G.O.N.O.

GRUPPO ONCOLOGICO NORD-OVEST

INDUCTION CHEMOTHERAPY WITH FOLFOXIRI PLUS CETUXIMAB AND MAINTENANCE WITH CETUXIMAB OR BEVACIZUMAB THERAPY IN UNRESECTABLE RAS AND BRAF WILD-TYPE METASTATIC COLORECTAL CANCER PATIENTS

*(*THE MACBETH STUDY*)*

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1. INTRODUCTION

CHEMOTHERAPY OF METASTATIC COLORECTAL CANCER

Colorectal carcinoma (CRC) is the second cause of cancer-related death in developed countries; in Italy during the 2000 about 30.000 new cases and over than 15.000 deaths have been recorded. Even if in the 90% of cases a radical resection of the primary tumour is possible, 25% of patients presents at diagnosis with metastatic disease and 50% of patients die from systemic disease (1).
For over than 30 years 5-fluorouracil (5-FU) has been the only standard treatment for patients with metastatic CRC (mCRC). A metanalysis (2) of various randomized trials has demonstrated that 5-FU alone versus best supportive care improves survival and quality of life of patients. Moreover a randomized trial demonstrated that the advantage is greater when chemotherapy is started early, in asymptomatic patients (3).
The introduction of irinotecan (CPT-11) and oxaliplatin (LOHP) improved antitumor activity and efficacy of chemotherapy in this disease (4,5). The combinations of CPT-11 + 5-FU/leucovorin (LV) (FOLFIRI and IFL) and LOHP + 5-FU/LV (FOLFOX) have demonstrated increased antitumor activity and efficacy compared with 5-FU/LV alone in phase III randomized studies (6,7,8,9). Of interest, phase III studies comparing CPT-11 + 5-FU/LV with 5-FU/LV alone suggested that a more active treatment administered upfront can prolong survival, even if active second-line therapies are offered to patients progressing on 5-FU/LV. Furthermore, studies with LOHP + 5-FU/LV have indicated that a highly active first-line chemotherapy regimen may permit, in a small subgroup of initially unresectable mCRC patients, a radical surgical approach to metastases after response to chemotherapy, and that approximately 30% to 40% of operated patients will survive without evidence of disease for >5 years (10,11). Therefore, these data indicate that, in mCRC, a more active first-line treatment can be more effective, and a meta-analysis of 25 randomized trials of first-line treatment also supports the relationship between tumor response to first-line chemotherapy and survival (12). A randomized study by the GERCOR (13) assigned 220 untreated mCRC patients to receive first-line FOLFIRI followed by FOLFOX-6 at progression (arm A), or the reverse (arm B). Both sequences achieved similar activity and efficacy, and, of interest, median overall survival (OS) was 21.5 months in arm A and 20.6 months in arm B, which are the highest survival times reported up to now in any randomized study of chemotherapy in mCRC. This study suggests that the exposure of mCRC patients to all three most active agents, 5-FU/LV, CPT-11 and LOHP, is associated with best survival outcomes. In addition, a study by Goldberg et al. (14) demonstrated the superiority of FOLFOX-4 regimen to IFL suggesting the importance of exposing patients to all the three agents to achieve prolonged survival, considering that in the IFL arm only 24% of patients could receive LOHP as second-line treatment, while in the FOLFOX-4 arm 60% of patients were able to receive salvage treatment with CPT-11. Furthermore, in a

sequential strategy, not all patients who progress after first-line chemotherapy are able to receive second-line treatment, and therefore not all are exposed to these three active agents. In fact, clinical trials suggest that approximately 20% to 40% of patients, mainly because of deterioration of their performance status and liver function, will not be fit enough to undergo further chemotherapy and will receive only supportive care (7,7,9,14). Moreover, a recent pooled analysis of seven phase III trials in mCRC demonstrated that survival is correlated with the proportion of patients who received all the three active drugs in the course of their disease, but not with the proportion of patients who received any second-line therapy (15).

A way to further improve the outcome of mCRC patients is a more aggressive first line polychemotherapy regimen containing all the three most active agents (LOHP, CPT-11 and 5-FU/LV). This strategy exposes all patients to the three active drugs. Falcone et al. conducted a phase I-II study in 42 metastatic chemotherapy-naive CRC patients to test feasibility and activity of the following regimen: CPT-11 125-175 mg/sqm day 1, LOHP 100 mg/sqm day 2, l-LV 200 mg/sqm day 1 and 5-FU 3800 mg/sqm 48h chronomodulated infusion; cycles repeated every 2 weeks (FOLFOXIRI). The combination regimen was feasible with acceptable toxicities: grade 3 diarrhea and grade 4 neutropenia in 21% and 55% of patients respectively. The regimen also revealed promising results in terms of activity (overall response rate = 71,4%) and efficacy (median progression- free survival/overall survival 10,4/26,5 months respectively) (17). Moving from these encouraging results the authors conducted a phase II study with a simplified FOLFOXIRI regimen in order to increase the tolerability and the feasibility of the regimen. Planned doses of CPT-11 and LOHP were 165 mg/sqm and 85 mg/sqm respectively while 5-FU was administered at a dose of 3200 mg/sqm 48 hours flat continuous infusion. Thirty-two patients were enrolled, all evaluable for activity and toxicity. Main grade 3-4 toxicities observed were: diarrhea (16% of patients), neutropenia grade 4 (34%), stomatitis grade 3 (6%) and peripheral neurotoxicity (37%). Response rate (RR) was 72%, median progression-free survival (PFS) 10,8 months and median OS 28,4 months. The simplified FOLFOXIRI regimen demonstrated a reduced toxicity while activity and efficacy were still very promising (18). The combined analysis of the 74 patients enrolled in the two above mentioned studies (17,18) demonstrated that a radical resection of residual metastatic disease after response to chemotherapy was performed in 19 patient (26%). The outcome of these resected patients is very promising, with a 4-years survival rate of 37%. Therefore, the Gruppo Oncologico Nord Ovest (G.O.N.O.) compared, in a phase III multicenter randomized trial (25), the simplified FOLFOXIRI regimen to the standard FOLFIRI regimen. A total of 244 patients with measurable, unresectable mCRC and previously untreated with chemotherapy for advanced disease, were randomly assigned to receive: CPT-11 180 mg/sqm d1, l-LV 100 mg/sqm d1+d2, 5FU 400 mg/sqm bolus d1+d2, 5FU 600 mg/sqm 22-h infusion on d1+d2 (FOLFIRI, arm A, n=122) or CPT-11 165 mg/sqm d1, LOHP 85 mg/sqm d1, l-LV 200 mg/sqm d1, 5FU 3200 mg/sqm 48-h infusion starting on d1 (FOLFOXIRI, arm B, n=122). Both treatments were repeated every 2 weeks and after progression to FOLFIRI, a LOHP-containing regimen was recommended. Patients

characteristics were (arm A vs arm B): median age 64 vs 62 yrs, ECOG PS 1-2 40% vs 39%, adjuvant CT 24% vs 24%, multiple sites of metastasis 45% vs 47%, liver metastases 77% vs 75%, liver involvement ≥ 25% 44% vs 40%. Main observed toxicities per patient were (arm A vs arm B): grade 3-4 diarrhea 11% vs 20% (p=NS), grade 3-4 vomiting 2% vs 7% (p=NS), grade 3-4 stomatitis 3% vs 5% (p=NS), grade 2-3 peripheral neurotoxicity 0% vs 19% (p<0.0001), grade 3-4 neutropenia 28% vs 50% (p<0.0006), febrile neutropenia 3% vs 5% (p=NS). Two patients in each arm died within 60 days, but no toxic deaths occurred. All the 244 pts enrolled were evaluated for activity and efficacy. Responses, assessed by investigators, were (arm A vs arm B): complete 6% vs 8%, partial 35% vs 58%, stable 33% vs 21% progression 24% vs 11%, not evaluable 2% vs 2%. RR (complete + partial) was significantly higher in the FOLFOXIRI arm (66% vs 41%, p=0.0002) and this difference was confirmed after a review performed by an external panel. This higher activity of FOLFOXIRI produced an increased rate of secondary R0 surgical resection of metastases compared to FOLFIRI (15% vs 6% of patients, p=0.033). At a median follow-up of 18.4 months 216 patients have progressed and median PFS and OS were significantly longer in the FOLFOXIRI arm (9.8 vs 6.9 months, p=0.0006 and 22.6 vs 16.7 months, p=0.032, respectively) with a hazard ratio of 0.60 in favor of FOLFOXIRI. Finally the rate of early progressions (within 6 months from treatment onset) was significantly lower in the FOLFOXIRI arm (18% vs 45%, p<0.0001). The authors concluded that the FOLFOXIRI regimen is feasible with manageable toxicities also in a multicenter setting; the incidence of grade 3-4 neutropenia and grade 2-3 peripheral neurotoxicity is increased with FOLFOXIRI, but febrile neutropenia, diarrhea and other toxicities are comparable to FOLFIRI; RR, prevention of early progressions, PFS and post-CT radical surgical resections are improved with FOLFOXIRI.

The results reported by the G.O.N.O. group with the FOLFOXIRI regimen are in contrast with those obtained by Souglakos et al. in a similar phase III trial comparing FOLFIRI to a combination of 5FU, CPT-11 and LOHP. The authors reported some improvements for the triplet combination in terms of RR (33.6% vs 43%), surgical R0 resections (4% vs 10%), PFS (median 6.9 vs 8.4 months) and OS (median 19.5 vs 21.5 months) but failed to demonstrate statistically significant benefits in favor of the experimental arm (26). The apparent discrepancy between the results of the aforementioned trials can be explained by two substantial differences in treatment schedules and study populations. First, the Hellenic Oncology Research Group (H.O.R.G.) maintained the 5-FU bolus, resulting in the consequent use of a dose of LOHP and CPT-11 significantly lower than in our study (65 vs 85 mg/sqm for LOHP and 150 vs 165 mg/sqm for CPT-11). Despite this, diarrhea was substantial (grade 3-4: 27.7%) and more frequent than with FOLFOXIRI regimen. Secondly, the study population in the H.O.R.G. was older (median age 66 vs 62 years, respectively) and with a poorer performance status (ECOG PS 0/1/2 36/53/11% in H.O.R.G. trial vs 61/37/2% in G.O.N.O. trial): in fact, patients older than 75 years or between 71 and 75 but with an ECOG PS >1 were not excluded, as in the G.O.N.O. trial. Indeed, Souglakos et al. reported a significantly higher incidence of toxicity in older and PS=2 patients that may have negatively influenced the

outcome results. Taken together these observations underline the importance of an optimal schedule for administering concomitantly the three cytotoxics and of rigorous patient selection criteria. Masi et al. recently published the results of a retrospective pooled analysis on 196 patients treated with first-line FOLFOXIRI in order to evaluate the long-term outcome of patients radically resected after chemotherapy (27). Thirty-seven (19%) could undergo a secondary R0 surgery on metastases (complete pathological response has been achieved in 4 patients) after a median of 5.5 months of treatment. The authors reported no intraoperative or post-operative mortality and a low rate of perioperative complications (27% of cases), all of which were transient and resolved completely without sequelae. After a median follow-up of 67 months, 5-years and 8 years survival were 42% and 33% respectively. At 5 years, 29% of the resected patients were free of progression. Such results demonstrated the feasibility and the efficacy of FOLFOXIRI as conversion therapy and were consistent with the observation of Folprecht et al. that more active first-line treatments resulted in a higher secondary resection rate of metastases (16).

CETUXIMAB IN THE TREATMENT OF COLORECTAL CANCER

Epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein with an intracellular tyrosine kinase domain (28). Binding of specific ligands, such as epidermal growth factor (EGF) or transforming growth factor alpha (TGF-α), to the receptor causes the dimerization of single-chain EGFR which subsequently activates receptor autophosphorylation through tyrosine kinase activity. These molecular events initiate a cascade of intracellular signaling pathways which ultimately regulate cancer-cell proliferation and differentiation, apoptosis and survival, invasion and metastatic potential and tumor-induced neovascularisation.

Considering that deregulation of EGFR-controlled pathways is a common phenomenon in human epithelial carcinogenesis, EGFR was the first growth factor receptor to be proposed as a target for cancer therapy. Up today, two different classes of EGFR-inhibitors have been developed and successfully tested in clinical trials for malignancies of different origin: anti-EGFR monoclonal antibodies (moAb) and small-molecule EGFR tyrosine kinase inhibitors (29).

Cetuximab belongs to the first of the above mentioned classes of EGFR-inhibitors. It is a chimeric IgG1 moAb that competitively inhibits endogenous EGF/TGF-α binding targeting the EGFR extracellular domain (30) with a consequent inhibition of cancer-cell proliferation and induction of apoptosis.

Van Cutsem et al. (31) 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 immunohistochemical EGFR-positive tumor, was 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 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 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 OS was found between the two treatment group: 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 paticular 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.

A randomized phase II study (32) evaluated the activity of cetuximab combined with FOLFOX-4 versus FOLFOX-4 alone in the first-line treatment of EGFR-expressing mCRC. Three hundred thirty-seven patients were enrolled: 169 patients received cetuximab (according to 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 the 0.6% reported in the FOLFOX-4-alone group. Infusional-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.

Preliminary safety results of the MRC COIN study (33), a phase III randomized trial comparing, in 804 mCRC patients, a first-line treatment with oxaliplatin and fluoropyrimidine with or without cetuximab, confirmed that addition of the anti-EGFR antibody to chemotherapy is feasible with manageable toxicities. As expected, patients receiving cetuximab presented a significant increase of grade 3-4 skin rash (12% and 10% for cetuximab added to 5-FU and capecitabine-based therapy respectively vs 0% and 1% for chemotherapy alone; p<0.001). The incidence of grade 3-4 diarrhea and nausea/vomiting was significantly higher in patients receiving cetuximab, in particular when it was combined with XELOX (cetuximab plus XELOX vs XELOX alone: diarrhea 25% vs 15%, p=0.005; nausea/vomiting 14% vs 7%, p=0.012). Hypomagnesemia, an effect due to the inhibition of EGFR in the kidney, was reported in 4-9% of patients treated with cetuximab versus the 2-3% observed in patients treated with chemotherapy alone (p<0.05). In the MRC COIN trial, the addition of cetuximab also increased the incidence of lethargy, that was reported in 17-21% of patients receiving chemotherapy plus cetuximab versus 7-8% of patients receiving only chemotherapy (p<0.001).

Recently, some experiences have been reported with the use of cetuximab plus a triplet chemotherapy regimen. At the 2009 ASCO Annual Meeting, Garufi et al. (34) presented definitive clinical results of the POCHER study testing the combination of cetuximab plus chrono-modulate CPT-11, LOHP and 5-FU in 43 patients with unresectable colorectal liver metastases. A partial response was achieved in 79% of patients (4 patients not evaluable because of toxicity) and

complete resection of liver metastases was obtained in 63% of patients, reporting a median PFS of 13 months. Major limiting toxicity was diarrhea (grade 3-4: 88%). At the last ASCO Gastrointestinal Cancer Symposium, Ychou et al. (35) reported the preliminary data of a phase II trial evaluating the combination of cetuximab (400 mg/sqm initial dose, then 250 mg/sqm/week) with FOLFIRINOX regimen (LOHP 85 mg/sqm d1, CPT-11 180 mg/sqm d1, LV 200 mg/sqm d1, 5-FU 400 mg/sqm bolus followed by 2400 mg/sqm infusion over 46 hours, d1,every 2 weeks) as first line treatment of mCRC patients. The most common grade 3-4 toxicities were neutropenia (28%), nausea/vomiting (10%), diarrhea (35%), anorexia (20%), asthenia (17%) and skin-toxicity (10%). Among 42 enrolled patients, 27 were evaluable for response assessment: RR was 82% with a complete RR of 15%. These results suggested that the new combination is promising and feasible.

KRAS MUTATIONS AS PREDICTORS OF RESISTANCE TO ANTI-EGFR MONOCLONAL ANTIBODIES

Many efforts have been made to identify potential predictors of benefit from anti-EGFR monoclonal antibodies. Since the evaluation of EGFR expression by immunohistochemistry was not demonstrated as an useful tool to predict the efficacy of the treatment (43), 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 (44-48), then corroborated by the results of *post-hoc* analyses of large phase III randomized studies (31,49,50), have evidenced 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 codon 12 and 13 in more than 90% of cases and abolish the intrinsic GTPase activity of Ras protein, leading to the constitutive activation of RAS/RAF/MAPKs cascade (51). Signalling events are thus independent from EGFR control.

The *post-hoc* analysis of CRYSTAL trial (Cetuximab Combined With Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer) according to *KRAS* mutational status showed that mainly 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 (31). Similarly, in the OPUS (Oxaliplatin and Cetuximab in first-line treatment of mCRC) study, a phase II randomized trial assessing the efficacy of FOLFOX plus cetuximab (vs FOLFOX) as 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) (32).

Such results are 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 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 *KRAS* wild-type group (hazard ratio [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) (50). On the basis of these results, panitumumab was initially approved by regulatory authorities for the treatment of mCRC patients with *KRAS* wild-type disease (52). Analogous results were obtained by the analysis of *KRAS* mutational status in samples from patients enrolled in CO.17 trial, that randomized

fluoropyrimidine-, irinotecan- and oxaliplatin-refractory mCRC patients to cetuximab vs BSC. The anti-EGFR antibody significantly improved PFS (3.7 months vs 1.9 months, HR=0.40, p<0.001) and OS (9.5 months vs 4.8 months, HR=0.55, p<0.001) only among patients with *KRAS* wild-type tumors (49).

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 (53).

Unfortunately, although the specificity of *KRAS* mutations as predictors of resistance to anti-EGFR monoclonal antibodies is quite high, the sensitivity of *KRAS* testing is less satisfactory (54), so that, while patients bearing such alterations do not respond to the treatment, also a percentage of patients with *KRAS* wild-type status does not achieve benefit from anti-EGFR antibodies.

KRAS activating mutations, occurring in codons other than 12 and 13, have been described in mCRC. Codon 61 and 146 mutations (55), that have been detected with frequencies ranging from 1 to 4%, determine the constitutive activation of RAS protein, by reducing its intrinsic GTPase activity or increasing its affinity for GTP (56). It has been recently reported that, among 87 patients with *KRAS* codon 12 and 13 wild-type disease, none of 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) (57).

BEVACIZUMAB IN THE TREATMENT OF COLORECTAL CANCER

Vascular endothelial growth factor (VEGF) plays a crucial role in the development of new blood vessels in both healthy tissues and tumors. It exerts its influence through binding principally to the VEGF receptor-2, found predominantly on the surfaces of vascular endothelial cells. Induction of the intracellular tyrosine kinase activity of receptor by VEGF binding, triggers the phosphorylation of a multitude of proteins with a subsequent cascade of intracellular signaling pathways. In the pathogenesis of cancer, VEGF has a number of key roles. It stimulates excessive angiogenesis, allowing the tumour to embark upon its exponential growth phase. This vascularisation also provides an exit route for haematogenous metastases and allows them to establish themselves at their final destination (36,37).

Bevacizumab is a recombinant humanized monoclonal IgG1 antibody that binds to and inhibits the biologic activity of human VEGF in *in vitro* and *in vivo* assay systems. Bevacizumab contains human framework regions and the complementarity-determining regions of a murine antibody that binds to VEGF (38).

Hurwitz et al. (39) have conducted a randomized phase III trial evaluating the addition of bevacizumab to first-line irinotecan-based treatment of mCRC patients. A total of 813 patients was randomly assigned to receive bolus-IFL (CPT-11 125 mg/sqm d1, 5-FU 500 mg/sqm d1 and LV 20 mg/sqm d1 given once weekly for 4 weeks every 6 weeks) plus placebo (Arm 1), bolus-IFL plus bevacizumab (5 mg/kg every 2 weeks) (Arm 2), or 5-FU/LV plus bevacizumab (5-FU 500 mg/sqm d1 and LV 500 mg/sqm d1 given once weekly for 6 weeks every 8 weeks; BV 5 mg/kg every 2 weeks) (Arm 3). Enrollment in Arm 3 was discontinued, as pre-specified, when the toxicity of bevacizumab in combination with the bolus-IFL regimen was deemed acceptable. The primary end point of this trial was overall survival. The addition of bevacizumab to IFL chemotherapy significantly improved RR (44.8% vs 34.8%; p = 0.004), PFS (10.6 months vs 6.2 months; HR=0.54; p <0.001) and OS (20.3 months vs 15.6 months; HR=0.66; p <0.001). In terms of toxicity profile, adding bevacizumab significantly increased the incidence of grade 3 hypertension (11% vs 2.3%, p <0.01), but interestingly it did not impact significantly upon the rates of proteinuria, thrombosis and bleeding. Six gastrointestinal perforations occurred in patients receiving IFL/bevacizumab. The results of this study demonstrated a significant improved activity and efficacy of the combination of bevacizumab with IFL in comparison to chemotherapy alone with manageable toxicities.

In the NO16966 study (40) about 1400 mCRC patients were randomized to receive either FOLFOX or XELOX plus bevacizumab or placebo as first-line treatment. The primary end point of this phase III study was PFS. The addition of bevacizumab to oxaliplatin-based regimens significantly increased PFS in comparison to chemotherapy alone (9.4 months vs 8.0 months; HR=0.83, 97.5% CI 0.72-0.95; p=0.0023). Median OS was 21.3 months in the bevacizumab group and 19.9 months in the placebo group: this difference didn't reach statistical significance (HR=0.89, 97.5% CI 0.76-

1.03; p=0.077). RR was similar in the two groups of treatment. The magnitude of the effect of bevacizumab seemed relatively less impressive if compared with that reported by Hurwitz. The main explanation, as specified by the authors (40) and suggested by Giantonio at the 2007 ASCO Annual Meeting (41), could be the frequent discontinuation (71% of patients) of bevacizumab plus chemotherapy before disease progression. The toxicity profile of bevacizumab was in line with data obtained in previous trials: the incidence of grade 3-4 thromboembolic events, hypertension and bleeding was 10%, 4% and 2% respectively, while grade 3-4 gastrointestinal perforations, proteinuria and wound healing complications were rare (<1%). Treatment discontinuation because of adverse events was reported in 30% of patients receiving bevacizumab.

The G.O.N.O. group conducted a phase II trial (42) to evaluate the combination of bevacizumab (5 mg/Kg d1) with FOLFOXIRI regimen (CPT-11 165 mg/sqm d1, LOHP 85 mg/sqm d1, LV 200 mg/sqm d1 and 5-FU 3200 mg/sqm infusion over 48h) repeated every 2 weeks, for a total of 12 cycles, followed by a maintenance treatment with bevacizumab +/- 5-FU/LV. A total of 57 unresectable mCRC patients was enrolled. Response was obtained in 77% of patients and radical surgery of metastases was performed in 26% of patients (43% in patients with liver-only metastases). After a median follow-up of 18.4 months, median PFS was 13.4 months. The most common grade 3-4 bevacizumab-related toxicities were deep venous thrombosis (5%) and hypertension (11%). Results of this study are very promising and suggest that bevacizumab can be safely combined with the G.O.N.O.-FOLFOXIRI regimen with manageable toxicities.

BIOLOGICAL RATIONALE FOR THE SEQUENTIAL INHIBITION OF EGFR AND VEGF

Different mechanisms have been hypothesized to be responsible of the phenomenon of acquired resistance, for which initially responsive patients become rapidly resistant to anti-EGFR antibodies. Great attention has been focused on alternative pathways, able to support cell growth and proliferation when EGFR signalling is blocked and, among these, on VEGF pathway. A significant increase in VEGF protein and specific mRNA was observed in human head and neck (A431) (59) and colorectal (GEO) (60) cancer cell lines, that become resistant to cetuximab. Interestingly, the treatment of resistant cells and xenografts with the multitargeted tyrosine kinase inhibitor ZD6474 (vandetanib), that inhibits VEGF pathway by targeting VEGFR1-3, was able to control cellular growth. A noticeable increase in VEGFR-1 and PlGF expression was also observed in resistant GEO cells, compared to parental lines (61). The silencing of VEGFR-1 through a specific siRNA partially restored sensitivity to anti-EGFR drugs by reducing the activation of VEGF pathway. This lead to reduced migration efficiency and thus impaired metastatic potential, sustained by VEGFR-1 hyperactivity. Benavente et al. have recently reported that the angiogenic potential of tumoral cells (SCC-1), that become resistant to anti-EGFRs, is much more relevant in comparison to that of sensitive cells, as demonstrated by the extensive vascularization and the aberrant growth of blood vessels observed in Matrigel plugs containing resistant cells, when subcutaneously implanted into nude mice (62).

Although only preclinical evidence is currently available, these data represent an interesting biologic rationale to further investigate the potential efficacy of the sequential administration of an anti-EGFR and an anti-VEGF targeted agent in clinical trials.

Taken together all the above reported evidence suggests that an innovative and promising strategy in the treatment of molecularly selected mCRC patients could be the following: to expose them promptly to the most active agents (i.e. the triplet combination of FOLFOXIRI plus cetuximab) with the aim to obtain a rapid disease control and the maximum tumoral shrinkage and then to treat patients with a less aggressive maintenance to inhibit tumoral regrowth.

2. STUDY RATIONALE

On the basis of all the above reported evidence and the following considerations we have designed a pilot study of 4-months induction first-line chemotherapy with FOLFOXIRI + cetuximab followed by maintenance with cetuximab or bevacizumab in patients affected by *RAS* and *BRAF*-wild-type (wt) mCRC:

- Results from a phase III multicenter study conducted by the G.O.N.O. group comparing FOLFOXIRI to FOLFIRI, demonstrated that FOLFOXIRI improves response-rate, progression-free survival, overall survival and post-chemotherapy radical surgical resections of metastases with mild and manageable toxicities.
- First-line FOLFIRI plus cetuximab, an anti-EGFR monoclonal antibody (MoAb), significantly improves response rate, progression-free survival (PFS) and overall survival (OS) in patients with *KRAS*-wt mCRC compared to FOLFIRI alone.
- The combination of cetuximab with FOLFIRI or FOLFOX in previously untreated mCRC is feasibile without a relevant increase in chemo-related side-effects and with a typical skin toxicity as most common adverse event.
- Several retrospective series and post-hoc analyses from randomized trials demonstrated that CRC bearing *KRAS* mutations are resistant to cetuximab. For this reason EMEA restricted its use to *KRAS*-wt patients.
- Bevacizumab, an anti-VEGF MoAb, added to conventional chemotherapy improves PFS of first-line mCRC patients irrespectively of the combination regimen adopted.
- A retrospective analysis of a first-line phase III trial of oxa-based chemotherapy +/- bevacizumab suggests that maintenance chemotherapy plus bevacizumab may be important in order to maximize the effect of delaying tumoral progression.
- Recently, an updated *post-hoc* analysis of phase III PRIME trial of first-line FOLFOX +/- panitumumab revealed a maximized OS benefit in the subgroup of patients not bearing *KRAS* or *NRAS* mutations at codons 12, 13, 59, 61, 117 and 146 (defined as *RAS* wild-type patients) or *BRAF* mutation at codon 600. Conversely, *RAS* mutant patients may derive a detrimental effect from the addition of panitumumab to first-line chemotherapy.

3. STUDY OBJECTIVES

PRIMARY OBJECTIVE

The main objective of this study is to explore the efficacy of induction FOLFOXIRI + cetuximab, followed by maintenance with cetuximab or bevacizumab as first-line treatment for *RAS* and *BRAF* wild-type mCRC patients, as proportion of patients free from disease progression after 10 months from the start of treatment.

SECONDARY OBJECTIVES

Secondary objectives of this study are to evaluate in each study arm:

- the distribution of best overall responses observed during the induction and the maintenance phases of treatment;
- the proportion of patients undergoing secondary R0 resection of metastases within 10 months from randomization;
- the distribution of time to strategy failure;
- the distribution of time to 2nd progressive disease;
- the distribution of time to progression-free survival (PFS);
- the duration of overall survival (OS);
- the safety profile.

PRIMARY Endpoint

Primary endpoint is described in Statistical Methods, section 10, page 38

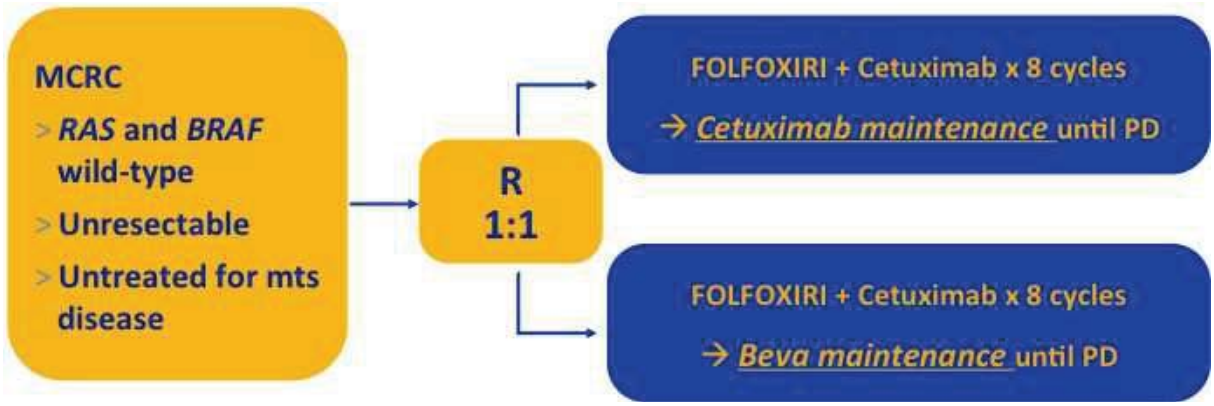
SECONDARY Endpoints

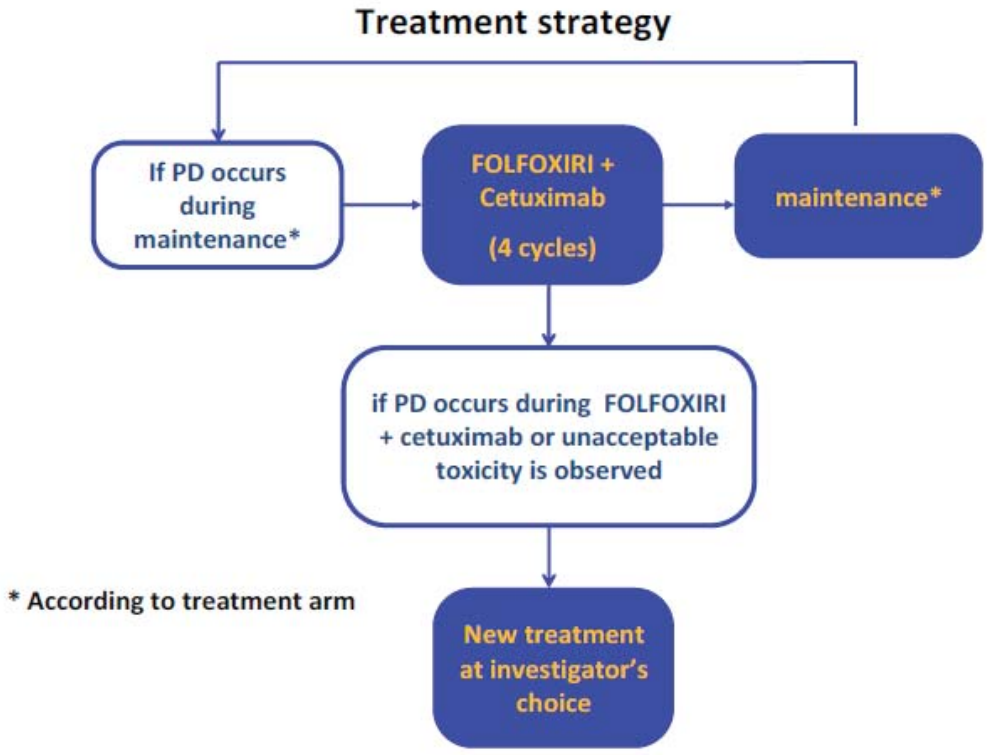
Secondary endpoints are described in Statistical Methods, section 10, page 38

4. STUDY DESIGN

This is a prospective, open-label, multicentric, phase II study in which patients will be randomized to receive induction treatment with FOLFOXIRI + cetuximab, followed by maintenance with cetuximab or bevacizumab, as first-line therapy for *RAS* and *BRAF* wild-type mCRC.

The study design and strategy are below described as flowcharts:





5. PATIENTS' SELECTION

INCLUSION CRITERIA

- Histologically confirmed colorectal adenocarcinoma;
- Availability of formalin-fixed paraffin embedded tumor block from primary and/or metastasis;
- *RAS* and *BRAF* wild-type status of primary colorectal cancer or related metastasis;
- Unresectable and measurable metastatic disease according to RECIST criteria;
- Male or female, aged > 18 years and < 75 years;
- ECOG PS $\leq$ 2 if aged < 71 years, ECOG PS = 0 if aged 71-75 years;
- Life expectancy of more than 3 months;
- Adequate haematological function: ANC ≥ 1.5 x 109/L; platelets ≥ 100 x 109/L, Hb ≥ 9 g/dL;
- Adequate liver and renal function: serum bilirubin ≤ 1.5 x ULN; alkaline phosphatase and transaminases ≤ 2.5 x ULN (in case of liver metastases < 5 x ULN); serum creatinine ≤ 1.5 x ULN;
- Previous adjuvant chemotherapy containing oxaliplatin is allowed if more than 12 months have elapsed between the end of adjuvant therapy and first relapse;
- Previous adjuvant chemotherapy with fluoropyrimidine monotherapy is allowed if more than 6 months have elapsed between the end of adjuvant and first relapse;
- At least 6 weeks from prior extended radiotherapy and 4 weeks from surgery;
- Written informed consent to experimental treatment and *RAS* and *BRAF* analysis.

EXCLUSION CRITERIA

- Prior palliative chemotherapy;
- Prior treatment with EGFR or VEGF inhibitors;
- Symptomatic peripheral neuropathy $\geq$ 2 grade NCIC-CTG criteria;
- Presence or history of CNS metastasis;
- Active uncontrolled infections; 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;
- Clinically significant cardiovascular disease: cerebrovascular accidents or myocardial infarction ≤ 12 months before treatment start, unstable angina, NYHA ≥ grade 2 chronic heart failure, uncontrolled arrhythmia, uncontrolled hypertension;

- Serious, non-healing wound, ulcer, or bone fracture;
- Evidence of bleeding diathesis or coagulopathy;
- Major surgical procedure or significant traumatic injury within 28 days prior to study treatment start;
- Current or recent (within 10 days prior to study treatment start) ongoing treatment with anticoagulants for therapeutic purposes or chronic, daily treatment with high-dose aspirin (>325 mg/day);
- Subtotal colectomy, malabsorption syndrome and chronic inflammatory bowel disease (i.e. ulcerative colitis, Chron syndrome);
- Fertile women (<2 years after last menstruation) and men of childbearing potential not willing to use effective means of contraception.
- Psychiatric disorder precluding understanding of information on trial related topics,
- Serious underlying medical condition (judged by the investigator) which could impair the ability of the patient to participate in the trial (e.g. uncontrolled diabetes mellitus, active autoimmune disease)
- Concurrent treatment with other experimental drugs or other anti-cancer therapy; treatment in a clinical trial within 30 days prior to trial entry
- Definite contraindications for the use of corticosteroids and antihistamines as premedication
- Known hypersensitivity to trial drugs or hypersensitivity to any other component of the trial drugs
- Any concomitant drugs contraindicated for use with the trial drugs according to the product information of the pharmaceutical companies
- Pregnancy
- Any psychological, familial, sociological or geographical condition potentially hampering compliance with the study protocol and follow-up schedule; those conditions should be discussed with the patient before registration in the trial
- Medical or psychological condition which, in the opinion of the investigator, would not permit the patient to complete the study or sign meaningful informed consent

6. ASSESSMENT OF *RAS* and *BRAF* MUTATIONAL STATUS

RAS mutational analysis will be centralized at the Unit of Pathology, Department of Surgery, University of Pisa, Prof. G. Fontanini. Formalin-fixed paraffin-embedded tumor blocks from primary tumor and/or metastasis should be sent to the Coordinating Center at screening for patients deemed eligible according to above reported selection criteria. A certification signed by the pathologist of *RAS* mutational status will be provided to each investigator within 15 days after the reception of the specimens. Dr. Loupakis will be responsible for their conservation and delivery to the laboratories as well as for their prompt return to each participating center. Adequate material will be stored for immunohistochemical analyses (up to 10 slides of 5-10 micron thickness).

KRAS mutational analysis

KRAS codon 12, 13, 59, 61, 117 and 146 mutations, *BRAF* V600E mutation and *NRAS* codon 12, 13 and 61 mutations, will be assessed by means of PCR and pyrosequencing.

7. PARTICIPATING CENTERS, ENROLLMENT AND STUDY DURATION

PARTICIPATING CENTERS

About 30 Italian Oncology Units.

ENROLLMENT

The registration and randomization procedures will be centralized at Centro Coordinamento Sperimentazioni Cliniche of Istituto Toscano Tumori – Azienda Ospedaliero-Universitaria Careggi.

Patients considered eligible and who have provided a written informed consent will be randomly assigned to one of the two treatment arms in a 1:1 ratio. Eligible patients will be stratified according to center, with a minimization algorithm.

The randomization will be performed by using an electronic WEB-based system.

The randomization code will consist of a unique identification code. This code must be used on all further documentation and correspondence, including E-CRFs.

It is responsibility of the principal investigator to ensure that each patient is eligible for the study before requesting randomization.

STUDY DURATION

Study length is planned to be 42 months since the enrollment is expected to be 30 months and the study closure will be approximately 12 months after the last patient was enrolled. Median follow-up will last approximately 18 months.

8. STUDY TREATMENT

Induction FOLFOXIRI plus cetuximab will consist of:

- **CETUXIMAB** 500 mg/sqm IV over 1-h* , day 1 followed by
- **IRINOTECAN** 130 mg/sqm IV over 1-h, day 1 followed by
- **OXALIPLATIN** 85 mg/sqm IV over 2-h, day 1 concomitantly with
- **l-LV** 200 mg/sqm IV over 2-h, day 1 followed by
- **5-FLUOROURACIL** 2400 mg/sqm IV 48-h continuous infusion, starting on day 1

repeated every 2 weeks for 8 cycles.

*Cetuximab will be administered over 2-h at cycle 1. If well tolerated, it will be administered over 90 minutes at cycle 2 and over 1-h by cycle 3.

Surgical revaluation will be performed after the induction phase (8 cycles).

Patients deemed unsuitable for surgery will received maintenance treatment as follows:

- **CETUXIMAB** 500 mg/sqm IV over 60-min, day 1

OR

- **BEVACIZUMAB** 5 mg/kg IV over 30-min, day 1

repeated every 2 weeks until PD, patient's refusal, unacceptable toxicity or consent withdrawal.

Doses of 5-Fluorouracil and irinotecan have been slightly reduced by comparison with standard GONO-FOLFOXIRI regimen. Such modification has been adopted given previous reports of a mild increase in grade 3-4 diarrhea when anti-EGFR antibodies are combined with chemotherapy, especially for irinotecan-based doublets (31, 63) or triplets (34, 35).

Patients undergoing secondary resection after “n” cycles of induction FOLFOXIRI plus cetuximab, will receive “12-n” cycles of FOLFOXIRI plus cetuximab as post-operative treatment, followed by maintenance with cetuximab or bevacizumab for 6 months according to their randomization arm.
Patients that progress during maintenance therapy (that is if PD does not occur during the induction phase and unacceptable toxicities are not observed), will be re-treated with FOLFOXIRI plus cetuximab or with a modified FOLFOXIRI plus cetuximab regimen (i.e. whatever modification to the regimen, such as dose reductions or every single drug interruptions provided that no new agents are adopted, excepted for capecitabine as substitute of 5-FU) for 4 cycles as a re-

challenge, followed by maintenance with a biologic (according to randomization arm). This therapy plan will be applied until PD occurs during FOLFOXIRI plus cetuximab or during a modified FOLFOXIRI plus cetuximab regimen or if the patient experiences unacceptable toxicity.

RESECTION OF METASTASES

The initiation of any non-protocol specific anti-tumour therapy or surgery is at the discretion of the investigator and in the best interest of the patient. In particular it is strongly recommended to have a multidisciplinary group with experience in the management of mCRC defining unresectability for each patient. Such team should include a medical oncologist, a general surgeon with experience in liver resections, a thoracic surgeon and an interventional radiologist. It is recommended to adopt standard predefined guidelines for resectability, such as Oncosurge criteria (64). Surgical radical resection of residual metastases in responsive patients is highly recommended and its feasibility should be evaluated every 2 months.

In case of a patient's eligibility for curative resection of metastatic disease during the induction treatment with FOLFOXIRI + cetuximab, at least 3 weeks should pass between the last administration of chemotherapy and the day of surgery. After resection, patients will restart the experimental treatment not earlier than 4 weeks after surgery, to receive a total of 12 cycles of FOLFOXIRI + cetuximab including those administered before surgery. Maintenance with cetuximab or bevacizumab will be continued until tumor progression, unacceptable toxicity or patient refusal, or for a maximum of 6 months.

DURATION OF TREATMENT

Maintenance treatment with cetuximab or bevacizumab should be continued until:

- Progressive disease;
- Unacceptable toxicities;
- Patients' refusal.

Baseline AND on-TREATMENT EVALUATIONS

Baseline evaluation

Within 1 month- prior to first infusion:

- Medical History;
- Cancer and treatment history;

- Vital signs (including blood pressure, pulse, body temperature, height and body weight), ECOG performance status, general physical examination (including cardiovascular function) and concomitant medications;
- ECG: a standard electrocardiogram will be performed at screening and as clinically indicated;
- Haematology: Haemoglobin, Platelet count, RBC, WBC including differential (Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils) INR and aPTT;
- Serum Chemistry: Na+, K+, Ca++, Cl–, Mg++, Urea (BUN), Uric Acid, total Protein, Albumin, Alkaline Phosphatase, ALT, AST, GGT, LDH, direct and total Bilirubin, Serum Creatinine, Glucose, CEA, Ca19.9;
- A serum pregnancy test will be performed within 7 days prior to treatment exposure if childbearing potential cannot be ruled out. In case the sampling date for pregnancy testing exceeds the 7 days time interval before treatment start, a urine test is acceptable for confirmation of the absence of pregnancy;
- Evaluation of disease (CT scan etc.);
- *RAS* and *BRAF* mutation testing.
- Baseline Adverse Events and/or symptomatology (grading by the common terminology criteria for adverse events (CTCAE version 4.0))
- Concomitant medications

During treatment evaluation

At day 1 of every planned cycle:

- Patients will be assessed for treatment toxicity (grading by the common terminology criteria for adverse events (CTCAE version 4.0));
- Haematology: Haemoglobin, Platelet count, RBC, WBC including differential (Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils);
- Serum Chemistry: Mg++, K^+, direct and total Bilirubin, Serum Creatinine.
- Concomitant medications; Body weight

Response evaluation

Patients should be evaluated for response every 4 cycles following the RECIST V1.1 criteria.

Evaluation after the end of chemotherapy

At 30 days (±2days) after last dose and every 2 months (±1 week)

- Medical History;

- Vital signs (including blood pressure, pulse, body temperature and body weight), ECOG performance status, general physical examination (including cardiovascular function) and concomitant medications;
- Patients will be assessed for treatment toxicity (grading by the common terminology criteria for adverse events (CTCAE version 4.0)).
- Haematology: Haemoglobin, Platelet count, RBC, WBC including differential (Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils) INR and aPTT;
- Serum Chemistry: Na+, K+, Ca++, Cl–, Mg++, Urea (BUN), Uric Acid, total Protein, Albumin, Alkaline Phosphatase, ALT, AST, GGT, LDH, direct and total Bilirubin, Serum Creatinine, Glucose, CEA, Ca19.9;
- Evaluation of disease (CT scan etc.).

CT SCAN IMAGING

Assessment of metastases and tumor progression will be based on contrast-enhanced spiral CT of the chest and abdomen (venous phase) with a contiguous slice thickness of ≤ 7mm performed in the radiology department of the study site. For quality reasons the scans will be stored digitally on CD-ROM.

For all patients a blinded copy of all CT scans will be forwarded to the coordinating center for central review. A central review of CT images will be performed by a Independent Radiologic Reviewer (IRR) who is not involved in the conduct of the study.

When there is suspicion of disease progression or evidence of clinical progression, radiographic evaluations should be performed immediately, that is within a maximum of 7 days, to confirm objective disease progression.

9. SAFETY ISSUES

DOSE REDUCTIONS AND DELAYS

Toxicities should be evaluated according to CTCAEv4.0.

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

Dose modifications for toxicities attributable to chemotherapy

TOXICITY AT THE START OF SUBSEQUENT CYCLES OF THERAPY	GRADE	CPT-11	OXALI	5FU
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			
Hand/foot syndrome	3 - 4	100%	100%	**STOP**
Neurotoxicity	≥ 3	100%	**STOP**	100%

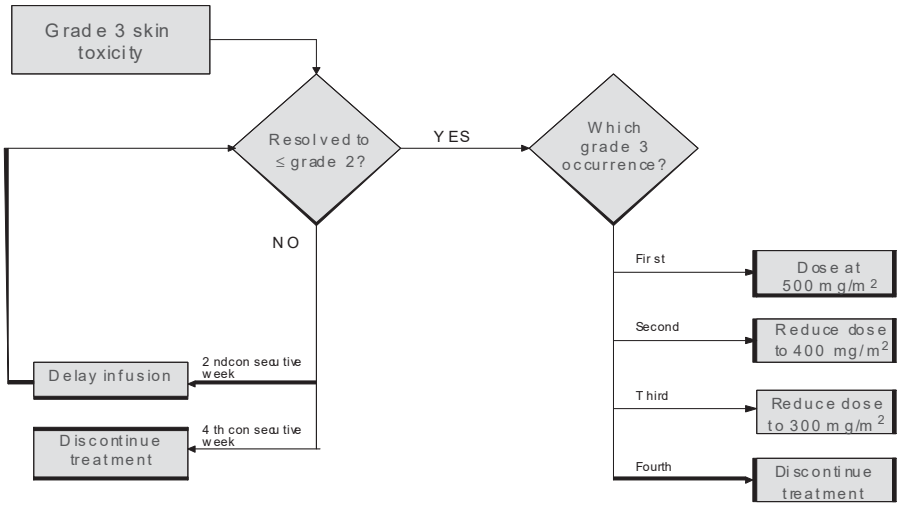
PREVIOUS TOXICITY	GRADE	CPT-11	OXALI	5FU
Neutropenia >5 days	4	**75%**	**75%**	100%
Febrile Neutropenia	4			
Thrombocytopenia	3-4			
Diarrhea	3	**75%**	100%	**75%**
Diarrhea	4	**50%**	100%	**50%**
Stomatitis	3	100%	100%	**75%**
Stomatitis	4	100%	100%	**50%**
Myocardial Ischemia	---	100%	100%	**STOP**

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). On resolution of toxicity, cetuximab doses may be re-escalated (see below). 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
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 hematologic or 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 scheme shown above. Even if the toxicity has resolved by the intervening week before the subsequent 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 re challenge with 100% dose).
- If toxicity recurs, subjects treated at 100% dose or 80% (400 mg/m2) dose should be restarted at 80% dose or 60% (300 mg/m2) dose, respectively, when the toxicity resolved after withholding 1 dose of cetuximab.
- Subjects who experience toxicity at the 60% dose level (300 mg/m2) 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 censored 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 has not disease progression, cetuximab monotherapy should be administered as soon as possible.

Delay of cetuximab administration greater 6 weeks from the previous dose of cetuximab are not allowed.

Dose modifications for toxicities attributable to bevacizumab

Event	Grade	Adjustment
Hypertension	3	If not controlled with triple-drug medication, discontinue bevacizumab
	4	Discontinue bevacizumab
Hemorrhage	≥2 (pulmonary or CNS)	Discontinue bevacizumab
	3 (non-pulmonary and non-CNS)	Hold bevacizumab until all of the following criteria are met: 1. The bleeding has resolved and haemoglobin is stable; 2. There is no bleeding diathesis that would increase the risk of therapy; 3. There is no anatomic or pathologic condition that significantly increases the risk of hemorrhage recurrence.
	4	Discontinue bevacizumab
Venous thrombosis	3 or 4	Discontinue bevacizumab
Arterial thromboembolic event	Any Grade	Discontinue bevacizumab
Congestive heart failure	3	Hold bevacizumab until ≤ Grade 2
	4	Discontinue bevacizumab
Proteinuria	3	Hold bevacizumab until ≤ Grade 2
	4	Discontinue bevacizumab
GI perforation	-	Discontinue bevacizumab
Bowel obstruction	1	Patients who experience partial obstruction not requiring medical intervention may continue on bevacizumab
	2	Hold bevacizumab in patients who experience partial obstruction requiring medical intervention. Resume upon complete resolution
	3 or 4	Discontinue bevacizumab
Wound dehiscence	-	Discontinue bevacizumab
Other unspecified bevacizumab-related adverse events	3	Hold bevacizumab until recovery to ≤ Grade 1
	4	Discontinue bevacizumab

Gastrointestinal Perforation

Bevacizumab should be permanently discontinued in patients who develop gastrointestinal perforation.

Surgical Procedures/Wound Healing Complications

Bevacizumab therapy should not be initiated for at least 28 days following major surgery or until the surgical wound is fully healed. In patients who experience wound healing complications during bevacizumab treatment, bevacizumab should be withheld until the wound is fully healed.

Bevacizumab therapy should be withheld 5 weeks before elective surgery. CVAD placement and complications will be monitored as an assessment of treatment-related complications. Date of placement of CVAD will be noted in the medical record and recorded in the eCRF. Episodes of CVAD removal or replacement will be recorded. Episodes of CVAD-related thrombosis, infection, or dysfunction will be recorded.

Hypertension

Patients should be monitored for the development or worsening of hypertension via frequent blood pressure measurement. Blood pressure measurements should be taken after the patient has been in a resting position for ≥ 5 minutes. Repeat measurements of blood pressure for verification should be undertaken if the initial reading is ≥ 140 mmHg systolic and/or ≥ 90 mmHg diastolic blood pressure.

• Grade 1 hypertension: Asymptomatic, transient (< 24 hrs) increase by > 20 mmHg (diastolic) or to > 150/100 mmHg if previously within normal limits. Intervention not indicated.

• Grade 2 hypertension: Recurrent or persistent (> 24 hr) or symptomatic increase by > 20 mmHg (diastolic) or to > 150/100 mmHg if previously within normal limits. Monotherapy with ACE-inhibitor may be indicated. Once controlled to < 150/100 mmHg, patients may continue bevacizumab therapy.

• Grade 3 hypertension: Requiring more than one anti-hypertensive or more intensive therapy than previously. Addiction of diuretic to ACE-inhibitor may be indicated; if hypertension is not controlled a third anti-hypertensive drug (calcium channel blocker) should be added.

Bevacizumab should be withheld for persistent or symptomatic hypertension and should be permanently discontinued if hypertension is not controlled with triple-drug medication.

Proteinuria

All patients will have a dipstick urinalysis or 24 hour protein determination performed within 48 hours prior to the first bevacizumab dose and thereafter every 8 weeks. Adjustment of bevacizumab administration for proteinuria of ≥ 2 g/24h will occur according to the following guidelines, listed below:

• < 2+ (dipstick): no additional evaluation is required.

• ≥ 2+ (dipstick): Collect 24-hour urine to determine the total protein within 3 days prior to the next scheduled dose:

- 24-hour proteinuria ≤ 2 g: Administer bevacizumab as scheduled.

- 24-hour proteinuria > 2 g: Bevacizumab treatment should be withheld pending next 24 hour total protein.

Repeat 24-hour urine protein ≤ 2 g: Administer bevacizumab as schedule. 24-hour protein should be further monitored prior to each administration of bevacizumab until it has decreased to ≤ 1 g/24h.

Repeat 24-hour urine protein > 2 g: Bevacizumab dose should be withheld until 24-hour protein has decreased to ≤ 2 g. 24-hour protein should be further monitored prior to each administration of bevacizumab until it has decreased to ≤ 1 g/24 h.

Nephrotic syndrome (Grade 4, CTCAEv4.0): Discontinue bevacizumab treatment.

Thrombosis/Embolism

All toxicity will be graded according to CTCAEv4.0 guidelines. For patients who develop thrombosis/embolism the following action is recommended:

Bevacizumab should be permanently discontinued in patients who develop arterial thromboembolic events of any grade and in patients to develop grade 3 or 4 venous thrombosis

Haemorrhage

All toxicity will be graded according to CTCAEv4.0 guidelines.

Patients who develop grade 3 pulmonary or CNS or grade 4 hemorrhage should discontinue bevacizumab treatment.

Patients who develop grade 3 non-pulmonary and non – CNS hemorrhage should hold bevacizumab until all of the following criteria are met:

The bleeding has resolved and haemoglobin is stable.

There is no bleeding diathesis that would increase the risk of therapy.

There is no anatomic or pathologic condition that significantly increases the risk of hemorrhage recurrence.

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 20 mg i.v.

– Day 2 in the morning: oral/i.m. 5HT antagonist + dexamethasone 8 mg i.m.

– Day 3 in the morning: oral/i.m. 5HT antagonist + dexamethasone 8 mg i.m.

– Days 1-14: metoclopramide orally o i.m. if necessary

- ***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 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.**
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 must 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, 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 (65).

Vitamin K1:

Much evidence has been presented on the beneficial effect of vitamin K1 cream on patients experiencing severe acne-like rash, anti-EGFr induced (66-70).

This evidence demonstrates that the twice a day use of Vitamin K1 cream in prophylactic (65-66, 68-70) 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 for 1 grade without reducing the cetuximab dose. No local or systemic toxicity of topical use of Vitamin K1 cream was observed.

The prophylactic approach showed (65) 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 through all 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/die 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/die, doxycycline 100 mg per os once/die 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 250 mg/m2. If there is no improvement by 14 days (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/die 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/die, doxycycline 100 mg per os once/die for ≥ 4 weeks and until the rash is symptomatic. Corticosteroids: according to investigator judjement, methylprednisolone 8 mg per os once or twice/die or prednisone 25 mg per os once/die , 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 immediately and definitively therapy
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 CPT-11 and oxaliplatin. 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:

- Precedent febrile neutropenia;
- Precedent 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. Subject's serum potassium and calcium parameters are recommended to be maintained, as per local medical practice, within the normal ranges during study treatment.

10. STATISTICAL METHODS

This is a prospective, open-label, multicentric, phase II study in which patients will be randomized to receive induction treatment with FOLFOXIRI + cetuximab, followed by maintenance with cetuximab or bevacizumab, as first-line therapy for *RAS* and *BRAF* wild-type mCRC.

No formal statistical comparisons between the results obtained in the two study arms are planned. All described statistical analyses will be performed independently for the two study arms.

PRIMARY ENDPOINT

The primary endpoint of this study is 10-month progression-free rate (10m-PFR).

10m-PFR is defined as the proportion of patients free from disease progression 10 months after randomization, relative to the total of enrolled patients. Patients whose disease status cannot be evaluated within 11 months after randomization and patients lost-to-follow-up or dead within 10 months after randomization will be considered as progressed for the purpose of the primary endpoint analyses.

The determination of disease progression will be based on investigator-reported measurements that will be subsequently confirmed by a central review. Disease status will be evaluated according to RECIST 1.1 criteria.

SECONDARY ENDPOINTS

Secondary endpoints of this study are the following:

Best overall response rate 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, during the induction and the maintenance phases of treatment. The determination of clinical response will be based on investigator-reported measurements that will be subsequently confirmed by a central review. Responses will be evaluated every 8 weeks. Patients who do not have an on-study assessment will be included in the analysis as non-responders.

10-month resection rate is defined as the percentage of patients, relative to the total of enrolled subjects, undergoing secondary R0 resection of metastases within 10 months after randomization.

Secondary R0 surgery is defined as microscopically margin-free complete surgical removal of all residual disease, performed during treatment or after its completion, allowed by tumoral shrinkage and/or disappearance of one or more lesions. Patients lost-to-follow-up, with disease progression or dead, within 10 months after randomization, will be considered as failures for the purpose of this secondary endpoint analysis.

Time to strategy failure is defined as the time from randomization to one of the followings:

1) progression during FOLFOXIRI + cetuximab or during a modified FOLFOXIRI + cetuximab regimen (i.e. whatever modification to the regimen, such as dose reductions or every single drug interruptions provided that no new agents are adopted, excepted for capecitabine as substitute of 5-FU); OR
2) progression and decision to not administer FOLFOXIRI + cetuximab or a modified FOLFOXIRI + cetuximab regimen (because of patient's refusal or according to medical decision made in the best patient's interest)
3) introduction of a new agent not included in the study treatment according to randomization arm; OR
4) death;

whichever occurs first.

For patients still on-treatment at the time of analysis, the time to strategy failure will be censored on the last date the patients were known to be alive.

Time to 2nd progressive disease is defined as the time from randomization to second documentation of objective disease progression or death due to any cause, whichever occurs first. Time to 2nd progressive disease 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 second progression-free at the time of the analysis. Alive patients having no tumor assessments after baseline will have time to event endpoint censored on the date of randomization.

Progression-free survival (PFS) is defined as the time from randomization to 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. Alive patients having no tumor assessments after baseline will have time to event endpoint censored on the date of randomization.

Overall survival (OS) is defined as the time from randomization to the date of death due to any cause. For patients still alive at the time of analysis, 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 of grade 3/4, according to National Cancer Institute Common Toxicity Criteria (version 4.0), during the induction and the maintenance phases of treatment.

Overall toxicity rate is defined as the percentage of patients, relative to the total of enrolled subjects, experiencing any adverse event of grade 3/4, according to National Cancer Institute Common Toxicity Criteria (version 4.0), during the induction and the maintenance phases of treatment.

ANALYSIS POPULATIONS

Modified Intention-to-treat population (mITT)

The mITT population will include all randomized patients who receive at least one dose of study medication, with study drug assignment designated according to initial randomization. The mITT population will be the population for evaluating all primary and secondary endpoints, with the exception of toxicity rate and overall toxicity rate.

Safety population (SP)

The SP population will include all patients who receive at least one dose of study medication, with treatment assignments designated according to actual study treatment. The SP will be the population for evaluating treatment administration/compliance and safety.

RAS and BRAF wild-type population

Exploratory analyses for all clinical outcome parameters will be conducted post-hoc to verify the efficacy of each treatment according to *RAS* and *BRAF* mutational status.

ANALYSES of ENDPOINTS

Analysis of primary endpoint

10m-PFR will be calculated as the number of patients free from disease progression 10 months after randomization divided by the total number of enrolled patients. The corresponding exact 2-sided 95% confidence interval will be calculated using a method based on the binomial distribution.

Analysis of secondary endpoints

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 exact 2-sided 95% confidence interval will be calculated using a method based on the binomial distribution.

10-month resection rate will be calculated as the number of patients undergoing secondary R0 resection of metastases within 10 months after randomization divided by the total number of enrolled patients. The corresponding exact 2-sided 95% confidence interval will be calculated using a method based on the binomial distribution.

Time to strategy failure, time to 2nd progressive disease, PFS and OS will be summarized using Kaplan-Meier method and will also be displayed graphically. The median event times and corresponding 2-sided 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 exact 2-sided 95% confidence interval will be calculated using a method based on the binomial distribution.

SAMPLE SIZE

Considering that the combination of cetuximab and FOLFIRI regimen achieved in the subpopulation of *KRAS* wild-type patients a median PFS of approximately 10 months (10m-PFR= 50%), the combination of FOLFOXIRI and cetuximab for 4 months, followed by maintenance treatment with the biologic drug until PD, will be considered promising if the 10m-PFR will increase from 50% to 70%. According to the Fleming single-stage design, and selecting the design parameters p0 (10m-PFR in the null hypothesis) = 0.50, and p1 (10m-PFR in the alternative hypothesis) = 0.70, and considering alpha and beta errors of 0.05 and 0.10 respectively, a total of 53 patients will be required per arm. The treatment of each arm will be judged promising if at least 33 patients will be alive and progression-free at 10 months.
Considering that in the first phase of the trial, also patients bearing rare *KRAS*, as well as *NRAS* and *BRAF* mutations were eligible and the incidence of rare *KRAS, NRAS* and *BRAF* mutations is about 30% among *KRAS* codon 12-13 wild-type patients, 68 patients per arm will be randomized. Therefore, 136 patients will be included. Nevertheless, the primary analysis for PFS in the ITT population will be conducted separately in each arm when 53 patients will be observed for 10 months.

11. 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 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 4 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).

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 (Centro Coordinamento Sperimentazioni Cliniche of Istituto Toscano Tumori – Azienda Ospedaliero-Universitaria Careggi) will be responsible for:

- reviewing the protocol
- centralizing databases
- centralizing data validation according to Data Validation Plan
- 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 economical support for costs related to data management, statistical analysis and the other activities of central and group coordinating centers.
- No funds can be provided to ethical committees and single participating centers.
- The study will be conducted according to the current regulations.

12. STUDY MONITORING

QUALITY ASSURANCE

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

RESPONSIBILITIES OF THE INVESTIGATORS

The Investigators undertake to 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 and legible manner according to the instructions provided.

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. The main duty of the Trial Monitor is to help the Investigator and the Coordinators to maintain a high level of ethical, scientific, technical and regulatory quality in all aspects of the study.

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.

During each monitoring visits, the following points will be scrutinized:

- subject informed consent
- subject recruitment and follow-up
- study drug allocation
- 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, the monitor of the study 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.

Considering the primary end point of the study, independent review of objective response will be performed by an external panel. For this reason, a copy (either on CD or radiological film) of each CT or RMN scan performed during the study will be required.

USE AND COMPLETION OF ELETTRONIC CASE REPORT FORMS (E-CRFs)

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

13. 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. In general, 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 (provided below).

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

A hospitalization meeting the regulatory definition for “serious” is any inpatient 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 an 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 after this 30 days period) 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 drug(s). 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 drug(s) (possible, probable). All deaths should be reported on the death report form section of the CRF regardless of cause.

REPORTING PROCEDURE

Reporting Procedures for All Adverse Events

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

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

Medically significant adverse events considered related to the investigational product 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 investigational product 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(s) in accordance with local procedures, statutes and the European Clinical Trial Directive (where applicable). 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(s) in accordance with local legal requirements, statutes and the European Clinical Trial Directive.

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 clinical recovery is complete 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.

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.

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

PLEASE FAX THE REPORT TO 050.992192

And mail a .pdf scan version to:

fotiosloupakis@gmail.com

m.morvillo@ao-pisa.toscana.it

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

14. REFERENCES

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15. LIST OF ACRONYMS/ABBREVIATIONS

5-FU: 5-fluoruracil

10m-PFR: 10 months Progression Free Rate

ACE: Angiotensin Converting Enzyme

ADR: Adverse Drug Reaction

AE: Adverse Event

ANC: Absolute Neutrophil Count

A.R.C.O.: Associazione Ricerca e Cure in Oncologia

BSC: Best Supportive Care

BV: Bevacizumab

CNS: Central Nervous System

CR: Complete Reponse

CT: Computed Tomography

CTCAE: Common Terminology Criteria for Adverse Events

CVAD: Central Vascular Access Device

EC: Ethics Committee

ECOG PS: Eastern Cooperative Oncology Group Performance Status

eCRF: elettronic case report form

EGF: Epidermal Growth Factor

EGFR: Epideraml Growth Factor Receptor

EMEA: European Medicine Agency

FDA: Food and Drug Administration

G.O.N.O.: Gruppo Oncologico del Nord-Ovest

H.O.R.G.: Hellenic Oncology Research Group

HR: Hazard Ratio

ITT: Intention to treat

IV: intravenous

LV: Leucovorin

mCRC: metastatic colorectal cancer

mITT: modified intention to treat

MoAb: monoclonal antibody

NYHA: New York Heart Association

OR: Odds Ratio

OS: Overall Survival

Oxa: Oxaliplatin

PCR: Polymerase Chain Reaction

PD: Progressive Disease

PFS: Progression Free Survival

PlGF: Placental Growth Factor

PR: Partial Response

RBC: Red Blood Cells

RECIST: Response Evaluation Criteria in Solid Tumors

RR: Response Rate

SAE: Serious Adverse Event

SP: safety population

TGF-a: Transforming Growth Factor-alpha

ULN: Upper Limit of Normal

VEGF: Vascular Endothelial Growth Factor

VEGFR: Vascular Endothelial Growth Factor Receptor

WBC: White Blood Cells

WT: wild-type

G.O.N.O.

GRUPPO ONCOLOGICO NORD-OVEST

INDUCTION CHEMOTHERAPY WITH FOLFOXIRI PLUS CETUXIMAB AND MAINTENANCE WITH CETUXIMAB OR BEVACIZUMAB THERAPY IN UNRESECTABLE RAS AND BRAF WILD-TYPE METASTATIC COLORECTAL CANCER PATIENTS

*(*THE MACBETH STUDY*)*

EUDRACT 2011-000840-70

APPENDICES

-- Protocol Vers. 3.0 – November 25th, 2013 --

APPENDIX I: 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 pure richiesta per coloro che non possono dare o che rifiutano il consenso

[*] *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.)*

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 II: 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

Per gentile concessione di 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.

The Appendices from III through IX will be provided in a separate booklet which are part of the protocol