variants before examining variants in breast cancer carrying a pathogenic mutation. Variants in genes such as BRCA1, BRCA2, PALB2, ATM, BARD1, and MRE11 are all involved in the DNA double-strand break repair pathways, which are crucial for genome stability and cancer susceptibility. Approximately 12.4% of women will be diagnosed with breast cancer in their lifetime. About 5-10% of these cases are hereditary. Variants in genes such as ATM, BRCA1, BRCA2, and PALB2 have been linked to hereditary breast cancer, with 3-3% of women with breast cancer carrying a pathogenic PALB2 mutation. These genes are all involved in the DNA double strand break repair pathways, providing an opportunity to assay groups of genes by repair pathway. I aim to assess the utility of the fluorescent Traffic Light Reporter (TLR) system for clinical interpretation of genetic variants. I will begin by examining a validation panel of previously studied BRCA2 variants before examining variants in PALB2.

Hypothesis
The readout of HDR/NHEJ is a physiologically relevant indicator of functionality of these genetic variants.

Clinical Interpretation of Variants
Benign | Likely benign | Uncertain significance | Likely pathogenic | Pathogenic

ACMG Variant Interpretation Guidelines consider:
- Population data
- Computational and predictive data
- Functional data
- Segregation data
- De novo inheritance
- Allelic data

The Problem
Our ability to generate data and identify genetic variants on a genome-wide scale has outpaced our ability to accurately interpret these findings, given limited data connecting them to disease. Even within genes that are known to play a role in disease, variants of uncertain significance (VUS) are numerous and complicate patient counseling.

Functional Studies and Breast Cancer
Normally, tumor suppressor genes may function in cell cycle regulation, apoptosis, or DNA damage repair. Loss-of-function mutations in these genes contribute to cancer susceptibility.

Validation Variant Panel
The Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) has published a panel of BRCA2 variants for use in validation of functional assays of BRCA2.

Anticipated Results
Known pathogenic and nonpathogenic controls will calibrate the assay’s dynamic range and permit functional interpretation of undiagnosed variants.

PAB2 Variant Selection
51% of clinically identified PAB2 variants are classified as VUS, and of these, 89% are missense. Most truncating variants in PAB2 are pathogenic and there is new functional evidence of pathogenic missense mutations in PAB2.

Anticipated Studies
Batches of these variants will be assessed, with each batch including at least one known pathogenic and one known benign as controls to be run alongside variants of uncertain significance.

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Figure adapted from Daly et al., NCCN (2017)