A triple drug combination targeting components of the nutrient-sensing network maximizes longevity

Jorge Iván Castillo-Quan\textsuperscript{a,b,c,1}, Luke S. Tain\textsuperscript{a,c,1}, Kerri J. Kinghorn\textsuperscript{a,c,1}, Li Li\textsuperscript{a,b,1}, Sebastian Grönke\textsuperscript{a,2}, Yvonne Hinze\textsuperscript{a,3}, T. Keith Blackwell\textsuperscript{b,2}, Ivana Bjedov\textsuperscript{a,1}, and Linda Partridge\textsuperscript{a,d,3}

\textsuperscript{a}Institute of Healthy Ageing, Department of Genetics, Evolution and Environment, University College London, WC1E 6BT London, United Kingdom; \textsuperscript{b}Section on Islet Cell & Regenerative Biology, Joslin Diabetes Center, Boston, MA 02215; \textsuperscript{c}Department of Genetics, Harvard Medical School, Boston, MA 02115; \textsuperscript{d}Department of Biological Mechanisms of Ageing, Max Planck Institute for Biology of Ageing, D-50931 Cologne, Germany; \textsuperscript{e}Department of Molecular Neuroscience, Institute of Neurology, WC1N 3BG London, United Kingdom; and \textsuperscript{f}Department of Cancer Biology, Cancer Institute, University College London, WC1E 6DD London, United Kingdom

Edited by Joseph S. Takahashi, The University of Texas Southwestern Medical Center, Dallas, TX, and approved September 16, 2019 (received for review August 1, 2019)

Increasing life expectancy is causing the prevalence of age-related diseases to rise, and there is an urgent need for new strategies to improve health at older ages. Reduced activity of insulin/insulin-like growth factor signaling (IIS) and mechanistic target of rapamycin (mTOR) nutrient-sensing signaling network can extend lifespan and improve health during aging in diverse organisms. However, the extensive feedback in this network and adverse side effects of inhibition imply that simultaneous targeting of specific effectors in the network may most effectively combat the effects of aging. We show that the mitogen-activated protein kinase kinase (MEK) inhibitor trametinib, the mTOR complex 1 (mTORC1) inhibitor rapamycin, and the glycogen synthase kinase-3 (GSK-3) inhibitor lithium act additively to increase longevity in Drosophila. Remarkably, the triple drug combination increased lifespan by 48%. Furthermore, the combination of lithium with rapamycin cancelled the latter’s effects on lipid metabolism. In conclusion, a polypharmacology approach of combining established, longevity-extending drugs in humans (2–4), and hence activates GSK3 (4), a potentially deleterious side effect of lowered IIS (19). We therefore tested whether lithium could have additive effects in combination with genetic inhibition of IIS upstream of Akt. Lithium was able to further extend the lifespan of flies lacking the insulin-like peptides 2, 3, and 5 (dilp2-3,5) (Fig. 1D) (20). In contrast, rapamycin or trametinib, neither of which inhibit GSK3, were not able to extend the lifespan of dilp2-3,5 flies (Fig. 1E and


This open access article is distributed under Creative Commons Attribution License 4.0 (CC BY).

1J.I.C.-Q. and L.S.T. contributed equally to this work.
2Present address: Department of Neurosurgery, School of Medicine, Stanford University, Palo Alto, CA 94304.
3To whom correspondence may be addressed. Email: l.partridge@ucl.ac.uk.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1913212116/-/DCSupplemental.

First published September 30, 2019.
**Methods**

**Fly Stocks, Husbandry, and Lifespan Analysis.** For all experiments, a wild-type white Dahoney (w^1118) stock, or, when noted, dilp2-3,5 mutant flies (w^1118 backcrossed), were used and raised as previously described (20). Licit (Sigma) in ddH2O, trametinib (LC laboratories) in dimethyl sulfoxide, and rapamycin (LC laboratories) in 100% ethanol were added to sugar–yeast–agar (SYA) medium to a final concentration of 1 mM, 15.6 μM, and 50 μM, respectively (5, 8, 11). Equivalent volumes and concentrations of vehicle were added to SYA medium for control treatments. Drug treatments were started 2 d posteclosion. Female flies (n = 130 to 200, 15 to 20 per vial) were sorted onto SYA medium that was replaced every 2 to 3 d throughout life.

**Fig. 2.** A triple drug combination maximizes longevity. (A) Representative survival curve and associated pairwise log-rank tests. (B) Replicated median/maximum lifespans plotted for all single (n = 4), double (n = 3), and triple (n = 2) combinations of rapamycin, trametinib, and lithium treatments. Each lifespans contained 130 to 200 flies per treatment. Numbers in parentheses show (total number of flies/number of censors). (C) Proboscis extension feeding behavior assay (1 and 15 d of treatment; Top and Middle) and quantification of ingested nonabsorbable (Bottom) blue dye (n = 8 replicates of 4 to 5 flies 15 d old, 1-way ANOVA with Dunnett’s test). (D) Mass spectrometry of systemic trametinib (Top) or rapamycin (Bottom) levels when other drugs were coadministered (n = 5, 1-way ANOVA). (E) Fecundity of treated (15 d) flies within a 24-h period (n = 8 replicates of 4 to 5 flies). Error bars show Tukey whiskers, and outlying data points are shown as dots. *P < 0.05, **P < 0.01, ***P < 0.001 (Kruskal–Wallis test and Dunn’s pairwise tests).
Lifespan raw data are provided as Dataset S1. Starvation assay was performed as previously described (11).

**Food Intake, Fecundity, and Triglyceride Measurements.** Feeding behavior (proboscis extension at 1 and 15 d of treatment) and food intake (quantified by dye-calibrated feeding) (4 to 5 flies per replicate, n = 8 to 10) were measured as previously described (23). Fecundity was quantified as number of eggs laid within 24 h (15 d), and triglyceride measurements (5 flies per replicate, n = 8) were performed as previously described (5, 11).

**Mass Spectrometry.** Flies (n = 5, 15 flies) were treated with drugs (15 d), their digestive system was allowed to void (1 h), they were snap frozen, drugs were extracted as previously described (5), and they were resuspended in 100 µL of acetonitrile/isopropanol 70:30 for measurement with an Acquity UPLC I-class MassLynx and absolute quantification.

ACKNOWLEDGMENTS. We are grateful to Prof. David Gems and Drs. Helena Cochemé, Natalie Moroz, and Filipe Cabreiro for advice and comments, and to Rachel Beltzhoover for proofreading. We thank Drs. Fiona Kerr, Anna Tillmann, and Giovanna Vinti for technical advice and assistance. We acknowledge funding from University College London Scholarships (J.I.C.-Q.), American Federation for Aging Research/Glenn Foundation for Medical Research Postdoctoral Fellowship (Grant PD18019 to J.I.C.-Q.), Max Planck Society (J.I.C.-Q., I.S.T., S.G., Y.H., and L.P.), and National Institutes of Health (Grants AG54215 and GM122610 to T.K.B.). This project has received funding from the European Research Council under the European Union’s Horizon 2020 research and innovation program (Grant Agreement 741989), European Research Council Starting Grant (Grant 311331 to I.B.), Research Into Ageing (I.B. and L.P.), Parkinson’s UK (L.L. and L.P.), Wellcome Trust Clinical Career Development Fellowship (Grant 214589/Z/18/Z to K.K.), Wellcome Trust Strategic Award (WT098565/Z/12/Z to L.P.), and Academy of Medical Sciences (K.J.K.).

18. V. P. Houde et al., Chronic rapamycin treatment causes glucose intolerance and hyperlipidemia by upregulating hepatic gluconeogenesis and impairing lipid deposition in adipose tissue. Diabetes 59, 1338–1348 (2010).