Mapping of long term effects on health :

Large scale testing in aged mice of the most promising therapies against aging and related diseases.

31/12/2014

We dedicate this presentation to L. Stephen Coles, M.D., Ph.D., January 19, 1941 – December 3, 2014, a pioneer in the field of gerontology and longevity research.

Stephen Coles had put in contact mouse experts in the USA with a few core volunteers of this project to engage technical discussions and see what could be achieved.

For many years, Dr. Coles managed the Gerontology Research Group mailing list, which has been an immense source of inspiration for this project.

Longevity: the revolution

PAST

A revolution has happened in nematodes (C. elegans)

- Revolutionary discovery in 1980: 8 ways to extend their life [1]
- In 1993, a mutation doubled the lifespan, in great vitality [2]
- In 2003 *thousands* of longevity tests performed; about 10% extend life.[3]
- In 2008, a mutation multiplied lifespan by nearly 10 !

Worms and mice age in different ways

- Worms have a fixed number of cells: prolonging cell life is key
- Mice are mammals and regeneration of the tissues is key

The longevity revolution is reaching mice and is promising for humans

- The revolution is reaching mice, but *few* tests are done.
- Mouse life-extending treatments known so far have actions on *human* aging (aspirin, metformin, metoprolol, rapamycin)
- Life-extending genetic patterns in mice were found in aging-protected *human* families (Larron-dwarfs, Foxo-3A, akt-1)

Let us perform many parallel longevity tests in mice, in ways that are transposable to humans.

NOW

Testing program overview

Timeline

• A set of lifespan tests will start with pre-aged animals and last for 18 months

•Another set needs mouse lines to be created and will last for 38 months

The results will be triplechecked and tuned in ways that can be transposed to humans. The major bulk of results will come in 5 years after testing is started.

People for the job

We have communicated with a number of researchers and have identified a group of labs with key animal conditions and key experience in lifespan tests and regenerative medicine.

Budget

The estimated cost of holding 1000 different tests is €20M.

There is also a direct correlation between funding, the number of tests and the schedule.

Expected results

We expect about 100 results out of 1000 tests to be positive:

- [illegible]todes, 10% of tests were positive (many tests were done)
- In mice, ~10% (difficult to estimate as few tests were done; ITP+Spindler)

Expected benefits:

1. Trigger a mind shift at all levels of society
2. The results should provide a general overview about what extends life.
3. We hope to achieve major life extension.
4. We hope that some apply to humans.

Application in human longevity studies

If we start our testing program this year we are expecting to see concrete impacts in less than 10 years.

What to test: interventions by source

Partial results already

Longevity DB: over 1000 interventions

Database lists human genes that are statistically correlated with longevity

➔ for many of them a causation could be tested in mice.

http://longevitydb.org/browse

Lifespan Observation Database

3359 life extensions reported: in rodents (114;often unclear but promising results), nematodes (2400), other species

➔ further confirmations should be done in mice.

http://lifespandb.sageweb.org

Numerous ideas

Facebook group: PotentialGerontodrugs

over 300 posts currently

➔ Each post is a potential life extending intervention to be tested in mice.

https://www.facebook.com/groups/gerontodrugs

Other sources

Various similar databases have been found. Numerous relevant forums and mailing lists are analyzed. We have also started to circulate a piece of paper in aging research congresses to list ideas.

.

Overall number of interventions is huge. Analysis is underway to set priorities

What to test: interventions by type

Human drug treatments

For treatments taken by human populations that are already thought to favor long healthy lives in humans. Concept: if the mice live longer and are healthier, it is a strong argument that the drugs are causally improving health in people.

Fundamental research results

Typically i*n vivo* and *in vitro* results from mice, rats, nematodes or fruit flies, needing robust lifespan & health span confirmations -- in some way that can be transposed to humans.

mouse drug treatments

various mouse therapies

Human genetic variants

For variants found in human populations that are already thought to favor long healthy lives in humans, and that can be transposed into a gene therapy in aged mice. Concept: if the mice leave longer and are healthier, it is a strong argument that the variant causally improves health in people and such a gene therapy may be likely to be effective in humans too.

mouse gene therapies

Interventions database

rs2292664
PCMT
rs9930200
HSPA9
cisd2
TERT
Plau
Thymus transplant

Overall schedule

Phase I
1 year

Formation of teams, fundraising, Investigation of techniques

TESTS STARTING WITH ALREADY-AGED MICE

Phase II	Phase III	Phase IV	Phase V
2 months	14 months	18 months	18 months
Equipment preparation	Test of 40 to 400 therapies	Confirmation and optimization of the results	Protocol initiation in humans

TESTS STARTING WITH SETTING A MOUSE LINE

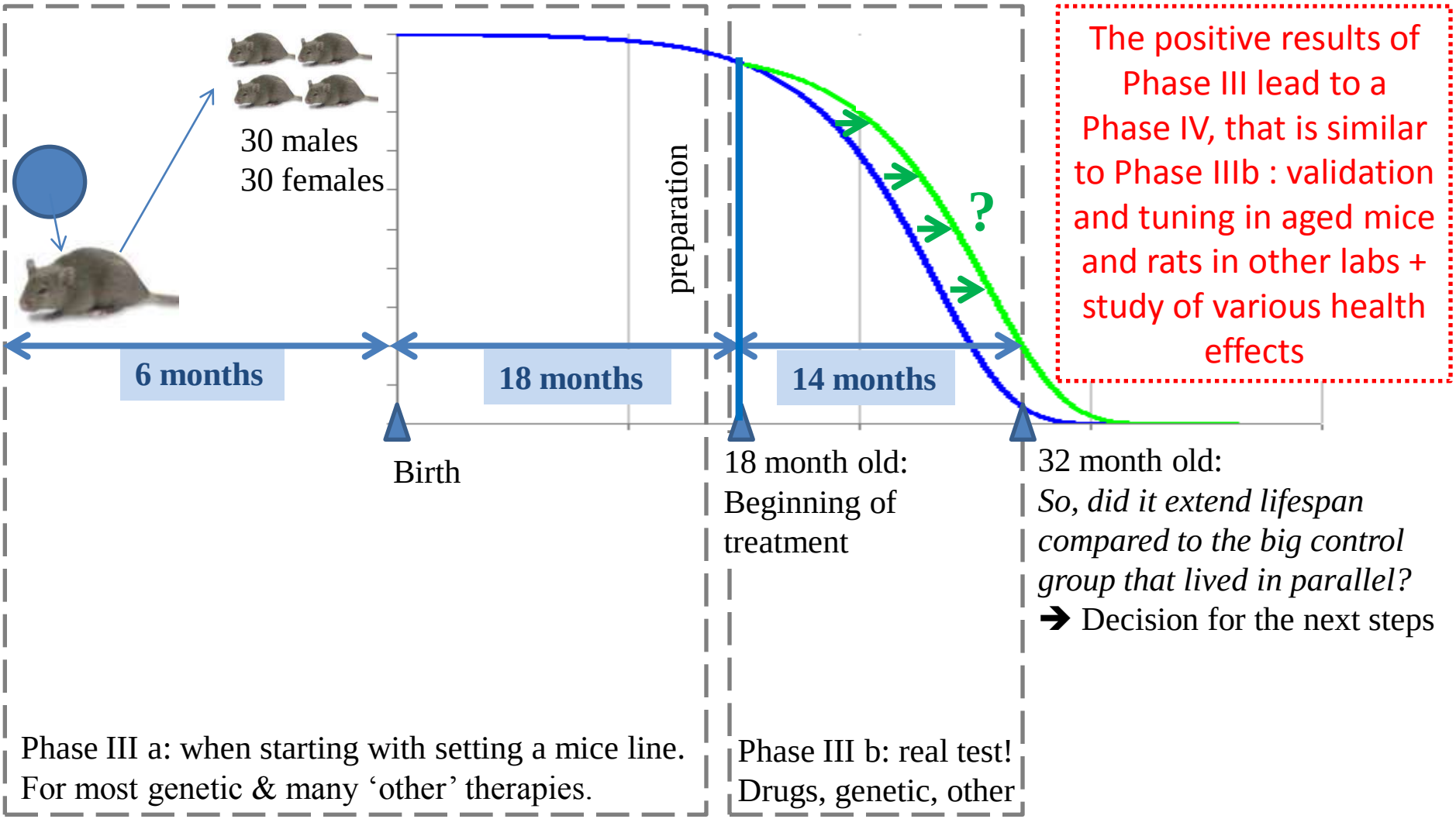
2 months	38 months	18 months	18 months
Equipment preparation	Test of 60 to 600 therapies	Confirmation and optimization of the results	Protocol initiation in humans

→ The schedule of Phase III is detailed next slide.

The number of interventions to test depends on funding

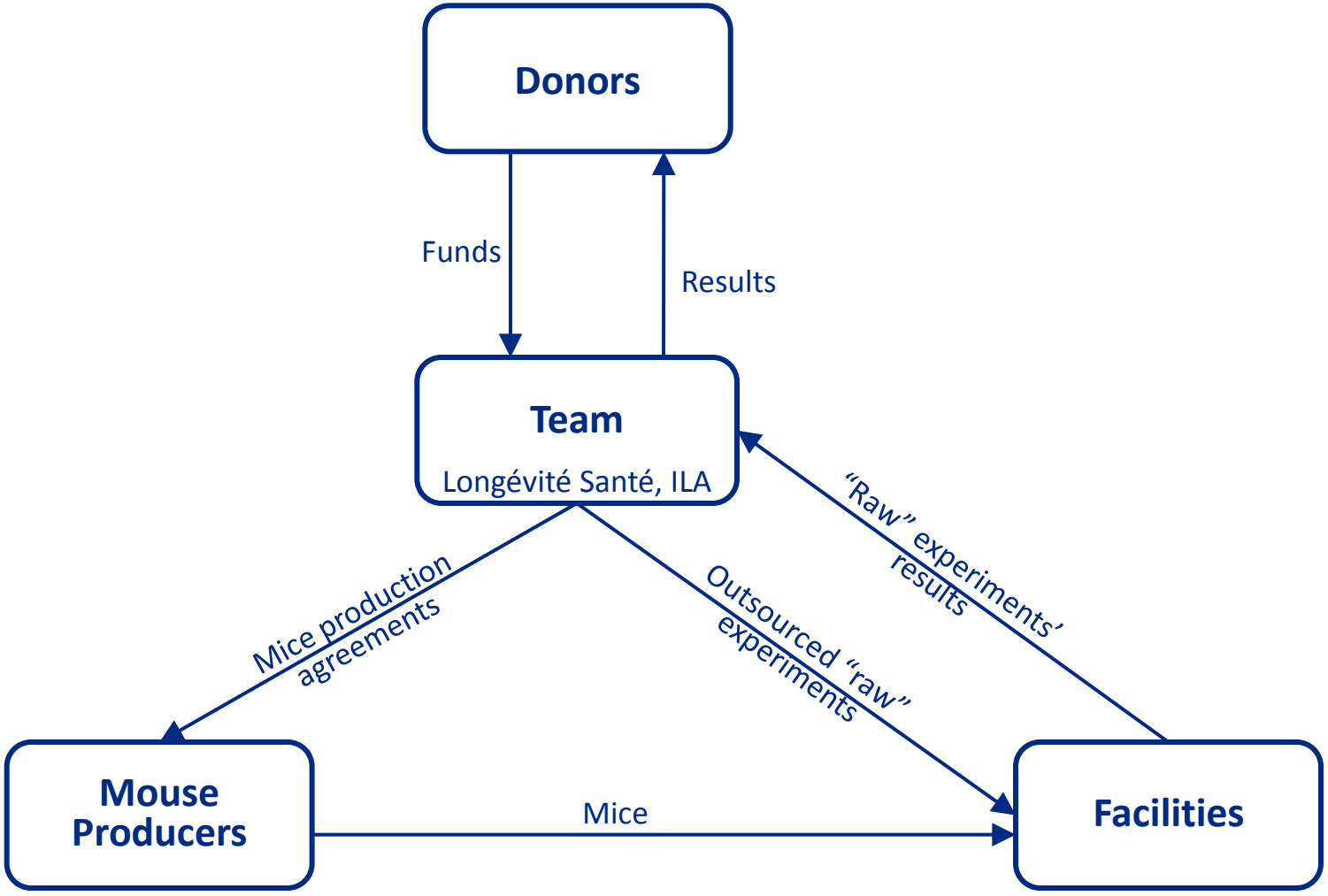
2 m€ → ≈100 therapies tested → ≈10 positive results
20 m€ → ≈1000 therapies tested → ≈100 positive results
The financial estimation is computed page 17. Besides the estimation, the number of tests performed will be that permitted by the funding.

Phase III layout



The positive results of Phase III lead to a Phase IV, that is similar to Phase IIIb : validation and tuning in aged mice and rats in other labs + study of various health effects

General organization



Current "To Do" - list

Today

Identify and sort potential interventions

Work in progress...

Identify funding sources

Refine testing methodology with the labs

Work in progress...

Locate labs suited for the job and discuss terms and conditions with them

DONE!!

Contact potential investors. Make general agreements

Calculate costs of experiments (equipment & personnel)

Work in progress...

Detailed funding negotiations

Receive the amount necessary to start the project

Future

Teams and labs

Laboratories equipped with the highest quality animal facilities (cages with individual filters and ventilation) and specialized in regenerative medicine and the research against aging processes

- Arlan Richardson
  - 40 years experience in long-term tests on rodents (compounds and gene therapies)
  - Laboratory USA (Oklahoma)

- Alexandra Stolzing
  - Regenerative medicine & stem cell aging
  - Translational Centre for Regenerative Medicine

    Leipzig University (Leipzig)

- Jean-Marc Lemaitre
  - Regenerative Medicine on mouse and human cells (stem cells and gene therapy)
  - Inserm in France (Montpellier)

Team of volunteers

Special thanks to the teams of volunteers:

Core team:

Edouard Debonneuil, Rached Messaoudi, Valentin Socolov, Martin Dinov, Daniel Krochmal, Maciej Michalak, Nicoleta Reinald

With assistance from:

Steve Coles (deceased), Johnny Adams, Daria Khaltourina, Matthew Scholz, Didier Coeurnelle, Anton Kulaga, Paul Spiegel, Liz Parrish, Alexandru Chircu, Alexandre Blanc, Dmitri Borisoglebsky, Victor Björk,… and so many other researchers and online volunteers.

▲Longévité & Santé, weekly evenings to organize the project at La Paillasse, Paris.

◀helpers from various countries and for various occasions

Thank you for your attention!

Please note that the updated version of these slides is at
http://longevityalliance.org/Projects/MajorMouseTestingProgram

Would you like to help,

- **Donate** via paypal at SLS_paypal@googlegroups.com
  - Or other means using the IBAN: FR7630003040400003726544034
- **Contact** mouseproject@longevityalliance.org to propose very specific help

  Specific volunteering help we have in mind:
  - If you have experience on mouse lifespan and healthspan testings, or in vivo gene therapy techniques, or drug incorporation in food, or anything that should make the design of the project more precise
  - If you know some potential sources of funding that you think may correspond, or are interested in spending time in writing grants please do not hesitate to suggest us
  - presenting the project at a conference or translating the slides in another language
  - Contacts for registered, non-lucrative, life-extension organisations that have fiscal advantages for donors in their country, who might want to help fund the project

Note: We are aware that various URLs and email addresses in this slide are not homogenous, and we are working to improve this in future versions. For clarity, SLS is Sceaux Longévité Santé, and ALA is the American Longevity Alliance, not-for-profit affiliates of the International Longevity Alliance located in France and the U.S., respectively.

Appendix

- Recap in 3 slides
- List of therapies to test in mice
- Techniques of gene therapy
- Other

Today there are less than 10 real rodent longevity studies per year. **Our goal: 1000 tests in less than 5 years**

Appendix

- In nematodes, 10% of thousands of tests were positive
- In mice, also about 10% [5/67 by Spindler.S and 5/15 tests by the ITP, 1-2 in common] :

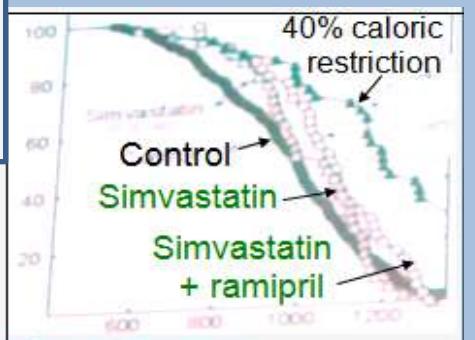
- **Interventions testing program, USA:**
  - Rapamycin (males, & females, +10-25%)
  - Aspirin (males, +10%)
  - NDGA (males, +10% lifespan)
  - Acarbose (males, +22%)
  - α-Estradiol (males, +10%)

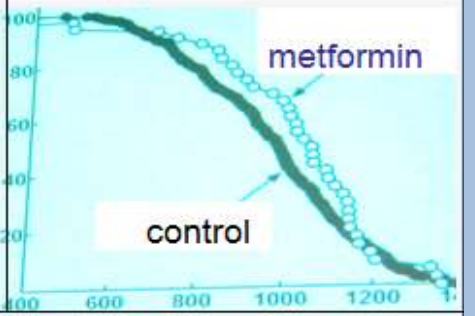
Aspirin: lower mortality confirmed in humans (80 mg/day, ≥5 years)

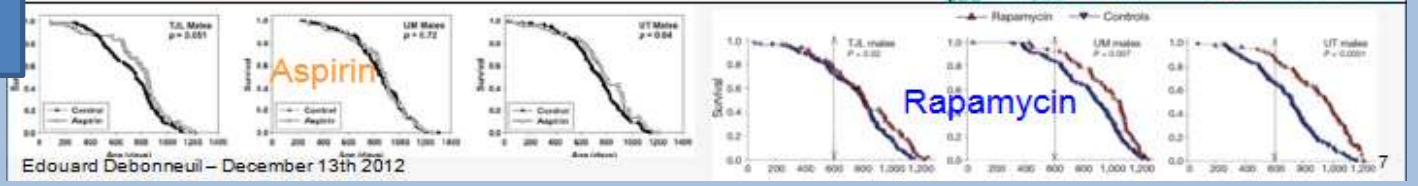
Everolimus: under test in the elderly (strengthening of the vaccine against flu)

Metformin: decrease in mortality confirmed in humans

- **Stephen Spindler et al., USA (EHA2012)**
  - α-Adrenergetic receptor blockers
    - Nebivolol, Metoprolol
      - Spindler et al. AGE. In press, 2012
  - Everolimus -- similar to rapamycin
  - Simvastatin -- statin
  - Ramipril -- ACE inhibitors
  - Metformin
  - NDGA -- small therapeutic window
  - Oxaloacetic acid







- Other results being checked in rodents (fullerenes)

Expected Results

Appendix

Discoveries in mice have a huge potential to lead to advances in human long term health

1

Trigger a mind shift at all levels of society

"Applied biology of longevity is upcoming" in view of numerous therapies that prevent or reverse/silence aging processes Can we obtaining several-fold life extensions in mice?

2

A rupture in life extension science

Guide longevity research through what indeed extends lifespan. Highlighting underlying mechanisms.

In conjunction with human statistics, we hope some results could apply perhaps as soon as in five years.

3

Strong life extension

Among the many expected results, the hope is that some lead to a very strong life extension

4

A new area of pharmacy: "long term health pharmaceuticals"

Discover new applications of existing human drugs on aging and long term health

Financing & Budget

Appendix

- Note:

Costs are being refined. In any case, the number of tests performed will be that permitted by the funding

Phase III

- Let us consider that 400 therapies are tested with aged mice and 600 therapies require a new mouse line; that each therapy needs 60 mice (30 males and 30 females) and that each of the two systems (aged mice/mouse lines) require a control of 1000 mice (asymmetric design; 50% male-female).
- Let us consider that handling mice costs about 1.5€ per mouse and week (depends on the housing conditions) and that mice live on average 27 months. A rapid calculation tells us that the overall cost of handling mice is 1.5*(27*(600*60+1000)+9*(400*60+1000))*(30.5/7) = 8 M€
- Let us consider that each of the aged mice costs 100€ and each of the young transgenic mice costs 30€ to produce. The initial cost of mice is (600*60+1000)*100+(400*60+1000)*30 = 4.5 M€
- The total for Phase III is 12.5 M€

Phase IV

- Let us consider that 10% of the therapies tested in Phase III lead to a Phase IV, and that on average for a given therapy Phase IV costs 6 times as much as Phase III (because the life extension needs to be confirmed and tuned). The cost of Phase IV is 12.5*10%*6 = 7.5 M€

Total

The total to test 1000 therapies would then be 12.5+7.5 = 20 M€, with the working hypothesis above. The main drivers of the cost are underlined above

Therapies to test in mice

- Examples of therapies extracted from the Facebook group

HIGH PRIORITY

- Control life extensions: aspirin, metformin, rapamycin, statins
- Snell, Ames, IGF-1 mutations at high age
- TA-65, other telomerase activator, and human telomerase variant
- TM5441 (quadrupled mouse healthy lifespan; decreases levels of senescence marker PAI-1)
- Centrophenoxin/Meclofenoxate: reduced lipofuscin levels & non published rat life extension
- Pharmacoperones (inverts the GNRHRmutation)
- Neuraminidase 1 supplementation (reported to partially reduce Alzheimer's disease)
- GDF11 injections (reported to partially reduce heart and brain aging in mice)
- D-Glucosamine (calorie restriction mimetics, reported to increase lifespan in aged mice)
- Dimethylaminoethanol (life extensions reported; lower doses to be tested)
- Trehalose (enhances autophagy; alters the IGF-1 axis; clears proteins related neurodegener.)
- LysoSENS's destruction of 7-ketoCholesterol (should soon be ready for test)
- Genetic stabilisation of transthyretin structures)
- ... [MANY OTHERS]

MID

- Biphosphonates (taken against osteoroporosis but seems to reduce mortality via other means)
- Fullerenes supplementation in rats (+90% lifespan reported)
- Tafamidis (treatment of familiar transthyretin disease)
- NAD+ supplementation
- ... [MANY OTHERS]

Some types of promising interventions

Appendix

interventions	name	type	biology	specific intervention to test	researchers in the field
gene therapy	rs9330200	SNP change	tubulin beta 4B	C->T	non disclosed here
gene therapy	rs2292664	SNP change	RIMS binding protein 2	C->T	non disclosed here
<...>	1027 such variants, with various degrees of pertinence				
gene therapy	Chromobacteri	insertion	microbial enzyme that degradates 7-keto-cholesterol		
gene therapy	HSPA9	overexpression	Increased proliferation potential of fibroblasts, more youthful morphology of		
gene therapy	TERT	overexpression	Reduced expression of SA-Beta-galactosidase lower senescense		
gene therapy	PCMT	overexpression	Lifespan extension in mouse		
gene therapy	Pck1	overexpression	Overexpression of Pck1 in skeletal muscles - increased number of mitochondr		
gene therapy	Plau	overexpression	Smaller apetite, reduced body weight and lenght, reduced temperature and p		
gene therapy	Cisd2	overexpression	mean,median and maximum lifespan increased, without any deleterious effe		
gene therapy	Cebpa->Cebp	gene change	fat metabolism transcription factor		non disclosed here
<...>	Etc.				
compound	biphosphonate	drug			
<...>					
other	Young thymus	transplantation			
<...>					

1. **Human genetic variants → mouse gene therapies**. For variants found in human populations that are already thought to favor long healthy lives in humans, and that can be translated into a gene therapy in aged mice. Concept: if the mice live longer and are healthier, it is a strong argument that the variant causally improves health in people and such a gene therapy may be likely to be effective in humans too.
2. **Human drug treatments → mouse drug treatments**. For treatments taken by human populations that are already thought to favor long healthy lives in humans. Concept: if the mice live longer and are healthier, it is a strong argument that the drugs are causally improving health in people.
3. **Fundamental research results → various mouse therapies.** Typically in vivo and in vitro results from mice, rats, nematodes or fruit flies, needing robust lifespan & health span confirmations -- in some way that can be transposed to humans.

An example: transpose rs9330200 to mice

Appendix

interventions	name	type	biology	specific intervention
gene therapy	rs9330200	SNP change	tubulin beta 4B	C->T
gene therapy	rs2292664	SNP change	RIMS binding protein 2	C->T

rs9330200 is a human variant of a gene that codes for the tubulin beta4b protein. The variant corresponds to having a "T" at some place. It was found in the Framingham Heart Study, in American citizens, that those with two copies of the gene with T **have less cardiovascular risks, less cancer risks and less premature death risks** than others, in a very statistically significant way (p_value = 6e-53)
Reference: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3592948/

Lab mice have that same gene! ("ortholog") indicating that it has the same function in mice. Naturally they have a "C", not a "T". **Therefore, we want to give the same variant:** very concretely, a part of the gene in lab mice is "GCCGCATGTCTATGAAGGAGGTGGA**C**GAACAGATGCTTAATGTCCAAAACA« , the goal is to replace it into "GCCGCATGTCTATGAAGGAGGTGGA**T**GAACAGATGCTTAATGTCCAAAACA" using CRISPR technologies. **if it makes them live longer…** (improves their health, etc) then it is a likely confirmation that it works in humans…

The goal is to test rs933020200 as well as many others candidates (we have more than 1400!). What will originally *come from human* statistics and is confirmed on mouse lifespan should have a good chance to work in humans so quite likely, if the project is not particularly stopped,

- in 5 years from now some persons (at first perhaps patients and techy elderlies) will start to take the gene therapy and will be monitored to check if it is good for their health,
- and potentially in 10 years it will become frequent to take those gene therapies to live longer healthily

Gene Therapy @old age: techniques

1. Drug-sensitive mouse lines

Appendix

Why set mouse lines? Why not treat 'normal', aged mice?

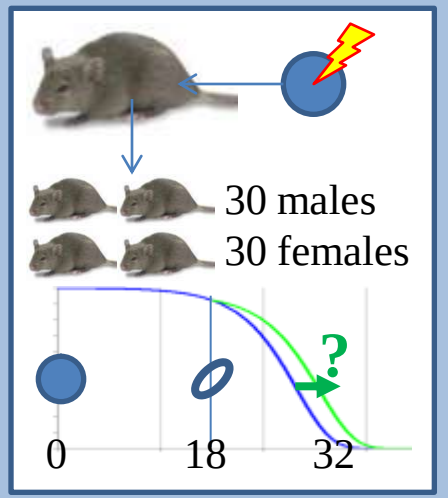
Experts tell us that current gene therapy techniques affect a *minor* portion of the body: even if a 'longevity gene' is introduced, it is uncertain to reach a noticeable effect; with such techniques, there is a risk to 'waste' the major testing program ➔ They rather suggest to make drug-sensitive transgenic mice (http://www.clontech.com/US/Products/Inducible_Systems/Protein)

Technique

Step 1 (6 months): for each gene variant to be tested, a mouse line is built: embryos incorporate the gene variant with a drug-sensitive promoter using the CRISPR technique and implanted into females. The progeny that contains the mutation is tested; if OK it is crossed to produce 30 males and 30 females for a survival analysis:

Step 2 (18 months): the mice are housed and fed normally. They should behave like the control because the gene variant is not expressed

Step 3 (14 months): at age 18 months, they are fed a drug that turns on the gene. Their survival curve is compared with that of the control group.



Note: we do not abandon the treatment of normal, aged, individuals:

a) After those 38 months, phase IV: for the cases (10%?) showing a statistical life extension, the gene variant is tested in aged mice and rats using different gene therapy techniques (next slides)

b) +In parallel with those 38 months, gene therapy techniques (next slides) are already tested for the most likely longevity genes today, to make them amenable to humans, and also to train for a)

Gene Therapy @old age: Techniques

2. True gene therapy at age 18 months: DNA change carried by a virus

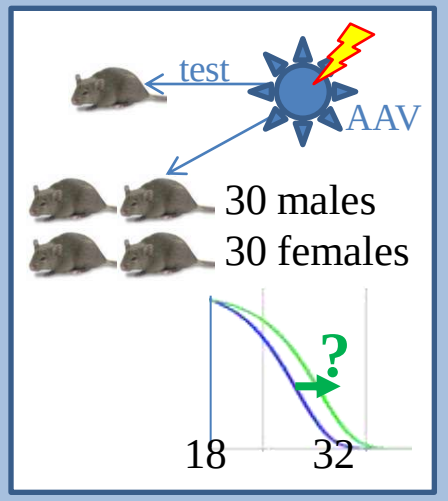
Appendix

Context

Several methods exist to change genes *in vivo*. Experts suggested us to use an "Adeno-Associated Virus" (AAV). Such viruses incorporate some of their DNA into the DNA of recipient that they infect. In the last decades, some of them were selected for scientific use to be applied to different organs: AAV1,AAV2..AAV9. *E.g.*, by default we'll choose AAV9, as it is tropic for neural tissue.

Technique

Step 1 (2 months): For each gene variant to be tested, the AAV incorporates the gene with the CRISPR technique and is injected, in young animals to check that it spreads, and in 30 males and 30 females of age 18 months
Step 2 (14 months): The survival of the aged mice is compared with that of the control group.



Limits

Low efficiency: A small fraction of cells receive AAVs and in a small fraction of them the construct enter the nucleus: the chance to sufficiently act to get clear life-extension seems low. Still, such a telomerase gene therapy in aged mice was reported (*)

Translation to the clinic: Frequently, adults have been infected by AAVs in the course of their life. For them, the therapy could lead to an immune response that would make the gene therapy inefficient. Healthy people may naturally hesitate to take this step.

(*) Bernardes de Jesus B. et al. (2002) http://onlinelibrary.wiley.com/doi/10.1002/emmm.201200245/full

Gene Therapy @old age: Techniques

3. Transient gene expression at ≥18 months: Minicircles carried by liposomal vectors

Appendix

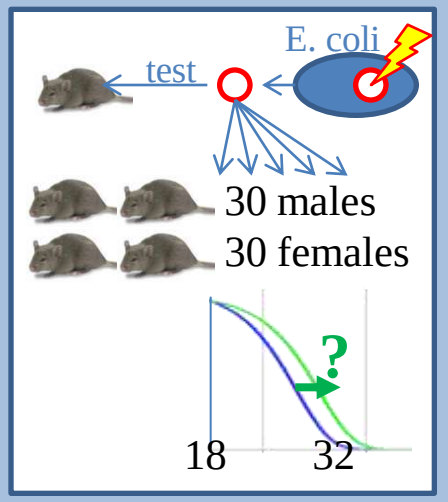
Context

Minicircles are DNA circles. In mice, they do not necessarily impact a larger number of cells than AAVs but they do not generate immune responses which makes the *human* translation more efficient. A drawback is that in dividing cells the therapy is transient, requiring regular treatments.

Technique

Step 1 (2 months): For each gene variant to be tested, minicircles that contain the gene are produced in sufficient quantity by modifying bacteria (a plasmid is created in E. coli, its prokaryotic parts are removed = minicircle: no immune response); they are then incorporated in liposomal vectors (to have minicircles enter cells) and injected in young animals to check that it spreads.

Step 2 (14 months): The minicircles are injected in 30 males and 30 females, every other week, starting at an age 18 months. Their survival is compared with that of the control group.



Of note

Evolutions: The field is evolving fast. These slides are therefore indicative: we may opt for better techniques as they emerge and become robust (microcircles, S/MAR elements…).

Gene Therapy @old age: Techniques

4. other

A matter of tissue-specificity

For most of the gene variants that we would like to test, we will not know *a priori* if targeting a specific organ makes sense. For example, a familial mutation may be associated with longevity in different populations without knowing if that could be through some action on a specific tissue.

Therefore the previous slides are about general gene therapies: *i.e.,* that are expressed, as much as possible, throughout the body. And in such cases, unless it has already been demonstrated that a gene therapy extends lifespan, we will start with the long technique #1: generating a mouse line and waiting for its whole lifespan.

For the specific cases where a specific organ should be targeted, gene therapy can be a lot more efficient. That is typically the case for cells of the blood, and the liver.

A fast growing field

As indicated in the last slide, techniques are improving fast in the field of gene therapy.

➔ It could happen that 6 months after the experiments start, some much better technique appears. But it would be worse to wait for decades for that to happen.

➔ It could well be that the long technique #1 is also an artificial way to model a future gene therapy technique that would be available to older people five or ten years from now. From that perspective, that technique would be much better than the other ones (AAVs, minicircles or other poorly effective therapies)

References

Appendix

The following references correspond to page 3.

C elegans "longevity revolution"

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Mouse upcoming "longevity revolution"

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