Characterisation of the mechanisms of tumour-induced dysfunction of clonal T cell expansions in multiple myeloma

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by

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CERTIFICATE OF ORIGINAL AUTHORSHIP

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Hayley Suen

2017

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PREFACE

The work described in this thesis has formed part of the following publications and presentations.

Publications

Joshua D, **Suen H,** Brown R, Bryant C, Ho PJ, Hart D, Gibson J (2016) The T cell in myeloma. *Clinical lymphoma myeloma and leukemia,* submitted.

Suen H, Brown R, Yang S, Weatherburn C, Ho, PJ, Woodland N, Nassif, N, Barbaro P, Bryant C, Hart, D, Gibson J, Joshua D (2016) Multiple myeloma causes clonal T cell immunosenescence: Identification of potential novel targets for promoting tumour immunity and implications for checkpoint blockade. *Leukemia***,** epub ahead of print.

Suen H, Brown R, Yang S, Ho PJ, Joshua D, Gibson J (2015) The failure of immune checkpoint blockade in multiple myeloma with PD-1 inhibitors in a phase 1 study. *Leukemia* **29,** 1621-1622.

Brown RD, Yang S, Weatherburn C, Gibson J, Ho PJ, **Suen H**, Hart D, Joshua DE (2015) Phospho-flow detection of constitutive and cytokine-induced pSTAT3/5, pAKT and pERK expression highlights novel prognostic biomarkers for patients with multiple myeloma. *Leukemia* 2015. 29:483-490.

Suen H, Joshua DE, Brown RD, Yang S, Barbaro PM, Ho PJ, Gibson J (2014) Protective Cytotoxic Clonal T-Cells in Myeloma Have the Characteristics of Telomere-Independent Senescence Rather Than an Exhausted or Anergic Phenotype: Implications for Immunotherapy. *Blood,* **124,** 3367.

Bryant C, **Suen H**, Brown R, Yang S, Favaloro J, Aklilu E, Gibson J, Ho PJ, Iland H, Fromm P, Woodland N, Nassif N, Hart D, Joshua D. Long-term survival in multiple myeloma is associated with a distinct immunological profile, which includes proliferative cytotoxic T-cell clones and a favourable Treg/Th17 balance. Blood Cancer Journal 2013; 3: e148.

Conference papers and presentations

Suen H, Brown R, Joshua D, Yang S, Weatherburn C, Nassif N, Woodland N, Hsu J, Bryant C, Hart D, Ho P, Gibson J. Multiple myeloma causes T cell ageing (immunosenescence) in tumour induced T cell clones (2015). Proceedings of the Annual Scientfic Meeting of the HAA.

Suen H, Joshua, DE, Brown RD, Yang S, Barbaro PM, Ho, PJ, Gibson, J (2014) Protective Cytotoxic Clonal T-Cells in Myeloma Have the Characteristics of Telomere-Independent Senescence Rather Than an Exhausted or Anergic Phenotype: Implications for Immunotherapy. *Blood,* **124,** 3367-3367. Proceedings of the American Society of Hematology.

Suen H, Brown R, Yang S, Weatherburn C, Ho PJ, Gibson J, Joshua D (2014). Pleiotropic tumour effects induce hypo-responsive senescence in T cells of patients with multiple myeloma. Proceedings of the Annual Scientfic Meeting of the HAA.

Suen H, Brown R, Yang S, Favaloro J, Woodland N, Nassif N, Gibson J, Ho PJ, Joshua D. Intracellular phospho-flow cytometry for studying signaling pathways in clonal T cell expansions. (2013) Proceedings of the 36th Annual Meeting of Australasian Flow Cytometry Group.

Suen H, Brown R, Yang S, Favaloro J, Woodland N, Nassif N, Gibson J, Ho PJ, Joshua D. Phosphoflow: analysis of intracellular signaling proteins using flow. (2013) Proceedings of the Sydflow Meeting.

Awards and Prizes

- **Young Investigator Awarrd**

Awarded to the best oral presentation at the 33rd Combined Health Sciences New Horizons Conference

- **UTS Paper of the Month Competition- April Winner**

Awarded to the best paper submitted by a higher degree research student at UTS

- **Sydney Catalyst Best Oral Presentation**

Awarded to the best oral presentation at the 2016 Sydney Catalyst Postgraduate & Early Career Researcher Symposium in Sydney, Australia.

- **Albert Baikie Medal**

Awarded to the best oral presentation by a young investigator at the 2015 HAA Annual Scientific Meeting. Presented by the Haematology Society of Australia and New Zealand in Adelaide, Australia.

- **Poster Prize**

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- **Travel Grants**

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AIF	Apoptosis-inducing factor
AIHW	Australian Institute of Welfare and Health
ALL	Acute lymphoblastic leukaemia
Allo-SCT	Allogeneic stem cell transplant
Anti-	Antibody
APAF1	Apoptotic protease-activating factor
APC	Antigen presenting cell
Auto SCT	Autologous stem cell transplant
Bak	Bcl-2-antagonist/killer
Bax	Bcl-2 associated X
Bcl	B cell lymphoma
BCMA	B cell maturation antigen
BID	BH3-interacting death domain agonist
BM	Bone marrow
Bort	Bortezomib
B_2M	Beta 2 microglobulin
C	Constant
Ca	Calcium
CAR	Chimeric antigen receptor
CD	Cluster of differentiation
CDKI	cyclin-dependent kinase inhibitor
CDR	Complementarity determining region
CFSE	Carboxyfluorescein succinimidyl ester
CLL	Chronic lymphocytic leukaemia
CML	Chronic myeloid leukaemia
CMV	Cytomegalovirus
CO_2	Carbon dioxide
CRAB	Hypercalcaemia, renal failure, anaemia and bone lesions
CRP	C reactive protein
CTL	Cytotoxic T lymphocytes
CTLA-4	Cytotoxic T-lymphocyte-associated protein-4
CyBORD	Cyclophosphamide, bortezomib and

	dexamethasone
DAPI	4’,6-diamidino-2-phenylindole
DC/s	Dendritic cell/s
Dex	Dexamethasone
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine tetra-acetic acid
EMRA	Effector memory T cell expressing CD45RA
ERK	Extracellular signal-related kinase
FACS	Fluorescence activated cell sorting
FADD	Fas-associated death-domain protein
FoxP3	Forkhead box P3
FISH	Fluorescence in situ hybridisation
FLIP	FADD-like IL-1β-converting enzyme-inhibitory protein
FMO	Fluorescence minus one
FR	Framework regions
G-CSF	Granulocyte colony stimulating factor
g/L	Grams/litre
GM-CSF	Granulocyte macrophage colony stimulating factor
h	hour
HePTP	Haematopoietic protein tyrosine phosphatase
HIV	Human immunodeficiency virus
HLA	Human leucocyte antigen
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IMiDs	Immunomodulatory drugs
IMWG	International Myeloma Working Group
ISS	International Staging System
ITAMS	Immunoreceptor tyrosine-based activation motifs
JNK	c-Jun N terminal kinase
K	Kinase
L	Leader sequence

LAG-3	Lymphocyte activation gene-3
Lat	Linker for activation of T cells
Lck	Lymphocyte specific protein tyrosine kinase
LDH	Lactate dehydrogenase
Len	Lenalidomide
LGL	Large granular lymphocytic leukaemia
M protein	Monoclonal protein; paraprotein
MAPK	Mitogen activated protein kinases
Mcl-1	Myeloid-cell leukaemia sequence 1
MDS	Myelodysplasia
MDSC	Myeloid derived suppressor cells
MGUS	Monoclonal gammopathy of undetermined significance
MHC	Major histocompatibility complex
Min	Minutes
MM	Multiple myeloma
mRNA	Messenger ribonucleic acid
MSAG	Medical Scientific Advisory Group
mSMART	Mayo stratification of Myeloma and Risk Adapted Therapy
n	(sample) number
N/A	Not applicable
NF-κB	Nuclear factor kappa B
ng	Nanograms
NK cells	Natural killer cells
NT	Not available for testing
nTreg	Natural T regulatory cells
p16	p16INK4a
p21	p21CIP1/WAF1
PB	Peripheral blood
PBMC/s	Peripheral blood mononuclear cell/s
PBS	Phosphate buffered saline
PBSC	Peripheral blood stem cell
PCR	Polymerase chain reaction
PD-1	Programmed cell death protein-1 or CD274

PI	Propidium iodide
PI3K	Phosphoinositide 3-kinase
PMA	Phorbol 12-myristate 13-acetate
Pom	Pomalidomide
pRb	Retinoblastoma tumor suppressor
PTP	Protein tyrosine phosphatase
PTPN7	Protein tyrosine phosphatase non-receptor type 7
qPCR	Quantitative polymerase chain reaction
RPAH	Royal Prince Alfred Hospital
RT	Room temperature
SARA	SMAD anchor for receptor activation
SASP	Senescence associated secretory phenotype
SCT	Stem cell transplant
SHP-2	Src homology 2 (SH2) domain containing protein tyrosine phosphatase (PTP)
SM	Smouldering myeloma
SMAD	Homologs of the *Caenorhabditis elegans* protein SMA and the *Drosophilia* protein, mothers against decapentaplegic (MAD)
SPE	Serum protein electrophoresis
STAT3	Signal transducer and activator of transcription
T cells	T lymphocytes
TCR	T cell receptor
TdT	Terminal deoxynucleotidyl transferase
TGF-β	Transforming growth factor β
Th	T helper cell
T-LGL	T-large granulocytic l
TNF	Tumour necrosis factor
Treg	T regulatory cell
T/S	Telomeric DNA quantity/single copy gene DNA quantity
U	International Units
U&E	Urea and electrolytes
UV	Ultraviolet
V	Variable

Vβ	Beta chain of the variable region (of the TCR)
WHO	World Health Organisation
WM	Waldenström macroglobulinaemia
ZAP-70	ζ-chain associated protein kinase of 70kDa
α	Alpha
β	Beta
γ	Gamma
δ	Delta
K	Kappa
λ	Lambda
°C	Centrigrade (degrees Celsius)
µL	Microlitres

ABSTRACT

Multiple myeloma is a cancer involving malignant plasma cells in the bone marrow. Despite advances in therapy, relapse is inevitable due to residual disease and myeloma remains incurable. New therapies are required to remove residual disease and maintain long term survival. Expanded clones of cytotoxic T cells have been detected in myeloma and their presence is associated with improved survival, suggesting a role in anti-tumour immunity. However, these cells are dysfunctional as they do not proliferate. Thus, tumour-induced dysfunction of T cell clones may be a tumour evasion mechanism that contributes to immune escape. The primary aim of this thesis was to elucidate the mechanism/s responsible for the observed dysfunction of these T cell clones, which may allow future development and implementation of novel strategies to restore clonal T cell function.

T cell clones were detected in 75% of a new cohort of myeloma patients (n=103) and their presence was associated with an improved survival, despite being non-proliferative. T cell clones were present in 100% of long term survivors of myeloma, providing further evidence that these cells prolong survival. In contrast, T cell clones from 10 year survivors were proliferative. Phospho-flow technology was used to investigate the differences in cell signalling pathways between T cell clones of 10 year and non-10 year survivors. The dysfunction in these cells was related to the upregulation of the SMAD pathway, promoting T cell inactivation and downregulation of the ERK pathway, which blocks proliferation of T cells.

Classification of T cell clones into an anergic, exhausted or senescence phenotype was carried out to determine if dysfunction is reversible, since reversal of dysfunction is phenotype dependent. The cells exhibited a senescent secretory effector phenotype: KLRG-1+/CD57+/CD160+/CD28- with normal telomere lengths for age, suggesting telomere-independent senescence. Importantly, the results demonstrate that dysfunction is potentially reversible. The p38-MAPK, p16 and p21 signaling pathways, which are known to induce

senescence were not upregulated. However, elevated telomerase levels may explain how senescent T cells maintain normal telomere lengths.

This thesis expands our understanding of the biology and clinical significance of T cell clones. It is the first to describe the dysfunction of T cell clones as telomere independent senescence, which is potentially reversible. Additionally, it has identified two novel mechanisms by which tumour cells induce dysfunction in T cell clones. These findings have implications for reversing tumour-induced dysfunction of T cell clones in patients with myeloma.