<table>
<thead>
<tr>
<th>TITLE:</th>
<th>A NON-INTERVENTIONAL MULTI-COUNTRY STUDY TO EVALUATE THE REAL-WORLD EFFECTIVENES OF AVASTIN (BEVACIZUMAB) FOR FIRST-LINE TREATMENT OF PATIENTS WITH METASTATIC COLORECTAL CANCER AND KNOWN KRAS STATUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROTOCOL NUMBER:</td>
<td>MO30177</td>
</tr>
<tr>
<td>VERSION NUMBER:</td>
<td>1.0</td>
</tr>
<tr>
<td>STUDIED MEDICINAL PRODUCT:</td>
<td>Bevacizumab / Avastin®</td>
</tr>
<tr>
<td>PRODUCT REFERENCE NUMBER:</td>
<td>RO4876646</td>
</tr>
<tr>
<td>DATE FINAL:</td>
<td>See electronic date stamp below</td>
</tr>
</tbody>
</table>

FINAL PROTOCOL APPROVAL

[This space is reserved for the electronic signature]  
Company Signatory  
17-May-2016 13:48:10

CONFIDENTIAL

This non-interventional study is managed by F. Hoffmann-La Roche Ltd of Basel, Switzerland. However, it may be implemented in individual countries by Roche’s local affiliates, including Genentech, Inc. in the United States. The information contained in this document, especially any unpublished data, is the property of F. Hoffmann-La Roche Ltd (or under its control) and therefore is provided to you in confidence. It is understood that this information will not be disclosed to others without written authorization from Roche except to the extent necessary.

Avastin ® — F. Hoffmann-La Roche Ltd
Protocol MO30177, Version 1.0

Based on protocol template Version 1.0 released on 10 Jan 2016
| **MARKETING AUTHORIZATION HOLDER(S) (MAH) or STUDY INITIATOR:** | Roche Registration Ltd  
6 Falcon Way  
Shire Park  
Welwyn Garden City AL7 1TW  
United Kingdom |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RESEARCH QUESTION AND OBJECTIVES:</strong></td>
<td>This study seeks to investigate overall survival in bevacizumab treated patients with mCRC, stratified by KRAS testing status. Primary outcome is a comparison of overall survival between patients with mCRC treated with bevacizumab containing regimens and patients treated with chemotherapy alone.</td>
</tr>
<tr>
<td><strong>COUNTRIES OF STUDY POPULATION:</strong></td>
<td>Australia, Denmark, Germany, United States of America</td>
</tr>
</tbody>
</table>
| **AUTHOR:** | PDB RWD-S  
Hoffman-La Roche  
Bldg 663  
Hochstrasse 16  
CH-4051 Basel, Switzerland |
TABLE OF CONTENTS

1. LIST OF ABBREVIATIONS ......................................................................................... 5
2. RESEARCH TEAM .................................................................................................. 6
3. SYNOPSIS ............................................................................................................... 7
4. AMENDMENTS AND UPDATES ............................................................................ 15
5. MILESTONES ......................................................................................................... 16
6. RATIONALE AND BACKGROUND ......................................................................... 16
7. RESEARCH QUESTION AND OBJECTIVES .......................................................... 17
8. RESEARCH METHODS ........................................................................................... 18
  8.1 Study Design ....................................................................................................... 18
  8.2 Setting .................................................................................................................. 19
  8.3 Variables .............................................................................................................. 21
  8.4 Data Sources ........................................................................................................ 22
  8.5 Study Size ............................................................................................................ 23
  8.6 Data Management ............................................................................................... 24
  8.7 Data Analysis ....................................................................................................... 24
9. STUDY DOCUMENTATION AND ADMINISTRATION ........................................... 25
10. LIMITATIONS OF THE RESEARCH METHOD ...................................................... 26
11. PROTECTION OF HUMAN PATIENTS ................................................................... 26
  11.1 Informed Consent ............................................................................................... 26
  11.2 Compliance with Laws and Regulations ............................................................ 26
  11.3 Institutional Review Board or Ethics Committee ................................................. 27
12. MANAGEMENT AND REPORTING OF ADVERSE EVENTS .................................. 27
  12.1 Adverse Events .................................................................................................. 27
  12.2 Serious Adverse Events ...................................................................................... 28
13. PLANS FOR DISSEMINATION AND COMMUNICATION OF STUDY RESULTS .................................................................................................................. 28
14. REFERENCES .......................................................................................................... 29
LIST OF APPENDICES

No table of figures entries found.
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 FU</td>
<td>Fluorouracil</td>
</tr>
<tr>
<td>CAPEOX</td>
<td>Chemotherapy regimen: Capecitabine, Folinic acid, Fluorouracil,</td>
</tr>
<tr>
<td>CRC</td>
<td>Colorectal Cancer</td>
</tr>
<tr>
<td>EC</td>
<td>Ethics Committee</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epidermal Growth Factor Receptor</td>
</tr>
<tr>
<td>EMR</td>
<td>Electronic Medical Records</td>
</tr>
<tr>
<td>ENCePP</td>
<td>European Network of Centers for Pharmacoepidemiology and Pharmacovigilance</td>
</tr>
<tr>
<td>ESMO</td>
<td>European Society for Medical Oncology</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>FOLFOX</td>
<td>Chemotherapy regimen: Folinic acid, Fluorouracil, Oxaliplatin</td>
</tr>
<tr>
<td>FOLFIRI</td>
<td>Chemotherapy regimen: Folinic acid, Fluorouracil, Irinotecan</td>
</tr>
<tr>
<td>GPP</td>
<td>Good Pharmacoepidemiological Practice</td>
</tr>
<tr>
<td>ICH</td>
<td>International Conference on Harmonisation</td>
</tr>
<tr>
<td>IRB</td>
<td>Institutional Review Board</td>
</tr>
<tr>
<td>ISPE</td>
<td>International Society of Pharmacoepidemiology</td>
</tr>
<tr>
<td>ISPOR</td>
<td>International Society For Pharmaco economics and Outcomes Research</td>
</tr>
<tr>
<td>KRAS</td>
<td>Kirsten Rat Sarcoma Viral Oncogene Homolog</td>
</tr>
<tr>
<td>mCRC</td>
<td>Metastatic Colorectal Cancer</td>
</tr>
<tr>
<td>NIS</td>
<td>Non-Interventional Study</td>
</tr>
<tr>
<td>NIS RTL</td>
<td>Non-Interventional Study Responsible Team Lead</td>
</tr>
<tr>
<td>NRAS</td>
<td>Neuroblastoma Rat Sarcoma Viral Oncogene</td>
</tr>
<tr>
<td>OS</td>
<td>Overall Survival</td>
</tr>
<tr>
<td>RAS</td>
<td>Rat Sarcoma Oncogene</td>
</tr>
<tr>
<td>RWD</td>
<td>Real World Data</td>
</tr>
<tr>
<td>TKK</td>
<td>Tumourregister Kolorektales Karzinom</td>
</tr>
<tr>
<td>TRACC</td>
<td>Treatment of Recurrent and Advanced Colorectal Cancer registry</td>
</tr>
</tbody>
</table>
2. **RESEARCH TEAM**

**Scientific Responsible**

Group International Medical Director  
Hoffman-La Roche  
Grenzacherstrasse 124  
CH-4070 Basel, Switzerland

**NIS Data Science Responsible**

Real World Data Scientist  
PDB RWD-S  
Hoffman-La Roche  
Grenzacherstrasse 124  
CH-4070 Basel, Switzerland

Complementary information is given in Appendix 1
3. SYNOPSIS

TITLE: A NON-INTERVENTIONAL MULTI-COUNTRY STUDY TO EVALUATE THE REAL-WORLD EFFECTIVENESS OF AVASTIN (BEVACIZUMAB) FOR FIRST-LINE TREATMENT OF PATIENTS WITH METASTATIC COLORECTAL CANCER AND KNOWN KRAS STATUS

PROTOCOL NUMBER: MO30177

VERSION NUMBER: 1.0

DATE OF SYNOPSIS: 24 February 2016

STUDIED MEDICINAL PRODUCT: Bevacizumab / Avastin®

MAIN AUTHOR Real World Data Science, Hoffman-La Roche, Basel.

INDICATION: Metastatic Colorectal Cancer (mCRC)

MARKETING AUTHORIZATION HOLDER or STUDY INITIATOR: Roche Registration Ltd
6 Falcon Way
Shire Park
Welwyn Garden City AL7 1TW
United Kingdom

Rationale and background

Incidence: A very common cancer affecting men and women worldwide

Colorectal cancer (CRC) is the third most common cancer in men (746,000 cases, 10.0% of the total) and the second most common cancer in women (614,000 cases, 9.2% of the total) worldwide (Ferlay 2014). There were 447,000 new cases of CRC in Europe in 2012, and despite improved survival over the last two decades, CRC was responsible for 215,000 deaths (Ferlay 2013). The American Cancer Society estimates that approximately 136,830 people in the USA were diagnosed with CRC in 2014, with approximately 50,310 people dying of the disease during that calendar year (American Cancer Society 2014).

Available options for patients with colorectal cancers

**Treatment guidance for patients with KRAS mutations**

Key tumour markers found in CRC are the RAS oncogenes, including KRAS and NRAS. KRAS is an oncogene in the downstream pathway of EGFR signaling, which when activated leads to increased cell proliferation, angiogenesis, migration, and cancer cell survival (Monson 2009). Several studies have shown that patients with a KRAS mutation do not benefit from anti-EGFR (cetuximab and panitumumab) treatment (Allegra 2015). Most recently, studies have suggested that patients with an NRAS mutation also do not benefit from these treatments (Douillard 2013, Van Cutsem 2015). Approximately 60% of patients with CRC have the wild-type KRAS gene (Monson 2009, Soulieres 2010, Benson 2007). KRAS-mutant patients may be treated with standard chemotherapy backbones, including monotherapy with 5FU or capecitabine, oxaliplatin-based regimens (FOLFOX, CAPEOX), irinotecan-based regimens (FOLFIRI), or other combination regimens (FOLFOXIRI). For patients who have good tolerance and who are likely to have a high tumour response rate, a range of chemotherapy regimens is advised. Bevacizumab is recommended for use with any of them, regardless of KRAS status (Van Cutsem 2014, ESMO consensus 2015).

**Scientific Gap**

No randomized studies specifically have compared bevacizumab
treatment combined with chemotherapy with chemotherapy alone in KRAS-mutant patients. There is evidence from subgroup analyses of randomized controlled trials that bevacizumab has a benefit compared to chemotherapy alone in patients with a KRAS mutation—in terms of progression-free survival and response rates. However, small sample sizes have limited the evaluation of overall survival (OS) in this patient population. (Hurwitz 2013, Kubicka 2013) Because pivotal studies of bevacizumab were completed before RAS testing was introduced into routine clinical practice, available RAS testing from these studies is limited. The recent ESMO consensus for first-line treatment includes patient fitness in addition to RAS status. Although RAS status is not the only criterion for selecting a first-line treatment with biologics, available treatments are limited to RAS wild-type patients. A better understanding of optimal treatment for RAS-mutant patients is important because of these patients’ limited treatment options.

Evidence from a large registry study (CORECT) showed a bevacizumab benefit for overall survival both for KRAS-mutant and wild-type patients. However, the lack of a control arm limited comparison with chemotherapy alone (Bencsikova 2015).

This study aims meta-analyze data from four cohorts on overall survival of patients with mCRC treated in first-line with bevacizumab containing regimens and comparators, stratified by KRAS status.

Research question and objectives

Primary Study Objective

The primary objective is to evaluate overall survival (from start of first-line treatment) for patients with metastatic colorectal cancer and a documented KRAS mutation who received bevacizumab-containing treatment or chemotherapy alone in routine clinical practice.
Secondary Study Objective
The secondary objective is to evaluate overall survival (from start of first-line treatment) for patients with metastatic colorectal cancer and documented KRAS wild-type status receiving bevacizumab-containing treatment or anti-EGFR (cetuximab and panitumumab) treatment in routine clinical practice.

Exploratory Objectives
The feasibility of analysing the following exploratory endpoints will be evaluated for each country and by treatment group, if available:

- Number and percentage of patients with a KRAS mutation
- Number and percentage of patients receiving second-line or later treatment
- Overall survival by resection of primary tumour (Yes/No)
- Overall survival by tumour location (Right vs. Left)
- Overall survival by secondary tumour resection rate
- Time from start of first-line therapy to start of second-line therapy

Study design
This is a non-interventional study (NIS) using aggregate secondary data from existing cohorts in the US, Germany, Australia, and Denmark. Summary data obtained through existing Roche collaborations and studies will be synthesized to meet study objectives.

Population
Study Eligibility Criteria

- Diagnosis of mCRC, first-line treatment with a bevacizumab-containing regimen or with chemotherapy alone, and KRAS-mutant status.

- Diagnosis of mCRC, first-line treatment with a bevacizumab-containing regimen or an anti-EGFR-containing regimen, and KRAS wild-type status.

Variables

- Overall survival (defined from start of first-line therapy to death from any cause, or to last date known that patient is still alive)
- Number and percentage of patients with KRAS mutations
Data sources

- Number and percentage of patients receiving second-line or later therapy

The data utilised in the analyses outlined in this protocol have already been collected for other research purposes. Therefore this study is classified as a "secondary data use NIS". Only aggregate data will be sourced from these data sources for the purpose of meta-analysis.

Summaries of demographic information, disease characteristics, treatment information, KRAS status, and survival outcomes will be provided by the data sources in custom table shells. Individual patient level information from these sources will not be made available and patient-level analyses informing the aggregate data reports supplied to Roche will be conducted by the data providers.

Following a feasibility assessment of potential cohorts, four cohorts were identified that met study eligibility criteria, with a sufficient number of patients to allow inter-cohort comparisons and pooling of study results. Each cohort is described below.

**United States - Vector Oncology Protocol Sponsored by US (Genentech) Roche Pharma Medical Affairs**

The Vector Oncology studies (Genentech/Vector Oncology protocols HPFSCRC1503 and HTPMC1411) are non-interventional studies, which employed existing data collected as part of routine clinical care in community oncology practices in the United States. This data has been aggregated by Vector Oncology into the Vector Oncology Data Warehouse or the Vector Oncology Electronic Medical Record partner. No data was collected prospectively and no patients were contacted as part of these studies. Electronic medical records and billing systems were used to identify eligible patients and to abstract patient demographic characteristics, KRAS status, treatment and outcomes.

**Germany - TKK Registry Study Supported by German Roche Pharma Affiliate**

The Tumourregister Kolorektales Karzinom (TKK) registry is a
large, ongoing, prospective, national registry run by a multicenter network of 269 currently practicing office-based medical oncologists in Germany. Details of this registry have been described elsewhere (Marschner 2015). Our planned study will use aggregate data already collected as part of this registry. No prospective data will be collected and no patients will be contacted.

**Australia - TRACC Registry Supported by Roche Pharma Australia**

The Treatment of Recurrent and Advanced Colorectal Cancer (TRACC) registry is an ongoing, multi-centre, prospective, non-interventional, cohort study that started in 2009. Details regarding the primary colorectal cancer, any adjuvant or neoadjuvant therapy, and outcomes are recorded using a BioGrid technology which de-identifies and aggregates data collected from participating sites across Australia. Details of this registry have been described elsewhere (Field 2015, Field 2013). Our study will make use of aggregate data already collected as part of this study. No prospective data will be collected and no patients will be contacted.

**Denmark – Roche Diagnostic Sponsored Study and Aarhus University Hospital, Department of Clinical Epidemiology**

The study ‘Impact of molecular profiling for metastatic colorectal cancer and clinical and economic outcomes’ conducted in collaboration with Aarhus University Hospital, Department of Clinical Epidemiology and Roche Diagnostics is a non-interventional study using routine health care data from linked registries in Denmark to examine treatment patterns and outcomes in relation to clinical characteristics and KRAS mutation testing status among Danish patients diagnosed with mCRC during 2009-2013. We will use aggregate data already collected as part of this study. No prospective data will be collected and no patients will be contacted.

**Study size**

Estimates of median overall survival (OS) in the target patient population with KRAS mutations range from approximately 20-25
months for patients receiving first-line bevacizumab-containing treatment and 14-19 months for patients receiving chemotherapy alone as first-line therapy (Hurwitz 2004, Loupakis 2014, Saltz 2008, Venook 2014, Heinemann 2014, Van Cutsem 2011, Douillard 2013, Kozloff 2009, Bendell 2012). Assuming an increase in median OS from 18.5 to 22 months for patients receiving bevacizumab-containing treatment (HR =0.84), approximately 1000 deaths will be required for 80% power and a 2-sided test at 0.05. If we assume a smaller treatment effect, given the greater heterogeneity of patients treated in routine clinical practice, an increase in OS from 18.5 to 21 months (HR=.88) requires approximately 1900 deaths to achieve the same power and type I error rate.

**Data Analysis**

This study will conduct meta-analyses of aggregate survival estimates from the four data sources outlined above. The following variables and analyses will be required from the summary reports included in the meta-analysis:

- Patient disposition, including number of patients enrolled, the number of patients receiving first-line treatment, and the number of patients with available KRAS status.

- Demographic and baseline disease characteristics including – where available - sex, age, Charlson Comorbidity Index score, and index year at start of first-line treatment. Depending on the level of information recorded, additional disease characteristics will be summarized, including any comorbidity, ECOG performance status, site of primary tumour, tumour stage, synchronous metastasis, resection of primary tumour, number of metastatic sites, location of metastatic sites, prior (neo) adjuvant treatment, time between initial diagnosis and start of first-line treatment, secondary resection, and number of later lines of treatment.

- Duration of follow up.

- Assessment of baseline and disease characteristics among treatment groups.
Overall survival for the primary endpoint will be defined as the duration of time from the date of the start of first-line treatment to the date of death from any cause. Subjects who are not known to have died will be censored at the date of last follow-up. All quartiles (including median) for OS will be presented together with a two-sided 95% confidence interval.

In order to be included in the meta-analyses, cox regression models adjusting for differences in patient characteristics between treatment groups will have to have been included in the summary data reports from the four data sources. The meta-analyses of aggregate data will be conducted by Hoffman-La Roche, Basel.

Summary statistics including demographic information, disease characteristics, treatment information, KRAS status, and survival outcomes, will be presented by data source.

Heterogeneity between the different data samples will be assessed by calculating Cochran’s Q and $I^2$ statistics. Hazard ratios and 95% confidence intervals will be combined using the inverse variance method in a random-effects model.

**Milestones**
4. AMENDMENTS AND UPDATES

Protocol amendments/updates so far: none.

<table>
<thead>
<tr>
<th>Amendment/Update Number</th>
<th>Date</th>
<th>Section of Study Protocol</th>
<th>Amendment or Update</th>
<th>Reason</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>{Date}</td>
<td>{Section no.}</td>
<td>{Short description}</td>
<td>{Reason}</td>
</tr>
<tr>
<td>2</td>
<td>{Date}</td>
<td>{Section no.}</td>
<td>{Short description}</td>
<td>{Reason}</td>
</tr>
<tr>
<td>{Number}</td>
<td>{Date}</td>
<td>{Section no.}</td>
<td>{Short description}</td>
<td>{Reason}</td>
</tr>
</tbody>
</table>
5. MILESTONES

<table>
<thead>
<tr>
<th>Milestone</th>
<th>Planned Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start of dataset creation</td>
<td>29 April 2016</td>
</tr>
<tr>
<td>End of dataset creation</td>
<td>29 April 2016</td>
</tr>
<tr>
<td>Final report of study results (CSR) and/or publication submission</td>
<td>29 March 2017</td>
</tr>
</tbody>
</table>

6. RATIONALE AND BACKGROUND

Incidence: A very common cancer affecting men and women worldwide
Colorectal cancer (CRC) is the third most common cancer in men (746,000 cases, 10.0% of the total) and the second most common cancer in women (614,000 cases, 9.2% of the total) worldwide (Ferlay 2014). There were 447,000 new cases of CRC in Europe in 2012, and despite improved survival over the last two decades, CRC was responsible for 215,000 deaths (Ferlay 2013). The American Cancer Society estimates that approximately 136,830 people in the USA were diagnosed with CRC in 2014, with approximately 50,310 people dying of the disease during that calendar year (American Cancer Society 2014).

Available options for patients with colorectal cancers

Treatment guidance for patients with KRAS mutations
Key tumour markers found in CRC are the RAS oncogenes, including KRAS and NRAS. KRAS is an oncogene in the downstream pathway of EGFR signaling, which when activated leads to increased cell proliferation, angiogenesis, migration, and cancer cell survival (Monson 2009). Several studies have shown that patients with a KRAS mutation do not benefit from anti-EGFR (cetuximab and panitumumab) treatment (Allegra 2015). Most recently, studies have suggested that patients with an NRAS mutation also do not benefit from these treatments (Douillard 2013, Van Cutsem 2015). Approximately 60% of patients with CRC have the wild-type KRAS gene (Monson 2009, Soulieres 2010, Benson 2007). KRAS-mutant patients may be treated with standard chemotherapy.
backbones, including monotherapy with 5FU or capecitabine, oxaliplatin-based regimens (FOLFOX, CAPEOX), irinotecan-based regimens (FOLFIRI), or other combination regimens (FOLFOXIRI). For patients who have good tolerance and who are likely to have a high tumour response rate, a range of chemotherapy regimens is advised. Bevacizumab is recommended for use with any of them, regardless of KRAS status (Van Cutsem 2014, ESMO consensus 2015).

Scientific Gap
No randomized studies have specifically compared bevacizumab treatment combined with chemotherapy with chemotherapy alone in KRAS-mutant patients. There is evidence from subgroup analyses of randomized controlled trials that bevacizumab has a benefit compared to chemotherapy alone in patients with a KRAS mutation — in terms of progression-free survival and response rates. However, small sample sizes have limited the evaluation of overall survival (OS) in this patient population. (Hurwitz 2013, Kubicka 2013) Because pivotal studies of bevacizumab were completed before RAS testing was introduced into routine clinical practice, available RAS testing from these studies is limited. The recent ESMO consensus for first-line treatment includes patient fitness in addition to RAS status. Although RAS status is not the only criterion for selecting a first-line treatment with biologics, available treatments are limited to RAS wild-type patients. A better understanding of optimal treatment for RAS-mutant patients is important because of these patients’ limited treatment options.

Evidence from a large registry study (CORECT) showed a bevacizumab benefit for overall survival both for KRAS-mutant and wild-type patients. However, the lack of a control arm limited comparison with chemotherapy alone (Bencsikova 2015).

This study aims to meta-analyze aggregate OS data from four cohorts of patients with mCRC treated in first-line with bevacizumab containing regimens and comparators, stratified by KRAS status.

7. **RESEARCH QUESTION AND OBJECTIVES**

This NIS intends to meta-analyze OS outcomes among patients with mCRC with available KRAS testing status, who received first-line treatment in routine clinical practice. The analyses aim to provide evidence comparing bevacizumab in combination with chemotherapy and chemotherapy alone among KRAS-mutant patients, among whom no randomized studies have been conducted. Our study will supplement results from randomized clinical trials comparing bevacizumab and treatments containing anti-EGFRs among KRAS wild-type patients.

This study will focus on KRAS rather than RAS mutation status since the number of patients who have been tested for their RAS-mutation status to date is insufficient for the purposes of this study, and historical data on KRAS status is superior. Nevertheless, if both KRAS and RAS status are available, the study will consider all RAS status. If
available the type of assay applied to determine (K)RAS status will be collected. To ensure a more homogenous patient population, the study will be limited to patients receiving bevacizumab or comparators in first-line therapy.

**Primary Study Objective**
The study’s primary objective is to evaluate overall survival (from start of first-line treatment) for patients with metastatic colorectal cancer and a documented KRAS mutation who received bevacizumab-containing treatment or chemotherapy alone in routine clinical practice.

**Secondary Study Objective**
The study's secondary objective is to evaluate overall survival (from start of first-line treatment) for patients with metastatic colorectal cancer and documented KRAS wild-type status receiving bevacizumab-containing treatment or anti-EGFR (cetuximab and panitumumab) treatment in routine clinical practice.

**Exploratory Objectives**
The feasibility of analysing the following exploratory endpoints will be evaluated for each country and by treatment group, if available:

- Number and percentage of patients with a KRAS mutation
- Number and percentage of patients receiving second-line or later treatment
- Overall survival by resection of primary tumour (Yes/No)
- Overall survival by tumour location (Right vs. Left)
- Overall survival by secondary tumour resection rate
- Time from start of first-line therapy to start of second-line therapy

---

**8. RESEARCH METHODS**

**8.1 STUDY DESIGN**
This is a non-interventional study (NIS) leveraging secondary data from existing cohorts in the US, Germany, Australia, and Denmark.

Due to the lack of single accessible data sources with sufficient eligible patients to investigate OS in mCRC patients treated with bevacizumab containing treatment regimens and comparators in first-line by KRAS status, the decision to meta-analyze aggregate results from four data sources was made. This will allow creating an aggregate sample size large enough to detect a clinically meaningful difference in OS.

Aggregate data summaries of demographic information, disease characteristics, treatment information, KRAS status, and survival outcomes will be provided by the data sources in custom table shells. Aggregate results from individual cohorts will be synthesized to meet study objectives. Individual patient-level data from the four countries
will not be collected by Hoffman-La Roche; individual patient-level analysis will have been or will be conducted by the data providers.

The analyses will be conducted in three stages:

- **First analysis - primary endpoint:** OS of mCRC diagnosed patients with KRAS mutant status treated in first-line with a bevacizumab containing regimen compared to chemotherapy alone.
- **Second analysis – secondary endpoint:** OS of mCRC diagnosed patients with KRAS wild-type status treated in first-line with a bevacizumab containing regimen compared to an anti-EGFR containing regimen.
- **Feasibility assessment of exploratory endpoints**

### 8.2 SETTING

This study is a secondary data use NIS meta-analyzing previously collected aggregate data from existing Roche Group analysis projects and collaborations. Individual patient level information from these sources will not be made available and patient-level analyses informing the aggregate data reports supplied to Roche will be conducted by the data providers.

Feasibility analyses identified four data sources in the following countries: Australia, Denmark, Germany, and the United States of America. The data from Denmark and the US are based on electronic medical records, whereas data from Australia and Germany are collected as part of ongoing prospective disease registries.

As the data were collected by different registries and by separate analysis projects, the inclusion criteria may differ slightly between the four data sources included in this study.

In order to maximise follow-up time, no enrolment date restrictions other than the cut-off points of availability of data and availability of KRAS testing status are employed. The following minimum criteria had to be satisfied for the data to be included in the study:

- Patients had to have been diagnosed with mCRC
- Patients had to have been treated in first line with a bevacizumab or cetuximab containing treatment regimens or chemotherapy alone.
- Patients have to have available data on OS, (K)RAS testing status, and right/left tumour location status.

Data source specific inclusion criteria are as follows:
United States - Vector Oncology Protocol Sponsored by US (Genentech) Roche Pharma Medical Affairs
Patients selected for inclusion will be patients represented in the Vector Oncology Data Warehouse who meet the following criteria:

- mCRC diagnosed at any point prior to March 2014
- Documentation of KRAS testing status
- Patients treated in first line with chemotherapy alone or chemotherapy with bevacizumab.
- Age ≥ 18 at metastatic diagnosis.

Germany - TKK Registry Study Supported by German Roche Pharma Affiliate
Patients were extracted from the TKK registry based on the following criteria:

- mCRC diagnosis between September 2006 and March 2015
- Documentation of KRAS testing status
- Patients treated in first line with chemotherapy alone or chemotherapy with bevacizumab or cetuximab

Australia - TRACC Registry Supported by Roche Pharma Australia

- mCRC diagnosis between September 2009 and December 2014
- Documentation of KRAS testing status
- Patients treated in first line with chemotherapy alone or chemotherapy with bevacizumab or cetuximab

Denmark – Roche Diagnostic Sponsored Study and Aarhus University Hospital, Department of Clinical Epidemiology
Patients with the following characteristics were included in the analysis:

- Primary or recurrent mCRC diagnosed between 2009 and 2013
- Documentation of KRAS status
- Patients treated in first line with chemotherapy alone or chemotherapy with bevacizumab or cetuximab
- Age ≥ 18 at metastatic diagnosis
8.3 VARIABLES

In order to align and meta-analyze the data from the four data sources, the variables outlined in table 1 are sought to be included in the aggregate data reports, where available:

Table 1. Variables to be included in aggregate data reports, where available

<table>
<thead>
<tr>
<th>Variable</th>
<th>Levels</th>
<th>Time of assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Female, male</td>
<td>Primary diagnosis</td>
</tr>
<tr>
<td>Age</td>
<td>Continuous</td>
<td>Start first-line treatment</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>Continuous</td>
<td>Primary diagnosis</td>
</tr>
<tr>
<td>Charlson comorbidity index</td>
<td>Continuous</td>
<td>Start first-line treatment</td>
</tr>
<tr>
<td>ECOG score</td>
<td>0, 1, 2, 3, 4, 5</td>
<td>Primary diagnosis</td>
</tr>
<tr>
<td>Any comorbidity</td>
<td>Yes, no</td>
<td>Start first-line treatment</td>
</tr>
<tr>
<td>Site of primary tumour</td>
<td>Colon, rectum</td>
<td>Primary diagnosis</td>
</tr>
<tr>
<td>Tumour stage</td>
<td>I, II, III, IV, TNM staging</td>
<td>Primary diagnosis</td>
</tr>
<tr>
<td>Resection of primary tumour</td>
<td>Yes, no</td>
<td>Primary diagnosis</td>
</tr>
<tr>
<td>Outcome of resection</td>
<td>R0, R1, R2, Rx</td>
<td>Primary diagnosis</td>
</tr>
<tr>
<td>Synchronous metastasis</td>
<td>Yes, no</td>
<td>Primary diagnosis</td>
</tr>
<tr>
<td>Number of metastatic sites</td>
<td>Continuous</td>
<td>Primary diagnosis</td>
</tr>
<tr>
<td>Location of metastatic sites</td>
<td>Liver, lung, peritoneum, other</td>
<td>Start first-line treatment</td>
</tr>
<tr>
<td>Prior (neo)adjuvant treatment</td>
<td>Yes, no</td>
<td>Start first-line treatment</td>
</tr>
<tr>
<td>BRAF mutation status</td>
<td>Yes, no</td>
<td>Primary diagnosis</td>
</tr>
<tr>
<td>Primary tumour site</td>
<td>Right, left</td>
<td>Primary diagnosis</td>
</tr>
<tr>
<td>Time between initial diagnosis</td>
<td>Continuous</td>
<td>Start first-line treatment</td>
</tr>
<tr>
<td>and start of palliative first-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>line treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment intent</td>
<td>Palliative, resectable, potentially</td>
<td>Primary diagnosis</td>
</tr>
<tr>
<td>Metastasectomy</td>
<td>Yes, no</td>
<td>Duration of follow-up</td>
</tr>
<tr>
<td>First-line treatment (type)</td>
<td>e.g. Chemotherapy alone, EGFR antibody</td>
<td>In first-line treatment</td>
</tr>
<tr>
<td>Index year start first-line</td>
<td>E.g. 2006, 2007</td>
<td>Start first-line treatment</td>
</tr>
<tr>
<td>treatment (type)</td>
<td></td>
<td>In second-line or any later line of</td>
</tr>
<tr>
<td>Index year start first-line</td>
<td></td>
<td>treatment</td>
</tr>
<tr>
<td>treatment (type)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vital status at last follow-up</td>
<td>Vital status – alive or dead, date of</td>
<td></td>
</tr>
<tr>
<td>Date of Death or date of last</td>
<td>cause of death (cancer vs other)</td>
<td></td>
</tr>
<tr>
<td>follow-up (reverse Kaplan-Meier</td>
<td></td>
<td></td>
</tr>
<tr>
<td>treatment until last contact (if</td>
<td>Death (censored event)</td>
<td></td>
</tr>
<tr>
<td>alive patient)</td>
<td>Date of Death or date of last contact</td>
<td></td>
</tr>
</tbody>
</table>
8.4 DATA SOURCES
As previously introduced, this study will leverage existing aggregate data from four independent data sources - two medical records data bases, based in the USA and Denmark, and two prospective registries, based in Australia and Germany. Only aggregate data will be requested from these data sources for the purpose of meta-analysis.

The data sources leveraged for the analyses outlined in this protocol are described below:

United States - Vector Oncology Protocol Sponsored by US (Genentech) Roche Pharma Medical Affairs
The Vector Oncology studies (Genentech/Vector Oncology protocols HPFSCRC1503 and HTPMC1411) are non-interventional studies, which employed existing data collected as part of routine clinical care in community oncology practices in the United States. This data has been aggregated by Vector Oncology into the Vector Oncology Data Warehouse or the Vector Oncology Electronic Medical Record partner. No data was collected prospectively and no patients were contacted as part of these studies. Electronic medical records and billing systems were used to identify eligible patients and to abstract patient demographic characteristics, KRAS status, treatment and outcomes.

Germany - TKK Registry Study Supported by German Roche Pharma Affiliate
The Tumourregister Kolorektales Karzinom (TKK) registry is a large, ongoing, prospective, national registry run by a multicenter network of 269 currently practicing office-based medical oncologists in Germany. Details of this registry have been described elsewhere (Marschner 2015). Our planned study will use aggregate data already collected as part of this registry. No prospective data will be collected and no patients will be contacted.

Australia - TRACC Registry Supported by Roche Pharma Australia
The Treatment of Recurrent and Advanced Colorectal Cancer (TRACC) registry is an ongoing, multi-centre, prospective, non-interventional, cohort study that started in 2009. Details regarding the primary colorectal cancer, any adjuvant or neoadjuvant therapy, and outcomes are recorded using a BioGrid technology which de-identifies and aggregates data collected from participating sites across Australia. Details of this registry have been described elsewhere (Field 2015, Field 2013). Our study will make use of aggregate data already collected as part of this study. No prospective data will be collected and no patients will be contacted.

Denmark – Roche Diagnostic Sponsored Study and Aarhus University Hospital, Department of Clinical Epidemiology
Avastin®—F. Hoffmann-La Roche Ltd
Protocol MO30177, Version 1.0
The study 'Impact of molecular profiling for metastatic colorectal cancer and clinical and economic outcomes' conducted in collaboration with Aarhus University Hospital, Department of Clinical Epidemiology and Roche Diagnostics is a non-interventional study using routine health care data from linked registries in Denmark to examine treatment patterns and outcomes in relation to clinical characteristics and KRAS mutation testing status among Danish patients diagnosed with mCRC during 2009-2013. We will use aggregate data already collected as part of this study. No prospective data will be collected and no patients will be contacted.

8.5 STUDY SIZE
Estimates of median overall survival (OS) in the target patient population with KRAS mutations range from approximately 20-25 months for patients receiving first-line bevacizumab-containing treatment and 14-19 months for patients receiving chemotherapy alone as first-line therapy (Hurwitz 2004, Loupakis 2014, Saltz 2008, Venook 2014, Heinemann 2014, Van Cutsem 2011, Douillard 2013, Arnold 2010, Kozloff 2009, Van Cutsem 2009, Bendell 2012). Assuming an increase in median OS from 18.5 to 22 months for patients receiving bevacizumab-containing treatment (HR =0.84), approximately 1000 deaths will be required for 80% power and a 2-sided test at 0.05. If we assume a smaller treatment effect, given the greater heterogeneity of patients treated in routine clinical practice, an increase in OS from 18.5 to 21 months (HR=.88) requires approximately 1900 deaths to achieve the same power and type I error rate.

Given the large number of deaths (1000-1900) required to detect a clinically meaningful increase in overall survival to meet our primary objective, our study plans to synthetize OS results from the cohorts in the US, Australia, Germany, and Denmark. Approximate numbers of patients in each cohort are provided in Table 1 below:

<table>
<thead>
<tr>
<th>KRAS Mutant</th>
<th>KRAS Wild type</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>United States</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>373</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td><strong>Germany</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>553</td>
<td>715</td>
<td></td>
</tr>
<tr>
<td><strong>Australia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>301</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td><strong>Denmark</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>924</td>
<td>636</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Approximate number of patients in each cohort.
8.6 DATA MANAGEMENT
No individual patient-level data will be collected and stored as part of this study. Aggregate data will be abstracted and validated from analysis reports by two separate researchers. Data will be entered in a single data file. Differences in abstraction will be queried and resolved.

8.7 DATA ANALYSIS
Hoffman-La Roche, Basel, will be conducting a pooled analysis of aggregate survival estimates to address the primary and secondary study objectives.

Individual patient-level data analyses will be conducted, or will have been conducted as existing prior studies, by the respective data sources (outlined in section 8.4) only; results from these analyses will be supplied in aggregate data reports to Hoffman-La Roche, Basel.

For the primary study objective, the following analysis populations will be compared:
- mCRC patients with KRAS mutant status treated with bevacizumab containing regimens
- mCRC patients with KRAS mutant status treated with chemotherapy alone.

The secondary study objective involves a comparison between the following analysis populations:
- mCRC patients with KRAS wild-type status treated with bevacizumab containing regimens
- mCRC patients with KRAS wild-type status treated with anti-EGFR containing regimens.

Data analyses conducted by the respective data sources providing aggregate data reports
The analyses outlined in this section will have been conducted, or will be conducted by the data providers in the completion of the aggregate data reports supplied to Hoffman-La Roche for meta-analysis.

Aggregate data summaries of demographic information, disease characteristics, treatment information, KRAS status, and survival outcomes will be provided.

Survival analyses – Kaplan-Meier estimates, hazard ratios, log-rank test, cox proportional hazards regression - on OS from the start of first-line treatment in mCRC to
death by any cause will be conducted by the data providers to compare OS endpoints between the comparison groups outlined in the primary and secondary study objectives, employing both unadjusted and adjusted models.

Subjects who are not known to have died will be censored at the date of last follow-up. All quartiles (including median) for OS will be presented together with a two-sided 95% confidence interval.

The Kaplan-Meier method will be used to estimate the distribution of OS. Number of patients at risk and 95%-confidence intervals will be displayed.

OS is defined as the duration of time from the date of the start of 1st-line treatment to the date of death from any cause. OS analysis will be analyzed for the four populations and be calculated as:

To facilitate meta-analysis, cox proportional hazards regression analyses adjusting for imbalances between treatment groups will have to be included in the aggregate data reports. Three models will be required:

- a pre-specified model, adjusting for a set of predefined variables
- a data-source specific model adjusting for variables that displayed a meaningful difference between the comparison groups in the respective data sets, as outlined in the descriptive analysis section above
- a model adjusting for a set of select variables based on identified meaningful differences between the comparison groups across all data sources

Meta-analyses

The meta-analyses of aggregate data will be conducted by Hoffman-La Roche, Basel.

Summary statistics will be presented for demographic information, disease characteristics, treatment information, KRAS status, and survival outcomes, by data source.

Heterogeneity between the different data samples will be assessed by calculating Cochran’s Q and I² statistics. Hazard ratios and 95% confidence intervals will be combined using the inverse variance method in a random-effects model.

Individual hazard ratios and combined OS estimate will be presented in a forest plot. A sensitivity analysis stratifying the pooled estimate by data source type – registry, EMR – will be conducted to assess a potential bias due to data collection differences.

9. STUDY DOCUMENTATION AND ADMINISTRATION

The study team must maintain adequate and accurate records to enable the conduct of the study to be fully documented, including but not limited to the protocol and protocol
amendments, and documentation of IRB/EC and Health Authority approval/notification (if applicable).

This study is managed by the Scientific Responsible and overseen by a Study Management Team.

10. **LIMITATIONS OF THE RESEARCH METHOD**

There are some limitations to this study design that should be considered. The analyses presented in this protocol regard aggregate data rather than individual patient level data, which may impact the findings of this study. Furthermore, the data sources included in this study employ different data collection methods – two utilize EMR data, and two are prospective registries, this may introduce heterogeneity and bias into the data. The total sample size may not be sufficient to conduct sub-population analyses to assess possible heterogeneity in the data.

11. **PROTECTION OF HUMAN PATIENTS**

11.1 **INFORMED CONSENT**

It will generally not be possible/practical to obtain informed consent for use of secondary data in a NIS; however, certain other precautions will be taken, including:

- Ensuring data are anonymised / pseudonymised
- Ensuring final analysis data are anonymised / pseudonymised
- Ensuring possibility of linkage back to individual identified patients is impossible or tightly controlled
- Obtaining ethical committee approval for use of data as proposed (e.g., the review of and extraction of information from individual medical charts) records for the proposed use ahead of study initiation. This will have been completed by data source from the respective vendors supplying the aggregate data included in this study.

11.2 **COMPLIANCE WITH LAWS AND REGULATIONS**

This study will be conducted in full conformance with the Guidelines for GPP published by the International Society of Pharmacoepidemiology (ISPE) and the laws and regulations of the country in which the research is conducted.

The study will comply with national and European Union requirements for ensuring the well-being and rights of participants in non-interventional post-authorization safety studies.
11.3 INSTITUTIONAL REVIEW BOARD OR ETHICS COMMITTEE

As this study presents a pooled analysis of aggregate data from four separate data sources, IRB or EC committee approval is not required.

IRB/EC committee approvals are in place for all individual data sources and studies contributing data to the analyses outlined in this protocol.

12. MANAGEMENT AND REPORTING OF ADVERSE EVENTS

Secondary data use NIS involve the use of secondary data and the reporting of adverse reactions in the form of ICSRs is not required. All adverse events extracted as per protocol will be summarized in any interim safety analyses and in the CSR and/or final publication.

As per protocol, these aggregate summaries may include the following adverse event types:

- Serious Adverse Events, including all deaths
- Adverse Events of Special Interest
- Non-serious Adverse Events
- Pregnancy
- Abnormal laboratory findings with or without associated AEs
- Overdose, abuse, misuse, medication error, occupational exposure, quality defect with or without associated AEs
- Reports of lack of efficacy
- Drug interactions

12.1 ADVERSE EVENTS

According to the International Conference of Harmonisation (ICH), an AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, regardless of causal attribution. An AE can therefore be any of the following:

- Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product
- Any new disease or exacerbation of an existing disease (a worsening in the character, frequency, or severity of a known condition)
- Recurrence of an intermittent medical condition (e.g., headache) not present at baseline
• Any deterioration in a laboratory value or other clinical test (e.g., electrocardiogram [ECG], X-ray) that is associated with symptoms or leads to a change in study treatment or concomitant treatment or discontinuation from study medicine.

12.2 SERIOUS ADVERSE EVENTS

An SAE is any AE that meets any of the following criteria:

• Is fatal (i.e., the AE actually causes or leads to death)
• Is life-threatening (NOTE: The term “life-threatening” refers to an event in which the patient was at immediate risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.)
• Requires or prolongs inpatient hospitalization
• Results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the patient’s ability to conduct normal life functions)
• Is a congenital anomaly/birth defect in a neonate/infant born to a mother exposed to study medicine
• Is a significant medical event in the physician’s judgment (e.g., may jeopardize the patient or may require medical/surgical intervention to prevent one of the outcomes listed above)

The terms “severe” and “serious” are not synonymous. Severity refers to the intensity of an AE (e.g., rated as mild, moderate, or severe, or according to NCI CTCAE criteria; the event itself may be of relatively minor medical significance (such as severe headache without any further findings).

13. PLANS FOR DISSEMINATION AND COMMUNICATION OF STUDY RESULTS

The primary and secondary outcome analyses, as well as exploratory analyses – if deemed feasible, are planned to be presented separately at European and North American conferences.

All available analyses will be presented in a single journal submission in the second quarter of 2017.
14. REFERENCES


Heinemann V, von Weikersthal LF, Decker T, et al. Randomized comparison of FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment of KRAS wild-type metastatic colorectal cancer: German AIO study KRK-0306 (FIRE-3); ASCO 2013. J Clin Oncol 31, 2013 (suppl; abstr LBA3506)


Venook A, Niedzwieky D, Lenz HJ, et al. 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 rectum (MCRC); ASCO 2014. J Clin Oncol 32:5s, 2014 (suppl; abstr LBA3)
Appendix 1
List of Stand-Alone Documents Not Included in the Protocol

- Hoffman-La Roche Study Management Team:

  **Australia - TRACC Registry**

  Royal Melbourne Hospital  
  300 Grattan St, Parkville VIC 3050, Australia

  Royal Melbourne Hospital  
  300 Grattan St, Parkville VIC 3050, Australia

  **Denmark – Department of Clinical Epidemiology, Aarhus University Hospital**

  Department of Clinical Epidemiology  
  Aarhus Universitetshospital  
  Olof Palmes Allé 43-45  
  DK-8200 Aarhus N

  Department of Clinical Epidemiology  
  Aarhus Universitetshospital  
  Olof Palmes Allé 43-45  
  DK-8200 Aarhus N

  Department of Clinical Epidemiology  
  Aarhus Universitetshospital  
  Olof Palmes Allé 43-45  
  DK-8200 Aarhus N
Germany - TKK Registry, iOMEDICO

iOMEDICO AG
Hanferstraße 28
79108 Freiburg im Breisgau

United States

Mayo Clinic
200 First St. SW
Rochester, MN 55905
United States

Duke Cancer Institute
10 Bryan Searle Drive
Seeley Mudd Building, 2nd floor
Durham, NC  27710
United States