Designing an Approach to Imaging MDSCs in Tumors

First in Human Trials: Analyzing Participant Selection Practices

A Thesis Prospectus

In STS 4500

Presented to

The Faculty of the

School of Engineering and Applied Science

University of Virginia

In Partial Fulfillment of the Requirements for the Degree

Bachelor of Science in Biomedical Engineering

By

Sophia Kerns

November 1, 2021

On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

ADVISORS

Rider Foley, Department of Engineering and Society

Tim Bullock, Department of Pathology

Richard Price, Department of Biomedical Engineering

Natasha Sheybani, Department of Biomedical Engineering

Introduction

The immune system is a complex network of different cell types. These cells and their signals work to fight infections, from the flu to cancer. Cancer has particularly complex interactions with the immune system, as certain immune cell types can promote tumor growth and inhibit other immune responses. In pre-clinical cancer research, it is important to be able to follow these cells as they react to the tumor. Imaging the cells enables a better understanding of how they traffic through the body. Without imaging, most of the data available is surrounding cell numbers, which gives an incomplete picture of how the cells are acting in the body. Imaging throughout treatment allows a more complete understanding of how cells move rather than just how many there are.

This capstone project is focused on developing an approach to imaging myeloid derived suppressor cells (MDSCs) as they traffic to tumors. MDSCs act to suppress other immune cell subtypes, especially T cells, and also impact other important physiological processes such as angiogenesis (blood vessel generation), which can aid tumor growth by providing more blood flow and nutrients to the region (Ostrand-Rosenberg et al., 2012; Youn & Gabrilovich, 2010). An imaging method that can be used actively during treatments would help researchers to better understand how MDSCs traffic in the body, especially in a tumor, and how they interact with the tumor microenvironment. An additional benefit of the development of this imaging approach is being able to image MDSCs within the body, not just following tumor removal. This is an important topic to investigate because MDSCs are detrimental to the tumor environment, but have not been studied as much as other cell types (Hoffmann et al., 2019). An accurate method of imaging could help inform research into cancer treatments and improve understanding of this cellular subtype.

This process includes the development of the probe, which entails choosing a target on the MDSC surface for attachment and linking a fluorescent signal. These elements together will allow imaging of where MDSCs are in the tumor as well as the number of cells present. The probe will then be tested on cultured MDSCs to determine if it works properly. Following confirmation of its efficacy, the probe will be tested in rats to determine if the efficacy translates to a small animal model. Probe fabrication requires use of previously described chemistries (Zhang et al., 2017). In addition to this technical aspect, the project also involves wet lab procedures such as cell culture and animal experimentation. In the technical project, a probe will be designed and validated to specifically image MDSCs in a mouse model. I will investigate the ethics of participant selection methods in First in Human (FIH) trials in terms of how they protect patients and science in parallel.

Imaging MDSCs in Tumors

The technical project is aimed at designing a probe to image MDSCs in tumors in the body. Current methods for imaging MDSCs do not produce as robust of data, as they often require tumor removal in order to be performed. One example of this is immunohistochemistry (IHC). This method takes tissue samples and stains cells for the desired target outside of the body, then cells can be counted using various programs (Duraiyan et al., 2012). One problem with an ex vivo imaging method is that it can only count cells following the conclusion of treatment. In vivo imaging solutions provide better data, as they can be performed at various time points throughout treatment. Solutions for in vivo imaging of different immune cells include either heavy metal labeling or fluorescent labeling. Heavy metal labeling requires imaging methods such as PET or MRI, which can be more intensive than the fluorescent alternative (Tavaré et al., 2014).

This project will be aimed at making a fluorescent probe imaging granulocytic MDSCs, which express Ly6g (Youn et al., 2008). Ly6G is a protein that is present exclusively on the cell surface of granulocytic MDSCs, although is does not have a known function (Wang et al., 2012). The probe will target the Ly6g on the cell based on this. There are three main components to the probe design: the targeting method, the conjugation method, and the fluorochrome. First of all, the probe will target MDSCs using an antibody specific for the Ly6G protein. There are several problems to consider when designing an antibody targeting method. The first problem is the Fc region of the antibody (Figure 1), which can bind to cells other than MDSCs. Removal of this region can prevent non-MDSC binding and make sure that only MDSCs are picked up by the probe (Buchwalow et al., 2011). The other region on the full antibody is the region that is specific for its individual target. Once the Fc region has been removed, this region can be further processed to result in a smaller fragment that is still specific for the target. The three fragment types that are in current use are shown at the bottom of figure 1. The second problem to consider is the target to background ratio of the probe. While larger molecules are retained better within the tumor, smaller molecules are able to be cleared from the blood more quickly and allow faster ability to clearly image the cells (Debie & Hernot, 2019). A third problem is that of affinity to the target. The antibody must have a high affinity for the target in order to give accurate measurements of cell levels. However, certain modifications to the antibody can result in lower affinity. One final problem to consider is that of the antibody half-life. Whole antibody molecules have a very long half-life as opposed to smaller molecules, but smaller molecules are advantageous for other reasons as mentioned before. Long half-life is advantageous because it allows the probe more time to attach to the target molecule from the blood.

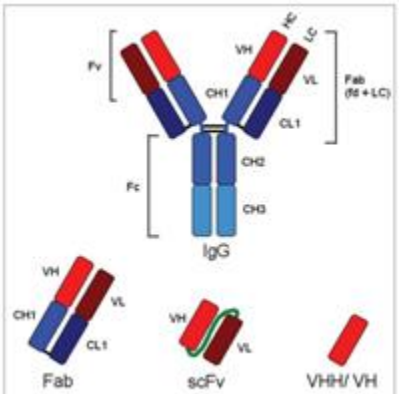

Figure 1: Antibody Diagram, along with antibody fragment types (Nelson, 2010).

The second major component is the conjugation method used to connect the fluorochrome to the antibody. This method has to be chosen carefully so as to avoid reducing the affinity of the antibody. This method also must vary with the type of antibody or antibody fragment used (Figure 1), as certain types of fragments have different potential bond sites. The third major component is the fluorochrome. This has to be chosen to allow for sufficient tissue penetration of both the excitation and emission wavelengths along with avoiding tissue damage. Previous literature has shown certain fluorochrome wavelengths to be best, specifically between 650 and 950 nm (Kobayashi et al., 2010; White et al., 2012). The fluorochrome strategy must also be compatible with the available fluorescence reading equipment, which is the Lago-X fluorescent reader.

These design solutions will first be evaluated through in vitro experiments, meaning that the imaging probe will first be evaluated in cultured cells. Once the probe has been perfected and is achieving specific targeting on the plate surface, an in vivo model will be used to evaluate efficacy in an animal model. This step will ensure that the probe not only targets the correct cells, but also provides usable information in the desired application of animal models.

Balancing all of these design requirements into one product will provide a solution for researchers to better image MDSCs in tumors. This solution allows for the cells to be imaged at different time points in treatment in vivo. It also provides an improvement over previous methods, which were primarily focused on final MDSC numbers following treatment. An understanding of MDSC trafficking, especially in certain tumor or treatment types, could help provide fuller knowledge of how treatments impact these cell numbers. Given how prevalent MDSC action is in tumor responses, knowledge of this particular cell type is critical for improving treatments and understanding cancer pathology.

This solution has downstream implications in society. Since the solution is aimed at allowing researchers better knowledge of their treatment effects, it will hopefully help treatments be improved to the point that they can be translated to clinical applications. It is therefore important to explore the societal implications of FIH trials, especially participant selection in these trials.

Participant Selection in FIH Clinical Trials

In some cases, clinical trials have resulted in patient harm and could potentially lead to death. One example of this is a clinical trial which produced a large cytokine storm in patients as a result of the treatment. A cytokine storm is a large overproduction of immune signaling molecules, which can lead to organ damage (Tisoncik et al., 2012). Some subjects in the trial had severe adverse effects, and some were even close to dying. Following analysis, it was observed that a key difference between the animal model used in preclinical research and human immune systems led researchers to miss a potential adverse effect (Eastwood et al., 2010). This is just one example of the importance of ensuring that patients are adequately protected by FIH trial practices.

One factor to consider when evaluating trials is that FIH trials are mainly geared towards evaluating dose selection of treatments (Satalkar et al., 2016). A key component is dose escalation, meaning that the earliest patient cohorts get a very small dose, then dosing increases with later cohorts (Figure 2). This means that FIH trial participants are not primarily expected to benefit in response to the treatment. It is important to remember this when considering patient treatment, as even those patients that are most sick and have not responded to other treatments will likely not see a personal benefit. FIH trials also bring up tensions between good scientific practice and patient treatment. The ideal experiment would be a randomized control trial, but these sorts of experiments do not work ethically with human subjects (Goldstein et al., 2018). Researchers are therefore placed in a constant struggle between performing the best scientific studies and taking care of the subjects, which is bound to produce ethical challenges (Hope & McMillan, 2004). This is especially poignant considering that non-robust data is essentially not helping anyone. One particularly challenging issue to consider is that of participant selection.

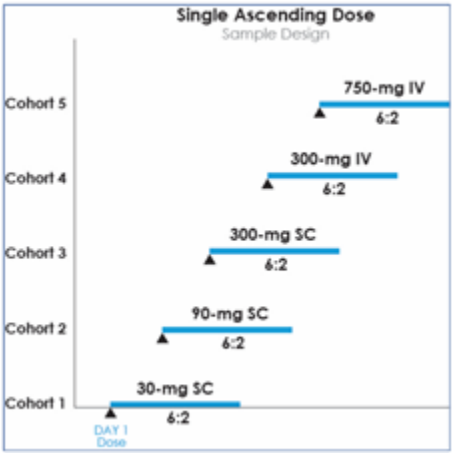

Figure 2: Graphic of dose escalation practices in FIH trials (Mel Affrime, 2020).

FIH trial ethics are based on overall social benefit to the populace rather than to the individual (Michelle Habets, 2017). This line of thought provides an ethical challenge as to what

the rights of the individual are when they interfere with overall societal benefit. The question that is often presented is whether the social benefit of the trial is worth the potential harm to the subjects. One of the largest potential issues with participant selection is payment for participation. Considering the risk involved in participation in FIH trials, payment tends to recruit volunteers who are desperate for money (Dresser, 2009). Whether intentional or not, this means that trials often take advantage of people who have a lower income. Trials also typically use healthy young to middle aged adults (Dresser, 2009). However, there is a history of exclusion of females. This exclusion is based on women being of childbearing age, and so researchers do not want to risk potential birth defects or infertility in women based on their treatments (Koonrungsesomboon et al., 2016). This exclusion results in data being skewed towards young to middle aged men, and therefore does not necessarily provide a good picture of how even a majority of people would respond to a new treatment.

The ethics of FIH trials will be evaluated through two ethical frameworks: Kantian ethics and care ethics. Kantian ethics is based around duty, stating that an action is moral if it falls in line with some central principle known as the categorical imperative (Ibo Van de Poel & Lamber Royakkers, 2011). This ethic believes that people are capable of arriving at morality through reason, and that immoral actions are therefore outside of reason (Johnson & Cureton, 2021). The standard for knowing if something can be considered morally acceptable is whether or not a person could wish the action to be the standard of the way people should behave. The ethic also emphasizes treating everyone as equals and treating people as and end in and of themselves rather than a means to a different end (Ibo Van de Poel & Lamber Royakkers, 2011). In the context of participation selection, it must be considered whether the current participant selection

and treatment practices should be the standard, and whether it would be acceptable to extend these practices to other arenas of partipant selection in other trials.

The second ethic to be explored is the care ethic. This ethic, first proposed by Carol Gilligan, examines more of the social context involved in ethical interactions. It explores how relationships between people are inevitably coupled to responsibilities and emphasizes the care that people give each other (Ibo Van de Poel & Lamber Royakkers, 2011). While this ethic was originally focused on the differences between gender interactions with the world, it provides a framework to examine relationships between players at hand rather than the actual actions performed (Skoe, 20141222). This can be connected to participant selection to explore relationships between clinicians and subjects.

Research Question and Methods

Are current practices of participant selection for FIH trials ethical?This will be evaluated using Kantian ethics and subsequently using care ethics. This can be achieved through several means. The first is looking at historical ethical codes such as the Nuremberg Code and Declaration of Helsinki which guide the development of research standards (Hope & McMillan, 2004). These codes were developed directly in response to Nazi trials involving horrific patient treatment, and are still used as standards for clinical trials patient treatment. Comparing current practices with the codes of conduct and also comparing these things to the ethical frameworks will provide an analysis of the standards of current practices in the context of the ethical frameworks. The second is through interviews with both clinical investigators and patients on their experiences with trials and their thoughts on the standards for translational research, as well as on the patient-clinician relationship. This can help give a better understanding of the human difficulties of designing these kinds of trials and the type of relationships that exist between

clinicians and patients. . The third is through case studies of both successful and unsuccessful FIH clinical trials. This can give insight into the most common reasons for trial failure, as well as the study design of most FIH trials. The final method is through investigation of prior research on participant selection in FIH trials. Together, these methods provide both personal and research-based insight into participant selection practices and their ethical implications.

Conclusion

Overall, this project is focused on making a research tool to image MDSCs in tumors. This will be accomplished by designing a targeting molecule, a conjugating method, and a fluorochrome to allow for fluorescent imaging of the cells. This design will solve the problem of being able to look at MDSCs in vivo, and hopefully also be able to translate into other cell types. As research tools can aid preclinical researchers in reaching a translational research stage, this design can aid downstream FIH trial design, as well as be used to provide information about potential immune responses to a treatment in humans. The STS topic will be focused on participant selection in FIH clinical trials. Through this analysis, FIH trial design ethics will be analyzed to determine if these practices need to be improved for best patient treatment. Together, these topics can help to address problems in the preclinical and clinical space.

References

Buchwalow, I., Samoilova, V., Boecker, W., & Tiemann, M. (2011). Non-specific binding of antibodies in immunohistochemistry: Fallacies and facts. *Scientific Reports*, *1*, 28. https://doi.org/10.1038/srep00028

Debie, P., & Hernot, S. (2019). Emerging Fluorescent Molecular Tracers to Guide Intra-Operative Surgical Decision-Making. *Frontiers in Pharmacology*, *10*, 510. https://doi.org/10.3389/fphar.2019.00510

Dresser, R. (2009). First-in-Human Trial Participants: Not a Vulnerable Population, but Vulnerable Nonetheless. *The Journal of Law, Medicine & Ethics : A Journal of the American Society of Law, Medicine & Ethics*, *37*(1), 38–50. https://doi.org/10.1111/j.1748-720X.2009.00349.x

Duraiyan, J., Govindarajan, R., Kaliyappan, K., & Palanisamy, M. (2012). Applications of immunohistochemistry. *Journal of Pharmacy & Bioallied Sciences*, *4*(Suppl 2), S307–S309. https://doi.org/10.4103/0975-7406.100281

Eastwood, D., Findlay, L., Poole, S., Bird, C., Wadhwa, M., Moore, M., Burns, C., Thorpe, R., & Stebbings, R. (2010). Monoclonal antibody TGN1412 trial failure explained by species differences in CD28 expression on CD4+ effector memory T-cells. *British Journal of Pharmacology*, *161*(3), 512–526. https://doi.org/10.1111/j.1476-5381.2010.00922.x

Goldstein, C. E., Weijer, C., Brehaut, J. C., Fergusson, D. A., Grimshaw, J. M., Horn, A. R., & Taljaard, M. (2018). Ethical issues in pragmatic randomized controlled trials: A review of the recent literature identifies gaps in ethical argumentation. *BMC Medical Ethics, 19*, 14. https://doi.org/10.1186/s12910-018-0253-x

Hoffmann, S. H. L., Reck, D. I., Maurer, A., Fehrenbacher, B., Sceneay, J. E., Poxleitner, M., Öz, H. H., Ehrlichmann, W., Reischl, G., Fuchs, K., Schaller, M., Hartl, D., Kneilling,

M., Möller, A., Pichler, B. J., & Griessinger, C. M. (2019). Visualization and quantification of in vivo homing kinetics of myeloid-derived suppressor cells in primary and metastatic cancer. *Theranostics*, *9*(20), 5869–5885. https://doi.org/10.7150/thno.33275

Hope, T., & McMillan, J. (2004). Challenge studies of human volunteers: Ethical issues. *Journal of Medical Ethics*, *30*(1), 110–116. https://doi.org/10.1136/jme.2003.004440

Ibo Van de Poel & Lamber Royakkers. (2011). *Ethics, Technology, and Engineering* (1st ed.). Wiley-Blackwell.

Johnson, R., & Cureton, A. (2021). Kant's Moral Philosophy. In E. N. Zalta (Ed.), *The Stanford Encyclopedia of Philosophy* (Spring 2021). Metaphysics Research Lab, Stanford University. https://plato.stanford.edu/archives/spr2021/entries/kant-moral/

Kobayashi, H., Ogawa, M., Alford, R., Choyke, P. L., & Urano, Y. (2010). New Strategies for Fluorescent Probe Design in Medical Diagnostic Imaging. *Chemical Reviews*, *110*(5), 2620–2640. https://doi.org/10.1021/cr900263j

Koonrungsesomboon, N., Laothavorn, J., & Karbwang, J. (2016). Ethical considerations and challenges in first-in-human research. *Translational Research*, *177*, 6–18. https://doi.org/10.1016/j.trsl.2016.05.006

Mel Affrime. (2020, May 12). *Planning Your (First) First-in Human Trial? Considerations for Success*. Xtalks. https://xtalks.com/planning-your-first-first-in-human-trial-considerations-for-success-2241/

Michelle Habets. (2017). The unique status of first-in-human studies: Strengthening the social value requirement | Elsevier Enhanced Reader. *Drug Discovery Today*, *22*(2), 471–475. https://doi.org/10.1016/j.drudis.2016.11.016

Nelson, A. L. (2010). Antibody fragments. *MAbs*, *2*(1), 77–83.

Ostrand-Rosenberg, S., Sinha, P., Beury, D. W., & Clements, V. K. (2012). Cross-talk between myeloid-derived suppressor cells (MDSC), macrophages, and dendritic cells enhances tumor-induced immune suppression. *Seminars in Cancer Biology*, *22*(4), 275–281. https://doi.org/10.1016/j.semcancer.2012.01.011

Satalkar, P., Elger, B. S., & Shaw, D. M. (2016). Stakeholder views on participant selection for first-in-human trials in cancer nanomedicine. *Current Oncology*, *23*(6), e530–e537. https://doi.org/10.3747/co.23.3214

Skoe, E. E. A. (20141222). Measuring care-based moral development: The Ethic of Care Interview. *Behavioral Development Bulletin*, *19*(3), 95. https://doi.org/10.1037/h0100594

Tavaré, R., McCracken, M. N., Zettlitz, K. A., Knowles, S. M., Salazar, F. B., Olafsen, T., Witte, O. N., & Wu, A. M. (2014). Engineered antibody fragments for immuno-PET imaging of endogenous CD8+ T cells in vivo. *Proceedings of the National Academy of Sciences*, *111*(3), 1108–1113. https://doi.org/10.1073/pnas.1316922111

Tisoncik, J. R., Korth, M. J., Simmons, C. P., Farrar, J., Martin, T. R., & Katze, M. G. (2012). Into the Eye of the Cytokine Storm. *Microbiology and Molecular Biology Reviews : MMBR*, *76*(1), 16–32. https://doi.org/10.1128/MMBR.05015-11

Wang, J.-X., Bair, A. M., King, S. L., Shnayder, R., Huang, Y.-F., Shieh, C.-C., Soberman, R. J., Fuhlbrigge, R. C., & Nigrovic, P. A. (2012). Ly6G ligation blocks recruitment of neutrophils via a β2-integrin–dependent mechanism. *Blood*, *120*(7), 1489–1498. https://doi.org/10.1182/blood-2012-01-404046

White, A. G., Gray, B. D., Pak, K. Y., & Smith, B. D. (2012). Deep-red fluorescent imaging probe for bacteria. *Bioorganic & Medicinal Chemistry Letters*, *22*(8), 2833–2836. https://doi.org/10.1016/j.bmcl.2012.02.078

Youn, J.-I., & Gabrilovich, D. I. (2010). The biology of myeloid-derived suppressor cells: The blessing and the curse of morphological and functional heterogeneity. *European Journal of Immunology*, *40*(11), 2969–2975. https://doi.org/10.1002/eji.201040895

Youn, J.-I., Nagaraj, S., Collazo, M., & Gabrilovich, D. I. (2008). Subsets of Myeloid-Derived Suppressor Cells in Tumor Bearing Mice. *Journal of Immunology (Baltimore, Md. : 1950)*, *181*(8), 5791–5802.

Zhang, C., Yu, X., Gao, L., Zhao, Y., Lai, J., Lu, D., Bao, R., Jia, B., Zhong, L., Wang, F., & Liu, Z. (2017). Noninvasive Imaging of CD206-Positive M2 Macrophages as an Early Biomarker for Post-Chemotherapy Tumor Relapse and Lymph Node Metastasis. *Theranostics*, *7*(17), 4276–4288. https://doi.org/10.7150/thno.20999