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The Cure VCP Scientific Conference 2021: Molecular and clinical insights into neurodegeneration and myopathy linked to multisystem proteinopathy-1 (MSP-1)

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Abstract

The 2021 VCP Scientific Conference took place virtually from September 9–10, 2021. This conference, planned and organized by the nonprofit patient advocacy group Cure VCP Disease, Inc. (<https://www.curevcp.org>), was the first VCP focused meeting since the 215th ENMC International Workshop VCP-related multi-system proteinopathy in 2016 (Evangelista et al., 2016). Mutations in VCP cause a complex and heterogenous disease termed inclusion body myopathy (IBM) with Paget's disease of the bone (PDB) and frontotemporal dementia (FTD) (IBMPFD), or multisystem proteinopathy 1 (MSP-1) Kimonis (n.d.), Kovach et al. (2001), Kimonis et al. (2000). In addition, VCP mutations also cause other age-related neurodegenerative disorders including amyotrophic lateral sclerosis (ALS), Parkinsonism, Charcot-Marie type II-B, vacuolar tauopathy among others (Korb et al., 2022). The objectives of this conference were as

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Declaration of Competing Interest
None.

Appendix A. Supplementary data

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follows: (1) to provide a forum that facilitates sharing of published and unpublished information on physiological roles of p97/VCP, and on how mutations of VCP lead to diseases; (2) to bolster understanding of mechanisms involved in p97/VCP-relevant diseases and to enable identification of therapeutics to treat these conditions; (3) to identify gaps and barriers of further discoveries and translational research in the p97/VCP field; (4) to set a concrete basic and translational research agenda for future studies including crucial discussions on biomarker discoveries and patient longitudinal studies to facilitate near-term clinical trials; (5) to accelerate cross-disciplinary research collaborations among p97/VCP researchers; (6) to enable attendees to learn about new tools and reagents with the potential to facilitate p97/VCP research; (7) to assist trainees in propelling their research and to foster mentorship from leaders in the field; and (8) to promote diversity and inclusion of under-represented minorities in p97/VCP research as diversity is critically important for strong scientific research. Given the range of topics, the VCP Scientific Conference brought together over one hundred and forty individuals representing a diverse group of research scientists, trainees, medical practitioners, industry representatives, and patient advocates. Twenty-five institutions with individuals from thirteen countries attended this virtual meeting. In this report, we summarize the major topics presented at this conference by a range of experts.

Keywords

Proteinopathy; VCP; p97; Ubiquitin; Autophagy; Clinical trial; Inhibitor; Inclusion body myopathy; Paget's disease of the bone; Frontotemporal dementia; Amyotrophic lateral sclerosis; Motor neuron disease

1. Introduction and objectives

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to learn about new tools and reagents with the potential to facilitate p97/VCP research; (7) to assist trainees in propelling their research and to foster mentorship from leaders in the field; and (8) to promote diversity and inclusion of under-represented minorities in p97/VCP research as diversity is critically important for strong scientific research. Given the range of topics, the VCP Scientific Conference brought together over one hundred and forty individuals representing a diverse group of research scientists, trainees, medical practitioners, industry representatives, and patient advocates. Twenty-five institutions with individuals from thirteen countries attended this virtual meeting. In this report, we summarize the major topics presented at this conference by a range of experts.

2. Background

Located on chromosome 9p13.3-p12, valosin containing protein (VCP) (also known as VCP, cdc48 in yeast, and Ter94 in drosophila) is an abundant homohexameric AAA+ ATPase that utilizes ATP hydrolysis to assist in various ubiquitin-dependent and -independent protein quality control mechanisms including (but not limited to) chromatin associated degradation (CAD), ER associated degradation (ERAD), and autophagy (van den Boom and Meyer, 2018; Meyer et al., 2012). The primary function of VCP in these pathways is to actively extract and unfold diverse substrates from various cellular compartments (Meyer and Weihl, 2014). Over 400 putative substrates have been identified for VCP (Heidelberger et al., 2018). VCP targets these various substrates via association with numerous co-factors that bind to the N or C-terminus of VCP (van den Boom and Meyer, 2018). However, the function of the cofactors, the pathways they regulate, and the substrates they target remain poorly understood.

Heterozygous missense mutations in VCP cause a complex and heterogenous disease termed inclusion body myopathy (IBM) with Paget's disease of the bone (PDB) and frontotemporal dementia (FTD) (IBMPFD), or multisystem proteinopathy 1 (MSP-1) (Kimonis, 2007; Kovach et al., 2001; Kimonis et al., 2000). Over 50 causative mutations have been mapped in VCP since 2004 with most residing in the N-terminal domain (Kimonis, 2007; Watts et al., 2004; Korb et al., 2021; Al-Obeidi et al., 2018; Al-Tahan et al., 2018). While VCP-related IBMPFD is a rare disease, it is highly heritable with over 80% of affected individuals reporting at least one affected parent (Kimonis, 2007). Available data from natural history studies of patients with VCP mutations reveal that the presentation of symptoms for patients is incredibly heterogenous (Al-Obeidi et al., 2018). As such, there is no standard set of recommendations for the management and care of MSP-1 patients.

3. Session 1. Introduction to VCP disease: discussion of clinical symptoms, diagnostics tools, and pathological features

3.1. Virginia Kimonis MD, MRCP. VCP multisystem proteinopathy disease: a genetic disorder involving muscle, bone and central nervous system

Session one opened with an overview of VCP related multisystem proteinopathy-1 (MSP-1) by Dr. Virginia Kimonis (University of California, Irvine). The Kimonis group identified the first autosomal dominant mutations in VCP related to MSP-1 in 2004 (Watts et al.,

2004). Subsequent studies have identified more than 50 mutations spread throughout *VCP* (Korb et al., 2021; Al-Tahan et al., 2018). While the majority of mutations cluster in the N-terminal domain, patients with *VCP* related *MSP-1* display extreme symptom heterogeneity (Al-Obeidi et al., 2018). It is estimated that 90% of patients have inclusion body myopathy, 50% have Paget's disease of the bone, 30% have frontotemporal dementia (FTD) and 10% have amyotrophic lateral sclerosis (ALS) (Weihl et al., 2008; Forman et al., 2006; Neumann et al., 2007; Kimonis et al., 2008; Johnson et al., 2010). Given this heterogeneity of the disease and the unique needs for each patient, Cure *VCP* Disease initiated a standard of care study (see section 9). This recently published study provides the first comprehensive set of recommendations for the management and care of *VCP* patients (Korb et al., 2022)

To study *VCP* dysfunction at the organismal level, Dr. Kimonis group generated a knock-in C57BL/6 mouse that is heterozygous for the R155H mutation (Nalbandian et al., 2012; Nalbandian et al., 2013; Badadani et al., 2010). These *VCP*^{R155H/+} mice express comparable levels of mutant *VCP* to wildtype mice and showed no difference in fertility or viability. However, the mice developed many features consistent with *MSP-1* including muscle weakness at 6 months of age, variable muscle fiber structure, muscle central nucleation, and cytoplasmic accumulation of ubiquitin and TDP-43 positive inclusion bodies (Badadani et al., 2010). Unfortunately, Dr. Kimonis reported that the heterozygous mice no longer display or express most of their phenotypes for reasons that are not well understood. Thus, alternative mouse models may need to be developed for future studies. In addition, the Kimonis group has developed primary mouse and human myoblasts and human iPSC-derived myoblasts that recapitulate various aspects of *VCP* pathology including large cytoplasmic vacuoles, increased lysosomal markers such as LAMP1, and cytoplasmic TDP-43 (Vesa et al., 2009; Llewellyn et al., 2017). These models may allow for expedited study of the role of *VCP* mutation on cellular health.

Most patients are heterozygous for *VCP* mutations, thus, genetic deletion of the mutant *VCP* allele through exon skipping may be a promising therapeutic strategy to reduce the amount of toxic protein product in affected cells (De Ridder et al., 2020). Dr. Kimonis described a proof-of-concept study in which the mutant *VCP* R155H allele was excised in the *VCP*^{R155H/+} mice via a tamoxifen-inducible Cre-mediated recombination system. Targeted deletion of the mutant allele improved muscle strength, muscle architecture, and decreased apoptosis (Nalbandian et al., 2015). In another study the Kimonis group made the serendipitous finding that a lipid enriched diet rescued the weight defect, improved survival past weaning, and improved muscle strength in the homozygous *VCP* R155H mouse model (Llewellyn et al., 2014). Other studies provide evidence that a lipid enriched diet can ameliorate some deficits in other neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease (Gasior et al., 2006). Targeted lipidomics on the *VCP*^{R155H/R155H} mice was performed to determine whether the lipid profile of these mice was altered. This revealed reduced palmitic acid and ceramide levels in the muscle tissue (Llewellyn et al., 2014). Dr. Kimonis also reported data suggesting that ceramide levels are elevated in primary myoblasts derived from both *VCP*^{R155H/+} mice and *VCP* *MSP-1* patients. Pharmacological interventions that target the condensation of palmitate with serine, the rate limiting step in de novo sphingolipid biosynthesis, can decrease ceramide levels in cells. In collaboration with Dr. Daniele Piomelli (University of California, Irvine), they

found they could inhibit ceramide biosynthesis by targeting serine palmitoyltransferase (SPT) to effectively reduce ceramide levels in mouse and human primary myoblasts. This inhibition reduced various markers of disease including reducing cytoplasmic TDP-43, autophagy markers, LC3B and p62, and ubiquitin (Weiss et al., 2021). It should be noted that mutations in SPT are linked to hereditary sensory neuropathy type 1. These mutations shift the substrate targeting of SPT, leading to the accumulation of metabolites that cannot be converted or easily degraded (Penno et al., 2010). Given that this disease arises from altered enzyme activity and not general loss of function, treating with SPT inhibitors should not induce harmful SPT related dysfunction. Overall, these studies suggest that modulating lipid levels may ameliorate disease for some patients, but further studies are needed.

3.2. Jordi Diaz-Manera MD, PhD. Muscle MRI as a useful tool for diagnosis of VCP muscle disease

Dr. Jordi Diaz-Manera (John Walton Muscular Dystrophy Research Centre) presented data from a new international VCP cohort study that aims to collect clinical, genetic, MRI, and muscle morphology data from a large cohort of patients. Currently, the study is composed of 223 patients in 175 families. 71% of patients are male with a mean age of symptom onset as 45.6 +/- 9.4 (18–71) years and mean disease progression is 11 +/- 6.7 (1–45) years. It should be noted that the study remains open, and physicians are encouraged to contact the study coordinators if they wish to collaborate with the cohort.

In the cohort, patient presentation was variable: 32.7% of patients presented with symmetric proximal lower limb involvement weakness, 14.8% of patients presented with distal lower limb symmetric weakness, 11.7% presented with another weakness combination, 10.8% presented with proximal unilateral symmetric weakness, 9.0% presented with proximal upper limb and lower limb weakness, 4% presented with PDB, and 0.4% presented with FTD. Patient progression revealed that 100% of patients developed weakness, 27% developed cognitive problems, 27% developed respiratory issues, and 25% developed PDB. Almost all patients developed proximal lower limb weakness followed by proximal upper limb weakness (88% and 81%, respectively). Given the disparities in patient phenotypes, the average amount of time to a positive diagnosis was 8.3 years from symptom onset.

Given the wide range of time to diagnosis confirmation (1–25 years from symptom onset), Diaz-Manera argued that muscle MRI may be a useful tool for monitoring disease progression and confirming diagnosis. Muscle MRI has been a useful tool for the diagnosis of other muscle-related conditions by characterizing fat infiltration (Morrow et al., 2018). Even though clinical phenotypes can be very similar, muscle fatty infiltration is not random. Currently, there are two MRI techniques that allow monitoring of muscle degeneration: Short-TI Inversion Recovery (STIR) to identify necrosis and inflammation (acute damage) and T1 to look at the fibrous fatty replacement (chronic damage). T1 has been utilized in the diagnosis of other disorders and reveals that there are three general patterns of fatty infiltration: general replacement, marbling, and patchy infiltration. The Diaz-Manera group aimed to determine whether the fatty infiltration in VCP patients produces a unique signature that can be used in diagnosis confirmation.

Dr. Diaz-Manera obtained 92 MRIs of the lower limbs from the international VCP cohort study. The T1 data from the pelvic and lower limb muscles of 21 patients (29% male) was presented. In this cohort, the mean age of disease onset was 45.6 years and the mean age at time of MRI was 52.3 years. The T1 MRI revealed that the patients showed the patchy infiltration pattern of specific muscle groups. The affected muscles were paraspinal, gluteus minor, vastus medialis and intermedius and the adductor magus, posterior muscles of the thigh, gastrocnemius medialis and soleus and/or tibialis anterior and extensor digitorum. The rarely affected muscles were pelvic floor, gracilis, rectus femoris, adductor longus, and tibialis posterior. It should be noted that other diseases such as spinal muscular atrophy can also present with a patchy infiltration pattern of fatty tissue. Therefore, clinicians should distinguish between diseases by determining which muscle groups are affected. Dr. Diaz-Manera also presented preliminary STIR data that showed about 50% of patients had increased STIR signal, suggesting these patients had increased necrosis and inflammation. Overall, the preliminary data presented by Dr. Diaz-Manera suggests that muscle MRI may be a useful tool in the diagnosis of VCP disease. Further data will need to be collected and new MRI sequences will need to be tested to determine if they provide a more diagnostic profile for VCP disease.

3.3. Gerald Pfeffer MD, CM, FRCPC, PhD. Genotype-phenotype correlation and genetic modifiers in VCP-MSP

Dr. Gerald Pfeffer (University of Calgary) began his talk by highlighting findings from the largest study of genotype-phenotype diversity in VCP patients. This study reported large inter- and intrafamilial symptom variability in VCP disease. Patients with the same mutation (i. e. R155H and R155C) commonly present with different symptoms potentially delaying patient diagnosis (Al-Obeidi et al., 2018). Some cases have presented with large phenotypic variation even between members of the same family (Abrahamo et al., 2016). These cases reveal the difficulty in understanding adult-onset genetic disorders as it remains a challenge to distinguish genetic disease from acquired or non-genetic conditions (Martens et al., 2020; Almomen et al., 2019). However, heterogeneity in VCP patient symptoms is not the only issue delaying diagnosis for patients. Past studies have also shown that other issues such as a later age of disease onset, presence of neurological comorbidities, and “luck factors” (i.e. what type of doctor a patient sees first) are associated with increased time to diagnosis (Palese et al., 2019; Kano et al., 2013; Westeneng et al., 2018). All these factors present a unique problem for VCP researchers. Nonetheless, there are some genotypes that show a clear genotype-phenotype correlation. Specifically, axonal Charcot-Marie-Tooth disease has only been associated with E185K, S171R, and G87E mutations and spastic paraplegia has only been associated with R93H and R159C. Further study will be required as these represent a small number of cases with late onset.

A possible explanation for the general lack of symptom homogeneity in VCP patients may be genetic modifiers. It is now estimated that about 5% of patients with a one monogenetic disease may have one or more additional monogenetic diseases as well (Posey et al., 2017). This can lead to composite or additive effects of the multiple diseases when the phenotypes of each disease modify each other resulting in increased or decreased severity of the disease (Rahit and Tarailo-Graovac, 2020). Unfortunately, true modifiers are very

difficult to identify as genetic modifier research is at an early stage and the list of known genetic modifiers is relatively short (Rahit and Tarailo-Graovac, 2020). Open resources that have compiled genetic modifier data for rare diseases are limited. One new resource highlighted by Dr. Pfeffer is PhenoModifier, a database that contains a curated list of genetic modifiers from the scientific literature (<https://www.biosino.org/PhenoModifier/>). Outside of these resources, genetic modifiers can be identified through various techniques such as genome wide association studies (GWAS) or family linkage studies, but these traditional methods require recruitment of a large number of patients, an almost impossible task for a rare disease. Thus, researchers may need to focus on using cases with either extreme variability or restricted/extreme phenotypes. These methods have been fruitful as *APOE4* and *SCA2* have been identified as modifiers in VCP disease (Mehta et al., 2007; Kirby et al., 2021). Other publications have discussed genetic modifiers that are relevant to multisystem proteinopathy and may have relevance to VCP disease, but further study is required (Rahit and Tarailo-Graovac, 2020; Mehta et al., 2007). Other candidates for genetic modifier effects in humans may eventually be discovered based on preliminary studies in animal models (Chalmers et al., 2020). A specific case from Dr. Pfeffer's clinic was presented of a possible novel modifier effect in a patient with distal myopathy, who had a family history of PDB. This case highlighted the importance of correlation of genetic testing with muscle pathology and muscle MRI, which are both investigations that can aid in providing additional phenotypic information.

4. Session 2. New molecular and cellular insight into VCP disease: role of VCP in protein quality control, proteasome degradation, endolysosomal function and autophagy regulation

4.1. Peter Shen PhD. How VCP unfolds proteins

Dr. Peter Shen (University of Utah) discussed insights gained from high-resolution structures of VCP that provide a glimpse into the inner workings of this cellular machine. The VCP monomer contains a N-terminal domain responsible for adaptor binding, and two ATPase domains (D1 and D2) that act as motor domains. Six VCP monomers assemble into a planar homohexameric ring with the D1 and D2 domains in a stacked symmetric structure. Two states had been mapped for the homohexameric structure: the active ATP bound state where the N-terminal domains are in an “up” confirmation and the inactive ADP bound state where the N-terminal domains are in a “down” confirmation. It was previously unknown how these states correlated with substrate unfolding. Dr. Shen's group determined the structure of VCP in the context of substrate unfolding using cryo-EM.

They introduced a FLAG tag into the endogenous locus of a Cdc48 (the yeast homolog of VCP) binding partner, Shp1, in order to purify native complexes directly from yeast cell lysates (Cooney et al., 2019). The purified material was imaged using cryo-EM and two general states of VCP were identified from the ensembles. The first state was the known planar homohexamer state with a vacant central pore. The second state was a novel asymmetrical staircase conformation with substrate bound in the central pore. In the substrate bound state, they found that both the D1 and D2 domains of five of the

six subunits wrapped around the substrate through a network of deep grooves formed by methionines (D1) and tryptophans (D2). These deep grooves acted as molecular footholds to accommodate a large variety of sidechains from the unfolding substrate. In contrast, the sixth subunit (or the lagging subunit) was found to be completely detached from the substrate and found at the bottom of the homohexamer. The lagging subunit was highly mobile which allowed it to move from the bottom to the top of the staircase. At the top, the lagging subunit binds the next exposed region of the unfolding substrate. Thus, Cdc48 unfolds substrate using a “hand-over-hand” mechanism that allows substrates to be pulled through the central pore similar to the mechanism of action of other ATPases. Preliminary studies of human VCP suggests that it processes substrate in a similar manner to the yeast homolog. Future work will focus on determining the structure of mutant VCP unfolding substrate to understand the effect of mutation on VCP unfolding capacity.

4.2. Hemmo Meyer PhD. A role of p97/VCP in lysophagy

VCP is reported to participate in both proteasome- and lysosome-mediated degradation (Ju et al., 2009; Tresse et al., 2010; Hill et al., 2021; Ritz et al., 2011; Papadopoulos et al., 2017; Hall et al., 2017). Dr. Meyer’s (University of Duisburg-Essen) group found that adapter proteins linked to sorting in the endolysosomal pathway such as Phospholipase A2 Activating Protein (PLAA) and UBXD1 form a complex with VCP. They hypothesized that this association may be due to autophagy of damaged lysosomes (lysophagy). Lysophagy is a necessary cellular process to prevent leaking lysosomes from releasing damaging enzymes that can lead to cell damage. Lysosomes can rupture due to exogenous (drugs, photodamage, viral proteins) and endogenous (ROS, unbalanced lipid composition, and neurotoxic aggregates) factors. Lysophagy is a stepwise process in which lysosome rupture leads to the influx of damage sensors (i.e. galectins 1, 3, 8, and 9) and ubiquitin machinery. Ubiquitination of the lysosome membrane recruits the components of the phagophore for removal of the damaged structure (Papadopoulos et al., 2017).

Dr. Meyer’s group found that treating cells with a chemical inducer of lysosomal stress, L-leucyl-L-leucine methyl ester (LLOMe), led to the accumulation of VCP and ubiquitin on the surface of the damaged lysosomes (Papadopoulos et al., 2017). Furthermore, they showed that the clearance of damaged lysosomes is dependent on VCP recruitment to K48 ubiquitin-conjugates to the surface of lysosomes as knockdown or inhibition of VCP inhibited clearance. Using mouse embryonic fibro-blasts and muscle tissue from R155H mutant mice they found that VCP disease mutations prevented damaged lysosome clearance. Thus, defects in lysophagy may contribute to VCP disease pathology.

Dr. Meyer’s group has recently determined the E2 enzyme that is responsible for ubiquitinating lysosome targets. They performed a siRNA screen for 40 E2 enzymes and identified UBE2QL1 as a possible ubiquitin conjugating enzyme important for lysophagy. Proximity biotinylation of UBE2QL1 identified VCP as a putative interactor. They found that UBE2QL is required for the K48-linked ubiquitination and VCP recruitment to damaged lysosomes. Their studies indicate that depletion of UBE2QL1 inhibits autophagosome formation around damaged lysosomes and decreases the clearance of damaged lysosomes (Koerver et al., 2019). Taken together, Dr. Meyer’s group has developed

a model by which UBE2QL1 ubiquitinates the damaged lysosome allowing recruitment of VCP to drive autophagophore formation and lysophagy. Future studies will focus on determining the importance of this pathway is to the maintenance of lysosomes in a diseased context.

4.3. Alyssa Johnson PhD. *Drosophila* VCP disease models reveal new cellular pathologies

Dr. Alyssa Johnson (Louisiana State University) discussed her recent work identifying a role for VCP in regulating tubular lysosomes (TLs). Her studies have revealed that lysosomes can form branched, tubular structures in the *Drosophila* muscle that are highly dynamic (Johnson et al., 2015). TLs are acidic in nature and co-localize with autophagosomes, suggesting that they are functional for degradation. Since the initial discovery, these structures have been identified in various species and tissues such as the epidermis and in immune cells. Depending on the cell type examined, TLs can be constitutively present or induced by specific stressors (Miao et al., 2020; Saric et al., 2016; Dolese et al., 2021). Importantly, Dr. Johnson's findings suggest that VCP is required for TL network integrity (Johnson et al., 2015).

Dr. Johnson's group identified small VCP interacting protein (SVIP) as the cofactor that that directly recruits VCP to TLs (Johnson et al., 2021). SVIP interacts with VCP through the first coiled-coiled domain and the TL network through its N-terminus. *Drosophila* SVIP co-localizes with the TL in vivo and overexpression of SVIP leads to a dramatic relocalization of VCP to the TL network, suggesting that SVIP is necessary for VCP localization to TL. Furthermore, they found that genetic deletion of SVIP leads to progressive motor neuron degeneration, muscle degeneration, thinned muscle fibers, defective organelle structures, and a reduction in total lifespan. Taken together, this data suggests that SVIP is required for maintaining TL integrity and organismal health.

Dr. Johnson presented preliminary data suggesting that flies harboring VCP patient mutations show TL disruption as well as many of the same defects found in VCP disease including locomotor defects, proteostasis decline, accumulation of TDP-43, and defective nuclear morphology (Wall et al., 2021). Interestingly, overexpression of SVIP in R152H/C (R155H in humans) mutant flies was able to rescue the TL network and improve locomotor defects. These studies suggest that the SVIP-VCP interaction may mediate MSP-1 disease pathogenesis and progression.

5. Session 3: new tools and large scale genomic and proteomic studies

5.1. Fabio Coppedè PhD. Epigenetics of amyotrophic lateral sclerosis

Fabio Coppedè (University of Pisa) opened the session discussing the role of epigenetics on gene expression in ALS. Epigenetic changes in gene expression are heritable but are not due to changes in DNA sequence and may include (1) DNA methylation and (2) histone tail modifications which alter chromosome condensation and subsequent transcription, as well as (3) non-coding RNA-mediated mechanisms via micro-RNAs and long non-coding RNAs. Dr. Coppedè's group studies how changes in DNA methylation alter gene expression in

patients with neurodegenerative diseases such as ALS. One of the first studies linking DNA methylation and ALS found increased expression and activity of DNA methyltransferases (DNMTs) and 5-methylcytosine in post-mortem brain tissue of ALS patients indicating increased global methylation of DNA (Chestnut et al., 2011). This data was corroborated by the EURALS Consortium which found similar results in whole-blood DNA methylation levels in ALS patients (Tremolizzo et al., 2014). Furthermore, a recent study compared discordant monozygotic twins where one sibling developed ALS while the other remains asymptomatic. This study found widespread changes in methylation patterns in the affected twin that were reminiscent of signatures found in individuals of older chronological age suggesting premature DNA aging (Young et al., 2017).

Dr. Coppedè's research focused on comparing global DNA methylation in familial cases of ALS caused by SOD1 mutations where he could compare symptomatic family members with family members that have the same gene mutation but are asymptomatic. Within families with SOD1 mutations, blood DNA had increased methylation in symptomatic patients when compared to asymptomatic carriers and non-carrier controls. Furthermore, DNA methylation increased with disease duration in patients (Coppedè et al., 2018). Genome wide methylation analysis from these patients allowed Dr. Coppedè to elucidate the specific genes which are being affected by DNA methylation. This study discovered 12 altered gene promoters which are involved with immune response and inflammation. His results were corroborated by additional studies in ALS patient cohorts (Figuroa-Romero et al., 2012; Tarr et al., 2019). Collectively these studies find a global increase in DNA methylation after disease onset which appear to impact genes involved in the immune response. Dr. Coppedè's current research is investigating the role of mitochondrial DNA (mtDNA) copy number and D-loop methylation and disease. He has previously shown that in contrast to genomic DNA, the D-loop region of mtDNA has decreased methylation in ALS cases carrying mutations in SOD1 as well as sporadic ALS patients (Stoccoro et al., 2018). Patients with mutations in other ALS genes such as *FUS*, *TDP-43* and *C9ORF72* did not have any difference in D-loop methylation (Stoccoro et al., 2020). Patients with VCP mutations have altered mitochondrial energy metabolism. Dr. Coppedè suggests that this may be due in part to changes in mtDNA methylation and that altering epigenetic changes may ameliorate disease phenotypes. However, preliminary studies in mouse models of ALS using epigenetic drugs had modest outcomes (Coppedè, 2020). He indicated that treating symptomatic individuals with these drugs may be too late in the disease course. Early intervention (in pre-symptomatic individuals) may be required for these drugs to have therapeutic benefit which highlights the need to develop biomarkers for early detection. He suggested that lifestyle modification and naturally occurring compounds that exert epigenetic changes could represent therapeutic opportunities.

5.2. Nicholas Seyfried PhD. Network based proteomics for biomarker discovery in Alzheimer's disease

Current biomarkers for neurodegenerative diseases are limited and do not capture patients early in disease progression. Dr. Nicholas Seyfried (Emory University) discussed Alzheimer's Disease (AD) research carried out as part of the Accelerating Medicine Partnerships (AMP), a joint venture between industry and the National Institutes of

Health. His group has been using multiplexed quantitative proteomics and weighted gene co-expression network analysis (WGCNA) to discover new biomarkers for AD (Wingo et al., 2021). Isobaric tandem mass tags that function as barcodes were used to label proteins from multiple samples. This method allows for thousands of proteins to be measured and samples to be directly compared to each other. Using this method, Dr. Seyfried identified proteins that were changing in 80 brain tissue samples derived from control, AD, Parkinson's Disease (PD), and AD-PD patients (Ping et al., 2020). Over 10,000 proteins were sequenced and clustered into up- and down-regulated modules that were associated with clinical and pathological phenotypes using WGCNA. These modules were grouped into panels based on cell types (neurons, astrocytes, oligodendrocytes, etc.) and biological function (synaptic organization, vascular function, myelination, glial immunity, and metabolism). Notably, changes in these panels correlate closely with A β /tau levels and clinical symptoms and were able to identify patients with asymptomatic Alzheimer's disease proving their usefulness in early detection of disease. Cerebrospinal fluid (CSF) is commonly used for AD diagnosis, however, changes in protein levels in CSF may not always correlate with those found in brain tissue. To determine differential changes between CSF and brain tissue, forty CSF samples were collected from normal and AD patients and processed through the TMT workflow. Over 500 differentially expressed proteins were detected including known biomarkers such as MAPT and neurogranin (NRGN). These hits were mapped to the biological function panels which found the synaptic, myelination, glial immunity and metabolic panels were enriched while the vascular panel was depleted in patients with AD compared to healthy controls. Furthermore, these panels showed similar changes in CSF taken from patients at risk for AD who have increased A β but do not yet have clinical symptoms. In particular, the metabolic panel was highly correlated with traditional biomarkers of AD. This study also identified control patients that scored similarly to AD patients on the panels. Continued follow-up with these patients will determine if they develop disease and may suggest that these panels are more sensitive than current biomarkers for early disease detection. Interestingly, these panel markers were able to differentiate AD from other neurodegenerative diseases such as FTD, PD, and ALS.

These panels may also be useful as theragnostic markers for patients undergoing treatment. Norepinephrine reuptake inhibitors including atomoxetine are currently in clinical trials for patients with mild cognitive impairment (<https://clinicaltrials.gov/ct2/show/NCT01522404>). In collaboration with this clinical trial, CSF was taken from patients taking the drug and placebo for TMT proteomics. Patients being treated with atomoxetine had significantly altered synaptic and metabolic panels scores indicating that this methodology may be used to monitor efficacy of therapeutic treatment.

Dr. Seyfried's current research is investigating how these panels perform in blood samples. CSF requires a lumbar puncture which is time intensive and is often uncomfortable for the patient. Blood draws are much more easily performed and thus these markers may be monitored in a broader population. Initial studies have shown that some of these proteins are found to change in the opposite direction from CSF samples (unpublished data), thus further studies are needed. While these tools have been developed for Alzheimer's disease, they may also be used for other diseases such as VCP disease though validation will be required.

5.3. Kalina Paunovska PhD. Delivering RNA therapeutics by testing thousands of nanoparticles in vivo

RNA-based therapeutics are gaining traction as exemplified by the current vaccines for the SARS-CoV-2 virus that utilize mRNA technology. However, delivery of RNA has proven difficult as naked RNA is rapidly degraded when administered necessitating vector-based methods. Dr. Kalina Paunovska (Georgia Institute of Technology) has been studying the use of lipid nanoparticles (LNPs) for the delivery of RNA therapeutics. LNPs consist of a lipid bilayer containing phospholipids, cholesterol, PEG lipids, and ionizable fatty acids surrounding a core of genetic material such as RNA. These LNPs may also form multi-laminar bodies composed of concentric spheres with alternating layers of lipids and RNA. Each of the constituents of LNPs may be altered to improve the efficiency of delivery. However, due to the massive number of possible combinations of lipid components and RNA, LNPs are often screened in vitro with only a few hits being carried forward to in vivo studies. Furthermore, delivery of LNPs may be affected by factors not captured in in vitro models such as immunogenicity, extracellular matrix penetration, and non-target tissue distribution.

To overcome this challenge, Dr. Paunovska has developed a novel method for high throughput screening of LNPs in vivo. Joint Rapid DNA Analysis of Nanoparticles (JORDAN) utilizes DNA barcoding to label pooled LNPs prior to administration into mice (Paunovska et al., 2018a). Tissue samples are then harvested and sequenced for the barcodes to identify LNPs that were taken up efficiently by the target tissue. Using this method, Dr. Paunovska found that cholesterol esters delivered LNPs better than oxidized cholesterol in mice. This finding may be used to design high fidelity LNPs for therapeutic delivery in the future (Paunovska et al., 2018b).

While JORDAN identifies LNPs that reach target tissues, it does not distinguish between LNPs that efficiently deliver their payload to the cytosol of cells within the target tissue versus LNPs that bind to the cell surface or are trapped within endosomes. To screen for functional delivery, Dr. Paunovska established a new method termed Functional in vivo Nanoparticle Delivery (Sago et al., 2018). This method utilizes a mouse model carrying a tdTomato gene cassette flanked by loxP sites. LNPs carrying Cre mRNA are injected into mice and functional delivery of the LNP can be screened by identifying tdTomato positive cells by flow cytometry or imaging. This method uncovered that using 25 α -hydroxy cholesterol enhances functional delivery to the liver, endothelial cells, and immune cells (Paunovska et al., 2019).

With RNA therapeutics on the rise and an increase in clinical trials utilizing RNA or ssDNA such as antisense oligomers, optimization of delivery methods such as LNPs is becoming essential to translational medicine. With the methods established by Dr. Paunovska, future development of clinically relevant LNPs may progress in a high-throughput manner.

6. Session 4. Resources for research –NIH NeuroBioBank

An essential part of translational medicine is validating scientific findings in patients. Biobanks facilitate this process by collecting patient samples for distribution to researchers

to help further our understanding of disease. The UMD Brain and Tissue Bank (BTB) was founded in 1991 by Dr. Ron Zielke as one of the first biobanks in the world (<https://www.medschool.umaryland.edu/btbank/>). The mission of the UMB BTB is to assist families for tissue donation, provide researchers access to human tissue, and accelerate research for the improvement, cure, and prevention of developmental and neurological disorders. The UMB BTB processes each hemisphere of brain tissue separately. The right hemisphere is fixed in formalin while the left hemisphere is frozen and is used for quality assurance. Every sample is reviewed to ensure RNA/DNA integrity, and proper pH. Samples from the UMB BTB may be used for a variety of assays including histology, single cell analysis, protein studies, and gene expression. Additionally, medical information is available and can be requested for most samples in the bank.

In 2013, the NIH established the NeuroBioBank (NBB) as a national resource which unified six biorepositories: UMD, University of Miami Brain Endowment Bank, Harvard Brain Tissue Research Center, Mount Sinai Brain Bank, UCLA Human Brain and Spinal Fluid Resource Center, and University of Pittsburgh Brain Tissue Donation Program (Freund et al., 2018). By combining the repositories from these institutions, NBB has become a national resource for brain tissue for researchers studying neurologic disease. UMD has been working with Cure VCP Disease to optimize data collection and patient sample donation. Previously, VCP disease patients without FTD/ALS were categorized as “non-brain disorder” and received the assignment of “unaffected control”. This would allow researchers seeking control samples for non-VCP disease research to request these samples and potentially deplete them. Dr. Blanchard has successfully lobbied the NBB to change the coding of VCP cases to “Genetic Carrier of Other Disease” preventing researchers seeking controls from receiving VCP disease samples and facilitating VCP disease researchers in finding relevant samples.

Researchers may request samples from the NBB through the NIH NBB website. These samples are provided to researchers at no cost other than shipping. There are over 10,000 patient cases available from a wide variety of neurological diseases as well as control samples. Most tissue samples are in a variety of formats to enable downstream studies. For patients seeking to donate tissue to the UMB Brain and Tissue Bank, Dr. Blanchard recommends either contacting the Brain Donor Project and requesting UMB as the banking institution or directly calling the UMB BTB. UMB collects donations from all 50 states. There is no cost to donors or their next of kin for participation in UMB BTB or any repository in the NBB.

7. Session 5. drug discovery: new inhibitors, small molecules, and anti-sense oligonucleotides targeting VCP

7.1. Donna Huryn PhD. Drug discovery: small molecules inhibitors of p97/VCP

Dr. Donna Huryn (University of Pittsburgh) gave an overview on small molecule inhibitors of VCP and their therapeutic potential in cancer and other disease models. Until about 2010, the main mechanism for downregulating VCP was through siRNA approaches. However, since 2010 there has been an interest in identifying small molecules that could specifically

inhibit VCP functions for the dual purpose of understanding VCP biology and to find drug-like compounds. In the last decade, different inhibitors have been identified that belong to various chemical classes, such as quinazolines (competitive inhibitors), phenyl indoles (uncompetitive inhibitors discovered by Dr. Arkin at UCSF), and triazoles (non-competitive inhibitors) (Chou et al., 2011; Chimenti et al., 2015; Alvarez et al., 2016). Active site inhibitors based on a quinazoline scaffold were identified as part of the NIH Molecular Libraries Program from labs at Caltech and University of Kansas, and has led to multiple VCP inhibitors, such as DBeQ, ML241, the covalent inhibitor LC-1028 and two compounds that advanced to clinical trials, CB-5083 and CB-5339 (Chou et al., 2011; Roux et al., 2021). More recently, members of the NCI Chemical Biology Consortium (CBC): the University of Pittsburgh Chemical Diversity Center, (UPCDC), Dr. Arkin from UCSF, and Dr. Chou from Caltech have initiated a project focusing on allosteric inhibitors of VCP.

In a collaboration between the Arkin (UCSF), Huryn and Subramanian (NIH) labs, a Cryo-EM structure of homohexameric VCP bound to an allosteric phenyl indole was solved and showed that it bound to the D2 domain of VCP at the D1 interface and locked the protein in an inactive down conformation (Banerjee et al., 2016). In subsequent studies, immobilized lipid conjugated phenyl indoles bound to VCP on cryo-EM grids. Structural analysis showed VCP assembly into a double hexamer (Hoq et al., 2021). These structures bring up the possibility that VCP function or biological interactions may utilize alternate assembly modes in vivo. Based on an earlier report by Nerviano and Genentech that described a series of triazoles (e.g. NMS-873), Dr. Huryn's and the UPCDC's collaborative efforts with members of the NCI CBC led to the identification of optimized compounds from this scaffold. These allosteric inhibitors had similar cellular effects as the competitive inhibitors as assessed by various biomarkers associated with VCP inhibition. The crystal structure of NMS-873 bound VCP indicated an overlap in the binding site of NMS-873 with the phenyl indoles albeit with slight variations in binding surfaces (Pan et al., 2021).

Can these various inhibitors be used in the treatment of VCP disease? While preliminary data suggests that quinazoline and triazoles may have activity in disease models, more research is needed to fine tune these compounds for clinical purposes. Furthermore, since not all VCP mutations exhibit a gain of function phenotype, small molecules activators may also be needed for specific mutants with a loss of function phenotypes. Dr. Huryn also suggested the possibility of developing inhibitors that disrupt specific VCP- adaptor complexes.

7.2. Tsui-Fen Chou Ph.D. Chemical proteomic profiling of VCP inhibitors

Dr. Tsui-Fen Chou (Caltech) discussed her group's efforts on chemical proteomic profiling of VCP inhibitors. She identified DBeQ, the first small molecule inhibitor of VCP when she was a post-doctoral scholar with Ray Deshaies (Chou et al., 2011). DBeQ was subsequently modified using structure activity relationship studies to yield the ML series of inhibitors (ML240 and ML241) which demonstrated higher potency and reduced IC₅₀ values (Chou et al., 2013). Cleave Biosciences further altered ML240 to develop CB-5083, which was highly selective for VCP and entered Phase I clinical trials as an anti-cancer compound. Off-target effects in patients stalled clinical trials, but further modification yielded CB-5339 which

eliminates the off-target effect and is currently in Phase 1 trials (Roux et al., 2021; Zhou et al., 2015).

Dr. Chou's group has been interested in finding covalent inhibitors for VCP that may be more potent in treating CB-5083-resistant cancers. They identified the covalent inhibitor, PPA (ACJI-99C) which modifies Cys 522 at the active site and shows inhibitory activity against human VCP, but not the yeast homolog Cdc48 (Zhang et al., 2021). In parallel, the group has also been characterizing VCP mutations (N660K, E470K) in cancers that are resistant to CB-5083. Characterization of these mutants indicated that they have higher affinity for ATP, thereby reducing the efficacy of the CB-5083 (Bastola et al., 2017). Allosteric inhibitors of VCP such as UPCDC-30245 and NMS-873 have been shown to overcome this resistance in colorectal cancer cells (Wang et al., 2020).

Dr. Chou's group has been investigating potential biomarkers of VCP disease by analyzing the proteomes of cells depleted of VCP, treated with VCP inhibitors (CB-5083, NMS-873, or UPCDC-30245) or the proteasome inhibitor MG132 (Wang et al., 2021). These studies found similarities in the profiles of differentially expressed proteins with VCP downregulation, depletion, or inhibition. In contrast, proteasome inhibition with MG132 revealed that cell cycle proteins and proteins associated with unfolded protein response (UPR) and ER processing were differentially impacted in VCP and proteasome inhibited cells. These observations highlight differences in substrate profiles between VCP and the proteasome and may explain why proteasome inhibition with MG132 is not as effective at treating solid tumors.

Dr. Chou's group has recently examined the VCP-independent effects of NMS-873 to understand off target effects of VCP inhibitors. NMS-873 (but not CB-5083, UPCDC-30245, or MG132) treatment caused an increase in glycolysis and inhibition of oxidative phosphorylation, which was rescued by using 2-deoxyglucose (2-DG), a synthetic analog of glucose and improved the anticancer properties of NMS-873. To further analyze if the effects of NMS-873 were VCP-dependent, NMS-873 resistant HCT116 cancer cells were generated. Pathway analysis of NMS-873 resistant HCT116 cells demonstrated that Golgi to ER traffic, ubiquitination, autophagy, ER quality control, and endosomal sorting were downregulated in the NMS-resistant lines. Interestingly, they found that extracellular lactate formation was VCP-independent. Bouwer et al. recently showed that NMS-873 inhibits.

mitochondrial oxidative phosphorylation targets Complex 1 and ATP synthase (Bouwer et al., 2021). This off-target effect could be beneficial to synergistically inhibit both VCP and OXPHOS. Dr. Chou concluded by reiterating the importance of finding specific inhibitors that target VCP-cofactor complexes to dissect the role of VCP in various gain of function diseases (Chou et al., 2011). Please see Table 1 for a full list of available p97 inhibitors.

8. Session 6. Gaps in current research areas

8.1. Edward Lee MD, PhD. From structure to function

Session 6 focused on summarizing the existing research about VCP structure and function and the gaps in the knowledge that may be probed to better understand the role of VCP in disease. The focus of the session was to understand how mutations in VCP lead to a wide variety of phenotypes and if it can be correlated with the ATPase/unfoldase activities. Dr. Edward Lee (University of Pennsylvania) gave an overview of reported VCP mutations that show an increase in ATPase/unfoldase activity in vitro and are perceived as gain of function. This hypothesis is supported by the observations that disease associated cellular phenotypes, such as higher cellular ubiquitin and p62 levels are rescued by VCP inhibitors (Zhang et al., 2017; Blythe et al., 2019). However, an increase in the ATPase/unfoldase activity does not necessarily equate to a gain-of-function as indicated by a loss of mitochondrial function, autophagosome maturation, and lysosomal function (Ju et al., 2009; Tresse et al., 2010; Hill et al., 2021; Ritz et al., 2011; Johnson et al., 2015). This suggests that ATPase/unfoldase activity alone cannot be linked to a gain-of-function or loss-of-function phenotype and more cellular parameters should be examined for making this correlation.

While in vitro studies have identified VCP as an unfoldase for ubiquitinated substrates, structural information alone cannot explain the dual roles for VCP in processes such as stress granules assembly and clearance (Buchan et al., 2013; Gwon et al., 2021; Maxwell Brian et al., 2021). Thus, to fully understand VCP function the field needs comprehensive assays that allow for genotype-phenotype correlation.

To support this hypothesis, he presented his group's work on the identification of a novel VCP hypomorphic mutation (D395G) in genetically unrelated families in the United States and Greece that presented with vacuolar tauopathy with FTD (Darwich et al., 2020). Patients displayed no clinical evidence of muscle or bone disease and TDP-43 mislocalization was not observed in brain tissue. However, the brain biopsies did show an increase in tau aggregate formation and vacuoles in the brain. The mutation does not overlap with the commonly observed MSP-1 mutations in the N-D1 linker region, suggesting that while FTD due to TDP-43 mislocalization could be attributed to a gain of function, a loss of function could also result in FTD associated with vacuolar tauopathy. More studies are needed to delineate if the gain of function ATPase mutations translate to gain of function in cellular pathways. These questions can be addressed effectively by equating the clinical phenotypes observed in patients to underlying cellular functions.

8.2. Michelle Arkin PhD. Why do mutations in VCP cause disease?

Michelle Arkin (University of California, San Francisco) discussed insights into VCP structure and function. The cryo-EM structure of VCP with the R155H mutation suggests that the mutation tends to favor the active 'up' conformation, indicating a gain of ATPase activity (Banerjee et al., 2016). However, it is still unclear if the R155H mutation can be considered a physiological gain of function mutation. VCP adaptors, such as p37, p47, FAF1, and UFD1-NPL4 demonstrate higher affinity towards the ATP-bound up conformation. In contrast, UBXD1, displays preferential association to the ADP-bound

‘down’ conformation, suggesting that changes in VCP-adaptor binding could substantially contribute to disease phenotypes. Understanding the preferential binding of adaptors to VCP will help in adapting suitable strategies for altering VCP function, such as inhibition of ATPase activity or altering the N–D1 linker region (Davies et al., 2008; Figuerola-Conchas et al., 2020).

The discussion following the talks highlighted the need for more research to correlate genotype and phenotype in VCP disease. Dr. Conrad Wehl (Washington University, St.Louis) and Dr. Arkin suggested the need for deep phenotyping of patients to establish the spectrum of VCP mutation phenotypes. Dr. Shen stressed the need to develop a broad spectrum of cellular assays that better mimic physiological phenotypes. Dr. Lee added that since VCP has opposing functions in many cellular processes, such as stress granule formation and clearance, the disease phenotypes could stem from a functional imbalance rather than a clear gain or loss of function which also needed investigation. Dr. Malavika Raman (Tufts University School of Medicine) suggested that this could be due to perturbed VCP-cofactor association and that cell-type specificity of adaptors needs to be understood to dissect diverse VCP disease phenotypes. The session highlighted the need for developing models and assays that will help researchers better understand the heterogeneity of VCP disease.

9. Session 7. Gaps in current clinical care

9.1. A panel discussion by Bhaskar Roy, MBBS, MBMS, MHS; Nupur Ghoshal MD, PhD; Meredith James DPT; Gerald Pfeffer, MDCM, FRCPC, PhD; Manisha Korb MD

Dr. Manisha Korb (University of California, Irvine) was the project lead for the Standard of Care study initiated by Cure VCP Disease that met in April 2021 (Korb et al., 2022). This study was initiated based on feedback from patients about the gaps in current clinical care, which included the long lead time to correct diagnosis, unclear screening guidelines for the different phenotypes of the disease and common symptoms that were not being addressed in clinical visits. To address these unmet needs and the disparities between clinics and institutions, Cure VCP Disease recruited over 50 clinicians from multiple countries, disciplines, and specialties to collaborate and establish a set of multidisciplinary standard of care recommendations. Clinicians were focused on outlining a clear set of clinical features, guidelines for diagnosis, treatment modalities and routine surveillance. This set of guidelines was developed for each major clinical presentation of MSP-1 and included myopathy, FTD, Paget’s disease of the bone, ALS, CMT and Parkinson’s disease/parkinsonism. Guidelines were also developed for cardiomyopathy, respiratory dysfunction, and additional supportive therapies such as physical or occupational therapy, speech language pathology and nutrition. Dr. Korb stressed the need for developing a clinical outcome assessment to monitor patients and inform future studies for diagnosis and treatment of MSP-1 patients.

In the following discussion, panel experts talked about what they see is the biggest gap in clinical care. Dr. Gerald Pfeffer stated that the biggest gap is in diagnosis as many individuals are still undiagnosed particularly those with late onset presentations, mild phenotypes, or atypical presentation. However, he was optimistic about faster diagnosis in the future given the increased use of sequencing panels in patients with different clinical

presentations. He stressed that due to the diversity of presentations in individuals with VCP-related disease, it is important that VCP should be included in as many relevant sequencing panels as possible. Dr. Pfeffer also talked about regional disparities in diagnosis. The gold standard is genetic testing as there is significant overlap in phenotypes and pathology between VCP disease and other conditions. In areas where genetic testing is not commonly available, sponsored testing programs, which are free of charge or subsidized should be considered.

Under-recognition of the heterogeneity in the phenotype of VCP disease is a major shortcoming in current clinical care according to Dr. Bhaskar Roy (Yale School of Medicine). VCP patients can present with diverse phenotypes such as proximal or distal muscle weakness, foot drop or scapular winging. A thorough understanding of these disparate phenotypes is needed so patients are not mis-diagnosed. He indicated that even EMG studies in patients display heterogeneous myopathic and neurogenic features and lead to delay in the diagnosis. Given that 10% of patients develop motor neuron disease (of which ALS is the major sub-type), EMG findings that are consistent with motor neuron disease should prompt clinicians to closely monitor these patients due to rapid progression of disease. There was debate about whether to use muscle MRI, needle muscle biopsy or open muscle biopsy during the development of the standard of care. In general, open muscle biopsy can better visualize morphological changes, such as rimmed vacuoles although needle biopsies can provide information on some molecular aspects.

Dr. Nupur Ghoshal (Washington University, St.Louis), the co-lead for the FTD group, stated that given the prevalence of FTD in about 30% of VCP patients, it is imperative that clinicians include FTD assessment of their patients. Without this, there will be delays in diagnosis and disease management. The Standard of Care study incorporated these guidelines for more efficient diagnosis. Dr. Ghoshal and her team also advocated for the use of readily accessible neuro-psychiatric evaluations such as the Montreal Cognitive Assessment (MoCA) or the Neuropsychiatric Inventory Questionnaire (NPI-Q) in the standard of care guidelines as they are simple, rapid, and useful for pre-screening for FTD. General neurologists can use these tests when they see patients and can then trigger a referral for rigorous neuropsychiatric testing, specialty evaluation and management of FTD.

Meredith James, DPT (John Walton Muscular Dystrophy Research Center), stressed the importance of bringing patients to a neuromuscular specialty clinic and the need for longitudinal clinical testing. Clinical outcome assessments of motor function, respiratory testing, upper limb function or mobility are essential to document patient progression. Due to the significant delay in diagnosis of VCP disease, there is usually only a short time to provide impactful care to patients. Therefore, providing access to multidisciplinary clinics, timely referral to occupational therapists and discussing mobility aids is critical. She also indicated that quantitative motor function testing be routinely used to monitor patients rather than manual muscle testing.

10. Session 8. Clinical trial readiness: biomarker discovery, toxicology consideration and patient longitudinal studies

10.1. Nelson Pace, PhD, SM. Insights from the allstripes platform

AllStripes Research has developed a rare disease platform that seeks to enable new treatments for people affected by rare disease. The platform allows patients to consolidate all their medical data into one centralized portal and provide consent to share this data in a de-identified manner with regulatory and research agencies. Currently, 40 different rare diseases are supported in their platform. Dr. Nelson Pace (Director of Epidemiology at AllStripes) shared insights gained from patients with VCP disease through their medical records on the AllStripes platform.

In the VCP cohort, 47 patients provided full research consent, of whom 17 patients had the most comprehensive medical data available which included confirmed VCP diagnosis, variant information and symptom onset. A number of features were identified within the 17 patients, the average age of diagnosis was ~51 years, while age of first symptom onset was ~44 years. Muscle weakness was apparent at ~46 years of age and on average was apparent approximately 6 years prior to diagnosis. Of the six different variants among the 17 patients 41% had the c.464G > A, p.Arg155His variant. IBM and Paget diagnosis was also documented in a subset of patients. Strikingly, while 50% of patients received a VCP diagnosis within 6.5 years after symptom onset, some patients were diagnosed much later (in one case 17 years after symptom onset). These findings highlight the need for a more streamlined protocol that will allow for faster diagnosis and earlier intervention.

Dr. Pace emphasized the need for increasing cohort size so that more information can be made available for research purposes. In the discussion following the presentation, Dr. Pace highlighted that the patient medical records can allow for longitudinal tracking of numerous metrics such as physical therapy outcomes and disease progression that will provide further granularity in managing symptoms and furthering research.

10.2. Lindsay Alfano, PT, DPT, PCS—Clinical trial readiness in VCP-MSP-1

Dr. Lindsay Alfano (Abigail Wexner Research Institute at Nationwide Children's Hospital) shared the outcomes of the longitudinal clinical trial readiness study on VCP-MSP-1. This one-year prospective longitudinal study was performed in collaboration with Cure VCP Disease and was designed to develop a protocol to determine what outcomes can be successfully measured in a future clinical trial. The study included two separate baseline visits that were conducted either on-site at Nationwide Children's or in the patient's home, followed by a remote visit at the 6-month timepoint, and culminated at 12 months with one remote and one onsite visit to determine how these outcomes perform cross-sectionally and their change over time. The study is currently at the baseline phase, with 24 patients enrolled (mean age: 51.2 years, 58% female). Mean age of symptom onset for this cohort is 42.5 years.

At the onsite visit at Nationwide Children's hospital a battery of motor assessments was administered which included: (i) the 10 or 100-m timed walk-run test, (ii) time to rise

test, (iii) North Star Assessment for Dysferlinopathy (NSAD), (iv) performance of upper limb (PUL) test, (v) a custom-designed video game known as ACTIVE (Ability Captured Through Interactive Video Evaluation) that measures workspace volume (WSV) and (vi) Spirometry. In addition to several patient-reported outcomes, serum and urine was also collected at the baseline and 12-month visits for future research. To minimize variability in data collection during remote assessments, Cure VCP Disease assembled and distributed home measurement kits and made pre-visit calls to all the families to prepare them for the visits. All the onsite assessments except 100-m timed test, 4-stair climb, ACTIVE WSV and biomarker collection were performed during the remote visit.

Preliminary study results were presented and as expected for VCP disease a degree of heterogeneity was observed in many of the assessments. Data collection is ongoing, and the study aims to better understand the feasibility of use and/or validate use of functional outcome measures in VCP disease, compare the validity and reliability of data collected onsite versus remotely, and evaluate the ability to measure meaningful change over time in this cohort. This ongoing study is an example of academic/patient group collaboration resulting in efficient study design, reduced burden of testing to boost enrollment in this rare cohort. A manuscript is currently in development describing the study outcomes.

10.3. Alison Skrinar, PhD. Clinical trial readiness: an industry perspective

Alison Skrinar (Vice President clinical outcomes research and evaluation at Ultragenyx) provided feedback on these studies from an industry perspective. She commented that the ability to integrate the types of data collected through Allstripes with observational studies can facilitate clinical development in the industry, because this type of data can be utilized to advance the clinical development program while the preclinical work is being done. It can be used to put the infrastructure in place to support study design, endpoint selection and preliminary discussions with the FDA, so that when viable treatment options become available, safety and efficacy can be established as quickly as possible.

One of the challenges of clinical trials is the desire to study a broad patient population while maintaining the ability to show statistically significant changes. This is particularly challenging for VCP disease where patient heterogeneity is significant and may necessitate a large trial to ensure that endpoints are met successfully.

Therefore, having information across the spectrum of disease severity is essential to identify assessments that can be applied to all patients. This will aid in designing an adequately powered single trial with clinically meaningful endpoints that can be reliably administered. She also stressed that the industry is avoiding the randomized, double blind, placebo-controlled trial for patients who have significant weakness, progressive decline and difficulty getting into the clinic, in favor of observational studies where patients serve as their own control in a clinical trial. Ultragenyx designs clinical trials to start earlier with an observational period so that researchers can evaluate patients in the absence of treatment. Observational studies help delineate disease specific manifestations as patients are monitored. For example, it can distinguish adverse events due to the drug/ treatment associated with the disease versus the safety profile of the drug. This is especially important in VCP disease since its multisystemic. The remote assessments in the study led by Dr.

Alfano can be advantageous in diseases with muscle weakness where it is difficult for patients to travel to clinical trial sites. These modalities help with recruitment and retention when patients can be evaluated in their home.

11. Session 9: looking forward: where do we go from here?

11.1. A panel discussion by Michelle Arkin PhD., Jordi Diaz-Manera MD, PhD., Conrad Wehl MD, and Tahseen Mozaffar MD

The 2021 Cure VCP Conference concluded with a panel discussion exploring the next steps in understanding VCP related MSP-1/IBMPFD. The panelists raised an assortment of topics that need to be addressed in the future. These include: 1) understanding the cause of patient heterogeneity, 2) identify disease predictors/novel biomarkers, 3) clarifying the full spectrum of VCP function, 4) establishing the relationship between VCP and sporadic disease, 5) identifying the functions of various cofactors, 6) identifying new models of disease pathology, and 7) identifying useful outcome measures for clinical trials. As such, multidisciplinary studies will need to be utilized for understanding and treating VCP disease.

12. Conclusion

The Cure VCP 2021 conference had nine sessions aimed at providing a forum for discussing recent insights into the underlying mechanisms of how VCP mutations cause disease (sessions 1 and 2), highlighting new tools and resources from cross-disciplinary research (session 3–5), identifying current gaps in knowledge to focus future research efforts (sessions 6, 7, and 9), and preparing for clinical trials (session 8). The poster sessions and short talks by trainees and investigators (not discussed in this review) provided an avenue for researchers to learn about new unpublished findings and allowed trainees an opportunity to present their work to leaders in the field. With close to 150 individuals in attendance (60% female, 40% male) from 25 institutions representing 13 countries the conference succeeded in meeting its goals. A full list of presented talks and poster presentations can be found in the Supplemental Information. Overall, the talks highlighted the heterogeneous nature of VCP related MSP-1, its complex disease pathology and showed the significant progress that has been made in understanding VCP function and disease. For example, recent *in vitro* studies provide insight into how ATP hydrolysis drives protein unfolding through the central pore and how this process is altered upon VCP mutation. Furthermore, Cure VCP Disease has partnered with clinicians to develop the first Standard of Care protocol for individuals with VCP disease and has also initiated clinical trial readiness studies (section 8). As such, the multidisciplinary and collaborative nature of this conference holds promise for the development of therapies that will mitigate disease phenotypes. Future meetings should continue to be multidisciplinary, highlighting both molecular and clinical advances made by a diverse group of investigators and their trainees. This will lead to a much deeper understanding of VCP disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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We would like to thank Cure VCP Disease for convening and facilitating this meeting. The speakers at each session were invited by Cure VCP. Short talks and posters were solicited from trainees, and all submitted work was invited to present at the meeting. At the time of the meeting five of the twenty-seven abstracts presented published studies. Thus, some of the presented data was not subject to peer review but represents the most current state of the field.

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References

- Abraham A, et al. , 2016. One family, one gene and three phenotypes: a novel VCP (valosin-containing protein) mutation associated with myopathy with rimmed vacuoles, amyotrophic lateral sclerosis and frontotemporal dementia. *J. Neurol. Sci* 368, 352–358. [PubMed: 27538664]
- Almomen M, et al. , 2019. High diagnostic yield and novel variants in very late-onset spasticity. *J. Neurogenet* 33 (1), 27–32. [PubMed: 30747022]
- Al-Obeidi E, et al. , 2018. Genotype-phenotype study in patients with valosin-containing protein mutations associated with multisystem proteinopathy. *Clin. Genet* 93 (1), 119–125. [PubMed: 28692196]
- Al-Tahan S, et al. , 2018. Novel valosin-containing protein mutations associated with multisystem proteinopathy. *Neuromuscul. Disord* 28 (6), 491–501. [PubMed: 29754758]
- Alvarez C, et al. , 2016. Allosteric indole amide inhibitors of p97: identification of a novel probe of the ubiquitin pathway. *ACS Med. Chem. Lett* 7 (2), 182–187.
- Badadani M, et al. , 2010. VCP associated inclusion body myopathy and paget disease of bone knock-in mouse model exhibits tissue pathology typical of human disease. *PLoS One* 5 (10).
- Banerjee S, et al. , 2016. 2.3 Å resolution cryo-EM structure of human p97 and mechanism of allosteric inhibition. *Science* 351 (6275), 871–875. [PubMed: 26822609]
- Bastola P, et al. , 2017. Specific mutations in the D1–D2 linker region of VCP/p97 enhance ATPase activity and confer resistance to VCP inhibitors. *Cell Death Dis.* 3 (1), 17065.
- Blythe EE, et al. , 2019. Multisystem proteinopathy mutations in VCP/p97 increase NPLOC4-UFD1L binding and substrate processing. *Structure* 27 (12), 1820–1829.e4. [PubMed: 31623962]
- Bouwer MF, et al. , 2021. NMS-873 functions as a dual inhibitor of mitochondrial oxidative phosphorylation. *Biochimie* 185, 33–42. [PubMed: 33727138]
- Buchan JR, et al. , 2013. Eukaryotic stress granules are cleared by autophagy and Cdc48/VCP function. *Cell* 153 (7), 1461–1474. [PubMed: 23791177]
- Chalmers MR, Kim J, Kim NC, 2020. Eip74EF is a dominant modifier for ALS-FTD linked mutant VCP phenotypes in *Drosophila*, but not miR-34. *bioRxiv*.
- Chestnut BA, et al. , 2011. Epigenetic regulation of motor neuron cell death through DNA methylation. *J. Neurosci* 31 (46), 16619–16636. [PubMed: 22090490]
- Chimenti MS, et al. , 2015. A fragment-based ligand screen against part of a large protein machine: the ND1 domains of the AAA+ ATPase p97/VCP. *J. Biomol. Screen* 20 (6), 788–800. [PubMed: 25690569]
- Chou T-F, et al. , 2011. Reversible inhibitor of p97, DBE-Q, impairs both ubiquitin-dependent and autophagic protein clearance pathways. *Proc. Natl. Acad. Sci* 108 (12), 4834. [PubMed: 21383145]
- Chou TF, et al. , 2013. Structure-activity relationship study reveals ML240 and ML241 as potent and selective inhibitors of p97 ATPase. *ChemMedChem* 8 (2), 297–312. [PubMed: 23316025]
- Cooney I, et al. , 2019. Structure of the Cdc48 segregase in the act of unfolding an authentic substrate. *Science* 365 (6452), 502–505. [PubMed: 31249134]
- Coppedè F, 2020. Epigenetics of neuromuscular disorders. *Epigenomics* 12 (23), 2125–2139. [PubMed: 33155830]

- Coppedè F, et al. , 2018. Increase in DNA methylation in patients with amyotrophic lateral sclerosis carriers of not fully penetrant SOD1 mutations. *Amyotroph Lateral Scler Frontotemporal Degener* 19 (1–2), 93–101. [PubMed: 28859526]
- Darwich NF, et al. , 2020. Autosomal dominant VCP hypomorph mutation impairs disaggregation of PHF-tau. *Science* 370 (6519).
- Davies JM, Brunger AT, Weis WI, 2008. Improved structures of full-length p97, an AAA ATPase: implications for mechanisms of nucleotide-dependent conformational change. *Structure* 16 (5), 715–726. [PubMed: 18462676]
- De Ridder W, et al. , 2020. Multisystem proteinopathy due to a homozygous p. Arg159His *VCP* mutation. *Neurology* 94 (8), e785. [PubMed: 31848255]
- Dolese DA, et al. , 2021. Degradative tubular lysosomes link pexophagy to starvation and early aging in *C. elegans*. *Autophagy* 1–12.
- Evangelista T, et al. , 2016. 215th ENMC international workshop VCP-related multi-system proteinopathy (IBMPFD) 13–15 November 2015, Heemskerk, *The Netherlands*. *Neuromuscul. Disord.*: NMD 26 (8), 535–547. [PubMed: 27312024]
- Figuerola-Romero C, et al. , 2012. Identification of epigenetically altered genes in sporadic amyotrophic lateral sclerosis. *PLoS One* 7 (12), e52672. [PubMed: 23300739]
- Figuerola-Conchas A, et al. , 2020. Small-molecule modulators of the ATPase VCP/p97 affect specific p97 cellular functions. *ACS Chem. Biol* 15 (1), 243–253. [PubMed: 31790201]
- Forman MS, et al. , 2006. Novel ubiquitin neuropathology in frontotemporal dementia with valosin-containing protein gene mutations. *J. Neuropathol. Exp. Neurol* 65 (6), 571–581. [PubMed: 16783167]
- Freund M, et al. , 2018. The NIH NeuroBioBank: creating opportunities for human brain research. *Handb. Clin. Neurol* 150, 41–48. [PubMed: 29496155]
- Gasior M, Rogawski MA, Hartman AL, 2006. Neuroprotective and disease-modifying effects of the ketogenic diet. *Behav. Pharmacol* 17 (5–6), 431–439. [PubMed: 16940764]
- Gwon Y, et al. , 2021. Ubiquitination of G3BP1 mediates stress granule disassembly in a context-specific manner. *Science* 372 (6549) eabf6548. [PubMed: 34739333]
- Hall EA, et al. , 2017. PLAA mutations cause a lethal infantile epileptic encephalopathy by disrupting ubiquitin-mediated Endolysosomal degradation of synaptic proteins. *Am. J. Hum. Genet* 100 (5), 706–724. [PubMed: 28413018]
- Heidelberger JB, et al. , 2018. Proteomic profiling of VCP substrates links VCP to K6-linked ubiquitylation and c-Myc function. *EMBO Rep.* 19 (4).
- Hill SM, et al. , 2021. VCP/p97 regulates Beclin-1-dependent autophagy initiation. *Nat. Chem. Biol* 17 (4), 448–455. [PubMed: 33510452]
- Hoq MR, et al. , 2021. Affinity capture of p97 with small-molecule ligand bait reveals a 3.6 Å double-hexamer cryoelectron microscopy structure. *ACS Nano* 15 (5), 8376–8385. [PubMed: 33900731]
- Johnson JO, et al. , 2010. Exome sequencing reveals VCP mutations as a cause of familial ALS. *Neuron* 68 (5), 857–864. [PubMed: 21145000]
- Johnson AE, et al. , 2015. VCP-dependent muscle degeneration is linked to defects in a dynamic tubular lysosomal network in vivo. *Elife* 4.
- Johnson AE, et al. , 2021. SVIP is a molecular determinant of lysosomal dynamic stability, neurodegeneration and lifespan. *Nat. Commun* 12 (1), 513. [PubMed: 33479240]
- Ju JS, et al. , 2009. Valosin-containing protein (VCP) is required for autophagy and is disrupted in VCP disease. *J. Cell Biol* 187 (6), 875–888. [PubMed: 20008565]
- Kano O, et al. , 2013. Limb-onset amyotrophic lateral sclerosis patients visiting orthopedist show a longer time-to-diagnosis since symptom onset. *BMC Neurol.* 13 (1), 19. [PubMed: 23394455]
- Kimonis V, 2007. Inclusion body myopathy with paget disease of bone and/or frontotemporal dementia. *GeneReviews®* [Internet] [cited 1993–2021; Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1476/>].
- Kimonis VE, et al. , 2000. Clinical and molecular studies in a unique family with autosomal dominant limb-girdle muscular dystrophy and Paget disease of bone. *Genet. Med* 2 (4), 232–241. [PubMed: 11252708]

- Kimonis VE, et al. , 2008. Clinical studies in familial VCP myopathy associated with Paget disease of bone and frontotemporal dementia. *Am. J. Med. Genet. A* 146a (6), 745–757. [PubMed: 18260132]
- Kirby AE, Kimonis V, Kompoliti K, 2021. Ataxia and parkinsonism in a woman with a VCP variant and long-normal repeats in the SCA2 allele. *Neurol. Genet* 7 (4), e595. [PubMed: 34395867]
- Koerver L, et al. , 2019. The ubiquitin-conjugating enzyme UBE2QL1 coordinates lysophagy in response to endolysosomal damage. *EMBO Rep.* 20 (10), e48014. [PubMed: 31432621]
- Korb MK, Kimonis VE, Mozaffar T, 2021. Multisystem proteinopathy: where myopathy and motor neuron disease converge. *Muscle Nerve* 63 (4), 442–454. [PubMed: 33145792]
- Korb M, et al. , 2022. Development of a standard of care for patients with valosin-containing protein associated multisystem proteinopathy. *Orphanet J Rare Dis* 17 (1), 23. [PubMed: 35093159]
- Kovach MJ, et al. , 2001. Clinical delineation and localization to chromosome 9p13.3–p12 of a unique dominant disorder in four families: hereditary inclusion body myopathy, Paget disease of bone, and frontotemporal dementia. *Mol. Genet. Metab* 74 (4), 458–475. [PubMed: 11749051]
- Llewellyn KJ, et al. , 2014. Lipid-enriched diet rescues lethality and slows down progression in a murine model of VCP-associated disease. *Hum. Mol. Genet* 23 (5), 1333–1344. [PubMed: 24158850]
- Llewellyn KJ, et al. , 2017. Myogenic differentiation of VCP disease-induced pluripotent stem cells: a novel platform for drug discovery. *PLoS One* 12 (6), e0176919. [PubMed: 28575052]
- Martens K, et al. , 2020. Case report: calpainopathy presenting after bone marrow transplantation, with studies of donor genetic content in various tissue types. *Front. Neurol* 11, 604547. [PubMed: 33505349]
- Maxwell Brian A, et al. , 2021. Ubiquitination is essential for recovery of cellular activities after heat shock. *Science* 372 (6549) eabc3593. [PubMed: 34739326]
- Mehta SG, et al. , 2007. APOE is a potential modifier gene in an autosomal dominant form of frontotemporal dementia (IBMPFD). *Genet. Med* 9 (1), 9–13. [PubMed: 17224685]
- Meyer H, Weihl CC, 2014. The VCP/p97 system at a glance: connecting cellular function to disease pathogenesis. *J. Cell Sci* 127 (Pt 18), 3877–3883. [PubMed: 25146396]
- Meyer H, Bug M, Bremer S, 2012. Emerging functions of the VCP/p97 AAA-ATPase in the ubiquitin system. *Nat. Cell Biol* 14 (2), 117–123. [PubMed: 22298039]
- Miao R, et al. , 2020. An ECM-to-nucleus signaling pathway activates lysosomes for *C. elegans* larval development. *Dev. Cell* 52 (1), 21–37.e5. [PubMed: 31735670]
- Morrow JM, et al. , 2018. Validation of MRC Centre MRI calf muscle fat fraction protocol as an outcome measure in CMT1A. *Neurology* 91 (12), e1125–e1129. [PubMed: 30120135]
- Nalbandian A, et al. , 2012. The homozygote VCP(R¹⁵⁵H/R¹⁵⁵H) mouse model exhibits accelerated human VCP-associated disease pathology. *PLoS One* 7 (9), 1–10.
- Nalbandian A, et al. , 2013. A progressive translational mouse model of human valosin-containing protein disease: the VCP(R155H/+) mouse. *Muscle Nerve* 47 (2), 260–270. [PubMed: 23169451]
- Nalbandian A, et al. , 2015. Targeted excision of VCP R155H mutation by Cre-LoxP technology as a promising therapeutic strategy for valosin-containing protein disease. *Hum Gene Ther Methods* 26 (1), 13–24. [PubMed: 25545721]
- Neumann M, et al. , 2007. TDP-43 in the ubiquitin pathology of frontotemporal dementia with VCP gene mutations. *J. Neuropathol. Exp. Neurol* 66 (2), 152–157. [PubMed: 17279000]
- Palese F, et al. , 2019. Predictors of diagnostic delay in amyotrophic lateral sclerosis: a cohort study based on administrative and electronic medical records data. *Amyotroph Lateral Scler Frontotemporal Degener* 20 (3–4), 176–185. [PubMed: 30656971]
- Pan M, et al. , 2021. Mechanistic insight into substrate processing and allosteric inhibition of human p97. *Nat. Struct. Mol. Biol* 28 (7), 614–625. [PubMed: 34262183]
- Papadopoulos C, et al. , 2017. VCP/p97 cooperates with YOD1, UBXD1 and PLAA to drive clearance of ruptured lysosomes by autophagy. *EMBO J.* 36 (2), 135–150. [PubMed: 27753622]
- Paunovska K, et al. , 2018a. A direct comparison of in vitro and in vivo nucleic acid delivery mediated by hundreds of nanoparticles reveals a weak correlation. *Nano Lett.* 18 (3), 2148–2157. [PubMed: 29489381]

- Paunovska K, et al. , 2018b. Analyzing 2000 in vivo drug delivery data points reveals cholesterol structure impacts nanoparticle delivery. *ACS Nano* 12 (8), 8341–8349. [PubMed: 30016076]
- Paunovska K, et al. , 2019. Nanoparticles containing oxidized cholesterol deliver mRNA to the liver microenvironment at clinically relevant doses. *Adv. Mater. (Deerfield Beach, Fla.)* 31 (14), e1807748.
- Penno A, et al. , 2010. Hereditary sensory neuropathy type 1 is caused by the accumulation of two neurotoxic sphingolipids*◆. *J. Biol. Chem* 285 (15), 11178–11187. [PubMed: 20097765]
- Ping L, et al. , 2020. Global quantitative analysis of the human brain proteome and phosphoproteome in Alzheimer's disease. *Sci. Data* 7 (1), 315. [PubMed: 32985496]
- Posey JE, et al. , 2017. Resolution of disease phenotypes resulting from multilocus genomic variation. *N. Engl. J. Med* 376 (1), 21–31. [PubMed: 27959697]
- Rahit KMT, Tarailo-Graovac M, 2020. Genetic modifiers and rare mendelian disease. *Genes* 11 (3).
- Ritz D, et al. , 2011. Endolysosomal sorting of ubiquitylated caveolin-1 is regulated by VCP and UBXD1 and impaired by VCP disease mutations. *Nat. Cell Biol* 13 (9), 1116–1123. [PubMed: 21822278]
- Roux B, et al. , 2021. Targeting acute myeloid leukemia dependency on VCP-mediated DNA repair through a selective second-generation small-molecule inhibitor. *Sci. Transl. Med* 13 (587).
- Sago CD, et al. , 2018. High-throughput in vivo screen of functional mRNA delivery identifies nanoparticles for endothelial cell gene editing. *Proc. Natl. Acad. Sci. U. S. A* 115 (42), E9944–E9952. [PubMed: 30275336]
- Saric A, et al. , 2016. mTOR controls lysosome tubulation and antigen presentation in macrophages and dendritic cells. *Mol. Biol. Cell* 27 (2), 321–333. [PubMed: 26582390]
- Stoccoro A, et al. , 2018. Mitochondrial DNA copy number and D-loop region methylation in carriers of amyotrophic lateral sclerosis gene mutations. *Epigenomics* 10 (11), 1431–1443. [PubMed: 30088417]
- Stoccoro A, et al. , 2020. Reduced mitochondrial D-loop methylation levels in sporadic amyotrophic lateral sclerosis. *Clin. Epigenetics* 12 (1), 137. [PubMed: 32917270]
- Tarr IS, et al. , 2019. Monozygotic twins and triplets discordant for amyotrophic lateral sclerosis display differential methylation and gene expression. *Sci. Rep* 9 (1), 8254. [PubMed: 31164693]
- Tremolizzo L, et al. , 2014. Whole-blood global DNA methylation is increased in amyotrophic lateral sclerosis independently of age of onset. *Amyotroph Lateral Scler Frontotemporal Degener* 15 (1–2), 98–105. [PubMed: 24224837]
- Tresse E, et al. , 2010. VCP/p97 is essential for maturation of ubiquitin-containing autophagosomes and this function is impaired by mutations that cause IBMPFD. *Autophagy* 6 (2), 217–227. [PubMed: 20104022]
- van den Boom J, Meyer H, 2018. VCP/p97-mediated unfolding as a principle in protein homeostasis and signaling. *Mol. Cell* 69 (2), 182–194. [PubMed: 29153394]
- Vesa J, et al. , 2009. Valosin containing protein associated inclusion body myopathy: abnormal vacuolization, autophagy and cell fusion in myoblasts. *Neuromuscul. Disord* 19 (11), 766–772. [PubMed: 19828315]
- Wall JM, et al. , 2021. CRISPR/Cas9-engineered *Drosophila* knock-in models to study VCP diseases. *Dis. Model. Mech* 14 (7) dmm048603. [PubMed: 34160014]
- Wang F, et al. , 2020. Allosteric p97 inhibitors can overcome resistance to ATP-competitive p97 inhibitors for potential anticancer therapy. *ChemMedChem* 15 (8), 685–694. [PubMed: 32162487]
- Wang F, et al. , 2021. Temporal proteomics reveal specific cell cycle oncoprotein downregulation by p97/VCP inhibition. *Cell. Chem. Biol* 29, 517–529. [PubMed: 34847375]
- Watts GD, et al. , 2004. Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. *Nat. Genet* 36 (4), 377–381. [PubMed: 15034582]
- Weihl CC, et al. , 2008. TDP-43 accumulation in inclusion body myopathy muscle suggests a common pathogenic mechanism with frontotemporal dementia. *J. Neurol. Neurosurg. Psychiatry* 79 (10), 1186–1189. [PubMed: 18796596]

- Weiss L, et al. , 2021. Ceramide contributes to pathogenesis and may be targeted for therapy in VCP inclusion body myopathy. *Hum. Mol. Genet* 29 (24), 3945–3953. [PubMed: 33410456]
- Westeneng HJ, et al. , 2018. Prognosis for patients with amyotrophic lateral sclerosis: development and validation of a personalised prediction model. *Lancet Neurol.* 17 (5), 423–433. [PubMed: 29598923]
- Wingo AP, et al. , 2021. Integrating human brain proteomes with genome-wide association data implicates new proteins in Alzheimer’s disease pathogenesis. *Nat. Genet* 53 (2), 143–146. [PubMed: 33510477]
- Young PE, et al. , 2017. Epigenetic differences between monozygotic twins discordant for amyotrophic lateral sclerosis (ALS) provide clues to disease pathogenesis. *PLoS One* 12 (8), e0182638. [PubMed: 28797086]
- Zhang T, et al. , 2017. Valosin-containing protein (VCP/p97) inhibitors relieve Mitofusin-dependent mitochondrial defects due to VCP disease mutants. *Elife* 6.
- Zhang G, et al. , 2021. A covalent p97/VCP ATPase inhibitor can overcome resistance to CB-5083 and NMS-873 in colorectal cancer cells. *Eur. J. Med. Chem* 213, 113148. [PubMed: 33476933]
- Zhou H-J, et al. , 2015. Discovery of a first-in-class, potent, selective, and orally bioavailable inhibitor of the p97 AAA ATPase (CB-5083). *J. Med. Chem* 58 (24), 9480–9497. [PubMed: 26565666]

Table 1

List of p97 small molecule inhibitors and mechanism of action. Please see associated references for full details.

Small Molecule Inhibitor	Mechanism of p97 inhibition	Type	Reference
DBeQ	Reversible, ATP-competitive inhibitor	Quinazoline	72
ML-240/241	Reversible, ATP-competitive inhibitor	Quinazoline	79
CB-5083	Reversible, ATP-competitive inhibitor	Quinazoline	80
CB-5339	Reversible second generation ATP-competitive inhibitor. In Phase 1 clinical trials (NCT04402541)	Quinazoline	75
PPA/ACJI-99C	Irreversible, active site covalent inhibitor	pyrazolo[3,4- <i>d</i>] pyrimidine	81
UPCDC-30245	Allosteric inhibitor	Phenylindole	76
NMS-873	Allosteric-non competitive inhibitor	Triazole	84

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