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CONTRACTING ORGANIZATION: Duke University, Durham, NC

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13. SUPPLEMENTARY NOTES

Initiating PI Calakos will continue award in extension period. Partnering/Collaborating PI Hall at NCATS/NIH will submit distinct Final report.

14. ABSTRACT

Dystonia is a movement disorder characterized by sustained involuntary postures and/or slow twisting movements. Dystonia is an unremitting and poorly treatable movement disorder that causes pain and motor disability. Dystonia can arise in many contexts – limb trauma, traumatic brain injury, stroke, neurodegenerative diseases, antipsychotic medication use, inherited syndromes, and "sporadically". Military personnel are at increased risk to develop dystonia because of exposure to risk factors such as limb trauma, traumatic brain injury, repetitive use tasks, and certain medications. Unfortunately, both the number of treatment options for dystonia and their efficacy are severely limited. Moreover, no disease-modifying options exist; all current treatments are symptomatic. To discover new drug treatments for dystonia, we developed a robust, cell-based, high-throughput assay to monitor dystonia-related cell pathology and screened >40,000 compounds. Here we will advance the most promising candidates through medicinal chemistry optimization to develop compounds with suitable drug-like properties. Drug-like molecules will be further characterized for their activity in neuronal dystonia models from mice and humans. Lastly, using the knowledge gained during the medicinal chemistry phase, modified molecules will be made in order to capture the binding partner of the drug and identify the "target protein" through which the drug exerts its beneficial effects. The outcome of this effort will be to generate first-of-kind drugs that can be used for testing in preclinical animal models and ultimately human studies. The activities herein will also generate a comprehensive surrounding data package in order to attract commercial partners. Success in this effort has the potential to generate affordable novel dystonia drugs that can overcome the significant limitations of the current therapy options for this chronic disease.

15. SUBJECT TERMS

Dystonia, DYT1, TorsinA, rare disease, drug discovery, movement disorder, brain disease

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1. Introduction

We aim to develop orally bioavailable medicines to treat DYT1 dystonia, an inherited form of a brain disorder characterized by involuntary slow, twisting and often painful movements and postures. This therapy development is of high impact because at present there are no highly effective oral medicines for dystonia. We hypothesize that drug-like compounds that normalize or reduce mutant DYT1-associated protein mislocalization, secretory pathway impairments and neuronal activity alterations are candidate molecules to treat the human disease, and thus we plan to develop novel compounds to do so. These compounds would then be used to help identify the protein target.

2. Keywords

Dystonia, DYT1, TorsinA, rare disease, drug discovery, movement disorder, brain disease

3. Accomplishments

What were the major goals of the project?

Specific aim 1 A-D: Perform Medicinal Chemistry Optimization on Top 3 Compounds

Major task 1: Create novel analogs (2 months per cycle, NCATS)

Major task 2: Test bioactivity on cellular phenotypes (2 months per cycle, NCATS/Duke)

Milestone 1: First round of complete SAR/ADME/cellular profile package reviewed and team decision on next steps made (4 months)

Progress: 100% completion.

Specific aim 2: Determine activity of top compounds in lower-throughput, more disease proximal assays

Major Task 3: Cholinergic interneuron response to D2R dopamine agonist using het DYT1 K1 mouse model (3 months per cycle, Duke)

Milestone 2: Bioactivity known in mouse brain of dystonia model for 1-3 top compounds (month 7, 10, 13…).

Completed March 2020 (3; ritonavir & 2 novel compounds); Completed August 2022 (additional 1 novel compounds & low dose (50nM) RTV)

Major task 4: Test bioactivity in iPSC-derived cells from patients and gene edited isogenic controls. (3 months per cycle, Duke)

Milestone 3: DYT1-genotype associated secretion impairment documented in NPCs, or alternative approach cell lines as indicated (6 months).

Progress 100% completion.

Milestone 4: Secretion phenotype in hu patient iPSC-derived lines known for top 4-6 compounds (month 9, 12, 15…)

Progress: 50% completion.

Milestone 5: Identification of DYT1 genotype-dependent neuronal activity phenotype (month 12)

Progress: 15% completion

Milestone 6: hu iPSC-derived neuronal phenotypes known for top 1-3 compounds (month 15, 18, 21…)

Progress: 0% completion

Milestone 7: Prepare and submit manuscript describing human DYT1 iPSC line generation and phenotypes (month 18)

Progress: 0% completion

Specific aim 1 E-F: Obtain in vivo PK/PD/toxicity for top drug-like compound with suitable properties in Aim 1 and 2

Major task 5: Send out compounds to test 1) permeability and efflux in caco-2 or MDR1-MDCK cells; 2) cardiotoxicity and genotoxicity in hERG and Ames assays; 3) selectivity in commercially available panels of kinases, GPCRs, ion channels etc. (month 24, NCATS)

Major task 6: Perform PK experiments via multiple routes with single and multi-day doses with appropriate formulation to determine schedule for adequate brain exposure (month 24, NCATS)

Milestone 8: In vivo PK/PD/Toxicity data in mice under acute and chronic dosing regimens (month 24)

Progress: 80% completion

Milestone 9: Patent application for novel composition of matter filed (month 30)

Progress: 0% completion

Specific Aim 3: Determine drug target for top compounds

Major task 7: Synthesize tagged compounds from top 1-3 compounds for elucidation of binding proteins (month 16-19, NCATS)

Milestone 10: Modified analog with parent compound's bioactivity developed (month 19)

Progress: 100% completion

Major task 8: Perform target deconvolution experiments and quantitative proteomic analysis with top 1-3 compounds (month 19-23, Duke)

Milestone 11: Quantitative differential proteomic analysis for lead compound and controls completed (23 months)

Progress: 100% completion

Major Task 9: Manipulate target protein expression to confirm effects on compound dose-response relationship (month 23-36, NCATS & Duke)

Milestone 12: Functional experiments supporting target ID completed (month 30)

Progress: 30% completion

Milestone 13: Submit manuscript describing target ID (month 33) Progress: 0% completion

Milestone 14: 1-4 optimized compounds with anti-dystonia bioactivity and drug-like properties suitable for in vivo testing (month 36)

Progress: 80% completion

What was accomplished under these goals?

Specific Aim 1: To perform medicinal chemistry optimization on top lead compounds

Specific Aim 1A-F:

We progressed the lead chemical series through more than 20 iterative rounds of medicinal chemistry to achieve key milestones in potency towards normalizing the delE Torsin A protein distribution and drug-like properties. These are highlighted in green in the exit criteria below and the properties of key milestone compounds are shown in Table 1.

Exit Criteria		
1: HC/HTS TorsinA mislocalization assay performance		EC_{50} < 100 nM Efficacy > 70%
2: Therapeutic Window		> 4
3: Cell morphology within normal limits		< 1 SD control means
4: Functional profiling criteria:		Activity in ISR/eIF2α assay
		Neuronal rescue in mouse brain slice assay (cholinergic interneuron physiology)
5: In vitro ADME/DMPK properties:	*Stability in assay media*	> 90% at 8h
	Aqueous solubility	>10x EC_{50}
	Stability in mouse and human microsomes, gastric fluid, and plasma	$t_{½}$ > 25 min
	PAMPA Papp	> 10 X 10-6 cm/s
	Efflux ratio in MDR1-MDCK cell monolayers	< 3
	$P_{A->B}$	> 5
6: In vivo ADME/DMPK properties		Compound after single and multiple dosing is able to provide brain concentration ≥ EC_{50} in the HC/HT assay for ≥ 2 hours every day.
7: Safety Profile:		Establish maximum tolerated dose from single and multiple day study.
		No activity at hERG at 4 x EC_{50}
		Activity at less than or equal to 3 known targets at less than or equal to 2x EC_{50} (detemined via screening in commerically available panels of kinases, GPCRs, ion channels etc.)
		No activity in a genotoxicity assay such as Ames.
8: IP/legal constraints		None
		green = criteria met

Detailed summary of med chem to date: We identified three hit compounds of differing chemotypes to advance through medicinal chemistry. These leads emerged from a screen of more than 40,000 compounds and were further selected based on performance in counter screens, consideration of med chem feasibility, lack of promiscuity within the NCATS internal screening database, and lack of surrounding IP claims. Preliminary SAR was most promising for the chemotype exemplified by the initial hit compound ("Hit", first compound in Table 1, Fig. 3). This chemotype consisted of a heterocyclic core (structure is blocked to protect IP), with two pendant methyl groups, attached to a western aromatic toluyl ring. The heterocyclic core was further attached in the east via an acyclic flexible linker that ended with a carboxamide of ortho, meta-dimethoxy benzylamine. To date, we have carried out 21 rounds of rigorous iterative med chem optimization (~5-50 analogs/round on average) over three years (with productivity severely affected for about 6 months due to the work restrictions imposed by the COVID19 pandemic); the activities and properties of key benchmark compounds and their partial chemical structures are shown on Table 1 and Fig. 1 respectively. Cyclization of the benzyl amine in the hit to tetrahydroisoquinoline NCGC00508967 ('967) led to a 4-fold activity improvement in the delE TorA assay. Changing the amide to an amine in NCGC00538447 ('447) led to

NCGC00#	EC_{50} (μM)	CC_{50} (μM)	fold shift vs hit	HLM $t_{1/2}$ (min)	MLM $t_{1/2}$ (min)	RLM $t_{1/2}$ (min)	PAMPA (10^{-6} cm/s) pH 7.4	Solubility (μg/mL)	MDCK $P_{A\ to\ B}$	MDCK $P_{B\ to\ A}$	MDCK ER	hERG IC_{50} (μM)	hERG IC_{50}/ delE EC_{50}	tPSA	cLogP	HBD	HBA
Initial Hit	5.26	13.21	1.0	3	1	1	573	4.76	ND	ND	ND	ND		96	2.6	1	7
508967	1.32	>40	4.0	2	2	3	976	3.23	ND	ND	ND	12.42	9.41	69	2.63	0	5
538447	0.418	33.17	12.6	26	6	5	1051	>44	4.6	13.2	2.84	0.5	1.20	52	4.56	0	5
685598	0.187	>40	28.1	105	26	7	849	30	5.7	34.0	6.0	ND		60	4.1	1	5
602080	0.132	>40	39.8	>120	14	6	609	>66	4.5	27.3	6.0	0.98	7.42	60	4.24	1	6
689055	0.187	16.63	28.1	5	2	3	ND	ND	2.3	6.6	2.9	3.99	21.34	60	4.7	1	7
689777	0.093	>40	56.6	35	24	20	1263	>67	2.1	7.7	3.7	1.99	21.40	60	3.7	1	5
687738	11.77	>40	0.4	120	120	120	666	33	11.8	34.5	2.9	ND		60	2.83	1	5
856560	0.023	18.65	228.7	62	9	2	1710	>48	1.8	11.8	6.59	ND		52	5.08	0	5

Table 1. Activity summary of key compounds. EC_{50} = Half Maximal Effective Concentration for rectification of delE Torsin A mislocalization; CC_{50} = Half Maximal Cytotoxicity Concentration in same cell line; LM $t_{1/2}$ = Rat Liver Microsomes half life (H=human, M=mouse, R=rat); PAMPA: Parallel Artificial Membrane Permeability; Solubility = Kinetic solubility in PBS buffer; ER = Efflux Ratio ($P_{B\ to\ A}/P_{A\ to\ B}$) in MDCK assay, where A and B are apical and basal sides of a monolayer of MDCK cells; ND: Not determined.

a 13-fold left shift in the potency over the hit. Compounds with the tetrahydroisoquinoline consistently exhibited low metabolic stability in mouse live microsomes (MLM); we couldn't improve this liability despite multiple attempts in medicinal chemistry such as increasing substitution around the nitrogen (data not shown). Opening the tetrahydroisoquinoline to the linear phenethyl amine generated leads such as NCGC00685598 ('598) and NCGC00602080 ('080) that were 30-40-fold more potent than the hit and importantly had a $t_{1/2}$ > 10 min in MLM making them candidates for evaluation in preliminary PK experiments. A single IP dose of '080 at 30 mpk in C57 BL mice afforded a high C_{max} of 160 μM in plasma at 15 min (Fig. 2). Concentrations above 100 nM were maintained in the plasma till 4 h with a calculated in vivo $t_{1/2}$ of 2.2 h. However, concentrations in the brain were quite low with a K_p (ratio of $AUC_{total,brain}$ to $AUC_{total,plasma}$) of 0.06. Testing analogs for bidirectional permeability through a monolayer of MDCK cells revealed that the compounds were subject to efflux. In this assay '080 and the closely related des-fluoro counterpart '598 had an ER of ~6 (Table 1). This efflux was attenuated to an ER of 1 in the presence of a Pgp inhibitor verapamil (data not shown) implying that Pgp might be the transporter responsible for the efflux. One encouraging result from protein binding experiments was that '080 had a 5-fold unbound free fraction in brain tissue over plasma (see Table in Fig. 2).

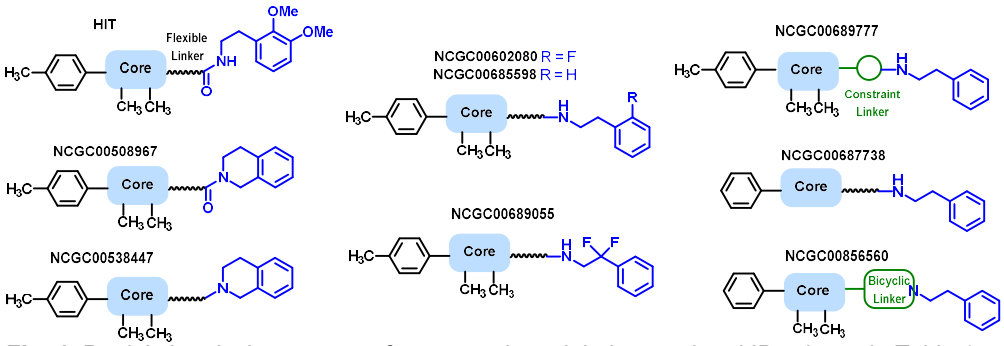


Fig. 1. Partial chemical structures of compounds and their associated IDs shown in Table 1.

The discovery of efflux in the MDCK assay led us to hypothesize that these high efflux ratios must be lowered to improve brain penetrance. To do so, we implemented several med chem strategies in our new analog designs: 1) reduce the nitrogen count, 2) reduce flexibility at the linker, 3) reduce the basicity of the amine present in the linker, and 4) increase steric hindrance around the amine. To that end, since the core was a bicyclic heterocycle with four embedded nitrogen atoms and two pendant methyl groups, we decided to 1) lower polarity by systematically removing nitrogen atoms or changing their positions in the rings, 2) reduce lipophilicity either by ring opening or contraction of either ring of the bicyclic core or by eliminating one or both pendant methyl groups, and 3) increase *sp*^{3} character to the core. To that end we synthesized ~30 analogs with novel cores, several of which demanded de novo synthetic design and execution. Unfortunately, none of these analogs showed comparable or improved potency in the primary ΔE-TorsinA mislocalization assay. Thus, we discovered that the original core structure is crucial for activity and our attempted structural variations led to either complete or partial loss in activity.

NCGC00#	602080	689055	689777
delE Torsin normalization EC_{50}	130 nM	148 nM	132 nM
% unbound plasma	0.39	0.515	2.1
% unbound brain	2.17	1	0.88
Fu brain/ Fu plasma	5.49	1.942	0.419
K_p ($K_{p,uu}$)	0.06 (0.33)	0.226 (0.439)	0.524 (0.220)
$t_{1/2}$ (hr) Plasma (Brain)	2.2 (0.6)	2.58 (?)	4.48 (?)
T_{max} (hr) Plasma (Brain)	0.083 (0.5)	0.25 (0.25)	0.25 (0.25)
C_{max} (μM) Plasma (Brain)	160 (1.3)	59 (21.16)	35 (17.92)
PAMPA ($x10^{-6}$ cm/s) >500 = good	608	ND	1263
C_u, max brain	28 nM	194 nM	158 nM
MDCK-MDR1 efflux ratio	6	2.9	3.7

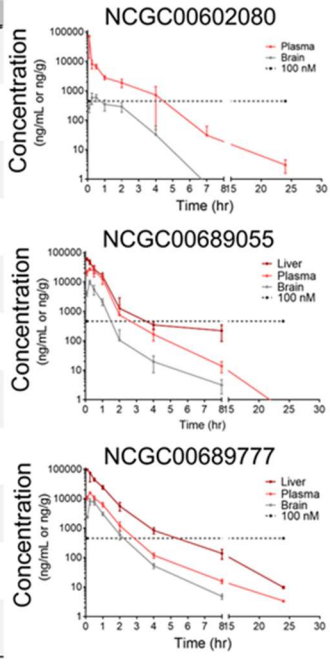


Fig. 2. PK profiles and parameters for three key compounds after 30 mpk IP single dose in male C57Bl6 mice. ND: not determined. "nM" = nmol/kg. n = 3.

From our structural modifications on the linker chain, we found that replacing the flexible linker with a constraint cyclic linker (NCGC00689777, Table 1) improved the ER 2-fold, from 6 to 3.7. Moreover, reducing the basicity of the amine by incorporating fluorine atoms beta to the nitrogen (NCGC00689055) improved the ER to 2.9. Importantly, both these analogs retained the potency of the previous leads. Unfortunately, '055 had low metabolic stability in MLM ($t_{1/2}$ ~3 min) and '777 had moderate metabolic stability with a $t_{1/2}$ of 20 min. Single IP doses at 30 mpk in mice reveal maximum unbound concentrations ($C_{u,max}$) of 194 and 158 nmol/g in brain, K_p ratios of 0.43 and 0.22 for analogs '055 and '777 respectively, and >100

nmol/kg brain concentrations for 1.5-2 h (Fig. 2). These improved brain exposures represent a significant milestone for the project because both analogs retain the potency of the previous leads. The compound '055 also has an acceptable $K_{p,uu}$ of 0.439 (an ideal value for $K_{p,uu}$ is 1) and a reduced hERG IC_{50} that is 20-fold higher than the EC_{50} in the delE TorA assay. However, we believe it will be important to have a tool compound that maintains sustained compound levels in the brain; this will be critical if a therapeutic effect is not realized with a high C_{max}. Thus, our focus is to improve metabolic stability and CNS penetrance.

Specific Aim 2:

Specific Aim 2A: Cholinergic interneuron physiology rescue in DYT1 knockin mouse model.

Completed for ritonavir (4uM) and 2 novel lead compounds. Since last update, another novel lead compound and a lower ritonavir dose (50nM) have been completed (Fig.3).

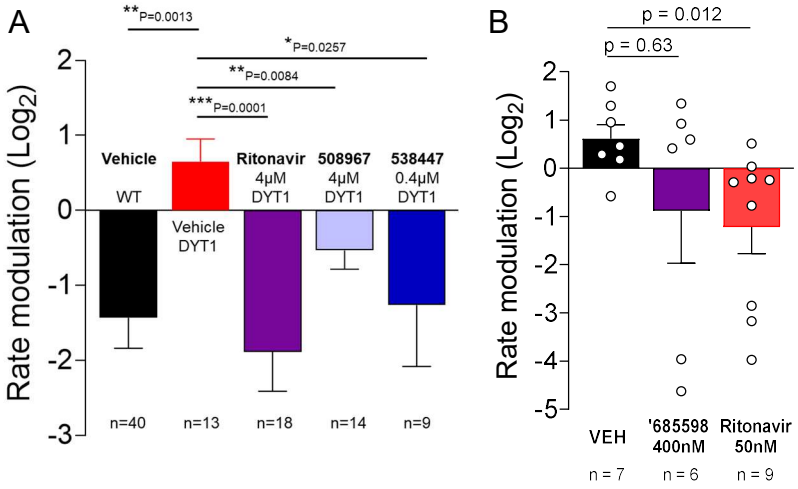


Fig. 3 Cholinergic interneuron physiology rescue in DYT1 knockin mouse model. (A) Ritonavir and 2 novel compound analogs tested at ~2X EC50 (B) Ritonavir at low dose (50nM) and more metabolically stable analog, '685598, tested at 400nM.

Specific Aim 2B: Development and deployment of patient derived iPSC models for drug testing pipeline.

(A) iPSC Line development from DYT1 affected patients and subsequent gene editing of the DYT1 mutation to create isogenic controls completed and lines onsite and in use. No updates in last funding cycle.

(B) A new opportunity to test ritonavir in a secretory phenotype in human cell lines arose from results from separately funded studies (**Proprietary Data**). In those studies, DYT1 genotype differences in secreted extracellular vesicle proteomes were identified in murine embryonic fibroblasts (MEFs; Fig 4). Additionally, the majority of differential EV proteins identified in DYT1/WT comparison was recapitulated through disruption if the integrated stress response in WT MEFs (Fig. 4). As a proof-of-concept for drugs modifying secretory phenotype(s) in relative DYT1 model systems, ritonavir was shown to correct this DYT1-specific defect in secretion of EVs (Fig. 5). Both, protein and miRNA components of secreted extracellular vesicles were normalized by ritonavir treatment.

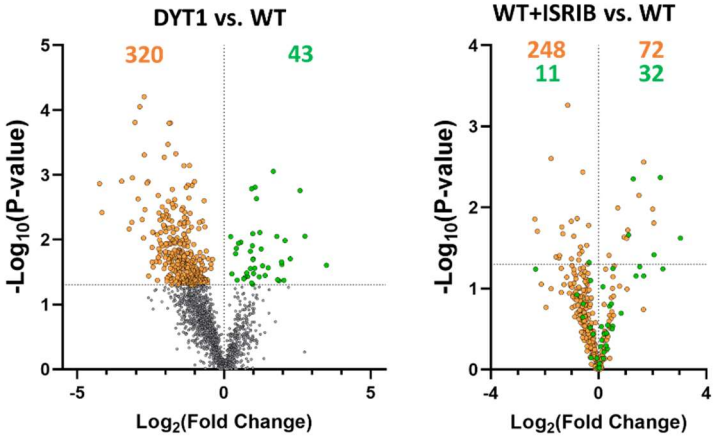


Fig. 4. (Left) Quantitative proteomics on DYT1 vs. WT EVs from MEFs. Horizontal dashed line indicates unadjusted p<0.05. Orange and green points represent proteins with lower or higher abundances, respectively, in DYT1 EVs compared to WT. (Right) Distribution of same 363 putative DYT1 genotype biomarkers in EVs from WT cells treated with ISR inhibitor (ISRIB) vs. WT (VEH) is non-random and biased towards reproducing DYT1 directionality. (n =3)

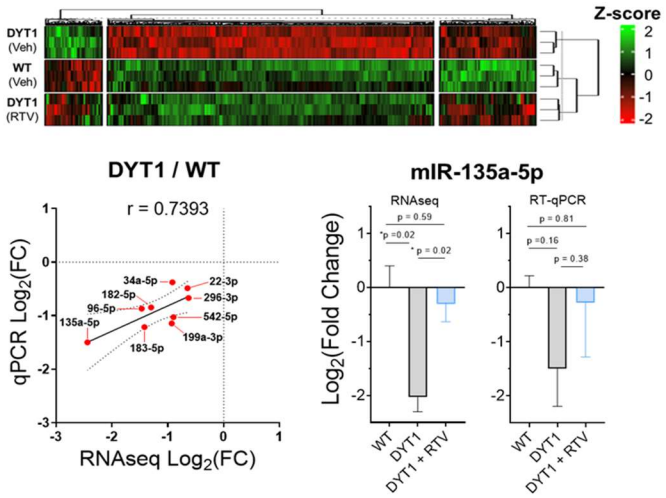


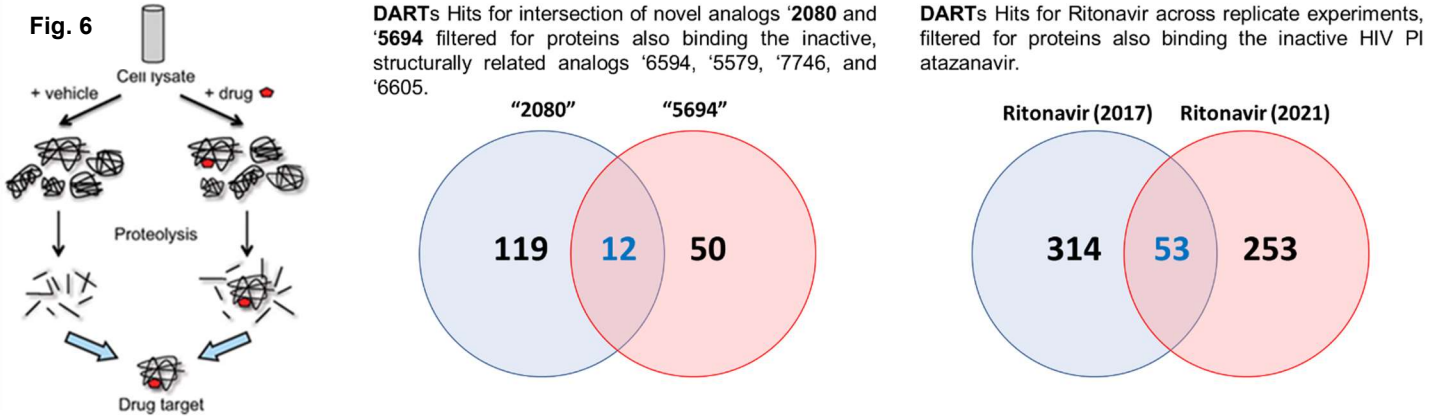
Fig. 5. (Top) Hierarchical clustering of EV proteins differential by genotype from WT, DYT1, or ritonavir treated DYT1 MEFs. Dendrogram shows DYT1 samples clustering with WT samples following ritonavir treatment. (bottom, left) Top differential miRNAs (DYT1/WT) identified by RNAseq compared to RT-qPCR replication in an independent experiment. (bottom, right) mIR-135a-5p, shown here, is one example of ritonavir normalizing DYT1 EV miRNAs as measured by RNAseq and RT-qPCR. *Welch's unpaired t-

(C) While working to establish patient-derived neuronal cell lines and identify phenotype to support drug discovery, we engaged in a collaborative opportunity with colleagues at the University of Florida to determine whether a brain MRI disturbance that has been observed in both human patients and mice was modified by our top tool compound, ritonavir. These results were strikingly positive and provide the first in vivo proof of concept data for ritonavir. Additionally, these data establish a path forward to include this phenotype for future compounds. These findings were published in the Caffall et al. *Sci.Trans.Med*. paper in August of 2021. One barrier is the time intensive nature of the effort and need for collaborator that is outside scope of original proposal. During the last funding period, we tested the ability of ritonavir to normalize white matter microstructures in DYT1 mice when administered in adult animals 2 weeks prior to DTI MRI acquisition. We have treated WT and DYT1 mice with 15mg/kg ritonavir +100mg/kg elacridar or vehicle + 100mg/kg elacridar daily for 14 days starting at P106. The mice were perfused with 10% formalin the day following final treatment and sent to our collaborators at University of Florida for DTI MRI acquisition. MRI acquisition has been completed and follow-on data analysis and interpretation is ongoing. As this represents an alternative treatment regime in adults, compared to our previous findings where treatment occurred during a discrete developmental window (~E10-P14), similar changes of DTI signals in DYT1 mouse brains would suggest a potential role for disease treatment following onset of symptoms. Proof-of-concept with ritonavir/elacridar adult treatment regime would establish another *in vivo* pipeline for testing novel compounds with increased potency/efficacy/brain availability than ritonavir. The modulation of DTI signals in the DYT1 mouse brain is a key assay for pre-clinical assessments in our expansion award application.

Specific Aim 3: Determine protein target of top compound.

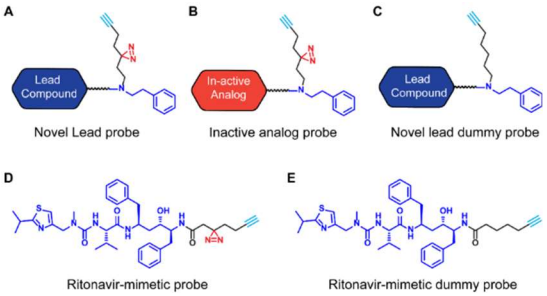
In prior funding cycle, DARTS (drug affinity responsive target stability) a drug target identification experiment was performed on top compounds (2 related compounds that were active in our assays, and 4 other related compounds that were inactive in our assay, as controls) as well as Ritonavir and Atazanavir (active and inactive in our assays, respectively). Duke's Proteomics Core ran TMT (tandem mass tag) mass spectrometry on the samples from these experiments. We have used trimmed normalized Cohen's D values for protected and de-protected peptides across multiple experiments (ritonavir x inactive atazanavir in 2017 and 2021, and novel compounds by inactive analogs in 2021). These normalizations allowed for directed comparison within and between separate proteomics data sets. Using this comparison, potential DYT1 specific drug targets have been identified for ritonavir and active novel compounds, '2080 and '5694.

Fig. 6



This final analysis has identified 12 protein hits for novel compounds '8020 and '5694 as well as 53 protein hits for the HIV protease inhibitor, ritonavir (Fig. 6). Small molecules putatively modulation 14 of these hit proteins were acquired and used for synergistic matrix testing between putative target modulating drugs and novel compound '2080 or ritonavir. None of the 14 putative protein target modulating drugs showed overt synergy or antagonism for the anti-DYT1 activity of '2080 or ritonavir in our primary torsinA mislocalization assay. As the directionality of the relationship between either '2080 or ritonavir is unknown, the inhibitory effect that is the predominant activity reported for the 14 compounds tested, may not be poised to enhance or impede the specific anti-DYT1 effect(s) of '2080 or ritonavir, further testing is ongoing, beginning with genetic knock-down/knock-out and overexpression of putative targets within the primary DYT1-TorsinA mislocalization assay. Identification of additional small molecules modulating these hits is ongoing.

In parallel to our label-free target deconvolution efforts (DARTs), consideration of other probe molecule approaches was underway, such as feasibility of “CLICK”-able and photoaffinity modifications to the parent compound to maintain potency while providing opportunities for co-precipitation of potential compound target molecules. Photo-reactive “Click”-able probes were synthesized based on ritonavir and a novel lead compound (‘685598) that were shown to maintain potency and efficacy in cell based anti-dyt1 assay (Fig. 7). Target crosslinking experiments in situ and in cellular lysates are currently ongoing (Fig. 8).



NCGC00#	Compound	EC_{50} µM
685598	Novel Lead	0.016
856376 (A)	Novel Lead Probe (5)	0.31
856373 (B)	Inactive Control Probe	N.D.
856375 (C)	NR Novel Probe (5)	0.026
159462	Ritonavir	7.34
856374 (D)	Ritonavir-mimetic Probe(2)	2.14
856372 (E)	NR Ritonavir-mimetic Probe(2	2.07

NR = Non-reactive; ND = Not detectable

Fig. 7. Photoaffinity probe designs based on novel lead compound (probe 5) and ritonavir (probe 2). EC_{50} chart showing retention of anti-DYT1 activity in primary assay.

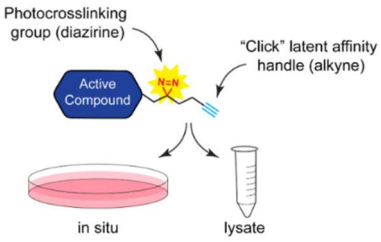


Fig. 8. Photocatalytic proximity labeling paradigm.

What opportunities for training and professional development has the project provided?

1. Poster presentation by Connor King of *Extracellular vesicle biomarker discovery for dystonia using proteomics* at the 2021 Annual Neurological Association Meeting.

How were the results disseminated to communities of interest?

Publications and seminars. In this funding cycle, results were presented at the American Neurological Association annual meeting 2021, Stanford University institutional seminar, and Tyler’s Hope for a Dystonia Cure disease foundation annual meeting.

The results from this project were disseminated in the form of a publication in a peer-reviewed journal (*Caffall et al., Sci. Transl. Med.* **13,** eabd3904 (2021)) and was selected as the journal’s cover story.

What do you plan to do during the next reporting period to accomplish the goals?

In the next reporting period, Duke will (a) photoaffinity capture of drug interactions using newly synthesized probes and validate drug targets identified in the quantitative proteomics screening of DARTS experiment in Milestone 11; (b) manipulate target protein expression to confirm effect on the dose response of the compound (Major task 9); (c) work on a manuscript for this once validated (Milestone 13); (d) dose animals in vivo with novel compounds (Milestone 14); (e) analyze proteomics results of EV biomarkers and incorporate into drug profiling efforts if suitable. (Milestone 4) (f) work on a manuscript for this once validated.

4. Impact

What was the impact on the development of the principal discipline(s) of the project?

The findings reported in Caffall et al., Sci Transl Med. article achieved a number of important advances for the field of dystonia. First, they established that a drug acting through the biochemical pathway known as the integrated stress response has corrective effects on preclinical DYT1 models. Second, they identified an FDA-approved compound with DYT1 corrective active, opening up repurposing opportunities. Third, they showed a very striking proof of principle that the discovered drug might have disease-modifying potential because drug was delivered for only a brief perinatal window to achieve sustained normalizing effects on brain microstructure as measured later by MRI in adulthood.

What was the impact on other disciplines?

The cell biology field concerning the integrated stress response, a cell homeostasis pathway, is advanced through knowledge of its impact on this particular disease, dystonia.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. Changes/Problems

Changes in approach and reasons for change

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

The NCATS and Duke laboratories were running at reduced capacity from August 2020 – July 2021 as a result of the COVID pandemic. All projects slowed as a result. Work has resumed, and revised timelines have been noted in the cases where the impact was the greatest.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects
None to report

Significant changes in use or care of vertebrate animals.
None to report

Significant changes in use of biohazards and/or select agents
None to report

6. Products

Publications, conference papers, and presentations

a. ***Journal publications*****.**

Zachary F. Caffall, Bradley J. Wilkes, Ricardo Hernández-Martinez, Joseph E. Rittiner, Jennifer T. Fox, Kanny K. Wan, Miranda K. Shipman, Steven A. Titus, Ya-Qin Zhang, Samarjit Patnaik, Matthew D. Hall, Matthew B. Boxer, Min Shen, Zhuyin Li, David E. Vaillancourt, Nicole Calakos, *The HIV protease inhibitor, ritonavir, corrects diverse brain phenotypes across development in mouse model of DYT-TOR1A dystonia***,** Science Translational Medicine; federal support acknowledged, yes.

b. ***Books or other non-periodical, one-time publications*****.** *None to report*

c. ***Other publications, conference papers, and presentations.*** *None to report*

Website(s) or other Internet site(s): *None to report*

Technologies or techniques: *None to report*

Inventions, patent applications, and/or licenses: *None to report*

Other Products: *None to report*

7. Participants & Other Collaborating Organizations

What individuals have worked on the project?

Name:	*Matthew Hall, Ph.D.*
Project Role:	*Project PI (NCATS)*
Researcher Identifier (e.g. ORCID ID):	0000-0002-5073-442X
Nearest person month worked:	1.5
Contribution to Project:	*Dr. Hall has directed and oversaw the project at NCATS in the area of biology, medicinal chemistry, informatics and project design.*
Funding Support:	NIH intramural funds

Name:	*Yaqin Zhang, M.S.*
Project Role:	*Biology research scientist (NCATS)*
Researcher Identifier (e.g. ORCID ID):	0000-0001-6067-980X
Nearest person month worked:	2.5
Contribution to Project:	*Ms. Zhang has performed work in the area of biology and assay development.*
Funding Support:	NIH intramural funds

Name:	*Min Shen, Ph.D.*
Project Role:	*Informatics project lead (NCATS)*
Researcher Identifier (e.g. ORCID ID):	0000-0002-8218-0433
Nearest person month worked:	1.5

Contribution to Project:	*Dr. Shen has led and oversaw the work in the area of informatics and project design.*
Funding Support:	NIH intramural funds

Name:	*Samarjit Patnaik, Ph.D.*
Project Role:	*Chemistry project lead (NCATS)*
Researcher Identifier (e.g. ORCID ID):	0000-0002-4265-7620
Nearest person month worked:	2.5
Contribution to Project:	*Dr. Patnaik has led and oversaw the work in the area of chemistry and project design.*
Funding Support:	NIH intramural funds

Name:	*Vinoth Kumar Chenniappan, Ph.D.*
Project Role:	*Chemistry research scientist (NCATS)*
Researcher Identifier (e.g. ORCID ID):	0000-0003-0844-1567
Nearest person month worked:	12
Contribution to Project:	*Dr. Chenniappan has performed work in the area of medicinal chemistry, project coordination and project design.*
Funding Support:	NIH intramural

Name:	*Diego Moya, Ph.D.*
Project Role:	*NIH IRTA postdoc fellow (Chemistry) (NCATS)*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	3
Contribution to Project:	*Dr. Moya has performed work in the area of medicinal chemistry.*
Funding Support:	NIH intramural

Name:	*Nicole Calakos, MD, PhD*
Project Role:	*Project PI (Duke)*
Researcher Identifier (e.g. ORCID ID):	0000-0002-9918-3294

Nearest person month worked:	2.0
Contribution to Project:	*Dr. Calakos oversees all her lab's work on this project, as the PI for the lab.*
Funding Support:	NIH, Foundation and Institutional discretionary funds

Name:	*Miranda Shipman*
Project Role:	*Research Analyst (Duke)*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	7
Contribution to Project:	*Ms. Shipman worked to determine a drug target for the top compounds and has performed the TorsinA localization assays to determine compound bioactivity.*
Funding Support:	NIH funds

Name:	*Zachary Caffall, MS*
Project Role:	*Research Analyst, Sr (Duke)*
Researcher Identifier (e.g. ORCID ID):	0000-0003-4601-6799
Nearest person month worked:	
Contribution to Project:	*Mr. Caffall has determined the importance of eIF2 alpha pathway signaling as a mechanism of action through which the HIV protease inhibitor, ritonavir, can modify in vitro, ex vivo, and in vivo phenotypes associated with human cell lines and mouse models of dystonia. He continues to oversee drug mechanism of action studies at Duke.*
Funding Support:	*Institutional discretionary funds*

Name:	*Josiah J. Sampson*
Project Role:	*Research Technician II (Duke)*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2.0
Contribution to Project:	*Technical support drug screening primary and secondary assays*
Funding Support:	

Name:	*Callie B. Eatman*
Project Role:	*Research Technician II (Duke)*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2.0
Contribution to Project:	*Technical support cell lines, ihc*
Funding Support:	Private Foundation

Name:	*Kaylee D. Bayles*
Project Role:	*Research Technician II (Duke)*

Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1.0
Contribution to Project:	*Animal colony care and genotyping*
Funding Support:	NIH funds

Name:	*Qiang Li, MD, PhD*
Project Role:	*Research Scientist (Duke)*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	*Dr Li is an accomplished electrophysiologist and is working to determine the drug effects on disease associated neuronal physiology including striatal cholinergic interneuron firing rates and hippocampal LTD.*
Funding Support:	NIH funds

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Since the start of this project, Dr Calakos has ended support on the following projects:

- None

She has begun work on the following projects:

- Title: Circuit Mechanisms For Dopamine Neuron Vulnerability and Resilience in PD
  Source of Support: Michael J. Fox Foundation for Parkinson's Research
  Project Start and End Date: 11/1/2021 - 10/31/2024
  Total Award Amount (including Indirect Costs): 8,999,577.50

- Title: Calakos: DCP3
  Source of Support: Dystonia Medical Research Foundation / NIH
  Project Number: U54-NS116025
  Project Start and End Date: 11/24/2021-8/31/2024
  Total Award Amount (including Indirect Costs): 75,000

What other organizations were involved as partners?

Nothing to Report

8. Special Reporting Requirements

Because only one institution is entering a no-cost extension period (Duke), an annual progress report will be submitted by the initiating PI (Duke/Calakos) and a final report will be submitted by Partnering PI (NCATS/Hall).

9. Appendices

None