

# Table of Contents

Certificate.....	ii
Declaration by the candidate.....	iii
Copyright transfer certificate.....	iv
Acknowledgements.....	v
Table of Contents .....	vii
List of Figures.....	xi
List of Tables.....	xiii
List of Abbreviations.....	xv
Preface.....	xvii
<b>1. Chapter 1: General Introduction.....</b>	<b>1</b>
<b>1.1. Introduction.....</b>	<b>2</b>
<b>1.2. Prevalence.....</b>	<b>2</b>
<b>1.3. Pathophysiology.....</b>	<b>3</b>
<b>1.4. Hallmarks of cancer.....</b>	<b>4</b>
1.4.1. Sustaining proliferative signaling.....	4
1.4.2. Resisting cell death.....	5
1.4.3. Evading growth suppressors.....	6
1.4.4. Enabling replicative immortality.....	6
1.4.5. Inducing angiogenesis.....	7
1.4.6. Tissue invasion and metastasis.....	8
1.4.7. Emerging hallmark: Reprogramming energy metabolism.....	9
1.4.8. Emerging Hallmark: Evading immune destruction.....	10
1.4.8.1 Factors that tumors exploit to avoid immune responses .....	11
<b>1.5. Management and treatment.....</b>	<b>12</b>
1.5.1. Surgery.....	12
1.5.2. Chemotherapy.....	13
1.5.3. Radiotherapy.....	13
1.5.4. Immunotherapy.....	14
1.5.4.1 Safety and toxicology of immune checkpoint inhibitors.....	15
<b>1.6. Caffeine.....</b>	<b>15</b>
1.6.1. Effect of caffeine on cell cycle progression.....	16
1.6.2. Effect of caffeine on apoptosis.....	17

1.6.3.	Effect of caffeine on drug efflux.....	19
1.6.4.	Effect of caffeine on anti-tumor immunity.....	21
1.6.5.	Effect of caffeine on tumor angiogenesis.....	23
<b>1.7.</b>	<b>Oxaliplatin.....</b>	<b>24</b>
1.7.1.	Immunologic Mechanisms: Immunogenic Cell Death (ICD).....	25
<b>1.8.</b>	<b>Aims.....</b>	<b>26</b>
<b>1.9.</b>	<b>Hypothesis.....</b>	<b>27</b>
<b>2.</b>	<b>Chapter 2: Evaluation of antitumor immune response of caffeine and anti-PD1 monoclonal antibody combination against carcinogen and cell line induced tumors in mice.....</b>	<b>30</b>
<b>2.1.</b>	<b>Introduction.....</b>	<b>31</b>
<b>2.2.</b>	<b>Materials and methods.....</b>	<b>32</b>
2.2.1.	Animals.....	32
2.2.2.	Drugs, Chemicals and Antibodies.....	32
2.2.3.	Carcinogen-induced tumor model.....	33
2.2.4.	Caffeine treatment protocol.....	33
2.2.5.	Body, heart, liver and kidney weight measurement.....	34
2.2.6.	Flow cytometric analysis of tumor infiltrating lymphocytes.....	34
2.2.7.	Analysis of intratumoral levels of TNF- $\alpha$ and IFN- $\gamma$ by ELISA.....	34
2.2.8.	Histopathological analysis of tumor tissue.....	35
2.2.9.	Statistical analysis.....	35
<b>2.3.</b>	<b>Results.....</b>	<b>35</b>
2.3.1.	Effect of caffeine on tumor incidence and growth.....	35
2.3.2.	Effect of caffeine on anti-tumor immune response.....	37
2.3.3.	Effect of caffeine on infiltration of T-lymphocytes.....	38
2.3.4.	Effect of caffeine on infiltration of CD8+ and CD4+CD25+ regulatory T cells.....	40
2.3.5.	Effect of caffeine on expression of PD-1 on CD8+ and CD4+CD25+ regulatory T cells.....	41
2.3.6.	Effect of caffeine on intratumoral levels of TNF- $\alpha$ and IFN- $\gamma$ .....	41
2.3.7.	Effect of caffeine on body, heart, liver and kidney weights.....	42
<b>2.4.</b>	<b>Discussion.....</b>	<b>43</b>
<b>2.5.</b>	<b>Conclusion.....</b>	<b>44</b>
<b>3.</b>	<b>Chapter 3: Evaluation of antitumor immune response of caffeine and anti-PD1 monoclonal antibody combination against carcinogen and cell line induced tumors in mice.....</b>	<b>46</b>

<b>3.1. Introduction.....</b>	<b>47</b>
<b>3.2. Materials and methods.....</b>	<b>47</b>
3.2.1. Animals.....	47
3.2.2. Cell line, Drugs and Chemicals.....	48
3.2.3. Carcinogen-induced tumor model.....	48
3.2.4. Cell line-induced tumor model.....	48
3.2.5. Tumor measurement and survival analysis.....	49
3.2.6. Body, heart, liver and kidney weight measurement.....	49
3.2.7. Flow cytometric analysis of tumor infiltrating lymphocytes.....	49
3.2.8. Analysis of intratumoral levels of TNF- $\alpha$ and IFN- $\gamma$ by ELISA.....	50
3.2.9. Statistical analysis.....	50
<b>3.3. Results.....</b>	<b>50</b>
3.3.1. Effects of caffeine, anti-PD1 mAb, or their combination on tumor progression and survival in carcinogen-induced tumor model.....	50
3.3.2. Effects of caffeine, anti-PD1 mAb, or their combination on tumor progression and survival in cell line-induced tumor model.....	52
3.3.3. Effects of caffeine, anti-PD1 mAb or their combination on T-lymphocyte infiltration into cell line-induced tumors.....	54
3.3.4. Effects of caffeine, anti-PD1 mAb or their combination on CD4 T-lymphocyte infiltration into cell line-induced tumors.....	55
3.3.5. Effects of caffeine, anti-PD1 mAb or their combination on CD8 T-lymphocyte infiltration into cell line-induced tumors.....	56
3.3.6. Effects of caffeine, anti-PD1 mAb or their combination on CD4+ CD25+ T-lymphocyte infiltration into cell-line induced tumors.....	57
3.3.7. Effects of caffeine or anti-PD1 mAb or their combination on intratumoral levels of TNF- $\alpha$ and IFN- $\gamma$ .....	58
3.3.8. Effect of caffeine or anti-PD1 mAb or their combination on body, heart, liver and kidney weights.....	59
<b>3.4. Discussion.....</b>	<b>59</b>
<b>3.5. Conclusion.....</b>	<b>62</b>
<b>4. Chapter 3: Evaluation of antitumor immune response of oxaliplatin and anti-PD1 monoclonal antibody combination against carcinogen and cell line induced tumors in mice.....</b>	<b>63</b>
<b>4.1. Introduction.....</b>	<b>64</b>
<b>4.2. Materials and methods.....</b>	<b>64</b>
4.2.1. Animals.....	64
4.2.2. Cell line, Drugs and Chemicals.....	65
4.2.3. Carcinogen-induced tumor model.....	65
4.2.4. Cell line-induced tumor model.....	65

4.2.5. Tumor measurement.....	66
4.2.6. Body, heart, liver and kidney weight measurement.....	66
4.2.7. Flow cytometric analysis of tumor infiltrating lymphocytes.....	66
4.2.8. Analysis of intratumoral levels of TNF- $\alpha$ and IFN- $\gamma$ by ELISA.....	67
4.2.9. Statistical analysis.....	67
<b>4.3. Results.....</b>	<b>67</b>
4.3.1. Effects of oxaliplatin, anti-PD1 mAb, or their combination on tumor progression and survival in carcinogen-induced tumor model.....	67
4.3.2. Effects of oxaliplatin, anti-PD1 mAb, or their combination on tumor progression and survival in cell line-induced tumor model.....	69
4.3.3. Effects of oxaliplatin, anti-PD1 mAb or their combination on CD4 T-lymphocyte infiltration into cell line-induced tumors.....	71
4.3.4. Effects of oxaliplatin, anti-PD1 mAb or their combination on CD4 8-lymphocyte infiltration into cell line-induced tumors.....	71
4.3.5. Effects of oxaliplatin, anti-PD1 mAb or their combination DAMPs.....	72
4.3.6. Effects of oxaliplatin or anti-PD1 mAb or their combination on intratumoral levels of TNF- $\alpha$ and IFN- $\gamma$ .....	73
4.3.7. Effect of oxaliplatin or anti-PD1 mAb or their combination on body, heart, liver and kidney weights.....	74
<b>4.4. Discussion.....</b>	<b>76</b>
<b>4.5. Conclusions.....</b>	<b>78</b>
<b>5. Chapter 5: Discussion and conclusions.....</b>	<b>79</b>
<b>5.1. Summary of major findings.....</b>	<b>84</b>
<b>5.2. Scope for further work.....</b>	<b>85</b>
<b>6. Chapter 6: References.....</b>	<b>87</b>

## List of Figures

Figure 1.1: Effect of caffeine on cell cycle progression.....	16
Figure 1.2: Effect of caffeine on apoptosis.....	18
Figure 1.3: Effect of caffeine on drug uptake.....	20
Figure 1.4: Effect of caffeine on anti – tumor immunity .....	22
Figure 1.5: Effect of caffeine on tumor angiogenesis.....	23
Figure 1.6: Mechanism of caffeine and anti-PD1 induced antitumor immune response...27	
Figure 1.7: Mechanism of oxaliplatin and anti-PD1 induced antitumor immune response.....	28
Figure 2.1: Mechanism of caffeine induced antitumor immune response.....	31
Figure 2.2: Caffeine treatment decreased carcinogen-induced tumor incidence.....	34
Figure 2.3: Caffeine treatment decreased the rate of carcinogen-induced tumor growth..	35
Figure 2.4: Caffeine treatment is associated with increased leucocytes infiltration in carcinogen-induced tumors .....	36
Figure 2.5: Caffeine treatment increased T lymphocyte infiltration in carcinogen-induced tumors.....	37
Figure 2.6: Caffeine treatment increased T lymphocyte infiltration in carcinogen-induced tumors- Flow cytometric results.....	38
Figure 2.7: Caffeine treatment increased TNF- $\alpha$ and IFN- $\gamma$ levels in carcinogen-induced tumors .....	40
Figure 3.1: Combination of caffeine and anti-PD1 mAb inhibited the carcinogen-induced tumor growth.....	49
Figure 3.2: Caffeine and anti-PD1 mAb combination therapy increased the overall survival period in carcinogen-induced tumor model .....	50
Figure 3.3: Caffeine and anti-PD1 mAb combination therapy inhibited cell line-induced tumor growth .....	51
Figure 3.4: Caffeine and anti-PD1 mAb combination therapy increased the T lymphocyte infiltration into cell line-induced tumors .....	52
Figure 3.5: Caffeine and anti-PD1 mAb combination therapy increased the CD4 T-lymphocyte infiltration into cell line-induced tumors .....	53
Figure 3.6: Caffeine and anti-PD1 mAb combination therapy increased the CD4 T-lymphocyte infiltration into cell line-induced tumors.....	54

Figure 3.7: Caffeine and anti-PD1 mAb combination therapy decreased the infiltration of CD4+ CD25+ T-lymphocytes in cell line-induced tumor model .....	55
Figure 3.8: Caffeine and anti-PD1 mAb combination therapy increased the intratumoral levels of TNF- $\alpha$ and IFN- $\gamma$ .....	56
Figure 4.1: Combination of oxaliplatin and anti-PD1 mAb inhibited the carcinogen-induced tumor growth .....	66
Figure 4.2: Combination of oxaliplatin and anti-PD1 mAb increased the overall survival period in carcinogen-induced tumor model .....	67
Figure 4.3: Oxaliplatin and anti-PD1 mAb combination therapy inhibited cell line-induced tumor growth .....	68
Figure 4.4: Effects of oxaliplatin, anti-PD1 mAb or their combination on CD4 T lymphocytes into cell line-induced tumor model .....	69
Figure 4.5: Effects of oxaliplatin, anti-PD1 mAb or their combination on CD8 T lymphocytes into cell line-induced tumor model.....	70
Figure 4.6: Effects of oxaliplatin, anti-PD1 mAb or their combination on DAMPs.....	71
Figure 4.7: Effects of oxaliplatin, anti-PD1 mAb or their combination on intra-tumoral levels of TNF- $\alpha$ and IFN- $\gamma$ .....	72

## List of Tables

Table 1: Effect of caffeine on body weight.....	40
Table 2: Effect of caffeine on major organs.....	41
Table 3: Effect of caffeine, anti-PD1 or their combination on body weight of mice with carcinogen induced tumors .....	57
Table 4: Effect of caffeine, anti-PD1 or their combination on body weight of mice with B16F10 melanoma induced tumors .....	57
Table 5: Effect of caffeine, anti-PD1 or their combination on major organs of mice with carcinogen induced tumors .....	58
Table 6: Effect of caffeine, anti-PD1 or their combination on major organs of mice B16F10 melanoma induced tumors .....	58
Table 7: Effect of oxaliplatin, anti-PD1 or their combination on body weight of mice with carcinogen induced tumors .....	73
Table 8: Effect of oxaliplatin, anti-PD1 or their combination on body weight of mice with B16F10 melanoma induced tumors .....	73
Table 9: Effect of oxaliplatin, anti-PD1 or their combination on major organs of mice with carcinogen induced tumors .....	74
Table 10: Effect of oxaliplatin, anti-PD1 or their combination on major organs of mice with B16F10 melanoma induced tumors .....	74