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Atorvastatin Treatment of Cavernous Angiomas with Symptomatic Hemorrhage Exploratory Proof of Concept (AT CASH EPOC)

NCT02603328

Protocol
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Version 5.0

Atorvastatin **T**reatment of **C**avernous **A**ngiomas with **S**ymptomatic **H**emorrhage **E**xploratory **P**roof **o**f **C**oncept (AT CASH EPOC)
Phase I-IIa Randomized, Placebo-Controlled, Double-Blinded, Single-Site Clinical Trial

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SYNOPSIS

Study Title

AT CASH EPOC: Atorvastatin Treatment of Cavernous Angiomas with Symptomatic Hemorrhage, Exploratory Proof of Concept (AT CASH EPOC) Trial

Objectives

The ***Primary Aim*** *of the study is to* assess whether the proposed intervention produces evidence of QSM biomarker activity (indicative of reduced or increased lesional bleeding) during 2 years of prospective follow-up of cavernous angioma (CA) after a recent symptomatic hemorrhage (SH).

Secondary Aims shall assess (1) a second biomarker (DCEQP permeability in lesion and brain), (2) Rho kinase (ROCK) activity on peripheral blood leukocytes and on any excised lesion specimens during the course of the study, (3) clinical event rates, (4) adverse events, and (5) functional outcomes in placebo versus treatment; and (6) we shall query pre-specified subgroups.

Design and Outcomes

This phase I/IIa randomized, placebo-controlled, double-blinded, single-site clinical trial is designed to investigate a proof of concept effect of atorvastatin versus placebo on CA lesional iron deposition assessed by validated quantitative susceptibility mapping (QSM) magnetic resonance imaging (MRI) studies in patients who suffered a symptomatic bleed within the preceding one year. Subjects will also be assessed by lesional and brain vascular permeability MRI using dynamic contrast enhanced quantitative perfusion (DCEQP) and a number of laboratory and clinical evaluation tools. Subjects shall be followed for 2 years from randomization, the period of highest likelihood of rebleed after a recent CA hemorrhage. Subjects shall undergo clinical and MRI evaluations at baseline, and at 12 and 24 months during the study period. Enrolled subjects and the treating team shall be blinded to treatment allocation.

Interventions and Duration

Enrolled subjects will be randomized 1-1 to atorvastatin (starting dose 80 mg PO daily) or placebo. Dosing shall continue for the 24-month follow-up period or until reaching a safety endpoint, whichever comes first. The study shall continue until all enrolled subjects, including those reaching safety endpoints, have undergone their 24 month (end of study) MRI and clinical assessment. Laboratory studies are also performed at 3 months after dose initiation. Dose de-escalation to 40 mg/day or identical placebo capsule shall be allowed at any time during the course of the study, in the case of pre-articulated side effects or laboratory abnormalities at the initial dose. At the 12 and 24-month follow up visits, clinical evaluations, and lesional QSM, brain and lesional DCEQP permeability, and peripheral blood leukocyte ROCK activity will be assessed to determine the response to atorvastatin or placebo.

Sample Size and Population

The study is designed to include 80 subjects, randomized 1:1 to receive either atorvastatin or placebo. Randomization is stratified to insure a balanced treatment allocation by gender. The sample size was expanded by 37% to accommodate a a highest possible estimate of missing data and potential attrition due to compliance, drug intolerance and safety endpoints, leaving at least 25 subjects in each of placebo and atorvastatin groups, which, per power calculations, are needed to detect a relevant difference in primary QSM biomarker. Subjects will be enrolled at the study investigator's institution where trial preparations have been optimized. Case referrals will be encouraged through a regional referral Chicago-wide academic and clinical consortium, and through the Angioma Alliance.

At midpoint of the trial, futility analysis is planned, and adaptive recalculation of sample size based on the actual observed primary outcome and treatment effect.

1. STUDY OBJECTIVES

The ***Primary Aim*** *of the study is to* assess whether the proposed intervention produces evidence of QSM biomarker activity (indicative of reduced or increased lesional bleeding) during 2 years of prospective follow-up after a recent symptomatic hemorrhage. **The primary outcome is the mean percent change in QSM (called the change score) per year.** Reduced CA hemorrhage would be a signal proof of concept of potential benefit, and increased hemorrhage a potential signal of risk. Primary analysis shall be based on intention to treat, and secondary analysis shall examine treatment rendered, considering attrition and compliance. In cases with multiple lesions, QSM measurements in the lesion with initial SH (index lesion) shall be considered for the primary outcome assessment.

Secondary Aims shall assess (1) a second biomarker (DCEQP permeability in lesion and brain), (2) ROCK activity on peripheral blood leukocytes and on any excised lesion specimens during the course of the study, (3) clinical event rates, (4) adverse events, and (5) functional outcomes in placebo versus treatment; and (6) we shall query pre-specified subgroups.

2. BACKGROUND

2.1 Supporting data

A Common Lesion, an Uncommon Disease. Cavernous angiomas (CAs) consist of clustered, giant, blood-filled capillary spaces ("caverns"), lined by endothelium, and separated by an amorphous matrix lacking mature vessel wall angioarchitecture (1). CAs occur in a sporadic form, manifesting a solitary lesion or a cluster of lesions associated with a venous developmental anomaly (2). The disease is familial in 20-30% of cases,

associated with multifocal lesions that develop over time throughout the patient's brain (3). The familial form (OMIM #116860) exhibits a Mendelian autosomal dominant inheritance due to a heterozygous loss-of-function mutation in one of 3 genes (*CCM1*/*KRIT1*, *CCM2*/*Malcavernin*, and *CCM3*/*PDCD10*) (4-10). Multiple cavernomas may also develop after cranial irradiation. Magnetic resonance imaging (MRI) is the hallmark diagnostic modality for these lesions, including sequences revealing chronic and acute hemorrhage, and tiny occult lesions.

CAs are often detected incidentally, or in association with seizures or non-specific neurologic symptoms (11-13). The prevalence of CA has been estimated at 0.46-0.63% of the population based on consecutive imaging studies in the MRI era (14, 15). A 0.5% prevalence was confirmed by the detection of 131 CAs among 24,535 consecutive autopsies (16). The natural history of such lesions is exceedingly benign, with less than <0.5% annual risk of clinically significant hemorrhage, and there is evidence-based consensus about not needing interventions beyond symptomatic management of such cases (17). But once a lesion has manifested a symptomatic hemorrhage (SH), its untreated clinical course is quite serious, with a 42.4% (CI 26.8-58.0) rate of recurrent bleed or focal neurologic deficit within 5 years (17-19). The highest likelihood of rebleeding in CAs with SH is in the following 2-3 years, with bleeding risk subsequently regressing to the low rate of asymptomatic lesions. **CA with SH is hence a singular clinical entity, distinguishing lesions impacting a patient's life and meriting clinical intervention.** The definition of SH in CA has been validated and adjudicated (20).

Based on <10-12% prevalence of SH among all CAs and assuming 0.5% of 325 million Americans harbor a CA, it is projected that fewer than 162,500 to 195,000 patients are living with a cavernoma that has bled at least once. Another recent population-based study in Olmsted County, MN identified CAs in 0.41% of adults undergoing MRI for non-clinical purposes. In that study, the prevalence of symptomatic disease was only 0.037% (21). *This would translate to an estimated prevalence of 120,000 symptomatic CAs in the U.S population. A fraction of these would have had a recent SH.* Thus, while overall CAs are common, those that are symptomatic and warranting therapeutic targeting, and particularly the **CA with SH meet the rare disease definition of <200,000 affected Americans, by any estimate**.

An Umet Clinical Need and Consensus Regarding the Therapeutic Target. CAs are typically observed expectantly, and surgically excised only *after significant clinical sequelae* (typically one or more SH). Healthcare costs of hemorrhagic stroke can reach up to $27,400/case in the first 90 days (2007 dollars), excluding socioeconomic impact of lost productivity (22).

Lesions in the brainstem and deep brain locations are more likely than other CAs to bleed (23), rebleed (19), and cause severe disability (18, 24). It is unclear if this greater propensity to SH simply reflects the clinical impact of bleeds in those locations, while lesions in less eloquent brain regions may bleed at similar rates but more likely without symptoms. While brain surgery for excision of CA may benefit some patients, it can be associated with significant cost and morbidity. A typical craniotomy costs $37,438 in 2011 dollars (25), excluding the cost of complications and morbidity. Surgical excision of CAs, particularly brainstem lesions, is associated with an alarming rate of surgical adverse events (Figure 1) (26). Hence, excision of CA is typically considered per current clinical

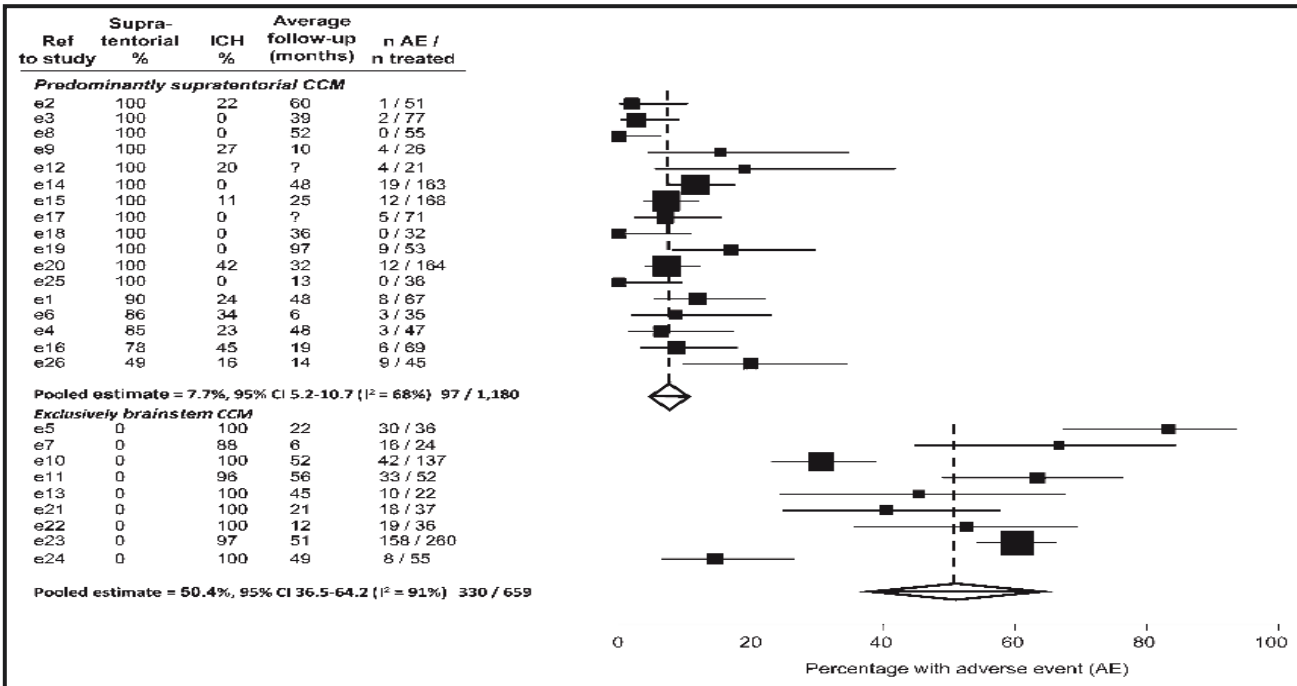

Figure 1. Incidence of adverse events after surgical treatment for CA. Note particularly high rate of morbidity in brainstem CAs, those reported with highest reported rebleed rates, and greatest disability. From Moultrie et al (26).

practice and evidence-based guidelines only **after one or more recurrent SHs, particularly in deep or brainstem locations (17, 27)**. In a non-randomized population-based cohort study, lesion excision was overall associated with significantly worse functional outcomes and greater complications compared to conservative management (26). Stereotactic radiosurgery has been used to treat CAs but there is controversy about its effectiveness and concern about complications and radiation-induced genesis of new CA lesions (28, 29).

The rates of development of a new CA (in familial cases), and of first SH in asymptomatic CAs, are far too low to allow meaningful testing or to compel primary prevention strategies. CAs with recent SH, where surgical resection is not undertaken (mostly in deep and brainstem locations), are the most likely to be followed expectantly per current evidence based guidelines (17), with **clinical equipoise for testing novel therapies aimed at preventing re-bleeding**. **It would be desirable to develop a drug that stabilizes the CA after a recent SH, and prevents recurrent bleeding. It would mitigate the costs and neurologic sequelae of re-bleeds and surgical resection in many patients.** Based on all natural history studies, the therapeutic benefit for secondary prevention (symptomatic re-bleed) would be greatest within 2-3 years after an SH (17-19).

2.2. Rationale

Preliminary Studies: Mechanistic Rationale, Preclinical Studies and Biomarker Validations

Defective Endothelial Barrier and Chronic Bleeding as Hallmarks of CA Pathology. **CAs invariably exhibit hemosiderin (and other non-heme iron) deposition indicating chronic hemorrhage (30, 31)**. Our group (32) and others (33, 34) documented that the endothelial cells (ECs) lining lesional caverns manifest **a "leaky" phenotype, with a defective EC barrier**. Loss of KRIT1 results in disruption of junctional integrity, resulting in increased endothelial permeability *in vitro* (35) and *in vivo* (36). Loss of Malcavernin and PDCD10 has similar effects (36-38). Importantly, although familial CCM patients may be *heterozygous* for *CCM1, CCM2* or *CCM3* mutations, the ECs *within* CA lesions show loss of the relevant protein (39), due to a *somatic mutation* of the remaining wild-type allele at the respective locus (40, 41). **Similar somatic mutations have been described in sporadic CA lesions as familial ones (42), indicating that an identical disease pathway resulting in lesional loss of CCM protein underlies familial and sporadic CAs**.

CCM Gene Loss Results in Endothelial Permeability, Mediated by RhoA/ROCK Activation. The loss of any of the 3 disease genes in ECs activates the GTPase protein RhoA, and results in increased actin stress fiber, decreased EC lumen formation and increased permeability (36, 37, 43, 44). **Recent evidence that CAs arise from endothelial gain of MEKK3-KLF2/4 signaling, also showed that *this activity is upstream of RhoA*, and that the causative effect on CA development is mediated via Rho activation** (45). The activity of Rho kinase (ROCK), an effector of RhoA, is also increased in these systems, reflected by phosphorylated myosin light chain (pMLC), a critical target of ROCK (36, 43, 44). **ROCK activation was demonstrated in surgically excised human CA lesion specimens from sporadic and all 3 familial genotypes** (36, 38, 42).

ROCK Inhibition Reverses CCM-Related Cellular Phenotypes. Work from our group in collaboration with Ginsberg at UCSD demonstrated that ECs from *Ccm1*$^{+/-}$ or *Ccm2*$^{+/-}$ mice exhibit a generalized vascular leakage *in vitro* and *in vivo* that is reversed by fasudil, a *specific* ROCK inhibitor (36). Others have reported that the vascular leakage in *Ccm2*$^{+/-}$ mice can be inhibited by statins which inhibit RhoA prenylation, a critical early step in RhoA/ROCK activation (37). **Thus inhibition of ROCK is a potential therapeutic target, reversing *CCM*-related junctional instability in sporadic and familial lesions which harbor similar somatic mutations and robust ROCK activity** (42).

Animal Models in the Heterozygous State Recapitulate the Human Disease. New opportunities to investigate CA pathogenesis arose from our discovery, and confirmation by Douglas Marchuk at Duke University and others, of biallelic somatic mutations in CA lesions (39-41). Based on the postulated two-hit Knudsonian mechanism, Marchuk and our team jointly developed *a robust mouse model of CCM, recapitulating the human disease in the heterozygous state* (46-48). When crossed into a background of elevated somatic mutation load (*Trp53*$^{-/-}$ or *Msh2*$^{-/-}$), *Ccm1, 2 or 3*$^{+/-}$ (heterozygous) mice manifest a rich repertoire of lesions with histology, phenotypic signatures and ultrastructure identical to the human lesions. These models helped us characterize the burden of **primordial and mature CCM lesions**, and **quantify lesional hemorrhage (integrated density of**

non-heme iron accumulated in lesions). We then hypothesized that *in vivo* ROCK inhibition with Fasudil or statins would inhibit lesion development and hemorrhage.

Fasudil but not Simvastatin Blunt CA Lesion Development. Both Decrease Hemorrhage in Murine Lesions. Our group was first to show that ROCK inhibitor fasudil prevents CA lesion development in murine *Ccm1* model (49). Further preclinical studies have confirmed these results in a larger cohort of mice using contemporaneous randomized treatment assignment versus placebo, and blinded outcome assessment per NINDS guidelines (50). Oral fasudil, administered at 100 mg/kg/day from 1-4 months of age significantly reduced the development of mature (multicavernous) CA lesions. Oral simvastatin 40 mg/kg/day had no significant effect on lesion development. Both fasudil and simvastatin significantly decreased non-heme iron deposits in CA lesions compared to placebo, indicating decreased lesional hemorrhage (51). Decreased iron burden by fasudil and simvastatin were also noted in the *Ccm2* model. ROCK inhibition also significantly decreased CCM iron leak after lesion development, when ROCK inhibitor was administered for only 1 month, later in the mouse's life.

Atorvastatin Recapitulates the Benefits of Fasudil, Better Than Simvastatin. As simvastatin dose escalation is currently limited by the U.S. Food and Drug Administration (FDA), **we sought to test the more potent drug atorvastatin** (52) in our models, particularly since **higher doses of atorvastatin have been shown to effect more robust ROCK inhibition in humans** (53, 54). The same *Ccm1*$^{+/-}$*Msh2*$^{-/-}$ mice were more recently treated with oral atorvastatin at 80 mg/kg/day for the same duration as with the prior experiements, and these achieved significant *robust inhibition of lesional development and hemorrhage, as with fasudil* (Figure 2). In these recent experiments, lesion burden was assessed by newer demonstrably more rigorous volumetric micro-CT technique (55). We then contemporaneously assessed pharmacologic ROCK inhibition in the more aggressive *Ccm3*$^{+/-}$*Trp53*$^{-/-}$ model (38), with **random treatment assignment to placebo, fasudil, simvastatin and atorvastatin**, at the same doses and for the same duration as with the other genotypes. **Atorvastatin again showed a significant and robust benefit in both lesion development and hemorrhage, similar to fasudil, but better than simvastatin** (Figure 3). This is the first evidence of therapeutic benefit of ROCK inhibition in the most aggressive *Ccm3* model. There were significantly fewer pMLC immunopositive ECs lining CA lesions, and fewer pMLC immunopositive leukocytes within the lesions in atorvastatin treated brains, consistent with blunted ROCK activity (Figure 4). Altogether, these results establish **a firm biologic premise of (1) therapeutic benefit by atorvastatin similar to specific ROCK inhibitor fasudil, and better than the lower potency simvastatin, and (2) effect of atorvastatin is associated with ROCK inhibition effect.**

There were neither deleterious effects on animal weights nor on the rate of attrition with any statin treatment of murine models of all genotypes from weaning to 4 months of age. Animals which died before completing statin therapy, had no greater prevalence of cerebral hemorrhage than those taking placebo or fasudil. These preclinical results reassure us that **chronic statin therapy, including higher potency atorvastatin effecting ROCK inhibition, decreases iron leak in murine CA lesions, with no hint of increased hemorrhage or attrition**. Interestingly, while the benefit of fasudil was limited to male cohorts (51), there was significant benefit of atorvastatin (lesion burden and iron

deposit) in both male and female mice in these latest studies.

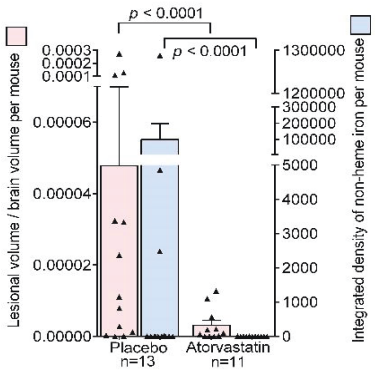

Figure 2. Atorvastatin 80 mg/kg/day decreased lesion burden (pink bars, left axis) and non-heme iron (blue bars, right axis) compared with placebos in *Ccm1+/-Msh2-/-* mice.

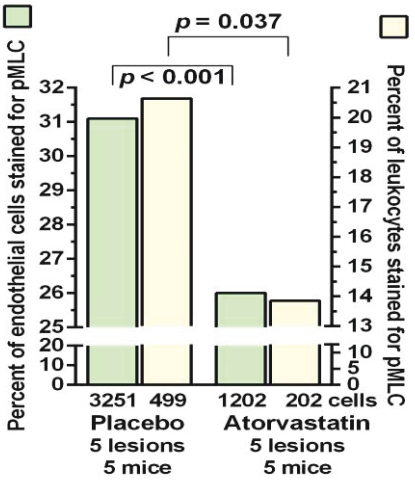

Figure 4. Chronic atorvastatin treatment significantly reduced the prevalence of pMLC (a marker of ROCK activity) immunopositive ECs lining CAs, and leukocytes within lesions in brains of *Ccm3+/-Trp53-/-* mice. Immunopostivity was assessed by two observers blinded to treatment assignment.

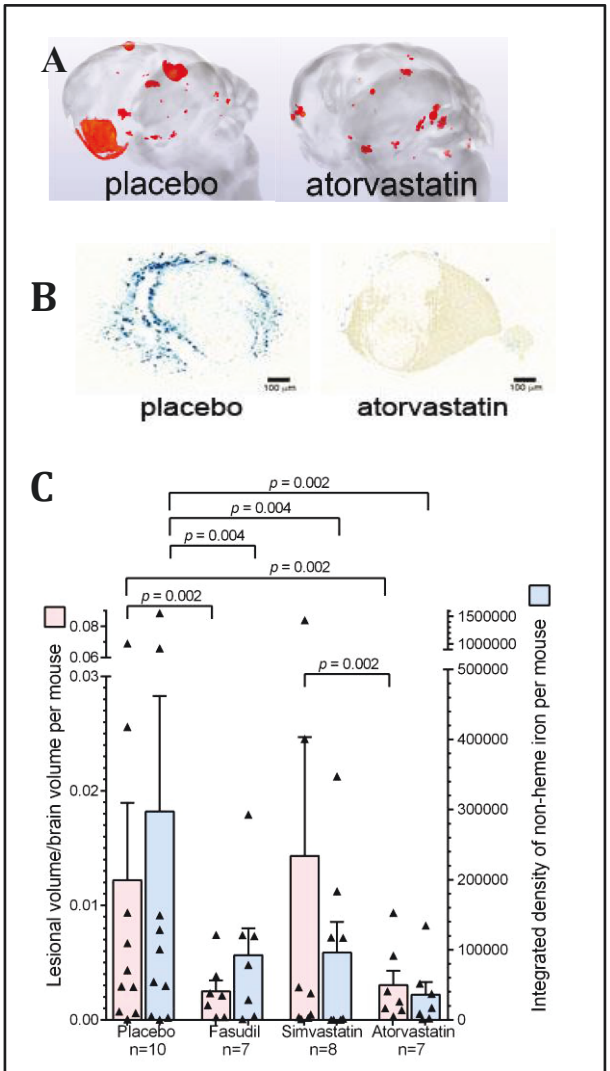

Figure 3. ROCK inhibition in *Ccm3+/-Trp53-/-* murine brains. Representative images show decreased (A) lesion burden by micro-CT volumetrics. (B) non-heme iron deposition by Perls staining with atorvastatin treatment, bar=100μm. (C) Fasudil and atorvastatin, but not simvastatin, decreased lesion burden (pink bars, left axis) compared with placebo. All 3 drugs decreased non-heme iron per brain (blue bars, right axis), an effect most robust with atorvastatin.

Quantitative Susceptibility Mapping (QSM) as a Biomarker of CA Lesional Hemorrhage. Since iron deposit in lesions is modified by ROCK inhibitor and statins in mice, the Awad group has recently applied **a novel technique for assessing iron concentration in CA lesions using**

quantitative susceptibility mapping (QSM) on MRI. We published validations of the technique including precise correlations of QSM with iron concentration in ferric, ferrous and ferumoxytol phantom solutions, and the exact prediction by QSM of iron content in excised CCM lesions assayed by mass spectroscopy (56). We later showed strong interobserver agreement in QSM measurements in human CAs, stability of QSM in clinically stable lesions, reproducibility of QSM across MRI instrument platforms at two hospitals, and greater lesional QSM in older patients and in cases with prior symptomatic bleeds (57).

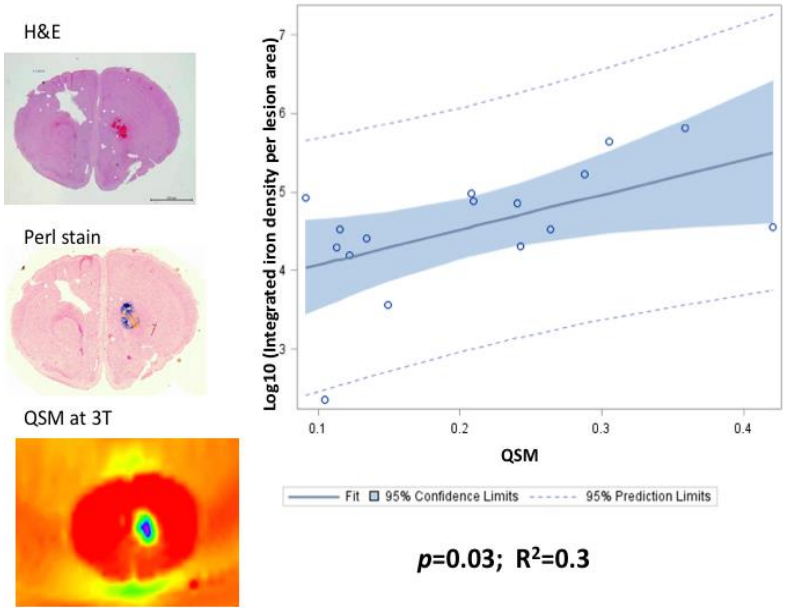

Figure 5. Correlation of mean lesional non-heme iron density on coronal hostologic images at largest lesion diameter (Y axis, log transformed), and mean lesional QSM by volumetric MRI at 3T (X axis, in ppm) in **murine brains with CA lesions**. At left are representative coronal histologic slices with H&E and Perls stain, and a matched coronal MRI QSM image of the same lesion obtained *ex vivo* prior to brain sectioning.

In other pilot work we show significant correlation of mean lesional QSM in murine brains imaged *ex vivo* in the human 3T MRI magnet, with histologic iron densitometry of Perls staining of the same lesions after brain sectioning (Figure 5). This demonstrates that **differences in QSM may be used to assess the same parameter of treatment effect (lesional non-heme iron density) impacted in preclinical studies**. The observed 87.8-100% range of artorvastatin treatment effect on Perls iron stain intensity in *Ccm1* and *Ccm3* models (Figures 2-3) would correspond to an effect size of 0.043 to 0.151 ppm difference in mean lesional QSM.

Linking QSM Change to Clinical Events in Longitudinal Follow-up of Previously Stable Human CAs.

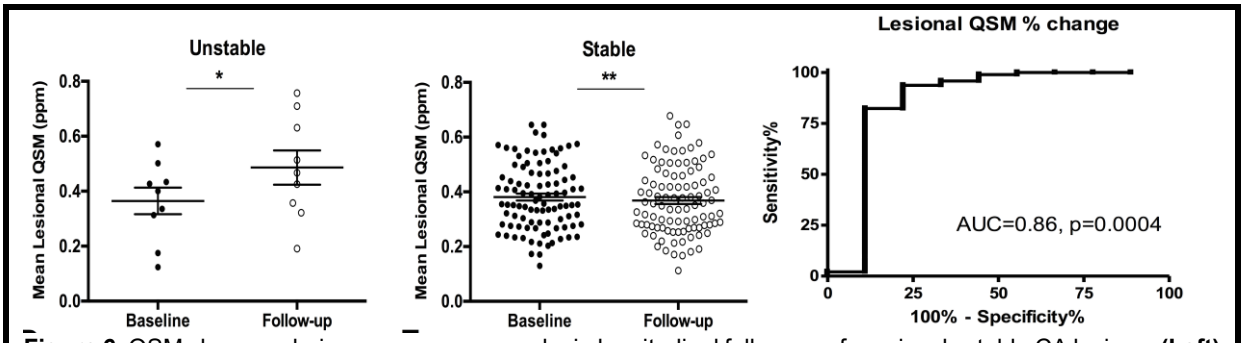

Figure 6. QSM changes during person-year epochs in longitudinal follow-up of previously stable CA lesions. **(Left)** Lesions manifesting growth or symptomatic bleeding during follow-up exhibited a significant increase in QSM (+ 44%, *p=0.01). **(Center)** Lesions which remained stable had a slight significant decrease in QSM (-3.2%, **p=0.003). In cases with multiple lesions, the unstable and stable lesions were analyzed separately. **(Right)** Receiver operating curve of sensitivity and specificity of QSM change in association clinical events.

We recently reported the first prospective assessment of QSM during longitudinal follow-up of human CCM subjects, and correlated paired QSM assessments during 1-year epochs with new clinical events (58). Among cases without recent bleeding or lesion growth within a year of the first measurement, CCM lesions that remained stable during longitudinal follow-up had a tiny average decrease (-3.2%) in mean lesional QSM. CCMs with demonstrated growth or symptomatic bleeding exhibited a significant increase (+44%) in mean lesional QSM (Figure 6). Receiver operating curves (ROC) of QSM change in individual subjects demonstrated a sensitivity of 82.29% and specificity of 88.89% at a +5.81% threshold change, highly significant (p = 0.0004), with a mere **+5.81% QSM change. These results link a robust QSM change directly to clinical events, and define a threshold QSM change as a sensitive and specific biomarker event.**

QSM change and clinical events in CAs after recent Symptomatic Hemorrhage. In further pilot studies, we assessed lesional QSM change during prospective **follow-up of a pilot cohort of CA patients with a clinical bleed within the prior year (meeting the proposed trial criteria) (59).**

Sixteen subjects followed for 1 or 2 years (total 22 person-year epochs), underwent paired QSM assessments at the beginning and end of each person-year epoch. Mean QSM in the index CA hemorrhagic lesion increased by an average of +7.93% per person-

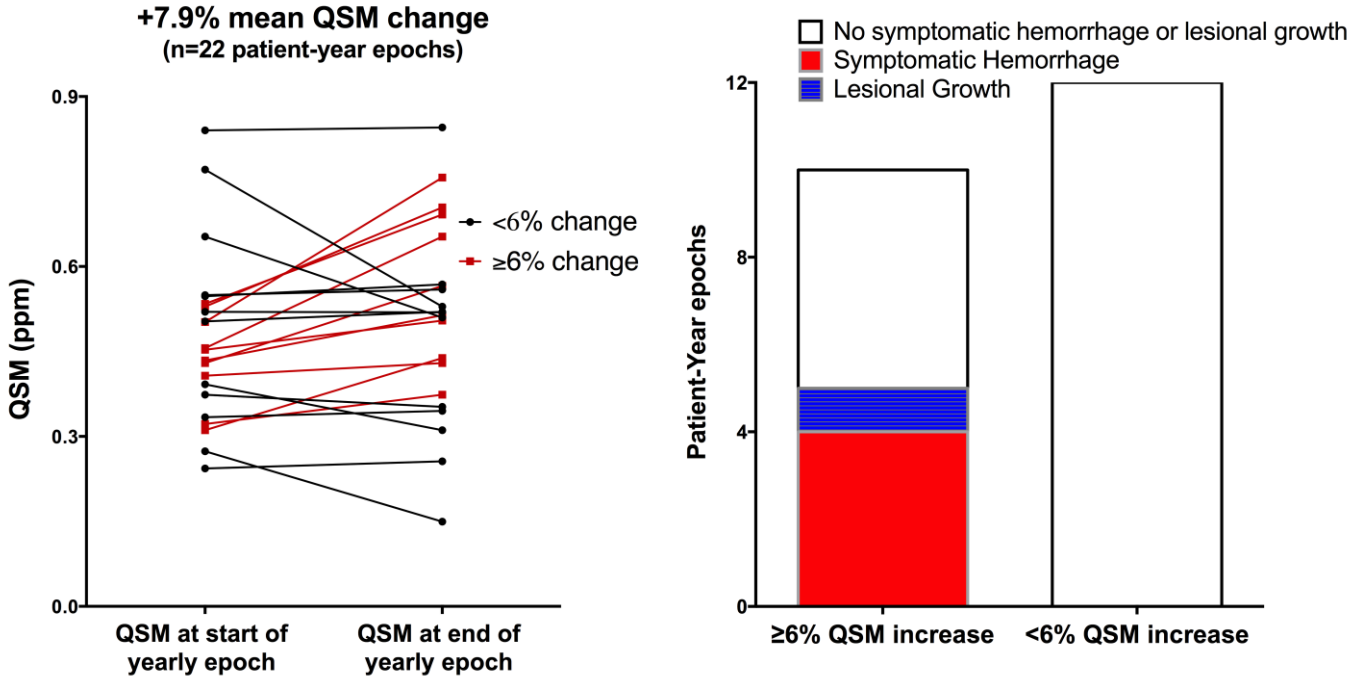

Figure 7. (Left) QSM measurements at the beginning and end of 22 person-year epochs of follow-up of cases with CA that had bled within 1 year, and followed for 1-2 years. Cases underwent annual QSM, allowing paired measurements at the beginning and end of each person-year epoch. In cases with multiple lesions, only QSM measurements in the lesion with initial hemorrhage (index lesion) were considered herein. Paired measurements in red identify epochs with a recorded threshold QSM increase by ≥6% in the index lesion. **(Right)** Number of person-year epochs with and without threshold QSM change, and clinical events during these epochs. All clinical events occurred in the setting of > 6% increase in QSM (59).

year epoch in the full cohort. Closer examination revealed 10/22 epochs with **a documented threshold biomarker event (≥6% QSM increase). This was twice as frequent as clinical events**. **No clinical event occurred without a threshold increase in QSM** (Figure 7). **A primary aim of the proposed trial shall explore whether the change in QSM is altered by statin treatment. A secondary aim shall explore the relationship of QSM change to clinical events in statin and placebo treated subjects.**

Quantitative Vascular Permeability in Lesion and Brain as a Potential Biomarker of Statin Therapy. Work by the New Mexico and Chicago groups demonstrated vascular hyperpermeability in human CA lesions, using dynamic contrast enhanced quantitative perfusion (DCEQP) MRI technique with gadolinium (60, 61). Our group further showed that more permeable CA lesions by DCEQP are associated with greater mean QSM (60). And we recently demonstrated, **as with QSM**, that **CAs that bleed during longitudinal surveillance also manifest significant increases in lesional permeability (mean change +85.9%; p = 0.005)** (58).

Our group has also reported greater permeability in normal brain white matter far (WMF) from lesions in familial cases (consistent with germline hemizygosity of *CCM* genes in those patients), than in sporadic cases (whose normal brain is unaffected by *CCM* genes) (62). **Nine CA subjects, already taking statins for routine cardiovascular indications unrelated to CA (5 familial, 4 sporadic), had lower mean WMF permeability than 60 cases not taking statins (34 familial, 26 sporadic) (Figure 8)** (62). This effect was observed **in both sporadic and familial cases**, and we can postulate that it may reflect ROCK inhibition by statin in background brain.

Figure 8. The effect of concomitant statin use on vascular permeability measured by DCEQP in white matter far from lesions (WMF). There was a trend towards lower WMF permeability (K_i) in patients taking statins for cardiovascular disease than those not taking statins, in sporadic and familial CA subjects (P=0.07) (62).

No differences in lesional permeability were noted with statin exposure in this retrospective cohort. Neither were consistent effects on lesional permeability noted in the first clinical trial of simvastatin (up to 40 mg/day) in familial patients with common Hispanic *CCM1* mutation (trials.gov# NCT0176445), performed by the New Mexico group (interim results, CCM Investigators Workshop, Washington, DC, November 2015). **However, it remains unclear if there would be an effect of statins on permeability of CA lesions after recent SH, where a large increase in mean lesional permeability is demonstrated (58), or whether higher statin doses would achieve greater permeability reductions consistent with more robust ROCK inhibition (53, 54).** These results and critical knowledge gaps justify continued exploration of vascular permeability biomarker in association with a treatment mechanistically targeting ROCK mediated hyperpermeability. **The impact on lesional and brain vascular permeability of statin, at doses effecting ROCK inhibition, shall be examined as a secondary aim in the proposed trial.**

Translation to Human Therapies and the Choice of Drug and Dose

Safety Considerations and Potential Translation to Human Therapies. Fasudil is a specific ROCK inhibitor (63) approved for human use in Japan with an intravenous dose of 90 mg/day, for a period of 2 weeks to prevent cerebral vasospasm after aneurysmal subarachnoid hemorrhage (64). Experience with more chronic administration of fasudil in man is limited, and serious adverse events have been reported (65). Because of the paucity of experience and questionable safety in chronic use, and the expired worldwide patent on the drug, **there is currently no pathway for the approval of fasudil for chronic use in man.** The pharmaceutical industry is developing a number of potential ROCK1 or ROCK2 inhibitors for more specific targeting of peripheral and brain vasculature, respectively. While a ROCK2 inhibitor might enhance therapeutic effects on brain vessels including CAs, and minimize peripheral side effects (more likely mediated by ROCK1), such drug has not been used in humans and is now undergoing preclinical development (R44NS095420 grant with Awad as coPI). **A pharma pipeline for safer or more effective chronic ROCK inhibitors will require several years before potential Investigational New Drug (IND) and clinical trials in man.**

Therapeutic clinical trials with statins have already been advocated in CAs based on **statin inhibition of RhoA prenylation, a critical early step in RhoA/ROCK pathway activation** (37, 66, 67). A clinical database at University of Chicago includes 24 human subjects with known CAs enrolled in Institutional Review Board (IRB) approved NINDS funded biomarker studies, who are *receiving various statins for routine cardiovascular indications unrelated to their CA disease* (atorvastatin 10-80 mg; simvastatin 10-60 mg; lovastatin 80 mg). Among those patients followed for 247 lesion-years (T_2-weighted lesions > 4 mm counted), there was only 1 documented SH (0.4 SH per 100 lesion-years). This rate is substantially lower than 173 bleeds during 1,604 lesion-years (T_2-weighted lesions > 4 mm) of follow-up of statin naïve cases in the same database (10.7 SH per 100 lesion-years). While this data does not control for treatment assignment or dose effect, it **does not raise any concern about hemorrhage tendency among CA patients receiving statins** nor in comparison to bleed rates reported in the literature (18). Nor have there been any cases in the New Mexico trial (see below, and letter by Leslie Morrison) nor any reported case in the published literature of CA hemorrhage after initiation of statin therapy (Search was performed on August 20th 2017 using MEDLINE portal for keywords “cerebral cavernous malformation”, “angioma” or “cavernoma” and “statin” or “atorvastatin” and “hemorrhage” or “bleeding”).

Statins have been widely used and well tolerated in neurosurgical patients, including (as with fasudil) for the treatment of vasospasm after subarachnoid hemorrhage (68). Statins have recently been reported to portend better outcome after intracerebral hemorrhage in man (69). In a meta-analysis of clinical trials of stroke prevention, including 83,205 patients, there was no evidence of increased hemorrhagic risk with long term statin use (70). Two clinical trials of stroke prevention (but not all others) did suggest a potentially increased hemorrhage risk with statin in secondary analyses (70). In one study, preconditioning and possibly the early withdrawal of statin were observed to worsen cerebral vasospasm after subarachnoid hemorrhage (71). Two recent reports associated statin use with cerebral aneurysm rupture (72) and with cerebral microbleeds in patients presenting with intracerebral hemorrhage (73), without establishing causation.

Considering all evidence class and level, current American Stroke Association guidelines on management and prevention of intracerebral hemorrhage include no restrictions on the use of statins in patients with hemorrhagic stroke (74). Altogether there is no hint of deleterious effects on statin-treated murine CA models (in fact statins resulted in *decreased* lesional hemorrhage), nor any suspected or reported harmful effects in humans with CA to date, **These cautious enthusiasm and concerns about other potentially harmful pleiotropic effects, create a veritable equipoise, motivating a need for specific and careful study of statins in hemorrhagic CA (75)**.

The Case for Atorvastatin as the Study Drug, at Doses Effecting ROCK Inhibition in Humans. A Phase I safety trial with simvastatin at dose up to 40 mg/day (trials.gov# NCT0176445) noted no deleterious effects in open label enrollment. At least one patient in that trial manifested a dramatic decrease in lesional and brain permeability upon initiating statin therapy (See letter by Leslie Morrison). That trial was limited to familial cases with common Hispanic *CCM1* mutation, and enrolled cases with largely stable CAs without recent SH. It was not designed to **explore higher statin doses effecting maximal ROCK inhibition**, nor was it designed to include **sporadic and familial cases after recent SH**. While that trial was underpowered, and a different case selection and enhanced biomarkers may have offered better sensitivity, the results argue for dose escalation or for testing another statin. Simvastatin limits dose escalation above 40 mg/day per current USFDA label. Atorvastatin is approved for higher doses, twice as potent as simvastatin in measurable effects (52). **Doses of atorvastatin of 40-80 mg/day are needed to achieve a robust pleiotropic ROCK inhibition effect, specifically assayed in human peripheral blood leukocytes** (53, 54). Rosuvastatin, which allows even higher dose escalation than atorvastatin has not been used as long or by as many patients as atorvastatin. **A systematic review of atorvastatin tolerance and side effects at those doses was carefully considered in the design and safety monitoring plan of the proposed trial.**

Equivalency of Mouse Versus Human Atorvastatin Dose. In humans the dosage of statins varies between approximately 0.1–1 mg/kg bodyweight, while most studies in rodents have used doses of 1–100 or even 500 mg/kg (76). An atorvastatin mouse dose of 80 mg/kg/day is equivalent to a calculated “human starting dose” of 44.8 mg/day according to USFDA guidelines: *Human starting dose = Mouse dose X 0.08 (Scaling factor) X 70 kg (Adult weight)* (77, 78). A plasma area under curve (0-24 hours) profile for atorvastatin indicates that a human dose of 96 mg/day replicates the mouse dose of 80 mg/kg/day (79). And per empiric evidence, at least 100 mg/kg/day dose is needed to decrease disease activity in a collagen induced arthritis murine model (80, 81) while atorvastatin at 40 mg/day decreases the disease activity score (DAS28) in arthritis patients (82, 83). And we noted ROCK inhibition effect in mice with atorvastatin 80 mg/kg/day, an effect observed in humans at 40-80 mg/day. Hence **the atorvastatin dose of 80 mg/kg/day in our preclinical experiments corresponds to a human dose range of 40-80 mg/day, commonly used in clinical practice and shown to achieve ROCK inhibition pleiotropic effect**. We propose to use these same doses in the proof of concept trial.

3 STUDY DESIGN

3.1 Patient Population

The study will enroll 80 adult patients 18-80 years of age (inclusive) with untreated CAs of all genotypes who suffered an adjudicated symptomatic lesional hemorrhage (20) within one year of enrollment. The hemorrhagic lesion would need to have been considered for surgery, and ultimately not resected. We are hence specifically targeting deep and brainstem lesions with greatest risk of rebleeding in the subsequent 2 years, most likely to benefit from stabilizing vascular leak. Given identical somatic mutations and demonstrated lesional ROCK activity (38, 42), cases with sporadic and familial CAs of all genotypes are included (except for post-irradiation cases). Inclusion and exclusion criteria are detailed in Protocol Section 4, aiming to exclude cases receiving prior statin therapy in any form or for any indication in the prior year, cases at high risk for statin intolerance, cases where the hemorrhagic lesion has been treated with surgery or radiation, and other customary exclusions in similar trials. Children will not be enrolled, per Investigational New Drug (IND) exemption restriction for this trial.

The Neurovascular Surgery Program at the U Chicago has an international reputation in CA clinical care and research (www.uchospitals.edu/ccm) and is the first recognized **Center of Excellence** by the Angioma Alliance (www.angioma.org). During the past 2 years (June 2015 - May 2017) the study PI's clinic evaluated 171 CA patients, and 149 cases, or **87% were enrolled in IRB approved clinical surveillance and biomarker validation projects**. Of these, 37 had suffered a SH during the prior year, 3 of whom were on statin therapy and 7 underwent surgery for lesion excision upon SH (including a case on statin). Hence, 28 subjects (14/ year) would have met inclusion criteria, and at least **12 cases/ year would be enrolled in At CASH EPOC trial from current U Chicago** case activity, based on our 87% enrollment record in IRB studies even without promised intervention. This in in addition to cases ("on the docket") who bled in the year prior to trial launch.

We plan to boost trial enrollment from **Chicago Consortium** sites (see letters from NU, Rush UIC Neurosurgery Chiefs, and Chicago StrokeNet PI Dr S Prabhakhan) targeting >30 CA cases/year with SH for screening, but need only enroll **10 additional cases/year though these regional referrals** and other healthcare professionals in the Chicago Neurological Society (including all practicing neurosurgeons and neurologists serving a 9 million regional population within driving distance of our center). The **Angioma Alliance** (see letter) has identified 48 CA cases throughout the U.S. with SH in the prior year, not taking statins, whose lesion was not resected and who expressed willingness to travel for a trial. We target screening of 20 such cases but need only enroll **5 additional cases/year through this national referral** to meet our enrollment trarget.

Our team has used similar Chicago consortia to recruit cases for the CLEAR and MISTIE trials. The Angioma Alliance has had a strong record of referring patients from outside Chicago and their enrollment in research studies at our site. We plan a **city wide**

seminar upon approval of the trial, and secured its funding from the Kluver Memorial fund at U Chicago, to present it to regional clinicians. **Trial brochure (attached) and trial status updates** shall be e-mailed to members of the Chicago Neurological Society, to the Society of Neurological Surgeons (including all U.S. neurosurgery academic chairs and program directors) and to all registered CA patients in the Angioma Alliance every 6 months. **We intend to facilitate the enrollment and retention** of trial subjects from outside the region, with stipends covering travel expenses for enrollment and follow-up visits. **We note that there is no other ongoing clinical trial of a competing therapy.**

Back up no-cost plan of **extending enrollments into year 4** can be considered, and a last resort option of enlisting another site with accredited biomarker validations, and a record of case enrollment through the **CASH Trial Readiness network** (U01 NS104157, 2017-2022) also led by the trial PI. **Retention is promoted** through scheduled calls every 3 months, and emails with trial status updates every 6 months to enrolled subjects.

3.2 Blinding

The study is designed as a double-blind, placebo-controlled clinical trial. Enrolled subjects will be blinded to treatment rendered, as will the PIs, outcomes assessors processing the radiological biomarkers, the trial staff at the Chicago site and the Data Coordinating Center (DCC) staff at the Baltimore site. Atorvastatin and placebo capsules will be formulated by a third party pharmacy representing two different doses of atorvastatin (40 and 80mg) or placebo. The Research Pharmacy at the Chicago site shall be informed electronically about the treatment allocation (see Section 3.3 Randomization), and it shall dispense the appropriate drug or placebo in a 6-month supply, given at enrollment then mailed by the study team biannually, one month ahead of expected date of exhausting the supply. Subjects in the treatment group will receive unlabeled color coded capsules containing pulverized atorvastatin tablets in the designated doses. Subjects in the control group will receive identical looking color coded capsules containing inactive filler. In the case of dose de-escalation, the Research Pharmacy shall be instructed about the decision to lower the dose, and it shall dispense capsules of different color indicating the lower dose, or identical colored placebo capsules, without breaking the blind to the patient or research team.

The Baltimore statistician shall prepare safety reports including comparison of adverse events (AEs) and interim outcome measures by treatment group labeled as A or B without identifying either as active treatment (See Sections 9.4 on Data Monitoring and 10.3 on Quality Assurance and Data and Safety Monitoring). If a concern is raised about safety and the need to suspend trial enrollment, the independent medical safety monitor (IMSM) may request to identify A or B groups as treatment or placebo. Individual subjects will not be identifiable in the safety reports, maintaining the double-blinding if the trial is resumed.

3.3 Randomization

Once a patient is determined to be potentially eligible for participation in the study, the site coordinator or a designated member of the study team at the Chicago site will enter the Inclusion/Exclusion information about the study subject into the VISION (Prelude Dynamics, Austin, Texas) web-based electronic case report form (eCRF) system. The VISION system will process these subject variables, determine eligibility and, if eligible, assign an identification number and treatment method to the patient. The randomization process is handled by an embedded software module designed using SAS (v.9.4 or higher). This module is part of the system and is invoked by the coordinator or investigator within the eCRF system. Randomization is triggered by the investigator or coordinator, via completion of a form in the eCRF, after all eligibility checks are performed. If the coordinator/investigator checks the randomize box and saves the form, the system will automatically set the enrollment date, assign a study ID and trigger the embedded randomization algorithm to determine the randomized treatment (including a final check to make sure the subject meets all eligibility criteria). The site coordinator is provided with a unique, blinded electronic treatment assignment number (randomized patient number) at the end of this process.

Enrolled subjects are randomized to the atorvastatin or placebo arm in a 1:1 ratio. Randomization shall be stratified by sex, with separate permuted blocks for each sex. A unique treatment assignment number (randomized subject number) and its corresponding treatment group allocation will be generated. This shall be communicated electronically through to the Research Pharmacy in Chicago, to appropriately dispense the capsules containing active drug or placebo.

3.4 Method of Dosing

After randomization, enrolled subjects in both groups will be given unlabeled color-coded capsules to be taken once daily. In the absence of signs or symptoms of statin-related adverse events (Table 2), subjects will be instructed to continue on the same regimen. The dose may be de-escalated based on subject's tolerance. Subjects reporting adverse events per pre-specified criteria (Category B detailed below) will be put onto the lower dose (40 mg/day) of atorvastatin, with a different color. Subjects randomized to the placebo arm will receive an equivalent supply of identical color unlabeled capsules, devoid of the active ingredient, to be administered in the same fashion as the active drug. They will undergo the same evaluations, dose adjustments, and follow up visits as the treatment cohort.

4. SELECTION AND ENROLLMENT OF SUBJECTS

4.1 Inclusion Criteria

4.1.1 Age 18-80 (inclusive).

4.1.2 Asymptomatic, mild or moderate disability requiring some help but able to attend own bodily needs without assistance. Defined as modified Rankin score (mRS) 0 – 3.
4.1.3 Diagnosis of CCM of any genotype supported by relevant imaging studies, where surgical resection is not performed after due consideration per current standard of care..
4.1.4 Symptomatic CCM bleeding event within 1 year prior to enrollment.
4.1.5 Must be willing/able to travel to the study site for study visits (baseline, 12 and 24 months) over the course of the study**.**

4.2 Exclusion Criteria

4.2.1 Pre-menopausal women who are breastfeeding, pregnant or likely to get pregnant during the study period.
4.2.2 Prior surgical treatment of the hemorrhagic CCM lesion.
4.2.3 Failure to pass MRI safety screening (claustrophobic, metal implant, etc.).
4.2.4 Known allergy or intolerance to gadolinium.
4.2.5 Severely impaired renal function (eGFR < 60ml/min), active renal disease or status post-kidney transplants.
4.2.6 Statin therapy, for any indication, for more than 7 continuous days or greater than 14 total days within 12 months preceding enrollment.
4.2.7 Indication to use statin medication for current approved indication, unrelated to CCM.
4.2.8 Known allergy or intolerance to statins.
4.2.9 Liver dysfunction or active liver disease (including chronic viral hepatitis) defined as baseline serum transaminases levels twice the upper range of normal.
4.2.10 Previous diagnosis of skeletal muscle disorders of any cause (myopathy), or baseline creatine kinase levels five times the upper range of normal.
4.2.11 Currently treated with or likely to need treatment with one or more of prohibited medications listed in section 5.3.
4.2.12 Active drug or alcohol use or dependence that, in the opinion of the site investigator, would interfere with adherence to study requirements.
4.2.13 Serious illness (requiring systemic treatment and/or hospitalization) until subject either completes therapy or is clinically stable on therapy, in the opinion of the site investigator, for at least 30 days prior to study entry.
4.2.14 Any other condition that the investigator believes would pose a significant hazard to the subject if the investigational therapy were initiated, including conditions resulting in or precipitating myopathy (e.g. HIV, uncontrolled hypothyroidism).

4.2.15 In the investigator's opinion, the patient is unstable, and would benefit from a specific intervention rather than treatment with atorvastatin.
4.2.16 Inability or unwillingness of subject or legal guardian/representative to give written informed consent.
4.2.17 No documentation of valid healthcare insurance.
4.2.18 No medical record confirmation of a primary care physician or other provider of ongoing medical care.
4.2.19 Previous cranial irradiation or radiosurgical treatment.
4.2.20 Use of ritonavir as an oral drug for COVID-19

4.3 Study Enrollment Procedures

4.3.1 Pre-screening:

Local CA Patients: Patients under the care of the study principal investigator will be pre-screened for eligibility. Pre-screening will include a review of medical records, prior imaging, and laboratory studies. Depending on the date of the last clinically symptomatic hemorrhagic event, eligible patients will be contacted and briefed about the study and if interested, will be invited to the screening/baseline visit. Subjects will be sent a copy of the consent form for their review prior to their first study visit. We estimate that at least 50% of subjects will come from this source.

Referred CA patients: patients referred through the Angioma Alliance, other healthcare professionals, or self-referred will undergo pre-screening evaluation over the phone with the Trial Nurse (TN) or coordinator to determine eligibility for the trial. Patients meeting the pre-screening criteria will be sent a copy of the consent form and will be asked to send a digital copy of their most recent MRI scan to confirm their diagnosis. Once diagnosis is confirmed by the study PI, subjects will be invited for the screening/baseline research visit at the University of Chicago Medicine.

4.3.2 Screening and Enrollment:

All potential subjects will be scheduled to meet with the principal investigator. The visit will involve detailed screening, including collection of medical history, prior and concomitant medications, and a neurological examination to confirm eligibility. Surgical resection will be considered in every case as an alternative to medical therapy, per current standard of care. As part of the screening, blood tests will be performed, which in the case of successful enrollment, will serve as baseline laboratory results (Section 6.3.6). With verification of eligibility criteria, informed consent will be finalized. ***A travel stipend for each trial related visit will be provided to subjects from outside the Chicago area who are required to travel greater than 200 miles to the study center.***

4.3.3 Baseline Imaging:

Randomized subjects will undergo a baseline MRI, which will include special sequences utilized throughout the trial (DCEQP and QSM). This will be pre-scheduled at the same time as the screening visit (particularly for subjects traveling from outside the Chicago area), or as soon as practical for local patients.

5. STUDY INTERVENTION

5.1 Intervention, Administration, and Duration

Study drug will be administered orally as a single daily dose in the morning. Subjects in the treatment group will be started on their corresponding dose 80mg of atorvastatin or placebo. Should the subject report an adverse event meeting pre-specified criteria (Adverse events Category B below), he/she will be put on the lower dose (40 mg) of drug or matched colored placebo capsule.

Administration of study drug shall continue once daily dose in the morning for 24 total months with scheduled visits and repeat imaging at 12 and 24 months from enrollment, unless the subject reaches attrition or a safety endpoint before then. If new symptoms arise at any point after starting the study intervention subjects will be contacted by the study-designated TN for further assessment and be provided with instructions regarding continuation of study intervention based on the study adverse events management protocol (Section 7).

5.2 Handling of Study Intervention

A third party pharmacy will be contracted to formulate and supply atorvastatin and placebo capsules for the trial. The Research Pharmacy at the University of Chicago will be responsible for storage, packaging and dispensing the study drug in conjunction with the research team.

5.3 Concomitant Interventions

5.3.1 Required Interventions

- Daily oral administration of the study drug.
- Laboratory blood testing at screening, 3, 12 and 24 months. Tests will include liver enzymes and creatinine kinase (to be repeated if clinically indicated).
- A total of three MRI scans involving special sequences for QSM and IV contrast material for DCEQP acquisition. IV contrast administration will be preceded by a blood test to check renal function as indicated per hospital policy.

5.3.2 Prohibited Interventions

The following drugs are contraindicated throughout the trial as they are associated with increased risk of rhabdomyolysis when combined with statins. If clinically indicated the decision whether or not these medications can be taken should be discussed with the study PI who will weigh the risks and benefits of staying on study drug.

- Cyclosporine
- Fibrates
- Niacin
- Azol antifungals
- HIV/HCV protease inhibitors
- Macrolide antibiotics
- Colchicine

5.3.3 Precautionary Interventions

The following drugs interact with Atorvastatin. If unavoidable, extra measures should be put in place for closer monitoring.

- OCP[1]
- Antacids[2]
- Digoxin[3]
- Lipid lowering resins[2]
- Rifampicin[4]
- Grapefruit juice[5]
- Calcium channel blockers[6]

[1] Values for norethindrone and ethinyl estradiol may be increased, co-administration of statins and an oral contraceptive increased serum levels for norethindrone and ethinyl estradiol. These increases should be considered when selecting an oral contraceptive for a woman taking atorvastatin.
[2]Interfere with absorption of atorvastatin from GI tract
[3]When multiple doses of atorvastatin and digoxin were co-administered, steady state plasma digoxin concentrations increased by approximately 20%. Patients taking digoxin should be monitored appropriately.
[4]As well as other known activators of the Cyt-P450(3A4) isoform which metabolizes most statins in the liver. Interaction affects the pharmacokinetic properties of the drug leading to changes in serum concentration.
[5]Contains one or more components that inhibit Cyt-P450(3a4) and can increase plasma concentrations of atorvastatin, especially with excessive grapefruit juice consumption (>1.2 liters per day).
[6]Weak inhibitors of Cyt-p(3a4) system

5.4 Adherence Assessments

The team is in the process of evaluating potential electronic monitoring devices, an eDiary and a smart bottle. Both devices are equipped with a SIM card and programmed to wirelessly transmit compliance data to the study database in real time. Both devices are linked to a closed loop electronic feedback system that is programmed to set off reminders in escalating manner and ultimately notify the study team. In addition to compliance, these innovative monitoring systems will also allow subjects to notify the team early on of any adverse events or change in concomitant medications. All compliance data will be continuously recorded in the study database and will be used to guide decision-making. In addition, members of the research team in liaison with the local pharmacy will perform pill counts to confirm adherence at the time of follow up visits.

In addition, the TN or research coordinagtor shall contact each subject for scheduled phone calls every three months (to be completed every 10-14 weeks), with a structured questionnaire about compliance and tolerance.

6. CLINICAL, LABORATORY AND IMAGING EVALUATIONS

All enrolled subjects will go through the same schedule of investigations and follow up visits throughout the study period (Table 1).

6.1 Schedule of evaluations

Evaluation	V1 Screening (0)	V2 Safety labs, Phone call (3m)	V3,4 Phone call (6, 9m)	V5 Follow up (12m)	V6-8 Phone call (15,18,21 m)	V9 End of Study (24m)
Informed consent	X					
Demographics	X					
Inclusion/Exclusion criteria	X					
CCM History	X					
Statin History	X					
Medical/Surgical History	X			X		X
Prior and concomitant medications	X	X	X	X	X	X
Physical and Neurological Examination	X			X		X
MMSE	X			X		X
mRS	X	X	X	X	X	X
Euro-QoL-5D Scale	X			X		X
Euro-QoL VAS	X			X		X
Neuro-QoL	X			X		X
Intervention adherence/ Adverse Events*		X	X	X	X	X
MRI	X			X		X
Laboratory investigations						
• CBC	X					
• Renal functions	X	X		X		X
• LFTs	X	X		X		
• TFTs	X					
• CK	X	X		X		
• Lipid profile	X			X		X
• Hepatitis viral profile	X					
• HIV	X					
• Pregnancy test	X					
Study intervention initiation/continuation assessment	X	***X***	X	X	X	
Randomization	X					

Table 1: AT CASH EPOC trial, schedule of evaluations.

*These are assessed at each follow-up, by automated weekly follow-up messages circulated by the electronic monitoring system, and in scheduled calls with each enrolled subject every three months (1-0-14 weeks), as described in section 5.4

6.2 Timing of Evaluations

6.2.1 Initial screening visit (V0) – up to 30 days prior to enrollment

This visit may occur on the same day as the baseline visit (V2) at the discretion of the investigator. Subjects will undergo the following assessments and sign an informed consent form in order to be randomized into this study:

- Informed consent
- Obtain subject demographics
- Eligibility criteria (inclusion/exclusion criteria)
- Neurosurgical review (confirmation of diagnosis, date of last symptomatic hemorrhage, statin use, past medical and surgical history)
- Laboratory blood tests (CBC, renal functions, LFT, CK, lipid profile, HIV, hepatitis viral profile and pregnancy test)
- Blood sample processed for ROCK activity assay on peripheral leukocytes, and plasma stored in biobank for future/ancillary biomarker studies.
- Characterization of genotype in cases with multiple lesions, or referral to Angioma Alliance for genotyping through the Alliance's program (www.angioma.org).

6.2.2 Second screening visit (V1) – Baseline

The screening visit (V0) and the baseline visit (V1) could occur on the same day at the investigator's discretion. The following will be performed at the baseline visit prior to each subject receiving study treatment:

- Eligibility criteria (inclusion/exclusion criteria)
- Physical, neurological and mini-mental state examination (MMSE)
- Modified Rankin score (mRS)
- Euro-QoL 5D, Euro-QoL VAS, Neuro-QoL SF Cog evaluations
- MRI
- Discuss results of MRI
- Meeting with Trial Nurse
- Evaluation for suitability of atorvastatin therapy
- Randomization
- Providing study monitoring device (eDiary/smart bottle)

6.2.3 Three month phone call and safety labs (V2)

The 3 month visit (V2) will consist of a phone call from the Trial Nurse or coordinator as well as laboratory tests. During the phone call, the TN or coordinator will collect information about the patient's current mRS, concomitant medications, and any adverse events. The following laboratory tests need to be completed as part of standard care at 3 months:

- Renal function
- LFT
- CK

6.2.4 Six and 9 month follow-up phonce call (V3 and V4)

The 6 and 9 month visits will consist of a phone call from the Trial Nurse or coordinator to collect the following information:

- Modified Rankin score (mRS)
- Concomitant medications
- Adverse events

6.2.5 Twelve month follow-up visit (V5) – 12m +/- 4wks

The following assessments will be performed on all subjects at 12 months from enrollment:

- Laboratory blood tests (Renal functions, LFT, CK and lipid profile)
- MRI with DCEQP and QSM
- mRS
- Euro-QoL 5D, Euro-QoL VAS, Neuro-QoL SF Cog evaluations
- Intervention adherence/ adverse events review: review results of laboratory investigations, review study monitoring device (eDiary/smart bottle), manual pill count, elicit drug-related adverse events, full medical examination, MMSE, assess suitability for continuation of statin therapy.
- Neurosurgical review: neurological examination, MMSE, discuss results of MRI, dose modification if necessary.
- Blood sample processed for ROCK activity assay on peripheral leukocytes, and plasma stored in biobank for future/ancillary biomarker studies.

6.2.6 Fifteen, 18, and 21 month follow-up phonce call (V6, V7, and V8)

The 15, 18, and 21 month visits will consist of a phone call from the Trial Nurse or coordinator to collect the following information:

- Modified Rankin score (mRS)
- Concomitant medications
- Adverse events

6.2.7 End of study visit (V9) – 24m +/- 4wks

The following interventions will be performed on all subjects at 24 months from enrollment:

- Laboratory blood tests (renal functions, LFT, CK and lipid profile)
- Intervention adherence/ adverse events review: review results of laboratory investigations, review study monitoring device (eDiary/smart bottle), manual pill count, elicit drug-related adverse events, full medical examination, MMSE, assess suitability for continuation of statin therapy
- MRI with DCEQP and QSM
- mRS
- Euro-QoL 5D, Euro-QoL VAS, Neuro-QoL SF Cog evaluations

- Neurosurgical review: neurological examination, MMSE and discuss results of MRI
- Blood sample processed for ROCK activity assay on peripheral leukocytes, and plasma stored in biobank for future/ancillary biomarker studies.

6.3 Special Instructions and Definitions of Evaluations

6.3.1 Neurosurgical Evaluation

Subjects will undergo a comprehensive neurological examination at every visit beginning with the screening visit (minus the 3-month lab test as that is done with their own PCP). Serial neurological examinations will objectively document subject's neurological status and monitor for any new deficits or changes from baseline neurological status. The comprehensive neurological evaluation will include the Mini-mental state examination (MMSE) and documentation of Modified Rankin Score (mRS), and a baseline quality of life assessments (Euro Quol 5D and Visual Analog Scale, and Neuro QOL Communications Short Form).

6.3.2 Confirmation of diagnosis and recent history

Diagnosis of cerebral cavernous malformation is based on radiological imaging, which utilizes high-resolution MRI sequences. Conventional sequences [T_1-weighted, T_2-weighted, FLAIR and susceptibility weighted imaging (SWI) sequences] are helpful in diagnosing clinically significant CCM lesions. Inactive lesions are depicted by regular to ovoid area >10 mm of homogenous signal loss surrounded by a hyperintense hemosiderin (iron) ring. Active lesions show evidence of blood at different ages within the lesion and are best seen on T_1-weighted images, to be correlated with clinical symptoms (18)

6.3.3 Informed Consent

The informed consent process can begin at any time during screening but must be obtained prior to randomization. A signature on the consent form does not translate into enrollment in the study. The study center will document the informed consent process and the signing of the consent form in a written progress note, place a signed copy of the consent form in the hospital medical records, and keep the signed original consent form in the subject study binder (paper or electronic). A signed copy must be given to the subject as well. Informed consent will include specific consideration of the alternative option of surgical lesion excision.

6.3.4 Evaluating suitability for statin therapy

This evaluation will be performed at screening, 3 and 12 months. These visits will include an evaluation of subjects' tolerance to study drug, a review of any new symptoms, a complete physical examination, and an evaluation of lab results. Subjects will also be reminded about the contraindications and precautions to statin therapy.

6.3.5 MRI

A magnetic resonance imaging protocol that includes DCEQP and QSM sequences will be performed at baseline, 12 and 24 months from enrollment. The imaging protocol will be performed using the 3T instrument, and involves intravenous contrast injection and utilizes special FDA approved sequences directed at assessing lesional/brain white matter permeability and iron deposition as detailed in Section 6.5. Prior to every MRI scan, subjects will have their renal functions checked if needed according to the local protocol.

6.3.6 Laboratory tests

These assessments will be performed at the baseline, 12 and 24 month follow up visits prior to every MRI scan according to the study schedule (Table 1) (84, 85). Assessments marked with an asterisk (*) will also be performed at the 3 month follow up visit as part of the subject's local, routine care to assess statin tolerance:

- **Complete blood count (CBC):** performed as part of baseline blood tests and repeated only if clinically indicated
- **Renal functions*:** Renal functions and electrolytes, performed at screening to determine subjects baseline renal function and safety of IV contrast material. Repeated prior to every scheduled MRI scan and as clinically indicated. Could be substituted with pin-prick tests prior to IV contrast administration according to local protocol.
- **Liver function tests (LFTs)*:** liver transaminases, alkaline phosphatase and bilirubin performed at baseline and at 6 months from enrollment on all subjects.
- **Thyroid function tests (TFTs)**: performed at screening and when investigating adverse events.
- **Serum creatine kinase (CK)*:** performed at baseline and at 6 months from enrollment on all subjects.
- **Lipid profile:** Total cholesterol, HDL and LDL.
- **Hepatitis viral profile**
- **HIV** (Known cause of myopathy, which is one of the most common side effects of statins resulting in cessation of treatment).
- **Pregnancy test**
- **Blood sample shall also be drawn for ROCK activity assay on peripheral leukocytes** as detailed in 6.4 below, and plasma freeze-stored for future or ancillary biomarker studies.

6.3.7 Unscheduled Evaluations

The electronic feedback system will allow for the exchange of notifications between the subject and study team. Unscheduled evaluations could be in the form of a telephone call or an actual visit to the study center. Evaluations could be triggered by the subject in response to any potential adverse events or requested by the study team if there are

any concerns about compliance or prohibited concomitant interventions. In the event of a possible drug-related adverse event an unscheduled visit will be arranged wherein a clinical evaluation and/or other relevant investigations will be performed.

6.3.8 Pregnancy

Because of the unknown teratogenicity profile of the study drug, women of childbearing age are excluded from the trial unless they are surgically sterilized or using a reliable method of contraception. In addition to formal laboratory testing at screening, negative pregnancy status will be confirmed for all women of childbearing potential prior to each MRI evaluation per local institutional policy. Pregnancy during the trial will be considered a safety endpoint; drug administration shall cease but patient will be followed for the duration of the study.

6.4 ROCK Activity Assays

6.4.1 ROCK Activity on Peripheral Leukocytes

Our collaborator James Liao has shown that ROCK activity may be quantified in human leukocytes isolated from peripheral blood [by assessing the ratio of pMBS/glyceraldehyde-3-phosphate dehydrogenase (GAPDH) protein], and that this activity is inhibited by statins (54, 86, 87). It is our intention to investigate whether ROCK activity in peripheral blood leukocytes is impacted by the treatment intervention at the proposed doses in CA subjects with recent hemorrhage. We have already performed these analyses in correlation with brain permeability measurements and disease severity in CAs, in conjunction with prior exploratory developmental project (R21NS087328), and data from those studies has been published (88).

In addition clinical laboratory tests to be performed at baseline, and at 12 and 24 months follow-up visits (V1, V3 and V4), 5-10 ml of blood are collected into heparinized vacutainer tubes. Leukocytes are isolated from the blood and frozen at -86°C after fixing in 50% trichloroacetic acid/50 mM dithiothreitol. Cells are thawed and relative ROCK activity in leukocytes is assessed by Western transfers as described by our collaborator James Liao (54, 86, 87, 89). Since ROCK phosphorylates MBS (MYPT1), relative ROCK activity will be assessed by the ratio of the intensity of pMBS staining/GAPDH staining. GADPH is chosen as reference since it was shown to be a more reliable internal control in Western blot analysis of leukocytes than other housekeeping proteins (90). For these quantitative assays, we shall use the pMBS antibody developed by our collaborator Liao, targeting the Thr^{853} of MBS site, known to be specific for only ROCK and not other serine/threonine kinases (91, 92). Lysophosphatidic acid-stimulated NIH3T3 fibroblast lysates will be run on all gels as a positive control to standardize results between experiments (89). We shall enter the pMBS/GADPH ratio in immunoblots for each subject/visit as “ROCK Activity on Peripheral Leukocytes” for subsequent secondary analyses as proposed in the SAP, comparing placebo and intervention groups at baseline, and at 1 and 2 year follow-up.

6.4.2 Ancillary Biomarker and Lesional Studies

Plasma Samples for Ancillary Studies. The plasma from the blood samples shall be stored at -86°C for subsequent/ancillary biomarker studies (88, 93). Subjects enrolled in this trial will be consented for plasma biomarker studies under separately approved IRB protocol.

ROCK Activity in Excised Lesions. Additional assessment of **lesional ROCK activity shall be performed on any excised lesion specimens** during the course of the study, as per current aim of ongoing program project studying signaling aberrations in CA (P01NS092521), and techniques published by our laboratory (36, 38, 42, 46, 49, 51, 94). Subjects enrolled in this trial who ultimately undergo lesion resection will be consented for these lesional studies under separately approved IRB protocol.

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6.5 Detailed Imaging Protocols

6.5.1 MRI Sequences for Characterizing the CA Lesion

The following standard clinical shall be conducted on a 3T instrument for comparability of sequences, consistent with evidence-based consensus recommendations for optimal imaging of cavernous angiomas (CA) (17).

Those shown below in **BOLD** are specifically relevant to biomarker measurements of iron content by quantitative susceptibility mapping (QSM), and permeability by dynamic contrast enhanced quantitative perfusion (DCEQP). Note that the proposed DCEQP scan will image the first pass of the contrast agent normally injected for the clinically indicated purpose of post-gadolinium (Gd) anatomic imaging. It does not require additional contrast burden (in fact it uses a lower contrast dose than for routine clinical indications). We will require that all imaging be performed on a 3T MRI scanner and an 8-channel head coil to allow for parallel imaging.

- Sagittal T_1-weighted sequence
- Coronal T_2-weighted sequence
- Axial VenBOLD
- Axial diffusion-weighted sequence
- Axial T_2-weighted sequence
- FLAIR long TR
- Axial T_1 -weighted sequence pre-Gd contrast
- **CCM Axial T_2*-weighted sequence**
- **AIF Localizer 3D TOF-MRA**
- **T_1–weighted mapping SR-SPGR**
- **DCE Multi-phase SR-SPGR +C**

The ***index CA with SH*** shall be characterized by the PI as to location (lobe, superficial/deep based on distance of lesion epicenter < or > along a line between cortical surface and ventricle, basal ganglia, thalamus, cerebellum, brainstem), and size (maximal diameter in mm on axial T_2- and T_1-weighted images). This information shall be entered on electronic data forms, referenced by subject number and scan date. In cases with

intra-lesional hemorrhage, the full lesion diameter shall be sized (including the "hemosiderin ring" on T_2–weighed sequences). In cases of extra-lesional hemorrhage, the maximum diameter of the CA and the hematoma shall be separately noted. These characterizations of lesion size and location are well standardized clinically, with the prescribed sequences at 3T field strength (17). Lesion size will not be considered on gradient echo or susceptibility sequences where blooming effects may introduce errors.

6.5.2 MRI Sequences for QSM and DCEQP Biomarker Measurements

In addition to the standard clinical scans (i.e. those un-bolded above), research scans will involve 1 additional image acquisition for QSM and 3 acquisitions for DCEQP (those in **bold** under MRI sequences above). These are performed at baseline and at two follow-up scans. These protocols are adapted from protocols previously published by many investigators, and they have been used and vetted extensively at The University of Chicago, included validations specifically in CA patients. The same protocols are undetgoing multi-site validation in conjunction with parallel CASH Trial Readiness Project (U01NS104157**).**

Quantitative Susceptibility Mapping (QSM)

A single three-dimensional, multi-echo, T_2*-weighted, spoiled gradient echo sequence will be used for data collection and create of the QSM images (Figure 9). The imaging parameters are: 3D, axial, FOV/matrix = 240 mm/256x256; repetition time (TR)= 55 ms/5 echos times uniform spacing, flip angle = 15°; partitions = 120,1.0 mm.

The stack will be oriented parallel to the corpus callosum before being repositioned in the S/I direction over the area of interest. The protocol design allows coverage of most if not all of the brain; ensure the area of interest is covered.

Localization for Whole Head Coverage for Quantitative Susceptibility Mapping

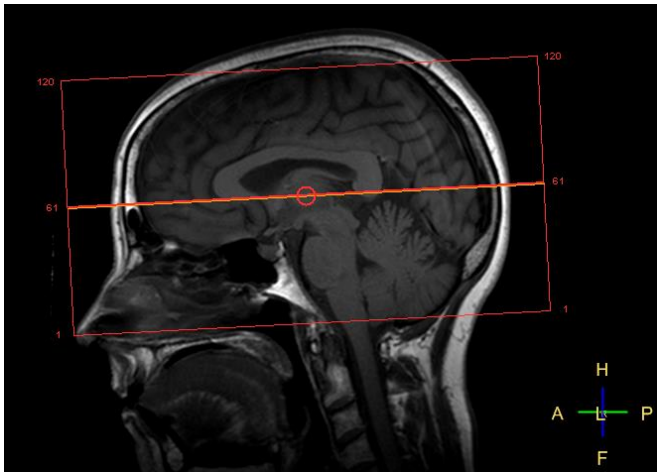

Figure 9: Multi-echo T_2*-weighted images cover the whole brain and are used to quantify magnetic susceptibility in cavernous angiomas.

QSM images will be de-identified, extracted in DICOM format and post-processed at University of Chicago. The QSM images will be reconstructed using a morphology-enabled dipole inversion (MEDI) algorithm (95, 96), as previously reported (60). Inspection of image quality is performed immediately by the technician, and this non-contrast acquisition may be repeated in the unlikely event that motion artifacts are identified.

Dynamic Contrast-Enhanced Quantitative Perfusion (DCEQP)

The DCEQP protocol consists of 3 components that are run in sequence: (1) Arterial Input Function (AIF) Localizer (2) T_1-weighted map and (3) Contrast Enhanced DCE dynamic scan.

Arterial Input Function (AIF) Localizer: A 3D Time-of-Flight acquired at the level of the lower brainstem and pons to cover the internal carotid arteries (Figure 10, yellow box). The reconstructed maximum intensity projection (MIP) images in both the coronal and sagittal views will be used to prescribe the slice to locating the artery for the AIF.

Arterial Input Function Localizer with Time-of-Flight Magnetic Resonance Angiogram

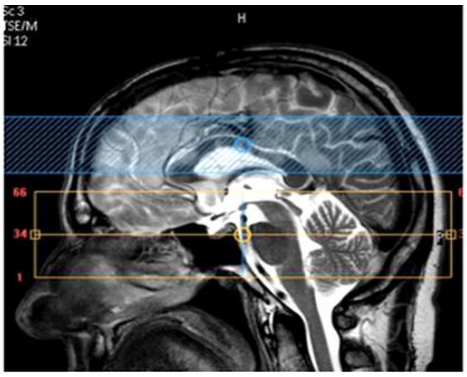

Figure 10: A Time-of-Flight magnetic resonance angiogram should cover the distal internal carotid artery and serves to aid in the prescription of the slices needed of proper acquisition of the arterial input function in the dynamic scans.

After the AIF localizer is acquired to image the position and angulation of the internal carotid arteries, a saturation recovery gradient recalled sequence (SPGR) will be acquired both for an initial T_1-weighted measurement and for the subsequent dynamic imaging (Figure 11). The prototypical acquisition parameters will be: echo time TE = 1.9 ms, repetition time TR = 3.9 ms, flip angle = 30°, field of view = 230 x 182 mm2, slice thickness = 8 mm, matrix size = 96×61, SENSE factor = 2, centric order phase encoding. The same base sequence is used for both the T1-weighted mapping and the dynamic series part. The base sequence consists of two imaging slabs (stacks):

Slab A - contains a single slice, used for locating the Internal Carotid Artery (ICA) used for determining the AIF (arterial input function).

Slab B - contains 4 slices covering the areas of interest in the brain. One of those slices should include coverage of the CASH lesion, and another the choroid plexus, to be used as an internal reference.

Important: The Slab A should be perpendicular (so the flow is perpendicular to the Slab A imaging plane) to the particular internal carotid artery (left or right) you wish to use for AIF. Please make a note during the data acquisition. Also, caution should be taken to avoid clinoid (c5) or petrous (c2) segments of the ICA.

Separate Slice Locations Prescribed for the Permeability Data and Arterial Input Function

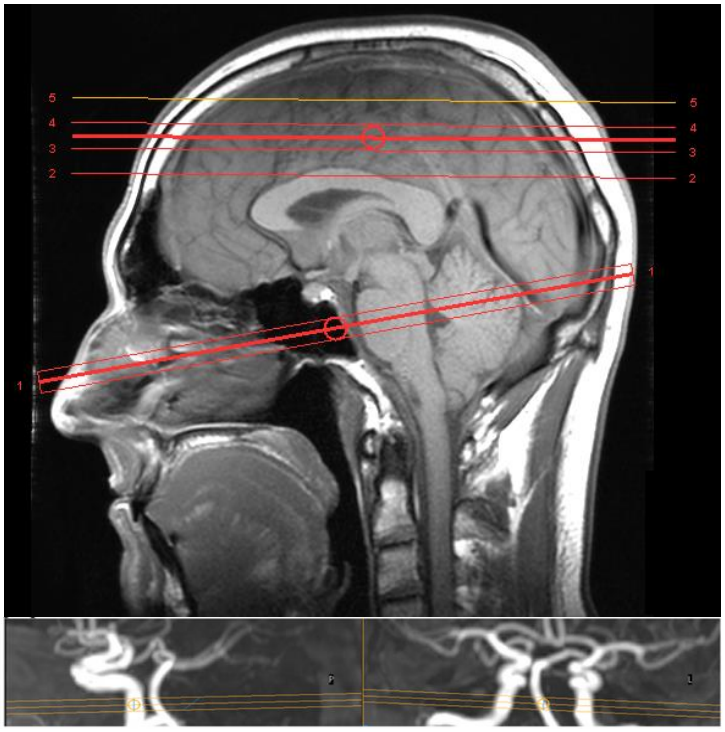

Figure 11: Top: Use a sagittal localizer to cover the relevant anatomy containing the cavernous angioma and choroid plexus (Slab A: top slices). Angulate the lower slice (Slab B), used for the arterial input function based on the 3D time of flight from both sagittal (lower left) and coronal orientations (bottom, right)

T_1-weighted mapping: For the purposes of T_1-weighted mapping, SPGR images should be acquired with the following inversion times Seven Time Delays (TD) values (120 ms, 300 ms, 600 ms, 1000 ms, 2000 ms, 4000 ms and 10,000 ms). A single image stack shall be acquired for each TD.

Dynamic Contrast-Enhanced (DCE) Scan: The passage of the bolus of the contrast agent will be imaged using a saturation TD of 120 ms to minimize the effect of water exchange in such measurements (97). A multi-phase, saturation recovery SPGR with identical coverage, localization and spatial resolution as the T_1-weighted mapping acquisition should be acquired next. This multi-phase acquisition should fix the delay time (TD = 120 ms) and acquire 250 phases (i.e. time points) in conjunction with an injection of a T_1-weighted shorting, Gd-based contrast agent. Co-localization is critical in order to spatially register the T_1-weighted map to the dynamic contrast kinetic scans.

Contrast Injection: MRI-based contrast kinetic will be mapped with a dynamic T_1-weighted scan acquired during the injection of the contrast agent which is normally used for post-Gd T_1-weighted imaging. Specifics are:

OMNISCAN (gadodiamide) will be administered http://www.rxlist.com/omniscan-drug.htm. Dosage will be based on the patient's weight (see chart included at

http://www.rxlist.com/omniscan-drug/indications-dosage.htm, use chart for 0.1 mMol/kg). The injection rate is performed using a computer controlled power injector through an 18 gauge catheter placed in the antecubital vein. Injection and saline flush (15 ml) should both be at 4.0 ml/sec and initiated after the 9th measurement (12 seconds after scan initiation) of the dynamic sequence (250 total,5:32 of total scan time). Since the scan will be performed as a part of routine clinical care with and without contrast, one half of the contrast agent for the permeability scan. After the permeability scan is complete, we inject the remaining half-dose (i.e. an additional 0.1 mmol/kg by body weight, for a total dose of 0.2 mml/kg) and perform routine post-contrast clinical MRIs. If the scan is to be performed only for research purposes, the remaining contrast need not be injected, and post-contrast sequences may be foregone.

Notes:

- The slice positions of the T_1-weighted mapping and the dynamic series need to be identical since a per-pixel based fit is performed during reconstruction
- Try to the tell subject to remain as still as possible to eliminate motion artifacts during this long scan - no additional motion correction post-processing is currently performed.

6.5.3 Data Export and Image Post-Processing

The raw data (images) relevant to biomarker postprocessing will be de-identified and extracted in the classical (not Philips “enhanced”) DICOM format. The needed sequences are:

- Axial VenBOLD,
- Axial T_2-weighted TSE matrix
- Axial T_2*-weighted
- AIF TOF localizer (called fast inflow angio)
- MIP fast inflow angio
- All images from the T_1-weighted mapping scans for each inversion [i.e. delay time, TI (TI_00120 to TI_10000)]
- Dynamic DCE images (called hperf120)

Additionally, the single QSM sequence will be extracted in DICOM format. The de-identified images shall be locally archived and uploaded into a secured shared server at the Magnetic Resonance Imaging Center (MRIC), traceable only to the assigned trial subject number and date of the scan. The University of Chicago *Research Computer Center Midway* computer shall be used for the storage and back up of raw imaging data, while deidentified calculated dynamic contrast enhanced quantitative perfusion (DCEQP) and quantitative susceptibility mapping (QSM) values are linked to enrolled subject number and submitted through the trial’s electronic case report forms (eCRP) to the Data Coordinating Center.

Image Post-Processing

All post-imaging processing will be completed locally at the MRIC using an existing image reconstruction pipeline on a computer running MATLAB software (Mathworks, Natick, MA). QSM images will be reconstructed by using a morphology-enabled dipole inversion algorithm (98), which generates the local susceptibility distribution by inverting the estimated tissue field map with prior information from the magnitude images. The tissue field map will be obtained by removing the background field induced by large susceptibility sources (i.e. air/tissue interface) from the field map derived from the gradient-echo phase images (60). Mean lesional susceptibility values will be generated by drawing a region of interest (ROI) around the lesion on the resulting QSM image in ImageJ for each slice in which the lesion is visible, then averaging all pixels within the ROIs for a lesion.

Final permeability values will be calculated in MATLAB by applying the Patlak method voxel-wise, represented by the following equation: $\frac{C_t(t)}{C_{a(t)}} = K_i \frac{\int_0^t C_a(\tau) d\tau}{C_a(t)} + V_b$, where each $C_t(t)$ is the concentration time-course of each voxel of tissue. The unidirectional influx constant, K_i, is estimated as the slope of the Patlak plot created by the above equation. This equation becomes valid after some time as the vascular phase vanishes, and data points from the first third of each time course are not included for this estimation. More detailed discussions of the methodology have been published (60).

Lesional permeability values will be generated similarly to QSM. For DCEQP studies, in addition to the lesion of interest, ROIs will be taken for either the choroid plexus (if covered by the imaging stack) or normal-appearing white matter far (> 5 mm) from the lesion, to be used as an internal reference value at follow up. Permeability values for White Matter Far (WMF) shall be generated in regions of interest (ROI) covering 3.6 X 3.6 mm (16 pixels or approximately 12.9 mm^2/ROI based on 256-256 matrix for axial brain MR slices). WMF regions are chosen in brain devoid of CA lesions on any MR sequence, as far as possible from and always >16 mm from the border of a visualized lesion, including the tiny SWI lesions.

7. MANAGEMENT OF ADVERSE EXPERIENCES

In the event of an adverse event (Table 2), the first concern will be for the safety of the subject. Subjects will be closely monitored and are required to notify the trial PI or designated TN of any new symptoms that occur following treatment onset. Managed adverse events include all new symptoms, whether expected or unexpected, which are assessed by the TN, in consultation with the on-site statin expert physician, to be reasonably or possibly related to the study intervention. All adverse events that the study team is notified about will trigger at least an unscheduled telephone evaluation. During the evaluation, a member of the study team will assess the seriousness of the reported event, its attributability to the study intervention and advise the subject regarding the treatment plan, including whether to continue on the study drug (99, 100). Serious criteria, definitions, and guidance for reporting follow in section 10.4: Adverse Experience Reporting.

Historically, very low levels of low-density lipoproteins (LDL) have been sporadically associated with some detrimental effects. However, clinical trials on statin therapy have not demonstrated any substantial correlation between lower LDL levels and safety. There is no evidence that lowering low-density lipoprotein levels in people with normal cholesterol profile is inherently unsafe (101).

7.1 Role of Trial Nurse (TN) and Study Coordinator

A trial nurse (TN) working closely with the PI (backed up and sharing call with a trained clinical research coordinator) will be available 24 hours a day, 7 days per week on a special pager and cell phone, and shall be the first portal of contact for study subjects. The TN will also be responsible for following up with subjects in between scheduled visits (in response ot calls by the subjects or at regular scheduled phone calls every three months). H/she will help assess attributability of any new symptoms to the study intervention (section 10.5) and instructing subjects when it is necessary to seek medical attention. The TN and study coordinator shall be blinded to the study group allocation. They will be supplied with management algorithms detailing the standards of care in dealing with the most recognized side effects of the study intervention (25, 102, 103). The TN and coordinator will be able to access subjects' electronic case report form (eCRF) to track their course throughout the study and document any events. The TN and study coordinator shall be overseen by and have unfettered access to the study PI regarding any trial related clinical questions. They shall have direct access to the External Medical Safety Monitor (see below) in the absence of the PI.

7.2 Adverse Event Management Protocol

Mild adverse events (Table 2, Category A) encountered by any subject regardless of assigned treatment will be recorded, relayed to the safety monitoring team and closely followed by the TN. More concerning adverse events (Category B) will be managed by withholding the study intervention for 2-4 weeks pending resolution of symptoms or biochemical abnormalities. Subjects will then be restarted on the lower dose (40 mg/day) of the study intervention (atorvastatin or placebo). Depending on the dose at which adverse events occurred, the process can be repeated, tapering the dose down to attrition as deemed appropriate by the PI in consultation with the external medical safety monitor (MSM). Intolerable or potentially serious adverse events that recur or persist in spite of dose reduction, along with other serious adverse events listed in Section 8 will lead to unblinding and determination of endpoint for that subject in the trial. A statin expert physician consultant is available at the Chicago site to advise regarding adverse event management in individual cases.

7.3 Common Adverse Events

7.3.1 CA hemorrhage

Best care criteria defined by the American Heart Association (AHA) guidelines for management of ICH will be the standards of care for all general medical care in this protocol. Specifically, the guidelines are (i) Stroke Council special writing group

guidelines for the management of ICH (74), (ii) European Stroke Initiative (EUSI) Guidelines for the management of intracranial hemorrhage (104), and (iii) the AANS guidelines for management of elevated intracranial pressure (105). Management may include blood pressure reduction, use of platelets and prothrombotic agents, and use of a surgical procedure. The care team is required to preserve life and functional outcomes in these instances. The management of each adverse event will be recorded and will be reviewed by the safety monitoring team.

If **a CA symptomatic bleed is verified as per adjudicated criteria (20),** including a rebleed of prior lesion or new bleed from another lesion, then a safety endpoint will have been reached, and the subject will be notified, and decision is made to cease the study intervention. Patients will remain in the study for the remaining scheduled follow-ups. The lesion that has hemorrhaged shall be considered for treatment as per current guidelines and standards of care.

7.3.2 Myositis/Rhabdomyolysis.

Skeletal muscle inflammation is a rare but well recognized adverse event in statin therapy. The severity of this inflammation ranges from mild myalgia to multi-organ system failure secondary to myoglobinuria (Table 2). Management should follow standard of care and is to be dictated by the PI based on symptom severity/degree of lab value elevation, in consultation with the on-site statin consultant. Management may include vigorous hydration, alkalinization of urine, correcting electrolytes, and in extreme cases hemodiafiltration. The decision to declare a safety endpoint in the case of myositis, versus the decision to decrease the dose of drug will depend on severity of the myositis as per established guidelines.

7.3.3 Hepatotoxicity

Drug-induced liver insult runs a spectrum from asymptomatic elevation of liver enzymes to acute fulminant hepatitis. However, it is now well recognized that statin-related hepatic adverse events are exceptionally rare and unpredictable. Analysis of safety data pooled from 49 clinical trials of atorvastatin in 14,236 patients showed that persistent elevation in hepatic transaminases does not exceed 0.6% (106). Statin-induced liver insult almost invariably occurs in cases with pre-existing chronic liver disease (e.g. viral/ alcoholic hepatitis, etc.) or concomitant ingestion of hepatotoxic drugs (25, 102, 103). All subjects in this study will be screened for risk of hepatic toxicity prior to enrollment, as per current recommendations by both the American College of Cardiology/American Heart Association (ACC/AHA) and FDA (107). The FDA has recently revised safety guidelines to remove the need for routine monitoring of liver enzymes in patients taking statins. To detect asymptomatic elevation of liver enzymes, enrolled subjects will also have their liver enzymes checked at 3 months and at each follow up visit. Additionally, subjects exhibiting any evidence of liver dysfunction at any point throughout the trial will undergo extensive hepatitis workup and further management in accordance with the trial adverse events management algorithms. (Table 2)

7.3.4 Other adverse events.

Other commonly reported adverse reactions (incidence ≥ 2% and greater than placebo) regardless of causality, reported in patients treated with atorvastatin in previous placebo-controlled trials, such as nasopharyngitis, diarrhea and urinary tract infection, will be individually reported and managed as elaborated in section 7.2.

Table 2 below summarizes potential adverse events by body systems that have been reported with the study intervention and other statin medications.

Adverse Event Category	Mild AE (Category A)	Moderate AE (Category B)	Serious AE (Category C)
General/Constitutional			
• Malaise / Fatigue	Tolerable/self-limited	Intolerable/persistent	Recurrence after dose reduction
• Pyrexia	38.0 - 39.0 degrees C (100.4 - 102.2 degrees F). self-limited	Intolerable/persistent	Recurrence after dose reduction
• Allergic reactions	Erythema multiform	Mild self-limited classic allergic responses such as pruritis, hives or urticaria, cutaneous vasodilation (“flushing”), development of edema (peripheral or airway), peripheral vasodilation (hypotension), bronchospasm, and/or tachycardia.	Recurrence after dose reduction/Anaphylactic reaction/Steven-Johnson syndrome / Toxic epidermal necrolysis
Muscular			
• Musculoskeletal pain (3.5%), muscle spasm (3.6%), arthralgia (6.9%)	Tolerable/self-limited	Intolerable/persistent	Recurrence after dose reduction
• Joint swelling	Asymptomatic; clinical or diagnostic observations only.	Intolerable/persistent	Recurrence after dose reduction
• Incipient Myositis	CK < 3 x ULN, no relevant clinical symptoms.	CK >3 x ULN ***Plus*** new elevation of ALT, irrespective of musculoskeletal symptoms.	Recurrence after dose reduction
• Definite Myositis (0.08%)		Myalgia ***Plus*** markedly elevated serum creatine kinase >10 x ULN	Recurrence after dose reduction
• Rhabdomyolysis			Myositis ***Plus*** evidence of new renal impairment +/- other organ system failure

Adverse Event Category	Mild AE (Category A)	Moderate AE (Category B)	Serious AE (Category C)
• Tendon Rupture			Tendon Rupture
GI/Hepatobiliary			
• Pharyngeal pain (2.3%) , dyspepsia (4.7%), nausea (4%), bloating, eructation, flatulence or diarrhea (6.8%)	Tolerable, self-limited	Intolerable/persistent	Recurrence after dose reduction
• Hepatitis (0.6%)	Elevated Transaminases (ALT, AST): <ULN - 3.0 x ULN, no relevant clinical symptoms.	Elevated Transaminases (ALT, AST): >ULN - 3.0 x ULN, no relevant clinical symptoms.	Recurrence after dose reduction / ALT >3.0 ULN; >3 x ULN with the appearance or worsening of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, or eosinophilia
• Acute Liver failure			Hepatitis plus abnormal plasma levels of ammonia, bilirubin, lactic dehydrogenase, and alkaline phosphatase
• Cholestasis		Serum conjugated bilirubin >1.5 x ULN; clinical jaundice.	Recurrence after dose reduction
• Pancreatitis			Severe abdominal pain; vomiting plus enzyme elevation (amylase 200 above ULN with elevated lipase levels) or radiologic findings
Nervous System			
• Dizziness, insomnia (3%), Depression, nightmares	Tolerable, self-limited	Intolerable/persistent	Recurrence after dose reduction

Adverse Event Category	Mild AE (Category A)	Moderate AE (Category B)	Serious AE (Category C)
• Cognitive impairment	1 points drop in MMSE; not interfering with work/school/life performance; specialized educational services/devices not indicated	>1 points drop in MMSE; interfering with work/school/life performance; specialized educational services/devices not indicated	Persistence after dose reduction
• Symptomatic Intracerebral Hemorrhage			New onset neurological symptoms ***Plus*** intraparenchymal or intralesional hemorrhage confirmed on imaging studies
Endocrine system			
• Hyperglycemia		New impaired glucose tolerance/glycemic control	Persistence after dose reduction
Respiratory			
• Nasopharyngitis (8.3%)	Tolerable, self-limited	Intolerable/recurrent	Recurrence after dose reduction
• Epistaxis	Mild episode, self-limited	Requiring medical attention, Recurrent episodes	Recurrence after dose reduction
Genitourinary			
• Urinary tract infection (5.7%)	Simple cystitis	Recurrent cystitis /pyelonephritis	Recurrence after dose reduction

Table 2: Statin-related adverse events.

AEs are classified based on body system, severity and reported incidences (102, 103, 106-108)

8. CRITERIA FOR INTERVENTION DISCONTINUATION

Atorvastatin/Placebo administration will be discontinued for any of the following reasons (designated as serious AEs or Category C in the preceding Table 2):

- Symptomatic intracerebral hemorrhage (ICH) whether primary, arising from index cavernous malformation that previously bled, or in other lesion, or other ICH of any kind
- Severe allergic reaction
- Rhabdomyolysis
- Markedly elevated liver enzymes
- Liver failure
- Pancreatitis
- Pregnancy
- Intolerable adverse events that persist or recur after serial dose reduction.
- In the investigator's judgment, withdrawal from the trial would be in the subject's best interest (Drug interaction/glycemic control).
- The subject or legal representative withdraws consent, or patient undergoes surgical resection or irradiation of CCM lesion, other brain surgery or irradiation for any cause.
- Patient develops clinical indications for statin use for cholesterol or cardiovascular indications unrelated to CCM.

If one or more of these events occur after commencing the study drug, the subject will be considered to have reached a safety endpoint. Study drug or placebo shall be discontinued. All subjects reaching safety endpoints will be followed through the 24-month follow up visit as a part of the outcome assessment for the study. An attempt will be made to contact subjects who withdraw from the study or who are lost to follow up for safety evaluation. If these subjects wish not to cooperate there will be no additional follow up.

9. STATISTICAL CONSIDERATIONS

9.1 General Design Issues

This shall be a double-blinded randomized, placebo-controlled, exploratory phase I-IIa proof of concept clinical trial. It shall include adults aged 18-80 years with untreated CA of any genotype, who suffered a symptomatic lesional hemorrhage within the preceding year. We plan to enroll 80 subjects during years 1-3, specifically targeting cases whose hemorrhagic lesion was not resected, with greatest risk of rebleeding in subsequent 2 years. Inclusion and exclusion criteria aim to exclude cases receiving prior statin therapy in any form or for any indication in the past year, cases at high risk for statin intolerance, and other customary exclusions in similar trials. Children will not be enrolled, per IND Exemption restriction for this trial.

Enrolled subjects will be blinded to treatment rendered, as will the investigators conducting and reporting biomarker studies (including QSM, the primary outcome measure),

and PIs, the clinical personnel caring for the patient, and DCC staff. The safety monitoring team will have access to A/B treatment designations but will be blinded to which group A and B are assigned, unless a concern is raised and consideration to suspend enrollment in the trial. Atorvastatin and placebo capsules will be formulated by a third party pharmacy in two color coded tiers (green and blue) representing different doses of atorvastatin (40 and 80 mg) or placebo. The 40 mg dose (or color matched placebo capsule) will be used in subjects who develop mild side effects on the initial dose target of 80 mg/day.

After satisfying the screening criteria, patients will be assigned a subject number and randomized to the atorvastatin or placebo arm in a 1:1 ratio (See section 3.3 Randomization). This will be implemented upon subject enrollment, and communicated to the Research Pharmacy at the Chicago site via the web-based Electronic Data Capture (EDC) system. Administration of study drug shall continue thereafter, at once daily oral dose for 24 months, with **scheduled visits and repeat imaging at 12 and 24 months from enrollment, and follow-up calls quarterly, and weekly feed back on compliance by electronic monitoring device**. Safety endpoints include a symptomatic intracranial hemorrhage, from the index CA lesion or another source, or a serious adverse event (SAE) necessitating drug discontinuation (Protocol Section 8). If new symptoms arise at any point after starting the study or relevant laboratory test abnormalities, subjects will be contacted by the TN for further assessment and instructions regarding continuation of study intervention, dose de-escalation or discontinuation, based on the study **adverse events management protocol** (See Protocol Section 7). This approach is similar to that used in other statin trials (84, 85, 99, 100, 102, 106, 107, 109).

9.2 Outcomes

The primary outcome is the mean percent change in lesional QSM per year (called the change score). Each patient contributes two outcome measurements (at year 1 and year 2) based on intention-to-treat. Evaluation of the intervention on this outcome will be performed as a time-averaged difference between two arms using a repeated measures analysis implemented as a linear mixed model, adjusted for gender (110). Patients with outcome measurements in both periods will be included in the initial intention-to-treat analysis. In cases with multiple lesions, only QSM measurements in the lesion with initial hemorrhage (index lesion) shall be considered for the primary outcome assessment. A secondary analysis of the percent QSM change per year shall be conducted per treatment rendered for all patients with at least one annual epoch of measurements.

Secondary outcomes shall include:

- Changes in DCEQP vascular permeability measurements in the index lesion and in brain (white matter far from lesion),
- Rates of QSM and DCEQP biomarker events, representing increases in index lesional QSM or DCEQP above previously articulated “biomarker thresholds” (58); rates of clinically overt hemorrhages in the index lesion per

adjudicated criteria; and rates of lesional expansion, defined as an increase in maximum lesion diameter on T_2-weighted sequences by 2 mm or more.

- Adverse event rates and serious adverse event rates, and drug compliance
- Changes in functional outcome (mRS, MMSE, Euro-QoL 5D, Euro-QoL VAS, Neuro-QoL evaluations)
- ROCK Activity Assay on Peripheral leukocytes (pMBS/GADPH ratio in immunoblots for each subject/visit)
- Impact of sex, genotype and lesion location on primary and secondary outcomes (pre specified subgroup analyses)

9.3 Sample Size and Accrual

9.3.1 Sample size and Treatment Effect Estimation

Sample size calculations were based on providing sufficient power to test the primary hypothesis, that the proposed intervention impacts QSM biomarker activity (indicative of reduced or increased lesional bleeding) during 2 years of prospective follow-up after a recent CA SH. **The primary outcome is the mean % change in lesional QSM per year (called the change score)**. Each patient contributes two outcome measurements (at year 1 and year 2, i.e. epochs). Evaluation of the intervention on this outcome will be performed as a time-averaged difference between two arms using a repeated measures analysis implemented as a linear mixed model and intention-to-treat principle (110).

Estimating a "First-in-Man" treatment effect on a biomarker can be difficult when such effect is demonstrated mechanistically but the translation to a human effect size remains unknown (111). Based on pilot data linking QSM change to clinical events, **we propose a minimum, clinically meaningful and mechanistically plausible treatment effect to be a 20% absolute difference in the mean change score.** It would be "a minimum expected effect", <1/2 the mean 44% QSM increase observed with CA bleed or growth during longitudinal 1-year follow-up of previously stable lesions; and "clinically meaningful", > 3X the threshold of QSM change associated with maximum sensitivity-specificity on ROC analyses (58). The absolute QSM effect size postulated herein is "plausible mechanistically". It would be 0.0068 ppm [20% of 0.0340 ppm mean QSM change observed during follow-up of CA with SH (59)], 6-22 folds lower than the observed range of artorvastatin treatment effect on Perls stain intensity in *Ccm1* and *Ccm3* murine models (Figures 2, 3, 5).

One-year %QSM change in 16 pilot study patients (Figure 7), followed for either 1 or 2 years (22 patient-year epochs), was used to estimate the 1-year/patient standard deviation and within-patient correlation of the primary outcome in trial candidates. A sample size calculation was performed using a repeated measures analysis of two annual percent QSM change scores, assuming a two-tailed test, power = 0.9 and alpha = 0.05 (112). The actual within person correction from the pilot data was estimated at approximately -0.6 (Spearman); however, as this was based on only 6 patients with two-year's

worth of data, we chose the more conservative within person correlation of 0 for our sample size estimates. In addition, we assumed a common standard deviation of 30 for the mean percent change score, which to be conservative is about 20% larger than the standard deviation in our pilot data. **To detect a 20% absolute difference between the mean change score in each arm, based on two annual change scores measured per patient (at years 1 and 2), 25 patients per arm would be required**. To detect a smaller or larger effect size, an absolute difference of 15% or 25%, 44 or 17 patients per arm would be required, respectively (Hintze 2014, PASS v13, NCSS, Kaysville, Utah, USA www.ncss.com). The sample size was further expanded by 37.5% to accommodate the highest estimate of missing data from all causes as discussed below. Hence we shall aim to enroll **40 patients/arm, 80 total subjects**.

9.3.2 Potential Missing Data, Attrition and Premature Trial Endpoint

We considered all potential causes of missing data and we developed strategies to minimize and mitigate them. In initial pilot studies, 34 of 177 (19%) QSM assessments in CA patients were unusable due to technical reasons (mostly patient movement during image acquisition). We eliminated this problem in the most recent 25 consecutive cases with specific patient instructions. We now implement a further step of reviewing the QSM acquisition, and rapidly processing it (10 minutes on local Matlab computer terminals in our laboratory). This allows us to repeat the QSM acquisition if needed, before proceeding with contrast injection for DCEQP measurements. This “chaperoned biomarker acquisition” reduces to virtually zero the rate of missing QSM measures (See letter by Dr Christoforidis and Protocol Section 6.5). We nevertheless project up to 10% missing biomarker data related to imaging (claustrophobia, etc.). We further project an additional 10% missing data due to imperfect follow-up, higher than that recorded in recent trials conducted by our teams (113, 114).

Up to 20% of subjects randomized to atorvastatin (10% of trial subjects) might not tolerate the 80 mg/day dose. Many will continue the drug at the lower dose. In fact, **serious side effects or intolerance of the lower dose are expected to result in attrition in less than 5% of cases**, per extensive meta-analyses of trial experience with the study drug (See Protection of Human Subjects; and Protocol section 7.3). Another **20-30% of cases might reach a prespecified study endpoint** because of SH, and they would also stop the drug before the end of the 2-years follow-up. All subjects who stop the drug, and those with non-compliance as defined, will still be followed for the full 2 years with scheduled clinical and imaging assessments, and will be included in the **primary analysis per intention-to-treat**.

To permit secondary analysis per treatment rendered, at the power and Type I error described above, we decided to **expand the sample size by an ample 37.5%, factoring the highest combined estimates of missing data and attrition.**

9.3.3 Futility Analysis

Due to concerns that the treatment may not be effective in reducing QSM measurements, we will perform a futility analyses once satisfactory primary outcome data (paired QSM assessment) has been logged on 50 of the planned 100 person-year epochs needed to test the trial hypothesis. At the point of the futility analysis, a mixture of patients

with two-year and one-year will be included in the data. Therefore, within person correlations between year 1 and year 2 will be controlled for in the futility assessment. As we have powered the study to see a 20% difference in percent QSM change values between treatment arms, we will call the trial intervention futile if we see a difference in QSM change scores of 10% or less between treatment. The futility analysis will be performed using a linear mixed-effects model adjusted for gender, and a one-sided confidence interval will be created around estimated regression coefficient from this model, which represents the mean group difference in the percentage change of QSM scores between treatment arms. In general, the futility analyses should not be too strict so as not to overlook a non-trivial effect size. Based on recommendations by Freidlin and Korn (115), we will consider a 99% one-side confidence on the difference in change scores between treatment groups. Under the futility analyses, three possible outcomes are possible: 1) the estimated group difference in change scores will be greater than 10%, 2) the estimate difference will be less than 10%, but the 99% one-sided confidence interval will include 7.5%, and 3) the estimate group difference will be less than 10% and the upper 99% confidence interval will exclude 10%. **A recommendation to stop the trial would be made under scenario 3, since this situation would indicate a 99% confidence that the true effect size is in fact less than 10%, and is therefore futile.**

9.3.4 Adaptive Adjustments to Sample size

In addition to looking at the futility of the treatment effect, we will also assess the actual within person correlation in the year 1 and year 2 outcome measures, and the variance in the change scores within each arm. Since the sample size is strongly related to these two statistics, estimating the true correlation and variances will allow us to propose adjustments to the sample size at the midpoint of the trial. **A larger than expected correlation and/or a smaller than expected variance of the change scores will allow us to reduce the final sample size but still retain the same estimated power.** We do not anticipate projecting a higher sample size than initially proposed, given our conservative assumptions regarding the within person correlation in the year 1 and year 2 outcome measures and variance in the change scores.

9.4 Data Monitoring

Data safety and monitoring procedures will be in place before enrollment begins and monitoring will be performed on a regular basis throughout the subject accrual and treatment periods.

9.4.1 Data and Safety Monitoring Team

The data and safety monitoring team was discussed and approved by the NINDS staff. It will comprise the local IRB, the trial's safety compliance officer (SCO) (Section 10.3.2), and an IMSM from outside the study and data management institutions, assisted by two external medical safety consultants with expertise in CCM disease and in statin therapy respectively. The data and safety monitoring team will provide an ongoing review

of the research, adjudicating adverse events, performing interim safety and efficacy analyses, and assessing progress towards achieving the goals of the study.

Safety assessment will take place at the end of the first year, after 30 participants have completed 12 months of follow-up and again after 60 subjects have completed 12 months of follow-up. Additional reviews will be decided on an ad hoc basis based on the frequency of safety endpoints encountered during the trial. The Brain Injury Outcomes (BIOS) statistician will provide the IMSM and the PI relevant tables including information regarding demographics, rebleeding rates, AEs, SAEs, and radiological and laboratory abnormalities (CT scans, liver function, etc.), per treatment assignments labeled A and B without unblinding. In addition, the IMSM will be provided with a copy of the SAE forms received. Further information will be given to the IMSM upon his/her request. The IMSM will provide written recommendation as to whether the study may continue, possible amendment(s) to the protocol, or whether the study should be stopped. The final decision will rest with the study PI, guided by protocol mandated safety thresholds to suspend and potentially revise or stop the trial (see below). Any protocol revision would require approval by the IRB, and shall be discussed with NINDS staff and external consultants prior to implementation.

9.4.2 Safety Threshold

While the proposed trial is underpowered to detect a significant difference in symptomatic clinical rebleeding rates between groups, **differential and absolute rates of hemorrhage in the placebo and treatment cohorts will be critically analyzed throughout the trial**. Continuous monitoring will be initiated with the enrollment of the tenth subject in the study. A symptomatic brain hemorrhage from the index hemorrhagic CA lesion or another source will be considered a safety endpoint for the respective subject. The natural history of untreated brainstem CA demonstrates a 32.1% (CI 1-59%) bleeding risk within two years after prior hemorrhage (18), and our own pilot data of 14 trial candidates undergoing biomarker studies showed 4 symptomatic rebleeds (28%) during 21 patient-years of follow-up (Figure 7). We will hence decide to implement a running safety threshold to preemptively suspend trial enrollment for a full safety review **if at any time, >40% of subjects in the treatment group suffer a symptomatic brain hemorrhage from the index CCM lesion or another source**. Subclinical bleeding is likely more common, and the proposed trial is designed and powered to identify increased subclinical iron leak in CCM lesions using our validated QSM biomarker, in addition to careful monitoring of clinically overt hemorrhages.

9.5 Data analysis

Exploratory analyses for quality assurance and understanding of missing data patterns and distributions will be performed on the outcomes and all measurements to be used in the primary and secondary analyses. For the **primary aim**, a linear mixed model will be used that adjusts for gender to evaluate the difference between the two arms in the QSM change of the index hemorrhagic lesion per patient. Adjustment for gender is necessary because the patients will be randomized by gender stratification. Not control-

ling for stratification variables can lead to upwardly biased standard errors and a decreased power (116). The model accounts for within-patient correlation between the measurements (117, 118). Patients with outcome measurements in both periods will be included in the initial intention-to-treat analysis. A secondary analysis of the percent QSM change per year shall be conducted per treatment rendered for all patients with at least one annual epoch of measurements. Significance levels are assumed as per sample size calculations.

To address **secondary aims**, each of the other outcomes will be analyzed with a similar approach, first per intention-to-treat sample in patients receiving two annual epochs of follow-up, and second by treatment-rendered in all patients with at least one annual epoch of measurements. To assess the difference in level of DCEQP permeability in lesion and brain between the two arms, a linear mixed model adjusted for gender as described for QSM will be used to evaluate the difference in percent change per year in lesional and WMF permeability. A similar adjusted linear logistic mixed model for the binary outcomes will be used to evaluate the difference between the two arms in the rates of symptomatic hemorrhage (per adjudicated definition), lesion expansion, and "biomarker event" (increase in QSM during an epoch above the pre-defined 6% threshold). To assess the difference of event rates for adverse events (AEs) per patient, an adjusted Poisson linear mixed model controlling for gender will be used to regress count outcomes in order to evaluate the difference in AE event rates between the two treatment arms. The AEs will be assigned preferred terms per body systems according to the Medical Dictionary for Drug Regulatory Affairs (MEDDRA) classification of the WHO terminology. AEs (categorized by body system) and abnormal laboratory data will be summarized and compared by treatment group. The compliance rate, general attrition rate and the attrition rate due to adverse events between the two groups shall also be compared. For analysis of leukocyte ROCK activity and functional outcomes (mRS, Euro QOL 5D and VAS, Neuro QOL Communication, and mini-MS score), an adjusted linear mixed model will be used to evaluate the difference in respective outcome score change per year between the two arms. We will report point-estimates, confidence intervals, and p-values for each analysis.

Demographic and baseline data, including age, gender, ethnicity, age at symptom onset, number of prior hemorrhagic events, number of T_2-weighted lesions, number of susceptibility weighted imaging (SWI) lesions, etc., will be summarized. As a secondary model, a linear mixed model that adjusts for those baseline factors shown to associated with the outcome will be created, and will consider the constraints of a small numbers in the respective sub-cohorts. Presenting the mean, standard deviation, median, minimum and maximum with the total number of patients contributing values, will summarize continuous endpoints. Presenting the frequency or proportion of patients in each category will summarize categorical endpoints. For all analyses, biased effects in three prespecified subgroups shall be queried (male/female, sporadic/familial genotype, and brainstem/other lesion location). If the numbers of genotyped subjects allow, we will also compare treatment effect in different genotypes among familial cases. Similar analyses were recently performed by our team in the MISTIE II and CLEAR III trials (113, 114).

Imputation of Missing Outcomes Data, and Sensitivity Analyses

As mentioned above, every effort will be made to minimize the number of patients will missing outcome measures. However, despite these best efforts, it is likely that some missing outcome data will be present in this data set. In order to account for these missing outcome values, we will perform a sensitivity analyses that uses a Markov chain Monte Carlo (MCMC) model to impute several possible measures for patients missing the primary outcome.

With multiple imputation (MI), we assume that the missing data points are missing at random (MAR) (119). We will use those baseline demographic variables, which are strongly associated with clinical outcome, to improve the missing outcome imputations. We will then generate an iteration series plot and autocorrelation function (ACF) plot for MCMC simulation. We shall plan 20 imputations with a 98% relative efficiency to accommodate a worst-case scenario, where 37.5% of the outcomes data points are missing (120). To verify if the missing data points occur at random we will utilize a tipping-point approach (121) that will follow MI procedure for sensitivity analysis and estimation of the MI departure from the MAR assumption. We shall use the sensitivity analysis to recalculate efficacy estimates from regression models described in the data analysis of the primary and secondary outcomes. This will help determine how sensitive efficacy estimates are under different assumed distributions for missing outcomes data. In other words, the results obtained by imputing missing data with several plausible outcomes will give a range of possible treatment effects under different assumptions of missingness.

We will have greater confidence in efficacy estimates if we obtain clinically and statistically significant results under the worst-case scenarios from the sensitivity analyses (e.g. imputing no improvement in the QSM change scores for all patients with missing data in the active treatment arm). In contrast, greater caution should be given to estimates of efficacy if results obtained from regression analyses are no longer clinically and statistically significant under extreme scenarios for the missing data. SAS 9.4 (SAS Institute Inc., Cary, NC, USA) will be used for MI and sensitivity analysis.

Analysis of Treatment Compliance

Treatment compliance is crucial to validate the study objectives. A compliance threshold of 90% will be implemented for proposed analyses per treatment rendered. Drug adherence will be rigorously monitored through an innovative electronic system that will provide continuously streamed real time data in addition to confirmatory manual pill count at each follow up call and scheduled visits. Assessment of compliance is an integral component at every scheduled or unscheduled evaluation and will be based on the **electronically acquired data as well as the missed doses log in the case report form (CRF)**. *A subject will be deemed noncompliant with the study intervention if he/she misses more than 21 consecutive doses at any point, or accumulate a log of 75 missed doses throughout the study*. Treatment compliance shall be compared in atorvastatin versus placebo cases as one of the secondary aims of the study.

10. DATA COLLECTION, SITE MONITORING, AND ADVERSE EXPERIENCE REPORTING

10.1 Records to Be Kept

Participation in this study requires that original study documents be retained for a minimum of 2 years following the end of the trial. This standard complies with U.S. FDA regulations (21 CFR §312.62[c]). Records must not be destroyed without first contacting *the study investigator to ensure that the time limits defined in the regulations have been met.*

For the purposes of this section, “original study documents” are defined as:

Subject records created at or available to the study center during the subject’s participation in the trial, or any other document that supports entries in the EDC system and represents the original source of that information, including but not limited to applicable sections of medical charts, patient correspondence, laboratory data, pharmacy logs and drug accountability forms, as well as any forms or documents used to compile or maintain original subject data or study procedural information.

All Essential Regulatory Documents [as defined under Good Clinical Practice (GCP) Regulations] including: all material communications with the IRB; all communications that are related to study subjects or which otherwise document material study-related procedures or safety issues. All study documents should be uploaded to the source documents section of the database. The database will be used as the master repository for all site and Sponsor regulatory documents

10.2 Role of Data Management

The trial investigator will permit trial related monitoring, audits, IRB review, and regulatory inspections by providing direct access to source data and documents. Data for each subject will be reported and recorded on electronic case report forms. Electronic case report forms (eCRF) must be completed for every randomized subject.

10.3 Quality Assurance

AT CASH EPOC will have a robust safety and data monitoring program in full compliance with NIH policy of 10-Jun-1998 as expanded by NINDS guidelines published 14-Sep- 2011 in addition to compliance with all applicable U.S. and international GCP regulations. This program, designed to safeguard the well-being of study participants and to ensure scientific integrity, will include the following components:

- Oversight by the DCC, Clinical Coordinating Center (CCC), and IRB
- A dedicated independent medical safety monitor (IMSM) and safety compliance officer;

- Automated data quality checks at the time of eCRF completion by the investigational site;
- Dedicated independent data monitoring and quality assurance (QA) teams (Emissary International);

The components of this multifaceted approach, including each dedicated team's specific responsibilities, are discussed below.

10.3.1. Data Coordinating Center (DCC)

The DCC will provide an independent review of the research, interim safety and efficacy data, and progress towards achieving the goals of the study. To enable the CCC to properly manage the trial, the project leadership, and key personnel will jointly work on a data monitoring plan early in the process. After the award but before the project begins, the CCC will work with the DCC to develop a detailed monitoring plan. The monitoring plan will describe the process for reporting adverse events to the IRB, IMSM, and NIH.

Interim safety analyses will be prepared for the DCC on a pre-arranged schedule to evaluate efficacy and safety. Safety events will trigger a "suspend recruitment and review" by the DCC. Prior to IMSM report generation, the case report forms will be finalized, data compiled, all requested analyses conducted, and suitable reports, tables and graphs prepared. All deaths will be reviewed.

10.3.2. Data and safety monitoring

The study is designed as a single-site trial investigating one minimal-risk intervention. Per approval by NINDS, subjects' safety will be monitored by the PI and safety monitoring team as described above. The PI will be actively involved in reviewing the progress of each subject and will notify the IRB as well as the external IMSM and the NINDS program official about adverse events and unexpected problems that may influence their decision to allow the trial to continue.

Independent Medical Safety Monitor (IMSM): The study PI will nominate an independent experienced physician with relevant expertise who is not in affiliation with the study site at University of Chicago Medicine or the data coordinating center at Johns Hopkins University and has no conflict of interest. The IMSM will oversee ongoing monitoring of reports of SAEs in real time to ensure GCP and to identify safety concerns quickly. The IMSM will also serve as a resource to the principal investigator to provide advice about management of AEs/SAEs but will not be involved in other aspects of the trial. The IMSM may suggest protocol modifications to prevent the occurrence of particular AEs. To maximize safety considerations, the IMSM will evaluate SAEs per treatment assignments labeled A or B. In the event of unexpected SAEs or an unduly high rate of SAEs, the IMSM may request identification of A or B as treatment versus placebo. And he/she will promptly contact the PI and the NINDS program officials. The IMSM will be assisted by two designated external safety consultants, with expertise in CCM disease and in statin therapy respectively.

Safety Compliance Officer (SCO): The study PI and IRB require a process for timely communication of adverse events and complication rates to regulatory agencies. Thus, an essential component of this process is the safety compliance officer (SCO). The SCO will have the day-to-day responsibility of ensuring that adverse events are promptly collected, medically reviewed by the PI or IMSM and appropriately reported to regulatory agencies in compliance with GCP standards. The SCO and the statistician at the Baltimore site shall be responsible for preparing and communicating incoming documents and reports to the PI and IMSM. Inclusion/exclusion criteria, review-of-system reports, medical history, medical events, and radiographic imaging entries will be cross-checked and reviewed by applying real- time database algorithms, crosschecking sentinel dates and times, procedures, AEs, SAEs, and protocol timelines. As aggregate data reports and quarterly and annual progress reports are prepared, the SCO will query the TN and study coordinator as needed and follow the data entry tasks to completion. The SCO will serve as the liaison between the IMSM and the PI and CCC personnel to garner additional information as needed and to elicit agreement or disagreement of the CCC with the IMSM's assessment. The SCO will manage the development and revision of trial protocols, Manual of Operations and Procedures (MOP), standard operating procedure (SOP), and protocol working guidelines with the trial leadership.

Reporting Dictionary and Data Standards: The SCO will work closely with the trial vendors to develop the EDC systems and the corresponding paper CRFs, used for all safety-related events. The Common Terminology Criteria for Adverse Events (CTCAE) dictionary has been adapted to allow for trial-specific reporting using standardized Medical Dictionary for Regulatory Activities (MedDRA) coding.

10.3.3 Automated Data Quality Checks in the EDC System

VISION® EDC System: The EDC system has a robust set of data quality checks that will be executed at the time of data entry at the investigational site. This includes the standard validations available with most EDC systems such as range checks (e.g., to flag a high liver function test as incompatible with inclusion criteria, or to ensure a temperature is not an impossible value) and data format checks (to flag an invalid date). Additionally, our VISION platform will perform very sophisticated cross-form computed calculations that would not be available in lesser EDC systems. For example, VISION will conduct a series of verifications looking at lab values, demographics, etc. and give the site guidance regarding the eligibility of the patient for randomization using complex cross-page computations. It will also detect when the CCC has made a protocol variance and present a list of such issues to the investigator and the monitoring team for evaluation and follow-up. Consequently, our EDC system will handle much of the consistency, completeness, and logic checks immediately at the time of data collection that normally would have to be done by the monitoring team and/or offline using statistical analysis that do not typically occur until weeks or months after the patient visit. This capability therefore will result in cleaner data that will be more likely to distinguish a treatment effect and significantly reduce the cost of, and delays for, monitoring and data cleaning activities.

10.3.4 Independent Data Monitoring and Quality Assurance Team

It is important to use an independent Contract Research Organization (CRO) for monitoring and quality assurance, to provide the highest level of integrity, industry- proven best practices, and professionally trained monitors. Our **CRO, Emissary International**, maintains a team of high-caliber, fully qualified monitors in the U.S. This team will be responsible for near real-time review of the clinical data entered into our EDC system against source medical records (i.e., Source Document Verification or SDV) as well as generation and resolution of the associated data queries.

QA Monitoring Plan: In accordance with recently drafted FDA recommendation for risk-based monitoring approaches, this trial will employ centralized monitoring and an EDC system to:

- Replace on-site monitoring for activities that can be done better using centralized reviewers;
- Verify source medical records remotely to ensure data integrity, reduce transcription errors, and identify any undocumented safety events;
- Utilize EDC real-time data quality checks to assess range, consistency, and completeness of data at the time of entry; and
- Employ frequent statistical analyses of study data to evaluate individual subject data for plausibility and protocol compliance.

10.3.5 Data Flow for the Data Coordinating Center

Correction of Automated Errors and Warnings: As data are entered, the EDC system will immediately generate automated warnings (yellow highlights) and errors (red highlights). Warnings will represent data that is outside expected limits, or where required data are missing. Errors will indicate conditions that are intolerable (such as an impossibly high body temperature) or that are unrealistic (such as an invalid date format).

In keeping with FDA requirements for electronic systems, the EDC system will not force the investigator/coordinator to immediately change the entered data (as that could be misconstrued as encouraging data falsification) but instead simply provide feedback via on-screen messages and red/yellow field highlighting. Warnings may be unavoidable, due to patient-specific issues, but are documented nonetheless so that they can be discussed with the monitoring team. Conversely, red errors must be resolved before the case form page can be advanced in the workflow (i.e., signed by the coordinator) so the data will be “clean” before it is exported for analysis.

Additionally, the EDC system will generate external email notifications in response to specific entries, such as immediately alerting the CCC of a possible dose/color-code error or notifying the safety officer of a new adverse event. The EDC system also produces various instantaneous reports that are useful for data quality and safety monitoring purposes by both the site staff and the CCC.

Monitoring Team Source Document Verification and Data Integrity Review: Emissary’s teams of monitors will review the online case forms for completeness, logic,

and consistency, then verify the entered data against the uploaded source medical records and data collection worksheets. Routine queries identified in this process will be entered into the EDC system (triggering an automated notice to the site). The monitors, working in conjunction with the DCC, will then work with the CCC personnel to obtain correction of all data errors and resolution of the corresponding queries.

Reviews will include data from the entire course of each patient's participation in the study. In accordance with a formal monitoring plan, this activity will include a review of all uploaded source documentation and will entail a 100% source verification of the primary safety and efficacy measures. Random sampling will be used to select secondary data for similar 100% source verification. Should the data accuracy for a patient exceed certain minimum expectations in this step, or if any material data integrity or regulatory compliance issues are identified, additional data from a patient will undergo intensive monitoring.

10.3.6 Other Components of Data Management Plan

Randomization and Data Collection: Each subject is assigned a unique study number by the EDC after randomization. Data collection of compliance, concomitant medications, and adverse events will assess the subject's clinical response to treatment as part of the clinical trial. This data will be used to assess compliance with the study protocol and intervention stopping rules.

Protocol Compliance: Procedures will be implemented to maximize adherence to the protocol (smart bottles). Early review of data is made possible by real- time entry of data into a database with validations and real-time monitoring. The study center is required to report a protocol deviation within 24 hours of occurrence or as soon as it is discovered. If the QA monitor discovers an undocumented major deviation during a monitoring activity, the monitor will notify the CCC immediately. A CCC personnel will report deviations as they are discovered to the local IRB in accordance with local requirements. Routine reporting of protocol deviations will be made to the NINDS and other regulatory agencies as required.

FDA Guidance for Electronic Data Entry Compliance: The design and development of the electronic database system will reflect the FDA Guidance for Industry for Computerized Systems Used in Clinical Trials (April 1999) as well as the Electronic Records/Electronic Signatures rule (21 CFR part 11). A secure, computer generated, time- stamped electronic record will allow reconstruction of the course of events relating to the creation, modification, and deletion of an electronic record. Source documents will be retained to enable a reconstruction and evaluation of the trial. The system will ensure that all applicable regulatory requirements for record keeping and record retention in clinical trials are met with the same degree of confidence as are provided with paper systems. Clinical investigators will retain the original copy of all source documents uploaded onto the eCRF. Query resolution correspondence will be maintained and eCRF edits will be tracked by the system. Changes to a required record will not obscure the

original information. The record will clearly indicate the time a change was made and clearly provide a means to locate and read the prior information through the audit trail. This audit trail will be in compliance with the 21 CFR 11.10(e). The record, along with supporting documentation, will also indicate who made the changes and when changes were made.

Security measures will be in place to prevent unauthorized access to the system and data. To ensure that individuals have the authority to proceed with data entry, the system will be designed to verify the electronic signature (user ID and password) at the start of a user session. Each entry to an electronic record, including any change, will be made under the electronic signature of the individual making that entry. A separate electronic signature will not be required for each entry or change; a single electronic signature will cover multiple entries or changes. Individuals who maintain the electronic record systems as well as the audit trail will carry the responsibilities to protect authenticity, integrity, and confidentiality of electronic records. Audit trails will be available at the study site or any other location where associated electronic study records are maintained. The system will be designed to contain the prompts, lookup values, cross-field validations, flags, and on-line help to encourage consistent use of clinical terminology and to alert the user in case that data entered are out of acceptable range. External safeguards will be in place to ensure that access to the computerized system and to the data is restricted to authorize personnel. Servers will be stored in a physically secured, guarded data center.

Security Measures: Users at both centers will be aware of system security measures and the importance of limiting access to authorized personnel. SOPs will be in place for handling and accessing the system to prevent unauthorized access. Access to the data at a clinical site will be restricted and monitored by the system through required log- on, security verification procedures, and audit trail. The data cannot be altered, browsed, queried, or reported via external software applications without entering through the protective software. Because the system will be largely through remote access, all data and applications used for the study will be logically and physically isolated in the servers in order to preclude unintended interaction with non-study use software. These servers will be strictly monitored and maintained by designated administrators at an independent third party (e.g., only Prelude Dynamics, the contracted EDC vender, has password access to the database and only its contracted commercial data center, On-Ramp Systems both of Austin, Texas, has access to the physical hardware); neither remote sites nor any member of the project team will have the ability to change such logical security of the system. Written procedures describing contingency plans for continuing the study by alternate means in the event of hardware or facilities failures with alternate hardware or at an alternate site will be provided to each site. It should be noted that the data management procedures will reflect the advanced use of computer and software technology; include database technology, and electronic file management principles; and therefore be of the highest possible standards achievable for data security and information integrity. Specifically, the data center is SAS-70 Type II compliant,

HIPAA-audited and certified for maintenance of banking, credit card, and protected health information (PHI).

Backup Recovery: Records will be backed up daily to prevent a catastrophic loss compromising the quality and integrity of the data. Data will be backed up onto digital media which will be stored at an offsite location. Backup and recovery logs will be maintained to facilitate an assessment of the nature and scope of data loss in the event of a system failure.

Limited Access: Each user will be assigned an individual account with a unique username and password. Any user will be locked out after 10 consecutive attempts, with any unauthorized access attempt recorded in a log file. Users will be required to exit the system upon leaving a workstation. The computer will automatically log off the current session when an idle period reaches 30 minutes. For short periods of inactivity, the automatic screensaver will be password protected to prevent unauthorized access to the system.

Audit Trails: All changes made to data in the electronic record are tracked and recorded in the audit trail. This audit trail will capture the date/time, the contents of the changes made, and the login id used to make the change. The audit trails will be created incrementally in chronological order, with prevention of overwrite, as such data overwriting is in violation of §11.10(e). Audit trail information will be reviewed by pre-authorized personnel if the need arises to verify the quality and integrity of the data.

Date and Time Stamps: All data will be saved on a central server carrying a time stamp, which will be documented in the audit trail. The EDC software will display the participating site's time zone. Individual users will be unable to change the time on the server.

10.4 Adverse Experience Reporting

10.4.1 Specification of Safety Variables: Safety assessments will consist of monitoring and reporting adverse events (AEs) and serious adverse events (SAEs), all events of death, and any study specific issue of concern.

10.4.1.1 Adverse Events (AEs):

Definition of AEs: An adverse event is any untoward medical occurrence in a patient entered into the study that does not necessarily have a causal relationship with the study intervention. An adverse event can be any unfavorable and unintended sign (including an abnormal lab finding), symptom, or disease temporally associated with the use of the study drug, whether or not related to the study drug. Adverse events will be triaged and managed at least

initially at the level of the TN. The investigator must follow adverse events to resolution whenever possible.

AEs include the following:

- AEs not previously observed in the subject that emerge during the protocol-specified AE reporting period, including signs or symptoms associated with intracerebral hemorrhage, myositis, or organ dysfunction that were not present prior to the AE reporting period.

- Complications that occur as a result of protocol-mandated interventions (e.g., contrast nephropathy).

- Preexisting medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period.

10.4.1.2 Serious Adverse Events (SAEs)

Definition of SAEs: A serious adverse event is an adverse event that results in any of the outcomes listed below. They generally correspond to Categories B and C adverse events in Table. 2.

- Results in death (i.e., the AE actually causes or leads to death). Study investigator agrees to adhere to FDA-defined guidelines and submit an expedited report of any death that is related (even remotely) to study drug.
- Life threatening (i.e., the AE, in the view of the investigator, places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.).
- Requires inpatient hospitalization or prolongs inpatient hospitalization.
- Persistent or significant disability or incapacity: a substantial disruption of a person's ability to conduct normal life functions.
- Considered a significant medical event by the investigator based on medical judgment (e.g., may jeopardize the subject or may require medical/surgical intervention to prevent one of the outcomes listed above).

Compulsory SAE: Certain AEs in the coding guide are defined as potentially serious SAEs if there is a medically qualifying factor. All AEs coded as a grade 4 or 5 will automatically require SAE reporting.

10.4.1.3 Medical events of interest (MEOI)

Definition of MEOIs: MEOIs must be reported to the PI for safety monitoring team review. The AE dictionary, which drives the drop down lists on the AE screen in the EDC system, pre-specifies which events are defined as MEOIs. A MEOI may or may not satisfy the definition of a SAE but will be reported using the same SAE reporting screen in the EDC system and will undergo the same review as a SAE. In summary, the MEOIs for the trial are as follows:

- Myositis/ Rhabdomyolysis
- Symptomatic Cerebral bleeding events
- Liver dysfunction.
- Pancreatitis.
- Any AE deemed by the site PI or safety monitoring team as possibly, probably, or related to the statin therapy.
- Any AE requiring discontinuation of study intervention or withdrawal from follow-up

10.4.2 Assessment of Adverse Events

All AEs and SAEs whether reported by the subject, elicited by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be recorded appropriately. Each reported AE or SAE will be described by its duration (i.e., start and end dates), regulatory seriousness criteria if applicable, suspected relationship to study drug administration, and actions taken.

10.4.2.1 Attributability of AEs to the study drug: The investigator in consultation with the MSM will define whether the event is best described as UNRELATED, POSSIBLY related, PROBABLY related, or DEFINITELY related to the study drug according to the following definitions. To ensure consistency of AE and SAE causality assessments, investigators will apply the following general guideline:

Yes

There is a plausible temporal relationship between the onset of the AE and the administration of the study drug, and the AE cannot be readily explained by the subject's clinical state, concomitant illness or therapies; and/or the AE follows a known pattern of response to the study drug; and/or the AE abates or resolves upon discontinuation of the study drug.

• **Possibly Related:** The adverse event has a temporal relationship to the study drug. However, an alternative etiology may be responsible for the adverse event. Information on drug withdrawal may be lacking or unclear.

• **Probably Related:** The adverse event has a temporal relationship to the study drug. The event is unlikely to be related to an alternative etiology. There is a reasonable response on withdrawal (de-challenge). Re-challenge information is not required.

• **Definitely Related:** The adverse event has a temporal relationship to the study drug and resolves when the study drug is discontinued. An alternative etiology is not apparent.

No

Evidence exists that the AE has an etiology other than the study drug (e.g., preexisting medical condition, underlying disease, concomitant illness or medication); and/or the AE has no plausible temporal relationship to the study drug administration

- **Unrelated:** There is evidence that the adverse event definitely has an etiology other than the study drug

10.4.2.2 Unexpected AE definition: Unexpected events are any adverse events in which the specificity or severity is not consistent with the natural history of CCM without the test intervention. Unexpected will be defined as the specificity or severity of an event that is not consistent with the risk information described in this protocol.

10.4.2.3 Adverse Event Reporting Period: The timeframe of reporting adverse events extends throughout the study period from randomization until the end of the study follow-up period for each enrolled subject. Adverse events must be recorded in the medical chart and in the EDC system.

10.4.2.4 Documentation: Documentation must include the event duration (start/stop). If a subject is discontinued early from study drug for any reason, CCC personnel must clearly report and document the circumstances and data leading to any discontinuation using the electronic case report forms. It must be determined if the reason for stopping study drug administration is an adverse event.

10.4.2.5 Follow up of ongoing AEs: For any untoward event(s) the subject should be followed until the event resolves or is explained with the frequency of follow up designated by the investigator.

10.4.2.6 Eliciting AEs during follow up visits: At each follow up visit, the subject will be asked about the occurrence of AEs since the last contact. AEs that were ongoing at the last contact will be updated with a stop date or confirmed as ongoing. This will continue until the end of study visit.

A consistent methodology for eliciting AEs at all subject evaluation time points will be adopted. This questionnaire will also be adopted for the automated weekly follow up messages circulated by the electronic monitoring system. Examples of non-directive questions include:

- Have you been well since your last clinical visit?
- Have you had any new health problems since you were last seen?
- Have you had any recent weakness or altered sensation affecting your arms or legs?
- Have you experienced any recent onset muscle or joint pain?
- Have you noticed any changes in urine or stool color?
- Have you had any unusual or unexpected worsening of your underlying medical condition?

10.4.3 Specific Instructions for Recording Adverse Events:

Investigators should use correct medical terminology/concepts when reporting AEs or SAEs. Avoid colloquialisms and abbreviations.

Diagnosis vs. Signs and Symptoms

If known at the time of reporting, a diagnosis should be reported rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, it is ok to report the information that is currently available. If a diagnosis is subsequently established, it should be reported as follow-up information.

Deaths

All deaths that occur during the protocol-specified AE reporting period, regardless of attribution, will be reported to the safety officer. When reporting a death, the event or condition that caused or contributed to the fatal outcome should be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, report “Unexplained Death.”

Preexisting Medical Conditions

A preexisting medical condition is one that is present at the start of the study. Such conditions should be reported as medical and surgical history. A preexisting medical condition should be re-assessed throughout the trial and reported as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When reporting such events, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., “more frequent headaches”).

Hospitalizations for Medical or Surgical Procedures

Any AE that results in hospitalizations or prolonged hospitalization should be documented and reported as an SAE. If a subject is hospitalized to undergo a medical or surgical procedure as a result of an AE, the event responsible for the procedure, not the procedure itself, should be reported as the SAE. For example, if a subject is hospitalized to undergo coronary bypass surgery, record the heart condition that necessitated the bypass as the SAE.

10.4.4 SAE Reporting

Any alarming, serious, or unexpected adverse event, including death due to any cause, which occurs during this study, inclusive of the follow up period, and whether or not thought to be related to the test article, must be reported immediately (within 24 hours of learning of the event) to the trial PI, the IMSM and to the local IRB as required. Completion of an SAE form for each SAE that occurs is required to formerly report the event to the trial PI. An expedited safety report will be used by the SCO to notify the PI and IMSM of each serious unexpected suspected adverse reaction, and a running total thereof. This protocol has a pre-specified monitoring plan for determining if subjects receiving the test article are at higher risk for mortality and will only report a death as an expedited safety report if there is evidence of a causal relationship between the test article and the event resulting in death. In addition, an expedited safety report will be used to notify the PI and IMSM if there is an imbalance between the arms suggesting there is a reasonable possibility that the test article caused any of the safety endpoints. Otherwise, the occurrence of these safety endpoints will be reported on an annual basis. The study leadership will submit events meeting the following criteria as expedited Safety Reports according to the guidance and timelines below. The completed case report will be emailed immediately upon completion to the PI and IMSM.

Calendar Day Telephone or FAX Report:

The study leadership will notify the IMSM and the IRB for review of any **fatal or life-threatening** adverse event that is **unexpected** (as defined above) and assessed by the PI (in consultation with the IMSM) to be **possibly, probably, or definitely related** to the test article. Such reports will be emailed or faxed within 7 calendar days of the study leadership first learning of the event. Relevant follow-up information will be submitted to all parties as soon as it becomes available.

Calendar Day Written Report:

The study PI will notify the IMSM and the IRB, in a written Safety Report, of any **serious, unexpected** AE (as defined above) that is considered **possibly, probably, or definitely related** to the test article. Such reports will be emailed or faxed within 15 calendar days of the study leadership first learning of the event. Relevant follow-up information will be submitted to all parties as soon as it becomes available.

Annual Written Report:

The study leadership will notify the IMSM and his designated consultants with a listing of all suspected serious adverse reactions which have occurred over this period and a report of the subjects' safety. Written Safety Reports will include an **Analysis of Similar Events** in accordance with 21 CFR §312.32. All safety reports previously filed concerning similar events will be analyzed and the significance of the new report in light of previous, similar reports commented on. Written safety reports with Analysis of Similar Events will be submitted to the IMSM within 15 calendar days of the study leadership first learning of the event.

11. HUMAN SUBJECTS

11.1 Institutional Review Board (IRB) Review and Informed Consent

This protocol and the sample informed consent document, and any subsequent modifications will be reviewed and approved by the University of Chicago Medicine IRB, responsible for oversight of the study. A signed consent form will be obtained from the subject. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject, parent, or legal guardian, and this process will be documented in the subject's record.

11.2 Subject Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified only by the Study Identification Number (SID) to maintain subject confidentiality. All records will be kept in a locked file cabinet. All computer entry and networking programs will be done using SIDs only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB, the NINDS, or the Office for Human Research Protections (OHRP).

11.3 Study Modification/Discontinuation

The study may be modified or discontinued at any time by the IRB, the NINDS, the PI, the OHRP, or other government agencies as part of their duties to ensure that research subjects are protected.

12. PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this trial will be governed by the policies and procedures developed by the study leadership. Any presentation, abstract, or manuscript will be made available for review by the study leadership and the funding agency prior to submission.

The results of the trial will be published regardless of its outcome. Publication regarding further analyses performed on the data will be by mutual agreement between the study leadership and the study investigators.

The investigators may publish or present at scientific meetings the results of this study, provided that confidential information is not disclosed, and only after obtaining advance written consent from the study leadership. Consent may be withheld at the sole discretion of study leadership.

In this regard, a copy of all public disclosures, including but not limited to publication manuscripts, abstracts, and seminar presentations by any member of the investigative team, should be provided to the study leadership, at least 30 days before the manuscript is submitted to the publisher or a presentation is made.

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