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Healthcare utilization databases as a powerful tool to generate evidence in real-world clinical practice: an application on the treatment of metastatic colorectal cancer with bevacizumab

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List of Abbreviations

HCU	Healthcare Utilization
RCT	Randomized Clinical Trial
NHS	National Health Service
CRC	ColoRectal Cancer
mCRC	Metastatic ColoRectal Cancer
FU	Fluorouracil
LV	Leucovorin
VEGF	Vascular Endothelial Growth Factor
AIFA	Agenzia Italiana del Farmaco
PFS	Progression-Free Survival
OS	Overall Survival
ORR	Overall Response Rate
CT	ChemoTherapy
CR	Cancer Registry
ATC	Anatomical Therapeutic Chemical

ICD9-CM	International Classification of Diseases, Ninth Revision, Clinical Modification
HR	Hazard Ratio
OR	Odds Ratio
CI	Confidence Interval
ML	Maximum Likelihood
PH	Proportional Hazard
PS	Propensity Score

Abstract

The efficacy of first-line bevacizumab added to chemotherapy (CT) in patients with metastatic colorectal cancer (mCRC) was assessed by several randomized clinical trials (RTC). However, data on the added value of bevacizumab in real-world post-marketing studies are scant. Moreover, the characteristics of patients included in RTCs are different from those of patients that physicians generally face in daily clinical practice, limiting the external validity of the results.

Healthcare utilization (HCU) databases, contrarily, allow the recruitment of unselected patients, including the elderly and those with co-morbidities, not always treated in highly specialised centres, reflecting the real clinical practice.

The present study aimed to evaluate the effectiveness of first-line bevacizumab in the Italian clinical practice of patients with mCRC. The overall survival (OS) of patients treated with first-line bevacizumab+CT was compared to the OS of patients treated with CT alone. Baseline characteristics of patients included in the cohort and the predictors of OS were also assessed.

Incident mCRC cases during the period 2010-2012 were selected from five Cancer Registries from Northern (Province of Varese, Mantova and Cremona) and Southern (Province of Palermo and Ragusa) Italy. Cases were linked to the Regional HCU databases of the five areas covered by the Cancer Registries, in order to obtain the entire pathway of health services provided by the National Health Service to each patient. The information collected from the HCU databases included the outpatient dispensations of high-cost drugs (among which bevacizumab), the diagnostic and intervention codes for admission to public or private hospitals and the outpatient services (including radiotherapies and diagnostic procedures).

A cohort of 1,118 incident mCRC cases was identified. After excluding subjects who did not meet the inclusion criteria, a final study cohort of 480 subjects was selected, of which 101 received first-line bevacizumab+CT and 379 received CT

alone. As compared to patients using CT alone, those using bevacizumab+CT were younger and received a surgical intervention before starting first-line treatment. The median OS was 22.5 and 14.6 months in patients treated with or without bevacizumab, respectively (p=0.011). The corresponding adjusted HR was 0.82 (95% CI 0.62-1.08). Young ages at baseline ($\leq$ 70 years) and experiencing surgery were significant protective factors.

Several sensitivity analyses were conducted, confirming the robustness of the results obtained from the main analysis.

The OS estimates were comparable to those coming from three large observational studies that assessed the OS of patients treated with first-line bevacizumab.

This study suggested a beneficial effect, even not statistically significant, of adding bevacizumab to CT in the real-world clinical practice of mCRC patients. HCU databases represented a powerful tool for conducting observational studies based on real-world data. However, they need to be handled carefully, taking into account the limitations associated to their use.

Chapter 1

Introduction

1.1 Healthcare Utilization Databases

1.1.1 What they are?

Healthcare Utilization (HCU) databases refer to the collection of data regarding health services dispensed to the whole population of a specific geographic area. In Italy they were primarily instituted for administrative purposes, with the aim of monitoring costs and planning health-care services. They are also used for reimbursement of health services from Regions to local health authorities [1]. Nevertheless, they actually represent a powerful tool in the field of pharmacoepidemiology. Their use is becoming increasingly common in the conduction of studies of pharmacoutilization, as well as for evaluating the effectiveness and the safety of drugs

used for the treatment of several diseases [2].

In the process of approval of drugs, HCU databases represent a valid tool for the conduction of postmarketing studies. Indeed, once a drug is approved and put on the marketplace by the health authorities on the basis of the results coming from phase 3 randomized clinical trials (RCTs), is it important to evaluate the effectiveness and safety in the real-world clinical practice, on a large sample of unselected patients followed up for a long period, allowing the detection of rare adverse drug reactions [3]. For this reasons, HCU databases are an important toll for integrating the results coming from RCTs, and for evaluating their external validity

The information stored in the HCU databases include the distribution of all drugs reimbursed by the National Health System (NHS), the hospitalizations in both public and private hospitals, the outpatient health services, the emergency room service, the certificates of delivery assistance, the exemptions for a specific disease. In Lombardy Region, the permission to access to the HCU databases is regulated by the Regional directorate and is permitted to the scientific institutions that own specific requirements, guaranteeing the ability and the expertise to handle such data and the intention to use them for scientific purposes [4].

1.1.2 Strengths

The use of the HCU databases is always more widespread due to several aspects. First of all, HCU databases cover the entire population they are referred to. In Italy, they cover health services of all patients located in a specific area, for example a single Region, in a given period of time. For this reason, sample sizes are generally high, even when considering relative rare exposures or outcomes. As a consequence, they include a wide and unselected population of patients of different ages, lifestyles and habits, socioeconomic characteristics, as well as clinical characteristics and comorbidities. It follows that the results derived from such kind of studies are highly generalized.

Second, they reflect the drug utilization in a real-world clinical practice. Indeed, drugs used for chronic conditions, such as diabetes, hypertension or hypercholesterolemia, are often administered by the patients without following rigorous conditions of use. The use of HCU databases allows to relate the outcome with the adherence to a specific treatment, as well as the persistence, the discontinuation and changes of therapies.

Third, they often contain health information referred to many years. Therefore, it is possible to study uncommon adverse events, or those who required a long latency period.

Fourth, the use of these databases are associated to low costs and relatively rapid

time of execution. Indeed, all the information are directly available for a specific population in a specific period of time, avoiding long time for collecting data [5].

1.1.3 Limitations

On the other hand, using the HCU databases is associated to some limitations. The most relevant is the lack of clinical information. Since the primary purpose for which the HCU databases were instituted is of administrative nature, they do not contain information about lifestyles, such as diet, smoking habits, physical activity, body mass index and alcohol consumptions. Moreover, they do not report information about the stage of the disease, complications, relapses, patient's performance status, diagnostic test results and specific comorbidities. The lack of these information may lead to confounding.

Secondly, the selection of the outcome through the HCU databases may lead to outcome misclassification. This kind of bias may occurs either when a patient is erroneously classified as having the disease (outcome), in case of no disease, or when a patient is classified has not having the disease, when the disease is actually present. In the first case, the bias arise from the generation of false positives, which reduces the specificity of the outcome selection. In the second case, false negatives will be generated, reducing the sensitivity of the outcome selection.

Finally, another source of bias is the misclassification of the exposure. It occurs when the true exposition is not correctly defined. [5].

All these three sources of uncertainty need to be handle with the appropriate statistical techniques, in order to obtain unbiased results.

1.2 An application on real-world data

1.2.1 Colorectal cancer

Adenocarcinoma of the colon and rectum (colorectal cancer, CRC) is the third most common cancer, the fourth most common cause of cancer death, and the second most common cancer in terms of the number of individuals living with cancer five years after diagnosis worldwide. An estimated 1,361,000 people are diagnosed with CRC and approximately 694,000 people die from CRC, annually, while 3,544,000 individuals are living with CRC [6]. In Italy, among all cancers, CRC is the one with the highest incidence, with about 52,000 estimated diagnoses in 2014 [7].

Approximately 20-25% of patients present metastatic CRC at initial diagnosis and another 25% will develop subsequent metastases [8]. For the majority of patients diagnosed with mCRC palliative chemotherapy is the most appropriate treatment option in order to achieve the goals of prolonging survival and improving quality of life [9]. The backbone of first- and second-line palliative chemotherapy for mCRC consists of a fluoropyrimidine based therapy combined in various combinations and schedules. Depending on clinical features, treatments for mCRC are generally based on fluoropyrimidine (5-FU or capecitabine) and leucoverin (LV), with or without irinotecan or oxaliplatin. In particular, currently used therapeutic schemes

are FOLFOX (5-FU, LV, oxaliplatin) FOLFIRI (5-FU, LV, irinotecan), XELIRI (capecitabine, irinotecan), XELOX (capecitabine, oxaliplatin), FOLFOXIRI (5-FU, LV, oxaliplatin, irinotecan). Bevacizumab may be combined with all the previous therapies [10]. Favourable survival has been shown to correlate with the patients receiving all active chemotherapeutic agents, emphasizing the importance of exposure to all active drugs during treatment [11].

1.2.2 Bevacizumab

Bevacizumab is a humanized recombinant monoclonal antibody that inhibits vascular endothelial growth factor (VEGF), a common target that plays an important role in the angiogenesis of the tumour [12]. It was the first antiangiogenic agent, approved in 2004 by both the Food and Drugs Administration and the European Medicines Agency, for the treatment of patients with metastatic colorectal cancer (mCRC). In Italy, it was approved by the Italian Medicine Agency (Agenzia Italiana del Farmaco, AIFA) in September 2005 as first-line treatment of mCRC in combination with fluorouracil (FU)-based chemotherapy with or without irinotecan [13]. In 2008, the indication was extended to the second-line setting [14]. The use of bevacizumab in combination with fluoropyrimidine-containing chemotherapy is a well-established first-line and second-line treatment for patients with mCRC [15–19]. Bevacizumab regulatory approval was based on the results of the pivotal AVF2107 phase III trial [20], in which 813 previously untreated patients were randomized to bolus 5-fluorouracil (5FU)/Leucovorin (LV) and irinotecan (the IFL regimen) plus placebo or IFL plus bevacizumab. Median OS improved from 15.6 to 20.3 months [HR=0.66, $p<0.001$], median progression-free survival (PFS) improved from 6.2 to 10.6 months (HR=0.54, $p<0.001$) and overall response rate (ORR) from 34.8% to 44.8% ($p=0.004$).

By the time of regulatory approval of bevacizumab, the IFL protocol was no longer the preferred first-line backbone regimen for mCRC. Several trials have

demonstrated that infusional fluoropyrimidine-based regimens with either oxaliplatin (FOLFOX) or irinotecan (FOLFIRI) were more efficacious and less toxic than IFL [21–23].

The combination of bevacizumab with oxaliplatin-based chemotherapy as first-line treatment was investigated in the NO16966 randomized study of 1400 patients [24]. Patients received oxaliplatin with capecitabine or 5FU/LV plus bevacizumab or placebo. Although the study was formally positive and the median PFS improved from 8.0 to 9.4 months (p=0.002), the results were still disappointing; neither the response rate (47% vs. 49%, p=0.31) nor the median OS time (21.3 months vs. 19.9 months, p=0.077) were significantly different between the groups.

1.2.3 Research question

Bevacizumab use has been shown to prolong progression-free survival (PFS) or overall survival (OS) in patients with mCRC treated in the first line. However, questions regarding its effectiveness when combined with currently used chemotherapy schemes still remain.

Only a few post-marketing observational cohort studies were conducted to evaluate the added value of bevacizumab in the real-world setting [25–27]; however, it is important, for both clinicians and health decision makers, to assess the effectiveness of bevacizumab under conditions that patients and physicians generally face in daily clinical practice. In this regard, observational studies based on real-world data allow the inclusion of patients usually under-represented in RCTs, such as the elderly, those with comorbidity, and those treated by non-expert healthcare providers [28, 29].

An evaluation of real-world efficacy outcomes and CT patterns associated with bevacizumab use for the treatment of first-line mCRC is therefore missing. This study will contribute to add evidence about the clinical impact of the use of bevacizumab for the treatment of mCRC in the real-world clinical practice.

1.2.4 Objectives

The main aim of this thesis was the conduction and the management of an Italian multi-regional retrospective cohort study in order to assess the effectiveness of first-line bevacizumab in a real-world setting of patients with mCRC.

The OS of patients using bevacizumab as first-line treatment was compared to the OS of patients using CT alone. The cumulative proportion of survivors at 1, 2, and 3 years after starting first-line treatment for mCRC and predictors of survival was evaluated. A stratified analysis by age was performed, in order to evaluate whether bevacizumab has a different impact on OS according to age groups. The latter aspect allowed to evaluate the effectiveness of bevacizumab also in older patients, that are usually excluded from RCTs.

Secondary aims were to assess the baseline characteristics of patients assigned to the different first-line treatments (bevacizumab+CT vs. CT alone) and the predictors of both bevacizumab use and OS. Moreover, duration of first-line treatment with bevacizumab and changes of therapies were calculated.

The activities planned for this project included the writing of a research protocol, the presentation of the protocol to an ethical committee, the coordination of the centres included in the project about the selection of mCRC cases and the linkage with the HCU databases, the performance of all the statistical analyses, the preparation of a final report and the writing of a manuscript to be submitted to a

peer-reviewed scientific journal. Regular meetings were planned with the scientific staff involved in the project.

Chapter 2

Methods

2.1 Data sources

Data were retrieved from the following two sources of data:

1. The Cancer Registries (CR) of five Italian Provinces: Varese, Mantova, Cremona, Ragusa and Palermo. They include the date of diagnosis of mCRC; the topographical code (ICDO3T code), which describes the anatomical site of origin of the tumour; the morphological code (ICDO3M code), which describes the cell type, or histology, of the tumour, together with the behaviour; the grading of the tumour, which describe the grade of differentiation of cancer cells as compared to normal cells; the staging of the tumour, which describes and classifies a cancer on the basis of the extent of cancer in the

body. The stage is based on the TNM staging system, where T describes the tumour size, N indicates the number of nearby involved lymph nodes and M refers to whether the cancer has metastasized; the subject's gender and date of birth; the vital status and the date of death, if applicable. An extract of the CR database is reported in Figure 2.1.

2. The Regional HCU databases of the five centres included in the study. The following databases were used:

   - The Hospital Discharge Forms database, which stores all the hospitalizations of all subjects accepted by both public and private hospitals. It contains the date of admission and discharge, the main and five secondary diagnoses (coded through the ICD9CM codes), the main and five secondary interventions (coded through the ICD9CM codes), the date of the main intervention (See Figure 2.4);
   - The database of drugs prescribed from hospital pharmacies, which contains all outpatient dispensations of high-cost drugs (including bevacizumab) reimbursed by the NHS. It includes the date of administration, the ATC code of the drug and the quantity dispensed (See Figure 2.2);
   - The outpatient's service database, which stores all the health service dispensed in the outpatient setting, including laboratory tests, imaging diagnostic procedures, radiotherapy and chemotherapy. Were available

information about the description and the date of the health service dispensed (See Figure 2.3).

All the previous databases were linked through an unique anonymous identification code.

mCRC cases

IDPZ	SESSO	DATA_NASC	DATAINCI	DATA_FW_L	STATO	STADIO	ETA	G	ICDO3_T	ICDO3_M
110900	2	26/04/1925	26/01/2011	19/03/2011	2	IV	85		C809	80703
113302	1	04/03/1951	19/07/2012	22/08/2013	2	IV	61	2	C186	81403
114070	1	17/12/1934	18/05/2010	04/01/2014	2	IV	75	2	C182	81403
116683	2	05/01/1939	28/03/2012	14/12/2013	2	IV	73	2	C185	81403
119545	2	25/04/1943	10/10/2012	31/12/2014	1	IV	69	3	C186	81403
120700	2	21/06/1957	23/05/2011	03/05/2012	2	IV	53	2	C187	81403
121318	1	15/09/1920	29/03/2011	30/07/2011	2	IV	90		C188	80003
130442	1	05/02/1928	02/11/2010	09/01/2013	2	IV	82		C187	84803
131196	2	09/06/1927	11/11/2010	29/11/2010	2	IV	83	2	C184	81403
139510	2	24/03/1956	13/12/2012	29/11/2014	2	IV	56	2	C210	81403
140163	1	22/06/1933	01/02/2010	18/07/2010	2	IV	76	2	C199	81403
140235	2	06/11/1922	31/08/2012	31/10/2012	2	IV	89		C183	80003
140726	1	19/03/1941	14/01/2011	11/08/2011	2	IV	69		C188	81403
143379	2	29/05/1945	06/02/2012	31/12/2014	1	IV	66	3	C199	84803
144556	1	27/05/1931	19/04/2011	23/07/2012	2	IV	79	3	C187	81403
146642	1	05/06/1941	02/01/2012	12/10/2014	2	IV	70	2	C182	81403
147996	1	05/01/1918	12/08/2010	08/10/2010	2	IV	92		C182	80003
148247	1	03/06/1939	22/03/2011	30/03/2011	2	IV	71	3	C183	82463
149071	1	23/10/1938	22/07/2011	06/10/2012	2	IV	72	3	C180	81403
156179	1	24/06/1947	06/12/2012	31/12/2014	1	IV	65	3	C209	84803
158451	1	03/03/1925	30/05/2011	26/07/2011	2	IV	86		C186	80003
159995	1	12/12/1928	14/07/2011	24/09/2011	2	IV	82	3	C182	81403

FIGURE 2.1: Extract of the Cancer Registry database

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IDPZ	codice farm	ATC	DESCRIZIONE	data erog fa
150813	036680027	L01XC07	AVASTIN*INFUS 1FL 100MG 4ML	05/04/2012
150813	036680027	L01XC07	AVASTIN*INFUS 1FL 100MG 4ML	19/04/2012
150813	036680027	L01XC07	AVASTIN*INFUS 1FL 100MG 4ML	03/05/2012
150813	036680027	L01XC07	AVASTIN*INFUS 1FL 100MG 4ML	17/05/2012
150813	036680027	L01XC07	AVASTIN*INFUS 1FL 100MG 4ML	31/05/2012
150813	036680027	L01XC07	AVASTIN*INFUS 1FL 100MG 4ML	14/06/2012
150813	036680027	L01XC07	AVASTIN*INFUS 1FL 100MG 4ML	28/06/2012
150813	038751020	L01XX19	IRINOTECAN ACC*1FL 5ML 20MG	07/03/2012
150813	038751020	L01XX19	IRINOTECAN ACC*1FL 5ML 20MG	21/03/2012
150813	038751020	L01XX19	IRINOTECAN ACC*1FL 5ML 20MG	04/04/2012
150813	038751020	L01XX19	IRINOTECAN ACC*1FL 5ML 20MG	18/04/2012
150813	038751020	L01XX19	IRINOTECAN ACC*1FL 5ML 20MG	02/05/2012
150813	038751020	L01XX19	IRINOTECAN ACC*1FL 5ML 20MG	16/05/2012
150813	038751020	L01XX19	IRINOTECAN ACC*1FL 5ML 20MG	30/05/2012
150813	038751020	L01XX19	IRINOTECAN ACC*1FL 5ML 20MG	13/06/2012
150813	038751020	L01XX19	IRINOTECAN ACC*1FL 5ML 20MG	27/06/2012
150871	035219029	L01BC06	XELODA*120CPR RIV 500MG	30/03/2012
150871	035219029	L01BC06	XELODA*120CPR RIV 500MG	20/04/2012
150871	035219029	L01BC06	XELODA*120CPR RIV 500MG	11/05/2012
150871	035219029	L01BC06	XELODA*120CPR RIV 500MG	01/06/2012
152659	035219029	L01BC06	XELODA*120CPR RIV 500MG	29/10/2010
152659	035219029	L01BC06	XELODA*120CPR RIV 500MG	19/11/2010
152659	035219029	L01BC06	XELODA*120CPR RIV 500MG	10/12/2010
152659	035219029	L01BC06	XELODA*120CPR RIV 500MG	30/12/2010
152659	035219029	L01BC06	XELODA*120CPR RIV 500MG	29/10/2010

FIGURE 2.2: Extract of the drug prescription database

RT prest ambul

IDPZ	DATA_COMI	PREST	DESC_PREST
110900	23/12/2005	91413	ES. ISTOCITOPATOLOGICO APP. DIGERENTE: Biopsia endosco
110900	23/12/2005	4516	ESOFAGOGASTRODUODENOSCOPIA [EGD] CON BIOPSIA
113302	12/05/2011	88761	ECOGRAFIA ADDOME COMPLETO
113302	29/07/2009	88798	ECOGRAFIA TRANSRETTALE
113302	29/07/2009	60111	BIOPSIA TRANSPERINEALE [PERCUTANEA] [AGOBIOPSIA] DEI
113302	05/01/2008	8622	RIMOZIONE ASPORTATIVA DI FERITA, INFEZIONE O USTIONE;
113302	30/09/2008	88761	ECOGRAFIA ADDOME COMPLETO
113302	29/07/2009	91441	ES. ISTOCITOPATOLOGICO APP. UROGENITALE: Agobiopsia p
113302	17/08/2007	88761	ECOGRAFIA ADDOME COMPLETO
113302	29/03/2003	90565	ANTIGENE PROSTATICO SPECIFICO (PSA) Incluso eventuale c
113302	11/08/2004	90565	ANTIGENE PROSTATICO SPECIFICO (PSA) Incluso eventuale c
113302	27/11/2003	87441	RADIOGRAFIA DEL TORACE DI ROUTINE, NAS; Radiografia sta
114070	13/05/2010	91413	ES. ISTOCITOPATOLOGICO APP. DIGERENTE: Biopsia endosco
114070	27/05/2010	91394	ES. CITOLOGICO URINE PER RICERCA CELLULE NEOPLASTICHE
114070	10/12/2010	8901F	VISITA ONCOLOGICA DI CONTROLLO
114070	25/10/2010	897B6	PRIMA VISITA ONCOLOGICA
114070	16/10/2010	88955	RISONANZA MAGNETICA NUCLEARE (RM) DELL'ADDOME INFI
114070	31/08/2011	90553	ANTIGENE CARBOIDRATICO 19.9 (CA 19.9)
114070	10/10/2011	88955	RISONANZA MAGNETICA NUCLEARE (RM) DELL'ADDOME INFI
114070	02/11/2011	8901M	VISITA RADIOTERAPICA DI CONTROLLO
114070	28/07/2011	9229K	RADIOTERAPIA CON TECNICHE AD INTENSITA' MODULATA AI
114070	08/11/2011	8901F	VISITA ONCOLOGICA DI CONTROLLO
114070	31/08/2011	90551	ANTIGENE CARBOIDRATICO 125 (CA 125)
114070	05/07/2012	8901F	VISITA ONCOLOGICA DI CONTROLLO
114070	05/06/2012	MAC05	Terapia di supporto (idratazione, alimentazione parenterale

FIGURE 2.3: Extract of the outpatient service database

RT sdo

IDPZ	DATA_RICV	DIAGN_PR	DIAGN_SEC:	DIAGN_SEC:	DIAGN_SEC:	DIAGN_SEC4	DIAGN_SEC:	COD_INT1	COD_INT2	COD_INT3	COD_INT4
113302	15/03/2005	6000	78791	4019				8875	8876	4525	8952
113302	15/12/2003	8072						9059	8952		
113302	20/11/2009	29532						9412	9423	9419	
113302	25/06/2012	1972	1970	1977	4019			5011	8741	8801	8703
113302	21/07/2012	1970	1977	1532	30000			8607	4525	9925	
113302	27/12/2012	V5811	1532	1977	1972	1961		9925	8952		
113302	06/09/2012	V5811	1977	1972	1532	2800		9925	8952		
113302	19/11/2012	V5811	1977	1970	1532			9925	8801	8741	
113302	11/08/2012	V5811	1533	03810				9925	9904		
113302	24/09/2012	V5811	1532	1977				9925			
113302	29/10/2012	V5811	1977	1970	1533			9925			
113302	09/10/2012	V5811	1977	1972	1532	30112		9925			
113302	05/03/2013	V5811	1538	1977				9925			
113302	13/02/2013	V5811	1977	1972	1532			9925	8952		
113302	13/05/2013	27651	5849	78701	1532	1977	1972	8952			
113302	14/01/2013	V5811	1539	1977				9925			
113302	05/05/2013	V5811	1538	1977	1972			9925			
113302	07/07/2013	03819	1532	1977	27651	5362	1972	4414	9925	8801	8703
113302	06/08/2001	600	5718	9093	30275			8952	8875	8744	8769
114070	14/04/2000	78651									
114070	12/11/2001	2824	V1051					8801	4523		
114070	23/04/2003	1519	V1051	42731				4391	5459	9089	8908
114070	15/05/2008	57461						5122	5199	5137	5111
114070	06/04/2008	41519	2859					8741	8872	8877	8876

FIGURE 2.4: Extract of the Hospital Discarge Forms database

2.2 Cohort selection

Incident cases of mCRC were selected from the CR of the five centres included in the study, during the period 2010-2012. Only CRC cases reporting a distant metastasis at initial diagnosis were selected, according to the objective of the study. Subjects with multiple cancers were excluded, in order to avoid the potential confounding introduced by having other cancers.

Cohort's mCRC cases were linked to the HCU database, in order to retrieve the health services provided to them from three year before the diagnosis of mCRC to the end of follow-up. In this way, it was possible to build up the entire diagnostic and therapeutic pathways of cohort's subjects.

2.3 Exposure assessment

Exposure to either bevacizumab+CT or CT alone as first-line treatment for mCRC was defined by a two-stage algorithm. Firstly, the first prescription of antineoplastic drug approved for the treatment of mCRC, subsequent to the date of mCRC diagnosis, was selected. The following drugs were considered: bevacizumab (ATC code: L01XC07), cetuximab (ATC code: L01XC06), panitumumab (ATC code: L01XC08), irinotecan (ATC code: L01XX19), oxaliplatin (ATC code: L01XA03), capecitabine (ATC code: L01BC06) and fluorouracile (ATC code: L01BC02). Secondly, because information on chemotherapy (i.e. irinotecan, oxaliplatin, capecitabine and fluorouracile) of inpatients were not available in our database, all hospitalisations subsequent to the date of mCRC diagnosis reporting a code of chemotherapy, either as diagnosis (ICD9-CM codes V58.1 and V58.11) or as intervention (ICD9-CM codes 99.25 and 99.28), were selected. Thus, the date of starting therapy (index date), was defined as the first date between the date of the first hospitalisation reporting a code of chemotherapy (if any) and the date of the first prescription of antineoplastic agent (if any). Finally, starting from the index date, all the prescriptions of antineoplastic drugs in the following 21 days (i.e., the duration of a chemotherapy cycle) were selected. Thus, first-line treatment was defined hierarchically: if any prescription of bevacizumab in the 21-day period, the subject was classified as exposed to bevacizumab+CT; if any prescription of

other biological drugs (i.e. cetuximab and panitumumab) in the 21-day period, the subject was classified as exposed to other drugs, and not included in the cohort study; if no prescriptions of biological drugs in the 21-day period, the subject was classified as exposed to CT only. Only subjects who started therapy within 90 days from the date of diagnosis of mCRC were included in the final cohort. Indeed, after consulting expert oncologists, it seems not likely that an individual with a diagnosis of mCRC is treated long time after the diagnosis. Even in case of surgical intervention before starting treatment, a time-lag of 90 days seems be reasonable. Moreover, this criteria was adopted in order to exclude potential errors in the compiling of the databases, or to exclude cases with severe complications who required to be treated in an unconventional way.

2.4 Covariates

Clinical information on cancer were available, such as tumour size and lymph node status (coded through the TNM Classification of Malignant Tumours [TNM, 2010]), grading, anatomical site (coded through the ICDO3T classification [30]), and histological characteristics (coded through the ICDO3M classification [30]). In addition, the following baseline characteristics were considered: year of mCRC diagnosis, gender, age, surgical intervention and Charlson comorbidity index [31], which was used as an indicator of subjects health condition. Outpatients procedures measured during the follow-up were also evaluated, such as number of computerized tomography of the abdomen, magnetic resonance, x-ray of digestive system, radiotherapies and surgical intervention. ICD9-codes of both comorbidities and procedures mentioned above are given in Appendix 1.

2.5 Sample size

In a study that compares the survival of two groups A and B, the sample size formula is usually based on the estimate of the number of deaths required, rather than the number of patients. Given a minimum detectable effect size, a type I error (α) and a type II error ((β)), the following formula can be used to estimated the required number of deaths [32]:

$$\frac{(z_\beta + z_{1-\alpha})^2}{P_A P_B \ln^2(\Delta)} \tag{2.1}$$

where:

- $z_(\beta)$ is in the β percentile of the Normal distribution;
- $z_{1-\alpha}$ is the $(1-\alpha)$ percentile of the Normal distribution;
- P_A is the proportion of patients exposed to group A;
- P_B is the proportion of patients exposed to group B;
- Δ is the minimum detectable effect size in the two groups, expressed in terms of HR.

To determine the required number of patients to include in the study, it is necessary to estimate the proportion of patients that will die. For this purpose, the following

formula can be used [32]:

$$d_B = 1 - \frac{1}{6}[S_B(f) + 4S_B(f + 0.5a) + S_B(f + a)] \tag{2.2}$$

where:

- d_B is the number of expected deaths in the group B (i.e. the unexposed, or control, group);
- $S_B(t)$ is the survival estimate in the group B, at time t;
- f is the length of the follow-up period;
- a is the length of the accrual period;

The expected deaths in the treatment A can be approximated by:

$$d_A = 1 - (1 - d_B)^{\frac{1}{\Delta}} \tag{2.3}$$

Thus, the total number of expected deaths is given by:

$$d = P_A d_A + P_B d_B \tag{2.4}$$

Finally, the total number of patients required can be calculated as the number of deaths, given by (2.1), divided by d.

In this study, the calculation of the sample size was based on the following considerations:

- in Italy, almost 52,000 cases of colorectal cancer are diagnosed every year [33];
- approximately 20-25% of them present metastases already at the time of diagnosis [8]. However, due to screening programmes, this percentage was low in the centres included in the study, accounting for about 15% of all cases of CRC (data to be published);
- cancer registries collaborating to the current study covered a population of almost 3,187,359 inhabitants, representing about 5% of the entire Italian population.

It followed that about 400 cases of mCRC were expected every year to be recorded from the considered registries. By considering a three-years period of recruitment (2010-2012), approximately 1,200 patients were expected to be recruited by the five Cancer Registries participating to the study. In order to calculate the minimum detectable effect size in terms of difference of median OS in patients treated with bevacizumab+CT or CT alone, the results deriving from the pivotal RCT by Hurwitz and colleagues [20] were considered. Indeed, the aforementioned RCT was the only one that directly compared OS of patients with mCRC treated with CT alone (i.e. IFL [irinotecan, 5-FU and leucovorin]) and with bevacizumab+CT

(i.e. bevacizumab+IFL). The authors estimated a median OS in patients treated with CT alone of 15.6 months. By assuming that:

- one third of patients were untreated;
- among treated patients, 20% of patients was treated with bevacizumab+CT and 80% was treated with CT alone

considering a one-tail first type error of 0.05 and a power of 0.80, the study was able to appreciate a gain in terms of median OS from 15.6 months, in the group treated with CT alone, to 20.0 months, in the group treated with bevacizumab+CT. The corresponding HR detectable under the aforementioned conditions was 0.78.
By assuming different distributions of subjects treated with bevacizumab+CT as first-line therapy, the gain in terms of overall survival is reported in table 2.1.
Finally, it should be considered that the sample size was calculated according to the crude comparison, based on the Log-Rank test. However, effect estimates of the therapy on the outcome were adjusted by several covariates (by using a Cox regression model), implicating a reduction of the precision of the estimates, and, consequently, a reduced ability of the study to appreciate a gain in the OS [32]. On this purpose, the variance of the parameter associated to the effect treatment estimated by the adjusted model ($\beta = ln[HR]$) was increased up to 50% as compared to the variance of the parameter associated to the effect treatment estimated by the

unadjusted model. By considering that using the unadjusted model the appreciable gain in OS was 4.4 months (from 15.6 to 20.0 months), the scenarios described above allowed to detect at most a gain in OS of 4.5 months. In order to compare the previous results, let consider that the RCT of reference on the use of bevacizumab for the first-line treatment of mCRC [20] reported an increased median OS from 15.6 months in patients treated with fluorouracil+leucovorin+irinotecan (IFL)+placebo to 20.3 months in patients treated with IFL+bevacizumab (corresponding to a gain in OS of 4.7 months). Although an observation study has not the objective to reproduce the evidence deriving from RCTs (but has the objective to evaluate the impact of different therapeutic schemes in the real clinical practice), the expected cohort size of the present study would have been sufficient to appreciate a potential gain on the OS in patients treated with bevacizumab+CT less than the one obtained in the RCT of reference [20].

% of patients treated with bevacizumab+CT	**HR**	**Median OS bevacizumab+CT group (months)**	**Gain in terms of median OS months**
30	0.806	19.4	3.8
25	0.796	19.6	4.0
20	0.781	20.0	4.4
15	0.758	20.6	5.0
10	0.720	21.7	6.1

TABLE 2.1: Detectable hazard ratios (HR) and corresponding median overall survival (OS) in the group of patients treated with bevacizumab+CT, assuming different distribution of exposure

2.6 Statistical analyses

Exposure to bevacizumab was evaluated through an intention-to-treat approach, according to the first treatment received after the diagnosis of mCRC (i.e. bevacizumab+CT or CT alone).

Baseline characteristic, according to first-line treatment, were assessed at diagnosis of mCRC. Comorbidities, such as diabetes, hypertension, ischemic heart disease, cerebrocardiovascular disease, respiratory disease, renal disease and liver diasease were assessed in the three year preceding the mCRC diagnosis. Surgical interventions were evaluated in the period between mCRC diagnosis (-180 days) and the start of first-line treatment

Predictors of bevacizumab use were evaluated through a multivariable logistic regression model, adjusted for baseline characteristics.

Clinical characteristics of the tumour, such as grading, cancer size (T) and lymph-nodal status (N), were not used in the model, because of missing values that would have resulted in a significant loss of information and potential biased estimates. Indeed, all observations with at least one missing value in any variable would have been excluded from the analyses, reducing the number of observation to be included in the analysis.

The primary outcome was OS, defined as the time from the index date (start of first-line treatment) to the first date among death, lost at follow-up or 31/12/2015.

OS was assessed by using the Kaplan-Meier method [34]. Data were censored at three years of follow-up, given the low number of subjects surviving longer. The log-rank test was used to test differences between groups [35]. Predictors of OS were assessed by using a Cox proportional hazard model [36]. The model included, other than the exposure to bevacizumab+CT or CT alone, baseline patients characteristics. The Charlson comorbidity index was used as an indicator of the subjects health condition, instead of adding to the model all the single comorbidities. Covariates measured during follow-up were included in the model as continuous time-dependent covariates (i.e. the number of diagnostic procedures and the number of radiotherapies). These variable were included in order to evaluate whether more frequent diagnostic test were associated with a worse OS, indicating a more severe disease. Results were expressed in terms of hazard ratios (HR) and corresponding 95% confidence intervals (CI).

Changes of therapies (switching) were considered only as a descriptive analysis. The number of subjects who started first-line treatment with bevacizumab+CT, and switched during the follow-up to other biologic drugs (i.e. cetuximab and panitumumab), as well as those who started first-line treatment with CT alone, and switched during follow-up to a biologic drug, were calculated, along with the median time-to-switch.

The addition of bevacizumab to first-line treatment based on CT alone was not considered as a time-dependent exposure, since the change of therapy is a signal of worsening of the disease. If considered in the model, the addition of bevacizumab

may erroneously result in a positive association with OS. Again, this may be due to the advanced stage of the disease of subjects who changed therapy, instead of a real harmful treatment effect.

2.6.1 Time-dependent variables

A time-dependent variable is defined as any variable whose value for a given subject may differ over time. Examples of such a variable include exposure level, employment status, smoking status, obesity level. All these examples consider variables whose values may change over time for any subject under study; the reason for a change in value depends on internal characteristics or behavior specific to the individual. In contrast, there may be variables whose value changes primarily because of external characteristics of the environment that may affect several individuals simultaneously. Examples of such a variable are air pollution index for a particular geographical area or heart transplant status for a person identified to have a serious heart condition, making him or her eligible for a transplant. Given a survival analysis situation involving both time-independent and time-dependent predictor variables, we can write the extended Cox model that incorporates both types:

$$h(t, \mathbf{X}(t)) = h_0(t) exp[\sum_{i=1}^{p_1} \beta_i X_i + \sum_{j=1}^{p_2} \delta_j X_j(t)] \tag{2.5}$$

where

$$\mathbf{X}(t) = (X_1, X_2, ..., X_{p_1}, X_1(t), X_2(t), ..., X_{p_2}(t)) \tag{2.6}$$

As with the Cox PH model, the extended model contains a baseline hazards function which is multiplied by an exponential function. However, in the extended model, the exponential part contains both time-independent predictors, denoted

by X_i variables, and time-dependent predictors, denoted by $X_j(t)$ variables. The entire collection of predictors at time t is denoted by $X(t)$.

As with Cox PH model, the regression coefficients in the extended Cox model are estimated using a maximum likelihood (ML) procedure. ML estimates are obtained by maximizing a (partial) likelihood function L. However, the computations for the extended Cox model are more complicated than for the Cox PH model, because the risk sets used to form the likelihood function are more complicated with time-dependent variables.

Methods for making statistical inferences are essentially the same as for the PH model. It can be use Wald test and likelihood ratio tests and large sample confidence interval methods.

An important assumption of the extended Cox model is that the effect of a time-dependent variable $X_j(t)$ on the survival probability at time t depends on the value of this variable at that same time t, and not on the value at an earlier or later time.

Proportional hazards assumption is no longer satisfied when using the extended Cox model. We consider the formula for the hazard ratio that derives from the extended Cox model:

$$\hat{HR}(t) = \frac{h(t, \mathbf{X}^\star(t))}{h(t, \mathbf{X}(t))} = exp[\sum_{i=1}^{p_1} \beta_i(X_i^\star - X_i) + \sum_{j=1}^{p_2} \delta_j(X_j^\star(t) - X_j(t))] \qquad (2.7)$$

Since the general hazard ratio formula involves differences in the values of the time-dependent variables at time t, this hazard ratio is a function of time. Thus, in general, the extended Cox model does not satisfy the PH assumption if any δ_j is not equal to zero [37, 38].

In patients with mCRC, diagnostic and intervention procedures during follow-up, such as computerized tomography of the abdomen, magnetic resonance, x-ray of digestive system, surgery and radiotherapies, can be a proxy of the severity of the disease. Patients who need more frequent diagnostic tests may be those with a more aggressive cancer. Since the number of these interventions are dependent from the length of follow-up (i.e. patients with longer follow-up may undergo more interventions), they were used as continuous time-dependent covariates in the Cox proportional hazard model.

2.7 Sensitivity analyses

Several sensitivity analyses were performed in order to assess the robustness of the results.

First, a more flexible algorithm for defining the first-line treatment was performed. For this purpose, a 42-days period, instead of a 21-days period, was used to define the first-line treatment of subjects with mCRC. Indeed, the guidelines of the European Medicine Agency about bevacizumab state that "*therapy should not be initiated for at least 28 days following major surgery or until the surgical wound is fully healed*" [39]. Since the surgical intervention might be preceded by a neo-adjuvant chemotherapy and bevacizumab might be started after surgery, a 42-day period seemed more appropriate for defining the first-line treatment in this setting. According to this alternative algorithm, a subject was defined as exposed to bevacizumab+CT if there was a prescription of bevacizumab in the 42 days following the index date.

Two other sensitivity analysis were conducted and shown below. The first one was a missing multiple imputation, in order to account for missing data. The second one was the propensity score method, in order to account for measurable confounding.

2.7.1 Multiple imputation of missing data

One of the peculiarity of this project, based on the use of HCU database, is the availability of clinical information about CRC. However, missing data are normally not handled from the standard procedure of analysis, and observations with any missing values in any of the variable required for the analysis (incomplete cases) are excluded from the analysis. Restricting the analyses to the subset of patients with all the data observed would result in a significant loss of information, which causes a loss of precision and power. Moreover, it may result in potential systematic difference between the incomplete cases and complete cases, leading to biased estimations. Missing data can be classified into three categories:

- Missing completely at random: there are no systematic differences between the missing values and the observed values;
- Missing at random: any systematic difference between the missing values and the observed values can be explained by differences in observed data;
- Missing not at random: even after the observed data are taken into account, systematic differences remain between the missing values and the observed values.

The bias introduce from the missing data is different according to the type of missing data. Unfortunately, it is not possible to know the category of missing

data using observed data. In some circumstances, the analyses of complete cases do not lead to bias. In particular, missing data in predictor variable do not cause bias, if the reason of missing is not not associated with the outcome [40]. Outside this specific situation, missing data should be handled with ad-hoc statistical techniques.

Multiple imputation is a bayesian method that allows to take into account incomplete cases (i.e. observations with any missing data) with a two-step approach. First, this method creates multiple imputed datasets, in which missing values are replaced by imputed values. These are sampled from their predictive distribution based on the observed data. The imputation procedure fully accounts for the uncertainty in predicting the missing values by conferring appropriate variability into the multiple imputed values.

Second, standard statistical methods are used to fit the model of interest to each of the imputed datasets. Estimates associated to each of the imputed datasets differ because of the variation introduced in the imputation of the missing values (stage 1), and they are, then, average together to give overall estimated associations. Valid inferences are obtained because they are based on the average of the distribution of the missing data given the observed data [41].

2.7.1.1 The MI Procedure with SAS

The statistical software SAS allows to perform a multiple imputation by means of the MI Procedure. Multiple imputation inference involves three distinct phases:

1. The missing data are filled in (imputed) m times to generate m complete data sets;
2. The m complete data sets are analyzed by using standard procedures;
3. The results from the m complete data sets are combined for the inference.

1. The missing data are filled in (imputed) m times to generate m complete data sets

The following syntax can be used to run a multiple imputation on both quantitative and categorical variables that present missing values(missing-at-random).

PROC MI data= out= nimpute= seed=;

by ;

var ;

class ;

fcs discrim nbiter= ;

run; Below in shown the meaning of the statment specified in the MI procedure:

The DATA option specifies the input data set.

The OUT option specifies the output data set with imputed values.

The NIMPUTE option specifies the number of imputations.

The SEED option specifies the seed to begin random number generator. This option is useful to have the same results each time the procedure is run.

The BY statement specifies groups in which separate multiple imputation analyses are performed.

The VAR statement lists the numeric variables to be analyzed.

The CLASS statement lists the classification variables in the VAR statement.

The FCS statement specifies a multivariate imputation by fully conditional specification methods.

The DISCRIM option specifies the discriminant function method of classification variables.

The NBITER option specifies the number of burn-in iterations.

2.The m complete data sets are analyzed by using standard procedures

The following syntax performs as many Cox proportional hazard models as the number of imputation specified through the option NIMPUTE in the MI procedure.

PROC PHREG data=;

class ;

*model time*status= / risklimits;*

by ;

ods output ParameterEstimates= ;

run;

The BY statment allows to performed separate analysis, one for each of the m dataset built up at step 1.

3.The results from the m complete data sets are combined for the inference

The MIANALYZE procedure combines the results of the analyses of imputations (step 2) and generates valid statistical inferences. Such procedure reads parameter estimates and associated standard errors or covariance matrix that are computed by the standard statistical procedure (e.g. the Cox proportional hazard model) for each imputed data set. Then, the MIANALYZE procedure derives valid univariate inference for these parameters. The corresponding syntax is shown below:

PROC MIANALYZE parms()= ;

modeleffects ;

class ;

ods output ParameterEstimates= ;

run;

The MODELEFFECTS statement lists the effects (covariates) to be analyzed.

The CLASS statement lists the classification variables in the MODELEFFECTS statement. The variables in the MODELEFFECTS statement that are not specified in a CLASS statement are assumed to be continuous.

The ODS OUTPUT statement specifies the name of the dataset where the parameter estimates generated by the MIANALYZE procedure has to be exported.

2.7.2 Propensity score

The propensity score (PS) method is a statistical technique used in observational study, in order take into account confounding. When conducting an observational study, that is, when the exposure is not randomized to two or more groups, different groups characteristics may introduce confounding, making the estimates of the treatment effect biased.

In a randomized experiment, if the sample size is large enough, the randomization guarantees that, on average, there are not systematic differences between the characteristics of the statistical units assigned to different treatments. However, in a non-randomized study, the investigator cannot control the treatment assignment. As a consequence, differences in covariates among different groups may exist, leading to biased results. Traditional methods for taking into account confounding, such as restriction, stratification, matching and covariates adjustment, can be generally applied. However, residual confounding may remain, due to the limited number of covariates of adjustment that can be used. At this purpose, PS represents a valid method that allow to take into account a huge number of measured covariates.

The PS for an individual represents the propension to be exposed to a treatment, given a series of given characteristics. Thus, the PS is a single value that summarize all the considered covariates. Formally, the PS is the probability of being exposed to treatment E, conditionally to a set of covariates [42]. Let's consider,

for an individual i, a set of covariates $\mathbf{X} = (X_1, ..., X_n)$. The corresponding PS will be given by:

$$PS(\mathbf{X}) = P(E = 1|X_1, ..., X_n) \tag{2.8}$$

which can be easly estimated by a logistic regression.

Theoretically, in case of no unmeasured confounding, the PS method creates an assignment of statistical units to different treatments similar to the randomization, the so-called "quasi-randomization". Indeed, let's consider two subjects with the same PS, one in the treatment group and the other in the control group. They have, a priori, the same probability to be treated. We can imagine they were "randomly" assigned to each group, in the sense they had the same probability to be treated or control [43].

Once the PS are estimated for each individual, they can be used according to different techniques. The most common are matching, stratification and regression adjustment. For each of the previous techniques, the PS is estimated in the same way, but it is applied differently. The following formula is referred to a Cox model conditioned to the PS estimates and the exposure.

$$\lambda(t|E, PS(\mathbf{X})) = \lambda_0(t)exp^{\beta_E E + \beta_X PS(\mathbf{X})} \tag{2.9}$$

In this way, the PS method should balance the treated and untreated groups for all the considered covariates, generating unbiased estimate of the treatment effect.

In this project, we decided to use a PS method as a sensitivity analysis, in order to assess the robustness of the main analysis. Additional variables measured at baseline, other than those used in the main survival analysis, were considered for the estimate of the PS. Among these, the presence of a diagnostic imaging procedure (i.e. x-ray of digestive system, computerized tomography of the abdomen and magnetic resonance), the presence of a therapeutic procedure (i.e. radiotherapy and surgery), the number of hospitalizations, the number of outpatient services, the number of drug prescriptions and the time-to-treat, defined as the number of days between the mCRC diagnosis and the start of first-line treatment. The PS, after being estimated through a multivariate logistic model, were used as a 1:1 matching variable. For each individual exposed to bevacizumab+CT, an individual exposed to CT alone was randomly selected, on the basis of the value of the PS, and tolerating a difference of ± 0.05. Exposed individual who did not match with any unexposed individual were excluded from the analysis. Since the survival times of matched subjects could be dependent, the standard error of the usual logrank test for independent samples was modified, in order to accommodate the possible correlation induced by matching [44]. HRs of death were estimated by using a conditional Cox proportional hazard model.

Chapter 3

Results

During the period 2010-2012, 1,118 incident mCRC cases were identified from the five areas included in the study. Twenty-three subjects were excluded because they reported a morphological code of the tumour (ICDO3M code) inconsistent with CRC. Among the remaining, 415 subjects were further excluded because they did not receive any pharmacological treatment, 74 subjects were excluded because they were treated in first-line with other drugs (e.g. cetuximab) and 126 subjects were excluded because they reported a date of starting therapy more than 90 days after the diagnosis of mCRC. Among a final study cohort of 480 subjects, 101 were treated with bevacizumab+CT and 379 were treated with CT alone as first-line treatment for mCRC. The flow-chart of the cohort selection is shown in Figure 3.1.

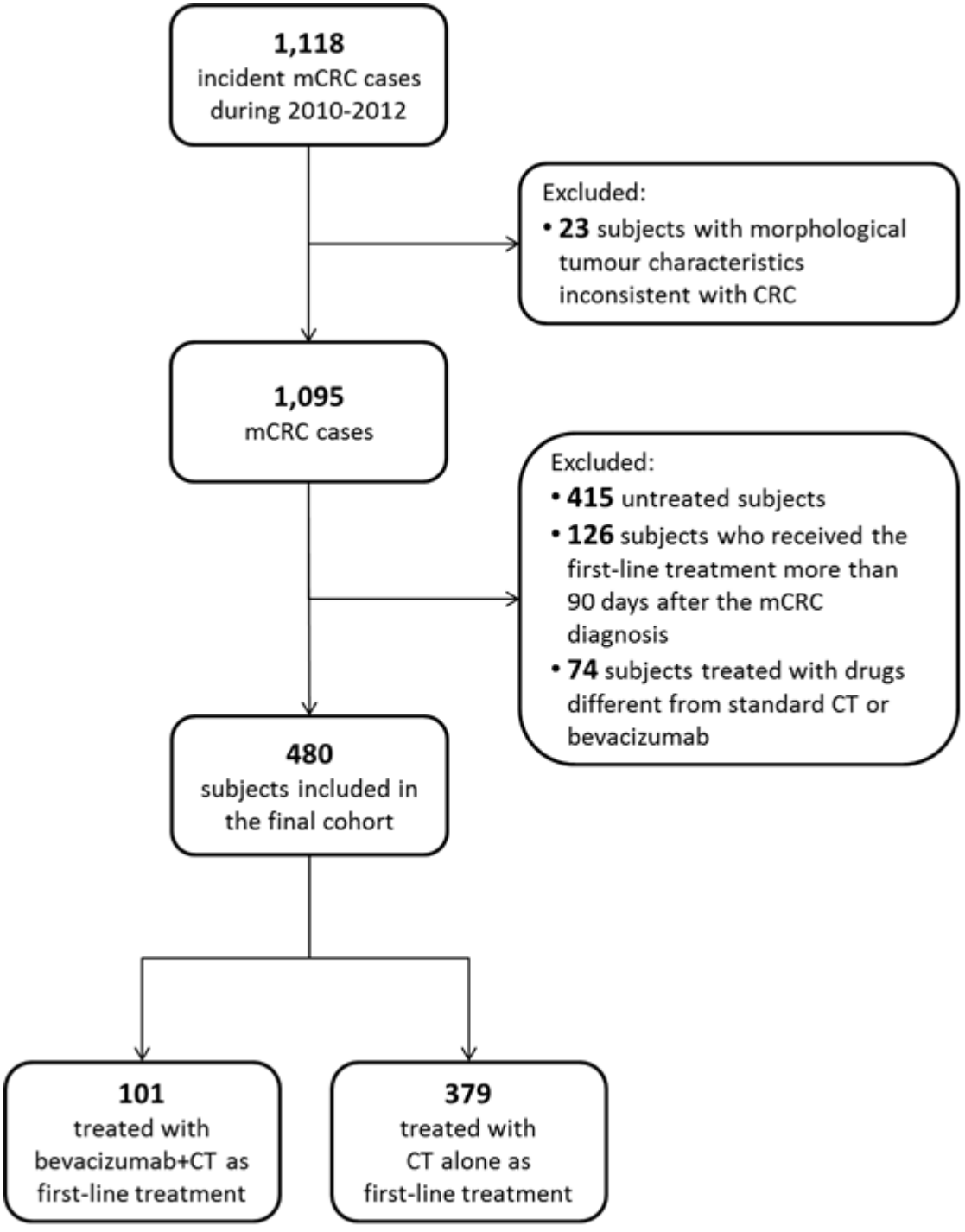


FIGURE 3.1: Flow-chart of cohort selection

3.1 First-line treatment

The proportion of mCRC cases among all CRC cases ranged from 11% in Varese Province to 20% in Mantova and Palermo province, probably reflecting the different impact on public heath of colorectal cancer screening.

Table 3.1 shows first-line treatments of the study cohort, overall and stratified by geographic area. Among 1,095 mCRC cases belonging to the initial cohort, 554 (50.6%) received at least one oncologic treatment after the diagnosis of mCRC. Among these, 101 (18.2%) used bevacizumab+CT as first-line treatment and 379 (68.4%) used CT alone.

The mean proportion of bevacizumab users among treated subjects in the whole study cohort was 18.2%, ranging from 13.8% in the Mantova Province to 30.2% in the Cremona Province.

The overall percentage of subjects treated with CT alone was 68.4%, varying from 55.6% in the Cremona Province to 72.4% in the Mantova Province.

Percentage of users of other drugs (mostly cetuximab) was about 14% in all centres, except for the Ragusa Province where it was 6.5%.

Overall, 415 (37.9%) mCRC cases resulted untreated, with the highest value in the Ragusa Province (51.0%). This may be due to several factors, including clinical characteristics of the disease and patients characteristics. When analysing characteristics of untreated subjects, it resulted that age was a strong discriminant

for being not treated. Indeed, around 80% of patients aged more than 80 years did not received any pharmacological treatment. Moreover, 197 (47.5%) untreated patients died within three months from the diagnosis of mCRC, indicating a terminal stage of disease.

It must be stated that the number of treated subjects in the Ragusa Province is underestimated, since the pharmacological treatment of 33 subjects was confirmed through the examination of the medical records, but no information were available on type and timing of prescriptions.

Treatment	**Overall** N (%)	**Cremona** N (%)	**Mantova** N (%)	**Palermo** N (%)	**Ragusa** N (%)	**Varese** N (%)
No. of mCRC cases	1,095	108	123	489	157	218
First-line treatment	554 (50.6)	63 (58.3)	58 (47.2)	265 (54.2)	46 (29.3)	122 (56.0)
Bevacizumab+CT	101 (18.2)	19 (30.2)	8 (13.8)	41 (15.5)	12 (26.1)	21 (17.2)
CT alone	379 (68.4)	35 (55.6)	42 (72.4)	187 (70.6)	31 (67.4)	84 (68.9)
Other	74 (13.4)	9 (14.3)	8 (13.8)	37 (14.0)	3 (6.5)	17 (13.9)
Start of treatment more than 90 days after mCRC diagnosis	126 (11.5)	19 (17.6)	4 (3.2)	50 (10.2)	31 (19.7)	22 (10.1)
Untreated	415 (37.9)	26 (24.1)	61 (49.6)	174 (35.6)	80 (51.0)	74 (33.9)

TABLE 3.1: First-line treatment for 1,095 mCRC cases, overall and by geographic area.

3.2 Baseline characteristics

Baseline characteristics of the study cohort are given in Table 3.2. About 55% of the cohort members were men. No difference by gender was observed between subjects treated with bevacizumab+CT and those treated with CT alone (p=0.967). Bevacizumab+CT users were younger than CT alone users (p¡0.001), had a lower Charlson comorbidity index (p=0.454), as well as a lower prevalence of diabetes (p=0.193), hypertension (p=0.681), ischemic heart disease (p=0.388) and cerebrocardiovascular disease (p=0.250).

TABLE 3.2: Baseline characteristics of 101 mCRC cases treated with bevacizumab+CT and 379 mCRC cases treated with CT alone.

	Bevacizumab+CT N=101	CT alone N=379
Year of mCRC diagnosis		
2010	38 (37.6)	145 (38.3)
2011	27 (26.7)	120 (31.6)
2012	36 (35.6)	114 (30.1)
	p-value=0.489	
Gender		
Male	56 (55.4)	211 (55.7)
Female	45 (44.6)	168 (44.3)
	p-value=0.967	
Age		
Median min-max	63 (33-79)	69 (32-88)
<50	19 (18.8)	32 (8.4)
50-59	21 (20.8)	64 (16.9)
60-69	30 (29.7)	103 (27.2)
70-79	31 (30.7)	141 (37.2)
≥80	0 (0.0)	39 (10.3)
	p-value<0.001	
Geographic area		
Cremona	19 (18.8)	35 (9.2)
Mantova	8 (7.9)	42 (11.1)
Palermo	41 (40.6)	187 (49.3)
Ragusa	12 (11.9)	31 (8.2)
Varese	21 (20.8)	84 (22.2)
	p-value=0.043	
Tumour site (ICDO3T)		
Colon	78 (77.2)	282 (74.4)
Rectosigmoid junction	11 (10.9)	27 (7.1)
Rectum	12 (11.9)	69 (18.2)
Anus and anal canal	0 (0.0)	1 (0.3)
	p-value=0.279	

Table 3.2: continue on the next page

Table 3.2: continued

	Bevacizumab+CT N=101	CT alone N=379
Grading		
Missing	3 (3.1)	20 (5.3)
Well differentiated	3 (3.1)	9 (2.5)
Moderately differentiated	47 (48.0)	194 (54.0)
Poorly differentiated	26 (26.5)	60 (16.7)
Undifferentiated	0 (0.0)	2 (0.6)
Unknown	22 (22.4)	94 (26.2)
	p-value=0.248	
Tumour size (T)		
Missing	23 (22.8)	93 (24.5)
T1	0 (0.0)	2 (0.7)
T2	2 (2.6)	5 (1.7)
T3	29 (37.2)	81 (28.3)
T4	20 (25.6)	53 (18.5)
Tx	27 (34.6)	145(50.7)
	p-value=0.095	
Nodal status (N)		
Missing	23 (22.8)	93 (24.5)
N0	8 (10.3)	31 (10.8)
N1	13 (16.7)	40 (14.0)
N2	24 (30.8)	60 (21.0)
Nx	33 (42.3)	155 (54.2)
	p-value=0.208	
Surgery		
No	26 (25.7)	169 (44.6)
Yes	75 (74.3)	210 (55.4)
	p-value=<0.001	
Charlson comorbidity index		
≤ 8	90 (89.1)	327 (86.3)
>8	11 (10.9)	52 (13.7)
	p-value=0.454	
Diabetes		
No	98 (97.0)	355 (93.7)
Yes	3 (3.0)	24 (6.3)
	p-value=0.193	

Table 3.2: continue on the next page

Table 3.2: continued

	Bevacizumab+CT N=101	CT alone N=379
Hypertension		
No	92 (91.1)	340 (89.7)
Yes	9 (8.9)	39 (10.3)
	p-value=0.681	
Ischemic heart disease		
No	99 (98.0)	363 (95.8)
Yes	2 (2.0)	16 (4.2)
	p-value=0.388	
Cerebrocardiovascular disease		
No	97 (96.0)	352 (92.9)
Yes	4 (4.0)	27 (7.1)
	p-value=0.250	
Respiratory disease		
No	94 (93.1)	353 (93.1)
Yes	7 (6.9)	26 (6.9)
	p-value=0.980	
Renal disease		
No	101 (100.0)	372 (98.1)
Yes	0 (0.0)	7 (1.9)
	p-value=0.354	
Liver disease		
No	98 (97.0)	376 (99.2)
Yes	3 (3.0)	3 (0.8)
	p-value=0.111	

3.3 Predictors of bevacizumab use

Age at diagnosis was a strong predictor of bevacizumab use. Patients aged less than 50 years had a significant higher probability to be treated with bevacizumab (OR=3.90, 95%CI 1.91-7.96), as well as those aged 50-59 years (OR=1.83, 95% CI 0.96-3.47) and those aged 60-69 year (OR=1.67, 95% CI 0.95-2.94), as compared to patients aged more than 70 years. Subjects who underwent a surgical intervention had a higher probability to be treated with bevacizumab, as compared to patients who did not (OR=2.57, 95%CI 1.54-4.27). No trend in the fraction of patients treated with bevacizumab was observed over time (p=0.296). Having a Charlson comorbidity index higher than 8 was associated with an lower probability to receive bevacizumab (OR=0.75, 95%CI 0.36-1.54), indicating that bevacizumab is more likely to be administered in patients with better health conditions (Table 3.3).

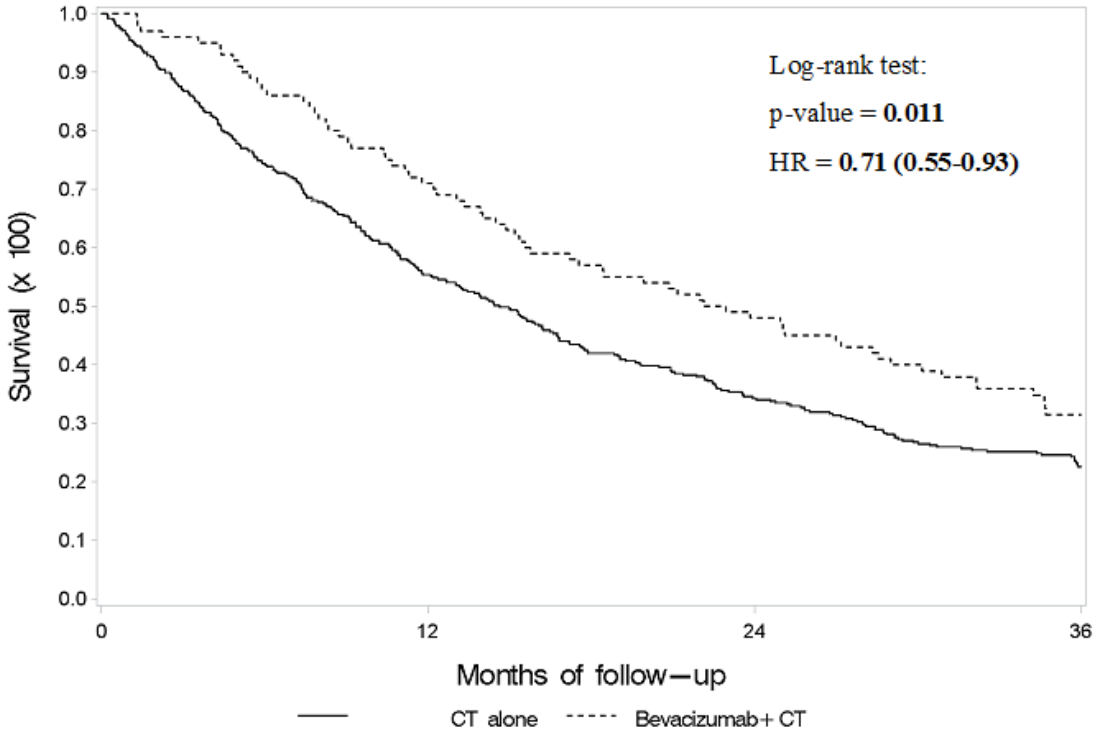
	N (% bevacizumab users)	**OR (95% CI)**
Year		
2010	183 (20.8)	1
2011	147 (18.4)	1.00 (0.57-1.77)
2012	150 (24.0)	1.34 (0.78-2.30)
		p-trend = 0.296
Gender		
Male	267 (21.0)	1
Female	213 (21.1)	0.91 (0.57-1.44)
Age		
<50	51 (37.3)	3.90 (1.91-7.96)
50-59	85 (24.7)	1.83 (0.96-3.47)
60-69	133 (22.6)	1.67 (0.95-2.94)
≥70	211 (14.7)	1
Surgery		
No	195 (13.3)	1
Yes	285 (26.3)	2.57 (1.54-4.27)
Charlson index		
≤8	417 (21.6)	1
>8	63 (17.5)	0.75 (0.36-1.54)

TABLE 3.3: Predictors of bevacizumab use. Odds Ratios (OR) and 95% Confidence Intervals (CI) estimated by a multivariable logistic regression model.

3.4 Kaplan-Meier survival estimates

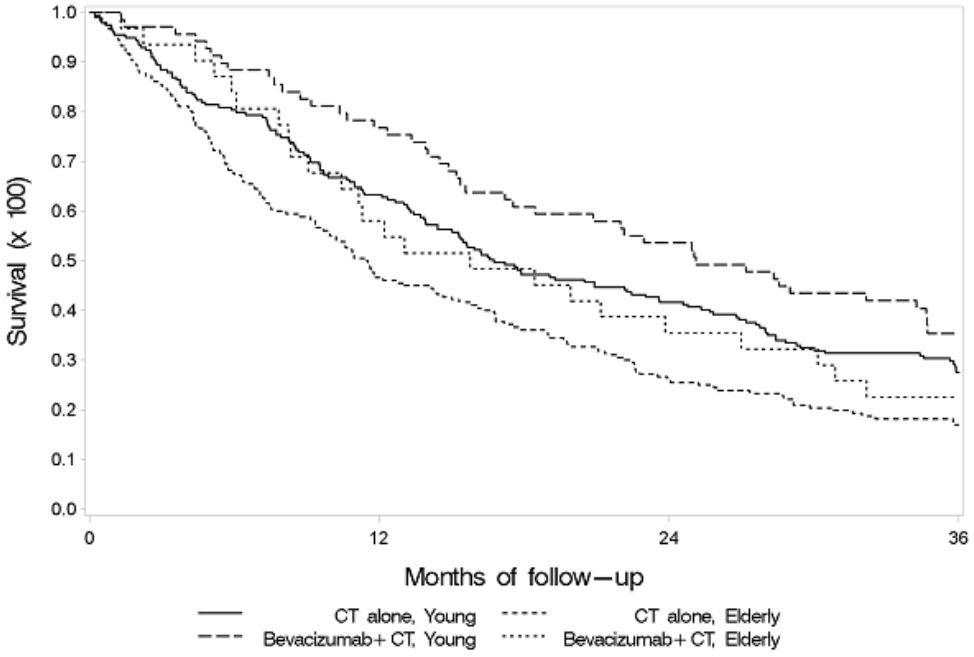
Median OS was 22.5 months in the bevacizumab+CT group and 14.6 months in the CT alone group (p-value log-rank test=0.011), corresponding to a crude HR of death of 0.71 (95%CI 0.55-0.93). Survival estimates at 1, 2 and 3 years were, respectively, 0.71, 0.48 and 0.32 in the bevacizumab+CT group and 0.55, 0.34 and 0.23 in the CT alone group (Figure 3.2). The follow-up was censored at three years, due to the small number of patients with a follow-up longer than three years.

A stratified analysis on OS was performed by age (Figure 3.3). As expected, age is a strong prognostic factor of survival. Bevacizumab was associated to an increased median OS in patients aged less than 70 years. Among these patients, median OS was 25.1 for those treated with bevacizumab+CT and 16.8 months for those treated with CT alone (p=0.087). The beneficial effect of bevacizumab on OS was also observed in patients aged more than 70 years. Median OS was 15.8 and 11.5 months in elderly patients using bevacizumab+CT and CT alone, respectively (p=0.221).



	Bevacizumab+CT No. = 101	**CT alone No. = 379**
Number of deaths	68 (67.3)	292 (77.0)
Median OS, months	**22.5**	**14.6**
Survival estimates		
1 year	0.71	0.55
2 year	0.48	0.34
3 year	0.32	0.23

FIGURE 3.2: Kaplan-Meier survival estimates stratified by first-line treatment.



	Young (<70 yrs)		Elderly (=70 yrs)	
	Bevacizumab+CT No.=70	**CT alone No.=199**	**Bevacizumab+CT No.=31**	**CT alone No.=180**
Number of deaths	44 (62.9)	143 (71.9)	24 (77.4)	149 (82.8)
Median OS, months	**25.1**	**16.8**	**15.8**	**11.5**
Log-rank test	p = 0.087		p = 0.221	

FIGURE 3.3: Kaplan-Meier survival estimates stratified by first-line treatment and age.

3.5 Cox PH model

The crude HR of death for bevacizumab+CT users as compared to CT alone users was 0.71 (95% CI 0.55-0.93). When adjusting for covariates measured at baseline, the corresponding HR was 0.82 (95% CI 0.62-1.07).

A survival analysis taking into account covariates measured during follow-up was performed. Number of radiotherapies, number of computerised tomography of the abdomen, number of magnetic resonances, number of x-rays of the digestive system and number of surgeries were considered and included to the model as time-dependent covariates. The corresponding Cox proportional hazard model adjusted by covariates measured both at baseline and during follow-up estimated an HR of deaths of 0.82 (95% CI 0.62-1.08) for bevacizumab+CT users, as compared to CT alone users. No differences were observed by gender (p=0.46). A decreased mortality was detected in patients who underwent a surgical intervention before the treatment onset (HR=0.49, 95% CI 0.39-0.61), as well as in patients aged less than 70 years (see Table 3.4). A positive trend in survival was observed over the years, even if not statistical significant (p=0.299). A unit increment in the number of diagnostic procedure (computerised tomography of abdomen, magnetic resonance and x-ray of digestive system) was associated with a worst prognosis, indicating that subjects followed-up more often are those with a worse health conditions.

	No.	**No. (%) of events**	**HR (95% CI)**
First-line treatment			
CT alone	379	292 (77.0)	1
Bevacizumab+CT	101	68 (67.3)	0.82 (0.62-1.08)
Year			
2010	183	143 (78.1)	1
2011	147	112 (76.2)	0.94 (0.73-1.21)
2012	150	105 (70.0)	0.87 (0.67-1.13)
			p- trend = 0.296
Gender			
Male	267	199 (74.5)	1
Female	213	161 (75.6)	1.01 (0.82-1.26)
Age			
<50	51	38 (74.5)	0.80 (0.55-1.15)
50-59	85	53 (62.4)	0.56 (0.41-0.77))
60-69	133	96 (72.2)	0.80 (0.62-1.04)
≥70	173 (82.0)	(14.7)	1
			p- trend = 0.005
Surgery			
No	195	166 (85.1)	1
Yes	285	194 (68.1)	0.49 (0.39-0.62)
Charlson index			
≤8	417	309 (74.1)	1
>8	63	51 (80.9)	1.23 (0.90-1.68)
Covariates measured during FU:			
Radiotherapy			1.05 (1.01-1.10)
Computerised tomography of abdomen			1.05 (0.98-1.13)
Magnetic resonance			1.24 (1.07-1.43)
X-ray of digestive system			2.22 (1.45-3.39)
Surgery			0.62 (0.49-0.77)

TABLE 3.4: Hazards ratios (HR) of death, and 95% confidence intervals (CI), estimated by a multivariable Cox proportional hazard model.

3.6 Patterns of treatment

Second-line treatments, defined as either switching from bevacizumab to another biologic drug or adding a biologic drug to standard chemotherapy, were evaluated. Among 101 patients starting therapy for mCRC with bevacizumab+CT, 17 (16.8%) switched to cetuximab during follow-up. Median time-to-switch, defined as the number of days from treatment onset with bevacizumab to the time of switch to cetuximab, was 300 days, and the median number of prescriptions of cetuximab was 23. Eleven subjects (10.9%) switched from bevacizumab to panitumumab. Median time-to-switch was 648 days, and the median number of prescriptions of panitumumab was eight.

Among 379 patients starting therapy for mCRC with CT alone, 105 (27.7%) switched to bevacizumab during follow-up. Median time-to-switch was 218 days, and median number of prescriptions of bevacizumab was seven. Thirty-six subjects (9.5%) switched to cetuximab during follow-up. Median time-to-switch was 288 days, and the median number of prescriptions of cetuximab was 12. Ten subjects (2.6%) switched to panitumumab during follow-up. Median time-to-switch was 586 days, and the median number of prescriptions of panitumumab was six (Table 5).

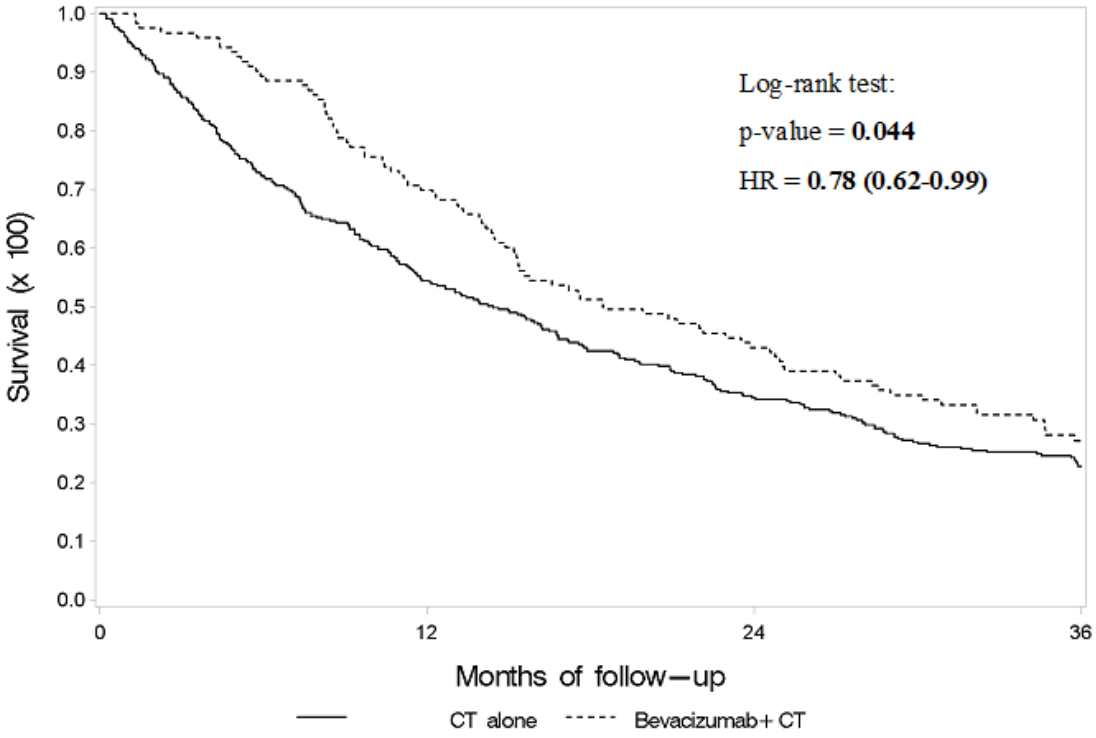
Duration of treatment with bevacizumab, defined as the number of days elapsed between the first and the last prescription of bevacizumab, was assessed. Among

101 patients starting therapy with bevacizumab+CT, the median duration of bevacizumab use was 176 days, and the median number of prescriptions of bevacizumab was nine. Timing of bevacizumab prescriptions, defined as the number of days elapsed from one prescription of bevacizumab to the following one, was also evaluated. Fifty-two percent of prescriptions were prescribed 14 days apart (±1 day), and 26% of prescriptions were prescribed 21 days apart (±1 day).

3.7 Sensitivity analyses

A 42-days period, instead of a 21-days period, was considered to define the first-line treatment.

In this setting, 124 subjects were classified as bevacizumab+CT users and 351 subjects were classified as CT alone users as first-line treatment for mCRC. Median OS was 18.5 and 14.4 months in the bevacizumab+CT and in the CT alone group, respectively (p=0.044). The corresponding crude HR was 0.78 (95% CI 0.62-0.99) (Figure 3.4). When adjusting for covariates measured both at baseline and during follow-up, the HR was 0.90 (95% CI 0.70-1.16).



	Bevacizumab+CT No. = 124	**CT alone No. = 351**
Number of deaths	89 (71.8)	270 (76.9)
Median OS, months	**18.5**	**14.4**
Survival estimates		
1 year	0.70	0.54
2 year	0.43	0.34
3 year	0.27	0.23

FIGURE 3.4: Sensitivity analysis. Kaplan-Meier survival estimates stratified by first-line treatment.

3.7.1 Multiple imputation of missing data

Missing data were observed on clinical characteristics of the tumour (i.e. tumour size(T), lymph-nodal status (N) and grading). In particular, the CR of Varese Province did not report any data about T and N. Considering the remaining four CR, the percentage of observations with missing data varied across among centres from 9% up to 68% (Table 3.5).

Using the imputed data on tumour grading, the multivariate HR of death was 0.77 (95% CI 0.58-1.01) for patients treated with bevacizumab+CT, as compared to those treated with CT alone. The corresponding HR, without adjusting for tumour grading, was 0.81 (95% CI 0.62-1.06).

When considering also imputed data on T and N, the model was restricted to 375 subjects, since the CR of Varese Province did not reported information to those variables, and, consequently, observations referred to such centre were excluded from the analysis. The model adjusted by tumour grading, T and N, other than baseline covariates, resulted in an HR of death of 0.83 (95% CI 0.61-1.14). The same model, without adjusting for tumour grading, T and N, gave an HR of 0.87 (95% CI 0.65-1.18) (Table 3.6).

	Geographic area				
	Cremona	**Mantova**	**Palermo**	**Ragusa**	**Varese**
	No.=54	**No.=50**	**No.=228**	**No.=43**	**No.=105**
T	37.0%	24.0%	61.0%	27.9%	100.0%
N	31.5%	26.0%	68.0%	32.6%	100.0%
Grading	24.1%	26.0%	40.8%	9.3%	15.2%

TABLE 3.5: Percentages of observation with missing value on cancer size (T), lymph nodal status (N) and grading.

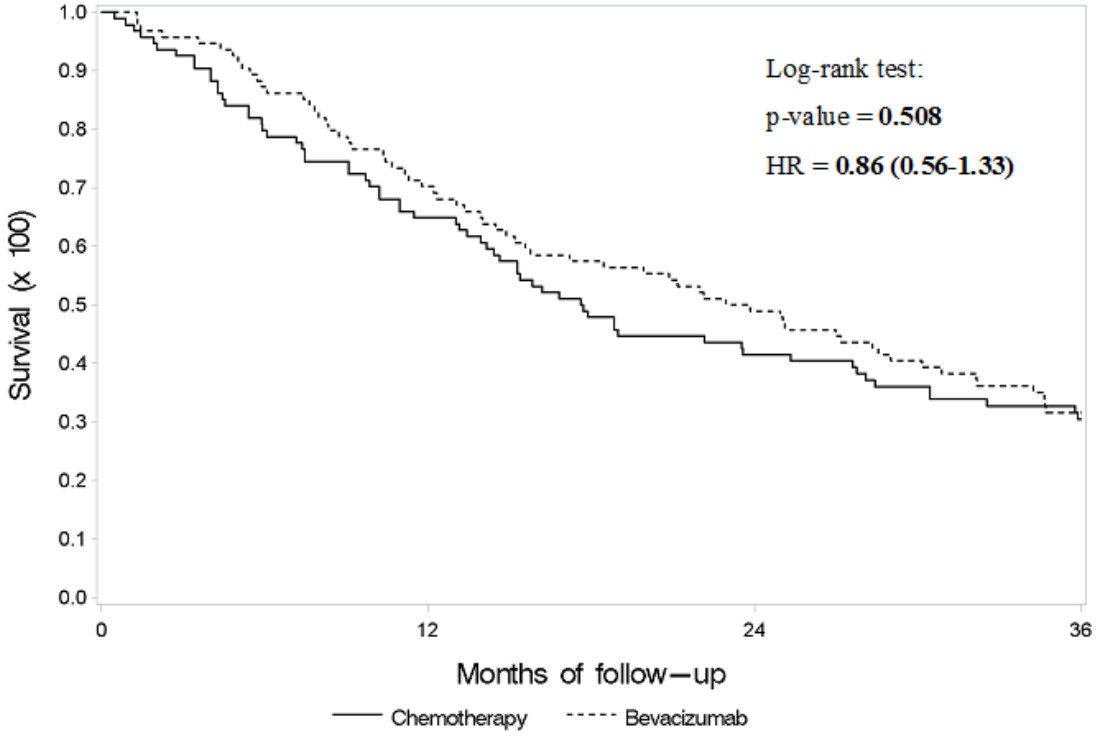
Model	**Study population**	**Number of subjects**	**HR (95% CI)**
Adjusted by grading	All centres	480	0.77 (0.58-1.01)
Not adjusted by grading	All centres		0.81 (0.62-1.06)
Adjusted by T, N and grading	Excluded Varese	375	0.83 (0.61-1.14)
Not adjusted by T, N and grading		375	0.87 (0.65-1.18)

TABLE 3.6: Percentages of subjects with missing value on cancer size (T), lymph nodal status (N) and grading.

3.7.2 Propensity score

Among 101 subjects treated with bevacizumab+CT, 94 were matched to a subjects treated with CT alone according to the PS value (with a 1:1 ratio). Seven subjects treated with bevacizumab+CT were excluded from this analysis, since they had an high PS which did not find any matching in the group of subjects treated with CT alone.

Median OS was 23.4 months and 17.7 months, respectively, in the bevacizumab+CT and in the CT alone group (p=0.747) .The corresponding HR was 0.86 (0.56-1.33) (Figure 3.5).



	Bevacizumab+CT No. = 94	**CT alone No. = 94**
Number of deaths	64 (68.1)	65 (69.2)
Median OS, months	**23.4**	**17.7**
Survival estimates		
1 year	0.70	0.55
2 year	0.49	0.41
3 year	0.32	0.30

FIGURE 3.5: Propensity score. Kaplan-Meier survival estimates stratified by first-line treatment.

Chapter 4

Discussion

The present study was conducted in order to evaluate the impact of first-line bevacizumab added to standard chemotherapy in the real-world clinical practice of patients with a diagnosis of mCRC.

The median OS of patients treated with first-line bevacizumab+CT was 22.5 months. This result was comparable to those coming from other post-marketing observational studies conducted to assess the effectiveness of bevacizumab in a real-world setting. In particular, the BEAT study [45] reported a median OS of 22.7 months in a large cohort of 1,914 mCRC cases treated with first-line bevacizumab. The BRiTE study [46] estimated a median OS of 22.9 months in 1,953 mCRC cases in the United States, treated with first-line bevacizumab combined with chemotherapy. In the ARIES study [47], a large US-based multicentre

prospective study, the median OS of 1,550 mCRC cases treated with first-line bevacizumab was 23.2 months. The ETNA observational study [48], conducted on 411 mCRC cases treated with first-line bevacizumab in several centres in France, estimated a median OS of 25.3 months.

In our study, the median gain in OS among patients treated with and without bevacizumab was 7.9 months, with a statistically significant reduction of the crude risk of death. However, given the observational nature of the study, the crude comparison among the risk in the two groups is not appropriate. Indeed, different patients characteristics in the two exposure groups may affect the relationship among the effect of bevacizumab and the risk of death, leading to biased estimates. In particular, we observed that bevacizumab was more likely administered in young patients, who underwent surgical intervention, and, perhaps, with less compromised health conditions. When considering the risk of death adjusted for a set of potential confounders, the benefit of adding bevacizumab to CT remained, although not statistically significant (HR=0.82, 95% CI 0.62-1.08). This result is consistent with those reported from the few observational studies that compared the OS of patients treated with and without bevacizumab. In particular, Meyerhardt [25] recruited a cohort of 1,526 mCRC cases from 2002 to 2007. First-line bevacizumab was associated with improved overall survival (adjusted HR 0.85, 95% CI 0.78-93). However, after restricting the study cohort to years 2004-2007 (i.e. from the year of the approval of bevacizumab), the adjusted HR was 0.93 (95% CI 0.84-1.02). Hammerman [26] compared the OS of 1,052 subjects treated

with first-line bevacizumab between 2006 and 2009 to 687 historical controls diagnosed between 2001 and 2004, when bevacizumab was not available yet. The adjusted HR of death was 0.75 (95% CI 0.68-0.84). Similarly, Renouf [27] selected 969 mCRC cases in the period 2003-2004 and 448 mCRC cases in 2006 (i.e. the date of first use of bevacizumab for mCRC). The median OS among the two cohorts was 13.8 and 17.3 months, respectively (p¡0.001). However, the observed protective effect of bevacizumab may be attributed, at least in part, to an improvement of the treatment of mCRC over the years.

The analysis stratified by age showed a statistical significant beneficial effect of bevacizumab in patients age less than 70 years. An median gain of OS of 4.3 months was observed also in elderly patients (i.e. those age more than 70 years), even if not statistically significant. This may be due to the small number of patients in this age strata. This result is coherent with the result reported on a recent meta-analysis of RCTs [49], in which the authors evaluated the efficacy of first-line bevacizumab in elderly or unfit patients with mCRC. The pooled HR of death was 0.79 (95% CI 0.64-0.98) for patients treated with bevacizumab in addition to CT.

The robustness of the results was evaluated though several sensitivity analysis. In the first one, a less strict algorithm was allowed in order to define the first-line treatment with bevacizumab. In this setting, the OS of patients treated with CT alone remained very similar to the one coming from the main analysis (14.4

vs 14.6 months, respectively). However, the OS of patients treated with bevacizumab+CT decreased from 22.5 to 18.5 months. This is due to the fact that, in this configuration, a quote of patients not using bevacizumab in the main analysis is became bevacizumab users, according the 42-days criteria. Since the patients not using bevacizumab were associated to a lower OS, these patients reduced the median OS of those using bevacizumab.

In the second sensitivity analysis, we performed a multiple imputation of missing data. Indeed, using the Cancer Registries to select the cohort, some characteristics of the tumour, usually not included in the HCU database, were available (i.e. cancer site, lymph nodal status and grading). However, several observations had missing values in such variables and, therefore, would have been excluded from the analyses. The comparison of the Cox models adjusted and unadjusted for cancer characteristics gave similar results, suggesting that such variables are not to be considered important confounders.

Finally, we performed a PS matching in order to take into account the potential bias introduced by residual measurable confounding. At this purpose, we built the PS based on baseline characteristics and additional variables available from HCU databases. In this way, we obtained two groups of patients with similar baseline characteristics and, by matching subjects on the PS, with the same probability to be treated with bevacizumab (DAgostino, 1998). The HR of death coming from the matching analysis on the PS was similar to the multivariable HR of death

coming from the main model. This result suggest that the residual confounding introduced by measured covariate not included in the main model has not a strong effect of the association between the exposure to bevacizumab and the risk of death.

We were not able to evaluate the effect of second-line bevacizumab use in patients starting first-line therapy with CT alone, since the second-line treatment is a proxy of disease progression. Thus, the exposure to second-line bevacizumab may result in a positive association with OS. However, this result may be due to the disease progression instead of a harmful effect of bevacizumab.

Progression-free survival (i.e. the time from first-line treatment starting to disease progression) was not considered in the analyses, because we were not able to detect such end-point.

Our study have several strengths. First, it involves a community-based population coming from different geographical areas in both Northern and Southern Italy, reflecting the prescribing habits of different physicians. In the study cohort were included unselected patients, diagnosed among the entire resident population of the five areas included in the study, without any restriction on age and on previous comorbidity, guaranteeing the representativeness of the routine clinical practice. Second, the selection of the cohort of patients with mCRC was based on the CR. This aspect allows to minimize the misclassification of the diagnosis, which typically affects those observational studies, based of HCU databases, in which the cancer diagnoses are based on the ICD9 codes reported in the hospital discharge

form database (Corrao, 2015). Moreover, clinical characteristics of the tumour were available, although incomplete for some observations.

This study has also some limitations. The administrative purpose for which the HCU databases were created may limit the accuracy and the completeness of the data recorded in there. In particular, we were not able to define the different therapeutic schemes (i.e. FOLFIRI, FOLFOX), and put them in relation with the OS. Another limitation is the lack of information on lifestyle habits, such as smoking and lifestyle habits, and specific information about cancer (i.e. k-ras and n-ras markers).

Chapter 5

Conclusions

The present study aimed to generate evidence about the impact of the use of bevacizumab for the treatment of mCRC in the real-world clinical practice, in five different geographic areas in Italy. The results suggest a beneficial effect of first-line bevacizumab treatment on overall survival in patients with mCRC, even not statistically significant when adjusting for potential confounders. A favourable prognosis of patients treated in first-line treatment with bevacizumab+CT, as compared to those treated with CT alone, was observed.

The median gain, in terms of overall survival, obtained by adding bevacizumab to standard chemotherapy was 7.9 months. Young ages at diagnosis of mCRC (¡70 years), undergoing a surgical intervention and having less comorbidities (i.e. having better health condition) were predictors of bevacizumab use. Being aged less than 80 years and having had a surgical intervention were associated with a

better prognosis.

Several sensitivity analyses were performed, confirming the robustness of the results. HCU databases are a powerful tool for conducting community-based observational studies.

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Appendix 1

ICD9-CM codes of procedures:

- Surgery: 32.3, 34.59, 42.86, 44.39, 45.41, 45.43, 45.49, 45.51, 45.61, 45.62, 45.71-45.76, 45.79, 45.8, 45.90-45.94, 46.01- 46.04, 46.10, 46.11, 46.13, 46.14, 46.20-46.23, 46.40, 46.43, 46.51, 46.52, 46.76, 46.79, 46.85, 46.93, 46.94, 48.33, 48.35, 48.49, 48.5, 48.62, 48.63, 48.69, 48.79, 50.22, 50.29, 50.3, 54.12, 54.4, 54.51, 54.59, 54.61, 68.8.
- Radiotherapy: 92.23.
- Computerized tomography of the abdomen: 88.01.
- Magnetic resonance: 88.91, 88.92, 88.93, 88.94, 88.95, 88.97.
- X-ray of digestive system: 87.6.

ICD9-CM codes of diagnoses:

- Diabetes: 250.*
- Hypertension: 401.*-405.*, 272.0-272.4
- Ischemic heart diseases: 410.*-414.*
- Cerebrocardiovascular diseases: 42*-43*

- Respiratory diseases: 460.*-519.*
- Renal diseases: 584.*-586.*
- Liver disease: 571.*, 573.8-573.9