Toward precision medicine in amyotrophic lateral sclerosis

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Abstract: Precision medicine is an innovative approach that uses emerging biomedical technologies to deliver optimally targeted and timed interventions, customized to the molecular drivers of an individual's disease. This approach is only just beginning to be considered for treating amyotrophic lateral sclerosis (ALS). The clinical and biological complexities of ALS have hindered development of effective therapeutic strategies. In this review we consider applying the key elements of precision medicine to ALS: phenotypic classification, comprehensive risk assessment, presymptomatic period detection, potential molecular pathways, disease model development, biomarker discovery and molecularly tailored interventions. Together, these would embody a precision medicine approach, which may provide strategies for optimal targeting and timing of efforts to prevent, stop or slow progression of ALS.

Keywords: Amyotrophic lateral sclerosis (ALS); precision medicine; customized therapies

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Introduction

Precision medicine is an innovative approach that applies recently developed biomedical technologies to optimize and individualize treatment to the molecular drivers of an individual's disease. It involves not creation of treatments that are unique to a patient, but rather classification of individuals into subpopulations that differ in their susceptibility to a particular disease, in the biology and/or prognosis of the disease they develop or in their response to a specific treatment (1,2). This approach of using tailored, mechanism-based therapies has been applied to cancer care and gained progressively greater impact, but it is only beginning to be considered in amyotrophic lateral sclerosis (ALS).

ALS is a neurodegenerative disease characterized by progressive deterioration mainly involving the corticospinal tract, brainstem and anterior horn cells of the spinal cord. Patients develop focal and then generalized weakness leading to paralysis. The incidence of ALS in the European population is 2–3 people per 100,000, and the overall lifetime risk of developing the condition is 1:400 (3-5). Approximately 5–10% of patients have familial ALS (FALS) and show a Mendelian pattern of inheritance; the remaining 90–95% of patients have sporadic ALS (SALS) (6). Over 60% of patients die within 3 years of presentation, usually from respiratory failure and about 10% survive for more than 10 years (7). There is no disease-modifying therapy for ALS, though riluzole slows the rate of progression and prolongs survival by 2 or 3 months (8).

ALS is a complex, multifactorial disease with variations in individual susceptibility and phenotype. The clinical and biological complexities of ALS have hindered development of effective therapeutic drugs. In this review we envision applying the key elements of precision medicine to ALS.

Comprehensive risk assessment

Genetic and environmental factors that influence susceptibility to ALS depend on multiple gene–gene and

gene–environment interactions and epigenetic effects, all of which also drive phenotypic individuality. It is important to identify these modifying factors, as they could be targets for therapeutic intervention.

Over the last two decades, a great deal of new knowledge has been gathered on ALS, especially on its underlying genetics. To date, about 23 genes have been implicated in FALS (*Table 1*). Mutations in these genes account for approximately two thirds of the genetic etiology of FALS and 10% of SALS (40). Chromosome 9 open reading frame 72 (*C9orf72*), Cu/Zn superoxide dismutase1 (*SOD1*), fused in sarcoma (*FUS*), and TAR DNA binding protein (*TARDBP*) are the most common mutated genes in both FALS and SALS in various patient populations (41). Thus, there is consensus that these four genes play a causal role in ALS, whereas further evidence is required to support the roles of the other genes. Multiple genome-wide association studies (GWAS) have been performed, identifying several candidate susceptibility genes for ALS, including *DPP6* (42), *ELP3* (43), *UNC13A* (44,45), *ZNF512B* (46), *ITPR2* and *SUNC1* (47). Some of these risk genes were proposed to modify phenotype, for example, age at onset (48) and survival (49,50). However, these associations should be interpreted cautiously, as attempts to replicate the observed effects have led to either conflicting or negative results. Future GWAS should involve larger case-control cohorts and should stratify GWAS data based on different populations and well-defined clinical categories to maximize the statistical power and minimize the false discovery rate. Genome sequencing will continue to drive research in ALS genetics forward, yielding even greater insight into the genetic architecture of ALS by providing a complete catalog of rare variants. It will also allow exploration of the role of noncoding and intergenic genetic variation in the pathogenesis of this disease.

Non-genetic factors, including environmental exposure to toxins, smoking, excessive physical activity, occupation, dietary factors and changes in immunity, have been proposed as increasing risk of developing SALS. These factors may drive epigenetic changes over many years, which then induce disease onset and progression. However, the only established risk factors so far are old age and male gender. A pooled analysis of five large cohorts found that smoking is the only probable environmental risk factor for ALS, but no dose-response relationship with either pack-years or duration of smoking was found (51). Recently, the gene-time-environment model of ALS was proposed, in which the genetic component of liability, time and environmental exposures all contribute to the development of ALS (52). Environmental exposure as a risk factor for ALS, though weak, is likely to be cumulative over time, exceeding a genetic-environmental threshold in those who, at some later time, develop ALS (53). Because ALS is a rare disease, single-center studies usually lack sufficient statistical power to assess the environmental risk in ALS with known genetic backgrounds, and thus test for gene–environment interactions. Future studies of environmental risk should enroll a large sample of patients from multiple centers stratified by age of onset and known genetic risk factors.

Phenotypic classification

High phenotypic variability of ALS is observed, with regard to site of onset, age at onset, familial occurrence, type of motor neuron involvement, extent of extramotor involvement and rate of progression. Primary muscular atrophy, which involves pure lower motor neurons (LMN), and primary lateral sclerosis, which involves pure upper motor neurons (UMN), constitute the ends of a spectrum. Intermediate phenotypes, such as UMN-predominant ALS, classical ALS and LMN-predominant ALS, are considered to be different expressions of ALS. Regionally isolated variants of ALS include flail arm syndrome, which involves bilateral proximal and typically predominant LMN arm weakness, and the flail leg syndrome, characterized by often asymmetric and primarily distal LMN involvement in the lower limbs.

Advances in ALS genetics have greatly broadened the known phenotype of this disease. The discovery of *TARDBP* and *FUS* enabled recognition that ALS and frontotemporal dementia (FTD) represent overlapping clinical syndromes (13,14,18,19) and this convergence has been strengthened by the discovery of *C9orf72*, Ubiquilin 2 (*UBQLN2*) and other genes (27,33,34). It is now accepted that ALS constitutes a continuum with FTD, with pure ALS and pure FTD at the ends of this spectrum of motor neuron and frontotemporal neuron involvement. Intermediate phenotypes include ALS with behavioral impairment, ALS with cognitive impairment, and ALS-FTD (7). In addition, discovery of mutations in valosin-containing protein (*VCP*), Sequestosome-1 (*SQSTM1*), heterogeneous nuclear ribonucleoprotein A2B1 (*HNRNPA2B1*) and heterogeneous nuclear ribonucleoprotein A1 (*HNRNPA1*) in subsets of patients with ALS, FTD, inclusion body myopathy and Paget disease of the bone showed that at least some forms of ALS are part of the 'multisystem proteinopathy',

Table 1 Summary of genes linked to amyotrophic lateral sclerosis

Gene	Location	FALS locus	Inheritance	Strategy	Associated phenotype	Protein function	References
SOD1	21q22.11	ALS 1	AD, AR	LA	ALS, PMA, juvenile ALS	Superoxide metabolism	(9)
ALS2	2q33.2	ALS 2	AR	LA	Juvenile ALS, infantile HSP	Vesicle trafficking	(10)
SETX	9q34.13	ALS 4	AR	LA	Juvenile ALS, dHMN, AOA2	RNA metabolism	(11)
SPG11	15q21.1	ALS 5	AR	LA, CGA	Juvenile ALS, HSP	DNA damage repair	(12)
FUS	16p11.2	ALS 6	AD, AR	LA, CGA	ALS, ALS-FTD, FTD	RNA metabolism	(13,14)
VAPB	20q13.33	ALS 8	AD	LA	ALS, PMA	Vesicle trafficking	(15)
ANG	14q11.1	ALS 9	AD	CGA	ALS, ALS-FTD	Angiogenesis	(16,17)
TARDBP	1p36.22	ALS 10	AD	LA, CGA	ALS, ALS-FTD, FTD	RNA metabolism	(18-20)
FIG4	6q21	ALS 11	AD, AR	CGA	ALS, PLS, CMT	Vesicle trafficking	(21)
OPTN	10p13	ALS 12	AD, AR	HM	ALS, FTD	Vesicle trafficking; autophagy	(22)
ATXN2	12q24.12	ALS 13	–	CGA	ALS, SCA2	Endocytosis; RNA translation	(23,24)
VCP	9p13.3	ALS 14	AD	WES	ALS, FTD, IBM, PDB	Proteasome; vesicle trafficking	(25,26)
UBQLN2	Xp11.21	ALS 15	XL	LA	ALS, ALS-FTD, juvenile ALS	Proteasome	(27)
SIGMAR1	9p13.3	ALS 16	AR	HM	juvenile ALS, dHMN	Proteasome	(28,29)
CHMP2B	3p11.2	ALS 17	AD	LA, CGA	ALS, FTD	Vesicle trafficking	(30,31)
PFN1	17p13.3	ALS 18	AD	WES	ALS	Cytoskeletal dynamics	(32)
C9orf72	9p21.2	ALF-FTD	AD	GWAS, LA	ALS, FTD, ALS-FTD	RNA metabolism	(33,34)
MATR3	5q31.2	–	AD	WES	ALS, distal myopathy	RNA metabolism	(35)
CHCHD10	22q11.23	–	AD	WES	ALS, FTD, cerebellar ataxia, myopathy	Mitochondrial dysfunction	(36)
SQSTM1	5q35.3	–	AD	CGA	ALS, FTD, IBM, PDB	Ubiquitination; autophagy	(37)
HNRNPA1	12q13.13	–	AD	WES	ALS, FTD, IBM, PDB	RNA metabolism	(38)
HNRNPA2B1	7p15.2	–	AD	WES	ALS, FTD, IBM, PDB	RNA metabolism	(38)
TBK1		–	AD	WES	ALS, FTD	Autophagy	(39)

AD, autosomal dominant; ALS, amyotrophic lateral sclerosis; AOA2, ataxia with oculomotor apraxia type 2; AR, autosomal recessive; CGA, candidate gene analysis; CMT, Charcot-Marie-Tooth disease; dHMN, distal heredity motor neuropathy; FTD, frontotemporal dementia; GWAS, genome-wide association studies; HSP, hereditary spastic paraplegia; HM, homozygosity mapping; IBM, inclusion body myopathy; LA, linkage analysis; PDB, Paget disease of bone; PLS, primary lateral sclerosis; PMA, progressive muscular atrophy; SCA2, spinocerebellar ataxia type 2; WES, whole exome sequencing; XL, X-linked.

a widespread disease process with muscle, bone and neuronal degeneration (54). Thus, there is also a spectrum of extramotor involvement in ALS, ranging from classic ALS with no or mild extramotor involvement to ALS with cognitive, extrapyramidal, cerebellar, sensory, autonomic, urinary, oculomotor, muscular or bone involvement, designated as 'ALS with multisystem degeneration' (7,55).

Different phenotypes of ALS may fit within a clinical and pathological continuum, or on the contrary, reflect heterogeneity of underlying pathophysiological mechanisms. Therefore, accurate disease categorization may help to explore the underlying pathophysiological

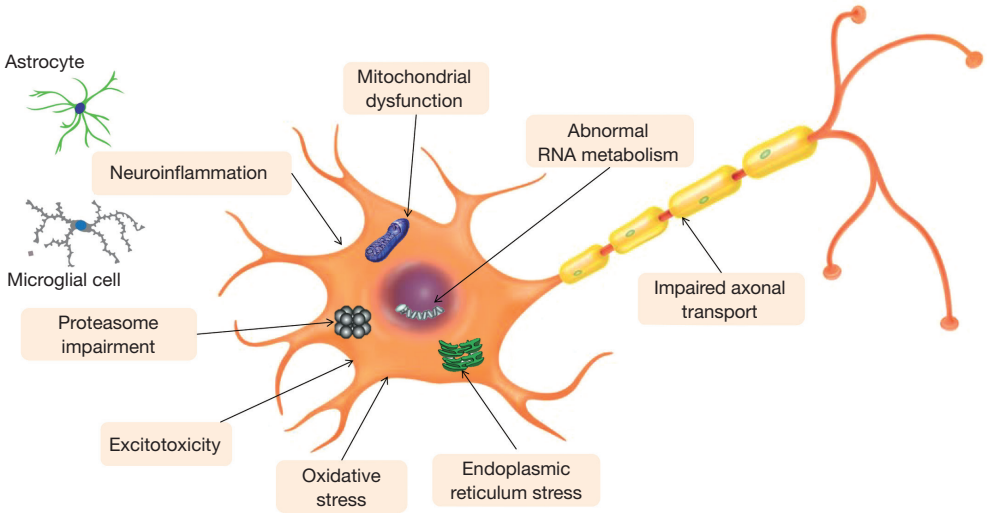

Figure 1 Potential molecular pathways of the pathogenesis of amyotrophic lateral sclerosis.

mechanisms and select candidates for clinical trials.

Confirming a presymptomatic period

It is unclear whether ALS, like many neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntington's diseases, is characterized by a presymptomatic period. However, the fact that patients with hereditary ALS do not present clinically until mid- to late-adulthood indicates that either these mutated genes are not 'switched on' until later in life or that there are decades of progressive cellular compromise, eventually culminating in catastrophic decline manifesting as presentation of clinically overt ALS (53). Recent progress in the genetic basis of ALS has led to the identification of increasing numbers of asymptomatic people at genetic risk for ALS, which will help to define the presymptomatic phase of the disease.

There is a compelling body of evidence to indicate that the onset of clinical symptoms is preceded by a long presymptomatic period. Longitudinal studies reported reduced motor unit number estimation (MUNE) and increased cortical excitability 3–10 months in advance of symptom onset in SOD1 mutation carriers (56,57). It is therefore imperative to identify the sensitive biomarkers at this preclinical stage by establishing presymptomatic diagnostic tools to identify those at high risk of developing ALS. This would open a potentially important window for neuroprotective intervention that might allow rescue of dysfunctional, but not yet dead, neurons and might even enable disease prevention (53). Abnormality of upper motor neuron function was clearly shown to precede clinical deficit in ALS carrying *SOD1* or *C9orf72* mutations (58-60). Moreover, surprisingly homogenous miRNA alterations were found in FALS patients and asymptomatic mutation carriers (60). These findings suggested the existence of biomolecular dysfunctions at a cellular level that are insufficient to cause clinical features and that such dysfunctions are potentially present and building for years or decades prior to the onset of clinical disease (53). Further studies are needed to confirm the presymptomatic period in patients with ALS and to establish presymptomatic diagnostic tools to identify those at high risk of developing the disease.

Studying potential molecular pathways

With advances in ALS genetics, several potential pathophysiological mechanisms have been implicated and proposed including oxidative stress, mitochondrial dysfunction, impairment of axonal transport, excitotoxicity, protein aggregation, endoplasmic reticulum stress, abnormal RNA processing and neuroinflammation (61) (*Figure 1*). Studies of ALS excitotoxicity mechanisms led to discovery of riluzole, the only FDA-proved drug for the disease. Recently, some additional molecular pathways have been identified by basic science research and therapeutic development efforts. The most notable of these include alterations in RNA metabolism and protein homeostasis.

The identification of ALS-causing mutations in the genes encoding TDP-43 and the RNA-binding protein

FUS, both of which are involved in pre-mRNA splicing, RNA transport and RNA translation, led to the proposal that aberrant RNA metabolism contributes to ALS pathogenesis (13,14,18,19). Besides TDP-43 and FUS, a surprising number of proteins linked to ALS are directly or indirectly involved in RNA processing and metabolism. These proteins include TAF15, EWSR1, ANG, SETX, ELP3, ataxin-1 and -2, hnRNPA1 and hnRNPA2B1 and C9orf72 (62). TDP-43 and FUS shuttle between the nucleus and cytoplasm. In response to stressors such as starvation or oxidative stress, TDP-43 and FUS exit from the nucleus and exist primarily in the cytoplasm, where they are incorporated into stress granules and form stress granule-based aggregates. ALS-associated mutations cause a shift in localization of TDP-43 or FUS from the nucleus to the cytoplasm and increase their propensity for aggregation (63,64). This increased cytoplasmic aggregation, a gain-of-function, results in nuclear depletion of TDP-43 and FUS, inducing abnormalities in RNA processing, a loss-of-function (65-67). The identification of *C9orf72* mutations as causing ALS made RNA toxicity a high profile researches focus for the disease. The *C9orf72* mutations causing ALS are GGGGCC hexanucleotide repeat expansions with several hundreds or even thousands of repeats (33,34). *C9orf72* mRNA levels were reduced by 50% in ALS patients with *C9orf72* abnormal expansions, suggesting that the expanded allele hinders generation of mature mRNA (33,68). Thus, *C9orf72* expansion may represent a loss-of-function mutation. However, the expanded hexanucleotide repeat was shown to form nuclear RNA foci in neurons in the frontal cortex and spinal cord in patients with *C9orf72* mutations. Pre-mRNA containing the expansion may thus also exert a deleterious gain-of-function effect (33). Another possible mechanism of ALS pathogenesis associated with *C9orf72* mutations would be repeat-associated non-ATG (RAN) translation (69). While medications to significantly reduce the gain-of-toxic function effect have not yet been discovered, targeting the production of toxic protein or RNA could achieve this aim (see discussion below).

Aggregates of mutant SOD1, TDP-43 or FUS are hallmarks of ALS. These aggregates or, more likely, their precursor oligomeric complexes, disturb normal protein homeostasis and induce cellular stress. Misfolded mutant SOD1 has toxic effects on the cell's degradation machinery, impairing its two major components, the proteasomal pathway and autophagy, thus circumventing this protective regulatory process in the cell (70,71). Mutant SOD1 then accumulates as oligomers and later as aggregates, leading to a stress response. More evidence for interference with normal proteasomal or autophagic protein degradation as a factor in ALS pathogenesis comes from the discovery of ALS-linked mutations affecting proteins that are directly involved in proteostasis or autophagy, such as mutations in *VCP*, *UBQLN2*, *SQSTM1*, charged multivesicular body protein 2b (*CHMP2B*), optineurin (*OPTN*), TANK-binding kinase 1 (*TBK1*), *TDP-43* and *FUS* (62). Recent studies showed that induction of autophagy might have therapeutic benefits for ALS. Molecules targeting mTOR dependent and mTOR independent autophagy pathways, including rapamycin (72,73), trehalose (74,75), spermidine, carbamazepine and tamoxifen (76), prolonged motor neuron survival or rescued motor dysfunction in mutant SOD1/TDP-43 transgenic mice, an effect correlating with increased autophagy. However, a recent phase III multicenter clinical trial reported no beneficial effects of lithium, which enhances autophagy, in ALS patient survival (77). Because most available drugs target many biological processes beyond autophagy, there is a need to explore new autophagy regulators with higher specificity and lower side effects.

Developing disease models

Laboratory models of ALS help researchers understand the basic processes of the disease, which is essential for developing new therapies. Though ALS has been modeled in cells, worms, flies, fish, mice and rats, no model is a perfect representation of the human disease, though each offers advantages for studying particular disease features. Rodents are especially important for testing potential therapies because their nervous systems are much larger and more complex than those of many other animal models. Development of transgenic animal models carrying genetic mutations identified in ALS patients has facilitated studying disease mechanisms and developing therapeutic strategies for ALS because these models recapitulate its key histopathological and biochemical features (78).

Discovery of *SOD1* mutations in FALS led to generation of the first transgenic mouse model of ALS (79). *SOD1* transgenic models reproduced many features of ALS, including motor deficits, reduced survival, fragmented and insoluble *SOD1* aggregates, reactive gliosis and neuronal loss (79-81). *SOD1* mouse models have been used extensively to study disease pathogenesis and for drug screening, but a substantial number of compounds that prolong survival and/or delay onset of paralysis in the

SOD1 mouse model showed disappointing results in clinical trials. Recently, identification of *TARDBP* mutations led to a number of transgenic mouse models expressing either wild-type or mutant TDP-43, which have a phenotype primarily consisting of cortical abnormalities and minor lower motor neuron involvement (82,83). Transgenic mice overexpressing wild-type or mutated human *FUS* developed ALS-like symptoms, with hindlimb paralysis and shortened life span, along with cytoplasmic FUS aggregation (84,85). Very recently, transgenic mice with *C9orf72* repeat-expansion was created, and mimicked both neuropathological and clinical C9orf72 mutated FTD/ALS phenotypes (86). These mice had neuronal loss, nuclear RNA foci, dipeptide repeat inclusions and TDP-43 pathologies in the brain, as well as such behavioral abnormalities as hyperactivity, anxiety, antisocial behavior and motor deficits. Given the high prevalence of *C9orf72* mutations in FALS/ALS-FTD, transgenic *C9orf72* mouse models are likely to contribute to studying the disease process and testing therapies against this form of the disease. With the number of genetic mouse models now available, compounds should be tested in models with various genetic backgrounds to determine the effects of genetic variability on therapeutic efficacy before advancement to clinical trials.

However, all of these animal models have limitations. They do not faithfully translate to human disease and each represents only a subset of FALS cases. Thus, most agents found effective in these models were not found to be of value in clinical trials (78). Reprogramming fibroblasts of ALS patients into induced pluripotent stem cells (iPSCs) appears to be a promising opportunity to develop new SALS models. These cells have been differentiated into ALS-relevant cell subtypes including motor neurons and astrocytes. Motor neurons derived from SALS patients recapitulate the major pathological features of the patients they were derived from, including TDP-43 aggregation (87). In addition, motor neurons derived from patients carrying *TARDBP* or *C9ORF72* mutations display abnormal physiological properties (88). Thus iPSC-differentiated neurons from ALS patients offer another platform to test therapeutic candidates. The emergence of powerful gene-editing tools such as clustered regularly interspaced short palindromic repeats (CRISPR) enables introduction of specific mutations into well-characterized iPSC lines and into endogenous genes in mice. The use of endogenous mouse genes might help overcome problems arising from expression of human rather than mouse proteins (89-91). Preclinical testing in these iPSC-based models might, therefore, identify promising candidate therapies with greater effectiveness in humans (92).

Discovering biomarkers

Biomarkers would facilitate diagnosis and thus might expedite initiation of neuroprotective therapies. Biomarkers could also help select patients for enrollment in clinical trials or identify subgroups that will benefit most from certain medications. Furthermore, robust biomarkers for disease activity might also help assess drug efficacy in trials. Biomarkers that have prognostic value for survival would be of value for decision-making and planning of care. Technological developments have led to the discovery of many candidate protein-based, neurophysiological, and neuroimaging biomarkers for ALS.

Dozens of candidate protein-based biomarkers were identified in the blood and/or cerebrospinal fluid (CSF) of patients with ALS [see review in (93)]. CSF neurofilaments have become leading candidate neurochemical biomarkers of diagnostic and prognostic value (94,95). Very recently, a prospective study showed the positive predictive value of elevated levels of CSF neurofilaments for diagnosis, distinguishing between patients with ALS and neurological disorder controls (94). Neurofilaments NF-L and pNF-H were at normal levels before onset of symptoms and were increased at early symptom onset in CSF and/or serum (96). In addition, neurofilament levels correlated moderately with motor neuron disease progression and duration (94). Thus blood and CSF neurofilament levels were linked to the symptomatic phase of ALS and might, therefore, serve as objective markers of structural damage to the nervous system, a promising surrogate in disease monitoring and clinical staging of ALS and an outcome measurement for future ALS therapeutic trials (97).

A number of global physiological features can be assessed that might differentiate ALS from other diseases and enable disease progression to be monitored. MUNE enables quantification and tracing of motor unit numbers, unaffected by compensatory reinnervation during disease progression. MUNIX was shown to be more rapidly recorded and have a better reproducibility compared with other more complex MUNE methods (98). Early studies of MUNIX showed index values to be reproducible in normal subjects, whereas those with ALS tended to decline over time with disease progression. In addition, in ALS patients, the index values showed greater changes than other metrics such as compound muscle action potential amplitude, ALS

functional rating scale (ALSFRS) values or forced vital capacity (99). Very recently, a multicenter prospective study performed longitudinal MUNIX measurements in multiple muscles of patients with ALS. The study demonstrated that MUNIX could track the loss of LMNs even when measuring clinically less affected muscles. In addition, MUNIX could accurately discriminate between faster or slower disease progressions. These results confirmed that MUNIX is a reliable electrophysiological biomarker to track lower motor neuron loss in ALS and might serve as a prognostic indicator (100). Electrical impedance myography, which assesses integrity and structure of a muscle, was shown to outperform other measures such as revised ALSFRS, MUNE and handheld dynamometry, in terms of its ability to detect deterioration (101), and might therefore serve as a meaningful measure of disease severity in ALS (102).

Imaging offers a noninvasive approach to biomarker discovery and disease monitoring. Voxel-based diffusion tensor imaging studies consistently showed reduced fractional anisotropy within the corticospinal tract and the corpus callosum. Voxel and surface-based MRI morphometry quantification demonstrated thinning of the primary motor cortex. However, the correlation of these imaging metrics with the absolute level of disability at the time of MRI, using ALSFRS or the estimated rate of disease progression, was inconsistent (103).

Further longitudinal studies are necessary to determine whether any protein-based candidate biomarkers, in combination with those obtained by neurophysiology and neuroimaging, will increase sensitivity and accuracy of diagnosis, help to monitor disease progression or predict prognosis in ALS (93).

Interventions tailored to individual molecular drivers

Genetic studies and outcomes from experimental models have provided the most compelling data on molecular mechanisms. Subsequent multiple clinical trials to test these proposed disease-altering interventions have been attempted but ultimately failed in treating ALS. In fact, virtually all such approaches neglected to consider the underlying clinical and biological complexities of this disease. A precision medicine approach has been overlooked until very recently, with some clinical trials aimed at specific patient populations (104). As the number of genes linked to ALS has increased, many patients have been found to harbor a mutant gene. Blocking expression of the mutant gene, therefore, stands out as a potentially definitive therapy. It might stop the complex cascade of events leading to motor neuron death before it starts. Antisense oligonucleotide (ASO) therapy has emerged as a highly promising approach for preventing mutant gene expression in neurodegenerative diseases. An interim analysis from a phase II clinical trial of ISIS-SMNRx in infants with type I spinal muscular atrophy demonstrated good tolerability and improved muscle function (105).

SOD1 targeted therapeutic strategies

Increasing evidence has indicated that the ALS-associated effects of mutant SOD1 are caused by a gain of toxic function rather than a loss of enzymatic function. Therefore, reducing concentrations of the mutant protein would be expected to slow progression of *SOD1*-linked ALS. In a *SOD1*-G93A rat model, treatment with ASOs at the time of symptom onset significantly slowed disease progression and increased survival (106). These results have led to an initial phase I clinical trial showing that intrathecal delivery of ASO targeting *SOD1* (SOD1Rx) to the CNS was well tolerated by patients harboring *SOD1* mutations and that CSF and plasma concentrations of ASOs were dose dependent, suggesting that intrathecal ASO delivery in patients is an effective route of delivery for ALS therapy (104). The next phase of testing will determine whether SOD1Rx can be given at a sufficiently high dosage and for a sufficiently long time to significantly reduce SOD1 protein levels, and whether such reduction will impact the course of disease in *SOD1*-mutated ALS. In addition to the ASO targeting SOD1, antibodies and small molecules targeting misfolded SOD1 are in preclinical testing stages (107).

C9orf72 targeted gene therapy

The SOD1Rx trial has supported pursuing potential applications of ASOs to other forms of genetically determined ALS. The likelihood that *C9orf72* mutations cause a toxic gain of function makes it a promising candidate for treatment with ASO. In fact, ASOs targeting *C9orf72* were shown to reduce *C9orf72* pathology in iPSC-differentiated neurons from *C9orf72*-mutated ALS patients, including RNA aggregation, aberrant transcription factor binding, dysregulated expression of other genes, susceptibility to glutamate excitotoxicity and neuronal firing abnormalities (108-110). Furthermore, administering a mouse-specific *C9orf72* ASO to the lateral

ventricle of adult mice, using a single intracerebroventricular stereotactic injection, resulted in a significant reduction of *C9orf72* RNA levels in the spinal cord and the brain 3 weeks after injection (110). In this study, the ASO was also detected throughout the CNS. However, no functional or behavioral alterations in strength, motor coordination, activity or anxiety were reported even at 17 weeks after the injection (110). These preliminary results indicated that the *C9orf72* suppression strategy might be safe and long-lasting in animal models. Given the high prevalence of *C9orf72* mutations in ALS patients of Caucasian origin, ASO could represent a promising approach for these *C9orf72*-mutated ALS patients.

ATXN2 targeted therapeutic strategies

Intermediate-length polyQ expansions in *ATXN2* were convincingly associated with an increased risk for ALS and functional screens identified ataxin-2 as a disease-modifying factor (23,24,111). Effects of intermediate-length repeat expansions may occur not only at the protein but also at the RNA level, similar to what has been observed in other disorders caused by expanded repeat regions. Given that therapeutic strategies aimed at targeting polyQ expansions have been successfully developed and applied in other disorders, such as Huntington's disease (112), ataxin-2 is likely to be a promising therapeutic target in ALS. ASOs and genome engineering techniques are promising approaches to target intermediate-length repeats in *ATXN2*. However, because manipulation of ataxin-2 may trigger unwanted side effects, such therapeutic approaches would require extensive preclinical testing (113).

TDP-43 targeted therapeutic strategies

RNA-binding proteins, such as TDP-43 and FUS, were recently shown to play a fundamental role in ALS pathogenesis. Several processes might be preferentially targeted to develop novel therapeutic or diagnostic approaches including aberrant aggregation processes, protein–protein interactions, RNA protein interactions or specific cellular pathways altered by disease (114). Compounds that decreases TDP-43 levels or reduce TDP-43 aggregation are very promising options. These include compounds activating the endoplasmic reticulum (ER) stress unfolded protein response, such as methylene blue (MB) (115), and other compounds such as cardiac glycosides, triptolide, CDK inhibitors and c-JNK inhibitors (87).

Conclusions

Together, the key elements of phenotypic classification, comprehensive risk assessment, detecting a presymptomatic period, studying potential molecular pathways, developing disease models, discovering biomarkers and tailoring interventions to molecular specifics embody a precision medicine approach. This approach should provide a strategy for optimal targeting and timing of efforts to prevent, stop or slow progression of ALS. Neuroscientists, neuropathologists and clinical researchers should work closely together to bring phenomics, genomics, proteomics, metabolomics and other unbiased approaches to bear on the problem, ensuring that future therapeutic strategies will be based on a solid molecular understanding of pathological mechanisms, identified in robust animal and cellular models.

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Footnote

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