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Daniel Yuan Qiang Wong

Rethinking Platinum Anticancer Drug Design: Towards Targeted and Immuno-chemotherapeutic Approaches

Doctoral Thesis accepted by
the National University of Singapore, Singapore

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Supervisor's Foreword

As Daniel Wong's graduate advisor during his Ph.D., I am pleased to write this foreword for this publication in Springer Theses. Daniel joined my laboratory as my first batch of final year project undergraduate students in 2010. He was working on the synthesis of targeted platinum(IV) anticancer complexes. He subsequently received a departmental scholarship and continued on to do his Ph.D. with me. In his 5 years with me, Daniel has been a self-motivated, independent and creative scientist. He has been highly curious and always discussing new potential project ideas with me. As a scientist, Daniel is willing to dive into the 'unknown' and take calculated risks in his research direction instead of sticking with the 'tried and proven'. I have watched him grown from a trainee student under my tutelage into a proficient scientist, himself mentoring another younger generation of scientists. In recognition of his research excellence, Daniel has won the prestigious Wang Gungwu Medal (2016) for the best Ph.D. thesis in the Natural Sciences in the National University of Singapore.

Broadly, this thesis is outstanding in its effort to propose new avenues for future platinum-based drug development. Platinum-based chemotherapeutics such as cisplatin has been a workhorse of chemotherapy regimens for decades. Nonetheless, clinical interest in new platinum-based chemical entities (NCEs) has been waning in recent years. In this era of cancer genomics and immunotherapy, traditional cytotoxic chemotherapeutics have seemingly been left behind. It is in this context that this thesis attempts to offer a rethink of platinum-based agents and to suggest new directions for the future research.

The first contribution of this thesis is a proof-of-concept, deploying a targeted drug while retaining a broad spectrum of action in the form of a targeted cisplatin-based derivative (Chap. 4). Daniel demonstrated that such a strategy could be therapeutically favourable because it could circumvent multi-factorial apoptosis resistance inherent in heterogeneous tumours, a problem which plagues conventional molecularly targeted drugs.

Daniel's second contribution is the conceptualisation of combined platinum-based immuno-chemotherapeutics which combines chemotherapy with immunotherapy. Back when this idea was first floated, it seemed like a longshot that platinum agents,

long held to be immunosuppressive, could modulate the immune microenvironment in a way that could be therapeutically favourable. There was some evidence supporting this hypothesis in literature but they were scarce and not widely recognized within the community. Daniel has made pioneering efforts in exploring this idea with some early promising results, and as two orthogonal approaches. The first approach is a 'macrophage-centric' approach which looks at activating macrophages directly with platinum agents (Chap. 5), while the second approach is a 'tumour-centric' approach of triggering immunogenic cell death of cancer cells (Chap. 6).

The discoveries of this work have been published in outstanding top-tiered journals, including two papers in Angewandte Chemie, one of which as a VIP paper, and another paper in Chemical Science. This Springer Theses award has been well-deserved.

Singapore, Singapore
February 2018

Prof. Ang Wee Han

Parts of this thesis have been published in the following articles:

1. D. Y. Q. Wong*, W. W. F. Ong*, W. H. Ang, Induction of immunogenic cell death by chemotherapeutic platinum complexes, *Angew. Chem. Int. Ed.* 2015
2. D. Y. Q. Wong*, J. H. Lim*, W. H. Ang, Induction of targeted necrosis with HER2-targeted platinum(IV) anticancer prodrugs, *Chem. Sci.* 2015, *6*, 3051–3056–Published by The Royal Society of Chemistry
3. D. Y. Q. Wong*, C. H. F. Yeo*, and W. H. Ang, Immuno-chemotherapeutic platinum(IV) prodrugs of cisplatin as multimodal anticancer agents, *Angew. Chem. Int. Ed.*, 2014, 26, 6752–6756
4. D. Y. Q. Wong, J. Y. Lau, W. H. Ang, Harnessing chemoselective imine ligation for tethering bioactive molecules to platinum(IV) prodrugs, *Dalton Trans.*, 2012, 41, 6104–6111

*** Equal contribution**

Acknowledgements

There are many who have contributed significantly towards the progress of my research in one way or another. Here, I will just like to acknowledge and thank them. First, I would like to express my unreserved appreciation towards my supervisor, Prof. Ang Wee Han. Without him, this research work would simply not have existed. Having been with the lab from its inception till now, I feel a sense of pride to be part of this competent, resource-rich and well-equipped lab. I'm grateful too, for Prof. Ang's confidence in me which has moulded me into a more self-assured researcher.

I'm also thankful for the presence of my fellow labmates. Thanks to Cheefei, for having shouldered the heavy burden of being the safety lead and also for being a great pal and the life of the lab. Thanks to Jianyu, the new safety lead, and Siewqi, the biological lead, for their sense of responsibility and their proactive efforts which have made the lab's working environment so much better for everyone. Thanks to Junxiang for constantly waiting for me to have lunch and whose work effort is commendable. Not forgetting—Mun Juinn, my lab 'BFF'. Thanks for being a great friend, whom I can confide in, and whose friendship I will always treasure. Finally, to the newcomers, Keefe, Sebastian and Marsha—I wish you all well.

It has been said that *the true credit belongs to the man in the arena, whose face is marred by dust and sweat and blood*. Thus, I cannot adequately thank and acknowledge the contributions of all my final year students enough. It is them, who have driven and achieved much of the work described here. Thanks to Lau Jiayi (Chap. 2, 2012), Charmian Yeo (Chaps. 3 and 5, 2013), Lim Jun Han (Chap. 4, 2014) and Wendy Ong (Chap. 6, 2014). Although not included in this thesis, Stephanie Loh and Valerie Chu (2015) made substantial progress on understanding macrophage activation and on immune checkpoint inhibitors respectively.

My family has been a constant unwavering pillar of support during these 4 years. Thanks to my dad for nudging me up every morning and for driving me to school. You are truly the most selfless and loving dad ever. Thanks to my mum for taking care of the household and for keeping me well-fed. No words are sufficient to express my gratitude. I thank my brother, Alex, and my sister, Jenny, for holding

the family together and supporting the family financially all these while throughout all the ups and downs. I also thank my sister-in-law, Meilin. It's amazing how she could juggle her research, become 'woman of the year' and still cook dinner for us! Thanks Sabbie, for your unconditional love and for everything, even though you are no longer with us. I will always miss you.

Finally, I thank my wife, Candice, for her unwavering belief and support. Without her, this Ph.D. journey would have been so much lonelier and the world would seem so much darker. Thanks for all the little words of encouragements to keep me going and for sticking with me through the tough times. I'm sorry you had to wait so long. I promise to cherish you for all the days of my life, together, forever.

feci quod potui, faciant meliora potentes
I have done what I could, let whoever can do more, do so.

Declaration

I hereby declare that this thesis is my original work and it has been written by me in its entirety, under the supervision of Dr. Ang Wee Han, Chemistry Department, National University of Singapore, between 8th Aug 2011 and 30th June 2015.

I have duly acknowledged all the sources of information which have been used in the thesis.

This thesis has also not been submitted for any degree in any university previously.

Contents

List of Figures

List of Tables

List of Schemes

Summary

Platinum-based chemotherapeutics such as cisplatin, carboplatin and oxaliplatin have been the mainstay of chemotherapy regimens for the treatment of many cancers for the last few decades. Nonetheless, clinical interest in new platinum agents appears to be waning with only a handful entering clinical trials recently. Till date, thousands of novel cytotoxic platinum complexes have been synthesized but only a few attain clinical relevance. This thesis highlights existing limitations of platinum drug design and proposes avenues for further exploration with focuses on molecularly targeted platinum chemotherapeutics with novel mechanisms of action and on combined immuno-chemotherapeutic platinum agents.

The work described in Chaps. 2 and 3 are the foundation underpinning the subsequent chapters. In Chap. 2, we developed a facile conjugation strategy via chemoselective imine ligation to tether relevant biomolecules to a platinum(IV) prodrug scaffold. This strategy was used repeatedly to synthesize the targeted platinum(IV)-peptide conjugates described in Chaps. 4 and 5. In Chap. 3, we sought to answer whether our class of asymmetrical platinum(IV) aryl scaffolds were really prodrugs of cisplatin. Validation of the platinum(IV) prodrug hypothesis is important because it is the underlying working assumption behind the targeted platinum(IV)-peptide strategies described in Chaps. 4 and 5.

The recognition that certain molecular pathways are critical to carcinogenesis and continued tumour progression and may therefore represent an Achilles' heel has triggered a revolution in cancer drug development. These molecularly targeted chemotherapeutics have made considerable progress in the clinical outcomes for many malignancies. In Chap. 4, we have developed highly potent and selective HER2-targeted platinum(IV)-AHNP prodrugs which demonstrate the feasibility of an idea of 'targeted necrosis' as a novel strategy to circumvent apoptosis resistance.

Finally, in Chaps. 5 and 6, we describe our conceptualization and realization of combined platinum-based immuno-chemotherapeutic agents which represents a significant paradigm shift from the conventional approach of directly targeting cancer. Historically, the role of the immune system towards chemotherapy outcomes has been neglected as anticancer drugs were believed to be immuno- and myelo-suppressive. Nonetheless, this has been challenged by contemporary evidence which

now suggests that many chemotherapeutics, including platinum-based agents, do stimulate the innate and/or adaptive immune system and that these ‘secret allies’ contribute tangibly towards clinical outcomes. While it has been generally accepted that platinum agents principally exert direct cytotoxic action against cancer cells via the formation of covalent platinum-DNA adducts, the recognition of platinum-induced antitumour immunomodulation is only beginning to gain traction. There have been compelling empirical evidences corroborating the immunomodulating capacity of platinum-based therapy with favourable chemotherapy outcomes. The work described here may ultimately pave the way towards superior immuno-chemotherapeutic agents and a better clinical outcome in patients.