Inhibition of Amyloid Aggregates in *C. Elegans* as a Model for Treatment of Alzheimer's and Parkinson's Disease

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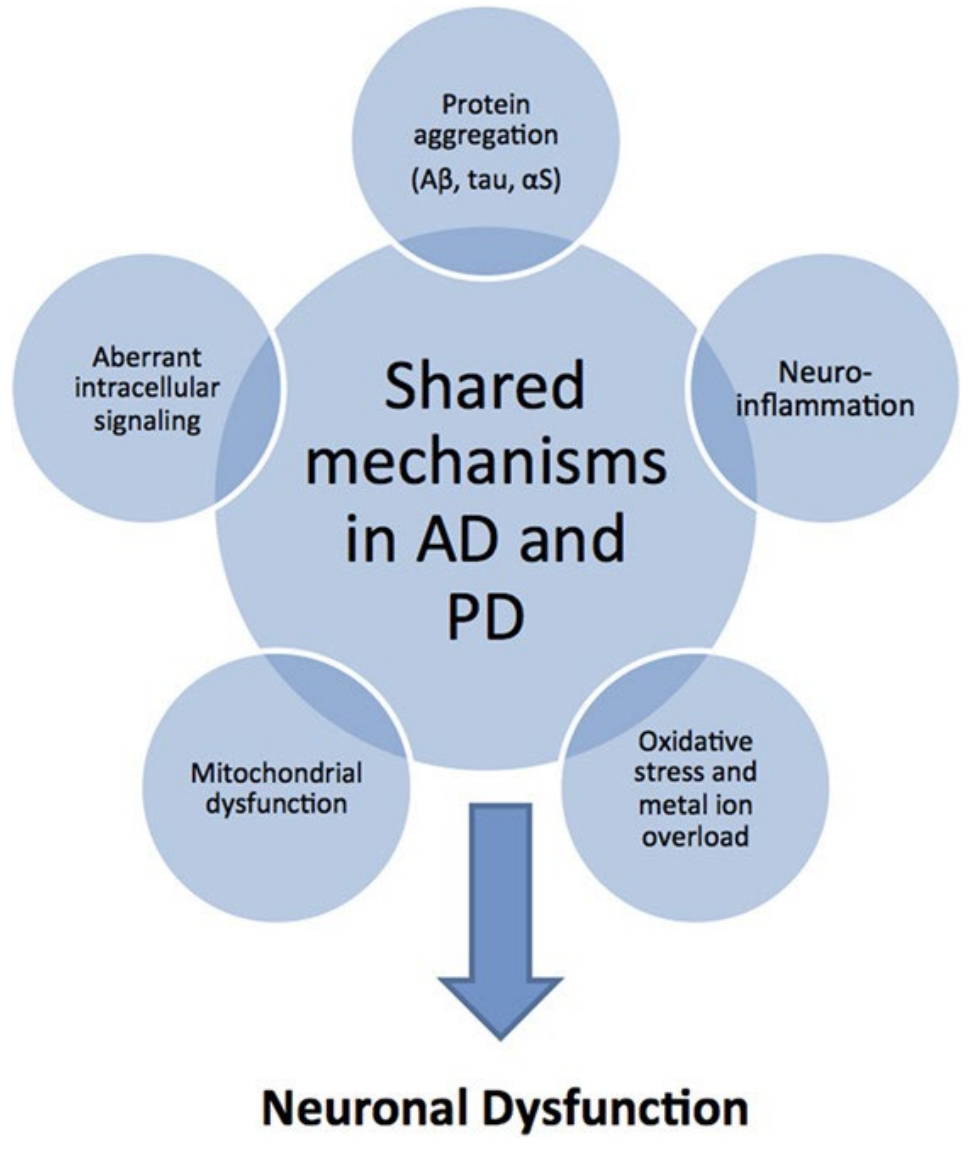
Abstract

Parkinson's Disease (PD) and Alzheimer's Disease (AD) are neurodegenerative diseases characterized by pathogenic amyloid aggregates that lead to loss of brain function, which globally affect more than 10 million[1] and 44 million[2] patients, respectively. PD is prevalent in 1% of adults over the age of 60[1] while 1 in 9 adults over 65 are diagnosed with AD[3]. Currently, these diseases cannot be cured and are therefore of high interest to scientists. This study utilizes three strains of *Caenorhabditis elegans*, a transparent nematode, as a model for the treatment of these pathologies. The GMC101 strain of genetically modified *C. elegans* overexpresses A-β 1-42 peptide in muscle cells. These peptides form amyloid aggregates over time associated with the onset and progression of AD in humans. Another strain, NL5901, overexpresses α-Synuclein peptide, leading to the aggregation of these amyloid peptides linked to PD in humans. This study applies small molecules to inhibit amyloid aggregation to slow or stop the progression of these diseases. The effectiveness of treatment is measured via 14-days motility assays of *C. elegans* strains with treatment, strains without treatment, and wild-type N2 strain serving as control. Results show that the FDA-approved anticancer drug Bexarotene inhibits A-β 1-42 peptide aggregation in GMC101 strains of *C. elegans* while the molecule SK-129, synthesized by Principal Investigator Dr. Sunil Kumar, inhibits α-Synuclein peptide aggregation in the NL5901 strains of *C. elegans*. These data suggested that future application of these treatments in human patients could hinder the development of these amyloid-related pathologies.

Keyword(s): Parkinson's Disease, Alzheimer's Disease, *C. Elegans*, Amyloid Aggregation

Background

- Parkinson's Disease and Alzheimer's Disease are neurodegenerative diseases characterized by pathogenic amyloid aggregates that lead to loss of brain function.
- This study utilizes small molecules that interfere with the nucleation step[4] of amyloid aggregation and therefore halt its progression.



https://doi.org/10.3389/fnut.2016.00031

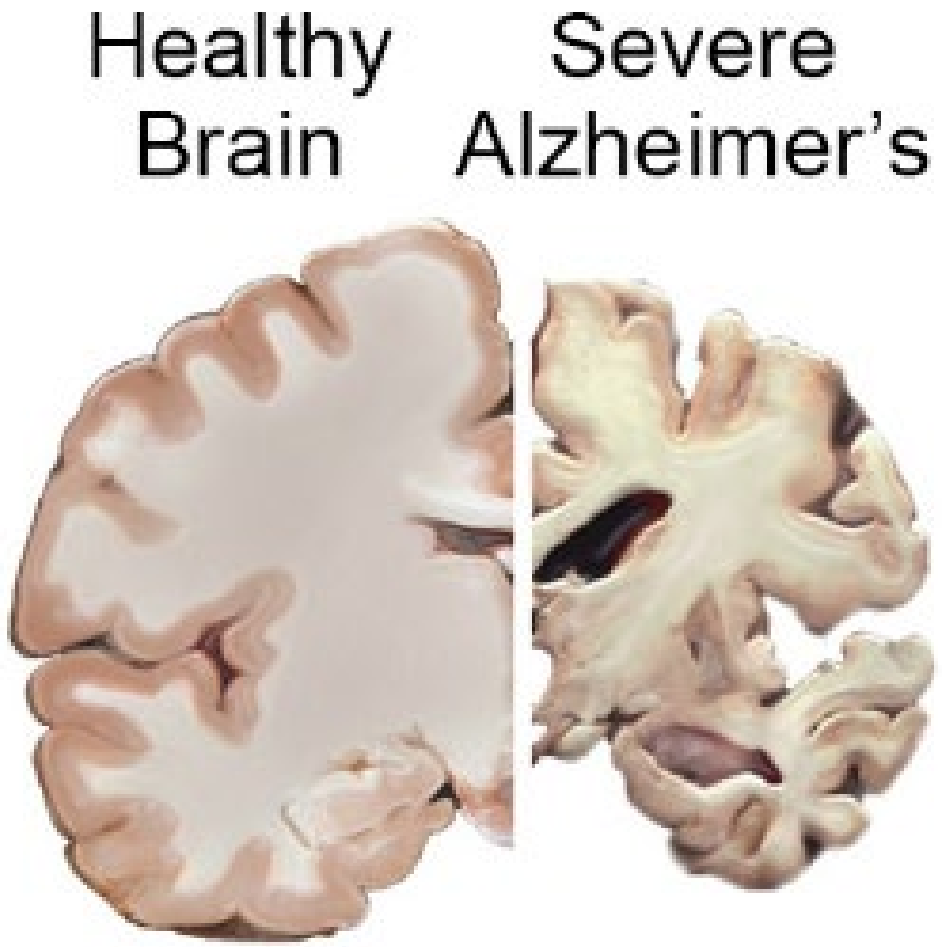
Background – Parkinson's Disease

- Parkinson's is caused by the brain's inability to produce dopamine, an essential neurotransmitter.
- Parkinson's affects more than 10 million[1] patients worldwide. It is prevalent in 1% of adults over the age of 60[1].

https://www.parkinson.org/blog/raise-awareness/spot-the-early-signs-of-parkinsons

Background – Alzheimer's Disease

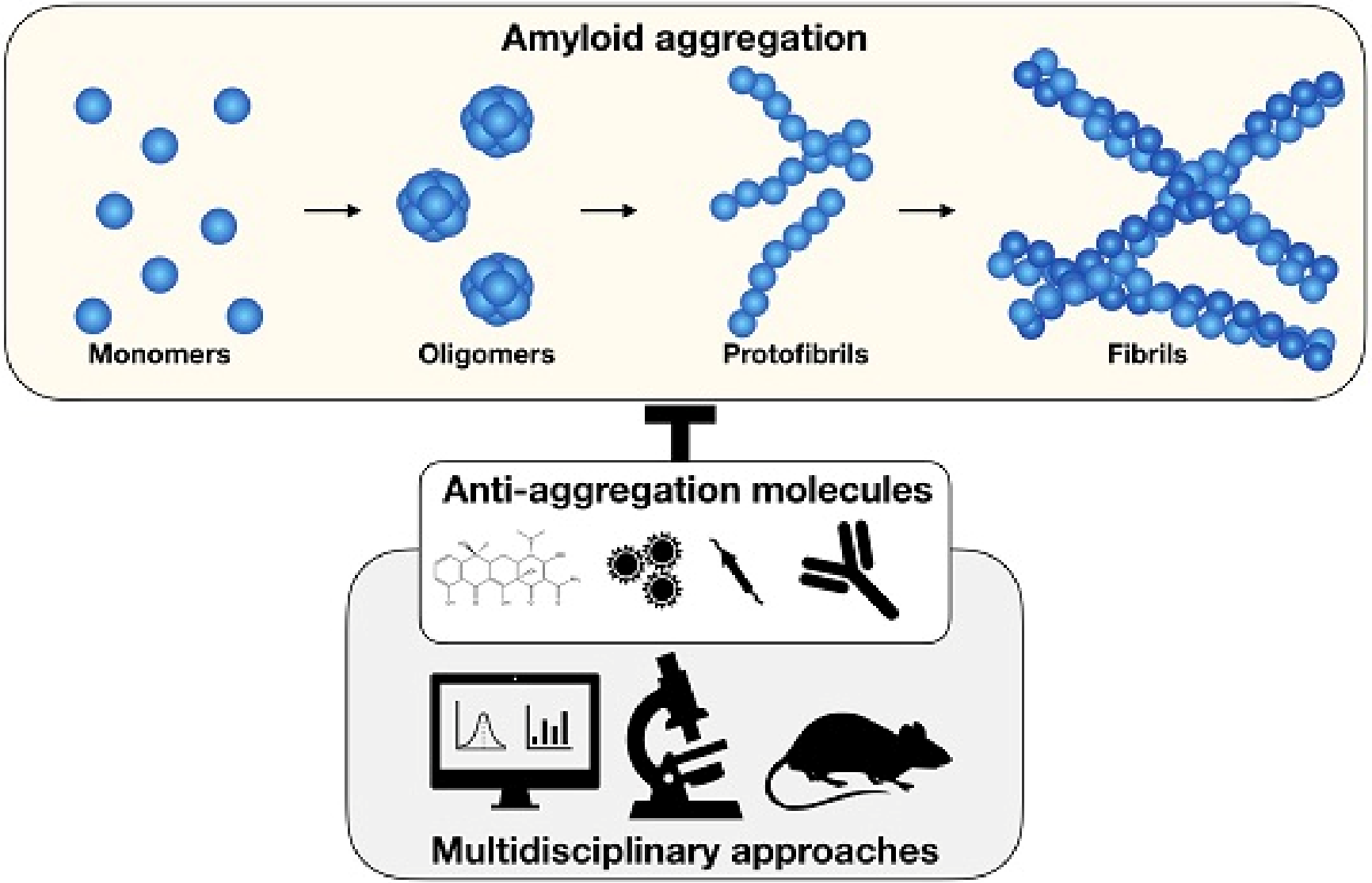
- Alzheimer's Disease is the most common form of dementia and is characterized by the progressive loss of memory and cognition.
- 44 million[2] patients globally have been diagnosed with Alzheimer's or similar forms of dementia. 1 in 9 adults (~11%) over the age of 65 are diagnosed with Alzheimer's[3].



https://www.nia.nih.gov/health/alzheimers-disease-fact-sheet

Research Questions

- What is the most effective treatment for inhibiting amyloid aggregation in *C. elegans*?
- How can we slow the onset and progression of Alzheimer's and Parkinson's Disease?



https://doi.org/10.3390/ijms19092677

Introduction

- Model organism = *C. elegans*
  - Transparent nematode about 1 mm in length
- 3 strains:

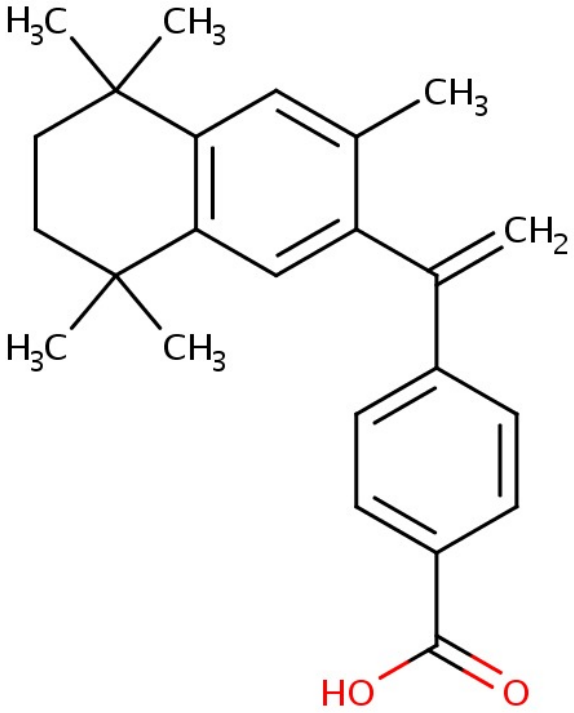
1. **N2 strain; wild type**
2. **GMC101 strain**
   - Overexpresses **A-β 1-42 peptide** and leads to these amyloid peptide aggregates linked to **Alzheimer's disease** in humans
   - Treatment with **Bexarotene** (photosensitive)
3. **NL5901 strain**
   - Overexpresses **α-Synuclein peptide** and leads to these amyloid peptide aggregates linked to **Parkinson's disease** in humans
   - Treatment with **SK-129** synthesized by Dr. Sunil Kumar et al.

Caenorhabditis elegans

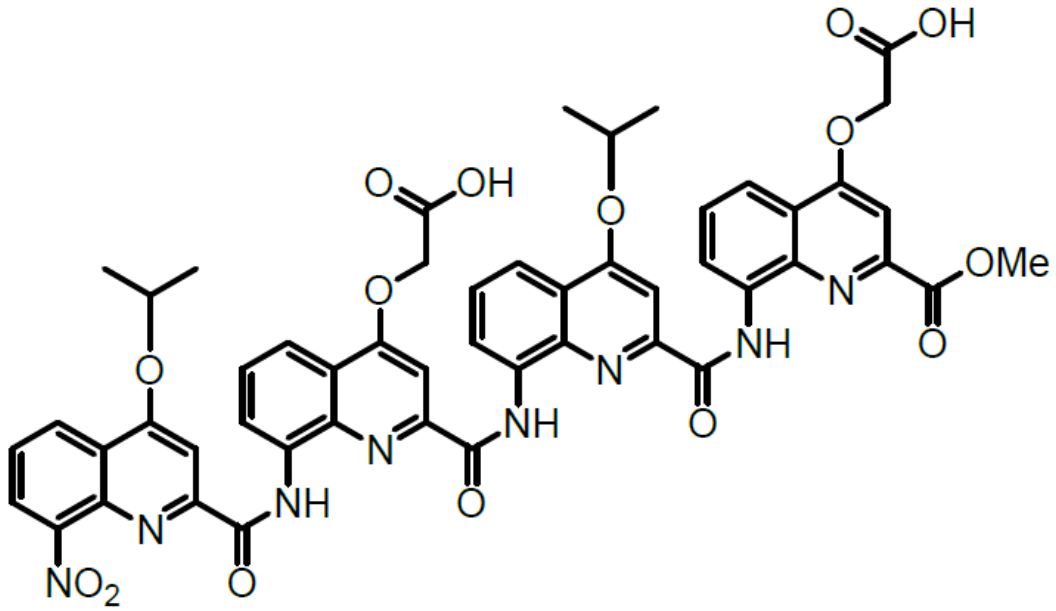
https://theconversation.com/animals-in-research-c-elegans-roundworm-14163

Structure of Treatment Molecules

Bexarotene for GMC101 strains[4]



SK-129 for NL5901 strains



Courtesy of Dr. Sunil Kumar

https://pubchem.ncbi.nlm.nih.gov/compound/Bexarotene

Methods[5] – Day 1

Strains are synchronized through a bleaching process including egglay[6] and incubation at 21°C for 30 hours on NGM plates with 0.5 OD OP50.

Both GMC101 and NL5901 strains

GMC101

75 µM bexarotene in M9 buffer is added to FUDR plates for the treatment strains.

100 µM SK129 is added to the 2 FUDR plates for the treatment strains.

NL5901

http://www.ipmscientific.com/Nematode%20Growth%20Agar%20Plates/Nematode%20Growth%20Agar%20Plates.html

Methods[5] – Days 2 and 3

Day 2

- Using M9 buffer, worms are transferred from NGM plates into FUDR solid media plates.

Day 3

- Worms are incubated at room temperature (21°C).

Day 3 for GMC101 only

- 75 µM bexarotene in M9 buffer is added to the FUDR plates for the treatment strains.

Methods[5] – Day 4

Step 1

- Liquid media is prepared using 75 µM FUDR, M9 buffer, 1M magnesium sulfate, 1M calcium chloride, and 1M potassium phosphate. 1 mL of liquid media is transferred into each of 6 empty 35mm plates with OP50, using 2 per strain.

  Strains:

  • Wild type N2, GMC101 + Bexarotene, and GMC101

  • Wild type N2, NL5901 + SK129, and NL5901

Step 2

Adding treatment molecules:

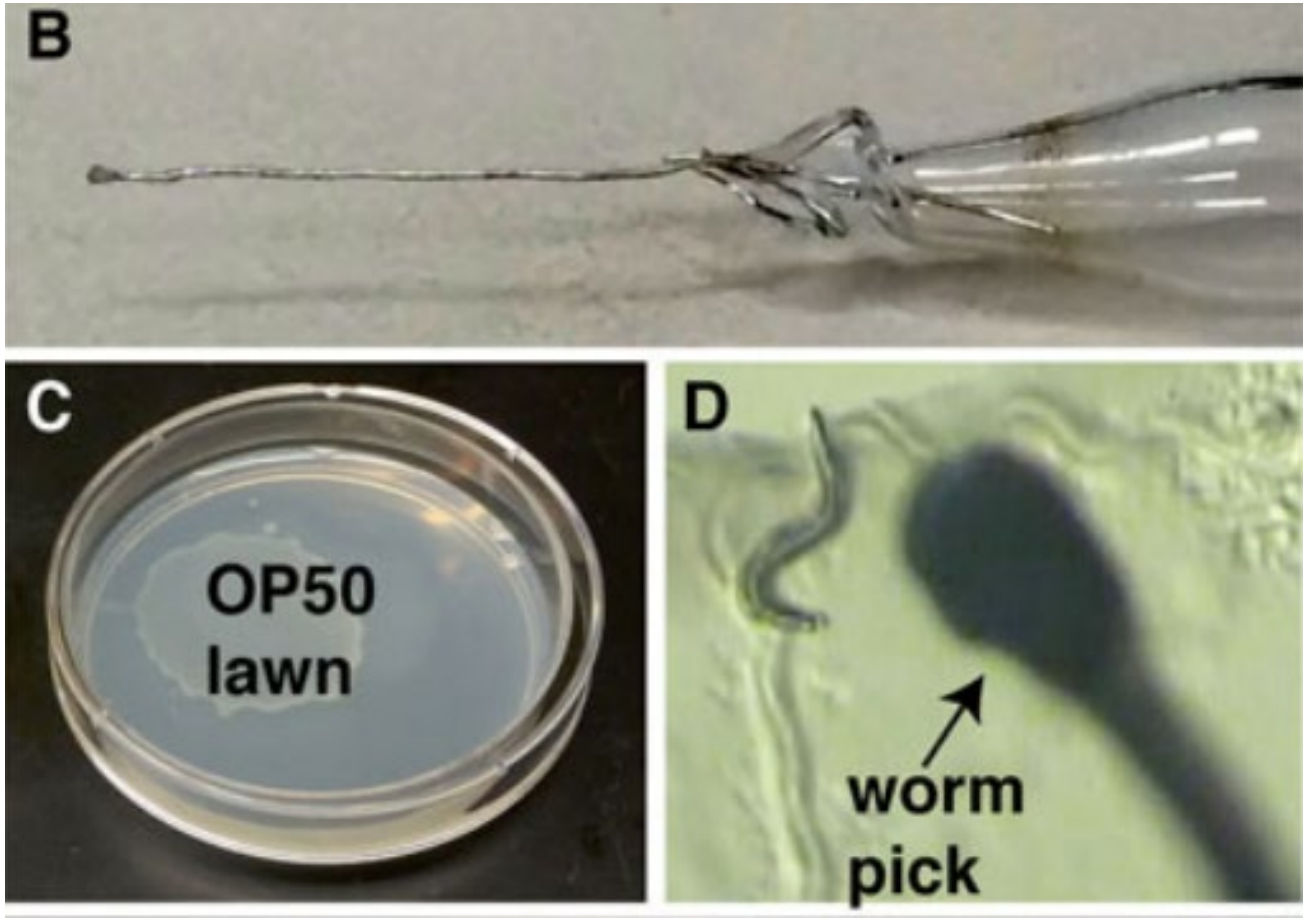
• 10 µM bexarotene is added to the 2 plates for the treatment strains.

• 50 µM SK129 is added to the 2 FUDR plates for the treatment strains.

Step 3

- Using a worm pick, 100 worms are transferred from the solid media to each of 6 liquid media plates.

Methods – Day 4



DOI: 10.1007/978-1-4939-3789-9_8

https://geneseesci.com/shop-online/product-details/59-AWP-B

Methods[5] – Day 5

Plates are tapped to activate worms in liquid media. The motility assay begins using Arena plate reader at 23°C . The assay runs for 1 hour for 14 consecutive days. 3 trials were completed.

After 14 days of data collection, data is analyzed using Microsoft Excel.

https://www.moleculardevices.com/products/microplate-readers/absorbance-readers/spectramax-abs-plate-readers

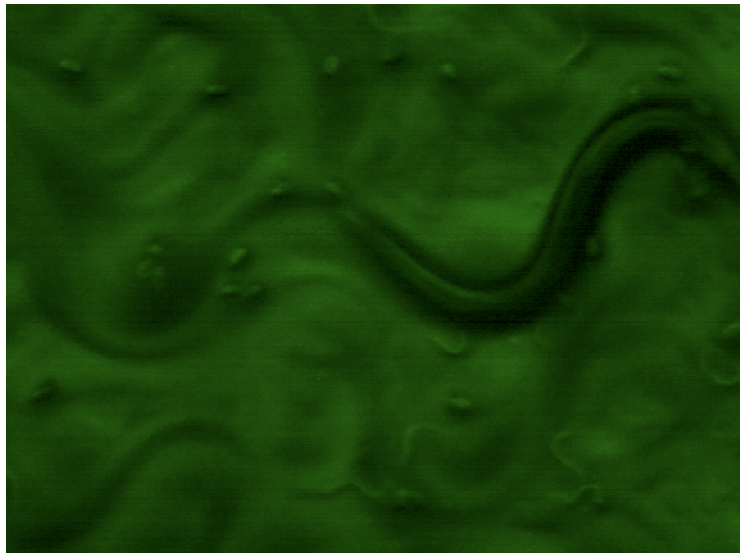
Fluorescence Microscopy

- **α-Synuclein** (NL5901) is tagged with YFP
  - Does not show up as bright as GFP under microscope
- **A-β 1-42** (GMC101) is tagged with GFP
- **N2** (wild type) has no fluorescence
  - It does not have any aggregates or fluorophores

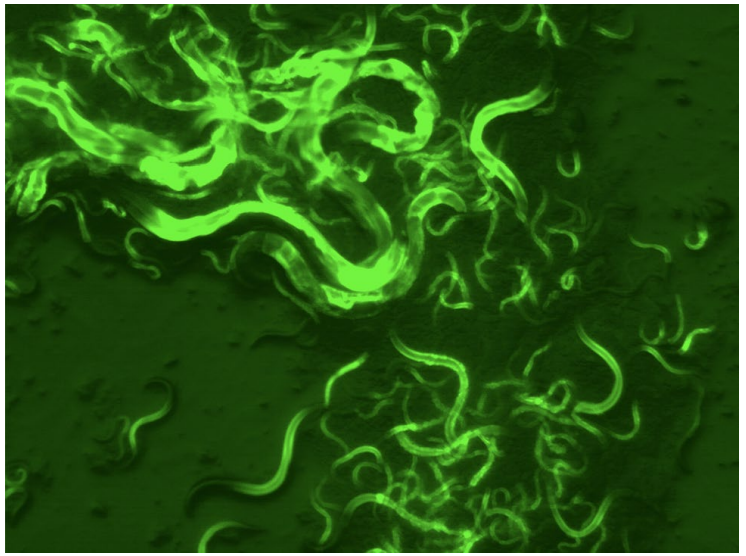
https://meijitechno.com/mt6000-m-motorized-fluorescence-microscope

Fluorescence Microscopy

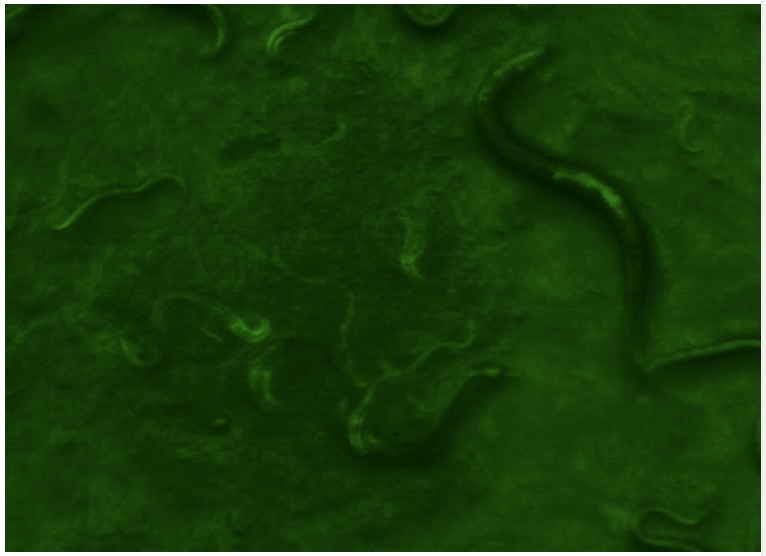
N2



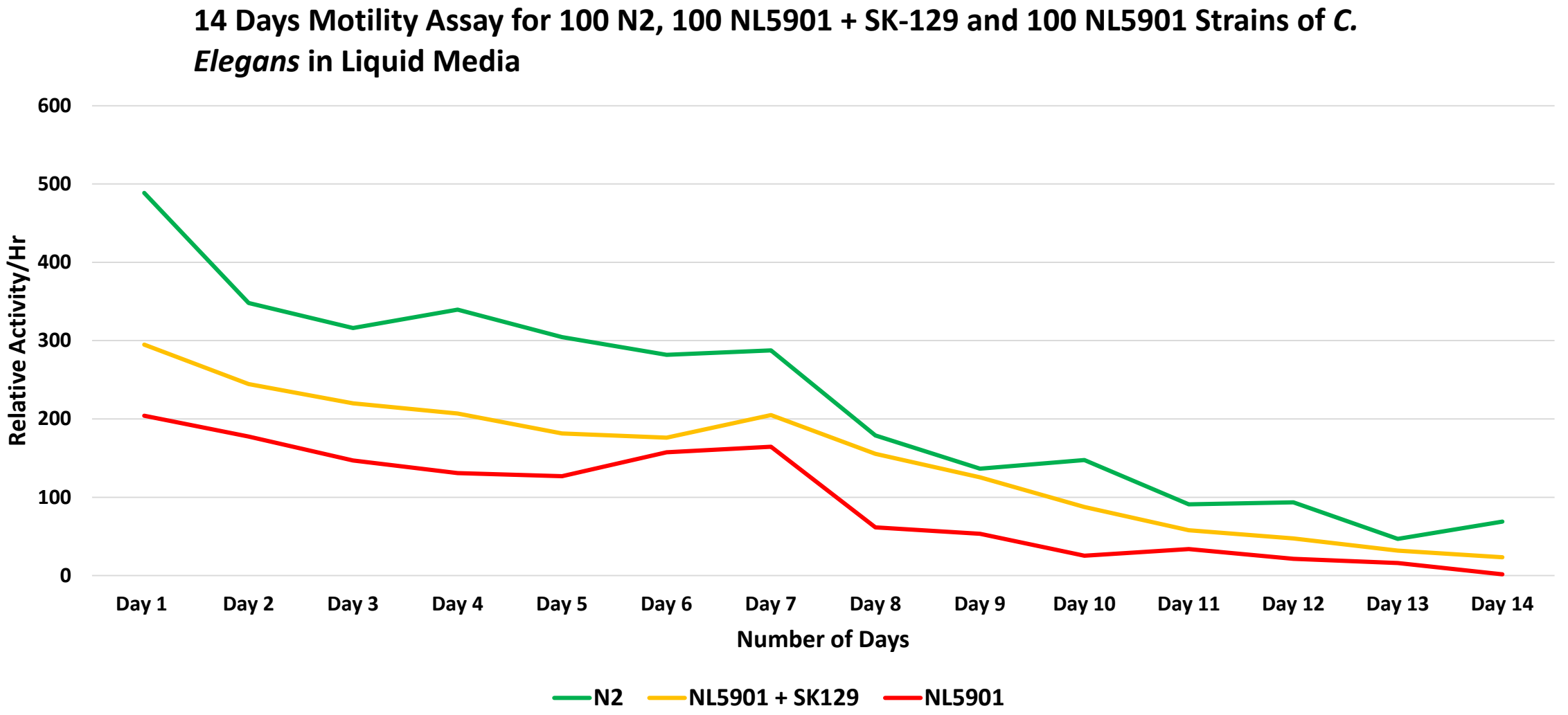
GMC101 (GFP)



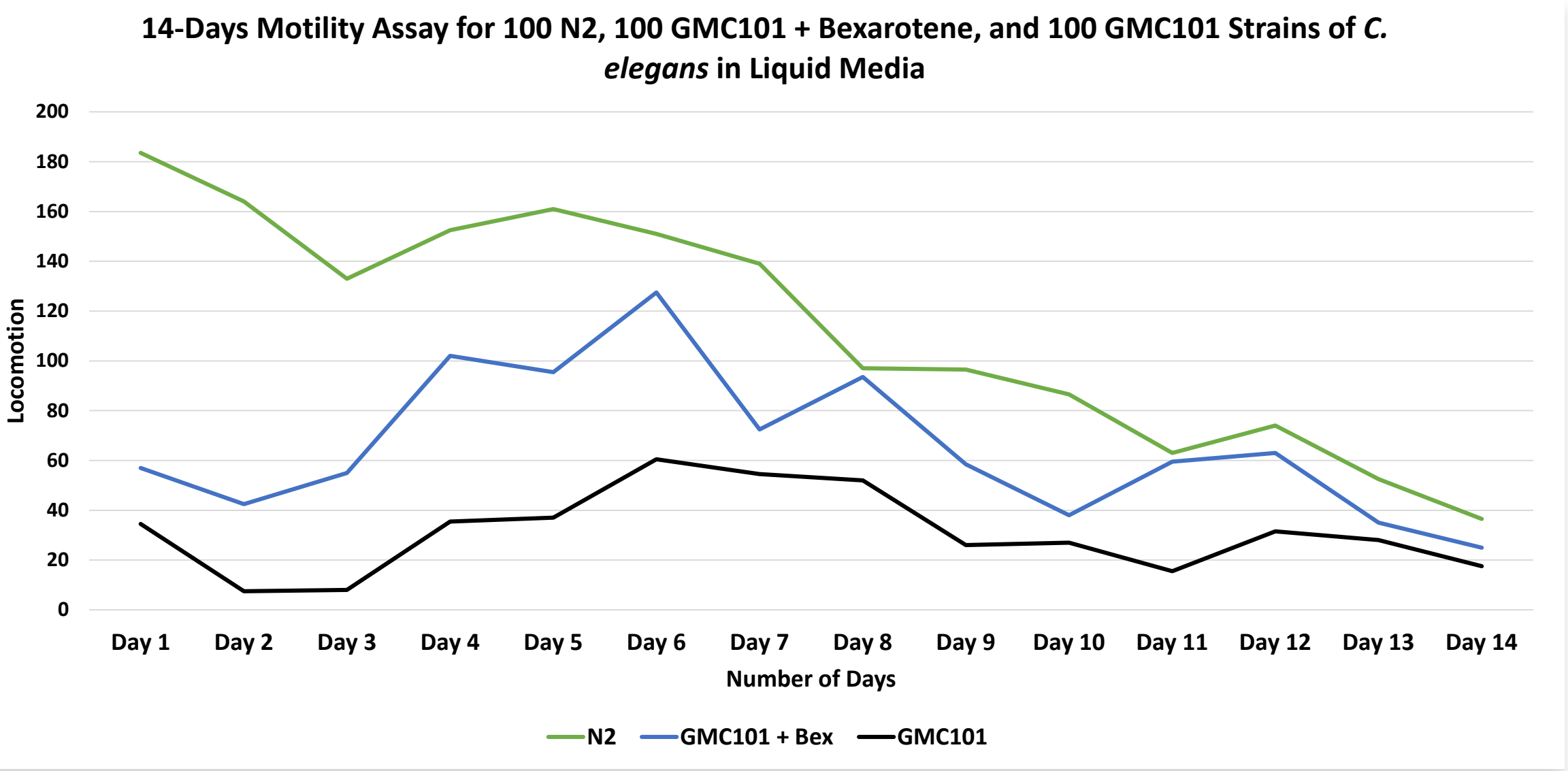
NL5901 (YFP)



Results – Data collected by Johnson Joseph



Results

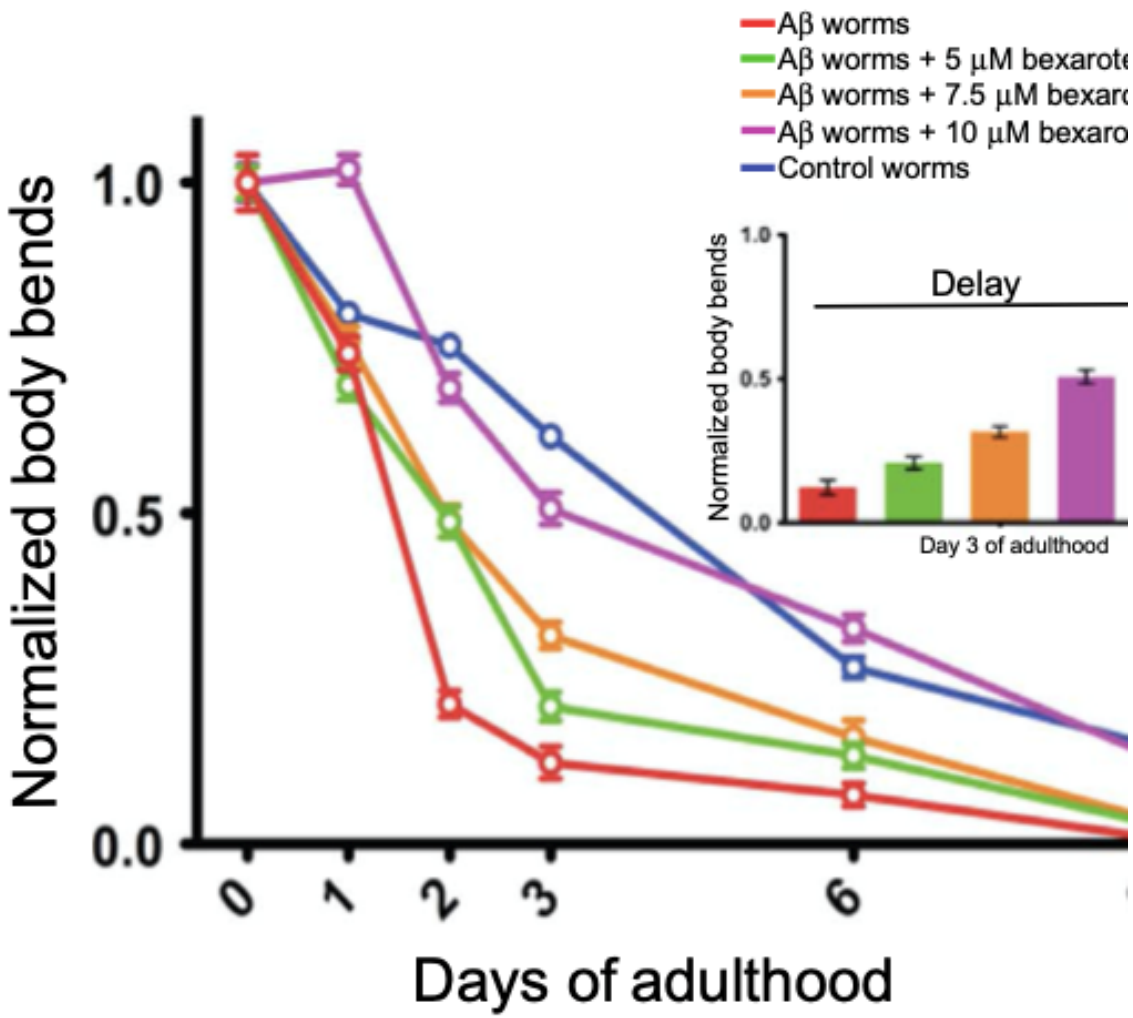

14-Days Motility Assay for 100 N2, 100 GMC101 + Bexarotene, and 100 GMC101 Strains of C. elegans in Liquid Media
Locomotion
200
180
160
140
120
100
80
60
40
20
0
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Day 9
Day 10
Day 11
Day 12
Day 13
Day 14
Number of Days
N2
GMC101 + Bex
GMC101

Discussion

- The locomotion declines for all strains in both the Parkinson's and Alzheimer's assays over time due to the natural loss of motility as the worms age and the accumulation of pathogenic amyloid plaques.
- The curve for NL5901 + SK129 demonstrates higher locomotion than the NL5901 (sick) strains, therefore SK129 is effective at inhibiting α-Synuclein aggregation.
- Bexarotene has high efficacy in the GMC101 strains because the curve for GMC101 + Bexarotene closely aligns with the curve for the wild type (N2) strains, so the molecule inhibits A-β 1-42 aggregation.

Previous Studies with GMC101

- Our data aligns with the 2016 Habchi et al. study[4] because both studies show that the GMC101 and N2 strains demonstrate similar locomotion.
- Furthermore, the Habchi et al. paper confirms what our study found in previous trials: that A-β 1-42 aggregation is better inhibited at higher concentrations of Bexarotene.



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