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Targeting NAD⁺ in translational research to relieve diseases and conditions of metabolic stress and ageing



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ABSTRACT

Nicotinamide adenine dinucleotide (NAD⁺) plays a fundamental role in life and health through the regulation of energy biogenesis, redox homeostasis, cell metabolism, and the arbitration of cell survival via linkages to apoptosis and autophagic pathways. The importance of NAD⁺ in ageing and healthy longevity has been revealed from laboratory animal studies and early-stage clinical testing. While basic researchers and clinicians have investigated the molecular mechanisms and translation potential of NAD⁺, there are still major gaps in applying laboratory science to design the most effective trials. This mini-review was based on the programme and discussions of the 3rd NO-Age Symposium held at the Akershus University Hospital, Norway on the 28th October 2019. This symposium brought together leading basic researchers on NAD⁺ and clinicians who are leading or are going to perform NAD⁺ augmentation-related clinical studies. This meeting covered talks about NAD⁺ synthetic pathways, subcellular homeostasis of NAD⁺, the benefits of NAD⁺ augmentation from maternal milk to offspring, current clinical trials of the NAD⁺ precursor nicotinamide riboside (NR) on Ataxia-Telangiectasia (A-T), Parkinson's disease (PD), post-sepsis fatigue, as well as other potential NR-based clinical trials. Importantly, a consensus is emerging with respect to the design of clinical trials in order to measure meaningful parameters and ensure safety.

1. Introduction

Ageing is a natural process which is increasingly being dissected for mechanisms that could be targeted with the hope of delaying or

limiting the rapid functional decline in resiliency seen in old age (Fang et al., 2015; Partridge et al., 2018). In order to address the global healthcare and socio-economic issues that are becoming apparent as medical advances extend human lives, research is aimed at exploring

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possible transformative interventional strategies and molecules that target ageing and age-predisposed diseases (Bakula et al., 2018; Campisi et al., 2019; Fang et al., 2016b; Kennedy et al., 2014; Lopez-Otin et al., 2013; Rubinsztein et al., 2011; Singh et al., 2019). Recently, nicotinamide adenine dinucleotide (NAD⁺) has entered the spotlight in ageing research, leading to many attempts to harness the potential of NAD⁺ and its precursors for use in the clinic. NAD⁺ coenzymes are the central catalysts of metabolism in all living cells, serving as critical components in energy production, metabolic transformations, detoxification of reactive oxygen species, and as a substrate for enzymes such as the PARPs, Sirtuins (SIRT1), CD38/CD157, and SARM1 (Bogan and Brenner, 2008; Canto et al., 2015; Fang et al., 2017; Lautrup et al., 2019c; Verdin, 2015; Yoshino et al., 2018).

Over the last decade, the importance of NAD⁺ in healthy ageing and longevity has been recognised, detailed molecular mechanisms unveiled, and many clinical trials explored. Studies from laboratory animals, such as in nematodes and mice, and in human primary cells and post-mortem tissues, as well as human brain imaging, indicate that there is an age-dependent reduction of NAD⁺ in cells and tissues (Fang et al., 2016a, 2014; Gomes et al., 2013; Hou et al., 2018; Lautrup et al., 2019c; Mouchiroud et al., 2013; Zhu et al., 2015). Mechanistically, it is suggested that ageing-induced NAD⁺ reduction may result from reduced production – as there is an age-dependent reduction of key enzymes involved in NAD⁺ metabolism – or increased consumption by NAD⁺-consuming enzymes, such as PARPs, CD38, and Sirtuins (Camacho-Pereira et al., 2016; Fang et al., 2016a, 2014; Lautrup et al., 2019c; Mouchiroud et al., 2013; Scheibye-Knudsen et al., 2014; Verdin, 2015). All three classes of enzymes compete for NAD⁺ during ageing, ultimately leading to a bioavailability level insufficient to sustain all NAD⁺-requiring cellular activities. Intriguingly, NAD⁺ repletion, by the supplementation of NAD⁺ precursors, such as nicotinamide riboside (NR) (Bieganowski and Brenner, 2004), nicotinamide mononucleotide (NMN), nicotinamide (NAM), or even NAD⁺ itself, delay ageing phenotypes and promote healthy longevity in both normal and accelerated ageing models in *Caenorhabditis elegans* (roundworms), *Drosophila melanogaster* (fruit flies), and mice (de Picciotto et al., 2016; Fang et al., 2019a, b; Fang et al., 2016a, 2014; Frederick et al., 2016; Gomes et al., 2013; Mills et al., 2016; Mitchell et al., 2018; Mouchiroud et al., 2013). Encouraged by animal studies, more than 20 clinical studies exploring whether NR may alleviate pathological ageing and age-predisposed diseases have been initiated. At least 5 clinical trials have been completed showing that 1–2 g/day of NR for up to 1–3 months is safe, as summarised recently (Lautrup et al., 2019c). While there were encouraging results in some NR-based phase 1 clinical trials aiming to reduce blood pressure in healthy middle-aged and older adults (Martens et al., 2018) and to slow disease progression in amyotrophic lateral sclerosis (ALS) (NR + pterostilbene) (de la Rubia et al., 2019), no effect was reported in trials of short-term (up to 2–3 months) NR supplementation in obese, insulin-resistant men (Dollerup et al., 2018) and nondiabetic males with obesity (Dollerup et al., 2019), nor in muscle-mitochondrial bioenergetics in aged men (Dollerup et al., 2019). Of note, all three reports were from the same study and reported different outcomes from the same set of obese men (NCT02303483). Possible considerations include a much higher dose of NR (2 g/day) than other trials (mostly 1 g/day) and a different NAD⁺ detection method. Thus, these studies emphasise some of the challenges with clinical trials of NAD⁺-boosting compounds with regards to dose and assessment of NAD⁺ bioavailability.

The Norwegian Centre on Healthy Ageing (NO-Age) aims to establish a multi-disciplinary research network to address the challenges of ageing and to foster translational studies to promote healthy ageing, healthy lifestyles and social participation in old age (Fang et al., 2019c). On the 28th of October 2019, the 3rd NO-Age Symposium took place, covering NAD⁺ in depth: from biochemistry to clinical trials. The event was hosted at the Akershus University Hospital (Ahus, Lørenskog, Norway), and was organised by Evandro F. Fang, Hilde Nilsen, Linda

Bergersen and Jon Storm-Mathisen, founding members of the NO-Age network. Here we provide a summary of the main points of the symposium.

2. An overview of the meeting

2.1. The basics of NAD⁺

NAD⁺ has a central function in metabolism, where it functions in redox reactions. NAD⁺ switches back and forth between its reduced (NADH), and oxidised forms (NAD⁺). NAD⁺ is reduced to NADH during catabolic processes – the burning of fats, carbohydrates, and proteins, while its reduced counterpart is used for anabolic purposes – building new molecules such as ATP, glucose, and ketone bodies (Bogan and Brenner, 2008; Lautrup et al., 2019c). NAD⁺ is also important as a substrate or coenzyme in certain non-redox reactions involved in several cellular processes including, but not limited to: pathways central in ageing, DNA repair, calcium signalling and gene regulation (Camacho-Pereira et al., 2016; Fang et al., 2016a, 2014; Lautrup et al., 2019c; Mouchiroud et al., 2013; Verdin, 2015).

Charles Brenner, the keynote speaker from the University of Iowa (Iowa, USA), started his lecture by explaining why we need a broader definition of metabolism. This argument is clearly emphasised by NAD⁺ having functions reaching far beyond anabolism and catabolism. Since his discovery of NR as an NAD⁺ precursor in 2004 (Bieganowski and Brenner, 2004), Brenner has expanded his research on NAD⁺ from metabolism to biomarker development and clinical trials for a broad variety of diseases. Failing metabolic homeostasis is observed in chronic heart failure (Diguët et al., 2018), Brenner and co-workers therefore investigated the expression of NAD⁺ biosynthetic enzymes in a mouse model of dilated cardiomyopathy and found a 30 % decrease in NAD⁺ levels, a downregulated recycling enzyme, and an upregulation of nicotinamide riboside kinase 2 (NRK2) (Diguët et al., 2018). By supplementing the mice with nicotinamide riboside (NR) they showed increased levels of NAD⁺ and several of its metabolites, which helped to stabilise their metabolism and attenuated the development of heart failure in a mouse model of dilated cardiomyopathy (Diguët et al., 2018).

Another metabolic stressor found to challenge the NAD⁺ metabolome is the postpartum period. A recent study by the Brenner group shows that postpartum rats have a suppressed NAD⁺ metabolome in the liver (Ear et al., 2019). Furthermore, raised blood levels of NAD⁺ were found, increasing the levels of NAD⁺ in the mammary glands at the liver's expense. By supplementing the mothers with NR, Brenner and colleagues observed improved nursing behaviour and nutrient transfer to the pups (Ear et al., 2019). Several positive, lasting changes in neurodevelopment and metabolic parameters were also noted (Ear et al., 2019). Brenner's experiment is one of many showing how stressors can affect the NAD⁺ pool at a physiological level. NAD⁺ seems to be redirected or shuttled to areas with high metabolic demand.

Elucidating just how dynamic the flux of NAD⁺ is on a cellular level has been a focus in recent work of Mathias Ziegler from the University of Bergen (Bergen, Norway), who has developed tools to identify the intracellular pools of NAD⁺. NAD⁺ is found in the mitochondria, nucleus, Golgi apparatus, peroxisomes and in the endoplasmic reticulum (ER) and there is a balance of NAD⁺ between the different sub-cellular compartments (Lautrup et al., 2019c; Stromland et al., 2019). By selectively depleting cell compartments of NAD⁺ with an EGFP-PARP1cd (the PARAPLAY system (Dolle et al., 2010)), Ziegler can examine the resulting effects on a single-compartment basis (VanLinden et al., 2015, 2017). By tracking the formation of NAD⁺ from stable isotope-labelled NAD⁺ precursors and simultaneously monitoring the disappearance of unlabelled NAD⁺, it is possible to determine cellular NAD⁺ turnover rates. Thereby, the impact of cellular insults on compartmental NAD⁺ metabolism can be established, even if the NAD⁺ concentration itself remains unchanged. Despite having a rather large NAD⁺ pool,

mitochondria are sensitive to direct changes to their NAD⁺ availability, both through depletion and satiety, and compensate by redistributing NAD⁺ from the cytosol to mitochondria. Mitochondria appear to play an important role in supporting NAD⁺-dependent processes throughout the cells under conditions that are accompanied by NAD⁺ depletion (VanLinden et al., 2015; Yang et al., 2007).

2.2. NAD⁺ in accelerated ageing and age-predisposed neurodegeneration

Hilde Nilsen from Ahus and UiO (Oslo, Norway) shared her understanding of NAD⁺ in Ataxia-Telangiectasia (A-T), a rare disease with features of premature ageing caused by defects in DNA repair (Fang et al., 2016b; Shiloh and Lederman, 2017; Shiloh and Ziv, 2013). Key features of A-T include impaired coordination of muscle movements, telangiectasia, cancer predisposition, sensitivity to DNA damaging agents (especially DNA double-strand break inducers), immunodeficiency, vestigial thymus and gonads, endocrine abnormalities, as well as neurodegeneration (Shiloh and Lederman, 2017). The majority of the A-T phenotypes can be explained by an impaired DNA damage response due to mutations in the gene *ATM* which encodes ATM (Shiloh and Ziv, 2013); Vilhelm Bohr and Evandro Fang, in collaboration with the Nilsen group and others, reported that a sustained DNA damage response drove NAD⁺ depletion in ATM-defective animal models, leading to mitochondrial dyshomeostasis and progressive neuronal loss (Croteau et al., 2017; Fang and Bohr, 2017; Fang et al., 2016a). In this study using *C. elegans*, Nilsen provided experimental evidence that hyper-PARYlation consumes NAD⁺ in *atm*-mutant nematodes. Moreover, it was demonstrated that augmentation of NAD⁺ levels through NR supplementation, PARP inhibition, or Sir2.1 activation, improved healthspan and extended lifespan in the mutants (Croteau et al., 2017; Fang and Bohr, 2017; Fang et al., 2016a). These findings were conserved from worms to mice, indicating the potential for therapeutic intervention in A-T.

Sofie Lautrup from Ahus and UiO (Oslo, Norway) presented a study on the autosomal recessive accelerated-ageing disorder Werner syndrome (WS), a classical DNA repair impairment-oriented premature ageing disease. From their second and third decade of life, WS patients develop a relatively short stature, display greying and loss of hair, prematurely aged faces, and juvenile cataracts, and are predisposed to cancer, dyslipidemia, premature atherosclerosis, and insulin-resistant diabetes (Oshima et al., 2017; Shamanna et al., 2017; Takemoto et al., 2013). Several major features of WS can be explained by a DNA repair deficiency caused by mutations in the gene encoding the Werner protein (WRN), an important DNA repair protein with unique helicase and exonuclease activities (Chan et al., 2019; Shamanna et al., 2016; Yu et al., 1996; Zhang et al., 2015). However, the molecular mechanisms underlying severe dysregulation of energy metabolism in WS is still not fully understood (Takemoto et al., 2013). Drs. Evandro Fang and Vilhelm Bohr led a project with data showing reduced NAD⁺ levels in WS patients and WS worms, possibly due to increased cellular NAD⁺ consumption by the PARPs, and reduced NAD⁺ production as evidenced by a decline in the levels of NMNAT1, which converts NMN to NAD⁺ (Fang et al., 2019a). The *D. melanogaster* WRN is a 353 amino acid protein with only the exonuclease domain (Cox et al., 2007; Lautrup et al., 2019a). Lautrup and colleagues showed that the knockdown of *WRN^{Nexo}* gene mutation of *WRN* in flies resulted in a short lifespan and impaired proliferation of intestinal stem cells (ISCs); NAD⁺ augmentation by supplying either NR or NMN was able to extend lifespan in both *WRN^{Nexo}* knockdown and WT flies, as well as restoring proliferative capacity of ISCs in the *WRN^{Nexo}* knockdown flies (Fang et al., 2019a). NAD⁺ augmentation also improved mitochondrial homeostasis and metabolism in both WS patient cells and WS animal models. Such data suggest reduced cellular NAD⁺ is a likely player in WS pathology, highlighting targeting of the NAD⁺ pool as an interventional target for WS patients. A clinical study in Werner patients is underway.

Yahyah Aman from Ahus and UiO (Oslo, Norway) presented on the

potential uses of NAD⁺ precursors in delaying the progression of Alzheimer's disease (AD). Although AD is most often characterised by accumulation of amyloid- β (A β) plaques and tau tangles, therapies targeting these proteinaceous deposits have given disappointing results in clinical trials, thus, there is a drive to develop novel therapeutic alternatives (Cummings et al., 2018; Kerr et al., 2017). Along with A β and tau, damaged mitochondria accumulate in the neurons of AD patients; a common pathological phenomenon partially due to compromised mitophagy (Fang, 2019; Fang et al., 2019b; Kerr et al., 2017; Lautrup et al., 2019b). Indeed, impaired mitophagy in AD was observed in human post-mortem tissue and in both iPSCs and animal models of AD. Moreover, pharmacological (including the use of NAD⁺ precursors) and genetic promotion of mitophagy alleviated memory loss in A β ₁₋₄₂ (CL2355) AD worms, tau (BR5270) AD worms, APP/PS1 AD mice and 3xTg AD mice (Fang et al., 2019b). The underlying mechanisms of NAD⁺-regulated mitophagy have been summarised by the Fang group elsewhere (Fang, 2019). Aman is now working on understanding the molecular mechanisms of how mitophagy eliminates p-tau in different laboratory model systems.

2.3. Clinical trials

Norway is being put on the map as a small country with an intense interest in NAD⁺ precursors. Several clinical trials are either ongoing or planned to start shortly.

Hilde Nilsen gave an update on an open-label observation trial on NR supplementation in children with A-T based on the results of the basic research she presented (mentioned above). Norway has established a nation-wide program for disease monitoring for this patient group that includes standardised longitudinal motoric testing. In this trial, A-T patients are given NR as a dietary supplement. Changes of blood NAD⁺ and related metabolites are monitored over a period of two years parallel to regular motoric testing and clinical follow-up.

Ole-Bjørn Tysnes from NeuroSysMed, Haukeland University Hospital (HUH) and the University of Bergen (UiO) (Bergen, Norway) gave a summary on the current literature on oxidative stress, NAD⁺ reduction and ALS. Major clinical phenotypes of ALS include impairment of voluntary muscle movement caused by the progressive degeneration of motor neurons in the spinal cord, brain stem, and motor cortex (Tang, 2017). ALS has complicated etiologies, with mitochondrial dysfunction, increased oxidative stress, NAD⁺ reduction, and impaired gut microbiota proposed as being implicated in some patients' groups (Blacher et al., 2019; Carri et al., 2017; Lautrup et al., 2019c). A recent study of the long-term ALS mortality trends in Norway between 1951 and 2014 revealed 5345 ALS deaths during this period, with a mean annual increase of 1.14 % (Nakken et al., 2016). Age-period-cohort analyses suggest that this increase was best explained by cohort effects due to environmental impact and improved case ascertainment.

Recent studies link impaired NAD⁺ metabolism to ALS pathology. A study reported that enhancement of the NAD⁺ salvage pathway protects astrocytes expressing an ALS-linked mutant SOD1, hereby promoting motor neuron survival (Harlan et al., 2016). Pharmacological (NR or NMN) or genetic (NAMPT overexpression) approaches to restore cellular NAD⁺ levels increased oxidative stress resistance in motor neurons (Harlan et al., 2019, 2016). Congruently, a recent study found low abundance of the protective bacterium *Akkermansia muciniphila*, associated with impaired NAD⁺ metabolism in ALS (Blacher et al., 2019). Compared with healthy controls, NAM depletion was evident in both serum and cerebrospinal fluid (CSF) samples from ALS patients; accordingly, NAM supplementation dramatically improved both behavioural and pathological phenotypes in ALS-prone SOD1-Tg mice (Blacher et al., 2019). Encouragingly, a recent preliminary clinical trial with EH301 (NR + pterostilbene) indicated potential benefits for ALS patients (de la Rubia et al., 2019). Tysnes is planning to run a similar trial, termed NO-ALS, from Bergen, evaluating the value of NAD⁺ augmentation in treating ALS.

Charalampos Tzoulis from the NeuroSysMed, Center, Haukeland University Hospital (HUH) and University of Bergen (UiB) (Bergen, Norway) gave updates on the randomised, double-blinded clinical trials NAD-PARK and NO-PARK. Parkinson's disease affects ~1.8 % of the population above the age of 65 years (de Rijk et al., 2000). The neuropathological hallmark of PD is the progressive loss of dopaminergic neurons in the *substantia nigra pars compacta* (SNc). Additional neurodegenerative changes occur in multiple regions of the nervous system and patients suffer progressive disability due to a combination of progressive motor impairment and non-motor dysfunction (Dickson, 2012).

Studies in post-mortem brain tissue from individuals with PD have shown impaired mitochondrial function, including complex-I deficiency (Flones et al., 2018) and defective mtDNA maintenance (Dolle et al., 2016). Furthermore, similar to AD and ALS, studies in patient iPSC-derived dopaminergic neurons, and animals modeling aspects of PD pathology have shown evidence of perturbed NAD⁺ metabolism. Moreover, NAD⁺ augmentation improved survival of dopaminergic neurons in fly-based models and patient iPSC-derived dopaminergic neurons (Lautrup et al., 2019c; Schondorf et al., 2018). For the nearly-completed NAD-PARK trial, 30 PD patients were recruited at the Department of Neurology, HUH, then randomly assigned (1:1) to one of two study groups (n = 15 per group): NR 500 mg x 2/day, or placebo (NCT03816020). While they are now assessing the results of NAD-PARK, Tzoulis is leading the phase 2 clinical trial (NO-PARK) where they are recruiting 400 patients with newly diagnosed PD and randomly assigning them to either NR (500 mg x 2/day) or placebo for a period of 52 weeks (NCT03568968).

Torbjørn Omland from Ahus and UiO (Oslo, Norway) gave a presentation of a planned clinical trial to evaluate the ability of NR to reduce chemotherapy-induced side effects in the cardiovascular system. Omland, together with Fang and Nilsen, is planning a clinical trial evaluating NR supplementation parallel to adjuvant therapy for breast cancer. Breast cancer is the most frequently occurring malignancy in Norwegian women, and the incidence has nearly doubled in the past 40 years (Cancer in Norway 2016). Unfortunately, many breast cancer survivors suffer from treatment-induced side effects, such as heart failure and other manifestations of cardiovascular malfunction. There is a limited understanding of the molecular mechanisms driving development of these. The study will extend and benefit from local infrastructure built through the PRADA (PREvention of cArDiac Dysfunction during Adjuvant breast cancer therapy) study program that encompasses women with early breast cancer receiving adjuvant therapy. PRADA III, the NR study, aims to show if NAD⁺ supplementation is capable of reducing the side effects of cancer patients receiving chemotherapy. The rationale for supplementing with NR is to restore NAD⁺ levels depleted following chemotherapy-induced PARP1-hyperactivation.

Arne Søråas from the Oslo University Hospital (OUS, Oslo, Norway) presented a planned clinical trial, also addressing PARP1-induced NAD⁺ depletion (NCT04110028). Arne Søråas is a post-doctoral fellow and project group leader at the research group for genome and epigenome regulation in embryo development, ageing, and disease. The research group studies geroscience with a translational aim and has set up the study in collaboration with several intensive care units at OUS to improve patient outcomes in the recovery phase after severe acute illness.

Recovery is often prolonged after a severe illness in aged patients, and even younger patients experience fatigue which leads to lengthy hospital stays, incomplete recovery, and increased mortality (Boyd et al., 2008; Hermans et al., 2014; Iwashyna et al., 2010). Currently optimal care, nutrition, and mobilisation are the best options for improving patient outcomes in this phase, and, to our knowledge, no pharmacological interventions are available. The severity of fatigue after acute illness is associated with the type of disease and extent of tissue damage suffered (Iwashyna et al., 2010). Tissue damage, usually

caused by infections, circulation disturbances, surgery, or trauma is a known activator of the nuclear NAD⁺ consuming PARP1 pathway (Luo and Kraus, 2012), and the study aims to show if NAD⁺ augmentation is capable of improving patient outcome during recovery.

In the trial it is planned that hospitalised patients will be randomised into groups and given either NR or placebo when they are clinically stabilised and still expected to stay in the hospital for at least one week. The primary endpoint is the duration of hospital stay after randomisation. Secondary endpoints include measures of the completeness of recovery. NR will be administered over three months to cover the whole recovery period. NR has not been supplemented in this group before, and the study is also a dose-ranging study where increasing doses of NR (from 250 mg to 2000 mg daily) are tested. The NAD⁺ metabolome of peripheral blood mononuclear cells will be measured in some of the participants, while epigenetic changes including changes to DNA methylation clocks and biochemical status will be measured in all patients.

Trygve Holmøy from Ahus and UiO (Oslo, Norway) chaired a panel discussion that tackled several emerging questions, spanning from how to determine appropriate doses of NR for clinical trials, to revealing/monitoring potential side effects, to reasons for some negative NR-based clinical trials. There is a strong need to implement reliable biomarkers, particularly in neurodegenerative diseases where clinical scoring systems are rather insensitive and require long follow-up. Neurofilament light chain is now emerging as a promising biomarker that reflects treatment effects in several neurodegenerative diseases (Gaetani et al., 2019). Moreover, there is a paucity of observational data from humans during health and disease that would allow us to predict potential treatment effects and thereby guide power calculations in clinical studies. Such discussions were very useful in view of the ongoing or planned NR-based clinical trials in Norway.

3. Emerging questions on how to design a better clinical study

While the majority of laboratory and clinical data suggest a strong translational potential for NAD⁺-boosting compounds, some studies have reported little-to-no effect, raising new questions to be addressed on challenges and barriers to the translation of NAD⁺-boosting compounds. For example, negative data from the study of NR in obese men (NCT02303483) suggests that further considerations on dose, treatment period, number and diversity of the patients need to be taken into account in future clinical studies, as well as the use of sensitive and stable NAD⁺ assessment methods. In the Q&A section, major questions and discussions related to how best to determine appropriate doses of NR in clinical trials, tips for experiment design, and the optimal timeline to watch for NAD⁺-related benefits. While there has been enough data on the pharmacokinetics and toxicity profile of NR in the healthy elderly, it is conceivable that children and patients with different diseases may have different responses to NAD⁺-boosting compounds, thus independent data on pharmacokinetics and toxicity should be established for each specific disease and age group. Accordingly, the aetiologies of each disease should be carefully considered to determine the optimal period for NAD⁺-boosting, compound-based treatment. The speakers also recommend including 'exercise' and 'exercise + NAD⁺ boosting compound' groups since exercise may play a synergistic role in NAD⁺-related benefits to metabolism and muscle functions.'

4. Closing remarks

Over the past decade, our understanding of NAD⁺ has expanded beyond its crucial role in bioenergetics and metabolism to its new role as a key component in healthy longevity. Growing evidence from laboratory animals to humans suggest an age-dependent reduction of NAD⁺, while NAD⁺ augmentation has been shown to improve healthspan and extend lifespan, as well as alleviate the symptoms of a broad range of age-related diseases, including AD, PD, ALS etc., in

laboratory animal models (Fang et al., 2017; Lautrup et al., 2019c; Verdin, 2015). Accordingly, several clinical trials of NR are planned or ongoing (<https://clinicaltrials.gov/>).

Despite the optimism there are still outstanding questions in the field: first, further study is needed on the detailed molecular mechanisms and links between NAD⁺, healthy ageing, and longevity, including its neuroprotective effects. Second, the relationship between NAD⁺ reduction and ageing in a large, human population setting needs to be determined. Third, while NR seems to be a promising supplement in a wide range of diseases, we must not forget that clinical trials are still small and in early phases, and that possible negative long-term effects could still become apparent. Furthermore, the precise relationships between NAD⁺ and different cancers should be addressed, since NAD⁺ augmentation has been shown to have tumour-promoting effects in laboratory animal model (Demarest et al., 2019; Nacarelli et al., 2019) but also to inhibit breast cancer in mice (Santidrian et al., 2013) and skin cancers in high-risk patients (Chen et al., 2015).

Declaration of Competing Interest

E.F.F. and H.N., have CRADA arrangements with ChromaDex. E.F.F., H.N., L.H.B., J.S.M., L.J.R. are consultants to Aladdin Healthcare Technologies. E.F.F. is a consultant to the Vancouver Dementia Prevention Centre. C.B. is Chief Scientific Adviser and a stockholder of ChromaDex.

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