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CRC Project – SAHA as a potential therapeutic in GRN+ FTD
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A. Study Purpose and Rationale.

Frontotemporal Dementia (FTD) is a disease that affects the frontal and temporal lobes of the brain, areas that are associated with high cognitive function, behavior, language, and personality. FTD occurs commonly; the disease affects 250,000 Americans and it accounts for 10-20% of cases of dementia below age 65 (Neary et al., 1998). The prevalence of both FTD and AD is 15 per 100,000 for the population aged 45-65 (Ratnavalli et al., 2002). Because of its earlier age of onset compared to a disease like Alzheimer's Disease (AD), its cost to society is relatively high.

FTD has two common presentations, involving either behavioral changes or dysfunction of language. In the former, patients may exhibit drastic personality changes and behave socially inappropriately, while in the latter patients may lose their ability to use and understand language. The clinical features of FTD can be variable, where the motor dysfunction can manifest with weakness, dysarthria, hyperreflexia, and parkinsonism. FTD is progressive, and the dementia often leads to immobility and total loss of speech. As opposed to other forms of dementia, memory loss is not a prominent feature of FTD. No clinical test for FTD exists and early diagnosis of FTD is often difficult due to its variable presentation (Mendez et al., 2007). There is currently no curative treatment for FTD, and FTD can be especially devastating to patients and their families because of the younger age of onset in comparison to other forms of dementia like AD. The median survival time for patients with FTD is seven years (Steenland et al.). In a small number of cases, FTD can occur in patients with amyotrophic lateral sclerosis (ALS).

FTD can be either sporadic or familial with the hereditary form occurring nearly half of the time. The first gene shown to cause FTD was tau, normally thought to stabilize microtubules. Mutations in tau cause FTD either by altering the relative concentrations of different isoforms of tau within the cell, or by causing tau species to preferentially adopt a pathologic hyperphosphorylated state. Thus tau is thought to cause FTD via toxic gain of function mutations (Neumann et al., 2009). The cause of the remainder of familial FTD had been unknown until 2006 when it was demonstrated that mutations in GRN create null alleles that can cause tau-negative FTD via haploinsufficiency. Remarkably, both MAPT and GRN lie in the same chromosomal region at 17q21. Haploinsufficency of *GRN* is the predominant mechanism leading to FTD, and these mutations may account for 25% of all cases of FTD (Mackenzie 2007).

At least 68 different mutations in the GRN gene have been identified. GRN mutations appear to create nonfunctional alleles, and these mutations are thought to cause FTD via a loss of function of progranulin. That makes GRN unique in that other types of neurodegeneration are typically caused by dominant negative gain of function mutations. Both nonsense and missense mutations can lead to an FTD phenotype. The feature common to all GRN mutations studied is a downregulation of functional GRN protein (Shankaran et al., 2008).

FTD caused by GRN haploinsufficiency is unique among dementias in that it is caused by a loss of function mutation rather than a toxic gain of function. Thus, if a loss of GRN is the cause of this phenotype and the underlying cause of disease, a novel treatment may lie in raising GRN levels either by endogenous GRN levels or alternatively by artificial supplementation with GRN or granulins. Given that the latter technique is plagued by the technical difficulty of large proteins being unable to cross the

blood brain barrier, significant research is currently ongoing to better understand cellular regulation, production, and degradation of GRN.

One such recent study (Cenik et al., 2011) sought to determine whether progranulin levels could be enhanced by small molecules, and they screened a library of compounds using a luciferase assay. Suberanilohydroxamic acid (SAHA) is a histone deacetylase inhibitor that is approved by the FDA, and further it has been shown to pass the blood brain barrier (Mielcarek et al., 2011). When human cells containing progranulin mutations were treated with SAHA, both mRNA and protein levels were shown to increase in a dose-dependent fashion. Given that SAHA has been shown to increase endogenous GRN levels, and further because of its relative safety because it is FDA approved, we seek to provide SAHA to individuals with FTD caused by mutations in GRN, in an effort to determine whether SAHA can slow the inevitable cognitive decline inherent to this disorder.

B. Study Design and Statistical Analysis.

A double-blinded study will be carried out to ascertain whether administration of SAHA can reduce the rate of cognitive decline in patients with known FTD. This study will have two arms, each with FTD patients with GRN mutations: (1) placebo and (2) SAHA. There will not be crossover between the two arms of the study. SAHA has been approved by the FDA and it will be given at established dosing and interval (for more details, see **Study Drugs**, below). The duration of the study will be one year.

The patients will be randomized into these two arms such that their average scores on mini-mental state examination (MMSE) are equal. The MMSE is a widely used tool for screening for dementia, which tests orientation, registration, attention, calculation, recall, language, repetition, and ability to follow complex commands. It is scored from 0-30, with a score of 27 or more being normal. The MMSE has high reliability and reproducibility, and has been in use for over 30 years (Molloy et al., 1991). The MMSE has normal T-tests will be performed between the two groups with an unpaired ttest, and standard values of $p<0.05$ and 80% power. Previous data show that MMSE scores decrease faster than in AD (Chow et al., 2006), more specifically with mean MMSE at diagnosis 23.2 with standard deviation of 3.8 and an average decline of 4.7 points per year (Rascovsky et al., 2005). Even a small change in rate of decline in MMSE would be indicate a significant slowing of disease, thus given that average decline is 4.7 points yearly, our desired effect is 2 points in one year on MMSE exam. Given that a one sided ttest will be used in this study, with 80% power we approximate to the following equation: Patients needed = $1 + 16 * (\text{effect size} / SD)^2$. Thus based on that formula 58 patients are needed in each arm of the study in order to achieve the statistical power necessary given the assumed variability as has been previously reported to achieve our desired effect size. Statistical analysis will be performed using SPSS.

C. Study Procedure.

Patients will be recruited at Columbia University Medical Center (and possibly at other academic medical centers as well) in outpatient Neurology clinic after initial diagnosis of FTD is made by a Neurologist. After being randomized, the patients will receive pills to take for the duration of the study. For the first two months of the study, they will return to the laboratory bimonthly to obtain CBC to screen for thrombocytopenia, and efforts will be made to be vigilant for systemic embolism as well. Patients will return to clinic every three months for one year (total 4 MMSE exams given) during which time each patient will be assessed for cognitive decline.

Should patients develop side effects from SAHA, each patient will be managed on a case-by-case basis. If a complication is severe, such as pulmonary embolism, they will be dropped from the study. However, if a side-effect is potentially surmountable, for example anemia, they will be offered 300mg rather than 400mg of SAHA with the hope that they will be able to complete 1 year of therapy with SAHA.

D. Study Drugs.

As described above, this study uses SAHA given that it was an extremely potent enhancer of GRN expression, and additionally that it is relatively safe as it has already been approved by the FDA. SAHA has been shown to increase GRN levels in cultured cells from patients with GRN mutations. Specifically, when cells from a GRN carrier and an unaffected family member were each treated with SAHA, GRN levels of both RNA and protein in the cells from the carrier were shown to approach levels of the unaffected individual.

SAHA will be given by mouth at a dose of 400mg once daily, the accepted dose and frequency for which it has been approved by the FDA for treatment of cutaneous T-cell lymphoma (CTCL). The drug has previously been shown to reach the CNS in acceptable concentrations in GBM studies (Galanis et al., 2009, Mann et al., 2007). Further, it had been shown to be well tolerated for nearly 4 months continuous treatment when used for treatment of CTCL, and for a maximal total treatment time of greater than one year. Typical side effects fall into four areas, and they include (1) GI symptoms (nausea, vomiting, and headache), (2) constitutional symptoms (fever, chills), (3) hematological (anemia, thrombocytopenia) and (4) taste disorders. Less common but more concerning side effects include thrombocytopenia (grade 3-5, 5.8% of patients) and pulmonary embolism (4.7% of patients).

E. Study Subjects.

Subjects will be recruited from dementia clinic, with a first prerequisite being that they must be diagnosed with FTD by a Neurologist specializing in behavioral Neurology, but importantly, this diagnosis needs to be made immediately before enrollment in this study. Patients will existing diagnosis of FTD will be excluded. Second, as it is necessary that all patients in this study have mutations in progranulin, this will be verified by sequencing using samples obtained from buccal swab.

F. Recruitment of Subjects.

Patients will be recruited from dementia clinic at Columbia University Medical Center, and if necessary, from frontotemporal dementia consortium clinics at Academic centers thtoughout the country.

G. Confidentiality of Study Data.

Data will be kept confidential on RSA encrypted disks at all times. Only the primary investigators will have access to the data, and the names of the patients will be deleted from the dataset and the patients will each be identified by a 6 digit number. Further, this entire study will be double-blinded, and an outside party (likely a member of the statistics department) will randomize the patients. Only after the study is completed will the investigator by unblinded to which patients are in which arm of the study.

H. Potential Conflict of Interest.

The author declares that there are no conflicts of interest.

I. Location of the Study.

The study will be performed in outpatient Neurology clinics associated with Columbia University Medical Center.

J. Potential Risks.
As described above, this drug has significant side effects, the most common of which include constitutional, GI, and gustatory symptoms, and less commonly but still very possible sequelae include thrombocytopenia and pulmonary embolism. However, given the grave nature of FTD, and the devastating speed at which FTD progresses, a drug with these potential side effects can and should be attempted as a treatment for FTD.

K. Potential Benefits
As described above, this study has the potential benefit of slowing the rate of cognitive decline in patients with FTD.

L. Alternative Therapies.
There are currently no known pharmacalogic therapies to treat FTD. Thus this treatment has the potential to offer a therapeutic option to patients with this disease.

M. Compensation to Subjects.
There will be no remuneration paid to the subjects involved in this research study.

N. Costs to Subjects.
All of the drugs will be given to the patients enrolled in this study free of charge.

O. Minors as Research Subjects.
All patients participating in this study will be over age 18, and minors will not be recruited as patients to this study.

P. References

Cenik B, Sephton CF, Dewey CM, Xian X, Wei S, Yu K, Niu W, Coppola G, Coughlin SE, Lee SE, Dries DR, Almeida S, Geschwind DH, Gao FB, Miller BL, Farese RV Jr, Posner BA, Yu G, Herz J. Suberoylanilide hydroxamic acid (vorinostat) up-regulates progranulin transcription: rational therapeutic approach to frontotemporal dementia. J Biol Chem. 2011 May 6;286(18):16101-8.

Chow TW, Hynan LS, and Lipton AM. MMSE Scores Decline at a Greater Rate in Frontotemporal Degeneration than in AD. Dement. Geriatr. Cogn. Disord. (2006); 22:194-199.

Galanis E, Jaeckle KA, Maurer MJ, Reid JM, Ames MM, Hardwick JS, Reilly JF, Loboda A, Nebozhyn M, Fantin VR, Richon VM, Scheithauer B, Giannini C, Flynn PJ, Moore DF Jr, Zwiebel J, Buckner JC. Phase II trial of vorinostat in recurrent glioblastoma multiforme: a north central cancer treatment group study. J Clin Oncol. 2009 Apr 20;27(12):2052-8.

Mackenzie IR. The neuropathology and clinical phenotype of FTD with progranulin mutations. Acta Neuropathol. 2007 Jul;114(1):49-54.

Mann BS, Johnson JR, Cohen MH, Justice R, Pazdur R. FDA approval summary: vorinostat for treatment of advanced primary cutaneous T-cell lymphoma. Oncologist. 2007 Oct;12(10):1247-52.

Mendez, M.F., Shapira, J.S., McMurtray, A., Licht, E., and Miller, B.L. (2007). Accuracy of the clinical evaluation for frontotemporal dementia. Arch Neurol *64*, 830-835.

Mielcarek M, Benn CL, Franklin SA, Smith DL, Woodman B, Marks PA, Bates GP. SAHA decreases HDAC 2 and 4 levels in vivo and improves molecular phenotypes in the R6/2 mouse model of Huntington's disease. PLoS One. 2011;6(11).

Molloy DW, Alemayehu E, Roberts R. Reliability of a Standardized Mini-Mental State Examination compared with the traditional Mini-Mental State Examination. Am J Psychiatry. 1991 Jan;148(1):102-5.

Neary, D., Snowden, J.S., Gustafson, L., Passant, U., Stuss, D., Black, S., Freedman, M., Kertesz, A., Robert, P.H., Albert, M.*, et al.* (1998). Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. Neurology *51*, 1546-1554.

Neumann, M., Sampathu, D.M., Kwong, L.K., Truax, A.C., Micsenyi, M.C., Chou, T.T., Bruce, J., Schuck, T., Grossman, M., Clark, C.M.*, et al.* (2006). Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science *314*, 130-133.

Ratnavalli, E., Brayne, C., Dawson, K., and Hodges, J.R. (2002). The prevalence of frontotemporal dementia. Neurology *58*, 1615-1621.

Rascovsky K, Salmon DP, Lipton AM, Leverenz JB, DeCarli C, Jagust WJ, Clark CM, Mendez MF, Tang-Wai DF, Graff-Radford NR, Galasko D. Rate of progression differs in frontotemporal dementia and Alzheimer disease. Neurology. 2005 Aug 9;65(3):397-403.

Shankaran, S.S., Capell, A., Hruscha, A.T., Fellerer, K., Neumann, M., Schmid, B., and Haass, C. (2008). Missense mutations in the progranulin gene linked to frontotemporal lobar degeneration with ubiquitin-immunoreactive inclusions reduce progranulin production and secretion. J Biol Chem *283*, 1744-1753.

Steenland, K., MacNeil, J., Seals, R., and Levey, A. Factors affecting survival of patients with neurodegenerative disease. Neuroepidemiology *35*, 28-35.