CHARACTERISATION OF PUTATIVE CIRCULATING MACROPHAGES BY IMAGING FLOW CYTOMETRY

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Introduction

Identified by Thomas Ashworth in 1869 circulating tumour cells (CTCs) and tumour associated macrophages (TAMs) are exceedingly rare cell populations present in the blood of some carcinoma patients, at concentrations of 1 per 1ml of whole blood.

CTCs are cells shed from the primary tumour site into the bloodstream and may permit the seeding of sites for development of metastases.

TAMs are derived from peripheral blood monocytes, which infiltrate the stroma of tumours via chemoattraction, and, through interaction with various growth factors present in the tumour microenvironment, differentiate. They are required for tumour cell migration, angiogenesis and metastasis formation . In others cases their action is tumouricidal or to stimulate the anti-tumour T cells. Their role is thought to differ dependent upon the type of tumour that induced them.

Since TAMs are linked directly to tumour progression if they can be isolated from blood samples and typed it may be possible to use them as a marker of disease progression and possibly diagnosis via a non-invasive method

Aims

- Proof of concept using a positive/negative control cell line (HL-60) treated with a transforming agent (PMA) to produce a model of macrophage like cells simulating TAMs for testing
- Use of controls to examine; viability of extraction procedure, identification of appropriate macrophage specific antibody dye, use of macrophage antibody in staining protocol, imaging flow cytometry protocols in order to produce the groundwork of a standardised diagnostic procedure.
- Incubation and subsequent analysis of healthy volunteer blood and patient ascites samples to assess real world viability and efficacy of developed methods in the detection of circulating tumour macrophages, and to determine if results are false positives which some literature suspects they may in fact be.

Results

Fig 1- **Example image** of how a purported circulating tumour macrophage appears under the effects of various cellular stains. The baseline image used for reference.

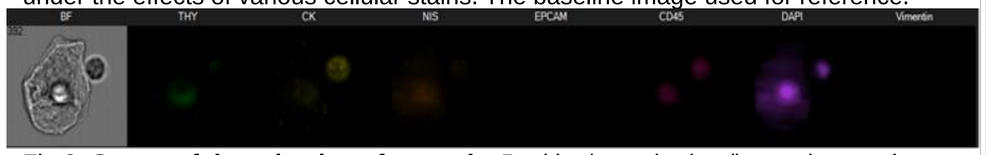


Fig 2- **Successful production of controls.** Positive/negative(top/bottom) controls indicating a distinction in size and morphology of the two populations. Macrophages are well defined as a population in the positive control. Furthermore -ve controls in the equivalent area do not appear similar to the baseline image whereas +ve controls do.

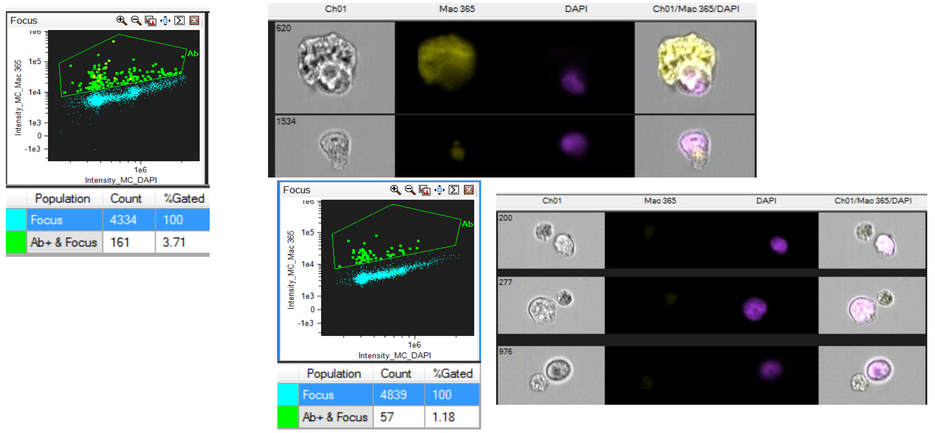


Fig 3-**Isolation of possible TAMs from blood-** Macrophage population is poorly defined and manual searching was required to isolate 23 suspected macrophages. Although the first two images in this search appear close to the baseline (and may in fact be the cells we are looking for) the others were poorly defined and are likely artefacts.

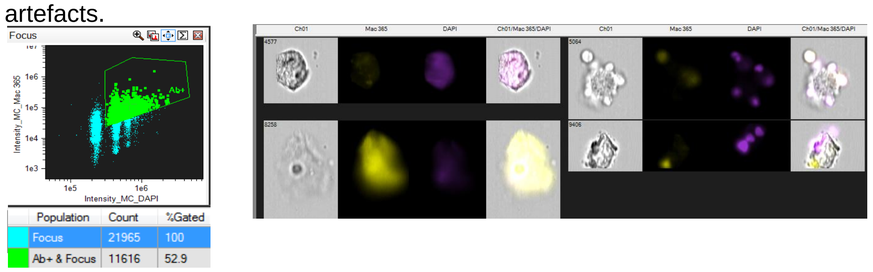
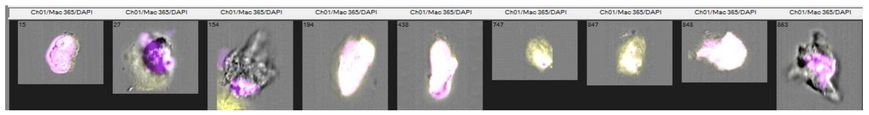


Fig 4- **Inconclusive test on ascites sample from patient**- Required visual isolation of images most of which appeared to be artefacts and cell debris, upon close inspection none were concordant with baseline. Results inconclusive.



Methods

- Culture of cryopreserved HL-60 cell line in 10% bovine serum albumin for 3 days
- Counting of cells by microscope and haemocytometer to determine volume required for six million cells
- Make up volume to 10ml for positive/negative control, inoculate positive control with 6nM PMA
- Incubate for 3 days in petri dishes to prevent adherence of transformed cells
- Collect cells in universal tubes, treating with trypsin to remove adherents
- Centrifuge at 500g for 15 min, collect pellet and resuspend in ice cold methanol to fix and permeabilize
- Stain with nuclear dye DAPI and macrophage dye MAC387
- Centrifuge and resuspend pellet in PBS
- Run sample on image stream microscope
- Analyse data and develop model
- Repeat experiment with blood and ascites samples

Conclusions

- Despite several failures of HL-60 cells to respond to PMA, HL-60 cells differentiated by PMA form an acceptable model for the TAMs, and permitted the isolation of a macrophage specific dye and development of a protocol for use on biological samples
- Somewhat successful analysis model produced for controls failed to isolate appropriate cells in patient samples requiring model refinement and time consuming manual searching. Although results were obtained refinement of protocol is required
- Cannot say with any certainty that isolated objects are in fact TAMs and not false positives.
- Current research by Adams et al may have outpaced this project in its aims

References: Ashworth, T. R (1869). "A case of cancer in which cells similar to those in the tumours were seen in the blood after death". *Australian Medical Journal* **14**: 146–7.
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