Exploration of factors that determine heterogeneity in efficacy and toxicities of anticancer drugs

By

Ganessan Kichenadasse

MBBS, FRACP, M Clin Edn

Thesis
Submitted to Flinders University
for the degree of

Doctor of Philosophy

College of Medicine and Public health

December 2020

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3. ABBREVIATIONS

5FU – 5- Fluorouracil

95%CI - 95% confidence intervals

ACEI – angiotensin-converting enzyme inhibitors

AEoSI - AEs of special interest

AEs – adverse events

AIC - Akaike information criterion

ARBs – angiotensin II receptor blockers

AUC – area under the curve

BB – beta blockers

BEV – bevacizumab

BMI – Body mass index

BOR – best overall response

BRAF - V-**R**apidly **A**ccelerated **F**ibrosarcoma Murine Sarcoma Viral Oncogene Homolog B

CAPOX – capecitabine/oxaliplatin

CCB – calcium channel blockers

CEA – carcinoembryonic antigen

C_{max} - maximum plasma steady state concentrations

CSR – clinical study reports

CR – complete response

CRC – colorectal cancer

CRP - C-reactive protein

$C_{ss,\ min}$ - steady state trough concentration

C-statistic - concordance statistic

CTLA-4 - cytotoxic T-lymphocyte antigen-4

CV – cardiovascular

CYP – cytochrome P450

DRI – direct renin inhibitors

DDIs - drug-drug interactions

ECOG – Eastern Co-operative Oncology Group

EGFR – epidermal growth factor receptor

FOLFIRI – folinic acid, 5FU, irinotecan

FOLFOX - folinic acid, 5FU, oxaliplatin

GDP - gross domestic product

GLOBOCAN – global cancer observatory

hENT1 - human equilibrative nucleoside transporter 1

HPB - Hepatobiliary and pancreatic

HR - hazard ratios

ICI - immune checkpoint inhibitors

IFL – irinotecan, 5FU, leucovorin

imAEs – immune mediated adverse events

IPD – individual patient data

irAEs - immune related adverse events

ITT - intention to treat

LDH - lactate dehydrogenase

LIPI - lung immune prognostic index

LTUC – lower tract urothelial cancer

MEK - mitogen activated protein kinase

MMR – mismatch repair

MSI – microsatellite instability

NCI-CTCAE v4.0 - National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0

NLR - neutrophil to lymphocyte ratio

NSCLC- non-small cell lung cancer

OATP1A/1B - organic anion-transporting polypeptides

OCT1/3 - organic cation transporters

OR - odds ratios

OS – overall survival

PD – progressive disease/pharmacodynamic

PD1 – programmed death -1

PDL1 – programmed death ligand 1

PFS – progression free survival

PK – pharmacokinetic

PPIs - proton pump inhibitors

PR – partial response

PS – Performance status

RAM – ramucirumab

RAS - Renin - angiotensin system

RASi - Renin - angiotensin system inhibitors

RECIST - Response Evaluation Criteria in Solid Tumours

ROC - receiver operating curve

SD – standard deviation/stable disease

TRAEs - treatment related adverse events

TCGA – the cancer genome atlas

UGT1A1 - uridine 5'-diphospho-glucuronosyltransferase enzyme 1A1

ULN - upper limit of normal

UTUC – upper tract urothelial cancer

VEGFi – vascular endothelial growth factor receptor inhibitors

WHO – World Health Organisation

4. SUMMARY

"Variability is the law of life, and as no two faces are the same, so no two bodies are alike, and no two individuals react alike and behave alike under the abnormal conditions which we know as disease"
(Osler 1905)

This thesis evaluates the previously under recognized factors that may contribute to the variations in response and toxicity from systemic cancer drugs. It was hypothesised that selected characteristics (patient anthropometric data (body mass index - BMI), use of concomitant medications such as renin-angiotensin inhibitors (RASi) and proton pump inhibitors (PPI), occurrence of immune-related adverse events (irAEs), primary site of origin of cancer and plasma concentration of the cancer drugs) can predict outcomes across a spectrum of systemic cancer drugs. All research studies in this thesis were conducted using individual patient data case report forms of 10,158 patients with five types of cancers who participated in 15 different clinical trials which were accessed using various data sharing platforms.

My research suggests that contrary to prior belief that obesity is a negative factor for drug therapy associated outcomes, patients with lung cancer (N = 1,548, from 4 trials) and high BMI had improved survival when treated with atezolizumab (pooled hazard ratios (HR) of 0.36 [95%CI, 0.21-0.62], P <0.001) for the group with obesity). While the proton pump inhibitors use was associated with worse survival (pooled HR 1.20, [95% CI 1.03-1.40], P = 0.02) in fluoropyrimidine-based chemotherapy treated colorectal cancer patients (N = 5,594 from 6 trials), the use of renin-angiotensin inhibitors were not (pooled HR 0.94, [95% CI 0.82-1.07], P = 0.38) in atezolizumab treated patients with lung, bladder, or kidney cancers (N = 2,539 from 7 trials). Both these findings on concomitant medications were surprising and unexpected as the pre-clinical data indicated the contrary with improved efficacy of fluoropyrimidine-based chemotherapy with proton pump inhibitors and renin-angiotensin inhibitors augmented immune response.

Additionally, using data from 2 clinical trials involving 830 patients with advanced melanoma, a new threshold as target steady-state trough concentration ($C_{ss,min}$ ≥ 50 mg/L) for optimal dosing of vemurafenib, a braf inhibitor, that was associated with improved survival (HR 0.67,[95% CI 0.52–0.88] *P* = 0.003). For the first-time, I have described a detailed evaluation of the incidence (5% of atezolizumab treated patients), type, severity and time-profile of multi-organ irAEs using a large cohort of atezolizumab treated patients (N = 1,548

from 4 trials). Multi-organ adverse events were associated with improved survival (pooled HR = 0.47, [95%CI 0.28 - 0.78], P <0.0001). My research also demonstrated that the occurrence of any irAE was associated with improved survival in both atezolizumab treated patients as well as those treated with taxanes or vinca alkaloid-based chemotherapies, indicating that they were prognostic rather than predictive of response to immunotherapy alone.

Among the primary site of origin of urothelial cancers, prior research reported that those with upper tract origin have different molecular characteristics that predict a lower benefit from immunotherapies when compared to the more common urinary bladder cancers. However, using data from 3 clinical trials (N = 1,331 patients), my research demonstrated that both upper and lower tract urothelial cancers have no significant differences in survival (HR was 0.99, [95%CI 0.82-1.21], P = 0.98) when treated with atezolizumab.

Based on these results several conclusions were drawn: 1. baseline BMI should be considered as a stratification factor in future clinical trials if these findings are confirmed in future studies; 2. clinicians should consider minimising the concomitant use of proton pump inhibitors in patients initiating chemotherapy for advanced colorectal cancer; 3. the new threshold concentration provides further evidence to support optimized dosing to reach the trough concentration to reduce inter-individual variability in vemurafenib survival; 4. new information generated to support treating clinicians to anticipate, recognise and treat multi-organ irAEs as well as trigger further research to better understand the pathophysiology of those toxicities; 5. primary site of origin of urothelial cancers may not affect outcomes from single agent immunotherapy. Future research that address these questions and challenges raised from my thesis should be conducted to improve our understanding of inter-individual variations in drug therapies thereby supporting dose optimisation and improving outcomes of patients.

5. PUBLICATIONS

5.1 Arising from candidature

1. **Kichenadasse G**, Miners JO, Mangoni AA, Rowland A, Hopkins AM, Sorich MJ. Association between Body Mass Index and overall survival with immune checkpoint inhibitor therapy for advanced non-small cell lung cancer. *JAMA Oncol.* 2020 Mar; 6(4):512-518.
2. **Kichenadasse G**, Hughes JH, Miners JO, Mangoni AA, Rowland A, Hopkins AM, Sorich MJ. Relationship between vemurafenib plasma concentrations and survival outcomes in patients with advanced melanoma. *Cancer Chemother Pharmacol.* 2020 Mar; 85(3):615-620.
3. **Kichenadasse G**, Miners JO, Mangoni AA, Rowland A, Hopkins AM, Sorich MJ. Multi-organ immune-related adverse events during treatment with atezolizumab. *J Natl Compr Can Netw.* 2020 Sep; 18(9).

5.2 Other publications during candidature

1. Hopkins AM, **Kichenadasse G**, Karapetis CS, Rowland A, Sorich MJ. Concomitant antibiotic use and survival in urothelial carcinoma treated with atezolizumab [published online ahead of print, 2020 Jul 11]. *Eur Urol*. 2020;S0302-2838(20)30527-3.
2. Hopkins AM, **Kichenadasse G**, Garrett-Mayer E, Karapetis CS, Rowland A, Sorich MJ. Development and validation of a prognostic model for patients with advanced lung cancer treated with the immune checkpoint inhibitor atezolizumab. *Clin Cancer Res.* 2020 Jul; 26(13):3280-3286.
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7. Hopkins AM, **Kichenadasse G**, McKinnon RA, Rowland A, Sorich MJ. Baseline tumour size and survival outcomes in lung cancer patients treated with immune checkpoint inhibitors. *Semin Oncol.* 2019 Aug - Oct;46(4-5):380-384.
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6. DECLARATION

I certify that this thesis:

1. does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and
2. that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Signed..... .....

Date.....17th of December 2020.....

7. ACKNOWLEDGEMENTS

Looking for new challenges in life, I commenced my research work towards Doctor in Philosophy in the field of Clinical Pharmacology, nearly a decade ago. I am extremely grateful for all the support and encouragement I have received from various people – supervisors, mentors, work colleagues, my parents, friends and finally the patients I treat in my clinical practice.

I have several people to thank and acknowledge for all their support. I was fortunate to have had Prof John Miners and then Prof Michael Sorich as my primary supervisors for this doctoral thesis. Over the years, Prof Miners has guided me through his detailed, focussed and constructive feedback on my research work and performance. His expert guidance, support and pragmatic approach have been of immense help for me to get to where I am now. Initially, I started this high degree with a plan of data generated through laboratory experiments for my research work. Trying to juggle part-time clinical load and laboratory-based research simultaneously along with personal life related requirements became difficult. Prof Miners' timely advice helped to change track and reach out to Prof Sorich to explore research projects using his big data-based research .

Prof Sorich came on as the primary supervisor in the latter part of this work. His enthusiasm, keen interest for developing novel and innovative ideas, constructive feedback, resourcefulness in getting access to big data and mentorship have greatly helped the conduct and completion of my research.

I am also grateful to my other supervisors, Prof A Mangoni for his attention to detail and editing skills, Assoc Prof Andrew Rowland for his enthusiasm, support in the laboratory aspects of my research and Dr Ashley Hopkins who has been of major support and guide for data access, statistical analysis of data and teaching me R-codes that were widely used in this project. Prof Matthew Doogue who has now moved to Christchurch, New Zealand, was one of my other original supervisors. He was the major force in encouraging me to pursue Clinical Pharmacology and the doctoral thesis in this field. I am immensely thankful for his support.

I would also like to thank my medical oncology colleagues, Professors, Bogda Koczwara and Christos Karapetis, who were instrumental in providing me with time away from usual work and for all their encouragement through the years.

Flinders University and the Australian Government Research Training Program Scholarship have supported this higher degree with their resources, guidance workshops and funding for which I am thankful. I also wish to acknowledge my parents, friends and other colleagues for their great support and encouragement. Finally, the patients and their carers who I treat(ed) in my oncology clinical practice have been greatly interested in my progress and encouraging of the research work. Moreover, the data used in this research were generously provided by the clinical trial participants and sponsors, to whom I am extremely grateful.

1. CHAPTER ONE: INTRODUCTION

1.1 Cancer

Cancer remains a significant health problem due to its survival, physical and mental health, quality of life and financial impact on the patients with cancer, and a broader effect on their families, the health system, and society at large. The Global Cancer Observatory (GLOBOCAN) data in 2018 indicates that there were more than 18 million new cancer cases diagnosed and 9.5 million deaths were reported from cancer (Ferlay et al. 2018). The impact of cancer and its treatment on quality of life is well recognized with significant deterioration identified in patients with advanced cancers especially towards the end of life. Similarly, the economic impact and financial burden of cancer and its treatment on the patients, family and society are increasingly reported. While the current global economic burden of cancer is unknown, it is expected to be substantially higher than the previously estimated US$ 1.6 trillion in 2010 reflected by the health care spending and loss of productivity caused by cancer related outcomes (Knaul et al. 2014). In 2017, the estimated spending on cancer care in the United States was 1.8% of gross domestic product (GDP) while it was 1.07% of GDP in the European Union (Yabroff et al. 2019). The worldwide cost for cancer care was projected to increase to $150 billion in 2020 (Prager et al. 2018). More recently, the projected increase in cancer related medical care costs to over $245 billion in the United States alone (Mariotto et al. 2020). A large proportion of the increase in health care costs is likely from an increase in the costs of new therapies. Despite these challenges, there is optimism that the major advances in our understanding of cancer biology, implementation of prevention and screening programs, early diagnosis, and improvement in cancer therapies over the last several decades have reduced overall mortality and improved outcomes for various types of cancers (Hashim et al. 2016; Siegel, Miller, and Jemal 2020). However, advanced cancers remain a difficult group of illnesses to treat.

One of reasons that make cancers difficult to treat is the fact that cancer is not one disease and can affect multiple tissues. The word “cancer”, especially “malignancy”, encompasses a group of diseases that are characterised by unlimited replication potential, invasion to surrounding tissues and metastasis to distant organs. Every cancer is different in multiple levels. The multi-layered variability introduced by the organ of

origin of the cancer, aetiology, mutational profile, inter and intra-tumoural heterogeneity, tumoural evolution with or without therapy and patient characteristics make it extremely difficult to individualise treatment decisions. Despite these challenges, cancer therapies have been successfully employed to cure, control, or palliate symptoms at an individual and population level that has significantly improved survival for most cancers (Arnold et al. 2019).

1.2 Cancer treatment

The treatment of cancer has evolved over the last several centuries - from the use of blunt tools of radical surgeries to improved precision in targeting the individual mutations within cancer cells. Such progress was achieved with improvement in better understanding of cancer biology through laboratory-based technologies for studying cancer cells, imaging tools, improved surgical/ medical care and resources, improved understanding of risk factors and heritability of cancers, better supportive care opportunities through antibiotics, anaesthetics, intensive care support, drug therapies, vaccines and screening programs. Cancers are treated with various modalities such as surgery, radiotherapy, systemic therapies or other physical interventions like (but not limited to) cryotherapy, radiofrequency ablation and microwaves. These treatment interventions are provided either on their own as single intervention or part of multi-modality approaches such as surgery followed by systemic therapies and radiotherapy for breast cancer depending on multiple factors including stage, cancer type and biology, patient's preference and available expertise. Among these treatment modalities, systemic therapies play a major role in the treatment of haematological and non-haematological (otherwise grouped as solid organ cancers).

1.3 Systemic cancer therapies

Systemic cancer therapies have been used since the 1940s when oestrogen and nitrogen mustards were introduced for the treatment of solid and haematological cancers in humans (Huggins and Hodges 1941; Farber et al. 1948). These therapies can be grouped as part of

- neoadjuvant treatment (before local treatment for the primary site – usually pre-surgical resection), adjuvant treatment (after surgical resection) or definitive treatment – when used as the main or only modality

- concurrent treatment (when given simultaneously with radiotherapy as part of combined modality approach) or sequential treatment (either before or after radiotherapy)
- based on the intention of treatment with curative or palliative approach; people with incurable cancers often receive multiple lines of systemic therapies
- single agent or as part of combination therapy

On the other hand, as cancer tissue is a mixture of various cells – cancer cells, host cells, infiltrating immune cells, endothelium, cancer associated fibroblasts and extracellular matrix which can be differentially affected by systemic cancer therapies, they also can be classified based on their mechanism of actions as (Espinosa and Raposo 2010; Palumbo et al. 2013):

- Traditional chemotherapy
- Targeted agents with kinase inhibitors or monoclonal antibodies
- Endocrine therapies
- Immunotherapies and
- Miscellaneous group

These agents can be used as single drugs or as part of combination approach with multiple chemotherapy drugs or with targeted agents or immunotherapies or even with radiotherapy (Seiwert, Salama, and Vokes 2007). With the rapid improvement in drug discovery, several systemic therapies have been approved in the last two decades for the treatment of cancer.

1.3.1 Traditional chemotherapy

Chemotherapy drugs are a group of systemic cancer drugs that target mechanisms and processes involved in cancer cell proliferation thereby inducing cell death (cytotoxic agents) or cell stasis (cytostatic agents). The established pathways affected for chemotherapy agents are DNA or RNA synthesis, DNA damage, and microtubule polymerisation or depolymerisation depending on mitosis phase specific or non-specific effects. Cancers are heterogeneous in their responsiveness to chemotherapy (Savage 2016; Savage et al. 2009) (Table 1).

Table 1: Cancer types and chemosensitivity

Chemosensitivity	Examples of cancer types
Highly responsive	Acute leukaemias, aggressive lymphomas, small cell lung cancer, choriocarcinoma, high grade sarcomas
Sensitive	Breast, colon, non-small cell lung, ovarian, urothelial cancers
Resistant	Clear cell renal cancer, melanoma, well differentiated thyroid cancer, low grade sarcomas

1.3.2 Targeted therapies

Targeted therapies are those drugs which abrogate one or more well-defined molecular abnormality that support cancer cell growth. These molecular abnormalities are typically driver mutations in cancer cells whose downstream pathways support cell division, proliferation, invasion and angiogenesis. While traditional chemotherapy predominantly affects DNA, RNA or microtubules, targeted therapies typically affect proteins. Monoclonal antibodies (drugs that end with suffix mab) target the extracellular domain of protein kinases in addition to other receptors on cell membranes. On the other hand, small molecules (drugs that end with suffix ib) target the kinase domains of protein kinases. In current clinical practice, testing for the presence of these gene (RNA or protein) targets that are either amplified or mutated (point mutations, translocations) in cancer cells or circulating tumour DNA is often mandatory prior to the initiation of the appropriate targeted drug. Such gene targets are often prognostic in addition to being predictive of response or lack of response to these targeted drugs. Some examples of drug-target pairs are in Table 2.

Table 2: Targeted drugs and their targets

Target	Drug
Her-2	Lapatinib, trastuzumab, ado-trastuzumab
Epidermal growth factor receptor	Afatinib, gefitinib, erlotinib cetuximab, panitumumab
Bcr-abl	Bosutinib, dasatinib, imatinib, nilotinib, omacetaxine, ponatinib
Anaplastic lymphoma kinase	Alectinib, ceritinib, crizotinib
Braf	Dabrafenib, encorafenib, vemurafenib
Bruton tyrosine kinase	Ibrutinib
c-kit	Imatinib

Antibody conjugates are unique group of drugs where a monoclonal antibody is conjugated with a payload using a cleavable linker (Shim 2020; Ponziani et al. 2020). The payload can be either a cytotoxic agent or radioactive moiety or a toxin. The monoclonal antibody binds to a specific target on the cancer cells and delivers the payload directly into the cells. The linker then cleaves and releases the payload which induces

cell death. Some examples of antibody drug conjugates include trastuzumab emtansine and brentuximab vedotin.

1.3.3 Endocrine therapies

Following the pioneering work of Beatson in the late 1800s and Huggins in the mid-1900s, endocrine control of cancer was recognized as an important modality for the treatment of breast and prostate cancers (Beatson 1896; Huggins and Hodges 1941). Since then, drugs that target androgen or oestrogen pathway have been developed successfully for endocrine responsive breast, prostate and gynaecological cancers. The expression of the oestrogen or progesterone receptors in cancers cells is predictive of response to endocrine treatment for breast cancers, hence testing is mandatory prior to initiation. Some examples of classical endocrine drugs and their mechanisms of action are shown in Table 3.

Table 3: Endocrine therapies

Target	Drug
Aromatase (Cytochrome P450 19A1)	Anastrazole, exemestane, letrozole
Selective oestrogen receptor modulator	Tamoxifen
Androgen receptors	Apalutamide, bicalutamide, cyproterone, darolutamide, enzalutamide, flutamide, nilutamide
Cytochrome P450 17A1	Abiraterone
Luteinizing hormone releasing hormone	Degarelix, goserelin, leuprolin, triptorelin

1.3.4 Immunotherapies

While immunotherapies that modulate both the innate and acquired immune system have been used for several years, it is only in the last decade that their true value has been recognized through the widespread use of immune check point inhibitors (ICI) (Table 4). Among the current clinically approved immunotherapies, ICI drugs are the most dominant which target the negative immune check points such as programmed death receptors (PD1) or ligand (PDL1) or cytotoxic T-lymphocyte antigen 4 (CTLA4) on immune-reactive T cells so as to reactivate suppressed cells to identify and kill cancer cells. The other major group of novel therapeutics that are rapidly evolving are the chimeric antigen receptor T cells (CAR-T) therapy, which is already approved for acute leukaemias in certain jurisdictions around the world.

Table 4: Immunotherapies

Immunotherapy group	Drugs
Cytokines	Interferon, interleukins
Vaccines	BCG, sipuleucel-T
CTLA-4 inhibitor	Ipilimumab
Immunomodulatory drugs	Lenalidomide, pomalidomide, thalidomide
PD-1/PD-L1	Atezolizumab, durvalumab, nivolumab, pembrolizumab
CAR-T cells	Tisagenlecleucel, axicabtagene

1.3.5 Miscellaneous agents

The last group of systemic cancer therapies includes a heterogeneous mix of agents. These agents cannot be grouped with the above therapies due to their varying mechanisms of actions. Some examples are shown in Table 5.

Table 5: Miscellaneous

Groups	Drugs
Somatostatin analogues	Octreotide, lanreotide
Radiopharmaceuticals	Radium 223, lutetium, radioiodine
Differentiation agents	All-trans retinoic acid, bexarotene
Bone directed therapies	Bisphosphonates, denosumab

1.3.6 Response and toxicities to systemic cancer therapies

While a wide variety of systemic cancer therapies are currently available, as with any drug therapy, only a proportion of treated patients respond, and a proportion develop adverse events in the form of drug toxicities. Moreover, most of the chemotherapy drugs have a narrow therapeutic index. In contrast to non-cancer drug therapies, cancer drugs are often administered at maximum tolerated doses which results in an increased incidence of adverse events. Such a higher degree of drug related toxicities is probably acceptable to derive benefit from cancer cell kill. As every patient and their individual cancers are biologically different, the response and toxicities to drugs exhibit substantial inter-individual variability. Hence, the “one-dose-fits-all” approach, at least for chemotherapy drugs with narrow therapeutic index, is not appropriate. To reduce variability in response and toxicity, some degree of personalisation of chemotherapy treatment occurs with body surface area-based dosing. However, significant inter-individual variability is noted across several systemic cancer therapies.

1.4 Variability

William Osler stated,

"Of the difficulties inherent in the art not one is so serious as this which relates to the cure of disease by drugs. There is so much uncertainty and discord even among the best authorities….One of the chief reasons for this uncertainty is the increasing variability in the manifestations of any one disease. As no two faces, so no two cases are alike in all respects and unfortunately it is not only the disease itself, which is so varied, but, subjects themselves have peculiarities which modify its action" (Osler 1914)

Inter-individual variability in cancer response and toxicity from anti-cancer drugs is well known. Variability in drug response is commonly defined as "*an effect of varying intensity occurring in different individuals at a specified dose of a drug", or as "a requirement of a range of concentrations (doses) in order to produce an effect of specified intensity in all of the patients*" (Rocca, Dragani, and Pagliaccia 2013).

Drug response in cancer is typically assessed by tumour response as measured by changes in two-dimensional size of cancerous lesions on imaging, changes in serum tumour markers, changes in radioactivity uptake in the lesions, presence or absence of detectable minimal residual disease, survival improvement (either as disease free or progression free or overall survival) and patient reported health related quality of life changes. Systemic cancer therapies are given to patients with the hope of inducing a drug response thereby improving survival and quality of life. However, the end results of a drug therapy depend on multiple variables. These factors are part of the complex interplay of three principal components - cancer, patient (or host) characteristics and drug-specific factors that are ultimately responsible for the beneficial and harmful outcomes from treatment. It is generally believed that cancer characteristics contribute towards pharmacodynamic related variability while patient and drug characteristics towards pharmacokinetic related variability. However, for certain groups of drugs such as immunotherapy drugs, patient characteristics may also impact pharmacodynamic related variability that may contribute towards response and toxicities that may appear from treatment.

1.4.1 Cancer characteristics that contribute to variability

The cancer characteristics as defined by genetic (e.g. drug sensitive or resistant mutations) and phenotypic (e.g. grade of cancer cells, angiogenesis, immune cell infiltration) heterogeneity are the main sources of pharmacodynamic (exposure-response) related variability that determine drug response. Moreover, within-

tumour heterogeneity (cell to cell differences in drug response within the same tumour) and between-tumour heterogeneity (differences in tumour characteristics between patients that determine varying response in the magnitude of drug response) are considered to be major impediments that contribute towards the variability in drug response (Palmer and Sorger 2017). In addition to the heterogeneity created by the phenotypic histological types of cancers within the same primary site, the cancer genome atlas (TCGA) project has highlighted the presence of multiple molecular subtypes within the same histology (TCGA 2020; Grossman et al. 2016). The differing genotypic mutational characteristics explain some of the variability in drug response between patients with the same tumour phenotype.

Matching the oncogenic driver mutations with drugs that target these mutation derived proteins has been the most effective in terms of controlling cancers (Roskoski 2020; Bedard et al. 2020). However, even among patients with matched target-targeted drug pairs, only a proportion respond to the treatment. Uniform response to a matched targeted therapy across a cohort of patients with similar subtype of cancer is unusual. For example, erlotinib treatment of patients with sensitive epidermal growth factor receptor (EGFR) mutated non-small cell lung cancer (NSCLC) induces a response of only in 60-70% (Park et al. 2016). It appears that primary resistance mechanisms such as Bcl-2 interacting mediator of cell death (BIM) gene mutations among other mechanisms may explain the lack of response in some patients (Ying et al. 2015).

In addition to the single time point snapshot of each tumour's genotypic and phenotypic characteristics, "within-tumour heterogeneity" contributes to varying response to the drug in the same patient through mutational evolution within the same clone of cancer cells and through the generation of multiple new clones. Such evolutionary changes are typified by secondary or acquired resistance to targeted therapies as in T790M EGFR mutations which are a major mechanism for resistance after first-line EGFR inhibitor therapies in NSCLC. Moreover, such secondary resistant mutations are seen across a variety of small molecule targeted therapies (Hamid and Petreaca 2020).

To overcome acquired resistance and within-tumour heterogeneity, drugs such as osimertinib, a specific EGFR T790M inhibitor, have been developed to counteract acquired resistance while a combination of drugs with additivity or synergy are considered for primary resistance. It is also recognized that combination

therapies can improve drug response within a population of patients through independent actions of each drug without improving response in an individual patient through additivity or synergy (Palmer and Sorger 2017). It appears that "between-tumour heterogeneity" provides the rationale for the dominant independent actions seen in a population of patients treated with combination therapies (Palmer, Chabner, and Sorger 2018).

While a significant number of research activities continues to be conducted to understand the cancer related heterogeneity through genotypic characteristics, limited published literature exists on factors such as patient and drug characteristics that may contribute to variability in drug response.

1.4.2 Patient (host) characteristics that contribute to variability

Patient characteristics such as age, sex, body weight, behavioural factors, co-existing diseases, pharmacogenetics, pharmacogenomics and structural changes at the site of drug absorption or drug delivery may contribute towards pharmacokinetic variability thereby affecting the drug concentration available at the target site. These host factors are not as widely studied in contrast to the cancer characteristics.

Among the measurable sources of variability attributed to the patient characteristics, age and body weight are important. Age as an indicator of variability in systemic cancer therapy response and toxicities is well established. Older patients (≥ 65 years of age), who often have reduced physiological reserve, polypharmacy and multiple comorbidities, have an increased risk of toxicities from traditional chemotherapy and targeted therapies when compared to people less than 65 years of age. Prior studies have reported that up to 50% of older adults have severe to fatal toxicities form traditional chemotherapy (Extermann et al. 2012). On the other hand, targeted therapies and immunotherapies have been associated with reduced survival benefit in the elderly with similar intensity of toxicities as in the younger age group (Bastiaannet et al. 2019; Feliu et al. 2020).

Similarly, the effect of body weight and body mass index (BMI) has been widely studied in the setting of traditional chemotherapy and targeted therapies. While the dose of chemotherapy for an individual patient is usually calculated using body surface area, increasingly fixed doses are being used for other systemic cancer therapies which may increase the risks of variability arising from overdosing or under-dosing. Obese patients

with cancer are often reported to have inferior outcomes and a lower incidence of toxicities due to under-dosing while people who were underweight often have increased toxicities from chemotherapy and targeted therapies from overdosing (Griggs et al. 2012; Miyahara et al. 2013). On the contrary, the impact of obesity on outcomes and toxicities from immunotherapy is still unclear with preliminary studies showing possible improved outcomes in obese patients (McQuade et al. 2018).

Other factors such as single nucleotide polymorphic variants of drug metabolising enzymes and transporters may contribute towards efficacy and toxicities of systemic cancer therapies. Genetic polymorphism of cytochrome P450 enzyme CYP2D6 variants and tamoxifen, uridine 5'-diphospho-glucuronosyltransferase enzyme 1A1 (UGT1A1) variants and irinotecan and kinase inhibitors such as erlotinib/nilotinib/pazopanib, and dihydropryimidine dehydrogenase and fluoropyrimidines are examples of the influence by genomic variability on the response and toxicities to chemotherapy, targeted therapies and endocrine therapies.

As cancers occur in older populations, there is an increased risk of drug-drug interactions (DDIs) arising from PK or PD effects from the concomitant medications used to treat comorbidities. Concomitant medications used for non-cancer indications may enhance or reduce cancer response or increase toxicities from cancer therapies. Similarly, systemic cancer therapies can modify the response/toxicities to concomitant medications. Concomitant medications such as aspirin, metformin and statins have been shown to have direct anti-cancer activities. In contrast, corticosteroids used as part of systemic cancer therapies may induce hyperglycaemia and may counteract the effect of anti-diabetic medications. Anti-VEGF inhibitors can induce hypertension that may necessitate the introduction or increased doses of concomitant anti-hypertensives. More recently, the negative effects of concomitant medications such as antibiotics causing gut dysbiosis on response to immunotherapy have been reported (Pinato et al. 2019). However, the impact of other concomitant medications on traditional chemotherapy or immunotherapy related cancer outcomes/toxicities is still unclear and warrants further studies.

1.4.3 Drug-specific factors that contribute to variability

The physicochemical properties of drugs and the drug regimen are the two categories of drug-specific factors that may drug response and toxicities (Turner, Park, and Pirmohamed 2015). The solubility, permeability,

target site binding affinity of a drug and its regimen of administration (dose, route, frequency and timing) all contribute towards a variable concentration of the administered drug at the target site in different individuals receiving the same dose.

One of the ways to reduce variability in drug response and toxicity that has attracted attention recently is the potential for the use of therapeutic drug monitoring, target concentration monitoring or plasma concentration guided dosing strategies for systemic cancer therapies (Gao et al. 2012; Hopkins, Menz, et al. 2020; Paci et al. 2014; Widmer et al. 2014). Among the traditional chemotherapy drugs, measuring plasma concentrations of methotrexate is part of standard care for the treatment of haematological malignancies and osteosarcomas. There is increasing evidence that dose individualisation is feasible and improves survival/reduces toxicities for targeted therapies that usually employ fixed dosing strategies. While much attention has been focussed on choosing the right drug for the patient with a certain type of cancer mutational profile, choosing the right dose using therapeutic drug monitoring or plasma concentration guide dosing strategies is important. Among the targeted therapies, there is evidence for these approaches from prospective studies for oral small molecule kinase inhibitors such as imatinib, sunitinib and pazopanib and everolimus (Groenland et al. 2019). However, for newer kinase inhibitors such as vemurafenib, a braf inhibitor, target threshold concentrations are poorly defined. Similarly, threshold concentrations are not well established for other kinase inhibitors.

It is likely that several of the above (cancer, patient or the drug) characteristics determine the total treatment effect and toxicities of a drug in an individual patient. While these characteristics are helpful, it is difficult to accurately predict the treatment effect of a drug at the individual patient level, as these are usually determined at the group level in clinical trials or observational studies within the paradigm of evidence generation. However, the results from these studies are described as “average” treatment effect or harms as toxicities which do not adequately address the variability in response/toxicities that arise due to variability at an individual patient level or subgroups of patients. The study of such variability across patients is called as “heterogeneity of treatment effect” (Kent, Steyerberg, and van Klaveren 2018; Dahabreh, Hayward, and Kent 2016).

1.5 Heterogeneity of treatment effects

"Heterogeneity of treatment effect (HTE) is the non-random, explainable variability in the direction and magnitude of treatment effects for individuals within a population which may arise from an underlying causal mechanism or artifacts of measurements or methods (e.g., chance, bias, or confounding)"
(Varadhan and Seeger 2013)

Treatment effects here mean both beneficial and harmful clinical outcomes. HTE exists if the average treatment effects depend on the samples and subgroups within the population studied. Non-random variability (HTE) often arises from patient factors that often influence whether the individual responds favourably or develops toxicities (Figure 1 & Figure 2). Evaluation of HTE has the main objective of understanding the treatment effects at an individual patient level. However, it is well-recognized that both beneficial and harmful treatment effects are determined only at the group level as the individual level HTE is unobservable due to their binary nature (Dahabreh, Hayward, and Kent 2016; Kent, Steyerberg, and van Klaveren 2018). The same individual cannot have a response and no response at the same time, hence the unobservable nature of HTE within an individual. To predict the HTE for an individual patient, they must be assigned to groups of patients with similarly defined characteristics (called as subgroups). While each individual is unique, a group of individuals with whom the individual resembles is required to make predictions on treatment effects and risks (Kohane 2009).

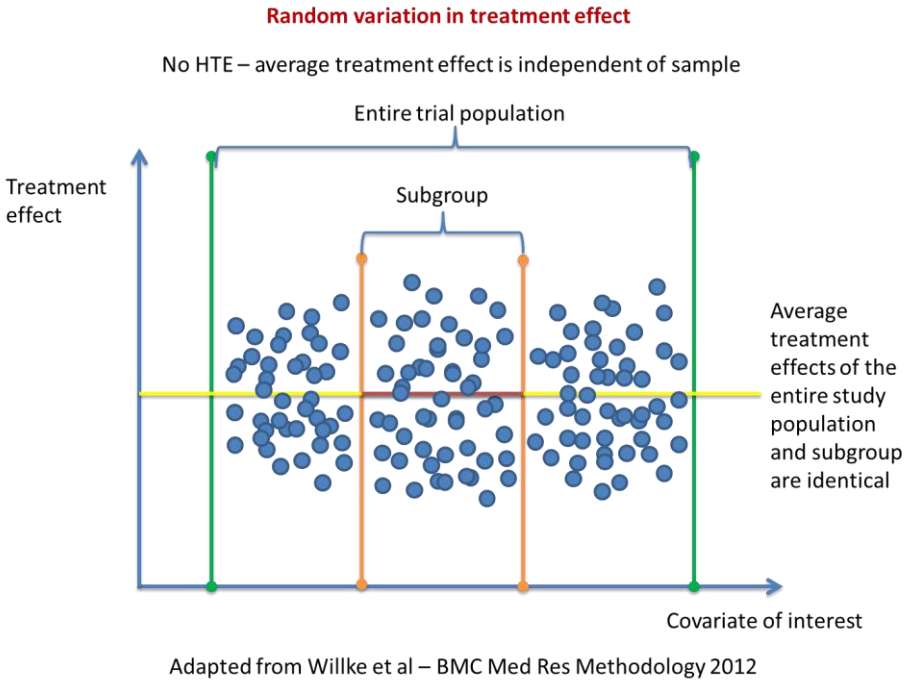
Data from participants involved in clinical trials are regularly used to evaluate group-level HTE to predict individualized treatment decisions. Subgrouping of baseline variables such as sex, age, and genetic biomarkers are regularly employed to derive personalised decisions for cancer treatment. However, each participant in a clinical trial may have several baseline characteristics; hence, each one can belong to more than one subgroup that were generated using these characteristics thereby making individual decisions highly complex.

1.5.1 Methods to study HTE

Among the HTE methods, subgroup analysis of clinical trial data and heterogeneity in meta-analysis of trials are well recognized and established. There are other methods such as predictive risk modelling, classification

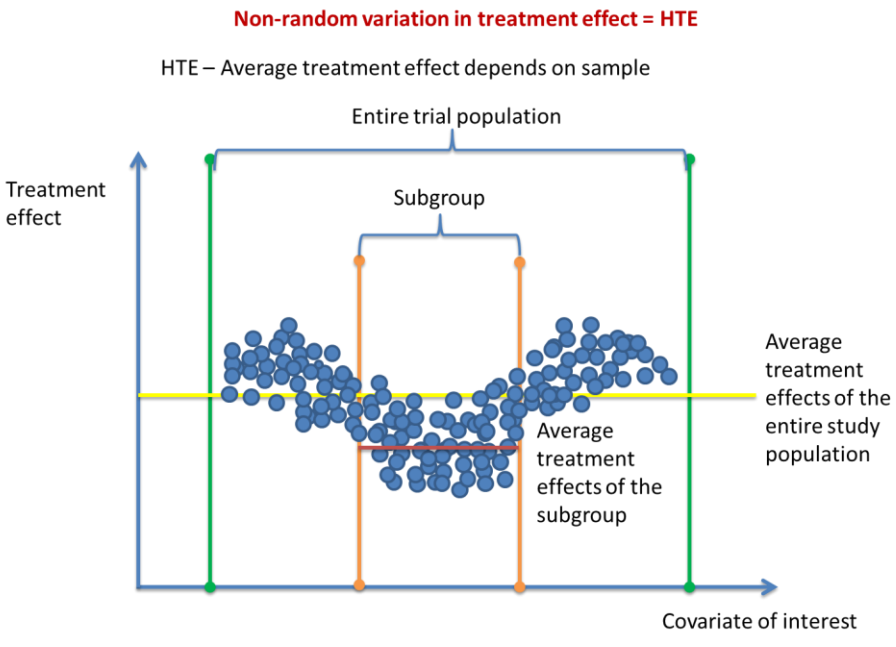
and regression tree analysis, series of "n-of-1 trials", quintile-based heterogeneity and non-parametric methods, that are increasingly being used (Willke et al. 2012). In this thesis, subgroup analysis of clinical trial data and meta-analysis of clinical trials were the HTE methodologies adopted.

Figure 1: Random variation in treatment effect



Adapted from Willke et al – BMC Med Res Methodology 2012

Figure 2: Non-random variation in treatment effect



Adapted from Willke et al – BMC Med Res Methodology 2012

1.5.2 Subgroup analysis of clinical trial data

HTE is often investigated using subgroup analyses where the whole trial cohort is divided into smaller groups based on one or more arbitrarily selected baseline patient or cancer characteristics. HTE related to the baseline variable are suggested to exist if the treatment effects vary across the levels of a baseline variable (Wang et al. 2007). Baseline patient attributes that have a "*strong a priori pathophysiological or empirical justification*" should be selected as the predictor variable in subgroup analysis (Kent et al. 2010). The selection criteria for the subgroups are usually pre-defined at the commencement of the trial as a stratification factor. However, subgroups can also be selected *post hoc* (nor previously specified or more commonly called as exploratory) after the trial is completed. Outcomes from the interventions are then compared between the subgroups to ascertain HTE.

While subgroup analyses are widely performed using clinical trial data, the credibility of most subgroup effects were considered to be low due to several reasons (Sun et al. 2012; Lagakos 2006). Among these, generation of smaller subgroups often leads to loss of statistical power for the analysis, creates imbalance between the subgroups due to lack of randomisation, and a high incidence of false positive and false negative findings (Brookes et al. 2001). The consequences of false discovery of a subgroup with differential benefit or harm from a treatment intervention may result in inappropriate treatment decisions.

Guidelines exist for proper planning, analysis, interpretation and reporting of subgroup analyses. These guidelines recommend selecting limited number of subgroups generated using the same dataset, must be pre-specified, have a strong biological rationale, adjustment for multiplicity and incorporation of statistical test for interaction (Tanniou et al. 2016; Sun et al. 2010; Oxman and Guyatt 1992). In addition, well conducted subgroup analyses can provide valuable information (Wang et al. 2007).

In this thesis, subgroups were chosen based on well-defined baseline patient or cancer characteristics that had strong biological plausibility and had supporting data from prior pre-clinical or clinical studies. Statistical power was maintained by pooling of data from two or more clinical trials and statistical tests for interaction was routinely performed.

1.5.3 Meta-analysis of trial data

Meta-analysis is a method for statistically combining treatment effects from two or more clinical trials thereby increasing the statistical power of the combined analysis compared to individual trials (Deeks JJ, Higgins JPT et al. 2019; Willke et al. 2012). Other advantages of a meta-analysis are the potential to address conflicting prior reports and those not addressed by individual studies. HTE can also be identified by testing across trials using tests of heterogeneity. However, HTE can also arise from differences in trial methodologies, design, interventions and outcomes assessed. Publication bias is another issue that may contribute towards bias in the data available for the meta-analysis. Hence, pooling of trials with similar design, patient population, intervention and outcomes should be carefully considered while performing meta-analysis.

As some of the research questions were not easily addressed using published literature, in this thesis, individual patient data from clinical trials was obtained in order to perform individual participant data (IPD) meta-analysis (Deeks JJ, Higgins JPT, and (editors). 2019; Tierney JF, Stewart LA, and M. 2019). Re-analysis of the original data from each trial participant was used to avoid publication bias and reduce missing information.

1.6 Aims and hypothesis

The overall aim of the research described in this thesis was to better understand and identify previously under-recognized sources that may contribute to the variability/HTE in the responses and toxicities to systemic cancer drugs. It was hypothesised that selected covariates (patient characteristics, treatment emergent toxicities, use of concomitant medications, primary site of cancer and plasma concentration) can predict outcomes (tumour response, survival and adverse effects) across a spectrum of systemic cancer drugs.

Among the patient characteristics, BMI was chosen as a covariate of interest in chapter 2 based on preliminary evidence from a prior report that obese patients with advanced melanoma who were treated with immunotherapy, had an improved survival (McQuade, Daniel et al. 2018). Advanced lung cancers are frequently treated with immunotherapy drugs and it was unclear if such an association existed between BMI

and outcomes in this context. It was hypothesised that the baseline BMI of patients who start on immunotherapy may be associated with survival in patients with advanced lung cancer.

While analysing data for the chapter 2, it became apparent that immunotherapy induced toxicities may be associated with survival outcomes. Moreover, from observations in my own clinical practice, a proportion of patients on immunotherapy develop multiple toxicities and there was limited literature on this issue. This gap in knowledge led to a descriptive analysis of the clinical profile of multiple immune adverse events and their association with immunotherapy outcomes which is described in chapter 3. Further analysis of the toxicity data from patients who were treated with chemotherapy as control arms in the clinical trials that were used in this chapter are described in chapter 4.

There are recent reports indicating that concomitant medications such as antibiotics and corticosteroids may reduce the efficacy of immunotherapy in patients with advanced cancers (Della Corte and Morgillo 2019; Elkrief et al. 2019; Maxwell et al. 2018; Petrelli et al. 2020). However, there are limited data on the impact of other concomitant medications such as anti-hypertensives such as renin-angiotensin inhibitors (RASi) on the outcomes from immunotherapy. RASi in particular have been reported to synergise with immunotherapy to improve their activity and decrease toxicity. Hence, it was hypothesised that concomitant use of RASi may affect the outcomes in patients being treated with immunotherapy. This research is reported in chapter 5.

While the data on the impact of concomitant medications on immunotherapy was being analysed, there was emerging evidence that concomitant proton pump inhibitors (PPI) may affect immunotherapy outcomes (Chalabi, Cardona et al 2020). However, there was limited information on the effect of concomitant PPIs on traditional chemotherapy drugs used for the treatment of gastrointestinal cancers. Prior studies reported possibly antagonistic effect of PPIs when given with capecitabine, an oral fluoropyrimidine (Cheng et al. 2019; Chu et al. 2017; Viñal et al. 2020). These observations led to the hypothesis that PPI use may negatively affect the benefit from fluoropyrimidine-based chemotherapy in patients with colorectal cancers for whom this type of chemotherapy is widely prescribed. The results from this research are described in chapter 6.

Furthermore, it was hypothesised that the trough plasma concentration of vemurafenib, a targeted therapy may be associated with survival outcomes in patients with melanoma, which provides the basis for the

research reported in chapter 7. Vemurafenib was chosen as the drug of interest due to the expertise available within the research team as well as limited information available on the dose individualisation for this drug.

Lastly, all advanced urothelial cancers are treated either chemotherapy or immunotherapy regardless of their primary site of origin. However, recently published literature and clinical observations indicate that urothelial cancers arising from upper tract (renal pelvis or ureters) may have differential response to immunotherapy (Yates and Catto 2013, Robinson, Vlachostergios et al. 2019, Hassler, Bray et al. 2020). It was hypothesised that upper tract urothelial cancer may have inferior outcomes from immunotherapy which forms the basis for the research described in chapter 8.

1.7 Approach for data analysis

"The variability of human beings in their illnesses and in their reactions to them is a fundamental reason for the planned clinical trial and not against it"(Hill 1971).

Clinical trials are one of the best ways to understand HTE using data from a population of patients being treated with similar illness and the same intervention. Evidence generated through human clinical trials help establish a new systemic cancer therapy as part of standard care in the treatment of patients with cancer in clinical practice. Moreover, regulatory approvals for new therapies require generation of evidence through clinical trials.

For most common cancers, one or more phase III comparative randomized controlled trials are required to confirm that the new systemic therapy intervention is either superior in terms of efficacy and/or safety, quality of life, ease of administration or as an inexpensive alternative over the existing standards. The optimal assessment of clinical benefits and an estimation of treatment effect is usually provided by phase III randomized controlled trials that provide unbiased data on the outcomes by comparing to an existing standard. Randomization results in reduced selection bias, balanced groups for potential confounders, and the optimal conduct of statistical tests (Suresh 2011). Phase II trials often provide early signals of activity of systemic therapy drugs in a uniform cohort of cancer population after the maximum tolerated dose or recommended phase II doses are established in the first-in-human phase I trials. In this thesis, individual

patient data from phase II and III clinical trials released by sponsors conducting the trials were used as the source material.

Most cancer clinical trials that evaluate systemic therapies are initiated, funded and sponsored by pharmaceutical companies. However, individual universities, governments and other organisations such as national/international cancer co-operative groups can also act as sponsors/funders for investigator-initiated trials. While sponsors and the contracted clinical research organisations act as the co-ordinating centres, individual patients are recruited from participating sites. Among various factors, the number of participants required in each trial determine the geographic location and number of participating sites in each clinical trial. Most phase III cancer trials are conducted in multiple sites, often in multiple countries, while the smaller phase II trials may be limited to a few sites within a single country. However, for rare cancers (with incidence less than 6 per 100,000 population), it is possible several sites will be required to enrol the required number of patients and collect data for meaningful interpretation.

Data are generated in clinical trials at different time points of its life cycle. The life cycle of a clinical trial that generates data can be divided into five major stages as follows (Institute of Medicine 2015):

1. Trial design and registration – clinical trial protocol and statistical analysis plan are useful information parts of which are commonly available in publicly available clinical trial registers such as www.clinicaltrials.gov, www.clinicaltrialsregister.eu and www.anzctr.org.au.
2. Participant enrolment – raw data generated from each participant throughout the course of the clinical trial is collated and transformed into an analysable format. These data form the collection of individual patient data (IPD).
3. Study completion – Usually, study completion occurs when "*last participant's last visit*" after which the investigating teams lock the dataset, clean and analyse the results using various statistical software tools. The initial analysis often involves endpoints that were prespecified in the trial protocol using parts of the data collected to generate results and reports.
4. Publication – Dissemination of results from clinical trials support clinical practice changes based on the new evidence generated. Although the results are expected to be published within 12 months of

their completion onto the clinical trial registers, compliance has been poor. One or more publications using information from the clinical trials in peer-reviewed journals can occur during any stages of the clinical trial life cycle.

5. Regulatory application – for clinical trials that were intended for regulatory approval for marketing of the new drugs, a detailed clinical study report (CSR) with additional information that is not available in published manuscripts and individual patient data are generated.

Data generated through clinical trials may vary across the trial teams that conduct these trials. Hence, to harmonize and standardize the clinical trial data being collected, the clinical data interchange standards consortium was formed in 1997 (Souza, Kush, and Evans 2007). Since then, the consortium has released standards for clinical trial data called as clinical data acquisition standards harmonization which specifies data fields, their labels and organization of the data onto a database (CDASHIGv2.1 2019). These standards are regularly used by clinical trials teams for the development of individual case report forms and electronic data capture systems. Once the data is populated onto a database, standard data tables are generated using multiple domains that describe various aspects of data such as demographics, laboratory values, imaging results, concomitant medications, adverse events and drug exposure which can be directly used for analysis (SDTMv1.7 2018). The analysis data model generated from the standard data tables provide support for dataset and metadata standards for clinical trial statistical analyses (ADaMv1.2 2019).

Until recently, access to data generated from clinical trials was limited only to the sponsors, study investigators and regulatory agencies. Several organisations have supported the push for making the data available for external researchers who are not directly involved in any of the stages of a clinical trial (Taichman et al. 2017). Clinical trial data sharing of has now been recognized as an important resource for the scientific community and the public.

1.7.1 Data sharing and access

Access to individual patient data from clinical trials to external researchers has several benefits including independent validation of results, analyses that were not previously planned, and support of meta-analyses (Keerie et al. 2018). Hence, data sharing from clinical trials after the primary analysis is completed is

considered as best practice by the World Health Organization (WHO), the International Committee of Medical Journal Editors (ICMJE) and other groups (Taichman et al. 2017). The other best practices for clinical trials are universal prospective registration and public disclosure of results. There are several platforms now available for clinical trial data sharing such as the

1. Yale University Open Data Access (YODA) project (https://yoda.yale.edu/),
2. Vivli (https://vivli.org/),
3. ClinicalStudyDataRequest (https://www.clinicalstudydatarequest.com/) and
4. Project data sphere (https://www.projectdatasphere.org/).

For the current research, de-identified data were obtained from the publicly available data sharing platforms, ClinicalStudyDataRequest and project datasphere (Clinicalstudydatarequest 2019; Projectdatasphere 2020). A research proposal with request for data from selected trials available on the platform was submitted in 2018 to the research committees of both platforms. The proposal included objectives, analysis and publication plans. Independent review panels and the sponsors reviewed the proposals. After approval from both groups, access to data was provided through a secure web portal for analysis. Data were released as data tables describing various clinical trial related parameters through a secure platform that required central approval prior to individual researcher access within the online environment.

1.7.2 Selection of clinical trials

Since its introduction in 2014, clinical trial data sharing is increasing; however, it is still not universal. Data from only 15% of completed pharmaceutical company sponsored clinical trials were shared within 2 years of the publication of the primary results (Hopkins, Rowland, and Sorich 2018). Roche, a pharmaceutical company with a large portfolio of cancer therapies, had submitted data from several clinical trials to the ClinicalStudyDataRequest.com (Clinicalstudydatarequest 2019). Data from clinical trials involving systemic cancer drugs from Roche made up the bulk of source material used in the current research (Table 6).

Table 6: Clinical trials data used in the current research

Trial (registration number)	Title

BIRCH (NCT02031458) (Peters et al. 2017)	A phase II, multicenter, single-arm study of atezolizumab in patients with PD-L1-positive locally advanced or metastatic non-small cell lung cancer
FIR (NCT01846416) (Spigel et al. 2018)	A phase II, multicenter, single-arm study of MPDL3280A in patients with PD-L1-positive locally advanced or metastatic non-small cell lung cancer
OAK (NCT02008227) (Rittmeyer et al. 2017)	A phase III, open-label, multicenter, randomized study to investigate the efficacy and safety of atezolizumab (anti-PD-L1 antibody) compared with docetaxel in patients with non-small cell lung cancer after failure with platinum containing chemotherapy
POPLAR (NCT01903993) (Fehrenbacher et al. 2016)	A phase II, open-label, multicenter, randomized study to investigate the efficacy and safety of mpdl3280a (anti−PD-L1 antibody) compared with docetaxel in patients with non−small cell lung cancer after platinum failure
IMvigor210 (NCT02951767, NCT02108652) (Balar, Galsky, et al. 2017)	A phase II, multicenter, single-arm study of atezolizumab in patients with locally advanced or metastatic urothelial bladder cancer (cohort 1) A phase II, multicenter, single-arm study of atezolizumab in patients with locally advanced or metastatic urothelial bladder cancer (cohort 2)
IMvigor211 (NCT02302807) (Powles et al. 2018)	A phase III, open-label, multicenter, randomized study to investigate the efficacy and safety of atezolizumab (anti-PD-L1 antibody) compared with chemotherapy in patients with locally advanced or metastatic urothelial bladder cancer after failure with platinum-containing chemotherapy
IMmotion150 (NCT01984242) (McDermott et al. 2018)	A phase II, randomized study of atezolizumab (anti-pd-l1 antibody) administered as monotherapy or in combination with bevacizumab versus sunitinib in patients with untreated advanced renal cell carcinoma
AVF2107 (NCT00109070) (Hurwitz et al. 2004)	A phase III, multicenter, randomized, active-controlled clinical trial to evaluate the efficacy and safety of RhuMAB VEGF (bevacizumab) in combination with standard chemotherapy in subjects with metastatic colorectal cancer
Carrato et al (NCT00457691) (Carrato et al. 2013)	A multicenter, randomised, double-blind, phase 3 study of sunitinib in metastatic colorectal cancer patients receiving irinotecan, 5-fluorouracil and leucovorin (FOLFIRI) as first line treatment
HORIZON III (NCT00384176) (Schmoll et al. 2012)	A randomised, double-blind, multicentre phase II/III study to compare the efficacy of cediranib (Recentin™, AZD2171) in combination with 5-fluorouracil, leucovorin, and oxaliplatin (FOLFOX), to the efficacy of bevacizumab in combination with FOLFOX in patients with previously untreated metastatic colorectal cancer
VELOUR (NCT00561470) (Van Cutsem et al. 2012)	A multinational, randomized, double-blind study, comparing the efficacy of aflibercept once every 2 weeks versus placebo in patients with metastatic colorectal cancer (mcrc) treated with irinotecan / 5-FU combination (FOLFIRI) after failure of an oxaliplatin based regimen
N016966 (NCT00069095) (Saltz et al. 2008)	A 2x2 factorial randomized phase III study of intermittent oral capecitabine in combination with intravenous oxaliplatin (q3w) ("Xelox") with/without intravenous bevacizumab (q3w) versus bolus and continuous infusion fluorouracil/intravenous leucovorin with intravenous oxaliplatin (q2w) ("FOLFOX-4") with/without intravenous bevacizumab (q2w) as first-line treatment for patients with metastatic colorectal cancer

RAISE (NCT01183780) (Tabernero et al. 2015)	A randomized, double-blind, multicenter phase 3 study of irinotecan, folinic acid, and 5-fluorouracil (FOLFIRI) plus ramucirumab or placebo in patients with metastatic colorectal carcinoma progressive during or following first-line combination therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine
BRIM-3 (NCT01006980) (Chapman et al. 2011)	BRIM 3: a randomized, open label, controlled, multicenter, phase III study in previously untreated patients with unresectable stage IIIc or stage IV melanoma with V600E BRAF mutation receiving vemurafenib (RO5185426) or dacarbazine
coBRIM (NCT01689519) (Larkin et al. 2014)	A phase III, double-blind, placebo-controlled study of vemurafenib versus vemurafenib plus GDC-0973 in previously untreated $BRAF^{V600}$-mutation positive patients with unresectable locally advanced or metastatic melanoma

1.7.3 Ethics

As de-identified data was used for all of the research reported in this thesis, Southern Adelaide Human Clinical Research Ethics Committee confirmed that the projects were of minimal risk to the participants. Hence, an exemption from ethics committee review was provided for the research activities performed.

1.8 Data analysis

A general statistical analysis plan was generated prior to each project. Descriptive and analytical statistics using data from trial participants were conducted using R-v3 (Team 2017). Pooling of data was performed where feasible for analyses and to generate results.

1.8.1 Statistical concepts and methods used in the thesis

A variety of descriptive and inferential statistical methods were used across the chapters in the current thesis. Descriptive statistics included tabulation of results for individual baseline characteristics including mean, median and interquartile range (IQR) for continuous variables, and percentages for binary or categorical variables. Standard deviation and standard errors of the mean were reported where required (Sedgwick 2015b). The distribution of baseline characteristics was compared between various subgroups across the thesis chapters and P-values for statistical difference between groups were calculated using the chi-squared test or Fisher's exact test for categorical data and the Kruskal-Wallis test or the Wilcoxon's test for continuous data. While the chi-squared test requires a large sample size, the Fisher's exact test can be applied for small

samples (Kim 2017). The Kruskal-Wallis test or the Wilcoxon's rank sum test/ Wilcoxon's signed rank test are non-parametric tests which are recommended when the continuous data are not normally distributed or are unknown (Nahm 2016). The Wilcoxon's rank sum test was used for the comparison of two independent groups, and the Kruskal-Wallis test was applied to investigate the difference in median values of three or more independent groups of samples (Sedgwick 2014b). 95% confidence intervals (95%CI) were also reported where appropriate.

"Statistical estimation is usually model-based" – Harrell F (Harrell 2001).

All inferential statistical analyses use statistical modelling that may help provide causal explanation, prediction of future events, or simply provide description of the variables within a dataset. Among the various statistical models, **regression analysis** is commonly used to provide a mathematically quantifiable estimate of the relationship or association between a **dependent** (otherwise called as outcome) variable and (one or more) **independent** (variously called as explanatory, regressor, predictor or covariate) variable(s).

Most of the analyses in the thesis required development of models that predict future health outcomes in patients with cancer using baseline characteristics as predictor variables. Guidance from previous authors indicate general areas that may perform well as predictor variables within a predictive model (Harrell 2001). Some examples of the predictor variables recommended include age, sex, type and severity of principal diagnosis, clinical status, comorbidities, and functional status (Iezzoni 2013). The development of such predictive models usually requires prospective data collection for improved accuracy. While prospective data collection provides the advantage of gathering data on appropriately defined variables, retrospective studies and clinical trial data have also been used to develop predictive models. Well conducted clinical trials in particular, have a pre-defined comprehensive data collection process that supports generation of predictive models. Data collection for the current thesis had already occurred through the selected clinical trials. Further, data from these trials provided the required information for optimal choice of predictor variables.

Several patient baseline characteristics were used in the thesis as the independent variables of interest to predict the dependent outcome variables of interest such as survival, response rates and adverse events. In selected chapters, plasma concentrations of the drug or therapy related adverse events that were

documented during treatment were also the independent predictor variables. Regression models were the primary methods that were used to develop predictive models. Several regression analyses methods were used in the thesis to investigate the association between predictor/explanatory variables and outcomes variables.

1.8.2 Regression analysis

The word "regression" was originally used in the context of regression towards the mean by Galton (Galton 1885). However, in the context of statistics, regression analysis includes a set of methods for the purposes of establishing causal relationships and predictions between variables. As a general rule of thumb, the type of dependent/outcome variable determines the method of regression analysis applied for a dataset (Lewis 2007). However, more than one regression method could be used for the same dataset to investigate the relationship between dependent and independent variables (Gelman 2020).

Among the regression methods, ***linear regression*** is one of the most used methods by researchers and statisticians. Linear regression, described as a mathematical equation, provides a quantitative estimate of the linear association between two variables (Sedgwick 2013d). When only one explanatory variable is used in the model, it is referred as ***simple linear regression,*** whereas when more than one explanatory variable is included, it is called as ***multiple linear regression***. However, both the dependent and explanatory variables are usually continuous in simple linear regression and multiple linear regression. If explanatory variables included are a mixture of categorical and continuous variables, then the method of analysis is called as multiple regression (Sedgwick 2013c). The dependent (outcome) variable must be continuous in nature for both simple and multiple linear regression methods. As in any statistical models, linear regression analysis requires several assumptions including validity, additivity, linearity, independence of errors, equal variance of errors and normality of errors (Gelman and Hill 2007).

In contrast, if the dependent variable is binary in nature, then ***logistic regression*** is commonly employed. Also called, logit regression, logistic regression quantifies the association between a binary dependent variable and one (***univariable logistic regression***) or more explanatory variables (***multivariable logistic regression)*** that are continuous, categorical or binary (Sedgwick 2013b). When the data are derived from

matched populations for the pre-defined confounding variables, then, a ***conditional logistic regression*** can be performed. The estimates from logistic regression analysis is reported as an **odds ratio** (OR) that reflects the probability of the dependent variable occurring for the given values of explanatory variables. Odds ratios can be unadjusted or crude when not adjusted for potential confounding explanatory variables or adjusted OR when the relevant confounding variables have been adjusted during the analysis. If the OR for the association remains statistically significant after adjusting for the confounding explanatory variables, the explanatory variable is believed to be an independent factor associated with the dependent outcome variable.

1.8.3 Cox proportional hazards regression

In contrast, if the dependent variable is "survival data" or "time to event data", the method usually adopted to quantify the association between a dependent variable and one or more explanatory variables is the ***Cox proportional hazards regression***. Although the dependent variable here (survival time or time to event) is continuous, the censored nature of survival time makes Cox proportional hazards regression different from the simple linear regression and multiple linear regression (Sedgwick 2013a). Censoring is said to exist when incomplete survival time data is available for some of the participants (Leung, Elashoff, and Afifi 1997). For example, if the participant in a trial is still alive at the end of the trial, their survival time is unknown. Similarly, for someone who exited the study before its completion, their survival time is unknown. Survival analysis techniques allow for these incomplete data to be used during analysis (Kollman 2018).

The explanatory variables can be binary, categorial or continuous in nature which occur at baseline, either trial entry or the start of the intervention or observation for the Cox proportional hazards regression analysis. The estimates from Cox proportional hazards regression are reported as **Hazards Ratio** (HR). Hazards of an event (e.g. death or recurrence) is the rate of the event as calculated by the probability of event within a time interval divided by the length of time interval (Sedgwick 2013a). HR is the ratio of event hazards in the population cohort of interest when compared with the reference cohort thereby providing a relative instantaneous risk of the event. HR are also generated when the dependent outcome (e.g. overall survival or progression free survival) is compared between the control and interventional arms within the context of a

clinical trial to provide efficacy information. Among several assumptions for Cox proportional hazards regression, the assumption that the hazards are proportional i.e. effect of each covariate is constant and HR does not change over time (i.e. proportional hazards assumption) is the most important.

In addition, if the explanatory variable is only one variable, the Cox model is called as **univariable model**. When two or more variables are evaluated, then it is called as the **multivariable Cox model**. Univariable Cox models generate unadjusted HR. On the contrary, multivariable Cox models provide adjusted HRs after the adjustment of relevant confounding factors. HRs are also produced when subgroups within an explanatory variable (e.g. men and women within the category of sex) are compared with a reference group whose HR is usually 1.

In cancer clinical trials, the explanatory variables often change, evolve or disappear over time on repeated measurements during the follow-up period. Such variables are called as **time-varying covariates** (Zhang et al. 2018). While evaluating the association between a baseline explanatory variable and outcome is useful, this fails to account for the changes in the explanatory variable which may introduce the issue of guarantee-time bias or immortal time bias. Moreover, some of the explanatory variables may have differential effect on short-term and long-term survival (**time-dependent effects**) (Dekker et al. 2008). Hence, variations in Cox proportional hazards regression models are required to adjust for these biases. **Conditional landmark analysis** and **time-dependent Cox regression** are some analytic techniques that can be used to account for such time-varying covariates and time-dependent effects (Giobbie-Hurder, Gelber, and Regan 2013).

1.8.4 Conditional landmark analysis

Although originally introduced to address the problem of guarantee-time or immortal time bias during survival analysis, the conditional landmark Cox analysis method is widely used across the medical literature (Dafni 2011). Immortal time here means the period during which the study outcome cannot occur. Immortal bias is if the immortal time is either misclassified to a different treatment status or excluded from analysis (Lévesque et al. 2010). A landmark time is pre-selected based on the event occurring during follow-up that helps generate two or more groups (e.g. response vs non-responder, those who develop toxicity vs no toxicity). The main purpose of this method is to estimate the outcome probabilities conditional on the

number of people in each group generated. Events that occur after this landmark time and those who cease participation in the trial or die prior to this landmark time are excluded from analysis thereby diminishing statistical power due to reduction in sample size available for analysis. One of the criticisms of this approach is the choice of landmark time which may be selected arbitrarily. Conduct and reporting of sensitivity analyses at various landmark time points as well as before the landmark time are considered to be some of the solutions (Dafni 2011).

1.8.5 Time-dependent Cox regression

Time-dependent Cox regression, otherwise known as extended Cox model with time-varying covariates, uses all patient data with the explanatory variables that change during the study and the analysis starts from the time of enrolment or randomisation. This model also allows for change in membership of the group with time without losing statistical power by including all eligible patients (Giobbie-Hurder, Gelber, and Regan 2013). In this thesis, emergence of treatment related adverse events and their association with survival outcomes was investigated using conditional landmark Cox regression and time-dependent Cox regression models.

1.8.6 Survival analysis methods

Survival analysis, otherwise called time-to-event analysis, provides an estimate of the time to occurrence of the event of interest. As the major outcomes of interest in various projects in this thesis were overall survival (OS) and progression-free survival (PFS), survival analysis methods were regularly employed. Among the statistical methods for the analysis of survival data, the **Kaplan-Meier method** (KM method) was employed for estimating the survival times and for the graphical display of survival curves. The Cox proportional hazards regression model was used to evaluate the effects of covariates on the hazards of the occurrence of outcome event of interest. As previously described (Chapter 1, section 8.3), one unique feature of survival/time-to-event data and survival analysis methods is the concept of “censoring” whereby the participants may not have experienced the event of interest (e.g. cancer progression or death) or lost to follow-up or withdrawal from trial participation prior to last follow-up (Sedgwick 2014a). Moreover, for those participants who were enrolled late in during the recruitment period, the duration of period of follow-up may not be adequate for

the event to occur. Such "censored" participants still provide useful information on the effect of the intervention. The KM plot provides information on median survival times as well as other landmark times. To compare the survival times between the treatment groups, the **log rank test** was employed to test for statistical significance. On the other hand, HR derived from the Cox proportional hazards regression model was used to describe the magnitude of the survival differences between treatment groups.

1.8.7 Statistical interaction tests

Subgroup analysis in randomized controlled trials to assess HTE often require evaluation of statistical interaction tests. **Stratification** based on baseline variables and generation of an **interaction model** are the two ways by which statistical interaction can be appraised (Brankovic et al. 2019). Interaction can be quantitative or qualitative. A quantitative interaction means one treatment is always better than the other regardless of the subgroups (Wang et al. 2007). A qualitative (or crossover) interaction is said to exist when one treatment is better than the other in one subgroup and worse than the other in another subgroup of patients (Gail and Simon 1985). This effect modification is identified in a statistical model as an interaction term between the treatment group and the subgroup variable (Wang et al. 2007).

Interaction modelling was routinely employed for assessing statistical interaction in this thesis. Interaction models were derived from regression analysis by the introduction of an interacting variable in the regression model when the treatment effects were evaluated. As both the logistic and Cox regression models have multiplicative scale of interaction testing, the final interaction model was "*treatment + baseline factor + (treatment × baseline factor)*" (Brankovic et al. 2019). In contrast, the linear regression models use an additive scale for interaction tests. Like other statistical tests, 95%CI for the magnitude and P-values for statistical significance were generated for the interaction term.

1.8.8 Meta-analyses

As previously described (Chapter 1, section 5.3), meta-analyses generally involve the synthesis of study level aggregate data to produce an estimate of treatment effects. The trials are usually identified after a systematic review process and study level aggregate data forms the basis of the analysis. In contrast, IPD meta-analyses

involves use of data from each participant across multiple studies. However, clusters of patients derived from each trial are usually retained while a meta-analysis is performed. Such an analysis may utilise a one-step or two-step approach (Riley, Lambert, and Abo-Zaid 2010).

Trials included in the current thesis were identified from data sharing platforms (chapter 1.7.2) and a systematic review method for study identification was not performed. **One-step IPD** was used for pooling of results except for the research work described in chapters 5 and 6, where a **two-step IPD** meta-analysis approach was used. In the first step, data from individual patients were analysed separately for each trial using Cox proportional hazards regression to produce trial level HR for the survival outcomes. This first step produced trial level aggregate data for the HR for OS and PFS. In the second step, the trial level aggregate data were synthesised to produce a pooled HR using a random effects model or fixed effects model (Riley, Lambert, and Abo-Zaid 2010). These models assume fixed or random treatment effects across studies. Forest plots were used for the display of results from the pooled meta-analysis.

As various trials that were conducted in different timespans and populations were combined in these analyses, HTE is expected (Higgins, Thompson, and Deeks 2002). Hence, statistical test of heterogeneity is usually performed to evaluate the extent of variation between individual trial HR estimates. Cochran's Q test and Higgin's I^2 test statistic were employed to test heterogeneity in the current thesis (Sedgwick 2015a). An I^2 value more than 50% indicates a high degree of heterogeneity in HR and effect size of the studies included in the meta-analysis. Random effects methods were used when heterogeneity in effect was anticipated between studies.

1.8.9 Clinical prediction models

Regression analysis methods lead to the development of clinical prediction models. For one of the projects in this thesis (Chapter 7), a clinical prediction model was developed using logistic regression and Cox proportional hazards regression to identify the best threshold plasma concentration of the drug that predicts response and survival outcomes. As there were several models generated with varying cut-offs for the threshold concentration, discriminative performance of each model was quantified using concordance statistic (**C-statistic**). Discriminative performance of a model means its ability to differentiate those patients

who had an outcome from those who did not have the outcome (Steyerberg and Vergouwe 2014). The higher the c-statistic, better the discriminative ability of the model. Model fitting was performed using Akaike information criterion (**AIC**) to facilitate the choice of the 'best' model (Akaike 1973). AIC is a commonly used measure of relative goodness of fit of a statistical model (Brewer, Butler, and Cooksley 2016). A lower AIC score is indicative of superior model. The most optimal plasma threshold concentration was defined using these techniques.

1.9 Thesis chapters

The thesis comprises the following chapters:

1. Patient characteristics – the association between baseline body mass index (BMI) and cancer outcomes in patients with lung cancer undergoing treatment with atezolizumab, an immune checkpoint inhibitor (chapter 2).
2. Patient characteristics - a description of the incidence of immune-related adverse events (irAEs), multi-organ irAEs, their predictors and their impact on cancer outcomes from atezolizumab or chemotherapy (chapters 3 and 4).
3. Use of concomitant medications - the association between the use of anti-hypertensives and cancer outcomes in patients with lung cancer undergoing treatment with atezolizumab (chapter 5).
4. Use of concomitant medications - the association between the use of proton pump inhibitors (PPI) and cancer outcomes in patients with colorectal cancer undergoing treatment with chemotherapy (chapter 6).
5. Plasma concentration - validate a plasma vemurafenib steady state trough concentration ($C_{ss,min}$) threshold that predicts survival outcomes of patients with $BrafV^{600}$ mutated melanoma (chapter 7).
6. Cancer characteristics – the relationship between the primary site of cancer and its effect on treatment outcomes from immunotherapy and chemotherapy (chapter 8).

2. CHAPTER TWO: BASELINE BMI AND OUTCOMES FROM IMMUNE CHECKPOINT INHIBITORS

This chapter has been derived and adapted with permission from the following publication:

Kichenadasse G, Miners JO, Mangoni AA, Rowland A, Hopkins AM, Sorich MJ. Association between Body Mass Index and overall survival with immune checkpoint inhibitor therapy for advanced non-small cell lung cancer. ***JAMA Oncol***. 2020; 6(4):512-518.

The accepted manuscript has been reproduced in Appendix 1.

As discussed in the previous chapter, variability in drug response and toxicities could arise from several factors. Among the baseline patient characteristics, age, sex, age, organ impairment and body weight are routinely explored when a drug is being developed. However, BMI is often poorly studied as a contributing factor towards variability in response to therapy especially for immunotherapy. In this chapter, the influence of BMI as a predictive variable with one of the systemic cancer therapies used in patients with lung cancer was evaluated.

2.1 Introduction

Lung cancer is one of the most common cancers around the world. As per GLOBOCAN 2018, the estimated age-standardized incidence in terms of incidence was 22.5 per 100,000 population and mortality of 18.5 per 100,000 worldwide (Ferlay et al. 2018). More than 80% of the patients with lung cancers have non-small cell lung cancer (NSCLC) and the rest present as small cell lung cancers as the main histological subtypes. Among the NSCLC, adenocarcinoma and squamous cell carcinoma are the main histological variants. However, with an improved understanding of the tumour biology, multiple different molecular subtypes of even within the adenocarcinoma type of NSCLC have also been recognized (Skoulidis and Heymach 2019).

Such molecular classification of NSCLC has led to the rapid evolution of its treatment options over the last two decades including chemotherapy, molecularly targeted drugs, ICI and combination approaches. ICI that target programmed death -1 (PD1) or its ligand 1 or (PDL1) monoclonal antibodies such as atezolizumab, durvalumab, nivolumab, and pembrolizumab are increasingly used for the treatment of both early and

advanced NSCLC. While durable responses were noted in advanced cancers, only a limited proportion of patients benefit from ICIs. Moreover, attempts to increase response using combination strategies incorporating multiple ICIs have a high incidence of irAEs resulting in early discontinuation. Predictive biomarkers for ICI therapy response are urgently required to identify patients who benefit or have adverse events from ICI.

Available predictive biomarkers for response, such as tumour mutation burden, PDL1 expression, and microsatellite instability, are generally focussed on cancer and its associated tumour infiltrating lymphocytes. As the patients who receive ICI therapies are highly heterogeneous and tumour-based biomarkers are resource intensive and not validated, several simple clinical and demographic characteristics are also being evaluated to predict response. One such characteristic is obesity.

The relationship between obesity (and its surrogate non-invasive measure – those with high BMI) and cancer is complex, with increased incidence, rapid disease progression, recurrence after treatment and mortality for some cancers but protection from other cancers ("obesity paradox") (Lennon et al. 2016). Previous literature showed that high BMI was associated with lower incidence of lung cancers and lower cancer specific mortality (Gupta et al. 2016; Hidayat et al. 2016; Morel et al. 2018; Yang et al. 2013). Moreover, high BMI is an independent positive prognostic factor for survival among those treated with surgery in early stage NSCLC, paclitaxel/carboplatin chemotherapy for advanced disease and radiotherapy for bone metastases (Yap et al. 2018; Sepesi et al. 2017; Masel et al. 2017; Dahlberg et al. 2013). However, it is unclear whether high BMI might also affect the association between ICI treatment and cancer outcomes.

In a recent retrospective study, McQuade *et al* (McQuade et al. 2018) reported that in patients with advanced melanoma treated with ICI and targeted therapies, obesity (BMI ≥ 30 kg/m^2) was associated with improved progression-free survival (PFS) and overall survival (OS) while, no such association was noted in patients treated with chemotherapy. Cortellini *et al* (Cortellini, Bersanelli, et al. 2019) reported that for patients with advanced cancers treated with ICI, PFS and OS were significantly longer for overweight/obese patients (BMI ≥ 25 kg/m^2) compared to non-overweight patients (BMI < 25 kg/m^2). Similarly, Richtig *et al* (Richtig et al. 2018) reported a higher response rate with ICI and longer survival in obese melanoma patients but not in

patients with normal body weight. While the pathophysiology behind the positive association between obesity with survival from ICI is unclear, leptin mediated T-cell dysfunction may be a contributing factor (Wang, Aguilar, et al. 2019).

In the current study, the relationship between high BMI and survival in advanced NSCLC patients treated with ICI was evaluated. The main objectives were to: (1) investigate the effect of BMI on the survival outcomes of patients initiating atezolizumab, or docetaxel; and (2) determine the effect of BMI on the incidence of treatment related adverse events (TRAEs) and irAEs in the same cohort.

2.2 Methods

2.2.1 Patients

A pooled post-hoc analysis of individual-participant data from the clinical trials OAK (Rittmeyer et al. 2017) (NCT02008227, 7 July 2016 data cut-off), POPLAR (Fehrenbacher et al. 2016) (NCT01903993, 8 May 2015 data cut-off), BIRCH (Peters et al. 2017) (NCT02031458, 28 May 2015 data cut-off), and FIR (Spigel et al. 2018) (NCT01846416, 7 Jan 2015 data cut-off) was conducted. Results for the primary analyses of data from all four trials were previously published (Fehrenbacher et al. 2016; Rittmeyer et al. 2017; Peters et al. 2017; Spigel et al. 2018). Secondary analysis of trial data was deemed to be negligible risk and exempt from the local Ethics Committee review. Data were accessed according to Roche's policy and process for clinical study data sharing plans (Clinicalstudydatarequest 2019).

OAK and POPLAR were randomized trials of atezolizumab 1,200 mg intravenous (IV) every 3 weeks versus docetaxel 75 mg/m² IV every 3 weeks for patients with advanced NSCLC that had failed platinum-containing therapy (Fehrenbacher et al. 2016; Rittmeyer et al. 2017). BIRCH and FIR were single-arm Phase II trials in PDL1 positive patients either first line or beyond therapy with atezolizumab (Peters et al. 2017; Spigel et al. 2018). Pooled analyses of OAK, POPLAR, BIRCH and FIR were used to demonstrate consistency of identified associations within an expanded cohort of patients treated with atezolizumab. PDL1 positive tumours were defined by PDL1 expression on 5% or more of tumour cells or tumour-infiltrating cells based on the VENTANA SP142 PDL1 immunohistochemistry assay (Ventana Medical Systems, Inc., Tucson, AZ, USA).

2.2.2 Predictor and outcome definitions

The primary outcome assessed was OS. The secondary outcomes were PFS and TRAEs and irAEs (any grade and grade 3 or 4 TRAEs/irAEs (using NCI CTCAEv4.0). PFS was investigator–assessed for POPLAR and OAK and defined by Response Evaluation Criteria in Solid Tumours (RECIST version 1.1) (Fehrenbacher et al. 2016; Rittmeyer et al. 2017). An independent review facility–assessed PFS via RECIST v1.1 for BIRCH (Peters et al. 2017) whereas, in FIR, the PFS was investigator-assessed as per modified RECIST (Spigel et al. 2018). Adverse events reported as related to the treatment interventions were considered as TRAEs and those reported as immune mediated were called as irAEs.

Baseline BMI was calculated using height and weight as recorded at study enrolment or first day of treatment (WHO 2019). BMI was categorised by WHO criteria: underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2), and obese (≥ 30 kg/m^2). Those with missing height and/or weight information for the calculation of BMI and those with underweight category of BMI were excluded from the analysis. As in the prior study of McQuade et al (McQuade et al. 2018), underweight patients were excluded from analyses because of low prevalence (<5%) and the focus was on comparing overweight and obese BMI categories to the normal weight BMI category.

Clinically relevant confounding factors evaluated included patient's age, sex, race (White/Asian/other), ECOG performance status, smoker status (current/previous/never), tumour histological type (squamous/non-squamous), number of tumour sites (<3 or ≥ 3), number of prior treatments in the advanced setting, PDL1 expression (positive/negative), serum lactate dehydrogenase (LDH; < or ≥ upper limit of normal [ULN]), blood C-reactive protein (CRP) level, and blood neutrophil to lymphocyte ratio (NLR; <3 or ≥ 3).

2.2.3 Statistical analysis

Associations between BMI and OS and PFS were modelled using Cox proportional hazards regression and reported as HR with 95%CI. Associations between BMI and TRAEs were modelled using logistic regression and reported as OR with 95%CI. All regression analyses were stratified by study. Survival curves for each category of BMI were estimated using the KM method.

Adjustment for potential confounding variables was undertaken by multivariable regression adjustment. Whether the association between BMI group and survival differed between males and females and between PDL1 positive and negative tumours was assessed using an interaction term in the Cox proportional regression model. Differences between BMI groups for the incidence of adverse events (both TRAEs and irAEs) were also evaluated using Cox proportional hazards. Evaluation of treatment benefit (atezolizumab vs docetaxel) by BMI subgroups was undertaken based on the intention to treat (ITT) populations of the two randomized trials – OAK and POPLAR. A treatment-by-BMI statistical interaction was evaluated in a Cox proportional regression model stratified by study. All analyses were conducted in R (version 3.4.3) using the survival package (Team 2017). Statistical tests were two-sided and a P value less than 0.05 was considered statistically significant.

2.3 Results

2.3.1 Association of BMI with survival outcomes and TRAEs for atezolizumab treated patients

Of the 1548 participants treated with atezolizumab across the four clinical trials, 114 (BMI unavailable in 40 participants and 74 underweight (BMI < 18.5 kg/m^2) were excluded from further analysis, leaving 1434 participants. Of these, 705 (49%) were normal weight, 490 (34%) were overweight and 239 (7%) were obese (Table 5). Compared to non-obese patients, a larger proportion of obese patients were white, male, previous smokers, and had NLR < 3 and lower CRP concentrations (Table 7).

Table 7: Baseline characteristics of atezolizumab treated patients

	Total No. 1,434	**BMI 18.5 - 24.9 No. 705**	**BMI 25.0 - 29.9 No. 490**	**BMI ≥30.0 No. 239**	**P-value**
Study					0.90
BIRCH	611 (43%)	301 (43%)	214 (44%)	96 (40%)	
FIR	122 (9%)	61 (9%)	44 (9%)	17 (7%)	
OAK	563 (39%)	274 (39%)	188 (38%)	101 (42%)	
POPLAR	138 (10%)	69 (10%)	44 (9%)	25 (10%)	
Age (years)	64 (57 - 70)	64 (56 - 70)	64 (58 - 71)	63 (57 - 70)	0.093
Sex					< 0.001*

Male	890 (62%)	388 (55%)	344 (70%)	158 (66%)	
Female	544 (38%)	317 (45%)	146 (30%)	81 (34%)	
Race					< 0.001*
White	1,132 (79%)	519 (74%)	407 (83%)	206 (86%)	
Asian	210 (15%)	146 (21%)	51 (10%)	13 (5%)	
Other	60 (4%)	30 (4%)	18 (4%)	12 (5%)	
Missing	32 (2%)	10 (1%)	14 (3%)	8 (3%)	
ECOG PS					0.067
0	507 (35%)	228 (32%)	188 (38%)	91 (38%)	
1	919 (64%)	474 (67%)	297 (61%)	148 (62%)	
2	6 (<1%)	2 (<1%)	4 (1%)	0 (0%)	
Missing	2 (<1%)	1 (<1%)	1 (<1%)	0 (0%)	
Histology					0.16
Non-squamous	1,033 (72%)	515 (73%)	358 (73%)	160 (67%)	
Squamous	401 (28%)	190 (27%)	132 (27%)	79 (33%)	
Tumour sites					0.13
<3	724 (50%)	349 (50%)	241 (49%)	134 (56%)	
≥3	676 (47%)	338 (48%)	240 (49%)	98 (41%)	
Missing	34 (2%)	18 (3%)	9 (2%)	7 (3%)	
Liver tumour site	273 (19%)	148 (21%)	82 (17%)	43 (18%)	0.17
Bone tumour site	406 (28%)	220 (31%)	126 (26%)	60 (25%)	0.058
Brain tumour site	65 (5%)	40 (6%)	20 (4%)	5 (2%)	0.055
Prior treatments[1]					0.53
0	157 (11%)	79 (11%)	54 (11%)	24 (10%)	
1	847 (59%)	402 (57%)	294 (60%)	151 (63%)	
2	430 (30%)	224 (32%)	142 (29%)	64 (27%)	
Smoking history					0.022*
Never	250 (17%)	136 (19%)	73 (15%)	41 (17%)	
Previous	1,012 (71%)	470 (67%)	365 (74%)	177 (74%)	
Current	172 (12%)	99 (14%)	52 (11%)	21 (9%)	
PD-L1 expression					0.91
Negative	491 (34%)	240 (34%)	166 (34%)	85 (36%)	
Positive	938 (65%)	462 (66%)	322 (66%)	154 (64%)	
Missing	5 (<1%)	3 (<1%)	2 (<1%)	0 (0%)	

Lactate dehydrogenase					0.78
≤ ULN	843 (59%)	412 (58%)	284 (58%)	147 (62%)	
> ULN	547 (38%)	268 (38%)	191 (39%)	88 (37%)	
Missing	44 (3%)	25 (4%)	15 (3%)	4 (2%)	
Neutrophil to lymphocyte ratio					0.004*
<3	491 (34%)	214 (30%)	177 (36%)	100 (42%)	
≥3	863 (60%)	450 (64%)	283 (58%)	130 (54%)	
Missing	80 (6%)	41 (6%)	30 (6%)	9 (4%)	
C Reactive Protein (mg/L)					< 0.001*
Median (IQR)	14 (4 - 42)	15 (4 - 48)	13 (5 - 45)	11 (4 - 32)	
Missing	43 (3%)	26 (4%)	9 (2%)	8 (3%)	

Data are median (IQR) or number of patients (%). P values per Fisher test for categorical data and Wilcoxon test for continuous data. [1]Number of prior treatments in the locally advanced or metastatic setting

OS differed significantly between normal, overweight and obese patients treated with atezolizumab (P = 0.0002), with improved OS for obese (HR 0.65) and overweight (HR 0.81) patients compared to patients with normal BMI (Table 8, Figure 3).

Table 8: BMI and OS/PFS for atezolizumab and docetaxel treated patients

	Atezolizumab treated patients		
BMI group (kg/m^2)	**HR for OS (95% CI)**		
	All patients	**PDL1 positive**	**PDL1 negative**
18.5 - 24.9	1.0	1.0	1.0
25.0 - 29.9	0.80 (0.68-0.95)	0.73 (0.58-0.91)	0.91 (0.71-1.16)
≥ 30.0	0.65 (0.51-0.81)	0.48 (0.34-0.66)	0.90 (0.66-1.22)
P value	0.0002	< 0.0001	0.68
BMI group (kg/m^2)	**HR for PFS (95% CI)**		
	All patients	**PDL1 positive**	**PDL1 negative**
18.5 - 24.9	1.0	1.0	1.0
25.0 - 29.9	0.89 (0.78-1.01)	0.86 (0.72-1.01)	0.93 (0.75-1.14)
≥ 30.0	0.86 (0.73-1.01)	0.78 (0.62-0.96)	1.01 (0.78-1.31)
P value	0.092	0.036	0.73
	Docetaxel treated patients		
BMI group (kg/m^2)	**HR for OS (95% CI)**		
	All patients	**PDL1 positive**	**PDL1 negative**
18.5 - 24.9	1.0	1.0	1.0

25.0 - 29.9	0.96 (0.78-1.18)	1.18 (0.83-1.69)	0.89 (0.72-1.11)
≥ 30.0	0.92 (0.70-1.21)	0.90 (0.55-1.45)	1.03 (0.77-1.37)
P value	0.82	0.48	0.51

This association remained significant after adjustment for potentially confounding variables (P=0.003, Table 9). The association between BMI groups and OS was consistent for males and females (P interaction = 0.764), but was significantly different between PDL1 positive and PDL1 negative tumours (P interaction 0.021). Specifically, the survival advantage associated with overweight and obese BMI groups was larger for PDL1 positive tumours than PDL1 negative tumours (Table 8, Figure 3).

Further, OS for patients with the highest PDL1 expression (≥50% of tumour cells or ≥10% of tumour-infiltrating immune cells; n=436) had HRs of 0.36 (95% CI 0.21 to 0.62) and 0.69 (95% CI 0.48 to 0.98) for obese and overweight groups, respectively. On the other hand, there was a trend towards improved PFS for the obese and overweight groups that did not reach statistical significance when analysed as separate groups (P=0.09, Table 8). The overweight and obese groups had similar PFS outcomes and in an exploratory analysis the combined overweight/obese BMI group demonstrated improved PFS compared to the normal BMI group (HR 0.88, 95% CI 0.78-0.99, P=0.03). The association between BMI and PFS was most apparent for the PDL1 positive tumours (PDL1 expression on ≥5% of tumour cells or tumour-infiltrating immune cells), and there was little indication of association for PDL1 negative tumours (Table 8). Patients with the highest category of PDL1 expression (on ≥50% of tumour cells or ≥10% of tumour-infiltrating immune cells; n=436) had PFS HRs of 0.68 (95% CI 0.49 to 0.94) and 0.72 (95% CI 0.56 to 0.92) for obese and overweight patient groups, respectively.

All-cause mortality and adverse events were then analysed for the trial population across the three BMI groups. As patients with high BMI have increased risks of deaths from cardiovascular diseases and other illnesses, non-cancer related deaths in the trial population was evaluated. Non-cancer related deaths were similar across the trials and BMI categories (Table 11 & Table 12).

Figure 3: OS as per BMI categories for atezolizumab and docetaxel treated patients

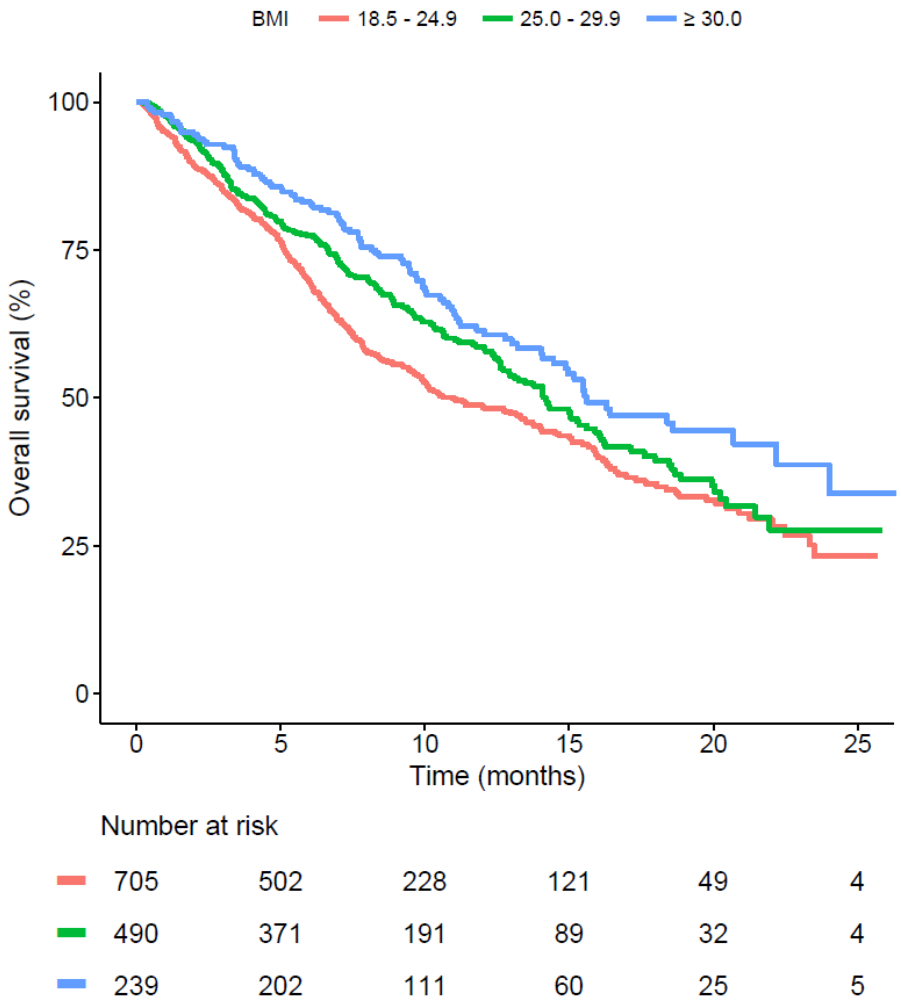


Fig 3A: All atezolizumab treated patients

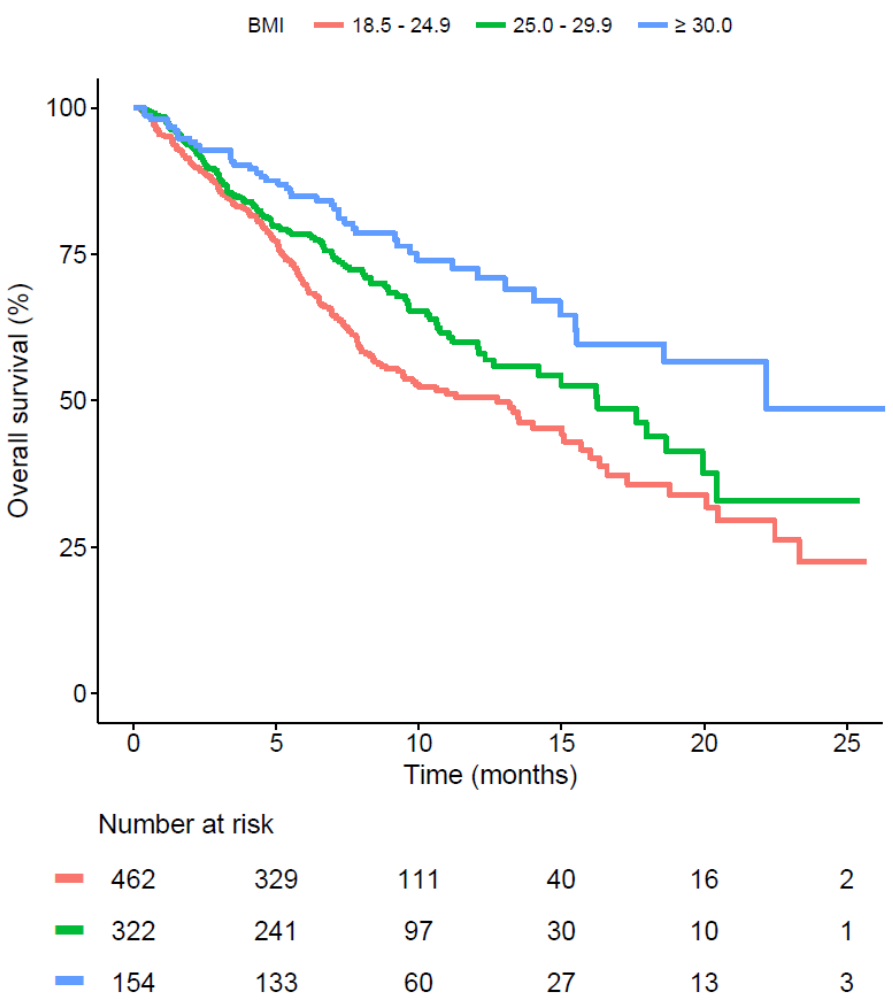


Fig 3B: PD-L1 positive atezolizumab treated patients

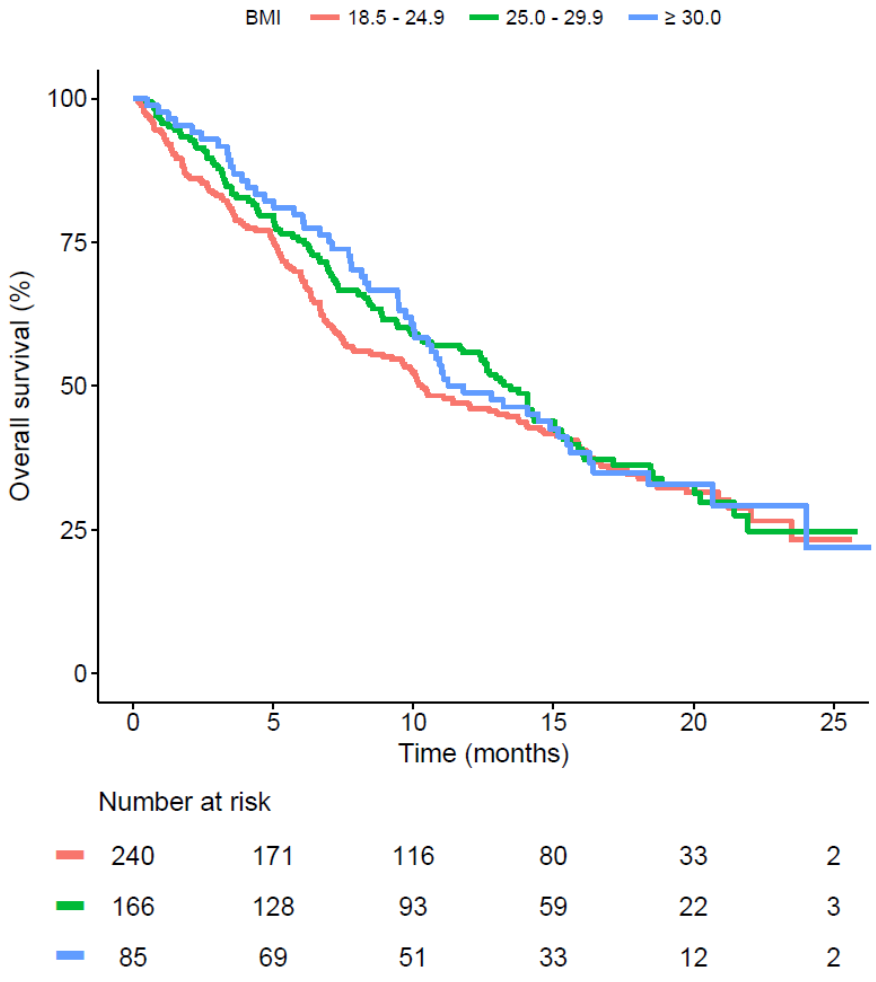


Fig 3C: PD-L1 negative atezolizumab treated patients

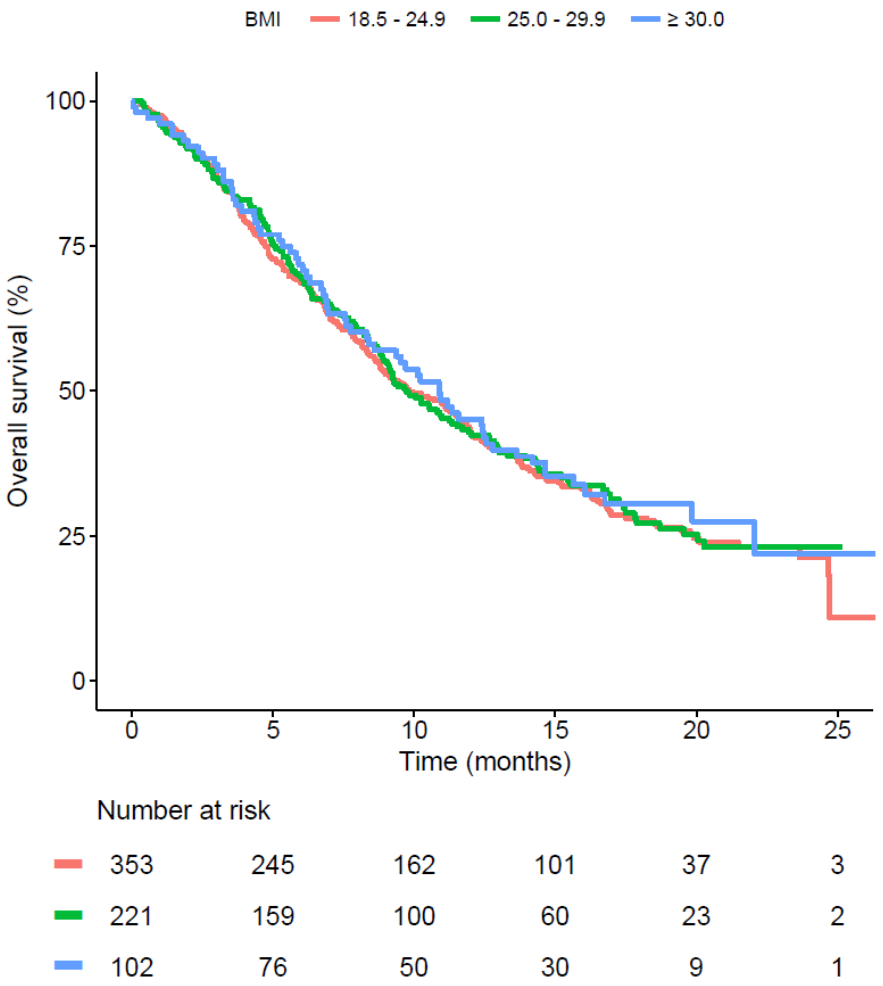


Fig 3D: Docetaxel

Table 9: Cox proportional hazards analysis for OS/PFS for atezolizumab treated patients

Variables	Overall Survival - HR (95% CI)				
	All trials	BIRCH	FIR	OAK	POPLAR
BMI (Kg/m^2)					
25.0-29.9	0.80 (0.67-0.96)	0.78 (0.56-1.09)	0.30 (0.09-0.96)	0.90 (0.69-1.16)	0.32 (0.16-0.67)
≥ 30	0.69 (0.54-0.87)	0.58 (0.35-0.95)	0.13 (0.01-1.23)	0.83 (0.60-1.14)	0.37 (0.18-0.75)
Age (years)	1.0 (0.99-1.01)	1.01 (0.99-1.03)	0.97 (0.97-1.08)	1.00 (0.99-1.01)	1.00 (0.97-1.03)
Female sex	1.02 (0.85-1.23)	1.17 (0.85-1.60)	0.68 (0.19-2.47)	1.02 (0.78-1.33)	0.77 (0.42-1.42)
Race					
Asian	0.81 (0.63-1.04)	1.61 (1.00-2.58)	2.78 (0.74-10.5)	0.69 (0.50-0.95)	0.40 (0.16-1.01)
Other	1.18 (0.82-1.69)	1.48 (0.73-3.00)	0.24 (0.02-2.66)	1.07 (0.65-1.78)	1.07 (0.41-2.27)
EOCG PS	1.42 (1.18-1.70)	1.50 (1.07-2.10)	2.08 (0.76-5.69)	1.38 (1.08-1.76)	1.30 (0.71-2.40)
Squamous histology	1.15 (0.96-1.38)	1.05 (0.76-1.45)	1.34 (0.40-4.47)	1.26 (0.97-1.63)	0.90 (0.51-1.60)
Count of tumour sites	1.52 (1.29-1.79)	1.24 (0.91-1.69)	1.14 (0.44-2.95)	1.73 (1.17-2.18)	1.03 (0.56-1.90)
Number of prior treatments	1.10 (0.95-1.27)	1.09 (0.89-1.34)	0.76 (0.21-2.79)	1.06 (0.82-1.36)	0.74 (0.43-1.28)
Smoking history					
Previous	0.77 (0.61-0.98)	0.72 (0.47-1.09)	0.69 (0.23-2.01)	0.77 (0.55-1.09)	0.97 (0.42-2.24)
Current	0.79 (0.58-1.10)	0.80 (0.42-1.51)	0.52 (0.11-2.79)	0.73 (0.46-1.16)	0.82 (0.29-2. 82)

PD-L1 high	0.59 (0.47-0.74)	N/A	NA	0.56 (0.43-0.73)	0.61 (0.34-1.09)
High LDH at baseline	1.39 (1.18-1.64)	1.57 (1.15-2.13)	1.42 (0.47-4.26)	1.36 (1.08-1.70)	1.79 (1.02-3.14)
Log CRP	1.42 (1.33-1.52)	1.59 (1.40-1.80)	1.43 (1.00-2.05)	1.34 (1.23-1.47)	1.62 (1.30-2.03)
Neutrophil Lymphocyte Ratio > 3	1.52 (1.25-1.85)	1.79 (1.19-2.70)	3.02(0.56-16.3)	1.42 (1.10-1.84)	1.48 (0.81-2.70)
Progression free survival - HR (95% CI)					
BMI (Kg/m^2)					
25.0-29.9	0.88(0.77-1.02)	0.84 (0.67-1.05)	0.50 (0.19-1.31)	1.00 (0.81-1.24)	0.68 (0.41-1.12)
≥ 30	0.87 (0.72-1.04)	0.77 (0.57-1.03)	0.52 (0.13-2.07)	1.09 (0.83-1429)	0.52 (0.29-0.94)
Age (years)	0.99 (0.99-1.00)	0.99 (0.98-1.01)	1.00 (0.97-1.04)	0.99 (0.98-1.00)	1.00 (0.97-1.02)
Female sex	0.99 (0.86-1.14)	1.01 (0.82-1.26)	0.93 (0.36-2.36)	0.94 (0.75-1.17)	1.20 (0.75-1.92)
Race					
Asian	1.12 (0.93-1.35)	1.34 (0.97-1.84)	2.89 (0.88-9.44)	1.02 (0.78-1.31)	0.80 (0.43-1.49)
Other	1.15 (0.85-1.57)	1.72 (0.95-3.15)	1.55 (0.27-8.83)	0.94 (0.61-1.46)	0.84 (0.40-1.77)
EOCG PS	1.12 (0.98-1.28)	1.08 (0.87-1.33)	1.04 (0.48-2.25)	1.02 (0.84-1.24)	1.54 (0.97-2.43)
Squamous histology	1.09 (0.95-1.26)	1.10 (0.88-1.38)	0.89 (0.34-2.29)	1.09 (0.87-1.37)	1.02 (0.65-1.60)
Count of tumour sites	1.20 (1.06-1.37)	0.98 (0.79-1.21)	1.07 (0.49-2.34)	1.41 (1.16-1.71)	1.39 (0.82-2.23)
Number of prior treatments	0.97 (0.87-1.08)	1.00 (0.88-1.15)	0.76 (0.24-2.37)	0.87 (0.70-1.08)	0.84 (0.54-1.32)
Smoking history					

Previous	0.69 (0.58-0.83)	0.65 (0.49-0.87)	1.21 (0.46-3.15)	0.64 (0.49-0.85)	0.77 (0.44-1.38)
Current	0.59 (0.46-0.76)	0.61 (0.40-0.93)	1.11 (0.28-4.44)	0.52 (0.35-0.77)	0.50 (0.23-1.09)
PD-L1 high	0.70 (0.58-0.85)	NA	NA	0.68 (0.55-0.84)	0.70 (0.46-1.08)
High LDH at baseline	1.33 (1.16-1.51)	1.46 (1.18-1.80)	2.21 (0.92-5.30)	1.28 (1.06-1.55)	1.05 (0.68-1.63)
Log CRP	1.15 (1.09-1.21)	1.20 (1.11-1.30)	1.44 (1.06-1.95)	1.11 (1.03-1.20)	1.13 (0.95-1.35)
Neutrophil Lymphocyte Ratio > 3	1.19 (1.03-1.37)	1.16 (0.92-1.46)	0.65 (0.19-2.24)	1.23 (1.00-1.51)	1.42 (0.90-2.24)

Table 10: Sex-specific association between BMI and OS for patients treated with atezolizumab

	Events/Patients	**Median, months (95% CI)**	**Univariable HR (95% CI)** [1]
Women			
BMI 18.5-24.9	155/317	13.3 (10.1-16.3)	1 (reference)
BMI 25.0-29.9	59/146	15.0 (14.1-NR)	0.74 (0.55-0.99)
BMI ≥ 30	27/81	NR	0.57 (0.38-0.86)
Men			
BMI 18.5-24.9	206/388	10.0 (8.2-13.5)	1 (reference)
BMI 25.0-29.9	165/344	13.2 (12.1-16.0)	0.80 (0.65-0.98)
BMI ≥ 30	73/158	14.9 (11.8-20.7)	0.65 (0.50-0.85)

[1]stratified for study

Table 11: Cause of death by trials

Cause	N (%)				
	All trials (n = 742)	**BIRCH** (n = 223)	**FIR** (n = 60)	**OAK** (n = 383)	**POPLAR** (n = 76)
Cancer related	673 (91%)	200 (90%)	50 (83%)	357 (93%)	66 (87%)
Non-cancer related	69 (9%)	23 (10%)	10 (17%)	26 (7%)	10 (13%)

Table 12: Cause of deaths by BMI groups

Cause of deaths	BMI groups (Kg/m^2) N (%) P = 0.44				
	Total (n = 742)	**18-5-24.9** (n = 361)	**25.0-29.9** (n = 224)	**≥30** (n = 100)	**Missing** (n = 57)
Cancer related	673 (91%)	326 (90%)	201 (90%)	94 (94%)	52 (91%)
Non-cancer related	69 (9%)	35 (9.7%)	23 (10%)	6 (6%)	5 (8.8%)

Toxicities from atezolizumab can be immune related or non-immune related adverse events. The incidence of all TRAEs was not significantly different between the BMI categories (all grades - 65%, 64% and 65%; P = 0.92 and grade 3 to 5 - 12%, 14% and 12%; P = 0.66, respectively for normal, overweight and obese categories). Similarly, no significant differences were seen in the frequency of irAEs across BMI categories except for skin related irAEs (Table 13 and Figure 4).

Table 13: Pooled adverse events related to atezolizumab across all trials

Adverse events	BMI groups (Kg/m^2) N (%)				P value
	Total (n = 1411)	**18-5-24.9** (n = 693)	**25.0-29.9** (n = 480)	**≥30** (n = 238)	
TRAEs	911 (64%)	450 (65%)	307 (64%)	154 (65%)	0.92
TRAEs (grade 3 or more)	178 (13%)	83 (12%)	67 (14%)	28 (12%)	0.66
irAEs (all grades)	390 (27%)	177 (26%)	138 (29%)	75 (32%)	0.73

2.3.2 Association of BMI with survival outcomes for docetaxel treated patients

Of the 713 participants treated with docetaxel in the OAK and POPLAR trials, 676 individuals (BMI unavailable in 9 and 28 were underweight) were included for further analysis. Characteristics of this cohort by BMI category are summarised in Table 14. For patients treated with docetaxel, there was no significant association between BMI and OS (P = 0.82) or PFS (P = 0.36; Table 8 and Figure 3). Additionally, the association between BMI and OS did not differ significantly between PDL1 positive and PDL1 negative tumours (P interaction = 0.41, Table 8).

2.3.3 Association of BMI with atezolizumab treatment efficacy

Prior analyses evaluated BMI as a prognostic marker of survival - the association between BMI and survival for patients treated with a specific treatment. Here, I report the evaluation of treatment effect modification in randomised clinical trials. In contrast to evaluating whether BMI is a prognostic marker of survival, we evaluate whether BMI is a 'predictive marker' of treatment benefit - the degree to which atezolizumab improves survival over docetaxel.

Exploratory analysis of atezolizumab treatment benefit (vs docetaxel) for BMI subgroups was restricted to the intention-to-treat (ITT) populations of the OAK and POPLAR randomized clinical trials (i.e. excluding the single arm studies BIRCH and FIR). The pooled ITT populations of OAK and POPLAR included 1,512 patients. However, as BMI was unavailable for 24 patients and 69 were underweight, these 93 were excluded, leaving 1,419 participants (707 randomly allocated to atezolizumab treatment and 712 randomly allocated to docetaxel treatment) in the ITT analysis population. Baseline characteristics were well balanced between the two treatment arms in the ITT analysis population (Table 15). The atezolizumab and docetaxel arms had median OS of 13.2 months and 9.8 months respectively, with a treatment efficacy HR of 0.79 (95% CI 0.69-0.90, P = 0.0004).

The estimated OS benefit of atezolizumab treatment (compared to docetaxel treatment) differed numerically between BMI groups (Figure 5 & 6), with HR values of 0.86, 0.79 and 0.68 for normal weight, overweight and obese patients, respectively. Atezolizumab survival benefit differences between BMI subgroups was most

pronounced for participants with PDL1 positive tumours, with treatment efficacy HR of 0.83, 0.59 and 0.46 for normal weight, overweight and obese patients, respectively (Figure 5). However, the test for statistical interaction between BMI subgroups and atezolizumab OS benefit over docetaxel did not reach statistical significance for the ITT analysis population (P interaction = 0.10) or the subset of PDL1 positive tumours (P interaction = 0.096). Notably, the HR values numerically favoured atezolizumab in all BMI subgroups (Figure 5).

2.4 Discussion

The present analyses, which pools data from multiple prospectively conducted clinical trials of atezolizumab, is the largest study to evaluate the relationship between obesity and ICI therapy outcomes. It was demonstrated that high BMI was associated with improved overall survival in patients with advanced NSCLC. Here, it was identified for the first time that there is a nearly linear relationship between BMI and OS from atezolizumab therapy when normal, overweight, and obese categories were compared. The association between BMI and OS remained significant after adjustment for trial specific stratification factors and a wide number of clinically relevant confounders. The strength of the association was further increased by the presence of PDL1 in the tumour/immune cells. While the current analysis is a post-hoc analysis of data from clinical trials, the results are consistent with prior studies that demonstrated high BMI being associated with improved survival outcomes from ICI across cancer types such as melanoma (Cortellini, Bersanelli, et al. 2019; McQuade et al. 2018; Wang, Aguilar, et al. 2019).

The current study adds to the emerging evidence that high BMI is associated with cancer survival from immunotherapy. However, the biological basis of the association is only just beginning to be understood. It is possible that obesity may induce a low-grade systemic meta-inflammation and impaired immune response. Moreover, obesity induces T-cell dysfunction and increases the exhausted PD-1 positive T-cell phenotype in fat and tumour microenvironment through leptin production, which may be the link between obesity and immune response (Wang, Aguilar, et al. 2019; Murphy et al. 2018). The identified association between high BMI and OS with atezolizumab was particularly strong in the PDL1 positive population, lending further

Figure 4: Forest plots for adverse events from atezolizumab

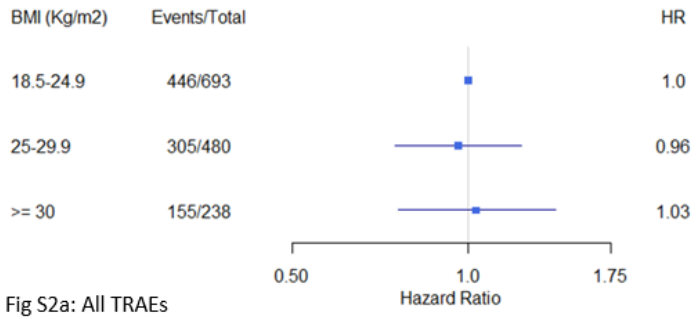


Fig S2a: All TRAEs

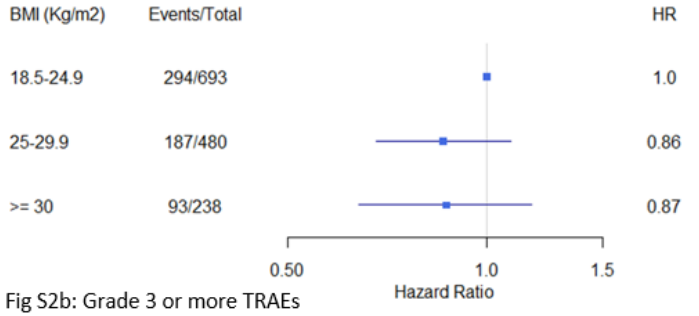


Fig S2b: Grade 3 or more TRAEs

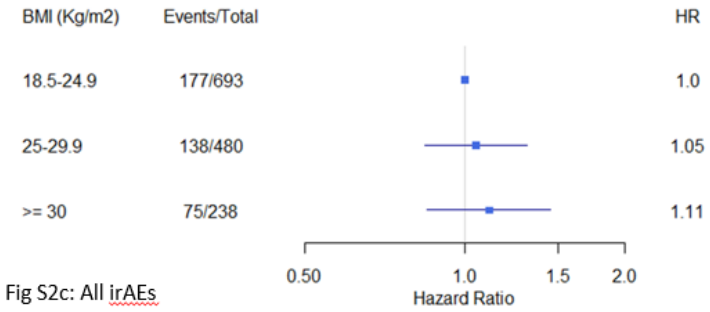


Fig S2c: All irAEs

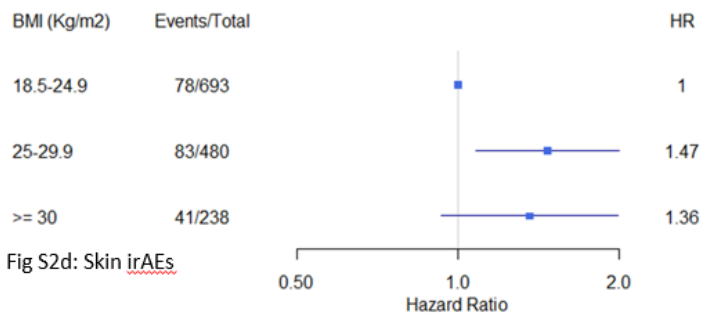


Fig S2d: Skin irAEs

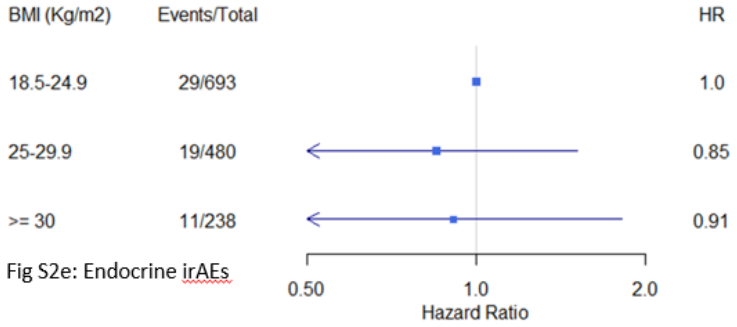


Fig S2e: Endocrine irAEs

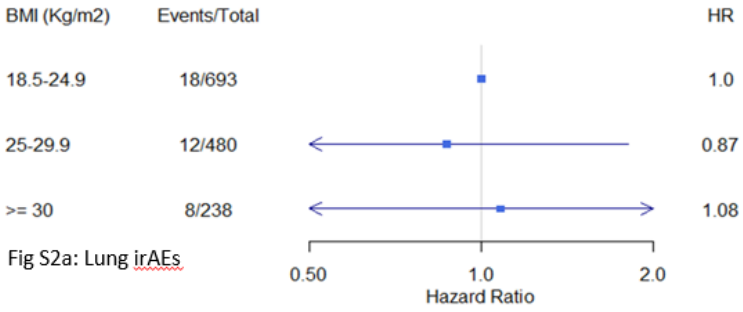


Fig S2a: Lung irAEs

Table 14: Baseline characteristics of docetaxel treated patients

	Total No. 676	BMI 18.5 - 24.9 No. 353	BMI 25.0 - 29.9 No. 221	BMI ≥ 30.0 No. 102	P-value
Study					0.055
OAK	548 (81%)	294 (83%)	180 (81%)	74 (73%)	
POPLAR	128 (19%)	59 (17%)	41 (19%)	28 (27%)	
Age (years)	63 (57 - 69)	62 (56 - 70)	65 (58 - 69)	62 (56 - 68)	0.064
Sex					0.53
Male	419 (62%)	212 (60%)	143 (65%)	64 (63%)	
Female	257 (38%)	141 (40%)	78 (35%)	38 (37%)	
Race					< 0.001*
White	497 (74%)	234 (66%)	169 (76%)	94 (92%)	
Asian	124 (18%)	87 (25%)	33 (15%)	4 (4%)	
Other	35 (5%)	21 (6%)	11 (5%)	3 (3%)	
Missing	20 (3%)	11 (3%)	8 (4%)	1 (1%)	
ECOG PS					0.72
0	250 (37%)	126 (36%)	84 (38%)	40 (39%)	
1	425 (63%)	227 (64%)	137 (62%)	61 (60%)	
Missing	1 (<1%)	0 (0%)	0 (0%)	1 (1%)	
Histology					0.17
Non-squamous	484 (72%)	263 (75%)	154 (70%)	67 (66%)	
Squamous	192 (28%)	90 (25%)	67 (30%)	35 (34%)	
Tumour sites					0.015*
<3	266 (39%)	123 (35%)	92 (42%)	51 (50%)	
≥3	410 (61%)	230 (65%)	129 (58%)	51 (50%)	
Liver tumour site	145 (21%)	80 (23%)	48 (22%)	17 (17%)	0.45
Bone tumour site	207 (31%)	112 (32%)	71 (32%)	24 (24%)	0.24
Brain tumour site	66 (10%)	42 (12%)	17 (8%)	7 (7%)	0.16
Prior treatments[1]					0.38
1	499 (74%)	258 (73%)	160 (72%)	81 (79%)	
2	177 (26%)	95 (27%)	61 (28%)	21 (21%)	
Smoking history					0.12
Never	110 (16%)	68 (19%)	30 (14%)	12 (12%)	
Previous	457 (68%)	225 (64%)	161 (73%)	71 (70%)	
Current	109 (16%)	60 (17%)	30 (14%)	19 (19%)	
PD-L1 expression					0.76
Negative	457 (68%)	242 (69%)	150 (68%)	65 (64%)	
Positive	216 (32%)	110 (31%)	71 (32%)	35 (34%)	
Missing	3 (<1%)	1 (<1%)	0 (0%)	2 (2%)	
Lactate dehydrogenase					0.76
≤ ULN	371 (55%)	193 (55%)	120 (54%)	58 (57%)	
> ULN	287 (42%)	152 (43%)	96 (43%)	39 (38%)	
Missing	18 (3%)	8 (2%)	5 (2%)	5 (5%)	
Neutrophil to lymphocyte ratio					0.97
<3	247 (37%)	128 (36%)	82 (37%)	37 (36%)	
≥3	418 (62%)	219 (62%)	139 (63%)	60 (59%)	
Missing	11 (2%)	6 (2%)	0 (0%)	5 (5%)	
C Reactive Protein (mg/L)					0.55
Median (IQR)	13 (4 - 38)	14 (4 - 39)	11 (4 - 36)	13 (5 - 31)	
Missing	7 (1%)	3 (1%)	2 (1%)	2 (2%)	

Data are median (IQR) or number of patients (%). P values per Fisher test for categorical data and Wilcoxon test for continuous data. [1]Number of prior treatments in the locally advanced or metastatic setting.

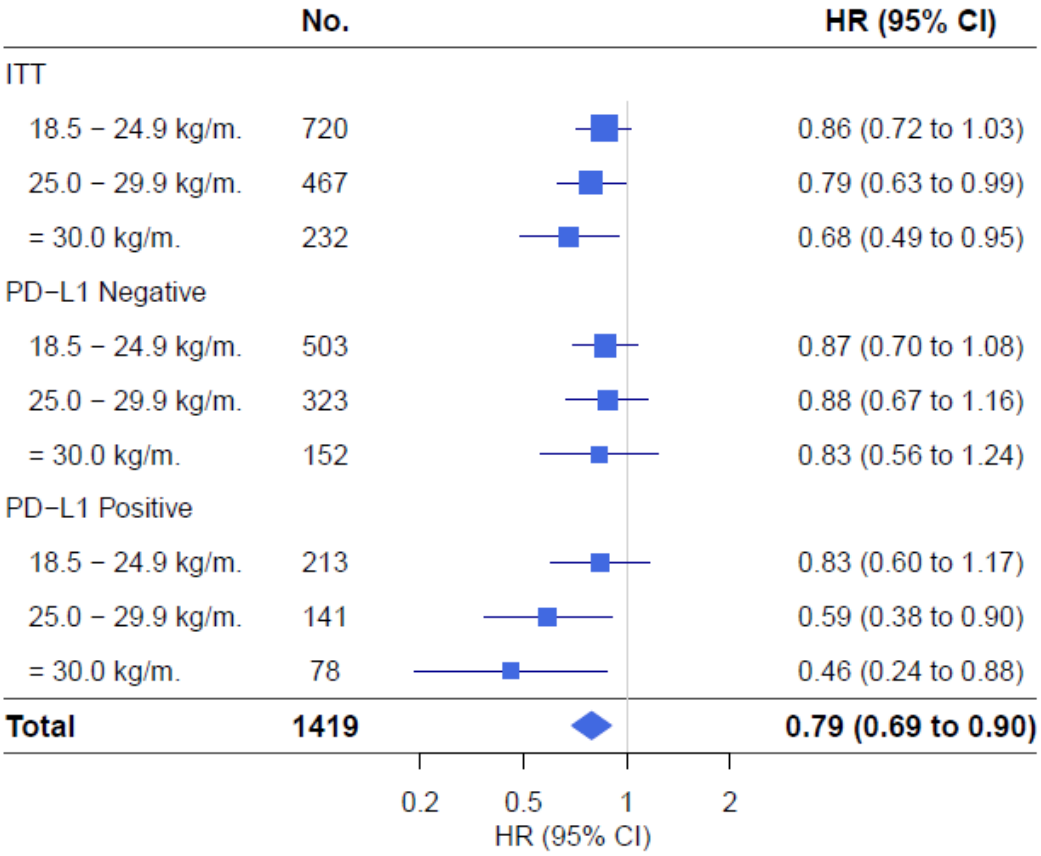
Table 15: Baseline characteristics from OAK and POPLAR trials

	Total No. 1,419	Docetaxel No. 712	Atezolizumab No. 707
Study			
OAK	1,144 (81%)	577 (81%)	567 (80%)
POPLAR	275 (19%)	135 (19%)	140 (20%)
Age (years)	63 (56 - 69)	63 (57 - 69)	63 (56 - 69)
Sex			
Male	886 (62%)	438 (62%)	448 (63%)
Female	533 (38%)	274 (38%)	259 (37%)
Race			
White	1,036 (73%)	522 (73%)	514 (73%)
Asian	263 (19%)	130 (18%)	133 (19%)
Other	73 (5%)	38 (5%)	35 (5%)
Missing	47 (3%)	22 (3%)	25 (4%)
ECOG PS			
0	524 (37%)	267 (38%)	257 (36%)
1	893 (63%)	444 (62%)	449 (64%)
Missing	2 (<1%)	1 (<1%)	1 (<1%)
Histology			
Non-squamous	1,027 (72%)	514 (72%)	513 (73%)
Squamous	392 (28%)	198 (28%)	194 (27%)
Tumour sites			
<3	594 (42%)	282 (40%)	312 (44%)
≥3	825 (58%)	430 (60%)	395 (56%)
Liver tumour site	299 (21%)	151 (21%)	148 (21%)
Bone tumour site	430 (30%)	218 (31%)	212 (30%)
Brain tumour site	126 (9%)	72 (10%)	54 (8%)
Prior treatments[1]			
1	1,041 (73%)	529 (74%)	512 (72%)
2	378 (27%)	183 (26%)	195 (28%)
Smoking history			
Never	251 (18%)	122 (17%)	129 (18%)
Previous	959 (68%)	476 (67%)	483 (68%)
Current	209 (165)	114 (16%)	95 (13%)
BMI (Kg/m^2)			
18.5-24.9	720 (51%)	377 (53%)	343 (49%)
25.0-29.9	467 (33%)	229 (32%)	238 (34%)
≥ 30	232 (16%)	106 (15%)	126 (18%)
PD-L1 expression			
Negative	978 (69%)	483 (68%)	495 (70%)
Positive	432 (30%)	225 (32%)	207 (29%)
Missing	9 (1%)	4(1%)	5 (1%)
Lactate dehydrogenase			
≤ ULN	777(55%)	372 (55%)	405 (57%)
> ULN	560 (39%)	287 (40%)	273 (39%)
Missing	82 (6%)	53 (7%)	29 (4%)
Neutrophil to lymphocyte ratio			
<3	515 (37%)	247 (35%)	268 (38%)
≥3	837 (59%)	419 (59%)	418 (59%)
Missing	67 (5%)	46 (6%)	21 (3%)
C Reactive Protein (mg/L)			
Median (IQR)	14 (4 - 41)	13 (4 - 38)	15 (5 - 43)
Missing	58 (4%)	42 (6%)	16 (2%)

Data are median (IQR) or number of patients (%). [1]Number of prior treatments in the locally advanced or metastatic setting.

Figure 5: Atezolizumab vs Docetaxel OS differences by ITT, PDL1 status

A



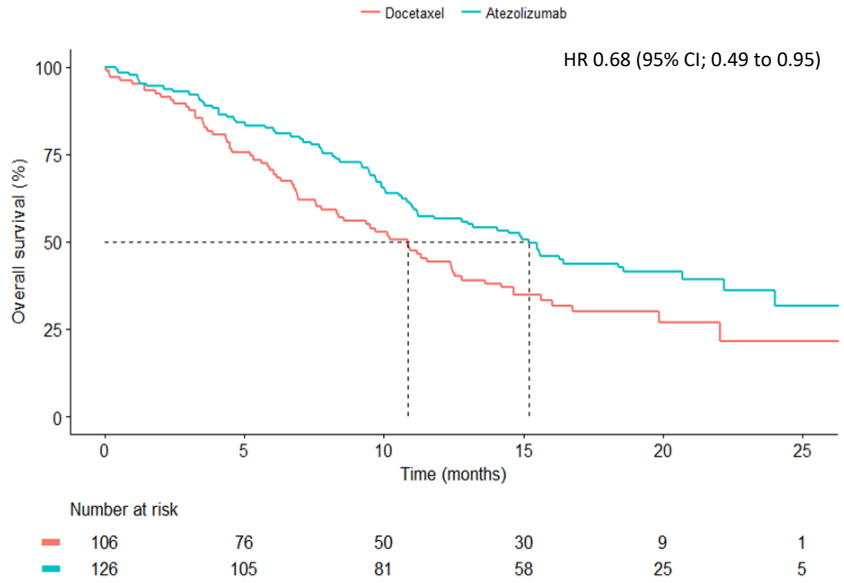
support to the presence of a T-cell dysfunction state in obese patients. Atezolizumab, by virtue of its mechanism of action of PD1/PDL1 axis inhibition on T-cells, might to induce a favourable response in obese patients with an established T-cell exhausted state.

The relationship between obesity and cancer prognosis is complicated. While obesity increases the risks of development of certain types of cancers such as breast cancers, it protects against worse outcomes in patients with advanced cancers such as lung cancers that are associated with wasting (Azvolinsky 2014). Obesity's association with improved survival in patients with lung cancer may not be specific to ICI therapy.

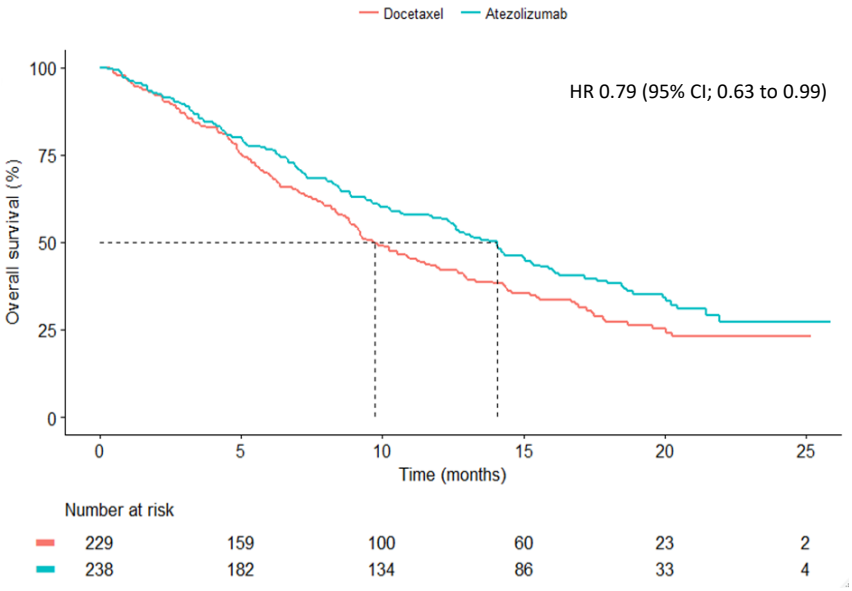
Previous observations indicate that high BMI is associated with better outcomes with surgery, radiotherapy and some types of chemotherapy in patients with early and advanced NSCLC (Yap et al. 2018; Sepesi et al. 2017; Masel et al. 2017; Dahlberg et al. 2013). In contrast, high BMI was not associated with survival benefit from chemotherapy with docetaxel. It appears that obesity may have a varying influence across the spectrum of treatment interventions for lung cancer.

Figure 6: OS as per BMI categories for pooled OAK and POPLAR trials

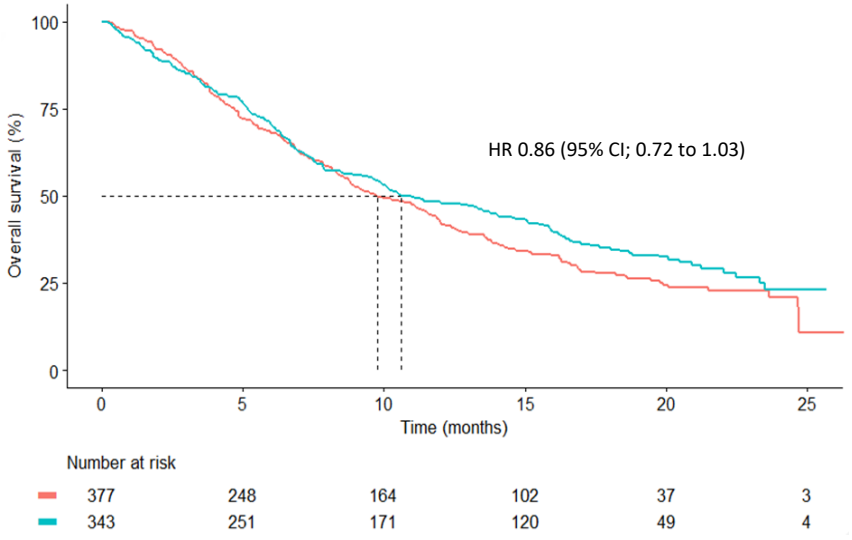
A) BMI ≥ 30 kg/m^2



B) BMI 25-29.9 kg/m^2



C) BMI 18.5-24.9 kg/m^2



The ITT comparison of atezolizumab versus docetaxel for BMI subgroups is novel. The observed signal of atezolizumab OS benefit between BMI subgroups in PDL1 positive tumours, should be re-evaluated in future studies with larger datasets of ICI treated patients. Moreover, it is unclear in this analysis if BMI could be considered as a treatment effect modifier due to lack of adequate power. Future research on the effect of BMI sub-groups across all ICI therapy trials may provide adequate power to evaluate this question.

It is well recognized that men and women have different body composition and adiposity with varying prevalence of obesity. However, the interaction between sex and ICI therapy outcomes is inconsistent. A recent report identified that sex may be a predictor of response to ipilimumab, a CTLA4 antibody, but not with PD1/PDL1 inhibitors, with males having better OS when compared to females due to sexual dimorphism in immune response (Conforti et al. 2018). An updated meta-analysis reported that both men and women had similar OS benefit with ICI therapies (Wallis et al. 2019). However, female patients with NSCLC have better overall outcomes than males, even after adjusting for smoking, cancer histology and oncogene mutations (Sagerup et al. 2011; Kawaguchi et al. 2010). Contrary to a previous report where obese men had a better outcome with immunotherapy in melanoma (McQuade et al. 2018), this research indicated that sex had no significant effect on the improved survival seen with obese males and females.

The relationship between BMI and treatment related and irAEs from ICI has been variably reported. In our dataset, we did not find that obese patients had an increased incidence of any grade of TRAEs when compared to normal BMI, similar to the results of McQuade et al (McQuade et al. 2018). However, a retrospective series by Cortellini et al (Cortellini, Bersanelli, et al. 2019) that included various cancer types and those with poor performance status, reported a higher incidence of any grade of irAEs in overweight/obese patients. Given the expected improved accuracy of data collected through prospective clinical trials in our analysis, it is unlikely that obesity is associated with increased TRAEs. Among the irAEs, except for skin irAEs, none of the other specific irAEs were consistently associated with high BMI possibly due to small sample size. Future research using a large using datasets from all ICI trials could robustly evaluate the relationship between obesity and the incidence of irAEs.

In the present post-hoc exploratory analysis, pooling of prospectively collected clinical trial data provided one of the largest cohorts of patients (>2,200 in total) who received uniform treatment with atezolizumab. The data were of high-quality and allowed analysis with adjustments for key clinical confounders. Further, the data contained only a small amount of missing information improving the accuracy of our analyses. The ITT analysis that compared atezolizumab and docetaxel arms for BMI sub-groups is quite unique in our study.

There are several limitations in our study. The results from this analysis should be considered as exploratory, not pre-planned, and need to be confirmed in subsequent clinical trials. Moreover, BMI alone as a measure of obesity is problematic due to its inability to differentiate fat and lean muscle mass, and to diagnose sarcopenia and its poor reflection of body fat distribution. It is likely a combination of clinical and biochemical markers may be required to characterise obesity more accurately.

Another known prognostic factor that influences survival in patients with NSCLC is pre-treatment weight loss, a measure of cachexia (Buccheri and Ferrigno 1994; Morel et al. 2018; Yang et al. 2011). Weight loss (either pre-treatment or during treatment) may variably influence treatment response. In the current analysis, one-time recorded height and weight at screening or day 1 of trial treatment for the calculation of BMI. As pre-treatment weight loss was variably recorded in the dataset provided, the effect of this important prognostic factor could not be assessed. It would be relevant to analyse data from other trials that have prospectively collected information on pre-treatment weight loss. Despite these limitations, the strength of the association between BMI and OS from atezolizumab especially in PDL1 positive patients cannot be ignored.

2.5 Conclusion

Baseline high BMI is independently associated with improved survival with atezolizumab in patients with advanced NSCLC and baseline BMI should therefore be considered as a stratification factor in future ICI therapy trials. As evidenced in this chapter, BMI, an under-studied baseline characteristic of patients undergoing ICI therapy, seems to strongly influence outcomes from atezolizumab. While this is an interesting and novel finding, future studies are warranted to confirm these in other cancer types as well as other non-atezolizumab ICI therapies.

The research work described in chapter 2 was published in a major peer-reviewed journal, initially as an early online version in December 2019 and as final publication in April 2020 (Kichenadasse et al. 2019b). As of 14th of August 2020, this paper had an altmetric attention score of 444 with nine citations, and 49 news outlets reporting on the obesity and outcome association (Altmetric 2020). Sanchez et al raised several questions on their commentary paper related to this publication and my responses to their comments addressing the complex relationship between body size and survival outcomes was subsequently published (Kichenadasse, Hopkins, and Sorich 2020; Sanchez and Furberg 2020) (Appendix 2). Since then, data from other studies including a meta-analysis have confirmed the association between BMI and ICI outcomes indicating that the results from my research were not statistical artefacts, but, likely a true association (An et al. 2020; Rogado et al. 2020; Martini et al. 2020).

3. CHAPTER THREE: VARIABILITY OF ADVERSE EVENTS FROM IMMUNE CHECKPOINT INHIBITORS

This chapter has been derived and adapted with permission from the following publication:

Kichenadasse G, Miners JO, Mangoni AA, Rowland A, Hopkins AM, Sorich MJ. Multi-organ immune-related adverse events during treatment with atezolizumab. ***J Natl Compr Can Netw***. 2020 Mar. [Accepted for publication]. The accepted manuscript has been reproduced in Appendix 3.

In the previous chapter, the relationship between baseline BMI and survival outcomes in patients with NSCLC being treated with ICI or chemotherapy were evaluated using data from clinical trials. During the analysis of data for the previous chapter as well as personal observations from treating patients with ICI in my own clinical practice, it became apparent some patients develop more than one organ type irAE. However, there was limited literature on this issue of multi-organ irAEs in the public domain. In chapter 3, I sought to characterise the pattern of multi-organ irAEs and identify those patients who develop multi-organ iRAEs from ICI using the same dataset that was analysed in chapter 2.

3.1 Introduction

As previously presented, ICI commonly target CTLA-4, PD1 and PDL1 that promote inhibitory signals on immune effector T cells against cancer cells (Wei, Duffy, and Allison 2018). While ICI have improved outcomes in several cancers, their use is also associated with significant adverse events (AEs) including death (Wang et al. 2018).

In contrast to other anti-cancer therapies, ICI cause heterogeneous toxicities through non-specific immune activation affecting tissues and organs. While guidelines exist for diagnosis and treatment of toxicities from ICI therapy, there is no international consensus on the terminology used for the definition, diagnostic criteria, grading, causal attribution, as well as little evidence supporting their management (Brahmer, Lacchetti, and Thompson 2018; Haanen et al. 2017; Puzanov et al. 2017). The European Society of Medical Oncology guidelines divide AEs from ICI therapy into infusion reactions and irAEs (Haanen et al. 2017). Others have

used AEs of special interest (AEoSI) or immune mediated adverse events interchangeably with irAEs (Wang, Chen, et al. 2017). Their pathophysiology is poorly understood with several proposed mechanisms leading to organ damage (Hirschhorn et al. 2018; Postow, Sidlow, and Hellmann 2018).

In addition, the reported incidence and time-course of irAEs from clinical trials varied between the type of checkpoint being targeted. A recent network meta-analysis identified that atezolizumab, an anti PD-L1 inhibitor, had the best safety profile (Xu et al. 2018). Other analyses reported an incidence of up to 30% with PD-1/PD-L1 inhibitors (Nishijima et al. 2017), 72% with ipilimumab (Bertrand et al. 2015), and 88% with combined therapies (Gu et al. 2019).

While more than one autoimmune disease can occur in the same patient (poly-autoimmunity) (Matusiewicz, Strozynska-Byrska, and Olesinska 2019), it is unclear whether multiple organ irAEs occurred in the same patient, and when they occur, simultaneously or serially. Moreover, the reported irAEs in clinical trials are often limited to single organs preventing accurate information regarding the incidence and time course of multiple irAEs (Maughan et al. 2017). Such an occurrence of multiple irAEs in the same patient may influence their treatment decisions both for the AEs and the cancer. There are additional complexities such as use of polypharmacy and involvement of various specialties for their management. Hence, it is important to have a thorough understanding of multi-organ irAEs from ICIs. Using pooled data from four prospective clinical trials in NSCLC patients treated with atezolizumab monotherapy, I analysed the incidence, severity, time-course, treatment, outcomes and risk factors of various organ-specific irAEs and their relationships with response rates/survival outcomes.

3.2 Methods

3.2.1 Study Population

Individual patient data from the clinical trials OAK (NCT02008227) (Rittmeyer et al. 2017), POPLAR (NCT01903993) (Fehrenbacher et al. 2016), BIRCH (NCT02031458) (Peters et al. 2017), and FIR (NCT01846416) (Spigel et al. 2018) were analysed via establish protocols for clinical data sharing

(Clinicalstudydatarequest 2019). This secondary analysis of trial data was exempted from review by the local Ethics Committee. Only atezolizumab treated cohorts were included in the analysis.

3.2.2 Definition of adverse events

AEs which the investigator attributed causality to atezolizumab and occurred while on atezolizumab or within 30 days after the last dose, were adjudicated treatment related AEs (TRAEs). irAEs were defined as per pre-specified study protocols (i.e. AEoSI as conditions suggestive of an autoimmune disorder from atezolizumab). These events were organ-specific and included endocrine, eye, gastrointestinal (GI), hepatobiliary and pancreatic (HBP), neurological, pulmonary, rheumatological, skin and other miscellaneous organ specific immune events. Definition terms for each organ specific irAEs as in Table 16. Single organ irAE cohort was defined as those with one organ system affected by irAE, while multi-organ irAE cohort was defined as those with more than one organ systems involved. Concurrent multi-organ irAE was defined as onset of irAEs within 7 days of each other, and sequential multi-organ irAEs by greater than 7 days between each. An irAE "episode" was recorded at the time of reporting and when the grade increases, decreases or resolves. An irAE which has not resolved or changed grade was counted as a single episode. AEs were graded using NCI CTCAEv4.0. Grades 3-5 were considered as severe irAEs.

Table 16: Definition of organ-specific irAEs

Organ specific irAE	Reported terms
Skin	Rash, maculo-papular rash, macular rash, papular rash, erythematous rash, dermatitis, psoriasis, eczema, lichen planus, generalised rash, pruritic rash, vitiligo, dermatomyositis, or pemphigoid
Endocrine	Hypothyroidism, hyperthyroidism, adrenal insufficiency, thyroiditis, hypophysitis, adrenal insufficiency, type 1 diabetes mellitus, and increased or decreased thyroid stimulating hormone
Gastrointestinal	Colitis or duodenitis

Neurological	Peripheral neuropathy, polyneuropathy, or Guillain-Barre Syndrome
Pulmonary	Pneumonitis, interstitial pneumonitis or organizing pneumonia
Hepatobiliary and pancreatic	Cholangitis, hepatitis or pancreatitis
Ocular	Episcleritis, uveitis, optic neuritis or endocrine ophthalmopathy
Rheumatological	Rheumatoid arthritis, polyarthritis, rheumatic disorder, or autoimmune arthritis
Laboratory	Elevated serum liver enzymes or bilirubin, lipase or amylase
Miscellaneous	Those which did not fit under the above organs such as cardiovascular system, kidneys and pericardium

3.2.3 Objectives

The primary objectives were the incidence, grades and time-course of irAEs from atezolizumab. Secondary objectives were incidence and grades of TRAEs, PFS, and OS. PFS was assessed using RECIST version 1.1 or modified RECIST. Exploratory analyses investigated risk factors for irAEs as well as the relationship between irAEs and survival outcomes.

Baseline age, sex, race, tumour characteristics, serum LDH and CRP, lung immune prognostic index (LIPI), neutrophil to lymphocyte ratio (NLR) and various sub-populations of white cells were evaluated for associations with incidence of irAEs.

3.2.4 Statistical analysis

Two-sided statistical tests were conducted in R (version 3.4.3) (Team 2017). A P value < 0.05 was considered statistically significant. Fisher test for categorical data and the Wilcoxon test for continuous data were utilised for association between baseline characteristics and irAEs. Timing of onset of irAE and was visually displayed using a Swimmer plot. Logistic regression analysis was performed to investigate association between irAE and best overall response. Simple Cox proportional hazards regression and time-dependent Cox proportional hazards regression as described in chapter 1.8 were used to model the association between the irAE cohorts

and survival outcomes (OS and PFS) and reported as HR with 95%CI. Survival curves were estimated using KM analysis.

3.3 Results

3.3.1 Overall incidence and severity of AEs

The analysis included 1,548 patients who received at least one dose of atezolizumab. One or more TRAEs occurred in 1,000 (65%) patients, with grade 3-5 TRAEs in 13% across the trials. 730 irAE episodes were reported across 424 (27%) patients who had one or more irAEs. The median time of onset was 49 days (Inter quartile range 21-130 days).

3.3.2 Baseline characteristics of patients who developed irAEs

Table 17 &Table 18 present the characteristics of patients with irAEs. Twelve percent of irAEs occurred within the first 42 days of atezolizumab. Those with irAEs were more likely to be Asian, with good performance status, lower CRP, differential changes in sub-populations of white cells, good LIPI score and lower NLR score. Details of organ-specific irAEs are described in Table 19. Figure 7 shows selected single organ specific irAEs and their time of onset. Skin irAEs were the most common single organ irAE, followed by laboratory abnormalities, and other organs. Of note, there was no reported myocarditis or severe cutaneous reactions such as bullous pemphigoid. Anaemia (36 episodes), thrombocytopenia (7 episodes), neutropenia (5 episodes) and lymphopenia (3 episodes) were not considered to be irAEs by the investigators.

Table 17: Baseline characteristics

	Total No. 1,548	No irAE No. 1,124	Single organ irAE No. 340	Multi-organ irAE No. 84	P-value
Study					0.61
BIRCH	659 (43%)	487 (43%)	138 (41%)	34 (40%)	
FIR	138 (9%)	108 (10%)	24 (7%)	6 (7%)	
OAK	609 (39%)	428 (38%)	145 (43%)	36 (43%)	
POPLAR	142 (9%)	101 (10%)	33 (10%)	8 (10%)	
Age (years)	64 (57 - 70)	63 (56 - 70)	66 (58 - 72)	62 (57 - 68)	0.14
Sex					0.75
Male	936 (60%)	673 (60%)	211 (62%)	52 (62%)	
Female	612 (40%)	451 (40%)	129 (38%)	32 (38%)	
Race					0.008*
White	1,216 (79%)	901 (80%)	245 (72%)	70 (83%)	

Asian	231 (15%)	149 (13%)	70 (21%)	12 (4%)	
Other	66 (4%)	48 (4%)	17 (5%)	1 (1%)	
Missing	35 (2%)	26 (2%)	8 (2%)	1 (1%)	
ECOG PS					0.031*
0	535 (35%)	366 (33%)	131 (39%)	38 (45%)	
1	1,002 (65%)	749 (67%)	208 (61%)	45 (54%)	
2	8 (1%)	6 (1%)	1 (<1%)	1 (1%)	
Missing	3 (<1%)	3 (<1%)	0 (0%)	0 (0%)	
Histology					0.20
Non-squamous	1,155 (72%)	797 (71%)	258 (76%)	60 (71%)	
Squamous	403 (28%)	327 (29%)	82 (24%)	24 (29%)	
Prior treatments[1]					0.067
0	170 (11%)	133 (12%)	31 (9%)	6 (7%)	
1	929 (60%)	661 (59%)	206 (72%)	62 (74%)	
2	449 (29%)	330 (29%)	34 (10%)	16 (19%)	
Smoking history					0.055
Never	270 (17%)	201 (18%)	61 (18%)	8 (10%)	
Previous	1,081 (70%)	777 (69%)	245 (72%)	59 (70%)	
Current	197 (13%)	146 (13%)	34 (10%)	17 (20%)	
Missing	1 (0.1%)	1 (0.1%)	0 (0%)	0 (0%)	

Data are median (IQR) or number of patients (%). P values per Fisher test for categorical data and Wilcoxon test for continuous data. [1]Number of prior treatments in the locally advanced or metastatic setting

Table 18: Baseline laboratory characteristics

	Total No. 1,548	**No irAE No. 1,124**	**Single organ irAE No. 340**	**Multi-organ irAE No. 84**	**P-value**
PD-L1 expression					0.69
Negative	528 (34%)	383 (34%)	119 (35%)	26 (31%)	
Positive	1,015 (65%)	737 (65%)	220 (64%)	58 (69%)	
Missing	5 (<1%)	4 (<1%)	1 (<1%)	0 (0%)	
Lactate dehydrogenase					0.23
≤ ULN	913 (59%)	649 (58%)	211 (62%)	53 (63%)	
> ULN	584 (38%)	439 (39%)	116 (34%)	29 (35%)	
Missing	51 (3%)	36 (3%)	13 (4%)	2 (2%)	
Neutrophil count (10^9/L)					0.002*
Median (IQR)	5 (3.7 – 6.7)	5.1 (3.8 – 7.0)	4.7 (3.7 – 6.0)	4.9 (3.5 – 6.3)	
Missing	28 (1.8%)	23 (2%)	5 (1.5%)	0 (0%)	
Lymphocyte count (10^9/L)					0.28
Median (IQR)	1.3 (0.9 – 1.8)	1.3 (0.9 – 1.8)	1.3 (1.0 – 1.8)	1.5 (1.1 – 2.0)	
Missing	90 (5.8%)	67 (6%)	18 (5.3%)	5 (6%)	
Basophil count (10^9/L)					0.14
Median (IQR)	0.02 (0 – 0.05)	0.02 (0 – 0.05)	0.03 (0 – 0.06)	0.03 (0 – 0.06)	
Missing	124 (8%)	93 (8.2%)	26 (7.6%)	5 (5.9%)	
Eosinophil count (10^9/L)					0.12
Median (IQR)	0.14 (0.09 – 0.24)	0.14 (0.09 – 0.23)	0.15 (0.10 – 0.29)	0.15 (0.10 – 0.30)	
Missing	108 (6.9%)	79 (7%)	24 (7%)	5 (5.9%)	
Monocyte count (10^9/L)					0.28
Median (IQR)	0.61 (0.48 – 0.84)	0.63 (0.48 – 0.85)	0.60 (0.48 – 0.80)	0.60 (0.48 – 0.80)	
Missing	96 (6.2%)	72 (6.4%)	19 (5.6%)	5 (5.9%)`	
CD3 count (10^9/L)					0.30
Median (IQR)	834 (561 – 1193)	812 (540 – 1182)	868 (600 – 1210)	952 (630 – 1297)	
Missing	75 (5%)	56 (5%)	15 (4%)	4 (5%)	

CD4 count (10^9/L)					0.13
Median (IQR)	486 (321 – 732)	470 (306 – 717)	510 (343 – 763)	559 (370 – 791)	
Missing	75 (5%)	56 (5%)	15 (4%)	4 (5%)	
CD8 count (10^9/L)					0.78
Median (IQR)	293 (177 – 455)	291 (175 – 450)	292 (184 – 446)	313 (218 – 468)	
Missing	75 (5%)	56 (5%)	15 (4%)	4 (5%)	
Neutrophil to lymphocyte ratio					0.007*
Median (IQR)	2.2 (1.6 – 3.2)	2.3 (1.7 – 3.3)	2.1 (1.6 – 2.8)	2.0 (1.4 – 2.8)	
Missing	29 (1.9%)	23 (2%)	6 (1.8%)	0 (0%)	
C Reactive Protein (mg/L)					< 0.001*
Median (IQR)	14 (4 - 42)	15 (4 - 48)	13 (5 - 45)	11 (4 - 32)	
Missing	43 (3%)	26 (4%)	9 (2%)	8 (3%)	
Lung Immune prognostic index (LIPI)					0.010*
0	678 (44%)	464 (41%)	172 (51%)	42 (50%)	
1	631 (41%)	472 (42%)	125 (37%)	34 (40%)	
2	180 (12%)	145 (13%)	29 (9%)	6 (7%)	
Missing	59 (4%)	43 (4%)	14 (4%)	2 (2%)	

Table 19: Details of organ-specific irAEs

Organ specific irAE	**Number of unique episodes of irAEs**	**Type of irAE (%)**	**Grades**	
			1/2	**≥3**
Skin	306	Skin rash (89%), Eczema (4.6%), Psoriasis (2.3%), lichen planus (1%); dermatomyositis, vitiligo and bullous pemphigoid (< 1% each)	86%	14%
Laboratory	197	Liver enzyme abnormalities were the majority followed by increased bilirubin, lipase, or amylase.	80%	20%
Endocrine	85	Thyroid gland related (hyper or hypothyroidism – 92.5%), adrenal gland related (4.7%), type 1 diabetes mellitus (2.4%) and hypophysitis (1.2%)	95.3%	4.7%
Neurological	56	Peripheral neuropathy (84%), polyneuropathy (9%) and Guillain-Barre syndrome (7%)	66%	7%
Pulmonary	45	Pneumonitis (95%) and organizing pneumonia (5%)	57.8%	42.2%*
Gastrointestinal	15	Colitis (93.3%) and duodenitis (6.7%)	66.7%	33.3%
Ocular	9	Uveitis (33.3%), optic neuritis (33.3%), endocrine ophthalmopathy (22.2%) and episcleritis (11.1%)	88.8%	11.2%
Rheumatological	8	Rheumatoid arthritis (37.5%) and polymyalgia rheumatica (37.5%), polyarthritis (12.5%) and rheumatic disorder (12.5%)	100%	0%
Miscellaneous	5	Pericarditis (40%), cytokine release syndrome (20%), Henoch-Schonlein purpuric nephritis (20%) and vasculitis (20%)	60%	40%

Hepatobiliary and pancreatic	4	Pancreatitis (75%) and cholangitis (25%)	100%	0%

*Includes 1 death

3.3.3 Treatment of irAEs and their outcomes

Overall, 7.5% of the 730 episodes of irAEs resulted in hospitalization which was required in almost half of grade 3 or 4 events and in 5.7% of grade 1 or 2 events. A total of 109 (15%) irAE episodes were treated with systemic corticosteroids with 20% being grade 1 or 2 (31 out of 109 episodes) and the rest being grade 3 or more. Atezolizumab was interrupted in 14% (102/730) of episodes and permanently discontinued in 2.5% (18/730 episodes). At the time of last follow-up, most irAE episodes (60% - 438/730 episodes) were resolved while 28% (204/730 episodes) had ongoing irAEs. Nine episodes resulted in sequelae despite resolution of the irAE. Only one death from irAEs (pneumonitis) was reported.

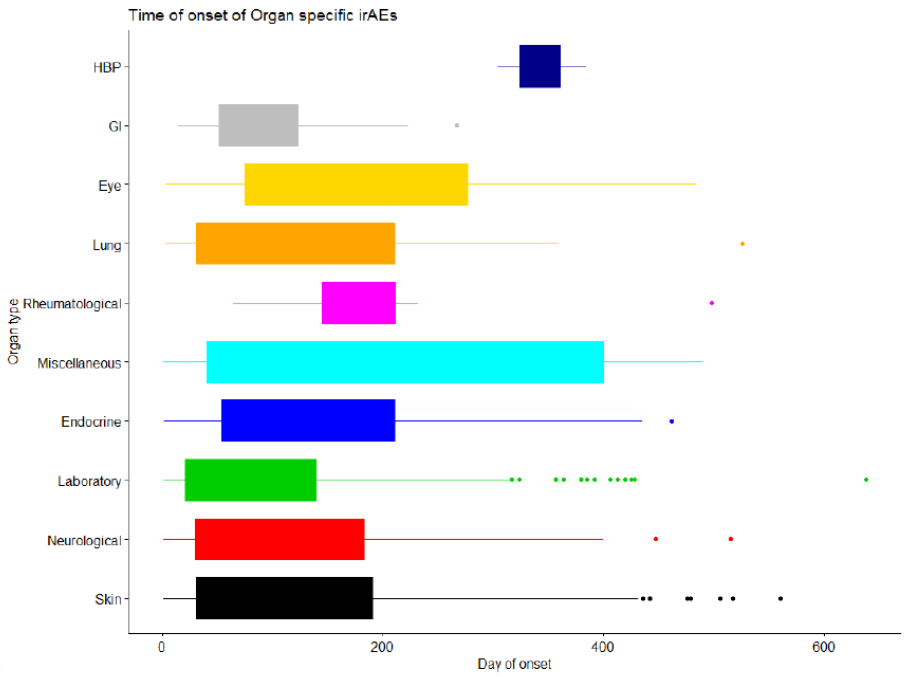
3.3.4 Second-line treatment irAEs

Second-line immunosuppressants for the treatment of irAEs, after systemic corticosteroid failure, were required in five patients. One received infliximab for colitis and had complete resolution; one required oral mycophenolate mofetil for hepatitis, one received anakinra for arthritis, and two had topical cyclosporine A for ocular irAEs. Atezolizumab was discontinued permanently in the patient with hepatitis. All other patients requiring second-line immunosuppressants received further atezolizumab. Of the 424 patients with irAEs, 340 had one organ specific irAEs (Table 17 and Table 18). The rest (84 patients, 5.4% (84/1548) of all atezolizumab treated patients and 19.8% (84/424) of all patients with irAEs) had multi-organ irAEs. Among those who had multi-organ irAEs, 70 out of 84 patients (83.3%) had two-organs, thirteen out of 84 patients (15.5%) had three organs, and one patient had four organ systems affected by irAEs.

3.3.5 Multi-organ irAEs

Of the 424 patients with irAEs, 340 had one organ specific irAEs (Table 17 and Table 18). The rest (84 patients, 5.4% (84/1548) of all atezolizumab treated patients and 19.8% (84/424) of all patients with irAEs) had multi-organ irAEs. Among those who had multi-organ irAEs, 70 out of 84 patients (83.3%) had two-organs, thirteen out of 84 patients (15.5%) had three organs, and one patient had four organ systems affected by irAEs (Figure 8). Twelve had concurrent onset irAEs while the rest (72 patients) had sequential onset.

Figure 7: Time of onset of all irAEs



HBP – hepatobiliary pancreatic

GI - Gastrointestinal

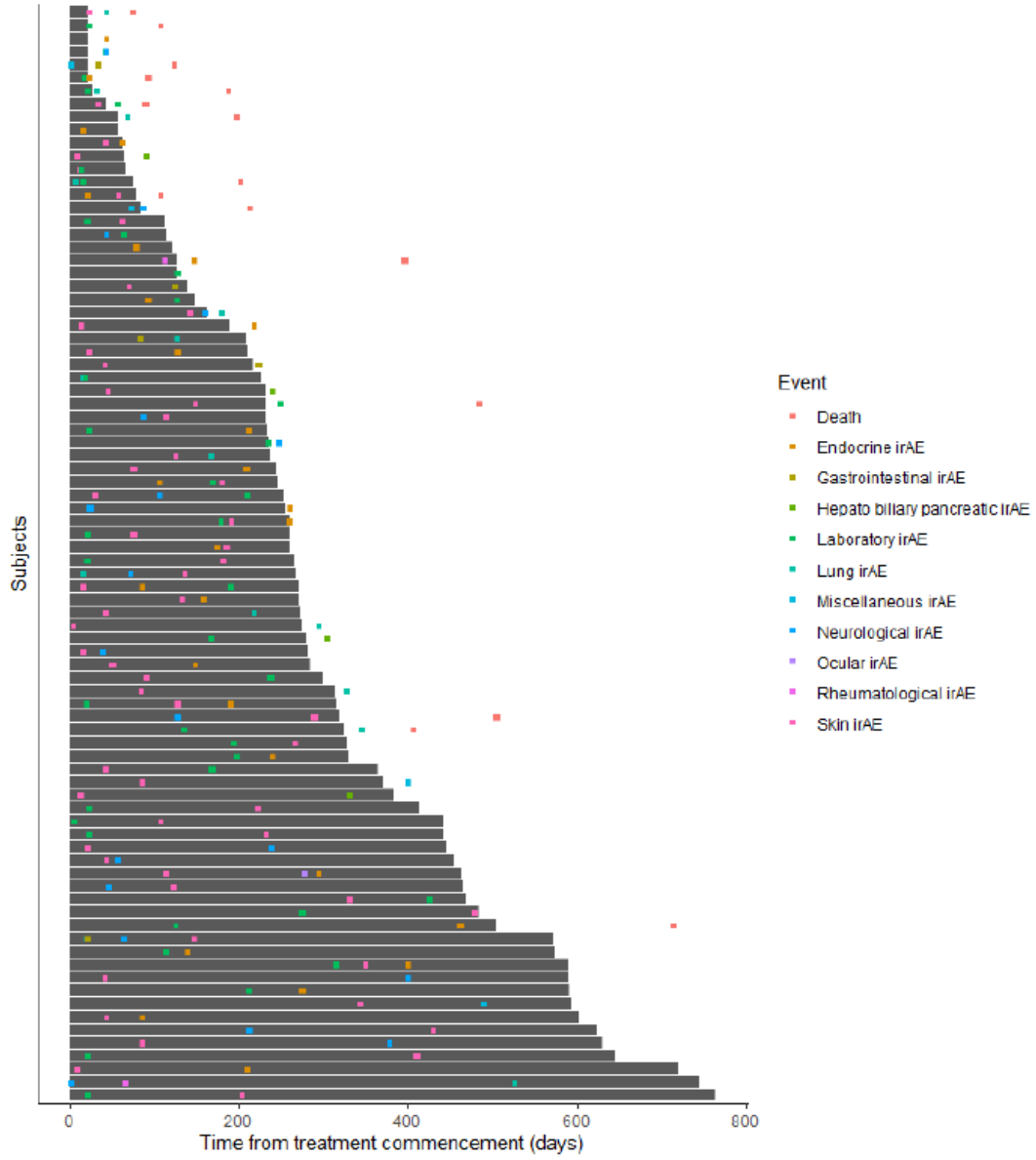
Those who had multi-organ irAEs were more likely to be white race, ECOG PS 0, lower baseline CRP and levels neutrophil-lymphocyte ratio than those who developed single-organ irAEs. The combination of organs affected by irAEs were “skin irAE plus”, “lab irAE plus without skin”, or “other combinations without skin or lab irAE”. Sixty patients were in the skin irAE plus cluster; 9 in the lab irAE plus without skin cluster, and the remaining 15 had other combinations.

3.3.6 Multi-organ irAEs and cancer outcomes

The development of multi-organ irAEs was associated with improved response rates, PFS and OS. The best overall response (combined complete and partial response) was significantly higher among those who developed multi-organ irAE than those with single organ or without irAEs (42%, 23%, 12% respectively) with OR of 2.18 (95% CI 1.5-2.9) for single-organ irAE (vs no irAE) and 5.32 (95% CI 3.3-8.5) for multi-organ irAEs (vs no irAE) (P = <0.0001). The median PFS using simple Cox model was significantly improved with multi-organ irAEs (7.2 months, 95% CI 5.4-12.4) vs single organ irAE (4.2 months, 95% CI 3.9-5.5) and no irAE cohort (2.7 months, 95% CI 2.5-2.8) (P = <0.001) (Figure 9). Similarly, the median OS with simple Cox model was significantly improved with multi-organ irAEs (not reached (NR) (95% CI 23.5-NR)) vs single organ irAE (20.1

months (95% CI 16.0-23.3)) and no irAE cohort (10.3 months (95% CI 9.5-11.4)) (P = <0.001) (Figure 10). HR calculated using a time-dependent Cox regression model for OS was 0.47 (95% 0.28 - 0.78) for multi-organ irAE (vs no irAE) and 0.69 (95% CI 0.57 – 0.85) for single organ irAE (vs no irAE) (P < 0.0001). However, the occurrence of multi-organ irAE was not associated with an improved PFS in the time-dependent Cox model (HR 0.92; 95% CI 0.62 – 1.35 for multi-organ iRAEs and HR 0.95; 95% CI 0.81 – 1.11 for single organ irAE, P = 0.74).

Figure 8: Swimmer's plot - time of onset of multi-organ irAEs and deaths in 84 patients



Each bar indicates duration of atezolizumab treatment

Figure 9: Association between irAEs and progression free survival

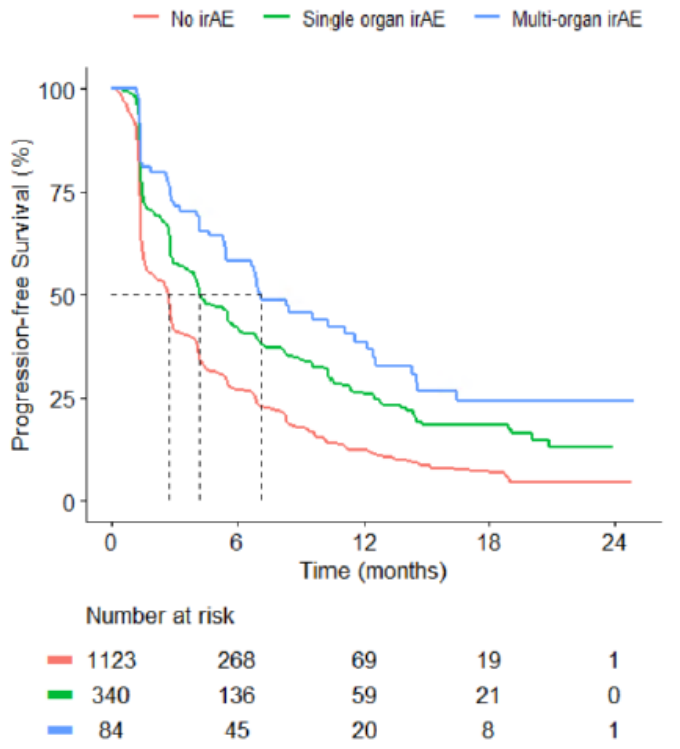
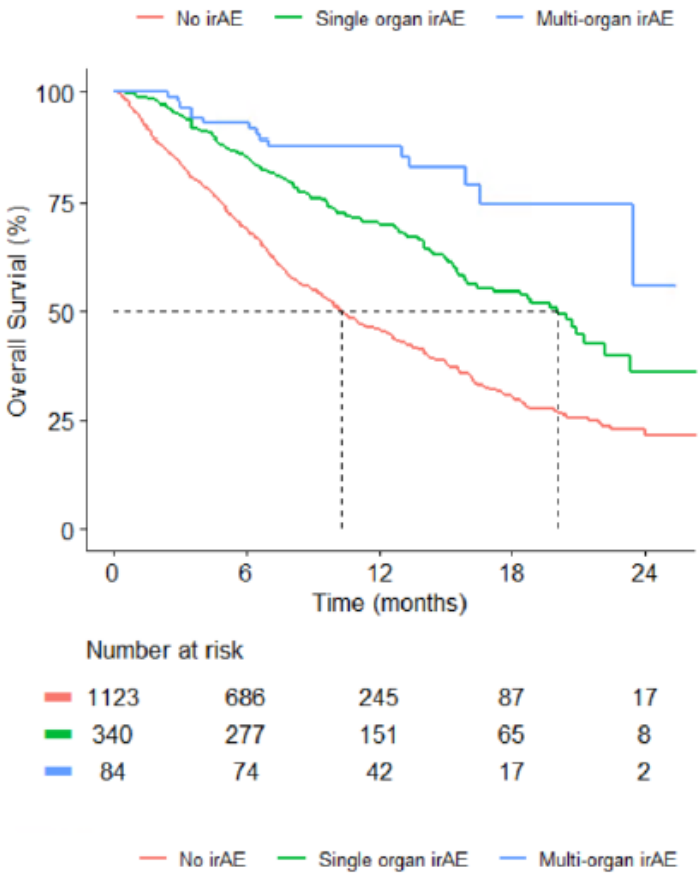


Figure 10: Association between irAEs and overall survival



3.4 Discussion

This chapter reported a comprehensively assessed multi-organ irAEs associated with an ICI using a large dataset from clinical trials. One-fifth of patients treated with atezolizumab developed at least one irAE whereas 5.4% had multi-organ irAEs. Mortality from irAEs and irAEs leading to permanent discontinuation of atezolizumab were uncommon, with most patients recovering without sequelae. Any irAE, including multi-organ irAEs, were significantly associated with better tumour response and survival outcomes.

While the occurrence of poly-autoimmunity is well known in rheumatological literature, there is little information on the occurrence of multi-system irAEs until recently, likely due to under recognition and lack of routine reporting. Shankar et al recently reported an incidence of 5% for multi-system irAEs among 319 lung cancer patients treated with anti PD1/PDL1 therapies, which is similar to our findings (Shankar et 2019). However, in contrast to this study, pneumonitis was the dominant irAE, with pneumonitis/dermatitis as the most common multi-organ irAE. In the current study, skin plus or laboratory plus clusters were the most dominant. Furthermore, this research included lung cancer patients treated with a single drug (atezolizumab) through clinical trials in contrast to Shankar et al where different ICI were given to patients in real world setting.

The differences in the incidence of organ specific irAEs between studies may be related to the lack of uniform definitions of organ specific irAEs across trials. Maughan et al have previously identified important differences in the definition of irAEs between atezolizumab, nivolumab and pembrolizumab trials and recommended standardized definitions (Maughan et al. 2017). For the current analysis, irAEs were defined according to trial protocols as autoimmune conditions based on known mechanisms of ICI drugs.

The type of single organ irAE was dominated by cutaneous irAEs, followed by laboratory abnormalities and endocrine irAEs in our study. This contrasts with previous analysis of anti PD1/ PDL1 drugs where any grade of endocrine irAE was the most common organ system affected, followed by pneumonitis (Wang, Zhou, et al. 2019). Of interest, neurological irAEs, especially neuropathy, emerged as an important irAE in our analysis. The reason for the relatively high incidence of neuropathy as an irAE from atezolizumab is unknown. It is also unclear whether the incidence of neurological irAEs is similar with other ICI drugs or whether they were

under-recognized or under-reported in previous trials. Future studies should consider prospective evaluation of neuropathy during treatment.

Among the baseline characteristics that are associated with the development of irAEs, Asian race and good LIPI score were statistically significant. Prior studies have similarly reported associations with Asian race (Peng and Wu 2018). However, the association with good LIPI score has not been previously reported.

Prior studies suggest a relationship between the occurrence of irAE and tumour response and survival from ICI therapies for various cancers (Cortellini, Buti, et al. 2019; Petrelli et al. 2019). It is possible that the presence of a generalized immunogenic state during ICI therapies may contribute towards better cancer control along with damage to normal organs which manifests as irAEs. This research identified a similar association between the occurrence of irAEs and best overall response, PFS and OS. Moreover, the occurrence of multi-organ irAEs was strongly associated with improved OS, but not for PFS with time-dependent Cox models indicating possible immortal time bias confounding results in simple Cox model.

In addition, the definition of abnormal blood results and their attribution to organ specific damage needs further consideration. Whether laboratory abnormalities alone constitute irAEs requires international consensus. None of the available guidelines provide definitions for organ specific irAEs. The current understanding of the pathophysiology, incidence, and management of irAEs can only be improved by using uniform definitions for the development of evidence-based guidance.

While the diagnosis and characterization of irAEs remain inconsistent and challenging (Hsiehchen et al. 2019), the expert consensus-based Trial Reporting of Immuno-Oncology (TRIO) recommendations are likely to improve the reporting of ICI in clinical trials. However, the TRIO guidance does not recognize or mandate reporting on multi-organ irAEs. As described in this research, a significant proportion of patients treated with ICI develops multi-organ toxicities and may have associations with treatment response and outcomes. Hence, their recognition and reporting should become part of published manuscripts and routine care of patients undergoing ICI therapies.

Poly-autoimmunity phenotypes often occur due to common genetic factors, immune pathogenic mechanisms and an established set of risk factors such female predominance, family history, smoking history and native American it is unclear if multi-organ irAEs have any risk factors (Anaya 2017). In the current study, white race and good ECOG PS were associated with multi-organ irAEs. In contrast to the poly-autoimmunity phenotype, sex and smoking were not associated with multi-organ irAEs. Further studies should evaluate any underlying genetic risk factors that may predispose multi-organ irAEs in certain individuals who receive ICI therapies using data through international collaboration (Khan et al. 2019).

Prior reports on poly-autoimmunity also identified that the treatment of multiple conditions adds complexity to their care (Anaya 2017; Bliddal, Nielsen, and Feldt-Rasmussen 2017; Matusiewicz, Strozynska-Byrska, and Olesinska 2019). Similarly, treatment of a patient with multi-organ irAEs poses other challenges such as need for endocrine replacement therapies use of immunosuppressants at the same time, possible need for second line immunosuppressants and secondary complications such as high blood glucose levels and infections from corticosteroids. In the current study, due to limited number of patients, it is unclear if patients with multi-organ irAEs have an increased need for second line immunosuppressants.

This research highlights a previously under-recognized issue of multi-organ irAEs arising from ICI therapies. The current study is the largest analysis of uniformly treated patients with NSCLC who developed multi-organ irAEs from an anti-PD-L1 inhibitor. Data was collected prospectively through multi-centre international clinical trials thereby improving the accuracy of the information. Moreover, the association between the occurrence of irAEs and survival outcomes were evaluated using time-dependent Cox models to avoid bias from different treatment duration (Eggermont et al. 2020).

There are several limitations in the current study. First, the analysis is a *post hoc* exploratory analysis and as such should be considered hypothesis generating and need to be confirmed in other studies. Second, the data was from lung cancer trials with atezolizumab. It is not clear if a similar pattern of multi-organ irAEs occurs with other cancer types or ICI drugs. Third, the data had investigator reported AEs and there was no external validation/confirmation of AEs. Lastly, the irAEs analysed were organ specific without the inclusion of constitutional symptoms such as fatigue and fever, which could be related to cytokines released by

activated immune cells. These toxicities are not traditionally regarded as irAEs although they may have immune aetiology. Despite these study limitations, this research demonstrated that multi-organ irAEs need appropriate recognition and evaluation.

3.5 Conclusion

Multi-organ irAEs were reported in 5.4% of patients treated with atezolizumab in lung cancer trials. Health professionals involved in the care of patients undergoing ICI therapies should recognize that irAEs can manifest as multiple organ system damage. Future trial reporting should consider incorporation of data on multi-organ toxicities in addition to single organ specific toxicities. As demonstrated in this chapter, toxicities from drug therapies (atezolizumab, here as an example) may be associated with survival outcomes. However, the severity, type and number of AEs a patient experience, is heterogeneous within a cohort of patients treated with the same intervention which highlights another level of variability during systemic cancer therapies.

4. CHAPTER FOUR: IMMUNE RELATED ADVERSE EVENTS AND SURVIVAL OUTCOMES

One of the interesting findings from chapter 3 was that the irAEs, especially, multi-organ irAEs were significantly associated with improved survival outcomes from atezolizumab in patients with NSCLC. However, it was unclear if such an association was unique to ICI type drugs and non-ICI type drugs were also associated with survival benefit. Hence, in this chapter 4, I planned to assess the relationship between irAE type toxicities from ICI or chemotherapy and survival outcomes using a data from randomized controlled trials that compared ICI and chemotherapy in two different cancer types.

4.1 Introduction

It is well recognized that all systemic cancer therapies are associated with some degree of short-term and long-term treatment-related adverse effects. These can be mild, severe enough to affect activities of daily living and quality of life, or, in extreme circumstances, lead to fatal outcome. Whether such toxicities are unavoidable to achieve beneficial clinical outcomes is a matter of ongoing debate.

It is also recognized that toxicities that arise from the direct pharmacodynamic effects of systemic cancer therapies may be associated with improved outcomes. For example, myelosuppression from chemotherapy drugs, endocrine symptoms from anti-hormonal drugs, skin rash from anti epidermal growth factor receptor inhibitors, hypertension from anti-angiogenic drugs, and more recently, irAEs from ICI have all been reported to be associated with improved outcomes across various cancer types (Abola, Prasad, and Jena 2014; Cortellini, Buti, et al. 2019; Di Maio et al. 2005; Khoja et al. 2014; Liu et al. 2013; Yoo et al. 2018).

ICI therapies are part of standard care in various cancers. Two-thirds or more of patients receiving ICI develop one or more AEs with 14% developing at least 1 severe AE (Wang, Zhou, et al. 2019). irAEs are specific toxicities arising through activation of the immune system with consequent organ damage. The pathophysiology of irAEs is poorly understood, although possible mechanisms include non-specific inflammatory cytokine release, T-cell activity against shared antigens of tumour and normal cells, complement mediated cytotoxicity, increased pre-existing autoantibodies, and the release of neutrophil

extracellular traps leading to organ damage (Berner et al. 2019; Hirschhorn et al. 2018; Postow, Sidlow, and Hellmann 2018). Several authors have previously reported that the occurrence of irAEs is associated with improved cancer outcomes (RR, PFS, OS) in advanced cancers and recurrence free survival in early stage cancers) (Baldini et al. 2020; Naqash et al. 2020; Xing et al. 2019; Zhou et al. 2020). However, most studies were retrospective and only a minority analysed data from clinical trials (Maher et al. 2019). Furthermore, they often used statistical modelling and analyses that do not account for the immortal time bias (Dall'Olio, Di Nunno, and Massari 2020; Anderson, Cain, and Gelber 2008).

Landmark analyses with events occurring prior to a fixed time-point, often performed to account for immortal time bias, result in decreased power. While the association points towards a predictive biomarker role of irAEs for ICI outcomes, it is unclear whether they might also have a prognostic role (FDA-NIH 2016; Simms, Barraclough, and Govindan 2013).

In addition, irAE like toxicities were also reported in control arms that included chemotherapy or placebo (Eggermont et al. 2020; Zhou et al. 2020). Most studies have reported associations between irAEs and outcomes using data from interventional ICI treated arms but not control arms. Thus, it is currently unknown whether a similar association between irAEs and outcomes also exists in the control arms treated with chemotherapy. Although the AEs from chemotherapy are not traditionally considered to be immune mediated, there is considerable overlap in the terminology used for the reported diagnosis of organs affected by toxicities. Trials use uniform criteria for defining irAEs across ICI and non-ICI arms.

In order to address these issues, this research evaluated the association between the incidence, severity and type of irAEs and cancer outcomes (RR, PFS and OS) using individual patient data from three randomized clinical trials comparing atezolizumab, with a microtubule inhibiting chemotherapy with either a taxane or vinflunine. The overall objective was to assess whether irAEs have a predictive or prognostic association with outcomes.

4.2 Methods

4.2.1 Patients

Deidentified IPD were released through data sharing processes and policies by Roche for the following three published trials: IMvigor 211, OAK and POPLAR (Fehrenbacher et al. 2016; Rittmeyer et al. 2017; Powles et al. 2018). All trials were randomized controlled trials comparing atezolizumab with chemotherapy in a second-line setting.

IMvigor211 was a phase III open-label trial that randomized atezolizumab with a control arm of chemotherapy (docetaxel, paclitaxel or vinflunine) in patients with locally advanced or metastatic urothelial cancers (UC) after progression with first-line platinum chemotherapy (Powles et al. 2018). OAK was a phase III open-label randomized trial comparing atezolizumab with docetaxel in patients with previously treated non-small cell lung cancer (NSCLC) (Rittmeyer et al. 2017). POPLAR was a phase II randomized trial that compared atezolizumab with docetaxel in previously treated patients with NSCLC (Fehrenbacher et al. 2016). The Southern Adelaide Clinical Human Research Ethics Committee provided exemption for review for this secondary analysis of deidentified trial data.

4.2.2 Assessments

The primary outcome for the current analysis was OS. Other outcomes assessed were PFS and RR. TRAEs were reported by the investigators in the participating sites as toxicities related to the intervention (atezolizumab or chemotherapy) and graded using the NCI CTCAEv 4.0. irAEs were uniformly defined and pre-specified in the study protocols as “adverse events of special interest that were related to an autoimmune condition from atezolizumab” and were graded similarly. The same definition was applied in the chemotherapy control arms as per the trial protocols.

irAEs were grouped based on the reported diagnosis in the case report forms as organ-specific or abnormal laboratory investigation with presumed immune aetiology. A definition of abnormal laboratory investigation with presumed immune aetiology was also used when organ specificity could not be attributed. To retain the comparison between the atezolizumab and the chemotherapy treated cohorts, the same set of toxicities (irAEs) were evaluated as the variable of interest.

4.2.3 Statistical analysis

Time-dependent Cox proportional hazards analyses were performed to assess the association between the occurrence of irAEs and survival outcomes. Specifically, either incident irAE or the grade of irAE (0 vs 1-2, vs 3-4) were included as a time-dependent covariate. Associations were reported as HR and 95%CI. Evaluation of whether the association between irAE and survival outcomes differed between treatments (atezolizumab vs chemotherapy) was evaluated using an interaction term between treatment and the time-dependent irAE covariate. The association between the irAE time-dependent covariate and survival outcomes was graphically displayed using the method of Simon and Makuch (Simon and Makuch 1984). Cox proportional hazard landmark analyses at 42 days (equivalent to 2 cycles of atezolizumab or chemotherapy) were conducted as a sensitivity analysis of the association between early irAEs and survival outcomes. All analyses were stratified for the individual study and cancer type and conducted using R 3.5.3 (Team 2017). A P-value < 0.05 was considered statistically significant.

4.3 Results

Data from a total of 2,366 patients from three clinical trials was available for analysis. The median age was 65 years (IQR of 57-71); 68% were men, the majority (72%) were white and had an ECOG PS of 1 (60%). 1,464 had NSCLC and the rest had urothelial cancer. Half the patients were from the OAK trial with the other 50% from the IMVigor211 (38%) and POPLAR (12%) trials. 1,210 patients in the atezolizumab treated cohort and 1,156 patients in the chemotherapy treated cohort received at least one dose of the planned treatment. Among the chemotherapy treated patients, 66% received docetaxel, 21% vinflunine and 13% paclitaxel. The baseline characteristics of the atezolizumab and chemotherapy treated cohorts were balanced Table 20.

Table 20: Baseline characteristics across all trials

Variable	Total N = 2,366	Atezolizumab N = 1,210	Chemotherapy N = 1,156	P-value
Study				0.98
IMvigor211	902(38%)	459 (38%)	443 (38%)	
OAK	1,187(53%)	609 (50%)	578 (50%)	
POPLAR	277(12%)	142 (12%)	135 (12%)	
Actual treatment				<0.001
Atezolizumab	1,210 (51%)	1,210 (100%)	0 (0%)	
Docetaxel	766 (32%)	0 (0%)	766 (66%)	
Paclitaxel	148 (6%)	0 (0%)	148 (13%)	
Vinflunine	242 (10%)	0 (0%)	242 (21%)	

Cancer type				0.87
Lung	1,464 (62%)	713 (62%)	751 (62%)	
Urothelial	902 (38%)	443 (38%)	459 (38%)	
Age (years)	65 (57 - 71)	64 (57 - 71)	65 (58 - 71)	0.53
Sex				0.86
Male	1,599 (68%)	820 (68%)	779 (67%)	
Female	767 (32%)	390 (32%)	377 (33%)	
Race				0.59
White	1,710 (72%)	873 (72%)	837(72%)	
Asian	395 (17%)	210 (17%)	185 (16%)	
Other	78 (3%)	37 (3%)	41 (4%)	
Missing	183 (8%)	90 (7%)	93 (8%)	
ECOG PS				0.97
0	937 (40%)	480 (40%)	457 (40%)	
1	1,426 (60%)	728 (60%)	698 (60%)	
Missing	3 (<1%)	2 (<1%)	1 (<1%)	
Histology				0.92
Non-squamous	1,053 (45%)	542 (45%)	511 (44%)	
Squamous	411 (17%)	209 (17%)	202 (17%)	
TCC	824 (35%)	417 (34%)	407 (35%)	
TCC with mixed	77 (3%)	42 (3%)	35 (3%)	
PD-L1 expression				0.31
Negative	873 (37%)	448 (37%)	425 (37%)	
Positive	1,484 (73%)	756 (63%)	728 (63%)	
Missing	9 (<1%)	6 (<1%)	3 (<1%)	
BMI				0.84
Median	25 (22 - 28)	25(22 - 29)	25 (22 - 28)	
Missing	49 (2%)	29 (2%)	20 (2%)	
Liver metastases	565 (24%)	294 (24%)	271 (23%)	0.63
Lung metastases	1,722 (73%)	864 (71%)	858 (74%)	0.13
Bone metastases	663 (28%)	337 (28%)	326 (28%)	0.85
Brain metastases	132 (6%)	59 (5%)	73 (6%)	0.13
Best overall response				<0.001*
PD	949 (40%)	570 (47%)	379 (33%)	
SD	10 (33%)	350 (29%)	438 (38%)	
PR	2 (7%)	146 (12%)	167 (14%)	
Non-CR/PD	2 (<1%)	2 (<1%)	0 (0%)	
CR	50 (2%)	27 (2%)	23 (2%)	
Missing	2 (7%)	115 (10%)	149 (13%)	
Disease control	1,153 (49%)	525 (43%)	628 (54%)	<0.001*

Data are median (IQR) or number of patients (%). P values per Fisher test for categorical data and Wilcoxon test for continuous data. EOCG PS – Eastern Co-operative Oncology Group performance status; TCC – transitional cell carcinoma; BMI = Body mass index; PD – progressive disease; SD – stable disease; PR - partial response; CR – complete response

4.3.1 Characteristics of irAEs

Among the atezolizumab treated patients, 804 (66%) had TRAEs and 351 (29%) had the selected irAEs. Of these, irAEs with an incidence of 5% or more included skin (18%), laboratory (8%) and endocrine (5%) irAEs. All other organ-specific irAEs were relatively uncommon (Table 21). In contrast, skin and neurological irAEs (12% each) were the most common among those who received chemotherapy. The median time for the

onset of irAEs from atezolizumab (92 days, IQR - 50-182) was significantly longer than that for the chemotherapy cohort (71 days, (IQR 46-134), P <0.001).

Table 21: irAEs across all trials

Variable	Total No. 2,366	Atezolizumab No. 1,210	Chemotherapy No. 1,156	P-value
No. of irAEs	616 (26%)	351 (29%)	265 (23%)	<0.001
Skin irAE				<0.001
Any grade	456 (15%)	286 (18%)	170 (12%)	
Mild	440 (14%)	272 (17%)	168 (12%)	
Severe	16 (1%)	14 (1%)	2 (<1%)	
Endocrine irAE				<0.001
Any grade	74 (3%)	69 (5%)	5 (1%)	
Mild	68 (3%)	64 (5%)	4 (<1%)	
Severe	6 (<1%)	5 (<1%)	1 (<1%)	
Neurological irAE				<0.001
Any grade	180 (8%)	39 (3%)	140 (12%)	
Mild	158 (7%)	35 (3%)	122 (10%)	
Severe	22 (1%)	4 (<1%)	18 (2%)	
Laboratory irAE				<0.001
Any grade	136 (6%)	95 (8%)	41 (4%)	
Mild	94 (4%)	62 (5%)	32 (3%)	
Severe	42 (2%)	33 (3%)	9 (1%)	
Lung irAE				<0.001
Any grade	28 (2%)	21 (2%)	7 (1%)	
Mild	16 (1%)	13 (1%)	3 (<1%)	
Severe	12 (<1%)	8 (<1%)	4* (<1%)	
GI irAE				0.26
Any grade	15 (1%)	11 (1%)	4 (<1%)	
Mild	8 (<1%)	6 (<1%)	2 (<1%)	
Severe	7 (<1%)	5 (<1%)	2 (<1%)	
HPB irAE				0.014
Any grade	13 (1%)	12 (1%)	1 (<1%)	
Mild	4 (<1%)	3 (<1%)	1 (<1%)	
Severe	9 (<1%)	9 (<1%)	0 (0%)	
Ocular irAE				0.50
Any grade	3 (<1%)	3 (<1%)	0 (0%)	
Mild	2 (<1%)	2 (<1%)	0 (0%)	
Severe	1 (<1%)	1 (<1%)	0 (0%)	
Rheumatological irAE				0.06
Any grade	8 (1%)	8 (1%)	0 (0%)	
Mild	6 (<1%)	6 (<1%)	0 (0%)	
Severe	2 (<1%)	2 (<1%)	0 (0%)	
Miscellaneous irAE				<0.001
Any grade	10 (1%)	9 (1%)	1 (<1%)	
Mild	5 (<1%)	4 (<1%)	1 (<1%)	
Severe	5 (<1%)	5 (<1%)	0 (0%)	
Time to 1st irAE (days)	78 (49 - 147)	92 (50 - 182)	71 (46 - 134)	<0.001

Data are median (IQR) or number of patients (%). P values per Fisher test for categorical data and Wilcoxon test for continuous data. mild irAE – grade 1 or 2; severe irAE – grade ≥3; GI – gastrointestinal; HPB – hepatic-pancreatic-biliary

4.3.2 Association of irAEs with survival

Using time-dependent Cox proportional analysis, the occurrence of any grade of irAEs in the atezolizumab treated cohort was significantly associated with improved OS (HR 0.70 [95%CI 0.59-0.84], P < 0.0001), but there was some evidence of improved PFS but it did not reach statistical significance (HR 0.87 [95%CI 0.74-1.02], P = 0.08) (Table 22, Figure 11). Similar associations with OS (HR 0.67 [95%CI 0.56-0.79], P < 0.0001) and PFS (HR 0.84 [95%CI 0.72-0.97], P = 0.02) were observed in the chemotherapy treated cohort (Table 22, Figure 12). The P-value for the interaction between treatment (atezolizumab vs chemotherapy) and irAE (yes vs no) was not statistically significant, indicating that the occurrence of irAEs was associated with favourable OS regardless of treatment with atezolizumab or chemotherapy.

Table 22: Time dependent analysis for OS and PFS

irAE	**Atezolizumab**		**Chemotherapy**	
	HR (95% CI)	**P-value**	**HR (95% CI)**	**P-value**
Overall Survival				
Any irAE		<0.0001		<0.0001
No	1.00		1.00	
Yes	0.70 (0.59-0.84)		0.67 (0.56-0.79)	
irAE grade		<0.0001		<0.0001
0	1.00		1.00	
1-2	0.63 (0.52-0.77)		0.65 (0.54-0.78)	
≥ 3	1.11 (0.81-1.53)		0.83 (0.53-1.28)	
Progression Free survival				
Any irAE		0.08		0.02
No	1.00		1.00	
Yes	0.87 (0.74-1.02)		0.84 (0.72-0.97)	
irAE grade		0.026		0.06

0 1-2 ≥ 3	1.00 0.82 (0.69-0.97) 1.19 (0.86-1.64)		1.00 0.83 (0.71-0.9) 0.88 (0.59-1.30)	

Figure 11: OS vs irAEs in Atezolizumab treated patients (Time-dependent analysis)

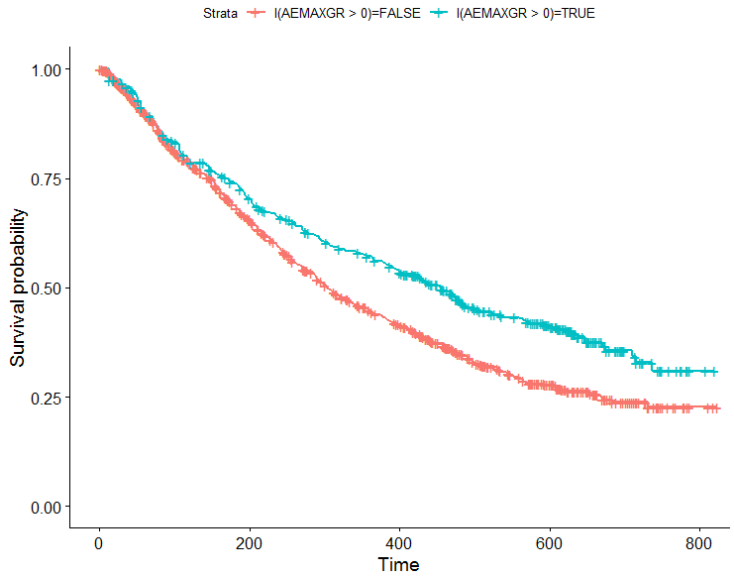
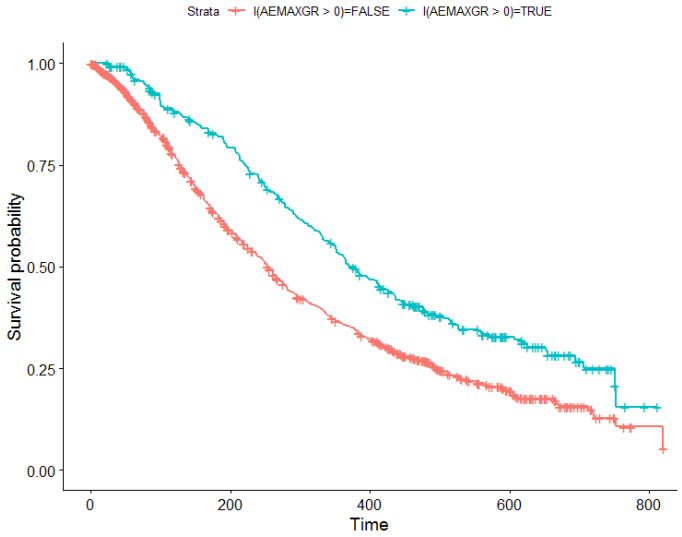


Figure 12: OS vs irAEs in chemotherapy treated patients (Time-dependent analysis)



A similar analysis was performed to investigate the association between irAE grades and survival. In both the atezolizumab and chemotherapy cohorts, when compared to patients without any irAEs, those with grade 1 or 2 irAEs had improved OS (HR 0.63 [95%CI 0.52-0.77], P < 0.0001; and HR 0.65 [95%CI 0.54-0.78], P < 0.0001, respectively). However, the presence of grade 3 or more irAEs was not associated with OS benefit in either cohort (HR 1.11 [95%CI 0.81-1.53] and (HR 0.83 [95%CI 0.53-1.28], respectively) (

Table 22). Similar associations were observed between the grades of irAEs and PFS in the atezolizumab, but not in the chemotherapy, cohort. A sensitivity day 42 landmark analysis showed a statistically significant association between the occurrence of any irAE and grade 1 or 2 irAEs in the first 42 days of therapy and subsequent OS, but not PFS, both in the atezolizumab and in the chemotherapy treated cohorts (Table 23, Figure 13 &Figure 14).

Table 23: Landmark analysis for OS and PFS – Day 42 (week 6)

irAE	**Atezolizumab**			**Chemotherapy**		
	Median (months)	**HR (95% CI)**	**P-value**	**Median**	**HR (95% CI)**	**P-value**
Overall Survival						
Any irAE			0.006			0.015
No	11.1			10.5		
Yes	16.3	0.74 (0.59-0.92)		13	0.76 (0.60-0.95)	
irAE grade			0.045			0.013
0	15	1.00		10.5	1.00	
1-2	21.3	0.76 (0.56-1.05)		13.3	0.72 (0.57-0.91)	
≥ 3	8	1.70 (0.97-2.97)		9.8	1.30 (0.67-2.53)	
Progression Free survival						
Any irAE			0.97			0.85
No	4.2			4.3		
Yes	4.2	0.99 (0.79-1.24)		5.6	0.98 (0.81-1.19)	
irAE grade			0.26			0.96
0	4.2	1.00		4.3	1.00	
1-2	4.4	0.92 (0.92-1.18)		5.6	0.80 (0.57-1.19)	
≥ 3	2.6	1.47 (0.91-2.39)		4.1	1.05 (0.57-1.91)	

Figure 13: OS vs irAE in Atezolizumab treated patients (landmark analysis at day 42)

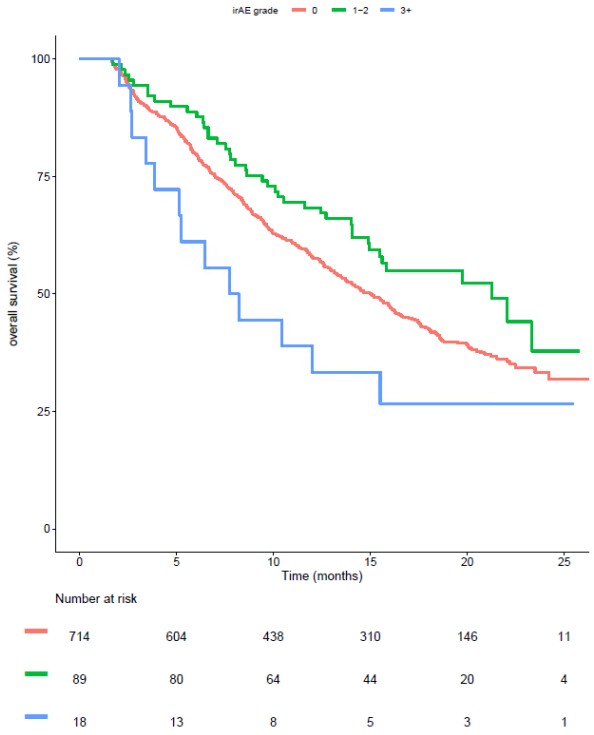


Figure 14: OS vs irAE in chemotherapy treated patients (landmark analysis at day 42)

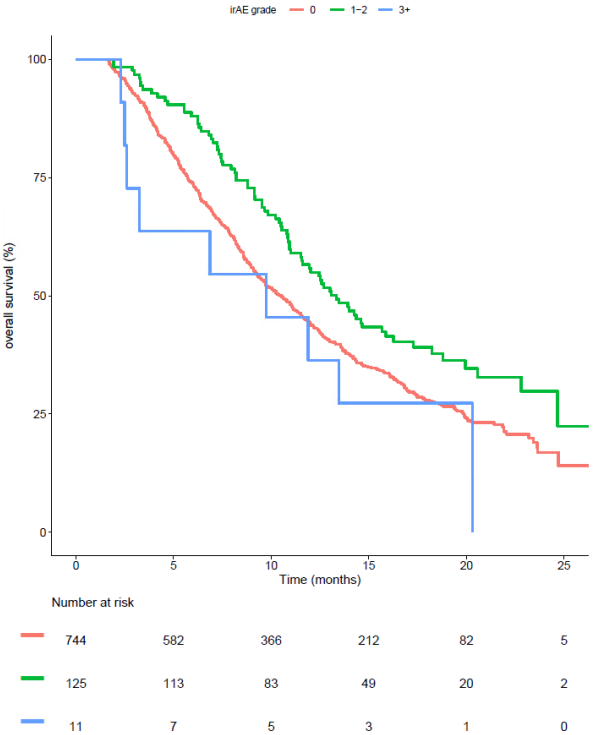


Table 24: Time dependent analysis for OS for selected organs (sub-groups)

irAE	Atezolizumab		Chemotherapy	
	HR (95% CI)	**P-value**	**HR (95% CI)**	**P-value**
Overall Survival				
Skin	0.52 (0.40-0.66)	<0.0001	0.55 (0.43-0.71)	<0.0001
Neurological	0.66 (0.41-1.96)	0.08	0.73 (0.59-0.91)	0.005
Lab	0.92 (0.68-1.24)	0.58	0.98 (0.65-1.48)	0.94

A sub-group analysis was conducted to evaluate the association between the most common organs affected by irAEs and survival outcomes (Table 24). The effect size of estimates for OS for skin irAEs were similar between the atezolizumab cohort (HR 0.52 [95%CI 0.40-0.66]) and the chemotherapy cohort (HR 0.55 [95%CI 0.43-0.71]). For neurological irAEs, the effect size estimate was slightly larger for atezolizumab (HR 0.66 [95%CI 0.41-1.96]) compared to chemotherapy (HR 0.73 [95%CI 0.59-0.91]).

4.4 Discussion

The results from this large cohort of patients with lung or urothelial cancers treated with either atezolizumab or mitotic spindle damaging chemotherapy indicate that treatment related irAEs were significantly associated with improved OS regardless of the type of treatment. The proposition that irAEs specifically predict outcomes in ICI-treated patients may warrant revision as a similar association with favourable OS was observed when irAE type toxicities occurred in patients treated with chemotherapy.

In addition, the results from this analysis indicates that irAEs as defined here (using the standard and all-encompassing definition) were a non-specific marker of favourable treatment outcomes regardless of treatment (atezolizumab or chemotherapy). This raises the hypothesis that the occurrence of irAEs should

be regarded as a prognostic, rather than predictive factor. The definition of a prognostic factor is any variable that provides information about a likely cancer outcome in the absence of any treatment or independent of the treatment received. By contrast, a predictive factor is a variable that is associated with a differential treatment effect between those with and without the variable (Ballman 2015; Group 2016; Simms, Barraclough, and Govindan 2013). As irAEs cannot occur in the absence of treatment, their prognostic nature in an untreated population cannot be evaluated. While the presence or absence of a variable at baseline prior to the initiation of treatment is often the prerequisite for studies assessing predictive factors, evolving treatment related variables such as toxicities (e.g. irAEs) are also often considered to be predictors of cancer outcomes. Notably, our analysis showed that using the same set of toxicities as the variable of interest, irAEs are associated with survival outcomes independent of the treatment administered. Therefore, irAEs might represent a general indicator of treatment benefit across multiple types of systemic therapies, rather than limited to ICI therapies. Eggermont et al also reported that irAEs occurred in the placebo arm of a trial albeit at a much lower frequency than in the ICI arm (Eggermont et al. 2020). In addition, a previous meta-analysis showed that AEs that were presumed to be immune mediated were reported in both ICI and chemotherapy treated patients (Magee et al. 2020). However, the rates of irAEs such as colitis, pneumonitis and hypothyroidism were higher in the ICI treated cohorts than the chemotherapy treated patients. Future studies should investigate the potential survival benefit of irAEs in control arm patients receiving chemotherapy and other types of systemic therapies to confirm our hypothesis that irAEs can be potentially prognostic factors across various treatment interventions.

It is a general belief that immune activation induced by ICI towards tumour antigens is also directed towards normal organs with shared antigens, resulting in irAEs. Both T-cell and B-cell mediated immune reactions have been postulated to be involved in irAEs in addition to other mechanisms (Postow, Sidlow, and Hellmann 2018). However, the organ damage manifesting as irAEs as surrogate for future improvement in OS, but not PFS in atezolizumab treated patients is intriguing. The mechanisms behind this association are unclear, however irAEs may simply reflect the off-target response to immune activation from ICI therapies.

While the association between irAEs and ICI outcomes has been described previously, the present study provides new evidence that irAE-like toxicities have similar association with OS in chemotherapy treated patients. Although the chemotherapy related toxicities are not typically defined as irAEs, it is increasingly being reported that chemotherapy induced toxicities can also have immunological mechanisms as their dominant aetiology. For example, the chemotherapy induced peripheral neuropathy observed with taxanes is often associated with neuroimmune responses with increased inflammatory cytokines, toll-like receptor 4 signalling and a reduction in anti-inflammatory cytokines in the spinal cord (Makker et al. 2017) (Li et al. 2014). Similarly, skin toxicities occur with varying incidence (6-81%) with docetaxel causing significantly worse toxicities than paclitaxel (Sibaud et al. 2016; Poi et al. 2013). Some of the skin toxicities of taxanes such as subacute cutaneous lupus erythematosus, have immunological aetiology including positive autoantibodies (e.g. anti-SSA/Ro antibodies through their respective antigen Ro52), and nucleosome release causing local autoimmune reaction directly related to microtubule damage (Sibaud et al. 2016). In addition, scleroderma like reactions have also been described with taxanes (Okada et al. 2015). However, the severe grade 3 or more reactions are secondary to a direct toxic effect through a non-immunoallergic mechanism (Poi et al. 2013). Another proposed mechanism involves the alteration of the gut microbiome by taxanes with consequent development of neurocognitive changes, a well-established toxicity from chemotherapy (Loman et al. 2019). Further studies are warranted to better understand the immunological basis of toxicities from all cancer therapies.

Recently, using data from IMvigor211 trial dataset, Khan et al reported a genome-wide association study showing that OS was associated with high vitiligo, high psoriasis and low atopic dermatitis polygenic risk scores in the atezolizumab cohort, but not in the chemotherapy cohort (Khan et al. 2020). These results indicate that it is possible that different immune pathophysiological mechanisms drive the skin irAEs from ICI and chemotherapy. Hence, the use of same definitions for irAEs in the chemotherapy arms requires reconsideration in future studies.

A previous meta-analysis found that only 5 out of 21 studies that reported the association between the occurrence of irAEs and outcomes used landmark analyses and time dependent analyses, and accounting for

immortal time bias was uncommon (Zhou et al. 2020). In the current study, time dependent and landmark analyses were conducted, both of which showed a favourable OS benefit. This indicates the consistency of the association between the occurrence of irAEs and OS.

The association between the grade of irAEs and survival outcomes has been reported previously by other authors (Zhou et al. 2020). We identified that those patients who had grade 1 or 2 irAEs, but not those without any irAE or those with severe (grade 3 or more) irAEs, had a favourable OS. It therefore appears that the severity of the irAE is not linearly associated with OS benefit. A similar association was also observed in the chemotherapy treated cohort. Severe toxicities are often secondary to direct toxic damage or non-immunogenic mechanisms of cell death (Poi et al. 2013). It can be inferred that both an absent and a too large activation of the immune response, or tissue damage, may foreshadow either lack of benefit or early cessation of therapies. Further studies on the quality and quantity of the immune response to ICI and their association with cancer outcomes and toxicities are justified.

Finally, there was a degree of specificity in terms of the organ affected by irAEs and survival benefits. Skin irAEs were the only group that was significantly associated with improved OS in the atezolizumab treated cohort whereas skin and neurological irAEs were both associated with significant OS benefit in the chemotherapy treated cohort. Other groups have similarly reported organ specificity with skin irAEs being the most common (Das and Johnson 2019; Eggermont et al. 2020; Zhou et al. 2020). In contrast to other reported findings, endocrine irAEs were not associated with improved OS. The reasons for this discrepancy are unclear and require further research.

The current study has several strengths and limitations. Strengths included: a large cohort of ICI and chemotherapy treated cohorts with pooled individual patient data from rigorously conducted randomized clinical trials; and the analyses included a time-dependent analysis accounting for immortal time bias as well as predefined sub-group analyses for the association between grade of irAEs/organ type and survival. The main limitations are that: a) the analyses were not pre-planned and should be considered hypothesis generating; b) the treatment included monotherapy interventions in relatively fit patients through clinical trials with stringent inclusion and exclusion criteria; and c) the applicability of the results among those treated

with combination therapies is uncertain. Further studies should explore these issues. Similarly, the trials included patients with lung and urothelial cancers treated with microtubule damaging chemotherapy drugs. It is unclear whether similar associations occur in other cancer types and with other chemotherapy agents. Despite these limitations, the results from the current study show significant irAE-associated OS benefits both with ICI therapies and chemotherapy.

4.5 Conclusion

irAEs, especially those of low-grade severity, were associated with improved overall survival both in atezolizumab and chemotherapy treated patient cohorts with lung or urothelial cancers. The similarity of the observed associations, independent of the treatment administered, raises the possibility that irAEs type events are prognostic, rather than predictive, factors.

The results from this research were unexpected as the analyses indicated that irAE type toxicities were associated with survival benefit even in chemotherapy treated cohort. Further studies that evaluate this association in other treatment settings should be conducted to confirm these findings. Moreover, pre-clinical and clinical studies should also explore immunological basis for chemotherapy related toxicities.

5. CHAPTER FIVE: CONCOMITANT ANTI-HYPERTENSIVES AND OUTCOMES FROM IMMUNE CHECKPOINT INHIBITORS

In this chapter, the effect of concomitant medications as another factor that may contribute to variability in systemic cancer therapy response and toxicities was evaluated. The use of concomitant non-cancer medications among patients with advanced cancer is common (prevalence of polypharmacy - 20-81% of patients) (McNeil et al. 2016; LeBlanc et al. 2015). Previously reported studies indicate that there is an increasing risk for cancer drug vs non-cancer drug interactions with potential drug interactions (seen up to 75% of patients) and clinically significant interactions (up to 6% of patients) (Goh, Lai, and Chew 2018; Ko et al. 2012; Popa et al. 2014). These interactions are usually due to either PK or PD effects. On the contrary, the currently available ICI therapies are monoclonal antibodies that act through immune activation. Their drug interactions are expected to be predominantly due to PD mediated. Among the concomitant medications, previous research has already highlighted that antibiotics and corticosteroids may influence response to other ICI therapies (Maxwell, Luksik et al. 2018, Petrelli, Grizzi et al. 2019, Pinato, Howlett et al. 2019, Chalabi, Cardona et al. 2020). However, there was little published information on the effect of concomitant use of anti-hypertensives in patients undergoing therapy with ICI.

The association between the concomitant use of anti-hypertensives with atezolizumab was investigated using data from seven clinical trials with a patient population that was not uniform, with three different cancer types, however, all treated with atezolizumab.

5.1 Introduction

Hypertension is common among patients with cancer (Małyszko et al. 2018). Current evidence suggests that antihypertensives, cancer and its treatment have a complex and conflicting relationship. The association between certain classes of antihypertensives and malignancies varies from increased risks, decreased risks or nil effects on the occurrence of cancers as well as beneficial effects, adverse effects, or nil effects on survival of some cancers (Battistoni et al. 2020; Tadic et al. 2019). With the significant improvement in survival from immune checkpoint inhibitors (ICI) and their widespread usage for cancer treatment, the

interaction between chronic conditions, concomitant medications and ICI therapy related outcomes are increasingly reported (Chalabi, Cardona, Nagarkar, Dhawahir Scala, Gandara, Rittmeyer, Albert, Powles, Kok, Herrera, et al. 2020; Herrscher and Robert 2020; Hopkins, Kichenadasse, et al. 2020; Kichenadasse et al. 2019a; Moujaess et al. 2019; Schmid et al. 2020). Recently, in a large cohort of patients with various types of cancers treated with ICI, it was reported that obesity and hypercholesterolemia were associated with lower all-cause mortality while hypertension, smoking and the use of beta blockers were associated with higher overall mortality especially in patients with lung cancer (Oren et al. 2020).

Among the antihypertensives, renin - angiotensin system inhibitors (RASi) are commonly used and have a more complex interaction with cancer and its treatment interventions than other drugs (Bangalore et al. 2011; Cui et al. 2019; Messerli et al. 2018; Sanidas et al. 2020). The three main classes of RASi include the angiotensin-converting enzyme inhibitors (ACEI), angiotensin II receptor blockers (ARBs) and direct renin inhibitors (DRIs). ACEI are generally first choice for cardiovascular risk management, ARBs are often prescribed if ACEI are contraindicated or not tolerated whereas aliskiren, the only approved DRI, is not commonly used due to the relatively high risk of adverse events (Whelton et al. 2018).

While the predominant physiological functions of RAS signalling are the modulation of fluid, electrolyte and blood pressure homeostasis, its role on carcinogenesis, proliferation and cancer progression through a multitude of mechanisms is receiving increased attention (Pinter and Jain 2017; Wegman-Ostrosky et al. 2015). Pre-clinical studies indicate that the components of the RAS pathway are differentially expressed in cancer types and that RAS signalling favours cancer cell proliferation, enhances pro-survival pathways, and induces neoangiogenesis, cell migration, invasion and metastasis (Wegman-Ostrosky et al. 2015).

Given the pleiotropic effects of RAS signalling in cancer, previous research has focused on repurposing RASi as anti-cancer drugs. Several *in vitro* and *in vivo* pre-clinical studies, retrospective clinical studies and small clinical trials have reported that RASi use can potentially reduce the incidence of certain types of cancers, potentiate the benefit of chemotherapy, anti-angiogenic agents and anti-epidermal growth factor receptor inhibitors, and prolong survival in particularly aggressive cancers such as pancreatic cancer (George, Thomas, and Hannan 2010; Hamy et al. 2020; Pinter and Jain 2017; Zhao et al. 2019). In a meta-analysis of cohort

studies, RASi were associated with improved survival in selected cancer types such as lung, renal, bladder, gastric and pancreatic cancers. However, this association was present with ARBs, but not with ACEI (Sun et al. 2017).

In addition, RAS signalling favours a pro-inflammatory state within the tumor microenvironment, thereby inducing an immunosuppressive milieu (Nakamura et al. 2018; Pinter and Jain 2017; Vallejo-Ardila et al. 2018; Xie et al. 2018). This might contribute towards resistance to agents such as ICI, a class of drugs that relies on intra-tumoral immune response. Therefore, the concomitant use of RASi might theoretically augment the therapeutic efficacy of ICI. In support of this hypothesis, Nakamura et al have recently reported that ARBs may abrogate resistance to ICI in mice (Nakamura et al. 2018). Others have also proposed that RASi may suppress chronic inflammation, thereby reducing or preventing ICI induced toxicities (Pinter, Kwanten, and Jain 2018). However, in a retrospective study of patients with non-small cell lung cancer (NSCLC), Medjebar et al reported that concomitant ACEI and ICI therapy resulted in inferior survival outcomes (Medjebar et al. 2019). In contrast, there was no association between the use of ACEI or ARBs and overall survival in another study (Oren et al. 2020). In view of these conflicting reports, it is important to better understand the effect of the concomitant use of RASi on the outcomes from ICI treated patients.

Given the contradictory results, further studies are required to better understand the biological and clinical significance of the interaction between RASi and ICI. Based on the available evidence, it was hypothesized that the concomitant use of RASi in cancer patients undergoing ICI therapy may improve survival benefit and reduce ICI related adverse events. Therefore, this research evaluated the association between concomitant use of RASi or other antihypertensive drug classes and the efficacy and safety of atezolizumab treatment, a PDL1 inhibitor, using pooled data from patients with NSCLC, urothelial or renal cancers across seven clinical trials.

5.2 Methods

5.2.1 Study definitions

The primary objectives were to evaluate the association between the concomitant use of RASi and: (i) survival (both overall survival (OS) and progression free survival (PFS)), and (ii) immune related adverse events (irAEs) during atezolizumab treatment for solid tumors. Exploratory analysis of the association between the concomitant use of other classes of antihypertensive agents with survival/ toxicity outcomes were conducted. Associations within the cohort of patients treated with chemotherapy were also explored. Relevant individual patient data were extracted from seven published trials – BIRCH (NCT02031458)(Peters et al. 2017), FIR (NCT01846416) (Spigel et al. 2018), OAK (NCT02008227) (Rittmeyer et al. 2017), POPLAR(NCT01903993) (Fehrenbacher et al. 2016); IMvigor 210 (cohort 1 NCT02951767 and cohort 2 NCT02108652) (Balar, Galsky, et al. 2017), and IMvigor 211 (NCT02302807) (Powles et al. 2018) and IMmotion 150 (NCT01984242) (McDermott et al. 2018). Only data from the monotherapy arms with atezolizumab or chemotherapy across the trials were included in the analysis. Specifically, the atezolizumab with bevacizumab and sunitinib arms from IMmotion 150 were not included. Deidentified data were collected and analysed through the data sharing program and policies ('ClinicalStudyDataRequest.com') after exemption by Southern Adelaide Clinical Health Research Ethics Committee.

Antihypertensives were categorized in the following classes: RASi (ACEI, ARBs or DRIs), beta blockers (BB), calcium channel blockers (CCB), and diuretics (thiazides and loop diuretics). Use of antihypertensives was considered concomitant if the patient was on any of these agents at enrolment to the respective clinical trials prior to the study drug administration. irAEs were defined as any investigator identified atezolizumab related organ specific immune related events in each trial. OS was the primary outcome, while PFS and any grade of irAEs were the secondary outcomes. PFS was reported using the RECIST version 1.1 or modified RECIST, while irAEs were graded according to the NCI-CTCAE v4.0. Baseline clinical characteristics, cardiovascular (CV) comorbidities and cause of death were also evaluated.

5.2.2 Statistical analysis

Pooled HR with 95%CI were reported for the association between the concomitant use of antihypertensives (any antihypertensive or specific use of RASi, BB, CCBs and diuretics) and survival (PFS and OS) as modelled using Cox proportional hazards regression. A two-stage meta-analysis of IPD was employed for the main

objective evaluating the association between RASi and PFS/OS (Burke, Ensor, and Riley 2017). HRs were generated from the IPD from each trial separately in the first stage, then the results were combined by a random-effects meta-analysis model in the second stage.

The association between concomitant use of antihypertensives and irAEs were assessed using logistic regression and reported as OR. Regression analyses were stratified by study and cancer type. Potential confounding variables (age, sex, race, body mass index, PD-L1 expression, and the number of sites of tumour metastases) were adjusted by multivariable regression analysis. A P value less than 0.05 was considered statistically significant. Analyses were conducted using R (version 3.4.3).

5.3 Results

Data from a total of 3,695 patients pooled from seven clinical trials were available for analysis. There were 2,539 atezolizumab treated patients and 1,156 chemotherapy treated patients across three cancer types available for further analysis (NSCLC: 1,548 atezolizumab treated and 713 docetaxel treated patients; urothelial cancers: 888 atezolizumab treated and 443 chemotherapy treated patients; renal cell cancer: 103 atezolizumab treated patients). Baseline clinical characteristics of the atezolizumab and chemotherapy treated cohorts is described in Table 25 and Table 26 respectively. Overall, 59% patients had one or more cardiac or vascular disorders at baseline. Hypertension was the most common (46% of all patients) cardiovascular risk factor at trial entry. Diabetes mellitus was noted in 528 (14%) patients.

A total of 1,601 (43%) trial participants were on one or more anti-hypertensives for various indications such as hypertension, cardiac failure or arrhythmias. RASi were used by 878 (24%) of all trial participants (12% each on ACEI or ARB with just 7 patients on both types of drugs). Aliskiren was used by only 3 atezolizumab treated patients. Therefore, aliskiren treated patients were not separately analysed as sub-groups of anti-hypertensive class. BB were the second most used concomitant anti-hypertensive class (19% of patients), whereas CCB were used in 12% and diuretics were used in 9 %. Overall, 43% of the patients were on at least one anti-hypertensive at trial entry.

Table 25: Atezolizumab treated population across all trials

On atezolizumab	Total No. 2,539	No RASi No. 1,935	RASi No. 604	P-value
Study				0.67
OAK	609 (24%)	464 (24%)	145 (24%)	
POPLAR	142 (6%)	111 (6%)	31 (5%)	
BIRCH	659 (26%)	511 (26%)	148 (25%)	
FIR	138 (5%)	104 (5%)	34 (6%)	
IMvigor211	459 (18%)	349 (18%)	110 (18%)	
IMvigor210	429 (17%)	325 (17%)	104 (17%)	
IMmotion150	103 (4%)	71 (4%)	32 (5%)	
Cancer type				0.18
NSCLC	1,548 (61%)	1,190 (61%)	358 (62%)	
RCC	103 (3%)	71 (4%)	32 (5%)	
Urothelial cancers	888 (35%)	674 (35%)	214 (35%)	
Age (years)	65 (58 - 72)	63 (56 - 70)	68 (63 - 74)	< 0.001*
Sex				0.002*
Male	1,701 (67%)	1,265 (65%)	436 (72%)	
Female	838 (33%)	670 (35%)	168 (28%)	
Race				0.11
White	2,024 (80%)	1,532 (79%)	492 (81%)	
Asian	304 (12%)	252 (13%)	52 (9%)	
Other	97 (4%)	72 (4%)	25 (4%)	
Missing	114 (4%)	79 (4%)	35 (6%)	
ECOG PS				0.38
0	948 (37%)	720 (37%)	228 (38%)	
1	1,554 (61%)	1,190 (61%)	364 (60%)	
2	33 (1%)	22 (1%)	11 (2%)	
Missing	4 (<1%)	3 (<1%)	1 (<1%)	
Tumour sites				0.26
Median	2 (1 - 3)	2 (1 - 3)	2 (1 - 3)	
Missing	34 (1%)	23 (1%)	11 (2%)	
PD-L1 expression				0.24
Negative	626 (25%)	477 (25%)	149 (25%)	
Positive	1,903 (75%)	1,451 (75%)	452 (75%)	
Missing	10 (<1%)	7 (<1%)	3 (<1%)	
BMI				< 0.001*
Median	25 (23 - 29)	25 (22 - 28)	27 (24 - 31)	
Missing	90 (4%)	71 (4%)	19 (3%)	
Diabetes	366 (14%)	197 (10%)	169 (28%)	< 0.001*
Hypertension	1,173 (46%)	599 (31%)	574 (95%)	< 0.001*
Cardiovascular disease	1,486 (59%)	889 (46%)	597 (99%)	< 0.001*
ACEI	299 (12%)	0 (0%)	135 (12%)	< 0.001
ARBs	311 (12%)	0 (0%)	140 (12%)	< 0.001
Beta blockers	488 (19%)	290 (15%)	198 (33%)	< 0.001
CCBs	364 (14%)	200 (10%)	164 (27%)	< 0.001
Diuretics	226 (9%)	115 (6%)	111 (18%)	< 0.001
Any anti-hypertensives	1,094 (43%)	490 (25%)	604 (100%)	< 0.001

Table 26: Chemotherapy treated population across all trials

On chemotherapy	Total No. 1,156	No RASi No. 882	Yes RASi No. 274	P-value
Study				0.66
OAK	578 (50%)	447 (51%)	131 (48%)	
POPLAR	135 (12%)	103 (12%)	32 (12%)	
IMvigor211	443 (38%)	332 (38%)	111 (41%)	
Cancer type				0.39

NSCLC	713 (62%)	550 (62%)	163 (59%)	
Urothelial cancers	443 (38%)	332 (38%)	443 (38%)	
Age (years)	65 (58 - 71)	64 (57 - 70)	67 (61 - 72)	< 0.001*
Sex				0.004*
Male	779 (67%)	575 (65%)	204 (74%)	
Female	377 (33%)	307 (35%)	70 (26%)	
Race				0.02*
White	837 (72%)	619 (70%)	218 (80%)	
Asian	185 (16%)	157(18%)	28 (10%)	
Other	41 (4%)	33 (4%)	8 (3%)	
Missing	93 (8%)	73 (8%)	20 (7%)	
ECOG PS				0.44
0	457 (37%)	326 (37%)	114 (42%)	
1	698 (60%)	538 (61%)	160 (58%)	
Missing	1 (<1%)	1 (<1%)	0 (0%)	
Tumour sites	2 (2 - 3)	2 (2 - 3)	2 (1 - 3)	0.21
PD-L1 expression				0.35
Negative	425 (37%)	326 (37%)	99 (36%)	
Positive	728 (63%)	555 (63%)	174 (63%)	
Missing	3 (<1%)	1 (<1%)	2 (1%)	
BMI				< 0.001*
Median	25 (22 - 28)	25 (22 - 27)	27 (24 - 30)	
Missing	20 (2%)	13 (1%)	7 (3%)	
Diabetes	162 (14%)	92 (10%)	70 (26%)	< 0.001*
Hypertension	510 (44%)	255 (29%)	255 (93%)	< 0.001*
Cardiovascular disease	879 (59%)	411 (47%)	268 (98%)	< 0.001*
ACEI	134 (12%)	0 (0%)	134 (12%)	< 0.001
ARBs	140 (12%)	0 (0%)	140 (12%)	< 0.001
Beta blockers	222 (19%)	131 (15%)	91 (33%)	< 0.001
CCBs	178 (15%)	98 (11%)	80 (29%)	< 0.001
Diuretics	91 (8%)	56 (6%)	35 (13%)	< 0.001
Any anti-hypertensives	507 (44%)	233 (26%)	274 (100%)	< 0.001

5.3.1 Concomitant RASi use and survival outcomes of atezolizumab treated cohort

A total of 604 (24%) atezolizumab treated patients were on a RASi at the start of the trial, 12% on ACEI and 12% on ARB. When compared to non RASi users, concomitant RASi users were more likely to be older, male, with high body mass index (BMI), higher prevalence of hypertension, diabetes and cardio-vascular diseases, and greater use of other anti-hypertensives (Table 25).

When the RASi users were compared to non-users, there was a borderline significant OS benefit was noted (pooled HR was 0.88, 95% CI 0.78-1.00, P = 0.05), and no significant PFS benefit was seen (pooled HR was 0.93, 95% CI 0.84-1.03, P = 0.15). When the two-stage meta-analysis of IPD was employed, there was no OS benefit (pooled HR 0.90, 95% CI 0.78-1.04, P = 0.15), or PFS benefit (pooled HR 0.95, 95% CI 0.85-1.05, P = 0.28) between RASi users non-users among the pooled atezolizumab treated cohort (Figure 15 & Figure 16).

On adjustment for confounding variables, there was no significant association with either OS (HR 0.94, 95% CI 0.82-1.07, P =0.38) or PFS (HR 1.003, 95% CI 0.90-1.12, P =0.95). On exploratory analysis, no associations between ACEI, BB, CCB or diuretic use and OS or PFS from atezolizumab were observed (Table 27). Concomitant ARB was the only class that was significantly associated with inferior PFS (pooled HR 1.16, 95% CI 1.01-1.33, P = 0.038), although there was no association with OS (pooled HR 0.98, 95% CI 0.82-1.16, P = 0.79) (Table 27).

Figure 15: RASi use and overall survival outcomes among atezolizumab treated patients

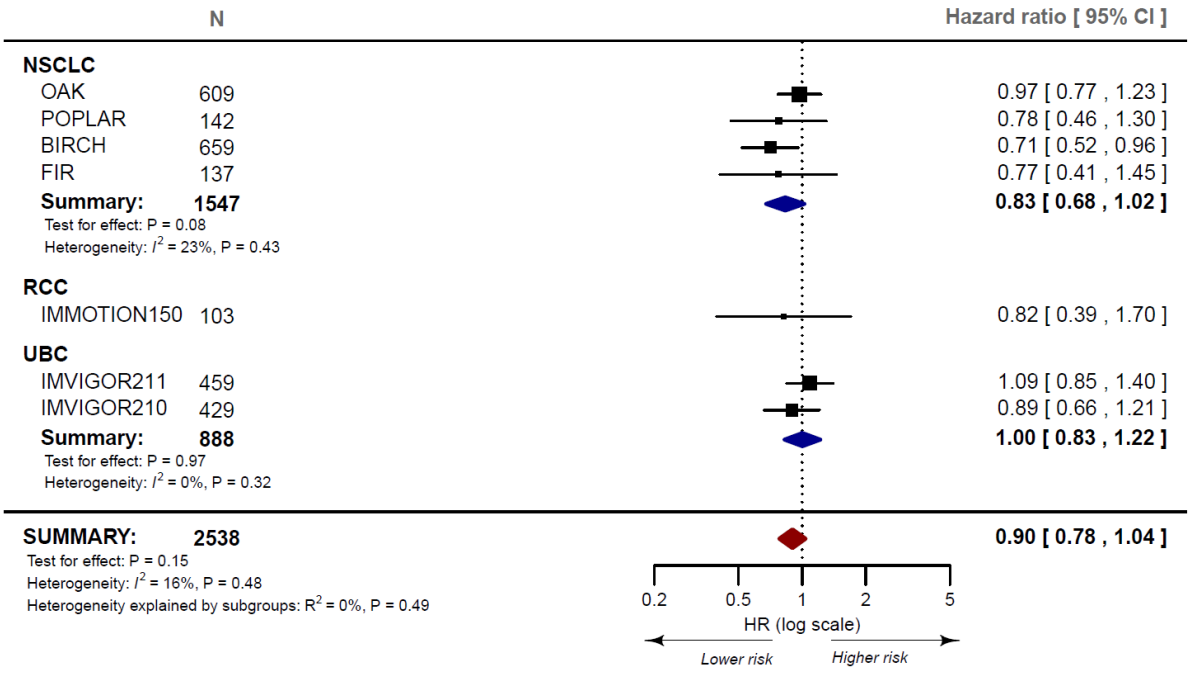


Figure 16: RASi use and PFS outcomes among atezolizumab treated patients

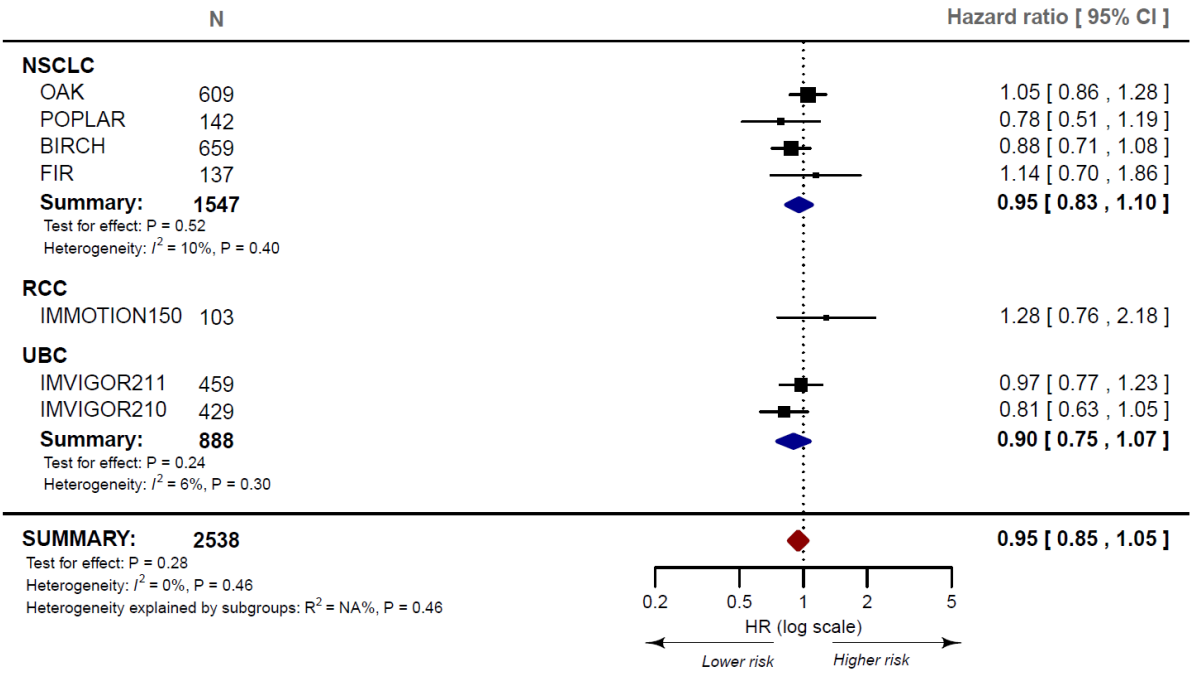


Table 27: Survival outcomes and sub-classes of anti-hypertensives

Concomitant drug class	Adjusted Pooled HR	95% CI	P- value
Overall survival for Atezolizumab treated cohorts			
ACEI	0.94	0.79-1.12	0.49
ARBs	0.98	0.82-1.16	0.79
BB	1.05	0.91-1.21	0.47
CCB	1.03	0.88-1.21	0.68
Diuretics	1.15	0.95-1.4	0.15
Any antihypertensive	1.01	0.89-1.13	0.92
Progression free survival for Atezolizumab treated cohorts			
ACEI	0.88	0.76-1.02	0.09
ARBs	1.16	1.01-1.33	0.038*
BB	1.01	0.90-1.13	0.47
CCB	1.04	0.88-1.21	0.68
Diuretics	1.15	0.92-1.19	0.51
Any antihypertensive	1.06	0.96-1.17	0.24
Overall survival for chemotherapy treated cohorts			
ACEI	0.99	0.79-1.12	0.49
ARBs	0.90	0.72-1.12	0.79
BB	0.91	0.76-1.09	0.31
CCB	1.07	0.89-1.30	0.46
Diuretics	1.53	1.20-1.95	<0.001*
Any antihypertensive	0.98	0.84-1.13	0.75
Progression free survival for chemotherapy treated cohorts			
ACEI	1.06	0.88-1.29	0.53
ARBs	1.01	0.89-1.23	0.94
BB	1.05	0.89-1.23	0.58
CCB	0.93	0.78-1.11	0.40
Diuretics	1.22	0.97-1.53	0.09
Any antihypertensive	1.01	0.89-1.16	0.84

5.3.2 Concomitant anti-hypertensive use and irAEs of atezolizumab treated cohort

Overall, 736 (25%) of atezolizumab treated patients had one or more irAEs during the study period. There was no significant difference in the incidence of irAEs with the concomitant use of a RASi (OR 0.94; 95% CI 0.76-1.15, P = 0.55), ARBs (OR 0.79; 95% CI 0.59-1.03, P = 0.09), or ACEI (OR 1.12; 95% CI 0.86-1.46, P = 0.39).

5.3.3 Concomitant anti-hypertensive use and survival outcomes of chemotherapy treated cohort

Among the cohort of chemotherapy treated patients, 274 (24%) were on RASi at baseline. Concomitant RASi users were more likely to be older, male, white race, with high BMI and hypertension, diabetes, cardiovascular disease, and greater use of other anti-hypertensives (Table 26). The concomitant use of RASi, ACEI, ARBs or any anti-hypertensive drug class was not significantly associated with PFS or OS, except for

diuretics, which were associated with significantly worse survival (pooled HR 1.53; 95% CI 1.20-1.95, P = <0.0001) (Table 27).

5.4 Discussion

In this pooled analysis of data from rigorously conducted clinical trials in patients with three different cancers, the concomitant use of antihypertensives, especially RASi, was not significantly associated with either improved or decreased survival or irAEs during treatment with the ICI, atezolizumab. Similarly, concomitant use of antihypertensives was not associated with any survival benefit during treatment with chemotherapy.

Previous epidemiological studies have reported that antihypertensives including RASi may differentially affect cancer incidence across multiple cancer types (Battistoni et al. 2020; Małyszko et al. 2018). However, there are limited data on the interaction between RASi and various systemic anti-cancer treatments, especially ICI (Aydiner, Ciftci, and Sen 2015; Li, Sun, and Hu 2017; Medjebar et al. 2019; Oren et al. 2020). The findings from the current study contrast with the available pre-clinical data and clinical observations from smaller studies, suggesting that RASi may improve survival and decrease irAEs from ICI (Medjebar et al. 2019; Pinter and Jain 2017; Pinter, Kwanten, and Jain 2018; Wegman-Ostrosky et al. 2015; Xie et al. 2018). Furthermore, exploratory analysis showed that concomitant use of ARBs was associated with an inferior PFS, but not with OS, during treatment with atezolizumab.

The results from the current study are similar to the large retrospective cohort study by Oren et al who reported no significant association between RASi and lung cancer outcomes (Oren et al. 2020). In contrast, Medjebar et al reported that concomitant ACEI use was associated with worse survival through an induction of intra-tumoral immunosuppressive state (Medjebar et al. 2019). However, limited information was provided regarding patient and cancer characteristics and the study was relatively small (27 patients received concomitant treatment with ACEI out of 283 pembrolizumab treated patients). The issues of different study design, sample size, and analysis between my research and that of Medjebar et al notwithstanding, it is possible that ARBs, similar to ACEI, induce intra-tumoral immunosuppression that may counteract

atezolizumab activity. Further experimental and clinical studies involving various types of cancer and other ICI are required to clarify the association between RASi and immunotherapies.

It is possible that the beneficial anti-cancer effects of RASi may be dependent on the primary site of the cancer, as previously reported (Roth et al. 2019). The current study analysed data from clinical trials involving lung, urothelial and renal cancers. All analyses were performed after stratification for the cancer types and study. There was no differential impact of concomitant RASi noted based on the primary site of cancer.

RASi are often used for the management of cardiac complications of several anti-cancer drugs such as anti-human epidermal growth factor receptor *2* (her-2), anti-angiogenesis and ICI. More recently, some authors have proposed that RASi could be combined with ICI to reduce or prevent a wide range of irAEs due to their immune modulatory effects, including suppression of circulating proinflammatory cytokines (Pinter and Jain 2017; Pinter, Kwanten, and Jain 2018). In our analysis, there was no significant difference in the incidence of irAEs between RASi users and non-users. However, it is unclear whether there was any effect on severity or specific organs affected by irAEs. Pending the results of further studies that address this issue, RASi remain an important option for the management of cardiac complications from ICI and other anti-cancer drugs.

Several reasons may explain the lack of observed effects of the combined treatment of RASi and ICI on cancer outcomes. There are limited data on the optimal *in vivo* concentration and the proportionate doses of RASi required to produce anti-cancer activity. Pre-clinical studies indicate that high concentrations of RASi were required to induce cancer cell apoptosis while such concentrations may not be achieved without significant toxicities in human clinical trials (Funao et al. 2008; Nakai et al. 2013; Stangier, Su, and Roth 2000). Moreover, the mild to moderate reduction noted in the concentrations of pro-inflammatory cytokines with RASi may not be adequate to prevent irAEs (Manabe et al. 2005). It is also possible that the effect size of RASi on patients being treated with ICI is relatively small, requiring a much larger RASi treated cohort to identify an interaction.

On the other hand, the association between the concomitant use of a diuretic and OS was an unexpected finding in chemotherapy, but not atezolizumab, treated patients. However, this observation requires

confirmation in larger studies given the relatively small sample size of our subgroup (8% of chemotherapy treated patients).

The current study has several strengths. It included the largest number of patients uniformly treated with atezolizumab using high quality data from clinical trials. The number of concomitant RASi users was relatively large which allowed optimal exploration of the interaction between RASi and ICI therapies. The analysis included stratification for the cancer types and adjustment for clinically relevant confounding variables, thereby improving its validity.

Limitations of the current study include lack of data on duration and dose changes of RASi, patients starting RASi after the commencement of trial, and adherence to concomitant medications. Moreover, the current study was a *post hoc* analysis and should therefore be considered as hypothesis generating for future prospective studies on the effect of the intervention of RASi on cancer outcomes. Similarly, the data included those patients treated with atezolizumab monotherapy. It is unclear if the lack of association between RASi and ICI will remain consistent when evaluated in combination strategies. Ongoing trials such as NCT03563248 - combining losartan, an ARB, with chemotherapy and nivolumab, an ICI, for the treatment of pancreatic cancer will generate useful knowledge in this context (NCT03563248 - Losartan and nivolumab in combination with chemotherapy and stereotactic body radiotherapy in localized pancreatic cancer).

5.5 Conclusion

There was no significant association between the concomitant use of RASi and atezolizumab in terms of survival or safety in patients with advanced malignancies using data from seven clinical trials. From this research, it appears that concomitant use of anti-hypertensives, especially from the RASi group, are unlikely to influence the activity or safety of atezolizumab indicating that the variability of response to atezolizumab is not explained by the concomitant use of RASi. This contrasts with other recent publications that concomitant drugs such as antibiotics, corticosteroids and proton pump inhibitors may negatively affect ICI therapy outcomes. Further larger studies should explore the relationship between concomitant RASi and ICI therapies using different agents and cancer types.

6. CHAPTER SIX: CONCOMITANT PROTON PUMP INHIBITORS AND OUTCOMES FROM CYTOTOXICS

As proton pump inhibitors (PPIs) were reported to be associated with negative survival outcomes with atezolizumab, I wanted to explore if PPIs similarly contribute towards any worse survival in patients undergoing cytotoxic chemotherapy. In this chapter, I report the results from an analysis involving more than 5,000 patients with a different cancer from previous chapters (colorectal cancers here), all being treated with fluoropyrimidine-based chemotherapy.

6.1 Introduction

PPIs are one of the most commonly used drugs by patients with cancer, especially gastrointestinal malignancies (Smelick et al. 2013). In observational studies in non-cancer populations, the long-term use of PPIs was associated with several adverse outcomes including increased all-cause mortality, cardiovascular and renal diseases, dementia, infections, fractures, hypomagnesaemia and cancers (Schoenfeld and Grady 2016; Xie et al. 2019). In contrast, in preclinical *in vitro* studies PPIs were initially reported to improve the efficacy of some anti-cancer agents through direct anti-cancer effects and altered acidity within the tumour microenvironment (Bellone et al. 2013; Ikemura, Hiramatsu, and Okuda 2017; Lugini et al. 2016; Pilon-Thomas et al. 2016). More recently, the potential for PPIs to adversely affect cancer outcomes when given concomitantly with oral anti-cancer drugs such as kinase inhibitors was described (Sharma et al. 2019; van Leeuwen et al. 2017; van Leeuwen et al. 2014; Hussaarts et al. 2019). An increased intragastric pH from PPI use, with consequent reduced absorption of kinase inhibitors, was considered the mechanism responsible for this interaction (van Leeuwen et al. 2017). Changes in gastrointestinal microbiome is another mechanism through which PPIs may affect the cancer outcomes and metabolism of drugs (Wedemeyer and Blume 2014).

Observational studies and retrospective analyses of trial data have also shown, albeit not consistently, that PPIs may decrease the efficacy of capecitabine, an oral fluoropyrimidine. As a result, patients with colorectal cancer (CRC), breast cancer and other malignancies treated with capecitabine might have an increased risk of cancer recurrence and/or shorter survival (Altundag 2017b, 2017a; Chu et al. 2017; Chu and Sawyer 2018; Graham et al. 2016; Rhinehart et al. 2018; Sun et al. 2016; Wong et al. 2019). However, pharmacokinetic (PK)

and *in vitro* studies have failed to identify the mechanisms of the interaction between PPIs and capecitabine (Cheng et al. 2019). Furthermore, it is unclear whether the potentially negative effects of PPIs might also involve intravenously administered cytotoxic agents.

In this project, it was sought to address these issues in this research by assessing the association between concomitant PPI use and survival outcomes in patients with advanced CRC treated with a fluoropyrimidine-based chemotherapy regimen using data from six completed CRC clinical trials. Furthermore, whether this association differed between oral and systemically administered fluoropyrimidines, and between other agents combined with the fluoropyrimidines were also evaluated.

6.2 Methods

6.2.1 Study population

A retrospective *post hoc* analysis was performed using anonymized individual patient data from six clinical trials in patients with advanced CRC obtained through the data sharing platforms, Project Data Sphere® (Projectdatasphere 2020) and *Clinical Study Data Request* (Clinicalstudydatarequest 2019)*:* AVF2107 trial (clinicaltrials.gov number NCT00109070) (Hurwitz et al. 2004), Carrato et al (NCT00457691) (Carrato et al. 2013), HORIZON III trial (NCT00384176) (Schmoll et al. 2012), VELOUR trial (NCT00561470) (Van Cutsem et al. 2012), N016966 trial (NCT00069095) (Saltz et al. 2008), and RAISE trial (NCT01183780) (Tabernero et al. 2015). Sponsors of the Carrato et al, HORIZON III and VELOUR trials released data from their respective control arms only while sponsors for the other four trials provided data for both control and intervention arms. Southern Adelaide Clinical Health Research Ethics Committee exempted review for this analysis.

6.2.2 Study definitions

A fluoropyrimidine-based regimen was defined as combination anti-cancer therapy including at least one of the fluoropyrimidines, 5-fluorouracil (5-FU) or capecitabine. Fluoropyrimidine-based chemotherapy, given either as first-line or second-line therapy, included either irinotecan or oxaliplatin as part of multi-agent combination therapy with fluoropyrimidines and leucovorin. Concomitant vascular endothelial growth factor receptor inhibitor (VEGFi) therapies administered were bevacizumab or ramucirumab, depending on the trial.

Concomitant PPIs used were esomeprazole, lansoprazole, omeprazole, pantoprazole, or rabeprazole. PPI use was defined as treatment with any PPI at the time of initiation of the respective trial intervention, for a minimum of 7 days.

6.3.3 Data collection and outcomes

Data extracted were age at trial enrolment, sex and race, tumour response, time to progression, and survival time. The outcome measures were PFS and OS among PPI users and non-users. PFS and tumour response were assessed using RECIST or RECIST version 1.1. Best overall response was defined as combined complete and partial responses. Sub-group analysis for the association between PPI use and OS included the type of chemotherapy (oxaliplatin vs irinotecan), oral vs intravenous fluoropyrimidine administration, addition of VEGFi and line of therapy.

6.3.4 Statistical analysis

Analysis was conducted using cohorts with fluoropyrimidine treated patients. HR with 95%CI for the association between PPI use and survival outcomes were estimated individually for each trial arm using Cox proportional hazards regression. HRs were adjusted for age, sex, race, ECOG performance status, and serum carcinoembryonic antigen (CEA) and LDH levels. Complete case analysis was undertaken due to minimal missing data for these covariates. Estimates were then pooled across all trials and arms using random-effects meta-analysis methods. A fixed-effect meta-analysis model was applied as a sensitivity analysis. Trial and summary HRs were visually displayed using forest plots and statistical heterogeneity described using the I^2 statistic. Analysis was performed using R (version 3.4.3) (Team 2017). A P-value of <0.05 was considered statistically significant.

6.3 Results

Data from 11 arms across six trials were available for analysis (Table 28). The N016966 trial had four arms, two trials had two arms and the rest had one arm. 5-FU with leucovorin and irinotecan (FOLFIRI or IFL) was the chemotherapy in all trials except N016966 and HORIZON III where a fluoropyrimidine was combined with oxaliplatin. Among the VEGFi therapies, bevacizumab was combined with chemotherapy (BEV+IFL,

BEV+FOLFOX or BEV+CAPOX) in the AVF2107g, HORIZON III and N016966 trials, while ramucirumab was combined with chemotherapy (RAM+FOLFIRI) in the RAISE trial. Data on sunitinib or aflibercept treated arms were not available for analysis. Most of the included trials were first-line interventions while VELOUR and RAISE were second-line therapies. From a total of 5,633 patients initially identified as intention to treat, data from 5,594 were available for further analysis as per-protocol treated population. Their baseline characteristics are shown in Table 30. The majority (58.8%) were men; the median age was 60 years.

6.3.1 PPI use

A total of 902 patients were on a PPI at the start of the trial chemotherapy intervention. The proportion of PPI users ranged between 11.3% and 25.8% across the trial cohorts (Table 29). PPI users had a similar median age and were more likely to be White or Caucasian when compared to non-PPI users. Omeprazole was the most frequently used PPI (39%).

6.3.2 Pooled analysis for association of PPI use and survival outcomes

Pooled analysis of the crude association between PPI use and survival outcomes indicated that PPI use was associated with statistically significant worse OS (random-effects pooled HR 1.23, 95% CI 1.07 to 1.43, Figure 17) and PFS (HR 1.22, 95% CI 1.07 to 1.38, Figure 18).

The association between PPI use and survival outcomes was then adjusted for age, sex, race, ECOG PS, and baseline CEA and LDH levels, where available, in 5,262 participants with complete data for the adjustment variables. Figure 19 shows the pooled estimates of adjusted HR for OS between PPI users and non-users during fluoropyrimidine-based chemotherapy. There was a statistically significant association between PPI use and worse OS outcomes with fluoropyrimidine-based chemotherapy (random-effects adjusted HR 1.20, 95% CI 1.03-1.40, P = 0.02) with substantial heterogeneity in effect size between studies (I^2 = 69%). A sensitivity analysis using a fixed effect model estimated a similar effect size (pooled HR 1.20, 95% CI 1.10-1.30). On pooled analysis, a significant effect of PPI use on PFS with fluoropyrimidine-based chemotherapy (overall pooled HR 1.20, 95% CI 1.05-1.37, P = 0.009 and I^2 = 65%) was observed (Figure 20).

Table 28: PPI and fluoropyrimidines – included trials

Trial	Population	Treatment intervention	Cohort size (N)	PPI use N (%)	Comments
First line trials					
AVF2107g	Advanced CRC, treatment naïve	IFL+ Placebo IFL + Bevacizumab	411 402	69 (16.7%) 89 (22.1%)	All arms included
N016966	Advanced CRC, treatment naïve	FOLFOX-4 ± Placebo CAPOX ± Placebo FOLFOX-4 + Bevacizumab CAPOX + Bevacizumab	668 667 350 350	123 (18.4%) 99 (14.8%) 47 (13.4%) 53 (15.1%)	All arms included
Carrato et al	Advanced CRC, treatment naïve	FOLFIRI	386	43 (11.1%)	Control arm only
HORIZON III	Advanced CRC, treatment naïve	FOLFOX-4 + Bevacizumab	713	88 (12.3%)	Control arm only
Second line trials					
VELOUR	Prior oxaliplatin chemotherapy	FOLFIRI	614	113 (18.4%)	Control arm only
RAISE	Prior oxaliplatin chemotherapy	FOLFIRI + Placebo FOLFIRI + Ramucirumab	536 536	136 (25.3%) 122 (22.7%)	All arms included

Table 29: PPI use

PPI Name	AVF2107g		N016966		Carrato et al		VELOUR		RAISE		HORIZON III	
	PPI Use (N = 159)		PPI Use (N = 327)		PPI Use (N = 43)		PPI Use (N = 113)		PPI Use (N = 258)		PPI Use (N = 88)	
	N	%	N	%	N	%	N	%	N	%	N	%
Esomeprazole	29	18.2	34	10.3	6	13.9	13	11.5	18	6.9	10	11.4
Lansoprazole	60	37.7	48	14.6	3	6.9	17	15.0	47	18.2	15	17.0
Omeprazole	46	28.9	115	35.1	22	51.1	59	52.2	119	46.1	36	40.9
Pantoprazole	31	19.4	38	11.6	12	2.9	21	18.5	56	21.7	22	24.9
Rabeprazole	10	6.2	8	2.4	0	0	3	2.6	18	6.9	5	5.7

Table 30: Demographics and outcomes

Variable	AVF2107		N016966		Carrato et al		VELOUR		RAISE		HORIZON III	
	PPI Use		PPI Use		PPI Use		PPI Use		PPI Use		PPI Use	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Cohort size	159	654	327	1,792	43	336	111	494	258	814	88	602
Age (years)	59(52-65)	60(52-69)	62(54-69)	61(53-67)	59(52-66)	58(51-65)	61(55-70)	61(54-68)	63(54-69)	61(53-67)	NA	NA
Sex												
Female	61(38%)	267(41%)	98(40%)	729(41%)	18(42%)	160(48%)	54(48%)	206(41%)	117(45%	340(42%)	41(47%)	249(41%)
Male	98(62%)	387(59%)	145(60%)	1062(59%)	25(58%)	176(52%)	59(52%)	291(59%)	141(55%)	474(58%)	47 (53%)	353(53%)
Race												
Caucasian	125(79%)	520(80%)	225(91%)	1485(83%)	29(67%)	216(64%)	104(92%)	416(84%)	206(80%)	609(75%)	85(97%)	559(93%)
Asian					10(23%)	103(31%)			42(16%)	172(21%)	2(2%)	38(6%)
Black	18(11%)	77(12%)	18(2%)	32(2%)	3(7%)	5(1%)	8(7%)	19(4%)	7(3%)	23(3%)		
Other/Missing	16(10%)*	57(9%)*	0(0%)*	275(15%)*	1(2%)	14(4%)	1(1%)	62(12%)*	3(1%)	10(1%)	1(1%)	5(1%)
ECOG PS												
0	84(54%)	376(57%)	122(50%)	1023(57%)	21(49%)	126(38%)	57(51%)	291(59%)	105(41%)	433(53%)	48(55%)	343(57%)
1 or 1+	75(47%)	277(42%)	121(50%)	762(43%)	13(30%)	162(48%)	54(49%)	203(41%)	152(59%)	378(46%)	40(45%)	257(43%)
Missing	0(0%)	1(1%)	0(0%)	7(0.2%)	0(0%)	2(1%)	0(0%)	0(0%)	19(0.2%)	3(0.2%)	0(0%)	0(0%)
CEA levels												
Median (IQR)	NA	NA	37 (8-264)	31 (7-162)	31 (7-155)	59 (15-225)	NA	NA	46 (10-242)	30 (8-147)	NA	NA
LDH levels												
Median (IQR)	225 (170-424)	219 (170-368)	342 (227-624)	346 (208-536)	324 (216-524)	412 (242-671)	392 (250 - 693)	307 (208 - 488)	High LDH 122(47%)	High LDH 318(39%)	High LDH 17(19%)	High LDH 21(3%)
Response												
CR	8(5%)	29(4%)	0(0%)	34(2%)	1(2%)	6(2%)	0(0%)	2 (<1%)	0(0%)	2(0.2%)	0(0%)	11(2%)
PR	78(49%)	281(43%)	102(42%)	828(46%)	10(23%)	128(38%)	8(7%)	48 (10%)	24(9%)	113(14%)	30(34%)	292(49%)
SD	58(36%)	227(35%)	74(30%)	578(32%)	20(47%)	159(47%)	62(55%)	320 (64%)	151(59%)	476(58%)	16(18%)	61(10%)
PD	12(8%)	49(7%)	32(13%)	147(8%)	6(14%)	29(9%)	28(25%)	99 (20%)	66(26%)	155(91%)	40(45%)	219(36%)
Missing/NE	3(2%)	68(10%)	35(14%)	205(11%)	6(14%)	14(4%)	15(14%)	28(6%)	17(7%)	68(8%)	2(2%)	19(3%)
Median PFS (months) [95%CI]	9.7 [8.3,10.8]	8.0 [7.7, 8,3]	7.6 [6.9,8.3]	8.4 [8.2,8.7]	7.4 [6.5,8.7]	9.3 [8.6,10.6]	3.1 [2.8,4.5]	5.5 [5.1,6.2]	4.2 [3.7,5.8]	5.5 [5.4,5.7]	8.4 [7.6,10.3]	10.9 [10.0,11.0]
Median OS (months) [95%CI]	19.8 [17.2,25.3]	16.9 [15.8,18.1]	18.4 [16.6,19.8]	19.9 [19.1,20.8]	14.7 [10.1,NA]	21 [19.2,NA]	9.3 [7.7,11.8]	12.5 [11.8,13.9]	10.6 [9.6,11.6]	13.4 [12.6,14.6]	18.4 [13.1,NA]	21.3 [19.6,25.7]

*Includes Asians; Age (median and IQR); NA – not available; NE – not evaluable.

Figure 17: Pooled OS using unadjusted HRs

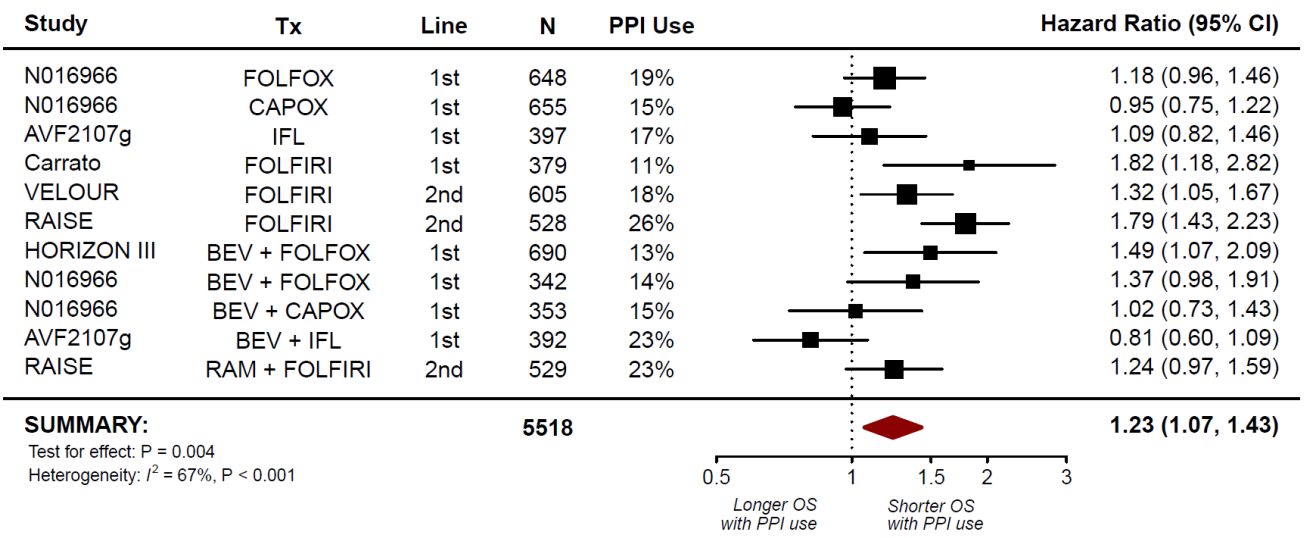


Figure 18: Pooled PFS using unadjusted HRs

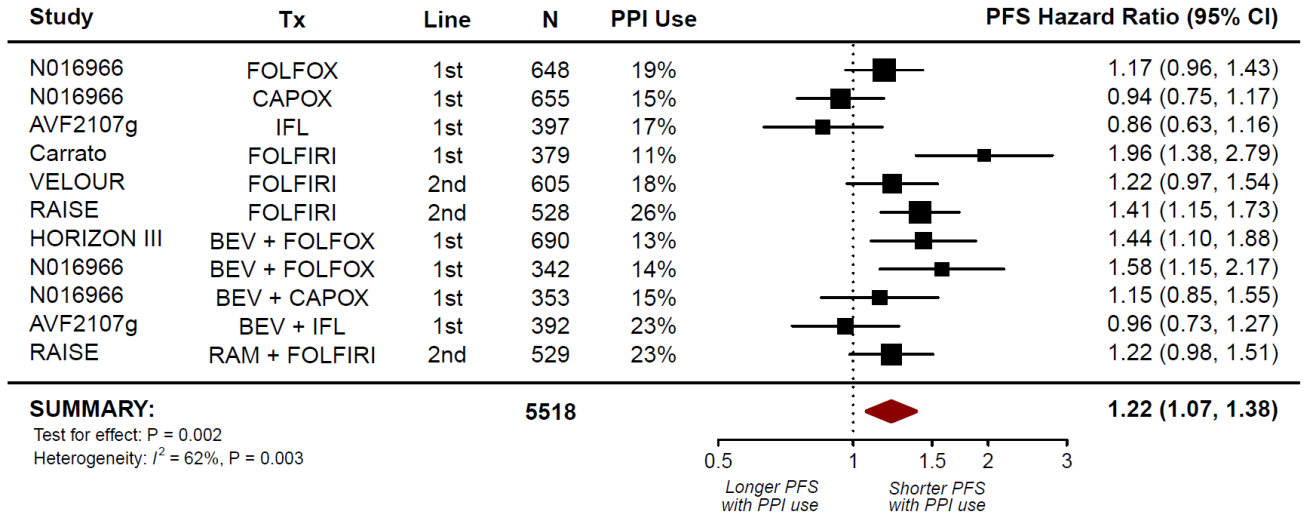


Figure 19: Pooled adjusted analysis of the association between PPI use and OS

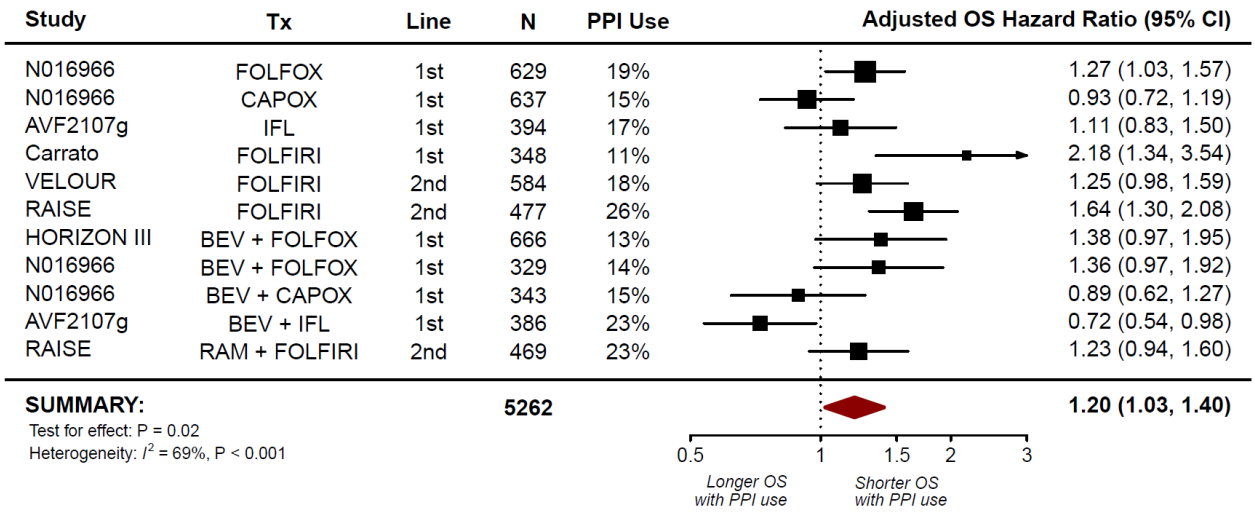
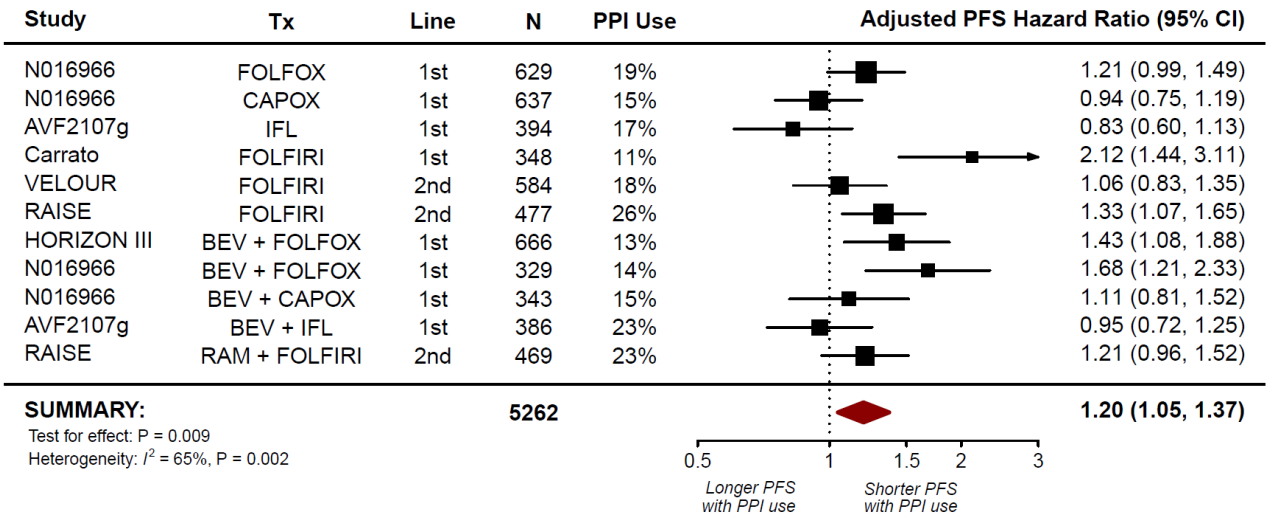


Figure 20: Pooled adjusted analysis of the association between concomitant PPI use and PFS



6.3.3 Sub-group analyses

Sub-group analyses were performed to assess whether the adjusted association between concomitant PPI use and survival outcomes differed across key subgroups. Generally, estimates of association between PPI use and survival outcomes were relatively consistent across treatment subgroups for both OS and PFS. There was little evidence to support heterogeneity of effect size on the basis of the chemotherapy agent (irinotecan vs oxaliplatin) combined with the fluoropyrimidine (Figure 21), the addition of a VEGFi to chemotherapy (Figure 22), or the line of therapy (Figure 23).

However, the comparison of subgroups treated with capecitabine vs intravenous 5-FU indicated a trend towards statistically significant heterogeneity of PPI effect size (P[heterogeneity] = 0.08, Figure 24). This exploratory analysis highlights that for patients treated with capecitabine the concomitant use of PPI may not be associated with inferior OS and PFS and that further study is warranted with respect to effects of PPI use in these subgroups.

6.3.4 PPI use and response rates

Complete response to the treatment intervention was uncommon (<5%). Wide variations across trials were observed in partial response, stable disease and progressive disease with ranges of 7 to 49%, 29 to 64% and 7 to 25%, respectively (Table 30). While the ORs for objective response rates with concomitant PPI use were

low in most cohorts, there was no statistically significant difference in response rates between PPI users and non-PPI users except in the HORIZON III trial (OR 0.51, 95%CI 0.32-0.82) (Table 31). No significant effect of concomitant use of PPI on adjusted overall response rates was observed (OR 0.83, 95% CI 0.66-1.05, P = 0.05 and I^2 = 45%) (Figure 25).

Figure 21: Subgroup analysis for OS using adjusted HRs - concomitant chemotherapy

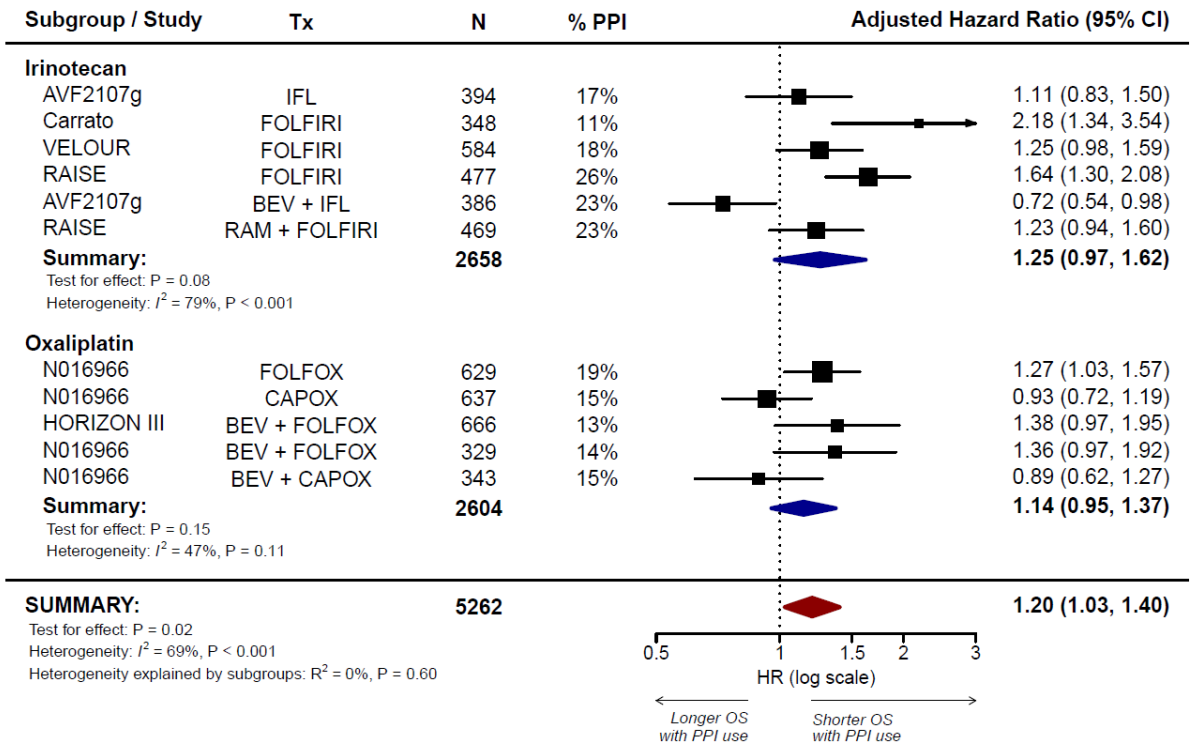


Figure 22: Subgroup analysis for OS using adjusted HRs - concomitant VEGFi use

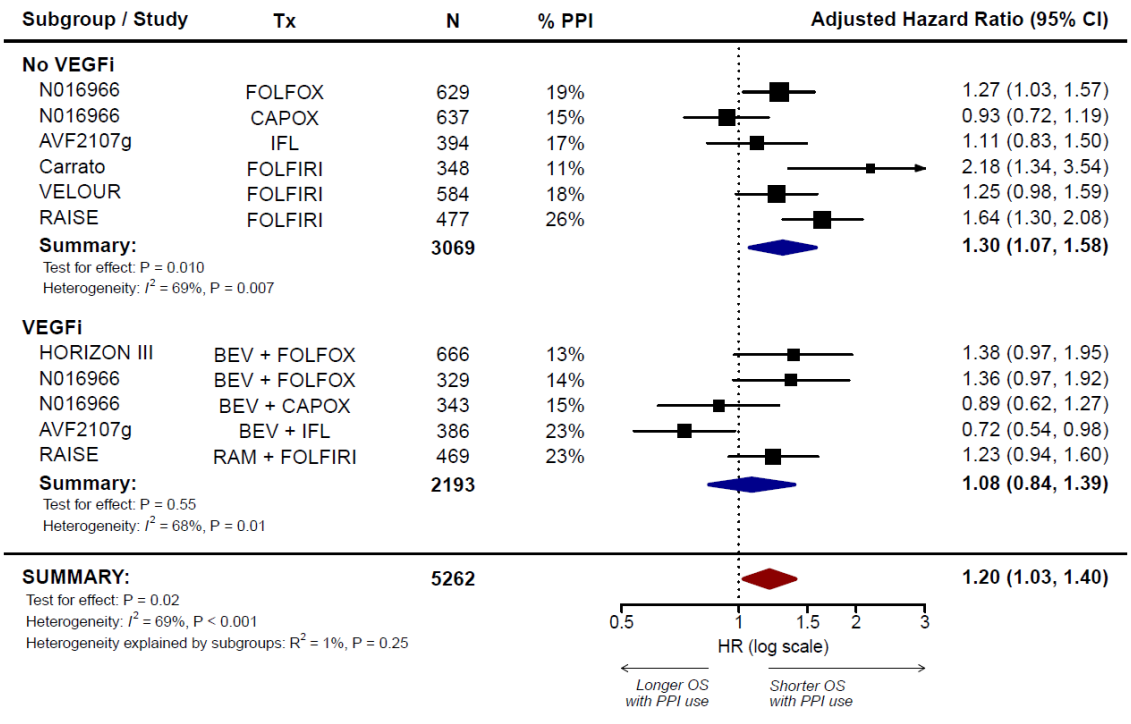


Figure 23: Subgroup analysis for OS using adjusted HRs - by line of use

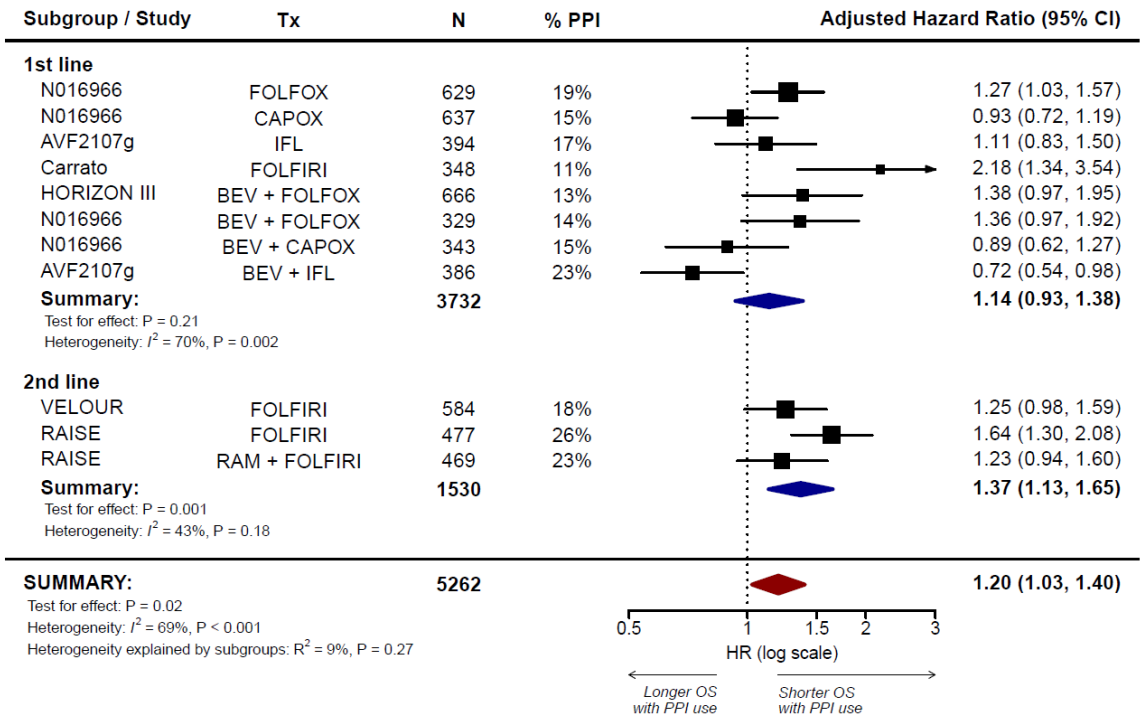


Figure 24: Subgroup analysis for OS using adjusted HRs - by capecitabine vs 5FU

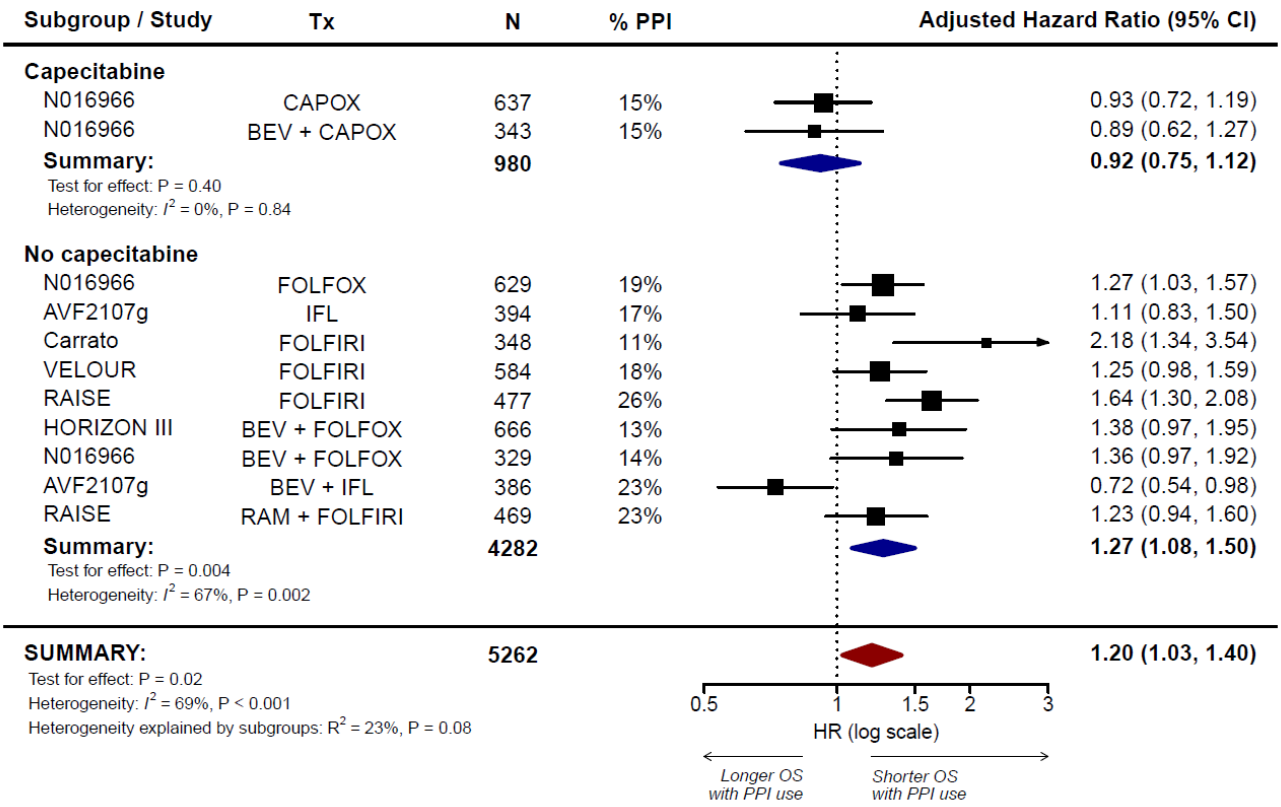
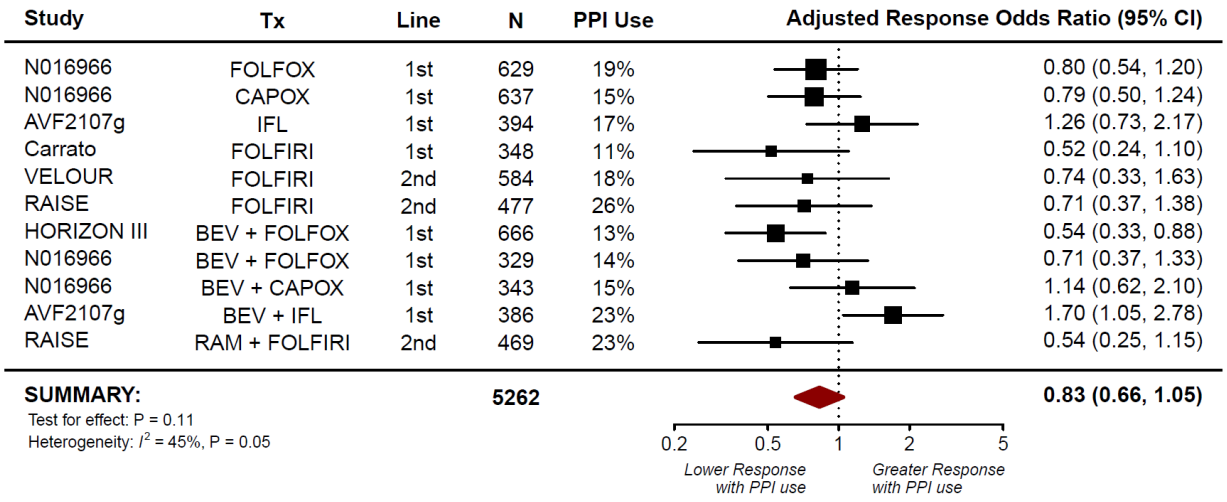


Table 31: PPI use and outcomes

Trial name	Unadjusted OS		Unadjusted PFS		Unadjusted Objective RR	
	HR	95% CI	HR	95% CI	OR	95% CI
AVG2107g						
IFL	1.09	0.82-1.46	0.86	0.63– 1.16	1.26	0.74-2.15
IFL + Bevacizumab	0.81	0.60-1.09	0.96	0.73 -1.27	1.59	0.98-2.55
N016966						
CAPOX	0.95	0.75-1.22	0.94	0.75-1.17	0.80	0.52-1.23
FOLFOX	1.18	0.96-1.46	1.17	0.96 -1.43	0.79	0.54 -1.18
CAPOX + Bevacizumab	1.02	0.73-1.43	1.15	0.85-1.55	1.01	0.56-1.81
FOLFOX + Bevacizumab	1.37	0.98-1.91	1.58	1.15-2.17	0.73	0.39-1.35
Carrato et al - FOLFIRI	1.82	1.18-2.82	1.96	1.38 - 2.78	0.52	0.25-1.06
VELOUR - FOLFIRI	1.32	1.05-1.67	1.22	0.97 - 1.54	0.66	0.30-1.45
RAISE						
FOLFIRI	1.79	1.43-2.23	1.41	1.15-1.72	0.66	0.35-1.25
FOLFIRI + Ramucirumab	1.24	0.97-1.59	1.22	0.98-1.51	0.56	0.28-1.11
HORIZON III - FOLFOX + Bevacizumab	1.49	1.07-2.09	1.44	1.10 – 1.88	0.51	0.32-0.82

Figure 25: Pooled response rates and concomitant PPI use



6.4 Discussion

The results from this pooled analysis of six clinical trials in patients with advanced CRC indicate that concomitant PPI use is associated with significantly worse survival outcomes with fluoropyrimidine-based combination chemotherapy.

There remains a substantial controversy on the potential negative effect of the concomitant use of PPIs on cancer outcomes in patients undergoing fluoropyrimidine (especially, capecitabine) based chemotherapy (Altundag 2017b, 2017a; Chu et al. 2017; Chu and Sawyer 2018; Graham et al. 2016; Rhinehart et al. 2018; Sun et al. 2016; Wong et al. 2019). A retrospective series of 671 CRC patients (474 on concomitant PPI) reported improved survival in the FOLFOX-treated cohort but not in the CAPOX-treated cohort (Wang, Liu, et al. 2017). On the other hand, Chu et al reported worse outcomes when PPIs were used concomitantly with CAPOX using data from a prospective trial in patients with gastroesophageal cancers (Chu et al. 2017). Worse outcomes when PPIs were given with capecitabine were additionally reported by other authors (Rhinehart et al. 2018; Sun et al. 2016). In contrast, sub-group analyses in the current study did not find a significant negative association with survival (crude or adjusted) of concomitant PPI use across 980 patients treated with CAPOX (with and without bevacizumab). However, there was a negative association with survival in the remaining patients treated with a range of 5FU based therapies.

The current study did not include a cohort with monotherapy interventions with fluoropyrimidines which prevents drawing any conclusions on the effect of PPIs on monotherapy. Moreover, the conflicting results between studies regarding an interaction between PPI use and add-on chemotherapy drugs indicate that the interaction is complex and may be context specific. Prospective studies with complete data on PPI use, including duration of PPI use and treatment adherence will help to better understand the association between PPI use of treatment outcomes. Similarly, the association between concomitant PPI use on the efficacy of other CRC drugs such as anti-epidermal growth factor receptor inhibitors and trifluridine/tipiracil warrants further evaluation.

Previous studies have identified that PPIs may have direct anti-cancer effects and also improve chemosensitivity of cancer cells by increasing extracellular pH (Ikemura, Hiramatsu, and Okuda 2017; Lugini et al. 2016; Wang, Liu, et al. 2017). However, the concentration required to induce CRC cell death may not be reached in vivo (Duncan et al. 2000). On the contrary, as we have observed in this study, concomitant PPI may be associated with negative effects on survival across the spectrum of combination chemotherapy with 5FU. Although the lack of association observed with oral capecitabine (CAPOX or CAPOX plus bevacizumab)

is intriguing, this association was based on data from a single study only (N016966) and the test for heterogeneity did not quite reach statistical significance.

The mechanistic basis for the negative effect of concomitant PPI use is unclear. We speculate that PPIs may inhibit uptake transporters in tumour cells as one possible mechanism. It is well established that the chemotherapy drugs evaluated in this study are substrates for various uptake transporters, some of which are expressed in CRC cells. PPIs, especially omeprazole, have been reported to inhibit several transporters at therapeutic concentrations. For example, PPIs inhibit oxaliplatin uptake transporters such as organic cation transporters (OCT 1 and 3) (Nies et al. 2011), while irinotecan and its active form, SN-38, inhibit transporters such as organic anion-transporting polypeptides (OATP1A/1B) (Han et al. 2015). On the other hand, PPIs may increase the expression of human equilibrative nucleoside transporter 1 (hENT1), potentially associated with poor response to fluoropyrimidines (Redzic, Hasan, and Al-Sarraf 2009; Phua et al. 2013). It is also unclear if there is a differential effect of PPIs on the intracellular uptake of oral vs intravenous fluoropyrimidines. In addition, the pH dependent uptake of chemotherapy drugs is well recognized (Kobayashi et al. 1999). It is possible that PPIs reduce intra-tumoral concentration of cytotoxic drugs through the inhibition of uptake transporters as well as altered pH in the tumour microenvironment. This hypothesis requires further testing in pre-clinical studies.

While chronic PPI use is associated with increased all-cause mortality in the general population (Xie et al. 2019), this study shows that both PFS and OS are negatively affected by concomitant PPI use with chemotherapy in CRC patients. The negative association between PFS and PPI use during FOLFIRI chemotherapy especially in the second-line setting, but not with IFL, indicates a possible interaction between irinotecan scheduling and anti-tumour response. A previous drug-drug interaction trial with short-term omeprazole and single agent irinotecan did not show any significant changes in PK parameters and toxicities of irinotecan (van der Bol et al. 2011). Hence, it is likely that other mechanisms such as alteration in gut microbiome, changes in tumour microenvironment and immune milieu by the PPIs, and their subsequent effects on irinotecan PK and pharmacodynamics, may play a role (Bellone et al. 2013; Imhann et al. 2016; Pilon-Thomas et al. 2016). PPI related microbiome changes can alter cancer outcomes either through

immunosuppression, through increased drug metabolism, altered autophagy or immunosuppression thereby increasing resistance to 5FU and oxaliplatin (Biswas et al. 2012; Macke et al. 2020; Wong and Yu 2019). Further *in vitro* and *in vivo* studies will be required to confirm these findings.

This study assessed the effect of PPI use in patients undergoing fluoropyrimidine-based chemotherapy in CRC. It is unclear whether the negative effects of PPI use occur in other cancers and/or with other drugs such as immunotherapies and other targeted agents such as anti-epidermal growth factor receptors. Moreover, the use of PPI may reflect the presence of other coexisting confounders such as symptomatic advanced cancer with liver metastases that may increase the need for PPI. Future studies should consider addressing these gaps by exploring PPI use in early stage CRC, non-CRC cancer types and other treatment settings.

The current study has significant strengths including a relatively large cohort size, comprehensive prospective clinical trial data from individual patients, analysis using individual participant data and strong generalisability resulting from pooling across multiple studies and treatments. However, there are also several limitations including lack of analysis of the effect of comorbidities on overall survival, the impact of chemotherapy dose modifications, lack of information on the duration of PPI use prior to the start of the trial, dose/adherence with PPI during the trial, initiation of PPI after chemotherapy was started, the influence of other acid suppressing drugs, access to data from other trials with anti-epidermal growth factor receptor inhibitors, and technical issues with data merging from various sharing platforms. None of the included trials had no chemotherapy or without fluoropyrimidine arms for evaluation, thus precluding direct comparison as well exploring prognostic relationship of concomitant PPI usage. Further studies including an analysis of PPI use and survival outcomes in patients with mCRC that are not receiving fluoropyrimidine based chemotherapy are warranted. Such relationships could also be explored in other cancer types.

While we adjusted for six clinically significant covariates for the calculation of pooled estimates, other contemporary prognostic factors such as right vs left sided primary location of the primary, molecular biomarkers such as RAS mutations and microsatellite instability were not uniformly available for inclusion. Moreover, we did not evaluate the association between PPI use and adverse events during fluoropyrimidine-based chemotherapy or the actual cause of death to assess competing risk-based outcomes. While future

studies should consider these issues accessing data from real world use of PPI during chemotherapy treatment may also provide further insights into this association.

6.5 Conclusion

In the current study with data from six clinical trials including more than 5,000 advanced CRC patients treated with fluoropyrimidine-based combination chemotherapy, the concomitant use of PPI was associated with worse OS and PFS. This association was significant after adjusting for age, sex, race, ECOG PS, baseline CEA levels and baseline LDH levels. The association effect size of PPI use and survival was similar across treatment subgroups, except for capecitabine-based therapies which requires further evaluation. Pending the identification of the mechanisms involved in this interaction and further confirmation in future studies, clinicians should cautiously consider the concomitant use of PPIs in advanced CRC patients treated with fluoropyrimidine-based combination chemotherapy.

The results reported in this chapter indicates that PPI can negatively influence the outcomes of patients undergoing chemotherapy for advanced CRC. In summary, research including the results from this chapter has demonstrated that PPIs are negative indicator of benefit from most systemic cancer therapies including traditional chemotherapy, ICI and targeted therapies which likely suggests the prognostic role of concomitant use of PPIs during cancer treatment.

7. CHAPTER SEVEN: PLASMA CONCENTRATION OF VEMURAFENIB AND SURVIVAL OUTCOMES

This chapter has been derived and adapted with permission from the following publication:

Kichenadasse G, Hughes JH, Miners JO, Mangoni AA, Rowland A, Hopkins AM, Sorich MJ. Relationship between vemurafenib plasma concentrations and survival outcomes in patients with advanced melanoma. *Cancer Chemother Pharmacol.* 2020 Mar; 85(3):615-620.

The accepted manuscript has been reproduced in Appendix 4.

In the preceding chapters, some examples of patient characteristics (baseline BMI, treatment emergent factors such as irAEs), and external factors such as use of concomitant medications (use of RASi and PPIs) that may contribute to variability in outcomes to systemic cancer therapies were evaluated. The relationship between the plasma concentration of a systemic cancer therapy and survival is the focus of the current chapter. Prior chapters had ICI or traditional chemotherapy as the drugs being investigated. In this chapter, the drug of interest is vemurafenib, a molecularly targeted therapy.

7.1 Introduction

Several kinase inhibitors are available for the management of both haematological and solid cancers. While a fixed dosage regimen is routinely used, these drugs are characterized by highly variable PK and hence exposure. However, relationships between exposure, response and toxicity are increasingly described indicating the potential for plasma concentration guided dosing strategies (Verheijen et al. 2017). Vemurafenib, a serine-threonine kinase inhibitor, is approved as monotherapy for the treatment of both BRAFV600 mutated advanced melanoma and BRAFV600 mutated Erdheim-Chester disease by the US Food and Drug Administration. It is also approved in combination with cobimetinib, a mitogen activated protein kinase (MEK) inhibitor, for the treatment of BRAFV600 mutated advanced melanoma. The current recommended starting dose of vemurafenib is 960 mg twice a day given orally, with dose modifications allowed for significant adverse events.

The clinical PK of vemurafenib is well established, with oral absorption showing high inter-patient variability (coefficient of variation 101%) (Zhang, Heinzmann, and Grippo 2017). Doses of 960 mg twice daily are associated with a median time to maximum drug concentration of 4-5 hours and mean (± SD) maximum plasma steady state concentrations (C_{max}) of 62 ± 23 mg/L. The elimination half-life of vemurafenib varies over time, changing from 25 hours after a single dose to 57 hours over multiple doses, with steady state being achieved by 15-22 days.(FDA 2017) Once at steady state, vemurafenib exhibits linear PK with the area under the concentration curve (AUC) over 8 hours being 392 ± 126 mg*h/L and the apparent oral clearance 31 L/day (coefficient of variation 32%) (Zhang, Heinzmann, and Grippo 2017). While the bioavailability of vemurafenib is unknown, food has been shown to have a significant effect on vemurafenib plasma concentrations with a 5-fold increase in AUC and a 2.5-fold increase in C_{max}.

Due to the high inter-patient and intra-patient variability reported for plasma vemurafenib concentrations, previous studies have evaluated exposure-response and exposure-toxicity relationships using a single plasma concentration threshold. Kramkimel et al. analysed 159 samples from 39 patients and reported that plasma vemurafenib concentrations below 40.4 mg/L at day 15 were associated with significantly shorter PFS and a lower incidence of grade ≥2 rash (Kramkimel et al. 2016). Goldwirt et al. (148 samples from 48 patients) identified a threshold of 42 mg/L during the first year of therapy that differentiated responders and non-responders (Goldwirt et al. 2016). Similar proportions of responders and non-responders were reported by another group using a threshold of 42 mg/L (data from 23 patients). Neither study identified an exposure-toxicity relationship (Funck-Brentano et al. 2015; Funck-Brentano et al. 2016). Moreover, Funck-Brentano et al suggested that a higher threshold may be required if lack of early response is noted (Funck-Brentano et al. 2016). The current study aimed to validate the proposed plasma vemurafenib steady state trough concentration ($C_{ss, min}$) threshold as a predictor of PFS and OS in patients with advanced melanoma.

7.2 Methods

Individual patient data from the previously published BRIM-3 (NCT01006980) and coBRIM (NCT01689519) clinical trials were accessed through Roche's data sharing policy. BRIM-3 was a monotherapy trial that compared vemurafenib and dacarbazine while coBRIM was a combination therapy trial of

vemurafenib/cobimetinib vs vemurafenib monotherapy for the treatment of advanced BRAFV600 mutated melanoma.

The primary outcome assessed for the current study was PFS while the secondary outcomes were OS and BOR. Patients who fulfilled all the following criteria were included in the primary analysis; had at least one $C_{ss,min}$ of vemurafenib available by day 23 of cycle 1 (D23); had no dose changes for at least 14 days prior to the sample collection; and had not progressed or died before D23. Vemurafenib concentrations were considered at steady state after 14 days at a consistent dose. A day 23 landmark was utilised (as opposed to day 15) to account for different sampling times between clinical trials. Sensitivity analysis was also performed with the addition of those patients who had no dose changes for a minimum of 7 days prior to sample collection.

Associations between plasma vemurafenib $C_{ss,min}$ and PFS/OS were modelled using Cox proportional hazards regression and reported as HR with 95% CI. Statistical tests were two-sided and a P value less than 0.05 was considered statistically significant. Clinically relevant confounders, including, age, gender, ECOG PS, stage of melanoma, BRAF V600 mutation type, LDH concentration and sites of metastases were accounted for in adjusted analyses. BOR included patients who achieved a complete or partial response using RECIST 1.1.

Potential non-linear associations were evaluated using restricted cubic splines with 3-5 knots and subsequent visual checks; an optimal $C_{ss,min}$ threshold was determined via assessment of discriminative performance (concordance statistic – C-statistic), model fit (Akaike information criterion (AIC)) and consistency of the PFS/ OS association. Various $C_{ss,min}$ thresholds were evaluated for association with survival outcomes through statistical significance and C-statistics.

Survival curves were estimated using the KM method. All analyses were conducted in R (version 3.4.3) using the survival package (Team 2017). Ethics approval was waived by the Southern Adelaide Clinical Health Research Ethics Committee.

7.3 Results

A total of 830 patients (583 on vemurafenib monotherapy and 247 on the vemurafenib/cobimetinib combination) from the two clinical trials were available for selection. After exclusion of 428 patients (62 for lack of plasma concentration data, 352 for dose adjustments 15 days prior to the cut-off, and 14 for disease progression, death or loss of follow-up prior to day 23), 402 were available for further analysis. A summary of patient characteristics is described in the Table 32. The median follow-up was 25.3 months, median PFS was 7.2 months and the median OS was 14.7 months. The median plasma vemurafenib $C_{ss,min}$ was 54.4 mg/L (interquartile range 42.5-69.7 mg/L).

The previously proposed plasma vemurafenib $C_{ss,min}$ threshold of 42 mg/L failed to demonstrate a significant association with PFS (HR 0.81, 95% CI 0.71-1.06; P = 0.12) or OS (HR 0.75, 95% CI 0.57-1.01, P = 0.054) at the D23 landmark. A significant linear relationship between plasma vemurafenib $C_{ss,min}$ and OS (HR 0.992, 95% CI 0.987-0.998, P = 0.01) was observed, while there was no significant association with PFS (HR 0.997, 95% CI 0.991-1.002, P = 0.22). Figure 26 describes the continuous association between plasma vemurafenib $C_{ss,min}$ and PFS / OS; HR is represented by a restricted cubic spline with three knots. To facilitate clinical utility cut-points were explored, with a $C_{ss,min}$ threshold of 50 mg/L identified as a consistent predictor for PFS and OS, with optimised performance based upon the c-statistic and AIC.

Table 32: Patient characteristics

	Total **N = 402**	**BRIM3** **N = 137**	**coBRIM** **N = 265**	**P-value**
Study treatment Vemurafenib Vemurafenib + Cobimetinib	 280 (70%) 122 (30%)	 137 (100%) 0 (0%)	 143 (55%) 122 (46%)	
Age (years)	55 (45-64)	56 (47-65)	55 (45-63)	0.34
Sex Female Male	 150 (37%) 252 (63%)	 46 (34%) 91 (66%)	 104 (39%) 161 (61%)	0.28
Race White Others Missing	 384 (96%) 3 (0.7%) 15 (3.3%)	 136 (99%) 1 (1%) 0 (0%)	 248 (93.5%) 2 (0.7%) 15 (5.8%)	1.00
ECOG PS 0 1 Missing	 271 (67.7%) 130 (32%) 1 (0.3%)	 87 (64%) 50 (36%) 0 (0%)	 184 (69%) 80 (30%) 1 (%)	0.22

Stage				0.25
Unresectable IIIc	26 (6%)	9 (7%)	17 (6%)	
M1a	54 (13%)	12 (9%)	42 (16%)	
M1b	66 (16%)	23 (17%)	43 (16%)	
M1c	256 (64%)	93 (68%)	163 (62%)	
BRAF V600 mutation				0.60
V600E	309 (77%)	121 (88%)	188 (71%)	
V600K	39 (10%)	13 (9%)	26 (10%)	
Missing	54 (13%)	3 (2%)	51 (19%)	
LDH at baseline				0.75
Elevated	173 (43%)	58 (42%)	115 (43%)	
Normal	223 (55%)	79 (58%)	144 (54%)	
Missing	6 (1%)	0 (0%)	6 (2%)	
Liver metastases at baseline				1.0
Yes	133 (33%)	45 (33%)	88 (33%)	
No	268 (67%)	91 (66%)	177 (67%)	
Missing	1 (0.3%)	1 (1%)	0 (0%)	
Lung metastases at baseline				0.09
Yes	224 (56%)	84 (61%)	140 (53%)	
No	177 (44%)	52 (38%)	125 (47%)	
Missing	1 (0.3%)	1 (1%)	0 (0%)	
Vemurafenib ($C_{min, ss}$) day 23 (mg/L)	54 (43-70)	57 (48-73)	52 (40-69)	0.008*

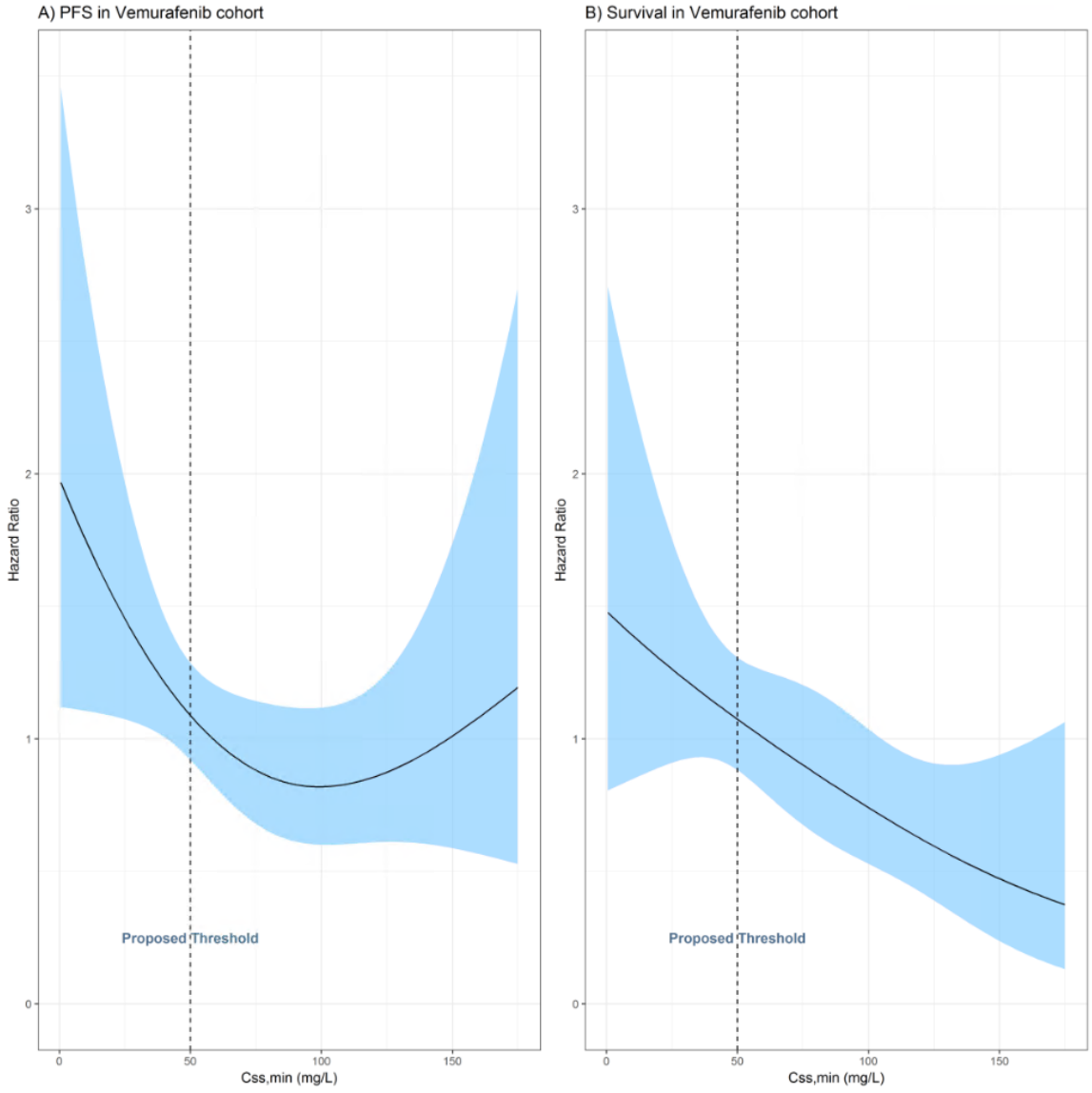
Data are median (interquartile range) or number of patients (%)

Moreover, the association between D23 plasma vemurafenib $C_{ss,min} \geq 50$ mg/L and PFS (P = 0.05) or OS (P = 0.008) remained statistically significant after adjusting for PS, LDH levels, sex, stage, and sites of metastatic disease (Table 33 and Table 34). A similar multivariate analysis was performed with the plasma vemurafenib $C_{ss,min}$ threshold of 42 mg/L. While there was a trend towards statistical significance for OS (HR 0.71, 95% CI 0.5-0.99, P = 0.046), the c-statistic was 0.666 and AIC was 1865 in contrast to the 50 mg/L threshold which had a higher c-statistic (Table 35). Sensitivity analysis performed by including an additional 68 patients with no dose adjustment 7 days prior to D23 (total cohort of 470) showed similar relationships between the plasma vemurafenib $C_{ss,min}$ threshold of 50 mg/L and survival outcomes. The D23 threshold of 50 mg/L was strongly associated with both PFS (HR 0.76, 95% CI 0.60-0.96; P = 0.023) and OS (HR 0.67, 95% CI 0.52-0.88; P = 0.003) (Figure 27).

In addition, there was no significant association between plasma vemurafenib $C_{ss,min}$, either at 42 mg/L or 50 mg/L, and BOR (Odds ratio 1.17, 95% CI 0.71-1.94; P = 0.53 and 1.38, 95% CI 0.880-2.15; P = 0.16, respectively))Figure 28). Next, the effect of cobimetinib to vemurafenib and outcomes as part of sub-group analysis was

evaluated. The addition of cobimetinib did not influence the concentration-response relationship (Figure 27). However, the median day 23 vemurafenib $C_{min, ss}$ was lower in the combination cohort when compared to vemurafenib alone (52 vs 57 mg/L, P = 0.008; Table 32). The threshold of 50 mg/L was associated with OS (HR 0.7, 95%CI 0.52-0.95, P = 0.02), but not PFS or BOR (Table 36).

Figure 26: Hazard ratio curves



7.4 Discussion

This analysis of prospectively collected data from 402 patients with advanced melanoma demonstrated that a plasma vemurafenib $C_{ss,min}$ threshold of ≥ 50 mg/L was associated with improved survival outcomes.

Figure 27: Vemurafenib concentration vs PFS/OS

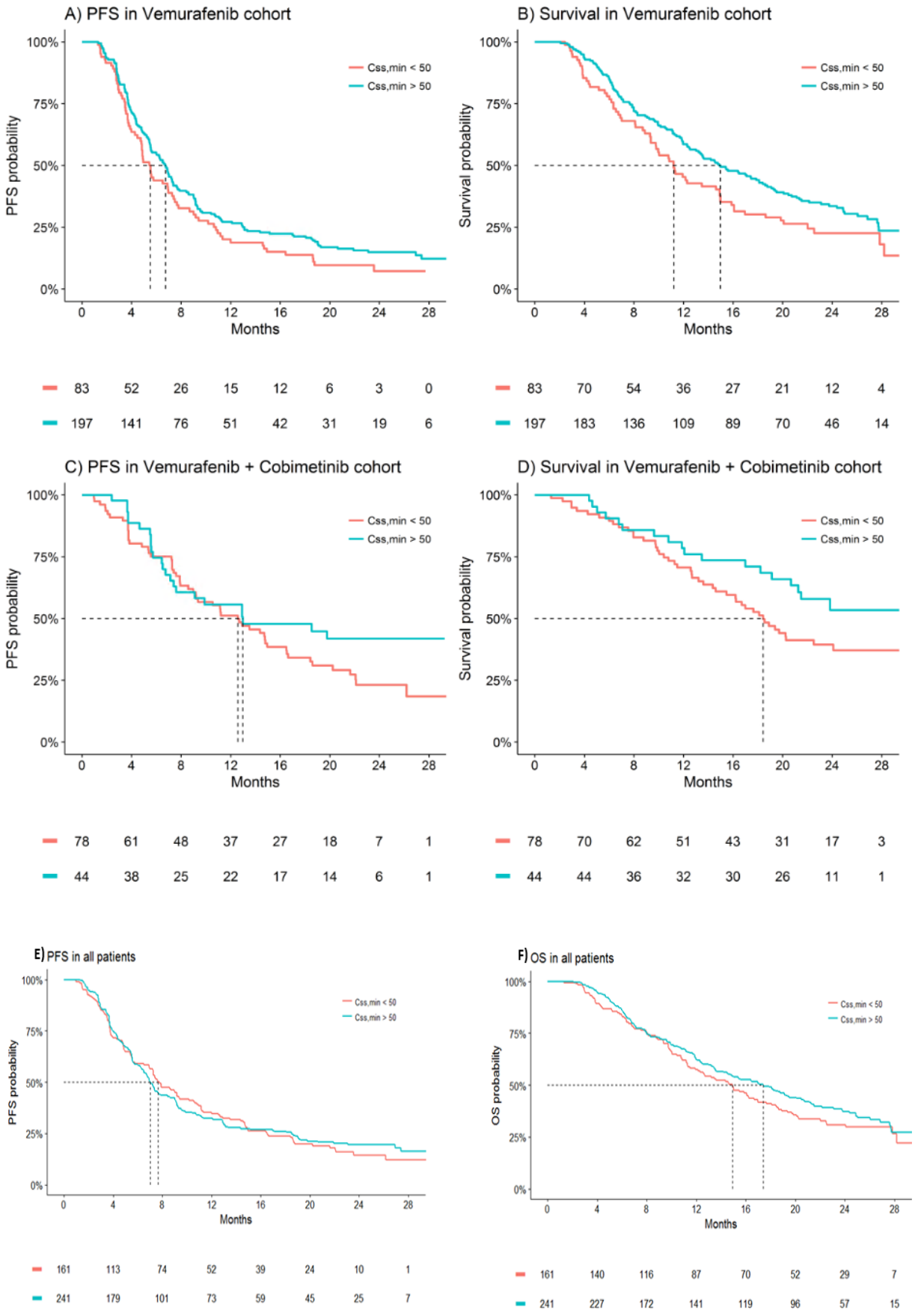


Table 33: Multivariate analysis for OS with plasma vemurafenib Css,min ≥ 50 mg/L

	HR	95% CI	P-value
Vemurafenib Css, min of 50 mg/L	0.67	0.50 – 0.91	0.008*
Age (years)	1.0	0.99 - 1.01	0.45
Female sex	0.98	0.73 – 1.32	0.89
ECOG PS	1.73	1.29 – 2.31	0.0002*
Stage			0.13
M1a	1.12	0.48 – 2.65	
M1b	1.98	0.89 – 4.36	
M1c	1.76	0.84 – 3.67	
BRAF V600K mutation	0.81	0.51 -1.28	0.36
Normal LDH at baseline	0.59	0.44 – 0.79	0.0004*
Liver metastases at baseline	1.08	0.74 – 1.37	0.96
Lung metastases at baseline	1.18	0.88 – 1.58	0.25

C-statistic = 0.668, AIC = 1862.1

Table 34: Multivariate analysis for PFS with plasma vemurafenib Css,min ≥ 50 mg/L

	HR	95% CI	P-value
Vemurafenib Css, min of 50 mg/L	0.76	0.58 - 1.0	0.05
Age (years)	1.0	0.99 - 1.01	0.72
Female sex	0.86	0.65 – 1.11	0.25
ECOG PS	1.74	1.33 – 2.26	< 0.0001*
Stage			0.59
M1a	1.08	0.56 – 2.08	
M1b	1.39	0.74 – 2.62	
M1c	1.33	0.76 – 2.34	
BRAF V600K mutation	0.88	0.59 -1.32	0.55
Normal LDH at baseline	0.63	0.49 – 0.82	0.0006*

Liver metastases at baseline	1.08	0.81 – 1.43	0.58
Lung metastases at baseline	1.10	0.85 – 1.44	0.44

C-statistic = 0.62, AIC = 2237.3

Table 35: Multivariate analysis for OS with plasma vemurafenib Css,min ≥ 42 mg/L

	HR	95% CI	P-value
Vemurafenib Css, min of 42 mg/L	0.71	0.50 – 0.99	0.046*
Age (years)	1.0	0.99 - 1.01	0.54
Female sex	0.96	0.71 – 1.28	0.77
ECOG PS	1.76	1.32 – 2.35	<0.001*
Stage			0.14
M1a	1.21	0.51 – 2.85	
M1b	2.02	0.92 – 4.46	
M1c	1.83	0.88 – 3.81	
BRAF V600K mutation	0.78	0.49 – 1.24	0.29
Normal LDH at baseline	0.57	0.43 – 0.76	<0.001*
Liver metastases at baseline	1.06	0.78 – 1.44	0.73
Lung metastases at baseline	1.16	0.87 – 1.56	0.31

C-statistic = 0.666, AIC = 1865

Clinically relevant confounding factors were systematically evaluated and adjusted in our analyses, thereby improving the validity of the association identified between plasma vemurafenib $C_{ss,min}$ and outcomes. Previous studies reported a lower concentration threshold, 40.4 mg/L or more than 42 mg/L (Funck-Brentano et al. 2015; Funck-Brentano et al. 2016; Kramkimel et al. 2016). However, these studies included fewer patients and did not address confounding variables potentially affecting survival outcomes, and the association was not explored for both PFS and OS (Funck-Brentano et al. 2015; Funck-Brentano et al. 2016; Goldwirt et al. 2016; Kramkimel et al. 2016). By contrast the present study used a large high-quality database

Figure 28: Plasma vemurafenib Css, min and response

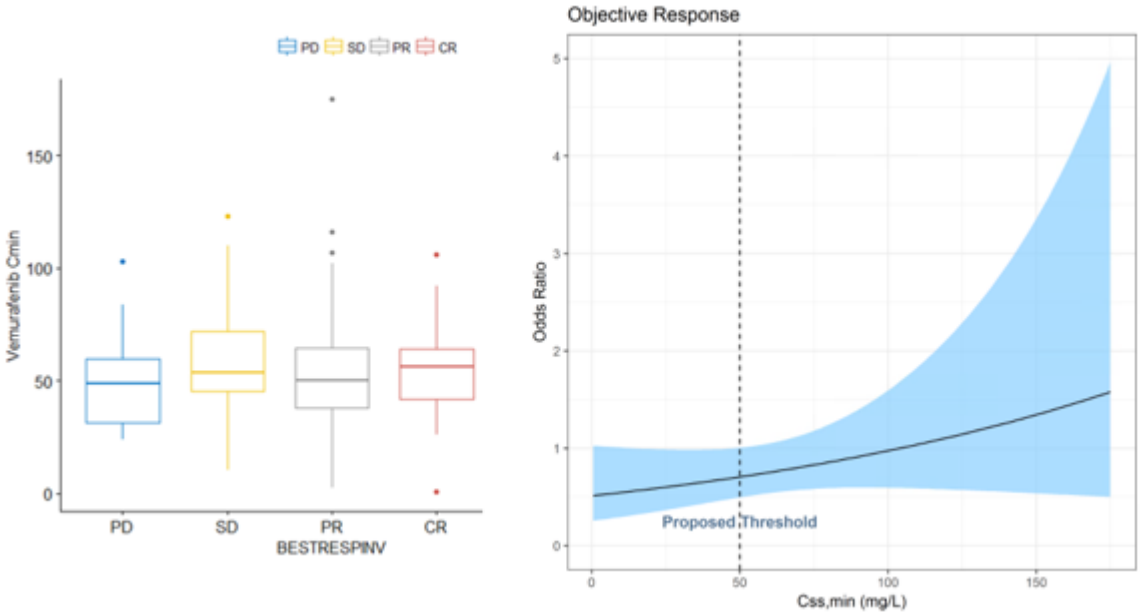


Table 36: Plasma vemurafenib Css, min and outcomes based on trials

Vemurafenib Css, min (mg/L)	**HR (95% CI)**		
	Total N = 402	**BRIM3 N = 137**	**coBRIM N = 265**
OS <50 ≥ 50	 1 0.67 (0.52-0.88) P = 0.003	 1 0.61 (0.35-1.05) P = 0.07	 1 0.7 (0.52-0.95) P = 0.02
PFS <50 ≥ 50	 1 0.76 (0.6-0.96) P = 0.023	 1 0.73(0.45-1.17) P = 0.19	 1 0.77 (0.58-1.01) P = 0.06
Best Overall Response <50 ≥ 50	 1 1.38 (0.88-2.15) P = 0.16	 1 1.17 (0.51-2.73) P = 0. 7	 1 1.46 (0.87-2.46) P = 0.15

and the vemurafenib $C_{ss,min}$ threshold (≥ 50 mg/L) was demonstrated as significantly associated with both OS and PFS. The association between vemurafenib $C_{ss,min}$ threshold (≥ 50 mg/L) and OS was seen for the combined population of two trials and for the combination therapy sub-group, while a non-significant trend was seen in the monotherapy group.

While it is common to use receiver operating curve (ROC) to define thresholds of concentrations, in our study we have used the c-statistic, which is equivalent to ROC. Both ROC and the c- statistic are used for

discrimination of a model generated to predict outcomes. While ROC is used for visualisation, the c-statistic quantifies the discriminative ability of a model. The higher the c-statistic, the better the model prediction for the outcome of interest (Steyerberg and Vergouwe 2014). This research found that a vemurafenib $C_{ss,min}$ threshold of ≥ 50 mg/L had the highest c-statistic among various cut-offs modelled to predict outcomes.

A population PK analysis for vemurafenib in a cohort of advanced melanoma patients was performed by Roche as part of the submission for regulatory review. The dataset comprised 5,515 plasma samples from 459 patients, including participants from the BRIM3 trial (Therapeutic Goods Administration 2012; Zhang, Heinzmann, and Grippo 2017). In contrast to our study where the plasma vemurafenib $C_{ss,min}$ was the PK parameter of interest, the relationship between mean $AUC_{0\text{-}8\ hours}$ on day 15 and response was explored. While an increase in tumour response with increasing exposure was noted, there was no clear exposure-response (PFS or OS) relationship at a dose of 960 mg bd. Similarly, another population PK model using 147 plasma samples from 26 patients with non-melanoma diseases with BRAFV600 mutations reported overlapping mean plasma vemurafenib concentrations across BOR categories (FDA 2017). It appears that no significant relationship between vemurafenib exposure and tumour response has been consistently described across all studies.

In this research, there was no association between the plasma vemurafenib $C_{ss,min}$ threshold of ≥ 50 mg/L and BOR. It is unclear why there was an association with survival outcomes in the absence of association with tumour response. The reason(s) behind the day 23 plasma vemurafenib concentration being associated with OS is not understood. It is unclear how achieving optimal vemurafenib therapeutic concentrations may influence subsequent post trial treatment and thereby OS. However, the improvement in PFS likely contributed by improved depth of response may influence survival outcomes (Lewis et al. 2019).

It was previously established that vemurafenib exposure was not altered by the addition of cobimetinib (Ribas et al. 2014). In the current study, a significantly lower median day 23 plasma concentration of vemurafenib when combined with cobimetinib than when given as monotherapy was noted. The mechanism(s) responsible for this potential drug-drug interaction is unclear.

There are several limitations in our study. The current study is an exploratory, post-hoc analysis. Hence, the new proposed threshold requires validation in prospective clinical trials with an adequate number of patients and plasma samples. A large proportion of patients were excluded from the current analysis due to dose adjustments within the 15 days prior to the D23 cut-off. Further, the current study evaluated plasma concentrations within the first month of starting vemurafenib. Although it is unclear if the threshold remains valid beyond this time, the association between D23 plasma vemurafenib $C_{ss,min}$ values and OS suggests that the relationship may persist beyond the first month. Moreover, the exposure-toxicity relationship was not evaluated to determine if there is an upper limit for survival benefit with acceptable toxicities.

Another limitation is the use of a single threshold trough plasma concentration ($C_{ss,min)}$ rather than area under the plasma concentration – time curve (AUC) approach. While the latter is increasingly considered as a better pharmacokinetic parameter for relationship with response/toxicity, the lack of access to the data to calculate vemurafenib AUC precluded its assessment in the current study. In addition, the measurement of a trough concentration may reduce the barriers for clinical translation of dose individualisation for kinase inhibitors (Lucas and Martin 2017).

7.5 Conclusion

A vemurafenib steady-state trough plasma concentration ($C_{ss,min}$) threshold of 50 mg/L is strongly associated survival outcomes for patients with advanced melanoma. This new threshold needs to be validated prospectively in future studies prior to implementation in routine clinical care.

In this chapter, my research demonstrated that an improved cut-off for the steady-state trough plasma concentration, a well-known PK parameter, as a predictor of variability in response as measured by survival for an oral targeted therapy. However, the use of target concentration guided dosing of systemic cancer therapies has not been implemented in routine clinical care due to various challenges (Bardin et al. 2014). For small molecule targeted therapies such as kinase inhibitors, the evidence such as results from this chapter, is rapidly evolving and thus support clinical implementation (Rowland et al. 2017).

8. CHAPTER EIGHT: PRIMARY SITE OF CANCER AND OUTCOMES WITH ATEZOLIZUMAB OR CHEMOTHERAPY

One of the well-known factors that is associated with heterogeneity in treatment response is the primary site of the origin of cancer. Moreover, within the same primary site, multiple histological subtypes and molecular subtypes within the same histology exist which may contribute towards differential treatment outcomes form the same therapy. Another layer of complexity is that the same histology may arise from different primary sites. For example, urothelial carcinomas can arise anywhere along the urothelium of the urinary tract. Despite arising from different primary sites, all urothelial cancers are treated with the same systemic therapies. In this chapter, the effect of the primary site of origin of the urothelial cancers on cancer outcomes with atezolizumab or chemotherapy was assessed.

8.1 Introduction

Urothelial cancers are less common group of cancers arising from bladder, renal pelvis, ureters, or urethra. Bladder primary site was the most common with an estimated age standardized incidence rate of 5.7 per 100,000 population and mortality of 1.9 per 100,000 population in 2018 (Ferlay et al. 2018). Among the urothelial cancers, those arising in the upper tract (renal pelvis and ureters, collectively referred to as upper tract urothelial cancers - UTUC) are rare (5-10% of all urothelial cancers) when compared to the lower tract (urinary bladder and urethral sites, collectively called as lower tract urothelial cancers - LTUC) (Roupret et al. 2018). Despite being grouped together with the more common bladder cancers, UTUC have several distinct features such as higher frequency of high-grade disease, more advanced at diagnosis, higher risk of recurrence and lower survival than LTUC (Taylor et al. 2019). Moreover, comprehensive genomic profiling indicates that a high proportion of UTUC have fibroblast growth factor receptor (FGFR3) mutations, higher frequency of microsatellite instability (MSI-H)/mismatch repair deficient (dMMR) tumours, a predominantly luminal-papillary type, a T-cell depleted tumour microenvironment and a lower mutational burden when compared to bladder cancers (Hassler et al. 2020; Meeks et al. 2020; Robinson et al. 2019; Yates and Catto 2013).

Despite these differences, patients with UTUC and LTUC are treated similarly either with platinum-based chemotherapy or ICIs (Flaig et al. 2020; Font et al. 2019; Ghatalia and Plimack 2020; Roupret et al. 2018). Over the last few years, at least five ICIs have been approved for the treatment of advanced urothelial carcinomas either as first-line in platinum ineligible patients or in those who progress after prior platinum-based chemotherapy (Ghatalia and Plimack 2020; Balar, Castellano, et al. 2017; Bellmunt et al. 2017; Powles et al. 2017; Sharma et al. 2017; Patel et al. 2018). ICIs include the PD-1 inhibitors, nivolumab and pembrolizumab and the PD-L1 inhibitors, atezolizumab, avelumab and durvalumab. Subgroup analyses from some of these trials indicate that patients with UTUC being treated with ICI may have inferior outcomes when compared to LTUC (Balar, Galsky, et al. 2017; Powles et al. 2018) . More recently, the results from the SAUL trial using real world population data for patients with UTUC treated with atezolizumab showed similar survival and response rates between the UTUC and LTUC (Sternberg et al. 2020). In view of the conflicting results, the efficacy and safety outcomes of patients with UTUC who were treated with atezolizumab using pooled individual patient data from two clinical trials were evaluated.

8.2 Methods

The primary objectives were to compare the survival (OS and PFS), response rates, and safety (specifically, irAEs) between UTUC and LTUC patients who were treated with atezolizumab. Similar analyses were also conducted using data from patients treated with chemotherapy in the control arms to identify heterogeneity in treatment effect that may be attributed to the upper tract primary sites. Deidentified individual patient data from IMvigor 210 (cohort 1 NCT02951767 and cohort 2 NCT02108652) and IMvigor 211(NCT02302807) clinical trials were used for this *post hoc* analysis (Balar, Galsky, et al. 2017; Powles et al. 2018). IMvigor 210 was a single arm phase II trial where atezolizumab was administered to patients with locally advanced or metastatic urothelial cancer while IMvigor 211 was a two-arm randomized phase III trial that compared atezolizumab with chemotherapy (either taxanes or vinflunine) in patients with locally advanced or metastatic urothelial cancer as second-line treatment trial after progression on a platinum containing chemotherapy. Data analysis was performed through Roche's data sharing policies. Ethics review was exempted by Southern Adelaide Clinical Health Research Ethics Committee.

8.2.1 Study definitions

In the dataset provided for analysis, those with primary sites documented as renal pelvicalyceal system or ureters were categorized as UTUC while those with urinary bladder or urethral primaries were grouped under LTUC. The primary outcome measure was OS. Secondary outcomes included PFS and any grade of irAEs. PFS was evaluated using RECIST version 1.1. Overall response rate was defined as combined complete response and partial response while disease control rate was defined as combined complete, partial responses and stable disease. irAEs were defined as presumed organ specific toxicity that may have auto-immune aetiology. irAEs were graded according to the NCI-CTCAE v4.0 (NCI CTCAE v4.0 2009).

8.2.2 Statistical analysis

Pooled data from the two clinical trials were used for analysis. Baseline characteristics were summarised using median, IQR or SD where applicable. Statistical differences between the UTUC and LTUC cohorts were reported using P-values derived from the chi-square test for categorical variables and the Kruskal-Wallis test for continuous data. A P-value less than 0.05 was considered statistically significant. Pooled HR with 95%CI were reported for survival (PFS and OS) differences between the cohorts using Cox proportional hazards regression. Logistic regression analysis was performed to compare the response rates between the two cohorts and reported as OR. All regression analyses were stratified by study. Potential confounding variables (performance status, PDL1 expression, and liver metastases) were adjusted by multivariable regression analysis. Survival plots were generated using the KM method. Analyses were conducted using R (version 3.5.3) (Team 2017).

8.3 Results

Data from a total of 1,331 patients with urothelial cancers from the two trials were available. Twenty-six patients did not have their primary site reported and were excluded from further analysis leaving a reminder of 1,305 patients. The UTUC cohort included 325 patients, 176 (54%) cancers arising from the renal pelvis and 149 (46%) from the ureters. The LTUC cohort included the reminder. Of whom, 950 (94%) had bladder primary, 30 (3%) had urethral primary and the remaining were lower genitourinary urothelial cancers. 51%

underwent radical cystectomy or nephroureterectomy and 92% received a prior platinum-based chemotherapy before trial enrolment. Overall, there were 868 atezolizumab treated patients and 437 chemotherapy treated patients across the 2 cohorts.

8.3.1 Atezolizumab treated patients

Among the patients treated with atezolizumab, 220 had UTUC while 648 had LTUC. Baseline characteristics of both the cohorts treated with atezolizumab are described in Table 37. The UTUC cohort had a higher proportion of patients who were Asians and those with lung metastases compared to the LTUC cohort. The remaining baseline characteristics were similar between the two cohorts.

Table 37: Atezolizumab treated patients with urothelial cancers

On atezolizumab	Total No. 868	Lower tract No. 648	Upper tract No. 220	P-value
Study				0.20
IMvigor210	417 (48%)	320 (49%)	97 (44%)	
IMvigor211	451 (52%)	328 (51%)	123 (56%)	
Cancer type				0.43
Transitional cell	787 (91%)	591 (91%)	196 (89%)	
Mixed histology	81 (9%)	57 (9%)	24 (11%)	
Age (years)	67 (60 - 74)	67 (60 - 74)	67 (59 - 73)	0.65
Sex				0.19
Male	673 (78%)	510 (79%)	163 (74%)	
Female	195 (22%)	138 (21%)	57 (26%)	
Race				<0.001*
White	702 (81%)	543 (84%)	159 (72%)	
Asian	70 (8%)	33 (5%)	37 (17%)	
Other	24 (3%)	18 (3%)	6 (3%)	
Missing	72 (8%)	54 (8%)	18 (8%)	
ECOG PS				0.56
0	370 (43%)	279 (43%)	91 (41%)	
1	474 (55%)	353 (54%)	121 (55%)	
2	24 (3%)	16 (1%)	8 (4%)	
Tumor sites				
Liver	247 (28%)	177 (27%)	70 (32%)	0.23
Lung	347 (40%)	244 (38%)	103 (47%)	0.02*
PD-L1 expression				0.22
Negative	267 (31%)	191 (29%)	76 (35%)	
Positive	601 (69%)	457 (71%)	144 (65%)	
Prior platinum therapy				
Yes	769 (89%)	580 (90%)	189 (86%)	
No	99 (11%)	68 (10%)	31 (14%)	
Prior radical surgery				0.05
Yes	472 (54%)	379 (58%)	93 (42%)	
No	139 (16%)	122 (19%)	17 (8%)	
Missing	257 (30%)	147 (23%)	110 (50%)	
Best overall response				0.08
PD	440 (51%)	309 (48%)	131 (60%)	

SD	165 (19%)	132 (20%)	33 (15%)	
PR	99 (11%)	78 (12%)	21 (10%)	
CR	39 (4%)	30 (5%)	9 (4%)	
Missing	125 (14%)	99 (15%)	26 (12%)	
Immune related adverse events (maximum grade)				0.42
0	625 (72%)	461 (71%)	164 (75%)	
1	117 (13%)	92 (14%)	25 (11%)	
2	73 (8%)	57 (9%)	16 (7%)	
3	43 (5%)	31 (5%)	12 (5%)	
4	10 (1%)	7 (1%)	3 (1%)	

There was no significant difference in OS between the two cohorts. The median OS for the UTUC and LTUC cohorts was 8.4 months and 8.8 months, respectively (Figure 29). The unadjusted HR for OS was 1.09 (95%CI: 0.90-1.31, P = 0.37) whereas the adjusted HR was 0.99 (95%CI: 0.82-1.21, P = 0.98). On the other hand, while the median PFS was similar (2.1 months each) between the 2 cohorts, the unadjusted HR for PFS was significantly worse for the UTUC cohort at 1.21 (95%CI: 1.03 -1.43, P = 0.02) (Figure 29). However, after adjusting for confounding factors, there was no significant difference despite a numerically worse PFS for the UTUC when compared to the LTUC cohorts (adjusted HR 1.16, 95%CI: 0.97-1.37, P = 0.09).

When the survival outcome analysis was limited to the PDL1 positive subset, the OS for the UTUC cohort was not significantly different to the LTUC cohort (adjusted HR 0.99, 95%CI: 0.82-1.21, P = 0.98). Similarly, the PFS, despite being numerically worse, was not statistically significantly different for the UTUC cohort after adjusting for confounding factors (adjusted HR 1.18, 95%CI: 0.97-1.37, P = 0.09).

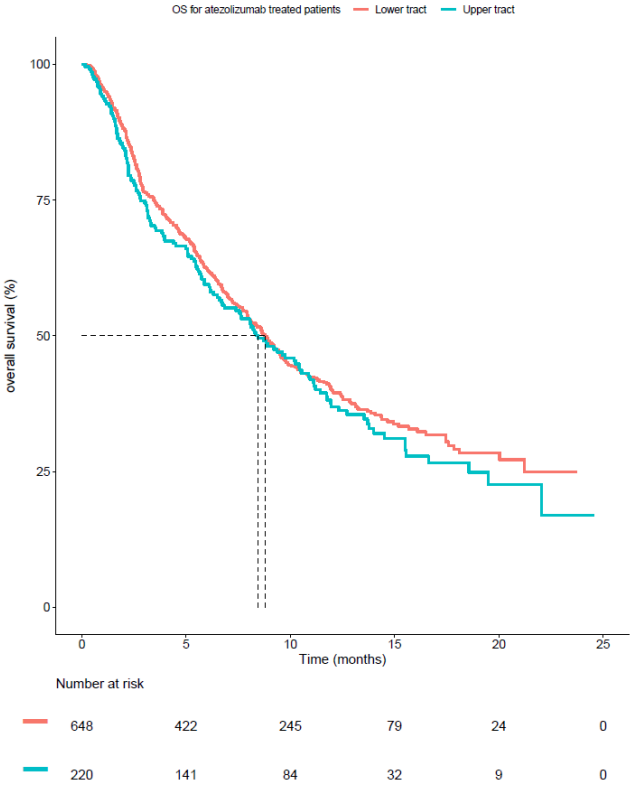
While the overall best response rates (ORR - combined complete response (CR) and partial response (PR)) were similar (14% vs 16%), the UTUC cohort had significantly lower disease control rates (DCR - combined CR, PR and stable disease (SD)) when compared with the LTUC cohort (29% vs 37%, respectively, OR 0.67, 95% CI 0.48-0.94, P = 0.02). In addition, the incidence of any grade irAEs was similar; 25% in the UTUC group and 29% in the LTUC group (OR 0.85, 95% CI 0.60-1.21, P = 0.37).

8.3.2 Chemotherapy treated patients

Among the 437 patients treated with chemotherapy, 105 had UTUC while 332 had LTUC. The majority received vinflunine (55%) followed by paclitaxel (33%) and docetaxel (12%). Baseline characteristics of both the cohorts treated with chemotherapy are described in Table 38. The UTUC cohort had a higher proportion

of patients who were Asians, lower renal function with glomerular filtration rate <60 ml/min, and distant metastasis at diagnosis when compared to LTUC.

Figure 29: OS and PFS in Atezolizumab treated patients



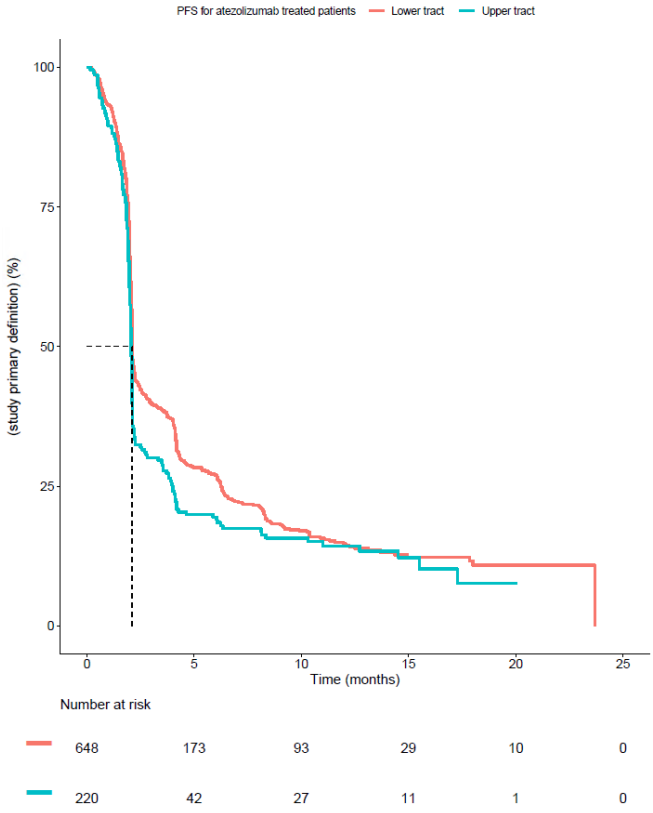


Table 38: Chemotherapy treated patients – Imvigor211 trial

On chemotherapy	Total No. 437	Lower tract No. 332	Upper tract No. 105	P-value
Cancer type				0.32
Transitional cell	402 (92%)	309 (93%)	93 (89%)	
Mixed histology	35 (8%)	23 (7%)	12 (11%)	
Chemotherapy type				0.068
Docetaxel	53 (12%)	46 (14%)	7 (7%)	
Paclitaxel	145 (33%)	113 (34%)	32 (30%)	
Vinflunine	239 (55%)	173 (52%)	66 (63%)	
Age (years)	67 (61 - 73)	67 (61 - 73)	68 (61 - 74)	0.55
Sex				0.08
Male	341 (78%)	266 (80%)	75 (71%)	
Female	96 (22%)	66 (20%)	30 (29%)	
Race				0.04*
White	315 (72%)	244 (73%)	71 (68%)	
Asian	53 (12%)	33 (10%)	20 (19%)	
Other	3 (1%)	3 (1%)	0 (3%)	
Missing	66 (15%)	52 (16%)	14 (13%)	
ECOG PS				0.09
0	194 (44%)	140 (42%)	54 (51%)	
1	243 (56%)	192 (58%)	51 (49%)	
Tumor sites				
Liver	118 (27%)	95 (29%)	23 (22%)	0.22
Lung	202 (46%)	147 (44%)	55 (52%)	0.18
PDL1 expression				0.24
Negative	130 (30%)	105 (32%)	25 (24%)	
Positive	307 (70%)	227 (68%)	80 (76%)	
Prior platinum therapy				
Yes	437 (100%)	332 (100%)	105 (100%)	
Prior radical surgery for primary				
Yes	193 (44%)	179 (54%)	14 (13%)	
No	0 (0%)	0 (0%)	0 (0%)	
Missing	244 (56%)	153 (46%)	91 (87%)	
Best overall response				0.61
PD	148 (34%)	109 (33%)	39 (37%)	
SD	125 (29%)	92 (28%)	33 (31%)	
PR	75 (17%)	60 (18%)	15 (14%)	
CR	22 (5%)	18 (5%)	4 (4%)	
Missing	67 (15%)	53 (16%)	14 (13%)	
Immune related adverse events (maximum grade)				0.32
0	345 (79%)	259 (78%)	86 (82%)	
1	47 (11%)	36 (11%)	11 (10%)	
2	32 (7%)	24 (7%)	8 (8%)	
3	12 (3%)	12 (4%)	0 (0%)	
4	1 (<1%)	1 (<1%)	0 (0%)	

There was no significant difference in OS between the two cohorts, with a median OS of 8.3 months in the UTUC cohort and 7.8 months in the LTUC cohort. The unadjusted HR for OS was 1.00 (95%CI: 0.78-1.28, P = 0.97) whereas the adjusted HR was 1.06 (95%CI: 0.82-1.37, P = 0.62). The median PFS was 3.9 months and 4 months for the UTUC and LTUC cohorts, respectively. The unadjusted HR for PFS for the UTUC cohort was

1.14 (95%CI: 0.90 -1.43, P = 0.28) and the adjusted HR was 1.18 (95%CI: 0.93-1.49, P = 0.17). The DCR were similar between UTUC and LTUC cohorts treated with chemotherapy, 50% vs 51%, respectively (OR 0.94, 95% CI 0.60-1.45, P = 0.77).

8.4 Discussion

This chapter reported the survival and safety outcomes for patients with UTUC treated either with atezolizumab or chemotherapy compared to LTUC using individual patient data from two clinical trials . This is one of the largest series of patients with UTUC uniformly treated with ICI as second-line therapy after platinum-based chemotherapy. From this analysis, it appears that patients with UTUC treated with atezolizumab have lower disease control rates, but similar survival and safety when compared to LTUC.

Data from previous trials indicated that UTUC patients may have lower ORR than LTUC patients when treated with ICI therapies. In the CheckMate 275 trial, nivolumab treated UTUC had a response rate of 11% in contrast to 22% for the LTUC group (Sharma et al. 2017). Similarly, in the JAVELIN trials, response rates of 11% vs 18% were reported for the UTUC and the LTUC cohorts respectively (Patel et al. 2018). In the first-line KEYNOTE-052 trial, 22% of the UTUC cohort had a response compared to 28% in the LTUC cohort (Balar, Castellano, et al. 2017). In contrast, patients in the SAUL study who received atezolizumab, had similar ORR of 12% and 14% respectively (Sternberg et al. 2020).

In the current study, atezolizumab treated UTUC patients had a similar ORR to LTUC patients, but significantly lower DCR. In addition, the adjuvant IMVigor010 trial reported worse disease-free survival for atezolizumab treated patients with UTUC when compared to observation (Hussain et al. 2020). It is possible that heterogeneity in patient populations across trials, differences in cancer characteristics and line of therapy may have influenced response rates. However, such low ORR indicates that monotherapy with ICI therapies might not benefit most patients, especially those who may need rapid response due to high disease burden related symptoms. Further analysis using data from the recently reported trials such as IMVigor130 and JAVELIN Bladder100 should be conducted to evaluate whether such low ORR persists in patients with UTUC

when treated with ICI in combination with chemotherapy or in maintenance setting after first-line chemotherapy (Galsky et al. 2020; Powles et al. 2020).

The short duration of PFS in those who achieve disease control with atezolizumab indicates that novel strategies such as combination with targeted agents such as FGFR inhibitors may be warranted. Although the MSI-high group of UTUC have a higher likelihood of response to ICI therapies, the sporadic non MSI-high group of UTUC are more common than the MSI-high cancers. As noted previously, most sporadic non MSI-high UTUC have the luminal-papillary gene expression profile that correlates with T-cell depleted immune microenvironment and higher incidence of FGFR3 mutations, all of which are likely explanations for such low ORR and short PFS with atezolizumab monotherapy (Robinson et al. 2019). Future clinical trials should also consider utilising combination strategies that may transform the immune "cold" microenvironment into immunogenic profile to facilitate reactive T-cell response.

Due to the relatively low incidence of UTUC, randomized clinical trials are rare. Given the established molecular, clinical, treatment and outcome differences in patients with UTUC, international collaboration will be required to prospectively conduct large multicentric clinical trials. The recently reported perioperative chemotherapy versus surveillance in upper tract urothelial cancer trial (POUT) was the first large phase III trial specifically conducted in the UTUC group of patients (Birtle et al. 2020). Data generated from this analysis provides a basis for the design of future clinical trials that address various treatment options for patients with advanced UTUC.

I acknowledge several strengths and limitations in the current study. This is one of the two largest cohorts of UTUC patients uniformly treated with ICI therapy (Sternberg et al. 2020). Individual patient data from prospectively conducted clinical trials were used as source material for the analysis. The data from chemotherapy treated control cohort was also reported to compare the heterogeneity in treatment effects from atezolizumab. The main limitations are this study was a secondary exploratory analysis with limited power to explore the subgroups of renal pelvis vs ureters as primary sites. Moreover, up to 20% of UTUC can have synchronous LTUC cancers which were not accounted for (Font et al. 2019; Roupret et al. 2018; Taylor et al. 2019). In addition, data on gene expression profiles and MSI status were not available for analysis.

8.5 Conclusion

In this large series of patients treated with atezolizumab, those with UTUC had worse disease control rates, but similar OS/PFS, overall response rates, and safety when compared to patients with LTUC. Future studies should consider combination approaches to improve outcomes of patients with this rare group of malignancies.

However, the disease control rates were significantly lower for the upper tract urothelial cancers when compared to lower tract cancers indicating some degree of heterogeneity in treatment effect which warrants confirmation in future studies. The results from this research also illustrates that despite having negative biological characteristics that may be associated with inferior outcomes UTUC had similar survival as the LTUC with atezolizumab. It is likely that the primary site of origin of the urothelial cancer may not contribute towards the variability in survival outcomes to ICI therapy among patients with urothelial cancers. However, studies with larger sample size would be required to confirm this lack of association. Future studies should also explore other factors that may contribute towards variability in response to ICI therapies for this group of patients.

9. CONCLUSION

9.1 Thesis summary

This thesis explored various factors such as baseline patient characteristics, the primary site of cancer, commonly used concomitant medications and threshold plasma concentration, that can determine the variability in efficacy and toxicities of anticancer drugs such as atezolizumab, chemotherapy and vemurafenib. In addition, variability in the manifestation of immune related adverse events, a special group of adverse events from immune check point inhibitors and their association with drug efficacy was also studied. All the research work for this thesis were conducted using large datasets from well conducted clinical trials which were accessed using various data sharing platforms. The data for analyses were extracted from individual patient data case report forms of 10,158 patients with five types of cancers who participated in 15 different clinical trials and their results were reported in the preceding chapters.

One of the major findings described in chapter 2 was that the previously under recognized baseline characteristics such as BMI, influence outcomes of patients undergoing atezolizumab monotherapy for patients with lung cancers. Contrary to prior belief that obesity is usually considered as a negative factor for drug therapy associated outcomes, the findings from this research indicated that those patients with high BMI had improved survival when treated with atezolizumab. While these findings were intriguing, the exact mechanisms behind the association was unclear. However, the consistency and strength of the effect size as reported in subsequent studies indicate that baseline BMI should be considered as a stratification factor in future clinical trials for advanced lung cancer being treated with single agent ICI drugs.

While it is well-known that type and severity of toxicities from systemic cancer therapies vary between individuals who receive the same drug for the same indication, there is little understanding of the incidence of multi-organ immune related adverse events from immunotherapy drugs like atezolizumab. Chapter 3 addresses the gap in this knowledge. For the first-time, this research described a detailed evaluation of the incidence, type, severity and time-profile of multi-organ immune related adverse events using a large cohort of atezolizumab treated patients. Moreover, it was also demonstrated that multi-organ adverse events were associated with improved survival from atezolizumab. Chapter 4 specifically evaluated if the immune related

adverse events were associated with survival in both chemotherapy and immunotherapy treated patients. It was found that the occurrence of irAEs were associated with survival in both atezolizumab as well as the taxanes or vinca alkaloid-based chemotherapies, indicating that they are prognostic rather than predictive of response to immunotherapy alone. The findings from these studies provide additional information to treating clinicians to anticipate, recognise and treat such adverse events as well as trigger further research to better understand the pathophysiology of those toxicities. In addition, their prognostic association even in chemotherapy treated patients requires further evaluation in future studies.

Chapters 5 and 6 explored the effect of baseline use of concomitant medications such as anti-hypertensives and PPI on the efficacy of atezolizumab and fluoropyrimidine-based chemotherapy, respectively. While the PPI were strongly associated with worse survival in fluoropyrimidine-based chemotherapy treated bowel cancer patients, the concomitant use of renin-angiotensin inhibitors was not in atezolizumab treated patients with lung, bladder, or kidney cancers. Both findings were surprising and unexpected as the pre-clinical data indicated the contrary. *In vitro* and *in vivo* animal studies reported that PPI improved the efficacy of fluoropyrimidine-based chemotherapy and renin-angiotensin inhibitors augmented immune response. Further studies are required to confirm these findings. However, the strength of the inferior outcomes with PPI raises red flag that the clinicians should consider minimising the concomitant use of these drugs in patients initiating chemotherapy for advanced bowel cancer. It is important to understand the medical indications for which PPI are being prescribed and consider alternatives if possible. In addition, future studies should also explore the effect of PPI in patients being treated with chemotherapy for early stage colorectal cancers.

In chapter 7, my research showed that a new threshold as target trough concentration for optimal dosing of vemurafenib, a BRAF inhibitor, commonly used for the treatment of melanoma and other diseases with braf mutations that was associated with improved survival. This chapter provided further evidence to support optimized dosing to reach the trough concentration to reduce inter-individual variability in vemurafenib survival. While this threshold needs confirmation in future studies, the upper threshold to reduce toxicities requires further evaluation. The application of results from this research would depend on future prospective

clinical trials that evaluate the efficacy, safety, and cost-economic analyses of plasma concentration guided dosing interventions.

In chapter 8, the effect of the primary site of origin of cancer was not significantly associated with survival outcomes among patients with urothelial cancers when treated with atezolizumab. However, the disease control rates were significantly lower for the upper tract urothelial cancers when compared to lower tract cancers indicating some degree of heterogeneity in treatment effect which warrants confirmation in future studies.

9.2 Future directions

It is important to highlight that the findings from the research work performed in this thesis involved data from previously completed clinical trials. All the analyses should be considered as unplanned and hypothesis generating. Hence, further studies will be required to confirm or refute the findings of this research. Moreover, as the data included patients who were participants of clinical trials, who usually have better health and performance status than patients in the general community, the generalisability of the research findings are limited. Future validation studies addressing these questions using data from real-world clinical practice should be conducted. The members of the current research have recently obtained access to a large electronic health record platform (CancerLinQ) that has real-world data involving more than 2,00,000 patients with lung cancer which may help clarify some of the questions raised in this research.

In addition, the hypotheses generated from this body of research work may provide basis for further pre-clinical work to better understand the mechanistic reasons. The interaction between PPI and fluoropyrimidine-based chemotherapy is not clearly understood. Similarly, the interaction between anti-hypertensives and immunotherapies as well as the causal factors and pathophysiology behind the 5% of treated patients having multi-organ immune related adverse events need to be clearly understood. The effect of the concomitant non-cancer drug therapies with all systemic cancer therapies including chemotherapy or immunotherapy should also be studied comprehensively in future studies.

The association of baseline high BMI with atezolizumab outcomes should be explored in future studies in patients with other types of cancer, in early stages of cancer, those receiving other types of immunotherapies and in patients who receive combination therapies. Future studies should also evaluate the effect of changes in BMI after the treatment is initiated on immunotherapy outcomes.

There were several challenges during the conduct of my research projects. One major challenge was accessing data from trials beyond those used in this thesis from all the sponsors. Despite international agreement and commitment for responsible data sharing of deidentified IPD for those trials that involve approved medications, a recent study found that only a small number of trials were eligible for data sharing for independent researchers after two years of publication of primary results (Hopkins, Rowland, and Sorich 2018). Some of the pharmaceutical sponsors were unwilling to release any data for their approved drugs, or release only limited data without information on concomitant medications, molecular information or extended survival. In addition, the data released by sponsors were in different software repositories that precluded merging of datasets. Some sponsors and data sharing platforms provided raw data without supporting documentation on data dictionaries or clinical study reports. Moreover, a long time (several months to 2 years) was taken by the sponsors and data sharing groups for the review of submitted projects and contractual agreement prior to data release. Once the data was released, data cleaning and data management for analysis was labour intensive. Addressing these challenging issues through a streamlined and standardised process for the request, review and data release may facilitate independent researchers to conduct interesting research using large data in future.

9.3 Conclusion

Understanding the factors that contribute towards the variability in response and toxicities to systemic cancer therapies will help dose optimisation for individual patients thereby improve patient outcomes. In this thesis, a variety of factors were explored to better understand the variability in response to systemic cancer therapies using clinical trial data from a large population of patients with a variety of cancers. The research work from this thesis identified that previously under-recognized patient factors such as baseline body mass index and use of concomitant medications such as proton pump inhibitors and anti-hypertensives

can influence outcomes with different types of systemic cancer therapies. Variability in the incidence of multi-organ toxicities and its effect on outcomes were reported for the first-time. A new threshold trough plasma concentration for a kinase inhibitor that showed improved association with survival was also identified. Future work that address the questions and challenges raised from this research should be continued to improve the understanding of inter-individual variations in drug therapies.

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11. APPENDICES

Appendix 1 – **Kichenadasse G**, Miners JO, Mangoni AA, Rowland A, Hopkins AM, Sorich MJ. Association between Body Mass Index and overall survival with immune checkpoint inhibitor therapy for advanced non-small cell lung cancer. *JAMA Oncol.* 2020 Mar; 6(4):512-518.

Appendix 2 – **Kichenadasse G**, Hopkins AM, Sorich MJ. Reply to Sanchez et al: “Obesity paradox in immunotherapy treated patients with NSCLC: a word of caution”. *JAMA Oncol.* 2020 Apr;6(6):941-942.

Appendix 3 – **Kichenadasse G**, Miners JO, Mangoni AA, Rowland A, Hopkins AM, Sorich MJ. Multi-organ immune-related adverse events during treatment with atezolizumab. *J Natl Compr Can Netw.* 2020 Sep; 18(9).

Appendix 4 – **Kichenadasse G**, Hughes JH, Miners JO, Mangoni AA, Rowland A, Hopkins AM, Sorich MJ. Relationship between vemurafenib plasma concentrations and survival outcomes in patients with advanced melanoma. *Cancer Chemother Pharmacol.* 2020 Mar; 85(3):615-620.