Of Mice and Medicine: Navigating the Leap from Preclinical Research to Clinical Practice

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Neurodegenerative diseases, such as Alzheimer's and Parkinson's, are devastating neurological ailments for which effective treatments, let alone cures, remain elusive. Over the past few decades, we had made leaps in understanding the pathology and clinical manifestation of these diseases. The pathophysiology of these diseases is also becoming increasingly clearer even though a complete picture is lacking. Therefore, these diseases pose a near tractable problem that can, with time, be potentially solved. What is one of the biggest challenges to finding a solution to a seemingly insoluble problem? The ominous metaphor of the "Valley of Death" describes the typical barriers in the discovery of new treatments for diseases. To find effective treatments for neurodegenerative diseases, one must cross the valley of death in the neurotherapeutics space, that is the translational gap from the laboratory to clinical practise. This gap is often encountered after the surge of early results, as many laboratories face a calamitous fall into the valley of death due to lack of funding, minimal industry engagement and collaboration, regulatory hurdles, among other reasons. This translational gap can be narrowed, if not closed, with the transformation of our pre-clinical testing approach for neurotherapeutics, including the use of appropriate humanized animal models such as rodents and non-human primates. This article explores the challenges scientists must navigate when utilizing one of the common animal models used to evaluate neurotherapeutics, i.e., mouse models.

We have made quantal leaps in engineering animals to model diseases, for instance, with the advent of gene editing technologies, we now have the resolution to edit even a single nucleotide, let alone the ability to engineer mouse models as per demand. Current mouse models have unravelled many complex intricate mechanisms of the neurodegenerative diseases, but they fall short of faithfully recapitulating the disease progression and symptom manifestation as they happen in humans. We argue that a critical rethinking of our pre-clinical testing architecture including mouse model generation methodologies is necessary to not only improve these models but also to enhance the drug evaluation process. Despite having great similarity to the human brain, the mouse brain still exhibits some key differences that needs to be accounted for when animal models are generated. For instance, myelin, the protective sheath insulating axons, plays a pivotal role in the proper functioning of the nervous system and is often compromised in neurodegenerative diseases. However, the composition and characteristics of myelin in mice are fundamentally different from those in humans, which can pose challenges for translating the results from mice to humans. Similarly, the immune system,

a crucial factor in neurodegenerative diseases, exhibits notable differences between mice and humans in the characteristics of both the innate and adaptive immune system and how these systems respond to an insult. Therefore, to cross the valley of death one must design models that will overcome many such disparities, which pose a threat to translation of results from mice to humans.

Despite the challenges to translation of results from animal models to humans, having models to closely study the pathophysiology is critically needed to decipher the underlying pathophysiology to design effective neurotherapeutics. For instance, from studies of the human brain, we have identified protein deposits called Lewy Bodies (aggregates of alpha synuclein, a protein that is mutated in disease brains), seen in diseases such as Parkinson's. Lewy bodies are traditionally thought to be pernicious and unequivocal markers of pathology. Further, studies from animal models showed that pathogenic Lewy bodies are the consequence of an evolutionary mechanism that otherwise protects the brain. Upon closer inspection, molecular modifications in alpha-synuclein can be seen in Lewy bodies, such as phosphorylation at serine 129, a critical molecular change in the context of Parkinson's disease. Identifying whether these molecular changes cause or a mere effect of the disease is yet to be identified. To study this, the ability to access the brain as it goes awry is critical, which is only possible to do in an animal model. With careful considerations, humanized animal models that faithfully replicate the disease can be created and would be one of the key requirements to solve the problem of translational gap.

Our current methodologies are less effective and hence most of the neurotherapeutics still have not overcome the valley of death. Perhaps what we need is a critical re-evaluation of the methodologies we currently employ for pre-clinical testing—a refinement that can close the gap between mouse models and humans and, by extension, lead to more success in finding neurotherapeutics for neurodegenerative diseases. To this end, novel, next generation humanized mouse models of Parkinson's disease are in development and will soon be available for testing. These models, unlike traditional ones, have humanized wild-type alpha synuclein protein that share the same characteristics and dynamics of the type of protein found in humans – This help to uncover normal functioning of the protein. Further to model disease pathophysiology, mouse models with mutated variants of humanized alpha synuclein are also in development. Collectively these models can cover both sporadic and familial forms of Parkinson's disease and other synucleinopathies caused by aggregation of the alpha-synuclein protein. Further, these humanized gene variants in mice are flanked with tags, allowing for easy genetic engineering that will be instrumental in understanding the mechanisms of disease. However, it is important to note that these are not the "ultimate" mouse models of disease, for many human aspects of Parkinson's disease will not be modelled with perfect fidelity. Rather, these models serve to steer us in the right direction to develop methodologies that are more relevant for biomedical research.

Nevertheless, making better mouse models is only the first step to improve the translation of current results from models to humans. Much attention is also needed to redesign other steps in our pre-clinical evaluation of therapeutics especially when it relates to our focus on biomarkers. Some of the failed neurotherapeutics have been very effective in fixing the late-stage endpoints of the disease, such as clearing the protein aggregates, but offered no significant symptomatic reprieve. Therefore, deciphering the impact of a drug on cognitive functions will serve as a more effective measure to evaluate the efficacy of the neurotherapeutics in addition to assessing the pathological and molecular betterment of the late-stage end points. However, traditional biomarkers of cognition in animal models often fall short and require the use of tasks that cannot be performed by human patients. For instance, a patient would never be forced to swim in a pool to evaluate drug effectiveness, a task often used with mice, known as the Morris Water Maze. Hence, identifying a cognitive biomarker of a disease that can be evaluated in the context of drug testing and be relevant to human clinical symptoms is a good starting point to further narrow the translational gap.

In searching for a translatable cognitive biomarker, one innovative approach involves the use of touchscreen cognitive testing for rodents. This method, inspired by touchscreen technology routinely used to assess higher order cognitive function in humans in clinics, offers a platform where cognitive abilities can be assessed in animal models in a manner that is both relevant and translatable to humans. The advantage of such cognitive tests lies in their capacity to bridge the gap between the two species, allowing for a more comprehensive evaluation of a drug's impact. By employing tests that both humans and mice can perform, researchers can gain insights into the cognitive aspects of neurodegenerative diseases that are often challenging to capture using traditional methods. Importantly, this sort of testing can expose rodents to a panoply of tasks that assess various cognitive domains like attention, learning, and memory, like how cognition is measured in human patients, bringing us closer to solving the issue of translational gap. What makes this approach particularly powerful is its ability to capture not only the immediate effects of a drug but also its impact on cognitive processes over time. This longitudinal assessment is crucial in understanding whether a drug not only alleviates symptoms temporarily but also exerts a sustained positive influence on the progressive cognitive decline associated with neurodegenerative diseases. In addition, this touchscreen system is fully automated, which can significantly reduce operator bias influence in research. In essence, the touchscreen cognitive testing method exemplifies a paradigm shift in preclinical drug evaluation. It moves beyond the traditional reliance on end-point molecular markers, neuronal death, or severe motor deficits.

Taken together, the current state of preclinical drug testing reveals a stark reality. Of the numerous drugs tested, only a fraction advance to clinical trials, and an even smaller percentage prove to be successful. This inefficient pipeline exacts a toll not only in terms of resources but, more crucially, in time. Approximately 90% of drugs that enter clinical trials fail to reach approval. We argue that the development of next generation human-relevant mouse models and a shift in our focus to study more tractable biomarkers of disease offers a more comprehensive and translatable means of assessing potential drug candidates, and brings us

close to crossing the “Valley of Death”. However, we must acknowledge potential limitations. Humanizing just one protein in a mouse will not provide a model that immaculately represents human biology but is an essential starting point that puts us in the right direction to tackle the issue of high failure of neurotherapeutics for neurodegenerative diseases. Once our approach is validated, we can further refine the approach to further improve the translation of preclinical testing to clinical success in humans.