G.O.N.O.

GRUPPO ONCOLOGICO NORD-OVEST

A PHASE II SINGLE-ARM STUDY OF CETUXIMAB PLUS IRINOTECAN AS RECHALLENGE 3RD-LINE TREATMENT OF *KRAS*, *NRAS* AND *BRAF* WILD-TYPE IRINOTECAN-PRETREATED METASTATIC COLORECTAL CANCER PATIENTS PROGRESSING AFTER AN INITIAL RESPONSE TO A 1ST-LINE CETUXIMAB-CONTAINING THERAPY AND A STANDARD 2ND-LINE

THE CRICKET STUDY

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-- Protocol Vers. 2.0 – August 2014 --

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1. ACRONYM

Cetuximab **R**echallenge in **I**rinotecan-pretreated m**C**RC, ***K****RAS, NRAS* and *BRAF* wild-type treated in 1st line with anti-**E**GFR **T**herapy **(CRICKET)**.

2. INTRODUCTION

Cetuximab is a chimeric IgG1 monoclonal antibody (moAb) that binds extracellular domain of epidermal growth factor receptor (EGFR) preventing its linkage with endogenous ligands such as transforming growth factor-*alfa* and epidermal growth factor.

EGFR is a transmembrane glycoprotein with an intracellular tyrosine kinase domain. EGFR activation leads to the activation of intracellular effectors involved in intracellular signaling pathways, such as the G protein KRAS. Moreover, oncogene *KRAS* mutations affect the clinical response to anti-EGFR therapy. Several phase II and phase III trials supported cetuximab combination in first-line treatment of metastatic colorectal cancer (mCRC). Van Cutsem et al. have conducted a randomized phase III trial to investigate the efficacy of cetuximab added to FOLFIRI as first-line treatment of metastatic colorectal cancer (mCRC) patients. A total of 1598 patients, with an immunohistochemical EGFR-positive tumor, were randomly assigned to receive FOLFIRI alone or in combination with cetuximab (at the initial dose of 400 mg/sqm, followed by a dose of 250 mg/sqm once weekly). The primary end-point of the study was progression-free survival (PFS): the addition of cetuximab significantly improved PFS (8.9 months vs 8.0 months; HR=0.85, 95% CI 0.72-0.99; p=0.048). Also in terms of response rate (RR), cetuximab plus FOLFIRI achieved a significant advantage in comparison to FOLFIRI alone (RR: 46.9% vs 38.7%; p=0.004) with a consequent improvement in the rate of radical surgery of metastases with curative intent (4.8% vs 1.7%; p=0.002). No significant difference in overall survival (OS) was found between the two treatment groups: 19.9 months vs 18.6 months in cetuximab-FOLFIRI and FOLFIRI group respectively (HR=0.93, 95% CI 0.81-1.07; p=0.31). The toxicity profile of the combination treatment, cetuximab plus FOLFIRI, was in line with that expected: the incidence of grade 3 skin reactions, and in particular acne-like rash, was significantly higher in patients receiving the anti-EGFR moAb in comparison with those receiving FOLFIRI alone (skin reactions: 19.7% vs 0.2%, p<0.001; acne-like rash: 16.2% vs 0.0%; p<0.001). None of the skin-related toxicities reported were grade 4 and, in the cetuximab-FOLFIRI group, grade of rash was shown to be associated with PFS. Also the incidence of grade 3-4 diarrhea (15.7% vs 10.5%, p=0.008) and infusion-related reactions (2.5% vs 0.0%, p<0.001) was significantly increased in cetuximab-FOLFIRI group. However, these toxicities were manageable and the combination treatment appeared feasible and well tolerated. [1, 2]

A randomized phase II study evaluated the activity of cetuximab combined with FOLFOX-4 versus FOLFOX-4 alone in the first-line treatment of EGFR-expressing mCRC. Three hundred and thirty-seven patients were enrolled: 169 patients received cetuximab (weekly schedule) plus FOLFOX-4 and 168 patients received FOLFOX-4 alone. The addition of cetuximab to chemotherapy showed an increase, even if not significant, in RR (46% vs 36%, p=0.064) and it was associated with an approximate doubling of R0 resection rate (4.7% vs 2.4%). In terms of PFS, no benefit was achieved by the combination of cetuximab with FOLFOX-4 in the intention-to-treat (ITT) population.

The combination treatment was well tolerated and the most frequent grade 3-4 adverse events reported were consistent with the well-known toxicity profile of cetuximab. Skin reactions (including xerosis, erythema, dermatitis acneiform, pruritus, skin exfoliation) were observed in 18% of patients treated with cetuximab plus FOLFOX-4 versus 0.6% reported in the FOLFOX-4-alone group. Infusion-related reactions occurred in 5% and 2% of patients receiving FOLFOX-4 with or without cetuximab respectively. Hypersensivity reactions and rash were the most common reasons for cetuximab discontinuation. The incidence of grade 3-4 neutropenia and grade 3 diarrhea was similar in the two groups of treatment (30% and 8%, respectively, in cetuximab plus FOLFOX-4; 34% and 7%, respectively, in FOLFOX-4 alone); no grade 4 diarrhea was reported. [3, 4]

The German phase III clinical trial FIRE-3 reports that first-line cetuximab plus FOLFIRI chemotherapy offers a roughly four-month survival advantage for patients with metastatic colorectal cancer, compared with bevacizumab plus FOLFIRI (28.8 vs 25.0 mo, HR 0.77, p=0.0164, 95% CI: 0.620-0.953), RR was comparable between arms in the ITT analysis (62% vs 57%, odds ratio 1.249). Median PFS of the ITT population was nearly identical (10.3 vs 10.4 mo, HR 1.04, p=0.69).[5]

Other studies support the use of cetuximab as a single agent or in combination with irinotecan for patients who have progressed on a previous chemotherapy. [6, 7]

Many efforts have been made to identify potential predictors of benefit from anti-EGFR moAbs.

Since the evaluation of EGFR expression by immunohistochemistry was not demonstrated as an useful tool to predict the efficacy of the treatment, attention has been focused on intracellular mediators, involved in the transduction of EGFR signal. Both KRAS/BRAF/MAPKs and PTEN/PI3K/pAKT pathways have been investigated.

Several retrospective experiences, subsequently corroborated by the results of *post-hoc* analyses of large phase III randomized studies, have demonstrated the role of *KRAS* activating mutations as predictors of resistance to anti-EGFR antibodies. Such mutations, that occur in about the 40% of CRCs, involve codons 12 and 13 in more than 90% of cases and lead to the constitutive activation of the RAS/RAF/MAPKs cascade. Signalling events are thus independent from EGFR control. The *post-hoc* analysis of the CRYSTAL trial according to *KRAS* mutational status showed that only patients with *KRAS* wild-type disease derived a significant advantage both in terms of PFS (9.9 months vs 8.7 months, HR=0.68, p=0.017) and RR (59.3% vs 43.2%, OR= 1.91), by the administration of cetuximab combined with chemotherapy. [2] Similarly, in the OPUS study, a phase II randomized trial assessing the efficacy of FOLFOX plus cetuximab (vs FOLFOX) as a first line regimen, the *post-hoc* analysis showed that among patients with *KRAS* wild-type disease, those treated with cetuximab experienced a better outcome both in terms of RR and PFS, in comparison with patients who had received only FOLFOX (RR: 60.7% vs 37.0%, p=0.011; PFS: 7.7 months vs 7.2 months, HR=0.57, p=0.016). [4, 8]

Such results were confirmed by the analysis of phase III trials that randomized heavily pretreated mCRC patients to anti-EGFR monotherapy vs best supportive care (BSC), whose results are thus

not affected by the potential confounding effect of the associated chemotherapy regimens. When compared to BSC, both cetuximab and panitumumab have demonstrated a survival benefit only for patients with *KRAS* wild-type tumors. No responders were identified among patients with *KRAS* mutated disease, treated with panitumumab, in comparison with the 17% of patients with *KRAS* wild-type tumors. Similar findings were reported in terms of PFS: the treatment effect in the *KRAS* wild-type group (HR, 0.45; 95% CI: 0.34 to 0.59) was significantly greater (P< .0001) than in the mutant group (HR, 0.99; 95% CI, 0.73 to 1.36). [9, 10]

As a result of the above reported results of *post-hoc* analyses and retrospectively collected series, demonstrating the negative predictive value of *KRAS* codon 12 and 13 mutations, the use of monoclonal antibodies is now restricted to patients with *KRAS* wild-type disease.

KRAS activating mutations, occurring in codons other than 12 and 13, have been described in mCRC. Codon 61 and 146 mutations, that have been detected with frequencies ranging from 1 to 4%, determine the constitutive activation of RAS protein. It has been reported that, among 87 patients with *KRAS* codon 12 and 13 wild-type disease, none of the patients bearing codon 61 or 146 mutations responded to cetuximab plus irinotecan, compared to 22 out of 68 wild type patients (p=0.096). *KRAS* mutations were also associated with shorter PFS (HR: 0.46, P=0.028). [11]

Analysis of a phase III panitumumab monotherapy study indicated that *KRAS* mutations beyond exon 2 and *NRAS* mutations may be predictive of panitumumab efficacy. [12] It has also been hypothesized that other mutations downstream of EGFR could affect its anti-EGFRs effectiveness such as *BRAF*. BRAF is a serine-threonine kinase and the principal effector of KRAS. *BRAF* V600E mutation in CRC occurs in 5%–12% of cases, and several retrospective studies have suggested that it is associated with a decreased response to anti-EGFR therapy. [13]

Douillard et al. recently published a retrospective analysis of the PRIME trial: the objective was to assess the effect of panitumumab + FOLFOX vs FOLFOX on OS in patients with mCRC based on *RAS* (*KRAS* or *NRAS*) or *BRAF* mutational status. A statistically significant OS benefit was observed in patients with wild-type *RAS* mCRC treated with panitumumab + FOLFOX vs FOLFOX (median gain of more than 7 months in the panitinumab arm), while patients with any RAS mutation derived a significantly detrimental effect from EGFR inhibition. On the basis of these results the use of panitumumab was restricted to *RAS (K*- and *N-RAS)* wt mCRC pts.. In this analysis, *BRAF* mutation showed an undisputed prognostic value, while its predictive value remains controversial even if the efficacy of EGFR inhibition in *BRAF* mutant patients is clearly of limited value. [14]

Results on extended *RAS* mutations as negative predictors were confirmed also by the retrospective analysis of the FIRE3 study, showing a lack of response to cetuximab in patients with any *RAS* mutation.[15]

3. STUDY RATIONALE

As just mentioned above, recent data emerging from literature clearly pointed out that activating mutations of *RAS* predict lack of response to cetuximab or panitumumab therapy. At the same time *BRAF* V600E mutant patients have an extremely poor prognosis[16] [17] and limited chances to benefit from anti-EGFR moAbs.[18] [11, 13]

The acquisition of secondary mutations in tumoral tissue, has not been shown yet to play a major role in resistance to anti-EGFRs in mCRC: the evaluation of *KRAS*/*BRAF* status before and after anti-EGFR antibody treatment carried out by Gattenlohner et al. resulted highly concordant (95% for *KRAS*, 100% for *BRAF*). However, 5% to 10% of mCRC show *KRAS* molecular heterogeneity between primary, lymph nodes, and distant metastases. [19]

Baldus et al. evaluated *KRAS*, *BRAF*, and *PI3K* gene status in the primary tumor, comparing the tumor center and the invasion fronts. The intratumoral heterogeneity of *KRAS*, *BRAF*, and PIK3CA mutations was observed in 8%, 1%, and 5% of primary tumors, respectively. [20] According to the evidence of intratumoral heterogeneity, the occurrence of disease progression after the initial response in a wild-type *KRAS* primary tumor could not be due to late acquisition of the mutation, but rather to the progressive prevalence of a mutated clone, caused by a sort of 'cetuximab-driven mutated genotype acquisition' occurring during therapy. The recourse to a cetuximab-based therapy in *KRAS* wild-type mCRC patients, even without modifing *KRAS* gene status, could lead to the destruction of wild-type cells and to the prevalence of mutated clones, which lead, after an initial tumor reduction, to the first progression of disease. A further line of therapy without cetuximab could restore *KRAS* wild-type clones, which may constitute the major part of the tumor mass at the time of a following progression of disease. At this point, a rescue through a cetuximab-based new line therapy may result in a further shrinkage of the disease. Moreover, the tumor cell entrance to epithelial-to-mesenchymal transition (EMT) or the reverse mesenchymal-to-epithelial transition may justify response or refractoriness, respectively, in patients retreated with cetuximab. EMT is characterized by the combined loss of epithelial cell junction proteins such as E-cadherin and the gain of mesenchymal markers such as vimentin. Therefore, it is likely that the epithelial cells are more susceptible to EGFR-targeted therapies due to their activation of AKT primarily through EGFR-ErbB3. Mesenchymal cells activate AKT through alternative pathways like integrin-linked kinase (ILK) and are largely resistant to EGFR inhibitors. Cetuximab-based therapy could lead during the time, after a first response, to activation of this alternative pathway, ILK-dependent, which favours EMT. [21] A further line without anti-EGFR therapy may down-regulate this process restoring cetuximab sensitivity. Santini et al. conducted a multicenter retrospective analysis that examined irinotecan-refractory patients who had a clinical benefit following a line of cetuximab-plus irinotecan-based therapy and then progression of disease, during cetuximab-based therapy, who underwent a new line chemotherapy and finally, after a clear new progression of disease, were retreated with the same or another cetuximab- plus irinotecan-based therapy. Thirty-nine

patients were enrolled, median age was 59 years: RR was 53.8 [95% confidence interval (CI) 39.1% to 63.7%] with 19 PRs (48.7%) and 2 CRs (5.1%). Stable disease (SD) was obtained in 35.9% of patients (95% CI 24.7% to 51.6%) for a clinical control rate of disease of 89.8%. Progression occurred in only four patients (10.2%). The median PFS was 6.6 months (95% CI 4.1% to 9.1%). Eighteen patients (46.1%) showed the same type of response (SD, PR or CR) during cetuximab retreatment when compared with the response obtained during the first cetuximab-based therapy, 2 patients (5.1%) had an increase in the quality of clinical result, transiting from PR to CR and from SD to PR, respectively. Both, SD lasting at least 6 months and PR during the first cetuximab-based therapy have been demonstrated to predict clinical benefit after cetuximab retreatment. [22]

Basing on these biological and clinical data, we designed a phase II prospective study with the aim of demonstrating that patients who responded and then progressed during a first-line irinotecan- and cetuximab-based therapy may benefit again, after a second-line of therapy, from a third-line treatment with cetuximab and irinotecan.

4. STUDY DESIGN

This is a multicentric, phase II single-arm study in which *KRAS*, *NRAS* and *BRAF* wild-type, irinotecan-resistant mCRC patients progressing after an initial response to a first-line cetuximab-containing therapy, receive a rechallenge third-line treatment with cetuximab plus irinotecan.

5. STUDY OBJECTIVES

PRIMARY OBJECTIVE

The main objective of this study is to evaluate, in terms of Overall Response Rate (ORR) the activity of cetuximab plus irinotecan as rechallenge third line treatment of *RAS (K*- and *N-RAS,* codons 12, 13, 59, 61, 117, 146*)* and *BRAF (V600E)* wild-type, irinotecan-resistant, mCRC patients progressing after an initial response to a first-line irinotecan- and cetuximab-containing therapy and a second-line with FOLFOXIRI/FOLFOX/XELOX plus bevacizumab.

SECONDARY OBJECTIVES

Secondary objectives of this study are:

- the duration of progression-free survival (PFS);
- the duration of overall survival (OS);
- the safety profile;
- the evaluation of potential predictive and/or prognostic biomarkers.

PRIMARY ENDPOINT

The primary endpoint of this study is **overall response rate (ORR)**

ORR is defined as the percentage of patients, relative to the total of enrolled subjects, achieving a complete (CR) or partial (PR) response, according to RECIST 1.1 criteria. The determination of clinical response will be based on investigator-reported measurements. Responses will be evaluated with a chest and abdominal computed tomography (CT) scan every 8 weeks. Patients who do not have an on-study assessment will be included in the analysis as non-responders.

SECONDARY ENDPOINTS

Secondary endpoints of this study are the following:

Progression-free survival (PFS) is defined as the time from the start of therapy until the first documentation of objective disease progression or death due to any cause, whichever occurs first. PFS will be censored on the date of the last evaluable on-study tumor assessment documenting absence of progressive disease for patients who are alive, on study and progression-free at the time of the analysis, or if lost to follow-up. Alive patients having no tumor assessments after baseline will have time to event endpoint censored on the date of treatment start .
The determination of disease progression will be based on investigator-reported measurements. Disease status will be evaluated according to RECIST 1.1 criteria.

Overall survival (OS) is defined as the time from the start of therapy until the date of death due to any cause. For patients still alive at the time of analysis, or if lost to follow up, the OS time will be censored on the last date the patients were known to be alive.

Toxicity rate is defined as the percentage of patients, relative to the total of enrolled subjects, experiencing a specific adverse event, according to National Cancer Institute Common Toxicity V 4.03

6. STATISTICAL METHODS

STATISTICAL CONSIDERATION AND SAMPLE SIZE

According to the Fleming single-stage design and selecting the design parameters p0 (RR in the null hypothesis) = 0.05*, and p1 (RR in the alternative hypothesis) = 0.20**, and considering alpha (one-sided) and beta errors of 0.05 and 0.20 respectively, a total of 27 patients will be required. Null hypothesis will be rejected if at least 4 patients have an objective response.

*A null hypothesis of 0.05 is based on the consideration that available therapies in later lines of treatment for mCRC lead to an extremely low RR. Second-line irinotecan based chemotherapy produces less than 5% RR. [23, 24]

The results of this study will also be subjected to a combined analysis with data from study 062202-264.

**The alternative hypothesis of 0.20 is what we consider as a potential target of interest for further studies in this setting with the experimental treatment.

ANALYSIS POPULATIONS

MODIFIED INTENTION TO TREAT POPULATION (mITT)

The mITT population will include all patients who receive at least one dose of study medication. The mITT population will be the population for evaluating all primary and secondary endpoints,

ANALYSES of ENDPOINTS

ANALYSIS OF PRIMARY ENDPOINT

Best overall response rate will be calculated as the number of patients with a best response of CR or PR divided by the total number of enrolled patients. The corresponding 95% confidence interval will be calculated using a method based on the binomial distribution.

ANALYSIS OF SECONDARY ENDPOINTS

PFS and OS will be described using the Kaplan Meier method for estimating time-to-events and will also be displayed graphically. The median event times and corresponding 95% CI for the median will be provided.

Toxicity rates and overall toxicity rate will be calculated as the number of patients experiencing a specific adverse event of grade 3/4 or any adverse event of grade 3/4 divided by the total number of enrolled patients. The corresponding 95% confidence interval will be calculated using a method based on the binomial distribution.

7. PATIENTS' SELECTION

INCLUSION CRITERIA

- Histologically proven diagnosis of colorectal adenocarcinoma;
- *RAS* and *BRAF* wild-type status of primary colorectal cancer and/or related metastasis;
- First-line irinotecan-based (FOLFIRI or FOLFOXIRI) cetuximab-containing therapy producing at least a partial response;
- First-line progression-free survival in response to cetuximab-containing therapy ≥ 6 months;
- Documentation of progression to first-line cetuximab within 4 weeks after last cetuximab administration;
- Time between the end of first-line therapy and the start of third-line treatment with cetuximab plus irinotecan ≥4 months;
- Second-line oxaliplatin-based (FOLFOXIRI, FOLFOX or XELOX) bevacizumab-containing therapy;
- Documentation of progression to second-line treatment;
- Measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST criteria, vers.1.1);
- Have tumor tissue (of primary tumor and metastases or at least one of the two) available for biomarker analysis;
- Male or female, aged > 18 years of age;
- ECOG Performance Status ≤ 2;
- Life expectancy of at least 3 months;
- Adequate bone marrow, liver and renal function assessed within 14 days before starting study treatment;
- Women of childbearing potential must have a negative blood pregnancy test at the baseline visit. For this trial, women of childbearing potential are defined as all women after puberty, unless they are postmenopausal for at least 12 months, are surgically sterile, or are sexually inactive;
- Subjects and their partners must be willing to avoid pregnancy during the trial and until 6 months after the last trial treatment. Male subjects with female partners of childbearing potential and female subjects of childbearing potential must, therefore, be willing to use adequate contraception as approved by the investigator, such as a two-barrier method or one-barrier method with spermicidal or intrauterine device. This requirement begins 2 weeks before receiving the first trial treatment and ends 6 months after receiving the last treatment;
- Signed informed consent obtained before any study specific procedures.

EXCLUSION CRITERIA

- Active uncontrolled infections or active disseminated intravascular coagulation;
- 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;
- Fertile women (<12 months after last menstruation) and men of childbearing potential not willing to use effective means of contraception;
- Women who are pregnant or are breastfeeding;
- Previous Grade 3/4 infusion related reaction to cetuximab.

8. PARTICIPATING CENTERS, ENROLLMENT AND STUDY DURATION

PARTICIPATING CENTERS

14 Italian Centers

ENROLLMENT AND DATA COLLECTION

Patient registration and data collection are centralized at Polo Oncologico Area Vasta Nord-Ovest – Azienda Ospedaliero-Universitaria Pisana (AOUP), Istituto Toscano Tumori (ITT).

STUDY DURATION

Planned accrual time is 18 months for a total study duration of 24 months.

WITHDRAWAL OF SUBJECTS FROM TREATMENT and FROM STUDY

The Investigator has the right to discontinue a patient from study treatment or withdraw a patient from the study at any time. In addition, patients have the right to voluntarily discontinue study treatment or withdraw from the study at any time for any reason. In instances where consent is withdrawn, the Investigator must clarify whether the patient is willing to continue to be followed (i.e. for survival).

Reasons for discontinuation of study treatment may include, but are not limited to, the following:

- Any medical condition that the Investigator or Sponsor determines may jeopardise the patient‘s safety if he or she continues study treatment;
- Major protocol violation (i.e. affecting the patients‘ safety);
- Investigator or Sponsor determines it is in the best interest of the patient;
- Patient non-compliance;
- Patient withdrawal of consent to receive further study treatment.

Reasons for withdrawal from the study may include, but are not limited to, the following:

- Patient withdrawal of consent to be followed up;
- Patient lost to follow-up;
- Death.

9. STUDY TREATMENT

Experimental Treatment: cetuximab plus irinotecan

- **Cetuximab** 500 mg/sqm iv over 1-h every 2 weeks

followed by

- **Irinotecan** 180 mg/sqm iv over 1-h every 2 weeks (or according to investigator's choice in the best interest of the patient at previously maximum tolerated dose, but no <130 mg/mq)

Treatment will be continued until:

- Disease progression;
- Death;
- Unacceptable toxicity*;
- Consent withdrawn.

*if irinotecan is stopped due to toxicity, cetuximab may be continued as monotherapy at physician's discretion.

If cetuximab is stopped due to toxicity, irinotecan may be continued as monotherapy at physician's discretion.

10. BASELINE AND ON-TREATMENT EVALUATIONS

AT BASELINE

- Complete medical history, ECOG PS, physical examination and vital signs;
- Complete blood chemistry: total bilirubin, AST, ALT, alkaline phosphatase, total proteins, albumin, LDH, creatinine, electrolytes (Na^+, K^+, Ca^{++}, Mg^{++}), aPTT, INR, CEA, CA19.9; pregnancy test (if clinically indicated);
- Complete blood count and differential;
- Chest and Abdominal computed tomography (CT) scan, or Abdomen MRI and Chest X-Ray if CT scan contraindicated;
- Written informed consent;
- Collection of formalin-fixed paraffin-embedded tumor blocks (or 10 slides of conventional thickness and polarity for IHC and 10 slides of 10 micron thickness for molecular biology) of primary and/or metastatic sites;
- Before the administration of the 1st cycle: collection of whole blood (two 6 ml K2-EDTA Vacutainer. Total volume withdrawn: 12 ml) and plasma samples (three 6 ml K2-EDTA Vacutainer. Total volume withdrawn: 18 ml) for pharmacogenetic, pharmacodynamic and circulating DNA analyses. Whole blood and plasma samples will be immediately maintained at 0-4°C (not more than 45 min) and then stored at -20°C and -80°C, respectively.

DURING TREATMENT - EVERY 2 WEEKS

- Before the administration of the 2nd cycle (i.e. cycle 1 day 14): collection of plasma samples (three 6 ml K2-EDTA Vacutainer. Total volume withdrawn: 18 ml) for pharmacodynamic analyses. Plasma samples will be immediately maintained at 0-4°C (not more than 45 min) and then stored at -80°C;
- Partial Blood chemistry: total bilirubin, AST, ALT, alkaline phosphatase, creatinine;
- Complete blood count and differential;
- Toxicity evaluation (CTCAE v4.03)ECOG PS, physical examination (including weight).

DURING AND AFTER TREATMENT UNTIL DISEASE PROGRESSION - EVERY 8 WEEKS

- At first CT-scan evaluation and at progression: collection of plasma samples (three 6 ml K2-EDTA Vacutainer. Total volume withdrawn: 18 ml) for pharmacodynamic and circulating DNA analyses. The obtained plasma samples will be immediately maintained at 0-4°C (not more than 45 min) and then stored at -80°C;
- Tumor evaluation (RECIST criteria v1.1) by using the same technique performed at baseline;

- Complete blood chemistry: total bilirubin, AST, ALT, alkaline phosphatase, total proteins, albumin, LDH, creatinine, electrolytes (Na^+, K^+, Ca^{++}, Mg^{++}), aPTT, INR, CEA, CA19.9.

FLOW CHART			
	Baseline	***Every 2 weeks***	***Every 8 weeks***
Informed Consent	X		
Demographics and Medical History	X		
General Physical Examination	X		X
Vital Signs and Physical Measurements Height, body weight, temperature, blood pressure, pulse, PS	X	X	X
Blood pregnancy test (if applicable)	X		
Hematology Hemoglobin, platelet count, RBC, WBC including differential	X	X	X
Blood Chemistry Creatinine, alkaline phosphatase, ALT, AST, total bilirubin		X	
Complete Blood Chemistry and Markers Creatinine, alkaline phosphatase, ALT, AST, GGT, LDH, total bilirubin, total proteins, albumin, Na^+, K^+, Ca^{++}, Mg^{++}, aPTT, INR, CEA, Ca19.9	X		X
Tumoral Samples Collection	X		
Whole Blood and Plasma Sampling	X	X (2^{nd} cycle)	X (1^{st} CT-scan and PD)
Toxicities Evaluation According to NCI CTCAEv4.03	X	X	X
Tumor Measurements According to RECIST Criteria 1.1	X		X

11. BLOOD, PLASMA AND TUMOR SAMPLES COLLECTION AND ANALYSES

In order to investigate the potential predictive and/or prognostic biomarkers, blood, plasma and tumor samples will be collected and analyzed as following:

Blood and plasma samples will be obtained for all study participants. Blood and plasma samples will usually be obtained at a time when the subject is having blood drawn for other trial purposes.

Preparation and storage of whole blood samples

- Venous blood will be obtained by any standard phlebotomy technique from a peripheral access point or from a central line by trained personnel (two 6 ml K2-EDTA Vacutainer) and stored at -20 °C as soon as possible (no later than 45 min). DNA will be extracted by means of standard commercial kits with the aim to run explorative pharmacogenetic analyses of candidate SNPs in selected genes directly or indirectly related to the EGFR pathway.

Preparation and storage of plasma samples

- Venous blood will be obtained by any standard phlebotomy technique from a peripheral access point or from a central line by trained personnel (three 6 ml K2-EDTA Vacutainer);
- K2-EDTA tubes will be centrifuged at room temperature for 10 min at 1600 (±150) g. Time between blood collection and plasma/blood cell processing should to be less than 4 hours;
- The supernatant of the three EDTA tubes will be transferred to one fresh 15 ml centrifuge tube without disturbing the cellular layer using a disposable 10 ml serological pipette or disposable bulb pipette (sufficient residual plasma should be left in the tubes after the centrifugation without disturbing the leukocyte layer when pipetting);
- The plasma will be centrifuged in the 15 ml centrifuge tube at room temperature for 10 min at 3000 (±150) g;
- The supernatant will be transferred to a fresh 15 ml centrifuge tube without disturbing the cellular layer using a disposable 5 ml or 10 ml serological pipette or disposable bulb pipette (a residual volume of about 0.3 ml (~7 mm) on the bottom of the 15 ml tube should be left in order to avoid contamination of the plasma with cells);
- Plasma tubes will be stored at -80°C.

Collection of tumor samples

Formalin-fixed paraffin-embedded tumour blocks (or 10 slides of conventional thickness and polarity for IHC and 10 slides of 10 micron thickness for molecular biology) of primary and/or metastatic sites will be collected according to standard guidelines. Tumor tissues will be sent to the Coordinating Center (U.O. Oncologia Medica 2 Universitaria-AOUP) for collection and anonymization. DNA will be extracted by means of standard commercial kits and analyzed as per

standard practice by means of Pyrosequencing or Sequenom MassARRAY.

Circulating cell-free tumor DNA analysis

Treatment with target-specific drugs may have the potential to select cells with acquired drug resistance due to the selective pressure on tumor growth. Therefore, treatment may require periodic monitoring finalized to the early identification of resistance at molecular level due to the occurrence of secondary oncogenic drivers mutations including *KRAS*, *NRAS*, *BRAF*, PIK3CA.

A plasma sample (5-10 ml, see previous indications) will be obtained at baseline, before the administration of the 2nd cycle, at first CT-scan evaluation and at progression. Circulating cell-free tumor DNA (cftDNA) will be extracted from plasma using the QIAamp Circulating Nucleic Acid Kit (Qiagen). DNA will be stored at -20°C until the cftDNA analysis, which will be performed by a digital droplet PCR (BioRad). This biomarker study will serve as an indication of the role of *RAS* mutations in treatment response and resistance.

12. SAFETY ISSUES

DOSE REDUCTIONS AND DELAYS

Toxicities should be evaluated according to CTCAEv4.03.

Once a dose has been reduced it should not be increased at a later time.

DOSE MODIFICATIONS FOR TOXICITIES ATTRIBUTABLE TO IRINOTECAN

EVENT AT THE START OF SUBSEQUENT CYCLES OF THERAPY	*GRADE*	*ADJUSTMENT*
WBC	<3.000/mm^3	Hold until resolution
Neutrophils	<1.000/mm^3	
Platelets	<100.000/mm^3	
Diarrhea	≥1	
Mucositis	≥1	
Any other non-hematological toxicity	≥2	

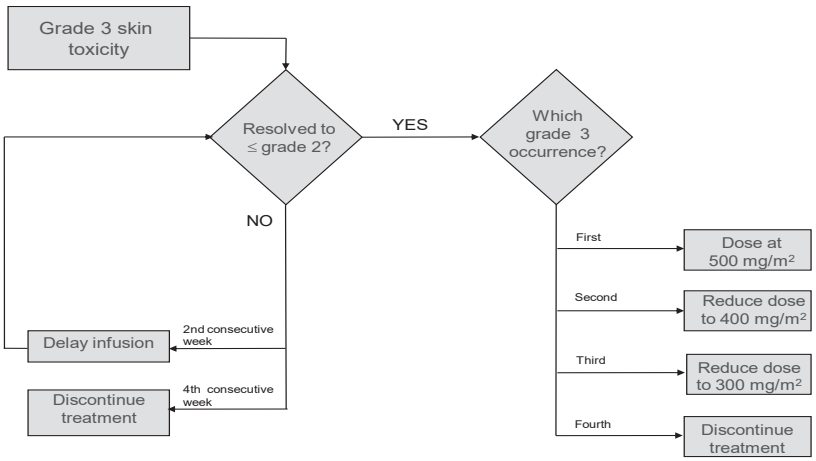
PREVIOUS EVENT	*GRADE*	*ADJUSTMENT*
Neutropenia >5 days	4	**75%**
Febrile Neutropenia	4	
Thrombocytopenia	≥3	
Diarrhea	3	**75%**
Diarrhea	4	**50%**

DOSE MODIFICATIONS FOR TOXICITIES ATTRIBUTABLE TO CETUXIMAB, ACCORDING TO INVESTIGATOR JUDGMENT

For subjects who experience toxicities while on study, one or more doses of cetuximab may need to be withheld, reduced or delayed (administered at >14 day intervals). Cetuximab dose reduction are listed in the table below.

EVENT	*GRADE*	*ADJUSTMENT*
Skin or nail toxicity – *First Occurrence*	3 or 4	Hold cetuximab until grade ≤ 2 and restart at 100% dose level
Skin or nail toxicity in patients treated at 100% or 80% dose level – *Recurring*	3 or 4	Restart cetuximab at 80% dose level or 60% dose level respectively
Symptomatic hypomagnesemia – *First Occurence*		Hold cetuximab until resolution and restart at 100% dose level Mg^{++} supplementation
Symptomatic hypomagnesemia in patients treated at 100% or 80% dose level – *Recurring*		Restart cetuximab at 80% dose level or 60% dose level respectively Mg^{++} supplementation
Diarrhea – *First Occurence*	3 or 4	Hold cetuximab until resolution and restart at 100% dose level
Diarrhea in patients treated at 100% or 80% dose level – *Recurring*	3 or 4	Restart cetuximab at 80% dose level or 60% dose level respectively
Any non-hematologic toxicity	4	Hold cetuximab until resolution

CETUXIMAB DOSE REDUCTION FOR SKIN REACTIONS SCHEME



CRITERIA FOR WITHHOLDING A DOSE OF CETUXIMAB

For subjects who experience a toxicity that meets the criteria for withholding a dose of cetuximab:

- Subjects are allowed to have one subsequent dose withheld for toxicity, as per the scheme shown above. Even if the toxicity has resolved by the intervening week before the next cycle of chemotherapy is due, cetuximab will be restarted along with chemotherapy.

The cetuximab dose (100% or reduced) will be defined according to the scheme shown above and described below:

- Subjects treated at 100% dose level whose toxicity resolves after 1 dose of cetuximab is withheld should be restarted at 100% dose level (recommended but not required, reduction to 80% dose is allowed as an alternative to rechallenge with 100% dose);
- If toxicity recurs, subjects treated at 100% dose or 80% (400 mg/m^2) dose should be restarted at 80% dose or 60% (300 mg/m^2) dose, respectively, if the toxicity has resolved after withholding 1 dose of cetuximab;
- Subjects who experience grade 3 toxicity at the 60% dose level (300 mg/m^2) will not be retreated with cetuximab.

Patients, who must have a delay of cetuximab administration beyond 4 weeks from the previous dose of cetuximab (2 consecutive missed doses) due to toxicity, will be considered unable to tolerate cetuximab and will not be retreated with cetuximab.

Cetuximab should be given on the first day of each chemotherapy cycle. If a cycle of chemotherapy is delayed, cetuximab administration should be also delayed. If chemotherapy is

delayed greater than 4 weeks from the previous chemotherapy dose, and the patient does not have disease progression, cetuximab monotherapy should be administered as soon as possible.

Delay of cetuximab administration greater than 4 weeks from the previous dose of cetuximab are not allowed.

If cetuximab is delayed greater than 4 weeks from the previous administration, and the patient does not have disease progression, irinotecan monotherapy could be continued at physician discretion.

CONCOMITANT MEDICATIONS AND MANAGEMENT OF SPECIFIC TOXICITIES

ACUTE CHOLINERGIC SYNDROME

Atropine sulfate can be used, at the discretion of the investigator, as secondary prophylaxis or therapy of early onset cholinergic syndrome induced by irinotecan. Secondary prophylactic or therapeutic administration of 0.25-1 mg of intravenous or subcutaneous atropine can be considered (unless clinically contraindicated) in patients experiencing rhinitis, increased salivation, miosis, lacrimation, diaphoresis, flushing, abdominal cramping, or diarrhea (occurring during or shortly after infusion of irinotecan).

ANTIEMETIC PROPHYLAXIS

- Day 1 before chemotherapy: 5HT antagonist i.v. + dexamethasone 16 mg i.v.
- Day 2 in the morning: oral/i.m. 5HT antagonist or metoclopramide + dexamethasone 8 mg i.m.

INFUSION RELATED REACTION PROPHYLAXIS

- Day 1 before Cetuximab: Anti-histamine and Corticosteroid (8mg dexamethasone or equivalent). This must be given for the first 3 cycles and is strongly recommended for all subsequent cycles.

TREATMENT OF DIARRHOEA

Irinotecan can induce both early and late forms of diarrhea that appear to be mediated by different mechanisms. Early diarrhea (occurring during or shortly after infusion of irinotecan) is cholinergic in nature. It is usually transient and only infrequently is severe. It may be accompanied by symptoms of rhinitis, increased salivation, miosis, lacrimation, diaphoresis, flushing, and intestinal hyperperistalsis that can cause abdominal cramping. Early diarrhea and other cholinergic symptoms may be ameliorated by administration of atropine (0.25 mg SC). Atropine should not be given prophylactically during cycle 1. Late diarrhea (generally occurring more than 24 hours after administration of irinotecan) can be prolonged, may lead to dehydration and electrolyte imbalance, and can be life-threatening. Patients and patients' caregivers should be carefully informed of possible severe toxic effects such as diarrhea and abdominal cramps. Each patient should be instructed to have loperamide readily available and to begin treatment for late diarrhea (generally

occurring more than 24 hours after administration of irinotecan) at the first episode of poorly formed or loose stools or the earliest onset of bowel movements more frequent than normally expected for the patient. The patient should also be instructed to notify the Investigator if diarrhea or abdominal cramps occur. If diarrhea persists for more than 24 hours despite loperamide, the patient should be instructed to take a fluoroquinolone antibiotic and to re-contact the treating Investigator. The patient should be hospitalised for parenteral support and loperamide should be replaced by another anti-diarrheal treatment (e.g. octreotide). Patients should have a supply of fluoroquinolone antibiotic available at home. The recommended dosage regimen for loperamide previously used in irinotecan clinical trials consists of the following: 4 mg at the first onset of late diarrhea and then 2 mg every 2 hours until the patient is diarrhea-free for at least 12 hours. Note: This dosage regimen exceeds the usual dosage recommendations for loperamide. Premedication with loperamide is not recommended. If diarrhea occurs it is of vital importance that measures are taken to avoid dehydration and electrolyte imbalance. Patients should be supported as clinically indicated. The use of drugs with laxative properties should be avoided because of the potential for exacerbation of diarrhea. Patients should be advised to contact their Investigator to discuss any laxative use. Abdominal cramps should be treated the same as for diarrhea.

CETUXIMAB SPECIFIC INFUSION RELATED REACTIONS

Cetuximab must be administered under the supervision of a physician experienced in the use of antineoplastic medicinal products. Close monitoring is required during the infusion and for at least one hour after the end of the infusion. Availability of resuscitation equipment must be ensured.

Symptoms usually occur during the first infusion and up to one hour after the end of infusion, but may occur after several hours or with subsequent infusions. Occurrence of a severe infusion related reaction requires immediate and permanent discontinuation of cetuximab therapy and may necessitate emergency treatment. In each case of an infusion related reaction, the investigator should implement treatment measures according to the best available medical practice. Based on previous experience with cetuximab infusion related reactions, the treatment guidelines as described in the following table may be applicable.

CTCAE v4.03 grades/symptoms	**Action**
NCI CTCAE grade 1: Mild transient reaction (transient flushing or rash, drug fever < 38°C)	Decrease cetuximab infusion rate by 50% and monitor closely for any worsening, decrease further if reactions persist as applicable: - 1st dose: decrease infusion rate by 50% - 2nd dose: decrease infusion rate by 50%, if infusion related reaction persists decrease infusion rate by another 25% - Subsequent doses: decrease infusion rate by 50%, if infusion related reaction persists decrease infusion rate by another 50% **The total infusion time for cetuximab should not exceed 4 hours.**

CTCAE v4.03 grades/symptoms	Action
NCI CTCAE grade 2: Rash, flushing, urticaria, dyspnea, drug fever ≥ 38°C. Promptly responsive to interruption of infusion and symptomatic treatment.	Stop cetuximab infusion. Administer bronchodilators, oxygen, i.v. fluids, antihistamines, etc. as medically indicated. Resume infusion at 50% of previous rate once infusion related reaction has resolved or decreased to grade 1 in severity, and monitor closely for any worsening. Prolongation of infusion duration should be performed as described for grade 1 reactions, as applicable. **The total infusion time for cetuximab should not exceed 4 hours.** **At second occurrence, cetuximab will be discontinued.**
NCI CTCAE grade 3: Symptomatic bronchospasm, allergy-related edema/angioedema, hypotension. Not rapidly responsive to brief interruption of infusion and/or to symptomatic medication; recurrence of symptoms following initial improvement; hospitalization, indicated for clinical sequelae.	Stop cetuximab infusion immediately and disconnect infusion tubing from the patient. Administer epinephrine, bronchodilators, antihistamines, glucocorticoids, intravenous fluids, vasopressor agents, oxygen, etc., as medically indicated. **The patient should not receive any further cetuximab treatment.**
NCI CTCAE grade 4: Anaphylaxis. Life-threatening consequences; urgent intervention indicated.	

Re-treatment following infusion related reactions: once a cetuximab infusion rate has been decreased due to an infusion related reaction, it will remain decreased for all subsequent infusions. If the patient has an infusion related reaction with the slowest infusion rate, the infusion should be stopped, and the patient must not receive any further cetuximab treatment. If a patient experiences a grade 3 or 4 infusion related reaction at any time, cetuximab should be discontinued.

If there is any question as to whether an observed reaction is an infusion related reaction of grades 1-4, one of the trial chairs should be contacted immediately to discuss and grade the reaction.

INTERSTITIAL PNEUMONITIS

Severe interstitial pneumonitis has been described in subjects treated with the EGFR-pathway targeting therapy gefitinib. To date, no increased risk of interstitial pneumonitis has been identified with cetuximab. Nevertheless, all subjects should have adequate chest imaging prior to commencing cetuximab therapy, as a safety precaution in order to document the baseline pulmonary condition. If there are respiratory symptoms at study entry, lung function tests and further diagnostic procedures should also be undertaken in order to diagnose pre-existing pulmonary fibrosis or interstitial pneumonitis. Furthermore, should pulmonary symptoms appear or worsen during or after cetuximab treatment, a detailed description is required and investigators should use their discretion in ordering such diagnostic procedures as are necessary to elicit an accurate diagnosis.

MANAGEMENT AND TREATMENT OF SKIN TOXICITY

Some educational and general interventions should be used in all patients:

- Sunscreen (avoid sun exposure, use protective products for the exposed areas)
- Avoid habits or products that cause dry skin (hot water, alcohol-based cosmetics)
- Try to maintain skin at maximum hydration (i.e using bath oils, etc.).
- Use warm water
- Frequent use of emollient creams alcohol free
- Use of Tocopherol acetate oil or gel
- Avoid tight shoes
- Do frequent checks
- Avoid the beard growth with regular shaving; use sharp razor multi-blade; use pre-shave creams, emollients and moisturizers after-shave, do not use alcoholic after-shave and electric shaver.

As general and prophylactic intervention maybe include the daily use of Vit K1 cream, beginning day-1 (one day before the administration of the first cetuximab dose) and continued through all the anti EGFR treatment period, applied to face, hands, feet, neck, back, and chest twice daily.

Vitamin K1:

Much evidence has been presented on the beneficial effect of vitamin K1 cream on patients experiencing severe anti-EGFr induced acne-like rash.

This evidence demonstrates that the twice a day use of Vitamin K1 cream in prophylactic or reactive approach improves cutaneous toxicity. The median improvement time in reactive K1 use is 8 - 18 days to observe down-staging in rash at least by 1 grade without reducing the cetuximab dose. No local or systemic toxicity from topical use of Vitamin K1 cream was observed.

The prophylactic approach showed that the twice daily use of Vitamin K1 cream from the beginning of anti EGFR treatment decreases the skin toxicity grade incidence (no grade 3 or 4 recorded in a group of 48 patients).

For these reasons, general and prophylactic intervention may include the daily use of Vit K1, beginning day-1 (one day before the administration of the first cetuximab dose) and continued throughout the anti EGFR treatment period, applied to face, hands, feet, neck, back, and chest twice daily.

Table 1: Management of skin rash grade 1

Skin lesions and symptoms	Papules, pustules, or symptom-free erythema
Cetuximab dose modifications	**No**
Topical treatment	**No**
Systemic treatment	**No**
Intervention	General educational and prophylactic measures

Table 2: Management of skin rash grade 2

Skin lesions and symptoms	Eruption with papules (Grade 2A) or pustules (Grade 2B) covering <50% of body surface, with moderate symptoms, and that does not interfere with daily activities
Cetuximab dose modifications	NO
Topical treatment	Antibiotics: clindamycin 1% gel, erythromycin 3% gel/cream, metronidazole 0.75-1% cream/gel, twice/day until regression to grade 1 (avoid benzoyl peroxide products). Lesions of the scalp: erythromycin 2% lotion
Systemic treatment	*Prevalence of papules (Grade 2A)* No *Prevalence of pustules (Grade 2B)* Antibiotics: minocycline 100 mg per os once/day, doxycycline 100 mg per os once/day for ≥ 4 weeks and until the rash is asymptomatic.

Table 3: Management of skin rash grade 3

Skin lesions and symptoms	Eruption with papules (Grade 3A) or pustules (Grade 3B) covering > 50% of body surface; severe symptoms that interfere with daily activities
Cetuximab dose modifications	*First occurrence*: delay cetuximab infusion for ≤ 14 days until the skin rash improves to grade ≤ 2. If there is an improvement, continue at 100% dose. If there is no improvement in 28 days since the previous infusion, discontinue therapy. *Second occurrence*: delay cetuximab infusion for ≤ 14 days until the skin rash improves to grade ≤ 2. If there is an improvement, continue at reduced dose of 400 mg/m2. If there is no improvement, discontinue therapy. *Third occurrence:* delay cetuximab infusion for ≤ 14 days until the skin rash improves to grade ≤ 2. If there is improvement, continue at reduced dose of 300 mg/m2. If there is no improvement, discontinue therapy. *Fourth occurrence*: discontinue therapy definitively.
Topical treatment	Antibiotics: clindamycin 1% gel, erythromycin 3% gel/cream, metronidazole 0.75-1% cream/gel, twice/day until regression to grade 1 (avoid benzoyl peroxide products). Lesions of the scalp: erythromycin 2% lotion
Systemic treatment	Antibiotics: minocycline 100 mg per os once/day, doxycycline 100 mg per os once/day for ≥ 4 weeks and until the rash is symptomatic. Corticosteroids: according to investigator judjement, methylprednisolone 8 mg per os once or twice/day or prednisone 25 mg per os once/day , for up to 10 days can be administered.
Systemic treatment in highly symptomatic/non-responsive patients	Retinoids: isotretinoin 0.3-0.5 mg/kg per os Corticosteroids: methylprednisolone, or dexamethasone iv Antihistamines: clorfenamine im/iv Antibiotics: amoxicillin/clavulanic acid, gentamicin iv Intravenous hydration

Table 4: Management of skin rash grade 4

Skin lesions and symptoms	Generalized rash; severe symptoms that require emergency treatment
Cetuximab dose modifications	Discontinue therapy immediately and definitively
Topical treatment	Antibiotics: clindamycin 1% gel, erythromycin 3% gel/cream, metronidazole 0.75 to 1% cream/gel, 2 times daily until regression to grade 1 (avoid benzoyl peroxide products). Lesions of the scalp: erythromycin 2% lotion
Systemic treatment	Retinoids: isotretinoin 0.3-0.5 mg/kg per os Corticosteroids: methylprednisolone, dexamethasone iv Antihistamines: clorfenamine im/iv Antibiotics: amoxicillin/clavulanic acid, gentamicin iv Intravenous hydration Hopsitalization

EXTRAVASATION

No severe extravasation reactions have been observed so far with irinotecan. As a general recommendation, in the event of extravasation, the following advice should be observed (like for any drug):

1. stop the infusion immediately,
2. do not remove the needle or cannula,
3. aspirate as much infiltrated drug as possible from the subcutaneous site with the same needle,
4. apply ice to the area for 15 to 20 minutes every 4 to 6 hours for the first 72 hours,
5. watch the area closely during the following days in order to determine whether any further treatment is necessary.

HEMATOPOIETIC GROWTH FACTORS

May be used to treat symptomatic neutropenia but should not be used prophylactically before the 1st cycle. The prophylactic use could be considered in case of:

- Previous febrile neutropenia;
- Previous grade 4 neutropenia for 5 days or more;
- More than 2 delays due to neutropenia

ELECTROLYTE MANAGEMENT

Subjects should be evaluated as outlined in Section "*Baseline and on-treatment evaluations* " and managed as per local medical practice. If hypomagnesemia is present, replacement should be managed with either oral or parental replacement, or both, according to institutional practice and to

the degree of hypomagnesemia present. It is recommended that subject's serum magnesium level should be maintained within the normal range during study treatment.

It is important to assess and manage serum potassium and calcium (adjusted for albumin) in subjects who have concomitant hypomagnesemia. A subject's serum potassium and calcium parameters are recommended to be maintained, as per local medical practice, within the normal ranges during study treatment.

13. ETHICAL ISSUES

This protocol is in accordance with the principles laid down by the 18th World Medical Assembly (Helsinki, 1964) and amendments laid down by the 29th (Tokyo 1975), the 35th (Venice, 1983), the 41st (Hong Kong, 1989), the 48th (Somerset West, 1996) and the 52nd (Edinburgh, 2000) World Medical Assemblies (see appendices).

INFORMED CONSENT

The investigator must explain to each patient (or legally authorised representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each patient must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect her subsequent medical treatment or relationship with physician. The informed consent will be given by means of standard written statement, written in non-technical language. The patient should read and consider the statement before signing and dating it, and should be given a copy of the signed document. If the subject cannot read or sign the document, oral presentation may be made or signature given by the subject's legally appointed representative, if witnessed by a person not involved in the study, mentioning that the patient could not read or sign documents. No patient can enter the study before his/her informed consent has been obtained. The informed consent is part of the protocol and must be submitted by the investigator with to the local ethical committee.

A copy of the patient's signed written consent will be kept by the center in the proper section of the Investigator Site File.

PATIENT PROTECTION

The names of patients will not be recorded; a sequential identification number will be attributed to each patient registered in the trial. This number will identify the patient and must be included on all Case Report Forms.

In order to avoid identification errors, patients initials (maximum of 3 letters) and date of birth will also be reported on the Case Report Forms.

Investigators will guarantee that all persons involved in this study will respect the confidentiality of any information concerning the trial subject.

All parties involved in this clinical trial will maintain the strict confidentiality to assure that neither the person nor the family privacy of the patient participating in the trial is violated; appropriate measures shall be taken to avoid the access of non authorized persons to the trial data. The processing of the personal data of patients taking part in the trial, and in particular regarding data concerning consent, shall comply with local law on the privacy (Legge delega 127/2001) and with the European Directive on the Privacy of data (95/46/EC).

The patient can withdraw consent whenever he wants and further data will not be collected, even if the already collected data will be used for the study's analyses.

CONFIDENTIAL SUBJECT INFORMATION FOR GENETIC ANALYSIS

For the storage of biological samples, e.g. of tumor tissue samples in a bio bank, specific means will be taken to ensure the subject's right to privacy and the pertinent guidance documents and regulations will be considered.

Subjects may withdraw their consent to store the biological samples .

If the patient withdraws his consent from the study within 5 years, the biological samples will be destroyed.

After 5 years, biological samples will be anonymized completely. At that time the samples cannot be identified in any way.

The samples will be maintained for potential analysis for 15 years from the acquisition. Samples will be destroyed according to GONO policies and procedures.

Samples will be collected and sent to the laboratory designated for the trial where they will be processed.

Blood and plasma samples will be processed, analyzed and stored at U.O. Farmacologia Clinica of Azienda Ospedaliero-Universitaria Pisana under the responsibility of Laboratory's Director.

Tumor tissue samples will be processed and analyzed at U.O. Anatomia e Istologia Patologica III of Azienda Ospedaliero-Universitaria Pisana under the responsibility of Laboratory's Director.

The tumor tissue samples will be stored at Dipartimento di Ricerca Traslazionale e delle Nuove Tecnologie (U.O. Oncologia Medica 2 Universitaria of Azienda Ospedaliero-Universitaria Pisana) under the responsibility of Laboratory's Director.

To maintain privacy of information collected from samples obtained for storage and future analysis, GONO has developed secure policies and procedures to maintain subject privacy. At the clinical site, a unique Code will be placed on the blood sample for transfer to the storage facility. The Code is a random number used only to identify the biosample of each subject. No other personal identifiers will appear on the sample tube. The first Code will be replaced with a Sample Code at the Central Laboratory or at the GONO designated facility. This sample is now a single coded sample. The Sample Code is stored separately from all previous sample identifiers. A secure code, hereinafter referred to as a "first coding key", will be utilized to match the Sample Code to the original blood code and subject number to allow clinical information collected during the course of the trial to be associated with the biosample. This "first coding key" will be transferred by the central laboratory or GONO designated facility under secure procedures to the GONO designated as the entrusted keyholder to maintain confidentiality of the biosamples. The Sample Code will be logged into the primary biorepository database, and in this database this identifier will not have identifying demographic data or identifying clinical information (i.e., race, sex, age, diagnosis, lab

values) associated with it. The sample will be stored in a designated repository site with secure policies and procedures for sample storage and usage.

ETHICS COMMITTEE (EC)

The Investigator must submit this protocol to the local Ethics Committee and is required to forward a copy of the written approval to the CRP.

The EC approval must report, the identification of the trial (title, protocol number and version), the documents evaluated (protocol, informed consent material, advertisement when applicable) and the date of their version.

ADMINISTRATIVE RESPONSIBILITIES

The Coordinating Center (U.O. Oncologia 2 Universitaria – Polo Oncologico Azienda Ospedaliero-Universitaria Pisana, AOUP) and the Data Center (U.O. Oncologia 2 Universitaria – Polo Oncologico Azienda Ospedaliero-Universitaria Pisana, AOUP) will be responsible for:

- reviewing the protocol;
- centralizing databases;
- centralizing data validation;
- controlling the quality of the reported data;
- emitting Data Query Forms;
- generating study program reports;
- generating the Statistical Analysis Plan;
- perform statistical analysis.

TRIAL SPONSORSHIP AND FINANCING

The present study is an investigator-initiated trial**,** carried out by participating clinicians, who have the intellectual ownership of the results.

The study is sponsored by:

- Gruppo Oncologico Nord-Ovest (G.O.N.O.) Cooperative Group Via G. Mameli, 3 – Genoa (ITALY), who will provide the economic support for costs related to data management, statistical analysis and the other activities of central and group coordinating centers.
- MERCK.

No funds can be provided to ethical committees and single participating centers.

The study will be conducted according to the current regulations.

14. STUDY MONITORING

QUALITY ASSURANCE

Each participating Investigator will be responsible for ensuring data quality. Each reported information will be systematically checked for consistency, completeness and accuracy by the Coordinating Center that will issue Data Query Forms in case of inconsistent data. Local quality control will be provided by each participating group, which will be responsible for monitoring the centers**.**

RESPONSIBILITIES OF THE INVESTIGATORS

The Investigators will perform the study in accordance with ICH Good Clinical Practice and Good Clinical Practice for Trials on Medicinal Products in the European Community (ISBN 92 - 825-9563-3).

The Investigator is required to ensure his compliance to the procedures required by the protocol with respect to the investigational drug schedule and visit schedule. The Investigator agrees to provide all information requested in the Case Report Form in an accurate manner.

The Investigator has responsibilities to the Health Authorities to take all reasonable steps to ensure the proper conduct of the study as regards ethics, protocol adherence, integrity and validity of the data recorded on the case report forms.

At regular intervals during the study, the center will be contacted, through site visits, letters or telephone calls, to review the study progress, the investigators and subjects adherence to protocol requirements. The following points will be scrutinized:

- subject informed consent, recruitment and follow-up;
- subject compliance to the study treatment;
- study treatment accountability;
- Adverse Event documentation and reporting.

SOURCE DOCUMENT REQUIREMENTS

According to the guidelines on ICH Good Clinical Practice, monitors from the Coordinating Center will check the case report form entries against the source documents. These personnel, bound by professional secrecy, will not disclose any personal identity or personal medical information.

USE AND COMPLETION OF CASE REPORT FORMS (CRFs)

It is the responsibility of the Investigator to prepare and maintain adequate and accurate CRFs for each patient enrolled in the study. All CRFs should be completed to ensure accurate interpretation of data.

15. ADVERSE EVENTS

DEFINITION OF AN ADVERSE EVENT

An adverse event is defined in the International Conference on Harmonisation (ICH) Guideline for Good Clinical Practice as “any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment.” (ICH E6:1.2).

The investigator is responsible for reviewing laboratory test results and determining whether an abnormal value in an individual study subject represents a change from values before the study. Abnormal laboratory findings without clinical significance (based on the investigator's judgment) should not be recorded as adverse events; however, laboratory value changes requiring therapy or adjustment in prior therapy are considered adverse events.

Patients will be instructed by the Investigator to report the occurrence of any adverse event.

ADVERSE DRUG REACTIONS (ADR)

All untoward and unintended responses to a medicinal product related to any dose administered.

The phrase “responses to a medicinal product” means that a causal relationship between the medicinal product and the adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out. A serious ADR (SADR) is an ADR that meets the definition of serious.

DEFINITION OF SERIOUS ADVERSE EVENT

A serious adverse event (SAE) is defined as an adverse event that:

• is fatal;

• is life-threatening (places the subject at immediate risk of death);

• requires in-patient hospitalization or prolongation of existing hospitalization;

• results in persistent or significant disability/incapacity;

• is a congenital anomaly/birth defect;

• other significant medical hazard.

’Hospitalization’ meeting the regulatory definition of “serious” is any in-patient hospital admission that includes a minimum of an overnight stay in a health care facility. Any adverse event that does not meet one of the definitions of serious (i.e., emergency room visit, outpatient surgery, or requires urgent investigation) may be considered by the investigator to meet the “other significant medical hazard” criterion for classification as a serious adverse event. Examples include allergic bronchospasm, convulsions, and blood dyscrasias.

Hospitalization for the performing of protocol-required procedures or administration of study treatment is not classified as a SAE.

All adverse events which do not meet any of the criteria for serious should be regarded as non-serious adverse events.

All serious adverse events occurring during the study treatment period must be reported according to the procedure described below. Any late SAE (occurring within 30 days after the last treatment administration) possibly or probably related to the study treatment should follow the same reporting procedure.

Progression of colorectal cancer leading to one of the above should not be reported as a serious adverse event.

DEATH ON STUDY

Any death occurring between the *registration* and 30 days following the *treatment* must be reported to the Sponsor within 24 hours, as a Serious Adverse Event, regardless of the relation to study treatment. The Sponsor must notify this SAE to *CE coordinating center* by fax within 1 working day. Deaths occurring during the study follow-up period (i.e. later than 30 days after the last infusion) need only to be reported as serious adverse event if it is thought that there is a possible relation to the study treatment (possible, probable). All deaths should be reported on the death report form section of the CRF regardless of cause.

RELATIONSHIP WITH TRIAL MEDICATION

Relationship	Description
UNRELATED	There is no evidence of any causal relationship
UNLIKELY	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatments).
POSSIBLE	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments).
PROBABLE	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
DEFINITELY	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
NOT ASSESSABLE	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

REPORTING PROCEDURE

REPORTING PROCEDURES FOR ALL ADVERSE EVENTS

The investigator is responsible for ensuring that all adverse events are properly captured in the subjects' medical records.

The following adverse event attributes must be assigned by the investigator: adverse event diagnosis or syndrome (if known), or signs/symptoms (if not known); appropriate event description; dates of onset and resolution; severity; assessment of relatedness to study treatment; actions taken.

Medically significant adverse events considered related to the study treatment by the investigator or the sponsor will be followed until resolved or considered stable.

It will be left to the investigator's clinical judgment to determine whether an adverse event is related and of sufficient severity to require the subject's removal from treatment or from the study. A subject may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable adverse event. If either of these situations arises, the subject should be strongly encouraged to undergo an end-of-study assessment and be under medical supervision until symptoms cease or the condition becomes stable.

SERIOUS ADVERSE EVENTS REPORTING PROCEDURES

Serious adverse events will be collected and recorded throughout the study period, defined as through to 30 days after the last dose of study treatment or the end of the study (including the follow-up period), whichever is longer.

The investigator should notify the Sponsor of all serious adverse events occurring at the site in accordance with local procedures, statutes and the European Clinical Trial Directive. The Sponsor will medically review all SAEs.

The Sponsor will ensure the notification of the appropriate Ethics Committees, Competent Authorities and participating Investigators of all serious adverse events occurring at the site in accordance with local legal requirements, statutes and the European Clinical Trial Directive.

Details should be documented on the specified Serious Adverse Event Form.

Mail a .pdf scan version to:

trials.office.pisa@gmail.com

Mail subject should report: SAE Cricket - "Name of Participating Center"

The Sponsor will also send the report to National Authorities, Ethic Committees (EC) and investigators as appropriate, according to local regulations.

FOLLOW-UP

Patients withdrawn from the study treatment due to any adverse event will be followed at least until the outcome is determined, even if it implies that the follow-up continues after the patients has left the trial, and where appropriate, until the end of the planned period of follow-up.

In case of serious adverse event, the patient must be followed until complete clinical recovery and laboratory results have returned to normal, or until symptoms have stabilized. This may imply that the follow-up will continue after the patient has left the trial.

Further information will be noted on the SAE form, by ticking the box marked “follow-up” and will be sent to the Coordinating Center as information becomes available.

The Sponsor shall supply Merck Serono with a copy of any serious individual case safety report regardless of the causality assessment concerning the Pharmaceutical Product administration. Serious individual case safety reports shall include reports whether or not associated with a technical complaint, reports on drug interaction, reports on suspected transmission of an infectious agent by the product. Further, Sponsor shall provide reports of the following special situations whether associated or not with an Adverse Event (“Adverse Events” or “AEs”): medication errors, overdose, abuse, misuse, off- label use, occupational exposure, pregnancy and breast feeding notification and lack of efficacy reports.

In addition, Merck Serono will perform a medical assessment for the purpose of signal detection and cumulative reporting of safety information and will also request follow-up information from the Sponsor as needed.

The Sponsor shall provide Merck Serono Global Drug Safety on a quarterly basis with information on Adverse Events (AEs), safety laboratory data, and any efficacy data necessary to assess the safety of the Pharmaceutical Product.

The Sponsor shall inform Merck Serono of any pregnancy occurring in a subject treated with the Pharmaceutical Product during the course of the Study. The Sponsor shall ensure that the case is followed up to the end of the pregnancy and provide all relevant documentation and a final report on the outcome to Merck Serono.

Merck Serono will perform regular signal detection on the Pharmaceutical Product in its global safety database. In case of any action arising from such signal detection activities which is relevant for the conduct of the Study, Merck Serono will inform the Sponsor in a timely manner.

16. REFERENCES

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G.O.N.O.

GRUPPO ONCOLOGICO NORD-OVEST

A PHASE II SINGLE-ARM STUDY OF CETUXIMAB PLUS IRINOTECAN AS RECHALLENGE 3RD-LINE TREATMENT OF *KRAS*, *NRAS* AND *BRAF* WILD-TYPE IRINOTECAN-PRETREATED METASTATIC COLORECTAL CANCER PATIENTS PROGRESSING AFTER AN INITIAL RESPONSE TO A 1ST-LINE CETUXIMAB-CONTAINING THERAPY AND A STANDARD 2ND-LINE

THE CRICKET STUDY

EUDRACT 2014-001126-15

APPENDICES

APPENDIX I: SYNOPSIS (ENGLISH VERSION)

A PHASE II SINGLE-ARM STUDY OF CETUXIMAB PLUS IRINOTECAN AS RECHALLENGE 3RD-LINE TREATMENT OF *KRAS*, *NRAS* AND *BRAF* WILD-TYPE IRINOTECAN-PRETREATED METASTATIC COLORECTAL CANCER PATIENTS PROGRESSING AFTER AN INITIAL RESPONSE TO A 1ST-LINE CETUXIMAB-CONTAINING THERAPY AND A STANDARD 2ND-LINE

(CRICKET) - EUDRACT 2014-001126-15

Version	2.0 – August, 2014
Sponsor	Gruppo Oncologico Nord-Ovest (G.O.N.O.)
Setting	Third line
Protocol Phase	Phase II single arm
Indication	Patients with unresectable, *KRAS, NRAS* and *BRAF* wild-type and irinotecan-pretreated metastatic colorectal cancer candidates for a 3rd-line treatment and having progressed on a 1st-line cetuximab-containing therapy.
Study Rationale	▪ Several phase II and III studies demonstrated that the combination of cetuximab with 1st-line chemotherapy improves response rate and progression-free survival in *KRAS* wild-type metastatic colorectal cancer patients. ▪ Cetuximab in combination with irinotecan is approved for the treatment of *RAS* wild-type, irinotecan-refractory metastatic colorectal cancer patients. ▪ A recent phase II study demonstrated a potential benefit from cetuximab rechallenge in irinotecan-refractory *KRAS* wild-type metastatic colorectal patients already progressed to a previous cetuximab-containing therapy. ▪ A recent retrospective analysis demonstrated the role of *RAS* mutations in the prediction of resistance to anti-EGFR treatment. Considering *BRAF* mutation, while its prognostic value is undisputed, its predictive role remains unclear.
Endpoints	**Primary:** • To evaluate the activity in terms of response rate (RR) **Secondary:** • Progression-free survival (PFS) • Overall survival (OS) • Safety profile • Evaluation of potential predictive and/or prognostic biomarkers
Inclusion Criteria	• Histologically proven diagnosis of colorectal adenocarcinoma; • *RAS* and *BRAF* wild-type status; • First-line irinotecan-based (FOLFIRI or FOLFOXIRI) cetuximab-containing therapy producing at least a partial response; • First-line progression-free survival in response to cetuximab-containing therapy ≥6 months; • Documentation of progression to first-line cetuximab within 4 weeks after last cetuximab administration; • Time between the end of first-line therapy and the start of third-line treatment with cetuximab plus irinotecan ≥4 months; • Second-line oxaliplatin-based (FOLFOXIRI, FOLFOX or XELOX)

Inclusion Criteria (Cont.)	bevacizumab-containing therapy; • Documentation of progression to second-line treatment; • Measurable disease according to RECIST criteria v1.1; • Have tumor tissue (of primary tumor and metastases or at least one of the two) available for biomarker analysis; • Male or female patients > 18 years of age; • ECOG Performance Status ≤ 2; • Life expectancy of at least 3 months; • Adequate bone marrow, liver and renal function assessed within 14 days before starting study treatment; • Women of childbearing potential must have a negative blood pregnancy test at the baseline visit. For this trial, women of childbearing potential are defined as all women after puberty, unless they are postmenopausal for at least 12 months, are surgically sterile or are sexually inactive; • Subjects and their partners must be willing to avoid pregnancy during the trial and until 6 months after the last trial treatment. Male subjects with female partners of childbearing potential and female subjects of childbearing potential must, therefore, be willing to use adequate contraception as approved by the investigator, such as a two-barrier method or one-barrier method with spermicidal or intrauterine device. This requirement begins 2 weeks before receiving the first trial treatment and ends 6 months after receiving the last treatment; • Signed informed consent obtained before any study specific procedure.
Exclusion Criteria	• Active uncontrolled infections or active disseminated intravascular coagulation; • Past or current history of malignancies other than colorectal carcinoma, except for curatively treated basal and squamous cell carcinoma of the skin cancer or in situ carcinoma of the cervix; • Fertile women (< 12 months after last menstruation) and men of childbearing potential not willing to use effective means of contraception; • Women who are pregnant or are breastfeeding; • Previous grade 3/4 infusion related reaction to cetuximab.
Main parameters of activity	• Response rate, evaluated according to RECIST 1.1 criteria
Main parameters of efficacy	• Progression-free survival (PFS) will be measured from the start of therapy until the first observation of disease progression or death due to any cause The determination of disease progression will be based on investigator-reported measurements. Disease status will be evaluated according to RECIST 1.1 criteria • Overall survival (OS) will be measured from the start of therapy until death due to any cause
Main parameters of safety	• Adverse events, laboratory parameters. • All toxicity will be graded using the NCI Common Toxicity Criteria CTCAE version 4.03
Study Treatment	*Experimental Treatment*: cetuximab plus irinotecan • Cetuximab 500 mg/sqm iv over 1-h every 2 weeks *followed by* • Irinotecan 180 mg/sqm iv over 1-h every 2 weeks (or according to investigator's choice in the best interest of the patient at previously maximum tolerated dose, but no <130 mg/mq)

Study Treatment (Cont.)	Treatment will be continued until: • Disease progression • Death • Unacceptable toxicity* • Consent withdrawn *if irinotecan is stopped due to toxicity, cetuximab may be continued as monotherapy at physician's discretion *if cetuximab will be stopped, irinotecan may be continued as monotherapy at physician's discretion
Study procedures	*At Baseline* • Complete medical history, ECOG PS, physical examination and vital signs • Complete blood chemistry and complete blood count and differential • Chest and Abdominal computed tomography (CT) scan • Written informed consent • Collection of formalin-fixed paraffin-embedded tumour blocks (or 10 slides of conventional thickness and polarity for IHC and 10 slides of 10 micron thickness for molecular biology) of primary and/or metastatic sites • Before the administration of the 1st cycle: collection of whole blood and plasma samples for pharmacogenetic, pharmacodynamic and circulating DNA analyses. Whole blood and plasma samples will be immediately maintained at 0-4°C (not more than 45 min) and then stored at -20°C and -80°C, respectively. *During Treatment - Every 2 weeks* • Before the administration of the 2nd cycle (i.e. cycle 1 day 14): collection of plasma samples for pharmacodynamic analyses. Plasma samples will be immediately maintained at 0-4°C (not more than 45 min) and then stored at -80°C • Partial Blood chemistry (i.e. total bilirubin, AST, ALT, alkaline phosphatase, creatinine) and complete blood count and differential • Toxicity evaluation (CTCAE v4.03) ECOG PS, physical examination (including weight). *During and After Treatment Until Disease Progression - Every 8 weeks* • At first CT-scan evaluation and at progression: collection of plasma samples for pharmacodynamic and circulating DNA analyses. The obtained plasma samples will be immediately maintained at 0-4°C (not more than 45 min) and then stored at -80°C • Tumor evaluation (RECIST criteria v1.1) • Complete blood chemistry, CEA, Ca19.9
Statistical Considerations	According to the Fleming single-stage design and selecting the design parameters p0 (RR in the null hypothesis) = 0.05, and p1 (RR in the alternative hypothesis) = 0.20, and considering alpha (one-sided) and beta errors of 0.05 and 0.20 respectively, a total of 27 patients will be required. Null hypothesis will be rejected if at least 4 patients will have an objective response.
Total Number of Centers	14 Italian Centers
Duration of the Study	Planned accrual time is 18 months for total study duration of 24 months

Enrollment and Data Management	Patients registration and data collection are centralized at Polo Oncologico Area Vasta Nord-Ovest – Azienda Ospedaliero-Universitaria Pisana (AOUP), Istituto Toscano Tumori (ITT)
Contacts Scientific Issues	Dr. Fotios Loupakis; Prof. Daniele Santini; Dr. Lisa Salvatore
Contact Administrative Issues	Dr. Laura Delliponti

FLOW CHART			
	Baseline	***Every 2 weeks***	***Every 8 weeks***
Informed Consent	X		
Demographics and Medical History	X		
General Physical Examination	X		X
Vital Signs and Physical Measurements Height, body weight, temperature, blood pressure, pulse, PS	X	X	X
Blood pregnancy test (if applicable)	X		
Hematology Hemoglobin, platelet count, RBC, WBC including differential	X	X	X
Blood Chemistry Creatinine, alkaline phosphatase, ALT, AST, total bilirubin		X	
Complete Blood Chemistry and Markers Creatinine, alkaline phosphatase, ALT, AST, GGT, LDH, total bilirubin, total proteins, albumin, Na^{+}, K^{+}, Ca^{++}, Mg^{++}, aPTT, INR, CEA, Ca19.9	X		X
Tumoral Samples Collection	X		
Whole Blood and Plasma Sampling	X	X (2^{nd} cycle)	X (1^{st} CT-scan and PD)
Toxicities Evaluation According to NCI CTCAEv4.03	X	X	X
Tumor Measurements According to RECIST Criteria 1.1	X		X

DOSE MODIFICATIONS FOR TOXICITIES ATTRIBUTABLE TO IRINOTECAN		
EVENT AT THE START OF SUBSEQUENT CYCLES OF THERAPY	***GRADE***	***ADJUSTMENT***
WBC	<3.000/mm^3	Hold until resolution
Neutrophils	<1.000/mm^3	
Platelets	<100.000/mm^3	
Diarrhea	≥1	
Mucositis	≥1	
Any other non-hematological toxicity	≥2	
PREVIOUS EVENT	**GRADE**	***ADJUSTMENT***
Neutropenia >5 days	4	**75%**
Febrile Neutropenia	4	
Thrombocytopenia	≥3	
Diarrhea	3	**75%**
Diarrhea	4	**50%**

DOSE MODIFICATIONS FOR TOXICITIES ATTRIBUTABLE TO CETUXIMAB		
EVENT	***GRADE***	***ADJUSTMENT***
Skin or nail toxicity – *First Occurrence*	3 or 4	Hold cetuximab until grade ≤ 2 and restart at 100% dose level
Skin or nail toxicity in patients treated at 100% or 80% dose level – *Recurring*	3 or 4	Restart cetuximab at 80% dose level or 60% dose level respectively
Symptomatic hypomagnesemia – *First Occurence*		Hold cetuximab until resolution and restart at 100% dose level Mg^{++} supplementation
Symptomatic hypomagnesemia in patients treated at 100% or 80% dose level – *Recurring*		Restart cetuximab at 80% dose level or 60% dose level respectively Mg^{++} supplementation
Diarrhea – *First Occurence*	3 or 4	Hold cetuximab until resolution and restart at 100% dose level
Diarrhea in patients treated at 100% or 80% dose level – *Recurring*	3 or 4	Restart cetuximab at 80% dose level or 60% dose level respectively
Any non-hematologic toxicity	4	Hold cetuximab until resolution

APPENDIX II: SINOSSI (ITALIAN VERSION)

STUDIO DI FASE II, A SINGOLO BRACCIO, DI TERAPIA DI III LINEA CON RECHALLENGE DI CETUXIMAB ED IRINOTECANO IN PAZIENTI CON CARCINOMA COLORETTALE METASTATICO *KRAS*, *NRAS* E *BRAF* WILD-TYPE E IRINOTECANO-PRETRATTATI PROGREDITI, DOPO AVER OTTENUTO UN' INIZIALE RISPOSTA, AD UNA TERAPIA DI PRIMA LINEA CONTENENTE CETUXIMAB

(CRICKET) - EUDRACT 2014-001126-15

Versione	2.0 – August 2014
Sponsor	Gruppo Oncologico Nord-Ovest (G.O.N.O.)
Setting	Terza linea
Fase	Fase II a singolo braccio
Indicazioni	Pazienti con tumore del colon-retto metastatico non resecabile, *KRAS, NRAS* e *BRAF* wild-type e irinotecano-pretrattati, candidati ad una terza linea di trattamento e progrediti ad una prima linea contenente cetuximab.
Razionale	▪ Diversi studi di fase II e III hanno dimostrato che la combinazione di cetuximab con una chemioterapia di prima linea migliora il tasso di risposte e la sopravvivenza libera da progressione nei pazienti con tumore del colon-retto metastatico *KRAS* wild-type. ▪ Cetuximab in combinazione con irinotecano è approvato per il trattamento dei pazienti con tumore del colon-retto metastatico *RAS* wild-type e irinotecano-refrattari. ▪ Un recente studio di fase II ha dimostrato il potenziale beneficio dal rechallenge con cetuximab in pazienti con tumore del colon-retto metastatico irinotecano-refrattari *KRAS* wild-type, già progrediti ad una precedente terapia contenente cetuximab. ▪ Una recente analisi retrospettiva ha dimostarto il ruolo delle mutazioni di *RAS* nel predire la resistenza agli anti-EGFR. Per quanto riguarda *BRAF* invece, mentre il suo valore prognostico negativo è indiscusso, il suo ruolo predittivo resta poco chiaro.
Endpoints	**Primario:** • Valutare l'attività in termini di tasso di risposta (RR) **Secondari:** • Sopravvivenza libera da progressione (PFS) • Sopravvivenza globale (OS) • Profilo di tossicità • Valutazione di potenziali biomarcatori predittivi e/o prognostici
Criteri di Inclusione	• Diagnosi istologica di adenocarcinoma del colon-retto; • *RAS* e *BRAF* wild-type; • Risposta ad una prima linea di terapia a base di irinotecan (FOLFIRI o FOLFOXIRI) e contenente cetuximab; • PFS alla prima linea di terapia contenente cetuximab ≥6 mesi; • Documentata progressione alla prima linea di terapia entro 4 settimane dall'ultima somministrazione di cetuximab; • Tempo tra la fine della terapia di prima linea e l'inizio della terza linea con cetuximab e irinotecano ≥4 mesi;

Criteri di Inclusione (Cont.)	• Seconda linea di trattamento a base di oxaliplatino (FOLFOXIRI, FOLFOX, XELOX) in associazione a bevacizumab; • Documentata progressione alla seconda linea; • Malattia misurabile secondo i criteri RECIST v1.1Avere tessuto tumorale (di tumore primario e metastasi o almeno uno dei due) disponibile per l'analisi dei biomarcatori; • Uomo o donna di età > 18 anni; • ECOG Performance Status ≤ 2; • Aspettativa di vita di almeno 3 mesi; • Adeguata funzionalità ematologica, epatica e renale valutata entro 14 giorni dall'inizio del trattamento in studio; • Donne fertili devono avere test di gravidanza negativo alla visita basale. Per questo studio sono considerate fertili tutte le donne dopo la pubertà, eccetto quelle che sono in menopausa da almeno 12 mesi, quelle chirurgicamente sterili o sessualmente inattive; • I pazienti e i loro partner devono evitare la gravidanza durante il trattamento e fino a 6 mesi dopo l'ultima somministrazione. I soggetti maschili con partner fertili e i soggetti femminili fertili devono quindi accettare l'uso di un adeguato metodo contraccettivo approvato dallo sperimentatore (es. doppia barriera o singola barriera con spermicida o dispositivo intrauterino). La contraccezione è richiesta 2 settimane prima dell'inizio del trattamento e fino a sei medi dopo l'ultima somministrazione; • Consenso informato scritto.
Criteri di Esclusione	• Infezioni in atto non controllate o coagulazione intravascolare disseminata in atto; • Storia passata o corrente di altri tumori oltre a quello del colon-retto, con l'eccezione di basaliomi o carcinomi squamocellulari della cute o carcinoma in situ della cervice trattati in modo curativo; • Donna (<12 mesi dall'ultima mestruazione) e uomo in età fertile che non utilizzano adeguati metodi contraccettivi; • Donne in gravidanza o allattamento; • Precedente reazione allergica di grado 3/4 correlata all'infusione di cetuximab.
Principali parametri di attività	• Tasso di risposta, valutato secondo i criteri RECIST 1.1
Principali parametri di efficacia	• Sopravvivenza libera da progressione (PFS): verrà misurata dall'inizio della terapia fino a progressione di malattia o morte dovuta a qualsiasi causa. La valutazione della progressione di malattia si baserà sulle misurazioni riportate dagli investigatori sulla base dei criteri RECIST 1.1 • Sopravvivenza globale (OS): verrà misurata dall'inizio della terapia fino a morte dovuta a qualsiasi causa
Principali parametri di safety	• Eventi avversi, parametri di laboratorio. • Tutte le tossicità verranno graduate secondo l' NCI Common Toxicity Criteria CTCAE versione 4.03.
Trattamento	*Trattamento sperimentale*: Cetuximab più irinotecano • Cetuximab 500 mg/mq ev in 1-h ogni 2 settimane *seguito da* • Irinotecano 180 mg/mq ev in 1-h ogni 2 settimane (o in accordo alla scelta dell'investigatore nel miglior interesse del paziente alla precedente massima dose tollerata, ma <130 mg/mq) Il trattamento verrà continuato fino a: • Progressione di malattia

Trattamento (Cont)	• Morte • Tossicità inaccettabile* • Ritiro del consenso *Se irinotecano viene sospeso per tossicità, cetuximab può essere continuato in monoterapia a discrezione dell'investigatore; Se cetuximab viene sospeso per tossicità, irinotecano può essere continuato in monoterapia a discrezione dell'investigatore.
Procedure dello studio	*Basale* • Anamnesi, ECOG PS, esame obiettivo e segni vitali • Emocromo e chimica completa • TC torace/addome • Consenso informato scritto • Raccolta di blocchetto in paraffina (o 10 vetrini con spessore e polarità convenzionali per IHC e 10 vetrini da 10 micron per biologia molecolare) del tumore primitivo e/o delle sedi metastatiche • Prima della somministrazione del primo ciclo: raccolta di campioni di sangue intero e di plasma per le analisi di farmacogenetica, farmacodinamica e DNA circolante. I campioni di sangue intero e di plasma devono essere immediatamente mantenuti a 0-4°C (per non più di 45 minuti) e poi conservati a -20°C e -80°C, rispettivamente *Durante il trattamento – Ogni 2 settimane:* • Prima della somministrazione del secondo ciclo (i.e. ciclo 1 giorno 14): raccolta di campioni di plasma per le analisi di farmacodinamica. I campioni di plasma devono essere immediatamente mantenuti a 0-4°C (per non più di 45 minuti) e poi conservati a -80°C • Chimica parziale (bilirubina totale, AST, ALT, fosfatasi alcalina, creatinina) ed emocromo • Valutazione delle tossicità • ECOG PS, esame obiettivo (incluso il peso) *Durante e dopo il trattamento fino a progressione – Ogni 8 settimane:* • Alla prima valutazione TC e alla progressione: raccolta di campioni di plasma per le analisi di farmacodinamica e DNA circolante. I campioni di plasma devono essere immediatamente mantenuti a 0-4°C (per non più di 45 minuti) e poi conservati a -80°C • Valutazione di malattia (RECIST v1.1) • Chimica completa, CEA, Ca19.9
Considerazioni statistiche	In accordo al disegno di Fleming single-stage, selezionando p0 (RR nell'ipotesi nulla) = 0.05, and p1 (RR nell'ipotesi alternativa) = 0.20, e considerando l'errore alpha (a 1 coda) e quello beta uguali a 0.05 e 0.20 rispettivamente, saranno necessari 27 pazienti. L'ipotesi nulla verrà rifiutata se almeno 4 pazienti avranno una risposta obiettiva.
Numero centri	14 centri Italiani
Durata dello studio	Accrual pianificato: 18 mesi; durata totale dello studio: 24 mesi
Arruolamento e gestione dati	La registrazione dei pazienti e la raccolta dei dati sono centralizzate presso il Polo Oncologico Area Vasta Nord-Ovest – Azienda Ospedaliero-Universitaria Pisana (AOUP), Istituto Toscano Tumori (ITT)
Contatti medici	Dr. Fotios Loupakis; Prof. Daniele Santini; Dr.ssa Lisa Salvatore
Contatti amminis.	Dr.ssa Laura Delliponti

FLOW CHART			
	Basale	***Ogni 2 settimane***	***Ogni 8 settimane***
Consenso informato	X		
Anamnesi	X		
Esame obiettivo	X		X
Segni vitali e parametri fisici Altezza, peso, temperatura, pressione, polso, PS	X	X	X
test gravidanza su sangue (se applicabile)	X		
Emocromo Emoglobina, piastrine, eritrociti, globuli bianchi e formula	X	X	X
Chimica Creatinina, fosfatasi alcalina, ALT, AST, bilirubina totale		X	
Chimica completa e Markers Creatinina, fosfatasi alcalina, ALT, AST, GGT, LDH, bilirubina totale, proteine, albumin, Na^{+}, K^{+}, Ca^{++}, Mg^{++}, aPTT, INR, CEA, Ca19.9	X		X
Raccolta campioni tumorali	X		
Raccolta sangue intero e plasma	X	X (ciclo 2)	X (prima TC e PD)
Valutazione delle tossicità Secondo NCI CTCAEv4.0	X	X	X
Valutazione di malattia Secondo i criteri RECIST v1.1	X		X

MODIFICAZIONI DI DOSE PER TOSSICITA' DOVUTE AD IRINOTECANO		
EVENTO ALL'INIZIO DI OGNI CICLO	***GRADO***	***MODIFICA***
Globuli bianchi	<3.000/mm^3	Sospendere fino a risoluzione
Neutrofili	<1.000/mm^3	
Piastrine	<100.000/mm^3	
Diarrea	≥1	
Mucosite	≥1	
Altre tossicità non ematologiche	≥2	
EVENTI PRECEDENTI	***GRADO***	***MODIFICA***
Neutropenia >5 giorni	4	**75%**
Neutropenia febbrile	4	
Piastrinopenia	≥3	
Diarrea	3	**75%**
Diarrea	4	**50%**

MODIFICAZIONI DI DOSE PER TOSSICITA' DOVUTE A CETUXIMAB		
EVENTO	***GRADO***	***MODIFICA***
Tossicità cutanea o ungueale – *Prima occorrenza*	3 o 4	Sospendere cetuximab fino a grado ≤ 2 e riprendere al 100% della dose
Tossicità cutanea o ungueale in pazienti trattati al 100% o 80% della dose – *Ricorrenza*	3 o 4	Riprendere cetuximab all' 80% o al 60% della dose rispettivamente
Ipomagnesemia sintomatica – *Prima occorrenza*		Sospendere cetuximab fino a risoluzione e riprendere al 100% della dose Supplementazione di Mg^{++}
Ipomagnesemia sintomatica in pazienti trattati al 100% o 80% della dose – *Ricorrenza*		Riprendere cetuximab all' 80% o al 60% della dose rispettivamente Supplementazione di Mg^{++}
Diarrea – *Prima occorrenza*	3 o 4	Sospendere cetuximab fino a risoluzione e riprendere al 100% della dose
Diarrea in pazienti trattati al 100% o 80% della dose – *Ricorrenza*	3 o 4	Riprendere cetuximab all' 80% o al 60% della dose rispettivamente
Qualsiasi tossicità non ematologica	4	Sospendere cetuximab fino a risoluzione

APPENDIX III: DICHIARAZIONE DI HELSINKI DELLA ASSOCIAZIONE MEDICA MONDIALE (AMM)

(Traduzione di Antonio G. Spagnolo)

PRINCIPI ETICI PER LA RICERCA MEDICA CHE COINVOLGE SOGGETTI UMANI

Adottata dalla 18° Assemblea Generale dall'AMM a Helsinki, Finlandia, nel giugno 1964 ed emendata dalla 29° Assemblea Generale a Tokyo, Giappone, nell'ottobre 1975, dalla 35° Assemblea Generale a Venezia. Italia, nell'ottobre 1983, dalla - 41ª Assemblea Generale a Hong Kong, nel settembre 1989, dalla 48° Assemblea Generale a Somerset West, Repubblica del Sud Africa, nell'ottobre 1996 e dalla 52° Assemblea Generale a Edimburgo, Scozia, nell'ottobre 2000.

a. Introduzione

1. L'AMM ha elaborato la Dichiarazione di Helsinki come dichiarazione di principi etici che forniscano una guida per i medici e per gli altri partecipanti ad una ricerca medica che coinvolge soggetti umani. La ricerca medica che coinvolge soggetti umani include la ricerca su materiale umano identificabile o su altri dati identificabili.
2. È dovere del medico promuovere e salvaguardare la salute delle persone. Le sue conoscenze e la sua coscienza sono finalizzate al compimento di questo dovere.
3. La Dichiarazione di Ginevra[*] dell'AMM impegna il medico con le parole «La salute del mio paziente sarà la mia preoccupazione principale», e il Codice Internazionale di Etica Medica[**] dichiara che «Un medico dovrà agire solo nell'interesse del paziente quando fornisca una cura medica che possa avere l'effetto di indebolire lo stato fisico e mentale del paziente».
4. Il progresso medico è fondato sulla ricerca la quale a sua volta si deve basare in qualche misura su una sperimentazione che coinvolga soggetti umani.
5. Nella ricerca su soggetti umani, le considerazioni correlate con il benessere del soggetto umano devono avere la precedenza sugli interessi della scienza e della società.
6. Lo scopo primario della ricerca medica che coinvolga soggetti umani è quello di migliorare le procedure preventive, diagnostiche e terapeutiche e di comprendere l'eziologia e la patogenesi della malattia. Anche i più comprovati metodi preventivi, diagnostici e terapeutici devono continuamente essere messi in discussione mediante la ricerca sulla loro efficacia, efficienza, accessibilità e qualità.
7. Nella pratica medica corrente e nella ricerca medica, la maggior parte delle procedure preventive, diagnostiche e terapeutiche implicano rischi ed aggravi.
8. La ricerca medica è sottoposta agli standard etici che promuovono il rispetto per tutti gli esseri umani e proteggono la loro salute e i loro diritti. Alcuni soggetti di ricerca sono vulnerabili e richiedono una speciale protezione. Devono essere riconosciuti le particolari necessità di coloro che sono economicamente e medicalmente svantaggiati. Una speciale attenzione e

[*] *Approvata a Ginevra nel 1948 e rivista a Sidney nel 1968 dalla XXII Assemblea Medica Mondiale (N.d.T.)*

[**] *Adottato a Londra dalla III Assemblea Generale dell'Associazione Medica Mondiale (N.d.T.)*

pure richiesta per coloro che non possono dare o che rifiutano il consenso personale, per coloro che possono essere esposti a dare il consenso sotto costrizione, per coloro che non beneficeranno personalmente dalla ricerca e per coloro per i quali la ricerca è associata alla cura.

9. I ricercatori devono essere al corrente dei requisiti etici, giuridici e regolatori della ricerca sui soggetti umani, sia i requisiti nazionali sia quelli internazionali, ove applicabili. Nessun requisito nazionale di natura etica, giuridica o regolatoria deve poter ridurre o eliminare alcuna delle protezioni per i soggetti umani esposte in questa Dichiarazione.

b. Principi basilari per tutta la ricerca medica

10. Nella ricerca medica è dovere del medico proteggere la vita, la salute, la riservatezza e la dignità del soggetto umano.
11. La ricerca medica che coinvolge soggetti umani deve essere conforme ai principi scientifici universalmente accettati e deve essere basata su una approfondita conoscenza della letteratura scientifica, di altre rilevanti fonti di informazione, e su un'adeguata sperimentazione in laboratorio e, ove appropriato, sull'animale.
12. Un'appropriata cautela deve essere posta nella conduzione di ricerche che possano incidere sull'ambiente, e deve essere rispettato il benessere degli animali utilizzati per la ricerca.
13. Il disegno e l'esecuzione di ogni procedura sperimentale che coinvolga soggetti umani devono essere chiaramente descritti in un protocollo di sperimentazione. Tale protocollo deve essere sottoposto ad esame, commenti, orientamenti e, dove previsto, all'approvazione da parte di un comitato etico di revisione appositamente istituito; che deve essere indipendente dal ricercatore, dallo sponsor e da qualsiasi altro tipo di indebita influenza. Questo comitato indipendente deve essere conforme alle leggi ed ai regolamenti della nazione in cui la sperimentazione è condotta. Il comitato ha titolo per monitorare i trial in corso. Il ricercatore ha l'obbligo di fornire le informazioni di monitoraggio al comitato, specialmente quelle relative agli eventi avversi seri. Il ricercatore deve anche sottoporre al comitato, per la revisione, le informazioni relative a finanziamento, sponsor, appartenenze a istituzione, altri potenziali conflitti di interesse e incentivi per i soggetti di sperimentazione.
14. Il protocollo di ricerca deve sempre contenere una esposizione delle considerazioni etiche implicate e deve recare l'indicazione di conformità con i principi, enunciati nella presente Dichiarazione.
15. La ricerca biomedica che coinvolge soggetti umani deve essere condotta solo da persone scientificamente qualificate e sotto la supervisione di un medico competente sul piano clinico. La responsabilità nei confronti del soggetto umano deve sempre ricadere sul personale medico qualificato e mai sul soggetto della ricerca, anche se questi ha dato il proprio consenso
16. Ogni progetto di ricerca medica che coinvolga soggetti umani deve essere preceduto dà un'attenta valutazione dei rischi e degli aggravi prevedibili in rapporto ai benefici attesi per il soggetto stesso o per altri. Ciò non preclude la partecipazione di volontarisani ad una ricerca medica. Il disegno di tutti gli studi deve essere pubblicamente disponibile.
17. I medici devono astenersi dall'intraprendere progetti di ricerca che coinvolgano soggetti umani a meno che non siano sicuri che i rischi implicati siano stati adeguatamente valutati e possano essere controllati in modo

soddisfacente. 1 medici devono interrompere ogni ricerca se i rischi si presentano superiori ai potenziali benefici o se si è raggiunta già una prova definitiva di risultati positivi e benefici.

18. La ricerca medica che coinvolga soggetti umani deve essere condotta solo se l'importanza dell'obiettivo prevalga sui i rischi e gli aggravi connessi per il soggetto. Ciò è particolarmente importante quando i soggetti umani siano volontari sani.

19. La ricerca medica è giustificata solo se vi è una ragionevole probabilità che le popolazioni in cui la ricerca è condotta possano beneficiare dei risultati della ricerca.

20. I soggetti devono essere volontari e partecipare informati al progetto di ricerca.

21. Il diritto dei soggetti di sperimentazione alla salvaguardia della loro integrità deve essere sempre rispettato. Deve essere adottata ogni precauzione per rispettare la privacy del soggetto, la riservatezza sulle informazioni relative al paziente e per minimizzare l'impatto dello studio sulla integrità fisica e mentale del soggetto e sulla sua personalità.

22. In ogni ricerca su esseri umani ciascun potenziale soggetto deve essere adeguatamente informato degli scopi, dei metodi, delle fonti di finanziamento, di ogni possibile conflitto di interessi, della appartenenza istituzionale del ricercatore, dei benefici previsti e dei rischi potenziali connessi allo studio, nonché dei fastidi che esso potrebbe comportare. Il soggetto deve essere informato del diritto di astenersi dal partecipare allo studio o della possibilità di ritirare il consenso alla partecipazione in qualsiasi momento senza ritorsioni. Solo dopo essersi assicurato che il soggetto abbia compreso le informazioni, il medico deve ottenere dal soggetto il consenso informato, liberamente espresso, pre feribilmente in forma scritta. Se il consenso non può essere ottenuto per iscritto, deve essere formalmente documentato e testimoniato un consenso non scritto.

23. Nell'ottenere il consenso informato al progetto di ricerca, il medico deve essere particolarmente attento quando il soggetto si trovi in una condizione di dipendenza nei suoi confronti o possa sentirsi costretto a dare il consenso. In questo caso il consenso informato deve essere ottenuto da un altro medico che conosca bene la ricerca ma non sia coinvolto in essa e che sia completamente indipendente nella relazione col soggetto.,

24. Per un soggetto di ricerca che sia legalmente, fisicamente o mentalmente incapace di dare il consenso, o per un minore legalmente incapace, il ricercatore deve ottenere il consenso informato dal tutore legale, in accordo con la legislazione specifica. Questi gruppi di soggetti non devono essere inclusi in una ricerca a meno che la ricerca stessa non sia necessaria per promuovere la salute della popolazione rappresentata e tale ricerca non possa essere invece attuata su persone legalmente capaci.

25. Quando un soggetto giudicato legalmente incapace, come un minore, sia capace di dare un assenso alla decisione di partecipare in una ricerca, lo sperimentatore deve ottenere tale assenso in aggiunta a quello del tutore legale.

26. La ricerca su individui dai quali non sia possibile ottenere un consenso, incluso quello rappresentato o anticipato, deve essere attuata solo se la condizione fisica o mentale che impedisce di ottenere il consenso è una caratteristica necessaria della popolazione in studio. Le ragioni specifiche per coinvolgere soggetti di ricerca che si trovino in condizioni tali da renderli incapaci di dare un consenso informato devono essere dichiarate nel

protocollo di sperimentazione per l'esame e l'approvazione da parte del comitato di revisione. Il protocollo deve dichiarare che il consenso a rimanere nella ricerca sarà ottenuto non appena possibile da parte dello stesso soggetto o da un rappresentante legalmente autorizzato.

27. Sia gli autori sia gli editori hanno obbligazioni etiche. Nella pubblicazione dei risultati della ricerca gli sperimentatori sono obbligati, a salvaguardare l'accuratezza dei risultati. Sia i risultati negativi sia quelli positivi devono essere pubblicati o resi in qualche modo pubblicamente disponibili. Le fonti del si devono essere dichiarati nella pubblicazione. Relazioni di sperimentazioni non conformi con i principi fissati in questa Dichiarazione non devono essere accettati per la pubblicazione.

c. Principi aggiuntivi per la ricerca medica associata alle cure mediche

28. Il medico può associare la ricerca medica con le cure mediche solo con il limite che la ricerca sia giustificata da un potenziale valore preventivo, diagnostico o terapeutico. Quando la ricerca medica è associata con le cure mediche si applicano degli standard addizionali per proteggere i pazienti che sono soggetti di ricerca.

29. I benefici, i rischi, gli aggravi e l'efficacia di un nuovo metodo devono essere valutati in confronto con quelli dei migliori metodi preventivi, diagnostici e terapeutici attualmente in uso. Ciò non esclude l'impiego di placebo, o l'assenza di trattamento, negli studi dove non esistono metodi comprovati di prevenzione, diagnosi o terapia.

30. A conclusione _dello studio, ad ogni paziente entrato nello studio deve essere assicurato l'accesso ai migliori metodi preventivi, diagnostici e terapeutici di comprovata efficacia identificati dallo studio.

31. Il medico deve informare pienamente il paziente di quali aspetti della cura sono correlati con la ricerca. Il rifiuto di un paziente a partecipare in uno studio non deve mai interferire con la relazione medico-paziente.

32. Nel trattamento di un paziente, laddove non esistano comprovati metodi preventivi, diagnostici e terapeutici o questi siano stati inefficaci, il medico, con il consenso informato del paziente, deve essere libero di usare mezzi preventivi, diagnostici e terapeutici non provati o nuovi, se a giudizio del medico essi offrono speranza di salvare la vita, ristabilire la salute o alleviare la sofferenza. Laddove possibile, tali mezzi dovrebbero essere fatti oggetto di una ricerca disegnata per valutare la loro sicurezza ed efficacia. In tutti i casi, le nuove informazioni devono essere registrate e, dove opportuno, pubblicate. Tutte le altre linee-guida di questa Dichiarazione devono essere seguite.

APPENDIX IV: SCALA PER LA VALUTAZIONE DEL PERFORMANCE STATUS

Scale di valutazione delle condizioni generali (performance status)
(Karnofsky ed ECOG)

ECOG	Karnofsky
0 In grado di svolgere le attività normali senza restrizioni	**100%** Normale nessun disturbo né evidenza di malattia
	90% In grado di svolgere le attività normali; modesti segni o sintomi di malattia
1 Presenta restrizioni alle attività fisiche strenue, ma deambula ed è in grado di svolgere attività lievi o sedentarie,quali lavori domestici	**80%** Attività normale con sforzo; alcuni segni o sintomi di malattia
2 Deambula, è autosufficiente, ma non può svolgere attività lavorative; in piedi per più del 50% del tempo	**60%** Richiede assistenza saltuaria, ma può soddisfare la maggior parte delle sue esigenze
	50% Richiede notevole assistenza e frequenti cure mediche
3 Appena autosufficiente, allettato o seduto per più del 50% del tempo	**40%** Disabile; richiede particolari cure e assistenza
	30% Gravemente disabile, sono opportuni il ricovero ospedaliero e un trattamento di sostegno efficace; il decesso non è imminente
4 Completamento disabile; non autosufficiente; sempre allettato o seduto	**20%** molto ammalato; sono necessari il ricovero ospedaliero e un trattamento di sostegno efficace
	10% Moribondo, i processi fatali progrediscono rapidamente
5 Deceduto	**0%** Deceduto

Karnofsky et al. The use of the nitrogen mustards in the palliation treatment of carcinoma with particular reference to bronchogenic carcinoma, Cancer 1:634-656, 1948, e di Oken MM et al: Toxicity and response criteria of the Eastern Cooperative Oncology Group, Am J Clin Oncol 5:649-655, 1982.

APPENDIX V: VALUTAZIONE RISCHIO/BENEFICIO

(STUDIO CRICKET) - EUDRACT 2014-001126-15

Versione 1.6 Aprile 2014

STUDIO DI FASE II, A SINGOLO BRACCIO, DI TERAPIA DI III LINEA CON RECHALLENGE DI CETUXIMAB ED IRINOTECANO IN PAZIENTI CON CARCINOMA COLORETTALE METASTATICO KRAS, NRAS E BRAF WILD-TYPE E IRINOTECANO-PRETRATTATI PROGREDITI, DOPO AVER OTTENUTO UN' INIZIALE RISPOSTA, AD UNA TERAPIA DI PRIMA LINEA CONTENENTE CETUXIMAB.

Il presente studio ha come obiettivo quello di valutare un ritrattamento con cetuximab e irinotecano in pazienti con tumore del colon-retto metastatico *KRAS, NRAS* e *BRAF* wild-type, irinotecano resistenti, progrediti ad una prima linea a base di irinotecano (FOLFIRI o FOLFOXIRI) contenente cetuximab dalla quale abbiano ricevuto un iniziale beneficio. Diversi studi hanno dimostrato che l'utilizzo di cetuximab in monoterapia o in combinazione con irinotecano migliora l'outcome di pazienti con tumore del colon-retto metastatico *KRAS* wild-type in cui gli altri trattamenti chemioterapici hanno fallito e che non hanno mai ricevuto un trattamento con anticorpi diretti contro il recettore per il fattore di crescita epidermico (EGFR). Un recente studio di fase II ha dimostrato un potenziale beneficio derivante da un ritrattamento con cetuximab in pazienti irinotecano-pretrattati che hanno già ricevuto una terapia contenente cetuximab. Inoltre recenti analisi hanno dimostrato che anche le mutazioni di *NRAS* hanno un ruolo nel predire la resistenza agli anticorpi anti-EGFR. Per quanto riguarda *BRAF*, benchè il suo ruolo predittivo non sia stato ancora definitivamente chiarito, è possibile affermare che la presenza di tale mutazione sia associata a ben poche possibilità di beneficiare di un trattamento con cetuximab o panitumumab, mentre il suo valore prognostico negativo resta indiscusso.

Dal punto di vista della safety, l'associazione di cetuximab e irinotecano presenta un profilo di tossicità maneggevole ed è ormai utilizzata routinariamente nella pratica clinica.

Questo studio di fase II ha lo scopo di gettare le basi per valutazioni future sul rechallenge di cetuximab e irinotecano in pazienti con tumore del colon-retto metastatico *KRAS, NRAS* e *BRAF* wild-type, che hanno beneficiato di un trattamento di prima linea a base di irinotecano contenente cetuximab.

Di seguito i riferimenti scientifici:

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