INITIATING DRUG SCREEN FOR MUSCLEBLIND (MBNL1) MODULATORS

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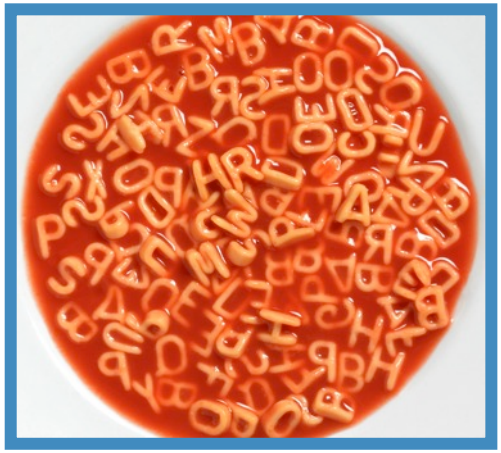
Disclaimer

This presentation may include forward-looking statements. Actual results could differ materially from those projected in forward-looking statements. The factors that could cause actual results to differ are discussed in Pfizer Inc.'s Annual Report on Form 10K and in Pfizer Inc.'s reports on Form 10Q and Form 8-K. These reports are available on Pfizer Inc.'s website at www.pfizer.com in the "Investor-SEC Filings" Section.

Talk outline

- Overview of the drug discovery and development pipeline with an emphasis on early activities
- Pfizer's approach to addressing CUG repeats pathology in DM
  - Our choice for a high-throughput screening (HTS) assay
  - Building and validating the HTS assay
- Initial screening results
  - Confirmation of biological activity in patient-derived fibroblasts



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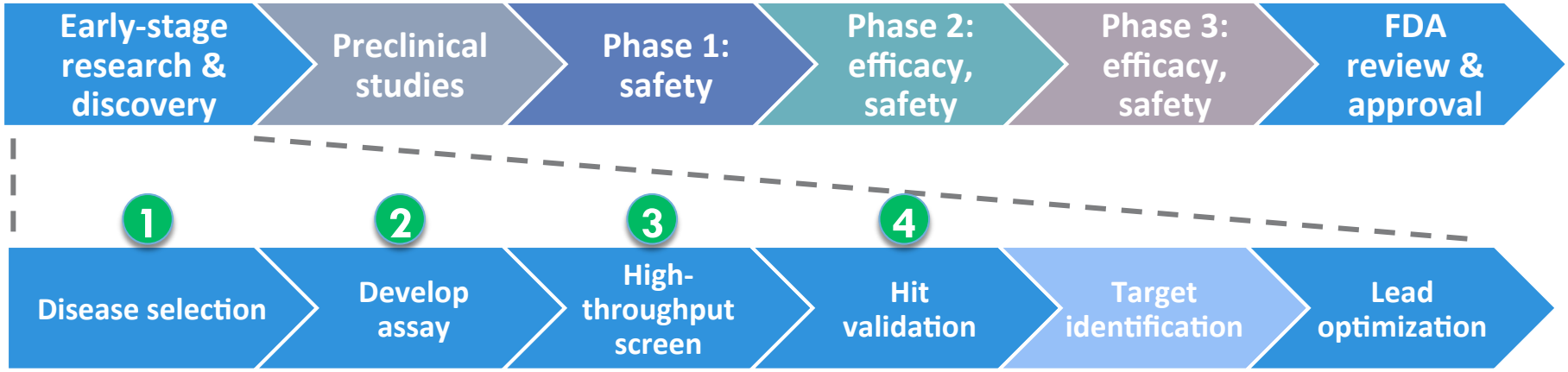
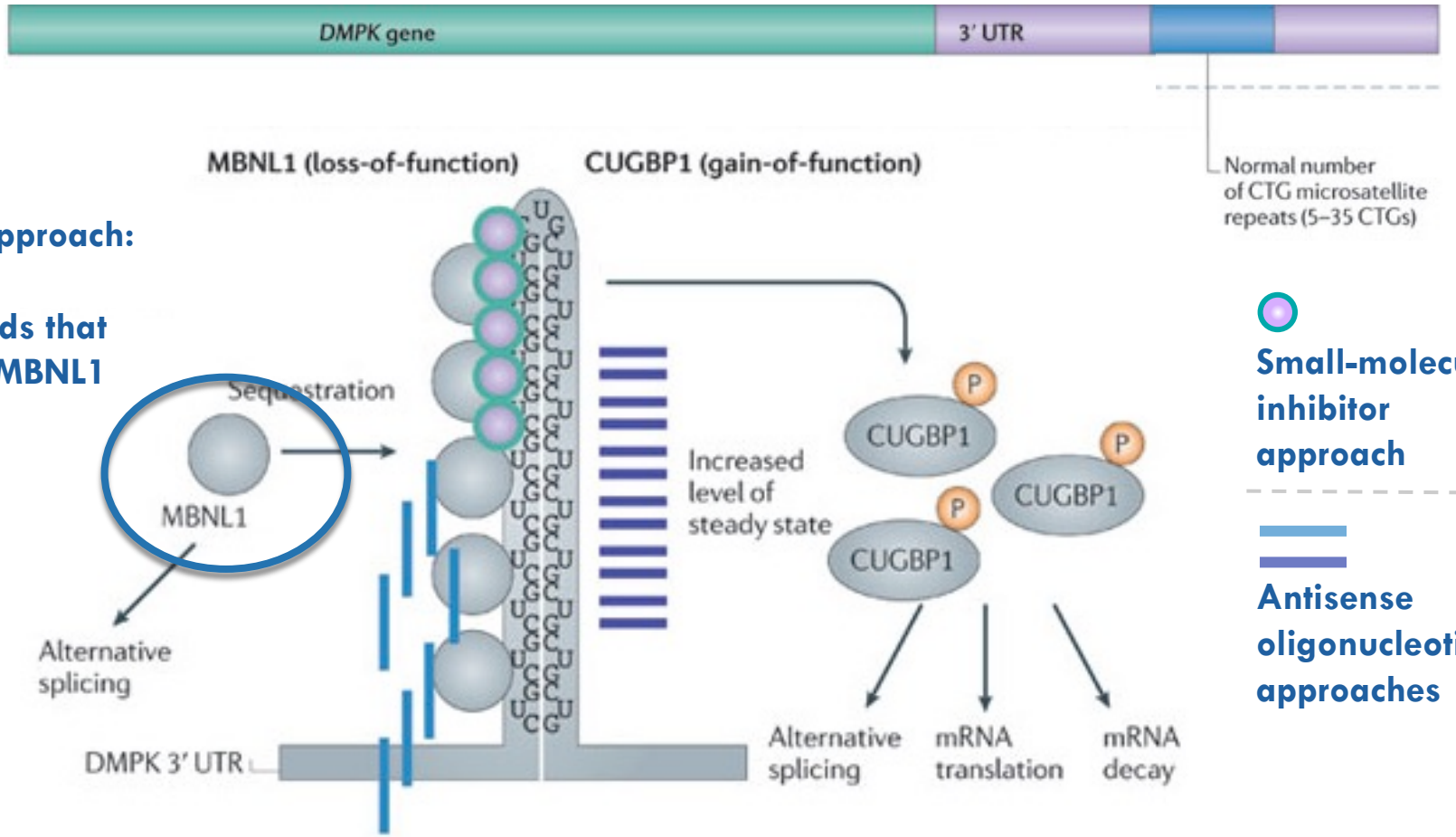
Drug discovery and development process



1. What is the disease aspect do you want to change (ie what do you want a chemical compound to do?)
2. Can you measure it? Is the assay fast? Is the assay reliable? Can assay be automated?
3. A chemical compound with a desired effect in an HTS screen is called a **hit**.
4. Hits serve as starting points for target identification, pharmacology studies (what does it do in an animal model?), ADMET/PK (how does the body absorb, distribute, metabolize and excrete the compound? How long does it stay in the body? Can the hit be chemically modified to improve on these parameters?)

Current status

Pathogenic consequences of 35–~4,000 CUG repeats in DMPK RNA



Pfizer's approach: identify compounds that increase MBNL1

Small-molecule inhibitor approach

Antisense oligonucleotide approaches

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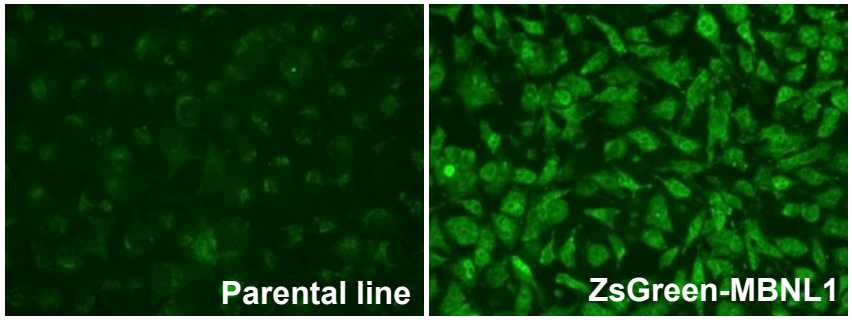
Nature Reviews Drug Discovery 10, 621-637 (August 2011)

Establish a reporter gene system amenable to high throughput screening

- Chose immortalized HeLa cells as cell system since they are easy to grow/expand and express MBNL1
- Marked the endogenous MBNL1 with a fluorescent tag
  - Used latest technology to precisely insert tag into only the MBNL1 gene
  - Very bright ZsGreen fluorescent tag
- Isolate ZsGreen-MBNL1 integrated cells



"Automated" the system to detect changes in ZsGreen-MBNL1 levels

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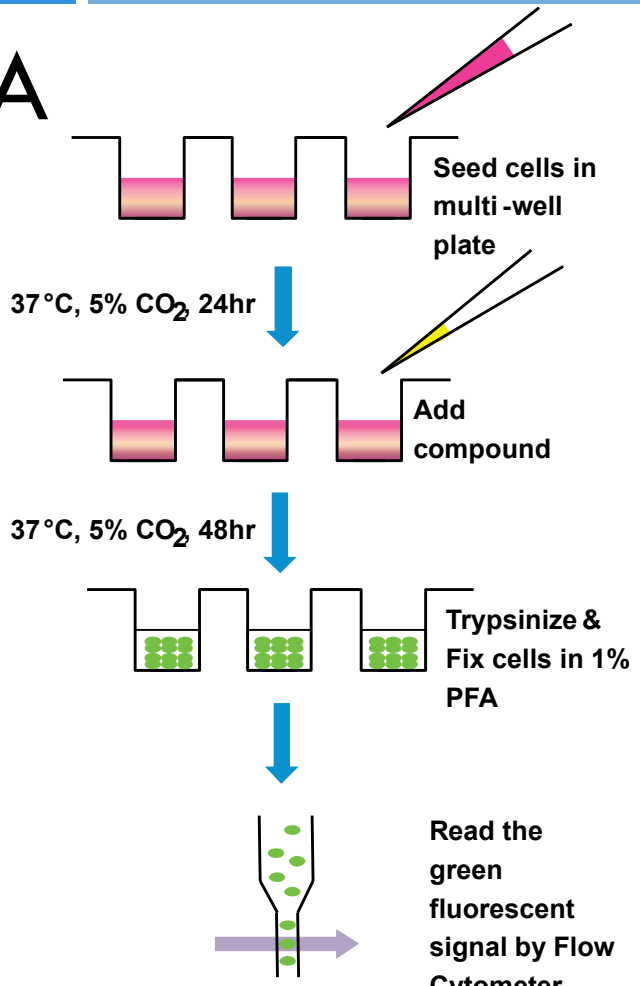

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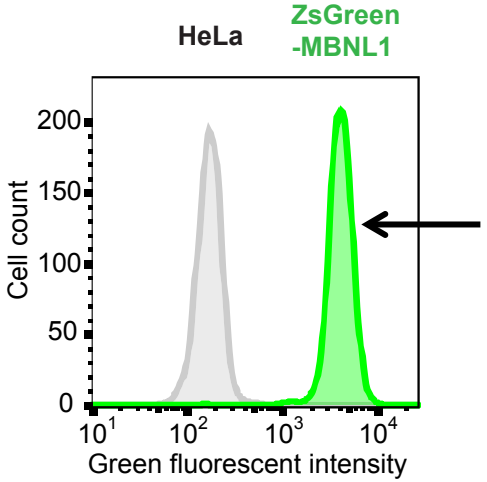

Identify compounds that increase that shift this signal

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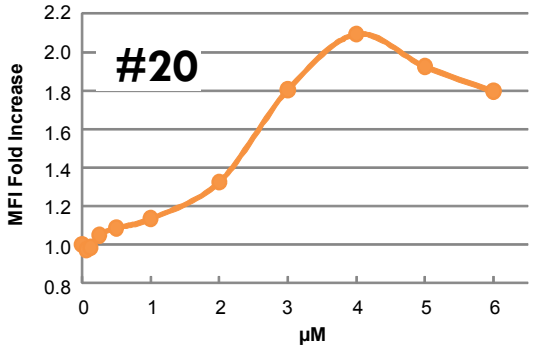
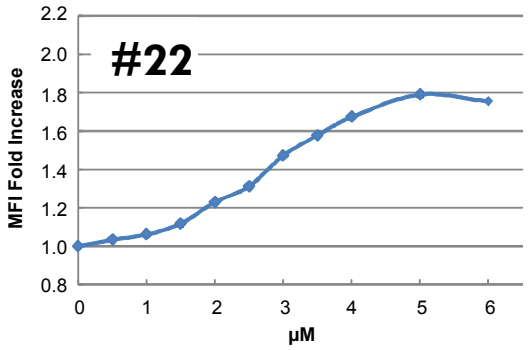
Initial screen identified compounds that demonstrated a dose-response in increasing ZsGreen-MBNL1 levels in the HeLa cell system





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Compound #20 and #22 are histone deacetylases (HDAC) inhibitors

- HDAC inhibitors open chromatin to allow genes to be expressed
- Importantly, these hits in DM1 patient-derived cells:
  A. Increased endogenous MBNL1 levels
  B. Partially rescued the aberrant splicing
- **These data help validate our screening system**

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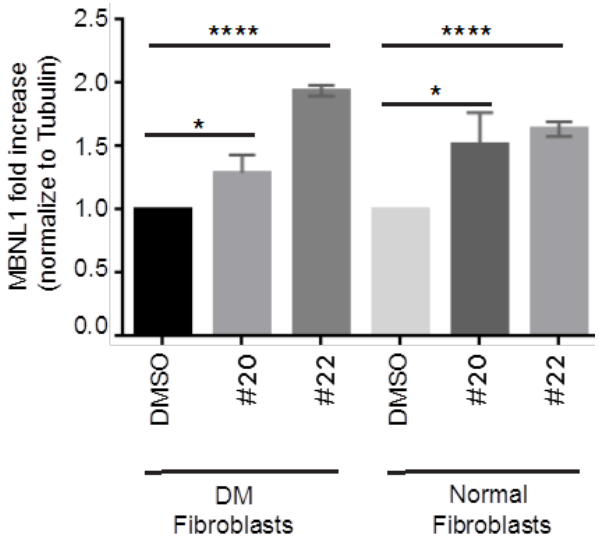

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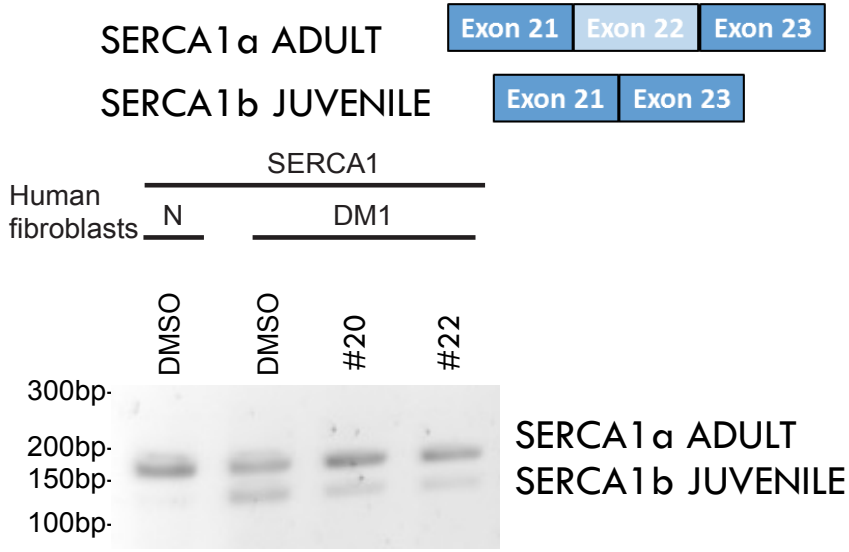
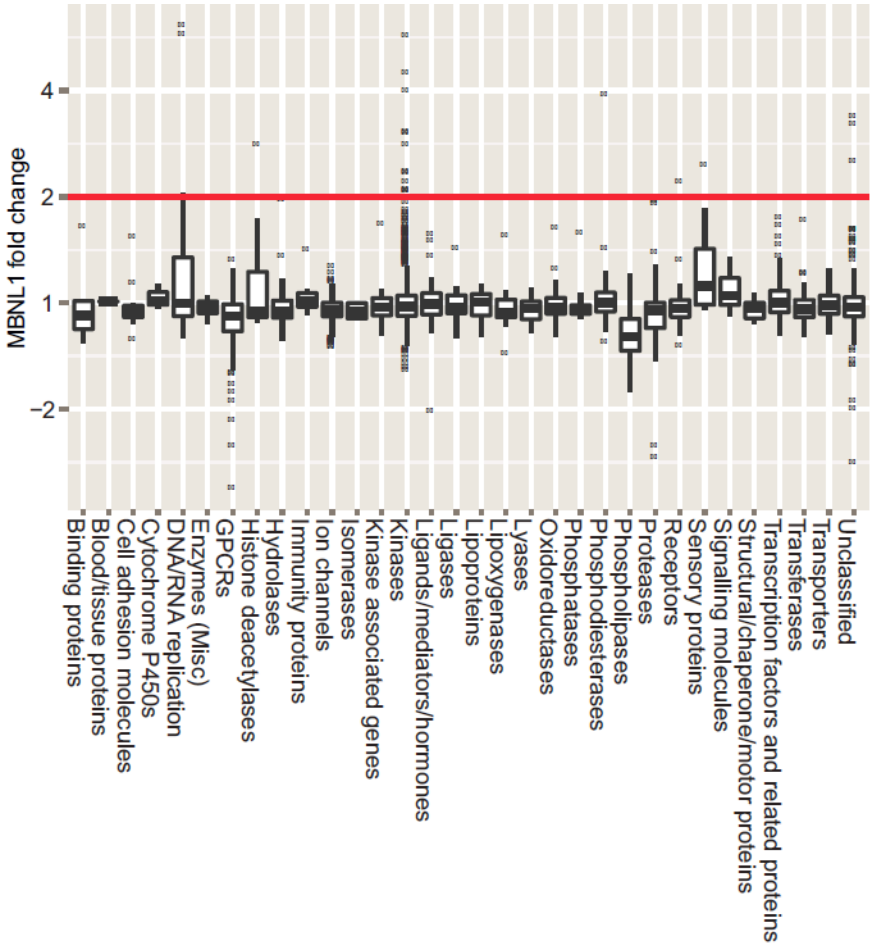


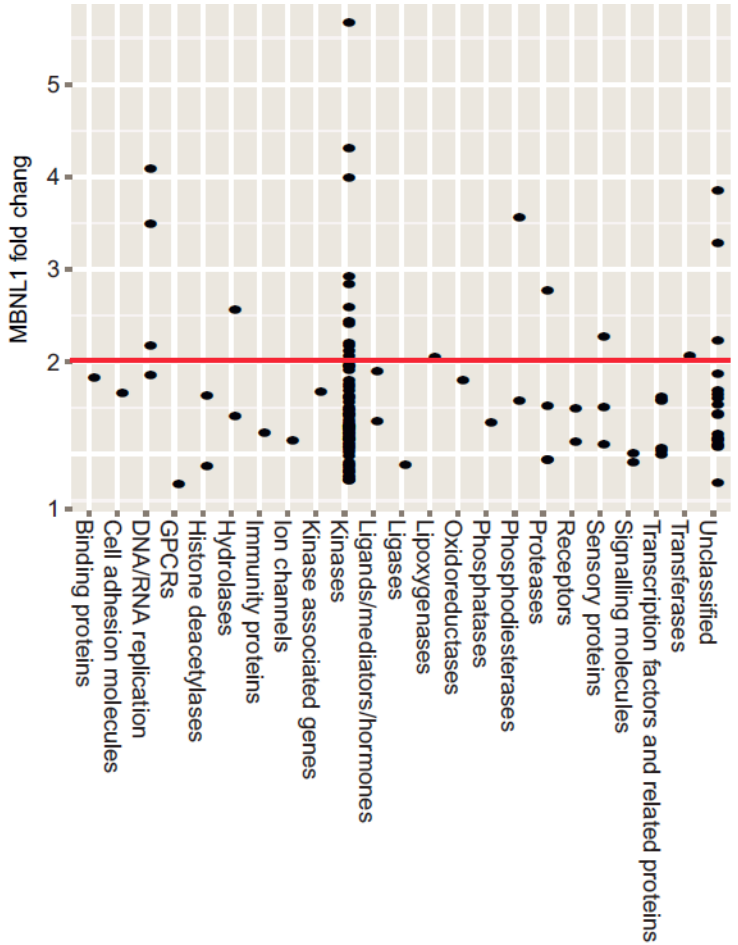
Expansion of screen to larger chemical compound libraries

- **Chemogenomic Library**
  - Well-defined, selective small molecules covering >1000 biological targets
- **FDA Drug Set**
  - Marketed drugs and clinical stage compounds with known biological activity

Compound Set	Screening Concentration	Total Compound Number	Compounds increase MBNL1 > 3×SD above the background		Compounds increase MBNL1 more than double	
			Hit Number	Hit Rate (%)	Hit Number	Hit Rate (%)
CGL	1μM	2753	128	4.6%	24	0.9%
FDA Drug Set	10μM	1040	34	3.3%	10	1.0%

Grouping Chemogenomic Library hits into targeting gene families





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Conclusions and future directions

- Conclusions:
  - We established a robust cell-based screening system for MBNL1 up-regulators
  - The initial screen identified HDAC inhibitors that increase MBNL1 level and partially rescue splicing
  - Preliminary hits from expanded library screens suggest several novel therapeutic targets
- Future Directions:
  - Validate and characterize compounds from Chemogenomics and FDA drug set screen
  - In vivo testing selected compounds in DM1 mouse model