LIMBIC-CENC BIOMARKERS CORE

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- **Main Aim**: Maintain the LIMBIC-CENC biorepository of blood and saliva biospecimens through collection, storage and distribution of well-curated samples to LIMBIC-CENC and collaborator approved biomarker studies that advance our knowledge of the chronic effects of neurotrauma.

- **Current Status**: Biorepository regulatory approvals in place and current. Continuing to receive and distribute biospecimens. DNA extraction and APOE testing ongoing. Carried out NED analysis (W Walker lead) on 1,500 participants at baseline.

- **Clinical Deliverables**: NED screening analysis suggests that NED dysfunction may not occur more frequently in combat-deployed SM/vets with mTBI than without.

- **1-year Goals**: Continue to carry out LIMBIC-CENC biorepository function and provide biospecimens to approved LIMBIC-CENC and external collaborator TBI biomarker studies.

- **End of Cycle Goal:** Make available longitudinal biospecimen samples to approved projects that include diagnostic and prognostic biomarkers of the chronic effects of neurotrauma

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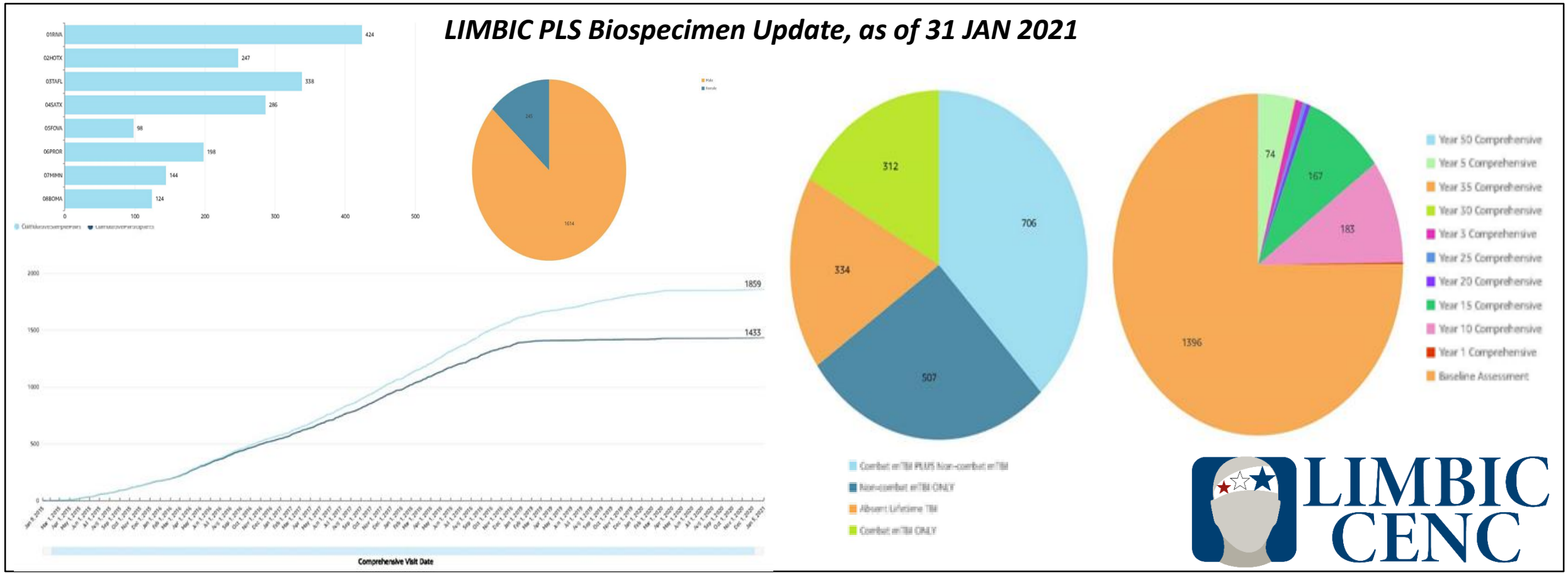
LIMBIC-CENC Biomarker Core

- Collated, stored, processed and aliquoted biospecimens from 1,859 Study 1 (426 F/U) participants and 163 samples from previous CENC studies for current total **28,223** aliquots of in the biorepository available for analysis.

- 4,202 have been distributed to LIMBIC Biomarker Discovery projects and to 3 collaborative projects approved by Research Committee.



Major Tasks Completed- 1

- Regulatory approvals, as follows: HRPO 2nd level BR approved 10-16-2020; CR approved-USUHS IRB, 3/16/2021; CR submitted HRPO 3/18/2021, approval pending.

- Received shipments from LIMBIC sites thru present, primarily from follow-up visits as VA sites resumes new enrollments post-COVID or new sites added.

- 3 collaborative projects under way (MTA's with 3 sites and shipping completed):
  - Roskamp Institute, Fiona Crawford, *Identifying APOE Related Lipid Biomarkers for Diagnosing Chronic Neurocognitive Deficits in TBI patients*.
  - Tampa VA, Drs. Patel/Nakase-Richardson, *Noncoding RNA in traumatic brain injury*.
  - MSD, Chris Campbell, *Ultrasensitive Blood Tests for Investigating Pathogenesis of Chronic TBI symptoms* (145 samples from Nick Davenport CENC Study 49).

- 1 Request under review:
  - MSD, Chris Campbell, *Ultrasensitive Blood Tests for Investigating Pathogenesis of Chronic TBI symptoms* (additional 500 samples from PLS requested)

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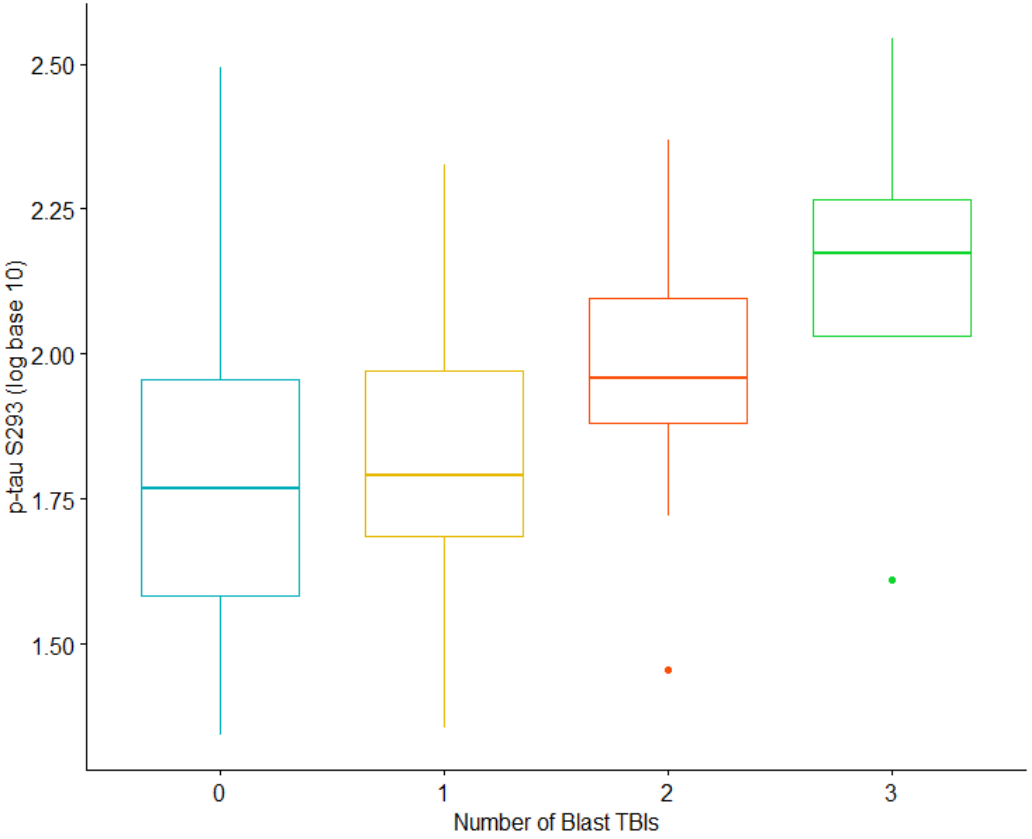
Serum p-tau Associates with Blast TBIs

p-tau S293 increases with number of blast TBIs



Box and whisker plot showing progressive increase in serum levels of p-tau S293 with number of blast TBIs. The serum levels of p-tau S293 were log transformed (base 10). Boxes indicate median and interquartile range. Whiskers extend to the 95th and 5th percentiles.

p-tau correlations with blast TBIs

Serum Biomarker	Odds Ratio (p-value)	Odds Ratio for p-tau levels normalized to total tau levels (p-value)
T175	3.4 (0.03)	3.7 (0.03)
T181	3.3 (0.11)	3.6 (0.11)
S214	9.5 (0.02)	4.7 (0.07)
cis T231	5.0 (0.02)	9.6 (0.005)
trans T231	5.5 (0.09)	2.8 (0.29)
S293	7.2 (0.006)	6.1 (0.01)
S396	4.9 (0.01)	10.4 (0.005)
Tau	1.2 (0.83)	

Results of ordinal logistic regression to assess correlation between serum levels of p-tau and tau with number of blast TBIs (0-3). In the right column, p-tau levels were normalized to total tau levels to compensate for cross-reactivity of p-tau assays with non-phosphorylated tau and log transformed (base 10). Biomarkers most closely associated with blast exposure are the p-tau assays S396, *cis* T231, S293, S214, and T175.

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Major Tasks Completed- 2

Collaborative analysis carried out with Bill Walker examining neuroendocrine dysfunction in LIMBIC PLS cohort

- No difference in NED rates across any of the mTBI groups including TBI negative controls, repetitive mTBI, blast-related mTBI, deployment duration, combat intensity, PTSD diagnosis, subject sleep dysfunction.
- There was a significant increased risk of hypothyroidism or male hypogonadism or any of the 3 pituitary disorders with advanced age and BMI, 2 already well-established risk factors in civilian cohorts.
- NED screening tests failed to differentiate among any of the clinical features often attributed to mTBI that might be exacerbated by these endocrinopathies, namely fatigue, depression, cognitive symptoms, or poorer executive function or processing speed.
- Manuscript submitted and under revision
- Abstract submitted to MHSRS

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Summary

- **Research Deliverables**:
  - Collected and stored biospecimens from 1,443 baseline and 446 follow-up PLS, 163 from two previous CENC Studies
  - Carried out NED screening (IGF-1, testosterone, TSH) on 1,500 PLS participants at baseline
  - Carried out DNA extraction, APOE genotyping on 1,206 PLS participants (with genetic testing consent) and entered results in FITBIR
  - Distributed samples to approved projects

- **1-Year Goal**:
  - Publish NED screening analysis results
  - Distribute additional requested samples to MSD for validation phase of project once approved by Research Committee
  - Continue ongoing DNA extraction and APO genotyping as received
  - Distribute samples to approved mTBI research projects

- **End of Cycle Goals**:
  - Well-curated Biorepository with full clinical data from participants from the LIMBIC PLS study
  - Complete baseline NED screening on all participants
  - Complete DNA extraction and APOE genotyping on all LIMBIC-CENC participants who give approval for genetic testing.
  - **Make available longitudinal biospecimen samples to approved projects that include diagnostic and prognostic biomarkers of the chronic effects of neurotrauma**

Questions

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LIMBIC-CENC BIOMARKERS DISCOVERY STUDY

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Kevin Wang, PhD

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- **Main Aim**: Identify novel biomarkers (protein, genetic, epigenetic) that can stratify and characterize mTBI subgroups and predict neurodegeneration and remote TBI outcomes or maintenance of symptoms.

- **Current Status**: DoD first and second level regulatory approvals in place and current. On schedule with Projects 1 and 2

- **Clinical Deliverables**: Biomarker characterization of 1) mTBI subgroups and 2) prognostic for neurodegeneration and remote TBI outcomes.

- **1-year Goals**: Complete multi-modal blood and imaging cross-sectional biomarker analysis in full cohort.

- **End of Cycle Goal:** Identified novel biomarkers that stratify/characterize mTBI subgroups and identify combat-deployed SM and veterans at increased risk for remote TBI-associated neurodegeneration and persistent TBI outcomes/symptoms

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Biomarkers Discovery Study Projects

- **Project 1 Proteomics (Y1-Y3)**: Identify biologic signatures that may be predictive (prognostic biomarkers) of long-term TBI outcomes or maintenance of symptoms. Identify novel biomarkers for chronic mTBI; characterize mTBI subgroups based on recovery and neurodegeneration.

- **Project 1 Proteomics (Y1-Y3)**: Collaborate with Kevin Wang (Gainesville VA) to develop and validate a rapid throughput multiplex immunoassay of candidate chronic TBI biomarkers.

- **Project 2 GWAS (Y2-Y4)**: Carry out GWAS within the CENC/LIMBIC cohort (N = 3,000) in collaboration with the Genetic Association in Neurotrauma (GAIN) consortium that has data from >10,000 participants.

- **Project 3 Epigenetics (Y4-Y5)**: Expand the miRNA study and to be the first to carry out a DNA methylation study in chronic TBI patients.

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Project 1 Proteomics (Years 1-3) - 1

- Completed assays of 4 neurodegeneration proteins (t-tau, NfL, GFAP & UCH-L1) on 1,200 PLS participants.
- Completed inflammation panel (IL-6, IL-10, TNF-α). Database locked and multiple analyses under way.
- Obtaining informative pre-injury serum samples from DoD serum biorepository
- Completing CENC Biomarker analyses and manuscript submission/publications
  - *Sleep dysfunction in chronic mTBI and Exosomal miRNA analysis* manuscript submitted to Sleep Medicine
  - *Blast TBI and Exosomal miRNA analysis* for Brain Injury special issue
  - *Poor Sleep in chronic mTBI, biomarkers and cognition* for Brain Injury special issue
  - *PTSD Symptoms and Exosomal miRNA analysis* for invited submission Biomedicine
  - *Sleep dysfunction and plasma/exosomal inflammatory markers* for invited submission, Frontiers Pharmacology
- Primary analysis of candidate protein biomarkers and TBI

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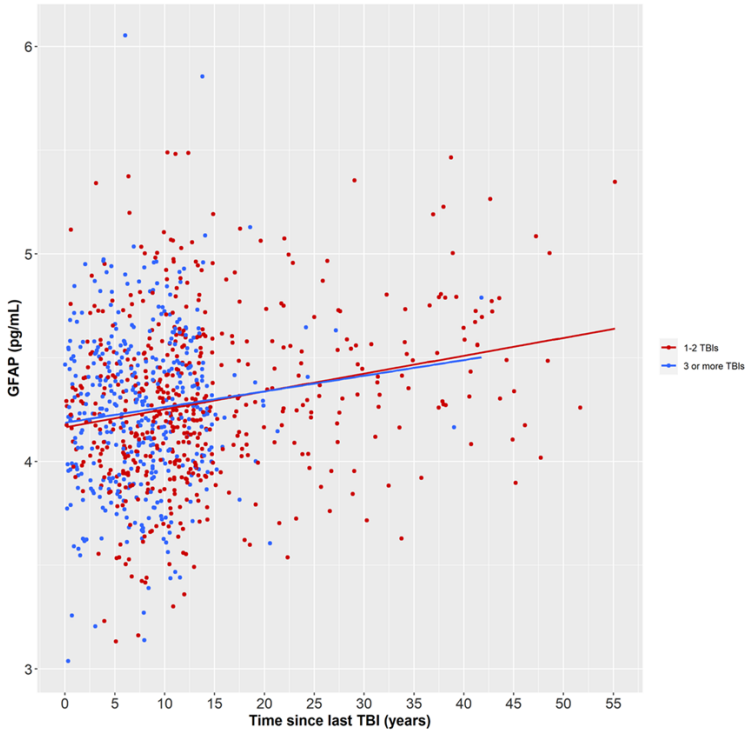
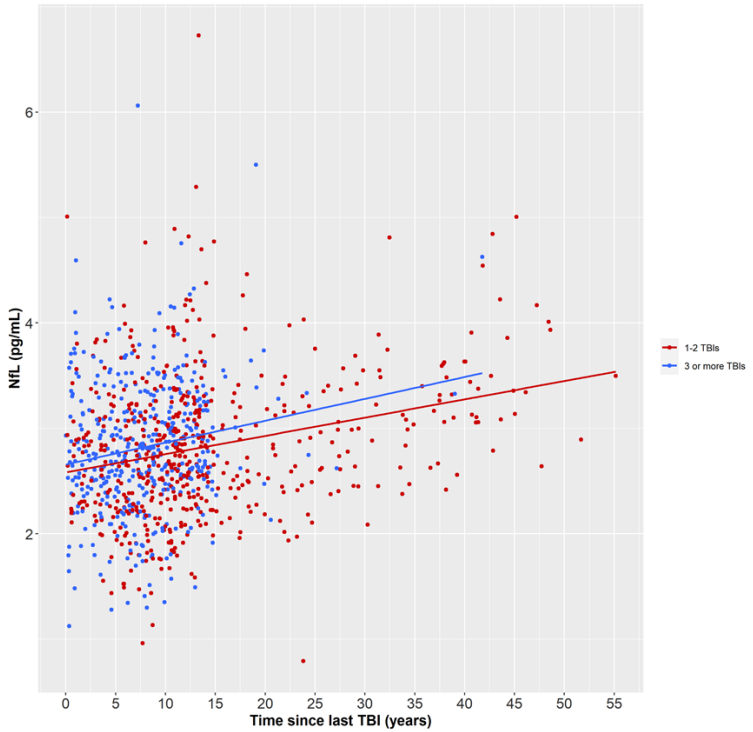
Preliminary Proteomic (4-plex + 3-plex) Analysis to date





	1-2 TBIS vs 3 or more TBIs			*1-2 TBIS vs 3 or more TBIs*		
Predictors	*Estimates*	*std. Error*	*p*	*Estimates*	*std. Error*	*p*
(Intercept)	0.49	0.07	**<0.001**	0.56	0.17	**0.001**
Time since last TBI (years)	-0.02	0.00	**<0.001**	-0.02	0.00	**<0.001**
NfL	0.08	0.03	**0.019**			
GFAP				0.02	0.04	0.643
Observations		947			955	
R^2 Nagelkerke		0.130			0.122	
AIC		1247.155			1264.066	

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Project 1 Proteomics (Years 1-5) - 2

- Submitted VA grant with Dr. Wang prime for novel sweat biomarker testing
- Collaborative multimodal Imaging/blood biomarker analyses
  - DTI-NfL
  - Volumetrics-NfL
  - rsfMRI and protein biomarkers
  - Random forest
- 1 manuscript published (Werner *Poor sleep correlates with biomarkers of neurodegeneration in mild traumatic brain injury patients: a CENC study*, Sleep 12/2020
- 1 manuscript under review (*Exosomal MiRNA of poor sleep symptoms Sleep Medicine*)
- 2 collaborative TBI biomarker review manuscripts working with Cohen's VA and InTBIR, submitted and under review
- Submitted 11 abstracts for national/international conferences.
  - 2 presented at the SfN global connectome in January 2021
  - 3 abstracts accepted at Sleep 2021
  - 6 submitted to 2021 MHSRS)

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Summary

- **Research Deliverables**:
  - Published association of plasma biomarker of neurodegeneration (NfL) and decreased executive function in CENC mTBI cohort with poor sleep, suggesting a potential pathomechanism of cognitive dysfunction.
  - Revising several other biomarker analyses of several chronic TBI outcomes (sleep dysfunction, behavioral health symptoms, cognition) and TBI characteristics (repetitive, blast) based on CENC Biomarker discovery project.
  - Completed baseline plasma proteomic measures of 7 candidate biomarkers from LIMBIC- PLS baseline cohort.

- **1-Year Goals**:
  - Publish 5 additional manuscripts of protein and miRNA biomarkers from CENC discovery project.
  - Complete characterization and multimodal imaging-blood proteomic analyses on LIMBIC PLS.
  - Complete acquisition of pre-injury specimens from DoD serum repository.
  - Continue to link PLS specimens with incident clinical outcomes of interest, such as dementia.
  - Continue DNA extraction/APOE genotyping and coordinate GWAS collaboratively through Broad Institute and GAIN.
  - Carry out collaborative, novel sweat patch biomarker project with Kevin Wang.

- **End of Cycle Goals**: Identify novel (blood, saliva, sweat-based) biomarkers (protein, genetic, epigenetic) that stratify/characterize mTBI subgroups and identify combat-deployed SM and veterans at increased risk for remote TBI-associated neurodegeneration and persistent TBI outcomes/symptoms.

Questions

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