

**EFFICACY OF PRO-APOPTOTIC RECEPTOR
AGONISTS IN THE TREATMENT OF PRIMARY
BREAST CANCER AND BONE METASTASIS**

IRENE ZINONOS B.Hlth.Sc. (Hons)

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DECLARATION

NAME: Irene Zinonos

PROGRAM: PhD

This work contains no material which has been accepted for the award of any other degree* or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously written by another person, except where due reference has been made in the text.

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*Due to the publication format of this thesis, Chapter 2 contains results also presented in my Honours Thesis.

SIGNATURE:.....**DATE:**.....

ACKNOWLEDGMENTS

“**Ithaca**”, a very famous poem by Konstantinos Kavafis (one of the greatest Greek poets) describes Odysseus’ long journey to return home after the Trojan War. Kavafis explains how important his journey was, even though it was beset by perils and misfortunes. Odysseus learnt valuable lessons and gained experience, he never gave up and he eventually arrived home to his beautiful wife Penelope and son Telemachus.

.....Η Ιθάκη σ' έδωσε το ωραίο ταξίδι

Χωρίς αυτήν δεν θά 'βγαινες στον δρόμο

Άλλο δεν έχει να σε δώσει πια

Κι αν πτωχική την βρεις, η Ιθάκη δεν σε γέλασε

Έτσι σοφός που έγινες, με τόση πείρα,

ήδη θα το κατάλαβες οι Ιθάκες τι σημαίνουν

Ithaca has given you the beautiful voyage

Without her you would never have taken the road

But she has nothing more to give you

And if you find her poor, Ithaca has not defrauded you

*With the wisdom you have gained, with so much experience,
you must surely have understood by then what Ithacas mean.*

The poem suggests that, had it not been for the desire of Odysseus to return to Ithaca, all the experiences along the way would have been missed. So, the journey AND the destination share the same importance in life, which brings me to my Ithaca, my PhD! I have finally arrived to my destination! The desire to complete my PhD, made the journey so much more enjoyable and the journey, with all the great things it taught me along the way, made the destination so much more important. However, experiencing this journey and eventually arriving to my destination would not have been possible if it wasn't for the support, guidance and love of many great people.

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ABSTRACT

Breast cancer is the most common malignancy among women which frequently metastasises to the bone. Despite the significant improvements in detecting and treating primary breast cancer, metastatic breast cancer remains a challenging condition to treat. The studies presented in this thesis were aimed to exploit the therapeutic potential of Pro-Apoptotic Receptor Agonists (PARAs) including the recombinant TNF-related apoptosis-inducing ligand, Apo2L/TRAIL, and its agonistic monoclonal antibody, drozitumab, for the treatment of primary breast cancer and bone metastases *in vitro* and *in vivo*.

Drozitumab is a fully human agonistic monoclonal antibody which binds to Apo2L/TRAIL death receptor DR5 and triggers apoptosis. The anticancer efficacy of drozitumab was evaluated using murine models of breast cancer xenografted at the orthotopic site and in bone. *In vitro*, drozitumab induced apoptosis in various human breast cancer cell lines, without being toxic to normal cells. *In vivo*, drozitumab exerted remarkable tumour suppressive activity as a single agent and co-operated with chemotherapeutic drugs, for increased efficacy against mammary tumours. In addition, drozitumab treatment completely inhibited tumour growth in bone, even in animal having well-advanced tibial tumours, leading to complete resolution of osteolytic lesions.

Osteoprotegerin (OPG) is a soluble member of the TNF receptor superfamily, which binds the receptor activator of NF- κ B (RANKL) and inhibits bone resorption. OPG can also bind and inhibit the activity of Apo2L/TRAIL, raising the possibility that the anticancer efficacy of Apo2L/TRAIL may be abrogated in the bone microenvironment, where OPG expression is high. *In vitro*, breast cancer cells engineered to overexpress OPG were protected from Apo2L/TRAIL-induced apoptosis. However, when mice were injected intratibially with cells overexpressing OPG, Apo2L/TRAIL treatment resulted in strong growth inhibition of

OPG overexpressing intratibial tumours indicating that OPG levels in bone, even in the face of supra-physiological concentrations, are unlikely to play a significant role in modulating Apo2L/TRAIL therapeutic potential.

Previous preclinical studies have shown that systemic administration of recombinant OPG inhibited tumour growth in bone and prevented cancer-induced osteolysis. However, the data presented in this thesis have demonstrated that while overexpression of OPG by breast cancer cells protected the bone from cancer-induced osteolysis, it was without effect on overall tumour burden. Despite the OPG-mediated bone protection, OPG overexpression led to a significant increase in the incidence of pulmonary metastasis. These results suggest that OPG-mediated inhibition of bone resorption modulates the bone microenvironment and it may affect the likelihood of cancer cells spreading elsewhere in the body. This also suggests that other anti-resorptive therapeutic agents including bisphosphonates (BPs), which have been the standard care for patients with skeletal malignancies, have the potential to harm by promoting cancer metastasis to other non-skeletal sites.

In conclusion, the data presented in this thesis demonstrate that drozitumab and Apo2L/TRAIL represent potent immunotherapeutic agents with strong activity as single agents and in combination with conventional chemotherapy against the development and progression of breast cancer. In addition, these studies provide important preclinical evidence that modulating the bone microenvironment by inhibiting osteoclastic bone resorption may not always be beneficial, a phenomenon which needs further investigation.

CONFERENCE PRESENTATIONS

- **Irene Zinonos**, Agatha Labrinidis, Michelle Lee, Vasilios Liapis, Shelley Hay, Vasilios Panagopoulos, Mark DeNichilo, Vladimir Ponomarev, Peter Diamond, Andrew CW Zannettino, David M Findlay, and Andreas Evdokiou.
Doxorubicin overcomes resistance to drozitumab based-immunotherapy in a mouse model of breast cancer. Research Day 12 October 2012, Basil Hetzel Institute, The Queen Elizabeth Hospital. Oral Presentation.
- **Irene Zinonos**, Agatha Labrinidis, Michelle Lee, Vasilios Liapis, Shelley Hay, Vladimir Ponomarev, Peter Diamond, Andrew CW Zannettino, David M Findlay, and Andreas Evdokiou.
Doxorubicin overcomes resistance to drozitumab based-immunotherapy in a mouse model of breast cancer. Faculty of Health Sciences 2012 Postgraduate Research Expo, University of Adelaide, Adelaide-SA. Poster presentation.
- **Irene Zinonos**, Agatha Labrinidis, Michelle Lee, Vasilios Liapis, Shelley Hay, Vladimir Ponomarev, Peter Diamond, Andrew CW Zannettino, David M Findlay, and Andreas Evdokiou.
Local production of Osteoprotegerin by breast cancer cells inhibits cancer-induced osteolysis but promotes pulmonary metastasis. Research Day 14 October 2011, Basil Hetzel Institute, The Queen Elizabeth Hospital. Oral Presentation.
- **Irene Zinonos**, Agatha Labrinidis, Michelle Lee, Vasilios Liapis, Shelley Hay, Vladimir Ponomarev, Peter Diamond, Andrew CW Zannettino, David M Findlay, and Andreas Evdokiou.

Local production of Osteoprotegerin by breast cancer cells inhibits cancer-induced osteolysis but promotes pulmonary metastasis. Faculty of Health Sciences 2010 Postgraduate Research Expo, University of Adelaide, Adelaide-SA. Poster presentation.

- **Irene Zinonos**, Agatha Labrinidis, Michelle Lee, Vasilios Liapis, Shelley Hay, Vladimir Ponomarev, Peter Diamond, Andrew CW Zannettino, David M Findlay, and Andreas Evdokiou.

Local production of Osteoprotegerin by breast cancer cells inhibits cancer-induced osteolysis and intra-osseous tumour burden but fails to restrain extra-medullary tumour growth. ECTS/IBMS 3rd Joint Meeting of the European Calcified Tissue Society and the International Bone and Mineral Society, 7-11 May 2001, Athens, Greece. Poster presentation.

- **Irene Zinonos**, Agatha Labrinidis, Michelle Lee, Vasilios Liapis, Shelley Hay, Vladimir Ponomarev, Peter Diamond, Andrew CW Zannettino, David M Findlay, and Andreas Evdokiou.

Anticancer efficacy of Apo2L/TRAIL is retained in the presence of high and biologically active concentrations of osteoprotegerin *in vivo*. Faculty of Health Sciences 2010 Postgraduate Research Expo, University of Adelaide, Adelaide-SA. Poster presentation.

- **Irene Zinonos**, Agatha Labrinidis, Michelle Lee, Vasilios Liapis, Shelley Hay, Vladimir Ponomarev, Peter Diamond, Andrew CW Zannettino, David M Findlay, and Andreas Evdokiou.

Apomab, a fully human agonistic DR5 monoclonal antibody, inhibits tumour growth and osteolysis in murine models of breast cancer development and progression. 20th

Annual Scientific Meeting of the ANZBMS 5-8 September 2010, Adelaide-SA. Oral Presentation.

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Anticancer efficacy of Apo2L/TRAIL is retained in the presence of high and biologically active concentrations of osteoprotegerin *in vivo*. 20th Annual Scientific Meeting of the ANZBMS 5-8 September 2010, Adelaide-SA. Poster Presentation.

- **Irene Zinonos**, Agatha Labrinidis, Michelle Lee, Vasilios Liapis, Shelley Hay, Vladimir Ponomarev, Peter Diamond, Andrew CW Zannettino David M Findlay, and Andreas Evdokiou.

Our Battle with Bone Cancer.3 minutes Thesis Competition. Faculty of Health Sciences, University of Adelaide. Oral Presentation.

- **Irene Zinonos**, Agatha Labrinidis, Michelle Lee, Vasilios Liapis, Shelley Hay, Vladimir Ponomarev, Peter Diamond, Andrew CW Zannettino David M Findlay, and Andreas Evdokiou.

Anticancer efficacy of Apo2L/TRAIL is retained in the presence of high and biologically active concentrations of osteoprotegerin *in vivo*. ASMR Medical Research Week, SA Scientific Meeting 2010, Adelaide-SA. Oral Presentation.

- **Irene Zinonos**, Agatha Labrinidis, Michelle Lee, Vasilios Liapis, Shelley Hay, Vladimir Ponomarev, Peter Diamond, Andrew CW Zannettino David M Findlay, and Andreas Evdokiou.

Apomab, a fully human agonistic DR5 monoclonal antibody, inhibits tumour growth and osteolysis in murine models of breast cancer development and progression. 6th Clare Valley Bone Meeting, 2010, Adelaide-SA. Oral Presentation.

- **Irene Zinonos**, Agatha Labrinidis, Michelle Lee, Vasilios Liapis, Shelley Hay, Vladimir Ponomarev, Peter Diamond, Andrew CW Zannettino David M Findlay, and Andreas Evdokiou.

Apomab, a fully human agonistic DR5 monoclonal antibody, inhibits tumour growth and osteolysis in murine models of breast cancer development and progression. 12th International TNF Conference, 26-29 April 2009, Madrid, Spain. Poster Presentation.

PRIZES AWARDED

- Best Oral Presentation in the category for Senior PhD Students (Laboratory), Research Day 12 October 2012, Basil Hetzel Institute, The Queen Elizabeth Hospital, Adelaide, SA.
- Best Poster Presentation for the School of Medicine. 2012 Postgraduate Research Expo, 31 August 2011, University of Adelaide, Adelaide-SA.
- Best Oral Presentation in the category for Senior PhD Students (Laboratory), Research Day 14 October 2011, Basil Hetzel Institute, The Queen Elizabeth Hospital, Adelaide, SA.
- Best Poster Presentation for the School of Medicine. 2011 Postgraduate Research Expo, 25 August 2011, University of Adelaide, Adelaide-SA.
- Best Poster Presentation for the Faculty of Health Sciences Faculty of Health Sciences. 2011 Postgraduate Research Expo, 25 August 2011, University of Adelaide, Adelaide-SA.
- Travelling Fellowship form the Faculty of Health Sciences Postgraduate, April 2011, University of Adelaide, Adelaide, SA.
- ECTS/IBMS Travel Award, 3rd Joint Meeting of the European Calcified Tissue Society and the International Bone and Mineral Society, 7-11 May 2001, Athens, Greece.
- Roger Melick Young Investigator Award, 20th Annual Scientific Meeting of the ANZBMS 5-8 September 2010, Adelaide-SA.

- Best Poster Presentation for the Faculty of Health Sciences 2010 Postgraduate Research Expo, University of Adelaide, 1 September 2010, Adelaide-SA.