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Spinal and bulbar muscular atrophy: From molecular pathogenesis to pharmacological intervention targeting skeletal muscle

Caterina Marchioretti[1,2,a], Roberta Andreotti[1,2,a], Emanuela Zuccaro[1,2], Andrew P. Lieberman[3], Manuela Basso[4] and Maria Pennuto[1,2]

Abstract

The clinical characteristics of SBMA, also known as Kennedy's disease (OMIM 313200), were initially documented by Dr. H Kawahara in the 18th century and a hundred years later by Dr. W. Kennedy. SBMA is a neuromuscular disease caused by expansions of a CAG microsatellite tandem repeat in exon 1 of the androgen receptor (*AR*) gene located on the X chromosome. These expansions result in the production of AR with an aberrantly expanded polyglutamine (polyQ) tract. In this review, we explore recent advancements in the significance of gene expression changes in skeletal muscle and discuss how pharmacological interventions targeting this aspect of disease pathogenesis can potentially be translated into therapies for SBMA patients.

Addresses

[1] Department of Biomedical Sciences, University of Padova, Padova, Italy

[2] Veneto Institute of Molecular Medicine (VIMM), Padova, Italy

[3] Department of Pathology, University of Michigan Medical School, Ann Arbor, MI, USA

[4] Department of Cellular, Computational and Integrative Biology (CIBIO), University of Trento, Trento, Italy

Corresponding authors: Pennuto, Maria (maria.pennuto@unipd.it); Lieberman, Andrew P. (liebermn@med.umich.edu); Basso, Manuela (manuela.basso@unitn.it)

[a] Equal contribution.

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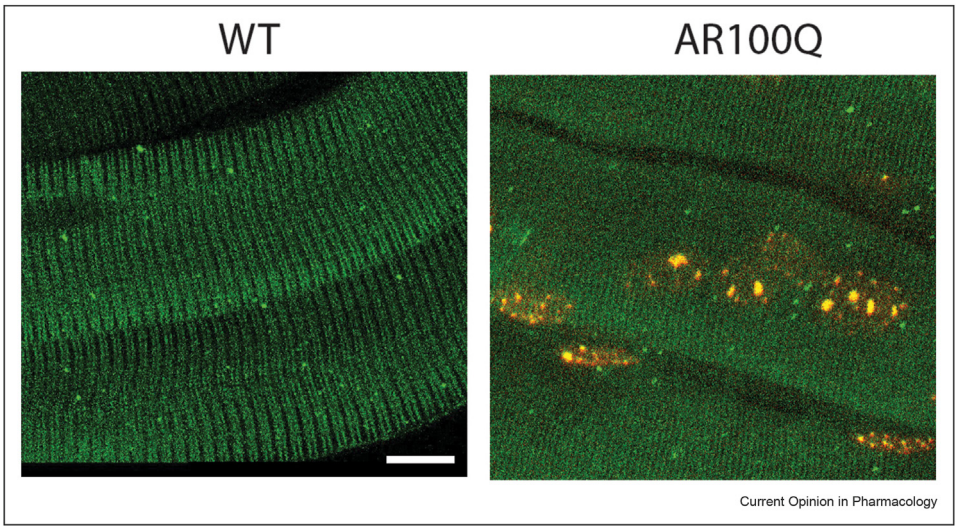
Introduction

SBMA is caused by the expansion of a CAG/glutamine (polyQ) tract in the androgen receptor (*AR*) gene [1]. The glutamine tract is polymorphic in length, ranging from 9 to 36 amino acids in unaffected individuals. Expansions to 38 or more repeats cause disease, and the longer the CAG repeat, the more severe the phenotype [2–4]. SBMA belongs to the family of polyQ diseases, including Huntington's disease (HD), dentatorubral-pallidoluysian atrophy, and six types of spinocerebellar ataxia (SCA). Despite their clinical differences, polyQ diseases exhibit several common features. These diseases typically manifest in mid-life, even though the disease proteins are expressed from development throughout adulthood. Furthermore, these disorders are characterized by the accumulation of misfolded proteins in the forms of micro-aggregates (amyloid fibrils) and inclusions bodies, which represent a pathological hallmark of neurodegeneration. Protein misfolding and aggregation are not limited to neurons but also occur in peripheral tissues, including skeletal muscle (Figure 1). These abnormal protein species are often regarded as a characteristic feature of SBMA and other neurodegenerative disorders. However, there is ongoing debate regarding whether these aggregated proteins exert toxic effects or have a protective role. Additionally, polyQ diseases are characterized by the loss of specific neuronal populations, despite the widespread expression of the disease proteins and the fact that sometimes these proteins serve essential cellular functions. This selective vulnerability accounts for the distinct clinical presentations observed in these diseases. The AR mutation results in the progressive degeneration of lower motor neurons and myofibers, leading to weakness, fasciculations, and atrophy of skeletal muscle [5]. Moreover, patients often present with peripheral symptoms, including endocrine and metabolic abnormalities, making SBMA a multisystem disease [6,7]. As a matter of facts, analysis of liver in 15 enrolled patients will reveal pathogenetic pathways occurring in this peripheral tissue (Table 1).

SBMA is an androgen-dependent disease

Among the polyQ diseases, SBMA has a unique feature: it exclusively presents its full symptoms in males, despite the AR being widely expressed in both sexes. There have been reports of four homozygotes sisters who exhibited cramps, fasciculations, and mild chronic neurogenic atrophy, with minimal disease progression

Figure 1



Inclusion bodies in SBMA myofibers. Immunohistochemical analysis in the quadriceps muscle of 8-week-old WT and AR100Q mice (n = 3). Green: RYR1 (MA3-925, 1:200); red: AR (H280, 1:200). Bar, 10 μm.

[8,9]. The molecular basis of the sex bias in SBMA became clearer upon development of animal models of the disease. Transgenic and knock-in SBMA mice exhibit a phenotype dependent on the sex, mirroring the sex bias observed in patients [10,11]. Transgenic female mice expressing polyQ-expanded AR develop a severe phenotype when treated with testosterone. Conversely, reducing testosterone levels in transgenic mice by surgical castration or leuprorelin treatment alleviates disease manifestations and progression. This indicates that males are affected because they have higher serum testosterone levels than females. The androgen-dependent nature of SBMA suggests a therapy based on the reduction of androgens in the serum. Results in preclinical models [12] and phase II clinical trials (Figure 2, Table 1) [13–16] with leuprorelin or other drugs targeting androgen signaling show encouraging results, further corroborating that SBMA is an androgen-dependent disease.

AR is a transcription factor activated by androgens

The relationship between androgen signaling and clinical manifestations of SBMA provides a molecular link between AR function, or better, mutant AR dysfunction and disease pathogenesis. AR belongs to the steroid hormone receptor family, which includes estrogen, progesterone, glucocorticoid, and mineralocorticoid receptors. These soluble receptors work as transcription factors once activated by their natural ligands, which are testosterone and its potent derivative dihydrotestosterone in the case of AR. Inactive AR mainly localizes to cytosol in association with heat shock proteins (HSPs) [17,18]. As a client of HSP90, AR can undergo two different fates depending on the complex it forms with its partners. It can either undergo rapid degradation through the ubiquitin-proteasome system shortly after synthesis or remain in an inactive and stable state, prepared to bind to ligand. Upon androgen binding, AR dissociates from HSPs, undergoes a conformational change that leads to intra- and inter-molecular interactions and translocates to the nucleus, where dimeric AR binds to specific sequences of DNA known as androgen-responsive elements (AREs). PolyQ expansions alter the native functions of AR [19], resulting in aberrant co-regulator (co-activator and co-repressor) expression [20], and recruitment [19,21], which is responsible for altered target gene expression both in motor neurons and myofibers [22–25].

PolyQ expansion confers toxic gain-of-function properties to mutant AR

AR mutations can be classified into two primary categories, enabling a clear differentiation between pure loss-of-function (LOF) from gain-of-function (GOF) mutations. Partial or complete AR LOF mutations (gene deletions and missense mutations) cause androgen insensitivity syndrome [26]. AR GOF mutations (gene amplifications and missense mutations) can cause hirsutism, prostate hyperplasia, and prostate cancer through hypermorphic and neomorphic GOF mechanisms [27]. Neurodegeneration is exclusively associated with the expansion of CAG repeats, representing the sole type of AR mutation linked to this condition. While patients with androgen insensitivity syndrome do not exhibit motor neuron degeneration or muscle atrophy, those with SBMA show mild indications of androgen insensitivity.

Table 1

SBMA clinical trials.

NCT_ID	Title	Status	Interventions	Gender	Phase	Study types	Study Designs
NCT00004771	Phase II Study of Leuprolide and Testosterone for Men With Kennedy's Disease or Other Motor Neuron Disease	Completed	Drug: Leuprolide	Male	Phase 2	Interventional	Primary Purpose: Treatment
NCT00303446	Dutasteride to Treat Spinal and Bulbar Muscular Atrophy (SBMA)	Completed	Drug: Dutasteride	Male	Phase 2	Interventional	Allocation: Randomized Intervention Model: Parallel Assignment Masking: Quadruple (Participant, Care Provider, Investigator, Outcomes Assessor) Primary Purpose: Treatment
NCT00851461	Effect of Goserelin (ZoladexÂ®) in Spinal and Bulbar Muscular Atrophy	Completed	Drug: Goserelin Procedure: Electrophysiologic study Procedure: tissue biopsy	Male	Phase 4	Interventional	Allocation: Randomized Intervention Model: Parallel Assignment Masking: Quadruple (Participant, Care Provider, Investigator, Outcomes Assessor) Primary Purpose: Treatment
NCT02024932	Safety, Tolerability, and Efficacy of BVS857 in Patients With Spinal and Bulbar Muscular Atrophy	Completed	Drug: BVS857	Male	Phase 2	Interventional	Allocation: Randomized Intervention Model: Parallel Assignment Masking: Quadruple (Participant, Care Provider, Investigator, Outcomes Assessor) Primary Purpose: Treatment
NCT02124057	Study of Hepatic Function in Patients With Spinal and Bulbar Muscular Atrophy	Completed		All	Phase 2	Observational	Observational Model: Cohort Time Perspective: Prospective
NCT02156141	High Intensity Training in Patients With Spinal and Bulbar Muscular Atrophy	Completed	Other: Supervised high intensity training Other: Optional training Other: Control period Other: Unsupervised High intensity training	Male		Interventional	Allocation: Randomized Intervention Model: Parallel Assignment Masking: None (Open Label) Primary Purpose: Supportive Care
NCT02501395	MRI in Patients With Kennedy Disease	Completed	Other: No intervention, observational	Male		Observational	Observational Model: Case-Control Time Perspective: Cross-Sectional
NCT03555578	Specified Drug-Use Survey of Leuprorelin Acetate Injection Kit 11.25 mg "All-Case Investigation: Spinal and Bulbar Muscular Atrophy (SBMA)"	Recruiting	Drug: Leuprorelin Acetate	All		Observational	Observational Model: Cohort Time Perspective: Prospective
NCT04944940	Clinical, Molecular and Imaging Biomarkers in Spinal and Bulbar Muscular Atrophy (SBMA)	Recruiting		Male		Observational	Observational Model: Cohort Time Perspective: Prospective

(*continued on next page*)

Table 1. (*continued*)

NCT_ID	Title	Status	Interventions	Gender	Phase	Study types	Study Designs
NCT05107349	Cell Signaling, Reinnervation and Metabolism in Kennedy Disease and Amyotrophic Lateral Sclerosis (ALS)	Not yet recruiting	Procedure: Muscle biopsy	All		Observational	Allocation: N/A Intervention Model: Single Group Assignment Masking: None (Open Label) Primary Purpose: Other
NCT05517603	A Study to Evaluate Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics Of AJ201 In Patients	Recruiting	Drug: AJ201 Drug: Placebo	Male	Phase 1/2	Interventional	Allocation: N/A Intervention Model: Single Group Assignment Masking: None (Open Label) Primary Purpose: Other
N.A.		Recruiting	Drug: NIDO-361		Phase 1		
N.A.		Recruiting	Drug: ASOs		Phase 1		

Completed and current clinical trials for SBMA.

Consequently, it is probable that CAG expansions in AR lead to neurodegeneration by combining toxic GOF and partial LOF mechanisms. Current therapeutic approaches for prostate cancer include suppression of androgen signaling. A similar therapy is approved in Japan for SBMA patients. Interestingly, prostate cancer patients treated with anti-androgens may rarely develop a neurological condition [28], and SBMA patients treated with leuprorelin may develop castration-resistant prostate cancer [29,30], thus suggesting converging pathways in these age-related diseases.

Therapeutic targets

The investigation of transcriptional dysregulation in SBMA skeletal muscle has yielded foundational insights into the pathogenetic pathways underlying this disease and has resulted in the discovery of distinct and potentially synergistic therapeutic approaches (Figure 2, Table 1). We explore several concepts that have recently surfaced from these studies, contributing to our current comprehension of disease mechanisms and emphasizing potential targets for therapeutic interventions.

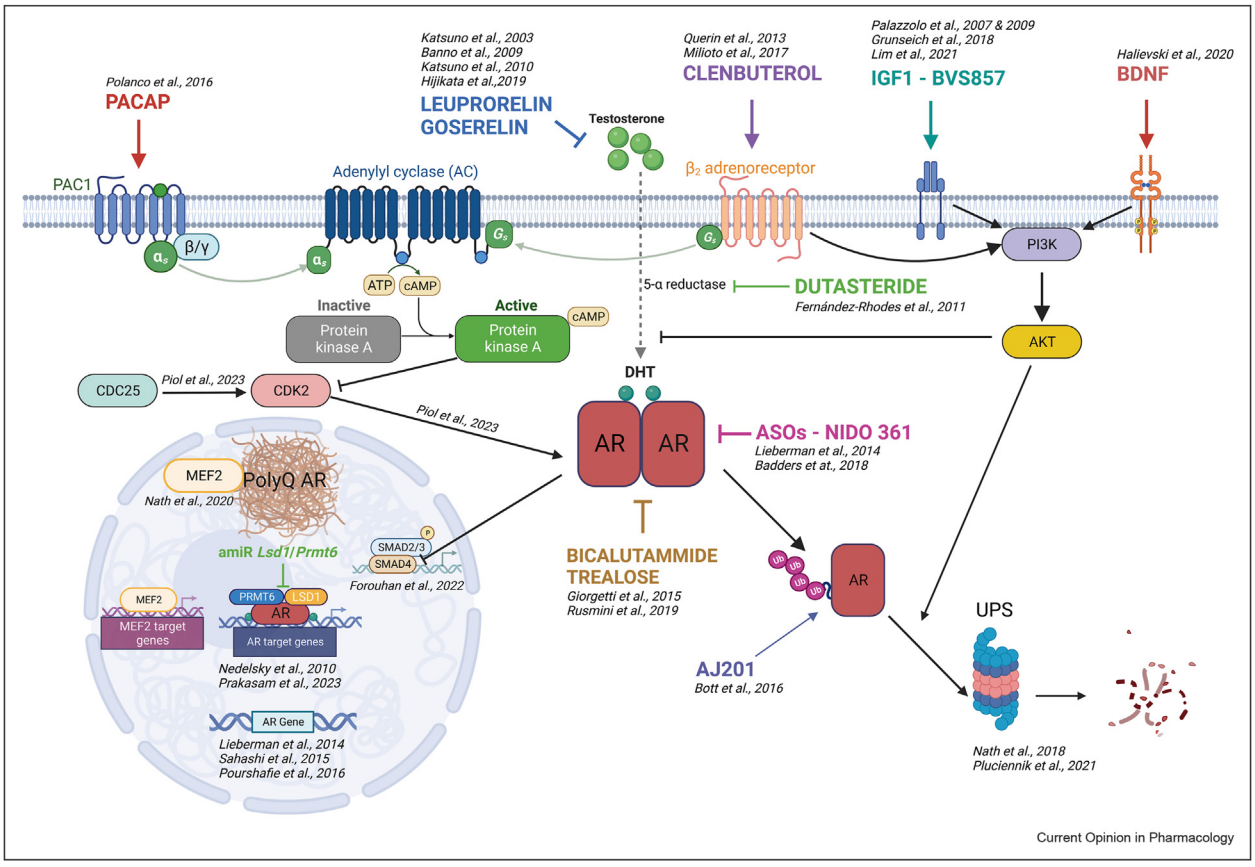
Metabolic dysregulation

SBMA muscle exhibits an early-onset shift from glycolytic to oxidative fiber types, accompanied by the downregulation of genes encoding glycolytic enzymes. Moreover, there are alterations observed in the signaling of mechanistic target of rapamycin (mTOR) and peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC1α) [24,31]. These changes culminate in functional limitations characterized by a substantial rise in energy expenditure during intensive exercise compared to control group. These results emphasize the role of skeletal muscle in the metabolic dysfunction observed in SBMA, which also manifests in some patients as insulin insensitivity, fatty liver, and metabolic syndrome [6,7]. It is worth mentioning that the metabolic alterations observed in the muscles of SBMA mice can be reversed through pharmacological intervention, such as the use of bicalutamide and/or trehalose [32], as well as through the subcutaneous administration of *AR*-targeted antisense oligonucleotides (ASOs) [33], establishing that expression of the polyQ AR in peripheral tissues including skeletal muscle underlies their occurrence (Figure 2).

Ubiquitin-proteasome pathway dysfunction

SBMA muscle is further characterized by the unanticipated downregulation of genes encoding ∼30% of constitutive proteasome subunits and ∼20% of E2 ubiquitin-conjugating enzymes [34]. This broad downregulation of a critical quality control pathway results from the diminished expression of the proteasome transcription factor nuclear factor erythroid 2-like 1 (NRF1/NFE2L1) (Figure 2) [35]. SBMA muscle exhibits significantly decreased levels of both the cleaved, active form of NRF1 and the aspartyl protease DNA-

Figure 2



Pathogenetic pathways and therapeutic targets in SBMA skeletal muscle. Summary of the main signaling pathways and factors involved in SBMA skeletal muscle pathology, and potential therapeutic approaches highlighted in this review.

damage inducible 1 homolog 2 (DDI2), which cleaves NRF1 at the endoplasmic reticulum membrane to allow for its nuclear translocation and transcriptional regulation of target genes [36]. A clinical trial will assess the efficacy of AJ201, which acts on genes involved in protein quality control, oxidative stress resistance and protein folding (Table 1). Degradation of polyQ AR mainly occurs through the ubiquitin-proteasome pathway, with the deubiquitinase ubiquitin-specific processing protease 7 (USP7) contributing to its efficient clearance [37]. The decrease in proteasome function in SBMA muscle impairs this process and accentuates the accumulation of toxic polyQ AR species.

Skeletal muscle atrophy

The dysfunction of the ubiquitin-proteasome pathway in SBMA is particularly striking, considering that skeletal muscle atrophy caused by various factors is typically associated with an upregulation of this pathway. This contrasts with the downregulation observed in SBMA. Additionally, the E3 ubiquitin ligases muscle RING-finger protein-1 (MuRF1), Atrogen-1, and muscle ubiquitin ligase of SCF complex in atrophy-1 (MUSA1) are typically associated with muscle atrophy [38], but are not as prominently expressed in SBMA [24,31]. Instead, the analysis of gene expression uncovered a widespread decrease in the expression of genes that encode structural proteins involved in the formation of the sarcomere [22], many of which are regulated by the transcription factor myocyte enhancer factor 2 (MEF2) (Figure 2) [39]. Comparisons with published datasets of target genes regulated by MEF2 or its endogenous inhibitor MRF4 demonstrated significant overlap with gene expression changes in SBMA muscle [39,40]. Indeed, MEF2 functional activity is significantly diminished in SBMA muscle, and overexpression of constitutively active MEF2 rescues atrophy. Similar changes are observed in skeletal muscle from R6/2 mice, a transgenic mouse model of HD, suggesting that functional impairment of MEF2 results from the expanded polyQ tract shared between these mutant proteins. The diminished function of additional transcriptional regulators, such as SMAD4, that promote the expression of anti-atrophy genes may also contribute to the muscle atrophy phenotype (Figure 2) [41].

Exercise and activation of anabolic signaling pathways in SBMA skeletal muscle

Various strategies have been pursued to stimulate anabolic and trophic signals in order to counterbalance the effects of impaired androgen signaling and toxic pathways caused by polyQ AR. One of these approaches is engaging in physical exercise, the effectiveness of which is contingent upon the specific type of training employed (Table 1). Aerobic training has shown limited effects on patients [42]. Conversely, high-intensity training [43], or weight lifting combined with functional training and dynamic balance [44] have yielded promising results. However, in a clinical trial functional exercise did not significantly modify primary outcome measures or overall quality of life, except for some effects observed in low-functioning patients [44].

An alternative strategy to reduce skeletal muscle atrophy involves the activation of specific anabolic pathways that may be compromised in SBMA muscle. For instance, the expression of brain-derived neurotrophic factor (BDNF) from muscle is diminished in SBMA, potentially playing a role in the development of muscle atrophy (Figure 2) [45]. Activation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway by insulin-like growth factor 1 (IGF-1) reduces androgen binding and protects cells from the toxicity of polyQ AR (Figure 2) [46]. Skeletal muscle-specific overexpression of an IGF-1 isoform induced by physical exercise remarkably ameliorates the phenotype of a severe mouse model of SBMA, thus providing a link between exercise and muscle homeostasis [47]. Moreover, the systemic administration of an IGF-1 mimetic (BVS857) of the liver-released isoform ameliorates SBMA mouse phenotypes (Figure 2, Table 1) [48]. However, regarding patients, this treatment shows limited effects on disease outcomes, although it does improve muscle volume [49].

Activation of the adenylyl cyclase/PKA pathway using the beta-agonist clenbuterol induces anabolic effects in skeletal muscle (Figure 2, Table 1). It has positive outcomes in both SBMA mice [50] and patients [51]. The neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) also ameliorates disease manifestations in SBMA mice [52]. The beneficial effects of clenbuterol and PACAP may be attributed to their ability to inhibit cyclin-dependent kinase 2 (CDK2) and reduce the phosphorylation of polyQ AR [53]. Clenbuterol also activates the PI3K/Akt pathway, and both IGF-1 and clenbuterol potentially exert their effects by phosphorylating specific serine residues of polyQ AR [46]. These pathways ultimately facilitate the clearance of mutant AR through proteasomal degradation. Consequently, these signaling pathways have dual effects in SBMA, with anabolic effects on muscle and direct actions on the disease protein at the post-translational level.

Impaired excitation-contraction coupling and mitochondrial pathology in SBMA muscle

Besides muscle atrophy, there is evidence that muscle contractility is altered in SBMA patients [54]. This aspect of muscle pathology is well recapitulated in animal models of the disease [22]. SBMA muscle is functionally impaired due to excitation-contraction coupling (ECC) disruptions. PolyQ AR alters the pattern of expression of genes encoding key components of the ECC machinery, and this alteration occurs before the onset of motor dysfunction in a severe mouse model of disease [22]. This phenomenon is associated with early alteration of mitochondrial respiration, followed by stimulus-dependent accumulation of calcium into mitochondria during disease onset and structural disorganization of muscle triads. Surgical castration and silencing of AR prevent these pathological processes, underscoring that the androgen-dependent deregulation of ECC and defective mitochondrial respiration can be reversed. These findings provide novel insights into the underlying pathological mechanisms in SBMA muscle and hold potential for the development of therapeutic strategies to counteract muscle wasting and fatigue.

Towards a gene therapy approach for SBMA

In the field of neurodegenerative diseases associated with toxic GOF mechanisms, gene silencing has emerged as a valuable therapeutic approach. Partial silencing of disease-related proteins has been investigated in both preclinical and clinical settings for monogenic diseases such as HD, SCA1, SCA3, and SCA7 [55]. Silencing the *AR* gene using antisense oligonucleotides (ASOs) in peripheral tissues [33] and in the central nervous system [56], as well as overexpressing a downregulated microRNA (miR-298) that specifically targets the 3′-untranslated region of *AR* transcripts [57], are all approaches that ameliorate SBMA mouse phenotypes (Figure 2). Clinical trials will be conducted to assess the effectiveness of these approaches in SBMA patients.

Another proposed strategy involves the overexpression of the AR45 isoform, which lacks the amino-terminal domain. This approach has shown promise in ameliorating the disease phenotype in SBMA mice by restoring the physiological transcriptional activity of AR [58]. An alternative approach aims to restore polyQ AR function by targeting transcriptional co-factors that mediate the toxic GOF caused by polyQ AR [19]. This approach seeks to intervene without interfering with the normal/basic functions of AR. Two compounds, tolfenamic acid (TA) and 1-[2-(4-methylphenoxy)ethyl]-2-[(2-phenoxyethyl)sulfanyl]-1H-benzimidazole (MEPB), have been shown to inhibit the recruitment of co-factors bearing an FXXLF or LXXLL motif (where L is leucine, F phenylalanine, and X any amino acid) and ameliorate the phenotype of SBMA mice [21]. A clinical

trial will test the efficacy of this approach in SBMA patients (NIDO-361) (Figure 2, Table 1). Interestingly, aberrant overexpression of two AR-coactivators with an LXXLL motif, protein arginine methyl transferase 6 (PRMT6) and lysine demethylase 1 (LSD1), has been observed in the skeletal muscles of SBMA patients and mice [20]. Silencing *Lsd1* and *Prmt6* in SBMA mice using artificial miRNAs (amiRs) delivered via adeno-associated virus subtype 9 (AAV9) attenuated the disease phenotype. While further analyses are required, these preliminary observations suggest that inhibiting the overexpression of AR co-activators holds promise to reduce the toxic GOF of mutant AR without exacerbating its LOF.

Conclusions

In the last decades, a collaborative effort between clinicians and scientists has been underway to investigate the underlying pathogenic pathways of SBMA and evaluate potential therapeutic strategies in both preclinical and clinical settings. These studies and the analysis of patients participating in clinical trials have revealed that SBMA is a multisystem disorder. This understanding is crucial when designing and developing therapies, as the treatment approach must address the progressive defects observed in multiple tissues and organs. It is noteworthy that toxic GOF mechanisms do not solely cause SBMA but also involve AR LOF. Therefore, any genetic or pharmacological interventions targeting AR expression and function may yield benefits but could also lead to unintended side effects. Moreover, a promising approach for therapy could involve combining *AR* silencing with drugs that have anabolic effects on skeletal muscle. This combination may benefit SBMA patients, particularly those experiencing pronounced muscle atrophy.

Author contribution

CM, RA, ALP, MB, MP wrote, reviewed and approved the manuscript.

Declaration of Competing Interest

MP and MB are named as co-inventors on the patent application Italian Priority N. 102022000026595 "New inhibitors of epigenetic regulators/nuovi inibitori di regolatori epigenetici". The other authors declare no competing interest.

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