Comparison of the effectiveness of integrative immunomodulatory treatments and conventional therapies on the survival of selected gastrointestinal cancer patients

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Table S1 Anamnestic data of colorectal cancer (CRC) patients receiving immunomodulating therapy (IMT), and 1:1, 2:1, 5:1 and 10:1 age, sex, side and metastasis matched CRC patients receiving conventional treatment only. Continuous and count data are presented as the mean ± standard deviation and the number of observations, respectively.

Parameter	**IMT (N = 21)**	**1:1 (N = 21)**	**2:1 (N = 42)**	**5:1 (N = 105)**	**10:1 (N = 210)**
Age (year)	49.00 ± 15.68	49.78 ± 12.98	51.73 ± 12.62	54.90 ± 10.85	59.48 ± 10.94
Sex (male : female)	7 : 14	8 : 13	14 : 28	46 : 59	107 : 103
TNM					
- T: 1/2/3/4/irresectable	2/2/8/7/2	2/0/11/8/0	4/2/19/14/3	5/11/53/29/7	10/26/108/45/21
- N: 0/1/2/3/irresectable	3/7/9/2	3/7/11/0	7/16/16/3	23/34/41/7	48/71/70/21
Location of the tumor					
- Cecum	2	1	4	10	19
- Ascending colon	2	2	5	12	23
- Transverse colon	1	0	1	7	11
- Descending colon	2	1	2	4	11
- Sigmoid colon	6	7	15	27	60
- Rectum	8	10	15	45	86
Sidedness					
- Left-sided	16	18	32	76	157
- Right-sided	5	3	10	29	53
Metastasis: synchronous / metachronous	11/9	11/8	21/17	58/41	127/69
Median survival since tumor diagnosis (month)	48.99	34.40	38.90	37.85	39.66
Survival rate since tumor diagnosis					
- 1-year (%)	100	100	100	100	100
- 2-year (%)	85.7	76.2	76.2	76.2	74.8
- 3-year (%)	61.5	47.6	52.4	51.4	53.3
- 4-year (%)	50.3	28.6	38.1	34.0	41.9
- 5-year (%)	22.4	19.0	24.3	29.9	33.4

TNM: Tumor – Node – Metastasis staging system.

Table S2 *p*-Values of the comparisons of the anamnestic data of colorectal cancer (CRC) patients receiving immunomodulating therapy (IMT), and 1:1, 2:1, 5:1 and 10:1 age, sex, side and metastasis matched CRC patients receiving conventional treatment only.

Parameter	**IMT vs 1:1**	**IMT vs 2:1**	**IMT vs 5:1**	**IMT vs 10:1**	**CMH-test**
Age (year)	0.8608 [a]	0.4929 [a]	0.1125 [a]	0.0067 [a]	–
Sex	1.0000 [b]	1.0000 [b]	0.4705 [b]	0.1693 [b]	0.1522
TNM					
- Stage T	0.4209 [b]	0.9300 [b]	0.6654 [b]	0.4894 [b]	0.4454
- Stage N	0.7518 [b]	0.9770 [b]	0.8259 [b]	0.7791 [b]	0.6200
Location of the tumor	0.9498 [b]	0.9530 [b]	0.9001 [b]	0.9556 [b]	0.7062
Sidedness	0.6965 [b]	1.0000 [b]	0.7984 [b]	0.5478 [b]	0.6448
Metastasis	1.0000 [b]	1.0000 [b]	0.9228 [b]	0.7047 [b]	0.5280
Median survival since tumor diagnosis (month)	0.4710 [c]	0.9830 [c]	0.9050 [c]	0.8980 [c]	–
Survival rate since tumor diagnosis	0.4658 [d]	0.9766 [d]	0.9107 [d]	0.9020 [d]	–

[a] Welch test; [b] Fisher's exact test; [c] Cox regression; [d] log-rank test; CMH-test: Cochran-Mantel-Haenszel test; TNM: Tumor – Node – Metastasis staging system.

Table S3 Anamnestic data of pancreatic cancer (PC) patients receiving immunomodulating therapy (IMT), and age, sex, tumor location and metastasis matched PC patients receiving conventional treatment with or without modulated electro-hyperthermia (mEHT). Continuous and count data are presented as the mean ± standard deviation and the number of observations, respectively.

Parameter	**IMT (N = 14)**	**Conventional (N = 14)**	**Conventional + mEHT (N = 14)**
Age (year)	55.83 ± 9.61	58.76 ± 7.53	59.64 ± 7.99
Sex (male : female)	5 : 9	4 : 10	4 : 10
Location of the tumor			
- Head	7	8	7
- Body	4	3	3
- Tail	3	3	4
Primary tumor irresectable	7	12	12
Metastasis: synchronous / metachronous	8/5	8/4	9/2
Median survival since tumor diagnosis (month)	28.99	11.71	14.08
Survival rate since tumor diagnosis			
- 1-year (%)	71.4	50.0	64.3
- 2-year (%)	50.0	0	28.6
- 3-year (%)	41.7	0	7.1

Table S4 *p*-Values of the comparisons of the anamnestic data of pancreatic cancer (PC) patients receiving immunomodulating therapy (IMT), and age, sex, tumor location and metastasis matched PC patients receiving conventional treatment with or without modulated electro-hyperthermia (mEHT).

Parameter	**IMT vs. Conventional**	**IMT vs. Conventional + mEHT**	**CMH-test**
Age (year)	0.3781 [a]	0.2649 [a]	–
Sex	1.0000 [b]	1.0000 [b]	0.7774
Location of the tumor	1.0000 [b]	1.0000 [b]	0.8257
Primary tumor irresectable	0.1032 [b]	0.1032 [b]	0.0114
Metastasis	1.0000 [b]	0.3179 [b]	0.3248
Median survival since tumor diagnosis (month)	0.0004 [c]	0.0462 [b]	–
Survival rate since tumor diagnosis	0.0002 [d]	0.0546 [d]	–

[a] Welch test; [b] Fisher's exact test; [c] Cox regression; [d] log-rank test; CMH-test: Cochran-Mantel-Haenszel test.

Literature data about the potential immunomodulators

Based on *in vitro* and animal model data, artesunate, curcumin, dichloroacetate, high-dose vitamin C, interferon-γ, and interleukin-2 have known anti-tumor effects. Without going into details, they can induce apoptosis or ferroptosis, inhibit tumor cell growth, suppress angiogenesis, inflammation and oxidative stress, reverse drug resistance, downregulate immunosuppression, and induce immune cell activity against cancer cells[1-7]. Moreover, it has been reported that a high dose of vitamin C in combination with anti-PD-1 checkpoint inhibition can increase cytotoxic T cell and NK cell activity in a lymphoma mouse model[8]. However, despite the wide knowledge regarding the effects of these treatment options on cancer cells and animal models, clinical study results are extremely limited, and largely only a single agent was used rather than any combinations.

Artesunate has been investigated in colorectal cancer (CRC) only: In a small-sized randomized trial, after a two-week pre-operative treatment with daily 200 mg *per os* artesunate, decreased Ki67 activity of the tumor has been found, and less recurrence has occurred compared to that of the placebo group (1 *vs*. 6)[9]. Similarly, a single study of CRC patients is available only in the case of interferon-γ, where it was concomitantly used with fluorouracil (5-FU) and interferon-α resulting in improved response rates, but with an increased 5-FU clearance[10]. Moreover, a limited number of data is available on dichloroacetate: 1-1 cholangiocellular cancer (CCC)[11], CRC[12], and gastric cancer (GC)[13] cases with prolonged stable disease after the introduction of dichloroacetate have been reported. No clinical results on gastrointestinal tumors could be found about ozone therapy[14].

Curcumin has been tested recently as a possible candidate in preventing the development of CRC[15,16], it is safe and well-tolerated by CRC patients[17-20], and it is able to reduce the side-effects of chemo(radio)therapy and improve patient's QoL[21,22]. Overall survival of metastatic CRC patients receiving FOLFOX (folinic acid + 5-FU + oxaliplatin) plus daily 2 g oral curcumin has improved, compared to those having FOLFOX only, without any difference in the progression-free survival[20]. It has to be noted that in another study[17], no difference in tumor response could be found. Moreover, curcumin has been found to be a potential booster for more effective immunotherapy[15], and there are a few randomized trials currently running, of which the results are eagerly awaited[16,23]. In pancreatic cancer (PC), poor bioavailability, even for the high, daily 12 g oral dose, and a high rate of serious adverse events caused by curcumin could have been reported[24,25]. The introduction of nanoparticle-coupled curcumin called

Theracurmin® and other newer curcumin analogs have shown more promising results[24-26], but the biological efficacy of curcumin is still low and the available clinical data is still scarce[27]. The same applies to GC: to date, no effective form of curcumin could have been found[28], and there is no clinical data on esophageal cancer (EC)[29] and CCC.

In the early '90s – including but not limited to the studies of Akiyoshi *et al.*[30], Atzpodien *et al.*[31,32], de Braud *et al.*[33], Lygidakis *et al.*[34], and Ridolfi *et al.*[35] –, subcutaneous and intravenous interleukin-2 have been tested in combination with standard chemotherapy regimens showing little improvements compared to treatments without interleukin-2. Later, in advanced and/or metastatic CRC, phase II and III studies investigated the efficacy and safety of the GOLFIG (gemcitabine + oxaliplatin + folinic acid + 5-FU followed by subcutaneous granulocyte-macrophage colony-stimulating factor + interleukin-2)[36-38] and the GILFICet (gemcitabine + irinotecan + folinic acid + 5-FU + cetuximab followed by subcutaneous interleukin-2)[39] chemoimmunotherapy regimens, and have found improved long-term progression-free survival, overall survival[36,37] and the treatment-related immunogenicity has been associated with increased antitumor activity[38,39]. A similar observation was confirmed in PC: neo- and adjuvant chemoimmunotherapy (gemcitabine + carboplatin + mitoxantrone + interleukin-2 + interferon-γ)[40,41] in combination with resective or palliative surgery have resulted in better and long-lasting response to treatment[41] and prolonged survival of patients for both types of surgery, compared to those with surgery alone[40,41].

High-dose vitamin C can reduce the inflammation in cancer patients[42], and its effect in combination with standard chemotherapy on CRC[43-46], PC[46-49], and GC[43] have been investigated in randomized trials. In a phase I study it was found that in terminal cancer patients disease progression had occurred on average after 8 weeks when administering a daily dose of 150 to 710 mg/kg vitamin C[50]. In advanced and metastatic CRC patients, no difference in response rates, overall-, and progression-free survival could have been justified[43-45]. In contrast, patients with RAS mutation have had better survival results if treated with high-dose vitamin C + FOLFOX with or without bevacizumab[44]. In metastatic PC, in combination with gemcitabine and erlotinib, the high-dose vitamin C treatment could have reduced the size of the tumor[47], and in combination with gemcitabine 3 months and 13 – 15.1 months progression-free and overall median survival have been reported, respectively[48,49].

The United States Food and Drug Administration (FDA) approved the use of 3 mg/kg nivolumab (normal dose) plus 1 mg/kg ipilimumab (low-dose) in CRC and in renal cancer, while a reversed dosing (1 mg/kg nivolumab + 3 mg/kg ipilimumab) was allowed for melanoma and hepatocellular carcinoma[51]. However, to date, the use of low-dose nivolumab (0.5 mg/kg)

in combination with low-dose ipilimumab (0.3 mg/kg) in the routine conventional oncological practice of any cancer is uncommon[51,52]. Some data are available from a limited number of phase I and II randomized clinical trials and from observational studies[51-54]: lower doses have been reported to have less toxicity, but the efficacy of the reduced doses is largely unknown. *In vitro*, animal models, and a few clinical studies have suggested that curcumin[55], dichloroacetate[56], high dose vitamin C[8,54,57], hyperthermia[54,58], interferon-γ[59], and interleukin-2[5,54,60] can/may increase the antitumor effects of ICIs, therefore, conducting randomized trials on the subject is urgently needed.

Studies investigating metronomic chemotherapy in gastrointestinal cancers have significantly emerged in recent years[61,62]. Although the toxicity of chemotherapeutic drugs improves during metronomic chemotherapy[61,62], the results of clinical studies are controversial. The most promising results have been found in PC[61,63], while the data on GC and EC is not conclusive[61,64]. In CRC, progression-free survival usually improves but no improvement in overall survival could have been justified[61,62,65], and there is virtually no data about CCC[61]. Similar to that of low-dose ICIs, randomized trials are required to fully investigate the effectiveness of metronomic chemotherapy in these tumors.

Whole-body hyperthermia (WBH) was investigated among the earliest studies of oncologic hyperthermia[66,67]. In most early studies, the number of cases was low, results have shown that WBH is a safe and effective treatment option[67], which can also improve the quality of life of patients[67,68], but in several cases, populations with mixed tumors were analyzed[68-72]. In addition to the improved disease response[73], WBH can prolong pain control for a longer duration in CRC/rectal cancer[74]. Moreover, post-treatment carcinoembryonic antigen (CEA) can be significantly lowered one month after WBH treatment in combination with ^{131}I anti-CEA monoclonal antibody[75]. Administering WBH preoperatively can improve the postoperative reactions of the immune system[76], while no conclusive data on systemic cancer multistep therapy (WBH plus induced hyperoxemia and hyperglycemia) could have been justified[77]. Improved disease response and prolonged survival have been also confirmed in GC[78] and in PC[79]. In the GC article[78], the authors could have also described improved Karnofsky Performance Status scores, decreasing the size of hepatic metastases, and decreasing the number of abdominal lymph nodes, and reduced rate of ascites development in the treatment arm[78].

The latest advancement in oncological hyperthermia is mEHT, which was developed later, and most data are available from the last decade[66,67,80]. In the studies of Fiorentini *et al.*[81,82], mEHT-treated PC patients had almost twice as long survival compared to those receiving

chemotherapy only. This strong observation could not be verified in a previous study conducted by our team[83]. Moreover, mEHT-treated PC patients having no metastasis and/or ascites benefit more from mEHT[83], and significantly better responses to treatment have also been described previously[81,82]. In addition, our team was able to justify, that the median overall survival time significantly improves in those PC patients, who receive more mEHT treatments, compared to matched non-mEHT treated PC patients[84]. Similar results have been described in three additional studies[85-87], where no comparison to control patients has been performed, but PC patients have had a better response to treatment and longer survival than normally expected[85-87]. In rectal cancer, preoperative concomitant mEHT has been associated with T- and N-downstaging[88-91] but with controversial survival data[90,91]. To our knowledge, only a few studies/case reports are available in CCC[92], CRC[93], EC[94], and GC[95], all with favorable responses to the treatment.

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