SYNOPSIS

Name of Company: Nerviano Medical Sciences S.r.l. **Name of Finished Product:** Not applicable **Name of Active Ingredient:** Danusertib Hydrochloride (PHA-739358)	*(For National Authority Use only)*
Title of Study: A pilot Phase II study of PHA-739358 in patients with Chronic Myeloid Leukemia relapsing on Gleevec or c-ABL therapy	
Protocol Number: AURA-6202-005	
Investigators: 1) Ronald Paquette; 2) Neil Shah; 3) Giovanni Martinelli;	
Study Centers: 1) UCLA (University of California, Los Angeles), Medical Hematology and Oncology Division, Los Angeles, California - USA; 2) UCSF (University of California, San Francisco), Department of Medicine, Hematology/Oncology, San Francisco, California - USA; 3) Azienda Ospedaliera Universitaria Policlinico S. Orsola-Malpighi, Istituto di Ematologia e Oncologia Medica "L. e A. Seràgnoli", Bologna – Italy	
Publication Reference: R. Paquette et al; PHA-739358, an Aurora Kinase Inhibitor, Induces Clinical Responses in Chronic Myeloid Leukemia Harboring T315I Mutations of BCR-ABL. Blood (ASH Annual Meeting Abstracts), Nov 2007; 110: 1030 R. Paquette. PHA-739358: An Aurora Kinase Inhibitor with Activity Against the BCR-ABL Kinase. "New drugs in hematology" (oral presentation). Bologna, Italy 5-7 October 2008.	
Studied Period (Years): Date of First Subject Enrolled: 21 June 2006 Date of Last Subject Completed: 18 January 2010	**Phase of Development:** Phase II
Objectives: To explore the clinical efficacy of PHA-739358 in the targeted population of CML patients in terms of hematological response lasting at least 4 weeks; To explore the safety profile of PHA-739358 in CML; To explore the pharmacokinetic profile of PHA-739358 and of its N-oxide metabolite PHA-816359 in plasma; To explore the modulation of histone H3 and CRKL phosphorylation after PHA-739358 administration; To explore the relationship between PHA-739358 levels in plasma and the modulation of histone H3 and CRKL phosphorylation; To explore the clinical efficacy of PHA-739358 in terms of cytogenetic response in bone marrow, when it applies; To explore response depending on status of T315I mutation in BCR-ABL kinase.	

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Methodology: This was a pilot Phase II, single-arm, open-label, multicenter study of PHA-739358 in adult patients with Chronic Myeloid Leukemia (CML), in Chronic, Accelerated or Blast Crisis Phase, relapsing on Gleevec or c-ABL therapy, who preferentially presented a T315I mutation in BCR-ABL kinase.
PHA-739358 was to be administered intravenously over a 6-hours infusion in a 4-week cycle at the following doses and schedules: 250 mg/m^2 on Days 1, 8 and 15 (as per original protocol); 330 mg/m^2 on Days 1, 8 and 15 (Amendment No. 1 and No. 2); 330/400 mg/m^2 on Days 1, 8, 15 and 22 (Amendment No. 3); 250 mg/m^2 on Days 1, 4, 8, 11, 15, 18, 22 and 25 (Amendment No. 4).

Safety assessments (vital signs, hematology, blood chemistry, and urinalysis) were to be performed at baseline and repeatedly at different time points during the treatment period, depending on the parameter, and at the end of treatment. To monitor cardiac events, a 12-lead electrocardiogram (ECG) was to be performed at baseline visit, at the end of infusion during Cycle 1 and at the end of treatment, and repeated as needed, under Investigator's judgment. A TTE was scheduled at baseline visit, at the end of Cycle 1 and at the end of every even cycle. Patients showing left ventricular ejection fraction (LVEF) value < 35% had to undergo a confirmatory multi gated acquisition (MUGA) scan. Patients were to be followed for adverse events (AE) from the signature of the Informed Consent form up to 28 days after the last dose of study treatment or until all drug-related toxicities had resolved or an alternative anticancer therapy was started.

Efficacy assessments were to be based on analyses of peripheral blood, bone marrow aspirate and, for patients with extramedullary disease, on clinical examinations and/or tumor imaging.
The hematologic response was to be evaluated according to Sawyers et al. [Blood 2002 May; 99(10): 3530-9] and assessed as: Complete Hematological Response (CHR), No Evidence of Leukemia (NEL), Return to Chronic Phase (RTC) or treatment failure (TF). All the hematologic responses had to be confirmed at least 4 weeks after they were first documented.
The cytogenetic response was to be evaluated according to the criteria described by Kantarjian et al.[N Engl J Med 2002 February; 346(9):645-5223] as Complete, Partial, Minor or No Response.
Blood and bone marrow aspirate were to be collected for cytogenetic, molecular and phenotypic characterization of the disease. Samples were to be used for measurements of the phosphorylation status of histone H3 and CRKL. Additional biomarkers of response or mechanism of action under evaluation at the time of protocol evaluation could be added once validated.
Plasma samples for evaluation of the pharmacokinetic profile of PHA-739358 and its N-oxide metabolite PHA-816359 were to be collected, from all patients treated, during Cycle 1, on Days 1, 2 and 8, according to the sampling schedule. Patients had to remain on treatment until disease progression, patient refusal, or unacceptable toxicity occurrence.

Four protocol amendments were implemented during the course of the trial and the main purposes were as follows:
Amendment No. 1 (26 June 2006) and *Amendment No. 2* (25 July 2006) were issued to increase the starting dose from 250 to 330 mg/m^2 (same schedule as original protocol);
Amendment No. 3 (2 March 2007) was implemented to increase the dose intensity of the CML patients on a phase-basis, according to the following dose-schedule:
- patients in Chronic Phase: 330 mg/m^2 (or 400 mg/m^2, provided no non-hematological Grade 3-4 toxicities were observed) over 6-hour infusion (on Days 1, 8, 15 and 22), in a q4wks cycle, corresponding to a total dose of 1320 mg/m^2 (or 1600) per cycle;
- patients in Advanced Phases (Accelerated and/or Blast Crisis Phase): 400 mg/m^2 (over 6-hour infusions weekly in a q4wks cycle (total dose corresponding to 1600 mg/m^2 per cycle);
Amended No. 4 (24 July 2008) was implemented to modify the current schedule in order to test if the preliminary evidence of clinical activity observed during this pilot study could have been additionally improved in advanced CML patients by increasing the frequency of administration. The new proposed schedule was 250

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mg/m^2 over a 6-hour infusion, twice a week (Days 1, 4, 8, 11, 15, 18, 22 and 25), in a q4wks cycle for patients in Advanced Phase; for patients in Chronic Phase, the dose schedule remained unchanged.

Number of Subjects (Planned and Analyzed): The original protocol foresaw to recruit and treat 16 patients. The size was not established on the basis of statistical considerations. Actually, as the original dose schedule was amended also sample size was increased, from 16 to 25 and eventually to 50 patients, in order to allow for similar size each of the dose schedule cohorts investigated.
Forty-four patients were enrolled and 42 were analyzed.

Diagnosis and Main Criteria for Inclusion:
Adult (≥ 18) consenting patients with confirmed diagnosis of CML (in Chronic, Accelerated or Blast Crisis Phases) relapsing on Gleevec or c-ABL therapy and preferably (≥40%) with T315I mutation in BCR-ABL kinase. Prior chemo-immunotherapy, with exception of hydroxyurea and/or steroid and/or anagrelide, was allowed, provided it had been completed 2 weeks before study treatment start.
Other main selection criteria included ECOG performance status ≤2, stage ≤1 hypertension (≤ 159/99 mmHg), with or without antihypertensive treatment; baseline laboratory data indicating acceptable liver and renal function; recovery from all acute toxic effects (excluding alopecia) of any prior therapy to Grade ≤ 1.
Patients with known history of HIV infection, CNS involvement in the malignancy, abnormal LVEF (i.e: <40% by TTE or <45% by MUGA), significant cardiovascular diseases, including cardiac dysrhythmias or major thromboembolic event occurred in the last 6 months, presence of CTC Grade 3 or 4 bleeding, unrelated to CML and pregnant and breast feeding females were to be excluded.

Test Product, Dose and Mode of Administration, Batch Number: PHA-739358 was dosed based on the patient's body surface area. The compound was administered in a 6-hour IV infusion over a 4-week cycle, at the following dose levels: 250 mg/m^2, on Days 1, 8 and 15; 330 mg/m^2 on Days 1,8, and 15; 330/400 mg/m^2 on Days 1,8, 15 and 22; and 250 mg/m^2 on Days 1,4,8,11,15,18, 22 and 25.
PHA-739358 batch numbers used in the study were: N0600048, N0600878, N0700058, N0700158, N0700292, N0700327, N0700422, N0800069, N0800165, N0800304 and N0800576.

Reference Therapy, Dose and Mode of Administration, Batch Number: Not Applicable

Duration of Treatment: Patients had to remain on treatment until disease progression, patient refusal, or unacceptable toxicity occurrence. Patients benefiting from treatment were to receive treatment for 24 weeks, with subsequent prolongation upon judgment of the Investigator. During the Follow-up period the assessments were to be performed every 3 months for a maximum of 6 months only in patients who discontinued the treatment for reason other than disease progression or until the start of a new treatment. Survival status was to be monitored every 3 months for one year. For the purpose of this study, the end of the trial was defined as 6 months after the end of treatment of the last patient or until all patients had progressed, whichever occurred first. For patients who continued to benefit from treatment with PHA-739358, NMS had to guarantee the supply of the drug at protocol study closure.

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Endpoints and Criteria for Evaluation:

Efficacy assessments were to be based on analyses of peripheral blood, bone marrow aspirate and, for patients with extramedullary disease, on clinical examinations and/or tumor imaging.
The hematologic response was to be assessed as Complete Hematological Response (CHR), No Evidence of Leukemia (NEL), Return to Chronic Phase (RTC) or Treatment Failure (TF). All the hematologic responses had to be confirmed at least 4 weeks after they were first documented.
The cytogenetic response was to be assessed in bone marrow aspirate and evaluated as Complete, Partial, Minor or No Response.
The modulation of histone H3 and CRKL phosphorylation, as measurement of PHA-739358 biological activity, was to be evaluated on blood and bone marrow samples.
Safety assessments included: vital signs, hematology, blood chemistry, urinalysis, ECG, LVEF, and type, frequency, severity, timing of AEs.
Other assessments included pharmacokinetic profile of PHA-739358 and its N-oxide metabolite PHA-816359 based on their concentrations in plasma at different time points after dosing.

Statistical Methods: All CML patients who received at least one dose of PHA-739358 were to be considered as target population. All patients data were to be presented by individual data listings arranged by domain or specifically designed to facilitate the exploration of data. Demographic and baseline characteristics were to be described by means of frequency tables or position and dispersion statistics. Treatment administration and exposure were to be characterized by presenting number of cycles delivered, overall and first cycles as opposed to following ones, maximum number of cycles per patients; mean and median absolute and relative dose intensity; number of cycles delayed and/or at a reduced dose.
Point estimate and 95% confidence interval of Response rate (complete response +/- partial) were to be provided both by considering the whole data set and by considering the two subpopulations identified by the phase of disease (i.e. Chronic Phase and Blast Crisis/Accelerated Phase). Efficacy evaluation was mainly focused at the detection of signs of association between response rate and collected biomarkers. In case of a substantial number of responses had occurred, association with the T315I mutation was to be explored by using Chi -Square test.
Adverse events were to be summarized by counting the number of patients with adverse events by body system, and by counting the number of patients with an adverse event as classified by the high level term of the MedDRA dictionary. In this analysis, a patient having the same event more than once was to be counted only once in the category of patient having experienced that event. Adverse events were to be summarized by worst CTCAE severity grade and closest relationship with study medication. In addition, adverse events were to be presented by treatment cycle (cycle 1 vs. cycles > 1) and overall. In frequency tables only treatment emergent adverse event were to be reported. Treatment emergent adverse events were defined as those ones that were not reported at baseline or that if reported at baseline had increased in severity or relationship over study.
Adverse events leading to death or to discontinuation from treatment, adverse events classified as severity grade 3 or higher, study-treatment-related events, and serious adverse events were to be presented in separate listings of individual events.
Laboratory values were to be graded according to CTC AE severity grade, where applicable. For each test, the worst CTC AE severity grade was to be summarized on a patient and on a cycle basis. For parameters not considered in the CTC AE scale, frequency of patients with values below, within, and above the normal ranges was to be summarized.

SUMMARY OF RESULTS:

Disposition of Subjects and Baseline Characteristics:

A total of 44 patients (33 CML and 11 ALL) were enrolled and treated at different starting doses /schedules,

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according to 4 protocol amendments implemented during the course of the trial. At that time of the database lock, 01 February 2011, all patients were off-study, except for patient No. 044 resulting still on treatment. Information reported in the present document relates to the data available in the company database from 42 treated patients, since, due to administrative reasons the clinical database of this study was locked without completing the medical review of the whole set of entered data and without solving all the emerged discrepancies.
Thirty-one patients had diagnosis of Chronic Myeloid Leukemia (CML) in Chronic Phase (6 patients), Accelerated Phase (7) or Blast Crisis Phase (18). Though as per protocol only patients with CML were to be enrolled in the study, one investigational site (Bologna, Italy) included 11 patients suffering from Acute Lymphoblastic Leukemia (ALL). Enrolment was closed at this site as soon as this violation was disclosed.
Sixteen patients (38.1%) presented extramedullary diseases at study entry, mostly located in the splenic area.
Overall, Philadelphia-chromosome was positive for 34 patients. All patients but one had T315I mutation status assessed at baseline, and 23 patients presented T315I mutation in BCR-ABL kinase at baseline. All patients were heavily pretreated (≥3 prior systemic therapy for hematologic malignancy).
The most frequent reason for study discontinuation was lack of efficacy (69.8%). Eight patients (18.6%) discontinued treatment due to adverse event, 3 patients (7.0%) completed treatment as per protocol, and for 1 patient (2.3%) the reason for withdrawal was missing.

The mean age at study entry was 50.5 years, and 50% of patients were 50 year-old or below. The enrolled patients were 34 male (81.0%) and 8 female (19.0%); 31 patients (73.8 %) were Caucasian, 2 patients were Black, 2 Asian (4.8% each) and for 7 patients (16.7%) the race was not listed.
ECOG performance status score was 0 in 19 patients (45.2%), 1 in 17 patients (40.5%). Six patients (14.3%), 5 of which in Blast Crisis Phase, had ECOG score 2 at study entry. Weight and height were within the limits of normality, with no particular observations.

PHA-739358 was dosed based on the patient's body surface area. The 42 patients received the 6-hour IV infusion of the compound as follows: 250 mg/m^2 on Days 1, 8 and 15 (3 patients, according to original protocol); 330 mg/m^2 on Days 1,8, and 15 (8 patients, as per Amendments No. 1 and No. 2), 330 mg/m^2 on Days 1,4,8,11,15 and 18 (1 patient, schedule foreseen in the original protocol); 330/400 mg/m^2 on Days 1,8, 15 and 22 (30 patients, as per Amendment No. 3), and 250 mg/m^2 on Days 1,4,8,11,15,18, 22 and 25 (1 patient, treated as per Amendment No. 4). Actually, this last patient was wrongly recorded in the CRF as treated according to Amendment No. 3 dose schedule, and, consequently he results allocated to the group of patients treated at 330 mg/m^2 on Days 1,8,15 and 22 in all the tables and listings of the present report.
The 42 patients received a total of 155 cycles. The median number of cycles per patient was 2 (range 1- 23). Twenty-seven out of 42 (64.3%) patients received >1 cycle of treatment. The median treatment duration was 8.0 weeks (range 0.4-88.1).

Most of the patients who started with the 330 mg/m^2 D 1, 8, 15 schedule actually switched to the weekly administration (i.e., 330/400 mg/m^2 D 1, 8,15, 22) for a significant period of their treatment. As a result, dose intensity of 330 mg/m^2 D 1, 8, 15 group is not far from the one of the group treated with the weekly administration.

Efficacy Results:
Two CML patients with T315I mutated BCR-ABL, both treated at a starting dose of 330 mg/m^2 administered on Days 1, 8 and 15 in a 4-week cycle, achieved a long-lasting response for 17 and 20 months, respectively: *Patient No. 004*, in Chronic Phase, with 100% of Ph-chromosome positive cells in bone marrow at study entry, achieved a complete hematological response (CHR) and a minor cytogenetic response after 3 cycles of therapy. The response resulted still present at cycle 12 with no evidence of leukemia (NEL) and it was maintained up to cycle 18. *Patient No. 005*, in Blastic Phase, with 40% of Ph-chromosome positive cells in bone marrow at

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baseline, achieved a NEL after three months of treatment and, afterwards, a CHR which was maintained up to Cycle 18 with return to Chronic Phase at Cycle 21. In addition, at month 3, the same patient reported a complete cytogenetic response which lasted up to month 9, and then only partially maintained up to 21 cycles of therapy. A molecular response was also reported by the Investigator. Clinical benefit, not fulfilling formal response criteria, was observed in three additional CML patients, two harboring the T315I BCR-ABL mutation and one with BCR-ABL mutation other than T315I. In addition, 3 patients who showed at the end of cycle 1 an initial response to therapy, interrupted treatment with PHA-739358 either due to adverse event or because they were addressed to bone marrow transplant.
At all the tested doses and schedules, a trend for inhibition of the CRKL phosphorylation and an occasional modulation of the histone H3 phosphorylation were observed among the evaluable patients receiving PHA-739358 as a 6-hour infusion.

Safety Results:
A total of 40 (out of 42) treated patients (95.2%) experienced at least 1 treatment emergent adverse event in the first or in subsequent cycles, and the most frequent were pyrexia (21 cases, 50.0%, 19% drug-related), diarrhea (19 cases, 45.2%, 31.0% drug-related), asthenia/fatigue (28 cases, 66.7%, 19.0% drug-related), nausea (10 cases, 23.8%, 14.3% drug-related), arthralgia and dyspnoea (9 cases each, 21.4%, 4.8% drug-related, respectively), night sweats (7 cases, 16.7%, 2.4% drug-related), stomatitis (6 cases, 14.3%, no drug-related), tachycardia (6 cases, 14.3%, 2.4% drug-related), headache (5 cases, 11.9%, 2.4% drug-related), vomiting and weight decrease (5 cases each, 11.9%, 2.4% drug-related, respectively) .
The events assessed as related to study treatment were mostly mild to moderate in severity. Nine CTC Grade 3-4 drug-related events were reported by 8 patients (19.0%) and consisted in: asthenia, neutropenia (2 cases), anemia, haematuria, pancytopenia, shock, tachycardia and pyrexia. No Grade 5 drug-related events were reported.

Treatment emergent hematologic abnormalities, and in particular neutropenia (76.2%), lymphocytopenia (66.7%), leucopenia and anemia (61.9%, each) and thrombocytopenia (47.6%) were quite frequent findings. Grade 4 neutropenia was reported as drug-related clinical adverse event in 2 cases, Grade 4 pancytopenia and Grade 3 anemia in one case each.

Treatment emergent abnormalities of blood chemistry parameters were mostly of Grade ≤2, except for two cases of Grade 3 increased bilirubin. In addition, few cases of Grade 3-4 alterations in blood electrolytes were reported, including 2 cases of hypercalcemia, 9 cases of hypophosphatemia, 3 cases of hypokalemia, 2 cases of hyperkalemia and 3 cases of hyponatremia.
Hypercalcemia and hypophosphatemia (one case each) were reported as adverse events unrelated to study treatment. None of the other reported blood chemistry abnormalities was considered related to study drug.

Occasional hypertensive episodes were observed in 26.2% of treated patients (11 cases). In particular, one CML patient in Blast Crisis had CTC Grade 4 increase in blood pressure. In this case hypertension was reported as not drug-related adverse event. The same patient also developed hypercalcemia with ventricular arrhythmias which led to patient's death. Few patients presented transient hypertensive episodes recovered to normal values at the following assessments.
A transient increase in pulse was observed 2 hours after the end of the Cycle 1 Day 14 infusion in one ALL patient, treated at 400 mg/m^2 on Days 1, 8 and 15. This episode was reported as a CTC Grade 3 tachycardia, possibly related to study drug.
Treatment emergent abnormalities of ECG tracings, including alterations in cardiac rhythm (3 cases) and conduction abnormalities (2 cases), occurred in five patients who had normal ECG condition at baseline. No cardiac alteration assessed on treatment was reported as drug-related adverse event.
No on treatment alteration of LVEF were reported. This abnormality was not reported as an adverse event by

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the Investigator and improved in the following assessments.

The company pharmacovigilance was notified of the occurrence of 36 SAEs reported in 21 patients. Drug-related SAEs occurred in 3 patients and included haemolysis, shock and haematuria.
Twelve patients died during the reporting period (defined as from patient's consent up to 28 days after the last treatment dose), 5 due to disease progression, and 5 due to adverse events unrelated to study treatment, including hypercalcemia with confusion and ventricular tachycardia, worsening of clinical conditions and concomitant pulmonary infection, interstitial pneumonitis, massive intracranial bleed and mucor fungal infection. In addition, two patients died due to respiratory arrest and septic shock (both not related to study medication), respectively, even if the reported cause of death was disease progression.

Concerning the pharmacokinetic evaluation, plasma concentration levels of PHA-739358, at all the tested doses were in agreement with those obtained in a previous pharmacokinetic study in patients with advanced/metastatic solid tumors who received PHA-739358 with the same infusion duration.

CONCLUSIONS:
Preliminary evidence of PHA-739358 activity, in patients suffering from CML in Chronic or Advanced Phase and harboring the T315I BCR-ABL mutation, was obtained in this pilot study. Efficacy and safety data, as well as the results coming from translational research, are indicators for possible further investigations in patients with multidrug resistant CML.

Date of the Report:
30 August 2011