Late-life targeting of the IGF-1 receptor improves healthspan and lifespan in female mice

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Diminished growth factor signaling improves longevity in laboratory models, while a reduction in the somatotropic axis is favorably linked to human aging and longevity. Given the conserved role of this pathway on lifespan, therapeutic strategies, such as insulin-like growth factor-1 receptor (IGF-1R) monoclonal antibodies (mAb), represent a promising translational tool to target human aging. To this end, we performed a preclinical study in 18-mo-old male and female mice treated with vehicle or an IGF-1R mAb (L2-Cmu, Amgen Inc), and determined effects on aging outcomes. Here we show that L2-Cmu preferentially improves female healthspan and increases median lifespan by 9% (P = 0.03) in females, along with a reduction in neoplasms and inflammation (P ≤ 0.05). Thus, consistent with other models, targeting IGF-1R signaling appears to be most beneficial to females. Importantly, these effects could be achieved at advanced ages, suggesting that IGF-1R mAbs could represent a promising therapeutic candidate to delay aging.
Diminished growth hormone (GH) and insulin/insulin-like growth factor-1 (IGF-1) signaling extends lifespan in many laboratory models, including mutations to *dag2* in worms, *Sch9* in yeast, and *Chico* in *drosophila*. Likewise, several dwarf models, including Ames, Snell and growth hormone receptor knockout (GHRKO) mice, are exceptionally long lived. A specific role for IGF-1 receptor (IGF-1R) signaling in the mediation of mammalian longevity was first established in IGF-1R haploinsufficient mice, which lived 33% longer than controls, but unlike other models of reduced somatotropic signaling, this effect was female specific. This unique sex difference was subsequently confirmed in two follow-up studies, though with more modest reported improvements in female lifespan, while a life shortening effect was observed in males. The underlying mechanism(s) linking reduced IGF-1 signaling to improved mammalian lifespan is thought to involve improved stress defenses and lower risk for proliferative diseases, though the reason for sex differences in this response remains unresolved.

Several examples have also now emerged suggesting the GH/IGF-1 signaling pathway is relevant to human aging, including the discovery of functional mutations in the *IGF-1R* gene in individuals with exceptional longevity, resulting in relative IGF-1 resistance, and in subjects lacking functional GH receptors (Laron dwarfs). Remarkably, low IGF-1 levels also predict better stress defenses and lower risk for proliferative diseases, whether beneficial effects associated with diminished IGF-1 signaling observed in animal models. In male-to-female transsexuals, IGF-1 levels in males were reduced by 33% and IGF-1 levels in females were increased by 20%, with more modest reported improvements in female lifespan, while a life shortening effect was observed in males. The underlying mechanism(s) linking reduced IGF-1 signaling to improved mammalian lifespan is thought to involve improved stress defenses and lower risk for proliferative diseases, though the reason for sex differences in this response remains unresolved.

Anti-IGF-1 receptor (IGF-1R) monoclonal antibodies (mAbs) were developed for clinical use in treating advanced stage cancers, including Ganitumab, which remains under investigation as a combination therapy in clinical trials targeting Rhabdomyosarcoma (NCT03401701) and Ewing Sarcoma (NCT02306161), for which it recently received orphan drug status. We therefore postulated that *IGF-1R* mAbs could represent a viable therapeutic tool to target IGF-1 action, and potentially mimic the beneficial effects associated with diminished IGF-1 signaling observed in animal models. In order to test this possibility, we engineered a murinized version of the anti-IGF-1R mAb, *L2-C* (*L2-Cmu*), in order to reduce effector function and enable chronic administration in mice. Here, we provide the first evidence of delayed aging with a therapeutic mAb, via long-term modulation of IGF-1 action. *L2-Cmu* proved feasible and well tolerated in older animals, and consistent with genetic models of *IGF-1R* heterozygosity, improves female healthspan and lifespan. Importantly, these effects were achieved even though treatment was not initiated until 18 mo of age. Thus, these data suggest that late-life targeting of IGF-1R signaling can recapitulate effects observed in genetic models of constitutive *IGF-1R* haploinsufficiency on lifespan. As IGF-1R mAbs are readily available for human use, these observations warrant further study into potentially harnessing these drugs to target at least some manifestations of aging.

**Results**

**L2-Cmu is a selective antagonist to the IGF-1R and hybrids.** *L2-Cmu* was developed as a murinized version of the *L2-C* mAb at Amgen Inc. (Thousand Oaks, CA). Western blotting and Biacore analysis confirmed that *L2-Cmu* binds to and inhibits IGF-1R activation by IGF-1 (Ki = 3.3 nM) and IGF-2 (Ki = 3.3 nM) (Fig. 1a; Supplementary Table 1), which was verified in the IGEN format (Fig. 1b, c). In NIH-3T3 mouse fibroblasts cells, pre-treatment with *L2-Cmu* led to an ~65% inhibition of IGF-1-mediated activation of IGF-1Rs and InsR/IGF-1R hybrid receptors (HybridRs) (Fig. 1d; *P* < 0.05). Moreover, when mice were pre-treated with vehicle or L2-Cmu (20 mg/kg) by intraperitoneal (i.p.) injection, and then challenged with an intravenous (i.v.) bolus of saline or 5 μg IGF-1, *L2-Cmu* was able to modulate IGF-1R activation in vivo, as demonstrated by a near complete inhibition of IGF-1R phosphorylation in lung and ~70% inhibition in heart (Fig. 1e, f; *P* < 0.05).

**Chronic L2-Cmu treatment is well tolerated in older mice.** We next performed a 6 mo feasibility study with weekly *L2-Cmu* i.p. injections (20 mg/kg) in 18-mo-old CB6F1 male and female mice, to carefully characterize the safety and efficacy of chronic IGF-1R modulation in aging animals. This dose of *L2-Cmu* was based in part on a prior pharmacodynamic (PD) study in which *L2-C* achieved similar suppression of tumor growth in vivo as Gani-tumab. A pharmacokinetic (PK) study confirmed *L2-Cmu* levels after a single injection were ~5-fold greater than the reported IC<sub>50</sub> values for Ganitumab (28 µg/mL) in both sexes for at least 7 days (Supplementary Fig. 1a), while chronic exposure levels of *L2-Cmu* were similarly increased to levels expected to provide sufficient coverage in vivo (Supplementary Fig. 1b). In females, *L2-Cmu* mAb treatment led to a slight, albeit significant reduction in red blood cells (RBCs), hemoglobin (Hb), hematocrit (Hct), white blood cells (WBCs), lymphocytes (Supplementary Table 2), and serum phosphorus (Supplementary Table 3), though most values, with the exception of WBCs, remained within the “normal range”, and were less severely affected than previously reported with Ganitumab in young CD1 mice. In males, no significant effects of *L2-Cmu* were observed on WBCs (Supplementary Table 4) or RBCs (Supplementary Table 4), but total protein, globulin, and ALT were slightly increased, while serum creatinine was reduced (Supplementary Table 5).

**L2-Cmu does not perturb glucose homeostasis in older mice.** We then evaluated effects of chronic IGF-1R mAb treatment on energy and glucose homeostasis in an initial cohort of older mice. In females, no significant effect on body weight, composition or energy balance was observed following 6 mo of *L2-Cmu* treatment (Supplementary Fig. 2a-f). In males, body weight was numerically decreased (*P* = 0.163) while lean mass was significantly reduced with mAb treatment (Supplementary Fig. 2g–h; *P* = 0.006), without effects on adiposity (Supplementary Fig. 2i), while energy expenditure was slightly reduced, without affecting food intake (Supplementary Fig. 2j–k; *P* ≤ 0.05). An increase in the respiratory exchange ratio (RER), indicative of increased carbohydrate utilization, was detected in mAb-treated males (Supplementary Fig. 2i; *P* ≤ 0.05). However, mAb treatment did not perturb glucose homeostasis (Fig. 2a–d) or insulin levels (Fig. 2e, g) in males or females. *L2-Cmu* led to a modest, numerical increase in circulating IGF-1 levels in older females (Fig. 2f; main effect *P* = 0.097), while IGF-1 levels in males were unaffected by treatment or age in this strain (Fig. 2h).

Interestingly, mAb treatment prevented the age-related rise in hypothalamic IGF-1R levels in both sexes, while resulting in reduced cortical IGF-1R levels in males only (Fig. 2i, j; *P* ≤ 0.05),
without significant effects on IGF-1R levels in lung or pancreas (Fig. 2i, j). Meanwhile, InsR levels were unaffected by mAb treatment in all tissues examined for either sex (Fig. 2k, l). Likewise, no effect was observed on downstream components of the Insulin/IGF-1 signaling pathway with mAb treatment in females (Supplementary Fig 3), but L2-Cmu attenuated S6 activation in male hypothalamus (Supplementary Fig 4c; Supplementary Fig 4d). We next evaluated effects on functional healthspan domains related to neuromuscular and physical performance following 5–6 mo of mAb treatment. With aging, females (Fig. 3a–c) and males (Fig. 3d–f) demonstrated characteristic declines in endurance, strength, and motor coordination. However, as compared to Old Con females, age-matched L2-Cmu-treated females had ~50% greater exercise tolerance (Fig. 3a; P ≤ 0.05), a two-fold increase in grip strength (Fig. 3b; P ≤ 0.05), and improved gross motor coordination, by reducing the number of slips on a medium and hard difficulty balance beam (Fig. 3c; P ≤ 0.05). In males, declining exercise tolerance was modestly mitigated with mAb treatment (~28% greater than Old Con males; Fig. 3d; P ≤ 0.05), but no effect was observed on strength (Fig. 3e), and coordination was only marginally improved on a medium (Fig. 3f; P ≤ 0.05), but not hard difficulty beam (Fig. 3i).

**L2-Cmu preferentially benefits female healthspan.** We next evaluated effects on functional healthspan domains related to neuromuscular and physical performance following 5–6 mo of mAb treatment. With aging, females (Fig. 3a–c) and males (Fig. 3d–f) demonstrated characteristic declines in endurance, strength, and motor coordination. However, as compared to Old Con females, age-matched L2-Cmu-treated females had ~50% greater exercise tolerance (Fig. 3a; P ≤ 0.05), a two-fold increase in grip strength (Fig. 3b; P ≤ 0.05), and improved gross motor coordination, by reducing the number of slips on a medium and hard difficulty balance beam (Fig. 3c; P ≤ 0.05). In males, declining exercise tolerance was modestly mitigated with mAb treatment (~28% greater than Old Con males; Fig. 3d; P ≤ 0.05), but no effect was observed on strength (Fig. 3e), and coordination was only marginally improved on a medium (Fig. 3f; P ≤ 0.05), but not hard difficulty beam (Fig. 3i).

**L2-Cmu treatment improves female cardiac function.** Given the reported importance of IGF-1 signaling to the myocardium,27,28 we next assessed the effects of IGF-1R antagonism on...
cardiovascular function by echocardiography (Fig. 4). Cardiac aging in mice is characterized by a decline in diastolic function29, which we confirmed by a reduction in the E/A ratio with age in both sexes (Fig. 4a, c; P ≤ 0.05). Importantly, L2-Cmu treatment in females did not adversely affect cardiac function, but instead restored diastolic function to more youthful levels (Fig. 4a), and this was associated with a reduction in measures of left ventricular posterior wall end diastole (LVPWd; Fig. 4b; P ≤ 0.05) and cardiac fibrosis (Fig. 4c; P ≤ 0.05). However, unlike a recent report where constitutive loss of IGF-1Rs specifically in the myocardium of male mice prevented age-related alterations to the heart19, late-life mAb treatment failed to preserve or restore these same parameters in male animals (Fig. 4e-g).

Because rapamycin, which extends mouse lifespan31, can reverse age-related diastolic dysfunction and restore a more youthful metabolome in the aged myocardium32, we next evaluated metabolomic changes in Young, Old Con, and Old mAb female-treated hearts. We observed that more than one-third of metabolites detected in heart were significantly altered with aging, which was predominantly characterized by a rise in the level of glycerophospholipids, and to a lesser extent, acylcarnitines (Fig. 4d; P ≤ 0.05). However, mAb treatment tended to oppose age-related changes in 30 of these metabolites (22 marginally significant after FDR correction), including a reduction in the age-related rise of many glycerophospholipids, and a distinct clustering of metabolites from heart tissue in Old Con versus Old mAb-treated animals by PCA (Supplementary Fig. 5), resulting in a more youthful metabolomic signature in heart.

Sexually-dimorphic effects of L2-Cmu on inflammatory markers. A rise in pro-inflammatory mediators is a hallmark of aging, thus we evaluated plasma inflammatory markers using a 25-plex immunoassay to determine if these parameters were affected by mAb treatment in male and female mice. Aging in females was
characterized by a significant rise in IL-1β, IL-4, IL-5, IL-6, IL-10, IL-12(p40), IL-12(p70), IL-17, CXCL-10, CXCL-1, MIP-1α, MIP-2, and TNFα, but several of these cytokines and chemokines were restored to a more youthful level with mAb treatment (Fig. 5a; Supplementary Table 6; P ≤ 0.05). In contrast, only G-CSF, IL-6, and RANTES were elevated in old male plasma, but mAb treatment led to a marked increase in the majority of these markers (Fig. 5b; Supplementary Table 7; P ≤ 0.05), demonstrating a clear exacerbation of systemic inflammatory status in male mice.

**L2-Cmu reduces cancer and improves survival in females.** In a 6 mo interim intervention trial with L2-Cmu in older mice, we performed an extensive histopathologic analysis and noted a reduction in endometrial hyperplasia severity (Supplementary Fig. 6a; P ≤ 0.05), whereas tumor burden in males tended to be increased (Supplementary Fig. 6b; P = 0.07). Furthermore, early indications of potentially improved survival to 24 mo was observed in female mice treated with L2-Cmu (Supplementary Fig. 6c; P = 0.41), while male interim survival with L2-Cmu was indistinguishable from Controls (Supplementary Fig. 6d; P = 0.77). Thus, to definitively determine if late-life pharmacologic modulation of IGF-1R signaling could improve survival, we performed a longevity study in female mice with lifelong i.p. injections of L2-Cmu once per week, beginning at 18 mo of age until death. As can be observed in this larger cohort (n = 45 group), long-term L2-Cmu treatment tended to reduce female body weight (Fig. 6a; P = 0.056) and lean body mass (Fig. 6b; P = 0.052), with no effect on adiposity (Fig. 6c). It is unclear to what extent the reduction in lean mass is attributable to loss of skeletal muscle mass per se, which comprises only one component of total body lean mass in mice, but the lack of effect on fat mass is consistent with the reported paucity of IGF-1Rs on mature adipocytes. Importantly, late-life L2-Cmu treatment improved mean lifespan (P = 0.023) and increased median lifespan by 9% (P = 0.03) in females (Fig. 6d), while the risk of death with late-life L2-Cmu treatment was 62.2% (40.7%, 95.2%) of that observed for controls (P = 0.029). Furthermore, end-of-life pathology confirmed that deaths due to cancer were significantly reduced, while those attributable to unknown causes were increased by mAb treatment (Table 1; P ≤ 0.05), an observation that is consistent with pathologic assessments in caloric-restricted and long-lived GHRKO mice33. However, despite improved survival and less cancers with mAb treatment, no significant effect was observed on maximum lifespan (P = 0.971).

**Discussion**

Across nature, diminished growth factor signaling is linked to improved longevity15. Importantly, this relationship is relevant to humans as individuals with exceptional longevity are enriched with functional IGF-1R mutations33, while low IGF-1 levels predict better survival in female nonagenarians16. Given the clear relationship between IGF-1 and aging, we reasoned that IGF-1R mAbs could provide a translational tool to mimic the beneficial effects reported by reduced signaling in this pathway. Here, we provide the first evidence of improved healthspan and age-related survival with a therapeutic mAb by demonstrating that treatment with an IGF-1R antagonist in older mice significantly and preferentially improves several indices of healthspan, reduced death
Fig. 4 L2-Cmu prevents age-related diastolic dysfunction in females. a-c Cardiac aging in CB6F1 female mice is characterized by a decline in diastolic function (E/A ratio), increased LVPWd, and accumulating amounts of fibrosis in the myocardium. However, 6 mo L2-Cmu treatment, beginning at 78 wks of age, was able to preserve diastolic function, reduce LVPWd measures in females [Young (n = 6), Old Con (n = 8), and Old mAb (n = 7)], and largely prevented the accumulation of cardiac fibrosis [Young (n = 4), Old Con (n = 5), and Old mAb (n = 6)]. d Metabolomic analysis of heart tissue in female mice [Young (n = 7), Old Con (n = 8), and Old mAb (n = 7)] revealed that the young and aged metabolome are distinct (see also Supplementary Fig. 5), and predominantly characterized by differences in glycerophospholipids, and to a lesser extent ceramides, while amino acids, biogenic amines, and acylcarnitines were largely unaffected. However, mAb treatment tended to oppose the age-related alterations in several metabolites, including the rise of several glycerophospholipids, resulting in a more youthful metabolomic signature in heart. Further metabolomic data analyses can be found in in Supplementary Fig. 5 and the dataset here: [https://doi.org/10.17605/OSF.IO/8QGX9]. e-g Remarkably, the favorable effects of L2-Cmu in males were absent for E/A ratio [Young (n = 5), Old Con (n = 6), and Old mAb (n = 5)] and fibrosis [Young (n = 8), Old Con (n = 8), and Old mAb (n = 8)], suggesting that the beneficial effects of modulating IGF-1 signaling in heart is specific to females. Bars represent mean ± s.e.m. Dot plots overlaid on bar graphs represent individual data points. Different letters denote a significant difference between groups by Tukey HSD, P ≤ 0.05 from neoplastic disease, and increased mean and median lifespan in females. Similar to rapamycin31, these effects were achieved even though not initiated until later in life, which we reasoned to be a safer therapeutic window for IGF-1R modulation than younger ages34.

In agreement with previous evidence from genetic models, these data show that chronic modulation of IGF-1R signaling may be most well suited for targeting aging in females6-8, rather than males. Such an indication is unique from most other drugs and compounds identified by the NIA-supported Intervention Testing Program (ITP) to improve lifespan, as acarbose, 17-α-estradiol, non-dihydrргuairetic acid (NDGA), and protandim, all preferentially improve male lifespan35,36. The potential explanation for sex differences in the IGF-1 signaling pathway on aging as well as response to other age-delaying interventions is unclear, but unique interactions of candidate pathways and targets with
sex hormones could explain in part these disparate effects. Likewise, sex differences in the way some of these drugs are absorbed and metabolized has been noted\(^\text{15}\), though we did not observe any differences in L2-Cmu PK or exposure levels between male and female animals.

Beyond the demonstration that pharmacologic blockade of IGF-1R signaling can favorably impact lifespan, these data also provide new insights into temporal and metabolic aspects regarding this pathway and aging. As previously mentioned, examples of longevity resulting from disruption of the GH/IGF-1 and/or insulin signaling pathway are mainly derived from early life manipulations\(^\text{17,18}\), whereas brief exposure to GH (and IGF-1) treatment early in development is sufficient to partially abrogate the exceptional longevity of Ames Dwarf mice\(^\text{20}\). In both male and female GHRKO mice, disruption of the GH/IGF-1 axis from birth results in mice that are remarkably long lived, but temporal disruption of this pathway in young adults (aGHRKO mice) improves lifespan only in females\(^\text{17}\), an observation which is consistent with the beneficial effects of targeting IGF-1R signaling post-development to female healthspan and aging in this study. Interestingly, adult mice with modest isolated elevations in GH and IGF-1 levels have greater lean mass, improved lipid oxidation and glucose clearance\(^\text{38}\), while specific, temporal reductions in GH and IGF-1 in adults have been linked to deleterious metabolic effects, including impaired hepatic insulin action\(^\text{39}\), and reduced β-cell mass and function\(^\text{40}\). Thus, specific targeting of IGF-1R signaling post development may prove to be the best approach to achieve the benefits of low growth signals on healthspan and aging, without the potential unwanted side effects of combined adult-onset GH/IGF-1 deficiency.

While identifying the optimal therapeutic window will require further investigation, we reasoned that starting later in life, a time in which function of the somatotropic axis is diminished, would be the best approach. Indeed, a rise in IGF-1 levels have been observed in multiple studies of IGF-1R blockade, which is attributed to the reduction in IGF-1-mediated inhibition of GH secretion\(^\text{36,40}\). For instance, administering IGF-1R mAbs in cancer trials was reported to result in increased adverse events in younger populations\(^\text{36}\), including insulin resistance, possibly due in part to disruption of the somatotropic axis and associated deleterious effects of excess GH\(^\text{11,12}\). However, we did not observe significant differences in circulating IGF-1 levels with mAb treatment in older mice, though levels tended to be higher in mAb-treated females, but glucose homeostasis was not adversely

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**Fig. 5** Sex differences in inflammatory markers with IGF-1R mAb treatment in aged mice. **a, b** A 25-plex cytokine/chemokine panel was performed on plasma obtained from female [Young (n = 8), Old Con (n = 15), and Old mAb (n = 16)] and male mice [Young (n = 8), Old Con (n = 14), and Old mAb (n = 16)]. Data were treated as nonparametric values and analyzed by the Kruskal-Wallis procedure and the Mann-Whitney U-test when appropriate. Any value below the lower limit of detection of the assay was replaced by the minimal detectable concentration (MOD)/√2 for the specific analyte. Therefore, undetectable values were treated as a tie for purposes of statistically ranking data. For generation of the heatmaps, values were normalized against Young Controls and log transformed. Group mean ± s.e.m. for all analytes are provided in Supplementary Tables 6, 7 and raw data found here: [10.17605/OSF.IO/BOQGX9]. In females, several inflammatory mediators, including IL-6, IL-12p-40, and MIP-1α, were significantly increased with aging, but were largely restored to more youthful levels with mAb treatment. Meanwhile, systemic inflammation in old male mice was markedly exacerbated by mAb treatment, as indicated by a marked increase in the majority of measured analytes over age-matched controls. *P < 0.05 for Old mAb versus Old Controls.
affected in either sex, suggesting minimal disruption of the somatotropic axis in this older cohort of mice.

Interestingly, a recent study examining the time-dependent effect of low IGF-1 levels on aging outcomes observed that a targeted reduction in liver-derived IGF-1 early (10 d or 5 mo) or later in life (15 mo) improved aspects of lifespan in females, though these effects were most pronounced when IGF-1 was reduced at 5 mo old. In contrast, male lifespan tended to be reduced by early-life IGF-1 deficiency, which is in agreement with a prior study in LID mice. Therefore, these findings further support the observation that low IGF-1 preferentially benefits females, and suggest that a potentially broad therapeutic window to target IGF-1R signaling may exist in females, with greater benefit possible by initiating at even younger ages than implemented in this study. Further optimization of the timing, dose, and duration of IGF-1R mAb treatment is warranted in order to maximize benefits while minimizing risks, particularly given recent evidence that transient administration of other drugs can have persistent benefits on age-related outcomes.

Beyond the observed increase in female lifespan, we show that pharmacologic blockade of IGF-1R signaling preserves, to various degrees, many aspects of healthspan, including neuromuscular function and endurance, and attenuates the age-related increase in diastolic dysfunction, systemic inflammation, and neoplasias in females. The observed sex dimorphism in inflammatory cytokines was a particularly intriguing and unexpected observation, which beyond serving as a marker of aging status, could be reflective of the relative increase and decrease in neoplasms, documented in male and female mice, respectively. However, it is not clear what contributed specifically to this effect on inflammation, though increasing evidence suggests that IGF-1 signaling is critical for maintaining immune cell homeostasis, but the prospects of sex differences in the requirement for IGF-1 signaling in these cells is not yet known and will require further study.

While the beneficial effects of low IGF-1 action on aging seems well supported by data in model systems and unique human populations, a broader examination of the literature suggests that a more nuanced, complex, and controversial relationship exists between IGF-1, aging, and disease, which is indicative of the pleiotropic actions attributed to IGF-1 in cells and tissues. Certainly, an increase in IGF-1 action is associated with greater risk for neoplastic disease, a link further supported by this study and others targeting IGF-1Rs, but IGF-1 has been paradoxically associated with protection against osteoporosis, type 2 diabetes, cerebrovascular and cognitive decline in pre-clinical studies, as well as in some human epidemiologic studies. Therefore, both temporal and spatial considerations of IGF-1R modulation will need to be considered in future trials with these drugs in order to clearly define the benefits and potential risks associated with this treatment, and guide development of strategies that allow for safe and effective long-term human use. Finally, while the focus of this investigation has centered on effects of inhibiting IGF-1 binding to the IGF-1R and HybridRs, L2-Cmu also interferes with IGF-2 binding to these receptors. However, as opposed to adult humans,
where IGF-2 levels are substantial, systemic IGF-2 levels are undetectable in adult rodents. Thus, the inability to account for inhibition of IGF-2 binding to the IGF-1R is an important limitation in this study, which should be considered when assessing outcomes from human trials using IGF-1R mAbs.

In summary, these data provide evidence that long-term IGF-1R blockade with mAbs is feasible, well tolerated, and can recapitulate effects observed with genetic disruption of IGF-1R signaling on lifespan. As IGF-1R mAbs are already in clinical trials, these observations warrant further exploration into the possible development of these drugs for safe and effective long-term use in humans as a strategy to delay at least some manifestations of aging. Finally, these observations provide a unique example of an intervention which appears to favor female healthspan and lifespan, thereby reinforcing the need for considering sex differences in devising therapeutic strategies to treat aging and its diseases.

Methods

Animals. Young (4 mo) and old (18 mo) male and female C57B1/6J mice were obtained from the Jackson Laboratory, Bar Harbor, ME. All animals were housed under standard temperature (22°C) and humidity-controlled conditions under a 14L:10D photoperiod and provided ad libitum access to water and a low-fat-puriﬁed diet upon arrival (10% calories from fat DiD2450H Research Diets Inc.). In vivo L2-Cmu validation studies were performed in 4–5-mo-old C57BL/6J male mice (Jackson Labs, Bar Harbor, ME). All experiments were approved by the Institutional Animal Care and Use Committee at the Albert Einstein College of Medicine (Protocol #20140107).

L2-Cmu development and validation. To target IGF-1R action in mice, we utilized the mAb, L2-Cmu (Amgen Inc, Thousand Oaks, CA), which is a murinized IgG1 version of the fully human L2-C mAb previously reported by Calzone et al. 25. Specifically, L2-Cmu was engineered as a human/murine chimera (i.e., reverse chimerism) so that the complementarity-determining regions (CDRs) were engineered into a new framework in which the entirety of the variable regions are human and the constant regions are mouse. Unlike the human IGF1 constant region of L2-C, which might induce effector functions, the murine IGF1 constant region would not be expected to elicit effector function activity. Validation of L2-Cmu was conﬁrmed by Biacore analysis and in murine fibroblasts (NIH-3T3) in vitro. For NIH-3T3 experiments, cells were grown in DMEM plus 10% fetal bovine serum (Invitrogen). At t = −4 h, cells were serum starved and at t = −1 h, pre-treated with vehicle, control IgG1, or L2-Cmu (100 µg/mL). Vehicle IGF1 (5 nM) or IGF2 (20 nM) was then added to the media for 2 min and cells were then rapidly harvested in ice, washed with PBS, and a 75% ethanol wash at −20°C. ITTs were performed in randomized-fed mice, early in their light cycle (~0700–0800h). Following a baseline glucose measurement, mice were injected i.p. with 0.75 U/kg insulin and blood glucose was measured at 15, 30, 45, and 60 min later.

Energy expenditure, substrate utilization, food intake, and spontaneous activity were assessed using an Oxymax for determining chronic exposure levels, plasma was isolated accordingly. Left ventricular diastolic function, presented as the E/A ratio, was measured at 15, 30, 45, and 60 min later.

Metabolic phenotyping. Body weight was monitored on a weekly basis and body composition was assessed at 2 mo intervals by qMR (ECHO MRS; Echo Medical Systems). Glucose tolerance tests (GTT) and insulin tolerance tests (ITT) were performed (n = 12 per group, per sex) to assess glucose metabolism and insulin sensitivity 46. For GTTs, animals were fasted for 4 h and a baseline blood glucose measurement was made prior to administering a 2 mg/kg i.p. glucose injection. Blood glucose was subsequently monitored at 15, 30, 60, 90, and 120 min post injection with a glucose meter (Accu-Check Contour). ITTs were performed in randomized-fed mice, early in their light cycle (~0700–0800h). Following a baseline glucose measurement, mice were injected i.p. with 0.75 U/kg insulin and blood glucose was measured at 15, 30, 45, and 60 min later.

For in vivo validation of L2-Cmu blockade, C57BL/6J mice were sedated with 100 mg/kg of ketamine (DOSItek, Inc., London, ON) and xylazine (10 mg/kg) followed by 5 min at a walking speed (8 m/min). Animals were then challenged with a graduated exercise test to voluntary fatigue on a motorized treadmill (Exer 3/6, Columbus Instruments) in females [Young (n = 10), Old Con (n = 17), and Old mAb (n = 22)] and males [Young (n = 8), Old Con (n = 10), Old mAb (n = 9)]. Mice were ﬁrst familiarized to the treadmill for three non-competitive days for 5 min at a speed of 8 m/min, followed by a 2-min run at 8 m/min. Mice were then exposed to a maximal exercise test to voluntary fatigue on a motorized treadmill (Exer 3/6, Columbus Instruments) in females [Young (n = 10), Old Con (n = 17), and Old mAb (n = 22)] and males [Young (n = 10), Old Con (n = 10), Old mAb (n = 9)]. Mice were ﬁrst familiarized to the treadmill for three non-competitive days for 5 min at a speed of 8 m/min, followed by a 2-min run at 8 m/min. Mice were then exposed to a maximal exercise test to voluntary fatigue on a motorized treadmill (Exer 3/6, Columbus Instruments) in females [Young (n = 10), Old Con (n = 17), and Old mAb (n = 22)] and males [Young (n = 10), Old Con (n = 10), Old mAb (n = 9)]. Mice were ﬁrst familiarized to the treadmill for three non-competitive days for 5 min at a speed of 8 m/min, followed by a 2-min run at 8 m/min. Mice were then exposed to a maximal exercise test to voluntary fatigue on a motorized treadmill (Exer 3/6, Columbus Instruments) in females [Young (n = 10), Old Con (n = 17), and Old mAb (n = 22)] and males [Young (n = 10), Old Con (n = 10), Old mAb (n = 9)]. Mice were ﬁrst familiarized to the treadmill for three non-competitive days for 5 min at a speed of 8 m/min, followed by a 2-min run at 8 m/min. Mice were then exposed to a maximal exercise test to voluntary fatigue on a motorized treadmill (Exer 3/6, Columbus Instruments) in females [Young (n = 10), Old Con (n = 17), and Old mAb (n = 22)] and males [Young (n = 10), Old Con (n = 10), Old mAb (n = 9)]. Mice were ﬁrst familiarized to the treadmill for three non-competitive days for 5 min at a speed of 8 m/min, followed by a 2-min run at 8 m/min. Mice were then exposed to a maximal exercise test to voluntary fatigue on a motorized treadmill (Exer 3/6, Columbus Instruments) in females [Young (n = 10), Old Con (n = 17), and Old mAb (n = 22)] and males [Young (n = 10), Old Con (n = 10), Old mAb (n = 9)].
methanol, and 20 µL of the extraction from each sample was used for the assay. A pooled quality control (QC) sample was added to the sample list. This QC sample was plated at different dilutions on the 96-well plate and injected six times to calculate the coefficient of variation (CV) for data quality control, and undetectable metabolites were excluded from the analysis. The dataset was then imported into R software [R version 3.4.2] and normalized using log transformation for multivariate analysis, unsupervised principle component analysis (PCA), and partial least squares-discretize analysis (PLS-DA).

**Histopathology.** Complete histopathology was performed in 24-mo-old female [Old Con (n = 16), and Old mAb (n = 16)] and male mice [Old Con (n = 15), and Old mAb (n = 17)] following 6 mo of mAb treatment, as well as in female mice at death from the longevity study [Old Con (n = 30), and Old mAb (n = 26) in brief, a gross evaluation was conducted when possible and then a complete necropsy was performed. Tissues were inflated with paraffin and HE sections were obtained. Slides were shipped to the University of Texas at San Antonio Pathology Core and evaluated by two pathologists who were blinded to the experimental groups (Y.L and G.B.H.). Diagnosis of each histopathological change was made using histological classifications for aging mice45,46. In brief, a list of lesions was compiled for each mouse that included both neoplastic and non-neoplastic diseases. Based on these histopathological data, tumor burden, disease burden, and severity of lesions in each mouse were assessed.

Tumor burden was calculated as the sum of the different types of tumors in each mouse. The disease burden was similarly calculated as the sum of the histopathological changes in a mouse and severity of neoplastic and renal lesions was assessed using an established grading system. The probable cause of death was determined independently by both pathologists based on the severity of the pathology found at necropsy. In cases with neoplastic lesions, mice with Grade 3 or 4 lesions were categorized as death by neoplastic disease. In more than 90% of cases, this was agreed upon by both pathologists. In cases where the two pathologists did not agree or where disease did not appear severe enough, the cause of death was categorized as unknown.

**Blood measures.** Clinical blood chemistry and related measures were determined in whole blood and serum of female [Old Con (n = 7) and Old mAb (n = 8)] and male mice [Old Con (n = 7) and Old mAb (n = 8)] by Antech Diagnostics (New Hyde Park, NY). Basal insulin was measured in plasma from female [Young (n = 16), Old Con (n = 26), and Old mAb (n = 25)] and male mice [Young (n = 17), Old Con (n = 32), and Old mAb (n = 28)] using a bead-based assay for mouse insulin (EMD Millipore, Inc) and detection was performed on a Bio-Plex MAGPIX Multiplex Reader (Biorad, Hercules, CA). Plasma IGF-1 levels were measured using the Mouse/Rat IGF-1 Quantikine ELISA Kit (MG100; R&D Systems) in plasma from female [Young (n = 8), Old Con (n = 16), and Old mAb (n = 15)] and male mice [Young (n = 8), Old Con (n = 16), and Old mAb (n = 16)]. In addition, a MAGPIX Multiplex Reader was used to measure 25 inflammatory mediators simultaneously in plasma from female [Young (n = 8), Old Con [HSD] method) were applied. Normality assumption was examined prior to analysis of variance for each individual measure.

**Immunoprecipitation.** For immunoprecipitation assays, NIH-3T3 cell or tissue protein, respectively, was extracted with a non-denaturing cell or tissue extraction buffer (Invitrogen/ThermoFisher, Carlsbad, CA). Immunoprecipitation was then performed using the Catch and Release Immunoprecipitation Kit (EMD Millipore), according to the manufacturer’s instructions with 10 µg of total protein from cells or 400 µg from tissues, and 1 µg of an anti-IGF-1R antibody (#9750, Cell Signaling). Following electrophoresis and transfer, membranes were blotted with either a pTyr antibody (1:1000; #8954, Cell Signaling) for IGF-1R activation, or an anti-IGF-1R antibody (#9758, Cell Signaling) for total levels. For IGF-1R/InsR HybridR activation and regulation of longevity and stress resistance by Sch9 in yeast. Science 292, 288–290 (2001).

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