

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

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Doctor of Philosophy

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.

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TABLE OF CONTENTS

CERTIFICATE OF ORIGINAL AUTHORSHIP	i
ACKNOWLEDGEMENTS	ii
PREFACE.....	iv
TABLE OF CONTENTS	vii
LIST OF FIGURES	xiii
LIST OF TABLES.....	xix
LIST OF ABBREVIATIONS.....	xxi
ABSTRACT	xxvi
CHAPTER 1 INTRODUCTION	1
1.1 Multiple Myeloma	2
1.1.1 Introduction to Multiple Myeloma.....	2
1.1.2 Clinical Presentation of MM.....	2
1.1.3 Diagnosis of MM	6
1.1.4 Classification and Staging of MM	8
1.1.1 T cells.....	11
1.2 The Defective Immune System in MM.....	16
1.2.1 The Immune System	16
1.2.2 TCR-V β subfamilies	17
1.2.3 Tumour induced suppression of the immune system in MM	24
1.3 Treatment for MM.....	28
1.3.1 Immunomodulatory drugs.....	28
1.3.2 Proteasome Inhibitors	29
1.3.3 Autologous Stem Cell Transplantation	29
1.3.4 Allogeneic Stem Cell Transplantation	30
1.3.5 Immunotherapy	30

1.4	Clonal CD8+ T cell expansions	32
1.4.1	Expansion of CD8+CD57+ T cells.....	32
1.4.2	Function of CD8+ CD57+ Cells	33
1.4.3	Clonal CD8+ T cell expansions in diseases	34
1.4.4	Clonal T cell expansions in MM.....	36
1.4.5	Specificity of T cell clones	38
1.4.6	Prognostic significance of T cell clones in MM	39
1.4.7	T cell clones in MM are hypo-responsive	40
1.4.8	T cell clones are present in long term survivors of MM and are responsive.....	42
1.5	Background and Aims of the Project	43
1.6	Clinical Significance of the Project	46
CHAPTER 2 Materials and Methods.....		47
2.1	General Chemicals and Reagents	48
2.2	Monoclonal Antibodies	50
2.3	Instruments, Equipment and Software	54
2.4	Patient Selection	56
2.5	Selection of the 10 year MM patient cohort.....	56
2.6	Collection of patient samples	61
2.7	General cell techniques.....	61
2.7.1	Isolation of PBMCs using Ficoll-Paque™	61
2.7.2	Cell washes.....	62
2.7.3	Cell counting	62
2.7.4	Preparation of cell culture media.....	62
2.7.5	Sterility	62
2.7.6	Preparation of frozen samples	62
2.7.7	Thawing of frozen PBMCs.....	63
2.7.8	Cell lines.....	63
2.8	Cell staining protocols for flow cytometry	64
2.8.1	Staining of Surface Antigens	64

2.8.2	Detection of intracellular antigens and cytokines	64
2.9	Flow cytometry protocols.....	64
2.9.1	Flow cytometry acquisition	64
2.9.2	Determination of cell viability by flow cytometry	65
2.9.3	Detection of clonal T cell expansions in patients.....	65
2.9.4	Cell sorting for purification of T cell clones	67
2.10	Statistical Analysis	67

CHAPTER 3 Detection and Characterisation of Clonal T cell Expansions 69

3.1	Introduction	70
3.1.1	Presence of clonal T cell expansions in MM	70
3.1.2	Clinical relevance of hypo-responsive MM T cell clones	70
3.1.3	Long term survivors of MM.....	73
3.1.4	Summary.....	74
3.2	Materials and Methods.....	74
3.2.1	Detection of clonal T cells expansions in MM patients by flow cytometry.....	74
3.2.2	Collection of patient information	75
3.2.3	Measurement of T cell proliferation using CFSE tracking dye.....	77
3.2.4	14 day <i>ex vivo</i> expansion of clonal T cells from MM patients.....	78
3.2.5	Stimulation of hypo-responsive T cell clones with immune modulators	79
3.2.6	IFN- γ production assay	79
3.3	Results	81
3.3.1	Screening of the TCR-V β repertoire.....	81
3.3.2	Clonal T cell expansions are detected in MM patients and have an increased incidence after IMiD therapy	84
3.3.3	MM patients do not show preferential expansion of any particular V β family.....	84
3.3.4	T cell clones in MM are associated with improved survival	85
3.3.5	Prognostic significance of large clonal T cell expansions.....	85
3.3.6	T cell clones are not related to ISS stage, treatment or disease status.....	86

3.3.7	Clonal T cell expansions are a universal feature of 10 year survivors of MM.....	87
3.3.8	Longitudinal analysis of clonal T cell expansions in 10 year survivors.....	87
3.3.9	Proliferative capacity of 10 year survivor T cell clones and T cell clones from other MM patients.....	91
3.3.10	Immune modulators failed to stimulate the proliferation of hypo-responsive T cell clones and did not augment proliferation of responsive T cell clones from 10 year survivors.....	93
3.3.11	T cell clones retain the ability to produce IFN- γ	94
3.4	Discussion.....	95

CHAPTER 4 ANALYSIS OF SIGNALLING PATHWAYS IN CLONAL T CELL EXPANSIONS..... 102

4.1	Introduction	103
4.1.1	Dysregulated pathways associated with anergy are found in T cell clones of WM patients.....	103
4.1.2	Apoptotic pathways	106
4.1.3	TGF- β pathway	111
4.1.4	Proliferation pathway.....	113
4.1.5	TCR signalling pathway.....	115
4.1.6	Phospho-flow cytometry	117
4.1.7	Summary.....	120
4.2	Materials and Methods.....	121
4.2.1	Fixation and Permeabilisation Methods.....	121
4.2.2	Controls.....	125
4.3	Results	125
4.3.1	Effect of fixation and permeabilisation on surface marker detection.....	126
4.3.2	Dilution of permeabilisation buffer to improve cell surface marker resolution	129
4.3.3	Effect of diluted permeabilisation buffer on phospho-protein detection.....	131

4.3.4	Effect of sequential staining on the detection of CD8+ T cells	133
4.3.5	Investigation of apoptotic pathways	136
4.3.6	Investigation of the TGF- β -SMAD signalling pathway	143
4.3.7	Investigation of the ERK Proliferation pathway	147
4.3.8	Investigation of the TCR-signalling pathway	151
4.4	Discussion.....	156

CHAPTER 5 UNDERSTANDING TUMOUR INDUCED T CELL DYSFUNCTION 164

5.1	Introduction	165
5.1.1	Anergic T cells.....	165
5.1.2	Exhausted T cells.....	166
5.1.3	Senescent T cells.....	169
5.1.4	Stem-like T cells.....	170
5.1.5	MM T cell clones have been described as three types of dysfunctional T cells that are found in cancer	171
5.1.6	Summary.....	173
5.2	Methods	174
5.2.1	Investigating cell surface phenotype by flow cytometry.....	174
5.2.2	Measurement of telomere length by q-PCR	176
5.2.3	Measurement of telomere length by Flow-FISH.....	176
5.2.4	Signalling pathways involved in the induction of senescence	178
5.2.5	Measurement of telomerase by flow cytometry	178
5.2.6	Flow detection of immune checkpoint proteins and other signalling pathways	179
5.2.7	Controls.....	179
5.3	Results	179
5.3.1	Introduction	179
5.3.2	T cell clones are neither exhausted nor anergic.....	180
5.3.3	T cell clones display the phenotype of senescent T cells.....	184
5.3.4	MM T cell clones have normal for age telomere lengths	186
5.3.5	Senescent T cells are not related to patient demographics.....	190
5.3.6	p16 and p21 levels are not upregulated in MM T cell clones.....	192

5.3.7	The p38-MAPK pathway is not responsible for inducing MM clonal T cell senescence	194
5.3.8	Summary of phenotypic features in MM T cell clones	194
5.3.9	MM T cell clones exhibit elevated levels of telomerase.....	197
5.3.10	p-Akt expression is elevated in MM T cells but is not related to telomerase activity	199
5.3.11	Checkpoint expression in MM	200
5.3.12	T-bet.....	203
5.4	Discussion.....	204
CHAPTER 6 FINAL DISCUSSION AND FUTURE DIRECTIONS		211
6.1	Key Findings from this work	212
6.2	Future Directions.....	221
6.3	Conclusions.....	229
REFERENCES		230

LIST OF FIGURES

Figure 1.1 Diagnostic features of MM	4
Figure 1.2 Revised IMWG diagnostic criteria for MM and SM	7
Figure 1.3 Hierarchy of human lymphocytes and their subsets.....	11
Figure 1.4 Structure of the T cell receptor.....	13
Figure 1.5 The genomic organisation of the TCR α and β loci.....	15
Figure 1.6 Germline rearrangement of the TCR- α and TCR- β genes	16
Figure 1.7 Detection of TCR β rearrangements by Southern blot analysis.	19
Figure 1.8 Analysis of the TCR-V β repertoire by flow cytometry using monoclonal antibodies against 24 V β families.....	21
Figure 1.9 Determination of T cell clonality by TCR CDR3 length analysis.....	22
Figure 1.10 CDR3 length analysis and sequencing of PCR products of sorted T cells to determine clonality	23
Figure 1.11 Proposed mechanisms associated with tumour-induced immunosuppression of cytotoxic T cells in MM	27
Figure 1.12 Spread of CD8+ T cell expansions in MM.....	37
Figure 1.13 CDR3 length analysis of T cell clones in MM patients	38
Figure 1.14 Survival curve for MM patients in the presence and absence of T cell clones.	40
Figure 1.15 Proliferative capacity of T cells in WM patients.....	41
Figure 2.1 Doublet discrimination.....	66
Figure 2.2 Representative gating strategy for identification of clonal T cell population in a MM patient.....	66
Figure 2.3 Sorting of T cell clones.....	68
Figure 3.1 Measurement of T cell proliferation by CFSE	78

Figure 3.2 Flow scattergrams of a TCR-V β repertoire screen of CD3+CD8+ T cells from a representative MM patient with an expanded V β 3 population	82
Figure 3.3 TCR-V β repertoire of a MM patient with a clonal expansion of the V β 3 family	83
Figure 3.4 Incidence of TCR-V β families in MM patients	84
Figure 3.5 Overall survival of MM patients with T cell clones.....	85
Figure 3.6 Overall survival of MM patients with large or small clonal T cell expansions	86
Figure 3.7 Incidence of TCR-V β families in 10 year survivors.....	90
Figure 3.8 Clonal T cell proliferation in non-10 year and 10 year survivors of MM	92
Figure 3.9 14 day <i>ex vivo</i> expansions of T cell clones from non-10 year and 10 year survivors of MM.....	92
Figure 3.10 Effect of immune modulators on the proliferation of T cell clones..	93
Figure 3.11 Effect of immune modulators on the proliferation of T cell clones from a 10 year survivor.....	94
Figure 3.12 IFN- γ production by T cell clones from non-10 year and 10 year survivors of MM.....	95
Figure 4.1 Dysfunctional pathways identified in T cell clones from patients with Waldenström's Macroglobulinaemia	105
Figure 4.2 Schematic diagram of the extrinsic and intrinsic pathways of apoptosis.....	107
Figure 4.3 Follow-up of the TCR-V β repertoire in a single MM patient over 18 months	108

Figure 4.4 Bcl-2 and Fas expression on MM T cells	110
Figure 4.5 The TGF- β signalling pathway	112
Figure 4.6 The MAPK signalling cascade	113
Figure 4.7 Inhibition of the ERK pathway by HePTP.....	114
Figure 4.8 The TCR signalling pathway	116
Figure 4.9 Outline of the generic generic phospho-flow technique	119
Figure 4.10 Comparison of the effects of traditional and sequential staining protocols on anti-CD3 antibodies conjugated to different fluorochromes	128
Figure 4.11 Effect of permeabilisation buffer on the detection of surface antibodies.....	130
Figure 4.12 Dilution of permeabilisation buffer compromises the ability to detect intracellular phospho-proteins	132
Figure 4.13 The effect of sequential staining on the detection of problematic surface antibodies	134
Figure 4.14 Flow diagram of the optimised phospho-flow cytometry method used to detect phosphorylated proteins.....	135
Figure 4.15 Detection of apoptotic proteins Fas, Fas-ligand and Bcl-xL after bead stimulations for up to 4 days.....	137
Figure 4.16 Fas expression on unstimulated T cells from MM patients	138
Figure 4.17 Fas ligand expression on T cells after 2 day bead stimulations ...	139
Figure 4.18 Bcl-xL expression on CD8+ T cells after 2 day bead stimulations	141
Figure 4.19 Bcl-xL expression on T cell clones from 10 year and non-10 year survivors of MM.....	142
Figure 4.20 p-SMAD expression on MM T cell clones	144

Figure 4.21 p-SMAD expression on MM T cell clones from 10 year and non-10 year survivors of MM.....	145
Figure 4.22 Optimisation of PMA concentration for the detection of p-ERK in T cells from a healthy control.....	147
Figure 4.23 p-ERK expression on MM T cell clones	149
Figure 4.24 p-ERK expression on MM T cell clones from 10 year and non-10 year MM survivors	150
Figure 4.25 CD3- ζ chain expression on MM T cells and T cells from normal controls.....	151
Figure 4.26 p-ZAP-70 expression on MM T cells and T cells from normal controls.....	152
Figure 4.27 Determination of optimal bead concentration for the induction of p-SHP-2 expression in CD8+ T cell subsets	153
Figure 4.28 p-SHP-2 expression on MM T cells and normal controls	155
Figure 5.1 Characteristics of anergic, senescent, exhausted and stem-like T cells found in the tumour microenvironment.....	167
Figure 5.2 Immune checkpoint blockade	168
Figure 5.3 Phenotypic features of dysfunctional T cells in cancer investigated in this study	175
Figure 5.4 Representative flow histogram gating for determination of cell surface phenotype of T cell clones.....	181
Figure 5.5 PD-1 expression on T cells from MM patients and normal controls	182
Figure 5.6 LAG-3 expression on T cells from MM patients and normal controls	182

Figure 5.7 TIM-3 expression on T cells from MM patients and normal controls	183
Figure 5.8 CTLA-4 expression on T cells from MM patients and normal controls	183
Figure 5.9 T cell clones in MM are mostly of the late differentiated stage.....	184
Figure 5.10 CD160 expression on T cells from MM patients and normal controls	185
Figure 5.11 KLRG-1 expression on T cells from MM patients and normal controls.....	185
Figure 5.12 Telomere length of MM T cell clones compared to the telomere lengths of PBMC from healthy individuals according to age.....	187
Figure 5.13 Telomere length of MM clonal and non-clonal T cells measured by qpCR.....	188
Figure 5.14 Representative flow histograms for Flow-FISH determination of telomere length of MM clonal T cells.....	189
Figure 5.15 Telomere length of MM clonal and non-clonal T cells measured by flow-FISH	190
Figure 5.16 p16 expression on T cells from MM patients.....	193
Figure 5.17 p21 expression on T cells from MM patients.....	193
Figure 5.18 p-p38-MAPK expression on T cells from MM patients and normal controls.....	195
Figure 5.19 Representative histogram for measurement of hTERT by flow cytometry.....	197
Figure 5.20 hTERT expression on T cells from MM patients	198
Figure 5.21 pAkt expression on T cells from MM patients	199

Figure 5.22 PD-1 expression on BM T cells from MM patients and normal controls.....	201
Figure 5.23 CTLA-4 expression on BM T cells from MM patients and normal controls.....	201
Figure 5.24 BTLA expression on T cells from MM patients and normal controls	202
Figure 5.25 T-bet expression on T cells from MM patients and normal controls	203
Figure 6.1 The process of cancer immunoediting from immune surveillance to tumour escape in MM.....	220
Figure 6.2 Preliminary data of the phenotype of 10 year survivor MM T cell clones.....	224
Figure 6.3 Preliminary data of telomere length and telomerase activity of 10 year survivor T cell clones.....	225

LIST OF TABLES

Table 1.1 Initial diagnostic workup on suspicion of MM or other monoclonal gammopathies.....	5
Table 1.2 The Durie-Salmon Clinical Staging System	9
Table 1.3 The Revised International Staging System	10
Table 1.4 Summary of the type and properties of CD8+ T cell expansions in normal aging individuals and some clinical conditions.	35
Table 2.1 Manufacturer details for chemicals and reagents.....	48
Table 2.2 Monoclonal antibodies for flow cytometry	50
Table 2.3 Isotype controls and secondary antibodies for flow cytometry	54
Table 2.4 Details for equipment and software.....	54
Table 2.5 Clinical characteristics of different patient cohorts analysed in this study.....	57
Table 2.6 Clinical characteristics of 10 year survivor cohort	60
Table 3.1 Incidence of V β expansion in different historical multiple myeloma cohorts at our institution	72
Table 3.2 TCR-V β families detected by the 8 antibody cocktails from the IO test BetaMark TCR-V β kit	76
Table 3.3 Clinical characteristics of MM patients screened for T cell clones	88
Table 3.4 List of TCRV β expansions identified in 10 year MM survivors and their proportions of CD3+ T cells.....	89
Table 3.5 Longitudinal analysis of clonal T cell expansions in 10 year survivors	90
Table 4.1 Fixation and permeabilisation methods for the detection of surface and intracellular proteins of interest	121

Table 4.2 Demographics of MM patients studied for p-SMAD expression	146
Table 5.1 Antibody panels for investigating cell surface phenotype of T cell clones.....	174
Table 5.2 Demographics for MM patients studied for T cell senescence phenotype and telomere length.....	192
Table 5.3 Identification and categorisation of dysfunctional T cells by phenotype and signalling pathways	196

LIST OF ABBREVIATIONS

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 ₂ M	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-1 β -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 <i>Caenorhabditis elegans</i> protein SMA and the <i>Drosophila</i> 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 I
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

V β	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
κ	Kappa
λ	Lambda
$^{\circ}\text{C}$	Centigrade (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.