Author/s:
Foroughi, Siavash

Title:
Optimising colorectal cancer therapies using clinical registries

Date:
2020

Persistent Link:
https://hdl.handle.net/11343/252844

Optimising colorectal cancer therapies using clinical registries

Siavash Foroughi

ORCID: 0000-0001-6916-8424

Doctor of Philosophy

August 2020

The Walter & Eliza Hall Institute of Medical Research

Department of Medical Biology

Faculty of Medicine, Dentistry and Health Sciences

The University of Melbourne

Submitted in total fulfilment of the requirements of the degree of

Doctor of Philosophy

Abstract

Colorectal cancer (CRC) remains one of the leading causes of cancer deaths worldwide. Advances in therapy have resulted in significant gains in survival, particularly in the metastatic setting. While the discovery of biomarkers, such as *RAS* mutations, have helped refine treatment selection to some degree, more accurate biomarkers are urgently needed.

Comparison of existing treatments, as well as the evaluation of the efficacy of new therapies, are informed by randomised controlled trials (RCTs), which form the evidentiary backbone of clinical practice guidelines and represent the gold standard of assessment. Despite their high internal validity, they can lack generalisability due to their highly selective inclusion criteria. Prospective, registry-based, randomised controlled trials (RRCTs) have the potential to bridge the gap between RCTs and real-world clinical practice in oncology.

The objective of this thesis is to explore how clinical registries can help to advance biomarker research. This thesis applies real-world data to examine the clinical utility and validity of emerging CRC biomarkers, and explores the feasibility of RRCTs in the oncology setting. In a cohort of 99 metastatic colorectal cancer (mCRC) patients, the role of the epidermal growth factor receptor (EGFR) and its ligands, amphiregulin and epiregulin, as potential prognostic and predictive biomarkers for mCRC patients is explored (Chapter 5). This study examines protein expression by immunohistochemistry and includes patients who were not treated with EGFR inhibitors, representing the largest such cohort reported to date.

The real-world validity of biomarker trials is explored in Chapter 6, where the characteristics of patients enrolled in these studies are compared to real-world patients. Using an established multi-centre CRC registry as the reference real-world cohort, clinical data was analysed for participants in three types of biomarker trials (retrospective, prospective observational and prospective interventional). This study provides novel insights into recruitment to, and potential validity of, biomarker trials.

Finally, Chapter 7 examines the feasibility of an Australian-first RRCT in oncology. This ongoing study is exploring chemotherapy sequencing in first-line treatment of mCRC and leverages an established multi-centre registry as the data collection platform. This study demonstrates the potential of RRCTs to accelerate progress in optimising patient treatments and outcomes.

This thesis demonstrates the power of high-quality clinical registries to facilitate prospective randomised trials, while providing opportunities to investigate and validate biomarkers in real-world settings.

Declaration

This is to certify that,

i. the thesis comprises only of my original work towards the Doctor of Philosophy, except where indicated in the preface;

ii. due acknowledgement has been made in the text to all other material used; and

iii. the thesis is fewer than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

Siavash Foroughi

5 August 2020

Preface

The work presented in this thesis was undertaken at the Walter and Eliza Hall Institute of Medical Research (WEHI) under the supervision of Professor Peter Gibbs, Associate Professor Jeanne Tie, and Professor Antony Wilks Burgess. For the duration of this work, the author was a recipient of an Australian Government Research Training Program (RTP) Scholarship and the WEHI top-up scholarship.

This thesis contains work that has already been published or is in preparation for publication. I duly acknowledge the important contributions of individuals to the work presented within this thesis which are outlined below. Where required, copyright permissions for inclusion of final article prints have been obtained. Contributions are listed below. Overall, I assess my contribution to the work presented in this thesis as being more than 80%.

Chapter 2

Foroughi et al., 2019, *Growth Factors.*

Published by Growth Factors on 26 December 2019.

Professor Antony Wilks Burgess supervised research, provided input into the direction of the review and edited the manuscript. Associate Professor Jeanne Tie and Professor Peter Gibbs provided intellectual input into the scope and direction of the review and edited the manuscript.

I estimate my contribution to this manuscript to be 85%.

Chapter 3

Foroughi et al., 2018, *Asia Pac J Clin Oncol.*

Published by Asia-Pacific Journal of Clinical Oncology on 26 June 2018.

Associate Professor Jeanne Tie and Professor Peter Gibbs supervised research, provided input into the direction of the review and edited the manuscript. Professor Antony Wilks Burgess provided intellectual input into the scope and direction of the review and edited the manuscript. Dr Hui-li Wong, Dr Lucy Gately, Dr Margaret Lee, and Dr Koen Simons edited the manuscript.

I estimate my contribution to this manuscript to be 80%.

Chapter 5

Submitted for publication to Growth Factors on 4 July 2020.

Associate Professor Jeanne Tie, Professor Peter Gibbs, and Professor Antony Wilks Burgess supervised research, provided input into the direction of the study, and edited the manuscript. Dr Hui-li Wong, Dr Michael Christie, Dr Rachel Wong, and Dr Margaret Lee edited the manuscript. Dr Ryan Hutchinson assisted with blinded scoring of the immunoreactive samples and edited the manuscript. Ms Ahida Batrouney assisted with optimisation of antibodies and sample processing and edited the manuscript.

I estimate my contribution to this manuscript to be 80%.

Chapter 6

Submitted for publication to Acta Oncologica on 1 July 2020.

Associate Professor Jeanne Tie and Professor Peter Gibbs supervised research, provided input into the direction of the study, and edited the manuscript. Professor Antony Wilks Burgess provided intellectual input into the scope and direction of the study and edited

the manuscript. Dr Hui-li Wong, Dr Rachel Wong, Dr Margaret Lee, Dr Belinda Lee, Professor Ian Jones, and Mr Iain Skinner edited the manuscript.

I estimate my contribution to this manuscript to be 80%.

Chapter 7

Manuscript in progress.

Associate Professor Jeanne Tie and Professor Peter Gibbs supervised research, provided input into the direction of the study, and edited the manuscript. Professor Antony Wilks Burgess and Dr Hui-li Wong provided intellectual input into the scope and direction of the study and edited the manuscript.

I estimate my contribution to this manuscript to be 80%.

Appendix

Foroughi et al., 2019, *Asia Pac J Clin Oncol.*

Published by Asia-Pacific Journal of Clinical Oncology on 12 February 2019.

Associate Professor Jeanne Tie, Professor Peter Gibbs, and Professor Antony Wilks Burgess provided intellectual input into the scope and direction of the letter and edited the letter. Dr Hui-li Wong, Dr Lucy Gately, Dr Margaret Lee, and Dr Koen Simons edited the letter.

I estimate my contribution to this letter to be 85%.

Dedication

For my parents, my wife, and my son.

Acknowledgements

When I began this PhD, I never imagined that I would be writing these words during a global pandemic. The COVID-19 pandemic has highlighted the importance of scientific discovery in the progress of human health as well as the immense value and fragility of the connections we share with the people around us.

This work would not have been possible without the generous contributions of many people, particularly the patients who have donated their time, data, and samples to research. I would like to acknowledge and thank them for their sacrifice and courage. While facing difficult life-changing circumstances, they have selflessly given us an opportunity to improve outcomes for the patients who will one day come after them.

I would like to express my deepest gratitude to my supervisors. My sincere thanks to my principal supervisor, Professor Peter Gibbs, who gave me the opportunity to undertake this research. Peter's foresight and leadership are nothing short of remarkable and inspiring. My time in the Gibbs laboratory over the past six years has allowed me to develop both academically and professionally, and I am very thankful to Peter for all the opportunities he has provided me.

I am grateful to my co-supervisors Associate Professor Jeanne Tie and Professor Tony Burgess for their commitment and excellent mentorship. Jeanne's clinical insight and guidance has been crucial to keeping my work on track. I am thankful to Tony for taking on a major role in my supervision, and for being one of the kindest and most generous scientists I have had the pleasure of working under. Thank you, Jeanne and Tony, for the significant roles you have played in my training as a scientist.

I would like to acknowledge and thank the members of my PhD committee: Associate Professor Andrew Webb for chairing my committee and providing me with invaluable direction and advice, and Dr Bridget Southwell for being a wonderful supporter and mentor for all these years. Also, a special thank you to the Division Coordinators, past and present, and Elizabeth Jessup who helped with my committee meetings. I would also like to thank Dr Keely Bumsted-O'Brien and Sue Hardy from the WEHI Education Office for their seemingly endless support.

It has been a privilege to work at the Walter and Eliza Hall Institute and it is truly something special to be a part of this community. During my PhD, I made many important connections with staff, both at the institute and across the Parkville precinct, and the calibre of science, passion and dedication was always a great source of inspiration.

I thank everyone in the Personalised Oncology Division (previously the Systems Biology and Personalised Medicine Division) and the many staff in the Division of Structural Biology, which was my second home for the duration of my studies. Thank you to all the members, past and present, of the two labs I have been a part of: the Gibbs and Burgess laboratories. At some point, each and every one of you has contributed to my training and experience by providing me with support, strength and confidence when I have needed it most.

I would especially like to acknowledge Matthew Chapman, Michael Harold, Tina Cavicchiolo and Nicole Ng from the Gibbs lab for their friendship and support over the years; thank you for the constant encouragement and for all the laughs. A special mention to Dr Maree Faux and Dr Ruth Mitchell from the Burgess lab; thank you for all your support, particularly in preparation for my final seminar. Also, thank you to my desk

buddy, Dr Katrina Black, for all the chats about life in science; it was always helpful to hear your perspective.

Many other staff at the Royal Melbourne Hospital, the University of Melbourne and the Walter and Eliza Hall Institute deserve thanks. Dr Michael Christie, Dr Ryan Hutchinson and Ahida Batrouney helped with all aspects of immunohistochemistry. Maria Bisignano and her team were instrumental in facilitating sample retrieval and I received expert support and advice from the histology team led by Ellen Tsui.

To my friends outside of WEHI, thank you for always being there, despite my constant distraction and general absence from your lives. Thank you for reminding me of life beyond this thesis and for being genuinely interested in my work; special thanks to Chris Senaratne and Christina Tee for patiently proof-reading my manuscripts before submission.

I thank my family for their patience and understanding. I am so grateful to my parents, Nepton and Mahmoud, who have always supported and believed in me. This thesis would not have been possible without your encouragement and love. Thank you to my sisters Roya, Rosita and Elly, and my brother, Cameron. You all inspired me in one way or another, especially Elly. To Ramin, my brother-in-law and WEHI colleague, thank you for first introducing me to WEHI for my high-school work experience. I am so thankful to you and Elly for always supporting me through the challenging times.

And finally, to my wonderful wife Hui-li, thank you for your unconditional support and never-failing encouragement throughout this journey. I am humbled by your kindness, generosity, and work ethic. To my unborn son, thank you for the added motivation over the last eight months; I hope this inspires you one day.

List of Publications and Presentations

Peer-reviewed articles published during candidature

Foroughi, Siavash, Hui-li Wong, Lucy Gately, Margaret Lee, Koen Simons, Jeanne Tie, Antony Wilks Burgess, and Peter Gibbs. 2018. "Re-Inventing the Randomized Controlled Trial in Medical Oncology: The Registry-Based Trial." Asia-Pacific Journal of Clinical Oncology 14 (6): 365–73. doi:10.1111/ajco.12992.

Foroughi, Siavash, Hui-li Wong, Lucy Gately, Margaret Lee, Koen Simons, Jeanne Tie, Antony Wilks Burgess, and Peter Gibbs. 2019. "Registry-Based Randomized Clinical Trials as a Method to Improve Cancer Care in Australia." Asia-Pacific Journal of Clinical Oncology 15 (3): 188-189. doi:10.1111/ajco.13122.

Foroughi, Siavash, Jeanne Tie, Peter Gibbs, and Antony Wilks Burgess. 2019. "Epidermal growth factor receptor ligands: targets for optimizing treatment of metastatic colorectal cancer." Growth Factors doi:10.1080/08977194.2019.1703702.

Manuscripts under review

Foroughi, Siavash, Ryan Hutchinson, Hui-li Wong, Michael Christie, Ahida Batrouney, Rachel Wong, Margaret Lee, Jeanne Tie, Antony Wilks Burgess, and Peter Gibbs. 2020. "Immunohistochemical evaluation of the prognostic and predictive power of epidermal growth factor receptor ligand levels in patients with metastatic colorectal cancer." Under review: *Growth Factors*

Foroughi, Siavash, Hui-li Wong, Jeanne Tie, Rachel Wong, Margaret Lee, Belinda Lee, Ian Jones, Iain Skinner, Antony Wilks Burgess, and Peter Gibbs. 2020. "Characteristics

and outcomes of participants in colorectal cancer biomarker trials versus a real-world cohort."

Under review: *Acta Oncologica*

Poster presentations

Foroughi, Siavash, Hui-li Wong, Lucy Gately, Margaret Lee, Jeanne Tie, Antony Wilks Burgess, and Peter Gibbs. "Re-inventing the randomized controlled trial in medical oncology: The registry-based trial." Australasian Gastro-Intestinal Trials Group Annual Scientific Meeting, Brisbane, November 2018.

Wong, Hui-li, Margaret Lee, **Siavash Foroughi**, Lucy Gately, Michael Harold, Evelien Rosens, Khic-Houy Prang, Margaret Kelahar, and Peter Gibbs. "ALT-TRACC: a registry-based randomised trial investigating alternating oxaliplatin and irinotecan doublets in treatment-naïve metastatic colorectal cancer." Clinical Oncology Society of Australia Annual Scientific Meeting, Perth, November 2018.

Foroughi, Siavash, Hui-li Wong, Jeanne Tie, Antony Wilks Burgess, and Peter Gibbs. "Are patients enrolled on biomarker trials representative of the real-world population? An analysis of stage II/III colorectal cancer patients." Australasian Gastro-Intestinal Trials Group Annual Scientific Meeting, Brisbane, August 2019.

Invited talks

How REDCap databases can facilitate registries and trials, Australasian Health & Research Data Managers Association Annual Scientific Meeting: The New Age of Clinical Trials: Registries and Biomarkers. Melbourne, 26 October 2018

Table of Contents

List of Tables

List of Figures

Acronyms and Abbreviations

5-FU	5-fluorouracil
ACCORD-CRC	Australian Comprehensive Cancer Outcomes and Research Database for Colorectal Cancer
AJCC	American Joint Committee on Cancer
AREG	Amphiregulin
ASA	American Society of Anesthesiologists
CRC	Colorectal cancer
ctDNA	Circulating tumour deoxyribonucleic acid
DFS	Disease-free survival
ECOG	Eastern Co-operative Oncology Group
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EGFRI	Epidermal growth factor receptor inhibitor
EREG	Epiregulin
FFPE	Formalin-fixed paraffin-embedded

FOBT	Faecal Occult Blood Test
FP	Fluoropyrimidine
H&E	Haematoxylin and eosin
HDI	Human Development Index
HREC	Human Research Ethics Committee
H-score	Histological score
IHC	Immunohistochemistry
IRSAD	Index of Relative Socioeconomic Advantage and Disadvantage
LV	Leucovorin
mCRC	Metastatic colorectal cancer
moAb	Monoclonal antibody
NBCSP	National Bowel Cancer Screening Program
NR	Not reported
OS	Overall survival
PFS	Progression-free survival
POS	Prospective observational study
RCS	Retrospective cohort study

RCT	Randomised controlled trial
RECIST	Response Evaluation Criteria in Solid Tumours
REDCap	Research Electronic Data Capture
RRCT	Registry-based randomised controlled trial
TNM	Tumor, nodes, metastasis
TRACC	Treatment of Recurrent and Advanced Colorectal Cancer Registry
UICC	Union for International Cancer Control
VEGF	Vascular endothelial growth factor

Chapter 1.

Introduction and Overview of Colorectal Cancer

This chapter contains a general overview of colorectal cancer (CRC) and the scope and aims of this thesis. In-depth literature reviews covering the relevant themes of this thesis (epidermal growth factor receptor ligands and registry-based randomised controlled trials) are included in the following two chapters.

Epidemiology

In Australia, approximately 16,000 new cases of CRC are diagnosed each year and up to 5,000 individuals will ultimately die of CRC (Australian Institute of Health and Welfare 2019a). On a global scale, the burden of CRC accounts for over 10% of all cancer incidence; it is the third most common malignancy and the second leading cause of cancer mortality in both men and women (Bray et al. 2018) (Figure 1).

The incidence of CRC, however, is not uniformly distributed throughout the world. CRC is most prevalent in developed countries and age-standardised rates of CRC in high and very-high Human Development Index (HDI) regions were over 3-fold higher than those in low and medium HDI regions: 51.2 versus 14.3 per 100,000, respectively (Bray et al. 2018). Furthermore, the incidence is predicted to increase in many high and very-high HDI countries, particularly in Australia, The United States, Ireland and Canada (Araghi et al. 2019). However, it is important to note that these incidence rates may be prone to ascertainment bias; there may be a high degree of underreporting in low HDI regions, as well as higher rates of screening in high HDI regions, leading to a higher incidence of asymptomatic cancers.

The variation in global CRC incidence may be largely due to multiple environmental risk factors such as sedentary lifestyle (Samad et al. 2005), obesity (Arnold et al. 2015), alcohol consumption (Bagnardi et al. 2015), and the adoption of a Western diet, typically with a high intake of red and processed meat, salt and fat, and low intake of whole grains and fibre (Bouvard et al. 2015; Magalhães, Peleteiro, and Lunet 2012), all of which are associated with an increased risk of colorectal carcinogenesis. The impact of these environmental factors is best highlighted by migration studies which demonstrate a rise in CRC rates in originally low-risk groups after migration to high-risk areas (Grulich, McCredie, and Coates 1995).

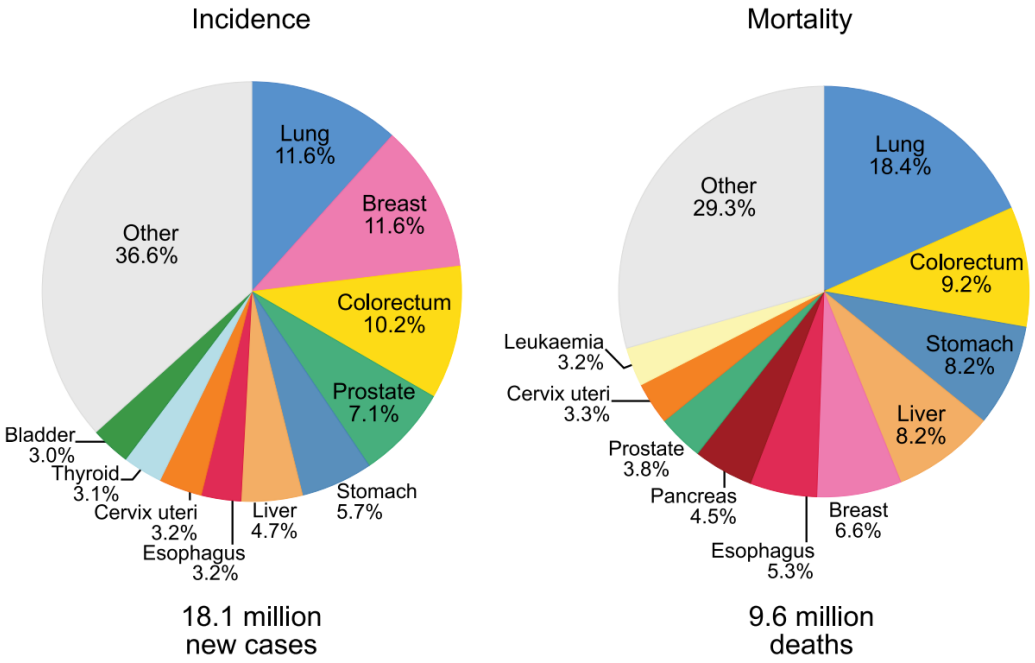


Figure 1. Incidence and mortality of the 10 most common cancers worldwide in 2018. The area of the pie chart reflects the proportion of the total number of cases or deaths. Source: GLOBOCAN 2018. Adapted from Bray et al. 2018.

Pathogenesis

Approximately 70% of CRCs occur sporadically, in people without a genetic predisposition or family history of CRC. Up to 25% have a familial component whereby a family history is observed but is inconsistent with known patterns of inherited disease,

and fewer than 10% of patients have a true inherited predisposition to CRC (Yamagishi et al. 2016). For most sporadic CRCs, polyps (or early adenomas) progress through the adenoma-carcinoma sequence (Hill, Morson, and Bussey 1978) to form an invasive CRC. This pathological process (Figure 2) occurs, in general, over five to ten years (Vogelstein et al. 1988), and involves the accumulation of multiple genetic events including activation of proto-oncogenes, such as *KRAS*, and inactivation of tumour suppressor genes, such as *APC* and *TP53* (Fearon and Vogelstein 1990).

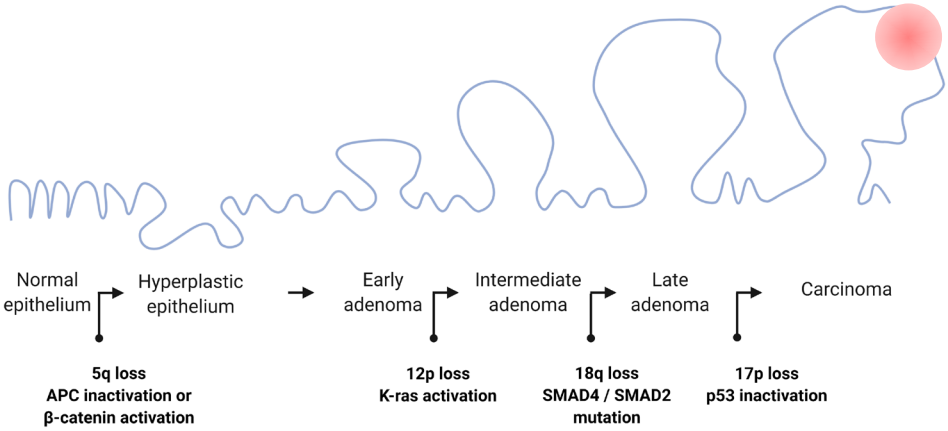


Figure 2. Genetic changes associated with the development of CRC. Adapted from Fearon and Vogelstein 1990.

TNM Classification

The American Joint Committee on Cancer (AJCC) staging manual is the benchmark for classifying patients with cancer as well as for defining prognosis and appropriate treatment (Amin et al. 2017). The combined AJCC/Union for International Cancer Control (UICC) tumor, lymph node, metastasis (TNM) system provides an internationally standardised classification method for the evaluation of cancer in terms of progression of the malignancy (Tables 1 and 2). TNM stages are defined according to the depth of primary tumour invasion, presence or absence of the malignancy in local lymph nodes as well as in distant organs.

Table 1. The Tumor, Nodes, Metastasis (TNM) staging system of colorectal carcinoma.

AJCC UICC 8th Edition	
Primary Tumor (T)	
Tx	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ, intraepithelial or invasion of lamina propria
T1	Tumor invading submucosa
T2	Tumor invading the muscularis propria
T3	Tumor invades through the muscularis propria into pericolorectal tissues
T4	Tumor directly invading other organs or structures
T4a	Tumor penetrating visceral peritoneum
T4b	Tumor directly invading or adhering to other organs or structures
Regional Lymph Nodes (N)	
Nx	Regional lymph nodes cannot be assessed
N0	No lymph node metastasis and no tumor deposits
N1	Metastasis in 1 - 3 regional lymph nodes
N1a	Metastasis in 1 regional lymph node
N1b	Metastasis in 2 - 3 regional lymph nodes
N1c	No regional lymph nodes are positive but there are tumor deposits in the subserosa, mesentery or nonperitonealized pericolic or perirectal/mesorectal tissues
N2	Metastasis in 4 or more regional lymph nodes
N2a	Metastasis in 4 - 6 regional lymph nodes
N2b	Metastasis in 7 or more regional lymph nodes
Distant Metastasis (M)	
M0	No distant metastasis by imaging; no evidence of tumor in other sites or organs (this category is NOT assigned by pathologists)
M1	Distant metastasis
M1a	Metastasis confined to 1 organ or site without peritoneal metastasis
M1b	Metastasis to 2 or more sites or organs is identified without peritoneal metastasis
M1c	Metastasis to the peritoneal surface is identified alone or with other site or organ metastases

Table 2. Prognostic stage grouping.

Staging	T	N	M
Stage 0	Tis	N0	M0
Stage I	T1 - T2	N0	M0
Stage IIA	T3	N0	M0
Stage IIB	T4a	N0	M0
Stage IIC	T4b	N0	M0
Stage IIIA	T1 - T2	N1 / N1c	M0
	T1	N2a	M0
Stage IIIB	T3 - T4a	N1 / N1c	M0
	T2 - T3	N2a	M0
	T1 - T2	N2b	M0
Stage IIIC	T4a	N2a	M0
	T3 - T4a	N2b	M0
	T4b	N1 - N2	M0
Stage IVA	any T	any N	M1a
Stage IVB	any T	any N	M1b
Stage IVC	any T	any N	M1c

Current Treatment

Symptoms of CRC can be vague and include changes in bowel habit such as alternating constipation and diarrhoea. In more advanced disease, fatigue, chronic bleeding and unintended weight loss are common (Majumdar, Fletcher, and Evans 1999). Rectal bleeding and change in bowel habit have a high predictive value for CRC (Hamilton and Sharp 2004). Colorectal cancer can remain asymptomatic for many years, particularly when localised to the proximal colon (Figure 3).

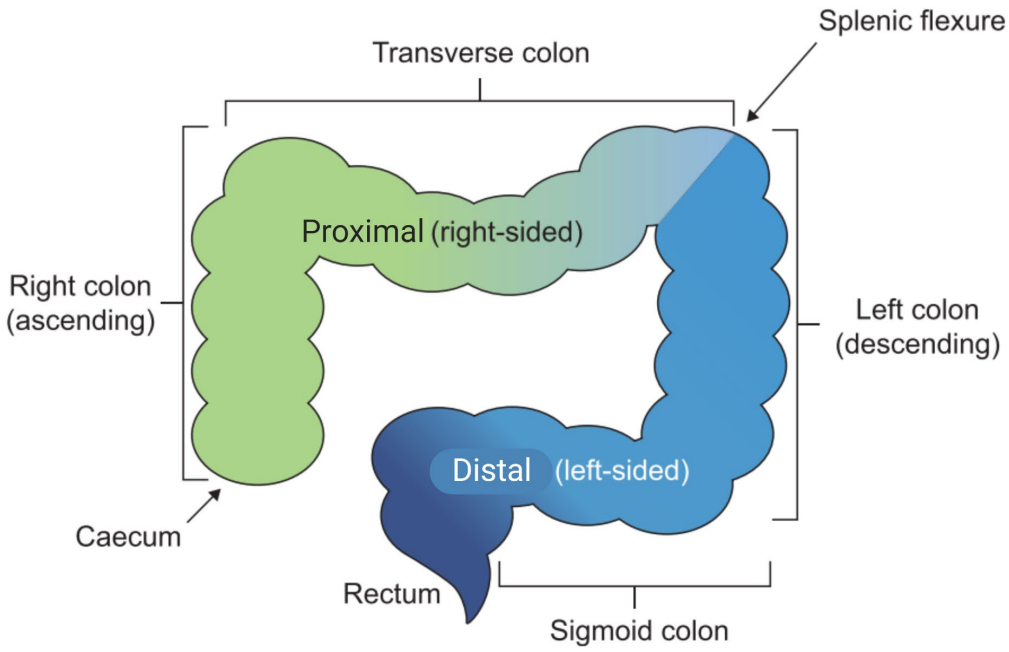


Figure 3. Schematic of proximal (right-sided) and distal (left-sided) colon and rectum. Figure adapted from Stintzing et al. 2017.

The prognosis of patients can be significantly improved with early detection (Table 3). The National Bowel Cancer Screening Program (NBCSP) was introduced in 2006 (Australian Institute of Health and Welfare 2019b) and initially offered free faecal occult blood test (FOBT) kits for eligible Australians aged 55-65. This age range has now been expanded to cover the 50-74 age group, since CRC incidence increases markedly from the age of 50 years. Early, and more recent, evaluation has indicated the NBCSP is

reducing CRC-related deaths through increased detection of early stage cancers (Ananda et al. 2009; Lew et al. 2017).

Table 3. Observed relative survival for colorectal cancer according to TNM staging at diagnosis (Australian Institute of Health and Welfare 2019a).

Stage	5-year relative overall survival
I	98.6%
II	88.6%
III	71.3%
IV	13.4%

Treatment of CRC varies and is highly dependent on stage at diagnosis. Surgery remains the definitive treatment for stage I-III CRCs. For localised disease, adjuvant chemotherapy can reduce the risk of CRC recurrence. In stage III disease, the use of adjuvant 5-fluorouracil chemotherapy provides a 30% relative risk reduction in disease recurrence and a 22 - 32% overall survival (OS) advantage, demonstrating a considerable survival benefit (Benson 2005). Further gains were achieved with the addition of oxaliplatin (André et al. 2004).

The role of adjuvant chemotherapy in the management of stage II CRC is less clear and in practice, use of adjuvant therapy in stage II disease has been limited to those with high risk features such as T4 disease or lymphovascular invasion (Kannarkatt et al. 2017). More recently, detection of tumour-derived fragmented DNA, or circulating tumour DNA (ctDNA), as a biomarker for disease recurrence and treatment impact in early stage CRC has shown great promise in transforming clinical decision-making for early stage CRC (Tie et al. 2016; 2019; Reinert et al. 2019). Analyses from the recently completed DYNAMIC randomised controlled trial (ACTRN12615000381583) and currently

recruiting DYNAMIC-III trial (ACTRN12617001566325) will evaluate the role of ctDNA analysis in guiding adjuvant treatment in patients with stage II and III CRC.

Approximately 25% of newly diagnosed CRC patients have metastatic disease and an additional 20% will develop metastases during clinical follow-up of an initially early stage cancer (Rodriguez-Bigas, Lin, and Crane 2003; Therkildsen et al. 2014). While resection of colorectal liver or lung metastases has the potential to be curative (Primrose 2010; Tomlinson et al. 2007), this is only suitable in a small proportion of the population and metastatic disease is generally managed with palliative intent systemic treatments. The backbone of palliative chemotherapy is a fluoropyrimidine (FP) [given intravenously as 5-fluorouracil (5-FU) or orally as capecitabine]. Standard of care for the majority of metastatic patients is the combination of 5-FU/leucovorin (LV) or capecitabine, with either oxaliplatin (FOLFOX, CAPOX) or irinotecan (FOLFIRI, CAPIRI), typically together with a monoclonal antibody (moAb) against either vascular endothelial growth factor (VEGF) or EGFR (Van Cutsem et al. 2014). The addition of these targeted therapies and exposure of patients to a broader range of cytotoxic agents has increased median survival of patients with mCRC from 6 months to upwards of 30 months (Venook et al. 2017). More recently, emerging data has shown that the use of pembrolizumab in microsatellite-instability high/mismatch repair deficient mCRC, demonstrates a clinically meaningful and statistically significant improvement in progression-free survival (PFS) versus chemotherapy as first-line therapy (Andre et al. 2020). Pembrolizumab also resulted in fewer treatment-related adverse events, providing further support for adoption of immune checkpoint inhibitors as standard of care for this subset of patients.

The introduction of targeted therapies adds further to the challenge of determining the optimal treatment combination for an individual patient. Predictive and prognostic

biomarkers could allow us to better personalise these therapies, thus improving outcomes with more informed treatment sequencing, reducing treatment-related toxicities from the use of ineffective therapies as well as reducing the economic burden associated with these costly therapies.

Thesis Scope and Aims

Colorectal cancer remains one of the most dominant and lethal tumour types in both men and women. While cytotoxic agents form the backbone of treatment for CRC, other therapies have been designed to target processes at the cellular level, such as angiogenesis, or individual proteins, such as EGFR. Despite these advances, there is a critical need to develop better strategies to characterise the patient subgroups most likely to benefit from these targeted therapies. Targeting of the EGFR pathway in the treatment of mCRC relied heavily on our comprehensive understanding of its signaling cascade, however, a greater understanding of CRC biology is required to identify biomarkers and new targets for the next generation of therapies.

Furthermore, for clinical research to be relevant, trials must enrol participants that reflect real-world patients (Mitchell et al. 2015). As few as 3% of adult patients with cancer take part in therapeutic trials (Unger et al. 2014). Selection bias through stringent trial eligibility criteria can skew the characteristics of the trial population (Kalata et al. 2009). There are multiple studies demonstrating that patients who participate in interventional clinical trials are younger and fitter than those in the general population (Jennens, Giles, and Fox 2006; Kennedy-Martin et al. 2015). Ultimately, trial populations may differ significantly from the broader population, diminishing external validity and potentially confounding the relevance and conclusions of oncology trials, with real-world patients

potentially achieving less benefit and experiencing more adverse events than trial participants.

With this background, the objective of this thesis is to explore how real-world data from clinical registries can help to advance biomarker research. The specific aims of this thesis are to:

i. measure tumour levels of the EGFR ligands amphiregulin (AREG) and epiregulin (EREG), to determine if they are associated with mCRC outcomes and investigate correlations with clinical features,
ii. explore the characteristics of CRC biomarker trial participants compared to real-world patients, and to
iii. evaluate the feasibility of conducting a prospective registry-based randomised controlled trial in a real-world mCRC population.

These three projects were selected to examine the utility of clinical registries in a variety of settings: translational research, comparative research, and to support randomised trials.

Chapter 2.

Epidermal Growth Factor Receptor Ligands: Targets for Optimizing Treatment of Metastatic Colorectal Cancer

Foroughi, Siavash, Jeanne Tie, Peter Gibbs, and Antony Wilks Burgess. 2019. "Epidermal growth factor receptor ligands: targets for optimizing treatment of metastatic colorectal cancer." *Growth Factors* doi:10.1080/08977194.2019.1703702.

Published review article

Abstract

The discovery of epidermal growth factor (EGF) and its receptor (EGFR) revealed the connection between EGF-like ligands, signaling from the EGFR family members and cancer. Over the next fifty years, analysis of EGFR expression and mutation led to the use of monoclonal antibodies to target EGFR in the treatment of metastatic colorectal cancer (mCRC) and this treatment has improved outcomes for patients. The use of the *RAS* oncogene mutational status has helped to refine patient selection for EGFR antibody therapy, but an effective molecular predictor of likely responders is lacking. This review analyzes the potential utility of measuring the expression, levels and activation of EGF-like ligands and associated processes as prognostic or predictive markers for the identification of patient risk and more effective mCRC therapies.

Introduction

Ever since the discovery of epidermal growth factor (EGF) (Cohen 1962, 1965; Taylor, Mitchell, and Cohen 1972; Savage, Inagami, and Cohen 1972; Savage, Hash, and Cohen 1973) and its receptor (EGFR) (Carpenter et al. 1975; Cohen, Carpenter, and King 1980; Cohen et al. 1982) there has been a connection between both the EGF-like ligands and the EGFR family members (also called the ErbB or HER family) (Yarden 2001) and cancer (Todaro, Fryling, and De Larco 1980; Osborne, Hamilton, and Nover 1982). Autocrine EGFR-ligands such as TGF-α appear to drive a number of cancers with oncogenic *RAS* mutations (Tang, Steck, and Yung 1997; Kenny and Bissell 2007), however, the autocrine, juxtacrine, paracrine (Wilson et al. 2012; Singh and Harris 2005), exosomal (Higginbotham et al. 2011) and cross-talk (Quesnelle, Boehm, and Grandis 2007) roles of EGFR-ligands suggests a much broader role for the EGF pathway in the progression of many cancers (Sigismund, Avanzato, and Lanzetti 2018; Tomoshige et al. 2018).

Over the last decade there has been a wide-ranging search for the association between particular EGFR-ligands, such as amphiregulin (AREG) and epiregulin (EREG), with the progression and treatment response of metastatic colorectal cancer (mCRC) (Jacobs et al. 2009; Jonker et al. 2014; Khambata-Ford et al. 2007; Seligmann et al. 2016; Stintzing et al. 2018). Whilst there are some clues that the tumor-associated levels of the EGF-like ligands AREG and EREG correlate with patient responses to treatment with EGFR inhibitors (Jacobs et al. 2009), more reagents, evidence, and a better understanding of the roles of the EGF-like ligands and receptor members in oncogenic signaling appear to be required before improvements to cancer treatments targeting this system are likely.

The EGFR family signaling pathways are among the most commonly dysregulated pathways in solid tumors, including colorectal cancer (CRC) (Han and Lo 2012). EGFR-family signaling plays a pivotal role in both tumor growth and progression (Tomas, Futter, and Eden 2014; Yewale et al. 2013). The EGFR family consists of four members belonging to the ErbB family of receptor tyrosine kinases (RTK): ErbB1/HER1, also known as EGFR; ErbB2/HER2; ErbB3/HER3 and; ErbB4/HER4 (Roskoski 2014). In the absence of ligand-binding, EGFR remains in a basal state whereby the native conformation of its extracellular domain suppresses its intracellular kinase activity (Markman et al. 2010; Kovacs et al. 2015). Activation of signaling from the EGFR family occurs through ligand binding (Walker et al. 2012), or oncogenic mutations (Purba, Saita, and Maruyama 2017).

The binding of ligands to an EGFR family member (except HER2) induces a conformational change allowing homo- or hetero-oligomerization (Burgess 2008). Ligands in the EGFR family include epidermal growth factor (EGF) (Cohen 1965), transforming growth factor-α (TGFα) (Derynck et al. 1984), AREG (Shoyab et al. 1989), EREG (Toyoda et al. 1995), betacellulin (BTC) (Shing et al. 1993), heparin-binding EGF-like growth factor (HB-EGF) (Higashiyama et al. 1991), epigen (EPGN) (Strachan et al. 2001) and a family of EGF-related ligands for ErbB3 and ErbB4: the neuregulins 1-4 (NRG-1, NRG-2, NRG-3 and NRG-4) (Holmes et al. 1992; Peles et al. 1992; Wen et al. 1992; Marchionni et al. 1993; Falls et al. 1993; Goodearl et al. 1993). All ligands, except the neuregulins which exclusively bind ErbB3 and ErbB4, are known to bind to the EGFR (Singh, Carpenter, and Coffey 2016). ErbB2 has no known ligand (Schneider and Wolf 2009). Although EGF-like precursor proteins are present in many tissues, these precursors require processing by enzymes such as the ADAM (a disintegrin and metalloprotease) family of proteins (Blobel 2005).

The role of the EGFR in malignant progression has been reviewed extensively (Goffin and Zbuk 2013; Schneider and Wolf 2009; Wee and Wang 2017). Here, we focus on the current understanding of the roles of EGFR-family signaling pathways, the ligands and their processing system components as prognostic and predictive factors and attempt to identify which proteins could be used to target mCRC. We discuss the evolution of colorectal tumors with an excess of an EGFR-family member and the role(s) the ligands or ligand processing might be playing in the progression of these tumors.

Epidemiology and incidence of colorectal cancer: the challenge

Colorectal cancer is the third most common cancer worldwide and the second highest cause of cancer mortality, accounting for approximately 1.8 million new cases and 881,000 deaths in 2018 (Bray et al. 2018). Incidence is particularly high in developed countries: in Australia, an estimated 16,398 new cases of CRC are expected to be diagnosed in 2019 and CRC remains the second most common cause of cancer mortality in Australia (Australian Institute of Health and Welfare 2019).

It has been reported that approximately 25% of newly diagnosed CRC patients have metastatic disease and an additional 20% will develop metastases during clinical follow-up of an initial early-stage cancer (Rodriguez-Bigas, Lin, and Crane 2003; Therkildsen et al. 2014). At present the overall 5-year survival for patients with metastatic, or stage IV, CRC is less than 10% and it is estimated that up to 50% of all CRC patients will ultimately die as a result of their metastatic disease (Rodriguez-Bigas, Lin, and Crane 2003).

Metastatic colorectal cancer treatment

Historically, CRC has been treated using a combination of surgery, radiotherapy and/or systemic treatments (Heinemann et al. 2013). In mCRC, the standard treatment for most

patients involves fluoropyrimidine-based chemotherapy, most often given in combination with oxaliplatin or irinotecan. Depending on size, number and location of CRC metastases, the aims of treatment are primarily to prolong life, palliate symptoms, improve quality of life, delay disease progression and, in limited cases, to cure (Salvatore et al. 2017). Surgical intervention is dependent on numerous factors such as patient performance status and extent of the metastatic disease, which most commonly involves liver and lung (Egner 2017). In recent times, the ability to target tumor vasculature and key oncogenic pathways in CRC has significantly altered the treatment paradigm for patients with mCRC, where agents that target angiogenesis or the EGFR have now been incorporated into routine care (Hutchinson et al. 2015). The introduction of these biologics has improved chemotherapy efficacy and, consequently, the outcomes of these patients (Hurwitz et al. 2004; Van Cutsem et al. 2011).

Selecting patients for specific targeted therapies

Biomarkers are measurable indicators that aim to precisely, reproducibly and objectively distinguish either a normal biological state from a pathological state, or to predict the response of specific patient sub-groups to particular interventions (Biomarkers Definitions Working Group. 2001). Biomarkers are used for many purposes including: (1) diagnostic markers, that allow early detection/confirmation of disease; (2) prognostic markers, that can describe the likely course of a given disease; and (3) predictive markers, that predict for treatment safety and/or efficacy outcomes (Heinemann et al. 2013).

The potential of predictive biomarkers to improve treatment selection make them a vital tool in the field of personalized medicine (Begg and Tavassoli 2017) and health economics (Seo and Cairns 2018). Predictive biomarkers allow us to define patient sub-populations who are the most or least likely to benefit from targeted therapies; thus,

improving the efficacy and/or cost-effectiveness of specific treatments (Seo and Cairns 2018; Harty, Jarrett, and Jofre-Bonet 2018) while avoiding potential toxicity in a patient population unlikely to benefit from treatment (Duffy and Crown 2008).

While detection of a *RAS* mutation is of high utility as a negative predictor for response to EGFR-inhibitors (Tan and Du 2012), absence of a mutation does not necessarily predict for treatment response (discussed in later sections). Additional biomarkers to accurately predict response to targeted therapy are required to further reduce toxicity and economic burden.

ErbB family receptors

The epidermal growth factor receptor (EGFR, or ErbB1) was characterized in the 1980's as a 170 kDA molecular weight transmembrane glycoprotein (Cohen et al. 1982), consisting of an intracellular domain; a single hydrophobic transmembrane domain; and a large extracellular domain. The intracellular domain includes a juxtamembrane domain, where feedback attenuation from protein kinase C occurs; a highly conserved, tyrosine kinase domain; and a tyrosine-rich carboxy-terminal tail. The extracellular domain is organized as a tandem repeat of two types of subdomains, (i) a leucine-rich segment responsible for ligand binding and (ii) a cysteine-rich domain, one of which is involved in homo- and hetero-dimerization with other ErbB family members (Cohen 1983; Carpenter and Cohen 1990). The ErbB family consists of four related membrane receptors, namely, EGFR (ErbB1), ErbB2, ErbB3 and ErbB4 which all share the overall primary structure of EGFR (Figure 1).

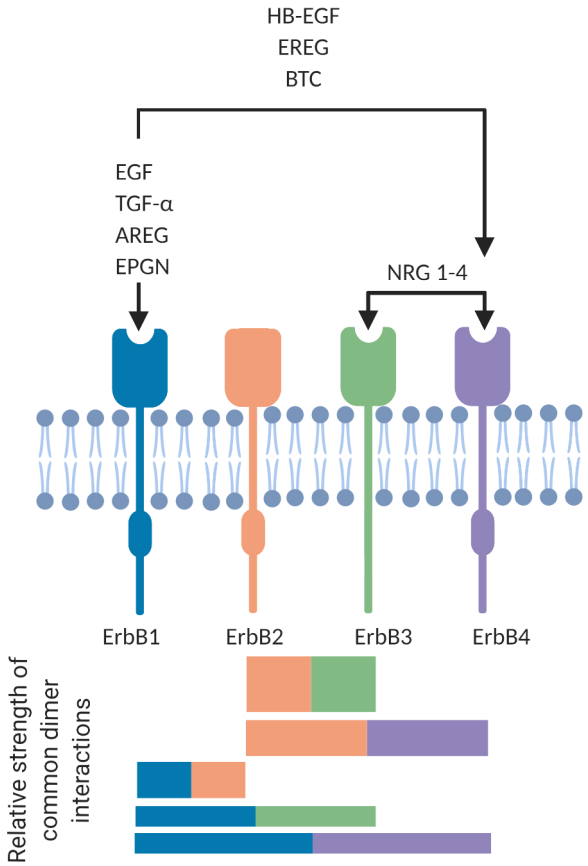


Figure 1. ErbB family members, their corresponding ligands and hierarchy of common dimerization partners. The ErbB family of receptor tyrosine kinases includes four structurally related members; ErbB1 (EGFR; HER1), ErbB2 (HER2), ErbB3 (HER3), ErbB4 (HER4). Despite commonalities in structure; ErbB2 has no known activating ligands and ErbB3 has no, or weak, tyrosine kinase activity. For simplicity, the schematic above shows receptor as monomers and their binding ligands are denoted above. Although all dimer combinations are possible, the mitogenic activity of these interactions varies. Common inter-receptor interactions are shown below the receptors where size of bands indicate the relative potency of interactions. Figure created with BioRender.

However, the ErbB family members have structural and functional differences which are important for their roles in signaling. ErbB1, ErbB2, and ErbB4 have functional tyrosine kinase domains, whereas ErbB3 has weak or no tyrosine kinase activity. Except for ErbB2, the extracellular regions can bind different ligands. Despite ErbB2 lacking the ability to bind a ligand, it is the preferred dimerization partner for the other ErbB family members and the ligand-activated dimers (or higher-order oligomers) are capable of

stimulating potent and prolonged signaling (Graus-Porta et al. 1997). ErbB2's native conformation resembles the EGFR-ligand-activated state: the ligand binding site is closed, but the dimerization loop protrudes to capture another EGFR-family member (Garrett et al. 2003). Different EGFR-family dimers or higher order oligomers are likely to drive CRC cells in different ways; where particular EGFR-family members predominate, the effects of activated autocrine or paracrine EGF-like ligands on cancer cells are likely to be different.

ErbB family members are often implicated in human cancer where EGFR, ErbB2, and ErbB3 are validated therapeutic targets (Lemmon, Schlessinger, and Ferguson 2014). Deficiencies of EGFR, ErbB2, ErbB3, and ErbB4 can have dramatic effects on physiology; these have been described in mouse model experiments (Sibilia et al. 1998; Sibilia and Wagner 1995; Strunk, Amann, and Threadgill 2004). Furthermore, mice lacking ErbB2, ErbB3, or ErbB4 display abnormal neuro and cardiac development and are all embryonic lethal. In most cases, even where an EGFR-family member is over-expressed, ligands are required for activation of the receptor kinase (Sibilia et al. 2007; Burgess 2008). An understanding of the stimulatory roles of the EGF-family ligands is not complete until we have identified the regulatory systems for expression, biosynthesis, processing and spatial distribution of the ligands (Singh, Carpenter, and Coffey 2016). Indeed, some of the ligand processing systems appear to be targets for controlling ligand stimulation of many cancer cells.

EGFR-family ligands

At least twelve different EGF-like growth factors have been identified (Roskoski 2014), seven of which regulate EGFR: EGF, TGF-α, BTC, HB-EGF, AREG, EREG, and EPGN (Singh, Carpenter, and Coffey 2016). The EGFR ligands are synthesized as type 1

transmembrane proteins, and proteolytic cleavage is required to release soluble, active ligands (Singh and Harris 2005). The transfer of the pro-ligands to the plasma membrane is controlled in part by the rhomboid proteins (Lee et al. 2001). The levels of pro-ligand available for cleavage will depend on the level of expression from the ligand gene and the activity of the particular rhomboid family member (Karachaliou and Rosell 2018). The level of ligand mRNA expression is controlled by transcriptional activation associated with the activation of p120 and/or β-catenin (Liang et al. 2017), which can occur when E-cadherin interactions are destroyed, e.g. during cell death (Georgopoulos, Kirkwood, and Southgate 2014) (Figure 2).

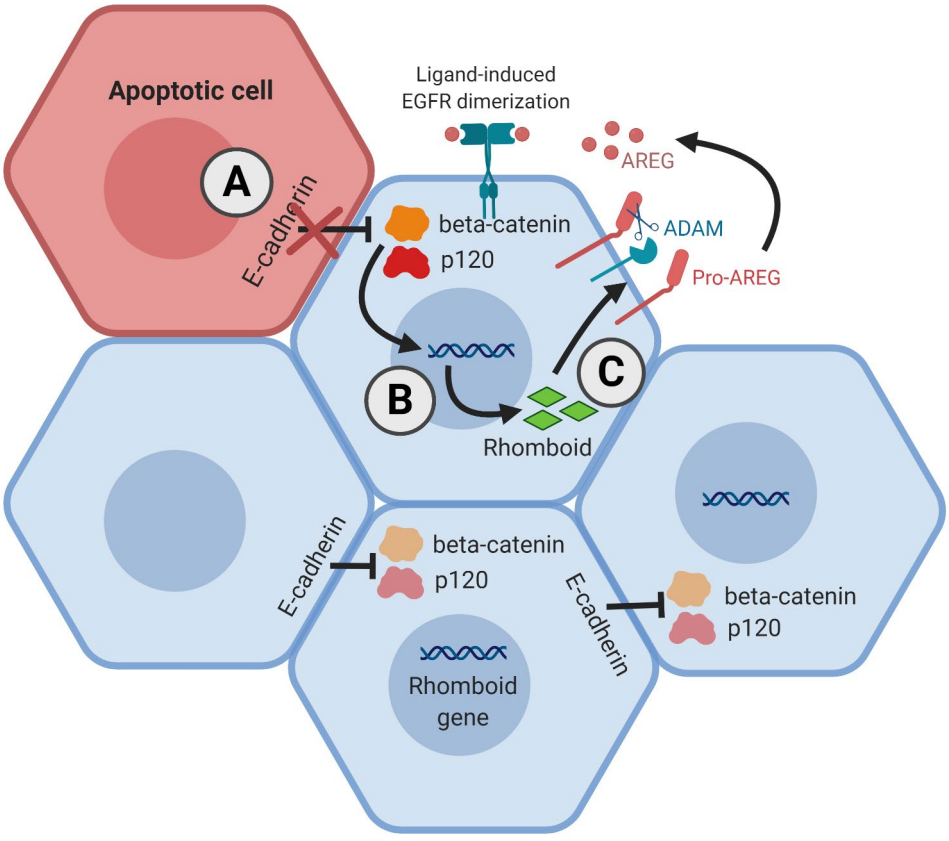


Figure 2. Disruption of E-cadherin interactions facilitates ligand mRNA expression, secretion and activation. Loss of E-cadherin, through cellular damage or death, releases p120 and/or β-catenin in nearby cells (**a**) which can induce rhomboid (rho) (**b**). Rho is involved in transfer of pro-ligands to the plasma membrane for processing (**c**). Figure created with BioRender.

In mammalian systems, ligand proteolysis at the cell surface is mediated by ADAM family metalloproteases (Singh, Carpenter, and Coffey 2016; Burgess 2008) (Figure 3). The ADAM proteases are membrane-anchored glycoproteins that also play a critical role in embryonic development (Blobel 2005). These proteases appear capable of activating several signaling systems during embryonic development so targeting these enzymes may have effects beyond signaling via EGFR-ligands e.g. activation of notch-ligands (Seals and Courtneidge 2003). However, targeting the rhomboid family members with elevated expression in mCRC, such as RHBDD2 (Lacunza et al. 2012), may lead to more specific inhibition of EGFR-ligand-activated proliferation in mCRCs.

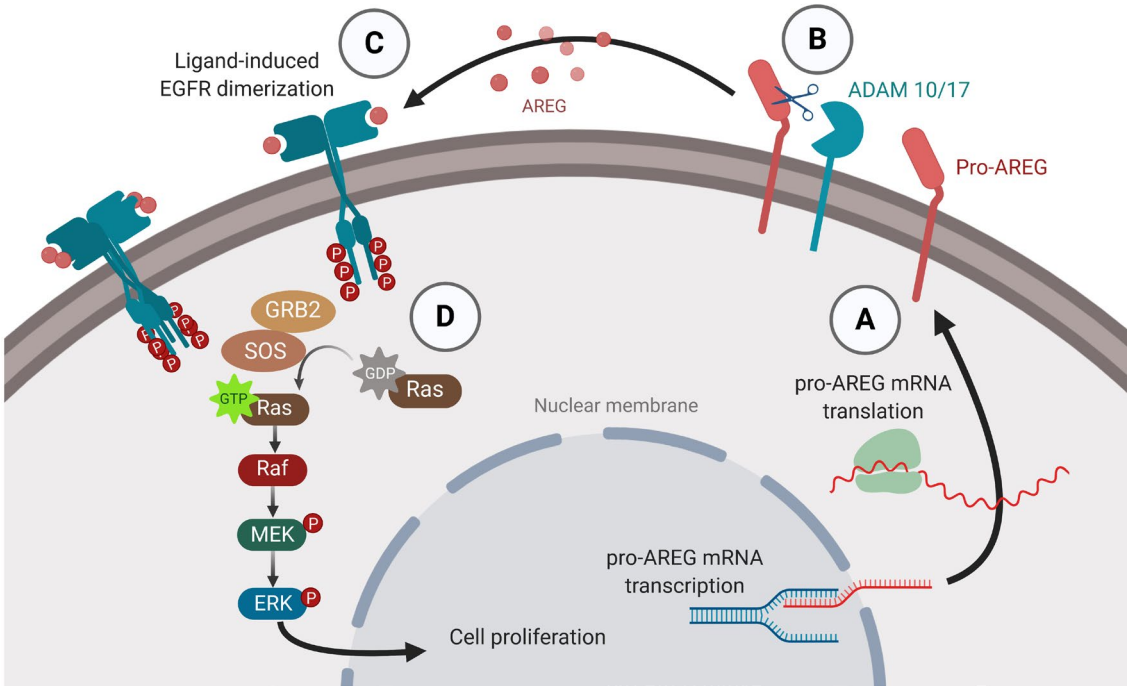


Figure 3. Mechanism of ErbB pro-ligand processing, receptor activation, and signaling cascade. Transcription, translation and transfer of pro-ligand is controlled by a combination of (i) endogenous ligand expression and (ii) activation and activity of associated rhomboid family members (**a**; refer Figure 2). ADAM-mediated shedding of pro-ligand, here shown as pro-AREG, releases the mature ligand which can bind the ErbB receptor through either paracrine or autocrine signalling (**b**). Autocrine signalling represented above (**c**). Receptor dimerization stimulates the intrinsic protein kinase activity of EGFR, resulting in the autophosphorlyation of 6 key tyrosine residues in the cytoplasmic domain (**d**). Five of these residues function as docking sites for downstream signalling proteins; the sixth residue is involved in downregulation of ligand-induced signalling. Figure created with BioRender.

In mouse embryonic cells, ADAM17, also known as Tumour-Necrosis Factor-α converting enzyme (TACE), is the main sheddase for EREG, TGF-α, AREG, HB-EGF, and the neuregulins, however, it is unclear if ADAM19 also plays a role in neuregulin processing (Horiuchi et al. 2005; Kurohara et al. 2004). ADAM10 appears to be responsible for the shedding and activation of EGF and BTC (Sahin et al. 2004). Although juxtacrine signaling by membrane-bound EGFR ligands, such as TGF-α, has been observed (Anklesaria et al. 1990), metalloprotease activity is required for autocrine and paracrine signaling to occur (Gee and Knowlden 2003). Metalloprotease-dependent EGFR-family signaling, can be disrupted by protease inhibitors, eloquently demonstrated using the metalloprotease inhibitor Batimastat (BB-94) (Dong et al. 1999). This inhibitor blocks EGFR-dependent cell proliferation and migration of human mammary epithelial cells, i.e. the growth of primary tumors and the migration of cells into metastatic sites. Cell proliferation was inhibited in direct proportion to the inhibition of EGF release and the consequential signaling, however, the inhibition of autocrine ligand stimulated proliferation and migration by the protease inhibitors is rescued by exogenous EGF (Dong et al. 1999).

AREG and EREG were first discovered by Shoyab et al., (Shoyab et al. 1989) and Toyoda et al., (Toyoda et al. 1995) respectively. Both genes are co-localized on chromosome 4q13.3. Although both ligands have relatively weak binding affinity for the EGFR, they stimulate potent and prolonged receptor activation (Shelly et al. 1998). Presumably, in these situations, the levels of the ligand processing enzymes are sufficient to allow ligand activation and subsequent receptor stimulation.

The precursor AREG protein (pro-AREG) is synthesized as a 252 amino acid transmembrane precursor that contains several cleavage and glycosylation sites which

can influence its biological activity (Brown et al. 1998). Through sequential ectodomain cleavage by TACE, which is also localized on the cell surface, AREG is released in its mature, soluble, form (i.e. an 84 amino acid glycoprotein) (Ohchi et al. 2012). The EREG precursor is also cleaved by TACE leading to the 46 amino acid form (Toyoda et al. 1995; Sahin et al. 2004). As with EGF, pro-AREG and -EREG can act as signal transducers through juxtacrine signaling (Singh and Harris 2005).

As ligands of EGFR and ErbB4, AREG and EREG mediate proliferation and differentiation in different cell types. In CRC, activation of EGFR by ligands stimulates transcriptional activation of genes associated with the oncogenic phenotype, i.e. proliferation, anti-apoptosis, angiogenesis and migration (Wee and Wang 2017). It is important to establish whether treatments aimed at interfering with the autocrine stimulation by AREG and/or EREG are likely to improve outcomes for patients and whether the levels of the ligands or their processing enzyme(s) might be useful predictors of tumor response to (i) EGFR, ErbB2 or ErbB3 inhibitor/antagonists; (ii) ADAM10- or ADAM17-inhibitors or (iii) EGFR-family signaling inhibitors (e.g. PI3K, BRAF, MEK or ERK inhibitor combinations).

Perturbation of growth factor signaling is a major driver of CRC (and many other cancers). In many patients, constitutive, elevated, autocrine signaling appears to be a major driver of the uncontrolled growth of the tumor cell population (Bernat-Peguera et al. 2019). However, other steps in the ligand/receptor stimulating system are also perturbed by mutation of transcriptional control or processing. Where these perturbations lead to ligand deprivation, the cellular responses inevitably up regulate other members of signaling pathways to compensate or overcome the availability of ligand (Sun and Bernards 2014). There is increasing evidence that the overexpression and dysregulation

of ADAMs play an important role in cancer. For example, overexpression of ADAMs 9, 10, 15, 17 and 19 have been observed in both primary and metastatic CRC tumors when compared with normal colonic tissue (Merchant et al. 2008). The processing of the pro-ligands relies on cleavage by the ADAM proteases (Zhou et al. 2005). If pro-ligand levels are reduced an increase in the levels of specific ADAMS may drive the production of more active ligand in the tumor environment. Analysis of the mRNA expression levels for CRC patients in the TCGA database (http://cancergenome.nih.gov/abouttcga) (National Cancer Institute 2018) indicates elevated levels for ADAMs 9, 15 and 19, so these enzymes might be significant targets for inhibiting autocrine ligand production and consequential EGFR activation in CRC.

The mRNA for the EGFR ligands AREG, EREG and HB-EGF are upregulated in many CRC cases, and likely to be drivers of tumor growth. Inhibitors of the processing enzymes for these ligands and EGFR inhibitors should be drug candidates for these patients (Richman and Jasani 2019). Although the mRNA for the EGFR is not elevated in the tumors of many CRC patients, it is interesting to note that there are patients with significantly elevated levels of ErbB2 mRNA (Figures 4 and 5; see also Duffy et al. 2011).

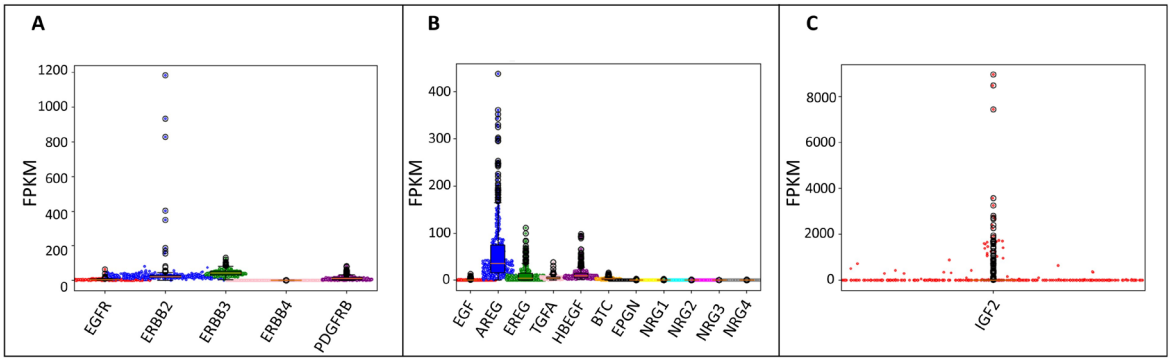


Figure 4. RNA Seq data of EGF receptor family members and related ligands and receptors. Data derived from 513 CRC patients from the TCGA database. FKPM values for EGFR, ErbB2, ErbB3, ErbB4 and PDGFRβ (**A**); EGF-family ligands (**B**) and; Insulin-like growth factor 2 (**C**). FKPM: Fragments Per Kilobase Million.

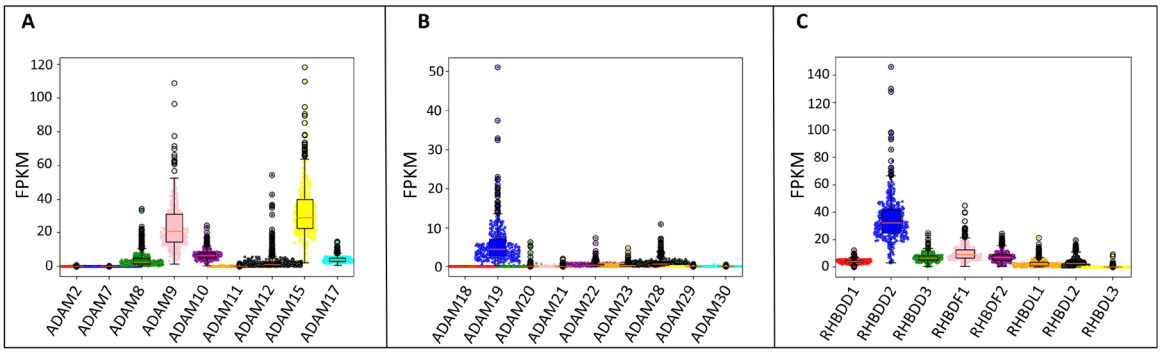


Figure 5. RNA Seq data of ADAMs and Rhomboid family members. Data derived from 513 CRC patients from the TCGA database. FKPM values for ADAM family members (**A** and **B**), and Rhomboid family members (**C**). FKPM: Fragments Per Kilobase Million.

Perhaps the EGF-like ligands normally stimulate EGFR:ErbB2 heterodimers and where the ligand availability is reduced, tumor cells compensate by elevating the ErbB2 levels. If this response is chronic and the tumor cells over-compensate, the elevated levels of the receptors likely lead to constitutive stimulation (Wang et al. 2014). The CRC with elevated ErbB2 mRNA should be sensitive to ErbB2 receptor or kinase inhibitors. Signaling from the EGFR family of receptors is complex: the receptor family members EGFR, ErbB2, ErbB3 and ErbB4 can form heterodimers and higher-order oligomers capable of stimulating cell viability, proliferation and migration (Mitchell, Luwor, and Burgess 2018). Although the EGFR appears to be a major component of CRC biology, other members of the EGFR family could drive signaling through heterodimers. In this context, it is interesting to note the correlation between the levels of the ErbB4 mRNA and the mRNA levels of one of its ligands – Neuregulin 3 (NRG3) in a significant number of patients (Figure 6). It would be interesting to know if anti-ErbB4 drugs (antibodies or kinase inhibitors) inhibit the growth of the CRC cells with ErbB4:NRG3 overexpression.

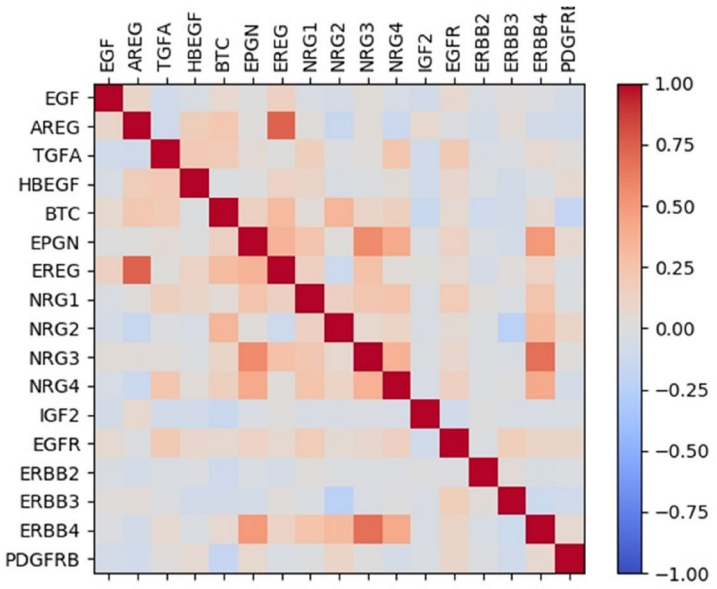


Figure 6. Correlation of the levels of mRNA for EGF receptor family members and related ligands and receptors. Data derived from 513 CRC patients from the TCGA database.

The EGFR family stimulates cellular processes via its ligand-regulated tyrosine kinase activity (Lemmon, Schlessinger, and Ferguson 2014). There are many receptor tyrosine kinases and although they have specific regulatory ligands and preferred substrates, there is a functional interaction between the signaling pathways which allows the activation of a different receptor kinase to compensate for the normal regulatory receptor (Lemmon and Schlessinger 2010; Sun and Bernards 2014). In some CRCs the levels of IGF2 mRNA are elevated significantly (Figure 4C); it would be interesting to know the sensitivity of these tumors to IGF2R antagonists. Apart from ErbB2, the only other receptor tyrosine kinase mRNA level elevated in the TCGA CRC tumors is PDGFRβ; the levels of PDGFRβ are elevated in a subset of glioblastomas and it has been proposed that this receptor drives the tumorigenicity of these tumors (Cantanhede and De Oliveira 2017; Cao 2013); again it would be interesting to know if the CRCs with elevated PDGFRβ could be inhibited by PDGFR antagonists.

Overexpression of ADAMs has been reported in the breast (Lendeckel et al. 2005), gastric (Carl-McGrath et al. 2005) and pancreatic ductal adenocarcinomas (Grützmann et al. 2004) as well as renal cell carcinomas (Roemer et al. 2004). Dysregulation of ADAMs may also result through the downregulation of endogenous inhibitors, such as the tissue inhibitor of metalloproteinases (TIMP) family members. In particular, TIMP-3 is often undetectable in tumors due to increased promoter methylation, correlating with disease progression (Hoque et al. 2008).

Twelve independent studies have reported on the importance of AREG and/or EREG as biomarkers for predicting treatment response and survival outcomes for cancer patients (Table 1) (Khambata-Ford et al. 2007; Jacobs et al. 2009; Saridaki et al. 2011; Pentheroudakis et al. 2013; Strimpakos et al. 2013; Cushman et al. 2015; Seligmann et al. 2016; Stahler et al. 2016; Llovet et al. 2015; Baker et al. 2011; Stintzing et al. 2018; Jonker et al. 2014; for a related meta-analysis please see Jing et al. 2016). However, predictive and prognostic effects in most of these studies (Khambata-Ford et al. 2007; Jacobs et al. 2009; Saridaki et al. 2011; Pentheroudakis et al. 2013; Strimpakos et al. 2013; Llovet et al. 2015) could not be separated due to lack of untreated (i.e. control) patients in the study populations. Two studies independently reported that the increased expression of AREG and EREG are strongly associated with therapeutic benefit of anti-EGFR therapy in mCRC (Khambata-Ford et al. 2007; Jacobs et al. 2009); high ligand expression has also been associated with better survival in *BRAF* wild-type patients treated with the anti-angiogenic agent bevacizumab (Genentech/Roche, South San Francisco, CA, USA) (Stintzing et al. 2018). However, many of these studies used mRNA expression levels or the total levels of AREG or EREG protein; it may be necessary to measure the levels of the activated (i.e. proteolytically cleaved) forms of these proteins, before their prognostic or predictive value can be fully realized.

Table 1. Summary of studies examining AREG and EREG expression in mCRC.

Author (publication date)	Therapy	ITT (Analyzed*)	Study Type & Population	Notable Population Characteristics	Hazard Ratio (p-value) AREG high vs low		Hazard Ratio (p-value) EREG high vs low	
					OS	PFS	OS	PFS
Khambata-Ford (2007)	CET	110 (80)	Prospective cetuximab monotherapy trial	38% *KRAS* mutant	NR	0.44 (<0.001)	NR	0.47 (0.0002)
Jacobs (2009)	CET±CT	220 (203^{A}; 200^{E})	Post-hoc analysis of four cetuximab trials	Chemorefractory metastatic colorectal cancer; EGFR IHC+	0.40 (<0.0001)	0.43 (<0.001)	0.42 (<0.0001)	0.41 (<0.001)
Saridaki (2011)	CT+CET	112 (106^{A}; 105^{E})	Single institution retrospective study	33% *KRAS* mutant	0.59 (0.013)	0.59 (0.018)	0.56 (0.004)	0.48 (0.002)
Baker (2011)	CET	326 (144)	Post-hoc analysis of three cetuximab monotherapy studies	*KRAS* wild-type	NR	NR	NR	NR
Pentheroudakis (2013)	CET±CT	226 (168^{A}; 160^{E})	Multi-center retrospective cohort study	32% *KRAS* mutant	0.47 (0.0002)	NR	0.45 (0.0009)	NR
Strimpakos (2013)	CET±CT	226 (168^{A}; 160^{E})	Multivariate analysis of Pentheroudakis (2013) cohort with additional mRNA biomarkers		0.17 (<0.0001)	NR	0.38 (0.006)	NR
Jonker (2014)	BSC±CET	572 (385)	Post-hoc analysis of CO.17 CET vs BSC RCT	42% *KRAS* mutant	NR	NR	0.82^ (0.24)	0.80^ (0.16)
Cushman (2015)*	CT±CET	238 (103)	Post-hoc analysis of CALGB 80203 RCT	43% *KRAS* mutant; Previously untreated mCRC	1.01 (0.923)	0.91 (0.144)	0.94 (0.212)	0.89 (0.016)
Llovet (2015)	CT+CET/PAN	105 (51)	Retrospective multi-center cohort study	*RAS* wild-type	0.50 (0.05)	0.30 (0.001)	0.80 (0.053)	0.60 (0.09)
Seligmann (2016)*	CT±PAN	696 (173)	Prospectively planned biomarker analysis of PICCOLO RCT	Analysis limited to *RAS* and *BRAF* wild-type	0.94 (0.18)	0.97 (0.50)	0.87 (0.001)	0.94 (0.16)
Stahler (2016)	CT	479 (192)	Post-hoc analysis of FIRE-1 RCT	51% *RAS* mutant; No exposure to targeted therapies	0.72 (0.11)	0.62 (0.03)	0.48 (<0.001)	0.62 (0.002)
Stintzing (2018)	CT+BEV	472 (331)	Post-hoc analysis of AIO KRK-020 RCT	50% *RAS* mutant	0.64 (0.001)	0.80 (0.18)	0.66 (0.001)	1.10 (0.65)

BEV, bevacizumab; BSC, best supportive care; CET, cetuximab; CT, chemotherapy; IHC, Immunohistochemistry; ITT, overall intention-to-treat population; NR, not reported; PAN, panitumumab; RCT, Randomised controlled trial. If the number of analyzed patients for each ligand differed, and was reported, they are denoted by ^{A} and ^{E} for AREG and EREG, respectively.

^ Hazard ratios reported for BSC group only.

* Expression of AREG and EREG analyzed as continuous variable.

ErbB mediated signaling

Upon ligand binding, activation of the intrinsic EGFR-tyrosine kinase activity occurs. This results in tyrosine phosphorylation of the receptor family members, as well as many proteins, in the intracellular signaling pathways. The phosphorylated form of the EGFR leads to the initiation of a mitogenic signaling cascade through three main axes: (1) the *RAS*/*RAF*/MEK/mitogen-activated protein kinase (MAPK) pathway, cell-cycle progression and cell proliferation (Yewale et al. 2013); (2) the phosphatidylinositol 3-kinase (PI3K) pathway, resulting in AKT-mediated activation of mTOR, which is responsible for anti-apoptosis and pro-survival signals (Tomas, Futter, and Eden 2014); and (3) the activation of the trafficking of the EGFR-family members and their associated ligands to endosomes, lysosomes and/or recycling (Sigismund, Avanzato, and Lanzetti 2018). All of these processes can affect the biology of the cancer cells.

The activation of various ErbB family member heterodimers as a result of ligand binding can determine the effect of the ligand on the cell in a wide range of malignant processes relevant to tumor growth, i.e. cell division, proliferation, angiogenesis, migration, metastases or apoptosis (Ullrich and Schlessinger 1990; Vallböhmer and Lenz 2005).

Epidermal growth factor receptor inhibitors: targeting cellular development and regulation

Cetuximab (Imclone Systems, New York, NY, USA) and panitumumab (Amgen, Thousand Oaks, CA, USA), are monoclonal antibodies against the extracellular domain of the EGFR; the former a murine/human chimeric protein and the latter a fully humanized antibody (Galizia et al. 2007; Yang et al. 1999, 2001). Cetuximab and panitumumab bind EGFR with high affinity, preventing endogenous ligands from

binding. For some tumors, these antibodies result in a decrease in signaling from the EGFR, inhibition of cell proliferation and in conjunction with other anti-cancer agents, induction of apoptosis and inhibition of cell migration and metastasis (Bou-Assaly and Mukherji 2010).

The introduction of targeted therapies such as EGFR inhibitors in first- and subsequent-line treatment has improved overall survival (OS) for patients diagnosed with mCRC (Van Cutsem and Köhne 2009; Van Cutsem et al. 2011; Douillard et al. 2010). Although the mutation status of the *RAS* oncogenes is a known predictive biomarker of resistance to anti-EGFR therapy (Lièvre et al. 2006), there is a need to further characterize the underlying biological and clinical factors that can effectively predict outcomes.

Predicting treatment responses for patients with metastatic colorectal cancer

Molecular features and primary tumor location

The intracellular signaling *RAS* proteins (*HRAS*, *KRAS* and *NRAS*) are proto-oncogenes that function as molecular switches regulating pathways involved in cell proliferation and survival (Han, Jeong, and Jang 2017). In many cancers, including CRC, the *RAS* proteins are frequently mutated to an active form at codon 12, 13 or 61 (Quinlan and Settleman 2009); *KRAS* is the most frequent (Prior, Lewis, and Mattos 2012). Where an activating mutation occurs, the resulting protein is constitutively active, driving cell proliferation and survival (Vecchione et al. 2011). Up to 40% of mCRC patients have *KRAS* mutations and these patients do not respond to treatment with EGFR inhibitors (Shaib, Mahajan, and El-Rayes 2013).

In 2008, exon two *KRAS* mutations were identified as the first predictive biomarker for the use of targeted treatment of mCRC (Richman et al. 2017). Oncogenic *KRAS* mutations

are strongly associated with resistance to anti-EGFR antibody therapy (Lièvre et al. 2006; Douillard et al. 2010; Lièvre et al. 2008). Further, there is evidence that in patients with *KRAS* mutated tumors, the addition of an EGFR antibody to their treatment may possibly yield inferior progression-free survival (PFS) outcomes (Douillard et al. 2014, 2010), but this has not been consistently seen. A further 20% of patients who are *KRAS* exon two wild-type will harbor other *RAS* mutations and thus are not expected to benefit from EGFR-directed therapy (Sorich et al. 2015). HER2 amplification and downstream alterations, such as *BRAF* mutation, are also associated with lack of response to treatment with anti-EGFR monoclonal antibodies, supporting broader molecular assessment before initiation of treatment (Pietrantonio et al. 2015; Raghav et al. 2019).

Although our understanding of *RAS* mutations as a biomarker of resistance to anti-EGFR antibody therapy has allowed the treatment to be restricted to *RAS* wild-type patients, only 10-17% of *KRAS* wild-type patients respond to targeted EGFR monotherapy (Karapetis et al. 2008; Cunningham et al. 2004; Van Cutsem et al. 2007). Notably, the response rate in these patients is relatively low, with some *KRAS* wild-type patients showing no response (Karapetis et al. 2008). The ineffectiveness of EGFR inhibitors in a large proportion of mCRC patients with wild-type *KRAS,* could be due to the sensitivity of detection of low-frequency *KRAS* mutations (Tougeron et al. 2013). However, other factors such as the induced expression of EGF-like ligands and the ligand processing enzymes might also interfere with the antibody responses. It is important to determine whether the targeting of these proteins might increase the sensitivity of CRC to EGFR antagonists or inhibitors and whether the levels of these proteins (i.e. the ligands and/or processing enzymes) can identify patients who are likely to respond to EGFR-based targeted therapies.

In CRC, primary tumor location is defined anatomically where right-sided cancers arise from the cecum to transverse colon and left-sided cancers are located from the splenic flexure to the rectum. The physiologic basis for this differentiation can be attributed to the different embryologic origins and microenvironments (Lee, Menter, and Kopetz 2017; Missiaglia et al. 2014; Zhang et al. 2018), further supporting that right- and left-sided colon cancers represent distinct genetic entities (Bufill 1990).

Emerging data suggest that primary tumor location may impact response to EGFR therapy. Post-hoc analyses of 3 randomized mCRC trials showed that patients with left-sided mCRC received greater benefit from the addition of cetuximab therapy compared to those with right-sided tumors (Heinemann et al. 2014; Venook et al. 2016; Brulé et al. 2015). Reanalysis of data from the NCIC CO.17 trial of cetuximab vs. best supportive care (BSC) in treatment-refractory mCRC (Jonker et al. 2007) found that among wild-type *KRAS* patients, those with left-sided mCRC had significantly improved PFS when treated with cetuximab compared to BSC (median 5.4 versus 1.8 months, HR 0.28 [0.18–0.45], $P < 0.0001$), whereas those with right-sided mCRC did not (median 1.9 versus 1.9 months, HR 0.73 [0.42–1.27], $P = 0.26$) (Brulé et al. 2015). Similarly, in the FIRE-3 (Heinemann et al. 2014) and CALGB-80405 (Venook et al. 2016) studies of first-line chemotherapy with bevacizumab or cetuximab, patients with left-sided primary tumors who received cetuximab appeared to have longer OS compared to those who received bevacizumab; this effect was not seen in patients with right-sided mCRC.

These findings were the first to show that in mCRC, primary tumor location is predictive of improved EGFR inhibition. While the underlying biological mechanism has not been fully elucidated, left-sided primary tumors have been shown to be more dependent on EGFR signaling. Right-sided primary, CpG island methylator phenotype (CIMP) high,

MSI-high and *BRAF*-mutated cancer demonstrated low AREG and EREG expression due to increased methylation of AREG and EREG loci (Lee et al. 2016). These findings offer a unifying explanation for the varied response to anti-EGFR therapy as well as a strong rationale for further investigation of the impact of primary tumor location, EGFR-ligand expression, CIMP-status and methylation patterns, ideally in prospective trials.

Conclusion

The growth of treatment options for patients suffering from mCRC has translated into meaningful improvements in survival for these patients. Over the last few decades, by combining and optimizing the sequencing of cytotoxics and targeted therapies, we have seen median survival grow from less than a year, to upwards of 30 months (Venook et al. 2014). These gains, unfortunately, come at the cost of physical and financial toxicity. *RAS* mutation testing has allowed us to restrict the use of anti-EGFR therapies in the 40% of mCRC patients harboring a mutation; however, despite this, a subset of wild-type patients still do not derive any benefit.

The need for effective and reliable predictive biomarkers for our existing targeted therapies in mCRC is obvious and much of our focus has been on the EGF-family of receptors and their ligands. While these are proving to be promising candidates, there is also the potential to reveal novel therapeutic targets, particularly related to the processing and regulation of these growth factors, changing the treatment paradigm and further improving outcomes.

References

Anklesaria, P., J. Teixido, M. Laihot, J. H. Pierce, J. S. Greenberger, and J. Massague. 1990. "Cell-Cell Adhesion Mediated by Binding of Membrane-Anchored Transforming Growth Factor a to Epidermal Growth Factor Receptors Promotes Cell Proliferation." *Proceedings of the National Academy of Sciences.* 87 (9): 3289–93. https://doi.org/10.1073/pnas.87.9.3289.

Australian Institute of Health and Welfare. 2019. "Cancer in Australia 2019." https://www.aihw.gov.au/reports/cancer/cancer-in-australia-2019/data.

Baker, J. B., D. Dutta, D. Watson, T. Maddala, B. M. Munneke, S. Shak, E. K. Rowinsky, et al. 2011. "Tumour Gene Expression Predicts Response to Cetuximab in Patients with KRAS Wild-Type Metastatic Colorectal Cancer." *British Journal of Cancer* 104 (3): 488–95. https://doi.org/10.1038/sj.bjc.6606054.

Begg, K., and M. Tavassoli. 2017. "Review: Biomarkers towards Personalised Therapy in Cancer." *Drug Target Review* 4 (2): 26–30. https://www.drugtargetreview.com/article/23631/biomarkers-personalised-therapy-cancer/.

Bernat-Peguera, A., P. Simón-Extremera, V. da Silva-Diz, M. López de Munain, L. Díaz-Gil, R. M. Penin, E. González-Suárez, et al. 2019. "PDGFR-Induced Autocrine SDF-1 Signaling in Cancer Cells Promotes Metastasis in Advanced Skin Carcinoma." *Oncogene* 38 (25): 5021–37. https://doi.org/10.1038/s41388-019-0773-y.

Biomarkers Definitions Working Group. 2001. "Biomarkers and Surrogate Endpoints: Preferred Definitions and Conceptual Framework." *Clinical Pharmacology and Therapeutics* 69 (3): 89–95. https://doi.org/10.1067/mcp.2001.113989.

Blobel, C. P. 2005. "ADAMs: Key Components in Egfr Signalling and Development." *Nature Reviews Molecular Cell Biology* 6 (1): 32. https://doi.org/10.1038/nrm1548.

Bou-Assaly, W., and S. Mukherji. 2010. “Cetuximab (Erbitux).” *American Journal of Neuroradiology* 31 (4): 626–27. https://doi.org/10.3174/ajnr.A2054.

Bray, F., J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre, and A. Jemal. 2018. “Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries.” *CA: A Cancer Journal for Clinicians* 68 (6): 394–424. https://doi.org/10.3322/caac.21492.

Brown, C. L., K. S. Meise, G. D. Plowman, R. J. Coffey, and P. J. Dempsey. 1998. “Cell Surface Ectodomain Cleavage of Human Amphiregulin Precursor Is Sensitive to a Metalloprotease Inhibitor: Release of a Predominant N- Glycosylated 43-KDa Soluble Form.” *Journal of Biological Chemistry* 273 (27): 17258–68. https://doi.org/10.1074/jbc.273.27.17258.

Brulé, S. Y., D. J. Jonker, C. S. Karapetis, C. J. O’Callaghan, M. J. Moore, R. Wong, N. C. Tebbutt, et al. 2015. “Location of Colon Cancer (Right-Sided versus Left-Sided) as a Prognostic Factor and a Predictor of Benefit from Cetuximab in NCIC CO.17.” *European Journal of Cancer* 51 (11): 1405–14. https://doi.org/10.1016/j.ejca.2015.03.015.

Bufill, J. A. 1990. “Colorectal Cancer: Evidence for Distinct Genetic Categories Based on Proximal or Distal Tumor Location.” *Annals of Internal Medicine* 113 (10): 779–88. https://doi.org/10.7326/0003-4819-113-10-779.

Burgess, A. W. 2008. “EGFR Family: Structure Physiology Signalling and Therapeutic Target.” *Growth Factors* 26 (5): 263–74. https://doi.org/10.1080/08977190802312844.

Cantanhede, I. G., and J. R. M. De Oliveira. 2017. “PDGF Family Expression in Glioblastoma Multiforme: Data Compilation from Ivy Glioblastoma Atlas Project Database.” *Scientific Reports* 7 (1): 1–9. https://doi.org/10.1038/s41598-017-

15045w.

Cao, Y. 2013. “Multifarious Functions of PDGFs and PDGFRs in Tumor Growth and Metastasis.” *Trends in Molecular Medicine* 19 (8): 460–73. https://doi.org/10.1016/j.molmed.2013.05.002.

Carl-McGrath, S., U. Lendeckel, M. Ebert, A. Roessner, and C. Röcken. 2005. “The Disintegrin-Metalloproteinases ADAM9, ADAM12, and ADAM15 Are Upregulated in Gastric Cancer.” *International Journal of Oncology* 26 (1): 17–24. https://doi.org/10.3892/ijo.26.1.17.

Carpenter, G., K. J. Lembach, M. M. Morrison, and S. Cohen. 1975. “Characterization of the Binding of 125-I-Labeled Epidermal Growth Factor to Human Fibroblasts.” *The Journal of Biological Chemistry* 250 (11): 4297–4304. http://www.ncbi.nlm.nih.gov/pubmed/1126952.

Carpenter, G., and S. Cohen. 1990. “Epidermal Growth Factor.” *The Journal of Biological Chemistry* 265 (14): 7709–12.

Cohen, S. 1962. “Isolation of a Mouse Submaxillary Gland Protein Accelerating Incisor Eruption and Eyelid Opening in the New-Born Animal.” *The Journal of Biological Chemistry* 237 (6): 1555–62. http://www.ncbi.nlm.nih.gov/pubmed/13880319.

———. 1965. “The Stimulation of Epidermal Proliferation by a Specific Protein (EGF).” *Developmental Biology* 12 (3): 394–407. https://doi.org/10.1016/00121606(65)90005-9.

———. 1983. “The Epidermal Growth Factor (EGF).” *Cancer* 51 (10): 1787–91. https://doi.org/10.1002/1097-0142(19830515)51:10<1787::AIDCNCR2820511004>3.0.CO;2-A

Cohen, S., G. Carpenter, and L. King. 1980. “Epidermal Growth Factor-Receptor-Protein Kinase Interactions. Co-Purification of Receptor and Epidermal Growth Factor-

Enhanced Phosphorylation Activity." *Journal of Biological Chemistry* 255 (10): 4834–42. http://www.ncbi.nlm.nih.gov/pubmed/6246084.

Cohen, S., H. Ushiro, C. Stoscheck, and M. Chinkers. 1982. "A Native 170 000 Epidermal Growth Factor Receptor-Kinase Complex from Shed Plasma Membrane Vesicles." *Journal of Biological Chemistry* 257 (3): 1523–31. http://www.ncbi.nlm.nih.gov/pubmed/ 6276390.

Cunningham, D., Y. Humblet, S. Siena, D. Khayat, H. Bleiberg, A. Santoro, D. Bets, et al. 2004. "Cetuximab Monotherapy and Cetuximab plus Irinotecan in Irinotecan-Refractory Metastatic Colorectal Cancer." *New England Journal of Medicine* 351 (4): 337–45. https://doi.org/10.1056/NEJMoa033025.

Cushman, S. M., C. Jiang, A. J. Hatch, I. Shterev, A. B. Sibley, D. Niedzwiecki, A. P. Venook, K. Owzar, H. I. Hurwitz, and A. B. Nixon. 2015. "Gene Expression Markers of Efficacy and Resistance to Cetuximab Treatment in Metastatic Colorectal Cancer: Results from CALGB 80203 (Alliance)." *Clinical Cancer Research* 21 (5): 1078–86. https://doi.org/10.1158/1078-0432.CCR-14-2313.

Derynck, R., A. B. Roberts, M. E. Winkler, E. Y. Chen, and D. V Goeddel. 1984. "Human Transforming Growth Factor-α: Precursor Structure and Expression in E. Coli." *Cell* 38 (1): 287–97. https://doi.org/10.1016/0092-8674(84)90550-6.

Dong, J., L. K. Opresko, P. J. Dempsey, D. A. Lauffenburger, R. J. Coffey, and H. S. Wiley. 1999. "Metalloprotease-Mediated Ligand Release Regulates Autocrine Signaling through the Epidermal Growth Factor Receptor." *Proceedings of the National Academy of Sciences* 96 (11): 6235–40. https://doi.org/10.1073/pnas.96.11.6235.

Douillard, J. Y., S. Siena, J. Cassidy, J. Tabernero, R. Burkes, M. Barugel, Y. Humblet, et al. 2010. "Randomized, Phase III Trial of Panitumumab with Infusional

Fluorouracil, Leucovorin, and Oxaliplatin (FOLFOX4) Versus FOLFOX4 Alone as First-Line Treatment in Patients with Previously Untreated Metastatic Colorectal Cancer: The PRIME Study." *Journal of Clinical Oncology* 28 (31): 4697–4705. https://doi.org/10.1200/JCO.2009.27.4860.

Douillard, J. Y., S. Siena, J. Cassidy, J. Tabernero, R. Burkes, M. Barugel, Y. Humblet, et al. 2014. "Final Results from PRIME: Randomized Phase III Study of Panitumumab with FOLFOX4 for First-Line Treatment of Metastatic Colorectal Cancer." *Annals of Oncology* 25 (7): 1346–55. https://doi.org/10.1093/annonc/mdu141.

Duffy, M. J., and J. Crown. 2008. "A Personalized Approach to Cancer Treatment: How Biomarkers Can Help." *Clinical Chemistry* 54 (11): 1770–79. https://doi.org/10.1373/clinchem.2008.110056.

Duffy, M. J., M. Mullooly, N. O'Donovan, S. Sukor, J. Crown, A. Pierce, and P. M. McGowan. 2011. "The ADAMs Family of Proteases: New Biomarkers and Therapeutic Targets for Cancer?" *Clinical Proteomics* 8 (1): 1–13. https://doi.org/10.1186/1559-0275-8-9.

Egner, J. R. 2010. "AJCC Cancer Staging Manual." *JAMA* 304 (15): 1726. https://doi.org/10.1001/jama.2010.1525.

Falls, D. L., K. M. Rosen, G. Corfas, W. S. Lane, and G. D. Fischbach. 1993. "ARIA, a Protein That Stimulates Acetylcholine Receptor Synthesis, Is a Member of the Neu Ligand Family." *Cell* 72 (5): 801–13. https://doi.org/10.1016/0092-8674(93)90407-H.

Galizia, G., E. Lieto, F. De Vita, M. Orditura, P. Castellano, T. Troiani, V. Imperatore, and F. Ciardiello. 2007. "Cetuximab, a Chimeric Human Mouse Anti-Epidermal Growth Factor Receptor Monoclonal Antibody, in the Treatment of Human

Colorectal Cancer." *Oncogene* 26 (25): 3654–60. https://doi.org/10.1038/sj.onc.1210381.

Garrett, T. P. J., N. M. McKern, M. Lou, T. C. Elleman, T. E. Adams, G. O. Lovrecz, M. Kofler, et al. 2003. "The Crystal Structure of a Truncated ErbB2 Ectodomain Reveals an Active Conformation, Poised to Interact with Other ErbB Receptors." *Molecular Cell* 11 (2): 495–505. https://doi.org/10.1016/S1097-2765(03)00048-0.

Gee, J. M. W., and J. M. Knowlden. 2003. "ADAM Metalloproteases and EGFR Signalling." *Breast Cancer Research* 5 (5): 223–24. https://doi.org/10.1186/bcr637.

Georgopoulos, N. T., L. A. Kirkwood, and J. Southgate. 2014. "A Novel Bidirectional Positive-Feedback Loop between Wnt-β-Catenin and EGFR-ERK Plays a Role in Context-Specific Modulation of Epithelial Tissue Regeneration." *Journal of Cell Science* 127 (Pt 13): 2967–82. https://doi.org/10.1242/jcs.150888.

Goffin, J. R., and K. Zbuk. 2013. "Epidermal Growth Factor Receptor: Pathway, Therapies, and Pipeline." *Clinical Therapeutics* 35 (9): 1282–1303. https://doi.org/10.1016/ j.clinthera.2013.08.007.

Goodearl, A. D. J., J. B. Davis, K. Mistry, L. Minghetti, M. Otsu, M. D. Waterfield, and P. Stroobant. 1993. "Purification of Multiple Forms of Glial Growth Factor." *Journal of Biological Chemistry* 268 (24): 18095–102. http://www.ncbi.nlm.nih.gov/pubmed/ 8394360.

Graus-Porta, D., R. R. Beerli, J. M. Daly, and N. E. Hynes. 1997. "ErbB-2, the Preferred Heterodimerization Partner of All ErbB Receptors, Is a Mediator of Lateral Signaling." *EMBO Journal* 16 (7): 1647–55. https://doi.org/10.1093/emboj/16.7.1647.

Grützmann, R., J. Lüttges, B. Sipos, O. Ammerpohl, F. Dobrowolski, I. Alldinger, S. Kersting, et al. 2004. "ADAM9 Expression in Pancreatic Cancer Is Associated with

Tumour Type and Is a Prognostic Factor in Ductal Adenocarcinoma." *British Journal of Cancer* 90 (5): 1053–58. https://doi.org/10.1038/sj.bjc.6601645.

Han, C. W., M. S. Jeong, and S. B. Jang. 2017. "Structure, Signaling and the Drug Discovery of the Ras Oncogene Protein." *BMB Reports* 50 (7): 355–60. https://doi.org/10.5483/ BMBRep.2017.50.7.062.

Han, W., and H.-W. Lo. 2012. "Landscape of EGFR Signaling Network in Human Cancers: Biology and Therapeutic Response in Relation to Receptor Subcellular Locations." *Cancer Letters* 318 (2): 124–34. https://doi.org/10.1016/j.canlet. 2012.01.011.

Harty, G., J. Jarrett, and M. Jofre-Bonet. 2018. "Consequences of Biomarker Analysis on the Cost-Effectiveness of Cetuximab in Combination with FOLFIRI as a First-Line Treatment of Metastatic Colorectal Cancer: Personalised Medicine at Work." *Applied Health Economics and Health Policy* 16 (4): 515–25. https://doi.org/10.1007/s40258-018-0395-5.

Heinemann, V., J. Y. Douillard, M. Ducreux, and M. Peeters. 2013. "Targeted Therapy in Metastatic Colorectal Cancer - An Example of Personalised Medicine in Action." *Cancer Treatment Reviews* 39 (6): 592–601. https://doi.org/10.1016/j.ctrv. 2012.12.011.

Heinemann, V., D. P. Modest, L. Fischer von Weikersthal, T. Decker, A. Kiani, U. Vehling-Kaiser, S.-E. Al-Batran, et al. 2014. "Gender and Tumor Location as Predictors for Efficacy: Influence on Endpoints in First-Line Treatment with FOLFIRI in Combination with Cetuximab or Bevacizumab in the AIO KRK 0306 (FIRE3) Trial." *Journal of Clinical Oncology* 32 (15_suppl): 3600–3600. https://doi.org/10.1200/jco.2014.32.15_ suppl.3600.

Higashiyama, S., J. Abraham, J. Miller, J. Fiddes, and M. Klagsbrun. 1991. "A Heparin-

Binding Growth Factor Secreted by Macrophage-like Cells That Is Related to EGF." *Science* 251 (4996): 936–39. https://doi.org/10.1126/science.1840698.

Higginbotham, J. N., R. E. McConnell, J. L. Franklin, G.-J. Kremers, D. W. Piston, M. J. Tyska, J. D. Gephart, et al. 2011. "Amphiregulin Exosomes Increase Cancer Cell Invasion." *Current Biology* 21 (9): 779–86. https://doi.org/10.1016/j.cub.2011.03.043.

Holmes, W. E., M. X. Sliwkowski, R. W. Akita, W. J. Henzel, J. Lee, J. W. Park, D. Yansura, et al. 1992. "Identification of Heregulin, a Specific Activator of P185erbB2." *Science* 256 (5060): 1205–10. https://doi.org/10.1126/SCIENCE.256.5060.1205.

Hoque, M. O., S. Begum, M. Brait, C. Jeronimo, M. Zahurak, K. L. Ostrow, E. Rosenbaum, et al. 2008. "Tissue Inhibitor of Metalloproteinases-3 Promoter Methylation Is an Independent Prognostic Factor for Bladder Cancer." *The Journal of Urology* 179 (2): 743–47. https://doi.org/10.1016/j.juro.2007.09.019.

Horiuchi, K., H. M. Zhou, K. Kelly, K. Manova, and C. P. Blobel. 2005. "Evaluation of the Contributions of ADAMs 9, 12, 15, 17, and 19 to Heart Development and Ectodomain Shedding of Neuregulins B1 and B2." *Developmental Biology* 283 (2): 459–71. https://doi.org/10.1016/j.ydbio.2005.05.004.

Hurwitz, H., L. Fehrenbacher, W. Novotny, T. Cartwright, J. Hainsworth, W. Heim, J. Berlin, et al. 2004. "Bevacizumab plus Irinotecan, Fluorouracil, and Leucovorin for Metastatic Colorectal Cancer." *The New England Journal of Medicine* 350 (23): 2335–42. https://doi.org/10.1056/NEJMoa032691.

Hutchinson, R. A., R. A. Adams, D. G. McArt, M. Salto-Tellez, B. Jasani, and P. W. Hamilton. 2015. "Epidermal Growth Factor Receptor Immunohistochemistry: New Opportunities in Metastatic Colorectal Cancer." *Journal of Translational Medicine*

13: 217. https://doi.org/10.1186/s12967-015-0531-z.

Jacobs, B., W. De Roock, H. Piessevaux, R. Van Oirbeek, B. Biesmans, J. De Schutter, S. Fieuws, et al. 2009. "Amphiregulin and Epiregulin MRNA Expression in Primary Tumors Predicts Outcome in Metastatic Colorectal Cancer Treated with Cetuximab." *Journal of Clinical Oncology* 27 (30): 5068–74. https://doi.org/10.1200/JCO.2008.21.3744.

Jing, C., Y. H. Jin, Z. You, Q. Qiong, and Z. Jun. 2016. "Prognostic Value of Amphiregulin and Epiregulin MRNA Expression in Metastatic Colorectal Cancer Patients." *Oncotarget* 7 (34): 55890–99. https://doi.org/10.18632/oncotarget.10151.

Jonker, D. J., C. S. Karapetis, C. Harbison, C. J. O'Callaghan, D. Tu, R. J. Simes, D. P. Malone, et al. 2014. "Epiregulin Gene Expression as a Biomarker of Benefit from Cetuximab in the Treatment of Advanced Colorectal Cancer." *British Journal of Cancer* 110 (3): 648–55. https://doi.org/10.1038/bjc.2013.753.

Jonker, D. J., C. J. O'Callaghan, C. S. Karapetis, J. R. Zalcberg, D. Tu, H. J. Au, S. R. Berry, et al. 2007. "Cetuximab for the Treatment of Colorectal Cancer." *New England Journal of Medicine* 357 (20): 2040–48. https://doi.org/10.1056/NEJMoa071834.

Karachaliou, N., and R. Rosell. 2018. "Rhomboids and Regulation of Receptor Tyrosine Kinase Ligands Shedding." *EBioMedicine* 37: 19–20. https://doi.org/10.1016/j.ebiom.2018.09.053.

Karapetis, C. S., S. Khambata-Ford, D. J. Jonker, C. J. O'Callaghan, D. Tu, N. C. Tebbutt, R. J. Simes, et al. 2008. "K-Ras Mutations and Benefit from Cetuximab in Advanced Colorectal Cancer." *New England Journal of Medicine* 359 (17): 1757–65. https://doi.org/10.1056/NEJMoa0804385.

Kenny, P. A., and M. J. Bissell. 2007. "Targeting TACE-Dependent EGFR Ligand

Shedding in Breast Cancer." *Journal of Clinical Investigation* 117 (2): 337–45. https://doi.org/10.1172/JCI29518.

Khambata-Ford, S., C. R. Garrett, N. J. Meropol, M. Basik, C. T. Harbison, S. Wu, T. W. Wong, et al. 2007. "Expression of Epiregulin and Amphiregulin and K-Ras Mutation Status Predict Disease Control in Metastatic Colorectal Cancer Patients Treated with Cetuximab." *Journal of Clinical Oncology* 25 (22): 3230–37. https://doi.org/ 10.1200/ JCO.2006.10.5437.

Kovacs, E., J. A. Zorn, Y. Huang, T. Barros, and J. Kuriyan. 2015. "A Structural Perspective on the Regulation of the Epidermal Growth Factor Receptor." *Annual Review of Biochemistry* 84: 739–64. https://doi.org/10.1146/annurev-biochem-060614-034402.

Kurohara, K., K. Komatsu, T. Kurisaki, A. Masuda, N. Irie, M. Asano, K. Sudo, Y. Nabeshima, Y. Iwakura, and A. Sehara-Fujisawa. 2004. "Essential Roles of Meltrin β (ADAM19) in Heart Development." *Developmental Biology* 267 (1): 14–28. https://doi.org/10.1016/ j.ydbio.2003.10.021.

Lacunza, E., R. Canzoneri, M. E. Rabassa, A. Zwenger, A. Segal-Eiras, M. V. Croce, and M. C. Abba. 2012. "RHBDD2: A 5-Fluorouracil Responsive Gene Overexpressed in the Advanced Stages of Colorectal Cancer." *Tumour Biology* 33 (6): 2393–99. https://doi.org/10.1007/s13277-012-0503-3.

Lee, J. R., S. Urban, C. F. Garvey, and M. Freeman. 2001. "Regulated Intracellular Ligand Transport and Proteolysis Control EGF Signal Activation in Drosophila." *Cell* 107 (2): 161–71. https://doi.org/10.1016/S0092-8674(01)00526-8.

Lee, M. S., D. G. Menter, and S. Kopetz. 2017. "Right versus Left Colon Cancer Biology: Integrating the Consensus Molecular Subtypes." *Journal of the National Comprehensive Cancer Network* 15 (3): 411–19. https://doi.org/10.6004/jnccn.

2017.0038.

Lee, M. S., E. J. McGuffey, J. S. Morris, G. Manyam, V. Baladandayuthapani, W. Wei, V. K. Morris, et al. 2016. “Association of CpG Island Methylator Phenotype and EREG/AREG Methylation and Expression in Colorectal Cancer.” *British Journal of Cancer* 114 (12): 1352–61. https://doi.org/10.1038/bjc.2016.87.

Lemmon, M. A., and J. Schlessinger. 2010. “Cell Signaling by Receptor Tyrosine Kinases.” *Cell* 141 (7): 1117–34. https://doi.org/10.1016/j.cell.2010.06.011.

Lemmon, M. A., J. Schlessinger, and K. M. Ferguson. 2014. “The EGFR Family: Not so Prototypical Receptor Tyrosine Kinases.” *Cold Spring Harbor Perspectives in Biology* 6 (4): a020768. https://doi.org/10.1101/cshperspect.a020768.

Lendeckel, U., J. Kohl, M. Arndt, S. Carl-McGrath, H. Donat, and C. Röcken. 2005. “Increased Expression of ADAM Family Members in Human Breast Cancer and Breast Cancer Cell Lines.” *Journal of Cancer Research and Clinical Oncology* 131 (1): 41–48. https://doi.org/10.1007/s00432-004-0619-y.

Liang, J., S. Balachandra, S. Ngo, and L. E. O’Brien. 2017. “Feedback Regulation of Steady-State Epithelial Turnover and Organ Size.” *Nature* 548 (7669): 588–91. https://doi.org/10.1038/nature23678.

Lièvre, A., J. B. Bachet, D. Le Corre, V. Boige, B. Landi, J. F. Emile, J. F. Côté, et al. 2006. “KRAS Mutation Status Is Predictive of Response to Cetuximab Therapy in Colorectal Cancer.” *Cancer Research* 66 (8): 3992–95. https://doi.org/10.1158/0008-5472.CAN-06-0191.

Lièvre, A., J.-B. Bachet, V. Boige, A. Cayre, D. Le Corre, E. Buc, M. Ychou, et al. 2008. “*KRAS* Mutations As an Independent Prognostic Factor in Patients With Advanced Colorectal Cancer Treated With Cetuximab.” *Journal of Clinical Oncology* 26 (3): 374–79. https://doi.org/10.1200/JCO.2007.12.5906.

Llovet, P., J. Sastre, J. S. Ortega, I. Bando, M. Ferrer, P. García-Alfonso, O. Donnay, et al. 2015. "Prognostic Value of BRAF, PI3K, PTEN, EGFR Copy Number, Amphiregulin and Epiregulin Status in Patients with KRAS Codon 12 Wild-Type Metastatic Colorectal Cancer Receiving First-Line Chemotherapy with Anti-EGFR Therapy." *Molecular Diagnosis & Therapy* 19 (6): 397–408. https://doi.org/ 10.1007/s40291-015-0165-0.

Marchionni, M. A., A. D. J. Goodearl, M. Su Chen, O. Bermingham-McDonogh, C. Kirk, M. Hendricks, F. Danehy, et al. 1993. "Glial Growth Factors Are Alternatively Spliced ErbB2 Ligands Expressed in the Nervous System." *Nature* 362 (6418): 312–18. https://doi.org/10.1038/362312a0.

Markman, B., F. Javier Ramos, J. Capdevila, and J. Tabernero. 2010. "EGFR and KRAS in Colorectal Cancer." *Advances in Clinical Chemistry*, 51:71–119. https://doi.org/10.1016/ S0065-2423(10)51004-7.

Merchant, N. B., I. Voskresensky, C. M. Rogers, B. LaFleur, P. J. Dempsey, R. Graves-Deal, F. Revetta, et al. 2008. "TACE/ADAM-17: A Component of the Epidermal Growth Factor Receptor Axis and a Promising Therapeutic Target in Colorectal Cancer." *Clinical Cancer Research* 14 (4): 1182–91. https://doi.org/10.1158/1078-0432.CCR-07-1216.

Missiaglia, E., B. Jacobs, G. D'Ario, A. F. Di Narzo, C. Soneson, E. Budinska, V. Popovici, et al. 2014. "Distal and Proximal Colon Cancers Differ in Terms of Molecular, Pathological, and Clinical Features." *Annals of Oncology* 25 (10): 1995–2001. https://doi.org/10.1093/annonc/mdu275.

Mitchell, R. A., R. B. Luwor, and A. W. Burgess. 2018. "Epidermal Growth Factor Receptor: Structure-Function Informing the Design of Anticancer Therapeutics." *Experimental Cell Research* 371 (1):1-19. https://doi.org/10.1016/j.yexcr.2018.

08.009.

National Cancer Institute. “The Cancer Genome Atlas.” 2018. *TCGA*. http://cancergenome.nih.gov/abouttcga.

Ohchi, T., Y. Akagi, T. Kinugasa, T. Kakuma, A. Kawahara, T. Sasatomi, Y. Gotanda, et al. 2012. “Amphiregulin Is a Prognostic Factor in Colorectal Cancer.” *Anticancer Research* 32 (6): 2315–21.

Osborne, C. K., B. Hamilton, and M. Nover. 1982. “Receptor Binding and Processing of Epidermal Growth Factor by Human Breast Cancer Cells.” *Journal of Clinical Endocrinology and Metabolism* 55 (1): 86–93. https://doi.org/10.1210/jcem-55-1-86.

Peles, E., S. S. Bacus, R. A. Koski, H. S. Lu, D. Wen, S. G. Ogden, R. Ben Levy, and Y. Yarden. 1992. “Isolation of the Neu/HER-2 Stimulatory Ligand: A 44 Kd Glycoprotein That Induces Differentiation of Mammary Tumor Cells.” *Cell* 69 (1): 205–16. https://doi.org/10.1016/0092-8674(92)90131-U.

Pentheroudakis, G., V. Kotoula, W. De Roock, G. Kouvatseas, P. Papakostas, T. Makatsoris, D. Papamichael, et al. 2013. “Biomarkers of Benefit from Cetuximab-Based Therapy in Metastatic Colorectal Cancer: Interaction of EGFR Ligand Expression with RAS/RAF, PIK3CA Genotypes.” *BMC Cancer* 13 (1): 49. https://doi.org/10.1186/1471-2407-13-49.

Pietrantonio, F., F. Petrelli, A. Coinu, M. Di Bartolomeo, K. Borgonovo, C. Maggi, M. Cabiddu, et al. 2015. “Predictive Role of BRAF Mutations in Patients with Advanced Colorectal Cancer Receiving Cetuximab and Panitumumab: A Meta-Analysis.” *European Journal of Cancer* 51 (5): 587–94. https://doi.org/10.1016/j.ejca.2015.01.054.

Prior, I. A., P. D. Lewis, and C. Mattos. 2012. “A Comprehensive Survey of Ras

Mutations in Cancer." *Cancer Research* 72 (10): 2457–67. https://doi.org/10.1158/0008-5472.CAN-11-2612.A.

Purba, E., E.-I. Saita, and I. Maruyama. 2017. "Activation of the EGF Receptor by Ligand Binding and Oncogenic Mutations: The 'Rotation Model.'" *Cells* 6 (2): 13. https://doi.org/10.3390/cells6020013.

Quesnelle, K. M., A. L. Boehm, and J. R. Grandis. 2007. "STAT-Mediated EGFR Signaling in Cancer." *Journal of Cellular Biochemistry* 102 (2): 311–19. https://doi.org/10.1002/ jcb.21475.

Quinlan, M., and J. Settleman. 2009. "Isoform-Specific Ras Functions in Development and Cancer." *Future Oncology* 5 (1): 105–16. https://doi.org/10.2217/14796694.5.1.105.

Raghav, K., J. M. Loree, J. S. Morris, M. J. Overman, R. Yu, F. Meric-Bernstam, D. Menter, et al. 2019. "Validation of HER2 Amplification as a Predictive Biomarker for Anti–Epidermal Growth Factor Receptor Antibody Therapy in Metastatic Colorectal Cancer." *JCO Precision Oncology* (3): 1–13. https://doi.org/10.1200/PO.18.00226.

Richman, S. D., J. Fairley, R. Butler, and Z. C. Deans. 2017. "How Close Are We to Standardised Extended RAS Gene Mutation Testing? The UK NEQAS Evaluation." *Journal of Clinical Pathology* 70 (1): 58–62. https://doi.org/10.1136/jclinpath-2016-203822.

Richman, S. D., and B. Jasani. 2019. "Predictive Biomarkers and Targeted Therapies in Colorectal Cancer." In *Predictive Biomarkers in Oncology*, edited by Badve S., Kumar G., 423–30. Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-95228-4_38.

Rodriguez-Bigas, M., E. Lin, and C. Crane. 2003. "Stage IV Colorectal Cancer." In

Holland-Frei Cancer Medicine, edited by DW Kufe, RE Pollock, RR Weichselbaum, RC Jr Bast, TS Gansler, JF Holland, and E III Frei, 6th ed. Hamilton (ON): BC Decker. http://www.ncbi.nlm.nih.gov/books/NBK13267/.

Roemer, A., L. Schwettmann, M. Jung, J. Roigas, G. Kristiansen, D. Schnorr, S. A. Loening, K. Jung, and R. Lichtinghagen. 2004. "Increased MRNA Expression of ADAMs in Renal Cell Carcinoma and Their Association with Clinical Outcome." *Oncology Reports* 11 (2): 529–36. http://www.ncbi.nlm.nih.gov/pubmed/14719095.

Roskoski, R. 2014. "The ErbB/HER Family of Protein-Tyrosine Kinases and Cancer." *Pharmacological Research* 79: 34–74. https://doi.org/10.1016/j.phrs.2013.11.002.

Sahin, U., G. Weskamp, K. Kelly, H.-M. Zhou, S. Higashiyama, J. Peschon, D. Hartmann, P. Saftig, and C. P. Blobel. 2004. "Distinct Roles for ADAM10 and ADAM17 in Ectodomain Shedding of Six EGFR Ligands." *The Journal of Cell Biology* 164 (5): 769–79. https://doi.org/10.1083/jcb.200307137.

Salvatore, L., G. Aprile, E. Arnoldi, C. Aschele, C. Carnaghi, M. Cosimelli, E. Maiello, et al. 2017. "Management of Metastatic Colorectal Cancer Patients: Guidelines of the Italian Medical Oncology Association (AIOM)." *ESMO Open* 2 (1): e000147. https://doi.org/10.1136/esmoopen-2016-000147.

Saridaki, Z., M. Tzardi, C. Papadaki, M. Sfakianaki, F. Pega, A. Kalikaki, E. Tsakalaki, et al. 2011. "Impact of KRAS, BRAF, PIK3CA Mutations, PTEN, AREG, EREG Expression and Skin Rash in ≥2nd Line Cetuximab-Based Therapy of Colorectal Cancer Patients." Edited by Meenhard Herlyn. *PLoS ONE* 6 (1): e15980. https://doi.org/10.1371/journal.pone.0015980.

Savage, C., T. Inagami, and S. Cohen. 1972. "The Primary Structure of Epidermal Growth Factor." *The Journal of Biological Chemistry* 247 (23): 7612–21. http://www.ncbi.nlm.nih.gov/pubmed/4636327.

Savage, C. R., J. H. Hash, and S. Cohen. 1973. “Epidermal Growth Factor. Location of Disulfide Bonds.” *Journal of Biological Chemistry* 248 (22): 7669–72. http://www.ncbi.nlm.nih.gov/pubmed/4750422.

Schneider, M. R., and E. Wolf. 2009. “The Epidermal Growth Factor Receptor Ligands at a Glance.” *Journal of Cellular Physiology* 218 (3): 460–66. https://doi.org/ 10.1002/ jcp.21635.

Seals, D. F., and S. A. Courtneidge. 2003. “The ADAMs Family of Metalloproteases: Multidomain Proteins with Multiple Functions.” *Genes and Development* 17 (1): 7–30. https://doi.org/10.1101/gad.1039703.

Seligmann, J. F., F. Elliott, S. D. Richman, B. Jacobs, G. Hemmings, S. Brown, J. H. Barrett, S. Tejpar, P. Quirke, and M. T. Seymour. 2016. “Combined Epiregulin and Amphiregulin Expression Levels as a Predictive Biomarker for Panitumumab Therapy Benefit or Lack of Benefit in Patients With RAS Wild-Type Advanced Colorectal Cancer.” *JAMA Oncology* 2 (5): 633. https://doi.org/10.1001/jamaoncol. 2015.6065.

Seo, M. K., and J. Cairns. 2018. “Do Cancer Biomarkers Make Targeted Therapies Cost-Effective? A Systematic Review in Metastatic Colorectal Cancer.” *PLoS ONE* 13 (9): e0204496. https://doi.org/10.1371/journal.pone.0204496.

Shaib, W., R. Mahajan, and B. El-Rayes. 2013. “Markers of Resistance to Anti-EGFR Therapy in Colorectal Cancer.” *Journal of Gastrointestinal Oncology* 4 (3): 308–18. https://doi.org/10.3978/j.issn.2078-6891.2013.029.

Shelly, M., R. Pinkas-Kramarski, B. C. Guarino, H. Waterman, L.-M. Wang, L. Lyass, M. Alimandi, et al. 1998. “Epiregulin Is a Potent Pan-ErbB Ligand That Preferentially Activates Heterodimeric Receptor Complexes.” *Journal of Biological Chemistry* 273 (17): 10496–505. https://doi.org/10.1074/jbc.273.17.10496.

Shing, Y., G. Christofori, D. Hanahan, Y. Ono, R. Sasada, K. Igarashi, and J. Folkman. 1993. “Betacellulin: A Mitogen from Pancreatic Beta Cell Tumors.” *Science* 259 (5101): 1604–7. https://doi.org/10.1126/science.8456283.

Shoyab, M., G. Plowman, V. McDonald, J. Bradley, and G. Todaro. 1989. “Structure and Function of Human Amphiregulin: A Member of the Epidermal Growth Factor Family.” *Science* 243 (4894): 1074–76. https://doi.org/10.1126/science.2466334.

Sibilia, M., and E. Wagner. 1995. “Strain-Dependent Epithelial Defects in Mice Lacking the EGF Receptor.” *Science* 269 (5221): 234–38. https://doi.org/10.1126/science. 7618085.

Sibilia, M., J. P. Steinbach, L. Stingl, A. Aguzzi, and E. F. Wagner. 1998. “A Strain-Independent Postnatal Neurodegeneration in Mice Lacking the EGF Receptor.” *The EMBO Journal* 17 (3): 719–31. https://doi.org/10.1093/emboj/17.3.719.

Sibilia, M., R. Kroismayr, B. M. Lichtenberger, A. Natarajan, M. Hecking, and M. Holcmann. 2007. “The Epidermal Growth Factor Receptor: From Development to Tumorigenesis.” *Differentiation* 75 (9): 770. https://doi.org/10.1111/j.1432-0436.2007.00238.x.

Sigismund, S., D. Avanzato, and L. Lanzetti. 2018. “Emerging Functions of the EGFR in Cancer.” *Molecular Oncology* 12 (1): 3–20. https://doi.org/10.1002/1878-0261.12155.

Singh, A. B., and R. C. Harris. 2005. “Autocrine, Paracrine and Juxtacrine Signaling by EGFR Ligands.” *Cellular Signalling* 17 (10): 1183–93. https://doi.org/10.1016/ j.cellsig. 2005.03.026.

Singh, B., G. Carpenter, and R. J. Coffey. 2016. “EGF Receptor Ligands: Recent Advances.” *F1000Research* 5: 2270. https://doi.org/10.12688/f1000research. 9025.1.

Sorich, M. J., M. D. Wiese, A. Rowland, G. Kichenadasse, R. A. McKinnon, and C. S. Karapetis. 2015. “Extended RAS Mutations and Anti-EGFR Monoclonal Antibody Survival Benefit in Metastatic Colorectal Cancer: A Meta-Analysis of Randomized, Controlled Trials.” *Annals of Oncology* 26 (1): 13–21. https://doi.org/10.1093/annonc/mdu378.

Stahler, A., V. Heinemann, C. Giessen-Jung, A. Crispin, A. Schalhorn, S. Stintzing, L. Fischer von Weikersthal, et al. 2016. “Influence of MRNA Expression of Epiregulin and Amphiregulin on Outcome of Patients with Metastatic Colorectal Cancer Treated with 5-FU/LV plus Irinotecan or Irinotecan plus Oxaliplatin as First-Line Treatment (FIRE 1-Trial).” *International Journal of Cancer* 138 (3): 739–46. https://doi.org/ 10.1002/ijc.29807.

Stintzing, S., B. Ivanova, I. Ricard, A. Jung, T. Kirchner, A. Tannapfel, H. Juette, S. Hegewisch-Becker, D. Arnold, and A. Reinacher-Schick. 2018. “Amphiregulin (AREG) and Epiregulin (EREG) Gene Expression as Predictor for Overall Survival (OS) in Oxaliplatin/Fluoropyrimidine Plus Bevacizumab Treated MCRC Patients—Analysis of the Phase III AIO KRK-0207 Trial.” *Frontiers in Oncology* 8: 7796–7807. https://doi.org/10.3389/fonc.2018.00474.

Strachan, L., J. G. Murison, R. L. Prestidge, M. A. Sleeman, J. D. Watson, and K. D. Kumble. 2001. “Cloning and Biological Activity of Epigen, a Novel Member of the Epidermal Growth Factor Superfamily.” *Journal of Biological Chemistry* 276 (21): 18265–71. https://doi.org/10.1074/jbc.M006935200.

Strimpakos, A., G. Pentheroudakis, V. Kotoula, W. De Roock, G. Kouvatseas, P. Papakostas, T. Makatsoris, et al. 2013. “The Prognostic Role of Ephrin A2 and Endothelial Growth Factor Receptor Pathway Mediators in Patients with Advanced Colorectal Cancer Treated with Cetuximab.” *Clinical Colorectal Cancer* 12 (4):

267-274.e2. https://doi.org/10.1016/ j.clcc.2013.07.001.

Strunk, K. E., V. Amann, and D. W. Threadgill. 2004. “Phenotypic Variation Resulting From a Deficiency of Epidermal Growth Factor Receptor in Mice Is Caused by Extensive Genetic Heterogeneity That Can Be Genetically and Molecularly Partitioned.” *Genetics* 167 (4): 1821–32. https://doi.org/10.1534/genetics.103.020495.

Sun, C., and R. Bernards. 2014. “Feedback and Redundancy in Receptor Tyrosine Kinase Signaling: Relevance to Cancer Therapies.” *Trends in Biochemical Sciences* 39 (10): 465. https://doi.org/10.1016/j.tibs.2014.08.010.

Tan, C., and X. Du. 2012. “KRAS Mutation Testing in Metastatic Colorectal Cancer.” *World Journal of Gastroenterology* 18 (37): 5171–80. https://doi.org/10.3748/wjg.v18.i37.5171.

Tang, P., P. A. Steck, and W. K. A. Yung. 1997. “The Autocrine Loop of TGF-α/EGFR and Brain Tumors.” *Journal of Neuro-Oncology* 35 (3): 303–14. https://doi.org/10.1023/ A:1005824802617.

Taylor, J. M., W. M. Mitchell, and S. Cohen. 1972. “Epidermal Growth Factor. Physical and Chemical Properties.” *The Journal of Biological Chemistry* 247 (18): 5928–34. http://www.ncbi.nlm.nih.gov/pubmed/5066542.

Therkildsen, C., T. K. Bergmann, T. Henrichsen-Schnack, S. Ladelund, and M. Nilbert. 2014. “The Predictive Value of KRAS, NRAS, BRAF, PIK3CA and PTEN for Anti-EGFR Treatment in Metastatic Colorectal Cancer: A Systematic Review and Meta-Analysis.” *Acta Oncologica* 53 (7): 852–64. https://doi.org/10.3109/0284186X.2014.895036.

Todaro, G. J., C. Fryling, and J. E. De Larco. 1980. “Transforming Growth Factors Produced by Certain Human Tumor Cells: Polypeptides That Interact with

Epidermal Growth Factor Receptors." *Proceedings of the National Academy of Sciences* 77 (9): 5258–62. https://doi.org/10.1073/pnas.77.9.5258.

Tomas, A., C. E. Futter, and E. R. Eden. 2014. "EGF Receptor Trafficking: Consequences for Signaling and Cancer." *Trends in Cell Biology* 24 (1): 26–34. https://doi.org/10.1016/ j.tcb.2013.11.002.

Tomoshige, K., M. Guo, T. Tsuchiya, T. Fukazawa, I. M. Fink-Baldauf, W. D. Stuart, Y. Naomoto, T. Nagayasu, and Y. Maeda. 2018. "An EGFR Ligand Promotes EGFR-Mutant but Not KRAS-Mutant Lung Cancer in Vivo." *Oncogene* 37 (28): 3894–3908. https://doi.org/10.1038/s41388-018-0240-1.

Tougeron, D., T. Lecomte, J. C. Pages, C. Villalva, C. Collin, A. Ferru, J. M. Tourani, C. Silvain, P. Levillain, and L. Karayan-Tapon. 2013. "Effect of Low-Frequency KRAS Mutations on the Response to Anti-EGFR Therapy in Metastatic Colorectal Cancer." *Annals of Oncology* 24 (5): 1267–73. https://doi.org/10.1093/annonc/mds620.

Toyoda, H., T. Komurasaki, D. Uchida, Y. Takayama, T. Isobe, T. Okuyama, and K. Hanada. 1995. "Epiregulin." *Journal of Biological Chemistry* 270 (13): 7495–7500. https://doi.org/10.1074/jbc.270.13.7495.

Ullrich, A., and J. Schlessinger. 1990. "Signal Transduction by Receptors with Tyrosine Kinase Activity." *Cell* 61 (2): 203–12. https://doi.org/10.1016/0092-8674(90)90801-K.

Vallböhmer, D., and H.-J. Lenz. 2005. "Epidermal Growth Factor Receptor as a Target for Chemotherapy." *Clinical Colorectal Cancer* 5: S19–27. https://doi.org/10.3816/CCC.2005.s.003.

Van Cutsem, E., M. Peeters, S. Siena, Y. Humblet, A. Hendlisz, B. Neyns, J.-L. Canon, et al. 2007. "Open-Label Phase III Trial of Panitumumab Plus Best Supportive Care

Compared With Best Supportive Care Alone in Patients With Chemotherapy-Refractory Metastatic Colorectal Cancer." *Journal of Clinical Oncology* 25 (13): 1658–64. https://doi.org/10.1200/JCO.2006.08.1620.

Van Cutsem, E., C.-H. Köhne, E. Hitre, J. Zaluski, C.-R. Chang Chien, A. Makhson, G. D'Haens, et al. 2009. "Cetuximab and Chemotherapy as Initial Treatment for Metastatic Colorectal Cancer." *New England Journal of Medicine* 360 (14): 1408–17. https://doi.org/10.1056/NEJMoa0805019.

Van Cutsem, E., C.-H. Köhne, I. Láng, G. Folprecht, M. P. Nowacki, S. Cascinu, I. Shchepotin, et al. 2011. "Cetuximab plus Irinotecan, Fluorouracil, and Leucovorin as First-Line Treatment for Metastatic Colorectal Cancer: Updated Analysis of Overall Survival According to Tumor KRAS and BRAF Mutation Status." *Journal of Clinical Oncology* 29 (15): 2011–19. https://doi.org/10.1200/JCO.2010.33.5091.

Vecchione, L., B. Jacobs, N. Normanno, F. Ciardiello, and S. Tejpar. 2011. "EGFR-Targeted Therapy." *Experimental Cell Research* 317 (19): 2765–71. https://doi.org/10.1016/ j.yexcr.2011.08.021.

Venook, A. P., D. Niedzwiecki, H.-J. Lenz, F. Innocenti, M. R. Mahoney, B. H. O'Neil, J. E. Shaw, et al. 2014. "CALGB/SWOG 80405: Phase III Trial of Irinotecan/5-FU/Leucovorin (FOLFIRI) or Oxaliplatin/5-FU/Leucovorin (MFOLFOX6) with Bevacizumab (BV) or Cetuximab (CET) for Patients (Pts) with KRAS Wild-Type (Wt) Untreated Metastatic Adenocarcinoma of the Colon or Re." *Journal of Clinical Oncology* 32 (15_suppl): LBA3–LBA3. https://doi.org/10.1200/jco.2014.32.15_suppl.lba3.

Venook, A. P., D. Niedzwiecki, F. Innocenti, B. Fruth, C. Greene, B. H. O'Neil, J. E. Shaw, et al. 2016. "Impact of Primary (1º) Tumor Location on Overall Survival (OS) and Progression-Free Survival (PFS) in Patients (Pts) with Metastatic Colorectal

Cancer (MCRC): Analysis of CALGB/SWOG 80405 (Alliance)." *Journal of Clinical Oncology* 34 (15_suppl): 3504–3504. https://doi.org/10.1200/JCO.2016.34.15_suppl.3504.

Walker, F., J. Rothacker, C. Henderson, E. C. Nice, B. Catimel, H. H. Zhang, A. M. Scott, et al. 2012. "Ligand Binding Induces a Conformational Change in Epidermal Growth Factor Receptor Dimers." *Growth Factors* 30 (6): 394–409. https://doi.org/10.3109/ 08977194.2012.739619.

Wang, X., G. Li, S. Koul, R. Ohki, M. Maurer, A. Borczuk, and B. Halmos. 2014. "PHLDA2 Is a Key Oncogene-Induced Negative Feedback Inhibitor of EGFR/ErbB2 Signaling via Interference with AKT Signaling." *Oncotarget* 9 (38): 24914–26. https://doi.org/ 10.18632/oncotarget.3674.

Wee, P., and Z. Wang. 2017. "Epidermal Growth Factor Receptor Cell Proliferation Signaling Pathways." *Cancers* 9 (12): 52. https://doi.org/10.3390/cancers9050052.

Wen, D., E. Peles, R. Cupples, S. V Suggs, S. S. Bacus, Y. Luo, G. Trail, et al. 1992. "Neu Differentiation Factor: A Transmembrane Glycoprotein Containing an EGF Domain and an Immunoglobulin Homology Unit." *Cell* 69 (3): 559–72. https://doi.org/10.1016/0092-8674(92)90456-M.

Wilson, K. J., C. Mill, S. Lambert, J. Buchman, T. R. Wilson, V. Hernandez-Gordillo, R. M. Gallo, L. M. C. Ades, J. Settleman, and D. J. Riese. 2012. "EGFR Ligands Exhibit Functional Differences in Models of Paracrine and Autocrine Signaling." *Growth Factors* 30 (2): 107–16. https://doi.org/10.3109/08977194.2011.649918.

Yang, X.-D., X.-C. Jia, J. R. F. Corvalan, P. Wang, and C. G. Davis. 2001. "Development of ABX-EGF, a Fully Human Anti-EGF Receptor Monoclonal Antibody, for Cancer Therapy." *Critical Reviews in Oncology/Hematology*. https://doi.org/10.1016/ S1040-8428(00)00134-7.

Yang, X. D., X. C. Jia, J. R. Corvalan, P. Wang, C. G. Davis, and A. Jakobovits. 1999. "Eradication of Established Tumors by a Fully Human Monoclonal Antibody to the Epidermal Growth Factor Receptor without Concomitant Chemotherapy." *Cancer Research* 59 (6): 1236–43. http://www.ncbi.nlm.nih.gov/pubmed/10096554.

Yarden, Y. 2001. "The EGFR Family and Its Ligands in Human Cancer. Signalling Mechanisms and Therapeutic Opportunities." *European Journal of Cancer* 37 Suppl 4: S3–8. https://doi.org/10.1016/S0959-8049(01)00230-1.

Yewale, C., D. Baradia, I. Vhora, S. Patil, and A. Misra. 2013. "Epidermal Growth Factor Receptor Targeting in Cancer: A Review of Trends and Strategies." *Biomaterials* 34 (34): 8690–8707. https://doi.org/10.1016/j.biomaterials.2013.07.100.

Zhang, L., Y. Zhao, Y. Dai, J.-N. Cheng, Z. Gong, Y. Feng, C. Sun, Q. Jia, and B. Zhu. 2018. "Immune Landscape of Colorectal Cancer Tumor Microenvironment from Different Primary Tumor Location." *Frontiers in Immunology* 9: 1578. https://doi.org/10.3389/ fimmu.2018.01578.

Zhou, B.-B. S., J. S. Fridman, X. Liu, S. M. Friedman, R. C. Newton, and P. A. Scherle. 2005. "ADAM Proteases, ErbB Pathways and Cancer." *Expert Opinion on Investigational Drugs* 14 (6): 591–606. https://doi.org/10.1517/13543784.14.6.591.

Chapter 3

Re-Inventing the Randomized Controlled Trial in Medical Oncology: The Registry-Based Trial

Foroughi, Siavash, Hui-li Wong, Lucy Gately, Margaret Lee, Koen Simons, Jeanne Tie, Antony Wilks Burgess, and Peter Gibbs. 2018. "Re-Inventing the Randomized Controlled Trial in Medical Oncology: The Registry-Based Trial." *Asia-Pacific Journal of Clinical Oncology* 14 (6): 365–73. doi:10.1111/ajco.12992.

Published review article

Abstract

Substantial progress has recently been made in optimizing the management of cancer patients, resulting in major gains in survival and quality of life. Much of this progress has resulted from the serial testing of promising treatment strategies, typically using prospective randomized controlled trials to compare outcomes achieved with the new approach versus the current standard(s) of care. However, there is an ever-expanding list of important questions that are difficult to investigate, particularly with respect to determining the optimal sequencing and combination of proven active agents. With the rapidly growing list of clinical, pathologic, and molecular characteristics that promise to predict treatment benefit and/or risk for defined patient subsets, many new questions regarding how best to personalize our approach to treatment selection are emerging. These questions can be investigated in the context of registry-based randomized clinical trials. Recently, the potential of registry-based randomized clinical trials was demonstrated in cardiology, highlighting the ability to rapidly recruit large numbers of patients to a trial addressing an important clinical question, with minimal cost and high external validity. In this review, we discuss the challenges and limitations of conventional clinical trials in multidisciplinary cancer care, describe the potential advantages of registry-based randomized trials, and highlight several registry-based oncology studies that are already underway to demonstrate the feasibility of this approach.

Introduction

Randomized controlled trials (RCTs) serve as a tool for comparing existing treatments, as well as evaluating the efficacy of new therapies. These can be conducted in a broad patient population or in a specific subset of patients. Fundamental to the success of RCTs are the inclusion and exclusion criteria that impact patient enrolment, stratification according to known prognostic and predictive factors, and the use of randomization. Ultimately, the aim is that any differences in outcome can be presumed to be due to the differential effects of the management strategies being compared. In an ideal world, each RCT would recruit a patient sample that is truly representative of the general population of cancer patients, thus maximizing the external validity of the results. However, the majority of conventional RCTs have highly selective inclusion and exclusion criteria, which can limit their true generalizability.

In this article, we aim to describe the challenges and limitations of RCTs in cancer medicine and the opportunity to bridge the gap between RCTs and real-world clinical practice using prospective, registry-based randomized clinical trials (RRCTs). We discuss how this approach can improve some of the prohibitive aspects of conducting an RCT and explore the drawbacks of using this study design. Finally, we introduce RRCTs that we are currently undertaking to demonstrate their feasibility and potential to accelerate progress in optimizing patient treatment and outcomes in medical oncology.

Strengths and limitations of conventional RCTs

Randomized controlled trials (RCTs) form the evidentiary backbone of clinical practice guidelines and represent the gold standard in the clinical research paradigm (James, Rao, and Granger 2015; Diamond 2014). RCTs are quantitative, comparative, controlled clinical experiments. The strength of the RCT rests on high internal validity, relying on

the combination of stratification and randomization to ensure that the only systematic difference between two treatment arms is the patients' exposure to the intervention of interest (Sullivan 2011). Stratification can be applied to eliminate confounding by known strong predictive factors. When properly executed, stratification guarantees perfect balance between treatment groups on the characteristics included in the study; however, it is limited by sample size: it is impossible to have more strata than observations. As such, it is impossible to achieve perfect balance on all characteristics that are potentially relevant. Instead, randomization is used to minimize the imbalance on all remaining characteristics of patients, measured or unmeasured, causally related or unrelated to the outcome of interest (Roberts and Torgerson 1998). Furthermore, RCTs are controlled, prospective trials and these properties help establish causality as opposed to mere correlation or reverse causality.

However, RCTs can be subject to extrapolation bias that limit their impact on the care of patients in routine clinical practice. Often the selective nature of inclusion criteria applied to clinical trials can restrict participation to a subset of patients (Rothwell 2005), typically those that are relatively young and fit and/or with minimal co-morbidities. In contrast, a large proportion of patients presenting for treatment advice in the real world are frail, of advanced age and/or have multiple comorbidities. Determining the relevance and generalizability of the results achieved in RCTs conducted in patients with narrow selection criteria and strict protocols is a continuous challenge for clinicians (Bahit et al. 2003; Khorsan and Crawford 2014).

Although generalization is nontrivial even in optimal circumstances, the reporting of selection criteria for RCTs is often incomplete and this further obstructs proper interpretation of the results (Ross et al. 2009). A study examining the reporting of

selection criteria from 52 clinical trial protocols and the resulting publications found discrepancies in all trials assessed. Of the 1,248 eligibility criteria pre-specified in the protocols, 479 were missing and 163 were modified in the final publication. Of concern, the vast majority of missing eligibility criteria (96%) and the majority of modified eligibility criteria (54%) suggested that broader populations than initially specified were included in the studies (Blumle et al. 2011). Modified, incomplete or inadequate reporting of eligibility criteria can drastically alter the context and applicability of trial results.

Another challenge associated with conventional clinical trials is the escalating cost of conducting RCTs, limiting the number of potential studies that can be undertaken in an increasingly resource-strained environment. Furthermore, the high cost of purchasing newer drugs prevent many of them from being tested in novel settings, such as a different patient group, a different disease or disease subset, or a different line of therapy. Exploring these new frontiers relies on substantial funding support being provided by the pharmaceutical industry in the form of free drug supply.

Increasingly, tumor types are being further sub-categorized according to clinical, pathologic, molecular, or prior treatment characteristics rather than simply the tumor site of origin. For example, studies of patients with metastatic colorectal cancer (mCRC) may focus on a clinical subset (e.g. resectable liver metastases) (Nordlinger et al. 2013), a molecular subset (e.g. *KRAS* wild-type) (Venook et al. 2017) or mandate a prior number of therapy(ies) (Mayer et al. 2015). Subclassification can limit the number of available patients that can be recruited and by extension, the feasibility of individual sites to participate in new trials. Where the focus is on even more uncommon subsets, such as *BRAF*-mutated mCRC (8-12% of patients) (Davies et al. 2002; Venderbosch et al. 2014), *HER2*-expressing mCRC (5%) (Sartore-Bianchi et al. 2016) or mismatch repair deficient

mCRC (5%) (Venderbosch et al. 2014) very few sites can contribute significant numbers of patients. Given the time, effort and costs associated with opening a trial at each site, it may not be feasible for the investigator or the sponsor to open a study that is predicted to recruit only one or two patients over the projected study period.

By this mechanism, centers contributing patients to clinical trials are also increasingly selected, with the centers recruiting the majority of patients likely to have the largest patient volumes and an interest or expertise in the disease type being studied (Gheorghe et al. 2013). Treatment outcomes achieved at these major clinical centers may possibly be significantly different to those achieved with the same approach at a smaller, non-specialist center. Factors contributing to this potential discrepancy include the treatment being provided by highly expert subspecialist clinicians who are supported by expert trial nurses, pharmacists and radiologists; and the patient population being fit and motivated to travel to the center to participate in a clinical trial that offers access to a promising new therapy (Kahan 2014). Consequently, for many reasons the gains achieved in clinical trials do not necessarily translate to the broader clinical population across the spectrum of health care systems (Localio et al. 2001). The outcomes of a drug given frequently at a major treatment center are conceivably quite different to the same drug being delivered infrequently at smaller centers (Haj Mohammad et al. 2016).

With a growing number of treatment options and potential biomarkers for cancer patients, there is a rapidly expanding number of unanswered questions related to the optimal combining or sequencing of treatment options and how best to tailor this for each individual patient and their circumstances. New approaches to advancing knowledge are needed, in particular approaches that deliver estimates that are unbiased and can readily

be used to impact practice without the need to extrapolate to a significantly different patient population.

Strengths and limitations of clinical registries to advance therapy knowledge

A clinical registry is defined as an organized system that uses observational study methods to systematically collect uniform data to evaluate specified outcomes for a population defined by a particular disease (Gliklich, Dreyer, and Leavy 2014).

The Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) is one of the best-known efforts to collect a standard dataset on consecutive cancer patients (National Cancer Institute 2017). Data collection for the SEER program began in 1973 and initially included data from seven geographic areas in the United States (Hankey, Ries, and Edwards 1999). Later this effort was expanded to include data collection across 20 geographic areas representing approximately 28% of the US population. SEER collects information on patient demographics, tumor characteristics, including primary tumor location and extent of disease, and the use of cancer-directed surgery, radiation therapy, systemic therapy and patient survival (National Cancer Institute 2017). The main limitation of SEER data is not its validity but rather the depth of clinical information (Nathan and Pawlik 2008); in SEER, treatment data are only collected for the first course of treatment (Baxter, Whitson, and Tuttle 2007), which can limit analysis of treatment outcomes across different centers. Linkage of SEER data to Medicare claims for health care services allows a more comprehensive examination of treatment information, but SEER-Medicare only captures data on patients aged 65 years and older (Warren et al. 2002), limiting its generalizability. Further, the SEER data on metastatic tumors is often inadequate (this data are only captured well if the tumor is metastatic at diagnosis); and SEER does not include detailed data on

comorbidities, potentially important confounders of treatment responses and outcomes (Nathan and Pawlik 2008; Hankey, Ries, and Edwards 1999).

The SEER program highlights some of the advantages and disadvantages associated with observational research based on analysis of clinical registry data. Broad inclusion criteria increase the probability that patient numbers are sufficiently large to explore outcomes in various subpopulations of interest, including those that are underrepresented in RCTs. Registries such as SEER can provide data on the benefit and safety of new therapies once they are adopted in the routine clinical setting (Cohen et al. 2015). However, in these analyses for efficacy and/or safety, therapy selection bias is always a confounding factor as almost inevitably it is the fitter patients with a better prognosis and potentially higher treatment tolerance that are more likely to receive the intervention (Krumholz 2009), and such patients have improved outcomes independent of treatment effect. Although some of this can potentially be accounted for, for example in a stratified analysis, using propensity scores or adjustment in multiple regression, in the absence of randomization it is not possible to be confident that observed differences are truly the result of treatment impact. It is impossible to adjust for characteristics that were not measured. Although all studies are limited to observed data, available registry data are limited by design choices that may not have been optimal for the research question. Lack of data availability is not limited to diagnostic or prognostic factors; efficacy analyses may also be influenced by nonstandardized endpoints or endpoints that are difficult to evaluate retrospectively, although reliable outcome data can be achieved for hard endpoints such as overall survival. Finally, data quality is a common and legitimate concern for registry-based research, particularly where data are collected retrospectively or for data points that may be poorly documented in the clinical notes, such as performance status, comorbidity, family history or patient preferences. Reporting specifications and standards may also be

modified over time or differ between regions, which may lead to differences in quality and incompatible categorisations.

Despite statistical advances (Lauer and D'Agostino 2013) comparative observational registry studies have generally not been accepted by the cancer research community as sufficient to change standards of care, mostly because they lack the credibility associated with pre-registration and randomization (Saad et al. 2017). The "intellectual trap" described by Lauer and D'Agostino (2013) between randomized trials that lack external validity (generalizability) and observational studies that lack internal validity, owing to unmeasured confounders, can be avoided using a randomized registry-based approach (Table 1).

Table 1. Comparison of conventional and registry-based randomized clinical trials.

	Conventional, randomized clinical trials	**Registry-based randomized clinical trials**
Key features	NHMRC Level I and II data Randomization Blinding Typically investigates new treatments Highly controlled and monitored	Identification and recruitment of eligible patients defined via a registry database search In-built, or easily accessible, randomization module with less selected and consecutive patient enrolment Standard agents in their usual setting, with standard of care investigations Generous inclusion criteria and contributions from centers that would otherwise not participate
Strengths	Quantitative, comparative, and controlled experiments with adequate power (gold-standard clinical design) Removes confounding factors High internal validity (robust comparison between intervention and control)	High external validity through inclusion of real-world patients and smaller centers Answer simple, pragmatic questions that are otherwise unlikely to be explored Rapid patient recruitment due to broad eligibility criteria Data collection can be part of routine care Substantially lower cost than conventional RCT
Limitations	Low external validity (highly selected populations due to strict inclusion and exclusion criteria) Very complex High cost	Requires existing high-quality registry Research question and study design limited by registry features; cannot address all clinical questions due to reliance on hard endpoints such as overall survival Uncertainty about data quality due to: Limited monitoring Incomplete or missing data Definition and accuracy of dataset

NHMRC, National Health and Medical Research Council.

These limitations of *post hoc* analyses of registry data can be overcome if comparisons are conducted prospectively by using high-quality registries that mandate capture of critical data points, stratify patients according to important prognostic variables and (most importantly) incorporate randomization to effectively eliminate selection bias. The registry RCT thus presents an opportunity to harness the "untapped potential of observational research to inform clinical decision making" (Visvanathan et al. 2017).

Registry-based randomized clinical trials

Registry-based randomized clinical trials (RRCTs) are a relatively new concept for clinical research in cardiology and other disease settings (James, Rao, and Granger 2015), and are yet to be explored as an alternative to conventional randomized studies in oncology. By combining the major strengths of a conventional RCT, namely patient stratification and randomization, important questions could be reliably addressed using RRCTs with greater ease and at significantly lower cost. Rapid patient recruitment is typically achieved by consecutive enrolment using generous eligibility criteria, which also enhances the generalizability of results due to inclusion of a real-world population (Rothwell 2005; Lauer and D'Agostino 2013).

Essentially replacing the case report form (CRF) used for a conventional RCT is the registry database, which captures the key data relevant to the patient group, the treatment administered and the outcome of interest. Typically, the registry database will have far less data for each patient than is captured for a conventional RCT, but where the treatments are with standard agents or common treatment combinations with well described adverse events, the focus of the RRCT can be upon a hard clinical endpoint such as overall survival. An exhaustive effort to collect all clinical and adverse event data is not made, but most cancer registries can capture key adverse event data, including

serious adverse events. In addition, linkage to population-based registry data (e.g. the National Death Index) and administrative data (e.g. pharmacy databases) can complement RRCTs and improve data accuracy (Nathan and Pawlik 2008).

Although lacking the credibility of a conventional RCT, registry-based RCTs can still retain key aspects of study conduct such as sample size calculations, interim analyses for futility and data audits. The economic advantage of RRCTs is largely due to the externalization of costs usually associated with conventional RCTs (Table 2).

Table 2. Costs associated with the conduct of conventional versus registry-based randomized clinical trials.

Costs	**Conventional RCT**	**Registry-based RCT**
Study setup		
Protocol development	✓	✓
Ethics and governance submissions	✓	✓
Case report form and database development	✓	×
Study conduct		
Investigational agent	✓	×
Study specific investigations	✓	×
Radiology disease assessments	✓	×
Additional pathology costs (e.g. sample retrieval)	✓	×
Personnel		
Research nurse	✓	×
Data manager	✓	✓[a]
Study monitor	✓	×
Project manager	✓	✓
Statistician	✓	✓

[a] Existing registry resource

Typically, these costs lie in establishing infrastructure for study databases, personnel costs associated with data capture and monitoring, as well as to conduct protocol specific procedures outside standard of care. By leveraging resources from an existing registry, these costs are indirectly transferred and result in a significant reduction in administrative and training costs. However, data quality may be affected as toxicity and dosing data are not always captured and radiological assessments are often not standardized, impacting

measurement of response rate and progression-free survival (PFS). In addition, other valuable endpoints such as quality of life are not usually captured as these are not typically included in standard registries. Thus, registry-based trials are most useful in pragmatic study designs that measure hard endpoints and compare treatments where toxicity profiles are already well known.

The Thrombus Aspiration in ST-Elevation Myocardial Infarction in Scandinavia (TASTE) trial was a landmark proof-of-concept RRCT designed to determine if routine intracoronary thrombus aspiration before percutaneous coronary intervention (PCI) reduced mortality in patients with ST-segment elevation myocardial infarctions (STEMI) (Fröbert et al. 2013). The study protocol was developed based on the Swedish Coronary Angiography and Angioplasty Registry (SCAAR), which formed the platform for randomization (Fröbert et al. 2010). SCAAR is one of four registries that form part of Sweden's national online cardiac registry: the Swedish Web-system for Enhancement and Development of Evidence-based care in Heart disease Evaluated According to Recommended Therapies (SWEDEHEART) (Taylor 2009; Jernberg et al. 2010). Using an established online registry as the basis for randomization, case record form data collection and follow-up made the trial economically and administratively feasible (Erlinge et al. 2016). A total of 7,244 patients, representing 60% of all STEMI patients referred for PCI in Sweden and Iceland during the study period, were randomized to receive PCI with or without thrombus aspiration. The study found no statistically significant difference in mortality at 30 days between the two groups. The study was completed in less than three years at an estimated cost of US$50 per randomized patient, a > 90% cost saving compared to a similar conventional RCT (James, Rao, and Granger 2015; Ramsberg and Neovius 2015).

Despite the benefits we have highlighted and the suitability of RRCTs to answer questions about effectiveness of treatments in real-world practice, challenges exist and care needs to be taken when determining if a registry-based approach is suitable (Table 1). A search of the clinicaltrial.gov website (NIH Clinical Trials Database 2017) identified at least nine ongoing RCTs implementing a registry-based approach (Table 3); notably none of these trials are enrolling patients with cancer.

Table 3. Active registry-based randomized clinical trials.

Trial	Status	Condition	Country of Origin	Clinicaltrials.gov identifier	Study start date
A registry-based randomized-controlled, double-blinded clinical trial of pimozide in patients with neuromuscular junction transmission dysfunction due to amyotrophic lateral sclerosis	Active, but not recruiting participants	Amyotrophic lateral sclerosis (ALS)	Canada	NCT02463825	April 2015
Bypass Equipoise Sleeve Trial, gastric bypass or sleeve gastrectomy: a randomized controlled national multicenter Study (BEST)	Recruiting	Severe obesity	Sweden	NCT02767505	September 2015
Ffr-gUidance for compLete Non-cuLprit REVASCularization: a registry-based randomized clinical trial	Recruiting	Coronary artery disease, ST-elevation myocardial infarction	Sweden	NCT02862119	August 2016
Timing of oral anticoagulant therapy in acute ischemic stroke with atrial fibrillation: a prospective multicenter registry-based non-inferiority randomized controlled clinical trial	Recruiting	Ischemic stroke, atrial fibrillation	Sweden	NCT02961348	February 2017
Randomized Evaluation of Decreased Usage of betablocCkErs after myocardial infarction in the SWEDEHEART registry: a registry-based, randomized, parallel, open-label, multicenter trial (REDUCE-SWEDEHEART)	Recruiting	Acute myocardial infarction, non-ST elevation myocardial infarction, ST elevation myocardial infarction	Sweden	NCT03278509	September 2017
Registry-based, prospective, single-blind, randomized controlled trial: robotic vs. laparoscopic ventral hernia repair with Intraperitoneal Onlay Mesh (IPOM)	Enrolling by invitation	Ventral hernia	United States of America	NCT03283982	September 2017

Trial	Status	Condition	Country of Origin	Clinicaltrials.gov identifier	Study start date
A multi-centre, open-label, nationwide registry-based, randomized, pragmatic trial comparing 2 post-PCI management strategies in high-risk PCI patients with complex clinical and lesion characteristics	Not yet recruiting	Coronary artery disease with myocardial infarction	Republic of Korea	NCT03217877	August 2017
Spironolactone Initiation Registry Randomized Interventional Trial in Heart Failure with Preserved Ejection Fraction, SPIRRIT-HFPEF	Not yet recruiting	Heart failure with preserved ejection fraction	Sweden	NCT02901184	December 2017
The effect of higher protein dosing in critically ill patients: a multicenter registry-based randomized trial	Not yet recruiting	Critical illness, Malnutrition	Canada	NCT03160547	January 2018

The potential of registry-based RCTs in medical oncology

In oncology practice, it is common to have multiple treatment options to select from, often with no clearly superior approach. Frequently this is because head-to-head RCTs have not been conducted successfully or that completed RCTs have not identified benefits in a specific patient subset, the RCT being essential to capturing the relative treatment effects of the available interventions (Zentner, Velasco-Garrido, and Busse 2005). As debates about comparative-cost effectiveness of clinical research intensify, it is becoming clear that many proposed clinical trials are too complex, difficult and expensive. The low cost of utilizing large-scale cancer registries as the platform for RCTs provides an opportunity to develop a new, and complementary, approach to clinical trial research (Figure 1). Logically, this requires the existence of suitable established clinical registries.

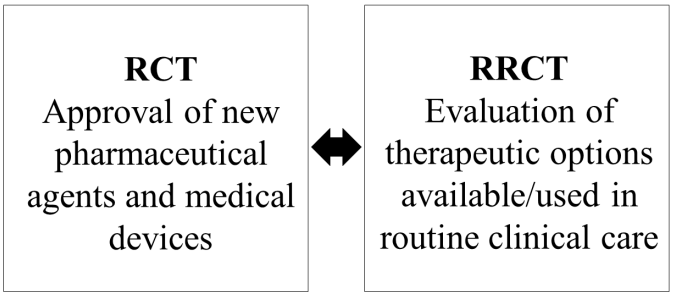


Figure 1. Registry RCTs complement traditional RCTs.

Oncology clinical registries in Australia: ready for primetime

Multiple cancer registries have been established in Australia. To support a registry-based RCT critical data items need to be captured, including known prognostic and predictive factors, and the data need to be captured in close to real-time as clearly patients cannot be randomized retrospectively after therapy has been initiated. The Treatment of Recurrent and Advanced Colorectal Cancer (TRACC) registry (Melbourne Health ethics number: 2009.113) (Field et al. 2013), is an Australian mCRC registry established in 2009 and expanded internationally to Hong Kong in 2016 (Biogrid Australia 2017). This multisite

prospective clinical registry captures baseline data, multidisciplinary treatment data across all lines of therapy, and outcome data on consecutive patients with mCRC managed at the participating centers. All information relevant to prognosis and treatment selection is recorded routinely, often at point of care, and typically at multiple important landmarks that occur well after the diagnosis. For example, the sites of metastatic disease and the Eastern Cooperative Oncology Group (ECOG) scale of performance status (PS) are recorded at the beginning of each new line of therapy. Details of the primary tumor and any adjuvant therapy are also available in the database. A general description of data items collected in the TRACC registry is provided in Table 4.

Table 4. Variables recorded in the TRACC registry.

Data items related to patient prognostic factors
Gender
Age
Patient medical history including comorbidities
Performance status
Data items related to disease factors
Primary tumor location
Metastatic disease sites
Tumor staging
Biomarker status (CEA, extended *RAS, PIK3CA, BRAF*, MMR)
Data items related to clinical decision-making
Treatment location and type
Treatment intent
Data items related to local and systemic therapy (captured across all lines of therapy)
Chemotherapy regimen
Biologic therapies
Treatment start and stop dates
Adverse events and serious adverse events
Best response to treatment
Date of progressive disease
Data items related to surgical interventions and outcomes
Resection of primary tumor
Resection of metastatic disease

Data related to supportive care
Referral to palliative care
Data related to survival outcomes
Date of death
Date of last follow-up

The TRACC registry provides a secure, 128-bit SSL encrypted, web-based platform allowing data to be entered directly by clinicians or data officers into an electronic database. The electronic platform is designed to minimize data errors through features such as mandatory fields, logic rules and built-in data dictionaries. As of the third quarter of 2017, patient enrolment is ongoing at 32 sites across Australia and Hong Kong and 2,870 participants have been recruited. De-identified data from TRACC is accessible to researchers by application to BioGrid Australia (https://www.biogrid.org.au), a process which includes ethics and scientific review as well as custodian approval (Biogrid Access Request 2017). TRACC has been used to examine the impact and safety of bevacizumab in routine care (Roohullah et al. 2014), and to explore whether the results are relevant in other populations of interest such as patients with *RAS* mutations, the elderly or those with peritoneal disease (Roohullah et al. 2015).

Similar registries have been established for prostate cancer (trial ID: ACTRN12616000585426), pancreatic cancer (trial ID: ACTRN12617001474347), HER2+ breast cancer (neoadjuvant and metastatic; trial ID: ACTRN12615000232538), and more recently efforts are underway in brain tumors, bladder cancer, testicular cancer, and gastro-esophageal cancer. Each of these new registries will involve sites across Australia, including both public and private settings, as well as metropolitan and regional centers. There is also increasing international engagement, now including multiple participating centers in the Asia Pacific region (New Zealand, Singapore, and Hong Kong) in many of the registries.

Recently initiated and proposed registry-based RCTs in Australia

With funding support from the Victorian Comprehensive Cancer Centre (VCCC), our group is initiating randomized registry-based trials in three different tumor types: mCRC (ALT-TRACC), glioblastoma (EX-TEM) and pancreatic cancer (PAN-PAL), the first two now having ethics approval and opening at multiple sites (Table 5). In each instance, all the required treatment and outcome data is being captured in existing registries coordinated by the Walter and Eliza Hall Institute of Medical Research (WEHI), with the only intervention being the need to randomize patients to receive treatments that are either current or emerging standard of care therapies. The disease types and trial designs are described later.

Metastatic colorectal cancer: ALT-TRACC

ALT-TRACC is an RRCT randomizing patients with treatment-naïve mCRC to alternating two cycles of oxaliplatin and irinotecan doublet chemotherapy versus standard continuous doublet chemotherapy. The hypothesis is that using alternating schedules of oxaliplatin and irinotecan doublets might increase efficacy by delaying the emergence of cell resistance and will also mean exposure to all active chemotherapy agents in the first-line setting, an alternative to giving all three drugs at once (FOLFOXIRI) where toxicity concerns have limited clinical uptake. Further justification for ALT-TRACC comes from recent phase II data from the Sequential and Concurrent FOLFOXIRI/Bevacizumab Regimens Versus FOLFOX/Bevacizumab in First-Line Metastatic Colorectal Cancer (STEAM) study, presented at the 2016 American Society of Clinical Oncology Gastrointestinal Cancers Symposium (Bendell et al. 2016). Two hundred and eighty patients were randomized in a 1:1:1 ratio to FOLFOXIRI plus bevacizumab versus alternating FOLFOX and FOLFIRI plus bevacizumab (as per the ALT-TRACC protocol) versus FOLFOX plus bevacizumab. The alternating FOLFOX-FOLFIRI regimen

appeared to have comparable efficacy to FOLFOXIRI without the additional toxicity associated with the triplet regimen. Compared to standard FOLFOX-bevacizumab, the alternating regimen resulted in an improved response rate and PFS, with no evident increase in toxicity. To validate this approach, a randomized phase III study powered to show an overall survival difference is required, hence the ALT-TRACC study.

Glioblastoma: EX-TEM

EX-TEM is an RRCT where recently diagnosed glioblastoma patients will be randomized to receive the standard 6 months of temozolomide following initial combination radiation therapy and temozolomide (as per the STUPP protocol (Stupp et al. 2005)) or to an additional 6 months of temozolomide. Notably, there has been little improvement in the survival outcomes of patients with newly diagnosed glioblastoma since the introduction of chemotherapy following the seminal trial results in 2005 (Stupp et al. 2005). The justification for a definitive randomized study is that several small retrospective studies and limited randomized phase II studies have suggested that extending post-radiation chemotherapy up to 12 cycles can improve survival, without an increase in toxicity (Seiz et al. 2010; Refae et al. 2015; Roldán Urgoiti, Singh, and Easaw 2012). The small numbers of patients included in the randomized studies, as well as the multiple confounders in any unselected clinical series create concern regarding the significance of the reported findings. Nevertheless, many oncologists in the United States and Canada have now adopted 12 cycles as their routine standard of care. To our knowledge EX-TEM will be the first randomized phase III clinical trial to examine the relative effectiveness of 6 months versus 12 months of post-radiation chemotherapy in this patient population. Recently, this study has been endorsed by the Cooperative Trials Group for Neuro-Oncology (COGNO), with a commitment to support this study at a large number of centers across Australia.

Pancreatic Cancer: PAN-PAL

PAN-PAL will randomize patients with recently diagnosed metastatic pancreatic cancer to early palliative care integrated with standard oncologic care or standard oncologic care alone, with palliative care referral in the standard arm at clinician discretion. Previous studies have indicated that late referrals to palliative care compromise the meaningful effect that these services can provide to quality of life and end-of-life care for patients with metastatic disease (Zimmermann et al. 2008). Along with this, however, is the seminal RCT in metastatic non-small cell lung cancer that demonstrated that early integration of palliative care resulted in clinically meaningful and statistically significant gains in overall survival (Temel et al. 2010). In the absence of an explanation for this survival gain, the clinical community remains uncertain about the survival impact of early palliative care referral, however it is clearly a study that is worth repeating, albeit in a different poor prognosis tumour type. A further RCT in pancreatic cancer demonstrated that early palliative care improved quality of life and had a significant positive impact on indicators of end-of-life treatment aggressiveness, however no difference in survival outcomes was found (Maltoni et al. 2016). This study was arguably limited by treating oncologists in the control arm being trained in palliative care delivery, thereby reducing the impact of the interventional arm. Furthermore, the palliative care intervention involved a single physician expert only. PAN-PAL will evaluate this intervention in a multi-centre fashion, utilizing an RRCT platform.

Each of these registry RCTs will be powered to show a difference in the same hard endpoint, that is overall survival, and linkage with the cancer registry and national death index will be undertaken to ensure accurate survival data are obtained.

Table 5. Key features of ALT-TRACC, EX-TEM, and PAN-PAL.

Trial name	Registry	Patient population	Treatment arms	Number of patients	Number of study centers	Endpoint
ALT-TRACC	Treatment of Recurrent and Advanced Colorectal Cancer (TRACC)	Metastatic colorectal cancer	Continuous versus alternating oxaliplatin and irinotecan doublet chemotherapy	Initially 140, to be expanded to 1,000	6 initially	Primary: Feasibility Secondary: OS, PFS, RR
PAN-PAL	Pancreatic cancer: Understanding Routine Practice and Lifting End results (PURPLE).	Pancreatic cancer	Early palliative care integrated with standard oncologic care, versus standard oncologic care alone	300	6 initially	Primary: Feasibility Secondary: OS, QOL indices including chemotherapy utilization
EX-TEM	The Brain Registry Australia: Innovation and traNslation (BRAIN)	Newly diagnosed glioblastoma	6 versus 12 months of post radiation chemotherapy	320	6 initially. Study is COGNO endorsed and new sites will be opened.	Primary: OS Secondary: PFS, SAEs

ALT-TRACC: Alternating oxaliplatin and irinotecan doublet schedules versus continuous doublet chemotherapy in previously untreated metastatic colorectal cancer: A Treatment of Recurrent and Advanced Colorectal Cancer registry-based prospective randomised trial; PAN-PAL: A randomised study of early referral of pancreatic cancer patients to palliative care; EX-TEM: phase III trial of Extended Temozolomide in newly diagnosed glioblastoma; OS: overall survival; PFS: progression-free survival; RR: response rate; SAE: serious adverse events.

Conclusions

Registry-based RCTs can provide a timely and cost-effective solution to answering clinical questions, which could be practice changing while bridging the gap between RCTs and observational studies, including phase IV clinical trials. Registry-based randomized trials can reduce costs and simplify trial conduct while achieving both high internal and external validity. However, they rely on the availability of extensive, accessible, high-quality registries and appropriate methods for the randomization for each trial question. We have identified several registries which provide a platform for oncology data collection, randomization and follow-up. These registries and the proposed registry RCTs provide a unique and significant opportunity for a new approach to clinical research in oncology.

References

Bahit, M. C., C. P. Cannon, E. M. Antman, S. A. Murphy, C. M. Gibson, C. H. McCabe, E. Braunwald, and R. J. Gibbons. 2003. "Direct Comparison of Characteristics, Treatment, and Outcomes of Patients Enrolled versus Patients Not Enrolled in a Clinical Trial at Centers Participating in the TIMI 9 Trial and TIMI 9 Registry." *American Heart Journal* 145 (1): 109–17. https://doi.org/10.1067/mhj.2003.43.

Baxter, N. N., B. A. Whitson, and T. M. Tuttle. 2007. "Trends in the Treatment and Outcome of Pancreatic Cancer in the United States." *Annals of Surgical Oncology* 14 (4): 1320–26. https://doi.org/10.1245/s10434-006-9249-8.

Bendell, J. C., B. R. Tan, J. A. Reeves, H. Xiong, B. G. Somer, H.-J. Lenz, H. S. Hochster, et al. 2016. "Overall Response Rate (ORR) in STEAM, a Randomized, Open-Label, Phase 2 Trial of Sequential and Concurrent FOLFOXIRI-Bevacizumab (BEV) vs FOLFOX-BEV for the First-Line (1L) Treatment (Tx) of Patients (Pts) with Metastatic Colorectal Cancer (MCRC)." *Journal of Clinical Oncology* 34 (4_suppl): 492–492. https://doi.org/10.1200/jco.2016.34.4_suppl.492.

"Biogrid Access Request." 2017. Accessed November 5, 2017. https://apps.biogrid.org.au/access/gui/Start.aspx.

"Biogrid Australia." 2017. Accessed November 5, 2017. https://www.biogrid.org.au/.

Blumle, A., J. J. Meerpohl, G. Rucker, G. Antes, M. Schumacher, and E. von Elm. 2011. "Reporting of Eligibility Criteria of Randomised Trials: Cohort Study Comparing Trial Protocols with Subsequent Articles." *British Medical Journal* 342: d1828–d1828. https://doi.org/10.1136/bmj.d1828.

Cohen, A. T., S. Goto, K. Schreiber, and C. Torp-Pedersen. 2015. "Why Do We Need Observational Studies of Everyday Patients in the Real-Life Setting?" *European Heart Journal, Supplement* 17: D2–8. https://doi.org/10.1093/eurheartj/suv035.

Davies, H., G. R. Bignell, C. Cox, P. Stephens, S. Edkins, S. Clegg, J. Teague, et al. 2002. "Mutations of the BRAF Gene in Human Cancer." *Nature* 417 (6892): 949–54. https://doi.org/10.1038/nature00766.

Diamond, G. A. 2014. "Randomized Trials, Observational Registries, and the Foundations of Evidence-Based Medicine." *American Journal of Cardiology* 113 (8): 1436–41. https://doi.org/10.1016/j.amjcard.2014.01.420.

Erlinge, D., S. Koul, P. Eriksson, F. Scherstén, E. Omerovic, R. Linder, O. P. Östlund, L. Wallentin, O. Fröbert, and S. James. 2016. "Bivalirudin versus Heparin in Non-ST and ST-Segment Elevation Myocardial Infarction—a Registry-Based Randomized Clinical Trial in the SWEDEHEART Registry (the VALIDATE-SWEDEHEART Trial)." *American Heart Journal* 175: 36–46. https://doi.org/10.1016/j.ahj.2016.02.007.

Field, K., H. L. Wong, J. Shapiro, S. Kosmider, J. Tie, S. Bae, D. Yip, et al. 2013. "Developing a National Database for Metastatic Colorectal Cancer Management: Perspectives and Challenges." *Internal Medicine Journal* 43 (11): 1224–31. https://doi.org/10.1111/imj.12230.

Fröbert, O., B. Lagerqvist, T. Gudnason, L. Thuesen, R. Svensson, G. K. Olivecrona, and S. K. James. 2010. "Thrombus Aspiration in ST-Elevation Myocardial Infarction in Scandinavia (TASTE Trial). A Multicenter, Prospective, Randomized, Controlled Clinical Registry Trial Based on the Swedish Angiography and Angioplasty Registry (SCAAR) Platform. Study Design And." *American Heart Journal* 160 (6): 1042–48. https://doi.org/10.1016/j.ahj.2010.08.040.

Fröbert, O., B. Lagerqvist, G. K. Olivecrona, E. Omerovic, T. Gudnason, M. Maeng, M. Aasa, et al. 2013. "Thrombus Aspiration during ST-Segment Elevation Myocardial Infarction." *New England Journal of Medicine* 369 (17): 1587–97. https://doi.org/

10.1056/NEJMoa1308789.

Gheorghe, A., T. E. Roberts, J. C. Ives, B. R. Fletcher, and M. Calvert. 2013. "Centre Selection for Clinical Trials and the Generalisability of Results: A Mixed Methods Study." Edited by Isabelle Boutron. *PLoS One* 8 (2): e56560. https://doi.org/ 10.1371/ journal.pone.0056560.

Gliklich, R. E., N. A. Dreyer, and M. B. Leavy. 2014. *Registries for Evaluating Patient Outcomes*. *Registries for Evaluating Patient Outcomes: A User's Guide*. Agency for Healthcare Research and Quality (US). https://www.ncbi.nlm.nih.gov/books/ NBK208643/?report=printable.

Haj Mohammad, N., N. Bernards, M. G. H. Besselink, O. R. Busch, J. W. Wilmink, G. J. M. Creemers, I. H. J. T. De Hingh, V. E. P. P. Lemmens, and H. W. M. van Laarhoven. 2016. "Volume Matters in the Systemic Treatment of Metastatic Pancreatic Cancer : A Population - Based Study in the Netherlands." *Journal of Cancer Research and Clinical Oncology* 142 (6): 1353–60. https://doi.org/ 10.1007/s00432-016-2140-5.

Hankey, B. F., L. A. Ries, and B. K. Edwards. 1999. "The Surveillance, Epidemiology, and End Results Program : A National Resource." *Cancer Epidemiology Biomarkers & Prevention* 8 (December): 1117–21. https://www.ncbi.nlm.nih.gov/pubmed/ 10613347.

James, S. K., S. V Rao, and C. B. Granger. 2015. "Registry-Based Randomized Clinical Trials - A New Clinical Trial Paradigm." *Nature Reviews Cardiology* 12 (5): 312–16. https://doi.org/10.1038/nrcardio.2015.33.

Jernberg, T., M. F. Attebring, K. Hambraeus, T. Ivert, S. James, A. Jeppsson, B. Lagerqvist, B. Lindahl, U. Stenestrand, and L. Wallentin. 2010. "The Swedish Web-System for Enhancement and Development of Evidence-Based Care in Heart

Disease Evaluated According to Recommended Therapies (SWEDEHEART)." *Heart (British Cardiac Society)* 96 (20): 1617–21. https://doi.org/10.1136/hrt.2010.198804.

Kahan, B. C. 2014. "Accounting for Centre-Effects in Multicentre Trials with a Binary Outcome – When, Why, and How?" *BMC Medical Research Methodology* 14 (1): 20. https://doi.org/10.1186/1471-2288-14-20.

Khorsan, R., and C. Crawford. 2014. "External Validity and Model Validity: A Conceptual Approach for Systematic Review Methodology." *Evidence-Based Complementary and Alternative Medicine* 2014 (March): 1–12. https://doi.org/10.1155/2014/694804.

Krumholz, H. M. 2009. "Registries and Selection Bias: The Need for Accountability." *Circulation. Cardiovascular Quality and Outcomes* 2 (6): 517–18. https://doi.org/10.1161/CIRCOUTCOMES.109.916601.

Lauer, M. S., and R. B. D'Agostino. 2013. "The Randomized Registry Trial — The Next Disruptive Technology in Clinical Research?" *New England Journal of Medicine* 369 (17): 1579–81. https://doi.org/10.1056/NEJMp1310102.

Localio, A. R., J. A. Berlin, T. R. Ten Have, and S. E. Kimmel. 2001. "Adjustments for Center in Multicenter Studies: An Overview." *Annals of Internal Medicine* 135 (2): 112. https://doi.org/10.7326/0003-4819-135-2-200107170-00012.

Maltoni, M., E. Scarpi, M. Dall'Agata, S. Schiavon, C. Biasini, C. Codecà, C. M. Broglia, et al. 2016. "Systematic versus On-Demand Early Palliative Care: A Randomised Clinical Trial Assessing Quality of Care and Treatment Aggressiveness near the End of Life." *European Journal of Cancer* 69: 110–18. https://doi.org/10.1016/j.ejca.2016.10.004.

Mayer, R. J., E. Van Cutsem, A. Falcone, T. Yoshino, R. Garcia-Carbonero, N.

Mizunuma, K. Yamazaki, et al. 2015. “Randomized Trial of TAS-102 for Refractory Metastatic Colorectal Cancer.” *New England Journal of Medicine* 372 (20): 1909–19. https://doi.org/10.1056/NEJMoa1414325.

Nathan, H., and T. M. Pawlik. 2008. “Limitations of Claims and Registry Data in Surgical Oncology Research.” *Annals of Surgical Oncology* 15 (2): 415–23. https://doi.org/10.1245/s10434-007-9658-3.

National Cancer Institute. 2017. “Surveillance Epidemiology and End Results (SEER).” 2017. http://seer.cancer.gov/.

“NIH Clinical Trials Database.” 2017. Accessed November 5, 2017. https://clinicaltrials.gov/ct2/home.

Nordlinger, B., H. Sorbye, B. Glimelius, G. J. Poston, P. M. Schlag, P. Rougier, W. O. Bechstein, et al. 2013. “Perioperative FOLFOX4 Chemotherapy and Surgery versus Surgery Alone for Resectable Liver Metastases from Colorectal Cancer (EORTC 40983): Long-Term Results of a Randomised, Controlled, Phase 3 Trial.” *The Lancet Oncology* 14 (12): 1208–15. https://doi.org/10.1016/S1470-2045(13)70447-9.

Ramsberg, J., and M. Neovius. 2015. “Register or Electronic Health Records Enriched Randomized Pragmatic Trials: The Future of Clinical Effectiveness and Cost-Effectiveness Trials?” *Nordic Journal of Health Economics* 5 (1): 62–76. https://doi.org/10.5617/njhe.1386.

Refae, A. A., A. Ezzat, D. A. Salem, and M. Mahrous. 2015. “Protracted Adjuvant Temozolomide in Glioblastoma Multiforme.” *Journal of Cancer Therapy* 6: 748–58. https://doi.org/10.4236/jct.2015.68082.

Roberts, C., and D. Torgerson. 1998. “Understanding Controlled Trials: Randomisation Methods in Controlled Trials.” *British Medical Journal* 317 (7168): 1301–10.

https://doi.org/10.1136/bmj.317.7168.1301.

Roldán Urgoiti, G. B., A. D. Singh, and J. C. Easaw. 2012. "Extended Adjuvant Temozolomide for Treatment of Newly Diagnosed Glioblastoma Multiforme." *Journal of Neuro-Oncology* 108 (1): 173–77. https://doi.org/10.1007/s11060-012-0826-3.

Roohullah, A., H. L. Wong, K. M. Sjoquist, P. Gibbs, K. Field, B. Tran, J. Shapiro, et al. 2015. "Gastrointestinal Perforation in Metastatic Colorectal Cancer Patients with Peritoneal Metastases Receiving Bevacizumab." *World Journal of Gastroenterology* 21 (17): 5352–58. https://doi.org/10.3748/wjg.v21.i17.5352.

Roohullah, A., H. Wong, K. M. Sjoquist, K. M. Field, B. Tran, J. D. Shapiro, J. J. McKendrick, et al. 2014. "Safety and Efficacy of Bevacizumab and Systemic Therapy in Metastatic Colorectal Cancer (MCRC) Patients with Peritoneal Disease in the Treatment of Recurrent and Advanced Colorectal Cancer (TRACC) Database." *Journal of Clinical Oncology* 32 (3_suppl): 569–569. https://doi.org/10.1200/jco.2014.32.3_suppl.569.

Ross, J. S., G. K. Mulvey, E. M. Hines, S. E. Nissen, and H. M. Krumholz. 2009. "Trial Publication after Registration in ClinicalTrials.Gov: A Cross-Sectional Analysis." Edited by Ida Sim. *PLoS Medicine* 6 (9): e1000144. https://doi.org/10.1371/journal.pmed.1000144.

Rothwell, P. M. 2005. "External Validity of Randomised Controlled Trials: To Whom Do the Results of This Trial Apply?" *The Lancet* 365 (9453): 82–93. https://doi.org/10.1016/S0140-6736(04)17670-8.

Saad, E. D., X. Paoletti, T. Burzykowski, and M. Buyse. 2017. "Precision Medicine Needs Randomized Clinical Trials." *Nature Reviews Clinical Oncology* 14 (5): 317–23. https://doi.org/10.1038/nrclinonc.2017.8.

Sartore-Bianchi, A., L. Trusolino, C. Martino, K. Bencardino, S. Lonardi, F. Bergamo, V. Zagonel, et al. 2016. “Dual-Targeted Therapy with Trastuzumab and Lapatinib in Treatment-Refractory, KRAS Codon 12/13 Wild-Type, HER2-Positive Metastatic Colorectal Cancer (HERACLES): A Proof-of-Concept, Multicentre, Open-Label, Phase 2 Trial.” *The Lancet Oncology* 17 (6): 738–46. https://doi.org/10.1016/S1470-2045(16)00150-9.

Seiz, M., U. Krafft, C. F. Freyschlag, C. Weiss, K. Schmieder, F. Lohr, F. Wenz, C. Thomé, and J. Tuettenberg. 2010. “Long-Term Adjuvant Administration of Temozolomide in Patients with Glioblastoma Multiforme: Experience of a Single Institution.” *Journal of Cancer Research and Clinical Oncology* 136 (11): 1691–95. https://doi.org/10.1007/s00432-010-0827-6.

Stupp, R., W. Mason, M. J. van den Bent, M. Weller, B. M. Fisher, M. J. B. Taphoorn, K. Belanger, et al. 2005. “Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma.” *New England Journal of Medicine* 352 (10): 987–96. https://doi.org/10.1056/NEJMoa043330.

Sullivan, G. M. 2011. “Getting off the ‘Gold Standard’: Randomized Controlled Trials and Education Research.” *Journal of Graduate Medical Education* 3 (3): 285–89. https://doi.org/10.4300/JGME-D-11-00147.1.

Taylor, J. 2009. “SWEDEHEART: Sweden’s New Online Cardiac Registry, the First of Its Kind.” *European Heart Journal* 30 (18): 2165–73. https://doi.org/10.1093/eurheartj/ehp299.

Temel, J. S., J. A. Greer, A. Muzikansky, E. R. Gallagher, S. Admane, V. A. Jackson, C. M. Dahlin, et al. 2010. “Early Palliative Care for Patients with Metastatic Non–Small-Cell Lung Cancer.” *New England Journal of Medicine* 363 (8): 733–42. https://doi.org/10.1056/NEJMoa1000678.

Venderbosch, S., I. D. Nagtegaal, T. S. Maughan, C. G. Smith, J. P. Cheadle, D. Fisher, R. Kaplan, et al. 2014. "Mismatch Repair Status and BRAF Mutation Status in Metastatic Colorectal Cancer Patients: A Pooled Analysis of the CAIRO, CAIRO2, COIN, and FOCUS Studies." *Clinical Cancer Research* 20 (20): 5322–30. https://doi.org/10.1158/1078-0432.CCR-14-0332.

Venook, A. P., D. Niedzwiecki, H. Lenz, F. Innocenti, B. Fruth, J. A. Meyerhardt, D. Schrag, et al. 2017. "Effect of First-Line Chemotherapy Combined With Cetuximab or Bevacizumab on Overall Survival in Patients With KRAS Wild-Type Advanced or Metastatic Colorectal Cancer: A Randomized Clinical Trial." *Journal of the American Medical Association* 317 (23): 2392–2401. https://doi.org/10.1001/jama.2017.7105.

Visvanathan, K., L. A. Levit, D. Raghavan, C. A. Hudis, S. Wong, A. Dueck, and G. H. Lyman. 2017. "Untapped Potential of Observational Research to Inform Clinical Decision Making: American Society of Clinical Oncology Research Statement." *Journal of Clinical Oncology* 35 (16): 6–8. https://doi.org/10.1200/JCO.2017.72.6414.

Warren, J. L., C. N. Klabunde, D. Schrag, P. B. Bach, and F. Riley. 2002. "Overview of the SEER-Medicare Data : Content , Research Applications , and Generalizability to the United States Elderly Population Source." *Medical Care* 40 (8). https://doi.org/10.1097/01.MLR.0000020942.47004.03.

Zentner, A., M. Velasco-Garrido, and R. Busse. 2005. "Methods for the Comparative Evaluation of Pharmaceuticals." *GMS Health Technology Assessment* 1: Doc09. http://www.ncbi.nlm.nih.gov/pubmed/21289930.

Zimmermann, C., R. Riechelmann, M. Krzyzanowska, G. Rodin, and I. Tannock. 2008. "Effectiveness of Specialized Palliative Care." *Journal of the American Medical*

Association 299 (14): 1698. https://doi.org/10.1001/jama.299.14.1698.

Chapter 4.

Materials and Methods

This chapter describes general materials and methods used; specific methods and reagents are described in greater detail in the relevant results chapters.

Clinical Registries

This work was undertaken using data from two prospective CRC registries: the Australian Comprehensive Cancer Outcomes and Research Database for Colorectal Cancer (ACCORD-CRC) established in 2000 (Kosmider et al. 2008), and the Treatment of Recurrent and Advanced Colorectal Cancer (TRACC) registry, established in 2009 (Field et al. 2013). These prospective multi-centre registries collect comprehensive clinicopathologic, treatment and outcome data from consecutive patients with mCRC from participating centres across Australia. Data fields encompass demographics; surgical, pathological, and staging information; treatment course and duration; recurrence; as well as follow-up and survival information. Raw data was obtained from the BioGrid Australia platform and analysed for each specific research question. Multiple data queries were required and errors were checked and corrected, for example, identification and consolidation of duplicate cases, impossible values, and missing data. Where required, cases were re-identified for chart review and data cleaning, then de-identified for analysis.

Cohort Study Tissue Samples

One of the projects in this thesis involved the retrieval and analysis of archival CRC specimens for EGF receptor and ligand expression by immunohistochemistry (further

detail in chapter 5). Suitable cases were selected from the TRACC registry, where eligibility was restricted to patients with known *KRAS* wild-type mCRC who had received combination chemotherapy with palliative intent. Formalin-fixed paraffin-embedded (FFPE) tumour blocks were retrieved from hospital anatomical pathology departments; where these were not available, unstained slides cut at 4µm thickness were retrieved instead.

Digital Microscope Slide Scanning

All stained specimens were digitised, at 20x objective lens, using the Panoramic Scan II at the Walter and Eliza Hall Institute of Medical Research (Parkville, VIC, AUS) and visualised with CaseViewer (both from 3DHISTECH Ltd, Budapest, Hungary).

Building a REDCap Framework for Registry Trials

Enrolment, randomisation and additional data points specific to the ALT-TRACC registry trial were developed in a standalone REDCap module. Data in the REDCap module can be linked to the disease registry via the unique registry ID, captured in the module at enrolment (Figure 1). The REDCap module facilitates registry-based trials by providing an online enrolment and randomisation portal, allowing live enrolment and randomisation allocation at study sites.

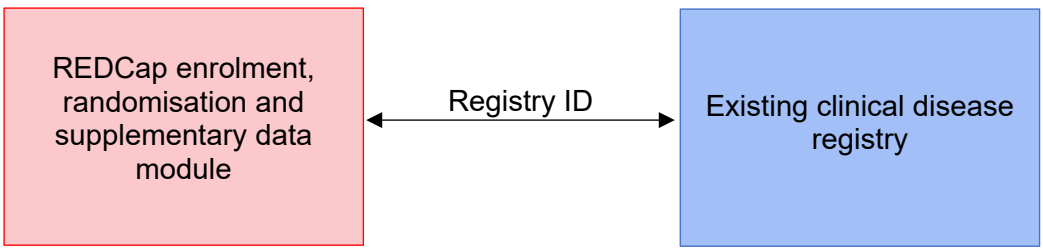


Figure 1. Framework for registry-based trials leveraging an existing clinical disease registry.

Ethics Statement

Ethical Use of Human Tissue and Data

This research utilised human tissue and data sourced from patients with mCRC treated at the Royal Melbourne Hospital, Melbourne Private Hospital, Western Hospital, Western Private Hospital, Box Hill Hospital and Epworth Eastern Private Hospital. De-identified patient clinicopathological data were obtained from BioGrid Australia. Patient cohorts and characteristics are described in detail in relevant thesis chapters.

The ethics approval to conduct research using these human tissues and/or data was obtained from the Melbourne Health Human Research Ethics Committee (HREC). Individual HREC approval numbers are included in the respective results chapters.

Patient Identifiers and Data Privacy

Patient privacy was protected throughout the duration of this research. All tissue and data used in the described projects were catalogued and coded with a unique identification number to protect individual privacy. This included storage of derived tissue components (such as tissue slides), thus eliminating the need for personally identifying information.

Data was stored in password-protected electronic databases, such as Research Electronic Data Capture (REDCap; Harris et al. 2009), which are hosted centrally on encrypted servers at the Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia.

Chapter 5.

Immunohistochemical Evaluation of the Prognostic and Predictive Power of Epidermal Growth Factor Receptor Ligand Levels in Patients with Metastatic Colorectal Cancer

Foroughi, Siavash, Ryan Hutchinson, Hui-li Wong, Michael Christie, Ahida Batrouney, Rachel Wong, Margaret Lee, Jeanne Tie, Antony Wilks Burgess, and Peter Gibbs. 2020. "Immunohistochemical evaluation of the prognostic and predictive power of epidermal growth factor receptor ligand levels in patients with metastatic colorectal cancer"

Manuscript under review

Abstract

Purpose

For patients with metastatic colorectal cancer (mCRC), selection for treatment with epidermal growth factor receptor (EGFR) inhibitors currently relies on the utility of *RAS* mutational status as a negative predictor for benefit. Patients with a left-sided primary benefit most from treatment with an EGFR inhibitor. Here we investigated the utility of immunohistochemical expression of the EGFR ligands amphiregulin (AREG) and epiregulin (EREG) as mCRC biomarkers.

Patients and Methods

This was a retrospective analysis of 99 mCRC patients with *KRAS* wild-type tumors. Expression levels of EGFR, AREG and EREG were determined by immunohistochemistry. The correlations between positive and negative levels of ligand immunoreactivity, clinicopathologic characteristics and overall survival (OS) were analyzed.

Results

AREG and EREG positivity was seen in 49% and 50% of cases, respectively. No difference in expression was observed by primary tumor side. Overall, there was no significant difference in OS by AREG or EREG expression. In the subset of patients who received an EGFR inhibitor, EREG positivity was associated with longer OS (median 34 vs. 27 months, P = 0.033), driven by a difference in patients with a left-sided primary (HR 0.37, P = 0.015).

Conclusion

Immunohistochemical analysis of the EGFR ligands (AREG and EREG) is not prognostic for mCRC survival. EREG protein expression is predictive for benefit from EGFR

inhibitor therapy in mCRC patients with a left-sided primary. Our study supports further investigation into EREG as a predictive biomarker in mCRC.

Introduction

In the first-line metastatic colorectal cancer (mCRC) setting, standard treatment options include combination chemotherapy plus an anti-angiogenic (bevacizumab) or combination chemotherapy plus an epidermal growth factor receptor (EGFR) inhibitor (cetuximab or panitumumab) (Venook et al. 2017; Stintzing et al. 2016; Rivera et al. 2017). Patient selection for treatment with EGFR inhibitors relies on the utility of *RAS* mutational status as a negative predictor for response (Lièvre et al. 2006) and while this has been helpful, the lack of a *RAS* mutation is not predictive of response to these therapies (Al-Shamsi, Alhazzani, and Wolff 2015). Patients with a right-sided primary also derive limited benefit from EGFR inhibitor treatment (Triest et al. 2019).

Several reports have suggested that increased mRNA expression of the genes encoding two of the EGFR ligands, amphiregulin (AREG) and epiregulin (EREG), are strongly associated with a therapeutic benefit from EGFR inhibitor therapy for patients with *RAS* wild-type mCRC (Khambata-Ford et al. 2007; Jacobs et al. 2009; Seligmann et al. 2016). However, the prognostic significance of the level of ligand expression is unknown, which could confound analysis of outcome data in a study population where all patients received an EGFR inhibitor. Also, previous reports that AREG and EREG expression (Lee et al. 2016) are decreased in right-sided colorectal cancers, which are known to have a worse prognosis (Lee, Menter, and Kopetz 2017), could also confound analyses of survival impact.

While AREG and EREG show promise as clinically relevant biomarkers, a validated patient selection strategy has not been developed. The majority of studies investigating AREG and EREG have used mRNA expression, with variability in methods and cutoffs used to define high and low expression (Khambata-Ford et al. 2007; Seligmann et al.

2016). Immunohistochemical (IHC) analysis offers multiple advantages, including not requiring an RNA purification step, being low cost, and being reliably performed on formalin-fixed paraffin-embedded (FFPE) tissue, which is routinely stored in anatomical pathology laboratories. Immunohistochemical analysis also offers the utility to evaluate intra-tumoral heterogeneity as well as the temporal-spatial distribution and the cellular localization of biomarkers. To date, there is limited data available assessing AREG and EREG expression in FFPE tissue sections from mCRC patients using IHC analysis.

In this study, we measured the levels of AREG and EREG expression using IHC in a cohort of *KRAS* wild-type mCRC patients. We then investigated the correlation of AREG and EREG expression levels with clinicopathologic features and clinical outcomes for mCRC patients. Our hypothesis was that AREG and EREG expression levels would be correlated to primary tumor location and outcomes, particularly in patients treated with EGFR inhibitors.

Patients and Methods

Patient selection and study design

This was a retrospective analysis of mCRC patients with *KRAS* wild-type tumors who all had initially received first-line, palliative intent, oxaliplatin- or irinotecan-based combination chemotherapy with a fluoropyrimidine backbone. Patients may also have received a biologic agent (bevacizumab, cetuximab or panitumumab) during their treatment course. Eligible patients were selected from the Australian Comprehensive Cancer Outcomes and Research Database for Colorectal Cancer (ACCORD-CRC) (Kosmider et al. 2008) and the Treatment of Recurrent and Advanced Colorectal Cancer (TRACC) (Field et al. 2013) registries. ACCORD-CRC and TRACC are prospective multicenter registries enrolling consecutive patients with mCRC from participating

centers across Australia. These registries capture comprehensive clinicopathologic, treatment and outcome data, which were extracted for analysis in this study. *KRAS* mutation data in the registries are from standard of care testing, meaning that not all patients underwent extended *RAS* testing, as this has only been funded in Australia since 2014.

Archival tissue from the primary tumor or a metastasis was retrieved for analysis. Samples collected after exposure to first-line chemotherapy were excluded. Where available, full-face resection specimens were used instead of biopsy samples. Hematoxylin and eosin (H&E)-stained sections for all tissue samples were assessed by a pathologist to ensure suitability for IHC analysis.

EGFR and EGFR-ligand IHC staining

Sections were freshly cut at 4 μm and left to dry at room temperature for 2 hours. The EGF-receptor (intracellular and extracellular domains), amphiregulin and epiregulin proteins were detected by IHC using CONFIRM anti-EGFR (5B7) rabbit monoclonal antibody (moAb) which binds to the internal domain of the EGFR, CONFIRM anti-EGFR (3C6) mouse moAb (both from Ventana Medical Systems/Roche Diagnostics, Tucson, AZ, USA) which binds to the external domain of the EGFR, anti-AREG (sc-74501) mouse moAb (Santa Cruz Biotech, Santa Cruz, CA, USA) and anti-EREG (D4O5I) rabbit moAb (Cell Signaling Technology, Danvers, MA, USA), respectively. Assays were performed using prediluted antibodies; EGFR antibodies are packaged ready-to-use, with the Ventana OptiView DAB IHC Detection Kit on the Ventana Benchmark ULTRA automated slide stainer (Ventana Medical Systems/Roche Diagnostics, Tucson, AZ, USA). Sectioning and staining of all samples were performed at the Department of Anatomical Pathology, The Royal Melbourne Hospital (Parkville, VIC, AUS). The

staining procedure included baking sections, on-board deparaffinization, pre-treatment using Ventana Ultra Cell Conditioning Solution 1 (ULTRA CC1), except for EGFR 3C6 which used Ventana Protease 1 digestion, and incubation with target antibody (Supplementary Table 1). In addition, H&E staining was performed for each case to aid in orientation of the IHC slides.

Scoring criteria

Staining intensity was scored independently by two observers (SF and RH) blinded to clinical information. The following IHC scoring scheme was used: membrane staining intensity was determined for tumor cells in a fixed field, where; 0, no staining; 1^+, faint membranous reactivity; 2^+, moderate membranous reactivity; and 3^+, strong membranous reactivity. The percentage of cells at each staining level was recorded and a histological score (H-score) was then calculated using the following formula: (% of 1^+ cells) × 1 + (% of 2^+ cells) × 2 + (% of 3^+ cells) × 3 (Pirker et al. 2012). All cases which were found to be discordant were rescored and discussed by both observers before a final score was given. Examples of immunostaining for each antibody are shown in Figure 1.

Statistical analysis

Cases were categorized into positive and negative staining according to a H-score of ≥ 1 versus 0, respectively. Descriptive statistics were used to summarize the clinicopathologic characteristics of the study cohort. The Chi-square and Mann-Whitney tests were used for categorical and continuous variables, respectively. Overall survival (OS) was defined as time from diagnosis of mCRC to death, censored at the date of last review for patients who were not known to have died or were lost to follow-up. Kaplan-Meier curves were used to illustrate the associations and non-associations between survival and biomarker status and were evaluated with the log-rank test. A two-tailed p-value of 0.05 was

considered statistically significant. Analysis was conducted in GraphPad Prism version 8.2.0 for Windows (GraphPad Software, La Jolla, CA, USA).

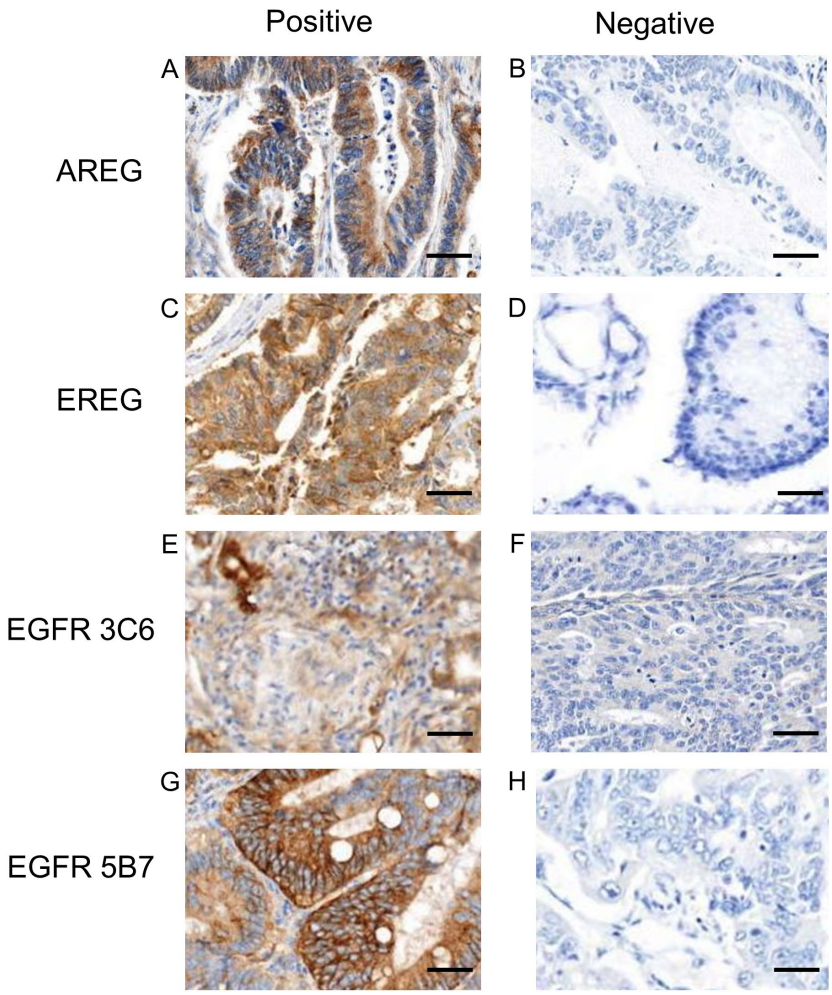


Figure 1. Examples of immunostaining for EGFR and EGFR ligands. (A, B) amphiregulin (AREG); (C, D) epiregulin (EREG); (E, F) EGFR external domain (EGFR 3C6); (G, H) EGFR internal domain (EGFR 5B7). Left panels show positive staining and the right panels show negative staining. Scale bars = 50 µm.

Ethical considerations

Ethical approval was obtained from the Melbourne Health Human Research Ethics Committee (HREC ID 2011.225). Waiver of consent was approved for this study, as only de-identified archival tissue and data were used.

Results

Clinicopathological features

The study cohort consisted of the 99/479 (21%) registry patients with *KRAS* wild-type mCRC who received palliative intent combination chemotherapy and had sufficient archival tissue available for analysis (Figure 2). Patient clinicopathologic characteristics are summarized in Table 1. Median age was 63 years and 63% of patients were male. More patients (59%) had metastatic disease at the time of CRC diagnosis. Seventy percent of patients had left-sided primary tumors (69 of 99 patients). The most common metastatic sites were liver (68%) followed by lymph node (29%) and lung (24%). Clinical characteristics were similar for the 479 eligible registry patients (median age 62 years, 61% male, 71% metastatic disease at CRC diagnosis, 69% left-sided primary tumors).

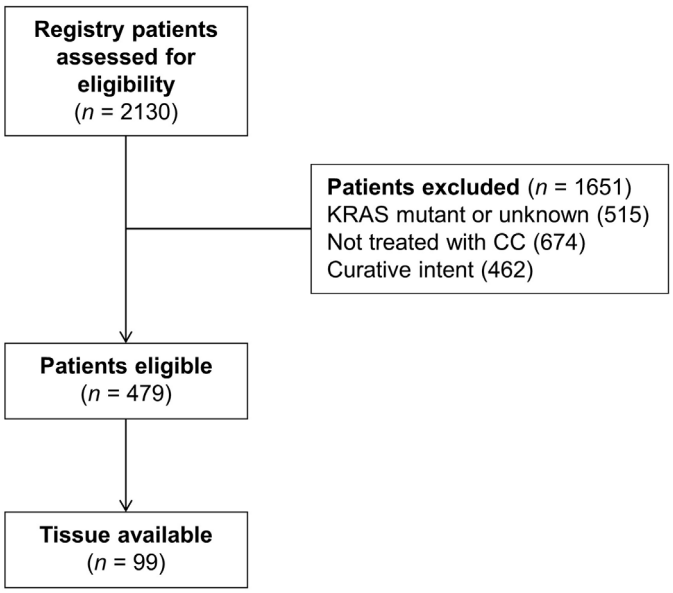
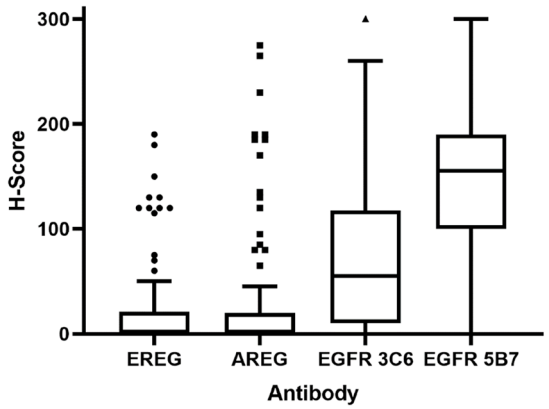


Figure 2. Patient selection process for inclusion in immunohistochemical assay cohort. CC, combination chemotherapy.

EGF Receptor, AREG and EREG protein levels

The majority of samples obtained were primary tumor samples (N=95, 96%) and full-face resections (N=82, 83%). Among the cases successfully analyzed, the median H-Scores were: EGFR 3C6 55 (range 0–300) and EGFR 5B7 155 (range 0–300) for external domain- and internal domain-specific antibodies, respectively, AREG 0 (range 0–275) and EREG 0.5 (range 0–190). The distribution of protein levels for the three proteins in these tissue biopsy specimens is illustrated in Figure 3.



	EREG	AREG	EGFR 3C6	EGFR 5B7
Successfully stained (n)	94	96	96	95
Median	0.5	0	55	155
Range	0 - 190	0 - 275	0 - 300	0 - 300

Figure 3. Distribution of H-Scores from IHC staining.

Receptor-ligand and ligand-ligand immunoreactivity were compared using Spearman correlation coefficients (Supplementary Figure 1). The strongest correlation was obtained between the internal and external EGFR domain antibodies (Spearman correlation coefficient = 0.66, $P < 0.001$). We note that no significant correlation was observed for the staining of EREG and AREG (Supplementary Figure 1).

Clinicopathologic features by ligand expression

The distribution of clinicopathologic features by AREG and EREG expression is described in Table 1. AREG positive patients were older (median age 65.4 vs. 60.9 years, P = 0.035) and EREG positive patients were less likely to have distant lymph node metastases (17 vs. 40%, P = 0.022), but no other significant differences were observed. There was no difference in ligand expression by primary tumor side: among left-sided tumors, 47% (31/66) expressed AREG and 55% (36/65) expressed EREG (p = 0.384); among right-sided tumors, 52% (15/29) expressed AREG and 39% (11/28) expressed EREG (p = 0.429). *BRAF* mutation and mismatch repair status were not formally compared, as this information was missing for up to two thirds of the patients.

Treatment and outcomes by ligand expression

The most common first-line chemotherapy regimen was an oxaliplatin doublet (83%). One patient received triplet chemotherapy with 5-fluorouracil, oxaliplatin and irinotecan. Almost half (41%) of patients received bevacizumab in combination with first-line chemotherapy and 44% received an EGFR inhibitor (cetuximab or panitumumab) at some point during their treatment course. There were no significant differences in treatments received among AREG and EREG positive or negative patients (Table 1).

At a median follow-up of 111.3 months (range 1.2-160.3), 86 patients (87%) had died. There was no significant difference in OS by AREG or EREG expression (Figure 4A and 4D). Median OS was 26.3 months for AREG positive and 24.7 months for AREG negative patients (P = 0.333); and 30.3 versus 23.6 months for EREG positive and negative patients, respectively (P = 0.055). When stratified according to EGFR inhibitor treatment, EREG positive patients who received an EGFR inhibitor had longer OS than negative patients (median 34 vs. 27 months, P = 0.033) (Figure 4E). Among patients who

did not receive an EGFR inhibitor, there was no difference in OS by AREG or EREG expression (Figure 4C and 4F). When further stratified by primary tumor location, the survival difference for EGFR inhibitor-treated patients by EREG status was limited to patients with a left-sided tumor (Figure 5).

Table 1. Association of AREG and EREG expression with clinicopathologic features.

	ALL	AREG			EREG		
		Negative	Positive	p-value	Negative	Positive	p-value
	N=99	N=49	N=47		N=47	N=47	
Median age at mCRC diagnosis (years)	63	60.9	65.4	0.035	63	62	0.581
Male gender	62	31 (63.3%)	29 (61.7%)	1.000	28 (59.6%)	31 (66.0%)	0.670
Year of mCRC diagnosis:				0.724			0.674
2002-2006	31	13 (26.5%)	16 (34.0%)		13 (27.7%)	16 (34%)	
2007-2011	31	16 (32.7%)	14 (29.8%)		14 (29.8%)	15 (31.9%)	
2012-2016	37	20 (40.8%)	17 (36.2%)		20 (42.6%)	16 (34%)	
Stage IV at CRC diagnosis	58	32 (65.3%)	24 (51.1%)	0.227	28 (59.6%)	28 (59.6%)	1.000
Primary side:				0.739			0.180
Left	69	35 (71.4%)	31 (66.0%)		29 (61.7%)	36 (76.6%)	
Right	29	14 (28.6%)	15 (31.9%)		17 (36.2%)	11 (23.4%)	
Unknown	1	0 (0.0%)	1 (2.1%)		1 (2.1%)	0 (0.0%)	
Metastatic site							
Liver	67	28 (57.1%)	36 (76.6%)	0.071	30 (63.8%)	35 (74.5%)	0.372
Lung	24	10 (20.4%)	14 (29.8%)	0.409	10 (21.3%)	12 (25.5%)	0.808
Lymph node	29	16 (32.7%)	13 (27.7%)	0.756	19 (40.4%)	8 (17.0%)	0.022
Peritoneum	18	8 (16.3%)	10 (21.3%)	0.719	12 (25.5%)	6 (12.8%)	0.189
Number of metastatic sites				0.220			0.445
1	62	34 (69.4%)	25 (53.2%)		26 (55.3%)	32 (68.1%)	
2	25	11 (22.4%)	14 (29.8%)		14 (29.8%)	10 (21.3%)	
3+	12	4 (8.2%)	8 (17%)		7 (14.9%)	5 (10.6%)	

	ALL	AREG			EREG		
BRAF status				-			-
Wild-type	27	11 (22.4%)	16 (34.0%)		13 (27.7%)	14 (29.8%)	
Mutant	7	3 (6.1%)	4 (8.5%)		5 (10.6%)	2 (4.3%)	
Unknown	65	35 (71.4%)	27 (57.4%)		29 (61.7%)	31 (66.0%)	
Mismatch repair status				-			-
Proficient	36	17 (34.7%)	18 (38.3%)		17 (36.2%)	18 (38.3%)	
Deficient	3	2 (4.1%)	1 (2.1%)		2 (4.3%)	1 (2.1%)	
Unknown	60	30 (61.2%)	28 (59.6%)		28 (59.6%)	28 (59.6%)	
First-line chemotherapy regimen				0.588			0.575
Oxaliplatin doublet	82	42 (85.7%)	37 (78.7%)		38 (80.9%)	40 (85.1%)	
Irinotecan doublet	16	7 (14.3%)	9 (19.1%)		9 (19.1%)	6 (12.8%)	
Triplet	1	0 (0.0%)	1 (2.1%)		0 (0.0%)	1 (2.1%)	
Bevacizumab with first-line chemotherapy	41	20 (40.8%)	20 (42.6%)	1.000	20 (42.6%)	19 (40.4%)	1.000
Received an EGFR inhibitor at any time	44	21 (42.9%)	22 (46.8%)	0.838	19 (40.4%)	22 (46.8%)	0.678

AREG: amphiregulin, EREG: epiregulin, EGFR: epidermal growth factor receptor, mCRC: metastatic colorectal cancer. Unknown values and triplet chemotherapy were excluded from p-value calculations. Due to rounding percentages may not add up to exactly 100%.

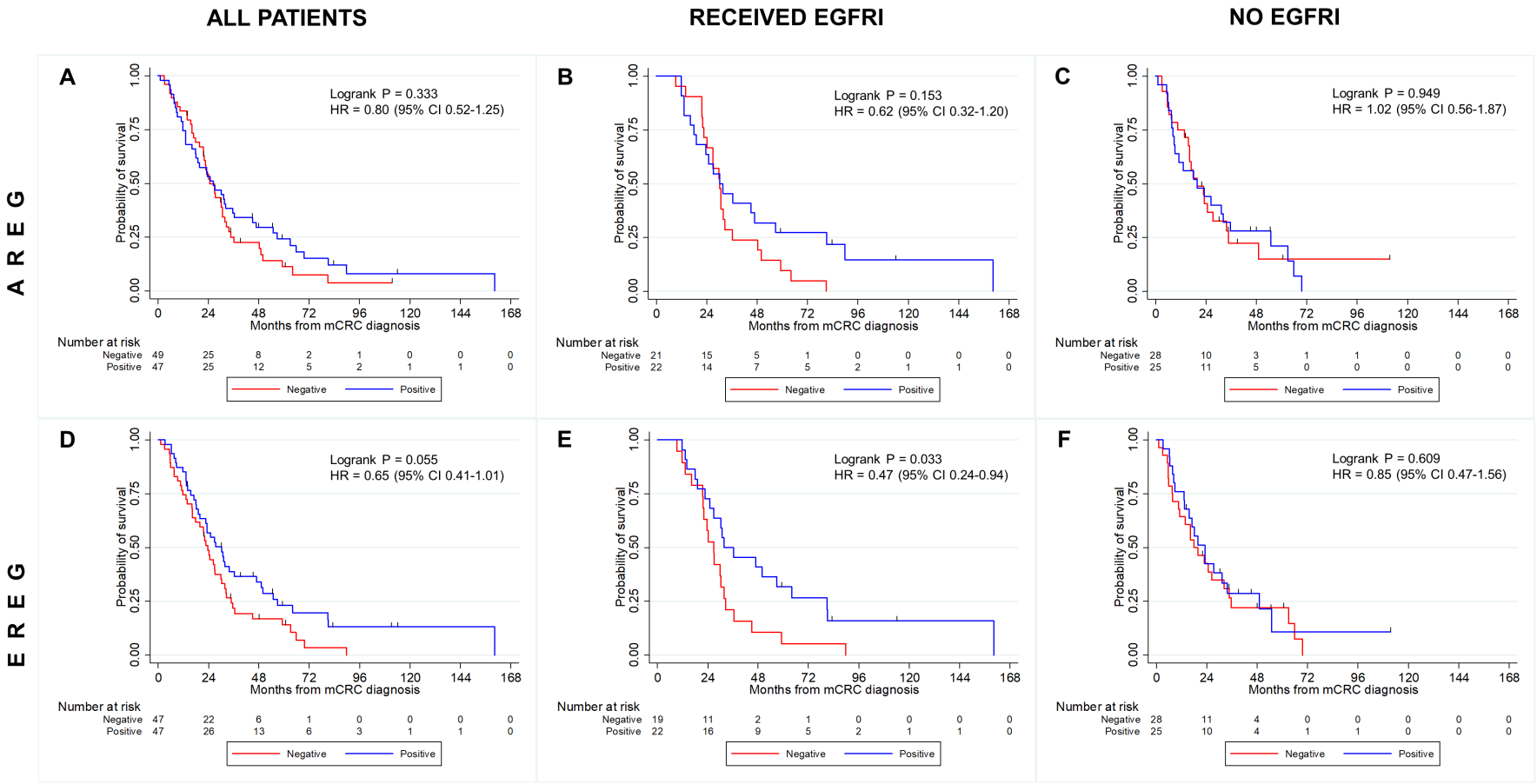


Figure 4. Overall survival (OS) according to AREG and EREG expression, stratified by receipt of epidermal growth factor receptor inhibitors (EGFRI). (A) Median OS for all patients by AREG expression: positive = 26.3 months (95% CI 18.1, 35.5), negative = 24.7 months (95% CI 21.6, 30.8). (B) Median OS for EGFRI-treated patients by AREG expression: positive = 31 months (95% CI 22.7, 32.5), negative = 30.3 months (95% CI 18.1, 56.8). (C) Median OS for non-EGFRI-treated patients by AREG expression: positive = 20months (95% CI 8.8, 35.5), negative = 19.9 months (95% CI 15.6, 33.8). (D) Median OS for all patients by EREG expression: positive = 30.3 months (95% CI 20, 46.8), negative = 23.6 months (95% CI 16.4, 29.9). (E) Median OS for EGFRI-treated patients by EREG expression: positive = 34months (95% CI 22.7, 64), negative = 27months (95% CI 21.6, 31.8). (F) Median OS for non-EGFRI-treated patients by EREG expression: positive = 23.1 months (95% CI 13.4, 33.8), negative = 18.9 months (95% CI 10.6, 32.3).

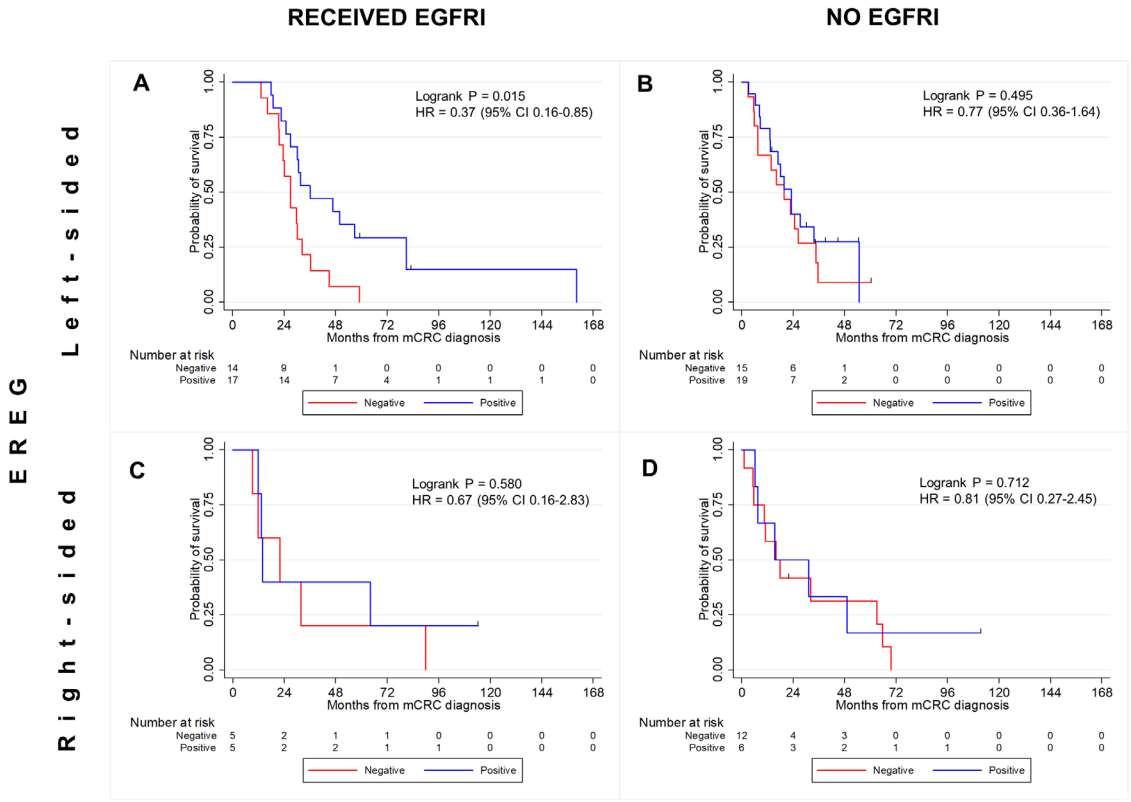


Figure 5. Overall survival (OS) according to EREG expression, stratified by receipt of epidermal growth factor receptor inhibitors (EGFRI) and primary tumor side. (A) Median OS for left-sided EGFRI-treated patients by EREG expression: positive = 36.4 months (95% CI 25, 80.9), negative = 27 months (95% CI 21.6, 32.5). (B) Median OS for left-sided non-EGFRI-treated patients by EREG expression: positive = 23.1 months (95% CI 13.3, 33.8), negative = 19.9 months (95% CI 6, 26.3). (C) Median OS for right-sided EGFRI-treated patients by EREG expression: positive = 14 months (95% CI 11.9, -), negative = 22.1 months (95% CI 9.2, -). (D) Median OS for right-sided non-EGFRI-treated patients by EREG expression: positive = 15.6 months (95% CI 6.2, -), negative = 16.1 months (95% CI 5.4, 63).

Discussion

The initial clinical trials demonstrating the activity of the anti-EGFR monoclonal antibodies cetuximab and panitumumab (Giusti et al. 2007; Cunningham et al. 2004) enrolled all patients with mCRC. Through binding to the ligand site in the extracellular domain of the EGFR, these anti-EGFR antibodies are able to prevent EGFR activation (Yang et al. 1999; Wong 2005). A major step forward in treatment selection was made when patients with a *RAS* mutation were shown not to benefit from treatment (Karapetis et al. 2008), with clinical benefit restricted to patients with wild-type *RAS* (Chan et al. 2017). While *RAS* mutations are the only established predictive tumor biomarker for selection of patients for EGFR inhibitor therapy, patients with a right-sided primary are known to derive limited benefit from treatment even when *RAS* wild-type (Wang et al. 2015; Grassadonia et al. 2019; Boeckx et al. 2018). Additional predictive markers for EGFR inhibitor therapy are urgently needed to aid clinical decision making.

In this context, we measured the levels of the EGFR ligands AREG and EREG in a cohort of 99 mCRC patients. Using a combination of percentage and intensity of IHC staining (the histological score) to quantitate the levels of AREG and EREG, we did not observe a significant difference in overall survival among patients with positive or negative AREG or EREG expression. When restricted to the patients who were not treated with an EGFR inhibitor, similar survival outcomes were also seen regardless of AREG or EREG expression status, demonstrating that in this registry cohort, AREG and EREG protein levels are not prognostic for mCRC patient survival.

To our knowledge, only two other studies have been published investigating the impact of IHC expression of EGFR ligands on mCRC outcomes (Yoshida et al. 2013; Khelwatty et al. 2017). Both were limited to patients who had received EGFR inhibitor therapy.

Yoshida et al. (2013) investigated the seven known EGFR ligands and defined staining as positive when > 30% of cancer cells were stained, without accounting for the intensity or location of the staining. In their cohort of 26 *KRAS* wild-type patients who received EGFR inhibitors in second or later lines of therapy, the expression of four ligands (including AREG and EREG) was associated with improved response rate and progression-free survival (PFS), with stronger associations observed if two or more ligands were co-expressed. For AREG, response rate was 50% in positive and 0% in negative cases (P = 0.007) and median PFS was 213 versus 85 days (P = 0.01); whereas for EREG, response rate for positive and negative cases was 58.3% versus 7.1% (P = 0.005) and median PFS was 238 versus 85 days (P = 0.0002). In contrast, Khelwatty et al. (2017) examined different cut-off percentages, intensity as well as location of ligand staining in their cohort of 60 *KRAS* wild-type patients, all of whom had treatment with an EGFR inhibitor. Notably, EREG was not detectable and only AREG cytoplasmic staining was observed and was reported to be associated with worse outcomes. Using a cut-off of > 10% tumor cells stained, AREG positive patients were more likely to have disease progression on cetuximab (100% versus 68.2%, P = 0.013). PFS was reported based on intensity of staining, with AREG 2+ intensity associated with worse PFS (HR 4.6, 95% CI 1.3-15.9, P = 0.018), although this was not significant on multivariate analysis.

The inclusion of patients who were not treated with an EGFR inhibitor in our cohort allowed us to examine, for the first time to our knowledge, the prognostic impact of AREG and EREG protein levels as assessed by IHC on the overall survival of mCRC patients. Previous studies that examined mRNA expression among EGFR inhibitor-untreated patients within clinical trial cohorts have reported conflicting results. In a post-hoc analysis of the CO.17 study of cetuximab versus best supportive care in chemotherapy-refractory mCRC, EREG mRNA expression level was not associated with

OS in the best supportive care arm (Jonker et al. 2014). Whereas in the PICCOLO study of second-line irinotecan alone or combined with panitumumab, EREG mRNA expression level was found to be prognostic for OS but not PFS, while AREG was not prognostic for either PFS or OS (Seligmann et al. 2016). Similarly, the FIRE1 study of first-line combination chemotherapy, which was conducted before EGFR inhibitor therapy was widely available, reported longer OS in patients with high levels of EREG mRNA, but no association between OS and levels of AREG mRNA (Stahler et al. 2016).

In our study using a registry cohort, EREG protein expression is predictive for benefit from EGFR inhibitor therapy in mCRC patients: EREG positive EGFR inhibitor-treated patients experienced the longest OS. Although AREG and EREG are co-localized to the same chromosome, bind the same receptor and are co-regulated, we did not observe a correlation in their immunoreactivity or their predictive power for the likely success of EGFR inhibitor treatment. Most data from mRNA studies show similar expression for AREG and EREG but appear to favor EREG as the stronger predictor of improved survival (Cushman et al. 2015; Stahler et al. 2016; Seligmann et al. 2016). Despite the co-expression of the genes, perhaps EREG is preferentially translated, processed, transported or activated (Foroughi et al. 2019) leading to increased stimulation of EGFR-dependent colorectal tumors. Our results do not support a role for AREG as a predictive or prognostic biomarker for patient responses to EGFR inhibitor treatment.

While we had hypothesized that primary tumor location would be a factor influencing the levels of AREG and EREG, there was no significant association between the AREG or EREG protein levels as measured by IHC and the location of the primary CRC. However, the predictive impact of EREG overexpression on EGFR inhibitor treatment appeared to be limited to left-sided cancers, a finding that should be confirmed in a larger independent

patient cohort. Previous studies reported that high mRNA expression of these ligands is significantly associated with primary tumor location in the left side of the colon (Seligmann et al. 2016; Missiaglia et al. 2014; Brulé et al. 2015; Lee et al. 2016). Khambata-Ford et al. (2007) postulated that higher gene expression of AREG and/or EREG may be due to a dependence of the tumor on autocrine EGFR-activation, and thus could predict for increased sensitivity to EGFR inhibitors. Since then, retrospective analyses have provided evidence that *RAS* wild-type right-sided tumors are associated with worse prognosis and outcomes with EGFR inhibitors, especially in first-line therapy (Wang et al. 2015; Grassadonia et al. 2019; Boeckx et al. 2018). Further, CIMP-driven tumors have been more frequently observed in right-sided tumors (Sugai et al. 2006; Koestler et al. 2014) while AREG and EREG expression have been observed to be strongly downregulated by methylation, suggesting a possible explanation for the association of right-sided primary tumor location and lack of EGFR inhibitor efficacy (Lee et al. 2016).

Our study has several limitations. Given its retrospective study design, it is subject to several potential biases, namely selection bias due to lack of randomization. A further limitation is the small sample size due to a large proportion of patients who had insufficient or unsuitable tissue for IHC analysis, despite meeting the clinical eligibility criteria. However, our study represents the largest IHC analysis of AREG and EREG protein levels in mCRC patients. Potentially, other biomarkers (*BRAF* mutation status, other *RAS* mutations or *PIK3CA* mutations) may be helpful in combination with AREG or EREG expression for predicting the likely outcomes for patients being treated with EGFR inhibitors. These studies would require many more patients with adequate tissue samples.

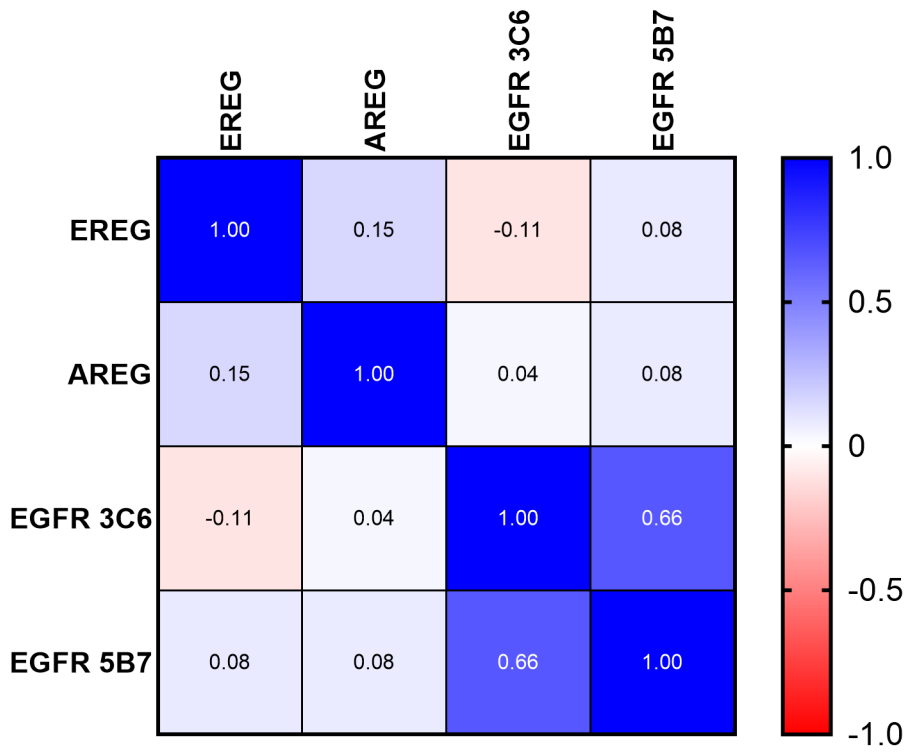
The variability in results from the published studies utilizing IHC (Khelwatty et al. 2017; Yoshida et al. 2013) or ligand mRNA levels (Foroughi et al. 2019; Jing et al. 2016), highlights the challenges associated with developing and validating EGFR ligand levels as clinically relevant biomarkers. In comparison to mRNA profiling, IHC analysis of primary tumor or biopsy samples may be more easily adapted to routine clinical practice, but there are yet no agreed standard protocols for measurement and reporting. Our findings offer support for further studies of AREG and EREG levels as determinants of EGFR inhibitor treatment choices, but it is important to consider the development of a robust, and perhaps automated, quantitative IHC or mass spectrometric scoring strategy which will reliably distinguish between intracellular, pro-AREG, pro-EREG and the corresponding levels of activated ligand or activated EGFR. Further, it would be useful to compare the predictive power of IHC-measured ligand levels with the predictive power of the corresponding levels of mRNA.

In conclusion, our study shows that IHC analysis of AREG and EREG is not prognostic for mCRC survival, however, EREG protein expression overall was predictive for benefit from EGFR inhibitor therapy, driven by this association in patients with a left-sided primary tumor. Our study supports further investigation of EREG as a predictive biomarker to optimize patient selection for EGFR inhibitor therapy in mCRC.

Supplementary Material

Supplementary Table 1. Dilution and incubation parameters for immunohistochemical analysis of the epidermal growth factor receptor, amphiregulin and epiregulin.

Antibody	Dilution	Incubation time at 36 deg C (min)	ULTRA CC1 at 100 deg C (min)	Protease 1 at 36 deg C (min)
EGFR 5B7	-	40	64	-
EGFR 3C6	-	40	-	8
AREG	1:300	16	8	-
EREG	1:100	60	64	-



Supplementary Figure 1. Spearman correlation analysis of the IHC staining by antibody pairs: EGFR 3C6 stains the external domain and EGFR 5B7 stains the internal domain of the EGFR.

References

Al-Shamsi, H. O., W. Alhazzani, and R. A. Wolff. 2015. “Extended RAS Testing in Metastatic Colorectal Cancer-Refining the Predictive Molecular Biomarkers.” *Journal of Gastrointestinal Oncology* 6 (3): 314–21. https://doi.org/10.3978/j.issn.2078-6891.2015.016.

Boeckx, N., K. Janssens, G. Van Camp, M. Rasschaert, G. Van Camp, M. Rasschaert, K. Papadimitriou, M. Peeters, and K. Op de Beeck. 2018. “The Predictive Value of Primary Tumor Location in Patients with Metastatic Colorectal Cancer: A Systematic Review.” *Critical Reviews in Oncology/Hematology* 121: 1–10. https://doi.org/10.1016/j.critrevonc.2017.11.003.

Brulé, S. Y., D. J. Jonker, C. S. Karapetis, C. J. O’Callaghan, M. J. Moore, R. Wong, N. C. Tebbutt, et al. 2015. “Location of Colon Cancer (Right-Sided versus Left-Sided) as a Prognostic Factor and a Predictor of Benefit from Cetuximab in NCIC CO.17.” *European Journal of Cancer* 51 (11): 1405–14. https://doi.org/10.1016/j.ejca.2015.03.015.

Chan, D. L. H., E. Segelov, R. S. H. Wong, A. Smith, R. A. Herbertson, B. T. Li, N. Tebbutt, T. Price, and N. Pavlakis. 2017. “Epidermal Growth Factor Receptor (EGFR) Inhibitors for Metastatic Colorectal Cancer.” *Cochrane Database of Systematic Reviews* 2017 (6). https://doi.org/10.1002/14651858.CD007047.pub2.

Cunningham, D., Y. Humblet, S. Siena, D. Khayat, H. Bleiberg, A. Santoro, D. Bets, et al. 2004. “Cetuximab Monotherapy and Cetuximab plus Irinotecan in Irinotecan-Refractory Metastatic Colorectal Cancer.” *New England Journal of Medicine* 351 (4): 337–45. https://doi.org/10.1056/NEJMoa033025.

Cushman, S. M., C. Jiang, A. J. Hatch, I. Shterev, A. B. Sibley, D. Niedzwiecki, A. P. Venook, K. Owzar, H. I. Hurwitz, and A. B. Nixon. 2015. “Gene Expression

Markers of Efficacy and Resistance to Cetuximab Treatment in Metastatic Colorectal Cancer: Results from CALGB 80203 (Alliance)." *Clinical Cancer Research* 21 (5): 1078–86. https://doi.org/10.1158/1078-0432.CCR-14-2313.

Field, K., H. L. Wong, J. Shapiro, S. Kosmider, J. Tie, S. Bae, D. Yip, et al. 2013. "Developing a National Database for Metastatic Colorectal Cancer Management: Perspectives and Challenges." *Internal Medicine Journal* 43 (11): 1224–31. https://doi.org/10.1111/imj.12230.

Foroughi, S., J. Tie, P. Gibbs, and A. W. Burgess. 2019. "Epidermal Growth Factor Receptor Ligands: Targets for Optimizing Treatment of Metastatic Colorectal Cancer." *Growth Factors* 37 (5–6): 209–25. https://doi.org/10.1080/08977194.2019.1703702.

Giusti, R. M., K. A. Shastri, M. H. Cohen, P. Keegan, and R. Pazdur. 2007. "FDA Drug Approval Summary: Panitumumab (Vectibix™)." *The Oncologist* 12 (5): 577–83. https://doi.org/10.1634/theoncologist.12-5-577.

Grassadonia, A., P. Di Marino, C. Ficorella, A. Cortellini, K. Cannita, A. Parisi, T. Gamucci, et al. 2019. "Impact of Primary Tumor Location in Patients with RAS Wild-Type Metastatic Colon Cancer Treated with First-Line Chemotherapy plus Anti-EGFR or Anti-VEGF Monoclonal Antibodies: A Retrospective Multicenter Study." *Journal of Cancer* 10 (24): 5926–34. https://doi.org/10.7150/jca.34550.

Jacobs, B., W. De Roock, H. Piessevaux, R. Van Oirbeek, B. Biesmans, J. De Schutter, S. Fieuws, et al. 2009. "Amphiregulin and Epiregulin MRNA Expression in Primary Tumors Predicts Outcome in Metastatic Colorectal Cancer Treated with Cetuximab." *Journal of Clinical Oncology* 27 (30): 5068–74. https://doi.org/10.1200/JCO.2008.21.3744.

Jing, C., Y. H. Jin, Z. You, Q. Qiong, and Z. Jun. 2016. "Prognostic Value of

Amphiregulin and Epiregulin MRNA Expression in Metastatic Colorectal Cancer Patients." *Oncotarget* 7 (34): 55890–99. https://doi.org/10.18632/oncotarget.10151.

Jonker, D. J., C. S. Karapetis, C. Harbison, C. J. O'Callaghan, D. Tu, R. J. Simes, D. P. Malone, et al. 2014. "Epiregulin Gene Expression as a Biomarker of Benefit from Cetuximab in the Treatment of Advanced Colorectal Cancer." *British Journal of Cancer* 110 (3): 648–55. https://doi.org/10.1038/bjc.2013.753.

Karapetis, C. S., S. Khambata-Ford, D. J. Jonker, C. J. O'Callaghan, D. Tu, N. C. Tebbutt, R. J. Simes, et al. 2008. "K-Ras Mutations and Benefit from Cetuximab in Advanced Colorectal Cancer." *New England Journal of Medicine* 359 (17): 1757–65. https://doi.org/10.1056/NEJMoa0804385.

Khambata-Ford, S., C. R. Garrett, N. J. Meropol, M. Basik, C. T. Harbison, S. Wu, T. W. Wong, et al. 2007. "Expression of Epiregulin and Amphiregulin and K-Ras Mutation Status Predict Disease Control in Metastatic Colorectal Cancer Patients Treated with Cetuximab." *Journal of Clinical Oncology* 25 (22): 3230–37. https://doi.org/10.1200/JCO.2006.10.5437.

Khelwatty, S., S. Essapen, I. Bagwan, M. Green, A. Seddon, and H. Modjtahedi. 2017. "The Impact of Co-Expression of Wild-Type EGFR and Its Ligands Determined by Immunohistochemistry for Response to Treatment with Cetuximab in Patients with Metastatic Colorectal Cancer." *Oncotarget* 8 (5): 7666–77. https://doi.org/10.18632/oncotarget.13835.

Koestler, D. C., J. Li, J. A. Baron, G. J. Tsongalis, L. F. Butterly, M. Goodrich, C. Lesseur, et al. 2014. "Distinct Patterns of DNA Methylation in Conventional Adenomas Involving the Right and Left Colon." *Modern Pathology* 27 (1): 145–55. https://doi.org/10.1038/modpathol.2013.104.

Kosmider, S., I. Jones, M. Hibbert, A. Hunter, S. McLaughlin, J. Johns, M. Chapman,

and P. Gibbs. 2008. “Towards Establishing a National Colorectal Cancer Database: Lessons Learnt from Bio21 Molecular Medicine Informatics Model.” *ANZ Journal of Surgery* 78 (9): 803–9. https://doi.org/10.1111/j.1445-2197.2008.04655.x.

Lee, M. S., E. J. McGuffey, J. S. Morris, G. Manyam, V. Baladandayuthapani, W. Wei, V. K. Morris, et al. 2016. “Association of CpG Island Methylator Phenotype and EREG/AREG Methylation and Expression in Colorectal Cancer.” *British Journal of Cancer* 114 (12): 1352–61. https://doi.org/10.1038/bjc.2016.87.

Lee, M. S., D. G. Menter, and S. Kopetz. 2017. “Right versus Left Colon Cancer Biology: Integrating the Consensus Molecular Subtypes.” *Journal of the National Comprehensive Cancer Network* 15 (3): 411–19. https://doi.org/10.6004/jnccn.2017.0038.

Lièvre, A., J. B. Bachet, D. Le Corre, V. Boige, B. Landi, J. F. Emile, J. F. Côté, et al. 2006. “KRAS Mutation Status Is Predictive of Response to Cetuximab Therapy in Colorectal Cancer.” *Cancer Research* 66 (8): 3992–95. https://doi.org/10.1158/0008-5472.CAN-06-0191.

Missiaglia, E., B. Jacobs, G. D’Ario, A. F. Di Narzo, C. Soneson, E. Budinska, V. Popovici, et al. 2014. “Distal and Proximal Colon Cancers Differ in Terms of Molecular, Pathological, and Clinical Features.” *Annals of Oncology* 25 (10): 1995–2001. https://doi.org/10.1093/annonc/mdu275.

Pirker, R., J. R. Pereira, J. Von Pawel, M. Krzakowski, R. Ramlau, K. Park, F. De Marinis, et al. 2012. “EGFR Expression as a Predictor of Survival for First-Line Chemotherapy plus Cetuximab in Patients with Advanced Non-Small-Cell Lung Cancer: Analysis of Data from the Phase 3 FLEX Study.” *The Lancet Oncology* 13 (1): 33–42. https://doi.org/10.1016/S1470-2045(11)70318-7.

Rivera, F., M. Karthaus, J. R. Hecht, I. Sevilla, F. Forget, G. Fasola, J. L. Canon, X. Guan,

G. Demonty, and L. S. Schwartzberg. 2017. “Final Analysis of the Randomised PEAK Trial: Overall Survival and Tumour Responses during First-Line Treatment with MFOLFOX6 plus Either Panitumumab or Bevacizumab in Patients with Metastatic Colorectal Carcinoma.” *International Journal of Colorectal Disease* 32 (8): 1179–90. https://doi.org/10.1007/s00384-017-2800-1.

Seligmann, J. F., F. Elliott, S. D. Richman, B. Jacobs, G. Hemmings, S. Brown, J. H. Barrett, S. Tejpar, P. Quirke, and M. T. Seymour. 2016. “Combined Epiregulin and Amphiregulin Expression Levels as a Predictive Biomarker for Panitumumab Therapy Benefit or Lack of Benefit in Patients With RAS Wild-Type Advanced Colorectal Cancer.” *JAMA Oncology* 2 (5): 633. https://doi.org/10.1001/jamaoncol.2015.6065.

Stahler, A., V. Heinemann, C. Giessen-Jung, A. Crispin, A. Schalhorn, S. Stintzing, L. Fischer von Weikersthal, et al. 2016. “Influence of MRNA Expression of Epiregulin and Amphiregulin on Outcome of Patients with Metastatic Colorectal Cancer Treated with 5-FU/LV plus Irinotecan or Irinotecan plus Oxaliplatin as First-Line Treatment (FIRE 1-Trial).” *International Journal of Cancer* 138 (3): 739–46. https://doi.org/10.1002/ijc.29807.

Stintzing, S., D. P. Modest, L. Rossius, M. M. Lerch, L. F. von Weikersthal, T. Decker, A. Kiani, et al. 2016. “FOLFIRI plus Cetuximab versus FOLFIRI plus Bevacizumab for Metastatic Colorectal Cancer (FIRE-3): A Post-Hoc Analysis of Tumour Dynamics in the Final RAS Wild-Type Subgroup of This Randomised Open-Label Phase 3 Trial.” *The Lancet Oncology* 17 (10): 1426–34. https://doi.org/10.1016/S1470-2045(16)30269-8.

Sugai, T., W. Habano, Y. F. Jiao, M. Tsukahara, Y. Takeda, K. Otsuka, and S. I. Nakamura. 2006. “Analysis of Molecular Alterations in Left- and Right-Sided

Colorectal Carcinomas Reveals Distinct Pathways of Carcinogenesis: Proposals for New Molecular Profile of Colorectal Carcinomas." *Journal of Molecular Diagnostics* 8 (2): 193–201. https://doi.org/10.2353/jmoldx.2006.050052.

Triest, L., C. Debeuckelaere, T. Vandamme, B. Van Den Heuvel, J. Van Den Brande, K. Papadimitriou, M. Rasschaert, H. Prenen, and M. Peeters. 2019. "Should Anti-EGFR Agents Be Used in Right-Sided RAS Wild-Type Advanced Colorectal Cancer?" *Current Colorectal Cancer Reports*. 15: 130-134. https://doi.org/10.1007/s11888-019-00439-x.

Venook, A. P., D. Niedzwiecki, H. Lenz, F. Innocenti, B. Fruth, J. A. Meyerhardt, D. Schrag, et al. 2017. "Effect of First-Line Chemotherapy Combined With Cetuximab or Bevacizumab on Overall Survival in Patients With KRAS Wild-Type Advanced or Metastatic Colorectal Cancer: A Randomized Clinical Trial." *Journal of the American Medical Association* 317 (23): 2392–2401. https://doi.org/10.1001/jama.2017.7105.

Wang, F., L. Bai, T. S. Liu, Y. Y. Yu, M. M. He, K. Y. Liu, H. Y. Luo, et al. 2015. "Right- and Left-Sided Colorectal Cancers Respond Differently to Cetuximab." *Chinese Journal of Cancer* 34 (9): 1–10. https://doi.org/10.1186/s40880-015-0022-x.

Wong, S. F. 2005. "Cetuximab: An Epidermal Growth Factor Receptor Monoclonal Antibody for the Treatment of Colorectal Cancer." *Clinical Therapeutics* 27 (6): 684–94. https://doi.org/10.1016/j.clinthera.2005.06.003.

Yang, X. D., X. C. Jia, J. R. Corvalan, P. Wang, C. G. Davis, and A. Jakobovits. 1999. "Eradication of Established Tumors by a Fully Human Monoclonal Antibody to the Epidermal Growth Factor Receptor without Concomitant Chemotherapy." *Cancer Research* 59 (6): 1236–43. http://www.ncbi.nlm.nih.gov/pubmed/10096554.

Yoshida, M., T. Shimura, M. Sato, M. Ebi, T. Nakazawa, H. Takeyama, and T. Joh. 2013.

"A Novel Predictive Strategy by Immunohistochemical Analysis of Four EGFR Ligands in Metastatic Colorectal Cancer Treated with Anti-EGFR Antibodies." *Journal of Cancer Research and Clinical Oncology* 139 (3): 367–78. https://doi.org/10.1007/s00432-012-1340-x.

Chapter 6.

Characteristics and Outcomes of Participants in Colorectal Cancer Biomarker Trials Versus a Real-World Cohort

Foroughi, Siavash, Hui-li Wong, Jeanne Tie, Rachel Wong, Margaret Lee, Belinda Lee, Ian Jones, Iain Skinner, Antony Wilks Burgess, and Peter Gibbs. 2020. "Characteristics and outcomes of participants in colorectal cancer biomarker trials versus a real-world cohort"

Manuscript under review

Abstract

Purpose

The restrictive eligibility criteria of therapy-focused cancer clinical trials can limit the external validity of the results. The characteristics and survival outcomes of patients enrolled in stand-alone biomarker studies has yet to be explored. We examined the characteristics of patients enrolled in a series of biomarker studies in stage II and III colorectal cancer (CRC) and of the broader patient population from which the study cohorts were recruited.

Patients and Methods

We examined three distinct trial scenarios; a retrospective cohort study (RCS) where archival tissue samples were analyzed, a prospective observational study (POS) where blood samples were collected but patients received standard treatment, and a randomized clinical trial (RCT) where biomarker analysis could inform clinical care. Clinical data for each study time period were extracted from a prospective registry.

Results

For all CRC patients (n=4,033) in this study, median age was 70 years and 54% were ECOG 0. For patients in the RCS (n=450), POS (n=284) and RCT (n=230), median age was 72, 65 and 64 years, with 45%, 74% and 79% being ECOG 0. For the POS and RCT, 33% and 36% of all patients with the relevant disease stage were enrolled over the study recruitment period. Survival outcomes were similar for patients in the RCS and POS. RCT outcome data are not available.

Conclusion

As for therapy-based trials, enrolment in prospective biomarker studies may be selective, despite relatively broad eligibility criteria. Characteristics and recruitment were similar

for POS and RCT patients, indicating study complexity may not necessarily limit patient recruitment. For the prospective biomarker study cohorts examined, the selective recruitment did not significantly impact survival outcomes, suggesting potential for high external validity.

Introduction

Prospective clinical trials have been the backbone of progress in cancer treatment, with randomized controlled trials the gold standard for evidence generation. Unfortunately, only a fraction of the whole patient population of interest are enrolled in most clinical trials (Murthy, Krumholz, and Gross 2004), in part due to the restrictive trial entry criteria, which potentially limits the external validity of trial results (Jennens, Giles, and Fox 2006; Kennedy-Martin et al. 2015). Other factors that can limit clinical trial participation include the complexity of some protocols that can be challenging for patients to comprehend, including the concepts of randomization and of control and experimental groups. Further, uncertainty about the treatment that the patient might receive in a multi-arm study can also be an issue, especially if there is reluctance on the part of the treating clinician or the patient about the allocation to one or more of the study arms. The extent to which each of these factors impacts on trial participation has not been well explored.

Analysis of tumor and/or blood samples, when combined with comprehensive clinical data, can lead to the discovery and validation of biomarkers that can be adopted into clinical care (Goossens et al. 2015). Appropriate biomarkers, e.g. *RAS* mutational status, have helped to refine estimates of prognosis or estimates of the likely benefit or harm of a potential therapy (De Roock et al. 2010). Traditionally such biomarker analyses are incorporated into therapeutic trial design, but stand-alone biomarker studies are becoming increasingly common. These can be retrospective analyses (Tie et al. 2011), utilizing archival tissue samples where matching clinical data is available, or prospective studies where patient consent is obtained to collect relevant biospecimens and outcome data. The latter can be observational studies where biomarker analysis does not inform patient treatment (Dawson et al. 2013) and study participants receive a standard of care treatment that would otherwise have been delivered, or interventional studies (Lee et al. 2017)

where the biomarker results inform patient treatment. Here the treatments can be selected from a range of more, or less, aggressive standard of care options or can be investigational treatments more likely to be active in a biomarker-defined subset.

Unlike therapy-focused clinical trials, the characteristics of patients enrolled in stand-alone biomarker studies have yet to be explored. The purpose of this study was to examine the characteristics and survival outcomes of patients enrolled in different types of biomarker studies, compared to a "real-world" patient population. To achieve this, we performed a retrospective analysis of data from a prospective Australian colorectal cancer (CRC) registry that captures all consecutive CRC diagnoses at participating centers (Kosmider et al. 2008). This registry also functions as the clinical data repository for a series of tissue (Turner et al. 2016; Ghosh et al. 2016; Rohr et al. 2017) and blood-based (Tie et al. 2015; 2016) biomarker studies, enabling comparison of the subsets of study participants within the broader registry population, which is considered representative of real-world patients. The biomarker studies range from a retrospective cohort study (RCS) using archival tissue, prospective observational studies (POS) where patients were consented for blood sample collection and received standard of care treatment blinded to the biomarker result, and randomized clinical trials (RCT) where patients were allocated to standard of care versus biomarker-informed chemotherapy options. Our aims were to describe the characteristics of patients within these three distinct trial scenarios and to compare them to non-participants captured in the registry over the same study period. Our hypothesis was that biomarker studies with broad eligibility criteria would enrol patients that are representative of a real-world population. We also sought to determine if recruitment rates were different for observational compared to randomized studies.

Patients and Methods

Registry Details

The Australian Comprehensive Cancer Outcomes and Research Database for Colorectal Cancer (ACCORD-CRC) registry was first established in 2000 to collect prospective data on consecutive patients diagnosed with CRC (Kosmider et al. 2008). Participating centers include a mix of public and private hospitals, as well as academic and community centers across Melbourne, Victoria. An audit of data from two participating hospitals reported a 95% case capture rate compared to the state-based population registry (Field et al. 2010).

Data are captured at the point of care and include demographics and patient characteristics, disease clinicopathological features, treatment history, outcomes and survival with follow-up data collected at 3- to 6-monthly intervals until death. Tumor stage is recorded according to the American Joint Committee on Cancer TNM staging system, with T and N stage derived from the histopathology report and M stage allocated based on review of baseline imaging. Consensus staging is agreed upon at the individual hospitals' multidisciplinary meetings. The histological and radiological assessment of patients has not changed over the study period.

Biomarker Study Details

Table 1 describes the five biomarker studies included in this analysis. A tissue-based retrospective cohort study included only stage II CRC patients where study eligibility was identified retrospectively (Ghosh et al. 2016; Rohr et al. 2017). As only archival tissue samples and data were used, ethics approval for waiver of consent had been obtained for this cohort. For the other four studies, eligible patients with stage II or III CRC were identified prospectively, and informed consent was obtained in accordance with ethics approval. These include two prospective observational studies (Tie et al. 2016; 2018),

with plasma samples collected from patients that were receiving standard of care treatment blinded to biomarker result, and two randomized clinical trials where the biomarker results could potentially inform treatment.

Study Population

For this analysis, we included registry patients who underwent upfront curative resection for stage II or III CRC at any of the six hospitals that participated in the biomarker studies described in Table 1 (Melbourne Health, Eastern Health, Western Health, Melbourne Private Hospital, Western Private Hospital and Epworth Eastern Hospital). Biomarker study participants were grouped into those included in the retrospective cohort study (RCS), prospective observational studies (POS) and randomized clinical trials (RCT). Thirteen patients participated in both an RCS and POS.

Real-world cohorts were derived from the registry and defined separately for analyses of the retrospective (RCS) and prospective biomarker studies (POS and RCT combined), accounting for differences in disease stage and recruitment dates across studies (Table 1). The real-world cohorts were defined as patients diagnosed within the study start and end dates, matched by disease stage. For the retrospective study analysis, the real-world cohort consisted of stage II CRC patients who underwent resection between 2000 and 2011. For the prospective study analysis, the real-world cohort comprised stage II CRC patients who underwent resection between 2011 and 2018, and stage III CRC patients who underwent resection between 2014 and 2018.

Table 1. Key characteristics of one retrospective tissue biomarker study and four prospective blood biomarker studies in early stage CRC.

CRC stage	Study type	Study enrolment timeframes	Key eligibility criteria	Informed consent
Stage II	Retrospective tissue cohort study	Jan 2000 – Dec 2011	1. Resected stage II colon cancer 2. Sufficient archival tissue available	No
Stage II	Prospective observational	Jul 2011 – Sept 2014	1. Resected stage II colon cancer 2. ECOG performance status 0-2 3. No major comorbidities 4. No other primary cancer ≤ 5 years 5. Registered within 8 weeks of surgery	Yes
Stage II	Randomized interventional	Aug 2015 – Jul 2019	1. Resected stage II colon cancer 2. ECOG performance status 0-2 3. No major comorbidities 4. No other primary cancer ≤ 3 years 5. No multiple primary CRCs 6. Fit for adjuvant chemotherapy 7. Registered within 4 weeks of surgery	Yes
Stage III	Prospective observational	Nov 2014 – May 2017	1. Resected stage III colon cancer 2. No other primary cancer ≤ 3 years 3. No multiple primary CRCs 4. Fit for adjuvant chemotherapy 5. Registered within 8 weeks of surgery	Yes
Stage III	Randomized interventional	Oct 2017 – ongoing	1. Resected stage III colon cancer 2. ECOG performance status 0-2 3. No major comorbidities 4. No other primary cancer ≤ 3 years 5. No multiple primary CRCs 6. Fit for adjuvant chemotherapy 7. Registered within 6 weeks of surgery	Yes

Data Analysis

Individual study logs were used to identify the patients in the ACCORD-CRC registry that were enrolled into one of the biomarker studies. Clinicopathologic and outcome data were extracted from the registry. Socioeconomic status was measured by the Index of Relative Socioeconomic Advantage and Disadvantage (IRSAD) score (Australian Bureau of Statistics 2016), where higher deciles indicate relative advantage. Patient fitness was assessed by the American Society of Anesthesiologists (ASA) physical status classification system (Dripps 1963) and the Eastern Co-operative Oncology Group (ECOG) performance status (Zubrod et al. 1960). Lifestyle data included smoking history and comorbidity data included diabetes. Post-operative complications were categorized as "yes" or "no" and whether patients commenced any adjuvant chemotherapy was recorded in the registry.

Recruitment rate for the prospective studies was defined as the number of enrolled patients divided by the number of registry patients with the same disease stage, in the time interval while the studies were open for recruitment. Patient numbers were first estimated for each individual hospital due to differences in study activation dates across sites, then combined for calculation. There were no competing trials open during the same time that might have impacted on recruitment rate for the biomarker studies.

Statistics

Descriptive statistics were used to summarize the clinicopathologic characteristics for all patients. Comparative statistics were used to examine differences between trial participants and non-participants, and those enrolled in prospective observational studies versus randomized clinical trials. The Chi-square and Mann-Whitney tests were used for categorical and continuous variables, respectively.

Median follow-up was calculated using the reverse Kaplan-Meier method. Overall survival (OS) and disease-free survival (DFS) were estimated using Kaplan–Meier curves to illustrate survival outcomes in real-world and trial participants. OS was defined as the time from surgery to all-cause death, censored at the date of last review. DFS was defined as the time from surgery to recurrence or all-cause death. Survival outcomes were not analyzed for the RCTs as these studies are still recruiting. Univariable and multivariable Cox proportional hazards analyses were performed to assess the relationship between survival and the clinical characteristics of patients. A two-tailed p-value of 0.01 was considered statistically significant. Analyses were conducted in STATA 12.1 (StataCorp LP, College Station, Texas, USA).

Ethical Considerations

The ethics approval to conduct this research was obtained from the Melbourne Health Human Research Ethics Committee (HREC) under project number HREC/54533/MH-2019 and BioGrid project number 201810/2. The ACCORD-CRC registry has ethics committee approval for waiver of consent for participation, as only de-identified and aggregated data is used for analysis.

Results

Study Cohorts

Between 2000 and 2018, 4,033 patients underwent upfront curative resection for stage II or III CRC (Figure 1). Median age for these patients was 70 years and 86% of patients were ECOG 0-2 (Supplementary Table 1). The retrospective cohort study (RCS) consisted of 450 out of a total of 1,095 stage II patients enrolled between 2000 and 2011. The prospective studies consisted of 514 patients, of whom 284 were enrolled in observational studies (POS) and 230 in randomized clinical trials (RCT) where the

biomarker result had the potential to inform management. The reference "real-world" cohort for these prospective studies consisted of 1,813 patients: 1,158 consecutive stage II patients enrolled for the period of study recruitment (2011 to 2018) and 655 consecutive stage III patients enrolled for the period of study recruitment (2014 to 2018) (see Figure 1).

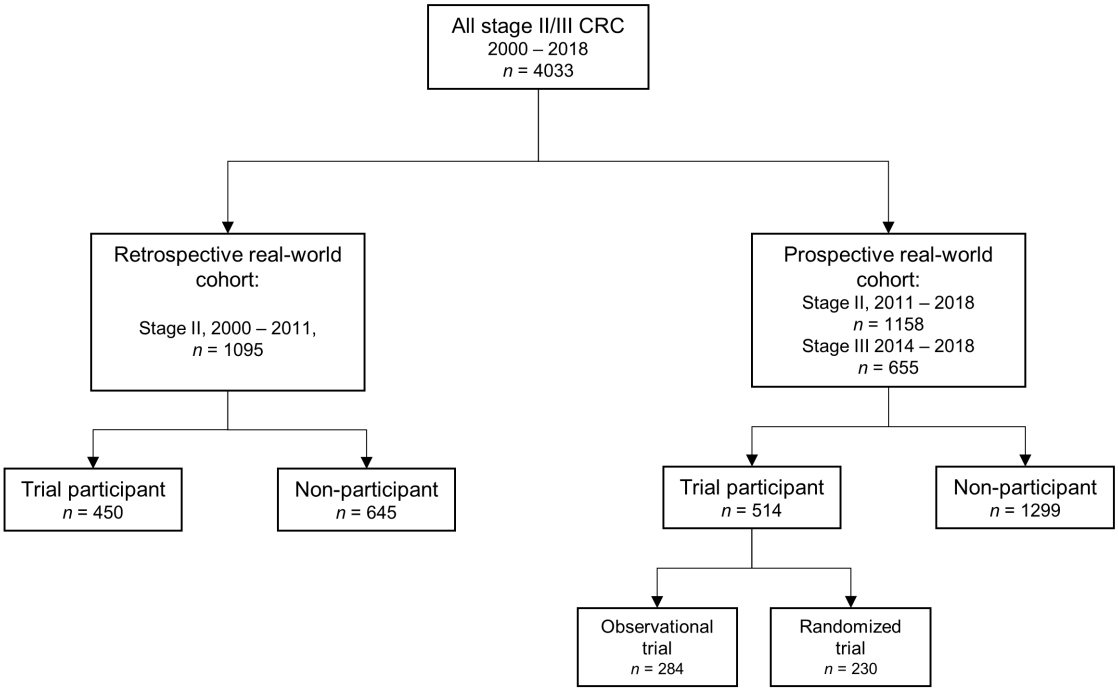


Figure 1. Number of stage II/III colorectal cancer patients captured in the ACCORD-CRC registry between January 2000 and December 2018 and enrolled in observational, randomized, or retrospective cohort studies. The enrolment periods for the retrospective cohort study and prospective observational study overlapped in 2011, hence 141 patients with stage II CRC were included in both real-world cohorts.

Retrospective Cohort Study

For the RCS, the trial participants and the corresponding real-world cohort are described in Table 2. Median age was similar for real-world patients, trial participants and non-participants: 71, 71.5 and 70 years, respectively. Trial participants were significantly more likely to have been treated at public centers ($P < 0.001$) and have lower socioeconomic status ($P < 0.001$), and less likely to have rectal primaries ($P < 0.001$). No

significant differences between the RCS participants and non-participants were observed regarding age, gender, ECOG, ASA, smoking history, presentation, diabetes status, post-operative complications or adjuvant chemotherapy (see Table 2).

Prospective Study Cohorts

For the prospective biomarker studies, trial participants were younger and fitter than their real-world counterparts (Table 3). Median age for the real-world cohort was 71 years; for trial participants versus non-participants the median age was significantly different (65 versus 74 years, $P < 0.001$). Trial participants also had a better ECOG performance status ($P < 0.001$), lower ASA scores ($P < 0.001$), more left colon primaries ($P < 0.001$), fewer post-operative complications ($P < 0.001$) and were more likely to receive adjuvant chemotherapy as their routine care treatment ($P < 0.001$). There were lower socioeconomic scores among trial participants ($P < 0.001$). Gender, treatment across hospital type, smoking history, urgency of presentation and stage distribution were similar for trial participants and non-participants (see Table 3).

Observational versus Randomized Prospective Trials

Analysis was performed to compare POS versus RCT participants (Table 3). Patients enrolled in RCTs had better ECOG performance status (79% versus 74% ECOG 0, $P = 0.010$), were slightly younger (median age 63.5 versus 65 years, $P = 0.023$) and more likely to have had a screen-detected cancer (23 versus 15%, $P = 0.011$), although the pre-specified threshold for statistical significance was not met for the latter. There were no significant differences with respect to gender, ASA score, primary tumor location, hospital type, socioeconomic score, smoking history, diabetes, stage distribution or post-operative complications.

Table 2. Characteristics of stage II colorectal cancer patients from 2000 to 2011.

	Retrospective Real-world cohort *n* = 1095	**Trial participants *n* = 450**	**Trial non-participants *n* = 645**	**P value**
Age				0.099
Median	71	71.5	70	
Range	20 - 101	24 - 101	20 - 95	
Gender				0.252
Male	609 (55.6%)	241 (53.6%)	368 (57%)	
Female	486 (44.4%)	209 (46.4%)	277 (43%)	
ECOG				0.169
0	516 (47.1%)	204 (45.3%)	312 (48.4%)	
1-2	370 (33.8%)	154 (34.2%)	216 (33.5%)	
3-4	30 (2.7%)	17 (3.8%)	13 (2%)	
Unknown	179 (16.4%)	75 (16.7%)	104 (16.1%)	
ASA				0.110
1-2	522 (47.7%)	213 (47.3%)	309 (47.9%)	
≥3	368 (33.6%)	170 (37.8%)	198 (30.7%)	
Unknown	205 (18.7%)	67 (14.9%)	138 (21.4%)	
Primary tumor location				<0.001
Right colon	488 (44.6%)	225 (50%)	263 (40.8%)	
Left colon	439 (40.1%)	223 (49.6%)	216 (33.5%)	
Rectum	167 (15.3%)	2 (0.4%)	165 (25.6%)	
Unknown	1 (0.1%)	0	1 (0.2%)	
Campus type				<0.001
Public	772 (70.5%)	375 (83.3%)	397 (61.6%)	
Private	323 (29.5%)	75 (16.7%)	248 (38.5%)	
IRSAD				<0.001
High (8-10)	421 (38.5%)	130 (28.9%)	291 (45.1%)	
Intermediate (5-7)	405 (37%)	191 (42.5%)	214 (33.2%)	
Low (1-4)	262 (23.9%)	127 (28.2%)	135 (20.9%)	
Unknown	7 (0.6%)	2 (0.4%)	5 (0.8%)	
Smoking history				0.984
Current smoker	129 (11.8%)	54 (12%)	75 (11.6%)	
Former or never smoker	905 (82.6%)	378 (84%)	527 (81.7%)	
Unknown	61 (5.6%)	18 (4%)	43 (6.7%)	
Presentation				0.132
Screen-detected	101 (9.2%)	35 (7.8%)	66 (10.2%)	
Other	895 (81.7%)	380 (84.4%)	515 (79.8%)	
Unknown	99 (9.1%)	35 (7.8%)	64 (9.9%)	
Diabetes				0.072
Yes	224 (20.5%)	106 (23.6%)	118 (18.3%)	
No	839 (76.6%)	341 (75.8%)	498 (77.2%)	
Unknown	32 (2.9%)	3 (0.7%)	29 (4.5%)	
Post-operative complications				0.155
Yes	411 (37.5%)	167 (37.1%)	244 (37.8%)	
No	580 (53%)	262 (58.2%)	318 (49.3%)	
Unknown	104 (9.5%)	21 (4.7%)	83 (12.9%)	
Adjuvant chemotherapy				0.690
Yes	232 (21.2%)	98 (21.8%)	134 (20.8%)	
No	863 (78.8%)	352 (78.2%)	511 (79.2%)	

Table 3. Characteristics of stage II colorectal cancer patients from 2011 to 2018, and stage III colorectal cancer patients from 2014 to 2018.

	Prospective real-world cohort ***n*** **= 1813**	**Trial participants** ***n*** **= 514**	**Trial non-participants** ***n*** **= 1299**	**P value**	**Prospective trial participants**		
					Observational trial ***n*** **= 284**	**Randomized trial** ***n*** **= 230**	**P value**
Age				<0.001			0.0231
Median	71	65	74		65	63.5	
Range	18 - 95	26 - 89	18-95		26-89	26-87	
Gender				0.219			0.7885
Male	985 (54.3%)	291 (56.5%)	694 (53.4%)		159 (56%)	132 (57.4%)	
Female	828 (45.7%)	223 (43.4%)	605 (46.6%)		125 (44%)	98 (42.6%)	
ECOG status				<0.001			0.010
0	1041 (57.4%)	392 (76.3%)	649 (50%)		211 (74.3%)	181 (78.7%)	
1-2	572 (31.6%)	103 (20%)	469 (36.1%)		70 (24.7%)	33 (14.4%)	
3-4	50 (2.8%)	0	50 (3.9%)		0	0	
Unknown	150 (8.3%)	19 (8.3%)	131 (10.1%)		3 (1.1%)	16 (7%)	
ASA score				<0.001			0.2656
1-2	811 (44.7%)	251 (48.8%)	560 (43.1%)		149 (52.5%)	102 (44.4%)	
≥3	771 (42.5%)	164 (31.9%)	607 (46.7%)		88 (31%)	76 (33%)	
Unknown	231 (12.8%)	99 (19.3%)	132 (10.2%)		47 (15.5%)	52 (22.6%)	
Primary tumor location				0.005			0.132
Right colon	882 (48.7%)	238 (46.3%)	644 (49.6%)		130 (45.8%)	108 (47%)	
Left colon	763 (42.1%)	242 (47.1%)	521 (40.1%)		140 (49.3%)	102 (44.4%)	
Rectum	164 (9.1%)	33 (6.4%)	131 (10.1%)		13 (4.6%)	20 (8.7%)	
Unknown	4 (0.2%)	1 (0.2%)	3 (0.2%)		1 (0.4%)	0	
Campus type				0.526			0.761
Public	1332 (73.5%)	383 (74.5%)	949 (73.1%)		210 (73.9%)	173 (75.2%)	
Private	481 (26.5%)	131 (25.5%)	350 (26.9%)		74 (26.1%)	57 (24.8%)	
IRSAD score				<0.001			0.0568
High (8-10)	843 (46.5%)	173 (33.7%)	670 (51.6%)		112 (39.4%)	61 (26.5%)	
Intermediate (5-7)	608 (33.5%)	211 (41%)	397 (30.6%)		114 (40.1%)	97 (42.2%)	
Low (1-4)	327 (18.1%)	108 (21%)	219 (16.9%)		57 (20.1%)	51 (22.2%)	
Unknown	35 (1.9%)	22 (4.3%)	13 (1%)		1 (0.4%)	21 (9.1%)	

	Prospective real-world cohort *n* = 1813	Trial participants *n* = 514	Trial non-participants *n* = 1299	P value	Prospective trial participants		
					Observational trial *n* = 284	Randomized trial *n* = 230	P value
Smoking history				0.038			0.8963
Current smoker	208 (11.5%)	70 (13.6%)	138 (10.6%)		41 (14.4%)	29 (12.6%)	
Former or never smoker	1544 (85.2%)	414 (80.5%)	1130 (87%)		237 (83.5%)	177 (77%)	
Unknown	61 (3.3%)	30 (5.9%)	31 (2.4%)		6 (2.1%)	24 (10.4%)	
Presentation				0.071			0.0112
Screen-detected	293 (16.2%)	95 (18.5%)	198 (15.2%)		42 (14.8%)	53 (23.1%)	
Other	1480 (81.6%)	403 (78.4%)	1077 (82.9%)		238 (83.8%)	165 (71.7%)	
Unknown	40 (2.2%)	16 (3.1%)	24 (1.9%)		4 (1.4%)	12 (5.2%)	
Diabetes				0.198			0.3638
Yes	389 (21.5%)	98 (19.1%)	291 (22.4%)		60 (21.1%)	38 (16.5%)	
No	1386 (76.5%)	395 (76.9%)	991 (76.3%)		221 (77.8%)	174 (75.7%)	
Unknown	38 (2.1%)	21 (4.1%)	17 (1.3%)		3 (1.1%)	18 (7.8%)	
Stage				0.137			0.9251
II	1158 (63.9%)	342 (66.5%)	816 (62.8%)		188 (66.2%)	154 (67%)	
III	655 (36.1%)	172 (33.5%)	483 (37.2%)		96 (33.8%)	76 (33%)	
Post-operative complications				<0.001			0.2615
Yes	613 (33.8%)	110 (21.4%)	503 (38.7%)		71 (25%)	39 (17%)	
No	971 (53.6%)	317 (61.7%)	654 (50.4%)		185 (65.1%)	132 (57.4%)	
Unknown	229 (12.6%)	87 (16.9%)	142 (10.9%)		28 (9.9%)	59 (25.6%)	
Adjuvant chemotherapy							
Stage II	145 (12.5%)	70 (20.5%)	75 (9.2%)	<0.001	NR	NR	
Stage III	465 (71%)	165 (95.9%)	300 (62.1%)	<0.001			

ASA; American Society of Anesthesiologists, ECOG; Eastern Co-operative Oncology Group, IRSAD; Index of Relative Socioeconomic Advantage and Disadvantage, NR; Not Reported.

Participation Rates in Prospective Trials

Participation rates for POS and RCTs were calculated based on individual site activation and close dates and the total number of stage II or III patients enrolled in the registry during that period. Stage II and III POS recruited 36% (188/522) and 28% (96/341) of patients diagnosed during the study enrolment periods, respectively, while RCTs recruited 37% (154/414) of stage II, and 33% (76/233) of stage III patients. There was no statistically significant difference observed (P = 0.297 for POS vs RCT; stage II and III studies combined).

Survival Outcomes

Median follow-up was 74.6 months for the RCS and 27.1 months for the prospective cohort studies (30.9 months and 22.3 months for stage II and stage III patients, respectively). 3-year DFS and 5-year OS rates were similar for patients included in the RCS relative to the total population of real-world patients (Figures 2A and 2B). For stage II and III patients in the POS, survival rates were similar to the real-world population (Figures 2C-2F). Survival was not reported for the RCS group as these studies are ongoing.

Univariable and multivariable analyses for DFS and OS for the prospective real-world cohort were performed (Supplementary Table 2). Among this cohort, poor ECOG or ASA and having post-operative complications were independently associated with poorer OS. Post-operative complications were also associated with poorer DFS. Treatment at a private campus was strongly associated with improved outcomes (P < 0.001). In stage III but not stage II CRC patients, receipt of adjuvant chemotherapy was independently associated with improved outcomes (P < 0.001). Trial participation did not impact DFS or OS.

In the retrospective real-world cohort, older age, poor ECOG or ASA and post-operative complications were associated with worse DFS and OS (Supplementary Table 3). Smokers and patients treated at a public campus also had worse OS.

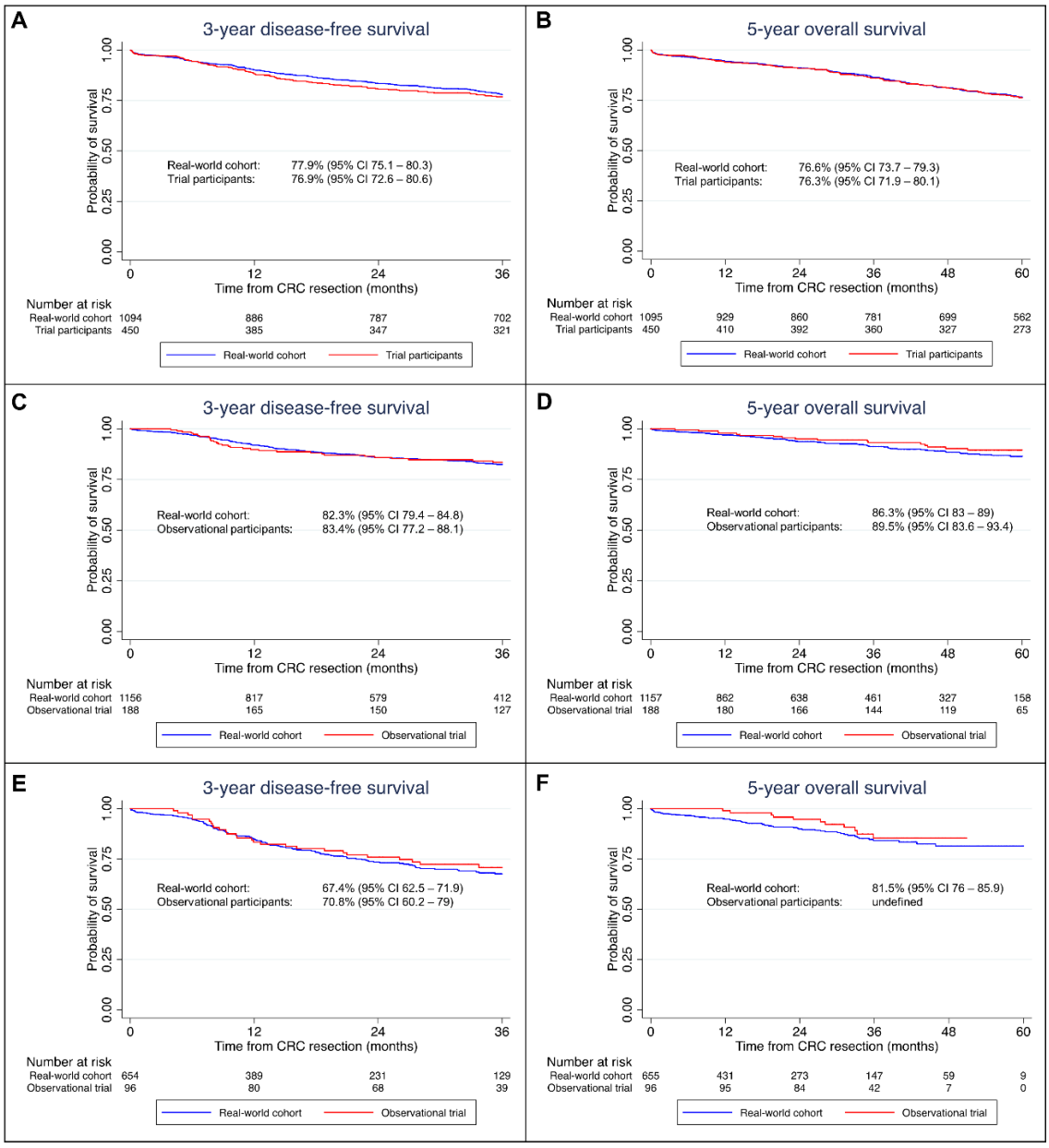


Figure 2. Three-year disease-free survival and 5-year overall survival for the retrospective real-world cohort (A, B), stage II prospective real-world cohort (C, D) and stage III prospective real-world cohort (E, F). Outcomes for observational trial participants were plotted alongside real-world cohorts for reference. Randomized trial participants are not shown as trials are ongoing.

Discussion

Biomarkers are of ever-increasing interest in cancer. They define specific subsets of patients with a differing prognosis and/or with a differing likelihood of deriving benefit or harm from a particular intervention (Lech, Slotwinski, and Krasnodebski 2014; Ruiz-Bañobre, Kandimalla, and Goel 2019). Until recently, biomarker research was largely conducted by incorporating biomarker studies into therapeutic clinical trial design or through retrospective analyses of cancer clinical trial data and specimens (Mandrekar and Sargent 2011), but increasingly, existing historical non-trial patient cohorts with matching clinical data are being used for biomarker discovery or validation. For the more promising predictive or prognostic markers, prospective studies are being pursued where the primary aim is to measure the clinical impact of biomarker-guided therapy. A search of ClinicalTrials.gov for the keywords “biomarker” and “cancer” returned 4,169 currently active studies.

In this analysis, we explore enrolment in a series of stand-alone CRC biomarker studies, the intent being to better understand recruitment to biomarker studies and how representative the trial participants are of real-world patients. This analysis also facilitates the study of the impact of trial design on study recruitment: e.g. comparing enrolment in a simple study involving a known standard treatment versus a complex randomized study with uncertainty regarding the treatment to be received.

For the initial study we examined, a retrospective cohort study, eligible patients with stage II CRC were identified by a search of the CRC registry. Beyond the requirement for sufficient follow-up data and the availability of formalin-fixed paraffin-embedded (FFPE) blocks, there were no other eligibility criteria. There was no requirement to contact patients as all data were de-identified prior to tissue analysis, allowing a waiver

of consent to be granted by the Institutional Review Board. As expected, with very broad eligibility and no need for patient consent, the clinical characteristics of the patients who ultimately contributed the samples and data to this study were reflective of the broader population of stage II CRC patients. The significant differences seen in campus and socioeconomic score are best explained by FFPE samples being more readily obtained at public institutions, whereas the lower proportion of rectal primaries among study participants likely reflects the use of tissue samples that had not been exposed to pre-operative therapy.

Enrolment into two prospective ctDNA studies was assessed for a stage II and a stage III trial where the biomarker result was not made available and patients received standard of care treatment. Eligibility for the two studies was very similar, with these eligibility criteria, including good ECOG performance status, suitability for adjuvant chemotherapy and the need to enrol patients within a trial-defined time window, likely a major contributor to the enrolment of a younger and fitter subset of patients. However, it is possible that clinicians were reluctant to offer the study to older and/or frailer patients even if they were eligible and it is also possible that older and/or frailer patients that were offered the study may have been less inclined to participate. We would anticipate that as the trial procedure was limited to a blood draw that could be performed immediately after the clinic visit, that this would not have dissuaded participation. However, there was no potential benefit for the individual patient, or patients may not have comprehended the study intent, and for both reasons may have declined participating, one possible factor in the selective enrolment. These patient characteristics are similar to those of recent therapeutic trials where older patients are commonly under-represented (Townsley, Selby, and Siu 2005; Lara et al. 2001; Jennens, Giles, and Fox 2006), and indeed the age

gap of real-world patients and participants in therapeutic studies appears to be widening (Abbasi 2019).

For the biomarker randomized controlled trial, where the biomarker may inform patient treatment, there are additional complexities as the clinician and the patient must be willing for the biomarker result to vary management from standard of care. Clinicians must be comfortable with offering the study to the patient knowing that the patient could be enrolled to any of the treatment arms. Patients must understand and consent to randomization, where the concepts of a control group and an experimental group may not be easily understood. A chance allocation to biomarker-driven versus standard of care treatment can be difficult for patients to comprehend. However, despite these additional barriers, as shown in Table 3, participants in the randomized trial appear largely similar to those in the observational study. In a separate analysis, a similar proportion of patients participated in both prospective and randomized studies: around one third of all stage II and III patients diagnosed over the time periods that the studies were recruiting. This participation rate compares favorably with reported recruitment (7 - 15.9%) in community and academic cancer therapy trials (Unger et al. 2019).

Despite these factors and some of the differences observed between the subset of trial participants and the total patient population, there was very limited impact observed when examining 3-year disease-free and 5-year overall survival. For many of these comparisons the survival curves are close together. Our results suggest, at least for the studies examined, that the external validity of any findings from these studies should be high, such that any conclusions made regarding the prognostic significance of ctDNA are relevant to the broader real-world population.

Our study has several limitations. We were unable to statistically measure differences between real-world comparator cohorts with the subgroup which participated in trials due to these being overlapping and non-independent populations. However, where trial participants and non-participants are not significantly different, it can be assumed that trial participants are also similar to the larger real-world cohort. There were missing data for some important clinicopathologic factors (e.g. ECOG, ASA) and in some cases potentially important factors were excluded from our analysis due to insufficient data (e.g. English as main language). More complete data would have strengthened the analysis and/or provided insight into other potentially important factors. Our study is also potentially subject to selection bias; although the ACCORD-CRC registry captures consecutive CRC diagnoses, the participating centers were all in metropolitan Melbourne and thus, regional patients are underrepresented in this analysis. We also further selected patients for the real-world cohorts according to the respective study enrolment periods, as we felt that this provided a homogenous group for comparison and minimized time-period effects. The recruitment period of the randomized and observational studies did not overlap; however, the characteristics of the registry population were similar across the time periods examined, suggesting that the registry population is stable over time. We were also unable to formally examine participation rates or compare characteristics of the subset of patients who met trial eligibility criteria, as this could not be conclusively determined retrospectively based on the data captured in the registry. Similarly, bias due to differences in recruiting processes cannot be determined; however, we did not observe any differences in treatment location among trial participants and non-participants, with a similar proportion treated in private and public hospitals. Finally, we chose to focus on stage II and III colorectal cancer in order to better explore the impact of patient factors on study recruitment in a defined and relatively homogenous population, but

acknowledge that similar studies in the metastatic disease setting would be of great interest.

In summary, clinical trials remain the gold standard for evidence generation in cancer management, regardless of whether the primary aim is to prospectively study and validate the role of a new therapeutic intervention or a promising biomarker. Here, in Stage II and III CRC, we have shown that for a retrospective cohort study, a cohort representative of real-world patients is likely to be recruited. In contrast, for prospective biomarker studies, similar to standard clinical trials, a younger and fitter patient population was enrolled. Likely this is largely driven by restrictive trial entry criteria as similar patients and a similar proportion of potentially eligible patients were enrolled in a simple observational study versus a complex and randomized interventional study. So, for prospective studies, even for simple observational studies, the eligibility criteria would seem to be the major driver of selective enrolment. At least for the study populations examined, the survival outcomes of the trial subset and all patients were similar, suggesting high external validity of any study evidence generated with respect to biomarker-driven treatment strategies.

Supplementary Material

Supplementary Table 1. Characteristics of stage II and III colorectal cancer patients from the ACCORD-CRC registry who underwent upfront curative surgical resection from 2000 to 2018.

	All patients *n* **= 4,033**	**Included in analysis** *n* **= 2,767**	**Not included in analysis** *n* **= 1,266**	**P value**
Age				<0.001
Median	70	71	67	
Range	14-101	18-101	14-99	
Gender				0.370
Male	2189 (54.3%)	1515 (54.8%)	674 (53.2%)	
Female	1844 (45.7%)	1252 (45.3%)	592 (46.8%)	
ECOG				0.067
0	2191 (54.3%)	1476 (53.3%)	715 (56.5%)	
1-2	1262 (31.3%)	898 (32.5%)	364 (28.8%)	
3-4	103 (2.6%)	72 (2.6%)	31 (2.5%)	
Unknown	477 (11.8%)	321 (11.6%)	156 (12.3%)	
ASA				0.006
1-2	1877 (46.5%)	1270 (45.9%)	607 (48.0%)	
≥3	1497 (37.1%)	1078 (39.0%)	419 (33.1%)	
Unknown	659 (16.3%)	419 (15.1%)	240 (29.0%)	
Primary tumor location				<0.001
Right colon	1772 (43.9%)	1304 (47.1%)	468 (37.0%)	
Left colon	1708 (42.4%)	1145 (41.4%)	563 (44.5%)	
Rectum	543 (13.5%)	313 (11.3%)	230 (18.2%)	
Unknown	10 (0.3%)	5 (0.2%)	5 (0.4%)	
Campus type				0.279
Public	2900 (71.9%)	2004 (72.4%)	896 (70.8%)	
Private	1133 (28.1%)	763 (27.6%)	370 (29.2%)	
IRSAD				0.052
High (8-10)	1719 (42.6%)	1198 (43.3%)	521 (41.2%)	
Intermediate (5-7)	1406 (34.9%)	967 (35.0%)	439 (34.7%)	
Low (1-4)	864 (21.4%)	562 (20.3%)	302 (23.9%)	
Unknown	44 (1.1%)	40 (1.5%)	4 (0.3%)	
Smoking history				0.119
Current smoker	448 (11.1%)	322 (11.6%)	126 (10.0%)	
Former or never smoker	3407 (84.5%)	2325 (84.0%)	1082(85.5%)	
Unknown	178 (4.4%)	120 (4.3%)	58 (4.6%)	
Presentation				0.008
Screen-detected	504 (12.5%)	371 (13.4%)	133 (10.5%)	
Other	3342 (82.9%)	2262 (81.8%)	1080 (85.3%)	
Unknown	176 (4.6%)	134 (4.8%)	53 (4.2%)	
Diabetes				0.241
Yes	828 (20.5%)	582 (21.0%)	246 (19.4%)	
No	3103 (76.9%)	2115 (76.4%)	988 (78.0%)	
Unknown	102 (2.5%)	70 (2.5%)	32 (2.5%)	
Stage				<0.001
II	2112 (52.4%)	2112 (76.3%)	0	
III	1921 (47.6%)	655 (23.7%)	1266 (100%)	

	All patients *n* = 4,033	Included in analysis *n* = 2,767	Not included in analysis *n* = 1,266	P value
Post-operative complications				0.389
Yes	1412 (35.0%)	973 (35.2%)	439 (34.7%)	
No	2184 (54.2%)	1475 (53.3%)	709 (56.0%)	
Unknown	437 (10.8%)	319 (11.5%)	118 (9.3%)	
Adjuvant chemotherapy				
Stage II	346 (16.4%)	346 (16.4%)	N/A	N/A
Stage III	1433 (74.6%)	465 (71.0%)	968 (76.5%)	0.009

Supplementary Table 2. Univariable and multivariable analyses of disease-free and overall survival for the prospective real-world cohort (N=1,813).

Variable	Groups	Disease-free survival				Overall survival			
		Univariable HR	P	Multivariable HR*	P	Univariable HR	P	Multivariable HR*	P
Age	≤70 >70	- 1.58 (1.26-1.98)	<0.001	- 1.24 (0.90-1.71)	0.182	- 2.48 (1.77-3.48)	<0.001	- 1.38 (0.87-2.21)	0.173
Gender	Male Female	- 0.75 (0.60-0.95)	0.015	- 0.85 (0.65-1.11)	0.227	- 0.75 (0.55-1.04)	0.086	- 0.96 (0.65-1.41)	0.823
ECOG	0 ≥1	- 1.65 (1.30-2.08)	<0.001	- 1.42 (1.05-1.93)	0.024	- 2.93 (2.09-4.12)	<0.001	- 2.14 (1.40-3.32)	0.001
ASA	1-2 ≥3	- 1.45 (1.14-1.84)	0.002	- 1.06 (0.80-1.42)	0.669	- 3.01 (2.11-4.30)	<0.001	- 1.77 (1.15-2.74)	0.010
Primary tumor location	Right colon Left colon or rectum	- 1.00 (0.80-1.25)	0.992	- 0.94 (0.72-1.23)	0.670	- 0.90 (0.66-1.24)	0.520	- 0.89 (0.61-1.31)	0.561
Campus type	Public Private	- 0.61 (0.46-0.80)	<0.001	- 0.52 (0.36-0.76)	0.001	- 0.26 (0.16-0.44)	<0.001	- 0.21 (0.10-0.43)	<0.001
IRSAD	Intermediate-High Low	- 0.88 (0.65-1.18)	0.387	- 0.85 (0.61-1.19)	0.335	- 1.07 (0.72-1.58)	0.752	- 0.88 (0.56-1.37)	0.570
Smoking history	Former or never Current	- 1.14 (0.82-1.58)	0.452	- 1.23 (0.84-1.80)	0.294	- 1.37 (0.89-2.13)	0.154	- 1.57 (0.93-2.64)	0.088
Presentation	Screen-detected Other	- 1.99 (1.35-2.94)	0.001	- 1.38 (0.90-2.11)	0.140	- 2.78 (1.46-5.28)	0.002	- 1.44 (0.72-2.87)	0.300
Diabetes	No Yes	- 1.29 (1.00-1.67)	0.054	- 1.10 (0.81-1.50)	0.537	- 1.67 (1.18-2.36)	0.004	- 1.11 (0.73-1.68)	0.622
Post-operative complications	No Yes	- 1.98 (1.56-2.52)	<0.001	- 1.65 (1.25-2.17)	<0.001	- 2.70 (2.10-4.19)	<0.001	- 1.90 (1.27-2.85)	0.002

Variable	Groups	Disease-free survival				Overall survival			
		Univariable HR	P	Multivariable HR*	P	Univariable HR	P	Multivariable HR*	P
Adjuvant chemotherapy									
Stage II	No	-		-		-		-	
	Yes	0.58 (0.34-0.99)	0.045	0.91 (0.47-1.76)	0.783	0.25 (0.09-0.67)	0.006	0.30 (0.07-1.30)	0.108
Stage III	No	-		-		-		-	
	Yes	0.37 (0.26-0.51)	<0.001	0.33 (0.20-0.53)	<0.001	0.19 (0.11-0.31)	<0.001	0.22 (0.11-0.45)	<0.001
Trial participation	No	-		-		-		-	
	Yes	0.67 (0.52-0.88)	0.003	0.81 (0.60-1.11)	0.198	0.52 (0.35-0.77)	0.001	0.69 (0.43-1.10)	0.120

*Only in 1,319 patients with complete data for all variables (225 DFS events, 112 OS events).

Supplementary Table 3. Univariable and multivariable analyses of disease-free and overall survival for the retrospective real-world cohort (N=1,095).

Variable	Groups	Disease-free survival				Overall survival			
		Univariable HR	P	Multivariable HR*	P	Univariable HR	P	Multivariable HR*	P
Age	≤70 >70	- 2.69 (2.15-3.36)	<0.001	- 1.91 (1.37-2.66)	<0.001	- 3.68 (2.84-4.76)	<0.001	- 2.50 (1.70-3.69)	<0.001
Gender	Male Female	- 0.82 (0.66-1.02)	0.071	- 0.75 (0.57-1.00)	0.046	- 0.84 (0.67-1.06)	0.153	- 0.72 (0.53-0.98)	0.038
ECOG	0 ≥1	- 2.17 (1.71-2.75)	<0.001	- 1.76 (1.31-2.36)	<0.001	- 3.10 (2.36-4.07)	<0.001	- 2.00 (1.44-2.79)	<0.001
ASA	1-2 ≥3	- 2.56 (2.02-3.25)	<0.001	- 1.58 (1.17-2.14)	0.003	- 3.22 (2.47-4.19)	<0.001	- 1.84 (1.31-2.58)	<0.001
Primary tumor location	Right colon Left colon or rectum	- 1.03 (0.83-1.27)	0.814	- 0.97 (0.73-1.27)	0.808	- 0.86 (0.69-1.08)	0.203	- 0.73 (0.53-0.99)	0.040
Campus type	Public Private	- 0.54 (0.41-0.72)	<0.001	- 0.70 (0.46-1.06)	0.094	- 0.39 (0.27-0.55)	<0.001	- 0.45 (0.27-0.76)	0.003
IRSAD	Intermediate-High Low	- 1.10 (0.87-1.39)	0.424	- 1.34 (1.00-1.80)	0.054	- 1.02 (0.79-1.32)	0.885	- 1.25 (0.91-1.73)	0.170
Smoking history	Former or never Current	- 1.21 (0.89-1.65)	0.221	- 1.57 (1.05-2.34)	0.027	- 1.46 (1.06-2.01)	0.019	- 2.08 (1.36-3.20)	0.001
Presentation	Screen-detected Other	- 1.77 (1.12-2.82)	0.015	- 1.69 (0.96-2.99)	0.069	- 1.96 (1.14-3.35)	0.014	- 1.49 (0.78-2.85)	0.228
Diabetes	No Yes	- 1.49 (1.17-1.88)	0.001	- 0.99 (0.73-1.36)	0.974	- 1.42 (1.10-1.84)	0.008	- 0.88 (0.62-1.23)	0.452
Post-operative complications	No Yes	- 1.87 (1.50-2.33)	<0.001	- 1.48 (1.11-1.98)	0.007	- 2.24 (1.76-2.85)	<0.001	- 1.73 (1.26-2.38)	0.001

Variable	Groups	Disease-free survival				Overall survival			
		Univariable HR	P	Multivariable HR*	P	Univariable HR	P	Multivariable HR*	P
Adjuvant chemotherapy	No Yes	- 0.57 (0.43-0.76)	<0.001	- 1.19 (0.80-1.78)	0.390	- 0.50 (0.36-0.70)	<0.001	- 1.15 (0.72-1.82)	0.562
Trial participation	No Yes	- 1.10 (0.89-1.36)	0.371	- 1.06 (0.81-1.40)	0.667	- 1.06 (0.84-1.33)	0.618	- 0.83 (0.61-1.12)	0.224

*Only in 701 patients with complete data for all variables (217 DFS events, 180 OS events).

References

Abbasi, J. 2019. “Older Patients (Still) Left Out of Cancer Clinical Trials.” *Journal of the American Medical Association* 322 (18): 1751. https://doi.org/10.1001/jama.2019.17016.

Australian Bureau of Statistics. 2016. “Census of Population and Housing: Socio-Economic Indexes for Areas (SEIFA) Australia.” 2016. https://www.abs.gov.au/ausstats/abs@.nsf/Lookup/bySubject/2033.0.55.001~2016~MainFeatures~IRSAD~20.

Dawson, S. J., D. W. Y. Tsui, M. Murtaza, H. Biggs, O. M. Rueda, S. F. Chin, M. J. Dunning, et al. 2013. “Analysis of Circulating Tumor DNA to Monitor Metastatic Breast Cancer.” *New England Journal of Medicine* 368 (13): 1199–1209. https://doi.org/10.1056/NEJMoa1213261.

De Roock, W., B. Claes, D. Bernasconi, J. De Schutter, B. Biesmans, G. Fountzilas, K. T. Kalogeras, et al. 2010. “Effects of KRAS, BRAF, NRAS, and PIK3CA Mutations on the Efficacy of Cetuximab plus Chemotherapy in Chemotherapy-Refractory Metastatic Colorectal Cancer: A Retrospective Consortium Analysis.” *The Lancet Oncology* 11 (8): 753–62. https://doi.org/10.1016/S1470-2045(10)70130-3.

Dripps, R. D. 1963. “New Classification of Physical Status.” *Anesthesiology* 24: 111.

Field, K. M., S. Kosmider, J. Johns, H. Farrugia, I. A. Hastie, M. Croxford, M. Chapman, M. Harold, N. Murigu, and P. Gibbs. 2010. “Linking Data from Hospital and Cancer Registry Databases: Should This Be Standard Practice?” *Internal Medicine Journal* 40 (8): 566–73. https://doi.org/10.1111/j.1445-5994.2009.01984.x.

Ghosh, P., J. Tie, A. Muranyi, S. Singh, P. Brunhoeber, K. Leith, R. Bowermaster, et al. 2016. “Girdin (GIV) Expression as a Prognostic Marker of Recurrence in Mismatch Repair-Proficient Stage II Colon Cancer.” *Clinical Cancer Research* 22 (14): 3488–

98. https://doi.org/10.1158/1078-0432.CCR-15-2290.

Goossens, N., S. Nakagawa, X. Sun, and Y. Hoshida. 2015. "Cancer Biomarker Discovery and Validation." *Translational Cancer Research* 4 (3): 256–69. https://doi.org/10.3978/j.issn.2218-676X.2015.06.04.

Jennens, R. R., G. G. Giles, and R. M. Fox. 2006. "Increasing Underrepresentation of Elderly Patients with Advanced Colorectal or Non-Small-Cell Lung Cancer in Chemotherapy Trials." *Internal Medicine Journal* 36 (4): 216–20. https://doi.org/10.1111/j.1445-5994.2006.01033.x.

Kennedy-Martin, T., S. Curtis, D. Faries, S. Robinson, and J. Johnston. 2015. "A Literature Review on the Representativeness of Randomized Controlled Trial Samples and Implications for the External Validity of Trial Results." *Trials* 16 (1): 495. https://doi.org/10.1186/s13063-015-1023-4.

Kosmider, S., I. Jones, M. Hibbert, A. Hunter, S. McLaughlin, J. Johns, M. Chapman, and P. Gibbs. 2008. "Towards Establishing a National Colorectal Cancer Database: Lessons Learnt from Bio21 Molecular Medicine Informatics Model." *ANZ Journal of Surgery* 78 (9): 803–9. https://doi.org/10.1111/j.1445-2197.2008.04655.x.

Lara, J., R. Higdon, N. Lim, K. Kwan, M. Tanaka, D. H. M. Lau, T. Wun, et al. 2001. "Prospective Evaluation of Cancer Clinical Trial Accrual Patterns: Identifying Potential Barriers to Enrollment." *Journal of Clinical Oncology* 19 (6): 1728–33. https://doi.org/10.1200/jco.2001.19.6.1728.

Lech, G., R. Slotwinski, and I. W. Krasnodebski. 2014. "The Role of Tumour Markers and Biomarkers in Colorectal Cancer." *Neoplasma*. https://doi.org/10.4149/neo_2014_003.

Lee, S. M., M. Falzon, F. Blackhall, J. Spicer, M. Nicolson, A. Chaudhuri, G. Middleton, et al. 2017. "Randomized Prospective Biomarker Trial of ERCC1 for Comparing

Platinum and Nonplatinum Therapy in Advanced Non-Small-Cell Lung Cancer: ERCC1 Trial (ET).” *Journal of Clinical Oncology* 35 (4): 402–11. https://doi.org/10.1200/JCO.2016.68.1841.

Mandrekar, S. J., and D. J. Sargent. 2011. “Design of Clinical Trials for Biomarker Research in Oncology.” *Clinical Investigation* 1 (12): 1627–36. https://doi.org/10.4155/cli.11.152.

Murthy, V. H., H. M. Krumholz, and C. P. Gross. 2004. “Participation in Cancer Clinical Trials: Race-, Sex-, and Age-Based Disparities.” *Journal of the American Medical Association* 291 (22): 2720–26. https://doi.org/10.1001/jama.291.22.2720.

Rohr, U.-P., P. Herrmann, K. Ilm, H. Zhang, S. Lohmann, A. Reiser, A. Muranyi, et al. 2017. “Prognostic Value of MACC1 and Proficient Mismatch Repair Status for Recurrence Risk Prediction in Stage II Colon Cancer Patients: The BIOGRID Studies.” *Annals of Oncology* 28 (8): 1869–75. https://doi.org/10.1093/annonc/mdx207.

Ruiz-Bañobre, J., R. Kandimalla, and A. Goel. 2019. “Predictive Biomarkers in Metastatic Colorectal Cancer: A Systematic Review.” *JCO Precision Oncology*, no. 3: 1–17. https://doi.org/10.1200/po.18.00260.

Tie, J., J. Cohen, Y. Wang, M. Lee, R. Wong, S. Kosmider, S. Ananda, et al. 2018. “Serial Circulating Tumor DNA (CtDNA) Analysis as a Prognostic Marker and a Real-Time Indicator of Adjuvant Chemotherapy (CT) Efficacy in Stage III Colon Cancer (CC).” *Journal of Clinical Oncology* 36 (15_suppl): 3516. https://doi.org/10.1200/JCO.2018.36.15_suppl.3516.

Tie, J., P. Gibbs, L. Lipton, M. Christie, R. N. Jorissen, A. W. Burgess, M. Croxford, et al. 2011. “Optimizing Targeted Therapeutic Development: Analysis of a Colorectal Cancer Patient Population with the BRAFV600E Mutation.” *International Journal*

of Cancer 128 (9): 2075–84. https://doi.org/10.1002/ijc.25555.

Tie, J., I. Kinde, Y. Wang, H. Wong, J. Roebert, M. Christie, M. Tacey, et al. 2015. "Circulating Tumor DNA as an Early Marker of Therapeutic Response in Patients with Metastatic Colorectal Cancer." *Annals of Oncology* 26 (8): 1715–22. https://doi.org/10.1093/annonc/mdv177.

Tie, J., Y. Wang, C. Tomasetti, L. Li, S. Springer, I. Kinde, N. Silliman, et al. 2016. "Circulating Tumor DNA Analysis Detects Minimal Residual Disease and Predicts Recurrence in Patients with Stage II Colon Cancer." *Science Translational Medicine* 8 (346): 346ra92. https://doi.org/10.1126/scitranslmed.aaf6219.

Townsley, C. A., R. Selby, and L. L. Siu. 2005. "Systematic Review of Barriers to the Recruitment of Older Patients with Cancer onto Clinical Trials." *Journal of Clinical Oncology* 23 (13): 3112–24. https://doi.org/10.1200/JCO.2005.00.141.

Turner, N., H. L. Wong, A. Templeton, S. Tripathy, T. Whiti Rogers, M. Croxford, I. Jones, et al. 2016. "Analysis of Local Chronic Inflammatory Cell Infiltrate Combined with Systemic Inflammation Improves Prognostication in Stage II Colon Cancer Independent of Standard Clinicopathologic Criteria." *International Journal of Cancer* 138 (3): 671–78. https://doi.org/10.1002/ijc.29805.

Unger, J. M., R. Vaidya, D. L. Hershman, L. M. Minasian, and M. E. Fleury. 2019. "Systematic Review and Meta-Analysis of the Magnitude of Structural, Clinical, and Physician and Patient Barriers to Cancer Clinical Trial Participation." *Journal of the National Cancer Institute* 111 (3): 245–55. https://doi.org/10.1093/jnci/djy221.

Zubrod, C. G., M. Schneiderman, E. Frei, C. Brindley, G. Lennard Gold, B. Shnider, R. Oviedo, et al. 1960. "Appraisal of Methods for the Study of Chemotherapy of Cancer in Man: Comparative Therapeutic Trial of Nitrogen Mustard and Triethylene Thiophosphoramide." *Journal of Chronic Diseases* 11 (1): 7–33. https://doi.org/

10.1016/0021-9681(60)90137-5.

Chapter 7.

Feasibility of a Registry-Based Randomised Controlled Trial Comparing First-Line Chemotherapy Sequencing Approaches in Metastatic Colorectal Cancer

Foroughi, Siavash, Hui-li Wong, Jeanne Tie, Antony Wilks Burgess, and Peter Gibbs. 2020. “Feasibility of a Registry-Based Randomised Controlled Trial Comparing First-Line Chemotherapy Sequencing Approaches in Metastatic Colorectal Cancer”

Manuscript in progress

Abstract

Purpose

We have investigated the feasibility of conducting a multi-centre prospective registry-based randomised controlled trial (RRCT) evaluating chemotherapy sequencing in treatment-naïve metastatic colorectal cancer (mCRC). This study uses an established Australian mCRC registry as the main platform for capturing clinical data. In this report, we describe our initial experience with the design and conduct of this Australian-first RRCT in oncology.

Patients and Methods

ALT-TRACC is an investigator-initiated RRCT examining alternating oxaliplatin and irinotecan doublet schedules versus continuous doublet chemotherapy in previously untreated mCRC, using the existing TRACC (Treatment of Recurrent and Advanced Colorectal Cancer) registry to capture clinical data. Eligible patients were identified by their treating oncologist during outpatient appointments. The primary outcome of feasibility was based on exceeding a pre-specified recruitment rate.

Results

While the current overall recruitment rate of 17% falls short of our pre-specified feasibility threshold of 33%, there was considerable variability across sites (2.8-28%), in part due to a higher than anticipated proportion of patients treated with curative intent or non-doublet therapy (single-agent or triplet chemotherapy) as a standard approach, more patients than expected declining participation, and enrolment in competing clinical trials that opened after ALT-TRACC was initiated. Notably, 41% of patients enrolled on ALT-TRACC would not have been eligible to participate in a conventional clinical trial. There

were no issues identified with trial compliance or with completeness of clinical data capture.

Conclusion

ALT-TRACC is the first Australian RRCT in oncology. The recruitment rate is currently below our pre-specified threshold, highlighting the need for more conservative accrual targets in future RRCTs in mCRC. The high proportion of enrolled patients that would have been excluded from traditional randomised controlled trials demonstrates the ability to enrol patients that otherwise would have not been trial eligible and the external validity of this model. The trial will be expanded to more sites to allow for faster recruitment and examine progression-free survival as the primary endpoint.

Introduction

Prospective, randomised controlled trials (RCTs) provide the highest level of scientific clinical evidence and are the gold standard for comparative studies, including of differing standard of care approaches. However, RCTs typically have narrow eligibility criteria that limit both recruitment and external validity and conventional RCTs are conducted at substantial per patient cost (Rothwell 2005; Emanuel et al. 2003). Clinical registries provide access to large pools of real-world patients and provide infrastructure for collecting baseline variables, treatment and outcome data at far lower cost (Wachtell et al. 2016). By incorporating the critical elements of conventional RCTs, including prospective identification of eligible patients, attainment of patient consent and random assignment of treatment, a registry-based randomised controlled trial (RRCT) also has high internal validity.

In treatment-naïve patients with metastatic colorectal cancer (mCRC), initial treatment with either FOLFOX (5FU plus oxaliplatin) or FOLFIRI (5FU plus irinotecan) chemotherapy results in similar overall survival (Colucci et al. 2005; Tournigand et al. 2004). Analysis of data from multiple clinical trials shows that the best survival outcomes are achieved in patients that receive all active agents (Grothey et al. 2004); however, the use of second-line chemotherapy where patients switch from one doublet to the other occurs in only 50% of patients, and hence, these patients do not derive the potential benefit that all active agents can provide (Grothey and Sargent 2005). Initial triplet chemotherapy, combining all active cytotoxic agents (5FU, oxaliplatin and irinotecan), has been demonstrated now in multiple randomised studies to improve overall survival compared to sequencing doublet chemotherapy (Marques et al. 2017), however concerns regarding adverse events have limited adoption into routine care. In order to reduce the toxicity associated with concurrent triplet chemotherapy whilst maintaining efficacy, a

sequential approach using alternating schedules of oxaliplatin and irinotecan with a fluoropyrimidine backbone has been proposed (Aparicio et al. 2005). A phase II randomised trial has now demonstrated similar efficacy with this sequential approach compared to concurrent triplet chemotherapy with respect to response rates and progression-free survival, while also demonstrating a more tolerable side effect profile (Hurwitz et al. 2019). Phase III data is awaited.

We sought to assess the feasibility of conducting a multi-centre prospective RRCT evaluating chemotherapy sequencing in treatment-naïve mCRC. The ALT-TRACC study was initiated in June 2018 using an established Australian mCRC registry as the main platform for capturing clinical data. The study continues to enrol patients across existing and new sites. In this report, we describe our experience with the design and conduct of this Australian-first RRCT in oncology.

Methods

Study Design

ALT-TRACC is a prospective, randomised phase II study examining alternating oxaliplatin and irinotecan doublet schedules versus continuous doublet chemotherapy in previously untreated mCRC, using the existing TRACC (Treatment of Recurrent and Advanced Colorectal Cancer) registry to capture clinical data. The TRACC registry was established in 2009 and captures consecutive patients with mCRC from 33 participating centres across Australia and Hong Kong (Field et al. 2013). Clinical data is captured at the point of care and includes mandatory fields for key prognostic characteristics including ECOG performance status, comorbidities, location of primary tumour and metastases, and resectability of metastatic disease. Systemic treatments across all lines of therapy are captured, including start and stop dates and best response as determined by

the treating clinician, guided by standard Response Evaluation Criteria in Solid Tumours (RECIST) (Eisenhauer et al. 2009). Treatment intent at the time of initial mCRC diagnosis and treatment discussions are also captured.

Study Cohort

Eligible patients were identified by their treating oncologist during outpatient appointments. Patients were eligible for enrolment if they met the following inclusion criteria: (1) age ≥18 years, (2) histologically confirmed, metastatic colorectal adenocarcinoma, (3) ECOG performance status of 0-2, (4) life expectancy of ≥ three months, and (5) adequate major organ function to receive doublet chemotherapy as judged by the treating clinician. Patients were excluded if they had a contraindication to any of the three cytotoxic agents (5FU, oxaliplatin and irinotecan), or had a concomitant medical condition that precluded the safe use of per-protocol treatment as judged by the treating clinician. Only treatment-naïve patients in the metastatic setting were included. Up to two cycles of doublet chemotherapy were allowed prior to consent and randomisation as treatment in both study arms was identical for the first two cycles.

Study Treatments

Participants were randomised to receive either clinician's choice continuous doublet chemotherapy, or an alternating schedule of two cycles of oxaliplatin doublet chemotherapy and two cycles of irinotecan doublet chemotherapy (study schema shown in Figure 1). Standard chemotherapy doses were recommended in the study protocol, but dose adjustments and addition of biologic agents (bevacizumab, cetuximab or panitumumab) were allowed according to the treating clinician's discretion. Similarly, de-escalation to maintenance fluoropyrimidine chemotherapy was allowed after four to

six months of initial doublet chemotherapy. Treatment was to be continued until disease progression, unmanageable toxicity or patient or treating clinician's request.

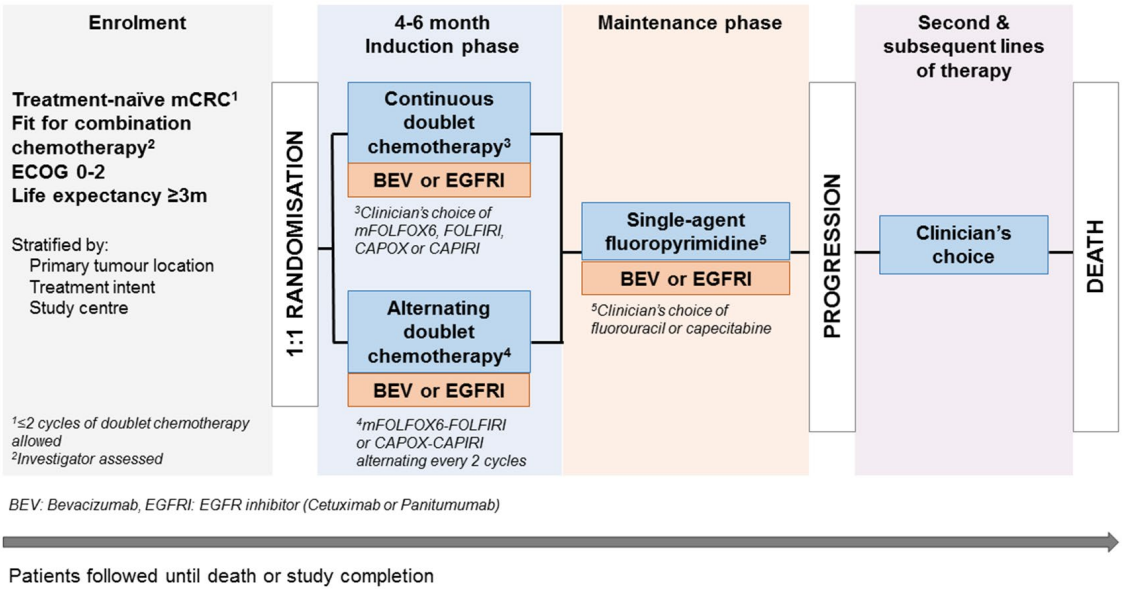


Figure 1. ALT-TRACC study schema.

Treatment Allocation and Data Management

The randomisation sequence was generated using R Studio (RStudio, Boston, MA, USA) and was stratified according to primary tumour location, treatment intent (palliative versus potentially curative) and study centre. Allocation concealment was maintained by using permuted blocks and was managed using a study-specific REDCap (Vanderbilt University, TN, USA) database with a randomisation module (Harris et al. 2009). The REDCap database included a simple electronic case report form to capture consent and eligibility data that was then linked with patient-specific clinical data collected in the TRACC registry using the participant's unique registry ID.

Study Outcome Measures

The primary objective of ALT-TRACC was feasibility and was evaluated by recruitment rate, defined as the proportion of eligible mCRC patients who enrolled onto this study.

Other feasibility measures were protocol compliance rate and completeness of follow-up data. Secondary endpoints were overall response rate, disease control rate, time to progression on first-line chemotherapy, overall survival, metastatic disease resection rate, proportion of patients who received all cytotoxic agents and serious adverse event rate.

Evaluation of recruitment rate for this report was performed using a combination of registry data and chart review. Eligible patients were initially defined as those considered for combination chemotherapy with non-curative intent who commenced treatment within the 14 days prior to the ALT-TRACC study opening for recruitment at that site. Chart review was then undertaken to assess clinical eligibility criteria not routinely captured in the TRACC registry, e.g. not suitable for either chemotherapy doublet. If this information was not evident from clinician notes, patients were assumed to be eligible.

Statistical Considerations

For the primary feasibility endpoint of recruitment rate, sample size was estimated using the Simon's two-stage optimal design, where 140 potentially eligible patients and at least 47 enrolled patients were required to reject an uninteresting recruitment rate of 20% in favour of the target recruitment rate of 33%, with over 90% power and type I two-sided error rate of 0.05. Recruitment rate was to be assessed after 50 potentially eligible patients had been registered in TRACC. For this report, descriptive statistics were used to summarise the clinicopathologic characteristics for ALT-TRACC study participants and all TRACC registry patients.

Ethics and Governance

The ALT-TRACC study was registered prospectively on the Australian and New Zealand Clinical Trials Register (ACTRN12618001480279). Study coordination, including registry linkage, was conducted by the Walter and Eliza Hall Institute of Medical

Research. All participating sites received human research ethics committee approval (HREC/17/MH/383) prior to commencement of participant recruitment. Written informed consent was obtained from all participants. The study was supported by the Victorian Comprehensive Cancer Centre Registry Trials Program.

Results

Study Status

As of July 2020, the ALT-TRACC study is open and recruiting patients in four centres across Victoria with local ethics applications underway in three further sites. The four participating sites are: (A) a large community public hospital, (B) a clinician's private practice, (C) a large academic public hospital, and (D) a regional hospital (mixed public and private practice). Sites A, B and C have contributed patients to the TRACC registry since 2009, whereas Site D began participating concurrently with the ALT-TRACC study.

Study Recruitment

Seventeen patients have consented and been randomised. Cumulative enrolment is shown in Figure 2. Recruitment rates were calculated for sites A, B and C individually to reflect differences in study initiation dates. Recruitment rate was not calculated for site D, which is only capturing data for ALT-TRACC patients in the TRACC registry. Overall recruitment rate was 17%, ranging from 2.8% to 28% across sites (Table 1).

Chart review was undertaken for all newly diagnosed mCRC patients who were considered for combination chemotherapy but not enrolled in ALT-TRACC (n = 126). Fifty-six patients (44%) were not eligible; reasons for ALT-TRACC ineligibility were treatment with curative intent (n = 34), contraindication to oxaliplatin or irinotecan such

as organ dysfunction, early progression after completion of adjuvant therapy or pre-existing neuropathy (n = 11), treatment initiated at or subsequently transferred to a different institution (n = 7) and significant concomitant medical conditions that precluded trial participation such as cognitive impairment (n = 4). Among the 70 potentially eligible patients who were not enrolled on ALT-TRACC, 27 received an alternative standard therapy (triplet chemotherapy (n = 16), single-agent chemotherapy (n = 8), FOLFOX with interdigitated radiotherapy (n = 3)), three enrolled in a competing pharmaceutical trial and four patients declined participation.

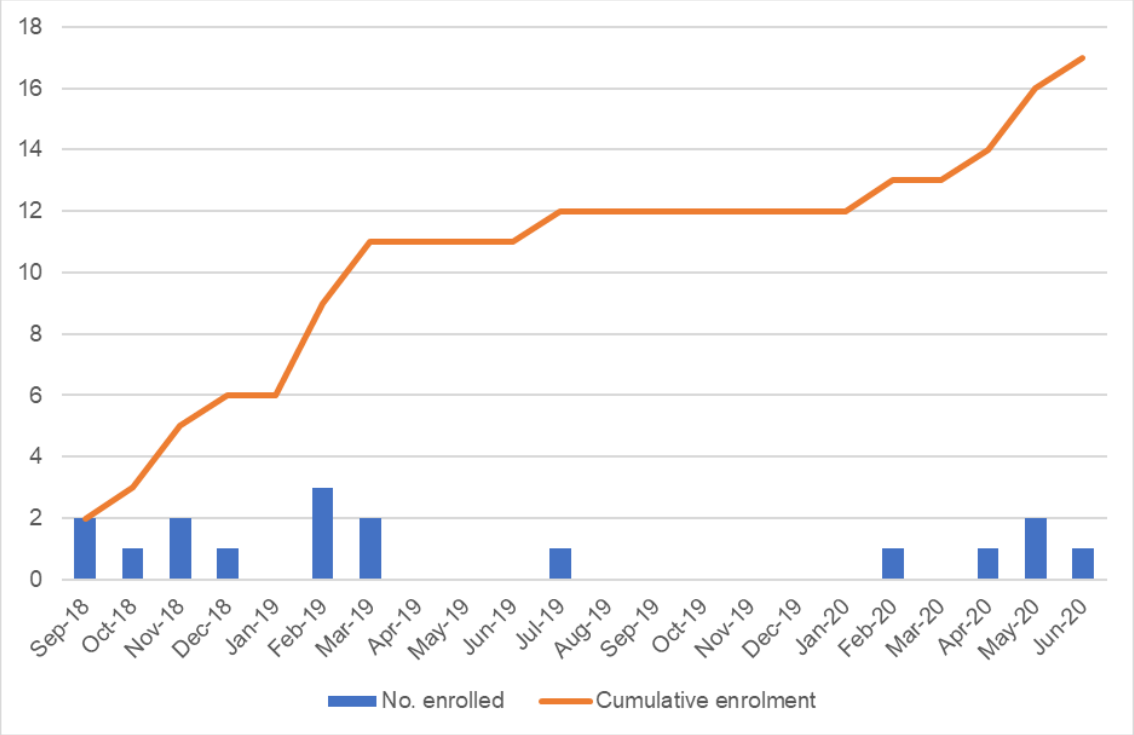


Figure 2. Cumulative enrolment over time.

Table 1. Recruitment rate of participants to the ALT-TRACC registry-based trial.

	Site A	Site B	Site C	TOTAL
No. of patients considered for combination chemotherapy	53	24	63	140
No. of eligible patients	36	12	36	84
Enrolled on ALT-TRACC	10	3	1	14[b]
Recruitment rate[a]	**28%**	**25%**	**2.8%**	**17%**
No. of patients not enrolled	43	21	62	126
Reasons not enrolled				
Not eligible				
Curative intent treatment	9	9	16	34
Contraindication to oxaliplatin or irinotecan	3	1	7	11
Treated elsewhere	3	2	2	7
Significant medical condition	2	0	2	4
Selected other treatment				
Triplet chemotherapy	10	2	4	16
Single-agent chemotherapy	1	3	4	8
FOLFOX with interdigitated radiotherapy	0	0	3	3
Other trial	1	0	2	3
Declined participation after discussion	2	0	2	4
Not documented	12	4	20	36

[a]Recruitment rate defined as proportion of eligible patients who were enrolled.
[b]One ALT-TRACC patient was not identified in the data query as their mCRC diagnosis was prior to 2018, with delayed initiation of systemic therapy.

Eligibility for Conventional Trials

Seven patients (41%) who were enrolled in the ALT-TRACC study would have been excluded from a conventional trial based on the clinical exclusion criteria that were used for a typical first-line mCRC phase III RCT involving doublet chemotherapy with biologic therapy (Venook et al. 2005). Furthermore, one patient met multiple exclusion criteria. Reasons for conventional trial exclusion were: comorbid condition that precluded study enrolment (n = 3), other malignancy within the previous 5 years (n = 2), adjuvant chemotherapy within the previous 12 months (n = 1), non-English speaking background (n = 1), and an ECOG performance status of 2 (n = 1). In total, six patients started chemotherapy before randomisation and three patients did not have *RAS* mutation results available at the time of randomisation.

Case Vignettes

Here we present a collection of case vignettes with the aim to demonstrate the broad and inclusive nature of this RRCT. We describe the presentation and main factors which would have resulted in exclusion of the patients from a conventional RCT. Cases are numbered consecutively, which is not reflective of recruitment or study ID.

Case 1

A 63-year-old male presented with a left-sided primary in-situ and bulky liver and peritoneal metastases. Clinical history included a prostate cancer diagnosed two years prior and idiopathic thrombocytopenic purpura controlled on azathioprine. Due to his disease symptoms, he started chemotherapy prior to randomisation and prior to molecular results being available. He consented to participate in the ALT-TRACC study when he attended for outpatient review prior to his second cycle of chemotherapy and was randomised immediately on-site.

Case 2

A 70-year-old male presented with bowel obstruction requiring emergency surgery for a *RAS*-mutated right-sided primary tumour. Two liver metastases and indeterminate lung lesions were seen on PET scan. Clinical history included a myocardial infarction one month prior, which precluded conventional trial participation and bevacizumab use. He consented to ALT-TRACC and was randomised prior to starting chemotherapy.

Case 3

A 63-year-old male from a non-English speaking background underwent elective resection of a stage III left-sided primary. Early post-operative imaging showed liver and lung metastases. Treatment options discussed included standard chemotherapy, ALT-

TRACC and a pharmaceutical trial. He was excluded from the pharmaceutical trial due to language and consented to ALT-TRACC instead.

Case 4

A 75-year-old man presented with iron deficiency anaemia and weight loss and was found on CT to have synchronous primaries in the right and left colon and multiple liver metastases. Biopsy of the left colon primary confirmed colorectal adenocarcinoma but the scope was unable to pass to obtain a biopsy of the right colon primary. He would have been excluded from a conventional trial due to the multiple primary tumours. He also needed to start chemotherapy urgently due to symptoms but was able to consent to ALT-TRACC.

Characteristics of Registry versus Registry Trial Patients

Table 2 shows clinicopathologic characteristics for the patients enrolled in ALT-TRACC and for all TRACC registry patients with mCRC diagnosed since 1st January 2018. There were 340 patients in the TRACC registry ($TRACC^{all}$), of whom 186 (55%) received combination chemotherapy ($TRACC^{chemo}$). Patients enrolled in ALT-TRACC had similar median age to the whole TRACC cohort (65 years and 63 years, respectively), whereas TRACC patients who received combination chemotherapy ($TRACC^{chemo}$) were younger (median age 58 years). ALT-TRACC patients had higher comorbidity scores than $TRACC^{all}$ registry patients who received combination chemotherapy (patients with age-adjusted Charlson Comorbidity Score > 3: ALT-TRACC 50%, $TRACC^{all}$ 27%, $TRACC^{chemo}$ 15%).

Table 2. Clinicopathologic characteristics of patients in the TRACC cohort: all patients, those who received combination chemotherapy and patients enrolled on the ALT-TRACC study.

	TRACC registry (all mCRC patients)	**TRACC registry (received combination chemotherapy)**	**ALT-TRACC study**
	N = 340	N = 186	N = 16[a]
Median age (range)	63 yrs (20-94)	58 yrs (20-85)	65 yrs (48-75)
Male sex	204 (60%)	123 (66%)	12 (75%)
Performance status			
ECOG 0-1	276 (81%)	173 (93%)	16 (100%)
ECOG 2	32 (9.4%)	6 (3.2%)	0
ECOG 3-4	14 (4.1%)	1 (0.5%)	0
Not recorded	18 (5.3%)	6 (3.2%)	0
Age-adjusted CCI score			
0-3	230 (68%)	153 (82%)	8 (50%)
>3	92 (27%)	27 (15%)	8 (50%)
Not recorded	18 (5.3%)	6 (3.2%)	0
Stage IV at diagnosis	200 (59%)	139 (75%)	13 (81%)
Primary tumour location			
Left	216 (64%)	119 (64%)	8 (50%)
Right	104 (31%)	56 (30%)	8 (50%)
Multiple/Occult	7 (2.1%)	5 (2.7%)	0
Not recorded	13 (3.8%)	6 (3.2%)	0
Metastatic sites			
Liver	203 (60%)	141 (76%)	14 (88%)
Lung	108 (32%)	51 (27%)	4 (25%)
Peritoneum	78 (23%)	47 (25%)	2 (13%)
Treatment location			
Public	294 (86%)	160 (86%)	11 (69%)
Private	44 (13%)	24 (13%)	3 (19%)
Not recorded	2 (0.6%)	2 (1.1%)	2 (13%)
RAS status			
Mutated	134 (39%)	82 (44%)	8 (50%)
Wild-type	120 (35%)	83 (45%)	7 (44%)
Unknown	86 (25%)	21 (11%)	1 (6.3%)
BRAF status			
Mutated	25 (7.4%)	23 (12%)	4 (25%)
Wild-type	224 (66%)	141 (76%)	11 (69%)
Unknown	91 (27%)	22 (12%)	1 (6.3%)
Mismatch repair status			
Proficient	284 (84%)	165 (89%)	14 (88%)
Deficient	14 (4.1%)	6 (3.2%)	1 (6.3%)
Unknown	42 (12%)	15 (8.1%)	1 (6.3%)
Treatment intent			
Curative	72 (21%)	34 (18%)	0
Potentially curative	39 (11%)	31 (17%)	1 (6.3%)
Palliative	193 (57%)	109 (59%)	15 (94%)
Not recorded	36 (11%)	12 (6.5%)	0

	TRACC registry (all mCRC patients)	TRACC registry (received combination chemotherapy)	ALT-TRACC study
	N = 340	N = 186	N = 16[a]
First-line chemotherapy			
Triplet	24 (7%)	24 (13%)	N/A
Doublet	162 (48%)	162 (87%)	16 (100%)
Fluoropyrimidine alone	64 (19%)	N/A	N/A
Other	1 (0.3%)	N/A	N/A
None	89 (26%)	N/A	N/A
First-line biologic agent			
Bevacizumab	98 (29%)	81 (44%)	7 (44%)
EGFRI	35 (10%)	29 (16%)	3 (19%)
Other	5 (1.5%)	3 (1.6%)	0
None	202 (59%)	73 (39%)	6 (38%)

[a]One ALT-TRACC patient was not identified in the data query as their mCRC diagnosis was prior to 2018.

Protocol Compliance

Compliance with protocol-defined treatment was assessed for the patients enrolled in ALT-TRACC. Three patients were not assessable as they had only been enrolled and started treatment in the month prior to this report. All seven patients randomised to the alternating arm correctly alternated between doublet chemotherapy regimens (all received FOLFOX-FOLFIRI doublets). Four patients received at least 16 cycles of doublet chemotherapy (i.e. 8 cycles of FOLFOX and 8 cycles of FOLFIRI) due to excellent tolerance. All seven patients randomised to the standard arm received 6-8 cycles of oxaliplatin doublet chemotherapy.

Timing of baseline imaging was also reviewed for the 14 patients where this was assessable. As per protocol, baseline imaging of chest, abdomen and pelvis was recommended within 28 days (and no more than eight weeks) of the first chemotherapy dose. Ten patients had baseline imaging within 28 days and the remaining four patients within eight weeks prior to starting chemotherapy.

Data Completeness

Completeness of follow-up data was not assessed for this report as the majority of patients remain on treatment or in follow-up. Completeness of clinicopathologic characteristics is reflected in Table 2: all ALT-TRACC patients had complete data for performance status, comorbidities, location of primary tumour and metastases, and treatment intent. Molecular profile was not recorded for one patient. *BRAF* mutation rate was higher than expected in the ALT-TRACC group; two of these patients were also recorded as having *RAS* mutations, suggesting a possible data entry error as *RAS* and *BRAF* mutations are typically mutually exclusive.

Discussion

We initiated the ALT-TRACC study to explore the feasibility of using an existing clinical registry (TRACC) to conduct a prospective randomised trial in the oncology setting. We have previously reviewed adopting this methodology in oncology and discussed its strengths and weaknesses (Foroughi et al. 2018). Briefly, RRCTs offer the potential for increased external validity of study data through pragmatic trial design and with the inclusion of real-world patients identified through existing clinical registries. Further, by utilising existing infrastructure the trial costs are externalised, reducing the cost of conducting a trial. We adopted a pragmatic study design for ALT-TRACC that allowed treating clinicians to determine treatment modifications such as dose adjustments, as would occur in real-world practice. While the study continues to recruit participants, our initial experience as described in this report highlights some of the benefits and challenges of RRCTs.

In this initial report, we found that recruitment rates were lower than anticipated and varied substantially across participating sites. This may be partly explained by higher than

expected rates of treatment with curative intent or selection of non-doublet chemotherapy as a standard approach. Among patients who would have been potentially eligible, other patient and/or clinician factors may have contributed, such as availability of competing trials (which differed by site) or impact on eligibility for subsequent trials (if randomised to the alternating arm) if a second-line industry-sponsored study was available. Patients may have been overwhelmed with the amount of trial-related information, although this would not be a limitation of the RRCT design but reflective of the challenges associated with obtaining informed consent for clinical trials (Behrendt et al. 2011). Similar challenges with recruitment were faced in other RRCTs (Litton et al. 2019), highlighting the need for more conservative accrual targets. Strategies to improve recruitment, such as dedicated study coordinators to identify patients in clinics, may also be helpful.

Notably, 41% of patients enrolled on ALT-TRACC would not have been eligible to participate in a conventional RCT, reflecting the broad eligibility criteria and pragmatic trial design. Several previous studies have shown that the majority of real-world patients would not meet eligibility criteria for clinical trials (Peixoto et al. 2017; Jung et al. 2020; Batra, Kong, and Cheung 2020; Al-Baimani et al. 2018). This demonstrates a significant opportunity to increase trial participation with the added advantage that the use of a registry to conduct an RCT enables assessment of generalisability by comparing outcomes for trial versus non-trial patients. For example, the landmark TASTE RRCT in cardiology was able to demonstrate that outcomes for randomised and non-randomised patients differed substantially, likely due to non-randomised patients being more medically unstable in this acute care setting, as they were largely excluded due to their inability to provide oral consent (Fröbert et al. 2013). Despite this, the RRCT results (i.e. no difference in mortality between the two treatment approaches under investigation) were similar in the non-randomised cohort, indicating that the study results are externally

valid. It is important to consider that even when using a population-based registry, an RRCT may not be fully representative of the wide range of real-world patients. While we have not assessed outcomes for ALT-TRACC as the study is still recruiting, the characteristics of ALT-TRACC patients are, so far, highly reflective of the overall TRACC registry population.

For ALT-TRACC, we introduced a randomisation module to complement an existing comprehensive clinical registry. This approach combines the benefits of randomised treatment allocation with the characteristics of a large-scale clinical registry and has many advantages, for example a simplified enrolment and data collection process, thereby increasing compliance and reducing burden on the participating hospitals. The simplicity of this approach has allowed the ALT-TRACC trial to continue recruiting during the COVID-19 pandemic while other conventional trial activity was significantly reduced (Fontana and Arkenau 2020; Weinkove et al. 2020), highlighting the benefit of leveraging clinical registries which are embedded into routine practice. To date, no major issues have been identified with protocol compliance or completeness of baseline patient and disease characteristics. It is important to ensure accuracy and completeness of follow-up data is maintained with regular audits. Certainly, by leveraging established registry infrastructure, beyond reducing the costs associated with performing an RCT, compliance and completeness of follow-up data are improved as data is being routinely captured irrespective of trial participation.

Triplet chemotherapy (FOLFOXIRI) has been shown to result in longer overall survival compared to doublet regimens (Cremolini et al. 2015; Falcone et al. 2007), but is associated with a higher rate of adverse events and in routine care is typically limited to young, fit patients. We have previously shown that FOLFOXIRI has been used

infrequently in real-world Australian practice (Semira et al. 2019). The randomised phase II STEAM trial (Hurwitz et al. 2019) demonstrated the potential of alternating doublet therapy to provide the activity of triplet therapy with less toxicity. These results need to be confirmed in a phase III study, hence our motivation to expand recruitment to ALT-TRACC and to power for survival endpoints, which is now being pursued with endorsement from the Australasian Gastro-Intestinal Trials Group. The importance of first-line treatment has been highlighted in a large meta-analysis supporting a relationship between tumour response to initial systemic chemotherapy and overall survival (Buyse et al. 2000). Presumably as more active therapies become available after standard first- and second-line chemotherapy, now including trifluridine/tipiracil as part of Australian practice, the importance of optimising first-line treatment will increase.

This report has several limitations. A major limitation of the current analysis is the small number of ALT-TRACC patients currently recruited, hence analyses may be subject to bias. While every effort was made to determine reasons for why patients were not enrolled onto study, our assessment was based on retrospective chart review and there may have been reasons clinicians felt patients were ineligible that were not evident from clinicians' notes. Finally, costs associated with conducting the ALT-TRACC study have not yet been estimated, although this analysis is planned.

Here we have demonstrated initial proof-of-concept for registry-based randomised controlled trials in oncology. Patients recruited to the ALT-TRACC RRCT to date are broadly representative of the registry population, suggesting that study results will be generalisable to real-world patients, unlike in conventional RCTs which tend to be highly selective. Most encouraging is the inclusion of a large proportion of patients who would

have otherwise been excluded from participating in a clinical trial. The trial protocol is currently being amended to examine progression-free survival as the primary endpoint.

References

Al-Baimani, K., H. Jonker, T. Zhang, G. D. Goss, S. A. Laurie, G. Nicholas, and P. Wheatley-Price. 2018. "Are Clinical Trial Eligibility Criteria an Accurate Reflection of a Real-World Population of Advanced Non-Small-Cell Lung Cancer Patients?" *Current Oncology* 25 (4): e291–97. https://doi.org/10.3747/co.25.3978.

Aparicio, J., C. Fernández-Martos, J. M. Vincent, I. Maestu, C. Llorca, I. Busquier, J. M. Campos, D. Pérez-Enguix, and M. Balcells. 2005. "FOLFOX Alternated with FOLFIRI as First-Line Chemotherapy for Metastatic Colorectal Cancer." *Clinical Colorectal Cancer* 5 (4): 263–67. https://doi.org/10.3816/CCC.2005.n.037.

Batra, A., S. Kong, and W. Y. Cheung. 2020. "Eligibility of Real-World Patients With Stage II and III Colon Cancer for Adjuvant Chemotherapy Trials." *Clinical Colorectal Cancer* 19 (4): e226–34. https://doi.org/10.1016/j.clcc.2020.05.005.

Behrendt, C., T. Gölz, C. Roesler, H. Bertz, and A. Wünsch. 2011. "What Do Our Patients Understand about Their Trial Participation? Assessing Patients' Understanding of Their Informed Consent Consultation about Randomised Clinical Trials." *Journal of Medical Ethics* 37 (2): 74–80. https://doi.org/10.1136/jme.2010.035485.

Buyse, M., P. Thirion, R. W. Carlson, T. Burzykowski, G. Molenberghs, and P. Piedbois. 2000. "Relation between Tumour Response to First-Line Chemotherapy and Survival in Advanced Colorectal Cancer: A Meta-Analysis." *Lancet* 356 (9227): 373–78. https://doi.org/10.1016/S0140-6736(00)02528-9.

Colucci, G., V. Gebbia, G. Paoletti, F. Giuliani, M. Caruso, N. Gebbia, G. Cartenì, et al. 2005. "Phase III Randomized Trial of FOLFIRI versus FOLFOX4 in the Treatment of Advanced Colorectal Cancer: A Multicenter Study of the Gruppo Oncologico Dell'Italia Meridionale." *Journal of Clinical Oncology* 23 (22): 4866–75. https://doi.org/10.1200/JCO.2005.07.113.

Cremolini, C., F. Loupakis, C. Antoniotti, C. Lupi, E. Sensi, S. Lonardi, S. Mezi, et al. 2015. “FOLFOXIRI plus Bevacizumab versus FOLFIRI plus Bevacizumab as First-Line Treatment of Patients with Metastatic Colorectal Cancer: Updated Overall Survival and Molecular Subgroup Analyses of the Open-Label, Phase 3 TRIBE Study.” *The Lancet Oncology* 16 (13): 1306–15. https://doi.org/10.1016/S1470-2045(15)00122-9.

Eisenhauer, E. A., P. Therasse, J. Bogaerts, L. H. Schwartz, D. Sargent, R. Ford, J. Dancey, et al. 2009. “New Response Evaluation Criteria in Solid Tumours: Revised RECIST Guideline (Version 1.1).” *European Journal of Cancer* 45 (2): 228–47. https://doi.org/10.1016/j.ejca.2008.10.026.

Emanuel, E. J., L. E. Schnipper, D. Y. Kamin, J. Levinson, and A. S. Lichter. 2003. “The Costs of Conducting Clinical Research.” *Journal of Clinical Oncology* 21 (22): 4145–50. https://doi.org/10.1200/JCO.2003.08.156.

Falcone, A., S. Ricci, I. Brunetti, E. Pfanner, G. Allegrini, C. Barbara, L. Crinò, et al. 2007. “Phase III Trial of Infusional Fluorouracil, Leucovorin, Oxaliplatin, and Irinotecan (FOLFOXIRI) Compared With Infusional Fluorouracil, Leucovorin, and Irinotecan (FOLFIRI) As First-Line Treatment for Metastatic Colorectal Cancer: The Gruppo Oncologico Nord Ovest.” *Journal of Clinical Oncology* 25 (13): 1670–76. https://doi.org/10.1200/JCO.2006.09.0928.

Field, K., H. L. Wong, J. Shapiro, S. Kosmider, J. Tie, S. Bae, D. Yip, et al. 2013. “Developing a National Database for Metastatic Colorectal Cancer Management: Perspectives and Challenges.” *Internal Medicine Journal* 43 (11): 1224–31. https://doi.org/10.1111/imj.12230.

Fontana, E., and H.-T. Arkenau. 2020. “Oncology Clinical Trials during the COVID-19 Outbreak: Lessons Learnt during the Crisis and Future Opportunities.” *Cancer*

Treatment Reviews 88: 102047. https://doi.org/10.1016/ j.ctrv.2020.102047.

Foroughi, S., H. Wong, L. Gately, M. Lee, K. Simons, J. Tie, A. W. Burgess, and P. Gibbs. 2018. "Re-Inventing the Randomized Controlled Trial in Medical Oncology: The Registry-Based Trial." *Asia-Pacific Journal of Clinical Oncology* 14 (6): 365–73. https://doi.org/10.1111/ajco.12992.

Fröbert, O., B. Lagerqvist, G. K. Olivecrona, E. Omerovic, T. Gudnason, M. Maeng, M. Aasa, et al. 2013. "Thrombus Aspiration during ST-Segment Elevation Myocardial Infarction." *New England Journal of Medicine* 369 (17): 1587–97. https://doi.org/10.1056/NEJMoa1308789.

Grothey, A., and D. Sargent. 2005. "Overall Survival of Patients with Advanced Colorectal Cancer Correlates with Availability of Fluorouracil, Irinotecan, and Oxaliplatin Regardless of Whether Doublet or Single-Agent Therapy Is Used First Line [11]." *Journal of Clinical Oncology* 23 (36): 9441–42. https://doi.org/10.1200/ JCO.2005.04.4792.

Grothey, A., D. Sargent, R. M. Goldberg, and H.-J. Schmoll. 2004. "Survival of Patients With Advanced Colorectal Cancer Improves With the Availability of Fluorouracil-Leucovorin, Irinotecan, and Oxaliplatin in the Course of Treatment." *Journal of Clinical Oncology* 22 (7): 1209–14. https://doi.org/10.1200/JCO.2004.11.037.

Harris, P. A., R. Taylor, R. Thielke, J. Payne, N. Gonzalez, and J. G. Conde. 2009. "Research Electronic Data Capture (REDCap) - A Metadata-Driven Methodology and Workflow Process for Providing Translational Research Informatics Support." *Journal of Biomedical Informatics* 42 (2): 377–81. https://doi.org/10.1016/ j.jbi.2008.08.010.

Hurwitz, H. I., B. R. Tan, J. A. Reeves, H. Xiong, B. Somer, H.-J. Lenz, H. S. Hochster, et al. 2019. "Phase II Randomized Trial of Sequential or Concurrent FOLFOXIRI-

Bevacizumab Versus FOLFOX-Bevacizumab for Metastatic Colorectal Cancer (STEAM).” *The Oncologist* 24 (7): 921–32. https://doi.org/10.1634/theoncologist.2018-0344.

Jung, H. A., J. M. Noh, J. M. Sun, S. H. Lee, J. S. Ahn, M. J. Ahn, H. Pyo, Y. C. Ahn, and K. Park. 2020. “Real World Data of Durvalumab Consolidation after Chemoradiotherapy in Stage III Non-Small-Cell Lung Cancer.” *Lung Cancer* 146: 23–29. https://doi.org/10.1016/j.lungcan.2020.05.035.

Litton, E., F. Bass, C. Dickson, G. Hillis, D. Inskip, T. Jacques, D. McGiffin, et al. 2020. “Prophylactic Intra-Aortic Balloon Counterpulsation in High Risk Cardiac Surgery: The PINBALL Pilot Multicentre, Registry-Linked, Randomised, Controlled Feasibility Trial.” *Heart, Lung and Circulation* 29 (5): 710–18. https://doi.org/10.1016/j.hlc.2019.04.006.

Marques, R. P., G. S. Duarte, C. Sterrantino, H. L. Pais, A. Quintela, A. P. Martins, and J. Costa. 2017. “Triplet (FOLFOXIRI) versus Doublet (FOLFOX or FOLFIRI) Backbone Chemotherapy as First-Line Treatment of Metastatic Colorectal Cancer: A Systematic Review and Meta-Analysis.” *Critical Reviews in Oncology/Hematology* 118: 54–62. https://doi.org/10.1016/j.critrevonc.2017.08.006.

Peixoto, R. D., M. Ho, D. J. Renouf, H. J. Lim, S. Gill, J. Y. Ruan, and W. Y. Cheung. 2017. “Eligibility of Metastatic Pancreatic Cancer Patients for First-Line Palliative Intent Nab-Paclitaxel Plus Gemcitabine Versus FOLFIRINOX.” *American Journal of Clinical Oncology* 40 (5): 507–11. https://doi.org/10.1097/COC.0000000000000193.

Rothwell, P. M. 2005. “External Validity of Randomised Controlled Trials: To Whom Do the Results of This Trial Apply?” *The Lancet* 365 (9453): 82–93.

https://doi.org/10.1016/S0140-6736(04)17670-8.

Semira, C., H. L. Wong, K. Field, M. Lee, B. Lee, L. Nott, J. Shapiro, et al. 2019. "Chemotherapy and Biologic Use in the Routine Management of Metastatic Colorectal Cancer in Australia: Is Clinical Practice Following the Evidence?" *Internal Medicine Journal* 49 (4): 446–54. https://doi.org/10.1111/imj.14115.

Tournigand, C., T. André, E. Achille, G. Lledo, M. Flesh, D. Mery-Mignard, E. Quinaux, et al. 2004. "FOLFIRI Followed by FOLFOX6 or the Reverse Sequence in Advanced Colorectal Cancer: A Randomized GERCOR Study." *Journal of Clinical Oncology* 22 (2): 229–37. https://doi.org/10.1200/JCO.2004.05.113.

Venook, A. P., C. D. Blanke, D. Niedzwiecki, H. J. Lenz, J. R. Taylor, D. R. Hollis, S. Sutherland, and R. M. Goldberg. 2005. "Cancer and Leukemia Group b/Southwest Oncology Group Trial 80405: A Phase III Trial of Chemotherapy and Biologics for Patients with Untreated Advanced Colorectal Adenocarcinoma." *Clinical Colorectal Cancer* 5 (4): 292–94. https://doi.org/10.3816/CCC.2005.n.043.

Wachtell, K., B. Lagerqvist, G. K. Olivecrona, S. K. James, and O. Fröbert. 2016. "Novel Trial Designs: Lessons Learned from Thrombus Aspiration During ST-Segment Elevation Myocardial Infarction in Scandinavia (TASTE) Trial." *Current Cardiology Reports* 18 (1): 11. https://doi.org/10.1007/s11886-015-0677-6.

Weinkove, R., Z. K. McQuilten, J. Adler, M. R. Agar, E. Blyth, A. C. Cheng, R. Conyers, et al. 2020. "Managing Haematology and Oncology Patients during the COVID-19 Pandemic: Interim Consensus Guidance." *Medical Journal of Australia* 212 (10): 481–89. https://doi.org/10.5694/mja2.50607.

Chapter 8.

Summary and Conclusions

This thesis describes a body of work using clinical registry data to examine a number of important questions regarding the management of CRC in a real-world population. Studies to date of the underlying tumour biology of CRC have resulted in patient subtyping according to biomarker status and enabled the development of targeted therapies (Graham et al. 2016). While this has contributed to improved survival outcomes, the increased availability of treatment options brings a new challenge for clinicians who must deal with the increasing complexity of optimal treatment sequencing and patient selection (Modest, Pant, and Sartore-Bianchi 2019). There remains an urgent need to identify and validate biomarkers that will facilitate the optimal use of these therapies, maximising clinical benefit and minimising adverse events through avoiding treatment options and their associated toxicities in patients who are unlikely to benefit from treatment. Equally important, is that evidence generation needs to expand beyond traditional randomised controlled trials, which are highly selective and can have limited generalisability to patients treated in real-world practice (Foroughi et al. 2018). Chapters 1 to 3 expand on these concepts and provide further background information for the original research work undertaken for this thesis.

The EGFR pathway is one of the most dysregulated pathways in CRC. Monoclonal antibodies targeting EGFR (cetuximab and panitumumab) are now widely adopted as part of standard treatment in mCRC (Van Cutsem et al. 2016). Efficacy of these drugs is limited to *RAS* wild-type tumours (Karapetis et al. 2008) but even so, a proportion of patients with *RAS* wild-type mCRC do not respond to EGFR inhibitors. EGFR ligands

have been investigated as potential biomarkers to better select patients for these therapies, with AREG and EREG being the most promising to date (Jing et al. 2016; Foroughi et al. 2019). Most of the existing research into EGFR ligand expression used mRNA techniques or only included patients who had received EGFR inhibitor therapy (Seligmann et al. 2016; Khambata-Ford et al. 2007). In chapter 5, we report on the largest immunohistochemical analysis to our knowledge, of AREG and EREG protein levels in a registry cohort of *KRAS* wild-type mCRC patients. By including patients who had not received EGFR inhibitor therapy, we were able for the first time to examine the prognostic impact of these proteins in this setting. While we have observed that these proteins are not prognostic for mCRC survival outcomes in this cohort, there was strong evidence that EREG protein expression is predictive for benefit from EGFR inhibitor therapy, an effect driven by the improved outcome achieved in left-sided primary tumours. These findings support further investigation of EREG as a biomarker to guide the use of EGFR inhibitor therapy.

In chapter 6, we explore the generalisability of biomarker studies by comparing characteristics of early-stage CRC patients who participated in these studies compared to a real-world cohort. Multiple previous studies have reported that participants in drug trials are younger and fitter than the general population (Abbasi 2019; Murthy, Krumholz, and Gross 2004), but this had not been examined for biomarker trials. Using data from an established CRC registry matched to five biomarker trial enrolment logs, we demonstrate that participants in retrospective biomarker trials, where the only requirement was available archival tissue, had similar characteristics and outcomes relative to the total real-world population. Whereas in prospective biomarker trials, even in simple observational studies with no impact on treatment delivery, a younger and fitter patient population was enrolled, matching what has been widely reported for therapy-based

studies. While there are multiple factors that impact on trial participation, the selective patient enrolment can be partly explained by trial eligibility criteria; although these were relatively broad, a certain level of patient fitness was required. These findings highlight the need for clinicians and researchers to consider the generalisability of any study evidence generated.

Finally, chapter 7 reports our progress in conducting an Australian-first multi-centre prospective registry-based randomised controlled trial in oncology, with the aim of providing proof-of-concept for a novel approach for conducting randomised trials that are cheaper than conventional trials and have conclusions that are more generalisable. The ALT-TRACC study was initiated in June 2018 using an established Australian mCRC registry as the main platform for capturing clinical data. Patients commencing doublet chemotherapy for mCRC are randomised to standard treatment versus a schedule of alternating doublet regimens, which allows for all active chemotherapy agents to be delivered in the first-line setting. This is a pragmatic study design that uses standard chemotherapy regimens, does not require biomarker results, and allows patients to be enrolled after starting chemotherapy. The study continues to enrol patients across existing and new sites. While still in its early stages, our ability to recruit patients that are representative of the registry cohort is very encouraging. Importantly, close to half of patients enrolled would not have been eligible for a conventional randomised trial. We are hopeful that this study design will pave the way for future oncology trials and indeed be adopted more broadly into other disciplines, where high-quality registries exist and can be leveraged. A widening gap exists between real-world practice and clinical trials, particularly in oncology (Abbasi 2019; Murthy, Krumholz, and Gross 2004), and we believe that RRCTs will help bridge this gap.

This thesis illustrates how clinical registries can be used to support different types of research, including translational research, comparative research and, for the first time in oncology, to support prospective randomised trials. To date, there is no overarching guidance on how to efficiently harness and optimise the use of clinical registries to enable improved outcomes for patients. Substantial investment is required in the design and support of clinical registries and further investment must be made into novel study designs and strategies to allow the use of registries to their full potential. While registry-based trials are more prominent in Europe and in the field of cardiology, this study represents the first oncology registry-based trial in Australia and shines a light on a new era of clinical research. Registry-based trials provide a cost-effective means to explore interesting, practice-changing clinical questions which may otherwise never be answered. With growing interest within several tumour streams and support from the Victorian Comprehensive Cancer Centre, registry-based research is positioned to become a powerful source of clinical evidence generation in Australia.

Limitations

As outlined in previous chapters, there are limitations to the research presented in this thesis. The acquisition of suitable amounts of well-preserved tumour tissue and the corresponding clinical data presents an immense challenge for clinical research. This is particularly relevant for chapter 5, where only around 20% of identified clinical cases had suitable archival tissue for analysis. Further, immunohistochemical analysis has some limitations due to the heterogeneity of staining and reporting. This was mitigated by examination of full-face resections specimens and use of a semi-quantitative scoring system. However, it is important to consider this analysis in the context of the disease

setting; frequently in mCRC, the only available tissue is a small biopsy core. These types of samples may be inaccurately assessed and were under-represented in our cohort.

With any retrospective analysis of real-world data, incomplete data can impact on the interpretation of analyses. Every effort was made to identify missing data and these have been reported where relevant. Further, when performing comparative analyses, the impact of any one factor on outcomes is difficult to ascertain due to potential confounders and biases. For example, while our data in chapter 5 suggests that EREG expression is predictive for EGFR benefit, the true effect cannot be fully ascertained from these studies and needs to be prospectively validated, ideally in a randomised trial.

Future Directions

Perhaps the most exciting future prospect is the development of an accurate predictive biomarker to help refine the subset of patients most likely to benefit from EGFR inhibitor therapies. While we have provided evidence that EREG protein levels are a promising candidate, the next step of a prospective randomised study would provide high level evidence relevant for its use. Furthermore, leveraging high quality clinical registries for randomised trials is a novel approach that is gaining traction across many disease types. Our initial efforts with the ALT-TRACC RRCT have been promising and expansion of the study to examine an efficacy endpoint is planned.

Conclusion

The data analyses and experiments presented in this thesis provide novel insights into the biology of EGFR ligands in mCRC and demonstrate the validity of the data from biomarker trials in oncology. Demonstrated here is the importance of developing pragmatic and inclusive study designs which allow for increased patient participation in

clinical trials. This research illustrates that registry-based trials are a feasible approach to increase the use of patient data as well as to answer important clinical questions in oncology.

References

Abbasi, J. 2019. "Older Patients (Still) Left Out of Cancer Clinical Trials." *Journal of the American Medical Association* 322 (18): 1751. https://doi.org/10.1001/jama.2019.17016.

Amin, M. B., F. L. Greene, S. B. Edge, C. C. Compton, J. E. Gershenwald, R. K. Brookland, L. Meyer, D. M. Gress, D. R. Byrd, and D. P. Winchester. 2017. "The Eighth Edition AJCC Cancer Staging Manual: Continuing to Build a Bridge from a Population-Based to a More 'Personalized' Approach to Cancer Staging." *CA: A Cancer Journal for Clinicians* 67 (2): 93–99. https://doi.org/10.3322/caac.21388.

Ananda, S. S., S. J. McLaughlin, F. Chen, I. P. Hayes, A. A. Hunter, I. J. Skinner, M. C. A. Steel, et al. 2009. "Initial Impact of Australia's National Bowel Cancer Screening Program." *Medical Journal of Australia* 191 (7): 378–81. https://doi.org/10.5694/j.1326-5377.2009.tb02843.x.

André, T., C. Boni, L. Mounedji-Boudiaf, M. Navarro, J. Tabernero, T. Hickish, C. Topham, et al. 2004. "Oxaliplatin, Fluorouracil, and Leucovorin as Adjuvant Treatment for Colon Cancer." *New England Journal of Medicine* 350 (23): 2343–51. https://doi.org/10.1056/NEJMoa032709.

Andre, T., K.-K. Shiu, T. W. Kim, B. V. Jensen, L. H. Jensen, C. J. A. Punt, D. M. Smith, et al. 2020. "Pembrolizumab versus Chemotherapy for Microsatellite Instability-High/Mismatch Repair Deficient Metastatic Colorectal Cancer: The Phase 3 KEYNOTE-177 Study." *Journal of Clinical Oncology* 38 (18_suppl): LBA4–LBA4. https://doi.org/10.1200/JCO.2020.38.18_suppl.LBA4.

Araghi, M., I. Soerjomataram, M. Jenkins, J. Brierley, E. Morris, F. Bray, and M. Arnold.

2019. “Global Trends in Colorectal Cancer Mortality: Projections to the Year 2035.” *International Journal of Cancer* 144 (12): 2992–3000. https://doi.org/10.1002/ijc.32055.

Arnold, M., N. Pandeya, G. Byrnes, A. G. Renehan, G. A. Stevens, M. Ezzati, J. Ferlay, et al. 2015. “Global Burden of Cancer Attributable to High Body-Mass Index in 2012: A Population-Based Study.” *The Lancet Oncology* 16 (1): 36–46. https://doi.org/10.1016/S1470-2045(14)71123-4.

Australian Institute of Health and Welfare. 2019a. “Cancer in Australia 2019.” https://www.aihw.gov.au/reports/cancer/cancer-in-australia-2019/data.

———. 2019b. “National Bowel Cancer Screening Program: Monitoring Report 2019.” Canberra: Cancer series no. 125. Cat. no. CAN 125. Canberra: AIHW.

Bagnardi, V., M. Rota, E. Botteri, I. Tramacere, F. Islami, V. Fedirko, L. Scotti, et al. 2015. “Alcohol Consumption and Site-Specific Cancer Risk: A Comprehensive Dose-Response Meta-Analysis.” *British Journal of Cancer* 112 (3): 580–93. https://doi.org/10.1038/bjc.2014.579.

Benson, A. B. 2005. “Adjuvant Chemotherapy of Stage III Colon Cancer.” *Seminars in Oncology*, 32:74–77. https://doi.org/10.1053/j.seminoncol.2005.04.016.

Bouvard, V., D. Loomis, K. Z. Guyton, Y. Grosse, F. El Ghissassi, L. Benbrahim-Tallaa, N. Guha, H. Mattock, and K. Straif. 2015. “Carcinogenicity of Consumption of Red and Processed Meat.” *The Lancet Oncology* 16 (16): 1599–1600. https://doi.org/10.1016/S1470-2045(15)00444-1.

Bray, F., J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre, and A. Jemal. 2018. “Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries.” *CA: A Cancer Journal for Clinicians* 68 (6): 394–424. https://doi.org/10.3322/caac.21492.

Cutsem, E. Van, A. Cervantes, R. Adam, A. Sobrero, J. H. Van Krieken, D. Aderka, E. Aranda Aguilar, et al. 2016. "ESMO Consensus Guidelines for the Management of Patients with Metastatic Colorectal Cancer." *Annals of Oncology* 27 (8): 1386–1422. https://doi.org/10.1093/annonc/mdw235.

Cutsem, E. Van, A. Cervantes, B. Nordlinger, and D. Arnold. 2014. "Metastatic Colorectal Cancer: ESMO Clinical Practice Guidelines for Diagnosis, Treatment and Follow-Up." *Annals of Oncology* 25 (September): iii1–9. https://doi.org/10.1093/annonc/mdu260.

Fearon, E. R., and B. Vogelstein. 1990. "A Genetic Model for Colorectal Tumorigenesis." *Cell* 61 (5): 759–67. https://doi.org/10.1016/0092-8674(90)90186-I.

Field, K., H. L. Wong, J. Shapiro, S. Kosmider, J. Tie, S. Bae, D. Yip, et al. 2013. "Developing a National Database for Metastatic Colorectal Cancer Management: Perspectives and Challenges." *Internal Medicine Journal* 43 (11): 1224–31. https://doi.org/10.1111/imj.12230.

Foroughi, S., J. Tie, P. Gibbs, and A. W. Burgess. 2019. "Epidermal Growth Factor Receptor Ligands: Targets for Optimizing Treatment of Metastatic Colorectal Cancer." *Growth Factors* 37 (5–6): 209–25. https://doi.org/10.1080/08977194.2019.1703702.

Foroughi, S., H. Wong, L. Gately, M. Lee, K. Simons, J. Tie, A. W. Burgess, and P. Gibbs. 2018. "Re-Inventing the Randomized Controlled Trial in Medical Oncology: The Registry-Based Trial." *Asia-Pacific Journal of Clinical Oncology* 14 (6): 365–73. https://doi.org/10.1111/ajco.12992.

Graham, D. M., V. M. Coyle, R. D. Kennedy, and R. H. Wilson. 2016. "Molecular Subtypes and Personalized Therapy in Metastatic Colorectal Cancer." *Current Colorectal Cancer Reports* 12 (3): 141–50. https://doi.org/10.1007/s11888-016-

0312-y.

Grulich, A. E., M. McCredie, and M. Coates. 1995. “Cancer Incidence in Asian Migrants to New South Wales, Australia.” *British Journal of Cancer* 71 (2): 400–408. https://doi.org/10.1038/bjc.1995.82.

Hamilton, W., and D. Sharp. 2004. “Diagnosis of Colorectal Cancer in Primary Care: The Evidence Base for Guidelines.” *Family Practice* 21 (1): 99–106. https://doi.org/10.1093/fampra/cmh121.

Harris, P. A., R. Taylor, R. Thielke, J. Payne, N. Gonzalez, and J. G. Conde. 2009. “Research Electronic Data Capture (REDCap) - A Metadata-Driven Methodology and Workflow Process for Providing Translational Research Informatics Support.” *Journal of Biomedical Informatics* 42 (2): 377–81. https://doi.org/10.1016/j.jbi.2008.08.010.

Hill, M. J., B. C. Morson, and H. J. R. Bussey. 1978. “Aetiology of Adenoma—Carcinoma Sequence in Large Bowel.” *The Lancet* 311 (8058): 245–47. https://doi.org/10.1016/S0140-6736(78)90487-7.

Jennens, R. R., G. G. Giles, and R. M. Fox. 2006. “Increasing Underrepresentation of Elderly Patients with Advanced Colorectal or Non-Small-Cell Lung Cancer in Chemotherapy Trials.” *Internal Medicine Journal* 36 (4): 216–20. https://doi.org/10.1111/j.1445-5994.2006.01033.x.

Jing, C., Y. H. Jin, Z. You, Q. Qiong, and Z. Jun. 2016. “Prognostic Value of Amphiregulin and Epiregulin MRNA Expression in Metastatic Colorectal Cancer Patients.” *Oncotarget* 7 (34): 55890–99. https://doi.org/10.18632/oncotarget.10151.

Kalata, P., P. Martus, H. Zettl, C. Rödel, W. Hohenberger, R. Raab, H. Becker, et al. 2009. “Differences between Clinical Trial Participants and Patients in a Population-Based Registry: The German Rectal Cancer Study vs. the Rostock Cancer Registry.”

Diseases of the Colon and Rectum 52 (3): 425–37. https://doi.org/10.1007/DCR.0b013e318197d13c.

Kannarkatt, J., J. Joseph, P. C. Kurniali, A. Al-Janadi, and B. Hrinczenko. 2017. "Adjuvant Chemotherapy for Stage II Colon Cancer: A Clinical Dilemma." *Journal of Oncology Practice* 13 (4): 233–41. https://doi.org/10.1200/jop.2016.017210.

Karapetis, C. S., S. Khambata-Ford, D. J. Jonker, C. J. O'Callaghan, D. Tu, N. C. Tebbutt, R. J. Simes, et al. 2008. "K-Ras Mutations and Benefit from Cetuximab in Advanced Colorectal Cancer." *New England Journal of Medicine* 359 (17): 1757–65. https://doi.org/10.1056/NEJMoa0804385.

Kennedy-Martin, T., S. Curtis, D. Faries, S. Robinson, and J. Johnston. 2015. "A Literature Review on the Representativeness of Randomized Controlled Trial Samples and Implications for the External Validity of Trial Results." *Trials* 16 (1): 495. https://doi.org/10.1186/s13063-015-1023-4.

Khambata-Ford, S., C. R. Garrett, N. J. Meropol, M. Basik, C. T. Harbison, S. Wu, T. W. Wong, et al. 2007. "Expression of Epiregulin and Amphiregulin and K-Ras Mutation Status Predict Disease Control in Metastatic Colorectal Cancer Patients Treated with Cetuximab." *Journal of Clinical Oncology* 25 (22): 3230–37. https://doi.org/10.1200/JCO.2006.10.5437.

Kosmider, S., I. Jones, M. Hibbert, A. Hunter, S. McLaughlin, J. Johns, M. Chapman, and P. Gibbs. 2008. "Towards Establishing a National Colorectal Cancer Database: Lessons Learnt from Bio21 Molecular Medicine Informatics Model." *ANZ Journal of Surgery* 78 (9): 803–9. https://doi.org/10.1111/j.1445-2197.2008.04655.x.

Lew, J. Bin, D. J. B. St John, X. M. Xu, M. J. E. Greuter, M. Caruana, D. R. Cenin, E. He, et al. 2017. "Long-Term Evaluation of Benefits, Harms, and Cost-Effectiveness of the National Bowel Cancer Screening Program in Australia: A Modelling Study."

The Lancet Public Health 2 (7): e331–40. https://doi.org/10.1016/S2468-2667(17)30105-6.

Magalhães, B., B. Peleteiro, and N. Lunet. 2012. "Dietary Patterns and Colorectal Cancer." *European Journal of Cancer Prevention* 21 (1): 15–23. https://doi.org/10.1097/CEJ.0b013e3283472241.

Majumdar, S. R., R. H. Fletcher, and A. T. Evans. 1999. "How Does Colorectal Cancer Present? Symptoms, Duration, and Clues to Location." *American Journal of Gastroenterology* 94 (10): 3039–45. https://doi.org/10.1111/j.15720241.1999.01454.x.

Mitchell, A. P., M. R. Harrison, M. S. Walker, D. J. George, A. P. Abernethy, and B. R. Hirsch. 2015. "Clinical Trial Participants With Metastatic Renal Cell Carcinoma Differ From Patients Treated in Real-World Practice." *Journal of Oncology Practice* 11 (6): 491–97. https://doi.org/10.1200/JOP.2015.004929.

Modest, D. P., S. Pant, and A. Sartore-Bianchi. 2019. "Treatment Sequencing in Metastatic Colorectal Cancer." *European Journal of Cancer* 109: 70–83. https://doi.org/10.1016/j.ejca.2018.12.019.

Murthy, V. H., H. M. Krumholz, and C. P. Gross. 2004. "Participation in Cancer Clinical Trials: Race-, Sex-, and Age-Based Disparities." *Journal of the American Medical Association* 291 (22): 2720–26. https://doi.org/10.1001/jama.291.22.2720.

Pentheroudakis, G., V. Kotoula, W. De Roock, G. Kouvatseas, P. Papakostas, T. Makatsoris, D. Papamichael, et al. 2013. "Biomarkers of Benefit from Cetuximab-Based Therapy in Metastatic Colorectal Cancer: Interaction of EGFR Ligand Expression with RAS/RAF, PIK3CA Genotypes." *BMC Cancer* 13 (1): 49. https://doi.org/10.1186/1471-2407-13-49.

Primrose, J. N. 2010. "Surgery for Colorectal Liver Metastases." *British Journal of*

Cancer 102 (9): 1313–18. https://doi.org/10.1038/sj.bjc.6605659.

Reinert, T., T. V. Henriksen, E. Christensen, S. Sharma, R. Salari, H. Sethi, M. Knudsen, et al. 2019. "Analysis of Plasma Cell-Free DNA by Ultradeep Sequencing in Patients With Stages I to III Colorectal Cancer." *JAMA Oncology* 5 (8): 1124. https://doi.org/10.1001/jamaoncol.2019.0528.

Rodriguez-Bigas, M., E. Lin, and C. Crane. 2003. "Stage IV Colorectal Cancer." In *Holland-Frei Cancer Medicine*, edited by DW Kufe, RE Pollock, RR Weichselbaum, RC Jr Bast, TS Gansler, JF Holland, and E III Frei, 6th ed. http://www.ncbi.nlm.nih.gov/books/NBK13267/.

Samad, A. K. A., R. S. Taylor, T. Marshall, and M. A. S. Chapman. 2005. "A Meta-Analysis of the Association of Physical Activity with Reduced Risk of Colorectal Cancer." *Colorectal Disease* 7 (3): 204–13. https://doi.org/10.1111/j.1463-1318.2005.00747.x.

Seligmann, J. F., F. Elliott, S. D. Richman, B. Jacobs, G. Hemmings, S. Brown, J. H. Barrett, S. Tejpar, P. Quirke, and M. T. Seymour. 2016. "Combined Epiregulin and Amphiregulin Expression Levels as a Predictive Biomarker for Panitumumab Therapy Benefit or Lack of Benefit in Patients With RAS Wild-Type Advanced Colorectal Cancer." *JAMA Oncology* 2 (5): 633. https://doi.org/10.1001/jamaoncol.2015.6065.

Stintzing, S., S. Tejpar, P. Gibbs, L. Thiebach, and H. J. Lenz. 2017. "Understanding the Role of Primary Tumour Localisation in Colorectal Cancer Treatment and Outcomes." *European Journal of Cancer* 84: 69–80. https://doi.org/10.1016/j.ejca.2017.07.016.

Therkildsen, C., T. K. Bergmann, T. Henrichsen-Schnack, S. Ladelund, and M. Nilbert. 2014. "The Predictive Value of KRAS, NRAS, BRAF, PIK3CA and PTEN for Anti-

EGFR Treatment in Metastatic Colorectal Cancer: A Systematic Review and Meta-Analysis." *Acta Oncologica* 53 (7): 852–64. https://doi.org/10.3109/0284186X.2014.895036.

Tie, J., J. D. Cohen, Y. Wang, M. Christie, K. Simons, M. Lee, R. Wong, et al. 2019. "Circulating Tumor DNA Analyses as Markers of Recurrence Risk and Benefit of Adjuvant Therapy for Stage III Colon Cancer." *JAMA Oncology* 5 (12): 1710–17. https://doi.org/10.1001/jamaoncol.2019.3616.

Tie, J., Y. Wang, C. Tomasetti, L. Li, S. Springer, I. Kinde, N. Silliman, et al. 2016. "Circulating Tumor DNA Analysis Detects Minimal Residual Disease and Predicts Recurrence in Patients with Stage II Colon Cancer." *Science Translational Medicine* 8 (346): 346ra92. https://doi.org/10.1126/scitranslmed.aaf6219.

Tomlinson, J. S., W. R. Jarnagin, R. P. DeMatteo, Y. Fong, P. Kornprat, M. Gonen, N. Kemeny, M. F. Brennan, L. H. Blumgart, and M. D'Angelica. 2007. "Actual 10-Year Survival after Resection of Colorectal Liver Metastases Defines Cure." *Journal of Clinical Oncology* 25 (29): 4575–80. https://doi.org/10.1200/JCO.2007.11.0833.

Unger, J. M., W. E. Barlow, D. P. Martin, S. D. Ramsey, M. LeBlanc, R. Etzioni, and D. L. Hershman. 2014. "Comparison of Survival Outcomes among Cancer Patients Treated in and out of Clinical Trials." *Journal of the National Cancer Institute* 106 (3). https://doi.org/10.1093/jnci/dju002.

Venook, A. P., D. Niedzwiecki, H. Lenz, F. Innocenti, B. Fruth, J. A. Meyerhardt, D. Schrag, et al. 2017. "Effect of First-Line Chemotherapy Combined With Cetuximab or Bevacizumab on Overall Survival in Patients With KRAS Wild-Type Advanced or Metastatic Colorectal Cancer: A Randomized Clinical Trial." *Journal of the American Medical Association* 317 (23): 2392–2401. https://doi.org/10.1001/jama.2017.7105.

Vogelstein, B., E. R. Fearon, S. R. Hamilton, S. E. Kern, A. C. Preisinger, M. Leppert, A. M. m. Smits, and J. L. Bos. 1988. “Genetic Alterations during Colorectal-Tumor Development.” *New England Journal of Medicine* 319 (9): 525–32. https://doi.org/10.1056/NEJM198809013190901.

Yamagishi, H., H. Kuroda, Y. Imai, and H. Hiraishi. 2016. “Molecular Pathogenesis of Sporadic Colorectal Cancers.” *Chinese Journal of Cancer* 35 (1): 4. https://doi.org/10.1186/s40880-015-0066-y.

Appendix

Registry-based randomized clinical trials as a method to improve cancer care in Australia

Foroughi, Siavash, Hui-li Wong, Lucy Gately, Margaret Lee, Koen Simons, Jeanne Tie, Antony Wilks Burgess, and Peter Gibbs. 2019. "Registry-based randomized clinical trials as a method to improve cancer care in Australia." *Asia-Pacific Journal of Clinical Oncology* 15 (3): 188–89. doi:10.1111/ajco.13122.

Published Letter to the Editor

Received: 15 November 2018 | Accepted: 7 December 2018 | Published on: 12 February 2019

DOI: 10.1111/ajco.13122

LETTER TO THE EDITOR

WILEY

Registry-based randomized clinical trials as a method to improve cancer care in Australia

We thank Tang et al[1] for endorsing our efforts to establish registry-based randomized clinical trials (RRCTs) to improve cancer care.[2] They raise some issues that are worthy of further discussion, especially, with respect to their summary Table where they describe differences between conventional randomized clinical trials (RCTs), RRCTs, and population-based observational research; this discussion also highlights some recent significant developments with our RRCT activity.

Regarding the "Strengths" listed in their summary Table, we believe it is important to note that the cost differential between conventional and RRCTs is substantial, simply stating that the costs of RRCTs are lower does not properly capture this differential. Conventional RCTs cost many thousands or tens of thousands of dollars per patient enrolled, versus as little as fifty dollars per patient for an RRCT.[3] The "Weaknesses" in their Table do not accurately capture the registry infrastructure that is supporting RRCTs. The registries being used, such as the treatment of recurrent and advanced colorectal cancer (TRACC) registry for metastatic colorectal cancer, established in 2009,[4] are long-standing registries so there is no need to establish or maintain a dedicated registry for the RRCT. There are many such resources that are already fully funded and capable of supporting a broad range of research activities. Using the current registry resources to capture treatment and outcome data for an RRCT adds further value to these existing activities.

We are collecting data in comprehensive disease-based registries such as capturing treatment and outcome data across multiple lines of therapy for specific disease types. These trials-oriented registries are distinctly different to traditional clinical quality registries that only capture limited datasets at the time of diagnosis. By using comprehensive disease registries, we believe there is no limit to the range of RRCT questions that can be explored effectively, whether they address treatment responses, adverse events, and/or survival outcomes. These registries may not have population-wide coverage in the strictest sense, but they do have a broad reach including private and public patients, and regional and metropolitan sites.

Like population-based observational research, our current projects are demonstrating that RRCTs can be conducted quickly and at low cost even with very large sample sizes. Long-term follow-up is also feasible, as the disease registries capture reliable data through death. We argue that the reliability and depth of data captured in administrative databases will always be inferior to a dedicated disease registry, at least in part due to lack of context for the administrative databases. Of course, it is possible to tap into these administrative data sources to obtain or validate data for the disease registries. Indeed, we have and will continue to link with multiple other datasets within and outside the hospital to maintain data accuracy and completeness.[5]

As acknowledged by Tang et al, there are many known and unknown factors that confound treatment decision making and patient outcomes. In the absence of randomization, any comparison of outcomes between different treatment groups is likely to be hypothesis generating only. RRCTs are an attempt to bridge the gap between conventional and observational trials, and while it may be difficult to match the quality of data captured in a conventional trial this is the reason for having a hard outcome, such as overall survival, as the primary endpoint of RRCTs.

Multiple RRCTs are now open and recruiting patients in Australia, further trials are in development across five tumor streams and extending beyond medical oncology. Significant progress has been made in engaging with several co-operative groups, such as the EXTEM study, a randomized study exploring the duration of temozolomide therapy for glioblastoma patients, has now been endorsed by Co-operative Trials Group for Neuro-Oncology (COGNO) with 15 centers across Australia. Discussions have been initiated with several other co-operative groups regarding their endorsement of the RRCT model.

ACKNOWLEDGMENTS

S.F. is supported by an Australian Government Research Training Program Scholarship.

ORCID

Siavash Foroughi https://orcid.org/0000-0001-6916-8424

Siavash Foroughi[1,2]
Hui-li Wong[1,2,3]
Lucy Gately[1,2]
Margaret Lee[1,2,4,5]
Koen Simons[6,7]
Jeanne Tie[1,2,3,5]
Antony Wilks Burgess[2,8,9]
Peter Gibbs[1,2,5]

[1]*Systems Biology and Personalised Medicine Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia*
[2]*Department of Medical Biology, The University of Melbourne, Parkville, Victoria, Australia*
[3]*Department of Medical Oncology, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia*

This article is accompanied by a Letter to the Editor by Tang et al., *Asia-Pac J Clin Oncol.* 2019;15:186–187. https://doi.org/10.1111/ajco.13121

  wileyonlinelibrary.com/journal/ajco *Asia-Pac J Clin Oncol.* 2019;15:188–189.

[4]*Department of Medical Oncology, Eastern Health, Box Hill, Victoria, Australia*
[5]*Department of Medical Oncology, Western Health, St Albans, Victoria, Australia*
[6]*Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Parkville, Victoria, Australia*
[7]*Western Centre for Health, Research and Education, Western Health, St Albans, Victoria, Australia*
[8]*Department of Surgery, Royal Melbourne Hospital, The University of Melbourne, Parkville, Victoria, Australia*
[9]*Structural Biology Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia*

Correspondence
Siavash Foroughi, Systems Biology and Personalised Medicine Division, The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria 3052, Australia.
Email: foroughi@wehi.edu.au

REFERENCES

1. Tang M, Schaffer A, Pearson S-A. Embracing the full spectrum of real-world data for cancer medicines research in Australia. *Asia-Pac J Clin Oncol*. 2019;15:186–187.
2. Foroughi S, Wong H, Gately L, et al. Re-inventing the randomized controlled trial in medical oncology: the registry-based trial. *Asia-Pac J Clin Oncol*. 2018:14:365–373.
3. Wachtell K, Lagerqvist B, Olivecrona GK, James SK, Fröbert O. Novel trial designs: lessons learned from thrombus aspiration during ST-segment elevation myocardial infarction in scandinavia (TASTE) trial. *Curr Cardiol Rep*. 2016;18:11.
4. Field K, Wong H-L, Shapiro J, et al. Developing a national database for metastatic colorectal cancer management: perspectives and challenges. *Intern Med J*. 2013;43:1224–1231.
5. Field K, Kosmider S, Johns J, et al. Linking data from hospital and cancer registry databases: should this be standard practice. *Intern Med J*. 2010;40:566–573.