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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

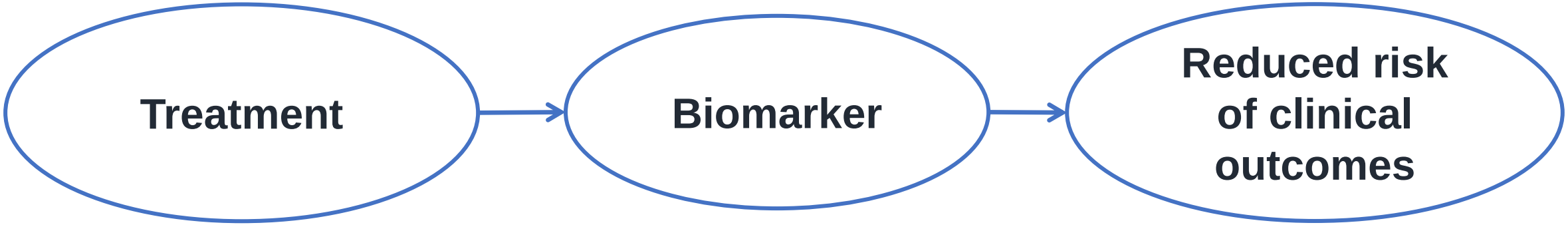
"It will take decades to establish whether treatments extend healthy aging.

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

REVIEW

Aging Cell WILEY

Measuring biological aging in humans: A quest

Luigi Ferrucci[1] | Marta Gonzalez-Freire[1] | Elisa Fabbri[1,2] | Eleanor Simonsick[1] | Toshiko Tanaka[1] | Zenobia Moore[1] | Shabnam Salimi[3] | Felipe Sierra[4] | Rafael de Cabo[1]

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

Genomic instability	• Single-cell/clonal NGS • Tests of DNA repair mechanisms • Measures of DNA modifications
Telomere shortening	• Telomere length • Markers of DNA damage response • Telomerase activity
Cellular senescence	• Senescent markers in blood and tissue • SASP proteins in blood and tissue
Epigenetic changes	• Methylation • Histone acetylation
Mitochondrial dysfunction	Mitochondrial volume/number/shape Mito respiration P^{31} MRI spectroscopy Markers of biogenesis mtDNA copy number and haplotypes
Decreased autophagy, proteostasis	Autophagy markers and flux (+ TEM) Chaperon proteins
Stem cell exhaustion	Proliferative capacity in vitro Resistance to stress

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

Lymphocytes (absolute number)
Monocytes (absolute number)
Granulocytes (absolute number)
Hemoglobin
Mean corpuscular volume
Red blood cell distribution width
Platelets
Mean platelet volume
25-Hydroxyvitamin D
Albumin
Alanine aminotransferase
Creatinine
Ferritin
Free thyroxine
High-sensitivity C-reactive protein
Cholesterol
High-density lipoprotein
Triglycerides
Thyroid-stimulating hormone
Systolic blood pressure
Diastolic blood pressure
Pulse
Ratio of forced expiratory volume after 1 s to forced
Forced vital capacity
Appendage lean mass
Waist to hip ratio
4-m walk test
Timed get up and go test
Chair rise test
Grip strength
Single leg balance test
Animal Fluency Test
Rey Auditory Verbal Learning Test (immediate recall)
Rey Auditory Verbal Learning Test (delayed recall)
Mental Alternation Test
Event-based Prospective Memory Test
Time-based Prospective Memory Test
Victoria Stroop Test
Choice Reaction Time Test
Controlled Oral Word Association Test

Lu, Aging 2019; 11:303
Waziry, European J Epidemiol 2019;34:793
Vershor, JGBS 2021;76:187–194

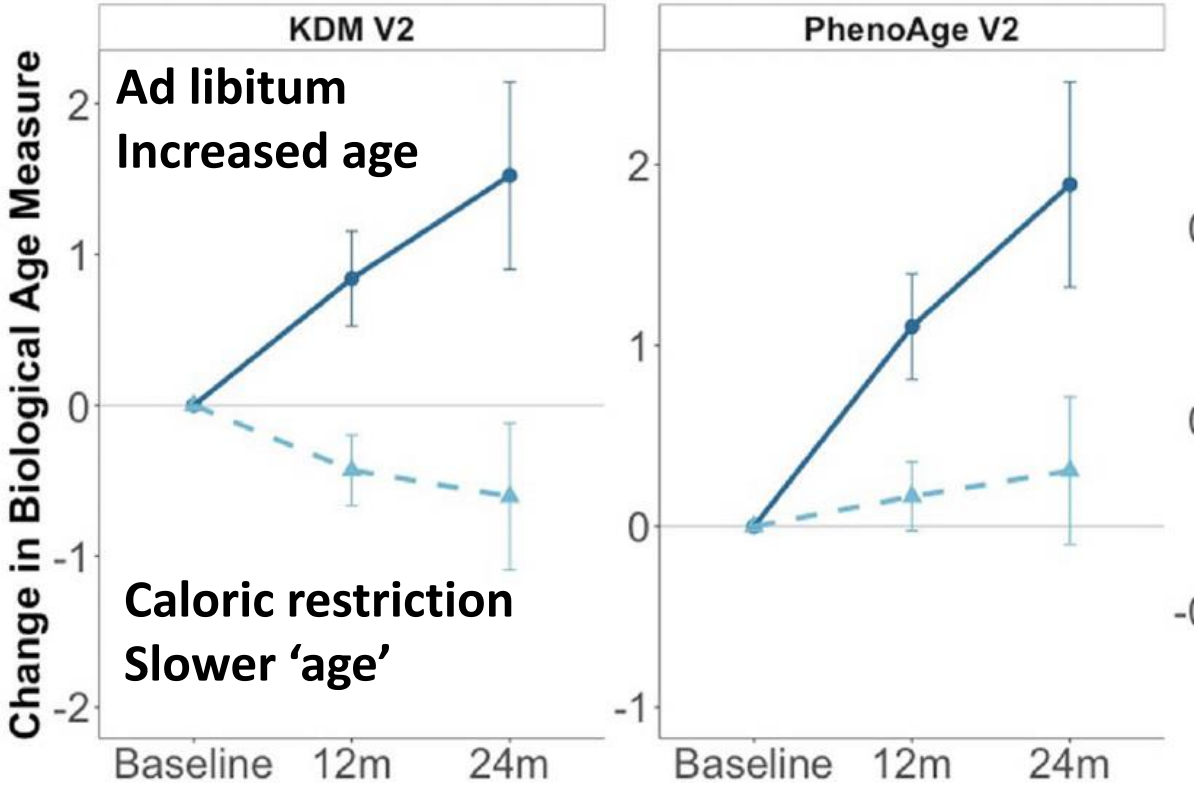
Composite measurements of biological age

- Advantages: better predictor than single markers?
  - A comprehensive assessment of features of ‘aging’
  - More markers might improve prediction of aging conditions
- Disadvantages: less effective surrogate?
  - Composites may not include the mechanism of action of a treatment
  - 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?

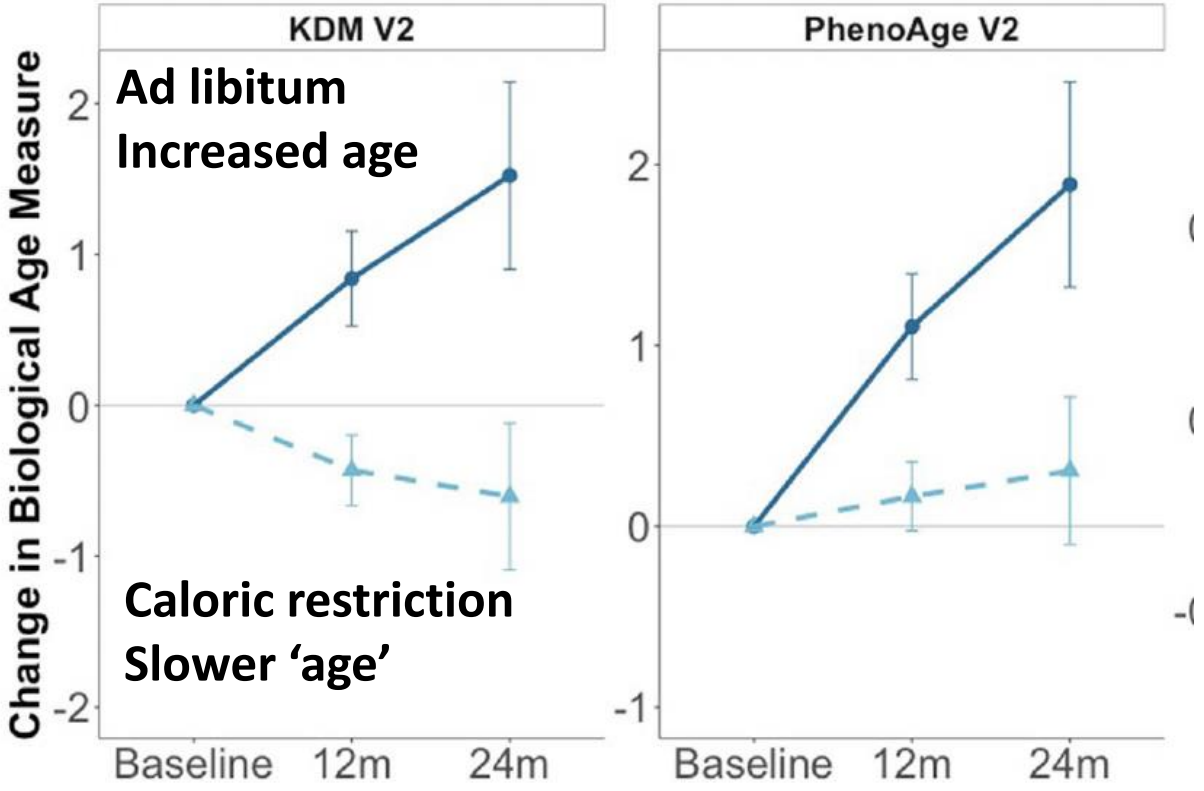
Markers

Influenced by CR

- HbA1c, (glucose)
- Systolic BP,
- Cholesterol
- CRP

Others

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

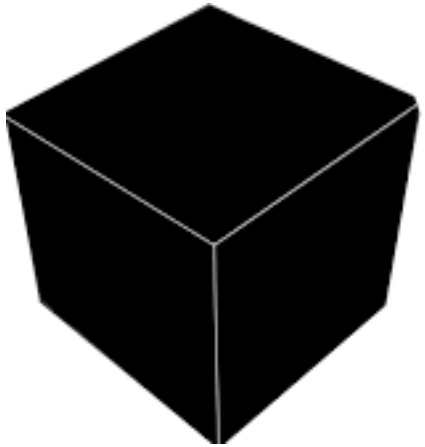


Kwon Geroscience 2021;43:2795–2808

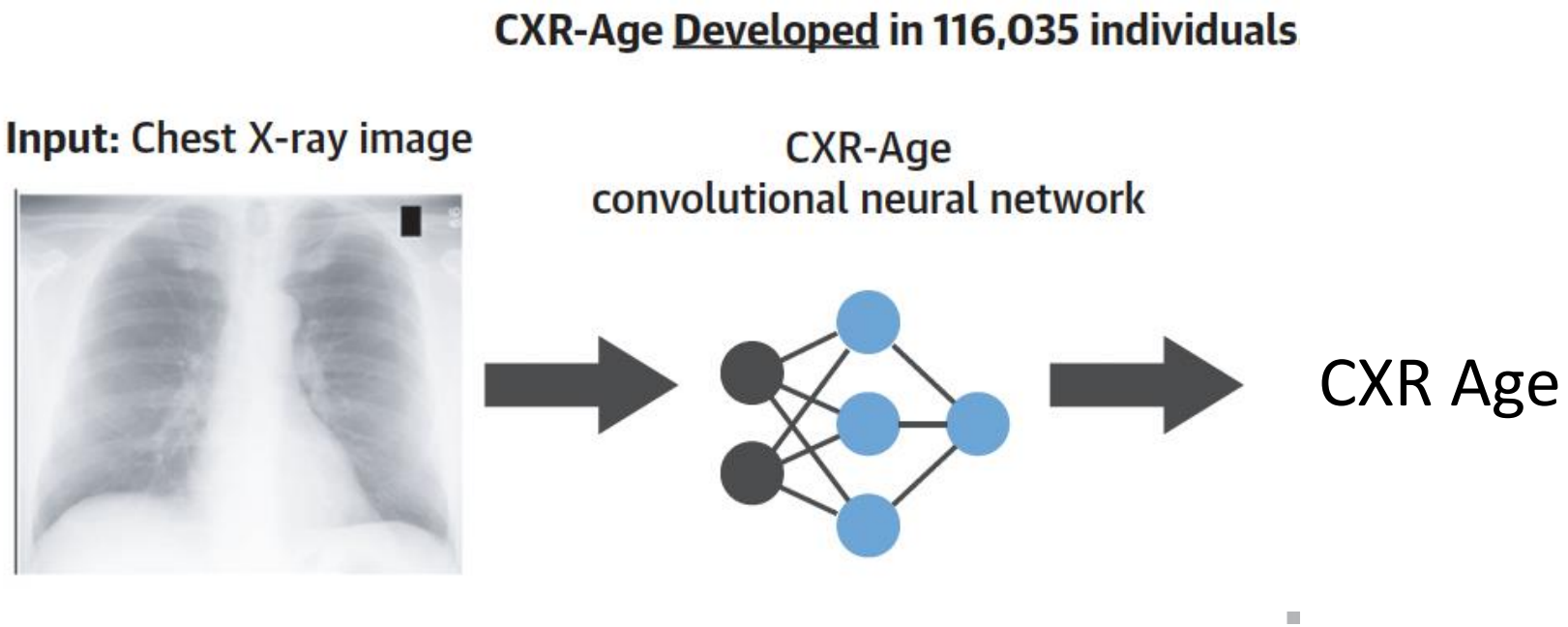
Black boxes

Measurements of Biological Age

Predictors with unknown mechanisms of action



Chest X-ray (CXR) Age developed by A.I. (deep learning) applied to CXR



Raghu et al. JACC Cardiovasc Imaging 2021

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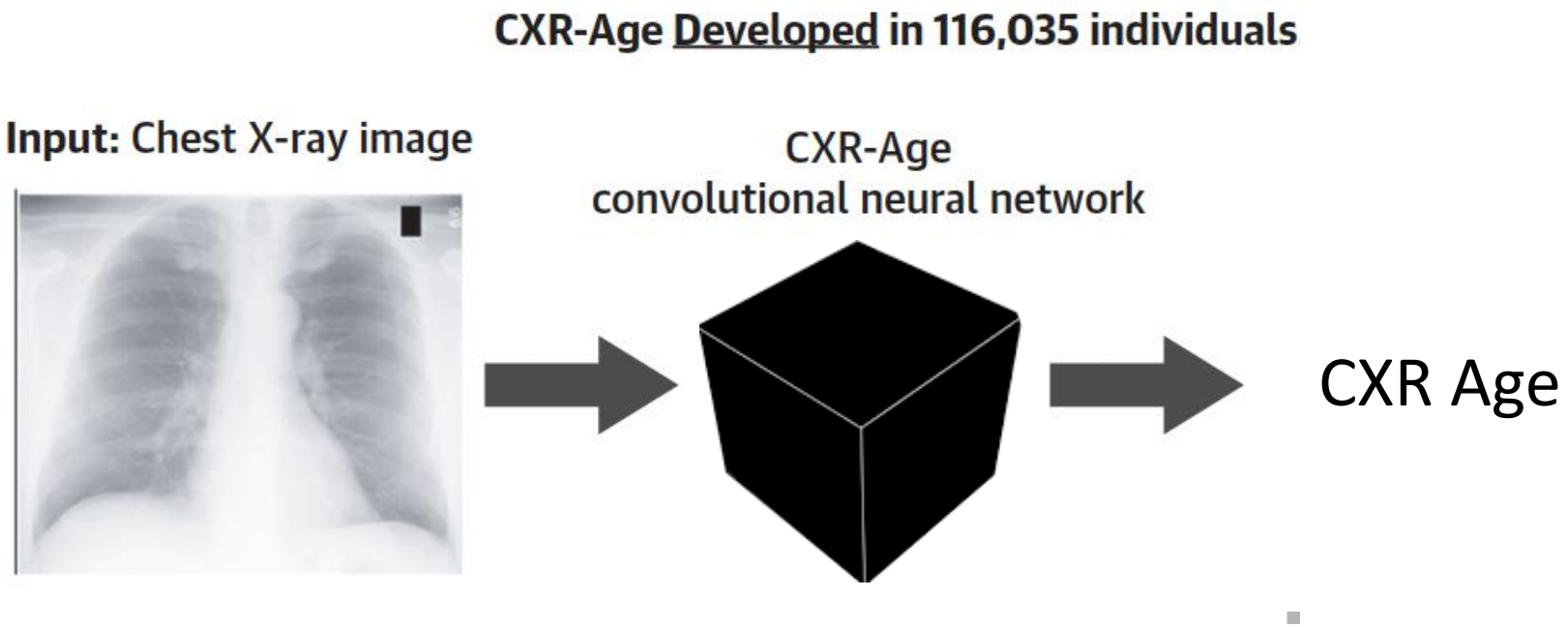
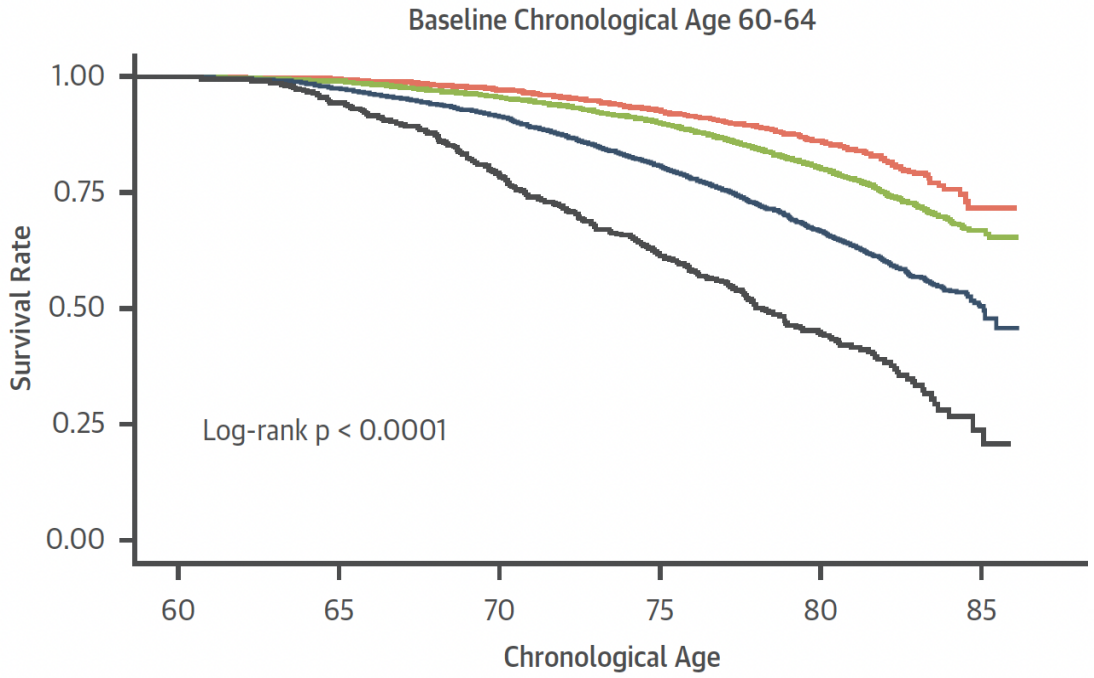
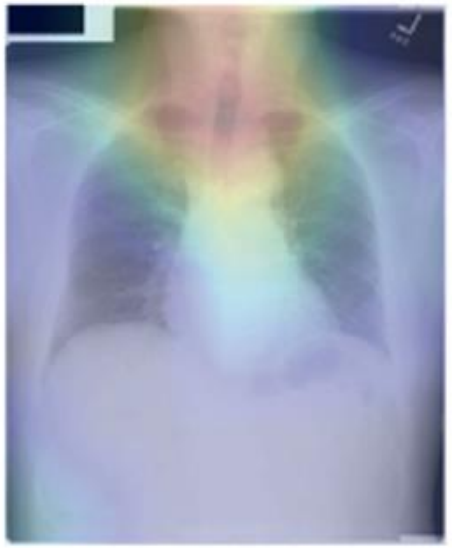


Raghu et al. JACC Cardiovasc Imaging 2021

CXR Age is powerful predictor of survival





Why?

- 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

Clock	No. CpGs
PhenoAge	513
→ GrimAge	1,113
Zhang Mortality Clock	10
→ DunedinPoAm	46
Telomere Clock	140

→ Strongest predictors

Predict many aging outcomes*

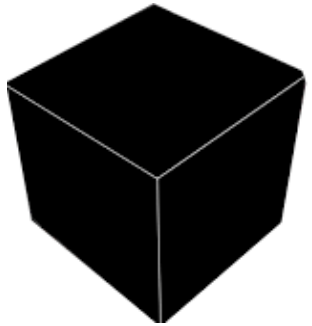
- Mortality
- Multimorbidity
- Diabetes
- Depression
- Impaired hearing
- and more…

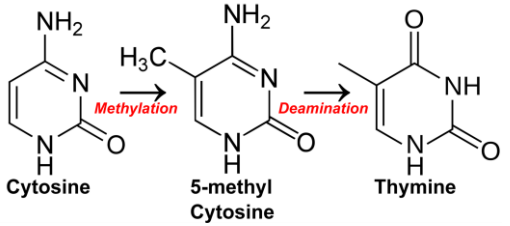
*Based on age acceleration: difference between biological & chronologic age

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

Biomarker definitions and their applications

Robert M Califf[1,2,3]

Exper Biol Med 2018; 243: 213–221

Previous and current FDA Commissioner

"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."

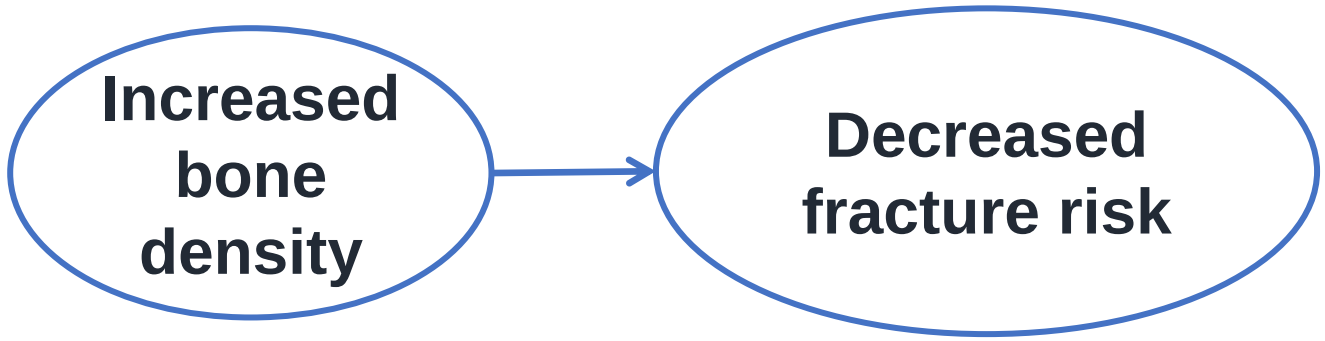
A surrogate marker

- 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

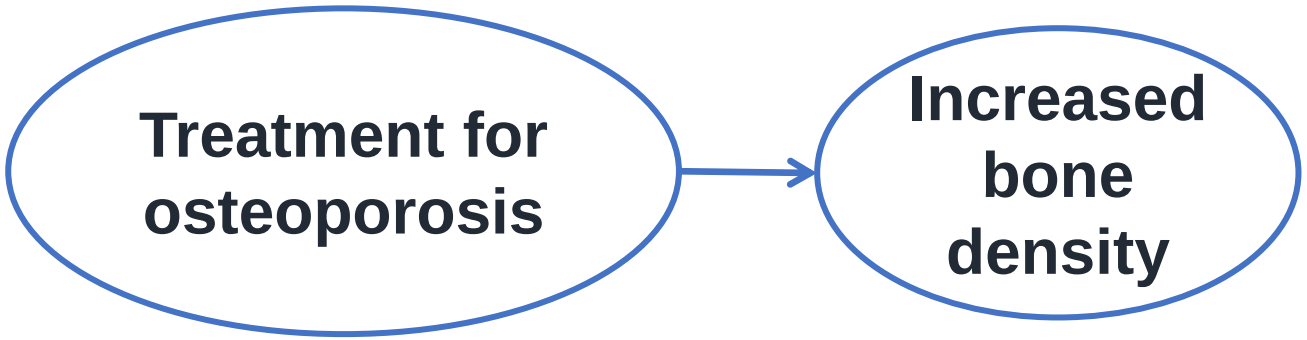
Browner, Newman, Cummings… Designing Clinical Research 5th ed. Chapter 11

Bone density is a predictive marker for fracture



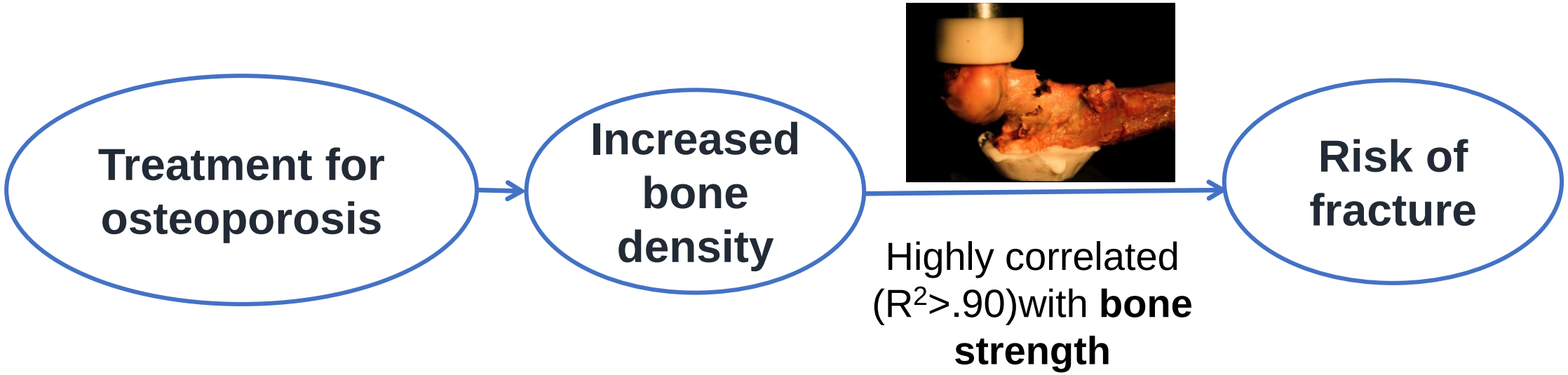
Does not make BMD
a surrogate

Treatments increase bone density



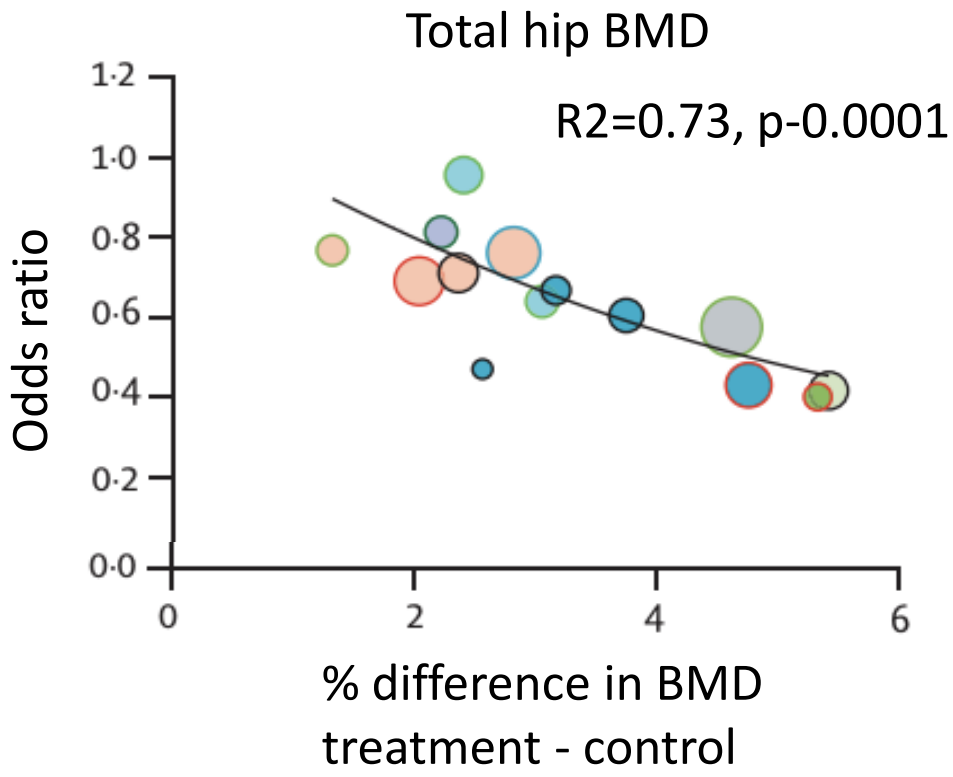
Still, not a surrogate

The marker (BMD) is in the pathway of the effect of treatment on risk of fracture



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

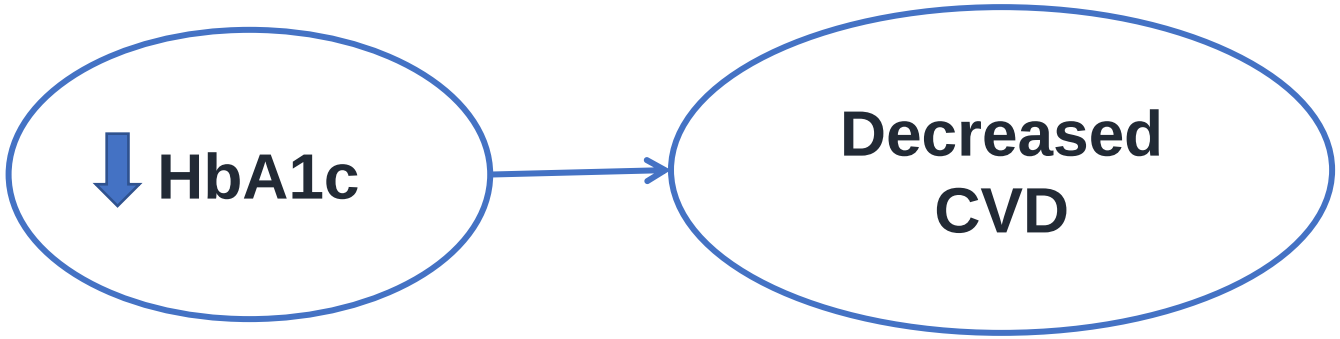


Black et al. Lancet Diabetes Endocrinol 2020; 8: 672–82

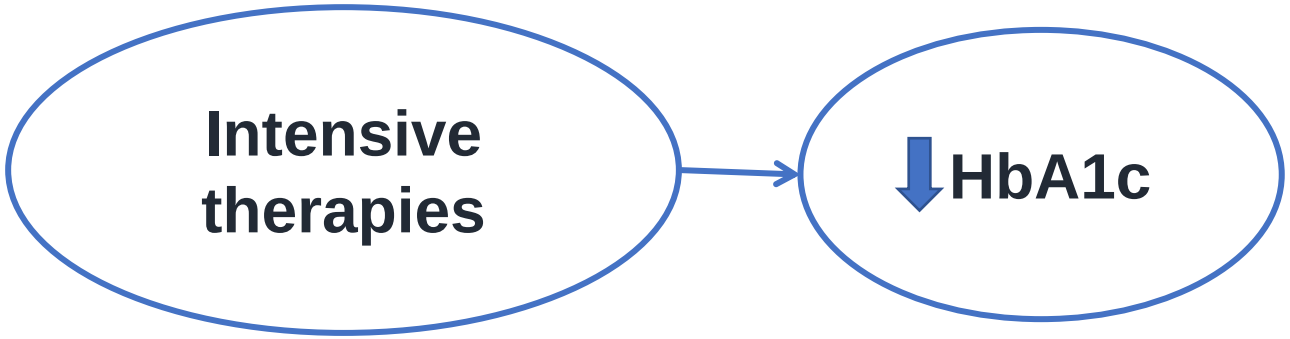
Predictive markers that failed as surrogates*

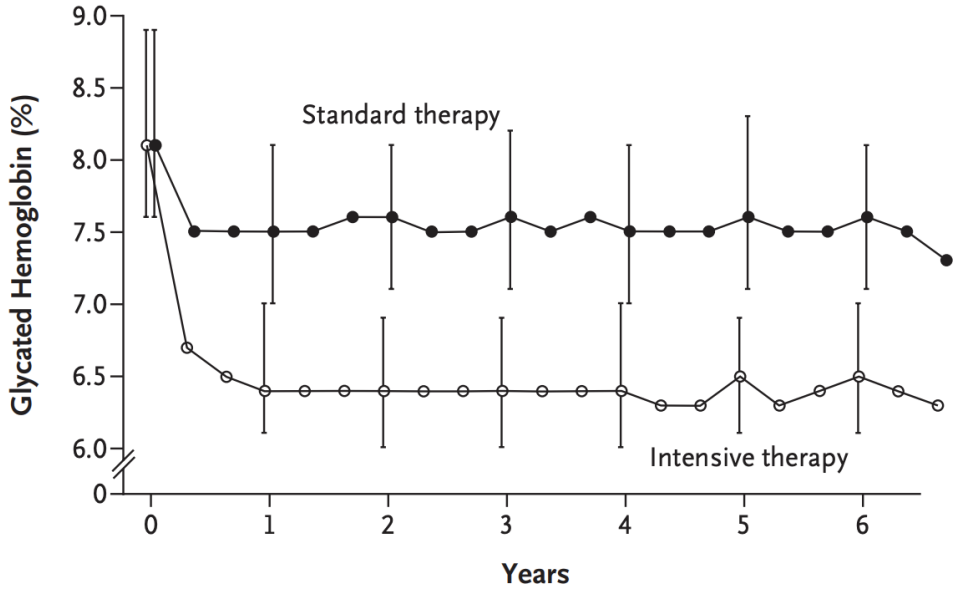
*Many other successful surrogates, e,g change in BP, HIV viral load

HbA1c predicts CVD and death

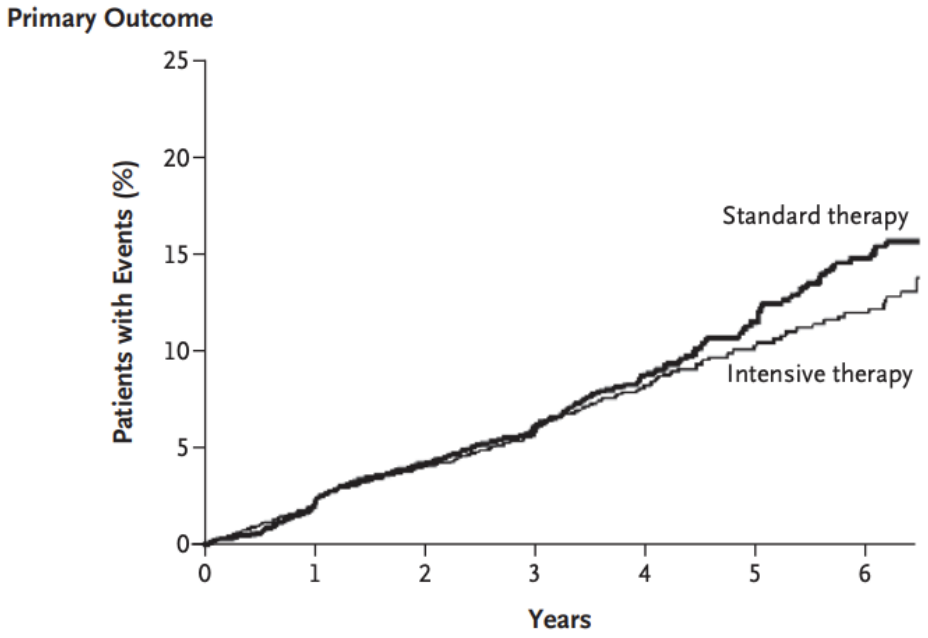


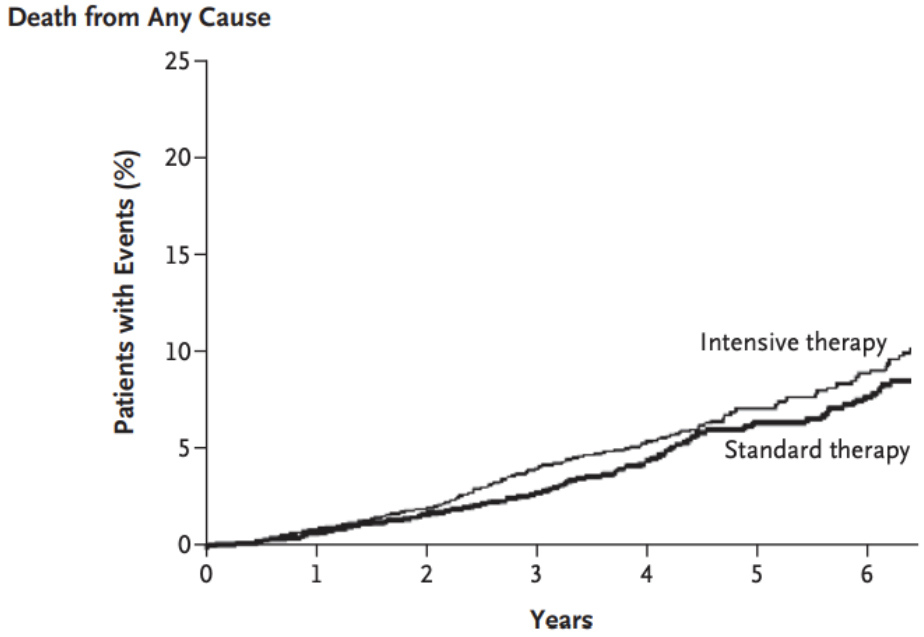
Intensive therapies (e.g. insulin) reduce HbA1c





Treatment increased cardiovascular events and total mortality





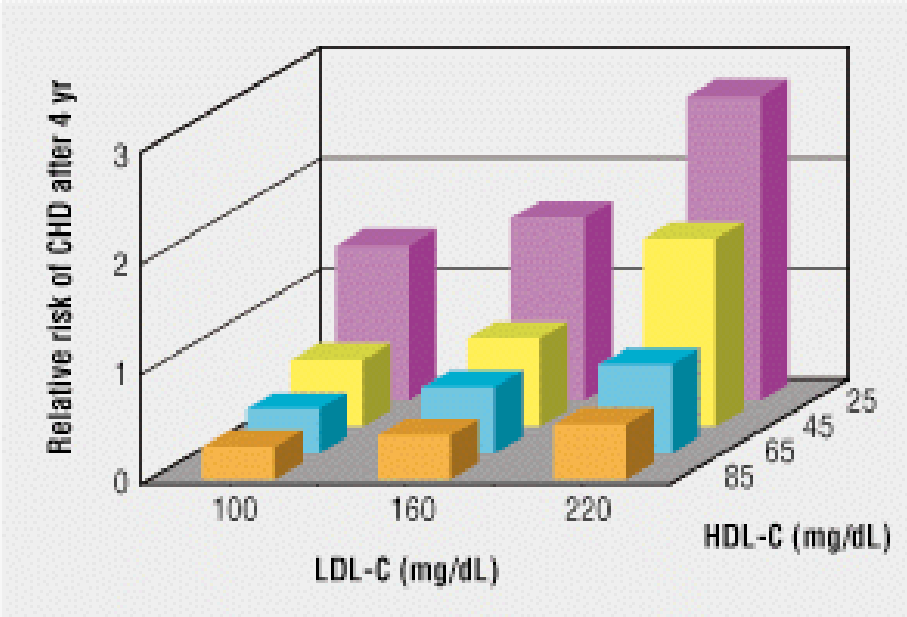
Primary composite outcome
nonfatal myocardial infarction, nonfatal stroke, or death from cardiovascular causes

ACCORD Study Group, NEJM 2008;358:24

HgA1c failed as a surrogate marker for intensive therapy

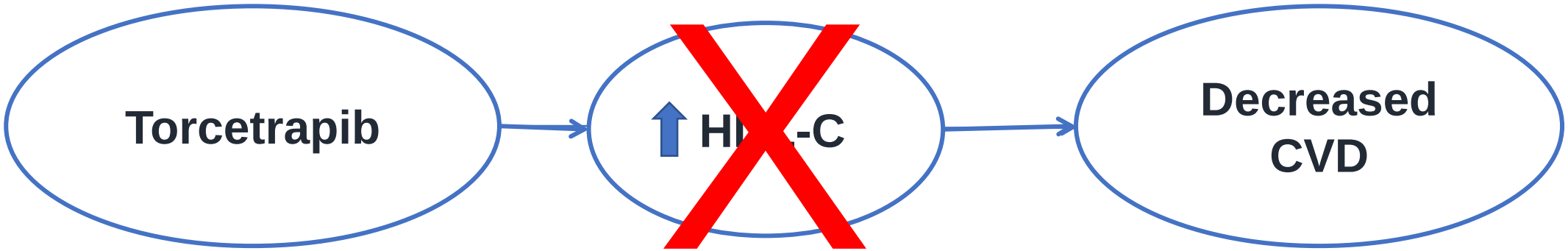
HDL-cholesterol

- 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



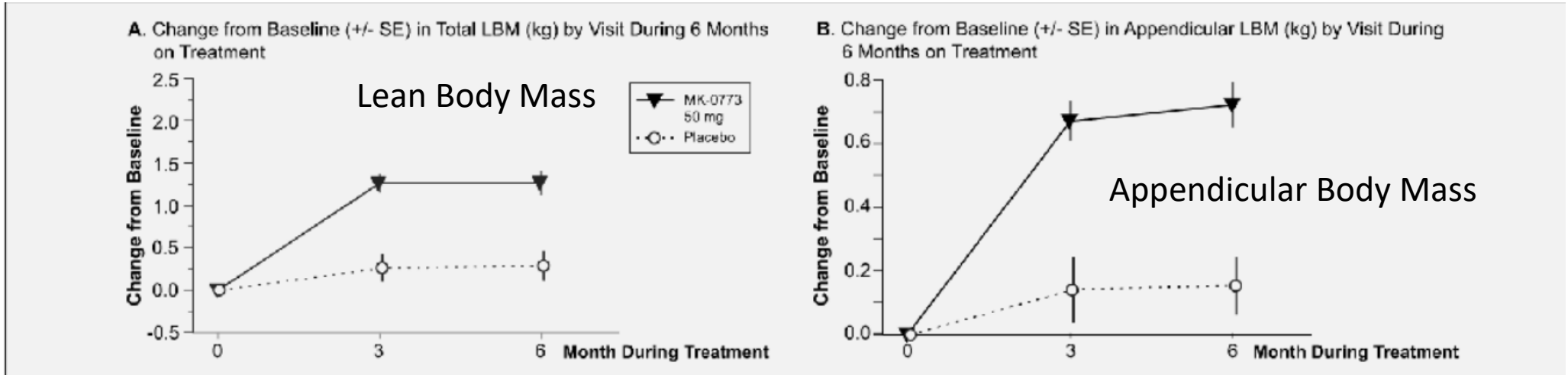
Illuminate Trial: Barter, N Engl J Med 2007;357:2109-22.

HDL-C failed as a surrogate marker for treatment to reduce CVD



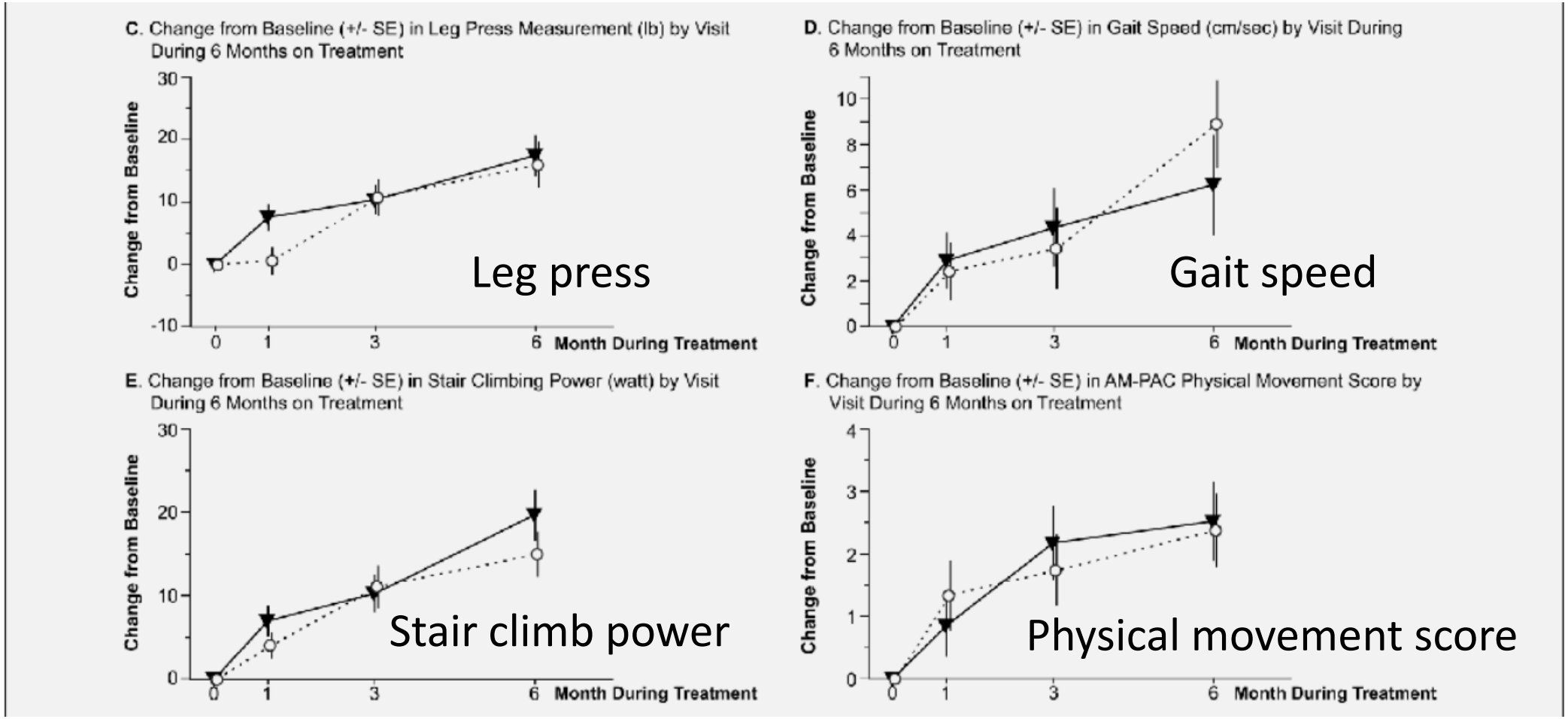
Treatment for sarcopenia - MK-0773

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



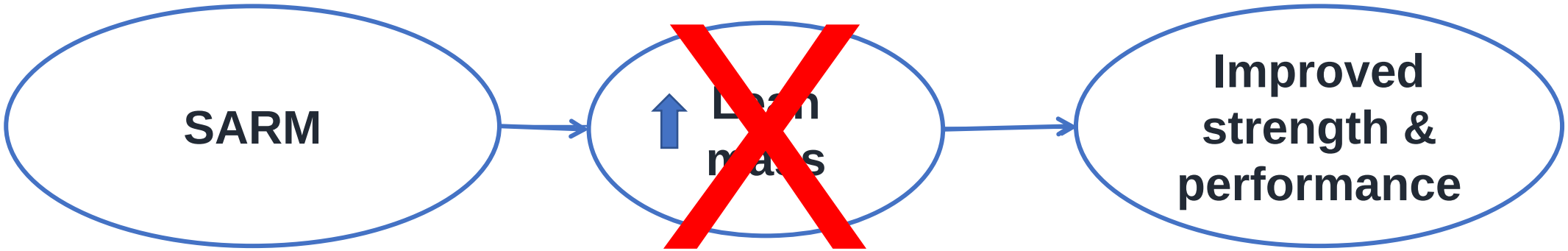
D.A. PAPANICOLAOU, J Nutrition Health Aging 2013;17

MK-0773 had no effects on clinical outcomes



Papanicolaou et al. J Nutrition Health Aging17;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

*Avgerinos, Ferrucci, Kapogiannis, Ageing Res Rev 2021; 68:101339

Lessons

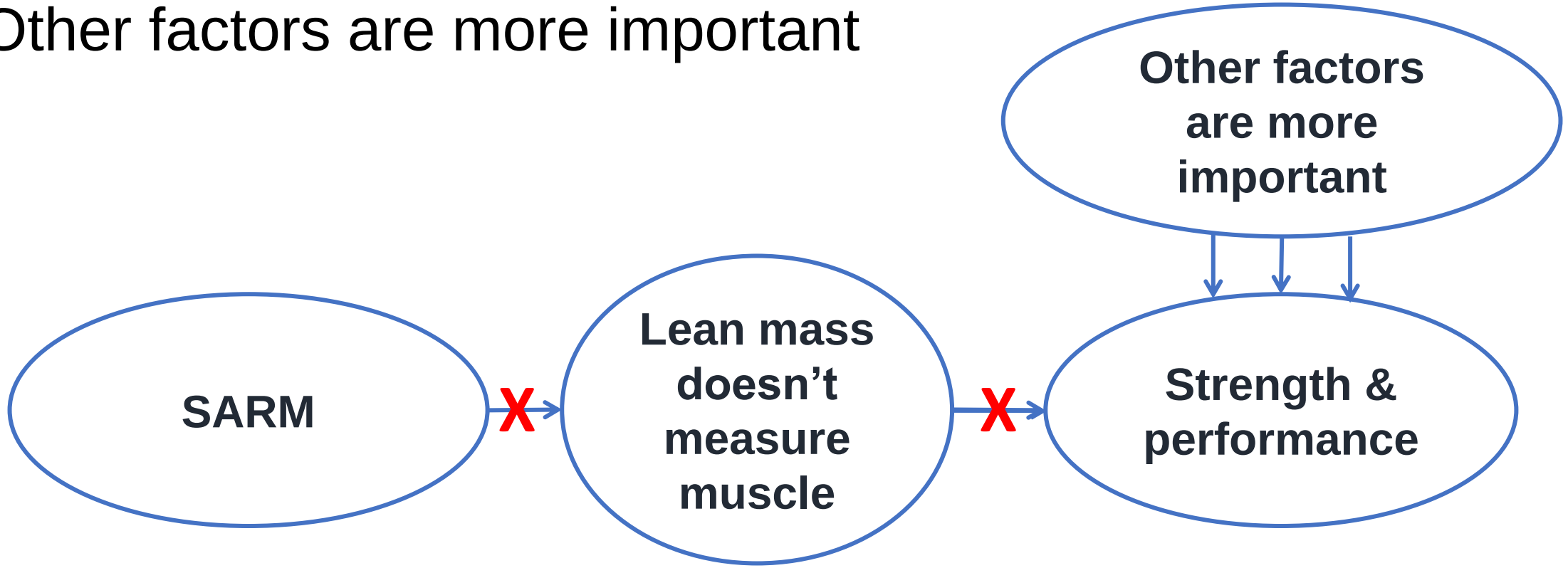
- Avoid (mis)using “surrogate maker”
- Aging research has many predictive markers
- We have no surrogate markers….yetd

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

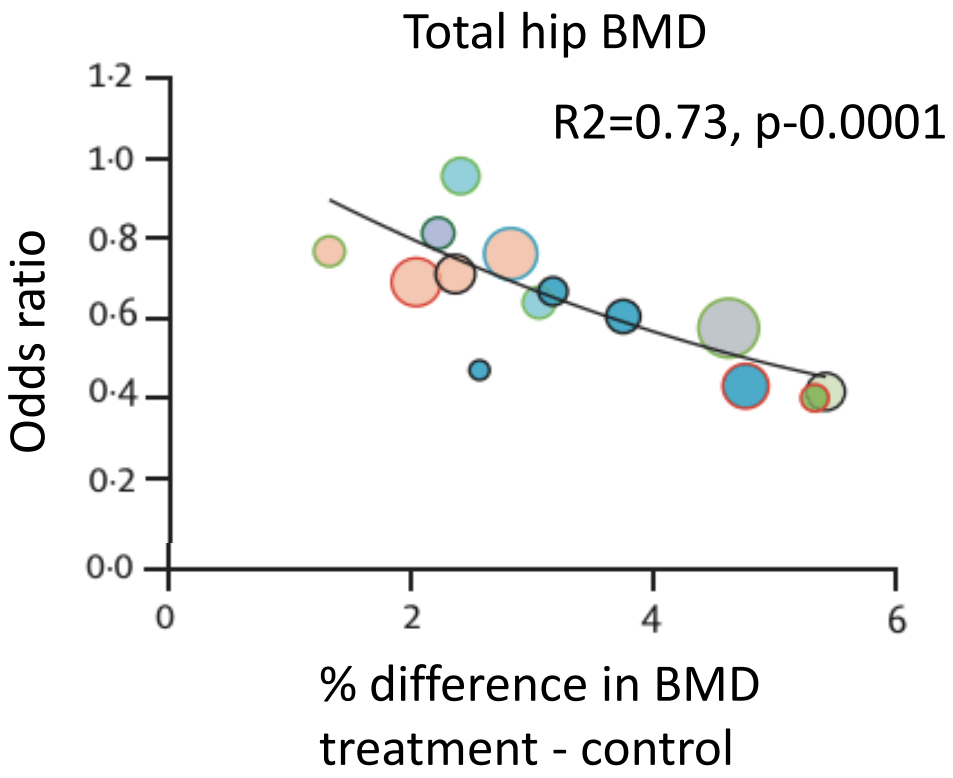
Prepare for analyses to establish surrogate markers for trials

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



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
  - Centrally collect all trial data
  - Standardize outcomes in clinical trials
  - Archive specimens at baseline, early, and at the end
- Meanwhile, avoid (mis)using “surrogate marker”
  - They are ’predictive’ or ‘potential’ surrogate markers

Special thanks

SAN FRANCISCO COORDINATING CENTER

Steve Kritchevsky

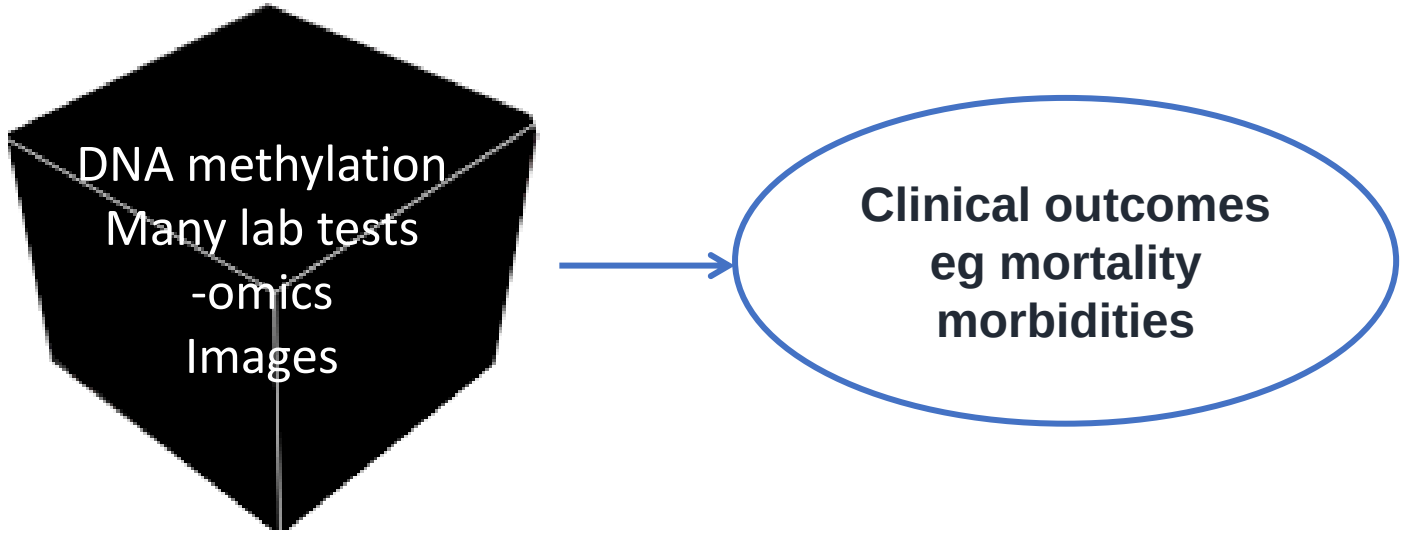
Dennis Black

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



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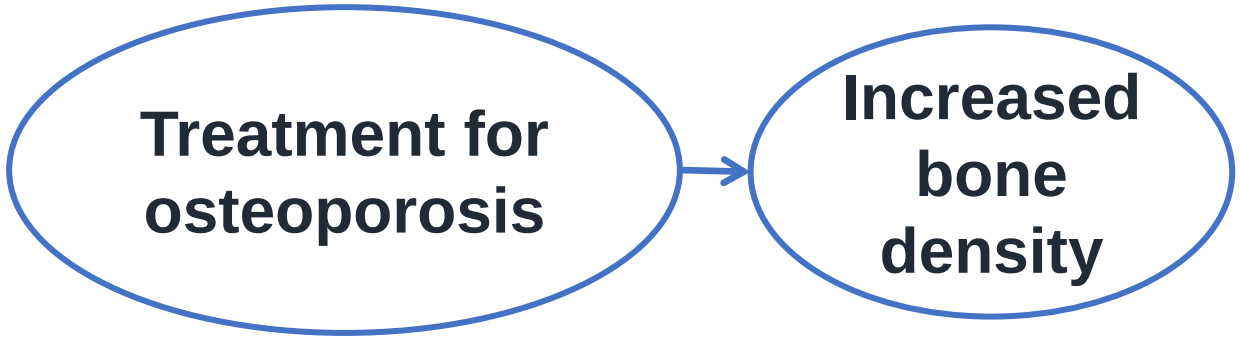
Endpoint markers: markers of *change*

- The biological mechanism is key.



- Key feature: precise measurement of *change* (not the cv%)

In clinical trials treatments increased BMD and decreased fracture rates

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