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Geroprotectors: A role in the treatment of frailty

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ABSTRACT

The proportion of the population over the age of 65 is growing the most rapidly due to the longevity revolution. Frailty is prevalent in this age group and strongly associated with disability and hospitalization, having a significant impact on the costs of health and social care. New effective interventions to delay or reverse frailty are urgently required. Geroprotectors are a new class of drugs, which target fundamental mechanisms of ageing and show promise in delaying the onset of or boosting resilience in frail older people. However, there are challenges to their clinical translation. Here we review the literature for evidence that frailty can be delayed or reversed and geroprotectors can improve frailty in murine models and in patients. We will then discuss the challenges, which make their clinical testing complex and propose potential options for moving forward.

1. Introduction

Although currently there is no universally agreed operational definition for frailty (Rodriguez-Manas et al., 2013), it is considered to be a distinctive clinical syndrome related to the ageing process in which multiple body systems gradually lose their in-built reserves, leading to an accumulation of deficits, and loss of resilience, i.e., the ability to recover from adverse events (Whitson et al., 2016). It is often associated with increased vulnerability and dependency. Around 10% of people aged over 65 years have frailty, rising to 25–50% of those aged over 85 years (Clegg et al., 2013). With the population of those aged > 65 years due to nearly double in the next 30 years, there is an urgent need to find interventions to delay or reverse frailty and improve resilience in older people so that they can maintain independence and good quality of life for longer.

Recent progress in the field of ageing research has led to the development of interventions with the potential to target frailty, also termed "geroprotectors" (Figueira et al., 2016). The main characteristic of this new class of drugs is the ability to target fundamental mechanisms of ageing, such as responses to oxidative damage, inflammation, senescence, which underpin multiple deficits occurring simultaneously (Bellantuono, 2018). For this reason, they are believed to have the potential to delay or even reverse frailty in older people and improve their responses to adverse events. Over 200 compounds have been classified as geroprotectors, each reported to slow ageing and/or extend lifespan in a variety of organisms (geroprotectors.org). Here we review the literature for evidence that frailty can be reversed and that geroprotectors can play an important role. We will then examine the barriers, which prevent their clinical testing and propose potential options to move forward.

2. Definition and assessment of frailty

Central to the assessment of whether frailty can be reversed is how frailty is defined and measured. There are two main definitions of frailty. The "Fried frailty phenotypic model" defines frailty as a distinct clinical syndrome meeting three or more of five phenotypic criteria: weakness, slowness, low level of physical activity, self-reported exhaustion, and unintentional weight loss (Fried et al., 2001). It describes patient characteristics, which, if present, can predict mortality risk. This model also allows for the possibility of fewer characteristics being present, in which case patients are considered pre-frail. By contrast, the cumulative deficit frailty model proposed by Rockwood assumes an accumulation of deficits (ranging from symptoms e.g., loss of hearing or low mood, signs such as tremoring, through to various diseases such as dementia) which can occur with ageing and, when combined, can be captured in a 'frailty index' (FI). A higher FI is associated with increased risk of death (Rockwood et al., 2005). Both Fried and Rockwood define frailty quite differently with a high impact of physical impairments in the Fried phenotype and accumulation of body deficits in the Rockwood

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model, describing frailty as almost two separate conditions. Both have limitations; the Fried frailty phenotype assessment is not very sensitive as a quantitative measure while the Rockwood FI was designed as a tool to measure severity of frailty after a comprehensive geriatric assessment. It is not validated for measuring improvement in individuals after an acute illness for example or an intervention. Even though the "Fried" frailty phenotype and the "Rockwood" FI are most accepted as a measure of frailty (for review see (von Zglinicki et al., 2016), they are not comprehensively used and often different investigators establish their own modified versions. In general, this makes a direct comparison of studies to assess the efficacy of interventions very difficult.

3. Is it possible to delay or reverse frailty?

Increased physical activity – from household activities to structured exercise training – has been proposed as a key intervention in treating frailty (Aguirre and Villareal, 2015; Fried, 2016; Liu and Fielding, 2011). Although a large number of trials have provided evidence of some beneficial effects of exercise interventions on populations with frailty (systematically reviewed in (Cadore et al., 2013; Chin A Paw et al., 2008; Daniels et al., 2008; Theou et al., 2011), it is very difficult to assess how meaningful these effects are for patients in terms of improving their ability to live independently or to overcome adverse events. This is due to the different definitions and measurements of frailty used, the large heterogeneity of the applied exercise protocols (i.e., type, frequency, duration), and the differences in age, gender, and ethnicity of the participants. Even when randomized control trials (RCT) were performed using a standardized operational definition of frailty as inclusion criteria, the differences in measures of physical performance resulted in variable outcomes (de Labra et al., 2015; Giné-Garriga et al., 2014; Lozano-Montoya et al., 2017).

Only few studies have assessed frailty longitudinally at baseline and post-intervention, but they appear to be more informative in addressing whether exercise can not only treat, but also reverse frailty (Apóstolo et al., 2018; Lee et al., 2012; Puts et al., 2017). Two separate studies, which have used Fried Frailty Criteria (FFC) at baseline and as an outcome measure, have shown significant reductions in the prevalence and level of frailty in community-dwelling elders after a multicomponent physical activity intervention of 6-months (Ng et al., 2015) and 12-months duration (Cesari et al., 2015), respectively. While both studies included pre-frail and frail participants as defined by FFC, Cesari et al. (Cesari et al., 2015) also included non-frail individuals. Cesari et al. (Cesari et al., 2015) reported that both the prevalence of frailty at 12 months and the number of frailty criteria was significantly reduced following physical activity. Ng et al., 2015, reported that the physical activity intervention lead to significant reductions in the mean frailty scores already at 3 months, which persisted to 6 months (end of intervention) and was maintained at 12-month follow-up assessments compared with the control group.

In other studies, exercise interventions have been combined with nutritional consultation (Chan et al., 2012; Serra-Prat et al., 2017) and/or supplementation (Kim et al., 2015). Chan et al., (Chan et al., 2012) selected Taiwanese older adults that were considered pre-frail or frail according to an adapted version of FFC and assessed frailty status directly after a 3-month intervention including exercise training (aerobic and strength exercises, 3x/week) and nutritional consultation, and at a 6-month follow-up. At 3 months, the intervention group had a significantly higher improvement rate in frailty status (pre-frail to robust/frail to pre-frail or robust) compared with the control group. However, in contrast to the study by Ng et al. (Ng et al., 2015), this was not the case at 6 or 12 months follow-up, suggesting that beneficial effects of exercise might not last when intensive interventions are terminated.

Similarily, Serra-Prat et al. (Serra-Prat et al., 2017) studied the effect of an exercise intervention (aerobic, strength, and balance training) combined with good nutrition on preventing frailty in community-dwelling pre-frail older people. Along with baseline FFC assessments, the participants were screened for malnutrition using the Short-Form Mini Nutritional Assessment questionnaire (MNA-sf) and those at risk were referred to the Nutritional Unit for further assessment, and the establishment of the usual dietary recommendations. On follow-up at 12 months, fewer participants in the intervention group had evolved from pre-frail to frail (4.9% vs. 15.3% respectively), and 15–20% of pre-frail participants returned to robustness, although this effect did not reach statistical significance.

The effects of a combined exercise and nutritional supplementation intervention have also been assessed (Kim et al., 2015). Frail (three or more criteria according to FFC) community-dwelling Japanese women were randomly divided into four groups: exercise (strength, balance, and gait training) without (placebo) or with nutritional supplementation of milk fat globule membrane (MFGM), or MFGM/placebo only. At post-intervention (3 months), the mean number of frailty criteria (out of five) significantly decreased in all four intervention groups. However, the effect was maintained at the 7-months follow-up only in the groups receiving exercise. The number of participants with frailty decreased in both exercise groups (exercise + placebo, exercise + MFGM) compared with control groups (placebo and MFGM only) with the combination of exercise and MFGM showing the greatest effect. Although not limited to interventions of physical activity/exercise, a very recent systematic review (Apóstolo et al., 2018) provides a comprehensive overview of the effects of different types of interventions (e.g., physical activity, multifactorial, psychosocial, cognitive, and nutritional) to prevent the progression of pre-frailty and frailty in older adults. All of these studies came to the conclusion that, amongst all interventions, exercise and nutrition were the most successful in reducing frailty. In agreement with Kim et al., (Kim et al., 2015), the combination of exercise and nutrition was the most beneficial for reducing frailty, while these improvements were not achieved when using either exercise or nutrition alone.

Until now, only few studies have investigated the effects of exercise interventions on frailty as defined by the Rockwood FI (Jones et al., 2004). Within the Beijing Longitudinal Study of Ageing (BLSA), both FFC and the Rockwood FI were assessed over a period of 8 years in order to investigate the relationship between frailty and physical activity (Ma et al., 2018). This study showed that low physical activity, defined by the self-reported questionnaire known as the "Beijing Longitudinal Study of Ageing leisure time Physical Activity Questionnaire" (BLSA-PAQ), was associated with increased FFC (Fried et al., 2001) and increased Rockwood FI (Jones et al., 2004), worse physical function, and higher risk of mortality in Chinese older adults.

The relationship between self-reported physical activity and Rockwood FI in older adults (above 50 years) has also been assessed within the English Longitudinal Study of Ageing (ELSA) (Rogers et al., 2017). More specifically, the Rockwood FI of participants classified as non-frail at baseline (Rockwood FI below or equal to 0.25) was assessed longitudinally over a period of 10 years in order to investigate how the change in FI over time varies by intensity of weekly physical activity. Compared to sedentary and mild physical activity (e.g., laundry, vacuuming), moderate physical activity (e.g., gardening, washing car) reduced the progression of frailty only in some age groups (particularly above 65 years) whereas vigorous activity (jogging, cycling at least 1x/week) significantly reduced the trajectory of frailty progression in all older adults. The results of this study thus suggest that high intensities of physical activity are needed in order to significantly reduce trajectories of frailty and should thus be encouraged, even at very old age. It also highlights that exercise in combination with other interventions can have additional effects in delaying or reversing frailty.

All together, these data suggest that it is possible to delay or even reverse frailty and that combination therapies may be better to maximize benefits.

4. Geroprotectors and frailty in preclinical studies

Both the FFC and the FI have been reverse translated into the emerging field of mouse frailty models (reviewed in (Kane et al., 2016b; Seldeen et al., 2015), with both methodologies showing an increase in frailty with age (Jones et al., 2004; Liu et al., 2014; Whitehead et al., 2014). Based on the Rockwood deficit accumulation model (Jones et al., 2004), a mouse frailty index was established in which 31 parameters are assessed non-invasively (Whitehead et al., 2014). A mouse frailty phenotype scale, mimicking the FFC, was also established (Liu et al., 2014). Four criteria (grip-strength, walking speed, physical activity, and endurance) were measured and for each criteria a scoring of 1.5 standard deviations below the group mean was considered positive. When a mouse presented with at least three positive criteria, it was categorized as frail. With this method, 9% of C57BL6/J mice were classified as frail at 28 months of age, which is a similar incidence to that observed in humans. As with studies in humans, researchers have used modified versions. The so-called "Valenica Score" based on the mouse equivalent of the FFC, is a measurement of five components (weight loss, weakness (grip-strength), poor endurance and slowness (incremental treadmill test) and low activity level (motor coordination)) (Gomez-Cabrera et al., 2017). Using this method, the frailty status of male C57BL/6 J mice, with and without free access to a running wheel, was assessed at ages 17, 20, 23, 26, and 28 months. The mice with free access to a running wheel performed significantly better than the sedentary animals in all the frailty criteria measured. At all ages, the percentage of frail mice was significantly higher in the sedentary mice compared to the wheel-runners. Graber et al. (Graber et al., 2015) measured frailty using the Frailty Intervention Assessment Value (FIAV) based on (Liu et al., 2014) and using the following four criteria: inverted cling grip test (overall muscle strength and endurance), rotarod speed (overall neuromotor function, walking speed), physical activity (mean km/week of voluntary wheel running), and a derived endurance score (mean of maximum time on rotarod and grip test). They measured FIAV before and after a 4-week intervention of voluntary wheel running in individual adult (6 months) and old (28 + months) male C57BL/6 J mice (Graber et al., 2015). Voluntary wheel running was able to maintain or improve the individual FIAV score in both age groups, however, the adult mice benefited more from the intervention. It should be noted that only two of the old mice (n = 11) were categorized as pre-frail and frail, respectively at baseline. Nevertheless, no mice were classified as frail after the exercise suggesting that aerobic exercise may revert frailty both at earlier and later stages of life in mice. This FIAV assessment tool was also used more recently to investigate the effect of high intensity interval training (10-minute uphill treadmill HIIT sessions 3x/week over 16 weeks) on frailty status in 24-month-old male C57BL/6 J mice (Seldeen et al., 2018). In this study, five of the six mice that were frail or pre-frail at baseline had reduced FIAV scores after the intervention, with four ultimately becoming non-frail/robust. Both studies showed improvements in multiple physical performance indicators (strength, endurance and gait-speed), as well as morphologic (e.g., fiber-type) composition (Graber et al., 2015), muscle mass and fiber cross-sectional area (Seldeen et al., 2018) and metabolic changes (e.g., mitochondrial biogenesis (Graber et al., 2015) and biomass (Seldeen et al., 2018)) in response to the exercise intervention. These data suggest that it is possible to measure frailty in mice and that these measurements are sufficiently discriminatory to measure improvement following interventions with similar outcomes to those seen in patients.

Geroprotectors have shown an ability to delay the onset of multiple tissues' dysfunction, multiple concurrent age-related diseases and boost resilience by modulating mechanisms of ageing such as senescence, autophagy, and inflammation (Bellantuono, 2018; Riera and Dillin, 2015). Drugs such as rapamycin, resveratrol, metformin, senolytics (e.g. fisetin, dasatininb, and querectin), which remove senescent cells, can improve the healthspan of multiple systems including cardiac, cognitive, neuromuscular, metabolic, and immune systems, and slow the development of cataracts, sarcopenia, osteoarthritis, osteoporosis, atherosclerosis, and Alzheimer's diseases in murine models (Baker et al., 2016; Childs et al., 2016; Farr et al., 2017; Jeon et al., 2017; Martin-Montalvo et al., 2013; Mitchell et al., 2018; Neff et al., 2013; Roos et al., 2016; Xu et al., 2018; Yousefzadeh et al., 2018). The evidence that geroprotectors can delay the onset of multiple age-related diseases suggest that they can improve aspects of frailty such as multimorbidity, when present. The fact that they can improve deficits in multiple systems and increase reserves support the notion that they can improve several clinically relevant measures associated with frailty, including strength, endurance, balance, and walking speed (Martin-Montalvo et al., 2013; Mitchell et al., 2018; Neff et al., 2013; Xu et al., 2015) and suggests that they have potential to delay or even reverse frailty and to boost the ability to resist or recover from adverse events. Indeed, the frailty index based on the Rockwood accumulation deficit model (FI) has been used in the testing of geroprotectors. Angiotensin II inhibition by either receptor blockade or synthesis blockade has been used as a primary and effective medication in cardiovascular diseases for decades. As levels of angiotensin II have been shown to increase with ageing and many of its negative actions occur in age-related diseases (Abadir, 2011; Dutka et al., 1996), these drugs have been revisited for various other indications including as a strategy to slow ageing (Benigni et al., 2010; de Cavanagh et al., 2011; Kosugi et al., 2006; Tuttle, 2006). Indeed, recently, the ACE inhibitor enalapril was shown to attenuate frailty in older mice (Keller et al., 2018). Interventions using both calorie restriction and resveratrol, known to confer improvement in healthspan across multiple organ systems (Bhullar and Hubbard, 2015; Lee and Longo, 2016), were also associated with a significant reduction in FI scores in C57BL/6 J mice, compared with age-matched controls (Kane et al., 2016a). Rapamycin, an inhibitor of mTOR, and a well characterized geroprotector, reduced frailty and improved long-term memory, neuromuscular coordination, and tissue architecture when administered to NFkb -/- mouse model of inflammageing (Correia-Melo et al., 2019).

More studies are required to understand what aspects of frailty may benefit from the use of geroprotectors. Differences were noticed between using the FI as an outcome measurement and specific phenotypic measurements, particularly those related to muscle performance. For example, there was no correlation between FI and rotarod performance when studying ageing in C57BL6/J mice (Kane et al., 2016a). In this study, the two tests were 3 months apart, but these results support similar findings in patient studies (Kulminski et al., 2008; Rockwood et al., 2007). Geroprotectors may be more suitable for improving the homeostasis or the reserve of multiple systems rather than focusing on improving muscle performance *per se*. Therefore, they may be beneficial in improving the patients' resistance or recovery to adverse events or their response to tissue regenerative interventions. This has implications when choosing the most suitable measure of outcome. FI, which covers a range of physiological systems including the integument and musculoskeletal system, vestibulocochlear/auditory systems, ocular/nasal systems, digestive system, urogenital system, and respiratory system, and signs of discomfort, may be more appropriate. One note of caution is that FI does not cover cognitive ability. Modifications to this index to assess cognitive function are required. In addition, experiments using FI require long-term studies and the changes in naturally ageing mice are relatively small, which may limit the ability to detect small improvements in a shorter time-frame. Beyond naturally ageing mice there are a number of models of accelerated ageing which may develop frailty more rapidly and with a more severe phenotype and thus may offer an opportunity to improve our mechanistic understanding of frailty while speeding up testing. Models of inflammation, such as the Interleukin (IL)-10 homozygous knock-out (KO) mouse, display signs of frailty with muscle weakness and increased mortality which occurred earlier than wild type mice (Ko et al., 2012; Walston et al., 2008). However, the mice did not appear to show all aspects of the frail

phenotype as there was no difference in weight, or activity level compared with wild-type controls. This may be due to the fact that some of the deficits start when the animals are still developing and may require deletion of the gene once they reach maturity to have a more comprehensive phenotype. Endurance and walking speed or any other parameter included in the FI were not assessed. Another model of inflammation, the NF-kB-/- mouse showed signs of frailty when measured by FI but there was no detailed analysis of the individual components to determine whether they accumulate specific defects (Correia-Melo et al., 2019). A detailed characterization of transgenic mouse models of premature ageing due to accumulation of DNA damage such as the Werner syndrome mice or the Hutchinson–Gilford mice (for a review see (Kõks et al., 2016), may also be informative. In addition, other models such as telomerase knock-out (*Terc-/-* and *Tert-/-*), *Atm* knock-out and *polG mutator* are deemed worthy of characterization. These mouse models are known to interfere with important mechanisms of ageing such as telomere shortening, oxidative damage, and mitochondrial dysfunction and to have multiple systems failure (Kõks et al., 2016). It would also be of interest to assess frailty in response to stressors such as administration of chemotherapy and radiotherapy, immobilization, surgery, or procedures such as ovariectomy to test resilience (reviewed in (Kirkland et al., 2016; Schosserer et al., 2019). Ovariectomy has been shown to produce an epigenetic pattern similar to those seen with age (Stubbs et al., 2017). All of these models could be useful in understanding the impact of frailty on resilience and the ability of geroprotectors to improve reserve and response to adverse events, in a way that would be more in line with clinical translation (Schosserer et al., 2019).

5. Clinical translation

While efforts to prevent frailty have mainly focused on lifestyle interventions, the International Conference on Frailty and Sarcopenia Research (ICFSR) Task Force has recognized that pharmacological approaches are likely required for individuals who meet frailty criteria or who have comorbid conditions that contribute to and complicate the frailty syndrome (Pahor et al., 2018). In this respect geroprotectors are promising candidates for a pharmacological approach. Indeed, in recent years geroprotectors have been used in a small number of exploratory clinical trials (Table 1). Rapamycin is among the most studied geroprotectors and, has been used in a safety clinical trial using non-immunosuppressive doses in patients with coronary artery disease undergoing cardiac rehabilitation. Although there was no improvement in measures of physical performance for frailty, there was an improvement in IL6 levels, a marker associated with inflammation and senescence (Singh et al., 2016) and reduced muscle function (Haddad et al., 2005). The negative outcome may be explained by the small number of patients enrolled in the study, and by the fact that most patients were not frail and they showed good quality of life at baseline as seen by robust grip strength and high scores on SF-12 questionnaire. Indeed the study by (Cesari et al., 2015) showed a larger effect in the more frail patients following intervention with exercise. A recently published pilot also confirmed similar results. Rapamycin was safely used in older volunteers (NCT02874924, (Kraig et al., 2018), but no significant effects were observed in grip strength or walking speed used as measure of frailty. This study also suffered from a small sample size and relatively short treatment (8 weeks). In addition, the inclusion criteria were very broad, enrolling participants in good health and clinically stable chronic diseases. However, the doses used in both studies were in the same range as those used in the study by (Mannick et al., 2014) where administration of an analogue of rapamycin, was shown to improve immune function in older people in response to influenza vaccine when given alone (Mannick et al., 2014) or in combination with BEZ235 (Mannick et al., 2018). The combination therapy proved even more efficacious. The effect of rapamycin on immune cells is thought to be due to an anti-ageing mechanism. No assessment of any parameter of frailty was performed in this study. However, aged immune cells are thought to be, at least in part, responsible for the pro-inflammatory phenotype which develops with age and that is associated with reduced muscle mass and strength. The differences in efficacy may be in the fact that this was a much larger study and the measure of outcome was focused on the immune system and not dependent on patient performance on the day, which is likely to introduce much variability.

Testing with metformin has shown more positive results. Metformin is used for the treatment of diabetes but is also one of the most widely studied geroprotectors (Burkewitz et al., 2014; Piskovatska et al., 2018) with effects on lifespan (Anisimov et al., 2005; Bulterijs, 2011; Menendez et al., 2011; Smith et al., 2010) and healthspan in animal models and humans (Martin-Montalvo et al., 2013; Thomas and Gregg, 2017). Based on a meta-analysis, metformin was shown to reduce all-cause mortality and diseases (Campbell et al., 2017). Clinical trial in older adults with diabetes showed reduced risk of cognitive decline and dementia (Ng et al., 2014) and reduced plasma cytokines in non-diabetic heart failure patients (TAYSIDE trial, (Cameron et al., 2016) including the ageing-associated cytokine CCL11. As it is relatively safe (Gregorio et al., 1996) and potentially off-patent, it is an attractive candidate. Sumantri et al (2014) (Sumantri et al., 2014)showed a protective effect of metformin on the risk of frailty, assessed using a 40-item FI, through improved grip strength and body balance. By contrast, (Wang et al., 2014) identified a beneficial effect of metformin on mortality, over other diabetes drugs administered in patients without frailty but there was no benefit to those patients already identified as frail. As there is a very strong correlation between frailty and mortality, this study suggests that there may be a critical window for intervention with metformin for the improvement of frailty. Both studies analysed a higher number of patients compared to the studies with rapamycin. When looking at combination therapy, one clinical study combined metformin with physical activity. Contrary to expectation, the study showed that metformin inhibited the improvement in skeletal muscle, mitochondrial respiration, and attenuated the increase in protein synthesis and whole-body insulin sensitivity after aerobic exercise training (Konopka et al., 2019). It suggests that additional studies are needed to understand the mechanisms that elicit positive and negative responses to metformin. Indeed, further clinical studies are underway assessing the effect of metformin directly on frailty alone or in combination with exercise (table1).

Much hope is pinned for the use of senotherapeutics, i.e. senolytics, which eliminate senescent cells and senostathics which neutralise the senescence-associated secretory phenotype (SASP) (Kirkland and Tchkonia, 2015; Kirkland et al., 2017; Tchkonia and Kirkland, 2018). Accumulation of senescent cells was shown to be a major contributor to frailty and age-related diseases in general (Tchkonia et al., 2013). The majority of senotherapeutics are repurposed drugs with well-known safety profiles. Their safety profile may also represent the most important limiting factor for systemic application as they interact with CYP450 enzymes which metabolise 90% of drugs and it can interfere with the metabolism and kinetic of other drugs in patients with complex medical needs and polypharmacy (Zhu et al., 2017). However, they can be used at intermittent dosing limiting the side effects. Studies with quercetin in combination with dasatinib and fisetin are ongoing (Yousefzadeh et al., 2018) in a variety of conditions and healthy older volunteers (Table 1). Of interest is the very recently published study using quercetin and dasatinib in Idiopathic Pulmonary Fibrosis where there was a significant improvement in 6-min walk test, 4-m gait speed, and chair-stand time (Justice et al., 2019). Eight of 14 participant's FI-LAB scores, (frailty index based on commonly used laboratory tests) (Rockwood et al., 2015) improved by at least 5%, but the mean difference did not reach significance and similarly trend to improvement of the SASP phenotype were seen but the sample size was too small to reach any meaningful conclusion. The major limitation of this study is the absence of a control group and therefore the functional improvements observed must be interpreted with caution. An appropriately

Table 1
List of clinical trials with geroprotectors, where frailty was a selection criteria or a primary or secondary endpoints. Abbreviations, PO, Primary outcome, 400 MWT, 400 m walking test, SO, secondary oucome, SPPB, Short Physical performance battery tests, QoL Quality of life, mo, months, wks, weeks, d, day, T2D, Type 2 diabetes, 6MWT, 6 min walking test, CAD, coronary artery disease, OGTT, oral glucose tolerance test, GH, growth hormone, MSC, mesenchymal stem cells, MMSE, minimal mental status test, AST, Aminotransferase, AP, Alkaine phosphatase, ALT, Alanine Amino Transferase, PCI, percutaneous coronary intervention, CABG, coronary artery bypass surgery, AE, adverse events, ECG, electrocardiogram, MRI, magnetic resonance imaging. SASP, senescence associated secretory phenotype, eGFR, estimated glomerular filatration rate, IL-6, interleukin 6, MCP-1, monocyte chemotactic protein 1, PAI-1, plasminogen activator inhibitor 1, MMPs metalloproteinases.

Clinical trial N	Condition*	Drug	Selection criteria	Endpoints	Design	References
ENRGISE Pilot (NCT02676466)	Inflammation	Losartan ascending 25-100 mg daily (8 + 8 + 8 weeks) ± fish oil Total 24wks	70 + IL6: 2.5 − 30 pg/ml walking speed and < 15 min 400 MWT	PO IL-6 400 MWT at 3,6,9,12mo SO: SPPB Frailty (Fried) Short form health survey (SF-36)	Placebo-controlled, randomized N = 300	(Cauley et al., 2018; Manini et al., 2017) Ongoing
NCT01989793	Sarcopenia	Losartan ascending 25-100 mg daily (8 + 8 + 8 wks) Total of 24	70-100 Pre-frail	PO Muscle strength & Fatigability at 8, 16, 24 wks SO Frailty score at 8, 16, 24 wks	Placebo controlled randomized N = 36 25 completers	Completed No significant changes
NCT02325245	Pre-frail elderly	Metformin 3 x 500 mg daily for 16 wks (+8 wks follow-up)	60 + Pre-frail (40-items list) excluding multiple diseases and depression	PO Frailty (40-items list) SO QoL (EQ-5D) Handgrip strength 15 feet Walking speed Serum myostatin levels	Placebo controlled randomized N = 150 Ph3	Status unknown, No data published
N/A	Elderly diabetics	Metformin usage	60 + Pre-frail (40-items list) excluding multiple diseases and depression	Frailty (40-items list)	Case control study N = 236	(Sumantri et al., 2014) Decreased frailty risk in Metformin users
N/A	Diabetic veterans	Metformin usage > 180d	65-89 veterans with T2D (from ~900k veteran population)	Frailty (40-items list)	Cohort study N = 2415	(Wang et al., 2014) Metformin may prevent frailty in diabetics
NCT02570672	Frailty	Metformin up to 1000 mg twice/d for 2 years	65-90 Prediabetic, non-frail, home-living excluding multiple diseases and depression	Frailty (Fried)	Placebo controlled randomized N = 150	Ongoing,
NCT03451006	Aging Inflammation Frailty	Metformin 500 mg every 6 to 8 hours for 1 year	60+, Stable CAD, prediabetic, frail (SPPB < 9) excluding multiple diseases and depression	PO: Frailty (SPPB) SO: Serum IL6, PAI, MCP1, Activin, MMPs	Placebo controlled randomized N = 12 Ph2	Ongoing
MASTERS (NCT02308228)	Aging	Metformin 1700 mg/d for 16 wks with RT after 2 wks	65 + With SPPB 3-12 excluding multiple diseases and depression	PO Muscle fibre size SO Muscle volume, strentgh Body composition Insulin sensitivity (OGTT)	Placebo controlled randomized N = 100	Ongoing (Long et al., 2017)
NCT02552355	Physical activity	Metformin 500-1500 mg/d + exercise 3d/wk for 12 wks	55 + with risk factors for diabetes	PO Mitochondrial function, protein & DNA synthesis, body composition, peak aerobic capacity, insulin sensitivity SO Glucose profile	Placebo controlled randomised, double blind N = 50	(Konopka et al., 2019) Attenuated cardiorespiratory fitness, insulin sensitivity with metformin. No difference in muscle protein synthesis
NCT01898611	Frailty syndrome	Ghrelin (7.5 microg/kg daily) 12 wks + resistance training	70 + With Fried FI 3-4 excluding multiple diseases and depression	PO SPPB SO Frailty (Fried) Physical measure QoL(SF-36)	Non controlled randomized N = 16	Completed No effect Very small and short trial, rather observational.
HORMA (NCT00183040)	Sarcopenia Muscle weakness Frailty	Testosterone (topical) and GH (3-5 microgram/kg/d) for 16 wks	65-90 male Low IGF-I and testosterone	PO Measure of actin and myosin heavy chain (contractile function) ubiquitin, and proteasome sub-units (muscle degradation) Local regulators of skeletal muscle synthesis (e.g. IGF-1, IGFBP4, myostatin) SO Muscle strength, mass, power and fatigability (endurance), physical performance	Placebo controlled randomized N = 108	(Sattler et al., 2011a, b) Durable improvement in muscle mass and function
NCT02874989	Idiopathic Pulmonary Fibrosis	Dasatinib 100 mg/day + Quercetin 1250 mg/d, 3 d/wk over 3-wks	50 + men and postmenopausal women	PO Reduction in the % of pro-inflammatory expressing cells	Open label study N = 15 Ph1	Completed (Justice et al., 2019) 6-W = MWT, 4-m gait speed, and

(*continued on next page*)

Table 1 (*continued*)

Clinical trial N	Condition*	Drug	Selection criteria	Endpoints	Design	References
				SO 6MWT, 4-m gait speed, chair-stands, clinical chemistries, frailty index (FI-LAB)		chair-stands time significantly improved. No change in FI-LAB, pulmonary function
NCT02848131	Chronic kidney disease (eGFR) 15-45 ml/min/ 1.73m^2) Diabetes and taking medication	Dasatinib 100 mg Quercetin 250 mg for 3 d (2 wks follow up)	40-80 Chronic kidney disease diabetes	PO Senescent cells in skin, fat blood (d14) SO Frailty index (Fried) and kidney function	Open label controlled N = 20	Ongoing
AFFIRM (NCT03430037)	Frailty elderly syndrome	Fisetin (20 mg/kg for 2d) for 2mo	70-90 postmenopausal women Various exclusion criteria due to treatment limitations	PO 6MWT	Placebo controlled randomized N = 40 Ph2	Ongoing
AFFIRM-LITE (NCT03675724)	Frailty elderly syndrome	Fisetin (20 mg/kg for 2 da) for 2 mo	70-90 Various exclusion criteria due to treatment limitations	PO Inflammation markers	Placebo controlled randomized N = 40 Ph2	Ongoing
NCT03325322	Chronic Kidney Diseases Diabetes Mellitus Diabetic Nephropathies	Fisetin (20 mg/kg for for 2d (2 wks follow up)	70-90 Various exclusion criteria due to treatment limitations	PO Inflammatory markers Effects on MSC function SO Frailty index (Fried) and kidney function	Placebo controlled randomized N = 30 Ph2	Ongoing
NCT02523274	Aging	Resveratrol (250 – 1000 mg daily) with exercise	65 + with physical limitations and sedentary lifestyle	PO Walking speed SO SPPB Muscle function 6MWT	Placebo controlled randomized N = 39	Ongoing
NCT01126229	Memory	Resveratrol (300 – 1000 mg/d) for 12 wks	65-100 Sedentary to moderate lifestyle (< 120 min aerobic activity/ week) Ability to walk 1mile MMSE > 24	PO safety complete blood count and complete metabolic count [(Na, K, Cl, CO2, BUN, Creatinine, Glucose, Total Protein, Albumin, Calcium, Phosphorous, Aspartate AST, AP, Total Bilirubin, ALT SO Memory tests 400 MWT, physical activity levels as measured by accelerometer.	Placebo controlled randomized N = 32	Completed No data published
CARE (NCT01649960)	Aging CAD	Rapamycin low-dose (0.5-2 mg) during cardiac rehabilitation for 12 wks	60 + eligible with CAD undergoing PCI or CABG or patients who are eligible for cardiac rehabilitation	PO Frailty (Fried-like physical performance tests, gait speed, and grip strength.) SO SASP(including interleukin 6, Matrix metalloproteinase 3, and Monocyte chemotactic protein 1) QoL (short-form 12) Preadipocyte (mitochondrial DNA and senescence)	Open label N = 13 single group Ph1 pilot	Completed (Singh et al., 2016) No improvement with rapamycin
NCT02874924	Aging	Rapamycin 1 mg/d for 8 wks (phase 2) to 4 mo (phase1)	70-95 In good health or clinically stable for chronic disease and cognitive function adequate (CLOX1 ≥ 10)	PO Immunologic (T cell count and function, cytokines) SO Physical function (40 foot walk speed) Cognitive function (EXIT25 interview) Other outcomes Cardiovascular effects Pulse Wave Velocity by ECG and Diastolic function by MRI	Placebo controlled, randomised, N = 34 completers (8 phase 1; 20 phase 2)	Completed (Kraig et al., 2018) No efficacy or AE. Longer treatment and bigger cohorts needed

* According to clinicaltrials.gov for "condition" refers to the disease, disorder, syndrome, illness, or injury that is being studied. Conditions may also include other health-related issues, such as lifespan, quality of life, and health risks.

powered randomized controlled trial is required.

Studies with fisetin and rosveretrol are ongoing and we will have to wait the outcome of those studies. However, the present completed trials have already highlighted the challenges in designing appropriate and cost-effective clinical trials. Firstly, the recruitment of a sufficient number of patients, and secondly the standardization of inclusion and exclusion criteria and measures of outcomes. Many of these patients have complex medical needs and are usually not included in clinical trials for this reason.

6. Challenges in clinical translation and way forward

The major challenge facing clinical trials for frailty is that there is no appropriate regulatory framework for the use of drugs targeting frailty. Frailty is not considered an indication the US Food and Drug Administration (FDA) or European Medicines Agency (EMA) and therefore drugs for frailty are not eligible for reimbursement by the health systems and regulatory bodies are reluctant to give approval to perform clinical trials for frailty. This does not give an incentive to the pharmaceutical companies to invest in the development of such drugs. It limits the resources available, the size of the trials, and the trial design. This in turn results in small trials with a mix of outcomes. For frailty to become an indication, there are a number of factors to address. The indication has to be well defined; animal models of the condition that reflect the majority of the aspects of the indication should be available; and there is also a need for clearly defined selection criteria and measurable outcomes, which show a benefit to the patient and ideally can be closely reproduced in the preclinical setting.

Although there is a general agreement on the necessity and usefulness of frailty as a clinical entity, there is still a lack of both a consensus definition and standardized assessments for use in clinical practice and research. The most common definition of frailty is a medical state with multiple causes and contributors that is characterized by diminished strength and endurance and reduced physiologic function that increase an individual's vulnerability to stressors. These can range from minor stressors, such as an infection or a new medication, to more serious events, such as surgical interventions, leading to potentially serious consequences, increased dependency and/or death. Frailty can be associated to multimorbidity but can also exist in absence of multimorbidity, complicating the definition even further (Marengoni et al., 2018; Palmer et al., 2018; Vetrano et al., 2018a, b; Zucchelli et al., 2018). There are efforts made in this direction, but a consensus definition is proving difficult to achieve. This may be due to the fact that under the same denomination there may be more than one condition and more knowledge on the biology of frailty and the molecular mechanisms driving is required to inform consensus.

In the meantime, there may be opportunities to develop more knowledge on frailty and how to treat it by studying the detrimental association of frailty with other pathologies. For example, in cancer the chemotherapy and radiotherapy treatments are significant stressors that have the potential to challenge physiological reserve. A systematic review has identified that prevalence of frailty, and pre-frailty in older cancer patients is high, with the median estimates of 42% and 43%, respectively (Handforth et al., 2015). Older people with frailty and pre-frailty (in which one or two criteria of the Fried Frailty phenotype are present and are at high risk of progressing to frailty) are at considerably increased risk of all-cause mortality, postoperative mortality, chemotherapy intolerance, and postoperative complications (Handforth et al., 2015). They could potentially benefit from pre-treatment with geroprotectors to decrease their vulnerability to a stressor and boost resilience. Similarly, patients with COPD show an increase in frailty associated with higher mortality (Maddocks et al., 2016). Frailty is one of the main causes of reduced compliance with pulmonary rehabilitation and therefore an improvement in frailty would be advantageous (Maddocks et al., 2016). Frailty is also common in older patients with hip fracture, where it is associated with increased time in hospital and postoperative complications. Time in hospital doubled between patients with an intermediate frailty index and a high frailty index, suggesting that improving frailty could have significant effects on the time spent in hospital and on the ability to recover (Krishnan et al., 2014). Geroprotectors are unlikely to be curative on their own but could be tested in combination with existing treatments for the underlying condition to provide a healthier status for patients. This could then boost responses and improve the outcomes of existing interventions for individual conditions, which would provide the indication for reimbursement. Improved responses with the same agent across multiple conditions associated with frailty as common denominator would support the concept that what had been targeted was the underlining condition of frailty.

The identification of standardized criteria for the selection of patients and the identification of endpoints to test the efficacy of geroprotectors to boost resilience in frail individuals is urgently required. In selecting a target population at risk of frailty, many factors need to be considered, including age, low physical activity, impaired physical function, impaired cognition, disability comorbidities, involuntary weight loss, incontinence, polypharmacy, and sensory deficits. In the LIFE study, the physical activity intervention was most effective in the most frail group (Cesari et al., 2015). However, the recent study combining metformin and exercise (Konopka et al., 2019) suggest that there may be a window when an intervention is most effective. Therefore, stratification of patients with different degree of frailty needs to be considered to understand who is benefiting the most.

Looking at the ongoing trials and considering the biology of these drugs which are designed to improve cellular and tissues' fitness and improve tissue reserves, this could be achieved by a combination of geriatric assessment, the Rockwood FI (Rockwood et al., 2005), and mobility tests such as those included in the Short Physical Performance Battery Tests. They could be used as both selection criteria and as a measure of outcomes. Measures of mobility are more acceptable to the regulatory authorities because mobility is widely recognized as an important prognostic factor in geriatric care. Mobility has been well documented as to its relation to multiple adverse outcomes in older persons, and has been studied as to how it relates to clinically meaningful change. The measures are taken with instruments that have reasonable validity, reliability, and sensitivity to change. However, mobility alone is not sufficient, as the advantage of geroprotectors is in in their multi-system approach, benefiting frailty – both in association with multiple chronic conditions or alone. The Rockwood FI is more likely to capture this aspect. Patient-related outcome measures (PROs) should also be considered as they better measure the impact of the intervention on the quality of life. However, such PROs need to be harmonized so that a direct comparison of results is possible. More exploratory measures of outcome are being investigated which include digital assessment by mobility devices, smart insoles and carpets, as well as smart phone applications. A big advantage of such technologies is that it is a continuous assessment in the home and/or normal environment and, thus, much more representative for the daily activity than any traditional assessment in the clinical environment. However, dealing with frail patients makes use of such digital devices challenging and patient compliance needs to be addressed together with caregivers. The search for "frailty" biomarkers is ongoing and no "unique" marker has yet been identified, which detects frailty across cohorts and populations. It has been recently proposed to build biomarker panels or indices for frailty similar to the Rockwood FI based on markers related to pathways involved in frailty and ageing (see (Cardoso et al., 2018)). Ideally, patients should be monitored using biomarkers, as they are less likely to be influenced by the patient state on the day of the test and also would allow closer monitoring over time.

7. Conclusions

Geroprotectors hold great promise for the treatment of frailty. New

treatments are urgently required as frailty impacts on the ability to maintain independent living and on quality of life of older people. This also has implications for health and social care spending, which are feared to become unsustainable in high-income countries at the projected rate of growth of those aged over 65. So far testing has been limited, mainly focused on safety as primary endpoint and with patchy results due to the small sample size and the great variability in study design. This makes direct comparison of the studies for efficacy difficult. Multi-centre, large international studies harmonized for inclusion criteria and outcome measures are required to better understand the potential and limitations of geroprotectors for frailty. They should be supported by large governmental investments and produce evidence of their efficacy. It is possible that more sensitive measure of frailty, based on the specific impaired mechanisms driving frailty, are required to better stratify patients. This may use a combination of functional and molecular biomarkers and may involve a much deeper understanding of the molecular pathways underpinning frailty. It is likely that under a common denomination there are different molecular determinants and syndromes. Such knowledge may lead to a new classification of frailty syndromes and may help finding consensus on how to define frailty. Demonstration of efficacy, combined with an agreed definition recognized by entities such as WHO, EMA and FDA will show a clear route to market. This is required to de-risk investments and enable the pharmaceutical industry to embrace this approach. This in turn will speed up progress.

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