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Valutazione della Velocità di Progressione di Malattia come biomarker e Sviluppo di Nomogramma Clinico per predire l'outcome alla seconda di linea di trattamento nei pazienti affetti da tumore del colon-retto metastatico trattati con bevacizumab beyond progression o aflibercept

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Colorectal cancer: Overview

1.1 Epidemiology and risk factors

Colorectal cancer (CRC) is the third most commonly occurring cancer in men and the second most commonly occurring cancer in women. There were over 1.8 million new cases in 2018. [1](Fig. 1.1)

Over the last 20 years, and the last decade in particular, the clinical outcome for patients with metastatic colorectal cancer (mCRC) has improved greatly due not only to an increase in the number of patients being referred for and undergoing surgical resection of their localized metastatic disease but also to a more strategic approach to the delivery of systemic therapy and an expansion in the use of ablative techniques.

In addition, mortality from CRC decreased by almost 35% from 1990 to 2007 [2] and is currently down by about 50% from peak mortality rates.[1]

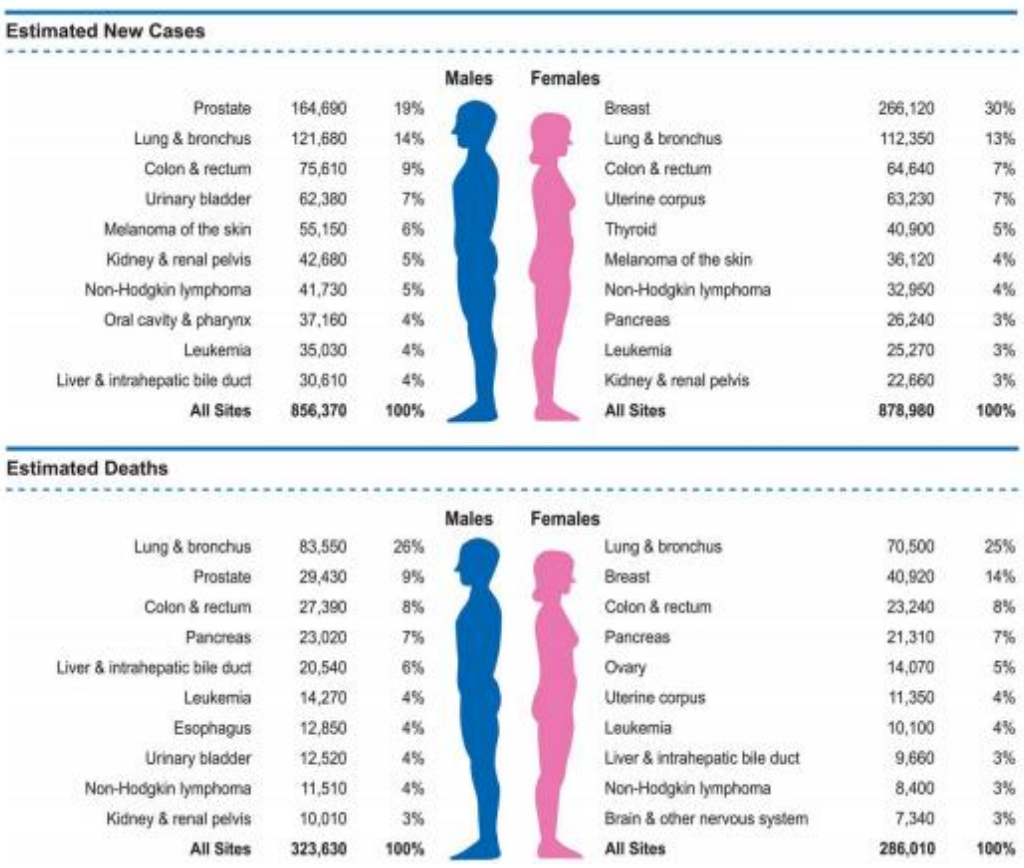

Fig 1.1 Cancer Types for the Estimated New Cancer Cases and Deaths by Sex, United States, 2018

15% of the neoplasms are localized at the level of the cecum and caecal appendix, 8% at the right ascending colon, 3% at the hepatic flexure, 5% at the transverse colon, 3% at the splenic flexure, descending or left colon 3%, sigma 20 %, sigmo-rectal junction 7%, rectum 27% and finally, anus 2%. [3](Fig 1.2)

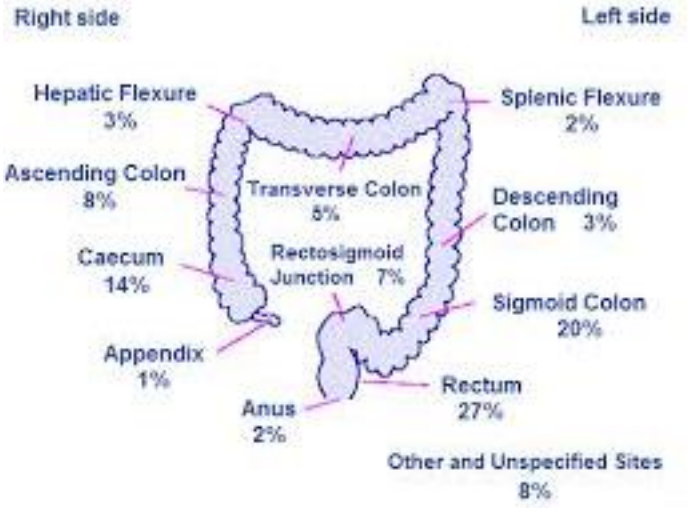

Figure 1.2 Colorectal cancer location

Colorectal cancer etiology is very complex and involves interactions between environmental factors and inherited susceptibility.

Several factors have been shown to put individuals at risk to CRC and these include age, the presence of polyps, inflammatory bowel disease, lifestyle, genetic background and family medical history.

Approximately 20% of cases of colon cancer are associated with familial clustering, and first-degree relatives of patients with colorectal adenomas or invasive colorectal cancer are at increased risk for colorectal cancer. [4-6] The most common genetic syndromes are familial adenomatous polyposis (FAP) syndrome and hereditary non polyposis colorectal cancer (HNPCC) syndrome, also known as Lynch syndrome, resulted from germline mutations in DNA mismatch repair (MMR) genes (MLH1, MSH2, MSH6, and PMS2).

Lynch syndrome is the most common form of genetically determined colon cancer predisposition, accounting for 2% to 4% of all colorectal cancer cases and often leads to cancers that occur at younger age.[7-11]

Recognized risk factors for the development of colorectal cancer include inflammatory bowel disease (ie, ulcerative colitis, Crohn's disease), smoking, the consumption of red and processed meats, alcohol consumption, diabetes mellitus, low levels of physical activity, metabolic syndrome, and obesity/high body mass index (BMI). [12-15]

In the EPIC cohort of almost 350.000 individuals, those who adhered to 5 healthy lifestyle factors (healthy weight, physical activity, non-smoking, limited alcohol consumption, and healthy diet) had an HR for the development of colorectal cancer of 0.63 (95% CI, 0.54–0.74) compared with those who adhered to ≤1 of the factors.[15]

There is good evidence that patient taking non-steroidal anti-inflammatory drugs (NSAIDs) reduce their risk of developing colorectal cancer. In 2003, a randomized controlled trial of over 1000 patients concluded that daily aspirin reduced the risk of colorectal adenoma formation in patients with a history of polyps. These findings are supported by epidemiological data which suggests that NSAIDs not only reduce the incidence of adenomas but also reduce the risk of progression to adenocarcinoma.[15] In addition, some data suggest that

smoking, metabolic syndrome, obesity, and red/processed meat consumption are associated with a poor prognosis. adenocarcinoma.[15-17]

Conversely, post-diagnosis fish consumption may be associated with a better prognosis. The relationship between diabetes and colorectal cancer is complex: although patients with colorectal cancer and diabetes appear to have a worse prognosis than those without diabetes [19], diabetic colorectal cancer patients treated with metformin seem to have a survival benefit over those not treated with metformin. [20-21]

1.2 Pathology and Staging

CRC is histologically divided into several types, suggested by World Health Organisation (WHO), with adenocarcinoma, mucinous adenocarcinoma and signet ring cell cancer being the most common subtypes in decreasing order (Table 1.1).

Adenocarcinoma
Mucinous adenocarcinoma (>50 % mucinous)
Signet-ring cell carcinoma (>50 % signet-ring cells)
Squamous cell carcinoma
Adenosquamous carcinoma
Small cell carcinoma
Medullary carcinoma
Undifferentiated carcinoma
Other

Table 1.1 The classification of CRC subtypes suggested by WHO

The most common histopathological classification of colorectal cancer is based on 3 parameters: the infiltrative growth of the primary tumour (T), spread to regional lymph node (N) or distant organs (M).[22] (Table 1.2)

The TNM-staging system provides broad prognostic information and facilitates decision-making in therapy. To predict the likelihood of detecting metastasis, at least 12 numbers of lymph nodes need to be examined, and the more lymph node that are examined by the pathologist is advantageous for survival in stage II CRC. [23] The distance between tumour and transverse margin is considered optimal over 5 cm in order to avoid recurrence in the anastomosis.

Stage	T	N	M
I	T1-2	0	0
IIA	T3	0	0
IIB	T4	0	0
IIIA	T1-2	1	0
IIIB	T3-4	1	0
IIIC	Any	N2	0
IV	Any	Any	1

Tx	Primary tumour cannot assessed
Tis	Carcinoma in situ
T1	Tumour invades submucosa
T2	Tumour invades muscularis propria
T3	Tumour invades through muscularis propria into subserosa or into non-peritonealized pericolonic or perirectal tissue
T4	Tumour directly invades other organs or structures and/or perforates visceral peritoneum
T4a	Perforates visceral peritoneum
T4b	Directly invades other organ or structures

Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1a	Metastasis in 1 regional lymph node
N1b	Metastasis in 2-3 regional lymph nodes
N1c	Satellites in subserosa, without regional lymph nodes
N2	Metastasis in 4 or more regional lymph nodes
N2a	Metastasis in 4-6 lymph nodes
N2b	Metastasis in 7 or more lymph nodes

M0	No distant metastases
M1a	Distant metastases in one organ
M1b	Distant metastases in more than one organ or peritoneum

Table 1.2 TNM colorectal cancer classification

1.3 Diagnosis

A clinical consciousness and a readiness to act on suspicious findings in patient history or tests are needed to find the colorectal cancer.

The work-up is commenced by taking the patient history, including heredity, followed by a physical exam including a digital rectal exam. A faecal occult blood test can confirm GI bleeding. The bowel can then be examined by colonoscopy or barium enema x-rays. An advantage of the latter is the possibility of taking biopsies or making smaller interventions. Whilst virtual colonoscopy is being developed we should not forget the simplicity of the rectoscopy, which also is a necessity to measure the level and height of the tumour in rectal cancer.

After the diagnosis additional investigations are made preoperatively for the purpose of cancer staging and operation planning.

Patients with invasive colon cancer appropriate for resection require a complete staging workup, including biopsy, pathologic tissue review, complete blood count, chemistry profile, carcinoembryonic antigen (CEA) determination, and baseline CT scans of the chest, abdomen, and pelvis. [24]

If the CT of the abdomen and pelvis is inadequate or if CT with IV contrast is contraindicated, an abdominal/pelvic MRI with contrast plus a non- contrast chest CT should be considered. The chest CT can identify lung metastases, which occur in approximately 4% to 9% of patients with colon and rectal cancer. [25-27] PET/CT scan is not indicated at baseline for preoperative workup, but PET/CT may be considered if abnormalities are detected on CT or MRI.

1.4 Prognostic Factors in Colorectal Cancer

Every patient affected by a malignant neoplasia shows several features that influence their prognosis. Some of them can be overcome by treatment efficacy, in that this latter can completely overrule the prognostic effect of a given parameter. Other features are only slightly affected by the primary treatment, unless they are part of associated therapies.

Others still, which account for the majority, are relatively unaffected by the administered therapies.

All these features as a whole are defined as prognostic factors:

1. patient related factors
2. disease related factors
3. treatment related factors

Among the patient related factors, we find:

- Age: it is not a predictive factor of the tumour's response to treatment [28]
- Gender: it seems to have an impact on overall prognosis of this disease as women have a median survival time higher than men, but this criterion does not appear to be predictive of response to treatment [28]
- Performance status (PS): it strongly affects the result of the treatment
- Presence of tumour-related symptoms: asymptomatic patients live longer and have a higher response to chemotherapy than symptomatic patients

Among the disease related factors, we find:

- infiltration of the visceral wall and the presence or absence of lymphonodal involvement are the starting point of all staging methods implemented for this condition.
- Venous or vascular invasion and perineural invasion [30]
- Tumor grading is connected to patients' overall survival, but the insufficient data do not allow to consider it a predictive factor of response to chemotherapy [31]
- Hystology type: the most frequent hysotype for large intestine cancer is adenocarcinoma, which accounts for 90-95% of all colon cancers. Colloidal or mucinous adenocarcinomas are 17% of large intestine tumours and are generally associated with poor prognosis. The highly uncommon signet-ring carcinoma (2-4% of mucous carcinomas) deserves its own classification.[32]
- Micro-satellite instability (MSI): in patients with a deficit of DNA mismatch reparation, genes which repair the transcriptive DNA alterations, there is a buildup of microsatellite mutations defined as micro-satellite

instability. These mutations are usually silent, as microsatellites reside in aeas which are codifying or promoting of genes involved in tumour growth.[33]

- Somatic loss of heterozygotes in colon cancer suggests that other tumour progression genes may be involved in colorectal oncogenesis before the formation of the polyp. Vogelstein et al. have examined the genetic alterations in colorectal cancer samples in several stages of neoplastic development and have found that alterations in chromosome 5q and in oncogene RAS tend to appear precociously[34]
- Site of the primary tumour: right versus left, where the right colon is the ascending tract as far as the splenic flexure; the tract that goes from the flexure to the rectum is considered left colon. Recent studies have shown that right colon and left colon tumours follow different pathogenetic molecular pathways[35], most likely related to a distinct embiologic origin and post-natal regulation. Right tumours are more frequently diploid, with a mucinous hystology, micro-satellite instability, CpG islands metilation, and BRAF mutated; conversely, left colon tumours are generally infiltrating, with a phenotype associated to chromosomal instability and aneuploidy[36] . Recent data have shown that the left site has a better prognosis than right tumours and that it would seem to be predictive of response to anti-EGFR inhibitors and RAS wt.[37]
- Deletion of chromosome 18: this area is often deleted in carcinomas and in advanced adenomas and only occasionally in early stage adenomas.[38] This gene has been named "deleted in colorectal cancer" (DCC) and the main structure of the product of the protein it codifies for is homologous to the adhesion molecule of neural cells (N-CAM). Volgenstein and his collaborators have found a fourth onco-suppressor gene, named "mutated in colorectal cancer" (MCC), also at 5q21, which determines mutations leading to function loss in sporadic colorectal carcinoma [38-39]
- CEA dosage can be useful as post-operative follow-up for relapse prevention, thanks to the potential benefit given by resection of liver metastases, which translates into survival gain.[40]

Treatment related factors include:

- previous adjuvant chemotherapies
- previous chemotherapies for advanced disease, which sometimes result into a resistance to second line treatment
- response, which seems to be an independent prognostic factor for survival [41]
- RAS mutational state, which is also predictive of response to anti_EGFR inhibitors in metastatic patients.
- BRAF mutational state. BRAF mutated tumours account for 5-10% of metastatic CRC, have a poorer prognosis, and show distinct clinical and pathological features[42], are more frequent in women, in right colon neoplasia, advanced age, high grade, and in MSI high tumours.
- LDH and fibrinogen: Silvestris et al [43] have examined the correlation between reduction of LDH levels compared to basal values and response to treatment.

Anti-VEGF drugs and VEGF blockade maintenance over the first line

2.1 Bevacizumab

Vascular endothelial growth factor A and B (VEGF-A, VEGF-B), and placental growth factor (PlGF) are members of the VEGF family of angiogenic factors that can act as potent mitogenic, chemotactic, and vascular permeability factors for endothelial cells. VEGF-A acts via two receptor tyrosine kinases, VEGFR-1 and VEGFR-2, present on the surface of endothelial cells. PlGF and VEGF-B bind only to VEGFR-1, which is also present on the surface of leucocytes. Excessive activation of these receptors by VEGF-A can result in pathological neo-vascularization and excessive vascular permeability. PlGF is also linked to pathological neo-vascularization and recruitment of inflammatory cells into tumours.[44] Bevacizumab is a humanized monoclonal antibody (IgG1) directed against VEGF-A and prevents its binding to the specific receptor, blocking its biological activity. [45]

Bevacizumab would seem to exert an indirect antitumor activity by enhancing chemotherapy, probably by improving the transport of chemotherapies through the vascular network of the tumor and reducing the interstitial pressure caused by the immaturity of the newly formed vessels.[46]

Bevacizumab was approved in first line setting in association with chemotherapy in mCRC patients improving response rate (RR: 44.8% vs 34.8%; p=0.004), median progression free survival (mPFS: 10.6 vs 6.2 months; p<0.001; HR=0.54) and median overall survival (mOS: 20.3 vs 15.6 months; p<0.001; HR=0.66).[47]

Bevacizumab was also investigated in first line setting in association with triplet.

TRIBE was a multicentre phase III trial randomizing unresectable and previously untreated mCRC patients to receive the triplet FOLFOXIRI plus bevacizumab or the doublet FOLFIRI plus bevacizumab. Longer progression-free survival (PFS) (median PFS 12.1 versus 9.7 months;HR= 0.75, 95% confidence interval (CI) 0.62–0.90; P<0.003), OS (median OS 29.8 versus 25.8 months; HR= 0.80, 95% CI 0.65–0.98; P¼0.03) and better objective response rate (65% versus 53%; p<0.006) were reported with the triplet plus bevacizumab. [48] Other trials more recently investigated the effect of the intensification of the chemotherapy with the triplet as compared to a standard doublet with consistent results in terms of both efficacy and toxicity [STEAM and CHARTA].[49-50]

The triplet was associated with increased grade 3/4 neutropenia (50% vs 20%), diarrhea (19% vs 11%) and stomatitis (9% vs 4%) but not with higher incidence of febrile neutropenia (9% vs 6%). Bev-related adverse events were in the expected range. The incidence of serious adverse events (20.4% vs 19.7%) and treatment-related deaths (2.4% vs 1.6%) was not significantly different between treatment arms.[51]

More than ten years ago, preclinical experiences suggested the potential efficacy of a sustained antiangiogenic strategy beyond the first occurrence of resistance. Results from the observational studies BRiTE and ARIES provided initial clinical data in support of this hypothesis. In particular, in the large US prospective observational cohort study BRiTE 642 (44.4%) out of 1445 patients who had experienced progressive disease, received bev beyond progression, while 531 (36.7%) received no bev beyond progression. [52]

A significant advantage in terms of survival beyond first progression (SBP) was noted with this strategy, that was still significant after adjusting for other prognostic factors (HR: 0.49 [0.41-0.58], p<0.001). Similar results were provided by the ARIES observational study. Among 539 out of 1097 patients who received bev beyond progression significantly longer SBP was observed, compared to 417 patients who did not. Results provided by the multivariate model were consistent with those from BRiTE trial (HR: 0.41 [0.34-0.49], p<0.001). [53]

More recently, a phase III trial, named TML (Treatment across Multiple Lines - ML18147) was conducted in Europe and Saudi Arabia, randomizing mCRC patients previously treated with bevacizumab plus standard first-line chemotherapy to cross-over chemotherapy with or without bevacizumab. Enrolled patients had experienced progressive disease less than 4 weeks prior to start of study treatment. Primary endpoint was OS. The use of bevacizumab beyond progression provided a significant advantage in terms of OS (11.2 vs 9.8 months, HR: 0.81 [0.69-0.94], p=0.0062) and PFS (5.7 vs 4.1 months, HR: 0.68 [0.59-0.78], p<0.0001), while no differences in response rate were reported (5.4% vs 3.9%, p=0.311). Adverse events were consistent with the expected toxicity profile of bev. As expected, the advantage provided by the addition of bev was independent of the KRAS mutational status [54-55]

Another phase III study with a similar design, the BEBYP (Bevacizumab BeYond Progression) trial, was contemporaneously conducted in Italy and prematurely stopped when results from TML were released. Primary endpoint was PFS. The continuation of bevacizumab beyond progression provided a significant advantage in terms of PFS (6.8 vs 5.0 months, HR: 0.72 [0.54-0.97], p=0.0029), while no differences in response rate (21% vs 18%, p=0.71) or OS (14.1 vs 15.5 months, HR: 0.77 [0.56-1.07], p=0.12) were reported. Nevertheless, the trial was clearly underpowered to detect an advantage in terms of survival[56]

Consistent results from both trials demonstrated the efficacy of a prolonged antiangiogenic strategy and identified the prosecution of bevacizumab in combination with a switched chemotherapy as a reasonable option for the second-line treatment of mCRC patients who have already received a bevacizumab-containing first-line regimen.

2.2 Aflibercept

Aflibercept, also known as VEGF TRAP in the scientific literature, is a recombinant fusion protein consisting of vascular endothelial growth factor (VEGF)-binding portions from the extracellular domains of human VEGF receptors 1 and 2, which are fused to the Fc portion of the human IgG1 immunoglobulin.[57]

Aflibercept acts as a soluble decoy receptor that binds to VEGF-A, with higher affinity than its native receptors, as well as the related ligands PlGF and VEGF-B. By acting as a ligand trap, aflibercept prevents binding of endogenous ligands to their cognate receptors and thereby blocks receptor mediated signaling. [57]

Acting on these factors, Aflibercept inhibits multiple pathways: VEGFR-2 mediated angiogenesis, VEGFR-1 mediated tumor growth, endothelial cell proliferation, myeloid progenitor cell recruitment. [57-58]

Aflibercept binds to human VEGF-A (equilibrium dissociation constant KD of 0.5 pM forVEGF-A165 and 0.36 pM for VEGF-A121), to human PlGF (KD of 39 pM for PlGF-2), and to human VEGF-B (KD of 1.92 pM) to form a stable, inert complex which has no detectable biological activity. The efficacy and safety of aflibercept were evaluated in a randomized, double-blind, placebo-controlled study in patients with mCRC who had previously been treated with an oxaliplatin-based treatment with or without prior bevacizumab. A total of 1.226 patients were randomized to receive either aflibercept or placebo in combination with FOLFIRI. The primary efficacy endpoint was overall survival. Treatment assignment was stratified by the ECOG performance status (0 versus 1 versus 2) and according to prior therapy with bevacizumab (yes or no).[59-60]

Demographics were well balanced between the treatment arms (age, race, ECOG performance status, and prior bevacizumab status). Of the 1226 patients randomized in the study, the median age was 61 years, 97.8 % had a baseline ECOG PS of 0 or 1, and 2.2 % had a baseline ECOG PS of 2. Among the 1226 randomized patients, 89.4 % and 90.2 % of patients treated with the placebo/FOLFIRI and aflibercept/FOLFIRI regimens, respectively, received prior oxaliplatin-based combination chemotherapy in the metastatic/advanced setting. Approximately 10 % of patients (10.4 % and 9.8 % of patients treated with the placebo/FOLFIRI and aflibercept/FOLFIRI regimens, respectively) received prior oxaliplatin-based adjuvant chemotherapy and progressed on or within 6 months of completion of adjuvant chemotherapy. Oxaliplatin-based regimens were administered in combination with bevacizumab in 373 patients (30.4 %). Overall efficacy results for the aflibercept/FOLFIRI regimen versus the placebo/FOLFIRI regimen are summarized in Figure 2.1

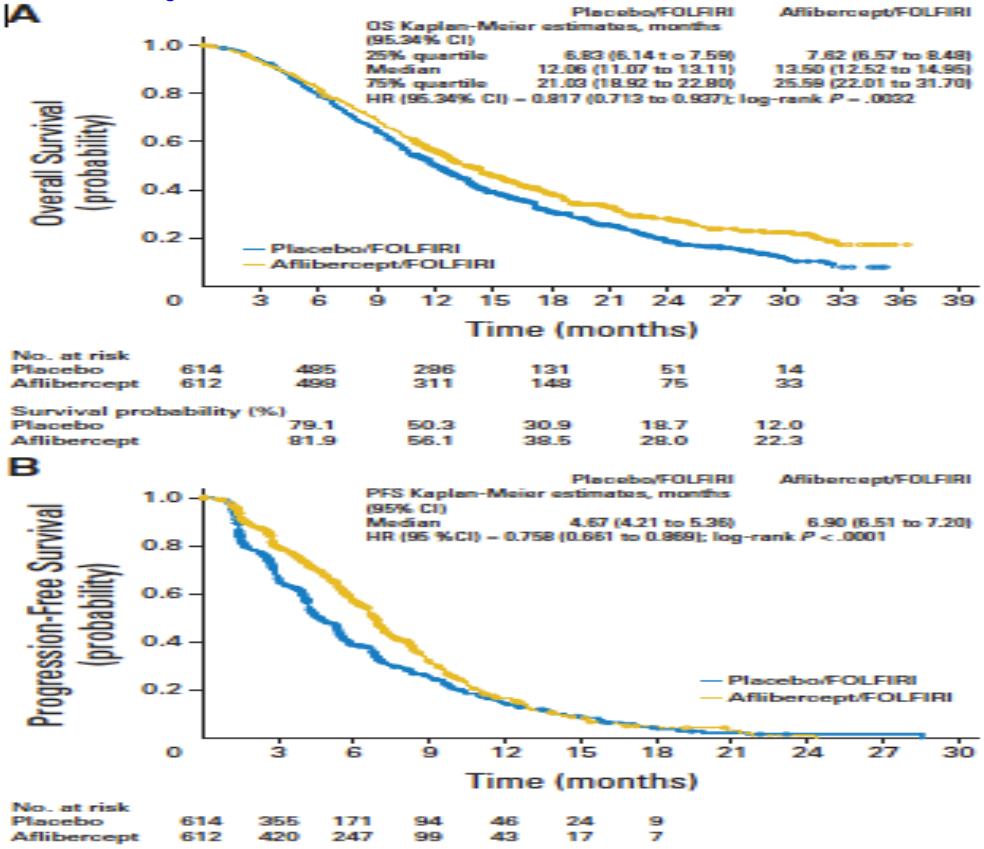

Figure 2.1: PFS and OS in VELOUR study

Other subgroup analyses for OS and PFS according to age (< 65; ≥ 65), gender, presence of liver metastasis only, history of prior hypertension, and number of organs involved, showed a treatment effect favouring the aflibercept/FOLFIRI regimen over the placebo/FOLFIRI regimen. In sub-group analysis of overall survival, a benefit consistent with the overall population was observed in patients < 65 years old and ≥ 65 years old who received the aflibercept/FOLFIRI regimen. Exploratory biomarker analyses were undertaken in the VELOUR trial including analyses of RAS mutational status in 482 of 1226 patients (n = 240 aflibercept; 242 placebo). In patients with RAS wild type tumours the HR (95 % CI) for OS was 0.7 (0.5-1.0) with a median OS of 16.0 months for patients treated with aflibercept, and 11.7 months for the patients treated with placebo. Corresponding data in patients with RAS mutant type tumours showed a HR for OS of 0.9 (0.7-1.2) with median 12.6 and 11.2 months for aflibercept and placebo, respectively. These data are exploratory and the statistical interaction test was non-significant (lack of evidence for heterogeneity in treatment effect between the RAS wild-type and RAS mutant subgroups).[61]

Differences between TML and Velour are shown in Table 2.1

Patient Population	VELOUR	TML
Prior treatment with Bevacizumab	Yes (approximately 30% of patients)	Yes (All patients)
1st line early progressors (1L PFS <3 months)	Eligible	Not eligible
Progression >3 months following the last dose of 1st line bevacizumab	Eligible	Not eligible
Patients having <3 consecutive months of 1st line bevacizumab	Eligible	Not eligible
Patients with non-measurable disease	Eligible	Not eligible
Patients relapsing within 6 months of completing oxaliplatin-based adjuvant chemotherapy	Eligible (10% of patients)	Not eligible
Placebo controlled	Yes	No
Chemotherapy regimen post-randomization	FOLFIRI (100%)	42% Irinotecan based (16% FOLFIRI)# 58% Oxaliplatin based (35% FOLFOX)#
Initial chemotherapy dosing post-randomization	Standard full-dose FOLFIRI	Investigator discretion taking into account 1st line tolerability
Regions	World-wide	Europe (Majority Germany & France)

*There are no head-to-head studies comparing aflibercept with bevacizumab
#Values presented for bevacizumab-treated TML patient arm

Van Cutsem, et al. Ann Oncol. 2011;22(suppl 5). Abstract O-0024 and presentation at: ESMO 13th WCGIC. June 22-25, 2011; Barcelona, Spain; Arnold et al, ASCO 2012. Presented June 3 2012

Table 2.1: Velour vs TML

Criteria of evaluation of response to anti-cancer therapies

3.1 WHO Criteria

In 1981, the World Health Organization published the first model of disease response criteria for the evaluation of the effects of antiblastic therapy. [62]

It introduced the concept of total disease load based on the sum of the products of the diameters (SPD) and the possibility of identifying a potential response to the systemic treatment through the changes of the afore-mentioned basal and following measurements.

Four groups could be identified:

- Complete response: the lesions are not identifiable for at least 4 weeks.
- Partial response: a minimum of 50% reduction of SPD versus basal (confirmed at 4 weeks).
- Progression disease: dimentional increase of at least 25% in at least one lesion
- Stable disease: the partial response or disease progression criteria are not met.

The maximum number of lesions to be identified is not agreed on, nor is the number of organs to be analyzed. The identification of non measurable/assessable lesions was also insufficient. Finally, the measurement of each lesion was bidimensional, deriving from the product of the larger diameters of it, or unidimensional, through a linear measurement

3.2 RECIST Criteria

The assessment of tumour response to systemic treatments most often used in oncology nowadays uses RECIST (Response Evaluation Criteria in Solid Tumors) criteria v 1.1 [63-64] which is based on the following concepts:

- measurable disease and non measurable disease
- target and non target lesion

At basal evaluation, tumoral lesions are considered *measurable* for a minimun size of 10 mm at CT (measured on larger diameter in at least one dimension). As for the pathological lymphnodes, they are measurable when they have a diameter of at least 15 mm shorter axis at CT.

Target lesions are chosen at baseline, with a maximum of five total lesions and a maximum of two per organ. All other lesions, or cancer sites, including pathological lymphnodes, should be identified as *non-target* lesions. These are reported at baseline, but will not be measured later and will just have to be indicated as present or absent at future evaluations.

Response criteria for target lesions:

- Complete response: total disappearance of all target lesions. Every pathological lymphnode is reduced in smaller axis to less than 10 mm.

- Partial response: a minimum of 30% descrease on the sum of the target lesions' diameters, taking into account the basal measurement.
- Disease progression: A minimum of 20% increase on the sum of the target lesions' diameters, taking into account the smallest sum obtained during the study. An absolute value increase of at least 5 mm is also needed. The appearance of one or more new lesions is obviously also considered as disease progression.
- Stable disease: both progression and partial response criteria are not satisfied, taking into account the smallest sum of the diameters obtained during the study.

Objective response of non target lesions:

- Complete response (CR): disappearance of all non target lesions and normalization of tumoral markers. All lymphnodes are non pathological (short axis < 10 mm)
- Non CR/Non DP: persisting of one or more non target lesions and/or tumoral markers above normal values.
- Disease progression (DP): appearance of one or more lesions. Unmistakable progression of pre-existing non target lesions.

The re-evaluations need to follow the timing of the chosen therapeutic protocol; as a matter of fact, in phase II studies, where the benefits of the treatment are not known, the measurement should be carried out every 6-8 weeks, coincidental with the end of a treatment cycle.

Differences between RECIST vs RECIST v1.1 criteria and WHO vs RECIST criteria are shown in Table 3.1 and Table 3.2 respectively.

RECIST 1.0	RECIST 1.1
Minimum size = 10 mm at spiral CT, 20 mm at conventional CT	Minimum size = 10 mm at CT
Longest diameter	Longest diameter (except in lymph nodes)
Unspecified	Short axis: target lesions ≥15 mm, nontarget lesions = 10–15 mm, nonpathologic lesions <10 mm
20% increase in SLD or new lesions, unequivocal progression considered to indicate progressive disease	>20% increase in SLD; ≥5-mm increase in size; new lesions; detailed description of unequivocal progression
10 lesions (≤5 in any one organ)	Five lesions (≤2 in any one organ)
N/A	Provides guidance as to when a lesion is considered new (ie, representative of progressive disease)
CT, MRI, chest radiography	CT, MRI, FDG PET

Table 3.1: Analysis of the key changes from RECIST 1.0 to RECIST 1.1

Criterion	WHO	RECIST 1.0
Definition of "measurable" lesions	Should be measurable in two dimensions, no minimum lesion size	Minimum size = 10 mm at spiral CT, 20 mm at conventional CT
Method of measurement	SPD	Longest diameter
Lymph nodes	Unspecified	Unspecified
Definition of progressive disease	≥25% increase in SPD	20% increase in SLD or new lesions, unequivocal progression considered to indicate progressive disease
Number of lesions measured	N/A	10 lesions (≤5 in any one organ)
New lesions	N/A	N/A
Guidance for imaging studies	N/A	CT, MRI, chest radiography

Note.—MRI = MR imaging, N/A = not applicable.

Table 3.2: comparison between WHO and RECIST 1.0 criteria

3.3 CHOI Criteria

Both WHO and RECIST criteria have not been satisfactory in the evaluation of response with the use of target therapy, as there is often a change in the size of the neoplasm, and in the shape and margins of the lesion itself, thus driving oncologists and radiologists to search for alternative criteria of response evaluation.

The first example was the use of CHOI criteria, employed in gastro-intestinal stromal tumours (GIST), after the introduction of Imatinib, a competitive Tirosin-Kinase *c-Kit* inhibitor. [65-66]

As an additional criterion of response to Imatinib, the CHOI criteria indeed include lesion abatement; this change can also be seen early in the treatment. The response definition is based on the change in tumour density (measured in Hounsfield Unit-HU) and size.

The tumour density of each target lesion is measured at every evaluation. The variation rate of the target lesions' density is calculated, and these are defined according to the RECIST 1.0 criteria. The total response is calculated on the average of the variation rates of tumour density for each target lesion.

PET scan has been recognized as a highly sensitive tool in the evaluation of both early and long term response. There is also a good correlation between response in terms of total disease load, measured at CT, and abatement, assessed with FDG-PET, through maximum SUV.

- Partial response: minimum 10% reduction of the lesion size at CT or minimum 15% reduction of abatement at FDG-PET; no new lesion. No new evidence of progression among non measurable lesions.
- Complete response: disappearance of all target lesions.
- Disease progression: increase of the cancer size of at least 10%. No met criteria of partial response. Appearance of new lesions. Appearance of intratumoral nodules or increase in size of pre-existing intratumoral nodules.
- Stable disease: neither partial response, nor disease progression criteria are met.

Comparison between WHO, RECIST and CHOI criteria are summarized in Table 3.3

Response	WHO*	RECIST 1.1	Choi†
Complete response	No lesions detected for at least 4 weeks	Disappearance of all target lesions or lymph nodes <10 mm in the short axis	Disappearance of all target lesions
Partial response	≥50% decrease in SPD (confirmed at 4 weeks)	>30% decrease in sum of longest diameters (SLD) of target lesions	≥10% decrease in tumor size or ≥15% decrease in tumor attenuation at computed tomography (CT); no new lesions
Progressive disease	≥25% increase in SPD in one or more lesions; new lesions	>20% increase in SLD of target lesions with an absolute increase of ≥5 mm; new lesions	≥10% increase in SLD of lesions; does not meet the criteria for partial response by virtue of tumor attenuation, new intratumoral nodules, or an increase in the size of the existing intratumoral nodules
Stable disease	None of the above	None of the above	None of the above

Table 3.3: Synoptic Table of Comparison between RECIST 1.0, RECIST 1.1, CHOI

3.4 Morphological Criteria of Response

In the last few years, oncologists have started Morphologic Criteria to respond to an unmet clinical need: to define tumor assessment when mCRC were treated with biologic drugs, especially antiangiogenic drugs. These criteria have been validated because they allow a high specificity in the evaluation of the response of liver metastases from colorectal cancer following post-operative anti-VEGF (Bevacizumab) target therapy. They have proved to be highly sensitive and specific in this context, as opposed to RECIST criteria[63-64], which have a high sensitivity in recognizing complete or larger pathological responses, but do not possess the same prediction efficacy towards smaller pathological responses. Among patients with colorectal liver metastases treated with bevacizumab containing chemotherapy, CT-based morphologic criteria had a statistically significant association with pathologic response and overall survival.

When using morphological criteria of response [67], no target lesions are identified. All liver metastases are evaluated, and such criteria are based on the assessment of:

1. Content: Homogeneous vs Heterogeneous (Figure 3.1).
2. Margin: Defined vs Irregular (Figure 3.2).

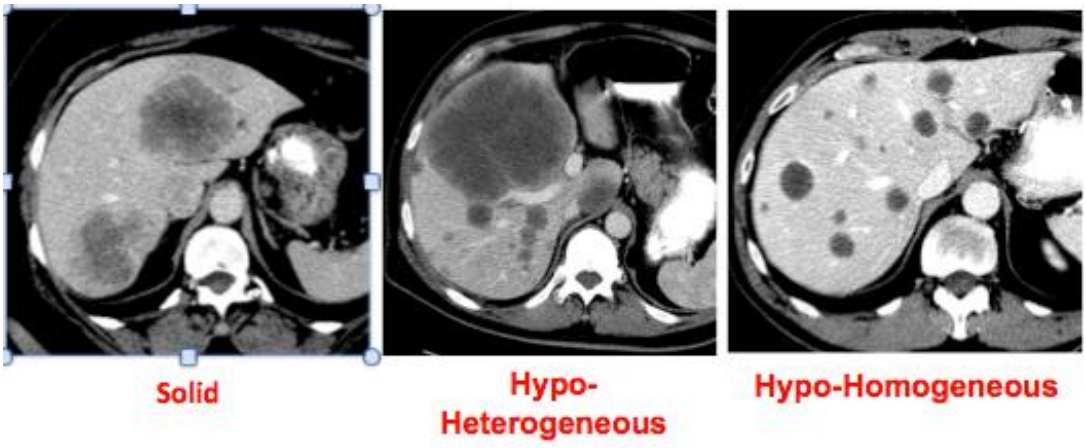

Figure 3.1: Internal features before treatment

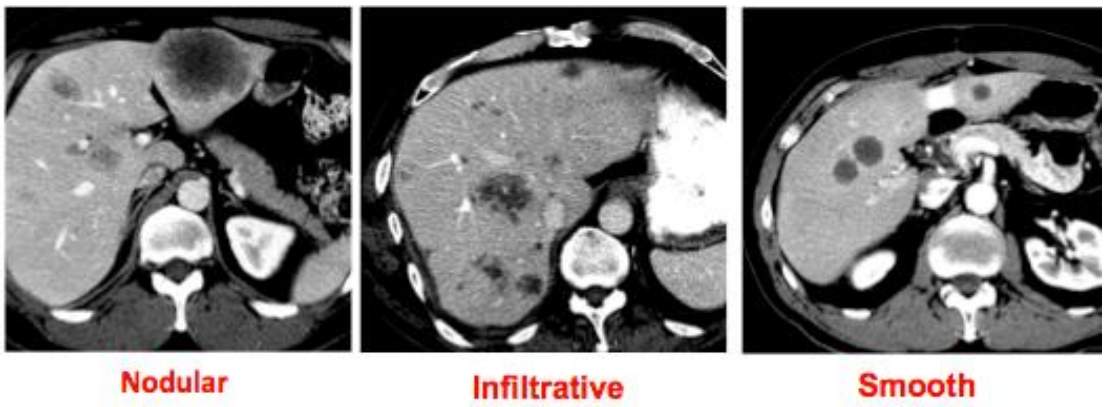

Figure 3.2: Margins before treatment

Each parameter (content/margin) is assessed subjectively. The lesion is evaluated on the overall appearance. In case of disagreement, the response is assessed based on dominant pattern.

The completely subjective assessment of the lesion's morphology allows the subdivision of neoplastic lesions into three groups, addressed using arabic numbers. (Figure 3.3)

Morphology Group	Computed Tomographic Tumor Characteristics		
	Overall Attenuation	Tumor-Liver Interface	Peripheral Rim of Enhancement
3	Heterogeneous	Ill defined	May be present
2	Mixed	Variable	If initially present, partially resolved
1	Homogeneous and hypoattenuating	Sharp	If initially present, completely resolved

Figure 3.3: Morphological groups at Computerized Tomography

Evaluation of VRPD as a new biomarker and development of a new clinical nomogram to predict outcome to 2nd -line therapy in mCRC treated with bevacizumab beyond progression or aflibercept

4.1 Background

Measuring the tumor response to a given treatment is often challenging, although the problems may differ from daily practice to clinical trials.

In everyday clinical practice, whether a treatment is deemed active or not is based on several considerations, including subjective judgment and arbitrary evaluations, more focused on patient's profile rather than on his/her metastatic lesions. Conversely, in clinical trials objective and reproducible criteria are required to assess and classify tumor response, and usually the evaluation is based on the radiological assessment of measurable/evaluable tumor lesions.

Therefore, in clinical practice, it is increasing the need to identify biomarkers that can show the progress of the disease and help in predicting patient outcome in subsequent lines. This biomarker should answer the question: how is evolving the disease? How fast?

Currently, to our knowledge, there aren't yet standardized prognostic or predictive parameters of outcome to the second line treatment that can predict at progression which are patients who can most benefit from systemic treatment next to the first line in mCRC patients treated with bevacizumab beyond progression or aflibercept.

Iacovelli et al investigated tumor growth rate (GR) and tumor flare (TF) after discontinuation of anti-VEGFR tyrosine kinase inhibitors (sunitinib or pazopanib), its prognostic role and outcome in terms of overall survival in metastatic renal cancer patients.[68]

Tumor growth rate (GR) was measured at different time points: at the time of discontinuation (t_0), before (t_{-1}) and after discontinuation (t_{+1}). Tumor flare was defined as the difference between the tumor growth rate values. Patients with tumor growth rate greater than the median had worse outcome in term of overall survival. This study showed that discontinuation of sunitinib or pazopanib results in acceleration of tumor growth rate and thus negatively affects the prognosis of metastatic renal cancer patients.

Based on these data, the aim of this study is to test a new clinical biomarker, calculated at first progression in metastatic colon cancer: velocity rate of disease progression (VRPD).

Velocity rate of disease progression (VRPD) would be a dynamic parameter that takes into account the boost proliferative disease at the time of progression to the first line giving an index that underlies the biology of the disease at the start of the second line.

Knowing how fast the disease progresses can help clinicians to understand what the next treatment can still have an impact on patient survival.

Therefore, the purpose of this study is to test if velocity rate of disease progression (VRPD) may be a new clinical biomarker that can predict, at time to first progression, in mCRC treated in first line with bevacizumab-based therapy, outcome to second-line therapy in patients treated with bevacizumab beyond progression or aflibercept.

4.2 Study Objectives

Primary objective

The main objective of this study is to evaluate whether the velocity rate of progressive disease (VRPD) at first line may be used as a new clinical biomarker to predict the outcome of second line treatment in terms of progression free survival (PFS) in metastatic colorectal patients treated with bevacizumab beyond progression or aflibercept. PFS was evaluated from start of second line to progression of disease or death; disease progression was defined as a > 20% increase in the sum of diameters of target lesions according to RECIST v.1.1.[64]

Secondary objective

The secondary objective of this study is to create a nomogram predictive of PFS in metastatic colorectal patients treated with bevacizumab beyond progression or aflibercept in second line.

4.3 Patients and Methods

We retrospectively analyzed data about 167 patients collected in the five institutional sites involved.
Data were collected between 01 Jan 2006 and 31 Jan 2019.
Data analyses were performed on February 13th 2019.

Patient's Selection

Inclusion Criteria

- Histologically proven diagnosis of colorectal adenocarcinoma
- Measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST criteria, vers.1.1)
- First-line bevacizumab-containing therapy
- Documentation of progression to first-line
- Computed tomography (CT) scans performed before (t_{-1}) and at the time of progression (t_0)
- Second-line bevacizumab or aflibercept-containing therapy
- Male or female, aged > 18 years of age
- ECOG Performance Status ≤ 2

Exclusion Criteria

- Past or current history of malignancies other than colorectal carcinoma, except for curatively treated basal or squamous cell carcinoma of the skin or in situ carcinoma of the cervix
- Contraindications to radiographic contrast agent (allergy, not adequate renal function)

Methods

- **VRPD analysis**

For each patient, VRPD was calculated as the ratio between the sum of the longest diameters (SLD) for target lesions evaluated by CT at the time of progression and the sum of the longest diameters (SLD) for target lesions evaluated by CT before progression multiplied for difference in months.

$$\mathbf{VRPD = SLD_0 / [SLD_{-1} (t_0 - t_{-1})]}$$

Target lesions were evaluated according to RECIST criteria v 1.1. [64]

SLD_0 = sum of the longest diameters of target lesions calculated at the time of progression

SLD_{-1} = sum of the longest diameters of target lesions evaluated by CT before progression

$t_0 - t_{-1}$= difference (in months) between CT before progression and CT at the time of progression

For VRPD cut-off point estimation a mixture model of two Gaussian distributions was used. This procedure is implemented using the function flexmix from the R package flexmix. The optimal cutoff is determined as the value where the probability density functions of the mixing distribution coincide.

- **Multivariate analysis and Nomogram construction**

We collected data on 22 variables, which were chosen based on the previous literature and the specific design of this study. Variables retaining a significant association to PFS in a multivariate analysis were automatically selected for the construction of a Nomogram using the statistical tools subsequently described.

We applied a Penalized Cox Regression Model based on adaptive elastic-net method with the penalty parameter selected by 5-fold cross-validation with λ selection rule set at 1 Standard Error.

The nomogram was evaluated in terms of discrimination and calibration. Discrimination was assessed with the area under the curve (AUC) at receiver operating characteristics analysis (with values ranging from 0.5 for no discrimination to 1.0 for perfect discrimination). To assess potential changes with time of the model discrimination performance, time-dependent AUC values were analyzed every 3 months from the month 0 to month 18 of follow-up using inverse-probability-of-censoring weights method. Calibration was investigated by plotting the predicted and observed probabilities of events at 6 months. All discrimination and calibration measures were internally validated using Repeated Cross Validation (Repeated cross-validation fold number:10; Repeated Times: 20). All analyses were conducted with R statistical software (version 3.3.2) equipped with the "glmnet," "hdnom". All tests were 2-tailed and a p value <0.05 was considered statistically significant.

4.4 Results

- **Descriptive analyses**

We enrolled 167 mCRC pts treated with bevacizumab beyond progression or aflibercept in second line.

Main patients' characteristics at baseline are summarized in Table 4.1.

In first line 41.6 % of pts were treated with Folfoxiri plus bevacizumab and 58.4% with bevacizumab in association with doublet (Folfiri or Folfox) or monotherapy (capecitabine). 57% of patients received maintenance therapy.

In second line 63% of pts were treated with bevacizumab plus doublet, 12% with bevacizumab in association with triplet and 25% of patients received aflibercept Folfiri. (Table 4.2)

Characteristic of pts	**Overall Population (N=167)**
Age	
Median	60 aa
Range	34-79
Sex	
F	58 (34.5 %)
M	109 (65.5 %)
ECOG PS	
0	131 (78 %)
1	36 (21 %)
Site of primary tumor	
Right colon	55 (33.3%)
Left colon/Rectum	112 (66.4 %)
Resected primary tumour	
YES	116 (69 %)
NO	51 (31 %)
Previous adjuvant therapy	
YES	15 (9.5 %)
No	152 (90.5 %)
Time to metastases	
Synchronous	122 (72.6 %)
Metachronous	45 (27.4 %)
***RAS*/*BRAF* status**	
RAS mutated	112 (66.6 %)
BRAF mutated	43 (25.6 %)
RAS/ BRAF wild type	42 (25 %)
Liver metastasis	
Yes	130 (78 %)
No	37 (22 %)

Table 4.1: Patients' characteristics

First line treatment	Overall Population
Bev triplet	69 (41.6 %)
Bev doublet/ monotherapy	98 (58.4%)
Maintenance therapy	
Yes	96 (57 %)
No	71 (43 %)
Second line treatment	
Bev doublet	104 (63 %)
Bev triplet	21 (12 %)
Aflibercept	42 (25 %)

Table 4.2: Patients' treatment in first and second line

- **VRPD analysis**

We observed that the VRPD parameter was largely homogenous in our population, with the exception of a relatively small population characterized by significantly larger values (Figure 4.1). We therefore decided to dichotomize our population based on a VRPD cut-off value.

A cut-off value of 1.19 was unbiasedly generated using a mixture model of two Gaussian distributions. This cut-off discriminated pts with low (VRPD < 1.19) vs high (VRPD >1.19) VRPD. In our population, 15 (9%) patients showed high VRPD, while 152 (91%) showed low VRPD. (Figure 4.1).

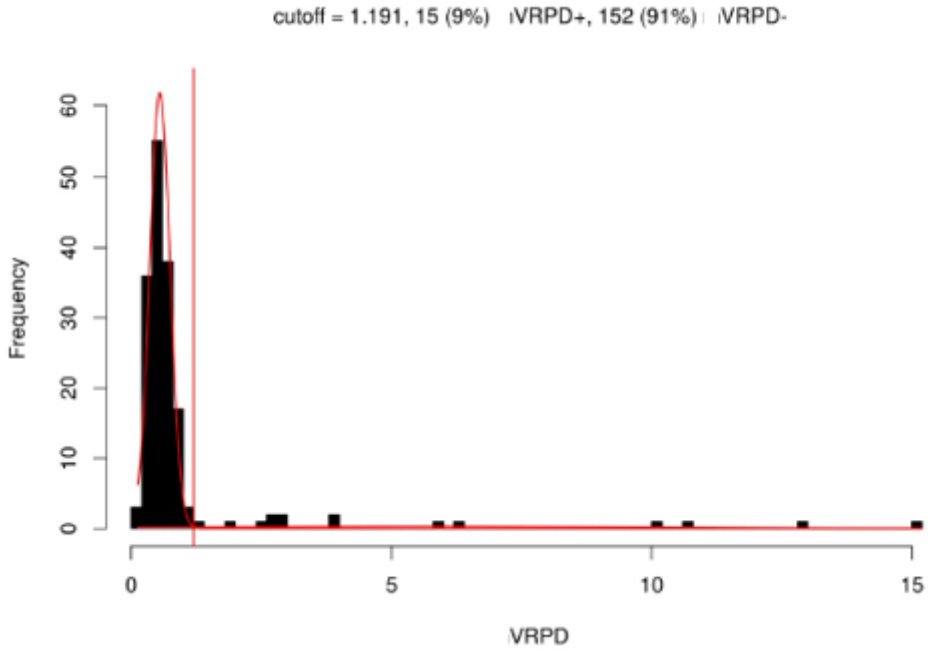

Figure 4.1: Distribution of VRPD

Considering VRPD in a univariate analysis, pts with high VRPD had a non-significant longer PFS (HR: 0,59 [95%CI: 0.34-1.03], p=0.06) than pts with low VRPD. This result is limited by the low number of high VRPD pts (Figure 2). No significantly different clinical or biological features were found between the two groups (data not shown).

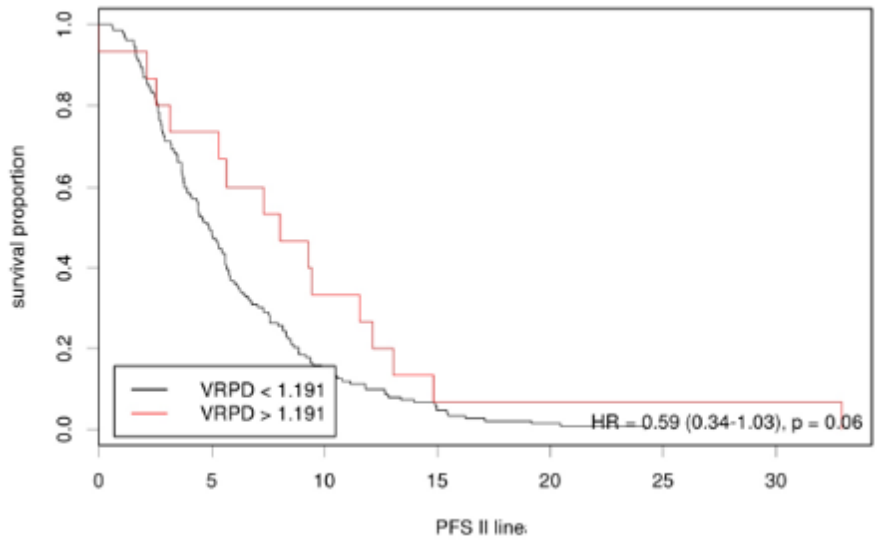

Figure 4.2: Second line PFS in high and low VRPD pts

- **Multivariate analysis and Nomogram construction**

All the 22 available variables, including VRPD (considered either as a continuous or as a dichotomized variable) were included in the multivariate analysis to determine which factors could better predict PFS in second line.

Six variables were selected by our statistical tools, based on their significance in the multivariate analysis, and used to build a Nomogram. These six variables were: gender (0= F; 1=M); primary tumour location (0= right; 1= left), baseline Performance Status (PS), presence of liver metastases (0=no; 1=yes), first line PFS in weeks, sum of the longest diameters at time of progression (SLD_0). (Figure 4.3)

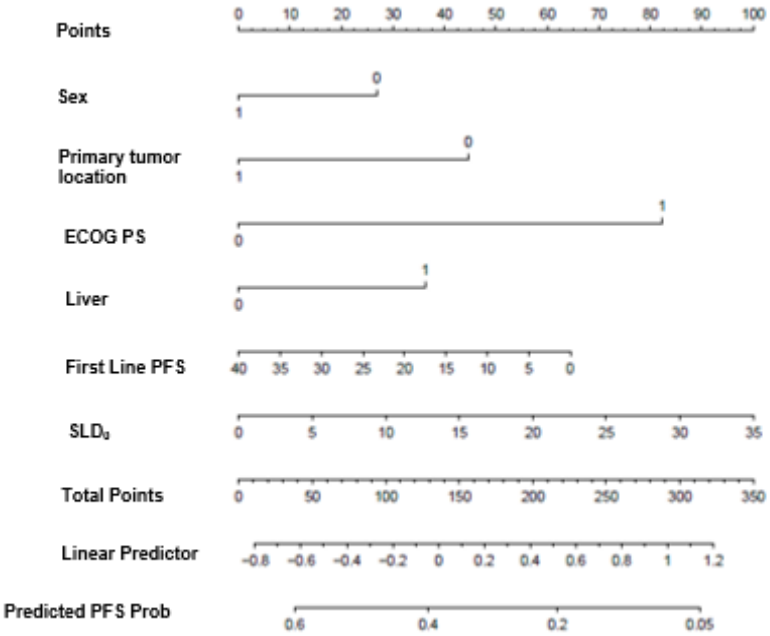

Figure 4.3: Plotted Nomogram

The nomogram showed good discrimination for the prediction of six months PFS with a significant difference in survival between two groups of pts with lower or higher score based on the median value ($p < 0.001$ at log-rank analysis; groups resulted of 83 patients each, as one patient was excluded because of missing data). Indeed, pts with a high score showed a worse PFS probability compared to pts with a low score. (Figure 4.4)

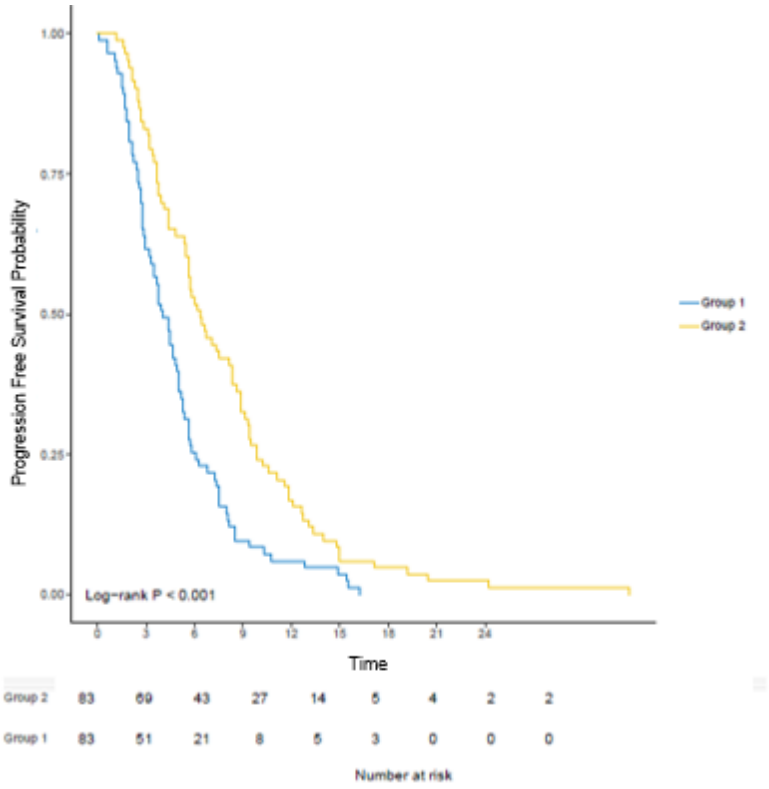

Figure 4.4: Kaplan-Meier survival curves and number at risk table for risk group

The nomogram was internally validated using Repeated Cross Validation showing a good performance in terms of discrimination and calibration. (Figure 4.5; Figure 4.6)

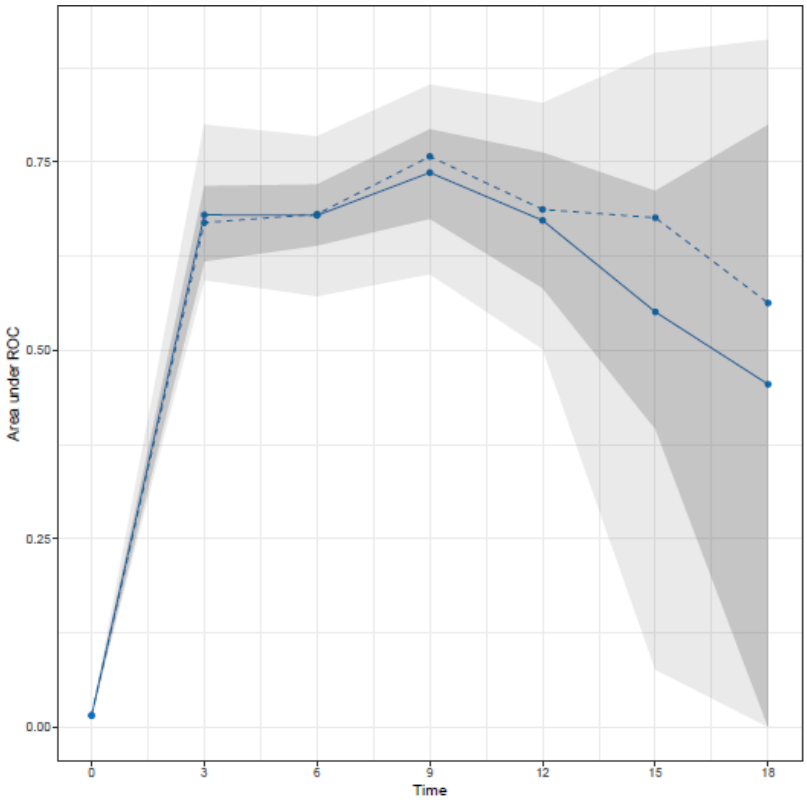

Figure 4.5: Internal Validation Plot

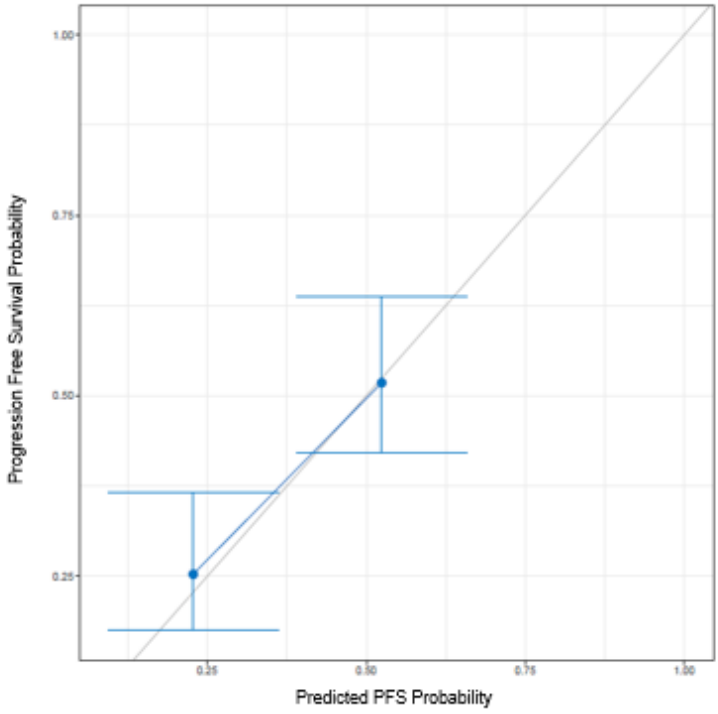

Figure 4.6: Internal Calibration Plot

4.5 Discussion

In the last few years both bevacizumab and aflibercept have been shown efficacy in second line therapy [55,59] following a first line therapy containing bevacizumab, but there is the unmet clinical need to predict outcome to second line therapy in order to distinguish pts who can benefit from further treatments more than others.

Therefore, we wanted test a new parameter, VRPD, to evaluate if the velocity of progression disease at first line could help clinicians to distinguish patients that can most benefit to subsequent treatments than others, but in our population, pts with high VRPD had a non-significant longer PFS (HR: 0,59 [95%CI: 0.34-1.03], p=0.06) than pts with low VRPD.

However, from our analysis, other parameters were statistically significant in multivariate and we used this data to build a nomogram that could predict six months PFS probability.

Nomograms have been developed for a variety of malignancies in order to improve outcome and response to treatment prediction and provide patients and physicians with a more understandable outcome measure when making treatment-related decisions.

Our nomogram included six variables: gender (0 = F; 1=M), primary tumour location (0= right; 1= left), baseline Performance Status (PS), presence of liver metastases (0=no; 1=yes), first line PFS, sum of the longest diameters at time of progression.

In our analyses, presence of liver metastases and sum of the longest diameters at time of progression retained a significant correlation in multivariate analysis and were included in our nomogram.

The greater is the sum, the worse is the outcome underlying that tumour burden, represented by tumour number and size, is an important prognostic factor, unlike the velocity rate with which the disease progresses.

Previous study showed that tumour burden was inversely correlated with overall survival and with primary resection for patients with mCRC at presentation [69] and is related with known prognostic factors for patients with mCRC, but its role will be performed to determine its independent impact.

Recent studies showed that early shrinking tumors under first-line chemotherapy responded better to subsequent lines, maintaining low tumor loads. Piessevaux et al. showed that early tumor shrinkage (ETS), defined as ≥ 20% shrinkage in tumor size per RECIST criteria at first-tumor assessment after baseline, is a favorable prognostic factor related to PFS and OS in cytotoxic treatment of mCRC. [70] FIRE-3, a phase III trial that compared first line therapy with FOLFIRI plus either cetuximab or bevacizumab, showed that FOLFIRI plus cetuximab induced a significantly higher overall response rate (ORR), a greater rate of early tumor shrinkage, and an increased depth of response compared to FOLFIRI plus bevacizumab. These response-related outcomes may in part explain the significant OS advantage of FOLFIRI plus cetuximab observed in this study.[71] These associations were investigated also in the phase III TRIBE trial that compared in first line FOLFOXIRI plus bevacizumab versus FOLFIRI plus bevacizumab. A significantly higher percentage of patients in the FOLFOXIRI plus bevacizumab arm achieved ETS ≥ 20%, when compared with the control arm (62.7% versus

51.9%, *P* = 0.025). In addition, the DpR was significantly higher in experimental arm (43.4% versus 37.8%, *P* = 0.003).

Both ETS and DpR were associated with PFS and OS showing that achieving rapid and deep tumor shrinkage consistently delays tumor progression and prolongs survival in patients treated with first-line chemotherapy plus bevacizumab.[72]

Based on our results, patients with a high tumor burden on the first line progression have a worse outcome on the second line. So we can consider tumor load as a relevant prognostic factor for second line PFS, unlike the velocity rate at which the tumor grow.

While in our population, men seem to have better outcome than women, previous studies have shown that women have a median survival time higher than men.[28] The effect of sex hormones, either endogenous or through hormonal replacement therapy, might be the most plausible explanation for the observed patterns.[73]

In a Dutch study, the survival advantage of women varied by CRC stage and age and was most pronounced for localized disease and in patients under 45 years of age. On the contrary, sex differences in survival did not vary by location of CRC. [74]

Also in our nomogram, left tumor location is associated with a better prognosis compared to right colon cancer, in according to an increasingly large amount of evidence that shows prognostic role of sidedness in colon cancer. Colon tumors proximal and distal to splenic flexure are distinct clinical and biological entities. Apart from having a different embryological origin—proximal colon from midgut and distal colon and rectum from hindgut—the right colon displays peculiar differences in mucosal immunology, probably owing to differences in gut microbiota.[75]

Tumors arising on the right side of the colon, in fact, seem to follow different molecular pathways of oncogenesis. These right colon cancers more commonly are diploid and characterized by mucinous histology, high microsatellite instability, CpG island methylation, and BRAF mutations.[76-78] Conversely, left colon cancers were found to have frequently p53 and KRAS mutations.[79]

Recently, Petrelli et al demonstrated that primary tumor location has a critical role in determining colorectal cancer prognosis, showing that the site of colon cancer as an independent prognostic factor in both early and advanced disease. Specifically, bearing a tumor originating in the left side of the colon was significantly associated with an absolute 19% reduced risk of death.[80]

4.6 Conclusion

VRPD is a parameter never tested in metastatic colorectal cancer, which highlighted a fast progressor subgroup with a surprising best outcome in second line PFS , but the result has not been validated and therefore further studies are needed to test VRPD in a larger validation set.

Other parameters, such as primary tumor location, baseline PS, presence of liver metastases, first line PFS and sum of the longest diameters at time of progression, resulted as prognostic factors for determine second line PFS.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018;68:7-30

2. Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: The impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA Cancer J Clin 2011;61:212-236

3. SEER Surveillance, Epidemiology, and End Results - - U.S. Department of Health and Human Services – National Cancer Institute - April 2000

4. Ahsan H, Neugut AI, Garbowski GC, et al. Family history of colorectal adenomatous polyps and increased risk for colorectal cancer. Ann Intern Med 1998;128:900-905; 12

5. Hemminki K, Chen B. Familial risk for colorectal cancers are mainly due to heritable causes. Cancer Epidemiol Biomarkers Prev 2004;13:1253-1256; 13

6. Quintero E, Carrillo M, Leoz ML, et al. Risk of advanced neoplasia in first-degree relatives with colorectal cancer: a large multicenter cross- sectional study. PLoS Med 2016; 13(5):e1002008

7. Hampel H, Frankel WL, Martin E, et al. Feasibility of screening for Lynch syndrome among patients with colorectal cancer. J Clin Oncol 2008;26:5783-5788; 15

8. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. N Engl J Med 2003;348:919-932; 16

9. Galiatsatos P, Foulkes WD. Familial adenomatous polyposis. Am J Gastroenterol 2006;101:385-398; 18

10. Guinney J, Dienstmann R, Wang X, et al. The consensus molecular subtypes of colorectal cancer. Nat Med 2015;21:1350-1356; 20

11. Hampel H, Frankel WL, Martin E, et al. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). N Engl J Med 2005;352:1851-1860)

12. Beaugerie L, Svrcek M, Seksik P, et al. Risk of colorectal high-grade dysplasia and cancer in a prospective observational cohort of patients with inflammatory bowel disease. Gastroenterology 2013;145:166-175 e168; 53

13. Johnson CM, Wei C, Ensor JE, et al. Meta-analyses of colorectal cancer risk factors. Cancer Causes Control 2013;24:1207-1222; 54

14. Lutgens MW, van Oijen MG, van der Heijden GJ, et al. Declining risk of colorectal cancer in inflammatory bowel disease: an updated meta-analysis of population-based cohort studies. Inflamm Bowel Dis 2013;19:789-799; 56

15. De Bruijn KM, Arends LR, Hansen BE, et al. Systematic review and meta-analysis of the association between diabetes mellitus and incidence and mortality in breast and colorectal cancer. Br J Surg 2013;100:1421-1429

16. Sinicrope FA, Foster NR, Yoon HH, et al. Association of obesity with DNA mismatch repair status and clinical outcome in patients with stage II or III colon carcinoma participating in NCCTG and NSABP adjuvant chemotherapy trials. J Clin Oncol 2012;30:406-412; 90

17. Yang B, Jacobs EJ, Gapstur SM, et al. Active smoking and mortality among colorectal cancer survivors: the Cancer Prevention Study II nutrition cohort. J Clin Oncol 2015;33:885-893

18. Song M, Zhang X, Meyerhardt JA, et al. Marine omega-3 polyunsaturated fatty acid intake and survival after colorectal cancer diagnosis. Gut 2016;66:1790-1796)

19. Mills KT, Bellows CF, Hoffman AE, et al. Diabetes mellitus and colorectal cancer prognosis: a meta-analysis. Dis Colon Rectum 2013;56:1304-1319

20. He XK, Su TT, Si JM, Sun LM. Metformin is associated with slightly reduced risk of colorectal cancer and moderate survival benefits in diabetes mellitus: A meta-analysis. Medicine (Baltimore) 2016;95:e2749

21. Mei ZB, Zhang ZJ, Liu CY, et al. Survival benefits of metformin for colorectal cancer patients with diabetes: a systematic review and meta- analysis. PLoS One 2014;9:e91818

22. Amin MB, Edge SB, Greene F, et al., eds. AJCC Cancer Staging Manual, 8th ed. New York: Springer

23. Swanson RS, Compton CC, Stewart AK, Bland KI. The prognosis of T3N0 colon cancer is dependent on the number of lymph nodes examined. Annals of surgical oncology. 2003;10(1):65-71

24. Balthazar EJ, Megibow AJ, Hulnick D, Naidich DP. Carcinoma of the colon: detection and preoperative staging by CT. AJR Am J Roentgenol 1988;150:301-306

25. Choi DJ, Kwak JM, Kim J, et al. Preoperative chest computerized tomography in patients with locally advanced mid or lower rectal cancer: its role in staging and impact on treatment strategy. J Surg Oncol 2010;102:588-592; 213

26. Grossmann I, Avenarius JK, Mastboom WJ, Klaase JM. Preoperative staging with chest CT in patients with colorectal carcinoma: not as a routine procedure. Ann Surg Oncol 2010;17:2045- 2050; 214

27. Qiu M, Hu J, Yang D, et al. Pattern of distant metastases in colorectal cancer: a SEER based study. Oncotarget 2015;6:38658- 38666212-214

28. Buyse M, Piedbois Y, Piedbois P, Gray R. Tumour site, sex, and survival in colorectal cancer. Lancet. 2000 Sep 2;356(9232):858. PubMed PMID: 11022959.)

29. Schmoll HJ, Van Cutsem E, Stein A, Valentini V, Glimelius B, Haustermans K, et al. ESMO Consensus Guidelines for management of patients with colon and rectal cancer. a personalized approach to clinical decision making. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 2012 Oct;23(10):2479-516.

30. Sternberg A, Sibirsky O, Cohen D, Blumenson LE, Petrelli NJ. Validation of a new classification system for curatively resected colorectal adenocarcinoma. Cancer. 1999 Sep 1;86(5):782-92

31. Silvestris N, Scartozzi M, Graziano G, Santini D, Lorusso V, Maiello E, et al. Basal and bevacizumab-based therapy-induced changes of lactate dehydrogenases and fibrinogen levels and clinical outcome of previously untreated metastatic colorectal cancer patients: a multicentric retrospective analysis. Expert opinion on biological therapy. 2015 Feb;15(2):155-62

32. Accinni R, Bartesaghi S, De Leo G, Cursano CF, Achilli G, Loaldi A, et al. Screening of homocysteine from newborn blood spots by high-performance liquid chromatography with coulometric array detection. Journal of chromatography A. 2000 Oct 27;896(1-2):183-9.

33. Saridaki Z, Papadatos-Pastos D, Tzardi M, Mavroudis D, Bairaktari E, Arvanity H, et al. BRAF mutations, microsatellite instability status and cyclin D1 expression predict metastatic colorectal patients' outcome. British journal of cancer. 2010 Jun 8;102(12):1762-8

34. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, et al. Genetic alterations during colorectal-tumor development. The New England journal of medicine. 1988 Sep 1;319(9):525-32

35. Loupakis F, Yang D, Yau L, Feng S, Cremolini C, Zhang W, et al. Primary tumor location as a prognostic factor in metastatic colorectal cancer. Journal of the National Cancer Institute. 2015 Mar;107(3)

36. Ang PW, Li WQ, Soong R, Iacopetta B. BRAF mutation is associated with the CpG island methylator phenotype in colorectal cancer from young patients. Cancer letters. 2009 Jan 18;273(2):221-4

37. Arnold D, Lueza B, Douillard JY et al Prognostic and predictive value of primary tumour side in patients with RAS wild-type metastatic colorectal cancer treated with chemotherapy and EGFR directed antibodies in six randomized trials. Ann Oncol. 2017 Aug 1;28(8):1713-1729

38. Lanza G, Matteuzzi M, Gafa R, Orvieto E, Maestri I, Santini A, et al. Chromosome 18q allelic loss and prognosis in stage II and III colon cancer. International journal of cancer. 1998 Aug 21;79(4):390-5

39. Kinzler KW, Nilbert MC, Su LK, Vogelstein B, Bryan TM, Levy DB, et al. Identification of FAP locus genes from chromosome 5q21. Science. 1991 Aug 9;253(5020):661-5

40. Moertel CG, Fleming TR, Macdonald JS, Haller DG, Laurie JA, Tangen C. An evaluation of the carcinoembryonic antigen (CEA) test for monitoring patients with resected colon cancer. Jama. 1993 Aug 25;270(8):943-7

41. Buyse M, Thirion P, Carlson RW, Burzykowski T, Molenberghs G, Piedbois P. Relation between tumour response to first-line chemotherapy and survival in advanced colorectal cancer: a meta-analysis. Meta-Analysis Group in Cancer. Lancet. 2000 Jul 29;356(9227):373-8

42. Souglakos J, Philips J, Wang R, Marwah S, Silver M, Tzardi M, et al. Prognostic and predictive value of common mutations for treatment response and survival in patients with metastatic colorectal cancer. British journal of cancer. 2009 Aug 4;101(3):465-72

43. Silvestris N, Scartozzi M, Graziano G, Santini D, Lorusso V, Maiello E, et al. Basal and bevacizumab-based therapy-induced changes of lactate dehydrogenases and fibrinogen levels and clinical outcome of previously untreated metastatic colorectal cancer patients: a multicentric retrospective analysis. Expert opinion on biological therapy. 2015 Feb;15(2):155-62

44. Carmeliet P Angiogenesis in health and disease Nat Med 2003; 9:653-660

45. Douillard JY, Cunningham D, Roth AD, et al. Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. Lancet 2000; 355:1041-7

46. Ferrara N, Hillan KJ, Novotny W. Bevacizumab (Avastin), a humanized anti-VEGF monoclonal antibody for cancer therapy. Biochem Biophys Res Commun 2005; 333:328-35

47. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus Irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004; 350: 2335-42

48. Loupakis F, Cremolini C, Masi G et al. Initial therapy with FOLFOXIRI and bevacizumab for metastatic colorectal cancer. N Engl J Med 2014; 371(17): 1609–1618

49. Stein A, Glockzin G, Wienke A et al. Treatment with bevacizumab and FOLFOXIRI in patients with advanced colorectal cancer: presentation of two novel trials (CHARTA and PERIMAX) and review of the literature. BMC Cancer 2012; 12(1):356

50. Bendell J, Benjamin R, Reeves J et al. Overall response rate (ORR) in STEAM, a randomized, open-label, phase 2 trial of sequential and concurrent FOLFOXIRI-bevacizumab (BEV) vs FOLFOX-BEV for the first-line (1L) treatment (tx) of patients (pts) with metastatic colorectal cancer (mCRC). J. Clin. Oncol. 2016; 34:492–492

51. Falcone A, Cremolini C, Masi G, et al. FOLFOXIRI/bevacizumab (bev) versus FOLFIRI/bev as first-line treatment in unresectable metastatic colorectal cancer (mCRC) patients (pts): Results of the phase III TRIBE trial by GONO group. J Clin Oncol 2013; 31

52. Grothey A, Sugrue MM, Purdie DM, Dong W, Sargent D, Hedrick E, et al. Bevacizumab beyond first progression is associated with prolonged overall survival in metastatic colorectal cancer: results from a large observational cohort study (BRiTE). J Clin Oncol. 2008 Nov 20;26(33):5326-34

53. Cohn AL, Bekaii-Saab T, Bendell JC, et al. Clinical outcomes in bevacizumab (BV)-treated patients (pts) with metastatic colorectal cancer (mCRC): Results from ARIES observational cohort study (OCS) and confirmation of BRiTE data on BV beyond progression (BBP). J Clin Oncol 2010;28:15s (suppl; abstr 3596)

54. Bennouna J, Sastre J, Arnold D, et al. Continuation of bevacizumab after first progression in metastatic colorectal cancer (ML18147): a randomised phase 3 trial. Lancet Oncol. 2013;14(1):29–37

55. Kubicka S, Greil R, André T, et al. Bevacizumab plus chemotherapy continued beyond first progression in patients with metastatic colorectal cancer previously treated with bevacizumab plus chemotherapy: ML18147 study KRAS subgroup findings. Ann Oncol. 2013 Sep;24(9):2342-9

56. Masi G, Loupakis F, Salvatore L, et al. A randomized phase III study evaluating the continuation of bevacizumab (bv) beyond progression in metastatic colorectal cancer (mCRC) patients (PTS) who received bv as part of first-line treatment: results of the bebyp trial by the gruppo oncologico nord ovest (GONO) Ann Oncol. 2012; 23: suppl 9 (LBA17)

57. Gaya A, Tse V. A preclinical and clinical review of aflibercept for the management of cancer. Cancer Treat Rev 2012;38:484-93

58. Chu QS. Aflibercept (AVE0005): an alternative strategy for inhibiting tumour angiogenesis by vascular endothelial growth factors. Expert Opin Biol Ther 2009; 9: 263-71

59. Van Cutsem E, Tabernero J, Lakomy R, et al. Addition of aflibercept to fluorouracil, leucovorin, and irinotecan improves survival in a phase III randomized trial in patients with metastatic colorectal cancer previously treated with an oxaliplatin- based regimen. J Clin Oncol 2012; 30:3499-506

60. Allegra CJ, Lakomy R, Tabernero J, et al. Effects of prior bevacizumab (B) use on outcomes from the VELOUR study: a phase III study of aflibercept (Afl) and FOLFIRI in patients (pts) with metastatic colorectal cancer (mCRC) after failure of an oxaliplatin regimen. J Clin Oncol 2012; 30 (abstract 3505)

61. E Maiello V Pomella P Wirapati M et al.Aflibercept efficacy according to sidedness, RAS and BRAF mutations. Findings from the VELOUR trial in second line therapy of advanced colorectal cancer patients. Annals of Oncology, Volume 28, Issue suppl_6, 1 October 2017

62. World Health Organization. WHO handbook for reporting results of cancer treatment. Geneva, Switzerland: World Health Organization, 1979

63. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors (RECIST Guidelines). J Natl Cancer Inst 2000;92:205–16

64. E.A. Eisenhauera, P. Therasseb, J. Bogaertsc, L.H. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1) European Journal of Cancer 45(2009)228–247

65. Choi H, Charnsangavej C, de Castro Faria S, et al. CT evaluation of the response of gastrointestinal stromal tumors after imatinib mesylate treatment: a quantitative analysis correlated with FDG PET findings. AJR Am J Roentgenol 2004;183(6):1619–1628

66. Choi H, Charnsangavej C, Faria SC, et al. Correlation of computed tomography and positron emission tomography in patients with metastatic gastrointestinal stromal tumor treated at a single institution with imatinib mesylate: proposal of new computed tomography response criteria. J Clin Oncol 2007;25(13):1753–1759

67. Yun Shin Chun, Jean-Nicolas Vauthey, Piyaporn Boonsirikamchai. Association of Computed Tomography Morphologic Criteria With Pathologic Response and Survival in Patients Treated With Bevacizumab for Colorectal Liver Metastasesc JAMA, December 2, 2009—Vol 302, No. 21

68. Iacovelli R, Massari F, Albiges L, et al. Evidence and clinical relevance of tumor flare in patients who discontinue tyrosine kinase inhibitors for treatment of metastatic renal cell carcinoma. Eur Urol 2015; 68:154-60

69. Ken Loh K W , Shapiro JD, Tran B et al Tumor burden (TB) as a prognostic indicator in patients with metastatic colorectal cancer (mCRC). *JCO* 32, no. 3_suppl (January 20 2014) 572

70. Piessevaux H, Buyse M, Schlichting M, et al. Use of Early Tumor Shrinkage to Predict Long-Term Outcome in Metastatic Colorectal Cancer Treated With Cetuximab. J Clin Oncol. 2013; 31: 3764-75

71. Piessevaux H, Buyse M, Schlichting M, et al. Use of Early Tumor Shrinkage to Predict Long-Term Outcome in Metastatic Colorectal Cancer Treated With Cetuximab. J Clin Oncol. 2013; 31: 3764-75

72. Cremolini C, Loupakis F, Antoniotti C, et al. Early tumor shrinkage and depth of response predict long term outcome in metastatic colorectal cancer patients treated with first line chemotherapy plus bevacizumab: results from phase III TRIBE trial by the Gruppo Oncologico del Nord Ovest. Ann Oncol. 2015; 26:1188-94

73. Majek O, Gondos A, Jansen L, et al Sex Differences in Colorectal Cancer Survival: Population-Based Analysis of 164,996 Colorectal Cancer Patients in Germany. PLoS One. 2013 Jul 5;8(7)

74. Majek O, Gondos A, Jansen L, et al Sex Differences in Colorectal Cancer Survival: Population-Based Analysis of 164,996 Colorectal Cancer Patients in Germany. PLoS One. 2013 Jul 5;8(7)

75. Gill SR, Pop M, Deboy RT, et al. Metagenomic analysis of the human distal gut microbiome. Science. 2006;312(5778):1355-1359

76. Tran B, Kopetz S, Tie J, et al. Impact of BRAF mutation and microsatellite instability on the pattern of metastatic spread and prognosis in metastatic colorectal cancer. Cancer. 2011;117(20):4623-4632

77. Hutchins G, Southward K, Handley K, et al. Value of mismatch repair, KRAS, and BRAF mutations in predicting recurrence and benefits from chemotherapy in colorectal cancer. J Clin Oncol. 2011;29(10):1261-1270

78. Iacopetta B. Are there two sides to colorectal cancer? *Int J Cancer*. 2002;101(5):403-408

79. Breivik J, Lothe RA, Meling GI, Rognum TO, Børresen-Dale AL, Gaudernack G. Different genetic pathways to proximal and distal colorectal cancer influenced by sex-related factors. Int J Cancer. 1997;74(6):664-669

80. Petrelli F, Tomasello G, Borgonovo K, et al Prognostic Survival Associated With Left-Sided vs Right-Sided Colon Cancer: A Systematic Review and Meta-analysis. JAMA Oncol. 2016 Oct 27. doi 10.1001/jamaoncol.2016.4227