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Early ageing after cytotoxic treatment for testicular cancer and cellular senescence: Time to act

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ABSTRACT

Treatment of testicular cancer (TC) has an exceptionally high success rate compared to other cancer types; even in case of metastasized disease, 80–90 % of TC patients can be cured. Consequently, attention has been drawn to a potential downside of this treatment success: late adverse treatment effects such as the accelerated development of otherwise age-associated features like cardiovascular disease and second malignancies. Underlying mechanisms are poorly understood. Emerging data suggest that cytotoxic treatment induces cellular senescence, resulting in secretion of inflammatory factors contributing to this early ageing phenotype. Molecular and cellular characterization of this early ageing will enhance understanding the pathogenesis of TC treatment-induced morbidity and contribute to better recognition and prevention of late effects.

In this review, we describe clinical manifestations of the early ageing phenotype among TC survivors, and subsequently focus on potential underlying mechanisms. We discuss the clinical implications and describe perspectives for future research and intervention strategies.

1. Introduction

Treatment of germ cell testicular cancer (TC) has an exceptional success rate compared to other cancer types; even in case of metastasized disease, 80–90 % of TC patients can be cured (Hanna and Einhorn, 2014). Consequently, attention has been drawn to a potential downside of this treatment success: late adverse treatment effects in long-term survivors of testicular cancer. Examples of these are the accelerated development of otherwise age-associated features such as cardiovascular risk factors and cardiovascular disease, chronic fatigue, cognitive impairment and secondary cancers (Haugnes et al., 2012). Currently, the mechanisms underlying late effects are poorly understood. Emerging data suggest that cytotoxic treatment induces cellular senescence via genomic instability and telomere shortening, and may be involved in the early ageing phenotype (Ness et al., 2018). Whereas ageing is defined as the time-dependent functional decline that affects most living organisms and is characterized by a number of loss-of-function diseases. Molecular and cellular characterization of this early ageing phenotype will enhance understanding of the pathogenesis of TC treatment-induced morbidity, contribute to better recognition and prevention of late effects, and might offer a novel target for combinatorial treatments aimed at reducing secondary morbidities.

In this review, we describe clinical manifestations of the early ageing phenotype among TC survivors, and subsequently focus on potential underlying molecular mechanisms. Finally, we discuss the clinical implications and describe perspectives for future research and intervention strategies.

2. testicular cancer treatments

Testicular cancer (TC) is the most common solid malignancy in men aged between 18 and 35 years (Bray et al., 2006). Since the introduction of platinum-based chemotherapy in the late 70 s, cure rates for metastatic TC have become exceptionally high: up to 80–90 % (Hanna and Einhorn, 2014). In combination with worldwide increasing incidences, the number of TC survivors is growing rapidly (Bray et al., 2006). In the US in 2016 there were 266.000 testicular cancer survivors, it is estimated that this number will increase to 335.000 in 2026 (Miller et al., 2016). According to estimates, in Europe over 430.000 males with a past diagnosis of TC are alive, and more than 220.000

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have survived longer than 15 years after diagnosis (Trama et al., 2012). Due to these high and increasing number of long-term survivors, TC is a good model to study the late effects of cancer treatment.

Treatment of TC usually starts with an orchidectomy of the affected testicle, which leads to cure in most patients with clinical stage I disease. These patients receive no follow-up treatment, but only active surveillance. However, patients with metastatic disease or at high risk of metastasis do undergo additional treatment. Stage I seminomas with elevated risk to develop metastatic disease in the past often were treated with 20 Gy radiotherapy on ipsilateral lymph nodes or one cycle of carboplatin chemotherapy. However currently surveillance is the most preferred option according to NCCN and EAU guidelines (NCCN, 2019; Albers et al., 2015). Stage I non-seminoma with vascular invasion can be treated with one cycle of BEP chemotherapy (Bleomycin, Etoposide and cisPlatin). For metastatic (≥stage II) seminoma or non-seminoma, the standard treatment consists of three or four cycles of BEP chemotherapy.

Radiotherapy is an alternative but not preferred over chemotherapy in low-volume stage II seminoma and can involve 30 – 36 Gy radiation to the para-aortic lymph nodes (NCCN, 2019; Albers et al., 2015; Oldenburg et al., 2013).

3. Ageing phenotype in testicular cancer survivors

After successful treatment, TC survivors are at risk of developing a spectrum of late adverse effects. Most published studies in this field have focused on cardiovascular risk factors or cardiovascular disease, secondary malignant neoplasms, infertility and sexuality, hypogonadism, neurotoxity and otoxicity, psychological aspects, quality of life and socioeconomic consequences (Travis et al., 2010). Fewer studies have focused on cognitive dysfunction, long-term renal toxicity, pulmonary dysfunction and skeletal health (Fung et al., 2018). Based on the type of late effects and the relatively young age at which these late effects occur, suggests that the cancer treatment – at least in some TC patients – leads to a phenotype resembling early ageing.

3.1. Vascular ageing

TC survivors have an increased risk of developing cardiovascular disease, a well-known age-associated phenomenon (Paneni et al., 2017). Four studies have investigated development of cardiovascular disease after TC treatment consisting of either chemotherapy, radiation therapy of both (Meinardi et al., 2000; Huddart et al., 2003; van den Belt-Dusebout et al., 2006; Haugnes et al., 2010) (Table 1). Van den Belt-Dusebout et al. found an approximately two-fold increased risk of myocardial infarction in TC survivors younger than 45 years compared to the general population (van den Belt-Dusebout et al., 2006). Furthermore, Haugnes et al. reported that BEP chemotherapy was significantly associated with a 5·7-fold higher risk for coronary heart disease (CHD) in age-adjusted analyses (Haugnes et al., 2010). Besides their increased risk of developing CHD at median 10–19 years after treatment, TC survivors are diagnosed with CHD at a remarkably young age. Median age at CHD diagnosis in the TC survivor population is between 39 and 60 years (Meinardi et al., 2000; van den Belt-Dusebout et al., 2006). The median age of first myocardial infarction in the general male population is 67 years (Canto et al., 2011). Together, these data suggest that in a portion of patients, TC treatment leads to CHD at a younger age than expected. Comparative data on the male background population are important but scarce.

Vascular toxicity after TC treatment is ascribed to the activation of endothelial cells in response to circulating cisplatin and/or bleomycin. The adverse effects of cisplatin and bleomycin on endothelial cells have been investigated in preclinical studies, which showed that both agents induce endothelial injury in vitro (Yu et al., 2008; Nuver et al., 2010). Several years after chemotherapy, TC survivors show signs of endothelial dysfunction, such as microalbuminuria and an unbalance in plasma levels of tissue-type plasminogen activator (tPA) and plasminogen activator inhibitor type 1 (PAI-1) (Nuver et al., 2004). Endothelial activation leads to increased adhesiveness and permeability of the endothelial wall for leucocytes and platelets, which can progress into atherosclerosis (Davignon and Ganz, 2004).

Another consequence of vascular ageing is microalbuminuria, which may be the result of vascular damage in the kidney. Nephrotoxicity is an important acute and long-term effect of both radiotherapy and chemotherapy for TC (Fosså et al., 2002; Lauritsen et al., 2015) (Table 1).

Besides direct endothelial damage from cisplatin and bleomycin administration, several studies have shown that TC survivors have an increased frequency of cardiovascular risk factors. Prevalent risk factors include overweight, insulin resistance, hypertension and dyslipidemia, which are collectively part of the metabolic syndrome (Willemse et al., 2013). This syndrome has been characterized as a proinflammatory and prothrombotic state, with elevated levels of interleukin-6 (IL-6), C-reactive protein (CRP) and PAI-1 and accumulation of senescent cells adipose tissue contributing to systemic metabolic alterations (Koh et al., 2005; Postmus et al., 2019). Approximately 25 % of TC survivors after chemotherapy have metabolic syndrome at a median age of 40 years (Nuver et al., 2005; de Haas et al., 2013; Haugnes et al., 2007) (Table 1). So both direct damage of the cardiovascular system as well as indirect metabolic changes are originating from the cytotoxic cancer therapy and influencing the ageing phenotype.

3.2. Secondary malignancies

The risk of developing a secondary non-TC malignancy is increased after TC treatment. Secondary solid tumor incidences are increased in TC survivors treated with chemotherapy and radiotherapy, but not in patients treated with surgery only (Fung et al., 2013). Development of secondary solid tumors can be partially explained as a direct treatment effect as most radiation-induced malignancies are located within or close to the initial radiation fields (bladder, pancreatic and gastric/duodenal cancers) (Albers et al., 2015; Horwich et al., 2014; Travis et al., 2005; van den Belt-Dusebout et al., 2007; Groot et al., 2018). Platinum residuals may induce damage to excreting organs: the renal clearance of the residuals may cause the increase in urinary tract cancers (bladder, urinary tract and kidney cancer) (Fung et al., 2013; Travis et al., 2005, 2005; van den Belt-Dusebout et al., 2007; Groot et al., 2018). The risk of secondary leukemia is associated with platinum-based chemotherapy, higher etoposide dose and with radiation dose to active bone marrow (Howard et al., 2008; Kollmannsberger et al., 1998; Travis et al., 2000). The increased risk for secondary cancer can also be related to malignant transformation in residual teratoma (Nettersheim and Schorle, 2017), of which soft tissue sarcoma and adenocarcinoma are the most frequent forms (Motzer et al., 1998). However, the increased risk for some solid tumor types, including lung cancer, prostate cancer, thyroid cancer and malignant melanoma has not been directly related to TC treatment (Fung et al., 2013; Travis et al., 2005). In the general population, however, increasing age also contributes to the risk of developing a malignancy (Siegel et al., 2015). We therefore hypothesize that some of the secondary malignancies related to healthy tissue damage are associated with the early ageing phenotype after TC treatment (Fung et al., 2013; Groot et al., 2018).

3.3. Other manifestations resembling an ageing phenotype

Subclinical hypogonadism after orchidectomy followed by chemotherapy is associated with the occurrence of metabolic syndrome (Table 1).

Even though most TC survivors do not have clinically significant chronic disease, around a quarter of them report from persisting mild to profound fatigue, which interferes with their ability to participate fully in normal life at home, at work or in the community (Orre et al., 2008;

Table 1
Ageing phenotype in testicular cancer (TC) survivors.

Late adverse effect	Specification of adverse effect	Association with ageing	References
Cardiovascular disease	Myocardial infarction	Age-adjusted analyses showed an 2.0 – 5.7 fold higher risk than the general population.	(Meinardi et al., 2000); (Huddart et al., 2003); van den Belt-Dusebout et al., 2006); (Haugnes et al., 2010)
	Coronary artery disease Atherosclerotic disease	Median age coronary heart disease ranging from 39 – 60 years in the TC population	
Secondary malignant neoplasms	Bladder cancer Kidney cancer Pancreatic cancer Upper and lower Gastro Intestinal cancer	Partly direct treatment effects (radiation field, platinum residuals in excreting organs), however not all tumor types can be explained this way.	(Kollmannsberger et al., 1998); (Travis et al., 2000); (Travis et al., 2005); (van den Belt-Dusebout et al., 2007); (Howard et al., 2008); (Fung et al., 2013); (Horwich et al., 2014); (Siegel et al., 2015); (Groot et al., 2018)
	Leukemia Lung cancer Prostate cancer Thyroid cancer Melanoma	Ageing is in general population contributing to cancer risk. Transformation in teratoma residuals harbor another mechanism not ageing related.	
Renal function decline	Decrease of renal function	Faster decline than expected when compared to physiological renal function decline in general population.	(Fosså et al., 2002); (Lauritsen et al., 2015)
Metabolic syndrome	Metabolic syndrome and its components: - Overweight - Insulin resistance - Hypertension - Dyslipidemia	- Compared to male reference population TC survivors treated with chemotherapy are 10 years younger at development of metabolic syndrome. - Associated with lower levels of testosterone and its decline with age.	(Nuver et al., 2005); (Haugnes et al., 2007); (de Haas et al., 2013); (Postmus et al., 2019)
Fatigue	Mild to profound fatigue, interfering with normal life	Fatigue is a marker of reduced physiologic capacity, fitting an ageing phenotype.	(Orre et al., 2008); (Sprauten et al., 2015)
Cognitive complaints	Lower cognitive performance in function tests, however not in every study corresponding with patient-reported complaints.	Mild cognitive impairment is also common among elderly.	(Schagen et al., 2008); (Pedersen et al., 2009); (Skoogh et al., 2012); (Wefel et al., 2014); (Amidi et al., 2015); (Stouten-Kemperman et al., 2015)
Decreased survival	Accelerated survival decline after 15 - > 30 years of follow up. Higher risk of dying from non-cancer causes than general population.	Ultimate feature of ageing is increased tendency to die.	(Fosså et al., 2007) Gandaglia et al. (2014), (Kvammen et al., 2016)

Sprauten et al., 2015).

Cognitive complaints have been reported after in TC patients both before and after treatment (Schagen et al., 2008; Skoogh et al., 2012). Several studies indicate that TC survivors treated with cisplatin based-chemotherapy had a lower cognitive performance during neuropsychological tests compared to normative data, although results were contradicted by others (Schagen et al., 2008; Stouten-Kemperman et al., 2015; Amidi et al., 2015; Wefel et al., 2014; Pedersen et al., 2009; Amidi et al., 2017) (Table 1).

3.4. Survival

The ultimate feature of ageing in humans is an increased tendency to die. Three studies have investigated long-term survival after TC treatment. The first study reported that TC survivors who survived at least one year after diagnosis had a 6 % higher risk of dying from non-cancer causes than the general population. Causes of death were infectious, digestive and circulatory diseases. However, the median follow-up duration was only 10 years (Fosså et al., 2007). Another study showed a non-cancer related mortality rate of 3·2 % after a median follow-up of 15 years, but did not perform a comparison to the general population (Gandaglia et al., 2014). In Norwegian long-term TC survivors, an accelerated survival decline beyond 15–30 years of follow-up was evident. Even after 30 years of follow-up the relative survival of TC survivors continued to decline (Wefel et al., 2014; Kvammen et al., 2016). These data point to reduced lifespan as a consequence of the treatment of TC (Table 1).

4. Early Ageing pathophysiology: Induction of Cellular Senescence

The clinical manifestations described above suggest that treatment for TC leads to an early ageing phenotype with features of a time-dependent functional decline. A general cause of ageing is the accumulation of excessive molecular and cellular damage (López-Otín et al., 2013). Importantly, much of this damage is directly promoted by interventions such as cytotoxic treatment for TC, indicating a possible association between cancer treatment and early ageing. In the following subsections we describe how cancer treatment administered to TC patients induces genomic instability and telomere shortening, eventually leading to apoptosis and cellular senescence in healthy tissue. Ultimately, this chain of events can lead to the ageing phenotype seen in TC survivors (Fig. 1).

4.1. TC treatment induces genomic instability and telomere shortening leading to senescence

4.1.1. Cellular senescence

Accumulation of unrepaired DNA damage, and telomere shortening are both potent inducers of cellular senescence a cellular state of stable growth arrest. The stable arrest of unstable cells represents an important tumor suppressor mechanism and guarantees tissue homeostasis (Campisi and d'Adda di Fagagna, 2007). Similarly, the growth arrest of the senescence program is a desired outcome of anti-cancer treatments, as it prevents tumor cell proliferation (Ewald et al., 2010). However, the number of senescent cells increases and accumulates with age in different organisms, including humans and mice. The presence of an excessive number of senescent cells has been associated with age-related diseases like atherosclerosis, COPD and Alzheimer's disease (Noureddine et al., 2011; Fyhrquist et al., 2013; Chinta et al., 2015). More recently, several studies in mice have reported that elimination of senescent cells alleviates a wide range of age-related symptoms, including cardiovascular and renal dysfunction, sarcopenia, osteoporosis, frailty, hypercholesterolemia and neurodegeneration (Baker et al., 2016; Roos et al., 2016; Baar et al., 2017; Xu et al., 2015a; Bussian et al., 2018; Xu et al., 2018).

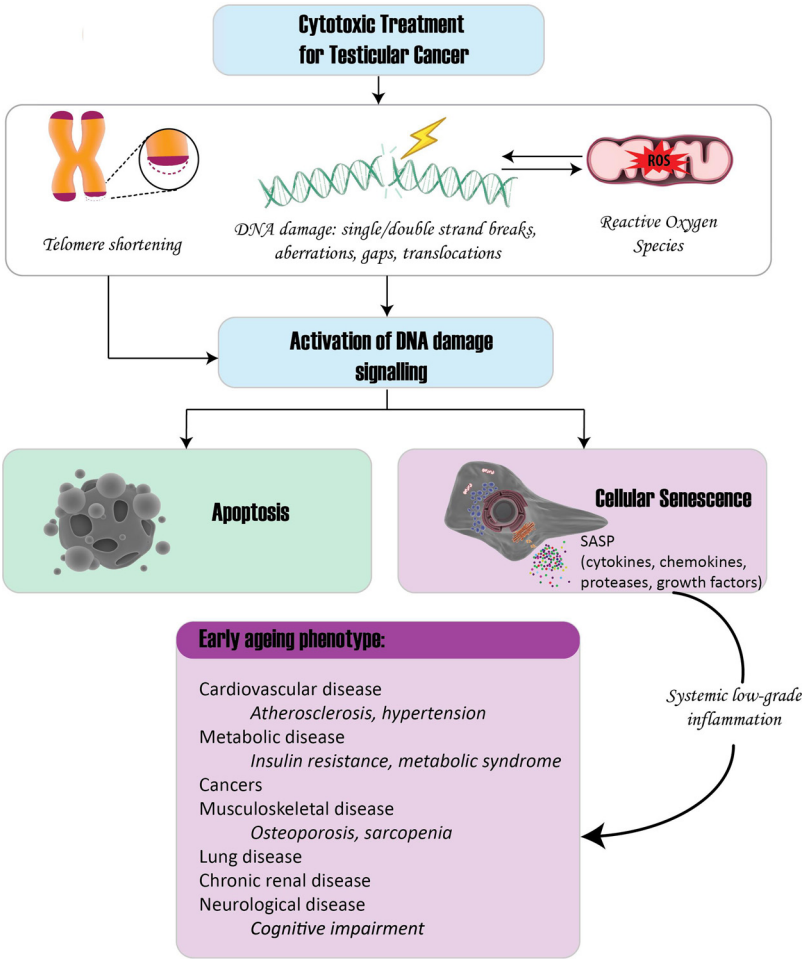

Fig. 1. Cytotoxic treatment for testicular cancer leads to both telomere shortening and DNA damage. DNA damage is accompanied by release of reactive oxygen species (ROS), leading to additional DNA damage. Both telomere shortening and DNA damage lead to activation of DNA damage signaling pathways, resulting in cellular apoptosis or cellular senescence. Senescent cells activate a 'senescence-associated secretory phenotype' (SASP), leading to a state of systemic low-grade inflammation, which has been associated with a phenotype of early ageing after testicular cancer treatment.

Senescent cells are thought to drive ageing pathology by means of cell and non-cell autonomous mechanisms. In a cell autonomous fashion, the senescence-associated growth arrest can lead to the depletion of important pools and niches of cells, such as stem cells. In a non-cell autonomous fashion, the senescence-associated secretory phenotype (SASP), a suite of several cytokines, chemokines, growth factors and proteases, has the ability to drive low-grade inflammation, which plays a key role in several age-related diseases (Davignon and Ganz, 2004; Campisi and d'Adda di Fagagna, 2007). Genotoxic agents used for TC treatment – ionizing radiation, bleomycin, etoposide and cisplatin – are well-known inducers of cellular senescence in vitro and *in vivo* (Aoshiba et al., 2003; Demaria et al., 2017). The increased burden of senescent cells causes upregulation of the SASP, resulting in loss of tissue homeostasis, functional decline and onset of pathology. It has been shown that TC treatment induces low-grade inflammation contributing to presence of the SASP (Nuver et al., 2004). Several chemotherapeutic drugs (doxorubicin, paclitaxel) induce cellular senescence in primary murine and human cells, and the genetic or pharmacological elimination of chemotherapy-induced senescent cells reduces several short-term and long-term adverse drug effects, including bone marrow suppression, kidney and cardiac dysfunctions, physical activity and strength in mice (Baar et al., 2017; Demaria et al., 2017).

4.1.2. Genomic instability leading to cellular senescence

Genetic damage accumulates throughout life, and can be caused by either endogenous or exogenous sources such as DNA replication errors, reactive oxygen species (ROS), UV radiation, X-rays or other radiation sources, or chemical agents like tobacco smoke (Hoeijmakers, 2009). DNA repair mechanisms are able to deal with most of the genetic damage resulting from these different threats, but repair deficiencies arise during ageing, leading to accumulation of DNA damage (López-Otín et al., 2013). Excessive accumulation of DNA damage leads to an increased rate of mutations, chromosomal aneuploidy or copy number variations and eventually results in cell death or senescence (Vijg and Suh, 2013).

TC treatment with radiotherapy and/or BEP-chemotherapy induces collateral damage to healthy tissue cells, being both an endogenous (induction of ROS) and exogenous (radiation, chemicals) source of DNA damage. For example, bleomycin acts through oxidation of deoxyribose of thymidylate and other nucleotides, eventually leading to ROS production (Froudarakis et al., 2013). Cisplatin also has DNA as its primary target and induces covalent bonds of purine bases, thus causing the formation of both intra- and inter-strand crosslinks (Wang and Lippard, 2005). Importantly, platinum levels remain detectable even 20 years after TC treatment, and are thereby a constant source of DNA damage (Gietema et al., 2000; Brouwers et al., 2008).

Various DNA repair mechanisms that modulate cisplatin and bleomycin cytotoxicity have been described (Wang and Lippard, 2005; Gietema et al., 2000; Brouwers et al., 2008; Sanz et al., 2002), but if the damage exceeds the repair capacity cells activate mechanisms associated to apoptosis or senescence. The amount of unresolved damage

seems to be a key determinant in the choice between these two states. For example, treatment with cisplatin induces cellular senescence at low doses and apoptosis at high doses (Demaria et al., 2017; Seluanov et al., 2001). Pre-clinical models show that upon treatment with cisplatin and other DNA damaging drugs, both senescence and apoptotic rates are increased (Demaria et al., 2017; Seluanov et al., 2001). This effect might be due to cell type-dependent differences in coping with stress and to the variable drug concentration that can reach different tissues. TC treatment may therefore induce an increased mutational load in early adult life, which can result into the generation of apoptotic and senescent cells.

However, while apoptotic cells will be only transiently present, senescent cells might persist in tissues for long periods and promote premature ageing phenotypes via SASP-mediated chronic inflammation and tissue dysfunction (Calcinotto et al., 2019).

4.1.3. Telomere shortening leading to cellular senescence

Each chromosome is 'capped' with at least a few hundred nucleotides of telomere repeats. The telomere loops protect DNA from being recognized as damaged and stabilizes the DNA complex. Progressive loss of telomere length is observed during serial replication as a consequence of DNA polymerases not able to replicate the full length and of loss of telomerase activity, the only enzyme able to add new telomere repeat sequences to chromosomes. Progressive loss of telomeres leads to chromosome instability and eventually to cellular apoptosis or senescence (Artandi, 2006). Chemotherapy and radiotherapy-induced cellular injury, followed by repair processes, leads to an increased amount of cellular replication and therefore shortening of the telomere (Li et al., 2012; Schröder et al., 2001; Gallicchio et al., 2018). Treatment with BEP chemotherapy shortened the telomere length of epididymal spermatozoa (germ cells) in rats (Liu et al., 2015). Until now, few patient studies have been performed regarding telomere shortening and the specific cancer treatment components (Scuric et al., 2017).

Decreased length of the telomere in peripheral blood cells is associated with poor survival, cardiovascular disease and cardiovascular risk factors (metabolic syndrome, diabetes mellitus), and higher cancer incidence and cancer mortality rate (Cawthon et al., 2003; Boonekamp et al., 2013; Samani et al., 2001; Weischer et al., 2012; Sampson et al., 2006; Huzen et al., 2014; Willeit et al., 2010).

In a case-control study involving childhood cancer survivors treated with different chemotherapy regimens and radiation fields, an inverse relationship was found between telomere length and development of secondary malignancies, especially thyroid cancer (Gramatges et al., 2014). In adult survivors of childhood ALL, telomere attrition, together with a cytokine profile of chronic inflammation, was shown to be consistently associated with the onset of accelerated ageing (Ariffin et al., 2017).

5. Time to act

Taken together, TC survivorship appears to be associated with early ageing. Successful treatment apparently comes at the cost of a shorter lifespan accompanied with age-related diseases and adverse health outcomes, at least in some young TC survivors. One of the most potentially life-threatening early ageing symptoms is cardiovascular disease development. Prevention measures, such as life style improvement with supporting physical activity, may play a key role in healthy cancer survivorship (Irwin, 2009; Westerink et al., 2016). After TC patients are discharged from regular oncological follow-up, general practitioners can have a role in monitoring risk factors like hypertension, dyslipidemia and impaired fasting glucose levels. However, in current European guidelines cardiovascular risk is calculated using the SCORE model, which predicts the risk to develop cardiovascular disease within the next 10 years (Perk et al., 2012). Since age is an important denominator for 10-year risk of cardiovascular disease, most TC survivors are placed in the lowest risk category (< 10 % risk) and therefore often have no indication for intervention regarding symptoms such as dyslipidemia or hypertension. To tackle this issue, practitioners could add a number of years, e.g. 15 years, to the chronological age of TC survivors when applying cardiovascular risk management, similar to patients with diabetes mellitus or rheumatoid arthritis. This roughly estimated 'additional age' could eventually be optimized with data on the biological and vascular age of TC survivors in updated results of landmark TC studies (such as the ongoing trials: clinicaltrials.gov no. NCT02276430 and NCT02572934).

TC mostly affects young men, and treatment successfully achieves long-term survival, making these patients an important model for the study of cancer survivorship. Accordingly, the early ageing phenotype observed after TC treatment could also be applicable to survivors of other tumor types, especially for patients treated with genotoxic therapies at young age such as Hodgkin's disease.

The current literature regarding adverse health outcomes late after TC treatment is mainly descriptive. Signs of treatment-induced endothelial damage are observed and it is known that circulating platinum residuals may cause damage even for years after treatment. How treatment causes endothelial damage and how platinum residuals stay present in the circulation are questions which remain to be answered. The time has come to look in depth into underlying mechanisms. Induction of senescent cells by cytotoxic treatment may be an important mechanism behind the development of late effects and an early ageing phenotype. Prospective studies should be performed to investigate markers such as presence of senescent cells, the SASP and telomere length before, during and after treatment for TC and compare them with healthy age-matched peers. Participants in these studies should be followed for many years to determine whether we are looking at a phenomenon in which ageing processes are accelerating after cancer treatment or whether cancer treatment causes the biological age to advance (Fig. 2).

In the future, therapies that can selectively target senescent cells (senolytics) and/or the SASP (senomorphics) might provide new opportunities to reduce late side effects (Soto-Gamez and Demaria, 2017) (Table 2). The senolytic agents ABT-263 and Foxo-DRI efficiently eliminated senescent cells via apoptosis and attenuated chemotoxicity in mice (Baar et al., 2017; Chang et al., 2016; Xu et al., 2015b). More recently, the combination of dasatinib and quercetin - was also shown to be a strong senolytic, effective in decelerating ageing phenotypes in mice, and safe for treatment of patients with idiopathic pulmonary

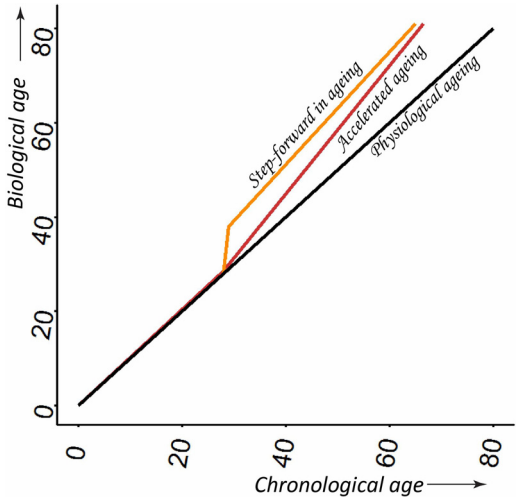

Fig. 2. Early ageing after testicular cancer treatment: are we looking at a phenomenon in which ageing processes are accelerated (red line) or does testicular cancer treatment causes biological age to make a step forward (orange line)? (For interpretation of the references to colour in the Figure, the reader is referred to the web version of this article).

Table 2
. Possible intervention strategies.

Prevention of cellular senescence	References
Avoiding life-style related stresses: - Quit cigarette smoking - Physical activity - Healthy diet	(Irwin, 2009); (Westerink et al., 2016)
mTOR inhibition: - Caloric restriction - mTOR inhibitors: rapamycin, metformin	(Inoki et al., 2012); (Soto-Gamez and Demaria, 2017); (Fontana et al., 2018a); (Fontana et al., 2018b); (Zhuo et al., 2009); (Noren Hooten et al., 2016)
Targeting cellular senescence	
Senolytic agents (ABT-263, p53-FOXO4 interaction targeting, dasatinib/quercetin) or senomorphics (targeting the SASP)	(Xu et al., 2015b); (Chang et al., 2016); (Baar et al., 2017); (Soto-Gamez and Demaria, 2017); (Xu et al., 2018) (Justice et al., 2019)

fibrosis (Xu et al., 2018; Justice et al., 2019). Nonetheless, pharmacological removal of senescent cells could be a promising approach in preventing and treating early ageing in cancer survivors, even if beneficial effects of senescent cells should be carefully considered (van Vliet et al., 2019). A more attainable intervention with clinical relevance for now is preventing additional cellular senescence by avoiding lifestyle-related stress like smoking and increase physical activity and a healthy diet. For example, in rodents caloric restriction extends lifespan, reduces oxidative stress and mitigates low-grade inflammation (Soto-Gamez and Demaria, 2017; Fontana et al., 2018a). In mice and humans, caloric restriction restrains the accumulation of senescent cells in the colon mucosa (Fontana et al., 2018b). Moreover, it is known that restricted caloric intake increases lifespan by activating the mTOR pathway (Inoki et al., 2012) (Table 2). Metformin, a well-known and widely used drug with minimal side effects has been shown to prevent the increase of senescent cells after irradiation and prevents the increase of the SASP (Noren Hooten et al., 2016). It is known that rapamycin protects cells against mTOR-induced cellular senescence (Zhuo et al., 2009).

Current indications suggest that, although the majority of testicular cancer patients who are cured with cytotoxic therapy, the long-term treatment effects cause patients to age earlier than chronologically expected. Presence of the first ageing signs, like development of hypertension or vascular damage, should not be disregarded in these young men. Research into cancer-treatment-induced ageing should be intensified in order to understand the underlying mechanisms and to identify intervention strategies for early ageing in young cancer survivors. TC survivors are an important model for this research. The time to act is now!

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CRediT authorship contribution statement

Sjoukje Lubberts: Conceptualization, Methodology, Writing - original draft, Writing - review & editing. **Coby Meijer:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing. **Marco Demaria:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing, Supervision. **Jourik A. Gietema:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing, Supervision.

Declaration of Competing Interest

Sjoukje Lubberts and Coby Meijer have nothing to disclose Marco Demaria is a co-founder of Cleara Biotech, a company devoted to develop agents against senescent cells. Jourik Gietema reports grants from Abbvie, Roche and Siemens, paid to the institution; outside the submitted work.

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