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Phase I Study of the Administration of Low-dose Perioperative Human Atrial Natriuretic Peptide in Patients With Resectable Colorectal Cancer

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Abstract. *Background/Aim: The aim of this single center, non-randomized, open-label, uncontrolled, interventional trial was to determine the feasibility of continuous administration of low-dose human atrial natriuretic peptide (hANP) perioperatively during curative operation for colorectal cancer patients without history of acute heart failure. Patients and Methods: The study included three males and two females ranging from 27 to 70 years old. Continuous intravenous injection of hANP solution was started before surgery. The primary endpoint was safety of hANP administration, and the secondary endpoints were perioperative changes in ANP, b-type natriuretic peptide, electrocardiogram (ECG), and lung function. Results: The American Society of Anaesthesiologists physical status was 1, 2, and 3 in three, one, and one patient, respectively. Grade 2 hypotension was observed in one case. No marked changes were observed between pre- and post-operation in all cases. Conclusion: Perioperative low-dose hANP administration is feasible and safe in patients with curative colorectal cancer.*

Colorectal cancer (CRC) is the third most common malignancy and the fourth leading cause of cancer-related mortality worldwide (1-3). Although surgical resection of the colon/rectum with lymph node dissection is the most effective therapy, additional therapies have been developed such as adjuvant therapy. Postoperative adjuvant fluoropyrimidine chemotherapy has been shown to prolong overall survival in patients with stage III CRC (4). The addition of oxaliplatin-containing chemotherapy to fluoropyrimidine-based chemotherapy has shown additional benefit for stage III CRC patients (5-7), and further studies have been performed to find the optimal adjuvant therapy for cancer recurrence prevention (8). The results of these studies indicated that postoperative adjuvant therapies benefit patients with CRC after curative surgery. However, no effective intraoperative intervention for the prevention of cancer metastasis is available in daily practice.

Human atrial natriuretic peptide (hANP) was identified in the human heart in 1984. It consists of 28 amino acids and exhibits a wide range of cardioprotective effects including anti-fibrosis, anti-hypertrophy, anti-inflammatory, and inhibition of sympathetic nerve activity, the renin–angiotensin–aldosterone system, and endothelin synthesis (9-11). ANP binds specifically to the guanylyl cyclase-A (GC-A) receptor (12). A previous study showed that metastasis of GC-A-nonexpressing tumor cells to the lung increased in vascular endothelium-specific GC-A knockout mice and decreased in vascular endothelium-specific GC-A transgenic mice compared with control mice (12). These results were also supported by a retrospective clinical analysis of patients with lung cancer (12). ANP may prevent cancer metastasis by inhibiting the adhesion of tumor cells to inflamed endothelial cells (12). This may be applicable in CRC; however, the feasibility of perioperative hANP administration in patients without severe cardiopulmonary dysfunction has not been established.

The aim of this interventional trial was to clarify the feasibility of intravenous administration of low-dose perioperative hANP in patients with resectable CRC.

Patients and Methods

Eligibility criteria. This study was a single-center, non-randomized, open-label, uncontrolled study, registered with UMIN-CTR,

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Key Words: Colorectal cancer, human natriuretic peptide, prevention of metastasis, feasibility.

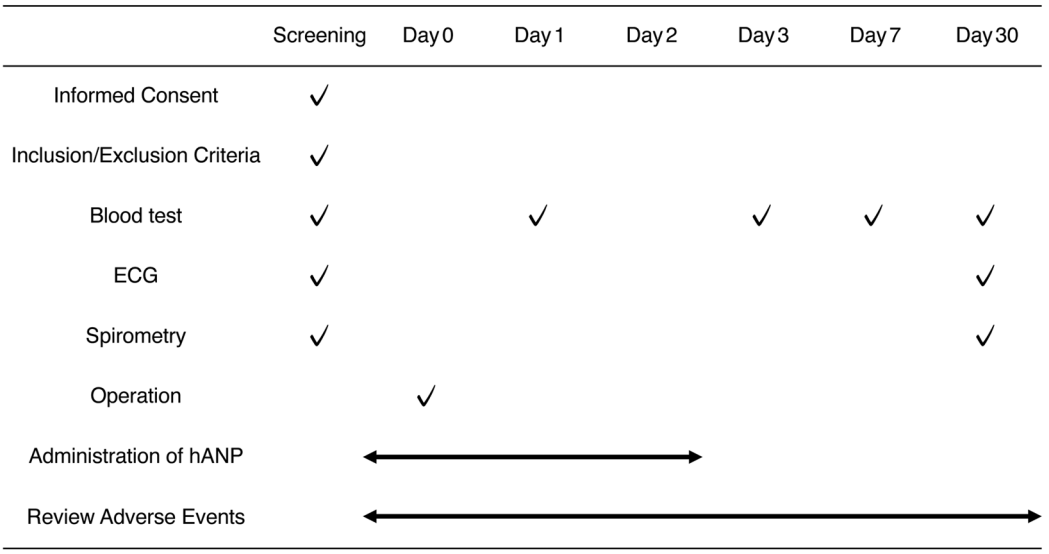

Figure 1. *Schedule of enrollment, interventions, and assessment. ECG: Electrocardiogram.*

Table I. *Patients enrolled in this study.*

Case	Age	Gender	Height (cm)	Weight (kg)	Past history	Smoking	ASA-PS	Spirogram	ECG
1	65	M	170.2	70.2		Never	1	WNL	WNL
2	33	F	157	44	Autonomic Ataxia	Never	1	WNL	WNL
3	27	M	170.6	66.4		Current smoker	1	WNL	WNL
4	70	F	149.4	62	DM, HT	Past smoker	2	Restrictive	WNL
5	67	M	180.7	77.8	COPD, Af*	Past smoker	3	Obstructive	WNL

COPD: Chronic obstructive pulmonary disease; DM: diabetes mellitus; Af: atrial fibrillation; HT: hypertension; ASA-PS: American Society of Anaesthesiologists physical status; WNL: within normal limits. Note: *treated with catheter ablation.

UMIN000025877. The study protocol was consistent with the principles of the Declaration of Helsinki, and the Ethics Committees of the Osaka University Hospital (Suita, Japan) approved the study. We enrolled five patients with primary CRC between April 1, 2017 and December 31, 2017. Inclusion criteria were: patients with histologically diagnosed CRC who planned to undergo curative surgery, Eastern Cooperative Oncology Group performance status of 0-1, age ≥20, major organs were functional [white blood cell count (WBC) >3000/mm^3, platelet count ≥100,000/mm^3, serum creatinine <1.5 mg/dl, total bilirubin <1.5 mg/dl, aspartate aminotransferase/alanine aminotransferase <100 IU/l], and provided written informed consent for study participation.

Registration. Eligible patients were registered preoperatively by calling the registration office after confirmation of the inclusion/exclusion criteria. An eligibility report form was sent to the Data Center at the Clinical Trial Coordinating Office at the Department of Gastroenterology, Osaka University Graduate School of Medicine. In this study, five patients received investigational treatment, and were analyzed according to the protocol. A total of seven cases were registered, but two patients withdrew their informed consent after registration.

Study design and treatment. This study was designed to determine the feasibility of continuous administration of low-dose hANP perioperatively during curative operation for CRC patients without a history of acute heart failure, which is the original indication for hANP. To prevent hypovolemia, extracellular fluid infusion was started at a speed of 80-100 ml/h at least 2 h before surgery. Carperitide (2000 μg, HANP FOR INJECTION®; Daiichi Sankyo Co., Ltd., Tokyo, Japan) was dissolved in 5 ml water for injection (Japanese Pharmacopoeia) and diluted in 45 ml saline, for a total volume of 50 ml hANP solution. Continuous intravenous injection of the hANP solution was started at a speed of 0.025 μg/kg/min at the start of extracellular fluid infusion. Continuous administration of hANP was continued for 72 h from the start of administration. If the attending physician determined that it was necessary, the dose was reduced by half. Administration of hANP was discontinued if hypotension persisted even with hANP reduction, if the attending physician determined that it was not desirable to continue hANP administration due to unexpected adverse events (AEs), or if the scheduled operation itself could not be performed.

Assessments. The primary endpoint of the study was the safety of hANP administration, and the secondary endpoints were perioperative plasma ANP (cut off line 0-43 pg/ml) and plasma B-

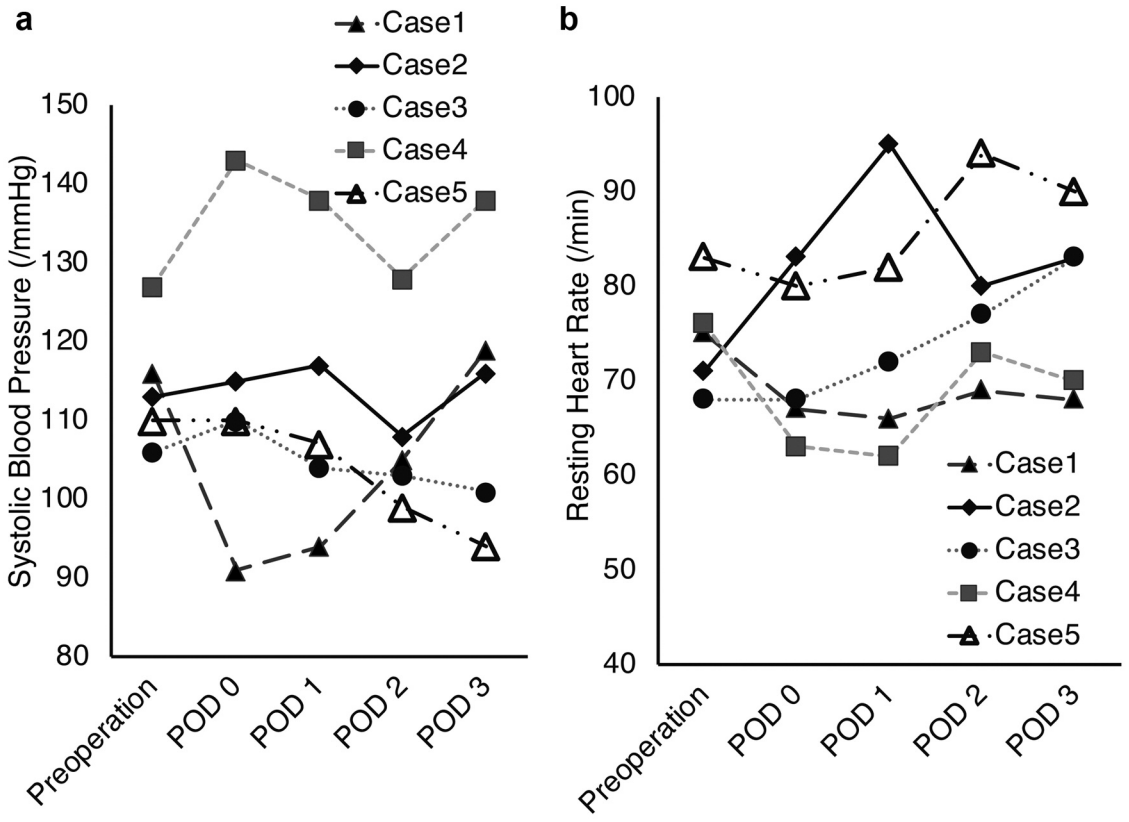

Figure 2. *Vital signs after surgery for CRC resection. Systolic blood pressure (a), and resting heart rate (b) were drawn using a line for each case.*

Table II. *Tumor characteristics and type of operation performed.*

Case	Tumor location	Clinical stage	CEA (ng/ml)	CA19-9 (U/ml)	Procedure	Operation time (min)	Blood loss (g)
1	Rectosigmoid	T3N0M0 Stage II	5	172	Anterior resection	217	40
2	Lower rectum	T3N2M0 Stage III	24	34	Abdominoperineal resection with pelvic and inguinal lymphadenectomy	949	450
3	Ascending colon	T3N2N0 Stage III	2	11	Right hemicolectomy	257	15
4	Sigmoid colon	T1N0M0 StageI	2	8	Sigmoidectomy	165	10
5	Upper rectum	T2N0M0 StageI	192	2	Low anterior resection	238	0

Clinical stage: according to the TNM classification 7th edition (12); Procedure: all cases received laparoscopic surgery; Blood loss: estimated blood loss.

type natriuretic acid (BNP, cutoff level 0-40 pg/ml) levels, electrocardiogram (ECG) changes, and lung function. The incidence of postoperative complications and whether or not catecholamines and diuretics were administered were evaluated. AEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v4.0 (13). The schedule of the study's assessment and evaluation including spirometry and ECG is described in Figure 1.

Results

Characteristics of patients enrolled in this study. Characteristics of the five patients prospectively enrolled in this study are summarized in Table I. The study enrolled three males and two females ranging in age from 27 to 70 years old (median, 65 years). The American Society of

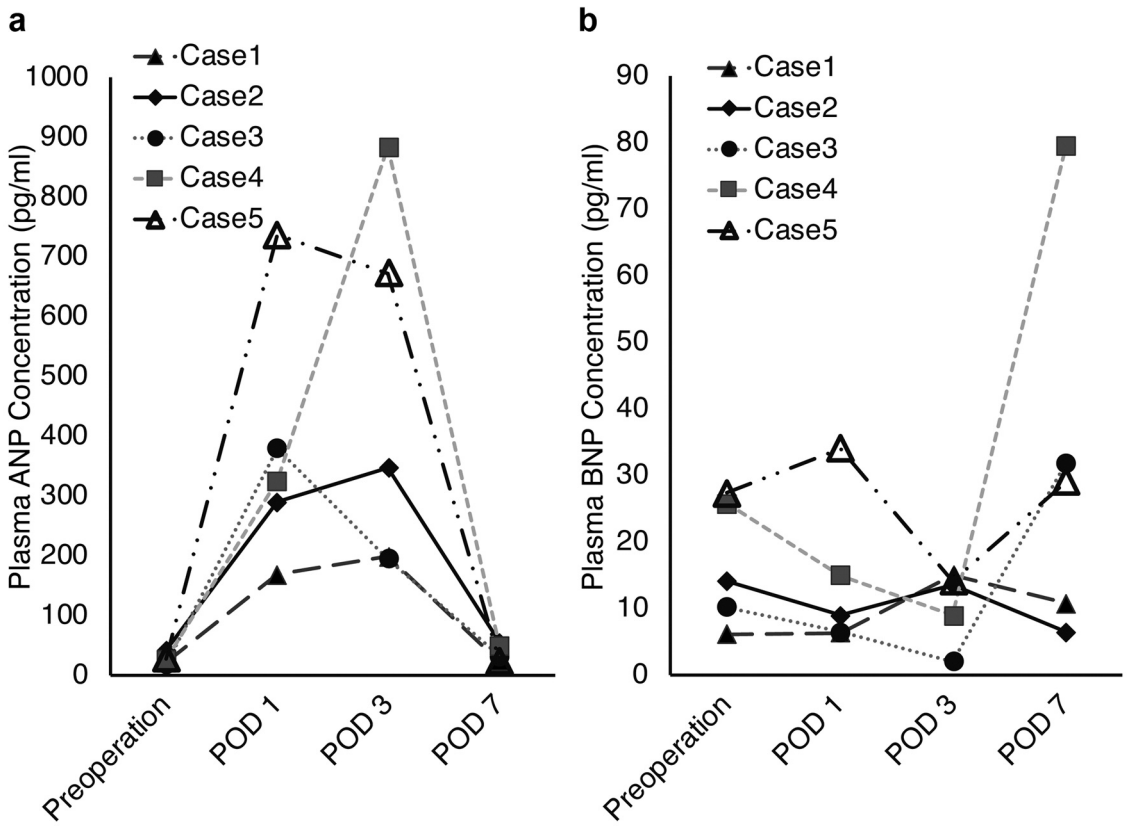

Figure 3. *Plasma ANP and BNP after surgery for CRC resection. Plasma ANP levels (a, cutoff line 0-43 pg/ml) and plasma BNP levels (b, cutoff line 0-40 pg/ml) were drawn using a line for each case.*

Anaesthesiologists physical status was 1 in three patients, 2 in one patient, and 3 in one patient. There were two never smokers, two past smokers, and one current smoker. Case 4 had restrictive pulmonary disease, and Case 5 had chronic obstructive pulmonary disease.

Operation findings. Operation findings including tumor location and clinical stage according to the tumor-node-metastasis classification (14) are summarized in Table II. Median operation time was 238 min (range=165-949 min), and median estimated blood loss was 15 g (range=0-450 g).

Trend of systolic blood pressure and resting heart rate. To understand perioperative vital signs, systolic blood pressure and heart rate were monitored. The trend of systolic blood pressure and heart rate were plotted in each case (Figure 2). In Case 1, Grade 2 hypotension was observed (Figure 2a), so the dose of hANP was reduced by half. In Case 4, systolic blood pressure was higher than in other cases, most likely due to the past history of hypertension. Resting heart rate in all cases was controlled between 60 to 100/min in the perioperation period (Figure 2b).

Trend of serum ANP and BNP levels. Plasma ANP and BNP levels were measured at pre-operation and post-operative day (POD) 1, POD 3, and POD 7, and plotted in each case (Figure 3a, b). Although, the dose of intravenous hANP administration was regulated at a speed of 0.025 μg/kg/min, plasma ANP levels were case-dependent. Peak plasma ANP levels were observed at POD 1 in two cases, and at POD 3 in three cases. Peak concentration of plasma ANP levels ranged from 199 to 884 pg/ml (median, 379 pg/ml). Plasma BNP levels in all cases changed within the normal range, except for Case 4 at POD 7 (79.4 pg/ml).

Change in cardiopulmonary tests. No marked changes were observed in spirometry and ECG findings between pre- and post-operation in all cases (data are not shown).

Trend of inflammatory response. To estimate AEs other than cardiopulmonary function, perioperative trend of WBC and plasma C-reactive protein (CRP) levels were monitored at pre-operation, POD 1, POD 3, POD 7, and 1 month after operation (Figure 4a, b). Peak WBC was observed on POD 1 in all cases. Prolonged CRP was observed in Case 2 compared to other

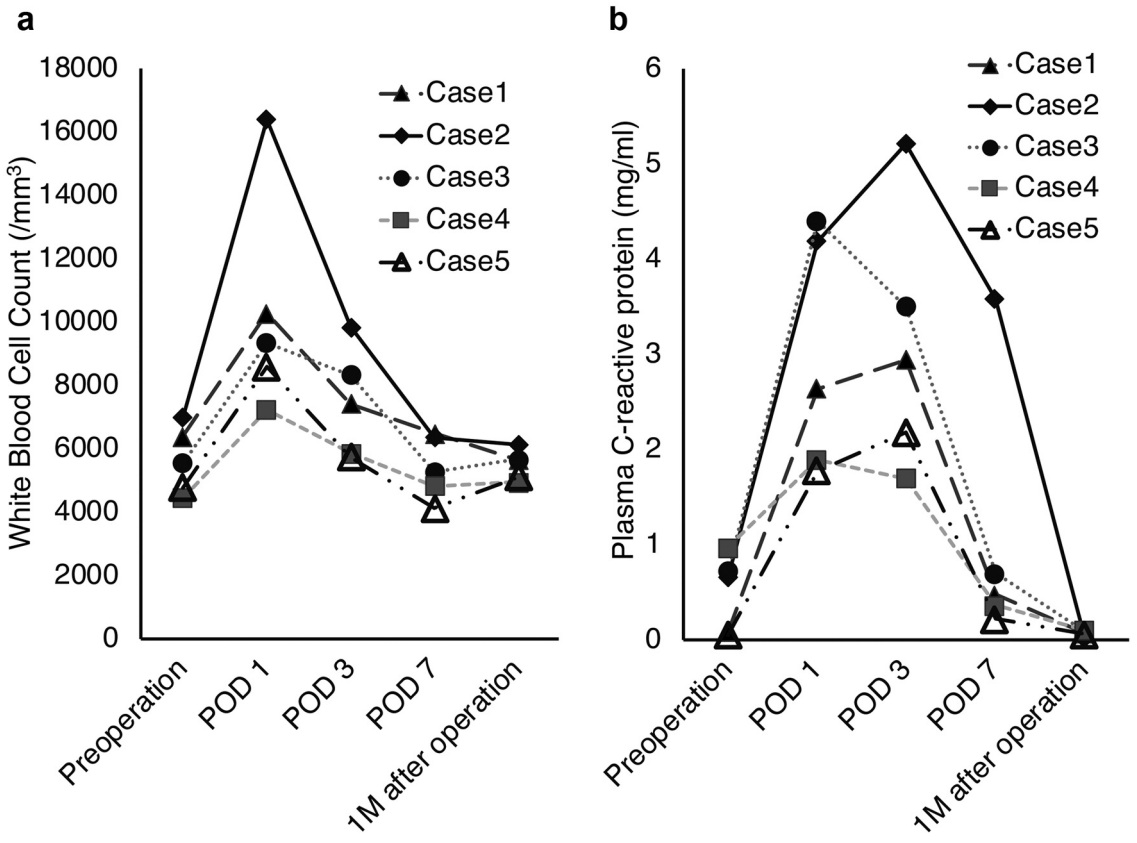

Figure 4. *Trend of inflammatory indicators after surgery for CRC resection. WBC count (a), and plasma CRP levels (b) were drawn using a line for each case.*

cases, probably due to the higher surgical invasion. There were no unexpected AEs that were attributed to hANP administration.

Discussion

There have been several reports about the scattering of cancer cells by surgical manipulation. As an approach to reduce cancer recurrence during surgery, Turnbull (15) proposed the "no touch" isolation technique for Dukes C CRC (15). A comparison of this technique with the conventional outer approach method for right hemicolectomy with D3 dissection revealed the superiority of the no touch isolation method for long-term survival (15-17). Evidence that cancer cells are being scattered during surgery have been proven by measurement of blood circulating tumor cells (18), cancer cells in ascites (19), and cell-free nucleic acid (20-22). Although the scattering of cancer cells has been detected intraoperatively, there are no available interventions to prevent intraoperative tumor scattering or engraftment of tumor cells, except for the careful manipulation of the organs to be resected.

Because hANP has beneficial effects on hemodynamics, neurohormonal balance, and inflammatory changes, it is reasonable to expect that hANP would protect against post-operative cardiopulmonary complications, and was approved by the Pharmaceuticals and Medical Devices Agency in Japan. In a retrospective analysis, lung cancer recurrence after curative surgery was significantly lower in ANP-treated (hANP administration) patients than in control patients (surgery alone) (12). In mouse models, metastasis of GC-A-nonexpressing tumor cells was increased in vascular endothelium-specific GC-A knockout mice and decreased in vascular endothelium-specific GC-A transgenic mice compared with control mice (12). These facts suggest that hANP might prevent cancer metastasis by inhibiting the adhesion of tumor cells to inflamed endothelial cells, even in CRC. However, evidence on the feasibility of intravenous hANP administration in patients who do not need it has not been previously reported, with the exception of one report on a 2 h administration in a healthy male (23). Therefore, this is the first report on the feasibility of continuous administration of hANP in CRC patients.

As shown in Tables I and II, hANP treatment was successfully performed in a wide variety of cases in this study. Except for Grade 2 hypotension, there were no other

AEs regardless of hANP involvement. In one of five patients (Case 1), blood pressure decreased on the day of surgery and the dose of hANP was decreased by half. One of the reasons for this hypotension might have been that the pre-operative BNP level in Case 1 was lower than that in the other cases (Figure 3b), but it is impossible to draw further conclusions from this study. Peak WBC counts were observed at POD 1, and peak CRP was mainly observed at POD 3 (Figure 4). These changes in inflammatory indicators were compatible with cases without inflammatory complications (24, 25). These findings also supported the feasibility of this trial treatment.

At present, no intervention is available as an adjuvant to prevent recurrence during curative surgery for CRC. The results of this study show that perioperative low-dose hANP administration is feasible and safe. As a limitation, carperitide has not been approved by the U.S. Food & Drug Administration or European Medicines Agency; however, the cost of hANP is about 1,800 Japanese yen per 1,000 μg, which is relatively inexpensive. Using this remedy currently used for acute heart failure to prevent cancer recurrence intraoperatively might be ideal in terms of drug repositioning (26).

Based on these results, we are currently completing a phase II trial to define the safety profile and evaluate the usefulness of perioperative low-dose hANP administration in preventing CRC recurrence.

Conflicts of Interest

The Authors declare that they have no conflicts of interest regarding this study.

Authors' Contributions

Hidekazu Takahashi contributed to the conception and design, acquisition of data, analysis and interpretation of data, drafting of the article and has made final approval of the manuscript. Takashi Takeda and Yujiro Nishizawa contributed to the conception and design, analysis and interpretation of data and has made final approval of the manuscript. Takayuki Ogino, Norikatsu Miyoshi, Chu Matsuda, Hirofumi Yamamoto, Tsunekazu Mizushima, Yuichiro Doki and Hidetoshi Eguchi contributed to the revision of the manuscript critically for important intellectual content and have made final approval of the manuscript.

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References

1 Siegel RL, Miller KD and Jemal A: Cancer statistics, 2018. CA Cancer J Clin *68(1)*: 7-30, 2018. PMID: 29313949. DOI: 10.3322/caac.21442

2 Ferlay J, Shin HR, Bray F, Forman D, Mathers C and Parkin DM: Estimates of Worldeide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer *127(12)*: 2893-2917, 2010. PMID: 21351269. DOI: 10.1002/ijc.25516

3 Matsuda T, Yamashita K, Hasegawa H, Oshikiri T, Hosono M, Higashino N, Yamamoto M, Matsuda Y, Kanaji S, Nakamura T, Suzuki S, Sumi Y and Kakeji Y: Resent updates in the surgical treatment of colorectal cancer. Ann Gastroenterol Surg *2(2)*: 129-136, 2018. PMID: 29863145. DOI: 10.1002/ags3.12061

4 Moertel CG, Fleming TR, Macdonald JS, Haller DG, Laurie JA, Goodman PJ, Ungerleider JS, Emerson WA, Tormey DC, Glick JH, Veeder MH and Mailliard JA: Levamisole and fluorouracil for adjuvant therapy of resected colon carcinoma. N Engl J Med *322(6)*: 352-358, 1990. PMID: 2300087. DOI: 10.1056/NEJM199002083220602

5 Andre T, Boni C, Mounedji-Boudiaf L, Navarro M, Tabernero J, Hickish T, Topham C, Zeninelli M, Clingan P, Bridgewater J, Tabah-Fisch I and de Gramont A: Multicenter International Study Group of Oxaliplatin/5-Fluorouracil/Leucovorin in the Adjuvant Treatment of Colon Cancer (MOSAIC) Investigators: Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. N Engl J Med *350(23)*: 2343-2351, 2004. PMID: 15175436. DOI: 10.1056/NEJMoa032709

6 Kuebler JP, Wieand HS, O'Connell MJ, Smith RE, Colangelo LH, Yothers G, Petrelli NJ, Findlay MP, Seay TE, Atkins JN, Zapas JL, Goodwin JW, Fehrenbacher L, Ramanathan RK, Conley BA, Flynn PJ, Soori G, Colman LK, Lecine EA, Lanier KS and Wolmark N: Oxaliplatin combined with weekly bolus fluorouracil and leucovorin as surgical adjuvant chemotherapy for stage II and III colon cancer: results from NSABP C-07. J Clin Oncol *25(16)*: 2198-2204, 2007. PMID: 17470851. DOI: 10.1200/JCO.2006.08.2974

7 Haller DG, Tabernero J, Maroun J, de Braud F, Price T, Van Cutsem E, Hill M, Gilberg F, Rittweger K and Schomoll HJ: Capecitabine plus oxaliplatin compared with fluorouracil and folinic acid as adjuvant therapy for stage III colon cancer. J Clin Oncol *29(11)*: 1465-1471, 2011. PMID: 21383294. DOI: 10.1200/JCO.2010.33.6297

8 Iveson TJ, Kerr RS, Saunders MP, Cassidy J, Hollander NH, Tabernro J, Haydon A, Glimelius B, Hakin A, Allan K, McQueen J, Scudder C, Boyd KA, Briggs A, Waterston A, Medley L, Wilson C, Ellis R, Essapen S, Dhadda AS, Harrison M, Falk S, Raouf S, Rees C, Olesen RK, Propper D, Bridgewater J, Azzabi A, Farrugia D, Webb A, Cunningham D, Hickish T, Weaver A, Gollins S, Wasan HS and Paul J: 3 *versus* 6 months of adjuvant oxaliplatin-fluoropyrimidine combination therapy for colorectal cancer (SCOT): an international, randomized, phase 3, non-inferiority trial. Lancet Oncol *19(4)*: 562-578, 2018. PMID: 29611518. DOI: 10.1016/S1470-2045(18)30093-7

9 Kangawa K and Matsuo H: Purification and complete amino acid sequence of α-human atrial natriuretic peptide (α-hANP). Biochemical Biophys Res Commun *118(1)*: 131-139, 1984. PMID: 6230082. DOI: 10.1016/0006-291x(84)91077-5

10 Nishikimi T, Maeda N and Matuoka H: The role of natriuretic peptides in cardioprotection. Cardiovasc Res *69(2)*: 318-328, 2006. PMID: 16289003. DOI: 10.1016/j.cardiores.2005.10.001
11 Woods RL: Cardioprotective function of atrial natriuretic peptide and B-type natriuretic peptide: a brief review. Clin Exp Pharmacol Physiol *31(11)*: 791-794, 2004. PMID: 15566395. DOI: 10.1111/j.0305-1870.2004.04073.x
12 Nojiri T, Hosoda H, Tokudome T, Miura K, Ishikane S, Otani K, Kishimoto I, Shintani Y, Inoue M, Kimura T, Sawabata N, Minami M, Nakagiri T, Funaki S, Takeuchi Y, Maeda H, Kidoya H, Kiyonari H, Shioi G, Arai Y, Hasegawa T, Takakura N, Hori M, Ohno Y, Miyazato M, Mochizuki N, Okumra M and Kangawa K: Atrial natriuretic prevents cancer metastasis through vascular endothelial cells. Proc Natl Acad Sci USA *112(13)*: 4086-4091, 2015. PMID: 25775533. DOI: 10.1073/pnas.1417273112
13 Division of Cancer Treatment & Diagnosis, National Cancer Institute: Common Terminology Criteria for Adverse Events (CTCAE) v4.0. https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40
14 Sobin LH, Gospodarowicz MK and Wittekind C: TNM classification of malignant tumors. 7th ed. New York: Wiley-Liss; 2011.
15 Turnbull RB, Jr., Kyle K, Watson FR and Spratt J: Cancer of the colon: the influence of the no-touch isolation technic on survival rates. Ann Surg *166(3)*: 420-427, 1967. PMID: 4967700. DOI: 10.3322/canjclin.18.2.82
16 Sugarbaker PH and Corlew S: Influence of surgical techniques on survival in patients with colorectal cancer. Dis Colon Rectum *25(6)*: 545-557, 1982. PMID: 6749455. DOI: 10.1007/bf02564164
17 Bruch HP, Schwandner O, Schiedeck TH and Roblick UJ: Actual standards and controversies on operative technique and lymph-node dissection in colorectal cancer. Langenbecks Arch Surg *384(2)*: 167-175, 1999. PMID: 10328170. DOI: 10.1007/s004230050187
18 Jiao LR, Apostolopoulos C, Jacob J, Szydlo R, Johnson N, Tsim N, Habib NA, Coombes RC and Stebbing J: Unique localization of circulating tumor cells in patients with hepatic metastases. J Clin Oncol *27(36)*: 6160-6165, 2009. PMID: 19884529. DOI: 10.1200/JCO.2009.24.5837
19 Runyon BA: Care of patients with ascites. N Engl J Med *330(5)*: 337-342, 1994. PMID: 8277955. DOI: 10.1056/NEJM19940 2033300508
20 Anker P, Mulcahy H, Chen XQ and Stroun M: Detection of circulating tumour DNA in the blood (plasma/serum) of cancer patients. Cancer Metastasis Rev *18(1)*: 65-73 1999. PMID: 10505546. DOI: 10.1023/a:1006260319913
21 Stroun M, Anker P, Lyautey J, Lederrey C and Maurice PA: Isolation and characterization of DNA from the plasma of cancer-patients. Eur J Cancer Clin Oncol *23(6)*: 707-712, 1987. PMID: 3653190. DOI: 10.1016/0277-5379(87)90266-5
22 Diehl F, Schmidt K, Choti MA, Romans K, Goodman S, Li M, Thornton K, Agrawal N, Sokoll L, Szabo SA, Kinzler KW, Vogelstein B and Diaz LA: Circulating mutant DNA to assess tumor dynamics. Nat Med *14(9)*: 985-990, 2008. PMID: 18670422. DOI: 10.1038/nm.1789
23 Richards AM, Tonolo G, Montorsi P, Finlayson J, Fraser R, Inglis G, Towrie A and Morton JJ: Low Dose Infusions of 26- and 28-Amino Acid Human Atrial Natriuretic Peptides in Normal Man. J Clin Endocrinol Metab *66(3)*: 465-472, 1988. PMID: 2965159. DOI: 10.1210/jcem-66-3-465
24 Warschkow R, Tarantino I, Torzewski M, Näf F, Lange J and Steffen T: Diagnostic accuracy of C-reactive protein and white blood cell counts in the early detection of inflammatory complications after open resection of colorectal cancer: a retrospective study of 1,187 patients. Int J Colorectal Dis *26(11)*: 1405-1413, 2011. PMID: 21701807. DOI: 10.1007/s00384-011-1262-0
25 Almeida AB, Faria G, Moreira H, Pinto-de-Sousa J, Correia-de-Silva P and Maia JC: Elevated serum C-reactive protein as a predictive factor for anastomotic leakage in colorectal surgery. Int J Surg *10(2)*: 87-91, 2012. PMID: 22222182. DOI: 10.1016/j.ijsu.2011.12.006
26 Ashburn TT and Thor KB: Drug repositioning: identifying and developing new uses for existing drugs. Nat Rev Drug Discov *3(8)*: 673-683, 2004. PMID: 15286734. DOI: 10.1038/nrd1468

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