Thesis Project Portfolio

Designing an Approach to Fluorescently Imaging MDSCs *in vivo*
(Technical Report)

First in Human Trials: Analyzing Ethical Standards
(STS Research Paper)

An Undergraduate Thesis

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Bachelor of Science, School of Engineering

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Sociotechnical Synthesis

Technical Project

The technical project is focused on designing an approach to fluorescently imaging Myeloid Derived Suppressor Cells (MDSCs). These immune cells are of particular interest because they are expressed at elevated levels in mice following Focused Ultrasound (FUS) treatment. MDSCs interact with the tumor microenvironment (TME) to promote tumor growth, and the TME encourages MDSC proliferation. MDSCs can also negatively impact other cells in the immune system. Current approaches to counting and imaging immune cells require that the tumor be removed, which does not allow for the observation of multiple time points or the conjunction of both spatial and cell count data. Designing a probe for *in vivo* use can help solve this problem by allowing for imaging at multiple time points and providing both spatial and count information. There are two aims to this project: first to fabricate the probe and second to do initial feasibility testing in vivo.

The primary components of this design include the targeting molecule, which ensures that the probe attaches specifically to MDSCs, and the fluorophore, which allows for imaging. The probe must have a high affinity for the target molecule, Ly6G, which can be achieved using an antibody. However, adjustments must be made to make sure that the antibody provides a good target to background ratio and is able to enter the TME. The fluorophore must both avoid tissue damage and be able to penetrate the tissue to allow for imaging. Based on these design criteria, a Fab antibody fragment and the Alexa Fluor 700 were chosen as the targeting molecule and fluorophore. These two can be conjugated using conjugating kits.

The first aim of fabricating the probe was done by making and purifying Fab fragments. These fragments were then tested on a Western Blot to determine if they had been properly fabricated. Due to some technical issues with Western Blotting throughout the project, this was not able to be completed. However, a BCA assay showed promising results with the correct amounts of protein showing up in both the pre-purified and the purified product.

The second aim was completed in a mouse study with a 5 mouse cohort. These mice were injected with 4T1 tumors and then allowed to grow out for about 20 days. The mice were then imaged as were their lungs, spleens, livers, and tumors. This was primarily done as a start to understanding the dosing and antibody kinetics.

Future work in this area includes completing probe fabrication and testing this probe both *in vitro* and *in vivo*. Another area of future work is fabricating other probe types and testing to determine the optimal targeting molecule/fluorophore combination.

STS Research Paper

The STS paper is focused on examining current First in Human (FIH) clinical trial practices through three ethical lenses: utilitarian ethics, Kantian ethics, and the care ethic. Utilitarian ethics is largely used to justify current clinical trial practices. The other two ethics are chosen as contrasts to this ethic, as they focus more on individual rights than the good of all society. This topic is examined as it related to historical codes of human experimentation, the preclinical to clinical translation, informed consent and patient populations.

Historical codes of ethics were developed in response to Nazi experimentation done in World War II, which had no regard for individual rights or suffering. These codes align with

Kantian and care ethics more than utilitarian ethics, further confirming that these are ethics perhaps more appropriate for evaluating FIH trials than the utilitarian ethic.

The preclinical to clinical translation is discussed in terms of how few treatments make it past the FIH trial stage due to unforeseen consequences. Lack of knowledge of the differences between animal and human immune systems can create issues in this translation from preclinical to clinical work. Through the Kantian and care ethics lenses, the understanding of these differences should be improved to ensure patient safety.

Informed consent poses significant challenges in current FIH trial practices. Making sure that patients understand trials is good and important. However, current standards have led to long and convoluted informed consent documents that are not accessible to the average person. These practices should be improved to live up to the standards of informed consent.

Patient populations in clinical trials pose an issue as they do not reflect the populations that will potentially be taking the drug. This can become an issue when treatments interact with these different groups differently. In order to create a culture of trust between the medical and general communities, clinical trials should reflect the populations of the target population.

Overall, FIH trial practices are in need of improvement. Combining the utilitarian ethic with the Kantian and care ethics can hopefully provide a route to improving these practices.

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