Accelerating Drug Discovery for Diseases of Ageing: *In Vivo* High Throughput Screening with *C. elegans*

In an era where rapid drug discovery is crucial, high throughput screening (HTS) emerged in the 1990s as a significant development, allowing for the swift and effective identification of active compounds and deeper insights into biological pathways. This process involves testing large libraries of compounds against biological targets to identify potential drug candidates. It relies on robotic automation and the use of standard format 96, 384 or 1536 well plates to screen tens of thousands of compounds per day.

Despite its transformative impact, current HTS methods face significant challenges in studying complex *in vivo* responses and age-related diseases. This article explores the potential of using *C. elegans* as a scalable and effective *in vivo* model for high throughput drug discovery in these therapeutic areas.

The Uses and Challenges of Traditional Models in HTS

In Vitro Screening

In vitro screening methods are widely used due to their cost-effectiveness and suitability for initial compound screening. These methods involve testing compounds in biochemical assays or with cultured cells to identify those that exhibit desired biological activity. However, *in vitro* models have limitations. They often fail to replicate the complex interactions that occur in living organisms, making it difficult to predict the efficacy and safety of compounds in humans. This limitation is particularly problematic for studying age-related diseases and other conditions where the cellular environment plays a critical role.[1]

Traditional 2D cell cultures, for instance, do not adequately mimic the three-dimensional architecture and microenvironment of tissues, which can significantly influence cell behaviour and drug responses. Moreover, cells in culture do not experience a comparable ageing process to whole organisms, which further limits their utility in age-related disease research.[2]

The only cells that show ageing are human primary fibroblasts and they are difficult to obtain in high numbers and vary between individual donors. Using these cells fails to replicate the systemic and tissue-specific ageing processes observed in whole organisms. This discrepancy can lead to misleading results when evaluating the efficacy of compounds to interfere with chronic diseases of ageing.

3D cell culture techniques have been developed to address some of these limitations by better replicating *in vivo* conditions. These advanced models incorporate more than one cell type and extracellular matrices to create a more physiologically relevant environment. However, despite their advantages, 3D cultures are less amenable to HTS, mostly because consistent establishment of cultures and microscopy is challenging in multi-well plates to the level required for large throughput screening.[1,2]

A related development is organoids, which are mini clusters of cells that mirror many properties of organs. Scaling this approach to HTS is possible but constrained by issues of consistently sorting large numbers into multiwell plates, and then performing microscopy on them.

Current Routes to *In Vivo* Data

For most drug discovery, the use of mammalian models, such as mice, provide the first time that a compound is tested *in vivo*. Mammalian models provide a closer approximation to human biology, making them invaluable for assessing drug efficacy and safety in complex living systems. These models can mimic human disease conditions more accurately than *in vitro* models. However, they are for obvious reasons, not amenable to HTS. Mammalian models are costly and time-consuming, requiring extensive resources and lengthy study periods just for experiments with a handful of conditions. For example, rodent testing in cancer therapeutics alone can add an estimated 4 to 5 years and cost $2 to $4 million per study.[3]

Additionally, the use of animals in research raises ethical concerns and involves complex regulatory requirements. These factors limit the scalability of mammalian models for high throughput applications. Ethical considerations and regulatory compliance add layers of complexity, making it challenging to expand the use of mammalian models in a high-throughput setting.

The average cost of developing a new drug, including the use of rodent models, is around $900 million, and the time to market is typically 10–15 years.[4,5] Moreover, the attrition rate in drug development is notably high. Out of every 5000–10000 compounds, only 250 make it to preclinical trials, five enter human trials, and just one reaches the market.[6] This high rate of failure further underscores the inefficiency and high cost associated with mammalian models.

Despite the significant advancements and potential of HTS in drug discovery, traditional methods face several critical challenges. *in vitro* models, while cost-effective, fail to replicate the complex interactions and ageing processes found in living organisms, limiting their predictive power for human efficacy and safety. Mammalian models, though more representative of human biology, are costly, time-consuming, and involve ethical and regulatory complexities that hinder their scalability.6 These challenges underscore the need for innovative approaches that can bridge the gap between *in vitro* and mammalian models, offering more efficient and scalable solutions to bring *in vivo* research earlier in drug discovery.

C. elegans as a Developing Model for High-Throughput Screening

The nematode worm Caenorhabitis elegans is being increasingly explored as a HTS model that bridges the gap between *in vitro* and mammalian models. With adults only a millimetre in length, several of these worms can easily fit into one well of 96 or 384 well plates and advanced microscopy or spectroscopy used to monitor them.

Historically used in basic biological research, *C. elegans* offers several distinct advantages, making it an attractive model organism in drug discovery. One significant advantage is its human translatability. *C. elegans* have significant homology with human cell types and organs, and it uses most of the neurotransmitters found in humans, such as dopamine, serotonin, acetylcholine, GABA, and glutamate. This biological similarity makes *C. elegans* a relevant model for studying human diseases. High conservation at the amino acid level of many targets makes it suitable for investigating drug responses.

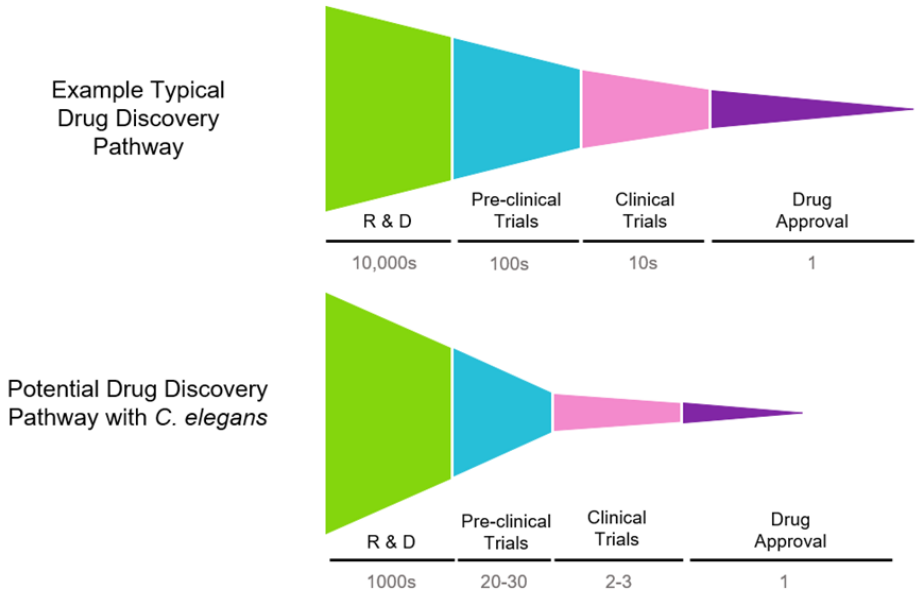
Understanding age-related processes is another area where *C. elegans* excels because it ages fast, and genetics can be used to determine which genes influence ageing. It was the first model organism in which single gene mutations were found to slow ageing, with key pathways such as insulin/PI3K/FOXO and mTOR, modulating ageing processes. In drug discovery, researchers can quickly observe the effects of drug treatments on the ageing process, accelerating the pace of discovery.

In addition to studying natural decline of function with age, several models have been developed to study the consequences of overexpressing human genes implicated in neurodegenerative diseases. This makes *C. elegans* particularly useful for studying the genetics of ageing and identifying compounds that influence longevity.

This makes *C. elegans* particularly useful for studying the genetics of ageing and identifying compounds that influence longevity.

Scalability is a crucial factor in HTS, and *C. elegans* excels in this regard. These worms can be easily maintained in large numbers, can be sorted using automatic sorting machines, fit into tiny wells and are transparent for microscopy. Ageing is intrinsically heterogeneous, so large test populations are required for robust data when studying the diseases of ageing. The *C. elegans* system and HTS can address this challenge and has been used to screen drugs that extend lifespan.[7,8]

Cost-effectiveness is another key advantage of *C. elegans*. Maintaining these worms is inexpensive compared to mammalian models, reducing the overall cost of large-scale screening projects. This economic benefit, combined with the other advantages, positions *C. elegans* as a powerful tool for HTS and drug discovery. The ability to bring *in vivo* testing earlier in the drug discovery pipeline can significantly de-risk the transition from *in vitro* studies to mammalian studies, providing a more efficient path to identifying promising drug candidates.



By leveraging these advantages, *C. elegans* studies can provide parallel *in vivo* data that significantly enrich and complement data from *in vitro* experiments, enhancing the overall robustness and predictive power of drug discovery efforts.

From Hits to Mechanisms

High throughput screens using *C. elegans* can identify compounds with potential, which can then be further studied to understand their mechanisms of action. This is particularly useful in areas like neurodegeneration, gut health, muscle health, weight management, toxicity, and reproductive health. *C. elegans* offers various disease models to test compounds, including models for ALS, Alzheimer's, and Parkinson's disease. By studying these models, researchers can gain insights into how compounds interact with specific biological pathways, facilitating the development of targeted therapies.

Successful Applications

C. elegans has been used in numerous studies to identify neuroprotective compounds, study aging processes, and assess toxicity. Research using *C. elegans* has led to the discovery of compounds that extend lifespan and improve health-span by modulating various genetic pathways.

One notable example of where *C. elegans* was used to find a new therapy is the PPM2-CDG case study. PPM2-CDG is a rare genetic disorder that affects multiple systems in the body. In this study, a model of PPM2-CDG was developed in *C. elegans* using mutations homologous to those found in humans. This mutant could not grow properly in the presence of a stress-inducing drug. Using HTS method, researchers conducted a drug repurposing screen of 4,000 drugs in 384 well plates using this model, and identified aldose reductase inhibitors as a class of drugs that modified the PPM2-CDG phenotype. One of these inhibitors, Epalrestat, was repurposed to treat a patient named Maggie, who had PPM2-CDG. Treatment with Epalrestat led to significant improvements in Maggie's speech, walking, fine motor skills, balance, and growth. The study has progressed to a Phase III clinical trial to evaluate the safety and efficacy of Epalrestat for PMM2-CDG patients, demonstrating the potential of *C. elegans* in drug discovery and the repurposing of existing drugs to treat rare diseases.[9]

Regulatory and Ethical Considerations

The regulatory landscape for HTS involves ensuring data integrity and reproducibility, particularly when using advanced technologies like AI. *C. elegans* models, due to their simplicity and ethical acceptability, face fewer regulatory hurdles compared to mammalian models. This makes *C. elegans* an attractive option for high throughput applications, as they align well with regulatory requirements while providing valuable *in vivo* data.

Ethical considerations in HTS include the use of animals and data privacy concerns related to AI. *C. elegans* provides an ethical advantage as a non-mammalian model, reducing the ethical concerns associated with higher animals. The use of *C. elegans* aligns with the principles of the 3Rs (Replacement, Reduction, and Refinement) in animal research, promoting more humane and responsible scientific practices.

Additionally, AI applications in HTS must address issues of data privacy and algorithmic transparency to ensure ethical integrity.

Emerging Trends in HTS

Technological Innovations: Recent advancements in HTS have significantly improved the efficiency and accuracy of the screening process. Automation and miniaturisation are key innovations that reduce costs and increase throughput. Automated systems can handle large libraries of compounds with minimal human intervention, ensuring consistent and reproducible results. Miniaturisation techniques, such as microfluidics, allow for the testing of smaller sample volumes, conserving valuable reagents and reducing overall costs.

AI and Machine Learning: AI-driven HTS leverages advanced machine learning algorithms to accurately predict active compounds, optimise lead compounds, and seamlessly integrate diverse datasets.[10] These technologies address traditional HTS challenges by reducing false positives and improving data analysis. Machine learning models can analyse complex biological data, identifying patterns and relationships that may not be apparent through traditional methods. This

enhances the efficiency and accuracy of HTS, making it a powerful tool in modern drug discovery.

Data Integration and Analysis: Advanced data processing techniques are crucial for extracting meaningful insights from the vast amounts of data generated by HTS. Automation and AI significantly enhance the efficiency and accuracy of data integration and analysis. By combining data from various sources, researchers can obtain a more comprehensive view of biological interactions and identify potential drug candidates more effectively. This integration enables better-informed decisions and accelerates the drug discovery process.

Integrated Research Frameworks: Integrating *C. elegans* models with other HTS frameworks could provide a more holistic approach to drug discovery, leveraging the strengths of various models and technologies. This integration would enable more comprehensive and accurate drug discovery, benefiting from the unique advantages of each model. For example, combining *C. elegans* with mammalian cell cultures and AI-driven data analysis can provide a multi-faceted understanding of drug effects, enhancing the overall robustness of the screening process. By leveraging AI, researchers can process large datasets more efficiently, identify patterns, and make data-driven decisions that accelerate the drug discovery process.

Alternative Model Organisms: In addition to *C. elegans*, other model organisms like zebrafish and fruit flies (Drosophila melanogaster) are gaining traction in HTS due to their genetic similarities to humans and ease of maintenance. Zebrafish, for example, are particularly useful for studying developmental processes and genetic mutations due to their transparency and small size during larval stages, allowing for non-invasive imaging and high-throughput screening.[11] Fruit flies offer advantages in studying neurological diseases and genetic interactions due to their well-mapped genome and short life cycle.

Human Cell Cultures and Organoids: Human cell cultures and organ-on-a-chip technologies are being increasingly adopted to replicate human tissues and organs, providing more relevant human data while reducing the reliance on animal models. These technologies allow for the study of complex cellular interactions in a controlled environment, improving the predictive power of HTS. For instance, 3D organoids can mimic the architecture and function of human organs, providing a more accurate model for drug testing and disease research.[12]

CRISPR and Gene Editing: Advances in gene editing technologies, such as CRISPR-Cas9, are revolutionising HTS by enabling precise genetic modifications in various model organisms and cell cultures. This allows researchers to create specific disease models and investigate the effects of genetic variations on drug responses, leading to more targeted and effective therapies.

Ethical and Regulatory Evolution: As the field evolves, so too will the ethical and regulatory landscape. There is a growing emphasis on reducing animal use in research and improving the ethical standards of HTS. Innovations like *C. elegans*, zebrafish, and human cell-based models align with these goals by providing alternative methods that meet regulatory requirements while minimising ethical concerns.

Conclusion

High throughput screening has revolutionised drug discovery by enabling rapid and efficient screening of compounds. Emerging trends and technological advancements, particularly in AI and machine learning, continue to enhance the effectiveness of HTS. *C. elegans*, and presents a scalable, *in vivo* alternative that, when integrated with advanced technologies, holds great promise for the future of drug discovery. The ongoing evolution of HTS methodologies will undoubtedly lead to more successful therapeutic discoveries and improved healthcare outcomes.

REFERENCES

1. Badr-Eldin, S. M., Aldawsari, H. M., Kotta, S., Deb, P. K., & Venugopala, K. N. Three-Dimensional *In Vitro* Cell Culture Models for Efficient Drug Discovery: Progress So Far and Future Prospects. Pharmaceuticals 15, 926 (2022)
2. Langhans, S. A. "Three-Dimensional *In Vitro* Cell Culture Models in Drug Discovery and Drug Repositioning." Frontiers in Pharmacology 9 (2018): 6. doi:10.3389/fphar.2018.00006.
3. DiMasi, J. A., Grabowski, H. G., & Hansen, R. W. "The price of innovation: new estimates of drug development costs." Journal of Health Economics 22, 151-185 (2003).
4. Tufts Center for the Study of Drug Development. "Tufts CSDD R&D cost study." (2016).
5. Baldrick, P. "Pharmaceutical Toxicology: Special Topics." (2008).
6. Paul, S. M., Mytelka, D. S., Dunwiddie, C. T., Persinger, C. C., Munos, B. H., Lindborg, S. R., & Schacht, A. L. "How to improve R&D productivity: the pharmaceutical industry's grand challenge." Nature Reviews Drug Discovery 9, 203-214 (2010)
7. Weinkove, D., & Zavagno, G. "Applying *C. elegans* to the Industrial Drug Discovery Process to Slow Aging." Frontiers in Aging 2, (2021). doi:10.3389/fragi.2021.740582.
8. Petrascheck, M., Ye, X., & Buck, L. B. "A High-Throughput Screen for Chemicals that Increase the Lifespan of Caenorhabditis elegans." Annals of the New York Academy of Sciences 1171, 1, 95-106 (2009). doi:10.1111/j.1749-6632.2009.04377.x
9. Maggie's Pearl. "Maggie's Pearl Phase 3 PMM2-CDG Trial." Maggie's Pearl (2024). Available at: https://www.maggiespearl.com/maggies-pearl-phase-3-pmm2-cdg-trial
10. Ofenbauer, Andreas. (2019). Characterisation of the KASH domain gene unc-83 and the pseudogene F55A3.7. 10.18452/20457.
11. NC3Rs. "Five Reasons Why Zebrafish Make Excellent Research Models." NC3Rs (2024). Available at: https://www.nc3rs.org.uk/news/five-reasons-why-zebrafish-make-excellent-research-models
12. Cruelty Free International. "Alternatives to Animal Testing." Cruelty Free International (2024). Available at: https://crueltyfreeinternational.org/about-animal-testing/alternatives-animal-testing

David Weinkove

David Weinkove is well respected in the academic fields of *C. elegans* and ageing biology. As a postdoc, he worked with David Gems, Ronald Plasterk and Erik Jorgensen. He is now Associate Professor at the Department of Biosciences, Durham University and Chair of the British Society for Research on Ageing. David is passionate about applying the strength of *C. elegans* research to industrial application and he co-founded Magnitude Biosciences to bring reliable automated technology together with experienced *C. elegans* scientists to bring increases in productivity to the whole field.

Email: david@magnitudebiosciences.com