



Acknowledgements

Drug screening assay



Heike Furhmann-
Stroissnigg



Yuan Yuan
Ling



Shannon Howard

Stem cells



Akaitz Dorronsoro



Lana Corbo

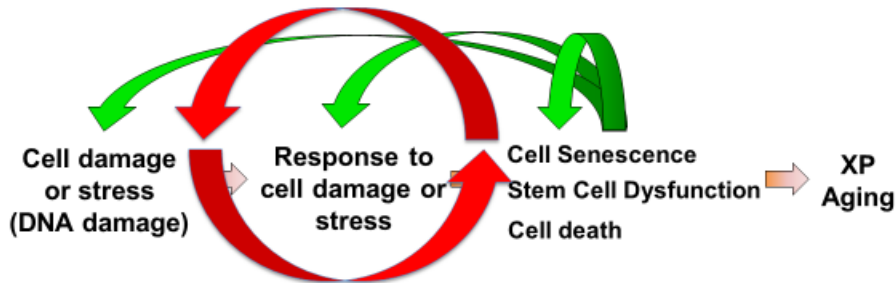


Matt Yousefzadeh



Sara McGowan

XP and Targets for Therapeutic Intervention



We continue to learn that one way that DNA damage causes problems is how your body responds to it.

Our current thinking is that DNA damage triggers activation of stress responses. These determine cell fate:

DNA damage can cause cell death – removing cells that are vital for organ function.

DNA damage can cause stem cells to have poor function – no longer able to help regenerate injured tissue.

It can cause cell senescence. Senescent cells secrete danger signals that promote inflammation and cause other cells in its vicinity to function less well.

Senescent cells can cause adjacent cells to senesce – snowballing the problem.

They can also sustain the danger signals, exacerbating the problem.

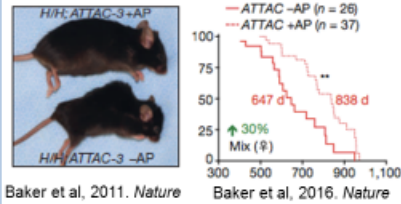
And they can cause additional DNA damage by increasing reactive oxygen species (ROS) production.

This creates a vicious cycle where DNA damage has launched a cascade of further damage that is hard to stop.

Acute and chronic senescence in aging and age-related diseases

Cellular senescence is defined as an irreversible state in which damaged cells become refractory to stimulation, but remain metabolically active.

Genetic trick to ablate senescent cells








Baker et al, 2011. *Nature*

Baker et al, 2016. *Nature*

Recently it was proven that if you can rid the body of senescent cells then mice live healthier and longer.

The question now is can we make a pill to do this?

Mouse models of XP	
<ul style="list-style-type: none"> + <i>Ercc1</i>^{-/-} mice age 30X faster + <i>Ercc1</i>^{-/Δ} mice age 6X faster ⇒ Rapid drug testing + Model a human progeria ⇒ Relevant to humans + Identical to very old normal mice <ul style="list-style-type: none"> + Biomolecular + Gene expression + Pattern of senescence + Histopathology + Organ function + Ultrastructural changes 	<div>5 wks</div>  <div>9 wks</div>  <div>14 wks</div>  <div>22 wks</div>  <div>25 wks</div> 

We use a model of XP that is very exaggerated to do drug discovery. The thinking is if a drug works in this severe disease model, it is apt to provide benefit in less severe situations.

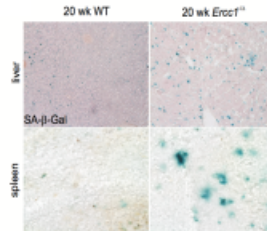
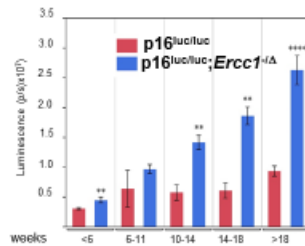
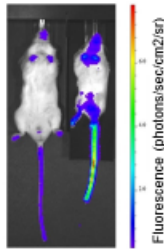
The mice, on top of having XP-like features, age very quickly. This makes drug discovery very rapid. But it also provides things we can treat that may be of interest to a broader population than XP patients (i.e., anyone who is aging). This may help with drug development.

The mice also model a progeroid syndrome (disease of accelerated aging) caused by mutations in XPF. This makes the model relevant to human health.

Finally, we compared the mice to naturally aged mice and found similarities at almost all levels. This emphasizes that there may be some aspects of XP that look like accelerated aging – aging of the skin, the reproductive system, perhaps the brain.

Increased senescence in *Ercc1*^{-Δ} mice

18 week *p16*^{luc/luc}
Wt *Ercc1*^{-Δ}



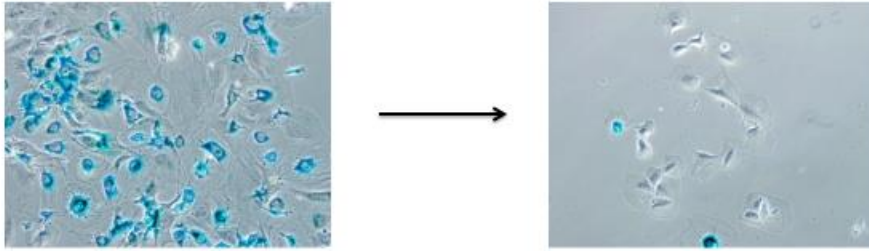
Yousefzadeh, Zhao et al, in preparation

The *Ercc1* mice that model XP have increased numbers of senescent cells in their bodies.

This shows that failure to repair endogenous DNA damage, when you are missing nucleotide excision repair, can lead to an increased burden of senescent cells.

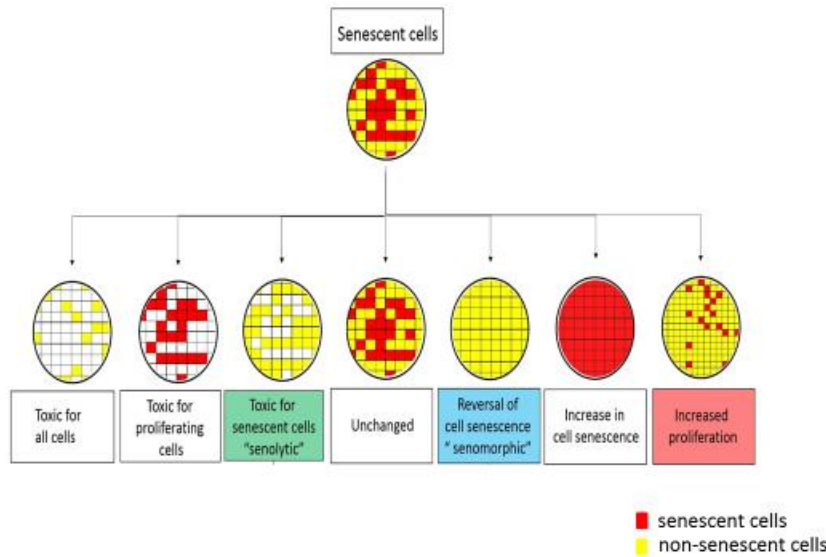
Senescent cells

Discovery of drugs that can rid us of senescent cells



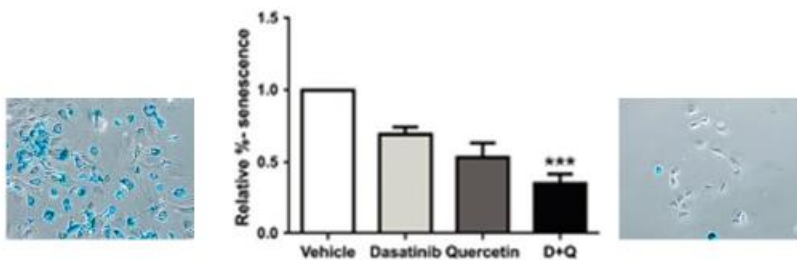
We are looking for drugs that reduce the burden of senescent cells. On the left is a dish of cells from an XP mouse model. Most cells are senescent (stained blue). When we add drugs, the goal is to get rid of the senescent (blue) cells.

Assay identifies different types of senotherapeutics



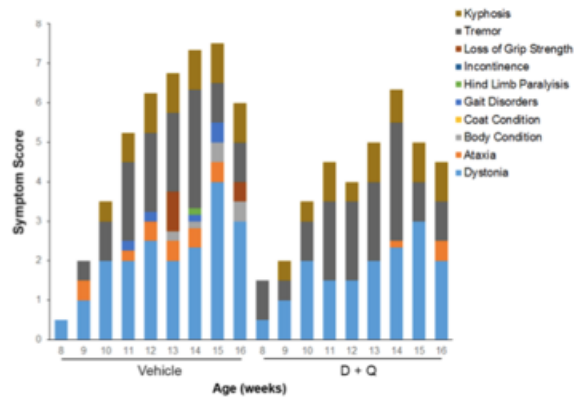
Here is what the test (assay) looks like. We start with a plate of cells that are mixed between healthy cells (yellow) and senescent cells (red). After testing a drug, there are several possible outcomes. The important ones are indicated in green: all of the senescent cells are killed, while the healthy cells remain fine; and blue: all of the senescent cells have reverted to a healthy status.

Dasatinib & Quercetin



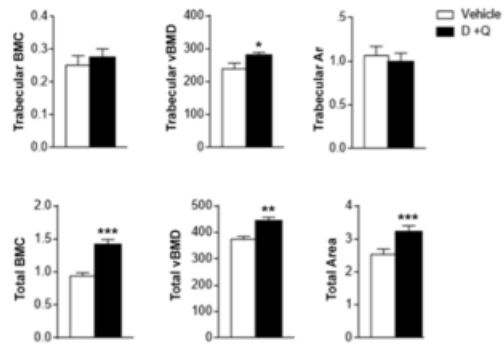
Here are some of the first drugs that we tested in the senescent cell assay. Dasatinib is a drug used to treat blood cancers. And quercetin is a natural product available from GNC. Each alone cleared senescent cells. But the combination was very potent. The fact that dasatinib is an FDA-approved drug and quercetin is a natural product will make it easier to get them approved for clinical use to remove senescent cells.

Weekly treatment with D+Q delays and lessens many symptoms of aging



When we treat the *Ercc1* mice with Dasatanib and quercetin, we see a significant delay in symptoms. Each color represents a symptom. Each week of life we measure all of the symptoms and determine if the mice have it yet and how severe it is. So the higher the bar, the sicker the mouse. On the right, side is the mouse that got the drugs. The bars are lower and shifted right for later onset. It looks like a subtle difference, but because this is an acute model, it equates to ~10 years delay in health problems in humans.

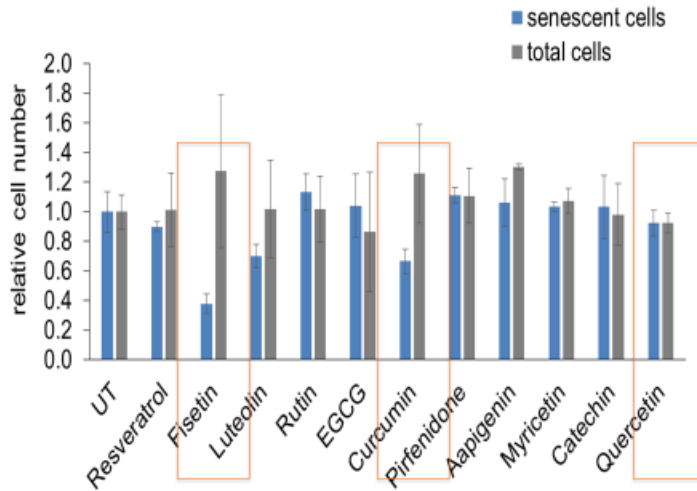
Weekly treatment of *Ercc1*^{-Δ} mice with D+Q improves bone



Zhu, Tchkonja et al. 2015. *Aging Cell*

Dasatanib and quercetin improved bone density significantly in the *Ercc1* mice

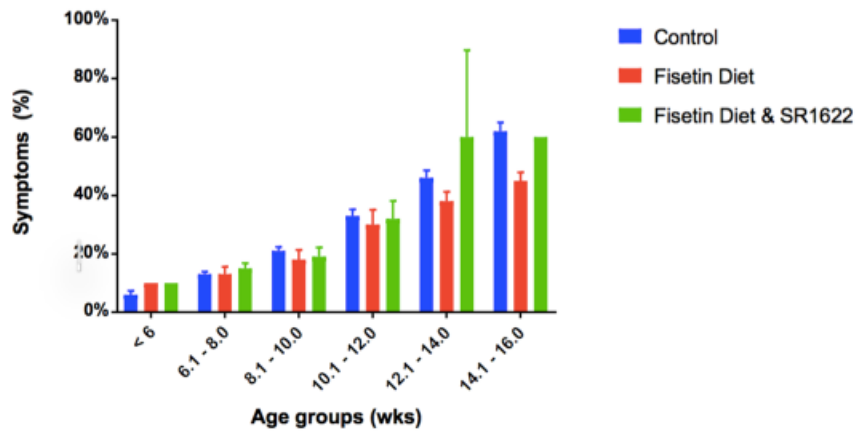
Senotherapeutic Polyphenols



We also tested a whole series of natural products related to quercetin for their ability to specifically kill senescent cells. Two that worked even better than quercetin (lower blue bar) were fisetin and curcumin. Curcumin is the yellow spice in Indian food. Fisetin is another natural product that you can buy from GNC or Amazon.

Fisetin extends healthy aging in *Ercc1*^{-Δ} mice.

Fisetin & SR1622



We put fisetin in the diet of *Ercc1* mice and it delayed their symptoms significantly (lower red bars).

Senolytics



How we view this is that when cells get damaged (DNA damage), many of them become senescent (yellow stars). It should be possible to take one of these drugs that clear senescent cells periodically to get rid of the damaged senescent cells. Clinical trials to test this are beginning at Mayo clinic. One of the test cases will be children who had cancer and were treated with high doses of chemotherapy. This is analogous to XP because these children experienced an extraordinary amount of DNA damage.

Targeting Aging with Metformin (TAME Trial) Glucophage

A TRIAL FOR THE AGES

Nir Barzilai wants to launch the first rigorous test
of a drug that could put the brakes on aging



Developed as part of a R24 NIH consortium,
focused on how to
design clinical trials for aging.

Patients enrolled in the study, between 65
and 79 years of age, will have one or two
of three conditions:

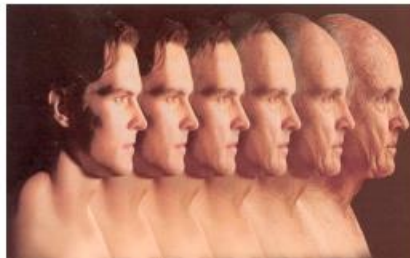
1. Cancer,
2. Heart disease
3. Cognitive impairment

The study will determine if metformin will
delay the onset of getting the next age
related disease.

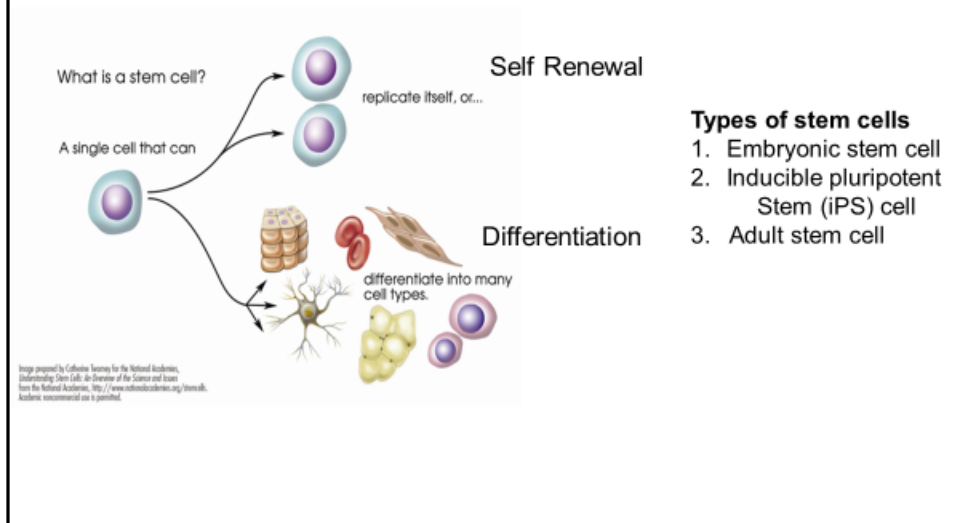
The first ever clinical trial to treat aging, as opposed to individual age-related diseases, will begin in 2017. If this is successful, which we will know in 3-5 years, then it will be possible to test drugs that target senescent cells very quickly. If you would like to know more about this trial, there is a National Geographic episode in the Breakthrough series that is on TV. Or google TAME.



Stem Cells and Aging



What is a stem cell?



Stem cells are special cells that are needed to regenerate injured tissue.

They have two properties: they make a copy of themselves (self-renewal) in order to maintain the number of stem cells. And they are able to make lots of types of cells (differentiation) to fix injured tissue.

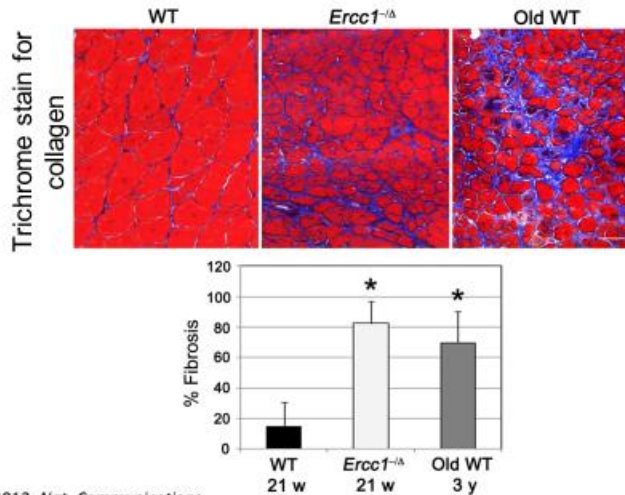
There are three general types of stem cells. Embryonic stem cells can make an entire new body, so they have a lot of value for tissue regeneration. But they are made from human fetuses. Thus they are highly controversial and banned from use by law.

IPs cells are a new technology (just awarded a Nobel Prize) in which you can take skin or muscle from anyone and turn it into the analogy of embryonic stem cells. This is very powerful, but not yet in clinical practice.

Adult stem cells are rare stem cells found in the tissue of all adults. These can be isolated from biopsies (fat, muscle, bone marrow). They are being investigated for healing powers.

Impaired muscle regeneration in progeroid and old mice

5 days post-cardiotoxin injection into the gastrocnemius muscle



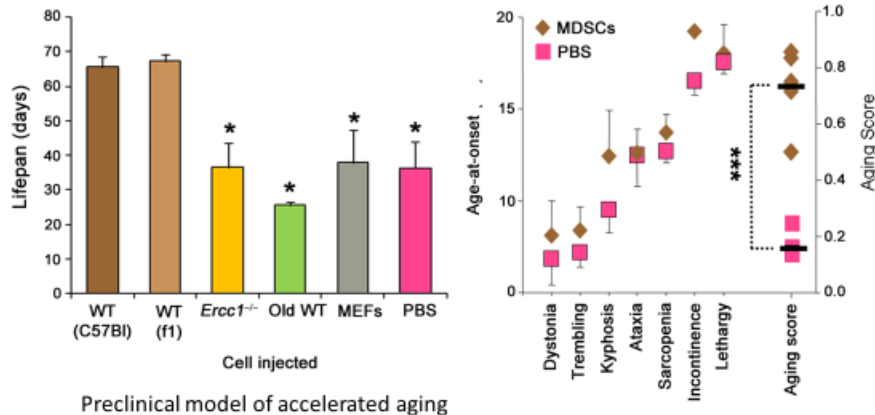
We showed in the *Ercc1* mice (severe XP) that adult stem cells appear to age more rapidly.

We cause muscle injury by injecting snake venom.

The normal mice (WT) heal their muscle within 5 days (lots of red myofibers). Old normal mice (old WT) heal poorly. There is a lot of fibrosis (blue) rather than new muscle. The *Ercc1* mice look more like the old mice than young. This means that muscle stem cell function declines with age or in response to unrepaired DNA damage.

First to show that stem cell attrition drives aging (not a consequence of)

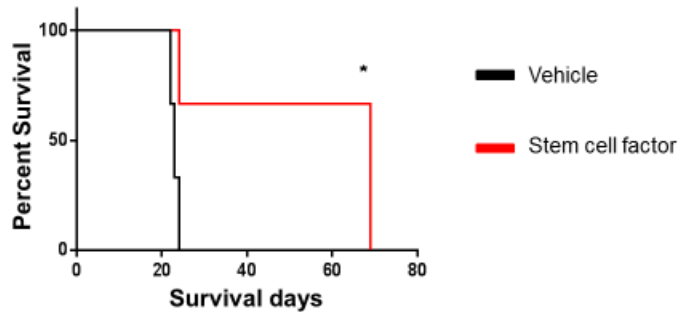
Big therapeutic implications !



We also showed that if you treat *Ercc1* mice with stem cells from a young adult mouse they lived longer (higher brown bars on left) and healthier (on right, brown diamonds are higher than pink squares, meaning that symptoms are delayed). This means that some day, stem cell therapy may help with some symptoms in XP patients.

The active ingredient in stem cells for extending lifespan is secreted

This improves efficacy and safety exponentially



Importantly, it is not stem cells themselves that are therapeutic, but something they secrete. The factor secreted by stem cells extend lifespan of Ercc1 mice (red line) compared to placebo (black line). This is important because getting stem cells from another person is the equivalent of doing a transplant. The recipient may have an immune response to the donor cells. This raises the question of a cost benefit ratio of trying stem cell therapy. But a factor secreted by stem cells should be much safer since there will be no immune response to it.

What does this mean for XP

- Fisetin is available at GNC, Amazon and Life Extension. However, the right dosing regiment is still unknown.
- Certain FDA approved anti-cancer drugs also could have benefit, but again, the dosing regiment has to be worked out. Here testing the compounds on skin first might be the quickest path to clinical use.
- The combination of a modified resveratrol (Pterostilbene) and a NAD⁺ precursor, important for at least one function of resveratrol, is available from Elysium. Improved resveratrols work better in our assay and a NAD⁺ precursor is entering a clinical trial for CS.
- Potent free radical scavengers work in the cell assay and in the mouse model. The most effective are not in clinical studies, but clearly taking anti-oxidants is a good thing.
- Metformin, although it has a marginal effect in mouse models, improves metabolism with aging. Might have a positive effect in XP.
- Additional new approaches, like factors from stem cells, are coming fast!