MarkVCID CSF Placental Growth Factor (PlGF) Fluid Biomarker Kit

1. Executive summary

We are proposing to validate a CSF fluid biomarker kit that measures PlGF as a small-vessel disease (SVD) biomarker with the aim of adding it to the existing AD CSF biomarkers that are validated and widely used. This will allow us to appropriately stratify patients into clinical trials as AD, VCID, or AD+VCID. SVD is a common cause of cognitive impairment in late-life. In addition, pathologic AD, characterized by amyloid plaques and neurofibrillary tangles, is also a frequent cause of late-life cognitive impairment. Unfortunately, many AD trials have failed and one likely explanation is that there are frequent co-morbidities; SVD being one of them. While acute vascular events and overt vascular changes on imaging have been precluded from recent AD trials, this likely misses a large portion of SVD cases. We have identified a single CSF biomarker indicative of SVD changes, both pathologic and cognitive. This biomarker is **placental growth factor (PlGF)**. PlGF is already a component of the approved endothelial signaling kit, which examines it in plasma, and we have found PlGF has a strong linear relationship with degree of white-matter hyperintensity pathology and also with some executive function-type cognitive outcomes.

Because CSF Aβ, tau and NfL are established biomarkers in the dementia field already, the overarching hypothesis to be tested for this kit is that PlGF is a biomarker of SVD.

We propose to carry out two types of validation for this CSF PlGF kit. The first type is methods validation, in other words, how reliably can PlGF can be measured across MarkVCID participating sites. We hypothesize that the same set of samples run at different sites will yield similar results such that an intraclass correlation coefficient will exceed .8 and the coefficient of variation in absolute values will be less than .25. The second type of validation will provide conceptual support for the biomarker. We hypothesize that at each participating site PlGF will be positively associated with SVD (WMH burden) and TRAILS-A after controlling for age. We anticipate completion of these two sets of validation studies within 12-months of initiation, but this absolute timeline is dependent upon participating sites completing recruitment sufficient to meet a sample size of 40.

Our primary proposed biomarker category is disease stratification for the purpose of patient selection and randomization. Enrichment of a VCID trial with patients who have SVD pathology will increase the likelihood of success.

Table 1: Quanterix Simoa assay on HD-1 instrument

	LLoD (pg/mL)	LLOQ-ULOQ (pg/mL)	Coefficient of Variance between days (%, mean)	Coefficient of Variance <10%*
PlGF	0.064	0.30 - 960	5.8	96.8%

2. Brief description of biomarker kit.

Our proposed "CSF PlGF kit" measures PlGF protein in CSF samples. We have found this informative regarding the pathologic process in brain of SVD. We propose the use of the consistent, sensitive Quanterix Simoa analysis system as described below. We have found these assays to be superior in their consistency over colorimetric ELISAs or Meso-Scale Discoveries. The below figure 1 highlights the key data we have collected in a sample size of 60 individuals who were either cognitively normal or have mild cognitive impairment (MCI).

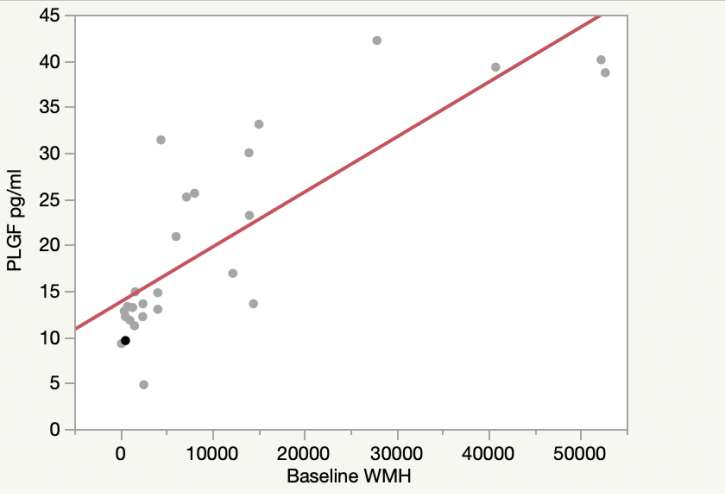

Figure 1: CSF PlGF associates with white matter hyperintensity volume. X axis shows WMH volume at the time of CSF collection. Y-axis shows PlGF levels detected in CSF using Quanterix Simoa assay. P<0.001 for regression; $R^2 = 0.679$.

Running this kit requires a Quanterix instrument, preferably the HD-1, although the new, benchtop, SR-X instrument is said to have the same accuracy and consistency as the HD-1.

Table 2. Lab supplies for CSF stratification kit
Pipette, 1-10uL
Pipette, 10-100uL
Pipette, 100-1000uL
Ice Bucket
Tube Rack
Quanterix HD-1
Quanterix PIGF assay kit
Quanterix consumables kits (discs, plates, tips, cuvettes)
Quanterix Buffer kit (system buffers 1 & 2)
Other Consumables: Pipette tips,1.5mL Eppendorf Tubes

3. Participating sites

Three sites, UKY, UCSF, and UTHSCA are capable of running the assays using the Quanterix HD-1 instrument. UKY, UTHSCA, UCSF, and UNM are collecting CSF samples that can be used for analysis. UNM may be acquiring a Quanterix instrument, however, if they do not, their samples will be shipped to UCSF for analysis.

4. Protocol for fluid sample acquisition

All prospective CSF samples will be collected according to MarkVCID fluid best practices determined by the Fluid Biomarker Subcommittee. We will require aliquoted CSF that has not undergone any freeze/thaw cycles. Shipping protocols will also follow the subcommittee best practices.

5. Additional data collection required for analysis

All additional data required for analysis are routine parts of standard MarkVCID data collection. We will use Uniform Data Set data as determined by the Clinical Data Subcommittee for our measures of cognition, history and presence of cerebrovascular and cardiac disease and vascular risk factors, and TRAILS-A. We also require that all subjects who participate in this validation study have at least one brain MRI with the potential to generate WMH volumes. We will require TRAILS-A data to be collected as our cognitive outcome measure.

6. Protocol for performing the analysis

The protocols for running CSF samples through the Quanterix HD-1 platform with the Simoa assays are very well established with extensive validation data available. We will be using the Quanterix Simoa kit “human PIGF”. The Simoa kits are bead-based, with the use of microfluidics to distribute beads into individual “wells” within the disc used for analysis. This method, in theory, permits the analysis of a single molecule. The benefit of the Quanterix HD-1 system, over other analytic systems, is that the majority of fluid handling is performed inside the instrument using robotics, eliminating any human error. This results in greater consistency across runs of a given assay. All kits include all the necessary diluents, blockers, and buffers kits plus wash buffers. Additional consumables kits must be purchased in order to obtain the discs and cuvettes to be loaded into the instrument. Additional materials required to run the assays include an ice bucket for thawing samples, pipettors, tips, and microfuge tubes. (see Table 2). Kit lot numbers and adherence to SOP processing will be recorded at each site for each Quanterix assay run. Best practices for running Quanterix assays are clearly spelled out in the kit documentation. Specific steps are outlined in Table 3. We are proposing that all samples are run in duplicate. Sensitivity and degree of agreement for PlGF are well documented and robust across a range of dilutions, although we have optimized dilution for the kit and this is detailed below. Our reliability and CVs are given in table 1 on page 1.

Table 3. CSF Stratification kit protocol steps
Thaw samples and calibrators on ice. Bring diluent, detector, SPG, RPG, and bead buffers to room temp.
Layout sample loading on plate diagram
Dilute calibrator curve according to lot specific concentration.
Transfer 240ul of PlGF calibrator to the 96 well plate
PlGF add 240ul of sample to the assigned well (NEAT)
Scan buffers into the machine
Load plate into the machine and set-up the run
Start run
Collect Data on USB drive

Although the protocol for running the Quanterix assays is well detailed and the process yields reliability data, we are proposing a training period to ensure optimal reliability of this kit within MarkVCID. UKY will provide eight identical CSF samples to each site performing analyses. Lab techs at those sites will run the PlGF assay on their instruments and send their data to the Coordinating Center. UKY will review and analyze these data to measure measurement reliability and interrater reliability. Any lab where more than 5% of CVs exceed .15 or whose absolute values are discrepant with the other sites (>25%) will be followed up with via video conference to review step-by-step protocols and troubleshoot the protocol with that site. In-person training with travel to UKY will be offered at the expense of UKY if the participating site and UKY determine that is the best approach.

After completion of the training, the three / four sites will carry out the larger reliability experiment. The Coordinating Center will assist with identifying 20 CSF samples (4 aliquots each), with one aliquot from each sample sent to UKY, UCSF, UTHSCA, and possibly UNM for analysis. Given the absence of a demendence on disease, these CSF samples could be from a commercial source. Sample size was based on achieving power of greater than .80 to detect an ICC of greater than .8. Analyses will be run in duplicate, requiring one kit per site. Upon completion, each site will digitally upload the values to the Coordinating Center for data storage and distribution. Cross-site variation in the distribution of obtained values will be analyzed by Bland-Altman analysis and cross-site correlation matrices to ensure cross-site reliability.

7. Step-by-step analytic plan

Aim 1. Methods validation (reliability across sites): We will first compute the CVs for each sample at each site. As samples are run in duplicate, If the CV >.20, the value for that analyte will be coded as missing and flagged for re-analysis. We have not experienced this in the several thousand samples we have analyzed through the Quanterix system.

The resulting dataset will have a minimum of three values (one from each site) for PlGF. The reliability of each of these measurements will be estimated using Intraclass correlation coefficients (ICC). Mixed effects regression models will be used to estimate the ICC by treating the three values for each sample as repeated measures.

Plate to plate variation: To establish plate-to-plate consistency, each site will identify six CSF samples that will be included in each of the first four plate runs to determine the plate-to-plate consistency. In addition to the CVs for duplicate samples described above, we will calculate the CVs for the four sets of samples across the plates. CVs <0.2 will be considered acceptable plate-to-plate variations. If we find that the plate-to-plate variability is greater than this we will assess variable factors such as pipetting in the initial steps, room temperature, or temperature increases in the instrument. We will also consult with Quanterix technical staff to help with the troubleshooting.

NOTE: We cannot realistically propose a within subject validation as we are currently doing in the plasma MarkVCID kits because the CSF collection is considered quite invasive and obtaining consent for repeated CSF collection will be highly unlikely. Test-retest as is occurring with plasma kits is not feasible for CSF.

Aim 2. Conceptual validation: Further validation for the PlGF kit will come from replicating early results from UKY at the other participating sites. In cross-sectional analyses, we found that the PlGF was significantly associated with WMH volume and TRAILS-A.

We propose to replicate and expand these findings using CSF samples from UNM, UCSF, UKY and UTHSCA. Each site is recruiting individuals and collecting all the necessary clinical data, MRI imaging, and CSF samples. Each site will study a minimum of 40 subjects (half normal and half with WMH and cognitive impairment) reflecting a range of WMH burden. Cross-sectional analyses will use the first time point in which there is a CSF sample and all the available clinical and imaging data available. We hypothesize that levels of the PIGF will be correlated with WMH volume and TRAILS-A.

8. Sample size calculation (individual site level)

Methods validation (reliability): With 20 samples each evaluated at the 3 sites, we will have over 80% power to detect an ICC of at least 0.8 assuming a null hypothesis ICC of 0.7.

Conceptual validation: For cross sectional analyses, with n=40, we will have 80% power to detect an association as small as R^2=0.30. For analyses of change in cognition, we will have 80% power to detect an additional contribution of as little as a 2-point drop on MoCA score, assuming other variables such as age, gender, and CDR are normally distributed.

9. Plan for longitudinal data collection and analysis

As a secondary hypothesis, we predict that bigger increases in PlGF over 2 years will be associated with faster decline in cognition and increased area of white matter hyperintensity. The longitudinal component of the proposal is the need for longitudinal cognitive assessment to determine the predictive value of PlGF for the 2-year change in TRAILS-A. The sites supporting this kit are actively recruiting patients and collecting CSF from their patients. Therefore, there will be longitudinal clinical data to determine our endpoints. Additional longitudinal data will provide further potential for associations of our kit with other key outcomes.

10. Plan for reporting outcomes

There is an explicit agreement that each site will share their data with the Coordinating Center to create the best opportunity to advance science. The merging of the PlGF kit analytes with the cross-sectional and longitudinal clinical imaging, and other biofluid data will offer an extraordinary opportunity to enhance not just our understanding of VCID but also to better prepare for clinical trials. This would undoubtedly promote analysis, presentation and publication of our results. The results of our work could also entice pharmaceutical company interest in the treatment of VCID, resulting in new clinical trials.

11. Plan for sharing data, samples/images, protocols

The detailed protocols and plan for validation will be shared with all sites. We also plan to share all data with the Coordinating Center so that it can be shared in accordance with protocols and agreements outlined by the MarkVCID consortium.