

## Peptide vaccines for hepatocellular carcinoma

Daisuke Nobuoka,<sup>1,2</sup> Toshiaki Yoshikawa,<sup>1</sup> Yu Sawada,<sup>1</sup> Toshiyoshi Fujiwara<sup>2</sup> and Tetsuya Nakatsura<sup>1,\*</sup>

<sup>1</sup>Division of Cancer Immunotherapy; Research Center for Innovative Oncology; National Cancer Center Hospital East; Kashiwa, Japan; <sup>2</sup>Department of Gastroenterological Surgery; Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences; Kita-ku, Okayama, Japan

**I**mmunotherapy is a potentially attractive treatment option for patients with hepatocellular carcinoma (HCC). We have reported that glypican-3 (GPC3) is an ideal target for anticancer immunotherapy against HCC because its expression is specifically detected in > 80% of HCCs, even during the early stages. Further, increased GPC3 expression is correlated with a poor prognosis. Based on results obtained from a preclinical study using mice, we conducted a phase I clinical trial using a GPC3-derived peptide vaccine. Phase I results showed that the GPC3-derived peptide vaccine was well tolerated. Furthermore, this was the first study to show that the frequency of peptide-specific cytotoxic T lymphocytes was correlated with overall survival in patients with HCC receiving a peptide vaccine. Next, we conducted a phase II clinical trial using the GPC3-derived peptide vaccine in patients with HCC after surgery or radiofrequency ablation (adjuvant setting). We are currently evaluating a third trial involving liver biopsies removed from patients with advanced HCC before and after GPC3-derived peptide vaccination. We expect that the results of these trials will result in future drug development.

### Introduction

Primary liver cancer, which predominantly consists of hepatocellular carcinoma (HCC), is an aggressive form of cancer that has a poor prognosis,<sup>1</sup> mainly due to the limited treatment options for advanced HCC. Although sorafenib, a targeted molecular therapeutic agent, has become the standard drug for first-line

systemic treatment,<sup>2,3</sup> no second-line treatment has been established for patients who do not respond to sorafenib treatment. Therefore, new treatment modalities are urgently required to prolong the survival of patients with advanced HCC.

Immunotherapy is a potentially attractive option for treating HCC, and the induction of tumor-specific reactions without autoimmunity is the ideal strategy. Recently, antigen-specific cancer immunotherapies against HCC, including peptide vaccines, dendritic cell vaccines and adoptive cell transfer therapies have attracted much interest. However, most previous clinical trials have not demonstrated adequate proof of immunotherapy efficacy for advanced HCC.<sup>4,5</sup> On the other hand, our clinical trial of glypican-3(GPC3)-derived peptide vaccine against advanced HCC demonstrated that the induction of peptide-specific cytotoxic T lymphocytes (CTLs) significantly correlated with patient survival.<sup>6</sup>

Here, we summarize recent advances and future directions of peptide vaccines for the treatment of HCC.

### Basic and Preclinical Studies Using GPC3 for the Treatment of HCC

GPC3 belongs to the glypican family of heparan sulfate proteoglycans, which are linked to the outer surface of the cell membrane through a glycosylphosphatidylinositol anchor.<sup>7</sup> We reported its identification as a carcinoembryonic antigen and an ideal target for anticancer immunotherapy against HCC due to its specific overexpression in HCC (> 80%).<sup>8-11</sup> Furthermore, we identified both HLA-A24(A\*24:02)

**Keywords:** peptide vaccine, glypican-3, clinical trial, hepatocellular carcinoma, cytotoxic T lymphocyte

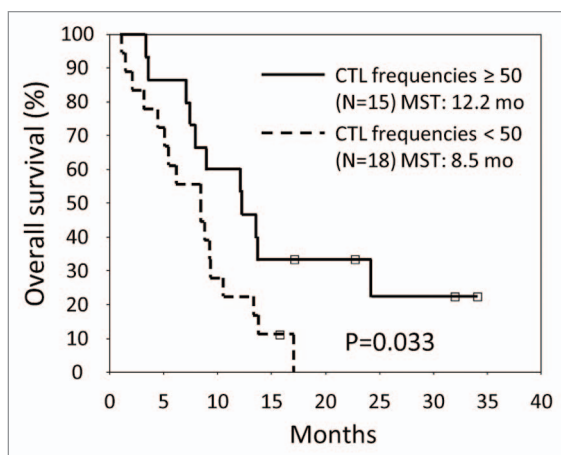
**Abbreviations:** HCC, hepatocellular carcinoma; GPC3, glypican-3; CTLs, cytotoxic T lymphocytes; IFA, incomplete Freund's adjuvant; PR, partial response; SD, stable disease; IFN- $\gamma$ , interferon- $\gamma$ ; ELISPOT, enzyme-linked immunospot; PBMCs, peripheral blood mononuclear cells

Submitted: 09/23/12

Accepted: 10/06/12

<http://dx.doi.org/10.4161/hv.22473>

\*Correspondence to: Tetsuya Nakatsura;  
Email: [tnakatsu@east.ncc.go.jp](mailto:tnakatsu@east.ncc.go.jp)



**Figure 1.** Kaplan-Meier curves for overall survival in patients undergoing GPC3-derived peptide vaccination. Patients with GPC3-specific CTL frequencies  $\geq 50$  had a longer survival than those with GPC3-specific CTL frequencies  $< 50$  ( $p = 0.033$ ). MST, median survival time. Adapted from Sawada et al.<sup>6</sup>

and H-2K<sup>d</sup>-restricted GPC3<sub>298–306</sub> (EYI LSL EEL), as well as HLA-A2(A\*02:01)-restricted GPC3<sub>144–152</sub> (FVG EFF TDV), as peptides that can induce GPC3-reactive CTLs without inducing autoimmunity.<sup>9,12</sup> Using binding assays, we confirmed that the HLA-A\*02:01-restricted GPC3<sub>144–152</sub> (FVG EFF TDV) peptide can also bind to HLA-A\*02:06 and HLA-A\*02:07. Using a mouse model to determine the optimal treatment schedule for the GPC3-derived peptide vaccines, we showed that incomplete Freund's adjuvant (IFA) is indispensable for peptide-based immunotherapy, and that the immunological effects of the peptide vaccine were dose-dependent.<sup>13</sup>

### Phase I Trial of the GPC3-Derived Peptide Vaccine for Advanced HCC

Based on the results from the preclinical studies, we conducted a phase I clinical trial of the GPC3-derived peptide vaccines in patients with advanced HCC.<sup>6</sup> This trial was a nonrandomized, open-label, phase I clinical trial with dose escalation (0.3–30 mg/patient) of GPC3 peptides. Peptides were emulsified with IFA and administered by intradermal injection for a total of three times on days 1, 15 and 29. In this trial, 33 patients with advanced HCC received peptide vaccines; 17 HLA-A24-positive patients were treated with the GPC3<sub>298–306</sub> (EYI LSL EEL) peptide and 16 HLA-A2-positive patients were

treated with the GPC3<sub>144–152</sub> (FVG EFF TDV) peptide.

The primary endpoint was peptide vaccination safety. The secondary endpoints were immunologic responses, clinical outcomes and determination of the optimal peptide dose for further clinical trials. The trial was registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR number, 000001395).

GPC3-derived peptide vaccination was well tolerated. One patient showed a partial response (PR) and 4 of the 19 patients with stable disease (SD) had tumor necrosis or regression that did not meet the criteria for a PR. The disease control rate (PR+SD) was 60.6% at 2 mo after the initiation of treatment. We also analyzed the GPC3-specific CTL frequency ex vivo using the interferon- $\gamma$  (IFN- $\gamma$ ) enzyme-linked immunospot (ELISPOT) assay. In most patients, GPC3 peptide-specific CTLs appeared in the peripheral blood. Furthermore, we established several GPC3<sub>144–152</sub> peptide-specific CTL clones with antigen-specific killing activity against tumor cells from peripheral blood mononuclear cells (PBMCs) obtained from patients vaccinated in this trial.<sup>14</sup> Tumor biopsies were performed in 7 patients to evaluate infiltration of CD8-positive T cells by immunohistochemical staining; marked infiltration of CD8-positive T cells into the tumor was detected after vaccination in 5 patients.

The GPC3 peptide-specific CTL frequency in peripheral blood was significantly correlated with overall survival in patients with HCC who received the peptide vaccination. In a multivariate analysis, the frequency of GPC3 peptide-specific CTLs was a predictive factor for overall survival. An analysis of all 33 patients showed that the median overall survival was 12.2 mo (95% CI, 6.5–18.0) in patients with high GPC3-specific CTL frequencies, as compared with 8.5 mo (95% CI, 3.7–13.1) in those with low GPC3-specific CTL frequencies ( $p = 0.033$ ) (Fig. 1). These observations suggest that GPC3-derived peptide vaccines represent a novel therapy for patients with HCC, with the potential to improve overall survival.

### Phase II Trial of the GPC3-Derived Peptide Vaccine for Treatment of HCC

In the phase I trial, the GPC3-derived peptide vaccines showed remarkable efficacy against advanced HCC. On the other hand, immunotherapy is expected to contribute toward cancer therapy, especially during the early stages or in recurrence prevention. Therefore, we conducted a phase II clinical trial of the GPC3-derived peptide vaccine in the adjuvant setting (UMIN-CTR: 000002614). Forty patients with initial HCC who had undergone surgery or radiofrequency ablation were enrolled in this phase II, open-label, single-arm trial. Ten 3-mg GPC3-derived peptide vaccinations were performed over 1 y following curative treatment. The primary endpoints were the 1- and 2-y recurrence rates. The secondary endpoints were immunological responses. Currently, the correlation between the time of recurrence and immunological responses is being analyzed.

In the phase I trial, we did not confirm whether the tumor-infiltrating lymphocytes detected after vaccination were GPC3-peptide-specific CTLs. Therefore, we are initiating a pilot study of liver biopsies removed from patients with advanced HCC before and after GPC3 peptide vaccination (UMIN-CTR: 000005093).

We expect that the results of these trials will provide a rationale for a larger

randomized clinical trial to determine the efficacy of the GPC3-derived peptide vaccine and to advance future drug development.

### Development of a Novel Strategy Using Peptide Vaccines for HCC Treatment

Although the peptide vaccine is a potentially attractive treatment modality, the antitumor effects of the peptide vaccine alone are not dramatic for advanced HCC. Therefore, we aim to develop combinational approaches<sup>15</sup> or strong antigen-specific immunotherapies, such as adoptive cell therapy following lymphodepletion.<sup>16</sup>

The density of endogenously presented antigen-derived peptides on tumor cells is generally sparse, resulting in the inability of antigen-specific CTLs to work effectively. To address this problem, we are performing intratumoral peptide injection in our ongoing study using mice. Intratumoral peptide injection enhances tumor cell antigenicity and may be a useful option for improving antigen-specific cancer immunotherapy against HCC. These translational studies involving innovative immunotherapeutic approaches will lead to the development of novel therapies for HCC.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

#### Acknowledgments

This work was supported in part by Health and Labor Science Research Grants for Research on Hepatitis, Clinical Research and Third Term Comprehensive Control Research from the Ministry of Health, Labor and Welfare, Japan.

#### References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; 55:74-108; PMID:15761078; <http://dx.doi.org/10.3322/canjclin.55.2.74>.
2. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al.; SHARP Investigators Study Group. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; 359:378-90; PMID:18650514; <http://dx.doi.org/10.1056/NEJMoa0708857>.
3. Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009; 10:25-34; PMID:19095497; [http://dx.doi.org/10.1016/S1470-2045\(08\)70285-7](http://dx.doi.org/10.1016/S1470-2045(08)70285-7).
4. Gretten TF, Forner A, Korangy F, N'Kontchou G, Barget N, Ayuso C, et al. A phase II open label trial evaluating safety and efficacy of a telomerase peptide vaccination in patients with advanced hepatocellular carcinoma. *BMC Cancer* 2010; 10:209; PMID:20478057; <http://dx.doi.org/10.1186/1471-2407-10-209>.
5. Olioso P, Giancola R, Di Riti M, Contento A, Accorsi P, Iacone A. Immunotherapy with cytokine induced killer cells in solid and hematopoietic tumours: a pilot clinical trial. *Hematol Oncol* 2009; 27:130-9; PMID:19294626; <http://dx.doi.org/10.1002/hon.886>.
6. Sawada Y, Yoshikawa T, Nobuoka D, Shirakawa H, Kuronuma T, Motomura Y, et al. Phase I trial of a glypican-3-derived peptide vaccine for advanced hepatocellular carcinoma: immunologic evidence and potential for improving overall survival. *Clin Cancer Res* 2012; 18:3686-96; PMID:22577059; <http://dx.doi.org/10.1158/1078-0432.CCR-11-3044>.
7. Filmus J. The contribution of in vivo manipulation of gene expression to the understanding of the function of glypicans. *Glycoconj J* 2002; 19:319-23; PMID:12975611; <http://dx.doi.org/10.1023/A:1025312819804>.
8. Nakatsura T, Yoshitake Y, Senju S, Monji M, Komori H, Motomura Y, et al. Glypican-3, overexpressed specifically in human hepatocellular carcinoma, is a novel tumor marker. *Biochem Biophys Res Commun* 2003; 306:16-25; PMID:12788060; [http://dx.doi.org/10.1016/S0006-291X\(03\)00908-2](http://dx.doi.org/10.1016/S0006-291X(03)00908-2).
9. Nakatsura T, Komori H, Kubo T, Yoshitake Y, Senju S, Katagiri T, et al. Mouse homologue of a novel human oncofetal antigen, glypican-3, evokes T-cell-mediated tumor rejection without autoimmune reactions in mice. *Clin Cancer Res* 2004; 10:8630-40; PMID:15623647; <http://dx.doi.org/10.1158/1078-0432.CCR-04-1177>.
10. Shirakawa H, Kuronuma T, Nishimura Y, Hasebe T, Nakano M, Gotohda N, et al. Glypican-3 is a useful diagnostic marker for a component of hepatocellular carcinoma in human liver cancer. *Int J Oncol* 2009; 34:649-56; PMID:19212669.
11. Shirakawa H, Suzuki H, Shimomura M, Kojima M, Gotohda N, Takahashi S, et al. Glypican-3 expression is correlated with poor prognosis in hepatocellular carcinoma. *Cancer Sci* 2009; 100:1403-7; PMID:19496787; <http://dx.doi.org/10.1111/j.1349-7006.2009.01206.x>.
12. Komori H, Nakatsura T, Senju S, Yoshitake Y, Motomura Y, Ikuta Y, et al. Identification of HLA-A2- or HLA-A24-restricted CTL epitopes possibly useful for glypican-3-specific immunotherapy of hepatocellular carcinoma. *Clin Cancer Res* 2006; 12:2689-97; PMID:16675560; <http://dx.doi.org/10.1158/1078-0432.CCR-05-2267>.
13. Motomura Y, Ikuta Y, Kuronuma T, Komori H, Ito M, Tsuchihara M, et al. HLA-A2 and -A24-restricted glypican-3-derived peptide vaccine induces specific CTLs: preclinical study using mice. *Int J Oncol* 2008; 32:985-90; PMID:18425324.
14. Yoshikawa T, Nakatsugawa M, Suzuki S, Shirakawa H, Nobuoka D, Sakemura N, et al. HLA-A2-restricted glypican-3 peptide-specific CTL clones induced by peptide vaccine show high avidity and antigen-specific killing activity against tumor cells. *Cancer Sci* 2011; 102:918-25; PMID:21281401; <http://dx.doi.org/10.1111/j.1349-7006.2011.01896.x>.
15. Suzuki S, Yoshikawa T, Hirotsawa T, Shibata K, Kikkawa F, Akatsuka Y, et al. Glypican-3 could be an effective target for immunotherapy combined with chemotherapy against ovarian clear cell carcinoma. *Cancer Sci* 2011; 102:1622-9; PMID:21668581; <http://dx.doi.org/10.1111/j.1349-7006.2011.02003.x>.
16. Rosenberg SA, Dudley ME. Adoptive cell therapy for the treatment of patients with metastatic melanoma. *Curr Opin Immunol* 2009; 21:233-40; PMID:19304471; <http://dx.doi.org/10.1016/j.coi.2009.03.002>.