Title of Project: Obesity and Cancer: Aromatase, the missing link

The majority of breast cancers in obese and older women depend on the lipid hormone oestrogen to grow. I have recently identified a molecular link that helps explain why obese women are at higher risk of developing breast cancer and it relies on the protein responsible for producing oestrogens. Current hormone therapy for breast cancer results in serious side-effects due to inhibition of oestrogen synthesis throughout the body. My work has identified potential breast-specific therapies that would obviate these problems.

My overall research strategy is based on the hypothesis that obesity is linked to an increased risk of oestrogen-dependent cancers as a direct result of the role of metabolic pathways in regulating oestrogen biosynthesis. Targeting these pathways may be of great benefit for the treatment and prevention of obesity-related postmenopausal cancers.

My vision for the next 4 yrs involves the expansion, diversification and translation of my current research program, which *is built around three research aims:*

Aim 1: To characterise the regulation of aromatase in obesity and breast cancer.
Aim 2: To identify and test novel therapeutics for obesity-related cancer.
Aim 3: To innovatively explore new regulators of aromatase in obesity and breast cancer.

The Ken Wynne Award was used to support a PhD stipend for work related to Aim 2 (Study 4) of my lab's research program.

Background
More than half of the Australian female population is now overweight or obese with an increased risk of developing a number of cancers later in life, including those of the breast and endometrium. The majority of these tumours are dependent on fat-derived oestrogens for growth. AMPK and its upstream kinase LKB1 are considered master regulators of energy homeostasis and have been shown to be downregulated in cases of obesity and breast cancer. My findings demonstrating that AMPK and AMPK-related metabolic pathways are key negative modulators of aromatase, responsible for the final and key step in oestrogen biosynthesis, serve as the basis for much of my current research program. This includes elucidating the role of novel AMPK-related metabolic pathways in regulating aromatase, identifying novel regulators of AMPK to aid in the development of potential therapeutics, and testing the AMPK-activating drug metformin in the context of obesity and hormone-dependent postmenopausal cancers.

Study 4: Ghrelin, a new breast-specific aromatase inhibitor
A number of peptide hormones, altered in obesity, are known to act via AMPK. One such factor is ghrelin, an orexigenic hormone produced by the gut that activates AMPK in the hypothalamus. My laboratory's studies in breast tissue confirmed that ghrelin activated AMPK, and also inhibited aromatase expression and activity. This has led to a new, exciting collaboration with Prof Furness (U. Melb) who has extensive expertise in ghrelin research. Our ESA and NBCF-funded research includes testing ghrelin receptor agonists for their effect on aromatase and oestrogen production as well as breast cancer cell growth *in vitro* and *in vivo*.

We found that ghrelin, des-acyl ghrelin and the des-acyl ghrelin mimetic, AZP531, were potent at inhibiting the growth of breast cancer cells in culture and in xenograft mouse models of breast cancer. Our interest in des-acyl ghrelin, over ghrelin, in the context of breast cancer therapy is multifaceted. Firstly, reported actions of ghrelin on breast cancer growth have been conflicting. Under certain circumstances, ghrelin has been shown to stimulate tumour growth, actions that are likely mediated via GHSR1a. Ghrelin has also been shown to stimulate IGF-1 secretion, a known stimulator of cancer cell growth. On the other hand, des-acyl ghrelin is more stable, does not bind to GHSR1a, has no effect on IGF-1 secretion and we have found that it is potent at inhibiting the growth of breast cancer cells *in vitro* and *in vivo*. Des-acyl ghrelin and AZP531 had

no effect on body weight and no adverse effects were observed during pathological assessment in these models. Current studies are aimed at understanding the mechanism of action of des-acyl ghrelin.

Aromatase expression in breast adipose stromal cells is also stimulated by macrophages that infiltrate obese adipose tissue. In an aim to examine whether des-acyl ghrelin may also impact the expression of aromatase via effects on the release of inflammatory mediators from adipose tissue macrophages, we undertook studies whereby adipose tissue macrophages or differentiated RAW cells (mouse macrophages) were pre-treated with des-acyl ghrelin. Conditioned media from these macrophages was then used to treat breast adipose stromal cells. Results demonstrate that pre-treatment of macrophages with des-acyl ghrelin inhibits the production of inflammatory mediators which in turn, prevents induction of aromatase expression. These results suggest that des-acyl ghrelin may be efficacious at breaking the obesity-breast cancer linkage.

Publications relating to this award:

In preparation

1) Au CC, Docanto MM, Zahid H, Ferrero R, **Brown KA** Des-acyl ghrelin inhibits the expression of aromatase via effects on macrophages.

2) Au CC, Britt K, Docanto MM, Callaghan B, Cain J, Furness JB, **Brown KA** Des-acyl ghrelin inhibits oestrogen production and the proliferation of breast cancer cells in vitro and in vivo.

Invited presentations relating to this award (ESA acknowledged):

International

1) Sex, Fat and Breast Cancer: the link between inflammation, dysregulated metabolism and estrogen production. (2016; Jan 6) *Meyer Cancer Center, Weill Cornell Medical College, NYC, USA*

2) Novel regulators of oestrogen production in obesity and breast cancer. (2015) *Congress on Steroid Research, Chicago, USA*

3) Breast-specific inhibition of aromatase in estrogen-dependent breast cancer. (2015) *Memorial Sloan Kettering Cancer Centre Medical Oncology Meeting, NYC, USA*

National

1) Targeting metabolic pathways to better treat oestrogen-dependent breast cancers (2015) *Australian & New Zealand Obesity Society (ANZOS) Annual Meeting, Melbourne VIC Australia*

2) Linking Inflammation, Dysregulated Metabolism & Oestrogen Biosynthesis in Obesity and Breast Cancer (2015) *ComBio2015, Melbourne VIC Australia*

3) Dysregulated metabolism in obesity and breast cancer: Clues to novel therapeutic strategies? (2015) *Endocrine Society of Australia (ESA) Annual Scientific Meeting, Adelaide, Australia*

4) Obesity and breast cancer: dysregulated metabolism as a driver of estrogen biosynthesis (2015*) Royal Melbourne Hospital Academic Centre Seminar Series, RMH, Melbourne VIC Australia*

5) Sex, Fat and Breast Cancer: the link between dysregulated metabolism and estrogen production. (2015) *Olivia Newton John Cancer Research Institute Seminar Series, Heidelberg VIC Australia*

6) Obesity and Breast Cancer: understanding the linkage to identify novel therapeutic strategies (2015) *Hudson Institute Seminar Series, Hudson Institute of Medical Research, Clayton VIC Australia*

7) Obesity and breast cancer: understanding the link to better treat the disease. (2015) *SOBS/MCCC seminar, Monash University, Clayton VIC Australia*

8) Dysregulated metabolism as a driver of oestrogen production in obesity and breast cancer. (2015) *1st Australian Cancer and Metabolism Meeting, Sydney, Australia*

9) Next generation of aromatase inhibitors for the treatment of oestrogen-dependent breast cancer. (2015) *Monash Health Research Week, Melbourne, Australia*

Platform presentations relating to this award:

1. **CheukMan Cherie Au**, Kara Britt, John B Furness, Sari Makela, Kristy A Brown '*Des-acyl ghrelin inhibits oestrogen production and breast cancer cells proliferation in vitro and in vivo*' BiomedLink 2015, oral presentation, 20thNovember 2015.
2. **CheukMan Cherie Au**, Kara Britt, John B Furness, Sari Makela, Kristy A Brown '*Des-acyl ghrelin inhibits oestrogen production and proliferation of breast cancer cells in vitro and in vivo*' Weizmann Australia - Making Connections Symposium 2015, oral presentation, 19th-20th October 2015.
3. **CheukMan C. Au**, Kara Britt, Maria M. Docanto, Zdenka Prodanovic, Beena Kumar, Brid Callaghan, Jason Cain, John B. Furness, Kristy A. Brown '*Des-acyl ghrelin inhibits estrogen production and the proliferation of breast cancer cells in vitro and in vivo'* The Annual Scientific Meeting of the Endocrine Society of Australia and the Society for Reproductive Biology, Novartis Junior Scientist Presentation, 24 Aug 2015.

Poster presentations relating to this award:

1. CheukMan Cherie Au, Kara Britt, John B Furness, Sari Makela, **Kristy A Brown** '*Des-acyl ghrelin inhibits oestrogen production and proliferation of breast cancer cells in 3D cultures, ex vivo and in vivo*' Obesity week in Los Angeles California, 2nd -7th November 2015.
2. **CheukMan C. Au**, Maria M. Docanto, Brid Callaghan, John B. Furness, Kristy A. Brown '*Targeting Oestrogen-Dependent Breast Cancer: Ghrelin Receptor Agonists As Novel Therapeutics*' Monash Health Research week (Melbourne, March 17th 2015).
3. **CheukMan C. Au**, Maria M. Docanto, Brid Callaghan, John B. Furness, Kristy A. Brown '*Targeting Oestrogen-Dependent Breast Cancer: Ghrelin Receptor Agonists As Novel Therapeutics*' Lorne cancer conference (Melbourne, February 12-14th 2015).

Funding relating to award:

Brown KA (2016-2019)

National Breast Cancer Foundation, Career Development Fellowship, $680,000

Brown KA (2015)

Komen Foundation; Junior Faculty Travel Award for Obesity & Breast Cancer Research, US$3,000

Brown KA (2015)

Endocrine Society of Australia Ken Wynne Memorial Award, $25,000

Au CC (2016) ESA Research Higher Degree Scholarship

Au CC (2016) ENDO Early Career Forum Travel Award

Au CC (2015) ESA Travel Grant

Expenditure summary:

	Amount
PhD Stipend (Miss Cherie Au)	$25,000