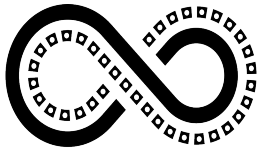

NORN GROUP

NIA Interventions Testing Program

Presented by Madison Ueland on 1/30/2022

Discussion Summary

Paper(s): https://www.nia.nih.gov/research/dab/interventions-testing-program-itp

Roadmap

1. Overview
2. Origins of the ITP
3. Testing strategy + SOPs
4. Criteria for drug selection
5. Notable findings

1. Overview

Core features of the ITP

- Three geographical sites
  - University of Michigan (Richard Miller)
  - Jackson Lab (David Harrison)
  - University of Texas (Randy Strong)
- Genetically heterogeneous mice
- Test in both sexes
- Statistical power: 80% for a 10% change in mean lifespan, in either sex, with data pooled from two or three sites
- Anyone can propose interventions
  - Must be single drug, dietary

2. Origins

September, 1999 NIA workshop

Motivation: Identify promising interventions in animals that might lead to clinical trials in humans

Historical context:

- CR only intervention widely accepted to extend maximum lifespan of mammals
  - CR known to extend lifespan in rats, mice, *C. elegans*… rhesus monkeys ongoing.
- Progress in identifying lifespan genes in invertebrates, less knowledge about mammals
  - Recall: Cynthia Kenyon *C. elegans* mutants from 1993
- Sporadic lifespan extending interventions in literature, but many compromised by design flaws
  - Too few animals, failure to control for possible CR, use of wrong animal model, poor housing conditions
- Little pathology or other biomarker assessment
- Infrequent publishing of negative results

Choice of animal model

Criteria in animal model selection (1999 workshop)

1. **External validity**: relevance to human aging
   - **Multiple causes of death**
2. **Inexpensive:** require large sample size for statistical power
   - **Relative ease of husbandry**
3. **Reproducible variability:** attain genetic diversity while maintaining reproducible genetic background for further studies. Test both genders.
4. **Responds to CR:** “CR is the only proven method to extend life span, any useful test model should be affected by CR”

Considered: dogs, birds, non-human primates, fruit flies or nematodes (for large scale screening), mice/rats

Choice of animal model (cont.)

- Inbred, genetically homogeneous mice have shorter lifespans and strain specific pathologies
- Four way cross mice enables reproducible genetic diversity - mice are sibs sharing a random 50% of their genome

Choice: UM-HET3 mice (cross of four inbred strains, generated by breeding two F1 hybrids, CB6F1 and C3D2F1)

Triplicate testing

- Arguments for multiple sites:
  - **Confidence** gained from obtaining similar results at 2-3 sites
  - **Specialized expertise** of each site
    - e.g. statistical analysis, pharmacokinetics, toxicology, optimal diet compounding
- Increased cost of each successful intervention
  - Offset by savings after phase I trial and ability to use fewer mice per tested compound per site

- Now we see **replicable, site-to-site differences despite standard operating procedures** (SOPs) illustrating importance of triplicate testing
  - **Lifespan**: Control male mice at UM consistently live longer than those at other two sites (little variation among females)
  - **Body weight**: Control mice of both sexes have lower body weight at UM than other two sites
- ITP has become a model for multi-site collaborative projects

3. Testing strategy and SOPs

Program structure

- Two stage program (+ pilot studies)
  - **Pilot studies:** Stability, bioavailability (blood levels after short term treatment), bioactivity (effect from short-term treatment), toxicity
  - **Stage I:** lifespan as primary endpoint
  - **Stage II:** follow-up on positive findings with further lifespan studies, dose-response studies, pathology and other biomarker analysis
- Risk of Type I errors
  - Could miss interventions that increase healthspan but do not change mortality outcomes in mice.
  - Deliberate trade-off. Inclusion of health-related outcomes in Stage I would reduce number of compounds tested per year

Standard Operating Procedures

- Some aspects easy to standardize, others harder
  - Easier: light/dark cycles, diet, pathogen control and choice of bedding
  - Harder: intestinal flora, light quality, minerals and organic compounds in water and air
- Mice protocol developed to detect 10% change in mean lifespan with 80% power with as few as two sites (i.e. even if systematic failure in one site)
  - 44 males and 36 females treated per site (more males due to expected losses from fighting)
  - Control group double size of test group (88 males, 72 females per site)
- Other
  - Mice for each year are bred over 6-8 months to reduce cohort effects
  - First litter is not used for lifespan studies to minimize effects of variation in early life nurturing
  - All sites use same batch of food
  - Body weight measured at six month intervals
  - Cages checked daily and mice are euthanized when they are classified as moribund (unlikely to survive 2 days)

Age of mice

- Default to begin treatment when mice are 4 months old
- Frequent divergence from this baseline
  - Sometimes pilot studies took longer than anticipated, or issues with drug stability e.g. Rapamycin (20 months)
    - 85% of rapamycin degraded by food preparation process - required microencapsulation to deliver stable doses inside chow
  - Drugs withheld to avoid undesirable biological effects e.g. 17-alpha-estradiol (10 months)

4. Criteria for drug selection

Intervention selection

- Anyone can apply! Proposals accepted from inside and outside US, from academics, commercial entities, and individuals without institutional affiliations.
- Two-stage review process:
  - Access Panel: independent review on scientific rationale and feasibility
  - Steering Committee: prioritizes proposals, advises on general protocol issues
- 10-15 proposals received per year, ~5 picked ($500k costs per intervention)
- Drugs, food or nutritional supplements, antioxidants, plant extracts etc

Intervention selection (2)

Core criteria: easily obtainable, reasonably priced, and can be delivered in the diet or water.

Nice to haves:

- **Preliminary data in mammalian models**: improves likelihood of acceptance since ITP has limited funding for dose-response studies
- **Human clinical evidence** or FDA approval = lower barriers to evaluate in humans

Reasons for rejection:

- **Feasibility:** proposals that require daily injections (may accept injections if administered for short periods of time or at infrequent intervals), gavage, chemically defined diets, removing components of diets
- **Rapid metabolism** e.g. trimethadione (approved in humans, lifespan extension in worms, but rapidly metabolised to dimethadione in mice)
- **Toxicity or instability** in food preparation or storage
- **IP issues**

5. Notable findings

Positive results

- Aspirin: males only
- Rapamycin: males and females (females > males)
- 17-alpha-estradiol: males only
- Acarbose: males and females (males >> females)
- Nordihydroguaiaretic acid (NDGA): males only
- Protandim: males only
- Glycine: males and females (males = females, but small effect)
- Canagliflozin: males only

→ **many sex differences!** 5/8 positive interventions only worked in males, others had sex-specific differences in effect size. Rapamycin only drug that has shown strong signal in females.

- Not entirely explained by different blood levels. Changing dosage to achieve ~equal blood levels of rapamycin did result in similar lifespan increases among males and females, but for NDGA, even similar blood levels saw no effect in females

Question for discussion:

Why do most of the positive interventions have greater effect in male mice?

Drugs tested late in life

- Rapamycin
  - 9 months, 20 months
  - Works equally as well late in life
- Acarbose
  - 4 months, 16 months
  - Works half as well at late middle age
- 17-alpha-estradiol
  - 4 months, 16 months
  - Works equally as well at late middle age

Notable negative results

- Resveratrol
  - Did not go through usual screening process. Rich Miller said ITP was “ordered” to test by Richard Hodes, director of NIA
  - Tested at very high does at 12 months and then 4 months, no change in median or maximum lifespan
- Metformin
  - Did not extend life in mice, but did when given with rapamycin
  - Rich Miller unsurprised because (1) could be good for people not mice (2) may have used wrong dose (3) dosing schedule may need adjusting
- Aspirin
  - Wait, wasn’t this positive? Only 8-10% increase in males. Initial sponsor gave low dose recommendation (1/100th of typical human dose, body weight adjusted)
  - Later higher dose showed no lifespan extension in either sex
- Nicotinamide riboside (NR)
  - No extension in 8 month old mice
- Green tea extract, methylene blue, curcumin

Caveats: usually only one dose tested, Phase I only monitors lifespan

Main takeaways

- You can achieve significant effects by just putting compounds in food
- Most effects are **sex-specific**
  - Sex specific steroid hormones probably do something relevant
- Most lifespan extending drugs work even when started in **middle age or late life**
- No interventions have caused significant shortening of lifespan
- Molecular clues
  - mTOR matters
  - Less glucose better than more

Table of ITP results (1)

Source: https://peterattiamd.com/richardmiller/

Compound	Cohort	Year	Concentration in food (ppm)	Age at Tx initiation (mo)	Median LS extension (males)?	% inc.	*P* value	P90 LS extension (males)?	% inc.	*P* value	Median LS extension (females)?	% inc.	*P* value	P90 LS extension (females)?	% inc.	*P* value
1,3-butanediol	Cohort 13	C2017	100000	6												
17-DMAG	Cohort 11	C2015	30	6												
17α-Estradiol	Cohort 5	C2009	4.8	10	YES	12%	0.002	NO	5%	0.13	NO	0%	0.80	NO	0%	0.90
17α-Estradiol	Cohort 7	C2011	14.4	10	YES	19%	0.000	YES	12%	0.000	NO	1%	0.98	NO	0%	0.86
17αEstradiol started in mid-life - Phase II	Cohort 12	C2016	14.4	16 + 20												
4-OH-PBN	Cohort 1	C2004	315	4	NO	3%	0.24	NO	-1%	1.00	NO	-4%	0.39	NO	0%	0.86
4-Phenylbutyrate (PBA)	Cohort 15	C2019	1000	9												
Acarbose	Cohort 5	C2009	1000	4	YES	22%	<0.0001	YES	11%	<0.001	YES	5%	0.01	YES	10%	0.001
Acarbose	Cohort 8	C2012	1000	16	YES	7%	<0.0001	YES	12%	0.0003	NO	3%	0.07	NO	6%	0.06
Acarbose Phase II	Cohort 9	C2013	1000	8	YES	17%	<0.0001	YES	11%	0.0008	YES	5%	0.003	YES	3%	0.007
Acarbose Phase II	Cohort 9	C2013	2500	8	YES	16%	<0.0001	YES	8%	<0.0001	YES	4%	0.006	NO	3%	0.13
Acarbose Phase II	Cohort 9	C2013	400	8	YES	11%	<0.0001	YES	11%	0.001	YES	0%	0.03	NO	2%	0.40
Aspirin	Cohort 1	C2004	20	4	YES	8%	0.01	NO	4%	0.17	NO	-4%	0.17	NO	0%	1.00
Aspirin	Cohort 10	C2014	200	11	NO	0%	0.36	NO	2%	0.61	NO	1%	0.86	NO	1%	0.86
Aspirin	Cohort 10	C2014	60	11	NO	-1%	0.74	NO	2%	1.00	NO	1%	0.75	NO	-1%	0.73
Astaxanthin	Cohort 15	C2019	400	12												
Bile Acids - UDCA	Cohort 7	C2011	5000	5	NO	7%	0.45	NO	0%	0.87	NO	-1%	0.76	NO	1%	0.49
Canagliflozin - SGLT2 inhibitor	Cohort 12	C2016	180	7	YES	14%	<0.0001	YES	9%	<0.0001	NO	1%	0.51	NO	1%	0.50
Candesartan Cilexetil	Cohort 12	C2016	30	8												
CAPE	Cohort 2	C2005	30	4	NO	3%	0.89	NO	-3%	0.17	NO	0%	0.84	NO	-1%	0.50
CAPE	Cohort 2	C2005	300	4	NO	2%	0.80	NO	-1%	0.54	NO	5%	0.07	NO	1%	0.87
Captopril	Cohort 13	C2017	180	5												
Curcumin	Cohort 4	C2007	2000	4	NO	3%	0.85	NO	-2%	1.00	NO	5%	0.44	NO	0%	0.39
Dimethyl Fumarate (DMF) 9 + 16	Cohort 15	C2019	120	9 & 16												

Table of ITP results (2)

Source: https://peterattiamd.com/richardmiller/

Compound	Cohort	Year	Concentration in food (ppm)	Age at Tx initiation (mo)	Median LS extension (males)?	% inc.	*P* value	P90 LS extension (males)?	% inc.	*P* value	Median LS extension (females)?	% inc.	*P* value	P90 LS extension (females)?	% inc.	*P* value
Enalapril Maleate	Cohort 2	C2005	120	4	NO	7%	0.22	NO	1%	1.00	NO	-2%	0.90	NO	0%	0.49
Fisetin on + cycling (3d on/11d off)	Cohort 14	C2018	600	20												
Fish Oil	Cohort 6	C2010	15000	9	NO	4%	0.26	NO	-2%	0.86	NO	-4%	0.09	NO	-3%	0.48
Fish Oil	Cohort 6	C2010	50000	9	NO	-6%	0.22	NO	-4%	0.32	NO	3%	0.25	NO	-3%	0.48
Geranylgeranyl acetone	Cohort 12	C2016	600	9												
Green tea extract	Cohort 4	C2007	2000	4	NO	5%	0.39	NO	1%	0.31	NO	7%	0.37	NO	-1%	0.73
HBX (2-(2-hydroxyphenyl)-benzoxazole)	Cohort 8	C2012	1	15	NO	1%	0.44	NO	1%	0.33	NO	-2%	0.43	NO	-1%	0.61
Hydrogen Sulfide - SG1002	Cohort 12	C2016	240	19												
Hydrogen Sulfide - SG1002	Cohort 15	C2019	240	6												
Hydrogen Sulfide - SG1002 midlife	Cohort 14	C2018	240	18												
INT-767 FXR/TG5R agonist	Cohort 8	C2012	180	10	NO	-3%	0.11	NO	-4%	0.24	NO	-1%	0.75	NO	0%	0.30
Inulin	Cohort 10	C2014	600	11	NO	-2%	0.73	NO	0%	0.09	NO	2%	0.41	NO	1%	0.86
L-Leucine	Cohort 13	C2017	40000	5												
Meclizine	Cohort 15	C2019	800	12												
Medium Chain Triglyceride Oil	Cohort 4	C2007	60000	4	NO	-1%	0.42	NO	2%	0.40	NO	2%	0.15	NO	3%	0.23
Metformin	Cohort 7	C2011	1000	9	NO	7%	0.35	NO	-2%	0.41	NO	0%	0.79	NO	0%	0.62
Metformin + Rapamycin	Cohort 7	C2011	1000 M + 14 R	9	YES	23%	0.000	YES	10%	0.000	YES	23%	0.000	YES	17%	0.000
Methylene Blue	Cohort 5	C2009	28	4	NO	-2%	0.27	NO	-5%	0.60	NO	1%	0.17	YES	6%	0.004
MIF098	Cohort 12	C2016	240	8												
Minocycline	Cohort 11	C2015	300	6												
MitoQ	Cohort 11	C2015	100	7												
Mycophenolic Acid (MPA)	Cohort 15	C2019	6.7	9												
NDGA	Cohort 1	C2004	2500	9	YES	12%	0.0006	NO	5%	0.16	NO	1%	0.43	NO	2%	0.86
NDGA cross-section study: Females	Cohort 8	C2012	5000	13												
NDGA cross-section study: Males	Cohort 8	C2012	2500	13												

Table of ITP results (3)

Source: https://peterattiamd.com/richardmiller/

Compound	Cohort	Year	Concentration in food (ppm)	Age at Tx initiation (mo)	Median LS extension (males)?	% inc.	*P* value	P90 LS extension (males)?	% inc.	*P* value	Median LS extension (females)?	% inc.	*P* value	P90 LS extension (females)?	% inc.	*P* value
NDGA Hi_Phase II	Cohort 6	C2010	5000	6 (M & F)	YES	7%	0.003	NO	2%	0.09	NO	-4%	0.42	NO	-1%	0.59
NDGA Lo_Phase II	Cohort 6	C2010	800	6 (M only)	YES	9%	0.02	NO	1%	0.86	N/A			N/A		
NDGA Med_Phase II	Cohort 6	C2010	2500	6 (M only)	YES	12%	0.01	NO	0%	0.86	N/A			N/A		
NFP	Cohort 1	C2004	200	4	NO	4%	0.56	NO	-2%	0.88	NO	0%	0.47	NO	-2%	0.86
Nicotinamide riboside	Cohort 12	C2016	1000	8												
Oxaloacetic acid	Cohort 4	C2007	2200	4	NO	4%	0.42	NO	3%	0.61	NO	3%	0.44	NO	3%	0.73
PB125	Cohort 13	C2017	100	5												
Protandim® *	Cohort 7	C2011	600	10	YES	7%	0.01	NO	6%	0.10	NO	3%	0.29	NO	6%	0.16
Rapamycin	Cohort 2	C2005	14	20	YES	20%	<0.0001	YES	9%	<0.0001	YES	13%	<0.0001	YES	14%	<0.0001
Rapamycin	Cohort 3	C2006	14	9	YES	8%	<0.0001	YES	11%	<0.0001	YES	17%	<0.0001	YES	16%	<0.0001
Rapamycin – intermittent Phase II	Cohort 11	C2015	42	20												
Rapamycin_HiPhase II	Cohort 5	C2009	42	9	YES	22%	<0.0001	YES	8%	0.0009	YES	28%	<0.0001	YES	20%	<0.0001
Rapamycin_LoPhase II	Cohort 5	C2009	4.7	9	NO	3%	0.20	NO	6%	0.18	YES	17%	<0.0001	YES	14%	<0.0001
Rapamycin_MidPhase II	Cohort 5	C2009	14	9	YES	10%	0.003	YES	8%	0.01	YES	22%	<0.0001	YES	21%	<0.0001
Rapamycin/Acarbose - Phase II	Cohort 13	C2017	14.7/1000	9 + 16												
Resveratrol	Cohort 3	C2006	1200	12	NO	7%	0.16	NO	2%	0.25	NO	3%	0.48	YES	3%	0.04
Resveratrol	Cohort 3	C2006	300	12	NO	0%	0.95	NO	-2%	0.42	NO	3%	0.64	NO	3%	0.48
Resveratrol	Cohort 4	C2007	300	4	NO	4%	0.97	NO	-1%	0.40	NO	4%	0.87	NO	0%	0.16
Simvastatin	Cohort 3	C2006	12	10	NO	4%	0.28	NO	2%	0.11	NO	4%	0.26	NO	1%	0.49
Simvastatin	Cohort 3	C2006	120	10	NO	-4%	1.00	NO	3%	0.11	NO	2%	0.55	NO	4%	0.22
Sulindac	Cohort 13	C2017	5	5												
Supplemental glycine	Cohort 10	C2014	80000	9	YES	6%	0.002	YES	5%	0.0005	YES	4%	0.01	NO	2%	0.70
Syringaresinol	Cohort 13	C2017	300	5												
TM5441 – inhibitor of PAI-1	Cohort 10	C2014	60	11	NO	-4%	0.69	NO	2%	0.17	NO	0%	0.32	NO	-1%	0.61
Ursolic Acid (bile acids)	Cohort 9	C2013	2000	10	NO	6%	0.38	NO	0%	1.00	NO	0%	0.49	NO	-1%	0.51

Sources

[format]

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\+ Intervention papers