Title

An invited review following *the Soujinkai Fujiu Memorial Award*:

Development of the Novel Immunotherapies Based on the Analyses of Suppressive Immunity in Gastrointestinal Cancer

A short running title

Novel Immunotherapies in Gastrointestinal Cancer

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Word count

1650 words

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.[1] As 2025, surgical resection is still the most effective treatment for gastrointestinal cancer. Treatment with cytocidal chemotherapy and molecular targeted drugs is used for unresectable and recurrent cases, but it rarely leads to a cure.[2] Although the effect of immunotherapy is characterized by long-term persistence[3] 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.[4-6] 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.[7] 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.[8, 9] 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^2) affected immunopotentiation in patients with advanced metastatic cancer[10] and induced cell-mediated immunity to autologous melanoma cells and regression of metastases after treatment with a

melanoma cell vaccine preceded by CY[11] through the depletion of suppressor-inducer T-cells.[12, 13] Histamine has an immunosuppressive effect on the in vitro human delayed hypersensitivity reaction.[14] Famotidine is a histamine type 2-receptor antagonist that has been reported to enhance cancer immunity[15] and augment cytotoxic activity by decreasing the population of suppressor T-cells.[16] Hence, histamine type 2-receptor antagonists attracted attention on cancer immunotherapy.[17] 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.[18] It was characterized by the combination of intra hepatic arterial infusion of interleukin (IL)-2[19-21] and OK-432,[22] 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.[18] 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.[23] Antigen-specific cytotoxic T lymphocytes (CTL) were generated for the management of effector cells.[24]

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.[25] 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.[26]

Dendritic cells (DCs) are antigen-presenting cells specialized for the induction of a primary T-cell response.[27] 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.[28] Next target of DC therapy was HCC. A Phase I trial was conducted on the overexpression of heat-shock protein (HSP)70[29] 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 1×10^7, 2×10^7, and 3×10^7. 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.[30] In the next phase I/II study, the safety and efficacy of this therapy as a postoperative adjuvant treatment after curative resection for HCC.[31] 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.[31] 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.[32, 33] To induce specific immunity to tumors, the applicant conducted basic research on immunogene therapy by introducing cytokine genes, such as IL-15[34, 35] and IL-18[36, 37] 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)[38] have been used to induce tumor-specific

123 immunity. At that time, we were conducting clinical research on peptide vaccine therapy for
124 colorectal cancer[39, 40] and pancreatic cancer[41, 42] in collaboration with Professor Yusuke Nakamura of
125 the Institute of Medical Science, University of Tokyo.[43, 44] In phase I trials for colorectal cancer,
126 antigen-specific immunity was frequently induced, and cases showed long-term antitumor effects.[39]
127 The phase II study was conducted as a first-line treatment for unresectable colorectal cancer in
128 combination with standard anticancer agents.[40, 45, 46] As a result, a significant increase in survival
129 was obtained in cases with a preserved immune status.[40, 47, 48] On the other hand, the effect was not
130 sufficient, and the reason why the effect was not noticeable was explored. Pretreatment blood cell
131 components, serum, and tumor tissues from each preserved case were comprehensively analyzed. As
132 a result, immunotherapy was found to be less effective in patients with a high inflammatory
133 response, such as lymphocytes of less than 15% [40] and high serum levels of IL-6.[49] In addition, by
134 the analysis of the patients with pancreatic cancer treated using vaccination, it was found that the
135 effect was low in cases with many regulatory T cells (Treg), bone marrow-derived suppressor cells,
136 and TIM3-positive cells in peripheral blood lymphocytes.[50] Next, we performed comprehensive
137 analysis of tumor and serum proteins using the SOMAscan (SomaLogic, Inc., Boulder, CO, USA) to
138 measure 1129 proteins related to the disease with colorectal cancer.[51, 52] As the result, some
139 inflammatory proteins were listed to relate the poor prognosis of treated colorectal cancer, Siglec-7
140 expression in intratumoral macrophages,[52] and serum lectin-like oxidized low-density lipoprotein
141 receptor-1, C-C motif chemokine ligand 7,[53] and proteinase-3[54] had the significant impact for poor
142 prognosis. Focusing on the serum as well as tumor tissue microRNA, serum microRNA-6826 was
143 the predictive biomarker for poor prognosis of colorectal cancer.[55] In the tumor microenvironment,
144 and microRNA in cancer cells[56, 57] as well as stromal cells,[56, 58] may be a novel predictive biomarker
145 for the efficacy of immunotherapy against metastatic colorectal cancer.
146
147 **Novel immunotherapy originating from Yamaguchi University**
148 Based on the analysis described in the previous section, we searched for a combination of immune
149 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),[59] which has a regulatory effect on the suppressive immunity (immune checkpoint), and Hiltonol (poly-ICLC),[60, 61] 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.[62] Furthermore, NEC's peptide binding capacity prediction AI system was used to identify breakthrough HSP70[29-31, 63] and GPC3-derived peptides[64] 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).[64] 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.[65] This immunotherapy could transform hepatocellular carcinoma, commonly referred to as a cold tumor, into a hot tumor. Hot tumors infiltrated by CD8+ T cells[66-70] and/or CD4+ T cells[71, 72] have a good prognosis and respond well to immune checkpoint inhibitors,[4, 73] as many studies have attempted to making hot tumor.[74, 75] Hence, novel vaccines are expected to be highly effective when combined with immune checkpoint inhibitors.

Acknowledgments

Thank you for receiving the Fujiu Prize and the chance for writing a related review. I would also like to express my gratitude to Professor Masaaki Oka, who has taught me since 1986. After graduation,

under the guidance of Professors Koichi Ishigami, Takashi Suzuki, Masaaki Oka, and Hiroaki Nagano, I was able to continue my research on cancer immunotherapy at Yamaguchi University until March 2022. I would like to express my heartfelt gratitude to the doctors in the Department of Gastroenterological, Breast and Endocrine Surgery and the research and administrative assistants for their full guidance and cooperation in these studies.

This study on the analysis of suppressive immunity was performed as a research program of the Project for Development of Innovative Research on Cancer Therapeutics (P-DIRECT; 11039020), The Japan Agency for Medical Research and Development (AMED; 15cm0106085h0005), and this study was supported in part by a grant for Leading Advanced Projects for Medical Innovation (LEAP; 16am0001006h0003) from AMED. The investigator-initiated clinical trial for pancreatic cancer using peptides vaccine was supported by The Health and Labour Sciences Research Grant from The Ministry of Health Labour and Welfare.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015 Mar 1;136(5):E359-86. doi: 10.1002/ijc.29210. Epub 2014 Oct 9.
2. Long J, Lin J, Wang A, Wu L, Zheng Y, Yang X, Wan X, Xu H, Chen S, Zhao H. PD-1/PD-L blockade in gastrointestinal cancers: lessons learned and the road toward precision immunotherapy. J Hematol Oncol. 2017 Aug 3;10(1):146. doi: 10.1186/s13045-017-0511-2.
3. Couzin-Frankel J. Breakthrough of the year 2013. Cancer immunotherapy. Science. 2013 Dec 20;342(6165):1432-3. doi: 10.1126/science.342.6165.1432.
4. Hazama S, Tamada K, Yamaguchi Y, Kawakami Y, Nagano H. Current status of

immunotherapy against gastrointestinal cancers and its biomarkers: Perspective for precision immunotherapy. Ann Gastroenterol Surg. 2018 Jun 22;2(4):289-303. doi: 10.1002/ags3.12180.

5. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollia GD, Gupta A, Wigginton JM, Sznol M. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012 Jun 28;366(26):2443-54. doi: 10.1056/NEJMoa1200690. Epub 2012 Jun 2.

6. Lipson EJ, Sharfman WH, Drake CG, Wollner I, Taube JM, Anders RA, Xu H, Yao S, Pons A, Chen L, Pardoll DM, Brahmer JR, Topalian SL. Durable cancer regression off-treatment and effective reinduction therapy with an anti-PD-1 antibody. Clin Cancer Res. 2013 Jan 15;19(2):462-8. doi: 10.1158/1078-0432.CCR-12-2625. Epub 2012 Nov 20.

7. Fujimoto S, Greene M, Sehon AH. Immunosuppressor T cells in tumor bearing host. Immunol Commun. 1975;4(3):201-17. doi: 10.3109/08820137409055774.

8. Tomazic V, Ehrke MJ, Mihich E. Augmentation of the development of immune responses of mice against allogeneic tumor cells after adriamycin treatment. Cancer Res. 1981 Sep;41(9 Pt 1):3370-6.

9. Arinaga S, Akiyoshi T, Tsuji H. Augmentation of the generation of cell-mediated cytotoxicity after a single dose of adriamycin in cancer patients. Cancer Res. 1986 Aug;46(8):4213-6.

10. Berd D, Maguire HC Jr, Mastrangelo MJ. Potentiation of human cell-mediated and humoral immunity by low-dose cyclophosphamide. Cancer Res. 1984 Nov;44(11):5439-43.

11. Berd D, Maguire HC Jr, Mastrangelo MJ. Induction of cell-mediated immunity to autologous melanoma cells and regression of metastases after treatment with a melanoma cell vaccine preceded by cyclophosphamide. Cancer Res. 1986 May;46(5):2572-7.

12. Berd D, Mastrangelo MJ. Effect of low dose cyclophosphamide on the immune system of cancer patients: depletion of CD4+, 2H4+ suppressor-inducer T-cells. Cancer Res. 1988 Mar

15;48(6):1671-5.

13. Kan S, Hazama S, Maeda K, Inoue Y, Homma S, Koido S, Okamoto M, Oka M. Suppressive effects of cyclophosphamide and gemcitabine on regulatory T-cell induction in vitro. Anticancer Res. 2012 Dec;32(12):5363-9.

14. Rigal D, Monier JC, Souweine G. The effects of histamine on leukocyte migration test in man. I. Demonstration of a LIF production inhibitor (LIF-PI). Cell Immunol. 1979 Sep 1;46(2):360-72. doi: 10.1016/0008-8749(79)90423-4

15. Smith T. Histamine type 2-receptor antagonists and cancer immunotherapy. Compr Ther. 1990 Jan;16(1):8-13.

16. Tsunoda T, Tanimura H, Yamaue H, Iwahashi M, Tani M, Tamai M, Arii K, Noguchi K. In vitro augmentation of the cytotoxic activity of peripheral blood mononuclear cells and tumor-infiltrating lymphocytes by famotidine in cancer patients. Int J Immunopharmacol. 1992 Jan;14(1):75-81. doi: 10.1016/0192-0561(92)90107-v.

17. Rizzo A, Cusmai A, Giovannelli F, Acquafredda S, Rinaldi L, Misino A, Montagna ES, Ungaro V, Lorusso M, Palmiotti G. Impact of Proton Pump Inhibitors and Histamine-2-Receptor Antagonists on Non-Small Cell Lung Cancer Immunotherapy: A Systematic Review and Meta-Analysis. Cancers (Basel). 2022 Mar 9;14(6):1404. doi: 10.3390/cancers14061404.

18. ka M, Hazama S, Yoshino S, Shimoda K, Suzuki M, Shimizu R, Yano K, Nishida M, Suzuki T. Intraarterial combined immunochemotherapy for unresectable hepatocellular carcinoma: preliminary results. Cancer Immunol Immunother. 1994 Mar;38(3):194-200. doi: 10.1007/BF01525641.

19. Rayner AA, Grimm EA, Lotze MT, Chu EW, Rosenberg SA. Lymphokine-activated killer (LAK) cells. Analysis of factors relevant to the immunotherapy of human cancer. Cancer. 1985 Mar 15;55(6):1327-33. doi: 10.1002/1097-0142(19850315)55:6<1327::aid-cncr2820550628>3.0.co;2-o.

20. Wadamori K, Oka M, Tokuda N, Fujikura Y, Hazama S, Fukumoto T, Suzuki T. Influence of continuous interleukin-2 administration via the portal vein on liver regeneration following

partial hepatectomy in rats. Hepatology. 1996 Jun;23(6):1578-83. doi: 10.1053/jhep.1996.v23.pm0008675180.

21. Mitchell MS, Kempf RA, Harel W, Shau H, Boswell WD, Lind S, Bradley EC. Effectiveness and tolerability of low-dose cyclophosphamide and low-dose intravenous interleukin-2 in disseminated melanoma [corrected]. J Clin Oncol. 1988 Mar;6(3):409-24. doi: 10.1200/JCO.1988.6.3.409. Erratum in: J Clin Oncol 1988 Jun;6(6):1067.

22. Oba MS, Teramukai S, Ohashi Y, Ogawa K, Maehara Y, Sakamoto J. The efficacy of adjuvant immunochemotherapy with OK-432 after curative resection of gastric cancer: an individual patient data meta-analysis of randomized controlled trials. Gastric Cancer. 2016 Apr;19(2):616-624. doi: 10.1007/s10120-015-0489-9. Epub 2015 Mar 25.

23. Mulé JJ, Shu S, Schwarz SL, Rosenberg SA. Adoptive immunotherapy of established pulmonary metastases with LAK cells and recombinant interleukin-2. Science. 1984 Sep 28;225(4669):1487-9. doi: 10.1126/science.6332379.

24. Kaufmann Y, Moscovitch M, Robb RJ, Rosenberg SA, Berke G. Antigen/mitogen induced cytolytic activity and IL-2 secretion in memory-like CTL-hybridomas. Adv Exp Med Biol. 1985;184:535-50. doi: 10.1007/978-1-4684-8326-0_35.

25. Kawaoka T, Oka M, Takashima M, Ueno T, Yamamoto K, Yahara N, Yoshino S, Hazama S. Adoptive immunotherapy for pancreatic cancer: cytotoxic T lymphocytes stimulated by the MUC1-expressing human pancreatic cancer cell line YPK-1. Oncol Rep. 2008 Jul;20(1):155-63.

26. Matsui H, Hazama S, Sakamoto K, Shindo Y, Kanekiyo S, Nakashima M, Matsukuma S, Tokuhisa Y, Iida M, Suzuki N, Yoshimura K, Takeda S, Ueno T, Yoshino S, Oka M, Nagano H. Postoperative Adjuvant Therapy for Resectable Pancreatic Cancer With Gemcitabine and Adoptive Immunotherapy. Pancreas. 2017 Sep;46(8):994-1002. doi: 10.1097/MPA.0000000000000880.

27. Kondo H, Hazama S, Kawaoka T, Yoshino S, Yoshida S, Tokuno K, Takashima M, Ueno T, Hinoda Y, Oka M. Adoptive immunotherapy for pancreatic cancer using MUC1 peptide-

pulsed dendritic cells and activated T lymphocytes. Anticancer Res. 2008 Jan-Feb;28(1B):379-87.

28. Shindo Y, Hazama S, Maeda Y, Matsui H, Iida M, Suzuki N, Yoshimura K, Ueno T, Yoshino S, Sakai K, Suehiro Y, Yamasaki T, Hinoda Y, Oka M. Adoptive immunotherapy with MUC1-mRNA transfected dendritic cells and cytotoxic lymphocytes plus gemcitabine for unresectable pancreatic cancer. J Transl Med. 2014 Jun 19;12:175. doi: 10.1186/1479-5876-12-175

29. Yoshida S, Hazama S, Tokuno K, Sakamoto K, Takashima M, Tamesa T, Torigoe T, Sato N, Oka M. Concomitant overexpression of heat-shock protein 70 and HLA class-I in hepatitis C virus-related hepatocellular carcinoma. Anticancer Res. 2009 Feb;29(2):539-44.

30. Maeda Y, Yoshimura K, Matsui H, Shindo Y, Tamesa T, Tokumitsu Y, Hashimoto N, Tokuhisa Y, Sakamoto K, Sakai K, Suehiro Y, Hinoda Y, Tamada K, Yoshino S, Hazama S, Oka M. Dendritic cells transfected with heat-shock protein 70 messenger RNA for patients with hepatitis C virus-related hepatocellular carcinoma: a phase 1 dose escalation clinical trial. Cancer Immunol Immunother. 2015 Aug;64(8):1047-56. doi: 10.1007/s00262-015-1709-1. Epub 2015 May 16.

31. Matsui HM, Hazama S, Nakajima M, Xu M, Matsukuma S, Tokumitsu Y, Shindo Y, Tomochika S, Yoshida S, Iida M, Suzuki N, Takeda S, Yoshino S, Ueno T, Oka M, Nagano H. Novel adjuvant dendritic cell therapy with transfection of heat-shock protein 70 messenger RNA for patients with hepatocellular carcinoma: a phase I/II prospective randomized controlled clinical trial. Cancer Immunol Immunother. 2021 Apr;70(4):945-957. doi: 10.1007/s00262-020-02737-y. Epub 2020 Oct 19. Erratum in: Cancer Immunol Immunother. 2021 Apr;70(4):959. doi: 10.1007/s00262-020-02819-x.

32. Traversari C, van der Bruggen P, Luescher IF, Lurquin C, Chomez P, Van Pel A, De Plaen E, Amar-Costesec A, Boon T. A nonapeptide encoded by human gene MAGE-1 is recognized on HLA-A1 by cytolytic T lymphocytes directed against tumor antigen MZ2-E. J Exp Med. 1992 Nov 1;176(5):1453-7. doi: 10.1084/jem.176.5.1453.

33. Brasseur F, Marchand M, Vanwijck R, Hérin M, Lethé B, Chomez P, Boon T. Human gene MAGE-1, which codes for a tumor-rejection antigen, is expressed by some breast tumors. Int J Cancer. 1992 Nov 11;52(5):839-41. doi: 10.1002/ijc.2910520528.

34. Hazama S, Noma T, Wang F, Iizuka N, Ogura Y, Yoshimura K, Inoguchi E, Hakozaki M, Hirose K, Suzuki T, Oka M. Tumour cells engineered to secrete interleukin-15 augment anti-tumour immune responses in vivo. Br J Cancer. 1999 Jul;80(9):1420-6. doi: 10.1038/sj.bjc.6690538.

35. Araki A, Hazama S, Yoshimura K, Yoshino S, Iizuka N, Oka M. Tumor secreting high levels of IL-15 induces specific immunity to low immunogenic colon adenocarcinoma via CD8+ T cells. Int J Mol Med. 2004 Oct;14(4):571-6.

36. Yoshimura K, Hazama S, Iizuka N, Yoshino S, Yamamoto K, Muraguchi M, Ohmoto Y, Noma T, Oka M. Successful immunogene therapy using colon cancer cells (colon 26) transfected with plasmid vector containing mature interleukin-18 cDNA and the Igkappa leader sequence. Cancer Gene Ther. 2001 Jan;8(1):9-16. doi: 10.1038/sj.cgt.7700277.

37. Higashi K, Hazama S, Araki A, Yoshimura K, Iizuka N, Yoshino S, Noma T, Oka M. A novel cancer vaccine strategy with combined IL-18 and HSV-TK gene therapy driven by the hTERT promoter in a murine colorectal cancer model. Int J Oncol. 2014 Oct;45(4):1412-20. doi: 10.3892/ijo.2014.2557. Epub 2014 Jul 22.

38. Chiarle R, Martinengo C, Mastini C, Ambrogio C, D'Escamard V, Forni G, Inghirami G. The anaplastic lymphoma kinase is an effective oncoantigen for lymphoma vaccination. Nat Med. 2008 Jun;14(6):676-80. doi: 10.1038/nm1769. Epub 2008 May 11.

39. Hazama S, Nakamura Y, Takenouchi H, Suzuki N, Tsunedomi R, Inoue Y, Tokuhisa Y, Iizuka N, Yoshino S, Takeda K, Shinozaki H, Kamiya A, Furukawa H, Oka M. A phase I study of combination vaccine treatment of five therapeutic epitope-peptides for metastatic colorectal cancer; safety, immunological response, and clinical outcome. J Transl Med. 2014 Mar 10;12:63. doi: 10.1186/1479-5876-12-63.

40. Hazama S, Nakamura Y, Tanaka H, Hirakawa K, Tahara K, Shimizu R, Ozasa H, Etoh R,

Sugiura F, Okuno K, Furuya T, Nishimura T, Sakata K, Yoshimatsu K, Takenouchi H, Tsunedomi R, Inoue Y, Kanekiyo S, Shindo Y, Suzuki N, Yoshino S, Shinozaki H, Kamiya A, Furukawa H, Yamanaka T, Fujita T, Kawakami Y, Oka M. A phase II study of five peptides combination with oxaliplatin-based chemotherapy as a first-line therapy for advanced colorectal cancer (FXV study). J Transl Med. 2014 Apr 30;12:108. doi: 10.1186/1479-5876-12-108.

41. Suzuki N, Hazama S, Ueno T, Matsui H, Shindo Y, Iida M, Yoshimura K, Yoshino S, Takeda K, Oka M. A phase I clinical trial of vaccination with KIF20A-derived peptide in combination with gemcitabine for patients with advanced pancreatic cancer. J Immunother. 2014 Jan;37(1):36-42. doi: 10.1097/CJI.0000000000000012.

42. Suzuki N, Hazama S, Iguchi H, Uesugi K, Tanaka H, Hirakawa K, Aruga A, Hatori T, Ishizaki H, Umeda Y, Fujiwara T, Ikemoto T, Shimada M, Yoshimatsu K, Shimizu R, Hayashi H, Sakata K, Takenouchi H, Matsui H, Shindo Y, Iida M, Koki Y, Arima H, Furukawa H, Ueno T, Yoshino S, Nakamura Y, Oka M, Nagano H. Phase II clinical trial of peptide cocktail therapy for patients with advanced pancreatic cancer: VENUS-PC study. Cancer Sci. 2017 Jan;108(1):73-80. doi: 10.1111/cas.13113. Epub 2016 Dec 19

43. Yagyu R, Furukawa Y, Lin YM, Shimokawa T, Yamamura T, Nakamura Y. A novel oncoprotein RNF43 functions in an autocrine manner in colorectal cancer. Int J Oncol. 2004 Nov;25(5):1343-8.

44. Shimokawa T, Matsushima S, Tsunoda T, Tahara H, Nakamura Y, Furukawa Y. Identification of TOMM34, which shows elevated expression in the majority of human colon cancers, as a novel drug target. Int J Oncol. 2006 Aug;29(2):381-6.

45. Tokuno K, Hazama S, Yoshino S, Yoshida S, Oka M. Increased prevalence of regulatory T-cells in the peripheral blood of patients with gastrointestinal cancer. Anticancer Res. 2009 May;29(5):1527-32.

46. Maeda K, Hazama S, Tokuno K, Kan S, Maeda Y, Watanabe Y, Kamei R, Shindo Y, Maeda N, Yoshimura K, Yoshino S, Oka M. Impact of chemotherapy for colorectal cancer on

regulatory T-cells and tumor immunity. Anticancer Res. 2011 Dec;31(12):4569-74.

47. Kitahara M, Hazama S, Tsunedomi R, Takenouchi H, Kanekiyo S, Inoue Y, Nakajima M, Tomochika S, Tokuhisa Y, Iida M, Sakamoto K, Suzuki N, Takeda S, Ueno T, Yamamoto S, Yoshino S, Nagano H. Prediction of the efficacy of immunotherapy by measuring the integrity of cell-free DNA in plasma in colorectal cancer. Cancer Sci. 2016 Dec;107(12):1825-1829. doi: 10.1111/cas.13085. Epub 2016 Dec 18.

48. Kanekiyo S, Hazama S, Takenouchi H, Nakajima M, Shindo Y, Matsui H, Tokumitsu Y, Tomochika S, Tsunedomi R, Tokuhisa Y, Iida M, Sakamoto K, Suzuki N, Takeda S, Yamamoto S, Yoshino S, Okuno K, Udaka K, Kawakami Y, Matsueda S, Ito K, Nagano H. IgG response to MHC class I epitope peptides is a quantitative predictive biomarker in the early course of treatment of colorectal cancer using therapeutic peptides. Oncol Rep. 2018 May;39(5):2385-2392. doi: 10.3892/or.2018.6288. Epub 2018 Mar 1.

49. Hazama S, Takenouchi H, Tsunedomi R, Iida M, Suzuki N, Iizuka N, Inoue Y, Sakamoto K, Nakao M, Shindo Y, Kanekiyo S, Tokumitsu Y, Yoshimura K, Maeda N, Maeda K, Maeda Y, Matsui H, Yoshino S, Nakamura Y, Fujita Y, Hamamoto Y, Okamoto M, Fujita T, Kawakami Y, Oka M. Predictive biomarkers for the outcome of vaccination of five therapeutic epitope peptides for colorectal cancer. Anticancer Res. 2014 Aug;34(8):4201-5.

50. Shindo Y, Hazama S, Suzuki N, Iguchi H, Uesugi K, Tanaka H, Aruga A, Hatori T, Ishizaki H, Umeda Y, Fujiwara T, Ikemoto T, Shimada M, Yoshimatsu K, Takenouchi H, Matsui H, Kanekiyo S, Iida M, Koki Y, Arima H, Furukawa H, Ueno T, Yoshino S, Fujita T, Kawakami Y, Nakamura Y, Oka M, Nagano H. Predictive biomarkers for the efficacy of peptide vaccine treatment: based on the results of a phase II study on advanced pancreatic cancer. J Exp Clin Cancer Res. 2017 Feb 28;36(1):36. doi: 10.1186/s13046-017-0509-1.

51. Nakashima-Nakasuga C, Hazama S, Suzuki N, Nakagami Y, Xu M, Yoshida S, Tomochika S, Fujiwara N, Matsukuma S, Matsui H, Tokumitsu Y, Kanekiyo S, Shindo Y, Maeda N, Tsunedomi R, Iida M, Takeda S, Yoshino S, Ueno T, Hamamoto Y, Ogihara H, Hoshii Y, Nagano H. Serum LOX-1 is a novel prognostic biomarker of colorectal cancer. Int J Clin

Oncol. 2020 Jul;25(7):1308-1317. doi: 10.1007/s10147-020-01673-2. Epub 2020 Apr 11.

52. Yamada K, Hazama S, Suzuki N, Xu M, Nakagami Y, Fujiwara N, Tsunedomi R, Yoshida S, Tomochika S, Matsukuma S, Matsui H, Tokumitsu Y, Kanekiyo S, Shindo Y, Watanabe Y, Iida M, Takeda S, Ioka T, Ueno T, Ogihara H, Hamamoto Y, Hoshii Y, Kawano H, Fujita T, Kawakami Y, Nagano H. Siglec-7 is a predictive biomarker for the efficacy of cancer vaccination against metastatic colorectal cancer. Oncol Lett. 2021 Jan;21(1):10. doi: 10.3892/ol.2020.12271. Epub 2020 Nov 3.

53. Chidimatsu H, Tsunedomi R, Nakagami Y, Xu M, Nakajima M, Nakashima-Nakasuga C, Tomochika S, Yoshida S, Suzuki N, Watanabe Y, Matsui H, Shindo Y, Tokumitsu Y, Iida M, Takeda S, Ioka T, Ueno T, Tanabe T, Hoshii Y, Hazama S, Nagano H. Serum CCL7 Is a Novel Prognostic Biomarker of Metastatic Colorectal Cancer. Anticancer Res. 2023 Jan;43(1):105-114. doi: 10.21873/anticanres.16139.

54. Furuya K, Nakajima M, Tsunedomi R, Nakagami Y, Xu M, Matsui H, Tokumitsu Y, Shindo Y, Watanabe Y, Tomochika S, Maeda N, Iida M, Suzuki N, Takeda S, Hazama S, Ioka T, Hoshii Y, Ueno T, Nagano H. High serum proteinase-3 levels predict poor progression-free survival and lower efficacy of bevacizumab in metastatic colorectal cancer. BMC Cancer. 2024 Feb 2;24(1):165. doi: 10.1186/s12885-024-11924-4.

55. Kijima T, Hazama S, Tsunedomi R, Tanaka H, Takenouchi H, Kanekiyo S, Inoue Y, Nakashima M, Iida M, Sakamoto K, Suzuki N, Takeda S, Ueno T, Yamamoto S, Yoshino S, Okuno K, Nagano H. MicroRNA-6826 and -6875 in plasma are valuable non-invasive biomarkers that predict the efficacy of vaccine treatment against metastatic colorectal cancer. Oncol Rep. 2017 Jan;37(1):23-30. doi: 10.3892/or.2016.5267. Epub 2016 Nov 22.

56. Tanaka H, Hazama S, Iida M, Tsunedomi R, Takenouchi H, Nakajima M, Tokumitsu Y, Kanekiyo S, Shindo Y, Tomochika S, Tokuhisa Y, Sakamoto K, Suzuki N, Takeda S, Yamamoto S, Yoshino S, Ueno T, Hamamoto Y, Fujita Y, Tanaka H, Tahara K, Shimizu R, Okuno K, Fujita K, Kuroda M, Nakamura Y, Nagano H. miR-125b-1 and miR-378a are predictive biomarkers for the efficacy of vaccine treatment against colorectal cancer. Cancer

Sci. 2017 Nov;108(11):2229-2238. doi: 10.1111/cas.13390. Epub 2017 Sep 22.

57. Shindo Y, Hazama S, Nakamura Y, Inoue Y, Kanekiyo S, Suzuki N, Takenouchi H, Tsunedomi R, Nakajima M, Ueno T, Takeda S, Yoshino S, Okuno K, Fujita Y, Hamamoto Y, Kawakami Y, Oka M, Nagano H. miR-196b, miR-378a and miR-486 are predictive biomarkers for the efficacy of vaccine treatment in colorectal cancer. Oncol Lett. 2017 Aug;14(2):1355-1362. doi: 10.3892/ol.2017.6303. Epub 2017 Jun 2.

58. Iida M, Hazama S, Tsunedomi R, Tanaka H, Takenouchi H, Kanekiyo S, Tokumitsu Y, Tomochika S, Tokuhisa Y, Sakamoto K, Suzuki N, Takeda S, Ueno T, Yamamoto S, Yoshino S, Fujita K, Kuroda M, Nagano H. Overexpression of miR-221 and miR-222 in the cancer stroma is associated with malignant potential in colorectal cancer. Oncol Rep. 2018 Sep;40(3):1621-1631. doi: 10.3892/or.2018.6575. Epub 2018 Jul 13.

59. Brignone C, Escudier B, Grygar C, Marcu M, Triebel F. A phase I pharmacokinetic and biological correlative study of IMP321, a novel MHC class II agonist, in patients with advanced renal cell carcinoma. Clin Cancer Res. 2009 Oct 1;15(19):6225-31. doi: 10.1158/1078-0432.CCR-09-0068. Epub 2009 Sep 15.

60. Levine AS, Sivulich M, Wiernik PH, Levy HB. Initial clinical trials in cancer patients of polyriboinosinic-polyribocytidylic acid stabilized with poly-L-lysine, in carboxymethylcellulose [poly(ICLC)], a highly effective interferon inducer. Cancer Res. 1979 May;39(5):1645-50.

61. Sabbatini P, Tsuji T, Ferran L, Ritter E, Sedrak C, Tuballes K, Jungbluth AA, Ritter G, Aghajanian C, Bell-McGuinn K, Hensley ML, Konner J, Tew W, Spriggs DR, Hoffman EW, Venhaus R, Pan L, Salazar AM, Diefenbach CM, Old LJ, Gnjatic S. Phase I trial of overlapping long peptides from a tumor self-antigen and poly-ICLC shows rapid induction of integrated immune response in ovarian cancer patients. Clin Cancer Res. 2012 Dec 1;18(23):6497-508. doi: 10.1158/1078-0432.CCR-12-2189. Epub 2012 Oct 2.

62. Kano Y, Iguchi T, Matsui H, Adachi K, Sakoda Y, Miyakawa T, Doi S, Hazama S, Nagano H, Ueyama Y, Tamada K. Combined adjuvants of poly(I:C) plus LAG-3-Ig improve antitumor

effects of tumor-specific T cells, preventing their exhaustion. Cancer Sci. 2016 Apr;107(4):398-406. doi: 10.1111/cas.12861.

63. Matsui H, Hazama S, Tamada K, Udaka K, Irie A, Nishimura Y, Miyakawa T, Doi S, Nakajima M, Kanekiyo S, Tokumitsu Y, Shindo Y, Tomochika S, Yoshida S, Iida M, Suzuki N, Takeda S, Yamamoto S, Yoshino S, Ueno T, Nagano H. Identification of a Promiscuous Epitope Peptide Derived From HSP70. J Immunother. 2019 Sep;42(7):244-250. doi: 10.1097/CJI.0000000000000274.

64. Nakajima M, Hazama S, Tamada K, Udaka K, Kouki Y, Uematsu T, Arima H, Saito A, Doi S, Matsui H, Shindo Y, Matsukuma S, Kanekiyo S, Tokumitsu Y, Tomochika S, Iida M, Yoshida S, Nakagami Y, Suzuki N, Takeda S, Yamamoto S, Yoshino S, Ueno T, Nagano H. A phase I study of multi-HLA-binding peptides derived from heat shock protein 70/glypican-3 and a novel combination adjuvant of hLAG-3Ig and Poly-ICLC for patients with metastatic gastrointestinal cancers: YNP01 trial. Cancer Immunol Immunother. 2020 Aug;69(8):1651-1662. doi: 10.1007/s00262-020-02518-7. Epub 2020 Mar 26.

65. Nakajima M, Hazama S, Tokumitsu Y, Shindo Y, Matsui H, Matsukuma S, Nakagami Y, Tamada K, Udaka K, Sakamoto M, Saito A, Kouki Y, Uematsu T, Xu M, Iida M, Tsunedomi R, Suzuki N, Takeda S, Ioka T, Doi S, Nagano H. Phase I study of a novel therapeutic vaccine as perioperative treatment for patients with surgically resectable hepatocellular carcinoma: The YCP02 trial. Hepatol Res. 2023 Jul;53(7):649-660. doi: 10.1111/hepr.13900. Epub 2023 Mar 30.

66. Kurebayashi Y, Ojima H, Tsujikawa H, Kubota N, Maehara J, Abe Y, Kitago M, Shinoda M, Kitagawa Y, Sakamoto M. Landscape of immune microenvironment in hepatocellular carcinoma and its additional impact on histological and molecular classification. Hepatology. 2018 Sep;68(3):1025-1041. doi: 10.1002/hep.29904. Epub 2018 Jul 25.

67. Pagès F, Mlecnik B, Marliot F, Bindea G, Ou FS, Bifulco C, Lugli A, Zlobec I, Rau TT, Berger MD, Nagtegaal ID, Vink-Börger E, Hartmann A, Geppert C, Kolwelter J, et. al. International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic

and accuracy study. Lancet. 2018 May 26;391(10135):2128-2139. doi: 10.1016/S0140-6736(18)30789-X. Epub 2018 May 10.

68. Mlecnik B, Bifulco C, Bindea G, Marliot F, Lugli A, Lee JJ, Zlobec I, Rau TT, Berger MD, Nagtegaal ID, Vink-Börger E, Hartmann A, Geppert C, Kolwelter J, Merkel S, et. al. Multicenter International Society for Immunotherapy of Cancer Study of the Consensus Immunoscore for the Prediction of Survival and Response to Chemotherapy in Stage III Colon Cancer. J Clin Oncol. 2020 Nov 1;38(31):3638-3651. doi: 10.1200/JCO.19.03205. Epub 2020 Sep 8.

69. Mlecnik B, Torigoe T, Bindea G, Popivanova B, Xu M, Fujita T, Hazama S, Suzuki N, Nagano H, Okuno K, Hirohashi Y, Furuhata T, Takemasa I, Patel P, Vora H et. al. , Clinical Performance of the Consensus Immunoscore in Colon Cancer in the Asian Population from the Multicenter International SITC Study. Cancers (Basel). 2022 Sep 6;14(18):4346. doi: 10.3390/cancers14184346.

70. Mlecnik B, Lugli A, Bindea G, Marliot F, Bifulco C, Lee JJ, Zlobec I, Rau TT, Berger MD, Nagtegaal ID, Vink-Börger E, Hartmann A, Geppert CI, Kolwelter J, Merkel S et. al. Multicenter International Study of the Consensus Immunoscore for the Prediction of Relapse and Survival in Early-Stage Colon Cancer. Cancers (Basel). 2023 Jan 8;15(2):418. doi: 10.3390/cancers15020418.

71. Kuwahara T, Hazama S, Suzuki N, Yoshida S, Tomochika S, Nakagami Y, Matsui H, Shindo Y, Kanekiyo S, Tokumitsu Y, Iida M, Tsunedomi R, Takeda S, Yoshino S, Okayama N, Suehiro Y, Yamasaki T, Fujita T, Kawakami Y, Ueno T, Nagano H. Intratumoural-infiltrating CD4 + and FOXP3 + T cells as strong positive predictive markers for the prognosis of resectable colorectal cancer. Br J Cancer. 2019 Oct;121(8):659-665. doi: 10.1038/s41416-019-0559-6. Epub 2019 Sep 6. Erratum in: Br J Cancer. 2019 Nov;121(11):983-984. doi: 10.1038/s41416-019-0605-4.

72. Nakagami Y, Hazama S, Suzuki N, Yoshida S, Tomochika S, Matsui H, Shindo Y, Tokumitsu Y, Matsukuma S, Watanabe Y, Iida M, Tsunedomi R, Takeda S, Fujita T, Kawakami Y, Ogihara

H, Hamamoto Y, Ioka T, Tanabe T, Ueno T, Nagano H. CD4 and FOXP3 as predictive markers for the recurrence of T3/T4a stage II colorectal cancer: applying a novel discrete Bayes decision rule. BMC Cancer. 2022 Oct 18;22(1):1071. doi: 10.1186/s12885-022-10181-7.

73. Mazloom A, Ghalehsari N, Gazivoda V, Nimkar N, Paul S, Gregos P, Rateshwar J, Khan U. Role of Immune Checkpoint Inhibitors in Gastrointestinal Malignancies. J Clin Med. 2020 Aug 6;9(8):2533. doi: 10.3390/jcm9082533.

74. Inoue Y, Hazama S, Suzuki N, Tokumitsu Y, Kanekiyo S, Tomochika S, Tsunedomi R, Tokuhisa Y, Iida M, Sakamoto K, Takeda S, Ueno T, Yoshino S, Nagano H. Cetuximab strongly enhances immune cell infiltration into liver metastatic sites in colorectal cancer. Cancer Sci. 2017 Mar;108(3):455-460. doi: 10.1111/cas.13162.

75. Xu M, Tsunedomi R, Kiyotani K, Tomochika S, Furuya K, Nakajima M, Matsui H, Tokumitsu Y, Shindo Y, Yoshida S, Iida M, Suzuki N, Takeda S, Ioka T, Hazama S, Nagano H. Anti-VEGF and Anti-EGFR Antibody Therapy on T-Cell Infiltration and TCR Variation in Metastatic Colorectal Cancer. Anticancer Res. 2023 Feb;43(2):613-620. doi: 10.21873/anticanres.16197.