Japanese phase I study of GC33, a humanized antibody against glypican-3 for advanced hepatocellular carcinoma

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GC33 is a humanized mAb against human glypican-3 (GPC3). In the first-in-human study carried out in the USA, GC33 was well tolerated and showed preliminary antitumor activity in patients with advanced hepatocellular carcinoma. This study aimed to assess the safety, tolerability, and pharmacokinetic characteristics of GC33 in Japanese patients with advanced hepatocellular carcinoma. The study design was a conventional 3 + 3 dose-escalation design to determine the maximum tolerated dose of GC33 given i.v. at 5, 10, or 20 mg/kg weekly. Immunohistochemistry was carried out on tumor biopsies to evaluate GPC3 expression. Thirteen patients were enrolled across the three dose levels, and no patients observed any dose-limiting toxicity up to the highest planned dose of 20 mg/kg. The most common adverse events were decreased lymphocyte count, decreased natural killer cell count, increased C-reactive protein, and pyrexia. Grade 3 adverse events (increased blood pressure, decreased lymphocyte count, and decreased platelet count) were observed in two or more patients. The AU_{inf} showed a dose-proportional increase from the 5 mg/kg dose group to the 20 mg/kg dose group. The trough concentrations of GC33 appeared to reach a steady state after the fourth to the sixth dose. Seven of the 13 patients showed stable disease, the other six showed progressive disease. Furthermore, three patients showed long-term stable disease of more than 5 months. In conclusion, GC33 given at up to 20 mg/kg weekly was well tolerated in Japanese patients with advanced hepatocellular carcinoma.

Hepatocellular carcinoma (HCC) is the sixth most common cancer in the world in terms of incidence, accounting for approximately 630,000 new patients per year, and the third most common cause of cancer death, with more than 600,000 people dying of HCC each year.1 2 Most patients present with the advanced stage of disease; however, the only approved therapy for advanced HCC is sorafenib, an oral multikinase inhibitor, and the benefits of sorafenib remain modest.3 4–6

Glypican-3 (GPC3) is a member of the glypican family. Glypicans are proteoglycans that are attached to the cell surface by a glycosyl–phosphatidylinositol anchor, and play an important role in cellular growth, cell differentiation, and cell migration.6–8 Immunohistochemical studies have shown that GPC3 is highly expressed in >70% of HCC tissues, whereas it is less detectable or not detectable in adjacent non-tumoral lesions.9 It has been reported that GPC3 expression is correlated with poor prognosis in HCC because membranous GPC3-positive HCC patients have a significantly lower disease-free survival rate than GPC3-negative HCC patients after surgical resection.10 Therefore, GPC3 represents a specific tumor marker and a potential therapeutic target in HCC.11

A recombinant humanized mAb against the C-terminal region of human GPC3,12 GC33 induced antibody-dependent cellular cytotoxicity against GPC3-positive HCC cell lines and caused tumor growth inhibition in human liver cancer xenograft models.13–15

The first-in-human (FIH) study of GC33 carried out in the USA was a phase I, open-label, multicenter, dose-escalation study in which GC33 was given at 2.5, 5, 10, or 20 mg/kg as monotherapy to patients with advanced HCC. GC33 was well tolerated, and no dose-limiting toxicity (DLT) up to the highest planned dose level was detected.14 In consideration of variations in the natural history and HCC treatment practices in Western countries versus Asian populations, and possible pharmacogenomic differences,15–18 in the current study we evaluated the safety, tolerability, and pharmacokinetic (PK) characteristics of GC33 in Japanese patients. Additionally, we assessed the preliminary antitumor activity, explored biomarkers, and compared a fully automated GPC3...
immunohistochemistry (IHC) test with a more laborious GPC3 IHC test that used previously.

Materials and Methods

Patients. Eligible patients had histologically or cytologically confirmed HCC (not including the fibrolamellar subtype) with no preferred alternative treatment, were ≥20 years old, had life expectancy of ≥3 months after enrolment, had an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1, had a Child–Pugh class of A or B, and had at least one radiographically evident lesion. Patients were required to have adequate hematologic, hepatic, and renal function as evidenced by a platelet count of ≥50 000/μL, absolute neutrophil count of ≥1500/μL, transaminase (aspartic aminotransferase and alanine aminotransferase) levels of ≤5.0 × ULN, total bilirubin level of ≤3.0 × ULN, prothrombin time-international normalized ratio of ≤2.0, alkaline phosphatase of ≤5.0 × ULN, and serum creatinine level of ≤2.0 × ULN. Tumor samples also had to be available, biopsied within 12 months before the required informed consent, for GPC3 IHC testing.

Patients were excluded if they were HIV antibody positive or had active infection requiring treatment, except for hepatitis B virus or hepatitis C virus. Exclusion criteria also included a history of transplantation, patients with brain metastases with symptoms, central nervous system diseases (including psychiatric diseases), other concurrent malignancies within the last 5 years, central nervous system manifestations of hepatic encephalopathy, severe or massive ascites, and a history of hypersensitivity to similar agents such as mAbs. Patients who had received surgery, locoregional therapy (ablative or transcatheter arterial [chemo] embolization) for HCC, chemotherapy other than sorafenib, radiotherapy, hormone therapy, immunotherapy, or any other investigational drug within 4 weeks prior to enrolment (2 weeks for sorafenib) were also excluded.

This trial was carried out in accordance with the Declaration of Helsinki and Good Clinical Practice. The trial protocol was approved by the Japanese regulatory agency PMDA and the institutional review board of each investigation site. Patients provided written informed consent according to institutional guidelines before enrolment.

Study design. The primary objective was to determine the safety and tolerability of GC33 in Japanese patients with advanced HCC, and the secondary objectives were to evaluate the PK, efficacy, and biomarkers. This trial was carried out at three sites across Japan, namely, the National Cancer Center Hospital East (Chiba), the Kanagawa Cancer Center Hospital (Kanagawa), and the National Cancer Center Hospital (Tokyo).

The patients enrolled were treated with GC33 at 5, 10, or 20 mg/kg given every week by i.v. infusion over a period of 30–90 min. Each cycle was defined by approximately 4 weeks of treatment. GC33 treatments were scheduled to continue until progressive disease (PD), occurrence of unacceptable toxicities, or the patient’s withdrawal of consent. The starting dose of 5 mg/kg was chosen based on the dose of GC33 estimated from xenograft mouse models that was expected to still have potential for some antitumor activity, while taking into account the safety and PK results from the phase I study carried out in the USA.

This study was a conventional 3 + 3 dose-escalation design. At least three patients in each of the 5 and 10 mg/kg cohorts, and at least six patients in the 20 mg/kg cohort were to be treated and monitored from the first dosing of GC33 until prior to the fifth dosing (cycle 1), as described previously. Dose-limiting toxicity was defined as any grade 3 or higher toxicity occurring during cycle 1 (as categorized by the NCT’s Common Terminology Criteria for Adverse Events version 4.03) other than grade 3 infusion reaction, transient electrolyte abnormalities, grade 3 decreased lymphocyte count, grade 3 decreased platelet count not requiring platelet transfusion, and <10 × ULN increase in hepatic enzymes (e.g., increased alanine aminotransferase, increased aspartic aminotransferase, increased alkaline phosphatase), grade 3 hepatic function abnormal (decreased serum albumin, increased total bilirubin, increased prothrombin time-international normalized ratio), and any non-drug-related toxicities. A medical monitor and an independent Data Safety Monitoring Board reviewed the safety data of each cohort and dose escalation.

Study assessment. Types and frequency of adverse events (AEs) and causal relationships between AEs and GC33 were evaluated. Incidence and severity of AEs were collected and graded according to the NCT’s Common Terminology Criteria for Adverse Events version 4.03. Particular attention was paid to infusion-associated symptoms possibly as a result of GC33 infusion. Laboratory evaluations during the study included hematology, blood biochemistry, blood coagulation, and natural killer (NK) cell count. Anti-GC33 antibodies were measured at screening and the end of cycle 1.

Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 was used to assess overall response and progression-free survival (PFS). Tumor burden and response to treatment were evaluated at the baseline and every 8 weeks by imaging (computed tomography or MRI), α-Fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP) were also measured at the baseline and during the study.

Tumoral expression of GPC3 was centrally examined and evaluated in biopsied specimens by two IHC methods, each using mouse anti-human GPC3 mAbs. Method 1, which was the method used in the previous FIH study, was carried out at Charles River Laboratories Inc. (Reno, NV, USA) and assessed by three qualified pathologists blinded to clinical outcomes using the scoring criteria shown in Table 1. Method 2, a fully automated IHC assay, was carried out at Ventana Medical Systems Inc. (Tucson, AZ, USA) using anti-glypican 3 (GC33) mouse monoclonal primary antibody (Catalog number 790–4564; Ventana Medical Systems) according to the manufacturer’s instructions. The immunostaining resulting from method 2 was assessed by two qualified pathologists blinded to clinical outcomes. Staining intensity was assessed in the cytoplasm and at the cell membrane separately. Staining intensity was scored on a four-point scale: 0, no staining was observed; 1 (weak), an intensity identified by the pathologist as higher than background and specific to the cells of interest; 2, moderate staining; and 3, maximal (strong) staining. The percentage of cells with specific antibody reactivity was scored for the tumor component of the specimen. The H score was derived by summing the percentage of cell staining at each intensity (weak, moderate, strong) multiplied by the weighted intensity of staining. Following the evaluation of GPC3 IHC staining intensity, the percentage of tumor cells stained, and the pattern of membrane and/or cytoplasmic positivity, a clinical score for method 2 was assigned according to the criteria described in Table 2.

Blood samples used for serum GC33 measurements were drawn at pre-dose, at the end of the first infusion, and at 1, 8, 24, 48, 72, and 120 h after the end of the first infusion. In addition, trough concentrations of serum GC33 were measured prior to each subsequent infusion until the end of cycle 3.
Table 1. Scoring system of immunohistochemical (IHC) staining of glypican-3 (GPC3) in hepatocellular tumor specimens using method 1

| Score | Positive cell rate (PR) | Staining intensity (SI)† | Staining pattern of cell membrane (SP-Cm)‡ |
|-------|--------------------------|--------------------------|------------------------------------------|
|       | No staining |<20%                      | No cell membrane pattern                  |
|       | ≤50%                     | 20–49%                   | Incomplete or partly complete (<20%)      |
|       | ≥50%                     | 50%                     | Rather complete (≥50%)                    |
|       |                           |                         | Complete staining, circular (ring-like) staining of tumor cells; incomplete staining, focal staining of rim of cells. |

Scoring method 1, used in a previous first-in-human study, involved assessment by three qualified pathologists blinded to clinical outcomes using the scoring criteria shown here. GPC3 staining categories: minimal, slight staining recognized at low-power objective, ×10; weak, slight staining recognized at low-power objective, ×4; moderate, staining found easily at ×4, but weaker than that of strong staining; strong, staining apparent strong with low-power objective, ×4.

†Complete staining, circular (ring-like) staining of tumor cells; incomplete staining, focal staining of rim of cells.

‡Pharmacokinetic parameters for GPC3 in serum were calculated by non-compartmental methods using data obtained after the first dosing.

Table 2. Scoring criteria of immunohistochemical staining of glypican-3 in hepatocellular tumor specimens using method 2

| Clinical score | Membrane staining | Cytoplasmic staining |
|---------------|-------------------|----------------------|
| 0             | Negative          | and                  |
| 1             | Positive staining in <10% of tumor cells | and/or |
| 2             | Weak or moderate staining in ≥10% of tumor cells | with or without |
| 3             | Strong staining in ≥10% of tumor cells | or |

Scoring method 2 used a fully automated immunohistochemical assay, with results assessed by two qualified pathologists blinded to clinical outcomes.
No anti-GC33 antibodies were detected in any of the patients at pre-treatment or post-treatment.

**Pharmacokinetics.** The PK data were evaluated for all 13 patients in all cohorts. The PK parameters of GC33 are shown in Table 6. At doses of 5, 10, and 20 mg/kg, the mean half-life (t1/2) was 4.17, 7.01, and 6.13 days and the mean total clearance was 0.566, 0.373, and 0.510 L/day, respectively.

The PK exposure (C max and AUC inf) after the first dose increased as the dose increased. The AUC inf showed a dose-proportional increase from the 5 mg/kg dose group to the 20 mg/kg dose group. The trough concentrations of GC33 appeared to reach a steady state after the fourth to the sixth dose. The trough concentrations after reaching a steady state in all patients in all cohorts were over 30 μg/mL, which was the predicted effective concentration in the xenograft models.

**Tumoral GPC3 expression.** All 13 unique core-needle biopsied specimens taken from tumor lesions in the liver were stained by both methods and were evaluated by using their respective scoring criteria. The representative GPC3-IHC features for both staining methods are shown in Figure 1(a) and all cases are shown in Figure S1. Twelve patients had a total GPC3 staining score of 7 or more by method 1, and 12 patients had positive clinical scores by method 2 (Fig. 1b). Both staining methods produced a similar staining pattern in the majority of specimens. The GPC3 staining score of method 1 was highly correlated with the clinical score of method 2 (Spearman’s correlation coefficient, 0.76; P = 0.00255). Higher clinical scores

| Characteristic                  | No. of patients (%) |
|--------------------------------|---------------------|
| Age, years                      |                     |
| Median                          | 66                  |
| Range                           | 48–78               |
| Weight, kg                      |                     |
| Median                          | 62.6                |
| Range                           | 55.2–81.5           |
| Sex                             |                     |
| Male                            | 12 (92)             |
| Female                          | 1 (8)               |
| ECOG PS                         |                     |
| 0                               | 9 (69)              |
| 1                               | 4 (31)              |
| Disease stage                   |                     |
| Stage III                       | 4 (31)              |
| Stage IVA                       | 3 (23)              |
| Stage IVB                       | 6 (46)              |
| Degree of differentiation       |                     |
| Well differentiated             | 3 (23)              |
| Moderately differentiated       | 8 (62)              |
| Poorly differentiated           | 2 (15)              |
| Child-Pugh                      |                     |
| A                               | 10 (77)             |
| B                               | 3 (23)              |
| Etiology                        |                     |
| HBV positive                    | 4 (31)              |
| HCV positive                    | 8 (62)              |
| Alcohol liver disease           | 1 (8)               |
| Vascular invasion               |                     |
| Yes                             | 7 (54)              |
| No                              | 6 (46)              |
| Extra-hepatic metastasis        |                     |
| Yes                             | 6 (46)              |
| No                              | 7 (54)              |
| Laboratory values               |                     |
| AFP, ng/mL                      |                     |
| Median                          | 361.1               |
| Range                           | 4.9–23.476         |
| DCP, U/mL                       |                     |
| Median                          | 3.619               |
| Range                           | 0.128–57.048       |
| Previous therapy                |                     |
| Surgical resection              | 4 (31)              |
| RFA/PEI                         | 1 (8)               |
| TACE/TAE                        | 10 (77)             |
| TAI                             | 5 (38)              |
| Other                           | 2 (15)              |
| Systemic chemotherapy           | 13 (100)            |
| No. of prior regimens           |                     |
| 1                               | 6 (46)              |
| 2                               | 5 (38)              |
| 3                               | 1 (8)               |
| 4                               | 1 (8)               |
| GPC3 IHC clinical score (method 2)|                 |
| 0                               | 1 (8)               |
| 1                               | 6 (46)              |
| 2                               | 4 (31)              |
| 3                               | 2 (15)              |

**Table 3. Patient characteristics at baseline**

**Table 4. Summary of common adverse events occurring in over 30% of patients with advanced hepatocellular carcinoma treated with GC33, a humanized antibody against glypican-3 (n = 13)**

| Adverse events                      | 5 mg/kg n = 4 | 10 mg/kg n = 3 | 20 mg/kg n = 6 | Total no. of patients n (%) |
|-------------------------------------|---------------|---------------|---------------|-----------------------------|
| Any adverse events                  | 4             | 3             | 6             | 13 (100)                    |
| Lymphocyte count decreased          | 4             | 3             | 3             | 10 (77)                     |
| Natural killer cell count decreased | 2             | 3             | 5             | 10 (77)                     |
| C-reactive protein increased        | 3             | 2             | 4             | 9 (69)                      |
| Pyrexia                             | 2             | 2             | 4             | 8 (62)                      |
| Aspartate                           | 1             | 2             | 3             | 6 (46)                      |
| aminotransferase increased         | 1             | 1             | 4             | 6 (46)                      |
| Blood alkaline phosphatase increased| 1             | 3             | 1             | 5 (38)                      |
| Blood lactate dehydrogenase increased| 1             | 0             | 4             | 6 (46)                      |
| Blood albumin decreased             | 1             | 0             | 3             | 4 (31)                      |
| Blood pressure increased            | 1             | 1             | 2             | 4 (31)                      |
| Fatigue                             | 0             | 3             | 1             | 4 (31)                      |
| Decreased appetite                  | 1             | 0             | 3             | 4 (31)                      |

**AFP, a-fetoprotein; DCP, des-C-carboxy prothrombin; ECOG PS, Eastern Cooperative Oncology Group Performance Status; HBV, Hepatitis B virus; HCV, Hepatitis C virus; PEI, Percutaneous ethanol injection; RFA, Radiofrequency ablation; TACE, Transcatheter arterial chemoembolization; TAE, Transcatheter arterial embolization; TAI, Transcatheter arterial infusion.**

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appeared to be associated with increased H scores for both the membrane and cytoplasm in this limited number of samples (Fig. 1c,d).

**Antitumor activity.** The efficacy analysis population consisted of all 13 patients who were treated with GC33. There were no patients whose best overall response was complete or partial response. Seven out of 13 patients (54%) showed stable disease (SD) (cohort 1, two out of four patients; cohort 2, two out of three patients; cohort 3, three out of six patients), the others showed PD. Three patients who had SD had received treatment for more than 5 months before progress (Fig. 2) and had a high GPC3 IHC score by method 1 (IHC score ≥7), similar to the previous phase I study. The median PFS for all patients was 2.1 months (1.6–3.5 months) (95% confidence interval). There was no significant difference in the best overall response or PFS among the three cohorts. Seven out of 11 patients (64%) evaluated showed a decrease in AFP (six patients with 20% reduction), and 11 out of 13 patients (85%) showed a decrease in DCP (six patients with 40% reduction) compared to their baseline levels (Fig. 3a,b). Moreover, computed tomography imaging showed shrinkage in one patient’s lung metastasis, in whom AFP and DCP levels were decreased (Fig. 3c). Two out of 13 patients were excluded from the AFP figure as their baseline AFP level was <10 ng/mL and deemed to be within the normal range.

### Discussion

This is the first study to evaluate the safety and tolerability of GC33 in Japanese patients. The results showed that GC33 was generally well tolerated, as no DLTs were reported in any cohort and an MTD was not reached. This safety profile is similar to that reported in the FIH study in which most AEs were mild to moderate and no DLTs were experienced.

The pharmacokinetics of GC33 showed that the clearance was larger and the half-life shorter at dose levels of 5.0 mg/kg than at dose levels of 10.0 mg/kg and 20.0 mg/kg suggesting non-linear elimination, which is similar to that found in the FIH. In general, antibody elimination is connected with non-specific linear elimination from the reticuloendothelial system and with antigen-specific non-linear elimination.

### Table 6. Pharmacokinetics parameters of GC33, a humanized antibody against glypican-3

| PK parameters for GC33 in serum were calculated by non-compartmental methods. Pharmacokinetic parameters estimated following the first GC33 infusion are: $t_{1/2}$, elimination half-life; CL, serum clearance; $C_{\text{max}}$, maximum drug concentration; $AUC_{\text{inf}}$, area under the concentration time curve from time 0 to infinity; and $V_{d,ss}$, volume of distribution at steady state. In addition, $C_{\text{trough}}$ (predose concentrations) at week 4 and 6 are summarized.

| (day) | CL (L/day) | $C_{\text{max}}$ (µg/mL) | $AUC_{\text{inf}}$ (day µg/mL) | $V_{d,ss}$ (L) | $C_{\text{trough}}$ (week 4; µg/mL) | $C_{\text{trough}}$ (week 6; µg/mL) |
|-------|------------|----------------|-----------------|---------------|----------------|----------------|
| 5 mg/kg |            |                |                 |               |               |               |
| No. of patients | 3 | 3 | 4 | 3 | 3 | 3 | 2 |
| Mean (min.–max.) | 4.17 | 0.566 | 141 | 584 | 3.13 | 61.7 | 61.2 |
| CV (%) | 29.9 | 27.9 | 22.3 | 29.2 | 20.0 | 32.0 | 63.0 |
| 10 mg/kg |            |                |                 |               |               |               |
| No. of patients | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Mean (min.–max.) | 7.01 | 0.373 | 258 | 2020 | 3.70 | 259 | 288 |
| CV (%) | 9.02 | 14.3 | 7.16 | 16.0 | 6.55 | 15.1 | 19.9 |
| 20 mg/kg |            |                |                 |               |               |               |
| No. of patients | 6 | 6 | 6 | 6 | 5 | 5 |
| Mean (min.–max.) | 6.13 | 0.510 | 395 | 2670 | 4.12 | 406 | 477 |
| CV (%) | 28.9 | 33.9 | 17.2 | 25.7 | 17.6 | 19.5 | 23.8 |
Although non-linear elimination at 5 mg/kg was observed, the elimination of GC33 may be attributable to non-specific linear elimination because a dose-proportional increase in AUC inf was also observed. In addition, similar to the results of the previous phase I study, a steady state appears to have been reached following four to six doses, and steady state trough concentrations of above 30 μg/mL were achieved in all patients in the 5–20 mg/kg groups. Preclinical data showed that a 5 mg/kg dose of GC33 had an effect on tumor growth inhibition, which was associated with trough serum antibody concentrations of 30 μg/mL.

In this study, no patient had a best response of complete or partial response, although three patients showed long SD of more than 5 months. Also in this study, 7 of 11 patients (64%) showed a decrease in AFP and 11 of 13 patients (85%) showed a decrease in DCP. In the FIH study of GC33, AFP values were either decreased or stabilized in patients who showed long SD. In the case of sorafenib, an early decrease of AFP has been postulated to be a good predictor of efficacy. In our study, however, no pharmacodynamic analysis was carried out as no post-treatment biopsy of the tumors was performed.

For the purposes of further developing a companion diagnostic test, we also used a fully automated IHC method and compared the results of that method with those of the method used previously in the FIH study. The results obtained from preclinical work and the FIH study of GC33 suggested that GPC3 expression would be a key parameter for antitumor activity of GC33. Both IHC methods used the same mouse mAb against human GPC3 and both produced similar staining patterns in
the majority of the specimens. Furthermore, the scoring system used in the previous method is based on the pattern and distribution of GC33 staining in both the membrane and cytoplasm, as is the new method for obtaining a clinical score that considers both membrane and cytoplasmic staining patterns and staining intensities. However, the scoring system of the previous method is somewhat more complex than that of the new method, in that the range of scoring is 0–14 in the previous method whereas in the fully automated method the clinical score range is 0–3. In this study, in addition to patients whose tumors had a high GPC3 IHC clinical score (2 and 3), there were patients whose tumor specimens had a GPC3 IHC clinical score of 1 but who showed long SD of more than 5 months and/or a decrease in tumor marker levels. Therefore, no definitive conclusions can be made at present, and a cut-off level for the GPC3 IHC clinical score to predict efficacy of GC33 has not been determined.

Recently, another therapeutic approach for HCC that targets GPC3 is being developed. A phase I clinical trial of a vaccine composed of two GPC3-derived peptides has been carried out in advanced HCC patients. The vaccination was well tolerated and induced GPC3-specific T cell responses that would be correlated with survival. As a therapeutic antibody, it can induce adaptive immunity against tumor-associated antigens and act to promote vaccine-like effects as well as direct activation of NK cells or macrophages to show Fc-mediated antitumor activity.

In conclusion, because no DLTs were reported for doses up to 20 mg/kg/week and because an MTD was not reached, GC33 is considered generally well tolerated in Japanese patients with advanced HCC. The correlation between antitumor activity and GPC3 expression is not clear, due to the limited sample sizes. Currently, a global, placebo-controlled, double-blind, multicenter phase II trial in previously treated patients with advanced HCC is being carried out that will provide more data with which to clarify the correlation between GC33 efficacy and GPC3 expression, as measured by the fully automated GPC3 IHC method in tumor specimens obtained by core needle biopsy.

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**Fig. 3.** Best changes from baseline in α-fetoprotein (AFP) (a) and des-γ-carboxy prothrombin (DCP) (b) in patients with advanced hepatocellular carcinoma treated with humanized antibody against glypican-3 (GC33). Best changes in AFP (11 patients) or DCP (13 patients) are shown as best percent changes from their baseline values. Two patients were excluded from AFP changes because their baseline AFP levels were within the normal range. (c) Computed tomography imaging of lung metastasis before and after (arrow) treatment with GC33. SD, Stable disease.
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Supporting Information

Additional supporting information may be found in the online version of this article:

Fig. S1. Immunohistochemical staining of glypican-3 (GPC3) in tumor biopsy specimens from patients with advanced hepatocellular carcinoma (n = 13) evaluated by two scoring methods, method 1 (score 0–14) (a) and method 2 (score 0–3) (b). Bar = 50 μm.