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Highlights

An in-silico approach to the dynamics of proliferation potential in stem cells and the study of different therapies in cases of ovarian dysfunction

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- The mean proliferation potential of the cell population was introduced as an indicator of ageing
- The influence of telomerase activity on the evolution of the mean proliferation potential was studied
- For people with POI disease, the impact of treatments with different telomerase levels was simulated
- Sexual steroids ameliorated cell proliferation potential but not to reach the levels of healthy population
- Gene therapy with adeno-associated virus made the proliferation potential similar to that of healthy people

An in-silico approach to the dynamics of proliferation potential in stem cells and the study of different therapies in cases of ovarian dysfunction

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ABSTRACT

A discrete mathematical model based on ordinary differential equations and the associated continuous model formed by a partial differential equation, which simulate the generational and temporal evolution of a stem cell population, are proposed. The model parameters are the maximum proliferation potential and the rates of mitosis, death events and telomerase activity. The mean proliferation potential at each point in time is suggested as an indicator of population ageing. The model is applied on hematopoietic stem cells (HSCs), with different telomerase activity rates, in a range of variation of maximum proliferation potential in healthy individuals, to study the temporal evolution of ageing. HSCs express telomerase, however not at levels that are sufficient for maintaining constant telomere length with aging (Zimmermann and Martens, 2008; Flores et al., 2008). Women with primary ovarian insufficiency (POI) are known to have low telomerase activity in granulosa cells and peripheral blood mononuclear cells (Xu et al., 2017). Extrapolating this to haematopoietic stem cells, the mathematical model shows the differences in proliferation potential of the cell populations when telomerase expression is activated using sexual steroids, though the endogenous promoter or with gene therapy using exogenous, stronger promoters within the adeno-associated virus. In the first case, proliferation potential of cells from POI condition increases, but when adeno-associated viruses are used, the proliferation potential reaches the levels of healthy cell populations.

1. Introduction

The telomere pathway links both aging (López-Otín et al., 2023) and fertility (Chico-Sordo et al., 2021; Córdova-Oriz et al., 2022; Ozturk, 2024). Telomeres are nucleoprotein structures located at the ends of linear chromosomes, important to prevent chromosomes fusions and degradation, safeguarding the genome (Martínez and Blasco, 2017; Varela et al., 2018; Córdova-Oriz et al., 2024). In mammals they consist of repetitions of the DNA sequence TTAGGG, bound by a protein complex called shelterin (de Lange, 2018) which is involved in telomere protection (de Lange, 2018). Telomeres extend to 10-15kb in humans (de Lange, 2018; Martínez and Blasco, 2017). However, on each cell division telomeres shorten (Olovnikov, 1973, 1971) as organisms age (Rossiello et al., 2022). The enzyme telomerase can counteract telomere shortening, by adding de novo repeats onto telomeres (Blackburn et al., 1989), but is only detectable in adult and embryonic stem cells, cancer cells and the germ line (Turner et al., 2010; Wright et al., 2001).

Stem cells are undifferentiated cells that can differentiate into the three germinal layers that give rise to all cell types of the organism and have the capacity for self-renewal (López-Otín et al., 2023). Bone marrow contains hematopoietic stem cells, which generate all the cell types of the blood tissue, including the immune system (Cui et al., 2023).

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Germline stem cells produce sperm and egg which are responsible for the reproduction and transmission of genetic information from generation to generation. Abnormal development of germline stem cells often causes severe diseases in humans, including infertility and cancer.

Infertility is a disease characterized by the impairment to conceive in a natural way after one year of unprotected sexual intercourse. One out of every six persons in the world suffer infertility at some point of their lives, according to the World Health Organization (2023). Regarding the female factor, premature ovarian failure (POI) is characterized by a drastic reduction of ovarian function before the age of 40 years, and affects to 1% of females (Silvén et al., 2022). Women with POI have different symptoms (Yang and Yang, 2023), such as hypoestrogenism and alterations in other hormones, amenorrhea and infertility due to termination of follicular development (Neumannová and Müllerová, 2018), some of which are common to mice with accelerated reproductive senescence (Polonio et al., 2020). POI leads to accelerated menopause (Wesevich et al., 2020), increasing the risk of suffering aging-associated diseases (Polonio et al., 2020).

Several lines of evidence link the telomere pathway with infertility (Xu et al., 2017; Butts et al., 2009), as women with ovarian failure have null or low telomerase activity and shorter telomeres in their granulosa cells and blood (Xu et al., 2017; Butts et al., 2009). Indeed, various strategies have been explored to restore telomere length and to increase tissue proliferative potential, with the aim of improving organismal fitness, and fertility in mice and humans (Townsley et al., 2016; Bernardes de Jesus et al., 2012; Jaskelioff et al., 2011). Telomerase reactivation in a model of accelerated aging, led to improved fertility outcomes (Jaskelioff et al., 2011). Danazol, a synthetic steroid can reactivate telomerase (Calado et al., 2009), improving the rate of mature oocytes with respect to untreated women (Córdova-Oriz et al., 2023). Another strategy is gene therapy, using adeno-associated virus (AVV) expressing telomerase, which can transduce several tissues and express the transgene for large periods of time (up to several years) (Büning et al., 2008). In mice, this therapy had many benefits, including improved organismal fitness, increased median lifespan without increasing cancer incidence and telomere lengthening (Bernardes de Jesus et al., 2012). Indeed, telomere lengthening of adult stem cells due to telomerase reactivation would be very important for tissue homeostasis. In mice, adult stem cells bear long telomeres (Flores et al., 2008) and telomerase activity (Marión and Blasco, 2010), but it is not enough as to maintain long telomeres with aging. Thus, even at stem cell compartments telomeres shorten with age (Flores et al., 2008). In women with POI, telomeres of peripheral blood mononuclear cells have short telomeres, opening the possibility to use telomere length in blood as a biomarker of infertility (Xu et al., 2017). The hematopoietic system has adult stem cells which divide to give rise other cell types. Stem cells divide symmetrically to self-renew, or in an asymmetric manner, to generate other cell types (Shahriyari and Komarova, 2013; Takano et al., 2004). In any case, adult stem cells are neither very accessible nor abundant, so, it is difficult to analyze their telomere length. Thus, the study of the dynamics of telomere length in stem cells of the hematopoietic system, is performed through measurements of telomere length of granulocytes and leukocytes, which are a few divisions apart from the stem cells (Boyle et al., 2023; Werner et al., 2015).

Tracking human stem cells is complicated for many reasons. That is why we are considering studying their evolution by means of an in-silico approach, which will help us to obtain clues to guide the work in the laboratory. There are many mathematical models dedicated to the dynamics of cell populations, for example, to the study of proliferation in clonal cancer cell populations (Ortega-Sabater et al., 2023). We focus on models that classify the population according to telomeric length, or in other words, proliferation potential, which allows us to study population ageing. Models of this type, with systems of ordinary differential equations (ODEs), applied to the study of granulosa cell ageing in the human follicle, are proposed in (Portillo and Peláez, 2021; Portillo et al., 2019), while in (Portillo et al., 2023) a continuous model formed by a partial differential equation (PDE) with zero-flux boundary conditions is derived from the discrete model. In the current work, we propose a discrete model with ODEs and a continuous model with a PDE to study the evolution of stem cells throughout life. Unlike in earlier models (Portillo et al., 2023; Portillo and Peláez, 2021; Portillo et al., 2019) in which the parameters remain constant, in the model of this work, the parameters are time-dependent, considering that the rate of stem cells divisions is higher in the early years and decreasing with age. This time dependence is assumed to be inversely proportional to the size of the stem cell pool as in (Boyle et al., 2023; Werner et al., 2015). In contrast to our model, in previous studies (Boyle et al., 2023; Werner et al., 2015) telomerase activity and cell death in HSCs are not considered. Thus, the model in this work intends to be more realistic. A multi-compartmental model for hematopoietic differentiation based on ODEs with several step of maturation has been proposed (Marciniak-Czochra et al., 2009). The model is based on the hypothesis that the percentage of self-renewal versus differentiation is regulated by a single external feedback mechanism depending on the level of mature

cells. This model also fails to consider telomerase activity on HSC. In our model, however, we take into account both telomerase activity and cell death.

Numerical experiments studied the generational-temporal evolution of the mean proliferation potential in healthy individuals with different telomere length percentiles and different levels of telomerase activity. Then, the evolution in patients with primary ovarian insufficiency, with low telomerase activity, following treatment to reactivate either endogenous or exogenous telomerase was simulated.

2. Mathematical model that classifies a population according to the generational age

We assumed that the average telomere length of a multi-potent stem cell, expressed as S, shortens by a constant factor during each division. The cell has a certain maximum and minimum telomeric length, being the Hayflick limit the minimal telomeric length that does not permit replication. We denoted by h the maximum proliferation potential of a cell, i.e. the maximum number of times that a cell can be divided before reaching the senescent state. The generational age of a cell was associated with its telomere length and therefore with its proliferation potential, regardless of when it was formed. The generational age of a cell subpopulation was indicated by subscript i, for $i = 0, 1, \dots, h-1, h$, grouping cells with the same proliferation potential. Thereby, cells with maximum telomeric length, and thus, maximum proliferation potential were considered as generational age of zero and were denoted by S_0. Then, cells whose telomeric length was strictly between the maximum and the minimum value $S_1, \dots, S_{h-1}$, can undergo mitosis. So, S_i can replicate $h - i$ times. Finally, senescent cells S_h, which have reached the Hayflick limit cannot replicate. The populations of cells of each generational age at a given time t was denoted by $N_i(t)$, for $i = 0, 1, \dots, h-1, h$.

Let m be the rate of mitotic replication per cell per unit of time. A symmetric cell division produces two stem cells, both entering the next subsequent state, while an asymmetric division of a stem cell in state i leads to one stem cell in the state $i + 1$ and one cell leaving the stem pool to give rise to more differentiated progeny; this can occur only when $i \neq h$. In this model it was assumed that stem cells divide symmetrically with probability p and asymmetrically with probability $1 - p$. Let d be the rate of mortality events per cell per unit of time. Cells of any generation are susceptible to death. Let r be the rate of telomerase activity per cell per unit of time which acts rejuvenating the cell, moving back to the previous generational age. Only $i \neq 0$ cells can be acted by telomerase.

In both, models (Werner et al., 2015) and (Boyle et al., 2023), it is assumed that there is no telomerase activity and no cell death. However, we believe that both telomerase activity and cell death must be taken into account on stem cell population dynamics.

The rate of stem cell divisions varies throughout life, being higher in the early years and decreasing with age. In (Werner et al., 2015), in relation to HSC, it is assumed to be inversely proportional to the size of the stem cell pool, considering the loss of telomere length at each division constant throughout life.

Refering $n(t) = \sum_{i=0}^{h} N_i(t)$ the total population, the average subpopulations $N_0, N_1, \dots, N_h$ satisfy

$$
\begin{aligned}
N_0'(t) &= \frac{1}{n(t)}[p(-mN_0(t)) + (1-p)(-mN_0(t)) - dN_0(t) + rN_1(t)],\\
N_i'(t) &= \frac{1}{n(t)}[p(2mN_{i-1}(t) - mN_i(t)) + (1-p)(mN_{i-1}(t) - mN_i(t)) - dN_i(t) - rN_i(t) + rN_{i+1}(t)],\ i = 1, \dots, h-1,\\
N_h'(t) &= \frac{1}{n(t)}[p2mN_{h-1}(t) + (1-p)mN_{h-1}(t) - dN_h(t) - rN_h(t)],
\end{aligned}
$$

grouping the terms together gives the following coupled non-linear ordinary differential equations

$$N_0'(t) = \frac{1}{n(t)}[-(m+d)N_0(t) + rN_1(t)], \tag{1}$$

$$N_i'(t) = \frac{1}{n(t)}[m(1+p)N_{i-1}(t) - (m+d+r)N_i(t) + rN_{i+1}(t)],\ i = 1, \dots, h-1, \tag{2}$$

$$N_h'(t) = \frac{1}{n(t)}[m(1+p)N_{h-1}(t) - (d+r)N_h(t)]. \tag{3}$$

Denoting by $\mathbf{N}(t) = [N_0, N_1, \dots, N_h]^T$, the system of equations (1)-(3) can be rewritten as

$$\mathbf{N}'(t) = \frac{1}{n(t)} \tilde{A}\, \mathbf{N}(t), \tag{4}$$

where

$$\tilde{A} = \begin{pmatrix} -(m+d) & r & 0 & \cdots & 0 \\ m(1+p) & -(m+d+r) & r & 0 & 0 \\ \vdots & \ddots & \ddots & \ddots & \vdots \\ 0 & \cdots & m(1+p) & -(m+d+r) & r \\ 0 & \cdots & 0 & m(1+p) & -(d+r) \end{pmatrix} \tag{5}$$

is a tridiagonal matrix of dimension $(h+1)\times(h+1)$.

The simulations in this paper were performed by numerically solving the system of ODEs (1)-(3) with the MATLAB function ode15s. However, we find it of qualitative interest to consider the associated continuous problem.

3. Qualitative study. Population density as a function of the generational age and time

Let x be the variable representing the generational age $x \in [0, h]$ and $N(x,t)$ the population density at generational age x and time t. Then, let $\mathcal{N}(t)$ be the total stem cell population at time t

$$\mathcal{N}(t) = \int_0^h N(x,t)dx. \tag{6}$$

We introduce the following non-local diffusion-advection equation

$$\mathcal{N}(t)N_t(x,t) = DN_{xx}(x,t) - vN_x(x,t) + \rho N(x,t), \quad 0 < x < h,\ t > 0, \tag{7}$$

where $D = (m(1+p)+r)/2$ is the diffusion constant, $v = m(1+p) - r$ the advection coefficient, and $\rho = mp - d$ can be interpreted as an *effective proliferation rate*. The equation (7) must be supplemented with suitable zero-flux boundary conditions

$$DN_x(0,t) - vN(0,t) = 0,\ t > 0, \tag{8}$$

$$DN_x(h,t) - vN(h,t) = 0,\ t > 0, \tag{9}$$

to guarantee that no cell population density $N(x,t)$ either leaves or enters the interval $x \in [0,h]$.

Considering second-order central finite differences to discretise the derivatives with respect to the variable x in the partial derivative equation (7)

$$\begin{aligned} N_{xx}(x,t) &= \frac{N(x-\Delta x,t) - 2N(x,t) + N(x+\Delta x,t)}{(\Delta x)^2} + \mathcal{O}((\Delta x)^2), \\ N_x(x,t) &= \frac{-N(x-\Delta x,t) + N(x+\Delta x,t)}{2\Delta x} + \mathcal{O}((\Delta x)^2), \end{aligned}$$

with step size equal to 1 and denoting by $N_i(t) \approx N(i,t)$, the discrete equations (2) are achived. In this sense the discrete model can be considered a type of discretisation in the x variable of the continuous model.

Notice that by defining the new variable

$$\tau(t) = \int_0^h \frac{ds}{\mathcal{N}(s)},$$

the partial differential equation (7) becomes a standard diffusion-advection equation with constant coefficients

$$N_\tau(x,\tau) = DN_{xx}(x,\tau) - vN_x(x,\tau) + \rho N(x,\tau), \tag{10}$$

that can be solved by using the following transformation,

$$N(x,\tau) = S(x,\tau)\exp\left[\left(\rho - \frac{v^2}{4D}\right)\tau + \frac{vx}{2D}\right]. \tag{11}$$

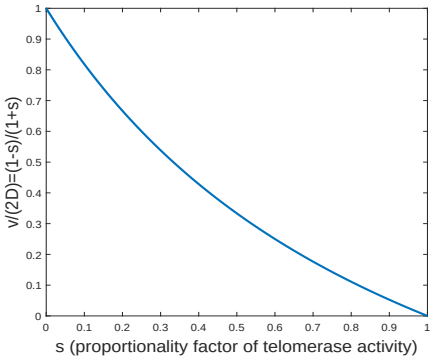


Figure 1: $v/(2D)$ versus proportionality factor of telomerase activity.

The equation for the auxiliary function $S(x,\tau)$ reads as

$$S_\tau(x,\tau) = DS_{xx}(x,\tau),\ 0 < x < h, \tau > 0. \tag{12}$$

Formally, once the solution of (10) is obtained, the complete solution in terms of the original variables (x,t) would be given implicitly.

Be advised that

$$\frac{v}{2D} = \frac{m(1+p)-r}{m(1+p)+r}$$

is a key value in (11). The parameter s was entered as the proportionality factor between r and $m(1+p)$, i.e. $r = s\,m(1+p)$, in order to study

$$\frac{v}{2D} = \frac{1-s}{1+s}$$

in terms of a single parameter. Small s values represent low telomerase activity, in particular $s = 0$ means no telomerase activity. Conversely, values of s close to 1 denote high telomerase activity. In particular, when $s = 1$ there is no advection. Figure 1 the proportionality factor of telomerase activity versus versus $\frac{v}{2D}$.

Figure 2 illustrates the generational-temporal evolution of a population of hematopoietic stem cells. Initially the population is concentrated at the left end of the x-range (generational age), but over the years the population shifts to the right of the x-range, i.e., it ages.

4. Mean proliferation potential as an indicator of aging

The population is distributed according to its proliferation potential in the interval $[0,h]$. We are looking for a single number that captures the average of the population's proliferation potential, which is an indicator of the ageing of the population.

Definition 1. *For h, the maximum proliferation potential of a cell, the centre of mass of the population density $N(x,t)$ at time t is*

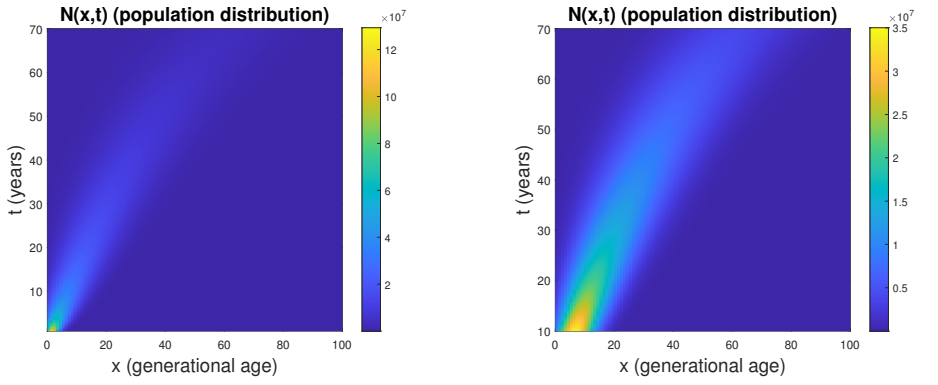
$$x_m(t) = \frac{\int_0^h xN(x,t)\,dx}{\int_0^h N(x,t)\,dx} \tag{13}$$

and the variance

$$\sigma^2(t) = \frac{\int_0^h (x-x_m(t))^2 N(x,t)\,dx}{\int_0^h N(x,t)\,dx}. \tag{14}$$

Let us define the mean proliferation potential as the distance between the point $x_m(t)$ and h, that is

$$h_m(t) = h - x_m(t). \tag{15}$$



(a) Population distribution from 1 up to 70 years

(b) Population distribution from 10 up to 70 years

(c) Population distribution from 40 up to 70 years

(d) Population distribution from 60 up to 70 years

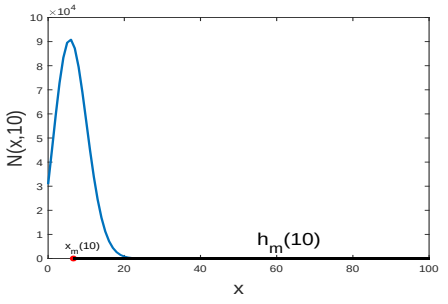
Figure 2: Generational-temporal distribution of a hematopoietic stem cells population with $s = 0.6$.

The mean proliferation potential h_m can vary between 0 and h. Values of h_m near zero represent aged populations, while values of h_m near h indicate young populations. We wanted to study the evolution over time of the proliferation potential of stem cells. In the numerical experiments, discrete counterparts of these values were calculated.

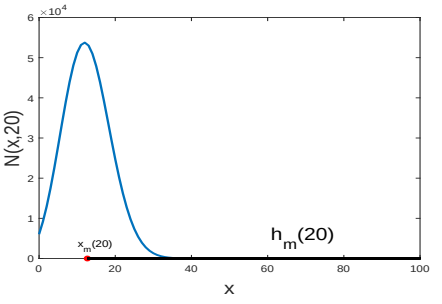
Figures 3 (a)-(g) depict the time evolution of a HSC population distribution from 10 to 70 years and the mean proliferation potential, with initial proliferation potential $h = 100$ and telomerase activity $s = 0.6$. Initially the entire population is at generational age 0. As the years go by, the population shifts to the right and so does the centre of mass x_m. Consequently, the distance between x_m and h, i.e. the mean proliferation potential h_m, decreases with age. Figure 3 (h) displays the temporal evolution of the mean of the population and bounds between the (mean−standard deviation) and the (mean+standard deviation) to give a sense of a plausible range of values for the generational age over time. Next we did a numerical simulation using the same parameters ($h = 100$, $s = 0.6$) but using the line method in the continuous problem (7)-(9). We first discretize the variable x using finite differences of order 2 and step size 0.2 and then discretize the variable t using the Matlab function ode15s. We took an initial density function of Gaussian (normal) type

$$N(x,0) = \frac{10^6}{\sqrt{2\pi\sigma^2}} e^{-(x-\nu)^2/2\sigma^2}, \tag{16}$$

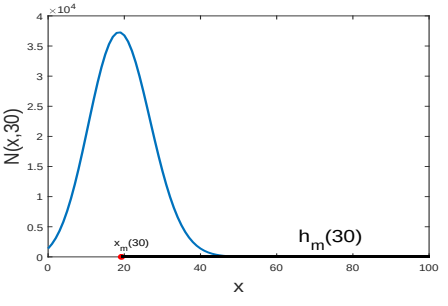
for $\nu = 0.6$ and $\sigma = 0.1$. Figure 4 displays the mean of the population between the bounds of the (mean−standard deviation) and the (mean+standard deviation). Figure 3(h), obtained with the discrete model and Figure 4, with the continuous model, were observed to be similar. From now on, we use the discrete model because of its close approximation to this continuous model.



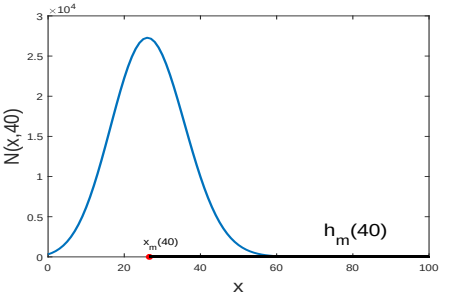
(a) Population distribution at 10 years, $x_m = 6.6$.



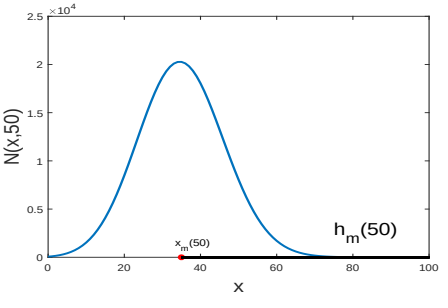
(b) Population distribution at 20 years, $x_m = 12.7$.



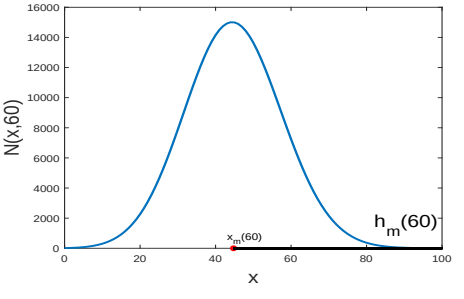
(c) Population distribution at 30 years, $x_m = 19.2$.



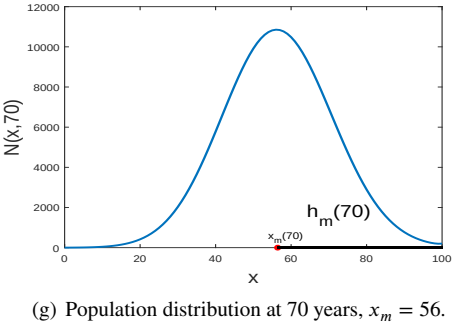
(d) Population distribution at 40 years, $x_m = 26.6$.



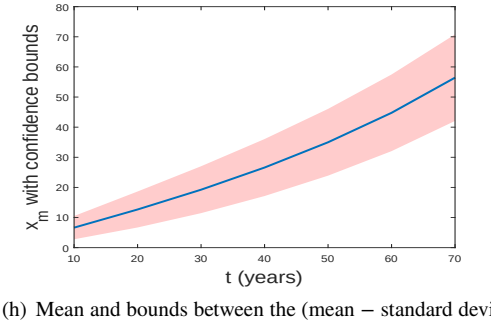
(e) Population distribution at 50 years, $x_m = 35$.



(f) Population distribution at 60 years, $x_m = 45$.



(g) Population distribution at 70 years, $x_m = 56$.



(h) Mean and bounds between the (mean − standard deviation) and the (mean+standard deviation).

Figure 3: Time evolution of hematopoietic stem cells population from 10 to 70 years with the discrete model (4). Temporal evolution of the mean with confidence bounds of the standard deviation of the population.

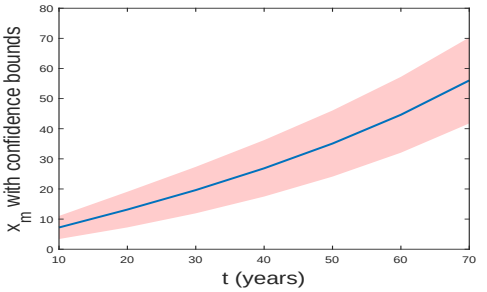


Figure 4: Mean and bounds between the (mean − standard deviation) and the (mean+standard deviation) using the method of lines in the continuous model (7)-(9) with size step equal to 0.2 in the discretization of the variable x.

Percentile	c	h
99	12.5	126.7
90	11.5	113.3
50	10.4	98.7
10	9.4	85.3
1	8.4	72.0

Table 1
Initial telomere length and initial proliferation potential for several percentils, according to data from (Armanios and Blackburn, 2012).

5. Hematopoietic stem cells. Numerical experiments

5.1. Model parameter estimation

Hematopoietic stem cells proliferate slowly (Foudi et al., 2009) to replace cells that regularly die as a result of wear and tear or injury. Monitoring HSCs directly is very challenging for numerous reasons. Since hematopoietic cells proliferate inside a hierarchically organised tissue with slowly dividing stem cells at its root, and cells leaving the stem cell pool have an approximately constant number of cell divisions before they reach maturation, the distribution of telomere lengths of mature cells is a good approach for the distribution of telomere lengths in stem cells (Rodriguez-Brenes et al., 2013). For this reason, in this work we assume that the values of the parameters measured for lymphocytes are similar to the values of the parameters for hematopoietic stem cells and in fact, these are the ones we will use in the numerical experiments.

The authors of (Cosgrove et al., 2021) estimate that the number of haematopoietic stem cells that are actively at any time ranges from $25,000$ to $1,300,000$. Then, in the numerical experiments, we assumed that initially the total population of $n_0 = 10^6$ stem cells is at generational age 0.

Some parameters of our model were computed according to data from (Werner et al., 2015): the probability of symmetrically division of stem cells $p = 0.35$, the effective proliferation rate $m/n_0 = 0.95\,(\text{year}^{-1})$ and the effective mortality rate $d/n_0 = 0.34\,(\text{year}^{-1})$.

Regarding the effective telomerase rate, we related it to the effective proliferation rate through the proportionality factor of telomerase activity s introduced in Section 2 in the following way: $r/n_0 = s\,(m/n_0)\,(1+p)$.

Finally, the estimation of the initial proliferation potential was made on the basis of the data from (Armanios and Blackburn, 2012). Table 1 displays the initial telomere length (c) from (Armanios and Blackburn, 2012) and the initial proliferation potential ($h = (c-3)/\triangle c$, for $\triangle c = 0.075$) for several percentiles. Therefore, in the numerical experiments we consider the initial proliferation potential for hematopoietic stem cells to range from 75 (lowest percentiles) to 125 (highest percentiles), assuming $h = 100$ the initial proliferation potential at the 50th percentile.

The ODE system (**??**) is then solved using the MATLAB function ode15s.

s	p_1	p_2	p_3
0.5000	−0.005742	−0.5686	99.2600
0.6000	−0.004425	−0.4759	99.2300
0.7000	−0.002889	−0.4007	99.2400
0.8000	−0.001285	−0.3408	99.2900

Table 2
Fitting the data of hematopoietic stem cells mean proliferation potential with $h = 100$ to a quadratic polynomial curve for several values of telomerase activity: $h_m(t) \approx p_1 t^2 + p_2 t + p_3$.

5.2. Evolution of healthy populations according to different percentiles of the initial proliferation potential and several telomerase activity

Our aim was to study the influence of telomerase activity on the evolution of the mean proliferation potential in hematopoietic stem cells, over time. We started by considering people at the 50th percentile, we assumed that $h = 100$. Then, we solve our model numerically for various values of the parameter s, the parameter varying between 0 and 1 indicating the amount of telomerase activity. Subsequently, the parabola $p_1 t^2 + p_2 t + p_3$ is fit to the data for $h_m(t)$ over time. The fitting for hematopoietic stem cells is shown in Table 2. To sum up, the quadratic polynomial that is fit to the data of the mean proliferation potential decreases with age depending on telomerase activity. Values $p_1 < 0$ and $p_2 < 0$ indicates the curves are decreasing. Moreover, the smaller s is, the greater the absolute values of p_1 and p_2 are. That is, the lower the telomerase activity is, the steeper the decrease of the average proliferation potential becomes.

We then repeated the same study covering the whole range of healthy individuals, from the initial proliferation potential corresponding to the lowest percentiles, represented by $h = 75$, the one corresponding to the 50th percentile associated with $h = 100$, and finally, the one of the highest percentile, related to $h = 125$. Figure 5 displays the time evolution of mean proliferation potential for hematopoietic stem cells, in a range of healthy people, for several values of telomerase activity. In all cases, the lower the telomerase activity, the lower the mean proliferation potential. Figure 5(a) depicts the time evolution of mean proliferation potential with initial potential equal to 75, simulating individuals at low percentiles. At $s = 0.5$ the mean proliferation potential becomes zero before the age of 79 and, for $s = 0.6$, after the age of 89. Figure 5(b) shows the time evolution of mean proliferation potential with initial potential equal to 100, associated to individuals at 50th percentile. The model predicts that with a value of $s = 0.5$ at 90 the mean proliferation potential is slightly above zero. Figure 5(c) displays the time evolution of mean proliferation potential with initial potential equal to 125, related to individuals at high percentiles. The values of mean proliferation potentials are higher and in no case become zero at the age of 90.

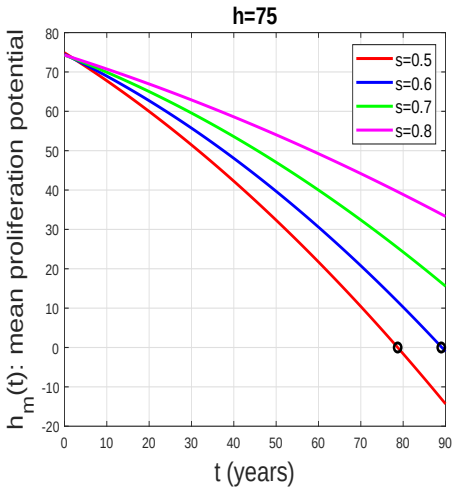
Finally, for each level of telomerase activity, the temporal evolution of the mean proliferation potential is displayed in Figure 6, in the range of the different percentiles. For the lowest telomerase activity value, at the age of 80 years, the mean proliferation potential in the low percentile was zero, while for the highest telomerase activity values even in the lowest percentiles the proliferation potential was above 30 even at the age of 90 years.

5.3. Shortened telomere length and diminished telomerase activity in patients with primary ovarian insufficiency

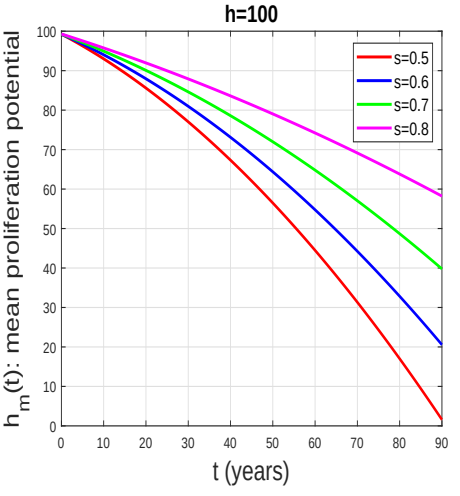
Human diseases due to mutations in telomerase components result in premature adult stem cell dysfunction (Blasco, 2007). The hematopoietic cells of patients with telomeropathy have very short telomeres, which cause defects in stem-cell number and tissue regeneration (Townsley et al., 2016). POI is a result of pathological reproductive aging and encompasses occult, biochemical and overt stages (Butts et al., 2009). Patients with occult POI have shortened telomere length and low telomerase activity in granullosa cells (Xu et al., 2017). Human lymphocytes and $CD34+$ hematopoietic cells up-regulate both telomerase reverse transcriptase (TERT) gene expression and telomerase enzymatic activity in response to androgens in vitro (Townsley et al., 2016).

In (Xu et al., 2017), telomere length from peripheral blood leukocytes and granulosa cells was shorter in patients compared with healthy controls. Moreover, telomerase activity seems to be diminished in granulosa cells of patients with POI compared with healthy women. We then made a simulation compatible with hematopoietic stem cell from POI patients, whose distribution at age 30 is similar to the distribution of healthy people at age 40 at the 50th percentile.

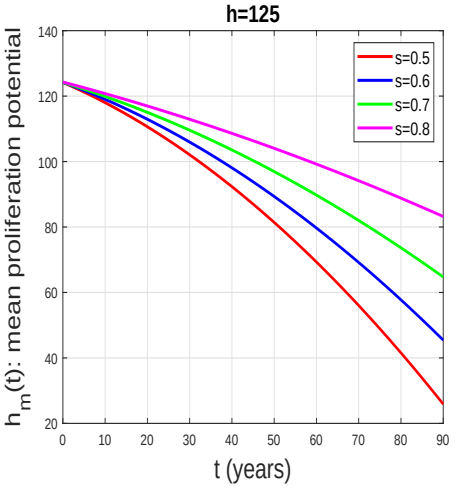
Figure 7 shows simulations of the temporal evolution of mean proliferation potential in POI patients, considering low values for telomerase activity $s = 0.4$ and $s = 0.2$, between 30 and 50 years of age, which is an interval of interest



(a) Low percentile.



(b) 50th percentile.



(c) High percentile.

Figure 5: Time evolution of mean proliferation potential for hematopoietic stem cells in healthy people with low percentile $h = 75$, 50th percentile $h = 100$ and high percentile $h = 125$ for several values of telomerase activity.

in the study of female fertility. Given the marked decrease in mean proliferation potential for women with POI observed in Figure 7, we asked whether telomerase treatments could mitigate the loss of proliferation potential.

5.3.1. Simulation of Danazol treatment to reactivate the expression of the endogenous telomerase gene

The synthetic sex hormone danazol administered orally for a total of 24 months led to the deceleration of the rate of telomere shortening in patients with telomere diseases (Townsley et al., 2016).

We intended to study the effect on the mean proliferation potential of cells with a two-year treatment with sexual steroids, to reactivate endogenous telomerase. The treatment was simulated at different ages: 30, 35, 40 and 45 years. Figure 8 displays simulations of time evolution of mean proliferation potential for POI women with relative low telomerase activity($s = 0.4$), with treatment to reactivate endogenous telomerase at different ages. If telomerase activity was increased to ($s = 0.8$) the mean proliferation potential runs closer and almost parallel to the mean proliferation potential of healthy people at the 50th percentile. However, the lower the telomerase reactivation after treatment ($s = 0.6$), the smaller the effect on improving the mean proliferation potential. Regarding age, proliferation

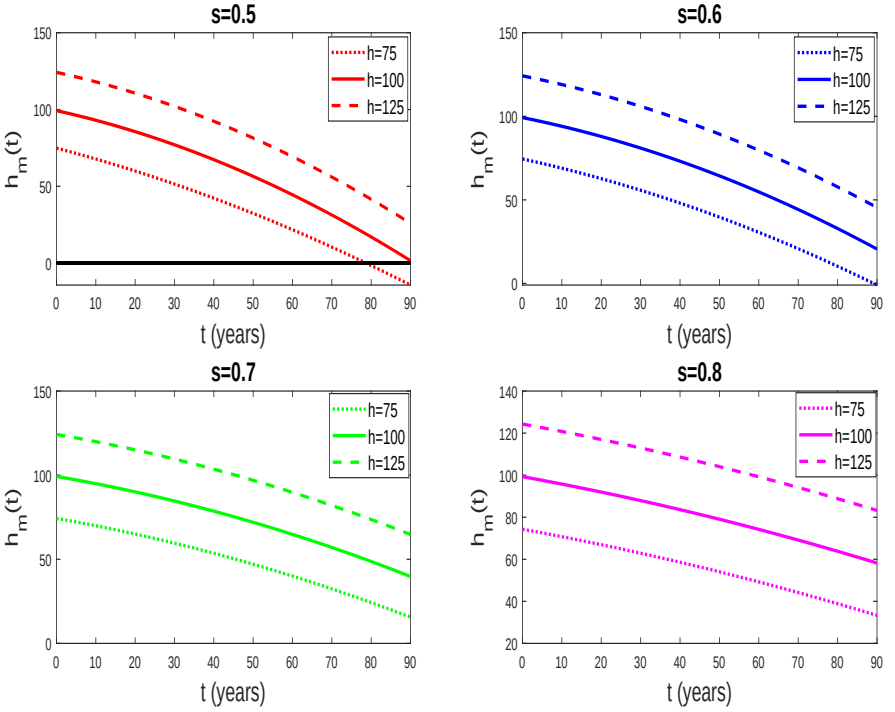


Figure 6: Time evolution of mean proliferation potential for hematopoietic stem cells, $h = 75$, $h = 100$ and $h = 125$.

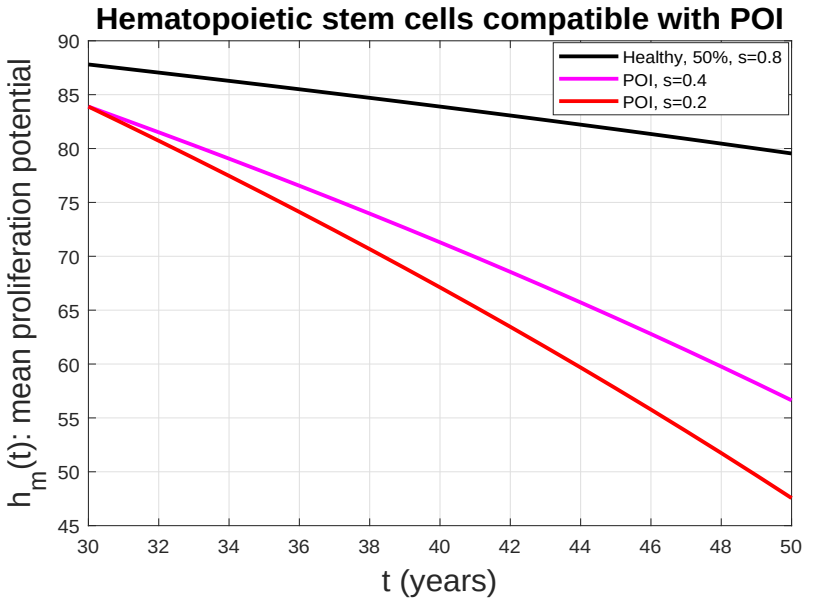


Figure 7: Time evolution of mean proliferation potential for hematopoietic stem cells compatible with POI.

potential is always increased compared to the situation prior to treatment, but as the age advances, the differences with respect to healthy people increase.

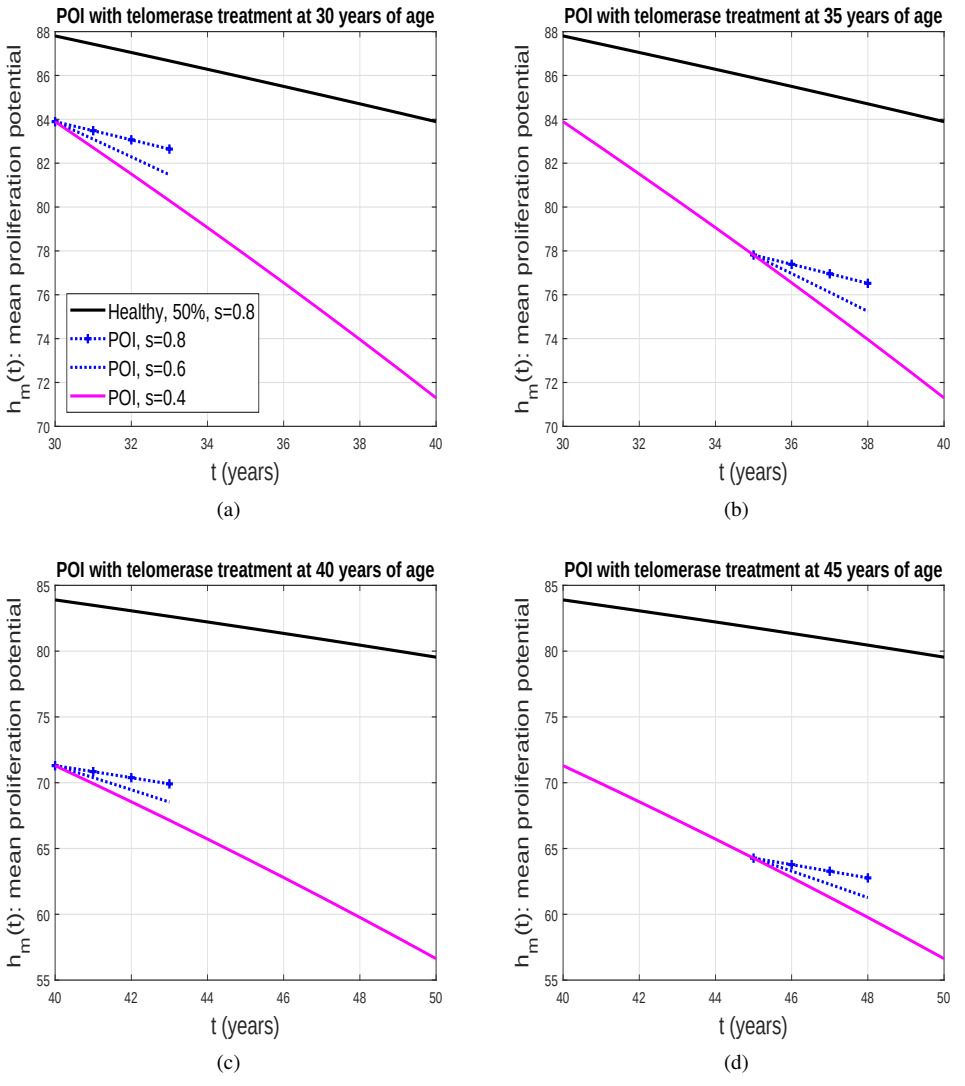


Figure 8: Time evolution of mean proliferation potential for patients with POI, considering relative low telomerase activity($s = 0.4$) with two–year treatment with sexual steroids, at different ages.

Because POI women have been reported to have very low telomerase activity (Xu et al., 2017), we also modelled the evolution of mean proliferation potential for these cases ($s = 0.2$), with treatment to reactivate endogenous telomerase at different ages (Figure 9). As in the previous case, but starting from a somewhat smaller mean proliferation potential, the lower the telomerase activity, due to poorer telomerase reactivation after treatment, ($s = 0.6, 0.4$), the smaller the effect on improving the mean proliferation potential. However, even in the best scenario ($s = 0.8$) the proliferation potential does not reach the capacity of healthy people. Overall, our results indicate that this type of treatment would not be enough as to reactivate the full ability of cells to proliferate showing only partial improvement of this variable. It is therefore necessary to look for treatments that allow to increase telomerase activity to favor cell proliferation potential without increasing the risk for cancer.

5.3.2. Simulation of telomerase gene therapy with AAV9

Telomerase gene therapy has been studied in mouse (Bär et al., 2016; Bernardes de Jesus et al., 2012). Telomerase expression from AAV9 vectors resulted in a several fold increase of telomerase protein compared to untreated mice

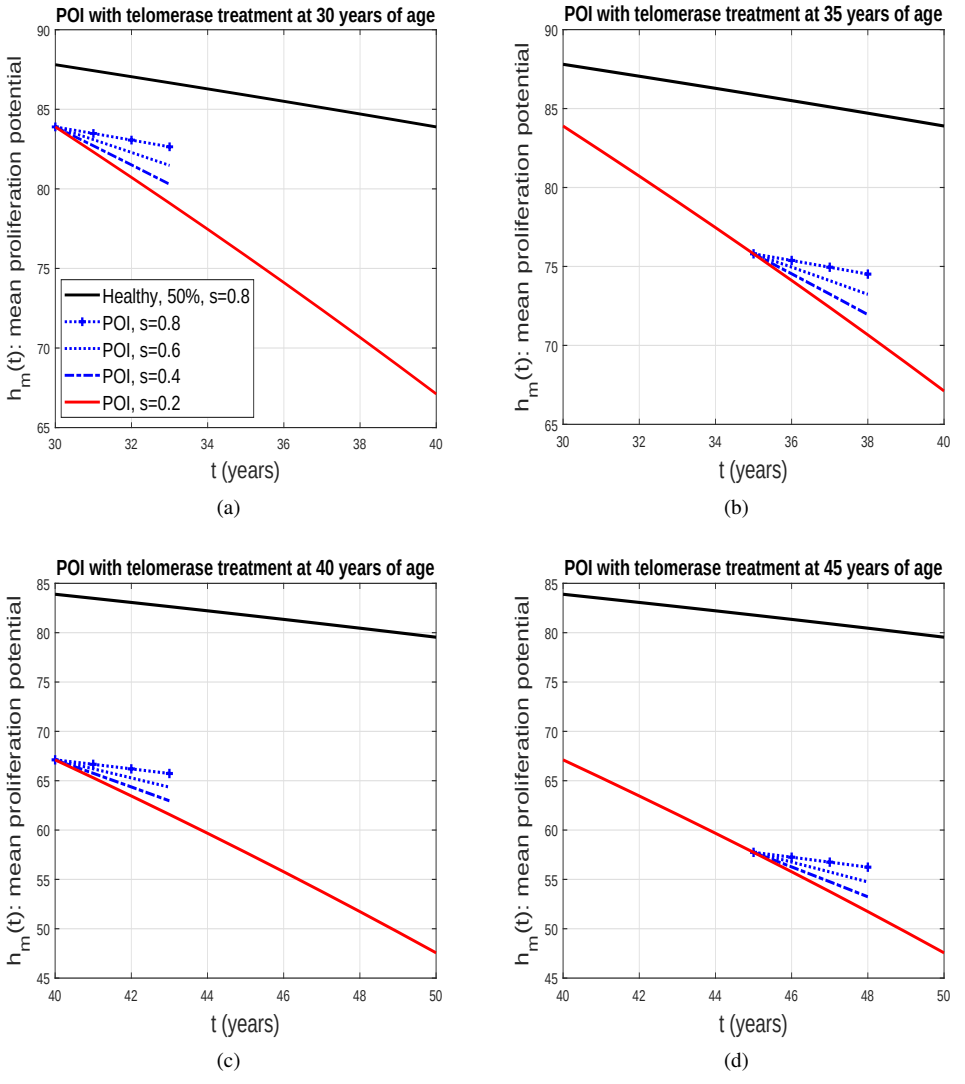


Figure 9: Time evolution of mean proliferation potential for POI considering low telomerase activity ($s = 0.2$) with two–year treatment with sexual steroids, at different ages.

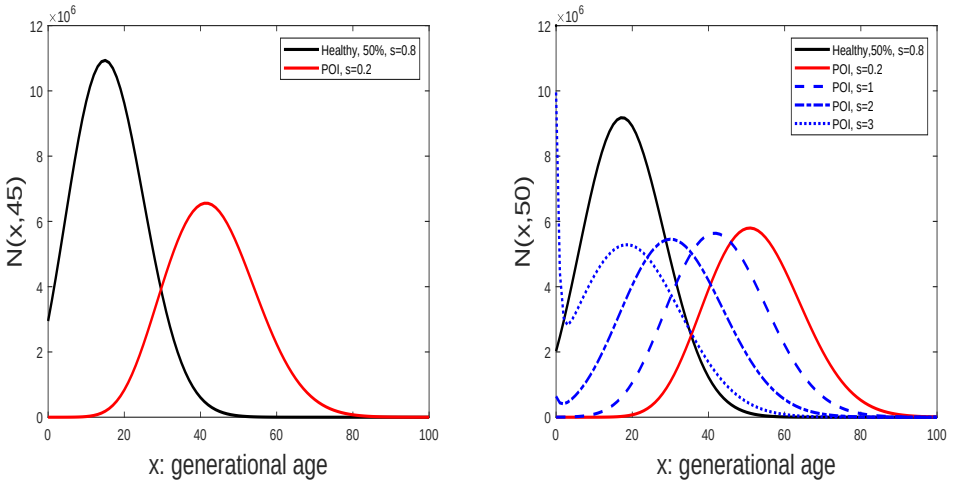
(Bernardes de Jesus et al., 2012) and a delay of aging without increasing the incidence of cancer in adult and old mice (Bernardes de Jesus et al., 2012).

We challenged our model with levels of telomerase above what would naturally be found in ovarian cells, which we represented as $s = 1$, $s = 2$ and $s = 3$. The center of mass of the initial population distribution in the cases of POI ($s = 0.2$) and Healthy ($s = 0.8$) individuals at the age of 45 years, for percentile 50th, is clearly separated (Figure 10 (a)), in a way that POI population is shifted towards higher generational age.

The cell population from POI patients, which was already older than that of the healthy person, at the age of 50 years has aged even further. Interestingly, after telomerase gene therapy, the evolution of the distribution of the cell population at the age of 50 years is shifted towards lower generational age (Figure 10 (b)). In the context of $s = 3$ in POI patients, the distribution of the cell population would be similar to what is found for healthy cells.

As in the previous subsection, the treatment is simulated at different ages: 30, 35, 40 and 45 years. This time with $s = 1$, $s = 2$, $s = 3$, $s = 4$ emulating increasing doses of exogenous telomerase.

Figure 11 displays simulations of time evolution of mean proliferation potential for POI women with relatively low telomerase activity ($s = 0.4$). It is noted that $s = 1$ is a maintenance dose, the average proliferation potential does not



(a) Healthy and POI at 45 years of age.

(b) POI with exogenous telomerase treatment at 50 years of age.

Figure 10: Population distribution corresponding to healthy women at 50th percentile with $s = 0.8$ and POI women with $s = 0.2$, at 45 and at 50 years after telomerase gene therapy with AAV9 (depected in blue), starting at the age of 45.

	POI $s = 0.4$			
	30	35	40	45
$s = 2$	2 years	> 4 years		
$s = 3$	1 year	> 2 years	> 4 years	5 years
$s = 4$	< 1 year	> 1 year	> 2 years	> 3 years
	POI $s = 0.2$			
	30	35	40	45
$s = 2$	2 years	5 years		
$s = 3$	1 year	3 years	> 4 years	> 5 years
$s = 4$	< 1 year	2 years	3 years	4 years

Table 3
Time that would be needed at each dose and at each starting time of treatment to achieve a mean proliferation potential of around 87.

change. If therapy starts at the age of 30 with dose $s = 2$, a situation similar to that of healthy people is reached in two years. When therapy starts at 35 years of age, a dose of $s = 3$ for two years is needed to reach healthy people. However, if therapy is delayed until the age of 40, it is necessary to continue with a dose of $s = 3$ for at least two and a half years. If therapy is delayed for another five years, the dose should be increased to $s = 4$ for more than two years. Similarly, Figure 12 does simulations of telomerase gene therapy with AAV9 for POI women with low telomerase activity ($s = 0.2$). The trend is similar to the previous case but with somewhat higher doses for longer periods of time. For example, in this case, if therapy is started at age 40, the $s = 4$ dose should be applied for more than 2 years and at age 45 for more than three years. Summarising, the better the starting situation and the earlier treatment begins, the less dose of telomerase gene therapy with AAV9 is needed and the shorter the duration of treatment to recover the mean proliferation potential to reach or even exceed that of healthy people.

We take as a benchmark for desirable mean proliferation potential that of healthy people in the 50th percentile at age 30, i.e. around 87. Table 3 shows the approximate time that would be needed at each dose and at each starting time of treatment to achieve a mean proliferation potential of around 87.

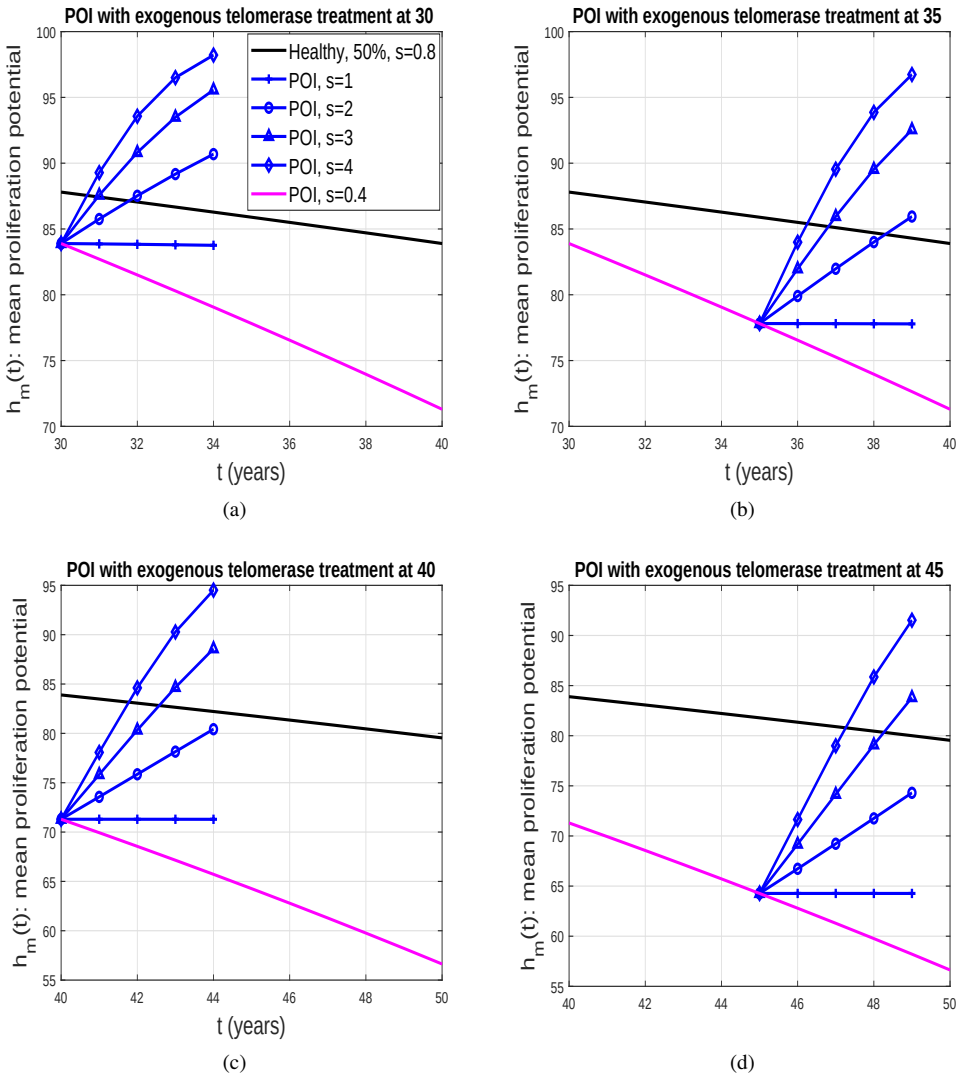


Figure 11: Time evolution of mean proliferation potential for POI with relative low telomerase activity($s = 0.4$) considering several doses of telomerase gene therapy with AAV9 at different ages.

6. Discussion

In this work we have used a mathematical model to analyze the evolution of telomere length of hematopoietic stem cells at reproductive age, considering different amount of telomerase activity. We have further studied the impact of different therapies on telomere length, both assuming endogenous gene activation of the telomerase gene promoter and gene therapy, where the exogenous gene is under the control of strong promoters, yielding higher telomerase expression. Unlike (Boyle et al., 2023; Werner et al., 2015), in our model it has been key to include telomerase activity in the generational-temporal dynamics of the population, as it has an important influence on evolution of the proliferation potential and, consequently, in the ageing of the population.

Starting from an initial Gaussian distribution, concentrated at the left end of the interval $[0, h]$, h the division potential of cells, the temporal and generational evolution of the population was simulated, taking the center of mass as reference of the population aging. Our results show that at older ages the possibilities of cells to divide are smaller, consistent with published data in mice (Martínez and Blasco, 2017; Yamada-Fukunaga et al., 2013) and humans (Uysal et al., 2021; Takubo et al., 2000). In fact, according to the mathematical model, the proliferation potential would

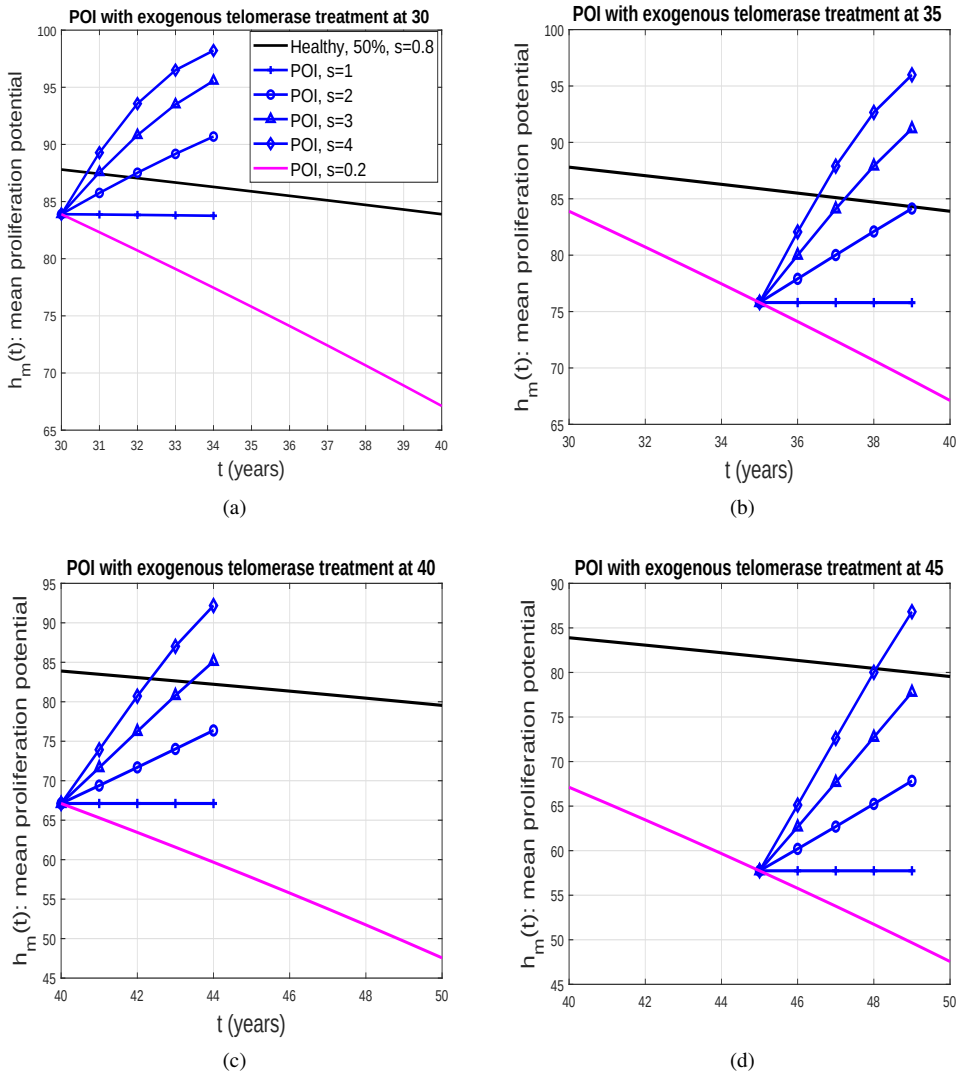


Figure 12: Time evolution of mean proliferation potential for POI with low telomerase activity($s = 0.2$) considering several doses of telomerase gene therapy with AAV9 at different ages.

decrease faster when telomere length was shorter from birth and telomerase activity is at lower levels, for any given age. The enzyme telomerase is a developmental gene, activated at the morula-to-blastocyst transition (Schaetzlein et al., 2004), time at which, the telomere length of the species is reset (Schaetzlein et al., 2004; Wright et al., 2001). The inner cell mass of the blastocyst, which will give rise to the embryo, has the longest telomeres (Varela et al., 2016, 2011) and afterwards, telomeres shorten as stem cells differentiate to give rise to different cell types (Liu et al., 2022). A recent metanalysis shows that telomeres shorten gradually, at a median rate of 23 bp per year until the age of 50, and then, TL shortening has a lower pace during elderly years (Ye et al., 2023).

However, mean telomere length for individuals of the same age is variable and can be affected by risk factors such as tobacco consumption, stress, obesity and other lifestyle choices (Chico-Sordo et al., 2021; Polonio et al., 2020; Valdes et al., 2005; Epel et al., 2004), as well as by genetic factors, such as mutations in telomerase and other genes involved in DNA repair (Martínez and Blasco, 2017; Kong et al., 2013). All these factors lead to different proliferation potential for specific individuals at the same age and also different telomerase activity. For instance, women with ovarian insufficiency have lower telomerase activity in their granulosa and blood cells (Xu et al., 2017; Butts et al., 2009).

To study the case of women with ovarian insufficiency, we used low values for telomerase activity (Xu et al., 2017; Butts et al., 2009), and the model found a dramatic decay of proliferation potential compared to healthy populations of cells. Thus, the question arises whether the decay of proliferation potential could be prevented in these cells. To this end we simulated the results of therapies directed to reactivate telomerase. The first part of the study was based on the fact that telomerase activity can be modulated. On one hand, there are several proteins, which interact with the telomerase RNA component (TERC). These proteins have different functions, for instance, Dyskerin in complex with NHP2, NOP10 and GAR1 (Meier, 2005), binds the RNA component of telomerase stabilizing it, but also participates in telomerase assembly and activity (Wong and Collins, 2006). Telomerase Cajal body protein 1 (TCAB1) binds the RNA component of telomerase and regulates its trafficking to that compartment (Venteicher et al., 2009). The proteins, hEST1 A can bind the protein component, independently of the RNA component. Its overexpression led to telomere lengthening (Snow et al., 2003). On the other hand, the regulation of gene expression, even in placenta (Chang et al., 2023) are critical for telomere maintenance. Transcription of the telomerase gene TERT has been shown to be regulated by the transcription factor cMyc (Wu et al., 1999) and also by sexual steroids (Calado et al., 2009) which will bind the promoter of telomerase gene and promote its expression (Calado et al., 2009). The mathematical model showed that at early ages, treatment with sexual steroids was more effective than treatment at older ages. Interestingly, at any age, the drastic diminution of the proliferation potential of cells was prevented, even if those cells had very low telomerase activity. However, after therapy, POI cells would never be close to the proliferation potential of healthy cells. These findings are in agreement with studies in humans with sexual steroids, in a population of individuals with short telomeres due to mutations in the telomerase gene (Townsley et al., 2016). Interestingly, the rate of telomere shortening in blood cells was reduced after treatment and the symptoms were alleviated, to the point of becoming independent of blood transfusions, even after the treatment was finished (Townsley et al., 2016).

The second part of the study was performed assuming that telomerase expression by gene therapy is much stronger (Bär et al., 2016; Bernardes de Jesus et al., 2012) and cause an improvement of organismal fitness and lifespan in mice (Whittemore et al., 2019; Bär et al., 2016, 2014; Bernardes de Jesus et al., 2012). When gene therapy with telomerase was modelled mathematically, the proliferation potential of cells with POI was recovered and reach the levels of healthy cells and could eventually, depending on the expression of telomerase ever surpass the proliferation potential of healthy cells. While these results are promising, telomerase gene therapy in humans raises ethical concerns. When telomerase is expressed in mice from birth, an increase incidence of tumor formation is found (García-Cao et al., 2006). This effect is not observed when telomerase is activated in adult mice (Bernardes de Jesus et al., 2012), perhaps future studies may clarify the effects of telomerase gene therapy in humans.

Conflict of interest statement

Nothing to declare.

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