1. Desired Needs

- **One proposed mechanism for the progression of Pulmonary Arterial Hypertension is the downregulation of connexin 40. Restoring the function adn expression of this protein is one of the main needs of this project**
- We have to develop a computational and experimental validation pipeline that can effectively measure Cx40 expression, formation/localization, permeability.

2. Constraints

- **Safety/Regulatory Affairs**: Lab safety techniques were followed (PPE, proper sanitation/hygiene). We used the FAIR standards to find scRNA-seq datasets for the in vitro functionality testing
- **Risks**: Since connexin 40 doesn't express as much this could lead to some risks with whether or not we secure funding in the long run to continue this research.
- **Global Impact:** Pulmonary Arterial Hypertension kills over 20,000 people each year. Therapies that exist right now do not solve the root of the issue but rather focus on looking at reducing symptoms
- **Manufacturability & Quality Control:** All computational pipelines are open, flow cytometry expression, Pearson correlation after cross-data set stability validation

3. Engineering Standards

- ISO 13485, 10993, and 5725 guided workflow advances and accurate and precise measurements of pipeline results (IF). FAIR principles were used for reproducibility and processing of transcriptomic data, and FDA 21 CFR Part 58 Good Laboratory Practices were used for wet lab validation and experimentation.

4. Ethical, Environmental, or Societal Concerns

- **Ethical**: Using AI and specific data could raise ethical concerns about the privacy of the source of the data.
- **Environmental**: Wet lab experimentation and machine use do have a negative impact on the environment, as they use significant energy to run.
- **Societal**: Changing and mutating proteins can be used for purposes beyond disease treatment, with potentially greater social implications.

5. Active Teamwork and Leadership

- **Each person had a specific task that dealt with a different part of research, whether it be quantification metrics, developing assays, or using data science techniques to inform wet lab experiments** (Nishant: permeability assay, Ben: IF imaging, Anirudh: BioEmu/AF3, Curtis: mutagenesis/flow cytometry, Satvik: ProteinMPNN, Aiden: scRNA-seq/kinase/PDZ)
- Additionally, Dr. Kufareva was pivotal with her weekly meetings, guiding the group towards making improvements in all pipelines and assays

6. Motivating Factors

- **New Knowledge:** I am transitioning to a more software-oriented career, so pursuing new knowledge in coding, general computer science, and SWE principles kept me motivated to learn.

- **Self-Initiating:** Dr. Kufareva handed the project over to us and provided guidance along the way. As a result, having questions and topics to discuss during these guided sessions was my motivation to continue advancing the project, ask questions, and ultimately take accountability for its progress.
- **Persist:** I think that the moment that I started seeing some of the pipelines I made work, and the results made me continue to persist to progress through the pipeline and get better results. This feeling of seeing partial results and continuing to progress kept me going.

7. Innovative and/or Entrepreneurial Ideas

- **For this project, I think making a website with a graphical interface that is clear for the Immunofluorescence pipeline will be necessary because it allows the user to see what each image means graphically and make sense of the different statistics behind IF quantification and what they mean. This will be much easier to use than running scripts and individually tweaking image clarity through json files (an interface would decrease the time it takes to run through experiments).**