1. Desired Needs

- Develop a physiologically relevant human model to study APOE biology in sporadic Alzheimer's disease, beyond the limitations of rodent or 2D cell culture systems.
- Create an isogenic platform that isolates the effects of distinct APOE alleles (ε2, ε4) on AD-relevant pathology with minimal confounding genetic background.
- Enable systematic phenotypic comparison across APOE genotypes including amyloid burden, tau phosphorylation, synaptic integrity, and apoptosis.
- Establish a reproducible and scalable organoid system that can be extended to therapeutic screening and translational applications.

2. Constraints

- **Safety/Regulatory:** BSL-2 standards govern all work with human-derived iPSC lines; ethical sourcing and IRB-compliant use of human stem cells required.
- **Risks:** Long-term culture is vulnerable to contamination, necrotic core formation, and batch-to-batch differentiation variability that may obscure genotype-specific phenotypes.
- **Global Impact:** Dementia affects 55+ million people worldwide; an improved preclinical model could accelerate globally relevant therapeutic development.
- **Manufacturability:** Organoid production is labor-intensive (12+ weeks/batch) and requires specialized equipment and trained personnel, limiting throughput and broad adoption.
- **Quality Control/Marketability:** Reproducibility standards (OSI v1.0, ANSI/ATCC ASN-0002-2022) and rigorous QC metrics are essential for the model to be adopted by external labs or industry.

3. Engineering Standards

- Organoid Standards Initiative (OSI) common guideline v1.0, informed reproducibility, quality assessment, and endpoint validation across batches.
- ANSI/ATCC ASN-0002-2022, governed iPSC authentication, cell line identity verification, and traceability of biological constructs.
- BSL-2 laboratory standards, constrained handling, containment, and waste disposal for all human-derived biological materials.
- Standardized immunofluorescence microscopy and western blot protocols, ensured quantitative comparability of protein-level readouts across genotypes.
- Future standards may emerge from this work for genotype-specific neurodegenerative disease modeling in 3D human organoid systems.

4. Ethical, Environmental, and Societal Concerns

- **Ethical:** Human iPSC-derived models require responsible handling, ethical sourcing, and honest communication of experimental limitations to avoid overstating clinical translatability.
- **Environmental:** Sustained organoid culture consumes significant laboratory reagents, plasticware, and energy; minimizing waste through efficient batch planning is a concern.
- **Societal:** Because organoids cannot fully recapitulate in vivo brain complexity, responsible science communication is essential to maintain public trust in emerging model systems.

5. Active Teamwork and Leadership

- **Collaboration:** My teammate and I contributed complementary expertise. I led data analysis pipelines of IF and WB; together we integrated findings across assays.
- **Delegation:** We divided subprojects by strength: differentiation protocol and pathology quantification (Taojin) and molecular characterization planning (Zhixuan), with shared responsibility for writing and interpretation.

- **Goals and Deadlines:** We set milestone-based timelines around culture timepoints (4, 8, 12 weeks) and coordinated deliverables around course deadlines and poster preparation.
- **Constructive Feedback:** We received ongoing feedback from our PI and senior lab members on experimental design, which we incorporated into protocol refinements and analytical decisions.

6. Motivating Factors

- **New Knowledge:** The complexity of APOE biology, spanning lipid transport, neuroinflammation, and synaptic function that pushed me to continuously expand my understanding of AD pathogenesis and 3D culture systems.
- **Self-Initiating:** The absence of a controlled isogenic human model for APOE-specific AD research meant there was no established roadmap; I took initiative in adapting protocols and troubleshooting independently.
- **Persistence:** Approximately 1 in 4 people carries at least one APOE ε4 allele, and two copies can increase AD risk 8-12 fold, yet no effective treatment exists. The belief that this platform could contribute, even incrementally, to Alzheimer's therapy kept me motivated through failed experiments, long timelines, and repeated troubleshooting.

7. Innovative and Entrepreneurial Ideas

- The isogenic APOE organoid platform is a foundational tool for APOE-targeted drug screening, a commercially underserved niche given that most preclinical AD models lack human-relevant genotype specificity.
- Integration of stimulated Raman spectroscopy (SRS) for label-free lipid imaging with the organoid model enables non-destructive, real-time biochemical profiling not feasible with conventional assays.
- The system could serve as the basis for an STTR/SBIR-fundable platform technology, potentially licensable to biotech companies developing APOEε4-targeting or APOE mimetic therapeutics.