This video series was made possible through a generous grant from the National Institute of General Medicines (Grant Number: 5 R25 GM133017-03), awarded to Cohen Veterans Bioscience (Principal Investigator: Chantelle Ferland-Beckham, PhD)

Module 3, Video 14: How preclinical research contributes to clinical research and development: the need for improved inclusion of sex as a biological variable by academic researchers

Preclinical researchers are often asked to put their research into the context of improving patient care. But sometimes the role of preclinical research in the drug development pipeline is not well understood by academic researchers. This becomes even more complicated when we consider how sex factors into this process. In this video, we will introduce the drug development process and provide recommendations for how and when sex as a biological variable should be considered in drug development.

It is widely acknowledged that there is a scientific and ethical imperative to include women in biomedical research. Female inclusion has been sparked by a paradigm shift in how we develop new thereapeutics. The old notion of one patient one treatment is no longer realistic. New therapeutics must be designed for a heterogenous patient population, accounting for the patient's unique characteristics, including their age, sex, race and pharmacogenomics. This paradigm shift has further highlighted the need for more inclusive study populations across the research spectrum [1].

At birth, the number of males and females is roughly equal. But as the population ages, the sex ratio skews further and further towards women. As a result, women are more likely to experience chronic health conditions that emerge later in life and make up a majority of the pharmaceutical consumer market [2].

Yet, the large majority of clinical treatments given to women are largely based on historical evidence derived from clinical studies predominantly conducted in men. As the field of sex differences research has grown and provided documented sex differences in metabolism, body fat distribution, and physiological mechanisms, industry-based research and development has made significant advances to improve the consideration of sex as a biological variable. But analyses of the inclusion of females in randomized controlled trials show that females are still not included at the same rates as males [3, 4], with some fields making better strides than others. Additionally, women are often excluded from clinical trials during many critical points in their lifespan, such as when pregnant, or included only if taking contraceptive medication, raising important questions about whether women's inclusion in clinical trials is representative of the true female population.

This video series was made possible through a generous grant from the National Institute of General Medicines (Grant Number: 5 R25 GM133017-03), awarded to Cohen Veterans Bioscience (Principal Investigator: Chantelle Ferland-Beckham, PhD)

The drug development process is long and expensive. Depending on the therapeutic area, the time to take a new drug target through to FDA approval may take between 9 to 15 years [5-7]. The average estimated cost of this process is 1 to 2.6 billion dollars [8, 9]. And there is a high attrition rate: only one compound in 5 to 10 thousand gains FDA approval [7]. While there are many reasons why drugs fail to reach the market, one of the most prominent is the quality of the preclinical research that the therapeutic is based on [10]. This includes the robustness and level of validation of the experimental findings as well as the predictive power of the animal model. In the context of sex as a biological variable, the limited inclusion and reporting of sex-specific effects in preclinical research, as well as the lack of validated animal models for diseases with known mechanistic or phenotypic differences in men and women, further limits investments by the pharmaceutical industry into sex-specific treatments. Thus, there is a need to reexamine HOW and WHEN to best integrate sex into drug development to improve treatment efficacy for both men and women and avoid the dangers of a sex mismatch between preclinical research and clinical trials.

In general, drug development involves a process by which pharmaceutical professionals rely on in vitro and animal research conducted by academic scientists to identify potential drug leads, such as a target pathway, enzyme, protein, receptor or gene. Pharmaceutical professionals also rely on preclinical research for animal model development AND validation. Ideally, this involves full characterization of the animal model, including the key outcome measures, and demonstrating that the results are highly replicable.

So, how and when should academic scientists include sex as a biological variable to best inform the clinical research and development process? Many of these steps have been covered in more detail throughout this video series. But to reiterate: In in vitro research, the earliest stage of drug development, the genetic sex of the cultured cells and cell lines should be noted and reported. Experiments should be conducted using both male and female cells whenever possible to identify sex differences at the earliest stage of drug development and potentially identify mechanistic insights. How the cells were maintained is also important to note, including the number of passages and the specific growth conditions. Both of these factors may lead to significant phenotypic differences in drug response. While limited in its translation to humans, it is also recommended that a single gonadal hormone, such as estrogen or testosterone, be added to cell cultures to examine the effect of the hormone on the outcome of interest.

As findings are moved to animals, the importance of addressing sex in preclinical findings to human therapeutics increases. The ARRIVE 2.0 Guidelines describe the minimum reporting requirements for animal research [12], and include transparently reporting the sex [and age] of

the animals. As already outlined throughout this video series, few reasons justify the exclusion of females from drug discovery research and the results should be properly analyzed by sex. Many diseases fluctuate in incidence, severity and symptomatology across the lifespan, especially in women. Therefore, it is also important to consider incorporating diverse animal cohorts into drug development experiments, such as animals of different ages or reproductive statuses. When a significant sex effect on your outcome measure of interest does exist, consider properly persuing the mechanism of that sex effect, as outlined in Video 13. Finally, as outlined in Video 7, a variety of other environmental factors related to housing and testing also influence differential effects by sex and should be clearly documented and accounted for statistically.

Some of the most important stages of the drug development process occur outside of the pharmaceutical industry, long before any compound is even considered for a clinical trial. The inclusion of both sexes is also critical as early as possible. Biomedical researchers in academia can assist in the development of potential therapeutics by making sure that they not only consider the effects of sex in their research program, but also accurately and completely report sex-based results. This will help improve translation and guide further therapeutic development by pharmaceutical scientists.

References

1. Collins, F.S. and H. Varmus, *A new initiative on precision medicine.* N Engl J Med, 2015. **372**(9): p. 793-5. DOI
2. Ritchie, H., *Gender Ratio*. 2019: Published online at OurWorldInData.org. p. https://ourworldindata.org/gender-ratio. DOI
3. Geller, S.E., et al., *Inclusion, analysis, and reporting of sex and race/ethnicity in clinical trials: have we made progress?* J Womens Health (Larchmt), 2011. **20**(3): p. 315-20. DOI
4. Liu, K.A. and N.A. Mager, *Women's involvement in clinical trials: historical perspective and future implications.* Pharm Pract (Granada), 2016. **14**(1): p. 708. DOI
5. DiMasi, J.A., et al., *Trends in risks associated with new drug development: success rates for investigational drugs.* Clin Pharmacol Ther, 2010. **87**(3): p. 272-7. DOI
6. Kaitin, K.I. and C.P. Milne, *A dearth of new meds.* Sci Am, 2011. **305**(2): p. 16. DOI
7. Mohs, R.C. and N.H. Greig, *Drug discovery and development: Role of basic biological research.* Alzheimer's & dementia (New York, N. Y.), 2017. **3**(4): p. 651-657. DOI
8. DiMasi, J.A., H.G. Grabowski, and R.W. Hansen, *Innovation in the pharmaceutical industry: New estimates of R&D costs.* J Health Econ, 2016. **47**: p. 20-33. DOI

This video series was made possible through a generous grant from the National Institute of General Medicines (Grant Number: 5 R25 GM133017-03), awarded to Cohen Veterans Bioscience (Principal Investigator: Chantelle Ferland-Beckham, PhD)

9. Wouters, O.J., M. McKee, and J. Luyten, *Estimated Research and Development Investment Needed to Bring a New Medicine to Market, 2009-2018.* Jama, 2020. **323**(9): p. 844-853. DOI
10. Drews, J., *Drug discovery: a historical perspective.* Science, 2000. **287**(5460): p. 1960-4. DOI
11. Tannenbaum, C. and D. Day, *Age and sex in drug development and testing for adults.* Pharmacol Res, 2017. **121**: p. 83-93. DOI
12. Percie du Sert, N., et al., *The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research.* PLOS Biology, 2020. **18**(7): p. e3000410. DOI