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RACP Foundation Research Awards

YEAR 1 PROGRESS REPORT

Project Title		Circulating Tumour DNA as a Personalised Biomarker in ER Positive Metastatic Breast Cancer
Name		Dr Louisa Lisa Lo
Award Received		2017 RACP NHMRC Kincaid Smith Excellence Award
Report Date		18 December 2018
Chief Investigator / Supervisor		Professor Sarah-Jane Dawson
Administering Institution		The University of Melbourne
Funding Period	Start Date:	1 January 2018
	Finish Date:	1 January 2022

PROJECT SUMMARY

When breast cancer spreads to other parts of the body (a process called metastasis), cancer cells break away from the main tumour and move through the blood stream - the DNA from these tumour cells then appears in the blood and is called circulating tumour DNA, or ctDNA. Researchers believe that analysing ctDNA could lead to the ability to predict, detect and monitor the return and spread of breast cancer.

As part of my PhD, I will use advanced technology to read the genetic code of genes (gene sequencing) originally found within tumour cells and identify those that have undergone changes, mutated in ctDNA. The advantage of analysing the ctDNA contained in a blood sample is that it contains information about all the genetic mutations that may occur at different metastatic sites in the body, information that can be missed by traditional tissue biopsies. This will allow mapping of the gene mutations that occur in late breast cancer. This type of information may help classify patients so they can receive more personalised treatment in the future.

ctDNA may also be used to monitor how patients are responding to treatment. Part of my PhD focuses on following this in groups of patients receiving different types of targeted treatments to compare the results. This study could also provide insights into how resistance to treatment for metastatic breast cancer develops at the genetic level.

ctDNA may also be used as a tool to detect residual cancer disease that is beyond the limit of detection by standard imaging after surgery and neo-/adjuvant chemotherapy in early breast cancer. The aim is to use the presence or absence of ctDNA after curative surgery and chemotherapy to predict with good accuracy which patients would have a high risk of cancer return. This may allow tailoring of more specific treatment to these patients to improve chances of cure.

Research in this area is very promising if successful would be a major break-through in understanding what breast cancer is doing at any given time - information that will be welcome for

patients who live in fear of their cancer returning and could provide doctors with another tool to help them save lives.

PROJECT AIMS / OBJECTIVES

Aim 1: Develop a comprehensive ctDNA analysis strategy for women with ER+ MSC (In progress) &

Aim 2: Prospectively investigate the clinical value of ctDNA in ER+ MSC through the implementation of a clinical trial (In Progress)

These aims will be investigated as part of a prospective study (TRAIL). I have designed and completed the study protocol for 'Tracking Residual Disease Using Circulating Tumour DNA In High- Risk Early Breast Cancer TrackinQ Residual Disease' (TRAIL) in Aug ~ 2018. It was ethically approved by PeterMac HREC in Sep 2018 with governance approval obtained in Oct 2018. 5 patients are currently registered on study. The study is designed to recruit high risk stage 3 ER+ and triple negative breast cancer (TNBC) who will be receiving neo-/adjuvant chemotherapy and surgery. Patients are consented for tumour tissue screening for trackable mutations at baseline and blood sample testing for ctDNA analysis. Eligible patients will have 3-monthly serial blood sampling to check for ctDNA as a marker of cancer minimal residual disease (MRD). If patients are detected to have ctDNA in the blood plasma for the first time, they will have CT staging and whole body bone scans to investigate for cancer relapse. This is otherwise not performed as part of standard of care. If cancer relapse is not observed on imaging., patients will continue to have blood sampling at 3-monthly interval for up to 2 years or until they are clinically indicated for further investigations as part of standard of care. As part of this aim, I will also look at using methylation profiling coupled with genomic information to see if this will improve the sensitivity of MRD detection. This study will open at two more sites in early 2019: Austin and Royal Melbourne Hospital with a target recruitment of 50 patients over 12 months in order for me to complete this PhD aim.

Aim 3: Utilise ctDNA analysis as tool for molecular disease monitoring in ER+ MBC

This aim has been investigated as part of 2 separate studies that used different treatments in ER+ MBC, specifically the

1) mBEP study- which is a phase IB study of the novel combination of Tamoxifen and Venetoclax in ER+ and BCL2-positive metastatic breast cancer and;

2) PIKNIC study- which is a phase 2 study on the single agent treatment Alpelisib (isoform specific PIK3CA inhibitor) in patients detected to have alteration in the Pl3K pathway from tumour or ctDNA

SIGNIFICANCE AND OUTCOMES

1) mBEP study

Using the mBEP study patient population, my ctDNA study has demonstrated that 42% and 30% of patients treated with the novel treatment combination Tamoxifen and Venetoclax have PIK3CA and ESR1 mutations detected in their plasma pre-treatment, respectively. I can confirm that this treatment combination can provide clinical benefit in patients regardless of whether they have any pre-treatment ESR1 or PIK3CA mutation in ctDNA. There is a significant fall in circulating levels of both ESR1 and PIK3CA mutations as early as 4 weeks after this treatment combination. A large decline in ctDNA level is significantly associated with tumour shrinkage or partial responses in patients with circulating ESR1 mutations. This work is now published as part of a manuscript in Cancer Discovery Journal in December 2018.

Future research:

1) mBEP study

Further work will be conducted as part of the mBEP study to look at the treatment resistance mechanism at end of treatment (EOT). The EOT plasma samples will be tested with a targeted sequencing panel to look for changes in the BCL2 gene family and SWI/SNF complex. SWI/SNF complex mutations were previously seen to be related to resistance developed in mantle cell lymphoma patients treated with Ibrutinib and Venetoclax. If a consistent pattern is observed, functional analyses will be undertaken to validate the role of these mutations.

2) PIKNIC study

Preliminary results from the PIKNIC study from 19 patients suggests that ctDNA PIK3CA mutants tend to obtain clinical benefit if there is a significant fall in ctDNA levels at 8 weeks after commencinQ Alpelisib. I will be completing the laboratory work and analyses in all recruited PIKNIC patients.

3) TRAIL study

Patients consented to the TRAIL study will have their blood taken at 3-monthly intervals as part of the main study and checked for the presence of ctDNA using digital droplet polymerase chain reaction (ddPCR). I will also be using 5-hydroxymethylcytosine (5hMC) sequencing given its tissue specificity to obtain epigenomic information from ctDNA and combine it with genomic information to try and improve on the sensitivity of MRD detection.

4) ELIMINATE study

The ELIMINATE study is phase 2 study that randomises ER+ patients with early breast cancer to either standard neoadjuvant chemotherapy or to neoadjuvant concurrent chemohormonal therapy to investigate its impact on downstaging of ER+ breast cancers at surgery. Using the same lab procedures as in the TRAIL study, I aim to look at the relationship between ctDNA presence and the result of downstaging of chemotherapy in ER+ breast cancer in the neoadjuvant setting.

PUBLICATIONS / PRESENTATIONS

Lindeman GJ, Lok SW, Whittle JR, Vaillant F, Lo LL, et al. A phase 1 b dose-escalation and expansion study of the BCL-2 inhibitor venetoclax combined with tamoxifen in ER and BCL-2-positive metastatic breast cancer. Cancer Discov. 2018 Dec 5. pii: CD-18-1151.