PD may be instrumental in achieving this goal. The neurological community has to reach a consensus on the design of clinical trials in ALS and proceed with a long follow-up to define the outcomes possibly using biomarkers.

## Summary

Future therapeutic strategies in ALS need to be grounded on robust data which are now evident from pre-clinical studies and from an in depth analysis of the complex pathology of ALS. The evolution of the concept of ALS from a selective motor neuron disease into a multi-system disorder is now well established. In this context motor neurons lost their uniqueness as therapeutic target. In fact, within motor areas cell types other than motor neurons contribute to the disease and multiple regions beyond cortico-spinal and cortico-nuclear systems are affected as well. Novel findings suggest that an altered protein clearance based on defective autophagy may increase the ability of prion-like proteins to spread away from motor neurons to produce such a multi-system disorder. This calls for novel therapies aimed beyond restoration of motor neuron loss in order to impede disease spreading and target a variety of cell types. In this scenario stem cells which still need to be investigated lost part of their

potential. Combined therapies are needed in which clinical evaluation needs to be grounded on ALS-specific guidelines for trial design.

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