Human Vaccines & Immunotherapeutics 9:5, 1069–1072; May 2013; © 2013 Landes Bioscience

Current status of immunotherapy for the treatment of biliary tract cancer

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Biliary tract cancer (BTC) is one of the most aggressive malignancies. Although various promising regimens of chemotherapeutic and/or molecular targeted agents have been developed, further treatment modalities, including immunotherapies, still remain to be established for refractory patients who are unresponsive to or relapse after currently available therapeutic options for BTC. Recently, several clinical trials of immunotherapies, including peptide-based vaccines and dendritic cell (DC)-based vaccines, have been reported with promising results. Here we summarize the data from phase I or phase II clinical trials of immunotherapies for BTC. In particular, we introduce our novel immunotherapeutic approach called personalized peptide vaccine (PPV), in which HLA-matched peptides were selected and administered based on the pre-existing host immunity before vaccination, for the treatment of advanced BTC. Further clinical trials would be recommended to prove clinical benefits of these novel immunotherapeutic approaches. Recently concomitant treatments, such as chemotherapies and immune checkpoint blockade, have been reported to enhance the therapeutic effects of cancer immunotherapies through multiple coordinated immune mechanisms. Additional therapies in combination with immunotherapies could produce synergistic effects in the treatment of advanced BTC.

Keywords: biliary tract cancer, peptide vaccine, dendritic cell vaccine, personalized vaccine, immunotherapy, clinical trial, biomarker

Abbreviations: BTC, biliary tract cancer; DC, dendritic cell; PPV, personalized peptide vaccine; GEM, gemcitabine; TAA, tumor-associated antigen; WT1, Wilms tumor gene 1; MUC1, mucin 1; SD, stable disease; PD, progressive disease; OS, overall survival; PFS, progression-free survival; HR, hazard ratio; CI, confidence interval

Submitted: 01/18/13

Accepted: 01/31/13

http://dx.doi.org/10.4161/hv.23844

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Introduction

Biliary tract cancer (BTC) is one of the most aggressive malignancies.[1,2] Only 10% of newly diagnosed patients present with early-stage disease and can be treated by a potentially radical excision of tumors. However, the remaining patients with unresectable, locally advanced and/or metastatic tumors show a poor prognosis, with a median survival of less than one year.[1,2] For advanced or recurrent BTC that are ineligible for surgery, various promising regimens of chemotherapeutic and/or molecular targeted agents have been studied.[1-4] For example, a combination of chemotherapeutic agents, gemcitabine (GEM) and cisplatin, has recently demonstrated a promising result in a randomized phase III trial in advanced BTC patients.[3] However, further treatment modalities still remain to be established for refractory patients who are unresponsive to or relapse after currently available therapeutic regimens for BTC.

Infiltration of different subsets of immune cells, including lymphocytes, macrophages, DCs and granulocytes, as well as immune-related microenvironments have been demonstrated to foster or inhibit tumor progression and/or metastatic potential in various types of cancers.[5,6] In BTC, higher frequencies of tumor-infiltrating $CD8^+$ cytotoxic T cells and/or $CD4^+$ T cells have been shown to be closely associated with favorable patient prognosis.[7,8] These findings have provided the rationale

Table 1. List of clinical trials of immunotherapies for biliary tract cancer

Type of vaccine	Disease condition	Phase of trial	Combined treatment	No. of patient	Clinical response	Median OS	Grade 3/4 toxicities (%)	Humoral response (%)	Cellular response (%)	Reference
MUC1 peptide	Advanced	I	(-)	3	PD 100%	NA	0	0	0	20
MUC1 peptide-loaded DCs	Adjuvant	I	(-)	2	No recurrence, 50%	NA	0	NA	NA	21
WT1 peptide	Advanced	I	GEM	16	SD 50%, PD 50%	288 d	0	NA	56	22
Tumor lysate-pulsed DCs plus activated T cell transfer	Adjuvant	I	(-)	36	PFS; 18.3M (vs 7.7M)	31.9M (vs 17.4M)	NA	NA	NA	24
Personalized peptide vaccine (PPV)	Advanced–(chemo-resistant)	II	chemotherapy	25	SD 80%, PD 20%	207 d	0	35	47	32

DCs, dendritic cells; GEM, Gemcitabine; OS, overall survival; PFS, progression-free survival; SD, stable disease; PD, progressive disease; M, months; NA, not available.

for further development of immunotherapies as a novel treatment modality against BTC. Here we summarize the current status of immunotherapies against BTC.

Recent Developments of Immunotherapeutic Approaches Against BTC

The field of cancer immunotherapy has drastically moved forward during these two decades since the first discovery of a tumor-associated antigen (TAA) recognized by cytotoxic T lymphocytes in 1991.[9-12] Advancement of molecular biological and immunological techniques has helped identify a large number of TAAs and peptide epitopes applicable as cancer immunotherapies.[13] For example, BTC has been reported to express a variety of TAAs, such as Wilms tumor gene 1 (WT1),[14] mucin 1 (MUC1)[15-17] and mutated K-RAS,[18,19] as potential targets for immunotherapies. Several clinical trials of immunotherapies targeting these molecules have recently been reported with promising results (**Table 1**).

Two groups employed a 100-mer peptide derived from MUC1 for the vaccination to BTC patients.[20,21] Yamamoto et al. reported a phase I clinical trial of vaccination with a 100-mer peptide consisting of the extracellular tandem repeat domain of MUC1 and incomplete Freund's adjuvant (Montanide ISA51) in patients with advanced pancreatic cancer (n = 6) or BTC (n = 3).[20] This study showed the safety of this vaccine formulation, but produced no substantial effects on antigen-specific immunological parameters or clinical outcomes in the vaccinated BTC patients. Lepisto et al. performed a Phase I/II clinical trial of vaccination with autologous DCs loaded with the 100-mer MUC1 peptide as an adjuvant therapy against pancreatic cancer (n = 10) or BTC (n = 2) patients following resection of their primary tumors.[21] The vaccine was well tolerated and no toxicity was observed. One of two patients with stage II intrahepatic cholangiocarcinoma had a long survival time without recurrence, although this patient showed no induction or boosting of MUC1 specific immune responses after vaccination.

Kaida et al. conducted an open-labeled, dose-escalation phase I trial of WT1 peptide vaccine combined with GEM to evaluate the safety and optimal immunological dose of this vaccine in HLA-A*0201, -A*0206, and/or -A*2402 positive patients with advanced pancreatic cancer (n = 9) or BTC (gallbladder carcinomas, n = 8; intrahepatic cholangiocarcinomas; n = 4; and extrahepatic cholangiocarcinomas, n = 4).[22] In this trial, 6 doses of GEM and 4 doses of WT1 peptide (1 or 3 mg) emulsified in incomplete Freund's adjuvant (Montanide ISA51) were administered. The adverse events were comparable to those with GEM alone, confirming the safety of this combination therapy. WT1-specific T cells in peptide-stimulated culture were detected by tetramer assay in 56% (9 of 16) of BTC patients. The clinical responses at 2 mo after vaccination showed 8 stable diseases (SD) and 8 progressive diseases (PD), and the median overall survival (OS) time for BTC was 288 d. Based on these promising data, the same group has started a phase I and randomized phase II study with WT1 peptide vaccine in combination with GEM and cisplatin for chemo-naive patients with unresectable or recurrent BTC.[23]

Shimizu et al. reported a phase I trial of autologous tumor lysate-pulsed DCs in combination with ex vivo CD3-activated T-cell transfer in an adjuvant setting for 36 postoperative patients with intrahepatic cholangiocarcinomas.[24] The median progression-free survival (PFS) and OS time of the patients receiving this adjuvant immunotherapy were 18.3 and 31.9 mo, respectively, which were significantly better than those of the control group receiving surgery alone [7.7 mo (p = 0.005) and 17.4 mo (0.022),

respectively]. In particular, patients with skin reactions (> 3 cm) at the vaccine site showed dramatically better prognosis. These results suggested a potential clinical benefit of this therapy for preventing recurrence and achieving long-term survival in intrahepatic cholangiocarcinoma patients, although a randomized trial will be needed for its confirmation.

Personalized Peptide Vaccine for BTC Patients

The anti-tumor immunity might differ widely among individual cancer patients, since the tumor cell characteristics and the host immune cell repertoires are reported to be quite diverse and heterogeneous among patients, even among those with identical HLA types and the same pathological types of cancer.[25-28] Considering the diversity of immune responses against heterogeneous tumor cells, tailored selections of vaccine antigens appropriate for individual patients could be a rational approach for developing effective cancer vaccines. We have developed a novel immunotherapeutic approach called personalized peptide vaccine (PPV), in which HLA-matched vaccine peptides are selected for vaccination based on the pre-existing host immunity from a list of vaccine candidates.[29,30] We have conducted a series of phase I and phase II clinical trials of PPV, which have shown better antigen-specific immune responses and promising clinical outcomes in patients with various types of advanced cancers.[31]

Recently, we conducted a phase II clinical trial of PPV for 25 chemo-resistant BTC patients (gallbladder carcinomas, n = 7; extrahepatic cholangiocarcinomas, n = 11; intrahepatic cholangiocarcinomas, n = 6; and periampullary carcinoma, n = 1) to evaluate the feasibility of this treatment and to identify potential biomarkers.[32] A maximum of 4 peptides were selected in consideration of the pre-existing host immunity before vaccination, as assessed by the titers of IgGs specific to each of the 31 different vaccine candidates [12 peptides for HLA-A2, 16 peptides for HLA-A24, 9 peptides for HLA-A3 supertypes (-A3, -A11, -A31, and -A33), and 4 peptides for HLA-A26], whose safety and immunological effects for other types of cancers were confirmed in previously conducted clinical studies. The selected peptides (3 mg/each peptide) were emulsified in incomplete Freund's adjuvant (Montanide ISA51) and subcutaneously administrated (weekly for 6 consecutive weeks and then bi-weekly thereafter) in combination with chemotherapeutic agents without severe adverse events. The median OS time was 207 d. In 10 patients who were radiologically evaluated before and after vaccination, the clinical response was classified as SD in 8 patients and PD in 2 patients. Humoral and T cell responses specific to the vaccine antigens were substantially induced in a subset of the vaccinated patients (35% and 47%, respectively). In the multivariate Cox regression analysis, lower IL-6 levels, higher albumin levels, and greater numbers of selected vaccine peptides were significantly favorable factors for OS [hazard ratio (HR) = 1.123, 95% confidence interval (CI) = 1.008 - 1.252, p = 0.035; HR = 0.158, 95% CI = 0.029 - 0.860, p = 0.033; HR = 0.258, 95% CI = 0.098 - 0.682, p = 0.006; respectively], suggesting that the evaluation of inflammation, nutritional status, and pre-existing antigen-specific immunity before vaccination could be useful for selecting appropriate BTC patients who would benefit from PPV. Based on this finding, we are planning an early phase clinical trial to reveal whether or not the blockade of IL-6-mediated inflammatory signaling with a humanized anti-IL-6 receptor monoclonal antibody, tocilizumab, would be beneficial for enhancing the immune and/or clinical responses after PPV in advanced BTC patients who show higher levels of plasma IL-6.[33,34]

Conclusions

Several clinical trials of immunotherapies for BTC have been reported with promising immunological responses and/or clinical outcomes. Further randomized trials would be essential to prove clinical benefits of these novel immunotherapies. Recently concomitant treatments, such as chemotherapies and immune checkpoint blockade, have been reported to enhance the therapeutic effects of cancer immunotherapies through multiple coordinated immune mechanisms, including activation of antigen-presenting cells or cytotoxic T cells and removal of suppressor cells.[35,36] Additional therapies in combination with immunotherapies could produce synergistic effects in the treatment of advanced BTC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This study was supported by the grants from the Regional Innovation Cluster Program of the Ministry of Education, Culture, Sports, Science and Technology of Japan, from the Project for Development of Innovative Research on Cancer Therapeutics (P-Direct) of the Ministry of Education, Culture, Sports, Science and Technology of Japan, and from the Sendai Kousei Hospital, Japan.

References

1. Yachimski P, Pratt DS. Cholangiocarcinoma: natural history, treatment, and strategies for surveillance in high-risk patients. J Clin Gastroenterol 2008; 42:178-90; PMID:18209589; http://dx.doi.org/10.1097/MCG.0b013e31806daf89.
2. Hezel AF, Deshpande V, Zhu AX. Genetics of biliary tract cancers and emerging targeted therapies. J Clin Oncol 2010; 28:3531-40; PMID:20547994; http://dx.doi.org/10.1200/JCO.2009.27.4787.
3. Valle J, Wasan H, Palmer DH, Cunningham D, Anthoney A, Maraveyas A, et al.; ABC-02 Trial Investigators. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. N Engl J Med 2010; 362:1273-81; PMID:20375404; http://dx.doi.org/10.1056/NEJMoa0908721.
4. Gruenberger B, Schueller J, Heubrandtner U, Wrba F, Tamandl D, Kaczirek K, et al. Cetuximab, gemcitabine, and oxaliplatin in patients with unresectable advanced or metastatic biliary tract cancer: a phase 2 study. Lancet Oncol 2010; 11:1142-8; PMID:21071270; http://dx.doi.org/10.1016/S1470-2045(10)70247-3.
5. Sasada T, Suekane S. Variation of tumor-infiltrating lymphocytes in human cancers: controversy on clinical significance. Immunotherapy 2011; 3:1235-51; PMID:21995574; http://dx.doi.org/10.2217/imt.11.106.
6. Senovilla L, Vacchelli E, Galon J, Adjemian S, Eggermont A, Fridman WH, et al. Trial watch: Prognostic and predictive value of the immune infiltrate in cancer. Oncoimmunology 2012; 1:1323-43; PMID:23243596; http://dx.doi.org/10.4161/onci.22009.
7. Nakakubo Y, Miyamoto M, Cho Y, Hida Y, Oshikiri T, Suzuoki M, et al. Clinical significance of immune cell infiltration within gallbladder cancer. Br J Cancer 2003; 89:1736-42; PMID:14583778; http://dx.doi.org/10.1038/sj.bjc.6601331.
8. Oshikiri T, Miyamoto M, Shichinohe T, Suzuoki M, Hiraoka K, Nakakubo Y, et al. Prognostic value of intratumoral CD8+ T lymphocyte in extrahepatic bile duct carcinoma as essential immune response. J Surg Oncol 2003; 84:224-8; PMID:14756433; http://dx.doi.org/10.1002/jso.10321.

9. Finn OJ. Cancer immunology. N Engl J Med 2008; 358:2704-15; PMID:18565863; http://dx.doi.org/10.1056/NEJMra072739.
10. Rosenberg SA. Cell transfer immunotherapy for metastatic solid cancer--what clinicians need to know. Nat Rev Clin Oncol 2011; 8:577-85; PMID:21808266; http://dx.doi.org/10.1038/nrclinonc.2011.116.
11. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. Nature 2011; 480:480-9; PMID:22193102; http://dx.doi.org/10.1038/nature10673.
12. Sasada T, Komatsu N, Suekane S, Yamada A, Noguchi M, Itoh K. Overcoming the hurdles of randomised clinical trials of therapeutic cancer vaccines. Eur J Cancer 2010; 46:1514-9; PMID:20413296; http://dx.doi.org/10.1016/j.ejca.2010.03.013.
13. Cheever MA, Allison JP, Ferris AS, Finn OJ, Hastings BM, Hecht TT, et al. The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. Clin Cancer Res 2009; 15:5323-37; PMID:19723653; http://dx.doi.org/10.1158/1078-0432.CCR-09-0737.
14. Nakatsuka S, Oji Y, Horiuchi T, Kanda T, Kitagawa M, Takeuchi T, et al. Immunohistochemical detection of WT1 protein in a variety of cancer cells. Mod Pathol 2006; 19:804-14; PMID:16547468.
15. Higashi M, Yonezawa S, Ho JJ, Tanaka S, Irimura T, Kim YS, et al. Expression of MUC1 and MUC2 mucin antigens in intrahepatic bile duct tumors: its relationship with a new morphological classification of cholangiocarcinoma. Hepatology 1999; 30:1347-55; PMID:10573510; http://dx.doi.org/10.1002/hep.510300609.
16. Matsumura N, Yamamoto M, Aruga A, Takasaki K, Nakano M. Correlation between expression of MUC1 core protein and outcome after surgery in mass-forming intrahepatic cholangiocarcinoma. Cancer 2002; 94:1770-6; PMID:11920540; http://dx.doi.org/10.1002/cncr.10398.
17. Mall AS, Tyler MG, Ho SB, Krige JE, Kahn D, Spearman W, et al. The expression of MUC mucin in cholangiocarcinoma. Pathol Res Pract 2010; 206:805-9; PMID:20947262; http://dx.doi.org/10.1016/j.prp.2010.08.004.
18. Ohashi K, Tstsumi M, Nakajima Y, Nakano H, Konishi Y. Ki-ras point mutations and proliferation activity in biliary tract carcinomas. Br J Cancer 1996; 74:930-5; PMID:8826860; http://dx.doi.org/10.1038/bjc.1996.459.
19. Tannapfel A, Sommerer F, Benicke M, Katalinic A, Uhlmann D, Witzigmann H, et al. Mutations of the BRAF gene in cholangiocarcinoma but not in hepatocellular carcinoma. Gut 2003; 52:706-12; PMID:12692057; http://dx.doi.org/10.1136/gut.52.5.706.
20. Yamamoto K, Ueno T, Kawaoka T, Hazama S, Fukui M, Suehiro Y, et al. MUC1 peptide vaccination in patients with advanced pancreas or biliary tract cancer. Anticancer Res 2005; 25:3575-9; PMID:16101182.
21. Lepisto AJ, Moser AJ, Zeh H, Lee K, Bartlett D, McKolanis JR, et al. A phase I/II study of a MUC1 peptide pulsed autologous dendritic cell vaccine as adjuvant therapy in patients with resected pancreatic and biliary tumors. Cancer Ther 2008; 6(B):955-64; PMID:19129927.
22. Kaida M, Morita-Hoshi Y, Soeda A, Wakeda T, Yamaki Y, Kojima Y, et al. Phase 1 trial of Wilms tumor 1 (WT1) peptide vaccine and gemcitabine combination therapy in patients with advanced pancreatic or biliary tract cancer. J Immunother 2011; 34:92-9; PMID:21150717; http://dx.doi.org/10.1097/CJI.0b013e3181fb65b9.
23. Okusaka T, Ueno M, Sato T, Heike Y. Possibility of immunotherapy for biliary tract cancer: how do we prove efficacy? Introduction to a current ongoing phase I and randomized phase II study to evaluate the efficacy and safety of adding Wilms tumor 1 peptide vaccine to gemcitabine and cisplatin for the treatment of advanced biliary tract cancer (WT-BT trial). J Hepatobiliary Pancreat Sci 2012; 19:314-8; PMID:22273718; http://dx.doi.org/10.1007/s00534-011-0495-1.
24. Shimizu K, Kotera Y, Aruga A, Takeshita N, Takasaki K, Yamamoto M. Clinical utilization of postoperative dendritic cell vaccine plus activated T-cell transfer in patients with intrahepatic cholangiocarcinoma. J Hepatobiliary Pancreat Sci 2012; 19:171-8; PMID:21874278; http://dx.doi.org/10.1007/s00534-011-0437-y.
25. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science 2011; 331:1565-70; PMID:21436444; http://dx.doi.org/10.1126/science.1203486.
26. Bei R, Scardino A. TAA polyepitope DNA-based vaccines: a potential tool for cancer therapy. J Biomed Biotechnol 2010; 2010:102758; PMID:20617190; http://dx.doi.org/10.1155/2010/102758.
27. Yewdell JW, Bennink JR. Immunodominance in major histocompatibility complex class I-restricted T lymphocyte responses. Annu Rev Immunol 1999; 17:51-88; PMID:10358753; http://dx.doi.org/10.1146/annurev.immunol.17.1.51.
28. Chen W, McCluskey J. Immunodominance and immunodomination: critical factors in developing effective CD8+ T-cell-based cancer vaccines. Adv Cancer Res 2006; 95:203-47; PMID:16860659; http://dx.doi.org/10.1016/S0065-230X(06)95006-4.
29. Itoh K, Yamada A. Personalized peptide vaccines: a new therapeutic modality for cancer. Cancer Sci 2006; 97:970-6; PMID:16984371; http://dx.doi.org/10.1111/j.1349-7006.2006.00272.x.
30. Sasada T, Noguchi M, Yamada A, Itoh K. Personalized peptide vaccination: a novel immunotherapeutic approach for advanced cancer. Hum Vaccin Immunother 2012; 8:1309-13; PMID:22894962; http://dx.doi.org/10.4161/hv.20988.
31. Noguchi M, Sasada T, Itoh K. Personalized peptide vaccination: a new approach for advanced cancer as therapeutic cancer vaccine. Cancer Immunol Immunother 2012; In Press; PMID:23197273; http://dx.doi.org/10.1007/s00262-012-1379-1.
32. Yoshitomi M, Yutani S, Matsueda S, Ioji T, Komatsu N, Shichijo S, et al. Personalized peptide vaccination for advanced biliary tract cancer: IL-6, nutritional status and pre-existing antigen-specific immunity as possible biomarkers for patient prognosis. Exp Ther Med 2012; 3:463-9; PMID:22969912.
33. Sansone P, Bromberg J. Targeting the interleukin-6/Jak/stat pathway in human malignancies. J Clin Oncol 2012; 30:1005-14; PMID:22355058; http://dx.doi.org/10.1200/JCO.2010.31.8907.
34. Nishimoto N, Kishimoto T. Interleukin 6: from bench to bedside. Nat Clin Pract Rheumatol 2006; 2:619-26; PMID:17075601; http://dx.doi.org/10.1038/ncprheum0338.
35. Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological aspects of cancer chemotherapy. Nat Rev Immunol 2008; 8:59-73; PMID:18097448; http://dx.doi.org/10.1038/nri2216.
36. Vanneman M, Dranoff G. Combining immunotherapy and targeted therapies in cancer treatment. Nat Rev Cancer 2012; 12:237-51; PMID:22437869; http://dx.doi.org/10.1038/nrc3237.