



From Worm to Pre-Clinical Data

How a Nutraceutical Company
Leveraged *C. elegans* to Predict the Most
Valuable Compound to Develop



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While mammalian models are currently the gold standard for drug development, the enforced 3Rs and animal welfare guidelines have forced us to re-evaluate our development pipeline to include early stage in-vivo hypothesis testing.

Understanding the biology of our compounds necessitates the use of an alternative model prior to going to more regulated, more resource and time-intensive models.

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How a Nutraceutical Company Leveraged *C. elegans* to Predict the Most Valuable Compound to Develop

**Disclaimer: The data and information contained in this case study have been altered to protect the identity of the customer.*

Traditionally, clients of NemaMetrix's in-vivo testing services are pharmaceutical companies investigating chemically synthesized small molecules for efficacy on a specific gene target of interest. However we have been seeing an increase in Nutraceutical clients wishing to investigate the impact of naturally derived compounds or mixtures.

Dr. Johnson, the CSO of a nutraceutical company, reached out to us recently with a question: "Can I make the claim that compounds A and B act as anti-aging catalysts?"

Since launching the company, Dr. Johnson has been focusing on developing a small number of natural extracts and compounds that may have the ability to promote healthy aging. The company's main focus is to move very rapidly from hypothesis to early proof of concept.

Most of the scientists on Dr. Johnson's team have a strong background in chemistry and biology. They understand the importance of choosing the right model to get meaningful results about their compounds. The team has a lot experience testing compounds and extracts in cell culture and in rodents.

They have gathered some promising preliminary data thanks to a collaboration with an academic lab. However, this preliminary study using mice ran for over two years, and Dr. Johnson now needs to move much faster to apply for patents and hopefully get those compounds on the market. Dr. Johnson has three main questions in mind in order to know where to put her resources for the next couple of years:

- Do compounds A and/or B increase lifespan in live animals?
- If so, do the increases in lifespan correlate with a better quality of life (healthspan)?
- What is the mechanism of action behind these effects? In particular, do compounds A and/or B act through the mitochondrial/cell respiration pathway?

Summary

Overview

Dr. Johnson, the CSO of a small nutraceutical company based in Germany is developing compounds that promote healthy aging.

Challenge

She needed to apply for patents and start to commercialize the compounds within the next 6-10 months. To do so, Dr. Johnson needed an *in-vivo* analysis of her compounds in an intact animal but using rodents as a model would be much too long and too costly at this stage. She needed fast data that is still relevant for human health.

Solution

We used the animal model *C. elegans* to perform a set of specific experiments designed by our experts to provide rapid and reliable data based on Dr. Johnson's priorities.

Benefits

NemaMetrix is the market leader for early preclinical in-vivo testing using small animal models to gain a better understanding of the efficacy, mode of action, toxicity and potential targets for novel compounds. Using the data that we provided, Dr. Johnson was able to apply for multiple patents in a timely manner.

Before calling us, Dr. Johnson had spent hours reading peer-reviewed publications, looking for a model that would give her the perfect combination of speed and relevance for human health. Dr. Johnson quickly realized that *C. elegans* has been considered a premium model for aging studies, thanks to its attractively short 2-week lifecycle and how much genetically similar to humans it is (over 80% of human disease genes are conserved between humans and *C. elegans*).

“Time is of the essence. We are trying to have faster models, but with short term accuracy”

We brainstormed the best approach to their project with Dr. Johnson and her team over the phone. Our team of experts weighed in to devise the most efficient approach based on her priorities. Within the week, we sent a preliminary proposal that included the assays we recommended and an estimation of the costs for this project. We walked through it with Dr. Johnson and after clarifying and tweaking the proposal, we landed on an experimental plan that would answer all 3 of Dr. Johnson's questions within 3-4 months.

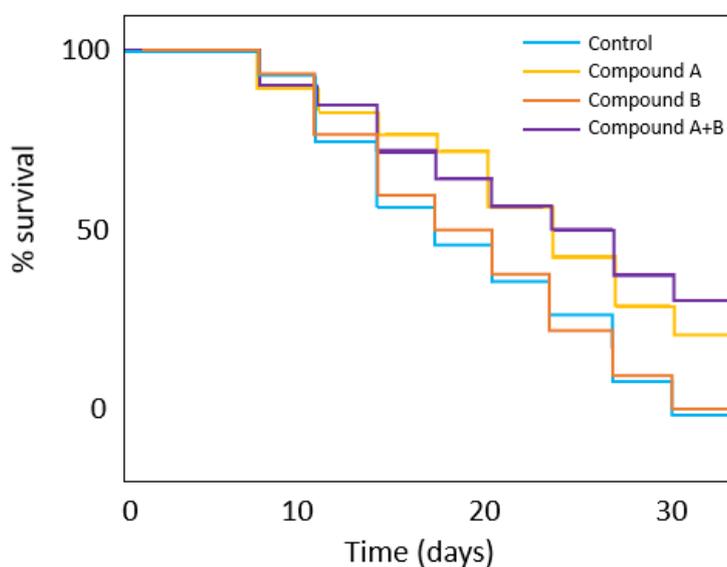
Within 3 weeks of Dr. Johnson's first email, we were collecting data for her project in our lab in Oregon.

We stayed in close contact with Dr. Johnson throughout the project, sending short email reports every 2-3 weeks to update her on the status of her project.

“Communicating with NemaMetrix was a breeze. I really appreciated the clarity, transparency and fast response time. It was truly reassuring to have such visibility throughout the process.”

Despite a short unexpected delay due to the lethality of the first concentration chosen for compound B, which we troubleshooted with her, Dr. Johnson's project was delivered within 5 months.

Dr. Johnson was happy to see that compound A and even compounds A and B together, have a strong lifespan extending effect.



(Left) Survival curve (Kaplan-Meier) showing that worms treated with Compound A (yellow) and a mix a compounds A+B (purple) live longer than untreated worms. Over 45% of the worms treated with A+B were still alive at a time point when all control worms had died.

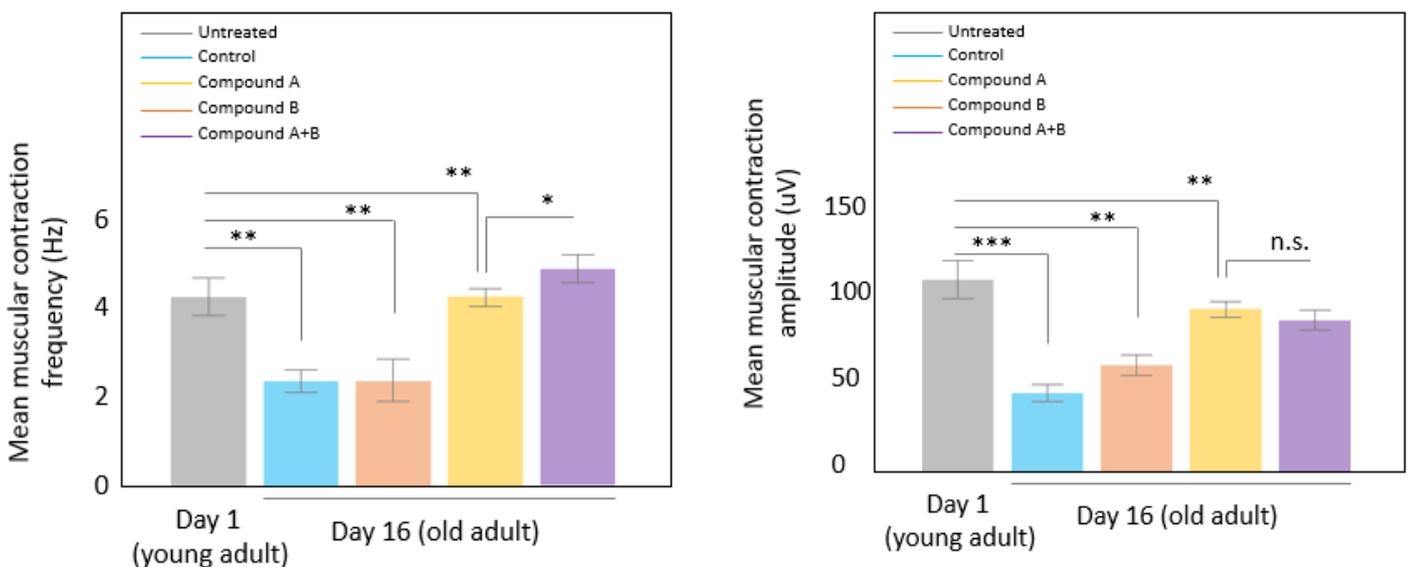
But living longer is not the only goal. In fact, it is always important to check whether extending life also extends the length of decrepitude. We need more information than just lifespan to evaluate the translational potential of any proposed anti-aging intervention to humans. In their review in a Nature journal in 2016, Uno and Nishida state “One of the goals of aging research is to not only extend lifespan but also extend healthspan. Worms display certain age-associated characteristics that resemble those observed in humans; therefore, worms also serve as a useful model for healthspan studies. Healthspan studies in worms include those that have examined mobility declines and pharyngeal pumping, fluorescent compound dynamics [...], and neuromuscular changes.”

“The revelation that compounds A and B act synergistically to extend lifespan was a good surprise and we will definitely take this into account as we move forward.”

To understand how healthy the long-lived worms are, we performed a series of experiments to test various aspects of health across the lifespan of the worms. Several studies show that mammals undergo a constellation of functional losses as they age, such as reduction in mobility and physical activity levels and loss of appetite.

We used state-of-the-art automated movement assays to determine not only the activity level of worm populations under each condition at multiple life stages, but also detect neuromuscular changes at a more granular, individual level.

We found that, in addition to living longer, worms exposed to compound A and the A+B mix also displayed a high activity level at an advanced age and retain a healthy level of neuromuscular contraction associated with feeding.



Measuring contraction of the pharyngeal muscle (feeding organ) at Day 1 and Day 16 of adulthood. (Left) Frequency of contraction of the pharyngeal muscle. (Right) Amplitude of contraction of the pharyngeal muscle (proxy for strength of muscle contraction). Older Worms that have been exposed to compound A or to a mix of compounds A+B are able to contract this muscle at a rate and with a strength similar to young untreated adult worms. This assay further supports the hypothesis that compound A alone or mixed with compound B promote neuromuscular health throughout the aging process.

Finally, Dr. Johnson wanted to quickly assess whether the mechanism of action of compound A involves the mitochondrial pathway. Mitochondrial dysfunction contributes to the pathology of many common disorders associated with aging, including neurodegeneration, metabolic disease and heart failure. Mitochondria therefore represent an important target for anti-aging drugs.

We took a molecular approach and used quantitative PCR on lyzed worms exposed to compound A to quantify the expression of 3 mitochondrial genes in young and old worms. Data showed that all 3 of those mitochondrial genes were expressed at a significantly higher level in old worms that had been exposed to compound A throughout their lives compared to untreated worms of the same age. Those levels were similar to the level of mitochondrial gene expression in young worms.

Many of the major mechanisms governing the aging process in mammals have been identified from studies in *C. elegans*, so Dr. Johnson felt very comfortable moving forward and applying for a patent for compounds A and B.

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I am very satisfied with this study. Being able to answer those preliminary questions so quickly allows us to accelerate the development of those compounds and move them down the pipeline. Our next step is to work with NemaMetrix to further our understanding of the mechanisms of action of our compounds, likely via a more genetic approach using transgenic worms.

Assays used for this study



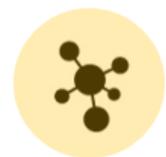
Population Movement

Quantify the activity of a worm population.



Individual Movement

Quantify detailed movement measures from individual worms.



Molecular Phenotyping

Quantify expression levels of a gene of interest.



Lifespan Assessment

Get survival curves for a cohort of worms.



Healthspan Assessment

Measure the vitality of worms as they age.