TRxA
CRITICAL PATH INSTITUTE

FUNDING OPPORTUNITY FOR DRUG DISCOVERY AND DEVELOPMENT

Pre-Proposal Example

1. **Project title**

   Neuregulin-1 type-III modulators for the treatment of Charcot-Marie-Tooth Type-1B.

2. **Names, contact information, and brief background** *(1000 characters maximum)* **of principal investigator(s) that highlights expertise available to the proposed project.**

   Jane Smith, Ph.D. Dr. Smith is Professor at the University in the Department of Pharmacology. She is a medicinal chemist with expertise in drug development, where she has advanced drug candidates from discovery through Phase 2 clinical trials. She has extensively studied TACE inhibitors in animal models in a range of disease models including cancer, CMT, and muscular dystrophies. Dr. Smith will oversee the experimental design, data analysis and reporting.

3. **Names and contact information for co-investigators within or outside your institution, as well as a brief explanation** *(1000 characters maximum)* **about the reason for the collaboration and the expertise of those listed.**

   Dr. Okeye, Ph.D., Assistant Scientific Investigator. Okeye is an associate professor in the department of Chemistry at the University. Dr. Okeye's group expertise includes X-ray crystallography and focuses on determining protein structure and its underlying function. Dr. Okeye has been involved with this project since inception and the group has developed co-crystallization routines for the small molecule TACE inhibitors.

4. **Name and email of Technology Transfer Office Rep**

   Laura Rychetsky, LRychetsky@university.edu

5. **Name and email of Award Notification Recipient**

   Richard Escobar, Awards@university.edu

6. **Type of award being requested (Stage 1, 2 or 3)**

   Stage 1

7. **Total funding being requested (in USD)**

   $250,000

8. **Please provide a non-confidential project abstract.** This non-confidential information may be shared with external reviewers and potential co-funders *(2200 characters maximum).*

Charcot–Marie–Tooth (CMT) neuropathies are a group of genetic disorders that affect the peripheral nervous system with heterogeneous pathogenesis. Type-1B CMTs are characterized by reduced levels of myelination. Axonal neuregulin 1 type III (Nrg1TIII) drives peripheral nerve myelination by activating downstream signaling pathways such as PI3K/Akt and MAPK/Erk that converge on master transcriptional regulators of myelin genes, such as Krox20. We propose to optimize our novel series of CNS penetrable non-hydroxamic acid small molecule modulators of Nrg1TIII as a therapeutic strategy to treat Type-1B CMTs. Our lead series of compounds suppress the Nrg1TIII inhibitor, tumor necrosis factor-α-converting enzyme (TACE/ADAM17) and has pronounced efficacy *in vitro* and *in vivo*. *In vivo* treatment stimulates Nrg1TIII signaling and ameliorates neuropathy in a mouse model of demyelinating CMT1B. Modulation of Nrg1TIII by TACE inhibition may represent a general treatment for other hypomyelinating neuropathies. We have established a screening tree to profile the newly proposed Nrg1TIII modulators; it is expected these modulators will have appropriate CNS penetrability, safety, and PK/PD profiles to be orally available candidate molecules for treating CMT1B.

9. What is the therapeutic indication and the target population of this new drug product *(375 characters maximum)*?

Early infantile onset Charcot-Marie-Tooth Type-1B caused by mutations in the *MPZ* gene (1q22), which account for approximately 6% of American CMT patients.

10. What is the biological target and/or pathway of the compound(s) *(260 characters maximum)*?

Tumor necrosis factor-α-converting enzyme (TACE/ADAM17), which inhibits neuregulin 1 type III (Nrg1TIII).

11. Is there structural information for the target? If yes, please describe. *(1500 characters maximum).*

TACE consists of a multidomain extracellular (EC) region, a transmembrane helix and an intracellular C-terminal tail. The EC region comprises an N-terminal pro domain, a 259-residue catalytic domain, and a Cys-rich moiety. TACE sequence indicates similarity with the MMPs. In comparison to MMPs, the peptide chain of the TACE catalytic domain is longer and is stable in the absence of calcium and exhibits a different inhibition pattern toward synthetic inhibitors. In contrast to the MMPs, TACE cleaves a 12-mer peptide spanning the cleavage site in pro-TNFα selectively between Ala and Val. There are multiple bound co-crystal structures of TACE in complex to inhibitors at <2.0 Å resolution available (RCSB PDB: 1ZXC, 2A8H, 2DDF). We have co-crystallized four of our lead inhibitors with the V353G mutant of TACE. The propensity of TACE to undergo autoproteolysis makes WT enzyme handling difficult and impedes the identification of inhibitor soakable crystal forms. V353G is located at the non-prime site of the substrate binding site. Because the inhibitors analyzed in our studies bind to the prime site, the crystal structures mimic the conformation remarkably similarly to the WT form. Co-crystal structures for the anti-target MMPs are available: MMP-1: 966C; MMP-2: 1HOV; MMP-3: 1CIZ; MMP-7: 2DDY; MMP-13: 3WV1. We can optimize potency against TACE while minimizing off-target activity against the MMPs by utilizing GLIDE docking scores versus all targets prior to chemical synthesis.

12. How does this project address an unmet medical need *(450 characters maximum)*?

CMT neuropathies have a prevalence of 1 in 2,500 persons and are one of the most common neurogenetic diseases. As with many neurogenetic disorders, the treatment of CMT has been symptomatic for over 100 years. There is no treatment for any CMT. Current management relies on rehabilitation therapy, surgery for deformities, and symptomatic treatment of pain and fatigue.

13. **Describe the novelty of the project's approach.** If there are marketed products available for the stated indication, or if similar research is being done in this area by competitors, what differentiates this project *(1000 characters maximum)*?

Mechanistically, the Zn^{2+} ion in TACE and other MMPs acts as an electrophile that activates the scissile C=O group, then a water nucleophile hydrolyses the peptide bond[1]. Clinical-stage inhibitors have been based almost entirely upon hydroxamic zinc-binding groups. Shortcomings of hydroxamates include poor pharmacokinetics[2], poor selectivity profiles across the MMPs[3], and lack of metal binding selectivity which is the putative cause of toxic side effects[4]. Clinical development of non-hydroxamates has been less intense, likely due to lower immediate potency. Our lead non-hydroxamate TACE inhibitors are equipotent to competitors but have significantly higher fold selectivity over MMPs, which will lead to a superior safety profile.

14. **Is there genetic evidence of the relevance of your target in the therapeutic indication you propose?** For example, are there naturally occurring mutations in humans that illustrate the role this target plays in the disease *(1000 characters maximum)*?

Murine CMT1B models present degrees of hypo-, de- and re-myelination of peripheral nerves, mechanisms include both loss and gain of function[5]. Scapin[6] demonstrated that Nrg1TIII signaling can ameliorate neuropathy. Axonal Nrg1TIII drives a Schwann cell choice between myelinating or non-myelinating phenotypes[7], levels of Nrg1TIII determine the amount of myelin and thickness[7,8]. Nrg1 acts through signaling pathways that control master regulators such as Krox20, which activates transcription controlling the synthesis of myelin proteins and lipids[9]. Nrg1TIII activity is regulated by TACE which cleaves Nrg1TIII to inhibit myelination[10]. Suppression of TACE should lead to increased Nrg1TIII signaling and amelioration of neuropathy.

15. **What scientific rationale, in addition to any genetic evidence mentioned earlier in this pre-proposal, is in place that manipulation of this target results in amelioration of disease?** *(2000 characters maximum)***?** Please provide figures as appropriate; figures must be readable as printed on a single 8.5" x 11" page at normal 100% scale, so please ensure appropriate resolution. If appropriate, upload this one page with up to four (4) figures to illustrate scientific concepts and findings.

Overexpression of Nrg1 overcomes impaired nerve development in murine models. In S63del mouse model[11] that capitulates hypomyelination followed by demyelination, misfolded $P0^{S63del}$ is retained in the ER and fails to be incorporated into myelin[12], Schwann cells mount a canonical unfolded protein response. Augmentation of Nrg1TIII overcomes the intoxicated process and ameliorate hypo-, de- and re-myelination[12]. Western analysis on spinal cord lysates in a S63del transgenic mice overexpressing an HA-Nrg1TIII (HANI) fusion protein probed with anti-HA antibody, confirms the expression of the HANI transgene in HANI/+ and HANI/+//S63del mice **(Fig 1)**. Neurophysiological analysis on 6m mice showed that overexpressing Nrg1TIII resulted in a significant increase of nerve conduction velocity, closer to WT values as compared with neuropathic mice **(Fig 2)**. These results indicate that overexpression of Nrg1TIII leads to a significant amelioration of neurophysiological parameters.

TACE inhibitors can promote myelination in *ex vivo* myelination model organotypic explant cultures of myelinating dorsal root ganglia (DRG). BMS-561392 (1 µM/14 day) treated WT and S63del DRGs **(Fig 3)** stained for myelin basic protein (MBP) show an increase in the number of myelinated segments in both S63del **(Fig 3C)** and WT treated samples **(Fig 3B)**, indicating that TACE inh. ameliorates myelination. BMS-561392 is not BBB permeable but demonstrates that a CNS penetrant candidate represents promising therapeutic candidates for hypomyelinating CMTs.

16. **Is there a validated biomarker or clinical assessment available that can be used in human trials and/or preclinical animal experiments that is reasonably likely to predict clinical outcome?** If yes, please describe. *(1500 characters maximum)*

The CMT neuropathy score (CMTNS) is a nine-item composite scale taking into account sensory and motor symptoms, that can be applied as a primary outcome measure in clinical trials[13]. The CMTNS ranges from 1 (good clinical performance) to 36 (severely affected) but is reported to increase only *ca.* 0.68 points per year. Therefore, insensitive outcome measures may increase the risk of false-negative results and a biomarker will be required to monitor therapeutic effect in a clinical trial. Since our therapeutics increase Nrg1TIII levels through TACE inhibition *via* ERK1/2 and Akt signaling, mechanistic biomarkers could be levels of p-Akt and p-ERK. Biomarkers of degenerating axons or Schwann cells that can measure disease severity and/or progression include neurofilament light chain (NfL) as a marker of axonal damage as it is elevated in blood samples from CMT patients and correlates with disease severity is also a possible marker. Of note, NfL has recently been used as a surrogate endpoint in accelerated approval of a new therapy for Amyotrophic Lateral Sclerosis[14]. Schwann cell-specific protein, TMPRSS5, is also a potential biomarker. In $P0^{S63del}$ transgenic mice, Schwann cells express transcription factor C/EBP homologous protein (CHOP), a protein previously reported to induce apoptosis in ER-stressed cells which could be used as a biomarker.

17. **Are there any predictable safety issues that need to be considered in light of the target, anticipated dosing regimen and/or any liabilities of the compound(s)** *(450 characters maximum)***?**

Previous TACE inhibitors have failed in the clinic due to musculoskeletal side effects. Though the exact reason for this side effect is unknown, literature suggests the toxicity of these molecules is due to their ability to inhibit MMP-1 and/or MMP-14. Analogues will be evaluated for toxicity in a mice model of spontaneous nociceptive and pain-like behaviors.

18. **What is the status of any IP associated with this project and this compound/compound series?** Provide patent or application numbers if published *(1500 characters maximum)*

A disclosure has been filed with the University Tech Transfer Office (TTO) informing of the chemotype. A provisional application covering a first generation of structurally distinct compounds was filed in June 2017 (US62/365168) and converted to PCT in June 2018 (published as WO2018017954A1). The PCT was nationalized only in the US (US20200188524A1) and is currently in prosecution at the USPTO. We will file a patent application to cover the new series of compounds, which show increased CNS penetration and higher-fold selectivity versus MMPs. IP will be owned by the University Board of Regents. The new compound series has not been previously disclosed (publication, conference proceeding or student thesis) and a prior art/competitive landscape search conducted by the TTO has not identified any disclosures of immediate concern. We would propose to delay patent filing until the scope of the structure-activity relationship is better understood to prevent generating prior art against our own future filings and to manage filing costs.

19. **What are the next steps needed to drive the project towards IND and/or commercial interest of potential licensing partners** *(1500 characters maximum)***?**

The project has garnered significant industrial engagement. Feedback received from industry suggests that *in vitro* measures of efficacy and surrogate biomarkers of endpoints relevant to TACE inhibition are aligned with the partners expectations and are considered the gold-standard at this point in time for CMT. Given the failure of TACE inhibitors in the clinic, a clear demonstration that musculoskeletal toxicity can be mitigated during development will be needed. Once a pre-clinical candidate has been identified with sufficient selectivity, potency and *in vitro* and *in vivo* ADME properties, we will initiate IND-enabling safety toxicology and CMC scale up. Ideally we would partner/license the program prior to completing GLP tox studies.

20. **List and describe activities to be performed with the funding requested, in light of the needed next steps mentioned above.** Per activity, indicate availability of assays/technology needed to evaluate compounds, location of the work to be performed (at your institution, a collaborator, or a CRO), anticipated timeline and funds needed to complete the work package. *(3300 characters maximum).*

a. To expand the SAR in the 'northern' region of the molecule. Docking studies (GLIDE) indicate that this pharmacophore binds in the S1' pocket and is expected to provide selectivity versus MMPs, in particular MMP-13. Manipulating substitution and complementing the existing interactions within the S1′ pocket either by hydrogen bond formation or hydrophobic interactions with Leu^{350}, Lys^{315} or Val^{314}. Current chemistry protocol offers a tractable synthesis to introduce substitution without the need for redesign. We will progress compounds through our screening tree to select the most appropriate candidates for non-clinical development. Candidate molecules will be designed to have high predicted CNS permeability (calculated MPO CNS score >4.0), <10 nM IC_{50} against TACE in the biochemical assay and <25 nM IC_{50} in the cellular assay. 100-fold selectivity against MMP-1, -2, -3, -7, -9 and -14. They should not be a substrate against any efflux transporter (≤2 in MDCK assay). Kinetic solubility of >10 mg/mL; SpectrumScreen Panel showing no target flags at 10 µM. Ames and MNT negative. Display low clearance and a demonstrative mechanism that would allow sustained coverage of IC50 in CSF by either QD or BID oral dosing.

b. We will advance up to two potent, selective and orally bioavailable compounds for *in-vitro* PK in mice, to establish target engagement and brain/CSF penetration and establish maximum tolerated dose (MTD). The most compelling compound from PK will be dosed in P0S63del mice by oral gavage, QD or BID, with vehicle, low (MTD/3) and high (MTD) dose for 12-weeks (n = 12 per group). Motor function will be assessed via accelerated RotoRod. Groups of 4-month-old $P0^{S63del}$ transgenic and control littermates will be evaluated in two sessions of three trials each, per day (6 h rest between the two daily sessions) on testing days (at weeks 2, 4, 6, 8, 10 and 12). During the test, the rod is accelerated from 4 to 40 rotations per minute, and the time that the animal remains on the rod (maximum 900 s) is measured. In-life endpoints will include clinical evaluation, serum biochemistry, DRG myelination assessment, histopathology (staining for MBP), necropsy and gross observations, tissue weight and body weight. This will inform our safety assessment strategy for IND-enabling safety studies.

21. **If applicable, list funding already secured related to the project that would complement TRxA support** (e.g., grants, institutional funds). *(1000 characters maximum).*

'The development of humanized murine models for the treatment of peripheral neuropathies' is supported through the Department of Defense award W72XPS2275336 to Dr. Smith's lab and is funded through May 2024.

This project received an award from The CMT Foundation for the screening of a commercial compound collection against TACE. The CMTF has expressed an interest in co-funding further development.

22. **References** *(10 000 characters maximum).*

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