Vaccination of biliary tract cancer patients with four peptides derived from cancer-testis antigens

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Biliary tract cancer (BTC) is not a highly prominent neoplasm, but its incidence has gradually increased over the past two decades. Early-stage BTCs are difficult to detect because of the lack of overt symptoms, implying that the prognosis of BTC patients is often extremely poor. Nowadays, the therapeutic options for BTC are limited to surgery (in a subset of patients) and/or chemotherapy based on gemcitabine plus cisplatin. The development of new modalities to treat BTC is therefore urgently needed. By means of the cDNA microarray technology coupled with laser microdissection, we have recently been investigating anticancer vaccines based on cancer-testis antigen-derived peptides for the treatment of several tumors. In a recent study, four peptides that are overexpressed by most BTC cells were selected as potential targets for vaccination. In the corresponding Phase I clinical trial, patients were vaccinated with these peptides on a continuous basis until their disease had progressed, a time point at which we assessed the safety of the procedure as a primary endpoint as well as antigen-specific immune responses and clinical benefit as secondary endpoints (Fig. 1). We focused on the fluctuations of the disease in the course of long-term vaccination until tumor progression.

Nine patients bearing unresectable, chemotherapy-refractory, advanced BTCs (intrahepatic bile duct cancer, extrahepatic bile duct cancer or gallbladder cancer) were enrolled in our trial. HLA-A*2402-restricted epitopes derived from four distinct cancer-testis antigens, namely, lymphocyte antigen 6 complex locus K (LY6K; RYCNLEGPI), TTK protein kinase (TTK; SYRNEIAYL), insulin-like growth factor-II mRNA binding protein 3 (IMP3; KTVNELQNL) and DEP domain-containing 1 (DEPDC1; EYYELFVNI), were admixed with incomplete Freund’s adjuvant (IFA, also known as Montanide ISA51) and injected (as a standalone intervention) s.c. in doses of 0.5, 1, or 2 mg into 3 patients, once a week until the eighth vaccination and once or twice a week after the ninth vaccination, until disease progression (UMIN-CTR number 000003229). Adverse events were assessed based on the Common Terminology Criteria for Adverse Events (CTCAE) v3.0 and immune responses were monitored by enzyme-linked immunosorbent spot (ELISPOT) and multimer assays. Clinical benefits were observed in terms of tumor response, progression-free survival (PFS) and overall survival (OS). The vaccination was well-tolerated, and no grade 3–4 adverse events were observed throughout the study. Peptide-specific T-cell responses were observed in 7 out of 9 patients, and favorable clinical responses (tumor regression or disease stabilization) were observed in 6 out of 9 patients. Although patients were enrolled after the failure of chemotherapy, the median PFS and OS were 5.2 mo and 12.7 mo, respectively, which are comparable with those achieved by standard chemotherapy, i.e., 5.8 mo and 11.2 mo, respectively. Peptide-specific cytotoxic T-lymphocyte (CTL) responses were documented by means of interferon γ (IFNγ)-specific ELISPOT assays in 7 out of 9 patients and appeared to constitute a prognostic factor for both OS and PFS, similar to grade 2 local skin reactions at the vaccination site. The prognostic importance of peptide-specific CTL responses has been determined in other clinical trials. However, the ability of peptide-based vaccines to induce specific CTLs in vivo has been shown to largely depend on the nature of peptides. The four peptides used in our trial turned out to be very effective. In particular, LY6K and DEPDC1 stand out as very promising candidates for the induction of robust CTL responses. Although peptide-based vaccines constitute a promising immunotherapeutic approach against cancer, their clinical
efficacy is currently limited. In particular, the therapeutic potential of immunotherapy is often restrained by the generalized immunosuppression of cancer patients. An "immune score" is currently being discussed as a novel possible approach to classify cancer based on immunological parameters. 

In our clinical trial, a vaccine combining peptides derived from four distinct cancer-testis antigens, namely, LY6K, TTK, IMP3, and DEPDC1, was admixed with incomplete Freund’s adjuvant and administered s.c., leading to successful antigen presentation by dendritic cells. Dendritic cells are capable of inducing peptide-specific cytotoxic T lymphocyte (CTLs) responses that mediate antineoplastic effects in vivo.

**Figure 1.** Vaccination of biliary tract cancer patients with four peptides derived from cancer-testis antigens. In our clinical trial, a vaccine combining peptides derived from four distinct cancer-testis antigens, namely, LY6K, TTK, IMP3, and DEPDC1, was admixed with incomplete Freund’s adjuvant and administered s.c., leading to successful antigen presentation by dendritic cells. Dendritic cells are capable of inducing peptide-specific cytotoxic T lymphocyte (CTLs) responses that mediate antineoplastic effects in vivo.

In summary, our multi-peptide vaccine was well-tolerated and appeared to provide (at least some) clinical benefit to BTC patients. Our results support the use of long-term vaccination as a means to ameliorate the prognosis of advanced BTC patients and call for subsequent studies to assess the PFS and OS of BTC patients receiving our vaccine in a randomized setting or as a postoperative, adjuvant intervention.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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