

**An invited review following *the Soujinkai Fujiu Memorial Award*:****Development of the Novel Immunotherapies Based on the Analyses of Suppressive Immunity in Gastrointestinal Cancer***Shoichi Hazama*<sup>1,2</sup><sup>1</sup> Department of Surgery, Gastroenterological Center, Shunan Memorial Hospital, 1-10-1, Ikunoya-minami, Kudamatsu, Yamaguchi, 744-0033, Japan<sup>2</sup> Department of Gastroenterological, Breast and Endocrine Surgery, Yamaguchi University Graduate School of Medicine, 1-1-1 Minami-Kogushi, Ube, Yamaguchi, 755-8505, Japan

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**Abstract** Surgical resection is the most effective treatment for gastrointestinal cancer; however, a novel treatment is needed for unresectable cases. We have developed an immunotherapy for gastrointestinal cancers. In 1990, we devised a combination immunotherapy for hepatocellular carcinoma using interleukin-2, OK-432, Adriamycin, cyclophosphamide, and famotidine, which resulted in complete response in 4 out of 24 patients. Subsequently, we developed immune cell therapies for gastrointestinal cancers. Tumor antigen-specific immunity was first reported in 1991. We administered cancer vaccines using epitope peptides derived from oncoantigens for colorectal and pancreatic cancers. As a result, antigen-specific immunity was induced at a high frequency, and cases showing long-term antitumor effects were observed, whereas cases that were ineffective were also observed; therefore, we searched for the cause. A comprehensive analysis of lymphocytes, serum, and tumor tissues revealed the presence of many immunosuppressive factors. Therefore, we developed an immune adjuvant that induces cytotoxic T lymphocytes without fatigue markers and tumor antigen-specific peptides. This vaccine was administered preoperatively in 20 cases of hepatocellular carcinoma; 12 cases showed significant lymphocyte invasion into tumors and tumor necrosis in 6 cases, and these results suggested that hepatocellular carcinoma changed into a hot tumor. This immunotherapy is expected to be useful in combination with checkpoint inhibitors.

**Key words:** Immunotherapy, gastrointestinal cancers, suppressive immunity

**Introduction**

Gastrointestinal (GI) cancers are the most common malignancies worldwide, and their incidence and mortality are increasing every

year.<sup>1</sup> As 2025, surgical resection is still the most effective treatment for gastrointestinal cancer. Treatment with cytotoxic chemotherapy and molecular targeted drugs is used for unresectable and recurrent cases, but it rarely

leads to a cure.<sup>2</sup> Although the effect of immunotherapy is characterized by long-term persistence<sup>3</sup> and is considered to be curative in many cases, the response rate of immunotherapy is 0-20% depending on the carcinoma, and further elucidation of the immunosuppressive mechanism is awaited.<sup>4,6</sup> After my graduation from Yamaguchi University in 1986, we have been trying to develop novel immunotherapies in our Department of Surgery II (Department of Gastroenterological, Breast and Endocrine Surgery), Yamaguchi University. In this short article, the author would like to present our approach to overcome gastrointestinal cancers.

### **Combination immunotherapy including hepatic arterial infusion against hepatocellular carcinoma**

Since the emergence of immune checkpoint inhibitors, the concept of overcoming suppressive immunity has become well-known to many clinicians. Suppressive immunity in cancer immunotherapy has long been investigated, and the thymus and spleen cells of tumor-bearing hosts have been shown to possess immunosuppressor cells that regulate the immune response to tumor.<sup>7</sup> Hence, we believe that novel immunotherapies, including immune-activating agents and agents that eliminate suppressive immunity, are crucial. Adriamycin, which is a cytotoxic anticancer agent, was reported to have the immunomodulating activity in cancer immunotherapy, and to exert different effects on the cell-mediated lytic response and complement-dependent cytotoxicity.<sup>8,9</sup> Although cyclophosphamide (CY) is a potent immunosuppressive cytotoxic drug, under the proper conditions, it can potentiate immune responses as well. A lower dose of CY (300 mg/m<sup>2</sup>) affected immunopotential in patients with advanced metastatic cancer<sup>10</sup> and induced cell-mediated immunity to autologous melanoma cells and regression of metastases after treatment with a melanoma cell vaccine preceded by CY<sup>11</sup> through the depletion of suppressor-inducer T-cells.<sup>12,13</sup> Histamine has an immunosuppressive effect on the *in vitro* human delayed hypersensitivity reaction.<sup>14</sup> Famotidine is a histamine type 2-receptor antagonist that has

been reported to enhance cancer immunity<sup>15</sup> and augment cytotoxic activity by decreasing the population of suppressor T-cells.<sup>16</sup> Hence, histamine type 2-receptor antagonists attracted attention on cancer immunotherapy.<sup>17</sup> Based on these reports, we devised a combination immunotherapy for hepatocellular carcinoma (HCC) using cytokines and biological response modifiers, which received attention at the time.<sup>18</sup> It was characterized by the combination of intra hepatic arterial infusion of interleukin (IL)-2<sup>19-21</sup> and OK-432,<sup>22</sup> which activate anticancer immunity, as well as adriamycin, cyclophosphamide, and famotidine, which have been reported to control suppressive immunity, resulting in the activation of anticancer immunity. Complete response (CR) was observed in 4 of 24 patients and partial response (PR) in 3 patients.<sup>18</sup> This combination immunotherapy was not covered by insurance because it was unknown what worked because it used multiple off-label drugs simultaneously. This is a difficult part of complex therapy.

### **Immune-cell therapy against gastrointestinal cancers**

Adoptive immunotherapy (AIT) for cancer treatment was presented in the form of IL-2 generated-lymphokine-activated killer cells.<sup>23</sup> Antigen-specific cytotoxic T lymphocytes (CTL) were generated for the management of effector cells.<sup>24</sup>

In this study, we assessed the efficacy of CTLs against pancreatic ductal adenocarcinoma (PDAC). Patients with curatively resected PDAC received AIT with CTLs stimulated using MUC1-expressing human cell lines (MUC1-CTLs), and the results demonstrated that MUC1-CTLs might prevent liver metastasis.<sup>25</sup> For the next step, combination therapy using MUC1-CTL and gemcitabine was performed. A total of 43 patients who underwent radical pancreatectomy received postoperative treatment with MUC1-CTLs and gemcitabine. The disease-free survival (DFS) was 15.8 months, and overall survival (OS) was 24.7 months. Combination therapy may prevent liver metastasis and local recurrence.<sup>26</sup>

Dendritic cells (DCs) are antigen-presenting

cells specialized for the induction of a primary T-cell response.<sup>27</sup> For patients with PDAC, the clinical efficacy of immunotherapy using DCs transfected with MUC1 mRNA (MUC1-DCs) and MUC1-CTLs was evaluated in a pilot study with gemcitabine. Forty-two patients with unresectable or recurrent PDAC were enrolled, with a median survival time of 13.9 months, one patient achieved CR (2.4%), three patients had PR (7.1%), and 22 patients had stable disease (SD) (52.4%). MUC1-DCs and MUC1-CTLs plus gemcitabine might offer an effective treatment for PDAC.<sup>28</sup> Next target of DC therapy was HCC. A Phase I trial was conducted on the overexpression of heat-shock protein (HSP)70<sup>29</sup> in HCC. The DCs transfected with HSP70 mRNA (HSP70-DCs) were injected intradermally via electroporation. Patients were treated with HSP70-DCs in a 3-patient manner, at  $1 \times 10^7$ ,  $2 \times 10^7$ , and  $3 \times 10^7$ . CR without any recurrence was achieved in 2 patients and SD in 5 patients. This study demonstrated that HSP70-DCs therapy is effective in patients with HCC.<sup>30</sup> In the next phase I/II study, the safety and efficacy of this therapy as a postoperative adjuvant treatment after curative resection for HCC.<sup>31</sup> Patients ( $n = 45$ ) with resectable stage II-IVa HCC were registered and randomly assigned into two groups (DC group: 31 patients, control group: 14 patients) before surgery. In the group with HSP70-expressing HCC, the DFS of the DC group tended to be better ( $p = 0.090$ ), and the OS of the DC group was significantly longer ( $p = 0.003$ ) than control group.<sup>31</sup> Immune-cell therapy against gastrointestinal cancers might be useful for novel combination immunotherapy.

#### Peptide vaccine therapy using Oncoantigens derived epitope peptides

In 1991, a tumor antigen (MAGE-1) was identified, demonstrating that specific immunity to tumor antigens can be induced in the body of cancer patients to achieve antitumor effects.<sup>32,33</sup> To induce specific immunity to tumors, the applicant conducted basic research on immunogene therapy by introducing cytokine genes, such as IL-15<sup>34,35</sup> and IL-18<sup>36,37</sup> into tumor cells and showed that specific antitumor immunity was efficiently induced

in mice and caused tumor rejection. In cancer patients, epitope peptides derived from oncoantigens (proteins that are highly expressed specifically in tumors and are essential for tumor growth and survival)<sup>38</sup> have been used to induce tumor-specific immunity. At that time, we were conducting clinical research on peptide vaccine therapy for colorectal cancer<sup>39,40</sup> and pancreatic cancer<sup>41,42</sup> in collaboration with Professor Yusuke Nakamura of the Institute of Medical Science, University of Tokyo.<sup>43,44</sup> In phase I trials for colorectal cancer, antigen-specific immunity was frequently induced, and cases showed long-term antitumor effects.<sup>39</sup> The phase II study was conducted as a first-line treatment for unresectable colorectal cancer in combination with standard anticancer agents.<sup>40,45,46</sup> As a result, a significant increase in survival was obtained in cases with a preserved immune status.<sup>40,47,48</sup> On the other hand, the effect was not sufficient, and the reason why the effect was not noticeable was explored. Pretreatment blood cell components, serum, and tumor tissues from each preserved case were comprehensively analyzed. As a result, immunotherapy was found to be less effective in patients with a high inflammatory response, such as lymphocytes of less than 15%<sup>40</sup> and high serum levels of IL-6.<sup>49</sup> In addition, by the analysis of the patients with pancreatic cancer treated using vaccination, it was found that the effect was low in cases with many regulatory T cells (Treg), bone marrow-derived suppressor cells, and TIM3-positive cells in peripheral blood lymphocytes.<sup>50</sup> Next, we performed comprehensive analysis of tumor and serum proteins using the SOMAscan (SomaLogic, Inc., Boulder, CO, USA) to measure 1129 proteins related to the disease with colorectal cancer.<sup>51,52</sup> As the result, some inflammatory proteins were listed to relate the poor prognosis of treated colorectal cancer, Siglec-7 expression in intratumoral macrophages,<sup>52</sup> and serum lectin-like oxidized low-density lipoprotein receptor-1, C-C motif chemokine ligand 7,<sup>53</sup> and proteinase-3<sup>54</sup> had the significant impact for poor prognosis. Focusing on the serum as well as tumor tissue microRNA, serum microRNA-6826 was the predictive biomarker for poor prognosis of colorectal cancer.<sup>55</sup> In the tumor microenvironment, and

microRNA in cancer cells<sup>56,57</sup> as well as stromal cells,<sup>56,58</sup> may be a novel predictive biomarker for the efficacy of immunotherapy against metastatic colorectal cancer.

### Novel immunotherapy originating from Yamaguchi University

Based on the analysis described in the previous section, we searched for a combination of immune adjuvants that could control suppressive immunity and induce effective activation of lymphocytes through joint research with Nihon Electric Corporation (NEC), Cytlimic Corporation, and the Department of Immunology at Yamaguchi University (Professor Koji Tamada). As a result, we found that the combination of IMP321 (LAG-3 Fc),<sup>59</sup> which has a regulatory effect on the suppressive immunity (immune checkpoint), and Hiltonol (poly-ICLC),<sup>60,61</sup> a TLR-3 agonist, which has an immune-enhancing effect, induces cytotoxic T cells that do not express fatigue markers of PD1 and LAG-3.<sup>62</sup> Furthermore, NEC's peptide binding capacity prediction AI system was used to identify breakthrough HSP70<sup>29-31,63</sup> and GPC3-derived peptides<sup>64</sup> that bind to HLA-A\*2402, \*0201, and \*0206 (HSP70p and GPC3p), and a new clinical trial of a new combination immunotherapy was conducted at the Department of Gastroenterological, Breast and Endocrine Surgery (Prof. Hiroaki Nagano), Yamaguchi University. In a phase I study of IMP321 + Hiltonol + HSP70p + GPC3p in multiple gastrointestinal cancers (YNP01 study) that have failed with standard treatments, tumor markers were reduced in 10 of 17 patients, and tumor control (SD) was observed in 6 patients for more than 2 months, and CTL induction was observed in 15 of 17 patients (HSP70) and 16 patients (GPC3).<sup>64</sup> In a phase II study in which 20 patients with resectable hepatocellular carcinoma were administered IMP321 + Hiltonol + HSP70p + GPC3p prior to surgery (YCP02 study). Analysis of the resected specimens revealed significant lymphocyte infiltration in the tumor tissues of 12 patients. In addition, significant tumor necrosis was observed in six cases after administration, all of which were cases of high lymphocyte invasion.<sup>65</sup> This immunotherapy

could transform hepatocellular carcinoma, commonly referred to as a cold tumor, into a hot tumor. Hot tumors infiltrated by CD8+ T cells<sup>66-70</sup> and/or CD4+ T cells<sup>71,72</sup> have a good prognosis and respond well to immune checkpoint inhibitors,<sup>4,73</sup> as many studies have attempted to making hot tumor.<sup>74,75</sup> Hence, novel vaccines are expected to be highly effective when combined with immune checkpoint inhibitors.

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### Conflict of Interest

The author declares no conflict of interest.

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