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Review

Drugging DNA Damage Repair Pathways for Trinucleotide Repeat Expansion Diseases

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Abstract. DNA damage repair (DDR) mechanisms have been implicated in a number of neurodegenerative diseases (both genetically determined and sporadic). Consistent with this, recent genome-wide association studies in Huntington's disease (HD) and other trinucleotide repeat expansion diseases have highlighted genes involved in DDR mechanisms as modifiers for age of onset, rate of progression and somatic instability. At least some clinical genetic modifiers have been shown to have a role in modulating trinucleotide repeat expansion biology and could therefore provide new disease-modifying therapeutic targets. In this review, we focus on key considerations with respect to drug discovery and development using DDR mechanisms as a target for trinucleotide repeat expansion diseases. Six areas are covered with specific reference to DDR and HD: 1) Target identification and validation; 2) Candidate selection including therapeutic modality and delivery; 3) Target drug exposure with particular focus on blood-brain barrier penetration, engagement and expression of pharmacology; 4) Safety; 5) Preclinical models as predictors of therapeutic efficacy; 6) Clinical outcome measures including biomarkers.

Keywords: Huntingtin (HTT), CAG repeat, polyglutamine (polyQ), somatic instability, mismatch repair (MMR), PARP, ATM

INTRODUCTION

A critical goal of biomedical research is to translate basic research findings into useful therapeutics. Regardless of the indication, there are several requirements in successfully identifying new treatments. First, we need a detailed knowledge of the natural history of the disease together with good understanding of underlying disease mechanisms. This informs selection of a molecular target and generation of a testable hypothesis on the target role in disease pathophysiology. A focussed drug discovery programme then goes through iterative rounds of optimisation to ultimately identify a candidate which satisfies a number of criteria such as appropriate metabolic stability for a practical dosing regimen, target engagement and dose-dependent efficacy in the disease model(s), together with a sufficient degree of animal safety. Carefully designed, tightly regulated clinical trials are initiated to determine the safety and efficacy of the new drug. This well-trodden path has been followed with respect to drug development for CNS diseases and yielded many successes in 1980s and 1990s. Relatively few new mechanisms have since been approved, especially for neurodegenerative disorders despite sustained investment. Many approved CNS treatments for neurodegenerative diseases (e.g., tetrabenazine, acetylcholinesterase inhibitors, riluzole)

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are palliative and provide temporary symptomatic relief in the face of the inexorable progress in the degeneration of remaining circuitry [1, 2]. These failures have curtailed the enthusiasm of pharmaceutical companies to undertake novel drug discovery in neurodegenerative diseases using their internal resources. Over the last decade many large companies have opted to 'externalise' their neuroscience R&D, which essentially means they wait to license in therapies that have already been advanced to a reasonable stage by others [3].

CNS drug discovery is evidently challenging, and at least partly driven by relatively poor understanding of disease pathophysiology and function of the brain compared with many other target organ systems [2, 4, 5]. The billions of highly interconnected, distinct cell types (predominantly but not limited to neuronal, glial and circulatory systems including vasculature) with numerous functional regional differences is inherently complex and not well understood. Similar disease phenotypes can result from very different underlying pathophysiologies as seen in Alzheimer's disease, frontotemporal dementia, amyotrophic lateral sclerosis, and Parkinson's disease [6–8]. Furthermore, the brain is a protected organ, and accordingly, entry of small molecule therapeutics into the CNS can be limited [9]. The difference in exposure levels in the systemic circulation versus the CNS can lead to reduced safety margins over adverse pharmacology and toxicity in peripheral systems. Indeed, toxicity is a major challenge for a disease requiring chronic treatment, although the levels of acceptable risk/benefit may be greater in diseases where the outcome is dire, and no effective treatments exist. In addition to peripheral toxicology, CNS therapies can often produce adverse pharmacological effects (e.g., dizziness, somnolence, cognitive impairment, nausea etc) that are difficult to predict from preclinical models. Such adverse effects (even if not toxic in the usual sense) can lead to tolerability issues, limiting dosing and ultimately terminate development of that drug [10].

Huntington's disease (HD) is in many ways a very attractive disease for CNS drug discovery and development because it is a comparatively common, monogenetic disorder that is increasingly well understood [11]. There are disease-modifying approaches being actively evaluated such as direct targeting of the expanded repeat at the DNA or RNA level, or the mutant protein [12–16]; which holds more promise than targeting other pathophysiological sequelae, at least some of which may be collateral damage rather than critical to the disease process [2, 11]. The causative mutation for HD, an expanded trinucleotide repeat sequence in the first exon of *HTT* is naturally polymorphic and indisputably associated with disease phenotypes such as age of motor onset above 39 CAG repeats [11]. Repeat length is the main driver for disease onset and progression, with longer repeats being associated with worsening prognosis. We also know that CAG repeats are somatically and intergenerationally unstable; with a bias toward expansion in a length, time, and tissue-dependent fashion (Fig. 1) [17–20]. Therefore, individuals with rapidly expanding repeats are more likely to have an earlier onset and faster progression associated with the higher mutation burden. Repeat stability (or lack of) has been shown experimentally to be mediated by proteins that function in DNA damage repair (DDR) processes [15, 16]. Pleasingly, human genetics data has indicated that variants in DDR proteins are associated with clinically relevant HD symptomatology including age at motor onset, rate of progression and somatic instability [21, 22]. This raises the tantalising potential for a novel disease modifying therapeutic: by modulating the function of such DDR proteins; could one significantly delay the onset of disease symptoms and slow the rate of progression through prevention of somatic expansion and increased mutational burden over time? Interestingly, many DDR proteins have been shown to be druggable, albeit in an oncology context [23]; indeed, very few DDR targets have been interrogated in a neurological disease context.

In this article, we consider how we can apply what we have learnt from historic approaches to neurodegenerative disease drug discovery to HD and to DDR targets. We will focus on a set of important determinants identified in a retrospective analysis by Astra Zeneca which were echoed as critical by other large pharmaceutical companies such as Pfizer [24, 25]. This can be summarised as the right target, the right molecule, the right tissue, the right safety, the right patient, and the right commercial potential. In the next six sections, we will consider the first five of these along with the right animal models and highlight them in the context of DDR and HD (though many points made here will be broadly applicable to other repeat expansion disorders).

THE RIGHT TARGET

Target selection is one of the key decision points in the drug discovery process. It is obvious that

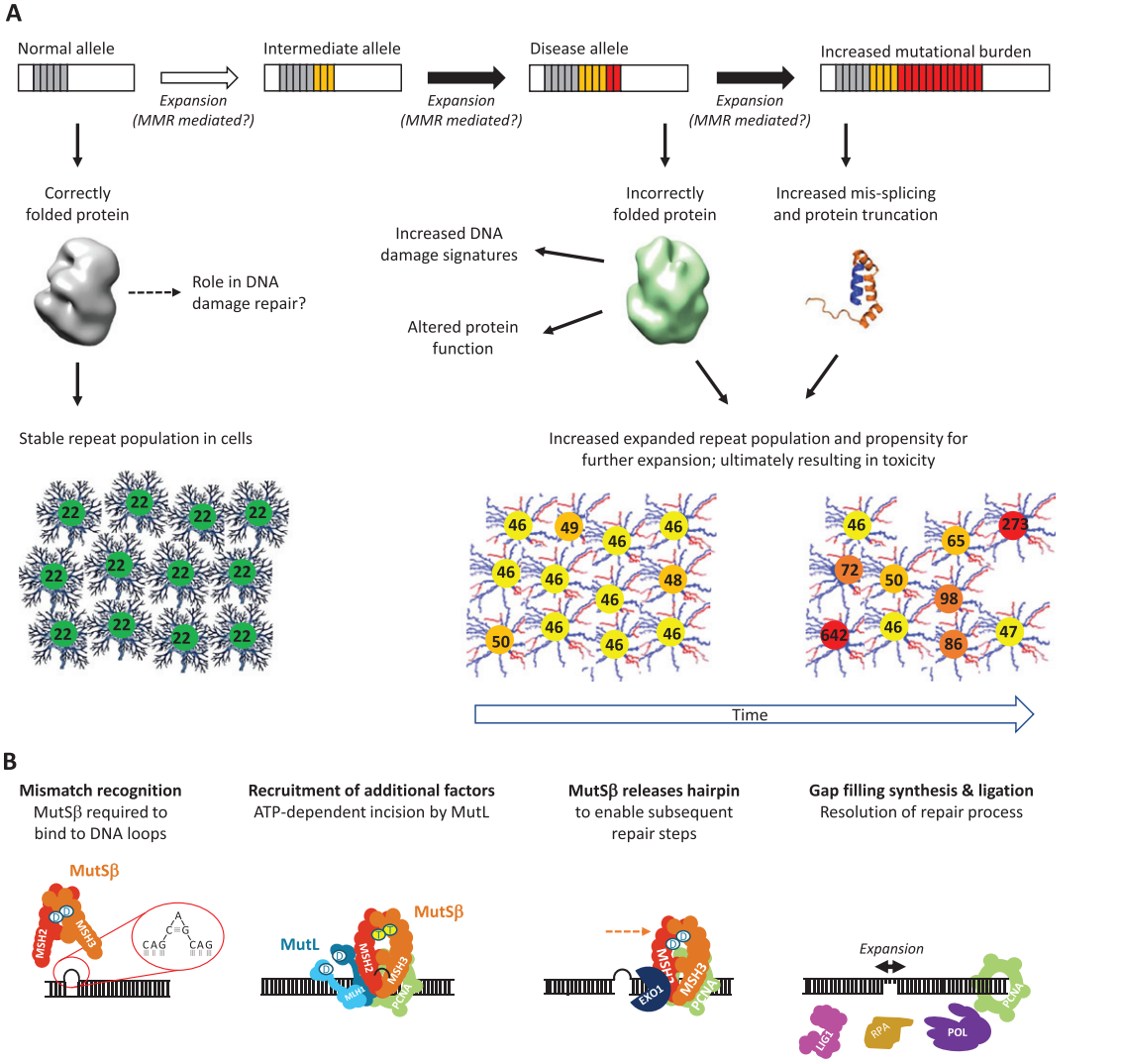

Fig. 1. Mechanisms of DNA damage and repair contributing to somatic and intergenerational expansion of the HTT CAG repeat is a critical aspect of HD pathophysiology. A) CAG repeats (grey boxes, 1 box = 4 CAG repeats; 20 CAG repeats shown) in normal HTT alleles are translated into a correctly folded protein (illustration from [117]) and are stable in cellular populations, represented below in schematic of striatal neurons (polyglutamine repeat lengths, assuming canonical allele configuration, are indicated in green circles). As the repeat sizes increase, there is increased propensity for somatic and/or intergenerational instability, with a notable bias toward expansion in a time- and tissue- dependent fashion; as represented by the second repeat schematic depicting intermediate alleles (grey and yellow boxes, 1 box = 4 CAG repeats; 32 CAG repeat shown). Disease associated repeat alleles (grey, yellow and red boxes, 1 box = 4 CAG repeats; 44 CAG repeats are shown) are translated into a protein that is incorrectly folded and accordingly has altered functionality. Importantly, the increased disease-associated repeats are more unstable at the DNA level which in turn increases the instability further, triggering additional pathophysiological sequelae including mis-splicing and premature truncation of the protein as a toxic N-terminal fragment (illustration from [118]). The overall mutational burden drastically increases over time; expansion in an individual cell is a stochastic event such that cells within a population may have vastly differing repeat lengths. This is illustrated in the schematic with impaired striatal neurons (polyglutamine repeat length numbers are in yellow/orange/red circles) ultimately progressing to toxicity and cell deaths (absence of cells within the population). Experimental data points to DDR pathways such as MMR as being critical for driving the repeat expansions. B) Summary of MMR pathway is illustrated as a sequence of steps where MutSβ is required for initial recognition of the mismatched DNA substrate (perhaps due to polymerase errors). MutSβ (MSH2 in red and MSH3 in orange) in turn mediates recruitment of additional factors including MutL heterodimers (blue) and other proteins including EXO1 (navy) and PCNA (green) to mediate the repair process. The detail of how this goes awry is unclear, but one mechanism could include MutL-mediated incision on the opposite strand of the additional CAG nucleotides [121] and subsequent gap-filling synthesis and ligation. Only a few proteins are shown for simplicity including LIG1 (pink), RPA (brown) and a polymerase (purple). Similarly, a gap of one trinucleotide repeat unit is shown here for simplicity. It is likely that this process occurs multiple times with single trinucleotide repeat unit "bubbles" which ultimately contributes to expansion of the repeats. Key: D in white circles = ADP; T in yellow circles = ATP.

selection of a biological target for a drug discovery programme is a critical decision: the most potent and safest molecule will do nothing if it is targeting something that has no relevance to the disease cascade. Confidence in target selection is derived from a range of evidence including direct linkage to human disease (typically through profiling technologies), an understanding of the biology underpinning the target and/or disease aetiology and demonstrating efficacy in animal models of disease. An ideal drug target is disease modifying and/or has a proven function in pathophysiology; modulation will have little impact in normal, non-disease physiology; expression profile is aligned with disease relevant processes; a target/disease-specific biomarker exists to monitor therapeutic efficacy; and the target should be amenable to pharmacological modulation by one or more of the common drug modalities: small molecules, antibodies and increasingly antisense oligonucleotides (ASOs) and gene therapies.

Over two decades of research has identified many common cellular pathway dysfunctions shared between HD and other neurodegenerative diseases (e.g., aggregation, autophagy, trafficking, mitochondrial dysfunction, expression changes, altered protein-interaction networks); at least some of which indicate effects that are further downstream from the initial disease trigger of an expanded trinucleotide repeat sequence [11]. Unfortunately, drug discoverers are sometimes guilty of selecting targets that are the most obvious or easiest to drug, rather than thinking about which targets are furthest upstream in the disease cascade in humans. Examples relevant to HD include modulation of aggregation or repurposing of existing PDE10A inhibitors, which have not been successful to date [2, 26, 27]. Arguably, the best target(s) are proximal to the causative mutation and hence would include the mutation itself (at the DNA, RNA, or mutated protein level) in addition to the proteins that directly modulate the mutation. For balance, we should however mention that a number of approaches seeking to modulate beta-amyloid in Alzheimer's disease have not been successful thus far though there are a number of reasons that could underpin this [28, 29].

The application of genome wide association (GWA) approaches to HD has been particularly informative with respect to novel therapeutic target identification with potential for clinically relevant impact in patients [20, 30–34]. These data have highlighted the critical role of DDR proteins which in turn links in with observations on trinucleotide stability (both intergenerational and somatic) and lead to a more comprehensive understanding of disease progression in a dynamic and temporal manner. In other words, target identification is being informed by human data, which increases the confidence that such drug targets are valid. We view the relationship between DDR and the *bona fide* cause of the disease (the trinucleotide repeat sequence) as highly compelling especially as these variant associations are not limited to HD and have been observed in the context of other trinucleotide repeat expansion diseases [35–39]. The observation that genetic inactivation of mismatch repair (MMR) pathway genes eliminates the somatic instability of trinucleotide repeats in disease models is in contrast to the increased di- and tetranucleotide instability typically associated with these genes although it could be argued that we are lacking a full characterisation of microsatellites in the human genome [40–42].

While the convergence of variant linkage on DDR pathways is compelling, the observed variants may not necessarily identify the best target for a drug discovery project. Variants need to be sufficiently frequent to enable detection within the population under study and furthermore, these variants need to have a measurable impact on expression levels or function. A GWA locus does not identify the genes nor the causal alleles: a relevant example here is rs557874766 which is associated with both *MSH3* and *DHFR* [30, 31, 43–45]. In addition, clinically relevant associations of variants in genes encoding drug targets do not predict the effect of modifying the same targets pharmacologically. For example, variants indicating increased expression of a target as being protective as is the case with *FAN1* does not automatically suggest agonism of the same target as the most parsimonious strategy; the nuclease-dependent function of FAN1 may not be relevant in the context of trinucleotide repeat expansion [46, 47]. Finally, while the MMR pathway has been highlighted through this approach, this does not negate the potential validity of other DDR pathways and targets [36, 48, 49]. Hence, a key outstanding question is "what is the best target in the 'DDR-ome' with respect to HD and other repeat expansion disorders for drug discovery?"

In addition to identifying new targets, we need to prioritise and validate them. The process of selecting new targets needs to include an evaluation of the risks associated with the mechanism under investigation. Many potential drug targets may be efficacious but also cause unacceptable toxicity or adverse effects.

Robust target validation is required in order to test the hypothesis that a particular molecular target is significant or even causative for disease pathophysiology; this can sometimes be comparatively straightforward (e.g., when drugs for a given target already exist) or extremely difficult (e.g., when the function of a disease target is virtually unknown). The only way to be certain that the target is instrumental in a given disease is to test the hypothesis in humans – which is usually impractical at the outset. Hence, preclinical target validation is necessary before committing to the lengthy and costly process of seeking suitable molecules against a given target (typically ∼12 years and ∼ US $1 billion to develop a drug from concept to approved therapy [50]). Validation activities include expression profiling, ideally in disease relevant cells/tissues/models, manipulation of the target in disease models with genetic tools and ideally complemented with pharmacological tools and functional analyses. So-called 'killer experiments' (i.e., experiments that show a target is not suitable) are advocated rather than experiments designed to provide more affirmative data. Killer experiments efficiently rule out targets with low probability of success, enable more focus on appropriate targets and reduce attrition in later stages when much more time, effort and money have been expended.

Pleasingly, in addition to human clinical genetic data, confidence in target selection in the context of DDR pathways has already been augmented by preclinical studies in HD models [51, 52]. Importantly, manipulation of specific MMR targets in mouse models have clearly demonstrated impacts on repeat stability profiles [44, 53–56]. Somatic and intergenerational instability have been observed in models and in clinical and/or post mortem samples in a time-, length- and tissue-dependent fashion [17–19, 33, 57–61]. These observations were performed prior to the GWA findings and showed concordance in that key pathways such as MMR were confirmed. Furthermore, the "directionality" of such manipulations has been consistent with respect to impact in models and clinical outcomes.

Taken together, the collective data link human genetic variants in DDR pathways with clinically relevant disease outputs for multiple repeat expansion diseases and highlight repeat instability as a key pathophysiological mechanism. This in turn gives us an exciting opportunity for selecting novel targets with genuine disease modifying potential and suitable for small molecule drug discovery.

THE RIGHT MOLECULE

Once the target is selected, the next big challenge is to find the right therapeutic to take into clinical development. This section is written in the context of selection of the right small molecule, though similar considerations apply to other therapeutic agents such as antibodies and ASOs. Discovering molecules with high affinity for the target is the most obvious challenge in drug discovery but is only one part of a much larger multi-parameter challenge. A suitable drug candidate is chosen based on having the right balance of properties across these key areas:

1. High affinity for the target to optimise pharmacological effects against many other factors;
2. Sufficient selectivity for the target to minimise adverse or toxic effects from other related targets;
3. Acceptable absorption and distribution, such that enough drug can reach the brain for pharmacological effect;
4. Sufficiently slow clearance from the body for a practical dosing regimen;
5. Acceptable safety profile leading to positive benefit versus risk outcome for the target patient population.

A drug discovery project requires a large multidisciplinary team to work on optimising all of these parameters more or less in parallel to ultimately select the best candidate molecule. Identifying high affinity molecules, typically in the low nanomolar or even sub-nanomolar range is one of the highest priority goals as this tends to help achieve some of the other required properties. Very high affinity for the desired target often increases the selectivity against similar targets in the same class/family because subtle differences in the shape of the binding site will be amplified for high affinity molecules. Additionally, a much lower concentration of a potent molecule is required to achieve its pharmacological effect, resulting in more flexibility in some of the absorption, distribution, metabolism, and excretion (collectively known as ADME) properties. A lower concentration also reduces the probability of 'off-target' safety issues that we will discuss in the section "The Right Safety Profile". Finally, there are also practical benefits: a more potent molecule requires a smaller dose (all other properties being equal), making it easier to formulate into a sensibly sized tablet and potentially reducing the cost of goods to manufacture

it. Drug affinity can be determined by binding or functional assays and both are usually employed at different points in the drug discovery process. Binding assays have the benefit of being relatively simple and high throughput and can be applied to fragments (very small molecules that are then combined to make more drug-like molecules) in addition to more drug-like molecules. When combined with structural information from crystallography or cryo-electron microscopy this can greatly aid the affinity optimisation and inform which changes can be tolerated whilst improving other parameters such as metabolic stability [62, 63]. Functional assays are usually more complex but provide valuable information that the molecule is having the desired pharmacological effect in addition to just binding to the target.

The various ADME properties of drugs are critical to their success in clinical development and can be a major reason for failure in clinical trials [64]. Over the last two decades great improvements have been made in our ability to measure many ADME properties *in vitro* and to predict human parameters with much better accuracy. A detailed discussion of ADME optimisation for CNS drugs is beyond the scope of this article but has been covered elsewhere [65, 66]. Small molecule drugs are usually intended for oral administration and therefore ensuring sufficient absorption from the gut is necessary. Metabolism of small molecules in the liver followed by excretion in the kidneys is the primary mechanism of removal from the body. Both cell-free and cell-based assays exist to examine the contribution of the various enzymes (chiefly cytochrome P450 subtypes) to these processes. Medicinal chemists are well versed in strategies to block metabolism at specific points within the structure of drug molecules to improve stability and therefore enhance overall pharmacokinetics of lead molecules. Biologics such as antibody therapies are dosed either intravenously or subcutaneously and so bypass issues of gut absorption, albeit at the detriment of convenient dosing for patients. Newer modality drugs such ASOs and gene therapies are typically dosed either intrathecally or via stereotaxic injection into the brain, again avoiding gut absorption but requiring specialist surgical facilities.

THE RIGHT TISSUE

To treat a neurodegenerative disease like HD, the right tissue is almost certainly going to include the brain, which represents a challenge for all the different therapeutic modalities. The brain is separated from the rest of the body by the blood-brain barrier (BBB) which is composed of capillary endothelium containing tight junctions and specific transporter systems to exclude pathogens and toxins, which unfortunately can also include therapeutic drugs [67]. There are other distinct compartments (retina, CSF) which also have their own barriers. The BBB is essentially impenetrable for viral-vector gene therapies and ASOs and hence stereotaxic injection and intrathecal dosing are required, respectively. These dosing routes create a concentration gradient of the therapeutic across different parts of the brain, a particular problem when the target brain nucleus is a deep structure such as the caudate putamen for HD. Antibody therapies do penetrate the brain to a minor extent, approximately 0.1–0.3%, and have been shown to have extracellular pharmacological effects, for example in lowering amyloid beta deposits levels in the brains of Alzheimer's patients [68, 69]. In the context of DDR for HD, antibodies are unlikely to be successful as the DDR targets are intracellular and predominantly confined to the nucleus. In contrast, small molecule drugs have a proven track record of CNS penetration across different brain regions, but they need to be designed with certain properties to avoid the BBB's efflux transporters and be permeable enough to traverse the endothelial membranes. Medicinal chemists working in CNS drug discovery have developed and refined a set of guidelines which enhance the probability of success, although ultimately penetrance still needs to be demonstrated empirically. These guidelines relate to physicochemical properties such as preferred values for molecular weight, lipophilicity, topological polar surface area (TPSA) and hydrogen bond donor (HBD) count (Table 1). Oral CNS drugs are generally smaller in size and more lipophilic compared with oral non-CNS drugs and to possess lower TPSA and fewer HBDs—with size and HBD capacity reported as being more critical [66, 70, 71]. Building upon this, medicinal chemists at Pfizer developed a CNS multiparameter optimization (MPO) to increase the probability of suitable compound identification on the basis of a set of six physicochemical properties: 1) lipophilicity using calculated partition coefficient (cLogP); 2) calculated distribution coefficient at pH = 7.4 (cLogD); 3) molecular weight (MW); 4) topological polar surface area (TPSA); 5) number of hydrogen bond donors (HBD); 6) most basic centre (pKa) [66]. Marketed CNS drugs are more

Table 1
Medicinal chemical properties of clinically precedented PARP, ATR and ATM inhibitors

Mechanism	Compound	MW	TPSA	HBD	HBA	CNS MPO	Evidence of CNS penetration
PARP	Olaparib	434	82	1	4	5.3	No blood brain barrier penetration in mice [119]
	Rucaparib	323	57	3	2	4.5	Restricted access to CNS [120]
	Niraparib	320	73	2	3	4.5	Reported to cross blood brain barrier in mice [119]
	Talazoparib	380	84	2	5	5.3	
	Veliparib	244	84	3	3	4.5	Brain penetrant in rat PK [82]
ATR	AZD6738	412	108	2	7	4.4	Activity in glioblastoma model and confirmed CNS penetration [122]
	M6620 (berzosertib)	463	124	2	7	3.1	
	M4344	542	134	2	9	3.5	
	BAY1895344	375	85	1	6	5.5	
ATM	AZD1390	478	62	0	5	3.9	$K_{p,uu}$ 0.33 cynomolgus macaque (PET data), in glioblastoma clinical trials [76]
	AZD0156	462	71	0	6	4.7	Reported as 6-7 fold less CNS penetrant than AZD1390
	Cpd 17 HD ATM ref	405	42	1	4	3.5	Mouse $K_{p,uu}$ 0.44 [78]

The CNS physicochemical property space is represented in these tables with respect to several parameters which intercalate with each other. These parameters are: 1) MW = molecular weight, molecules less than <450 are more likely to be able to penetrate the blood brain barrier through free diffusion. 2) TPSA = topological polar surface area, the surface sum over all polar atoms, which influences BBB permeability, upper limit is estimated between 60–90 $Å^2$. 3) HBD and HBA = hydrogen bond donor and acceptor respectively, increased hydrogen binding decreases BBB penetration, so 0-1 HBDs are preferred. 4) CNS MPO = Central Nervous System MultiParameter Optimisation, an algorithm to score compounds with respect to CNS penetration and is built on six physicochemical properties: MW, TPSA, HBD, cLogP (measure of hydrophilicity), cLogD (measure of lipophilicity), and pKa (acidic strength). Higher scores >5 being deemed to be more likely to be CNS penetrant as determined by unbound partition coefficient (Kpu,u). No single parameter can be used to explain or predict the pharmacokinetic properties related to brain exposure and there are several exceptions to the "preferred range" guidelines. This drives the need to empirically determine CNS penetration. It can be clearly seen from the table that predicting CNS exposure is not straightforward: AZD0156 has a higher MPO score but reportedly lower CNS penetration than AZD1390.

likely to show higher scores (range of 4–6) with this algorithm.

Brain penetration and therefore exposure at the site of action is an key pharmacokinetic (PK) property an efficacious drug must possess, but industry analysis of clinical attrition found that two other fundamental PK/pharmacodynamic (PD) principles are also required: binding to the pharmacological target as expected for its mode of action and expression of functional pharmacological activity commensurate with the demonstrated target exposure and binding [25]. Although all three may seem fairly obvious, actually measuring these in patients with CNS diseases is far from trivial. Lack of data on these three principles not only increased the probability of a drug failing in clinical trials, but often made it difficult to decide whether the hypothesis was adequately tested.

Demonstration of free drug exposure at the target at levels that exceeds pharmacological potency over the required period of time increases the confidence that adequate exposure has been achieved to test the hypothesis. Drugs cannot typically be sampled from the human brain for PK measurement during clinical assessment. This is particularly pertinent for approaches seeking to modulate the genetic sequence as it is challenging to ascertain whether the sequence has been appropriately modulated in every cell without destroying that cell [14, 15]. Using preclinical animal models (typically rodents such as mice and rats but other species can be used including dogs or primates) as a predictor for human brain exposure levels is not always straightforward due to differences in BBB permeability, drug metabolising enzymes and transporters. Nonetheless, both *in vitro* and *in vivo* model assays and systems are utilised to measure the many different parameters to optimise and integrate into a coherent model of brain penetration and distribution. It is worth noting here that measurement of whole brain drug concentrations alone can be misleading as the majority of the drug is usually bound to proteins, whereas the unbound drug concentration in the brain better represents the concentration of drug actually available to bind to the target, especially for cell surface targets. In contrast when drugging the DDR for repeat expansion diseases, we also need to consider the predominantly nuclear site of the target proteins. In other words, the unbound intracellular drug concentration may have more relevance than unbound extracellular drug concentration assuming these are in equilibrium with each other [72].

Target occupancy is a prerequisite for expression of pharmacology and target modulation. Target

occupancy and binding affinity are related in that the latter is an *in vitro* measure of the concentration of ligand resulting in drug-target complex formation while occupancy is the proportion of targets that have formed a complex relative to the total number of targets *in vivo*. Direct evidence can be gained from PK/PD studies of *in vivo* occupancy measurements with positron emission tomography (PET) or radiolabelled ligands. The use of the PDE10A PET ligand in HD patients is an excellent example of a CNS-penetrant ligand that not only informed of target occupancy with the drug candidate but also illuminated the pronounced decrease in expression levels in prodromal stages [73]. However, it can be technically challenging to develop a suitable PET ligand and furthermore PET ligands often take as long and cost as much as the main drug project to develop. One can also consider indirect evidence such as good understanding of binding properties and potency against the target including potential impact of species differences, polymorphisms, alternative isoforms, or other relevant target phenotypes. Feeding into target occupancy considerations are target binding kinetics which include on- and off-rates of drugs [74]. This has been applied to the design of identifying kinase inhibitors with slow off-rates from the target as a means of mitigating potency shifts arising from high cellular ATP concentrations and selectivity concerns.

Functional modulation of the target is necessary—something that binds but has no pharmacological effect will not be efficacious. The use of biomarkers that reflect the primary pharmacology gives the highest level of confidence and direct evidence that sufficient levels of target modulation is being achieved. This is where we anticipate the greatest challenges lie with respect to DDR targets for HD, particularly in earlier phases of clinical development. Direct measurement of primary pharmacology related to target function may be better assessed using peripheral surrogates such as PBMCs; this could include disease-relevant outputs such as somatic instability measures in addition to target-relevant pharmacodynamic measures. This, together with an understanding of exposure/occupancy levels is required to drive these measurable outcomes and can be incorporated into CNS-relevant calculations.

More recent CNS drug discovery comprising "non-traditional" neurodegenerative disease targets such as kinases have indicated that there is potential for optimism: there are now examples showing it is possible to generate molecules that fall within favourable CNS property space [75]. Few therapeutics developed against DDR targets for oncology indications have been developed with CNS penetration in mind (Table 1). Published data with AZD1390, an ataxia telangiectasia mutated (ATM) inhibitor for glioblastoma represents a good example of a small molecule optimisation for a kinase target (which also happens to have DDR activity) with CNS penetration [76, 77]. Similarly, CHDI has sponsored a campaign to generate brain-penetrant ATM inhibitors for HD achieving good target exposures and engagement of pharmacology [78]. Unfortunately, disease-relevant effects such as blocking somatic instability have not been reported.

Another relevant case study that leverages existing DDR targeting approaches for HD is exemplified by poly (ADP-ribose) polymerase (PARP). As for ATM, the interest around PARP in HD derives from altered reactive oxygen species profiles [49, 79]. Unfortunately, published PARP inhibitor work *in vivo* does not detail compound exposure, target engagement or expression of target pharmacology in brain regions or surrogate peripheral cells including PARP activity assays (e.g., PAR formation) or whether the increased PARP1 immunoreactivity was modified with compound treatment [80, 81]. More detailed assessments with an alternative tool molecule may be warranted: while most PARP inhibitors do not appear to have properties commensurate with brain penetration, one exception is veliparib, which has reported CNS activity, precedented PK and efficacy together with low PARP trapping [82].

Finally, and most importantly is the safety consideration of how much inhibition of DDR activity can be tolerated with chronic administration. Significantly higher systemic exposure compared with central exposure for a therapeutic effect in the brain may result in unacceptably high DDR inhibition and cellular toxicity in the periphery.

THE RIGHT SAFETY PROFILE

Safety of any experimental therapy is absolutely paramount, and all clinical trials are designed to carefully balance potential safety risks against possible therapeutic benefits. For HD, which has no effective therapies and is invariably fatal, the risk/benefit equation will be different than for milder diseases where effective therapies already exist. Safety risks can take the form of frank toxicity (e.g., cardiovascular, hepatic, renal) as well as tolerability (e.g., sedation,

ataxia, dizziness, nausea), both of which limit maximum exposures in patients. It is also important to consider both acute safety issues (e.g., seizures) and more chronic toxicity (e.g., tumour formation) and critically whether any adverse effect reverses when the drug is removed. Reversibility is much easier to assess with small molecules which are typically dosed daily compared with monthly (or less frequent) ASO administration. Gene therapies cannot typically be removed once administered and hence a great deal of safety data is required before human administration. Finally, toxicity issues can be 'on-target' or 'off-target.' On-target toxicity is caused by the target mechanism, such as the potential for accumulated DNA damage by DDR-targeting drugs, whereas the cause of off-target toxicity is not usually identified but is likely caused by hitting other biological target(s).

As with ADME predictions, drug safety science has advanced over the past couple of decades such that many forms of toxicity can be predicted from *in vitro* studies (e.g., cardiovascular toxicity can be assessed by looking at the potency of a test drug to modulate ion channels required for cardiovascular function), thus reducing the number of molecules that require testing in whole animals [83]. However, regulatory rules still require all novel therapies to be tested in animal toxicology studies before entering human trials. The studies are carried out at multiples of the drug exposure planned for human studies so that "no-adverse-effect levels" can be determined and used to limit the maximum human exposure.

DDR proteins, by their very nature, are part of a complex system whose role is to safeguard genetic integrity. DNA damage, if unrepaired, can lead to mutations in somatic and/or germline cells which in turn can alter cellular phenotype and cause cell dysfunction, death, and disease: several cancers have acquired DNA repair defects. Furthermore, there are a number of syndromes associated with mutations in DNA repair factors that are characterised by cancer and/or neurodegeneration [84]. Similarly, various HD genetic modifier hits are better known in an oncogenic context. Some of the strongest GWA signals were found associated with *FAN1* (FANCD2/FANCI-associated nuclease 1), a structure-specific endonuclease associated with Fanconi Anaemia, a recessive autosomal disorder characterised by cancer predisposition and bone marrow failure [85]. A number of MMR pathway genes including *MLH1* and *PMS2* are also associated with increased microsatellite instability when mutated, which can in turn lead to colorectal and endothelial cancers in particular [86]. Therefore, there is a possibility that inhibition of specific DDR proteins could increase the risk of oncogenic outcomes. This risk will need careful assessment using relevant genetic toxicology assays as part of the drug discovery program to analyse transient or permanent defects of the genetic material and can include bacterial or cell-based reporter assays. Functional tests (e.g., micronucleus, comet and γH2AX assays) assessing the level of induced DNA damage and DNA repair capacity may be used clinically as well as preclinically. Interestingly, subjects with a defect in DDR genes such as *ATM* can be identified by their reduced DDR kinetics after *ex vivo* cell radiation by comet and γH2AX assays [87]. Due to the debilitating nature of HD, and the currently limited therapeutic options, the increased risk of carcinogenesis may be at least partly outweighed by the potential benefit of a DDR inhibition strategy in patients, although careful long-term monitoring would likely be required to identify any problems early. This risk versus benefit equation would be different for healthy volunteers who receive no benefit. Accordingly, a positive genetic toxicity signal may limit the study design of a phase 1 first in human trial in healthy volunteers.

Intriguingly, HD patients may be less susceptible to oncogenic initiation but the basis for this is not clear [88–91]. Huntingtin (HTT) is known to interact with several proteins with a defined role in DDR including ATM, PARP, p53 and PNKP (polynucleotide kinase 3'-phosphatase), and has been postulated to have a more direct role in DDR itself [79, 92–94]. It is also worth bearing in mind that unlike cancer cells, HD cells are less likely to be dependent on specific repair pathways to survive or proliferate and therefore there may be sufficient robustness in the system to offset the effects of a small molecule inhibitor against a particular target. Hence, this is distinct from a synthetic lethality approach that is being employed in cancer which seeks to target the dependency on a particular repair pathway. PARP inhibitors such as olaparib have been a success story for synthetic lethality in cancers with hereditary or somatically acquired mutations in *BRCA1/2* resulting in defective DNA repair by homologous recombination [95]. In addition to being effective, olaparib has been reportedly well tolerated by patients (although it still has notable side effects, particularly in the context of long term treatment, such as decreased red and white blood cell counts), which strengthens confidence that at least some DDR approaches may be suitable to treat HD [96, 97].

THE RIGHT PRECLINICAL MODELS

A primary challenge for any neurodegenerative disease is the development of disease models that accurately recapitulate key features of pathophysiology in a practical timeframe for experimentation. This enables preclinical validation of the therapeutic targets and/or efficacy testing with compounds. Genetic models of HD have been generated using different model organisms ranging from invertebrates (*Caenorhabditis elegans* and *Drosophila melanogaster*) to rodent (transgenic and knock-in mouse and rat) to large animal (minipig, sheep and monkey) [52, 98]. The mouse models are the most utilised for a number of reasons including their small size and short generation time together with rich genetic resources such as multiple inbred strains in conjunction with a range of genetic manipulation tools. The three main classifications of HD mouse models used in multiple labs are: 1) overexpressing a fragment of the human protein sequence; 2) genetic modification of the endogenous murine homologue; and 3) human genomic locus transgenics. Some of these are available with different trinucleotide repeat lengths but are otherwise genetically matched (i.e., an allelic series). Most of these mouse models have good construct validity with recapitulation of the fundamental genetic lesion of an expanded CAG repeat [99]. A common caveat is that extremely long CAG repeats (typically 100+) are necessary to facilitate measurement of any mutation-associated phenotypes in the lifespan of the mouse and therefore may be a closer model for the juvenile form of the disease. Each mouse model has also been well characterised with respect to face validity (how well the phenotypes recapitulate clinical observations). The ultimate test of predictive validity (whether key mechanistic findings and therapeutic efficacy in a model can predict clinical outcomes and vice versa) is lacking in the absence of a proven clinically effective disease-modifying therapy for HD. Indeed, it may be the case that seeking to use a preclinical model is not the best strategy of predicting clinical efficacy; an alternate route could comprise a demonstration of a robust PKPD relationship in animal(s) together with suitable safety margins prior to testing the hypothesis in human patients.

Preclinical mouse models will likely play a role in identifying promising therapeutic candidates that can be translated to the clinic in a shorter timeframe than those needed for large animal models. A critical first question is which model(s) should be used with respect to DDR therapeutics given the need for a robust, progressive somatic instability phenotype that mirrors clinical parameters with relevant tissue specificity (in itself a challenge given the incomplete information from patients for the latter). Available data suggest either fragment or knock-in models are likely to be suitable given clear demonstration of somatic instability in disease-relevant tissues [58]. A dual model approach (i.e., both fragment and full-length knock in) may be warranted together with assessment of other early molecular phenotypes such as transcriptional dysregulation or mis-splicing rather than behavioural phenotypes. The number of animals required in a study to robustly determine whether somatic instability is significantly altered is not yet known. Indeed, the primary outcomes will need to be carefully considered as it is not clear what the inflection point is for initiating pathophysiology [100]. In other words, we still need to ascertain whether modulation of somatic instability will impact on behavioural phenotypes in the context of a repeat that is already highly expanded. This needs to be combined with work on target engagement and expression of pharmacology to understand whether the drug mechanism modulates somatic instability and other HD relevant phenotypes/putative biomarkers in disease-relevant cells and accessible biofluids.

THE RIGHT PATIENT—AND THE RIGHT BIOMARKER

The ultimate goal is to develop candidates that provide patient benefit in clinical trials. This is not trivial: a phase III therapeutic trial for a neurodegenerative disease could take 2–3 years of dosing a participating patient and take 4–5 years to run a complete trial, whereas in many other diseases 3–6 months of dosing would be sufficient. Therefore, earlier stage clinical trials in HD are more likely to establish an effect on their primary pharmacology (blocking somatic instability for DDR approaches or lowering mHTT for huntingtin-targeted therapies) first. If that succeeds the clinical benefit for patients will then be determined in later, longer, and more expensive trials.

There are several unique advantages for HD as a disease for clinical trials. Diagnosis of gene carriers prior to the onset of clinical manifestation is relatively straightforward and enables appropriate subject recruitment with confidence, even at

early disease stages. The heritable basis for this disease contributes to the highly engaged and actively mobilised patient populations which is enhanced through organisations such as the Huntington's Disease Society of America and the European Huntington's Disease Network, which is supported by CHDI. Clinical research platforms based on worldwide registries such as Enroll-HD enhance the natural history information, which informs clinical trial design (selection of trial endpoints, number of participants, duration and so on). Additionally, they facilitate greater participation of the patient population which speeds up recruitment and reduces costs overall. It is not yet clear whether these registries will be able to provide sufficient participants for the different trials, an issue that could hinder overall progress as more potential candidates enter clinical development. Finally, HD is classified as a rare disease by regulatory agencies, such as the Food and Drug Administration in the USA, which means it can benefit from various schemes designed to accelerate approval. The regulatory path is further aided by initiatives such as the Huntington's Disease Regulatory Science Consortium, which seek to gain support for certain trial designs and clinical endpoints to increase the chance of success. The faster disease progression in patients with the juvenile form of the disease may permit the identification of a more robust efficacy signal in a shorter trial duration, but these patients are very rare and so practical challenges may out-weigh the benefit of running a trial in this sub-population.

In addition to primary pharmacology, objective measures of efficacy and side effects and responders versus non-responders are required in later phases—ultimately, we need the patient to measurably benefit. With respect to HD, "classical" clinical measures such as the Unified Huntington's Disease Rating Scale are comparatively insensitive to change over typical clinical trial durations. Whether these traditional approaches can be enhanced through the use of technology and more quantitative measures is an ongoing area of study. Emerging data suggests that imaging and biofluid based quantitative surrogate measures (discussed further in subsequent paragraphs) can detect disease initiation or progression more sensitively than "traditional" clinical measures, revealing effects earlier and with fewer patients which would ultimately impact on phase 2/3 trials [101, 102]. The benefit of these markers still needs to be proven by adequately powered, well designed clinical trials considering a range of parameters including gender-, age- and tissue-specific variations.

A more robust set of structural and functional imaging approaches look promising with respect to evaluating longitudinal changes in the disease state such as striatal volume [101]. One interesting example is represented by the PET ligand ([^{18}F]MNI-659) originally developed to interrogate PDE10A inhibitor target engagement. It has revealed early and significant decreases in PDE10A enzyme levels in the caudate putamen and globus pallidus of disease gene expansion carriers versus healthy controls across all stages [73, 103]. This ligand appears more sensitive than the dopamine receptor and volumetric methods currently used and has also been used preclinically [14, 104].

Biofluid biomarkers under development may offer complementary information relating to changes in at least some biological processes at the molecular level. Mutant HTT measurement is being assessed as a pharmacodynamic biomarker for lowering therapies; if modulating somatic instability ultimately reduces mutant HTT aggregation (a length- and time-dependent phenomenon), it may be worth considering for DDR target modulation [101, 105, 106]. A more direct output, currently under active investigation, relates to somatic instability of the trinucleotide repeats themselves. Developing routes to measure and analyse repeat expansions in a population of cells with sufficient sensitivity and throughput will clearly enhance preclinical and clinical decision making. One challenge is that there is no standardised method to measure somatic instability over time in a population of cells [51, 107]. This is further amplified by the nature of the data: repeat expansion is a stochastic event in individual cells yielding an unusual distribution of repeat lengths in a population. Accordingly, there are gaps in our understanding around the onset, extent, and duration of repeat expansion in different cell types although there are ongoing studies which are aimed at addressing these. We anticipate this will ultimately inform on the true mutational burden in different cell types with respect to disease staging and progression. A related consideration is that there are currently no standardised, statistically appropriate methods in place to determine whether somatic instability has been significantly modulated by an intervention.

Another potential disease-relevant biomarker is the detection of elevated DNA damage, which has been reported in HD mouse models, cell lines and in patient PBMCs [93, 108–112]; this warrants further investigation to determine how robust and reproducible these observations are. DDR biomarkers such as the

comet and γH2AX assays are leveraged in precision cancer therapy as is the pharmacogenetic analyses of particular mutations as a so-called "companion diagnostic". These can be used to identify populations who are 1) likely to benefit; 2) be at increased risk for serious adverse effects for a particular treatment; or 3) to improve safety or efficacy by monitoring treatment response [113]. One correlate in the HD field could be the proactive assessment of known DDR variants including those identified through GWAS.

In addition to disease-specific measures, biomarkers that detect activity of a specific drug candidate on its target can determine the level of engagement during a clinical trial. The successful example of PARP1/2 inhibitors in the context of a synthetic lethal approach in BRCA1/2-deficient carriers is worth considering here. Olaparib, rucaparib, niraparib, veliparib and talazoparib are PARP inhibitors that are in development or have been approved for maintenance treatment of recurrent cancer in the context of BRCA1/2 mutations that are responding to platinum-based chemotherapy (Table 1). Each of these molecules possess unique pharmacokinetic profiles and therapeutic indices despite sharing a common binding motif – the NAD+ active site pocket of PARP1. The clinical development of PARP inhibitors has been greatly facilitated by the use of target engagement measures including PARP1 expression level and activity measurement (via poly adenosine ribose chain producing activity) in tumour tissue and PBMCs [114]. This approach could be applied to other DDR drug discovery targets for HD whereby expression level and subcellular localisation of the target can be measured together with *ex vivo* functional assessments such as activity-based diagnostic assays configured for the target in question [115].

The current HD therapeutic pipeline is comparatively rich and promising with disease-specific therapies in development. There are currently 34 ongoing trials in clinical assessment for treatment of HD, spread across various stages of development and modalities [116]. Excitingly, there are a number of approaches in active discovery or development targeting the underlying mutant genetic sequence, the messenger (both allele- and non-allele selective) or the mutant protein directly rather than the consequences of the mutation. Many (but not all) of these approaches require some of the emerging novel therapeutic modalities such as RNA interference (e.g., ASOs) and gene therapy-based adeno-associated virus vectors. The caveat associated with at least some of these approaches is the requirement for more invasive approaches to delivery such as direct infusion into the CNS or repeated intrathecal deliveries; both of which require specialist facilities. This contrasts with small molecules which have the potential for oral delivery (ideally once daily in tablet or capsule form), which are likely to be more appealing to patients. Regardless, these new symptomatic treatment modalities will almost certainly inform further clinical investigations.

CONCLUSIONS

Here, we have sought to give an overview of how to approach drug discovery using a compelling set of targets (DDR) for a specific disease population (HD). We have distilled this into a set of principles aligned with a "traditional" small molecule drug discovery and development route. We believe that in order to successfully treat HD, we need to address the fundamental underlying cause of the disease: the expanded trinucleotide repeats. The ideal approach would be a therapeutic that specifically and safely edits these repeats below a disease-causing threshold; or at the least prevents further expansion and increased mutational burden. In this context, at least some DDR proteins are highly compelling as potential disease modifying targets through converging lines of evidence: 1) clinical variants in DDR proteins are associated with relevant quantifiable disease output measures such as age of onset, rate of progression and somatic instability; 2) increased somatic instability and expansion of the repeats increases the mutational burden and disease severity; 3) expansion of the repeats *in vitro* and *in vivo* is driven by several proteins with a defined role in DDR, particularly MMR; 4) manipulation of the same proteins (i.e., by mutating or altering the expression) modulates repeat expansion. Therefore, we know that DDR proteins can affect relevant phenotypes associated with an expanded trinucleotide repeat; hence targeting these proteins can be disease modifying by definition. There are still some gaps in our knowledge and we therefore need to seek to address these gaps in order to drive better decision making and to ensure our confidence continues to improve as we move from research to clinical development (Box 1). Ultimately, this will help us identify the right patient and the best way of performing clinical studies prior to starting phase 1 development and enrolling participants into clinical trials.

We need to develop the best therapeutic modality for this set of targets—which equates to the right molecule in the right tissue in the right patients. We would argue that small molecules offer the best opportunity with respect to exposure at key sites of action—nuclear-localised proteins in multiple cell types including those in deep brain structures such as medium spiny neurons in the caudate putamen. Small molecule assessment in relevant preclinical models will facilitate the campaign to drive efficacy whilst minimising liabilities. Small molecules also offer patients a more convenient regimen of oral treatment as opposed to invasive delivery routes. They can also be readily combined with other therapeutics or discontinued quickly should adverse effects be observed. Another important consideration is that if we identify suitable small molecules that are efficacious with respect to stabilising repeats and well-tolerated by patients, this means that gene-positive individuals will be able to initiate and sustain treatment at a very early point in the pathophysiological cascade prior to measurable deficits. We would agree that another route, albeit a more challenging one, is to enable selective editing of the expanded repeats to below the disease-causing threshold.

The development of novel DDR drugs for neurodegeneration is also facilitated by understanding of challenges with DDR drugs for oncology and key liabilities associated with specific targets. We need to maintain a watching brief, continue to address the gaps in our understanding, and ensure we continue to work to realise the potential to increase therapeutic benefit and reduce risk. Taken together, we believe success is possible, with close collaboration between patients, academic investigators, preclinical drug discoverers, clinicians, diagnostic developers, and regulatory bodies.

Box 1: Gaps in our understanding with respect to DDR and HD that still need addressing

– The detailed molecular mechanisms underpinning the MMR mediated expansion bias of trinucleotide repeats;
– Assessment of rate of change of somatic instability in different tissues and cell types across multiple models and clinical samples;
– Impact of modulating somatic instability (e.g., with interruptions of the pure CAG repeat tracts) on defined molecular and behavioural sequelae;
– "Inflection point", i.e., what is the critical threshold/point of no return with respect to an expanded repeat? If we prevent repeats expanding to this inflection point, would we derail disease pathophysiology completely?
– Systematic and unbiased assessment of the contribution of individual proteins in DDR pathways to HD pathophysiology together with validation follow-up studies to identify the best target for drug discovery;
– Whether biological and/or pharmacological interrogation will mirror the effect of a constitutive genetic manipulation with respect to somatic instability and other relevant phenotypes;
– To what extent do the same mechanisms also apply to other disease-causing expanded trinucleotide repeats (e.g., CTG, CGG and GAA) or even other microsatellite expansions such as the hexanucleotide expansion in c9orf72 that causes frontotemporal dementia and amyotrophic lateral sclerosis?

Box 2: Drug Discovery Glossary

ADME: Absorption, distribution, metabolism, and elimination of a drug following administration to a human or model mammalian system.

Affinity: A measure of how potently a ligand binds to its biological target.

Attrition: Failure of a therapeutic project or target for a variety of reasons, e.g., lack of efficacy, lack of safety, inability to develop a suitably potent or selective drug molecule, etc.

Drug Exposure: The concentration a drug achieves in the body, or a body compartment, following administration. Drug exposure for a given dose can vary depending on dose route, dose formulation, species and of course time after administration. The measurement of exposure levels allows more accurate comparison of different compounds within a drug discovery project and helps dose setting when translating results from preclinical species to human clinical trials. Drug exposure is most commonly measured in plasma, although for neurological diseases measurement of brain exposure is more representative because exposure can be restricted by the BBB.

Free Drug Concentration: Most drugs are bound to serum proteins such that the unbound aqueous concentration is much lower than the total concentration measured in that compartment. Likewise, in brain tissue much of the drug will be bound

non-specifically to brain proteins. In order to exert a pharmacological response, the drug must bind to its target. In the majority of cases, only unbound drug is available to interact with the target. Usually the total drug concentration is measured in plasma/brain samples and then the free fraction is calculated based on determining the percentage of plasma/brain protein binding.

High-Throughput Screening: Automated techniques for analysing thousands of chemical compounds to discriminate between active and inactive substances; commonly uses robotics and 96-, 384- or 1536-well formats.

IND: Investigational New Drug application, filed with the Food and Drug Administration in the USA at the completion of preclinical studies to initiate human clinical trials. This contains information about the chemical structure and manufacture of a drug, its mode of action, the results of preclinical testing including toxicity from animal studies and outlines plans for clinical protocols to demonstrate safety and efficacy in humans.

Lead: A compound that displays reasonable specificity and potency against a desired pharmacological target but is not sufficiently optimised to be taken forward for clinical studies.

Lead Optimisation: This is an iterative process whereby structural modifications are introduced to lead molecules to improve their pharmacological potency or selectivity and ideally enhance other drug properties including solubility, permeability and ADME all of which must be optimised to produce a suitable drug candidate for clinical studies.

NCE: New chemical entity

Target Engagement: The interaction of ligands with their target receptor/enzyme/channel, etc.

Preclinical Studies: Testing performed using laboratory animals to provide information about mode of action of a drug, efficacious dosage levels, ADME, adverse effects and toxicology.

PKPD Relationship: Pharmacokinetics (the ADME properties of a drug)-Pharmacodynamic (the pharmacological properties of a drug); this relates drug effects to a measure of drug concentration in a body compartment.

SAR (structure-activity relationships): Information that relates chemical structure to biological activity, potency, or toxicity.

Safety Margins: The relationship between the efficacious drug exposure and toxicological drug exposure

Target Validation: Evaluation of the function of the target in the disease process to determine whether alteration of that function will provide a useful therapeutic effect.

CONFLICT OF INTEREST

CLB and DSR are employees of LoQus23 Therapeutics Ltd. KRG is an employee of Sandexis Medicinal Chemistry and employed as a chemistry consultant by LoQus23 Therapeutics.

REFERENCES

[1] Gribkoff VK, Kaczmarek LK. The need for new approaches in CNS drug discovery: Why drugs have failed, and what can be done to improve outcomes. Neuropharmacology. 2017;120:11-9.

[2] Travessa AM, Rodrigues FB, Mestre TA, Ferreira JJ. Fifteen years of clinical trials in Huntington's disease: A very low clinical drug development success rate. J Huntingtons Dis. 2017;6:157-63.

[3] Schuhmacher A, Gassmann O, Hinder M. Changing R&D models in research-based pharmaceutical companies. J Transl Med. 2016;14:105.

[4] Finkbeiner S. Bridging the Valley of Death of therapeutics for neurodegeneration. Nat Med. 2010;16:1227-32.

[5] Pankevich DE, Altevogt BM, Dunlop J, Gage FH, Hyman SE. Improving and accelerating drug development for nervous system disorders. Neuron. 2014;84:546-53.

[6] Chornenkyy Y, Fardo DW, Nelson PT. Tau and TDP-43 proteinopathies: Kindred pathologic cascades and genetic pleiotropy. Lab Invest. 2019;99:993-1007.

[7] Pang SY-Y, Ho PW-L, Liu H-F, Leung C-T, Li L, Chang EES, et al. The interplay of aging, genetics and environmental factors in the pathogenesis of Parkinson's disease. Transl Neurodegener. 2019;8:23.

[8] Abramzon YA, Fratta P, Traynor BJ, Chia R. The overlapping genetics of amyotrophic lateral sclerosis and frontotemporal dementia. Front Neurosci. 2020;14:42.

[9] Mikitsh JL, Chacko A-M. Pathways for small molecule delivery to the central nervous system across the blood-brain barrier. Perspect Med Chem. 2014;6:11-24.

[10] Walker AL, Imam SZ, Roberts RA. Drug discovery and development: Biomarkers of neurotoxicity and neurodegeneration. Exp Biol Med. 2018;243:1037-45.

[11] Bates GP, Dorsey R, Gusella JF, Hayden MR, Kay C, Leavitt BR, et al. Huntington disease. Nat Rev Dis Primer. 2015;1:15005.

[12] Tabrizi SJ, Leavitt BR, Landwehrmeyer GB, Wild EJ, Saft C, Barker RA, et al. Targeting huntingtin expression in patients with Huntington's disease. N Engl J Med. 2019;380:2307-16.

[13] Denis HL, David LS, Cicchetti F. Antibody-based therapies for Huntington's disease: Current status and future directions. Neurobiol Dis. 2019;132:104569.

[14] Zeitler B, Froelich S, Marlen K, Shivak DA, Yu Q, Li D, et al. Allele-selective transcriptional repression of mutant HTT for the treatment of Huntington's disease. Nat Med. 2019;25:1131-42.

[15] Nakamori M, Panigrahi GB, Lanni S, Gall-Duncan T, Hayakawa H, Tanaka H, et al. A slipped-CAG DNA-binding small molecule induces trinucleotide-repeat contractions *in vivo*. Nat Genet. 2020;52:146-59.
[16] Tabrizi SJ, Ghosh R, Leavitt BR. Huntingtin lowering strategies for disease modification in Huntington's disease. Neuron. 2019;101:801-19.
[17] Kennedy L, Evans E, Chen C-M, Craven L, Detloff PJ, Ennis M, et al. Dramatic tissue-specific mutation length increases are an early molecular event in Huntington disease pathogenesis. Hum Mol Genet. 2003;12: 3359-67.
[18] Shelbourne PF, Keller-McGandy C, Bi WL, Yoon S-R, Dubeau L, Veitch NJ, et al. Triplet repeat mutation length gains correlate with cell-type specific vulnerability in Huntington disease brain. Hum Mol Genet. 2007;16: 1133-42.
[19] Swami M, Hendricks AE, Gillis T, Massood T, Mysore J, Myers RH, et al. Somatic expansion of the Huntington's disease CAG repeat in the brain is associated with an earlier age of disease onset. Hum Mol Genet. 2009;18:3039-47.
[20] Ciosi M, Maxwell A, Cumming SA, Hensman Moss DJ, Alshammari AM, Flower MD, et al. A genetic association study of glutamine-encoding DNA sequence structures, somatic CAG expansion, and DNA repair gene variants, with Huntington disease clinical outcomes. EBioMedicine. 2019;48:568-80.
[21] Massey TH, Jones L. The central role of DNA damage and repair in CAG repeat diseases. Dis Model Mech. 2018;11:dmm031930.
[22] Jones L, Houlden H, Tabrizi SJ. DNA repair in the trinucleotide repeat disorders. Lancet Neurol. 2017;16:88-96.
[23] Pilié PG, Tang C, Mills GB, Yap TA. State-of-the-art strategies for targeting the DNA damage response in cancer. Nat Rev Clin Oncol. 2019;16:81-104.
[24] Cook D, Brown D, Alexander R, March R, Morgan P, Satterthwaite G, et al. Lessons learned from the fate of AstraZeneca's drug pipeline: A five-dimensional framework. Nat Rev Drug Discov. 2014;13:419-31.
[25] Morgan P, Van Der Graaf PH, Arrowsmith J, Feltner DE, Drummond KS, Wegner CD, et al. Can the flow of medicines be improved? Fundamental pharmacokinetic and pharmacological principles toward improving Phase II survival. Drug Discov Today. 2012;17:419-24.
[26] Delnomdedieu M, Tan Y, Ogden A, Berger Z, Reilmann R. J06 A randomized, double-blind, placebo-controlled phase ii efficacy and safety study of the PDE10A inhibitor PF-02545920 in huntington disease (amaryllis). J Neurol Neurosurg Psychiatry. 2018;89:A99-100.
[27] Takeuchi T, Nagai Y. Protein misfolding and aggregation as a therapeutic target for polyglutamine diseases. Brain Sci. 2017;7:128.
[28] Chen G, Xu T, Yan Y, Zhou Y, Jiang Y, Melcher K, et al. Amyloid beta: Structure, biology and structure-based therapeutic development. Acta Pharmacol Sin. 2017;38:1205-35.
[29] Mehta D, Jackson R, Paul G, Shi J, Sabbagh M. Why do trials for Alzheimer's disease drugs keep failing? A discontinued drug perspective for 2010-2015. Expert Opin Investig Drugs. 2017;26:735-9.
[30] Genetic Modifiers of Huntington's Disease (GeM-HD) Consortium. Identification of genetic factors that modify clinical onset of Huntington's disease. Cell. 2015;162:516-26.
[31] Moss DJH, Pardiñas AF, Langbehn D, Lo K, Leavitt BR, Roos R, et al. Identification of genetic variants associated with Huntington's disease progression: A genome-wide association study. Lancet Neurol. 2017;16:701-11.
[32] Genetic Modifiers of Huntington's Disease (GeM-HD) Consortium. CAG repeat not polyglutamine length determines timing of Huntington's disease onset. Cell. 2019;178:887-900.e14.
[33] Monckton DG. Somatic expansion of the CAG repeat in Huntington disease: An historical perspective. J Huntingtons Dis. 2020; doi: 10.3233/JHD-200429
[34] Hong EP, MacDonald ME, Wheeler VC, Jones L, Holmans P, Orth M, Monckton DG, Long JD, Kwak S, Gusella JF, Lee J-M. Huntington's disease pathogenesis: Two sequential components. J Huntingtons Dis. 2020; doi: 10.3233/JHD-200427
[35] Bettencourt C, Hensman-Moss D, Flower M, Wiethoff S, Brice A, Goizet C, et al. DNA repair pathways underlie a common genetic mechanism modulating onset in polyglutamine diseases. Ann Neurol. 2016;79:983-90.
[36] Iyer RR, Pluciennik A, Napierala M, Wells RD. DNA triplet repeat expansion and mismatch repair. Annu Rev Biochem. 2015;84:199-226.
[37] Schmidt MHM, Pearson CE. Disease-associated repeat instability and mismatch repair. DNA Repair. 2016;38:117-26.
[38] Zhao X, Gazy I, Hayward B, Pintado E, Hwang YH, Tassone F, et al. Repeat instability in the Fragile X-related disorders: Lessons from a mouse model. Brain Sci. 2019;9:52.
[39] Zhao X, Kumari D, Miller CJ, Kim G, Hayward B, Vitalo AG, Pinto RM, Usdin K. Modifiers of somatic repeat instability in mouse models of Friedreich ataxia and the Fragile X-related disorders: Implications for the mechanism of somatic expansion in Huntington disease. J Huntingtons Dis. 2020; doi: 10.3233/JHD-200423
[40] Vieira MLC, Santini L, Diniz AL, Munhoz C de F. Microsatellite markers: What they mean and why they are so useful. Genet Mol Biol. 2016;39:312-28.
[41] Baudrin LG, Deleuze J-F, How-Kit A. Molecular and computational methods for the detection of microsatellite instability in cancer. Front Oncol. 2018;8:621.
[42] Raeker MÖ, Pierre-Charles J, Carethers JM. Tetranucleotide microsatellite mutational behavior assessed in real time: Implications for future microsatellite panels. Cell Mol Gastroenterol Hepatol. 2020;9:689-704.
[43] Drummond JT, Genschel J, Wolf E, Modrich P. DHFR/MSH3 amplification in methotrexate-resistant cells alters the hMutSα/hMutSβ ratio and reduces the efficiency of base-base mismatch*β*repair. Proc Natl Acad Sci U S A. 1997;94:10144-9.
[44] Tomé S, Manley K, Simard JP, Clark GW, Slean MM, Swami M, et al. MSH3 polymorphisms and protein levels affect CAG repeat instability in Huntington's disease mice. PLoS Genet. 2013;9:e1003280.
[45] Flower M, Lomeikaite V, Ciosi M, Cumming S, Morales F, Lo K, et al. MSH3 modifies somatic instability and disease severity in Huntington's and myotonic dystrophy type 1. Brain. 2019;142:1876-86.
[46] Goold R, Flower M, Moss DH, Medway C, Wood-Kaczmar A, Andre R, et al. FAN1 modifies Huntington's disease progression by stabilizing the expanded HTT CAG repeat. Hum Mol Genet. 2019;28:650-61.
[47] Deshmukh AL, Porro A, Mohiuddin M, Lanni S, Panigrahi GB, Caron MC, Masson JY, Sartori AA, Pearson

CE. FAN1, a DNA repair nuclease, as a modifier of repeat expansion disorders. J Huntingtons Dis. 2020; doi: 10.3233/JHD-200448

[48] Iyer RR, Pluciennik A. DNA mismatch repair and its role in Huntington's disease. J Huntingtons Dis. 2020; doi: 10.3233/JHD-200438

[49] Maiuri T, Hung CLK, Suart CE, Begeja N, Barba-Bazan C, Peng Y, Savic N, Wong T, Truant R. DNA repair in neurodegeneration: Somatic expansion and alternative hypotheses. J Huntingtons Dis. 2020; doi: 10.3233/JHD-200414

[50] Mohs RC, Greig NH. Drug discovery and development: Role of basic biological research. Alzheimers Dement (N Y). 2017;3:651-7.

[51] Massey T, McAllister B, Jones L. Methods for assessing DNA repair and repeat expansion in Huntington's disease. Methods Mol Biol. 2018;1780:483-95.

[52] Wheeler VC, Dion V. Modifiers of CAG repeat instability: Insights from model systems. J Huntingtons Dis. 2020; doi: 10.3233/JHD-200426

[53] Wheeler VC. Mismatch repair gene Msh2 modifies the timing of early disease in HdhQ111 striatum. Hum Mol Genet. 2003;12:273-81.

[54] Dragileva E, Hendricks A, Teed A, Gillis T, Lopez ET, Friedberg EC, et al. Intergenerational and striatal CAG repeat instability in Huntington's disease knock-in mice involve different DNA repair genes. Neurobiol Dis. 2009;33:37-47.

[55] Kovalenko M, Dragileva E, St. Claire J, Gillis T, Guide JR, New J, et al. Msh2 acts in medium-spiny striatal neurons as an enhancer of CAG instability and mutant huntingtin phenotypes in Huntington's disease knock-in mice. PLoS One. 2012;7:e44273.

[56] Pinto RM, Dragileva E, Kirby A, Lloret A, Lopez E, St. Claire J, et al. Mismatch repair genes Mlh1 and Mlh3 modify CAG instability in Huntington's disease mice: Genome-wide and candidate approaches. PLoS Genet. 2013;9:e1003930.

[57] Shelbourne PF, Killeen N, Hevner RF, Johnston HM, Tecott L, Lewandoski M, et al. A Huntington's disease CAG expansion at the murine Hdh locus is unstable and associated with behavioural abnormalities in mice. Hum Mol Genet. 1999;8:763-74.

[58] Gonitel R, Moffitt H, Sathasivam K, Woodman B, Detloff PJ, Faull RLM, et al. DNA instability in post-mitotic neurons. Proc Natl Acad Sci U S A. 2008;105: 3467-72.

[59] Neto JL, Lee J-M, Afridi A, Gillis T, Guide JR, Dempsey S, et al. Genetic contributors to intergenerational CAG repeat instability in Huntington's disease knock-in mice. Genetics. 2017;205:503-16.

[60] Telenius H, Almqvist E, Kremer B, Spence N, Squitieri F, Nichol K, et al. Somatic mosaicism in sperm is associated with intergenerational (CAG)n changes in Huntington disease. Hum Mol Genet. 1995;4:189-95.

[61] Mangiarini L, Sathasivam K, Mahal A, Mott R, Seller M, Bates GP. Instability of highly expanded CAG repeats in mice transgenic for the Huntington's disease mutation. Nat Genet. 1997;15:197-200.

[62] Jhoti H, Williams G, Rees DC, Murray CW. The "rule of three" for fragment-based drug discovery: Where are we now? Nat Rev Drug Discov. 2013;12:644.

[63] Murray CW, Blundell TL. Structural biology in fragment-based drug design. Curr Opin Struct Biol. 2010;20:497-507.

[64] Kola I, Landis, J. Can the pharmaceutical industry reduce attrition rates? Nat Rev Drug Discov. 2004;3:711-6.

[65] Wager TT, Chandrasekaran RY, Hou X, Troutman MD, Verhoest PR, Villalobos A, et al. Defining desirable central nervous system drug space through the alignment of molecular properties, *in vitro* ADME, and safety attributes. ACS Chem Neurosci. 2010;1:420-34.

[66] Wager TT, Hou X, Verhoest PR, Villalobos A. Central nervous system multiparameter optimization desirability: Application in drug discovery. ACS Chem Neurosci. 2016;7:767-75.

[67] Villabona-Rueda A, Erice C, Pardo CA, Stins MF. The evolving concept of the blood brain barrier (BBB): From a single static barrier to a heterogeneous and dynamic relay center. Front Cell Neurosci. 2019;13:405.

[68] Banks WA, Terrell B, Farr SA, Robinson SM, Nonaka N, Morley JE. Passage of amyloid β protein antibody across the blood-brain barrier in a mouse model of Alzheimer's disease. Peptides. 2002;23:2223-6.

[69] Levites Y, Smithson LA, Price RW, Dakin RS, Yuan B, Sierks MR, et al. Insights into the mechanisms of action of anti-Aβ antibodies in Alzheimer's disease mouse models. FASEB J. 2006;20:2576-8.

[70] Rankovic Z. CNS drug design: Balancing physicochemical properties for optimal brain exposure. J Med Chem. 2015;58:2584-608.

[71] Rankovic Z. CNS physicochemical property space shaped by a diverse set of molecules with experimentally determined exposure in the mouse brain. J Med Chem. 2017;60:5943-54.

[72] Fridén M, Gupta A, Antonsson M, Bredberg U, Hammarlund-Udenaes M. *In vitro* methods for estimating unbound drug concentrations in the brain interstitial and intracellular fluids. Drug Metab Dispos. 2007;35:1711-9.

[73] Fazio P, Fitzer-Attas CJ, Mrzljak L, Bronzova J, Nag S, Warner JH, et al. PET molecular imaging of phosphodiesterase 10A: An early biomarker of Huntington's disease progression. Mov Disord. 2020;35:606-15.

[74] Copeland RA, Pompliano DL, Meek TD. Drug-target residence time and its implications for lead optimization. Nat Rev Drug Discov. 2006;5:730-9.

[75] Benn CL, Dawson, LA. Review of rationale for clinically precedented kinase targets for neurodegenerative disease. Front Aging Neurosci. 2020; doi: 10.3389/fnagi.2020.00242

[76] Durant ST, Zheng L, Wang Y, Chen K, Zhang L, Zhang T, et al. The brain-penetrant clinical ATM inhibitor AZD1390 radiosensitizes and improves survival of preclinical brain tumor models. Sci Adv. 2018;4:eaat1719.

[77] Karlin J, Allen J, Ahmad SF, Hughes G, Sheridan V, Odedra R, et al. Orally bioavailable and blood-brain-barrier penetrating ATM inhibitor (AZ32) radiosensitizes intracranial gliomas in mice. Mol Cancer Ther. 2018;17:1637-47.

[78] Toledo-Sherman L, Breccia P, Cachope R, Bate JR, Angulo-Herrera I, Wishart G, et al. Optimization of potent and selective ataxia telangiectasia-mutated inhibitors suitable for a proof-of-concept study in Huntington's disease models. J Med Chem. 2019;62:2988-3008.

[79] Maiuri T, Suart CE, Hung CLK, Graham KJ, Barba Bazan CA, Truant R. DNA damage repair in Huntington's disease and other neurodegenerative diseases. Neurotherapeutics. 2019;16:948-56.

[80] Cardinale A, Paldino E, Giampà C, Bernardi G, Fusco FR. PARP-1 inhibition is neuroprotective in the R6/2

mouse model of Huntington's disease. PLoS One. 2015;10:e0134482.
[81] Paldino E, Cardinale A, D'Angelo V, Sauve I, Giampà C, Fusco FR. Selective sparing of striatal interneurons after poly (ADP-Ribose) polymerase 1 inhibition in the R6/2 mouse model of Huntington's disease. Front Neuroanat. 2017;11:61.
[82] Donawho CK, Luo Y, Luo Y, Penning TD, Bauch JL, Bouska JJ, et al. ABT-888, an orally active poly(ADP-Ribose) polymerase inhibitor that potentiates DNA-damaging agents in preclinical tumor models. Clin Cancer Res. 2007;13:2728-37.
[83] Weaver RJ, Valentin J-P. Today's challenges to de-risk and predict drug safety in human "Mind-the-Gap." Toxicol Sci. 2019;167:307-21.
[84] Madabhushi R, Pan L, Tsai L-H. DNA damage and its links to neurodegeneration. Neuron. 2014;83:266-82.
[85] Jin H, Cho Y. Structural and functional relationships of FAN1. DNA Repair (Amst). 2017;56:135-43.
[86] Li G-M. Mechanisms and functions of DNA mismatch repair. Cell Res. 2008;18:14.
[87] Sordet O, Redon CE, Guirouilh-Barbat J, Smith S, Solier S, Douarre C, et al. Ataxia telangiectasia mutated activation by transcription- and topoisomerase I-induced DNA double-strand breaks. EMBO Rep. 2009;10:887-93.
[88] Sørensen SA, Fenger K, Olsen JH. Significantly lower incidence of cancer among patients with Huntington disease. Cancer. 1999;86:1342-6.
[89] Ji J, Sundquist K, Sundquist J. Cancer incidence in patients with polyglutamine diseases: A population-based study in Sweden. Lancet Oncol. 2012;13:642-8.
[90] Thion MS, Tézenas du Montcel S, Golmard J-L, Vacher S, Barjhoux L, Sornin V, et al. CAG repeat size in Huntingtin alleles is associated with cancer prognosis. Eur J Hum Genet. 2016;24:1310-5.
[91] Coarelli G, Diallo A, Thion MS, Rinaldi D, Calvas F, Boubkiza OL, et al. Low cancer prevalence in polyglutamine expansion diseases. Neurology. 2017;88:1114-9.
[92] Steffan JS, Kazantsev A, Spasic-Boskovic O, Greenwald M, Zhu Y-Z, Gohler H, et al. The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription. Proc Natl Acad Sci U S A. 2000;97:6763-8.
[93] Maiuri T, Mocle AJ, Hung CL, Xia J, van Roon-Mom WMC, Truant R. Huntingtin is a scaffolding protein in the ATM oxidative DNA damage response complex. Hum Mol Genet. 2016;26:395-406.
[94] Gao R, Chakraborty A, Geater C, Pradhan S, Gordon KL, Snowden J, et al. Mutant huntingtin impairs PNKP and ATXN3, disrupting DNA repair and transcription. ELife. 2018;8:e42988
[95] Patel M, Nowsheen S, Maraboyina S, Xia F. The role of poly(ADP-ribose) polymerase inhibitors in the treatment of cancer and methods to overcome resistance: A review. Cell Biosci. 2020;10:35.
[96] Friedlander M, Matulonis U, Gourley C, du Bois A, Vergote I, Rustin G, et al. Long-term efficacy, tolerability and overall survival in patients with platinum-sensitive, recurrent high-grade serous ovarian cancer treated with maintenance olaparib capsules following response to chemotherapy. Br J Cancer. 2018;119:1075-85.
[97] Robson ME, Tung N, Conte P, Im S-A, Senkus E, Xu B, et al. OlympiAD final overall survival and tolerability results: Olaparib versus chemotherapy treatment of physician's choice in patients with a germline BRCA mutation and HER2-negative metastatic breast cancer. Ann Oncol. 2019;30:558-66.
[98] Pouladi MA, Morton AJ, Hayden MR. Choosing an animal model for the study of Huntington's disease. Nat Rev Neurosci. 2013;14:708-21.
[99] Yang XW, Gray M. Mouse models for validating preclinical candidates for Huntington's disease. In Lo DC, Hughes RE, editors. Neurobiology of Huntington's Disease: Applications to Drug Discovery. Boca Raton (FL): CRC Press/Taylor & Francis; 2011. Chapter 7.
[100] Donaldson J, Powell S, Rickards N, Holmans P, Jones L. What is the pathogenic CAG expansion length in Huntington's disease? J Huntingtons Dis. 2020;doi: 10.3233/JHD-200445
[101] Zeun P, Scahill RI, Tabrizi SJ, Wild EJ. Fluid and imaging biomarkers for Huntington's disease. Mol Cell Neurosci. 2019;97:67-80.
[102] Scahill RI, Zeun P, Osborne-Crowley K, Johnson EB, Gregory S, Parker C, et al. Biological and clinical characteristics of gene carriers far from predicted onset in the Huntington's disease Young Adult Study (HD-YAS): A cross-sectional analysis. Lancet Neurol. 2020;19:502-12.
[103] Wilson H, Niccolini F, Haider S, Marques TR, Pagano G, Coello C, et al. Loss of extra-striatal phosphodiesterase 10A expression in early premanifest Huntington's disease gene carriers. J Neurol Sci. 2016;368:243-8.
[104] Wijeratne PA, Johnson EB, Eshaghi A, Aksman L, Gregory S, Johnson HJ, et al. Robust markers and sample sizes for multicenter trials of Huntington disease. Ann Neurol. 2020;87:751-62.
[105] Byrne LM, Rodrigues FB, Johnson EB, Wijeratne PA, Vita ED, Alexander DC, et al. Evaluation of mutant huntingtin and neurofilament proteins as potential markers in Huntington's disease. Sci Transl Med. 2018;10:eaat7108.
[106] Aviolat H, Pinto RM, Godschall E, Murtha R, Richey HE, Sapp E, et al. Assessing average somatic CAG repeat instability at the protein level. Sci Rep. 2019;9:19152.
[107] Ciosi M, Cumming SA, Chatzi A, Larson E, Tottey W, Lomeikaite V, Hamilton G, Wheeler VC, Pinto RM, Kwak S, Morton AJ, Monckton DG. Approaches to sequence the HTT CAG repeat expansion and quantify repeat length variation. J Huntingtons Dis. 2020; doi: 10.3233/JHD-200433
[108] Giuliano P. DNA damage induced by polyglutamine-expanded proteins. Hum Mol Genet. 2003;12:2301-9.
[109] Illuzzi J, Yerkes S, Parekh-Olmedo H, Kmiec EB. DNA breakage and induction of DNA damage response proteins precede the appearance of visible mutant huntingtin aggregates. J Neurosci Res. 2009;87:733-47.
[110] Disatnik M-H, Joshi AU, Saw NL, Shamloo M, Leavitt BR, Qi X, et al. Potential biomarkers to follow the progression and treatment response of Huntington's disease. J Exp Med. 2016;213:2655-69.
[111] Askeland G, Dosoudilova Z, Rodinova M, Klempir J, Liskova I, Kuśnierczyk A, et al. Increased nuclear DNA damage precedes mitochondrial dysfunction in peripheral blood mononuclear cells from Huntington's disease patients. Sci Rep. 2018;8:9817.
[112] Castaldo I, Rosa MD, Romano A, Zuchegna C, Squitieri F, Mechelli R, et al. DNA damage signatures in peripheral blood cells as biomarkers in prodromal huntington disease. Ann Neurol. 2019;85:296-301.
[113] Reddig A, Rübe CE, Rödiger S, Schierack P, Reinhold D, Roggenbuck D. DNA damage assessment and potential applications in laboratory diagnostics and

precision medicine. J Lab Precis Med. 2018;3: doi: 10.21037/jlpm.2018.03.06

[114] Carney B, Kossatz S, Lok BH, Schneeberger V, Gangangari KK, Pillarsetty NVK, et al. Target engagement imaging of PARP inhibitors in small-cell lung cancer. Nat Commun. 2018;9:176.

[115] Soleimany AP, Bhatia SN. Activity-based diagnostics: An emerging paradigm for disease detection and monitoring. Trends Mol Med. 2020;26:450-68.

[116] Rodrigues FB, Wild EJ. Huntington's Disease Clinical Trials Corner: April 2020. J Huntingtons Dis. 2020;9:185-97.

[117] Bravo-Arredondo JM, Kegulian NC, Schmidt T, Pandey NK, Situ AJ, Ulmer TS, et al. The folding equilibrium of huntingtin exon 1 monomer depends on its polyglutamine tract. J Biol Chem. 2018;293:19613-23.

[118] Vijayvargia R, Epand R, Leitner A, Jung T-Y, Shin B, Jung R, et al. Huntingtin's spherical solenoid structure enables polyglutamine tract-dependent modulation of its structure and function. eLife. 2016;5:e11184.

[119] Sun K, Mikule K, Wang Z, Poon G, Vaidyanathan A, Smith G, et al. A comparative pharmacokinetic study of PARP inhibitors demonstrates favorable properties for niraparib efficacy in preclinical tumor models. Oncotarget. 2018;9(98):37080-96.

[120] Durmus S, Sparidans RW, van Esch A, Wagenaar E, Beijnen JH, Schinkel AH. Breast cancer resistance protein (BCRP/ABCG2) and P-glycoprotein (P-GP/ABCB1) restricted oral availability and brain accumulation of the PARP Inhibitor Rucaparib (AG-014699). Pharm Res. 2015;32:37-46.

[121] Kadyrova LY, Gujar V, Burdett V, Modrich PL, Kary-rov FA. Human MutLg, the MLH1-MLH3 heterodimer, is an endonuclease that promotes DNA expansion. PNAS 2020:117(7):3535-3542.

[122] Fròsina G, Profumo A, Marubbi D, Marcello D, Ravetti JL, Daga A. ATR kinase inhibitors NVP-BEZ235 and AZD6738 effectively penetrate the brain after systemic administration. Radiat Oncol. 2018;13:76.