PRODIGE 16 (FFCD 0905)

Randomized phase II/III trial of Transcatheter Arterial Chemoembolization plus Sunitinib or placebo in patients with hepatocellular carcinoma (SATURNE study)

A study of the PRODIGE-AFEF Group (FFCD-FNCLCC-AFEF)

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PROMOTION AND RANDOMISATION

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Rational	**1.** Transcatheter arterial chemoembolization (TACE) is widely used for selected patients with isolated hepatocellular carcinoma (HCC) **2.** Most of the patients undergoing TACE will relapse within two years **3.** HCC is one of the most vascularized tumors, with a crucial role for Vascular Endothelial Growth factor (VEGF) **4.** Angiogenesis inhibition is theoretically a logical approach to limit the risk of tumor relapse after TACE. **5.** Sunitinib malate is a novel angiogenesis inhibitor directed against VEGF receptors, with additional direct anti-tumor effect (by inhibiting PDGFR) **6.** Addition of sunitinib malate to TACE could reduce the risk of relapse
Objectives (Pilot phase)	Inacceptable bleeding or hepatic failure 10 weeks after last administration of the TACE-sunitinib combination
Objectives (phase II)	Phase II : Primary endpoint : Inacceptable bleeding or hepatic failure 10 weeks after last administration of the TACE-sunitinib combination Secondary end-points Tumor stabilisation rate Relapse-free survival Overall survival Safety Quality of life
Objectives (phase III)	Primary end-point Overall survival Secondary end-points Overall survival rate at 2 years Relapse-free survival Disease-free survival Tumor stabilisation rate Safety Quality of life
Design	Multicentre, prospective randomized, double-blind, placebo-controlled, phase II- III study
Treatments	**Arm A** Sunitinib 37.5 mg/day (3 tablets of 12.5 mg) orally on the 4/2 schedule (4 weeks on treatment followed by 2 weeks off treatment) during one year **Arm B** Placebo 3 tablets/day for 4 weeks over 6 weeks during one year **Treatment started 7 to 10 day before each TACE course for a total of 28 days per cycle, with a 3 days interruption (day preceding to day following TACE)**
Patients' nb	Non-randomized pilot phase: 10 chemoembolization sessions, regardless of the number of patients Phase II step: 70 patients (35 in each arm), Phase III step: 190 (95 in each arm), including the patients of the phase II step
Inclusion criteria	• Histologically proven HCC or liver tumor responding to the Barcelona criteria (18) • Child-Pugh score 5-6 (class A) • No portal vein thrombosis • Tumor suitable for TACE (one or more than one planned TACE courses allowed) • A prior radiofrequency ablation is allowed if the interval between radiofrequency and planned TACE is $\geq$ 3 months • Tumor not suitable for surgical resection • ECOG performance status $\leq$ 2 • Age $\geq$ 18 years • Adequate hematologic, renal and hepatic functions (neutrophil count $\geq$ 1.5 10^9/L, platelet count $\geq$ 70 x 10^9/L, haemoglobin level $\geq$ 10 g/dL, prothrombin activity $\geq$ 50 %, creatinine $\leq$ 120 µmol/L, normal bilirubin level ($\leq$ 15 mg/L), alanine and aspartate transaminases (ALT and AST) $\leq$ 4 times the upper limit of normal (ULN), alkaline phosphatases $\leq$ 5 times the ULN, fibrinogen level $\geq$ 1.5 g/L) • Ability for patient to comply with scheduled follow-up and management of toxicity • Written informed consent

Non inclusion criteria	• History of chemoembolization • Portal thrombosis • Extrahepatic metastases including brain metastases • Concomitant participation of the patient in another clinical trial • Uncontrolled hypertension or requiring at least 2 classes of antihypertensive agents • Concomitant illness or uncontrolled severe clinical situation • Patient treated with a CYP3A4 inhibitor within 7 days prior to treatment • Patient treated with a CYP3A4 potentiator within 12 days • Patient requiring long-term anticoagulant therapy • Patient with a contraindication to vascular exclusion procedures • Pregnancy or breastfeeding • Lack of effective contraception (for men or women of childbearing age) • Pretreatment with sunitinib, sorafenib or any other angiogenesis inhibitor • History of other cancers excluding cancers known to have been cured for more than 5 years (in this case, histological evidence for HCC is required), or basocellular skin tumors or cervical cancer in situ treated adequately and with curative intent • Patient who for psychological, social, family or geographic reasons could not be followed regularly • Patient with a contraindication to vascular occlusion procedures
Stratifications	• Main tumour diameter < *vs.* ≥ 5 cm • Uninodular vs. multinodular • Centre
Duration	Accrual duration : 24 months ; overall study duration : 60 months

I. RATIONALE

Transcatheter arterial chemoembolization (TACE) is widely used for selected patients with isolated or limited hepatocellular carcinoma (HCC) not suitable for surgical resection. Recent randomized trials have demonstrated a survival benefit for patients treated with TACE, by comparison to symptomatic treatment (1-4). TACE is justified by the fact that HCC is one of the most vascularized tumors. Advances in the understanding of the mechanisms underlying HCC development confirm that angiogenesis plays a key role. After TACE, most of the patients will relapse within two years, and this relapse is associated with a tumor re-vascularisation. Especially, Vascular Endothelial Growth Factor (VEGF) has emerged as a prominent factor (5). In patients undergoing TACE, high pre-treatment serum VEGF concentrations are associated with a shorter survival (6, 7). Moreover, VEGF concentrations rise after TACE, probably as a consequence of tumor ischemia, and reach a peak after 1 day (8). In a murine model, VEGF has been demonstrated to play a crucial role in establishment of collateral circulation and reconstruction of blood supply of residual cancer tissue after TACE (9). In human, increase of VEGF levels during the month following TACE was associated with a higher risk of metastatic relapse (10). Altogether, these data suggest that neoadjuvant plus adjuvant therapy by angiogenesis inhibitors could represent a logical way to reduce the risk of relapse after TACE.

Sunitinib malate (SUTENT) is a novel oral multitargeted receptor tyrosine kinase inhibitor that has shown antiangiogenic and antitumor activities. Antiangiogenic properties of sunitinib are based on its action on VEGFR-1, VEGFR-2 and VEGFR-3 (11). Additionally, sunitinib inhibits PDGFR, which is also involved in HCC growth (11-12).

Based on these properties, we postulate that sunitinib might yield clinical benefit when administered before and after TACE in patients with HCC. Sunitinib might interfere with tumor re-vascularisation as well as tumor growth, and reduce the risk of relapse. Additionally, a recent experimental study performed on a glioma model, showed that sunitinib, by improving tumoral hemodynamics and blood flow, increases the delivery of doxorubicin to tumors (13).

Some angiogenesis inhibitors are associated with a significant risk of bleeding, and this may raise concern regarding their use in patients with HCC developed on cirrhosis, since cirrhosis is frequently complicated by portal hypertension (14). In a retrospective study was assessed the risk of complications in 66 patients receiving sunitinib (n=21) or imatinib (n=45)

Gastrointestinal Stromal Tumors, and who underwent a surgical debulking (15). Sunitinib was stopped a median of 5 days prior to surgery (range 0-26). Only 2 patients experienced postoperative bleeding requiring reoperation, but it was not mentioned if these patients were receiving sunitinib or imatinib. In patients with advanced HCC and cirrhosis, phase II studies have demonstrated a good tolerability of bevacizumab, an anti-VEGF monoclonal antibody (16, 17). This was confirmed by another phase II study assessing the combination gemcitabine-oxaliplatin-bevacizumab (18). In a pilot study performed in patients undergoing TACE, adjuvant treatment with bevacizumab was well tolerated and seemed to increase the disease control duration (19). More recently, were reported the results of two phase II studies assessing safety and efficacy of sunitinib in patients with advanced HCC. In the study by Faivre et al, sunitinib was administered at a dose of 50 mg/day for 4 weeks every 6 weeks in 37 patients with advanced HCC with Child-Pugh A or B cirrhosis (20). Patients received a median of two cycles. According to the RECIST criteria, only one objective response was observed, but interestingly, a decrease of tumor density was observed in 68% of the patients. Moreover, tumor activity, as assessed by volumetric measurement of decrease in tumor enhancement percent, showed minor (< 50%) and major (≥ 50%) post-treatment tumor necrosis in 25 % and 46 % of the patients, respectively. Grade 3–4 toxicities included thrombocytopenia (43 %), neutropenia (24 %), central nervous system symptoms (24 %), asthenia (22 %) and haemorrhage (14 %). A dose reduction was required in 27 % of the patients. This safety profile suggested that patients should be better selected and sunitinib dose revised. In the study by Zhu et al, 26 patients with advanced HCC and ≤ 3 Carcinoma of the Liver Italian Program (CLIP) score received sunitinib at a lower dose (37.5 mg/day for 4 weeks every 6 weeks) (21). Only one objective response has been observed but the median progression-free and overall survivals were 4.1 and 11.6 months, respectively. Moreover, the mean tumor permeability, assessed by dynamic contrast-enhanced magnetic resonance imaging, decreased from a mean average of 38 %. The treatment was generally well tolerated. Grade 3/4 toxicities included neutropenia (12 %), lymphopenia (15 %), SGOT/SGPT (23/12 %), fatigue (8 %), rash (8 %), and thrombocytopenia (12 %).

Since TACE is restricted to patients without portal vein thrombosis, and without severe cirrhosis, sunitinib therapy should be even safer. Peri-TACE setting could so represent one of the most appropriate situations to assess efficacy and safety of sunitinib in patients with HCC. We have chosen to use sunitinib at a dose of 37.5 mg/day for 4 weeks every 6

weeks. Treatment will be started 7 to 10 days before the first TACE course, for a total duration of one year.

II. STUDY DESIGN

- Prospective, multicentre, randomized, double-blind, placebo-controlled, parallel-group, phase II-phase III clinical trial
- Accrual period : 24 months
- Study duration : 48 months

III. PROMOTION

The study will be run in the PRODIGE-AFEF cooperative group and sponsored by the ‘‘Fédération Francophone de Cancérologie Digestive’’ (FFCD).

IV. END-POINTS

PILOT PHASE STUDY

- End-point : Inacceptable bleeding or hepatic failure 10 weeks after last administration of the TACE-sunitinib combination

INITIAL PHASE II STUDY

- Primary end-point:

  Inacceptable bleeding or liver failure 10 weeks after last administration of the TACE-sunitinib combination

- Secondary end-points:

  Tumor stabilisation rate

  Relapse-free survival

  Overall survival

  Safety

  Quality of life

PHASE III STUDY

- Primary end-point:

  Overall survival

- Secondary end-points:

  Overall survival rate at 2 years

Relapse-free survival

Disease-free survival

Tumor stabilisation rate (stable size of the lipiodol deposition zones by two consecutive CT scans or prolonged normalisation of serum Alpha-Foetoprotein (AFP)

Safety

Quality of life

V. INCLUSION CRITERIA

- Age 18 to 75 years
- ECOG performance status ≤ 2
- Histologically proven HCC or liver tumor responding to the Barcelona criteria (22)
- Child-Pugh score 5-6 (class A)
- No portal vein thrombosis
- Tumor not suitable for surgical resection
- Tumor suitable for TACE (one to three courses allowed)
- A prior radiofrequency ablation is allowed if the interval between radiofrequency and planned TACE is ≥ 3 months
- Adequate haematological, renal and hepatic functions (neutrophil count ≥ 1.5 10^9/L, platelet count $\geq 100 \times 10^9$/L, haemoglobin level ≥ 10 g/dL, prothrombin activity ≥ 50 %, creatinine ≤ 120 µmol/L, normal bilirubin level (≤ 12 mg/L), alanine and aspartate transaminases (ALT and AST) ≤ 3.5 times the upper limit of normal (ULN), alkaline phosphatases ≤ 4 times the ULN, fibrinogen level ≥ 1.5 g/L)
- Ability for patient to comply with scheduled follow-up and management of toxicity
- Written informed consent

VI. NON-INCLUSION CRITERIA

- History of chemoembolization
- Portal thrombosis

- Extrahepatic metastases including brain metastases
- Concomitant participation of the patient in another clinical trial
- Uncontrolled hypertension or requiring at least 2 classes of antihypertensive agents
- Concomitant illness or uncontrolled severe clinical situation
- Patient treated with a CYP3A4 inhibitor within 7 days prior to treatment
- Patient treated with a CYP3A4 potentiator within 12 days
- Patient requiring long-term anticoagulant therapy
- Patient with a contraindication to vascular exclusion procedures
- Pregnancy or breastfeeding
- Lack of effective contraception (for men or women of childbearing age)
- Pretreatment with sunitinib, sorafenib or any other angiogenesis inhibitor
- History of other cancers excluding cancers known to have been cured for more than 5 years (in this case, histological evidence for HCC is required), or basocellular skin tumors or cervical cancer in situ treated adequately and with curative intent
- Patient who for psychological, social, family or geographic reasons could not be followed regularly
- Patient with a contraindication to vascular occlusion procedures

VII. RANDOMIZATION

After checking the eligibility criteria, randomisation will be performed at the Fédération Francophone de Cancérologie Digestive (FFCD) data centre in Dijon. A minimization technique will be used.

VIII. STRATIFICATIONS

- Main tumour diameter $< vs. \geq 5$ cm
- Uninodular vs. multinodular
- Centre

IX. STUDY TREATMENTS

- Treatment arm : Chemoembolization (1 to 3 sessions) + sunitinib (SUTENT®) 37.5 mg / day (3 cps 12.5 mg) orally 4 weeks out of 6 (4 weeks of treatment then 2 weeks of rest) during 1 year

- Control arm : Chemoembolization (1 to 3 sessions) + placebo 3cps / d4 weeks out of 6 for 1 year

Treatment will be started 7 to 10 days before each TACE course for a total of 28 days per cycle with a three days interruption: from the day preceding to the day following each TACE. Total treatment duration will be one year (8 cycles). Treatment will be discontinuated in case of documented tumor relapse or progression.
Study drugs will be given in the morning with water and without regard to meals beginning.

TACE modalities

The use of Doxorubicin Coated Beads (DC Beads) has been retained on the basis of the PRECISION V study. This randomised study compared conventional TACE with doxorubicin to TACE with DC Beads (23). Although the overall tumor response rate achieved by DC Beads was not significant superior ($p = 0.11$), it was significantly higher in some subgroups of patients (especially ECOG 1 patients and patients with bilobar disease). Additionally, the use of DC Beads appeared safer, especially with significantly reduced liver toxicity (3 vs. 9 %). Finally, a theoretical advantage for using DC Beads is that this method is standardized and more reproducible than conventional TACE.
The investigators must perform pre-treatment angiography to confirm the vascular anatomy and exclude significant arteriovenous shunting and are encouraged to identify and protect the cystic artery and gastric arteries.
TACE in HCC using DC beads should respect the following guidelines: 1 vial 300-500 micron followed by 1 vial 500-700 micron with a target dose of 150mg doxorubicin.

Beads are mixed with non-ionic contrast media or saline at a ratio of one to 5 to ensure smooth delivery through the catheter. The beads should be delivered slowly, approximately 1mL per minute, to minimise reflux and clogging within the catheter. A microcatheter is

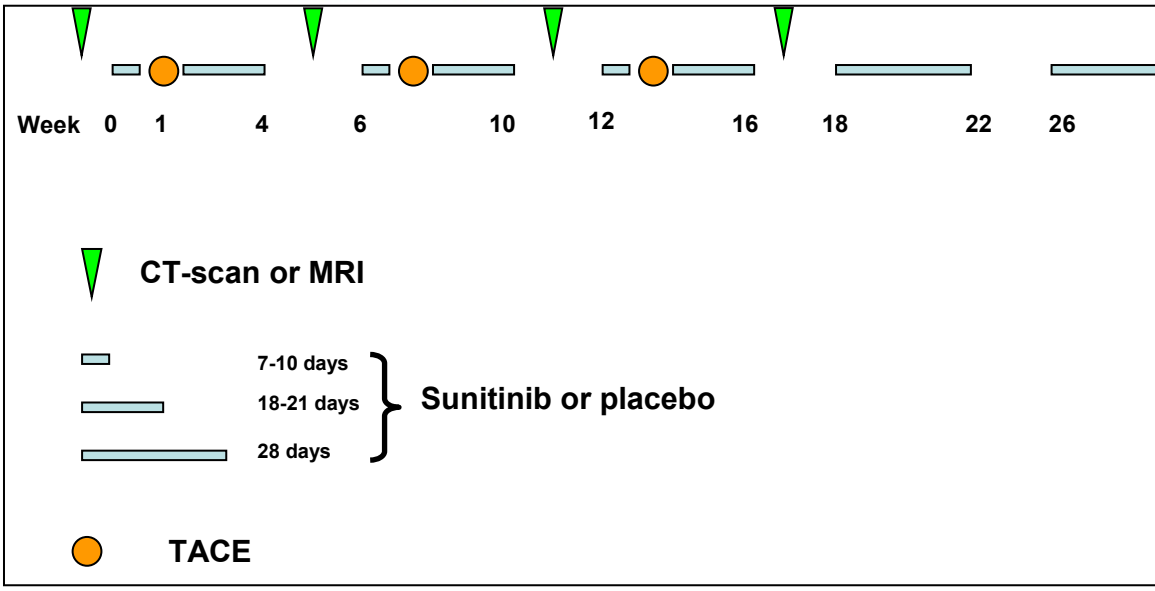
recommended to obtain as superselective position as possible, namely in case of patients with finite number of tumors.

Patients with finite number of tumors should be treated in order to deliver the full dose of doxorubicin to the target lesion if single, or to divide the dose between them, if several. Treatment must be repeated with the same schedule at least twice. The use of a microcatheter to obtain selective catheterization of the feeding artery(ies) is recommended. No additional embolization is recommended after the use of DC beads in order to leave the artery patent for a second course.

Patients with diffuse bilobar disease must be treated for each lobe in separate treatments. In such setting, a catheter is placed in one lobar hepatic artery and the full dose of treatment is injected. Second course will target the other lobe. Embolization to nearly complete stasis in the 2nd to 3rd order branches must be obtained with additional embolic material if needed.

The embolization technique should always include adequate pain management as well as prophylactic antibiotics and steroids at the physician's discretion.

Repetition of TACE courses Three courses are scheduled 6 to 8 weeks apart when patient is enrolled in the study.
The choice of performing imaging between the two initial treatments is left on investigator preference
Additional treatment after the two initial ones will be done according to tumor response and treatment schedule provide in the study. No further treatment is recommended if complete response according to EASL criteria is obtained or if a tumor progression exists on tumors already targeted with TACE.



X. DOSES ADJUSTMENTS

The main expected toxicities are:

- Fatigue
- Diarrhoea
- Anorexia
- Vomiting
- Hand-foot syndrome
- Skin rash
- Mucosal inflammation
- Hypertension
- Neutropenia
- Thrombopenia
- Anaemia
- Hypothyroidism
-

<u>In case of any toxicity, the following guidelines will be used</u>

Toxicity grade (NCI-CTC)

1	no dose modification
2	discontinuation of treatment for 1 week - recovery : no dose modification - non-recovery, 1 additional week rest - recovery : 2 tablets per day (25 mg) - non-recovery : withdraw of study
3	discontinuation of treatment for 1 week - recovery : 2 tablets per day (25 mg) - non-recovery : withdraw of study
4	withdraw of study

In case of lymphopenia (of any grade), treatment can be continuated without dose modification.

Particular case of sunitinib-induced hypothyroidism: results from the largest clinical series indicate that 53% to 85% of patients treated with sunitinib develop thyroid test abnormalities, and that 30% will develop clinical manifestations of thyroid dysfunction (24). In other hand, this hypothyroidism seems to be associated with a higher efficacy of sunitinib, relationship observed in patients treated for a renal cancer. In this trial, it is recommended to test TSH at baseline, at day 1 (+/- 7) of cycles 2, 3, 4, 6, 8, and 3 months after sunitinib or placebo retrieval:

- if TSH > ULN but < 10 mIU/L: replacement hormone therapy only in case of symptoms of hypothyroidism
- TSH > 10 mIU/L: hormone replacement therapy aiming at normalization of THS

In case of biological or clinical hypothyroidism, the patient must be addressed to an endocrinologist.

XI. FOLLOW-UP

- AFP concentration measurement before entry, every month until one month after the last TACE course, every 3 months for 2 years and every 6 months thereafter. TSH measurement at baseline, and every 3 months subsequently. Plasma samples will be simultaneously collected and stored for measurement of circulating soluble angiogenic factors.

- Liver CT-scan and/or MRI before entry, one month after each TACE course, every 3 months for 2 years and every 6 months thereafter, until tumor progression. All CT-scan and MRI will be reviewed by an external independent radiologist panel.
- Additionally, a clinical examination, measurement of haematological, renal and hepatic parameters will be performed before initiation of each sunitinib or placebo course (every 6 weeks for one year), then every 3 month the second year and every 6 months thereafter.
- **Haematological, renal and hepatic parameters will be also measured at each TACE course: d-1 or d0, d1, d2, d3.**

XII. RANDOMIZED PILOT AND PHASE II STEP

The aim of this initial study is specially to assess safety of the combination TACE-sunitinib. Independently of the respective common side effects of TACE and sunitinib, the phase II study will focus on the acute (1 week after last TACE and sunitinib combination administration) potent complications related to the combination of both treatments. Patients will be specially monitored for the detection of severe **bleeding** or **liver failure**.

A severe bleeding is defined by

Any bleeding (inguinal, tumoral, gastro-intestinal…) following TACE and requiring a local treatment (other than inguinal compression)

Any bleeding requiring a systemic treatment (e.g. blood transfusion)

An inguinal bleeding during more than 24 hours.

A severe liver failure is defined by the occurrence of any of the following complications:

Encephalopathy

Ascitis

Increase of bilirubin level $\geq$ 10mg/L

Decrease of Prothrombin Time $\geq$ 50 %

Increase of ALT and/or ALT $\geq$ 6 x baseline value

During this phase II step, patients will be hospitalized up to 5 days after each TACE course for clinical and biological monitoring. The biological monitoring will consist in the daily measurement of haematological, liver and kidney functions.

XIV. TRANSLATIONAL STUDY

- Plasma samples will be collected and stored for measurement of circulating soluble angiogenic factors during the first TACE course: d-1 or d0, d1, d2, d3.
- After specific written informed consent, a blood sample will be collected on EDTA for DNA extraction and assessment of polymorphisms within genes implicated in the angiogenesis pathway (e.g. VEGF receptors). It is recommended to collect this blood sample at entry in the study, but this can be performed thereafter.

XV. DISCONTINUATION CRITERIA

Patients will be informed that they have the right to withdraw from the trial at any time, without prejudice to their medical care, and are not obliged to state their reasons. Any discontinuations must be fully documented in the CRF and should be followed up by the Investigator.

Additionally, the Investigator may withdraw a patient at any time if he/she considers this to be in the patient's best interest.

The treatment must be stopped for the following reasons:

- Documented tumor progression
- Initiation of other anti-tumor treatment,
- Unacceptable toxicity (in the Investigator's opinion)
- Non-compliance with the treatment schedule, defined as the patient missing: two successive cycles or three cycles during the entire treatment phase. Yet, the patient should be followed and will be included in the intent-to-treat analysis.
- Changes in medical status of the patient such that the investigator believes that patient safety will be compromised (e.g. worsening of cirrhosis).

Additionally, patients may be discontinuated for any of the following reasons:

- Protocol violations, including non-compliance with trial procedures, patient lost to follow-up and patient refusal
- Serious intercurrent illness or significant worsening of intercurrent illness
- Adverse events.

If a patient fails to return for a scheduled visit/follow up, attempts should be made to contact the patient to ensure that the reason for not returning is not an adverse event. Likewise if a patient declares his/her wish to discontinue from the trial e.g. for personal reasons, an attempt should be made to establish that the true reason is not an adverse event (bearing in mind the patient is not obliged to state his/her reason).

XVI. STATISTICAL METHOD

PILOT step

During this stage, patients will not be randomized and will all receive sunitinib. After a total of 10 chemoembolization sessions, inclusions will be discontinued and tolerance results will be reviewed by a committee of independent experts.

Patients included in this pilot phase will be particularly monitored for the purpose of detecting two potential complications associated with the combination of sunitinib-chemoembolization: severe bleeding and liver failure. The observed rate of bleeding or liver failure after chemoembolization is in the order of 10 to 15% in recent studies . One or two toxicities are expected among the 10 chemoembolization sessions. If 4 or more toxicities occur, then the toxicity of the sunitinib-chemoembolization combination is considered unacceptable and the trial is discontinued. If 3 or fewer toxicities occur, the toxicity is considered acceptable and the trial is continued in phase II with randomization. Patients included in the pilot phase will not be included in the final analysis of the SATURN trial.

The results of the pilot phase will be sent to AFFSAPS for information.

Phase II step

Patients included during this first step will be specially monitored for the detection of two complications: severe bleeding and liver failure (as defined above) in the sunitinib arm. The expected rate of severe haemorrhage and/or liver failure in patients undergoing TACE is approximately 10 % to 15%, as observed in recent studies with a TACE alone arm (25). We defined the unacceptable toxicity rate as 30 % in patients undergoing TACE plus sunitinib treatment.

Using Fleming one step design with the following hypotheses:

H0: An occurrence rate of bleeding or liver failure 1 week after last administration of the TACE-sunitinib combination of 30 % is unacceptable

H1: An occurrence rate of bleeding or liver failure 1 week after last administration of the TACE-sunitinib combination of 15% is expected

It would be required to include 35 patients in each arm.

Among the 35 patients in the Sunitinib arm:

If we observed 8 or more than 8 patients (22.9 %) with severe bleeding or liver failure 1 week after last administration of the TACE-sunitinib, the toxicity rate is not statistically different from 30%. This treatment would be declared unacceptable and inclusions will not be pursued for phase III trial.

If we observed 7 or less than 7 patients (20 %) with severe bleeding or liver failure 1 week after last administration of the TACE-sunitinib, the toxicity rate is statistically different from 30 %. This treatment would be declared promising and inclusion will be pursued for phase III,

Calculated power is 85.6% and alpha type one error is 13.3 %

Decision rules for phase III trial continuation:

Despite randomization and inclusion of 35 patient in the TACE alone arm we will not use Fleming decision rules for TACE alone arm to pursue randomization since this is the standard arm in the phase III trial.

1. We observed 7 or less than 7 patients (20%) with severe bleeding or liver failure 1 week after last administration of the TACE-sunitinib

We will pursue randomization whatever occurrence of these toxicities in the TACE alone arm since this is the standard arm in the phase III trial.

2. We observe 8 or more than 8 patients (22.9 %) with severe bleeding or liver failure 1 week after last administration of the TACE-sunitinib.

We will check amongst the 35 patients receiving TACE alone:

- If we observed also 8 or more than 8 patients (22.9 %) with severe bleeding or liver failure 1 week after last administration of the TACE alone. Then we will ask an independent data monitoring committee (IDMC) about the opportunity to pursue randomization for phase III trials since these results could reflect the “noise” of TACE toxicities alone.

- If we observed also 7 or less than 7 patients (20%) with severe bleeding or liver failure 1 week after last administration of the TACE alone. Then we will ask an independent data monitoring committee (IDMC) about the opportunity to stop randomization for phase III trials.

These 70 patients will be theoretically recruited in 9 months (7.5 pts/ month) and at least 1 week follow-up after last administration of the TACE-sunitinib combination is required then Phase II step will be analyses about 9 months and 1 week after the inclusion of the first patient.

Phase III step

As primary end-point of the phase III study, we have chosen the overall survival for the following reasons:

- The overall survival is generally the primary end-point of trials assessing TACE.
- The relapse-free survival has been recently recommended for trials performed in an adjuvant setting in patients treated for HCC (26). Yet, TACE is not a curative approach in most cases. Moreover, after TACE, it is frequently difficult to accurately define when an HCC is relapsing, and commonly used criteria (e.g. RECIST criteria) are probably not appropriate enough.
- Management of patients with HCC requires a global approach in order to control the tumour progression, but also the underlying cirrhosis. After TACE, a significant part of the patients will not die from the cancer, but from a complication of the cirrhosis. Only the overall survival takes into account both parameters. If an investigational anticancer drug administered after TACE has an objective effect regarding the tumour growth, but is accompanied by a worsening in the cirrhosis outcome, its global contribution will be relative.

In recent studies assessing TACE, the median overall survival was approximately 60%, but these studies mainly included selected patients, especially with post-viral cirrhosis (1, 2, 25, 27). In France, the main cause of cirrhosis is alcohol. In a recent phase III trial conducted by the FFCD group, tamoxifen alone was compared to tamoxifen plus TACE (28). The cause of cirrhosis was alcohol in 73 % of the patients allocated to the combined therapy

arm. In this arm, the 2-year survival rate was 32 %, but 26 % of included patients had a Child-B cirrhosis. In another French randomised study comparing TACE and conservative treatment in patients with Child-A cirrhosis, the survival rate at 2 years was 38 % in the TACE arm (29). Since the present study is also restricted to patients with Child-A cirrhosis, we estimated that the survival rate at 2 years will be 40 % in the TACE plus placebo arm.

Hypothesis : to improve OS rate at 2 years from 40 % vs. 60% for Sunitinib arm, with a 90% power and an alpha type I error (bilateral) of 0.05 it is required to observe 130 events and to include 180 pts during 2 years (+5 % lost of follow-up = 10 pts + 180 = 190 pts).

Based on the inclusion of 70 patients in the phase II study, then it will be required to include 190 – 70 = 120 extra patients for the phase III trial.

The minimum follow-up to observe the required number of deaths for final analysis of phase III trial will be about 36 months after the inclusion of the last included patients for total study duration of 5 years.

Two interim analyses are planed to reject H0 or H1. The p value will be done according to East software (Version 5).Using the alpha-spending function (Lan and DeMets, 1983) and O'Brien-Fleming method (O'Brien and Fleming, 1979).

The first interim analysis is planned at the final analysis (9 to 10 months after the inclusion of the first patient) of the phase II study with an expected number of events equal to 10 deaths representing 8 % of the total number of deaths required for the final analysis.

The second interim analysis is planned 34 months after the inclusion of the first patient with an expected number of events equal to 80 deaths representing 2/3 of the total number of deaths required for the final analysis

Overall survival: Time interval between randomization and death (all causes). Alive patients will be censored at the last follow-up

Relapse-free survival: Time interval between randomization and local or distant relapse or death (all causes). Alive patients without relapse will be censored at the last follow-up. Alive patients with secondary cancer will be censored at the last follow-up

Disease-free survival: Time interval between randomization and local or distant relapse or second cancer or death (all causes). Alive patients will be censored at the last follow-up.

An analysis plan will produced before the database is frozen. All of the analyses will be conducted with a 5% (bilateral) type one error. Analyses of the principal and secondary objectives will be conducted on an intent-to-treat basis and will bear on all of the patients enrolled:

The data for each arm will be presented as means (standard deviation), median and range or percentages and will be compared using the Student t test, Anova or Kruskal Wallis and Wilcoxon for quantitative variables or the Chi 2 and Fisher exact test for qualitative variables.

Median follow up will be calculated according to the so-called « reverse Kaplan Meier » method for each of the Arms, with its 95% confidence interval.

Survival curves will be estimated using the Kaplan-Meier analyses from time to randomization, and compared by the log-rank test and stratified log rank test. Cox proportional hazard models will be used to estimate univariate and multivariate hazard ratios (HR) with 95 % confidence intervals (CI).

The incidence rate of the side effects and of the unexpected serious side effects will be reported using frequencies and percentages for:

- Number and percentage of patients with at least one side effect
- Number and percentage of patients with at least one grade 3-4 side effect
- Number and percentage of patients with at least one SAE
- Number and percentage of patients with at least one side effect and grade 3-4 side effect due to the treatment.
- Number and percentage of patients with at least one side effect that lead to cessation of the treatment

Time to the first occurrence of grade 3-4 toxicity will be estimated by the Kaplan-Meier method.

XVII. POPULATIONS FOR ANALYSIS

The populations for the analysis are defined as follows:

Intention to treat: The intention to treat population will be composed by all included patients who were randomised to one of the two treatment groups, and patients will be analysed according to the treatment to which they were randomised. This will be the primary population for all analyses of efficacy data and baseline characteristics.

Per protocol: The per protocol population excludes patients randomised who did not receive any trial medication or who had a major violation of the protocol inclusion or exclusion criteria. Patients will be analysed according to the treatment to which they were randomised.

Safety: The safety population will include all patients who received at least one dose of trial medication. Only patients with clear documentation that no trial medication was received may be excluded. Patients will be analysed according to the treatment, which they actually received. This will be the population for the analysis of all safety data.

XVIII. ADVERSE EVENT REPORTING

1. Definition

An adverse event (AE) is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

AE include the following:

- All suspected adverse medication reactions,
- All reactions from medication overdose, abuse, discontinuation, sensitivity, or toxicity.
- Apparently unrelated illnesses, including the worsening of a pre-existing illness.
- Injury or accidents. Note that if a medical condition is known to have caused the injury or accident, the medical condition and the accident should be reported as two separate AE.
- Abnormalities in physiological testing or physical examination findings that require clinical intervention or further investigation (beyond ordering a repeat [confirmatory] test).
- Laboratory abnormalities that require clinical intervention or further investigation (beyond ordering a repeat [confirmatory] test) unless they are associated with an already reported

clinical event. Laboratory abnormalities associated with a clinical event (e.g. elevated liver enzymes in a patient with jaundice) should be described in the comments of the report of the clinical event rather than listed as a separate AE.

2. Reporting

All AE, as defined above, encountered during the clinical trial as well as any **Serious Adverse Events** (SAE) will be reported in the appropriate section of the CRF. It is important that this includes the duration of the AE (onset/resolution dates), the severity, the relationship to the drug and any concomitant treatment dispensed (or other action taken). Adverse event data should be obtained through observation of the patient, from any information volunteered by the patient, or through patient questioning. The general type of question asked could be similar to: "Do you have any health problems?" or "Have you had any health problems since your last clinic visit?"

2.1. Definition of Relationship of AE to the Investigational Medicinal Product

The Investigator will also be asked to assess the possible relationship between the AE and the investigational medication. The relationship should be assessed according to the following criteria:

Relationship of the Adverse Event to the Investigational Medicinal Product

None (Intercurrent Event)

An event that is not and cannot be related to the Investigational Medicinal Product, e.g. patient is a passenger in a road traffic accident.

Unlikely (remote)

Relationship is not likely e.g. a clinical event including laboratory test abnormality with temporal relationship to drug administration which makes a causal relationship improbable and in which other drugs, chemicals or underlying disease provide plausible explanations.

Possible Relationship may exist, but could have been produced by the patient's condition or treatment or other cause.

Probable

Relationship is likely; the adverse event abates upon discontinuation of Investigational Medicinal Product and cannot be due to the patient's condition.

Highly Probable

Strong relationship, the event abates upon discontinuation of Investigational Medicinal Product and, if applicable, re-appears upon repeat exposure.

2.2. Definition of Severity of Adverse Events

Severity of any AE will be graded according to National Cancer Institute (NCI) Common Toxicity Criteria (CTC) version 3.0, where applicable. For each episode, the highest severity grade attained should be reported. If an AE occurs that is not listed in the CTC, the Investigator will evaluate its severity using the following criteria:

Definition of Severity of Adverse Events

Mild Grade 1 - Does not interfere with subject's usual function (awareness of symptoms or signs, but easily tolerated [acceptable]).

Moderate Grade 2 - Interferes to some extent with subject's usual function (enough discomfort to interfere with usual activity [disturbing]).

Severe Grade 3 - Interferes significantly with subject's usual function (incapacity to work or to do usual activities [unacceptable])

Life Threatening Grade 4 - Results in risk of death, organ damage, or permanent disability (unacceptable)

Note the distinction between the seriousness and the intensity of an AE. **Severe** is a measure of intensity; thus, a **severe** reaction is not necessarily a **serious** reaction. For example, a headache may be severe in intensity, but would not be classified as serious unless it met one of the criteria for serious events listed in section 3.

2.3. Frequency of Adverse Events

Once The AE occurred only once and cleared in < 24 hours.

Occasionally The AE occurred sporadically or episodically between the onset and clearance dates.

Continuously The AE was present for the entire time between onset and clearance dates and was > 24 hours duration.

3. Serious Adverse Events

3.1. Definition of a Serious Adverse Event

A Serious Adverse Event is defined as any untoward medical occurrence that at any dose:

- Results in death.
- Is life-threatening (i.e. the patient was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe).
- Requires inpatient hospitalisation or prolongation of existing hospitalisation.
- Results in persistent or significant disability/incapacity.
- Is a congenital anomaly/birth defect.

Medical and scientific judgement should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately lifethreatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalisation; or development of drug dependency or drug abuse.

3.2. SAE Reporting Procedure for Investigators to Sponsor

The Investigator must report all SAE, regardless of presumed causal relationship, to the Promotor by fax on a Serious Adverse Event form within 24 hours of learning of the event. Details of the relevant fax number for SAE will be provided as a separate document.

Information on Serious Adverse Events (SAE) will be recorded on a specific Non Carbon Repeat (NCR) SAE form. Blank copies are included in the trial Investigator's File. The Investigator should follow up the event until resolution or stabilisation of the condition. Follow-up reports (as many as required) should be completed and faxed following the same procedure above.

A final report is required in any case once the condition is resolved or stabilised and no more information about the event is expected. The final report should be completed and faxed following the same procedure above.

4. Adverse Event Reporting Period

All AE that occur during the treatment period i.e. from the date of written informed consent to the final trial visit will be recorded in the CRF. In addition, any known untoward event that occurs subsequent to the AE reporting period that the Investigator assesses as possibly, probably or highly probably related to the Investigational. Medicinal Product should also be reported as an AE.

XIX. QUALITY OF LIFE

Quality of life (QOL) will be measured by the general thirty-question EORTC quality of life questionnaire QLQ-C30. QOL is measured at baseline and every 3 months the first year (treatment period) and every 6 months the second year. Patients who withdraw will have a QOL assessment at discontinuation. These discontinuation data will be listed, but will not be included in tables or analyses. Every effort must be made to complete the QOL questionnaire. Where a whole questionnaire has not been completed but the patient has survived to the appropriate time, one of the following options will be recorded on the CRF.

1 = patient felt too ill
2 = clinician or nurse felt the patient was too ill
3 = patient felt it was inconvenient, takes too much time
4 = patient felt it was a violation of privacy
5 = patient didn't understand the actual language/ illiterate
6 = administrative failure to distribute the questionnaire to the patient
7 = other, please specify

For codes 1 or 2, data will be regarded as 'missing too ill' whereas for any of the other

Firstly mean differences of QoL scores between last available measurement and inclusion will be compared using Wilcoxon test. Secondly Time until definitive score deterioration will estimated using Kaplan Meier, and compared using log-rank tests. They will be defined as the time interval between randomization and the first occurrence of a decrease in QLQ-C30 score of ≥ 5 points without any further improvement in QoL score of ≥ 5 points or any further available QoL data..

These analyses were repeated using a 10 points MCID, and by including deaths as event

XX. REFERENCES

1. Llovet JM, Real MI, Montana X, et al. Arterial embolization or chemoembolization *versus* symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. Lancet 2002, 359: 1734-9

2. Lo CM, Ngan H, Tso WK, Liu CL, Lam CM, Poon RT, Fan ST, Wong J. Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. Hepatology 2002; 35:1164-71.

3. Bruix J, Sala M, Llovet JM. Chemoembolization for hepatocellular carcinoma. Gastroenterology 2004, 127 (5 Suppl 2): S179-188

4. Llovet JM. Updated treatment approach to hepatocellular carcinoma. J Gastroenterol 2005, 40:225-35.

5. Poon RT, Ho JW, Tong CS, Lau C, Ng IO, Fan ST. Prognostic significance of serum vascular endothelial growth factor and endostatin in patients with hepatocellular carcinoma. Br J Surg 2004, 91:1354

6. Poon RT, Lau C, Yu WC, Fan ST, Wong J. High serum levels of vascular endothelial growth factor predict poor response to transarterial chemoembolization in hepatocellular carcinoma: a prospective study. Oncol Rep. 2004, 11:1077-84.

7. Sergio A, Cristofori C, Cardin R, Pivetta G, Ragazzi R, Baldan A, Girardi L, Cillo U, Burra P, Giacomin A, Farinati F. Transcatheter arterial chemoembolization (TACE) in hepatocellular carcinoma (HCC): the role of angiogenesis and invasiveness. Am J Gastroenterol 2008, 103: 914-21.

8. Li X, Feng GS, Zheng CS, Zhuo CK, Liu X. Expression of plasma vascular endothelial growth factor in patients with hepatocellular carcinoma and effect of transcatheter arterial chemoembolization therapy on plasma vascular endothelial growth factor level. World J Gastroenterol 2004, 10: 2878-82

9. Li X, Feng GS, Zheng CS, Zhuo CK, Liu X. Influence of transarterial chemoembolization on angiogenesis and expression of vascular endothelial growth factor and basic fibroblast growth factor in rat with Walker-256 transplanted hepatoma: an experimental study. World J Gastroenterol 2003, 9:2445-9.

10. Xiong ZP, Yang SR, Liang ZY, et al. Association between vascular endothelial growth factor and metastasis after transcatheter arterial chemoembolization in patients with hepatocellular carcinoma. Hepatobiliary Pancreat Dis Int 2004, 3:386-90.

11. Mendel DB, Laird AD, Xin X, et al. In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. Clin Cancer Res 2003; 9: 327-37.

12. Stock P, Monga D, Tan X, Micsenyi A, Loizos N, Monga SP. Platelet-derived growth factor receptor-alpha: a novel therapeutic target in human hepatocellular cancer. Mol Cancer Ther 2007,6: 1932-41.

13. Czabanka M, Vinci M, Heppner F, Ullrich A, Vajkoczy P. Effects of sunitinib on tumor hemodynamics and delivery of chemotherapy. Int J Cancer. 2009,124:1293-300.

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

15. Raut CP, Posner M, Desai J, Morgan JA, George S, Zahrieh D, Fletcher CD, Demetri GD, Bertagnolli MM. Surgical management of advanced gastrointestinal stromal tumors after treatment with targeted systemic therapy using kinase inhibitors. J Clin Oncol. 2006;24:2325-31

16. Siegel AB, Cohen EI, Ocean A, Lehrer D, Goldenberg A, Knox JJ, Chen H, Clark-Garvey S, Weinberg A, Mandeli J, Christos P, Mazumdar M, Popa E, Brown RS, Rafii S, Schwartz JD. Phase II trial evaluating the clinical and biologic effects of bevacizumab in unresectable hepatocellular carcinoma. J Clin Oncol 2008, 26:2992-8.

17. B. Benatsou, N. Lassau, L. Chami, S. Koscielny, A. Roche, M. Ducreux, D. Malka, V. Boige. Dynamic contrast-enhanced ultrasonography (DCE-US) with quantification for the early evaluation of hepatocellular carcinoma treated by bevacizumab in phase II. J Clin Oncol 26: 2008 (May 20 suppl; abstr 4588)

18. Zhu AX, Sahani D, Norden-Zfoni, et al. A phase II of gemcitabine, oxaliplatin in combination with bevacizumab in patients with hepatocellular carcinoma. Proc Am Soc Clin Oncol 2005, 23: abs 4120

19. Britten CD, Finn RS, Gomes AS, et al. A pilot study of IV bevacizumab in hepatocellular cancer patients undergoing chemoembolization. Proc Am Soc Clin Oncol 2005, 23: abs 4138

20. Faivre S, Raymond E, Douillard JY, et al. Assessment of safety and drug-induced tumor necrosis with sunitinib in patients (pts) with unresectable hepatocellular carcinoma (HCC). Proc Am Soc Clin Oncol 2007, 25 (18S): abs 3546.

21. Zhu AX, Sahani DV, di Tomaso E, et al. A phase II study of sunitinib in patients with advanced hepatocellular carcinoma. Proc Am Soc Clin Oncol 2007, 25 (18S): abs 4637.

22. Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodés J; EASL Panel of Experts on HCC. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. J Hepatol 2001, 35: 421-30.

23. Lencioni R, Malagari K, Vogl T, Pilleul F, Denys A, Watkinson A, Lammer J. A randomized phase II trial of a drug eluting bead in the treatment of hepatocellular carcinoma by transcatheter arterial chemoembolization. 2009 Gastrointestinal Cancer Symposium. Abs 116.

24. Wolter P, Stefan C, Decallonne B, Dumez H, Bex M, Carmeliet P, Schöffski P. The clinical implications of sunitinib-induced hypothyroidism: a prospective evaluation. Br J Cancer. 2008, 99: 448-54.

25. Cheng BQ, Jia CQ, Liu CT, Fan W, Wang QL, Zhang ZL, Yi CH. Chemoembolization combined with radiofrequency ablation for patients with hepatocellular carcinoma larger than 3 cm: a randomized controlled trial. JAM 2008, 299: 1669-77.

26. Llovet JM, Di Bisceglie AM, Bruix J, Kramer BS, Lencioni R, Zhu AX, Sherman M, Schwartz M, Lotze M, Talwalkar J, Gores GJ; Panel of Experts in HCC-Design Clinical Trials. Design and endpoints of clinical trials in hepatocellular carcinoma. J Natl Cancer Inst 2008, 100: 698-711.

27. Yamagiwa K, Shiraki K, Yamakado K, Mizuno S, Hori T, Yagi S, Hamada T, Iida T, Nakamura I, Fujii K, Usui M, Isaji S, Ito K, Tagawa S, Takeda K, Yokoi H, Noguchi T. Survival rates according to the Cancer of the Liver Italian Program scores of 345 hepatocellular carcinoma patients after multimodality treatments during a 10-year period in a retrospective study. J Gastroenterol Hepatol 2008, 23: 482-90.

28. Doffoël M, Bonnetain F, Bouché O, Vetter D, Abergel A, Fratté S, Grangé JD, Stremsdoerfer N, Blanchi A, Bronowicki JP, Caroli-Bosc FX, Causse X, Masskouri F, Rougier P, Bedenne L; Fédération Francophone de Cancérologie Digestive. Multicentre

randomised phase III trial comparing Tamoxifen alone or with Transarterial Lipiodol Chemoembolisation for unresectable hepatocellular carcinoma in cirrhotic patients (Fédération Francophone de Cancérologie Digestive 9402). Eur J Cancer 2008, 44: 528-38.

29. Groupe d'Etude et de Traitement du Carcinome Hepatocellulaire. A comparison of lipiodol chemoembolization and conservative treatment for unresectable hepatocellular carcinoma. Groupe d'Etude et de Traitement du Carcinome Hépatocellulaire. N Engl J Med 1995, 332: 1256-61.

XXI. APPENDICES

List of participating centres

Declaration of Helsinki

ICH-Good Clinical Practice-investigator's responsabilities

Example of Performance Status (ECOG/Karnofski scale)

BCLC classification

Child-Pugh score

CLIP score

Information notice to the patient

Informed consent

Specific informed consent for genetic study

The World Medical Association Declaration of Helsinki

World Medical Association Declaration of Helsinki: Recommendations Guiding Medical Doctors in Biomedical Research Involving Human Subjects

Adopted by the 18th World Medical Assembly, Helsinki, Finland, 1964 and as revised by the World Medical Assembly in Tokyo, Japan in 1975, in Venice, Italy in 1983, and in Hong Kong in 1989.

Introduction

It is the mission of the physician to safeguard the health of the people. His or her knowledge and conscience are dedicated to the fulfilment of this mission.

The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

The Purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the aetiology and pathogenesis of disease.

In current medical practice most diagnostic, therapeutic or prophylactic procedures involve hazards. This applies especially to biomedical research.

Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

In the field of biomedical research a fundamental distinction must be recognized between medical research in which the aim is essentially diagnostic or therapeutic for a patient, and medical research, the essential object of which is purely scientific and without implying direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, the World Medical Association has prepared the following recommendations as a guide to every physician in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Physicians are not relieved from criminal, civil and ethical responsibilities under the laws of their own countries.

I. Basic Principles

1. Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.

2. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted for consideration, comment and guidance to a specially appointed committee independent of the investigator and the sponsor provided that this independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.

3. Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.

4. Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.

5. Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseeable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interests of science and society.

6. The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

7. Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.

8. In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

9. In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely-given informed consent, preferably in writing.

10. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.

11. In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation. Whenever the minor child is in fact able to give a consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.

12. The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

II. Medical Research Combined with Professional Care (Clinical Research)

1. In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgment it offers hope of saving life, reestablishing health or alleviating suffering.

2. The

potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.

3. In any medical study, every patient--including those of a control group, if any--should be assured of the best proven diagnostic and therapeutic method.

4. The refusal of the patient to participate in a study must never interfere with the physician-patient relationship.

5. If the physician considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (I,2).

6. The physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

III. Non-Therapeutic Biomedical Research Involving Human Subjects (Non-Clinical Biomedical Research)

1. In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.

2. The subjects should be volunteers--either healthy persons or patients for whom the experimental design is not related to the patient's illness.

3. The investigator or the investigating team should discontinue the research if in his/her or their judgment it may, if continued, be harmful to the individual.

4. In research on man, the interest of science and society should never take precedence over considerations related to the well-being of the subject.

4. INVESTIGATOR

4.1 Investigator's Qualifications and Agreements

4.1.1 The investigator(s) should be qualified by education, training, and experience to assume responsibility for the proper conduct of the trial, should meet all the qualifications specified by the applicable regulatory requirement(s), and should provide evidence of such qualifications through up-to-date curriculum vitae and/or other relevant documentation requested by the sponsor, the IRB/IEC, and/or the regulatory authority (ies).

4.1.2 The investigator should be thoroughly familiar with the appropriate use of the investigational product(s), as described in the protocol, in the current Investigator's Brochure, in the product information and in other information sources provided by the sponsor.

4.1.3 The investigator should be aware of, and should comply with, GCP and the applicable regulatory requirements.

4.1.4 The investigator/institution should permit monitoring and auditing by the sponsor, and inspection by the appropriate regulatory authority (ies).

4.1.5 The investigator should maintain a list of appropriately qualified persons to whom the investigator has delegated significant trial-related duties.

4.2 Adequate Resources

4.2.1 The investigator should be able to demonstrate (e.g., based on retrospective data) a potential for recruiting the required number of suitable subjects within the agreed recruitment period.

4.2.2 The investigator should have sufficient time to properly conduct and complete the trial within the agreed trial period.

4.2.3 The investigator should have available an adequate number of qualified staff and adequate facilities for the foreseen duration of the trial to conduct the trial properly and safely.

4.2.4 The investigator should ensure that all persons assisting with the trial are adequately informed about the protocol, the investigational product(s), and their trial-related duties and functions.

4.3 Medical Care of Trial Subjects

4.3.1 A qualified physician (or dentist, when appropriate), who is an investigator or a sub-investigator for the trial, should be responsible for all trial-related medical (or dental) decisions.

4.3.2 During and following a subject's participation in a trial, the investigator/institution should ensure that adequate medical care is provided to a subject for any adverse events, including clinically significant laboratory values, related to the trial. The investigator/institution should inform a subject when medical care is needed for intercurrent illness(es) of which the investigator becomes aware.

4.3.3 It is recommended that the investigator inform the subject's primary physician about the subject's participation in the trial if the subject has a primary physician and if the subject agrees to the primary physician being informed.

4.3.4 Although a subject is not obliged to give his/her reason(s) for withdrawing prematurely from a trial, the investigator should make a reasonable effort to ascertain the reason(s), while fully respecting the subject's rights.

4.4 Communication with IRB/IEC

4.4.1 Before initiating a trial, the investigator/institution should have written and dated approval/favourable opinion from the IRB/IEC for the trial protocol, written informed consent form, consent form updates, subject recruitment procedures (e.g., advertisements), and any other written information to be provided to subjects.

4.4.2 As part of the investigator's/institution's written application to the IRB/IEC, the investigator/institution should provide the IRB/IEC with a current copy of the Investigator's Brochure. If the Investigator's Brochure is updated during the trial, the investigator/institution should supply a copy of the updated Investigator's Brochure to the IRB/IEC.

4.4.3 During the trial the investigator/institution should provide to the IRB/IEC all documents subject to review.

4.5 Compliance with Protocol

4.5.1 The investigator/institution should conduct the trial in compliance with the protocol agreed to by the sponsor and, if required, by the regulatory authority(ies) and which was given approval/favourable opinion by

the IRB/IEC. The investigator/institution and the sponsor should sign the protocol, or an alternative contract, to confirm agreement.
4.5.2 The investigator should not implement any deviation from, or changes of the protocol without agreement by the sponsor and prior review and documented approval/favourable opinion from the IRB/IEC of an amendment, except where necessary to eliminate an immediate hazard(s) to trial subjects, or when the change(s) involves only logistical or administrative aspects of the trial [e.g., change in monitor(s), change of telephone number(s)].
4.5.3 The investigator, or person designated by the investigator, should document and explain any deviation from the approved protocol.
4.5.4 The investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB/IEC approval/favourable opinion. As soon as possible, the implemented deviation or change, the reasons for it, and, if appropriate, the proposed protocol amendment(s) should be submitted:
(a) to the IRB/IEC for review and approval/favourable opinion,
(b) to the sponsor for agreement and, if required,
(c) to the regulatory authority(ies).

4.6 Investigational Product(s)
4.6.1 Responsibility for investigational product(s) accountability at the trial site(s) rests with the investigator/institution.
4.6.2 Where allowed/required, the investigator/institution may/should assign some or all of the investigator's/institution's duties for investigational product(s) accountability at the trial site(s) to an appropriate pharmacist or another appropriate individual who is under the supervision of the investigator/institution.
4.6.3 The investigator/institution and/or a pharmacist or other appropriate individual, who is designated by the investigator/institution, should maintain records of the product's delivery to the trial site, the inventory at the site, the use by each subject, and the return to the sponsor or alternative disposition of unused product(s). These records should include dates, quantities, batch/serial numbers, expiration dates (if applicable), and the unique code numbers assigned to the investigational product(s) and trial subjects. Investigators should maintain records that document adequately that the subjects were provided the doses specified by the protocol and reconcile all investigational product(s) received from the sponsor.
4.6.4 The investigational product(s) should be stored as specified by the sponsor and in accordance with applicable regulatory requirement(s).
4.6.5 The investigator should ensure that the investigational product(s) are used only in accordance with the approved protocol.
4.6.6 The investigator, or a person designated by the investigator/institution, should explain the correct use of the investigational product(s) to each subject and should check, at intervals appropriate for the trial, that each subject is following the instructions properly.

4.7 Randomization Procedures and Unblinding
The investigator should follow the trial's randomization procedures, if any, and should ensure that the code is broken only in accordance with the protocol. If the trial is blinded, the investigator should promptly document and explain to the sponsor any premature unblinding (e.g., accidental unblinding, unblinding due to a serious adverse event) of the investigational product(s).

4.8 Informed Consent of Trial Subjects
4.8.1 In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki. Prior to the beginning of the trial, the investigator should have the IRB/IEC's written approval/favourable opinion of the written informed consent form and any other written information to be provided to subjects.
4.8.2 The written informed consent form and any other written information to be provided to subjects should be revised whenever important new information becomes available that may be relevant to the subject's consent. Any revised written informed consent form, and written information should receive the IRB/IEC's approval/favourable opinion in advance of use. The subject or the subject's legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information should be documented.
4.8.3 Neither the investigator, nor the trial staff, should coerce or unduly influence a subject to participate or to continue to participate in a trial.

4.8.4 None of the oral and written information concerning the trial, including the written informed consent form, should contain any language that causes the subject or the subject's legally acceptable representative to waive or to appear to waive any legal rights, or that releases or appears to release the investigator, the institution, the sponsor, or their agents from liability for negligence.

4.8.5 The investigator, or a person designated by the investigator, should fully inform the subject or, if the subject is unable to provide informed consent, the subject's legally acceptable representative, of all pertinent aspects of the trial including the written information given approval/favourable opinion by the IRB/IEC.

4.8.6 The language used in the oral and written information about the trial, including the written informed consent form, should be as non-technical as practical and should be understandable to the subject or the subject's legally acceptable representative and the impartial witness, where applicable.

4.8.7 Before informed consent may be obtained, the investigator, or a person designated by the investigator, should provide the subject or the subject's legally acceptable representative ample time and opportunity to inquire about details of the trial and to decide whether or not to participate in the trial. All questions about the trial should be answered to the satisfaction of the subject or the subject's legally acceptable representative.

4.8.8 Prior to a subject's participation in the trial, the written informed consent form should be signed and personally dated by the subject or by the subject's legally acceptable representative, and by the person who conducted the informed consent discussion.

4.8.9 If a subject is unable to read or if a legally acceptable representative is unable to read, an impartial witness should be present during the entire informed consent discussion. After the written informed consent form and any other written information to be provided to subjects, is read and explained to the subject or the subject's legally acceptable representative, and after the subject or the subject's legally acceptable representative has orally consented to the subject's participation in the trial and, if capable of doing so, has signed and personally dated the informed consent form, the witness should sign and personally date the consent form. By signing the consent form, the witness attests that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the subject or the subject's legally acceptable representative, and that informed consent was freely given by the subject or the subject's legally acceptable representative.

4.8.10 Both the informed consent discussion and the written informed consent form and any other written information to be provided to subjects should include explanations of the following:

(a) That the trial involves research.

(b) The purpose of the trial.

(c) The trial treatment(s) and the probability for random assignment to each treatment.

(d) The trial procedures to be followed, including all invasive procedures.

(e) The subject's responsibilities.

(f) Those aspects of the trial that are experimental.

(g) The reasonably foreseeable risks or inconveniences to the subject and, when applicable, to an embryo, fetus, or nursing infant.

(h) The reasonably expected benefits. When there is no intended clinical benefit to the subject, the subject should be made aware of this.

(i) The alternative procedure(s) or course(s) of treatment that may be available to the subject, and their important potential benefits and risks.

(j) The compensation and/or treatment available to the subject in the event of trial-related injury.

(k) The anticipated prorated payment, if any, to the subject for participating in the trial.

(l) The anticipated expenses, if any, to the subject for participating in the trial.

(m) That the subject's participation in the trial is voluntary and that the subject may refuse to participate or withdraw from the trial, at any time, without penalty or loss of benefits to which the subject is otherwise entitled.

(n) That the monitor(s), the auditor(s), the IRB/IEC, and the regulatory authority (ies) will be granted direct access to the subject's original medical records for verification of clinical trial procedures and/or data, without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations and that, by signing a written informed consent form, the subject or the subject's legally acceptable representative is authorizing such access.

(o) That 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. If the results of the trial are published, the subject's identity will remain confidential.

(p) That the subject or the subject's legally acceptable representative will be informed in a timely manner if information becomes available that may be relevant to the subject's willingness to continue participation in the trial.

(q) The person(s) to contact for further information regarding the trial and the rights of trial subjects, and whom to contact in the event of trial-related injury.

(r) The foreseeable circumstances and/or reasons under which the subject's participation in the trial may be terminated.
(s) The expected duration of the subject's participation in the trial.
(t) The approximate number of subjects involved in the trial.
4.8.11 Prior to participation in the trial, the subject or the subject's legally acceptable representative should receive a copy of the signed and dated written informed consent form and any other written information provided to the subjects. During a subject's participation in the trial, the subject or the subject's legally acceptable representative should receive a copy of the signed and dated consent form updates and a copy of any amendments to the written information provided to subjects.
4.8.12 When a clinical trial (therapeutic or non-therapeutic) includes subjects who can only be enrolled in the trial with the consent of the subject's legally acceptable representative (e.g., minors, or patients with severe dementia), the subject should be informed about the trial to the extent compatible with the subject's understanding and, if capable, the subject should sign and personally date the written informed consent.
4.8.13 Except as described in 4.8.14, a non-therapeutic trial (i.e., a trial in which there is no anticipated direct clinical benefit to the subject), should be conducted in subjects who personally give consent and who sign and date the written informed consent form.
4.8.14 Non-therapeutic trials may be conducted in subjects with consent of a legally acceptable representative provided the following conditions are fulfilled:
(a) The objectives of the trial can not be met by means of a trial in subjects who can give informed consent personally.
(b) The foreseeable risks to the subjects are low.
(c) The negative impact on the subject's well-being is minimized and low.
(d) The trial is not prohibited by law.
(e) The approval/favourable opinion of the IRB/IEC is expressly sought on the inclusion of such subjects, and the written approval/favourable opinion covers this aspect. Such trials, unless an exception is justified, should be conducted in patients having a disease or condition for which the investigational product is intended. Subjects in these trials should be particularly closely monitored and should be withdrawn if they appear to be unduly distressed.
4.8.15 In emergency situations, when prior consent of the subject is not possible, the consent of the subject's legally acceptable representative, if present, should be requested. When prior consent of the subject is not possible, and the subject's legally acceptable representative is not available, enrollment of the subject should require measures described in the protocol and/or elsewhere, with documented approval/favourable opinion by the IRB/IEC, to protect the rights, safety and well-being of the subject and to ensure compliance with applicable regulatory requirements. The subject or the subject's legally acceptable representative should be informed about the trial as soon as possible and consent to continue and other consent as appropriate should be requested.

4.9 Records and Reports
4.9.1 The investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the sponsor in the CRFs and in all required reports.
4.9.2 Data reported on the CRF, that are derived from source documents, should be consistent with the source documents or the discrepancies should be explained.
4.9.3 Any change or correction to a CRF should be dated, initialed, and explained (if necessary) and should not obscure the original entry (i.e., an audit trail should be maintained); this applies to both written and electronic changes or corrections. Sponsors should provide guidance to investigators and/or the investigators' designated representatives on making such corrections. Sponsors should have written procedures to assure that changes or corrections in CRFs made by sponsor's designated representatives are documented, are necessary, and are endorsed by the investigator. The investigator should retain records of the changes and corrections.
4.9.4 The investigator/institution should maintain the trial documents as specified in Essential Documents for the Conduct of a Clinical Trial (see 8.) and as required by the applicable regulatory requirement(s). The investigator/institution should take measures to prevent accidental or premature destruction of these documents.
4.9.5 Essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period however if required by the applicable regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.
4.9.6 The financial aspects of the trial should be documented in an agreement between the sponsor and the investigator/institution.
4.9.7 Upon request of the monitor, auditor, IRB/IEC, or regulatory authority, the investigator/institution should make available for direct access all requested trial-related records.

4.10 Progress Reports

4.10.1 The investigator should submit written summaries of the trial status to the IRB/IEC annually, or more frequently, if requested by the IRB/IEC.

4.10.2 The investigator should promptly provide written reports to the sponsor, the IRB/IEC and, where applicable, the institution on any changes significantly affecting the conduct of the trial, and/or increasing the risk to subjects.

4.11 Safety Reporting

4.11.1 All serious adverse events (SAE) should be reported immediately to the sponsor except for those SAEs that the protocol or other document (e.g., Investigator's Brochure) identifies as not needing immediate reporting. The immediate reports should be followed promptly by detailed, written reports. The immediate and follow-up reports should identify subjects by unique code numbers assigned to the trial subjects rather than by the subjects' names, personal identification numbers, and/or addresses. The investigator should also comply with the applicable regulatory requirement(s) related to the reporting of unexpected serious adverse drug reactions to the regulatory authority (ies) and the IRB/IEC.

4.11.2 Adverse events and/or laboratory abnormalities identified in the protocol as critical to safety evaluations should be reported to the sponsor according to the reporting requirements and within the time periods specified by the sponsor in the protocol.

4.11.3 For reported deaths, the investigator should supply the sponsor and the IRB/IEC with any additional requested information (e.g., autopsy reports and terminal medical reports).

4.12 Premature Termination or Suspension of a Trial

If the trial is prematurely terminated or suspended for any reason, the investigator/institution should promptly inform the trial subjects, should assure appropriate therapy and follow-up for the subjects, and, where required by the applicable regulatory requirement(s), should inform the regulatory authority (ies). In addition:

4.12.1 If the investigator terminates or suspends a trial without prior agreement of the sponsor, the investigator should inform the institution where applicable, and the investigator/institution should promptly inform the sponsor and the IRB/IEC, and should provide the sponsor and the IRB/IEC a detailed written explanation of the termination or suspension.

4.12.2 If the sponsor terminates or suspends a trial, the investigator should promptly inform the institution where applicable and the investigator/institution should promptly inform the IRB/IEC and provide the IRB/IEC a detailed written explanation of the termination or suspension.

4.12.3 If the IRB/IEC terminates or suspends its approval/favourable opinion of a trial, the investigator should inform the institution where applicable and the investigator/institution should promptly notify the sponsor and provide the sponsor with a detailed written explanation of the termination or suspension.

4.13 Final Report(s) by Investigator

Upon completion of the trial, the investigator, where applicable, should inform the institution; the investigator/institution should provide the IRB/IEC with a summary of the trial's outcome, and the regulatory authority (ies) with any reports required.

PERFORMANCE STATUS (ECOG/KARNOFSKY SCALE)

DESCRIPTION	ECOG GRADE	KARNOFSKY EQUIVALENT	
Fully active, able to carry on all pre-disease performance without restriction.	0	100	Normal, no complaints; no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature, i.e. light housework, office work.	1	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self but unable to carry on normal activity or to do work.
Ambulatory and capable of self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	2	60	Requires occasional assistance but is able to care for most of personal needs.
		50	Requires considerable assistance and frequent medical care.
Capable of only limited self care, confined to bed or chair more than 50% of waking hours.	3	40	Disabled; requires special care and assistance.
		30	Severely disabled; hospitalisation is indicated although death not imminent.
Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	4	20	Very ill; hospitalisation and active supportive care necessary.
		10	Moribund.

CHILD-PUGH SCORE

	1	2	3
Encephalopathy	**Absent**	**Confusion**	**Coma**
Ascitis	**Absent**	**Mild**	**Abundant**
Albumine (g/l)	**> 35**	**28-35**	**< 28**
Bilirubine (µmol/l)	**< 35**	**35-50**	**>50**
Prothrombin activity (%)	**>50**	**40-50**	**<40**

Child A: score 5 or 6
Child B: score 7- 9
Child C: score 10-15