Fluorescence imaging of sarcoma cells for potential targeted fluorescence-guided surgery

Kolhe SN[1*], Rankin KS[2]

[1]Newcastle University Medical Student, s.kolhe@newcastle.ac.uk | [2]Honorary Consultant Orthopaedic Surgeon, Newcastle University

1. Introduction

- The aim of surgery for bone and soft-tissue cancers (sarcomas) is a wide local excision with clear margins
- Complications can occur if resection margins are inadequate, increasing the chance of recurrence; or risk increased patient morbidity if excessive amounts of healthy tissue is removed
- There are currently no options for intra-operative fluorescence guidance in routine practice
- The ability to employ targeted fluorescence guidance during sarcoma surgery would be a step forward in improving tumour resection margins and patient outcomes

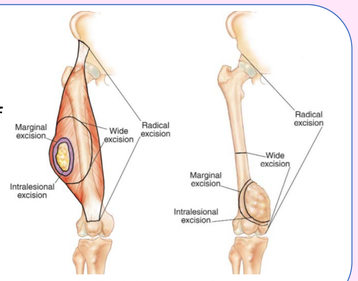


Figure 1: *Excision types for sarcoma*

2. Aims

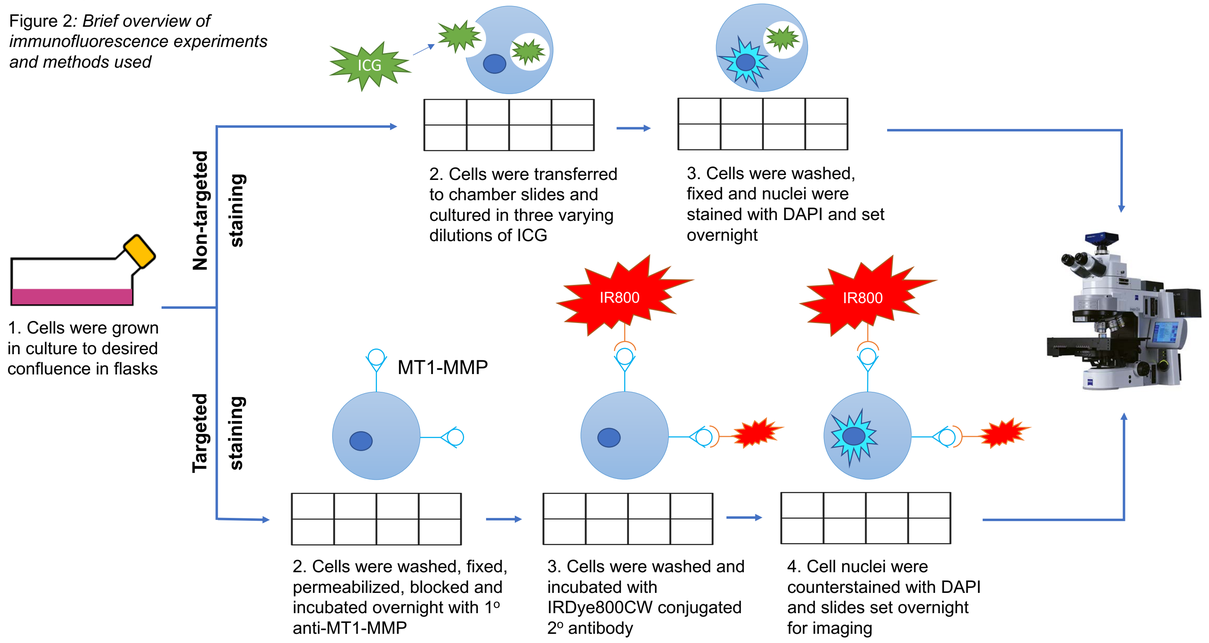
i. To compare non-targeted and targeted fluorescence uptake between fibrosarcoma (HT-1080) and breast cancer (MCF-7) cells by using indocyanine green (ICG) and IRDye800CW conjugated secondary antibodies

ii. Demonstrate proof of concept that antibodies labelled with fluorescent tags targeted to highly-expressed sarcoma cell surface markers (MT1-MMP) allow visualisation of sarcoma cells at a microscopic level

3. Methods

- Immunofluorescence experiments were conducted on HT-1080 and MCF-7 cells lines using standard techniques (Figure 2)
- ICG and IRDye800CW conjugated secondary antibodies were used to demonstrate non-targeted and targeted fluorescence respectively. MCF-7 cells are negative for MT1-MMP, hence were used as a negative control for targeted fluorescence
- Images were taken using the Zeiss AxioImager 1 fluorescence microscope and analysed qualitatively

Figure 2*: Brief overview of immunofluorescence experiments and methods used*



4. Results

➢ **Non-targeted** fluorescence uptake using ICG:
- Lowest [ICG] – **increased** fluorescence observed in **HT-1080 cells**
- Medium/High [ICG] - similar levels of fluorescence observed between both cell lines

➢ **Targeted** (tumour-specific) fluorescence using MT1-MMP antibody-conjugated IRDye800CW:
- **No fluorescence** observed in MCF-7 cells
- **Clear fluorescence** observed in HT-1080 cells

Non-specific ICG uptake by tumour cells relies on enhanced permeability, uptake and retention. Increased ICG fluorescence in HT-1080 cells supports the hypothesis that fibrosarcoma cell lines proliferate faster than breast cancer, therefore their endocytic pathways (uptake mediator) are more active.

MT1-MMP was utilized as a target on HT-1080 cells for fluorophore-conjugated secondary antibody to bind and allow detection. MCF-7s are MT1-MMP negative, therefore fluorescence was not observed, demonstrating targeted and tumour-specific fluorescence in relation to sarcoma.

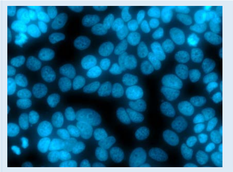
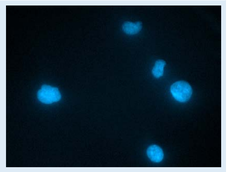
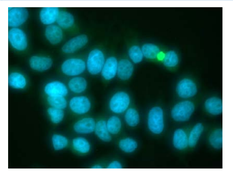
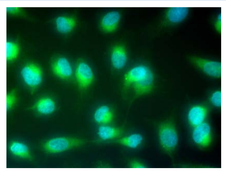
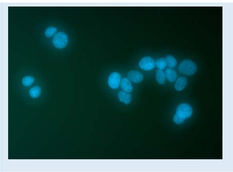
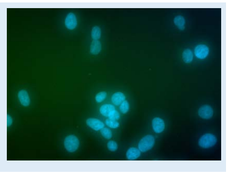
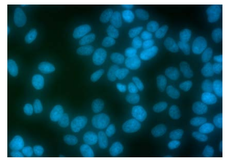
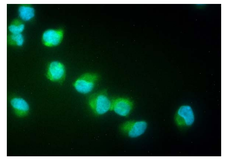
	MCF-7	HT-1080
DAPI only control (no primary or secondary antibodies)		
ICG (non-targeted)		
Secondary antibody only control		
Primary + IRDye800CW conjugated secondary antibody (targeted)		

Table 1: *Fluorescence microscopy images taken of MCF-7 and HT-1080 cells with different fluorophores.* ***Blue*** *represents DAPI stained nuclei.* ***Green*** *represents secondary antibody-fluorophore conjugate or ICG respectively*

5. Conclusion

This project compared two distinct approaches in which near infra-red (NIR) fluorescence guidance can be used to detect sarcoma cells: non-targeted and targeted fluorescence.

✓ **Targeted fluorescence allowed visualization of sarcoma cells in a more specific way than non-targeted fluorescence.**

✓ **This concept of using tumour-specific fluorescence to visualize sarcoma was demonstrated successfully on a cellular level - this could increase accuracy of surgical excision margins if developed clinically.**

Targeted fluorescence guidance in sarcoma surgery is a promising avenue for improving resection margins without additional unnecessary morbidity.

The project findings will be incorporated and taken forward into further sarcoma research at the NICR as we aim to continue the optimisation of targeted fluorescence, both *in vitro* and *in vivo,* with a view of improving clinical practice.

References

Fig 1. https://link.springer.com/chapter/10.1007%2F0-306-48407-2_1

Acknowledgements

Funding: Newcastle University