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Study Title: Study on Safety and Efficacy of NMS-01940153E in Adult Patients With Unresectable Hepatocellular Carcinoma (HCC) Previously Treated With Systemic Therapy

Protocol Reference Number: MPSA-153-001

NCT Number: NCT05630937

Phase I/II study on safety and efficacy of NMS-01940153E in adult patients with unresectable hepatocellular carcinoma (HCC) previously treated with systemic therapy

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Therapeutic Area:	Oncology
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SPONSOR REFERENCES AND APPROVAL

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Sponsor Medical Expert:	[redacted]
Sponsor Statistician:	[redacted]
Sponsor Representative:	[redacted]

INVESTIGATOR'S SIGNATURE

Title	Phase I/II study on safety and efficacy of NMS-01940153E in adult patients with unresectable hepatocellular carcinoma (HCC) previously treated with systemic therapy
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I declare that I will carry out the study in accordance with the referenced protocol, the ICH E6 R2 (Good Clinical Practices), Declaration of Helsinki and in accordance to local legal and regulatory requirements.

Investigator's Name	Investigator's Title
Investigational Site Name	
Investigational Site Address	
Investigator's Signature	**Date**

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1.1. Protocol Identifiers

Therapeutic Area: Oncology

Product: NMS-01940153E

Indication: Unresectable Hepatocellular Carcinoma (HCC)

Protocol Number: MPSA-153-001

Title of Study: Phase I/II study on safety and efficacy of NMS-01940153E in adult patients with unresectable hepatocellular carcinoma (HCC) previously treated with systemic therapy

1.2. Background Information and Study Rationale

Primary liver cancer is the 6th most common neoplasm and among the leading cause of cancer-related deaths worldwide. Hepatocellular carcinoma (HCC) constitutes 80–90% of all primary liver cancers. The incidence is increasing and will soon surpass one million annual cases worldwide.

At early stages of HCC (BCLC stage 0/A), treatment strategies are potentially curative and include liver resection, liver transplantation, and ablation. At intermediate stages (BCLC stage B), palliative locoregional therapies such as transarterial chemoembolization (TACE) remain the gold standard for treatment. However, most HCC cases are diagnosed at advanced stages (BCLC stage C) when curative therapies or TACE are no longer indicated.

Until recently, sorafenib was the only approved systemic therapy for HCC. The therapeutic landscape changed remarkably in 2017 with the approval of regorafenib as second-line treatment for patients with HCC who have been previously treated with sorafenib. When well tolerated, treatment with sorafenib followed by regorafenib (at radiologic progression on sorafenib) results in a median OS of 26 months. However, when this sequence is not possible due to lack of efficacy or intolerance to these drugs, the prognosis remains poor. In 2018, based on a Phase III non-inferiority trial, lenvatinib was approved in Japan, Europe and the USA as a first-line treatment in HCC. In 2019, cabozantinib and ramucirumab received regulatory approval for HCC patients previously treated with sorafenib. In addition, the United States Food and Drug Administration (FDA) approved pembrolizumab and, recently, ipilimumab in combination with nivolumab, for use in the second line following sorafenib.

A phase III trial of atezolizumab combined with bevacizumab demonstrated a significantly better overall survival and progression-free survival outcomes than sorafenib in patients with advanced unresectable HCC not previously treated with systemic therapy. The combination was approved in 2020 and is now the preferred 1st line treatment option both for NCCN and ESMO guidelines.

Regardless of the availability of therapeutic agents in first and second lines, the failure of systemic treatment remains a major therapeutic challenge and relapsed/refractory HCC still represents an area of unmet medical need. Patients with good performance status and compensated liver function who progress after all approved treatments are considered suitable for new investigational drugs or strategies.

NMS-01940153E, also known as S81694 (from now on called NMS-01940153E), is a dihydropyrazoloquinazoline derivative, potent small molecule inhibitor of Monopolar Spindle 1 kinase (MPS1 which is also known as TTK kinase). MPS1 is a dual tyrosine/threonine kinase expressed only in proliferating cells. Upon activation by phosphorylation, MPS1 plays a critical role in the control of mitosis regulating the Spindle Assembly Checkpoint (SAC) through proper kinetochore recruitment of other essential SAC proteins.

Most solid tumors are characterized by complex chromosome rearrangements. Aneuploidy is a common feature of cancer cells: approximately 90% of solid tumors are aneuploid, with the majority (> 70%) having a gain rather than a loss of chromosomes. MPS1 has been found highly expressed in a number of tumors of different origins.

Non-clinical data
In tumor cells exposed to NMS-01940153E, anti-proliferative activity is associated with the expected mechanism of action of an MPS1 inhibitor. It causes mitotic checkpoint override, accelerates mitosis, induces chromosomal misalignment, and promotes aneuploidy, apoptosis and cancer cell death, accompanied by reduction of mitotic markers such as phospho-histone H3 and MPS1 auto-phosphorylation.

In vitro, NMS-01940153E is active on a broad panel of cancer cell lines. [redacted].

Nonclinical safety of NMS-01940153E was characterized in single-dose toxicity studies after IV administration in rats, dogs and monkeys and in repeated toxicity studies after once weekly IV administration for 4 weeks in rats and monkeys. In the pivotal GLP 4-week toxicity studies the major target organs observed following the repeated administration of NMS-01940153E were the haemolymphopoietic system (HLP) in rats and monkeys, the intestinal tract in rats and the male reproductive organs particularly in rats at high doses. In addition, injection site alterations were seen in both species.

NMS-01940153E induced haemolysis was observed *in vitro* on human blood at high concentrations (≥ 320 µM); no haemolysis occurred up to a concentration of 200 µM.

According to the toxicity studies, NMS-01940153E safety profile was considered manageable, with target organ toxicity in line with its pharmacological mechanism of action.

Clinical data
NMS-01940153E originated in Nerviano Medical Sciences and was licensed out to Servier in 2013. Servier conducted a phase I, First in Human trial (FIH), with NMS-01940153E as single-agent, in patients with advanced/metastatic solid tumors (EudraCT 2014-002023-10) and a Phase I-II study of NMS-01940153E in combination with paclitaxel in patients with advanced/metastatic breast cancer (EudraCT 2017-002459-27). In 2019 Servier decided to stop the clinical development of NMS-01940153E due to internal portfolio decisions and the compound was returned to Nerviano Medical Sciences.

The Phase I, FIH study, was a dose-escalation study of NMS-01940153E administered to sequential cohorts of adult patients with advanced/metastatic solid tumors; the primary objective of the study was to define the Maximum Tolerated Dose (MTD) and the associated Dose-Limiting Toxicities for NMS-01940153E administered as IV infusion on days 1, 8, 15 in repeated 4-week cycles.

The study was stopped before the primary objective was met. Thirty-eight patients were treated according to nine different dose levels (4, 6, 9, 13.5, 20, 30, 45, 67.5 and 135 mg/m^2/week); among 36 patients evaluable for first cycle Dose Limiting Toxicities, 3 DLTs were reported: grade 3 anemia, at 4 mg/m^2/week; grade 4 hypertensive crisis, at 20 mg/m^2/week; grade 3 fatigue, at 135 mg/m^2/week.

The most frequent treatment emergent adverse events (TEAEs) reported in at least 15% of patients were fatigue (57.9%), anaemia (44.7%), nausea (31.6%), decreased appetite (28.9%), constipation, pain, vomiting (23.7% each), cough (21.1%), diarrhea (18.4%) and dyspnea (15.8%).

The most frequent TEAEs related to NMS-01940153E reported in at least 10% of patients were anaemia (36.8%), fatigue (28.9%), nausea (21.1%), decreased appetite (15.8%), neutropenia (13.2%) and diarrhea (10.5%).

Thirty-six out of 38 treated patients were evaluable for efficacy; one patient, with metastatic clear renal cell cancer, achieved a durable complete tumour response (CR); 13 patients showed stable disease (SD), including 2 patients with HCC.Although only 2 patients with HCC refractory to multiple prior therapies were enrolled in the study, both remained stable for 6 months and one of them had a transient decrease in tumor size. On these grounds, we hypothesize that monotherapy treatment in previously treated patients with advanced HCC might be further explored.
Refer to the Investigator's Brochure for current clinical status.

1.3. Trial Objectives and Endpoints

Primary Objective:

- To determine the Maximum Tolerated Dose (MTD) and Recommended Phase 2 Dose (RP2D) of NMS-01940153E administered as single agent to adult patients with unresectable hepatocellular carcinoma (HCC) previously treated with systemic therapy (Phase I).
- To assess the anti-tumor efficacy of NMS-01940153E administered as single agent to adult patients with unresectable hepatocellular carcinoma previously treated with systemic therapy (Phase II).

Secondary Objectives:

- To define the safety and tolerability profile of NMS-01940153E.
- To evaluate the pharmacokinetics of NMS-01940153E and its main metabolite NMS-03593478 in plasma and, limited to Phase I, also in urine.
- To evaluate additional measures of antitumor efficacy of NMS-01940153E.

Exploratory Objective:

- To explore correlation among Alpha-Fetoprotein (AFP) levels changes with NMS-01940153E exposure and clinical outcomes;
- To explore correlation among baseline tumoral and clinical prognostic factors with clinical outcome;
- To explore the potential correlation of selected molecular alterations in circulating free DNA (cfDNA) at baseline with clinical outcome.

Primary Endpoints:

- Drug related Dose Limiting Toxicities (DLTs) (Phase I).
- Objective Response Rate (ORR). The ORR will be calculated as the proportion of evaluable patients who have achieved, as best overall response (BOR), confirmed complete response (CR) or partial response (PR) measured by investigator-assessed RECIST 1.1 (Phase II).

Secondary Endpoints:

- Overall safety profile of NMS-01940153E characterized by type, severity (graded using NCI CTCAE Version 5.0), duration of the adverse events (AEs) and laboratory and ECG abnormalities, and relationship of the AEs to study treatment in the first and subsequent cycles of therapy.
- Pharmacokinetic parameters of NMS-01940153E and its main metabolite NMS-03593478 in plasma and urine (urine only in Phase I).
- Objective Tumor Response (Partial and Complete Response) as measured by investigator-assessed RECIST 1.1 (Phase I).
- Objective response rate as measured by investigator-assessed mRECIST (Phase II).
- Duration of response (DoR) as measured by investigator-assessed RECIST 1.1. and investigator-assessed mRECIST.
- Progression Free Survival, including landmark analyses, as measured by investigator-assessed RECIST 1.1.
- Overall survival (OS).

Exploratory Endpoint:

- Evaluation of AFP plasma levels;

- Evaluation of tumoral and other clinical prognostic features at baseline;
- Assessment of selected molecular alterations in circulating free DNA (cfDNA) at baseline

1.4. Study Design and Methods

Study Design:

This is a Phase I/II, open-label, non-randomized, multicenter study to explore safety, tolerability and antitumor activity of NMS-01940153E as single agent in adult patients with unresectable HCC previously treated with systemic therapy.

An independent Data Safety Monitoring Board (DSMB) will provide study oversight through periodic safety evaluations and make recommendations on study progress in the transition from Phase I to Phase II and at the planned interim analysis for the futility of Phase II.

The Phase I portion is designed as a dose-escalation study in sequential cohorts of patients aimed to obtain the MTD that is defined based on the DLTs observed in the first cycle of treatment. The selection of the starting dose and the dose escalation scheme is based on the safety information gained in the FIH study (EudraCT 2014-002023-10) and in consideration of the possible different tolerability in patients with compromised liver.

Once the MTD is identified and the safety profile of NMS-01940153E has been reviewed by the Investigators, the Sponsor and the independent DSMB and is considered adequate based on the entire Phase I data available, the Phase II portion of the study started. The Phase II portion is designed as an exploratory two stage study with an interim analysis for futility and stopping criteria for unacceptable toxicity to assess the antitumor activity of NMS-01940153E in adult patients with unresectable HCC previously treated with systemic therapy measured as objective response rate.

Treatment:

NMS-01940153E is supplied as labeled type I glass vials containing 30 mg/vial of powder for solution for infusion. Each labeled vial (primary packaging) is packaged in a labeled single small carton (secondary packaging).

Patients will receive treatment with NMS-01940153E administered as intravenous (IV) infusion for 3 consecutive weeks on days 1, 8, 15 followed by 1 week of rest, in a 28-day cycle. The duration of infusion will be dependent on the total dose to be administered:

- 1 hour infusion if $\leq$ 255 mg are administered
- 1 hour 30 minutes infusion if > 255 mg but < 375 mg are administered

Within one cycle, in case the patient cannot be retreated on the day foreseen because of toxicity, the dose will be skipped and the patient will be retreated at the next foreseen time-point.

Dose reduction/omission is foreseen based on toxicities observed.

Patients that cannot be retreated for any reason within 1 week from day 28 (up to day 35) will go off-treatment, unless the investigator believes treatment continuation is in the patient's best interest. In this case a maximum delay of 2 weeks (up to day 42) will be allowed. Otherwise patients may continue on investigational drug until disease progression, unacceptable toxicity, withdrawal of consent by the patient or other discontinuation criteria described in the protocol are met.

Phase I Dose Escalation

The safety profile of NMS-01940153E administered IV on days 1, 8, 15 every 4 weeks has been characterized in the FIH, dose escalation study, conducted in adult patients with advanced/metastatic solid tumors up to the dose of 135 mg/m^2/week. This dose was not defined as MTD in study 2014-002023-10 (EudraCT), since only 1 patient experienced DLT (Grade 3 fatigue) out of 6 evaluable patients.

Considering the possible different tolerability in patients with compromised liver, the proposed starting dose for the Phase I portion of the study is 100 mg/m^2/week, which is an intermediate dose level between the highest (135 mg/m^2/week) and the next lower dose level (67.5 mg/m^2/week) tested in the FIH study. According to the observed safety profile, dose increments of 25-35% will be applied to the proposed starting dose, until the MTD is reached. In presence of unacceptable toxicity observed at 100 mg/m^2/week, a lower dose level by 25-35% may be tested.

Patients experiencing DLT during the DLT window can be retreated according to the time delay and dose modification schema.

No intra-patient dose escalation will be allowed.

Phase II

In the Phase II portion of the study, patients will be treated at the RP2D defined in the Phase I portion, as starting dose.

Phase I Dose Escalation

A conventional 3+3 study design with inter-individual dose-escalations will be adopted. Patients will be allocated to sequential cohorts of progressively higher dose levels of NMS-01940153E based on the presence of Dose Limiting Toxicities (DLT).

At each dose level, a minimum of 3 patients will be included.

If 0/3 patients experiences first cycle DLT, the next cohort will start one dose level higher.

If 1/3 patients experiences first cycle DLT, up to three more patients will receive the study medication at the same dose level; if 1/6 patients experiences first cycle DLT the next cohort will start one dose level higher.

If ≥2/3 or ≥2/6 patients experience DLTs in the first cycle of treatment, the MTD is considered to have been exceeded. Three more patients will be entered at the previous dose level (if only 3 patients were previously treated at that prior dose) or, in case the MTD is exceeded at the starting dose level, six patients will be entered to a lower dose level.

Only one dose level will be open for enrollment at any time.

The first patient enrolled on a dose level should be observed for DLT for at least 2 weeks before entering any other patient at that dose level.

In absence of 1st cycle DLT in the first patient, the third patient can be enrolled at any time.

In presence of 1st cycle DLT in the first patient, the third patient can be enrolled only after the second patient has completed the first cycle without DLT.

In case a cohort needs to be expanded to more than 3 patients, the additional patients can be enrolled simultaneously.

All patients must be observed for one cycle before subsequent patients are enrolled at the next higher dose level. If a patient fails to receive at least 66% of the first cycle of treatment, for reasons other than treatment-related toxicities, an additional patient must be enrolled at the same dose level.

After the completion of each cohort, available safety and pharmacokinetic (PK) data will be reviewed and new dose levels will be decided jointly by the Investigators and the Sponsor.

Definition of DLTs

For this protocol, a DLT will be defined as any of the following events for which the relationship to NMS-01940153E cannot be definitely excluded. Toxicities are to be graded according to the NCI CTCAE version 5.0.

Hematological toxicities	
Neutropenia	Grade 4 lasting >7 days or Grade 4 of any duration associated with Grade ≥2 infection
Febrile neutropenia	ANC <1000/mm^3 and a single temperature of >38.3 degrees C (101 degrees F) or a sustained temperature of >38 degrees C (100.4 degrees F) for more than one hour.
Thrombocytopenia	Grade 3 associated with any grade bleeding or Grade 4
Anaemia	Grade ≥ 4
Non-Hematological toxicities	
Nausea, vomiting or diarrhea	Grade ≥3 despite optimal treatment
Signs of potential severe drug-induced liver injury as assessed according to Hy's law	Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >3 ULN and total bilirubin >2 ULN in the absence of cholestasis (alkaline phosphatase [ALP] <2 ULN) and no other reason can be identified.
Any other non-hematological toxicities;	- Grade ≥ 3 toxicities unless clear alternate etiology is documented by the investigator except: - asymptomatic Grade 3 electrolyte changes that can be successfully supplemented (e.g., hypokalemia) and resolve/recover to baseline within 72 hours - Grade 3 fatigue that improves to ≤ Grade 2 in no more than 5 days - alopecia
Other significant toxicity	
Failure to complete the first cycle of treatment with at least 66% of the planned total dose due to toxicity possibly related to the study medication	-
Failure to start Cycle 2 for more than 1 week (day 36 or later) due to persistent Grade ≥ 2 toxicities possibly related to the study medication, excluding fatigue	-
Any toxicity that is possibly related to the study medication and requires the withdrawal of the patient from the study during the DLT observation period	-
Any on-study death not clearly due to the underlying disease or extraneous causes	-

DLT window

The DLT window is defined as the time interval between the day of the first dose administration in Cycle 1 and the day of the first dose administration in Cycle 2 which is expected to be 28 days or up to 35 days in case of dose delay. For patients who do not receive Cycle 2, 35 days will be the time interval considered for the

evaluation of DLT, unless no toxicities are observed or recovery of toxicities occurs earlier or unless the patient has disease progression and/or starts a new anti-cancer therapy earlier.

Definition of MTD and RP2D

The MTD is the dose level at which 0/6 or 1/6 patients experience DLT in the first cycle of treatment with the next higher dose having at least 2/3 or 2/6 patients encountering DLT. Effectively, the MTD is the highest dose associated with first cycle DLT in < 33% of patients.

This dose or an inferior one may be the dose recommended for further Phase II investigation (RP2D).

Phase II

In the Phase II portion, patients will receive, as starting dose, the RP2D defined in the Phase I portion of the study.

The Phase I part of the trial ended on May 30th, 2022 with the declaration of an RP2D of 100 mg/m^2/week, and the study has already received a unanimous recommendation from an independent Data Safety Monitoring Board (DSMB) to continue the trial, moving to Phase II study.

The RP2D of 100 mg/m^2/week, 3 of 4 weekly schedule, is justified based on safety, efficacy and pharmacokinetic considerations. The trend of dose-related neutropenia as the major safety feature points to the 100 mg/m2/week level as more tractable relative to 135 mg/m^2 (refer to full safety summaries in the IB). In terms of anti-tumor activity, the fact that, out of 6 evaluable, one confirmed RECIST partial response (despite one missed dose during the first two cycles) plus one highly prolonged stable disease were observed at 100 mg/m^2 as well as, out of 4 evaluable, 2 patients with substantial AFP reduction were observed points to reasonable activity at this dose level. Furthermore, pharmacokinetics at the 100 mg/m2 dose level are in a meaningful range relative a continuous mouse tumor stabilization model predicting 0.2 uM average concentration threshold relative to the 100 mg/m2 mean Cmax in HCC patients which is 7 fold higher at 1.35 uM and with AUC just slightly below the mouse threshold, 32.5 uM*h versus 35. Refer to IB for full PK descriptions. RECIST response and AFP reductions were observed at the 135 mg/m^2/w level and PK was slightly higher but overall the anti-tumor pattern at 135 was similar to 100 mg/m^2. Therefore, overall, the 100 mg/m^2 level is justifiable as the RP2D.

Patients will be enrolled in continuous, and continuously evaluated for endpoints, with a futility analysis and stopping criteria for unacceptable toxicity performed as described in Section 12. The enrollment will be stopped in the event of futility as described in Section 12, with no more than 20 subjects treated overall until non-futility can be declared, if appropriate. For the safety monitoring, if two or more patients on study experience Grade 4 adverse events the study will be put on hold to evaluate whether to continue or if additional risk mitigation is warranted. In any case, the Sponsor may stop or pause enrollments any time during the study based on safety, efficacy or strategic reasons.

Statistical Methods:

Analysis Sets

The following analysis sets are defined according to ICH E9 guidelines.

Phase I Dose Escalation

Enrolled Set (ES): this population will include all patients who are enrolled in Phase I part, regardless of whether patients receive study treatment or not. This population will be evaluated in the analysis of patients' disposition.

Treated Set (TS): this set will include all enrolled patients who take at least one dose of NMS-01940153E. This population will be evaluated in the analysis of patients' disposition, baseline characteristics, treatment exposure, safety and efficacy.

Dose-Limiting Toxicity Evaluable Set (DLTES): this set will include all patients who receive in the first cycle at least 66% of study drug, unless the reason for non-compliance is drug-related toxicity, and undergo a DLT

assessment within the DLT window. Patients not fulfilling one or more of the aforementioned criteria will not be considered evaluable for MTD determination and will be substituted.

Phase II

Enrolled Set (ES): this population will include all patients enrolled in Phase II, regardless of whether patients receive study treatment or not. This population will be evaluated in the analysis of patients' disposition.

Treated Set (TS): this set will include all enrolled patients who actually receive at least one dose of NMS 01940153E. This population will be evaluated in the analysis of patients' disposition, baseline characteristics, treatment exposure and safety.

Evaluable Set (ES): this is the patient population for the primary efficacy analysis. This set will include patients of the TS who have measurable disease at baseline assessed according to RECIST 1.1 and at least one tumor evaluation on treatment, unless they die before the first tumor on-treatment assessment, in which case they will be considered treatment failure.

Primary Endpoint

Phase I Dose escalation

The primary endpoint, i.e. first cycle DLT, will be evaluated in the DLT Evaluable Set. All enrolled patients who received at least one dose of NMS-01940153E will be included in the analysis of safety.

Phase II

The primary endpoint, i.e. the ORR and its confidence interval (CI), will be evaluated in the Evaluable Set as the proportion of patients who have achieved as best overall response, confirmed complete response or partial response as defined by investigator-assessed RECIST 1.1.

Secondary Efficacy Endpoints

Objective response rate as measured by investigator-assessed mRECIST, and its CI will be provided for Phase II part only.

Duration of response, Progression Free Survival and Overall Survival will be provided for both phases. Time-to-event endpoints will be also summarized using Kaplan Meier (KM) curves and further characterized in terms of median and landmark estimates with the corresponding CI, for Phase II part only.

Safety analysis

Safety analyses will be carried out on the Treated Set by assigned dose level and overall for phase I part and overall for phase II part.

For both phases, adverse events and laboratory assessments will be scored according to the NCI CTCAE (version 5.0) grading system. The Medical Dictionary for Regulatory Activities (MedDRA) will be used to code the reported adverse events.

Pharmacokinetic analysis

Phase I Dose Escalation

The pharmacokinetics of NMS-01940153E and its metabolite NMS-03593478 in plasma and urine will be assessed by standard non-compartmental analysis. The following pharmacokinetic parameters will be provided on both Day 1 and Day 15: maximum concentration (Cmax) and related time of achievement (Tmax), last detectable concentration (Clast) and related time of observation (Tlast), area under concentration versus time up to 168 hours (dosing interval), half-life of the terminal phase ($t_{½,z}$), plasma clearance (CL), volume of distribution (Vss) and renal clearance (CLR).

Phase II

For PK plasma samples collected during Phase II, a sparse sampling procedure will be adopted.

Details of the sampling procedures as well as population PK model development description and population PK data analysis will be detailed in the population PK Statistical Analysis Plan.

Pharmacokinetic data from Phase II portion of the study will be analyzed using mixed-effect modeling approach. Data may be also pooled with PK data from previous Phase I studies.

The objective of the population pharmacokinetic data analysis will be to describe the pharmacokinetics of NMS-01940153E in the subject population and explore the role of demographic and pathophysiological covariates influencing the pharmacokinetics of the compound.

Sample Size Determination:

An overall sample size of approximately 55 patients may be anticipated considering both phases of the study.

For the Phase I, approximately 12-15 treated patients may be expected. Since the trial design foresees that sequential dose-escalation steps are applied to cohorts of 3 to 6 patients up to the identification of the MTD, the number of patients who will be enrolled and treated may vary, depending upon the toxicity observed that will influence cohort size and number of dose levels tested.

For the Phase II, the investigator-assessed objective response rate will be the primary outcome measure. The sample size is based on precision considerations for the estimate of the primary efficacy endpoint. A single-group design will be used to obtain a one-sided 95% lower-limit confidence interval for a single proportion. The sample proportion is assumed to be 0.3. To produce a confidence interval with a distance from the sample proportion to the lower limit of no more than 0.12, 38 patients will be needed.

A lower bound of 18% for ORR by RECIST 1.1 would be meaningful relative to second line therapies in HCC. The emergence of atezolizumab with bevacizumab in first line has obscured true current second line outcomes with regorafenib, lenvatinib or sorafenib but it is logical to assume second line ORR would be no better relative to the pre-atezolizumab/bevacizumab era in which sorafenib was the first line standard. Prior to the atezolizuman/bevacizumab era, regorafenib in second line showed ORR of 11% by mRECIST and cabozantinib of 4%, nivolumab 15%, pembrolizumab 18% and apatinib 10.7% assessed by RECIST 1.1. Therefore, in the third line setting following failure of atezolizumab/bevacizumab in first line followed by TKI in second line, and where current third line standards are not yet defined, it is reasonable to expect historical ORR no higher than 18%. Accounting for a 10% proportion of non-evaluable patients, up to 43 patients could be required for completing the trial, however, the Phase II part of the study will not enroll beyond 40 subjects unless discussed and agreed with FDA or relevant health authority based on emerging response rates.

Interim Analyses:

In the Phase II part of the study an interim evaluation for futility will be undertaken as soon as the first 10 evaluable patients will be enrolled and the data for primary efficacy endpoint analysis will be available including observations through at least 3 months with at least 1 on-treatment tumor assessment by RECIST 1.1. If less than 1 responder i.e. patients who achieved CR or PR as best overall response, are observed, the trial will be terminated for futility. This futility decision must be taken before 20 subjects total are enrolled otherwise enrollment will pause at 20 subjects until non-futility can be declared (if applicable). If at least 1 unconfirmed or confirmed responder is observed and there are no safety limitations, patients' enrollment will continue and proceed up to an overall enrollment of 38 evaluable patients or 40 total patients.
For the safety monitoring, if two or more patients on study experience Grade 4 adverse events the study will be put on hold to evaluate whether to continue or if additional risk mitigation is warranted. The independent DSMB will review the interim results and provide a recommendation on the study progress.

1.5. Subject Selection

Subject Inclusion Criteria: Subjects must meet all of the following inclusion criteria to be eligible for enrollment into the study:

1. Histological, cytological or radiological diagnosis of HCC, according to the American Association for the Study of Liver Diseases (AASLD) / European Association for the Study of the Liver (EASL) criteria, in

subjects that are refractory or not able to tolerate the standard therapy, or subjects for whom the standard therapy is not considered appropriate by the physician.

2. The subject has disease that is not amenable to a curative treatment approach (e.g., transplant, surgery, radiofrequency ablation) and unsuitable for or refractory to locoregional treatments (e.g., TACE).
3. At least one uni-dimensional measurable lesion by CT or MRI according to RECIST 1.1 which is either not previously treated by local therapy or, if treated, it has clearly progressed before the subject is recruited.
4. Phase I only: subjects must have disease relapsed or refractory to the standard of care treatment not exceeding 3 lines of prior systemic treatment. Subjects intolerant to previous treatment with TKIs are eligible.
   Phase II: subjects must have disease relapsed or refractory to the standard of care treatment including an immunocheckpoint inhibitor as first line and at least a tyrosine kinase inhibitor, not exceeding 3 lines of prior systemic treatment.
   A minimum of 14 days of treatment with prior TKI would be required to qualify as line of therapy.
5. Child-Pugh score ≤6 (class A, see Appendix 2).
6. Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1.
7. Age ≥18 years old on day of consent.
8. No history of liver transplantation or not listed for high urgent transplantation.
9. Required laboratory data:

Absolute neutrophil count (ANC)	≥ 1,500/mm^3 (≥ 1.5 x 10^9/L)
Platelets	≥ 60,000/mm^3 (≥ 60.0 x 10^9/L)
Hemoglobin*	≥ 10.0 g/dL
Albumin	≥ 3.0 g/dL (≥ 30 g/L)
Total serum bilirubin	≤ 1.5 x ULN and no more than 2 mg/dl, unless abnormality considered due to Gilbert's syndrome
Creatinine clearance**	> 60 mL/min
ALT/AST	≤ 5 x ULN
INR	≤ 1.5
*Transfusions of red blood cells or other hemocomponents not allowed within 72 h before treatment start.	
**Calculated using the Cockroft-Gault equation (mL/min): (140 – age) x weight (kg) / (serum creatinine [mg/dL]× 72) for males. (For females multiply by 0.85)	

10. In case of active hepatitis B (HBV) or chronic HIV infection the patient should receive antiviral therapy per local standard of care.
11. Patients must use effective contraception or abstinence. Female subjects must be surgically sterile or, if subjects of childbearing potential, must agree to use effective contraception (see Appendix 6) or abstinence during the period of therapy and in the following 180 days after discontinuation of study treatment. Male subjects must be surgically sterile or must agree to use effective contraception or abstinence during the period of therapy and in the following 90 days after discontinuation of study treatment.
12. With the exception of alopecia, resolution of all acute toxic effects of any prior systemic therapy, surgery or radiotherapy to NCI CTC (Version 5.0) Grade ≤1 or to the baseline laboratory values as defined in Inclusion Criteria Number 9.

13. Able and willing to comply with scheduled visits, therapy plans, and laboratory tests required in this protocol.

14. Signed and dated IEC-approved Informed Consent Form indicating that the subject is aware of the neoplastic nature of his/her disease and has been informed of the procedures to be followed, the investigational nature of the therapy, potential benefits, side effects, discomforts, risks and alternative treatments.

Subject Exclusion Criteria: The presence of any of the following will exclude a subject from study enrollment:

1. Known fibrolamellar HCC or mixed hepato-cholangiocarcinoma.
2. Subjects with untreated or incompletely treated gastro-esophageal varices with bleeding or at high risk for bleeding are excluded with the following clarification: subjects with history of prior variceal bleeding must have been treated with adequate endoscopic therapy without any evidence of recurrent bleeding for at least 6 months prior to study entry and must be stable on optimal medical management (e.g. non-selective beta blocker, proton pump inhibitor) at study entry.
3. Subjects with QTcF interval ≥480 milliseconds or with risk factors for torsade de pointes (e.g., heart failure, uncontrolled hypokalemia, family history of long QT syndrome) or receiving treatment with concomitant medications known to prolong the QT/QTc interval that cannot be replaced with another treatment.
4. Ascites defined as CTCAE Grade ≥2. Subjects who have been on a stable medication regimen for at least 2 months to manage ascites are eligible if they show ascites Grade <2. Subjects with clinically undetectable ascites who are Child A with detectable ascites at CT/MRI are eligible.
5. Uncontrolled high blood pressure (systolic blood pressure, SBP >150 mmHg and/or diastolic blood pressure, DBP >95 mmHg, despite optimal treatment, on at least 2 out of 3 determinations repeated at 30 minutes interval and done in case that the first one meets the criterion for exclusion).
6. Direct-Acting Antivirals (DAA) at the time of treatment start; previous HCV treatment with DAAs is allowed.
7. Clinical evidence of hepatic encephalopathy.
8. Known brain metastases or evidence of leptomeningeal disease.
9. Known history of allergic reactions to polysorbate 80.
10. Any of the following in the past 6 months: myocardial infarction, uncontrolled cardiac arrhythmia, unstable angina, coronary/peripheral artery bypass graft, symptomatic congestive heart failure, cerebrovascular accident or transient ischemic attack, pulmonary embolism, deep vein thrombosis (except chronic/stable portal vein thrombosis).
11. Major surgery, other than diagnostic surgery, within 4 weeks before treatment start.
12. Any anticancer agent within 4 weeks or, in absence of toxicity, 5 half-lives (within 6 weeks for nitrosureas, mitomycin C and liposomal doxorubicin) before treatment start.
13. Radiation therapy within 4 weeks or radionuclide treatment (e.g., I-131 or Y-90) within 6 weeks before treatment start.
14. Untreated uncontrolled bacterial, viral, or fungal infections including acute HIV infection or acquired immunodeficiency syndrome (AIDS), untreated uncontrolled HBV, untreated uncontrolled HCV, untreated uncontrolled concomitant HBV and HCV; patients who are seropositive following HBV vaccine are eligible.
15. Subjects under treatment with therapeutic dose of anticoagulants (e.g., warfarin or warfarin-related agents, low-molecular weight heparin, or similar agent such as anti Xa and anti-thrombin agents) or antiplatelet

agents (e.g., clopidogrel) or with coagulation disorders. Aspirin at dose up to 100 mg is permitted. Prophylaxis with anticoagulants is allowed to meet the INR value range as cited in inclusion criterion 9.

16. Uncontrolled diabetes mellitus.
17. Pregnant or breast-feeding women.
18. Known second malignancy that is progressing or requiring active treatment. Exceptions include adequately treated basal cell or squamous cell skin cancer or in situ carcinoma of the cervix uteri.
19. Current enrollment or participation in another interventional clinical trial.
20. Clinically significant respiratory or metabolic diseases uncontrolled by medication.
21. Subjects with active alcohol and/or substances abuse.
22. Any known organ dysfunction, serious illness, acute or chronic medical or psychiatric condition, or laboratory abnormality which, in the Investigator's opinion, may increase the risk associated/interfere with study participation, or with the interpretation of the results.
23. Subjects who, within 7 days prior to the first NMS-01940153E intake, are receiving or received strong inducers of FMO1 and FMO3.
24. Subjects who are receiving sensitive CYP3A4 substrates, CYP3A4 and BCRP substrates with narrow therapeutic index (NTI).

1.6. Schedule of Events

See protocol Section 7

2. ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BCLC	Barcelona clinic liver cancer
BCRP	Breast cancer resistance protein
BOR	Best overall response
BSA	Body surface area
BUN	Blood Urea Nitrogen
Ca	Calcium
CI	Confidence interval
CL	Clearance
Clast	Last detectable concentration
CLR	Renal clearance
Cmax	Maximum concentration
CR	Complete response
CRF	Case report form
CT scan	Computed Tomography
CTCAE	Common terminology criteria for adverse events
CYP	cytochrome
DAA	Direct-acting antivirals
DBP	Diastolic blood pressure
DLT	Dose limiting toxicity
DNA	Deoxyribonucleic acid
DoR	Duration of response
DSMB	Data safety monitoring board
ECG	Electrocardiogram
ECOG PS	Eastern cooperative oncology group performance status
eCRF	Electronic case report form
EoT	End of treatment
FIH	First in human
FMO	Flavin-containing monooxygenase
FU	Follow up

G	Grade
Gamma(γ)-GT	Gamma-glutamyl transferase
GCD	Global Clinical Development
G-CSF	Granulocyte-Colony stimulating factor
h	Hour
Hb	Hemoglobin
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HR	Hazard ratio
IB	Investigator brochure
ICF	Informed consent form
IMP	Investigational medicinal product
INR	International normalized ratio
IV	Intravenous
K	Potassium
LDH	Lactate dehydrogenase
MedDRA	Medical dictionary for regulatory activities
MPS1	Monopolar spindle 1
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose
Na	Sodium
NCI	National cancer institute
NE	Not evaluable
NMS	Nerviano Medical Sciences
NTI	Narrow therapeutic index
ORR	Overall response rate
OS	Overall survival
P	Phosphorus
PD	Progressive disease
PET	Positron emission tomography
PFS	Progression free survival
PK	Pharmacokinetic

PLT	Platelet
PR	Partial response
RBC	Red blood cell
RECIST	Response evaluation criteria in solid tumours
RNA	Ribonucleic acid
RP2D	Recommended phase 2 dose
SAC	Spindle assembly checkpoint
SAE	Serious adverse event
SBP	Systolic blood pressure
SD	Stable disease
SOC	System organ class
$t_{1/2, z}$	Half-life of the terminal phase
TACE	Transarterial chemoembolization
TEAE	Treatment emergent adverse event
TKI	Tyrosine kinase inhibitor
TTK	Tyrosine/threonine kinase
ULN	Upper limit normal
USA	United State of America
Vss	Volume of distribution
WBC	White blood cell

3. BACKGROUND INFORMATION AND STUDY RATIONALE

3.1. Disease background

Primary liver cancer is the 6th most common neoplasm and among the leading cause of cancer-related deaths worldwide. Hepatocellular carcinoma (HCC) constitutes 80-90% of all primary liver cancers [1, 2].

The incidence is increasing and will soon surpass one million annual cases worldwide with an age-adjusted incidence of 10.1 cases per 100,000 person-years. In 2019 the estimated incidence in US was 42.030 new cases/year diagnosed with estimated disease related deaths of 31.780. The disease is more frequent in males than in females, in the ratio of 2.4:1 [1, 2].

According to the Italian Cancer Registry, in 2018 12.800 new cases of HCC were documented in Italy. The prognosis of the disease is poor with a 5-years survival rate of only 18-20% based on US and Italian cancer registry data [1, 2].

HCC most common etiological factor in the world is hepatitis B virus (HBV) infection. The worldwide incidence is heterogeneous because of the variable prevalence of risk factors. Almost 80% of HCC cases occur in sub-Saharan Africa and eastern Asia and the major risk factors here are hepatitis B and aflatoxin exposure, whereas hepatitis C and Non-alcoholic fatty liver disease (NAFLD) is the primary risk factor in the USA, Europe, and Japan. The development of cirrhosis is associated with high risk for developing HCC [3, 4].

Coexistence of cancer and cirrhosis in HCC is an essential hallmark that has shaped clinical trial design over the time and is outlined in the Barcelona Clinic Liver Cancer (BCLC) algorithm.

The BCLC staging system is an effective, integrated system that takes into account the patient performance status (PS), tumor burden, vascular involvement, potential hepatic dysfunction, and portal hypertension [5].

At early stages of HCC (BCLC stage 0/A), treatment strategies are potentially curative and include liver resection, liver transplantation, and ablation. At intermediate stages (BCLC stage B), palliative locoregional therapies such as transarterial chemoembolization (TACE) remain the gold standard for treatment. However, most HCC cases are diagnosed at advanced stages (BCLC stage C) when curative therapies or TACE are no longer applicable [5].

Data available in the literature highlight the need to limit prior locoregional therapies in these patients to avoid irreversible deterioration of liver function or general health status and switch to systemic therapy if no response is observed after TACE. Despite the advances in disease care and treatment strategies, patients with advanced HCC still present a poor survival outcome [6-7].

Until recently, sorafenib, an oral multikinase inhibitor, was the only approved systemic therapy for HCC. The first Phase III trial of sorafenib versus placebo demonstrated a

significant, yet modest improvement in median overall survival (OS; 10.7 vs. 7.9 months, respectively; hazard ratio [HR], 0.69; 95% confidence interval [CI], 0.55–0.87; $p < 0.001$) [8,9].

The therapeutic landscape changed remarkably in 2017 with the approval of regorafenib as second-line treatment for patients with HCC who have been previously treated with sorafenib. In the Phase III trial, regorafenib moderately improved median OS over placebo (10.6 months vs. 7.8 months; HR, 0.63; 95% CI: 0.50–0.79; $p < 0.0001$) and progression-free survival (PFS; 3.1 months vs. 1.5 months; HR, 0.46; 95% CI: 0.37–0.56; $p < 0.0001$). When well tolerated, treatment with sorafenib followed by regorafenib (at radiologic progression on sorafenib) results in a median OS of 26 months. However, when this sequence is not possible, due to lack of efficacy or intolerance to these drugs, the prognosis remains poor [10 - 13].

The Phase III trial of lenvatinib demonstrated non-inferiority to sorafenib in the first-line treatment of advanced HCC, with a median OS of 13.6 months compared to 12.3 months, respectively (HR, 0.92; 95% CI: 0.79–1.06). Based on these data, in 2018 lenvatinib was approved in Japan, Europe and the USA as a first-line treatment in HCC [12 - 13].

In 2019, also cabozantinib and ramucirumab received regulatory approval for HCC patients previously treated with sorafenib. In addition, the United States Food and Drug Administration (FDA) approved pembrolizumab and, recently, ipilimumab in combination with nivolumab, for use in the second line following sorafenib [12, 13].

A Phase III trial of atezolizumab combined with bevacizumab demonstrated significantly better overall survival and progression-free survival outcomes than sorafenib in patients with advanced unresectable HCC not previously treated with systemic therapy. The combination was approved in 2020 and is now the preferred 1st line treatment option [14] both for NCCN and ESMO guidelines. Despite the availability of these new therapeutic options, the overall survival of patients with HCC who have failed a first-line therapy remains dismal, in the range of only 8-10 months in randomized studies [12-14].

Regardless of the availability of therapeutic agents in first and second lines, the failure of systemic treatment remains a major therapeutic challenge and relapsed/refractory HCC still represents an area of unmet medical need.

Patients with good performance status and compensated liver function who progress after all approved treatments are considered suitable for new investigational drugs or strategies.

3.2. Study rationale

NMS-01940153E, also known as S81694 (from now on called NMS-01940153E), is a dihydropyrazoloquinazoline derivative, potent small molecule inhibitor of Monopolar Spindle 1 kinase (MPS1 which is also known as TTK kinase). MPS1 is a dual tyrosine/threonine kinase expressed only in proliferating cells. Upon activation by phosphorylation, MPS1 plays a critical role in the control of mitosis regulating the Spindle Assembly Checkpoint (SAC)

through proper kinetochore recruitment of other essential SAC proteins. The SAC complex regulates a mitotic mechanism required for proper chromosome alignment, influencing the stability of the kinetochore-microtubule interaction and ensuring that cells do not divide until all sister chromatids correctly align to the metaphase plate.

Most solid tumors are characterized by complex chromosome rearrangements. Aneuploidy is a common feature of cancer cells: approximately 90% of solid tumors are aneuploid, with the majority (> 70%) having a gain rather than a loss of chromosomes. MPS1 has been found highly expressed in a number of tumors of different origins.

3.3. Non-clinical data

In tumor cells exposed to NMS-01940153E, anti-proliferative activity is associated with the expected mechanism of action of an MPS1 inhibitor. It causes mitotic checkpoint override, accelerates mitosis, induces chromosomal misalignment, and promotes aneuploidy, apoptosis and cancer cell death, accompanied by reduction of mitotic markers such as phospho-histone H3 and MPS1 auto-phosphorylation.

In vitro, NMS-01940153E is active on a broad panel of cancer cell lines. Particularly high sensitivity was observed in [redacted] hepatocellular carcinoma cell lines, whereas normal cell lines resulted to be less affected by the treatment. Confirming the slow dissociation rate of NMS-01940153E, a relatively brief period of exposure to the compound is sufficient to cause cancer cell death.

In hepatocellular carcinoma cell lines, single agent antiproliferative activity was observed. Moreover, MPS1 is expressed in all tested HCC cell lines.

In vivo, in various xenograft or transgenic tumor models [redacted] after intravenous (IV) administration, the compound demonstrated significant tumor growth inhibition, up to 95% with an intermittent schedule at well-tolerated doses from 15 to 30 mg/kg [15].

3.4. Toxicology

Nonclinical safety of NMS-01940153E was characterized in single-dose toxicity studies after IV administration in rats, dogs and monkeys and in repeated toxicity studies after once weekly IV administration for 4 weeks in rats and monkeys. In the pivotal GLP 4-week toxicity studies the major target organs observed following the repeated administration of NMS-01940153E were the haemolymphopoietic system (HLP) in rats and monkeys, the intestinal tract in rats and the male reproductive organs particularly in rats at high doses. In addition, injection site alterations were seen in both species.

NMS-01940153E was not genotoxic in the Ames test but, as expected based on its mechanism of action, induced micronuclei in human peripheral lymphocytes *in vitro*.

NMS-01940153E induced haemolysis was observed *in vitro* on human blood at high concentrations (≥ 320 µM); no haemolysis occurred up to a concentration of 200 µM.

NMS-01940153E was not irritating to the skin of rabbits while it induced severe ocular irritation following the application to the rabbit of 100 mg/eye for 1 hour.

In the Safety pharmacology investigations, a dose-related inhibition of the IKr potassium channel was observed in the hERG assay with a calculated inhibitory concentration 50 (IC50) of 13 µM (about 13- to 650-fold the anti-proliferative IC50 and well above the Cmax measured in vivo). In a telemetry-monitored cardiovascular study in monkeys, the dose of 4 mg/kg induced a transient moderate increase in arterial pressure but had no effect on heart rate, ECG intervals (including QT and QTc) and body temperature.

According to the toxicity studies, NMS-01940153E safety profile was considered manageable, with target organ toxicity in line with its pharmacological mechanism of action [15].

3.5. Clinical data

NMS-01940153E originated in Nerviano Medical Sciences and was licensed out to Servier in 2013. Two clinical studies were performed by Servier. A Phase I, First in Human trial (FIH) was conducted with NMS-01940153E as single-agent, in patients with advanced/metastatic solid tumors (EudraCT 2014-002023-10); a Phase I-II study of NMS-01940153E in combination with paclitaxel was conducted in patients with advanced/metastatic breast cancer (EudraCT 2017-002459-27). In 2019 Servier decided to stop the clinical development of NMS-01940153E due to internal portfolio decisions and the compound was returned to Nerviano Medical Sciences.

The Phase I, FIH study, was a dose-escalation study of NMS-01940153E administered to sequential cohorts of adult patients with advanced/metastatic solid tumors who had exhausted standard treatment options or for whom no standard treatment was available. The objective of the study was to define the Maximum Tolerated Dose (MTD) and the associated Dose-Limiting Toxicities for NMS-01940153E administered as IV infusion on days 1, 8, 15 in repeated 4-week cycles [16].

The study started in September 2015 and was stopped on July 2019, following the Sponsor's (Servier) decision to stop the development of NMS-01940153E as a single agent. As a consequence, the primary objective of the study (to define the Maximum Tolerated Dose) was not met.

Overall 39 patients were enrolled and 38 received treatment with NMS-01940153E at nine different dose levels: 4 (6 patients), 6 (3 patients), 9 (3 patients), 13.5 (4 patients), 20 (6 patients), 30 (3 patients), 45 (3 patients), 67.5 (4 patients), and 135 mg/m^2/wk (7 patients). The median age at study entry was 58.5 years; ECOG performance status was 0 in 16 patients (42.1%) and 1 in 22 (57.9%).

The most frequent cancer types at study entry were: cancers of the gastrointestinal tract (23.7%), cancers of lung (13.2%), cancer of head and neck (10.5%), sarcomas of the soft tissue and bone (7.9%), benign and malignant mesothelioma, cancer of the breast and cancers of the endocrine system (5.3% each) [16].

3.6. Safety results

Among 36 patients evaluable for first cycle Dose Limiting Toxicities, 3 DLTs were reported: grade 3 anemia, at 4 mg/m^2/week; grade 4 hypertensive crisis, at 20 mg/m^2/week; grade 3 fatigue, at 135 mg/m^2/week [16].

The most frequent treatment emergent adverse events (TEAEs) reported in at least 15% of patients were fatigue (57.9%), anaemia (44.7%), nausea (31.6%), decreased appetite (28.9%), constipation, pain, vomiting (23.7% each), cough (21.1%), diarrhea (18.4%) and dyspnea (15.8%) [16].

The most frequent TEAEs related to NMS-01940153E reported in at least 10% of patients were anaemia (36.8%), fatigue (28.9%), nausea (21.1%), decreased appetite (15.8%), neutropenia (13.2%) and diarrhea (10.5%) [16].

Among the 4 patients treated at 67.5 mg/m^2/week, the TEAE related to NMS-01940153E reported were nausea (2 patients G1), anemia (1 patient G2), decreased appetite (1 patient G1), fatigue (1 patient G1) and platelet count decreased (1 patient G3) [16].

Among the 7 patients treated at 135 mg/m^2/week, the TEAE related to NMS-01940153E reported were anemia (1 patient G3, 3 patients G2), neutropenia (4 patients G3), decreased appetite (2 patients G2, 1 patient G1), diarrhoea (2 patients G2, 1 patient G1), fatigue (1 patient G3, 1 patient G2), nausea (1 patient G2, 1 patient G1), haemolysis (1 patient G1), keratitis (1 patient G3), mucosal inflammation (1 patient G3) and pain (1 patient G2) [16].

3.7. Efficacy results

The exploration of efficacy was a secondary endpoint for the study and based on objective tumour response as defined by the Response Evaluation Criteria in Solid Tumours (RECIST version 1.1) [17].

The best overall response (BOR) was defined as the best response recorded from the start of the investigational drug until the end of treatment, according to Investigator's assessments.

Out of the 39 enrolled patients, 3 patients were excluded from the efficacy analysis due to: absence of measurable disease at baseline, no dose of NMS-01940153E received, no on treatment tumour assessment performed. Out of the 36 patients evaluable for efficacy, one patient could not be evaluated since he/she died before the first scheduled tumour assessment.

Out of the 35 patients for whom a BOR was assessed, one patient, with metastatic clear renal cell cancer, achieved a complete tumour response, with response duration of 112.7 weeks; 13

patients showed stable disease, including 2 patients with HCC. The duration of the stabilization ranged from 6.43 (censored observation) to 67.29 weeks, with a median duration of 24.29 weeks (95%CI: 12.29-67.29).

Although only 2 patients with HCC refractory to multiple prior therapies were enrolled in the study, both remained stable for 6 months while on NMS-01940153E and one of them, treated at 135 mg/m^2/week, had a transient decrease in tumor size. On these grounds, we hypothesize that monotherapy treatment in previously treated patients with advanced HCC might be further explored.

4. TRIAL OBJECTIVES AND ENDPOINTS

4.1. Objectives

4.1.1. Primary Objective

- To determine the Maximum Tolerated Dose (MTD) and Recommended Phase 2 Dose (RP2D) of NMS-01940153E administered as single agent to adult patients with unresectable hepatocellular carcinoma (HCC) previously treated with systemic therapy (Phase I).
- To assess the anti-tumor efficacy of NMS-01940153E administered as single agent to adult patients with unresectable hepatocellular carcinoma previously treated with systemic therapy (Phase II).

4.1.2. Secondary Objective(s)

- To define the safety and tolerability profile of NMS-01940153E.
- To evaluate the pharmacokinetics of NMS-01940153E and its main metabolite NMS-03593478 in plasma and, limited to Phase I, also in urine.
- To evaluate additional measures of antitumor efficacy of NMS-01940153E.

4.1.3. Explorative Objective:

- To explore correlation among Alpha-Fetoprotein (AFP) levels changes with NMS-01940153E exposure and clinical outcomes;
- To explore correlation among baseline tumoral and clinical prognostic factors with clinical outcome;
- To explore the potential correlation of selected molecular alterations in circulating free DNA (cfDNA) at baseline with clinical outcome.

4.2. Endpoints

4.2.1. Primary Endpoint

- Drug related Dose Limiting Toxicities (DLTs) (Phase I).

- Objective Response Rate (ORR). The ORR will be calculated as the proportion of evaluable patients who have achieved, as best overall response (BOR), confirmed complete response (CR) and partial response (PR) measured by investigator-assessed RECIST 1.1 (Phase II).

4.2.2. Secondary Endpoint(s)

- Overall safety profile of NMS-01940153E characterized by type, severity (graded using NCI CTCAE Version 5.0), duration of the adverse events (AEs) and laboratory and ECGs abnormalities, and relationship of the AEs to study treatment in the first and subsequent cycles of therapy.
- Pharmacokinetic parameters of NMS-01940153E and its main metabolite NMS-03593478 in plasma and urine (urine only in Phase I).
- Objective Tumor Response (Partial and Complete Response) as measured by investigator-assessed RECIST 1.1 (Phase I).
- Objective response rate as measured by investigator-assessed mRECIST (Phase II).
- Duration of response (DoR) as measured by investigator-assessed RECIST 1.1. and investigator-assessed mRECIST
- Progression Free Survival, including landmark analyses, as measured by investigator-assessed RECIST 1.1.
- Overall survival (OS).

4.2.3. Exploratory Endpoint:

- Evaluation of AFP plasma levels;
- Evaluation of tumoral and other clinical prognostic features at baseline;
- Assessment of selected molecular alterations in circulating free DNA (cfDNA) at baseline.

5. TRIAL DESIGN AND DESIGN RATIONALE

This is a Phase I/II, open-label, non-randomized, multicenter study to explore safety, tolerability and antitumor activity of NMS-01940153E as single agent in adult patients with unresectable HCC previously treated with systemic therapy.

An independent Data Safety Monitoring Board (DSMB) will provide study oversight through periodic safety evaluations and make recommendations on study progress in the transition from Phase I to Phase II and at the planned interim analysis for the futility of Phase II.

The Phase I portion is designed as a dose-escalation study in sequential cohorts of patients aimed to obtain the MTD that is defined based on the DLTs observed in the first cycle of treatment. The selection of the starting dose and the dose escalation scheme is based on the safety information gained in the FIH study (EudraCT 2014-002023-10) and in consideration of the possible different tolerability in patients with compromised liver.

Once the MTD is identified and the safety profile of NMS-01940153E has been reviewed by the Investigators, the Sponsor and the independent DSMB and is considered adequate based on the entire Phase I data available, the Phase II portion of the study started.

The Phase II portion is designed as a two-stage study with an interim analysis for futility and stopping criteria for unacceptable toxicity to assess the antitumor activity of NMS-01940153E in adult patients with unresectable HCC previously treated with systemic therapy measured as objective response rate.

5.1. Treatment administration:

NMS-01940153E is supplied as labeled type I glass vials containing 30 mg/vial of powder for solution for infusion. Each labeled vial (primary packaging) is packaged in a labeled single small carton (secondary packaging).

Patients will receive treatment with NMS-01940153E administered as intravenous (IV) infusion for 3 consecutive weeks on days 1, 8, 15 followed by 1 week of rest, in a 28-day cycle. The duration of infusion will be dependent on the total dose to be administered:

- 1 hour infusion if ≤255 mg are administered
- 1 hour 30 minutes infusion if >255 mg but <375 mg are administered

Within one cycle, in case the patient cannot be retreated on the day foreseen because of toxicity, the dose will be skipped and the patient will be retreated at the next foreseen time-point.

Dose reduction/omission is foreseen based on toxicities observed (see Section 9.2.3.1).

Patients that cannot be retreated for any reason within 1 week from day 28 (up to day 35) will go off-treatment, unless the investigator believes treatment continuation is in the patient's best

interest. In this case a maximum delay of 2 weeks (up to day 42) will be allowed. Otherwise patients may continue on investigational drug until disease progression, unacceptable toxicity, withdrawal of consent by the patient or other discontinuation criteria described in the protocol are met.

Phase I Dose Escalation

The safety profile of NMS-01940153E administered IV on days 1, 8, 15 every 4 weeks has been characterized in the FIH, dose escalation study, conducted in adult patients with advanced/metastatic solid tumors up to the dose of 135 mg/m^2/week. This dose was not defined as MTD in study 2014-002023-10 (EudraCT), since only one patient experienced DLT (Grade 3 fatigue) out of 6 evaluable patients.

Considering the possible different tolerability in patients with compromised liver, the proposed starting dose for the Phase I portion of the study is 100 mg/m^2/week, which is an intermediate dose level between the highest (135 mg/m^2/week) and the next lower dose level (67.5 mg/m^2/week) tested in the FIH study [16]. According to the observed safety profile, dose increments of 25-35% will be applied to the proposed starting dose, until the MTD is reached. In presence of unacceptable toxicity observed at 100 mg/m^2/week, a lower dose level by 25-35% may be tested.

Patients experiencing DLT during the DLT window can be retreated according to the time delay and dose modification schema.

No intra-patient dose escalation will be allowed.

Phase II

In the Phase II portion of the study, patients will be treated at the RP2D defined in the Phase I portion as starting dose.

5.2. Phase I Dose Escalation

A conventional 3+3 study design with inter-individual dose-escalations will be adopted. Patients will be allocated to sequential cohorts of progressively higher dose levels of NMS-01940153E based on the presence of Dose Limiting Toxicities (DLT).

At each dose level, a minimum of 3 patients will be included.

If 0/3 patients experiences first cycle DLT, the next cohort will start one dose level higher.

If 1/3 patients experiences first cycle DLT, up to three more patients will receive the study medication at the same dose level; if 1/6 patients experiences first cycle DLT the next cohort will start one dose level higher.

If ≥2/3 or ≥2/6 patients experience DLTs in the first cycle of treatment, the MTD is considered to have been exceeded. Three more patients will be entered at the previous dose level (if only 3 patients were previously treated at that prior dose) or, in case the MTD is exceeded at the starting dose level, six patients will be entered to a lower dose level.

Only one dose level will be open for enrollment at any time.

The first patient enrolled on a dose level should be observed for DLT for at least 2 weeks before entering any other patient at that dose level.

In absence of 1st cycle DLT in the first patient, the third patient can be enrolled at any time.

In presence of 1st cycle DLT in the first patient, the third patient can be enrolled only after the second patient has completed the first cycle without DLT.

In case a cohort needs to be expanded to more than 3 patients, the additional patients can be enrolled simultaneously.

All patients must be observed for one cycle before subsequent patients are enrolled at the next higher dose level. If a patient fails to receive at least 66% of the first cycle of treatment, for reasons other than treatment-related toxicities, an additional patient must be enrolled at the same dose level.

After the completion of each cohort, available safety and pharmacokinetic (PK) data will be reviewed and new dose levels were decided jointly by the Investigators and the Sponsor.

5.2.1. Definition of DLTs

For this protocol, a DLT will be defined as any of the following events for which the relationship to NMS-01940153E cannot be definitely excluded. Toxicities are to be graded according to the NCI CTCAE version 5.0 [21].

Table 1. DLT criteria	
Hematological Toxicity	
Neutropenia	Grade 4 lasting >7 days or Grade 4 of any duration associated with Grade ≥2 infection
Febrile neutropenia	ANC <1000/mm^3 and a single temperature of >38.3 degrees C or a sustained temperature of ≥38 degrees C for more than one hour.
Thrombocytopenia	Grade 3 associated with any grade bleeding or Grade 4
Anaemia	Grade ≥4
Non-Hematological Toxicity	
Nausea, vomiting or diarrhea	Grade ≥3 despite optimal treatment
Signs of potential severe drug-induced liver injury as assessed according to Hy's law	Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >3 ULN and total bilirubin >2 ULN in the absence of cholestasis (alkaline phosphatase [ALP] <2 ULN) and no other reason can be identified.
Any other non-hematological toxicities;	Grade ≥ 3 toxicities unless clear alternate etiology is documented by the investigator and except: - asymptomatic Grade 3 electrolyte changes that can be successfully supplemented (e.g., hypokalemia) and resolve/recover to baseline within 72 hours - Grade 3 fatigue that improves to ≤ Grade 2 in no more than 5 days - alopecia
Other significant toxicity	
Failure to complete the first cycle of treatment with at least 66% of the planned total dose due to toxicity possibly related to the study medication	-
Failure to start Cycle 2 for more than 1 week (day 36 or later) due to persistent Grade ≥ 2 toxicities possibly related to the study medication, excluding fatigue	-
Any toxicity that is possibly related to the study medication and requires the withdrawal of the patient from the study during the DLT observation period	-
Any on-study death not clearly due to the underlying disease or extraneous causes	-

5.2.2. DLT window

The DLT window is defined as the time interval between the day of the first dose administration in Cycle 1 and the day of the first dose administration in Cycle 2 which is expected to be 28 days or up to 35 days in case of dose delay. For patients who do not receive Cycle 2, 35 days will be the time interval considered for the evaluation of DLT, unless no toxicities are observed, or recovery of toxicities occurs earlier or unless the patient has disease progression and/or starts a new anti-cancer therapy earlier.

5.2.3. Patient replacement

During the first cycle of the dose-escalation phase, patients who meet any of the following criteria will not permit the DLT evaluation and therefore will be replaced:

- Did not receive at least 66% of cycle study drug, unless treatment was stopped due to toxicity possibly related to the study medication;
- Did not undergo DLT assessment in the DLT window.

5.2.4. Definition of MTD and RP2D

The MTD is the dose level at which 0/6 or 1/6 patients experience DLT in the first cycle of treatment with the next higher dose having at least 2/3 or 2/6 patients encountering DLT. Effectively, the MTD is the highest dose associated with first cycle DLT in < 33% of patients.

This dose or an inferior one may be the dose recommended for further Phase II investigation (RP2D).

5.3. Phase II

The Phase I part of the trial ended on May 30th, 2022 with the declaration of an RP2D of 100 mg/m2/week, and the study has already received a unanimous recommendation from an independent Data Safety Monitoring Board (DSMB) to continue the trial, moving to Phase II study.

The RP2D of 100 mg/m2/week, 3 of 4 weekly schedule, is justified based on safety, efficacy and pharmacokinetic considerations. The trend of dose-related neutropenia as the major safety feature points to the 100 mg/m2/week level as more tractable relative to 135 mg/m2 (refer to full safety summaries in the IB). In terms of anti-tumor activity, the fact that, out of 6 evaluable, one confirmed RECIST partial response (despite one missed dose during the first two cycles) plus one highly prolonged stable disease were observed at 100 mg/m2 as well as, out of 4 evaluable, 2 patients with substantial AFP reduction were observed points to reasonable activity at this dose level. Furthermore, pharmacokinetics at the 100 mg/m2 dose level are in a meaningful range relative a continuous mouse tumor stabilization model predicting 0.2 uM average concentration threshold relative to the 100 mg/m2 mean Cmax in HCC patients which is 7 fold higher at 1.35 uM and with AUC just slightly below the mouse threshold, 32.5 uM*h versus 35. Refer to IB for full PK descriptions. RECIST response and AFP reductions were observed at the 135 mg/m2/w level and PK was slightly higher but overall the anti-tumor pattern at 135 was similar to 100 mg/m2. Therefore, overall, the 100 mg/m2 level is justifiable as the RP2D.

In the Phase II portion, patients will receive the RP2D defined in the Phase I portion of the study as starting dose.

As soon as signs of toxicity are observed requiring a dose de-escalation, the dose will be decreased as described in Section 9.2.3.1, Table 6). A maximum of two dose decreases will be allowed, otherwise patient will have to be withdrawn from the study.

Patients will be enrolled in continuous, and continuously evaluated for endpoints, with a futility analysis and stopping criteria for unacceptable toxicity performed as described in Section 12. The enrollment will be stopped in the event of futility as described in Section 12, with no more than 20 subjects treated overall until non-futility can be declared, if appropriate. In any case, the Sponsor may stop or pause enrollments any time during the study based on safety, efficacy or strategic reasons.

6. SUBJECT SELECTION

This clinical trial can fulfill its objectives only if appropriate subjects are enrolled. The following eligibility criteria are designed to select subjects for whom protocol treatment is considered appropriate. All relevant medical and non-medical conditions should be taken into consideration when deciding whether this protocol is suitable for a particular subject. Prospective approval of protocol deviations to recruitment and enrolment criteria, also known as protocol waivers or exemptions, is not permitted.

6.1. Subject Inclusion Criteria

Subjects must meet all of the following inclusion criteria to be eligible for enrollment into the study:

1. Histological, cytological or radiological diagnosis of HCC, according to the American Association for the Study of Liver Diseases (AASLD) / European Association for the Study of the Liver (EASL) criteria, in subjects that are refractory or not able to tolerate the standard therapy, or subjects for whom the standard therapy is not considered appropriate by the physician.
2. The subject has disease that is not amenable to a curative treatment approach (e.g., transplant, surgery, radiofrequency ablation) and unsuitable for or refractory to locoregional treatments (e.g., TACE).
3. At least one uni-dimensional measurable lesion by CT or MRI according to RECIST 1.1 which is either not previously treated by local therapy or, if treated, it has clearly progressed before the subject is recruited.
4. Phase I only: subjects must have disease relapsed or refractory to the standard of care treatment not exceeding 3 lines of prior systemic treatment. Subjects intolerant to previous treatment with TKIs are eligible.
   Phase II: subjects must have disease relapsed or refractory to the standard of care treatment including an immunocheckpoint inhibitor as first line and at least a tyrosine kinase inhibitor, not exceeding 3 lines of prior systemic treatment.
   A minimum of 14 days of treatment with prior TKI would be required to qualify as line of therapy;
5. Child-Pugh score ≤ 6 (class A, see Appendix 2).
6. Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1.
7. Age ≥18 years old on day of consent.
8. No history of liver transplantation or not listed for high urgent transplantation.

9. Required laboratory data:

Absolute neutrophil count (ANC)	≥ 1,500/mm^3 (≥ 1.5 x 10^9/L)
Platelets	≥ 60,000/mm^3 (≥ 60.0 x 10^9/L)
Hemoglobin*	≥ 10.0 g/dL
Albumin	≥ 3.0 g/dL (≥ 30 g/L)
Total serum bilirubin	≤ 1.5 x ULN and no more than 2 mg/dl, unless abnormality considered due to Gilbert's syndrome
Creatinine clearance**	> 60 mL/min
ALT/AST	≤ 5 x ULN
INR	≤ 1.5
*Transfusions of red blood cells or other hemocomponents not allowed within 72 h before treatment start.	
**Calculated using the Cockroft-Gault equation (mL/min): (140 – age) x weight (kg) / (serum creatinine [mg/dL]× 72) for males. (For females multiply by 0.85)	

10. In case of active hepatitis B (HBV) or chronic HIV infection the patient should receive antiviral therapy per local standard of care.

11. Patients must use effective contraception or abstinence. Female subjects must be surgically sterile or, if subjects of childbearing potential, must agree to use effective contraception (see Appendix 6) or abstinence during the period of therapy and in the following 180 days after discontinuation of study treatment. Male subjects must be surgically sterile or must agree to use effective contraception or abstinence during the period of therapy and in the following 90 days after discontinuation of study treatment.
12. With the exception of alopecia, resolution of all acute toxic effects of any prior systemic therapy, surgery or radiotherapy to NCI CTC (Version 5.0) Grade ≤1 or to the baseline laboratory values as defined in Inclusion Criterion Number 9.
13. Able and willing to comply with scheduled visits, therapy plans, and laboratory tests required in this protocol.
14. Signed and dated IEC-approved Informed Consent Form indicating that the subject is aware of the neoplastic nature of his/her disease and has been informed of the procedures to be followed, the investigational nature of the therapy, potential benefits, side effects, discomforts, risks and alternative treatments.

6.2. Subject Exclusion Criteria

The presence of any of the following will exclude a subject from study enrollment:

1. Known fibrolamellar HCC or mixed hepato-cholangiocarcinoma.
2. Subjects with untreated or incompletely treated varices with bleeding or high risk for bleeding are excluded with the following clarification: subjects with history of prior variceal bleeding must have been treated with adequate endoscopic therapy without any evidence of recurrent bleeding for at least 6 months prior to study entry and must be stable on optimal medical management (e.g. non-selective beta blocker, proton pump inhibitor) at study entry.

3. Subjects with QTcF interval ≥480 milliseconds or with risk factors for torsade de pointes (e.g., heart failure, uncontrolled hypokalemia, family history of long QT syndrome) or receiving treatment with concomitant medications known to prolong the QT/QTc interval that cannot be replaced with another treatment.
4. Ascites defined as CTCAE Grade ≥2. Subjects who have been on a stable medication regimen for at least 2 months to manage ascites are eligible if they show ascites Grade < 2. Subjects with clinically undetectable ascites who are Child A with detectable ascites at CT/MRI are eligible.
5. Uncontrolled high blood pressure (systolic blood pressure, SBP >150 mmHg and/or diastolic blood pressure, DBP >95 mmHg, despite optimal treatment, on at least 2 out of 3 determinations repeated at 30 minutes interval and done in case that the first one meets the criterion for exclusion).
6. Direct-Acting Antivirals (DAA) at the time of treatment start; previous HCV treatment with DAAs is allowed.
7. Clinical evidence of hepatic encephalopathy.
8. Known brain metastases or evidence of leptomeningeal disease.
9. Known history of allergic reactions to polysorbate 80.
10. Any of the following in the past 6 months: myocardial infarction, uncontrolled cardiac arrhythmia, unstable angina, coronary/peripheral artery bypass graft, symptomatic congestive heart failure, cerebrovascular accident or transient ischemic attack, pulmonary embolism, deep vein thrombosis (except chronic/stable portal vein thrombosis).
11. Major surgery, other than diagnostic surgery, within 4 weeks before treatment start.
12. Any anticancer agent within 4 weeks or, in absence of toxicity, 5 half-lives (within 6 weeks for nitrosureas, mitomycin C and liposomal doxorubicin) before treatment start.
13. Radiation therapy within 4 weeks or radionuclide treatment (e.g., I-131 or Y-90) within 6 weeks before treatment start.
14. Untreated uncontrolled bacterial, viral, or fungal infections including acute HIV infection or acquired immunodeficiency syndrome (AIDS), untreated uncontrolled HBV, untreated uncontrolled HCV, untreated uncontrolled concomitant HBV and HCV; patients who are seropositive following HBV vaccine are eligible.
15. Subjects under treatment with therapeutic dose of anticoagulants (e.g., warfarin or warfarin-related agents, low-molecular weight heparin, or similar agent such as anti Xa and anti-thrombin agents) or antiplatelet agents (e.g. clopidogrel) or with coagulation disorders. Aspirin at dose up to 100 mg is permitted. Prophylaxis with anticoagulants is allowed to meet the INR value range as cited in inclusion criterion 9.
16. Uncontrolled diabetes mellitus.
17. Pregnant or breast-feeding women.

18. Known second malignancy that is progressing or requiring active treatment. Exceptions include adequately treated basal cell or squamous cell skin cancer or in situ carcinoma of the cervix uteri.
19. Current enrollment or participation in another interventional clinical trial.
20. Clinically significant respiratory or metabolic diseases uncontrolled by medication.
21. Subjects with active alcohol and/or substances abuse.
22. Any known organ dysfunction, serious illness, acute or chronic medical or psychiatric condition, or laboratory abnormality which, in the Investigator's opinion, may increase the risk associated/interfere with study participation, or with the interpretation of the results.
23. Subjects who, within 7 days prior to the first NMS-01940153E intake, are receiving or received strong inducers of FMO1and FMO3.
24. Subjects who are receiving sensitive CYP3A4 substrates, CYP3A4 and BCRP substrates with narrow therapeutic index (NTI).

7. SCHEDULE OF EVENTS

Table 2. Phase I Schedule of Events

Protocol activities and forms to be completed	Screening (day)		Cycle 1 (day)										Cycle >1 (day)					Last cycle	Off Treatment assessments [25]	
	≤28	≤7	1	2	3	4	8	15	16	17	18	22	1	8	15	22	28	EoT[24]	28D FU	Bi-mth FU
Informed Consent [1]	X																			
Relevant medical/oncologic history [2]		X																		
ECOG PS [3]		X	X*										X					X	X	
Height	X																			
Weight [4]		X	X										X							
Temperature [5]		X	X				X	X					X	X	X					
Blood pressure and heart rate [6]		X	X				X	X					X	X	X			X	X	
Gastro-esophageal endoscopy [7]		X																		
12-lead ECG [8]		X	As medically indicated										As medically indicated					X		
Laboratory assessments																				
Pregnancy test [9]		X																X		
Serology [10]		X																		
Hematology [11]		X	X*				X	X				X	X	X	X	X		X	X	
Blood chemistry [12]		X	X*				X	X				X	X	X	X	X		X	X	
Coagulation [13]		X	X*				X	X				X	X	X	X	X		X	X	
AFP [14]		X															X	X		
Urinalysis [15]		X	As medically indicated										As medically indicated					X		
Other clinical assessments																				
Concomitant medications [16]	X	X	X										X					X	X	
Adverse Events [17]	X	X	X										X					X	X	
Study treatment																				
Enrolment [18]		X																		
NMS-01940153E infusion [19]			X				X	X					X	X	X					
Disease assessment																				
Tumor imaging [20]	X																X	X		X
Survival follow up [21]																			X	X
Investigational assessments																				
PK blood sample [22]			X°	X°	X°	X°	X°	X°	X°	X°	X°	X°	X°	X°	X°					
PK urine sample [23]			X°					X°												

See Footnotes for Schedule of Events on following page

Footnotes for Phase I Schedule of events
1. Informed consent: to be obtained before the starting of any trial related procedure; it may occur also before the 28-day screening period. After signature, a subject Screening number is assigned centrally.
2. Relevant medical/oncologic history: to be assessed at screening, within 7 days from treatment start. Medical history includes history of relevant medical conditions and concomitant illnesses either present (active or controlled) or resolved. Oncologic history includes information on initial diagnosis, prior regimens and best response to prior regimens.
3. ECOG PS: to be recorded at screening, within 7 days from treatment start; on day 1 of each treatment cycle (before treatment administration); at end of treatment (EoT) and at 28-days follow up.
4. Weight (kilograms): to be recorded at screening, within 7 days from treatment start, and on day 1 of each treatment cycle (before treatment administration).
5. Temperature (Celsius degree): to be recorded at screening, within 7 days from treatment start, and on days 1, 8, 15 of each treatment cycle (before treatment administration).
6. Blood pressure and heart rate (supine): to be recorded at screening, within 7 days from treatment start; on days 1, 8, 15 before the treatment start and within 30 minutes after the end of infusion; at EoT and 28-days follow up. At each determination, if SBP > 150 mmHg and/or DBP > 95 mmHg is registered, repeat determinations two more times at 30 minutes interval.
7. Gastro-esophageal endoscopy: to be performed to all patients with known history for gastro-esophageal varices. For these patients, the assessment is mandatory at screening, within 7 days from treatment start, unless an endoscopy is available within 3 months before the treatment start.
8. 12-lead ECG: to be performed to determine QT interval using Fridericia standard (QTcF) at screening, within 7 days from treatment start, and at EoT visit. To be repeated at any time it is medically indicated.
9. Pregnancy test (serum/urine): for women of childbearing potential only, to be performed at screening, within 72 hours from treatment start and at EoT visit.
10. Serology: in case of positive HBV anamnesis, includes HBV DNA; in case of positive HCV anamnesis, includes HCV RNA; in case of HBV unknown anamnesis, includes HbsAg, HbeAg, HbcAg, Anti-HBc-IgM; in case of HCV unknown anamnesis, includes liver function test as by local standards and HCV Ab. Anti-HIV test. To be performed at screening, within 7 days from treatment start.
11. Hematology: includes hemoglobin (Hb), red blood cells (RBC), platelet (PLT) and white blood cells (WBC) with differential count (neutrophils, lymphocytes, monocytes, eosinophils and basophils). To be performed at screening, within 7 days from treatment start; from cycle 1 to cycle 3, on days 1, 8, 15, 22; from cycle 4 onward, on days 1, 8, 15; at EoT and 28-days follow up. A time window of -1 day is allowed; on days of treatment administration, sampling should be performed and results should be available before the start of NMS-01940153E infusion. In case of cycle delay due to toxicity and/or if clinically indicated, additional assessments should be done as per medical judgment.
12. Blood chemistry: includes glucose, electrolytes (Na, K, Ca, P, magnesium), urea, serum creatinine, creatinine clearance (calculated with Cockroft-Gault equation), total protein, albumin, aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), gamma-glutamil transferase (γ-GT), total bilirubin, unconjugated bilirubin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH). To be performed at screening, within 7 days from treatment start; from cycle 1 to cycle 3, on days 1, 8, 15, 22; from cycle 4 onward, on days 1, 8, 15; at EoT and 28-days follow up. A time window of -1 day is allowed; on days of treatment administration, sampling should be done and results should be available before the start of NMS-01940153E infusion. In case of cycle delay due to toxicity and/or clinically indicated, additional assessments should be done as per medical judgment.
13. Coagulation: includes international normalized ratio (INR) of prothrombin. To be performed at screening, within 7 days from treatment start; from cycle 1 to cycle 3, on days 1, 8, 15, 22; from cycle 4 onward, on days 1, 8, 15; at EoT and 28-days follow up. A time window of -1 day is allowed; on days of treatment administration, sampling should be done and results should be available before the start of NMS-01940153E infusion. In case of cycle delay due to toxicity and/or clinically indicated, additional assessments should be done as per medical judgment.
14. AFP: to be performed at screening, within 7 days from treatment start, at the end of even cycles at disease evaluation and at EoT. A time window of -2/+5 days is allowed.
15. Urinalysis: includes pH, glucose, protein and blood. To be performed at screening, within 7 days from treatment start, and at EoT. To be repeated as medically indicated.
16. Concomitant medications: includes all treatment, supportive care taken (started or completed), transfusion, procedures and hospitalization up to 28 days before treatment start. Once the patient has ended the study treatment, concomitant medications should be recorded for 28 days (in case, up to 35 days) after the last on-study treatment administration or until all study drug-related toxicities have resolved, whichever comes first, or until alternative anticancer therapy is initiated. In this case, the reporting period will end at the time the new treatment is started.
17. Adverse events: to be documented at each scheduled visit since patient signs the Informed Consent until 28 days after the last treatment administration, or until all serious or study drug-related toxicities have resolved or are determined to be “chronic” or “stable”, whichever comes first, or until alternative anticancer therapy is initiated. In this case, the reporting period will end at the time the new treatment is started.
18. Enrolment: the investigator request of subject enrolment is the guarantee that all the eligibility criteria are met; Enrolment number and dose level are assigned centrally. It is recommended to start the treatment within 1-4 days from patient enrolment.

19. NMS-01940153E infusion: patients will receive treatment with NMS-01940153E administered as IV infusion for 3 consecutive weeks on days 1, 8, 15 followed by 1 week of rest, in a 28-day cycle. The duration of infusion will be dependent on the total dose to be administered: 1 hour infusion if ≤255 mg are administered; 1 hour 30 minutes infusion if >255 mg but <375 mg are administered. Within one cycle, in case the patient cannot be retreated on the day foreseen because of toxicity, the dose will be skipped and the patient will be retreated at the next foreseen time-point. Dose reduction/omission is foreseen based on toxicities observed. Patients that cannot be retreated, for any reason, within 1 week from day 28 will go off-treatment, unless the investigator believes treatment continuation is in the patient best interest. In this case a maximum delay of 2 weeks (up to day 42 from cycle start) will be allowed. Otherwise patients may continue on investigational drug until a withdrawal criterion is met.
20. Tumor imaging: to be performed at screening, within 28 days from treatment start (in case a tumor imaging is already available in the specified time window, it has not to be repeated); at the end of even months (even cycles) of treatment and at EoT. The EoT assessment is to be performed only if it was not performed in the previous 4 weeks. In patients withdrawn for reasons other than progressive disease (PD), during follow up, tumor assessment should be performed every 2 months, until PD or until a new antitumor therapy starts. On treatment, a time window of -2/+5 days is allowed. Patients with responding tumors (complete or partial response) must have response confirmed by CT scan at least 4 weeks after the 1st documentation of response. The same method of assessment and technique should be used to characterize and follow the same lesion at screening, during treatment and at EoT. CT scan is the desirable method for lesion measurement (see Section 11.3.1).
21. Survival follow up: post-treatment survival status, including additional information (e.g. new anticancer therapy), will be collected at the 28-days follow up visit and thereafter every 2 months from last treatment administration until death. Telephone contacts are acceptable for these assessments.
22. PK blood sample: during the Phase I, blood sampling for PK is to be performed at cycles 1 and 2 only (refer to Table 3 for details). During the Phase II, a sparse sampling procedure will be adopted.
23. PK urine sample: urine collection for PK is to be performed at cycle 1, only, on days 1, 15. Urine will be collected before the treatment administration and in four different portions between 0-6h, 6-24h, 24-48h, 48-72h from the start of study drug administration.
24. End of Treatment (EoT): visit to be performed within 7 days from the decision of discontinuing the study drug.
25. Off treatment assessments: 28 days, and no more than 35 days, after discontinuation of study treatment patients will return for the following assessments: ECOG PS, blood pressure and heart rate, hematology, blood chemistry, coagulation, concomitant medications, adverse events and survival follow up. Further follow up contacts will be scheduled every 2 months from last treatment administration, until death, to assess the survival status, including possible additional information. 28 day follow up for laboratory abnormalities: in case hematology, blood chemistry, coagulation, study drug-related and clinically significant abnormalities persist beyond the 28-days follow up assessment, they should be followed until they resolve or the Investigator assess them as chronic or stable, or until alternative anticancer therapy is initiated. In this case, the reporting period will end at the time the new treatment is started. 28 days follow up for adverse events: the AEs reporting period ends 28 days after the last study treatment administration. The following events should be followed after the end of the reporting period: serious adverse events (SAEs) with outcome “not recovered” or “unknown” and study drug-related AEs with outcome “not recovered” or “unknown”. They have to be followed until they resolve or the Investigator assess them as chronic or stable, or until alternative anticancer therapy is initiated. In this case, the reporting period will end at the time the new treatment is started.
* Cycle 1 Day 1 assessments: evaluations do not need to be repeated before study drug administration if they were performed at screening within 24 hours from treatment start.
° refer to Table 3

Table 3. Investigational Assessments details for Phase I

	Cycle 1 (Day)															Cycle 2 (Day)
	D1, D15											D2, D16	D3, D17	D4, D18	D8, D22	D1, D8, D15
Timing related to EoI (hour)	Pre	-	0.0[§]	0.15	0.30	1.0	2.0	4.0	6.0	7.0	8.0	24.0	48.0	72.0	168.0	Pre-dose
Study drug infusion		X														
PK blood sample[a]	X		X[§]	X	X	X	X	X	X		X	X	X	X	X	X
Blood pressure[b]	X[€]				X[€]										X[+€#]	X[+€]

a. PK blood samples: all patients enrolled in the Phase I portion of the study will undergo blood draws for PK analysis (central laboratory).
Phase I Dose-Escalation
Cycle 1:
Day 1: before the start of infusion, 5 minutes before the end of infusion (0.0§), 15 minutes, 30 minutes, 1h, 2h, 4h, 6h, 8h after the end of study drug administration
Day 2: 24h after the end of study drug administration
Day 3: 48h after the end of study drug administration
Day 4: 72h after the end of study drug administration
Day 8: before the start of infusion
Day 15: before the start of infusion, 5 minutes before the end of infusion (0.0§), 15 minutes, 30 minutes, 1h, 2h, 4h, 6h, 8h after the end of study drug administration
Day 16: 24h after the end of study drug administration
Day 17: 48h after the end of study drug administration
Day 18: 72h after the end of study drug administration
Day 22: 168h after the end of study drug administration
Cycle 2:
Day 1: before the start of infusion
Day 8: before the start of infusion
Day 15: before the start of infusion
Phase II
During the Phase II portion of the study, a sparse sampling procedure will be adopted.

b. Blood Pressure
Phase I Dose-Escalation
Cycle 1:
Day 1: before the start of infusion and within 30 minutes after the end of study drug administration; before the blood sample for PK is taken.
Day 8: before the start of infusion and within 30 minutes after the end of study drug administration; before the blood sample for PK is taken.
Day 15: before the start of infusion and within 30 minutes after the end of study drug administration; before the blood sample for PK is taken.
Cycle 2:
Day 1: before the start of infusion and within 30 minutes after the end of study drug administration; before the blood sample for PK is taken.
Day 8: before the start of infusion and within 30 minutes after the end of study drug administration; before the blood sample for PK is taken.
Day 15: before the start of infusion and within 30 minutes after the end of study drug administration; before the blood sample for PK is taken.

+ before the treatment start and within 30 minutes after the end of infusion
§ 5 minutes before the end of infusion (EoI)
 # on D8 only
€ before the blood sample is taken

Table 4. Phase II Schedule of Events

Protocol activities and forms to be completed	Screening		Cycle 1 (day)				Cycle > 1 (day)				Last cycle	Off treatment assessments [26]	
	≤ 28	≤ 7	1	8	15	22	1	8	15	28	EoT [25]	28D FU	Bi- mth FU
Informed Consent [1]	X												
Relevant medical/oncologic history [2]		X											
Physical examination and ECOG PS [3]		X	X*				X				X	X	
Height	X												
Weight [4]		X	X				X						
Temperature [5]		X	X	X	X		X	X	X				
Blood pressure and heart rate [6]		X	X	X	X		X	X	X		X	X	
Gastro-esophageal endoscopy [7]		X											
12-lead ECG [8]		X	X		X		As medically indicated				X		
Laboratory assessments													
Pregnancy test [9]		X	X*				X				X		
Serology [10]		X											
Hematology [11]		X	X*	X	X	X	X	X	X		X	X	
Blood chemistry [12]		X	X*	X	X	X	X		X		X	X	
Coagulation [13]		X	X*	X	X	X	X		X		X	X	
AFP [14]		X								X•	X		
Urinalysis [15]		X	As medically indicated				As medically indicated				X		
Other clinical assessments													
Concomitant medications [16]	X	X	X				X				X	X	
Adverse events [17]	X	X	X				X				X	X	
Study treatment													
Enrolment [18]		X											
NMS-01940153E infusion [19]			X	X	X		X	X	X				
Disease assessment													
Tumor imaging [20]	X									X•	X		X
BCLC and TNM staging[21]	X												
Survival follow up [22]												X	X
Investigational assessments													
Biomarkers sampling[23]		X											
PK blood sample [24]			X°	X°	X°	X°	X°		X°	X°			

See Footnotes for Schedule of Events on following page

Footnotes for Phase II Schedule of events
1. Informed consent: to be obtained before the starting of any trial related procedure; it may occur also before the 28-days screening period. After signature, a subject Screening number is assigned centrally.
2. Relevant medical/oncologic history: to be assessed at screening, within 7 days from treatment start. Medical history includes history of relevant medical conditions and concomitant illnesses either present (active or controlled) or resolved. Oncologic history includes information on initial diagnosis, prior regimens and best response to prior regimens.
3. Physical examination and ECOG PS: to be recorded at screening, within 7 days from treatment start; on day 1 of each treatment cycle (before treatment administration); at EoT and at 28-days follow up.
4. Weight (kilograms): to be recorded at screening, within 7 days from treatment start, and on day 1 of each treatment cycle (before treatment administration).
5. Temperature (Celsius degree): to be recorded at screening, within 7 days from treatment start, and on days 1, 8, 15 of each treatment cycle (before treatment administration).
6. Blood pressure and heart rate: to be recorded at screening, within 7 days from treatment start; on days 1, 8, 15 before the treatment start and within 30 minutes after the end of infusion; at EoT and 28-days follow up. At each determination, if SBP > 150 mmHg and/or DBP > 95 mmHg is registered, repeat determinations two more times at 30 minutes interval.
7. Gastro-esophageal endoscopy: to be performed to all patients with known history of gastro-esophageal varices. For these patients the assessment is mandatory, at screening within 7 days from treatment start, unless an endoscopy is available within 3 months before the treatment start. For all other patients the assessment is not required, granted that an endoscopy is available within 24 months before the treatment start, or PI would justify the absence of clinical need for this assessment;
8. 12-lead ECG (12-lead, in triplicate, includes qualitative and quantitative assessment): to be performed to determine QT interval using Fridericia standard (QTcF) at screening, within 7 days from treatment start, at cycle 1 day 1 (first dose) and at cycle 1 day 15 (dose 3) triplicate ECG will be collected prior to dosing (30 min before the start of infusion and at least 10 minutes after any blood drawn or intravenous access procedure) and at peak concentration, prior to and as close as possible to the PK timepoint planned 5 minutes before the end of infusion (e.g. ≤ 10 minutes),, and at EoT visit. To be repeated at any time it is medically indicated. If the ECG assessment is not feasible on cycle 1 day 1 or cycle 1 day 15, a different timing or other solutions should be discussed with Sponsor. Additional assessments to be performed if clinically indicated. At each time point, three consecutive supine 12-lead ECGs will be performed approximately 2 minutes apart to determine mean QT interval using Fridericia standard (QTcF). The following parameters will be collected: synus rithm, heart rate, RR interval, PR interval, QRS interval, QT interval and QTcF interval. For all patients enrolled in the study, the Sponsor may request the ECG tracing and reports collected during the study be submitted to a third-party for retrospective independent review of the ECGs reading and interpretation.
9. Pregnancy test (serum/urine): for women of childbearing potential only, to be performed at screening, within 72 hours from treatment start, on day 1 of each treatment cycle (before treatment administration) and at EoT visit.
10. Serology: in case of positive HBV anamnesis, includes HBV DNA; in case of positive HCV anamnesis, includes HCV RNA; in case of HBV unknown anamnesis, includes HbsAg, HbeAg, HbcAg, Anti-HBc-IgM; in case of HCV unknown anamnesis, includes liver function test as by local standards and HCV Ab. Anti-HIV test. To be performed at screening, within 7 days from treatment start.
11. Hematology: includes hemoglobin (Hb), red blood cells (RBC), platelet (PLT) and white blood cells (WBC) with differential count (neutrophils, lymphocytes, monocytes, eosinophils and basophils). To be performed at screening, within 7 days from treatment start; on cycle 1, on days 1, 8, 15, 22; from cycle 2 onward, on days 1, 8, 15; at cycle 2, in case neutropenia Grade ≥2 is observed, repeat the hematological evaluation also at day 22; at EoT and 28-days follow up. A time window of -1 day is allowed; on days of treatment administration, sampling should be performed and results should be available before the start of NMS-01940153E infusion. In case of cycle delay due to toxicity and/or if clinically indicated, additional assessments should be done as per Investigator judgment.
12. Blood chemistry: includes glucose, electrolytes (Na, K, Ca, P, magnesium), urea or BUN, serum creatinine, creatinine clearance (calculated with Cockroft-Gault equation), total protein, albumin, aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), gamma-glutamil transferase (γ-GT), total bilirubin, unconjugated bilirubin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH). To be performed at screening, within 7 days from treatment start; on cycle 1, on days 1, 8, 15, 22; from cycle 2 onward, on days 1, 15; at EoT and 28-days follow up. A time window of -1 day is allowed; on days of treatment administration, sampling should be done and results should be available before the start of NMS-01940153E infusion. In case of cycle delay due to toxicity and/or clinically indicated, additional assessments should be done as per Investigator judgment.
13. Coagulation: includes international normalized ratio (INR) of prothrombin. To be performed at screening, within 7 days from treatment start; on cycle 1, on days 1, 8, 15, 22; from cycle 2 onward, on days 1, 15; at EoT and 28-days follow up. A time window of -1 day is allowed; on days of treatment administration, sampling should be done and results should be available before the start of NMS-01940153E infusion. In case of cycle delay due to toxicity and/or clinically indicated, additional assessments should be done as per Investigator judgment.
14. AFP: to be performed at screening, within 7 days from treatment start; at the end of even cycles and at EoT. A time window of -2/+5 days is allowed.
15. Urinalysis: includes pH, glucose, protein and blood. To be performed at screening, within 7 days from treatment start and at EoT. To be repeated as medically indicated.

16. Concomitant medications: includes all treatment, supportive care taken (started or completed), transfusion, procedures and hospitalization up to 28 days before treatment start. Once the patient has ended the study treatment, concomitant medications should be recorded for 28 days (in case, up to 35 days) after the last on-study treatment administration or until all study drug-related toxicities have resolved, whichever comes first, or until alternative anticancer therapy is initiated. In this case, the reporting period will end at the time the new treatment is started.
17. Adverse events: to be documented at each scheduled visit since patient signs the Informed Consent until 28 days after the last treatment administration, or until all serious or study drug-related toxicities have resolved or are determined to be "chronic" or "stable", whichever comes first, or until alternative anticancer therapy is initiated. In this case, the reporting period will end at the time the new treatment is started.
18. Enrolment: the investigator request of subject enrolment is the guarantee that all the eligibility criteria are met; Enrolment number is assigned centrally. It is recommended to start the treatment within 1-4 days from patient enrolment.
19. NMS-01940153E infusion: patients will receive treatment with NMS-01940153E administered as IV infusion for 3 consecutive weeks on days 1, 8, 15 followed by 1 week of rest, in a 28-day cycle. The duration of infusion will be dependent on the total dose to be administered: 1 hour infusion if ≤255 mg are administered; 1 hour 30 minutes infusion if >255 mg but <375 mg are administered. Within one cycle, in case the patient cannot be retreated on the day foreseen because of toxicity, the dose will be skipped, and the patient will be retreated at the next foreseen time-point. Dose reduction/omission is foreseen based on toxicities observed. Patients that cannot be retreated, for any reason, within 1 week from day 28 will go off-treatment, unless the investigator believes treatment continuation is in the patient best interest. In this case a maximum delay of 2 weeks (up to day 42 from cycle start) will be allowed. Otherwise patients may continue on investigational drug until a withdrawal criterion is met.
20. Tumor imaging: to be performed at screening, within 28 days from treatment start (in case a tumor imaging is already available in the specified time window, it has not to be repeated); at the end of even months (even cycles) of treatment and at EoT. The EoT assessment is to be performed only if it was not performed in the previous 4 weeks. In patients withdrawn for reasons other than progressive disease (PD), during follow up, tumor assessment should be performed every 2 months, until PD or until a new antitumor therapy starts. On treatment, a time window of -2/+5 days is allowed. Patients with responding tumors (complete or partial response) must have response confirmed by CT scan at least 4 weeks after the 1st documentation of response. The same method of assessment and technique should be used to characterize and follow the same lesion at screening, during treatment and at EoT. Ct scan is the desirable method for lesion measurement (see Section 11.3.1).
21. BCLC and TNM Staging: TNM and BCLC staging will be requested at enrollment for all patients, including collection of clinical related additional info
22. Survival follow up: post-treatment survival status, including additional information (e.g. new anticancer therapy), will be collected at the 28-days follow up visit and thereafter every 2 months from last treatment administration until death. Telephone contacts are acceptable for these assessments.
23. Biomarkers sampling: peripheral blood samples will be collected at Enrollment (≤7 days before Cycle1 Day1) for analysis of selected molecular alterations in circulating free DNA (cfDNA) from plasma
24. PK blood sample: during the Phase II, a sparse sampling procedure will be adopted; blood sampling for PK is to be performed at cycles 1, 2, 3 and 4 (refer to Table 5 for details).
25. End of Treatment (EoT): visit to be performed within 7 days from the decision of discontinuing the study drug.
26. Off treatment assessments: 28 days, and no more than 35 days, after discontinuation of study treatment patients will return for the following assessments: ECOG PS, blood pressure and heart rate, hematology, blood chemistry, coagulation, concomitant medications, adverse events and survival follow up. Further follow up contacts will be scheduled every 2 months from last treatment administration, until death, to assess the survival status, including possible additional information. 28 day follow up for laboratory abnormalities: in case hematology, blood chemistry, coagulation, study drug-related and clinically significant abnormalities persist beyond the 28-days follow up assessment, they should be followed until they resolve or the Investigator assess them as chronic or stable, or until alternative anticancer therapy is initiated. In this case, the reporting period will end at the time the new treatment is started. 28 days follow up for adverse events: the AEs reporting period ends 28 days after the last study treatment administration. The following events should be followed after the end of the reporting period: serious adverse events (SAEs) with outcome "not recovered" or "unknown" and study drug-related AEs with outcome "not recovered" or "unknown". They have to be followed until they resolve or the Investigator assess them as chronic or stable, or until alternative anticancer therapy is initiated. In this case, the reporting period will end at the time the new treatment is started.
* Cycle 1 Day 1 assessments: evaluations do not need to be repeated before study drug administration if they were performed at screening within 24 hours from treatment start.
• only even cycles

Table 5. Investigational Assessments details for Phase II																				
	Cycle 1 (Days)										Cycles 2 and 4 (Days)						Cycle 3 (Days)			
	D1			D8		D15				D22	D1	D15				D26 - 28	D15			
Timing related to EoI (hour)	0.0§	15min – 2h	>2h – 6h	Pre	0.0§	Pre	0.0§	15min – 2h	>2h – 6h	Any	Pre	Pre	0.0§	15min – 2h	>2h – 6h	Any	Pre	0.0§	15min – 2h	>2h – 6h
PK blood sample[c]	X	X^{Od}	X^{Ev}	X^{Od}	X^{Ev}	X	X	X^{Ev}	X^{Od}	X	X	X	X	X^{Od}	X^{Ev}	X	X	X	X^{Ev}	X^{Od}

c. PK blood samples: all patients enrolled in the Phase II portion of the study will undergo blood draws for PK analysis (central laboratory) based on their enrollment number (odd or even), as detailed below.

Odd patients (X^{Od})
Cycle 1:
Day 1: 5 minutes before the end of infusion (0.0§), from 15 minutes to 2h after the end of study drug administration
Day 8: before the start of infusion (pre-dose)
Day 15: before the start of infusion (pre-dose), 5 minutes before the end of infusion (0.0§), from >2h to 6h after the end of study drug administration
Day 22: one sample, at any time
Cycles 2 and 4:
Day 1: before the start of infusion (pre-dose)
Day 15: before the start of infusion (pre-dose), 5 minutes before the end of infusion (0.0§), from 15 minutes to 2h after the end of study drug administration
One samples on day 26 or day 27 or day 28, at any time.
Cycle 3:
Day 15: before the start of infusion (pre-dose), 5 minutes before the end of infusion (0.0§), from >2h to 6h after the end of study drug administration

Even patients (X^{Ev})
Cycle 1:
Day 1: 5 minutes before the end of infusion (0.0§), from >2h to 6h after the end of study drug administration
Day 8: 5 minutes before the end of infusion (0.0§)
Day 15: before the start of infusion (pre-dose), 5 minutes before the end of infusion (0.0§), from 15 minutes to 2h after the end of study drug administration
Day 22: one sample, at any time
Cycles 2 and 4:
Day 1: before the start of infusion (pre-dose)
Day 15: before the start of infusion (pre-dose), 5 minutes before the end of infusion (0.0§), from >2h to 6h after the end of study drug administration
One samples on day 26 or day 27 or day 28, at any time.
Cycle 3:
Day 15: before the start of infusion (pre-dose), 5 minutes before the end of infusion (0.0§), from 15 minutes to 2h after the end of study drug administration

Note: blood pressure is to be taken on days 1, 8, 15 before the treatment start and within 30 minutes after the end of infusion, before the blood sample for PK is taken (if the case).

8. ENROLLMENT PROCEDURES

Patients considered suitable will be informed about study and procedures, both verbally and by written information. Patients will be given all the time they need for reading information provided, discuss and question them and ask others' opinion. Patients who will decide to participate in the study will sign and date the informed consent form (ICF) before starting any trial related procedures; concomitantly, the Investigator will also sign and date the form.

After the signature of the ICF, a screening number will be centrally assigned to the patient, allowing the screening process for eligibility to begin. All screening evaluations must be completed and reviewed to confirm that potential suitable patients meet all eligibility criteria. Upon completion of the screening evaluation, patients meeting all the eligibility criteria will be enrolled and, for Phase I patients, dose level assigned; the enrollment number will be centrally assigned and maintained throughout all the study duration. Patients who do not meet all the eligibility criteria will be declared screening failure.

Screening and enrollment numbers assignment and, for Phase I patients only, also dose level, will be centrally managed by the Sponsor within one working day. The investigator will maintain a screening and enrollment log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

No study drug will be dispensed to a patient until the entire registration/enrollment process is completed. It is recommended to start the treatment within 1-4 days from patient enrollment.

For the phase I portion of the study, the Sponsor will notify all the participant sites of the inclusion of a new patient and will inform them of the next possible enrollment date. The enrollment will be competitive among the participating sites.

8.1. Screening Procedures

Following the obtaining of the ICF, in order to get the patient's screening number, the Investigator will enter the information required in a web application made available to centers by the Sponsor (details are reported in the Study Manual).

The screening number will be sequential for the center where the patient has been recruited. The screening number assignment is automatically notified by e-mail.

In case the web application is not available because of any reason, the Investigator will enter the information required in a paper registration/enrollment form and fax/email it to the Sponsor (details are reported in the Study Manual).

8.2. Enrollment procedures

Upon completion of the screening evaluation, the Investigator will enter the information in the web application, confirming patient's eligibility or declaring the patient as screening failure; patients meeting all the eligibility criteria will be enrolled. The enrollment number will be assigned centrally and automatically notified by e-mail (details are reported in the Study Manual).

The enrollment number will be sequential regardless of the center where the patient has been recruited.

In case the web application is not available because of any reason, the Investigator will enter the information required in a paper registration/enrollment form and fax/email it to the Sponsor (details are reported in the Study Manual).

The eligibility of a patient is an Investigator's responsibility. Verification of the confirmed eligibility will be performed by the Sponsor or authorized personnel delegated by the Sponsor during the study.

9. TREATMENT

9.1. Product

9.1.1. Description

NMS-01940153E is a [redacted], potent small molecule inhibitor of MPS1 kinase, critical player in the control of mitosis.

NMS-01940153E is provided as 30 mg salt, [redacted] Each vial (primary packaging) is packaged in a labeled single small carton (secondary packaging). The labeling of packages complies with regulatory requirements of countries involved in the study as well as recommendations provided in Annex 13 of the European Guide to Good Manufacturing Practice.

9.1.2. Drug Preparation/Administration/Dispensing

NMS-01940153E will be administered only at the investigational site and for no reason will be delivered to the patient for administration outside the reference investigational site. The appropriate amount of NMS-01940153E to be administered will be calculated based on the body surface area (BSA) of each individual patient, calculated based on Mosteller formula:

$BSA\ (m^2) = [height\ (cm) \times weight\ (kg)/3600]^{1/2}$

The 30 mg/vial powder for solution for infusion will be reconstituted with 10 mL of sterile water for injection; the freeze-dried cake will easily dissolve by manual shaking in not more than one minute. The obtained solution will be further diluted with 0.9% Sodium Chloride for

injection, according to the dose of NMS-01940153E to be administered. Details are reported in the Investigational Medicinal Product (IMP) module of the study manual.

Only qualified personnel familiar with procedures that minimize undue exposure to them and to the environment should undertake the preparation, handling, and safe disposal of chemotherapeutic agents.

9.1.3. Procedure for Handling Drug Spills

As NMS-01940153E is a cytotoxic agent, drug handling precautions as for cytotoxic agents should be followed. Avoid contact or inhalation. In case of skin contact, the affected area should be washed with soap and plenty of water. Any contaminated clothing should be removed. In case of eye-contact, rinse thoroughly with plenty of water. Seek medical advice as soon as possible. In case of accidental spill, caregivers should be instructed to wear protective (waterproof) gloves. Collect the spilled substance in a suitable container for disposal in accordance with the local regulations concerning handling and disposal of cytotoxic materials. Once the spilled drug has been collected, use a detergent and then wash thoroughly with plenty of water for removal.

9.1.4. Storage and Stability

The IMP must be stored in a locked room that can be accessed only by the Pharmacist, the Investigator, or another duly designated person, in the original packaging, at controlled room temperature (not above +25°C and 77°F) conditions.

On the basis of the available stability results, a shelf-life of 60 months at controlled room temperature not above +25°C and 77°F is assigned to the IMP.

The NMS-01940153E reconstituted/diluted solutions can be stored for not more than 4 hours at room temperature and ambient light conditions prior to patient administration. However, it is recommended to prepare the reconstituted/diluted solutions just before or close to the infusion time (further details are reported in the IMP module of the study manual).

9.1.5. Source of drug

NMS-01940153E will be manufactured, packaged and distributed by NerPharMa S.r.l., (Nerviano, Italy) in compliance with good manufacturing practice (GMP) guidelines and under Sponsor’s direction. NMS-01940153E re-supplies may be obtained by contacting the Sponsor, according to instructions detailed in the IMP module of the study manual.

9.1.6. The study product

The study product must not be used outside the context of this protocol. Under no circumstances should the Investigator or site personnel supply study product to other Investigators or clinics, or allowed the supplies to be used other than as directed by this protocol without authorization from the Sponsor.

The study drug must be administered to patients, according to study schedule, until the criteria for withdrawal are met, as detailed in Section 10. Drug accountability should continue to be performed and documented until the patient's last dose of NMS-01940153E.

Adequate records on receipt, use, return, destruction or loss, or other disposition of medication must be maintained. Drug accountability will be provided by the investigational site by using either specific drug accountability forms provided by the Sponsor or records used by the pharmacy at the investigational site and agreed upfront with the Sponsor. In either case, information describing study drug supplies and their disposition, patient by patient, must be provided, signed by the Investigator (or the pharmacist or other person who dispensed the drug). Details are provided in the IMP module of the study manual.

After verification by the study monitor and upon authorization of the Sponsor, the unused drug vials may be destroyed at the site as dictated by the appropriate standard procedures of the participating institution and destruction must be documented; alternatively, all unused drug vials will be returned to the Manufacturer who will provide, on behalf of the Sponsor, to send them to destruction. All used drug vials should be discarded according to the standard institutional policy.

9.2. Treatment Administration

9.2.1. Treatment Dose and Schedule

NMS-01940153E will be administered IV for 3 consecutive weeks on days 1, 8, 15 followed by 1 week of rest, in a 28-day cycle. The total dose to be administered will be calculated based on patient's BSA (see Section 9.1.2). The duration of infusion will be dependent on the total dose to be administered:

- 1 hour infusion if ≤255 mg are administered
- 1 hour 30 minutes infusion if >255 mg but <375 mg are administered

The starting dose for the Phase I portion of the study is 100 mg/m^2/week, then according to the observed safety profile, dose increments of 25-35% will be applied until the MTD is reached. In presence of unacceptable toxicity observed at 100 mg/m^2/week, a lower dose level by 25-35% may be tested.

In the Phase II portion of the study, NMS-01940153E will be administered at the RP2D defined in the Phase I, as starting dose (see Section 5.2 for definitions).

9.2.2. Duration of Treatment

Patients may continue on study treatment until disease progression, unacceptable toxicity, withdrawal of consent by the patient or other discontinuation criteria are met (see Section 10). The study will be closed once that the last patient has left the study.

9.2.3. Dose Modifications

9.2.3.1. Dose Reductions/Treatment discontinuations

Dose reductions/treatment discontinuations may occur as a consequence of drug-related toxicities, during a cycle or at the start of a new cycle. All dose reductions/treatment discontinuations should be based on the most severe toxicity observed in the previous cycle and attributable to the study drug (according to the NCI CTCAE v 5.0). In the event of multiple toxicities, the dose reduction/treatment discontinuation should be based on the worst toxicity observed.

Any modification to the NMS-01940153E dose should be documented in the eCRF.

During treatment, doses can be de-escalated only twice, otherwise patients would have to be withdrawn.

Doses reduced for drug-related toxicity should generally not be re-escalated, even if there is minimal or no toxicity with the reduced dose according to Table 6. However, intra-patient re-escalation back to the previous dose level may be permitted at the discretion of the Investigator after discussion with the Sponsor.

Recommended dose reductions or discontinuation criteria during the treatment cycle and at the start of the subsequent cycle are shown in Table 6.

9.2.3.2. Re-treatment Criteria and Cycle Delay

A new cycle of treatment may begin when hematological and non-hematological toxicities are Grade ≤1 or have recovered to baseline values.

If these conditions are not met, treatment should be delayed for 1 week to allow for recovery. If, after 1-week delay, re-treatment criteria are met, then the new cycle can be initiated with a starting dose defined based on the toxicities observed in the previous cycle as outlined in the Table 6.

If, after this period, toxicities do not recover to the pre-defined level, the patient will be withdrawn from the treatment, unless the Investigator believes treatment continuation is in the patient best interest. In this case, a maximum delay of 2 weeks (up to day 42 from cycle start) will be allowed.

Table 6. Dose Reductions/Treatment Discontinuations for NMS-01940153E Based on the Worst Grade (as per NCI CTCAE Criteria, Version 5.0) Observed During a Treatment Cycle and in the Prior Treatment Cycle

Toxicity	Dose Adjustment during treatment cycle	Next cycle Starting Dose
Hematological Toxicities		
Neutropenia Grade 3	Omit dose; when resolved to ≤ Grade 1 within the same cycle, decrease one dose level.	Start at or maintain the decreased dose level.
Neutropenia Grade 4	Omit dose; when resolved to ≤ Grade 1 within the same cycle, decrease one dose level.	Start at or maintain the decreased dose level.
Febrile Neutropenia	Omit dose; when resolved, decrease one dose level.	Start at or maintain the decreased dose level.
Thrombocytopenia Grade 3 associated with bleeding or thrombocytopenia Grade 4	Omit dose; when resolved to ≤ Grade 2 within the same cycle, decrease one dose level.	Start at or maintain the decreased dose level.
Anemia Grade 3	Omit dose; when resolved to ≤ Grade 2 within the same cycle, decrease one dose level.	Start at or maintain the decreased dose level.
Anemia Grade 4	Study treatment discontinuation.	Study treatment discontinuation.
Other Grade 3 Hematologic Toxicities	Omit dose; when resolved to ≤ Grade 2 within the same cycle, decrease one dose level.	Start at or maintain the decreased dose level.
Other Grade 4 Hematologic Toxicities	Study treatment discontinuation.	Study treatment discontinuation.
Nausea and/or Vomiting		
Grade ≥ 3 despite optimal management of the event §‡	Omit dose; when resolved to ≤ Grade 2 within the same cycle, decrease one dose level.	Start at or maintain the decreased dose level.
Diarrhea		
Grade 2 despite optimal management of the event §	Omit dose; within the same cycle decrease one dose level.	Start at or maintain the decreased dose level.
Grade ≥ 3 despite optimal management of the event §	Omit dose; when resolved to ≤ Grade 2 within the same cycle, decrease one dose level.	Start at or maintain the decreased dose level.
Neurologic Toxicities (except insomnia/paraesthesia)		
Grade 2	Omit dose; when resolved to ≤ Grade 1, decrease one dose level.	Start at or maintain the decreased dose level.
Grade > 2	Consider discontinuation of study treatment based on risk/benefit assessment #.	Consider discontinuation of study treatment based on risk/benefit assessment #.
Other Non-Hematological Toxicities (except alopecia)		
Intolerable or persistent Grade 2 adverse events	Omit dose; when resolved to ≤ Grade 2, maintain the dose level at first occurrence; at second occurrence, omit dose; when resolved to ≤ Grade 2, decrease one dose level. .	At second occurrence, start at or maintain the decreased dose level.

Table 6. Dose Reductions/Treatment Discontinuations for NMS-01940153E Based on the Worst Grade (as per NCI CTCAE Criteria, Version 5.0) Observed During a Treatment Cycle and in the Prior Treatment Cycle

Toxicity	Dose Adjustment during treatment cycle	Next cycle Starting Dose
Grade 3	Omit dose; when resolved to ≤ Grade 2, decrease one dose level.	Start at or maintain the decreased dose level.
Grade 4	Omit dose; when resolved to ≤ Grade 2, decrease one dose level or consider study treatment discontinuation #	Start at or maintain the decreased dose level or consider study treatment discontinuation #
Grade ≥ 3 hypersensitivity reaction suggestive of anaphylactic reaction #	Study treatment discontinuation	Study treatment discontinuation.
§ For prophylaxis and management of the events, see details in Supportive Care Sections 9.2.7. ‡ lasting longer than 48h without resolution to grade 2 or better and judged at least possibly related to NMS-01940153E. # Investigator to discuss risk/benefit with Sponsor, if positive, decrease one dose level.		

9.2.4. Extension Treatment

Not applicable.

9.2.5. Overdose Instructions

There are no known antidotes for NMS-01940153E overdose. All cases of overdose [associated or not to an adverse event/serious adverse event (AE/SAE)] and medication errors accompanied by an AE/SAE, should be notified, using the SAE form, to the Sponsor Pharmacovigilance immediately (i.e. within 24 hours) according to procedures described in part 11.5.1.7. Study monitor should be also contacted to discuss the details of any overdose.

Whether or not the overdose/medication error is accompanied by an AE, as determined by the Investigator, the overdose/medication error and, if applicable, any associated AE must be captured on the AE eCRF page (refer to Safety Assessments, Section 11.5, for further details).

9.2.6. Unblinding

Not applicable.

9.2.7. Concomitant Medications and Other Therapy

Any medication the patient takes other than the study treatment, including herbal and other non-traditional remedies, is considered a concomitant medication. All information concerning concomitant medication taken (started or completed) up to 28 days before the start of treatment with NMS-01940153E and within 28 days (and, if applicable, no more than 35 days) from last study drug administration, should be collected on the electronic case report form (eCRF): name, active ingredient, reason for use, route of administration, strength and unit, frequency or total daily dose, start and stop dates. Any change in the dosage or regimen of a concomitant medication must be recorded in the eCRF.

Prior treatments for the disease under study are to be collected on separate and appropriate forms of the eCRF and should cover the entire tumour history.

Directives for supportive care of the patients are outlined below and could be adapted according to institutional practices; however they cannot ever include strong inducers of flavin-containing monooxygenase 1 (FMO1), FMO3 as well as sensitive cytochrome 3A4 (CYP3A4) substrates, CYP3A4 and BCRP (breast cancer resistance protein) substrates with narrow therapeutic index (NTI). See Appendix 3 and Appendix 4 for details.

9.2.7.1. Antiemetics Support

Mild nausea and vomiting have been reported in the FIH study (EudraCT 2014-002023-10). Among 38 patients who received NMS-01940153E, nausea was reported in 12 patients (31.6%), with only one patient experiencing grade 3. Grade 1-2 vomiting was reported in 9 patients (23.7%).

Prophylactic therapy with antiemetics, administered according to hospital guidelines, is allowed including during cycle 1, if deemed appropriate.

Antiemetic therapy can be administered at Investigator's discretion whenever needed, including during cycle 1.

9.2.7.2. Antidiarrheal Support

The use of antidiarrheal agents is allowed according to hospital guidelines along with antibiotic treatment and IV hydration, if indicated. Patients should be instructed to refer to the centre in case of: diarrhoea lasting > 24 hours despite optimal supportive care or diarrhea with fever; black or bloody stools; symptoms of dehydration such as lightheadedness, dizziness, or faintness and inability to take liquids by mouth due to nausea and vomiting.

9.2.7.3. Hematopoietic Support

Red blood cell and/or platelets transfusion: during treatment should be considered at the Investigator's discretion in patients with significant anaemia and/or thrombocytopenia; transfusions are however not allowed within 72h before treatment start. Folates may be indicated also for anaemia treatment.

Hematopoietic growth factors
In the dose escalation part, prophylactic use of G-CSF or initiation of erythropoietin in Cycle 1 is not permitted but may be instituted in Cycle ≥ 2 in patients who are having difficulty with severe neutropenia or anemia. Patients who have been treated for ≥ 4 weeks with erythropoietin prior to the first cycle may continue the existing treatment.

Growth factors may be used as medically indicated in patients with relevant neutropenic complications, such as tissue infection, sepsis, etc., or anemia, at the Investigator's discretion. Patients with neutropenic fever or infection should be hospitalized promptly for IV antibiotic

therapy and may receive therapeutic CSFs as appropriate. Treatment of relevant neutropenic complications (e.g. tissue infection, sepsis, etc.) is left at the Investigator's discretion and should be done according to the hospital guidelines for treating these types of events.

9.2.7.4. Prophylaxis for Polysorbate 80 intolerance

Intolerance: prophylactic treatment is not allowed before Cycle 1. In case the toxicity profile emerging during the study suggests that there might be signs of intolerance to polysorbate 80, prophylaxis with antihistaminic or corticosteroids according to centre practices could be implemented.

9.2.7.5. Other Permitted Concomitant Medications

Therapies considered necessary for the patient's wellbeing may be given at the discretion of the Investigator, i.e. chronic treatments for concomitant medical conditions, as well as agents required for life-threatening medical problems, analgesics etc. according to the drug-drug interaction information. In particular, strong inhibitors of FMO1 and FMO3, moderate substrates of CYP3A4 and CYP3A4/BCRP substrates with no NTI should be used with caution (see Appendix 4).

Precaution should be used in treatment of NMS-01940153E with concomitant drugs that are known to prolong QT or QTc intervals, since the potential effect of study drug on QT prolongation has not been fully evaluated in humans. The Investigator should consult individual labels for concomitant medication. If medically indicated, a cardiology consult should be obtained.

Whenever possible the administration of drugs that may cause drug-drug interaction should not be done concomitantly with NMS-01940153E.

Patients should be advised to contact the treating physician before starting any new drug.

9.2.7.6. Other Anticancer or Experimental Therapy

No other approved or investigational anticancer treatment will be permitted during the study treatment period, including chemotherapy, immunotherapy, biological response modifiers, and hormones.

9.2.7.7. Concomitant Radiotherapy

Palliative radiotherapy to specific sites is permitted if considered medically necessary by the treating physician. It is recommended to avoid radiotherapy for at least 5 days after the last dose of NMS-01940153E and to contact the Sponsor. Also, the irradiated area should be as small as possible, and ≤25% of the bone marrow reserve (see Appendix 5). During the period of irradiation and for 2 weeks after, therapy with NMS-01940153E should be withheld. If irradiation-related toxicities (other than xerostomia) have not normalized to pre-irradiation levels after these 2 weeks of rest, the patient should be removed from the study. Irradiated lesions will be followed for disease progression. In case radiotherapy involving >25% bone

marrow reserve is needed, the patient will be considered as having progressed and should be removed from treatment. During the study, radiotherapy for active brain metastases will represent a progression.

10. SUBJECT WITHDRAWAL FROM STUDY PARTICIPATION

Patients may withdraw from the study at any time at their own request, or they may be withdrawn at any time at the discretion of the Investigator or Sponsor for safety, behavioral, or administrative reasons.

If the patient does not return for a scheduled visit, every effort should be made to contact him/her. In any circumstance, every effort should be made to document patient outcome, if possible. The Investigator should inquire about the reason for withdrawal, request the patient to return for a final visit, if applicable, and follow-up with the patient regarding any unresolved adverse events.

If the patient withdraws consent for disclosure of future information, no further evaluations should be performed and no additional data should be collected. The Sponsor may retain and continue to use any data collected before such withdrawal of consent.

Patients may continue with therapy unless any of the following occurs:

- Disease progression by RECIST 1.1 at any time, unless there is reasonable evidence of clinical benefit to justify continuation on treatment, to be discussed with the Sponsor;
- Unacceptable drug-related toxicities incompatible with continuation of treatment with NMS-01940153E according to the judgment of the Investigator, even at a reduced dose;
- Any medical event requiring administration of an unauthorised concomitant treatment if it interferes with the study evaluations and/or if it jeopardises patient's safety (see Section 9.2.7);
- Change in the patient's medical status (including pregnancy) such that the Investigator believes that patient safety may be compromised or that it would be in the best patient's interest to stop treatment;
- Occurrence of permanent or significant incapacity or disability;
- Treatment delay for >1 week from day 28 of any cycle due to treatment related toxicity, unless the investigator believes treatment continuation is in the best patient's interest. In this case a maximum delay of 2 weeks is allowed;
- More than two dose de-escalations required;
- Substantial deviation from specified inclusion or exclusion criteria or non-compliance by the patient with protocol requirements;
- Patient's refusal to continue study treatment;
- Withdrawal of consent;

- Patient lost to follow up;
- Death;
- Study terminated by the Sponsor.

Patients will be withdrawn from the study in case of:

- Withdrawal of consent;
- Patient lost to follow up;
- Death;
- Study terminated by the Sponsor.

Data to be collected for the end of study treatment/withdrawal are described in the Schedule of Events, reported in Section 7.

After treatment discontinuation patients will be followed for progression, if not progressed earlier, until the start of a new anticancer therapy, and for survival. Patients will be followed for at least 28 days after the last dose of study drug for adverse events and every 2 months up to death, for secondary efficacy endpoint purpose.

11. ASSESSMENTS

11.1. Timing of Assessments

Section 7 (SCHEDULE OF EVENTS) summarizes information on the timing of study assessments to be performed during Phase I and Phase II. Protocol waivers or exemptions are not allowed.

11.2. Disease Assessments

11.2.1. TNM Staging

TNM Staging will be performed at enrollment according to "American Joint Committee on Cancer (AJCC) TNM Staging for Hepatocellular Cancer (8th ed., 2017)" [18];

Histological grading and fibrosis score, as defined by Ishak, will be requested, if available.

Table 7. Definitions for T, N, M

T	Primary Tumor
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Solitary tumor ≤2 cm, or >2 cm without vascular invasion
T1a	Solitary tumor ≤2 cm
T1b	Solitary tumor >2 cm without vascular invasion
T2	Solitary tumor >2 cm with vascular invasion, or multiple tumors, none >5 cm
T3	Multiple tumors, at least one of which is >5 cm
T4	Single tumor or multiple tumors of any size involving a major branch of the portal vein or hepatic vein, or tumor(s) with direct invasion of adjacent organs other than the gallbladder or with perforation of visceral peritoneum
N	**Regional Lymph Nodes**
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis
M	
M0	No distant metastasis
M1	Distant metastasis

11.2.2. Barcelona Clinic Liver Cancer (BCLC) Staging System

BCLC [19] (Table 8) will be collected at enrollment:

Table 8. Definitions for prognostic groups

Very early stage (0)	• Single ≤2 cm • Preserved liver function, PS 0
Early stage (A)	• Single, or ≤3 nodules each ≤3 cm • Preserved liver function, PS 0
Intermediate stage (B)	• Multinodular • Preserved liver function, PS 0
Advanced stage (C)	• Portal invasion and/or extrahepatic spread • Preserved liver function, PS 1-2
Terminal stage (D)	• Any tumor burden • End stage liver function, PS 3-4

Additional info on tumoral characteristic will be requested when available.

11.3. Efficacy Assessments

The determination of antitumor efficacy will be based on objective tumor assessments made according to RECIST guideline (version 1.1) [17] and to the modified Response Evaluation Criteria in Solid Tumors (mRECIST) guideline [20].

A summary of RECIST 1.1 and mRECIST is reported in the following sections.

11.3.1. Tumor Imaging

Baseline tumor imaging (by CT scan, PET or MRI) has to be performed at screening within 28 days prior to the treatment start. On treatment, tumor assessments will be performed every even month (i.e., end of Cycle 2, 4, 6 etc) or whenever a clinical deterioration is observed and at the end of last cycle, unless a tumor assessment was performed within the previous 4 weeks. In patients withdrawn for causes other than disease progression, during follow up, regular tumor imaging assessments will be performed every 2 months, until PD or until a new antitumor therapy starts. On treatment, a time window of -2/+5 days is allowed.

Patients with responding tumors (complete or partial response) must have response confirmed at least 4 weeks after the first documentation of response.

CT scan is the desirable method for lesion measurement. MRI is acceptable provided that the size of the measurable lesion is twice the slice thickness of the MRI. MRI should be performed at pre-treatment in case of known or suspected CNS involvement.

According to RECIST, the same method and technique (CT scan or MRI) should be used to characterize each identified and reported lesion at baseline and during the following assessments.

Imaging-based evaluation should be preferred to clinical examination when both could be used to assess the antitumor effect of the treatment.

According to mRECIST, tumor assessment should be performed with the administration of intravenous contrast to obtain a dual-phase imaging of the liver. To prevent possibly allergic reactions to intravenous Contrast Medium (CM), patients with previous reaction(s) to CM, asthma even drug controlled and/or food or drug allergies will be treated with a premedication (corticosteroid).

Patients can be followed with either contrast-enhanced spiral CT (preferably with use of multi-slice scanners) or contrast-enhanced dynamic MRI. Each modality has advantages and disadvantages, and mRECIST does not recommend one modality over another.

However, it is recommended that the same imaging modality be used throughout the study. Ultrasound – including contrast-enhanced ultrasound – is not recommended for the general use of mRECIST.

PET/CT is not accepted for treatment assessment in guidelines.

Obtaining a pre-contrast scan is useful but not mandatory for CT. Conversely, establishing the intrinsic T1 intensity of tumour lesions at baseline is recommended in MRI, in order to infer subsequent contrast enhancement or perform subtraction imaging.

The administration of intravenous contrast is required for all CT or MRI studies if not medically contraindicated. In contrast-enhanced studies, it is crucial to time the contrast administration so that high-quality arterial-phase imaging is obtained on the first run, and high-quality portal venous-phase imaging is obtained on the second run. An arterial phase that is acquired too early (i.e., when the hepatic arterial branches are fully enhanced but the portal vein is not yet enhanced) may be inadequate since the degree of enhancement of HCC is usually higher in the late arterial phase (i.e., when the hepatic arterial branches are fully enhanced, the portal vein is also enhanced, but the hepatic veins are not yet enhanced).

Delayed imaging acquired two to five minutes after injection may be useful, but it is not mandatory and should be done only if it is part of clinical practice. In MRI studies, when using a hepatobiliary contrast agent instead of an extracellular contrast agent, the hepatobiliary phase acquired about 20 min after injection of gadoxetate disodium may be used to aid lesion detection and diagnostic confidence [20].

All patients' files and scans must be available for source verification.

11.3.1.1. Central Imaging Facility

A central imaging facility may evaluate imaging studies and supportive clinical data in a central and independent fashion as central review of tumor response assessments. The facility will not perform clinical evaluations. The central imaging facility may be chartered to establish radiographic progression and may consider selected clinical data solely to aid in the

interpretation of radiographic images. All radiological scans, acquired at all scheduled time points and any additional (unscheduled) radiological images acquired to evaluate for potential progressive disease, must be maintained as a copy of digital data for the retention period applicable to the protocol duration, GCPs, and federal, international and/or state legal as well as medical requirements. The Sponsor and/or designee may retain the media until or beyond study completion.

11.3.1.2. Measurability of Tumor Lesions

At baseline tumor lesions will be categorized by the Investigator as measurable or non-measurable by the RECIST guideline (version 1.1) criteria as described below:

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). In case of MRI, the size of the measurable lesion must be twice the slice thickness of the MRI and a minimum of 10 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: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung and 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, Positron Emission Tomography (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.

According to mRECIST, the measurement of the longest viable tumor diameter for the assessment of response can be only applied in case of typical lesions. Every effort should be made to time the contrast administration so that high-quality arterial-phase imaging is obtained throughout the liver on the first run, and high-quality portal venous-phase imaging is obtained throughout the liver on the second run. Delayed imaging obtained in the equilibrium phase may be useful, but it is not mandatory and should be done only if it is part of clinical practice.

Conversely, for non-enhancing atypical lesions, as well as for any extra-hepatic neoplastic niches, the measurements of the longest overall tumor diameter as per conventional RECIST should prevail.

11.3.1.3. Recording Tumor Measurements

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.

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 × 30 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.

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.

According to mRECIST a target lesion should meet all the following criteria:

- The lesion can be classified as a RECIST measurable lesion (i.e., the lesion can be accurately measured in at least one dimension as 1 cm or more).
- The lesion is suitable for repeat measurement.
- The lesion shows intratumoral arterial enhancement on contrast-enhanced CT or MRI.

The relevant number of target lesions for the investigator-assessed mRECIST assessment is recommended [20] as a maximum of 2 target lesions per organ and 5 target lesions in total. Other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline.

It is important to point out that only well-delineated, arterially enhancing lesions can be selected as target lesions for mRECIST.

Conversely, for non-enhancing atypical lesions, as well as for any extrahepatic neoplastic niches, the measurements of the longest overall tumor diameter for the assessment of response as per conventional RECIST should prevail.

Non-target lesions. All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline, including pathological lymph nodes with short axis ≥10 mm but <15 mm (nodes that have a short axis <10 mm are considered non-pathological and should not be recorded or followed).

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”).

According to mRECIST, infiltrative-type HCC should be considered as a non target lesion when the mass shows ill-defined borders and therefore does not appear to be suitable for accurate and repeat measurements.

11.3.1.4. Target Lesions Tumor Response

Complete response (CR) is defined as disappearance of all target lesions and reduction in short axis to <10 mm of any pathological lymph nodes (whether target or non-target).

Partial response (PR) is defined as at least 30% decrease in the sum of the diameters of the target lesions, taking as a reference the baseline sum diameters.

Progressive disease (PD) is defined as at least 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) is defined as neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

According to mRECIST treatment response for target lesions will be defined as follows:

CR: the disappearance of any intratumoral arterial enhancement in all target lesions.

PR: at least a 30% decrease in the sum of diameters of viable (contrast enhancement in the arterial phase) target lesions, taking as reference the baseline sum of the diameters of target lesions.

PD: an increase of at least 20% in the sum of the diameters of viable (enhancing) target lesions, taking as reference the smallest sum of the diameters of viable (enhancing) target lesions recorded since the treatment started. To note that, consistent with RECIST and mRECIST criteria, only radiographic progression, not clinical progression, determines the calculation of RECIST PD or mRECIST PD.SD: any cases that do not qualify for either partial response or progressive disease.

Since for HCC the measurement of the longest diameter of the viable tumor may be challenging in lesions showing partial internal necrosis, according to mRECIST, the following points should be taken into account in such cases:

- The measurement of the viable tumor should be performed on CT or MRI obtained in the arterial phase, when the contrast between viable vascularized tumor tissue and non enhancing necrotic tissue is the highest.

- The longest diameter of the viable tumor is not necessarily located in the same scan plane in which the baseline diameter was measured: a thorough careful evaluation of the CT or MRI scans is required.

- The measurement of the viable tumor diameter should not include any major intervening areas of necrosis.

11.3.1.4.1. 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. 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 is 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 retro-peritoneum; 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”.

11.3.1.5. Non Target Lesions Tumor Response

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) is defined as the 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 is defined as a persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive disease (PD) is defined as unequivocal progression of existing non-target lesions. (Note: the appearance of one or more new lesions is also considered progression).

When effusions are known to be a potential adverse effect of treatment, the cytological/histological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or SD is mandatory to differentiate between response or SD and PD.

Tumor necrosis should be taken into account when assessing the response of non-target lesions. According to mRECIST the disappearance of intratumoral arterial enhancement in non-target lesions should be considered equivalent to the disappearance of non-target lesions, and therefore, should declare complete response of non-target lesions. The persistence of intratumoral arterial enhancement in one or more non-target lesions should be considered equivalent to persistence of one or more non-target lesions, and therefore, should declare incomplete response/stable disease. The appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions should declare progressive disease.

For patients with HCC and cirrhosis the mRECIST provides the following special recommendations for the assessment of tumor response in non-target lesions:

1. Portal vein thrombosis. Malignant portal vein thrombosis should be considered a non measurable lesion due to the difficulty in performing consistent measurements of the malignant thrombus during the course of the treatment. Measurements of the extent of the malignant thrombus may be impaired by the possible presence of a bland component of the thrombosis.

2. Portal hepatic lymph node. Lymph nodes detected at the portal hepatic can be considered as malignant if the lymph nodes short axis is at least 20 mm. Evidence of reactive lymph nodes at the porta hepatis, in fact, is a common finding in patients with cirrhosis regardless of the presence of an HCC. The short axis of the node is the diameter normally used by radiologists to judge if a node is involved by solid tumor.

3. Pleural effusion and ascites. As per original RECIST, cytologic confirmation of the neoplastic nature of any effusion that appears or worsens during treatment is required when the measurable tumor has met criteria for response or stable disease. Under such circumstances, the cytologic examination of the fluid collected will permit differentiation between response or stable disease (an effusion may be aside effect of the treatment) and progressive disease (if the neoplastic origin of the fluid is confirmed). The mRECIST for HCC panel of experts considered this issue to be of high importance in the setting of HCC in cirrhosis. The emergence or the increase in ascites is a common event during the course of treatment in a cirrhotic patient, which may be due to worsening of the underlying chronic liver disease and be unrelated to cancer progression. Other effusions, such as pleural effusion, may also be unrelated to cancer progression and be caused by the liver insufficiency. Thus, the mRECIST for HCC emphasizes that cytopathologic confirmation of the neoplastic nature of any effusion (particularly ascites) that appears or worsens during treatment is required when the measurable tumor has met criteria for response or stable disease. It has to be underlined that peritoneal carcinomatosis is a very rare event in HCC.

11.3.1.5.1. Special notes on assessment of progression of non-target disease

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.

11.3.1.6. 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 tumour (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. A lesion identified on a follow-up study in anatomic allocation that was not scanned at baseline is considered a new lesion and will indicate disease progression. 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.

Characterization of a newly detected focal liver lesion as true HCC is a challenging issue in the setting of cirrhosis because pathologic abnormalities related to cirrhosis changes, such as large regenerative nodules and dysplastic nodules, may be indistinguishable from a small

tumor. Moreover, the presence of perfusion abnormalities resulting in areas of abnormal liver enhancement in a cirrhotic liver may ultimately mimic or conceal focal liver lesions; hence, perfusion abnormalities represent an additional major source for interpretation errors.

According to mRECIST the assessment of tumor progression was observed when:

a) A newly detected hepatic nodule will be classified as HCC, and therefore will be declared as evidence of progression, when its longest diameter is at least 1 cm and the nodule shows the typical vascular pattern of HCC on dynamic imaging, that is, hypervascularization in the arterial phase with washout in the portal venous or late venous phase.

b) Liver lesions larger than 1 cm that do not show a typical vascular pattern can be diagnosed as HCC by evidence of at least 1-cm-interval growth in subsequent scans.

c) An individual radiologic event will be adjudicated in retrospect as progression at the time it was first detected by imaging techniques, even if strict criteria were fulfilled only on subsequent radiologic testing.

11.3.1.7. Confirmation of Tumor Response

To be assigned a status of PR or CR, changes in tumor measurements in patients with responding tumors must be confirmed ≥4 weeks after the criteria for response are first met by the same tumor imaging technique used at baseline and on treatment (e.g., CT scan or MRI).

In case of SD, measurements must have met the SD criteria at least once after study entry at the minimum interval of 8 weeks.

11.3.1.8. Determination of Overall Response by RECIST (version 1.1)

When both target and non-target lesions are present, individual assessments will be recorded separately. The overall assessment of response will involve all parameters as depicted in Table 9.

Table 9. Response Criteria by RECIST 1.1*

Target Lesion[1]	Non-Target Lesion[2]	New Lesion[3]	Overall Response[4]
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any response	Yes or No	PD
Any response	PD	Yes or No	PD
Any response	Any response	Yes	PD

[1] Measurable lesions only.
[2] May include measurable lesions not followed as target lesions or non-measurable lesions.
[3] Measurable or non-measurable lesions.
Abbreviations: CR = complete response, PD = progressive disease, PR = partial response, SD = stable disease, NE = not evaluable.

** According to RECIST 1.1, patients with non-target disease only will be classified as CR if no new lesions appear and baseline lesions completely disappear;*
non-CR/non-PD, in case of no new lesions and neither CR or unequivocal PD in baseline lesions;
PD if new lesions appear or baseline lesions show unequivocal progression

11.3.1.9. Determination of Overall Response by mRECIST

When both target and non-target lesions are present, individual assessments will be recorded separately. The overall assessment of response will involve all parameters as depicted in the table here below.
In mRECIST, identical to conventional RECIST, overall patient response is a result of the combined assessment of target lesions, non target lesions, and new lesions.

Table 10. Response Criteria in mRECIST*

Target Lesions [1]	Non-Target Lesions[2]	New Lesions [3]	Overall Response
CR	CR	No	CR
CR	IR/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any response	Yes or No	PD
Any response	PD	Yes or No	PD
Any response	Any response	Yes	PD

[1] Measurable lesions only
[2] May include measurable lesions not followed as target lesions or non-measurable lesions
[3] Measurable or non-measurable lesions
Abbreviations: mRECIST = modified Response Evaluation Criteria in Solid Tumors, CR = complete response, IR = incomplete response, PD = progressive disease, PR = partial response, SD = stable disease

**According to mRECIST for HCC, in absence of target lesions, the following definitions of the criteria used to determine the objective tumor response for nontarget lesions apply: complete response is the disappearance of all nontarget lesions; incomplete response/stable disease is the persistence of one or more nontarget lesions; and progressive disease is the appearance of one or more new lesions and/or unequivocal progression of existing non target lesions.*

According to mRECIST for HCC, tumor necrosis should be taken into account when assessing the response of nontarget lesions. The disappearance of intratumoral arterial enhancement in nontarget lesions should be considered equivalent to the disappearance of nontarget lesions, and therefore, should declare complete response of nontarget lesions

It is important to point out that appearance of one or more new lesions declares progression whatever the response of target and non-target lesions.

11.3.1.10. Best Overall Response

The best overall response is the best response recorded from the start of the study treatment until the end of treatment taking into account any requirement for confirmation (achievement of both measurement and confirmation criteria, depending on the nature of the study and the protocol requirement). The patient's best overall response assignment will depend on the findings of both target and non-target disease and will also take into consideration the appearance of new lesions.

Table 11. Best overall response when confirmation of CR and PR required

Overall response First time point	Overall response Subsequent time point	BEST overall response
CR	CR	CR
CR	PR	SD, PD or PR[a]
CR	SD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	NE	SD provided minimum criteria for SD duration met, otherwise NE
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
PR	NE	SD provided minimum criteria for SD duration met, otherwise NE
NE	NE	NE

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, and NE = not evaluable.
[a] If a CR is truly met at first time point, then any disease seen at a subsequent time point, even disease meeting PR criteria relative to baseline, makes the disease PD at that point (since disease must have reappeared after CR). Best response would depend on whether minimum duration for SD was met. However, sometimes 'CR' may be claimed when subsequent scans suggest small lesions were likely still present and in fact the patient had PR, not CR at the first time point. Under these circumstances, the original CR should be changed to PR and the best response is PR.

In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of a CR depends upon this determination, it is recommended that the residual lesion be investigated by fine needle aspirate or biopsy before assigning a status of CR.

For equivocal findings of progression (e.g., very small and uncertain new lesions; cystic changes or necrosis in existing lesions), treatment may continue until the next scheduled assessment. If at the next scheduled assessment, progression is confirmed, the date of progression should be the earlier date when progression was suspected.

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" (see Study Manual specific data collection instructions). Of note, every effort should be made to document the objective progression even after discontinuation of treatment. Symptomatic deterioration is not a descriptor of an objective response: it is a reason for stopping study therapy. The objective response status of such patients is to be determined by evaluation of target and non-target disease.

11.4. Outcomes Research Assessments

Not applicable.

11.5. Safety Assessments

Safety assessments include collection of AEs, SAEs, 12-lead ECGs, pregnancy tests, vital sign measurements, physical examinations and safety laboratory tests as outlined in the Schedule of events, Section 7.

Additional safety assessments may be performed if deemed necessary by the Investigator.

Assessment of adverse events will include type, incidence, severity (graded by the National Cancer Institute [NCI] Common Terminology Criteria for Adverse Events [CTCAE], Version 5.0) [21], timing, seriousness, and relatedness.

11.5.1. Adverse Event Assessment

11.5.1.1. Definition of Adverse Events

11.5.1.1.1. Adverse Event

According to ICH definition and EU Regulation (No. 536/2014), an AE is defined as any untoward medical occurrence in a subject to whom a medicinal product is administered and which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or diagnosis temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Any untoward medical occurrence, which occurs outside the period of patient follow-up defined in the protocol, is not considered an AE. Symptoms or medically significant laboratory or instrumental (e.g., by electrocardiography) abnormalities of a pre-existing condition should not be considered an AE. However, occurrence of new symptoms, laboratory or instrumental abnormalities, as well as worsening of pre-existing ones, is considered AEs.

11.5.1.1.2. Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that at any dose falls into one or more of the following categories:

- Results in death
- Is life-threatening, i.e., an event which, in the view of the Investigator, placed the patient at risk of death at the time of event (it does not include an event which hypothetically might have caused death if it was more severe)
- Requires inpatient hospitalization or prolongation of existing hospitalization

- Results in persistent or significant disability/incapacity, where disability is defined as a substantial disruption of a person's ability to conduct normal life functions, either reported or defined as per clinical judgment
- Is a congenital anomaly/birth defect (if exposure to product just before conception or during pregnancy resulted in an adverse outcome in the child)
- Is any other important medical event, i.e., may not result in death, be life-threatening, or require hospitalization, but based upon appropriate medical judgment, it may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in the points above. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, and blood dyscrasias or convulsions that do not result in inpatient hospitalization.

A non-serious adverse event is any adverse event that does not meet the criteria listed above or the outcome cannot be determined with the information provided.

Each adverse event has to be classified by the Investigator as serious or non-serious.

In this study the following do not have to be classified as SAEs:

- Admission to hospital required by protocol.
- Hospitalization for routine treatment or monitoring of the studied indication not associated with any deterioration in patient's clinical condition.
- Hospitalization or prolongation of hospitalization for technical, practical, or social reasons, in absence of an AE.
- A procedure requiring hospitalization planned prior to starting the study treatment; must be documented in the Source Documents and the e-Case Report Form (e-CRF). Prolonged hospitalization for a complication remains a reportable SAE.
- Hospitalization for an elective treatment of a pre-existing condition unrelated to the studied indication.
- Events definitely related to disease progression.

However, all deaths occurring during the reporting period have to be reported as SAEs, even if due to disease progression.

11.5.1.2. Unexpected Adverse Event

An unexpected AE is one, the nature or severity of which is not consistent with the applicable product information that, for the present trial, is the Investigator Brochure (IB) [15].

11.5.1.3. Eliciting Adverse Event Information

The Investigator has to report all directly observed AEs and all AEs spontaneously reported by the trial patient using concise medical terminology. In addition, each trial patient will be

questioned about adverse events at each clinic visit following signature of the Informed Consent Form. The question asked will be “Since your last clinical visit have you had any health problems?”

11.5.1.4. Adverse Event Reporting Period

The adverse event reporting period for this trial begins upon signing of informed consent and ends 28 days after the last study treatment administration.

However, if a patient begins a new anticancer therapy earlier than 28 days after the last dose of study treatment administration, the adverse event reporting period will end at the time the new anticancer therapy starts.

All the adverse events that occur in trial patients during the adverse event reporting period must be reported to the Sponsor, whether or not the event is considered related to the study treatment.

11.5.1.5. Adverse Event Follow Up after the End of the Reporting Period

The following events should be followed after the end of the reporting period (i.e., 28 days after the last study treatment administration):

1. SAEs with outcome ‘not recovered’ or ‘unknown’ at the end of the reporting period.

2. Non-serious events classified as related to the investigational study treatment with outcome ‘not recovered’ or ‘unknown’ at the end of the reporting period.

These events should be followed until they resolve or until the Investigator determines, whenever possible, that they have become “chronic” or “stable”. Resolution of such events is to be documented on the SAE Form and/or on e-CRF.

In addition, if after the end of the reporting period, suspected serious adverse reactions or deaths are reported to the Investigator and he/she believes that they are related to the IMP, it is the Investigator’s responsibility to report these suspected serious adverse reactions to the Sponsor. Such suspected serious adverse reactions will be reported using a Serious Adverse Event Report or by any other way chosen by the Investigator.

11.5.1.6. Relationship to the Investigational Medicinal Product

The relationship of the adverse event will be assessed by means of the question “Is there a reasonable possibility that the event may have been caused by the study drug?” The Investigator should respond to this question with either ‘Yes’ or ‘No’.

‘No’ equals to Unrelated/Unlikely, i.e. there is no reasonable possibility that the study drug caused the event; ‘Yes’ equals to Related, i.e. there is a reasonable possibility that the study drug caused the event.

11.5.1.7. Reporting Requirements

Each adverse event has to be classified by the Investigator as SERIOUS or NON-SERIOUS (as per paragraph 11.5.1.1.2). This classification of the event determines the reporting procedures to be followed. If a serious adverse event occurs, reporting will follow local and international regulations, as appropriate.

If a serious adverse event occurs the Sponsor Pharmacovigilance has to be notified, using the designated form, within 24 hours of awareness of the event by the Investigator, by e-mail or by fax (here below Pharmacovigilance address/contacts).

e-mail: Drugsafety-MPSA@nervianoms.com

[redacted]

The initial report should be followed by submission of more detailed adverse event information as soon as it is available. Additional follow-up should be provided up to the resolution of the SAE, or the Investigator determines it has become chronic or stable (not recovered) or a new anticancer therapy is initiated.

Reporting requirements for adverse events are summarized in the following table.

REPORTING REQUIREMENTS FOR ADVERSE EVENTS

Seriousness	Reporting Time	Type of Report
SERIOUS	Within 24 hours	Initial report on designated SAE form
	As soon as available	Follow-up/Final report on designated SAE form
NON-SERIOUS	As per eCRF procedure	Appropriate section of eCRF

Suspected Unexpected Serious Adverse Reactions (SUSARs) will be reported by the Sponsor to all competent Regulatory Authorities, to the Ethics Committees and to all the Investigators involved, according to local regulations and requirements stated in ICH Good Clinical Practice.

In the rare event that the Investigator does not become aware of the occurrence of a serious adverse event immediately (for example, if an outpatient trial patient initially seeks treatment elsewhere), the Investigator has to report the event within 24 hours after learning of it and document his/her first awareness of the adverse event.

Serious adverse events should also be consistently recorded in the appropriate section of e-CRF.

Non-serious adverse events have to be reported in the appropriate section of e-CRF only.

11.5.1.8. Recording Adverse Events in the Case Report Forms

Information on AEs must be evaluated by a physician and recorded in source documents such as the hospital file. AEs have to be reported in the e-CRF as aforementioned. The Investigator will also be asked to assess the relationship between the AE and the investigational medication.

- Preexisting Conditions

In this trial, a preexisting condition (i.e., a disorder present before the AE reporting period started and recorded in the pretreatment medical history form) should not be reported as AE unless the condition worsens, or episodes increase in frequency during the AE reporting period.

- Procedures

Diagnostic and therapeutic non-invasive and invasive procedures, such as surgery, should not be reported as AE. However, the medical condition for which the procedure was performed should be reported if it meets the definition of AE. For example, an acute appendicitis that begins during the AE reporting period should be reported as AE and the resulting appendectomy should be recorded in the source documents.

- Symptoms of the Disease

The specific symptoms of the disease have to be reported as adverse events if they correspond to new occurrence or to worsening of severity or frequency versus the baseline visit. Note: the general wording “Progression of the disease” should not be reported as adverse event. In case of global deterioration of health status requiring discontinuation, further data entry instructions are specified in the Study Manual.

- Abnormal Laboratory Findings

Abnormal laboratory findings have to be reported as adverse events when they cause study drug administration change (dose omission, dose delay, dose reduction, infusion interruption, drug discontinuation) or when they require clinical intervention (e.g., hospitalization for further investigation or management of the laboratory abnormality).
Note: uncomplicated and asymptomatic abnormal laboratory findings have not to be reported as adverse events. The corresponding values have to be reported only in the relevant e-CRF section (e.g., hematology, biochemistry).

11.5.1.9. Grading of Adverse Event Severity

In this study the severity/intensity of AEs will be graded using the Common Terminology Criteria for Adverse Events (CTCAE, Version 5.0) of the US National Cancer Institute [21].

For each event, changes in severity grade and seriousness criteria should be recorded.

For adverse events not reported in the CTCAE Version 5.0, the Investigator will use the Grade or adjectives reported in Table 12

Table 12. Grading of Adverse Event Severity for events not reported in the CTCAE v 5.0

Grade	Adjective	Description
Grade 1	Mild	Does not interfere with patient's usual function
Grade 2	Moderate	Interferes to some extent with patient's usual function
Grade 3	Severe	Interferes significantly with patient's usual function
Grade 4	Life-threatening	Results in threatening to life or in an incapacitating disability
Grade 5	Death	Results in death

Note the distinction between the term "serious" and "severe", which are not synonymous. The term "severe" is used to describe the intensity of a specific event (i.e., severe headache) while the term "serious" is based on patient/event outcome or action criteria. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations. For example, vomiting Grade 1 for which a hospitalization occurred is a serious event even if the event is not severe.

11.5.1.10. Exposure In Utero

If a female patient becomes or is found to be pregnant while receiving the study drug or within 180 days from its discontinuation, the Investigator should transmit this information using the Part I of Exposure in Utero (EIU) Form to the Sponsor Pharmacovigilance. This must be done irrespective of whether an adverse event has occurred and within 24 hours of awareness of the pregnancy. However, if the patient begins a new anticancer therapy before 90 days after the last dose of study treatment administration, the exposure "in utero" reporting period will end at the time the new treatment is started.

The Investigator will follow the patient until completion of the pregnancy or until pregnancy termination (i.e., induced abortion) and then notify the Sponsor of the outcome within 5 days or as specified below. The Investigator will provide this information completing Part II of EIU form. The reason(s) for an induced abortion must be specified.

If the outcome of the pregnancy meets the criteria for immediate classification as a serious adverse event (i.e., spontaneous abortion, stillbirth, neonatal death, or congenital anomaly [including that in an aborted fetus]), the Investigator should follow the procedures for reporting serious adverse events, i.e., report the event to the Sponsor (as described in Section 11.5.1.7)

In the case of a live birth, the "normality" of the newborn can be assessed at the time of birth (i.e., no minimum follow-up period of a presumably normal infant must pass before an Exposure in Utero Form can be completed). The "normality" of an aborted fetus can be assessed by gross visual inspection unless pre-abortion laboratory findings are suggestive of a congenital anomaly.

Other pregnancy outcomes that are classified as SAEs:

- "Spontaneous abortion" includes miscarriage and missed abortion.
- All neonatal deaths that occur within 1 month of birth should be reported, without regard to causality. In addition, any infant death after 1 month that the Investigator assesses as possibly related to the "in utero" exposure to the study drug should also be reported.

If a female partner of a male patient taking the IMP becomes pregnant while the male patient is still on IMP or within 90 days after last dose of IMP, the male patient taking IMP should notify the Investigator, and the pregnant female partner should be advised to call her healthcare provider immediately. If a pregnancy related event is reported in a female partner of a male patient, the Investigator should ask if the female partner is willing to share information with the Sponsor and allow the pregnancy related event to be followed up to completion.
The Investigator has to submit the information of pregnancy or suspect pregnancy of a female partner of a male patient to the Sponsor Pharmacovigilance by e-mail or by fax, using the EIU Form, within 24 hours from his/her awareness of the event.

11.5.2. Laboratory Safety Assessments

Laboratory safety assessments will include repeated evaluation of hematology, biochemistry and coagulation parameters, urinalyses and pregnancy test, according to timing reported in Section 7, Schedule of Events (Table and related footnotes). Investigators may order additional blood tests for planning treatment administration, dose modification, or further evaluation of adverse events.

The following laboratory parameters will be used to monitor safety:

- *Hematology* (local laboratory) includes: hemoglobin (Hb), red blood cells (RBC), platelet (PLT) and white blood cells (WBC) with differential count (neutrophils, lymphocytes, monocytes, eosinophils and basophils). The assessment is mandatory, at screening (≤7 days from treatment start). During treatment on Phase I, hematology should be done from cycle 1 to cycle 3, on days 1, 8, 15, 22 and, from cycle 4 onward, on days 1, 8, 15; during treatment on Phase II, hematology should be done on cycle 1, on days 1, 8, 15, 22; from cycle 2 onward, on days 1, 8, 15; at cycle 2, in case neutropenia Grade ≥2 is observed, repeat the hematological evaluation also at day 22. In case of cycle delay due to toxicity and/or if clinically indicated, additional assessments should be done as per medical judgment; hematology should be repeated at the start of the delayed cycle. All hematology assessments performed during the treatment period can be done the day before the scheduled one; on days of treatment administration, sampling should be performed and results should be available before the start of NMS-01940153E infusion. Hematology should be performed also at end of treatment (EoT) and 28-days follow up visits. Note: at Cycle 1 Day 1, hematology does not need to be repeated before study drug administration if it was performed at screening within 24 hours from treatment start.

- *Blood chemistry* (local laboratory) includes: glucose, electrolytes (Na, K, Ca, P, magnesium), urea or BUN (Phase II), serum creatinine, creatinine clearance (calculated with Cockroft-Gault equation), total protein, albumin, aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), gamma-GT, total bilirubin, unconjugated bilirubin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH). The assessment is mandatory, at screening (≤7 days from treatment start). During treatment on Phase I, blood chemistry should be done from cycle 1 to cycle 3, on days 1, 8, 15, 22 and, from cycle 4 onward, on days 1, 8, 15; during treatment on Phase II, blood chemistry should be done on cycle 1, on days 1, 8, 15, 22 and, from cycle 2 onward, on days 1, 15. In case of cycle delay due to toxicity and/or if clinically indicated, additional assessments should be done as per medical judgment; blood chemistry should be repeated at the start of the delayed cycle. All blood chemistry assessments performed during the treatment period can be done the day before the scheduled one; on days of treatment administration, sampling should be performed, and results should be available before the start of NMS-01940153E infusion. Blood chemistry should be performed also at EoT and 28-days follow up visits. Note: at Cycle 1 Day 1, blood chemistry does not need to be repeated before study drug administration if it was performed at screening within 24 hours from treatment start.
- *Coagulation* (local laboratory) includes international normalized ratio (INR) of prothrombin. The assessment is mandatory, at screening (≤7 days from treatment start). During treatment on Phase I, coagulation should be done from cycle 1 to cycle 3, on days 1, 8, 15, 22 and, from cycle 4 onward, on days 1, 8, 15; during treatment on Phase II, blood chemistry should be done on cycle 1, on days 1, 8, 15, 22 and, from cycle 2 onward, on days 1, 15. In case of cycle delay due to toxicity and/or if clinically indicated, additional assessments should be done as per medical judgment; coagulation should be repeated at the start of the delayed cycle. All coagulation assessments performed during the treatment period can be done the day before the scheduled one; on days of treatment administration, sampling should be performed, and results should be available before the start of NMS-01940153E infusion. Coagulation should be performed also at EoT and 28-days follow up visits. Note: at Cycle 1 Day 1, coagulation does not need to be repeated before study drug administration if it was performed at screening within 24 hours from treatment start.
- *Urinalysis* (local laboratory) includes: pH, glucose, protein and blood. The assessment is mandatory, at screening (≤7 days from treatment start) and at EoT visit. If clinically indicated, additional assessments should be done as per medical judgment.
- *Serum/Urine Pregnancy Test* (local laboratory). It is indicated only for women of child-bearing potential. Phase I: the assessment should be done at screening, within 72 hours from treatment start, and at EoT visit. Phase II: the assessment should be done at screening, within 72 hours from treatment start, on day 1 of each treatment cycle (before treatment administration), and at EoT visit. Note: at Cycle 1 Day 1, pregnancy test does not need to be repeated before study drug administration if it was performed at screening within 24 hours from treatment start.

- *Serology*: in case of positive HBV anamnesis, includes HBV DNA; in case of positive HCV anamnesis, includes HCV RNA; in case of HBV unknown anamnesis, includes HbsAg, HbeAg, HbcAg, Anti-HBc-IgM; in case of HCV unknown anamnesis, includes liver function test as by local standards and HCV Ab. Anti-HIV test. To be performed at screening, within 7 days from treatment start.

In case hematology, blood chemistry and coagulation have not recovered at the end of last cycle, they should be followed-up for 28 days after the last study treatment administration or until they resolve or the Investigator assesses them as chronic or stable, or until alternative anticancer therapy is initiated. In the latest case, the reporting period will end at the time the new treatment is started. If the laboratory value is abnormal, clinically significant and evaluated as drug related after the 28 days follow-up assessment, it has to be reported only as adverse event and as such followed until it resolves or the Investigator assesses it as chronic or stable, or until alternative anticancer therapy is initiated.

11.5.3. Other Safety Assessments

The following safety assessments will also be performed, according to timing reported in Section 7, Schedule of Events:

- *Physical examination*: on Phase II should be performed at screening, within 7 days from treatment start, on day 1 of each treatment cycle, at EoT and at 28-days follow up. Note: at Cycle 1 Day 1, it does not need to be repeated before study drug administration if it was performed at screening within 24 hours from treatment start.
- *ECOG PS*: should be recorded at screening, within 7 days from treatment start; on day 1 of each treatment cycle (before treatment administration); at EoT and at 28-days follow up visits. Note: at Cycle 1 Day 1, ECOG PS does not need to be repeated before study drug administration if it was performed at screening within 24 hours from treatment start.
- *Weight*: should be recorded at screening, within 7 days from treatment start, and on day 1 of each treatment cycle (before treatment administration).
- *Height*: should be recorded at screening only.
- *Temperature*: should be recorded as Celsius degree, at screening within 7 days from treatment start, and on days 1, 8, 15 of each treatment cycle (before treatment administration).
- *Blood pressure and heart rate*: the assessment is mandatory, at screening within 7 days from treatment start; on days 1, 8, 15 before the treatment start and within 30 minutes after the end of infusion (before any blood samples is taken); at EoT and 28-days follow up visits. At each determination, if SBP >150 mmHg and/or DBP >95 mmHg is registered, repeat determinations two more times at 30 minutes interval.
- *Gastro-esophageal endoscopy*: should be performed to all patients with known history of gastro-esophageal varices. For these patients the assessment is mandatory, at screening within 7 days from treatment start, unless an endoscopy is available within 3 months

before the treatment start. For all other patients the assessment is not required, granted that an endoscopy is available within 24 months before the treatment start, or PI would justify the absence of clinical need for this assessment;

12-lead EC(12-lead, in triplicate, includes qualitative and quantitative assessment): the assessment is mandatory at screening, within 7 days from treatment start, at cycle 1 day 1 (first dose) and at cycle 1 day 15 (dose 3) triplicate ECG will be collected prior to dosing (30 min before the start of infusion and at least 10 minutes after any blood drawn or intravenous access procedure) and at peak concentration, prior to and as close as possible to the PK timepoint planned 5 minutes before the end of infusion (e.g. ≤ 10 minutes), and at EoT visit. It includes QT interval using Fridericia standard (QTcF) calculation. If clinically indicated, additional assessments should be done as per medical judgment. To be repeated at any time it is medically indicated. If the ECG assessment is not feasible on cycle 1 day 1 or cycle 1 day 15, a different timing or other solutions should be discussed with Sponsor. At each time point, three consecutive supine 12-lead ECGs will be performed approximately 2 minutes apart to determine mean QT interval using Fridericia standard (QTcF). The following parameters will be collected: synus rithm, heart rate, RR interval, PR interval, QRS interval, QT interval and QTcF interval. For all patients enrolled in the study, the Sponsor may request the ECG tracing and reports collected during the study be submitted to a third-party for retrospective independent review of the ECGs reading and interpretation.

- *AFP*: to be performed at screening, within 7 days from treatment start, at the end of even cycles at disease evaluation and at EoT. A time window of -2/+5 days is allowed.
- *Survival follow up*: the post-treatment survival status, including additional information (e.g., new anticancer therapy), will be collected at the 28-days follow up visit and thereafter every 2 months from last treatment administration until death. Telephone contacts are acceptable for these assessments.

11.6. Other Assessments

11.6.1. Blood sampling

Plasma samples will be used for pharmacokinetic evaluations of NMS-01940153 and its main metabolite NMS-03593478.

During the dose escalation part of the study (Phase I), blood samples will be collected from all patients during Cycle 1 and Cycle 2. The sample schema for pharmacokinetic (PK) collection is outlined in Table 13. During the Phase II, blood samples will be collected from all patients, based on their enrollment number (odd or even), during Cycles 1, 2, 3 and 4. The sample schema for PK collection is outlined in Table 14.

Blood (4 mL/timepoint) will be collected into sodium heparin Vacutainer tubes for PK analysis at the designated time. Additional blood samples for PK analysis may be collected if, in the opinion of the Investigator and of the Sponsor, an evaluation of PK parameters outside the schedule above is needed for safety reasons.

Table 13. Phase I PK Blood Sample Collection

Cycle	Day	Sample number	Sample timepoint
1	1	#1	Pre-dose
		#2	5 minutes before the end of infusion
		#3	15 minutes after the end of infusion
		#4	30 minutes after the end of infusion
		#5	1 h after the end of infusion
		#6	2 h after the end of infusion
		#7	4 h after the end of infusion
		#8	6 h after the end of infusion
		#9	8 h after the end of infusion
	2	#10	24 h after the end of infusion
	3	#11	48 h after the end of infusion
	4	#12	72 h after the end of infusion
	8	#13	Pre-dose
	15	#14	Pre-dose
		#15	5 minutes before the end of infusion
		#16	15 minutes after the end of infusion
		#17	30 minutes after the end of infusion
		#18	1 h after the end of infusion
		#19	2 h after the end of infusion
		#20	4 h after the end of infusion
		#21	6 h after the end of infusion
		#22	8 h after the end of infusion
	16	#23	24 h after the end of infusion
	17	#24	48 h after the end of infusion
	18	#25	72 h after the end of infusion
	22	#26	168 h after the end of infusion
2	1	#27	Pre-dose
	8	#28	Pre-dose
	15	#29	Pre-dose

On Phase I, during Cycle 1, the total volume of blood withdrawn for pharmacokinetic determinations from each patient will be 104 mL over 22 days (26 samples); during Cycle 2, the total volume of blood withdrawn for pharmacokinetics determinations from each patient will be 12 mL over 15 days (3 samples).

Table 14. Phase II PK Blood Sample Collection

Cycle	Day	Sample number	Enrollment number	Sample timepoint
1	1	201	All	5 minutes before the end of infusion
		202	Odd	From 15 min to 2h after the end of infusion
		203	Even	From >2h to 6h after the end of infusion
	8	204	Odd	Pre-dose
		205	Even	5 minutes before the end of infusion
	15	206	All	Pre-dose
		207	All	5 minutes before the end of infusion
		208	Even	From 15 min to 2h after the end of infusion
		209	Odd	From >2h to 6h after the end of infusion
	22	210	All	At any time
2	1	211	All	Pre-dose
	15	212	All	Pre-dose
		213	All	5 minutes before the end of infusion
		214	Odd	From 15 min to 2h after the end of infusion
		215	Even	From >2h to 6h after the end of infusion
	26 or 27 or 28	216	All	At any time
3	15	217	All	Pre-dose
		218	All	5 minutes before the end of infusion
		219	Even	From 15 min to 2h after the end of infusion
		220	Odd	From >2h to 6h after the end of infusion
4	1	221	All	Pre-dose
	15	222	All	Pre-dose
		223	All	5 minutes before the end of infusion
		224	Odd	From 15 min to 2h after the end of infusion
		225	Even	From >2h to 6h after the end of infusion
	26 or 27 or 28	226	All	At any time

On Phase II, during Cycle 1, the total volume of blood withdrawn for PK determinations from each patient will be 28 mL over 22 days (7 samples); during Cycle 2, the total volume of blood withdrawn for PK determinations from each patient will be 16 mL over 26/28 days (4 samples); during Cycle 3, the total volume of blood withdrawn for PK determinations from each patient will be 12 mL over 1 day (day 15; 3 samples); during Cycle 4, the total volume of blood withdrawn for PK determinations from each patient will be 16 mL over 26/28 days (4 samples).

Details on PK samples collection, processing, storage and shipping instructions will be provided in the Study Manual.

11.6.2.1. Bioanalytical method

NMS-01940153E and its main metabolite NMS-03593478 will be assayed in plasma using a validated LC-MS/MS method. Pharmacokinetics analysis will be performed by Accelera S.r.l. (located in Italy) identified by the Sponsor as the entity in charge of samples shipment, storage and analysis.

11.6.3. Urine Sampling

Urine samples will be used for determination of the urinary excretion of the parent drug and its main metabolite.

Urine samples will be collected only during the phase I of the study, at Cycle 1, on days 1 and 15. Urine will be collected before NMS-01940153E administration and in four different portions between 0-6h, 6-24h, 24-48h, 48-72h from the start of study drug administration.

At the end of the urine collection period, for each portion, the total volume of urine will be measured, and total volume recorded in eCRF. The urine will then be mixed aliquots withdrawn for PK analysis, as outlined in Table 15.

Table 15. PK Urine Sample Collection

Cycle	Day	Sample number	Sample timepoint
1	1	#1	Pre-dose
		#2	0-6h from the start of infusion
		#3	6-24h from the start of infusion
		#4	24-48h from the start of infusion
		#5	48-72h from the start of infusion
	15	#6	Pre-dose
		#7	0-6h from the start of infusion
		#8	6-24h from the start of infusion
		#9	24-48h from the start of infusion
		#10	48-72h from the start of infusion

Additional urine samples for PK analysis may be collected if, in the opinion of the Investigator and of the Sponsor, an evaluation of PK parameters outside the schedule above is needed for safety reasons.

Details on sample collection and handling and shipping instructions will be provided in the Study Manual.

All the samples for pharmacokinetic determinations will be sent to Accelera (located in Italy), or other identified by the Sponsor as the entity in charge of samples shipment, storage and analysis.

11.6.4. Exploratory Assessments

11.6.4.1. cfDNA Sampling

For cfDNA analysis, 20 ml of peripheral blood will be collected for all patients at screening. Sponsor might decide, at any time, to stop collection of these samples based on scientific or logistic reasons.

12. STATISTICAL METHODS

12.1. Sample Size Calculation

An overall sample size of approximately 55 patients may be anticipated considering both phases of the study.

For the Phase I, approximately 12-15 treated patients may be expected. Since the trial design foresees that sequential dose-escalation steps are applied to cohorts of 3 to 6 patients up to the identification of the MTD, the number of patients who will be enrolled and treated may vary, depending upon the toxicity observed that will influence cohort size and number of dose levels tested.

For the Phase II, the investigator-assessed objective response rate by RECIST 1.1. will be the primary outcome measure.

The sample size is based on precision considerations for the estimate of the primary efficacy endpoint. A single-group design will be used to obtain a one-sided 95% lower-limit confidence interval for a single proportion. The sample proportion is assumed to be 0.3. To produce a confidence interval with a distance from the sample proportion to the lower limit of no more than 0.12, 38 patients will be needed. A lower bound of 18% for ORR by RECIST would be meaningful relative to second line therapies in HCC. The emergence of atezolizumab with bevacizumab in first line has obscured true current second line outcomes with regorafenib, lenvatinib or sorafenib but it is logical to assume second line ORR would be no better relative to the pre-atezolizumab/bevacizumab era in which sorafenib was the first line standard. Prior to the atezolizumab/bevacizumab era, regorafenib in second line showed ORR of 11% by RECIST1.1 [10] and cabozantinib 4% [22], nivolumab 15% [23], pembrolizumab 18% [24] and apatinib 10.7% [25]. Therefore, in the third line setting following failure of atezolizumab/bevacizumab in first line followed by TKI in second line, and where current third line standards are not yet defined, it is reasonable to expect historical ORR no higher than 18%. Accounting for a 10% proportion of non-evaluable patients and if the stopping rule for futility is not met, up to 43 patients could be required for completing the trial, as reported in detail in Section 12.4; however, the Phase II part of the study will not enroll beyond 40 subjects unless discussed and agreed with FDA or relevant health authority, based on emerging response rates.

12.2. Definition of Analyzed Study Populations

The following analysis sets are defined according to ICH E9 guidelines [26].

Phase I Dose Escalation

Enrolled Set (ES): this population will include all patients who are enrolled in Phase I part, regardless of whether patients receive study treatment or not. This population will be evaluated in the analysis of patients' disposition.

Treated Set (TS): this set will include all enrolled patients who take at least one dose of NMS-01940153E. This population will be evaluated in the analysis of patients' disposition, baseline characteristics, treatment exposure, safety and efficacy.

Dose-Limiting Toxicity Evaluable Set (DLTES): this set will include all patients who receive in the first cycle at least 66% of study drug, unless the reason for non-compliance is drug-related toxicity, and undergo a DLT assessment within the DLT window. Patients not fulfilling one or more of aforementioned criteria will not be considered evaluable for MTD determination and will be substituted.

Phase II

Enrolled Set (ES): this population will include all patients enrolled in Phase II, regardless of whether patients receive study treatment or not. This population will be evaluated in the analysis of patients' disposition.

Treated Set (TS): this set will include all enrolled patients who actually receive at least one dose of NMS 01940153E. This population will be evaluated in the analysis of patients' disposition, baseline characteristics, treatment exposure and safety.

Evaluable Set (ES): this is the patient population for the primary efficacy analysis. This set will include patients of the TS who have measurable disease at baseline assessed according to RECIST 1.1 and at least one tumor evaluation on treatment, unless they die before the first tumor on-treatment assessment, in which case they will be considered treatment failure. In detail, the evaluable population for primary efficacy endpoint will consist of a subset of the evaluable population up to the total number of subjects pre-determined by the interim for futility and final analyses. This means that, if the number of evaluable subjects exceeds the planned total number of subjects at either stage, the exceeding subjects will not be considered for primary efficacy analysis. Subjects will be counted into the primary evaluable population, consecutively, based on the date of first NMS-01940153E administration.

12.3. Analyses

A Statistical Analysis Plan [26] will be written and completed before the planned interim analysis and locking the Database. These specifications will detail the implementation of all the planned statistical analyses in accordance with the principal features stated in the following sections.

Patients from the Phase I part and from the Phase II part will be analyzed separately.

12.3.1. Study Conduct and Subject Disposition

Patients' disposition and reasons for ending the treatment and the study will be presented in frequency distribution tables and individual data listings. The patients not meeting the eligibility criteria, and who are considered protocol violators will be identified and described by individual data listing. Reasons for stopping treatment will be summarized as frequency

distribution in the treated patient population and, if clinically interesting, in other patient subsets. Untreated patients will be identified and described separately.

12.3.2. Baseline Characteristics and Treatment Group Comparability

Descriptive statistics of the baseline characteristics will be generated across all treated patients and, if clinically interesting, in other patient subsets. Frequency distributions will be presented for the categorical/categorized variables. Summary statistics including mean, standard deviation, median, minimum, maximum and the number of assessed patients will be calculated, as appropriate, for the quantitative variables. Individual data will be presented in listings.

The study has no comparative arm.

12.3.3. Treatment Administration/Compliance

The treatment exposure and the compliance with study treatment will be descriptively analyzed in the treated patient population and, if clinically interesting, in other patient subsets. Descriptive statistics (e.g. min, max, mean, standard deviation, and median value) will be calculated on a per-patient basis for the following variables: the number of cycles administered, the overall duration of treatment, the actual and total doses administered, and the absolute and relative dose intensity. Frequency distributions of patients and/or cycles will be used to describe dose modifications, delays and omissions, as well as the reasons for deviation from the planned therapy. Individual data will be presented in listings as reported in the relevant CRF sections.

12.3.4. Efficacy Analyses

The efficacy of NMS-01940153E will be assessed on the treated set for Phase I and evaluable set for Phase II, respectively, as defined in Section 12.2. For Phase II, only if deemed of interest and only as supportive analysis, efficacy could be also evaluated on the treated population.

12.3.4.1. Primary Efficacy Endpoint

For Phase II part, the primary efficacy endpoint ORR will be evaluated as the proportion of patients who have achieved as best overall response, CR or PR as defined by investigator-assessed RECIST 1.1. In addition to the point estimate, binomial exact confidence intervals will be provided.

The decision rules for interim analysis for futility are specified in Section 12.1.

12.3.4.2. Secondary Efficacy Endpoints

Objective response rate will be also calculated for Phase II part only, as the percentage of evaluable patients with best response equal to complete response (CR) or partial response (PR) as defined by investigator-assessed mRECIST. Binomial exact confidence intervals will be also provided.

Time-to-event endpoints, i.e. Duration of Response (DoR), Progression Free Survival and Overall Survival: they will be carried out on treated set for Phase I and evaluable set for Phase II, respectively. Time-to-event endpoints will be also summarized using Kaplan Meier (KM) curves and further characterized in terms of median and landmark estimates with the corresponding confidence intervals, for Phase II part only.

12.3.5. Outcomes Research Analyses

Not applicable

12.3.6. Safety Analyses

Unless otherwise specified, the safety analyses will be performed on the treated set by assigned dose level and overall for Phase I and overall for Phase II.

Adverse events will be coded by Medical Dictionary for Regulatory Activities (MedDRA) and their severity will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE, version 5.0). The analysis will focus on the events reported after the start of treatment (treatment emergent adverse events). For each dose level and overall, the incidence of AEs will be grouped by System Organ Class (SOC) and by preferred term. Each patient will be counted once according to the worst grade reported throughout the whole treatment period for each SOC and/or for each preferred term. If clinically indicated, selected AEs will be presented by treatment cycle (Cycle 1 vs. Cycles >1). In addition, SAEs, AEs with severity grade 3-5, AEs leading to treatment withdrawal and AEs related to study treatment, will be reported separately.

The DLT occurrence during the first treatment cycle will be reported for the Dose-Limiting Toxicity Evaluable Set (DLTES).

Frequency of deaths will be presented overall and by dose level, by relationship to the study treatment and according to the time since treatment discontinuation.

Laboratory data will be graded according to the NCI CTCAE scale (version 5.0) whenever possible. Laboratory abnormalities will be summarized in frequency distribution tables by dose level and treatment period (i.e. treatment Cycle 1, Cycle >1 and the whole treatment period). For each parameter, patients will be classified based on the maximal severity grade observed during the analyzed time-window. Changes of severity grade vs. baseline will be evaluated by shift tables. Selected laboratory parameters will be further explored by analyzing the nadir/zenith values, the time to nadir/zenith and the time to recovery after nadir/zenith, as clinically indicated.

For the laboratory data not graded by the NCI CTCAE scale, a cross tabulation of on treatment worst finding versus baseline finding will be presented reporting the number of patients with values within or out of the normal range.

All collected safety data (including vital signs, ECG, laboratory assessments and adverse events, etc.) will be presented in individual patients' data listings. Identification of clinically relevant values and calculation of changes from baseline will be provided for those parameters and conditions deemed of clinical relevance.

12.3.7. Analyses of Other Endpoints

12.3.7.1. Alpha-Fetoprotein as Surrogate Marker of Efficacy

Exploratory analysis on the relationship between AFP levels and treatment efficacy variables in patients treated with NMS-01940153E will be performed if sufficient data are collected.

12.3.7.2. Pharmacokinetic

12.3.7.2.1. Phase I Dose Escalation

The pharmacokinetics of NMS-01940153E and its metabolite NMS-03593478 in plasma and urine will be assessed by standard non-compartmental analysis using Phoenix WinNonlin 8.1 or later version (Certara Company).

The following pharmacokinetic parameters will be provided on both Day 1 and Day 15: maximum concentration (C_{max}) and related time of achievement (T_{max}), last detectable concentration (C_{last}) and related time of observation (T_{last}), area under concentration versus time up to 168 hours (dosing interval), half-life of the terminal phase (t½,z), plasma clearance (CL), volume of distribution (Vss) and renal clearance (CLR).

On Day 1, for NMS-01940153E and NMS-03593478, maximum concentration, Cmax,D1, and related time of achievement, Tmax,D1, last detectable concentration, Clast,D1, and related time of observation, Tlast,D1, will be taken directly from the raw data.

Area under concentration versus time up to the last detectable concentration (AUClast) will be calculated using the linear trapezoidal rule. The half-life of the terminal phase, t½,z, will be determined by linear regression analysis of the natural-log concentration versus time curve according to the formula:

$$t_{1/2,z} = \frac{\ln(2)}{\lambda_z}$$

where λz is the slope of the regression line. A minimum of three data points will be used in calculating λz.

AUC∞ (D1) will be calculated by adding the portion of the area calculated as $C_{last,D1}/\lambda_z$ to AUClast,D1.

The clearance (CL) of NMS-01940153E will be calculated according to the formula:

$$CL = \frac{Dose}{AUC\infty_{D1}}$$

The volume of distribution at steady state (Vss) of NMS-01940153E will be calculated according to the formula:

$$V_{ss} = CL \cdot MRT \text{ , where } MRT = \frac{AUMC_{D1}}{AUC_{D1}} - \frac{t_{inf}}{2}$$

On Day 15, Cmax,D15, Tmax,D15, Clast,D15 and Tlast,D15 will be taken directly from the raw data.

AUC0-168,D15, AUClast,D15 and AUCD15 will be calculated as on Day 1.

The clearance (CL) of NMS-01940153E will be calculated according to the formula:

$$CL = \frac{Dose}{AUC_{\tau, D15}}$$

where AUCτ is the area under the curve in the dosing interval.

Accumulation ratios for NMS-01940153E and NMS-03593478, based on C_{max} (R_A,C_{max}) and AUC_{last} (R_A,AUC_{last}) will be calculated as the ratio between the individual parameter obtained on Day 15 to the corresponding one on Day 1.

On Day 1 and Day 15, metabolite to parent ratio will be calculated based on C_{max} and AUC_{last} values.

On Day 1 and Day 15, the amount of NMS-01940153E and its metabolite NMS-03593478 excreted in urine in the time interval 0-72 h (Ae_{0-72}) will be calculated multiplying the urinary concentration by the urinary volume in the collection times: 0-6, 6-24, 24-48 and 48-72 hours. The parameter will be also expressed as percent of dose (fe%). For the metabolite, the parameter expressed as percent of dose will be calculated after correction of the dose for the molecular weight of the compound.
Renal clearance of NMS-01940153E and its metabolite NMS-03593478 will be calculated as:

CLR = Ae0-72 / AUC0-72

Statistical analysis
Descriptive statistics (mean ±SD, CV%, min, max, median) will be calculated for plasma and urine concentration data and pharmacokinetic parameters.

Statistical analysis will be performed after a log-transformation of the data of NMS-01940153E treatment. For each group separately, a mixed effect model will be fitted with day

as fixed effect and subject as a random effect. Day 15 will be compared to Day 1 for each dose level. The ratio will be calculated by back-transforming the difference between the Least Square means. Using the estimate of variance, 90 % confidence intervals will be calculated for the difference and back-transformed. Results of the analyses will be reported in the paragraph of the statistical results.

The power method will be used to evaluate the dose proportionality of NMS-01940153E and possibly its metabolite. C_{max}, AUC_{last}, AUC_{0-168} and AUC data on Day 1 and Day 15. Dose *versus* Cmax and AUCs relationship will be performed using the power model

$$y = a \cdot Dose^{b}$$

This relationship becomes linear after a logarithmic transformation

$$\log(y) = \log(a) + b \cdot \log(dose)$$

where a is the intercept to y (AUC) axis and b is the slope of the regression line. Dose proportionality required slope to be close to unity for dose dependent parameters, and decision on dose proportionality will be made based on the inclusion of the theoretical slope of 1 in the 90 % confidence intervals.

12.3.7.2.2. Phase II

For PK plasma samples collected during Phase II, a sparse sampling procedure will be adopted. For each patient, a total of 20 plasma samples will be collected; the blood sample intervals are designed to patients to grant homogeneous distribution of the samplings in different time intervals (refer to Table 14).

Pharmacokinetic data from Phase II portion of the study will be analyzed using mixed-effect modeling approach. Data may be also pooled with PK data from previous Phase I studies.

Details of the sampling procedures as well as population PK model development description and population PK data analysis will be detailed in the population PK Statistical Analysis Plan.

The objective of the population pharmacokinetic data analysis will be to describe the pharmacokinetics of NMS-01940153E in the subject population and explore the role of demographic and pathophysiological covariates influencing the pharmacokinetics of the compound. The main pharmacokinetic parameters and the influence of covariates (e.g., gender, age, body weight, histopathological covariates) will be estimated using a population PK approach. The key guidances of FDA and EMA will be followed in the population PK analysis. The population PK analysis will be performed using NONMEM software (version VII or higher).

The population PK model will consist of the following:

- identification of the structural model that best describe the pharmacokinetic data in absence of covariates.
- Identification of the variance component/s characterizing between-patient variability (BSV) in model parameters. The between-patient variability (BSV) will be modeled as an exponential random-effect model in order to positively constrain the individual parameter values, which will be thus assumed to follow the following log-normal distribution:

  $$\theta_i = \theta_{Typical} \cdot \exp(\eta_i)$$

  where θi is the PK parameter for the *ith* individual, θTypical is the typical value (geometric mean) of the parameter in the population, ηi is a random inter-patient effect with mean 0 and estimated variance $\omega 2$.
- Residual unexplained variability will be modeled using additive or proportional or additive and proportional models, or other error models considered suitable. The residual error εi,j will be assumed to be normally distributed with mean 0 and the variance σ^2.

12.3.7.2.3. Covariate Analysis

Covariate analysis will be performed using visual inspection in order to identify relevant covariates explaining the variability of NMS-01940153E. The most relevant covariates will be then formally evaluated using a stepwise forward additive approach using a p-value of 0.05 and a backward elimination using a p-value of 0.01 (chi-squared distribution).

12.3.7.3. Translational Research

Exploratory analyses on biomarkers in blood and tumor will be conducted based on the completeness of data collected.

12.4. Interim Analysis Plan

In the Phase II part of the study an interim evaluation for futility will be undertaken as soon as the first 10 evaluable patients will be enrolled and the data for primary efficacy endpoint analysis will be available including observations through at least 3 months with at least 1 on-treatment tumor assessment by RECIST 1.1. If less than 1 responder i.e., patients who achieved CR or PR as best overall response, are observed, the study will be terminated for futility. This futility decision must be taken before 20 subjects total are enrolled otherwise enrollment will pause at 20 subjects until non-futility can be declared (if applicable). If at least 1 unconfirmed or confirmed responder is observed and there are no safety limitations, patients' enrollment will continue and proceed up to an overall enrollment of 38 evaluable patients or 40 total patients. For the safety monitoring, if two or more patients on study experience Grade 4 adverse events the study will be put on hold to evaluate whether to continue or if additional risk mitigation is warranted. The independent DSMB will review the interim results and provide a recommendation on the study progress.

Notification of the temporarily interruption of recruitment as well as restart of enrollment will be notified to the IECs.

12.5. Data Monitoring Committee

An independent DSMB will provide study oversight through periodic safety evaluations and make recommendations on study progress in the transition from Phase I to Phase II and at the planned interim analysis for the futility of Phase II.

During the Phase I part, teleconferences will be held at the end of each cohort between the Investigators and the Sponsor to review all relevant safety and PK data and take decision on the dose escalation to be applied to the next cohort of patients. Once the MTD is identified, and the safety profile of NMS-01940153E has been reviewed by the Investigators, the Sponsor and the independent DSMB and is considered adequate based on the Phase I data available, the RP2D will be defined and the Phase II portion of the study will start.

Two clinical experts in the field of hepatology or onco-hepatology and a statistician, who are not involved in the study and have no conflict of interest with respect to study results, will compose the independent DSMB.

Tasks of the independent DSMB will be:

- To provide a recommendation on study progress in the transition from Phase I to Phase II and at the planned interim analysis for the futility of Phase II, based on risk-benefit evaluation;
- To periodically review adverse events, serious adverse events and all other safety and pharmacokinetic data collected during the study;
- To suggest other toxicities to be recorded/monitored in the group of patients who will be enrolled after the independent DSMB safety evaluation.

13. END OF THE TRIAL

For the purpose of this study, the end of the trial is defined as the date of the last visit of the last patient, including follow up.

14. QUALITY CONTROL AND QUALITY ASSURANCE

The Sponsor assumes accountability for actions delegated to other individuals (e.g., Contract Research Organizations).

Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

The Investigator must permit study-related monitoring, audits performed by the Sponsor or designee, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.

It is important that the Investigator and his/her relevant personnel are available during the monitoring visits and possible audits or inspections and that sufficient time is devoted to the process.

The Sponsor or designee is responsible for the data management of the study including quality checking of the data. That means that following data entry or electronic receipt of data, data validation will take place and Forms/Reports for data clarifications will be addressed to the Investigator. Data management activities will address the coding and review of terms by scientific and clinically qualified staff.

15. DATA HANDLING AND RECORD KEEPING

15.1. Electronic Case Report Forms (eCRFs)

An eCRF must be filled in by the Investigator or authorized delegate for each enrolled and treated patient. Refer to Study Manual data entry instructions about data required for patients classified as screening failure or enrolled but untreated.

It is the Investigator's or authorized delegate's responsibility to ensure completion and to review and authorize release of the eCRF for each enrolled Clinical Trial Patient. The signature on the eCRF serves to attest that the information contained in the eCRF is true, accurate and reliable. At all times, the Investigator has full responsibility for the accuracy, legibility, completeness, and timeliness of all data (e.g., clinical data, laboratory results) reported in the eCRF and in the related data clarification tools (e.g., Data Clarification, Discrepancy Notes, Queries).

Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the Investigational Site. Source documents would include, but are not limited to, hospital/clinic records, physicians' and nurses' notes, appointment books, original laboratory result reports, ECG, x-rays, signed informed consent forms (ICF).

15.2. Record Retention

Any study records and documents, including signed ICFs, pertaining to the conduct of the study, must be retained by the Investigator for the period of time specified in the Clinical Trial Agreement, unless local regulations or institutional policies require a longer retention period.

No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

15.3. Confidentiality of Clinical Trial or Study Documents

All documents and information given to the Investigator by the Sponsor with respect to NMS-01940153E and study MPSA-153-001 are strictly confidential.

The Investigator agrees that he/she and the members of his/her team will use the information only in the framework of this Clinical Trial, for carrying out the Clinical Trial Protocol. This agreement is binding as long as the confidential information has not been disclosed to the public by the Sponsor.

The Investigator must not disclose any information of Clinical Trial Protocol as well as any information extracted from it to other parties without the prior written authorization of the Sponsor. The only exception is upon request of the representatives of the Competent Authorities; in the latter case, the Investigator commits himself to informing the Sponsor prior to disclosure of information to these authorities.

16. ETHICS

16.1. Institutional Review Board (IRB)/Independent Ethics Committee (IEC)

Clinical Study Protocol, Protocol Amendments, Informed Consent Forms, Investigator Brochure and other relevant documents (e.g., advertisements) must be submitted to IRB(s)/IEC(s) by the Investigator(s) and/or Sponsor/Delegate Party in accordance with local regulations reviewed and approved by the IRB(s)/IEC(s) before the study is initiated.

Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants. In that case, the Investigator must notify the IRB/IEC, the Sponsor or Delegated Party and Regulatory Authority, as applicable, as soon as possible after the implementation.

The Investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC

- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures.

16.2. Ethical Conduct of the Trial

This study will be conducted in accordance with and with the following:

- The Clinical Trial Protocol
- Ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
- Applicable ICH Good Clinical Practice (GCP) Guidelines.
- Current and applicable laws and regulations.

The Investigator will be responsible for:

- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies, as applicable, and all other applicable local regulations.

16.3. Informed Consent Process

It is the responsibility of the Investigator (or a person designated by the Investigator when accepted by local regulations) to give each individual (or his/her legally authorized representative) full and adequate explanation regarding the nature of the study (e.g., objectives, aims, methods and procedures of the study, anticipated benefits, possible risks and potential hazards involved). The Investigator or his/her delegate will answer all questions regarding the study.

Participants must be informed that their participation is voluntary and that they have the right to withdraw from the study at any time.

Participants or their legally authorized representative will be required to sign before any trial-related procedure is undertaken, a statement of informed consent (ICF) that meets the requirements of ICH guidelines, 21 CFR 50 and Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, local regulations, and the IRB/IEC or study center.

The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.

A copy of the signed ICF(s) must be provided to the participant or the participant's legally authorized representative.

16.4. Personal Data Protection

Participants will be assigned a unique identifier by the Sponsor. Any participant records or datasets that are transferred to the Sponsor or designee will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.

The participant must be informed that his/her personal study-related data will be used by the Sponsor or designee in accordance with local data protection law. The level of disclosure must also be explained to the participant.

The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor or designee, by appropriate IRB/IEC members, and by inspectors from Regulatory Authorities.

17. SPONSOR DISCONTINUATION CRITERIA

Nerviano Medical Sciences reserves the right to discontinue the trial prior to inclusion of the intended number of patients but intends only to exercise this right for valid scientific or administrative reasons. After such a decision, the Investigator must contact all participating patients. As directed by Nerviano Medical Sciences or the Delegated Party, all study materials must be collected and all case report forms completed to the greatest extent possible.

18. DISSEMINATION AND PUBLICATION OF RESULTS

Results of the study may be presented or published. The conditions regulating dissemination of the information derived from this clinical study are described in the Clinical Trial Agreement.

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27. Drug Development and Drug Interactions: Table of Substrates, Inhibitors and Inducers. FDA Web site

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APPENDICES

Appendix 1. ECOG Performance Status

Grade	ECOG Performance Status
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work.
2	Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours.
3	Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours.
4	Completely disabled; cannot carry on any selfcare; totally confined to bed or chair.
5	Dead

Appendix 2. Child Pugh Score and Classification

Child Pugh Score			
Measure	**1 point**	**2 points**	**3 points**
Total bilirubin, μmol/l (mg/dl)	<34 (<2)	34-50 (2-3)	>50(>3)
Serum albumin, g/dl	>3.5	2.8-3.5	<2.8
PT INR	<1.7	1.70-2.30	>2.30
Ascites	None	Mild	Moderate to severe
Hepatic encephalopathy	None	Grade I-II (or suppressed with medication)	Grade III-IV (or refractory)

Chronic liver disease is classified into Child_Pugh class A to C, employing the added score from above.

Child-Pugh Classification	
Points	Class
5-6	A
7-9	B
10-15	C

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Appendix 3. Contra-indicated drugs

The following lists are not intended to be exhaustive lists [27-28]. For updated interaction information please refer to drug summary of product characteristics. Investigators should verify case by case.

No strong inducers of FMO1 and FMO3 are currently known; they are contraindicated as a theoretical rule.

Sensitive CYP3A4 substrates	
	CYP3A4
Sensitive substrates*	Alfentanil, Avanafil, Budesonide, Buspirone, Conivaptan, Darifenacin, Darunavir, Dasatinib Dronedarone, Ebastine, Eletriptan, Eplerenone, Everolimus, Felodipine, Ibrutinib, Indinavir, Lomitapide, Lovastatin, Lurasidone, Maraviroc, Midazolam, Naloxegol, Nisoldipine, Quetiapine,Saquinavir, Sildenafil, Simvastatin, Sirolimus, Tacrolimus, Ticagrelor, Tipranavir, Tolvaptan, Triazolam, Vardenafil

* Substrates metabolized at least at 80% by CYP3A4

CYP3A	
Substrates with NTI	Alfentanil**, Astemizole, Cisapride, Cyclosporine, Dihydroergotamine, Fentanyl, Pimozide, Quinidine, Sirolimus**, Tacrolimus**, Terfenadine.

** also sensitive CYP3A4 substrates

No BCRP substrates with NTI are currently known; they are contraindicated as a theoretical rule.

FDA, Drug Development and Drug Interactions: Table of Substrates, Inhibitors and Inducers

Appendix 4. Concomitant treatments to be used with caution

The following lists are not intended to be exhaustive lists [27-28]. For updated interaction information please refer to drug summary of product characteristics. Investigators should verify case by case.

No strong inhibitors of FMO1 and FMO3 are currently known.

Moderate sensitive CYP3A4 substrates	
	CYP3A
Moderate substrates*	Alprazolam, Aprepitant, Atorvastatin, Colchicine, Eliglustat, Rilpivirine, Rivaroxaban, Tadalafil

* Substrates metabolized from 50% to 80% by CYP3A4.

BCRP	
Substrates with no NTI	Atorvastatin, Hematoporphyrin, Imatinib, Pitavastatine, Rosuvastatin, Sofosbuvir, Sulfasalazine, Tizanidine

FDA, Drug Development and Drug Interactions: Table of Substrates, Inhibitors and Inducers

Appendix 5. The Distribution of Active Bone Marrow in the Adult

Site	Marrow Weight (g)	Fraction Red Marrow - Age 40	Red Marrow Weight -Age 40 (g)	% Total Red Marrow
Head			**136.6**	**13.1**
Cranium	165.8	0.75	124.3	
Mandible	16.4	0.75	12.3	
Upper Limb Girdle			**86.7**	**8.3**
2 Humerus, head & neck	26.5	0.75	20.0	
2 Scapulae	67.4	0.75	50.5	
2 Clavicles	21.6	0.75	16.2	
Sternum	39.0	0.6	**23.4**	**2.3**
Ribs			**82.6**	**7.9**
1 pair	10.2	All 0.4	4.1	
2	12.6		5.0	
3	16.0		6.4	
4	18.6		7.4	
5	23.8		9.5	
6	23.6		9.4	
7	25.0		10.0	
8	24.0		9.6	
9	21.2		8.5	
10	16.0		6.4	
11	11.2		4.5	
12	4.6		1.8	
Vertebrae (Cervical)			**35.8**	**3.4**
1	6.6	All 0.75	5.0	
2	8.4		6.3	
3	5.4		4.1	
4	5.7		4.3	
5	5.8		4.4	
6	7.0		5.3	
7	8.5		6.4	
Vertebrae (Thoracic)			**147.9**	**14.1**
1 pair	10.8	All 0.75	8.1	
2	11.7		8.8	
3	11.4		8.5	
4	12.2		9.1	
5	13.4		10.1	
6	15.3		11.5	
7	16.1		12.1	
8	18.5		13.9	
9	19.7		14.8	
10	21.2		15.9	
11	21.7		16.3	
12	25.0		18.8	
Vertebrae (Lumbar)			**114.1**	**10.9**
1 pair	27.8	All 0.75	20.8	
2	29.1		21.8	
3	31.8		23.8	
4	32.1		24.1	
5	31.4		23.6	
Sacrum	194.0	0.75	**145.6**	**13.9**
Lower limb girdle			**273.0**	**26.1**
2 os coxae	310.6	0.75	233.0	
2 femoral head & neck	53.0	0.75	40.0	
Total	**1497.7**		**1045.7**	**100.0**

R.E. ELLIS - The Distribution of Active Bone Marrow in the Adult. Phy. Med. Biol. 5, 255 – 258, 1961.

Appendix 6. Recommended Effective Contraception

Since NMS-01940153E has potential induction of CYP3A4, hormonal contraceptives might lose efficacy while on treatment with NMS-01940153E and therefore this should specially be taken into account for female patients. It is advisable, unless total abstinence is applied, to use two contraceptive methods, of which at least one barrier method.

Methods that can achieve a failure rate of less than 1% per year when used consistently and correctly are considered as highly effective birth control methods and therefore recommended. Such methods include:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
  - oral
  - intravaginal
  - transdermal
- progestogen-only hormonal contraception associated with inhibition of ovulation:
  - oral
  - injectable
  - implantable
- intrauterine device (IUD)
- intrauterine hormone-releasing system (IUS)
- bilateral tubal occlusion
- vasectomy
- sexual abstinence

In case of male patients who have not had a vasectomy, the partner with a childbearing potential should use one of the highly effective contraceptive methods reported above.

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