UNIVERSITI PUTRA MALAYSIA

POLYMER COATED GENETICALLY MODIFIED Salmonella enterica SEROVAR AGONA AS TUMOUR TARGETING AGENT

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By

UBAIDAH NAIM BINTI TARAQ NAEM ZIA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

March 2021

DEDICATION

To my parents, husband, son and sister:

Thank you for your helping hands during dark times,
Thank you for being my pillar of support that I can trust,
Thank you for encouraging me to work towards my dreams.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

POLYMER COATED GENETICALLY MODIFIED *Salmonella enterica* SEROVAR AGONA AS TUMOUR TARGETING AGENT

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Chairman : Professor Ts. Cheah Yoke Kqueen, PhD
Faculty : Medicine and Health Sciences

Conventional tumour therapies pose significant adverse effects to the patients and this brings the need for the development of therapy that is more tumour specific while not affecting the patients negatively. Bacterial mediated tumour therapy is now studied intensely in hope of finding a therapy that accumulate in tumours specifically while activating the patient's system to eliminate tumours more efficiently. Utilising bacteria as tumour therapy is found to be attractive, as these organisms could be genetically modified to be less pathogenic and express tumour suppressing proteins and in case of severe infections antibiotics could be used. *Salmonella* Agona (*S.* Agona) had been shown to have similar tumour suppression capabilities as *Salmonella* Typhimurium (*S.* Typhimurium) with reduced systemic effects to the tumour-bearing mice. *S.* Agona was then genetically modified to have its Δsop*B*Δsop*D*Δleu*B*Δarg*D* genes attenuated (BDLA *S.* Agona) which showed no clinical signs of systemic infections in dogs, better efficacy to suppress tumours compared to other *S.* Agona auxotrophs and reduced virulence when tested in tumour-bearing mice. However, it was observed that multiple administrations of the treatment did not increase tumour suppression efficacy, suggesting immunity of the patients reduces the tumour suppression capacity of the BDLA *S.* Agona strain. The ubiquitous nature of the strain could mean that most of individuals are exposed to the strain and have *Salmonella*-specific antibodies that could reduce tumour targeting and suppression capabilities. A strategy to overcome this is by encapsulating the bacteria in biodegradable polymers, such as Poly(allylamine hydrochloride) (PAH) to allow the strain to escape neutralising antibodies and possibly improve accumulation in tumours. This study aims to investigate the use of PAH coating on the BDLA *S.* Agona to enhance its capabilities as tumour targeting and suppressing agent and to evaluate the cytokine profiles and histopathology following the PAH-coated BDLA *S.* Agona treatment in naïve and immunised tumour-bearing mice. The 5mg/mL PAH-BDLA *S.* Agona treatment showed improvement in tumour targeting capabilities in naïve mice with 1.038

times more accumulation in tumours compared to BDLA *S.* Agona treated naïve mice. The 5mg/mL PAH-BDLA *S.* Agona treatment also showed improved tumour suppression capabilities in immunised mice, especially on day 12 (T/C ratio of 0.65) and day 15 with mean tumour volume (0.6 fold) and relative tumour growth (0.77 fold) compared with BDLA *S.* Agona treated group. From the H&E analysis, it is observable that immunised subjects receiving 5mg/mL PAH-BDLA *S.* Agona showed no inflammation and few microabscesses in liver, smallest lymphoid follicles in the spleens and reduced lymphoid aggregate in small intestines. 5mg/mL PAH-BDLA *S.* Agona treatment showed the least increment in cytokines systemically (mean of IL-1β: 0.3147 and TNF-α: 0.1540 pg/gm) in immunised subjects, while statistically significant at $p < 0.05$ for induction of TNF-α (3.079 pg/gm) in tumours was observed in naïve subjects which is beneficial in tumour suppression. This study justifies the use of 5mg/mL PAH-BDLA *S.* Agona as tumour therapy and future development as drug delivery agent as it showed improved tumour targeting and suppressing capabilities with lesser systemic effects to subjects.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

Salmonella enterica SEROVAR AGONA YANG DIUBAH SECARA GENETIK DAN DILAPISI POLIMER SEBAGAI AGEN MENYASAR TUMOR

Oleh

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Mac 2021

Pengerusi : Profesor Ts. Cheah Yoke Kqueen, PhD
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Terapi tumor konvensional memberi kesan buruk kepada pesakit dan ini membawa kepada perlunya pengembangan terapi tumor yang lebih spesifik terhadap tumor dan tidak memberi kesan negatif kepada pesakit. Terapi tumor yang dimediasi oleh bakteria kini dikaji secara mendalam dengan harapan untuk menemukan terapi yang terkumpul pada tumor secara khusus dan mengaktifkan sistem pesakit untuk menghapuskan tumor dengan lebih berkesan. Penggunaan bakteria sebagai terapi tumor dianggap menarik, kerana organisma ini dapat diubah secara genetik untuk menjadi kurang patogenik dan mengekspresikan protein penindas tumor secara khusus kepada tumor. Kesan buruk bakteria juga mudah dikawal kerana antibiotik dapat digunakan jika terjadi jangkitan yang teruk. *Salmonella* Agona (*S.* Agona) terbukti mempunyai kemampuan penindasan tumor yang serupa dengan *Salmonella* Typhimurium (*S.* Typhimurium) tetapi dengan pengurangan kesan sistemik pada tikus yang menghidap tumor. *S.* Agona kemudian diubahsuai secara genetik agar gen Δsop*B*Δsop*D*Δleu*B*Δarg*D* dilemahkan dan diistilahkan sebagai BDLA *S.* Agona yang tidak menunjukkan tanda-tanda klinikal jangkitan sistemik berikutan pemberian kepada anjing, keberkesanan yang lebih baik untuk menindas tumor berbanding dengan *S.* Agona auxotroph lain dan mengurangkan virulensi ketika diuji pada tikus yang menghidap tumor. Walau bagaimanapun, diperhatikan bahawa penambahan jumlah rawatan tidak meningkatkan keberkesanan penindasan tumor, menunjukkan imuniti pesakit mengurangkan kapasiti penindasan tumor dari strain BDLA *S.* Agona. Sifat strain yang sentiasa ada di mana-mana mungkin bermaksud bahawa kebanyakan individu terdedah kepada strain dan menghasilkan antibodi khusus *Salmonella* yang dapat mengurangkan penyasaran dan penindasan terhadap tumor oleh strain yang dikaji. Strategi untuk mengatasinya adalah dengan memasukkan bakteria ke dalam polimer yang terbiodegradasi, seperti Poly(allylamine hydrochloride) (PAH) untuk membolehkan strain itu terselamat dari antibodi peneutralan dan mungkin meningkatkan pengumpulan tumor. Kajian

ini dijalankan bertujuan untuk meniliti penggunaan salutan PAH pada BDLA *S.* Agona untuk meningkatkan keupayaannya sebagai agen penyasaran dan penindasan tumor serta menilai profil sitokin dan histopatologi berikutan rawatan BDLA *S.* Agona yang dilapisi PAH pada tikus naif dan yang diimunisasi yang menghidap tumor. Rawatan 5mg/mL PAH-BDLA *S.* Agona menunjukkan peningkatan keupayaan menyasar tumor bagi tikus naif dengan pengumpulan 1.038 kali lebih banyak pada tumor berbanding dengan tikus naif yang dirawat BDLA *S.* Agona. Tikus yang diimunisasi dan dirawat dengan 5mg/mL PAH-BDLA *S.* Agona menunjukkan peningkatan keupayaan penindasan tumor, terutama pada hari ke-12 (nisbah T/C 0.65) dan pada hari ke-15 dimana purata isipadu tumor (0.6 kali ganda) dan pertumbuhan tumor relatif (0.77 kali ganda) berbanding tikus yang dirawat BDLA *S.* Agona. Kajian histologi menunjukan bahawa tikus yang diimunisasi tidak menunjukan sebarang keradangan dengan jumlah mikroabses di hati yang berkurangan, saiz folikel limfoid yang terkecil di limpa dan kewujudan agregat limfoid yang berkurangan di usus kecil. Rawatan 5mg/mL PAH-BDLA *S.* Agona juga menunjukkan kenaikan sitokin (purata IL-1β: 0.3147 dan TNF-α: 0.1540 pg/gm) bagi tikus yang telah diimunasasi, dan kenaikan yang ketara secara statistik dengan $p < 0.05$ bagi TNF-α (3.079 pg/gm) di dalam tumor bagi tikus naif yang bermanfaat dalam penindasan terhadap tumor. Penemuan ini menunjukan bahawa 5mg/mL PAH-BDLA *S.* Agona dapat dikembangkan dengan lebih lanjut sebagai vektor penyampaian ubat yang menyasarkan tumor kerana ia menunjukan penambahbaikan dalam penindasan dan penyasaran terhadap tumor dengan kesan sampingan yang kurang.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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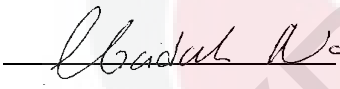
Signature:  Date: _____

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

arg*D*	arginine*D* gene
AST	Antibiotic Susceptibility Test
ATCC	American Type Culture Collection
BCG	Bacillus Calmette-Guerin
BDLA *S*. Agona	Δsop*B*Δsop*D*Δleu*B*Δarg*D* *Salmonella* Agona
BMTT	Bacterial mediated tumour therapy
BSA	Bovine serum albumin
CFU	Colony forming unit
CAR	Chimeric antigen receptor
CTLA4	Cytotoxic T-lymphocyte-associated protein 4
Cx43	Connexin 43
DC	Dendritic cells
DMEM	Dulbecco's Modified Eagle Medium
DNA	Deoxyribonucleic acid
E. coli	Escherichia coli
ELISA	Enzyme-linked immunosorbent assay
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
FRET	Förster Resonance Energy Transfer
H&E	Haematoxylin and Eosin
IACUC	Animal Care and Use Committee
ICB	Immune checkpoint blockers
IDO	Indoleamine 2,3-dioxygenase
IP	Intraperitoneal

IL-1β	Interleukin 1 beta
IL-6	Interleukin 6
IL-18	Interleukin 18
IFN-γ	Interferon-γ
LbL	layer-by-layer
leu*B*	LeucineB gene
LPS	Lipopolysaccharides
min	Minute
mL	Milliliter
mM	Millimeter
MOI	Multiplicity of infection
MYD88	Myeloid differentiation primary response 88
NA	Nutrient agar
NB	Nutrient broth
NK	Natural killer cells
OD	Optical density
PAH	Poly(allylamine hydrochloride)
PBS	Phosphate Buffered Saline
PD-1	Programmed cell death 1
PD-L1	Ligand for programmed cell death 1
PEM	Polyelectrolyte multilayer
PI	Post-inoculation
PSS	Polystyrene sulfonate
rpm	Revolutions per minute
S. Agona	*Salmonella* Agona
shRNA	Small hairpin ribonucleic acid

SPIs	*Salmonella* Pathogenicity Islands
SPI-1	*Salmonella* Pathogenicity Island 1
S. Typhimurium	*Salmonella* Typhimurium
T/C	Treated-to-control
Th2	T-helper type 2
TIL	Tumour-infiltrating lymphocytes
TME	Tumour microenvironment
TNF-α	Tumour necrosis factor alpha
TLR	Toll-like receptors
TLR4	Toll-like receptor 4
Treg	Regulatory T-cells
T3SS1	Type III Secretion System 1
TUNEL	Terminal deoxynucleotidyl transferase–mediated nick-end labeling
WHO	World Health Organization
XLD	Xylose lysine deoxycholate
Δ	Deletion of gene
°C	Degree Celsius
%	Percentage
µL	Microliter

CHAPTER 1

INTRODUCTION

1.1 Research Problem

Solid tumours refer to abnormal masses, which generally consists of a solid centre rather than filled with cyst or liquid. Solid tumours are classified either as malignant (cancerous) or benign (non-cancerous), and the names derived from the type of cells that forms them (National Cancer Institute, 2019a). Both benign and malignant tumours often require conventional interventions such as surgery, radiotherapy and chemotherapy. These interventions often pose significant adverse effects to the patients since it is very invasive, unspecifically targets tumour tissues and chemotherapeutic drugs had been reported to cause cardiotoxicity in patients (Han, Kee, & Hong, 2018; Polk, Vaage-Nilsen, Vistisen, & Nielsen, 2013). Besides that, these interventions are only successful in the eradication of localised and primary tumours (Saga & Kaneda, 2013; Lee, Wu, & Shiau, 2005a). These limitations account for continuous research and development for tumour therapeutics that are less invasive, targets the tumours specifically and could be used as a tool for drug delivery directly into the tumours.

Bacterial cancer therapeutics offers advantages over conventional therapies as it is able to migrate to regions of tumours that are not possible for passive chemotherapeutics drugs, have the ability to specifically target tumours and can be genetically modified to increase its effectiveness (Forbes, 2010; Forbes, 2006). The intentional use of bacteria as tumour therapy agent dates to the early 20th century when American physician William B. Coley also known as the 'father of immunotherapy of cancer' developed Coley's toxin, which is a bacterial mixture as tumour therapy (Forbes et al., 2018). A wide range of bacteria species, for example, *Bifidobacterium, Salmonella* and *Clostridium* spp. had been studied for its potential as tumour therapy agent and even for imaging purposes as an ideal tumour therapy agent should be inexpensive, target and replicates in the tumour specifically as well as have the capacity for therapeutic delivery (Morrissey, Sullivan, & Tangney, 2010). *Salmonella*, being an obligate anaerobe, have the advantage to colonise both big tumours with hypoxic centres and small tumours with a non-hypoxic condition (Leschner & Weiss, 2010). Up to date, only Bacillus Calmette-Guerin (BCG), a live bacterial therapy is approved by the FDA and used for the treatment of non-muscle invasive bladder cancer (Lehouritis, Hogan, & Tangney, 2017). The VNP 20009 is the only *Salmonella* strain evaluated in phase I clinical study for the treatment of nonresponsive metastatic melanoma or renal cell carcinoma (Wang, Kazmierczak, & Eisenstark, 2016). However, from the clinical trial, it was reported that this strain showed poor tumour colonisation (Leschner & Weiss, 2010).

As the bacteria infiltrate the tumours and start replicating, the tumours would be starved to death and intracellular replication of *Salmonella* specifically would

cause the tumours to burst or induce cell death via apoptosis or autophagy (Forbes et al., 2018; Uchugonova et al., 2015; Lee et al., 2014; Wall, Srikanth, & McCormick, 2010). The outer components of *Salmonella*, such as lipopolysaccharides (LPS) and flagellin elicits responses at the cellular level which controls proliferation, apoptosis and even enhance antigenicity of tumours to trigger the immune response against tumours (Patyar et al., 2010). The LPS had also been shown to induce tumour necrosis factor-alpha (TNF-α) and interleukin 1 beta (IL-1β) secretion which is essential for efficient tumour colonisation and induction for haemorrhagic necrosis which is essential for bacterial invasion and even have early and strong anti-tumour response (Frahm et al., 2015; Kim et al., 2015).

The properties that allow such bacteria to invade tumours creates concern of the possibility of invading major organs and causing infection to the patients, especially immunosuppressed patients and a strategy to overcome this is by developing ideal tumour targeting bacterial strain that has reduced virulence and colonise tumours specifically (Zia et al., 2021; Taniguchi et al., 2010). Another strategy is by using a less pathogenic strain, which could solve the issue of pathogenicity of the bacterial therapy towards the patients, and this is where *Salmonella* Agona (*S.* Agona) comes into context. *S.* Agona was recovered from *'ulam'*, which are normally eaten raw and taking into consideration the rarity of an outbreak related to this strain this suggests the possibility of lower pathogenicity of this strain compared to *Salmonella* Typhimurium (*S.* Typhimurium) (Khoo, Sim, Salleh, & Cheah, 2015; Salleh et al., 2003).

The Δsop*B*Δsop*D*Δleu*B*Δarg*D* attenuated *Salmonella* Agona (BDLA *S.* Agona) strain had shown better efficacy to suppress tumours as compared to other auxotrophs and reduced virulence, however, it was noted that multiple administration of the treatment did not increase the tumour suppression efficacy of the treatment (Gwee, Khoo, Yeap, Tan, & Cheah, 2019). Since *Salmonella* is ubiquitous and found everywhere, approximately 20% of the human population displays an active antibody titer against *Salmonella* (Felgner et al., 2018). This results in the development of immunity against this pathogen and the production of *Salmonella*-specific immunity reduces the tumour targeting activity of the strains (Lee, Wu, Chen, & Shiau, 2009). Felgner et al. (2018) reported that *Salmonella* SL7207 and *E. coli* Symbioflor-2 strains lost more than 50% of its tumour clearing efficacy in immunised mice compared to naïve mice.

A strategy to overcome this is by encapsulating the bacteria with a biodegradable polymer, such as Poly(allylamine hydrochloride) (PAH) which renders inactivation of the bacteria by existing neutralising antibodies present in the subjects hence improving the target of the strains to tumours (Lee, Lin, Hsieh, Chen, & Kuo, 2013). Lee et al. (2013) reported that the PAH capsulated *Salmonella* Choleraesuis (*S.* Choleraesius) was shown to be shielded from the neutralising antibodies and was showed to display lower toxicity and improved efficacy and safety (Lee et al., 2013). This method was adopted in this study to observe the effect of PAH encapsulation on the *S.* Agona strains, both wild and the BDLA *S.* Agona strains.

Hence, it is necessary to evaluate tumour therapy capabilities and effects of PAH-coated BDLA *S.* Agona towards tumour-bearing mice in the hope of finding a better tumour therapy agent, hence this study was conducted.

This thesis comprises of six chapters, starting with Chapter 1, which includes the introduction to the research topic, the problem statement, the hypothesis and objectives of the study. It is followed by Chapter 2, which discusses the history, advantages and current literature regarding bacterial mediated tumour therapy (BMTT), which mainly utilises the *Salmonella* strain. Chapter 3 describes the first working chapter of the thesis, which answers the first objective of the thesis. By utilising BacterioScan™ and gentamicin assay, the effects of PAH coatings on the basic phenotypes (growth, size and invasiveness) of the strains are investigated. The outcomes of this chapter justify the decision to proceed with the study by utilising 5mg/mL PAH-coated BDLA *S.* Agona to further study its capabilities for tumour targeting and tumour reduction capabilities. Chapter 4 describes the second working chapter which answers the second objective of the thesis, which is to evaluate the tumour targeting and suppression capabilities of 5mg/mL PAH-coated BDLA *S.* Agona in mice with different immune profiles. The tumour latency due to immunisation, changes of tumour volumes, relative tumour volume and biodistributions of 5mg/mL PAH-coated BDLA *S.* Agona *in vivo* were studied and discussed in this chapter. The following chapter (Chapter 5) answers the third objective of the thesis, which is to evaluate the cytokines profile, IL-1β and TNF-α in serum and tumours. The Haematoxylin and Eosin (H&E) sections of various organs and utilisation of terminal deoxynucleotidyl transferase-mediated nick-end labelling (TUNEL) assay on tumour sections allowed the effects on multiple tissues and the percentage of apoptosis of tumour cells were evaluated following the treatments. Figure 1.1 represents the methodologies used throughout the study, respective to the chapters.

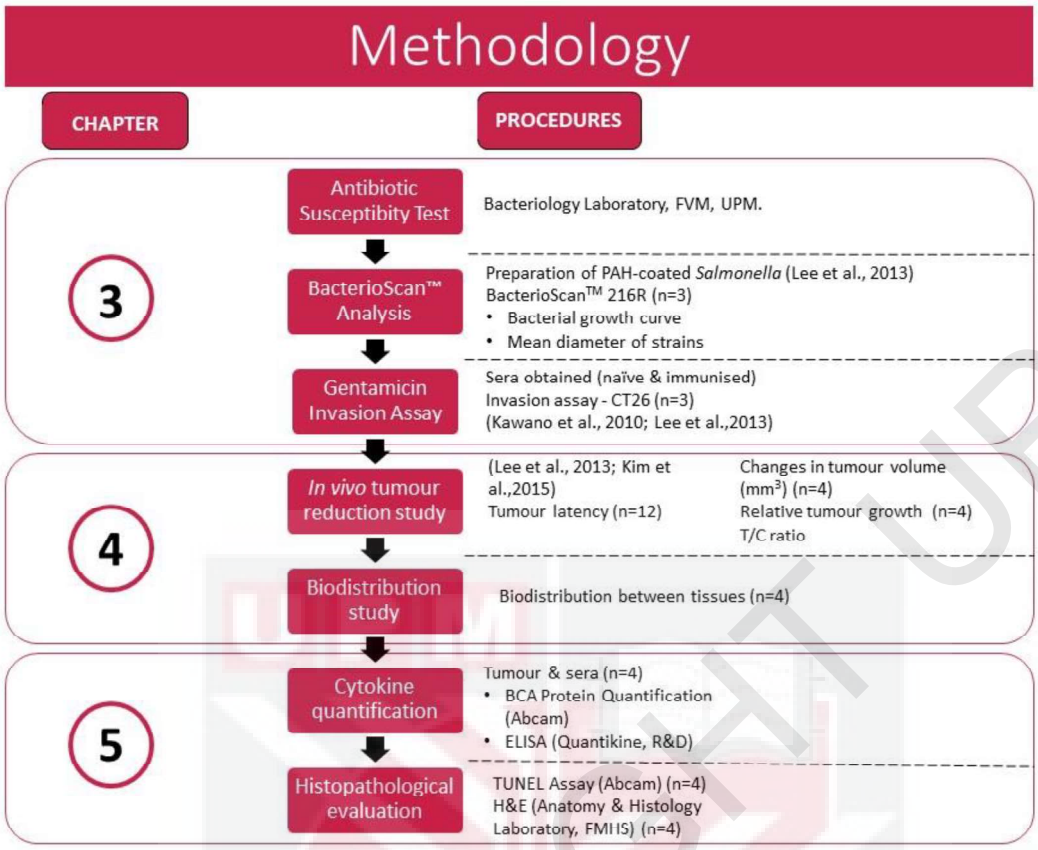

Figure 1.1 : Flowchart of the methodologies in this study
The flowchart summarises the methodologies used throughout the study.

Chapter 6 summarises and conclude the thesis by discussing the outcomes of the study, describing the limitation of the study and providing future recommendations for research to enhance knowledge in this area. Figure 1.2 is the conceptual framework that describes the studies that were previously carried out, furthering into the current research under the same project.

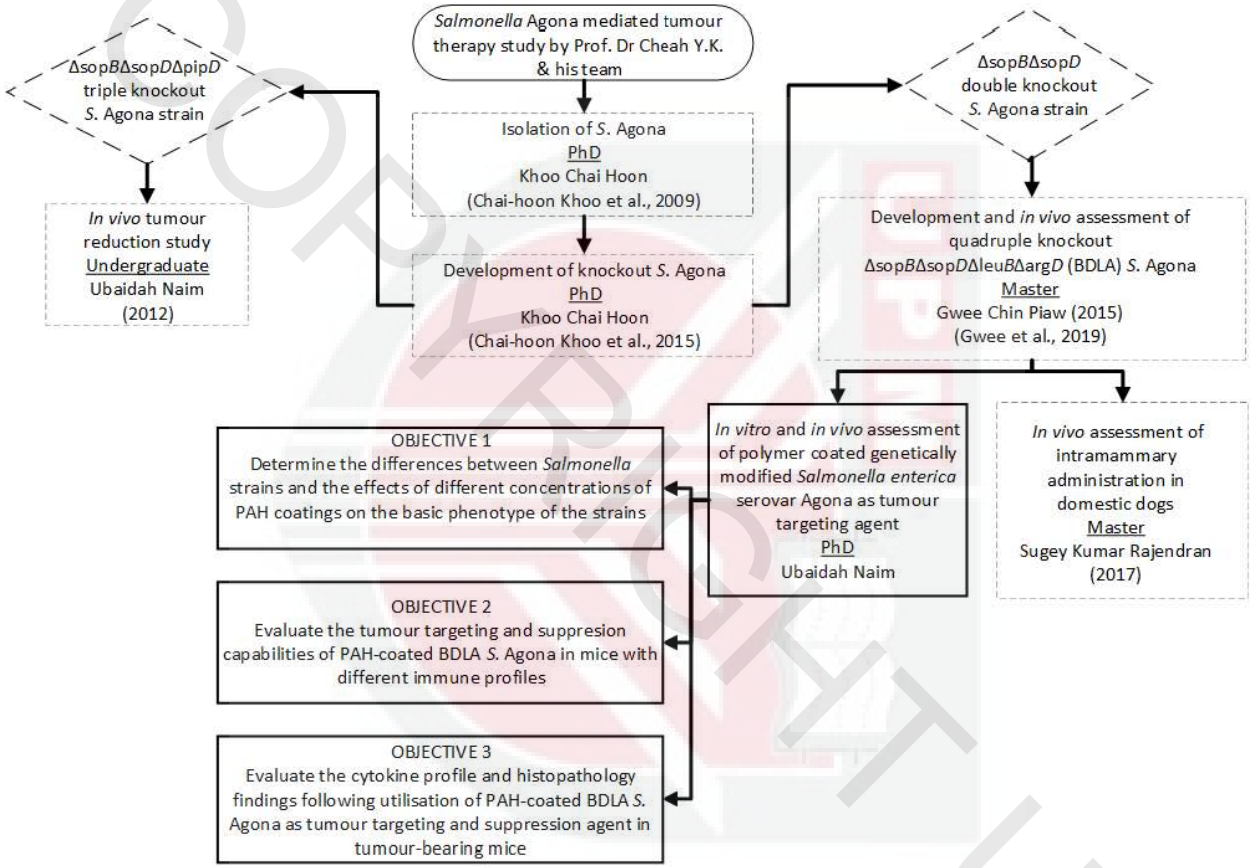

Figure 1.2 : Conceptual framework illustrates the overview flow of previous and current study relating to the utilisation of genetically modified *Salmonella* Agona

Boxes with bold line indicate parts studied in this current study. Dotted lined boxes are related studies carried out by other students in the research group, under the same project.

1.2 Hypothesis

We hypothesised that the PAH-coated BDLA *S.* Agona might show improved tumour targeting and suppressing capabilities as well as reduced pathogenicity and systemic adverse effects towards the subjects.

1.3 Objectives

1.3.1 General Objectives

This study aimed to investigate the use of PAH-coated BDLA *S.* Agona as an improved tumour targeting and suppressing tumour therapy agent and to study the effects of administration of the treatment.

1.3.2 Specific Objectives of the Study

The specific objectives of this study were:

a. to determine the differences between *Salmonella* strains and the effects of different concentrations of PAH coatings on the basic phenotype of the strains (Chapter 3).

b. to evaluate the tumour targeting and suppression capabilities of PAH-coated BDLA *S.* Agona in mice with different immune profiles (Chapter 4).

c. to evaluate the cytokine profile and histopathology findings following utilisation of PAH-coated BDLA *S.* Agona as tumour targeting and suppression agent in tumour-bearing mice (Chapter 5).

1.4 Significance of the Study

The current study will provide a better understanding of the effects of utilisation of BDLA *S.* Agona as a tumour targeting and suppressing agent as well as the possibility of improvement of these capabilities and the utilisation of PAH coating on the strain to reduce adverse effects on the subjects.

REFERENCES

Acheson, D., & Hohmann, E. L. (2001). Nontyphoidal salmonellosis. *Clinical Infectious Diseases*, *32*(2), 263-269.

Agarwal, A., Lvov, Y., Sawant, R., & Torchilin, V. (2008). Stable nanocolloids of poorly soluble drugs with high drug content prepared using the combination of sonication and layer-by-layer technology. *Journal of Controlled Release*, *128*(3), 255-260.

Amar Ng, E. S. N. (2012). *Assessment of Wild Type And Salmonella Pathogenicity Island (Spi) Knock-Out Salmonella Typhimurium And Salmonella Agona Effects on Testis and Testosterone Levels in Cancer Model Mice* (Bachelor's dissertation). Universiti Putra Malaysia.

American Cancer Society. (2020). *Cancer Facts & Figures 2020*. Retrieved from https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2020/cancer-facts-and-figures-2020.pdf

Anderson, K. G., Stromnes, I. M., & Greenberg, P. D. (2017). Obstacles posed by the tumor microenvironment to T cell activity: a case for synergistic therapies. *Cancer Cell*, 31(3), 311-325.

Arzanlou, M., Chai, W. C., & Venter, H. (2017). Intrinsic, adaptive and acquired antimicrobial resistance in Gram-negative bacteria. *Essays in Biochemistry, 61*(1), 49-59.

Baban, C. K., Cronin, M., O'Hanlon, D., O'Sullivan, G. C., & Tangney, M. (2010). Bacteria as vectors for gene therapy of cancer. *Bioengineered Bugs*, *1*(6), 385-394.

Balkwill, F., & Mantovani, A. (2001). Inflammation and cancer: back to Virchow? *The Lancet, 357*(9255), 539-545.

Barbé, S., Van Mellaert, L., & Anné, J. (2006). The use of clostridial spores for cancer treatment. *Journal of Applied Microbiology*, *101*(3), 571-578.

Berraondo, P., Sanmamed, M. F., Ochoa, M. C., Etxeberria, I., Aznar, M. A., Pérez-Gracia, J. L., ... & Melero, I. (2019). Cytokines in clinical cancer immunotherapy. *British Journal of Cancer*, *120*(1), 6-15.

Bindea, G., Mlecnik, B., Tosolini, M., Kirilovsky, A., Waldner, M., Obenauf, A. C., ... & Bruneval, P. (2013). Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. *Immunity*, *39*(4), 782-795.

Binnewies, M., Roberts, E. W., Kersten, K., Chan, V., Fearon, D. F., Merad, M., ... & Vonderheide, R. H. (2018). Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nature Medicine*, *24*(5), 541-550.

Bonaventura, P., Shekarian, T., Alcazer, V., Valladeau-Guilemond, J., Valsesia-Wittmann, S., Amigorena, S., ... & Depil, S. (2019). Cold tumors: a therapeutic challenge for immunotherapy. *Frontiers in Immunology*, *10*, 168.

Bourdeau, R. W., Lee-Gosselin, A., Lakshmanan, A., Farhadi, A., Kumar, S. R., Nety, S. P., & Shapiro, M. G. (2018). Acoustic reporter genes for noninvasive imaging of microorganisms in mammalian hosts. *Nature*, *553*(7686), 86-90.

Broadway, K. M. (2018). *Novel Perspectives on the Utilization of Chemotactic Salmonella Typhimurium VNP20009 as an Anticancer Agent* (Doctoral dissertation). Virginia Tech.

Broadway, K. M., Denson, E. A., Jensen, R. V., & Scharf, B. E. (2015). Rescuing chemotaxis of the anticancer agent Salmonella enterica serovar Typhimurium VNP20009. *Journal of Biotechnology*, *211*, 117-120.

Brogden, K. A. (2009). Cytopathology of Pathogenic Prokaryotes. In N. F. Cheville (Ed.), *Ultrastructural Pathology The Comparative Cellular Basis of Disease* (pp. 424–523). Wiley-Blackwell.

Bugrysheva, J. V., Lascols, C., Sue, D., & Weigel, L. M. (2016). Rapid antimicrobial susceptibility testing of Bacillus anthracis, Yersinia pestis, and Burkholderia pseudomallei by use of laser light scattering technology. *Journal of Clinical Microbiology*, *54*(6), 1462-1471.

Buisseret, L., Garaud, S., de Wind, A., Van den Eynden, G., Boisson, A., Solinas, C., ... & Craciun, L. (2017). Tumor-infiltrating lymphocyte composition, organization and PD-1/PD-L1 expression are linked in breast cancer. *Oncoimmunology*, *6*(1), e1257452.

Cai, Z., Sanchez, A., Shi, Z., Zhang, T., Liu, M., & Zhang, D. (2011). Activation of Toll-like receptor 5 on breast cancer cells by flagellin suppresses cell proliferation and tumor growth. *Cancer Research*, *71*(7), 2466-2475.

Cann, S. H., Van Netten, J. P., & Van Netten, C. (2003). Dr William Coley and tumour regression: a place in history or in the future. *Postgraduate Medical Journal*, *79*(938), 672-680.

Carpenter, S., Rigaud, M., Barile, M., Priest, T. J., Perez, L., & Ferguson, J. B. (2006). *The Ebers Papyrus*. Bard College.

Chandra, A., Rick, J., Yagnik, G., & Aghi, M. K. (2019, August). Autophagy as a mechanism for anti-angiogenic therapy resistance. In *Seminars in Cancer Biology*. Academic Press.

Chang, W. W., & Lee, C. H. (2014). Salmonella as an innovative therapeutic antitumor agent. *International Journal of Molecular Sciences*, *15*(8), 14546-14554.

Chang, W. W., Lai, C. H., Chen, M. C., Liu, C. F., Kuan, Y. D., Lin, S. T., & Lee, C. H. (2013). Salmonella enhance chemosensitivity in tumor through connexin 43 upregulation. *International Journal of Cancer*, *133*(8), 1926-1935.

Chen, H. M., Wang, Y., Su, L. H., & Chiu, C. H. (2013). Nontyphoid Salmonella infection: microbiology, clinical features, and antimicrobial therapy. *Pediatrics & Neonatology*, *54*(3), 147-152.

Chiu, C. H., & Su, L. H. (2019). Salmonella, Non-Typhoidal Species (S. Choleraesuis, S. Enteritidis, S. Hadar, S. Typhimurium). Retrieved from http://www.antimicrobe.org/b258.asp

Chiu, C. H., Lin, T. Y., & Ou, J. T. (1999). A clinical trial comparing oral azithromycin, cefixime and no antibiotics in the treatment of acute uncomplicated Salmonella enteritis in children. *Journal of Paediatrics and Child Health*, *35*(4), 372-374.

Clairmont, C. L. K. P. J., Lee, K. C., Pike, J., Ittensohn, M., Low, K. B., Pawelek, J., ... & Li, Z. (2000). Biodistribution and genetic stability of the novel antitumor agent VNP20009, a genetically modified strain of Salmonella typhimuvium. *Journal of Infectious Diseases*, *181*(6), 1996-2002.

Cruz-Tapias, P., Castiblanco, J., & Anaya, J. M. (2013). Major histocompatibility complex: antigen processing and presentation. In J. M. Anaya, Y. Shoenfeld, A. Rojas-Villarraga, et al. (Eds.) *Autoimmunity: From Bench to Bedside* (pp. 169-184). El Rosario University Press.

Din, M. O., Danino, T., Prindle, A., Skalak, M., Selimkhanov, J., Allen, K., ... & Hasty, J. (2016). Synchronized cycles of bacterial lysis for in vivo delivery. *Nature*, *536*(7614), 81-85.

Dinarello, C. A. (1997). Proinflammatory and anti-inflammatory cytokines as mediators in the pathogenesis of septic shock. *Chest*, *112*(6), 321S-329S.

Doherty, J., & Baehrecke, E. H. (2018). Life, death and autophagy. *Nature Cell Biology*, *20*(10), 1110-1117.

Donlan, R. M. (2000). Role of biofilms in antimicrobial resistance. *ASAIO Journal, 46*(6), S47-S52.

Dranoff, G. (2004). Cytokines in cancer pathogenesis and cancer therapy. *Nature Reviews Cancer, 4*(1), 11-22.

Duan, Q., Zhang, H., Zheng, J., & Zhang, L. (2020). Turning Cold into Hot: Firing up the Tumor Microenvironment. *Trends in Cancer*.

Dunlop, R. J., & Campbell, C. W. (2000). Cytokines and advanced cancer. *Journal of Pain and Symptom Management, 20*(3), 214-232.

Eng, S. K., Pusparajah, P., Ab Mutalib, N. S., Ser, H. L., Chan, K. G., & Lee, L. H. (2015). Salmonella: a review on pathogenesis, epidemiology and antibiotic resistance. *Frontiers in Life Science, 8*(3), 284-293.

Fàbrega, A., & Vila, J. (2013). Salmonella enterica serovar Typhimurium skills to succeed in the host: virulence and regulation. *Clinical Microbiology Reviews, 26*(2), 308-341.

Felfoul, O., Mohammadi, M., Taherkhani, S., De Lanauze, D., Xu, Y. Z., Loghin, D., ... & Gaboury, L. (2016). Magneto-aerotactic bacteria deliver drug-containing nanoliposomes to tumour hypoxic regions. *Nature Nanotechnology*, *11*(11), 941-947.

Felgner, S., Kocijancic, D., Frahm, M., & Weiss, S. (2016). Bacteria in cancer therapy: renaissance of an old concept. *International Journal of Microbiology*, *2016*.

Felgner, S., Kocijancic, D., Frahm, M., Heise, U., Rohde, M., Zimmermann, K., ... & Weiss, S. (2018). Engineered Salmonella enterica serovar Typhimurium overcomes limitations of anti-bacterial immunity in bacteria-mediated tumor therapy. *Oncoimmunology*, *7*(2), e1382791.

Fernández, L., Breidenstein, E. B., & Hancock, R. E. (2011). Creeping baselines and adaptive resistance to antibiotics. *Drug Resistance Updates, 14*(1), 1-21.

Finn, O. J. (2012). Immuno-oncology: understanding the function and dysfunction of the immune system in cancer. *Annals of Oncology: Official Journal of the European Society for Medical Oncology*, *23*, viii6-9.

Forbes, N. S. (2006). Profile of a bacterial tumor killer. *Nature Biotechnology, 24*(12), 1484–1485.

Forbes, N. S. (2010). Engineering the perfect (bacterial) cancer therapy. *Nature Reviews Cancer, 10*(11), 785–794.

Forbes, N. S., Coffin, R. S., Deng, L., Evgin, L., Fiering, S., Giacalone, M., ... & Kaur, B. (2018). White paper on microbial anti-cancer therapy and prevention. *Journal for Immunotherapy of Cancer*, *6*(1), 1-24.

Frahm, M., Felgner, S., Kocijancic, D., Rohde, M., Hensel, M., Curtiss, R., ... & Weiss, S. (2015). Efficiency of conditionally attenuated Salmonella enterica serovar Typhimurium in bacterium-mediated tumor therapy. *MBio*, *6*(2).

Gajewski, T. F. (2015, August). The next hurdle in cancer immunotherapy: Overcoming the non–T-cell–inflamed tumor microenvironment. In *Seminars in Oncology* (Vol. 42, No. 4, pp. 663-671). WB Saunders.

Galluzzi, L., Bravo-San Pedro, J. M., Vitale, I., Aaronson, S. A., Abrams, J. M., Adam, D., ... & Baehrecke, E. H. (2015). Essential versus accessory aspects of cell death: recommendations of the NCCD 2015. *Cell Death & Differentiation*, *22*(1), 58-73.

Galluzzi, L., Buqué, A., Kepp, O., Zitvogel, L., & Kroemer, G. (2017). Immunogenic cell death in cancer and infectious disease. *Nature Reviews Immunology*, *17*(2), 97.

Galluzzi, L., Chan, T. A., Kroemer, G., Wolchok, J. D., & López-Soto, A. (2018). The hallmarks of successful anticancer immunotherapy. *Science Translational Medicine*, *10*(459).

Galluzzi, L., & Kroemer, G. (2017). Secondary necrosis: accidental no more. *Trends in Cancer*, *3*(1), 1-2.

Galluzzi, L., Vitale, I., Abrams, J. M., Alnemri, E. S., Baehrecke, E. H., Blagosklonny, M. V., ... & Gottlieb, E. (2012). Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. *Cell Death & Differentiation*, *19*(1), 107-120.

George, L., Bavya, M. C., Rohan, K. V., & Srivastava, R. (2017). A therapeutic polyelectrolyte–vitamin C nanoparticulate system in polyvinyl alcohol–alginate hydrogel: An approach to treat skin and soft tissue infections caused by Staphylococcus aureus. *Colloids and Surfaces B: Biointerfaces*, *160*, 315-324.

Goldberg, M. S. (2019). Improving cancer immunotherapy through nanotechnology. *Nature Reviews Cancer*, *19*(10), 587-602.

Gotwals, P., Cameron, S., Cipolletta, D., Cremasco, V., Crystal, A., Hewes, B., ... & Engelman, J. A. (2017). Prospects for combining targeted and conventional cancer therapy with immunotherapy. *Nature Reviews Cancer*, *17*(5), 286-301.

Green, D. R., & Llambi, F. (2015). Cell death signaling. *Cold Spring Harbor Perspectives in Biology*, *7*(12), 1-24.

Grivennikov, S. I., Greten, F. R., & Karin, M. (2010). Immunity, inflammation, and cancer. *Cell*, *140*(6), 883-899.

Guillerey, C., Huntington, N. D., & Smyth, M. J. (2016). Targeting natural killer cells in cancer immunotherapy. *Nature Immunology, 17*(9), 1025-1036.

Gwee, C. P., Khoo, C. H., Yeap, S. K., Tan, G. C., & Cheah, Y. K. (2019). Targeted inactivation of Salmonella Agona metabolic genes by group II introns and in vivo assessment of pathogenicity and anti-tumour activity in mouse model. *PeerJ*, *7*, e5989.

Han, Y. H., Kee, J. Y., & Hong, S. H. (2018). Rosmarinic acid activates AMPK to inhibit metastasis of colorectal cancer. *Frontiers in Pharmacology*, *9*, 68.

Hedges, A. J. (2002). Estimating the precision of serial dilutions and viable bacterial counts. *International Journal of Food Microbiology*, *76*(3), 207-214.

Hoffman, R. M. (Ed.). (2016). *Bacterial therapy of cancer: methods and protocols*. Humana Press.

Hoffman, R. M., & Zhao, M. (2014). Methods for the development of tumor-targeting bacteria. *Expert Opinion on Drug Discovery*, *9*(7), 741-750.

Ichim, G., & Tait, S. W. (2016). A fate worse than death: apoptosis as an oncogenic process. *Nature Reviews Cancer*, *16*(8), 539.

Idelevich, E. A., & Becker, K. (2019). How to accelerate antimicrobial susceptibility testing. *Clinical Microbiology and Infection, 25*(11), 1347-1355.

Iheagwara, U. K., Beatty, P. L., Van, P. T., Ross, T. M., Minden, J. S., & Finn, O. J. (2014). Influenza virus infection elicits protective antibodies and T cells specific for host cell antigens also expressed as tumor-associated antigens: a new view of cancer immunosurveillance. *Cancer Immunology Research*, *2*(3), 263-273.

Jessy, T. (2011). Immunity over inability: The spontaneous regression of cancer. *Journal of Natural Science, Biology, and Medicine*, *2*(1), 43.

Jones, M. A., Wood, M. W., Mullan, P. B., Watson, P. R., Wallis, T. S., & Galyov, E. E. (1998). Secreted effector proteins of Salmonella dublin act in concert to induce enteritis. *Infection and Immunity*, *66*(12), 5799-5804.

Kahraman, M., Zamaleeva, A. I., Fakhrullin, R. F., & Culha, M. (2009). Layer-by-layer coating of bacteria with noble metal nanoparticles for surface-enhanced Raman scattering. *Analytical and Bioanalytical Chemistry*, *395*(8), 2559.

Kawano, M., Manabe, T., & Kawasaki, K. (2010). Salmonella enterica serovar Typhimurium lipopolysaccharide deacylation enhances its intracellular growth within macrophages. *FEBS letters*, *584*(1), 207-212.

Keestra-Gounder, A. M., Tsolis, R. M., & Bäumler, A. J. (2015). Now you see me, now you don't: the interaction of Salmonella with innate immune receptors. *Nature Reviews Microbiology*, *13*(4), 206-216.

Kelderman, S., & Kvistborg, P. (2016). Tumor antigens in human cancer control. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*, *1865*(1), 83-89.

Khoo, C. H., Cheah, Y. K., Lee, L. H., Sim, J. H., Salleh, N. A., Sidik, S. M., ... & Sukardi, S. (2009). Virulotyping of Salmonella enterica subsp. enterica isolated from indigenous vegetables and poultry meat in Malaysia using multiplex-PCR. *Antonie van Leeuwenhoek*, *96*(4), 441.

Khoo, C. H., Sim, J. H., Salleh, N. A., & Cheah, Y. K. (2015). Pathogenicity and phenotypic analysis of sopB, sopD and pipD virulence factors in Salmonella enterica serovar Typhimurium and Salmonella enterica serovar Agona. *Antonie Van Leeuwenhoek*, *107*(1), 23-37.

Kim, J. E., Phan, T. X., Nguyen, V. H., Dinh-Vu, H. V., Zheng, J. H., Yun, M., ... & Hwang, W. (2015). Salmonella typhimurium suppresses tumor growth via the pro-inflammatory cytokine interleukin-1β. *Theranostics*, *5*(12), 1328.

Konnova, S. A., Danilushkina, A. A., Fakhrullina, G. I., Akhatova, F. S., Badrutdinov, A. R., & Fakhrullin, R. F. (2015). Silver nanoparticle-coated "cyborg" microorganisms: rapid assembly of polymer-stabilised nanoparticles on microbial cells. *RSC Advances*, *5*(18), 13530-13537.

Kroschinsky, F., Stölzel, F., von Bonin, S., Beutel, G., Kochanek, M., Kiehl, M., & Schellongowski, P. (2017). New drugs, new toxicities: severe side effects of modern targeted and immunotherapy of cancer and their management. *Critical Care*, *21*(1), 89.

Kucerova, P., & Cervinkova, M. (2016). Spontaneous regression of tumour and the role of microbial infection–possibilities for cancer treatment. *Anti-Cancer Drugs*, *27*(4), 269.

Kupz, A., Curtiss III, R., Bedoui, S., & Strugnell, R. A. (2014). In vivo IFN-γ secretion by NK cells in response to Salmonella typhimurium requires NLRC4 inflammasomes. *PloS One*, *9*(5), e97418.

Kwon, S. Y., & Min, J. J. (2014). Genetically engineered Salmonella typhimurium for targeted cancer therapy. In *Gene Therapy of Cancer* (pp. 443-452). Academic Press.

Laerd Statistics. (2017a). Testing for normality in SPSS Statistics. Statistical tutorials and software guides. Retrieved from https://statistics.laerd.com/premium/spss/tfn/testing-for-normality-in-spss.php

Laerd Statistics (2017b). One-way ANOVA using SPSS Statistics. Statistical tutorials and software guides. Retrieved from https://statistics.laerd.com/

Lalsiamthara, J., Senevirathne, A., So, M. Y., & Lee, J. H. (2018). Safety implication of Salmonella based Brucella vaccine candidate in mice and in vitro human cell culture. *Vaccine*, *36*(14), 1837-1845.

Lee, C. H., Lin, S. T., Liu, J. J., Chang, W. W., Hsieh, J. L., & Wang, W. K. (2014). Salmonella induce autophagy in melanoma by the downregulation of AKT/mTOR pathway. *Gene Therapy*, *21*(3), 309-316.

Lee, C. H., Lin, Y. H., Hsieh, J. L., Chen, M. C., & Kuo, W. L. (2013). A polymer coating applied to Salmonella prevents the binding of Salmonella-specific antibodies. *International Journal of Cancer*, *132*(3), 717-725.

Lee, C. H., Wu, C. L., Chen, S. H., & Shiau, A. L. (2009). Humoral immune responses inhibit the antitumor activities mediated by Salmonella enterica serovar Choleraesuis. *Journal of Immunotherapy*, *32*(4), 376-388.

Lee, C. H., Wu, C. L., & Shiau, A. L. (2005a). Systemic administration of attenuated Salmonella choleraesuis in combination with cisplatin for tumor therapy. *Molecular Therapy, 11*, 707–716.

Lee, C. H., Wu, C. L., & Shiau, A. L. (2005b). Systemic administration of attenuated Salmonella choleraesuis carrying thrombospondin-1 gene leads to tumor-specific transgene expression, delayed tumor growth and prolonged survival in the murine melanoma model. *Cancer Gene Therapy*, *12*(2), 175-184.

Lehouritis, P., Hogan, G., & Tangney, M. (2017). Designer bacteria as intratumoural enzyme biofactories. *Advanced Drug Delivery Reviews*, *118*, 8-23.

Leigh, N. D., Bian, G., Ding, X., Liu, H., Aygun-Sunar, S., Burdelya, L. G., ... & Cao, X. (2014). A flagellin-derived toll-like receptor 5 agonist stimulates cytotoxic lymphocyte-mediated tumor immunity. *PloS One*, *9*(1), e85587.

Leschner, S., & Weiss, S. (2010). Salmonella—allies in the fight against cancer. *Journal of Molecular Medicine*, *88*(8), 763-773.

Leschner, S., Westphal, K., Dietrich, N., Viegas, N., Jablonska, J., Lyszkiewicz, M., ... & Weiss, S. (2009). Tumor invasion of Salmonella enterica serovar Typhimurium is accompanied by strong hemorrhage promoted by TNF-α. *PloS One*, *4*(8), e6692.

Li, Y., Wang, S., Xin, W., Scarpellini, G., Shi, Z., Gunn, B., ... & Curtiss, R. (2008). A sopB deletion mutation enhances the immunogenicity and protective efficacy of a heterologous antigen delivered by live attenuated Salmonella enterica vaccines. *Infection and Immunity*, *76*(11), 5238-5246.

Lin, H. C., Yang, C. J., Kuan, Y. D., Wang, W. K., Chang, W. W., & Lee, C. H. (2017). The inhibition of indoleamine 2, 3-dioxygenase 1 by connexin 43. *International Journal of Medical Sciences*, *14*(12), 1181.

Liu, Z., Han, C., & Fu, Y. X. (2019). Targeting innate sensing in the tumor microenvironment to improve immunotherapy. *Cellular & Molecular Immunology*, 1-14.

Maciejowski, J., & de Lange, T. (2017). Telomeres in cancer: tumour suppression and genome instability. *Nature Reviews Molecular Cell Biology*, *18*(3), 175-186.

Mah, L. Y., & Ryan, K. M. (2012). Autophagy and cancer. *Cold Spring Harbor Perspectives in Biology*, *4*(1), a008821.

Majowicz, S. E., Musto, J., Scallan, E., Angulo, F. J., Kirk, M., O'Brien, S. J., ... & International Collaboration on Enteric Disease "Burden of Illness" Studies. (2010). The global burden of nontyphoidal Salmonella gastroenteritis. *Clinical Infectious Diseases, 50*(6), 882-889.

Mangalmurti, N., & Hunter, C. A. (2020). Cytokine storms: understanding COVID-19. *Immunity*.

Manickam, A., & Sivanandham, M. (2011). Mycobacterium bovis BCG and purified protein derivative-induced reduction in the CD80 expression and the antigen up-take function of dendritic cells from patients with cervical cancer. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, *159*(2), 413-417.

Martínez-Lostao, L., Anel, A., & Pardo, J. (2015). How do cytotoxic lymphocytes kill cancer cells?. *Clinical Cancer Research*, *21*(22), 5047–5056.

Mastrangelo, G., Grange, J. M., Fadda, E., Fedeli, U., Buja, A., & Lange, J. H. (2005). Lung cancer risk: effect of dairy farming and the consequence of removing that occupational exposure. *American Journal of Epidemiology*, *161*(11), 1037-1046.

McCarthy, E. F. (2006). The toxins of William B. Coley and the treatment of bone and soft-tissue sarcomas. *The Iowa Orthopaedic Journal*, *26*, 154.

Michael, A., Kelman, T., & Pitesky, M. (2020). Overview of Quantitative Methodologies to Understand Antimicrobial Resistance via Minimum Inhibitory Concentration. *Animals, 10*(8), 1405.

Mohd Idris, M. F. (2012). *Effects on Kidney and Liver Enzyme Levels after Treatment with Wild Type And Knock-Out Salmonella Typhimurium and Salmonella Agona on Mice Induced With Cancer Model* (Bachelor's dissertation). Universiti Putra Malaysia.

Morrissey, D., O'Sullivan, G. C., & Tangney, M. (2010). Tumour targeting with systemically administered bacteria. *Current Gene Therapy*, *10*(1), 3-14.

Motulsky, H. J. (2020a). How it works: ROUT method. Retrieved from https://www.graphpad.com/guides/prism/7/statistics/stat_how_it_works_rout_method.htm

Motulsky, H. J. (2020b). Interpreting results: Welch and Brown-Forsythe tests. Retrieved from https://www.graphpad.com/guides/prism/8/statistics/interpreting_welch_browne-forsythe_tests.htm

Mu, H., Bai, H., Sun, F., Liu, Y., Lu, C., Qiu, Y., ... & Duan, J. (2019). Pathogen-targeting glycovesicles as a therapy for salmonellosis. *Nature Communications, 10*(1), 1-8.

Murakami, T., Hiroshima, Y., Zhao, M., Zhang, Y., Chishima, T., Tanaka, K., ... & Hoffman, R. M. (2015). Therapeutic efficacy of tumor-targeting Salmonella typhimurium A1-R on human colorectal cancer liver metastasis in orthotopic nude-mouse models. *Oncotarget*, *6*(31), 31368.

Naim, U. (2012). *Assessment of Wild Type and Salmonella Pathogenicity Islands (SPIs) Knockout Salmonella Enterica Serovar Typhimurium (S. Typhimurium) and Salmonella Enterica Serovar Agona (S. Agona) as s Tumour Reduction Agent using Mice as Model* (Bachelor's dissertation). Universiti Putra Malaysia.

National Cancer Institute. (2019a). Definition of solid tumor - NCI Dictionary of Cancer Terms - National Cancer Institute. Retrieved from https://www.cancer.gov/publications/dictionaries/cancer-terms/def/solid-tumor

National Cancer Institute. (2019b). Immunotherapy for Cancer. Retrieved from https://www.cancer.gov/about-cancer/treatment/types/immunotherapy

National Cancer Institute. (2019c). Side Effects of Immunotherapy - National Cancer Institute. Retrieved from https://www.cancer.gov/about-cancer/treatment/types/immunotherapy/side-effects

Nguyen, C. T., Hong, S. H., Sin, J. I., Vu, H. V. D., Jeong, K., Cho, K. O., ... & Rhee, J. H. (2013). Flagellin enhances tumor-specific CD8+ T cell immune responses through TLR5 stimulation in a therapeutic cancer vaccine model. *Vaccine*, *31*(37), 3879-3887.

Onwuezobe, I. A., Oshun, P. O., & Odigwe, C. C. (2012). Antimicrobials for treating symptomatic non-typhoidal Salmonella infection. *Cochrane Database of Systematic Reviews*, (11).

Patyar, S., Joshi, R., Byrav, D. P., Prakash, A., Medhi, B., & Das, B. K. (2010). Bacteria in cancer therapy: a novel experimental strategy. *Journal of Biomedical Science*, *17*(1), 21.

Pfeffer, C. M., & Singh, A. T. (2018). Apoptosis: a target for anticancer therapy. *International Journal of Molecular Sciences*, *19*(2), 448.

Phan, T. X., Nguyen, V. H., Duong, M. T. Q., Hong, Y., Choy, H. E., & Min, J. J. (2015). Activation of inflammasome by attenuated Salmonella typhimurium in bacteria-mediated cancer therapy. *Microbiology and Immunology*, *59*(11), 664-675.

Polk, A., Vaage-Nilsen, M., Vistisen, K., & Nielsen, D. L. (2013). Cardiotoxicity in cancer patients treated with 5-fluorouracil or capecitabine: a systematic review of incidence, manifestations and predisposing factors. *Cancer Treatment Reviews*, *39*(8), 974-984.

Popowicz, N. D., Lansley, S. M., Cheah, H. M., Kay, I. D., Carson, C. F., Waterer, G. W., ... & Lee, Y. G. (2017). Human pleural fluid is a potent growth medium for Streptococcus pneumoniae. *PLoS One*, *12*(11), e0188833.

Pui, C. F., Wong, W. C., Chai, L. C., Nillian, E., Ghazali, F. M., Cheah, Y. K., ... & Radu, S. (2011). Simultaneous detection of Salmonella spp., Salmonella Typhi and Salmonella Typhimurium in sliced fruits using multiplex PCR. *Food Control, 22*(2), 337-342.

Rajendran, S. K. (2017). *Clinicopathological assessment of intramammary administration of genetically engineered Salmonella Agona (4KA32) in domestic dogs* (Master's dissertation). Universiti Putra Malaysia.

Rathman, M., Barker, L. P., & Falkow, S. (1997). The unique trafficking pattern of Salmonella typhimurium-containing phagosomes in murine macrophages is independent of the mechanism of bacterial entry. *Infection and Immunity*, *65*(4), 1475-1485.

Rodloff, A., Bauer, T., Ewig, S., Kujath, P., & Müller, E. (2008). Susceptible, intermediate, and resistant–the intensity of antibiotic action. *Deutsches Ärzteblatt International*, *105*(39), 657.

Roland, K. L., & Brenneman, K. E. (2013). Salmonella as a vaccine delivery vehicle. *Expert Review of Vaccines*, *12*(9), 1033-1045.

Rolls, G., & Sampias, C. (2019). An Intro to H&E Staining: Protocol, Best Practices, Steps & More. Retrieved from https://www.leicabiosystems.com/knowledge-pathway/he-staining-overview-a-guide-to-best-practices/

Rosenberg, S. A., Spiess, P. J., & Kleiner, D. E. (2002). Antitumor effects in mice of the intravenous injection of attenuated Salmonella typhimurium. *Journal of Immunotherapy (Hagerstown, Md.: 1997)*, *25*(3), 218.

Rubio, C. A., Ásmundsson, J., Silva, P., Illies, C., Hartman, J., & Kis, L. (2013). Lymphoid aggregates in Crohn's colitis and mucosal immunity. *Virchows Archiv*, *463*(5), 637-642.

Russell, B. A., Jachimska, B., & Chen, Y. (2018). Polyallylamine hydrochloride coating enhances the fluorescence emission of Human Serum Albumin encapsulated gold nanoclusters. *Journal of Photochemistry and Photobiology B: Biology*, *187*, 131-135.

Saccheri, F., Pozzi, C., Avogadri, F., Barozzi, S., Faretta, M., Fusi, P., & Rescigno, M. (2010). Bacteria-induced gap junctions in tumors favor antigen cross-presentation and antitumor immunity. *Science Translational Medicine*, *2*(44), 44ra57-44ra57.

Saga, K., & Kaneda, Y. (2013). Virosome presents multimodel cancer therapy without viral replication. *BioMed Research International*, *2013*.

Sakoulas, G., Kumaraswamy, M., Kousha, A., & Nizet, V. (2017). Interaction of antibiotics with innate host defense factors against Salmonella enterica serotype newport. *Msphere, 2*(6).

Salleh, N. A., Rusul, G., Hassan, Z., Reezal, A., Isa, S. H., Nishibuchi, M., & Radu, S. (2003). Incidence of Salmonella spp. in raw vegetables in Selangor, Malaysia. *Food Control*, *14*(7), 475-479.

Schiavoni, G., Gabriele, L., & Mattei, F. (2013). The tumor microenvironment: a pitch for multiple players. *Frontiers in Oncology*, *3*, 90.

Schmidt, H., & Hensel, M. (2004). Pathogenicity islands in bacterial pathogenesis. *Clinical Microbiology Reviews*, *17*(1), 14-56.

Schuerle, S., & Danino, T. (2020). Bacteria as Living Microrobots to Fight Cancer. Retrieved from https://www.the-scientist.com/features/bacteria-as-living-microrobots-to-fight-cancer-67305

Séon, L., Lavalle, P., Schaaf, P., & Boulmedais, F. (2015). Polyelectrolyte multilayers: a versatile tool for preparing antimicrobial coatings. *Langmuir*, *31*(47), 12856-12872.

Serrano, M. P., Rafti, M., Thomas, A. H., & Borsarelli, C. D. (2019). Photosensitizing properties of hollow microcapsules built by multilayer self-assembly of poly (allylamine hydrochloride) modified with rose Bengal. *RSC Advances*, *9*(33), 19226-19235.

Sever, R., & Brugge, J. S. (2015). Signal transduction in cancer. *Cold Spring Harbor Perspectives in Medicine*, *5*(4), a006098.

Sfondrini, L., Rossini, A., Besusso, D., Merlo, A., Tagliabue, E., Mènard, S., & Balsari, A. (2006). Antitumor activity of the TLR-5 ligand flagellin in mouse models of cancer. *The Journal of Immunology*, *176*(11), 6624-6630.

Shapiro, M. G., Goodwill, P. W., Neogy, A., Yin, M., Foster, F. S., Schaffer, D. V., & Conolly, S. M. (2014). Biogenic gas nanostructures as ultrasonic molecular reporters. *Nature Nanotechnology*, *9*(4), 311.

Shurin, M. R., Zong, J., & Keskinov, A. A. (2015). Infection and Cancer: Multi-directorial Relationship. In *Infection and Cancer: Bi-Directorial Interactions* (pp. 1-10). Springer, Cham.

Shutava, T. G., Fakhrullin, R. F., & Lvov, Y. M. (2014). Spherical and tubule nanocarriers for sustained drug release. *Current Opinion in Pharmacology*, *18*, 141-148.

Sidik, M. A. (2012). *Effects on Brain and Neutrophil Count after Treatment with Wild Type and Knock-out Salmonella Typhimurium and Salmonella Agona in Induced Cancer Model Mice* (Bachelor's dissertation). Universiti Putra Malaysia.

Silva, J., Leite, D., Fernandes, M., Mena, C., Gibbs, P. A., & Teixeira, P. (2011). Campylobacter spp. as a foodborne pathogen: a review. *Frontiers in Microbiology, 2*, 200.

Stern C., Kasnitz N., Kocijancic D., Trittel S., Riese P., Guzman C.A., Leschner S., Weiss S.. Induction of CD4(+) and CD8(+) anti-tumor effector T cell responses by bacteria mediated tumor therapy (2015). *International Journal of Cancer, 137*(8):2019-28.

Syal, K., Mo, M., Yu, H., Iriya, R., Jing, W., Guodong, S., ... & Tao, N. (2017). Current and emerging techniques for antibiotic susceptibility tests. *Theranostics*, *7*(7), 1795.

Tait, S. W., Ichim, G., & Green, D. R. (2014). Die another way–non-apoptotic mechanisms of cell death. *Journal of Cell Science*, *127*(10), 2135-2144.

Tang, A. C., Rahavi, S. M., Fung, S. Y., Lu, H. Y., Yang, H., Lim, C. J., ... & Turvey, S. E. (2018). Combination therapy with proteasome inhibitors and TLR agonists enhances tumour cell death and IL-1β production. *Cell Death & Disease, 9*(2), 1-13.

Taniguchi, S. I., Fujimori, M., Sasaki, T., Tsutsui, H., Shimatani, Y., Seki, K., & Amano, J. (2010). Targeting solid tumors with non-pathogenic obligate anaerobic bacteria. *Cancer Science*, *101*(9), 1925-1932.

Toso, J. F., Gill, V. J., Hwu, P., Marincola, F. M., Restifo, N. P., Schwartzentruber, D. J., ... & Freezer, L. J. (2002). Phase I study of the intravenous administration of attenuated Salmonella typhimurium to patients with metastatic melanoma. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, *20*(1), 142.

Toussaint, B., Chauchet, X., Wang, Y., Polack, B., & Gouëllec, A. L. (2013). Live-attenuated bacteria as a cancer vaccine vector. *Expert Review of Vaccines*, *12*(10), 1139-1154.

Tsujimoto, Y. (2012). Multiple ways to die: non-apoptotic forms of cell death. *Acta Oncologica*, *51*(3), 293-300.

Ubezio, P. (2019). Beyond The T/C Ratio: Old And New Anticancer Activity Scores In Vivo. *Cancer Management and Research*, *11*, 8529.

Uchugonova, A., Zhang, Y., Salz, R., Liu, F., Suetsugu, A., Zhang, L., ... & Zhao, M. (2015). Imaging the different mechanisms of prostate cancer cell-killing by tumor-targeting Salmonella typhimurium A1-R. *Anticancer Research*, *35*(10), 5225-5229.

Uribe-Querol, E., & Rosales, C. (2015). Neutrophils in cancer: two sides of the same coin. *Journal of Immunology Research*, *2015*.

Vakkila, J., & Lotze, M. T. (2004). Inflammation and necrosis promote tumour growth. *Nature Reviews Immunology*, *4*(8), 641-648.

Valdez Y., Ferreira R.B.R., Finlay B.B. (2009) Molecular Mechanisms of *Salmonella* Virulence and Host Resistance. In Sasakawa C. (eds) *Molecular Mechanisms of Bacterial Infection via the Gut*. Current Topics in Microbiology and Immunology, vol 337. Springer, Berlin, Heidelberg.

Vinay, D. S., Ryan, E. P., Pawelec, G., Talib, W. H., Stagg, J., Elkord, E., ... & Kwon, B. S. (2015). Immune evasion in cancer: Mechanistic basis and therapeutic strategies. *In Seminars in Cancer Biology* (Vol. 35, pp. S185-S198). Academic Press.

Vitalis, R. E., Regidor-lll, D., Haresh, K. K., Nishibuchi, M., Nor-Khaizura, M. A. R., & Son, R. (2016). Methicillin-resistant Staphylococcus aureus strains isolated from human and environmental surfaces in a research laboratory. *International Food Research Journal, 23*(6), 2710.

Waldmann, T. A. (2018). Cytokines in cancer immunotherapy. *Cold Spring Harbor Perspectives in Biology, 10*(12), a028472.

Wall, D. M., Srikanth, C. V., & McCormick, B. A. (2010). Targeting tumors with salmonella Typhimurium-potential for therapy. *Oncotarget*, *1*(8), 721.

Wang, C. Z., Kazmierczak, R. A., & Eisenstark, A. (2016). Strains, mechanism, and perspective: Salmonella-based cancer therapy. *International Journal of Microbiology*, *2016*.

Wang, J., Li, D., Chang, H., & Guo, B. (2019). Crosstalk between cancer and immune cells: Role of tumor-associated macrophages in the tumor microenvironment. *Cancer Medicine, 8*(10), 4709-4721.

Wang, W. K., Kuan, Y. D., Kuo, C. Y., & Lee, C. H. (2014). Connexin 43 gene therapy delivered by polymer-modified Salmonella in murine tumor models. *Polymers*, *6*(4), 1119-1128.

Westphal, K., Leschner, S., Jablonska, J., Loessner, H., & Weiss, S. (2008). Containment of tumor-colonizing bacteria by host neutrophils. *Cancer Research*, *68*(8), 2952-2960.

Wisner, A., Desin, T., White, A., Potter, A., & Köster, W. (2012). The Salmonella pathogenicity island-1 and-2 encoded type III secretion systems. *Salmonella–A Diversified Superbug,* 469-494.

World Health Organization Agency for Research on Cancer (IARC). (2018). GLOBOCAN 2018: estimated cancer incidence, mortality and prevalence worldwide in 2018. Retrieved from https://gco.iarc.fr/today/data/factsheets/populations/458-malaysia-fact-sheets.pdf

World Health Organization. (2020). WHO report on cancer: setting priorities, investing wisely and providing care for all.

World Organization for Animal Health (OIE) (2012). Guideline 2.1, Laboratory methodologies for bacterial antimicrobial susceptibility testing. Retrieved from https://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/GUIDE_2.1_ANTIMICROBIAL.pdf.

Xie, L., Tong, W., Yu, D., Xu, J., Li, J., & Gao, C. (2012). Bovine serum albumin nanoparticles modified with multilayers and aptamers for pH-responsive and targeted anti-cancer drug delivery. *Journal of Materials Chemistry*, *22*(13), 6053-6060.

Yoke-Kqueen, C., Learn-Han, L., Noorzaleha, A. S., Son, R., Sabrina, S., Jiun-Horng, S., & Chai-Hoon, K. (2008). Characterization of multiple-antimicrobial-resistant Salmonella enterica subsp. enterica isolated from indigenous vegetables and poultry in Malaysia. *Letters in Applied Microbiology*, *46*(3), 318-324.

Yong, A. Y., Shabahang, S., Timiryasova, T. M., Zhang, Q., Beltz, R., Gentschev, I., ... & Szalay, A. A. (2004). Visualization of tumors and metastases in live animals with bacteria and vaccinia virus encoding light-emitting proteins. *Nature Biotechnology*, *22*(3), 313-320.

Yoon, W. S., Chae, Y. S., Hong, J., & Park, Y. K. (2011). Antitumor therapeutic effects of a genetically engineered Salmonella typhimurium harboring TNF-α in mice. *Applied Microbiology and Biotechnology*, *89*(6), 1807-1819.

Zhang, J. M., & An, J. (2007). Cytokines, inflammation and pain. *International Anesthesiology Clinics, 45*(2), 27.

Zhang, M., Swofford, C. A., & Forbes, N. S. (2014). Lipid A controls the robustness of intratumoral accumulation of attenuated Salmonella in mice. *International Journal of Cancer*, *135*(3), 647-657.

Zhao, M., Yang, M., Ma, H., Li, X., Tan, X., Li, S., ... & Hoffman, R. M. (2006). Targeted therapy with a Salmonella typhimurium leucine-arginine auxotroph cures orthotopic human breast tumors in nude mice. *Cancer Research*, *66*(15), 7647-7652.

Zhou, S., Gravekamp, C., Bermudes, D., & Liu, K. (2018). Tumour-targeting bacteria engineered to fight cancer. *Nature Reviews Cancer*, *18*(12), 727-743.

Zheng, D. W., Chen, Y., Li, Z. H., Xu, L., Li, C. X., Li, B., ... & Zhang, X. Z. (2018). Optically-controlled bacterial metabolite for cancer therapy. *Nature Communications*, *9*(1), 1680.

Zheng, J. H., & Min, J. J. (2016). Targeted cancer therapy using engineered Salmonella typhimurium. *Chonnam Medical Journal*, *52*(3), 173-184.

Zia, U.N.T.N., Ng, E.S.N.A., Sidik, M.A., Idris, M.F.M., Khoo, C.H., Sukardi, S., … & Cheah, Y.K. (2021). Tumour Growth Inhibition and Systemic Responses of ΔsopBΔsopDΔpipD Disrupted Salmonella Agona and Salmonella Typhimurium in Mice. *Malaysian Journal of Medicine and Health Sciences, 17*(2), 63-71.