

Biomarkers of human ageing: The MARK-AGE Study and beyond

Alexander Bürkle, MD

Professor and Chair of Molecular Toxicology

Department of Biology, University of Konstanz, Germany

27 September 2019

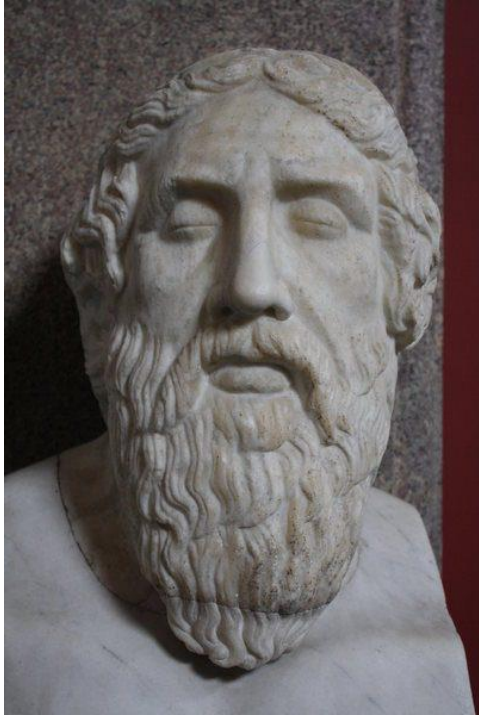
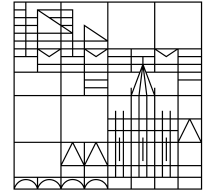
COST CLINIMARK TRAINING SCHOOL

Approaches for Biomarker Discovery and Validation

Spetses 23-27 September 2019

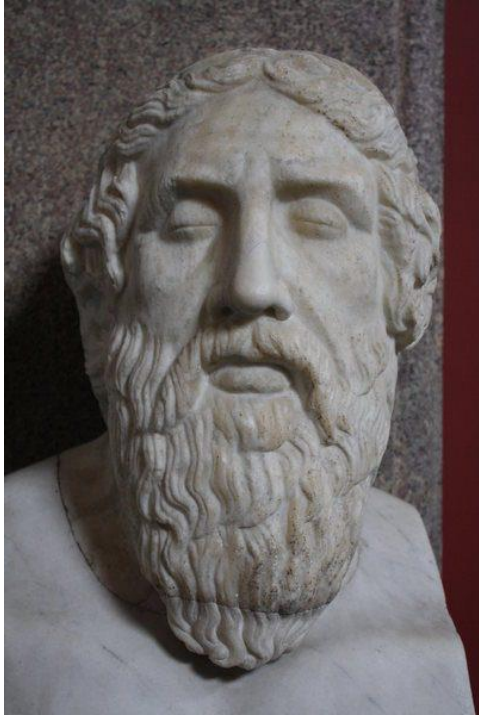
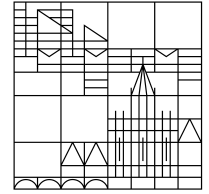


CA16113 - CliniMARK



Homer, Odyssey

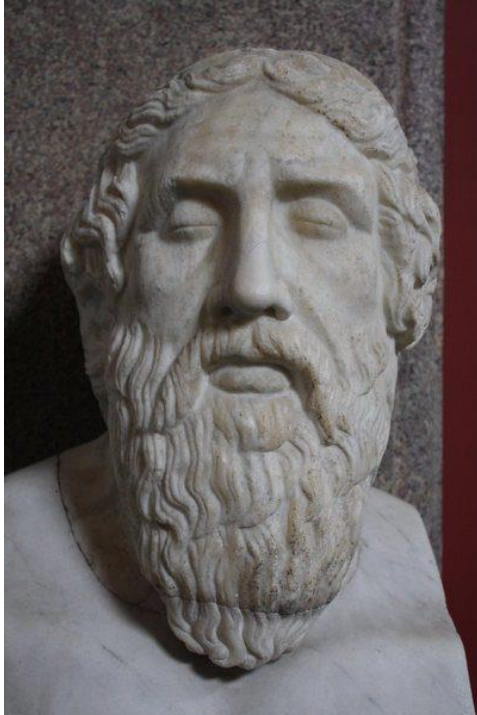
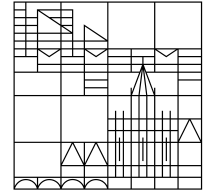
Ἦμος δ' ἡριγένεια φάνη ῥοδοδάκτυλος Ἥως [...]



Homer, Odyssey

Ἦμος δ' ἠριγένεια φάνη ῥοδοδάκτυλος Ἥως [...]

When but early-born appeared rosy-fingered Dawn



Homer, Odyssey

Ἦμος δ' ἠριγένεια φάνη ῥοδοδάκτυλος Ἥως [...]

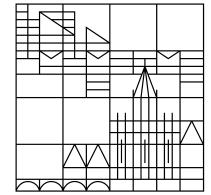
When but early-born appeared rosy-fingered Dawn

“But when early-born rosy-fingered Dawn appeared...”



Hotel Spetses, 25 Sept 2019

Universität
Konstanz



Homer, *Odyssey*

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Hotel Spetses, 25 Sept 2019

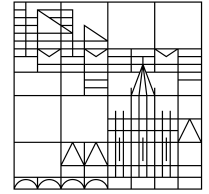
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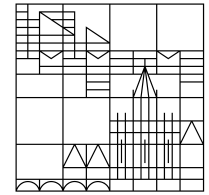
“But when early-born rosy-fingered Dawn appeared...”

Universität
Konstanz



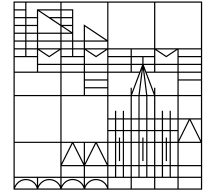
**Homer
must have
visited the
island of
Spetses**





Brian F.C. Clark, 1936-2014
(photo: Lisbeth Heilesen)

- Professor of Biostructural Chemistry, University of Aarhus, Denmark
- An early breakthrough for his department was solving the structure of the GTP-binding domain of the tRNA-binding protein EF-Tu
- President of the International Union of Biochemistry and Molecular Biology (IUBMB),
- Chairman of the Federation of European Biochemical Societies (FEBS),
- [...]
- In the 1990s, Coordinator of the **EU-funded Concerted Action Programme MOLGERON**
- Honorary citizen of the Spetses, where he has been organising Advanced Courses in Molecular and Cell Biology for 40 years
- In 2012, **a lecture hall at the Spetses Hotel was named after Brian Clark.**



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Human Ageing -- basic facts

- Rate of ageing in humans is not uniform (genetic heterogeneity; environmental factors)

Centenarians (>100 yrs) and Supercentenarians (>110 yrs)



**Madame Jeanne Calment
(died in 1997 at the age of 122 yrs)**

Centenarians (>100 yrs) and Supercentenarians (>110 yrs)



**Madame Jeanne Calment
(died in 1997 at the age of 122 yrs)**

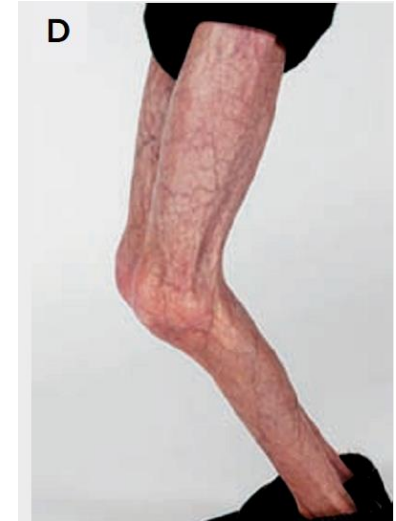
Quit smoking at age 119

Life span record holders

Rank	Name	Sex	Birth date	Death date	Age	Country of death or residence
1	<u>Jeanne Calment</u> ^[1]	F	21 Feb 1875	4 Aug 1997	122 y, 164 days	<u>France</u>
2	<u>Sarah Knauss</u> ^[5]	F	24 Sep 1880	30 Dec 1999	119 y, 97 days	<u>United States</u>
3	<u>Nabi Tajima</u> ^[6]	F	4 Aug 1900	21 Apr 2018	117 y, 260 days	<u>Japan</u>
4	<u>Lucy Hannah</u> ^[7]	F	16 July 1875	21 Mar1993	117 y, 248 days	United States
5	<u>Marie-Louise Meilleur</u> ^[8]	F	29 Aug 1880	16 Apr 1998	117 y, 230 days	<u>Canada</u>
6	<u>Violet Brown</u> ^[6]	F	10 Mar 1900	15 Sep 2017	117 y, 189 days	<u>Jamaica</u>
7	<u>Emma Morano</u> ^[6]	F	29 Nov 1899	15 Apr2017	117 y, 137 days	<u>Italy</u>
8	<u>Chiyo Miyako</u> ^[9]	F	2 May 1901	22 July 2018	117 y, 81 days	Japan
9	<u>Misao Okawa</u> ^[6]	F	5 Mar 1898	1 Apr 2015	117 y, 27 days	Japan
10	<u>María Capovilla</u> ^[10]	F	14 Sep 1889	27 Aug 2006	116 y, 347 days	<u>Ecuador</u>

From: Wikipedia

Progeria Hutchinson Gilford



Merideth et al (2008) N Engl J Med; Kieran et al. (2007) Pediatrics

Human Ageing -- basic facts

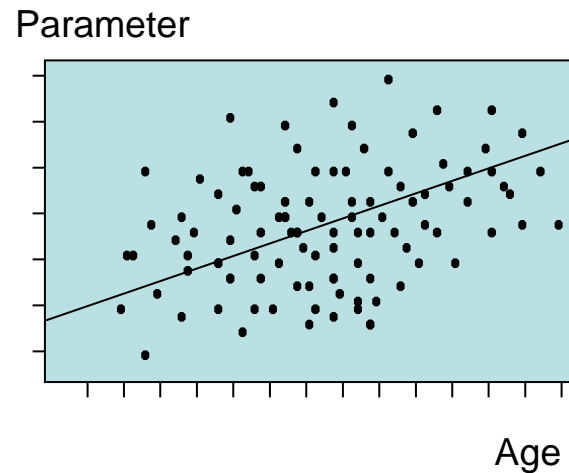
- Rate of ageing in humans is not uniform (genetic heterogeneity; environmental factors)
- Ageing status of individuals cannot be assessed directly

Human Ageing -- basic facts

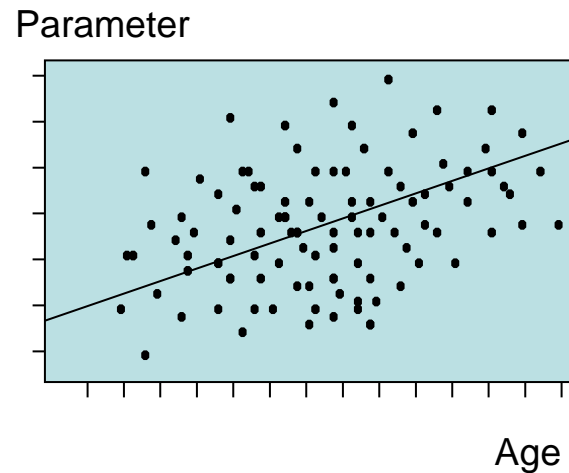
- Rate of ageing in humans is not uniform (genetic heterogeneity; environmental factors)
- Ageing status of individuals cannot be assessed directly

=> biomarkers could be a step forward

Single Parameter Showing Some Correlation with Age in Population



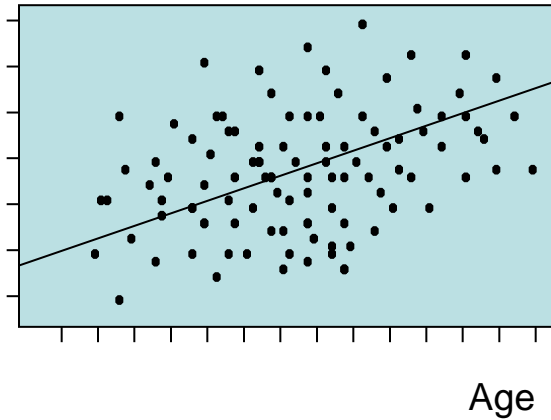
Single Parameter Showing Some Correlation with Age in Population



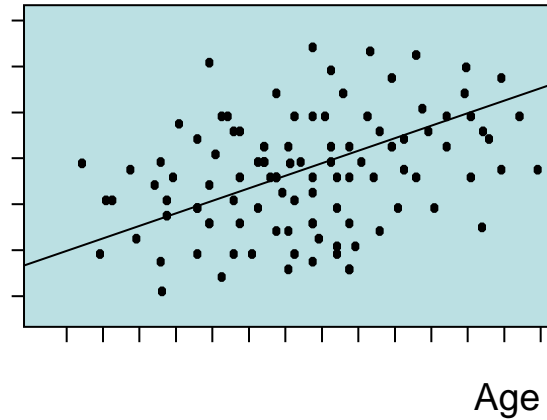
.... Not good enough!

Strategy for the establishment of a Biological Age Score

Parameter 1

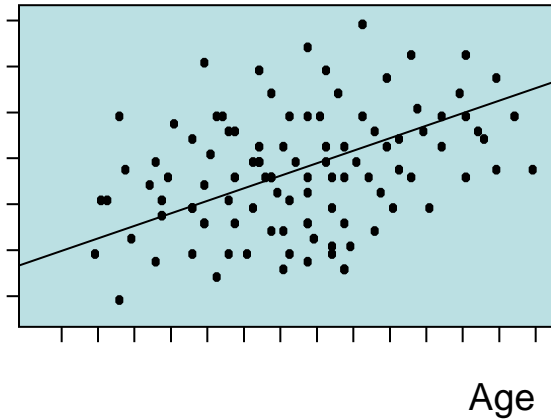


Parameter 2

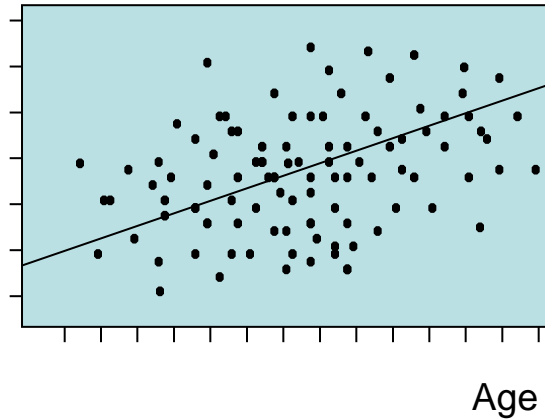


Strategy for the establishment of a Biological Age Score

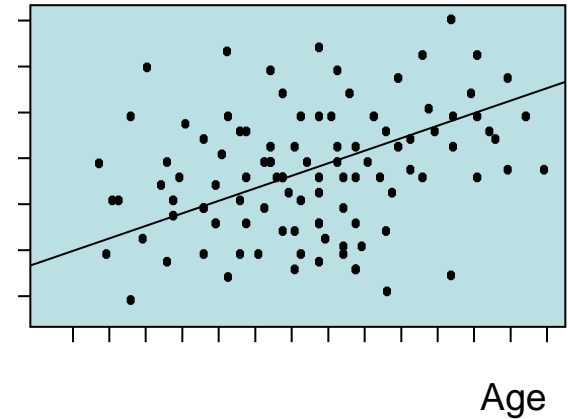
Parameter 1



Parameter 2

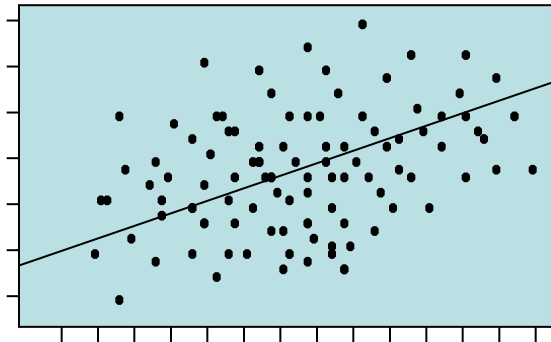


Parameter 3



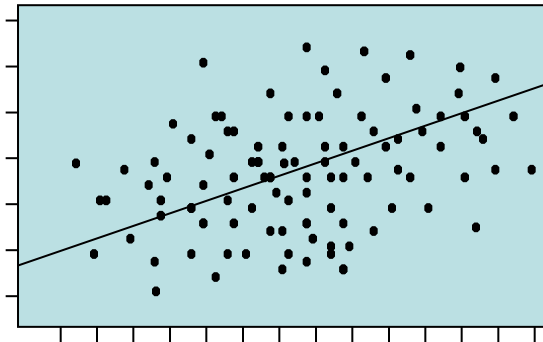
Strategy for the establishment of a Biological Age Score

Parameter 1



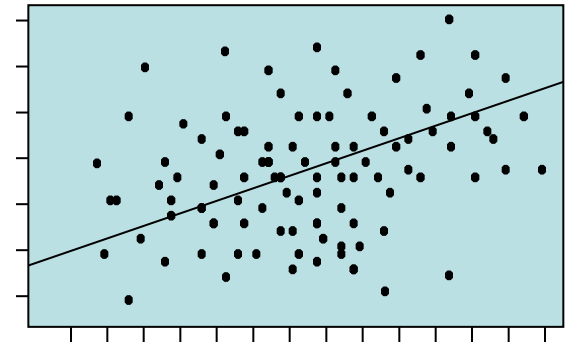
Age

Parameter 2



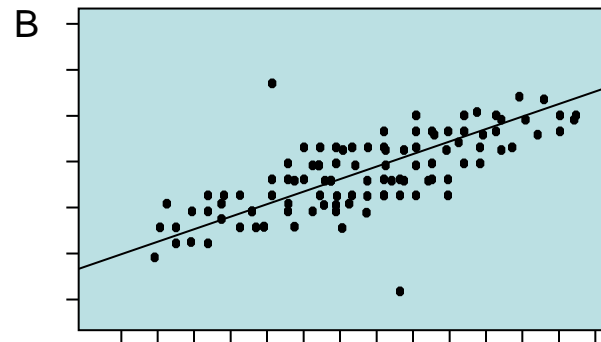
Age

Parameter 3



Age

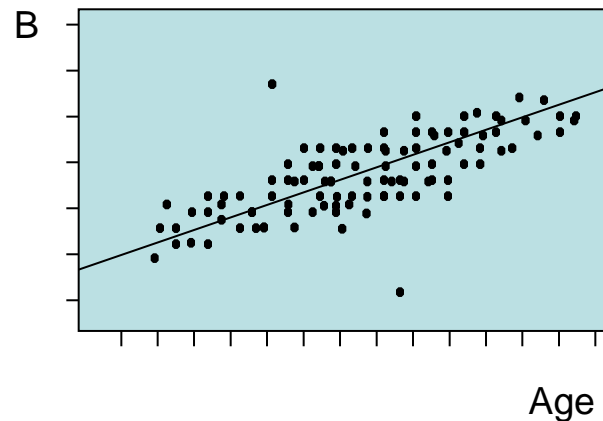
Biological age score $B = a \times P_1 + b \times P_2 + c \times P_3 \dots$



Age

Use of the Biological Age Score

Biological age score $B = a \times P_1 + b \times P_2 + c \times P_3 \dots$



- Collectively, the *Biological age scores* of the members of the MARK-AGE population model chronological age of these individuals via biochemical / molecular analyses.
- The *Biological age score* of a certain new individual indicates the **predicted chronological age** of this person, and we define this value as „biological age“ of the person.

MARK-AGE Project

Project full title:

European Study to Establish Biomarkers of Human Ageing

(HEALTH-F4-2008-200880)

1 April 2008 – 30 September 2013

Scientific Co-ordinator:

Alexander Bürkle
University of Konstanz,
Konstanz, Germany



www.markage.eu



The MARK-AGE Consortium

#	Beneficiary name	PI	Country
1	Universitaet Konstanz	Alexander Bürkle	Germany
2	BioTeSys GmbH	Jürgen Bernhardt, Christiane Schön	Germany
3	Fundación Centro Nacional de Investigaciones Oncológicas Carlos III	Maria Blasco	Spain
4	DNage B.V.	Gerben Zondag	The Netherlands
5	Erasmus Universitair Medisch Centrum Rotterdam	Jan Hoeijmakers	The Netherlands
6	Facultés Universitaires Notre-Dame de la Paix de Namur	Olivier Toussaint	Belgium
8	Universitaet Innsbruck	Beatrix Grubeck-Loebeinstein	Austria
9	Istituto Nazionale Riposo e Cura per Anziani	Eugenio Mocchegiani	Italy
10	NESTEC SA	Sunil Kochar	Switzerland
11	National Hellenic Research Foundation	Stathis Gonos	Greece
12	Instytut Biologii Doświadczalnej im. M. Nenckiego PAN	Ewa Sikora	Poland
13	Institutul National de Gerontologie si Geriatrie Ana Aslan	Daniela Gradinaru	Romania
14	Rijksinstituut voor Volksgezondheid en Milieu	Martijn Dollé	The Netherlands
15	StratiCELL Screening Technologies SA/NV	Michel Salmon	Belgium
16	Aarhus Universitet	Peter Kristensen	Denmark
17	Aston University	Helen Griffiths	UK
18	Vlaams Instituut voor Biotechnologie vzw	Valerie Vanhooren, Claude Libert, Chitty Chen	Belgium
19	Universitaet Hohenheim	Tilman Grune	Germany
20	Martin-Luther Universitaet Halle-Wittenberg	Andreas Simm	Germany
21	Alma Mater Studiorum – Università di Bologna	Claudio Franceschi	Italy
22	Unilever UK Central Resources Limited	Duncan Talbot	UK
23	Università degli Studi di Roma “La Sapienza”	Paola Caiafa	Italy
24	Université Pierre et Marie Curie – Paris 6	Betrand Friguet	France
25	Academisch Ziekenhuis Leiden - Leids Universitair Medisch Centrum	Rudi Westendorp, Eline Slagboom, Ton de Craen	The Netherlands
26	Tampereen Yliopisto	Antti Hervonen	Finland
27	Cranfield University	Richard Aspinall	UK

Purpose of MARK-AGE

Population study (about 3,300 subjects) to identify a set biomarkers of ageing which, as a **combination of parameters with appropriate weighting**, should measure biological age better than any marker in isolation.

Implementation

WP1 – Recruitment

From all subjects enrolled, anthropometric, clinical and social data will be collected in a standardised fashion. Upon written informed consent, the following set of information will be obtained by using a questionnaire:

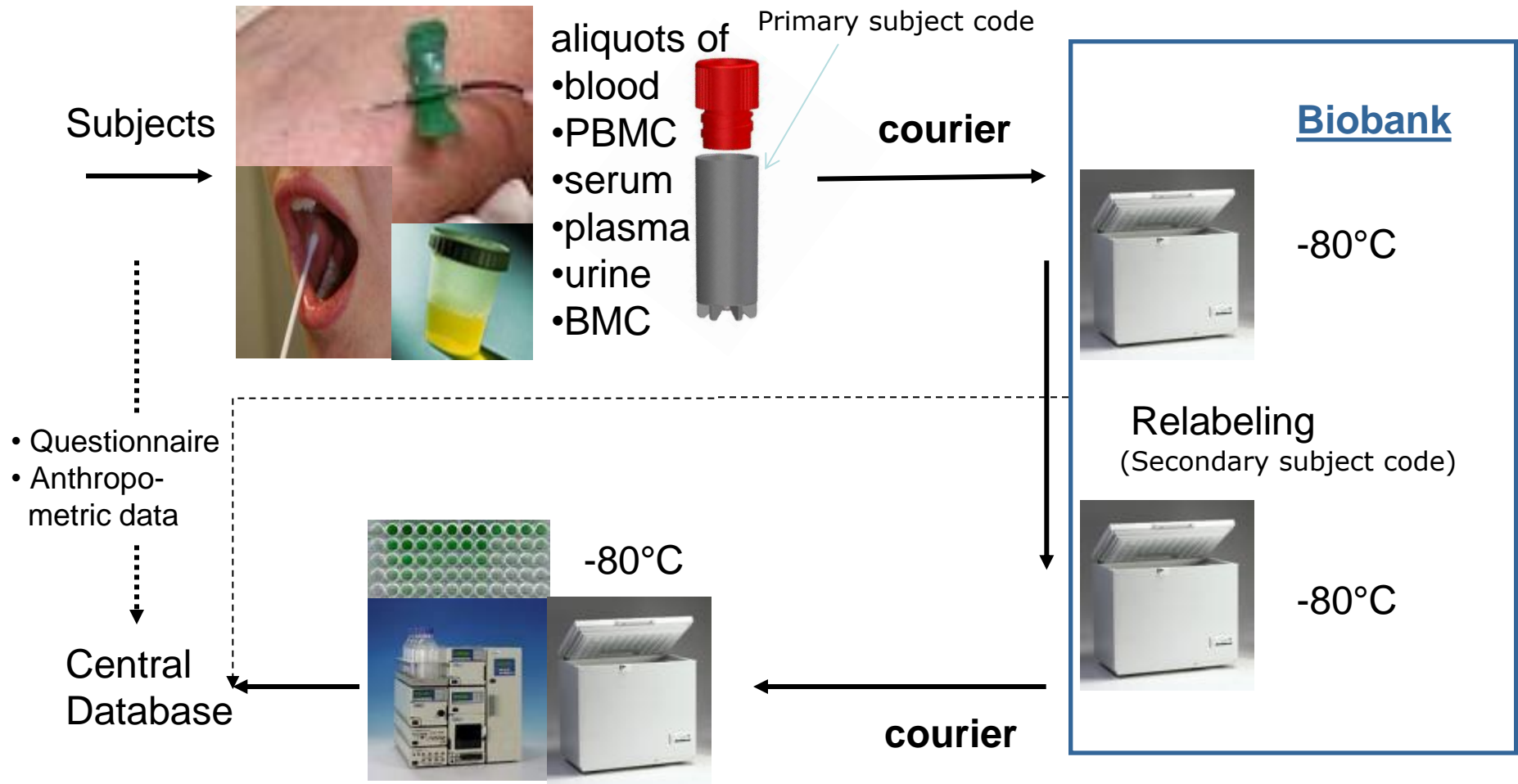
- **Demographic information:** family composition, marital status, education, occupation, and housing conditions.
- **Lifestyle:** use of tobacco and alcohol, daily activities.
- **Functional status:** Activities of Daily Living (ADL) and Norton Scale
- **Cognitive status:** STROOP test, 15-picture learning test
- **Health status:** present and past diseases, self-perceived health, number and type of prescribed drugs.
- **Mood:** ZUNG depression scale.

A **physical examination** for all probands will comprise the following:

- Body mass index
- Waist and hip circumference
- Blood pressure at rest
- Heart rate at rest
- Lung capacity- FEV1
- Lung capacity- FVC (forced expiratory vital capacity)
- Handgrip strength

Mechanisms of Ageing and Development (2015) 151, 1-122

Biological samples => Biobank



MARK-AGE
Partners

Mechanisms of Ageing and Development (2015) 151, 1-122

WP 2 – DNA-based markers

Global and subtelomeric DNA methylation
DNA methylation of the chromosomal region 11p15.5 (including <i>SIRT3</i> , <i>PMSD13</i> , <i>HRAS1</i> , <i>IGF1</i> , <i>TH</i> , <i>INS</i>)
Expression of <i>DNMT1</i> , <i>IGF2</i> , <i>PARP</i> , <i>1 TERT</i>
Telomere length
DNA repair upon X-irradiation
Cellular poly(ADP-ribosylation) capacity
mtDNA mutation
APOE genotype

Mechanisms of Ageing and Development (2015) 151, 1-122

WP3 – Markers based on proteins and their modifications

N-glycans in serum	Serum
N-glycans in urine	Urine
N-glycan of IgG fraction	Serum
ApoJ/CLU	Serum
AGEs in plasma (fluorescence spectroscopy)	Plasma
Carboxymethyllysine, pentosidine, arg-pyrimidine and imidazolone	Plasma
Proteasome chymotrypsin-like activity	PBMC
Protein levels of proteasome catalytic subunit	PBMC
Protein levels of proteasome regulatory subunit	PBMC
Total methionine sulfoxide reductase (Msr) activity	PBMC
Protein levels of Msr A	PBMC
Protein levels of Msr B	PBMC

Mechanisms of Ageing and Development (2015) 151, 1-122

WP4 - Immunological markers

IgG	Serum
IgM	Serum
IgA	Serum
IgE	Serum
IL-1 β , IL-2, IL-6, IL-8, IL-10, IL-12, IL-13, TNF- α , b-FGF, IP-10, MCP-1, IFN- γ , GM-CSF, CD40	Serum
Phenotyping of T cells (CD3/CD4, CD3/CD8), B cells (CD19), NK cells (CD3/CD16+56), monocytes (CD14/CD16)	PBMC
sjTRECs	PBMC
HHV-6A, HHV-6B, HHV-7, CMV viral loads	PBMC (Plasma, Urine)
CMV antibodies	Serum
Influenza A IgG antibodies	Serum
Influenza B IgG antibodies,	Serum
Measles IgG antibodies	Serum
Tetanus IgG antibodies	Serum
Interferone- γ production upon stimulation with Influenza A antigen	PBMC
Interferone- γ production upon stimulation with Influenza B antigen	PBMC
Interferone- γ production upon stimulation with Measles antigen	PBMC
Interferone- γ production upon stimulation with Tetanus antigen	PBMC
Autoantibodies against thyroglobulin	Plasma-EDTA
Autoantibodies against antinuclear antibodies	Serum
Blood counts and differential blood counts	Whole blood
Dead/apoptotic T cells (non-treated, PHA- or curcumin-treated)	PBMC
Caspase-3 positive T cells cells (non-treated, PHA- or curcumin-	PBMC

WP 5 – Clinical Chemistry, Hormones and Markers of Metabolism

Blood urea nitrogen	Plasma (EDTA)
Creatinine	Plasma (EDTA)
Creatinine	Urine
Adiponectin concentration	Serum
Serum amyloid A and P	Serum
Pentraxin 3	Serum
Lipoprotein particle size classes	Serum
Transferrin	Serum
Ferritin	Serum
Alpha2-macroglobulin	Serum
Ceruloplasmin	Serum
Fasting glucose	Serum
Fasting insulin	Plasma (EDTA)
Glycosylated hemoglobin A _{1c}	Blood
Albumin	Plasma (EDTA)
Total protein	Serum
Total protein	Plasma (EDTA)

Mechanisms of Ageing and Development (2015) 151, 1-122

WP 6 – Oxidative stress markers

Malondialdehyde	Plasma (EDTA)
Protein carbonyls	Plasma (EDTA)
Nitrated proteins	Plasma (EDTA)
Protein oxidation	Plasma (EDTA)
LDL oxidation	Serum
Nitric Oxide (NO ₂ - + NO ₃ -)	Plasma (EDTA)
8-Isoprostane prostaglandin F ₂ -alpha	Urine, Plasma (heparin)
2,3-dinor 8-isoprostane prostaglandin F ₁ -alpha	Urine
Creatinine	Urine
Total glutathione	Blood
Total cysteine	Blood
Oxidized and reduced glutathione	Blood
Total glutathione	BMC
Retinol	Serum

Mechanisms of Ageing and Development (2015) 151, 1-122

WP 6 – Oxidative stress markers, con't

Total lutein	Plasma (EDTA)
Total zeaxanthin	Plasma (EDTA)
Beta-cryptoxanthin	Plasma (EDTA)
Total lycopene	Plasma (EDTA)
Alpha-carotene	Plasma (EDTA)
Beta-carotene	Plasma (EDTA)
Gamma-tocopherol	Plasma (EDTA)
Alpha-tocopherol	Plasma (EDTA)
Ascorbatic acid	Plasma (EDTA)
Uric acid	Plasma (EDTA)
Ascorbate	BMC
Alpha-tocopherol	BMC
Beta-carotene	BMC
DNA amount	BMC
Total Zn, Cu, Se and Fe in plasma	Plasma (Heparin)
Distribution/ amount of Zn, Cu, Se and Fe among plasma proteins	Plasma (Heparin)
Metallothioneins	PBMC

Mechanisms of Ageing and Development (2015) 151, 1-122

WP 8 - Data Analysis and Bioinformatics

[...]

MARK-AGE Publications

Special Issue on “**Biomarkers of Human Ageing**” Edited by Bürkle A, Grune T, Gonos ES, Bohr VA **Mechanisms of Ageing and Development (2015) 151, 1-122**

1. Bürkle A et al., Editorial. Mech Ageing Dev. 2015 Nov;151:1
2. Bürkle A, et al. MARK-AGE biomarkers of ageing. Mech Ageing Dev. 2015 Nov;151:2-12.
3. Capri M, et al., MARK-AGE population: From the human model to new insights. Mech Ageing Dev. 2015 Nov;151:13-7
4. Moreno-Villanueva M, et al., MARK-AGE standard operating procedures (SOPs): A successful effort. Mech Ageing Dev. 2015 Nov;151:18-25
5. Moreno-Villanueva M et al., The MARK-AGE phenotypic database: Structure and strategy. Mech Ageing Dev. 2015 Nov;151:26-30.
6. Baur J, et al. The MARK-AGE extended database: data integration and pre-processing. Mech Ageing Dev. 2015 Nov;151:31-7.
7. Baur J, et al., MARK-AGE data management: Cleaning, exploration and visualization of data. Mech Ageing Dev. 2015 Nov;151:38-44.
8. Giampieri E et al.. Statistical strategies and stochastic predictive models for the MARK-AGE data. Mech Ageing Dev. 2015 Nov;151:45-53
9. Jansen E et al., Quality control data of physiological and immunological biomarkers measured in serum and plasma. Mech Ageing Dev. 2015 Nov;151:54-9.
10. Zampieri M et al., Reconfiguration of DNA methylation in aging. Mech Ageing Dev. 2015 Nov;151:60-70.
11. Vanhooren V, et al. Protein modification and maintenance systems as biomarkers of ageing. Mech Ageing Dev. 2015 Nov;151:71-84.
12. Sikora E, Activation-induced and damage-induced cell death in aging human T cells. Mech Ageing Dev. 2015 Nov;151:85-92
13. Malavolta M, et al. Serum copper to zinc ratio: Relationship with aging and health status. Mech 14 Dev. 2015 Nov;151:93-100
14. Gradinaru D, et al., Oxidized LDL and NO synthesis--Biomarkers of endothelial dysfunction and ageing. Mech Ageing Dev. 2015 Nov;151:101-13
15. Griffiths HR et al., Novel ageing-biomarker discovery using data-intensive technologies. Mech Ageing Dev. 2015 Nov;151:114-21.

MARK-AGE Original Publications:

- Pinchuk I et al (2019). Gender- and age-dependencies of oxidative stress, as detected based on the steady state concentrations of different biomarkers in the MARK-AGE study. Redox Biol 24:101204
- Rietman ML et al (2019). Antioxidants linked with physical, cognitive and psychological frailty: Analysis of candidate biomarkers and markers derived from the MARK-AGE study. Mech Ageing Dev. 177:135-43.
- Ciccarone F et al. (2017) DNA hydroxymethylation levels are altered in blood cells from Down syndrome persons enrolled in the MARK-AGE project. J Gerontol A Biol Sci Med Sci. 2017 Oct 21. doi: 10.1093/gerona/glx198.
- Giacconi R et al. (2017) Zinc-induced Metallothionein in centenarian offspring from a large European population: the MARK-AGE Project. J Gerontol A Biol Sci Med Sci. 2017 Oct 17. doi: 10.1093/gerona/glx192 [Epub ahead of print]
- Weinberger B et al. (2017) Protection against Tetanus and Diphtheria in Europe: the impact of age, gender and country of origin based on data from the MARK-AGE Study. Exp Gerontol 2017 Oct 7. pii: S0531-5565(17)30516-8.
- Weber D et al. (2017) Associations between specific redox biomarkers and age in a large European cohort: the MARK-AGE Project. Oxid Med Cell Longev, 2017:1401452
- Ciccarone F et al. (2016) Age-dependent expression of DNMT1 and DNMT3B in PBMCs from a large European population enrolled in the MARK-AGE study. Aging Cell 15:755-65

MARK-AGE Original Publications:

- Valentini E et al. **(2016)**. Analysis of the machinery and intermediates of the 5hmC-mediated DNA demethylation pathway in aging on samples from the MARK-AGE Study. *Aging* (Albany NY) 29;8:1896-1922
- Stuetz W et al. **(2016)** Plasma carotenoids, tocopherols, and retinol in the age-stratified (35-74 years) general population: cross-sectional study in six European countries. *Nutrients*. 2016;8(10). pii: E614.
- Dunston CR et al. **(2012)** Terminal galactose residues on transferrin are increased in midlife adults compared to young adults. *Proteomics*. 2012 Nov;12(21):3147-53.
- Zampieri M et al. **(2010)** Validation of suitable internal control genes for expression studies in aging. *Mech Ageing Dev* 131:89-95.

Recent MARK-AGE Review Article:

Moreno-Villanueva M, Bürkle A. Epigenetic and redox biomarkers: Novel insights from the MARK-AGE study. *Mech Ageing Dev*. **2019**;177:128-134



COBRA

Co-morbidity in relation to AIDS

Biomarkers of ageing in
HIV-positive individuals and
matched controls



GÖTEBORGS UNIVERSITET



Universität
Konstanz



Erasmus MC
Universitair Medisch Centrum Rotterdam



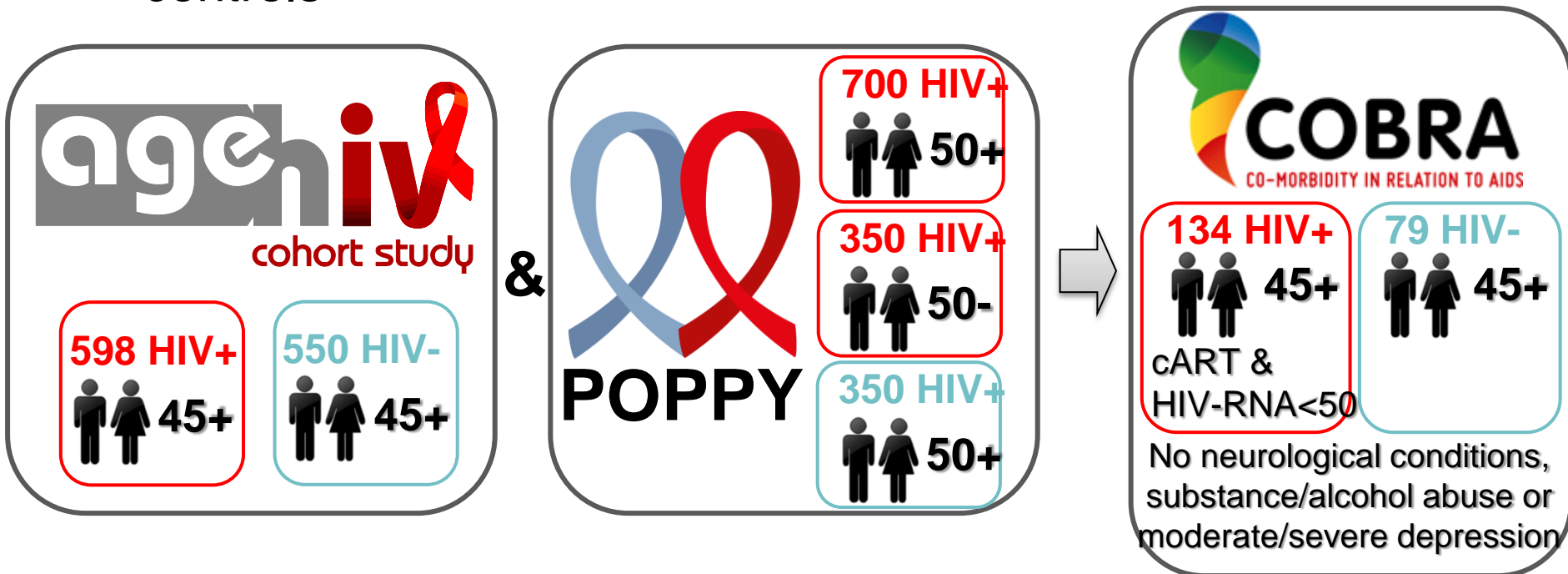
ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA



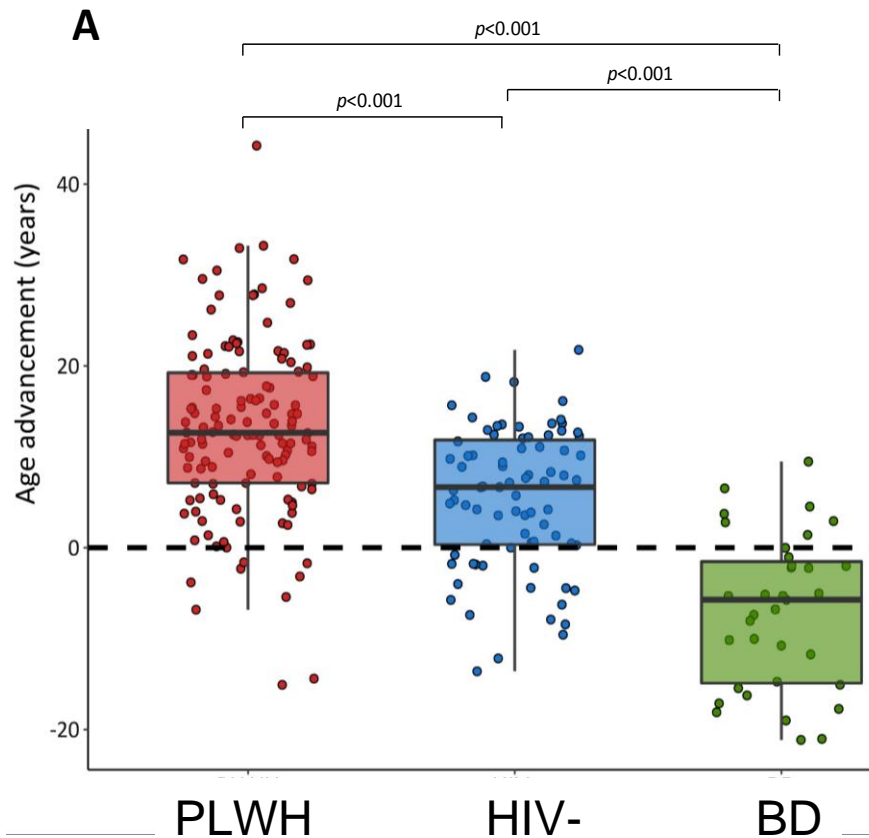
UNIVERSITÀ DEGLI STUDI
DI MODENA E REGGIO EMILIA

Methods: the cohort

- Longitudinal HIV cohort study built on established infrastructures in the Netherlands and UK
- Appropriately chosen and comparable HIV-negative controls



Age advancement in **Persons Living with HIV (PLWH)**, in **HIV-negative lifestyle controls** and in **blood donors [BD]**



- **134 PLWH** aged ≥ 45 , on cART and with a plasma HIV viral load < 50 copies/mL for ≥ 12 months prior to enrolment were recruited. Median (IQR) duration of HIV was 15 (9-21) years and nadir $CD4^+$ was 180 (90-250).
- **79 similarly aged HIV-negative controls** with comparable socio-demographic and lifestyle characteristics, were recruited from sexual health centres.
- **35 age-matched healthy BD** were recruited from the Dutch national blood bank.

De Francesco D, et al. Do people living with HIV experience greater age advancement than their HIV-negative counterparts? AIDS. **2019 Feb 1**;33(2):259-268

Summary

- **MARK-AGE:** 10 blood parameters in men / women used to calculate the predicted age of a person: „Biological Age“
- Down Syndrome subjects display an age advancement
- Male smokers display an age advancement
- Postmenopausal women taking HRT display an age retardation
- **COBRA:** Relative to the MARK-AGE population, **PLWH** / **HIV-** / **BD** display an age advancement of about **12** / **6** / **- 4** years

Future Directions

- MARK-AGE should be turned into a **longitudinal study**: How predictive is „biological age“ for morbidity and mortality?

Acknowledgements: The MARK-AGE Consortium...

List of Beneficiaries

#	Beneficiary name	PI	Country
1	Universitaet Konstanz	Alexander Bürkle	Germany
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13	Institutul National de Gerontologie si Geriatrie Ana Aslan	Daniela Gradinaru	Romania
14	Rijksinstituut voor Volksgezondheid en Milieu	Martijn Dollé	The Netherlands
15	StratiCELL Screening Technologies SA/NV	Michel Salmon	Belgium
16	Aarhus Universitet	Peter Kristensen	Denmark
17	Aston University	Helen Griffiths	UK
18	Vlaams Instituut voor Biotechnologie vzw	Valerie Vanhooren, Claude Libert, Chitty Chen	Belgium
19	Universitaet Hohenheim	Tilman Grune	Germany
20	Martin-Luther Universitaet Halle-Wittenberg	Andreas Simm	Germany
21	Alma Mater Studiorum – Università di Bologna	Claudio Franceschi	Italy
22	Unilever UK Central Resources Limited	Duncan Talbot	UK
23	Università degli Studi di Roma “La Sapienza”	Paola Caiafa	Italy
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25	Academisch Ziekenhuis Leiden - Leids Universitair Medisch Centrum	Rudi Westendorp, Eline Slagboom, Ton de Craen	The Netherlands
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27	Cranfield University	Richard Aspinall	UK

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The Konstanz Team

Dr María Moreno
Scientific Manager

Sebastian Oehlke
PhD Student

Prof Michael Berthold
Bioinformatics

Gudrun von Scheven
Lab Technician

Thilo Sindlinger
Database manager

Prof Michael Junk
Math. Modelling

Barbara Bausch
Lab Technician

Jennifer Baur
PhD Student

Dr Zhaoxia Yang
Math. Modelling

The Bologna Team

Prof Claudio Franceschi
WP1 Leader

Dr Miriam Capri
Recruitment of subjects

Prof Gastone Castellani
Math. Modelling

The Hohenheim / Jena Team

Prof Tilman Grune
Deputy Co-ordinator

Dr Nicolle Breusing
Biobank Manager

The COBRA Consortium

Imperial College of Science, Technology and Medicine - *Department of Medicine, Division of Infectious Diseases*: A. Winston, J. Underwood, L. Tembo, L. McDonald, M. Stott, K. Legg, A. Lovell, O. Erlwein, N. Doyle, C. Kingsley, P. Norsworthy, Scott Mullaney. *Department of Medicine, Division of Brain Sciences, The Computational, Cognitive & Clinical Neuroimaging Laboratory*: D.J. Sharp, R. Leech, J.H. Cole.

University College London - *Research Department of Infection and Population Health*: C. Sabin, D. De Francesco.

GGD Amsterdam/Public Health Service Amsterdam - *Cluster of Infectious Diseases, research department*: M. Prins, M. Martens, S. Moll, J. Berkel, G.R. Visser., M. Totté, S. Kovalev.

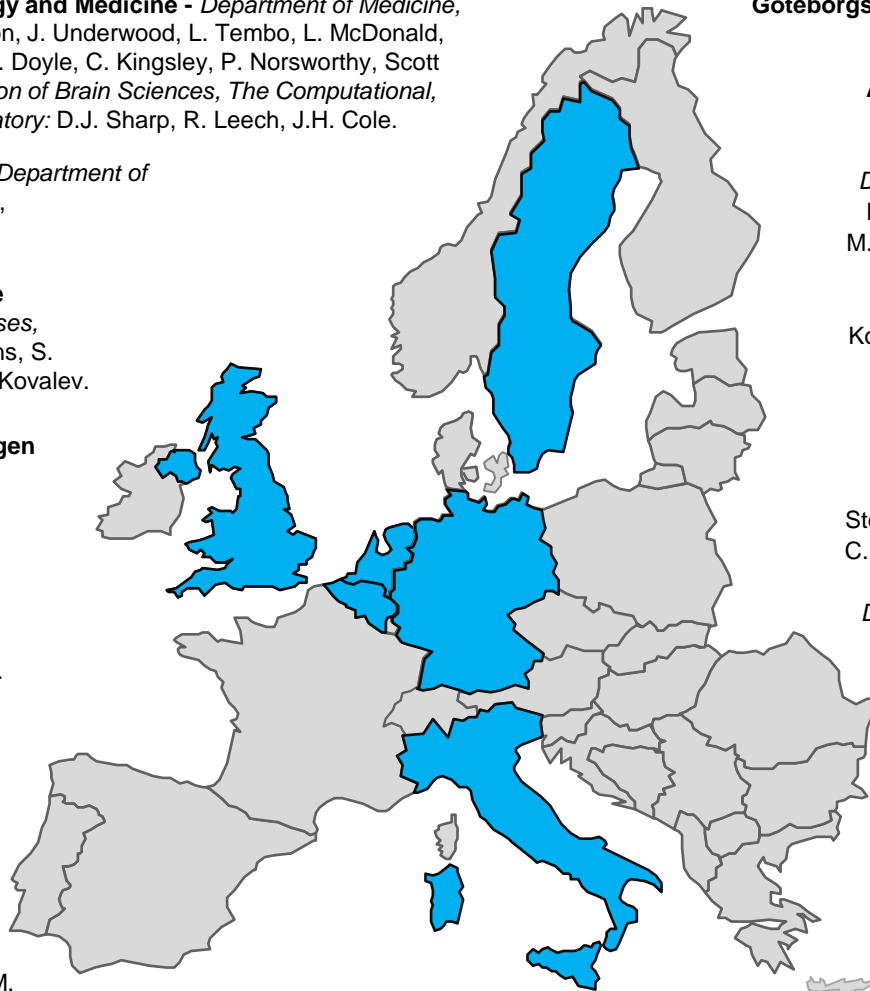
Stichting Katholieke Universiteit Nijmegen
D. Burger, M. de Graaff-Teulen.

Erasmus Universitair Medisch Centrum Rotterdam - *Department of Genetics*: J. Hoeijmakers, J. Pothof.

Vlaams Instituut voor Biotechnologie - *Inflammation research center*: C. Libert, S. Dewaele.

Universität Konstanz - *Department of Biology*: A. Bürkle, T. Sindlinger, S. Oehlke

Alma Mater Studiorum Università di Bologna - *Department of Experimental, Diagnostic and Specialty Medicine*: C. Franceschi, P. Garagnani, C. Pirazzini, M. Capri, F. Dall'Olio, M. Chiricolo, S. Salvioli.



Göteborgs Universitet - M. Gisslén, D. Fuchs, H. Zetterberg.

Academisch Medisch Centrum, Universiteit van Amsterdam - *Department of Global Health and Amsterdam Institute for Global Health and Development (AIGHD)*: P. Reiss, J. Schouten, K.W. Kooij, R.A. van Zoest, B.C. Elsenga, F.R. Janssen, M. Heidenrijk, W. Zikkenheiner. *Division of Infectious Diseases*: M. van der Valk, A. Henderiks, *Department of Experimental Immunology*: N.A. Kootstra, A.M. Harskamp-Holwerda, I. Maurer, M.M. Mangas Ruiz, A.F. Girigorie, T. Booiman. *Department of Medical Microbiology*: J. Villaudy, E. Frankin, A. Pasternak, B. Berkhout, A. van der Kuyl. *Department of Neurology*: P. Portegies, B.A. Schmand, G.J. Geurtsen, J.A. ter Stege, M. Klein Twennaar. *Department of Radiology*: C.B.L.M. Majoie, M.W.A. Caan, T. Su. *Department of Cell Biology*: K. Weijer, E. Siteur-Van Rijnstra. *Division of Endocrinology and Metabolism*: P.H.L.T. Bisschop. *Department of Experimental neuroendocrinology*: A. Kalsbeek. *Department of Ophthalmology*: M. Wezel. *Department of Psychiatry*: I. Visser, H.G. Ruhé.

Stichting HIV Monitoring - F.W.N.M. Wit, S. Zaheri, M.M.J. Hillebregt, Y.M.C. Ruijs, D.P. Benschop.

Università degli studi di Modena e Reggio Emilia - *Department of Medical and Surgical Sciences for Children & Adults*: G. Guaraldi.

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