

Finding The Rare Cells in Liver Cancer

Exploring the Potential of Prognostic and Predictive Biomarkers in Circulating Tumour Cells (CTC) in Patients with Hepatocellular Carcinoma.

Rabbiaatul Addawiyah Binti Imawana, Misti McCain, Dr Helen Reeves & Dr David Jamieson

Stage 2 MBBS, 16000809, Medical School Newcastle University Medicine Malaysia R.A.Binti-Imawana2@Newcastle.edu.my

Introduction

Liver cancer is the second commonest cause of cancer deaths after lung cancer. WHO reported a staggering number of 788 000 deaths worldwide due to liver cancer in 2015¹. Cancer Research UK reported 5100 deaths in 2014 alone which equals to 19 deaths per day in the country².

What is Circulating Tumour Cells (CTCs)?

Cancers grow rapidly and can shed their cells into the blood. These cells are called circulating tumour cells (CTCs).

Why study Circulating Tumour Cells (CTCs)?

Currently, liver cancer is detected and confirmed by scans although this only offers physical information. Taking real liver tissue in a procedure called biopsy is not preferred as it involved sticking a needle into the liver and can seed cancer cells along the way. However, through less invasive liquid biopsy patient blood can be collected instead and screened for CTC.

Moreover, CTCs have varied protein material attached to them called biomarkers. These biomarkers can be used to target a specific pathway for drug development, stratifying treatment based on which CTCs were found in each patient for a more personalised treatment.

Aims

To detect the presence of CTCs in liver cancer patients and quantify the prevalence of specific biomarkers on these CTCs. These biomarkers are the varied material attached to CTCs. Specific biomarker studied in this project were DNA-PK, panCK, FGFR4, c-Met and pERK.

Research question: Among DNA-pk, panCK, FGFR4, c-Met and pERK which type of biomarkers are most prevalent in liver cancer circulating tumour cells?

Methods

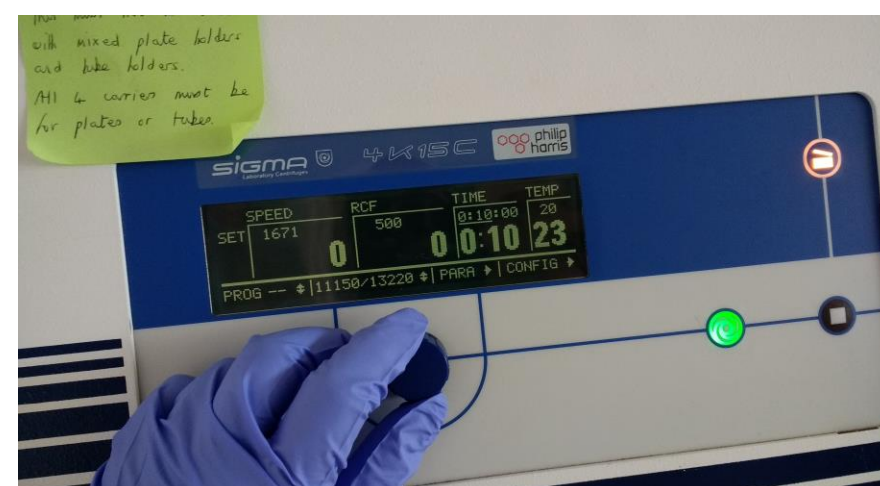


Red Blood Cells Rupture

A chemical called lyse solution was added and blood samples were warmed at 37 °C. Then, samples were spun in a machine called centrifuge. This would concentrate remaining cell at the bottom of the tubes while the ruptured ones can be discarded.

Blocking

Since the CTCs are extremely rare, careful steps must be taken to prevent losing them. A blocking solution was used and tubes were left on the rolling platform to coat all the surfaces evenly.



White Blood Cells Depletion

Magnetic particles were added to the sample which attracted white blood cells. Then, the tubes were placed inside magnets. Both tubes and magnets were inverted to collect their contents leaving behind the magnet-bound white blood cells.



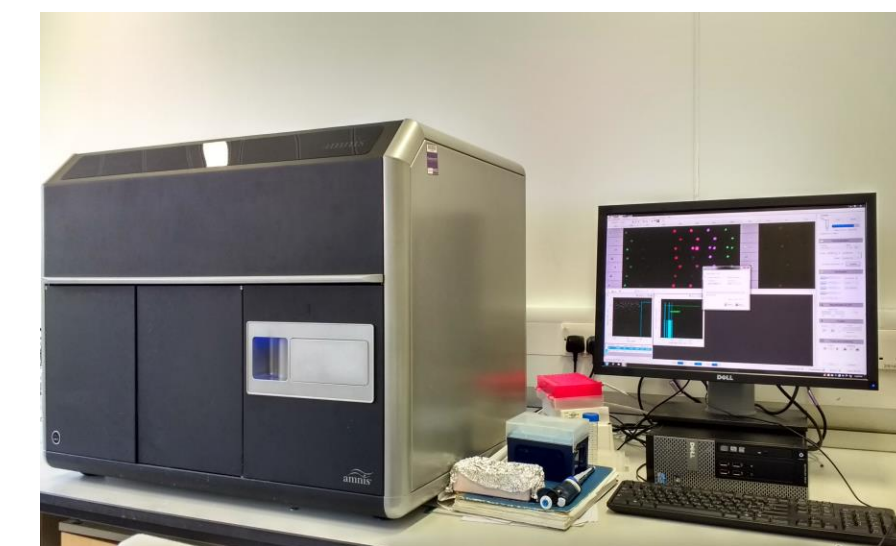
Antibody Staining

Antibodies are large Y-shape proteins that can bind to the biomarkers on the CTCs. These antibody stains have materials which will turn fluorescent or light up when excited by light energy. Some of these antibodies are very sensitive to light. Hence, they must be protected by wrapping the sample with aluminium foil.

Running Samples on ImageStream®

The samples were loaded onto the ImageStream® where the data recorded can show what the cells look like and biomarkers are on the cells.

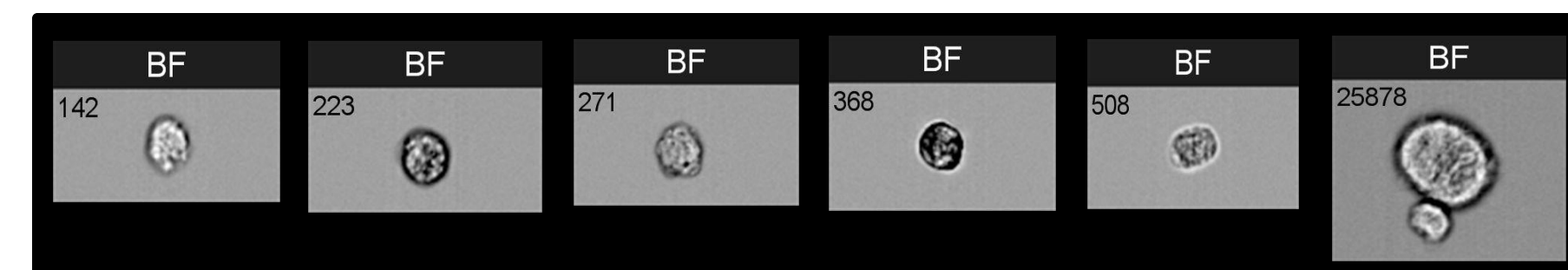
ImageStream® works by passing the cells one by one through laser which hit the cells and scattered in different directions based on the cell size and the excitation of the stains that were attached to it.



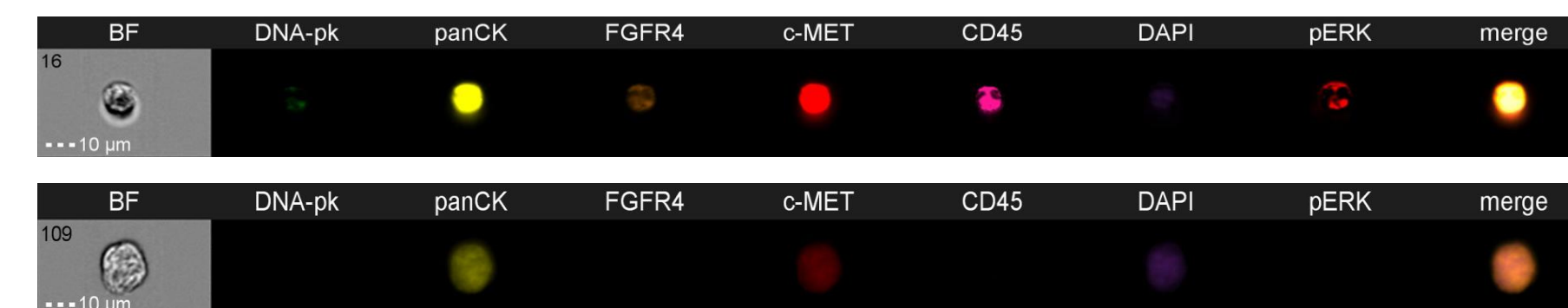
Data Analysis

How To Distinguish CTC?

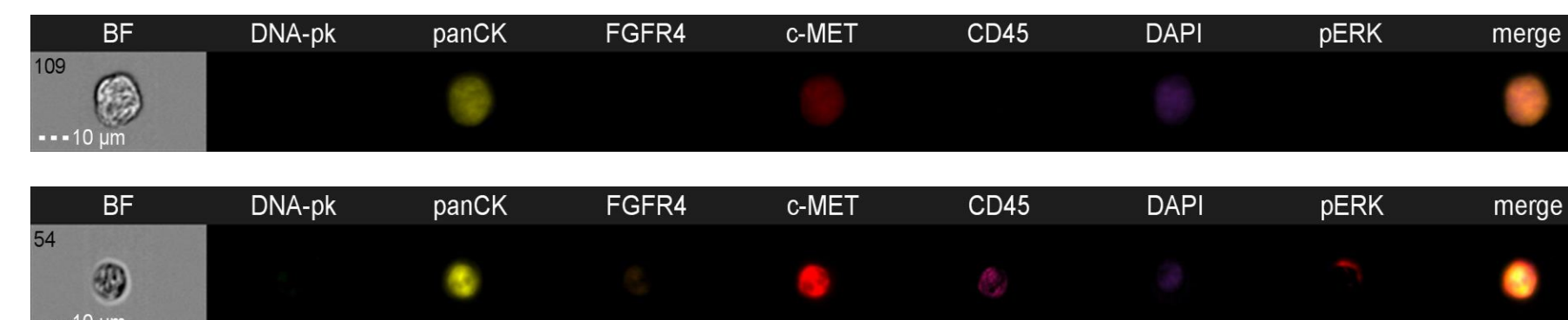
Firstly, CTCs are distinguished by size. Although previous steps had eliminated most of the other cells, there are still tens of thousands of white blood cells left. Through the IDEAS® software all the cells detected can be compared and analysed. CTCs have bigger size than white blood cells. Notice how cell number 25878 is gigantic in size as it is a CTC compared to the rest which are white blood cells.



Next, to rule out white blood cells which can sometimes appear large, a stain called CD 45 is used which light up only in white blood cells. For instance 16 is a white blood cell hence its CD 45 stain light up while CD 45 is absent in a CTC, 109.

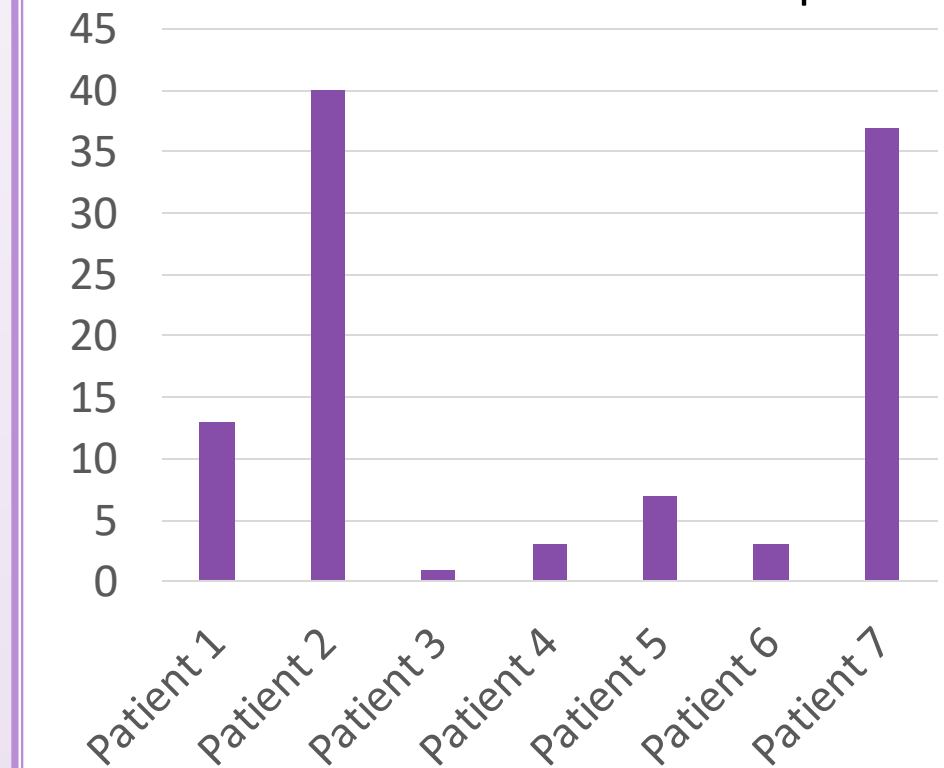


Another property of CTCs is intense DAPI signal which correspond to the cell DNA. Cancer cells proliferate at much higher rate than normal cells, thus having more DNA copies, a material replicated during cell division. Notice how 109 has a more intense DAPI signal in purple compare to 54.



Results and Discussions

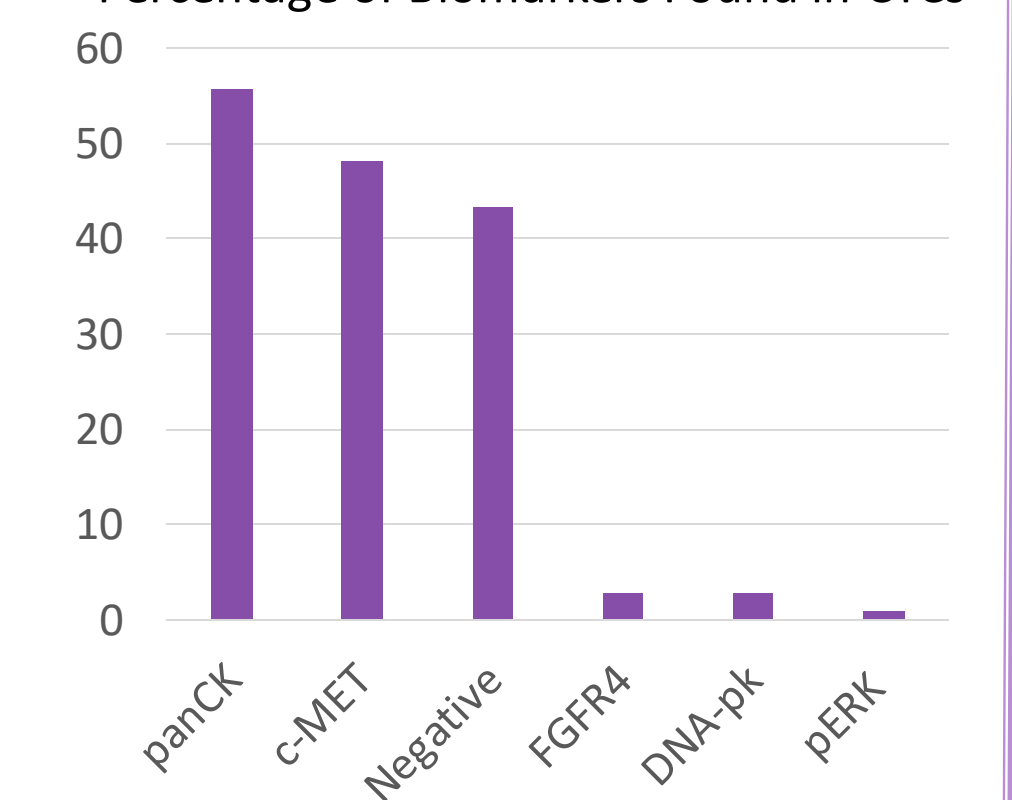
The numbers of CTCs in each patient



This first graph showed that each patient has different number of CTCs with highest number of CTCs found is 40 CTCs and lowest is only 1 CTC. Further studies can be done to investigate whether patients at different stages of liver cancer would have a certain range of CTCs in their blood circulation.

This second graph showed that pan-CK and c-MET are two of the highest percentage biomarkers, 55.77 % and 48.08 % respectively. Interestingly, the third highest proportion of biomarkers are negative which means these CTCs do not have any biomarkers detected on them.

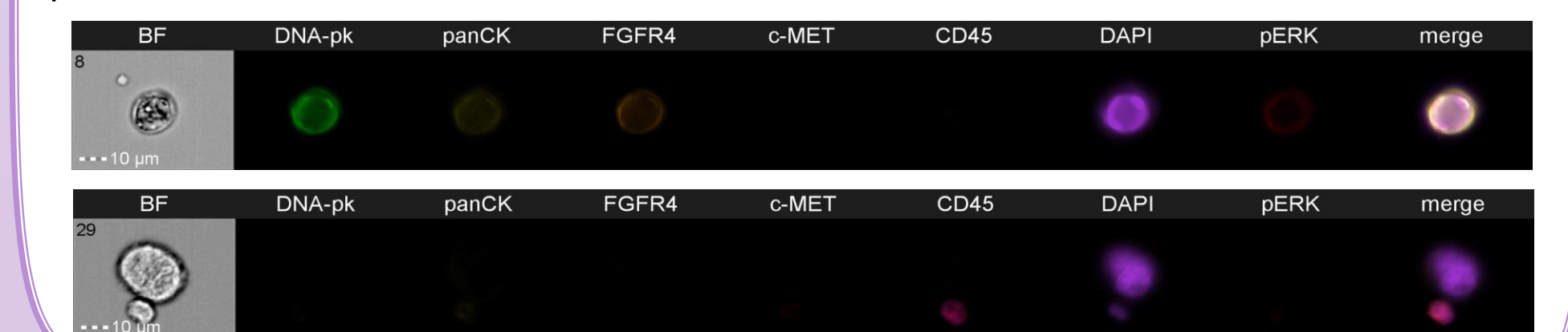
Percentage of Biomarkers Found in CTCs



What can future studies look into?

This project had shown that certain biomarkers do have higher prevalence than others such as pan-CK and c-MET. Nevertheless, each patient showed different patterns of biomarkers. For instance, CTC labelled 8 below has many types of biomarkers attached to it while another patient CTC labelled 29 had no biomarkers detected. This leads to a question whether these patterns of biomarkers on CTCs could translate into meaningful stratifying strategy for treatment or drug development?

Future study of longer duration and more samples can be conducted to investigate these patterns and how they relate to patients' response to drugs. Besides whether certain patients with or without specific type of biomarkers would respond better to certain treatment? This could lead to a more personalised treatment for liver cancer patients.



Conclusions

Pan-CK and c-Met were found to be the highest prevalence of biomarkers in these samples. These two biomarkers can be focussed on for future studies on early detection of liver cancer and as drug target for liver cancer. However, further research is needed to study the complex pattern of biomarkers on liver cancer CTCs.

References:

- 1) Cancer Research UK. *Liver Cancer statistics*. <http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/liver-cancer#heading-One>
- 2) World Health Organization. (2017). *Cancer*. <http://www.who.int/mediacentre/factsheets/fs297/en/>