Omics Sciences and Amyotrophic Lateral Sclerosis

Subjects: Neurosciences

Contributor: Francesca Luisa Conforti , Paola Ruffo , Sebastiano Cavallaro

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease caused by the progressive loss of motor neurons (MNs) resulting in weakness and paralysis of voluntary muscles. The main clinical feature of ALS is the upper and lower MN involvement. The age of onset is about 60 years, and the incidence is 5 per 100,000 inhabitants. Although important research progress has been made, the etiopathology of ALS is mostly unknown. The mechanisms underlying the development of the disease are multiple, with the involvement of a complex interaction between genetic and molecular characteristics. The major ALS-related genes include superoxide dismutase 1 (*SOD1),* FUSed in sarcoma *(FUS)*, TAR DNA binding protein *(TARDBP)* and chromosome 9 open reading frame 72 (*C9Orf72)*. Due to the high complexity of the disease, the diagnosis is made by exclusion and there are no effective drug therapies that can stop or significantly slow down the progression of the disease. To date, the drugs used to slow down the course of the disease are Riluzole, which works by reducing excitotoxicity, and Edaravone that decreases oxidative stress. Neuropathology and medical genetics have led to the discovery that ALS and Frontotemporal Dementia (FTD) are related diseases and form a broad neurodegenerative continuum. Both of these pathologies can be caused by mutations in the same gene that can lead to different clinical pictures. The discovery of hexanucleotide expansion involving the *C9Orf72* gene helped to define a genetic basis to explain the spectrum ALS/FTD.

clinical trials omics personalized medicine neurodegenerative disease motor neuron disease

1. Drug Development

The application of omic sciences has enabled the development of novel approaches to understand the molecular nature of ALS. As described in the following sections, their use has potential clinical implications to stratify patients and identify effective and safe treatments [1][2].

1.1. Omics Approach for Patients Stratifications

A genomic approach has been used in different clinical trials to select patients with specific drug-targetable gene mutations. Trial NCT01041222 was the first to use an intrathecally injected antisense oligonucleotide (ASO) designed to inhibit *SOD1* expression in *SOD1*-fALS mutation carriers. The results revealed a successful strategy showing that the drug (ISIS 333611) was well tolerated [3]. A similar genetic stratification was applied in trial

NCT00706147, where a genotype-phenotype homogeneous population of *SOD1*-fALS mutation carriers were used to test the safety, tolerability and efficacy of Arimoclomol, a drug promoting the correct folding of proteins. The entry demonstrated drug tolerability and safety but did not show therapeutic efficacy [4]. In trial NCT04494256, subjects with *ATXN2* expansion were enrolled to assess the safety, tolerability and pharmacokinetic profile of BIIB105, an ASO designed to bind and degrade the *ATXN2* mRNA.

1.2. Omics Approach for Monitoring

A transcriptomic approach has been used to evaluate pre- and post-treatment gene expression changes in ALS patients [5]. In trial NCT04632225, RNA sequencing was used to determine and compare transcription profiles in patients receiving Engensis or placebo. Engensis is a gene therapy based on a plasmid that allows direct expression of the hepatocyte growth factor (HGF) in nerve cells of ALS patients. In trial NCT03359538, the effects of rapamycin in addition to riluzole in ALS patients was assessed by evaluating pathways related to immune response in plasma and cerebrospinal fluid (CSF). A transcriptomic and metabolomic approach was used in trial NCT00875446 to test the safety, pharmacokinetics and pharmacodynamics of the monoclonal antibody GSK1223249 (Ozanezumab), targeting the myelin-associate neurite outgrowth inhibitor (NOGO-A) in ALS patients. The results conducted on muscle biopsy and plasma by DNA microarray technology, demonstrated the drug is well tolerated although no drug-dependent gene expression patterns in muscle or plasma were identified [6]. The same approach was exploited in trial NCT03456882 to identify pharmacodynamic biomarkers following treatment with RNS60, an electrokinetically altered aqueous fluid.

In several clinical trials, the drug's impact was determined by comparing the expression of different markers in patients exposed to drugs or placebo (NCT01854294, NCT03800524, NCT04505358, NCT03693781) or observing pre- and post-treatment clinical outcomes (NCT01884571, NCT02525471, NCT02469896, NCT05193994, NCT04788745). Notably, the results of trial NCT01854294 showed that the master regulator peptide GM604 altered plasma expression levels of SOD1, TAU and TDP-43 proteins, slowing down disease progression [7]. In trial NCT01884571, analysis of mRNA expression profiles in blood T-cells was used to assess the effect of immunosuppression and showed no disease-modifying effect following this treatment [8]. By evaluating the effects of Tocilizumab, a monoclonal antibody that inhibits Interleukin-6, trial NCT02469896 demonstrated dysregulation of pro-inflammatory genes in peripheral blood mononuclear cells of sporadic ALS patients [9].

Although discoveries related to the molecular basis of a disease may offer unprecedented opportunities to translate into new drugs, their development requires an enormous amount of time, money, and effort. For this reason, some trials have the objective to repurposing existing drugs to new disease areas [10]. Based on this strategy, the pharmacological properties of Enoxacin were assessed in ALS patients in trial NCT04840823. By acting against bacterial DNA topoisomerase II, this antibacterial agent is used in the treatment of urinary tract infections [11]. Since Enoxacin may regulate the expression of miRNAs, this trial aims to evaluate the ability of this drug in modulating miRNA species in CSF and plasma of ALS patients. Another repurposing drug strategy has been exploited in trial NCT02437110 where the combination of Darunavir, Ritonavir, Dolutegravir, and Tenofovir alafenamide (an

antiretroviral therapy approved for human immunodeficiency virus infection) has been tested to suppress the activation of Human Endogenous Retrovirus-K (HERV-K) in ALS patients.

Clinical trial NCT04066244 proposed a multi-omics approach, the NCT04066244 to characterize the safety, tolerability and response of BLZ945, a Colony Stimulating Factor 1 (CSF-1) inhibitor. The outcomes of this trial will be based on genotyping cytochrome P4502C8 (CYP2C8), an enzyme involved in metabolism of xenobiotics.

1.3. Multi-Omics Approach for Both Stratification and Monitoring

A genomic, metabolomic and transcriptomic approach has been used to select patients and monitoring therapy. In trials NCT03626012 and NCT04288856, C9Orf72 patients were enrolled to evaluate the safety, tolerability and pharmacokinetics of BIIB078, an ASO targeting C9Orf72 mRNA. A similar approach was applied in trials NCT05163886 and NCT05053035, where C9Orf72 patients were recruited to test the safety, tolerability and biological effect of LAM-002A, (apilimod) an inhibitor of the PIKfyve kinase that works by clearing toxic protein aggregates within lysosomes. The outcome of this trial was to evaluate LAM-002 levels, metabolites and neurofilament light chain (NfL) in plasma and CSF.

Clinical trial NCT04993755 aimed to characterize the safety and tolerability of the reverse transcriptase enzyme inhibitor TPN-101 (censavudine) in C9Orf72 patients with ALS/FTD, ALS or FTD. A similar patient's classification was used in trial NCT04931862 to test the effect of WVE-004, an ASO designed to mediate the degradation of C9Orf72 mRNA.

A genetic stratification was applied in trial NCT02623699, where SOD1-patients were enrolled to test BIIB067 (tofersen), an ASO designed to degrade *SOD1* mRNA and to prevent protein synthesis. The results confirmed drug safety and revealed drug-dependent *SOD1* expression levels in CSF [12]. The same cohort of patients was used in the extension study NCT03070119 to test long-term treatment with BIIB067. The placebo-controlled trial NCT04856982, proposed to initiate BIIB067 treatment in clinically presymptomatic patients. A similar genetic stratification was applied in trial NCT01083667, where *SOD1* mutation carriers were used to test the antiparasitic drug, Pyrimethamine (Daraprim).

A genomic and metabolomic approach was used in trial NCT04768972 to evaluate the safety, pharmacokinetics and pharmacodynamics of ION363 in FUS-mutation carriers. This drug is an ASO designed to reduce the production of mutated neurotoxic form of *FUS* protein. This successful strategy showed a reduction in CSF of NfL concentration. Using a repurposing drug strategy, *FUS* patients were recruited in trial NCT03707795 to evaluate the effect of Betamethasone by assessing levels of different biomarkers in plasma. A similar strategy was exploited in trial NCT05189106, where the reduction of CSF inflammatory biomarkers was assessed in patients with Alzheimer's disease and C9Orf72-ALS carriers following treatment with Baricitinib, a JAK inhibitor approved for rheumatoid arthritis and COVID-19 treatment [14].

Clinical trial NCT04220021 used a genomic and transcriptomic approach to evaluate safety and tolerability of Metformin in C9Orf72- patients. Since this drug, approved as a diabetes mellitus remedy, demonstrated beneficial

effects through different signalling pathways [15], the trial aim was to evaluate CSF deregulated Repeat-associated non-AUG (RAN) protein levels.

2. Not Drug Related Clinical Trials

A multi-omics approach has been used in trail NCT02590276 to identify disease signatures in FTD/ALS C9Orf72-carriers [16]. The results implicated dysregulated circulating miRNAs as biomarkers of disease progression [7]. In trial NCT03984708, analysis of metabolic status and associated metabolic pathways in skin biopsy fibroblasts of ALS patients was used to find new therapeutic strategies.

A transcriptomic approach has been used in several clinical studies. In trial NCT01984957, DNA microarray technology was used to identify dysregulated gene expression in muscle biopsies of ALS patients. Combined omics technologies were applied in trial NCT02670226 to identify novel biomarkers in ALS patients by exploration of blood, muscle and satellite cells metabolomes and transcriptomes. A similar approach was applied in clinical trial NCT03851302, where mRNA expression of metabolism genes was assessed in muscle of ALS patients following neuromuscular magnetic stimulation (NMMS).

References

1. Giovanna Morello; Salvatore Salomone; Velia D'Agata; Francesca Luisa Conforti; Sebastiano Cavallaro; From Multi-Omics Approaches to Precision Medicine in Amyotrophic Lateral Sclerosis. *Frontiers in Neuroscience* **2020**, *14*, 577755, 10.3389/fnins.2020.577755.
2. Giovanna Morello; Antonio Gianmaria Spampinato; Sebastiano Cavallaro; Molecular Taxonomy of Sporadic Amyotrophic Lateral Sclerosis Using Disease-Associated Genes. *Frontiers in Neurology* **2017**, *8*, 152, 10.3389/fneur.2017.00152.
3. Timothy M Miller; Alan Pestronk; William David; Jeffrey Rothstein; Ericka Simpson; Stanley H Appel; Patricia L Andres; Katy Mahoney; Peggy Allred; Katie Alexander; et al.Lyle W OstrowDavid SchoenfeldEric A MacklinDaniel A NorrisGeorgios ManousakisMatthew CrispRichard SmithC Frank BennettKathie M BishopMerit E Cudkowicz An antisense oligonucleotide against SOD1 delivered intrathecally for patients with SOD1 familial amyotrophic lateral sclerosis: a phase 1, randomised, first-in-man study. *The Lancet Neurology* **2013**, *12*, 435-442, 10.1016/s1474-4422(13)70061-9.
4. Michael Benatar; Joanne Wuu; Peter M. Andersen; Nazem Atassi; William David; Merit Cudkowicz; David Schoenfeld; Randomized, double-blind, placebo-controlled trial of arimoclomol in rapidly progressive *SOD1* ALS. *Neurology* **2018**, *90*, e565-e574, 10.1212/wnl.0000000000004960.

5. Benjamin Rix Brooks; Robert G Miller; Michael Swash; Theodore L Munsat; El Escorial revisited: Revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotrophic Lateral Sclerosis* **2000**, *1*, 293-299, 10.1080/146608200300079536.
6. Antoine Moulignier; Antoine Moulonguet; Gilles Pialoux; Willy Rozenbaum; Reversible ALS-like disorder in HIV infection. *Neurology* **2001**, *57*, 995-1001, 10.1212/wnl.57.6.995.
7. Mark Kindy; Paul Lupinacci; Raymond Chau; Tony Shum; Dorothy Ko; A Phase 2A randomized, double-blind, placebo-controlled pilot trial of GM604 in patients with Amyotrophic Lateral Sclerosis (ALS Protocol GALS-001) and a single compassionate patient treatment (Protocol GALS-C).. *F1000Research* **2017**, *6*, 230, 10.12688/f1000research.10519.1.
8. Christina N. Fournier; David Schoenfeld; James D. Berry; Merit E. Cudkowicz; James Chan; Colin Quinn; Robert H. Brown; Johnny S. Salameh; Malu G. Tansey; David R. Beers; et al.Stanley H. AppelJonathan D. Glass An open label study of a novel immunosuppression intervention for the treatment of amyotrophic lateral sclerosis. *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration* **2018**, *19*, 242-249, 10.1080/21678421.2017.1421666.
9. Carol Milligan; Nazem Atassi; Suma Babu; Richard J. Barohn; James B. Caress; Merit E. Cudkowicz; Armineuza Evora Ba; Gregory A. Hawkins; Marlena Wosiski-Kuhn; Eric A. Macklin; et al.Jeremy M. Shefner MdZachary SimmonsRobert P. BowserShafeeq S. Ladha Tocilizumab is safe and tolerable and reduces C -reactive protein concentrations in the plasma and cerebrospinal fluid of ALS patients. *Muscle & Nerve* **2021**, *64*, 309-320, 10.1002/mus.27339.
10. Sudeep Pushpakom; Francesco Iorio; Patrick A. Eyers; K. Jane Escott; Shirley Hopper; Andrew Wells; Andrew Doig; Tim Guilliams; Joanna Latimer; Christine McNamee; et al.Alan NorrisPhilippe SanseauDavid CavallaMunir Pirmohamed Drug repurposing: progress, challenges and recommendations. *Nature Reviews Drug Discovery* **2018**, *18*, 41-58, 10.1038/nrd.2018.168.
11. L A Jaber; E M Bailey; M J Rybak; Enoxacin: a new fluoroquinolone.. *Clinical pharmacy* **1989**, *8*, 97-107.
12. Timothy Miller; Merit Cudkowicz; Pamela J. Shaw; Peter M. Andersen; Nazem Atassi; Robert C. Bucelli; Angela Genge; Jonathan Glass; Shafeeq Ladha; Albert L. Ludolph; et al.Nicholas J. MaragakisChristopher J. McDermottAlan PestronkJohn RavitsFrançois SalachasRandall TrudellPhilip Van DammeLorne ZinmanC. Frank BennettRoger LaneAlfred SandrockHeiko RunzDanielle GrahamHani HoushyarAlexander McCampbellIvan NestorovIh ChangManjit McNeillLaura FanningStephanie FradetteToby A. Ferguson Phase 1–2 Trial of Antisense Oligonucleotide Tofersen for *SOD1* ALS. *New England Journal of Medicine* **2020**, *383*, 109-119, 10.1056/nejmoa2003715.
13. Dale J. Lange; Mona Shahbazi; Vincenzo Silani; Albert C. Ludolph; Jochen H. Weishaupt; Senda Ajroud-Driss; Kara G. Fields; Rahul Remanan; Stanley H. Appel; Claudia Morelli; et al.Alberto DorettiLuca MadernaStefano MessinaUlrike WeilandStefan L. MarklundPeter M. Andersen

Pyrimethamine significantly lowers cerebrospinal fluid Cu/Zn superoxide dismutase in amyotrophic lateral sclerosis patients with *SOD1* mutations. *Annals of Neurology* **2017**, *81*, 837-848, 10.1002/ana.24950.

14. Johannes Wohlrab; David Stintzing; Luise Schultz; Konstantin Jügelt; Olaf H.-U. Schroeder; Influence of Janus Kinase Inhibitors on the Neuronal Activity as a Proof-of-Concept Model for Itch. *Skin Pharmacology and Physiology* **2021**, *35*, 94-101, 10.1159/000519669.
15. Ziquan Lv; Yajie Guo; Metformin and Its Benefits for Various Diseases. *Frontiers in Endocrinology* **2020**, *11*, 191, 10.3389/fendo.2020.00191.
16. Giorgia Querin; Peter Bede; Mohamed Mounir El Mendili; Menghan Li; Mélanie Pélégrini-Issac; Daisy Rinaldi; Martin Catala; Dario Saracino; François Salachas; Agnes Camuzat; et al.Véronique Marchand-PauvertJulien Cohen-AdadOlivier ColliotIsabelle Le Ber MdPierre-François Pradat Mdfor The Predict to Prevent Frontotemporal Lobar Degeneration and Amyotrophic Lateral Sclerosis (PREV-DEMALS) Study Group Presymptomatic spinal cord pathology in *c9orf72* mutation carriers: A longitudinal neuroimaging study. *Annals of Neurology* **2019**, *86*, 158-167, 10.1002/ana.25520.