Choosing Measurements of Biological Age
Measurements of biological age have many uses

- Comprehensively characterize the physiology of aging
- Improve health care decisions
- Endpoints in trials to discover new treatments
We need **surrogate markers** to test treatments to extend healthy life

“It will take decades to establish whether treatments extend healthy aging. Biomarkers have the potential to enables early tests of treatment effectiveness over months to years.”

Kwon and Belsky Geroscience 2021;43:2795–2808
A surrogate marker

- Predicts clinical outcome
- Treatment-induced changes in the marker consistently predict effects of treatment on the clinical outcome

The marker is in the pathway of the treatment’s biological mechanism of action

Browner, Newman, Cummings... Designing Clinical Research 5th ed. Chapter 11
Outline

Biomarkers of mechanisms of aging
Predictive markers
Surrogate markers
  • Successes and failures
  • How to validate surrogate markers
A menu: biomarkers of mechanisms of aging

See also
Justice, Geroscience 2018; 40:419–436
LeBrasseur, J. Frailty Aging 2021;3:196
Markers to test the effect of treatment on hallmarks of aging. Few are feasible for trials.

<table>
<thead>
<tr>
<th>Genomic instability</th>
<th>Mitochondrial dysfunction</th>
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<tbody>
<tr>
<td>• Single-cell/clonal NGS</td>
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<td>• Tests of DNA repair mechanisms</td>
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<td>• Measures of DNA modifications</td>
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<thead>
<tr>
<th>Telomere shortening</th>
<th>Mitochondrial volume/number/shape</th>
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<td>• Telomere length</td>
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<tr>
<td>• Markers of DNA damage response</td>
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<tr>
<td>• Telomerase activity</td>
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<tr>
<th>Cellular senescence</th>
<th>Mito respiration</th>
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<td>• Senescent markers in blood and tissue</td>
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<td>• SASP proteins in blood and tissue</td>
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<tr>
<th>Epigenetic changes</th>
<th>P^31 MRI spectroscopy</th>
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<td>• Methylation</td>
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<td>• Histone acetylation</td>
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<th>Mitochondrial dysfunction</th>
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<tr>
<td>• Markers of biogenesis</td>
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<td>• mtDNA copy number and haplotypes</td>
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<tr>
<th>Decreased autophagy, proteostasis</th>
<th>Autophagy markers and flux (+ TEM)</th>
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<tr>
<td>• Chaperon proteins</td>
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<tr>
<th>Stem cell exhaustion</th>
<th>Proliferative capacity in vitro</th>
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<tr>
<td>• Resistance to stress</td>
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</table>
Predictive biomarkers
Most measurements of biological age are based on composites of clinical tests & research biomarkers

DNAm based surrogate
- adrenomedullin
- beta-2-microglobulin
- CD56
- ceruloplasmin
- cystatin-C
- EGF fibulin-like ECM protein1
- growth differentiation factor 15
- leptin
- myoglobin
- plasminogen activator inhibitor 1
- serum paraoxonase/arylesterase 1
- tissue Inhibitor Metalloproteinases 1
- smoking pack-years

Rotterdam
- c-reactive protein
- Creatinine
- Urea nitrogen
- Albumin
- Total cholesterol
- Cytomegalovirus
- Alk. phosphatase
- FEV
- Systolic BP

Lu, Aging 2019; 11:303
Waziry, European J Epidemiol 2019;34:793
Vershhor, JGBS 2021;76:187–194
Composite measurements of biological age

• Advantages: better predictor than single markers?
  o A comprehensive assessment of features of ‘aging’
  o More markers might improve prediction of aging conditions

• Disadvantages: less effective surrogate?
  o Composites may not include the mechanism of action of a treatment
  o If the composite includes a marker of the mechanism, then adding more markers may dilute the responsiveness of the measurement as a surrogate marker
Does caloric restriction slow biological aging? Measured biological age based on a composite of markers.

CR slowed biological aging.

**Markers**

- HbA1c, (glucose)
- Systolic BP,
- Cholesterol
- CRP
- Albumin
- Alk. Phos.
- CBC
- RDW
- CMV density

Kwon GeroScience 2021;43:2795–2808
Some markers are known to be influenced by CR. Did adding other markers dilute the measurement of the effect of CR?

**Markers**

- **Influenced by CR**
  - HbA1c, (glucose)
  - Systolic BP,
  - Cholesterol
  - CRP

- **Others**
  - Albumin
  - Alk. Phos.
  - CBC
  - RDW
  - CMV density

Kwon Geroscience 2021;43:2795–2808
Black boxes

Measurements of Biological Age
Predictors with unknown mechanisms of action
Chest X-ray (CXR) Age developed by A.I. (deep learning) applied to CXR

CXR-Age Developed in 116,035 individuals

Input: Chest X-ray image

CXR-Age convolutional neural network

CXR Age

Raghu et al. JACC Cardiovasc Imaging 2021
Chest X-ray (CXR) Age developed by A.I. (deep learning) applied to CXR

CXR-Age Developed in 116,035 individuals

Input: Chest X-ray image

CXR-Age
convolutional neural network

CXR Age

Raghu et al. JACC Cardiovasc Imaging 2021
CXR Age is powerful predictor of survival

- Maximizes prediction
- The biologic mechanism is opaque
- It does not matter for prediction

Raghu et al. JACC Cardiovasc Imaging 2021
Epigenetic Age
Many epigenetic age clocks

<table>
<thead>
<tr>
<th>Clock</th>
<th>No. CpGs</th>
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<tbody>
<tr>
<td>PhenoAge</td>
<td>513</td>
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<tr>
<td>GrimAge</td>
<td>1,113</td>
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<td>Zhang Mortality Clock</td>
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<tr>
<td>DunedinPoAm</td>
<td>46</td>
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<tr>
<td>Telomere Clock</td>
<td>140</td>
</tr>
</tbody>
</table>

Strongest predictors

Predict many aging outcomes*

- Mortality
- Multimorbidity
- Diabetes
- Depression
- Impaired hearing
- and more…

*Based on age acceleration: difference between biological & chronologic age

Simpson, Aging Cell 2021;20:e13452.
What does epigenetic age measure?

- Unknown
- Inflammation? Metabolic dysfunction?
- A fundamental process in all cells?
- DNA damage?
  - Methylation of Cytosine → can lead to G-T mismatch
  - Could epigenetic age reflect accumulation of mismatch DNA mutations?

R. Holliday, Mutation Research, 1993;28: 61-67
What does epigenetic age measure?

• The mechanism may not be important for prediction
• However, the mechanism may be important to understand the value of epigenetic age as a “surrogate marker” for a treatment
Surrogate markers
“The single most common and serious error in the evaluation of biomarkers is the assumption that a correlation between the measured level of a biomarker and a clinical outcome means that the biomarker constitutes a valid surrogate.”
A surrogate marker

• Predicts clinical outcome
• Treatment-induced changes in the marker consistently predict effects of treatment on the clinical outcome
• Treatments can be approved for the clinical outcome
• That does not validate the ‘surrogate’ marker

Browner, Newman, Cummings... Designing Clinical Research 5th ed. Chapter 11
Bone density is a predictive marker for fracture

Increased bone density → Decreased fracture risk

Does not make BMD a surrogate
Treatments increase bone density

Still, not a surrogate
The marker (BMD) is in the pathway of the effect of treatment on risk of fracture.

1. Treatment for osteoporosis
2. Increased bone density
   - Highly correlated ($R^2 > .90$) with bone strength
3. Risk of fracture
Bone density is a valid surrogate for effects of treatment to reduce fracture risk

- Based on many trials. Drugs were approved to prevent fracture.
- Strong correlations between change in BMD by treatment and reductions in fracture risk.
- Took years of compiling and standardizing data from many trials.
- Instead of trials of 7-20,000 for 3+ years, FDA will approve drugs based on change in BMD in small short trials.

Black et al. Lancet Diabetes Endocrinol 2020; 8: 672–82
Predictive markers that failed as surrogates* 

*Many other successful surrogates, e.g. change in BP, HIV viral load
HbA1c predicts CVD and death
Intensive therapies (e.g. insulin) reduce HbA1c
Treatment increased cardiovascular events and total mortality

Primary composite outcome
nonfatal myocardial infarction, nonfatal stroke, or death from cardiovascular causes

ACCORD Study Group, NEJM 2008;358:24
HgA1c failed as a surrogate marker for intensive therapy
HDL-cholesterol

- HDL-C and LDL-C predict CHD
- Torcetrapib increased HDL 72% and decreased LDL 25%
- Trial: Torcetrapib + atorvastatin vs. atorvastatin alone
  - 25% increased CV events
  - 58% increased mortality

HDL-C failed as a surrogate marker for treatment to reduce CVD
Treatment for sarcopenia - MK-0773

- Selective androgen receptor modulator (SARM)
- 170 women ≥ age 65 years randomized vs. placebo
- Improved lean mass

D.A. PAPANICOLAOU, J Nutrition Health Aging 2013;17
MK-0773 had no effects on clinical outcomes

- Leg press
- Gait speed
- Stair climb power
- Physical movement score

Papanicolaou et al. J Nutrition Health Aging 17;2013:6
Lean mass failed as a surrogate for SARMs to increase strength physical performance
Other ‘potential surrogates’ that failed

- HbA1c: Rosiglitazone improved HbA1c but increased risk of CVD events and heart failure
- Aβ Amyloid: treatments large reductions in amyloid with no or small improvements in cognition*
- 25(OH)D levels
  - Low levels predict mortality, cancer, CVD, fractures, falls and more
  - Large trials of Vitamin D3 supplements failed to reduce cancer, CVD, fractures, falls ….and more
  - High doses increase the risk of falls

Lessons

- Avoid (mis)using “surrogate maker”
- Aging research has many predictive markers
- We have no surrogate markers….yetd
Adverse effects of using false ‘surrogates’

• Promotion of ineffective treatments
• Potential adverse effects not discovered in small trials using only biomarker
Why do biomarkers fail to be surrogates?

• They do not accurately measure the mechanism
• Other factors are more important
How to validate that a biologic age is a ‘surrogate marker’ for clinical outcomes
Look ahead

Prepare for analyses to establish surrogate markers for trials
What is needed to find and validate surrogate measurements of biological age?

• Randomized trials of a treatment that reduce aging-related condition
• The treatment influences the biomarker of aging
• The biomarker of aging predicts the aging outcome
• Treatment-induced change in ‘biological age’ predicts change in the age-related clinical outcome
Validating that a marker of biological age is a surrogate marker

- Requires several randomized trials with significant effects on aging-related clinical outcomes
- Measure change in biomarker at baseline, early, and the end
- Show that change in the marker consistently predicts change in the outcome

\[ R^2 = 0.73, \ p < 0.0001 \]
To prepare for validating surrogate markers

• Standardize outcomes and biomarkers in trials
• Create repository of trial data
• In trials archive biological specimens to test new potential surrogate markers
Include standardized clinical outcomes in all trials for meta-analyses

Suggested clinical outcomes:
- Multimorbidity – a standard instrument
- Mortality and healthy (disease and disability-free) survival
- Frailty – standard definitions
- Common diseases: CHD, cancer, hip fracture

Safety / adverse events
- MedDRA or equivalent
Summary

• Many measures of biological age predict aging outcomes
• We need validated surrogate measurements
• Plan ahead
  o Centrally collect all trial data
  o Standardize outcomes in clinical trials
  o Archive specimens at baseline, early, and at the end
• Meanwhile, avoid (mis)using “surrogate marker”
  o They are ’predictive’ or ‘potential’ surrogate markers
Special thanks

Steve Kritchevsky  
Dennis Black  
Dan Evans

Staff and scientists of the SF Coordinating Center
Predictive markers of biological age

• Goal: to maximize the accuracy of prediction of an outcome
• Many markers, methylation sites, -omics data
• Some use machine learning or deep learning of large datasets
• The biological basis may not be knowable
Endpoint markers: markers of *change*

- The biological mechanism is key.

- Key feature: precise measurement of *change* (not the cv%)
In clinical trials treatments increased BMD and decreased fracture rates

Treatment for osteoporosis → Increased bone density → Decreased fracture risk

BMD is a valid surrogate for the effect of treatment on risk of fracture