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Harding JJ, Awada A, Roth G, Decaens T, Merle P, Kotecki N, Dreyer C, Ansaldi C, Rachid M, Mezouar S, Menut A, Bestion EN, Paradis V, Halfon P, Abou-Alfa GK, Raymond E

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Research Article

First-in-human effects of PPT1 inhibition using the oral treatment with GNS561/* Ezurpimtrostat *in patients with primary and secondary liver cancers

Authors: James J. Harding[1,2], Ahmad Awada[3], Gael Roth[4], Thomas Decaens[4], Philippe Merle[5], Nuria Kotecki[3], Chantal Dreyer[6], Christelle Ansaldi[7], Madani Rachid[7], Soraya Mezouar[7], Agnes Menut[7], Eloïne Nadeige Bestion[7], Valérie Paradis[8], Philippe Halfon[7], Ghassan K. Abou-Alfa[1,2]* and Eric Raymond[6,7]*

1. Department of Oncology, Memorial Sloan Kettering Cancer Center, New York, United States.
2. Weill Medical College at Cornell University, New York, United States.
3. Department of Oncology, Institute Jules Bordet, Brussels, Belgium.
4. University Grenoble Alpes, Department of Hepatology and Gastroenterology, CHU Grenoble Alpes, Institute for advanced biosciences Research Center Inserm U 1209/CNRS 5309, Grenoble, France.
5. Department of Hepatology and Gastroenterology, Hospices Civils de Lyon, Lyon, France.
6. Department of Oncology, Hospital Saint Joseph, Paris, France.
7. Genoscience Pharma, Marseille, France.
8. Department of Pathology, Hospital Beaujon, Paris, France.

Short Title: GNS561 phase I clinical trial

Corresponding Author:
Dr Ghassan K. Abou-Alfa, MD
Memorial Sloan Kettering Cancer Center
300 East 66th Street
New York, NY 10065
United States of America
+1 646 888 4184
abou-alg@mskcc.org

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Abstract

Introduction - GNS561/Ezurpimtrostat is a first-in-class, orally bioavailable, small molecule that blocks cancer cell proliferation by inhibiting late-stage autophagy and dose-dependent build-up of enlarged lysosomes by interacting with the palmitoyl-protein thioesterase 1 (PPT1).

Methods - This phase I, open-label, dose-escalation trial (3+3 design) explored two GNS561 dosing schedules: one single oral intake three times a week (Q3W) and twice daily (BID) continuous oral administration in patients with advanced hepatocellular carcinoma, cholangiocarcinoma, and pancreatic adenocarcinoma or colorectal adenocarcinomas with liver metastasis. The primary objective was to determine GNS561 recommended phase II dose (RP2D) and schedule. Secondary objectives included evaluation of the safety/tolerability, pharmacokinetics, pharmacodynamics, and antitumor activity of GNS561.

Results - Dose escalation ranged from 50-400mg Q3W to 200-300mg BID. Among 26 evaluable patients for safety, 20 were evaluable for efficacy and no dose-limiting toxicity was observed. Adverse events (AEs) included gastrointestinal grade 1-2 events, primarily nausea and vomiting occurred in 13 (50%) and 14 (54%) patients respectively, and diarrhea in 11 (42%) patients. Seven (7) grade 3 adverse events were reported (diarrhea, decreased appetite, fatigue, ALT and AST increased). Q3W administration was associated with limited exposure and the BID schedule was preferred. At 200 mg BID GNS561, plasma and liver concentrations were comparable to active doses in animal models. Liver trough concentrations were much higher than in plasma a median time of 28 days of administration with a mean liver to plasma ratio of 9559 (Min 149-Max 25759), which is in accordance with rat preclinical data observed after repeated administration. PPT1 expression in cancer tissues in the liver was reduced upon GNS561 exposure. There was no complete or partial response. Five patients experienced tumor stable diseases (25%), including one minor response (-23%).

Conclusion - Based on a favourable safety profile, exposure, and preliminary signal of activity, oral GNS561 RP2D was set at 200mg BID. Studies to evaluate the antitumor activity of GNS561 in HCC and iCCA are to follow.

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Introduction

Lysosomes have been shown to play a major role in autophagy and cancer cell death either alone or in connection with several other cell death pathways [1]. Dysregulated autophagic-lysosomal activity and mammalian target of rapamycin complex (mTORC)-1 signaling, composed of mTOR itself and regulatory-associated protein of mTOR were also shown to allow cancer cells to become resistant to the cellular stress induced by chemotherapy and targeted therapy [2]. Among proteins involved in lysosomal degradation, the palmitoyl protein thioesterase 1 (PPT1), a glycoprotein that belongs to the palmitoyl protein thioesterase family, was shown to play important roles in the catabolism of lipid-modified proteins during lysosomal degradation by removing thioester-linked fatty acyl groups such as palmitate from cysteine residues [3]. PPT1 was shown to palmitoylate proteins, enabling protein degradation and intracellular trafficking of membrane bound proteins. This process was shown to play a central role in the control of cellular autophagy and PPT1 was reported as highly expressed in several cancer cell lines as well as in advanced cancers in patients [4].

Thereby, PPT1 has been regarded as a target for cancer therapy [5]. The antisense strategy against PPT1 was shown to inhibit PPT1 activity and increases death in neuroblastoma cells [6,7].

Chloroquine and quinacrine have also been used as lysosomal autophagy inhibitors in laboratory experiments [8]. However, the molecular target of chloroquine derivatives remaining unknown, and there was limited proof-of-concept to develop clinical trials in patients with cancer [9]. More recently, the screening for novel antimalarial dimeric quinacrines led to the identification of small molecules with potent lysosomal effects and anticancer activity [10]. Novel dimeric quinacrines were shown to target PPT1, leading to subsequent inhibition of lysosomal catabolism through the rapid accumulation of palmitoylated proteins, impairing mTOR function, increasing autophagy and cancer cell death [10,11]. More recently, preclinical data suggested that PPT1 inhibition enhances the antitumor activity of checkpoint inhibitors in melanoma offering opportunities for further development of clinical trials [12].

GNS561/Ezurpimtrostat, a novel chemical entity that was identified from the screen of quinoline derivatives that inhibit autophagy and induce antiproliferative activity in cultured cancer cells. In preclinical experiments, we demonstrated that GNS561 displays lysosomotropism and targets PPT1 [13]. Exposure to GNS561 was shown to block the PPT1-dependent autophagic activity and the relocalization of mTOR in hepatocarcinoma cells (HCC). Similar results were obtained in intrahepatic cholangiocarcinoma (iCCA) cells where GNS561 inhibits cellular proliferation by inhibiting late-stage autophagy and dose-dependent build-up of enlarged lysosomes [14]. The GNS561-induced PPT1 inhibition leads to lysosomal deacidification, which induces lysosomal unbound zinc accumulation and then the permeabilization of lysosomal membranes was shown to activate caspases and cancer cell lethality. GNS561 also inhibits TGF-β1 functions and hepatic fibrosis in preclinical models [15]. Animal models in rats and mice with hepatocarcinoma showed antitumor activity of oral GNS561 at the doses of 15 mg/kg. In animal studies, High liver tropism was reported in mouse after single oral dosing at 50mg/kg and in dog 5 days after daily oral repeated dosing at 15mg/kg. The threshold dose associated with antitumor activity in rats was 15 mg/kg/day and was associated with a mean C-through plasma concentration of 50 ng/mL. Interestingly, animal models showed that liver concentrations were consistently above 600 times higher than plasma concentration. Nevertheless, despite high GNS561 liver concentrations in rats and dogs, toxicology studies showed no major liver toxicity at low and mid dose levels (Data on file, Genoscience).

Given the high concentrations of GNS561 in the liver at non-toxic doses using oral dosing in toxicology studies and the preclinical data showing antiproliferative activity of GNS561 in HCC, iCCA, pancreatic, and colon cancer models, we selected these tumor types for patient enrolment in this first-in-human clinical trial. In this phase I trial, we report the safety, pharmacokinetic analyses, antitumor activity, and PPT1 expression under GNS561 therapy given as oral tablets in patients with primary and secondary liver malignancies.

Materials and Methods

Study design

This phase I, open-label, dose-escalation trial (3+3 design) explored two dosing schedules: one single oral intake thrice weekly and twice daily continuous oral capsule intake of GNS561 (IND 133561 and EUDRACT 2017-003585-27) in patients with advanced primary (HCC and iCCA) and secondary liver cancer (metastasis from distant carcinomas).
The primary objective was to determine recommended phase II dose (RP2D) and schedule of GNS561 for further clinical development. The secondary objectives included an evaluation of the safety profile, pharmacokinetics (PK), pharmacodynamics (PD), and preliminary antitumor activity of GNS561.
The protocol was approved and registered in France, Belgium, and the USA with the appropriate regulatory authorities and ethics committee and performed in accordance with the Declaration of Helsinki and applicable regulatory requirements (ClinicalTrials.gov NCT03316222).

Eligibility criteria
All patients provided written informed consent. Patients ≥18 years old with a pathologically confirmed, locally advanced/metastatic primary (HCC and iCCA) or secondary (metastatic colon or pancreas carcinoma) liver solid tumor (tumor burden <50% per investigator judgment) that was refractory after standard therapy for the disease and for which no curative therapy was available. Patients were required to receive no other anticancer therapy in the 4 weeks or 5 half-lives, whichever is greater, prior to the first dose of GNS561.
Patients were required to be willing to have liver biopsy at the beginning of Cycle 2 (Day 1 ± 1 day). Tumors had to be measurable per Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 using CT-scan and/or MRI [16] and patients had to have a performance status $\leq$ 1 by Eastern Cooperative Oncology Group (ECOG) [17] with a life expectancy ≥ 12 weeks.
Patients were required to have adequate liver function defined as aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ≤ 5 x ULN and Child-Pugh score A ≤ 6), with no evidence of prior cirrhotic decompensation within last 12 months prior to enrolment. Other criteria included adequate hematologic and renal functions prior to the first dose of GNS561.
Exclusion criteria included: Pregnant or breast-feeding mothers, any history of encephalopathy, known oesophageal varices with recent history of bleeding, clinically significant ascites or paracentesis, concurrent hematologic malignancies or other malignancy, known allergic reaction to quinoline derivatives (e.g., quinine, chloroquine, mefloquine) as well as intolerance or hypersensitivity to components of the capsules, presence of residual toxicities of ≥ Grade 2 after prior antitumor therapy ≤ 4 weeks prior to first dose, malabsorption, any clinically significant cardiovascular condition as judged by the Investigator, and untreated chronic hepatitis B. Patients with chronic hepatitis C and/or controlled chronic hepatitis B could be enrolled in this study.

Drug administration and dose escalation
Study drug was provided as oral capsules containing 50 or 200 mg of GNS561. GNS561 was given without any antiemetic prophylaxis at first dosing but oral ondansetron as needed was allowed prior to drug intake in case of occurrence of nausea and vomiting. No intra-patient dose escalation was permitted.
The starting dose of 50 mg was based on the calculation of 1/10 of dose inducing 10% lethality in the most sensitive species. The first cohort received 50 mg once daily three times per week (Monday, Wednesday, and Friday – Q3W). One cycle was 4 weeks. Doses for subsequent cohorts were planned to be escalated by no more than doubling (100% increase) the previous dose until ≥ 1 patient experiences a dose limiting toxicity (DLT) or ≥ 2 patients in a cohort experience a drug related Grade ≥ 2 toxicity. In such instances, and for doses above 400 mg, dose escalation cohorts increased by increments not greater than 50% of the prior dose. If a DLT was observed in ≥ 1 patient in a cohort, an additional 3 patients had to be enrolled in that cohort for a total of 6 patients. The maximum tolerated dose (MTD) was defined as the dose level below the one at which DLT occurred in >1/3 or >1/6 patients. Dose escalation continued to the RP2D. The RP2D was defined as the highest dose level in which DLTs have occurred in ≤ 1/3 or ≤ 2/6 patients in a cohort in conjunction with review of the safety, clinical activity, and available PK/PD data from each cohort by a safety monitoring

committee (SMC) consisting of the Investigators and Sponsor representatives adjudicated all dose escalation.

Study assessments

To be considered evaluable for safety, patients had to be exposed to at least one dose of GNS561. Patients came at hospital every week during first cycle, then every 2 weeks after Cycle 1 (ECGs, lab samples, vital signs and physical examination). Treatment-emergent adverse events (TEAEs) were graded according to NCI-CTCAE, version 4.3. The DLT observation period was the first cycle of treatment, so 28 days. DLTs were classified as any grade ≥3 non-hematologic toxicity excluding: nausea, vomiting and diarrhea lasting ≤ 48 hours, any grade 4 laboratory abnormalities that last > 24 hours, any liver toxicity (aspartate aminotransferase or alanine aminotransferase > 10 ULN or baseline value, total bilirubin > 5 ULN or baseline or >2 ULN in addition of AST or ALT > 5 ULN/baseline not reversible within 14 days) and any adverse event (AE), in the judgment of the investigator, presents a substantial clinical risk to the patient to continue GNS561.
Patients who experienced DLT that resolves to Grades 0 or 1 within 14 days might resume treatment with GNS561 at the next lower dose level. If a patient experienced DLT in Cohort 1, treatment with GNS561 was permanently discontinued.
To be considered evaluable for antitumor activity, patients had to perform baseline and tumour assessments after at least 2 cycles (8 weeks) of treatment unless discontinuation as due to early disease progression. Tumor response was assessed according RECIST, version 1.1 [17] with cross sectional imaging at the end of every two cycles.

Pharmacokinetic analyses

Following initial assessments and collection of the pre-dose PK blood sample, patients were fed a meal 30 to 60 minutes prior to dosing with GNS561. Following dosing, patients were required to remain at the clinic until the 10-hour PK blood sample was collected (PK sampling times: Predose, 1, 2, 4, 6, 8, and 10 hours) and then return on Cycle 1 Day 2 for collection of the 24-hour PK sample, and on Cycle 1 Day 3 for the collection of the 48 hour PK sample. The same procedure was applied for Cycle 2 (from D1 to D3 Cycle 2).
For PK blood analysis, the analytical method to quantify GNS561 in human plasma was validated using a liquid chromatography coupled with MS/MS detection (multiple reaction monitoring) in positive ion electrospray mode.
For PK liver samples, the analytical method to quantify GNS561 in human non tumoral liver tissues was qualified using a liquid chromatography coupled with MS/MS detection (multiple reaction monitoring) in positive ion electrospray mode.

PPT1 Immunofluorescence analysis

Liver biopsies from non-tumor and tumor tissues were performed one month after the start of GNS561 exposure. Tissue slides were deparaffinized and then a heat induced epitope retrieval was performed in a pressure cooker for 10 minutes using a preheated TRIS EDTA buffer (10 mM Tris base, 1 mM EDTA solution, 0.05% Tween 20, pH 9.0).

Statistical analysis

The study followed a classical phase I 3+3 design, and no formal statistical hypothesis was tested. The analyses presented are therefore descriptive.

Results

Patient characteristics

A total of 35 patients were screened. An inform consent was obtained for each patient before their participation in the study. Twenty-six patients were enrolled, received at least one dose of GNS561, and were therefore evaluable for safety. Six patients were not fully evaluable for activity due to early treatment discontinuation (two physician decisions for early withdrawal, four for non-drug related

adverse events). As a result, a total of 20 patients were considered fully evaluable for antitumor activity (supplementary figure 1).
Patient characteristics at baseline are displayed in Table 1. The median age was 60-year-old. Tumor types consisted of iCCA (11 patients), HCC (10 patients), metastatic pancreatic adenocarcinoma (4 patients), and metastatic colorectal cancer (1 patient). Three (3) patients had a chronic hepatitis B controlled with treatment (entecavir and/or tenofovir) and five (5) patients had positive hepatitis C serology. Six patients had a cirrhosis among 10 patients with HCC and one patient among 11 with iCCA. The causality of cirrhosis was hepatitis B virus (2 patients), Hepatitis C virus (2 patients), alcohol (1 patient), steatosis (1 patient) and alcohol plus steatosis (1 patient). Regarding prior treatments, 18% of iCCA patients, 60% of HCC patients, 75% of pancreatic adenocarcinoma patients and 100% of colorectal cancer patient had received 3 or more prior therapies.

Dose escalation
GNS561 was given as a single oral daily intake three times a week in 4 cohorts of 3 patients at doses of 50 mg, 100 mg, 200 mg, and 400 mg. No dose limiting toxicity was observed over the first 28 days in those cohorts of patients, but pharmacokinetic data suggested that this schedule yielded suboptimal exposures.
Human data evaluated based on preclinical HCC rat model suggested that optimal exposures may be obtained using a BID dose administration of GNS561 in subsequent cohorts. At the dose of 300mg BID, no DLT was observed but none of three patients enrolled at this dose level received one full 28-day cycle of treatment. The frequent occurrence of mild to moderate nausea and vomiting was more difficult to manage than in previous dose levels despite optimal daily prevention with antiemetics, making clinically impossible to recommend this dosing for further phase II trials. Furthermore, the high plasma exposure of GNS561 at the dose of 300mg BID was considered beyond expectation, potentially jeopardizing the exploration of higher dose levels. Based on clinical and pharmacokinetic data, the safety monitoring committee took the decision to stop the dose-escalation and, although the MTD was not achieved, it was considered safe to consider the dose of 300mg BID as near-MTD, preventing to further explore higher dose, and leading to explore the immediate lower dose level of 200mg BID as the potential recommended dose. Therefore, three additional patients were schedule to enrol at the dose level of 200mg BID among which 5 were entered due to concomitant accrual in two centers.

Adverse events
The mean duration of GNS561 treatment was 55 days (ranging 28-169 days). Among 26 patients evaluable for safety (Table 2), no DLT was observed, and grade 3 adverse events transiently observed did not meet predefined DLT conditions. Grade 1-2 nausea (13 patients; 50%, vomiting (14 patients; 54%), and diarrhoea (9 patients grade 1-2; 35% and two patients grade 3; 8%) were predominantly observed as treatment related adverse events. Among other gastrointestinal adverse events, decreased appetite (grade 3 for one patient treated at the dose of 200mg BID), abdominal pain, and distension were each observed in two patients (8%), while dyspepsia, constipation and weight decreased were each reported in one patient (4%). Grade 1-2 asthenia/fatigue was reported in 6 patients (23%) and two patients reported grade 3 asthenia/fatigue. Grade 1-2 hypertension, blurred vision, hypercalcemia, nephritic pain, mucositis, and cough were each observed respectively in one patient.
Low zinc plasma levels are often observed in patients with advanced malignancies (19). In our study, zinc plasma levels were clinically monitored due to the observed lysosomal unbound zinc accumulation in *in vitro* preclinical studies. As anticipated in this advanced stage patient population, only four patients had normal zinc plasma levels before starting GNS561. Two patients in the 50mg cohort, who already had baseline low zinc plasma levels prior to treatment, experienced a grade 1-2 decrease of zinc plasma levels during treatment with GNS561 and were treated by zinc supplementation. These two patients experienced no subsequent worsening of zinc plasma levels requesting no further zinc supplementation. Six patients with low zinc plasma levels at the time of screening had zinc supplementation before starting GNS561 treatment with no further requirement

of supplementation during treatment with GNS561. Sixteen other patients with low zinc plasma levels had no zinc supplementation prior to GNS561 therapy with no worsening of zinc plasma levels during treatment. Altogether, no patient developed clinically relevant zinc plasma levels and/or symptoms that would suggest any consistent effect of GNS561 on zinc plasma level.
Among other biological disorders, transient grade 3 ALT increase was reported in one patient at the dose of 200mg Q3W in a late-stage patient with biliary stent obstruction, grade 3 AST in one patient at the dose of 400mg Q3W after the end of GNS561 treatment and bilirubin level elevation grade 1-2 in two patients. Grade 1-2 anemia was reported in one patient.
Neither significant changes in electrocardiogram parameters nor cardiac toxicity were observed.
Twenty-eight serious adverse events not related to GNS561 were reported among the 26 patients.
Two suspected unexpected serious adverse reactions (SUSARs) were reported to health authorities as possibly related to GNS561: one patient with diarrhea (grade 3) and one patient with vomiting (grade 2). No treatment related deaths occurred.

PK analysis
GNS561 showed quantifiable plasma concentrations at every tested dose (Table 3). The plasma exposures were linear as the dose increase from 50 mg/Q3W up to 200 mg/BID on C1D1 and C2D1. GNS561 plasma exposure tended to be supra-linear from 200 to 300 mg/BID on C1D1 based on C_{max}, $AUC_{0\text{-}10h}$, $AUC_{0\text{-}24h}$ and AUC_{last}. The maximum median time to reach maximum plasma concentration was approximately 4h and 5.5h (all patients regrouped) on C1D1 and C2D1 respectively. Based on AUC_{last}, mean plasma exposures were 3 to 9 times higher on C2D1 than on C1D1. A dose level of 200 mg/BID would allow plasma trough concentrations to be above the 50 ng/mL that was previously estimated as an active exposure in the rat cancer model.
Liver trough concentrations (Table 4) were much higher than in plasma samples a median time of 28 days of administration with mean liver to plasma ratios (C_{trough} concentrations) of 9559 (Min 149-Max 25759) which is in accordance with rat preclinical data observed after repeated administration (C_{max} based ratio: 4580 to 26600, $AUC_{0\text{-}last}$ based ratio: 3070 to 20800).
GNS561 displayed favourable bioavailability with interpatient variability (CV%: 13 to 223% and 21 to 98.2% on plasma concentrations on cycle 1 day 1 and cycle 2 day 1 respectively), and dose proportional exposure in plasma. GNS561 concentrations accumulated after multiple administration (2.6-9.0-fold). This accumulation process may be due to a high volume and distribution and long half-life, nevertheless, these parameters could not be evaluated during this study. Plasma and liver concentrations at doses ranging 100-200 mg BID were comparable to therapeutic exposures in preclinical models.

PPT1 expression in liver biopsy
Among patients who underwent liver biopsy, eight (8) tumor liver tissues after one month of GNS561 exposure were available for analyses. We compare these samples with three non-tumoral and four tumor liver archival tissues from patients with liver tumors. As shown in Figure 1, expression of PPT1 was higher in baseline liver tumor tissues as compared to non-tumoral liver tissues. Interestingly, PPT1 expression is reduced under GNS561 exposure and although numbers of cases remain limited, PPT1 expression is more reduced in stable diseases compared with patient who presented tumor progression (not statistically significant).

Antitumor activity
Among 20 patients evaluable for antitumor activity, we observed absence of objective response rate (ORR), 5 stable diseases, and 15 progressive diseases, including 5 patients with tumor progression of a non-target lesion (Figure 2). Of 5 patients with stable disease, three of them were sorafenib pretreated for advanced HCC and experienced tumor stabilization ranging 5-16 months. The remaining 2 patients had iCCA previously treated with gemcitabine-cisplatin (GEMCIS) chemotherapy. One patient had stable disease during 12 months of GEMCIS and one patient had partial response with 5 months of GEMCIS. This last patient had stable disease according RECIST 1.1 with tumor size reduction by 23% and that was considered as a “minor response”.

Discussion

GNS561 is an oral quinolone derivative that was shown to induce lysosomal disruption and inhibit PPT1 function in several preclinical models. GNS561 displays antiproliferative and antitumor activity in animal and human models. In this first-in-human, first-in-class, phase I clinical trial evaluating oral GNS561 in patients with advanced solid tumors. We evaluated oral capsules of GNS561 in patients with primary and secondary liver cancers. The selection of patients with predominant liver cancer deposits was based on the preclinical observations of high liver concentrations of GNS561 after oral intake. Pharmacokinetic data showed that the three times a week schedule was safe but associated with suboptimal plasma concentrations during the first 4 weeks of therapy. Therefore, the study was continued using a continuous twice daily dosing. In this study, we demonstrated that GNS561 at doses up to 200 mg BID (400mg per day) displays a safe and tolerable toxicity profile. No dose-limiting toxicity was reported. More specifically and despite high liver concentrations, GNS561 was not associated with liver toxicity. Moreover, based on the chloroquine moiety of GNS561, a particular attention was paid to electrocardiogram (ECG) changes and cardiac toxicity but neither significant change in ECG nor cardiac toxicity were reported.

The RP2D of GNS561 is 200 mg BID continuous dosing. As per the protocol definition, we did not reach the MTD as no DLT were reported up to 300 mg BID. Nevertheless, we observed a high frequency of mild to moderate digestive toxicities consisting primarily of nausea and vomiting that were considered by the investigators as a limiting factor to seek for higher dosing. Indeed, nausea and vomiting as well as other gastrointestinal side effects could hamper the observance and the absorption of the oral formulation using tablets of GNS561 in phase II/III trials. A pharmacokinetic study phase I with different oral route formulations (capsules, tablets, and gastro-resistant tablets) are ongoing. The dose of 200 mg BID was associated with plasma exposures ranging from 91.1 and 1314 µg*h/L on C1D1 and 3639 to 6449 µg*h/L. GNS561 appears to be associated with long plasma half-life. In the liver, the dose of 200 mg BID led to concentrations ranging 910 to 2970 µg/g (N=2) on C2D1. Based on animal data, we previously observed that plasma and liver ratio reached in human were higher than that observed in rat and mouse models. Our data also showed that despite no limiting toxicity, the dose of 200 mg BID was the GNS561 dose recommended for any potential next clinical trials in oncology.

As a first-in human clinical trial investigating GNS561/Ezurpimtrostat, this study may have several limitations. In this study, the efficacy was not a primary endpoint and could not be satisfactorily addressed due to the low number of evaluable patients, receiving GNS561 at doses below optimal dosing during the dose-escalation. Moreover, the studied population was highly pre-exposed and/or resistant to prior therapies in respective indications. Nevertheless, five patients experienced sustained tumor stabilizations according RECIST 1.1 with biomarker stabilizations (alfa fetoprotein [AFP] for HCC and CA19.9 for iCCA), including one patient with iCCA who experienced a minor response. Another limit of this trial is related to the interpatient variability in the pharmacokinetic results among patients in the same cohort. The oral dosing, fasting/non-fasting absorption, and first liver passage may have been responsible of interpatient pharmacokinetic variability. Other studies are ongoing or planned to complete these first-in-human data.

The study confirms that PPT1 expression is higher in tumor tissues compared to non-tumoral liver tissues with a lowering expression in tumor tissues from patient exposed to GNS561. One of the limits of our study is the low number of evaluable patients and liver biopsies available to analyse PPT1 expression. The preliminary data encourage further exploration of the role of PPT1 tumor expression in other clinical trials using GNS561/Ezurpimtrostat.

Conclusion

GNS561/Ezurpimtrostat a clinical first-in-class PPT1 inhibitor, lysosome interacting agent displays a favourable safety profile and is associated with potentially active plasma and liver concentration at the recommended dose of 200 mg continuous BID. Further studies are scheduled in patients with advanced HCC and iCCA to evaluate the expression of PPT1 and the antitumor activity of GNS561. Recent preclinical data have shown that inhibition of PPT1 using GNS561 potentiates the effects of anti-PD-1 immunotherapy by increasing the expression of major histocompatibility complex-1 and

the cytotoxicity effect of $CD8^+$ lymphocytes [18, manuscript submitted]. Based on this favourable safety profile and plasma exposure, GNS561 will be further evaluated in combination with checkpoint inhibitors.

Statements

Statement of Ethics

All patients provided written informed consent. The protocol was approved and registered in France, Belgium, and the USA with the appropriate regulatory authorities and ethics committee and performed in accordance with the Declaration of Helsinki and applicable regulatory requirements (ClinicalTrials.gov NCT03316222).

Conflict of Interest Statement

JJH received research support from Bristol Myers Squibb and consulting fees from Bristol Myers Squibb, Merck, Eli Lilly, Eisai, Exelexis, CytomX, Imvax, Adaptiimmune, QED, Zymeworks.
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Author Contributions

Study concepts: Philippe Halfon, Christelle Ansaldi, Eric Raymond
Study design: Eric Raymond, Philippe Halfon
Data acquisition: James J. Harding, Ahmad Awada, Gaël Roth, Thomas Decaens, Philippe Merle, Nuria Kotecki, Chantal Dreyer, Christelle Ansaldi, Eloïne Bestion, Valerie Paradis, Ghassan K. Abou-Alfa, Eric Raymond
Quality control of data and algorithms: Madani Rachid, Christelle Ansaldi
Data analysis and interpretation: Ghassan K. Abou-Alfa, James Harding, Soraya Mezouar, Eric Raymond
Statistical analysis: Agnes Menut
Manuscript preparation: Eric Raymond
Manuscript editing: James J. Harding, Eric Raymond, Thomas Decaens, Gael Roth, Valerie Paradis
Manuscript review: James J. Harding, Eric Raymond, Thomas Decaens, Gael Roth, Valerie Paradis

Data Availability Statement

The data that support the findings of this study are not publicly available due to their containing information that could compromise the privacy of research participants but are available from the corresponding author E.R .

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Figure Legends

Fig. 1. PPT1 expression (Immunofluorescence) in human liver biopsies prior and after GNS561 exposure in patients with cholangiocarcinoma (iCCA) and hepatocellular carcinoma (HCC).

Fig. 2. Water fall plot of GNS561 antitumor activity at various dose levels.
Three patients who experienced tumor progression on non-target lesions are not displayed in this diagram.

Table Legends

Table 1. Demographics and baseline assessment of efficacy-evaluable participants
Table 2. Safety of escalated doses of GNS561
Table 3. Pharmacokinetic evaluation of GNS561**:** PK tables C1D1 (Cycle 1 Day 1) plasma and PK tables C2D1 (Cycle 2 Day 1) plasma on 26 screened patients
Table 4. Concentrations of GNS561 in the liver

Figure 1. PPT1 expression (Immunofluorescence) in human liver biopsies prior and after GNS561 exposure in patients with cholangiocarcinoma (iCCA) and hepatocellular carcinoma (HCC)

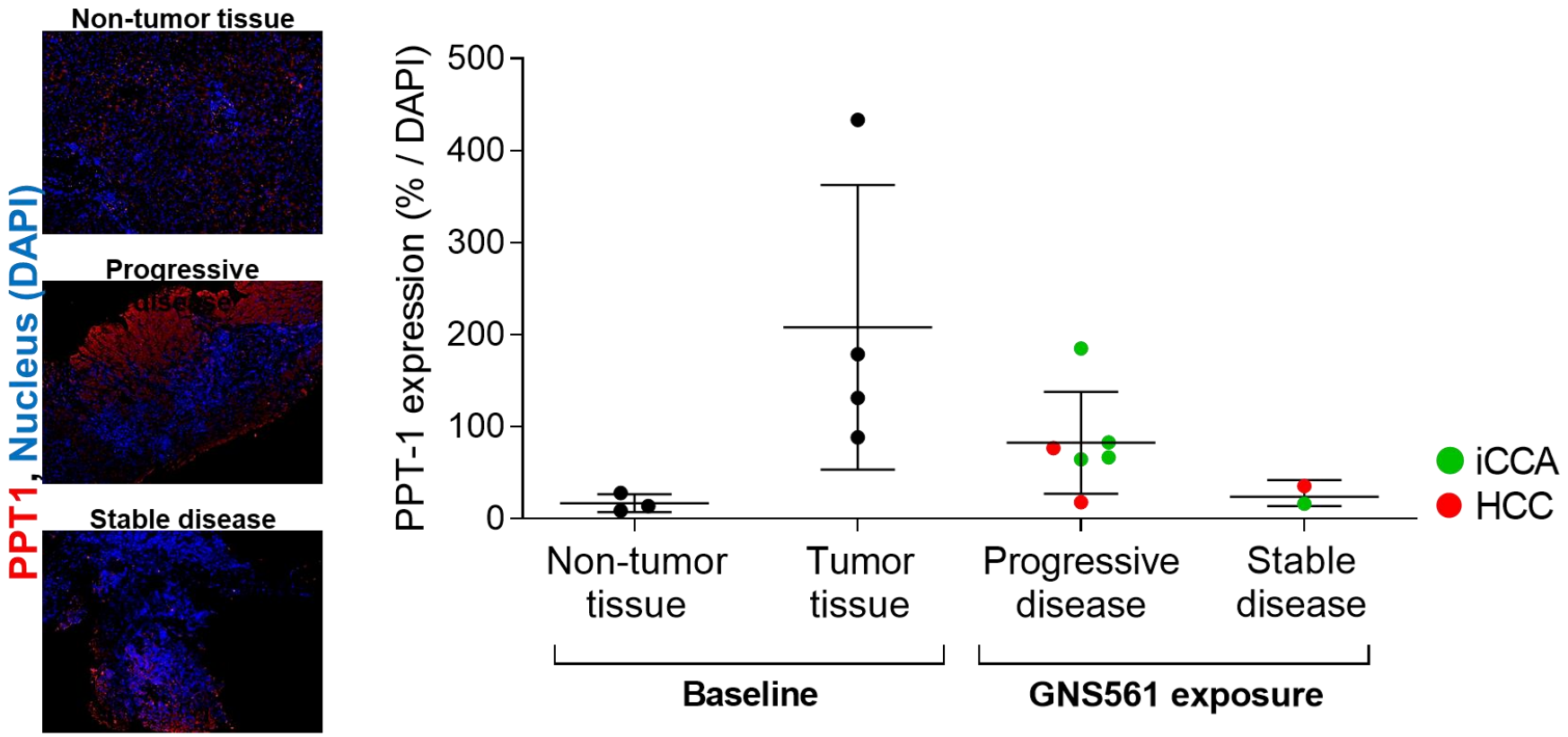
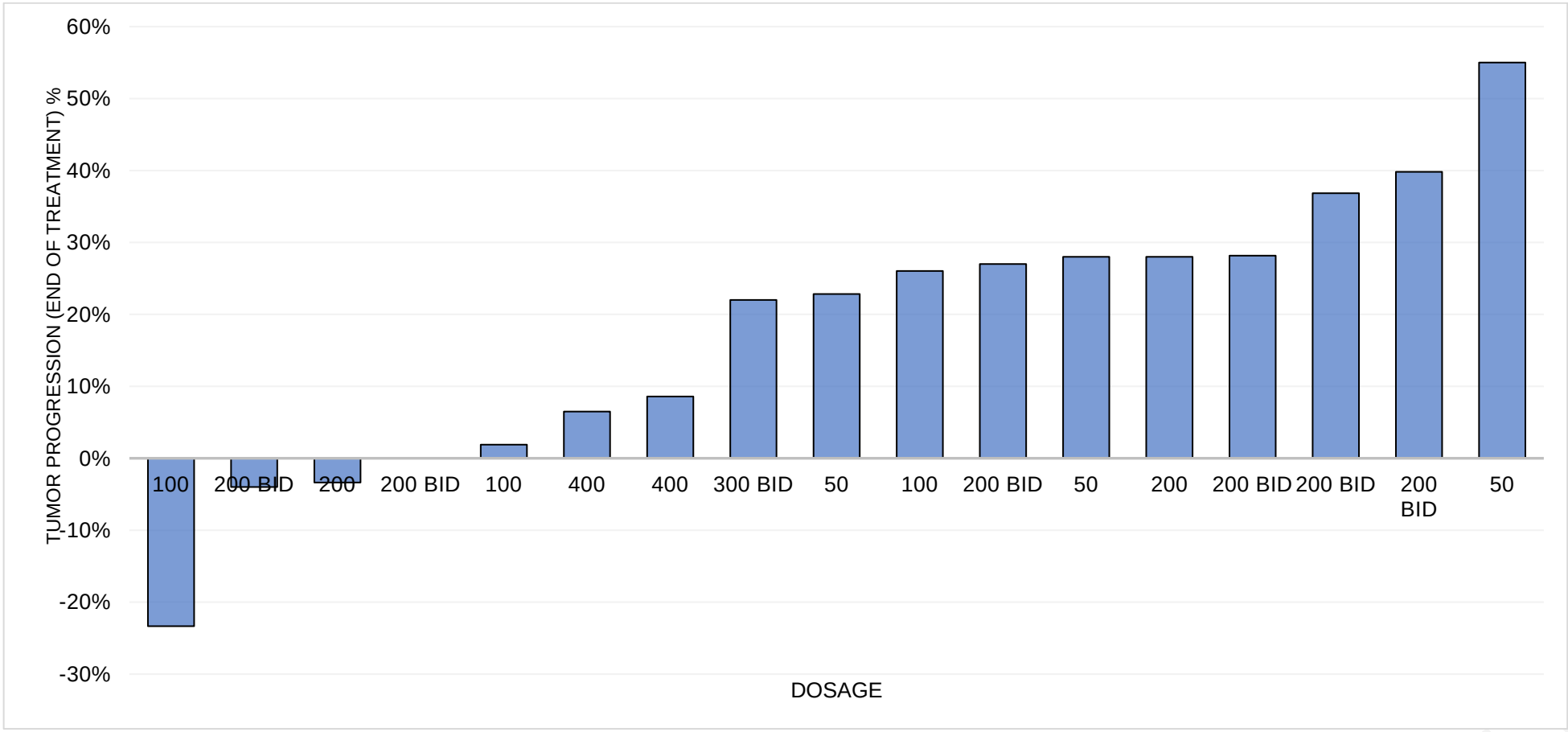


Figure 2. Water fall plot of GNS561 antitumor activity at various dose levels.

Three patients who experienced tumor progression on non-target lesions are not displayed in this diagram.



	Three times a week (Q3W)								Twice a day (BID)				All		Range
Doses	**50mg**		**100mg**		**200mg**		**400mg**		**200mg**		**300mg**		**All**		**Range**
	***n* (4)**	**%**	***n* (3)**	**%**	***n* (3)**	**%**	***n* (5)**	**%**	***n* (8)**	**%**	***n* (3)**	**%**	***n* (26)**	**%**	
Age															
< 65	2	50	0	0	1	33	3	60	8	100	1	33	15	58	
> 65	2	50	3	100	2	67	2	40	0	0	2	67	11	42	
Median (range)	54		70		66		54		59		57		60		(23-80)
Gender															
Female	1	25	1	33	0	0	1	20	2	25	2	67	7	27	
Male	3	75	2	67	3	100	4	80	6	75	1	33	19	73	
BMI															
< 29	3	75	2	67	1	33	5	100	8	100	3	100	22	85	
≥ 29	1	25	1	33	2	67	0	0	0	0	0	0	4	15	
Median (range)	22		28		28		23		23		23		24		(16-33)
ECOG															
0	2	50	0	0	1	33	3	60	4	50	2	67	12	46	
1	2	50	3	100	2	67	2	40	4	50	1	33	14	54	
Tumor type															
HCC	1	25	2	67	1	33	3	60	2	25	1	33	10	39	
iCCA	3	75	1	33	2	67	2	40	3	38	0	0	11	42	
PDAC	0	0	0	0	0	0	0	0	2	25	2	67	4	15	
CCR	0	0	0	0	0	0	0	0	1	12	0	0	1	4	
Tumor size (mm)															
≤ 50	0	0	1	33	1	33	0	0	1	12	0	33	3	12	
50 < x < 100	1	25	2	67	1	33	1	20	2	25	1	67	8	30	
≥ 100	3	75	0	0	1	33	4	80	5	62	2		15	58	
Median (range)	107		67		89		150		119		105		107		(30-200)
Number of prior therapy lines															
≤1	3	75	1	33	2	67	3	60	2	25		0	11	42	
2	0	0	1	33	0	0	1	20	1	12		0	3	12	
≥3	1	25	1	33	1	33	1	20	5	62	3	100	12	46	
Median (range)	1		2		1		1		3		3		2		

Table 2. Safety of escalated doses of GNS561

Treatment related adverse events *n* (%)			
Grade	**1-2**	**3**	**Total**
Nausea	**13**(50%)	-	13(50%)
Vomiting	**14**(54%)	-	14(54%)
Diarrhea	**9 (35%)**	**2**(8%)	11(42%)
Decreased appetite	**2**(8%)	**1**(4%)	3(12%)
Abdominal pain	**2**(8%)	-	2(8%)
Abdominal distension	**2**(8%)	-	2(8%)
Constipation	**1**(4%)	-	1(4%)
Fever	**1**(4%)	-	1(4%)
Dyspepsia	**1(4%)**	-	1(4%)
Regurgitation	**1(4%)**	-	1(4%)
Weight decreased	**1(4%)**	-	1(4%)
Fatigue	5(19%)	1(4%)	6(23%)
Dizziness	**1**(4%)	-	1(4%)
Occasional weakness	**1**(4%)	-	1(4%)
Asthenia	**1**(4%)	**1**(4%)	2(8%)
Sweating	**1**(4%)	-	1(4%)
Blood zinc decreased	2(8%)	-	2(8%)
Anemia	**1**(4%)	-	1(4%)
ALT increased	-	**1**(4%)	1(4%)
AST increased	**1**(4%)	**1**(4%)	2(8%)
Increased bilirubin level	**2**(8%)	-	2(8%)
Blood albumin decreased	**1(4%)**	-	1(4%)
hypertension	**1**(4%)	-	1(4%)
dyspnea	**1**(4%)	-	1(4%)
blurred vision	**1**(4%)	-	1(4%)
hypercalcemia	**1**(4%)	-	1(4%)
Nephritic pain	**1**(4%)	-	1(4%)
Dry mouth	**1**(4%)	-	1(4%)
Cough	**1**(4%)	-	1(4%)
Peripheral sensory neuropathy	**1**(4%)	-	1(4%)
Mucosal inflammation	**1(4%)**	-	1(4%)

Table 3. Pharmacokinetic evaluation of GNS561

PK tables C1D1 (Cycle 1 Day 1) plasma.

Dosing	ID	Cmax	Tmax	Clast	Tlast	AUC_0_10	AUC_0_24	AUClast
		ug·L⁻¹	h	ug·L⁻¹	h	h·ug·L⁻¹	h·ug·L⁻¹	h·ug·L⁻¹
50 mg Q3W	Mean (median for Tmax/last)	1.47	5.04	0.645	47.71	9.38	21.2	37.3
	SD	1.56	na	0.949	na	10.3	25.4	48.4
	CV%	106%	na	147%	na	110%	120%	130%
	n	4	4	4	4	4	4	4
100 mg Q3W	Mean (median for Tmax/last)	3.50	4.05	0.991	47.3	21.4	28.4	38.7
	SD	2.39	na	1.162	na	15.3	na	na
	CV%	68%	na	na	na	71%	na	na
	n	3	3	2	3	3	2	2
200 mg Q3W	Mean (median for Tmax/last)	8.35	8.00	1.09	48	42.8	82.4	115
	SD	3.99	na	0.570	na	22.5	40.0	56.9
	CV%	48%	na	52%	na	53%	49%	50%
	n	3	3	3	3	3	3	3
400 mg Q3W	Mean (median for Tmax/last)	30.5	3.58	6.21	47.1	156	357	616
	SD	28.1	na	7.5	na	163	416	685
	CV%	92%	na	115%	na	104%	116%	111%
	n	5	5	5	5	5	5	4
200 mg BID	Mean (median for Tmax/last)	21.2	47.2	17.0	47.5	46.1	173	519
	SD	13.8	na	15.6	na	38.1	86	373
	CV%	65%	na	92%	na	83%	50%	72%
	n	8	8	8	8	8	8	8
300 mg BID	Mean (median for Tmax/last)	93.8	46.9	78.9	47.5	328	1037	2630
	SD	5.93	na	28.7	na	57.9	553	1285
	CV%	6%	na	36%	na	18%	53%	49%
	n	3	3	3	3	3	3	3

Underlined: individual data excluded from descriptive statistics as 10, 24 and or 48h timepoint not available

PK tables C2D1 (Cycle 2 Day 1) plasma on 26 screened patients

Dosing	ID	Cmax	Tmax	Clast	Tlast	AUC_0_10	AUC_0_24	AUClast
		ug·L⁻¹	h	ug·L⁻¹	h	h·ug·L⁻¹	h·ug·L⁻¹	h·ug·L⁻¹
50 mg Q3W	Mean (median for Tmax/last)	3.54	3.43	2.33	47.5	31.1	72.7	133
	n	2	2	2	2	2	2	2
100 mg Q3W	Mean (median for Tmax/last)	8	23.6	7.3	48	63.1	160	329
	SD	6.1	na	5.6	na	47.5	139	277
	CV%	76%	na	77%	na	75%	87%	84%
	n	3	3	3	3	3	3	3
200 mg Q3W	Mean (median for Tmax/last)	32.3	14.65	20.40	35.75	250.0	768.0	1351
	n	2	2	2	2	2	1	1
400 mg Q3W	Mean (median for Tmax/last)	55.9	2.02	25.1	48.1	418	911	1604
	n	1	1	1	1	1	1	1
200 mg BID	Mean (median for Tmax/last)	117.4	10	96.1	47.3	970	2542	4857
	SD	33.7	na	20.7	na	650	728	1442
	CV%	29%	na	22%	na	36%	29%	30%

n	5	5	5	5	5	5	3

Underlined: individual data excluded from descriptive statistics as 10, 24 and or 48h timepoint not available

Table 4. Concentrations of GNS561 in the liver

Dose Level	Subject Id	Liver concentration (µg/g)
50 mg Q3W	01-002	0.376 (1) (2)
50 mg Q3W	01-004	6.12 (1)
50 mg Q3W	02-001	39.33
200 mg Q3W	02-003	56.7
200 mg Q3W	02-002	116
400 mg Q3W	04-002	259
200 mg BID	03-002	2970
200 mg BID	03-003	910

(1): Quantification did not meet the acceptance criteria
(2): Value below the limit of quantification (-6% of the LLOQ i.e. 0.1µg/g)