Research Article

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Randomized phase II placebo controlled study of codrituzumab in previously treated patients with advanced hepatocellular carcinoma

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Background & Aims: Codrituzumab, a humanized monoclonal antibody against Glypican-3 (GPC3) that is expressed in hepatocellular carcinoma (HCC), interacts with CD16/FcγRIIIa and triggers antibody-dependent cytotoxicity. Codrituzumab was studied *vs.* placebo in a randomized phase II trial in advanced HCC patients who had failed prior systemic therapy.

Methods: Patients with advanced HCC who had failed prior systemic therapy, ⩾18 years, Eastern cooperative oncology group (ECOG) 0-1, Child-Pugh A were randomized 2:1 to biweekly codrituzumab 1600 mg *vs.* placebo. Patients were stratified based on GPC3 immunohistochemical expression: 2+/3+, 1+, and 0. Primary endpoint was progression free survival. Secondary endpoints include overall survival (OS), tolerability, pharmacokinetics, and an exploratory endpoint in biomarkers analysis.

Results: 185 patients were enrolled: 125 received codrituzumab and 60 placebo: Median age 64/63, 85/75% male, 46/42% Asian, ECOG 0 65/63%, 74/77% having vascular invasion and/or extrahepatic metastasis. 84%/70% had prior sorafenib. Drug exposure was 98.4% of planned dose, with an identical adverse events profile between the 2 groups. The median progression free survival and overall survival in the codrituzumab *vs.* placebo groups in months were: 2.6 *vs.* 1.5 (hazard ratios 0.97, $p = 0.87$), and 8.7 *vs.* 10 (hazard ratios 0.96, $p = 0.82$). Projected C_{trough} at cycle 3 day 1 based exposure, high CD16/FcγRIIIa on peripheral immune cells, and GPC3 expression in the tumor, were all associated with prolonged progression free survival and overall survival.

Conclusions: Codrituzumab did not show clinical benefit in this previously treated HCC population. Whether higher codrituzumab drug exposure or the use of CD16 and GPC3 as potential biomarkers would improve outcome remain unanswered questions.

Lay summary: Codrituzumab is a manufactured antibody against a liver cancer protein called glypican-3. In this clinical trial, codrituzumab was not found be effective against liver cancer. It was suggested though that a higher dose of codrituzumab or selecting patients with high level of glypican-3 or its mediator CD16 might improve outcome.

Clinical trial registration: This trial is registered at Clinicaltrials.gov (NCT01507168).

Keywords: Hepatocellular carcinoma; Codrituzumab; Glypican-3; CD16.
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Introduction

Glypican-3 (GPC3) is a member of the glypican family, a group of heparan sulfate proteoglycans linked to the cell surface and which plays an important role in cell growth, differentiation, and migration [1,2]. GPC3 is highly expressed in HCC and has become a useful diagnostic marker for HCC by immunohistochemical (IHC) studies since the adjacent non-tumoral tissue does not express GPC3 [3–8]. GPC3 may promote HCC growth by stimulating the canonical Wnt pathway, and/or interacting

with the IGFII-IGF1R pathway, or it may play a role in fibroblast growth factor (FGF) signaling [9–12]. Therefore, GPC3 may represent a specific tumor marker and a potential target for therapy in HCC [13].

Codrituzumab is a recombinant, humanized monoclonal antibody that binds to human GPC3 with high affinity [14–18]. Codrituzumab interacts with CD16/FcγRIIIa and triggers antibody-dependent cytotoxicity (ADCC) [15]. Non-clinical characterization of codrituzumab demonstrates that it elicits ADCC against GPC3-positive human hepatoma cells lines (SK-03: SK-HEP-1 HCC line engineered to overexpress GPC3; HepG2: hepatoblastoma), using human peripheral blood mononuclear cells (PBMCs) as effector cells [18]. Phase I studies in US [19] and Japan [20] showed that codrituzumab was well tolerated up to 20 mg/kg/wk without dose limiting toxicity.

In this phase II study, codrituzumab was compared with placebo in a randomized way in advanced HCC patients who had failed at least one prior systemic therapy.

Patients and methods

Study population

Patients with histologically confirmed unresectable advanced or metastatic HCC following Barcelona clinic liver cancer (BCLC) classification, who received at least one prior systemic therapy, were eligible. Subjects had to be ⩾18 years of age, with an ECOG score [21] of 0–1, a Child-Pugh score of A, measurable disease as defined by RECIST version 1.1 [22], and adequate organ function defined by platelet count $\geqslant 50 \times 10^9$/L, absolute neutrophil count ⩾1,500/μL, hemoglobin ⩾8.0 g/dl, alanine transaminase (ALT or SGPT) and aspartate transaminase (AST or SGOT) $\leqslant 5 \times$ upper limit of normal, total bilirubin ⩽2 mg/dl and creatinine $\leqslant 2 \times$ ULN or calculated Creatinine Clearance ⩾60 ml/min using Cockcroft and Gault formula [23]. Patients with prior organ transplantation or known positive HIV infection were excluded. The study was approved by institutional review boards of participating centers and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Study design, dose administration, randomization, and cohort assignment

Patients were randomized in a 2:1 ratio to codrituzumab 1,600 mg intravenously every two weeks after two weekly loading doses *vs.* placebo. Prior to randomization, patients were assigned into 3 cohorts based on the immunohistochemistry (IHC) analysis of GPC3 expression: cohort A (GPC3 IHC 2+/3+), B (GPC3 IHC 1+) and C (GPC3 IHC 0) (Supplementary Fig. 1). Patients were stratified based on the following factors: GPC3 expression status by IHC, region, ECOG performance status (0 *vs.* 1), and presence or absence of macroscopic vascular invasion or extra-hepatic spread. A two-stage adaptive design was used with intention to collect more information in patients who have high GPC3 expression levels and therefore, likely to benefit from codrituzumab treatment.

Efficacy and safety analysis

The primary efficacy endpoint was progression free survival (PFS) based on investigator assessment. Secondary endpoints included overall survival (OS), time to progression, tolerability and safety of codrituzumab *vs.* placebo. Tumor assessment was done by computed tomography at weeks 6, 12 and 18, and every 8 weeks afterwards until progression. Response evaluation was based on investigator assessment using the criteria of RECIST version 1.1 [22]. Human anti-human immunoglobulin test (HAHA) was evaluated in pretreatment, cycles 1, 6, 10, final visit and 28-day off-study visit. The NCI-CTCAE version 4.0 was used to evaluate adverse events.

Pharmacokinetics and exposure-response analysis

Pharmacokinetic (PK) samples were collected from all patients participating in the study. An extensive PK sampling schedule was performed for 40 patients in cycle 1 days 1, 2, 5, 8, 9 and 12 and cycle 6 (days 1, 2, 5 and 11), as well as at the predose in cycles 2, 3, 7, 9, 10, and 11. A sparse PK sampling was performed in cycle 1 (days 1, 3 and 8) and cycle 6 (day 1) as well as at the predose in cycles 9, 10 and 11 for the remaining patients. Additional samples were obtained at the final visit, the 28-day follow-up visit, and at the time of progression of disease for all patients. A population pharmacokinetic (popPK) model was developed using the PK data from 120 patients with evaluable PK data. Individual predose concentrations at Cycle 3 day 1 (C3D1) that correspond to Day 29 were simulated using the popPK model. The target saturation was derived from Michaelis-Menten constant (K_m) within the model [24].

In a post hoc analysis, the codrituzumab arm was divided into low and high exposure subgroups by the trough level on C3D1 and the exposure-efficacy relationship on PFS was explored. To reduce the bias introduced by potentially unbalanced confounding risk factors among the different groups (high exposure, low exposure and placebo), a nearest available Mahalanobis metric matching within calipers defined by the propensity score method was used to create balanced groups of high exposure and placebo and low exposure and placebo separately [25]. These matched groups were then compared for the treatment effects. Hazard ratios (HR) for PFS were calculated for the propensity score matched high *vs.* placebo, and low exposure group *vs.* placebo, respectively.

Biomarkers

GPC-3 IHC was performed in fresh tissue or in tissue prepared within 3 months from formalin-fixed paraffin-embedded blocks of the primary or the metastatic tumor. The percentage of tumor cells stained and the pattern of membrane and/or cytoplasmic positivity were used to infer a clinical score (range 0–3+) assigned according to the criteria described in Supplementary Table 1. CD16MESF (Molecules of Equivalent Soluble Fluorochrome), which represents the level of expression of CD16 in natural killer (NK) cells in the PBMCs, was determined by flow cytometry. Additional biomarker methodological details are presented in the Supplementary material.

Statistical design

All patients enrolled were included in the intent-to-treat population (ITT), and all patients who received at least one dose of codrituzumab were included in the safety population. A data review committee including both internal Roche and external members with expertise in oncology and statistics, not involved in the study, was established to help evaluate the outcome of the first stage of the study and to monitor safety.

The primary analysis of PFS was planned to take place after approximately 112 PFS events in GPC3 IHC 1+/2+/3+ and approximately 79 PFS events in GPC3 IHC 2+/3+ populations have been observed. In case of early termination of tumor GPC3 IHC 1+ at futility analysis, approximately 90 PFS events in tumor GPC3 IHC 2+/3+ would have been required at the final analysis. The analysis was expected approximately 24 months after the first patient was randomized. The clinical study report will be based on this final analysis of PFS.

For biomarker analysis, we used a cutoff value at median or any other percentile to define "biomarker high" *vs.* "biomarker low". We calculated HR, 95% confidence intervals (CI) and corresponding two-sided *p* values for treatment effect for both "biomarker high" and "biomarker low" groups separately, based on Cox proportional hazard regression models, with OS as response variable, and adjusting clinical baseline covariates. Since multiple biomarkers were tested at the same time, False Discovery Rate (FDR) was computed to adjust statistical significance via the Benjamini and Hochberg method [26]. For a given biomarker, we defined significance when FDR <0.05. Kaplan-Meier curves were employed to visualize marginal treatment effect. Different cutoffs (33rd, 50th, and 67th percentiles) of CD16 MESF were explored by calculating HR, CI and *p* values based on 5-fold cross-validation and determined if they remained significant.

Results

Study population

Between February 2012 to March 2013, 259 patients with HCC diagnosis were screened for the study and 185 patients were enrolled in three cohorts: 105 in Cohort A (GPC3 IHC 2+/3+), 56 in Cohort B, (GPC3 IHC 1+), and 24 in Cohort C (GPC3 IHC 0). The main reasons for screening failure include lack of tumor tissue, out of range of Child-Pugh scores and laboratory parameters,