CLINICAL STUDY PROTOCOL

Title: A phase II, open label, non-randomized study of second or third line treatment with the combination of sorafenib and everolimus in patients affected by relapsed and non-resectable high-grade osteosarcoma.

Trial name: S.E.R.I.O. (Sorafenib plus Everolimus in Relapsed Inoperable Osteosarcoma)

Test Drug: Sorafenib, Everolimus

Sponsor’s Name and Address: Italian Sarcoma Group
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Sponsor’s Telephone Number: 051.6366757

Study Number/Version/Date: S.E.R.I.O. TRIAL vers. 2.2, 16th November 2010

Development Phase: Phase II

Type: No Profit

The undersigned confirm that they agree to conduct the study under the conditions described in this protocol

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1. INTRODUCTION

1.1 BACKGROUND

Neo-adjuvant and adjuvant chemotherapy has dramatically changed the prognosis of patients affected by osteosarcoma. Nevertheless, complete surgical removal with wide margins is still the most critical aspect in the multimodal approach to this disease. Indeed, localized Osteosarcoma of the extremity shows an overall survival around 65% in different series \(^1\), whilst localized Osteosarcoma of the pelvis or of the spine has an overall survival of 27% \(^2\). The reason for this difference is mainly due to the difficulty in reaching appropriate margins due to anatomical structures. Therefore, local relapse is more frequent in central Osteosarcoma, and metastatic relapse occurs more often in Osteosarcoma of the extremity.

In general, metastases affect from 30 to 60% of patients affected by Osteosarcoma and lung ranks first among metastatic sites followed by bone\(^7\). There are some well recognized prognostic factors at relapse. The most important ones are the interval to relapse (cut-off 24 months) and the number of metastatic nodules to the lung (less than 3 pulmonary lesions). According to these prognostic factors, we can identify patients having a 5-year post-relapse overall survival of 72% (relapse interval longer than 24 months and less than 3 pulmonary metastases) and patients whose long term probability to survive is around 5% (relapse before 24 months from the end of therapy and more than 2 lung metastases) \(^7,8\). Surgery plays a critical role again in achieving long term control of metastatic disease. It has been shown that a second surgical complete remission is a key factor to increase the chance of cure in this subset of patients: the risk of death is 5 times higher in patients who are not eligible to surgery \(^8,9\). On the contrary, the role of chemotherapy in this setting of often heavily pre-treated patients is somewhat controversial. Adjuvant chemotherapy was not shown to increase overall survival in patients undergone to complete surgical removal of metastases. However, in retrospective series, chemotherapy was shown to be effective in increasing overall survival of patients who were not disease free because of surgery\(^10\). Ifosfamide in the range of 14 g per squared meter was shown to be effective \(^11\). Another regimen that proved to be effective is the association of cyclophosphamide and etoposide. Unfortunately, even in patients achieving a response, the duration of response is short and long-term survivors are exceptional\(^12,13\). Therefore, new therapeutic tools are urgently needed.

The advent of imatinib, as well as other molecules, has proven that target therapy is effective in both hematological and solid tumors. So, formerly incurable and chemo-refractory diseases such as gastrointestinal stromal tumors can now be cured sometimes with extraordinary long-lasting results\(^14,15\). This success has generated an enormous interest to explore approaches based on specific molecular targets in other “difficult” neoplasms such as osteosarcoma. The chemotherapeutic “enpass” explains the intensive researches aimed to identify molecular targets to make this disease eligible to new therapeutic drugs. Osteosarcoma specimens were shown to express different potential targets KIT, EGFR, HER2, VEGFR, PDGFR and IGFR-1 are among the most studied ones\(^16-24\). If imatinib and monoclonal antibodies targeting IGFR-1 have failed to show any activity in advanced Osteosarcoma\(^25,26\), recent data from our group demonstrated that MAPKs are expressed and represent a suitable target to sorafenib\(^27\). On this basis, the Italian Sarcoma Group conducted a phase II trial with sorafenib in non-resectable either relapsed or metastatic Osteosarcoma after failure of standard multimodal therapies. 35 patients affected by advanced Osteosarcoma were treated with sorafenib and final results will be presented in 2010 CTOS meeting\(^28\). Briefly, sorafenib had an unprecedented activity in
terms of progression-free survival at four and six months of 46% and 29%, respectively. Furthermore, RECIST responses and non dimensional responses clearly showed that the study drug objectively halted tumor progression. Notwithstanding, sorafenib caused responses lasting more than six months in only few patients and no response at all in almost 50% of Osteosarcoma patients. Under this light, it is clear that sorafenib is a step forward, but it needs to be improved.

Our preclinical team, as well as other researchers, has recently focused on other transduction cellular pathways involved in Osteosarcoma progression. In particular, the PI3-K and the mTOR pathways have been shown to contribute to several of the tumor processes: angiogenesis, cellular division, protein synthesis and migration. Indeed, the inhibition of these two key enzyme pathways caused tumor arrest and regression in the preclinical setting (Osteosarcoma cell lines and xenograft). The role of mTOR pathway is well established and not only in osteosarcoma. In the clinical setting, several groups have already tested the combination of sorafenib and everolimus in the kidney and in the neuroendocrine cancers. The results of these phase I studies consistently showed that the two drugs can be safely combined. The proposed daily doses are: sorafenib 800 mg and everolimus 5 mg.

On the basis of our clinical and preclinical data as well as on these studies showing the safety of sorafenib and everolimus, we think it is reasonable to evaluate the activity of this combination in the lack of alternative effective therapies in non-resectable, relapsing or progressive metastatic high-grade Osteosarcoma patients after standard multimodal therapies.

1.2 SORAFENIB (BAY-43-9006)

Osteosarcoma relapsing and/or not resectable in a radical way is still an incurable disease. At the same time, data from different groups have shown that many tyrosine kinase receptors (KIT, EGFR, HER2, VEGFR and PDGFR) are implicated in survival advantage of Osteosarcoma cells. Our data showing MAPKs involvement in Osteosarcoma specimens support the hypothesis that a multikinase inhibitor as sorafenib may prove active and, eventually, effective in this disease. Our preliminary data are hints of sorafenib potential activity in this tumor. The available results on sorafenib activity and toxicity in renal cell carcinoma supported our previous trial with this drug in a subset of patients without any further available treatment.

The preliminary results of our phase II study with sorafenib at the dose of 800 mg daily have been encouraging: PFS at 4-months and 6-months were 46% and 29%, respectively. These data are unprecedented in this context and are strengthened by the observed tumor regression which is a direct proof of sorafenib activity.

Structure of compound

![Structure of compound](image)

BAY 43-9006 is a novel orally-active bi-aryl urea with a molecular formula of C 21 H 16 CIF 3 N 4 O 3 x C 7 H 8 O 3 S and a molecular weight of 637 g/mol. Sorafenib (BAY 43-9006) is an oral multi-kinase inhibitor targeting several serine/threonine and receptor tyrosine kinases. An inhibitor of signal transduction, sorafenib prevents tumor progression in Osteosarcoma patients. Under this light, it is clear that sorafenib is a step forward, but it needs to be improved.
cell proliferation and angiogenesis via its effects on the RAF/MEK/ERK (MAPK/ERK Kinase) pathway at the level of Raf kinase and tyrosine kinases vascular endothelial growth factor receptor-2 (VEGFR (vascular endothelial growth factor receptor -2) and platelet derived growth factor receptor (PDGFR-β))\(^{42}\).

Sorafenib has undergone evaluation in vitro in HCT-116 (colon) and MiaPaca-2 (pancreas) cell lines. Further in vivo evaluation was carried out in the HCT-116 xenograft model which contains a K-ras mutation. Sorafenib significantly inhibited tumour growth in these models in a dose-dependent fashion. Sorafenib has also demonstrated direct inhibition of the MAP kinase pathway (as measured by ERK phosphorylation in several sorafenib-treated tumour cell lines) in addition to VEGFR-2 and -3 and PDGFR-B inhibition in cell culture system biochemical assays. Xenograft models including MDA-MB-231 human mammary adenocarcinoma, colon and non-small cell lung cancer (NSCLC) models treated with daily sorafenib demonstrated significant inhibition of tumour growth and tumour microvessel density, as measured by anti-CD31 immunostaining\(^{42,43}\).

In vitro data indicates that sorafenib is metabolized by two pathways: oxidative metabolism mediated by cytochrome P450 (CYP)3A4 and glucuronidation mediated by UGT1A9. Following administration of sorafenib 100 mg, 96% of the dose is recovered: 77% in the faeces (51% of the total dose unchanged) and 19% in the urine as glucuronidated metabolites. The main metabolite of sorafenib, pyridine N-oxide, comprising 9 – 16% of analytes at steady state, shows in vitro potency similar to sorafenib. A limited dosing study in healthy volunteers demonstrated that blocking CYP3A4-mediated sorafenib metabolism (via concomitant administration of ketoconazole, which also inhibits UGT1A9) did not lead to an increase in the mean AUC of a single 50 mg dose of sorafenib. Therefore, CYP3A4 inhibitors are not predicted to significantly affect sorafenib drug concentrations, although further study is needed. There is no information of the effect of CYP3A4 inducers on the metabolism of sorafenib. Additional studies with CYP isoform-selective substrates indicated that sorafenib is unlikely to alter the metabolism of substrates of CYP2C19, CYP2D6 and CYP2C9 (including warfarin)\(^{44,45}\).

Sorafenib drug concentrations measured in the Phase I studies identified several consistent features of sorafenib metabolism. Sorafenib pharmacokinetics are characterized by a relatively slow absorption phase with C max achieved 6–12 h after dosing. The mean t\(\frac{1}{2}\) is a 24 – 36 h. All studies identified considerable inter-patient variability in sorafenib plasma concentrations with no clear dose dependency. Although small numbers preclude definitive statements, there is an apparent increase in sorafenib concentration at 400 mg b.i.d., with no further increase at higher dose levels. Steady state plasma concentrations of sorafenib are achieved within 7 days. Intake of food has no appreciable impact of sorafenib metabolism, with the exception of a high fat meal reducing sorafenib bioavailability by 29%. There is no impact of renal function on sorafenib steady state in patients with a creatinine clearance >30 ml/min, and there is no data on patients with creatinine clearance <30 ml/min or on dialysis. Sorafenib metabolism is not altered in patients with Child–Pugh class A or B hepatic impairment, and there is no data in patients with Child–Pugh class C. There is no clear relationship between plasma concentration of drug and incidence of drug-related toxicity. Over 5900 patients have been treated with single-agent sorafenib in company sponsored clinical trials so far. The approved labeled single-agent dose is 400 mg, twice daily given orally on a continuous, uninterrupted schedule. Preliminary antitumor activity has been reported in a variety of tumor types with tumor shrinkage seen in colorectal carcinoma, thyroid, sarcoma, pancreatic carcinoma, renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), melanoma, and non-small cell lung carcinoma (NSCLC)\(^{46-55}\). Sorafenib is
generally well-tolerated in adults. Drug-related adverse events were mainly mild-to-moderate and included hand-foot skin reaction, diarrhea, fatigue, hypertension, pain and rash. Sorafenib has been approved in 2005 by regulatory agencies for the treatment of metastatic renal cell carcinoma. After, the positive results of a phase III trial in hepatocellular carcinoma, sorafenib was approved by EMEA and FDA in 2007 for the treatment of hepatocellular carcinoma.

1.3 EVEROLIMUS (RAD001)

Structure of compound

RAD001 (everolimus) is a novel derivative of rapamycin. RAD001 has been in clinical development since 1996 as an immunosuppressant in solid organ transplantation. Since 2003, RAD001 is approved in Europe (trade name: Certican®) via the Mutual Recognition Procedure (MRP) for the prevention of organ rejection in patients with renal and cardiac transplantation. Certican® is also approved in Australia, South Africa, the Middle East, Central and South America, the Caribbean and some Asian countries.

RAD001 is being investigated as an anticancer agent based on its potential to act: directly on the tumor cells by inhibiting tumor cell growth and proliferation; indirectly by inhibiting angiogenesis leading to reduced tumor vascularity (via potent inhibition of tumor cell VEGF production and VEGF-induced proliferation of endothelial cells). In 2009, the European Committee approved everolimus for the treatment of patients with advanced renal cell carcinoma, whose disease has progressed on or after treatment with VEGF-targeted therapy.

1.3.1 mTOR pathway and cancer

Everolimus (RAD001) is a macrolide and a new derivative of rapamycin that has been hydroxyl-ethylated to increase its polarity and facilitate its formulation for oral administration. At the cellular and molecular level RAD001 acts as a signal transduction inhibitor. RAD001 selectively inhibits mTOR (mammalian target of rapamycin), a key protein kinase present in all cells which regulates cell growth, proliferation and survival. mTOR is mainly activated via the PI-3 kinase pathway through AKT/PKB and the tuberous sclerosis complex (TSC1/2). TOR is an ubiquitous protein kinase involved in cell cycle
control and specifically in the progression of cells from the G1 to S phase. It is considered to be located downstream of PI-3-K and AKT, its own primary downstream substrates being the eIF-4E-binding protein (4E-BP1) and p70 S6 kinase (p70s6k) both of which play a role in the translational regulation of mRNAs encoding proteins participating in G1-phase progression. Mutations in these components or in PTEN, a negative regulator of PI-3-K may result in their dysregulation. Abnormal functioning of various components of the signaling pathways contributes to the pathophysiology of numerous human cancers. Various preclinical models have confirmed the role of this pathway in tumor development

The main known functions of mTOR include the following: mTOR functions as a sensor of mitogens, growth factors and energy and nutrient levels, facilitating cell-cycle progression from G1-S phase in appropriate growth conditions; the PI-3-K (mTOR) pathway itself is frequently dysregulated in many human cancers, and oncogenic transformation may sensitize tumor cells to mTOR inhibitors; the mTOR pathway is involved in the production of pro-angiogenic factors (i.e., VEGF) and inhibition of endothelial cell growth and proliferation; through inactivating eukaryotic initiation factor 4E binding proteins and activating the 40S ribosomal S6 kinases (i.e. p70S6K1), mTOR regulates protein translation, including the HIF-1 proteins. Inhibition of mTOR is expected to lead to decreased expression of HIF-1

1.3.2 Preclinical studies

RAD001 inhibits the proliferation of a range of human tumor cell lines in-vitro including lines originating from lung, breast, prostate, colon, melanoma and glioblastoma. IC50’s range from sub/low nM to μM. RAD001 also inhibits the proliferation of human umbilical vein endothelial cells (HUVECS) in vitro, with particular potency against VEGF-induced proliferation suggesting that RAD001 may also act as an antiangiogenic agent. The antiangiogenic activity of RAD001 was confirmed in vivo. RAD001 selectively inhibited VEGF-dependent angiogenic response at well tolerated doses. Mice with primary and metastatic tumors treated with RAD001 showed a significant reduction in blood vessel density when compared to controls.

RAD001 when administered orally once daily was a potent inhibitor of tumor growth, at well tolerated doses, in 11 different mouse xenograft models (including pancreatic, colon, epidermoid, lung and melanoma) and two syngeneic models (rat pancreatic, mouse orthotopic melanoma). These models included tumor lines considered sensitive and “relative resistant” in vitro. In general, RAD001 was better tolerated in mouse xenograft models than standard cytotoxic agents (i.e., doxorubicin and 5-fluorouracil), while possessing similar antitumor activity. Additionally, activity in a VEGF-impregnated s.c implant model of angiogenesis and reduced vascularity (vessel density) of RAD001-treated tumors (murine melanoma) provided evidence of in vivo effects of angiogenesis. It is not clear which molecular determinants predict responsiveness of tumor cells to RAD001. Molecular analysis has revealed that relative sensitivity to RAD001 in vitro correlates with the degree of phosphorylation (activation) of the AKT/PKB protein kinase and the S6 ribosomal protein. These effects occurred within the dose range of 2.5 to 10 mg/kg, administered orally, once a day. In preclinical models, the administration of RAD001 is associated with reduction of protein phosphorylation in target proteins downstream of mTOR, notably phosphorylated (p)-S6 (p-S6) and p-4E-BP1, and occasionally with an increase in phosphorylation AKT, a protein upstream of mTOR signaling pathway

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1.3.3 Clinical experience

1.3.3.1 RAD001 pharmacokinetics

The pharmacokinetic characteristics of RAD001 have been extensively investigated in the context of the drug's development as an immunosuppressant in solid organ transplantation where RAD001 was administered twice daily as a part of an immunosuppressant, multi-drug regimen including cyclosporine A and glucocorticoids. More recent Phase 1 studies provide steady-state pharmacokinetics for both the weekly and daily schedules at varying dose levels in patients with advanced cancers. RAD001 is rapidly absorbed after oral administration, with a median time to peak blood levels (tmax) of 1-2 hours post dose. The extent of absorption is estimated at above 11%. The area under the blood concentration-time curve (AUC) is dose-proportionate for the dose ranges tested while maximum blood concentration (Cmax) appears to plateau at dose levels higher than 20 mg. The terminal half-life in cancer patients averaged 30 hours, which is similar to that in healthy subjects. Inter-patient variability is moderate with the coefficient variation (CV) approximately 50%. A high-fat meal altered the absorption of RAD001 with 1.3 hour delay in tmax, a 60% reduction in Cmax and a 16% reduction in AUC.

In whole blood, approximately 80% of RAD001 is contained in red blood cells. Of the fraction of drug contained in plasma, 74% is protein-bound. The apparent distribution volume (Vz/F) after a single dose was 4.7 L/kg. RAD001 is eliminated by metabolism, mainly by hydroxylation, then excreted into the feces >80%. RAD001 is mainly metabolized by CYP3A4 in the liver and to some extent in the intestinal wall. RAD001 is also a substrate of P-glycoprotein. Therefore, absorption and subsequent elimination of systematically absorbed RAD001 may be influenced by concomitant medications that interact with CYP3A4 and/or P-glycoprotein. In vitro studies showed that RAD001 is a competitive inhibitor of CYP3A4 and of CYP2D6 substrates, potentially increasing the concentrations of concomitant medications eliminated by these enzymes. In two phase III clinical trials in patients following kidney transplantation, strong inhibitors of CYP3A4 (azole’s, antifungal’s, cyclosporine, erythromycin) have been shown to reduce the clearance of RAD001 thereby increasing RAD001 blood levels. Similarly, Rifampicin, a strong inducer of CYP3A4, increases the clearance of RAD001 thereby reducing RAD001 blood levels. Caution should be exercised when co-administering RAD001 with CYP3A4 inhibitors or inducers. Co-administration of RAD001 did not influence pharmacokinetics of gemcitabine, imatinib, letrozole and specifically of sorafenib. In subjects with mild-moderate hepatic impairment, mean AUC to RAD001 is increased by 2-fold whilst renal impairment does not affect the pharmacokinetics of RAD001. Age, weight (both over the adult range) and gender do not affect the pharmacokinetics of RAD001 to a clinically relevant extent. AUC was positively correlated with serum bilirubin concentration and with prolongation of prothrombin time and negatively correlated with serum albumin concentration61-62.

1.3.3.2 Pharmacodynamic studies

Pharmacokinetic/pharmacodynamic modeling based on inhibition in a peripheral biomarker (S6 kinase inhibition in peripheral blood mononuclear cells) suggests that 5-10 mg daily should be an adequate dose to produce a high-degree of sustained target inhibition. Furthermore, molecular pharmacodynamic (MPD) studies using IHC in biopsied tumor tissue assessed the degree of inhibition and its duration (for p-S6, p-4E-BP1 and p-Akt expression) with the daily and weekly dosing. The pathologist was blinded for the biopsy sequence. There was almost complete inhibition of p-S6 at all doses and schedules studied (p=0.001).
Preliminary results suggest a dose-related decrease in p-4E-BP1 and increase in p-Akt expression with maximal effect at 10 mg daily and ≥ 50 mg weekly. In the monotherapy part of [Study C2101]/[Study C2102], the inhibition of S6K1 activity in PMBCs was measured in patients on the weekly regimen (5, 10, 20, 30 mg) by radioimmunoassay.\textsuperscript{63}

Subsequently, pharmacokinetic-pharmacodynamic modeling was carried out, extrapolating from preclinical findings in order to predict the likely inhibitory effect of RAD001 on its known target in the tumor of patients at these doses in the weekly regimen, as given, at postulated higher weekly doses, and in a postulated daily regimen. For the weekly regimen, the modeling suggested 20 mg/week as a minimum inhibitory dose but with decline of inhibition (over 50%) between administrations only marginally improved by increased dosage. The model indicated that a greater degree of sustained inhibition should be obtained with a daily regimen at comparable total drug consumption\textsuperscript{61-62}.

1.3.3.3 Phase I oncology studies

Seven single agent Novartis sponsored trials have or are being conducted in various advanced malignancies in a total of 826 patients enrolled as of 31Aug07. Data are available from three monotherapy phase I clinical studies (RAD001C2101, RAD001C2102 and RAD001C2107) given to 147 patients with advanced solid tumors. Such studies included various doses and schedules (weekly dosing, range 5-70 mg and daily dosing 5-10 mg). Approximately, 46% of patients reported rash and erythema. In some cases rash was accompanied by other symptoms: pruritus (16%), skin dryness (10%), nail disorders (6%). Maximum severity was Grade 1/2 in all but one case, a 10 mg/day melanoma patient with Grade 3 severity. This was the only patient to interrupt treatment or reduce dosage because of rash, the severity falling to Grade 1 after interruption and dose reduction to 5 mg/day. No patient discontinued study because of rash. Stomatitis/mucositis/mouth ulceration was reported in 40% of patients. In most cases these appeared rapidly after the start of therapy (51% within two weeks, 73% within four weeks). Mostly Grade 1/2, the drug was interrupted in patients with Grade 3 severity (8/59, 14% of patients with stomatitis), of whom one discontinued permanently; a second patient was discontinued due to stomatitis. Confounding factors make it possible to assess the value of reducing dosage on the attenuation of symptoms in only four patients, this occurring successfully in two of them. Hematology abnormalities, considered drug-related, were recorded in a total of 28 patients. Myelosuppression is a recognized effect of rapamycin, in line with their antiproliferative properties, but severe, suspected drug-related cytopenia was not frequent at these doses. It was a reason for discontinuation in 3 patients. Grade 4 thrombocytopenia (20x10^9/L) was recorded as a final value in a patient (70mg/wk) subsequent to an acute GI hemorrhage requiring multiple red cell transfusions. Hyperlipidemia, a recognized side-effect of rapamycin, was recorded as a suspected adverse event in 16 patients, mostly as hypercholesterolemia. Grade 3 hypertriglyceridemia was noted in 2 patients. Eleven patients initiated lipid-lowering drug therapy while on the study. Hyperglycemia was recorded as a suspected drug effect in 12 patients being of Grade 3 severity in five\textsuperscript{58,61,62}.

1.3.3.4 Pneumonitis

The term ‘pneumonitis’ describes non-infectious, non-malignant infiltration in the lungs which is evident radiologically. More precise diagnosis should follow histo-cytological examination following lung biopsy, generally during bronchoscopy which may or may not be symptomatic. In oncology studies with RAD001, severe pneumonitis suspected as drug-
related has been reported as a serious adverse event on 13 occasions. Data from two investigator-initiated trials, which included serial lung scans in patients, has suggested a high frequency of patients with ‘low-Grade’ pneumonitis, either Grade 1 (asymptomatic, evident radiologically only) or Grade 2 (mild symptoms not interfering with activities of daily living). The majority of patients continued to be treated, even without dose-reduction. An advisory board, including several leading oncology pulmonologist’s recently reviewed the findings and recommended implementation of specific measures in clinical studies for the detection and management of the disorder. Adherence to these recommendations should ensure detection of clinically relevant pneumonitis occurring in patients and its appropriate management.

1.4 POTENTIAL FOR PHARMACOKINETIC DRUG INTERACTIONS

There are six different phase I studies which studied the interaction between sorafenib and everolimus. None of these trials reported any pharmacokinetic interaction between the two drugs. In detail, Harzstark and colleagues have demonstrated that the steady state AUC 0-24h of everolimus + sorafenib was 193.3 (+/- 32.9) ng h/mL comparable to the steady state AUC 0-24h of everolimus single agent of 238 (+/- 77) ng h/mL. At the same time, the authors have shown that sorafenib steady state AUC 0-24h was not influenced by everolimus concomitant use, being the AUC 0-24h of sorafenib in combination and alone of 134600 (+/- 42072) and 131451 (+/- 53838) ng h/mL, respectively.

1.5 RATIONALE SUPPORTING THE PROPOSED STUDY

As above mentioned, chemotherapy has at most slightly improved the scenario of relapsed and non-resectable high-grade osteosarcoma. Thus, we have run and recently completed a phase II study with sorafenib after failure of standard chemotherapy in patients with relapsed/metastatic Osteosarcoma (abstract # .... CTOS, Oct 2010). The study was based on our previous preclinical data supporting the rationale to use MAPKs inhibitors in Osteosarcoma (Pignochino Y, et al, 2009). The trial accrued 35 patients, median age 21 years treated with sorafenib 800 mg daily until progression or unacceptable toxicity. Progression-free survival at six-months was 29%. RECIST v1.0 objective response was 8% and non dimensional responses by FDG-PET assessment and Choi criteria, was 8%. These results are unprecedented compared to other published data available and therefore of high interest. Unfortunately, as in other targeted therapy settings, both primary and secondary resistance were major causes of treatment failure. Our translational research team has continued the preclinical work on Osteosarcoma specimens and cell lines. We have explored other targetable pathways to improve sorafenib activity and we ended up focusing on mTOR. Beyond some theoretical reasons supporting the potential role of mTOR in Osteosarcoma, we have shown that the mTOR pathway is activated in Osteosarcoma and, moreover, that there is a more than additive inhibitory effect of the combination of mTOR and MAPKs inhibitors in Osteosarcoma preclinical models (Pignochino Y, et al 2010). The combination of sorafenib and everolimus has already been explored by several groups in renal cell carcinoma, thyroid cancer and neuroendocrine tumors showing the feasibility of the combination (P Cen et al. 2009. Chan JA et al. 2010. Nogova L et al. 2010. Brose MS, et al. 2010. Harzstark AL, et al. 2010).

The various phase I studies have come up with three different doses to be explored in subsequent phase II studies. The first combination is sorafenib 800 mg and everolimus 10 mg both daily (Cen P 2009). The second and most frequently suggested doses are sorafenib 800 mg and everolimus 5 mg daily (Harzstark AL 2010). The third one is sorafenib 200 mg twice
daily and everolimus 10 mg daily (Chan JA 2010). Of course, the observed toxicities are slightly different according to the chosen schedule and an analytical description is beyond the purpose of this document. Our previous experience with sorafenib in Osteosarcoma patients showed that the dosage of 800 mg/d is feasible in most patients with a manageable toxicity. The majority of our patients reported at least one CTC grade 1 or 2 adverse events requiring dose reductions or short drug holidays. In light of these observations, the combination regimen chosen for this study is sorafenib 400 mg bid and everolimus 5 mg daily. We believe that this regimen has the lowest risk of additive toxicities at dosages proven effective and approved in other indications for both drugs.

The clinical setting of this trial is represented by relapsed and unresectable osteosarcoma patients older than 17 years old having already received at least standard chemotherapy (cisplatin, doxorubicin and methotrexate +/- ifosfamide). This novel combination strategy in Osteosarcoma will assess if inhibition of two downstream signaling pathways affects tumor progression and survival. Any observed activity would represent a significant achievement. Indeed, since there are no standard therapies in relapsed and non-resectable osteosarcoma, the potential benefits may be the disease control with an increase in progression-free survival.
2. STUDY OBJECTIVES

2.1 PRIMARY OBJECTIVE
Primary objective of the study will be to assess the antitumor activity of sorafenib 400 mg twice a day in combination with everolimus 5mg/die as second or third line treatment of relapsed unresectable/metastatic high-grade osteosarcoma

2.1.1 Primary endpoint
- Progression Free Survival at 6 months

2.2 SECONDARY OBJECTIVES
Secondary objectives of the study will be to explore the activity of the combination sorafenib and everolimus in this unfavorable Osteosarcoma subset. This will be accomplished by both recording overall survival, progression-free survival, duration of response, RECIST response rate (dimensional reduction), non-dimensional response rate (metabolic or functional responses), safety and oncogene activation and correlation with outcomes parameters. Any improvement in patients’ quality of life will be captured by the Pain and Analgesic scale and recorded as clinical benefit. Finally, a specific effort will be conducted to evaluate the pattern of response, if any, given the peculiar patterns of response observed in solid tumors with molecular-targeted therapy.

2.2.1 Secondary endpoints
- Overall survival (OS)
- Progression-free survival (PFS)
- Overall response rate (ORR)
- Duration of response
- Non-dimensional pattern of response (i.e. metabolic or functional)
- Clinical benefit
- Safety (according to CTC version 4.03)

2.2.2 Exploratory objectives
- Expression of MAPKs pathway, VEGFR, PDGFR, Ezrin/Moesin and mTOR pathway (pS6 expression).
- Correlation (if any) between the above reported oncogenes/metabolic pathways and clinical outcome parameters.
- Predictive and prognostic role of serum lactate dehydrogenase (LDH) and serum alkaline phosphatase (ALP).
3. INVESTIGATOR(S) AND OTHER STUDY PARTICIPANTS

IRCC Candiolo: Prof Massimo Aglietta, Dr Giovanni Grignani

IOR Bologna: Dr Stefano Ferrari, Dr.ssa Emanuela Palmerini, Dr Piero Picci

INT Milano: Dr Paolo Casali, Dr.ssa Palma Di Leo, Dr.ssa Rossella Bertulli

IEO Milano: Dr Filippo De Braud, Dr Tommaso De Pas

Istituto Nazionale Tumori Regina Elena Roma: Prof. Francesco Cognetti, Dr.ssa Virginia Ferraresi

4. INVESTIGATIONAL PLAN

4.1 STUDY DESIGN AND PLAN

This is an open label, non-randomized, phase II clinical study of sorafenib 800 mg p.o./day in combination with everolimus 5mg/die until progression or unacceptable toxicity as second or third line treatment in a population of patients who have already received a treatment consisting of doxorubicin, methotrexate, cisplatin +/- ifosfamide and have failed first or second line treatment for relapsing disease.

From a methodological viewpoint, this study is designed and conducted as a standard phase II trial\textsuperscript{65}. Nevertheless, it is foreseeable, in the light of the type of response to therapy that is observed in this disease and, specifically to targeted therapies, that there will be some uncertainties in assessing response\textsuperscript{66}. This is due to the lack of validated criteria for assessing tumor responses that occur very slowly, often in the absence of measurable reductions in the size of the tumoral lesion over long periods, and that are best detected and monitored with radio metabolic approaches or with variations in either tumor density or minimal (10%) tumor dimensional reduction. In Osteosarcoma as well as in other tumors, pathological response has been of great value in order to understand whether dimensionally unchanged lesions were responding to the drug. Accordingly, we believe that only freedom from progression can detect the activity of this kind of combination therapies and response evaluation will be a great challenge for clinicians involved. Therefore, patients will be continuously treated according to clinical judgment, in the absence of progression, toxicity, patient’s willingness to discontinue the therapy, clinical decision to discontinue the therapy, accumulating evidence suggesting discontinuing the therapy and/or that other treatment options are more effective. Tumor response will be assessed before employing any other treatment modality capable of eliminating any evidence of disease, such as surgery and/or radiation therapy.

Accrual is expected to start in April 2011 and stage I is expected to be completed in approximately 10 months. If 6 patients among the first 17 enrolled, will reach the primary endpoint the study will be carried on to stage II. Accrual is expected to be complete after 2 years from the start of the study. The statistical analysis will be performed 6-7 months after last patient enrollment.
4.2 SELECTION OF STUDY POPULATION

37 patients with advanced Osteosarcoma (not resectable, unlikely resectable in a radical fashion, metastatic) after first or second line treatment for relapsing disease will be enrolled in Italian centers of the Italian Sarcoma Group.

4.2.1 Inclusion Criteria

1. Patients with histologically documented and not surgically resectable or metastatic high-grade osteosarcoma which progressed after first or second line treatments for relapsing disease.

2. Measurable disease as defined by having at least one uni-dimensional (RECIST v1.1 / bone lesions are allowed) measurable lesion that can be accurately measured by means of CT or MRI. Baseline evaluations must be completed within 28 days prior to enrollment.

3. Eastern Cooperative Oncology Group (ECOG) Performance Status of 0/1 and an estimated life expectancy of at least 3 months. Patients with and ECOG PS 2 are eligible if the PS 2 depends solely on orthopedic problems.

4. Estimated life expectancy of at least 3 months.

5. Age ≥ 18 years.

6. Adequate bone marrow, liver and renal function as assessed by the following laboratory requirements to be conducted within 7 days prior to start of treatment:
   - Hemoglobin > 9.0 g/dl
   - Absolute neutrophil count (ANC) ≥ 1,500/mm³
   - Platelet count ≥ 100,000/µl
   - Total bilirubin ≤ 1.5 times the upper limit of normal
   - ALT and AST ≤ 2.5 x upper limit of normal (≤ 5 x upper limit of normal for patients with liver involvement of their cancer)
   - PT-INR/PTT < 1.5 x upper limit of normal (Patients who are being therapeutically anticoagulated with an agent such as warfarin or heparin will be allowed to participate provided that no prior evidence of underlying abnormality in these parameters exists)
   - Serum creatinine ≤ 2 x upper limit of normal.

7. Written informed consent.

4.2.2 Exclusion Criteria

Patients who meet the following criteria at the time of screening will be excluded:

1. Dementia or significantly altered mental status that would prohibit the understanding or rendering of informed consent and compliance with the requirements of this protocol.

2. Patients with any severe and/or uncontrolled medical conditions such as unstable angina pectoris, symptomatic congestive heart failure, myocardial infarction ≤ 6
months, serious uncontrolled cardiac arrhythmia, uncontrolled hyperlipidemia, active or uncontrolled severe infection, cirrhosis, chronic or persistent active hepatitis or severely impaired lung function. In particular for history of cardiac disease: congestive heart failure >NYHA class 2; active CAD (MI more than 6 months prior to study entry is allowed); cardiac arrhythmias requiring anti-arrhythmic therapy (beta blockers or digoxin are permitted) or uncontrolled hypertension.

3. History of HIV infection and active clinically serious infections (> grade 2 NCI-CTC version 4.03).

4. Symptomatic metastatic brain or meningeal tumors (unless the patient is > 6 months from definitive therapy, has a negative imaging study within 4 weeks of study entry and is clinically stable with respect to the tumor at the time of study entry).

5. Patients with seizure disorders requiring medication (such as steroids or anti-epileptics).

6. Pregnant or breast-feeding patients. Women of childbearing potential must have a negative pregnancy test performed within 7 days of the start of treatment. Both men and women enrolled in this trial must use adequate barrier birth control measures during the course of the trial and 8 weeks after last dose of study drug.

7. Patients with evidence or history of bleeding diathesis.

8. Patients undergoing renal dialysis.

9. Patients unable to swallow oral medications.

10. Uncontrolled diabetes (fasting glucose > 2 x ULN).

11. Patients receiving chronic, systemic treatment with corticosteroids or another immunosuppressive agent (except corticosteroids with a daily dosage equivalent to prednisone ≤ 20 mg for adrenal insufficiency). Patients receiving corticosteroids must be on a stable dose for ≥ 4 weeks prior to the first dose of RAD001. Topical or inhaled corticosteroids are permitted.

12. Patients with a history of another malignancy within 5 years prior to study entry, except curatively treated non-melanotic skin cancer or in-situ cervical cancer skin or other solid tumors curatively treated with no evidence of disease for ≥3 years. Patients with severe and/or uncontrolled concurrent medical disease that in the opinion of the investigator could cause unacceptable safety risks or compromise compliance with the protocol (e.g. impairment of gastrointestinal (GI) function, or GI disease that may significantly alter the absorption of the study drugs).

13. Anticancer chemotherapy or immunotherapy during the study or within 4 weeks of study entry.

14. Radiotherapy during study or within 3 weeks of start of study drug. (Palliative radiotherapy will be allowed). Major surgery within 4 weeks of start of study.

15. Investigational drug therapy outside of this trial during or within 4 weeks of study entry.

16. Prior exposure to the study drugs or their analogues.
17. Patients with known hypersensitivity to sorafenib, RAD001 or other rapamycin analogs (sirolimus, temsirolimus), or to its excipients.

18. Substance abuse, medical, psychological or social conditions that may interfere with the patient’s participation in the study or evaluation of the study results.

19. A history of noncompliance to medical regimens or inability or unwillingness to return for scheduled visits.

4.3 REMOVAL OF SUBJECTS FROM STUDY

Subjects may be withdrawn from the study for the following reasons:

- At their own request or at the request of their legally acceptable representative.
- If, in the investigator's opinion, continuation in the study would be detrimental to the subject's well-being.

Also subjects must be withdrawn for the following reasons:

- Substantial non-compliance with the requirements of the study.
- Patients with a beta-HCG test consistent with pregnancy. Pregnancy will be reported along the same time lines as a serious adverse event.
- Use of illicit drugs or other substances that may, in the opinion of the investigator, have a reasonable chance of contributing to toxicity or otherwise skewing results.
- Development of an intercurrent illness or situation which would, in the judgment of the investigator, affect assessments of clinical status and study endpoints to a significant degree.
- Progression or recurrence of the underlying cancer.
- The development of a second cancer.
- Patient who is lost to follow-up.
- Interruption in study drug administration for greater than 30 days.
- Patient death.

In all cases, the reason for withdrawal must be recorded in the case report form and in the subject's medical records.

All patients who discontinue because of adverse events or clinical laboratory abnormalities should be followed up until they recover or stabilize, and the subsequent outcome recorded. The cause of death should be recorded in detail within 24 hours on a SAE form.

4.3.1 Replacement of Patients:

Patients who discontinue due to toxicity related to one or both study drugs will NOT be replaced. Patients who discontinue during the first week due to reasons other than toxicity will be replaced.
4.4 TREATMENTS

4.4.1 Treatments to be Administered

Sorafenib tablet 200 milligrams packed in bottle containing 140 tablets. Sorafenib will be provided by Bayer.

Everolimus is formulated in tablets of 2.5 or 5 mg strength, blister-packed under aluminum foil in units of 10 tablets. Everolimus will be supplied to the study investigators by Novartis.

The storage conditions for study drug will be described on the medication label. Sorafenib will be administered orally twice daily at the same time every day. Two 200 mg tablets will be taken either one hour before or two hours after a meal followed by a glass of water in the morning and in the evening. In general, patient should have a low to moderate fat meal.

Everolimus will be administered orally once daily at the same time every day immediately after a meal, as a single dose of 5 mg. Patients should have a low-fat breakfast. After this light meal, study medication of Everolimus is to be taken. The tablets of Everolimus should not be chewed or crushed.

Patients should wait at least two hours before any subsequent meal. Patients will receive Everolimus and Sorafenib until progression, toxicity, withdrawal of informed consent, clinical investigator decision. If vomiting occurs, no additional trial medication should be taken that day in an effort to replace the material that has been vomited. Next intake of drug should be when scheduled.

4.4.2 Identity of Investigational Product

Medication will be labeled according to the requirements of local law and legislation. Label text will be approved according to agreed the Sponsor procedures, and a copy of the labels will be made available to the study site upon request.

4.4.3 Selection of Doses in the Study

All patients will be treated at the initial dose of 800 mg dose per day (400 mg b.i.d.) for Sorafenib and 5 mg dose per day for Everolimus.

4.4.4 Prior and Concomitant Therapy

All concomitant medications (including start/stop dates, and indication) must be recorded in the patient’s source documentation as well as in the appropriate pages of the CRF (electronic or paper).

Permitted:

- Patients may receive palliative and supportive care for any underlying illness. Patients receiving bisphosphonates for bone metastases may continue while on treatment.

- Palliative radiotherapy within this period and during the study will be allowed for local pain control provided that 1) in the opinion of the Investigator, the patient does not have progressive disease, 2) no more than 10% of the patient’s bone marrow is irradiated and 3) the radiation field does not encompass a target lesion. Study drug may be continued during palliative radiotherapy in the face of tumor progression at the discretion of the Investigator, in collaboration with the sponsor.
• G-CSF and other hematopoietic growth factors may be used in the management of acute toxicity such as febrile neutropenia when clinically indicated or at the discretion of the investigator, however they may not be substituted for a required dose reduction.

• Patients taking chronic erythropoietin are permitted provided no dose adjustment is undertaken within 2 months prior to the study.

• Primary prophylaxis with erythropoietin is not permitted, however secondary prophylaxis is permitted as long as it does not substitute a necessary dose reduction (this text is not recommended for phase I protocols).

4.4.5 *Inhibitors of CYP3A4 and/or PgP*

• Co-administration with strong inhibitors of CYP3A4 (e.g., ketoconazole, itraconazole, ritonavir) or P-glycoprotein (PgP) should be avoided.

Co-administration with moderate CYP3A4 inhibitors (e.g., erythromycin, fluconazole) or PgP inhibitors should be used with caution. If patient requires co-administration of moderate CYP3A4 inhibitors or PgP inhibitors, reduce the dose of everolimus to half the currently used dose. Additional dose reductions to every other day may be required to manage toxicities. If the inhibitor is discontinued the everolimus dose should be returned to the dose used prior to initiation of the moderate CYP3A4/PgP inhibitor. Seville orange, star fruit, grapefruit and their juices affect P450 and PgP activity. Concomitant use should be avoided.

4.4.6 *Inducers of CYP3A4 and/or PgP*

• Avoid the use of strong CYP3A4 inducers.

There is no clinical information on the effect of CYP3A4 inducers on the pharmacokinetics of sorafenib. Substances that are inducers of CYP3A4 activity (e.g. rifampin, St. John’s wort, phenytoin, carbamazepine, phenobarbital, and dexamethasone) are expected to increase metabolism of sorafenib and thus decrease sorafenib concentrations. There are no clinical data evaluating the effect of chronically co-administered CYP3A4 inducers on sorafenib's efficacy.

Since there is a possibility of decreased sorafenib efficacy upon chronic co-administration of CYP3A4 inducers with sorafenib, chronic co-administration of CYP3A4 inducers with sorafenib, should be avoided to the extent possible.

Patients taking narrow therapeutic index medications should be monitored proactively. These medications include warfarin, phenytoin, quinidine, carbamazepine, phenobarbital, cyclosporin and digoxin.

If patient requires co-administration of strong CYP3A4 inducers (i.e., phenytoin, carbamazepine, rifampin, rifabutin, phenobarbital, St. John’s wort), an increase in the dose of everolimus up to twice the currently used daily dose should be considered, using 2.5mg increments. Enzyme induction usually occurs within 7-10 days, therefore everolimus dose should be increased by one increment 7 days after the start of the inducer therapy. If no safety concerns are seen within the next 7 days, the dose can be increased again one additional increment up to a maximum of twice the daily dose used prior to initiation of the strong CYP3A4 inducer.

This dose adjustment of everolimus is intended to achieve similar AUC to the range observed without inducers. However, there are no clinical data with this dose adjustment in patients receiving strong CYP3A4 inducers. If the strong inducer is discontinued the everolimus dose should be returned to the dose used prior to initiation of the strong CYP3A4/PgP inducer.
However any dose adjustment should be considered carefully taking into considerations the drug-drug interactions between Sorafenib and Everolimus.

Table 4-1 Clinically relevant drug interactions: substrates, inducers, and inhibitors of isoenzyme CYP3A

<table>
<thead>
<tr>
<th>SUBSTRATES</th>
<th>Calcium channel blockers:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotics: clarithromycin, erythromycin, telithromycin</td>
<td>amlodipine, diltiazem, felodipine, lercanidipine, nifedipine, nisoldipine, nitrendipine, verapamil</td>
</tr>
<tr>
<td>Anti-arrhythmics: Quinidine</td>
<td>HMG CoA reductase inhibitors: cerivastatin, lovastatin, simvastatin</td>
</tr>
<tr>
<td>Benzodiazepines: alprazolam, diazepam, midazolam, triazolam</td>
<td>Steroid 6beta-OH: estradiol, hydrocortisone, progesterone, testosterone</td>
</tr>
<tr>
<td>Immune modulators: cyclosporine, tacrolimus (FK506)</td>
<td>Miscellaneous: alfentanil, aprepitant, aripiprazole, buspirone, cafergot, caffeine, cilostazol, cocaine, codeine-N-demethylation, dapsone, dexamethasone, dextromethorphan, docetaxel domperidone, eplerenone, fentanyl, finasteride, gleevac/imitinib, haloperidol, irinotecan, LAAM, lidocaine, methadone, nateglinide, ondansetron, pimozide, propranolol, quetiapine, quinine, risperidone, salmeterol, sildenafil, sirolimus, sorafenib, sunitinib, tamoxifen, taxol, terfenadine, torisel, trazodone, vincristine, zaleplon, ziprasidone, zolpidem</td>
</tr>
<tr>
<td>HIV Antivirals: indinavir, nelfinavir, ritonavir, saquinavir</td>
<td></td>
</tr>
<tr>
<td>Prokinetic: Cisapride</td>
<td></td>
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<tr>
<td>Antihistamines: astemizole, chlorpheniramine, terfenadine</td>
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</table>

<table>
<thead>
<tr>
<th>INDUCERS</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Barbiturates, carbamazepine, glucocorticoids, modafinil, oxcarbazepine, phenobarbital, phenytoin, pioglitazone, rifabutin, rifampin, St. John’s wort, troglitazone, efavirenz, nevirapine</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>INHIBITORS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Strong inhibitors: indinavir, nelfinavir, ritonavir, clarithromycin, itraconazole, ketoconazole, nefazodone, saquinavir, telithromycin, posaconazole (Krishna et al, 2009)</td>
<td></td>
</tr>
<tr>
<td>Moderate inhibitors: aprepitant, diltiazem, erythromycin, fluconazole, grapefruit juice, verapamil</td>
<td></td>
</tr>
<tr>
<td>Weak inhibitors: cimetidine, seville orange (Malhotra et al, 2001)</td>
<td></td>
</tr>
</tbody>
</table>
Unclassified as per the Indiana University DDI listing:
ciprofloxacin, delavirdine, troleandomycin, mibebradil, amiodarone, chloramphenicol,
diethyldithiocarbamate, fluvoxamine, starfruit, gestodene, imatinib, mifepristone, norfloxacin,
norfloxetine, voriconazole*

Based on http://medicine.iupui.edu/clinpharm/ddis/table.asp as of December 01, 2009

*Voriconazole (unclassified as per the Indiana University DDI table)
Strong inhibitor according to the following reference:
(http://www.nature.com/clpt/journal/v80/n5/pdf/clpt2006438a.pdf)

Table 4-2 List of clinically relevant drug interactions mediated by PgP substrates

| Digoxin, fexofenadine, indinavir, vincristine, colchicine, topotecan, paclitaxel |

Table 4-3 List of clinically relevant drug interactions mediated by PgP inhibitors

| ritonavir, cyclosporine, verapamil, erythromycin, ketoconazole, itraconazole, quinidine, elacridar (GF120918), LY335979, valspodar (PSC833) |

Table 4-4 List of clinically relevant drug interactions mediated by PgP inducers

| Rifampin, St John’s worth |

NOTES:
5. SCHEDULE OF ASSESSMENT AND PROCEDURES

5.1 SCREENING PERIOD

The following should be collected during the screening period:

**Within 28 days prior to start of study drug:**

- Tumor assessment radiological evaluation using RECIST (v1.1). This radiological evaluation which meets the standard of care for imaging of lesions in the respective organ system(s) i.e. CT and/or MRI scans. All suspected sites of disease should be imaged.
- Chest CT scan;
- Electrocardiogram (ECG), Echocardiogram;
- Bone scan;
- Signed Informed Consent; enrollment in the study is defined as the signing of the Informed Consent;
- Demographic data: age, sex, weight, height, race;
- ECOG performance status;
- Documentation of the primary diagnosis, including confirmation of the relevant biopsy/histology slides by the institutional pathologist. On these slides, the following molecular analysis will be performed: expression of the oncogenes/pathways PI3K, mTOR, ERK1 and 2, ERM, PDGR, VEGFR, FGFR, HOH,. No further biopsy will be required, except when clinically indicated. All the above procedures should be done only after signature of Informed Consent;

**OPTIONAL**

- Fresh frozen tissue collection for tumor characterization (optional). After the signature of an appropriate informed consent a needle or core biopsy samples of 5 mm or greater size should be collected and immediately snap frozen upright in a cryo-vial in liquid nitrogen or dry ice using proper safety precautions. All tumor tissue should be frozen within 60 minutes of collection from the patient. If for any reason the samples cannot be kept at -70°C or colder, then approval from the Clinical Trial Head should be sought to ship the samples on dry ice to the central lab on the same day of collection. This procedure will be done in order to investigate the correlation between the molecular activated pathways (i.e. PI3K, mTOR (pS6), ERK1 and 2, ERM, PDGR, VEGFR, FGFR, HOH, (refer to the previous point) of each sample and the clinical outcomes. It is suggested but not mandatory at the physician’s discretion.
- PET total body (optional). It is suggested but not mandatory at the physician’s discretion.

**Within 14 days prior to start of study drug:**

- A complete physical exam with medical history, vital signs and ECOG performance status assessment should be performed within 14 days of start of study medication. Record all concomitant prescribed and over-the-counter medications. Record past cancer chemotherapy, radiotherapy and surgery. Detailed examination of the exact size and location of all tumor lesions (if visible or palpable) should be done at this time. Signs and
symptoms that existed prior to enrolment (signing of informed consent) and are ongoing at the time when the informed consent is signed as well as any sign or symptom that begins or worsens after enrollment (even if prior to start of study medication should be recorded Adverse Events at Screening page using the NCI-CTC v 4.03 guidelines.

- **Vital signs** should be assessed. Blood pressure measurement will be performed in a consistent manner using a manual cuff using the same arm with the patient sitting for 5 minutes prior to the evaluation. (see Appendix )

**Within 7 days prior to start of study drug:**

- Complete Blood Count (CBC): hemoglobin, hematocrit, platelet count, white blood cell count (WBC). WBC should include differential neutrophil, lymphocyte, monocyte, basophil and eosinophil counts.
- Electrolyte panel: sodium, potassium, chloride, calcium, phosphate.
- Chemistry Panel (need not be repeated if done within 14 days prior to start of study drug): glucose, creatinine, Blood Urea Nitrogen (BUN). Aspartate Amino-Transferase (AST), Alanine Amino-Transferase (ALT), bilirubin, Alkaline Phosphatase (ALP), uric acid, total protein, albumin, lipase, amylase, Lactic Dehydrogenase (LDH), Creatin Kinase (CK), triglycerides, cholesterol As hyperglycemia has been reported in clinical trials with Everolimus, monitoring of fasting serum glucose is recommended prior to the start of everolimus therapy and periodically thereafter. Optimal glycemic control should be achieved before trial therapy.
- Coagulation panel (need not be repeated if done within 14 days prior to start of study drug unless the patient is anticoagulated and an adjustment was made in the anticoagulant medication dose within that time): Prothrombin Time (PT) or the International Ration of PT (PT-INR), Partial Thromboplastin Time (PTT).
- Urinalysis (need not be repeated if done within 14 days prior to start of study drug): specific gravity, pH, glucose, protein, ketones, microscopic analysis for RBC, WBC, bacteria, crystals, casts.
- Urine [or serum] pregnancy test for women of child bearing potential (must be negative) (need not be repeated if done within 14 days prior to start of study drug).

Signs and symptoms that existed prior to enrollment (signing of informed consent) and are not ongoing at the time when the informed consent is signed, should be recorded in the Medical History page.

Any sign or symptom that begins, or is ongoing after enrollment (even if prior to start of study medication) must be documented on an Adverse Event page.

**Screening for hepatitis B:** Prior to starting study drugs, the following three categories of patients will be tested for hepatitis B viral load and serologic markers, that is, HBV-DNA, HBsAg, HBs Ab, and HBc Ab.

1. All patients who currently live in (or have lived in) Asia, Africa, Central and South America, Eastern Europe, Spain, Portugal, and Greece. [http://wwwnc.cdc.gov/travel/yellowbook/2010/chapter-2/hepatitis-b.aspx#849 ]
2. Patients with any of the following risk factors:
- known or suspected past hepatitis B infection;
- blood transfusion(s) prior to 1990;
- current or prior IV drug users;
- current or prior dialysis;
- household contact with hepatitis B infected patient(s);
- current or prior high-risk sexual activity;
- body piercing or tattoos;
- mother known to have hepatitis B;
- history suggestive of hepatitis B infection, e.g., dark urine, jaundice, right upper quadrant pain.

3. Additional patients at the discretion of the investigator

The management guidelines are provided according to the results of the baseline assessment of viral load and serological markers for hepatitis B.

Screening for hepatitis C: Patients with any of the following risk factors for hepatitis C should be tested using quantitative RNA-PCR

- known or suspected past hepatitis C infection (including patients with past interferon ‘curative’ treatment);
- blood transfusions prior to 1990;
- current or prior IV drug users;
- current or prior dialysis;
- household contact of hepatitis C infected patient(s);
- current or prior high-risk sexual activity;
- body piercing or tattoos;

At the discretion of the investigator, additional patients may also be tested for hepatitis C.

5.2 TREATMENT PERIOD

The following testing should be performed at each visit during the treatment period:

- Physical examination and vital signs;
- Toxicity and adverse events documentation (including start/Stop dates, relationship to study drug, outcome and action taken);
- Concomitant medication (including start/stop dates, dose, frequency, route of administration and indication);
- Drug accountability and dispensing;
- ECOG performance status;
- Laboratory Evaluations;
- Complete Blood Count (CBC);
• Electrolyte panel;
• Chemistry panel;
• Coagulation panel;

Clinical examination, blood tests, CT and/or MRI scans at base-line and every 2-month interval thereafter, ECG and Echocardiography at base-line, after 2 months of therapy and then every 4-months interval. Bone scan is mandatory at baseline. After that, bone scan should be performed every 2 months, if clinically indicated. PET total body, which is suggested but not mandatory at the physician’s discretion, will be performed at base-line, after 15 days, and then every 2 months in order to evaluate the prognostic relevance of an early PET response or the confirmatory role of PET in non-dimensional response.

Each patient will be followed-up regularly until progression thereafter, whenever possible physician will record any further line treatment and date of death.

End of treatment visit
When a patient is to be taken off treatment, the following assessment should be done 30 days after treatment has stopped:

• Brief medical history and complete physical examination including review of all organ system + vital signs and weight;
• ECOG performance status;
• Laboratory evaluations: complete blood count, electrolyte panel chemistry panel, coagulation panel urinalysis, pregnancy test;
• Toxicity and adverse events documentation;
• Concomitant medication.

After study drug treatment ends, patients will be evaluated approximately every 3 months to determine survival status until death is documented (telephone follow up is acceptable). Adverse events should be collected past 30 days after the treatment stop for all AEs that were ongoing at the end of treatment as well as new adverse events that in the opinion of the investigator could be related to study treatment.

Long Term Follow Up
This type of follow up can be done by telephone and its purpose is to determine the survival status of the patient.

All patients that progressed and/or start new anti cancer therapies must enter this type of follow up. This type of follow up will be done approximately every 3 months until death of patient.

During this period, there will be no protocol related visits. Information on survival must be collected. The collection of information on other anti cancer therapies is strictly enforced but is not mandatory.

All data collection during this period of time must be defined in this section.
5.3 DATA QUALITY
Monitoring and auditing procedures defined/agreed by the sponsor will be followed, in order to comply with GCP guidelines. Each center will be visited at regular intervals by a monitor to ensure compliance with the study protocol, GCP and legal aspects. This will include on-site checking of the case report forms (CRF) for completeness and clarity, cross-checking with source documents, and clarification of administrative matters.

5.4 DOCUMENTATION
Entries made in the CRF must be verifiable against source documents. The source data parameter to be verified and the identification of the source document must be documented. The study file and all source data should be retained until notification given by the sponsor for destruction.

6. ETHICAL AND LEGAL ASPECTS

6.1 ETHICS COMMITTEE (EC) OR INSTITUTIONAL REVIEW BOARD (IRB)
Documented approval from appropriate Ethics Committee(s)/IRBs will be obtained for all participating centers prior to study start, according to ICH, GCP, local laws, regulations and organizations. When necessary, an extension, amendment or renewal of the Ethics Committee approval must be obtained and also forwarded to the sponsor. The Ethics Committees must supply to the sponsor, upon request, a list of the Ethics Committee members involved in the vote and a statement to confirm that the Ethics Committee is organized and operates according to GCP and applicable laws and regulations.

6.2 ETHICAL CONDUCT OF THE STUDY
The procedures set out in this protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the sponsor and investigator abide by Good Clinical Practice Guidelines and under the guiding principles detailed in the Declaration of Helsinki. The study will also be carried out in keeping with applicable local law(s) and regulation(s). This may include an inspection by the sponsor representatives and/or Regulatory Authority representatives at any time. The investigator must agree to the inspection of study-related records by the Regulatory Authority/sponsor representatives, and must allow direct access to source documents to the Regulatory Authority/sponsor representatives.
Modifications to the study protocol will not be implemented by either the sponsor or the investigator without agreement by both parties. However, the investigator may implement a deviation form, or a change of, the protocol to eliminate an immediate hazard(s) to the trial subjects without prior EC/IRB/Sponsor approval/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it and if appropriate the proposed protocol amendment should be submitted to the EC/IRB/Sponsor. Any deviations from the protocol must be fully explained and documented by the investigator.

6.3 SUBJECT INFORMATION AND CONSENT
A core information and Informed Consent Form will be provided. Prior to the beginning of the study, the investigator must have the ECs/IRB written approval/favorable opinion of the written Informed Consent Form and any other written information to be provided to subjects.
The written approval of the EC/IRB together with the approved subject information/Informed Consent Forms must be filed in the study files.

Subjects will be informed that their participation is voluntary and that they may withdraw consent to participate at any time. They will be informed that choosing not to participate will not affect the care the subject will receive for the treatment of his/her disease. The subject or legally acceptable representative will be given sufficient time to read the informed consent form and the opportunity to ask questions. After this explanation and before entry to the study, consent should be appropriately recorded by means of either the subject's or his/her legally acceptable representative's dated signature. After having obtained the consent, a copy of the informed consent form must be given to the subject.

If the subject or legally acceptable representative is unable to read or write, an impartial witness should be present for the entire informed consent process (which includes reading and explaining all written information) and personally date and sign the informed consent form after the oral consent of the subject or legally acceptable representative is obtained. Written informed consent must be obtained before any study specific procedure takes place. Participation in the study and date of informed consent given by the subject should be documented appropriately in the subject’s files.

### 6.3.1 Optional biopsy informed consent.

Each patient will be given an option to participate in the extension part of the study (additional biopsy). A separate informed consent form will be covering this part of the study and will be submitted for ethical approval together with the Study Protocol and the main informed consent form.

### 6.4 INSURANCE

All subjects participating in the study will have insurance coverage by the sponsor, which is in line with applicable laws and/or regulations.

### 6.5 CONFIDENTIALITY

All records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. Subject names will not be supplied to the sponsor. Only the subject number and subject initials will be recorded in the case report form, and if the subject name appears on any other document (e.g., pathologist report), it must be obliterated before a copy of the document is supplied to the sponsor. Study findings stored on a computer will be stored in accordance with local data protection laws. The subjects will be informed in writing that representatives of the sponsor, EC/IRB, or Regulatory Authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws. If the results of the study are published, the subject’s identity will remain confidential. The investigator will maintain a list to enable subjects’ records to be identified. The principal investigator will be responsible for the respect of the confidentiality and privacy. The collection and processing of personal data from subjects enrolled in this study will be limited to those data that are necessary to investigate the efficacy, safety, quality, and utility of the investigational product(s) used in this study.
These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. The investigator ensures that the personal data will be

- processed fairly and lawfully
- collected for specified, explicit, and legitimate purposes and not further processed in a way incompatible with these purposes
- adequate, relevant, and not excessive in relation to said purposes
- accurate and, where necessary, kept current

Explicit consent for the processing of personal data will be obtained from the participating subject (or his/her legally acceptable representative) before collection of data. Such consent should also address the transfer of the data to other entities and to other countries. The subject has the right to request through the investigator access to his/her personal data and the right to request rectification of any data that are not correct or complete. Reasonable steps should be taken to respond to such a request, taking into consideration the nature of the request, the conditions of the study, and the applicable laws and regulations.

The same precautions will be performed for the samples obtained with the optional biopsy procedure. These samples will be stocked according to the procedures enumerated in paragraph 5.1. The principal investigator will be responsible for all these procedures. The subject has the right to request through the investigator access to his/her personal samples and the right to request the destruction of his/her tissue sample at any time. Reasonable steps should be taken to respond to such a request, taking into consideration the nature of the request, the conditions of the study, and the applicable laws and regulations.

In the absence of patient’s request the tissue samples will be conserved according to local law and no further molecular analysis will be performed in absence of explicit patient’s consent.
7. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

The trial is aimed to evaluate the activity of the combination sorafenib and everolimus in patients affected by advanced Osteosarcoma who already failed standard first, or second line therapies for relapsing disease. It is designed as an open label, non randomized, multicenter, phase II trial. To assess tumor response due to targeted therapy is rather difficult. This is because responses may occur very slowly, quite often in the absence of measurable or significant reductions in the size of the tumoral lesions over long periods; secondly responses are best detected and monitored with radio metabolic approaches and/or by studying changes in signal intensity, contrast uptake/enhancement and tumor density at CT/MRI. Moreover, it is of the greatest interest to gather any correlation between activity of the combination sorafenib and everolimus and either protein expression or their genotypes. Even a completely negative trial could raise some insights for future studies. Eligible patients will be prospectively enrolled, treated and followed according to the study protocol, and the observed results, in terms of toxicity and activity, will be continuously updated and monitored.

The study sample size was calculated according to a phase II design – 2-optimal stage (Simon) with PFS as the main endpoint. There is a surprising lack of published data on PFS in patients with high-grade osteosarcoma relapsed or not eligible to standard treatments (surgery + chemotherapy). This is possibly due to the low-absent rates of responses to 1st line chemotherapy (unless second complete surgical remission is achieved), that, in the various studies (Navid F., Cancer, 2008; O’Day K and Gorlick R., Expert Rev Anticancer Ther, 2009), cluster at about 15%, with few studies (Bacci G., Acta Oncol, 2005) reporting response rates around 20 to 30%. Reported median Overall Survival (OS) is below 6 months and seldom greater than 12 months. Little information (Navid F., Cancer, 2008) is available on response rate, PFS and OS in patients progressing after 1st line therapy such as those included in the present trial, mostly because no 2nd line anticancer treatment is usually administered. It is reasonable to expect that the large majority (>90%) of these patients, in the absence of further treatments, will show further disease progression within 3-4 months. Accordingly, patients who survive without progression at 4 months can be defined as successes. In our above reported trial, less than 30% of patients were free from progression at six months. On this basis, we chose as primary endpoint the progression-free survival at six months.

The data obtained will be compared (with an historical analysis) with the data that have been derived from the phase II study with Sorafenib alone in a population with inclusion criteria comparable for age and status of the disease.

7.1 Efficacy

Progression Free Survival (PFS) refers to the time from registration into the study to the date of progressive disease or death. In the absence of progression time will be censored at the date of last tumor assessment or follow-up. PFS will be used to evaluate the main study objective. Subjects who die for any reason would be included in the analysis with their date of death. Note, it is the date of the test (actual or scheduled) and not the date of assessment that is used to calculate time to progression.

Overall survival (OS) is the time interval between date of registration into study and the date of death. For alive patients, time will be censored at the date of last follow-up.
Objective response (OR) refers to the number of patients with complete, partial or minimal responses, according to RECIST.
Modified Response Criteria (MRC) refers to any consistent variation in radio metabolic diagnostic test (i.e. PET or Bone scan) and/or changes in signal intensity, contrast uptake/enhancement and tumor density at CT/MRI. As already mentioned above, there are at least other two settings (GIST and Chordoma) in which this kind of response has been shown useful and reproducible to detect targeted therapy activity. From this point of view, patients will be considered in response if there has been an objective response or at least ONE of the following criteria are met:

a) An unequivocal reduction in tumor density at CT scan;
b) An unequivocal reduction in signal intensity and/or contrast enhancement at MRI;
c) An unequivocal reduction in SUV at PET scan;
d) An unequivocal reduction in bone scan uptake.

Clinical Benefit will be prospectively evaluated by means of Pain and Analgesic Scale recording of analgesic consume and as lack of progression of disease at six months. Besides, Clinical Benefit will be assessed according to CTOS as any lack of progression at 24 weeks. Biological studies. Immunohistochemistry will be performed to assess MAPKs, VEGFR, PDGFR expression in paraffin embedded Osteosarcoma specimens. PDGFR and MAPKs positive specimens will be processed to genotype analyses.
Safety will be captured by recording: physical examinations, vital signs, performance status/body weight; blood tests and chemistry tests; intensity and severity of adverse events, use of analgesic medication.

**7.2 STUDIED POPULATIONS**

The **Safety Population** includes all patients who receive at least one dose of study medications.

The **Intention To Treat (ITT) Population** will include all enrolled patients who received at least one dose of study medications. Patients going off study due to AEs or toxicity prior to the key response evaluation will be considered treatment failures.

The per Protocol Population (PP) will consist of all patients who: a) did not violate inclusion criteria and exclusion criteria; b) completed the treatment study phase or withdrew from the study for progression or death; c) withdrew from the study for toxicity (AE related to study drug) and had at least one post baseline response evaluation.

All the evaluation criteria will be computed in the ITT and in the PP

As the primary objective of the study is to assess the antitumor activity of the combination Sorafenib and Everolimus, the primary analysis will be run primarily on the PP and confirmed on the ITT population; all secondary efficacy analyses will be run on the ITT population only. Replacement of patients who are not in the PP is in general not foreseen. Patients may be replaced in individual cases after discussion between the Sponsor and the investigators if a patient is felt not to provide sufficient information for the assessment of safety and efficacy of the combination Sorafenib and Everolimus.
7.3 SAMPLE SIZE

7.3.1 Determination of Sample Size

The Simon’s Optimal 2-stage design will be used, and the study should be able to discriminate whether the proportion of successes is 50% (P1 according to Simon’s terminology, i.e. the minimal proportion of successes that makes the experimental treatment worth further studies) or 25% (P0, the proportion of successes that, if true, implies no clinically worthwhile activity of the experimental treatment). Setting Alfa error at 5% and Beta error at 10%, 17 patients need to be enrolled in the 1st stage and at least 6 successes need to be observed in order to proceed to the second stage where 20 more patients need to be enrolled, for a total of 37 pts. A total of 14 or more successes (i.e. patients alive at 6 months without signs of progression) should be observed among these 37 patients, in order to consider the experimental treatment worth further studies.

7.3.2 Statistical and Analytical Plans

The efficacy and safety analyses will be performed on an intention-to-treat population and safety population, respectively.

The PFS e OS will be computed according to the Kaplan and Meier method and compared by means of the log-rank test. Progression-free interval will also be compared against historical controls. Exploratory, hypothesis generating aimed analyses will be performed stratifying patients according to line of therapy. We will explore also the pattern of response according to metastatic site (visceral vs bony).

The OR probability and the corresponding 95% confidence interval will be estimated by dividing the number of patients showing an “objective” response (according to RECIST criteria) to drug by the total number of patients in the population. For each patient, the best response observed at any time during treatment with the experimental drug will be considered. All data concerning adverse events, either discrete or continuous (lab values) will be analyzed in detail. Other indicators will be analyzed by descriptive analyses calculating frequency distributions. Continuous data will be summarized by mean, standard deviation, median, minimum and maximum. All statistical tests will be two-sided and p values below 0.05 will be considered as significant. Correlation with factors of probable prognostic value (e.g. alkaline phosphatase levels, performance status, …) will be searched.
8. ADVERSE EVENTS

8.1 ADVERSE EVENT MONITORING
Subjects must be carefully monitored for adverse events. This monitoring also includes clinical laboratory tests. Adverse events should be assessed in terms of their seriousness, severity, and relationship to the study drug.

8.2 ADVERSE EVENT DEFINITIONS

8.2.1 Adverse Event
An adverse event is any untoward medical occurrence in a subject or clinical investigation subject administered with a pharmaceutical product. The adverse event does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not considered related to the medicinal product.
Adverse events associated with the use of a drug in humans, whether or not considered drug related, include the following:

- An adverse event occurring in the course of the use of a drug product in professional practice;
- An adverse event occurring from an overdose whether accidental or intentional;
- An adverse event occurring from drug abuse;
- An adverse event occurring from drug withdrawal;
- An adverse event where there is a reasonable possibility that the event occurred purely as a result of the subjects participation in the study (e.g. adverse event or serious adverse event due to discontinuation of anti-hypertensive drugs during wash-out phase) must also be reported as an adverse event even if it is not related to the investigational product.

The clinical manifestation of any failure of expected pharmacological action is not recorded as an adverse event if it is already reflected as a data point captured in the CRF. If, however, the event fulfills any of the criteria for a “serious” adverse event, it must be recorded and reported as such. If Progressive Disease leads to signs and symptoms that meet the "serious" criteria (i.e. hospitalization, disability, death or important medical event), the signs and symptoms should be reported as a SAE and not as underlying cause (i.e. Progressive Disease). AEs must be collected until the end of pre defined study treatment. If AEs are not completely resolved at that time they must be collected until resolution (resolution must be defined in the protocol as chronicity or complete resolution).

8.2.2 Serious Adverse Event
A serious adverse event is any untoward medical occurrence that at any dose:

- Results in death;
- Is life-threatening;
• Requires in-patient hospitalization or prolongation of existing hospitalization;
• Results in persistent or significant disability or incapacity;
• Is a congenital anomaly or birth defect;
• Is an important medical event;

**Life-threatening**: The term “life-threatening” in the definition of “serious” refers to an adverse event in which the subject was at risk of death at the time of the event. It does not refer to an adverse event which hypothetically might have caused death if it were more severe.

**Hospitalization**: Any adverse event leading to hospitalization or prolongation of hospitalization will be considered as Serious, UNLESS at least one of the following exceptions are met:

- The admission results in a hospital stay of less than 12 hours
- The admission is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study)

**Disability** means a substantial disruption of a person’s ability to conduct normal life’s functions.

**Important medical event**: Any adverse event may be considered serious because it may jeopardize the subject and may require intervention to prevent another serious condition. As guidance for determination of important medical events refer to the “WHO Adverse Reaction Terminology – Critical Terms List”. These terms either refer to or might be indicative of a serious disease state.

Such reported events warrant special attention because of their possible association with a serious disease state and may lead to more decisive action than reports on other terms.

### 8.2.3 Unexpected Adverse Event

An unexpected adverse event is any adverse drug event, the specificity or severity of which is not consistent with the current Investigator Brochure (or Package Insert for marketed products). Also, reports which add significant information on specificity or severity of a known, already documented adverse event constitute unexpected adverse events. For example, an event more specific or more severe than described in the Investigator Brochure would be considered “unexpected”. Specific examples would be: (a) acute renal failure as a labeled adverse event with a subsequent new report of interstitial nephritis and (b) hepatitis with a first report of fulminant hepatitis.
8.2.4 Relationship of Adverse Event to Investigational Product

The assessment of the relationship of an adverse event to the administration of study drug is a clinical decision based on all available information at the time of the completion of the case report form.

An assessment of “No” would include:

- The existence of a clear alternative explanation e.g., mechanical bleeding at surgical site.

OR

- Non-Plausibility e.g., the subject is struck by an automobile when there is no indication that the drug caused disorientation that may have caused the event; cancer developing a few days after the first drug administration.

An assessment of “Yes” indicates that there is a reasonable suspicion that the adverse event is associated with the use of the investigational drug.

Factors to be considered in assessing the relationship of the adverse event to study drug include:

- The temporal sequence from drug administration: The event should occur after the drug is given. The length of time from drug exposure to event should be evaluated in the clinical context of the event;
- Recovery on discontinuation (de-challenge), recurrence on reintroduction (re-challenge): Subject’s response after drug discontinuation (de-challenge) or subjects response after drug re-introduction (re-challenge) should be considered in the view of the usual clinical course of the event in question;
- Underlying, concomitant, intercurrent diseases: Each report should be evaluated in the context of the natural history and course of the disease being treated and any other disease the subject may have;
- Concomitant medication or treatment: The other drugs the subject is taking or the treatment the subject receives should be examined to determine whether any of them may be suspected to cause the event in question;
- The pharmacology and pharmacokinetics of the test drug: The pharmacokinetic properties (absorption, distribution, metabolism and excretion) of the test drug(s), coupled with the individual subject’s pharmacodynamics should be considered.

8.2.5 Severity of the Adverse Event

The following classification should be used:

The intensity or severity of adverse events should be documented using the National Cancer Institute-Common Toxicity Criteria, (NCI-CTC v. 4.03).

The severity of adverse events should be graded as follows:

- Mild: usually transient in nature and generally not interfering with normal activities;
- Moderate: sufficiently discomforting to interfere with normal activities;
8.2.6 Adverse Event Documentation

All adverse events occurring after the subject has signed the informed consent must be fully recorded in the subject’s case record form. Documentation must be supported by an entry in the subject’s file. A laboratory test abnormality considered clinically relevant, e.g., causing the subject to withdraw from the study, requiring treatment or causing apparent clinical manifestations, or judged relevant by the investigator, should be reported as an adverse event. Each event should be described in detail along with start and stop dates, severity, relationship to investigational product, action taken and outcome.

8.3 Reporting of Serious Adverse Events/Pregnancy

Serious adverse events (SAEs), including laboratory test abnormalities fulfilling the definition of serious, after signing the informed consent and during follow-up period must immediately (within 24 hours of the investigator’s awareness) be reported to the person detailed in the study file. A serious adverse event Form must also be completed within 24 hours of the investigator awareness and forwarded to the designated person as detailed in the study file. Each serious adverse event must be followed up until resolution or stabilization by submission of updated reports to the designated person. An isolated laboratory abnormality that is assigned grade 4, according to CTC definition, is not reportable as a SAE, unless the investigator assesses that the event meets standard ICH criteria for an SAE. CTC grade 4 baseline laboratory abnormalities that are part of the disease profile should not be reported as a SAE, specifically when they are allowed or not excluded by the protocol inclusion/exclusion criteria. If an investigator is in doubt about the applicable reporting obligations, he/she should consult with the study monitor for the Sponsor.

CTC grade 4 lab abnormalities will be documented in the lab data and will be reviewed on a regular basis.

When required, and according to local law and regulations, serious adverse events must be reported to the Ethics Committee and Regulatory Authorities.

Pregnancy occurring during a clinical investigation, although not considered a serious adverse event, must be reported to the Sponsor within the same timelines as a serious adverse event on a Pregnancy Monitoring Form. The outcome of a pregnancy should be followed up carefully and any abnormal outcome of the mother or the child should be reported. This also applies to pregnancies following the administration of the investigational product to the father prior to sexual intercourse.

9. Dose Modification for Toxicity

9.1 Sorafenib Dose Modification

The recommendations for dose modifications which may become necessary due to various significant toxicities related in a possible, probable or definite way to sorafenib, are described below.

Definition of dose levels for sorafenib:

- Dose level 1: 400 mg (2 tablets) orally, twice a day;
- Dose level 2: 400 mg (2 tablets) orally, once a day;
- Dose level 3: 400 mg (2 tablets) orally, once every other day.

**Table 9-1: Hematological Criteria for Dose Delay and Dose Modification of sorafenib**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Dose Delay</th>
<th>Dose Modification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0-2</td>
<td>Treat on time</td>
<td>No Change</td>
</tr>
<tr>
<td>Grade 3</td>
<td>Treat on time</td>
<td>DECREASE one dose level b</td>
</tr>
<tr>
<td>Grade 4</td>
<td>DELAY a until ≤ Grade 2</td>
<td>DECREASE one dose level b</td>
</tr>
</tbody>
</table>

a If no recovery after 30 day delay, treatment will be discontinued unless patient is deriving clinical benefit;
b If more than 2 dose reductions are required, treatment will be discontinued.

**Table 9-2: Non-hematological Criteria for Dose Delay and Dose Modification of sorafenib (except skin toxicity, stomatitis and fatigue)a**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Dose Delay</th>
<th>Dose Modification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0-2</td>
<td>Treat on time</td>
<td>No Change</td>
</tr>
<tr>
<td>Grade 3</td>
<td>DELAY b until ≤ Grade 2</td>
<td>DECREASE one dose level c</td>
</tr>
<tr>
<td>Grade 4</td>
<td>OFF protocol therapy</td>
<td>OFF protocol therapy</td>
</tr>
</tbody>
</table>

a Also excludes nausea/vomiting that has not been pre-medicated, and diarrhea
b If no recovery after 30 day delay, treatment will be discontinued unless patient is deriving clinical benefit
c If more than 2 dose reductions are required, treatment will be discontinued
The following is recommended for agents that are known to cause HFS:

**Table 9-4: Grading for Hand-Foot Syndrome**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>Minimal skin changes or dermatitis (e.g., erythema, edema, or hyperkeratosis) without pain</td>
</tr>
<tr>
<td>Grade 2</td>
<td>Skin changes (e.g., peeling, blisters, bleeding, edema, or hyperkeratosis) with pain; limiting instrumental ADL</td>
</tr>
<tr>
<td>Grade 3</td>
<td>Severe skin changes (e.g., peeling, blisters, bleeding, edema, or hyperkeratosis) with pain; limiting self care ADL</td>
</tr>
</tbody>
</table>

According to the grade and incidence of skin toxicity (including rash and hand-foot syndrome) for a given patient, the following dose modification schedule will be followed (see Table 9-5).
### Table 9-5: Skin Toxicity Criteria for Dose Delay and Dose Modification

<table>
<thead>
<tr>
<th>Toxicity Grade</th>
<th>During a Course of Therapy</th>
<th>Dose for Next Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>Maintain dose level</td>
<td>Maintain dose level</td>
</tr>
<tr>
<td>Grade 2</td>
<td>1st appearance, Interrupt until resolved to grade 0-1</td>
<td>Maintain dose level</td>
</tr>
<tr>
<td></td>
<td>2nd appearance, Interrupt until resolved to grade 0-1</td>
<td>Consider decreasing dose frequency or level</td>
</tr>
<tr>
<td></td>
<td>3rd appearance, Interrupt until resolved to grade 0-1</td>
<td>Consider decreasing dose frequency or level</td>
</tr>
<tr>
<td></td>
<td>4th appearance, Discontinue treatment</td>
<td>Permanently</td>
</tr>
<tr>
<td>Grade 3</td>
<td>1st appearance, Interrupt until resolved to grade 0-1</td>
<td>Consider decreasing dose frequency or level&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2nd appearance, Interrupt until resolved to grade 0-1</td>
<td>Consider decreasing dose frequency or level</td>
</tr>
<tr>
<td></td>
<td>3rd appearance, Discontinue treatment</td>
<td>Permanently</td>
</tr>
</tbody>
</table>

<sup>a</sup> For patients who require a dose reduction for grade 3 rash or hand-foot syndrome, the dose of study drug may be increased to the starting dose after one full cycle of therapy has been administered at the reduced dose without the appearance of rash or hand foot syndrome ≥ grade 1.

Patients with discomfort due to hand foot syndrome may be treated with topical emollients, low potency topical steroids, or urea-containing cream. For patients who require a dose reduction for grade 3 rash or hand-foot syndrome, the dose of study drug may be increased to the starting dose after one full cycle of therapy has been administered with the reduced dose, without the appearance of rash or hand foot syndrome ≥ grade 1.

### 9.2 Everolimus Dose Modification

If administration of everolimus must be interrupted because of unacceptable toxicity, study drug dosing will be interrupted or modified according to the guidelines in the following tables. If the toxicity is intolerable to the patient at the original 5 mg daily dose, interrupt everolimus until recovery to Grade ≤ 1 then reintroduce everolimus at the initial dose or lower dose level depending on toxicity type and Grade. If a patient has already decreased two dose levels, no further dose reduction is permitted. Any dose interruption requiring ≥ 28 days to resolve will result in permanent discontinuation of the treatment.
Table 9-6: Everolimus dose level modification guidelines

<table>
<thead>
<tr>
<th>Dose levels</th>
<th>Dose and schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting dose</td>
<td>5 mg daily</td>
</tr>
<tr>
<td>Decrease one level</td>
<td>2,5 mg daily</td>
</tr>
<tr>
<td>Decrease two dose levels (schedule)</td>
<td>2,5 mg every other day</td>
</tr>
</tbody>
</table>

9.3 KNOWN UNDESIRABLE ADVERSE EVENTS OF EVEROLIMUS

Adverse events most frequently observed with Everolimus are rash, stomatitis/oral mucositis, fatigue, headache, anorexia, nausea, vomiting, diarrhea, and infections. Non-infectious pneumonitis has also been observed. Overall, the most frequently observed laboratory abnormalities include neutropenia, thrombocytopenia, hypercholesterolemia, and/or hypertriglyceridemia. The majority of these AE’s have been of mild to moderate severity (CTC Grade 1-2).

Table 9-7 Criteria for dose-modification in case of suspected Everolimus toxicity and re-initiation of Everolimus treatment

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-hematological toxicity</strong></td>
<td></td>
</tr>
<tr>
<td>Grade 2 (except pneumonitis)</td>
<td>If the toxicity is tolerable, maintain the same dose. If the toxicity is intolerable, interrupt Everolimus until recovery to ≤ Grade 1 then reintroduce Everolimus at 5 mg. If event returns to Grade 2, interrupt Everolimus until recovery to ≤1 Grade and reintroduce Everolimus at the lower dose level.</td>
</tr>
<tr>
<td>Grade 3 (except hyperlipidemia – see below)</td>
<td>Interrupt Everolimus until recovery to ≤ Grade 1 and reintroduce Everolimus at the lower dose level. (For pneumonitis see table 9 and consider a short course of corticosteroids).</td>
</tr>
<tr>
<td>Grade 3 hyperlipidemia (hypercholesterolemia and/or hypertriglyceridemia)</td>
<td>Should be managed using standard medical therapies. <strong>No dose interruption of Everolimus required</strong></td>
</tr>
<tr>
<td>Grade 4</td>
<td>Discontinue Everolimus.</td>
</tr>
</tbody>
</table>
9.3.1 Management of stomatitis/oral mucositis/mouth ulcer

Stomatitis/oral mucositis/mouth ulcers considered related to EVEROLIMUS should be treated using local supportive care. A general guideline to treat stomatitis/oral mucositis/mouth ulcers is provided.

### Table 9-8: Criteria for dose-modification in case of suspected Everolimus toxicity and restarting of Everolimus treatment

<table>
<thead>
<tr>
<th>Hematological toxicity</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 2 Thrombocytopenia</td>
<td>Interrupt Everolimus until recovery to ≤ Grade 1 (≥75 x10^9/L) and reintroduce Everolimus at 5 mg.</td>
</tr>
<tr>
<td>(platelets &lt;75 x10^9/L, ≥ 50 x10^9/L)</td>
<td></td>
</tr>
<tr>
<td>Grade 3 Thrombocytopenia</td>
<td>Interrupt Everolimus until recovery to ≤ Grade 1 (platelets ≥ 75 x10^9/L) and resume Everolimus at lower dose level. If Grade 3 thrombocytopenia recurs, discontinue Everolimus.</td>
</tr>
<tr>
<td>(platelets &lt;50 x10^9/L, ≥ 25 x10^9/L)</td>
<td></td>
</tr>
<tr>
<td>Grade 4 Thrombocytopenia</td>
<td>Interrupt Everolimus until recovery to ≤ Grade 1 (platelets ≥ 75 x10^9/L) and resume Everolimus at (-2) dose level. If Grade 3 thrombocytopenia recurs, discontinue Everolimus.</td>
</tr>
<tr>
<td>(platelets &lt;25 x10^9/L)</td>
<td></td>
</tr>
<tr>
<td>Grade 3 Neutropenia</td>
<td>Interrupt Everolimus until recovery to ≤ Grade 1 (neutrophils ≥ 1.5 x 10^9/L) and resume Everolimus at 5 mg. If neutrophil count again returns to Grade 3, hold Everolimus until neutrophil count ≥ 1.5 x10^9/L and resume Everolimus at the lower dose level. Discontinue Everolimus for a third episode of Grade 3 neutropenia.</td>
</tr>
<tr>
<td>(neutrophils &lt;1x10^9/L, ≥0.5 x10^9/L)</td>
<td></td>
</tr>
<tr>
<td>Grade 4 Neutropenia</td>
<td>Interrupt Everolimus until recovery to ≤ Grade 1 (neutrophils ≥ 1.5 x 10^9/L) and resume Everolimus at the lower dose level. If Grade 3 or 4 neutropenia occurs despite dose reduction, discontinue Everolimus.</td>
</tr>
<tr>
<td>(neutrophils &lt;0.5x10^9/L)</td>
<td></td>
</tr>
<tr>
<td>Grade 3 febrile neutropenia</td>
<td>Interrupt Everolimus until resolution of fever and neutropenia to ≤ Grade 1. Hold Everolimus until the neutrophil count ≥ 1.5 x 10^9/L and fever has resolved and resume Everolimus at the lower dose level. If febrile neutropenia recurs, discontinue Everolimus.</td>
</tr>
<tr>
<td>(not life-threatening)</td>
<td></td>
</tr>
<tr>
<td>Grade 4 febrile neutropenia</td>
<td>Discontinue Everolimus.</td>
</tr>
<tr>
<td>(life-threatening)</td>
<td></td>
</tr>
<tr>
<td>Any hematological or non-</td>
<td>Discontinue Everolimus.</td>
</tr>
<tr>
<td>hematological toxicity requiring</td>
<td></td>
</tr>
<tr>
<td>interruption for ≥ 28 days</td>
<td></td>
</tr>
</tbody>
</table>
1. For Grade 1, use conservative measures such as non-alcoholic mouthwash or salt water (0.9%) rinses several times a day until resolution.

2. For Grade 2 or 3 the suggested treatments are topical analgesic mouth treatments (i.e., local anesthetics such as, benzocaine, butyl aminobenzoate, tetracaine hydrochloride, menthol, or phenol) with or without topical corticosteroids.

3. Agents containing hydrogen peroxide, iodine, and thyme derivatives may tend to worsen mouth ulcers. It is preferable to avoid these agents.

4. Antifungal agents must be avoided unless a fungal infection is diagnosed. In particular, systemic imidazole antifungal agents (ketoconazole, fluconazole, itraconazole, etc.) should be avoided in all patients due to their strong inhibition of EVEROLIMUS metabolism leading to higher EVEROLIMUS exposures. Therefore, topical antifungal agents are preferred if an infection is diagnosed. Similarly, antiviral agents such as acyclovir should be avoided unless a viral infection is diagnosed.

9.3.2 Management of non-infectious pneumonitis

Both asymptomatic radiological changes (Grade 1) and symptomatic non-infectious pneumonitis (Grade 2 = not interfering with activities of daily living and Grade 3 = interfering with activities of daily living and oxygen indicated) have been noted in patients receiving EVEROLIMUS therapy.

Non-infectious pneumonitis has been associated with EVEROLIMUS and other mTOR inhibitors. If non-infectious pneumonitis develops, a consultation with a pneumologist should be considered. If the patient develops Grade 3 pneumonitis, treatment with EVEROLIMUS must be interrupted and the patient treated as medically indicated (short course corticosteroids, oxygen, etc). Management of non-infectious pneumonitis suspected to be associated with EVEROLIMUS and dose modification instructions are provided.

Table 9-9: Management of non-infectious pneumonitis

<table>
<thead>
<tr>
<th>Worst Grade</th>
<th>Required Investigations</th>
<th>Management of Pneumonitis</th>
<th>EVEROLIMUS Dose adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>CT scans with lung windows. Repeat chest x-ray/CT scan every 2 Cycles until return to baseline.</td>
<td>No therapy required</td>
<td>Administer 100% of EVEROLIMUS dose.</td>
</tr>
<tr>
<td>G2</td>
<td>CT scan with lung windows. Repeat each subsequent Cycle until return to baseline. Consider a bronchoscopy.</td>
<td>Symptomatic only. Prescribe corticosteroids if cough is troublesome.</td>
<td>Reduce EVEROLIMUS dose until recovery to ≤ Grade 1. EVEROLIMUS may also be interrupted if symptoms are troublesome.</td>
</tr>
<tr>
<td>G3</td>
<td>CT scan with lung windows.</td>
<td>Prescribe corticosteroids</td>
<td>Hold treatment until</td>
</tr>
</tbody>
</table>
Repeat each subsequent Cycle until return to baseline. A bronchoscopy is recommended.

**G4**  
CT scan with lung windows and required pulmonary function testing including: spirometry, DLCO, and room air O2 saturation at rest. A bronchoscopy is recommended.

Prescribe corticosteroids if infective origin ruled out. Taper as medically indicated.

Recovery to ≤Grade 1. May restart treatment within 28 days at a reduced dose if evidence of clinical benefit. Discontinue treatment.

### 9.3.3 Mixed origin adverse event.

There are some adverse events which may occur as a consequence of each drug or due to the combination of both of them. It follows the expected rate of some of the toxicities which may be caused by both drugs.

**Stomatitis:**  
≥ 1/10 everolimus; ≥1/100, <1/10 sorafenib

**Skin rash:**  
≥ 1/10 everolimus; ≥ 1/10 sorafenib

**Fatigue:**  
≥ 1/10 everolimus; ≥ 1/10 sorafenib

**Anemia:**  
≥ 1/10 everolimus; ≥1/100, <1/10 sorafenib

**Thrombocytopenia:**  
≥ 1/10 everolimus; ≥1/100, <1/10 sorafenib

These kinds of toxicities must be regarded and managed along the suggested dose reduction rules enumerated in Table 9-10. When therapy is restarted dose should be adjusted according to the suggested dose reductions. However, since there may be a certain degree of acquired tolerance to each drug, the physician may increase one dose level for each drug at the time aiming to the maximum tolerated dose for both drugs in that specific patient.
Table 9-10: Dose reductions recommended for toxicities occurring due to the combined use of both drugs

<table>
<thead>
<tr>
<th></th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fatigue</strong></td>
<td>no dose adjustment</td>
<td>check after 1 wk</td>
<td>STOP both drugs</td>
<td>STOP both drugs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-check after 1 wk</td>
<td>-check after 1 wk</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>and if resolution:</td>
<td>and if resolution:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(-1) dose everol</td>
<td>(-2) dose everol</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(-1) dose soraf</td>
<td>(-2) dose soraf</td>
</tr>
<tr>
<td><strong>Skin rash</strong></td>
<td>check after 1 wk</td>
<td>check after 1 wk and:</td>
<td>STOP both drugs</td>
<td>STOP both drugs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-1) dose everol</td>
<td>-check after 1 wk</td>
<td>-check after 1 wk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-1) dose soraf</td>
<td>and if resolution:</td>
<td>and if resolution:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(-1) dose everol</td>
<td>(-2) dose everol</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(-1) dose soraf</td>
<td>(-2) dose soraf</td>
</tr>
<tr>
<td><strong>Stomatitis</strong></td>
<td>check after 1 wk and</td>
<td>check after 1 wk and</td>
<td>STOP both drugs</td>
<td>STOP both drugs</td>
</tr>
<tr>
<td></td>
<td>reduce:</td>
<td>reduce:</td>
<td>-check after 1 wk</td>
<td>-check after 1 wk</td>
</tr>
<tr>
<td></td>
<td>(-1) dose everol</td>
<td>(-2) dose everol</td>
<td>and if resolution:</td>
<td>and if resolution:</td>
</tr>
<tr>
<td></td>
<td>(-1) dose soraf</td>
<td>(-2) dose soraf</td>
<td>(-1) dose everol</td>
<td>(-2) dose everol</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(-1) dose soraf</td>
<td>(-2) dose soraf</td>
</tr>
<tr>
<td><strong>Anemia</strong></td>
<td>no dose adjustment</td>
<td>check after 1 wk and</td>
<td>STOP both drugs</td>
<td>STOP both drugs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>reduce:</td>
<td>-check after 1 wk</td>
<td>-check after 1 wk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-1) dose everol</td>
<td>and if resolution:</td>
<td>and if resolution:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-1) dose soraf</td>
<td>(-1) dose everol</td>
<td>(-2) dose everol</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(-1) dose soraf</td>
<td>(-2) dose soraf</td>
</tr>
<tr>
<td><strong>Thrombocytopenia</strong></td>
<td>check after 1 wk</td>
<td>check after 1 wk and</td>
<td>STOP both drugs</td>
<td>STOP both drugs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>reduce:</td>
<td>-check after 1 wk</td>
<td>-check after 1 wk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-1) dose everol</td>
<td>and if resolution:</td>
<td>and if resolution:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-1) dose soraf</td>
<td>(-1) dose everol</td>
<td>(-2) dose everol</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(-1) dose soraf</td>
<td>(-2) dose soraf</td>
</tr>
</tbody>
</table>

10. USE OF DATA AND PUBLICATION

All data and results and all intellectual property rights in the data and results derived from the study will be the property of the sponsor (Italian Sarcoma Group). Authorship for publications/scientific communications will be based (besides the study principal investigator role), on the enrollment performance (number of randomized subjects) of the participating study centers. Balanced representation of sites will be considered (e.g. top enrolling centers) and will take into account the publication policy of the targeted journal/scientific meeting. The investigator, whilst free to utilize data derived from the study for scientific purposes, must discuss any publication with the sponsor prior to release and obtain written consent of the sponsor on the intended publication. The sponsor recognizes the right of the investigator to publish the results upon completion of the study. However, the investigator must send a draft manuscript of the publication or abstract to the sponsor thirty days in advance of submission in order to obtain approval prior to submission of the final version for publication. This will be reviewed promptly and approval will not be withheld unreasonably. In case of a difference of opinion between the sponsor and the investigator(s), the contents of the publication will be discussed in order to find a solution which satisfies both parties. In case of publication/presentation of the study results, the Sponsor engages to mention that they were obtained with the contribution of Bayer and Novartis. The Sponsor also engages to
acknowledge to Bayer and Novartis the reasonable opportunity of reviewing and expressing comments on the proposed publication.
11. REFERENCES


12. APPENDICES

12.1 APPENDIX 1. EFFICACY AND SAFETY ASSESSMENTS

Primary efficacy parameter – Tumor response

Tumor response will be evaluated in all patients in the center itself. The tumor response will be assessed every 2 months, with an imaging technique (CT scan or MRI) according to local facilities, and at the discretion of the local Investigator, depending on the characteristics of tumor lesions. The original article “New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1)” published in the European Journal of Cancer (2009; 45: 228-247) is accessible at the URL address: http://www.ejcancer.info/article/S0959-8049%2808%2900873-3/pdf

Tumor response will be recorded according to RECIST 1.1 criteria (a maximum of 2 lesions per organ, and 5 overall will be taken as disease indicators for tumor response).

Secondary efficacy parameter – Tumor metabolic response

Tumor metabolic response, which is suggested but not mandatory at the physician’s discretion, will be evaluated in each center itself. The tumor metabolic response will be assessed every 2 months, with a 18-FDG-PET, and at the discretion of the local Investigator, depending on the characteristics of tumor lesions. The original article on PERCIST criteria published in the Journal of Nuclear Medicine (2009; 50:122S–150S) is accessible at the URL address: http://jnm.snmjournals.org/cgi/reprint/50/Suppl_1/122S.

Tumor metabolic response will be recorded according to PERCIST criteria (a maximum of 2 lesions per organ, and 5 overall will be taken as disease indicators for tumor metabolic response).

Safety assessments

Safety assessments will consist of evaluation of adverse events and serious adverse events, laboratory parameters including haematology, chemistry, vital signs, physical examinations, and documentation of all concomitant medications and/or therapies. Toxicity will be evaluated according to the NCI Common Toxicity Criteria, v. 4.03, accessible at the URL address: http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf
### 12.2 Appendix 2. Eastern Cooperative Oncology Group (ECOG) Performance Status Scale

<table>
<thead>
<tr>
<th>Description</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fully active, able to carry on all pre-disease activities without restriction.</td>
<td>0</td>
</tr>
<tr>
<td>Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature e.g. light housework, office work.</td>
<td>1</td>
</tr>
<tr>
<td>Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.</td>
<td>2</td>
</tr>
<tr>
<td>Capable of only limited self care, confined to bed or chair more than 50% of waking hours.</td>
<td>3</td>
</tr>
<tr>
<td>Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.</td>
<td>4</td>
</tr>
</tbody>
</table>
### 12.3 APPENDIX 3. EVALUATION SCALES

#### 12.3.1 SARC-CTOS Clinical Benefit Evaluation Scale

<table>
<thead>
<tr>
<th>CR (1)</th>
<th>Clinical benefit</th>
<th>CR</th>
<th>Clinical Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR (2)</td>
<td>Clinical Benefit</td>
<td>PR</td>
<td>Clinical Benefit</td>
</tr>
<tr>
<td>SD (3)</td>
<td>No Benefit</td>
<td>SD</td>
<td>Clinical Benefit</td>
</tr>
<tr>
<td>PD (4)</td>
<td>No Benefit</td>
<td>SD</td>
<td>No Benefit</td>
</tr>
<tr>
<td>2-months</td>
<td>6-months</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(1) CR: complete remission  
(2) PR: partial remission  
(3) SD: stable disease  
(4) PD: progressive disease


#### 12.3.2 RECIST 1.1 Evaluation Scale

<table>
<thead>
<tr>
<th>Target lesions</th>
<th>Non-target lesions</th>
<th>New lesions</th>
<th>Overall response</th>
<th>Best Response for this Category also requires</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>CR</td>
<td>No</td>
<td>CR</td>
<td>&gt;4 wk. Confirmation</td>
</tr>
<tr>
<td>CR</td>
<td>Non-CR/non-PD</td>
<td>No</td>
<td>PR</td>
<td>&gt;4 wk. Confirmation</td>
</tr>
<tr>
<td>CR</td>
<td>Not evaluated</td>
<td>No</td>
<td>PR</td>
<td>&gt;4 wk. Confirmation</td>
</tr>
<tr>
<td>PR</td>
<td>Non-PD or not all evaluated</td>
<td>No</td>
<td>PR</td>
<td>&gt;4 wk. Confirmation</td>
</tr>
<tr>
<td>SD</td>
<td>Non-PD or not all evaluated</td>
<td>No</td>
<td>SD</td>
<td>Documented at least once &gt; 6 wk. from baseline</td>
</tr>
</tbody>
</table>

Not all evaluated  
PD  
Any  
PD  
Any  
Any

*Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "**symptomatic deterioration**". Every effort should be made to document the objective progression even after discontinuation of treatment.*
12.4 **APPENDIX 4. REPORT FORM FOR SEVERE ADVERSE EVENT**

---

**Serious Adverse Event Report Form**

**Study Information**

1. **Country:**
   - Centre Number: 
   - Indication: 
   - Study ID: S.E.R.I.O. TRIAL

2. **Initial:**
   - Follow-up: Recurrent events or complications of a previously reported event should be reported as follow-up

   **Argus case ID (if known):**

3. **Was the treatment code broken?**
   - Yes, please enter in section 6
   - No
   - Not applicable (i.e. open study)

**Subject Information**

4. **Subject ID:**
   - **Randomisation No:**
   - **Date of Birth:**
   - **Age:**

   **Sex:**
   - Male
   - Female

   **Ethnicity:**
   - Caucasian
   - Hispanic
   - Asian
   - Other
   - Unknown

   **Weight:**
   - **Height:**

   **Weight:**
   - kg
   - lbs
   - Please tick which unit is appropriate

   **Height:**
   - cm
   - in
   - Please tick which unit is appropriate

5. **Medical history relevant to the SAE including concurrent and pre-existing conditions (please provide dates):**

   **Condition**
   - **Onset date**
   - **Ongoing at time of SAE?**
   - **If no, End date**

<table>
<thead>
<tr>
<th>Condition</th>
<th>dd</th>
<th>mm</th>
<th>yyyy</th>
<th>Yes</th>
<th>No</th>
<th>dd</th>
<th>mm</th>
<th>yyyy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

S.E.R.I.O. TRIAL SAE form

---

Page 1 of 5
### Serious Adverse Event Report Form

Summarize all pertinent information from the health records on this form in BLOCK CAPITALS

#### Information on Serious Adverse Event(s)

<table>
<thead>
<tr>
<th>Mark if diagnosis (x)</th>
<th>Onset date</th>
<th>Outcome</th>
<th>Is there a reasonable possibility that the study treatment caused the event?</th>
<th>Is there a reasonable possibility that any other medications contributed to the event?</th>
<th>Other possible contributory factors:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dd  mm  YYYY</td>
<td>1-7 dd  mm YYYY</td>
<td>Y/N</td>
<td>Treatment name</td>
<td>Y/N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Seriousness criteria

1. Death, 2. Life threatening, 3. Involved or prolonged inpatient hospitalization, 4. Results in persistent or significant disability/incapacity, 5. Congenital anomaly/birth defect, 6. Medically significant event (For definition please refer to SAE Quick Reference Guide)

* Please note that progression of study indication should usually be assessed as not suspected because the event occurred in spite of study treatment administration

### 9. Please provide rationale for causality assessment to study treatment

* * *
Serious Adverse Event Report Form

Summarize all pertinent information from the health records on this form in BLOCK CAPITALS

10. Description of the event(s) including all hospitalization start and stop dates

11. If subject died, was an autopsy performed?

<table>
<thead>
<tr>
<th>Yes*</th>
<th>No</th>
<th>Unknown</th>
<th>If yes, date of autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>dd mm yy</td>
</tr>
</tbody>
</table>

Please provide the primary cause of death and any relevant findings as determined by the autopsy, if performed, in section B

Treatment of the reported event(s)

<table>
<thead>
<tr>
<th>Details of drug &amp; non-drug treatment</th>
<th>Treatment dates</th>
<th>Dosage (amount, unit, route, frequency)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Start dd mm YYYY Stop dd mm YYYY</td>
<td>(e.g. 20mg oral bid)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Laboratory, test or scan data relevant to the reported SAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date dd mm YYYY</td>
</tr>
</tbody>
</table>

*If CTCAE grade 4 or above, or considered to be serious for any other reason, please also list in section B

14. Comments on laboratory and test data findings

S.E.R.I.O. TRIAL SAE form

Page 4 of 5
15. Please provide additional information from any previous section here. Please do not attach discharge summaries, copies of medical records or examination results unless specifically requested.

Please check this box if the causality between study treatment and all the events reported on this form are NOT SUSPECTED.

Reporter information

16. Name and address of investigator/reporter (please print)

Investigator name

Report name (if different)

Address

Title

First name

Last name

Tel

Fax

Signature of investigator

Date of signature: dd mm YYYY

Thank you very much for your co-operation in this matter.

Please fax the completed form to your local DS&E office – n°0119933290 or 0119933299.

For office use only

Manufacturer Receipt Date: dd mm YYYY

Local reference number:
12.5 **APPENDIX 5. THE BRIEF PAIN INVENTORY SHORT FORM (BPI)** *

**Brief Pain Inventory (Short Form)**

**Question 1:** Throughout our lives, most of us have had pain from time to time (such as minor headaches, sprains, and toothaches). Have you had pain other than these everyday kinds of pain today?

1. Yes
2. No

**Question 2:** On the diagram, shade in the areas where you feel pain. Put an X on the area that hurts the most.

**Question 3:** Please rate your pain by circling the one number that best describes your pain at its worst in the last 24 hours.

<table>
<thead>
<tr>
<th>No Pain</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
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<tbody>
<tr>
<td>Pain as bad as you can imagine</td>
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</tbody>
</table>

**Question 4:** Please rate your pain by circling the one number that best describes your pain at its least in the last 24 hours.

<table>
<thead>
<tr>
<th>No Pain</th>
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<th>2</th>
<th>3</th>
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<th>5</th>
<th>6</th>
<th>7</th>
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</table>

**Question 5:** Please rate your pain by circling the one number that best describes your pain on the average.

<table>
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<tr>
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<th>2</th>
<th>3</th>
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<th>6</th>
<th>7</th>
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<tbody>
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</tbody>
</table>

**Question 6:** Please rate your pain by circling the one number that tells how much pain you have right now.

<table>
<thead>
<tr>
<th>No Pain</th>
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<th>2</th>
<th>3</th>
<th>4</th>
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<th>6</th>
<th>7</th>
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<tbody>
<tr>
<td>Pain as bad as you can imagine</td>
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</tbody>
</table>
7. What treatments or medications are you receiving for your pain?

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8. In the last 24 hours, how much relief have pain treatments or medications provided? Please circle the one percentage that most shows how much relief you have received.

<table>
<thead>
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<th>Percentage</th>
<th>Relief</th>
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<td>0%</td>
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</tr>
<tr>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>20%</td>
<td></td>
</tr>
<tr>
<td>30%</td>
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<td>80%</td>
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<td>90%</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>Complete Relief</td>
</tr>
</tbody>
</table>

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9. Circle the one number that describes how, during the past 24 hours, pain has interfered with your:

| Activity/Interference | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|-----------------------|--|--|--|--|--|--|--|--|--|--|--|---|
| A. General Activity   |   |   |   |   |   |   |   |   |   |   |   |   |
| Does not Interfere    |   |   |   |   |   |   |   |   |   |   |   |   |
| Completely Interferes |   |   |   |   |   |   |   |   |   |   |   |   |
| B. Mood               |   |   |   |   |   |   |   |   |   |   |   |   |
| Does not Interfere    |   |   |   |   |   |   |   |   |   |   |   |   |
| Completely Interferes |   |   |   |   |   |   |   |   |   |   |   |   |
| C. Walking Ability    |   |   |   |   |   |   |   |   |   |   |   |   |
| Does not Interfere    |   |   |   |   |   |   |   |   |   |   |   |   |
| Completely Interferes |   |   |   |   |   |   |   |   |   |   |   |   |
| D. Normal Work (includes both work outside the home and housework) |   |   |   |   |   |   |   |   |   |   |   |   |
| Does not Interfere    |   |   |   |   |   |   |   |   |   |   |   |   |
| Completely Interferes |   |   |   |   |   |   |   |   |   |   |   |   |
| E. Relations with other people |   |   |   |   |   |   |   |   |   |   |   |   |
| Does not Interfere    |   |   |   |   |   |   |   |   |   |   |   |   |
| Completely Interferes |   |   |   |   |   |   |   |   |   |   |   |   |
| F. Sleep              |   |   |   |   |   |   |   |   |   |   |   |   |
| Does not Interfere    |   |   |   |   |   |   |   |   |   |   |   |   |
| Completely Interferes |   |   |   |   |   |   |   |   |   |   |   |   |
| G. Enjoyment of life  |   |   |   |   |   |   |   |   |   |   |   |   |
| Does not Interfere    |   |   |   |   |   |   |   |   |   |   |   |   |
| Completely Interferes |   |   |   |   |   |   |   |   |   |   |   |   |

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12.6  **APPENDIX 6. ANALGESIC SCORE**

Modified from the Radiation Therapy Oncology Group analgesic score*.

Type of pain medication administered

0 = None

1 = Minor analgesics (aspirin, NSAID, acetaminophen, propoxyphene, etc.)

2 = Tranquillisers, antidepressants, muscle relaxants, and steroids

3 = Mild narcotics (oxycodone, meperidine, codeine, etc.)

4 = Strong narcotics (morphine, hydromorphone, etc.)

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12.7 APPENDIX 7. TUMOR RESPONSE EVALUATION CRITERIA

Definitions of measurable, non-measurable, target and non-target lesions and objective response criteria based on the new (January 2009) response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1).

12.7.1 Definition of Measurable and Non-Measurable Lesions

At baseline, tumor lesions/lymph nodes will be categorized measurable or non-measurable as follows:

Measurable

*Tumor lesions*: Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:
- 10 mm by CT scan (CT scan slice thickness no greater than 5 mm).
- 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable).
- 20 mm by chest X-ray.

*Malignant lymph nodes*: To be considered pathologically enlarged and measurable, a lymph node must be 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable

All other lesions, including small lesions (longest diameter <10 mm or pathological lymph nodes with 10 to <15 mm short axis) as well as truly non-measurable lesions.

Lesions considered truly non-measurable include:
- *leptomeningial disease*
- *ascites*
- *pleural or pericardial effusion*
- *inflammatory breast disease*
- *lymphangitic involvement of skin or lung*
- *abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.*

Special considerations regarding lesion measurability

Bone lesions, cystic lesions, and lesions previously treated with local therapy require particular comment:

Bone lesions:
- Bone scan, PET scan or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.
- Lytic bone lesions or mixed lytic-blastic lesions, with *identifiable soft tissue components*, that can be evaluated by cross sectional imaging techniques such as CT or MRI can be
considered as measurable lesions if the *soft tissue component* meets the definition of measurability described above.

- Blastic bone lesions are non-measurable.

**Cystic lesions:**

- Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.
- Cystic lesions thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

**Lesions with prior local treatment:**

- Tumor lesions situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion. Study protocols should detail the conditions under which such lesions would be considered measurable.

### 12.7.2 Specifications by methods of measurements

**Measurement of lesions**

All measurements should be recorded in metric notation, using calipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

**Method of assessment**

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging based evaluation should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

**Clinical lesions:** Clinical lesions will only be considered measurable when they are superficial and 10 mm diameter as assessed using calipers (e.g. skin nodules). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is suggested. As noted above, when lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study.

**Chest X-ray:** Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.

**CT, MRI:** CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. When CT scans have
slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Cytology, histology: These techniques can be used to differentiate between PR and CR in rare cases if required by protocol (for example, residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain). When effusions are known to be a potential adverse effect of treatment (e.g. with certain taxane compounds or angiogenesis inhibitors), the cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment can be considered if the measurable tumor has met criteria for response or stable disease in order to differentiate between response (or stable disease) and progressive disease.

12.7.3 Tumor response evaluation

Assessment of overall tumor burden and measurable disease
To assess objective response or future progression, it is necessary to estimate the overall tumor burden at baseline and use this as a comparator for subsequent measurements. Only patients with measurable disease at baseline should be included in protocols where objective tumor response is the primary endpoint. Measurable disease is defined by the presence of at least one measurable lesion (as detailed above in Section 12.7.1). In studies where the primary endpoint is tumor progression (either time to progression or proportion with progression at a fixed date), the protocol must specify if entry is restricted to those with measurable disease or whether patients having non-measurable disease only are also eligible.

Baseline documentation of ‘target’ and ‘non-target’ lesions
When more than one measurable lesion is present at baseline all lesions up to a maximum of five lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline (this means in instances where patients have only one or two organ sites involved a maximum of two and four lesions respectively will be recorded). Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected.

Lymph nodes merit special mention since they are normal anatomical structures which may be visible by imaging even if not involved by tumor. As noted in Section 12.7.1, pathological nodes which are defined as measurable and may be identified as target lesions must meet the criterion of a short axis of 15 mm by CT scan. Only the short axis of these nodes will contribute to the baseline sum. The short axis of the node is the diameter normally used by radiologists to judge if a node is involved by solid tumor. Nodal size is normally reported as two dimensions in the plane in which the image is obtained (for CT scan this is almost always the axial plane; for MRI the plane of acquisition may be axial, sagittal or coronal). The smaller of these measures is the short axis. For example, an abdominal node which is reported
as being 20 mm \times 30 \text{ mm} has a short axis of 20 mm and qualifies as a malignant, measurable node. In this example, 20 mm should be recorded as the node measurement. All other pathological nodes (those with short axis 10 mm but <15 mm) should be considered non-target lesions. Nodes that have a short axis <10 mm are considered non-pathological and should not be recorded or followed.

A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then as noted above, only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

All other lesions (or sites of disease) including pathological lymph nodes should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as ‘present’, ‘absent’, or in rare cases ‘unequivocal progression’. In addition, it is possible to record multiple non-target lesions involving the same organ as a single item on the case record form (e.g. ‘multiple enlarged pelvic lymph nodes’ or ‘multiple liver metastases’).

12.7.4 Response criteria

This section provides the definitions of the criteria used to determine objective tumor response for target lesions.

**Evaluation of target lesions**

**Complete Response (CR):**
Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

**Partial Response (PR):**
At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.

**Progressive Disease (PD):**
At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).

**Stable Disease (SD):**
Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

**Special notes on the assessment of target lesions**

**Lymph nodes**
Lymph nodes identified as target lesions should always have the actual short axis measurement recorded (measured in the same anatomical plane as the baseline examination), even if the nodes regress to below 10 mm on study. This means that when lymph nodes are included as target lesions, the ‘sum’ of lesions may not be zero even if complete response criteria are met, since a normal lymph node is defined as having a short axis of <10 mm. Case
report forms or other data collection methods may therefore be designed to have target nodal lesions recorded in a separate section where, in order to qualify for CR, each node must achieve a short axis <10 mm. For PR, SD and PD, the actual short axis measurement of the nodes is to be included in the sum of target lesions.

**Target lesions that become ‘too small to measure’**

While on study, all lesions (nodal and non-nodal) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (e.g. 2 mm). However, sometimes lesions or lymph nodes which are recorded as target lesions at baseline become so faint on CT scan that the radiologist may not feel comfortable assigning an exact measure and may report them as being ‘too small to measure’. When this occurs it is important that a value be recorded on the case report form. If it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0 mm. If the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned *(Note: It is less likely that this rule will be used for lymph nodes since they usually have a definable size when normal and are frequently surrounded by fat such as in the retroperitoneum; however, if a lymph node is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned in this circumstance as well). This default value is derived from the 5 mm CT slice thickness (but should not be changed with varying CT slice thickness). The measurement of these lesions is potentially non-reproducible, therefore providing this default value will prevent false responses or progressions based upon measurement error. To reiterate, however, if the radiologist is able to provide an actual measure, that should be recorded, even if it is below 5 mm.

**Lesions that split or coalesce on treatment**

When non-nodal lesions ‘fragment’, the longest diameters of the fragmented portions should be added together to calculate the target lesion sum. Similarly, as lesions coalesce, a plane between them may be maintained that would aid in obtaining maximal diameter measurements of each individual lesion. If the lesions have truly coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximal longest diameter for the ‘coalesced lesion’.

**Evaluation of non-target lesions**

This section provides the definitions of the criteria used to determine the tumor response for the group of non-target lesions. While some non-target lesions may actually be measurable, they need not be measured and instead should be assessed only qualitatively at the time points specified in the protocol.

**Complete Response (CR):**
- Disappearance of all non-target lesions and normalization of tumor marker level.
- All lymph nodes must be non-pathological in size (<10 mm short axis).

**Non-CR/Non-PD:**
- Persistence of one or more non-target lesion(s) and/or maintenance of tumour marker level above the normal limits.

**Progressive Disease (PD):**
- *Unequivocal progression* (see comments below) of existing non-target lesions. *(Note: the appearance of one or more new lesions is also considered progression).*
Special notes on assessment of progression of non-target disease

The concept of progression of non-target disease requires additional explanation as follows:

When the patient also has measurable disease

In this setting, to achieve ‘unequivocal progression’ on the basis of the non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest ‘increase’ in the size of one or more non-target lesions is usually not sufficient to qualify for unequivocal progression status. The designation of overall progression solely on the basis of change in non-target disease in the face of SD or PR of target disease will therefore be extremely rare.

New lesions

The appearance of new malignant lesions denotes disease progression; therefore, some comments on detection of new lesions are important. There are no specific criteria for the identification of new radiographic lesions; however, the finding of a new lesion should be unequivocal: i.e. not attributable to differences in scanning technique, change in imaging modality or findings thought to represent something other than tumor (for example, some ‘new’ bone lesions may be simply healing or flare of pre-existing lesions). This is particularly important when the patient’s baseline lesions show partial or complete response. For example, necrosis of a liver lesion may be reported on a CT scan report as a ‘new’ cystic lesion, which it is not.

A lesion identified on a follow-up study in an anatomical location that was not scanned at baseline is considered a new lesion and will indicate disease progression. An example of this is the patient who has visceral disease at baseline and while on study has a CT or MRI brain ordered which reveals metastases. The patient’s brain metastases are considered to be evidence of PD even if he/she did not have brain imaging at baseline.

If a new lesion is equivocal, for example because of its small size, continued therapy and follow-up evaluation will clarify if it represents truly new disease. If repeat scans confirm there is definitely a new lesion, then progression should be declared using the date of the initial scan.

While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible ‘new’ disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a) Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.

b) No FDG-PET at baseline and a positive FDG-PET at follow-up:

- If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD.
- If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan).
- If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
### 12.8 APPENDIX 8. TUMOR METABOLIC RESPONSE EVALUATION CRITERIA

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PERCIST 1.0</th>
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</table>
| **Measurability of lesions at baseline** | 1. Measurable target lesion is hottest single tumor lesion SUL of ‘‘maximal 1.2-cm diameter volume ROI in tumor’’ (SUL peak). SUL peak is at least 1.5-fold greater than liver SUL mean + 2 SDs (in 3-cm spherical ROI in normal right lobe of liver). If liver is abnormal, primary tumor should have uptake > 2.0 x SUL mean of blood pool in 1-cm-diameter ROI in descending thoracic aorta extended over 2-cm z-axis.  
2. Tumor with maximal SUL peak is assessed after treatment. Although typically this is in same region of tumor as that with highest SUL peak at baseline, it need not be.  
3. Uptake measurements should be made for peak and maximal single-voxel tumor SUL. Other SUV metrics, including SUL mean at 50% or 70% of SUV peak, can be collected as exploratory data; TLG can be collected ideally on basis of voxels more intense than 2 SDs above liver mean SUL (see below).  
4. These parameters can be recorded as exploratory data on up to 5 measurable target lesions, typically the 5 hottest lesions, which are typically the largest, and no more than 2 per organ. Tumor size of these lesions can be determined per RECIST 1.1. |
| **Normalization of uptake** | Normal liver SUL must be within 20% (and <0.3 SUL mean units) for baseline and follow-up study to be assessable. If liver is abnormal, blood-pool SUL must be within 20% (and <0.3 SUL mean units) for baseline and follow-up study to be assessable. Uptake time of baseline study and follow-up study 2 must be within 15 min of each other to be assessable. Typically, these are at mean of 60 min after injection but no less than 50 min after injection. Same scanner, or same scanner model at same site, injected dose, acquisition protocol (2- vs. 3-dimensional), and software for reconstruction, should be used. Scanners should provide reproducible data and be properly calibrated. |
| **Objective response** | **CMR:** complete resolution of 18F-FDG uptake within measurable target lesion so that it is less than mean liver activity and indistinguishable from surrounding background blood-pool levels. Disappearance of all other lesions to background bloodpool levels. Percentage decline in SUL should be recorded from measurable region, as well as (ideally) time in weeks after treatment was begun (i.e., CMR –90, 4). No new 18F-FDG–avid lesions in pattern typical of cancer. If progression by RECIST, must verify with follow-up.  
**PMR:** reduction of minimum of 30% in target measurable tumor 18F-FDG SUL peak. Absolute drop in SUL must be at least 0.8 SUL units, as well. Measurement is commonly in same lesion as baseline but can be another lesion if that lesion was previously present and is the most active lesion after treatment. ROI does not have to be in precisely same area as baseline scan, though typically it is. No increase, >30% in SUL or size of target or nontarget lesions (i.e., no PD by RECIST or IWC) (if PD anatomically, must verify with follow-up). Reduction in extent of tumor 18F-FDG uptake is not requirement for PMR. Percentage decline in SUL should be recorded, as well as (ideally) time in weeks after treatment was begun (i.e., PMR –40, 3). No new lesions.  
**SMD:** not CMR, PMR, or PMD. SUL peak in metabolic target lesion should be recorded, as well as (ideally) time from start of most recent therapy, in weeks (i.e., SMD –15, 7).  
**PMD:** 30% increase in 18F-FDG SUL peak, with >0.8 SUL unit increase in tumor SUV peak from baseline scan in pattern typical of tumor and not of infection/treatment effect. OR: Visible increase in extent of 18F-FDG tumor uptake (75% in TLG volume with no decline in SUL. OR: New 18F-FDG–avid lesions that are typical of cancer and not related to treatment effect or infection. PMD other than new visceral lesions should be confirmed on follow-up study within 1 mo unless PMD also is clearly associated with progressive disease by RECIST 1.1. PMD should be reported to include percentage change in SUV peak, (ideally, time after treatment, in weeks) and whether new |
lesions are present/absent and their number (i.e., PMD, +35, 4, new: 5). Because SUL is continuous variable, dividing response criteria into limited number of somewhat arbitrary response categories loses much data. For this reason, PERCIST preserves percentage declines in SUV peak in each reported category. Because rapidity with which scan normalizes is important (faster appears better), PERCIST asks for time from start of treatment as part of reporting. For example, CMR 90, 1, is probably superior to CMR 90, 10, especially if latter patient were SMD 20, 1. More than one measurement of PET response may be needed at differing times, and it may be treatment type–dependent. PERCIST 1.0 evaluates SUV peak of only hottest tumor. This is possible limitation of approach, but lesions and their responses are highly correlated in general. Additional data are required to determine how many lesions should be assessed over 1. A suggested option is to include the 5 hottest lesions, or the 5 observed on RECIST 1.1 that are most measurable. Percentage change in SUV can be reported for single lesion with largest increase in uptake or smallest decline in uptake. Additional studies will be needed to define how many lesions are optimal for assessment.

**Nontarget lesions:** CMR, disappearance of all 18F-FDG–avid lesions; PMD, unequivocal progression of 18F-FDG–avid nontarget lesions or appearance of new 18F-FDG–avid lesions typical of cancer; non-PMD: persistence of one or more nontarget lesions or tumor markers above normal limits.

**Overall response**

1. Best response recorded in measurable disease from treatment start to disease progression or recurrence.
2. Non-PMD in measurable or nonmeasurable nontarget lesions will reduce CR in target lesion to overall PR.
3. Non-PMD in nontarget lesions will not reduce PR in target lesions.

**Duration of response**

1. Overall CMR: from date CMR criteria are first met; to date recurrent disease is first noted.
2. Overall response: from date CMR or PMR criteria are first met (whichever status came first); to date recurrent disease is first noted.
3. SMD: from date of treatment start to date PMD is first noted.

TLG = total lesion glycolysis; CMR = complete metabolic response; PMR = partial metabolic response; PD = progressive disease; SMD = stable metabolic disease; PMD = progressive metabolic disease; CR = complete remission; PR = partial remission; NC = no change; ROI = Region Of Interest; 18F-FDG PET = F-18 fluoro-2-deoxy-D-glucose positron emission tomography.

SUV can be normalized to body mass, lean body mass (SUL), or body surface area. Body surface area and SUL are less dependent on body habitus across populations than is SUV based on total body mass.

For PERCIST: Single-voxel SUL is commonly used but has been reported to be less reproducible than SUV peak, especially with very small single-voxel values. It is suggested, but not required, that lesions assessed on PERCIST be larger than the 1.5-cm-diameter volume ROI used to minimize partial-volume effects. Percentage changes are proposed to deal with SUV peak changes. Use of maximal SUV could be explored. If 5 lesions are used as exploratory approach, it is suggested that sum of SUVs of baseline 5 lesions serve as baseline for study. After treatment, sum of same 5 lesions should be used. Percentage change in SUV is based on change in these sums from study 1 to study 2. Exploratory analysis can include calculating percentage change in SUV in individual lesions and averaging them. This may produce different result. We believe summed SUV approach will be less prone to minor errors in measurements.

For total lesion glycolysis: Exploratory analysis can include either all foci of tumor with maximal SUL > 2 SDs above normal liver, 5 lesions with highest SUV, or lesion with highest SUV. It is suggested that threshold approach, typically at 2 SDs above normal liver SUV, be used to generate lower bounds of ROI (3 SDs could be used for very active tumors). We believe this approach will be less variable than methods based on maximal SUV with percentage of maximal cutoff. Criteria for progression include 75% growth in TLG for SUV and are conservatively placed at 75% increase. Progression is judged from best response if being assessed after first scan was performed. For response by TLG, we propose 45% reduction as useful starting point, but more data are needed to make firm recommendations. If TLG is determined, explicit methodologic details should be provided. It should not be a primary metric, but a secondary endpoint at this time.