Final Study Report

Study Title: The effect of Metformin on biomarker activity in primary breast cancer.

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The aim of this phase IV clinical trial was to see whether the effects of metformin on cancer cells seen in vitro, can be seen in women with breast cancer. This phase IV clinical trial was run by a multidisciplinary team including representation from the Tayside Breast Cancer and Diabetes Teams, was based on collaborative work between Life Sciences and the Medical School and sponsored by the University of Dundee Research and Innovation Services. The trial was conducted locally under the supervision of physicians and surgeons recognized as experts in this field of medicine.

It took 10 months (October 2006 – July 2007) from putting the trial paperwork together to get the trial up and running. To start with, I did literature search and collected all the evidence in support of the trial, then I put them together and wrote a review article, which was then published in *Critical Reviews in Oncology and Haematology* (July 2008. 67 (2008) 1-7), titled: Targeting AMPK: a new therapeutic opportunity in primary breast cancer. This literature review also helped me in writing all the other trial paperwork, including applications to the MHRA, local ethics committee and funding agencies. Accomplishing this major work in this relatively short period of time was down to the help I received from my supervisors and colleagues; as well as my special commitment and persistence to get the project up and running in time. Breast Cancer Research Scotland was our primary sponsor, however I also managed to get further grants from TENOVUS Tayside (£10,000) and Anonymous Trust Grant (£13,000).

The major hurdles in the process of recruitment were first getting other doctors and health care providers, who were not co-investigators but were in the breast cancer team, to know about the trial and remember when they needed to cooperate. To start with, I had to be present at every breast outpatient clinic to identify the potentially eligible candidates and ask them, or ask the surgeon or the radiologist who was doing the diagnostic cores to obtain consent and take extra core biopsies for research. Despite that, it turned out that the major reason for no recruitment was not taking

extra core biopsies at presentation [49/154 (31.8%)]. Breast care nurses also needed to be reminded continuously about the whole process of recruitment. I was also offering to take the research cores to the Tayside Tissue Bank in order to avoid delays in processing the samples. I had to do this for about a year until I was fairly confident that things were going smoothly and my presence in the outpatient clinics was not necessary. I had to be present in all Breast MDT meetings to make sure all eligible candidates were identified. If I was not able to be present then I delegated the job to my supervisor, Alastair Thompson, or one of our research nurses.

The second major hurdle was getting patients into the first arm of the trial. Eligible candidates were approached and offered the trial on their follow-up appointment after the MDT, which was the same appointment when they were given the bad news about cancer diagnosis. It was extremely difficult to ask a newly diagnosed breast cancer patient who just knew about her illness to offer an extra core biopsy, when otherwise it is not necessary.

The third hurdle was when eligible patients were offered an operation date, which was earlier than the two weeks "window of opportunity" from when they could enrol [21/154 (13.6%)]. It was ethically inappropriate to delay surgery for the purpose of our research.

As it proved difficult to recruit many patients to the first arm of the trial, samples from the first arm patients were collectively analysed then that arm closed (n=8). The second arm of the trial, which comprised randomly assigning participants to either take metformin or not in the same neoadjuvant setting, without the need to have an intermediate core biopsy, had more people participating. For patients in this arm, the second core biopsy was taken during their definitive surgery, on the operating table at least 2 weeks from recruitment. Because of potential variation between patients it was required to recruit a larger number of patients to observe significant effects.

Moreover, 18 months on, it was decided to change the target number recruited to the 40 to 55 in total, in order to compensate for participants who withdrew from the trial (2 out of 34 by then), and paticipants who were going to be excluded because of insufficient tissue in their core biopsy samples.

Some 8/55 (14.5%) patients who were in the second arm withdrew. 7/55 (12.7%) who were in the metformin group withdrew because of intolerable nausea, diarrhoea or a "bloated feeling". None of them required hospitalization and all symptoms resolved on stopping the drug. These unwanted effects were expected especially with increasing the dose. Supported by published work, unpublished work by Alessi's and Hardi's group and our previous experience with metformin, high dose of metformin was recommended; however, to reduce gastrointestinal symptoms to minimum, a "gentle" low-dose start was advised by British National Formulary and the team. As the two weeks "window of opportunity" was too short to increase the dose gradually, the team decided to increase the dose straightaway from 500mg to 2000mg o.d. after a week of taking the drug. Having seen the number of patients withdrew because of gastrointestinal symptoms, which were worse after increasing the dose, we would have designed the trial differently as such that the dose escalaltion would have been more gradual.

The ALMAC Diagnostics Breast Cancer DSA™ research tool was chosen to analyse the samples because it offers the most comprehensive gene expression analysis platform for the study of breast cancer. Howerver, as they had no FFPE cutting facility, we had to do this ourselves. Then using immunohistochemistry, confirmation will be sought for protein expression on FFPE sections corresponding to this transcriptome data.

The results of gene analysis showed that four genes were significantly down regulated by metformin: PDE3B, a critical regulator of cAMP which affects the activation/

phosphorylation of AMPK; SSR3, TP53 and CCDC14. By Ingenuity Pathway Analysis with t-test, the TNFR1 signalling pathway was most significantly affected by metformin: TGFB, MEKK were commonly upregulated and cdc42 downregulated. By Gene Set Analysis the p53, BRCA1 and cell cycle pathways were underexpressed following metformin.

It remains unclear why some of the *in vitro* effects of metformin shown in this thesis could not be demonstrated *in vivo*, such as activation of AMPK and ACC. A key question concerns whether the effects of metformin treatment are direct effects on the tumour itself or are due to effects elsewhere (e.g. on the liver), which may indirectly alter the hormonal or nutritional environment of the tumour. Suppression of cell proliferation and G1 cell cycle arrest demonstrated on cell line studies in this thesis would not show up in gene expression profiling method used to analyse our clinical trial samples; however, we will follow the trial participants for at least 5 years. Any significant differenes in the tumour behavior or survival between the treatment and control group of the second arm should show up, but with such small numbers are not likely to be conclusive.

In conclusion, this window of opportunity trial presents the first evidence of direct mechanisms of action of metformin on primary breast cancer and provides a biological rationale for the use of metformin in the neoadjuvant and adjuvant treatment of breast cancer.

This clinical trial was part of my PhD thesis, which was submitted to the University of Dundee and been accepted. I was awarded PhD degree on the 31 March 2010. However, publications arising from this trial are underway.